Supplementary Information

An essential role for TASL in mouse autoimmune pathogenesis and Toll-like receptor signaling

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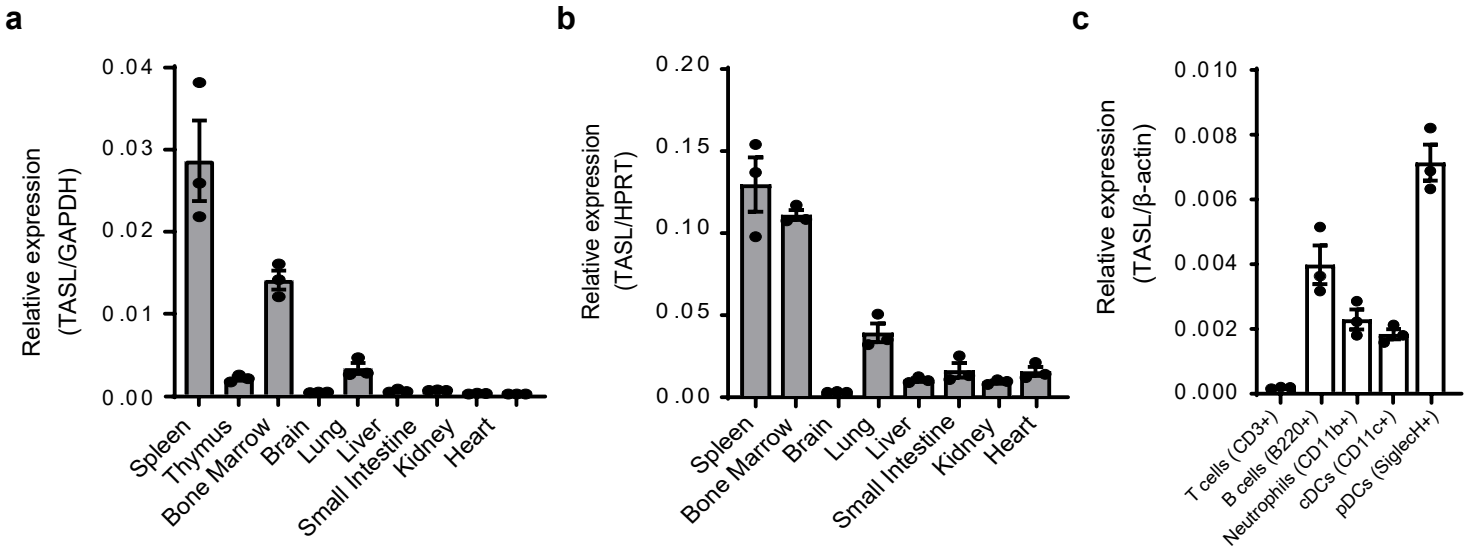
Supplementary Fig. 1



d **Target Guide Design**

Target sequence sgRNA 1 **PAM**

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5′ - AAAATTATAAGAAGACGCTGTGG - 3′
3′ - TTTTAATATTCTTCTGCGAC    - 5′
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Target sequence sgRNA 2 **PAM**

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5′ - AAGGCCTACCATTCCAGTGCAGG - 3′
3′ - TTCCGGATGGTAAGGTCACG    - 5′
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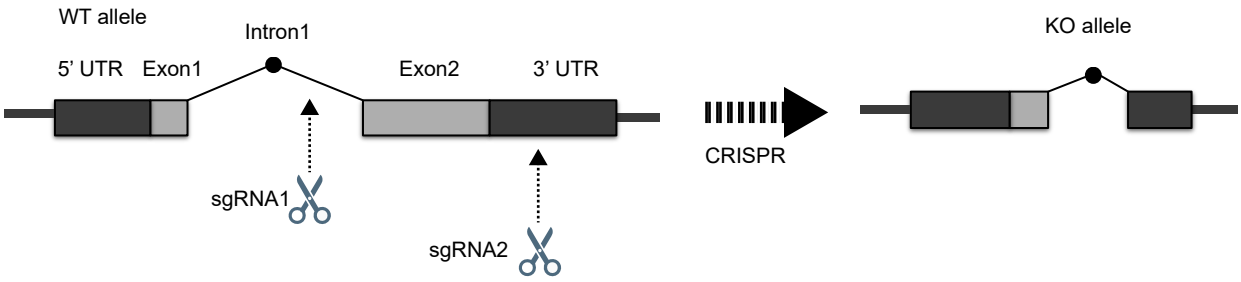
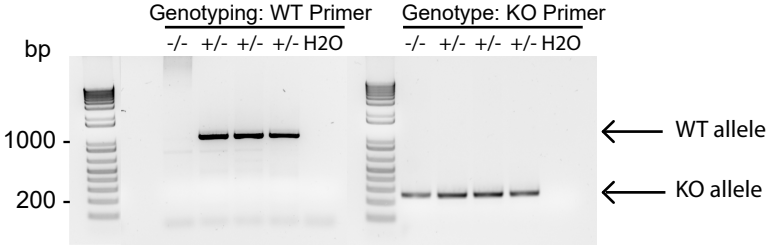
e **Targeting Schematic**

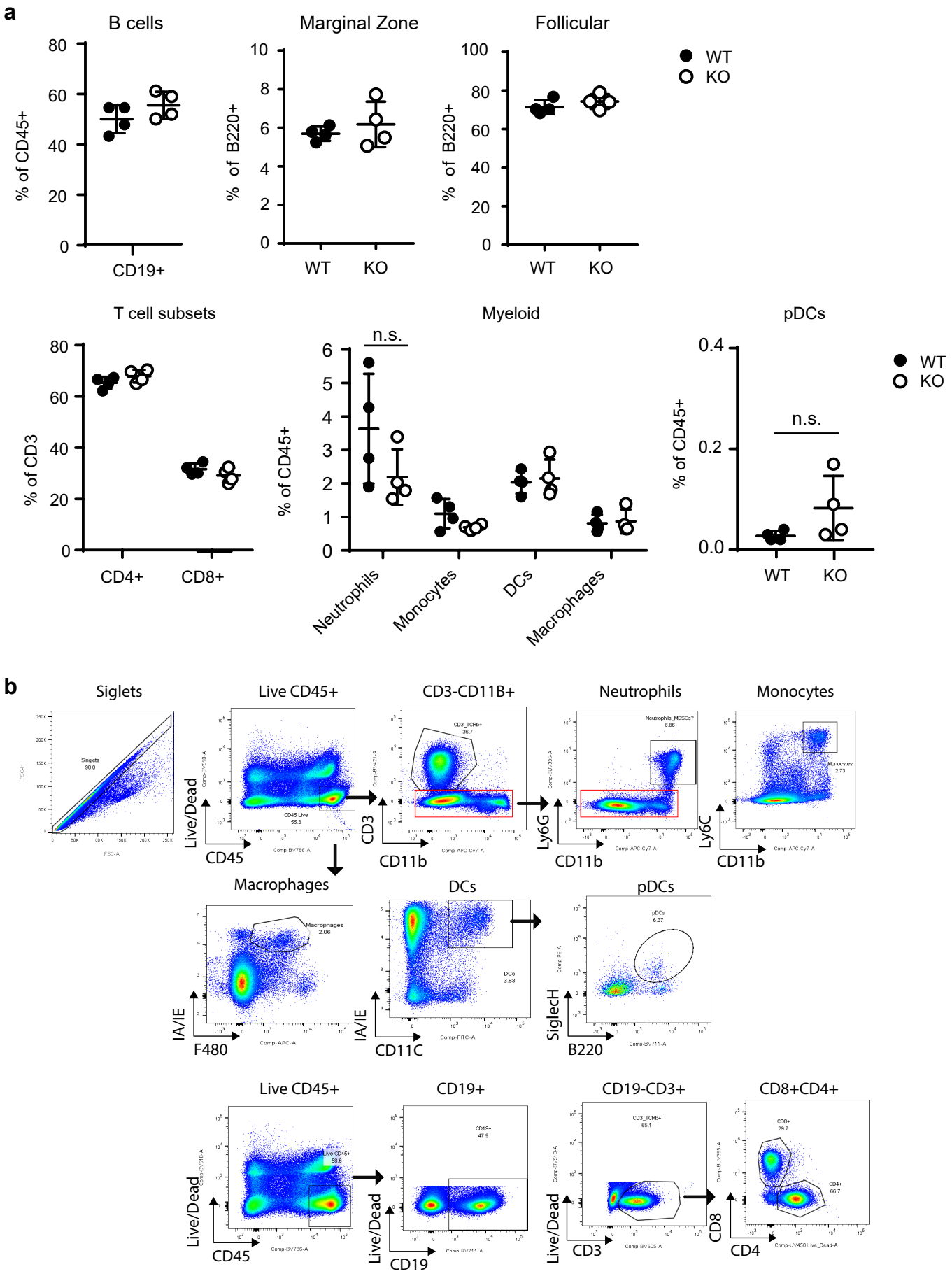


f **Genotyping**



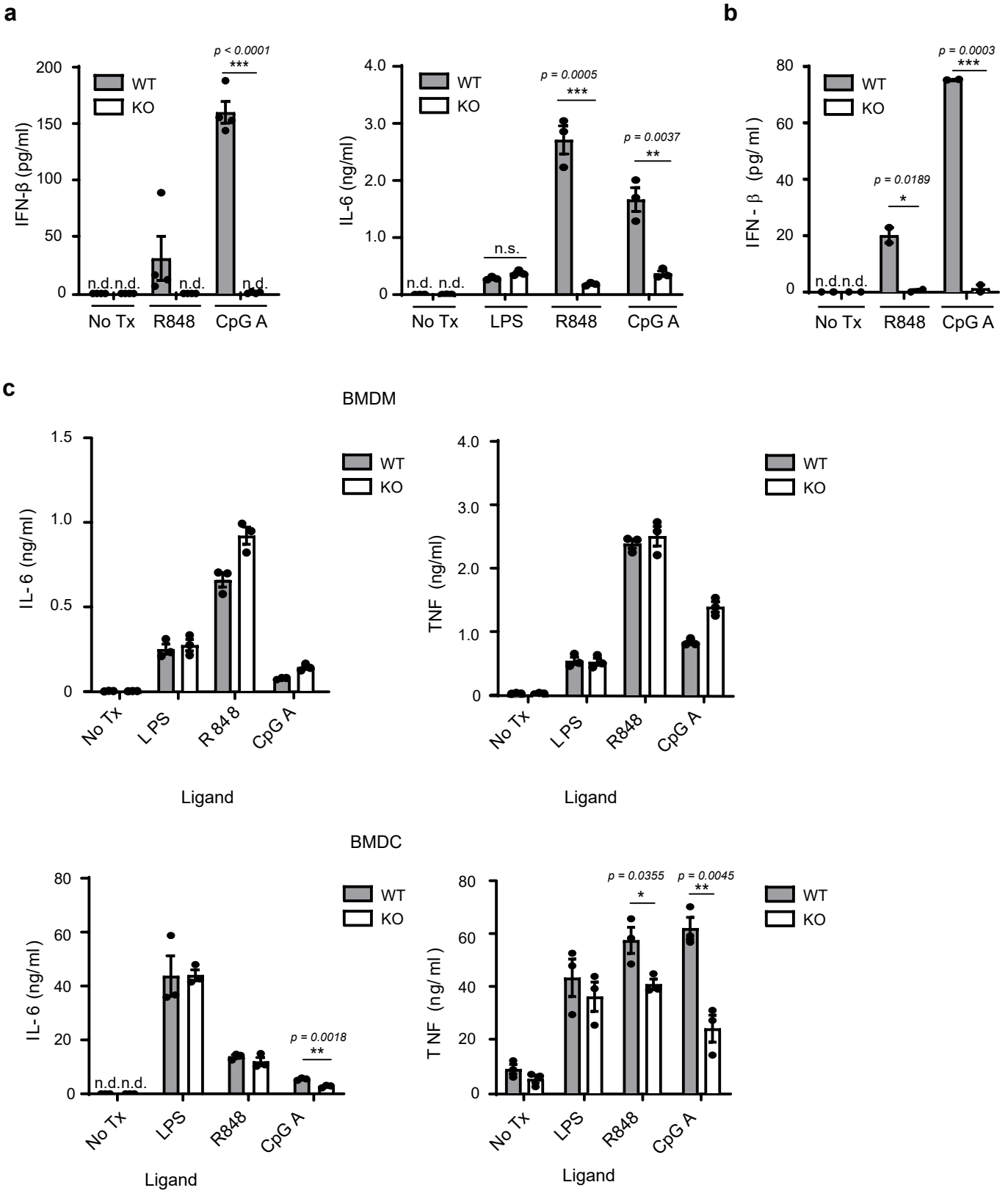
Supplementary Figure 1. *TASL* expression and knockout mouse strategy. RT-qPCR for *TASL* expression in (**a**) indicated human tissues. (**b**) indicated mouse tissues. (**c**) RT-qPCR analysis of *Tasl* expression from various immune cells sorted from mouse (n=3) spleens. (**d**)**.** Target sequence (red) and PAM site(blue) used to generate sgRNA oligos against *Tasl* loci. (**e**)**.** Schematic representation of the generation of *Tasl*$^{-/-}$ mice illustrating sgRNA1 targeting the intronic region and sgRNA2 targeting 3'UTR region for removal of exon 2 of *Tasl*. (**f**) Agarose gel depicting genotyping results from DNA samples obtained from knockout mice (-/-) or heterozygote mice (+/-). Arrows indicate presence of wild type (WT) or knockout (KO) specific alleles. Samples loaded with H_20 was used as a negative control. In (a-c), data are means ± SEM. In (a-c) and (f) data are representative of 2 independent experiments.

Supplementary Figure 2



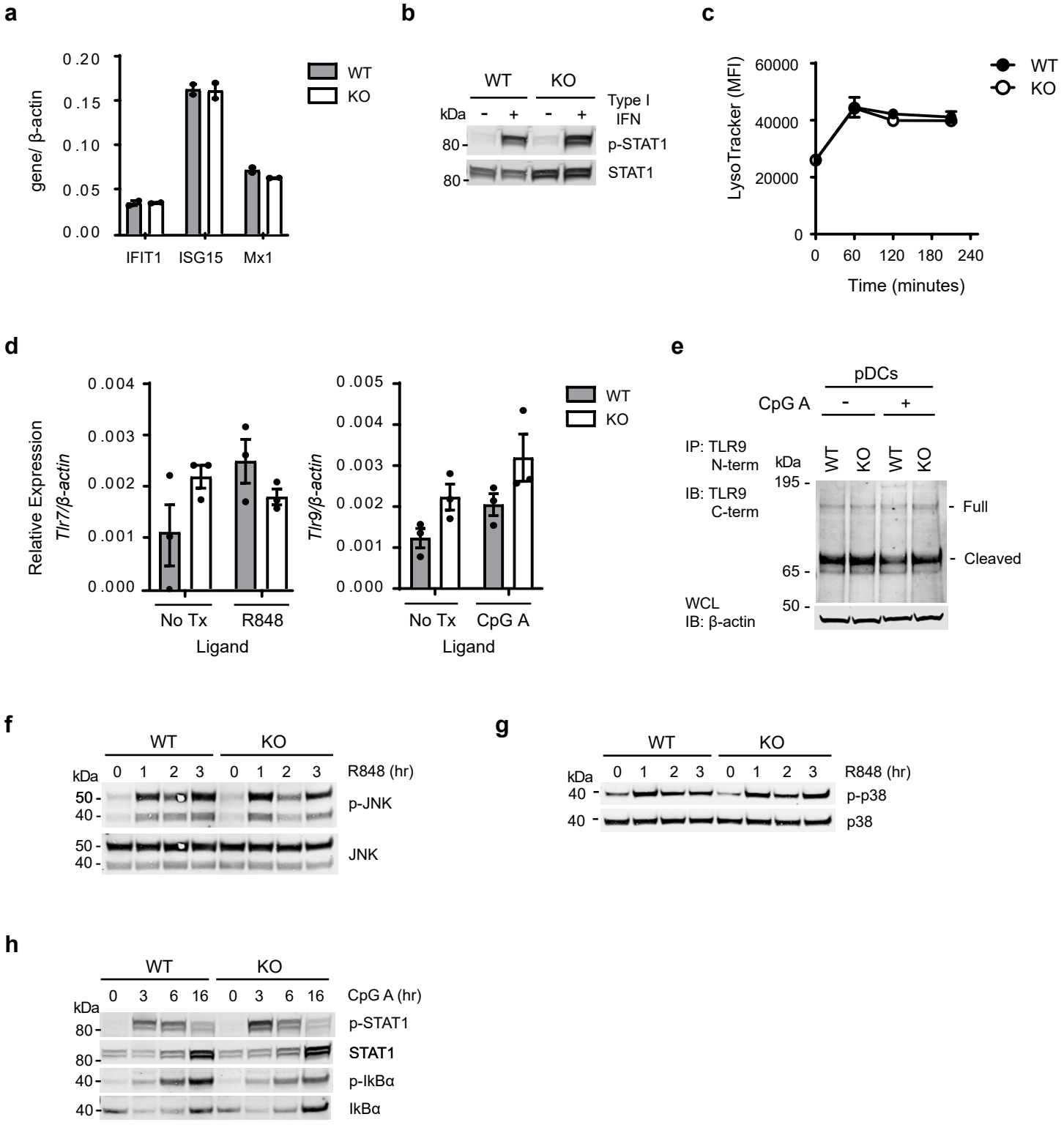
Supplementary Figure 2. Immune cell populations in *Tasl*$^{-/-}$ mice. (**a**) FACs analysis of spleens from wildtype (WT) and *Tasl*$^{-/-}$ (KO) mice was performed and gated on CD45+(immune cells) or CD3+ (T cells) positive cells. Shown are percentage of B cells, marginal zone B cells, follicular B cells, T cells, myeloid cell populations, and pDCs. Data are means ± SEM and are representative of 2 independent experiments with 4 mice per genotype individually represented. (**b**) Representative FACs gating strategy for defined populations represented in (a).

Supplementary Figure 3



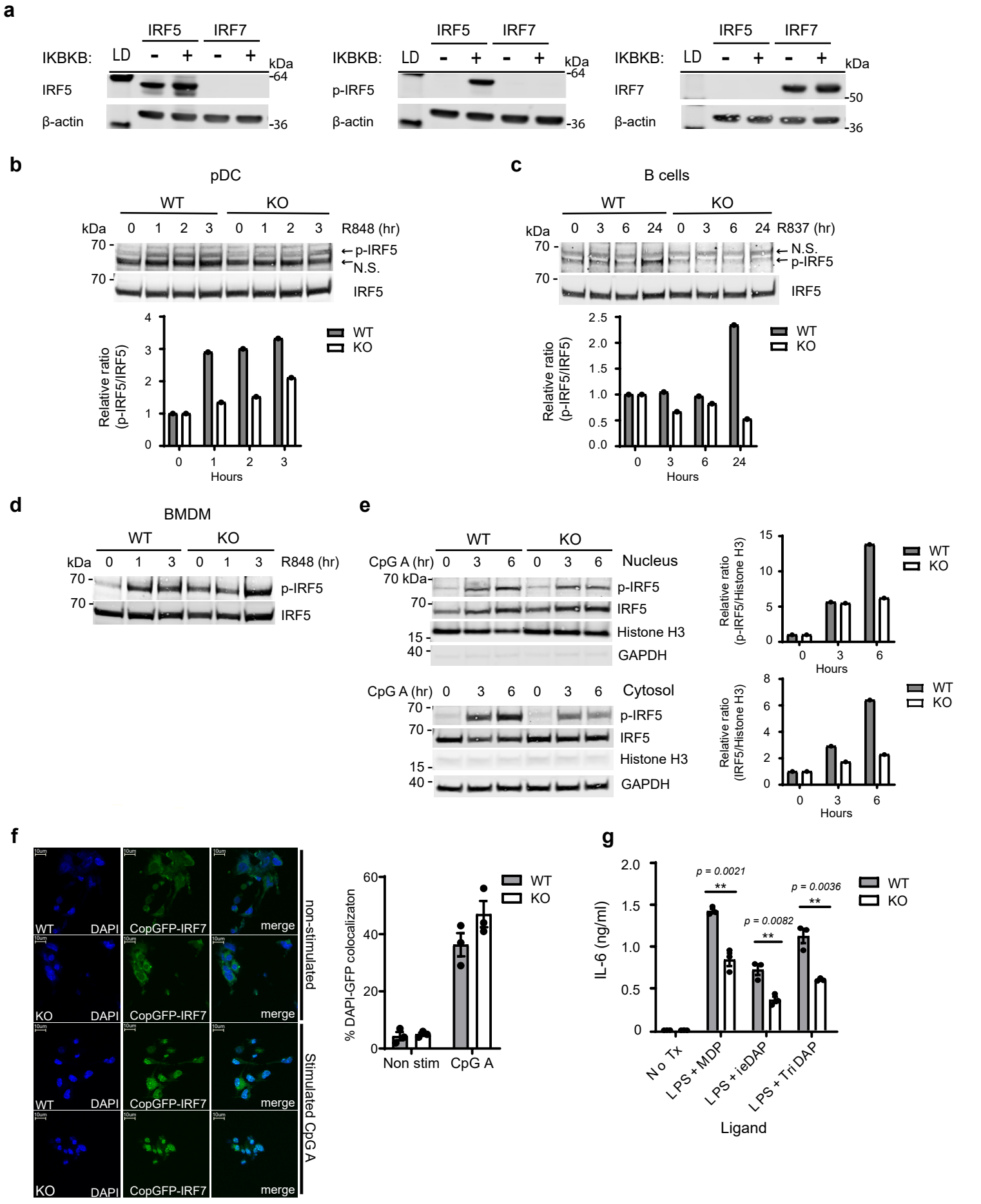
Supplementary Figure 3. Cytokine responses from *Tasl*$^{-/-}$ mice. IFN-β and IL-6 levels from supernatants of (**a**) total mouse splenocytes (**b**) total mouse marrow cells stimulated with either LPS (5μg/ml), R848 (10μg/ml) or CpG A (3μM) overnight. (**c**) M-CSF bone marrow-derived macrophages (BMDM) and GM-CSF conventional dendritic cells (BMDC) isolated from wildtype (WT) and *Tasl*$^{-/-}$ (KO) mice were stimulated with LPS (500ng/ml), R848 (1μg/ml) and CpG A (10μg/ml) and IL-6 and TNF were measured 24 hours later by CBA. Representative of 2 independent experiments. Data are means ± SEM and are representative of at least 2 independent experiments, 5 mice per genotype were pooled per experiment. Unpaired t- test (2-paired) *** $P<0.0005$; ** $P<0.005$; * $P<0.05$, n.d. not detected.

Supplementary Figure 4



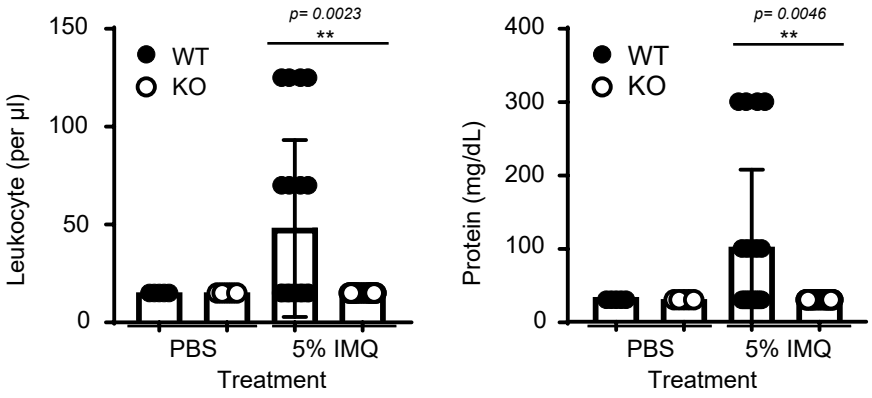
Supplementary Figure 4. TLR7/9 expression and signaling pathways in *Tasl*$^{-/-}$ mice. (**a**) RT-qPCR analysis of ISG expression of pDCs isolated from wildtype (WT) or *Tasl*$^{-/-}$ (KO) spleens and treated with Type I IFN (500U) for 5 hours. (**b**) Western blot analysis of pSTAT1 and STAT1 in splenic pDCs with Type I IFN for 30 mins. (**c**) WT or KO Flt3L pDCs treated with LysoTracker were stimulated with CpG A (3μM) for the indicated time periods and fluorescence was analyzed by flow cytometry. (**d**) RT-qPCR analysis for TLR7 and TLR9 expression in spleens from wild type (WT) or *Tasl*$^{-/-}$ (KO) mice stimulated with R848 (10μg/ml) or CpG A (μM) (n=3, biological replicates). (**e**) Endogenous TLR9 from Flt3L derived pDCs from wild type (WT) or *Tasl*$^{-/-}$ (KO) mice was immunoprecipitated and lysates were subjected to western blot analysis with TLR9 antibody. β-actin in whole cell lysate (WCL) was detected as internal control. (**f**) Immunoblots of p-JNK and JNK (**g**) p-p38 and p38 and (wild type and knockout Flt3L pDCs stimulated with R848 (10μg/ml) for the indicated time periods (0-3hr). Representative of at least 2 independent experiments, 5 mice per genotype were pooled per experiment. (**h**) Immunoblots of pIkBa, IkBa, pSTAT1, and STAT1 in CpG A (3μM) stimulated Flt3L pDCs. In (a) and (c-d), data are means ± SEM and in (a-c), and (e-h), 5 mice per genotype were pooled per experiment. All data are representative of at least 2 independent experiments.

Supplementary Figure 5



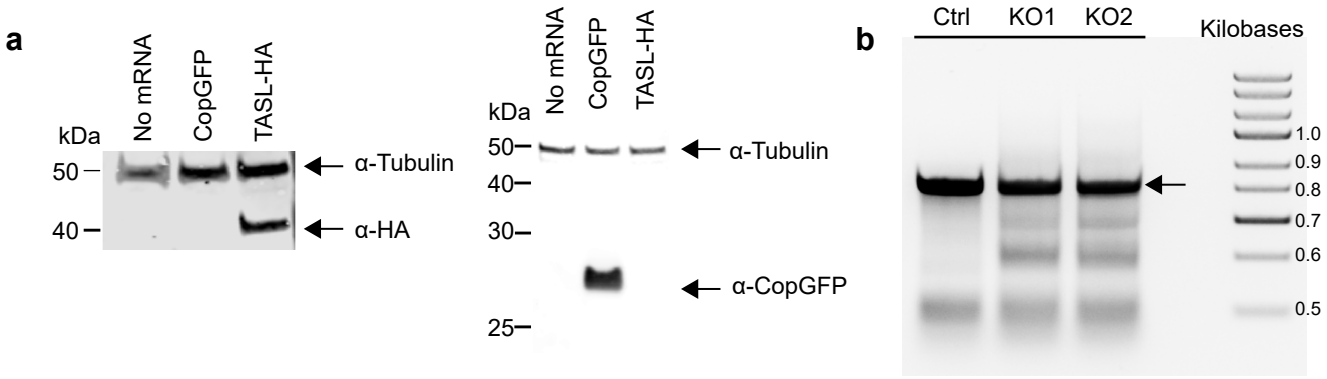
Supplementary Figure 5. IRF5 expression in *Tasl*$^{-/-}$ mice. (**a**) Western blot analysis of HEK293T cells transfected in combination with vectors encoding mouse *Irf5*, *Irf7*, and *IKbkb*. L.D. indicates protein ladder. Beta actin was used as a loading control. Western blot analysis of p-IRF5 and total IRF5 in (**b**) Western blot analysis of pIRF5 and IRF5 in Flt3L pDCs stimulated with R848 (10μg/ml) for the indicated time periods (0-3hr). N.S. indicates a non-specific band. Bottom panel indicates quantification of p-IRF5 band normalized to total IRF5 protein band and representative of two independent experiments. (**c**) Western blot analysis of p-IRF5 and IRF5 in splenic B cells stimulated with R837 (10μg/ml) for the indicated time periods (0-24hr). N.S. indicates a non-specific band. Bottom panel indicates quantification of p-IRF5 band from two independent experiments normalized to total IRF5 protein band. (**d**) Western blot analysis of pIRF5 and IRF5 in M-CSF derived BMDMs stimulated with R848 (1μg/ml) for the indicated time periods (0-3hr). (**e**) Western blot analysis of cytosolic and nuclear fractions obtained from Flt3L pDCs stimulated with CpG A (3μM) for the indicated time periods (0-6hrs). Graphs indicate quantification of p-IRF5 or IRF5 bands normalized to Histone H3 protein band. Quantification is normalized to zero time point and is representative of two independent experiments. (**f**) Microscopy image of Flt3L pDCs transfected with mRNA encoding CopGFP-IRF7(Green) and stimulated with CpG A (3μM) for 6 hrs followed by DAPI staining for nuclei (Blue). Data plotted is the average percent colocalization of CopGFP-IRF7 and nuclei from three cover slips and is representative of two independent experiments. (**g**) Flt3L derived pDCs were stimulated with LPS (5ng/ml) and the indicated NOD1/2 ligands (10μg/ml). IL-6 secretion was measured 24hrs post stimulation (n=5 pooled, 3 replicates per condition) Data are means ± SEM. p-values; unpaired *t*-test (2-tailed), $^{**}P<0.005$.

Supplementary Figure 6



Supplementary Figure 6. *Tasl*$^{-/-}$ mice are resistant to Aldara model of SLE. Urinalysis of leukocytes and protein levels from wild type (WT) and *Tasl*$^{-/-}$ (KO) mice that were treated with either PBS (n= 5) or 5% imiquimod (IMQ; Aldara) (n = 20) for 8 weeks. Data are means ± SEM and representative of 2 independent experiments. Unpaired t- test (2 tailed) $^{**}P<0.005$.

Supplementary Fig. 7



Supplementary Figure 7. *TASL* mRNA and CRISPR Analysis. (**a**) HEK293T cells were transfected with in vitro synthesized mRNA encoding either CopGFP or TASL-HA. Westernblot analysis was performed using anti-tublin, anti-HA, and anti-CopGFP antibodies (**b**) Surveyor mutation analysis of *TASL* loci in BLCL line transfected with Cas9 complexed with either control gRNAs (ctrl) or gRNAs targeting TASL (KO1, KO2). Arrow indicates full length TASL product.

Supplementary Table 1 Source and catalog numbers of BLCL lines used in this study

Catalog Number	Description	Product	SEX
HG01500	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01606	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01625	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01669	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01682	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01756	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01761	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02224	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02231	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02238	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01503	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01608	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01624	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01672	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01680	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01765	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01767	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02221	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02233	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG02236	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	M
HG01786	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01746	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01702	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01628	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01784	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01757	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01704	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG01632	1000 GENOMES PROJECT - IBERIAN POPULATIONS IN SPAIN	LCL from B-Lymphocyte	F
HG00111	1000 GENOMES PROJECT - BRITISH FROM ENGLAND AND SCOTLAND, UK	LCL from B-Lymphocyte	F
HG00102	1000 GENOMES PROJECT - BRITISH FROM ENGLAND AND SCOTLAND, UK	LCL from B-Lymphocyte	F
GM20795	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM20799	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM20832	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM20804	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM20797	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM20822	1000 GENOMES PROJECT - PANEL OF 114 TOSCANI IN ITALIA	LCL from B-Lymphocyte	F
GM12006	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F
GM12761	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F
GM11994	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F
GM12004	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F
GM12763	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F
GM11993	1000 GENOMES PROJECT-CEPH	LCL from B-Lymphocyte	F