Inflammation regulates long non-coding RNA-PTTG1-1:1 in myeloid leukemia

Sébastien Chateauvieux,[1,2] Anthoula Gaigneaux,[1°] Déborah Gérard,[1] Marion Orsini,[1] Franck Morceau,[1] Barbora Orlikova-Boyer,[1,2] Thomas Farge,[3,4] Christian Récher,[3,4,5] Jean-Emmanuel Sarry,[3,4] Mario Dicato[1] and Marc Diederich[2]

°Current address: University of Luxembourg, Faculty of Science, Technology and Communication, Life Science Research Unit, Belvaux, Luxemburg.

[1]Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, Luxembourg, Luxembourg; [2]College of Pharmacy, Seoul National University, Gwanak-gu, Seoul, Korea; [3]Cancer Research Center of Toulouse, UMR 1037 INSERM/ Université Toulouse III-Paul Sabatier, Toulouse, France; [4]Université Toulouse III Paul Sabatier, Toulouse, France and [5]Service d'Hématologie, Centre Hospitalier Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopôle, Toulouse, France

Correspondence: MARC DIEDERICH - marcdiederich@snu.ac.kr

doi:10.3324/haematol.2019.217281

Supplementary data

Inflammation regulates long non-coding RNA-PTTG1-1:1 in myeloid leukemia

Sébastien Chateauvieux[1,2], Anthoula Gaigneaux[1*], Déborah Gérard[1], Marion Orsini[1], Franck Morceau[1], Barbora Orlikova-Boyer[1,2], Thomas Farge[3,4], Christian Récher[3,4,5], Jean-Emmanuel Sarry[3,4], Mario Dicato[1] and Marc Diederich[2]

[1] Laboratoire de Biologie Moléculaire et Cellulaire du Cancer, Hôpital Kirchberg, 9, rue Edward Steichen, 2540 Luxembourg, Luxemburg;

[2] College of Pharmacy, Seoul National University, 1 Gwanak-ro, Gwanak-gu, Seoul 08826, Korea;

[3] Cancer Research Center of Toulouse, UMR 1037 INSERM/ Université Toulouse III-Paul Sabatier, 2 avenue Hubert Curien, Oncopôle, 31037 Toulouse, France;

[4] Université Toulouse III Paul Sabatier, Toulouse, France;

[5] Service d'Hématologie, Centre Hospitalier Universitaire de Toulouse, Institut Universitaire du Cancer de Toulouse Oncopôle, 31059 Toulouse, France;

Materials and methods

Cell culture and treatment

Leukemia cell lines were purchased from Deutsche Sammlung für Mikroorganismen und Zellkulturen (DSZM; Braunschweig, Germany) and cultured in RPMI medium (Lonza, Basel, Switzerland) supplemented with 10% (v/v) fetal calf serum (FBS) (Biowest, Nuaille, France) and 1% (v/v) antibiotic–antimycotic (Lonza, Basel, Switzerland) at 37 °C and 5% of CO_2. TF-1 cells require the presence of growth factors like granulocyte-macrophage colony-stimulating factor (GM-CSF) to survive or erythropoietin (EPO) to differentiate. TF-1 cells were grown in the presence of 5 ng/mL human recombinant GM-CSF (Tebu-Bio, PeproTech, Boechout, Belgium) or 10 U/mL human recombinant EPO (Epoetin Beta, NeoRecormon, Roche Pharma AG, Grenzach-Whylen, Germany). Human umbilical cord blood was obtained from the Clinique Bohler, Luxembourg with a written informed consent in agreement with the National Committee of Research Ethics in Luxembourg. The blood was collected in heparinized tubes. The mononucleated cell fraction was isolated using Ficoll™ (GE Healthcare, Roosendaal, The Netherlands) density gradient medium. $CD34^+$ hematopoietic stem cells (HSPC) cells were selected using magnetic cell sorting following the manufacturer's instructions (MACS Miltenyi, Utrecht, The Netherlands). The average purity of $CD34^+$ cells was +- 96%. After isolation, cells were cultured in serum-free culture medium for cell expansion (Stem Cell II, Sigma–Aldrich, Bornem, Belgium), to which a cytokine cocktail containing interleukin (IL)-3 (Reliatech, Wolfenbüttel, Germany) and stem cell factor (SCF) (Reliatech) was added. IL-3 (10 ng/mL) was added together with SCF (10 ng/mL) for 3 days following $CD34^+$ cell enrichment.

Compounds for cell treatments: TNFα (20 ng/mL); EPO (TF-1 cells: 10 U/ml; HSPC: 2 U/mL); IL-1β (10 ng/mL); lipopolysaccharide (LPS) (100 ng/mL); and polyinosinic-polycytidylic acid (Poly(I:C)) (25 μg/mL), goniothalamin (GTN) (20 μM), cycloheximide (CHX) (5 μM), MG132 (5 nM). Pretreatment with inhibitors (GTN, MG132 or CHX) were performed 1 h before TNFα treatments. Treatment of transfected cells were performed 24 h after transfection. For cell viability and Mitotracker Red (Molecular Probes/Invitrogen, Thermo Fisher Scientific, Belgium) assays performed on transfected cells, cells were transfected by Si623+Gap01, SiCT+GapCT, pl-PTTG1-1:1 or empty pUC19 and medium was changed after 24 h. Cells were then treated at 66 h with TNFα during 6 h for the 72 h treatment, and at 72 h for 24 h for the 96 h treatment.

Microarray analysis

A custom 2-color Agilent array (Prof. Jo Vandesompele, Center for Medical Genetics, Ghent, Belgium; Agilent-039714 lincRNA SurePrint G3 Human GE 8x60K Microarray PVD 028004) was used. Our experimental design compared cell samples treated with EPO to cells pretreated with TNFα (EPO+TNFα) or cells treated with GM-CSF to cells treated with GM-CSF and TNFα (GM-CSF+TNFα), at different time points (0, 6, 24, 48 h). Gene expression data were analyzed using the Bioconductor (1) package "LIMMA" (2) in the R statistical programming environment (3). A quality control step was performed to increase the power of differential expression analysis by identifying measures from lower reliability spots. Two measures were used as lower quality markers (*i.e.* "gIsPosAndSignif" column provided by the Agilent scan software and the signal/noise ratio). Spots flagged as bad in every array were removed from the analysis. Preprocessing was performed without background subtraction to keep correlation between replicates. "Within-array" normalization was performed using "loess" algorithm, and "between-array" normalization was performed using "Aquantile."

A linear model and contrasts were used to extract differently expressed probes between conditions of interest. Specifically, the following differences were extracted: (1) TNF effect: EPO+TNFα *vs* EPO (6, 24, 48 h), GM-CSF+TNFα *vs* GM-CSF (6 h), (2) GM-CSF effect (GM-CSF 6 h *vs* EPO 0 h), EPO effect (EPO 6, 24, 48 h *vs* EPO 0 h). Significant spots were selected based on an FDR-adjusted p-value cut-off of 0.01 and a fold change of at least 1.5. Data are available on GEO (Accession number 104371). Long non-coding RNAs were defined based on available annotations. More specifically, probes annotated with an Ensembl transcript ID were defined as "Long_noncoding" when they belong to noncoding transcript biotype (as defined in www.ensembl.org/Help/Glossary), and probes without Ensembl transcript ID were defined as "Long_noncoding" based on an "lnc-" named lncipedia parent (lncipedia_2_1).

Functional analysis of LncRNA

Functional analysis was performed using the guilt-by-association method (4). Co-expressed coding genes were obtained from GeneFriends (5), using Ensembl transcript name as ID. We kept co-expressed genes with a defined ENTREZID, correlation >= 0.5 (absolute value), and Mutual Rank < 50. Functional enrichment and graphing was performed using clusterProfiler (6) and DOSE (7) libraries, in R environment (ver. 3.3.2, (3)). We used hallmark genesets and canonical pathways (c2.cp) genesets from mSigDB, version 5.2, restricting to genesets with a min size of 20. Significant results were selected on the basis of an FDR adjusted p-value cutoff of 0.05.

RNA extraction

RNA was extracted using TRIzol Reagent (Invitrogen, Thermo Fisher Scientific, Belgium) from 0.5 to 2 million cells, according to the manufacturer's instructions. Cytoplasmic and Nuclear RNA extraction were performed using the Qiagen protocol "Purification of cytoplasmic RNA form animal cells" which allows separation of cytoplasmic and nuclear RNA. For overexpression analysis following transfection with the pl-PTTG1-1:1 expression plasmid (Supplementary Fig 15), RNAs were cleaned up with RNeasy Mini Kit (Qiagen, Venlo, The Netherlands) including a DNAse digestion according to the manufacturer's instructions. For samples designed for microarray analysis, RNA integrity was assessed by the Agilent Bioanalyzer and RNA Integrity Number (RIN) was > 8.5. For AML patient samples, RIN was > 6.

Reverse transcription and real time PCR

Reverse transcription was performed with the Superscript™ III kit (Invitrogen). For mRNA/lncRNA, 2-5 µg total RNA were used with random hexamer primers from the SuperScript™ III first-strand synthesis system for RT-PCR (Invitrogen). Real-time PCR analysis were performed using the SYBR Green Master Mix according to the manufacturer's protocol. For miRNA, reverse transcription was performed using the miScript II RT Kit (Qiagen) and the real-time PCR using the miScript SYBR® Green PCR Kit (Qiagen). Real-time PCR was performed using a 7300 Real-Time PCR System (Applied Biosystems, Thermo Fisher Scientific, Belgium). Quantifications were performed in triplicate, and expression levels were normalized using β-actin (Actb) or non-coding RNA RNU6b (RNU6b) as internal standards. Relative gene expression levels correspond to fold induction compared to untreated cells.

Primers used were:

ACTB F: CTCTTCCAGCCTTCCTTCCT;

ACTB R: AGCACTGTGTTGGCGTACAG;

lncPTTG1-11 F: GAGAACAGCGGCTGAATTGG;

lncPTTG1-11 R: AGATGGTGCAAAGACCCCTC;

SP1 F: GTGGAGGCAACATCATTGCTG
SP1 R: GCCACTGGTACATTGGTCACAT
SP3 F: AGTGGGCAGTATGTTCTTCCC
SP3 R: GACTGGATCTGTGGTATCACTTG
p65 F: CCCACGAGCTTGTAGGAAAGG
p65 R: GGATTCCCAGGTTCTGGAAAC
has-mir146a-5p: UGAGAACUGAAUUCCAUGGGUU;

Flow Cytometry

Cells were washed three times in phosphate buffered saline (PBS) before incubation with 5 µL of antibody for 90 min at room temperature. PE-labeled CD11b (clone D12), FITC-labeled CD15 (clone W6D3) (BD Biosciences, Erembodegem, Belgium) and PE-labeled CD14 (clone TÜK4) (Miltenyi Biotec, Leiden, Netherlands) antibodies were used. The corresponding isotype control antibodies (PE mouse Isotypic control IgG1κ and FITC mouse isotypic control IgG2a from BD Biosciences) were used to set the gating levels. Cells were washed three times and analyzed with a FACS Calibur flow cytometer (BD Biosciences). The corresponding isotype control antibodies (BD Biosciences) were used to set the gating levels. Every result was reported to the corresponding isotypic control, then presented as ratio of transfected / non-transfected cells.

Cell cycle analysis was performed using propidium iodide (PI) staining, cells were collected and fixed in 70% ethanol, and DNA was stained with a PI solution (1 µg/mL, Sigma-Aldrich, St. Louis, Missouri, USA) in 1x PBS, supplemented with RNase A (100 µg/mL; Roche, Basel, Switzerland).

The loss of mitochondrial membrane potential was analyzed by MitoTracker Red staining (Molecular Probes/Invitrogen). Briefly, 1 x 10^6 cells were incubated for 20 minutes at 37°C with 50 nM of MitoTracker Red.

Analysis were performed on a FACS Calibur flow cytometer (BD Biosciences). The statistical analysis was based on 10,000 events per sample using FlowJo® software (version 8.8.7, Tree Star, Ashland Oregon, USA).

Assays of cell proliferation, viability, morphology, and differentiation

Cell number and viability were assessed using trypan blue dye exclusion. For evaluation of cell morphology, cytospin preparations were stained with May-Grünwald/Giemsa solution (MGG) (Merck, Leuven, Belgium).

For transfected cells, cell proliferation was assessed in parallel by Incucyte videomicroscopy (Essen BioScience, Hertfordshire, United Kingdom) on plates previously coated with Poly-D-Lysine and transfected with control (pUC19) or plPTTG1-1:1 plasmids.

Plasmids and transfection

The plPTTG1-11 plasmid was constructed with the pcDNA3.3-TOPO TA Cloning Kit (Invitrogen), amplified in competent *E. coli* (Invitrogen) and purified by NucleoBond® PC-500 columns (Macherey-Nagel, Filter Service, Eupen, Belgium) according to the manufacturers' protocols. Transfection by electroporation used 500 ng of plasmid per million of cells at 1.5 x 10^7 cells/mL and at 250 V and 500 µF (BioRad Gene PULSER® II) (Bio-Rad Laboratories N.V., Temse, Belgium). 72h after transfection, PTTG1-1:1 expression was assessed by qPCR (Supplementary figure 21). GapmeR (Exiqon, Qiagen) and siRNA (Stealth RNAi, Thermo Fisher Scientific, Belgium) targeting PTTG1-1:1 were transfected at 500 nM and 200 nM respectively, using HighPerFect (Qiagen) transfection kit according to the provider's protocol. The double-knockdown approach aims to target lncRNAs in both cellular compartments, nucleus and cytoplasm (GapmeRs are more efficient in the nucleus whereas siRNA are more efficient in the cytoplasm). This approach increases the efficiency of silencing, avoids successive transfections, and preserves cell viability. SiRNA targeting p65 (Qiagen) were transfected in K562 and U937 at 5 and 40 nM respectively, using HighPerFect (Qiagen) transfection kit.

Si623 stealth_623-S AGACUCUUGGGAGAAAUCACUUUAA
Si623 stealth_623-AS UUAAAGUGAUUUCUCCCAAGAGUCU
SiCt stealth_control_623-S AGAGUUCGAGGUAAAUCACUUCUAA
SiCt stealth_control_623-AS UUAGAAGUGAUUUACCUCGAACUCU
siRNA-p65 (HS_RELA_5 SI00301672): AAGATCAATGGCTACACAGGA
siRNA-p65 (HS_RELA_7 SI02663094): CCGGATTGAGGAGAAACGTAA
AS-LNA-GapmeR_01 TAAACGAGTAGCAGC
AS-LNA-GapmeR_Ct AACACGTCTATACGC

For NF-κB activity assays in cells over-expressing PTTG1-1:1, cells were co-transfected with plPTTG1-1:1 (10 µg of plasmid per million of cells) + pGL4-NF-κB reporter (5 µg of plasmid per million of cells) + pRL-SV40 *Renilla* plasmid for normalization (5 µg of plasmid per million of cells) using an Amaxa® Cell Line Nucleofector® Kit V (Lonza). For NF-κB activity assays in PTTG1-1:1 knockdown cells, cells were pre-transfected with GapmeR (Exiqon, Qiagen) and siRNA (Stealth RNAi, Thermo Fisher Scientific, Belgium) targeting PTTG1-1:1 as previously defined with HighPerFect (Qiagen). 48h later, cells were transfected with pGL4-NF-κB reporter plasmid (5 µg of plasmid per million of cells) + SV40-Renilla plasmid for normalization (5 µg of plasmid per million of cells). 24 hours after Amaxa transfection, cells were counted and treated for 3 and 6 hours with TNF-α. Then NF-κB activity was assessed with the Dual-Glo® Luciferase Assay System (Promega), according to the manufacturer protocol.

Fluorescence in Situ Hybridization

Fluorescence *in situ* Hybridization (FISH) was performed using 3'End Biotinylated probes (Eurogentec, Sereing, Belgium) (Supplementary Table 2), designed with www.singlemoleculefish.com. Prior to staining, cells were treated for 6 h with TNFα, then cells were washed in PBS, and centrifuged on slides with a Cytofuge 2 (StatSpin, Laméris, Belgium). Cells were fixed with Triton 0.2%, formaldehyde 4% in PBS for 5 min, washed in PBS, then fixed a second time in formaldehyde 4% for 5 min. Then cells were washed in PBS and dehydrated in successive baths of ethanol at 75%, 90%, 100%, for 5 min each. Cells were hybridized with hybridization buffer (10% Dextran, 50% formamide, 2X saline-sodium citrate (SSC), 1 mM EDTA, 50 mM sodium phosphate buffer, 10 ng/µl Cot-1 DNA, 0.5 µg/µl tRNA) and 5 µM of probes,_in a wet chamber, overnight. Cells were incubated in 2X SSC for 5 min at 37°C, then Tris-NaCl (TN) buffer (0.1 M Tris-HCl, 0.15 M NaCl) for 5 min at room temperature (RT), then TNT buffer (TN+0.05% Tween 20) for 10 min at room temperature, then saturated in TNT buffer with 5% bovine serum albumin (BSA) for 20 min. Endogenous peroxidase activity was inactivated by incubation with 1% H_2O_2 in PBS for 30 minutes at RT. FISH were reveled using Alexa Fluor™ 594 Tyramide Reagent (Thermo Fisher Scientific) according to the manufacturer's instructions. Images were collected with an Olympus IX81 microscope controlled by Olympus Xcellence ® Software (Olympus, Antwerpen, Belgium).

RNASeq

K562 cell were transfected with Si623/Gap01 or SiCt+GapCt (Mock transfection) under the experimental conditions mentioned above, then cells were treated or not with TNFα

(20 ng/mL). RNA was extracted using TRIzol (Invitrogen) from 0.5 to 2 x 10^6 cells, according to the manufacturer's instructions. RNA cleanup was performed using the RNeasy Purification Kit (Qiagen). RNA quantity and quality were assessed by Nanodrop (Isogen, De Meern, Netherlands) and Agilent Bioanalyzer (Agilent Technologies, Machelen, Belgium) (RIN>9), respectively. RNASeq analysis was done by Eurofins GATC Biotech GMBH (Konstanz, Germany). The RNASeq reads were aligned to the reference genome or reference transcriptome using Bowtie generating genome/transcriptome alignments. TopHat identified the potential exon-exon splice junctions of the initial alignment. Cufflinks identified and quantified the transcripts from the preprocessed RNASeq alignment assembly. Cuffmerge merged the identified transcript fragments to full-length transcripts and annotated the transcripts based on the given annotations. Finally, merged transcripts from two or more samples/conditions are compared using Cuffdiff to determine the differential expression levels at transcript and gene levels including a measure of significance between samples/conditions.

Public data analysis

Data were loaded and analyzed in R environment, version 3.4 [3], using Bioconductor [1]. Three public patient datasets were used: TCGA AML (GSE62944), Verhaak (GSE6891) and Rapin (GSE42519) datasets were imported using GEOquery (1) (Supplementary Tables 5 and 6). TARGET dataset (https://ocg.cancer.gov/programs/target) was imported using TCGAbiolinks (2) (Supplementary Table 7). Expression values were log-normalized. Correlations between gene expression levels were assessed by Pearson correlation, and association with French-American-British (FAB) classes were estimated by ANOVA. Bloodpool cohort was imported from Bloodspot website (8) (Supplementary Table 4). PTTG1-1:1 gene expression of each AML patient's karyotype subclasses were pooled to be compared to healthy hematopoietic stem cells. Expression values were log-normalized, and P-values were determined by the Mann-Whitney test.

Normalized "Reads Per Kilobase of transcript per Million mapped reads" (RPKM) for PTTG1-1:1 (mir-146A) (GRCh37 genome version) from AML patients and healthy controls were downloaded from http://www.vizome.org/ (9). Boxplot of PTTG1-1:1 (mir-146A) expression was generated using the ggboxplot function from the ggpubr (10) package in R 3.5.2 (11) and RStudio (12). Significant differences in PTTG1-1:1 expression was assessed by a Kruskal-Wallis test and P-values were determined by the Mann-Whitney test.

Long non-coding RNA-Protein interaction prediction

Prediction of putative target proteins of PTTG1-1:1 was computed using the SFPEL-LPI web server (13) and drawn in Cytoscape 3.7.1 (14).

Enrichment analysis

Gene enrichment analysis was performed using EnrichR (15, 16) and the results were represented using GOplot (17) in R 3.5.2 (11) and RStudio (12).

Negative control for NF-κB independent gene expression

In order to determine genes independent of the NF-κB pathway, the p65 binding motif was searched within the promoter, defined as transcriptional start site (TSS) ± 500 bp, of protein-coding genes that were not differentially expressed in K562 cells treated with TNFα and further filtered for genes that do not contain a p65 motif into their promoter sequence. This analysis was performed in R 3.5.2 (11) and RStudio (12) using the MotifDb (18), seqLog (19) and motifStack (20) packages.

Statistical analysis

Data were expressed as the mean ± SEM and significance was estimated by using one-way or two-way ANOVA tests using Prism 8.2 software, GraphPad Software (La Jolla, CA, USA). P-values <0.05 were considered as significant.

Supplementary references

1. Gentleman RC, Carey VJ, Bates DM, Bolstad B, Dettling M, Dudoit S, et al. Bioconductor: open software development for computational biology and bioinformatics. Genome Biol. 2004;5(10):R80.
2. Smyth GK, Michaud J, Scott HS. Use of within-array replicate spots for assessing differential expression in microarray experiments. Bioinformatics. 2005 May 1;21(9):2067-75.
3. Team RC. A Language and Environment for Statistical Computing. Vienna, Austria: R Foundation for Statistical Computing, 2016.
4. Wolfe CJ, Kohane IS, Butte AJ. Systematic survey reveals general applicability of "guilt-by-association" within gene coexpression networks. BMC Bioinformatics. 2005 Sep 14;6:227.
5. van Dam S, Craig T, de Magalhaes JP. GeneFriends: a human RNA-seq-based gene and transcript co-expression database. Nucleic Acids Res. 2015 Jan;43(Database issue):D1124-32.
6. Yu G, Wang LG, Han Y, He QY. clusterProfiler: an R package for comparing biological themes among gene clusters. OMICS. 2012 May;16(5):284-7.
7. Yu G, Wang LG, Yan GR, He QY. DOSE: an R/Bioconductor package for disease ontology semantic and enrichment analysis. Bioinformatics. 2015 Feb 15;31(4):608-9.
8. Bagger FO, Sasivarevic D, Sohi SH, Laursen LG, Pundhir S, Sonderby CK, et al. BloodSpot: a database of gene expression profiles and transcriptional programs for healthy and malignant haematopoiesis. Nucleic Acids Res. 2016 Jan 4;44(D1):D917-24.

9. Tyner JW, Tognon CE, Bottomly D, Wilmot B, Kurtz SE, Savage SL, et al. Functional genomic landscape of acute myeloid leukaemia. Nature. 2018 Oct;562(7728):526-31.

10. Kassambara A. ggpubr: 'ggplot2' Based Publication Ready Plots. 0.2. ed, 2018.

11. Team RDC. R: A language and environment for statistical computing. R Foundation for Statistical Computing, 2010.

12. Team R. RStudio: Integrated Development for R. 2015.

13. Zhang W, Yue X, Tang G, Wu W, Huang F, Zhang X. SFPEL-LPI: Sequence-based feature projection ensemble learning for predicting LncRNA-protein interactions. PLoS Comput Biol. 2018 Dec;14(12):e1006616.

14. Shannon P, Markiel A, Ozier O, Baliga NS, Wang JT, Ramage D, et al. Cytoscape: a software environment for integrated models of biomolecular interaction networks. Genome Res. 2003 Nov;13(11):2498-504.

15. Kuleshov MV, Jones MR, Rouillard AD, Fernandez NF, Duan Q, Wang Z, et al. Enrichr: a comprehensive gene set enrichment analysis web server 2016 update. Nucleic Acids Res. 2016 Jul 8;44(W1):W90-7.

16. Chen EY, Tan CM, Kou Y, Duan Q, Wang Z, Meirelles GV, et al. Enrichr: interactive and collaborative HTML5 gene list enrichment analysis tool. BMC Bioinformatics. 2013 Apr 15;14:128.

17. Walter W, Sanchez-Cabo F, Ricote M. GOplot: an R package for visually combining expression data with functional analysis. Bioinformatics. 2015 Sep 1;31(17):2912-4.

18. Shannon P, Richards M. MotifDb: An Annotated Collection of Protein-DNA Binding Sequence Motifs. 1.24.1 ed, 2018.

19. Bembom O. seqLogo: Sequence logos for DNA sequence alignments. 1.48.0 ed, 2018.

20. Ou J, Wolfe SA, Brodsky MH, Zhu LJ. motifStack for the analysis of transcription factor binding site evolution. Nat Methods. 2018 Jan 3;15(1):8-9.

21. Fukuda Y, Wang Y, Lian S, Lynch J, Nagai S, Fanshawe B, et al. Upregulated heme biosynthesis, an exploitable vulnerability in MYCN-driven leukemogenesis. JCI Insight. 2017 Aug 3;2(15).

22. Guertin DA, Sabatini DM. Defining the role of mTOR in cancer. Cancer Cell. 2007 Jul;12(1):9-22.

23. Ghosh J, Kapur R. Role of mTORC1-S6K1 signaling pathway in regulation of hematopoietic stem cell and acute myeloid leukemia. Exp Hematol. 2017 Jun;50:13-21.

24. Schepers H, Wierenga AT, Vellenga E, Schuringa JJ. STAT5-mediated self-renewal of normal hematopoietic and leukemic stem cells. JAKSTAT. 2012 Jan 1;1(1):13-22.

25. Smits EL, Anguille S, Berneman ZN. Interferon alpha may be back on track to treat acute myeloid leukemia. Oncoimmunology. 2013 Apr 1;2(4):e23619.

26. Anguille S, Lion E, Willemen Y, Van Tendeloo VF, Berneman ZN, Smits EL. Interferon-alpha in acute myeloid leukemia: an old drug revisited. Leukemia. 2011 May;25(5):739-48.

Supplementary table legends

Supplementary Table 1

Table summarizing the analysis of peptides longer than 10 amino acids resulting from the different reading frames of the PTTG1-11 sequence and analyzed with WebServer Pfam 30.0.

Supplementary Table 2

Table presenting the sequence of the different probes used for Fluorescence in Situ Hybridization (FISH).

Supplementary Table 3

Table summarizing genes that are up- or down-regulated by PTTG1-1:1 silencing and the link between pathways associated with the extinction of PTTG1-1:1 (Si623+GAP01: SG) and AML (with associated references).

Supplementary Table 4

Bloodpool Adult AML patient clinical characteristics.

Supplementary Table 5

TCGA adult patient characteristics by group of expression.

Supplementary Table 6

Verhaak dataset patient clinical characteristics.

Supplementary Table 7

TARGET dataset patient clinical characteristics.

Supplementary Table 8

AML patient dataset used for the qPCR analysis of PTTG1-1:1 expression.

Supplementary Table 9

Differentially expressed genes in RNAseq experiment: (SiRNA+GapmeR)-PTTG1-1:1 *vs* (SiRNA+GapmeR)-Control

Supplementary Table 10

Differentially expressed genes in RNAseq experiment: (SiRNA+GapmeR)-PTTG1-1:1 *vs* (SiRNA+GapmeR)-Control in the presence of TNFα.

Supplementary Tables

Supplementary Table 1

5'3' Frame 1	**Nb**	**Result**
MDRRQ	5	
MGCVSVRPLKFSSSAGISLSSWA	23	No Pfam-A Matches
MLSRVPSS	8	
MNDLSKSLLLLLLLVYIY	18	No Pfam-A Matches
MIQVLL	6	
MLLYETLGRNHFNEA	15	No Pfam-A Matches
MIFPPSSRDQRELIECDNDSLAGTAEASGSFLRSAVKEDEKHGYALLLVPLFLYLISTSFYKYIRATLSLCISNKAKRGCNYTLLQSSVSPGNRNAKALKASLFADMVSWVPWAKSFCCPPLSPSKLGPF	130	No Pfam-A Matches
MGNKGCDLSFGNSSLIHHCLLVSWNFHVITVYRMKE	36	No Pfam-A Matches
MCQALCSGLSLYA	13	No Pfam-A Matches
MKAPSQACGTWVCSLGCTDQIKDLPHPSHTLPTSALCLSDP	41	No Pfam-A Matches
MEKIQNIY	8	
MLWVSAPLCCGH	12	No Pfam-A Matches
MKYGIHRYTHTLKN	14	No Pfam-A Matches
MYVGIRVLKDVHQFANNGYL	20	No Pfam-A Matches
5'3' Frame 2		
MIK	3	
MCILSFEN	8	
MISASLSCCCCYSFTFIDYLR	21	No Pfam-A Matches
MKLETHGIAMQ	11	No Pfam-A Matches
MIL	3	

MRNMDMPFFWSPSSFI	16	No Pfam-A Matches
MQRL	4	
MDFIRL	6	
MSLQSTE	7	
MHDLSESHQASSGKRGQINNRMAN	24	No Pfam-A Matches
MERGAWLL	8	
MEFTDTHTH	9	
MLHFIENLC	9	
MLQNNLVSMIQFM	13	No Pfam-A Matches
MYINLLTMVISDVVGFEMCFSFWLYFSLLFDLTQNMFGYNNKVIED	46	No Pfam-A Matches
5'3' Frame 3		
MAPAGPIGVVNPGPEGMPKGGQDGQETVAQRGGGEQRLNWK	41	No Pfam-A Matches
MKF	3	
MALPCNDFSPLFTGSEGTNRM	21	No Pfam-A Matches
MGQVLLLPSSVTK	13	No Pfam-A Matches
MMITFRQKVRGLMSAIWTLLGFRFDGE	27	No Pfam-A Matches
MLILILSSQ	9	
MISVNLTKPHLENGDKLTTGWQIEKHVTMFYRWKGVLGYYEGPLASVWDMGVFSGLY	57	No Pfam-A Matches

Supplementary Table 2

Control Probes

Control probe	ccagtgaatccgtaatcatg
U1 probe	ctcccctgccaggtaagtat
U2 probe	ccaaaaggccgagaagcgat

EVEN Probes		ODD Probes	
PTTG1-11-Probe01	catccaggaaaggaagagcg	PTTG1-11-Probe02	tatcatttccaattcagccg
PTTG1-11-Probe03	cacatcggcttttcagagat	PTTG1-11-Probe04	actgacacaacccatggaat
PTTG1-11-Probe05	agagatatcccagctgaaga	PTTG1-11-Probe06	ttcttcaggatctactctct
PTTG1-11-Probe07	tctgtctccagtcttccaag	PTTG1-11-Probe08	tctcacaggaactcacactc
PTTG1-11-Probe09	aaccctgcttagcatagaat	PTTG1-11-Probe10	cagcaagagagacttgctga
PTTG1-11-Probe11	tcggtgtcactgagaagaca	PTTG1-11-Probe12	gttaatttgtccccgttttc
PTTG1-11-Probe13	gtagaacatggttacgtgtt	PTTG1-11-Probe14	cgagggggccttcataataa
PTTG1-11-Probe15	tgatcagtacaacccagaga	PTTG1-11-Probe16	agtgggcagagtgtgagaag
PTTG1-11-Probe17	aatgagtcactggctaaggg	PTTG1-11-Probe18	tctggtgtcggttgagattt
PTTG1-11-Probe19	attctggattttctccatca	PTTG1-11-Probe20	agaggagctgaaacccagag
PTTG1-11-Probe21	ctaaatcggagttctgagcc	PTTG1-11-Probe22	gtttgctttaggaagagtgc
PTTG1-11-Probe23	atgtgtgtgtgtatctgtga	PTTG1-11-Probe24	acagtgaatttctgcctact
PTTG1-11-Probe25	accaagttttggtaaagcaa	PTTG1-11-Probe26	aaccaaacatgttctgtgtt

Supplementary Table 3

Pathway	Effect of Si623+Gap01		AML pathology-related function
	SG/Ctl	TNFα-Ctl /TNFα	
Heme metabolism	**Down-regulated:** UCP2, FAM46C, CAST, TNRC6B, NEK7, AQP3, BTG2, HBZ, MKRN1, KLF3, GCLM, TFRC, CDC27	**Down-regulated:** CAST, FAM46C, AQP3, TNRC6B, BTG2, HBZ, MKRN1, NEK7, KLF3, GCLM	- Heme is involved in electron transfer and sustains AML progression (21).
mTOR complex 1 signaling	**Down-regulated:** GGA2, BTG2, SLC2A3, PNO1, M6PR, ADIPOR2, MTHFD2, INSIG1, BUB1, ACTR2, RRM2, CDC25A, TFRC	**Down-regulated:** DHCR24, BTG2, SLC2A3, M6PR, PNO1, ADIPOR2, LDLR, RRM2, MTHFD2, UBE2D3	- Regulates ribosomal biogenesis, autophagy, and metabolism (22). - Plays a critical role in leukemia initiation and progression and in regulation of HSCs/LSCs (23).
IL2-Stat5 signaling		**Down-regulated:** TNFRSF4, POU2F1, SLC2A3, RNH1, MYC, KLF6, LTB, PIM1, CISH, CDC42SE2	- Induces genes related to proliferation, apoptosis, differentiation and inflammation (24). - Constitutively phosphorylated Stat5 is implicated in LSCs self-renewal.
IFNα response		**Up-regulated** LGALS3BP, SAMD9, UBE2L6, IFIH1, SP110, GBP4, RSAD2	- Tested for induction of AML remission for the treatment of patients relapsing upon hematopoietic stem cell transplantation, and for post-remission strategy to prevent recurrence (25, 26).

Abbreviations: ACTR2: Actin-related protein 2, ADIPOR2: Adiponectin receptor protein 2, AQP3: Aquaporin-3, BTG2: B-cell translocation gene 2, BUB1: Budding uninhibited by benzimidazoles 1, CAST: Calpastatin, CDC25A: cell division cycle 25A, CDC27: cell division cycle 27, CDC42SE2: cell division cycle 42 small effector 2, CISH: cytokine inducible SH2 containing protein, DHCR24: 24-dehydrocholesterol reductase, FAM46C: family with sequence similarity 46, member C, GBP4: Guanylate Binding Protein 4, GCLM: Glutamate--cysteine ligase regulatory, GGA2: golgi associated, gamma adaptin ear containing, ARF binding protein 2, HBZ: Hemoglobin subunit zeta, IFIH1: Interferon-induced helicase C domain-containing protein 1, INSIG1: Insulin-induced gene 1, KLF3: Krueppel-like factor 3, KLF6: Krueppel-like factor 6, LDLR: low-density lipoprotein receptor, LGALS3BP: galectin 3 binding protein, LTB: Leukotriene B4 receptor 1, M6PR: Mannose-6-Phosphate Receptor, MKRN1: Makorin Ring Finger Protein 1, MTHFD2: Methylenetetrahydrofolate Dehydrogenase (NADP+ Dependent) 2, MYC: c-Myc, NEK7: Serine/threonine-protein kinase NEK7, PIM1: Serine/threonine-protein kinase pim-1, PNO1: Partner Of NOB1 Homolog, POU2F1: POU Class 2 Homeobox 1, RNH1: Ribonuclease inhibitor 1, RRM2: Ribonucleotide Reductase Regulatory Subunit M2, RSAD2: Radical SAM domain-containing 2, SAMD9: sterile alpha motif domain-containing protein 9, SLC2A3: Solute Carrier Family 2 Member 3, SP110: Sp110 nuclear body protein, TFRC: Transferrin receptor protein 1, TNFRSF4: TNF receptor superfamily member 4, TNRC6B: Trinucleotide repeat-containing gene 6B protein, UBE2D3: Ubiquitin-conjugating enzyme E2 D3, UBE2L6: Ubiquitin/ISG15-conjugating enzyme E2 L6, UCP2: uncoupling protein 2

Supplementary Table 4

Bloodpool adult AML patient characteristics

Characteristics	**All patients**
No. of patients	1309
Normal karyotype (No.)	989
Complex karyotype (No.)	87
inv(16) (No.)	77
t(8;21) (No.)	98
t(11q23)/MLL (No.)	58

Supplementary Table 5

TCGA adult AML patient characteristics

Characteristics	**All patients**	**Low LOC285628**	**High LOC285628**
No. of patients	178	89	89
Median PTTG1-1:1 (range)	2.665 (0.58-6.02)	1.69 (0.58-2.65)	3.61 (2.68-6.02)
Median age, y (range)	58 (18-88)	59 (18-82)	56 (21-88)
Median WBC, 10^9/L (range)	17 (1-297)	14 (1-297)	27 (1-203)
Median BM blasts, % (range)	34.5 (0-98)	18 (0-98)	49 (0-97)
Median OS, d (range)	350 (0-2861)	303.5 (0-2496)	470.5 (0-2861)
Cytogenetic risk			
Favorable (%)	33 (18.5%)	10 (11.2%)	23 (25.8%)
Intermediate (%)	103 (57.9%)	57 (64.0%)	46 51.7%)
Unfavorable (%)	40 (22.5%)	21 (23.6%)	19 (21.3%)
Not available (%)	2 (1.1%)	1 (1.1%)	1 (1.1%)
Dead vs alive	116/62	66/23	50/39

Abbreviations: BM, bone marrow; OS, overall survival; WBC, white blood cells.

Supplementary Table 6

Verhaak dataset: Clinical characteristics

Characteristics	**All patients**
Patients	461
Age (range)	43 (15-61)
Median PTTG1-1:1 (range)	5.01346226 (4.906890596-9.327777171)
Female/Male	230/231
Cytogenetic risk (Good/Intermediate/Poor/Unknown)	97/261/93/10
FAB	
M0	16
M1	95
M2	106
M3	24
M4	79
M4e	5
M5	104
M6	6
Mx	1
Score: Raeb	4
Score: Raeb-T	13
Unknown	8
Mutation	
CEBPA (Double/Single/WT)	24/10/427
NPM (Neg/Pos)	321/140
FLT3-ITD (Neg/Pos)	335/126
FLT3-TDK (Neg/Pos)	411/50
NRAS (Neg/Null/Pos)	415/1/45
KRAS (Neg/Pos)	457/4
EVI-1 (Neg/Pos)	430/31

Abbreviations: CEBPA, CCAAT/enhancer-binding protein alpha; EVI1, Ecotropic viral integration site-1; ITD; internal tandem duplication; FLT3, Fms-like tyrosine kinase 3; KRAS, Kirsten RAt Sarcoma virus; NPM, nucleoplasmin; NRAS, neuroblastoma RAt Sarcoma virus; Raeb, Refractory anemia with excess blasts; TDK, mutation in the activation loop of the second tyrosine kinase domain

Supplementary Table 7

TARGET dataset patient clinical characteristics

Characteristics	**All patients**
Patients	168
Female/Male	91/77
Age (range)	8.952 (0.375-22.551)
BM leukemic blast percentage (range)	74.8 (14-100)
Event Free Survival Time, year (range)	1.1015 (0.233-9.945)
Median PTTG1-1:1 (range)	13.025 (0-18.426)
Median OS, yr (range)	3.393 (0.307-11.019)
Median WBC, 10^9/L (range)	46.15 (1.3-519)
Dead vs Alive	85/83
FAB	
M0	4
M1	21
M2	45
M4	52
M5	35
M6	3
M7	8
Mutation	
FLT3-ITD positive (No/Yes)	153/15
FLT3 (No/Yes)	158/10
NPM (No/Unknown/Yes)	153/8/7
CEBPA (No/Unknown/Yes)	158/2/8
WT1 (No/Unknown/Yes)	150/6/12
c-Kit Exon8 (No/Unknown/Yes)	35/119/14
c-Kit Exon 17 (No/Unknown/Yes)	36/119/13
Monosomy.5 (No/Unknown)	156/12
Monosomy.7 (No/Unknown)	156/12
Trisomy.8 (No/Unknown/Yes)	146/12/10
Trisomy.21 (No/Unknown/Yes)	151/12/5

Abbreviations: BM, bone marrow; CEBPA, CCAAT/enhancer-binding protein alpha; c-Kit, KIT proto-oncogene receptor tyrosine kinase**;** ITD; internal tandem duplication; FLT3, Fms-like tyrosine kinase 3; NMP, nucleoplasmin; OS, overall survival; WBC, white blood cells; WT1, Wilms tumor 1.

Supplementary Table 8: Clinical characteristics of AML patients tested in this study.

#	Sex/ age	FAB	Cytogenetic abnormalities	FLT3-ITD mutation	CEBPA	NPMc
BOU	F/75	5	47, XX, +8	1		0
BES	M/39	1	Normal Karyotype	0	0	1
CHA	F/35	4	Normal Karyotype	1	0	0
MON	F/21	5	Normal Karyotype	1	0	1
BAS	M/74	1	47,X,-Y,t(2;8)(p22;q24),+4,+8x3,add(9)(q21),-11,-17,2~21 dmin[10] / 46,X,-Y,add(3)(q28),+8,+add(8)(q24),-11,-17,+der(?)hsr(?)[cp3]	0		0
ALA	M/54	2	Normal Karyotype	1		1
BAN	M/-	2	Normal Karyotype	1		
TRE	M/68	1	Normal Karyotype	0		0
FER	F/57	4	Normal Karyotype	1	0	1
ARJ	M/77	2	Normal Karyotype	1		
VIC	M/71	5	Normal Karyotype	0		1
WAC	F/25	5	Normal Karyotype	0		0
LAN	M/63	2	Normal Karyotype	1	1	0
NOG	F/63	5	46, XX, t(11;12)(p15;p13)	1	0	0

Abbreviations: (FAB, French-American-British, FLT3-ITD, FMS-like tyrosine kinase-3-internal tandem duplication; CEBPA, CCAAT/enhancer-binding protein alpha; NPMc, cytoplasmic nucleophosmin 1).

Supplementary Table 9: Differentially expressed genes in RNAseq - Basal expression

Symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
AC026369.1	70.69336776	1.225274603	0.233759137	5.132194006	2.86E-07	2.17E-04
AFF4	2943.666231	-0.558674874	0.133391033	-4.188249784	2.81E-05	0.006890832
AHCYL2	420.1885723	0.805687173	0.174203419	4.618078307	3.87E-06	0.001635709
AKAP11	2774.745023	-0.545379742	0.128387201	-4.24651134	2.17E-05	0.00547545
AKAP12	1112.394674	0.70335263	0.171767941	4.100957458	4.11E-05	0.009070663
ANLN	4125.806047	-0.767739332	0.145875105	-5.263774358	1.41E-07	1.21E-04
ARFRP1	938.9671444	0.706067275	0.140091588	5.040486133	4.64E-07	3.01E-04
ARHGEF10	678.0412627	-0.885821559	0.180213781	-4.913114143	8.96E-07	5.24E-04
ARNTL2	1037.482703	-0.767652043	0.179983129	-4.268824354	1.97E-05	0.005218274
ATAD2B	1744.305512	-0.460222643	0.108299181	-4.249709852	2.14E-05	0.005452389
BAG5	1608.565503	-0.53856602	0.120552739	-4.46704883	7.93E-06	0.002836647
BICD2	1830.991089	-0.687853103	0.097245594	-7.072156136	1.53E-12	4.46E-09
BTG2	3025.99532	-0.599467605	0.135108214	-4.449660055	8.60E-06	0.003014779
C19orf66	281.5340007	0.868823045	0.184503322	4.722923666	2.32E-06	0.001101224
C5orf30	582.5399575	-0.448158989	0.10950915	-4.090837089	4.30E-05	0.009345722
CACNA1I	202.5487239	0.656912429	0.179985556	4.367599848	1.26E-05	0.003795979
CARD8	806.6074097	0.562471409	0.130931252	4.295998813	1.74E-05	0.004800012
CAST	1824.080465	-0.551371176	0.115513566	-4.771009921	1.83E-06	9.18E-04
CAV2	1100.320044	-0.806870665	0.105962049	-7.610004089	2.74E-14	1.37E-10
CD46	8449.062218	-0.818364746	0.085229492	-9.601206599	7.90E-22	9.23E-18
CDCA4	1727.338155	-0.458173631	0.111725002	-4.101498709	4.10E-05	0.009070663
CDCA7L	948.5965525	-0.602728183	0.136582093	-4.409155225	1.04E-05	0.003399637
CECR2	979.1027221	-0.61164629	0.144638031	-4.230666366	2.33E-05	0.005792447
CERS2	4781.3223	-0.450405145	0.10585272	-4.254476907	2.10E-05	0.005452389
CETN2	815.0389754	-0.442626742	0.100098275	-4.421044187	9.82E-06	0.003279127
CHGB	33.78491633	1.161470097	0.275320362	4.252079717	2.12E-05	0.005452389
CKMT1A	796.6482666	0.652600284	0.149205708	4.370605841	1.24E-05	0.003795979
CLIC4	4012.586241	-0.568122415	0.137127461	-4.146889867	3.37E-05	0.008091531
CPLX1	276.3349715	1.527387933	0.243523655	6.308815377	2.81E-10	4.93E-07
CRTC1	461.7773289	0.530201861	0.125817355	4.213277509	2.52E-05	0.006213058
CTB-131K11.1	275.429101	-1.022801026	0.152907628	-6.679021899	2.41E-11	6.02E-08
CTNNBIP1	791.871389	0.495886133	0.119999161	4.132063627	3.60E-05	0.008345884
CYTH1	1238.304035	0.468770099	0.109655989	4.275225487	1.91E-05	0.005109281
DDI2	4668.192337	-0.685183422	0.1636367	-4.184031255	2.86E-05	0.006923202
DDIAS	835.8989613	-0.605103633	0.14645854	-4.129230368	3.64E-05	0.0083938
DDX18	5719.295389	-0.53760045	0.122124326	-4.401094865	1.08E-05	0.003495758
DHX29	2122.625567	-0.503573861	0.106325353	-4.735250055	2.19E-06	0.001080152
DHX33	1587.52818	-0.530122191	0.115453865	-4.589873508	4.44E-06	0.001829002
DISP2	108.2413496	1.179261849	0.269917928	4.437697721	9.09E-06	0.003094404
DNAJC25-GNG10	342.4383828	-1.13337151	0.171994331	-6.5768248	4.81E-11	1.05E-07
DSCR4	191.3522709	0.646569501	0.158696503	4.067421867	4.75E-05	0.009845225
DSG2	3098.111431	-0.490984386	0.118479089	-4.143786894	3.42E-05	0.00814606
DUXAP8	915.578017	0.45312946	0.105440659	4.296750302	1.73E-05	0.004800012
DYNLL2	2549.185984	-0.394609334	0.075912343	-5.197668738	2.02E-07	1.65E-04
DYRK2	805.6404822	-0.893244872	0.217491577	-4.101402018	4.11E-05	0.009070663
E2F3	4453.809883	-0.658443066	0.113217645	-5.816443296	6.01E-09	7.53E-06
EZH2	5923.471064	-0.417803908	0.069644057	-5.999167655	1.98E-09	2.78E-06
FAM133B	1973.754519	-0.795709785	0.145766946	-5.456268094	4.86E-08	5.01E-05
FAM168B	5497.699544	-0.576481379	0.123029969	-4.685634357	2.79E-06	0.001287248
FAM199X	3319.471584	-0.385204609	0.075094927	-5.129451836	2.91E-07	2.17E-04
FAM208B	5716.007335	-0.512770335	0.118706877	-4.31965124	1.56E-05	0.004430273
FAM3C	1095.917856	-0.740345817	0.145912897	-5.068465317	4.01E-07	2.76E-04
FAM8A1	1290.405213	-0.76352363	0.175573388	-4.344105846	1.40E-05	0.004084962
FBXO28	2342.749855	-0.783521069	0.122869083	-6.376566787	1.81E-10	3.34E-07

FBXW7	730.0237591	-0.7826858	0.158410928	-4.940334488	7.80E-07	0.000471334
FTL	30872.23199	-0.334469535	0.072927371	-4.586393539	4.51E-06	0.001838115
FUT4	1303.131395	-0.668281946	0.125637582	-5.322107604	1.03E-07	9.24E-05
FZD4	439.3053299	-0.550963377	0.122629272	-4.487916336	7.19E-06	0.002740351
FZD6	379.8432338	-0.62341713	0.152502629	-4.086676728	4.38E-05	0.009345722
GAPDH	52662.88963	-0.305416077	0.059671038	-5.118334871	3.08E-07	2.25E-04
GLO1	10575.05692	-0.378285295	0.088983626	-4.251171958	2.13E-05	0.005452389
GMPR	1419.976031	-0.623012639	0.146498172	-4.249075181	2.15E-05	0.005452389
GNG10	503.7196804	-1.50434814	0.125038191	-12.01130075	3.10E-33	1.09E-28
GPD2	466.2275578	-1.06511104	0.248907179	-4.276324491	1.90E-05	0.005109281
GRK3	539.6784745	0.747734349	0.181774653	4.11750849	3.83E-05	0.008717509
GTF2A1	1680.225034	-0.623231976	0.136943857	-4.551690738	5.32E-06	0.002119771
GTF3C4	3548.615976	-0.353680161	0.085495387	-4.136632467	3.52E-05	0.008345884
GXYLT1	968.3548556	-0.582395431	0.132851712	-4.383305797	1.17E-05	0.00369136
HEATR1	7898.089295	-1.209715802	0.163778755	-7.380393238	1.58E-13	6.15E-10
HIC2	8957.804962	-0.31653236	0.076951273	-4.113138233	3.90E-05	0.008826945
HIST1H2AG	1208.208735	0.663700681	0.142325981	4.6660682	3.07E-06	0.001384726
HIST1H2AI	318.2237588	0.732372046	0.151060294	4.847680484	1.25E-06	7.03E-04
HIST1H3H	1123.847563	0.526122028	0.104474995	5.03576024	4.76E-07	3.01E-04
HLTF	2709.437451	-0.615919446	0.096008311	-6.414928433	1.41E-10	2.74E-07
HOXA11-AS	31.16973637	1.147174335	0.2707624	4.084045272	4.43E-05	0.009345722
IKBKE	1166.426436	0.769598672	0.178006162	4.409618671	1.04E-05	0.003399637
INIP	1245.960757	-0.538550025	0.12581992	-4.279873554	1.87E-05	0.005081313
IPMK	282.5940749	-0.634757253	0.140672499	-4.511242745	6.44E-06	0.002482558
ITK	85.98096379	1.04482082	0.239815215	4.373067631	1.23E-05	0.003795979
KCNC3	910.7410962	0.599185718	0.145772276	4.109970757	3.96E-05	0.008891536
KLHL15	982.5276483	-0.739833148	0.154656201	-4.780880827	1.75E-06	8.87E-04
LARP7	1579.849761	-0.745913643	0.159912807	-4.665325059	3.08E-06	0.001384726
LEPROT	1702.379464	-0.63721233	0.131683082	-4.839314132	1.30E-06	7.13E-04
LSM12	1881.625313	-0.624347966	0.123982047	-5.033999584	4.80E-07	3.01E-04
MAGEC1	3639.371644	-0.695192624	0.128785475	-5.397841222	6.74E-08	6.57E-05
MAK16	2616.733955	-0.630347262	0.105532133	-5.970331282	2.37E-09	3.19E-06
MANEAL	371.8953649	1.090081487	0.182309396	5.963929941	2.46E-09	3.20E-06
MAPK8IP2	331.799075	0.959058695	0.206471248	4.641028532	3.47E-06	0.001519018
MBD6	957.5331218	-0.778223816	0.16805977	-4.632498035	3.61E-06	0.001563449
MBNL3	630.8873905	-1.05662289	0.24302568	-4.356896711	1.32E-05	0.00388586
MBTPS1	2334.326514	-0.622830971	0.13917363	-4.476287187	7.60E-06	0.002832294
MED1	2132.891729	-0.977444012	0.093019701	-10.50540087	8.16E-26	1.43E-21
METTL14	790.5478656	-0.707132285	0.133285107	-5.30372875	1.13E-07	9.94E-05
MFN1	1086.257601	-0.850500724	0.151551573	-5.610766637	2.01E-08	2.28E-05
MFSD13A	345.8252317	0.915357241	0.212806276	4.319023742	1.57E-05	0.004430273
MIR3142HG	447.612206	-0.901224715	0.260784242	-4.890318292	1.01E-06	5.79E-04
MKRN1	2358.86747	-0.580810024	0.127429692	-4.557439054	5.18E-06	0.002086303
MOCS2	1483.781649	-0.509032833	0.095643233	-5.32166152	1.03E-07	9.24E-05
MPI	1056.210263	0.600248634	0.130269401	4.609644491	4.03E-06	0.001683204
MTHFD2	6841.52529	-0.443818917	0.10412958	-4.262960562	2.02E-05	0.005316885
MZT1	1284.36158	-0.630678601	0.125141864	-5.03858074	4.69E-07	3.01E-04
NAB2	869.5885864	-0.656817561	0.159774502	-4.118159165	3.82E-05	0.008717509
NDUFA7	839.863346	0.789436418	0.164206879	4.811055394	1.50E-06	7.85E-04
NET1	9959.364901	-0.462108701	0.082799652	-5.581493963	2.38E-08	2.61E-05
NPTXR	1011.578244	0.578606377	0.112082106	5.161762779	2.45E-07	1.95E-04
NUBP1	612.184135	-0.652886894	0.148547526	-4.393415681	1.12E-05	0.00355576
OTULIN	1514.790359	0.409093185	0.087861817	4.656352287	3.22E-06	0.001428126
PACSIN1	221.607155	0.790755785	0.166983792	4.7271648	2.28E-06	0.001093253
PAIP1	3963.481757	-0.595622812	0.080979739	-7.354944902	1.91E-13	6.70E-10
PEA15	1571.566406	-0.652414096	0.115417035	-5.651721654	1.59E-08	1.92E-05
PLEKHB2	3139.932104	-0.513142297	0.119649927	-4.288048032	1.80E-05	0.004936178
PNO1	1731.167975	-0.633147372	0.139847272	-4.523707464	6.08E-06	0.002389712

POC1B	646.4225934	0.875063113	0.132466593	6.603326302	4.02E-11	9.39E-08
POLR2F	1264.912196	-0.53858706	0.128624349	-4.186516971	2.83E-05	0.006895406
POMP	3486.914139	-0.460767965	0.074604893	-6.176127681	6.57E-10	1.05E-06
PPTC7	2179.910062	-0.310423402	0.076141134	-4.077043184	4.56E-05	0.009559973
PTER	953.4890551	-0.689600015	0.152429223	-4.521659028	6.14E-06	0.002389712
PTPN12	2702.748934	-0.861218039	0.18648092	-4.626633587	3.72E-06	0.001588744
PTPRVP	276.0043486	1.114025525	0.197630013	5.637963388	1.72E-08	2.01E-05
R3HDM2	935.282274	0.752824814	0.184027945	4.094586846	4.23E-05	0.009265425
RAP1B	7100.896628	-0.454072263	0.102308029	-4.438652312	9.05E-06	0.003094404
RNF138	2587.031962	-0.390806368	0.088830206	-4.398968472	1.09E-05	0.003497795
RNH1	2091.607726	-0.537763434	0.120282025	-4.470572238	7.80E-06	0.002833899
ROCK2	3385.706234	-0.490079899	0.119982335	-4.084068781	4.43E-05	0.009345722
RP11-334C17.5	120.2184333	0.957189918	0.202006016	4.727822386	2.27E-06	0.001093253
RP11-603J24.9	3869.862138	-0.521408393	0.120637584	-4.321853591	1.55E-05	0.004430273
RP11-875O11.3	9.948627239	0.74604013	0.26778873	4.133771507	3.57E-05	0.008345884
RPL10	49495.71977	-0.26920369	0.061775774	-4.357787006	1.31E-05	0.00388586
RTN4	8188.411943	-0.506666206	0.080449085	-6.298075257	3.01E-10	5.03E-07
SCG3	152.3543929	1.822803914	0.267797019	6.780598787	1.20E-11	3.23E-08
SCRT1	31.43058098	1.357431633	0.269604573	4.979464988	6.38E-07	3.92E-04
SCRT2	45.84299153	1.926214354	0.248029893	7.436529329	1.03E-13	4.53E-10
SEC11A	3647.011231	0.680117375	0.087031654	7.814216133	5.53E-15	3.23E-11
SEC24A	2141.49027	-0.657664718	0.11798447	-5.575434993	2.47E-08	2.62E-05
SHKBP1	1857.144723	0.45039076	0.103201963	4.364332563	1.28E-05	0.003820222
SHOC2	782.4408882	-1.209070327	0.199707615	-6.039606688	1.54E-09	2.26E-06
SLC35B3	350.5819821	-0.862582374	0.178289303	-4.836335039	1.32E-06	7.13E-04
SPAST	1071.569298	-0.605911466	0.117856711	-5.138744286	2.77E-07	2.15E-04
SRP68	3439.752812	-0.571391622	0.14047189	-4.066388266	4.77E-05	0.009845225
SSR1	5059.530248	-0.326178565	0.075633507	-4.312504284	1.61E-05	0.004526485
ST3GAL3	405.142172	0.856733278	0.191580748	4.483122147	7.36E-06	0.002772534
STAT5B	3301.838107	-0.783171063	0.176041274	-4.445995121	8.75E-06	0.003036282
STX6	1067.6539	-0.745258398	0.153811853	-4.845275075	1.26E-06	7.03E-04
STYX	722.7263161	-0.649911439	0.146882443	-4.422378747	9.76E-06	0.003279127
SYN1	72.78659605	1.211898226	0.228733781	5.249729378	1.52E-07	1.27E-04
SYP	373.849388	0.806137838	0.180063097	4.469450716	7.84E-06	0.002833899
TBP	866.322084	-0.633323743	0.131067961	-4.830084071	1.36E-06	7.25E-04
TCEB3	3527.689321	-0.463463736	0.103629224	-4.471933444	7.75E-06	0.002833899
TDG	3247.17139	-0.762901355	0.093593222	-8.150576758	3.62E-16	2.54E-12
TEX2	864.0219057	-0.9023587	0.102553834	-8.794472622	1.44E-18	1.26E-14
TJP2	2936.890677	-0.424407055	0.086411947	-4.912925329	8.97E-07	5.24E-04
TMEM198	493.0061422	0.76397826	0.174880305	4.368854163	1.25E-05	0.003795979
TMEM38B	3035.972139	-0.46899103	0.115103923	-4.075992532	4.58E-05	0.009559973
TOPORS	973.3195968	-0.539016083	0.106556108	-5.058166748	4.23E-07	2.85E-04
TRAPPC9	784.5683384	0.881250554	0.216293913	4.085747495	4.39E-05	0.009345722
UBE2D1	546.0303502	-0.832475167	0.129277151	-6.437928386	1.21E-10	2.50E-07
UBE2D3	9159.712555	-0.385190697	0.075887529	-5.075916294	3.86E-07	2.70E-04
UBE2K	5570.087248	-0.481275419	0.108018628	-4.455174768	8.38E-06	0.002967994
UBE2L6	353.1212634	0.856611233	0.183198943	4.692576528	2.70E-06	0.001260906
UNC13A	1216.337622	1.297242699	0.242273158	5.351513659	8.72E-08	8.26E-05
URI1	3893.079644	-0.464686655	0.096867689	-4.796699175	1.61E-06	8.31E-04
UTP6	2523.994631	-0.834354319	0.135792358	-6.14203848	8.15E-10	1.24E-06
WASL	1190.83742	-0.513056084	0.118505359	-4.32935599	1.50E-05	0.004332262
WWOX	161.7687959	0.829524065	0.203201765	4.087225223	4.37E-05	0.009345722
XKR7	35.72936522	2.258283127	0.274789527	7.313730453	2.60E-13	8.28E-10
YTHDF3	2775.416422	-0.467494505	0.106817012	-4.376216689	1.21E-05	0.00377937
ZDHHC11	33.03221586	1.114376644	0.265263671	4.239427469	2.24E-05	0.005610748
ZFP36L2	2722.944202	-0.43894394	0.080560072	-5.448517787	5.08E-08	5.09E-05

Supplementary Table 10: Differentially expressed genes in RNAseq - TNFα

Symbol	baseMean	log2FoldChange	lfcSE	stat	pvalue	padj
AC018462.2	133.0046429	1.645110631	0.277036172	8.973066971	2.88E-19	2.02E-15
AFG3L1P	1422.560617	-0.519787121	0.107460556	-4.836754729	1.32E-06	7.71E-04
ANLN	4125.806047	-0.675847771	0.145787768	-4.635016563	3.57E-06	0.001603939
AP000477.3	18.45508301	1.21758497	0.268783969	4.251200081	2.13E-05	0.006370302
AP3D1	4708.850784	-0.417051515	0.087580483	-4.761810341	1.92E-06	0.001034678
BICD2	1830.991089	-0.643431602	0.097448681	-6.602942207	4.03E-11	1.09E-07
BTG2	3025.99532	-0.769826962	0.133292728	-5.763120686	8.26E-09	1.11E-05
C19orf66	281.5340007	1.150500558	0.179926474	6.3706451	1.88E-10	4.40E-07
CALB1	151.2261885	0.821764614	0.19392355	4.234514015	2.29E-05	0.006690639
CAST	1824.080465	-1.04176985	0.115596413	-9.012925015	2.01E-19	1.76E-15
CAV2	1100.320044	-0.896365465	0.107127624	-8.36816114	5.85E-17	3.42E-13
CCNB2	3378.363739	-0.339894595	0.081592587	-4.165649765	3.10E-05	0.008847805
CD46	8449.062218	-0.850939386	0.085274685	-9.979035218	1.88E-23	2.20E-19
CDK18	25.78075742	1.161702019	0.274122708	4.359461562	1.30E-05	0.004663582
CECR2	979.1027221	-0.624410742	0.144003999	-4.33380042	1.47E-05	0.004892633
CEP120	802.861589	-0.795764546	0.153611426	-5.180944991	2.21E-07	1.80E-04
CHST3	197.8533322	1.297728991	0.22367842	5.698249748	1.21E-08	1.52E-05
CPLX1	276.3349715	1.063145083	0.243266263	4.348707177	1.37E-05	0.004752715
CSNK1A1	5586.719889	-0.389197532	0.085167049	-4.569901699	4.88E-06	0.002111632
CTB-131K11.1	275.429101	-0.750782366	0.152026144	-4.935849005	7.98E-07	4.91E-04
DCTPP1	2080.414904	0.547292511	0.094454023	5.793937923	6.88E-09	1.01E-05
DDI2	4668.192337	-0.676307019	0.163740106	-4.133560347	3.57E-05	0.009858627
DLK1	4040.676426	-0.431121955	0.079736458	-5.406730177	6.42E-08	6.62E-05
DNAJC25-GNG10	342.4383828	-1.366590078	0.172620354	-7.90873216	2.60E-15	1.14E-11
DSG2	3098.111431	-0.50552785	0.118451272	-4.267885957	1.97E-05	0.006014917
DUXAP8	915.578017	0.451485404	0.106217376	4.251471287	2.12E-05	0.006370302
DYNLL2	2549.185984	-0.334400316	0.076301409	-4.38279451	1.17E-05	0.004369193
E2F3	4453.809883	-0.593560397	0.113005963	-5.251687027	1.51E-07	1.42E-04
EFR3A	1115.33376	-0.772676021	0.149908513	-5.154327871	2.55E-07	1.94E-04
ERO1A	1719.691359	-0.569929492	0.118261261	-4.81910831	1.44E-06	8.29E-04
EZH2	5923.471064	-0.311299324	0.06947976	-4.480374365	7.45E-06	0.002976282
FAM114A2	447.3981296	0.701833385	0.133711576	5.247363339	1.54E-07	1.42E-04
FAM168B	5497.699544	-0.591038792	0.123035101	-4.803782471	1.56E-06	8.80E-04
FAM3C	1095.917856	-0.911237499	0.14614973	-6.238944286	4.41E-10	9.08E-07
FBXO28	2342.749855	-0.554105089	0.122624503	-4.51843879	6.23E-06	0.002599651
FTL	30872.23199	-0.399371943	0.072891611	-5.478909522	4.28E-08	4.69E-05
FUT4	1303.131395	-0.544131855	0.124386159	-4.372045528	1.23E-05	0.004541682
GAPDH	52662.88963	-0.33044397	0.059657096	-5.539047069	3.04E-08	3.44E-05
GMPR	1419.976031	-0.895020191	0.147007982	-6.092327899	1.11E-09	2.17E-06
GNG10	503.7196804	-1.314667587	0.122533763	-10.71281344	8.86E-27	3.11E-22
GPD2	466.2275578	-1.060022715	0.248798809	-4.283715681	1.84E-05	0.005752377
GTF2A1	1680.225034	-0.626067627	0.136573113	-4.58312331	4.58E-06	0.002007142
GTF3C2-AS1	64.05092851	1.167524865	0.278732522	4.33164582	1.48E-05	0.004894155
GXYLT1	968.3548556	-0.676776545	0.133087499	-5.08496667	3.68E-07	2.69E-04
HEATR1	7898.089295	-0.960585015	0.16382231	-5.868597731	4.39E-09	7.59E-06
HIC2	8957.804962	-0.386447235	0.077122652	-5.011089047	5.41E-07	3.65E-04

HIST1H2AG	1208.208735	0.592168109	0.141561843	4.179981149	2.92E-05	0.008376324
HIST1H2AI	318.2237588	0.651450179	0.14974549	4.346309016	1.38E-05	0.004757843
HMGA1	31933.69578	-0.343974256	0.072984105	-4.712973129	2.44E-06	0.001205272
HN1	2451.656655	-0.348101089	0.077853668	-4.471072543	7.78E-06	0.003065298
HOOK1	1452.428162	0.811254325	0.171290197	4.741735296	2.12E-06	0.001108592
IPMK	282.5940749	-0.832052985	0.139334169	-5.965705383	2.44E-09	4.49E-06
KIAA1324L	1502.524222	0.617672862	0.122379338	5.047290138	4.48E-07	3.14E-04
KLHL15	982.5276483	-0.796758424	0.154804452	-5.148003464	2.63E-07	1.96E-04
KLHL8	1193.164707	-0.814669454	0.174753109	-4.670332709	3.01E-06	0.001424442
KMT5A	5602.971356	-0.402868411	0.068714121	-5.863056995	4.54E-09	7.59E-06
LAMP1	3248.775194	-0.606451823	0.141811697	-4.277072101	1.89E-05	0.005822718
LEPROT	1702.379464	-0.565960213	0.131271859	-4.310597595	1.63E-05	0.005284368
LIN54	1131.177515	-0.526317124	0.122011533	-4.314763649	1.60E-05	0.005234158
LINC01358	57.06563437	1.261021497	0.262779392	5.186934881	2.14E-07	1.78E-04
LRRC61	2681.29977	-0.521403249	0.116317278	-4.479782616	7.47E-06	0.002976282
LSM12	1881.625313	-0.650840861	0.124263127	-5.238616121	1.62E-07	1.45E-04
MAFG	3137.570726	-0.585696653	0.137998137	-4.242776473	2.21E-05	0.006503106
MAGEC1	3639.371644	-0.532070384	0.128706534	-4.133941423	3.57E-05	0.009858627
MAK16	2616.733955	-0.528768425	0.106320507	-4.975372658	6.51E-07	4.08E-04
MANEAL	371.8953649	1.176077793	0.183486279	6.413000775	1.43E-10	3.57E-07
MAPK8IP2	331.799075	0.966903405	0.20634696	4.684019458	2.81E-06	0.001350757
MDM4	2479.93453	-0.513461328	0.105625908	-4.861016897	1.17E-06	6.94E-04
MECOM	937.5492965	-0.745722287	0.172069299	-4.340806666	1.42E-05	0.004784754
MED1	2132.891729	-0.943931718	0.092994256	-10.14973188	3.32E-24	5.82E-20
MEX3D	822.0181737	-0.663644787	0.131115655	-5.061538807	4.16E-07	2.98E-04
MFN1	1086.257601	-0.855935034	0.151346232	-5.655420777	1.55E-08	1.88E-05
MGLL	50.14494022	1.0213309	0.241395961	4.246943128	2.17E-05	0.006437482
MIB1	4167.061858	-0.508165721	0.101582896	-5.002193426	5.67E-07	3.68E-04
MIR3142HG	447.612206	-1.848189362	0.240416177	-7.087327914	1.37E-12	4.58E-09
MKRN1	2358.86747	-0.665832963	0.127365022	-5.227807408	1.72E-07	1.50E-04
MMP24	17.17392989	1.256686036	0.278206478	4.435784112	9.17E-06	0.003572982
MYBPHL	81.57151661	1.154009015	0.267681239	4.413868512	1.02E-05	0.003877051
OR51B4	143.0919339	0.881594413	0.204921515	4.303737485	1.68E-05	0.005351658
OTULIN	1514.790359	0.439190983	0.087941232	4.993380913	5.93E-07	3.78E-04
PACSIN1	221.607155	0.795110508	0.167430345	4.751856341	2.02E-06	0.001070484
PAIP1	3963.481757	-0.405866392	0.080963407	-5.012985103	5.36E-07	3.65E-04
PDZD8	2241.882894	-0.630272384	0.140388255	-4.490020261	7.12E-06	0.002936881
PEA15	1571.566406	-0.721199277	0.115276743	-6.256063421	3.95E-10	8.65E-07
PER2	380.0617157	-0.857612741	0.166145105	-5.164179246	2.41E-07	1.89E-04
PFDN2	1652.368886	0.458698392	0.098744546	4.645524396	3.39E-06	0.001564534
PKD1	2087.800899	-0.509527738	0.113561037	-4.486536491	7.24E-06	0.002950579
PNO1	1731.167975	-0.612799189	0.140340329	-4.369260963	1.25E-05	0.00455206
POLR2F	1264.912196	-0.616965448	0.128607931	-4.797561975	1.61E-06	8.80E-04
POMP	3486.914139	-0.318109374	0.074337662	-4.279164507	1.88E-05	0.0058193
POU2F1	1308.596644	-1.123366868	0.194196017	-5.793011666	6.91E-09	1.01E-05
PTER	953.4890551	-0.694666272	0.152463466	-4.557205088	5.18E-06	0.002215982
RIMBP3C	26.60914149	0.916462803	0.263965033	4.404363467	1.06E-05	0.003998866
RNF138	2587.031962	-0.38750686	0.089249571	-4.342059977	1.41E-05	0.004784754
RP11-351I24.3	59.26634588	-1.403132058	0.243267276	-5.733556097	9.83E-09	1.28E-05

SCG3	152.3543929	1.2823906	0.267799227	4.87668506	1.08E-06	6.52E-04
SCRT2	45.84299153	1.413747993	0.236970017	5.769892098	7.93E-09	1.11E-05
SDSL	164.3620754	0.880412464	0.202021085	4.353844098	1.34E-05	0.00468907
SEC11A	3647.011231	0.720030832	0.086959121	8.279729598	1.23E-16	6.18E-13
SH3BGRL	2021.487657	0.607894636	0.130611101	4.653755797	3.26E-06	0.00152337
SHC2	103.4811458	1.084590265	0.224421465	4.798245126	1.60E-06	8.80E-04
SHOC2	782.4408882	-1.41178561	0.199848767	-7.080376188	1.44E-12	4.58E-09
SPIRE2	710.9655051	0.472021482	0.108232007	4.361582612	1.29E-05	0.004663582
SPRED1	751.0186963	-0.807563892	0.187461039	-4.304980913	1.67E-05	0.005351658
SPTBN4	83.07772542	1.047739187	0.222522687	4.715265328	2.41E-06	0.001205272
STAT5B	3301.838107	-0.825487434	0.176106816	-4.690401103	2.73E-06	0.001327486
STX6	1067.6539	-0.866523744	0.153861052	-5.630640846	1.80E-08	2.10E-05
SYN1	72.78659605	1.188750269	0.22898385	5.162970147	2.43E-07	1.89E-04
TAP2	1189.230643	0.785930577	0.143988349	5.454120756	4.92E-08	5.23E-05
TBP	866.322084	-0.655856643	0.131108219	-5.003295386	5.64E-07	3.68E-04
TDG	3247.17139	-0.48781356	0.093551269	-5.214510147	1.84E-07	1.58E-04
TEX2	864.0219057	-0.75603036	0.104464519	-7.240293201	4.48E-13	1.74E-09
TGIF1	705.3243339	-0.523257796	0.120038046	-4.357185145	1.32E-05	0.004664734
UBE2D1	546.0303502	-0.554339029	0.129213868	-4.288783637	1.80E-05	0.005673352
UBE2D3	9159.712555	-0.334366385	0.075760054	-4.413405893	1.02E-05	0.003877051
UBE2L6	353.1212634	0.832153851	0.180266667	4.599325713	4.24E-06	0.001880707
UNC13A	1216.337622	1.40894858	0.24218703	5.839828704	5.23E-09	8.33E-06
USP15	8753.017934	-0.692457188	0.164883937	-4.208330847	2.57E-05	0.007452789
UTP6	2523.994631	-0.714620829	0.135939537	-5.258500326	1.45E-07	1.41E-04
XKR7	35.72936522	2.276534423	0.275335243	6.686301739	2.29E-11	6.69E-08
ZBED6CL	4680.405075	-0.517774072	0.111632236	-4.635487226	3.56E-06	0.001603939
ZFP36L2	2722.944202	-0.381160814	0.080447019	-4.738018634	2.16E-06	0.00111251
ZNF410	780.2148912	-0.713092048	0.171134731	-4.163361211	3.14E-05	0.008864923
ZNF487	51.81741499	-1.42681756	0.267540719	-5.311966274	1.08E-07	1.09E-04
ZNF598	3481.476668	-0.386107791	0.092821373	-4.159609727	3.19E-05	0.008939688
ZNF777	1126.353094	-0.376870992	0.082844424	-4.549428781	5.38E-06	0.002271761

Supplementary figure legends

Supplementary figure 1

Gene annotation of the PTTG1-1:1 Locus, and conservation in vertebrates. A. Gene annotation of the PTTG1-1:1 (ENST00000517927) locus in the UCSC genome browser (https://genome.ucsc.edu) showing Multiz Alignment and Conservation in vertebrates. Bottom panel: zoom on chromatin structure and transcription factor binding sites in the promoter region for nucleotides -2000 to +500 relative to the transcriptional start site (TSS). **B.** Encode V3 regulation tracks in K562 and CD14$^+$ cells: localization of DNase hypersensitivity clusters (darker gray indicates higher signal, relative to the maximum signal); levels of activating histone methylation (H3K4me1, H3K4me3, H3K36me3), acetylation (H3K27Ac) and repressing histone marks (H3K27me3) in K562 cells (blue) and CD14$^+$ monocytes (black). All putative conserved transcription factor binding sites: CCAAT/enhancer binding protein (CEBP_01), NF-κB (NFKB_C, NFKAPPAB_01), NF-κB p65 (NFKAPPAB65_01), NF-κB cRel (CREL_01), octamer binding site (OCT_C), heat shock factor 1 and 2 (HSF1_01, HSF1_02).

Supplementary figure 2

Functional pathway analysis of PTTG1-1:1. Significant pathways associated with co-expressed genes (adjusted $p < 0.05$), obtained from the clusterProfiler library. Counts depict the number of associated co-expressed genes in each pathway. Larger gene ratio in pathways are represented by larger points and show a stronger impact on inflammatory pathways. FDR-adjusted P-values are color-coded.

Supplementary figure 3

Basal expression of PTTG1-1:1. A. Basal expression levels of PTTG1-1:1 relative to β-actin in three different HSPC samples and various cell lines (in triplicate), as indicated. **B.** Average expression levels of PTTG1-1:1 relative to β-actin in HSPCs, leukemic (K562, MEG01, U937, TF-1, KG1, MV4-11, MOLM13, THP1, KG1, HL60, Raji and Jurkat) and solid tumor cell lines (LnCAP and PC3). Data were expressed as the average ± SEM. Significance: * $P<0.05$; ** $P<0.01$.

Supplementary figure 4

Effect of pro-inflammatory mediators on PTTG1-1:1 expression. K562, U937 and TF-1 cells were induced by pro-inflammatory mediators IL-1β, LPS and poly(I:C) after 6, 24 and 48 h. Results are expressed relative to β-actin and as a ratio of treated compared to untreated cells (CT). Significant results were obtained with IL-1β in K562 and TF-1, and with LPS in U937. Data were expressed as the average ± SEM. Significance: ns: Not Significant; * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

Supplementary figure 5

Localization and comparison of the expression of PTTG1-1:1. A. Barplot of the quantification of PTTG1-1:1 in the cytoplasm and nucleus of K562, U937 and TF-1 cells after TNFα treatment. The results are normalized to RNU6b in the nucleus and β-actin in the cytoplasm and show stronger induction in the cytoplasm. **B.** Fluorescence in Situ Hybridization (FISH) using DAPI (Blue) for nuclear staining on cells treated for 6 hours with TNFα. Biotinylated probes were localized using an antibody conjugated to horseradish peroxidase (HRP)-streptavidin and revealed by Alexa Fluor 594 Tyramide reagent (Red). The positive control consists of a mix of U1 and U2 snRNAs probes. The positive control shows a strong fluorescence in the nucleus. Samples stained by even and odd probes targeting PTTG1-1:1 show a diffuse fluorescence in the cytoplasm. Data were expressed as the average ± SEM. Significance: ns: Not Significant; * P<0.05; ** P<0.01.

Supplementary figure 6

Effect of NF-κB inhibition. A. by MG132 (1 h of pretreatment) on the expression of SP1 and SP3 mRNA (NF-κB independent genes) and PTTG1-1:1 in K562, U937 and TF-1 cells treated with and without TNFα and IL-1β for 6 hours. Results are expressed relative to β-actin as a ratio of treated *vs.* untreated cells (CT). Results show the inhibitory effect of MG132 on NF-κB-induced PTTG1-1:1 expression in all cell lines and the IL-1β-induced PTTG1-1:1 expression in TF-1 cells. MG132, TNFα and IL-1β did not affect the expression of SP1 and SP3, except in U937 cells. **B.** Expression of p65, SP1 and SP3 mRNA as well as PTTG1-1:1 in K562 cells transfected with siRNA against p65 (RELA) with TNFα treatment for 6 hours. Results are expressed relative to β-actin and normalized to the control (CT). Data were expressed as the average ± SEM. Significance: ns: Not Significant; * P<0.05; ** P<0.01; *** P<0.001, **** P<0.0001..

Supplementary figure 7

Pathways affected by TNFα or PTTG1-1:1 knockdown in RNAseq experiments. A. Gene Set Enrichment Analysis: Comparison of gene sets associated with genes that are up- or down-regulated by PTTG1-1:1 silencing, either without TNF α (basal) or in TNF α-induced samples. **B.** Consistency of pathways affected by TNFα in RNAseq experiments in K562 cells. Larger gene proportions in pathways are represented by larger points. P-values are represented as a color.

Supplementary figure 8

Expression of PTTG1-1:1 in HSPC, AML patients and cell lines. A. PTTG1-1:1 expression was assessed in hematopoietic stem progenitor cells (HSPC), in 14 AML patients and in U937 and TF-1 cells. Dot plots present PTTG1-1:1 quantification in each sample. AML Patient sample data summarized on Supplementary Table 8. **B.** Distribution of PTTG1-1:1 (Named MIR146Ain this database*)* expression was plotted in AML patients (*n* = 437), healthy bone marrow mononuclear cells (BM MNC, *n* = 19) and healthy $CD34^+$ cells (*n* = 12). An ANOVA was performed using a Kruskal-Wallis test and a Wilcoxon test was conducted to assess the significance between the different groups.

Supplementary figure 9

Multivariable analysis of the dataset associated with the patients' survival. A. TCGA dataset and **B.** Verhaak dataset. Forest plot that showing hazard ratios estimates as well as confidence interval and p-values. AIC: Akaike Information Criterion. Significance: * P<0.05; ** P<0.01; *** P<0.001

Supplementary figure 10

Relative expression levels of PTTG1-1:1 in K562 and TF-1 cells co-transfected with Si623 and Gapmer01 (targeting PTTG1-1:1) with and without TNFα. Co-transfection inhibits TNFα-induced PTTG1-1:1 expression with higher efficiency in both cellular compartments (GapmeR target the nuclear RNA and siRNA target the cytoplasmic RNA). Results are expressed relative to β-actin and normalized to the control (CT). Data were expressed as the average ± SEM. Significance: * P<0.05; ** P<0.01; *** P<0.001; **** P<0.0001.

Supplementary figure 11

Quantification of the effect of PTTG1-1:1 knockdown on the K562 phenotype. A. Relative amounts (in percentage) of K562 cells showing a monocyte/macrophage-like phenotype after MGG staining, 72 h after transfection by siRNA-control + GapmeR-control (SiCt + GapA) or by siRNA and GapmeRs targeting PTTG1-1:1 (Si623 + Gap01) in K562 cells. **B.** Average size (in pixels, measured with ImageJ software) of cells with a basal phenotype (Undiff) compared to those with a monocyte/macrophage-like phenotype (Diff). Data were expressed as the average ± SEM. Significance: **** $P<0.0001$.

Supplementary figure 12

PTTG1-1:1 expression during myeloid differentiation. PTTG1-1:1 (MIR3142HG) expression represented as normalized log2-transformed intensity values in hematopoietic cells throughout myeloid differentiation (GSE42519). Results show a progressive decrease of PTTG1-1:1 expression during myeloid differentiation.

Supplementary figure 13

Effect of PTTG1-1:1 over-expression on proliferation, cell cycle and cell differentiation. A. Determination of K562 cell proliferation by Incucyte videomicroscopy on plates coated with Poly-D-Lysine. **B.** Cell cycle analysis of K562 cells by flow cytometry after propidium iodide staining. Cells were transfected with control (pUC19) or plPTTG1-1:1 plasmids after 24, 48 and 72 h. **C.** Expression of CD11b, CD14 and CD15 markers in K562 and HL60 cells three days after transfection with plPTTG1-1:1 or pUC19 plasmids. Results were obtained by flow cytometry and are expressed as the ratio of the mean fluorescence intensity of the cells transfected with the plasmid overexpressing the lncRNA (plPTTG1-1:1) compared to the expression after transfection with the control plasmid (pUC19). Results are normalized to isotypic controls and show a decrease of CD15 expression in K562 cells as well as a decrease of CD11b and CD15 expression in HL60 cells. Data were expressed as the average ± SEM. Significance: ns: not significant;* $P<0.05$; ** $P<0.01$; *** $P<0.001$.

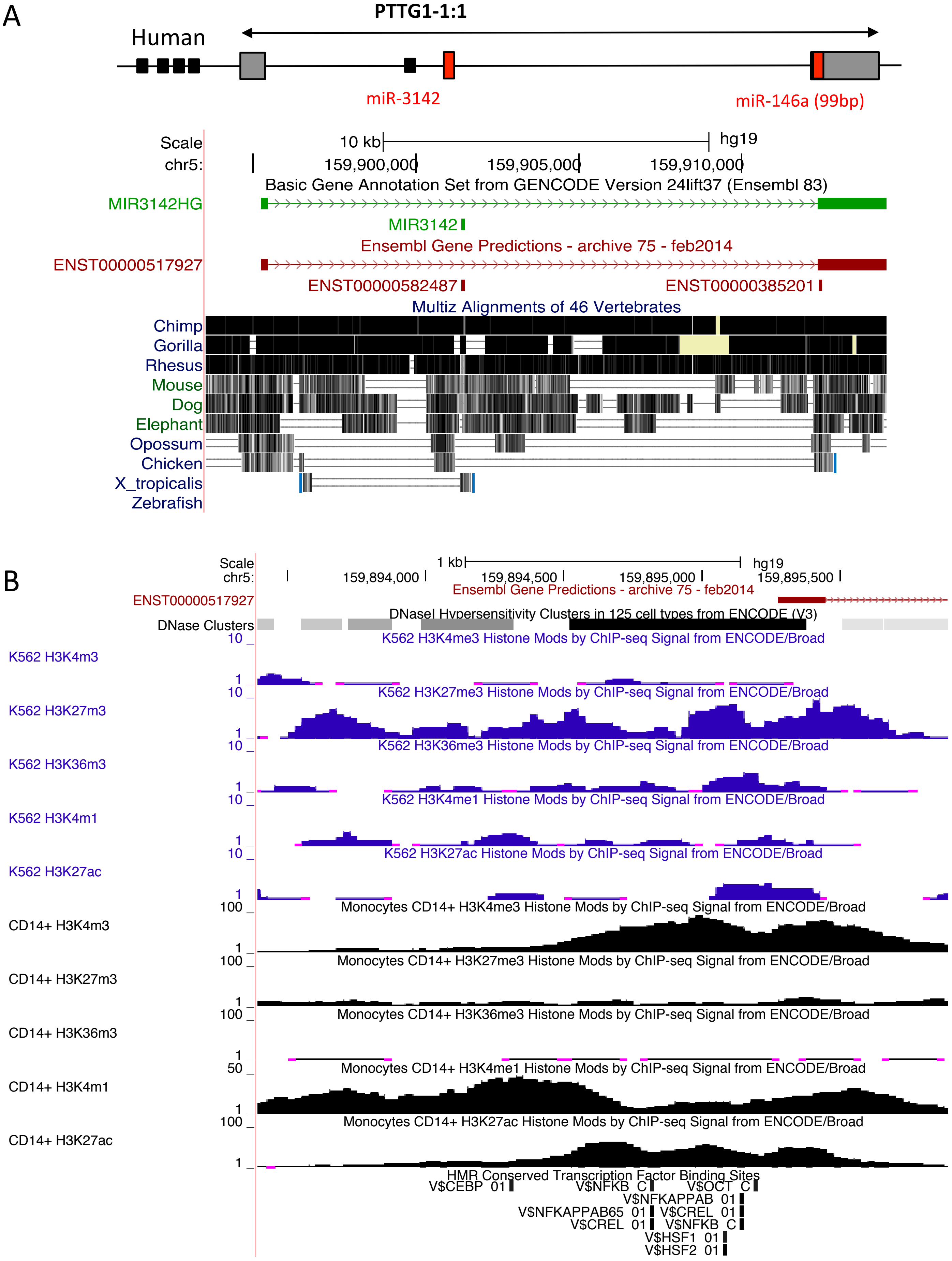
Supplementary figure 14

Most affected genes by PTTG1-1:1 knockdown. A. Heatmap showing the 168 significant differentially expressed genes (absolute fold change (FC) of 1.2, FDR < 0.01) after PTTG1-1:1 (MIR3142HG) knockdown in K562 cells. **B.** Heatmap showing the 126 significant differentially expressed genes (absolute fold change (FC) of 1.2, FDR < 0.01) after PTTG1-1:1 (MIR3142HG) knockdown in K562 cells treated with TNFα.

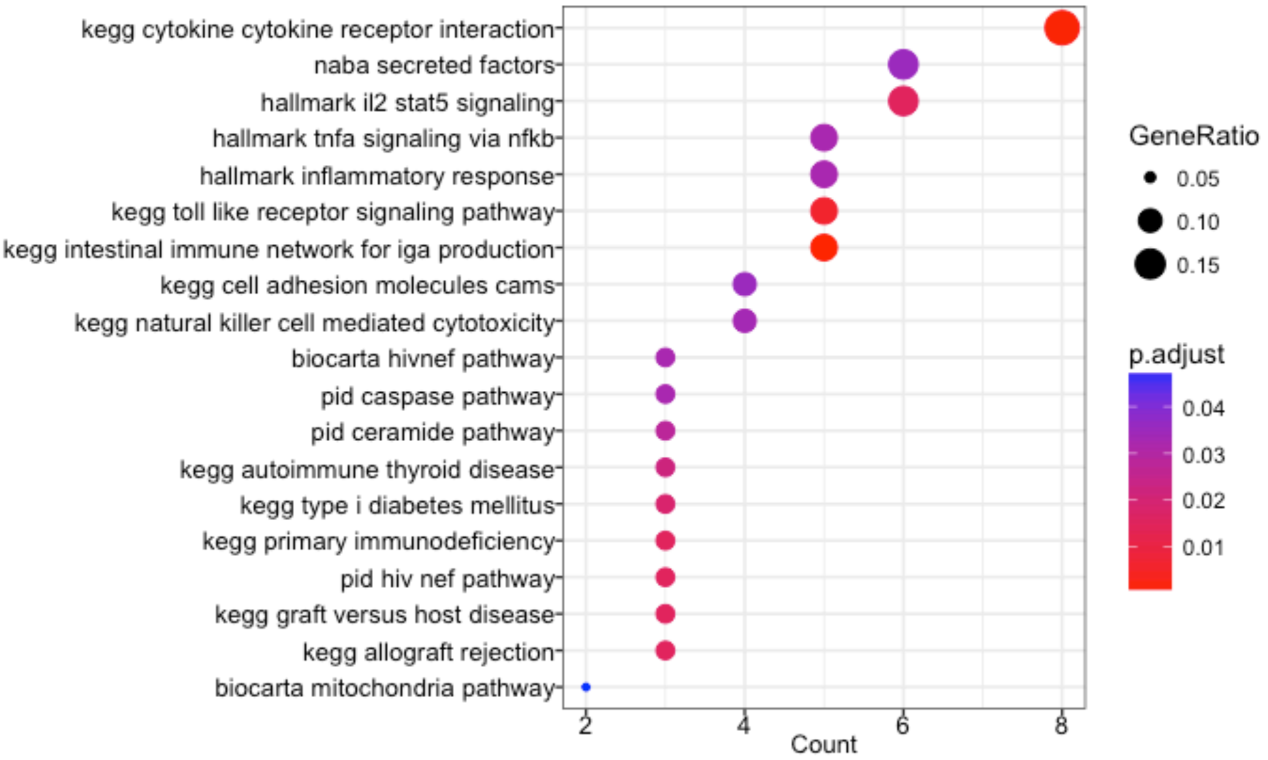
Supplementary figure 15

Effect of the PTTG1-1:1 knock down. Impacted gene ontology pathways and putative target proteins. **A.** Enrichment analysis of significant differentially expressed genes after PTTG1-1:1 (MIR3142HG) knockdown in K562 cells using the GOplot R package. Depicted genes are connected to their biological or molecular functions through colored ribbons. The outer circle represents $\log_2$FC of upregulated (red) or downregulated (blue) genes. The figure presents an over-representation of genes implicated in mRNA stabilization/processing and protein maturation/stability. **B.** Predicted PTTG1-1:1 target proteins. Putative target proteins (light blue squares) and their associated GO terms (orange circles) of PTTG1-1:1 (violet octagon). This result presents an over-representation of pathways implied in mRNA/siRNA stabilization, processing and interaction.

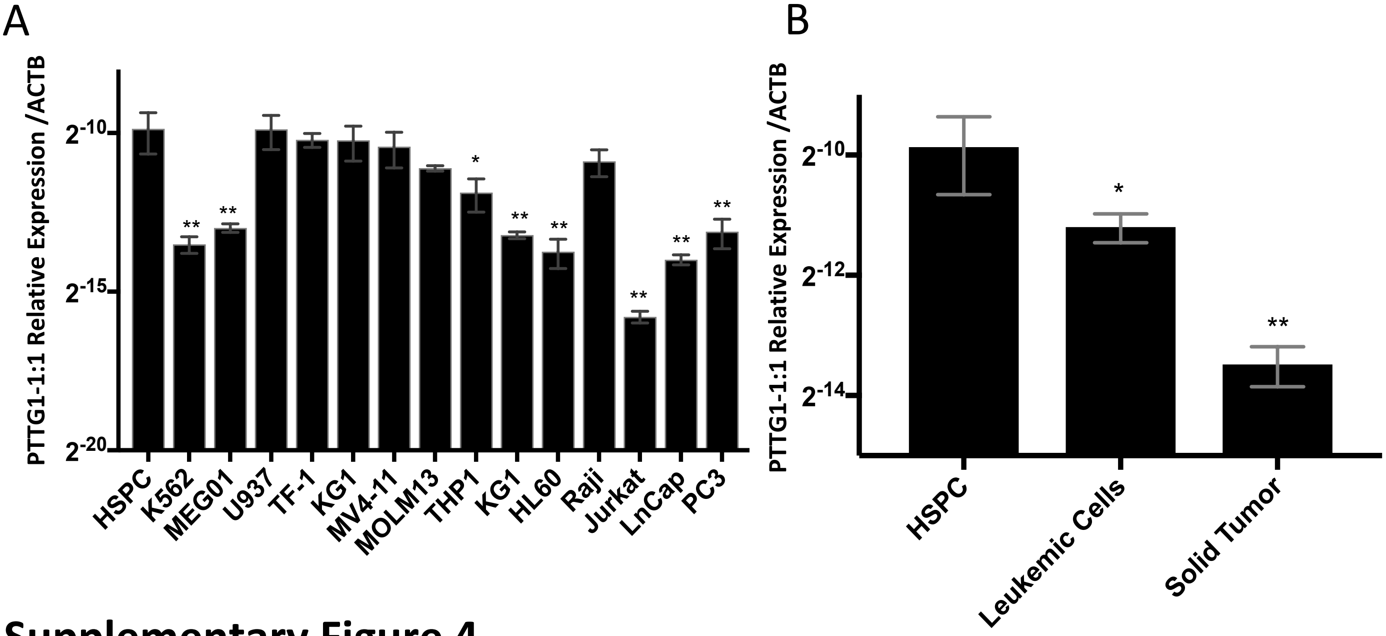
Supplementary Figure 1



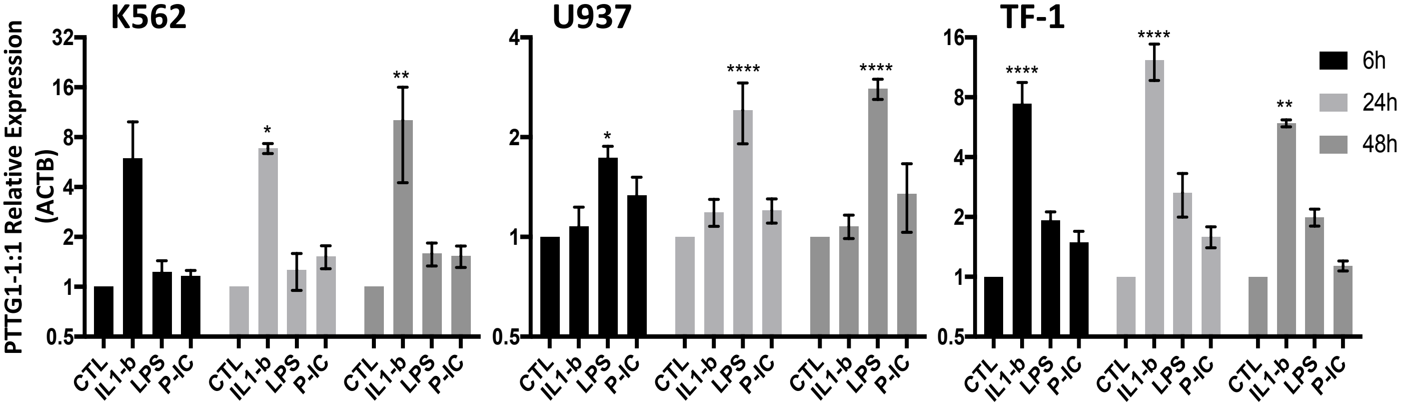
Supplementary Figure 2



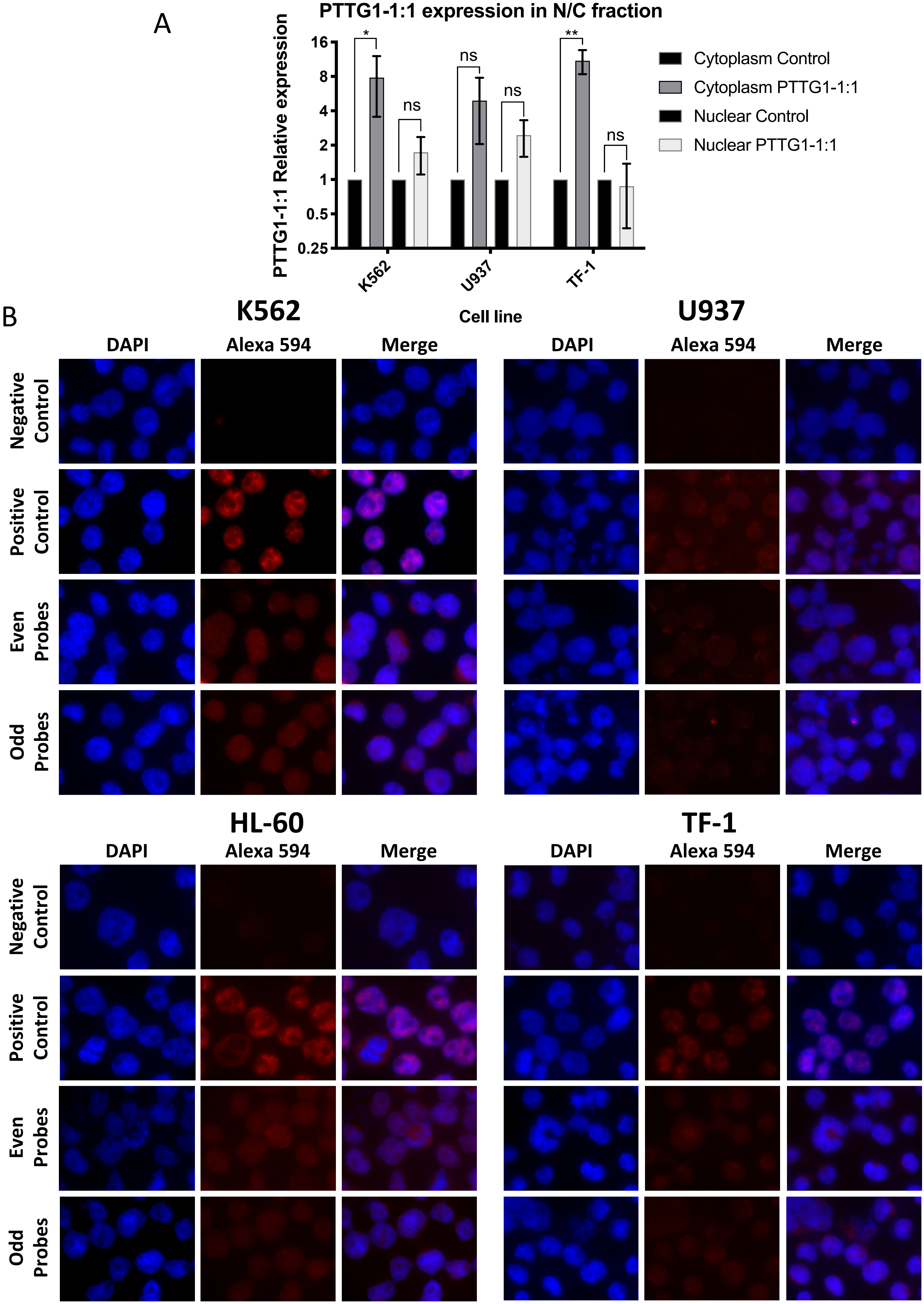
Supplementary Figure3



Supplementary Figure 4

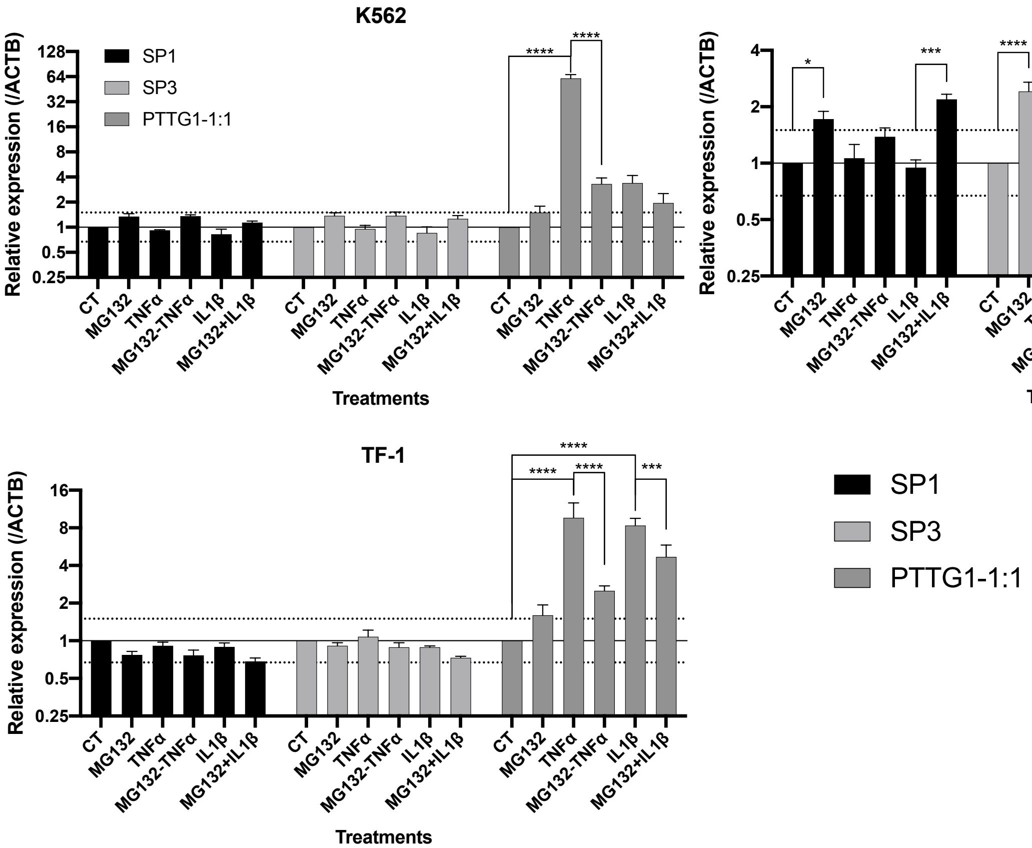


Supplementary Figure 5

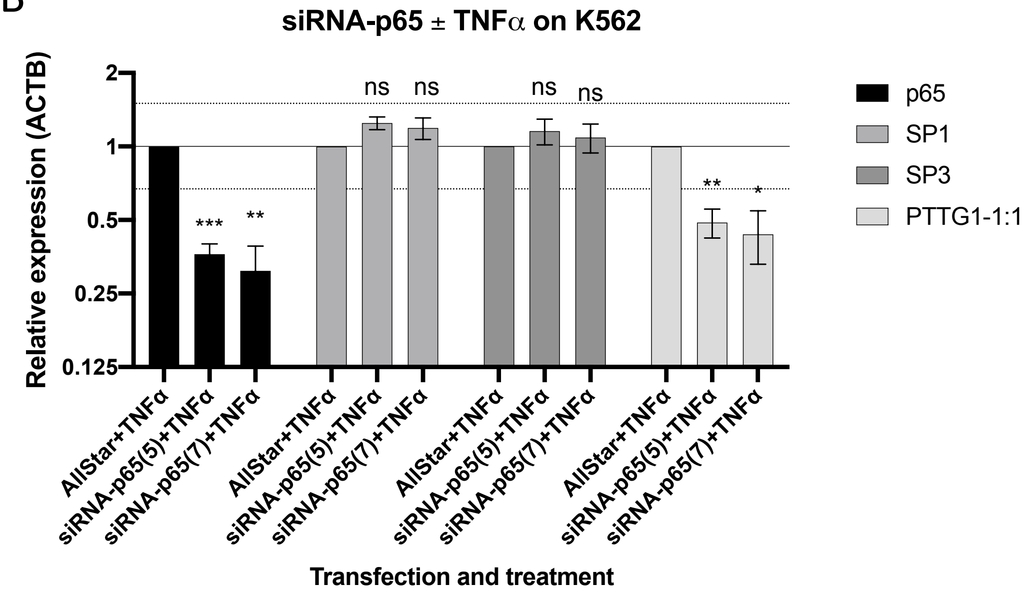


Supplementary Figure 6

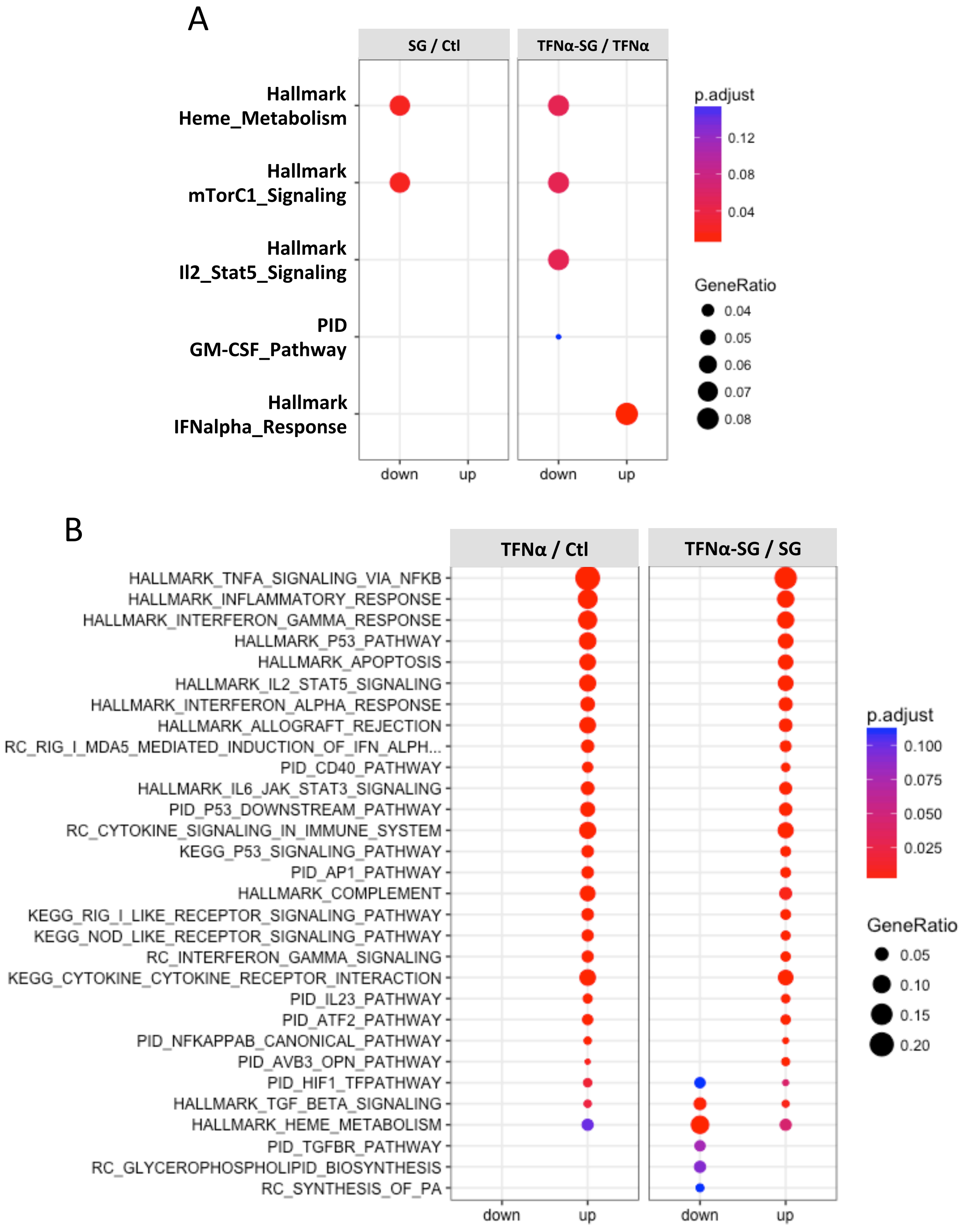
A



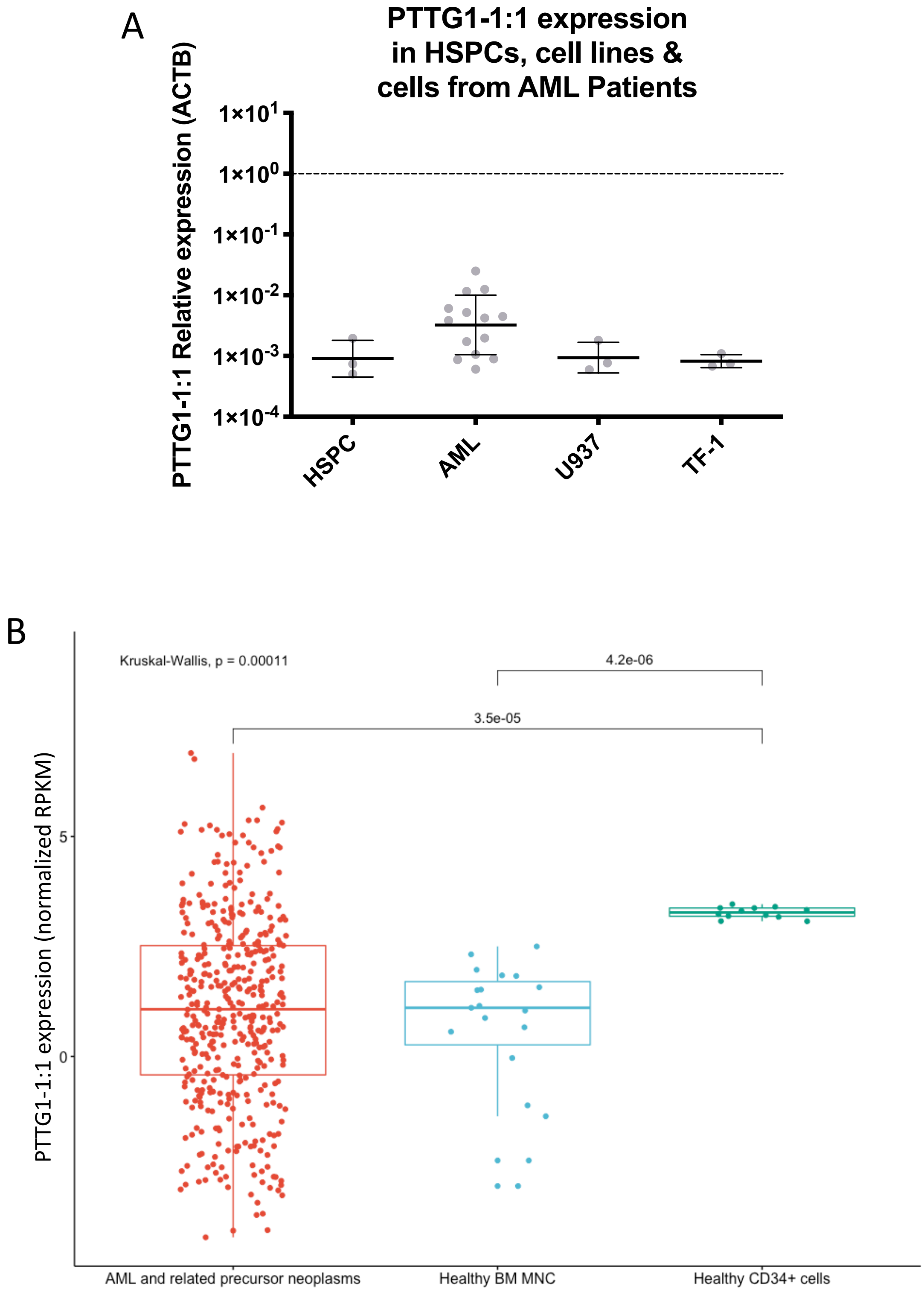
B



Supplementary Figure 7

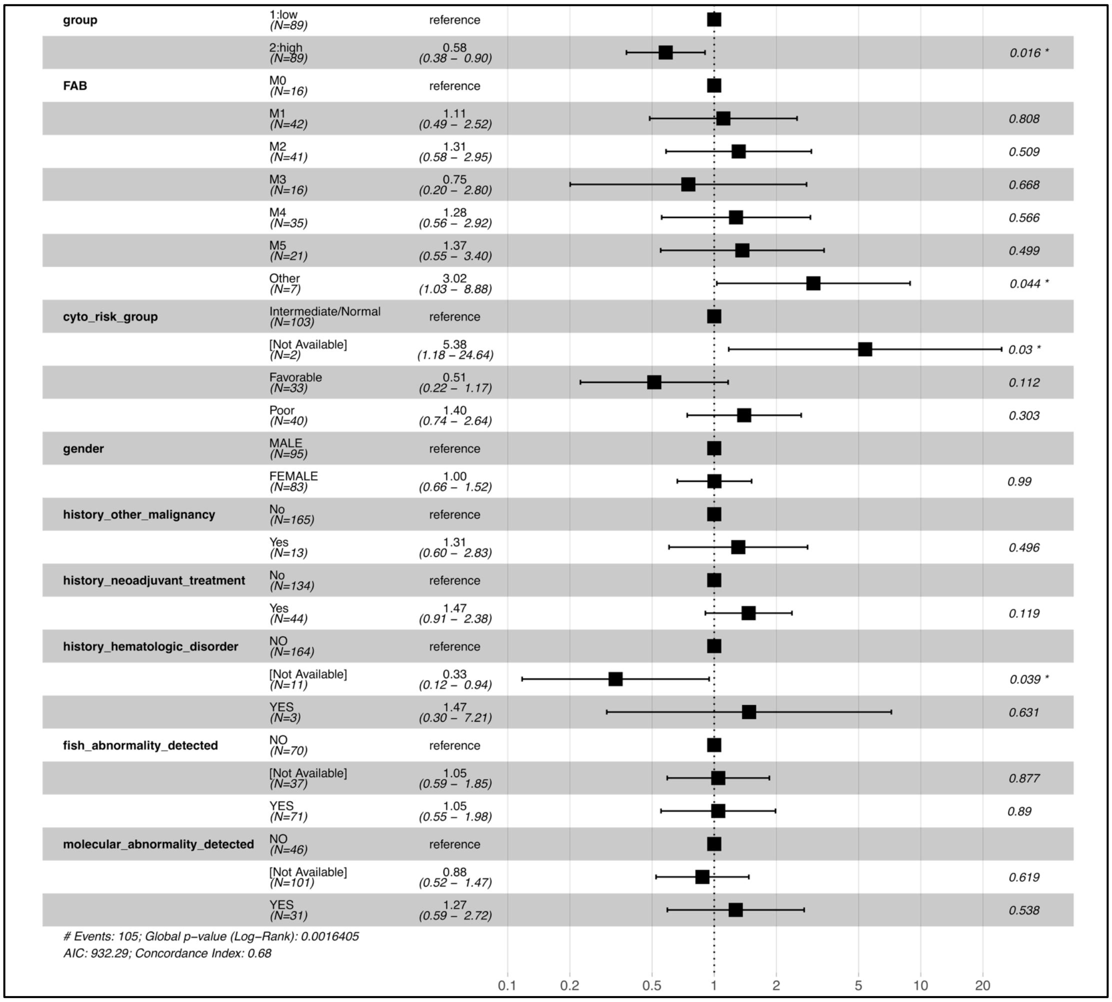


Supplementary Figure 8

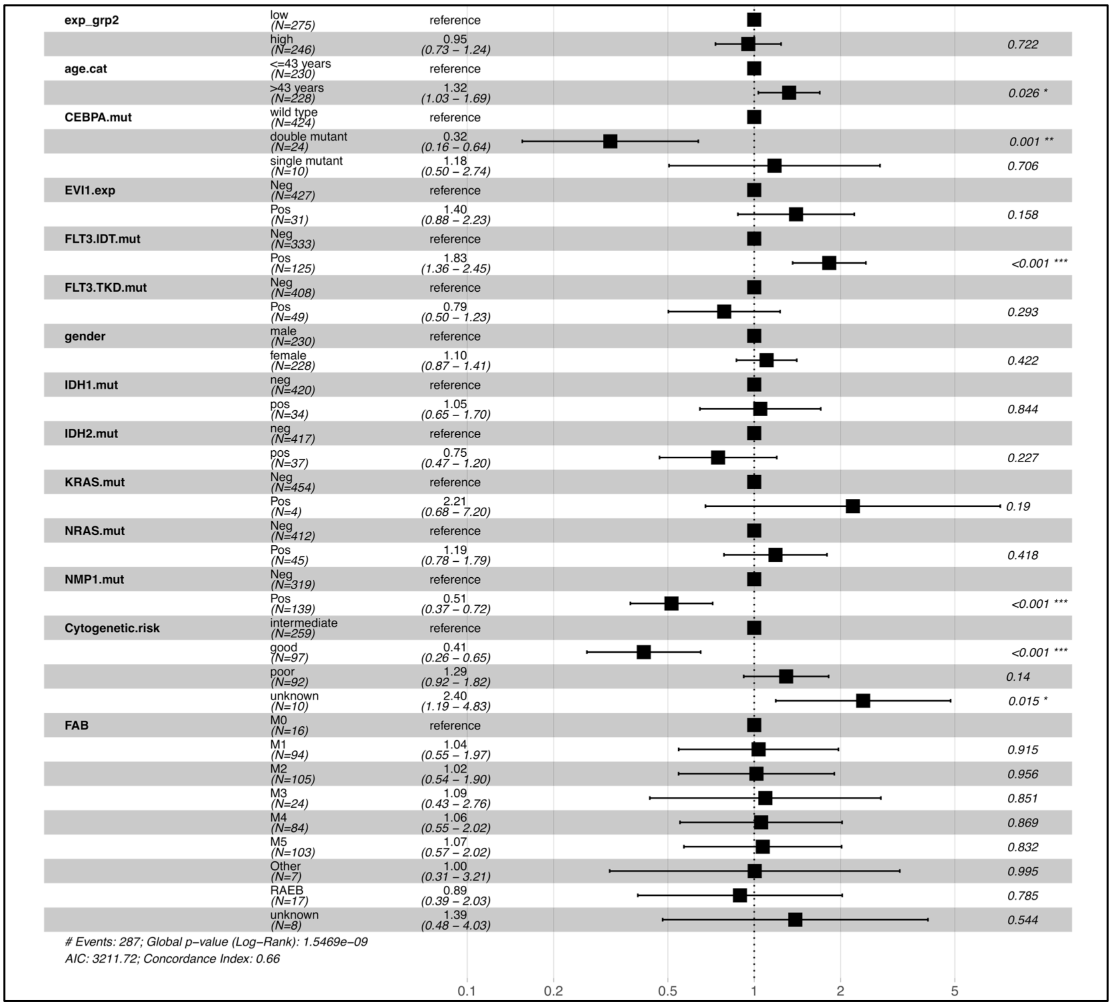


Supplementary Figure 9

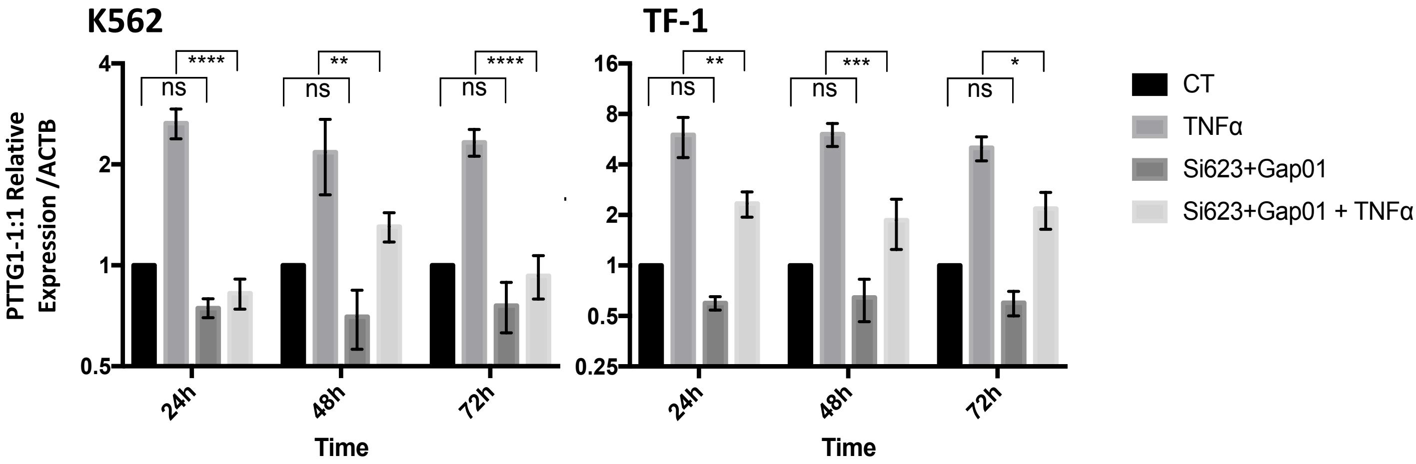
Multivariate analysis in the TCGA Dataset



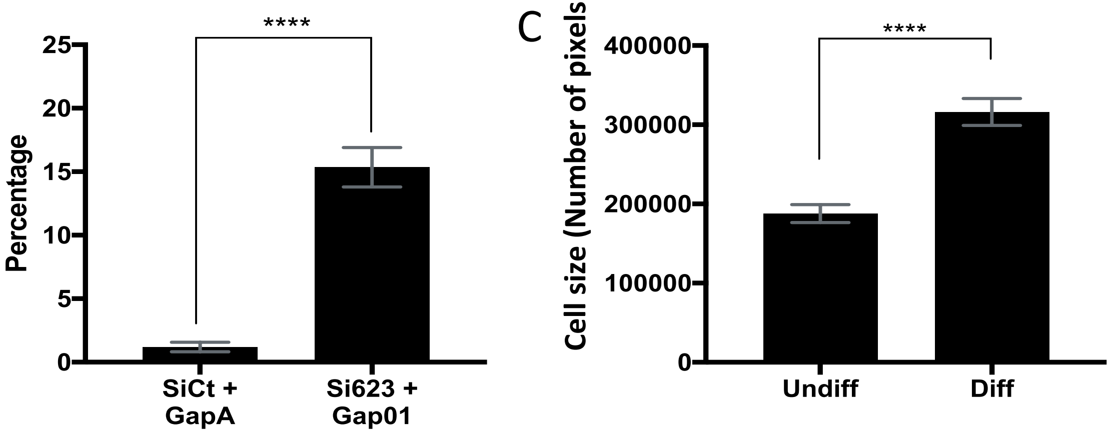
Multivariate analysis in the Verhakk Dataset



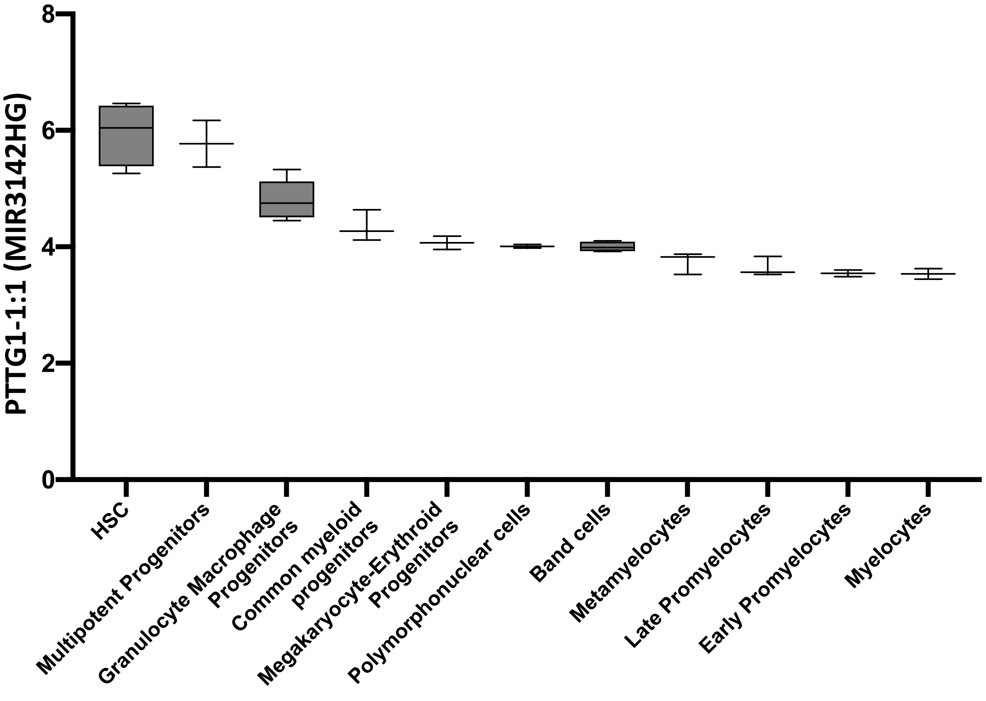
Supplementary Figure 10



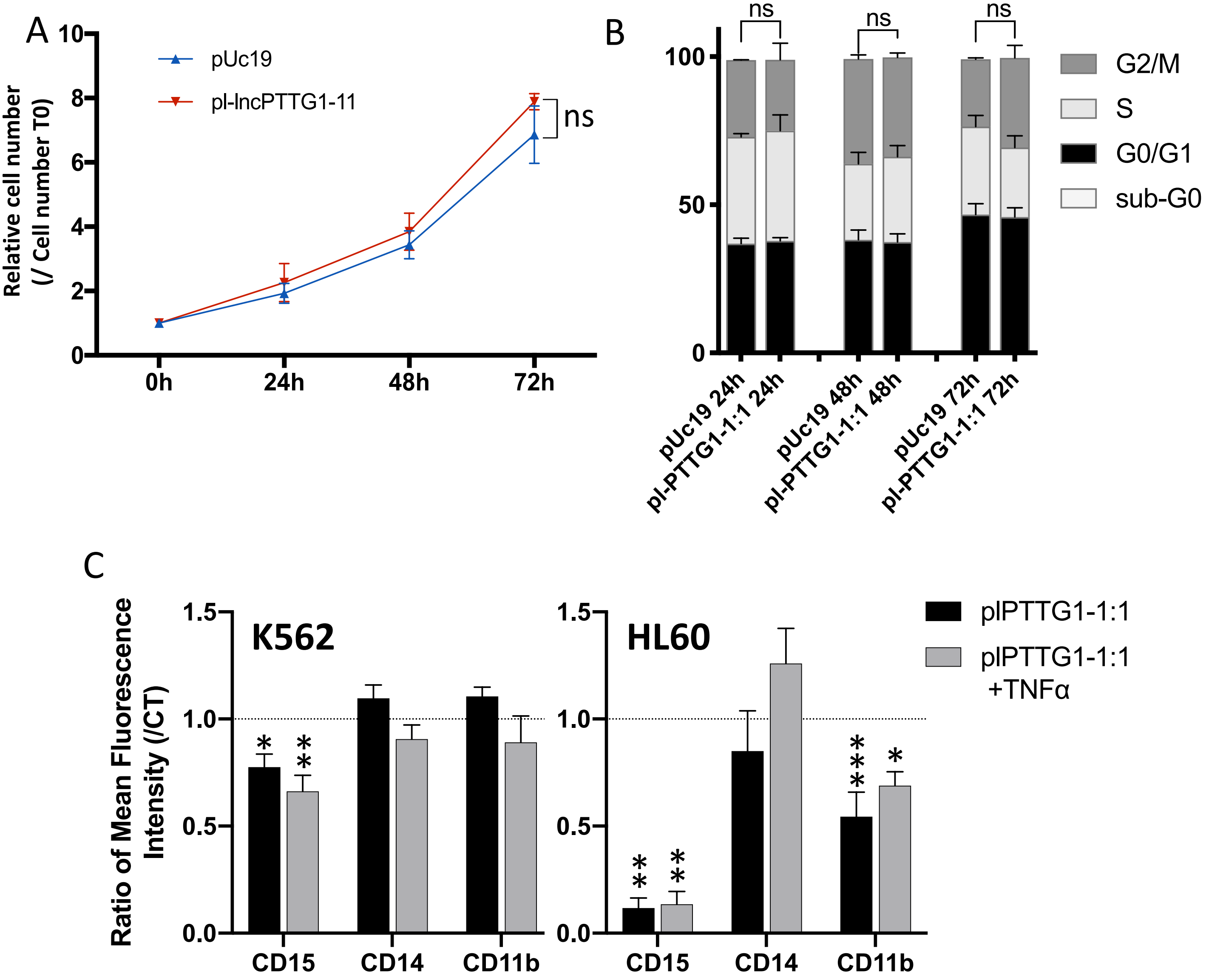
Supplementary Figure 11



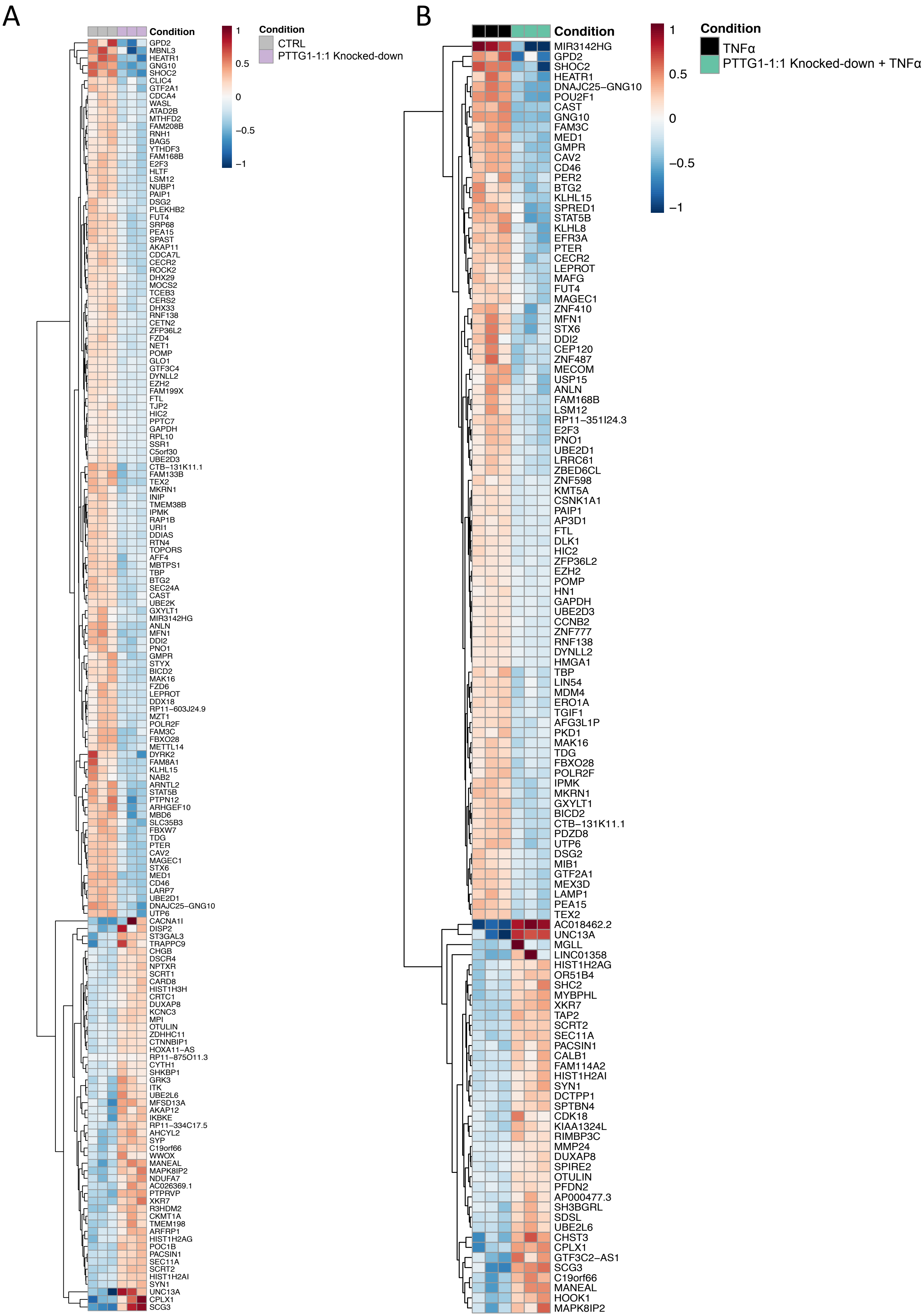
Supplementary Figure 12



Supplementary Figure 13



Supplementary Figure 14



Supplementary Figure 15