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by Holly R. Foster, Nina Herbert, Christian A. Di Buduo, Anna P. Schmidt, Juan Fang, Ratnashree Biswas, Momal Taimoor, Amie K. Waller, Daniel Howard, Thomas M. Vallance, Moyra Lawrence, Annett Mueller, Thomas Moreau, Amanda L. Evans, Ernest Turro, Roman Fischer, David A. Wilcox, Karin M. Hoffmeister, Alessandra Balduini and Cedric Ghevaert

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Title

Thyroid hormones induce an acute platelet release mechanism via integrin $\alpha V\beta 3$

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Thyroid hormones induce platelet production via $\alpha V\beta 3$

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Contributions

Conceptualization: HRF, NH, CADB, AB, CG Methodology: HRF, NH, CADB, JF ET, RF, DAW, KMH, AB, CG Investigation: HRF, NH, CADB, APS, JF, RB, MT, AKW, DH, TMV, ML, AM, TM, ALE, ET, RF Visualization: HRF, NH, CADB, ET, RF, CG Supervision: CG, AB, KMH, DAW, RF Writing-original draft: HRF, NH, CADB, ET, RF, DAW, KMF, AB, CG Writing-review & editing: HRF, NH, CADB, ET, AB, CG

Data-sharing statement

For original data please contact cg384@cam.ac.uk. Additional methods used in this study are in the Supplemental Material.

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Abstract

Understanding how mature megakaryocytes (MKs) release their platelets, and crucially, what are the triggers that facilitate this process is of huge impact on human medicine. Controlling this biological process, as well as being able to utilise *in vitro* produced platelets will be a major therapeutic advancement. Unfortunately, the exact mechanism and mediators that drive thrombopoiesis remain elusive. Here, we seek to identify such mediators through studying the dynamics of platelet production after an acute loss of platelets. Analysis of plasma taken from 19 plateletpheresis donors at various timepoints pre and post donation, identified peak platelet production timepoints (4-8 hours). Analysis of these timepoints by proteomic and metabolomic techniques allowed for the identification of Triiodothyronine (T3), as well as its analogues, GC-1 (Sobetirome), MGL-3196 (Resmetirom) and KB2115 (Eprotirome), as having a direct effect on *in vitro* platelet production in human cord blood (T3 3 hours 100nM 1.26±0.24 and GC-1 100µM 5.54±1.58, MGL-3196 300µM 6.92±1.38, KB2115 75µM 17.90±5.25 fold change at 12 hours, mean±SD) and iPSC-derived MKs (viral A1ATD1 KB2115 36.1µM 3.36±0.38, inducible QOLG1.1H KB2115 75µM 1.85±0.46 fold change, mean±SD). Receptor specific antagonists revealed that thyroid hormone induced platelet production primarily signals via the non-genomic signalling pathway, integrin $\alpha V\beta 3$ (CD51/61, vitronectin receptor), of which MKs highly express. When combined with silk-based-3-dimensional scaffold bioreactor technology, we observe a significant upscaling of platelets (KB2115 2.8±0.79 fold change±SD) that respond positively to agonist stimulation (P-selectin exposure). This shows the direct impact of thyroid on platelet production through integrin $\alpha V\beta 3$, which offers interesting therapeutic potential in the field of transfusion medicine.

Introduction

Platelets are a critical component of haemostasis and are now known to play a role in the innate immune system and tissue repair. They are derived from megakaryocytes (MKs) in the bone marrow. Mature MK lie close to the bone marrow sinusoids where they release platelets into the vasculature by budding off the ends of extensions, known as proplatelets or possibly via an “MK rupture” process^{1, 2}. Platelets then terminally mature in the circulation (marked by a decrease in size and loss of their mRNA content) and remain in the circulating system for approximately 10 days^{3, 4}. Platelet transfusions represent a crucial therapeutic tool for patients who are either actively bleeding (following trauma or surgery) or for patients with a severely reduced platelet count (thrombocytopenia) as a result of genetic disorders or malignancies and the (often) myelosuppressive treatment thereof. For a healthy adult, platelet counts range from 150-450x10⁹/L, with a third of all platelets being pooled in the spleen. To maintain these numbers an adult must produce approximately 1x10¹¹ platelets every day⁵. This production can increase 10-fold in times of stress and acute demand (e.g. bleeding).

We are totally reliant on blood donors to generate platelets which creates a range of issues. Platelets have a short shelf life, between 5-7 days (as opposed to 35 days for red blood cell units which can be refrigerated), making the supply management of platelets complicated in instances when there are acute changes to donor availability such as national holidays, natural disasters, and pandemics⁶. In addition, multi-transfused patients or multiparous women can become immunised against non-self HLA Class I antigens, relying on HLA matching. Finally, any allogeneic blood component exposes the recipient to the risk of transfusion-transmitted infection with bacteria (platelets, unlike other blood products, have to be kept at room temperature) and viruses, necessitating strict donor selection and microbiological screening programs that have to be constantly adapted to emerging new infectious agents. The generation of *in vitro*-derived platelets has the potential to address issues of supply, microbiological safety, and allo-immunogenicity (using for example genome-edited universal cells). Although the publication of highly efficient culture systems for the production of MKs from pluripotent stem cells has made this a possibility, the rate of release of functional platelets from the mature MKs even with advanced bioreactor systems remains several orders of magnitude below the estimated rates of release of platelets per MK *in vivo* (30-80 platelets per MKs vs 1000 to 2000 platelet per MK respectively)⁷⁻¹².

In this manuscript, we use platelet donation as a model of acute platelet loss in order to identify soluble mediators in the blood that can improve the upscaling of *in vitro* platelet production.

Methods

For more information, please see Supplementary Materials and Methods

Blood sampling of apheresis/plasmapheresis donors and plasma isolation

19 healthy male plateletpheresis donors who donate regularly were recruited, and full consent was given in accordance with East of England-Cambridge Central Research Ethics Committee (14/EE/0194). 5 healthy male plasmapheresis donors were recruited, and collections done under the IRB approved “Healthy Donor Bank” protocol (PRO00026243).

Cord blood-derived megakaryocytes

CD34+ cells were isolated from cord blood obtained with full consent in accordance with Cambridgeshire 4 Research Ethics Committee (07/MRE05/44), the Ethical Committee of the I.R.C.C.S. Policlinico San Matteo Foundation of Pavia, and the principles of the Declaration of Helsinki.

Proplatelet assay

In brief, proplatelet assays were performed on 200 μ g/ml fibrinogen coated slides and incubated for 37 °C for indicated timepoints. Cells were permeabilised with 0.1% saponin/0.2% gelatin and stained with mouse anti-human α -Tubulin (1/250, clone B-5-1-2, Merck), phalloidin CruzFluor-555 (sc-363784, Santa Cruz) and DAPI.

Platelet production assay

In brief, platelet production assays were performed in high-glucose RPMI (Thermo Fischer Scientific, A10491) at 37 °C and analysed at indicated timepoints. For experiments with antagonists, cells were pre-incubated for 30mins at 37 °C prior to start of assay. Platelets were analysed by flow cytometry using CD41a-APC H7 (1/200, clone HIP8, BD Pharmingen), CD42a-APC (1/100, clone REA209, Miltenyi Biotec) and Calcein AM (C3100MP, Thermo Fisher Scientific).

Silk bone marrow model

Silk fibroin aqueous solution was obtained from Bombyx mori silkworm cocoons according to previously published literature¹⁶. MKs were stained with anti-CD61 (1/100, Beckman Coulter) and Alexa Fluor secondary antibody (1/500, Invitrogen) for imaging. For ex vivo platelet production, a custom flow chamber was produced and perfused as previously described.¹³ The perfusion of the silk scaffold was performed with a basic medium (DMEM, Euroclone) containing KB2115 and analysed by flow cytometry.

Platelet activation assay by flow cytometry

In brief, platelet activation assays were performed in high-glucose RPMI and stimulated with ADP and Thrombin (Merck) for 30mins at 37 °C in the presence of 1mM CaCl₂ (Merck), CD41-APC H7 (1/200), P-selectin-APC (1/30, clone AK4, 304910 Biolegend), Calcein violet (for platelets 2.5pg/ml, for whole blood 12.5pg/ml, Thermo Fischer Scientific). Samples were fixed with 0.2% formal saline and analysed with a flow cytometer (Gallios).

Results

Platelet donation is followed by the rapid release of newly formed platelets

Nineteen Caucasian male individuals who regularly give platelets by apheresis were recruited (Supplementary Table 1). To analyse the dynamics of change in the blood indices, blood samples were taken pre-, immediately post-donation and subsequently at 4-8 hours and day 1, 3, 7 and 14 post-donation. As expected, the platelet counts dropped significantly by 0.65 \pm 0.06 fold, immediately post-donation (mean \pm SD, p <0.001, Fig. 1a). This was accompanied by a significant increase in both immature platelet fraction (IPF) and mean platelet volume (MPV) that peaked at 4-8 hours post donation (Fig.1b-c, Supplementary Table 2). The recovery in the platelet count showed a lag with the rise in IPF, with only a marginal increase in the platelet count

(non-significant) between day 1 and 3 followed by a much more rapid and significant rise between day 3 and 7, by which point the platelet count had recovered back to baseline levels ($p < 0.001$, Supplementary Table 3). There was also an increase in lymphocyte counts and red cell distribution width at the 4–8 hour time point (Supplementary Table 2).

To rule out an effect of the apheresis process itself, 5 healthy male plasmapheresis donors were recruited and sampled at the same time points (Supplementary Table 4). Although there were no significant changes in the platelet counts or the IPF post plasma donation (Fig. 1d-e), we noted a small decrease in MPV 4-8 hours after donation (Fig. 1f). There was also a small increase in the neutrophil and lymphocyte counts in the 4-8 hours samples post plasma donation (Supplementary Table 5).

The rapid release of newly formed platelets is triggered by signals contained within the plasma

We hypothesized that the signal(s) that trigger an acute release of platelets would be contained within the plasma compartment. To verify this, we added plasma isolated from platelet apheresis donor samples taken at baseline, 4-8 hours and day 14 post-donation, to cord blood-derived MK (CBMK) cultures. All following experiments were carried out using serum or plasma from donors who exhibited high fold changes at the 4-8 hour timepoint in MPV and IPF compared to the pre-donation control (Supplementary Fig. 1). Incubation of CBMKs for 48 hours with 4-8 hour plasma increased the percentage of MKs forming proplatelets by 1.49 ± 0.57 fold compared to MKs cultured with pre-donation plasma, although not significantly (mean \pm SD, $p = 0.061$, Fig. 1g-h and Supplementary Fig. 2). Platelet production increased 1.19 ± 0.09 fold at 4-8 hours plasma compared to cultures incubated with the pre-donation plasma (mean \pm SD, $p < 0.01$, Fig. 1i, Supplementary Fig. 3a shows gating strategy and Supplementary Fig. 3b).

Identifying candidate triggers for platelet release

We first focused on known signals that may promote platelet production. Analysis by ELISA showed no statistically significant changes in serum thrombopoietin (TPO) with any timepoint post-donation compared to the pre-donation control (Supplementary Fig. 4a).

To identify potential novel candidates that drive the acute release of newly formed platelets, 3 types of analyses were performed on the plasma samples: mass spectrometry, metabolomics (Metabolon) and Luminex-based quantitation of 56 candidate growth factors/cytokines (R&D). The analyses were carried out on three timepoints, the point at which there was the biggest increase in both IPF, MPV and effect on platelet production *in vitro* (4-8 hours) compared to baselines, pre-donation and Day 14. Volcano plots of all the analytes at time point 4-8 hours analysed against the baseline (pre-donation and Day 14) highlight that most do not see significant variation compared to baseline measurements (Fig. 2a-c). Significant increases at the 4-8 hours timepoint were observed in: mass spectrometry, 26 proteins; metabolomics, 111 metabolites and multiplex bead assay, 3 cytokines/growth factors (Supplementary Tables 6-11).

Nine analytes were selected to be screened in platelet production assays using CBMKs, based on novelty, links to platelet activation (for exclusion) and receptor expression. Alpha 1-acid glycoprotein, L-Carnitine, Leu-Gly, Succinimide, ECM-1 and Chemerin all showed no significant increases in platelet production compared to the control (Supplementary Fig. 4b-g). SerpinA7 (thyroid binding globulin, TBG) had

a significantly increased plasma concentration in both the mass spectrometry analyses and the Luminex screen (Fig. 2a and 2c). SerpinA7 is the major carrier protein for the thyroid hormones thyroxine (T4) and triiodothyronine (T3). CBMKs stimulated with biologically relevant concentrations of T3 (1-100pM), showed a significant increase in platelet production (Fig. 2d). There were no significant increases in platelet production with T4 or its metabolite, diiodo-L-thyronine (T2, Fig. 2e-f, Supplementary Fig. 5).

Free T3 levels were analysed in our plateletpheresis donors (Fig. 3a and Supplementary Table 12). A rise of free T3 serum levels can be observed at D1 after platelet donation, reaching a peak and significance at D3. No differences were observed in thyroid stimulating hormone (TSH) levels in the plateletpheresis donor samples and no differences of free T3 levels were observed in plasmapheresis donors post plasma donation (Supplementary Fig. 6a and Fig. 3b).

A mouse model of acute platelet depletion was also tested in wild type male C57BL/6J mice using an antibody against mouse CD42b (Fig. 3c). Administration of 0.6µg/g BW anti-CD42b led to a significant decrease in platelet count within the first 24 hours post-injection with a return to baseline by D5 (Fig. 3d, Supplementary Table 13). Significant increases in free T3 levels were observed D3 post depletion compared to the mice injected with vehicle control (Fig. 3e). Incidentally, analysis of serum TPO concentrations post depletion showed an increase in TPO levels 24 and 48 hours post depletion, returning to baseline levels at 72 hours (Supplementary Fig. 6b).

Thyroid hormones acutely increase platelet production

To further verify our findings, commercially available thyroid hormone analogues were also tested. CBMKs were incubated with three different thyroid hormone analogues; GC-1 (Sobetirome), MGL-3196 (Resmetirom) and KB2115 (Eprotirome, Supplementary Fig. 5). All analogues induced a robust dose-dependent increase in platelet production after 6-8 hours (Fig. 4a-c). This rise in the platelet count was not seen when using an albumin-containing culture medium which binds the thyroid hormone and analogues (Supplementary Fig. 7a). Time-course experiments of all 3 analogues revealed that all induced a very rapid increase in platelet production (Fig. 4d-f) reaching statistical significance as early as 8 hours, with maximum fold changes being observed after 12 hours (GC-1 100µM 5.54±1.58, MGL-3196 300µM 6.92±1.38, KB2115 75µM 17.90±5.25 fold change at 12 hours, mean±SD). We also observed dose-dependent increases in platelet production with MKs from two different hPSC sources using two different differentiation protocols (viral A1ATD1 KB2115 36.1µM 3.36±0.38 and inducible QOLG1.1H KB2115 75µM 1.85±0.46 fold change, mean±SD, Supplementary Figure 7b-e).

Flow cytometry analysis of the size and density (forward scatter and side scatter) of the platelet-sized events within our thyroid hormone stimulated CBMK cultures revealed a 'new population' of platelets, not observed with hPSC-derived MKs (population 'B', Supplementary Fig. 8a and Supplementary Fig. 7d-e) larger than the platelet sized events observed in the vehicle control (population 'A'). Population B represented 37.1±11.40% of the total platelet population in the KB2115 treated sample as opposed to 17.5±10.91% in the vehicle control (Supplementary Fig. 8b, mean±SD) and had a significant increase in both viability (calcein-AM, Supplementary Fig. 8c) and purity (CD41a⁺/42a⁺, Supplementary Fig. 8d) over platelets in population A.

Proplatelet formation assays revealed a rapid activation of MKs with over 60% of the MK population exhibiting signs of spreading by 2 hours (indicated by striated F-actin

patterns, Supplementary Fig. 9a-b), compared to <5% in the vehicle control. By 8 hours, KB2115 stimulated MKs showed a 2-fold increase in the percentage of proplatelet forming MKs (Fig. 5a-b).

Thyroid hormone analogues promote platelet release primarily through a pathway downstream of integrin $\alpha V\beta 3$

We sought to investigate which receptors and downstream pathways are responsible for the phenotype observed. Quantitative RT-PCR and flow cytometry data confirmed expression of both thyroid hormone receptors (THRA/B) and the vitronectin receptor (integrin $\alpha V\beta 3$, Fig. 6a-c).

To confirm which receptor the thyroid hormone agonists promoted platelet formation, CBMKs were preincubated for 30 minutes with either tetrac, an inhibitor of $\alpha V\beta 3$ or 1-850, a THRA/B antagonist and then stimulated with the most potent of the T3 analogues, KB2115. Antagonism of the $\alpha V\beta 3$ receptor caused a 0.41 ± 0.06 fold inhibition of the KB2115-induced platelet production, while the THRA/B antagonists had only a limited effect (mean \pm SD, tetrac $p < 0.05$, Fig. 6d, controls shown in Supplementary Fig. 10a-b). Analysis of the different FS/SS A and B platelet populations from CBMKs, described above, showed that tetrac significantly inhibited the appearance of the newly produced platelet almost exclusively in population “B” (Supplementary Fig. 8e-f). To further confirm that thyroid hormones signal downstream of the $\alpha V\beta 3$ receptor in MKs, primary MKs were differentiated from $\beta 3^{-/-}$ KO and control mouse bone marrows and stimulated with $75 \mu\text{M}$ KB2115 for 6 hours. The wild type controls exhibited a 1.21 ± 0.08 -fold increase in platelet production (mean \pm SD, $p < 0.05$) with KB2115 but this response was completely abrogated in the $\beta 3^{-/-}$ KO MKs (Fig. 6e).

To gain a measure of how significant a contribution signalling downstream of $\alpha V\beta 3$ plays into the platelet count recovery post-acute platelet depletion, wild type mice we treated intravenously with daily doses of tetrac or vehicle control. Untreated mice platelet counts returned to baseline levels 4 days after depletion, Fig. 6f. Tetrac treated mice showed a much slower recovery rate, with platelet counts returning to baseline levels between day 8-10.

The signalling components responsible for the promotion of platelet release following binding of thyroid hormones to integrin $\alpha V\beta 3$ were investigated by pre-incubating CBMK cultures for 30 minutes with specific inhibitors for intracellular calcium mobilisation (BAPTA-AM), PKC (Gö6983), PI3K (LY294002) or MEK (U-0126), and then stimulated with KB2115. BAPTA-AM, Gö6983 and U-0126 all significantly inhibited KB2115 induced platelet production, whilst LY294002 had a very limited effect (Fig. 6g).

Application to the production of platelets in vitro: platelet production from pluripotent stem cells and 3D culture systems

We assessed whether thyroid hormone analogues promote *ex vivo* platelet production by CBMKs in the silk-based bone marrow model functionalized with $25 \mu\text{g/mL}$ fibronectin to support cell adhesion as previously published¹³⁻¹⁵. The silk bone marrow model was enclosed into a flow chamber which allowed the perfusion of medium containing $75 \mu\text{M}$ KB2115, or vehicle as control (Fig. 7a).

Confocal microscopy imaging of the 3D culture, after 4 hours of perfusion, demonstrated the presence of proplatelet forming MKs (Fig. 7b). After flow through of the culture medium, the analysis of platelet count demonstrated significantly increased numbers of $\text{CD41}^+ \text{CD42b}^+$ platelets in medium containing KB2115

(2.8 ± 0.79 fold change \pm SD, Fig. 7c). The morphological characterisation of the released particles highlighted the presence of both disc-shaped β 1-tubulin⁺ platelets of 1–4 μ m diameter and pre-platelets intermediates of >4 μ m diameter (Fig. 7d). Stimulation of these platelets with ADP and thrombin showed an increase in P-selectin exposure as assessed by flow cytometry analysis, compared to the untreated controls (Fig. 7e), and similar to peripheral blood platelets (Supplementary Fig. 11).

Discussion

Understanding how mature MKs release their platelets, and crucially, what are the triggers that facilitate this process are of huge impact on human medicine. Controlling this biological process therapeutically could lead either to an increase of platelet release for patients who are thrombocytopenic (and at risk of bleeding) or reduce platelet release for patients suffering with myeloproliferative diseases such as essential thrombocythemia where the platelet count is increased to the point that the major morbidity is from thromboembolic events

In this study, we demonstrate through serial sampling of human platelet apheresis donors that the sudden drop in the platelet count (around 30%) over the donation period (1-1.5 hour) leads to the very prompt release of newly formed platelets (in a matter of hours) which cannot reflect an increase in megakaryopoiesis, but very likely the sudden increase of platelet production from a pre-existing pool of mature MKs. Consistent with other studies, IPF and MPV values immediately increase post-donation and this is followed by a concerted platelet count recovery between D3 and D7 post donation¹⁶⁻¹⁸. Platelet donation is an intense process, see Supplementary Fig. 12, and it is not unreasonable to speculate that the process itself, the return of processed blood, could initiate proinflammatory responses post donation. We indeed see an immediate rise in white cells reflecting this in samples taken from both platelet apheresis donors and donors undergoing plasmapheresis (Supplementary Table 2 and 5). However, the rise in IPF and MPV is only seen in the donors who give platelets showing that the sudden release of newly formed platelets is a specific response to the drop in the platelet count and not the result of the apheresis process itself.

We hypothesized that the signal that triggers this sudden platelet release is contained within the plasma compartment, an idea reinforced by our observation that the addition of plasma taken post-donation promoted both proplatelet formation by cultured MKs as well as their platelet release *in vitro*. Screening of selected upregulated analytes, or their cargo (in the case of carrier proteins), in platelet production assays identified that biologically relevant concentrations of T3 (1-10pmol/L) exhibited a subtle but significant increase in platelet production. An effect, interestingly not seen with other biologically active native thyroid hormones, T4 or T2. Analysis of free T3 in both human and mouse acute platelet depletion models showed a significant increase in serum levels but interestingly not at the earlier timepoints when IPF and MPV are at their peak. This increase in free T3 levels coincides with the timepoint where we observe the most significant difference in platelet numbers. This causal link between thyroid hormones rise and increased platelet production was confirmed *in vitro* with thyroid hormone analogues, which, when incubated with cultured MKs, produced from multiple stem cell sources, robustly induced dose-dependent increases in platelet production as well as increased proplatelet formation. This effect was shown to signal mainly through the non-classical α V β 3 receptor rather than the classical THRA/B receptor. Moreover, in the

murine model of acute platelet depletion we showed that blocking $\alpha V\beta 3$ signalling significantly delayed platelet recovery.

Interestingly, Xu B *et al.* have previously shown that $CD34^+$ cells differentiated into MKs in the presence of low dose T3 can have an effect on MK development and by extension platelet production although no direct effects of T3 on platelet production were studied, i.e. T3 as an agonist of terminal differentiation,¹⁹. This however supports that MKs truly have the signalling capabilities to be modulated by thyroid hormones.

A second morphologically distant (FS/SS) platelet population was observed in response to KB2115 treatment but this phenomena was exclusively observed in CBMK static 2D cultures. In static, 2D systems, CBMKs baseline ability to generate platelets is considerably lower than that of hPSC-derived MKs, ~ 0.2 platelets/MK as opposed to $0.5-1$ platelets/MK respectively¹⁴. This difference is extended further when CBMK cultures are moved from a static system to a more dynamic, 3D system that we encounter with the silk scaffolds (>1 platelet/MK)¹³⁻¹⁵. The baseline MK 'state' will significantly affect how it responds to outside stimuli, whether this is maturity or a physical environment that is conducive to platelet production, which may explain why we see differences in the platelets produced. Therefore, the observation of 2 distinct platelet populations may be a feature of a sub-optimal culture system rather than a true reflection of KB2115 actions.

Taken together, our observation *in vitro* of T3 promoting platelet production and increases in free T3 *in vivo* at timepoints where platelet production is actively increasing, strongly suggests that T3 is a direct regulator of platelet production. It is very likely that T3 acts in concert with other signals to increase the release of platelet from MKs. First, its peak does not correspond with the early time point during which the IPF reaches a maximum. Second, blocking $\alpha V\beta 3$ integrin and thereby T3 effect on MKs merely delayed the platelet recovery in mice, but did not abrogate it.

Previous studies have reported platelet count changes in patients with either hypo- or hyperthyroidism and in response to treatment with either thyroid hormones supplement or anti-thyroid drugs. Ijaz *et al.* analysed healthy individuals with no history of thyroid disease and found that increase serum total T4 levels correlated with increase platelet counts²⁰. No link was made between T3 or TSH levels with platelet count and no pituitary-thyroid axis hormones with MPV. Gullu *et al.* studied overt and subclinical hypothyroidism patients before and after treatment with Levothyroxine²¹. They found that both patients subsets had increased platelet activity that could be reversed with Levothyroxine, with overt hyperthyroidism patients showing increases in platelet count post treatment. Conversely, Panzer *et al.* have shown that anti-thyroid drugs given to hyperthyroidism patients increase the platelet counts. Sullivan *et al.* and Kurata *et al.* have both shown platelet counts dropping after thyroid hormone treatment in mice and hyperthyroid rats, respectively²²⁻²⁴. These conflicting results may reflect the fact that thyroid hormones may have an effect on both haemopoietic stem cells and their differentiation into MKs (which we did not examine in this study given the immediate platelet release seen in the donors) and, additionally, on the very last stage of thrombopoiesis and the acute release of platelets. The former effect on stem cell differentiation would correlate with chronic changes in thyroid hormones, whilst the latter would reflect acute changes such as those analysed in this study.

The endeavour of producing clinically relevant quantities of platelets in culture for transfusion (each unit used for adult patients contains 3×10^{11} platelets) has long been impaired by the enormous cost-of-goods associated with the manufacturing process.

As demonstrated in this study, thyroid hormone analogues can be integrated into this process by applying it to a 3-dimensional bone marrow system. The gains made (2-fold increase) do not bring the amount of platelet released per MKs to the estimated levels *in vivo* (1000 platelets per MK) but represent a significant step forward reducing the timing and volume needed for *ex vivo* human platelet production which represent a large proportion of the manufacturing cost-of-goods²⁵.

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Figure legends

Figure 1. Analysis of the dynamics of platelet production post-acute loss of platelets. Analysis of (a, d) platelet count, (b, e) immature platelet factor (IPF), (c, f) mean platelet volume (MPV), at the indicated timepoints (pre pheresis donation [pre], immediately post pheresis [post], days post pheresis [D]), of healthy volunteers donating (a-c) platelets, n=19 or, (d-f) plasma, n=5, data expressed as relative to pre-donation. CBMK incubated with 10% plateletpheresis plasma from the indicated timepoints for 48 hours, (g) representative image of a proplatelet forming MK, α -tubulin (green), DAPI (blue), buds at end of extensions (red arrows), swellings along shaft (white arrows), scale bar = 10 μ m, n=3; quantification of (h) proplatelet formation by IF, n=5, and (i) platelet production by flow cytometry, n=5, both expressed as relative to MKs incubated with pre-donation plasma, each dot represents a different donor. All data expressed as mean \pm SD, (a-f) one-way ANOVA with Dunnett's multiple comparison test, (h-i) paired student T-test, *p<0.05, **p<0.01, ***p<0.001, not statistically significant (ns).

Figure 2. Identification and analysis of modulated analytes post-acute loss of platelets. Analysis of plasma from plateletpheresis donors for (a) proteins by mass spectrometry, n=5, (b) metabolites by metabolomics (Metabolon), n=11 and, (c) growth factors, chemokines and cytokines by multiplex bead assay, n=5, data represents -log₁₀(P) versus the effect size at the middle point (pre vs. D14, gamma). Analysis of platelet production from CBMKs incubated for 3 hours with either (d) T3, n=4, (e) T4, n=3 or (f) T2, n=3, data expressed as relative to control, either vehicle (VC) or no agonist (NA). (d-f) one-way ANOVA with Dunnett's multiple comparison test, *p<0.05

Figure 3. Free T3 serum levels increase post an acute loss of platelets. Analysis of serum free T3 concentration at indicated timepoints from (a) plateletpheresis, n=5, or (b) plasmapheresis donors, n=5, data expressed as relative to pre-donation (pre). C57BL/6J mice i.p. injected with 0.6 μ g/g BW anti-CD42b at day (D) 0 timepoint, analysis at indicated timepoints, (baseline, B) (c) diagram of mouse experiment indicating timepoints of injection and analysis, (d) platelet count at indicated timepoints, n=3-9, (e) concentration of free T3 within the plasma, n=3-8. All data expressed as mean \pm SD, (a-b) one-way ANOVA with Dunnett's multiple comparison test, (d) two-way ANOVA with Šídák's multiple comparison test, (e) paired student T-test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, not statistically significant (ns).

Figure 4. Thyroid hormones are potent inducers of *in vitro* platelet production. Analysis of platelet production by flow cytometry of CBMKs stimulated for 6-8 hours with (a) GC-1, n=4, (b) MGL-3196, n=4, (c) KB2115, n=3-5, data expressed as relative to vehicle control (VC), no agonist (NA). Analysis of platelet production by flow cytometry at indicated timepoints of CBMK stimulated with (d) 125 μ M GC-1, n=3-5, (e) 300 μ M MGL-3196, n=3, and (f) 75 μ M KB2115, n=3, with appropriate vehicle control and no agonist control, data expressed as relative to vehicle control at 2 hour timepoint. All data expressed as mean \pm SD, (a-c), one-way ANOVA with Dunnett's multiple comparison test, (d-f) two-way ANOVA with Tukey's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p>0.0001.

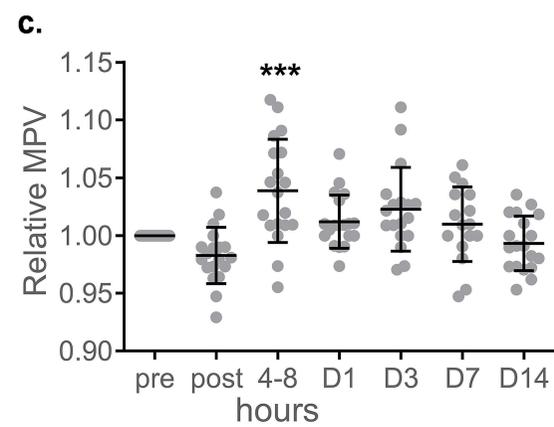
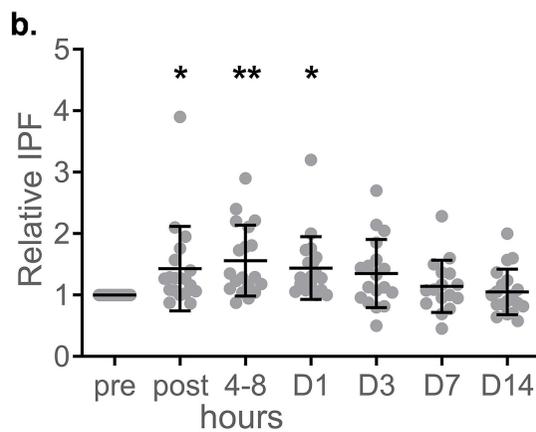
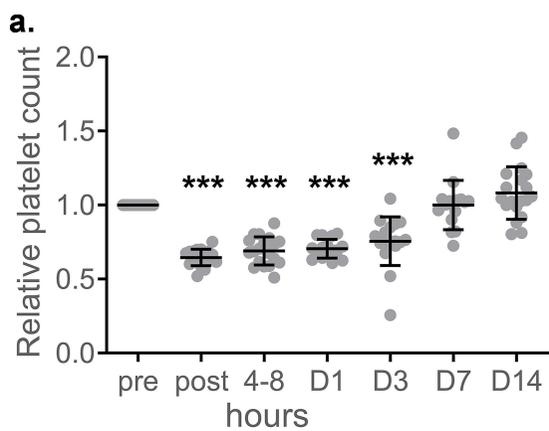
Figure 5. Thyroid hormone analogues promote proplatelet formation. Analysis of proplatelet formation at indicated timepoints of CBMKs stimulated with 75 μ M KB2115 or vehicle control, **(a)** percentage of proplatelet forming MKs, n=3, **(b)** representative images of CBMK on either fibrinogen or fibronectin stimulated for 8 hours with either 75 μ M KB2115 or vehicle control, DAPI (blue), α -tubulin (green), F-actin (red), buds at end of extensions (pink arrows), swellings along shaft (white arrows). All data expressed as mean \pm SD, **(a)** two-way ANOVA with Šídák's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p>0.0001.

Figure 6. Thyroid hormone analogue signalling is mediated by integrin α V β 3 in megakaryocytes. Receptor expression analysed in CBMK by **(a)** RT-PCR, data expressed as Δ Ct, n=3, **(b-c)** representative images of flow cytometry histograms, isotype control (white), indicated receptor (grey), n=3. Analysis of platelet production by flow cytometry of CBMKs stimulated for 6-8 hours with 75 μ M KB2115 with or without 30min preincubation with 1 μ M Tetrac, 1 μ M 1-850 or vehicle control (VC), **(d)** data expressed as percentage inhibition of KB2115 induction platelet production, n=3-4. **(e)** Analysis of platelet production by flow cytometry of bone marrow-derived MKs from either integrin β 3 knock out mice(B3 KO) or wild type mice (WT) stimulated for 6-8 hours with 75 μ M KB2115, n=3, data expressed as relative to vehicle control. **(f)** Analysis of platelet count in 0.6 μ g/g body weight (BW) anti-CD42b platelet depleted WT mice at indicated timepoints with daily i.p. of either 1 μ g/g BW tetrac or vehicle control (Ctl), n=5, Baseline (B). **(g)** Analysis of platelet production by flow cytometry of CBMK stimulated for 6-8 hours with 75 μ M KB2115 with a 30min pre-incubation with either 30 μ M BAPTA-AM, 5 μ M Go6983, 5 μ M LY294002, 15 μ M U-0126 or vehicle control, data expressed a percentage inhibition of KB2115 induced platelet production, n=3-4. All data expressed as mean \pm SD, **(d + g)** one-way ANOVA with Dunnett's multiple comparison test, **(e)** two-way ANOVA with uncorrected Fisher's LSD, **(f)** two-way ANOVA with Šídák's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001.

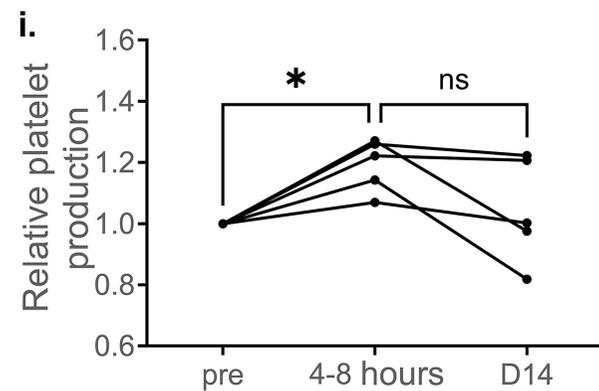
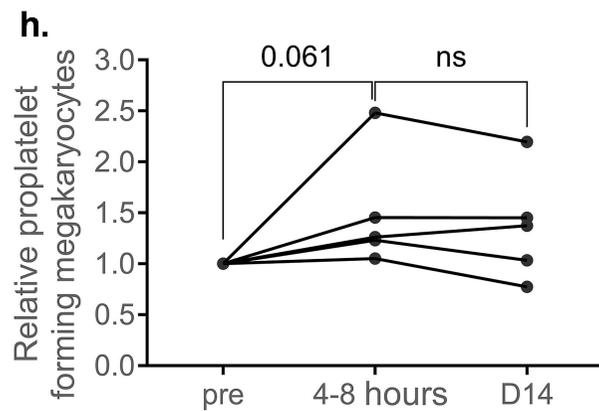
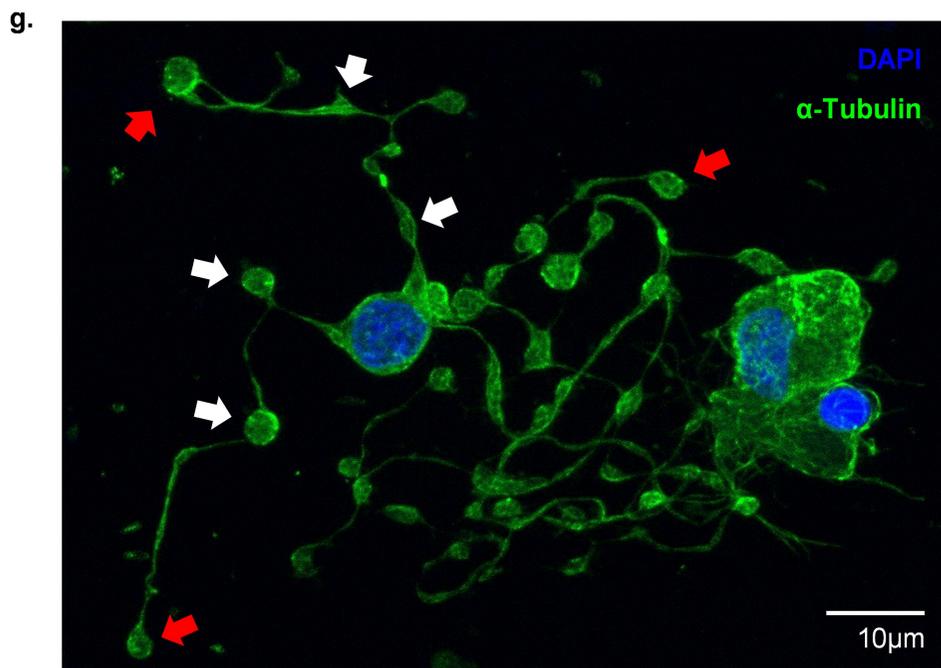
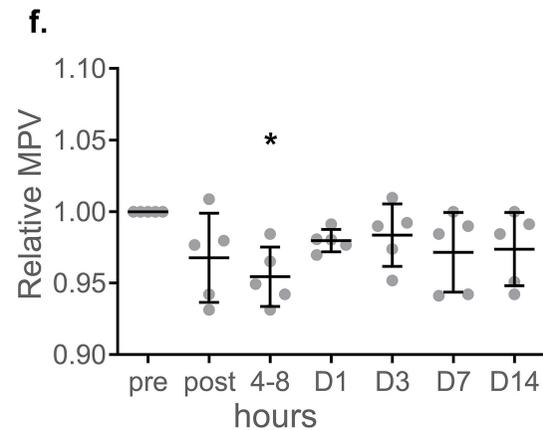
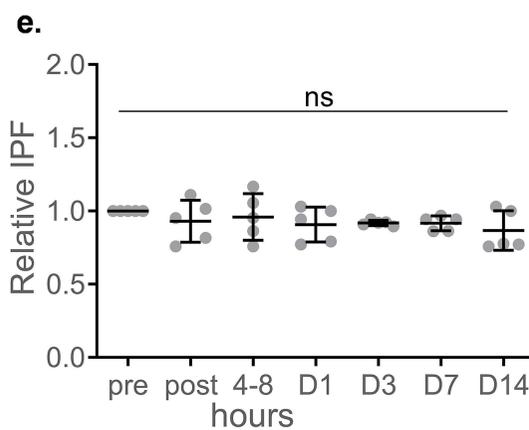
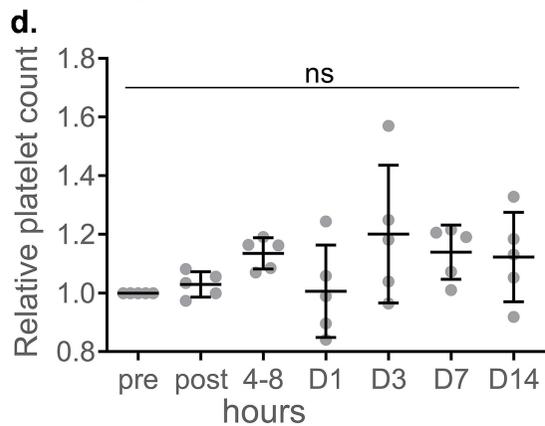
Figure 7. Ex vivo platelet production within the silk bone marrow model. **(a)** The bone marrow model consisted of a flow chamber connected to a syringe pump and gas-permeable tubing to allow perfusion of medium to the system. A silk-based spongy scaffold with interconnected pores mimicking bone marrow microcirculation was modelled into the chamber. After megakaryocyte seeding, the system was connected to a gas-permeable collection bag, containing anticoagulant, and placed into an incubator at 37°C and 5% CO₂, and perfused for 4 hours (Syringe pump and transfusion bag items are from Biorender.com). **(b)** Confocal microscopy analysis of megakaryocytes seeded into the silk bone marrow. **(bi)** 3D culture of megakaryocytes perfused in the medium containing vehicle control. **(bii)** 3D culture of megakaryocytes perfused in the medium containing 75 μ M KB2115. The box highlights a proplatelet-forming megakaryocyte in adhesion to silk scaffold, elongating highly branched proplatelet shafts, which assemble nascent platelets at their terminal ends, within the hollow space of silk pores. Arrows indicate proplatelets and platelet-like particles released into the perfused medium (green = megakaryocytes, blue = silk) (scale bars = 50 μ m). **(c)** Platelet count was assessed by flow cytometry by mixing samples with counting beads. Perfusion with medium containing 75 μ M KB2115 significantly increased the number of collected platelets. **(d)** Immunofluorescence staining of β 1-tubulin (red) highlighted the presence of the microtubule coil typically present in resting platelets (scale bars = 5 μ m). **(e)** Flow cytometry analysis of the activation of

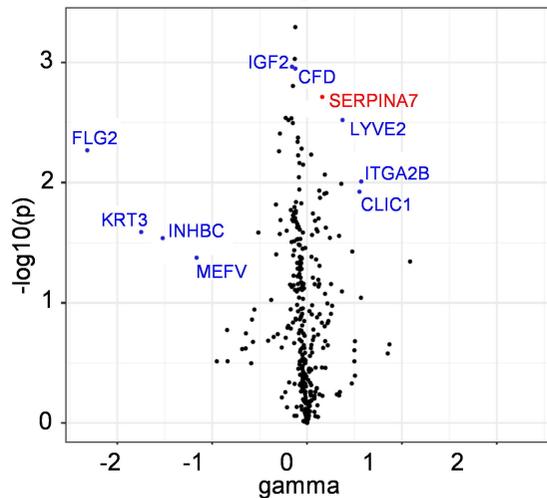
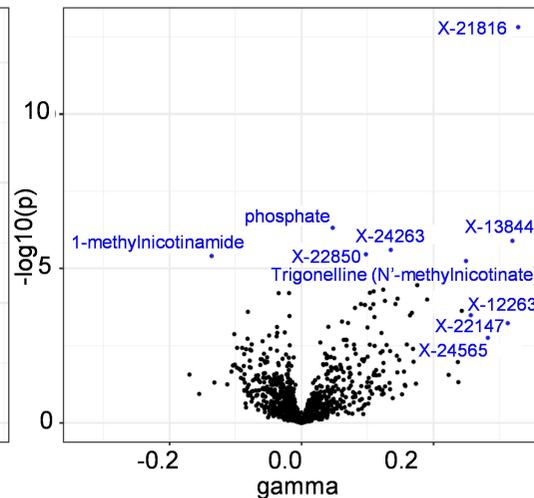
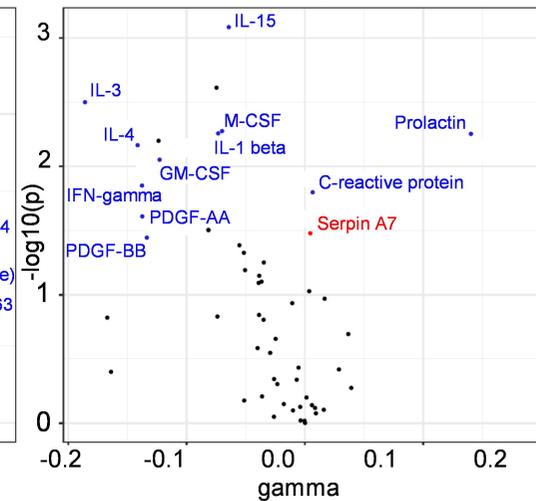
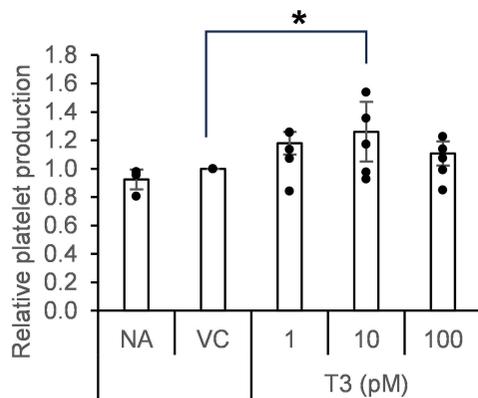
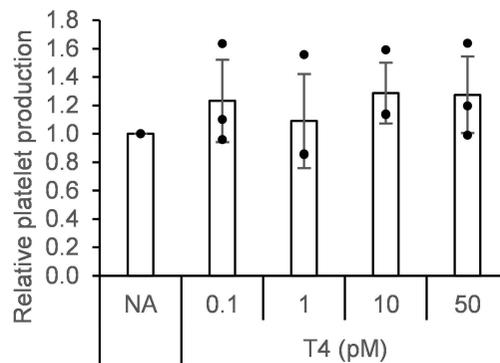
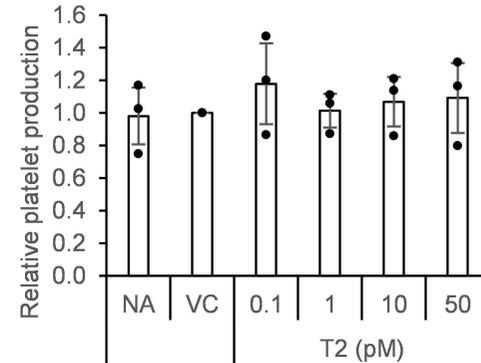
vehicle control (VC) or 75 μ M KB2115 *ex vivo* collected platelets demonstrated increased P-Selectin exposure upon treatment with 25 μ M ADP or 3 U/mL thrombin indicative of physiological functionality, red dotted line indicates basal level. All data expressed as mean \pm SD, (c)(e) paired Student T-test, *p<0.05.

Plateletpheresis Donors



Plasmapheresis Donors

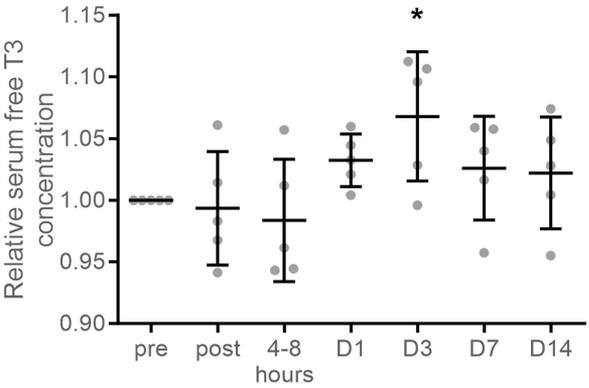


a.**Mass spectrometry****b.****Metabolon****c.****Multiplex bead assay****d.****e.****f.**

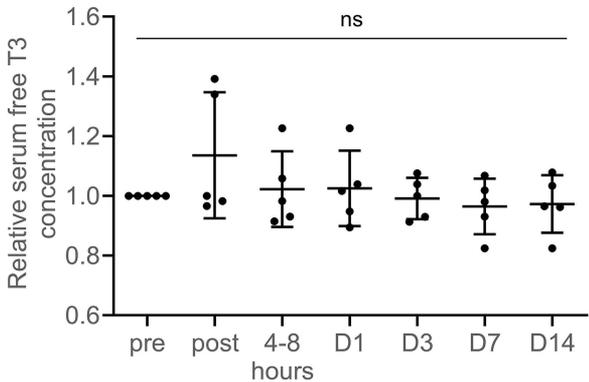
Human model of acute platelet loss



a. Plateletpheresis

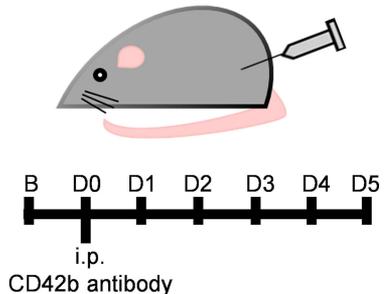


b. Plasmapheresis

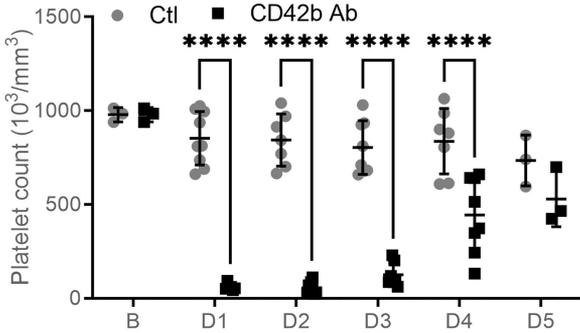


Mouse model of acute platelet loss

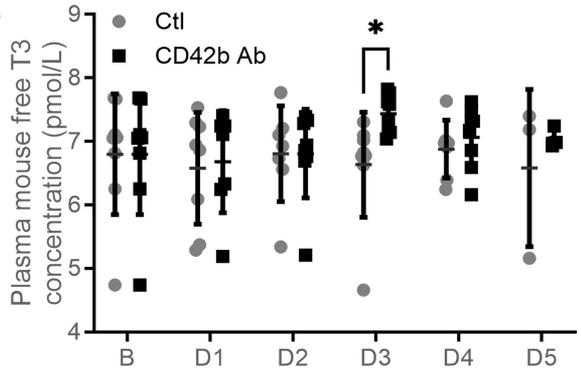
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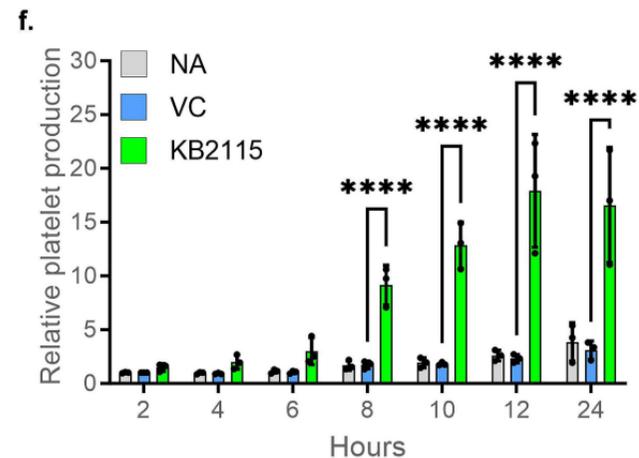
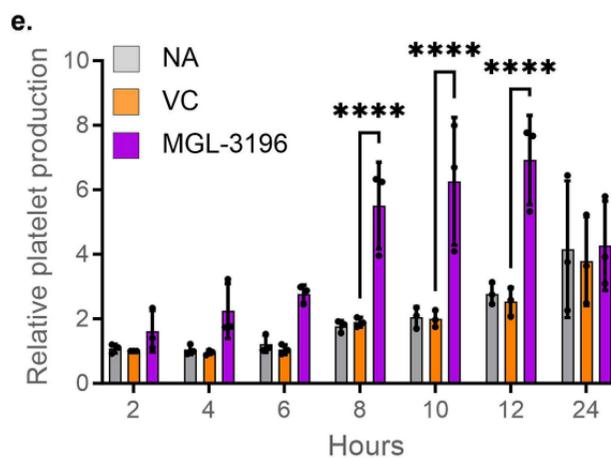
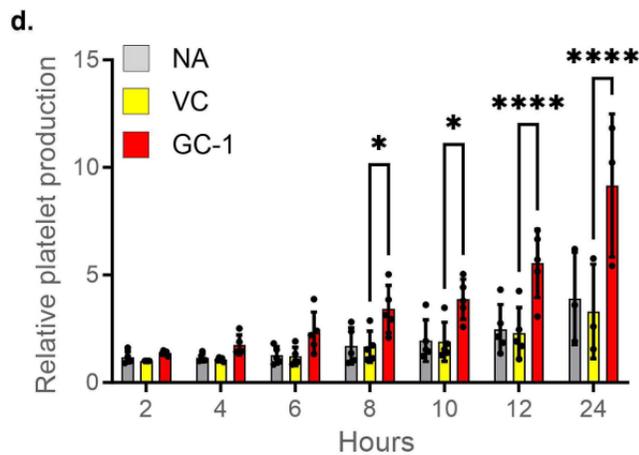
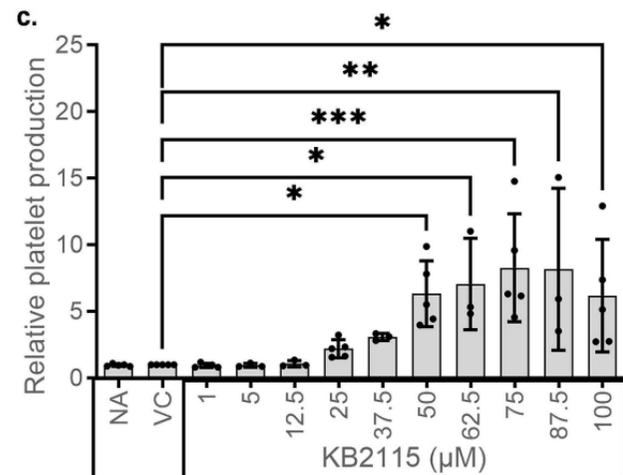
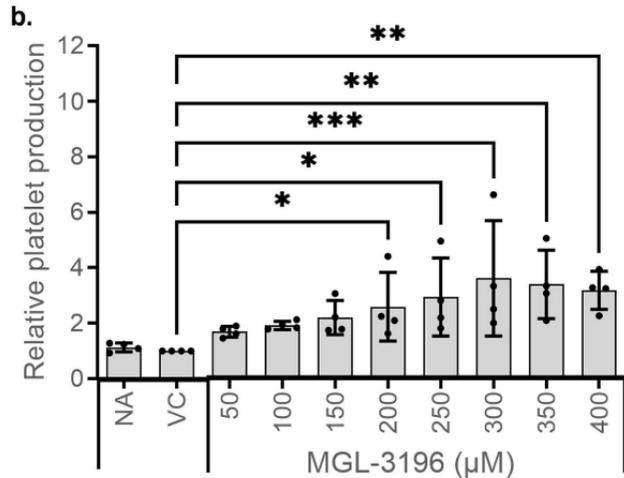
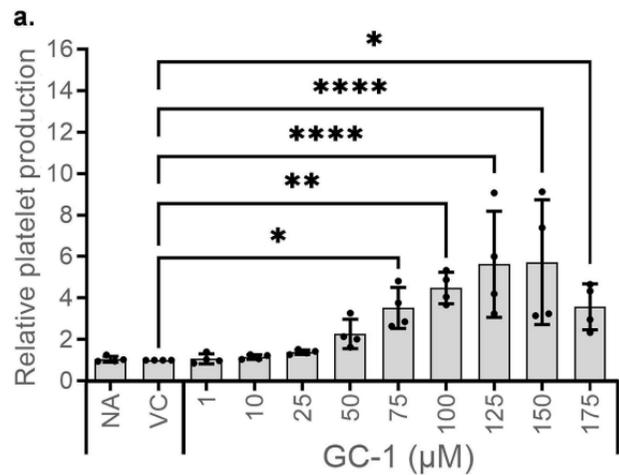


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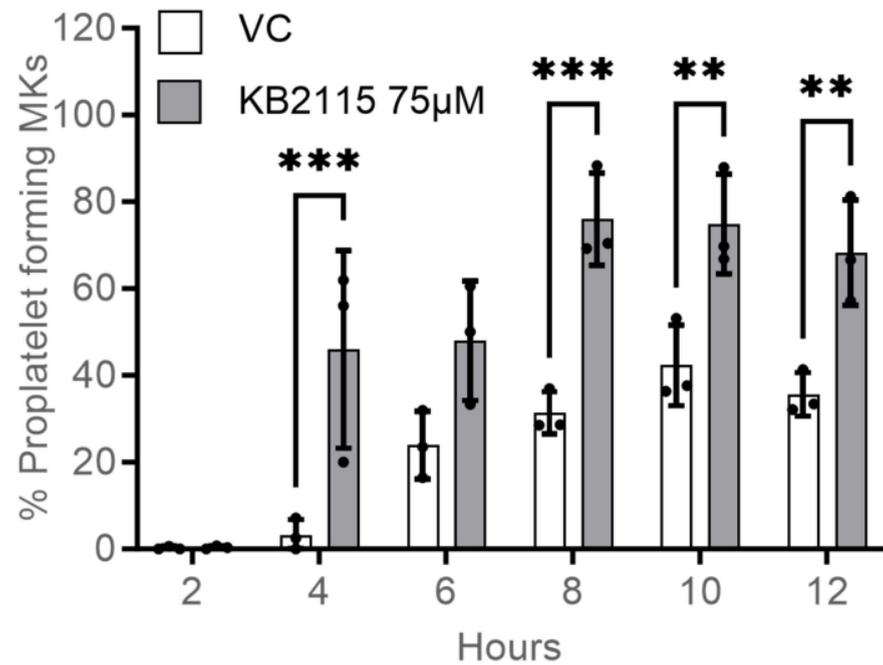


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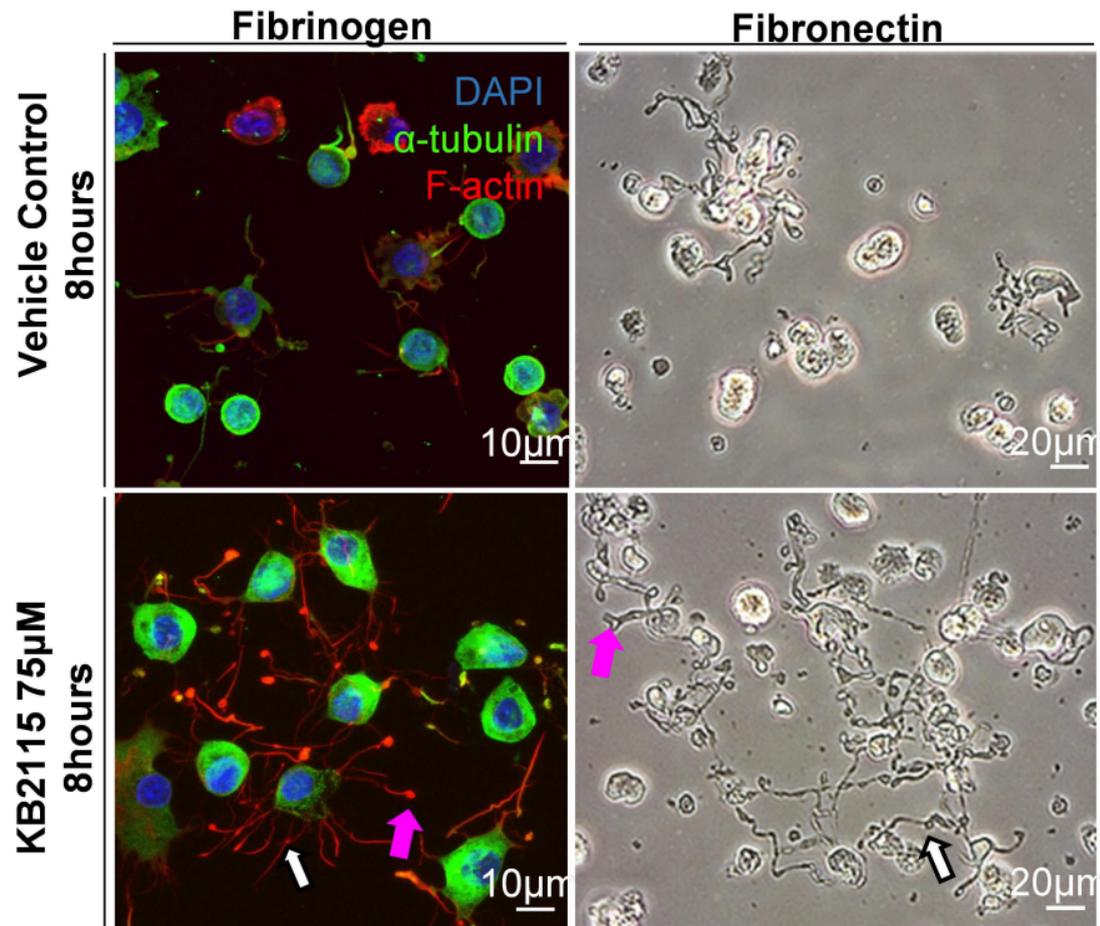


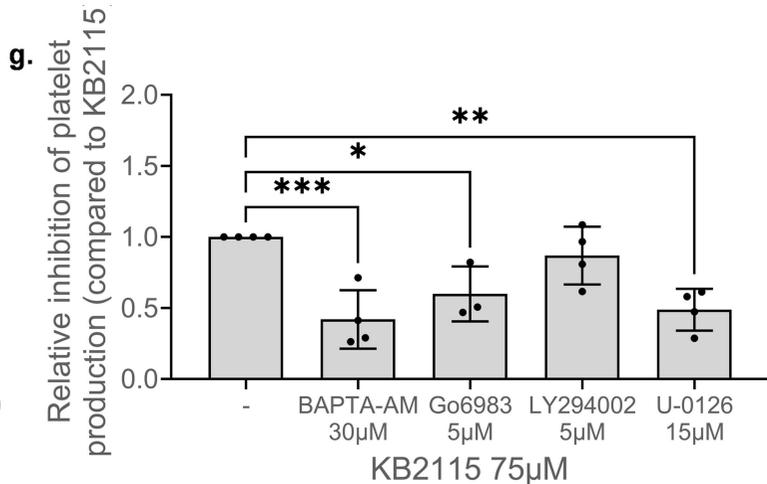
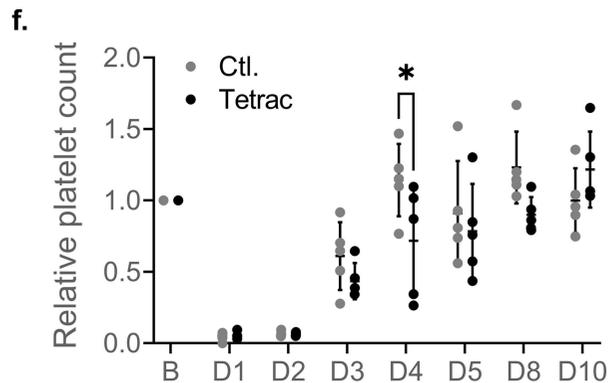
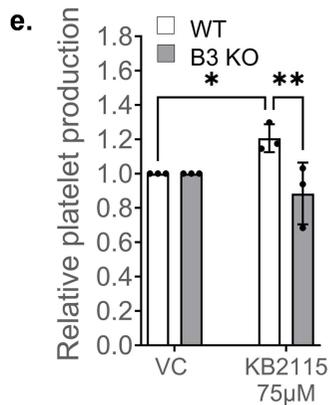
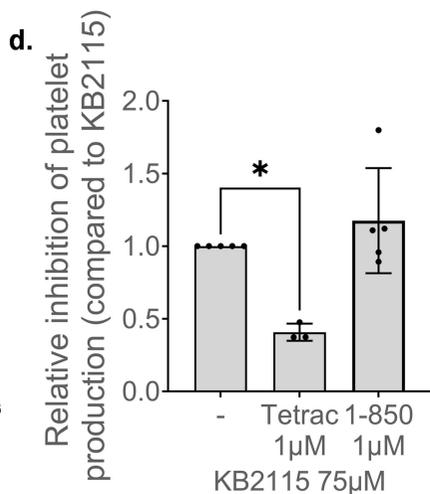
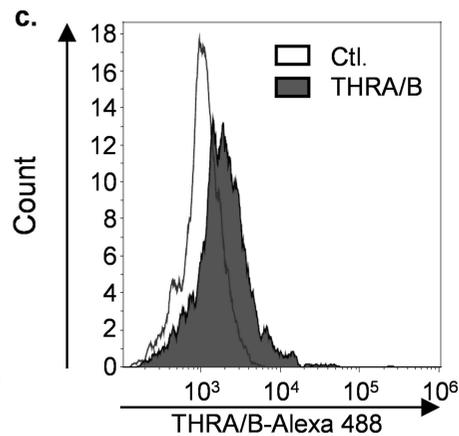
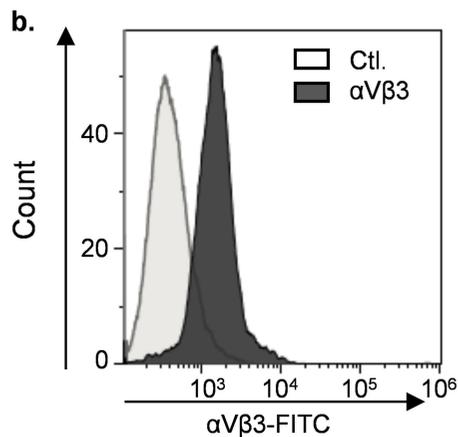
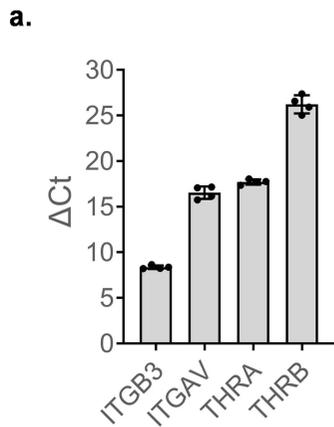


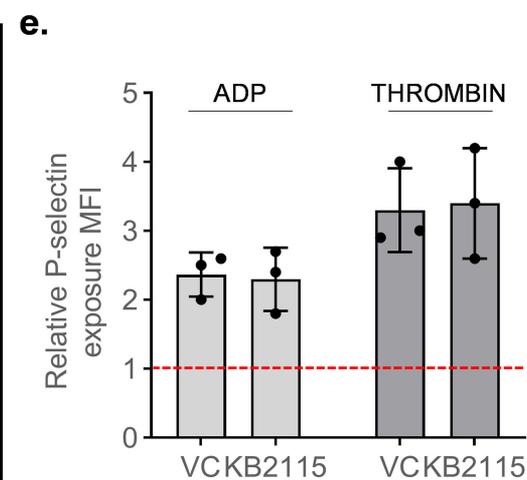
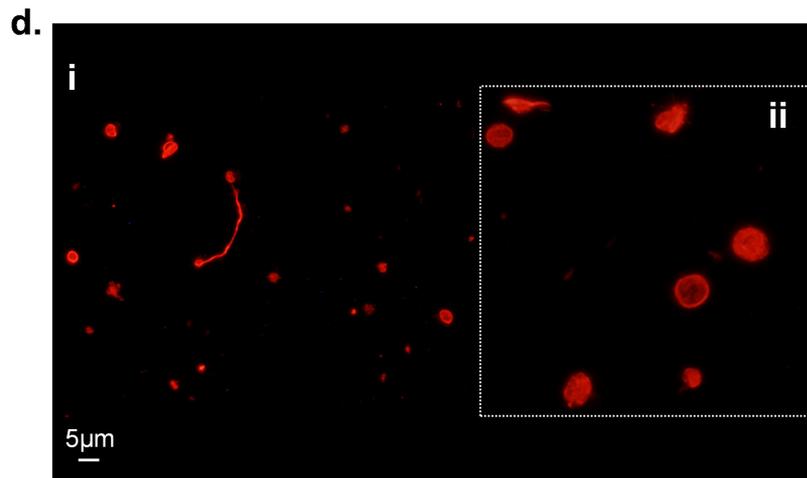
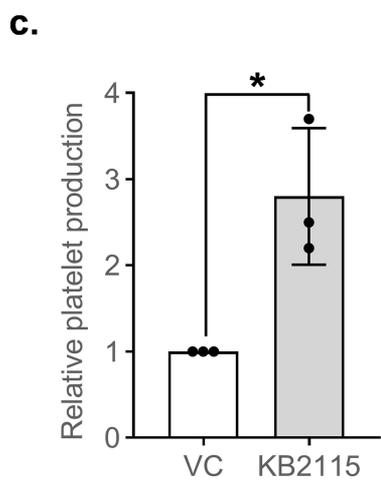
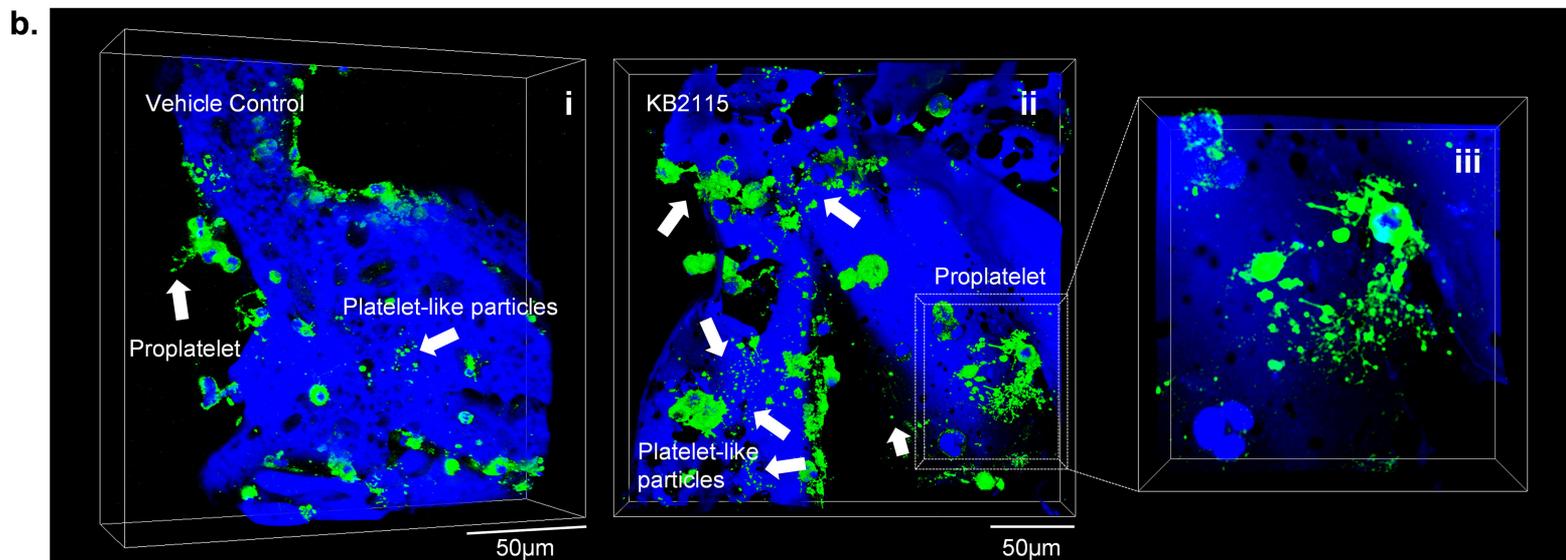
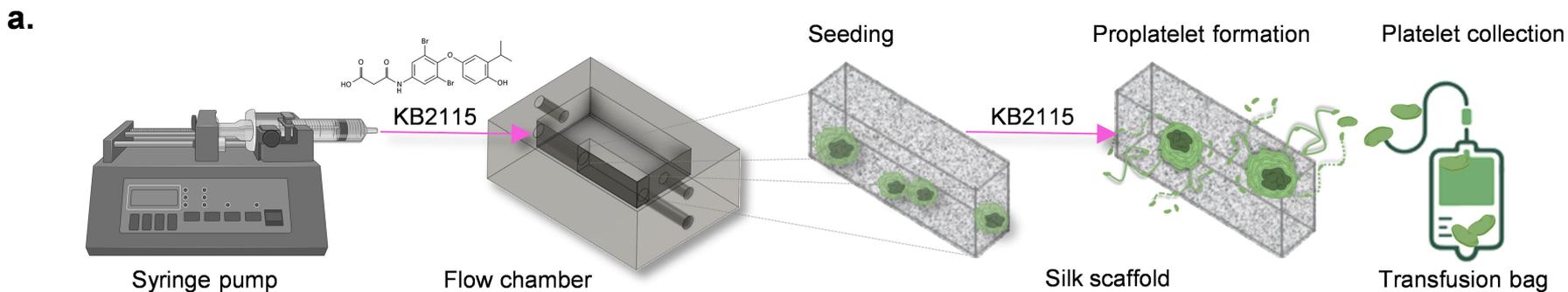
a.



b.







Supplementary Methods

Blood sampling of apheresis/plasmapheresis donors and plasma isolation

All studies involving the use of human samples were conducted in accordance with the Declaration of Helsinki.

Apheresis donors. 19 male donors who donate regularly were recruited, and full consent was given in accordance with East of England-Cambridge Central Research Ethics Committee (14/EE/0194). Platelet donations were carried out using a Trima Accel automated blood collection system (TerumoBCT) and performed by trained nurses in the Cambridge Blood Donor Centre (NHSBT, Cambridge, UK).

Plasmapheresis donor. 5 healthy male donors were recruited, and collections done under the IRB approved “Healthy Donor Bank” protocol (PRO00026243). Full donor consent was given according to this protocol and processing of samples was performed under the IRB approved protocol “Analysis of Plasma Proteins after Platelet donation” (PRO00031603). Plasma donations were carried out and performed by trained phlebotomists at Versiti Headquarters (Milwaukee, USA).

Sampling. Blood was sampled from the opposite arm usually used for donation and was drawn into EDTA, heparin and serum BD vacutainers. Full blood counts were performed within 30mins of being taken with the EDTA blood samples using either Sysmex XN-2000 (UK) or Sysmex XN-1000 (USA) blood counter (Sysmex). To isolate plasma, EDTA or heparinised blood was centrifuged at 1000g for 15mins at 22 °C 15mins after collection. The top plasma was collected, immediately aliquoted and stored at -80 °C until analysis.

To isolate serum, blood in the serum BD vacutainers were allowed to stand at room temperature (r.t.) for 30-60mins then centrifuged at 1000g for 15mins at 22 °C. The top serum was collected, immediately aliquoted and stored at -80 °C until analysis.

Cord blood-derived megakaryocytes

CD34⁺ cells were isolated from cord blood obtained with full consent in accordance with Cambridgeshire 4 Research Ethics Committee (07/MRE05/44), the Ethical Committee of the I.R.C.C.S. Policlinico San Matteo Foundation of Pavia, and the principles of the Declaration of Helsinki.

In brief, cord blood diluted 1:1 with PBS was layered over Ficoll-Paque premium (GE Healthcare) and centrifuged at 400g for 30mins. The mononuclear cell layer was collected and washed once in PBS 510g 6mins then in MACS buffer (PBS with 0.5% BSA, 2mM EDTA) 200g, 10mins. Pellets were resuspended in MACS buffer and incubated for 20mins with FC block and CD34 magnetic microbeads (Miltenyi Biotec). Magnetic separation was employed with MACS columns (Miltenyi Biotec) and a magnet. Columns were washed 3 times with MACS buffer and finally removed from the magnet where the CD34 cells were eluted. Cells were centrifuged at 510g for 10mins, and pellets resuspended in CBMK culture media (Cellgro supplemented with 50ng/ml TPO (Cellgenix) and 5ng/ml interleukin (IL)1 β (Cellgenix)) to a concentration of 10⁴ cells/ml. Cells were seeded in 1ml per well of a 12 well plate and incubated at 37 °C in a 5% CO₂ humidified incubator. On day 3, cells were fed by the addition of 1ml CBMK culture media. On day 6 1ml of media was removed from each well and 1ml of fresh CBMK culture media was added. Cells were further incubated at 37 °C until day 9/10.

Alternative protocols were also tested¹. Briefly, MKs were cultured for 13 days in StemSpan media (STEMCELL Technologies, Vancouver, Canada) supplemented with 10 ng/ml TPO and IL-11 (Peprotech, London, UK), 1% penicillin-streptomycin, and 1% L-glutamine (Euroclone). The culture medium was renewed every 3 days for two weeks.

Mouse bone marrow-derived megakaryocytes

This research was regulated by the UK Animals (Scientific Procedures) Act 1986 Amendment Regulations 2012 (project license P667BD734) and $\beta 3(-/-)$ mice (Kindly provided by R.O.Hynes, Howard Hughes Medical Institute, Cambridge, MA) studies approved by Medical College of Wisconsin's Animal Care Committee project number AUA00000488².

Wild type (+/+) colony littermates were sacrificed, bone marrow cells were collected from the tibias and femurs and stored on ice in DMEM Glutamax supplemented with 10% FBS, 100U/ml penicillin/streptomycin, then centrifuged at 200g for 5mins at 4 °C. Erythrocytes were lysed in buffer containing 150mM ammonium chloride, 10mM potassium bicarbonate and 0.1mM EDTA (pH7.3) and filtered through a 70 μ m cell strainer. MK progenitors were negatively selected with rat anti-mouse CD11b biotin-conjugated (clone M1/70, 1/500, eBioscience), rat anti-mouse Ly6G (clone RB6-8C5, 1/500, eBioscience), rat anti-mouse CD45R/B220 biotin (clone RA3-6B2, 1/500, BD Biosciences), rat anti-mouse CD16/CD32 (clone 2.4G2, 1/500, BD Biosciences) and magnetic beads against rat IgG (Dynabeads, ThermoFisher). Cells were centrifuged at 200g for 5mins, and pellets were resuspended in Cellgro supplemented with 100ng/ml mouse TPO. Cells were incubated for 4 days at 37 °C in a 5% CO₂ humidified incubator.

MKs were isolated by BSA gradient. In brief, 4ml 1.5% BSA was layered over 4ml 3% BSA. Cells were collected and centrifuged for 5mins at 120g, and pellets were resuspended in 4ml PBS which was then layered over the 1.5% BSA. The gradient was incubated at r.t. for 45 mins, then the top 10ml was centrifuged at 200g for 5 mins and resuspended with Cellgro supplemented with 100ng/ml thrombopoietin (TPO), 100ng/ml stem cell factor (SCF), 10ng/ml IL-3, 100ng/ml IL-6 and 100ng/ml IL-11 (all recombinant mouse, Peprotech). These cells were further incubated for 3 days at 37 °C for a second round of MK isolation. The bottom 2 ml was centrifuged at 120g for 5mins and resuspended in appropriated media for the assay of choice.

iPSC-derived megakaryocytes

The differentiation of human pluripotent stem cells (hPSC) into megakaryocytes was carried out using forward programming techniques (FOP) either virally or using a doxycycline-inducible system, as previously described^{3,4}.

Viral FOP. A1ATD1 hPSC were obtained from the Cambridge Biomedical Research Centre iPSC Core Facility. In brief, A1ATD1 hPSC were lentivirally transduced with 3 transcription factors; FLI1 (MOI20), TAL1 (MOI20) and GATA1 (MOI20), in an in-house media DMEM/F12 (Thermo Fisher Scientific) 7.5% w/v NaHCO₃ (Thermo Fisher Scientific), 2x Insulin-Transferrin-Selenium premixed solution (Thermo Fisher Scientific), 6.4mg/ml L-Ascorbic acid 2-phosphate sesquimagnesium salt hydrate (Sigma) supplemented with 10ng/ml recombinant human BMP4 (QKine), 20ng/ml recombinant human FGF₂ and 10 μ g/ml protamine sulphate for 24 hours. Cells were then washed with PBS and placed in a mesoderm media (above in-house media supplemented with 20ng/ml FGF₂ and 10ng/ml BMP4) for 24 hours. Media was then replaced with a MK culture media (Cellgro supplemented with 20ng/ml TPO and

25ng/ml SCF), with semi-depletion of media every 2-3 days. On Day 10, cells were harvested with TrypLE (Thermo Fisher Scientific), centrifuged at 300g 5mins and then seeded back in MK culture media in TC treated plates (no vitronectin). Cells were fed by semi-depletion every 2-3 days until mature (greater than 70% CD41⁺/42⁺).

Inducible FOP. QOLG1 hPSC were obtained from the Human Induced Pluripotent Stem Cell Initiative (HipSci). QOLG1 hPSC, had been modified in-house with a doxycycline-inducible overexpression cassette containing FLI1, TAL1 and GATA1, as previously described but using the next generation construct containing genomic insulators in the dual genomic harbour system, denoted inducible (i)QOLG1.1H⁴. In brief, iQOLG1.1H were cultured in the above mesoderm in-house media supplemented with 125ng/ml doxycycline, 20ng/ml FGF₂ and 10ng/ml BMP4 for 24 hours. Cells were then incubated for 24 hours with 3μM CHIR99021 (Cayman Chemicals). Cells were incubated for a further 24 hours in mesoderm media supplemented with doxycycline, FGF₂ and BMP4, then transferred to an in-house MK culture media (AMK) containing IMDM (without phenol red), 0.5% BSA (BioSera), 1x Insulin-Transferrin-Selenium premixed solution, 50μM β-mercaptoethanol, 1x Chemically Defined Lipid Concentrate (Gibco) supplemented with 125ng/ml doxycycline, 20ng/ml TPO and 25ng/ml SCF and fed via semi-depletion every 2-3 days⁴. On day 10, cells were harvested as above and cultured in the in-house AMK culture media until mature.

Proplatelet assay

Cell purity was first analysed using CD41a-APC H7 (1/200, clone HIP8, BD Pharmingen), CD42a-APC (1/100, clone REA209, Miltenyi Biotec) and 0.5μg/ml DAPI (Cell Signalling). Briefly, cells were incubated with appropriate antibodies for 15mins at r.t. then diluted with PBE (PBS containing 0.2% BSA, Biosera, 5mM EDTA, Sigma) and DAPI. Analysis was performed on a Gallios flow cytometer using Kaluza software (Beckman Coulter). Experiments were only performed on CBMKs that had a purity above 70% (CD41⁺/CD42⁺) and a viability above 80% as indicated by the DAPI staining. Chamber slides (μ-slide, Ibidi) or sterile coverslips were washed three times with PBS, then incubated with 200μg/ml Fibrinogen (FIB3, Enzyme Research) overnight at 4 °C or for 1 hour at r.t. Cells were centrifuged at 100g for 6mins, and the pellets were resuspended to 0.25x10⁶ cells/ml in Cellgro. Wells were washed once with Cellgro, then cells were seeded. Cells were fixed at indicated time points using 1% formaldehyde (FA). For staining, wells were incubated with 50μM NH₄Cl for 5 mins then washed 3 times with a permeabilisation buffer containing 0.1% saponin (Quillaja Bark, Sigma), 0.2% gelatin (cold water fish skin, Sigma) and 0.02% sodium azide in PBS. Cells were incubated with mouse anti-human α-Tubulin (1/250, clone B-5-1-2, Sigma) prepared in permeabilisation buffer for at least 1 hour at r.t. Wells were washed again 3 times with permeabilisation buffer and then incubated with goat-anti-mouse IgG1 Alexa Fluor 488 (1/1000, A21121, Thermo Fisher Scientific) and phalloidin CruzFluor-555 (sc-363784, Santa Cruz) in permeabilisation buffer for at least 1 hour in the dark at r.t. Cells were then washed 3 times with PBS and incubated with 1μg/ml DAPI for 5mins in the dark at r.t. Finally, cells were washed 3 times with PBS, wells were mounted using Prolong Diamond anti-fade mounting media (Thermo Fisher Scientific) or coverslips applied to slides with said mounting media. Slides were cured overnight in the dark at r.t. and then stored at 4 °C.

Cells were imaged either with a 40x objective on a Leica DM4000 with LAS software or with a 63x oil objective on an SP5 using this software. Assays were run with a minimum of 2 technical replicates. 5 images were taken per technical replicate and the percentage proplatelet-forming MKs or spreading MKs were averaged.

Platelet production assay

Experiments were only performed on CBMKs above 80% DAPI⁻ and above 70% 41⁺/42⁺, and for hPSC-MKs above 30% DAPI⁻ and above 70% 41⁺/42⁺, see above for details of staining protocol. Cells were centrifuged 120g /100g 5mins and pellets were resuspended to 0.5x10⁶ cells/ml (CBMK/hPSC-MK) or 0.2x10⁶ cell/ml (mouse MKs) in high-glucose RPMI (Thermo Fischer Scientific, A10491). For experiments with plasma, to reduce the amount of contaminating platelets within this experiment, plasma was centrifuged 4000g 15mins 4 °C to pellet platelets and no MK controls were used to ascertain the correct amount of platelet contamination from each different timepoint. For experiments involving antagonists, cells were preincubated with the appropriate concentration of antagonist; 1μM 3,3',5,5'-Tetraiodothyroacetic acid (Tetrac, Sigma), 1μM 1-850 (17248, Cayman Chemicals), 30μM BAPTA-AM (A1076, Sigma), 5μM Gö6983 (228T, Tocris, Bio-technie), 5μM LY294002 (SM24, Cell Guidance Systems) or 15μM U-0126 (70970, Cayman Chemicals), for 30mins at 37 °C. Cells were seeded at 50,000 cells/well (CBMK/hPSC-MK) or 20,000 cells/well (mouse MKs) in a 96-well flat bottomed plate and incubated at 37 °C in a 5% CO₂ humidified incubator for the indicated time points. All cells/platelets were harvested and stained with CD41a-APC H7 (1/200), CD42a-APC (1/100) for human-derived platelets and CD41-APC/Cy7 (1/200, clone 1C2, Biolegend), CD42d-PerCP/Cy5.5 (1/200, clone MWReg30, Biolegend) for mouse MK-derived platelets. Both were also stained with 200ng/ml calcein-AM (C3100MP, Thermo Fisher Scientific) for 15mins at 37 °C. Samples were diluted with PBE containing 0.5μg/ml DAPI and flow-count fluorospheres (Beckman Coulter) and analysed by flow cytometry (Gallios, Beckman coulter). Events that were of a platelet size/density (FS/SS) set by peripheral blood platelets and calcein⁺/CD41⁺/CD42⁺ were defined as platelets, Supplementary Fig. 3. All platelet production assays were run in triplicate and platelet numbers calculated using flow-count fluorospheres.

Mass Spectrometry

10μl of plasma was subjected to depletion of the two most abundant protein components (Albumin, using affinity spin columns as per the instruction manual (Thermo Scientific/Pierce), followed by reduction/alkylation with DTT/Iodoacetamide, Chloroform/Methanol precipitation and tryptic digest. Peptide samples were desalted using SOLA SPE Cartridges (Thermo Scientific/Pierce) and resuspended at a concentration of 1μg/μl. Samples were analysed on a LC-MS/MS platform consisting of Dionex Ultimate 3000 UPLC and Q-Exactive mass spectrometer (both Thermo Fisher Scientific), using a linear gradient from 2-35% ACN in 0.1% Formic Acid/5% DMSO over 120mins with an EASY-Spray PepMap C18 column (500mm x 75um, 2um particle size). Survey scans were acquired at a resolution of 70000 (@200 m/z) and the most 15 abundant precursors were selected for MSMS analysis.

Resulting data was imported into Progenesis LCMS (V4.1.4832.42146, Waters) using default settings. MS/MS spectra were identified with Mascot (V2.3, Matrix Science). Peptide FDR was adjusted to 1% and peptides hits with scores lower than 20 were excluded.

Metabolon

Plasma samples isolated from plateletpheresis donor blood were submitted to Metabolon, Morrisville, NC, for metabolomic analysis.

Multiplex bead assay

Plasma samples isolated from plateletpheresis blood were analysed using a custom premixed multiplex human magnetic Luminex assay (R&D Systems, Bio-technie) following the manufacturer's protocol. Briefly, samples were diluted according to manufacturer's recommendations and then incubated with the microparticle cocktail for 2 hours at room temperature on a plate shaker (800rpm). Plates were washed three times using a 96-well plate magnet and then incubated for 1 hour at r.t. on a plate shaker with the biotin antibody cocktail. Microparticles were washed again three times using a magnet and then incubated for 30mins at r.t. on a plate shaker with the streptavidin-PE. After a final wash, the microparticles were resuspended in wash buffer and analysed using a Luminex 100 and xPONENT v3.1 software (Luminex).

Immunoassay analysis

Thyroid stimulating hormone (TSH) and human Free T3 (all ADVIA Centaur, Siemens) were analysed using a clinically accredited laboratory at Addenbrookes Hospital, Cambridge, UK (National Institute for Health Research, Cambridge Biomedical Research Centre, Core Biochemistry Assay Laboratory, Tissue Bank and Histology). Human plasma was also analysed with ELISAs for TPO (DY288, Bio-technie) following manufacturer's instructions. Plasmapheresis serum samples were analysed for Free T3 using ab108663, Abcam following manufacturer's instructions. Mouse plasma was isolated from blood collected from the inferior vena cava (IVC). In brief, mice were terminally anaesthetised intraperitoneally with 100mg/ml ketamine and 10mg/ml xylazine. IVC blood was drawn over 50 μ l 0.5M EDTA and centrifuged at 2000g 15mins at 4 °C. Plasma was isolated and immediately stored at -20 °C. Plasma was analysed for Free T3 (NBP2-60012, NovusBio) and TPO (MTP00, Bio-technie) following manufacturer's instructions.

RT-PCR

RNA was isolated from cells using RNeasy Plus mini kit (Qiagen) following manufacturer's instructions. RNA was incubated with random hexamers for 65 °C for 5mins then with SuperScript III reverse transcriptase, dNTPs and RNase inhibitors (all Thermo Fisher Scientific) following manufacturers protocols. RT-PCR was performed using SYBR green Brilliant II low ROX (Agilent), with 10 μ M primers (optimised to 90-110% primer efficiency with clean single Tm peaks); THRA forward-GTGGGGCACTCGACTTTC reverse-TGACTGACCTCCGCATGAT, THRB forward-CATAAATTCCATTTGTCAGTGAG reverse-GCTCCACTGTGGGGAGATATT, ITGAV forward-TCCATTTCAATTTTGTGGATAAA reverse-TCCCAGGACTGTATTGCTGA, ITGB3 forward-CATGGCCGGAACACATCT reverse-GCAAGTACTGTGAGTGCGATG and housekeeping gene 18s forward-GCTTAATTTGACTCAACACGGGA reverse-AGCTATCAATCTGTCAATCCTGT, on a MX3000 (Stratagene) using a 1 plateau, normal 2 step protocol. Data expressed Δ CT (target CT – housekeeping CT).

Flow cytometry analysis of receptor expression

For extracellular staining, MKs were stained with mouse anti-human integrin $\alpha V\beta 3$ -FITC (1/50, clone LM609, Millipore) for 20mins at r.t. Samples were diluted with PBE (described above) and analysis was performed on a Gallios flow cytometer. For intracellular staining, MKs were fixed with 0.2% formyl saline for 10mins at r.t. MKs were washed twice with PBE at 120g 5mins and incubated with a saponin/gelatin/PBS permeabilisation buffer (described above) and mouse anti-human THRA/THRB antibody (1/20, clone C3, Thermo Fisher Scientific) for 30mins at r.t. Samples were washed three times in permeabilisation buffer at 120g 5mins and incubated in permeabilisation buffer with a goat anti-mouse Alexa Fluor 488 (1/1000, Thermo Fisher Scientific) for 30mins at r.t. in the dark. Samples were finally washed three times in PBE at 120g 5mins and analysis was performed on a Gallios flow cytometer.

In vivo assays-platelet depletion

JAX C57BL/6J (Charles River Laboratories) male, aged-matched mice were intraperitoneally injected once at 'Day 0' with either 0.6 μ g/g body weight (BW) CD42b antibody (R300, Emfret) in PBS or vehicle control (PBS). For experiments involving the antagonist tetrac, mice were intraperitoneally injected with 1 μ g/g BW tetrac in PBS or vehicle control (equivalent volume of DMSO in PBS) daily from Day 1-5 inclusive. Blood samples were taken at indicated intervals by tail vein bleeds and terminal inferior vena cava bleed in the presence of EDTA, as previously described above. Platelet counts, as well as other blood cell indices, were analysed by veterinary haematology analyser (Scil Vet abc plus+, Horiba).

Preparation of the silk bone marrow model

Silk fibroin aqueous solution was obtained from *Bombyx mori* silkworm cocoons according to previously published literature⁵. A silk solution (8% w/v) was mixed with 25 μ g/ml fibronectin and dispensed into a moulding system. NaCl particles (approximately 500 μ m in diameter) were then sifted into the solution in a ratio of 1 mL/2 g of NaCl particles. The scaffolds were then placed at r.t. for 48 hours and then soaked in distilled water to leach out the NaCl particles. The scaffolds were sterilized in 70% ethanol and finally rinsed in culture medium before any cell-based experiments.

Imaging of MK cultures within the 3D silk bone marrow model

For immunofluorescence imaging in the silk bone marrow tissue model, samples were fixed in 4% paraformaldehyde (PFA) for 20mins and then blocked with 5% bovine serum albumin (BSA, Sigma) for 30 min at r.t.. Samples were probed with anti-CD61 (1/100, Beckman Coulter) overnight at 4°C and then immersed in Alexa Fluor secondary antibody (1/500, Invitrogen) for 2 hours at r.t.. Nuclei were stained with Hoechst. Samples were imaged by a TCS SP8 confocal laser scanning microscope (Leica, Heidelberg, Germany). For silk fibroin scaffold imaging, we took advantage of silk auto-fluorescence in UV light. Silk fluorescence was brightened by staining with Hoechst. For all immunofluorescence imaging, the acquisition parameters were set on the negative controls. 3D reconstruction and image processing were performed using Leica licensed softwares.

Ex vivo platelet production

A custom flow chamber was produced and perfused as previously described⁶. The perfusion of the silk scaffold was performed with a basic medium (DMEM, Euroclone) containing KB2115.

Collected platelets, produced *ex vivo*, were analysed by flow cytometry using the same forward and side scatter pattern as human peripheral blood and identified as CD41a⁺CD42a⁺ events. Human peripheral blood platelets were isolated from whole blood that was centrifuged at 200 × g for 10 min to obtain platelet-rich plasma (PRP). Platelets were washed in Tyrode's buffer (134 mM NaCl; 0.34 mM Na₂HPO₄; 2.9 mM KCl; 12 mM NaHCO₃; 20 mM HEPES; 5 mM glucose) in the presence of 0.2 U/ml apyrase and 1 μM PGE 1 (Sigma, Milan, Italy) and allowed to rest at room temperature for 1 h, before being used.

Isotype controls were always used as a negative control to exclude non-specific background signals. The platelet number was calculated using a TruCount bead standard. All samples were acquired with a BD FACS Lyric (Becton Dickinson).

Platelet activation assay by flow cytometry

For platelet activation studies, MKs were seeded at 1x10⁶/ml in A10491 RPMI overnight in 12 well plates at 37 °C with the indicated ligand. Whole blood controls, blood was first drawn over EDTA with tourniquet. The tourniquet was removed, then blood was drawn into sodium citrate. The sodium citrate blood was diluted 1/25 with Tyrodes solution (134mM NaCl, 2.9mM KCl, 0.34mM Na₂HPO₄.2H₂O, 12mM NaHCO₃, 20mM HEPES, 1MM MgCl₂.6H₂O, 5mM D-Glucose, pH 7.3, all Merck). 250μg/ml PGI₂ (Merck) was added to both the diluted whole blood and MK cultures. MKs/platelets were collected and centrifuged 120g 5min r.t. to reduce MK contamination. Supernatants were collected and centrifuged 1400g 10mins r.t. to pellet the platelets. Pellets were resuspended with Tyrodes solution supplemented with 1mM CaCl₂ (Merck). Platelets and whole blood were incubated for 30mins at 37 °C in the presence of CD41-APC H7 (1/200), P-selectin-APC (1/30, clone AK4, 304910 Biolegend), Calcein violet (for platelets 2.5pg/ml, for whole blood 12.5pg/ml, Thermo Fischer Scientific) with or without the indicated concentrations of ADP (Merck) or thrombin (Merck). Samples were fixed with 0.2% formyl saline (water with 0.85% NaCl, and 0.2% formaldehyde, Merck). Gates were set compared to unstained samples (calcein violet), appropriate isotype controls (CD41 and P-selectin exposure) and 4.2mM EDTA (Fibrinogen binding). The sample were analysed by flow cytometry (Gallios). Relative MFI of platelet sized events that were calcein and CD41 positive were compared to no agonist controls. All samples were run in duplicate.

Statistical Analysis

For analysis of Mass spectrometry, Metabolon and Multiplex bead assay. Using three different assays, we quantified molecules in plasma samples obtained pre-donation, 4-8 hours post donation and D14 post-donation. The number of donors for each assay were as follows: mass spectrometry (n=5), Luminex bead array (n=5) and Metabolon (n=11). We fitted the following linear mixed model for each molecule captured by each assay:

$$\log(y_{ij} + 1) = \alpha + x_j\beta_j + (1 - |x_j|)\gamma + v_i + \epsilon_{ij},$$

where:

- y_{ij} is the raw intensity measurement for donor i at time point j ,
- $x = (-1, 0, 1)$,
- v_i is a normally distributed random effect, and

- ϵ_{ij} is a normally distributed residual.

Using a likelihood ratio test, we tested the null hypothesis $\gamma = 0$, which represents a model in which there is no significant deviation from a linear trend across the three consecutive time points.

For all other. Values were either expressed as mean plus or minus the standard deviation (mean \pm SD). A two-tailed paired t-test was performed for statistical analysis of data from samples tested in parallel under different experimental conditions. One and two-way ANOVAs were also performed with indicated post-hoc analysis. A p-value of less than 0.05 was considered statistically significant. All N numbers represent biological replicates. For experiments involving cord blood-derived MK, different donors are used and for iPSC-derived MK, different FOP experiments are used.

Data availability

Numerical data analysis for the multi-omic study of the platelet apheresis donors are detailed in Supplementary Tables 6-11. Non-identifiable donor information is also detailed in Supplementary Tables 1 + 4. Correspondence and requests for data or information should be addressed to Cedric Ghevaert, Wellcome-MRC Cambridge Stem Cell Institute, Jeffrey Cheah Biomedical Centre, Cambridge Biomedical Campus, University of Cambridge, Puddicombe Way, Cambridge, CB2 0AW, UK. Request will be responded to within 10 working days

Supplementary Figure Legends

Supplementary figure 1. (a) Analysis of plateletpheresis donor mean platelet volume (MPV) and immature platelet fraction (IPF) at 4-8 hours compared to the pre-donation control, each data label indicates a donor reference ID, red dots indicate donor plasma or serum that was utilised in further experiments.

Supplementary figure 2. Representative images of proplatelet-forming megakaryocytes from cord blood-derived megakaryocyte cultures incubated with plasma from the indicated timepoints for 6-8 hours, staining indicates α -tubulin (green) and DAPI (blue), scale bar 10 μ m, n=3.

Supplementary figure 3. (a) Representative flow cytometry sequential gating strategy for the analysis of platelets. Firstly, platelet sized particles are gated on based on forward scatter (FS) and side scatter (SS) of a peripheral blood platelet sample. Then calcein-AM positive events are gated on using an unstained sample as the control, calcein stained sample (light grey), unstained sample (dark grey). Finally, CD41 and CD42a positive events are gated on based on the isotype controls. Platelets sized events that are calcein-AM/CD41/CD42a positive are considered 'true' platelets. **(b)** Representative flow cytometry data from cord blood-derived megakaryocyte cultures incubated for 6-8 hours with the indicated 10% plateletpheresis plasma, n=5.

Supplementary figure 4. (a) Analysis of plateletpheresis serum by ELISA for thrombopoietin (TPO) at the indicated timepoints, data expressed as relative to pre-donation control for each donor, each line represents a different donor, n=6. Analysis of platelet production from CBMKs incubated for 6-8 hours with the indicated concentrations of either **(b)** alpha-1-acid glycoprotein, n=3, **(c)** L-carnitine, n=3, **(d)** Leu-Gly, n=3, **(e)** Succinimide, n=3-4, **(f)** Extracellular matrix-1 (ECM), n=5, **(g)** Chemerin, n=5, data expressed as relative to control, mean \pm SD. **(a-g)** one-way ANOVA with Dunnett's multiple comparison test, not statistically significant (ns).

Supplementary figure 5. Representative chemical structures of thyroid hormones, thyroxine (T4), triiodothyronine (T3), diiodothyronine (T2). Chemical structures of thyroid hormone analogue agonists GC-1 (Sobetirome), MGL-3196 (Resmetirom), KB2115 (Eprotirome) and antagonists, tetraiodothyroacetic acid (tetrac).

Supplementary figure 6. (a) Analysis of plateletpheresis serum thyroid stimulating hormone (TSH) concentration at indicated timepoints, pre donation (pre), immediately post donation (post), day (D), mean \pm SD, n=5. **(b)** Analysis of TPO plasma concentration at indicated timepoints in wildtype mice with either 0.6 μ g/g BW CD42b Ab i.p. (black circles) or vehicle control i.p. (VC, grey circles), mean \pm SD, n=4-5. **(a)** one-way ANOVA with Dunnett's multiple comparison test, no significant difference (SD), **(b)** two-way ANOVA with Šidák's multiple comparison test, *p<0.05, ***p>0.001, ****p>0.0001.

Supplementary figure 7. Analysis of platelet production by flow cytometry of **(a)** CBMK with or without 1% BSA incubated for 6-8 hours with either 75 μ M KB2115 or vehicle control (Ctl), **(b)** Virally differentiated A1ATD1-MKs incubated overnight with the indicated concentrations of KB2115 or vehicle control (VC), **(c)** Inducible

QOLG1.1H-MK incubated overnight with the indicated concentrations of KB2115, all data expressed as mean±SD, relative to vehicle control, n=3. Representative forward scatter (FS) and side scatter (SS) flow cytometry dot plots of **(d)** viral A1ATD1 MKs and **(e)** inducible QOLG1.1H. **(a)** one-way ANOVA with Bonferroni's multiple comparison test, **(b-c)** one-way ANOVA with Dunnett's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, not statistically significant (ns).

Supplementary figure 8. **(a)** Representative flow cytometry dot plots, forward scatter (FS) vs. side scatter (SS), of CBMK stimulated with vehicle control or 75µM KB2115 for 6-8 hours. Analysis of platelet populations, Platelet 'A' (A) and Platelet 'B' (B) by flow cytometry of CBMKs stimulated for 6-8 hours with 75µM KB2115 or vehicle control **(b)** percentage of platelet sized events, n=4, **(c)** percentage of calcein positive, platelet sized events, n=4, and **(d)** percentage of CD41a/CD42a positive, platelet sized events, n=4. Analysis of platelet production by flow cytometry of CBMKs stimulated for 6-8 hours with 75µM KB2115 with or without 30min preincubation with 1µM Tetrac, **(e)** representative dot plots of forward scatter (FS) and side scatter (SS), n=3, **(f)** platelet numbers in platelet gates, Platelet 'A' (A) and Platelet 'B' (B), n=3. All data expressed as mean±SD, **(b + d)** two-way ANOVA with uncorrected Fisher's LSD, **(c)** paired Student T-test **(e)** one-way ANOVA with Dunnett's multiple comparison test, **(f)** two-way ANOVA with uncorrected Fisher's LSD, *p<0.05, **p<0.01, ***p<0.001, ****p>0.0001

Supplementary figure 9. Analysis of spreading of CBMK incubated for 6-8 hours with either 75µM KB2115 or vehicle control (VC), **(a)** representative images at 2 hours, DAPI (blue), F-actin (red), n=3, **(b)** percentage of spreading MKs at indicated timepoints, n=3, mean±SD, two-way ANOVA with Šidák's multiple comparison test, ****p<0.0001.

Supplementary figure 10. Analysis of platelet production by flow cytometry of CBMK incubated for 6-8 hours with vehicle control and pre-incubated for 30mins with either vehicle control (VC) or **(a)** 1µM 1-850, n=4, **(b)** 1µM Tetrac, n=3. All data mean±SD, all data expressed as relative to no agonist control (NA), one-way ANOVA with Dunnett's multiple comparison test, not statistically significant (ns).

Supplementary figure 11. Analysis of activation of peripheral blood platelets by flow cytometry by 3 U/mL thrombin. **(a)** representative flow plots of unactivated (basal) and activated (Thrombin) platelets showing FS/SS and P-selectin gating, n=4 **(b)** Relative MFI of P-selectin of stimulated (3 U/ml thrombin) and unstimulated platelets, n=4, Mean±SD, Paired Student T-test, ****p<0.0001.

Supplementary figure 12. Schematic of the pheresis process. Blood is collected from the donor where anti-coagulant is added before the blood is centrifuged to separate out three layers, red blood cells (RBC, red), platelet rich plasma (orange, for platelets) and platelet poor plasma (yellow, for plasma). Each layer is continually syphoned from the centrifuge, platelet rich plasma and platelet poor plasma is passed through a leukocyte reduction device. Depending on what is being collected, each layer can either be diverted to a collection bag or a reservoir. Blood in the reservoir is then returned to the donor through the same collection needle.

Supplementary Table Legends

Supplementary Table 1. showing plateletpheresis donor and donation information, negative (-ve), positive (+ve), unknown (?), British including Welsh/Scottish/English/Northern Irish (A), double platelet donation (D), triple platelet donation (T), single homozygous donation (HZD), body mass index (BMI).

Supplementary Table 2. showing plateletpheresis donors full blood counts relative to the pre-donation control (Pre), bold text indicates statistical significance, n=19, average (Av.), one-way ANOVA with Tukey's multiple comparison test, not statistically significant (ns), *p<0.05, **p<0.01, ***p<0.001.

Supplementary Table 3. showing plateletpheresis donors **(a)** platelet count (PLT), **(b)** immature platelet fraction (IPF) and **(c)** mean platelet volume (MPV), at the indicated timepoints, data expressed as relative to pre-donation control (Pre), n=19, one-way ANOVA with Bonferroni's multiple comparison test, not statistically significant (ns), *p<0.05, **p<0.01, ***p<0.001.

Supplementary Table 4. showing plasmapheresis donor and donation information, negative (-ve), positive (+ve), body mass index (BMI).

Supplementary Table 5. showing plasmapheresis donors full blood counts relative to the pre-donation control (Pre), bold text indicates statistical significance, average (Av.), n=5, one-way ANOVA with Tukey's multiple comparison test, not statistically significant (ns), *p<0.05, **p<0.01, ***p<0.001.

Supplementary Table 6. showing proteins identified with mass spectrometry that are down-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=5.

Supplementary Table 7. showing proteins identified with mass spectrometry that are up-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=5.

Supplementary Table 8. showing metabolites identified with Metabolon that are up-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=11.

Supplementary Table 9. showing metabolites identified with Metabolon that are down-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=11.

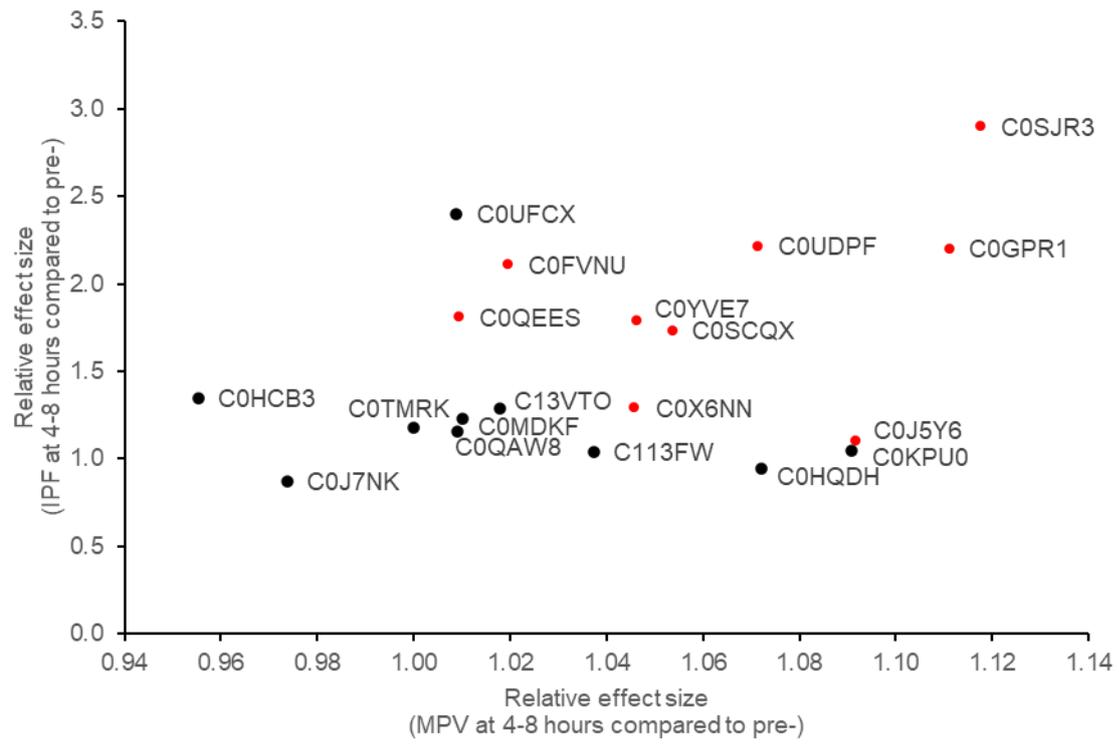
Supplementary Table 10. showing cytokines and growth factors identified with a multiplex bead assay that are down-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=5.

Supplementary Table 11. showing cytokines and growth factors identified with a multiplex bead assay that are up-regulated at the 4-8 hour timepoint compared to the middle point (pre vs. D14), difference in timepoints pre and D14 (2x beta, 1x beta indicated in table), difference in the middle point (pre vs. D14) and the 4-8 hour timepoint (gamma), p value (p), bold text indicated statistically significant proteins, n=5.

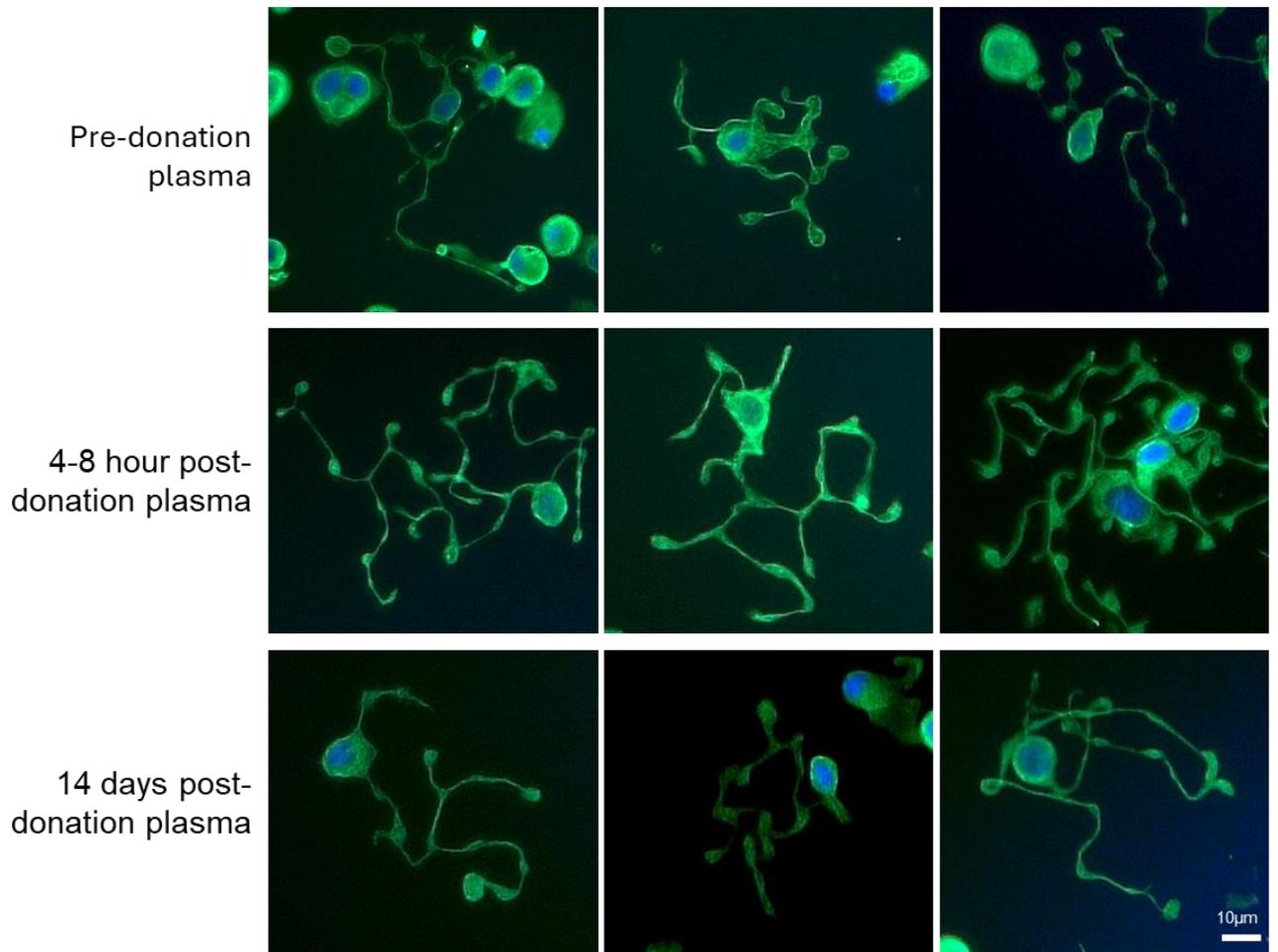
Supplementary Table 12. showing the average serum concentration (pmol/L) of free T3 in plateletpheresis samples at the indicated timepoints, n=5, pre-donation (pre), immediately post donation (post), day (D), standard deviation (SD).

Supplementary Table 13. showing full blood counts for wildtype mice at the indicated timepoints either i.p. injected with 0.6µg/g body weight (BW) anti-CD42b Ab (Depletion) post baseline measurement, or vehicle control (control), n=3-4, two-way ANOVA with with Šídák's multiple comparison test, *p<0.05, **p<0.01, ***p<0.001, ****p<0.0001, not statistically significant (ns).

Supplementary Figure 1

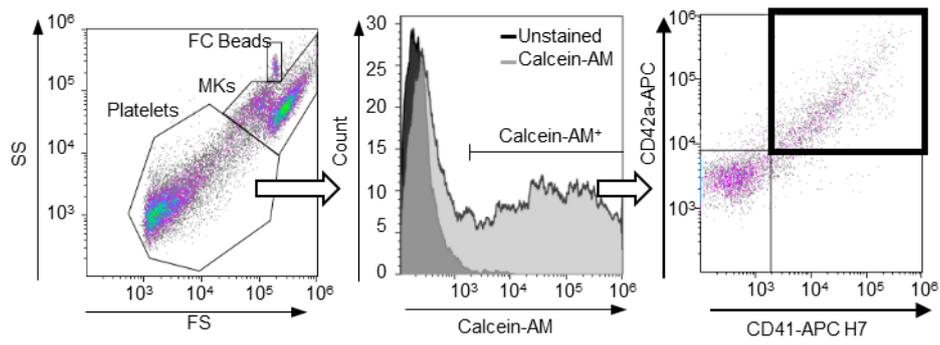


Supplementary Figure 2

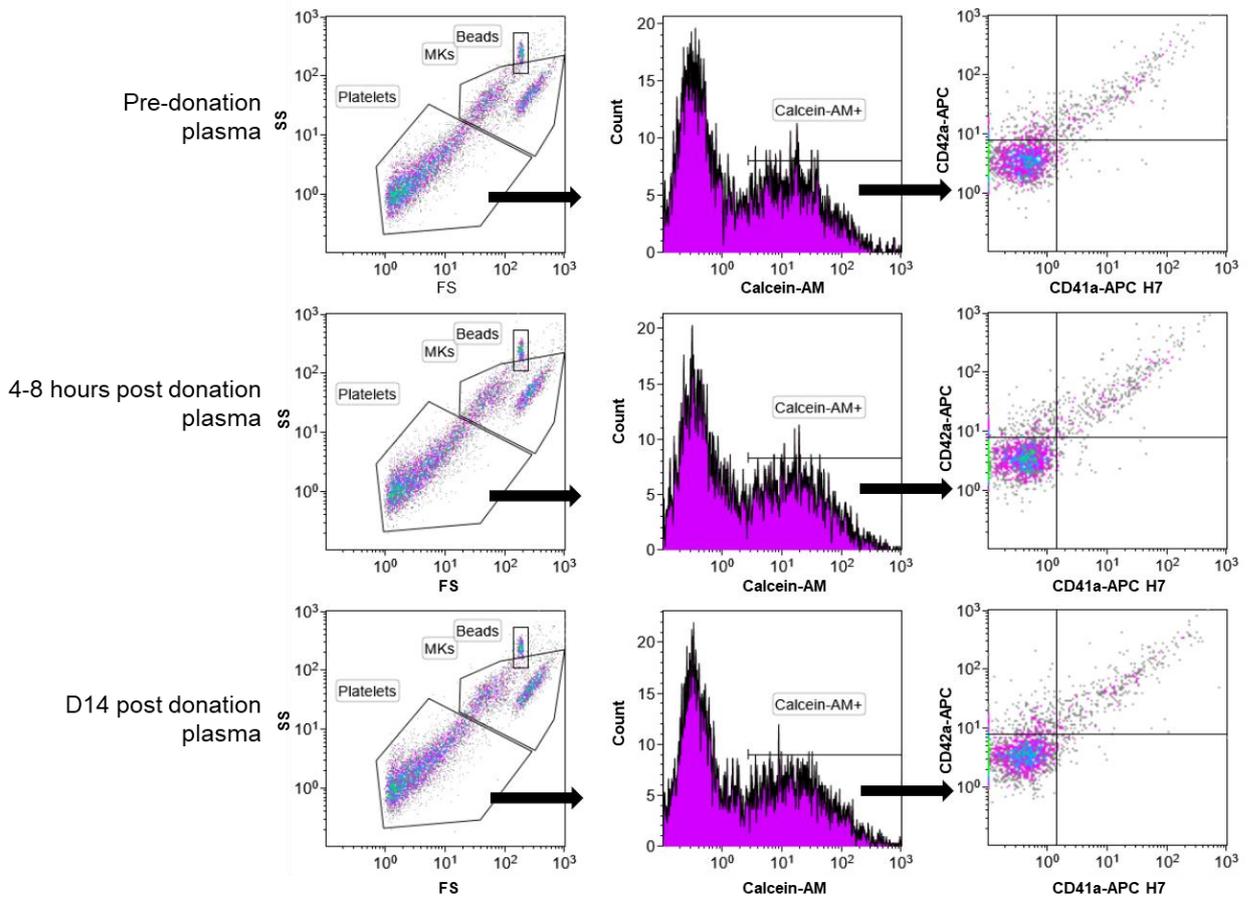


Supplementary Figure 3

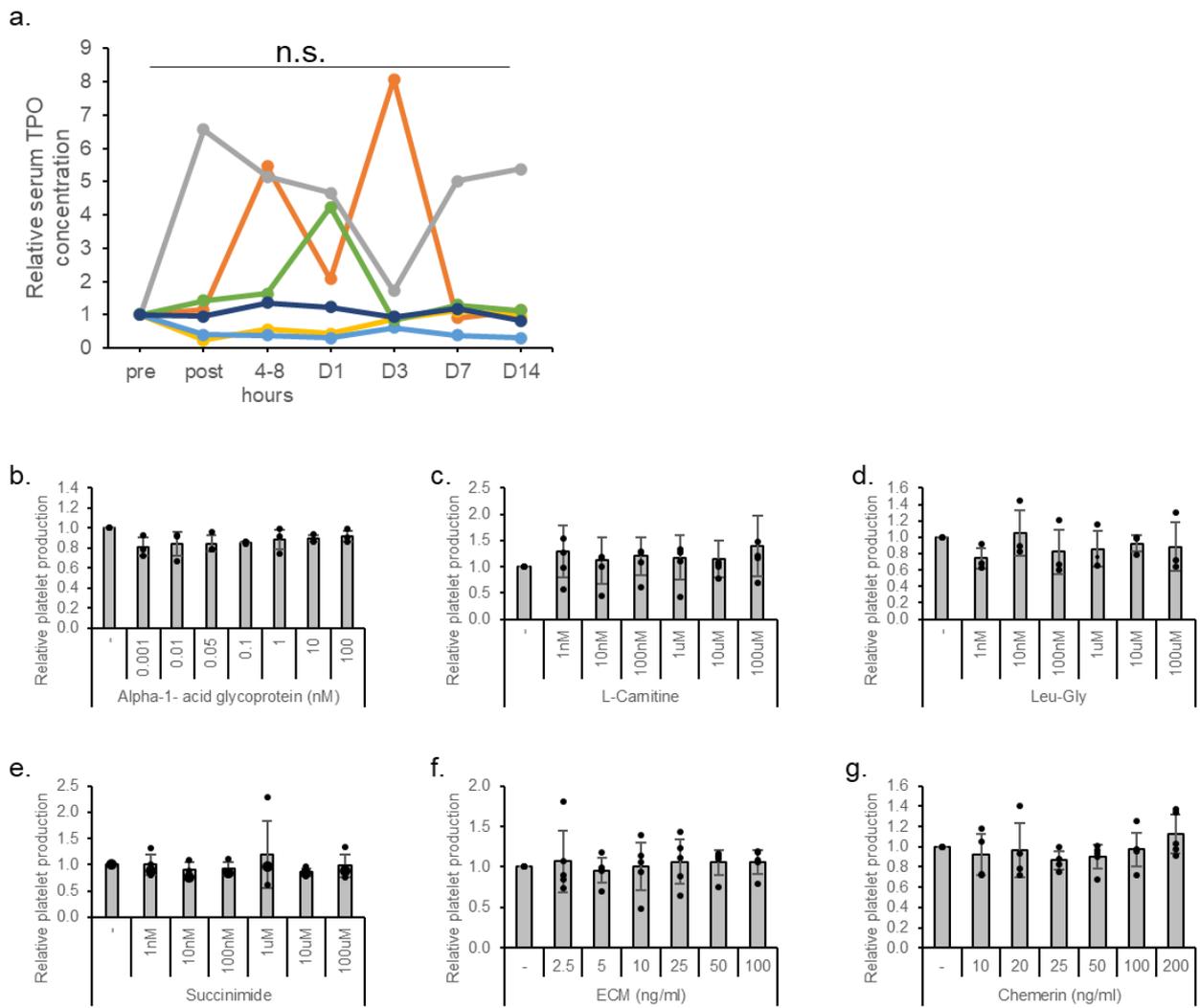
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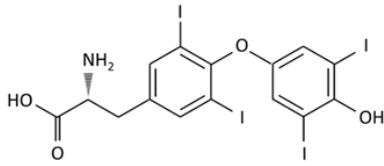


Supplementary Figure 4

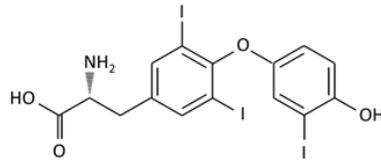


Supplementary Figure 5

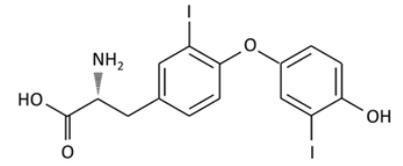
Thyroid hormones



Thyroxine (T4)

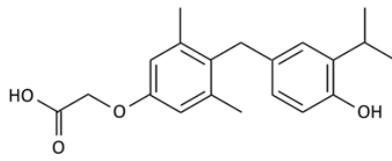


Triiodothyronine (T3)

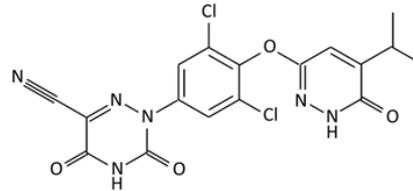


Diiodothyronine (T2)

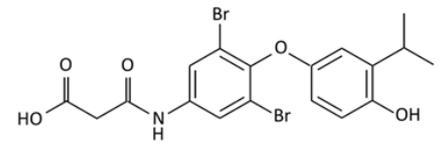
Thyroid hormone analogues- agonists



GC-1 (Sobetirome)

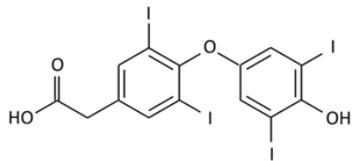


MGL-3196 (Resmetirom)



KB2115 (Eprotirome)

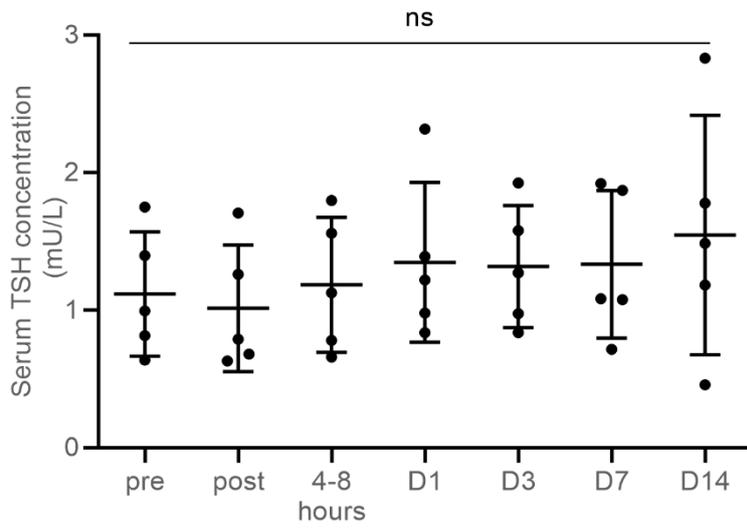
Thyroid hormone analogues- antagonists



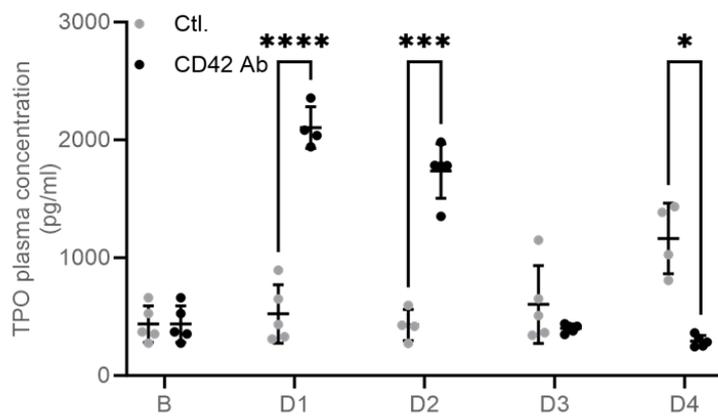
Tetraiodothyroacetic acid (Tetrac)

Supplementary Figure 6

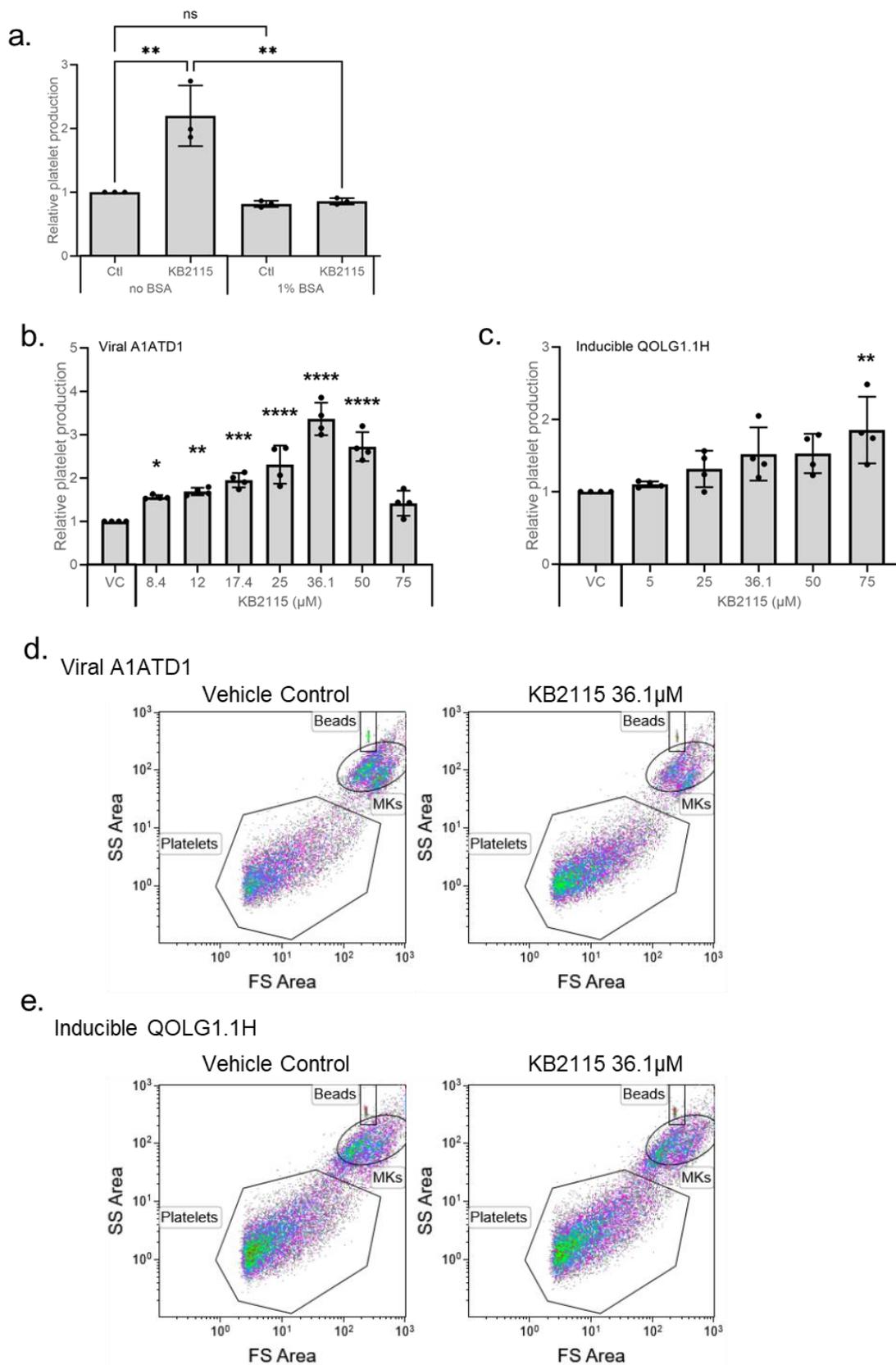
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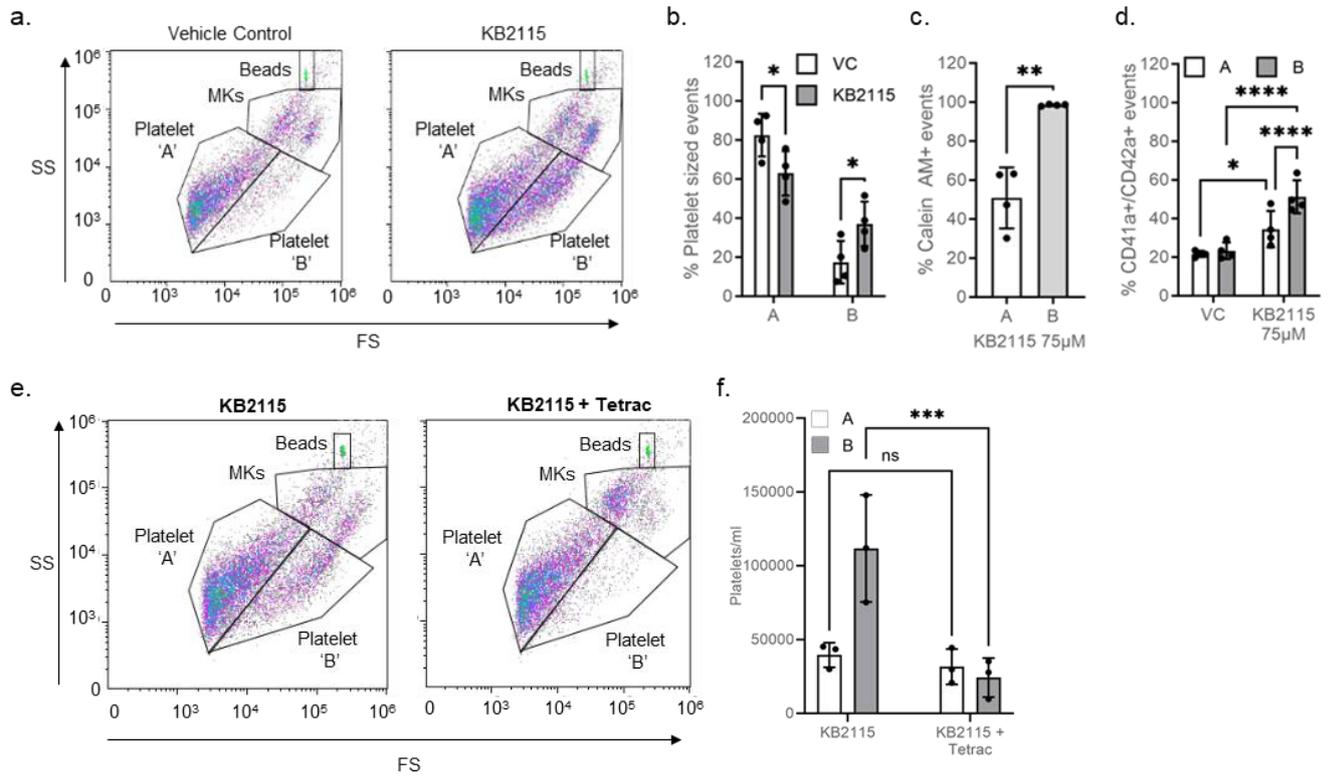
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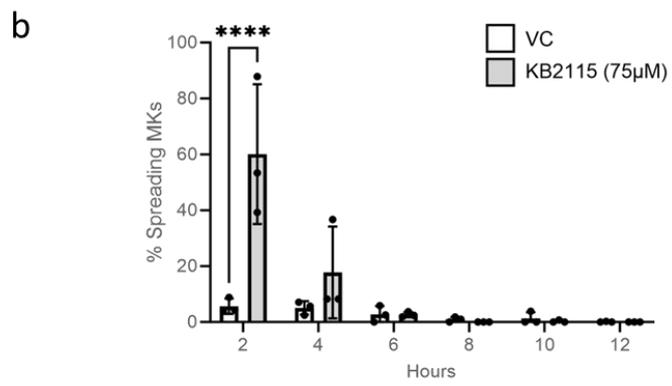
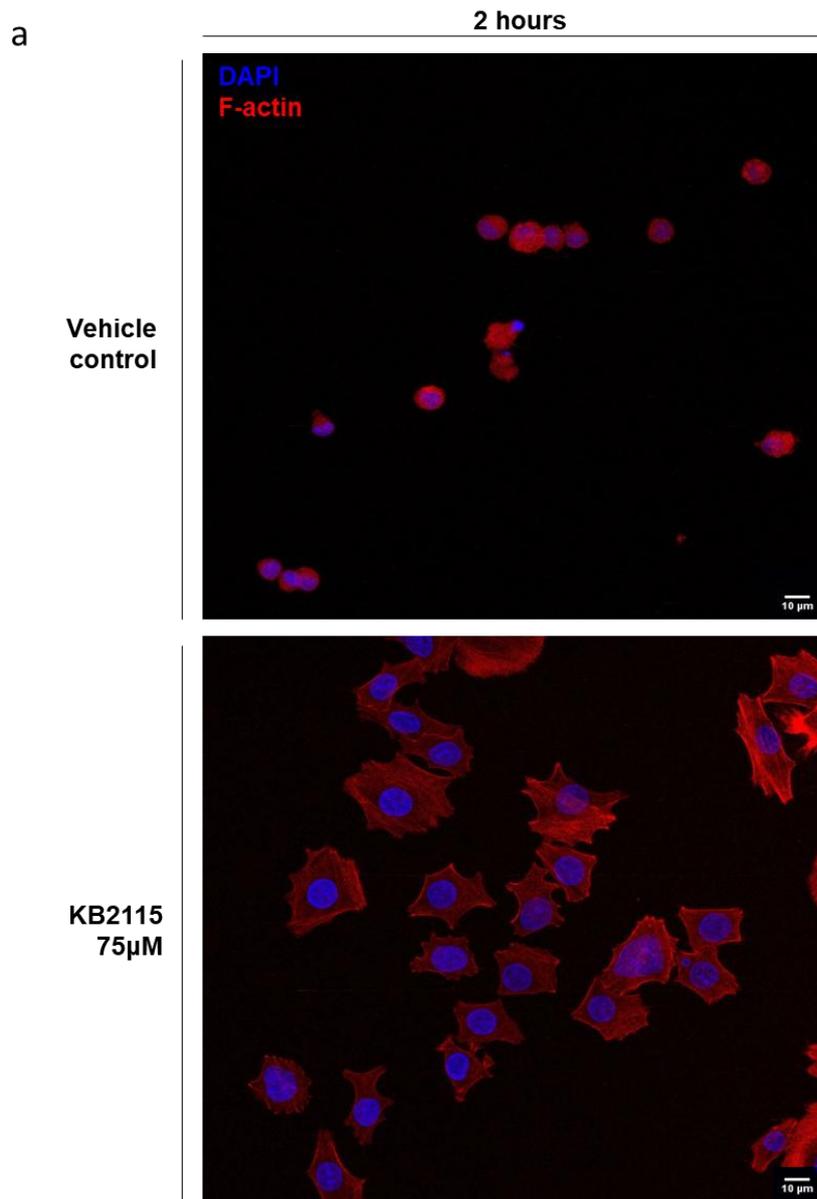
Supplementary Figure 7



Supplementary Figure 8

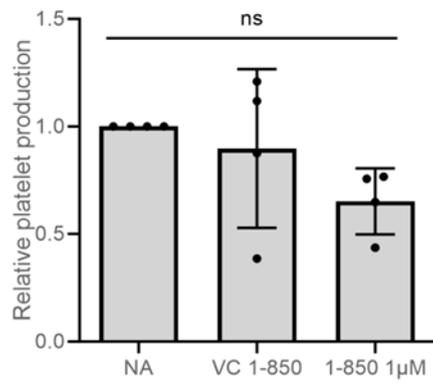


Supplementary Figure 9

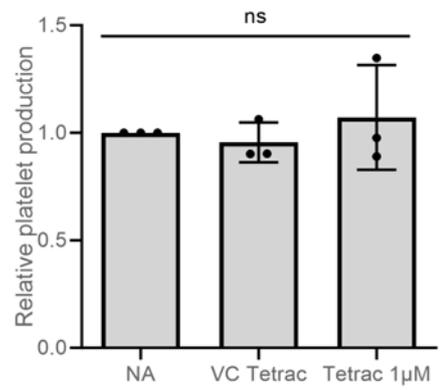


Supplementary Figure 10

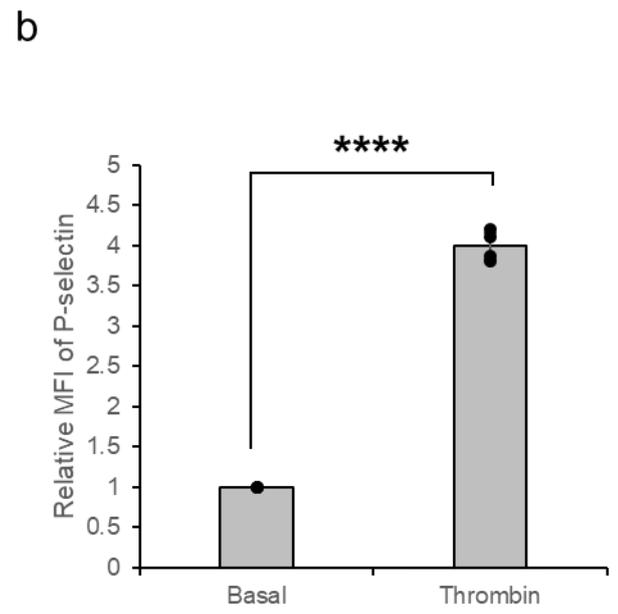
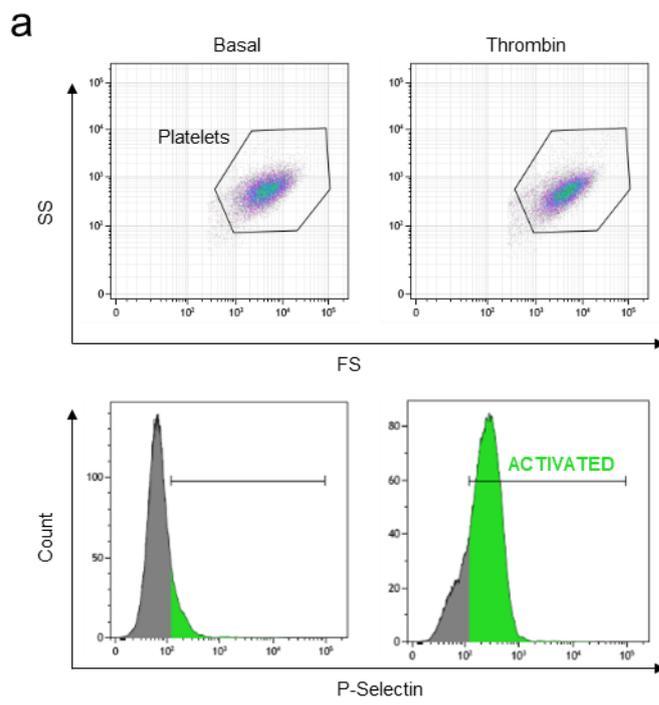
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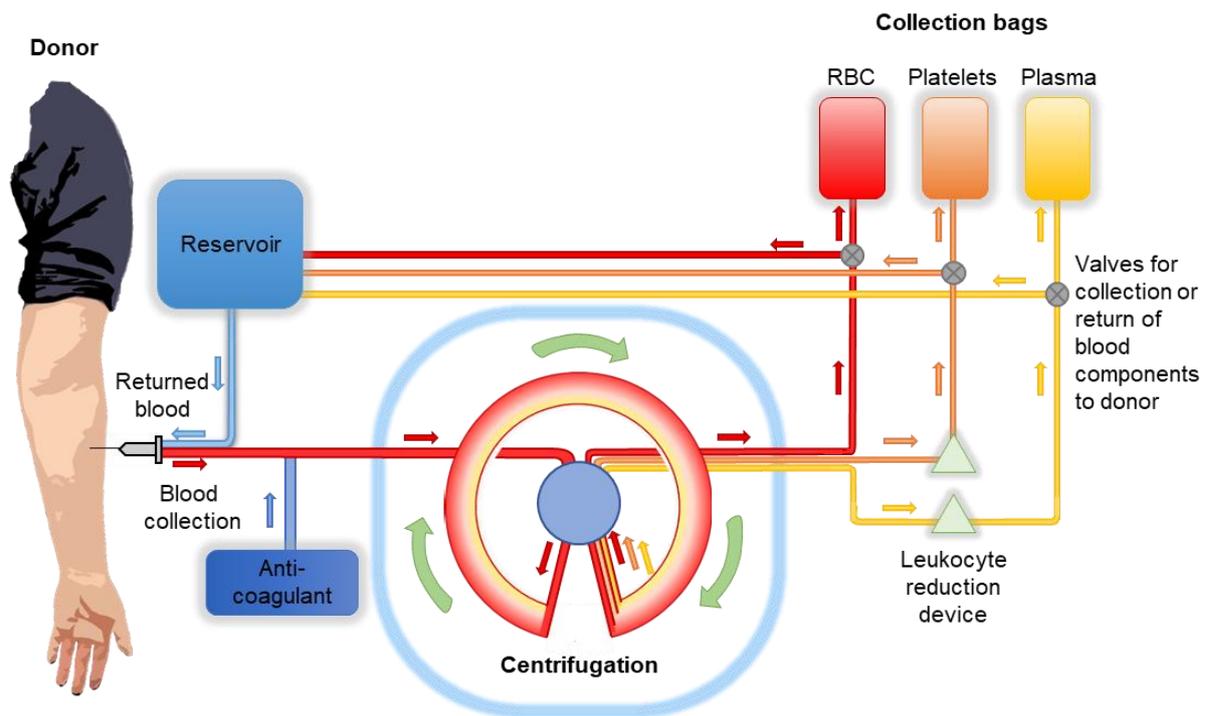
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Supplementary Figure 11



Supplementary Figure 12



Supplementary Table 1

Donor ID	Age	Gender	weight KG	BMI	height CM	initial count 10 ⁹ /L	POST COUNT 10 ⁹ /L	yield 10 ¹¹	Procedure time MIN	CMV	blood type	No. Platelet donation prior to study	No. whole blood donation prior to study	Type of donation on day of study	Ethnicity
C0FVNU	50	Male	89.5	31	170	289	197	8.1	82	-ve	A+	12	2	D	A
C0SCQX	71	Male	88	31.2	168	256	157	8	89	?	O+	96	37	T	A
C0SJR3	69	Male	80	23.4	185	231	145	5.4	65	-ve	O+	47	20	HZD	A
C0GPR1	56	Male	92	30.7	173	297	198	4.9	52	-ve	B+	16	33	D	A
C0J5Y6	70	Male	74	27.2	165	324	200	8.6	85	-ve	O+	48	47	D	A
C0YVE7	69	Male	81	26.4	175	229	158	4.9	67	?	A+	41	37	D	A
C0TMRK	64	Male	89	29.7	173	268	188	5.4	48	+ve	A-	101	35	D	A
C0J7NK	74	Male	95	31	175	207	142	4.8	67	+ve	B-	43	36	D	A
C0X6NN	62	Male	79	24.9	178	294	178	5.8	62	?	A+	61	37	D	A
C13VT0	60	Male	95	29.3	185	286	215	5.3	58	?	B+	112	29	D	A
C0HCB3	56	Male	82	25.9	178	211	124	4.9	78	-ve	O+	93	23	D	A
C0QEES	50	Male	81	24.2	183	300	156	8.1	84	+ve	A+	59	14	T	A
C113FW	53	Male	77	23	183	182	123	4.2	75	?	O+	177	40	D	A
C0UDPF	55	Male	92	30.7	173	248	165	5.4	62	?	O+	122	26	D	A
C0UFCX	34	Male	68	22.7	173	225	127	5.8	85	-ve	A+	52	26	D	A
C0KPU0	48	Male	88	27.8	178	237	152	4.9	79	+ve	O-	90	17	D	A
C0QAW8	70	Male	65	22.7	170	302	207	5.8	59	?	B+	27	7	D	A
C0HQDH	68	Male	85	26.2	180	324	252	5.5	63	+ve	A+	32	24	D	A
C0MDKF	64	Male	80	25.2	178	280	196	5.6	58	+ve	A+	75	17	D	A

Supplementary Table 2

	Timepoints																			
	Pre		Post			4-8 hours			Day 1			Day 3			Day 7			Day 14		
	Av.	SD	Av.	SD	p	Av.	SD	p	Av.	SD	p									
WBC (10 ³ /ul)	1	0	1.06	0.122	ns	1.26	0.234	***	1.09	0.185	ns	1.01	0.117	ns	1.00	0.147	ns	0.99	0.180	ns
RBC (10 ⁶ /ul)	1	0	1.01	0.045	ns	1.02	0.073	ns	0.98	0.053	ns	0.96	0.060	ns	0.96	0.059	ns	0.98	0.086	ns
HGB (g/dL)	1	0	1.02	0.051	ns	1.01	0.070	ns	0.95	0.115	ns	0.96	0.065	ns	0.90	0.218	ns	0.93	0.218	ns
HCT (%)	1	0	1.02	0.048	ns	1.04	0.081	ns	0.98	0.058	ns	0.97	0.079	ns	0.97	0.062	ns	0.98	0.096	ns
MCV (fL)	1	0	1.00	0.009	ns	1.03	0.020	***	1.00	0.013	ns	1.01	0.043	ns	1.00	0.010	ns	1.00	0.013	ns
MCH (pg)	1	0	1.00	0.019	ns	0.99	0.017	ns	1.00	0.014	ns	1.00	0.021	ns	0.99	0.034	ns	1.00	0.023	ns
MCHC (g/dL)	1	0	1.00	0.014	ns	0.97	0.025	ns	0.98	0.100	ns	0.99	0.038	ns	0.99	0.033	ns	1.00	0.018	ns
PLT (10 ³ /ul)	1	0	0.65	0.055	***	0.69	0.093	***	0.71	0.062	***	0.74	0.160	***	1.00	0.161	ns	1.08	0.172	ns
RDW-SD (fL)	1	0	1.00	0.012	ns	1.07	0.048	***	0.99	0.020	ns	1.02	0.075	ns	1.00	0.020	ns	1.00	0.024	ns
RDW-CV (%)	1	0	1.00	0.005	ns	1.04	0.026	***	1.00	0.010	ns	1.01	0.029	ns	1.00	0.017	ns	1.01	0.015	ns
PDW (fL)	1	0	0.98	0.050	ns	1.06	0.069	**	1.02	0.041	ns	1.02	0.050	ns	1.02	0.050	ns	0.98	0.043	ns
MPV (fL)	1	0	0.98	0.024	ns	1.04	0.043	***	1.01	0.022	ns	1.02	0.035	ns	1.01	0.031	ns	0.99	0.023	ns
P-LCR (%)	1	0	0.97	0.104	ns	1.14	0.150	***	1.04	0.080	ns	1.06	0.108	ns	1.04	0.106	ns	0.98	0.079	ns
PCT (%)	1	0	0.66	0.135	***	0.74	0.114	***	0.74	0.124	***	0.83	0.149	*	1.06	0.251	ns	1.12	0.268	ns
NEUT# (10 ³ /ul)	1	0	1.15	0.157	ns	1.35	0.454	ns	1.10	0.238	ns	0.94	0.233	ns	0.97	0.161	ns	1.50	2.188	ns
LYMPH# (10 ³ /ul)	1	0	0.98	0.220	ns	1.25	0.194	**	1.09	0.279	ns	1.06	0.207	ns	1.08	0.240	ns	1.00	0.251	ns
MON# (10 ³ /ul)	1	0	0.94	0.341	ns	1.17	0.337	ns	1.13	0.427	ns	1.02	0.403	ns	1.07	0.472	ns	1.05	0.455	ns
EO# (10 ³ /ul)	1	0	0.87	0.178	ns	1.04	0.373	ns	1.07	0.299	ns	1.19	0.204	ns	1.25	0.402	ns	1.09	0.383	ns
BASO# (10 ³ /ul)	1	0	0.99	0.390	ns	1.03	0.362	ns	1.05	0.317	ns	1.18	0.456	ns	1.13	0.444	ns	1.16	0.485	ns
RET% (%)	1	0	0.97	0.088	ns	0.94	0.218	ns	1.04	0.148	ns	1.13	0.241	ns	1.17	0.213	*	1.20	0.198	**
IRF (%)	1	0	1.15	0.760	ns	1.43	0.928	ns	1.47	0.747	ns	1.80	2.059	ns	1.64	0.950	ns	2.05	2.095	ns
LFRR (%)	1	0	0.96	0.201	ns	1.00	0.018	ns	1.00	0.018	ns	1.00	0.019	ns	0.99	0.015	ns	0.99	0.028	ns
MFR (%)	1	0	1.07	0.583	ns	1.39	0.855	ns	1.45	0.754	ns	1.75	1.997	ns	1.66	0.964	ns	1.83	1.495	ns
RET-He (pg)	1	0	1.00	0.032	ns	1.01	0.037	ns	1.01	0.038	ns	1.01	0.041	ns	0.98	0.049	ns	0.99	0.047	ns
IPF (%)	1	0	1.43	0.669	*	1.56	0.561	**	1.44	0.498	*	1.35	0.539	ns	1.14	0.412	ns	1.05	0.360	ns
NEUT% (%)	1	0	1.09	0.109	ns	1.05	0.169	ns	1.01	0.099	ns	1.02	0.161	ns	0.97	0.112	ns	1.00	0.112	ns
LYMPH% (%)	1	0	0.92	0.150	ns	1.04	0.197	ns	1.02	0.235	ns	1.06	0.173	ns	1.08	0.172	ns	1.01	0.201	ns
MONO% (%)	1	0	0.89	0.269	ns	0.94	0.211	ns	1.02	0.253	ns	1.70	3.027	ns	1.05	0.320	ns	1.07	0.301	ns
EO% (%)	1	0	0.82	0.156	ns	0.81	0.277	ns	0.99	0.295	ns	1.20	0.186	ns	1.26	0.393	*	1.13	0.417	ns
BASO% (%)	1	0	0.86	0.324	ns	0.80	0.276	ns	0.95	0.314	ns	1.13	0.454	ns	1.06	0.352	ns	1.15	0.516	ns

Supplementary Table 3

a

PLT count	Timepoints																				
	Pre			Post			4-8hours			D1			D3			D7			D14		
	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p
Pre	-	-	-	0.65	0.055	***	0.69	0.093	***	0.71	0.062	***	0.76	0.160	***	1.00	0.161	ns	1.08	0.172	ns
Post	-	-	-	-	-	-	1.07	0.124	ns	1.09	0.054	ns	1.18	0.258	ns	1.56	0.253	***	1.69	0.337	***
4-8hours	-	-	-	-	-	-	-	-	-	1.03	0.108	ns	1.12	0.258	ns	1.48	0.296	***	1.60	0.359	***
D1	-	-	-	-	-	-	-	-	-	-	-	-	1.08	0.223	ns	1.43	0.219	***	1.55	0.293	***
D3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.44	0.517	***	1.54	0.529	***
D7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1.06	0.117	ns

b

IPF	Timepoints																				
	Pre			Post			4-8hours			D1			D3			D7			D14		
	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p
Pre	-	-	-	1.43	0.669	ns	1.56	0.561	*	1.44	0.498	ns	1.35	0.539	ns	1.14	0.412	ns	1.05	0.360	ns
Post	-	-	-	-	-	-	1.14	0.279	ns	1.06	0.217	ns	1.00	0.273	ns	0.88	0.261	ns	0.77	0.198	ns
4-8hours	-	-	-	-	-	-	-	-	-	0.98	0.287	ns	0.90	0.223	ns	0.80	0.278	ns	0.71	0.214	*
D1	-	-	-	-	-	-	-	-	-	-	-	-	0.94	0.276	ns	0.88	0.365	ns	0.76	0.238	ns
D3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.93	0.279	ns	0.81	0.234	ns
D7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.95	0.247	ns

c

MPV	Timepoints																				
	Pre			Post			4-8hours			D1			D3			D7			D14		
	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p	relative	SD	p
Pre	-	-	-	0.98	0.024	ns	1.04	0.043	**	1.01	0.022	ns	1.02	0.035	ns	1.01	0.031	ns	0.99	0.023	ns
Post	-	-	-	-	-	-	1.06	0.043	***	1.03	0.019	ns	1.04	0.046	**	1.03	0.035	ns	1.01	0.032	ns
4-8hours	-	-	-	-	-	-	-	-	-	0.98	0.039	ns	0.99	0.054	ns	0.97	0.048	ns	0.96	0.036	***
D1	-	-	-	-	-	-	-	-	-	-	-	-	1.01	0.037	ns	1.00	0.027	ns	0.98	0.023	ns
D3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.99	0.023	ns	0.97	0.037	ns
D7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.98	0.022	ns

Supplementary Table 4

Donor No.	Age	Gender	Weight Kg	BMI	Height cm	Yield ml	Procedure time MIN	CMV Status	Blood Type	Ethnicity
#1	29	Male	70.3	22.9	175	450	37	-ve	A+	Caucasian
#2	27	Male	70.3	21.6	180	450	37	-ve	A+	Caucasian
#3	22	Male	77.1	24.4	178	450	36	+ve	O+	African American
#4	38	Male	104.3	31.2	183	450	44	+ve	O+	Caucasian
#5	63	Male	88.5	24.4	191	450	35	+ve	A+	Caucasian

Supplementary Table 5

	Pre		Post			4-8 hours			Day 1			Day 3			Day 7			Day 14		
	Av.	SD	Av.	SD	p	Av.	SD	p	Av.	SD	p	Av.	SD	p	Av.	SD	p	Av.	SD	p
WBC (10 ³ /ul)	1.00	0	0.94	0.062	ns	1.34	0.171	***	1.03	0.083	ns	0.95	0.064	ns	1.01	0.075	ns	0.97	0.118	ns
RBC (10 ⁶ /ul)	1.00	0	1.00	0.087	ns	1.03	0.063	ns	1.02	0.055	ns	0.94	0.103	ns	0.96	0.106	ns	0.91	0.124	ns
HGB (g/dL)	1.00	0	1.00	0.082	ns	1.02	0.051	ns	1.00	0.040	ns	0.93	0.113	ns	0.96	0.102	ns	0.90	0.132	ns
HCT (%)	1.00	0	1.00	0.083	ns	1.03	0.059	ns	1.01	0.042	ns	0.94	0.102	ns	0.96	0.101	ns	0.91	0.115	ns
MCV (fL)	1.00	0	1.00	0.005	ns	1.00	0.010	ns	0.99	0.014	ns	1.01	0.010	ns	1.00	0.013	ns	1.00	0.021	ns
MCH (pg)	1.00	0	1.00	0.010	ns	0.99	0.015	ns	0.98	0.024	ns	0.99	0.012	ns	0.99	0.007	ns	0.99	0.025	ns
MCHC (g/dL)	1.00	0	1.00	0.012	ns	0.99	0.008	ns	1.00	0.018	ns	0.98	0.016	ns	0.99	0.014	ns	0.99	0.029	ns
PLT (10 ³ /ul)	1.00	0	1.03	0.039	ns	1.14	0.048	ns	1.01	0.141	ns	1.20	0.210	ns	1.14	0.083	ns	1.12	0.136	ns
RDW-SD (fL)	1.00	0	1.00	0.008	ns	0.99	0.013	ns	0.98	0.011	ns	1.00	0.013	ns	1.01	0.022	ns	1.00	0.026	ns
RDW-CV (%)	1.00	0	1.00	0.003	ns	0.99	0.006	ns	1.00	0.014	ns	1.00	0.003	ns	1.01	0.013	ns	1.01	0.011	ns
PDW (fL)	1.00	0	0.93	0.057	ns	0.91	0.049	*	0.95	0.093	ns	0.92	0.041	ns	0.95	0.071	ns	0.93	0.057	ns
MPV (fL)	1.00	0	0.97	0.028	*	0.95	0.019	**	0.98	0.007	ns	0.98	0.019	ns	0.97	0.025	ns	0.97	0.023	ns
P-LCR (%)	1.00	0	0.90	0.091	*	0.87	0.076	**	0.96	0.024	ns	0.96	0.057	ns	0.93	0.088	ns	0.94	0.076	ns
PCT (%)	1.00	0	0.99	0.048	ns	1.07	0.040	ns	1.06	0.059	ns	1.19	0.201	ns	1.10	0.103	ns	1.16	0.175	ns
NEUT# (10 ³ /ul)	1.00	0	0.99	0.067	ns	1.46	0.386	***	1.06	0.115	ns	0.95	0.067	ns	1.01	0.104	ns	1.09	0.117	ns
LYMPH# (10 ³ /ul)	1.00	0	0.90	0.098	ns	1.26	0.147	**	1.00	0.117	ns	0.94	0.044	ns	0.99	0.061	ns	0.82	0.159	ns
MON# (10 ³ /ul)	1.00	0	0.95	0.043	ns	1.20	0.311	ns	1.03	0.219	ns	1.02	0.249	ns	1.07	0.206	ns	0.97	0.155	ns
EO# (10 ³ /ul)	1.00	0	0.84	0.082	ns	1.00	0.201	ns	0.88	0.285	ns	0.90	0.219	ns	1.03	0.224	ns	0.74	0.208	ns
BASO# (10 ³ /ul)	1.00	0	0.77	0.160	ns	1.00	0.158	ns	0.87	0.108	ns	0.97	0.166	ns	1.06	0.171	ns	0.76	0.224	ns
NEUT% (%)	1.00	0	1.05	0.030	ns	1.07	0.152	ns	1.03	0.103	ns	1.01	0.044	ns	1.00	0.060	ns	1.13	0.081	ns
LYMPH% (%)	1.00	0	0.95	0.046	ns	0.97	0.214	ns	0.97	0.077	ns	0.99	0.046	ns	0.98	0.062	ns	0.84	0.092	ns
MONO% (%)	1.00	0	1.02	0.065	ns	0.89	0.178	ns	0.99	0.154	ns	1.06	0.211	ns	1.05	0.163	ns	1.00	0.121	ns
EO% (%)	1.00	0	0.90	0.137	ns	0.77	0.200	ns	0.86	0.289	ns	0.96	0.214	ns	1.02	0.233	ns	0.75	0.130	ns
BASO% (%)	1.00	0	0.82	0.155	ns	0.74	0.178	ns	0.86	0.124	ns	1.02	0.155	ns	1.09	0.232	ns	0.78	0.164	ns
PLT-I (10 ³ /ul)	1.00	0	1.03	0.030	ns	1.12	0.056	ns	1.07	0.068	ns	1.20	0.206	ns	1.12	0.091	ns	1.20	0.165	ns
PLT-F (10 ³ /ul)	1.00	0	1.03	0.039	ns	1.14	0.048	ns	1.01	0.141	ns	1.20	0.210	ns	1.14	0.083	ns	1.12	0.136	ns
PLT-O (10 ³ /ul)	1.00	0	1.01	0.038	ns	1.13	0.031	ns	0.99	0.204	ns	1.20	0.185	ns	1.13	0.091	ns	1.08	0.148	ns
RET# (10 ⁶ /ul)	1.00	0	1.01	0.095	ns	1.06	0.104	ns	1.08	0.089	ns	1.00	0.215	ns	1.14	0.317	ns	1.09	0.279	ns
RET-He (pg)	1.00	0	1.00	0.008	ns	0.99	0.010	ns	1.01	0.020	ns	1.00	0.024	ns	1.00	0.031	ns	1.00	0.017	ns
RET% (%)	1.00	0	1.02	0.056	ns	1.03	0.058	ns	1.06	0.048	ns	1.06	0.136	ns	1.16	0.201	ns	1.19	0.195	ns
IRF (%)	1.00	0	0.96	0.201	ns	0.94	0.140	ns	1.05	0.182	ns	1.12	0.169	ns	1.41	0.220	**	1.25	0.123	ns
LFR (%)	1.00	0	1.00	0.022	ns	1.00	0.014	ns	1.00	0.015	ns	0.99	0.010	ns	0.97	0.018	**	0.98	0.008	ns
MFR (%)	1.00	0	0.92	0.180	ns	0.94	0.105	ns	1.04	0.185	ns	1.06	0.112	ns	1.30	0.184	*	1.20	0.095	ns
HFR (%)	1.00	0	1.72	1.191	ns	1.87	2.090	ns	1.66	1.098	ns	1.93	0.922	ns	3.19	2.202	ns	3.39	4.326	ns
IPF (%)	1.00	0	0.93	0.128	ns	0.96	0.143	ns	0.91	0.106	ns	0.92	0.016	ns	0.92	0.045	ns	0.87	0.121	ns

Supplementary Table 6

Protein ID	Gene name	beta	gamma	p	Protein ID	Gene name	beta	gamma	p
Q8BUN1	MENT	0.053010	-0.150506	0.000017	Q8UX71	PLXDC2	-0.016404	-0.102270	0.114109
P00746	KFD	0.009653	-0.143482	0.000333	P00738	C1R	0.015048	-0.046903	0.117918
P35527,Q99456	KRT9/KRT12	-0.047904	-0.327047	0.000351	P02787	TF	0.026728	-0.075289	0.119717
O95445	APOM	0.036652	-0.124114	0.000379	P10203,Q16880,Q6Y2X3	A2M/UGT8/DNAJC14	-0.003594	-0.141373	0.120914
P02656	APOC3	-0.007128	-0.128913	0.000426	P36955,Q86VW0	SERPINF1 /SEST1	0.038909	-0.095170	0.128135
P55056	APOC4	0.022663	-0.110175	0.000948	P10119	AGT	0.011830	-0.116881	0.128258
P07358	C8B	0.026642	-0.171459	0.001190	Q06033	ITI3	0.028433	-0.100147	0.133303
P01344	IGF2	0.038817	-0.177723	0.001190	A6NIZ1	RPI1BL	-0.024819	-0.379329	0.133365
P55290	CDH13	0.078384	-0.246829	0.001206	P24592	IGFBP6	0.000265	-0.067966	0.137917
Q6EMK4	VASN	0.030141	-0.174524	0.001721	Q92954	PRG4	0.023908	-0.056005	0.144099
Q9UGM5	FETUB	0.012908	-0.070828	0.002028	P05160	F13B	0.081549	-0.123462	0.144309
P07360	C8G	0.000116	-0.050536	0.002150	P10031	C5	0.011848	-0.056402	0.155716
Q96PD5	PGLYRP2	0.058090	-0.143639	0.002243	Q8UX07	FERMT3	0.056592	-0.056225	0.155995
P02774	GC	0.051062	-0.104835	0.002965	P03951	F11	-0.001527	-0.070182	0.157497
Q16270	IGFBP7	0.037773	-0.180923	0.003042	P02538	KRT6A	-0.172748	-0.592837	0.167913
P09871	C1S	0.046375	-0.109928	0.003048	P62979	RPS27A	-0.022870	-0.190748	0.175438
P26927,Q2TV78	MST1 /MST1L	0.024124	-0.102285	0.003362	P00734	F2	0.020173	-0.050155	0.180812
O75665	OFD1	0.009535	-0.114315	0.004373	P13645,Q16703,Q7Z3Y7,Q7Z3Y8	KRT10 /KRT36/KRT28/KRT27	-0.002648	-0.114676	0.192173
P02654	APOC1	0.024545	-0.168580	0.004640	P02775	PPBP	0.014118	-0.170380	0.192176
Q5T200	ZC3H13	0.095267	-0.186418	0.004878	P10591	UCHAIN	0.001524	-0.160005	0.194005
P04264	KRT1	0.023742	-0.277159	0.005425	Q03003	DST	-0.055066	-0.118200	0.195087
P00740	F9	-0.005444	-0.115383	0.005605	P09172	DBH	0.090844	-0.108105	0.196560
P00742	GLIPR2	0.050013	-0.073642	0.005938	P02788	LTF	0.025762	-0.864843	0.211476
Q9H4G4	GLIPR2	0.042555	-0.213843	0.006401	Q80VB7	CD183	0.054531	-0.714534	0.211838
P05106	ITGB3	0.109645	-0.376223	0.008716	Q8UX07	DHR513	-0.185026	-0.445963	0.231940
Q5SYC1	CLVS2	0.058650	-0.377484	0.009230	P04278	SHBG	-0.020344	-0.065770	0.243510
P00450	CP	0.021417	-0.146056	0.009264	P04259,Q96678,P05877,Q5KKE5	KRT6B /KRT75/KRT8/KRT79	-0.238438	-0.291491	0.251682
Q8D862	FLG2	0.484999	-2.477125	0.009802	P15169	CPN1	0.016445	-0.026384	0.266713
P04211	IGLV7-43	0.040006	-0.227328	0.009904	P04406	SAPDH	0.116911	-0.445361	0.269041
O00533	CHL1	0.102108	-0.227888	0.009928	P00488,P16452	F13A1 /EPB42	-0.007462	-0.115010	0.279322
P05362	ICAM1	0.024211	-0.185796	0.010766	Q96390	PEBP4	-0.054304	-0.292020	0.281980
P06276	BCHE	0.047148	-0.081661	0.012260	Q9QNV8	HIST2H2BF	-0.030633	-0.732824	0.283644
P02743	APC3	0.017164	-0.086379	0.012453	P02749	APOH	0.000945	-0.050478	0.287910
P15144	ANPEP	0.061637	-0.166745	0.012723	Q14560	LECT2	-0.648069	-0.648069	0.297546
P35542	SA4A	0.026308	-0.134859	0.012773	Q9P0K7	RAI14	-0.034413	-0.125976	0.297593
P02747	C1QC	0.011487	-0.092527	0.013021	P16999	GLV1-44	0.524721	-0.550632	0.300447
P35908	KRT2	-0.011625	-0.139527	0.013065	P00747,Q02325,Q15195,Q6ZU55	PLG /PLGLB1 /PLGLA /CCDC121	0.031252	-0.035793	0.316813
Q8TF61	FBXO41	-0.010862	-0.067167	0.013566	P19013	KRT4	0.046815	-0.887381	0.326643
P02746	C1QB	0.021295	-0.097371	0.013612	Q43866	CD5L	-0.075561	-0.150266	0.339972
Q9Z539	LPIN2	0.582709	-0.684145	0.013699	P18085	IGFBP2	0.032223	-0.055564	0.341369
P02760	AMPB	0.005316	-0.052528	0.014218	Q8TEA8	DTD1	0.034696	-0.073974	0.342562
P51884	LUM	0.036549	-0.085922	0.014681	P11023	HSPA5	0.025439	-0.065998	0.344200
P03952	KLKB1	0.032019	-0.097574	0.015284	Q9UK55	SERPINA10	0.009687	-0.034662	0.349246
P02649	APOE	0.027763	-0.125238	0.016932	P36915	SNL1	0.668297	-0.624255	0.359004
P54108,P16562	CRISP3/CRISP2	0.044430	-0.098389	0.017198	P02655	APOC2	-0.093714	-0.025652	0.384935
P01042	KNG1	0.040832	-0.095272	0.018220	P04275	VWF	0.028819	-0.046741	0.398335
O43307	ARHGFE9	0.036695	-0.171106	0.019017	P01814	IGKV2D-40	-0.053467	-0.057957	0.405844
P10643	C7	0.044389	-0.149170	0.020847	Q99969	RARRES2	-0.003675	-0.054265	0.430145
P01024,Q95568,Q13620	C3 /METTL18 /CUL4B	0.082326	-0.130818	0.021174	Q75083	WDR1	-0.045472	-0.451089	0.432778
O15553	MEFV	0.499569	-1.452560	0.021360	P17936,Q14717	IGFBP3 /TRDMT1	0.013604	-0.016821	0.433690
Q9NQ79	CRTAC1	0.003469	-0.023121	0.023801	P18423	LBP	-0.035591	-0.018981	0.436633
Q9UJJ9	GNPTG	0.096146	-0.182259	0.025788	Q981Y4,Q8WWZ3	CFB2 /EDATADD	0.010940	-0.008280	0.437238
Q8N6C8	LILRA3	-0.010154	-0.133348	0.026691	P02750	LRG1	0.011871	-0.046307	0.450800
P13671	C6	0.022147	-0.133309	0.029207	Q9NR20	DYRK4	-0.207219	-0.076741	0.452415
P02765	AHSG	0.043974	-0.084660	0.029761	P02768	ALB	0.012993	-0.072031	0.463776
P07357	C8A	0.029158	-0.127420	0.031481	P15582	TGFB1	0.002423	-0.048767	0.477416
Q86Y23	HRNR	-0.005866	-0.259878	0.032449	P06396	SSN	0.013951	-0.033378	0.484428
P27918	CFP	0.020897	-0.173165	0.035025	P04217	A1BG	-0.000253	-0.019973	0.486089
Q00403	GFIT2B	0.090325	-0.374701	0.040716	P00738	HP	-0.051059	-0.050977	0.489025
P49908	SELENOP	0.035676	-0.125706	0.041030	Q14520	HABP2	0.022582	-0.019405	0.500153
P12035	KRT3	0.539349	-1.803273	0.042487	P54289	CACNA2D1	0.278375	-0.138857	0.502465
P13591	NCAAM1	-0.009528	-0.216896	0.042970	P22892	IGFBP4	-0.039267	-0.017650	0.508836
P06727,Q43290	APOA4 /SART1	0.041541	-0.095431	0.043066	Q9NZP8	C1RL	-0.011534	-0.011198	0.512225
P00748	F12	0.042301	-0.089553	0.043417	P25092	GUCY2C	-0.008256	-0.027469	0.518823
P12259	F5	0.014735	-0.082928	0.044444	P25311,A8MT79	AZGP1	0.056334	-0.022149	0.523025
P62805	HIST1H4A	0.106124	-0.285096	0.047021	B9A064,P0CG04	IGLL5 /IGLC1	-0.023558	-0.038187	0.545777
P55103	INHBC	-0.045465	-1.465406	0.047359	P02652	APOA2	-0.025764	-0.036330	0.565220
P07225	PROS1	0.017287	-0.072433	0.048573	P04196,Q08031,Q9NRN5	HRG /PRAF2 /OLFML3	0.016995	-0.011681	0.567176
Q08380	LGAL S3BP	0.024554	-0.042260	0.048632	P13646	KRT13	-0.217117	-0.159750	0.582994
P12955	FEPD	0.041589	-0.120145	0.050929	P01762,P01763,P01765	IIGHV3-11 /IIGHV3-48 /IIGHV3-23	-0.061599	-0.058781	0.588629
P00751	CFB	0.017772	-0.083305	0.052269	Q00391	SOX1	0.025636	-0.010069	0.601894
P22891	PROZ	-0.053403	-0.099274	0.053916	P22105	TNXB	0.008042	-0.016178	0.628120
Q13790	APOF	0.017277	-0.101630	0.059836	P22792	CPN2	-0.005770	-0.009363	0.630170
P01860	IGHG3	-0.030645	-0.149894	0.060006	P19320	VCAM1	0.001671	-0.020308	0.633200
P01623,P01620,P18135	IGKV3-20	-0.006318	-0.288340	0.060081	Q92698	RAD54L	-0.981059	-0.425195	0.646580
P0DJ18	SAA1	-0.021769	-0.121371	0.060624	Q88UD1	OAF	0.032777	-0.052303	0.654074
O43790	KRT8B	0.004491	-0.082283	0.061652	P01834,Q175747	IGKC /PIK3C2G	-0.036170	-0.031970	0.654214
P02647	APOA1	-0.001419	-0.044679	0.063531	P41222	PTGDS	0.017528	-0.021569	0.669555
P04114,Q43147,Q8WUJ1	APOB /SGSM2 /CYB5D2	-0.003314	-0.062112	0.064901	P07359	GF1BA	-0.022460	-0.020405	0.671310
Q96KN2	CNDP1	0.039853	-0.055142	0.067880	P01871,P04220	SP1	0.051836	-0.034408	0.695857
Q14624	ITI4	0.024457	-0.048561	0.067811	P0CCG05,A0M8Q6,P0CF74,P0CCG06	IGLC7 /IGLC8 /IGLC2 /IGLC3	-0.030259	-0.025113	0.704931
P15311,P26038	EZR /MSN	-0.161375	-0.453358	0.067955	P19827,P07148	ITI1H /FABP1	0.021101	-0.011988	0.718420
P05156,Q5SWL8	CFI /PRAMER19	0.004731	-0.063377	0.069527	Q95810	CAVIN2	0.0173679	-0.109602	0.720469
Q9C073	FAM117A	-0.000744	-0.063927	0.069636	Q75636	FCN3	0.017419	-0.015389	0.722895
Q8NGU4	OR21P	0.070850	-0.037398	0.070547	P0C0L5	C4B	-0.005906	-0.014889	0.723482
Q96Q42	ALS2	0.001705	-0.086908	0.071177	P23142	FBLN1	0.045428	-0.038394	0.724120
O14791	APOL1	0.008764	-0.078140	0.071677	P35658	IRS1	-0.022424	-0.009498	0.727515
P06310	IGKV2-30	0.016270	-0.133686	0.072647	P0C0L4	C4A	-0.001854	-0.010625	0.741522
Q96GE4	CEP95	0.020033	-0.055309	0.080929	Q5T955	CCDC18	-0.018526	-0.035100	0.753859
P01008	SERPINC1	0.062180	-0.057401	0.081499	P19823	ITI2	-0.007379	-0.010038	0.776744
O00187	MASP2	0.009213	-0.107793	0.081959	P02741	CRP	-0.055362	-0.015339	0.791133
P04004	VTN	0.040955	-0.078486	0.083396	Q9NR81	SARS2	0.316209	-0.193637	0.801621
P06681,Q14814	C2 /MEF2D	0.005314	-0.074631	0.084425	P10909	CLU	0.050499	-0.022266	0.804664
P08493	MG	0.021801	-0.154207	0.085181	P08571	CD14	-0.011339	-0.008990	0.805076
O14786	NRP1	0.003481	-0.031862	0.086382	P07737	PFN1	-0.296120	-0.189972	0.839312
P82930	MRFPS34	0.691927	-0.837888	0.086631	P02745	C1QA	0.007584	-0.004705	0.853009
P0C055	H2AFZ	0.012287	-0.263325	0.087049	Q14028	CNGB1	0.005085	-0.006015	0.868314
P04070	PROC	0.000280	-0.074416	0.088489	Q08294	SOD3	0.021971	-0.022500	0.869943
P49747	COMP	-0.009832	-0.037807	0.088828	P40197	SP5	-0.115416	-0.019806	0.885589
P13796,P13797	LCP1 /PLS3	0.063814	-0.096218	0.0900					

Supplementary Table 7

Protein ID	Gene name	beta	gamma	p	Protein ID	Gene name	beta	gamma	p
Q722Q7	LRRC70	-0.135505	0.246776	0.000215	P00915	CA1	-0.150708	0.219822	0.273900
P67936;P06753;P07951	TPM4/TPM3/TPM2	-0.055633	0.088530	0.000516	P01859	IGHG2	-0.058793	0.089523	0.284524
P05543	SERPINA7	-0.026199	0.185964	0.000702	P01009;P20848	SERPINA1 /SERPINA2	-0.032818	0.057181	0.314205
Q9Y5Y7	LYVE1	-0.062107	0.444443	0.002835	P08803;P36980	CFH/CFHR2	-0.010545	0.042302	0.325649
P01011	SERPINA3	-0.061226	0.169012	0.003714	P82937;A2BFH1;Q9Y536	PFIA/PPIAL4G/PPIAL4C/PPIAL4A	-0.047672	0.049194	0.325989
P02763	ORM1	-0.059553	0.226023	0.003738	Q86SQ7	SDCCAG8 5	-0.041156	0.058438	0.326300
P49913	CAMP	-1.296828	1.563307	0.003924	P43251	BTD	-0.006320	0.013873	0.366403
P22352	GPX3	-0.038917	0.162956	0.003975	P30519	HMOX2	0.024388	0.039227	0.372812
P04180	LCAT	-0.037060	0.048257	0.004266	P08519	LPA	-0.005252	0.034309	0.397559
P02753	RBP4	-0.044482	0.125089	0.005709	P33908	MAN1A1	-0.000473	0.042830	0.430610
O00299	CLIC1	-0.168893	0.513368	0.007195	Q92673	SORL1	0.513752	0.515549	0.440040
P02766	ITTR	-0.001570	0.054278	0.008069	P07333	CSF1R	-0.053191	0.083464	0.442549
P35579;A7E2Y1	MYH9/MYH7B	-0.004509	0.254080	0.008610	Q8NBP7	PCSK9	-0.054094	0.074253	0.442765
P14625;Q58FF3	HSP90B1/HSP90B2P	-0.031370	0.132403	0.009597	P08727	KRT19	-0.207865	0.128201	0.474523
P02679	FGG	-0.064832	0.173457	0.010620	P06733	ENO1	-0.011131	0.060019	0.485392
P08514	ITGA2B	-0.223769	0.531014	0.013452	P01877	IGHA2	-0.013051	0.054017	0.500383
Q8TEC5	SH3RF2	-0.037090	0.122580	0.014576	P01593	IGKV1D-33	-0.155788	0.055405	0.500644
Q8IXR9	C12orf56	-0.053137	0.182158	0.019551	P02790;Q5T013	HPX/HY1	-0.005389	0.015932	0.504960
P27105	STOM	-0.198343	0.437632	0.023486	P01876	IGHA1	-0.021969	0.050836	0.526860
P61626	LYZ	-0.036738	0.089050	0.031481	P02675	FGF	-0.020424	0.034113	0.564963
Q9H098	FAM107B	-0.227116	0.364377	0.032091	Q02985	CFHR3	-0.068838	0.043201	0.573408
P05452	CLEC3B	-0.015602	0.113860	0.032458	Q9H299	SH3BGR3	-0.006227	0.058824	0.584178
P08709	F7	-0.037540	0.087396	0.037961	P48740	MASP1	-0.016635	0.013189	0.595734
Q92820	GGH	0.011099	0.166546	0.038676	P65058	PLTP	0.003059	0.014293	0.617961
P08185	SERPINA6	-0.033627	0.098287	0.039513	P08637;O75015	FCGR3A/FCGR3B	-0.050399	0.055610	0.621219
P60709;A5A3E0;P0CG38;P0CG39;P6236;Q9BYX7	ACTB/POTEF/POTEI/POTEJ/ACTA2/POTEKP	-0.022370	0.164307	0.044597	P18208	VCL	0.059985	0.041633	0.624871
P19652	ORM2	-0.017679	0.194669	0.050827	P07996;P35442	THBS1/THBS2/HSPA1A	-0.008713	0.039403	0.632442
P05546	SERPIND1	0.007950	0.050393	0.052595	P01857	IGHG1	-0.044570	0.024778	0.647385
P16610	ECM1	-0.008464	0.115548	0.053497	P08107	HSPA1B	-0.081104	0.076324	0.664021
P27169	PON1	-0.022661	0.048354	0.053537	P02776	PF4	-0.056265	0.042955	0.664524
Q9NTM9	CUTC	-0.025287	0.136227	0.061933	P23280	CA6	0.056893	0.139280	0.671515
P21333;O75369	FLNA/FLNB	-0.047405	0.220544	0.062362	P02042	HBD	-0.125195	0.069836	0.678138
O75882;Q5VV63	ATRN/ATRN1	-0.003635	0.043144	0.079724	Q03591	CFHR1	-0.012673	0.026769	0.680966
Q13464	ROCK1	-0.052125	0.069286	0.080262	Q9BWP8	COLEC11	0.512448	0.259394	0.699839
P11597	CETP	-0.106933	0.290574	0.085169	P08253	MMP2	0.002043	0.022729	0.704301
P20851	C4BPB	-0.024620	0.132230	0.095920	P68871;P02100	HBB/HBE1	-0.114095	0.069617	0.723894
Q9NPH3	IL1RAP	-0.071645	0.153552	0.100134	P24593	IGFBP5	-0.026396	0.023990	0.726844
P16070	CD44	-0.017791	0.025221	0.101535	Q04756	HGFAC	0.002818	0.007828	0.739023
P29622	SERPINA4	-0.026264	0.061668	0.104124	P01625;P06312	IGKV4-1	-0.058933	0.038118	0.744907
Q15166	PON3	-0.002253	0.081549	0.106988	P15151	PVR	-0.315893	0.294437	0.749826
P08697	SERPINF2	-0.002641	0.029059	0.119933	P01742	IGHV1-69	-0.041101	0.026695	0.784026
P78417	GSTO1	-0.081726	0.168348	0.123668	Q6PCE3	PGM2L1	0.179223	0.160199	0.786000
P61769	B2M	-0.015043	0.057268	0.126796	P05155;O94892	SERPING1 /ZNF432	-0.040171	0.005509	0.794592
Q12805	EFEMP1	0.029770	0.197421	0.127871	P13224	GP1BB	-0.164045	0.119378	0.804796
P33151	CDH5	0.012815	0.233970	0.134704	P04003	C4BPA	-0.002371	0.008146	0.810191
P02751	FN1	-0.091067	0.150462	0.134724	P69905;P02008	HBA1/HBZ	-0.084453	0.038289	0.813927
P05090	APOD	-0.024843	0.073720	0.139684	Q95497	VNN1	0.006054	0.062404	0.814378
Q8WWZ7	ABCA5	-0.004262	0.091101	0.142725	P04040	CAT	-0.072308	0.040618	0.816750
P32119	PRDX2	-0.277357	0.527659	0.149864	Q6UXB8	PI16	-0.026702	0.020960	0.834029
Q9Y490;Q9Y4G6	TLN1/TLN2	-0.008478	0.101504	0.157214	P04075	ALDOA	-0.039277	0.016375	0.842700
P88032	ACTC1	-0.005500	0.081494	0.175401	Q9NQC3	RTN4	-0.095678	0.132668	0.851186
P37802	TAGLN2	0.057643	0.172038	0.191164	Q8PIJ6	FBXO38	0.000180	0.004928	0.856742
P31946	YWHAB	-0.033742	0.204521	0.199972	P01861	IGHG4	-0.047351	0.010884	0.864956
Q15848	ADIPOQ	-0.017802	0.225318	0.200546	P01700;P01707	IGLV1-47/IGLV2-11	-0.072882	0.013259	0.879743
P05154	SERPINA5	-0.015487	0.033343	0.222027	P08567	FLEK	0.059905	0.004266	0.957749
P43652	AFM	-0.022832	0.035250	0.224333	P23528;P60981;Q9Y281	CFL1/DSTN/CFL2	0.030104	0.006835	0.962635
P12814;Q08043	ACTN1/ACTN3	0.237880	0.956892	0.230372	P24387	CRHBP	-0.156637	0.033533	0.967799
Q9GZY4	COA1	0.594911	0.668160	0.236514	P43121	MCAM	0.186472	0.004195	0.985319
P80108	GPLD1	-0.008509	0.027302	0.237468	P07195;P00338	LDHB/LDHA	0.032478	0.000256	0.992547
Q9UNW1	MINPP1	0.287285	0.631809	0.241893					

Supplementary Table 8

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
X - 21816	0.00126906	0.32827940	1.53E-13	X - 18913	-0.03856053	0.12383672	0.02097786
phosphate	0.00417408	0.04732036	0.00000048	1-docosahexaenoyl-GPE (22:6)*	-0.01273584	0.04060959	0.02112258
X - 13844	0.02079956	0.31958435	0.00000127	X - 21295	-0.01233898	0.15529584	0.02189443
X - 24263	0.00748611	0.13530432	0.00000250	docosadiolate	-0.01776549	0.06367887	0.02274160
X - 22850	-0.00561565	0.09761055	0.00000347	X - 12543	-0.00357006	0.11020941	0.02311600
trigonelline (N-methylnicotinate)	-0.00023801	0.24920101	0.00000571	3-methyl catechol sulfate (1)	0.00828346	0.14518559	0.02314123
X - 12193	-0.00085946	0.17536067	0.00003462	X - 17676	-0.02302836	0.07344448	0.02387603
paraxanthine	0.00798642	0.12382142	0.00004834	1-palmitoyl-2-arachidonoyl-GPI (16:0/20:4)*	-0.00664301	0.04007402	0.02510009
1-linoleoyl-GPE (18:2)*	-0.01189420	0.10933113	0.00005491	X - 17367	-0.01606771	0.08805028	0.02523094
1,7-dimethylurate	-0.00405108	0.10362675	0.00006090	X - 24261	-0.02264036	0.07458923	0.02645976
1-methylurate	-0.02313245	0.14559953	0.00009439	gamma-glutamylglutamate	-0.02507359	0.03740099	0.02690770
quininate	-0.00872287	0.19035111	0.00010182	X - 18345	0.05074531	0.22293817	0.02782646
1-oleoyl-GPE (18:1)	-0.01802285	0.12642914	0.00011163	theobromine	-0.00610119	0.11773945	0.02827701
N-palmitoyl-sphinganine (d18:0/16:0)	0.02622085	0.14233740	0.00014140	X - 17685	-0.05109448	0.10916257	0.02837464
1-palmitoyl-3-linoleoyl-glycerol (16:0/18:2)*	-0.01171499	0.09010455	0.00014175	glycochenodeoxycholate	0.02303047	0.11327861	0.02892740
X - 24278	-0.01669117	0.10883845	0.00014339	1-docosapentaenoyl-GPC (22:5n6)*	-0.01252227	0.05634444	0.03295738
X - 24023	-0.02354320	0.09613152	0.00015193	X - 16938	-0.00117539	0.07231462	0.03323081
X - 24260	-0.01770948	0.08101343	0.00018913	7-methylxanthine	-0.00822650	0.02550535	0.03400311
5-acetylamino-6-amino-3-methyluracil	-0.01066174	0.10433498	0.00019561	1-eicosapentaenoyl-GPE (20:5)*	-0.00934858	0.05218617	0.03547493
1,2-dilinoleoyl-GPE (18:2/18:2)*	0.00778601	0.24280191	0.00023447	X - 23263	-0.00007331	0.05199715	0.03593769
X - 13723	-0.03644339	0.16727372	0.00027253	2-arachidonoyl-GPE (20:4)*	0.00381715	0.03895219	0.03735512
X - 12263	-0.03682151	0.25652510	0.00032544	4-guanidinobutanoate	0.00464843	0.05217765	0.03762188
X - 24811	-0.02664157	0.16440309	0.00032722	X - 13737	-0.00507153	0.05082913	0.04009536
1-methylxanthine	-0.00562165	0.11050481	0.00037360	N-acetylglutamate	-0.01531835	0.06380267	0.04043709
1-palmitoyl-2-linoleoyl-glycerol (16:0/18:2)*	-0.01223803	0.06138818	0.00052305	citruiline	-0.00961219	0.02056008	0.04170863
X - 22145	0.01719765	0.07057096	0.00058062	X - 16570	-0.02682192	0.04477413	0.04314603
X - 22147	-0.02021271	0.31248222	0.00058996	X - 24542	-0.03474424	0.07678139	0.04567663
glucuronate	-0.00008704	0.04070805	0.00077438	1-oleoyl-2-linoleoyl-GPI (18:1/18:2)*	-0.01322638	0.04881539	0.04650925
1,3,7-trimethylurate	-0.00601442	0.09989898	0.00081439	o-cresol sulfate	0.00550887	0.09976767	0.04785931
X - 23196	-0.03321844	0.12112001	0.00122346	X - 16947	0.07964384	0.23767797	0.04785931
X - 24565	0.02863484	0.28233143	0.00175994	3-methoxycatechol sulfate (1)	-0.03817071	0.10589680	0.04930035
1-oleoyl-2-linoleoyl-glycerol (18:1/18:2)	-0.01811760	0.06730961	0.00202186	caffeic acid sulfate	-0.05824695	0.13402050	0.05073179
theophylline	-0.00803423	0.06397383	0.00204295	erythronate*	-0.00014450	0.01756743	0.05078034
1-oleoyl-3-linoleoyl-glycerol (18:1/18:2)	-0.01685534	0.06829720	0.00207078	eugenol sulfate	0.03819877	0.17337733	0.05371513
succinylcarnitine	-0.00663655	0.02854200	0.00248572	X - 17673	-0.04549284	0.15646473	0.05873330
X - 17346	-0.01349670	0.15957030	0.00288990	3-methylxanthine	-0.01220969	0.07472977	0.06222773
1,3-dimethylurate	-0.00682432	0.07953375	0.00292039	1-oleoyl-2-dihomo-linolenoyl-GPC (18:1/20:3)*	-0.01773910	0.02796075	0.06265160
1-oleoyl-2-linoleoyl-GPE (18:1/18:2)*	-0.00395007	0.10351799	0.00292383	maltose	0.01392008	0.05218806	0.06267324
azelate (nonanedioate)	0.00605497	0.12893964	0.00300357	X - 17350	-0.07409996	0.15744517	0.06381012
cyclo(leu-pro)	-0.01627831	0.06825271	0.00315328	ursodeoxycholate	0.00083158	0.09060428	0.06414807
2-oleoyl-GPE (18:1)*	-0.01122529	0.11076604	0.00320886	X - 12701	0.00442251	0.12659832	0.06588402
1-arachidonoyl-GPE (20:4n6)*	-0.00919915	0.04843906	0.00398156	3-hydroxyhippurate	-0.01128872	0.10221584	0.06618654
X - 12814	-0.05604330	0.16896617	0.00401080	2-linoleoyl-GPC (18:2)*	0.00496993	0.03064236	0.06666955
caffeine	-0.00877439	0.08012532	0.00403734	1-palmitoyl-2-oleoyl-GPE (16:0/18:1)	-0.01265318	0.03972719	0.06798572
X - 12199	-0.04873014	0.13423874	0.00471554	X - 21729	-0.00150531	0.06987044	0.06799383
5-acetylamino-6-formylamino-3-methyluracil	-0.02604253	0.12277925	0.00474392	chenodeoxycholate	-0.02293206	0.09962018	0.06935909
3,7-dimethylurate	0.00496932	0.11988881	0.00534864	X - 12123	-0.01265785	0.11783832	0.06982676
X - 12748	-0.00362488	0.03566719	0.00570401	phenylalanyltryptophan	-0.01281988	0.04796109	0.07003791
X - 24514	-0.04765994	0.08213134	0.00577391	N-acetylmethionine	0.00290366	0.02280131	0.07043706
1-linoleoyl-GPC (18:2)	0.00394874	0.03857397	0.00661722	pyrraline	-0.00574952	0.07196106	0.07829926
suberate (octanedioate)	-0.00976713	0.14440432	0.00672482	maleate	-0.02345044	0.11388458	0.07834501
prolylglycine	-0.00644465	0.10020678	0.00720240	Cys-gly, oxidized	0.00544581	0.02307221	0.07748785
1-palmitoyl-2-oleoyl-GPI (16:0/18:1)*	-0.00720899	0.04444626	0.00730414	X - 12411	-0.02764653	0.06408269	0.07932672
7-alpha-hydroxy-3-oxo-4-cholestenoate (7-Hoca)	-0.01198307	0.04033113	0.00760925	X - 11538	-0.02583959	0.05563303	0.08051242
2-aminoheptanoate	-0.01129579	0.03818723	0.00794936	trans-urocanate	-0.02138651	0.04652620	0.08132022
X - 13553	-0.00506262	0.04973207	0.00799797	1,2,3-benzenetriol sulfate (2)	0.08274317	0.13418881	0.08132952
X - 24337	-0.01110584	0.06946339	0.00845153	sucrose	0.04756075	0.11990267	0.08339137
X - 21411	-0.00127017	0.09528429	0.00871412	glutarate (pentanedioate)	0.00813402	0.03921413	0.08570823
2-linoleoyl-GPE (18:2)*	-0.01946679	0.09172425	0.00885022	X - 23649	-0.01655551	0.12251987	0.08644935
lanthionine	-0.03213817	0.14805418	0.00912822	propionylglycine	-0.04227946	0.04259761	0.08811480
X - 12733	-0.01226368	0.09422846	0.01009874	1-stearoyl-2-docosahexaenoyl-GPS (18:0/22:6)*	-0.00408931	0.03889789	0.09154348
X - 23655	-0.02415125	0.16978920	0.01046319	X - 17185	-0.01052096	0.10446596	0.09262836
X - 17765	-0.04247652	0.23693133	0.01069728	3-methylglutarate/2-methylglutarate	0.00721036	0.04851855	0.09394366
X - 24757	-0.01050348	0.12419600	0.01145592	glycooxycholate glucuronide (1)	-0.00019183	0.08112030	0.09489197
choline phosphate	0.00079772	0.03277776	0.01251293	2-hydroxyoctanoate	0.00386529	0.04338724	0.09483631
pimeloylcarnitine/3-methyladipoylcarnitine	0.01644107	0.03772839	0.01251678	X - 24024	-0.02014958	0.05046474	0.09493982
1-stearoyl-2-linoleoyl-GPE (18:0/18:2)*	-0.01252827	0.05871558	0.01313166	X - 23641	0.00840795	0.02912899	0.09776959
1-stearoyl-2-oleoyl-GPE (18:0/18:1)	-0.01374271	0.08740097	0.01318414	X - 21959	-0.00720823	0.03454703	0.09851508
X - 23636	-0.00713470	0.03316775	0.01321523	glycooursodeoxycholate	0.01348135	0.07823266	0.10100379
glycerol 3-phosphate	0.00390933	0.04464327	0.01549387	3-(3-hydroxyphenyl)propionate	-0.00874906	0.08780647	0.10469657
X - 24449	-0.01689897	0.05993384	0.01566462	N-acetylchitruiline	-0.00985213	0.04258873	0.10564697
X - 17010	0.02049147	0.05245243	0.01590684	1-palmitoleoyl-GPC (16:1)*	-0.01000963	0.01903371	0.10601116
glycochenodeoxycholate glucuronide (1)	-0.00661739	0.07308374	0.01620181	carboxyethyl-GABA	-0.00582177	0.02696960	0.10833364
hydantoin-5-propionic acid	-0.02034148	0.07093518	0.01667825	1-stearoyl-2-oleoyl-GPC (18:0/18:1)	-0.01442154	0.02465677	0.10904972
cysteine-glutathione disulfide	0.02380515	0.05004215	0.01678605	3-methylglutaconate	-0.00392691	0.04475959	0.11140293
1-dihomo-linolenoyl-GPE (20:3n3 or 6)*	-0.00677354	0.06021318	0.01832524	2,3-dihydroxypridine	0.00580558	0.12440891	0.11251682
pyroglutamylvaline	-0.00420337	0.05394157	0.01922318	vanillylmandelate (VMA)	-0.00697871	0.02789678	0.11310002
X - 12680	0.00059225	0.11306510	0.01944880	alpha-ketobutyrate	-0.02600693	0.08113711	0.11352721
1-palmitoyl-2-linoleoyl-GPI (16:0/18:2)	-0.00410744	0.04503098	0.02034077				

Supplementary Table 8 continued

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
X - 24571	0.00842875	0.10698856	0.11479165	X - 14314	-0.02557802	0.02540148	0.26663743
1-oleoyl-2-linoleoyl-GPC (18:1/18:2)*	-0.00078201	0.01576409	0.11595108	X - 24438	-0.00740629	0.04526420	0.27269146
1-linoleoyl-2-docosahexaenoyl-GPC (18:2/22:6)*	-0.00305622	0.01664474	0.11760083	ellanoin	-0.01089588	0.01377124	0.01532751
cholate	-0.01845610	0.15233686	0.11887712	X - 12170	0.00033845	0.04035963	0.27604898
gamma-glutamylhistidine	-0.00276379	0.01667895	0.11951376	1-oleoyl-GPC (18:1)	-0.00600393	0.01189586	0.27629938
2-methoxyresorcinol sulfate	-0.05189102	0.12194599	0.11996767	X - 23776	0.01508568	0.09459037	0.27831586
cis-aconitate	0.00532885	0.02141941	0.12213203	glutamate	-0.01296190	0.01799211	0.28098390
X - 21442	-0.00128106	0.06203339	0.12609280	2-methylbutyrylglycine	-0.01007660	0.02902600	0.28173884
X - 24812	0.02394117	0.03498062	0.12932274	1-linoleoyl-GPA (18:2)*	-0.00180554	0.02435260	0.28196832
1,2-dilinoleoyl-GPC (18:2/18:2)	-0.00002699	0.02866893	0.13169140	X - 22035	-0.01770873	0.05569375	0.28413552
methylsuccinate	0.00751602	0.02564730	0.13280527	3-hydroxysebacate	0.01936199	0.05609489	0.28770850
taurochenodeoxycholate	0.06198503	0.08135881	0.13504838	N-formylmethionine	0.00115748	0.00997520	0.28835886
N-acetylglucosaminylasparagine	0.00845244	0.02083489	0.13621619	crotonide	0.00303364	0.00893140	0.29211124
1-palmitoyl-2-linoleoyl-GPE (16:0/18:2)	-0.00769619	0.03524001	0.14175510	N-acetylproline	-0.00574612	0.03116665	0.29320565
5,6-dihydrothymine	0.01394786	0.02900075	0.14238014	12,13-DIHOME	-0.01536612	0.05392180	0.29560908
alanine	0.01023199	0.04115658	0.14257815	X - 24439	-0.00804084	0.02585464	0.29619220
4-hydroxyphenylacetate	0.01733667	0.08166243	0.14391944	1-linoleoyl-2-linolenoyl-GPC (18:2/18:3)*	-0.01138650	0.02875966	0.30148067
hexanoylglycine	0.03112138	0.06725684	0.14628993	1-palmitoyl-2-dihomo-linolenoyl-GPE (16:0/20:3)*	-0.01659129	0.02569155	0.30336272
linoleoylcarnitine*	0.01396976	0.02007924	0.14816056	1-dihomo-linolenoyl-GPC (20:3n3 or 6)*	-0.00686604	0.01423705	0.30449816
1-oleoylglycerol (18:1)	-0.00458096	0.07791004	0.14829828	X - 23729	-0.02979327	0.06533262	0.30938662
N-acetylaspartate (NAA)	0.01405655	0.01802866	0.14987119	heme	-0.03451566	0.04193774	0.31693774
myo-inositol	-0.00061354	0.02581487	0.15202013	dihydroorotate	0.00914783	0.01959477	0.32651634
1-stearoyl-2-linoleoyl-GPI (18:0/18:2)	0.00143272	0.02596737	0.15291711	1-linoleoyl-GPI (18:2)*	0.00461952	0.02756985	0.32665648
sphingosine 1-phosphate	0.01018726	0.01723707	0.15658928	X - 12339	-0.00549201	0.03249262	0.33615029
X - 21807	-0.00341206	0.02629411	0.15780109	dodecanedioate	0.01209636	0.03823278	0.33853374
3-hydroxypyridine sulfate	0.00248374	0.13823978	0.15794614	X - 24293	-0.07546178	0.06083691	0.33791166
2'-deoxyuridine	-0.03742097	0.04847943	0.16631738	Nε-carboxymethyllysine	0.00199110	0.05336799	0.34486747
X - 23984	0.00475900	0.01947511	0.16700712	X - 12753	-0.03141020	0.09120367	0.34681349
aspartate	-0.00603074	0.01579333	0.16717420	N-acetylphenylalanine	0.00548696	0.01349809	0.34756695
X - 24049	0.00812912	0.02989259	0.16726797	taurocholate	0.09647912	0.04831836	0.34911015
X - 12712	-0.00346883	0.09149945	0.16834139	3-methyl catechol sulfate (2)	0.01756290	0.05067359	0.34965068
1-linolenoyl-GPC (18:3)*	-0.01883615	0.02830799	0.16951124	1-palmitoyl-2-meadoyl-GPC (16:0/20:3n9)*	-0.01946336	0.01949174	0.35262012
hipurate	0.01119426	0.05885519	0.17478032	X - 20529	-0.00758349	0.04035664	0.35756102
arabitolxylitol	-0.01160444	0.01755104	0.17479411	ornithine	0.00609858	0.00709041	0.36691828
N-acetyl-aspartyl-glutamate (NAAG)	0.01470062	0.03811293	0.17736978	3-methoxycatechol sulfate (2)	-0.00574312	0.01499327	0.36755979
N-acetyltryptophan	0.00496361	0.02372013	0.17803032	N-acetylariginine	-0.00400441	0.01174029	0.36978233
laurate (12:0)	-0.01791118	0.04769494	0.18044757	sebacate (decanedioate)	0.01319830	0.04091894	0.37315799
1-palmitoyl-2-adrenoyl-GPC (16:0/22:4)*	-0.02238147	0.02554988	0.18062717	3-hydroxybutyrate (BHBA)	-0.00761576	0.06809803	0.37441545
5-oxoproline	0.00540496	0.01131841	0.18378118	X - 17354	-0.01324654	0.03215804	0.37732674
X - 12410	-0.00161120	0.08848166	0.18547836	fumurate	0.02039727	0.01075282	0.37807657
sphingosine	0.00703188	0.01935579	0.18603363	X - 22102	-0.00614457	0.02265719	0.38026951
X - 12906	0.01627238	0.04890152	0.18797574	X - 12221	0.03821762	0.04700549	0.38116814
methionine sulfoxide	-0.00420503	0.02206615	0.19521275	X - 24306	-0.00312220	0.02305646	0.38561282
1-palmitoleoyl-GPE (16:1)*	-0.03466762	0.04487529	0.19743168	X - 21265	-0.00785520	0.01247675	0.38618619
7-methylguanine	-0.00701852	0.01134521	0.20335626	N-acetyliserine	0.00700525	0.00951437	0.38924757
erucate (22:1n9)	0.02015674	0.06186920	0.20382426	X - 19141	-0.01360128	0.01516026	0.39437811
glycocholate	0.05308883	0.06368455	0.20664513	N-acetyl-cadaverine	-0.00054157	0.03687853	0.39449446
5-hydroxylysine	0.00396477	0.03949746	0.20862226	N2,N2-dimethylguanosine	-0.00080159	0.00642966	0.39607931
gulonic acid*	0.00647332	0.01821500	0.21377108	X - 21796	-0.00527392	0.00956487	0.39690124
X - 24760	-0.05244523	0.07912161	0.21663582	X - 21935	-0.00321632	0.02076194	0.39728923
X - 23297	-0.02010743	0.05367288	0.21779538	1-arachidonoyl-GPC (20:4n6)*	-0.00293558	0.00871469	0.39987349
X - 23590	-0.00748534	0.02804857	0.22058934	1-linoleoyl-2-arachidonoyl-GPC (18:2/20:4n6)*	-0.00138536	0.01657629	0.40125196
valerate	0.03269046	0.05072268	0.22308124	X - 11261	-0.01736305	0.01545731	0.40408514
adenosine	-0.04416717	0.04464793	0.22395669	1-palmitoyl-2-palmitoleoyl-GPC (16:0/16:1)*	-0.01799775	0.01521053	0.40595222
1-stearoyl-2-arachidonoyl-GPI (18:0/20:4)	0.00073535	0.01604025	0.22870180	linoleate (18:2n6)	-0.00662636	0.03971364	0.41147249
1-stearoyl-2-dihomo-linolenoyl-GPC (18:0/20:3n3 or 6)*	-0.01233130	0.01496468	0.23212396	undecanedioate	0.01719869	0.03441764	0.41433143
arabonate/xylonate	-0.00623502	0.01855231	0.23244904	1-linoleoyl-2-eicospentaenoyl-GPC (18:2/20:5)*	-0.00153663	0.01893541	0.41500870
alpha-ketoglutarate	0.00656906	0.01218095	0.23380457	1-eicospentaenoyl-GPC (20:5)*	-0.00360624	0.01473724	0.41726433
1,2,3-benzenetriol sulfate (1)	-0.04381749	0.10127595	0.23814286	adenosine 5'-monophosphate (AMP)	0.00459042	0.01233665	0.41916732
1-methylguanidine	0.02860116	0.03830340	0.23898265	21-hydroxypregnenolone disulfate	0.00498167	0.01228254	0.42302680
1-stearoyl-2-linoleoyl-GPC (18:0/18:2)*	-0.00267826	0.00945479	0.24152256	X - 12707	-0.00873817	0.01424241	0.42340450
creatine	0.00178319	0.01185374	0.24190913	imidazole propionate	-0.01016497	0.02374842	0.42515157
1-oleoyl-2-dihomo-linolenoyl-GPC (18:1/20:2)*	-0.00197598	0.01333363	0.24363864	X - 12730	0.01957955	0.06738023	0.42822326
1-palmitoyl-GPG (16:0)*	-0.01101868	0.03283663	0.24441697	uracil	0.00521498	0.01406568	0.43063548
X - 23659	-0.02209295	0.03304123	0.24552521	phosphatidylcholine (18:0/20:2, 20:0/18:2)*	-0.02045724	0.03337430	0.44301206
N-methylpicoalate	0.01560744	0.02322390	0.24826458	tyramine O-sulfate	-0.02019680	0.04819788	0.44605772
N-acetylucine	0.00342401	0.02173065	0.25116708	X - 12714	-0.01027329	0.04701433	0.45268759
X - 16397	0.00687375	0.03148675	0.25137768	1-palmitoleoyl-2-linoleoyl-GPC (16:1/18:2)*	-0.00065295	0.01145542	0.45662911
X - 22836	0.06636620	0.09624334	0.25291267	X - 17343	0.04594565	0.04046686	0.45783986
arachidate (20:0)	-0.00819907	0.02679614	0.25310523	X - 17351	0.00705245	0.03032670	0.46190833
N-alpha-acetylorithine	-0.00078319	0.01427107	0.25362021	1-stearoyl-2-docosapentaenoyl-GPC (18:0/22:5n6)*	-0.01092618	0.00793960	0.46943285
1-stearoyl-2-meadoyl-GPC (18:0/20:3n9)*	-0.02159409	0.02450989	0.25412874	X - 22509	-0.02703000	0.03769017	0.47397677
X - 23291	-0.02615070	0.04279296	0.25636667	1-palmitoyl-2-oleoyl-GPC (16:0/18:1)	-0.00815365	0.00617622	0.47727651
homovanillate (HVA)	-0.00552065	0.04019391	0.26007669	dopamine sulfate (1)	-0.01290416	0.01389000	0.48000568
2-acetamidophenol sulfate	-0.04374284	0.07298522	0.26098219	N1-methylinosine	0.00017935	0.00945258	0.48289442
1-docosahexaenoyl-GPC (22:6)*	-0.00172385	0.01873969	0.26102861	1-margoyl-2-linoleoyl-GPC (17:0/18:2)*	0.00564522	0.00798673	0.48505566
3-(3-hydroxyphenyl)propionate sulfate	-0.02005946	0.05812412	0.26419025	4-acetamidobutanate	0.00070147	0.00744636	0.48665710
isoleudine	0.00502386	0.01266561	0.26505512	phosphatidylcholine (14:0/14:0, 16:0/12:0)	-0.00781678	0.02363447	0.48938634
3-hydroxy-3-methylglutarate	0.01886966	0.03403469	0.26539486	glycerophosphoglycerol	-0.03485821	0.02605800	0.48990479

Supplementary Table 8 continued

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
serylalanine	-0.00146840	0.01009260	0.49582444	X - 24803	0.00133727	0.00293414	0.65882105
3-acetylphenol sulfate	-0.02061477	0.03638030	0.49792356	3-methoxytyrosine	0.00249430	0.00531719	0.66084487
X - 13891	0.02918362	0.03846399	0.50163058	X - 24411	-0.05304342	0.01420889	0.66280512
N-methylproline	0.05140231	0.07712617	0.50368744	X - 15497	-0.00488254	0.01266876	0.66397371
13-HODE + 9-HODE	-0.03670852	0.02842347	0.51173905	1-palmitoyl-2-eicosapentaenoyl-GPE (16:0/20:5)*	-0.01565683	0.01131330	0.66696375
X - 21849	-0.02557521	0.02515485	0.51176348	gamma-glutamylleucine	-0.01328678	0.00680815	0.66816065
X - 24804	0.00523168	0.00736760	0.51508220	picolinine	0.00749588	0.00957189	0.67107533
X - 24738	0.07216696	0.09359042	0.51735597	cytidine	0.00004925	0.00612395	0.67342543
X - 21752	-0.01979528	0.01614991	0.51981755	adrenate (22:4n6)	0.0088979	0.02308711	0.67475206
ribonate	0.00009966	0.01341737	0.52063697	X - 12212	0.01912266	0.02187637	0.67518294
X - 24125	-0.03141547	0.03122821	0.52333987	X - 23314	0.03485787	0.03859720	0.67741090
linoleylethanolamide	0.01061454	0.01589712	0.52445005	8-oxopiperidine-2-carboxylic acid	0.01208755	0.01051777	0.67776535
methyl glucopyranoside (alpha + beta)	0.04533613	0.03654807	0.52579734	X - 14904	0.05406931	0.01994093	0.67969501
X - 11491	-0.04506213	0.01496623	0.52743870	X - 24054	-0.00434368	0.00469592	0.68675520
X - 15666	-0.01925385	0.01510799	0.52745710	alpha-tocopherol	0.00644484	0.00479150	0.68795915
1-(1-enyl-stearoyl)-2-linoleoyl-GPC (P-18:0/18:2)*	0.04657708	0.02789717	0.52815415	X - 18779	-0.00313196	0.00630717	0.68829018
dopamine sulfate (2)	-0.00509717	0.05500627	0.52851795	2-palmitoleyl-GPC (16:1)*	-0.00882157	0.0115294	0.69578856
X - 13529	-0.01303848	0.01371227	0.53296550	sulfate*	-0.00378011	0.00282495	0.69622045
X - 24307	-0.00375229	0.01374532	0.53430701	X - 12007	0.01700998	0.02369090	0.69787013
1-palmitoyl-2-linoleoyl-GPC (16:0/18:2)	0.00113459	0.00419991	0.53671038	linolenate [alpha or gamma; (18:3n3 or 6)]	-0.01469936	0.02019675	0.70045134
1-stearoyl-2-arachidonoyl-GPE (18:0/20:4)	-0.01283669	0.00929579	0.53647828	X - 17704	-0.01742063	0.02323787	0.70151360
X - 24806	0.00414562	0.00614287	0.53714460	4-androsten-3beta,17beta-diol monosulfate (1)	-0.01180118	0.00490095	0.70458877
N-acetylisoleucine	0.02859154	0.01081068	0.54114893	X - 18779	0.0128721	0.02672191	0.70476521
X - 12117	-0.00440692	0.01212031	0.54124200	1-palmitoleyl-2-docosahexaenoyl-GPC (16:1/22:6)*	-0.01380853	0.00708956	0.70774504
X - 16654	-0.01214791	0.02857051	0.54235851	X - 17325	-0.02474014	0.01246464	0.70954681
N-acetylneuraminic acid	-0.00647693	0.00524452	0.54257233	homostachydrine*	-0.00607452	0.01581137	0.71372794
X - 17438	-0.02148981	0.02041416	0.54339826	thyroxine	-0.00289171	0.02329327	0.71511415
phosphoethanolamine	-0.00242399	0.00790651	0.54491574	phenol sulfate	-0.00480566	0.00697031	0.71594886
guanidinooxalate	-0.00404935	0.00493796	0.54850663	2-oleoylglycerol (18:1)	0.05447689	0.01950511	0.71967857
X - 22771	-0.01614752	0.01396980	0.55118016	X - 11905	-0.01074592	0.00898828	0.72217760
suberoyl carnitine	0.03246663	0.01840223	0.55280939	1-palmitoyl-2-stearoyl-GPC (16:0/18:0)	0.00452785	0.00374195	0.72548388
X - 12230	0.00005498	0.03848874	0.55279004	1-myristoyl-2-palmitoyl-GPC (14:0/16:0)	0.00478384	0.00688629	0.73014074
methionine sulfone	-0.00672820	0.00678798	0.55403114	N-acetylputrescine	0.00555756	0.00359642	0.73046111
1,2-dilinolenoyl-GPC (18:3/18:3)*	-0.01368546	0.01381791	0.55536766	1-myristoyl-2-docosahexaenoyl-GPC (14:0/22:6)*	-0.00780943	0.00523676	0.73133877
stachydrine	0.00636331	0.03736102	0.56162143	beta-hydroxyisovalerate	-0.00478622	0.00461370	0.74357944
4-allylphenol sulfate	-0.07432724	0.03943183	0.56767220	X - 24027	-0.00477467	0.00549773	0.74659541
citrate	0.00316210	0.00514837	0.57039273	X - 12930	-0.00938904	0.01607722	0.75155274
1-oleoyl-GPI (18:1)*	-0.00110417	0.01994652	0.57166631	1-oleoyl-2-docosapentaenoyl-GPC (18:1/22:5n6)*	-0.01359801	0.00389607	0.76106689
N4-acetylcytidine	-0.02099527	0.01984957	0.57236872	X - 19551	-0.03227794	0.01222585	0.76643476
1-oleoyl-2-eicosapentaenoyl-GPC (18:1/20:5)*	0.00690967	0.01755250	0.57583839	X - 12329	0.00343847	0.03086143	0.76804603
X - 07765	-0.02568744	0.03471363	0.57827934	X - 23145	0.01177371	0.00628444	0.77174436
1-palmitoyl-2-arachidonoyl-GPC (16:0/20:4)	-0.00348575	0.00413186	0.57982074	gamma-tocopherol/beta-tocopherol	0.01471377	0.00774994	0.77233030
eicosanodiolate	-0.01530158	0.01144333	0.58012868	2-isopropylmalate	0.01380422	0.02676441	0.77798941
X - 21829	0.00684524	0.01752195	0.58620434	4-hydroxyphenyllaetoyl carnitine	0.01856350	0.01396163	0.78228244
levulinate (4-oxovalerate)	0.04027980	0.02110067	0.58895946	1-palmitoyl-GPC (16:0)	0.00306929	0.00401919	0.78273613
1-stearoyl-2-arachidonoyl-GPC (18:0/20:4)	-0.00595417	0.00466326	0.59013081	2-arachidonoyl-GPC (20:4)*	-0.00002465	0.00450912	0.78273761
EDTA	0.00277133	0.00552222	0.59237189	X - 24512	-0.00629274	0.00401415	0.79047137
2-methylmalonyl carnitine	0.04160108	0.02583032	0.59377933	X - 23293	0.01417915	0.01293810	0.80105526
cystathionine	-0.00699954	0.01088511	0.59449422	2-palmitoyl-GPE (16:0)*	-0.00269002	0.00347518	0.80573683
X - 24455	-0.00544165	0.01065571	0.59624489	X - 16480	-0.00031229	0.01348865	0.81040600
cysteine s-sulfate	0.03159142	0.00963098	0.60084513	alpha-hydroxyisocaproate	0.00320709	0.00306249	0.81001257
1-stearoyl-GPE (18:0)	-0.01177499	0.00703450	0.60384601	X - 12824	0.03206360	0.01214200	0.81063600
X - 24766	0.00627602	0.00344920	0.60497363	2-hydroxydecanoate	-0.02815633	0.01022161	0.81749944
X - 24425	0.00539910	0.01007559	0.60639852	X - 18901	-0.01789693	0.00368798	0.81759662
hypoxanthine	0.04655791	0.01636308	0.60836511	1-myristoyl-2-arachidonoyl-GPC (14:0/20:4)*	-0.01318517	0.00388790	0.81936262
1-arachidonoyl-GPI (20:4)*	0.00571396	0.01336931	0.61018016	X - 11849	-0.00747522	0.00573072	0.82232780
1-linoleoylglycerol (18:2)	0.00900076	0.02025689	0.61054609	palmitate (16:0)	-0.00475683	0.00824790	0.82628123
pro-hydroxy-pro	0.01171825	0.01580117	0.61461410	1-(1-enyl-stearoyl)-2-oleoyl-GPE (P-18:0/18:1)	-0.01605755	0.00276054	0.82689859
X - 19438	0.02606671	0.01163056	0.61502038	X - 19434	-0.05451649	0.00918401	0.82809900
1-stearoyl-2-docosapentaenoyl-GPC (18:0/22:5n3)*	-0.00699634	0.00585563	0.61550962	adenosine 3',5'-cyclic monophosphate (cAMP)	-0.01262401	0.00657425	0.83110892
X - 12015	-0.00219409	0.01958364	0.61642692	5-methylthioadenosine (MTA)	-0.00538323	0.00294861	0.83279596
1-(1-enyl-stearoyl)-2-arachidonoyl-GPE (P-18:0/20:4)*	-0.01310248	0.00570434	0.61814942	X - 11847	-0.02280260	0.01011701	0.83335635
1-margaroyl-2-oleoyl-GPC (17:0/18:1)*	-0.00369286	0.00719026	0.61935388	1-palmitoyl-2-eicosapentaenoyl-GPC (16:0/20:5)*	-0.00355935	0.00369899	0.83781384
pyridoxal	-0.04844635	0.02926166	0.62313632	X - 21441	-0.02302758	0.00332678	0.84168653
X - 23587	0.01681239	0.02091403	0.62472432	phosphatidylcholine (15:0/18:1, 17:0/16:1)*	-0.00143361	0.00319613	0.84473882
diglylglycine	-0.01490461	0.01659178	0.63132354	1-linolenoylglycerol (18:3)	-0.02052134	0.00777518	0.84772765
cysteinylglycine	0.01917614	0.00652823	0.63138369	X - 12729	0.03328658	0.01250893	0.84822494
tetradecanedioate	0.00964483	0.01664497	0.63363148	O-acetylhomoserine	0.08496658	0.01532451	0.84863403
N-palmitoyl-sphingosine (d18:1/16:0)	0.00166861	0.00521545	0.63405247	X - 12450	-0.01441989	0.00927655	0.84978757
X - 11549	-0.00982517	0.01195474	0.63457162	1-palmitoyl-2-dihomo-linolenoyl-GPC (16:0/20:3n3 or 6)*	-0.00973019	0.00183987	0.84988196
methylmalonate (MMA)	0.01573659	0.01224177	0.63789099	N6-acetylysine	0.00126307	0.00236207	0.85331866
X - 24097	-0.02711040	0.01450128	0.64263983	ferulic acid 4-sulfate	-0.03421883	0.00432021	0.86183848
isovalerate	0.00059244	0.02497856	0.64482488	deoxycholate	-0.00226284	0.00586008	0.86220968
X - 24204	-0.00280137	0.00736680	0.64555171	X - 24334	-0.00823657	0.00535837	0.86318345
1-(1-enyl-palmitoyl)-2-oleoyl-GPE (P-16:0/18:1)*	-0.01040552	0.00482668	0.64614022	1-myristoyl-GPC (14:0)	0.00070027	0.00250118	0.86509844
4-hydroxyhippurate	0.04181825	0.02349320	0.65300595	X - 11795	-0.01624141	0.00184654	0.86725029
X - 12127	-0.01547483	0.01007637	0.65318800	piperine	-0.03543113	0.00961601	0.86854432
succinate	0.00396561	0.00660728	0.65566315	valine	0.00619956	0.00228698	0.87438472
S-allylcysteine	-0.04521718	0.03216294	0.65650188	X - 24452	-0.00451137	0.00252563	0.87789437

Supplementary Table 8 continued

Metabolite	beta	gamma	p
X - 09789	-0.05290979	0.00497216	0.87943721
glycerophosphoethanolamine	-0.00425034	0.00118012	0.88655120
X - 13846	0.01607790	0.01040206	0.88939256
N-(2-furoyl)glycine	0.01436806	0.01206743	0.89380806
X - 16944	-0.00208491	0.00455304	0.89519169
stearidonate (18:4n3)	-0.02561476	0.00559232	0.89715017
histidine	0.00068775	0.00087548	0.89993265
1-(1-enyl-stearoyl)-2-linoleoyl-GPE (P-18:0/18:2)*	-0.00639113	0.00192839	0.90133737
hexadecanedioate	0.01786494	0.00420191	0.90675867
N-acetylthreonine	-0.00045801	0.00168061	0.90844895
18-hydroxypalmitate	-0.01832897	0.00301709	0.91253349
phenylacetylcarbitine	0.01011614	0.00335177	0.91544103
orotate	-0.00775907	0.00116231	0.91715703
hyocholate	-0.01322636	0.00333772	0.91843385
X - 24588	-0.01052077	0.00164404	0.92036995
X - 23637	0.00825839	0.00157140	0.92403459
3-methylglutaryl carnitine (2)	0.00592052	0.00152064	0.92636713
xanthurenate	-0.00717803	0.00174345	0.93028152
X - 12738	0.02093255	0.00631699	0.93078369
X - 13703	-0.04130593	0.00341429	0.93114339
caproate (6:0)	-0.02656440	0.00327044	0.93491135
1-oleoyl-GPG (18:1)*	0.02887394	0.00401209	0.93543331
1-nonadecanoyl-GPC (19:0)	-0.00163840	0.00104122	0.93543596
X - 15486	-0.01530886	0.00313836	0.93649727
X - 10458	0.03983445	0.00324022	0.93739225
betaine	-0.00251611	0.00061803	0.93903944
X - 11564	0.00142372	0.00067637	0.94302565
urea	0.00102787	0.00087899	0.94534926
ribitol	-0.01800361	0.00131757	0.94644327
3-phosphoglycerate	-0.01362501	0.00095534	0.94678718
1-palmitoleoyl-2-linolenoyl-GPC (16:1/18:3)*	-0.02360962	0.00124215	0.95568827
X - 24747	-0.00386285	0.00225430	0.95570949
X - 24736	0.02172492	0.00166291	0.95924295
X - 11632	0.01154126	0.00232711	0.95954667
X - 11315	0.00878238	0.00056697	0.96029232
phenylalanylphenylalanine	0.01884065	0.00101740	0.96157482
gamma-glutamylphenylalanine	-0.00863187	0.00070008	0.96225271
phenylalanine	0.00073759	0.00038317	0.96385256
palmitoleate (16:1n7)	-0.01933849	0.00278784	0.96654646
2-hydroxyglutarate	0.00986311	0.00054442	0.96668282
creatinine	0.00170595	0.00025989	0.96805163
leucine	0.00237471	0.00044999	0.96849262
phosphatidylcholine (16:0/22:5n3, 18:1/20:4)*	-0.00177457	0.00031585	0.97013041
X - 18899	-0.01301755	0.00035346	0.97503895
2-docosahexaenoyl-GPE (22:6)*	-0.00406028	0.00110651	0.97574661
phosphatidylcholine (18:0/20:5, 16:0/22:5n6)*	-0.00071546	0.00039799	0.97957687
X - 12283	-0.02246657	0.00092753	0.98028560
X - 12544	0.00881179	0.00136296	0.98033128
X - 23583	-0.01135436	0.00080678	0.98059263
oleate/vaccenate (18:1)	-0.00658379	0.00063405	0.98962395
X - 23756	-0.00541034	0.00032016	0.99003539
X - 12726	0.01315208	0.00055685	0.99022008
X - 11308	-0.00600851	0.00007613	0.99067832
1-methylimidazoleacetate	0.01673859	0.00017316	0.99266075
X - 22776	0.05041996	0.00029815	0.99331112
homocitrulline	0.05009876	0.00025390	0.99640834
glycerate	0.00411006	0.00003240	0.99706306
X - 13837	0.02389407	0.00014605	0.99720239
catechol sulfate	0.01535008	0.00008876	0.99736778
octadecanedioate	0.07493443	0.00009171	0.99800334
pyroglutamylglutamine	0.01112019	0.00001979	0.99953142
X - 21444	0.03364300	0.00000091	0.99996595

Supplementary Table 9

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
1-methylnicotinamide	0.03851064	-0.13613881	3.93E-06	adipoylcarnitine	0.03477817	-0.03683178	0.03797085
palmitoyl sphingomyelin (d18:1/16:0)	0.00100050	-0.01884885	6.25E-05	S-1-pyrroline-5-carboxylate	-0.03230766	-0.07061103	0.03837111
kyurenine	0.00531380	-0.03462273	6.28E-05	X - 13866	0.03872458	-0.08793208	0.03863519
4-androsten-3alpha,17alpha-diol monosulfate (2)	0.00077492	-0.08119179	0.00025347	gamma-glutamyltryptophan	-0.00709962	-0.06585015	0.03867255
sphingomyelin (d18:1/20:1, d18:2/20:0)*	0.00334720	-0.01822163	0.00034603	2-hydroxypalmitate	-0.00413888	-0.03480981	0.03873384
2-palmitoyl-GPC (16:0)*	0.00067819	-0.03709882	0.00078491	X - 21383	0.03549887	-0.04157926	0.03991656
deoxycarnitine	0.00713625	-0.02531116	0.00117275	sphingomyelin (d18:1/17:0, d17:1/18:0, d19:1/16:0)	0.00509040	-0.01728345	0.04022783
X - 02249	0.00983569	-0.03675545	0.00121545	5alpha-pregnan-3beta,20alpha-diol disulfate	0.00749733	-0.02935738	0.04064754
X - 12104	-0.03810467	-0.10183186	0.00133595	X - 23997	-0.02135043	-0.07352406	0.04215188
X - 21662	0.00822719	-0.04903097	0.00147676	X - 12459	0.03950299	-0.06433920	0.04242816
1-palmitoyl-2-docosahexaenoyl-GPE (16:0/22:6)*	-0.00623320	-0.03768556	0.00158347	sphingomyelin (d18:1/24:1, d18:2/24:0)*	-0.00077179	-0.01816658	0.04527990
biliverdin	-0.02538100	-0.08171334	0.00188174	inosine	0.02133895	-0.04739466	0.04628634
X - 24585	0.01022212	-0.05899818	0.00227056	10-undecenoate (11:1n1)	0.00508404	-0.04543130	0.04668506
X - 12846	0.00684752	-0.07542775	0.00285759	X - 16946	-0.02599442	-0.06294092	0.04751976
sphingomyelin (d18:1/18:1, d18:2/18:0)	0.00190981	-0.01781850	0.00306667	sphingomyelin (d18:1/20:0, d16:1/22:0)*	0.00099561	-0.01379513	0.04763221
X - 17340	0.00491631	-0.09756707	0.00371157	pregn steroid monosulfate*	0.00410394	-0.03482458	0.04785533
gamma-glutamyl-2-aminobutyrate	0.02720510	-0.08987932	0.00372165	X - 21448	-0.02294179	-0.05656121	0.04837816
2-aminobutyrate	0.02285056	-0.04702740	0.00397270	2-stearoyl-GPE (18:0)*	-0.00709352	-0.02147122	0.04840109
X - 15492	0.00703339	-0.08394311	0.00408648	corticosterone	0.03521168	-0.13196877	0.05221598
cortisol	0.01460038	-0.07785969	0.00424544	tauroithocholate 3-sulfate	0.07209428	-0.08583190	0.04975592
nicotinamide	-0.00229072	-0.07622266	0.00453797	butyrylcarnitine	-0.00101453	-0.04538267	0.05141480
tylglycarnitine	0.00838263	-0.03711404	0.00458089	X - 24431	0.01397308	-0.03906916	0.05174078
bilirubin (Z,Z)	-0.01295753	-0.05689402	0.00575633	N6-succinyladenosine	0.01034345	-0.03805909	0.05229952
X - 11470	0.03430162	-0.08679064	0.00589583	5alpha-androstan-3alpha,17beta-diol disulfate	-0.00081297	-0.03102522	0.05249275
palmitoyl dihydroxyphingomyelin (d18:0/16:0)*	0.01049994	-0.02344337	0.00610028	glycosyl-N-palmitoyl-sphingosine	0.00101312	-0.01678678	0.05284702
X - 22379	0.00613276	-0.05208333	0.00754337	O-sulfo-L-tyrosine	-0.00554345	-0.03214347	0.05290375
carnitine	0.00139304	-0.01184230	0.00758545	5-methyluridine (ribohydridine)	0.00459643	-0.01700979	0.05370996
1-[1-enyl-palmitoyl]-2-docosahexaenoyl-GPE (P-16:0/22:6)*	-0.00130552	-0.01911974	0.00776518	1-[1-enyl-palmitoyl]-2-docosahexaenoyl-GPC (P-16:0/22:6)*	0.00028803	-0.01730131	0.05373444
indolelactate	-0.00209138	-0.02865303	0.00838413	X - 11843	0.05447290	-0.11244100	0.05649839
6-hydroxyindole sulfate	0.00260813	-0.06181305	0.00860779	AIcA ribonucleotide	-0.00252357	-0.02795358	0.05651275
pregnanediol-3-glucuronide	0.02084170	-0.06025968	0.00868192	alpha-hydroxyisovalerate	-0.01122865	-0.02935385	0.05746842
X - 15469	0.01119162	-0.07837700	0.00956578	X - 17337	0.04868177	-0.04572516	0.05834826
N2,N5-diacetylornithine	0.00517620	-0.05602280	0.00936651	X - 22475	0.03299366	-0.08006425	0.05853044
3-iodoxyl sulfate	0.00574842	-0.05525031	0.01034039	X - 14568	0.00294898	-0.00915822	0.05863196
5alpha-androstan-3alpha,17beta-diol monosulfate (2)	0.00210024	-0.05170800	0.01058424	xanthine	0.00592960	-0.03763921	0.05883621
X - 13431	0.03411986	-0.05484861	0.01132647	S-methylcysteine	0.002221668	-0.04305784	0.05884997
1-[1-enyl-oleoyl]-GPE (P-18:1)*	-0.01117177	-0.03530931	0.01153230	1-linoleoyl-2-docosapentaenoyl-GPC (18:2/22:5n3)	0.00317213	-0.02976936	0.06190535
11-androstosterone sulfate	0.00138259	-0.01907235	0.01161673	bilirubin (E,Z or Z,E)	-0.00460995	-0.02978178	0.06430659
X - 12216	0.03168547	-0.10101390	0.01295235	1-[1-enyl-palmitoyl]-GPE (P-16:0)*	-0.00742714	-0.02833452	0.06457203
X - 24558	0.03207227	-0.06235419	0.01325610	X - 24020	0.01419261	-0.02716266	0.06550319
N1-Methyl-2-pyridone-5-carboxamide	0.01056387	-0.03444623	0.01350739	indolin-2-one	0.01876073	-0.05225873	0.06687051
X - 24220	0.05018018	-0.10373949	0.01381345	1-pentadecanoyl-GPC (15:0)*	0.00494325	-0.01753604	0.07031962
decanoylcarnitine	0.03094428	-0.10331693	0.01391302	5alpha-pregnan-3beta,20beta-diol monosulfate (1)	0.00625156	-0.03202382	0.07058398
stearoyl sphingomyelin (d18:1/18:0)	0.00270080	-0.01547419	0.01428833	4-ethylphenylsulfate	0.00654504	-0.08199498	0.07136831
X - 12126	0.01043501	-0.08742620	0.01435411	glycine	0.00351188	-0.01830171	0.07151065
5-bromotryptophan	0.00032110	-0.02156946	0.01447032	threonine	0.00183906	-0.02076373	0.07180477
laurylcarnitine	0.03228819	-0.07844445	0.01559099	1-pentadecanoyl-2-docosahexaenoyl-GPC (15:0/22:6)*	0.01250542	-0.02710042	0.07253528
octanoylcarnitine	0.01534662	-0.07698568	0.01628217	cis-4-decenoyl carnitine	0.02984248	-0.05784365	0.07360846
4-methylcatechol sulfate	0.01543352	-0.09765668	0.01638207	X - 12101	0.01128175	-0.02887347	0.07547122
phenylacetylglutamine	-0.01955760	-0.09411983	0.01783226	1-[1-enyl-palmitoyl]-2-arachidonoyl-GPC (P-16:0/20:4)*	-0.00092511	-0.01522413	0.07589308
androsterone sulfate	-0.00214547	-0.01913726	0.01957013	X - 16124	0.00518623	-0.05925736	0.07939623
X - 21733	0.02280660	-0.09173845	0.01998363	4-hydroxychlorothalonil	0.00548053	-0.01140035	0.07971077
X - 11850	0.06959652	-0.10614251	0.02178925	X - 24849	-0.02403613	-0.04653668	0.08126714
benzoylcarnitine*	0.03240820	-0.05531079	0.02452469	4-androsten-3beta,17beta-diol monosulfate (2)	-0.00146702	-0.04769561	0.08187423
X - 23669	-0.00765917	-0.05607582	0.02475677	X - 02269	-0.00513246	-0.01369599	0.08210785
X - 22775	0.00465285	-0.03784420	0.02506481	propionylcarnitine	0.00538810	-0.02269128	0.08220370
p-cresol sulfate	0.00117609	-0.05737419	0.02512276	1-dihomo-linoleoyl-GPC (20:2)*	-0.00507657	-0.04654075	0.08229670
X - 12462	-0.02844674	-0.05610317	0.02539144	glycochenodeoxycholate sulfate	0.03289010	-0.06532010	0.08239437
urate	0.00099026	-0.02079136	0.02636658	17-methylstearate	-0.00000110	-0.05515531	0.08276959
17alpha-hydroxypregnenolone sulfate	0.00991548	-0.08635875	0.02646021	N-acetylmurine (2)	0.01832885	-0.04039215	0.08347407
X - 23765	0.00223563	-0.02261547	0.02698636	X - 11433	0.01683933	-0.04874596	0.08429405
X - 21310	0.00396659	-0.04140640	0.02718406	X - 12063	0.00464466	-0.01742813	0.08512174
X - 12731	0.05460482	-0.16974151	0.02720416	adenine	-0.00649853	-0.04402057	0.08608829
X - 12798	-0.00343061	-0.02157604	0.02751778	X - 11540	-0.00091090	-0.08680614	0.08687370
dimethyl sulfone	0.00013134	-0.02123326	0.02854852	X - 12816	0.03263075	-0.08442490	0.08768137
3b-hydroxy-5-cholenoic acid	0.01431403	-0.06577299	0.02878717	cholesterol	0.01025358	-0.01607877	0.08848842
X - 17145	0.03833633	-0.06862296	0.02937457	gentisate	0.06556716	-0.06924938	0.08910266
X - 12688	-0.00130435	-0.03018544	0.03092649	X - 17327	0.04080122	-0.08380590	0.08919905
X - 23585	0.02069338	-0.04095364	0.03131341	X - 22143	0.00953955	-0.08639226	0.09123230
etiocolanone glucuronide	-0.02032497	-0.04986680	0.03249190	1-[1-enyl-stearoyl]-2-oleoyl-GPC (P-18:0/18:1)	-0.00095007	-0.01437807	0.09143568
3-(4-hydroxyphenyl)lactate	-0.00203789	-0.03010190	0.03307050	X - 11530	-0.02808001	-0.04775712	0.09222390
5alpha-pregnan-3beta,20alpha-diol monosulfate (2)	0.01320931	-0.03477241	0.03355027	vanillic alcohol sulfate	0.00942240	-0.07373627	0.09316570
beta-alanine	0.00427682	-0.05793875	0.03421486	X - 21286	0.00109735	-0.03152049	0.09326975
4-androsten-3alpha,17alpha-diol monosulfate (3)	-0.00573179	-0.02032712	0.03475526	threonate	0.01190049	-0.01836569	0.09713372
2-stearoyl-GPI (18:0)*	-0.03989633	-0.09173860	0.03498951	X - 12013	0.05880121	-0.07575343	0.09769680
salicylate	0.02914563	-0.05405879	0.03536965	X - 17335	0.02258336	-0.05093205	0.09866435
2-hydroxystearate	0.00161620	-0.04024675	0.03636039	X - 21736	0.00118011	-0.02751894	0.10069493
5alpha-androstan-3alpha,17beta-diol-17-glucosiduronate	-0.01542518	-0.04680435	0.03754072	1-[1-enyl-stearoyl]-GPE (P-18:0)*	-0.005942711	-0.02340420	0.10110681

Supplementary Table 9 continued

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
5alpha-androstan-3alpha,17alpha-diol monosulfate	0.00376776	-0.04026744	0.10114818	X - 21834	-0.02691118	-0.03736912	0.20767985
taurine	0.00021640	-0.01297508	0.10180154	kynurenate	0.01058331	-0.01978663	0.20785410
lactosyl-N-palmitoyl-sphingosine	0.00144714	-0.01233849	0.10191425	arginine	-0.00771087	-0.01640510	0.21139427
glycocholate sulfate*	-0.01583872	-0.02404548	0.10422267	X - 12028	0.00091093	-0.00993198	0.21156043
3-hydroxy-5-cholestenic acid	0.00573333	-0.02041438	0.10480730	X - 13835	0.01911418	-0.04108514	0.21323328
p-oresol-glucuronide*	0.02041540	-0.08081571	0.10650519	quinolinate	-0.00642300	-0.01486094	0.21324306
gamma-glutamylalanine	0.00255628	-0.02439570	0.10989776	doosapentaenoate (n6 DPA; 22:5n6)	-0.02289914	-0.05279691	0.21474970
3-hydroxy-2-ethylpropionate	-0.01740035	-0.03939925	0.11144761	palmitoylcarnitine	0.00444440	-0.01381132	0.21644287
X - 13729	0.01963489	-0.02526936	0.11158252	X - 13684	-0.01512730	-0.03659682	0.21787375
X - 23652	0.00772090	-0.04587540	0.11263418	isobutyrylcarnitine	0.00721436	-0.01461811	0.21797228
X - 23749	0.00666303	-0.01824656	0.11307914	lactate	0.00150513	-0.02099608	0.22090702
1-stearoyl-2-docosahexaenoyl-GPE (18:0/22:6)*	-0.01222698	-0.01937952	0.11327035	X - 24832	-0.00400132	-0.03066533	0.22209504
X - 17299	0.01128214	-0.02662517	0.11373149	1-palmitoyl-2-alpha-linolenoyl-GPC (16:0/18:3n3)*	0.01294491	-0.02726685	0.22305824
X - 12849	-0.06809895	-0.15498268	0.11539769	X - 12524	0.00001437	-0.02018762	0.22509471
X - 24748	-0.00084915	-0.01768460	0.11580449	X - 24432	-0.01291768	-0.04618364	0.22574193
glycerophosphorylcholine (GPC)	-0.00287119	-0.02572389	0.11582786	X - 15503	0.00648077	-0.01790105	0.22979868
1-stearoyl-GPI (18:0)	-0.01778713	-0.04447661	0.11643213	malate	0.01448492	-0.01416699	0.23395710
pseudouridine	0.00089713	-0.01515661	0.11842424	X - 17328	0.04849766	-0.08479937	0.23515438
bilirubin (E.E)*	-0.03174731	-0.03590198	0.12065195	ectoine	0.06229590	-0.06567846	0.23749025
alanine	0.00108618	-0.01731745	0.12074918	X - 12206	-0.00334465	-0.01223907	0.24400882
X - 14658	0.04159291	-0.05840037	0.12132690	S-adenosylhomocysteine (SAH)	0.01110721	-0.03262717	0.24505472
pregnenolone sulfate	0.00276746	-0.03267034	0.12156444	tryptophan betaine	-0.00093955	-0.01249898	0.24831188
myristoleylcarnitine*	0.01484190	-0.06677727	0.12271273	cystine	-0.01089664	-0.04393422	0.24963204
X - 21658	-0.01138518	-0.01798802	0.12440477	1-adrenoyl-GPC (22:4)*	-0.01195994	-0.02229828	0.24987315
sphingomyelin (d18:1/14:0, d16:1/16:0)*	-0.00448965	-0.01571797	0.12521047	doosahexaenoate (DHA; 22:6n3)	0.00459009	-0.04128479	0.25077810
X - 11522	-0.02475003	-0.04240521	0.12751613	2-hydroxyhippurate (salicylurate)	0.03261219	-0.04666468	0.25412963
X - 17469	-0.01673341	-0.08075837	0.12860985	hexanoylcarnitine	-0.01691053	-0.03174794	0.25603288
lactose	-0.00180391	-0.07736687	0.12913576	X - 12708	-0.01925845	-0.02548989	0.25681814
X - 21358	0.00432157	-0.02039305	0.12931181	isovalerylcarnitine	0.00681768	-0.03288323	0.25764249
X - 24527	0.02136474	-0.06846300	0.13272904	gamma-glutamylvaline	0.00001519	-0.01611644	0.26206288
sphingomyelin (d18:1/14:0, d16:1/16:0)*	0.00204083	-0.01441113	0.13315130	X - 17890	0.02971055	-0.06035226	0.26359178
X - 18914	-0.00267881	-0.01710940	0.13397492	1-margaroyl-GPC (17:0)	0.00288091	-0.01062814	0.26479964
X - 11880	0.00236721	-0.01333734	0.13445157	1-(1-enyl-palmitoyl)-2-linoleoyl-GPC (P-16:0/18:2)*	-0.00337658	-0.01082946	0.26528985
X - 11381	0.02828028	-0.06884509	0.13468155	tyrosine	-0.00485326	-0.01380301	0.26775279
sphingomyelin (d18:1/15:0, d16:1/17:0)*	-0.00102999	-0.01252553	0.13469504	1-(1-enyl-palmitoyl)-2-myristoyl-GPC (P-16:0/14:0)*	0.00834821	-0.01577885	0.27103778
5alpha-androstan-3beta,17alpha-diol disulfate	0.00495462	-0.02367647	0.13482795	uridine	0.02845369	-0.02327837	0.27226415
3-hydroxydecanoate	0.00878577	-0.01052515	0.13532458	X - 24041	0.01333846	-0.02204017	0.27254648
taurocholate sulfate	0.01950607	-0.03241177	0.13550078	imidazole lactate	0.00230683	-0.01883497	0.27424673
dimethylarginine (SDMA + ADMA)	-0.00138567	-0.01335401	0.13906724	succinimide	-0.02217724	-0.02741536	0.27465412
3-hydroxylaurate	-0.00972905	-0.05465544	0.14113346	gamma-glutamyltyrosine	-0.00714310	-0.02098760	0.27632505
tryptophan	0.00044675	-0.01696919	0.14324463	3-methylhistidine	0.00345984	-0.06848098	0.27640939
X - 24699	0.00161134	-0.01725828	0.14460468	siocosapentaenoate (EPA; 20:5n3)	0.00374613	-0.03118611	0.27731256
methionine	0.00402421	-0.02607442	0.14609800	glycosyl-N-stearoyl-sphingosine	-0.00265008	-0.02244868	0.27764714
propylphenylalanine	0.00363172	-0.01798685	0.14668262	X - 24813	0.00454471	-0.01082451	0.27801409
myristoylcarnitine	0.01239379	-0.03105511	0.14677801	X - 18249	-0.00355079	-0.01102652	0.27826622
phenylacetylglutamine	0.00022732	-0.03252784	0.14925705	4-vinylphenol sulfate	-0.00164618	-0.05999645	0.28203565
ethylmalonate	-0.00765994	-0.01936110	0.14955903	1-arachidoyl-GPC (20:0)	-0.00589432	-0.01639330	0.28292115
N-palmitoylglycine	0.00795487	-0.03644149	0.15087606	caprylate (8:0)	0.04427954	-0.05414125	0.28670003
arachidonate (20:4n6)	-0.00407929	-0.04104648	0.15195999	N-formylphenylalanine	0.04575777	-0.04377473	0.28805597
1-(1-enyl-stearoyl)-2-docosahexaenoyl-GPC (P-18:0/22:6)*	-0.00283296	-0.01384138	0.15439365	X - 12851	0.00439671	-0.02733565	0.28926007
C-glycosyltryptophan	-0.00596450	-0.01602914	0.15481758	3-hydroxybutyrylcarnitine (1)	0.00554342	-0.03312610	0.29070752
X - 12739	0.02965818	-0.07411358	0.15671865	indoleacetylglutamine	-0.01882525	-0.02038953	0.29256024
3-carboxy-4-methyl-5-propyl-2-furanpropanoate (CMPF)	0.00814925	-0.02342520	0.15712290	1-(1-enyl-palmitoyl)-2-palmitoyl-GPC (P-16:0/16:0)*	-0.00102093	-0.00993004	0.29379890
1-palmitoyl-GPA (16:0)	-0.00685876	-0.03304480	0.15855987	1-(1-enyl-stearoyl)-2-arachidonoyl-GPC (P-18:0/20:4)	-0.00210454	-0.00886323	0.30408813
2-keto-3-deoxy-gluconate	0.00702602	-0.03715988	0.15947297	serine	0.00858854	-0.01153729	0.30934021
X - 24728	-0.01029224	-0.03619733	0.16178017	2-docosahexaenoyl-GPC (22:6)*	-0.01546235	-0.03045101	0.31050031
3-methyltyridine	-0.00399490	-0.01439301	0.17041700	3-hydroxyisobutyrate	0.00550500	-0.03240738	0.31063011
X - 11378	-0.00044017	-0.01236178	0.17057275	sarcosine	0.00945222	-0.01869777	0.31235648
X - 24309	-0.00241026	-0.02284545	0.17176065	X - 16935	-0.00362685	-0.01102871	0.31404933
1-stearoyl-GPS (18:0)*	0.00322457	-0.01531014	0.17254818	X - 21735	-0.00688710	-0.05040496	0.31461793
sphingomyelin (d18:2/23:0, d18:1/23:1, d17:1/24:1)*	-0.00165945	-0.01045159	0.17259851	X - 23644	0.03745252	-0.08394702	0.31671074
X - 24544	0.00398301	-0.02384040	0.17282899	X - 12715	0.01843241	-0.05069806	0.31920510
asparagine	0.00734842	-0.01528884	0.17358043	2-methylbutyrylcarnitine (C5)	0.02789371	-0.03898818	0.31922247
lysine	-0.00035036	-0.01479197	0.17557105	methyl indole-3-acetate	-0.00293801	-0.02545745	0.31924292
sphingomyelin (d18:2/14:0, d18:1/14:1)*	-0.00348635	-0.01370547	0.17589105	behenoyl sphingomyelin (d18:1/22:0)*	-0.00277014	-0.00760166	0.32184115
pyroglutamine*	0.00521408	-0.01183520	0.17706096	X - 14939	0.00364941	-0.02587897	0.32215247
sphingomyelin (d18:2/16:0, d18:1/16:1)*	-0.00056365	-0.01197337	0.18405237	X - 11442	-0.03836620	-0.03342365	0.33336785
X - 21410	0.01065000	-0.01516364	0.18831682	3-phenylpropionate (hydrocinnamate)	0.04772870	-0.04205449	0.33473833
X - 24749	-0.03270979	-0.02493010	0.18887695	glycolithocholate sulfate*	0.01121920	-0.04068455	0.33605304
X - 17359	-0.01039482	-0.02628986	0.19081586	X - 21668	0.03175395	-0.03226075	0.33796948
sphingomyelin (d18:1/22:1, d18:2/22:0, d16:1/24:1)*	-0.00482188	-0.01747236	0.19115304	glucose	0.01678804	-0.01136655	0.34137618
5-hydroxyindoleacetate	-0.00683223	-0.05726947	0.19244886	dihomone-linolenate (20:3n3 or n6)	-0.00393687	-0.03447356	0.34311977
2-hydroxy-3-methylvalerate	0.00781316	-0.02004937	0.19384130	glycoedoxycholate sulfate	0.00762025	-0.03375724	0.34364966
X - 14626	0.03149167	-0.04574587	0.19690356	X - 24061	-0.00132100	-0.00783523	0.34448586
acisoga	0.00541857	-0.03659876	0.19693886	15-methylpalmitate	-0.00015248	-0.03170714	0.34481696
1-(1-enyl-stearoyl)-2-docosahexaenoyl-GPE (P-18:0/22:6)*	-0.00256589	-0.01064537	0.19892276	X - 24549	0.02939183	-0.03230092	0.34531279
umbelliferone sulfate	-0.02927872	-0.07557811	0.20291907	X - 14838	0.00070561	-0.03684215	0.34921521
homocarnitine	0.00269103	-0.01532202	0.20498154	X - 12847	0.00605220	-0.05609317	0.34973442

Supplementary Table 9 continued

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
X - 01911	-0.02882470	-0.04438862	0.35005102	tricosanoyl sphingomyelin (d18:1/23:0)*	0.00177100	-0.00658430	0.51803387
gamma-glutamylthreonine*	-0.01169498	-0.01643441	0.35168097	3-methyladipate	0.05941997	-0.02278011	0.52275828
X - 23705	-0.00442285	-0.00620424	0.35198241	X - 12879	-0.00289001	-0.03903508	0.52290822
N-acetyl-beta-alanine	0.02113140	-0.02427682	0.35364398	X - 21666	0.00387720	-0.03342379	0.52307662
pantothenate	-0.00200128	-0.01251741	0.35432771	stearyl carnitine	0.02162569	-0.00970964	0.52504128
X - 18886	0.01969843	-0.03523483	0.35799788	iminodiacetate (IDA)	0.00830583	-0.01051407	0.52661372
retinol (Vitamin A)	-0.00795561	-0.00624026	0.35904365	X - 17653	-0.00502652	-0.00711586	0.52762126
isovalerylglycine	0.00371505	-0.03892754	0.35928377	malonate	-0.02453809	-0.01745156	0.52984758
dihomo-linolenoyl-choline	-0.04615659	-0.04210440	0.36018896	X - 21319	0.00884836	-0.01282868	0.53559368
4-androsten-3beta,17beta-diol disulfate (2)	-0.00342255	-0.00741193	0.36289274	cysteine sulfonic acid	0.01287124	-0.00661139	0.53610186
trans-4-hydroxyproline	0.00644543	-0.02025149	0.36780904	X - 12844	-0.00613969	-0.01158944	0.53657035
N-acetyl-3-methylhistidine*	-0.00401745	-0.04383689	0.36872449	1-methylhistidine	0.00346175	-0.01138480	0.53729505
N6-methyladenosine	-0.01047577	-0.01548801	0.37183514	X - 23276	-0.00607361	-0.01336277	0.53777995
X - 11852	0.08033695	-0.06570213	0.37719371	2,3-dihydroxyisovalerate	0.00688449	-0.04879599	0.54088390
X - 11334	0.00695880	-0.02225274	0.38002925	X - 12100	-0.00501569	-0.00519523	0.54130217
gamma-glutamylmethionine	0.00121564	-0.01807486	0.38323801	3-hydroxybutyryl carnitine (2)	0.00992796	-0.01276335	0.54240639
1-arachidonylglycerol (20:4)	0.00002267	-0.02757485	0.38336291	O-methylcatechol sulfate	0.01628148	-0.01298574	0.54532775
sphingomyelin (d18:2/24:1, d18:1/24:2)*	-0.00157730	-0.00664682	0.38370405	2-myristoyl-GPC (14:0)*	0.00156630	-0.01676484	0.54965733
oxalate (ethanedioate)	0.00852010	-0.01077287	0.38573773	X - 24645	0.02052549	-0.01133907	0.55447198
2-oleoyl-GPC (18:1)*	-0.00274677	-0.01083057	0.38598232	malonyl carnitine	0.00678441	-0.00904934	0.55704356
N-acetylvaline	0.00755915	-0.01013282	0.38697316	1-palmitoyl-2-palmitoleoyl-GPE (16:0/16:1)*	-0.02927420	-0.01441831	0.55953292
pipecolate	0.02746996	-0.03345980	0.38870823	catechol glucuronide	0.01544787	-0.04041530	0.56273022
N6-carbamoylthreonyl adenosine	-0.00680914	-0.01127355	0.39689576	benzoate	-0.03202519	-0.01857227	0.56345277
X - 14056	0.01450854	-0.01069342	0.40489857	gamma-CEHC	0.00289604	-0.01400237	0.56641827
glutaryl carnitine (C5)	0.00387351	-0.00851083	0.40572837	pentadecanoate (15:0)	0.00609503	-0.01392252	0.56697335
X - 22764	-0.01098905	-0.04866319	0.40712851	X - 15245	-0.00408837	-0.00320227	0.56849023
X - 21792	-0.00094544	-0.02909325	0.41006315	X - 11485	0.00710869	-0.02520969	0.58190114
sphingomyelin (d18:1/21:0, d17:1/22:0, d16:1/23:0)*	-0.00541003	-0.00616997	0.41404637	phenyllactate (PLA)	0.00720188	-0.00654305	0.58283355
2-linoleoylglycerol (18:2)	-0.04640589	-0.03463774	0.41540112	1-margaroyl-2-arachidonoyl-GPC (17:0/20:4)*	-0.00052167	-0.00408737	0.58915685
1-stearoyl-GPC (O-18:0)*	-0.00706344	-0.01080201	0.41950182	X - 24645	0.00508750	-0.00876204	0.59410232
dimethylglycine	0.00247115	-0.00961510	0.41952541	sphinganine	0.00353935	-0.00823269	0.59545296
1-lignoceroyl-GPC (24:0)	-0.00558808	-0.01664515	0.42025907	1-palmitoyl-GPC (16:0)	0.00488375	-0.00484008	0.59897760
X - 22162	-0.01944824	-0.01021546	0.42289158	X - 23639	0.01127178	-0.00714859	0.60069553
X - 11372	-0.00356630	-0.00622385	0.42673100	N-methyltaurine	-0.00831378	-0.04237815	0.60070587
X - 24329	-0.01020528	-0.01139544	0.42746245	gamma-glutamyl-epsilon-lysine	0.00700426	-0.01648400	0.60076933
coritzone	-0.00598755	-0.01163698	0.42927846	X - 12718	-0.00971879	-0.01703534	0.60179324
caprate (10:0)	0.00762428	-0.02585060	0.42993585	X - 23295	0.00334471	-0.02215769	0.60249924
10-nonadecanoate (19:1n9)	-0.00190927	-0.03881645	0.43308850	1-palmitoyl-GPI (16:0)*	-0.00355170	-0.02058577	0.60447298
pyridoxate	-0.01665532	-0.03927845	0.43447947	glycohyocholate	0.05673170	-0.01630124	0.60494313
X - 11871	-0.06538149	-0.03876624	0.43636407	1-behenoyl-GPC (22:0)	0.00391273	-0.01020089	0.60676406
X - 24765	-0.00555730	-0.00847859	0.43776504	1-palmitoyl-GPC (O-16:0)	-0.01630325	-0.00905381	0.60696855
4-methyl-2-oxopentanoate	0.00835178	-0.01432373	0.44371039	cysteine	0.003654187	-0.00391583	0.60894277
1-oleoyl-2-eicosapentaenoyl-GPE (18:1/20:5)*	-0.04868128	-0.03006798	0.44468203	3-methyl-2-oxobutyrate	0.00582147	-0.00855804	0.61143770
4-vinylguaiaacolsulfate	0.00957931	-0.05566620	0.44980428	phenylpyruvate	-0.02347707	-0.01051087	0.61535400
5alpha-androstan-3beta,17beta-diol monosulfate (2)	-0.00197489	-0.01233010	0.45694009	1-oleoyl-2-docosapentaenoyl-GPC (18:1/22:5n3)*	0.00028940	-0.00605141	0.61574705
pyroglutamylglycine	0.01295918	-0.02334120	0.45788305	X - 21353	0.00221939	-0.02016042	0.62463523
X - 17654	-0.00180527	-0.00734474	0.46016637	N-acetyl glycine	0.00046175	-0.01015058	0.62512354
leuoylglutamine*	0.00026215	-0.00026215	0.46017583	X - 11407	-0.00604269	-0.02315420	0.62584113
glycolithocholate	0.01710442	-0.04403436	0.46090082	lindoleacetate	0.00060377	-0.00746131	0.62616749
X - 21365	-0.00491348	-0.00805415	0.46296981	1,2-dipalmitoyl-GPC (16:0/16:0)	0.00661184	-0.00286319	0.62683483
1-margaroyl-GPE (17:0)*	-0.02686623	-0.02026343	0.46503710	docosadienoate (22:2n6)	0.00848018	-0.01796261	0.62815466
X - 11305	-0.00354721	-0.00521020	0.46933064	X - 11483	-0.00307971	-0.01431755	0.62864910
1-meadoyl-GPC (20:3n9)*	-0.01256175	-0.03173511	0.47115604	1-pentadecanoyl-2-arachidonoyl-GPC (15:0/20:4)*	0.01221582	-0.00517991	0.63061479
X - 11429	-0.00310355	-0.00574651	0.47601420	mannitol/sorbitol	0.01061182	-0.02001237	0.63119934
X - 24475	0.00539791	-0.01642334	0.47737023	N6,N6,N6-trimethyllysine	0.00944211	-0.00744989	0.63192080
X - 16071	0.00903378	-0.00857310	0.48082732	oleoylcholine	-0.07353377	-0.02770390	0.63246081
pyruvate	0.01185584	-0.00942620	0.48205514	N-acetylglucosamine/N-acetylgalactosamine	0.00254078	-0.00713466	0.63737736
X - 16580	0.01988122	-0.01907367	0.48220980	1-oleoyl-2-docosahexaenoyl-GPE (18:1/22:6)*	-0.00964282	-0.00797302	0.64405555
palmitoleoyl carnitine*	0.00660176	-0.01803461	0.48281710	X - 12740	-0.01201994	-0.03434984	0.64500003
N1-methyladenosine	0.00074699	-0.00590215	0.48326291	N-acetyl carnosine	0.00294124	-0.00709983	0.64517271
X - 23680	0.00727170	-0.01508700	0.48602539	2-methylserine	-0.00153147	-0.00998728	0.64557850
1-(1-enyl-palmitoyl)-2-oleoyl-GPC (P-16:0/18:1)*	-0.00083097	-0.00568288	0.49097875	gamma-glutamylglycine	-0.00118471	-0.00465823	0.64642866
1-palmitoyl-2-arachidonoyl-GPC (O-16:0/20:4)*	-0.00520239	-0.00594554	0.49224849	3-hydroxyhexanoate	0.01049482	-0.01202485	0.64859882
1-(1-enyl-stearoyl)-GPC (P-18:0) *	-0.00510773	-0.00597931	0.49291101	X - 12231	-0.01367757	-0.02660474	0.65514282
X - 16964	-0.01799172	-0.02167515	0.49450571	X - 17146	0.03518514	-0.05060652	0.65848987
X - 24240	-0.00483362	-0.00991704	0.49916445	X - 21470	-0.01527561	-0.00791545	0.65870993
X - 24831	-0.00770461	-0.00896178	0.49957156	1-palmitoyl-2-arachidonoyl-GPE (16:0/20:4)*	-0.00876487	-0.00724648	0.65910545
docosapentaenoate (n3 DPA; 22:5n3)	0.00836296	-0.03786573	0.50193166	1-(1-enyl-palmitoyl)-2-arachidonoyl-GPE (P-16:0/20:4)*	-0.01317834	-0.00554540	0.66046134
galactonate	-0.00887796	-0.02400401	0.50299725	indolepropionate	-0.00726949	-0.01491409	0.66116997
X - 23782	-0.01056108	-0.02115049	0.50555258	phenylacetylglutamate	0.04309964	-0.02920925	0.66129082
dihomo-linoleate (20:2n6)	-0.00294862	-0.03503608	0.50748835	1-margaroyl-2-docosahexaenoyl-GPC (17:0/22:6)*	0.01379279	-0.00520982	0.66183483
andro steroid monosulfate (1)*	-0.00536988	-0.00769722	0.50960348	N-acetyl-1-methylhistidine*	-0.01407169	-0.01072499	0.66543401
1-pentadecanoyl-2-linoleoyl-GPC (15:0/18:2)*	0.01379387	-0.00723168	0.51082164	5alpha-androstan-3beta,17beta-diol disulfate	-0.00936844	-0.00501625	0.67050273
X - 11441	-0.03589375	-0.02195692	0.51093247	acetyl carnitine	-0.00226089	-0.00719482	0.67142745
X - 24422	-0.01564232	-0.01142245	0.51246170	glutamine	0.00124294	-0.00293058	0.67454780
tartronate (hydroxymalonate)	-0.00124601	-0.01296524	0.51606600	indole-3-carboxylic acid	0.01017737	-0.01185286	0.67898738
cinnamoylglycine	0.02679813	-0.04127874	0.51620462	X - 11637	-0.08408246	-0.02746985	0.68175225
mannose	-0.01094022	-0.01463761	0.51779165	sphinganine-1-phosphate	0.00936833	-0.00684206	0.68198015

Supplementary Table 9 continued

Metabolite	beta	gamma	p	Metabolite	beta	gamma	p
methylsuccinoylcarnitine(1)	0.00537197	-0.00421560	0.68420901	N-oleoyltaurine	-0.02003734	-0.00683370	0.86579760
oleoylcarnitine	0.01495357	-0.00688208	0.68598987	fructose	0.05265752	-0.00640918	0.87135720
palmitoylcholine	-0.06122235	-0.02312828	0.68816273	1-(1-enyl-oleoyl)-GPC (P-18:1)*	-0.00297703	-0.00137485	0.87763280
X - 18921	0.00398824	-0.01020112	0.69135955	1-(1-enyl-palmitoyl)-2-palmitoleoyl-GPC (P-16:0/16:1)*	0.00399736	-0.00147905	0.87779518
1,5-anhydroglucitol(1,5-AG)	-0.00278274	-0.00266684	0.69321044	1,2-distearoyl-GPC (18:0/18:0)	0.00412222	-0.00316483	0.87934924
N-delta-acetylmethionine	0.00779281	-0.00687205	0.69566925	6alpha-androstan-3alpha,17beta-diol monosulfate (1)	-0.01356198	-0.00211541	0.88168775
X - 11478	-0.00682558	-0.00756323	0.69936473	1-palmityl-2-oleoyl-GPC (O-16:0/18:2)*	-0.01281198	-0.00226731	0.88472310
X - 11444	0.00555940	-0.00742760	0.70371702	N-acetyltaurine	-0.00061178	-0.00143653	0.89602835
X - 21364	-0.01145412	-0.00373627	0.70553320	2-aminoadipate	-0.00070001	-0.00301784	0.89797185
hypotaurine	0.00785528	-0.00519035	0.70852663	erythritol	0.00806422	-0.00239117	0.89888340
1-eicosenoyl-GPC (20:1)*	-0.00291823	-0.00465906	0.70859623	X - 23739	-0.00302219	-0.00192179	0.90026069
4-hydroxyxoumarin	-0.01606203	-0.01871011	0.71217694	X - 11452	-0.02089809	-0.00790354	0.90167455
X - 14662	0.00618684	-0.01461408	0.71302314	X - 23369	-0.00551356	-0.00492509	0.90619250
X - 21258	-0.02735412	-0.01162249	0.71798862	X - 21607	0.00875184	-0.00311718	0.90785434
X - 24809	-0.00673698	-0.00349759	0.72083274	X - 12442	-0.00048006	-0.00576567	0.91441247
nonadecanoate (19:0)	-0.00187785	-0.00941568	0.72263279	ergothioneine	-0.01458636	-0.00121333	0.91660368
1-docosapentaenoyl-GPC (22:5n3)*	0.00425466	-0.00485091	0.72340110	X - 18889	0.00061484	-0.00282499	0.91662740
1-palmityl-2-oleoyl-GPC (O-16:0/18:1)*	-0.00560776	-0.00311765	0.72770198	X - 12812	-0.00041459	-0.00558236	0.92749708
X - 24243	-0.02912277	-0.01325526	0.73072603	X - 12306	0.03853690	-0.00437536	0.92766520
gamma-glutamylisoleucine*	-0.00493475	-0.00445584	0.73170182	N-acetylhistidine	-0.01147475	-0.00211020	0.93162203
2-hydroxybutyrate/2-hydroxyisobutyrate	-0.00085847	-0.00858487	0.73228636	3-hydroxyoctanoate	0.02388632	-0.00228826	0.93465887
proline	-0.00123758	-0.00270094	0.73613836	2-aminophenol sulfate	-0.02107107	-0.00403882	0.93829847
3-ureidopropionate	0.00853617	-0.00562418	0.73882877	methyl-4-hydroxybenzoate sulfate	0.00227439	-0.00440308	0.94399599
X - 11299	-0.00342117	-0.01201387	0.74169061	X - 12681	-0.00273021	-0.00118319	0.94439553
dehydroisoandrosterone sulfate (DHEA-S)	-0.00797965	-0.00265574	0.74193955	X - 24513	-0.00723745	-0.00061706	0.94454273
pregnen-diol disulfate*	-0.00196820	-0.00399937	0.74242604	myristate (14:0)	-0.00733484	-0.00299932	0.94462723
4-androsten-3beta,17beta-diol disulfate (1)	-0.01037759	-0.00336342	0.74261429	4-acetylphenol sulfate	0.02445648	-0.00282415	0.94894090
oleoyl ethanolamide	0.02072725	-0.00687088	0.74298936	X - 23780	0.03342989	-0.00202585	0.96464387
16a-hydroxy DHEA-3 sulfate	0.01264954	-0.00325227	0.74558063	ethyl paraben sulfate	-0.00065480	-0.00061037	0.97859570
5-dodecanoate (12:1n7)	-0.01482713	-0.01421916	0.74609179	N-acetyltyrosine	0.00353725	-0.00035043	0.97949425
1-palmitoyl-2-docosahexaenoyl-GPC (16:0/22:6)	-0.00148380	-0.00318488	0.74709302	X - 17677	0.07454653	-0.00180481	0.98048293
spermidine	-0.00307212	-0.00668958	0.74870285	hydroquinone sulfate	0.02794816	-0.00095747	0.98329557
S-methylmethionine	0.02534342	-0.01976979	0.74906810	10-heptadecanoate (17:1n7)	-0.00549782	-0.00067654	0.98930925
gamma-carboxyglutamate	-0.00464030	-0.01115775	0.74955942	margarate (17:0)	-0.00087450	-0.00038801	0.99175981
X - 18922	-0.00625066	-0.00475853	0.74966065	11-ketotriocholanolone glucuronide	0.01203528	-0.00013450	0.99407234
1-stearoyl-GPC (18:0)	-0.00231403	-0.00431133	0.75185994				
1-myristoyl-2-linoleoyl-GPC (14:0/18:2)*	-0.00475303	-0.00519486	0.75238387				
1-stearoyl-2-docosahexaenoyl-GPI (18:0/22:6)*	0.01339271	-0.00666470	0.75784362				
1-oleoyl-2-docosahexaenoyl-GPC (18:1/22:6)*	-0.00329798	-0.00327071	0.75877421				
eicosanoate (20:1)	-0.00051404	-0.01669483	0.75934077				
X - 24546	-0.00351690	-0.00684707	0.76032071				
choline	0.00241955	-0.00292953	0.76067725				
1-palmitoleoylglycerol (16:1)*	0.00621863	-0.01246135	0.76323933				
flavin adenine dinucleotide (FAD)	0.00463491	-0.00609612	0.76734973				
gamma-glutamylglutamine	-0.00654649	-0.00398159	0.76958571				
X - 21343	-0.00430727	-0.01190617	0.77491456				
thiopropine	0.00280621	-0.00263376	0.77513069				
X - 11787	-0.00426201	-0.00152713	0.77669678				
X - 21339	-0.00498082	-0.00204118	0.77735058				
X - 21821	-0.01914956	-0.01018618	0.78110032				
2-piperidinone	-0.00734550	-0.00317660	0.78151372				
arachidonoylcholine	-0.00690445	-0.01506780	0.78174754				
trimethylamine N-oxide	0.00792002	-0.01179025	0.78200091				
thymol sulfate	0.02665816	-0.02601018	0.78429916				
X - 12822	-0.02409037	-0.00882893	0.79023693				
X - 11440	0.00243094	-0.00449791	0.79024214				
5-hydroxymethyl-2-furoic acid	-0.00177616	-0.01632435	0.79250296				
1-(1-enyl-palmitoyl)-2-linoleoyl-GPE (P-16:0/18:2)*	-0.00326001	-0.00339257	0.79644485				
X - 12818	-0.00742279	-0.00650763	0.79829171				
3-methyl-2-oxovalerate	0.01235576	-0.00435597	0.80042723				
nicotinamide riboside	0.03461714	-0.00773001	0.80425476				
gluconate	0.00715450	-0.00304927	0.80979702				
3-aminoisobutyrate	0.00377285	-0.00313878	0.81515711				
2-aminooctanoate	-0.00443012	-0.00353595	0.81769754				
1-stearoyl-2-docosahexaenoyl-GPC (18:0/22:6)	-0.00185726	-0.00259448	0.81963526				
glycerol	0.00391208	-0.01088497	0.82260411				
X - 17668	0.00730230	-0.01559883	0.82361976				
1-stearoyl-2-arachidonoyl-GPS (18:0/20:4)	-0.01910288	-0.00505738	0.83316760				
docosahexaenoylcholine	-0.05264805	-0.00941507	0.83631703				
X - 24518	-0.00590642	-0.00685181	0.84057422				
DSGEGDFXAEAGGVR*	0.16536058	-0.02048015	0.84212911				
myristoleate (14:1n5)	-0.02213397	-0.01220277	0.84450299				
N-acetylglutamine	-0.00331855	-0.00413769	0.85014801				
stearate (18:0)	-0.00308399	-0.00547882	0.85051646				
X - 24678	-0.01580943	-0.00663624	0.85271901				
4-hydroxyphenylpyruvate	-0.01043960	-0.00443490	0.85273188				
X - 12472	0.02839592	-0.00876236	0.85427391				
N-acetyllalanine	-0.00308098	-0.00152758	0.86013253				

Supplementary Table 10

Analyte	beta	gamma	p
IL-15	-0.00241	-0.0643	0.00082
Serpin E1	0.00289	-0.0747	0.00243
IL-3	0.00508	-0.1859	0.00316
M-CSF	-0.02366	-0.07	0.0053
IL-1 beta	9.88E-05	-0.0733	0.00553
BDNF	-0.01785	-0.1238	0.00631
IL-4	-0.00679	-0.1415	0.00683
GM-CSF	-0.03834	-0.1228	0.00886
IFN-gamma	-0.00225	-0.1378	0.01408
PDGF-AA	-0.00206	-0.1375	0.02448
Fas Ligand	-0.0297	-0.0818	0.0307
PDGF-BB	-0.04469	-0.1337	0.03584
IL-36/IL-1F8	-0.01586	-0.0554	0.04108
RANTES/CCL5	-0.01768	-0.0516	0.04701
IL-1 alpha	-0.03248	-0.0347	0.05591
Midkine	-0.01649	-0.0506	0.06413
FGF basic	-0.01238	-0.0386	0.07095
CD44	-0.01698	-0.0366	0.07887
TNF-alpha	-0.01484	-0.0391	0.08069
Serpin C1	0.00687	-0.0107	0.11601
VEGF	-0.00701	-0.0388	0.14322
EPO	0.00994	-0.074	0.14741
Fractalkine	-0.12318	-0.1671	0.1505
IL-8/CXCL8	-0.02672	-0.0349	0.15623
SCF	-0.02221	-0.0248	0.22
IL-33	-0.01802	-0.04	0.25979
TPO	-0.02216	-0.0294	0.28284
ICAM-1/CD54	-0.00944	-0.0054	0.36875
LIF	-0.01181	-0.026	0.45259
ErbB2/Her2	-0.00271	-0.0069	0.45801
IL-6	-0.01213	-0.0233	0.49516
MMP-9	-0.00647	-0.0362	0.6182
EGF	-0.03236	-0.0513	0.66476
MIP 3a	0.02168	-0.0178	0.70866
Vitamin D binding protein	0.00222	-0.004	0.7457
CD40 Ligand	-0.01561	-0.0101	0.7945
HB-EGF	-0.06959	-0.0262	0.89039
SDF-1 alpha	0.01116	-0.0036	0.95166
MMP-2	0.01367	-0.0003	0.95679

Supplementary Table 11

Analyte	beta	gamma	p
Prolactin	0.010383	0.140419	0.005571
C-reactive protein	-0.00528	0.00658	0.015884
Serpin A7	-0.00014	0.004531	0.033133
CD14	-0.00116	0.003658	0.093644
Chermin	0.00388	0.016703	0.107158
Angiopoietin-like 4	-0.00239	0.036718	0.201916
Lumican	-0.02705	0.028794	0.380844
SOST	-0.04443	0.039184	0.530263
Beta 2-Microglobulin	0.000255	0.001377	0.630343
VCAM-1	-0.01458	0.005949	0.722365
MIP-3b/CCL19	-0.04202	0.00861	0.759651
Chitnase 3-like 1	-0.03273	0.015974	0.78517
Procalcitonin	-0.05394	0.009315	0.83682
CD23	-0.00852	0.000115	0.993565

Supplementary Table 12

Plateletpheresis serum samples

Timepoint	Free T3 (pmol/L)	
	Average	SD
pre	4.81	0.36
post	4.79	0.57
4-8 hours	4.74	0.49
D1	4.96	0.33
D3	5.13	0.41
D7	4.94	0.42
D14	4.92	0.49

Supplementary Table 13

Blood cell indices		Baseline	D1	D2	D3	D4	D5
PLT 10 ³ /mm ³	Control mean	978.8	756.4	885.5	755.6	771.8	734.8
	Depletion mean	978.8	76.85	89.28	87.64	507.9	529.9
	p	ns	****	****	****	ns	ns
MPV μm ³	Control mean	5.3	5.425	5.9	5.5	5.233	5.4
	Depletion mean	5.3	6.75	6.8	6.4	6	6.133
	p	ns	*	ns	ns	ns	ns
HGB g/dL	Control mean	12.04	10.82	13.62	14.2	13.65	14.1
	Depletion mean	12.04	11.56	19.01	13.6	14.35	15.71
	p	ns	ns	ns	ns	ns	ns
MCHC g/dL	Control mean	36.48	39.59	37.14	34.61	36.84	36.12
	Depletion mean	36.48	39.19	35.18	54.76	35.87	36.63
	p	ns	ns	ns	ns	ns	ns
MCH pg	Control mean	17.31	18.33	17.85	16.46	17.3	17.1
	Depletion mean	17.31	18.71	17.26	25.61	16.97	18.03
	p	ns	ns	ns	ns	ns	ns
RBC 10 ⁶ /mm ³	Control mean	7.473	6.778	8.243	9.143	8.507	8.753
	Depletion mean	7.473	7.145	11.87	7.1	9.057	9.413
	p	ns	ns	ns	ns	ns	ns
RDW %	Control mean	15.83	14.83	16.13	16.07	15.23	15.17
	Depletion mean	15.83	15.25	15.8	15.47	15.27	17.3
	p	ns	ns	ns	ns	ns	*
MCV μm ³	Control mean	47.67	46.25	48	47.67	47	47.33
	Depletion mean	47.67	47.5	49.33	47.33	47.33	49
	p	ns	ns	ns	ns	ns	ns
HCT %	Control mean	33.03	27.9	36.83	41.03	37.17	39.1
	Depletion mean	33.03	29.45	54.57	31.03	40.07	42.83
	p	ns	ns	ns	ns	ns	ns
WBC 10 ³ /mm ³	Control mean	3.257	5.1	4.58	4.81	3.983	4.347
	Depletion mean	3.257	4.765	5.54	3.847	5.32	8.577
	p	ns	ns	ns	ns	ns	**
LYM 10 ³ /mm ³	Control mean	2.397	4.363	3.635	2.933	3.37	3.5
	Depletion mean	2.397	3.84	3.17	3.09	4.147	6.56
	p	ns	ns	ns	ns	ns	*
LYM %	Control mean	75.47	86.68	78.55	64.3	85.6	81.87
	Depletion mean	75.47	82.05	61.9	81.43	79.4	76.73
	p	ns	ns	ns	ns	ns	ns
MON 10 ³ /mm ³	Control mean	0.07333	0.1125	0.165	0.3167	0.11	0.1433
	Depletion mean	0.07333	0.175	0.5033	0.11	0.2167	0.3967
	p	ns	ns	ns	ns	ns	ns
MON %	Control mean	3.767	3.375	3.85	7.733	4.333	4.433
	Depletion mean	3.767	4.5	9.467	4.433	5.5	5.5
	p	ns	ns	**	ns	ns	ns
GRA 10 ³ /mm ³	Control mean	0.79	0.6225	0.965	1.557	0.5033	0.7067
	Depletion mean	0.79	0.75	1.867	0.6467	0.96	1.62
	p	ns	ns	ns	ns	ns	ns
GRA %	Control mean	20.77	9.95	17.6	27.97	10.07	13.7
	Depletion mean	20.77	13.45	28.63	14.13	15.1	17.77
	p	ns	ns	ns	ns	ns	ns
EOS 10 ³ /mm ³	Control mean	0.03333	0.1025	0.235	0.05	0.1133	0.02667
	Depletion mean	0.03333	0.085	0.06667	0.06333	0.04667	0.16
	p	ns	ns	ns	ns	ns	ns
EOS %	Control mean	1.333	2.375	4.65	1.2	3	0.8333
	Depletion mean	1.333	1.8	1.367	3.233	1	2.067
	p	ns	ns	ns	ns	ns	ns

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