Mutant *Samd9l* expression impairs hematopoiesis and induces bone marrow failure in mice

Sherif Abdelhamed¹#, Melvin E. Thomas III¹#, Tamara Westover¹, Masayuki Umeda¹, Emily Xiong¹, Chandra Rolle¹, Michael P. Walsh¹, Huiyun Wu², Jason R. Schwartz³, Virginia Valentine⁴, Marcus Valentine⁴, Stanley Pounds², Jing Ma¹, Laura J. Janke¹,⁵, Jeffery M. Klco¹$.

Supplemental Methods

Patient materials

Commercially available human cord blood-derived CD34+ cells (Lonza, Switzerland) were cultured in human medium (StemSpan SFEM-II (StemCell Technologies, Canada) enriched with human cytokines (PeproTech, NJ) IL-6 (100ng/ml), FLT3 (100ng/ml), SCF (100ng/ml), TPO (100ng/ml), 1uM Stem Regenin-1, and 35nM UM171 (StemCell Technologies, Canada). Patient samples harboring *SAMD9L*-S626L mutations were obtained with informed consent using a protocol approved by the St. Jude Children's Research Hospital Institutional Review Board. Bone marrow aspirates were collected, mononucleated cells were isolated by FicollTM (GE Healthcare) and cryopreserved in the St. Jude Biorepository.

Intracellular flow cytometry
Cell surface staining was first performed using fluorescently labeled antibodies (Table S2). Cell cycle and protein synthesis were assessed by Click-iT™ EdU or Click-iT™ Plus O-propargyl-puromycin (OPP) assays, respectively (Invitrogen, CA). Cells were incubated with 10uM EdU for 2h or 10uM OPP for 30 minutes in RPMI (ThermoFisher, MA) with 15% FBS and supplemented with murine cytokines including interleukin-3, interleukin-6, SCF, Thrombopoietin, and Flt3-l (PeproTech, NJ) as previously reported (1, 2). After incubation, cells were fixed in 2% paraformaldehyde, permeabilized in 0.5% Saponin and Click-iT reactions were performed with the appropriate reagents. For intracellular phospho-SMAD2/3 staining, cells were fixed in 2% paraformaldehyde, permeabilized with 0.5% triton X-100, and stained with pSMAD2/3 antibody (BD Biosciences, CA). All data were analyzed using FlowJo software (TreeStar, OR).

Sanger DNA and Samd9l targeted sequencing

For Sagner sequencing, genomic DNA was harvested using Quick-DNA MiniPrep (Zymo Research). Using the indicated primers (Table S2), gDNA was used to amplify the Samd9l-KI construct. The product bands (~5kb) were gel-cleaned and sequenced using Janus liquid handling robotics system (Perkin Elmer), Veriti thermal cyclers for sample preparation (Applied Biosystems), and 3730xl DNA Analyzers (Applied Biosystems). For Samd9l targeted sequencing, fragments of interest were amplified (~5kb) using the indicated primers in table S2, and libraries were performed using Illumina Nextera XT part number FC-131-1096 and sequenced using NovaSeq 6000 S4 flowcell, paired end 100 cycle.
Western blot

Cells were harvested by washing several times with PBS and lysed in denaturation lysis buffer (50mM Tris-HCl pH8.0, 150mM NaCl, 4% SDS, 0.5% triton x-100, 10% glycerol, and 5% BME), heated at 99°C for 5-10 minutes and briefly sonicated. Protein concentration was calculated by Bradford assay and 20ug total protein was loaded per sample onto a 4-20% gradient agarose gel. Immunoblotting was performed by transferring the gel to the PVDF membrane. Blots were then blocked with 5% non-fat milk in TBST (blocking buffer) for 2h at room temperature and stained with indicated primary antibodies (1:500 or 1:1000 dilution) in blocking buffer overnight at 4°C with gentle rocking. Blots were washed 3 times with TBST for 5 minutes and stained with HRP-conjugated secondary antibodies (1:2000 dilution) for 2h at room temperature with gentle rocking. Blots were washed 3 times with TBST for 5 minutes and visualized using chemiluminescence.

Microscopy

HEK293T cells were plated on 22mm diameter poly-L-lysine coated coverslips (Neuvitro, WA) and treated with IFN-α (1000U) for 24h. These cells were washed twice with PBS, fixed with 4% paraformaldehyde for 15min, permeabilized in 0.3% triton X-100 for 10 minutes, and blocked with 5% rat serum for 1h. Cells were then stained with anti-SAMD9 or anti-SAMD9L primary antibodies (Table S2) for 2h at room temperature and washed with PBS. Cells were then stained with donkey anti-rabbit for 2h at room temperature, washed with PBS, and stained with DAPI for 5 minutes. Coverslips were mounted using ProLong Diamond Antifade (Invitrogen, CA). Images were acquired on a Nikon C2 laser
scanning confocal microscope using a 60X oil-objective lens controlled by NIS-Elements software (Nikon, Japan).

**Supplemental Tables**

**Supplemental Table S1:** quantification of the bone marrow smears.

| Series          | Population    | Samd9l-WT Veh | Samd9l-WT pI:pC | Samd9l-Mut Veh | Samd9l-Mut pI:pC |
|-----------------|---------------|---------------|----------------|---------------|-----------------|
| Myeloid Series  | Myeloblast    | 9.2           | 9.2            | 13.5          | 20.3            |
|                 | Promyelocyte  | 0.2           | 4.7            | 1.1           | 3.7             |
|                 | Myelocyte     | 25.6          | 18.2           | 31.0          | 40.4            |
|                 | Metamyelocyte | 11.8          | 18.7           | 18.7          | 18.1            |
|                 | Neutrophil    | 17.2          | 16.3           | 24.4          | 3.5             |
|                 | Monocytic     | 9.9           | 13.4           | 3.4           | 9.6             |
| Lymphocyte Series | Lymphoblast | 2.4           | 1.3            | 0.2           | 0.2             |
|                 | Lymphocyte    | 23.7          | 18.2           | 7.6           | 4.1             |

**Supplemental Table S2:** reagents and resources.

| REAGENT or RESOURCE | SOURCE         | IDENTIFIER |
|---------------------|----------------|------------|
| Antibodies          |                |            |
| BV605 anti-mouse/human CD45R/B220 | Biolegend | 103244   |
| PE/Cy7 anti-mouse CD3ε | Biolegend | 100320   |
| AF700 anti-mouse/human CD11b | Biolegend | 101222   |
| PerCP-Cy5.5 Ly-6A/E (Sca-1) Monoclonal Antibody (D7) | Thermo Fisher | 45-5981-82 |
| PE/Cy5 anti-mouse CD127 (IL-7Rα) | Biolegend | 135016   |
| PE/Cy7anti-mouse CD34 | Biolegend | 128618   |
| AF700 anti-mouse CD48 | Biolegend | 103426   |
| APC-ef780 c-Kit (2B8) | eBioscience | 47-1171-82 |
| BV605 anti-mouse CD150 | Biolegend | 115927   |
| BV711 CD16/CD32 | eBioscience | 56-0161-82 |
| PE anti-mouse CD3ε | Biolegend | 100308   |
| PE anti-mouse CD4 | Biolegend | 130310   |
| PE anti-mouse/human CD45R/B220 | Biolegend | 103208   |
| PE anti-mouse/human CD11b | Biolegend | 101208   |
| PE anti-mouse Ly-6G/Ly-6C (Gr-1) | Biolegend | 108408   |
| PE anti-mouse TER-119/Erythroid Cells | Biolegend | 116208   |
| PerCP-cy5.5 anti-mouse CD71 | Biolegend | 113816   |
| Reagents                                                                                                           | Supplier                                      | Catalog Number |
|-------------------------------------------------------------------------------------------------------------------|-----------------------------------------------|----------------|
| AF647 anti-Smad2 (pS465/pS467)/Smad3 (pS423/pS425)                                                               | BD Biosciences                                | 562696         |
| APC AffiniPure F(ab')₂, Fragment Donkey Anti-Rabbit IgG                                                         | Jackson ImmunoResearch                        | 711-136-152    |
| LIVE/DEAD™ Fixable Aqua Dead Cell Stain Kit                                                                     | Thermo Fisher                                 | L34957         |
| AF700 anti-mouse CD45.1                                                                                           | Biolegend                                     | 110724         |
| eFluor 450 monoclonal Antibody (104) CD45.2                                                                    | Thermo Fisher                                 | 48-0454-82     |
| Polyclonal antibody to Glycophorin A (CD235a)                                                                   | Cloud-Clone                                  | PAB704Mu01     |
| Anti-Gata1                                                                                                        | Abcam                                         | ab131456       |
| Anti- Myeloperoxidase (MPO)                                                                                        | Dako                                          | A0398          |
| Anti-Mouse CD45R/B220 Clone RA3-6B2                                                                               | PharMingen                                    | 553084         |
| Recombinant monoclonal PAX5                                                                                       | Abcam                                         | ab109443       |
| Anti-CD3-ε                                                                                                        | SantaCruz                                     | sc-1127        |
| Recombinant Anti-SAMD9                                                                                             | Abcam                                         | ab180575       |
| Rabbit Polyclonal anti-SAMD9L                                                                                      | Proteintech                                   | 25173-1-AP     |
| Anti-GAPDH (14C10)                                                                                                 | Cell Signaling                                | 2118S          |
| APC Annexin V                                                                                                     | BD Biosciences                                | 550474         |

**Biological samples**

- **Human cord blood-derived CD34+cells**
  - Lonza, 2C-101
- **Patient samples with SAMD9L-p.S626L mutations**
  - St. Jude Children's Research Hospital [https://www.stjude.org/](https://www.stjude.org/)

**Chemicals, peptides, and recombinant proteins**

| Chemical/Peptide                                                                 | Supplier                                      | Catalog Number |
|---------------------------------------------------------------------------------|-----------------------------------------------|----------------|
| SD-208 TGF-βRI (ALK5) inhibitor                                                | Selleckchem                                   | S7624          |
| Murine IL-3                                                                     | PeproTech                                     | 213-13         |
| Murine IL-6                                                                     | PeproTech                                     | 216-16         |
| Murine Flt-3 Ligand                                                             | PeproTech                                     | 250-31L        |
| Murine SCF                                                                      | PeproTech                                     | 250-03         |
| Recombinant Mouse IFN-alpha                                                     | R&Dsystems                                    | 12100-1        |
| Polyinosine-polyctydidylic acid (pi:pC)                                         | Invivogen                                     | tlrl-pic-5     |

**Critical commercial assays**

| Assay                                                                 | Supplier                                      | Catalog Number |
|----------------------------------------------------------------------|-----------------------------------------------|----------------|
| Click-iT™ EdU Cell Proliferation Kit                                  | Thermofisher                                  | C10337         |
| MethoCult™ GF                                                        | StemCell Technologies                          | M3434          |

**Deposited data**

| Type       | Depository        | Accession Number |
|------------|-------------------|------------------|
| RNA-seq    | GEO               | GSE190566        |
| scRNA-seq  | GEO               | pending          |

**Experimental models: Organisms/strains**

| Model Number  | Organism/Strain                                      |
|---------------|------------------------------------------------------|
| Samd9l/-      | (Samd9l-KO)                                          |
| B6.Samd9l(cKI)| Ingenious Targeting Laboratory                      |
| B6.Cg-Tg(Vav1-cre)A2Kio/J (Vav1- Cre) | Jackson Laboratory | 008610 |
| B6.SJL-Ptprca Pepcb/BoyJ (Cd45.1) | Jackson Laboratory | 002014 |
| C57BL/6J     | Jackson Laboratory                                   | 000664         |

**Oligonucleotides**

**Genotyping**

| Oligo Name   | Sequence                     | Source            | Accession |
|--------------|------------------------------|-------------------|------------|
| Vav1-Cre F   | CAGGTTTTGGTGACAGTCA          | This paper        | N/A        |
| Vav1-Cre R   | GGTGTTGTAGTTGTCCCCCT        | This paper        | N/A        |
| Internal F   | CTAGGCCACAGAAATGGAAGATCT    | This paper        | N/A        |
| Internal R   | GTAGGGGAAATTTCTAGCATCATCC   | This paper        | N/A        |
| LOX1         | TCC CGA TTT CCA CAC AGA TTA GTC | This paper   | N/A        |
| SEQ1         | GCG TTT TAT CAG AAG TTA GTC ACC C | This paper   | N/A        |
**Sanger sequencing**

| Primer                        | Sequence                        | Source       | Notes |
|-------------------------------|---------------------------------|--------------|-------|
| GFPWW1 F                      | CCGCATCGAGCTGAAGGGCATCGAC       | This paper   | N/A   |
| mCh SQ1 F                     | AGACCGCCAAGCTGAAGGTGAC          | This paper   | N/A   |
| SEQ1 R                        | GCGTITTTATCAGAAGTGCTGGACCC      | This paper   | N/A   |
| Samd P3 F                     | TCTGGCCCAAAAGAAAGCACCTAAG       | This paper   | N/A   |
| Samd9l SF 33                 | CAAAAGACTGGACCAAAGA            | This paper   | N/A   |
| Samd9l SF 404:               | AAAACATGTAGGTGATGTGG           | This paper   | N/A   |
| Samd9l SF 815:               | AAATCAGTGAAAGCCAGGG            | This paper   | N/A   |
| Samd9l SF 1205:              | GCTCAGTGTGAATCTTTAC            | This paper   | N/A   |
| Samd9l SF 1600:              | GTTTTGGGTGTTCTCTTT             | This paper   | N/A   |
| Samd9l SF 2023:              | GAAGAACACTTTATCGAGG            | This paper   | N/A   |
| Samd9l SF 2396:              | AAGAGAAGCCTATATTCTG            | This paper   | N/A   |
| Samd9l SF 2745:              | CCTGGCATATTCACTCTCTT            | This paper   | N/A   |
| Samd9l SF 3093:              | TACAGACAACGGCAAGGAAC           | This paper   | N/A   |

**Samd9l targeted sequencing**

| Primer                        | Sequence                        | Source       | Notes |
|-------------------------------|---------------------------------|--------------|-------|
| WT Samd9l-GFP F               | CAAAGACCCCAACGGAAGGC            | This paper   | N/A   |
| Mutant Samd9l-mCherry F       | GGACATCACCTCCCACAACGG           | This paper   | N/A   |
| Samd9l R                     | GGGCTAGAAAGAGTAAGTAC            | This paper   | N/A   |

**Software and algorithms**

| Software                  | Version | Source       | Notes |
|---------------------------|---------|--------------|-------|
| R package Limma           | 3.32.10 | (4) SCR_010943 |       |
| R package pheatmap       | 1.0.12  | NA SCR_016418 |       |
| R package ggplot         | 2.0.0   | (5) SCR_014601 |       |
| GSEA                      | 4.1.0   | (6) SCR_003199 |       |
| R environment            | 4.0.2   | (7) SCR_001905 |       |
| R package Seurat         | 3.2.1   | (8) SCR_007322 |       |
| UMAP                      |         | (McInnes and Healy, 2018) <arXiv:1802.03426> SCR_018217 |       |
| DAVID                     |         | (9, 10) SCR_001881 |       |
| R package emmeans        |         | (11) SCR_018734 |       |
| FlowJo version 10        |         | TreeStar N/A |       |
Figure S1: *SAMD9L* mutant mice have altered hematopoietic differentiation and proliferation. A. Schematic showing the conditional insertion cassette. GFP-fused *SAMD9L-WT* (inserted at exon 2) flanked by LoxP sites and a neomycin selection cassette flanked by FRT sites upstream of a stop codon and a mCherry-fused mutant *SAMD9L* containing the W1171R mutation. The Neo cassette was removed before the Cre expression. After Cre recombination, the GFP-fused *SAMD9L-WT* gene is removed and mCherry-fused *SAMD9L-Mut* is expressed. B. Polymerase chain reaction (PCR) analysis of GFP-*SAMD9L-WT* or mCherry-*SAMD9L-Mut* fusions after recombination using *SAMD9L*
targeted sequencing primers (Table S2). The gels show PCR products of 4900bp from Samd9l-WT, Samd9l-Mut, and C57BL/6 mice. Recombination was confirmed by deletion of the GFP-Samd9l-WT band in the Samd9l-Mut mouse. C. Graphical representation for the sequence coverage of the Samd9l-KI locus. Using overlapping primers (Table S2) insertion of the Samd9l-KI locus was confirmed by sanger sequencing. D-E. Flow cytometric analysis of C57BL/6, Samd9l-KO, Samd9l-WT, and Samd9l-Mut mice. (D) Gating strategy for HSPC populations. (E) Percentage of lymphoid or myeloid progenitors in the spleens (n=3). F. EdU incorporation assay by flow cytometry showing the contribution of HSPC and progenitor populations in the total proliferating cells from Samd9l-KO, Samd9l-WT, and Samd9l-Mut mice (n=3). G-H. Flow cytometric analysis of C57BL/6, Samd9l-KO, Samd9l-WT, and Samd9l-Mut mice. (G) Gating strategy for mature hematopoietic cells: B cells (B220+CD3-), T cells (B220-CD3+), and Myeloid (B220-CD3-CD11b+) (H) Percentages of mature cells in the spleens (n=4). I. EdU incorporation in the mature cells of Samd9l-KO, Samd9l-WT, and Samd9l-Mut mice (n=3). For statistical analysis, groups were initially compared by Kruskal-Wallis test, significant results were followed by pairwise comparisons with the Wilcoxon rank-sum test (p-values, *p<0.05, **p<0.01, ***p<0.001). Error bars indicate standard error (SEM) of the mean for biological replicates. For representation, C57BL/6 (grey), Samd9l-KO (blue), Samd9l-WT (black), and Samd9l-Mut (red).
Figure S2: Higher expression of *Samd9l* in mature B cells may account for their increased sensitivity to the effects of the mutation.

A-B. Violin plots of the expression of *Samd9l* in different hematopoietic lineages from publicly available expression profiling arrays from human GSE19599 (A) and mouse GSE6506 (B) datasets. C. Expression of *Samd9l* from C57BL/6 sorted B cell (B220+, CD3e-), T cell (CD3e+, B220-), Myeloid (CD11b+, B220-, CD3e-), and Lin-Kit+ (cKit+, CD11b-, B220-, CD3e-) by qPCR (n=4). D. Violin plots showing the expression of *Samd9l* in different murine B cell lineages from publicly available gene expression data GSE38463. E. Flow cytometric gating strategy for Hardy fractions of B cell maturation stages as follows: Pre-pro-B (Fr.A) B220+ CD43+ BP1- CD24-; Pro-B (Fr.B) B220+ CD43+ BP1- CD24+; Pre-B (Fr.C-C’) B220+ CD43+ BP1+ CD24+; Pre-B (Fr.D) B220+ CD43- IgM- IgD-; Immature B (Fr.E) B220+ CD43+ IgM+ IgD-; and Mature B (Fr.F) B220+CD43- IgM+ IgD+. F. Flow cytometric analysis comparing the Hardy fractions in the BM of *Samd9l*-WT and *Samd9l*-Mut (n=6). For statistical analysis, groups were initially compared by Kruskal-Wallis test. Significant Kruskal-Wallis results were followed by pairwise comparisons with the Wilcoxon rank-sum test (p-values, *p<0.05, **p<0.01). Error bars indicate SEM of the mean for biological replicates.
Figure S3: Samd9l mutant mice have a distinct profile of hematopoietic progenitors in single-cell analysis. A. A heatmap of single-cell RNA-seq data showing 11 clusters identified by well-established markers (12, 13). The top 5 uniquely expressed marker genes in each cluster were annotated. The color represents the z-scored expression level of each gene. B-D. A heatmap (B) or pie-charts (C) illustrating the proportion of the 11 clusters identified from single-cell RNA-seq analysis, (D) Bar graphs showing the proportion of the main populations in Samd9l-KO, Samd9l-WT, and Samd9l-Mut mice. In the heatmap, red or blue colors indicate high or low values among the groups, respectively, and numbers represent the percentages in total cells. E. UMAP plots demonstrating the differentiation trajectories of hematopoietic progenitors identified by
the indicated markers. F. A Rich factor plot showing the results of GO (Gene Ontology) term analysis for the DEGs between HSPC populations of \textit{Samd9l-Mut} and \textit{Samd9l-WT} mice. The horizontal axis represents logged FDR, and the color and size of circles represent the number and relative enrichment of genes in each GO term. G. A Rich factor plot showing the results of GO term analysis for the DEGs. The horizontal axis represents logged FDR, and the color and size of each dot represent numbers and relative enrichment of genes in each GO term. H. A Rich factor plot showing the results of GO term analysis for the DEGs between B cell populations of \textit{Samd9l-Mut} mouse and \textit{Samd9l-WT} mouse.
Figure S4: Samd9l mutant cells are less fit than normal counterparts. A. Number of cells per colony for C57BL/6 (grey), Samd9l-KO (blue), Samd9l-WT (black), and Samd9l-Mut (red) BM cells cultured for one week in methylcellulose (n=3). B. Relative distribution of the indicated colony subtypes in the tested groups (n=3). C. Mature cell populations (B, T, and Myeloid cells) in the peripheral blood of CD45.2 cells (C57BL/6, Samd9l-KO, Samd9l-WT, or Samd9l-Mut) from competitive transplants injected via tail-vein injections. Blood was collected at week 8 post-injection and assessed by flow cytometer (n=12). D. BM and spleen CD45.2 chimerism (Samd9l-WT or Samd9l-Mut) from competitive transplants via intrafemoral (I.F.) injections versus CD45.1 competitor cells (n=10). E-G. Flow cytometric analysis (n=10) showing the proportions of different mature cell populations in the (E) BM (left) and spleen (right); and (F) BM hematopoietic progenitors in CD45.2 cells (Samd9l-WT or Samd9l-Mut) from I.F. injected competitive transplants versus CD45.1. (G) Fold change of weekly peripheral blood percentages of B, T, or Myeloid cells in CD45.2 over CD45.1. For panels A-C, groups were initially compared by Kruskal-Wallis test, and if significant, followed by pairwise comparisons with Wilcoxon rank-sum test. For panel D-F, a two-way ANOVA global test was performed and followed by Wilcoxon rank-sum test between genotypes. For panel G, a longitudinal mixed effects
regression model was used for statistical analysis. A significant result was tested by

evaluating the equality of effect at pre-specified time points between the two groups. (p-

values, *p<0.05, **p<0.01, ***p<0.001). Error bars indicate SEM of the mean for biological
replicates.
Figure S5: Inflammation regulates Samd9l expression and furthers the decrease in mutant cell fitness. A. Immunofluorescent microscopy of HEK293T treated with IFN-α or vehicle for 24h. Cells were labeled with anti-SAMD9 or anti-SAMD9L (red) and the DAPI nuclear stain (blue). Images were acquired on a Nikon C2 laser scanning confocal microscope (60x). B. SAMD9 or SAMD9L protein expression in human cord-blood CD34+ cells treated with vehicle or IFN-α (1000U) for 24h. C. Volcano plots of DEG in hCD34+ cells with and without IFN-α. SAMD9 and SAMD9L genes were annotated. D. Cell count of BM cells treated twice with IFN-α (1000U) or vehicle for 48h from the indicated mice in Figure 4B (n=5). E-F. EdU (E) or O-propargyl-puromycin (OPP) incorporation (F) in BM of Samd9l-KO, Samd9l-WT, or Samd9l-Mut cells treated twice with IFN-α (1000U) or vehicle for 48h (n=4 per group). G. CD45.2 chimerism in the
spleens of Samd9l-WT and Samd9l-Mut treated with vehicle or pl:pC. The data shows the percentage of CD45.2 cells of the donor cells from 5:1 competitive transplants versus CD45.1 as described in Figure 4H. H. Mature cells percentage of CD45.2 cells in BM (left) or spleen (right) as described in Figure 4H. I-J Competitive transplants of Samd9l-Mut treated with or without pl:pC (CD45.2) versus CD45.1 (1:1 ratio). The data shows CD45.2 chimerism in (I) PB, (J) BM, and spleens. K. Annexin V percentage in CD45.1 or CD45.2 cells from BM of the 1:1 transplants. For panel I, a longitudinal mixed effects regression model was followed by evaluating the equality of effect at the pre-specified timepoints. For all other panels, pairwise comparisons Kruskal-Wallis test followed by multiple Wilcoxon rank-sum tests was used. Data show mean ±SEM. P-value: *, p<0.05, **, p<0.01, ###, ***, p<0.001, color indicate the comparison group). For representation, Samd9l-WT (black), and Samd9l-Mut (red) (strips/dotted lines for pl:pC or IFN-α).
**Figure S6: Transcriptional changes of LK cells after inflammation.**

**A.** A tSNE plot of vehicle- and pl:pC-treated Samd9l-WT and Samd9l-Mut LK cells (n=2 mice per condition).

**B.** Pathways upregulated in pl:pC-treated relative to vehicle-treated Samd9l-Mut mice. The rich factor is determined by statistically significant genes divided by the total gene set, the size and color of dots represent gene count and fold enrichment, respectively. The position of the dots indicates the false discovery rate (FDR) significance for the indicated pathways.

**C.** A plot of pathway enrichments of DEG from comparisons of vehicle or pl:pC treated Samd9l-Mut against Samd9l-WT groups and vice versa. The size of the circles is proportional to the significance (FDR) of the enrichment. The color is dependent on the rich factor of the analysis.
Figure S7: A. GSEA showing TGF-β pathway enrichment in hCD34+ cells overexpressing SAMD9L-W1180R relative to control hCD34+ cells transduced with empty vector (CL20). B. Intra-cellular phospho-SMAD2/3 expression in WBM form Samd9l-KO, Samd9l-WT, and Samd9l-Mut BM treated twice with IFN-α (1000U) or vehicle for 48h and assessed by flow (n=4). C-D. Intracellular phospho-SMAD2/3 expression in (C) WBM or (D) mature cells of Samd9l-WT (n=4), and Samd9l-Mut (n=3) BM treated with pl:pC or vehicle as described in Figure 5A. E. Annexin V percentage in cells after week 1 and week of CFU from Samd9l-WT or Samd9l-Mut treated with vehicle or SD-208 (n=4 per group). Statistics were measured by Kruskal-Wallis test followed by multiple Wilcoxon rank-sum tests for pairwise comparisons (p-values, *p<0.05, **p<0.01, ***p<0.001). Error bars indicate SEM of the mean for biological replicates. For representation, Samd9l-KO (blue), Samd9l-WT (black), and Samd9l-Mut (red). IFN-α or pl:pC (dotted lines) and vehicle (solid). Brown or grey colors were used for 1D11 mAb treated Samd9l-Mut or Samd9l-WT mice, respectively.
**Figure S8:** Inflammation exacerbates the pathogenesis of the *Samd9l* mutant mice. **A.** BM cytospins at 60x magnification stained with a modified Romanowsky stain. Red arrows = immature myeloid precursors and green arrowheads = lymphocytes. **B-C.** BM sections (H&E stain) at 40x magnification (**B**) and spleen at 4x magnification (**C**). **D.** Thymus sections (10X and 40X) from *Samd9l-Mut* mice treated with pl:pC and show either apoptosis in the cortex (left, n=2) or atypical hyperplasia (right, n=2).
Figure S9: Inflammation further changes lineage composition in *Samd9l* mutant mice. **A.** A heatmap of the single-cell RNA-seq data from *Samd9l*-WT or *Samd9l*-Mut mice treated with either vehicle or pl:pC showing 17 clusters identified from the top 5 uniquely expressed marker genes in each cluster. The color represents the z-scored expression level of each gene. **B.** UMAP plots of representative markers for the main populations. The colors of each dot represent the normalized expression level of genes indicated above. **C.** A heatmap showing the proportion of the identified 17 clusters as well as the major 5 populations from each WBM sample. The red or blue colors indicate high or low values compared to the average of the groups, respectively, and numbers...
represent the percentages in total cells. D. Pie charts showing the distribution of the identified 17 clusters in Samd9l-WT and Samd9l-Mut mice with or without pl:pC treatment. E. Rich factor plot showing the GO term analysis for the DEGs between HSPC populations of vehicle-treated and pl:pC-treated Samd9l-Mut mice. F-G. UMAP plots demonstrating the differentiation trajectories of Myeloid (F), or B cells (G) in Samd9l-WT and Samd9l-Mut mice treated with vehicle or pl:pC. H. Violin plots of the expression levels of differentially expressed genes between B cell populations of vehicle-treated or pl:pC-treated Samd9l-Mut mice. Representative genes involved in proliferation (Mki67 and Stat1), pro-apoptotic response (Bax), and inflammatory response (Nfkb2) are shown.
Figure S10: Inflammation exacerbates mutant Samd9l phenotypes. A. Cross sections of BM (40x magnification) of vehicle- and pI:pC-treated Samd9l-WT and Samd9l-Mut mice showing expression of CD235a. B-C. Cross section of spleens (4x magnification) from vehicle- and pI:pC-treated Samd9l-WT and Samd9l-Mut mice stained with (B) anti-Gata1 (C) anti-CD235a. D-E. Stages of erythroid maturation (ProE, EryA, EryB, and EryC) in the spleen (n=10) (D) or PB (E) of pI:pC or vehicle-treated Samd9l-WT (n=7) and Samd9l-Mut (n=8) mice assessed by flow cytometry after 4 weeks after pI:pC or vehicle treatment. F. CBC showing changes in red blood cells (RBC) and platelets (PLT) after 4 weeks post-pI:pC or vehicle treatment in Samd9l-WT and Samd9l-Mut mice (n=8). G. CBC (n=3) showing the RBC counts in lethargic Samd9l-Mut mice (red) relative to Samd9l-WT mice (black). H. Disease burden in the spleens of the lethargic mice showing spleen size in grams of the tested mice (n=3). For panels G and H, Wilcoxon test was performed to test the distribution difference between the 2 genotypes. For all other panels, Kruskal-Wallis test was performed and followed by multiple Wilcoxon rank-sum tests for pairwise comparisons. Error bars indicate SEM of the mean for biological replicates. For representation, Samd9l-WT (black), and Samd9l-Mut (red). (Dotted lines, p-values, #p<0.05, ##p<0.01, ###p<0.001, color indicate the comparison group) and vehicle (solid, p-values, *p<0.05, **p<0.01, ***p<0.001, color indicate the comparison group).
Figure S11: Inflammation induces non-random chromosome deletion in Samd9l mutant mouse. A. Heatmap of RNA-seq data from Samd9l-WT and Samd9l-Mut mice treated with vehicle or pI:pC (n=1 per group). The data shows the expression pattern of the expressed genes within the affected region at chromosome 6 (see also Figure 8E). B. FISH analysis of spleens from a Samd9l-Mut mouse treated with pI:pC showing the affected locations. Left image is showing the proximal at chr6:3,496,083-3,687,193 (red), and distal probe at chr6:28,129,437-28,303,622 (green). The right image is showing the same cell with an additional intermediate probe at chr6:22,116,691-22,428,747 (red, Wnt16). Nuclei were stained by DAPI and were outlined by white dashed lines. Images were captured using a Nikon E800 microscope with a 60X PlanApo objective lens. The imaging software used was Nikon NIS-Elements AR with 3D deconvolution.
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