Supplemental Methods

Competitive bone marrow transplantation

Whole bone marrow (BM) cells (2×10^6) from wild-type and *Asc*$^{-/-}$ mice were transplanted via the tail vein into congenic recipient mice (Ly5.1) irradiated with lethal doses of radiation (9.5 Gy) with the same number of competitive BM cells (Ly5.1) (primary transplant). PB cells were analyzed every month after transplantation, and BM cells were analyzed 4 months later. Whole BM cells (5×10^6) from primary recipient mice were transplanted via the tail vein into secondary recipient mice (Ly5.1) irradiated with lethal doses (9.5 Gy) (secondary transplantation). The PB cells were analyzed monthly after transplantation.

Immunoblot analysis

Wild type (WT) and *Asc*$^{-/-}$ mice were intraperitoneally injected with 1.5 mL of 10% thioglycolate. Three days later, the peritoneal cavity was washed with PBS, and the peritoneal fluid was collected. Cells in the peritoneal fluids were resuspended in RPMI (FUJIFILM Wako) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin/streptomycin/glutamine (Gibco) and cultured on cell culture dishes. After 30 min of culture, the medium was replaced, and macrophages adhered to the cell culture dishes, which were used for the following experiments. Peritoneal macrophages were treated with 200 ng/mL lypopolysaccharide (LPS) (InvivoGen) at 37°C in 5% CO2 for 4 h followed by treatment with 10 mM nigericin (InvivoGen) for 1 h. Cells were collected using a cell scraper, dissolved in 2% sodium dodecyl sulfate (SDS) lysis buffer (20 mM Tris-HCl pH 8.0, 2% SDS), and sonicated (High, 30 s ON, 30 s OFF, 10 times) using a BIORUPTOR (Sonicbio). SDS sample buffer (100 mM Tris-HCl pH.6.8, 20% Glycerol, 2% SDS, 0.04% BPB, 10% 2-mercaptoethanol) was then added, and the cells were denatured by heating at 95°C for 10 min to make cell lysates. To collect proteins in supernatants, the culture medium was agitated vigorously with four times the volume of ice-cold acetone and allowed to stand overnight at -20°C. The precipitate was collected by centrifugation, resuspended in SDS sample buffer, and heat-denatured. These samples were electrophoresed using SDS-PAGE, followed by western blotting. The following

antibodies were used: anti-ASC/TMS1 (D2W8U) (Cell Signaling Technology, 67824S, 1:1000), anti-mouse IL-1β/IL-1F2 (R&D Systems, AF-401-NA, 1:800), anti-NLRP3 (Abcam, ab272702, 1:1000), anti-Cleaved Caspase-1 (Cell Signaling Technology, 89332S, 1:1000), and anti-β-actin (C4) (Santa Cruz, sc-47778, 1:1000).

Detection of active Caspase-1

Active Caspase-1 was detected using the FAM-FLICA® Caspase-1 Assay Kit (Immunochemistry Technologies). FLICA was dissolved in dimethyl sulfoxide and made into 150 x stock, then diluted with PBS (30 x FLICA solution) immediately before use. One μL of 30 x FLICA solution was added to 30 μL of cell suspension and allowed to react for 1h at 37°C. After the reaction, cells were washed with 1 x Cellular Wash Buffer twice and treated with 0.5 μg/mL propidium iodide for 5 min. The stained cells were analyzed using FACS Celesta.

Immunofluorescence staining

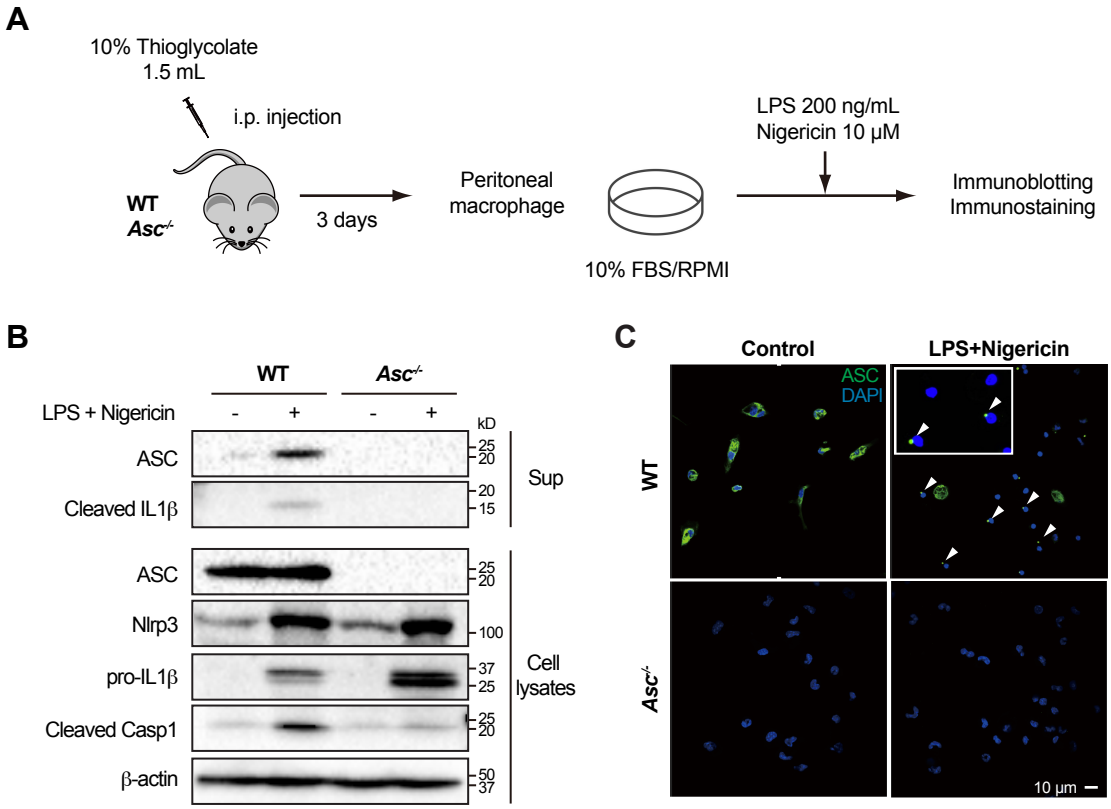
After treatment of intraperitoneal macrophages with LPS and Nigericin obtained in the same way as for Immunoblot analysis, cells were transferred onto glass slides and allowed to stand for 30 min at room temperature; after fixation with 4% paraformaldehyde for 15 min, cells were washed with PBS and permeabilized with 0.1% Triton X-100 for 10 min. After washing with PBS, the cells were blocked with 3% BSA for 1 h and washed again. The cells were incubated with anti-ASC primary antibody at 4°C overnight. Subsequently, the cells were incubated with an anti-rabbit IgG secondary antibody at room temperature for 2 h. Moreover, they were treated with 1 μg/mL DAPI for 5 min, washed again, and then mounted with Glass Antifade Mountant. Asc localization was observed using a live cell imaging microscope DragonFly201 (Andor), and images were analyzed using the Imaris Microscopy Image Analysis Software (Oxford Instruments). The antibodies used for immunostaining were anti-ASC/TMS1 (D2W8U) (Cell Signaling Technology, 67824S; 1:400) and CF488A Donkey Anti-Rabbit IgG (H&L) (Biotium, 20015; 1:1000).

Cell death analysis

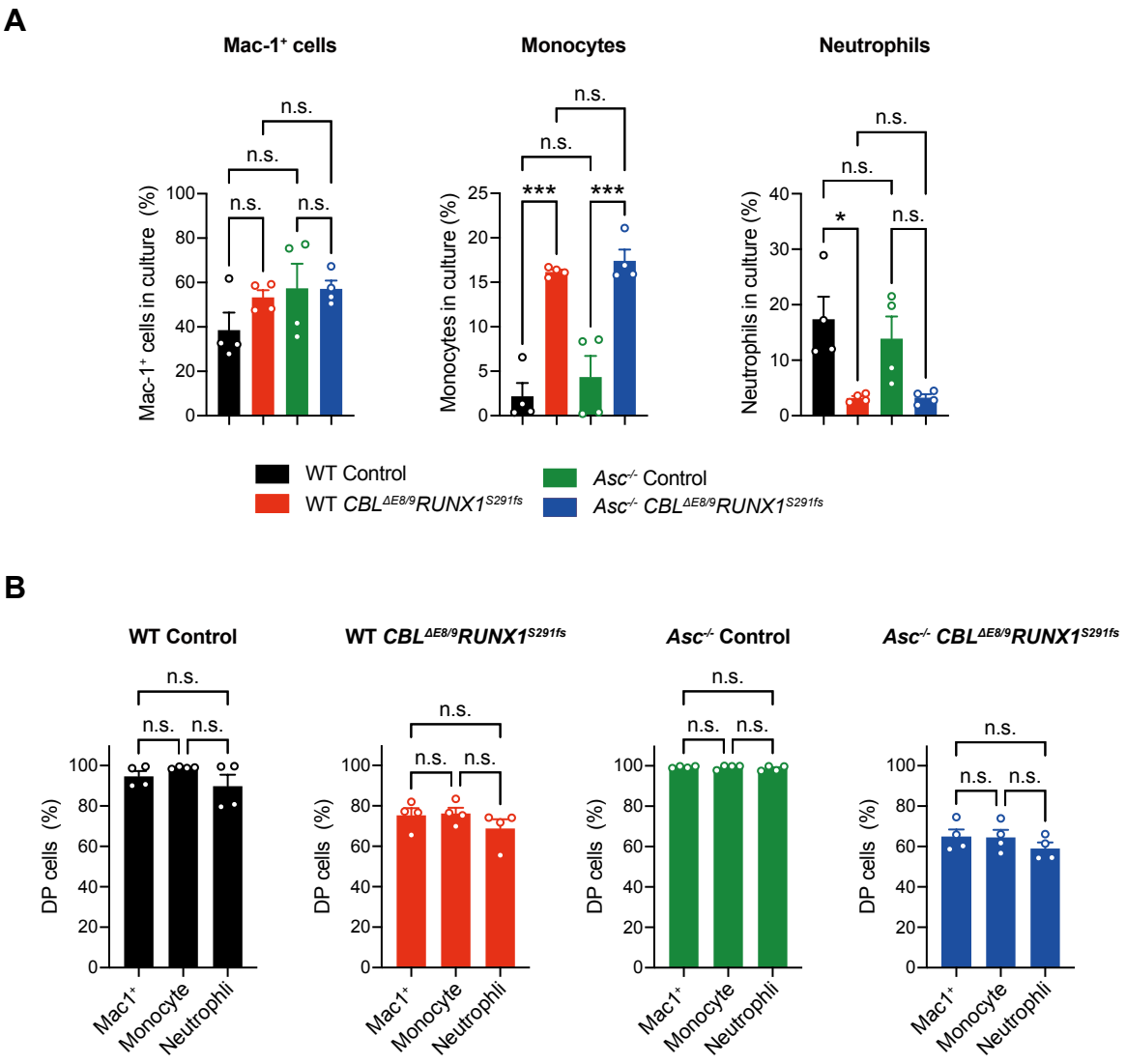
Cell death analysis was performed on cultured or transplanted cells using an Annexin V Apoptosis Detection Kit (BD Pharmingen).

Cytokine measurement

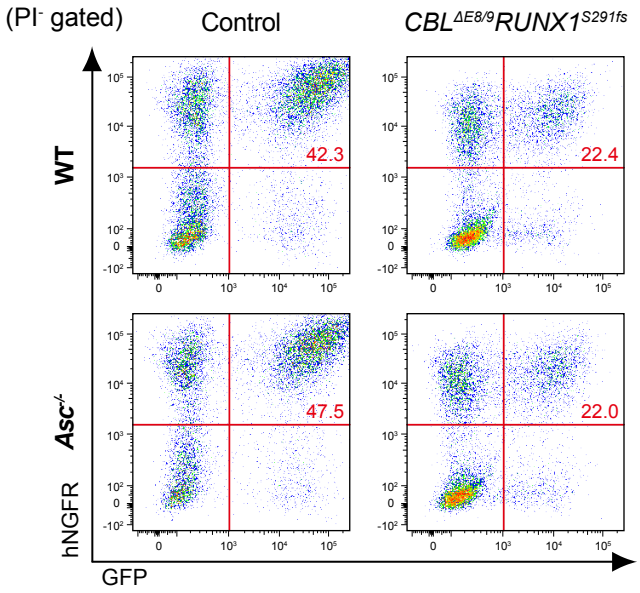
For BM fluid collection, the femur and tibia from one hind limb of each mouse were flushed with 200 μL of PBS using a 1 mL syringe with a 26-gauge needle. The collected BM fluid was centrifuged at 300 × g for 5 min at 4℃ to remove BM cells. The supernatants were centrifuged at 12,000 × g for 10 min, and the resulting supernatants were stored at –80°C until analysis. For collection of culture supernatants conditioned medium was collected from $Lin^{-}hNGFR^{+}GFP^{+}$ cell cultures on day 8 and clarified by centrifugation before analysis. The concentrations of IL-1β, IL-18, and TNF-α were measured by enzyme-linked immunosorbent assay using Mouse IL-1 beta/IL-1F2 DuoSet (R&D Systems, DY401-05), Mouse IL-18 DuoSet (R&D Systems, DY7625-05), and Mouse TNF-alpha DuoSet (R&D Systems, DY410-05), respectively, according to the manufacturer's instructions. Absorbance was measured at 450 nm with wavelength correction at 540 nm using a CLARIOstar Plus microplate reader (BMG LABTECH). The corrected absorbance was calculated by subtracting the absorbance at 540 nm from that at 450 nm. Cytokine concentrations were calculated by interpolation from the corresponding standard curves using a four-parameter logistic regression model and were multiplied by the appropriate sample dilution factors.



Supplementary Figure 1. Impaired activation of inflammasomes in *Asc*$^{-/-}$ peritoneal macrophages
(A) Experimental schema. Macrophages were collected from the peritoneal cavity of wild-type and *Asc*$^{-/-}$ mice injected with 10% thioglycolate. Peritoneal macrophages were primed with LPS for 4 h, followed by stimulation with nigericin for 1 h to induce inflammasome activation.
(B) Detection of inflammasome-associated molecules by immunoblotting. After stimulation in (A), cell lysates of peritoneal macrophages and culture supernatants concentrated with acetone were prepared and subjected to SDS-PAGE electrophoresis, followed by western blot to detect ASC, Nlrp3, activated Caspase-1, pro-IL-1β, and activated IL-1β (cleaved IL-1β).
(C) Analysis of ASC speck formation by immunostaining. Cells stimulated in (A) were immunostained with an anti-ASC antibody and observed by confocal fluorescence microscopy. Arrows indicate Asc specks. Enlarged view is depicted in the upper left panel.

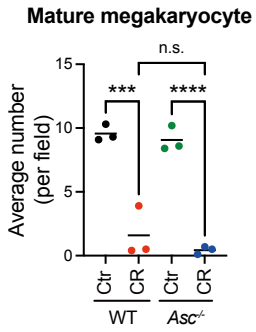


Supplementary Figure 2. Differentiation of culture cells
(A) Proportions of mature myeloid cells on day 15 of culture.
(B) Proportions of *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} (DP) cells within Mac-1$^+$ myeloid cells, CD115$^+$ monocytes and Ly6G$^+$ neutrophils. Significant differences between the two groups by *one-way* ANOVA are indicated with an asterisk. *p <0.05, ***p<0.001, n.s., not significant.

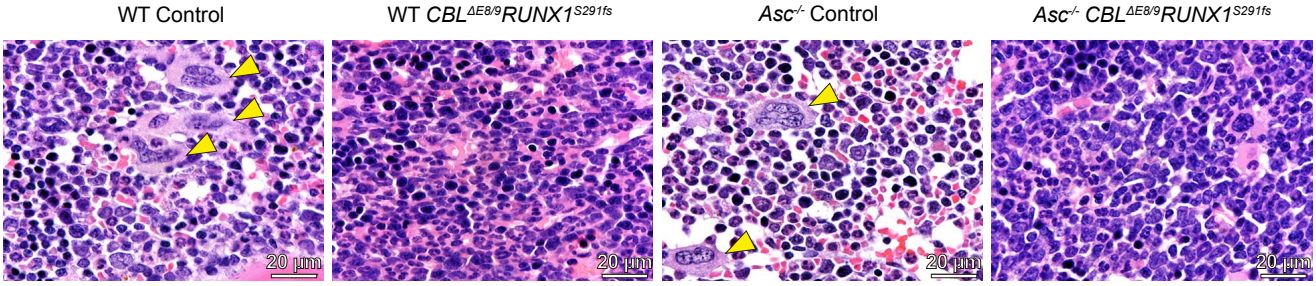


Supplementary Figure 3. Flow cytometric profiles of transduced c-kit-positive cells used for transplantation
Representative flow cytometric profiles of c-kit-positive cells from WT and *Asc*$^{-/-}$ mice transduced with *CBL*$^{\Delta E8/9}$ and *RUNX1*^{S291fs} retroviruses immediately before transplantation. *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} cells accounted for more than 10% of the bulk c-kit-positive cells in all transplantation experiments.

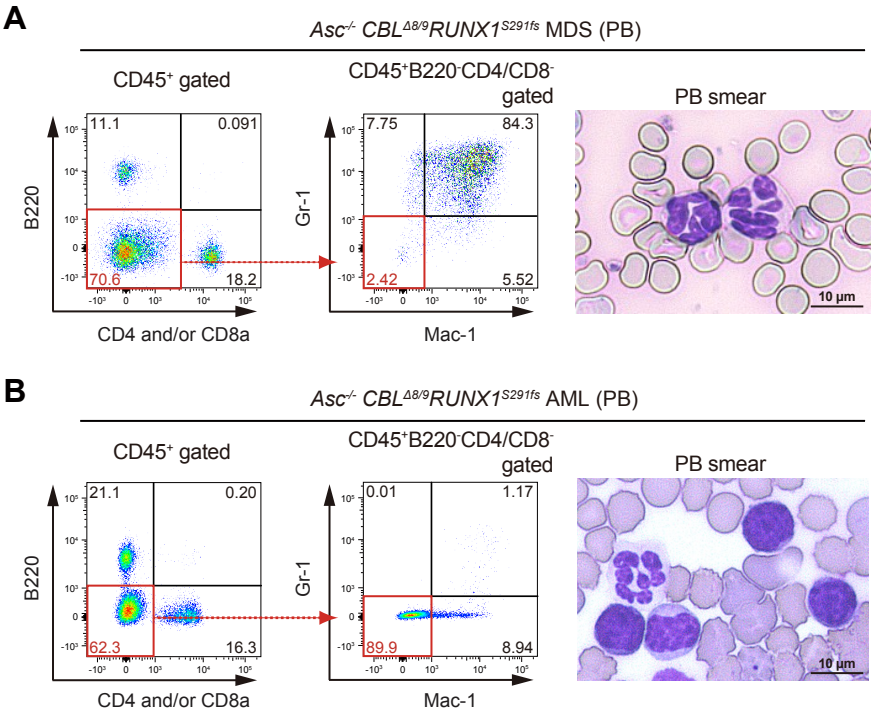
A



B

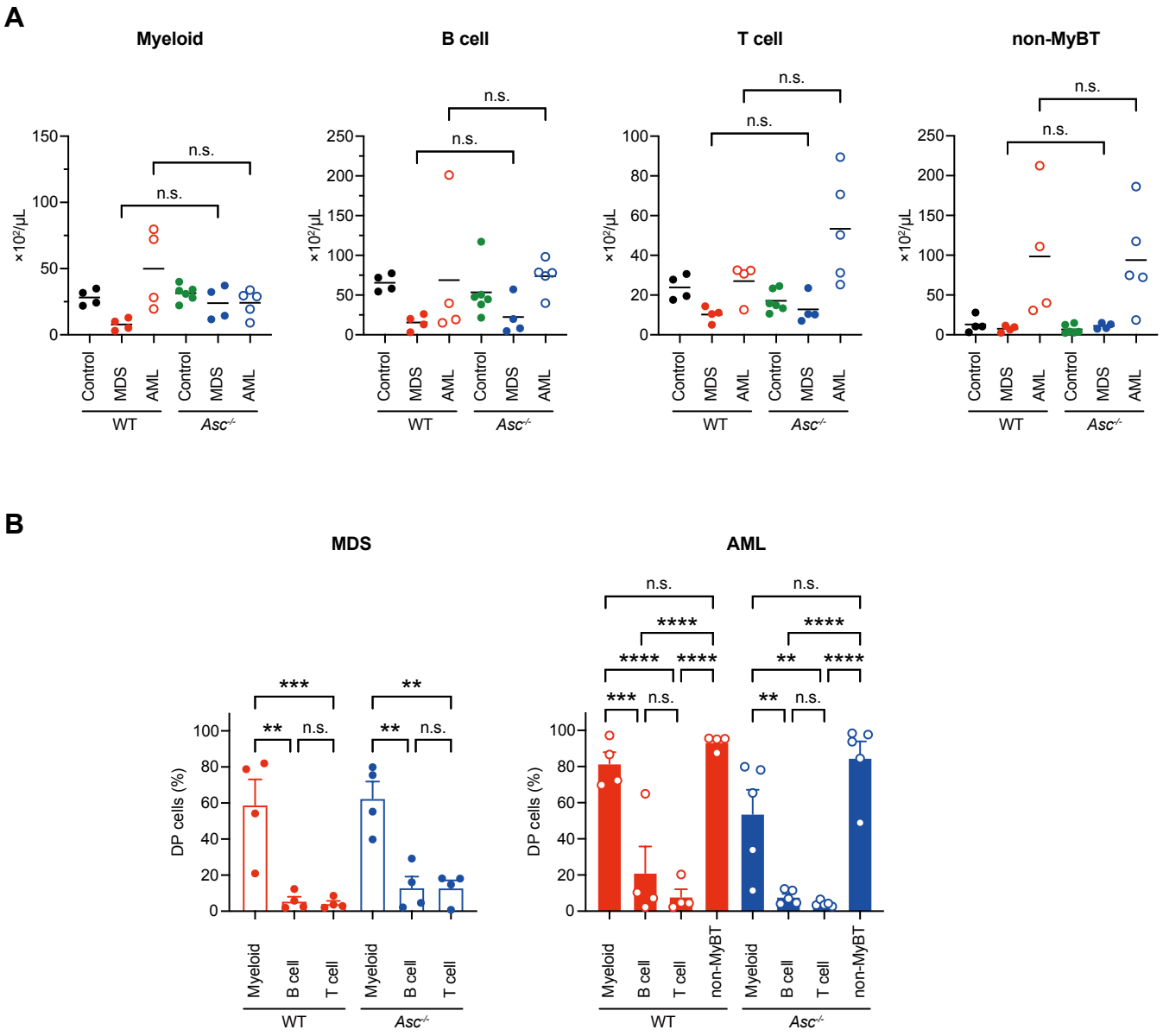


Supplementary Figure 4. Histological analyses of BM from WT and *Asc*$^{-/-}$ control and *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} mice
(A) Numbers of mature megakaryocytes in the BM of WT and *Asc*$^{-/-}$ control and *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} mice. Each dot represents the average number of mature megakaryocytes per mouse, calculated from 10 high-power fields (HPFs; ×40 objective). Significant differences between groups were determined by *one-way* ANOVA and are indicated with asterisks. ***p <0.001, ****p <0.0001; n.s., not significant.
(B) Representative hematoxylin and eosin (H&E)-stained sections of femurs from WT and *Asc*$^{-/-}$ control and *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} mice at 3 months post-transplantation. Mature megakaryocytes are indicated by yellow arrowheads.

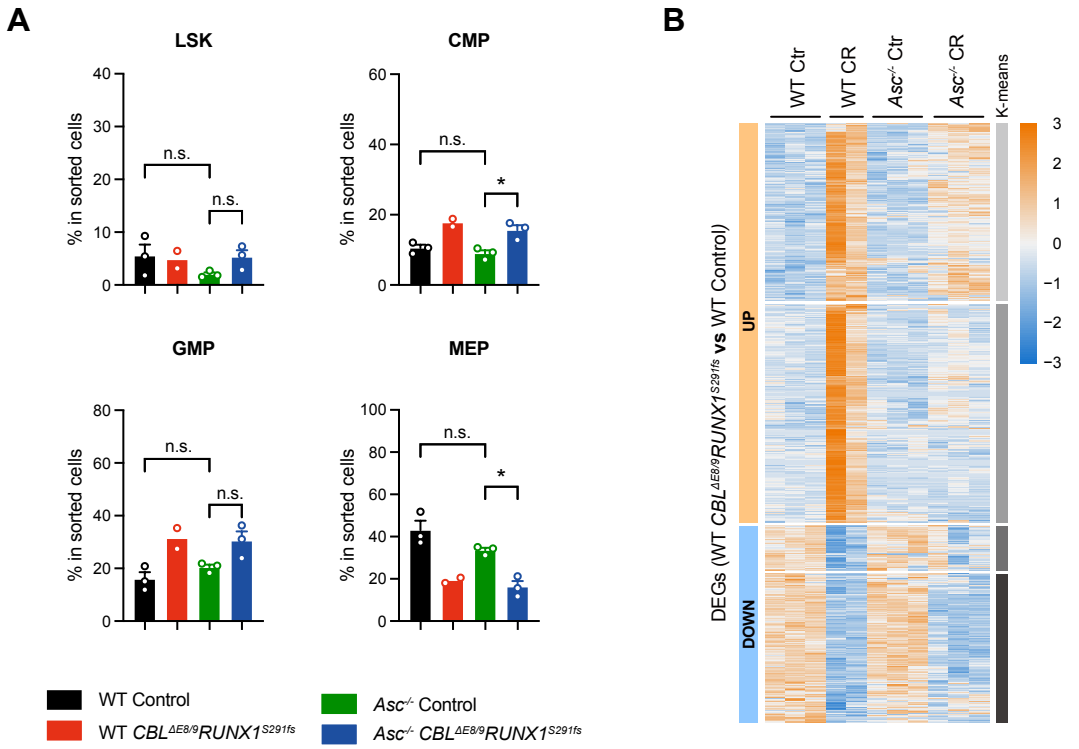


Supplementary Figure 5. Representative *Asc*$^{-/-}$ *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} MDS and AML mice

(A, B) Representative *Asc*$^{-/-}$ *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} MDS mice that died of advanced MDS (A) and AML (B). Flow cytometry plots of PB (left and middle) and images of PB smears (right) are depicted.



Supplementary Figure 6. Lineage composition of PB from moribund MDS and AML mice.
(A) Absolute numbers of myeloid cells, B cells, T cells and non-MyBT cells in MDS and AML mice PB at their moribund stages and control mice at 7 months post-transplantation.
(B) Proportions of *CBL*$^{\Delta E8/9}$*RUNX1*^{S291fs} (DP) cells within myeloid cells, B cells, T cells and non-MyBT cells in MDS and AML mice PB at their moribund stages.
Significant differences between the two groups by *one-way* ANOVA are indicated with an asterisk. *p <0.05, **p<0.01, ***p<0.001, ****p<0.0001, n.s., not significant.



Supplementary Figure 7. Expression of DEGs in WT *CBL$^{\Delta E8/9}$RUNX1^{S291fs}* compared to WT control
(A) Proportions of HSPC subpopulations within the Lin$^-$c-kit$^+$ progenitors sorted for RNA-seq analysis. For the *CBL$^{\Delta E8/9}$RUNX1^{S291fs}* groups, Lin$^-$c-kit$^+$ progenitors were sorted from GFP$^+$hNGFR$^+$ double positive (DP) cells. For the control groups, because of the low frequency of DP cells, Lin$^-$c-kit$^+$ progenitors were sorted from GFP$^+$ and/or hNGFR$^+$ cells. Statistical analysis was performed using oneway ANOVA. Note that the WT *CBL$^{\Delta E8/9}$RUNX1^{S291fs}* group contained only two biological replicates. * p<0.05, **p<0.01.
(B) Heatmap showing the expression of DEGs in WT *CBL$^{\Delta E8/9}$RUNX1^{S291fs}* compared to WT control in WT and *Asc$^{-/-}$* control (Ctr) and WT and *Asc$^{-/-}$ CBL$^{\Delta E8/9}$RUNX1^{S291fs}* (CR) Lin$^-$c-kit$^+$ cells.