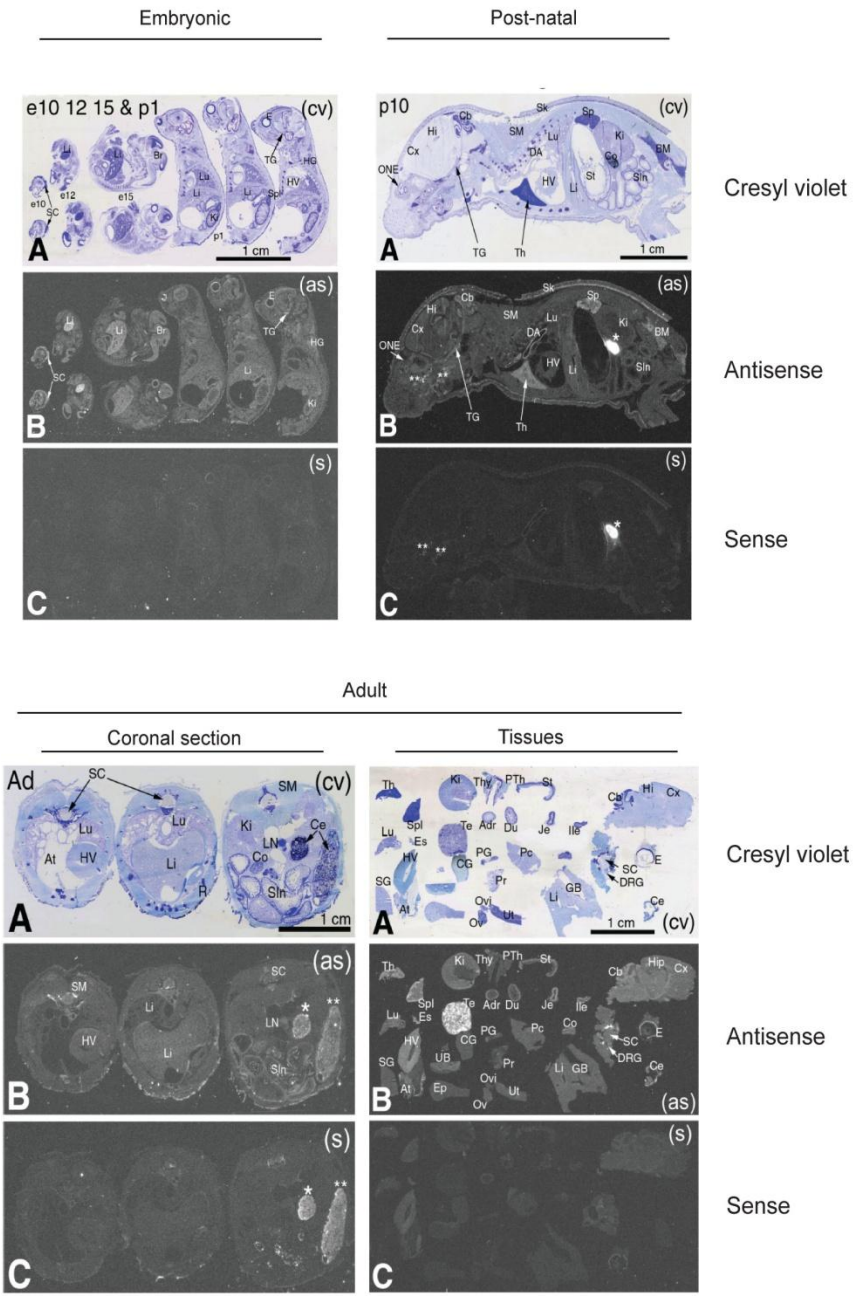
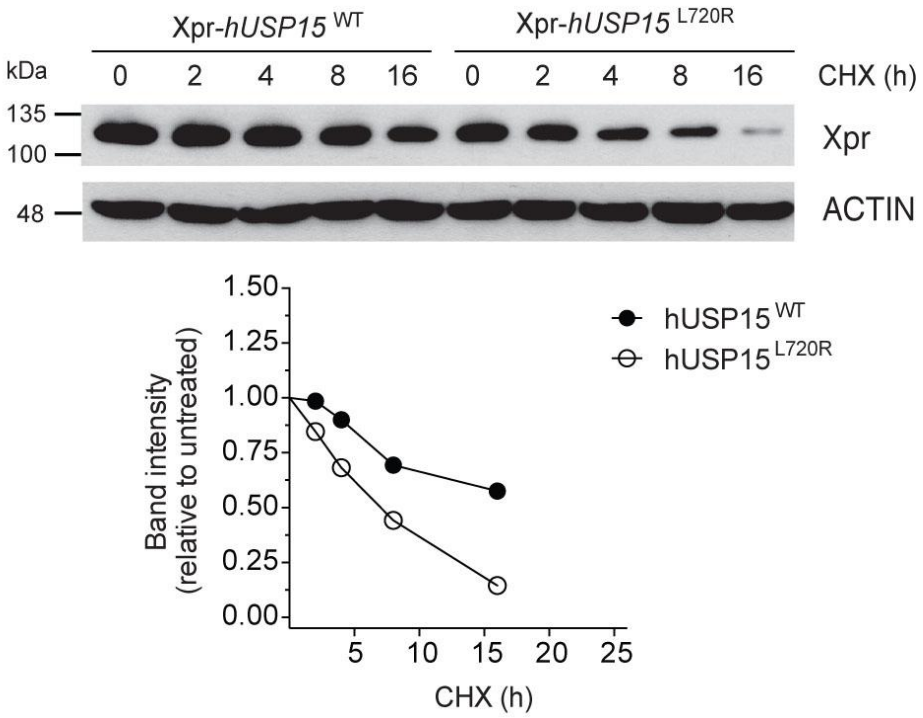


Supplementary Figure 1

Ubiquitous pattern of Usp15 mRNA expression in embryonic, post-natal and adult mice

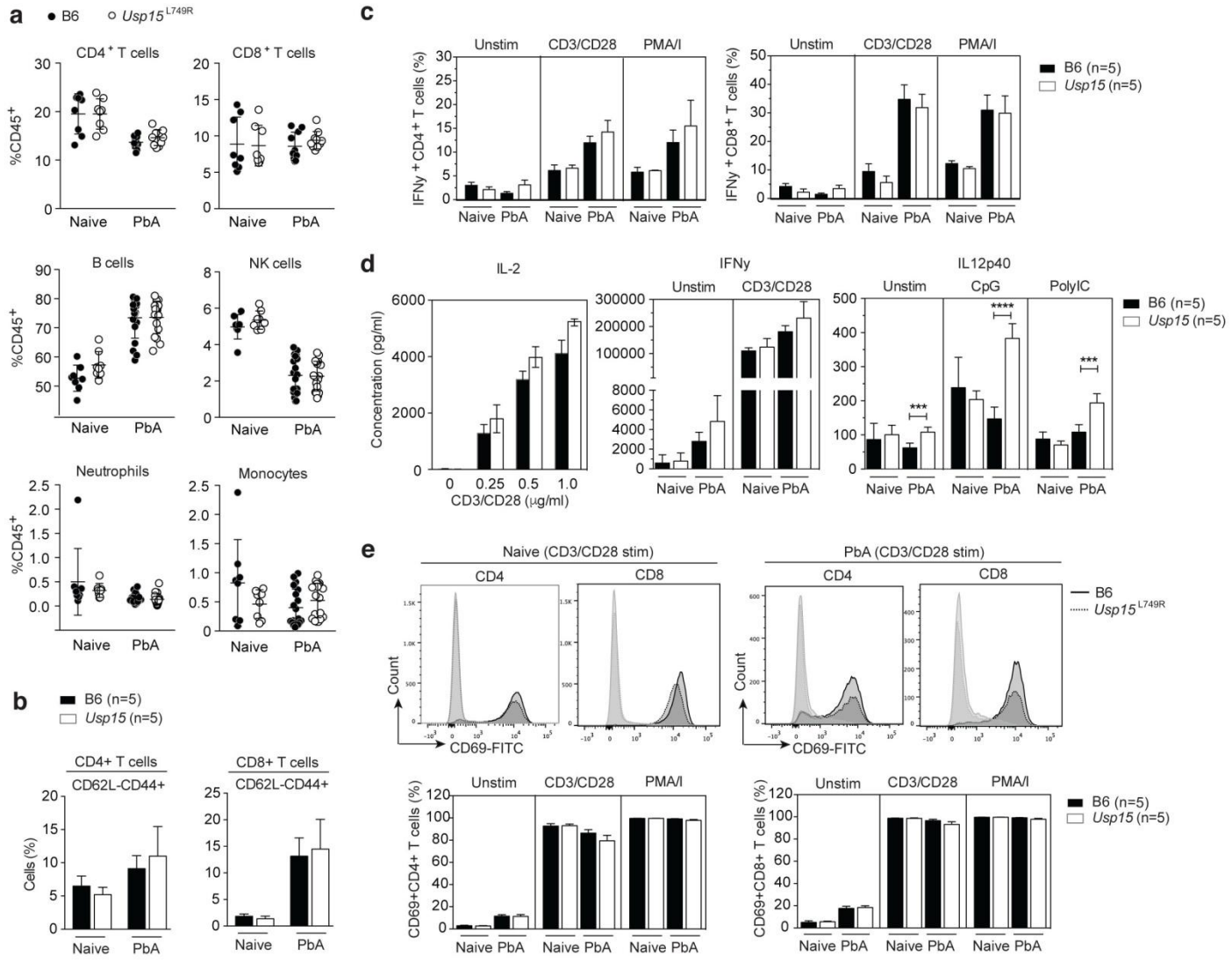
Mouse sections were stained with cresyl violet to localize *Usp15* RNA to specific organs and structures. *In situ* hybridization was carried out using radiolabelled antisense (as) and sense (s) probes. The results shown are from X-ray film autoradiography obtained following 5-days exposure. Non-specific localized signals (visible with sense and anti-sense probes) are indicated with an asterisk (*); in the teeth (p10) and the large intestine lumen (p10 and adult). (Magnification: Embryonic x2.4, Post-natal x3, Adult x2.4). Abbreviations: Adr–adrenal gland; At–heart atrium; Br–brain; Bro–bronchcus; Car– cartilage; Cb–cerebellum; Co–colon; Cx–cerebral cortex; Du – duodenum; E – eye; Ep – epididymis; Es – esophagus; GB – gallbladder; HV–heart ventricle; Il–ileum; Je–jejunum; Ki–kidney; Li–liver; LI–large intestine; Lu–lung; OL–olfactory lobe; Ov–ovary; Ovi–oviducts; PB–pelvis bone; Pc–pancreas; PG–pituitary gland; Pr–prostate; PTh–parathyroid gland; R–ribs; Sk–skin; Spl–spleen; St–stomach; SV–seminal vesicle; Te–testis; Th–thyroid gland; UB–urinary bladder; Ut-uterus; CA–central artery; GC-germinal center; LN–lymphatic nodule; RP–red pulp; Tr–trabeculum; V-vein; LF–lymphoid follicle; Me–medulla; MG–mammary glands; Cx–cortex.



Supplementary Figure 2

Reduced protein stability of the USP15 L720R human variant *in vitro*

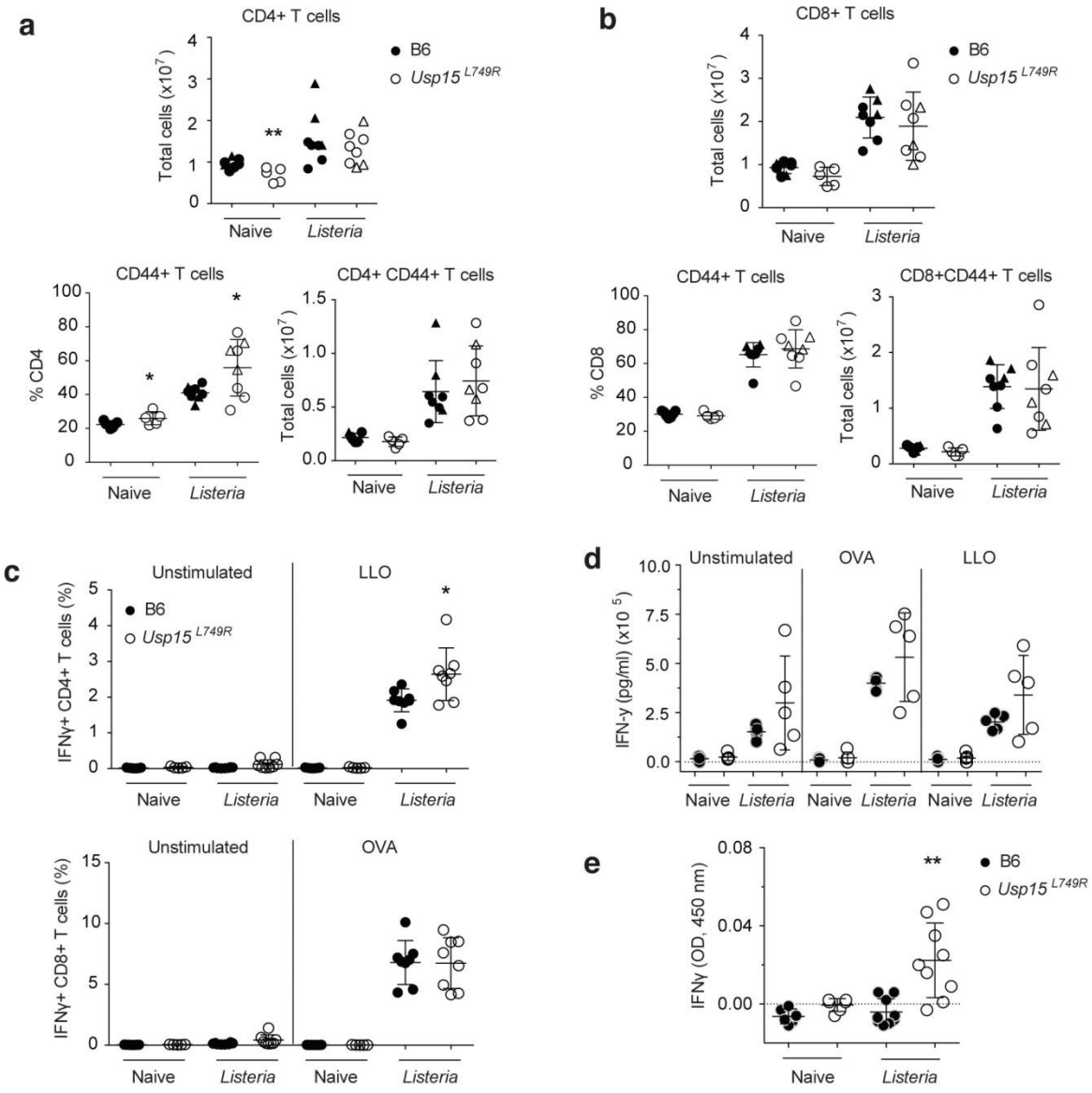
HEK293 cells stably expressing HA-tagged WT or USP15^{L720R} proteins were treated with cycloheximide (CHX, 20 μg/ml) for 2, 4, 8, and 16h, and equal amounts of protein were analyzed by immunoblotting. Data is from a single experiment.



Supplementary Figure 3

Immunophenotyping of *Usp15*^{L749R} mutants at steady-state and following *P. berghei* ANKA infection

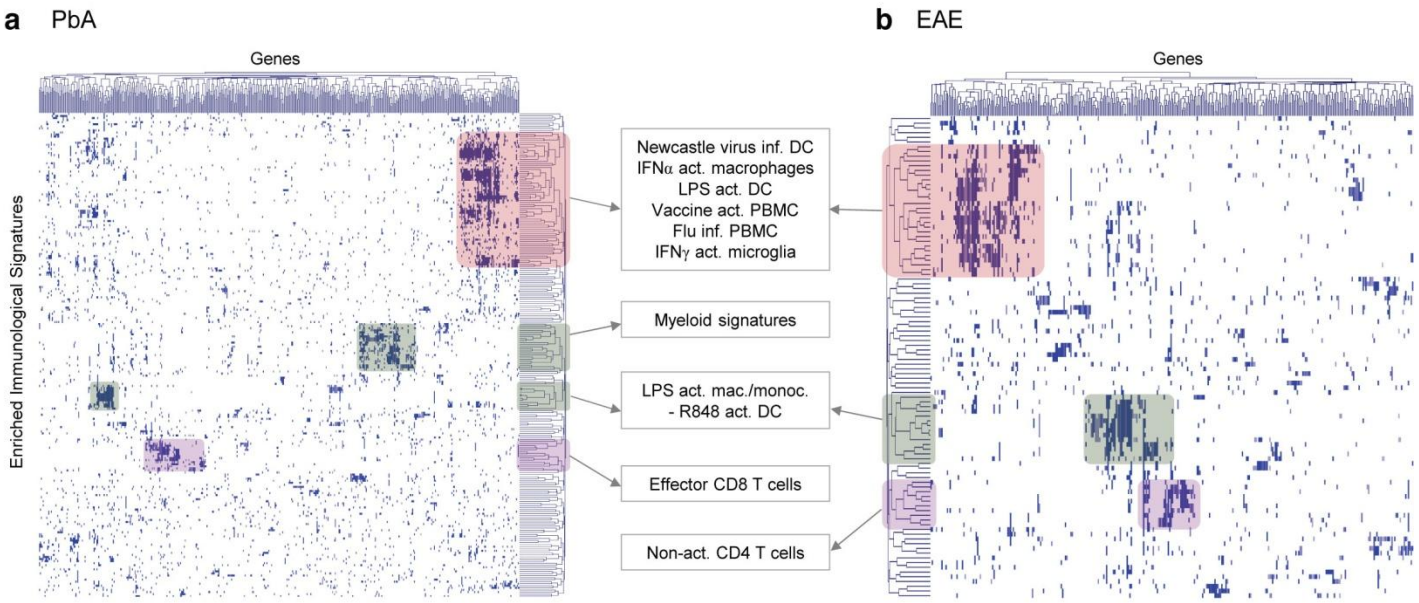
(a) The number and proportions of different spleen cell types from naïve and from day 5-PbA infected animals, were established by flow cytometry with markers for T cells (CD4, CD8), B cells (B220), NK cells (NK1.1), monocytes and neutrophils (CD11b, Ly6G). Results are pooled from 5 independent experiments. (**b**) The percentage of splenic $CD4^+$ and $CD8^+$ effector T cells ($CD62L^-CD44^+$) were also assessed. Data represents a single experiment with 5 mice per group, and are expressed as a mean ± SD. **(c-d)** Cells were re-stimulated *in vitro* with either media alone (unstimulated, US), with anti-CD3 and anti-CD28 (TCR engagement), with PMA/Ionomycin, with CpG, or with Poly:IC and cytokine production was assessed by flow cytometry (C, intracellular staining), or by ELISA (D, culture supernatants). Data is a representation of two independent experiments with 5 mice per group, and is expressed as a mean ± SD. (**e**) The activation state of $CD4^+$ and $CD8^+$ T cells were assessed by analysis of CD69 cell surface expression in response to TCR engagement (anti-CD3/anti-CD28). Data represents a single experiment with 5 mice per group, and is expressed as a mean ± SD.



Supplementary Figure 4

USP15 negatively regulates $CD4^+$ T cell activation during *Listeria monocytogenes* infection

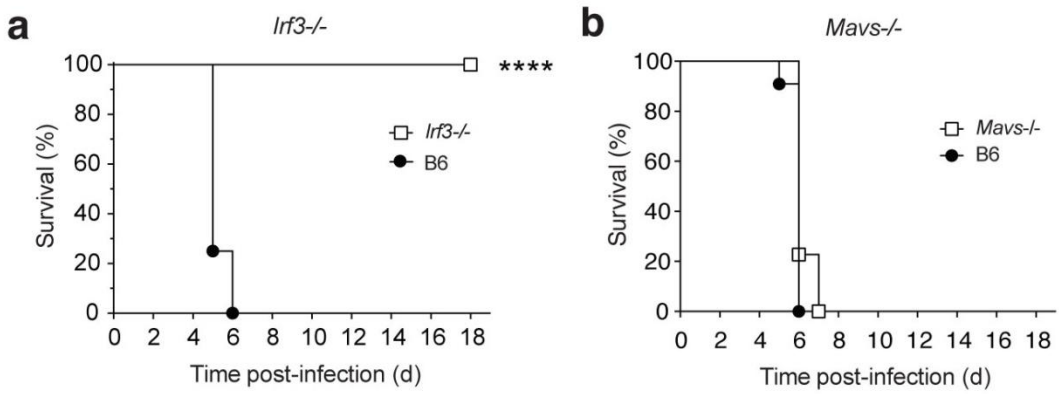
Wild type B6 mice and *Usp15*^{L749R} mutants infected with $1x10^4$ CFU of *Listeria monocytogenes* (strain 10403s) expressing ovalbumin (OVA) were sacrificed on day 7 post-infection, and phenotyped for the activation of the T cell response in spleen cell populations. **(a, b)** CD44 expression (T cell activation) on $CD4^+$ T cells (A), or $CD8^+$ T cells (B), expressed as percentage and total cell numbers. **(c, d)** Cells were re-stimulated *in vitro* with *Listeria*-specific antigens, LLO or OVA, and IFN-γ production was assessed by flow cytometry (C, intracellular staining), or by ELISA (D, culture supernatants) for $CD4^+$ and $CD8^+$ T cells. **(e)** Serum IFN-γ levels were measured by ELISA, and plotted as optical density absorbance (OD) at 450 nm. **(a-e)** Data is a combination of two independent experiments. All data are expressed as a mean ± SD for each group, and all statistical analyses were performed using the two-tailed unpaired Student's t-test.



Supplementary Figure 5

Cell populations and associated molecular pathways differentially regulated in a USP15-dependent fashion

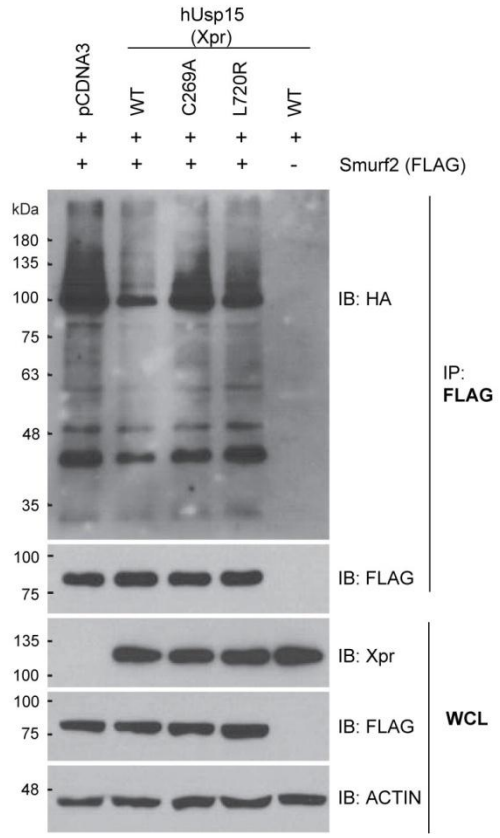
(a) LEA dendogram for genes with reduced expression in *Usp15*^{L749R} mutant mice compared to B6 (day 5 post-PbA infection) and that drive significant enrichment (FDR<0.01) of immunological expression signatures (GSEA). Enriched immunological signatures and functions are highlighted by color boxes: red = signatures of IFN activation, green = myeloid signatures and responses, and purple = T cell signatures. Refer to Online Methods for details on LEA analysis. **(b)** LEA clustering analysis as described in (A) for immunological signatures depleted in *Usp15*^{L749R} mutant mice during EAE neuroinflammation progression.



Supplementary Figure 6

Mouse mutants bearing a loss of function mutation in *Irf3* are protected against neuroinflammation

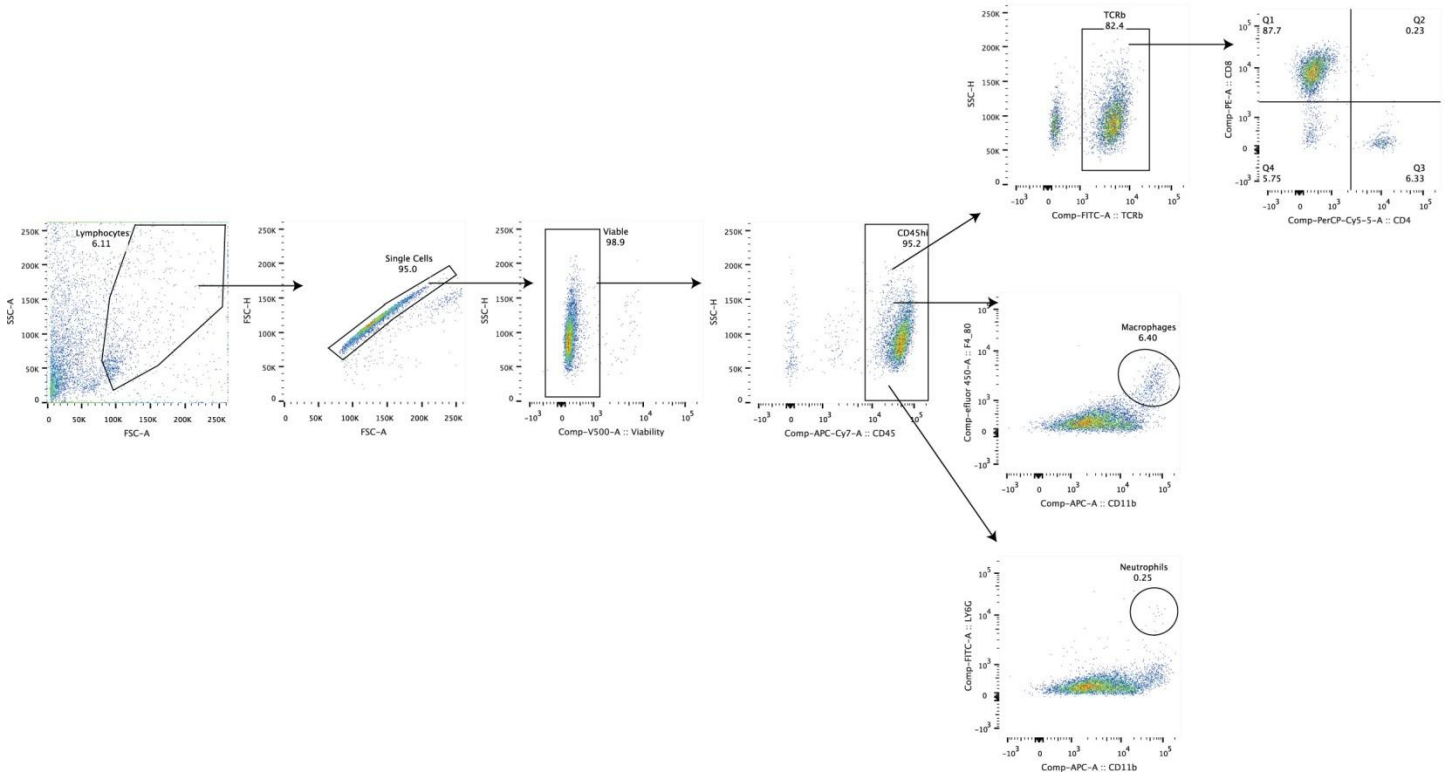
Survival plots for PbA-infected (**a**) *Irf3* mutants (*Irf3*$^{-/-}$) (n=13) and B6 controls (n=8), and (**b**) *Mavs* mutants (*Mavs*$^{-/-}$) (n=22) and B6 (n=11). Statistical significance for survival between groups of mice was determined by the Log-rank test (* $P<0.05$, **** $P<0.0001$).



Supplementary Figure 7

The L720R mutation affects the ability of USP15 to deubiquitinate SMURF2

HEK293T cells transiently expressing *Smurf2*-FLAG with or without *Usp15*-Xpress construct plus HA-tagged ubiquitin (Ub) were lysed 48h post-transfection, SMURF2 was immunoprecipitated using anti-FLAG antibody and immunoblotting analysis was performed as indicated. Construct expression in whole cell lysates (WCL) was confirmed by western blot



Supplementary Figure 8

Full gating strategy for flow cytometry analysis of brain cellular infiltration

Five days post-PbA infection, infiltrating cells were isolated from perfused brains and stained for flow cytometry analyses. Viable cells were selected for based on their exclusion of the Zombie Aqua Dye. Leukocytes were gated as $CD45^{hi}$ cells. Populations of leukocytes gated from the $CD45^{hi}$ gate were analyzed as follows: CD4 T cells ($TCRb^{+}CD4^{+}CD8^{-}$), CD8 T cells ($TCRb^{+}CD4^{-}CD8^{+}$), macrophages ($Cd11b^{+}F4/80^{+}$) and neutrophils ($Cd11b^{+}Ly6G^{+}$).