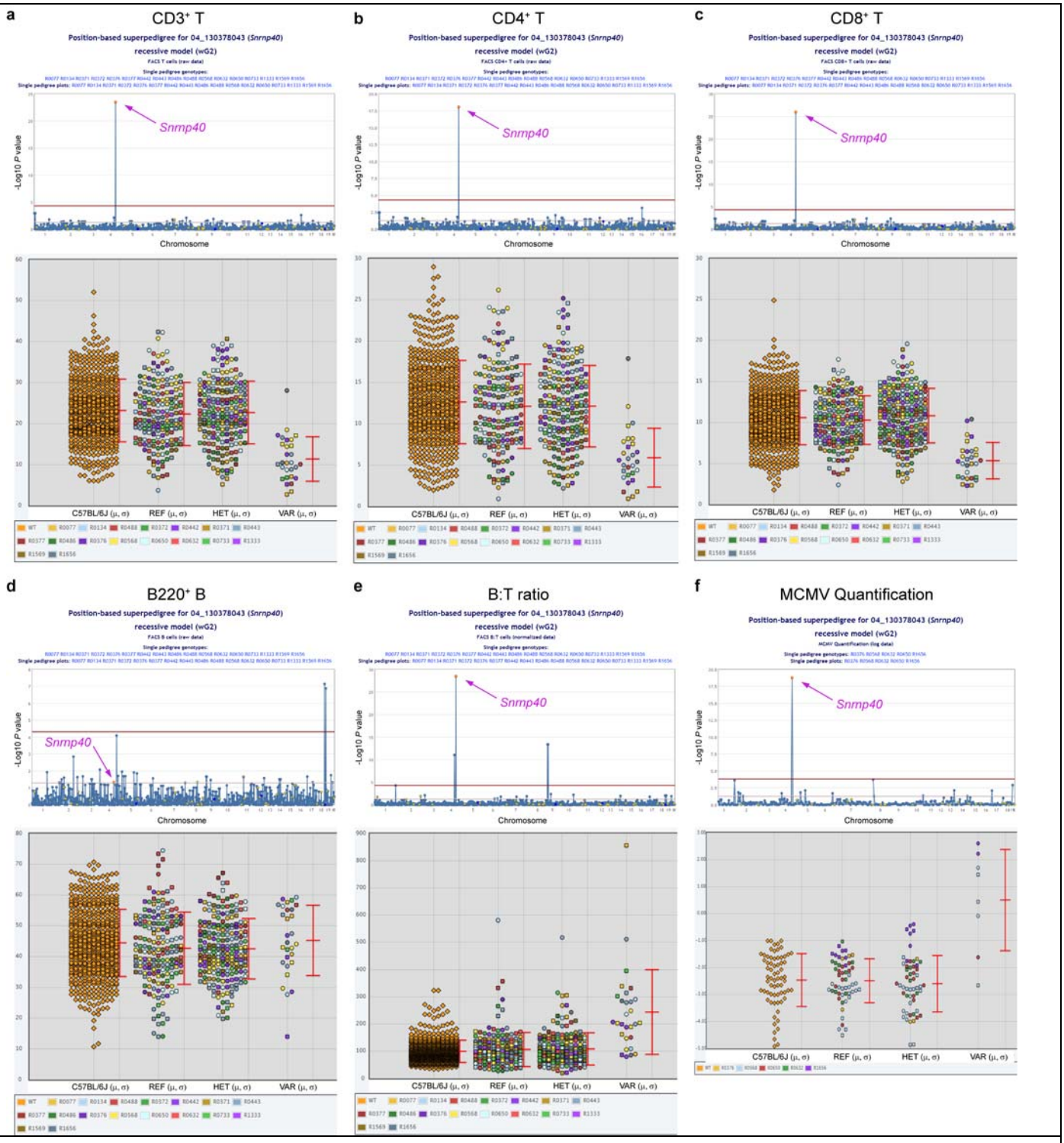


Supplementary Figure 1

Immune phenotypes detected in *skywarp* mice from 17 unrelated pedigrees derived from the C57BL/6J stock.

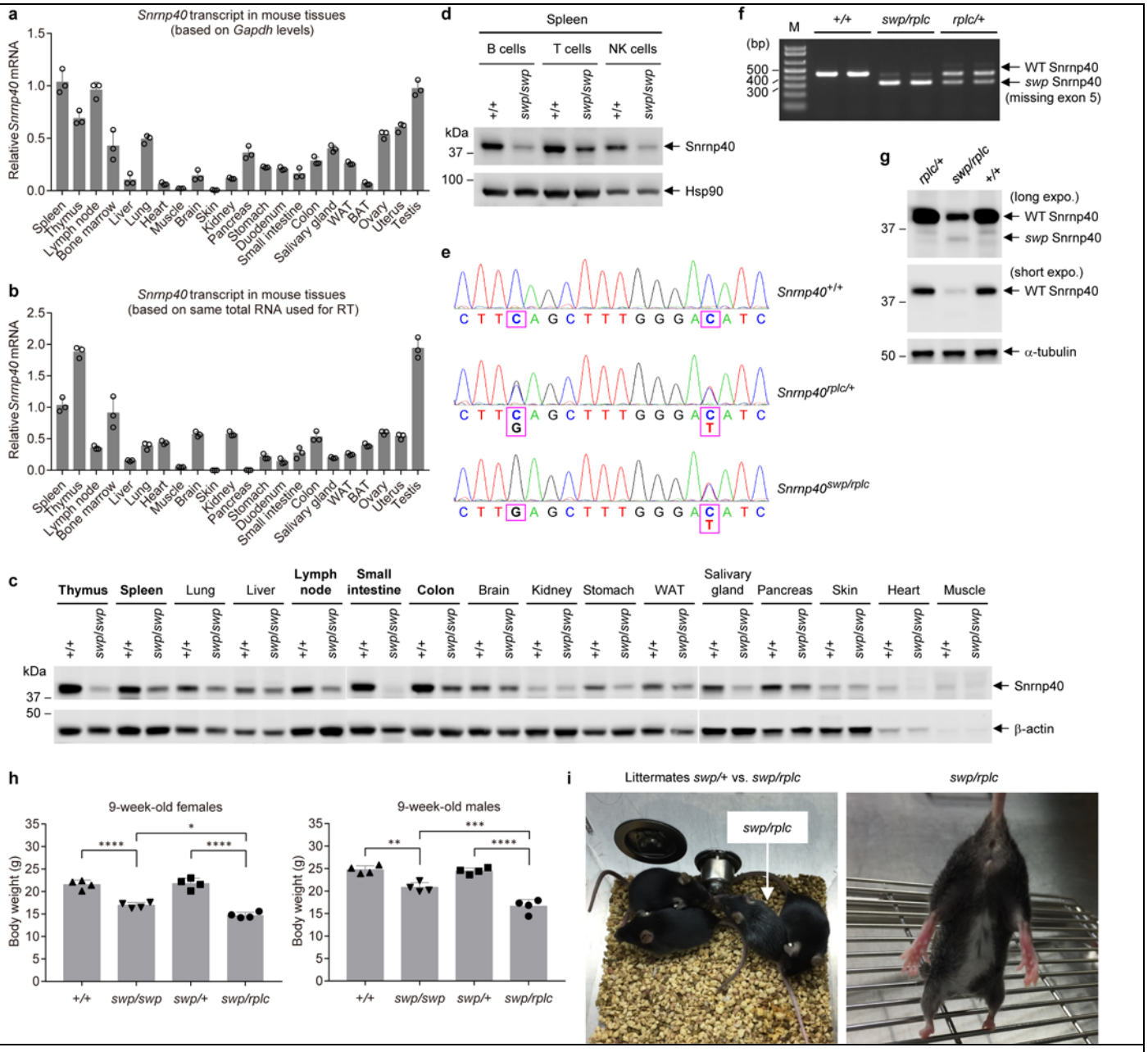
a-**d**, Lower panels, frequency of (**a**) $CD3^+$ T cells, (**b**) $CD4^+$ T cells, (**c**) $CD8^+$ T cells, and (**d**) $B220^+$ B cells among total white blood cells isolated from the blood of mice from 17 pedigrees carrying ENU-induced mutations. Upper panels, Manhattan (Mht) plots showing *P* values of association between the phenotypes in the lower panels and mutations (ENU-induced and spontaneous) identified in the 17 pedigrees, calculated using a recessive model of inheritance. **e**, Lower panel, B cell to T cell ratio in the blood of mice from the same 17

pedigrees. Upper panel, Mht plot showing the *P* values of association between the elevated B:T ratio and mutations identified in the 17 pedigrees, calculated using a recessive model of inheritance. **f**, Lower panel, MCMV titer in the spleen of mice from 5 of the 17 pedigrees five days after infection with MCMV. Upper panel, Mht plot showing the *P* values of association between elevated MCMV titer and mutations identified in the 5 pedigrees, calculated using a recessive model of inheritance. For scatter plots: C57BL/6J, REF (*Snrnp40*$^{+/+}$), HET (*Snrnp40*$^{swp/+}$), and VAR (*Snrnp40*$^{swp/swp}$). Each data point represents one mouse. Mean (μ) and SD (σ) are indicated. For Mht plots: The $-\text{Log}_{10}$ *P* values (Y axis) were plotted versus the chromosomal positions of mutations (X axis). Horizontal red and purple lines represent thresholds of *P* = 0.05 with or without Bonferroni correction, respectively. *n*=168 REF, *n*=212 HET, *n*=29 VAR in **a**-**e**; *n*=56 REF, *n*=55 HET, *n*=8 VAR in **f**. Ten to twenty C57BL/6J wild-type mice (total *n* in scatter plots not counted) were included as controls in each screening experiment but were not used for *P* value calculation (**a**-**f**). All *P* values in upper panels (**a**-**f**) were calculated using generalized linear model. *P*=3.274e-24 in **a** (raw data, wG2, recessive model), *P*=8.547e-19 in **b** (raw data, wG2, recessive model), *P*=1.044e-26 in **c** (raw data, wG2, recessive model), *P*=0.045939 in **d** (raw data, wG2, recessive model), *P*=3.22e-29 in **e** (normalized data, wG2, recessive model), *P*=1.614e-19 in **f** (log data, wG2, recessive model).

Supplementary Figure 2

Detection of a spontaneous mutation in *Snrnp40* in the C57BL/6J stock.

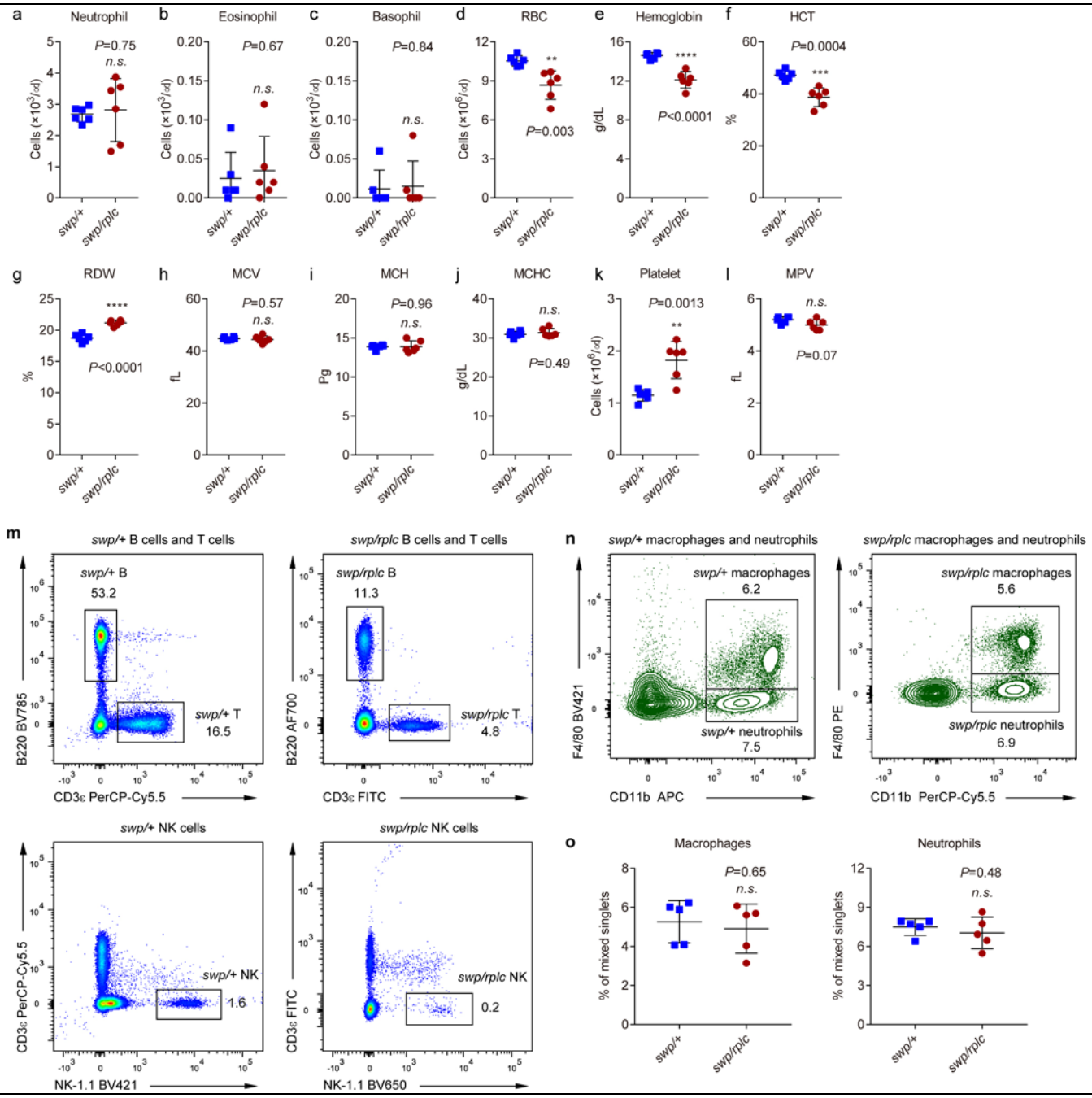
a, DNA sequencing chromatograms. Direct capillary sequencing of 250 non-mutagenized C57BL/6J stock mice from JAX detected heterozygosity for the *swp* mutation in two animals. The region containing the *swp* mutation in the two C57BL/6J stock mice is shown. **b**, Multiple sequence alignment of Snrnp40 amino acid sequence in 11 species. Alignment was performed using Clustal Omega program, and reformatted by MView tool. cov, percent coverage; pid, percent identity with respect to the first sequence (human). Identities normalized by aligned length. Colors indicate identical sequence. *Red box denotes the missing sequence caused by the *swp* mutation.



Supplementary Figure 3

Snrnp40 expression profile and the *Snrnp40^{rplc}* allele.

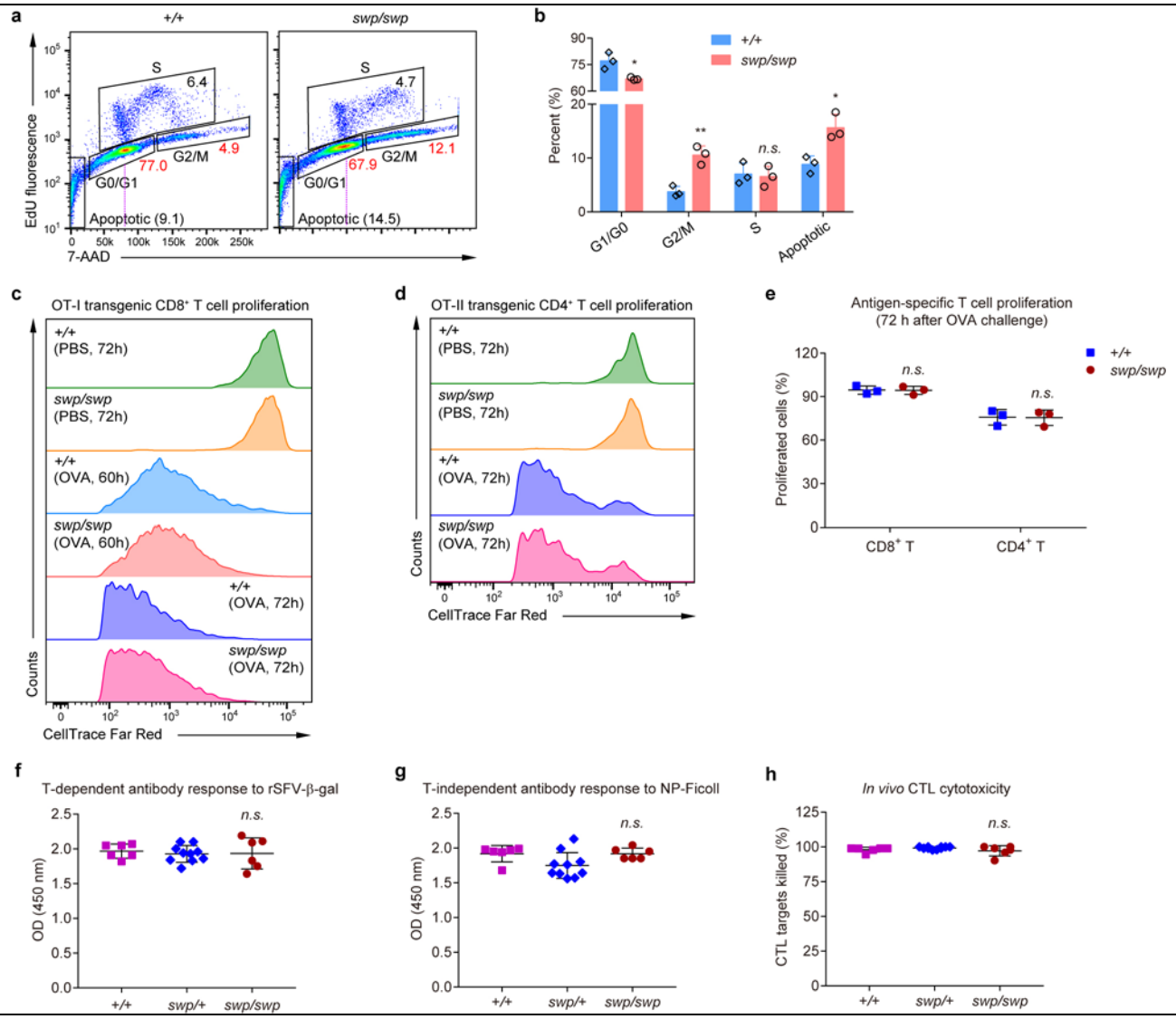
a,**b**, RT-qPCR analysis of *Snrnp40* mRNA levels in various mouse tissues normalized by *Gapdh* mRNA levels using $2^{-\Delta\Delta Ct}$ method (**a**), or by total RNA levels used for reverse transcription using $2^{-\Delta Ct}$ method (**b**). *n*=3 wild-type C57BL/6J littermate mice in **a**,**b**. **c**, Immunoblot analysis of Snrnp40 in various mouse tissues. **d**, Immunoblot analysis of Snrnp40 in splenic B cells, T cells, and NK cells. **e**, DNA sequencing chromatograms. The region containing the *swp* mutation (C→G) and the marker mutation (C→T) in *Snrnp40$^{+/+}$*, *Snrnp40$^{rplc/+}$*, and *Snrnp40$^{swp/rplc}$* mice is shown. **f**, RT-PCR amplification across exon 5 of *Snrnp40* mRNA from splenocytes of *Snrnp40$^{+/+}$*, *Snrnp40$^{rplc/+}$*, and *Snrnp40$^{rplc/swp}$* mice. **g**, Immunoblot analysis of Snrnp40 in splenocytes of the indicated genotypes. Data are representative of five independent experiments with similar results. **h**, Body weight of female and male mice at 9 weeks of age (*n*=4 for each genotype). *P* values were determined by one-way ANOVA with Sidak's multiple comparisons test. **P*=0.031, ***P*=0.0015, ****P*=0.0009, *****P*<0.0001. **i**, Representative images of *Snrnp40$^{swp/rplc}$* mice and *Snrnp40$^{swp/+}$* littermates. *Snrnp40$^{swp/rplc}$* mice display reduced body size, some white back hairs, and a patch of white hair on the belly. Data are representative of two (**a**-**c**), three (**d**,**f**,**h**),or five (**g**) independent experiments (mean ± s.d. in **a**,**b**,**h**).



Supplementary Figure 4

Analysis of immune cells in the blood of *Snrnp40*$^{swp/rplc}$ mice.

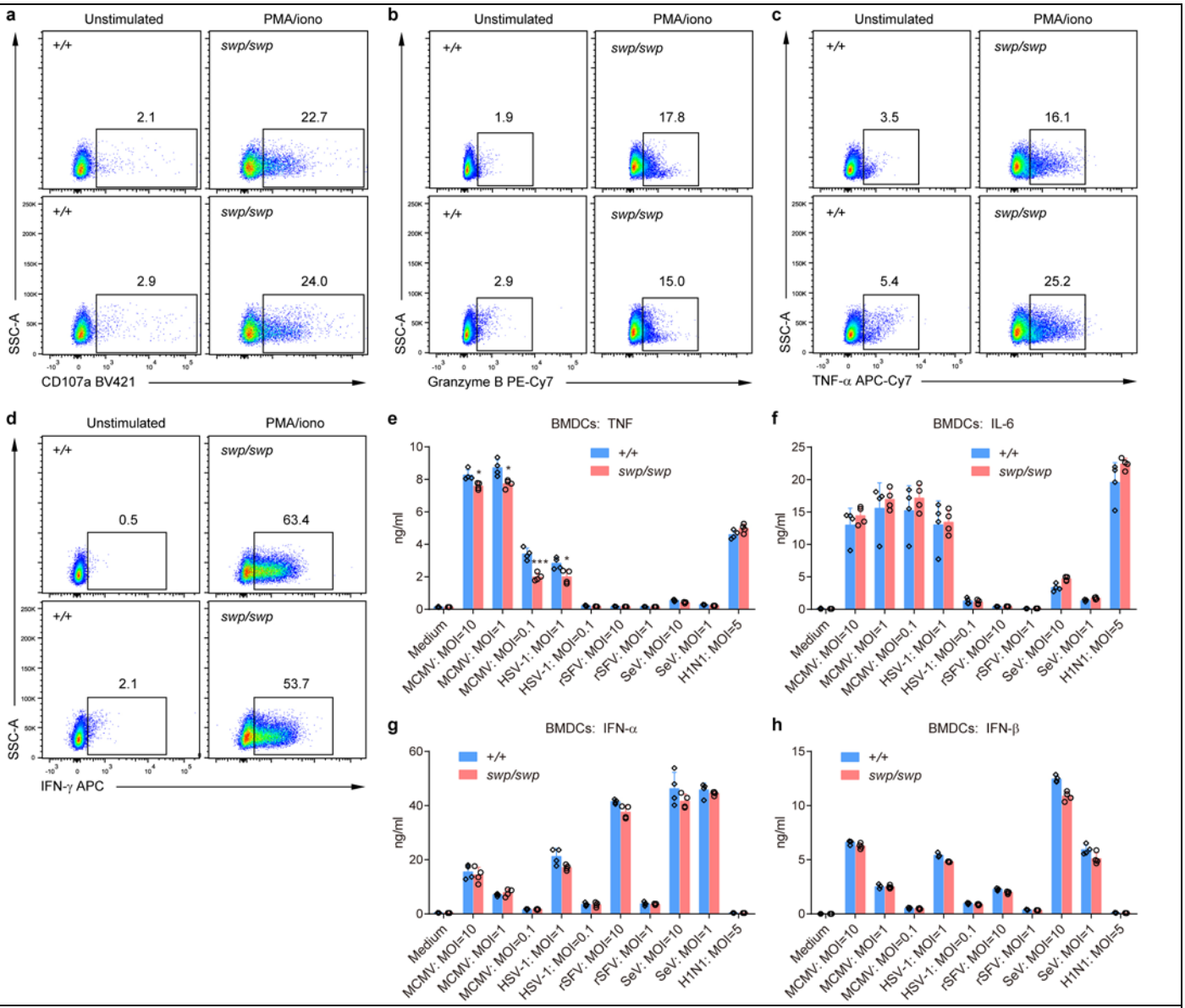
a-**l**, Hematological analysis of blood from *Snrnp40*$^{swp/rplc}$ and *Snrnp40*$^{swp/+}$ littermates (*n*=6 mice for each genotype). Neutrophil (**a**), eosinophil (**b**), basophil (**c**), red blood cells (RBC) (**d**), hemoglobin (**e**), hematocrit (HCT) (**f**), red cell distribution width (RDW) (**g**), mean cell volume (MCV) (**h**), mean cell hemoglobin (MCH) (**i**), mean corpuscular hemoglobin concentration (MCHC) (**j**), platelet (**k**), and mean platelet volume (MPV) (**l**) were determined. The indicated *P* values were determined by unpaired, two-tailed Student's *t*-test. **m**,**n**, Flow cytometry of cells in samples containing equal volumes of blood isolated from *Snrnp40*$^{swp/rplc}$ and *Snrnp40*$^{swp/+}$ mice, assessing the absolute number ratio of B cells (B220$^+$), T cells (CD3ε$^+$), and NK cells (NK1.1$^+$CD3ε$^-$) (**m**), or macrophages (CD11b$^+$F4/80$^+$) and neutrophils (CD11b$^+$F4/80$^-$) (**n**) in *Snrnp40*$^{swp/rplc}$ and *Snrnp40*$^{swp/+}$ littermates. Numbers indicate percent cells in outlined areas. **o**, Frequency of macrophages and neutrophils in the mixed blood samples. *n.s.*, not significant (*P* values indicated; unpaired, two-tailed Student's *t*-test). Data are representative of two (**n**,**o**), three (**m**), or four (**a**-**l**) independent experiments (mean ± s.d. in **a**-**l**,**o**).



Supplementary Figure 5

Defective cell cycle progression of *Snrnp40*$^{swp/swp}$ T cells and normal adaptive immune responses in *Snrnp40*$^{swp/swp}$ mice.

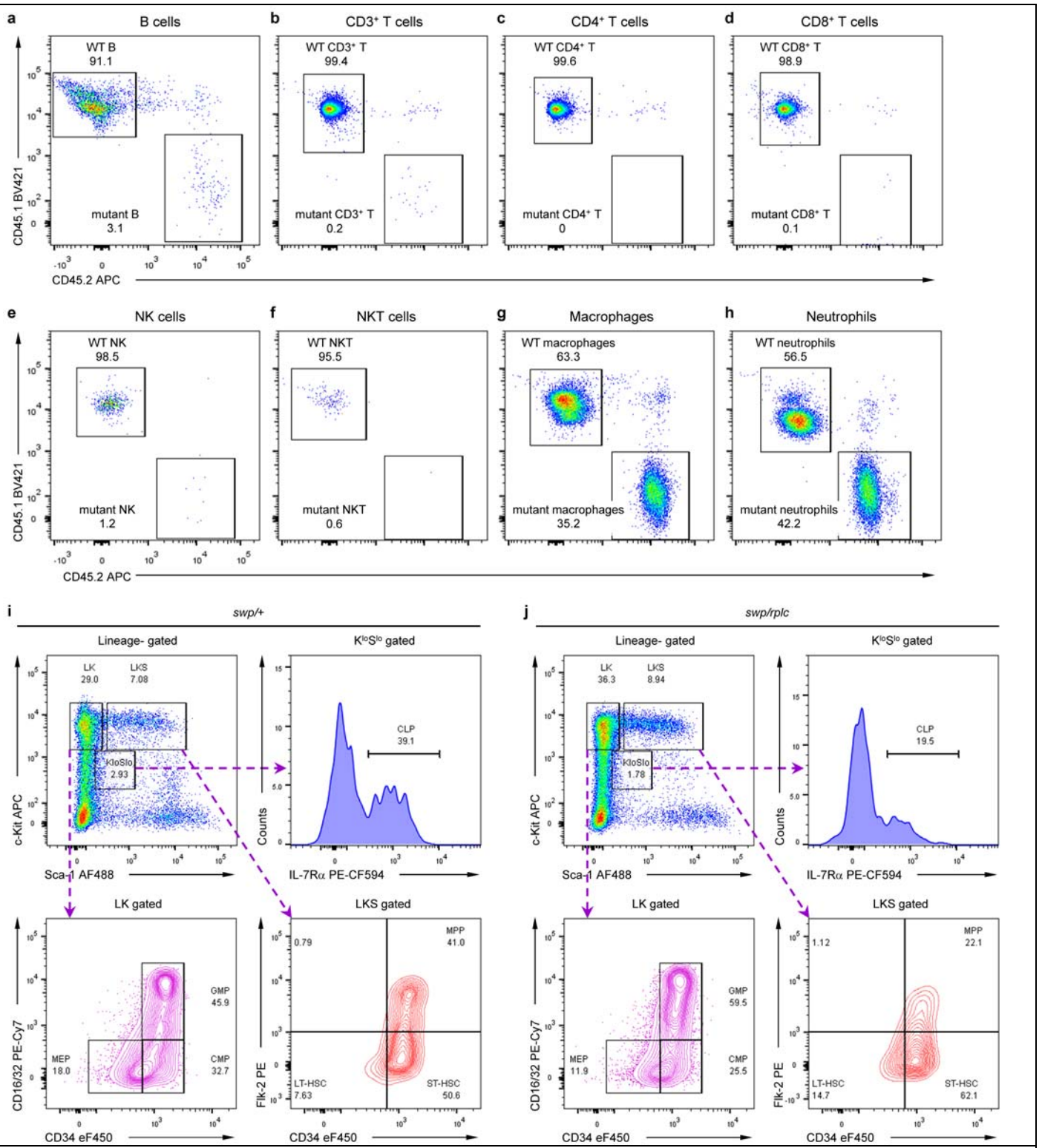
a, Mitotic cell cycle analysis. Flow cytometry of splenic T cells activated by TCR stimulation for three days and then labeled with EdU for 6 h, assessed for EdU incorporation and 7-AAD staining. Dotted line denotes the average size of T cells at G0/G1 stage. Numbers indicate percent cells in outlined areas. **b**, Quantification of the percentage of T cells in the indicated stage of the cell cycle, or the percentage of apoptotic cells determined as in (**a**). *n.s.*, not significant (P=0.80), *P=0.021, **P=0.0039, *P=0.016 (unpaired, two-tailed Student's *t*-test). **c**, Wild-type recipient mice (C57BL/6J, CD45.1) adoptively transferred with CellTrace^{TM} Far Red-labeled *Snrnp40*$^{swp/swp}$ or wild-type OT-I CD8$^+$ T cells (CD45.2). Flow cytometry of CellTrace^{TM} Far Red dilution in *Snrnp40*$^{swp/swp}$ or wild-type OT-I CD8$^+$ T cells isolated from the spleens of recipient mice 60 or 72 h after immunization with OVA or PBS. **d**, Wild-type recipient mice (C57BL/6J, CD45.1) adoptively transferred with CellTrace Far^{TM} Red-labeled *Snrnp40*$^{swp/swp}$ or wild-type OT-II CD4$^+$ T cells (CD45.2). Flow cytometry of CellTrace^{TM} Far Red dilution in *Snrnp40*$^{swp/swp}$ or wild-type OT-II CD4$^+$ T cells isolated from the spleens of recipient mice 72 h after immunization with OVA or PBS. **e**, Quantification of the percentage of OT-I (CD8$^+$) and OT-II (CD4$^+$) cells that underwent proliferation in response to OVA immunization measured 72 h after immunization (n=4 mice per genotype; *n.s.*, not significant (P=0.94, 0.95); unpaired, two-tailed Student's *t*-test). **f**,**g**, Antibody responses to immunization. T-dependent antibody response to rSFV-β-gal (**f**) and T-independent antibody response to NP-Ficoll (**g**) in *Snrnp40*$^{+/+}$ (n=6), *Snrnp40*$^{swp/+}$ (n=10), and *Snrnp40*$^{swp/swp}$ (n=6) mice were measured 13 days and 6 days after immunization, respectively. *n.s.*, not significant (P=0.87 in **f**, P=0.05 in **g**); one-way ANOVA with Sidak's multiple comparisons. **h**, *In vivo* CTL cytotoxicity in *Snrnp40*$^{+/+}$ (n=6), *Snrnp40*$^{swp/+}$ (n=10), and *Snrnp40*$^{swp/swp}$ (n=6) mice. *n.s.*, not significant (P=0.19); one-way ANOVA with Sidak's multiple comparisons. Data are representative of two (**f**-**h**) and three (**a**-**e**) independent experiments (mean ± s.d. in **b**,**e**,**f**-**h**).



Supplementary Figure 6

Normal *Snrnp40*$^{swp/swp}$ NK cell degranulation and BMDC cytokine production.

a-**d**, Flow cytometry of NK cells from the spleens of *Snrnp40*$^{swp/swp}$ and wild-type mice assessing the expression of Lamp-1 (CD107a) (**a**), granzyme B (**b**), TNF (**c**), and IFN-γ (**d**) in response to stimulation with PMA/ionomycin (50 ng/ml PMA and 1 μg/ml ionomycin) or no stimulation. **e**-**h,** Concentration of TNF (**e**), IL-6 (**f**), IFN-α (**g**), and IFN-β (**h**) in the supernatants of *Snrnp40*$^{swp/swp}$ and wild-type BMDCs 16 hours after infection with the indicated viruses and multiplicities of infection (MOI) (*n*=4 independent BMDCs, each generated from independent mice; **P*=0.015, 0.014, 0.022, ****P*=0.00066, unpaired, two-tailed Student's *t*-test). Data are representative of three independent experiments (**a**-**h**; mean ± s.d. in **e**-**h**).

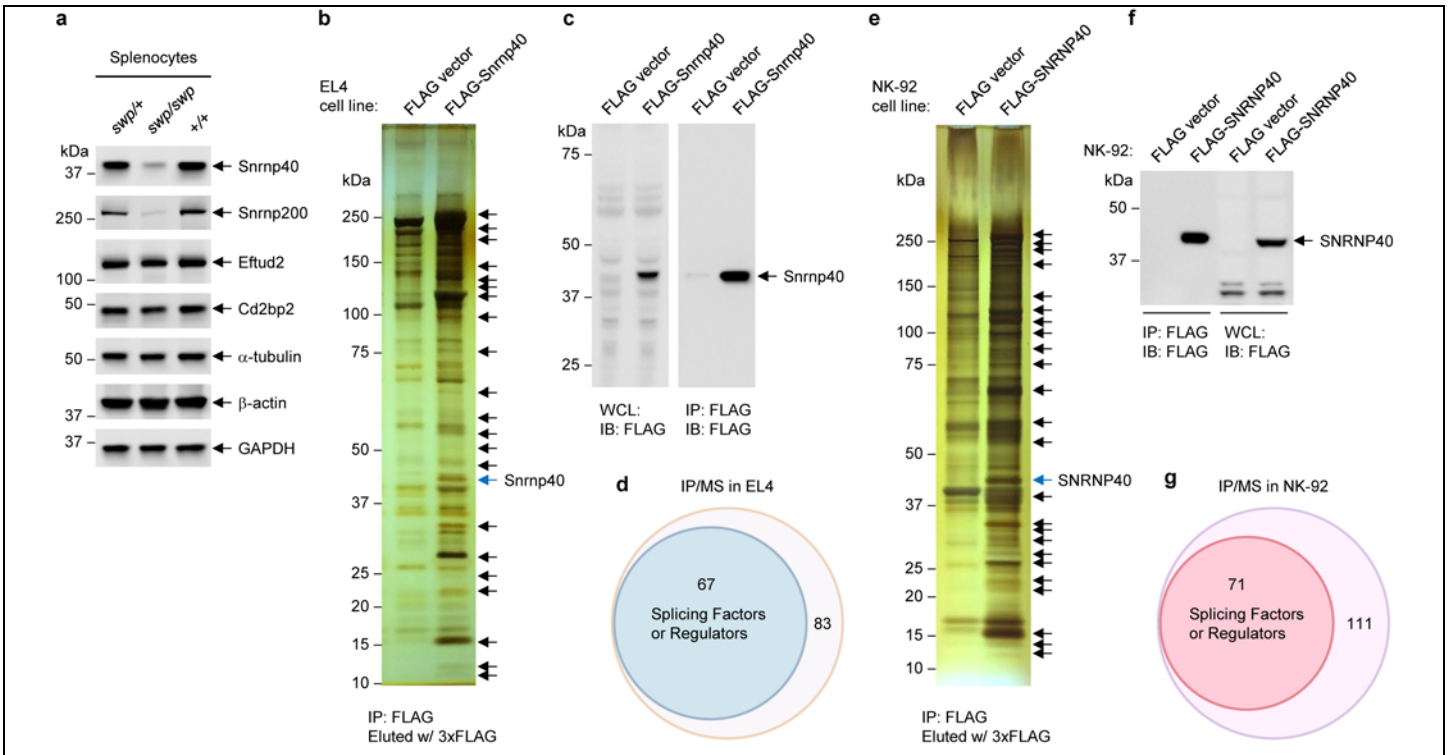


Supplementary Figure 7

Hematopoietic cell-intrinsic defect in lymphoid development in *Snrnp40*$^{swp/rplc}$ mutant mice.

a-**h**, Flow cytometry of immune cells in the blood of mixed bone marrow chimeras reconstituted with a 1:1 mixture of wild-type (CD45.1) and *Snrnp40*$^{swp/rplc}$ (CD45.2) bone marrow cells, assessing the percentage of B cells (**a**, B220^{+}), CD3^{+} T cells (**b**, CD3ε^{+}), CD4^{+} T cells

(**c**, $CD3\varepsilon^+CD4^+$), $CD8^+$ T cells (**d**, $CD3\varepsilon^+CD8\alpha^+$), NK cells (**e**, $NK\text{-}1.1^+CD3\varepsilon^-$), NKT cells (**f**, $NK\text{-}1.1^+CD3\varepsilon^+$), macrophages (**g**, $CD11b^+F4/80^+$), and neutrophils (**h**, $CD11b^+F4/80^-$) derived from wild-type (CD45.1) and *Snrnp40*$^{swp/rplc}$ (CD45.2) bone marrows respectively. Representative results from one of ten chimeras in two experiments are shown (**a**-**h**). **i**-**j**, Gating strategy for flow cytometry analysis of hematopoietic stem and progenitor cells in *Snrnp40*$^{swp/+}$ (**i**) and *Snrnp40*$^{swp/rplc}$ (**j**) bone marrow. Single cells negative for Lineage expression ($Lineage^-$) were further classified into three populations based on c-Kit and Sca-1 expression: LKS ($c\text{-}Kit^+Sca\text{-}1^+$), LK ($c\text{-}Kit^+Sca\text{-}1^-$), and $K^{lo}S^{lo}$ ($c\text{-}Kit^{lo}Sca\text{-}1^{lo}$). The LKS population was classified into three populations based on Flk-2 and CD34 expression: long-term hematopoietic stem cells (LT-HSC, $CD34^-Flk\text{-}2^-$), short-term hematopoietic stem cells (ST-HSC, $CD34^+Flk\text{-}2^-$), and multipotent progenitor cells (MPPs, $CD34^+Flk\text{-}2^+$). The LK population was classified into three populations based on CD16/32 and CD34 expression: megakaryocyte–erythroid progenitors (MEPs, $CD34^-CD16/32^-$), common myeloid progenitors (CMPs, $CD34^+CD16/32^-$), and granulocyte-macrophage progenitors (GMPs, $CD34^+CD16/32^+$). The $K^{lo}S^{lo}$ population expressing IL-7Rα was defined as common lymphoid progenitors (CLPs, IL-7Rα^+). Representative results from one of three independent experiments are shown (**i**,**j**).



Supplementary Figure 8

Coimmunoprecipitation of Snrnp40-interacting proteins for analysis by mass spectrometry.

a, Immunoblot analysis of Snrnp40, Snrnp200, Eftud2, and Cd2bp2 in mouse splenocytes of the indicated genotypes. Experiment was repeated three times independently with similar results. **b**-**d**, Identification of Snrnp40-interacting proteins by mass spectrometry using mouse EL4 cells. **b**, Silver stained gel containing proteins immunoprecipitated from EL4 cells stably expressing FLAG-tagged mouse Snrnp40 (FLAG-Snrnp40) or vector alone. Arrows indicate bands present exclusively or with greater intensity in FLAG-Snrnp40 immunoprecipitate. **c**, Analysis of FLAG-Snrnp40 expression in EL4 cells by immunoblot of whole cell lysate or by immunoprecipitation and immunoblot. **d**, Venn diagram for top 83 proteins identified by mass spectrometry that coimmunoprecipitated with FLAG-Snrnp40 (top 25 candidates present only in Snrnp40 sample, and top 58 candidates with spectral index ratio > 7 in Snrnp40 vs. vector samples). 80.7% (67 proteins) were known splicing factors or regulators. **e**-**g**, Identification of SNRNP40-interacting proteins by mass spectrometry using human NK-92 cells. **e**, Silver stained gel containing proteins immunoprecipitated from NK-92 cells stably expressing FLAG-tagged human SNRNP40 (FLAG-SNRNP40) or vector alone. Arrows indicate bands present exclusively or with greater intensity in FLAG-SNRNP40 immunoprecipitate. **f**, Analysis of FLAG-SNRNP40 expression in NK-92 cells by immunoblot of whole cell lysate or by immunoprecipitation and immunoblot. **g**, Venn diagram for top 111 proteins identified by mass spectrometry that coimmunoprecipitated with FLAG-SNRNP40 (top 63 candidates present only in SNRNP40 sample, and top 48 candidates with spectral index ratio > 24 in SNRNP40 vs. vector samples). 64.0% (71 proteins) were known splicing factors or regulators. Experiment was performed one time (**b**-**g**).