Supplemental Material and Methods

Animals. Exons 2-8 of the Wt Scribble locus were conditionally targeted to create Scribble $^{flox/flox}$ alleles. LoxP sites were introduced into the introns 1 and 8 flanking Scribble exons 2 through 8 in mouse embryonic stem cells, using a selectable neo cassette flanked by frt sites. Targeted stem cells were injected into blastocysts of C57BL/6J mice to obtain chimeric floxed mice. After germline transmission, the mice were crossed to C57BL/6J mice expressing flp recombinase to remove the neo cassette. Progeny containing floxed Scribble alleles lacking the neo cassette (Scribble$^{flox/flox}$) were further backcrossed to C57BL/6J mice. Construction of the targeting vector, generation and injection of targeted stem cells and subsequent generation of chimeric mice were performed by inGenious Targeting Laboratory (New York, United States of America).

Scribble$^{flox/flox}$ mice were crossed to Mx1-Cre$^{Tg/-}$ (1, 2) and Vav1Cre$^{Tg/-}$ (3) to generate hematopoietic specific genotypes. The Scribble $^{flox/flox}$ mice were also crossed to an inducible Rosa26Cre-ERTi2$^{Tg/-}$ background for inducible ubiquitous deletion. Cre-mediated recombination causes a frame shift and early stop of Scribble protein translation.

Taz$^{flox/flox}$;Yap1$^{flox/flox}$ (4) were crossed with Vav-Cre$^{Tg/-}$ and Mx1-Cre$^{Tg/-}$ animals. Yap1 (S112A)$^{Tg/-}$ (5) animals were crossed with Rosa26Cre-ERTi2$^{Tg/-}$ mice.

Littermate mice from the same breeding pair were used in all experiments. All mouse strains were maintained at an Association for Assessment and Accreditation of

Laboratory Animal Care accredited, specific-pathogen–free animal facility at Cincinnati Children's Hospital Medical Research Foundation, Cincinnati, under an Institutional Animal Care and Use Committee approved protocol. All mice were between 6 and 12 wk of age at the time of experimentation. Mice genotypes were determined by PCR analysis. Sequences of genotyping primers are available upon request.

Induction of Cre recombinase expression in inducible transgenic animals was achieved by either 6 injections of polyinositide/cytidine (polyI:C) at an intraperitoneal dose of 10 mg/kg every other day in Mx1-Cre$^{Tg/-}$ animals or by administration of 1mg tamoxifen (10mg/mL) intraperitoneal twice a day for 4 days for the Rosa26Cre-ERTi2$^{Tg/-}$ animals.

Fluorescence activated cell sorting (FACS) and Immunophenotypic analysis of HSC/P. Bone Marrow cells were stained using a mixture of biotin-conjugated monoclonal anti-mouse lineage antibodies against CD45R (B220, Clone RA3-6B2), Gr-1 (Ly6G, Clone RB6-8C5), CD4 (Clone RM4-5), CD8a (Ly-2, Clone 53–6.7), Mac-1 (CD11b, CloneM1/70), CD3ε (Clone 145–2C11), and TER119 (Ly-76) (all from BD). In a subsequent labeling step, the cells were incubated with a combination of streptavidin-allophycocyanin (APC)-Cy7 (BD), phycoerythrin (PE) Cy7 anti-mouse Sca-1 (Ly6A/E, clone D7; BD), APC anti-mouse CD117 (c-kit, Clone 2B8; BD), PerCP-cy5.5 anti-mouse CD16/32 (Clone 2.4G2;BD) FITC anti-mouse CD48 (Clone HM481; BD) or BV605 anti-mouse CD48 (Clone HM481;BD), eFluor 450 anti-mouse CD34 (Clone RAM34; eBioscience), PE anti-mouse CD135 (FLT3, CloneA2F10.1) or PE anti-mouse CD150 (Clone mShad150; eBioscience) antibodies. Analysis of bone marrow nuclear cells harvested from all genotypes were immunophenotypically defined by differential

expression of cell surface antigens: Lin^{-} C-kit^{+} Sca-$1^{+/-}$ $CD135^{+/-}$ $CD34^{+/-}$ $CD48^{+/-}$ $CD150^{+/-}$ $CD16/32^{+/-}$.

Cell cycle analysis. Cell cycle analysis of LSK $CD48^{-}$ $CD150^{+}$ HSC harvested from the BM was performed by FACS analysis of immunophenotypically identified cells (see above for staining procedure) and linear-mode quantification of incorporation of Hoechst 33342 (2mg/mL) and PyroninY (0.25 mg/mL). Verapamil (100 µM) was added during staining and analysis to prevent extrusion of Hoechst 33342.

Immunofluorescence microscopy analysis. HSC sorted (using BD FACSAria II) from all geneotypes were seeded onto fibronectin (RetroNectin catalog T100B, TAKARA BIO INC.) coated glass chamber slides in culture medium containing mouse stem cell factor (100ng/mL) and Thrombopoietin (100ng/mL). Cells were cultured overnight (10-12 hours) at 37 degree Celsius and then fixed in 4% paraformaldehyde for 20 minutes at 4°C, permeabilized with 0.1% Triton X-100 (catalog T9284, Sigma-Aldrich) for 10 minutes at room temperature, and blocked with 5% protease free bovine serum albumin (BSA) in PBS for one hour. The slides were stained with primary antibodies in 5% BSA at 4°C overnight: Goat anti-Scribble (Santa Cruz, sc11048), Rabbit anti-phospho-Lats1 (Thr1079) (Cell Signaling Technologies, 8654), Mouse anti-Yap1 (Santa Cruz, sc398182), Rabbit anti-Yap1/Taz (Cell Signaling Technologies, 8418), Rabbit anti-Phospho-Yap1 (Ser127)(Cell Signaling Technologies, 13008), Mouse anti-Cdc42-GTP (New East Biosciences, 26905) or Rabbit anti-Cdc42 (Cell Signaling Technologies,

11A11). The cells were washed with PBS twice and then labeled with secondary antibodies (from Invitrogen): Donkey anti–rabbit Alexa Fluor 488 (catalog A21206), donkey anti-mouse Alexa Flour 546 (A10036), donkey anti-goat Alexa Fluor 568 (A11057), donkey anti-goat Alexa Fluor 647 (A21447) or donkey anti-mouse Alexa Flour 647 (A31571) at 1:500 v/v concentration for 2 hours at room temperature. Cells were washed with PBS and then slides were mounted using Prolong Gold Antifade mounting media (Thermo Fisher Scientific, P36935) containing DAPI. The stained cells were analyzed by a LSM 710 confocal microscope system (Carl Zeiss) equipped with an inverted microscope (Observer Z1, Zeiss) using a Plan Apochromat ×63 1.4 NA oil immersion lens. Stained cells were also imaged with a Nikon Ti-E Inverted A1R Confocal Microscope with GaAsp PMTs, Resonant Scanner, Piezo Z-Drive, Andor iXon 888 EMCCD Widefield Camera. Images were analyzed and processed using NIS-Elements and Adobe Photoshop v7.

Fate determination as assessed by nuclear Myc expression was analyzed by immunofluorescence of paired daughter HSC was performed as mentionedabove with the addition of nocodazole to HSC cultures for 24 hours at 10nM (Millipore, 487928). Following fixation, permeabilization and blocking (as described previously), primary antibody staining was conducted with a Rabbit anti-Myc (Cell signaling Technologies, 13987) monoclonal antibody. A donkey anti–rabbit Alexa Fluor 488 (Invitrogen, A21206) secondary antibody was then used and subsequent mounting and imaging/analysis was performed as previously described.

Immunofluorescence of apoptosis in dividing HSC was achieved using the IncuCyte Caspase-3/7 red reagent (Essen Bioscience, 4704) at 0.5µM, in cultures of LSK $CD48^{-}$

$CD150^+$ SLAM HSC in Stemspan media supplemented with 10% FBS and100ng/mL of SCF and TPO. When combined with time-lapse cinematography, this reagent couples the activated caspase-3/7 recognition motif (DEVD) to a DNA intercalating dye and fluoresces (excitation peak at 630 nm and emission peak at 650 nm) to indicate the development of apoptosis in real time. IncuCyte images were taken by a Nikon inverted microscope at 20-60 hours of culture with both phase contrast and red fluorescence emission using a 20x optimal magnification objective (imaging intervals were taken every 30 min at a rate of 3 frames -90 min- per second of video imaging) Images were compiled and representative videographs were generated using IncuCyte Zoom basic software (Essen Bioscience. Ann Arbor, MI).

Proximity Ligation Assay (PLA). HSC were sorted onto fibronectin coated glass chamber slides and cultured overnight at 37 degree Celsius as previously indicated under Confocal Immunofluorescence Microscopy Analysis. Cells were subsequently fixed and permeabilized in preparation for PLA (Sigma, Duolink® In Situ Detection Reagents Red - DUO92008). After blocking with 5% BSA, primary antibodies were added (Goat anti-Scribble (Santa Cruz, sc11048), Mouse anti-Yap1 (Santa Cruz, sc398182), Rabbit anti-Yap1/Taz (Cell Signaling Technologies, D24E4), Rabbit anti-Cdc42 (Cell Signaling Technologies, 11A11), Mouse anti Cdc42-GTP (New East Biosciences, 26905) and incubated at 37°C for 2 hours. The cells were washed and then treated with the PLA probe, a secondary antibody directly conjugated with oligonucleotides (PLA probe MINUS and PLA probe PLUS). A ligation solution, consisting of two oligonucleotides and Ligase, is added and the oligonucleotides will

hybridize to the PLA probe and join to form a circle if in close proximity. An amplification solution is added containing fluorescently labeled oligonucleotides and a polymerase. Here, the oligonucleotide arm of one PLA probe acts as a primer for a rolling-circle amplification (RCA) reaction using the ligated circles as a template, generating a repeated sequence product. The fluorescently labeled oligonucleotides then hybridize to the RCA product which is analyzed by fluorescent microscopy.

Paired Daughter Cell Assay. Isolate BMNC and preform Lineage cell depletion following the protocol for magnetic activated cell sorting (MACS) from Miltenyi Biotec (130-090-858). Stain lineage depleted HSC/P as mentioned above for LSK $CD48^-$ $CD150^+$ SLAM HSC. Sort single HSC, using BD FACSAria II, into individual wells of a Terasaki plate (Greiner Bio-One, 653108) with 17ul of Stemspan (Stem Cell Technologies, 09650) medium containing SCF and TPO (100ng/ml). After several hours of incubation at 37C, wells with single cells were marked and monitored for cellular division at 24 and 40 hours post sort. At 40 hours, paired daughter cells were physically separated and deposited into individual wells of a 96 well round bottom tissue culture plate (Falcon, 351177) containing 200 µL of Iscove's Modified Dulbecco's Medium (IMDM) (Thermo Fisher Scientific, 12440053) supplemented with 10% Fetal Bovine Serum (GE Healthcare Lifesciences, Hyclone SH30396.03) plus SCF, TPO, G-CSF (20ng/ml each), IL3 (50 ng/ml) and EPO (4 U/ml) to allow four lineage myeloid differentiation. After 14 days of culture, paired clones were harvested for cytospin and stained with hematoxylin and eosin. Clones were examined for the presence of neutrophils (n), erythroid cells (e), macrophages (m) and megakaryocytes (M).

Colony-forming-cell assays. Hematopoietic stem and progenitor cells isolated from BM were grown on methylcellulose medium supplemented with cytokine mixtures (Methocult GF M3434; Stem Cell Technologies) and colony-forming progenitors were scored on days 7-10.

Cobblestone Area Forming Cell (CAFC) assay. Murine BM cells are overlaid on Flask Bone Marrow Derived (FBMD-1) stromal cell layers in 12 dilutions, 2-fold apart (15 wells/dilution), to allow limiting dilution analysis of the precursor cells forming hematopoietic clones. Cultures are fed weekly by changing half of the medium [MyeloCult M5300 base (Stem Cell Technologies, 05350) supplemented with 100 IU/mL penicillin, 0.1 mg/mL streptomycin and 10µM Hydrocortisone (hydrocortisone-21-hemisuccinate, Sigma-H4001)] while frequencies of cobblestone area forming cells are assessed at weekly intervals. Wells were scored positive if at least one phase-dark hematopoietic clone (containing 5 or more cells) is observed. The frequency of CAFC was calculated using limiting dilution analysis software (L-Calc, version 1.1) and Poisson statistics.

Long term BrdU incorporation and retention assay (6). Animals freely imbibed water containing 5-Bromodeoxycytidine (BrdU: 1mg/mL) and 5% glucose for 10 days. Animals were euthanized after 10 days of BrdU administration to assess levels of incorporation into HSC/P populations as well as 80 days post BrdU administration to quantify

quiescence within HSC/P populations determined by BrdU retention (BD Pharmingen intracellular staining kit: Anti-BrdU-Alexa 488).

Serial competitive repopulation transplantation. Equal amounts of CD45.2^{+} BM (Vav1Cre;Wt and Vav1;Scribble$^{\Delta/\Delta}$, Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ or Rosa26Cre;Wt and Rosa26Cre;Yap1 S112A) were mixed with congenic CD45.1^{+} B6.SJL$^{PtprcaPep3b/BoyJ}$ competitor BM cells and were transplanted at a 1:1 ratio into lethally irradiated B6.SJL$^{PtprcaPep3b/BoyJ}$ recipients. Peripheral blood chimera (measured by CD45.2^{+} leukocytes) of primary, secondary and tertiary recipients were measured every 4 weeks and absolute blood counts were recorded. Peripheral blood FACS analysis of CD45.2 chimera and lineage reconstitution was performed by staining peripheral blood cells using monoclonal anti-mouse lineage antibodies against CD45.2 (Clone 104), CD45.1 (Clone A20), CD45R (B220, Clone RA3-6B2), CD11b (M1/70) and CD3ε (Clone 145-2C11). After 16 weeks of reconstitution, recipients were sacrificed and PB, BM and spleen were analyzed for total CD45.2 chimera, lineage reconstitution/ absolute lineage positive cellularity and HSC/P analysis was performed on BM and spleen. BM cells were pooled from each recipient for each genotype and serially transplanted into secondary recipients. This process was terminated after 16-20 weeks of tertiary reconstitution.

5-FU administration and survival analyses. Kaplan-Meier survival analysis of mice after myeloablation with serial injections of 5-fluorouracil (150 mg/kg) five days apart.

Absolute neutrophil, platelet and reticulocyte counts were recorded every 5 days from retro-orbital blood draws. Histology of femurs and spleens were assessed at the time of death or sacrifice.

Generation of Scribble structure/function mutants and lentiviral transduction. Scribble structure and function mutants were generously provided by Jean-Paul Borg of Centre de Recherche en Cancérologie de Marseille, in a pET-431b(+) vector fused at the amino terminus with the 491aa Nus•Tag™ protein (7). We used Polymerase Chain Reaction (PCR) amplification to exclude the Nus tag from our Scribble sequences and subcloned into a stem cell specific EF1α-MCS-IRES-RFP lentiviral vector (System Bioscience, CD531A-2). The full length Scribble sequence would not PCR amplify from the pET-431b expression vector with high purity and therefore, we digested the full length cDNA from another Scribble containing plasmid (using EcoR I and Not I), and pasted it into our EF1α-MCS-IRES-RFP lentiviral vector. All plasmids were verified by sequencing and confirmed to be in frame and have correct start and stop codons corresponding to the original constructs and cDNA. Sequences of cloning and genotyping primers are available upon request. We made virus with these plasmids with high titers and transduced hematopoietic stem and progenitor cells (HSP/C) with an MOI of 20.

Rac1/Cdc42 glutathione-S-transferase (GST) p21 activated kinase (PAK) effector pull down assay. Active Rac1/Cdc42 pulldowns were performed using the Rac1/Cdc42

Activation Magnetic Beads Pulldown Assay Kit (Millipore, 2718273) on Lineage depleted BM cells.

Transcriptome and Bioinformatics Analysis. Total RNA was extracted from sorted HSC derived from Mx1Cre;Wt or Scribble$^{\Delta/\Delta}$, Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ and Scribble$^{\Delta/\Delta}$;Taz $^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ mice one week following polyinositide/cytidine (polyI:C) administration and gene deletion using RNeasy Mini Kit (QIAGEN). RNA quality and concentration were measured by Bioanalyzer 2100 using the RNA 6000 Nano Assay. The initial amplification step was performed with the Ovation RNA-Seq System v2 (Tecan Genomics) and the cDNA concentration and size were measured using the Qubit dsDNA BR assay and a DNA 1000 Chip, respectively. The subsequent libraries were prepared with the Nextera XT DNA Sample Preparation kit (Illumina Technologies). Briefly, 1ng of cDNA was suspended in Tagment DNA Buffer, and tagmentation (fragmentation and tagging with the adaptors) was performed with the Nextera enzyme (Amplicon Tagment Mix), incubating at 55C for 10 min. NT Buffer was then added to neutralize the samples. Libraries were prepared by PCR with the Nextera PCR Master Mix, and 2 Nextera Indexes (N7XX, and N5XX) according to the following program: one cycle of 72C for 3min, one cycle of 98C for 30s, 12 cycles of 95C for 10s, 55C for 30s, and 72C for 1min, and one cycle of 72C for 5min. The purified cDNA was captured on an Illumina flow cell for cluster generation and Libraries were sequenced on the Illumina HiSeq2500 following the manufacturer's protocol. The concentration of the pool was optimized to acquire at least 25-30 million reads per sample using Paired-End Reads with a read length of 75 bps.

Reads were aligned with TopHat software, using hg19 as the reference genome and Reads Per Kilobase of transcript per Million mapped reads (RPKM) as output. RPKM were log2-transformed and baselined to the median expression of the average of each class of samples. Analysis of differential gene expression and gene ontology (GO) pathway analyses using Altanalyze software (developed by Cincinnati Children's Research Foundation).

Statistical analysis.

Data are presented as average ± standard deviation. Comparisons were performed with Student's t test, chi-squared test and one-way or two-way ANOVA when required. Statistical significance levels were established at 5%, 1% and 0.1%.

References

1. Kuhn R, Schwenk F, Aguet M, Rajewsky K. Inducible gene targeting in mice. Science (New York, NY). 1995;269(5229):1427-9.
2. Mikkola HK, Klintman J, Yang H, Hock H, Schlaeger TM, Fujiwara Y, et al. Haematopoietic stem cells retain long-term repopulating activity and multipotency in the absence of stem-cell leukaemia SCL/tal-1 gene. Nature. 2003;421(6922):547-51.
3. Wong PK, Egan PJ, Croker BA, O'Donnell K, Sims NA, Drake S, et al. SOCS-3 negatively regulates innate and adaptive immune mechanisms in acute IL-1-dependent inflammatory arthritis. J Clin Invest. 2006;116(6):1571-81.
4. Sakabe M, Fan J, Odaka Y, Liu N, Hassan A, Duan X, et al. YAP/TAZ-CDC42 signaling regulates vascular tip cell migration. Proc Natl Acad Sci U S A. 2017;114(41):10918-23.
5. Chen Q, Zhang N, Xie R, Wang W, Cai J, Choi KS, et al. Homeostatic control of Hippo signaling activity revealed by an endogenous activating mutation in YAP. Genes & development. 2015;29(12):1285-97.
6. Wilson A, Laurenti E, Oser G, van der Wath RC, Blanco-Bose W, Jaworski M, et al. Hematopoietic stem cells reversibly switch from dormancy to self-renewal during homeostasis and repair. Cell. 2008;135(6):1118-29.

7. Audebert S, Navarro C, Nourry C, Chasserot-Golaz S, Lecine P, Bellaiche Y, et al. Mammalian Scribble forms a tight complex with the betaPIX exchange factor. Current biology : CB. 2004;14(11):987-95.

Supplemental Video Legends

Supplemental Video 1. Wt HSC primary division and subsequent daughter (secondary) divisions. Representative video-clip that display a single Wt HSC primary division yielding two daughter cells that subsequently undergo successful secondary divisions. Individual HSC were imaged using combination of phase contrast and optimized red fluorescence filter (20x optical magnification) and traced throughout bulk cultures to analyze division kinetics and apoptosis. A cell permeable IncuCyte Caspase-3/7 Reagent was added to the cultures (0.5μM) after 20 hours to detect active caspase activity and apoptotic events post division. The video-clip spans 17 seconds at a rate of 3 frames/second and represents imaging at times 24.5 through 50 hours of cultures of Wt HSC. Primary HSC division occurs during the fifth second of the videograph which represents time = 32 hours of culture. Successful secondary divisions of daughter HSC occurred at 47 hours of culture (corresponding to frames after 17 seconds of the video-clip). Two apoptotic events were identified out of 60 cells analyzed (see Supplemental Video 2 for imaging).

Supplemental Video 2. Scribble$^{\Delta/\Delta}$ HSC primary division followed by daughter cell apoptosis. Representative video-clip that displays a single Scribble$^{\Delta/\Delta}$ HSC primary division that yields two daughter cells in which one daughter cell undergoes programed cell apoptosis. Individual HSC were cultured and analyzed as for the Supplemental Video 1. Video-clip spans 19 seconds at a rate of 3 frames/second and represents times 24.5 through 53 hours of culture of Scribble$^{\Delta/\Delta}$ HSC. Primary HSC division occurs

at 2 seconds and corresponds to a division during hour 27.5 of culture. One daughter HSC undergoes an apoptotic event, evidenced by the acquisition of the cleaved caspase-3/7 nuclear intercalating dye and the morphological nuclear fragmentation observed at 16 seconds or 48.5 hours of culture. The surviving Scribble$^{\Delta/\Delta}$ daughter cell successfully divides a few seconds later (18 second frame) after 51.5 hours of culture. Fifteen apoptotic events were identified out of 60 cells analyzed.

Supplemental Figure Legends

Supplemental Figure 1. Yap1 and Taz are necessary for HSC function (A) Non-Mendelian inheritance of Vav1Cre$^{Tg/-}$;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ mice. Predicted Vav1Cre$^{Tg/-}$;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ mice are not viable. **(B)** Genomic PCR showing inducible (Mx1Cre) deletion of Yap1 and Taz in bone marrow. **(C)** Hematopoietic cellularity within the peripheral blood (PB) and bone marrow of Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ mice one week after induced deletion. PB counts were measured by white blood cell (WBC), absolute neutrophil (ANC), platelet and reticulocyte counts. **(D)** Number of Colony Forming Units (CFU) from Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ LSK BM cells. **(E)** Serial Competitive Repopulation Assay between Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ deficient hematopoietic reconstitution. Deletion was induced by Poly I:C injections (10mg/kg) 4 weeks after transplant and subsequent data time points are normalized to the pre-Poly I:C chimera at week 4. **(F)** Absolute number of BM CD45.2^{+} cellularity 16 weeks after primary and secondary competitive reconstitution. **(G)** PB ANC of Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ mice at different points after serial 5-FU administration. **(H)** Kaplan-Meier survival analysis of mice reconstituted with Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ BM following induced deletion and serial 5-fluorouracil (150 mg/kg) administration five days apart. **(I)** PB ANC of Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ reconstituted mice at different time points during serial 5-FU administration. **(J)** RNA-sequencing heat map clustering significant differential regulation between Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1 $^{\Delta/\Delta}$ HSC. **(K)** Normalized expression values (mRNA) for pre-specified Yap1 driven transcripts between

Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ HSC from RNAseq analysis. n.d.= not detected. **(L)** Immunofluorescence showing Flag expression from Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ hematopoietic progenitor cells transduced with Empty vector or Yap1 S127D lentivirus. **(M)** Number of CFU from Mx1Cre;Wt and Mx1Cre;Taz$^{\Delta/\Delta}$;Yap1$^{\Delta/\Delta}$ Lin^{-} BM cells transduced with Empty vector or Yap1 S127D containing mutant lentivirus. Nuclei are counterstained with DAPI and merged images are shown in the right micrographs. Scale bar is 5 μm. **(N)** Quantification of HSC fate defined by Myc allocation in paired daughter cells. *p<0.05, **p<0.01, ***p<0.001.

Supplemental Figure 2. Scribble deficient HSC lose polarized Yap1 expression in the cytosol. (A) Immunofluorescence depicting Proximity Ligation Assay on Wt HSC using IgG control primary antibodies (anti-goat IgG and anti-Mouse IgG) subsequently targeted with corresponding probes for oligomerization. Nuclei are counterstained with DAPI and merged images are shown in the right micrographs. Scale bar is 5 μm. **(B)** Generation of Scribble$^{\Delta/\Delta}$ mice: Exons 2-8 of the Wt Scribble locus were conditionally targeted to create Scribble$^{flox/flox}$ alleles and crossed with Mx1-Cre$^{Tg/-}$ and Vav1Cre$^{Tg/-}$ to generate hematopoietic specific genotypes. **(C)** qRT-PCR of *Scribble* mRNA from bone marrow (BM) HSC (immunphenotypically defined and sorted by differential expression of cell surface antigens: Lin^{-} c-kit^{+} Sca-1^{+} CD48^{-} CD150^{+}) isolated from Vav1Cre$^{Tg/-}$ Wt or Scribble$^{\Delta/\Delta}$ animals. *** p<0.001. **(D)** Western blot analysis of Scribble protein in bone marrow nucleated cells (BMNC) isolated from Vav1Cre$^{Tg/-}$ Wt and Scribble$^{\Delta/\Delta}$ mice. Top arrow denotes the full-length protein and the bottom two arrows are likely expression of translated splice variants. **(E-G)** Quantification of HSC Immunofluorescence depicting

frequency of HSC with polarized Scribble **(F)** Scribble-Yap1 co-localization **(G)** and Scribble-pLats co-localization. **(H)** Immunofluorescence showing Scribble localization in Wt and $Taz^{\Delta/\Delta}$;$Yap1^{\Delta/\Delta}$ HSC. Nuclei are counterstained with DAPI and merged images are shown in the right micrographs. Scale bar is 5 μm. **(I)** Quantification of the frequency of HSC with Scribble expression and polarization.

Supplemental Figure 3. Scribble binds to and modulates Cdc42 expression and activation. (A) RNA-sequencing heat map clustering significant differential regulation between Mx1Cre;Wt and Mx1Cre;$Scribble^{\Delta/\Delta}$ HSC. **(B)** Normalized expression values (mRNA) for pre-specified Yap1 driven transcripts between Mx1Cre;Wt and Mx1Cre;$Scribble^{\Delta/\Delta}$ HSC from RNAseq analysis. n.d.= not detected, *p<0.05, **p<0.01, #p<0.08. **(C)** Gene Ontology analysis of differentially regulated pathways between Mx1Cre;Wt and Mx1Cre;$Scribble^{\Delta/\Delta}$ HSC, 1.5 fold. Numbers represent the number of genes within each GO pathway that are differentially regulated. **(D)** Heatmap clustering statistically significant genes from small GTPase binding and Rho guanyl-nucleotide exchange factor activity signatures. **(E)** Cdc42 effector PAK pull down from of Wt and Scribble deficient Lin^- BM cells. **(F)** Quantification of total and active Cdc42 expression from Lin- BM cells measuring band intensity preformed using Image J software comparing Cdc42 or Cdc42-GTP to β-actin levels. **(G-H)** PLA detection of endogenous Scribble/Cdc42 (G) and Scribble/Cdc42-GTP (H) interactions in HSC. The specificity of the interactions is revealed by the reduced signal detected in Scribble deficient HSC. Nuclei are counterstained with DAPI and merged images are shown in the right

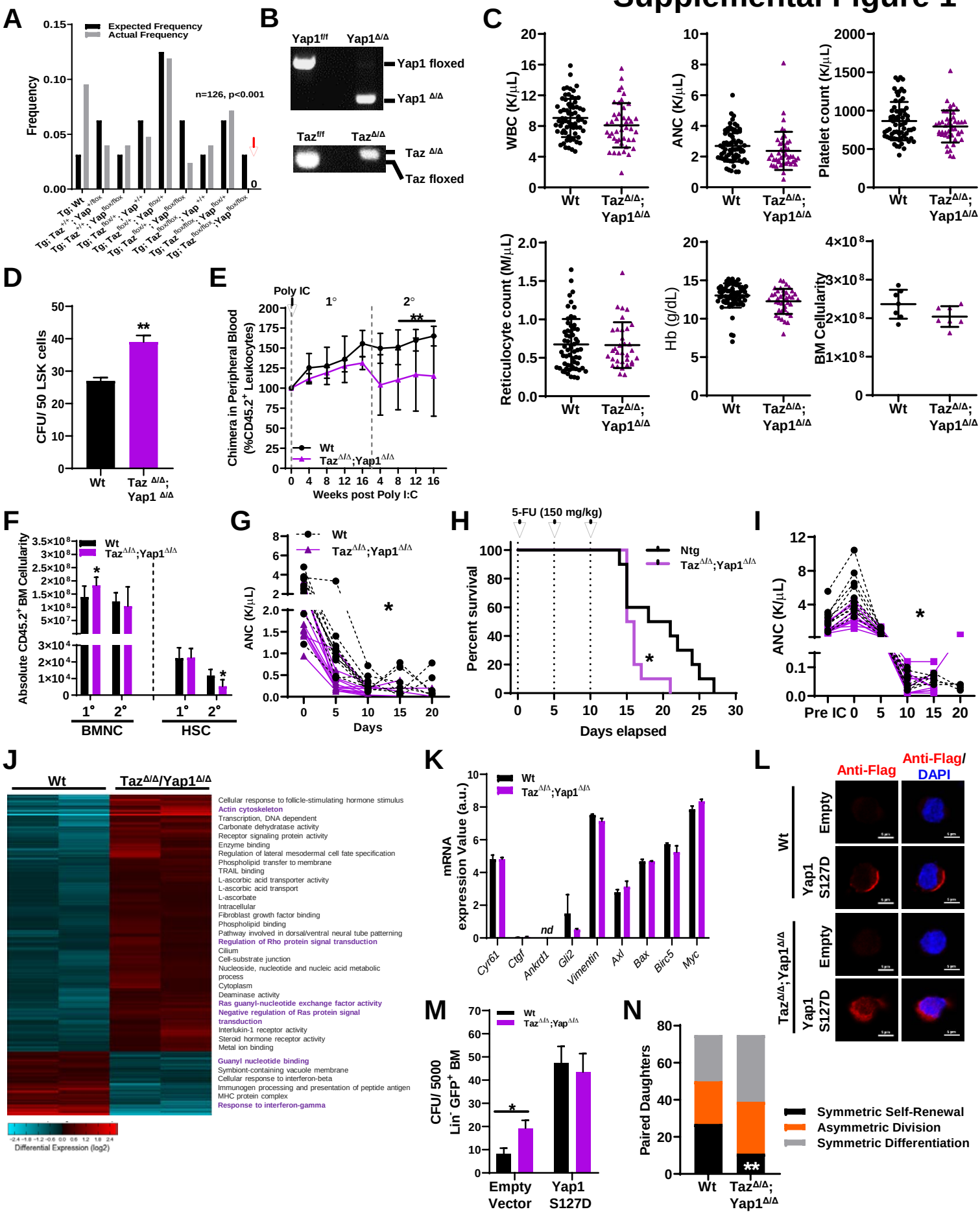
micrographs. Scale bar is 5 µm. Scale bar is 5µm. **(I)** Quantification of PLA signal from F and G. *p<0.05, **p<0.01, *** p<0.001.

Supplemental Figure 4. Scribble deficiency increases repopulation and self-renewal of HSC independent of acquired nuclear Yap1 localization. (A) Bone Marrow (BM) chimerism measured by CD54.2^{+} BM nuclear cells (BMNC) from primary recipients of Vav1Cre serial transplant. **(B)** BM CD45.2^{+} LSK cellularity from primary recipients. **(C)** BM chimerism from tertiary recipients of Vav1Cre serial transplant. **(D)** BM CD45.2^{+} LSK cellularity from tertiary recipients. **(E-G)** Lineage reconstitution among tertiary recipients from the PB (E), BM (F) and Spleen (G). **(H)** Spleen weight (grams) from the tertiary recipient mice. (**I**) Schematic depicting a cobblestone area forming cell (CAFC) assay where murine BM cells are overlaid on a Flask Bone Marrow Derived (FBMD-1) stromal cell monolayer in 12 dilutions, 2-fold apart, to allow limiting dilution analysis of the precursor cells forming hematopoietic clones. **(J)** Cobblestone area forming ability after several weeks in culture. The frequency of CAFC was calculated using Poisson statistics. **(K)** Immunofluorescence showing overexpression and nuclear accumulation of Yap1 (S112A) in HSC. Nuclei are counterstained with DAPI and merged images are shown in the right micrographs. Scale bar is 5 µm. **(L)** Quantification for the frequency of HSC with nuclear Yap1 expression. **(M)** Kaplan-Meier survival analysis of Wt and Yap1 (S112A) transgenic mice after serial myeloablation with 5-fluorouracil (150mg/kg). **(N)** Serial Competitive Repopulation Assay between Wt and Yap1 (S112A) overexpressing hematopoietic reconstitution. *p<0.05, **p<0.01, ***p<0.001.

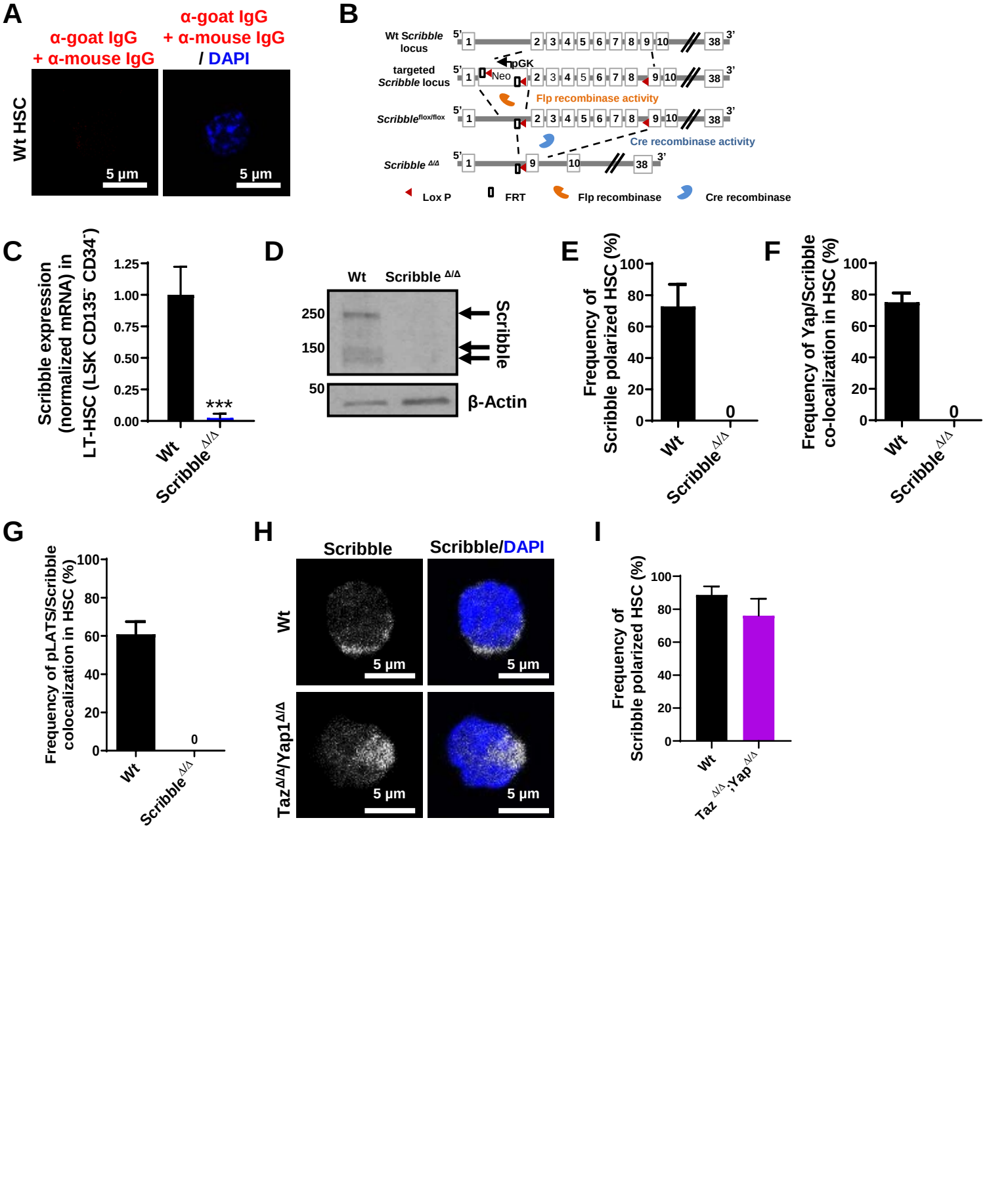
Supplemental Figure 5. Scribble deficiency decreases quiescence of HSC and modulates fate. (A) Cell cycle analysis of Lin- CD48- CD150+ HSC harvested from the BM of Wt and Scribble$^{\Delta/\Delta}$ mice. Stages of cell cycle were assessed by FACS analysis with incorporation of DNA binding Hoechst 33342 (2 mg/mL) and nucleotide binding PyroninY (0.25mg/mL). **(B)** Absolute BrdU retaining (BrdU^{+}) LSK cells assessed by FACS analysis of BM from mice described in Figure 5E. ***p<0.001. **(C)** Division kinetics of Vav1Cre;Wt and Vav1Cre;Scribble$^{\Delta/\Delta}$ SLAM HSC measured using IncuCyte and time-laps imaging. **(D)** Relative number (%) of wells containing the indicated number of cells after 40 hours individual HSC cultures. (Four independent experiments, n>450 HSC). **(E)** Representative cytospin images of HSC fate or division modality, m=macrophage, n=neutrophil, e=erythrocyte and M=megakaryocyte. Symmetric Self-Renewal division is indicated by the presence of all 4 lineages (macrophage, neutrophil, erythrocyte and megakaryocyte) in each clone/ daughter; Asymmetric Division occurs when one clone is missing one or more of the 4 lineages; while symmetric Differentiation / Commitment is determined when both clones are incomplete and missing one or more lineage output populations. **(F)** Relative frequency of paired daughter cells analyzed for division modality, assessed as the presence or absence of full multi-lineage differentiation potential among individual paired daughter clones. n of 50 individual pairs. Chi Square analysis; ** p<0.01. **(G)** Quantification of HSC fate between Vav1Cre;Wt and Vav1Cre;Scribble$^{\Delta/\Delta}$ HSC defined by Myc allocation in paired daughter cells. Paired daughters were imaged following 24 hours of Nocodazol (10nM) treatment. n of 120 individual pairs. Fisher's exact test; * p<0.05.

Supplemental Figure 6. Triple deficiency of Yap, Taz and Scribble identifies a change in the RhoGTPase activation gene transcriptome and Scribble deficiency results in diminished Cdc42 expression. (A) RNA-sequencing analysis highlighting differential regulation between Mx1Cre Wt, $Taz^{\Delta/\Delta}$;$Yap1^{\Delta/\Delta}$ and $Scribble^{\Delta/\Delta}$;$Taz^{\Delta/\Delta}$;$Yap1^{\Delta/\Delta}$ HSC. **(B)** Expression (mRNA) of *Prex1* and *Fgd4* in Mx1Cre Wt, $Scribble^{\Delta/\Delta}$, $Taz^{\Delta/\Delta}$;$Yap1^{\Delta/\Delta}$ and $Scribble^{\Delta/\Delta}$;$Taz^{\Delta/\Delta}$;$Yap1^{\Delta/\Delta}$ HSC. * $p<0.05$; ** $p<0.01$; *** $p<0.001$. **(C)** Cartoon depicting the major functional consequences on HSC activity upon loss of Yap1/Taz (top panel), Scribble (middle panel) and Scribble/Yap1/Taz (bottom panel).

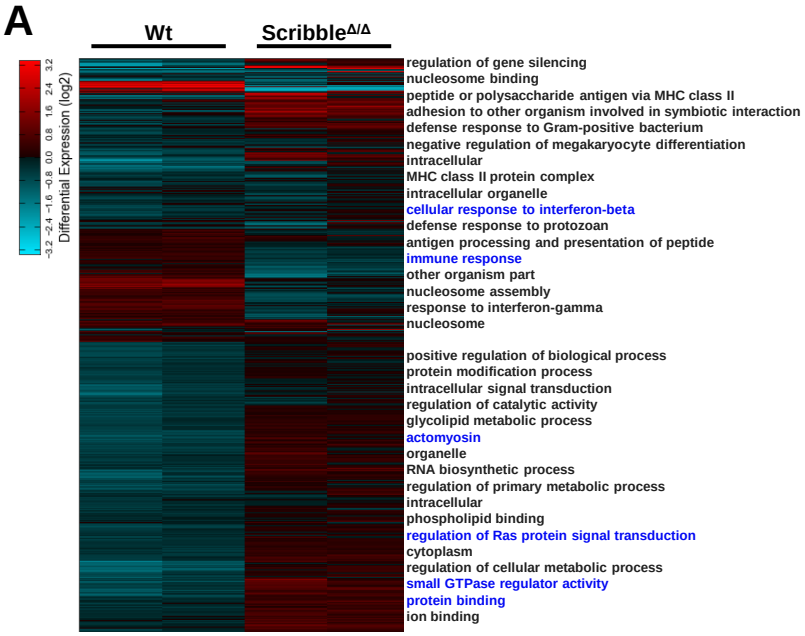
Supplemental Figure 1

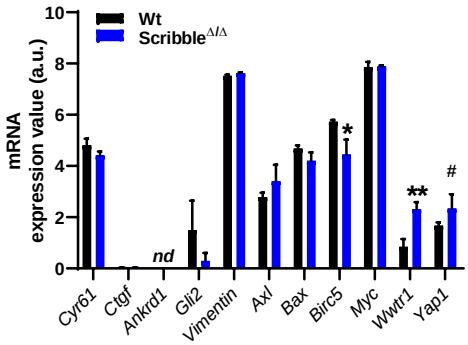


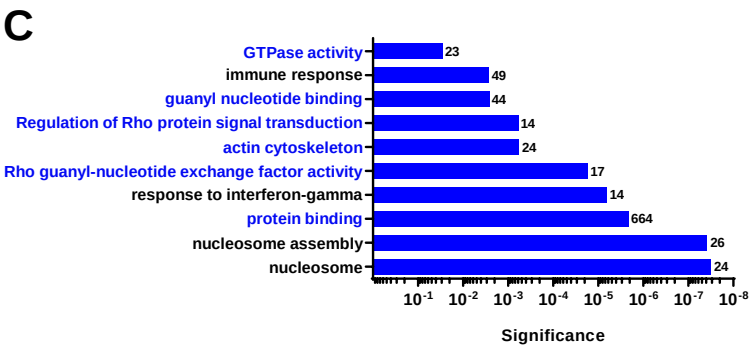
Supplemental Figure 2

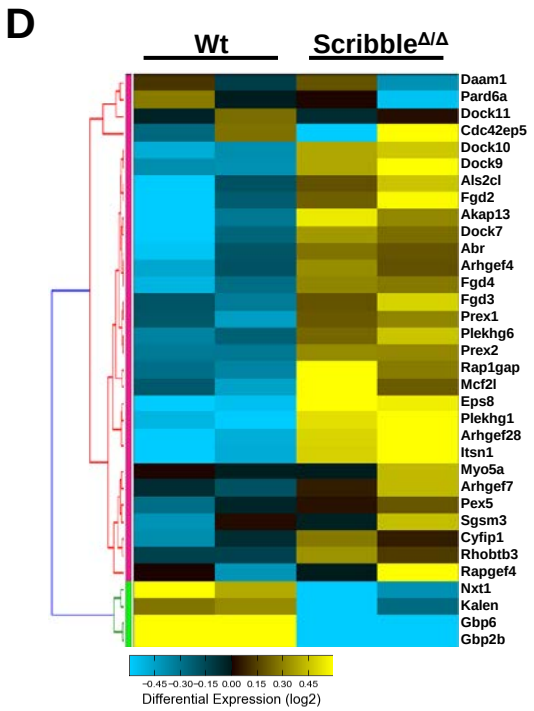


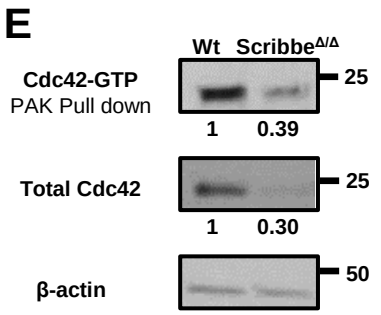
Supplemental Figure 3

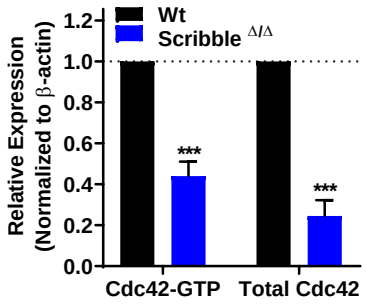


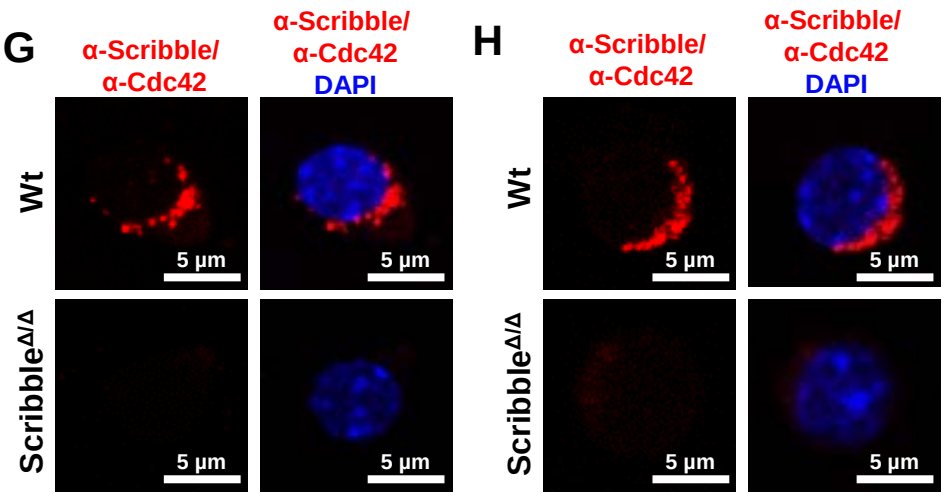


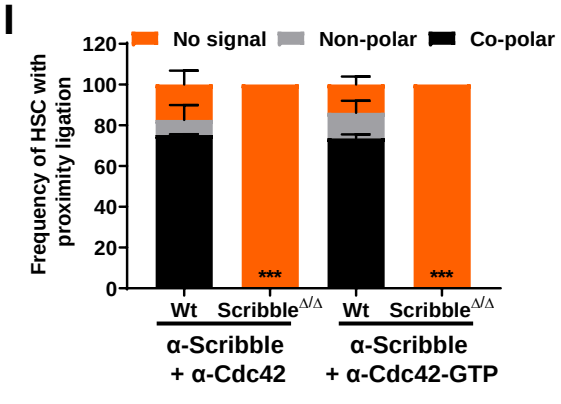




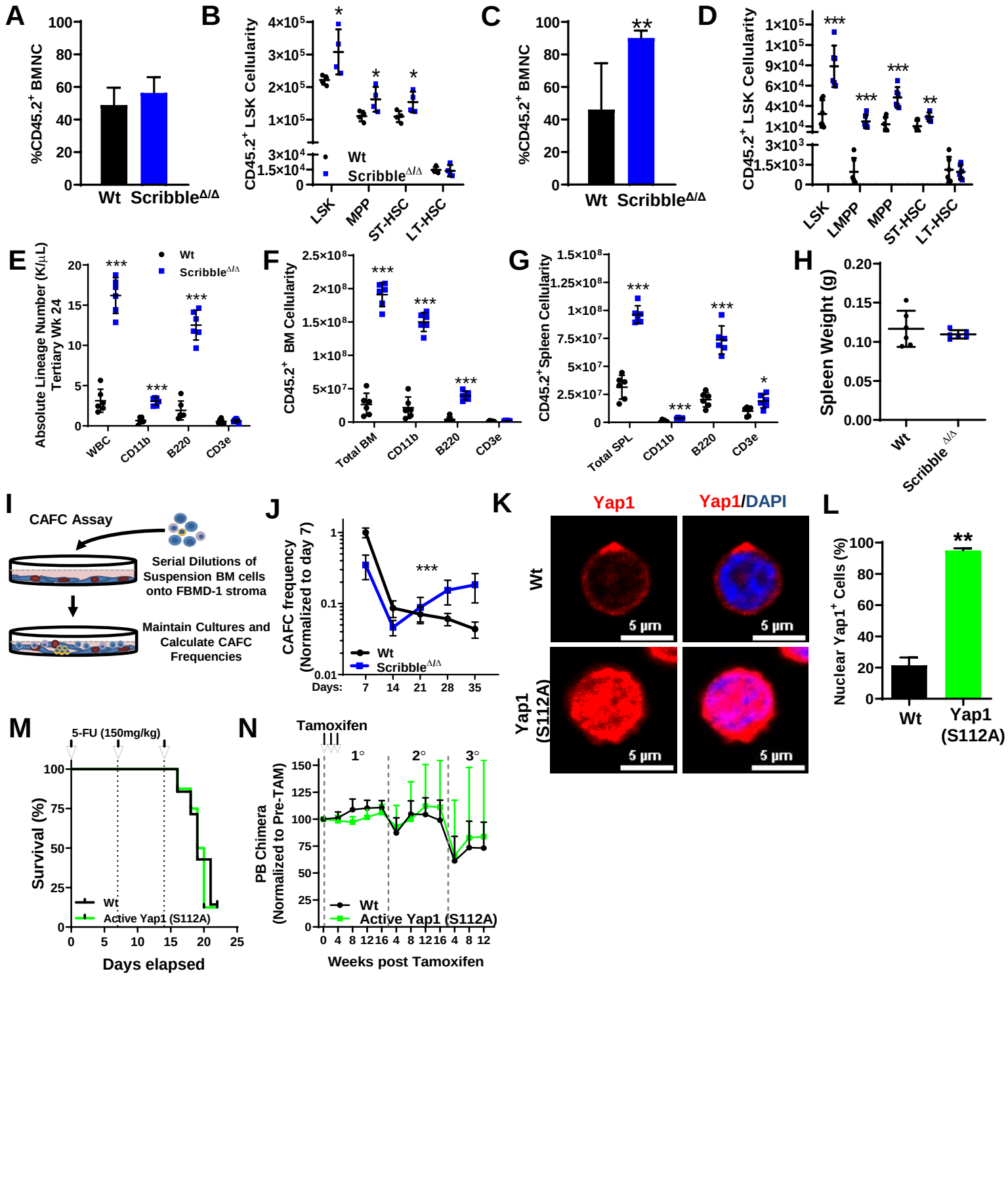




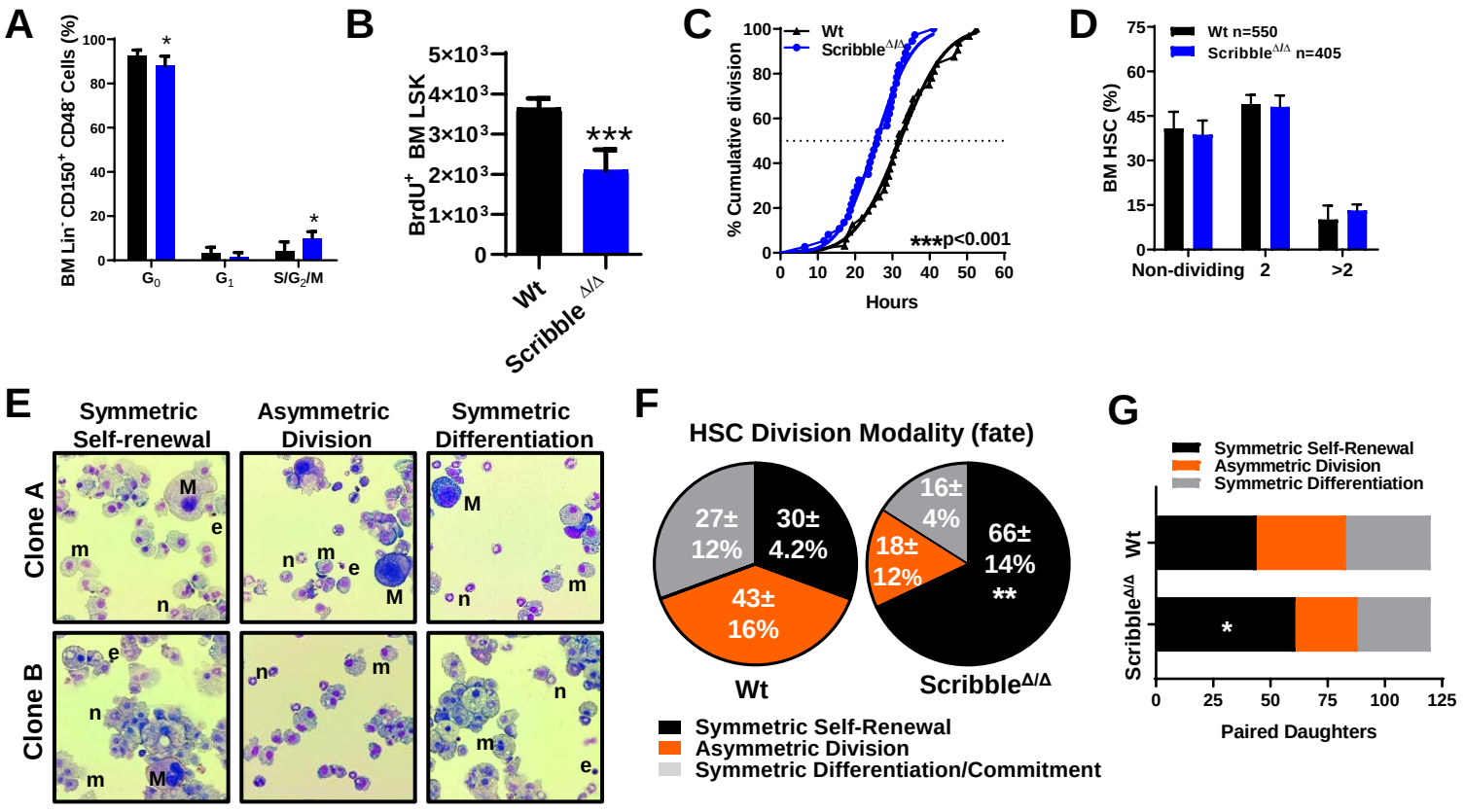




Supplemental Figure 4



Supplemental Figure 5



Supplemental Figure 6