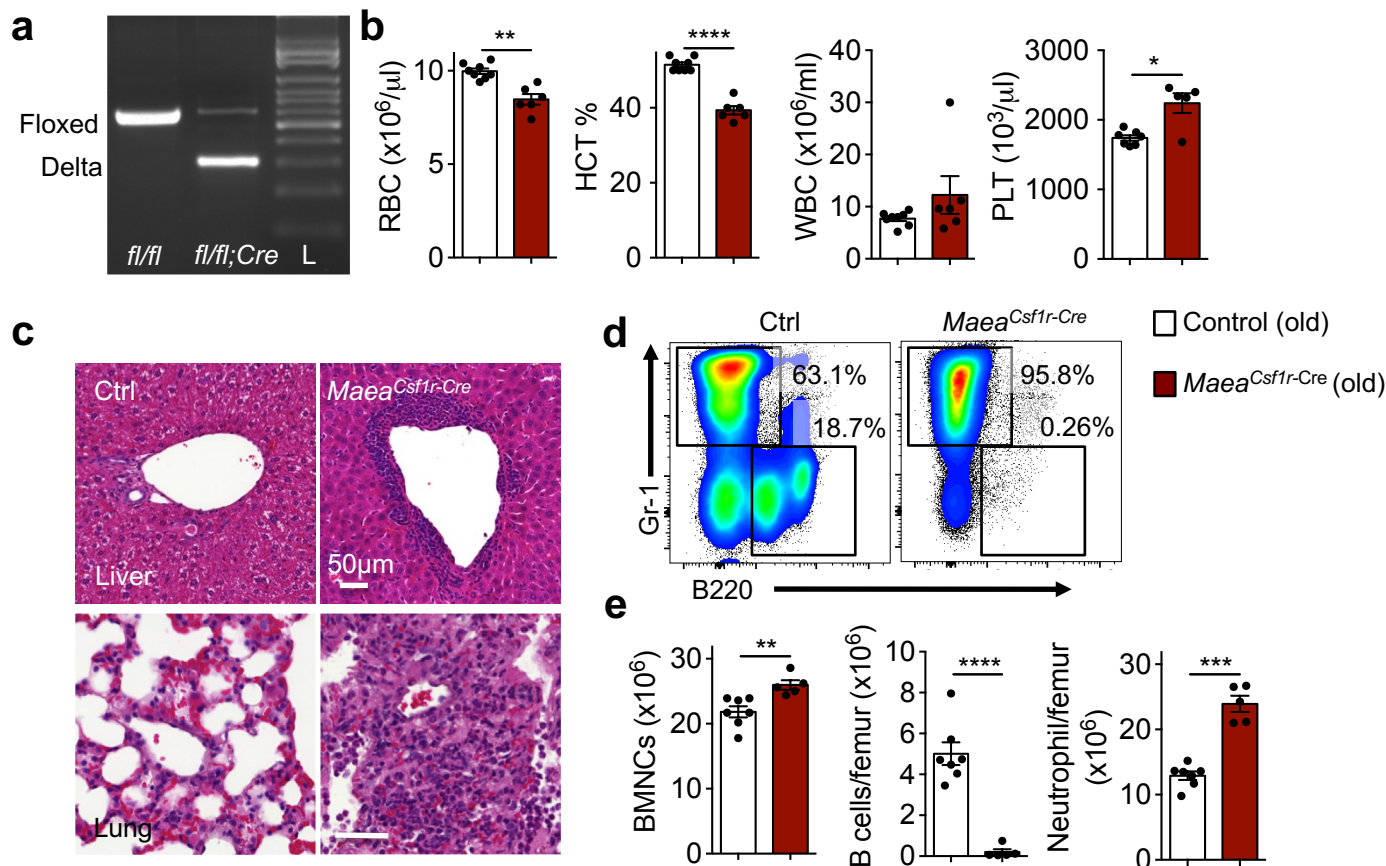


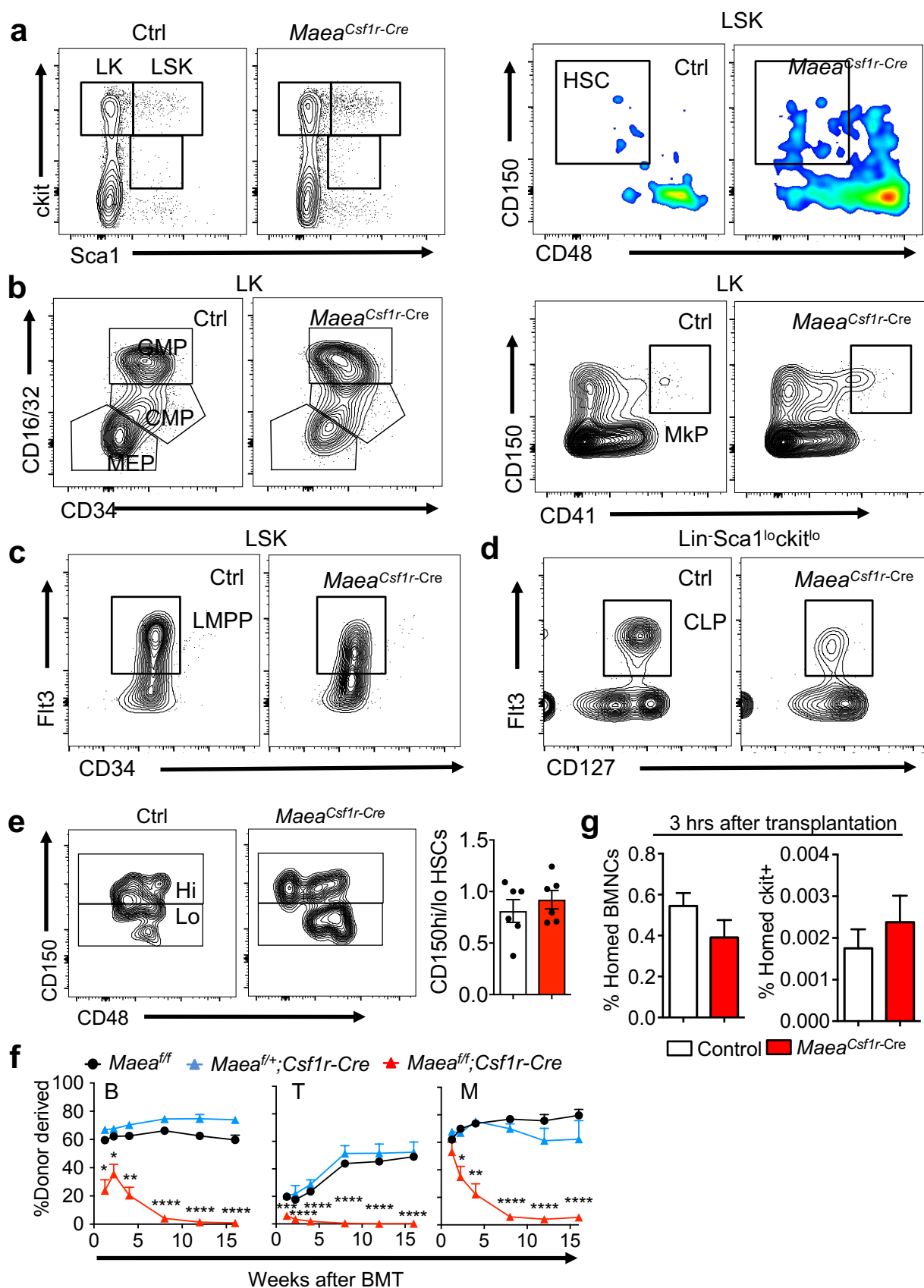
Supplementary Fig. 1



Supplementary Fig. 1. *Maea^{Csf1r-Cre}* mice develop myeloproliferation and lymphopenia.

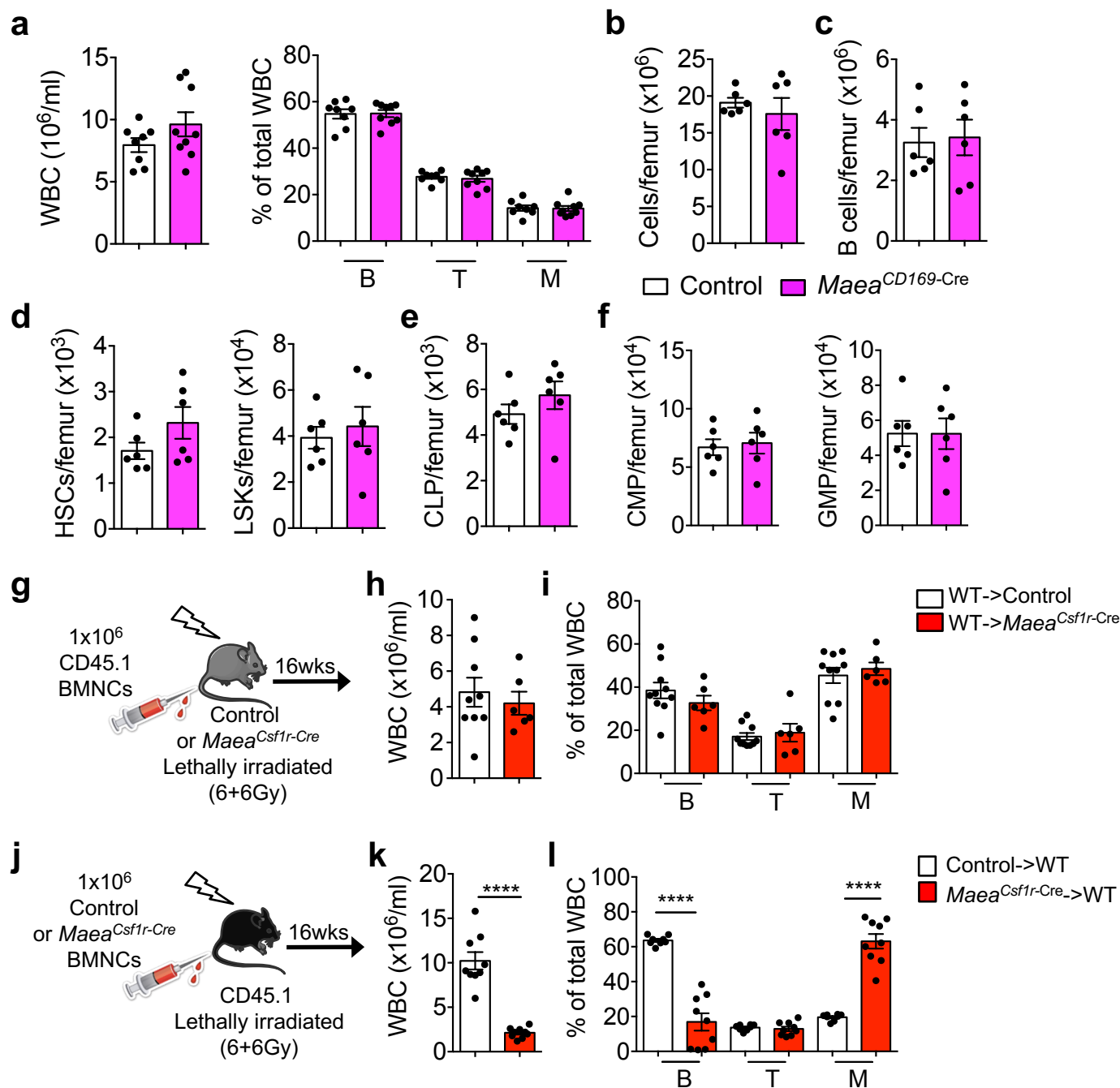
a, PCR on genomic DNA isolated from BM HSCs of *Maea^{flxed}* (*fl/fl*) control and *Maea^{Csf1r-Cre}* (*fl/fl;Cre+*) mice. L=100bp DNA ladder. **b**, Peripheral blood indices of 7 months old control and *Maea^{Csf1r-Cre}* mice. RBC, red blood cells; HCT, haematocrit; WBC, white blood cells; PLT, platelets. **c**, Representative H&E stained paraffin sections from the livers and lungs of 7 months old control and *Maea^{Csf1r-Cre}* mice. Scale bar=50μm. **d** and **e**, Representative FACS plots (**d**) and quantification (**e**) of BM cellularity, Gr-1⁺ neutrophils and B220⁺ B cells in total BM nucleated cells in 7 months old control and *Maea^{Csf1r-Cre}* mice. Data are shown as mean $\pm$ s.e.m. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ by unpaired Student's *t* test.

Supplementary Fig. 2



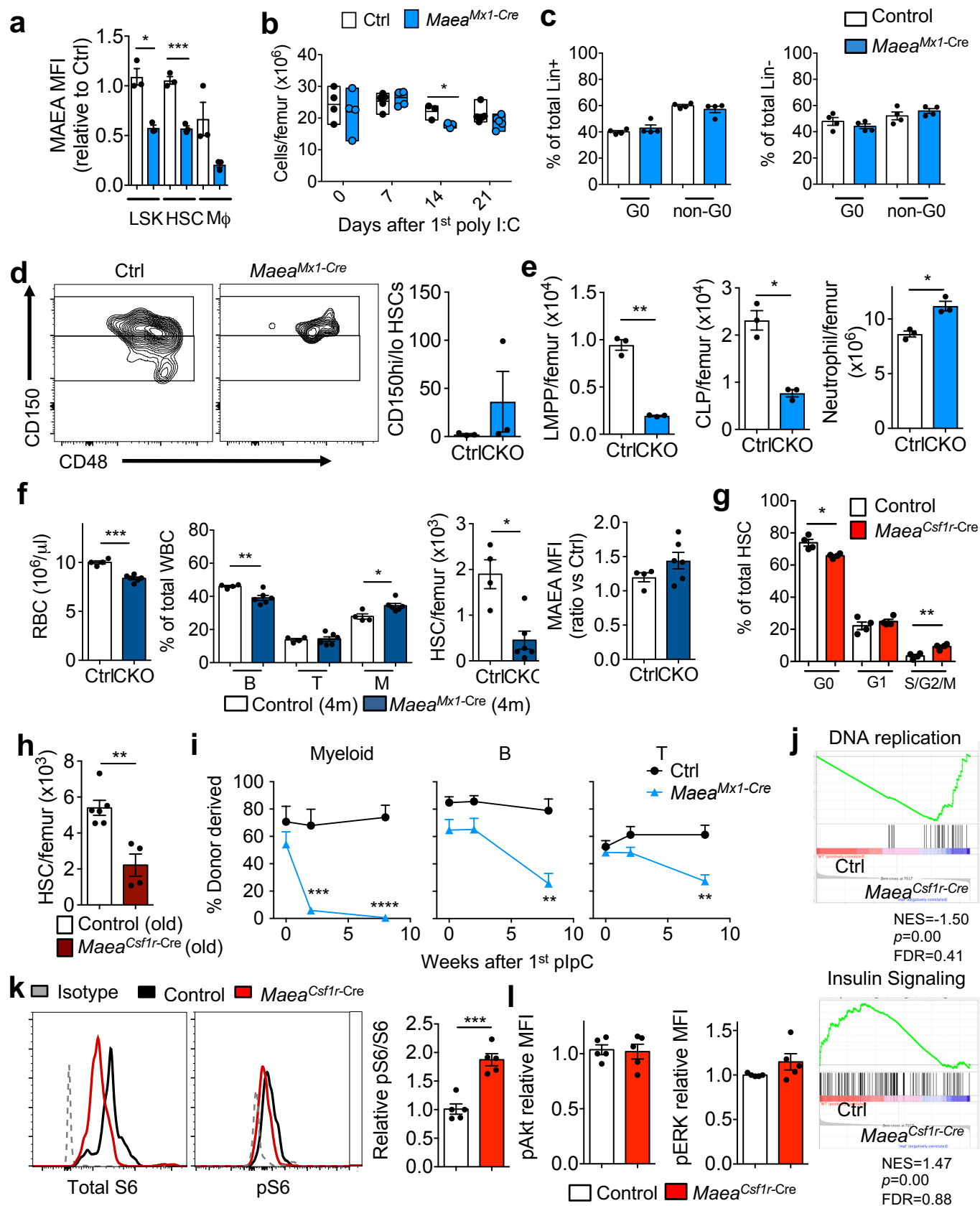
Supplementary Fig. 2. Altered hematopoiesis in *Maea^{Csf1r-Cre}* mice. **a**, Representative FACS plots and quantifications showing increased LSK, LK and HSCs in the *Maea^{Csf1r-Cre}* mice. Events are pre-gated on the lineage- populations. **b**, Representative gating strategy for GMP, CMP, MEP and MkP from the LK population in **a**. **c**, Representative FACS plots showing decreased frequencies of LMPPs in the LSK population from the *Maea^{Csf1r-Cre}* mice. **d**, Representative FACS plots showing decreased frequencies of CLPs in the Lin⁻Sca1^{lo}ckit^{lo} population from the *Maea^{Csf1r-Cre}* mice. **e**, Quantification of CD150 high vs low myeloid-biased vs lymphoid competent HSCs in *Maea^{Csf1r-Cre}* mice. **f**, Peripheral blood donor chimaerism at indicated time points after competitive BM transplantation of equal number (1×10^6) of CD45.1 wild type competitor BM cells and CD45.2 donor BM cells from indicated genotypes into lethally irradiated CD45.1 wild type recipients (n=4~6 each group). **g**, Homed control and *Maea^{Csf1r-Cre}* total BM cells and ckit⁺ cells in recipient BM 3 hours after transplantation (n=5 each group). Data are shown as mean $\pm$ s.e.m. **p< 0.01, ***p< 0.001, ****p<0.0001 by unpaired Student's *t* test.

Supplementary Fig.3



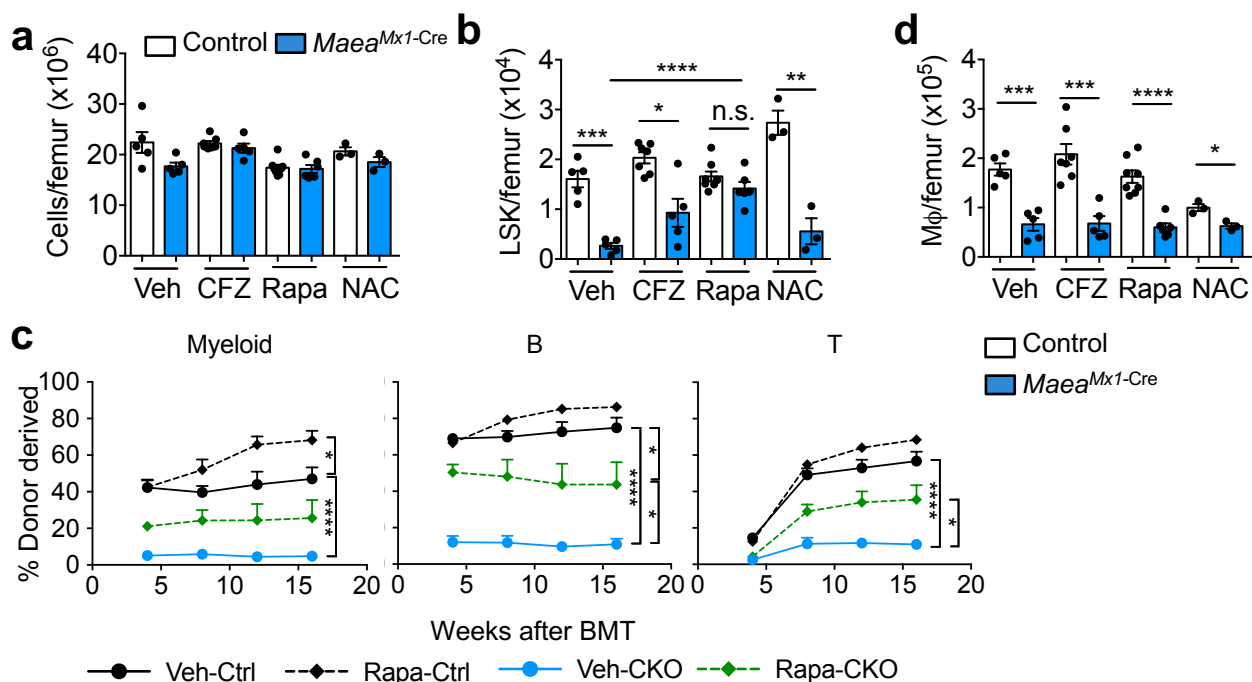
Supplementary Fig.3 Lymphopenia in *Maea*^{Csf1r-Cre} mice is caused by hematopoietic cell-intrinsic defects but not by macrophages. **a**, Analysis of white blood cells (WBCs) in *Maea*^{CD169-Cre} mice. **b**, Quantification of *Maea*^{CD169-Cre} BM cellularity. **c**, Total B cell numbers in the BM of *Maea*^{CD169-Cre} mice. **d-f**, Quantification of HSPC numbers (d), lymphoid progenitors (e) and myeloid progenitors (f) in *Maea*^{CD169-Cre} mice. **g**, Experimental design to examine the contribution of the microenvironment to the lymphoid defects in the *Maea*^{Csf1r-Cre} mice. **h**, Quantification of leukocytes in peripheral blood of control and *Maea*^{Csf1r-Cre} mice reconstituted with wild-type BM cells. **i**, Frequencies of B, T, and myeloid cells in peripheral blood of control and *Maea*^{Csf1r-Cre} mice reconstituted with wild-type BM cells. **j**, Experimental design to examine the contribution of the hematopoietic cells to the lymphoid defects in the *Maea*^{Csf1r-Cre} mice. **k**, Quantification of leukocytes in peripheral blood of wild-type mice reconstituted with control and *Maea*^{Csf1r-Cre} BM cells. **l**, Frequencies of B, T, and myeloid cells in peripheral blood of wild-type mice reconstituted with control and *Maea*^{Csf1r-Cre} BM cells. Data are shown as mean $\pm$ s.e.m. ****p<0.0001 by unpaired Student's *t* test.

Supplementary Fig.4



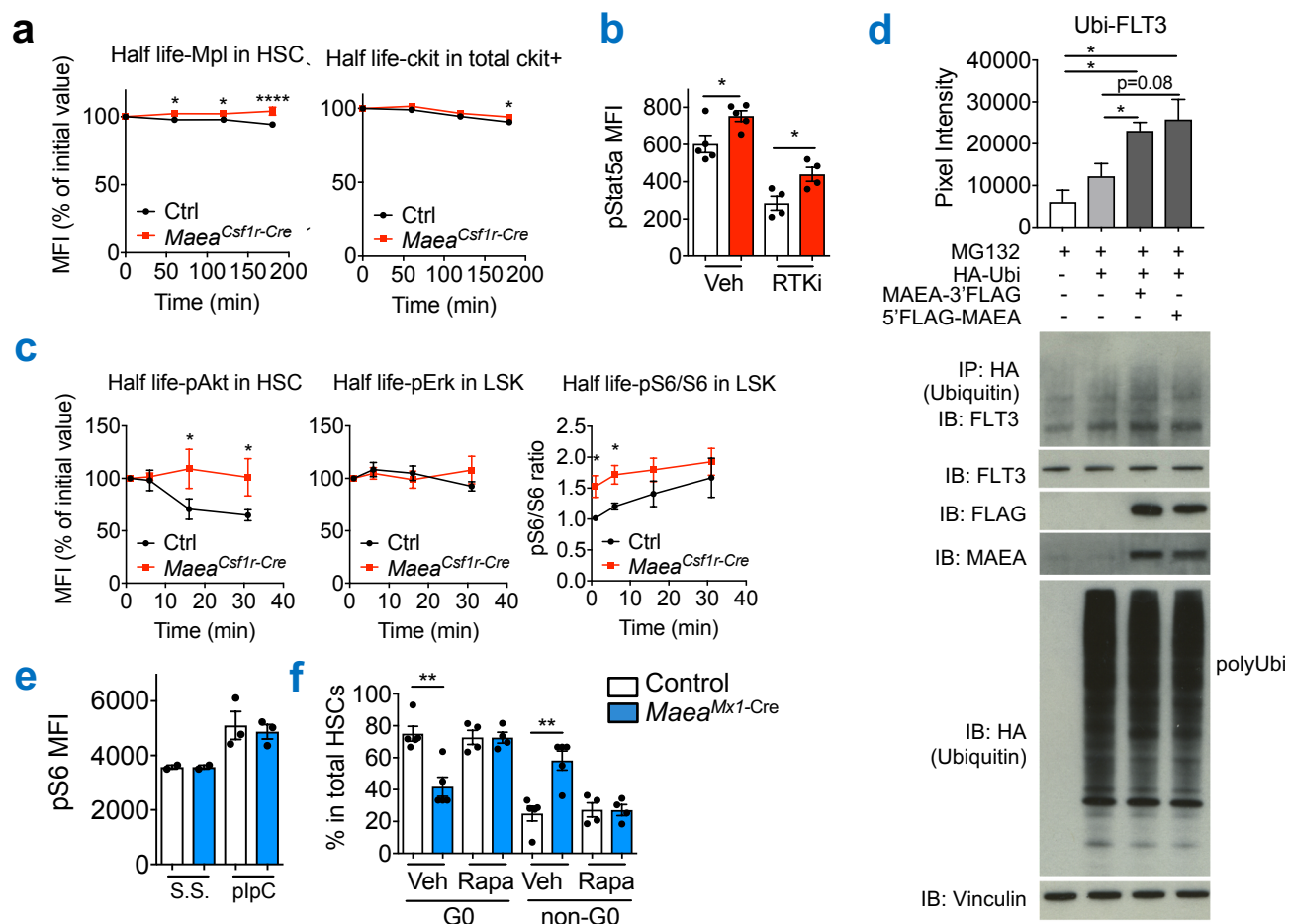
Supplementary Fig.4 *Maea*-deletion impairs HSC quiescence and function. **a**, *Maea* expression in control and *Maea*^{Mx1-Cre} BM cells at 3wks after 1st poly I:C injection. **b**, BM cellularity of control and *Maea*^{Mx1-Cre} mice at indicated time points after 1st poly I:C injection. **c**, Cell cycle profiles of control and *Maea*^{Mx1-Cre} total lineage+ and lineage- cells at 21days after 1st poly I:C injection. **d**, Representative FACS plots and quantification of CD150 high vs low myeloid-biased vs lymphoid competent HSCs in BM of *Maea*^{Mx1-Cre} mice 14days after 1st poly I:C injection. **e**, Quantification of lymphoid progenitor cells and neutrophils in BM of *Maea*^{Mx1-Cre} mice 14days after 1st poly I:C injection. **f**, Analysis of red blood cell (RBC) counts, white blood cell (WBC), HSC numbers and MAEA expression in control and *Maea*^{Mx1-Cre} mice 4 months after plpC injections. **g**, Cell cycle analysis of BM HSCs from control and *Maea*^{Csf1r-Cre} mice by Ki-67 and H33342 dye staining (n=4). **h**, Quantification of HSC numbers in 7 months old control and *Maea*^{Csf1r-Cre} BM. **i**, Donor chimaerism in peripheral blood myeloid, B and T lineage leukocytes from control and *Maea*^{Mx1-Cre} mixed chimeric mice at indicated time points after 1st poly I:C injection. **j**, GSEA enrichment plot showing significant up-regulation of DNA replication pathway and down-regulation of insulin signaling pathway in *Maea*^{Csf1r-Cre} HSCs. **k**, Representative histograms showing total S6 and pS6 levels and pS6/S6 ratio in control and *Maea*^{Csf1r-Cre} HSCs. **l**, pAKT (S473) and pERK1/2 (p44/42 MAPK, T202/Y204) levels in control and *Maea*^{Csf1r-Cre} HSCs. Data are shown as mean $\pm$ s.e.m. *p< 0.05, **p< 0.01, ***p< 0.001, ****p<0.0001 by unpaired Student's *t* test.

Supplementary Fig.5



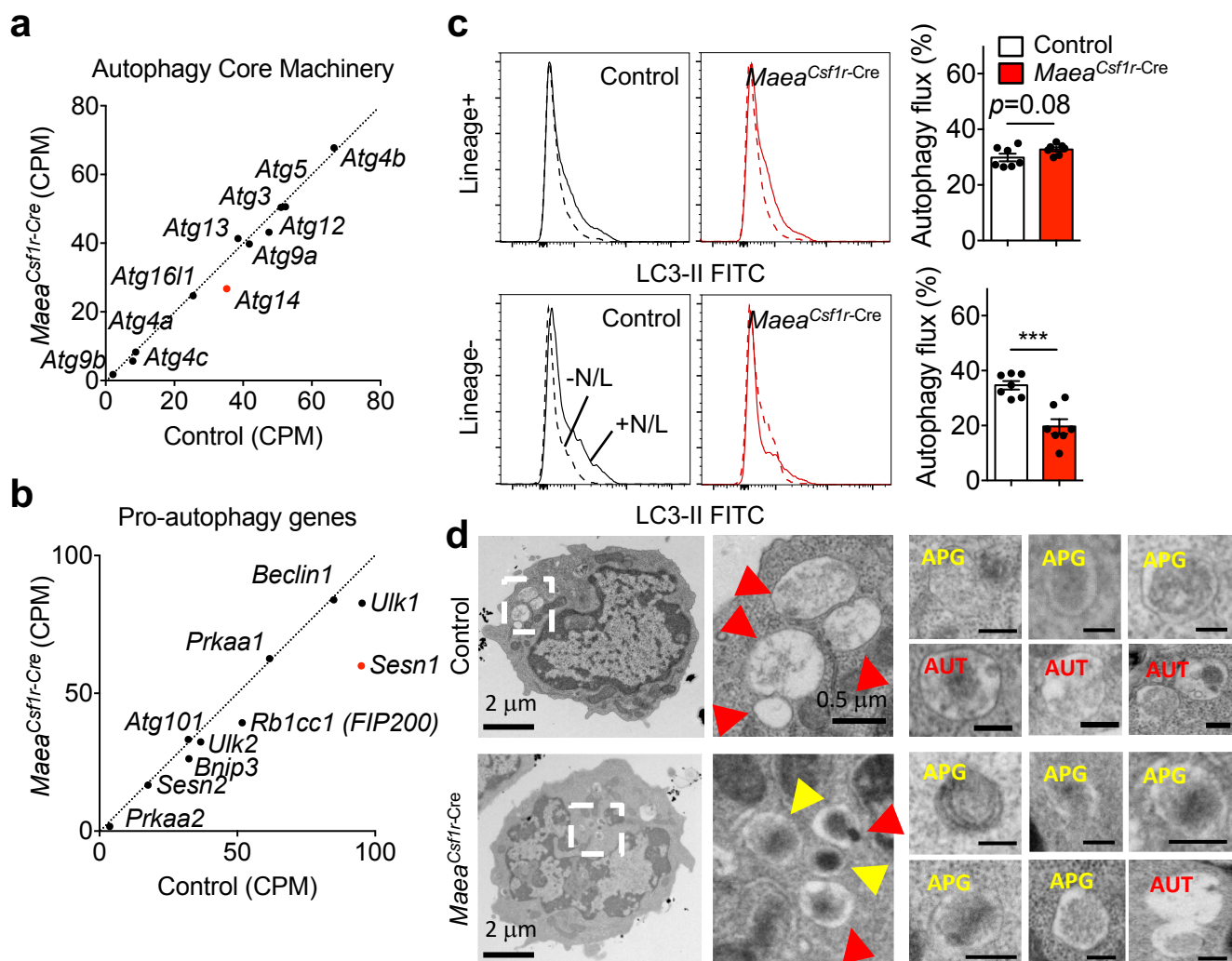
Supplementary Fig. 5. Deletion of *Maea* results in aberrant HSC activation and depletion in a mTOR-dependent manner. **a** and **b**, Quantification of BM cellularity (a) and BM LSK numbers (b) in control and *Maea*^{Mx1-Cre} mice treated with vehicle, carfilzomib, rapamycin or NAC 3wks after poly I:C induction. **c**, Donor chimaerism in peripheral blood of CD45.1 lethally irradiated wild type recipients at indicated time points after competitive BM transplantation of equal number of CD45.1 wild type competitor BM cells and CD45.2 donor BM cells from indicated groups (n=5 each group). **d**, Quantification of BM macrophage numbers in control and *Maea*^{Mx1-Cre} mice treated with vehicle, carfilzomib, rapamycin or NAC 3wks after poly I:C induction. Data are shown as mean $\pm$ s.e.m. n.s., not significant. *p< 0.05, **p< 0.01, ***p< 0.001, ****p<0.0001 by unpaired Student's *t* test.

Supplementary Fig.6



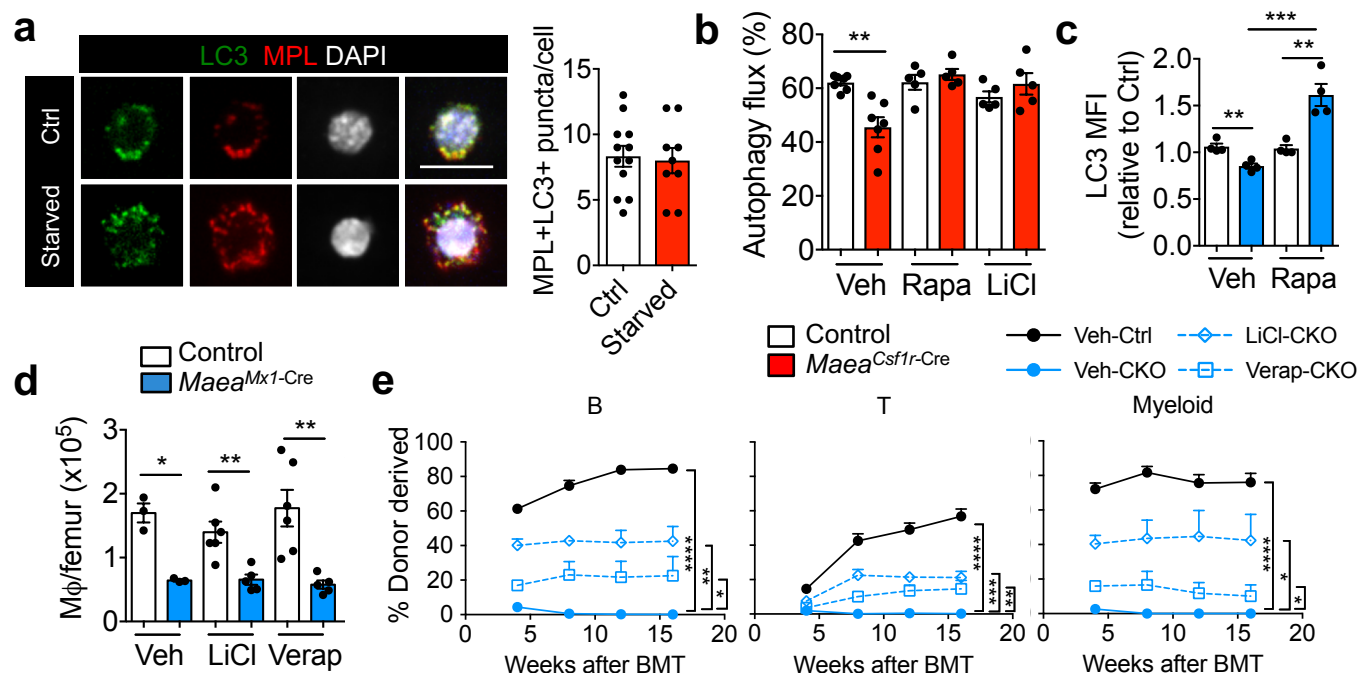
Supplementary Fig. 6. MAEA regulates receptor stability and signaling. **a**, FACS evaluation of surface receptor Mpl and ckit half-lives in control and *Maea^{Csf1r-Cre}* HSPCs incubated in the presence of 50μM cycloheximide (n=4). **b**, Phospho-flow evaluation of pSTAT5a in control and *Maea^{Csf1r-Cre}* LSKs from mice treated with vehicle or RTKi PKC412. **c**, Phospho-flow evaluation of signaling molecules pAkt, pErk and pS6/S6 ratio in control and *Maea^{Csf1r-Cre}* HSPCs at indicated time points after cytokine (TPO, FLT3 and SCF 20ng/ml each) stimulation (n=4~5). In **a** and **c**, data are shown as mean ± s.e.m. *p< 0.05 and ****p<0.0001 by two-way ANOVA multiple comparisons. **d**, Ubiquitination of FLT3 by MAEA shown by immunoprecipitation of HA-Ubiquitin in 293T cells transfected with constructs expressing HA-Ubiquitin, MAEA-3'FLAG and 5'FLAG-MAEA and cultured in the presence of 10 μM MG132 for 4 hours prior to harvest 24 hours post transfection. The whole cell lysates and immunoprecipitates were analysed by the indicated immunoblots. Ubiquitinated FLT3 was quantified using the gel analyzer tool in ImageJ. Data are shown as mean ± s.e.m. *p< 0.05 by Welch's t test. IB, immunoblotting. IP, immunoprecipitation. **e**, pS6 staining in BM HSCs isolated from control and *Maea^{Mx1-Cre}* mice without plpC injection (steady state, S.S.) and 1 day after the last plpC injection. **f**, Cell cycle profile, by Ki67 and H33342 staining, of BM HSCs isolated from control and *Maea^{Mx1-Cre}* mice treated with vehicle or rapamycin (Rapa) for 3 weeks after plpC. Data are shown as mean ± s.e.m. **p< 0.01 by Welch's t test.

Supplementary Fig.7



Supplementary Fig.7. Normal expression of autophagy machinery but impaired autophagy flux in *Maea^{Csf1r-Cre}* HSCs. **a**, Expression of core autophagy machinery in control and *Maea^{Csf1r-Cre}* HSCs from RNA-seq analysis. **b**, Expression of pro-autophagy genes in control and *Maea^{Csf1r-Cre}* HSCs from RNA-seq analysis. **c**, Representative histograms and FACS quantifications showing autophagy flux by LC3-II in control and *Maea^{Csf1r-Cre}* lineage+ (upper) and lineage- (lower) BM cells, measured at the same time as in Fig. 4c. **d**, Additional micrographs of whole cells (left) and examples of autophagosomes (APG, yellow arrows) and autolysosomes (AUT, red arrows) from control and *Maea^{Csf1r-Cre}* HSCs. Bars in inserts = 0.1mm. Data are shown as mean $\pm$ s.e.m. *** $p < 0.001$ by unpaired Student's *t* test.

Supplementary Fig.8



Supplementary Fig.8. MAEA regulates receptor stability and HSC maintenance via autophagy. **a**, Representative immunofluorescence images and quantification showing subcellular colocalization of MPL and LC3 in freshly isolated (Ctrl) and starved (cultured ex vivo in StemSpan with no cytokines but in the presence of lysosome inhibitors N/L for 3hrs to induce autophagy) HSPCs. Scale bar=10 μ m. **b**, Evaluation of autophagy flux in control and *Maea^{Csf1r-Cre}* HSCs incubated with 0.2 μ M rapamycin and 10mM LiCl. **c**, LC3-II staining in BM HSCs isolated from control and *Maea^{Mx1-Cre}* mice treated with vehicle or rapamycin (Rapa) for 3 weeks after plpC. Statistic significance determined by 2-way ANOVA multiple comparisons. **d**, Quantification of BM macrophages in control and *Maea^{Mx1-Cre}* mice treated with vehicle, lithium chloride, or verapamil 3wks after poly I:C induction. **e**, Donor chimaerism in peripheral blood of CD45.1 lethally irradiated wild type recipients at indicated time points after competitive BM transplantation of equal number of CD45.1 wild type competitor BM cells and CD45.2 donor BM cells from indicated groups (n=5 each group). Data are shown as mean $\pm$ s.e.m. *p< 0.05, **p< 0.01, ***p< 0.001, ****p<0.0001 by unpaired Student's *t* test unless otherwise indicated.