

***Gfi1b* controls integrin signaling-dependent cytoskeleton dynamics and organization in megakaryocytes**

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Methods

Mice

The generation of *Gfi1b*^{fl/fl} conditional knockout and *Gfi1b*^{EGFP} knock-in mice has been described previously.^{1,2} *Rosa*-Cre-ERT mice were obtained from The Jackson Laboratory (Bar Harbor, Maine, USA), strain B6;129-Gt(ROSA)26Sortm1(cre/ERT)Nat/J Stock Nb. 004847. *Pf4*-Cre transgenic mice used for megakaryocyte-specific ablation of *Gfi1b* expression were obtained from The Jackson Laboratory, strain C57BL/6-Tg(Pf4-cre)Q3Rsko/J Stock Nb. 008535. *Rosa*^{mT/mG} two colour Cre reporter mice were obtained from The Jackson Laboratory, strain Gt(ROSA)26Sortm4(ACTB-tdTomato,-EGFP)Luo/J Stock Nb 007576. All mice were backcrossed with C57BL/6 mice for at least 8 generations and were housed under specific pathogen-free conditions.

Activation of the inducible *Rosa*-Cre-ERT and assessment of the Cre efficiency

The activation of the Cre in the *Rosa*-Cre-ERT mice was achieved by intraperitoneal administration of a first dose (100 mg/kg) of tamoxifen (Sigma-Aldrich) at day 0 and a second dose (50 mg/kg) the day after as described previously.³ Mice were sacrificed for analysis either at day 1 to 8 after first injection (short term) or after 2 or 9 months (long term).

To assess the efficiency of both *Pf4*-cre and *Rosa*-cre-ERT using the *Rosa*^{mT/mG} two color Cre reporter mice, bone marrow cells were depleted of lin⁺ (CD3, B220, Gr1, CD11b - BD Biosciences) cells on an AutoMACS Pro (Miltenyi Biotec). The negative fraction was labeled with lineage cocktail (B220, CD3, CD4, CD8, Gr1, CD11b, NK1.1, Il7R, CD19 - all from BD Biosciences or eBioscience), PE/Cy5-streptavidin, PerCP/ef710-CD71 (eBioscience), APC-CD150, BV421-cKit, PE/Cy7-CD41, BV711-Sca1, APC/Cy7-CD16/32 (BioLegend), AF700-CD9 (Novus), BV510-CD48, BV605-Ter119 and PE/CF594-CD105 (BD Biosciences) antibodies. A minimum of 2 million events were acquired on a customized BD LSRFortessa (BD Biosciences) and GFP⁺ cells were recorded as deleted whereas Tomato⁺, GFP⁻ were recorded as non-deleted. Alternatively, cells were sorted on a BD FACSAria III sorter (BD Biosciences) for GMPs, PreMegEs, HSCs and Megakaryocytes and analyzed by PCR to assess the efficiency of the Cre as described previously.¹

Platelets

Circulating platelet counts were measured on an Advia 120 cell analyzer (Bayer) using the mouse archetype of multispecies software version 2.2.06. Mature and reticulated platelets were further analyzed by FACS. 3 μ l of EDTA-treated blood were incubated with PE-CD41 (BD Biosciences) and either AF647-CD61 (Biolegend) or APC-CD42b (ebioscience) antibodies, then counterstained with Thiazole Orange (Sigma-Aldrich; 1 μ g/ml PBS) and analyzed on a BD FACSCalibur (BD Biosciences). For platelet cytopins, EDTA-treated blood samples were diluted in PBS/EDTA and centrifuged at 160xg. Plasmas were re-centrifuged at 800xg and pellets suspended in PBS then centrifuged on slides using a Shandon Cytospin 4 (Thermo Scientific). Blood smears were prepared from fresh blood. Both blood smears and platelet cytopins were stained with May-Grünwald/Giemsa (MGG) as per standard procedure. To assess platelet half-life, mice were injected i.v. with 35 μ g/g of a 4 mg/ml Sulfo-NHS-biotin (Pierce/Fisher) solution in PBS. 10 μ l of blood were collected on a daily basis for six consecutive days starting at day 0, four hours after the injection. Blood samples were stained with PerCP/Cy5.5-streptavidin, PE-CD41 and AF647-CD61 (BD Biosciences) antibodies then analyzed on a BD FACSCalibur.

Megakaryocytes characterization *in vivo*

Quantification of megakaryocytes in bone marrow was done by FACS using biotinylated lineage cocktail (CD3, B220, Gr1, CD11b, Ter119), PerCP/Cy5.5-streptavidin, FITC-CD61; PE-CD41 and APC-cKit antibodies. Cells were acquired on a BD FACSCalibur and megakaryocytes were identified as lin^- , $cKit^+$, $CD41^+$ and $CD61^+$.

To assess megakaryocyte ploidy, cells were lineage depleted as described earlier and put in culture under 5% CO_2 in DMEM/10%FBS supplemented with 1% Hoechst 33342 (Life Technology) for 1 hour. Ten minutes before the end of the incubation, a second lineage-antibody cocktail (CD3, B220, Gr1, CD11b, Ter119, CD19, II7R, NK1.1) was added to the culture. Cells were harvested and washed in DMEM/10%FBS and incubated with PerCP/Cy5.5-streptavidin, AF700-CD9, PE/Cy7-CD41 (BioLegend), PE-CD61 (BD Biosciences) and APC/Cy7-cKit (eBioscience) antibodies. A minimum of two million events was acquired on a BD LSRFortessa and megakaryocytes were defined as Lin^- , $CD9^{high}$, $CD41^{high}$, $CD61^{high}$ $cKit^+$ and ploidy was evaluated by Hoechst fluorescence intensity.

Megakaryocytic progenitors were assessed by acetylcholinesterase staining using a murine CFU-Mk Assay on MegaCult-C (StemCell Technology) as per manufacturer's and standard protocols.⁴

Megakaryocytes characterization *in vitro*

To assess megakaryocyte motility *in vitro*, cells were prepared as above but were plated on fibrinogen-, fibronectin- or collagen-coated coverslips and allowed to attached for 1 hour then put upside-down on a Dunn chamber containing StemSpan SFEM/2.6% FBS/20 ng/ml mSCF/50 ng/ml rmTPO then followed by time-lapse microscopy over a period of 6 to 9 hours in 5% CO₂ at 37°C.

To assess megakaryocytes *in vitro* based on their maturity, bone marrow cells from *Pf4-Cre x Gfi1b^{flox} x Rosa-Tomato* were lineage depleted using the MojoSorttm magnetic separation system (Biolegend) using a B220, Mac-1, Gr-1, CD16/21 antibody cocktail and put in culture in StemSpan SFEM (StemCell Technologies) supplemented with 2.6% FBS, 1% L-Glutamine and SCF (20 ng/ml) then cultured two days at 37°C and 5% CO₂. Cells were transferred into fresh media containing TPO (50 ng/ml) and cultures for 4 more days. 1 hour prior of harvesting, Hoechst 33342 was added at a final concentration of 1%. Cells were stained with APC-conjugated cKit antibody and sorted at a low flow rate on a FACSAria III cell sorter using a 130 µm nozzle for cells that are GFP⁺ and based on their ploidy (2N and 8N+) on the Hoechst channel. Cells were recovered in RPMI/10% FBS and analyzed for the spreading, motility and cytoskeleton as describe earlier.

***In vitro* proplatelet formation assay**

Total bone marrow cells were harvested and positively selected on an AutoMACS using a biotin-labelled anti-CD41 antibody and anti-biotin magnetic beads, and cultured in DMEM/10% FBS + human recombinant TPO (50 µM) for 5 days either in presence of ligand (fibronectin) or without ligand.⁵ Cells were then split into 96-wells plate in presence of inhibitors and allowed to grow for 12 hours, then observed under microscope under a 5% CO₂ atmosphere at 37°C.

Microscopy

May-Grünwald/Giemsa-stained blood smear were recorded with a MicroPublisher 3.3 RTV color camera (QImaging) mounted on an Axiophot Microscope (Zeiss) using light transmission and a 100X oil-immersion Plan-NeoFluar lens (Zeiss). The images were acquired with the Northern Eclipse v7.0 software (Empix) and flat-field corrected using ImageJ v1.46r (NIH).

Images of Acetylcholinesterase stained CFU-Mks were recorded with a high resolution DP72 color camera (Olympus) mounted on a DM4000B Microscope (Leica) using light transmission and the CellSens Entry v1.8.1 software (Olympus).

Immunofluorescence imaging of Mks in vitro were recorded either with a DCX-950P DP72 camera (Sony) mounted on a Leitz DMRB Microscope (Leica) using fluorescence transmission and the Matlab software (MathWorks) or by taking z-stacked images on a LSM 700 confocal microscope (Zeiss) and the Zen software (Zeiss). 3D reconstitution of the images was done with ImageJ v1.46r (NIH) using the 3D viewer plugin.

For Mk motility assessment by time-lapse microscopy, cells were acquired either by dark field or phase contrast using a DMIRE2 inverted microscope (Leica) mounted with an ORCA-ERA 1394 camera (Mamamastu) in a CO₂/temperature-controlled chamber and recorded using the Volocity v5.5.1 software (PerkinElmer). Movie was analyzed using the Volocity v6.0 software and cellular velocity measured using ImageJ v1.46r using the Chemotaxis Tool plugin (ibidi) and the Manual Tracking plugin (Institut Curie).

Western blot of megakaryocytes

For western blot analysis of Mk proteins, Mks were cultured in vitro and enriched on a BSA gradient as described in the Material and methods section of the main text. Mk total proteins were isolated using Nonidet P-40 lysis buffer containing the protease inhibitors cocktail (Roche) and phosphatase inhibitor cocktail (Sigma). Total protein was quantified using the Quick Start™ Bradford Protein Assay (Bio-Rad) to load equal amount of protein for each sample on a 10 % SDS-PAGE and transferred to PVDF Immobilon-P membrane (Millipore) at 4° C. After blocking in PBS/0.1% Tween 20 and 5% milk for 1 hour at room temperature, membranes were incubated with the primary antibodies for 1 hour. They were then washed in PBS/0.1% Tween 20 and incubated with the appropriate HRP-conjugated secondary antibody for 1 hour. Bands were visualized using the SuperSignal West Dura kit (Thermo Scientific) according to the

manufacturer's instructions. The primary antibodies used were: HPRT (EPR5298; Abcam), PAK4 (Cell Signaling), PAK4-P (EPR2471(2); Abcam), Itga2b/CD41 (MAB4118, R&D Systems), Integrin β_3 (D7X3P; Cell Signaling), alpha tubulin (TU-02; Santa Cruz), acetylated alpha tubulin (6-11B-1; Santa Cruz), beta I tubulin (SAP.4G5; Abcam) and the secondary antibodies used: chicken anti-rabbit HRP (sc-2963; Santa Cruz), chicken anti-mouse HRP (sc-2962; Santa Cruz) and chicken anti-goat HRP (sc-2961; Santa Cruz).

Inhibitors

The inhibitors used were: PF-3758309 (10 μ M; MedChem), PF-573228 (10 μ M; Selleckchem), Y-27632 (10 μ M; Selleckchem), NSC-23766 (10 μ M; Tocris), ML-141 (10 μ M; Tocris), FRAX486 (10 μ M; Tocris), W56 and F56 peptides (200 μ M; Tocris) and (-)-Blebbistatin (25 μ M; Selleckchem).

Gene expression profiling by RNA-Seq analysis

To prepare RNA from megakaryocytes for RNA-Seq, the bone marrow from the 2 tibiae, 2 femora and 2 humeri (from 3 *Pf4-cre Gfilb^{fl/fl}* and 6 *Pf4-cre Gfilb^{w/fl}* mice; as well as from 4 *Rosa-Cre-ERT Gfilb^{fl/fl}* and 6 *Rosa-Cre-ERT Gfilb^{w/fl}* treated with tamoxifen 2 weeks prior the experiment) was harvested in RPMI/10% FBS and pooled prior to lysis in Red Blood Cell Lysis buffer (Sigma) for 5 minutes. Cells were incubated with a lineage antibody cocktail (B220, CD3, CD4, CD8, Gr1, CD11b, NK1.1, Il7R, CD19) and then labeled with PE/Cy7-conjugated streptavidin, FITC-conjugated anti-CD41, PE-conjugated anti-CD61 and APC-conjugated anti-cKit antibodies. $\text{Lin}^- \text{cKit}^+ \text{CD41}^+ \text{CD61}^+$ (PF4) or $\text{Lin}^- \text{cKit}^+ \text{CD41}^+ \text{CD9}^+$ (ROSA) megakaryocytes were sorted on a MoFlo cell sorter. RNA was extracted using a MagMax-96 Total RNA Isolation kit (Ambion) and quality-checked with a RNA 6000 Pico kit (Agilent). RNA-Seq libraries were prepared using the Illumina TruSeq Stranded mRNA Kit according to the manufacturer's instructions, and sequenced using the TruSeq PE Clusterkit v3-cBot-HS on an Illumina HiSeq 2000 system. Approximately 100 million pair-ended, 50bp sequencing reads were aligned to the mm10 genome using Tophat v2.0.10. Reads were processed with Samtools and mapped to Ensembl genes using HTSeq. Differential expression was tested using the DESeq R package (R Coding Team). A genome coverage file was generated and scaled to RPM using Bedtools. RNA-Seq results were deposited on the GEO Database (accession number GSE71310).

Functional Analysis

The DAVID online tool was used to identify over-represented biological functions among genes significantly differentially expressed between *Gfilb* knockout vs. control samples.⁶ The enrichment of selected biological functions of interest was also analyzed using the GSEA tool.⁷ Read counts for Ensembl genes from HTSeq were used and enrichment calculated using 1000 Gene Set permutations.

Statistical methods.

Values are expressed as means \pm standard deviations (SD) or as medians when the distribution did not pass the D'Agostino and Pearson omnibus normality test. Unpaired two-sample Student's t test or the Mann-Whitney test was used for statistical analysis when two groups were compared, an analysis of variance (ANOVA) and Tukey's multiple comparison test or the Kruskal-Wallis test and Dunn's posttest were used when three or more groups were compared. The χ^2 test was used to assess the number of cells per CFU-Mks. In all statistical tests, a $P < 0.05$ was considered significant.

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Supplementary Figure 1. Expansion of Mks in the *Rosa-Cre-ERT Gfi1b^{fl/fl}* mouse.

(A) Representative FACS plots showing the rapid expansion of Mks upon tamoxifen treatment of *Rosa-Cre-ERT Gfi1b^{fl/fl}* knockout mice.

(B) Representative image of MGG-stained collagen-based colony forming assay obtained from lineage-negative cells purified from *Rosa-Cre-ERT Gfi1b^{fl/fl}* knockout and *Rosa-Cre-ERT Gfi1b^{wt/fl}* controls two months after tamoxifen administration (LT: long term) showing typical non-Mk CFUs, multipotent CFU-GEMMs, CFU-Mks and mixed-CFUs (at least one CFU-Mks in the vicinity of a non-Mk CFU).

(C) A typical CFU-Mk from *Rosa-Cre-ERT Gfi1b^{fl/fl}* knockout mice presented at a higher magnification, clearly showing the presence of several small cells exhibiting an immature phenotype despite staining positive for the acetylcholinesterase activity.

Supplementary Figure 2. Ploidy defects in *Gfilb*-null Mks.

(A) Representative FACS plots and histograms of Hoechst-stained lineage-depleted bone marrow showing the gating strategy to identify the most phenotypically mature Mks.

The histograms show the Hoechst profile of CD41⁻ CD9⁻ non-Mk cells (shaded grey) to define the 2N and 4N populations and mature Mks (solid black line) with gates delineating cells with different ploidy.

(B) Representative overlaid FSC/SSC backgating of 8N, 16N and 32N Mks from both *Pf4*-Cre-driven and *Rosa*-Cre-ERT-driven conditional *Gfilb* knockouts for the forward scatter (FSC) and side scatter (SSC) quantitation shown in Figure 3F.

Supplementary Figure 3. Defects of *Gfi1b*-null Mks in the *Pf4*-cre mice are not reminiscent of an immature state.

(A) Strategy to isolate untouched and viable mature (8N+) and immature (2N) megakaryocytes based on their DNA content for functional assays. Cells from the *Pf4-Cre Gfi1b^{fl/fl} or wt/fl Rosa^{mT/mG}* mice were expanded *in vitro* then sorted for GFP (megakaryocytes) and their ploidy following staining with Hoechst that allows cells to be put back into culture⁸. In the Hoechst histogram, Megakaryocytes are shown in blue (the gray area represents non-megakaryocytes) to identify 2N and 4N cells.

(B) Roundness index of immature (2N) and mature polyploid (8N+) megakaryocytes that were allowed to spread on fibronectin for 5 hours. (WT) *Gfi1b^{wt/wt}* controls (2N $n = 24$; 8N+ $n = 88$) and (KO) *Gfi1b^{fl/fl}* knockout (2N $n = 20$; 8N+ $n = 88$). Kruskal–Wallis test: $P < 0.0001$. The *post hoc* Dunn's multiple comparison test identifies statistical significance between control and knockout cells but no difference between 2N and 8N+ cells for either the controls or the knockout.

(C) Low ploidy immature (Ctrl: $n = 30$; KO: $n = 31$) and high ploidy mature (Ctrl: $n = 32$; KO: $n = 35$) megakaryocytes were tracked by time-lapse microscopy over a period of 10 hours and their velocity was measured as in Figure 5 E-G. Results are presented as mean velocity \pm SD in μm per minute.

(D) Analysis of a cross section passing through the center of 30 Mks ($n = 30$) prepared as in (A) (*Pf4-Cre Gfi1b^{flx/flx}* (KO) and *Pf4-Cre Gfi1b^{wt/wt}* (WT) mice. The diameter was subdivided in 100 sections and the AF555 intensity was measured as in Figure 6E.

Supplementary Figure 4. GFI1B-deficient Mk-derived platelets exhibit an increase of several surface integrin receptors.

(A) Representative FACS histograms of integrins measured at the surface of circulating platelets from *Pf4-Cre Gfi1b^{fl/fl}* knockout mice (red line) and *Pf4-Cre Gfi1b^{w^t/fl}* controls (blue line).

(B) Mean fluorescence intensity (MFI) of integrin receptors present on the surface of platelets analyzed in (A) from *Pf4-Cre Gfi1b^{fl/fl}* knockout (KO) and *Pf4-Cre Gfi1b^{w^t/fl}* control (Ctrl) mice. Results are presented as mean \pm SD ($n = 2$ for both genotypes).

(C) Heat map of surface integrin receptors. The first column (Ligands) of this heat map presents the different ligands associated with integrin signaling. The second column (Receptor) identifies the different integrin receptors specific to the given ligands based on their alpha chain (α) and beta chain (β). The third column (Protein) indicate the relative fold change in surface presentation for all corresponding integrin subunits (first is the α -chain; second is β -chain) in the knockout compared to the controls using heat map colour as shown below the table; fold change from 2 fold decrease (-2) to two fold increase (2). The fourth column (Inferred surface receptor) presents the inferred fold change of each whole receptor based on the change observed in the limiting subunit (α -chain or β -chain). The last column (inferred ligand-specific receptor units) represents the inferred fold change in all receptor specific to the given ligand weighted based on their relative expression (i.e. Making the inference that a receptor of a ligand that is expressed at higher level than another receptor for the same ligand will have a greater impact on the global fold change of the amount of receptors for this ligand than the second one).

Supplementary Figure 5. Integrin signature at the surface of *Gfi1b* knockout and control Mks .

Megakaryocytes ($\text{Lin}^- \text{cKit}^+ \text{CD41}^+ \text{CD61}^+$) from *Pf4-Cre Gfi1b^{flox/flox}* (KO) and *Pf4-Cre Gfi1b^{wt/wt}* (WT) from the experiment presented in Figure 6F where stained for integrins ITGAV, ITGAL, ITGA2, ITGA4, ITGA6, ITGB2, ITGB5, ITGB7 and ITGB1. Because cell surface integrin expression is not homogenous, cells were divided into low (blue), medium (red) and high (green) integrin expressing subgroups and the mean fluorescence intensity (MFI; bar graphs) was measured for each subgroup. The proportion of cells expressing either low, medium or high levels of integrin is presented for each integrin in a pie chart. The integrin α_{Iib} (ITGA2b; CD41) and the integrin β_3 (ITGB3; CD61) are not presented as they are used as markers to identify megakaryocytes and are therefore all integrin-high by definition.

Supplementary Figure 6. Gene set enrichment analyses (GSEAs) from the RNA-Seq analysis performed on *Pf4*-Cre or *Rosa*-Cre-ERT *Gfi1b*^{fl/fl} and *Gfi1b*^{wt/fl} Mks.

(A) Correlation between the gene expression profiles from the RNA-Seq on *Gfi1b*-null Mks (*Pf4*-Cre *Gfi1b*^{fl/fl}) presented in this study and the microarray on MKPs published by Foudi et al⁹. The Pearson correlation coefficient is shown. The P value was determined using a permutation with 1 million re-samplings.

(B) List of the top 10 most deregulated genes in *Pf4*-Cre- and *Rosa*-Cre-ERT-driven *Gfi1b* knockout Mks.

(C) Venn diagram comparing the combined 500 most overexpressed genes in Mks from *Pf4*-Cre *Gfi1b*^{fl/fl} and *Rosa*-Cre-ERT *Gfi1b*^{fl/fl} mice relative to their respective controls showing the proportion of shared genes between the two models and the proportion of genes that are specifically highly overexpressed in each model. An analysis of the two gene sets performed through the Database for Annotation, Visualization and Integrated Discovery (DAVID) software revealed pathways that are prominently affected in Mks from *Pf4*-cre *Gfi1b*^{fl/fl} mice (left) or the *Rosa*-Cre-ERT *Gfi1b*^{fl/fl} animals (right).

Supplementary Figure 7. Gene set enrichment analyses (GSEAs) from the RNA-Seq analysis performed on *Pf4-Cre* or *Rosa-Cre-ERT* *Gfi1b*^{fl/fl} and *Gfi1b*^{wt/fl} Mks.

(A-B) GSEAs of the microtubule destabilization (A) stability and microtubule stability (B) pathways. Only the top over-expressed and under-expressed genes are identified on the dot plot.

(C-D) GSEAs of the KEGG hematopoietic cell lineage gene set (C) and of a megakaryocytic differentiation gene set (D) based on data published by Lim et al¹⁰ for both *Pf4-Cre*- and *Rosa-Cre-ERT*-driven *Gfi1b* knockouts. *Gfi1b* mRNA expression (yellow dot) itself is up-regulated, which is expected since GFI1B auto-represses its own transcription. Consequently, in absence of GFI1B, transcription from the locus is enhanced, but a protein is not produced since exons 2-4 including the translation initiation codon are deleted (see Figure S1C and original description of the *Gfi1b* knockout in supplementary reference 1).

Normalized Enrichment Scores (NES) and Nominal *p*-values (*P*) are given for each GSEA plot.

Supplementary Figure 8. Full and partial rescue of the *Gfi1*-null phenotype by small molecule inhibitors in vitro.

(A) Representative immunofluorescence microscopy of *Pf4*-Cre *Gfi1b*^{wt/fl} (Ctrl) and *Pf4*-Cre *Gfi1b*^{fl/fl} (KO) Mks that were allowed to spread on fibronectin for 3 hours in the absence (untreated) or presence of different inhibitory small molecules: PF573288 (FAK inhibitor); PF3758309 (PAK inhibitor); Y27632 (ROCK inhibitor); and blebbistatin (myosin II inhibitor). Cells were stained for the nucleus (DAPI), integrin α_{Iib} (CD41-FITC) and F-actin (phalloidin-AF555) and Mks identified as being CD41 positive.

(B) Representative immunofluorescence microscopy of *Pf4*-Cre *Gfi1b*^{wt/fl} (Ctrl) and *Pf4*-Cre *Gfi1b*^{fl/fl} (KO) Mks that were allowed to spread on fibronectin for 3 hours in presence of different inhibitory small molecules: PF3758309 and FRAX486 (PAK inhibitors); ML141 (CDC42 inhibitor); and NSC23766 and W56 peptide (RAC1 inhibitors). Cells were stained for the nucleus (DAPI), integrin α_{Iib} (CD41-FITC) and β -tubulin (AF555) and Mks identified as being CD41 positive.

Supplementary Video 1. Phase contrast time-lapse microscopy of a control Mk.

Primary wild-type Mk cultures were put on fibrinogen and recorded by phase-contrast microscopy at 37°C/5% CO₂ for several hours. The video reveals Mks with a dynamic behavior and good reactivity to the fibronectin-covered slides.

Supplementary Video 2. Phase contrast time-lapse microscopy of a *Gfilb* knockout Mk.

Primary knockout Mk cultures isolated from *Pf4-Cre Gfilb^{fl/fl}* were put on fibrinogen and recorded by phase-contrast microscopy at 37°C/5% CO₂ for several hours. The video reveals Mks with a poor reactivity to the fibronectin-covered slides and an overall stillness punctuated with the formation of bleb-like structures, suggesting an incapacity to form pseudopodia.

Supplementary Video 3. High motility of control Mks put on fibrinogen.

The individual path travelled by 11 control Mks put on fibrinogen during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

Supplementary Video 4. Low motility of *Gfilb* knockout Mks put on fibrinogen.

The individual path travelled by 16 *Gfilb* knockout Mks put on fibrinogen during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

Supplementary Video 5. High motility of control Mks put on fibronectin.

The individual path travelled by 25 control Mks put on fibronectin during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

Supplementary Video 6. Low motility of *Gfilb* knockout Mks put on fibronectin.

The individual path travelled by 9 *Gfilb* knockout Mks put on fibronectin during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

Supplementary Video 7. High motility of control Mks put on collagen.

The individual path travelled by 6 control Mks put on collagen during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

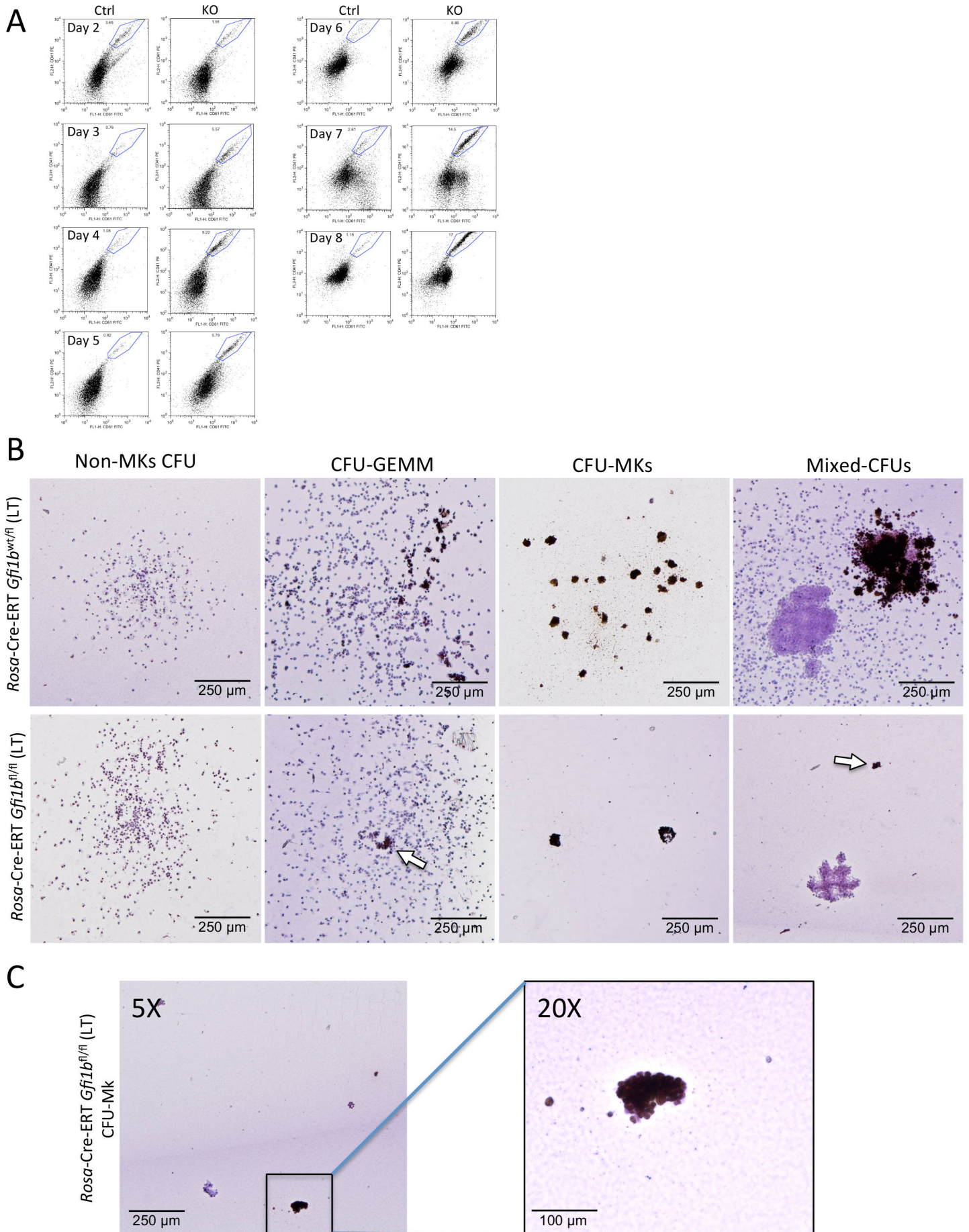
Supplementary Video 8. Impaired motility of *Gfilb* knockout Mks put on collagen.

The individual path travelled by 5 *Gfilb* knockout Mks put on collagen during the time-lapse experiment is shown. The path of a cell turns red as soon as the cell dies or gets out of frame.

Supplementary Video 9. A 360° 3D view of the nucleus of a *Gfilb* knockout Mk with the tubulin restrained in the center.

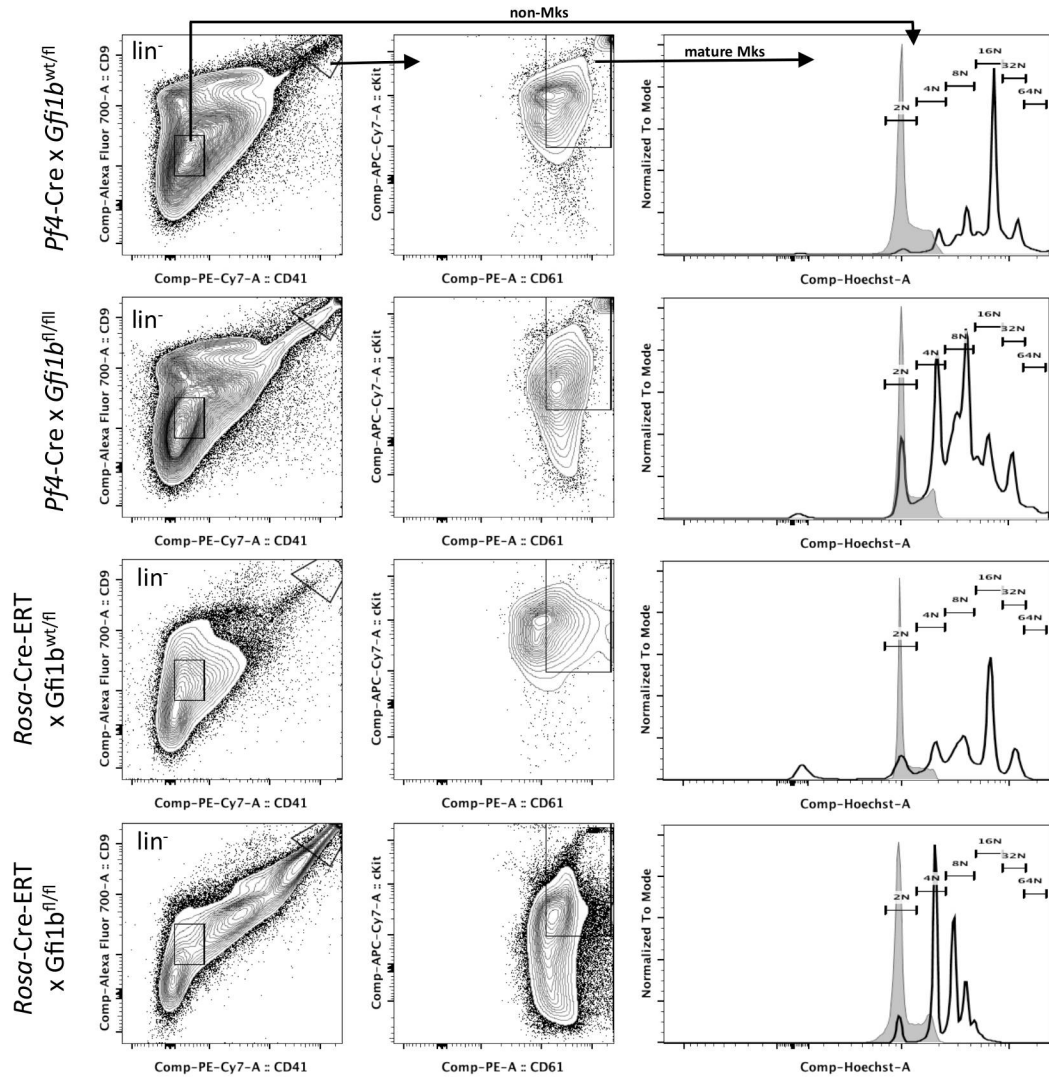
Z-staked images taken by confocal microscopy were assembled to reconstitute a 3D view of the nucleus of a *Gfilb* knockout Mk stained with a AF555-conjugated anti- β -tubulin antibody. We can clearly see that almost the totality of the tubulin network (red) is localized in a pocket at the center of the staghorn-shaped nucleus (blue) of the Mk.

Supplemental Figure 1 (Beauchemin *et al*)

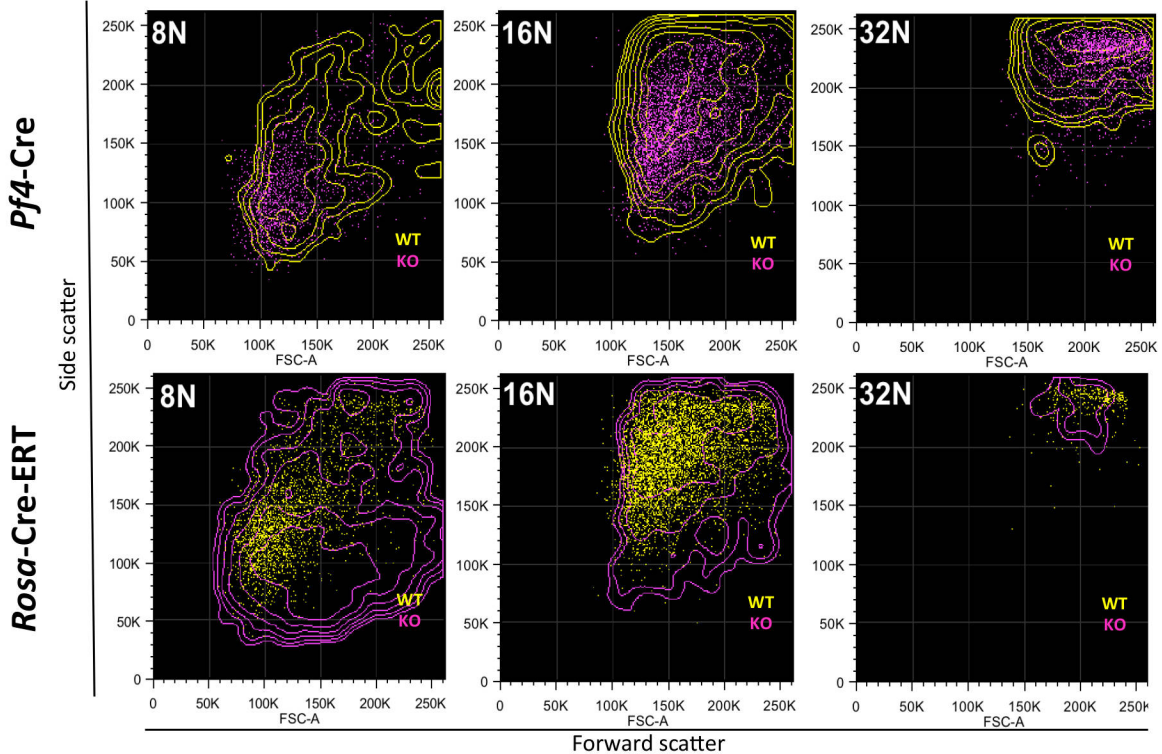


Supplemental Figure 2 (Beauchemin *et al*)

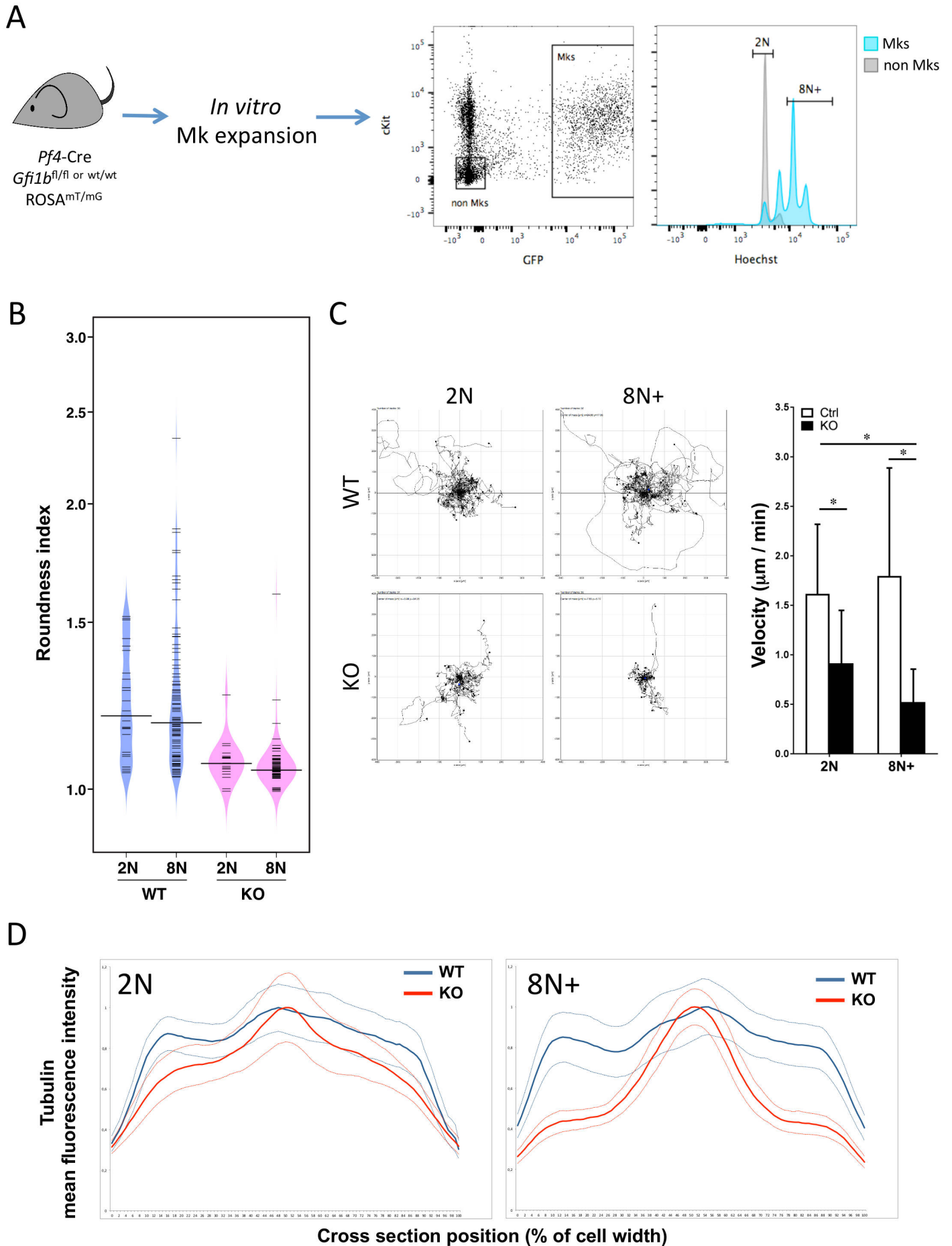
A



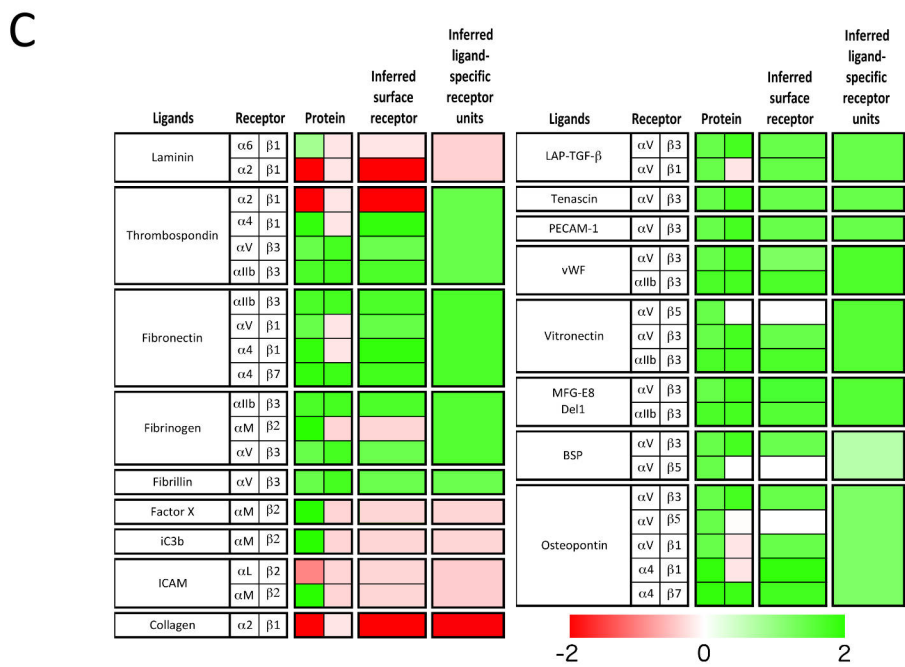
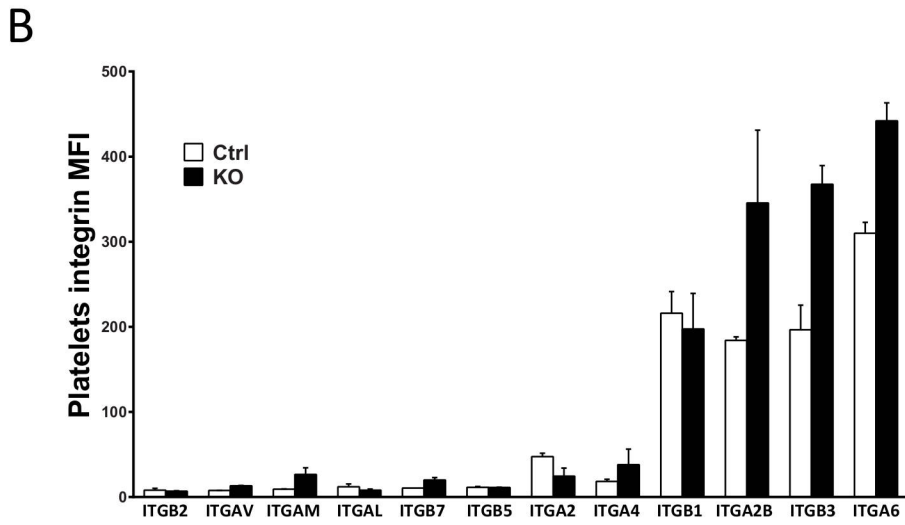
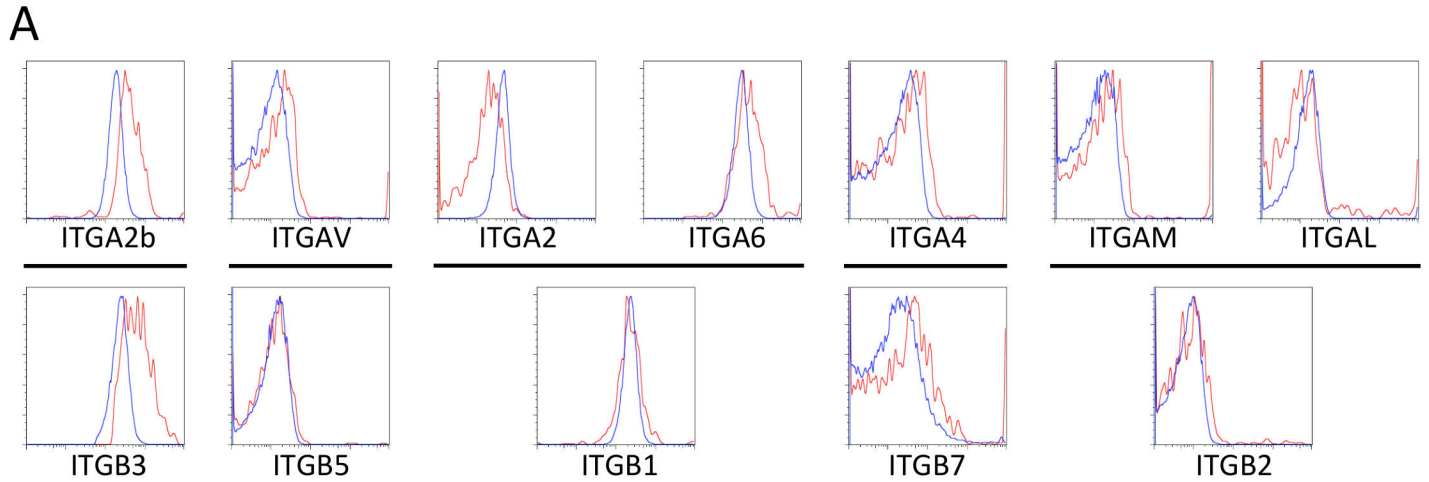
B



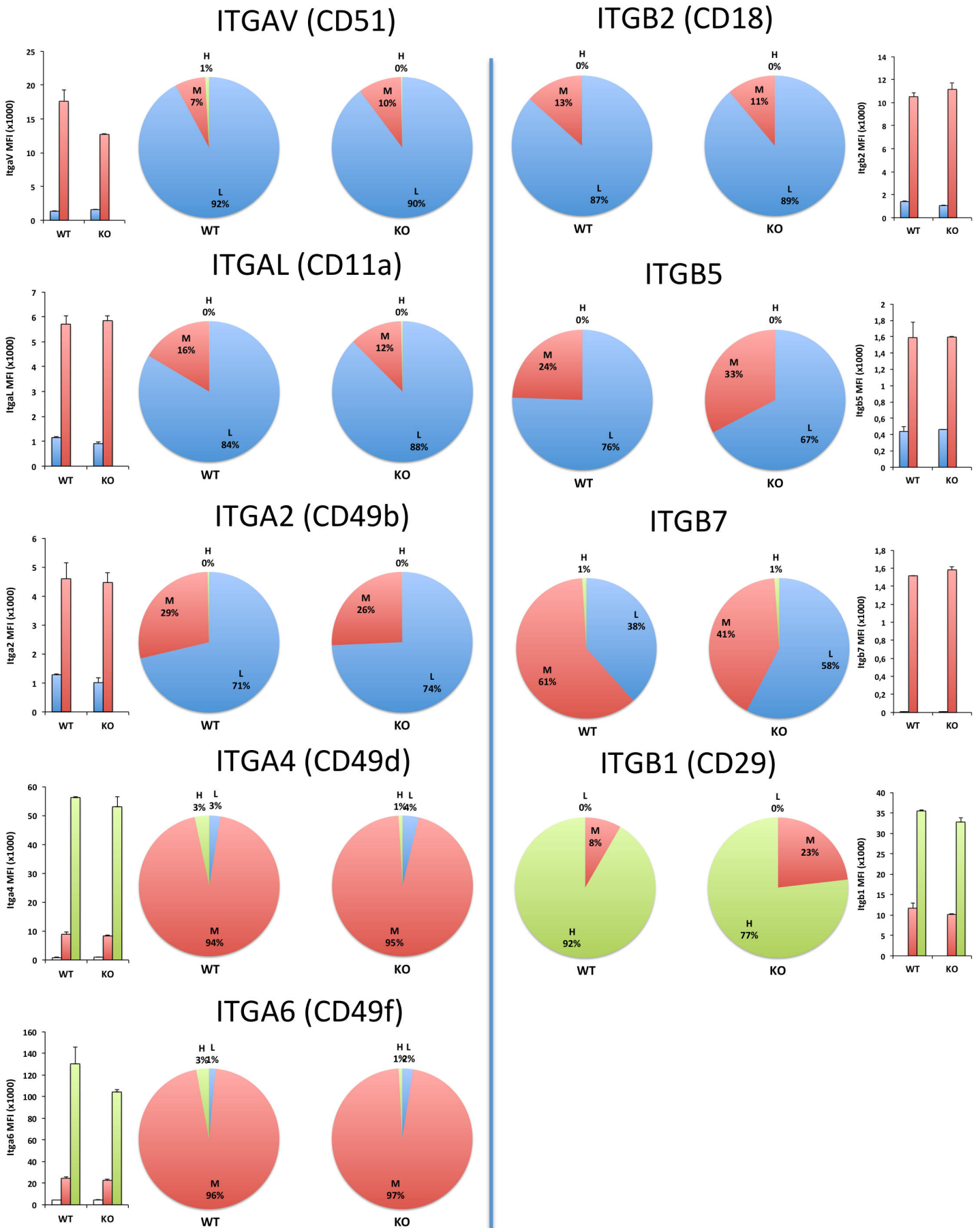
Supplemental Figure 3 (Beauchemin *et al*)



Supplemental Figure 4 (Beauchemin *et al*)

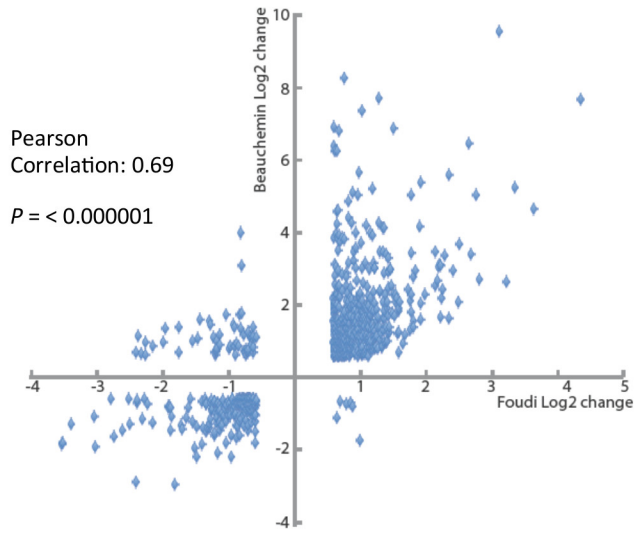


Supplemental Figure 5 (Beauchemin *et al*)



Supplemental Figure 6 (Beauchemin *et al*)

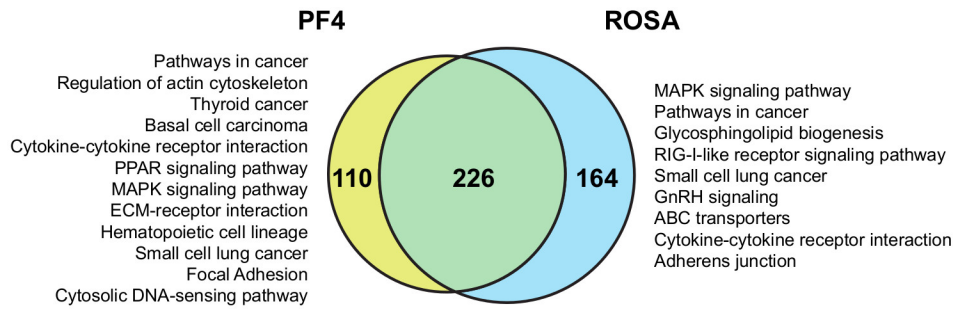
A Correlation between Beauchemin & Foudi gene expression profiles in Pre-Megakaryocytes

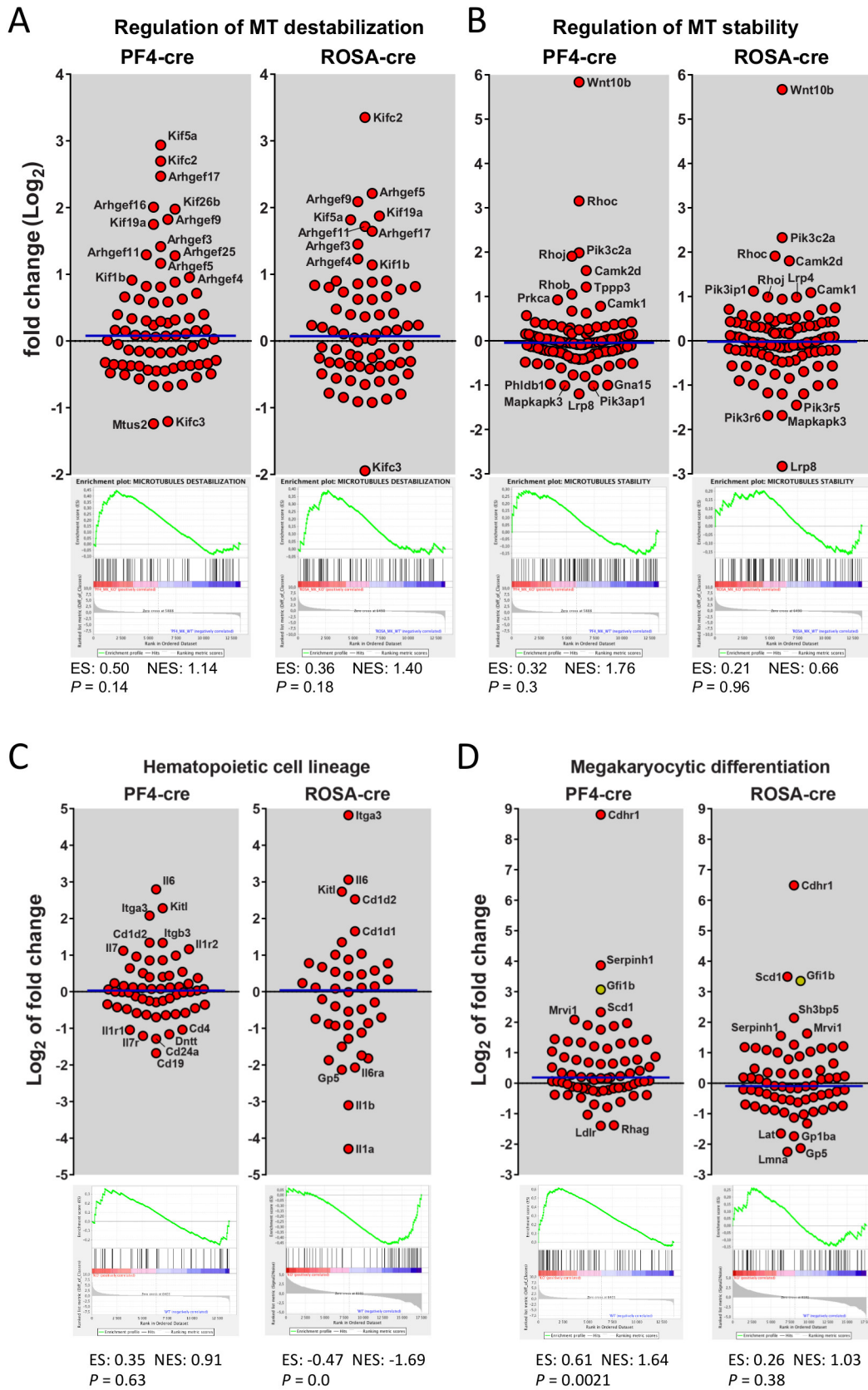


B

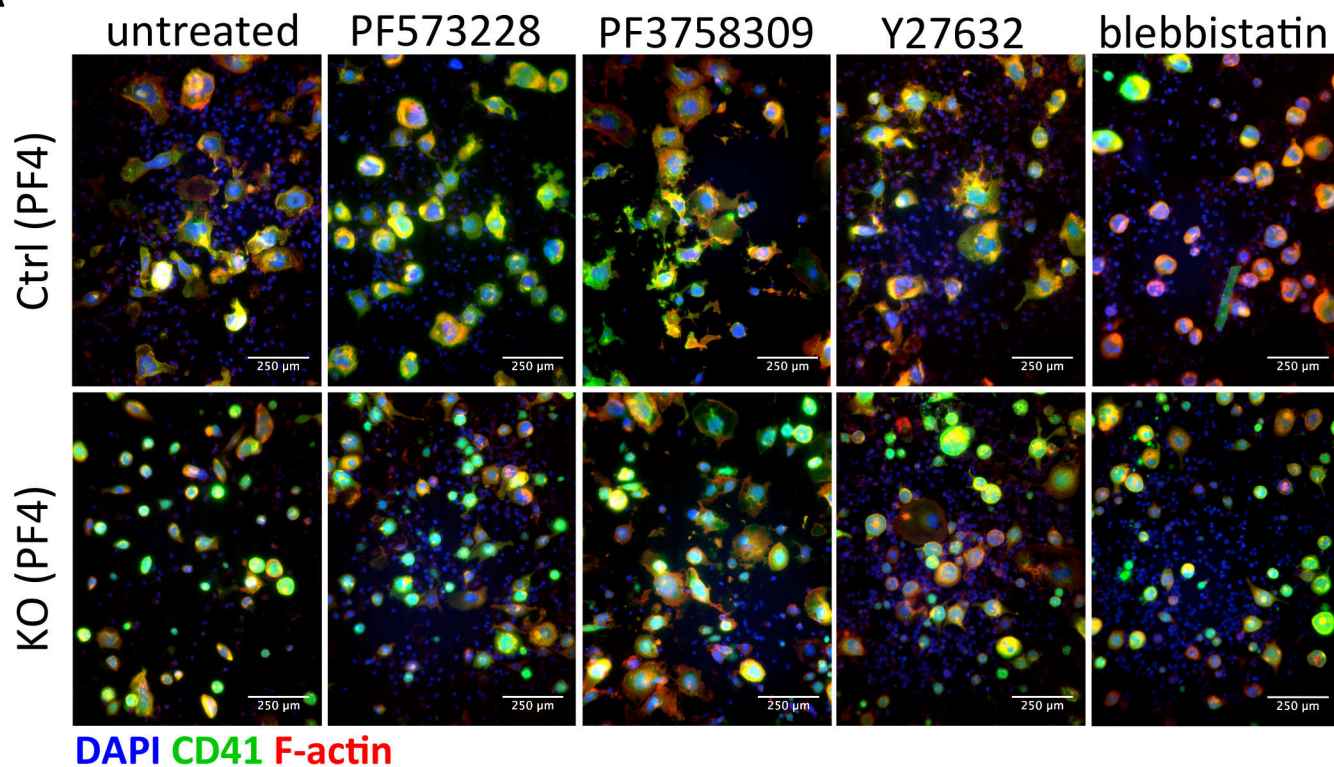
| <i>Pf4-Cre</i> | | | <i>Rosa-Cre-ERT</i> | | |
|-----------------|-------------|-------------|---------------------|-------------|-------------|
| Gene | Fold change | Adj P value | Gene | Fold change | Adj P value |
| <i>Tmem215</i> | 749.86 | 3.07E-55 | <i>Tmem215</i> | 556.53 | 2.51E-12 |
| <i>Plagl1</i> | 203.06 | 1.27E-39 | <i>Procr</i> | 190.58 | 4.13E-09 |
| <i>Cacna1e</i> | 131.45 | 1.23E-34 | <i>Plagl1</i> | 159.75 | 4.13E-09 |
| <i>Ctnna3</i> | 92.34 | 2.32E-24 | <i>Lamb2</i> | 133.37 | 3.36E-07 |
| <i>Hist1h4m</i> | 0.003 | 2.32E-24 | <i>Cnrip1</i> | 72.44 | 1.10E-05 |
| <i>Cdhr1</i> | 448.19 | 8.36E-24 | <i>Fbn1</i> | 65.14 | 1.10E-05 |
| <i>Rbm20</i> | 34.65 | 3.69E-23 | <i>Cacna1e</i> | 62.60 | 4.56E-05 |
| <i>Adamts9</i> | 307.27 | 7.35E-22 | <i>Ablim1</i> | 38.41 | 6.76E-05 |
| <i>Rasgrp3</i> | 86.62 | 8.08E-22 | <i>Chl1</i> | 36.99 | 7.23E-05 |
| <i>Procr</i> | 47.73 | 2.48E-20 | <i>Laptm4b</i> | 42.87 | 0.00011 |

C





A



B

