Supplemental material

Chen et al., https://doi.org/10.1084/jem.20172026

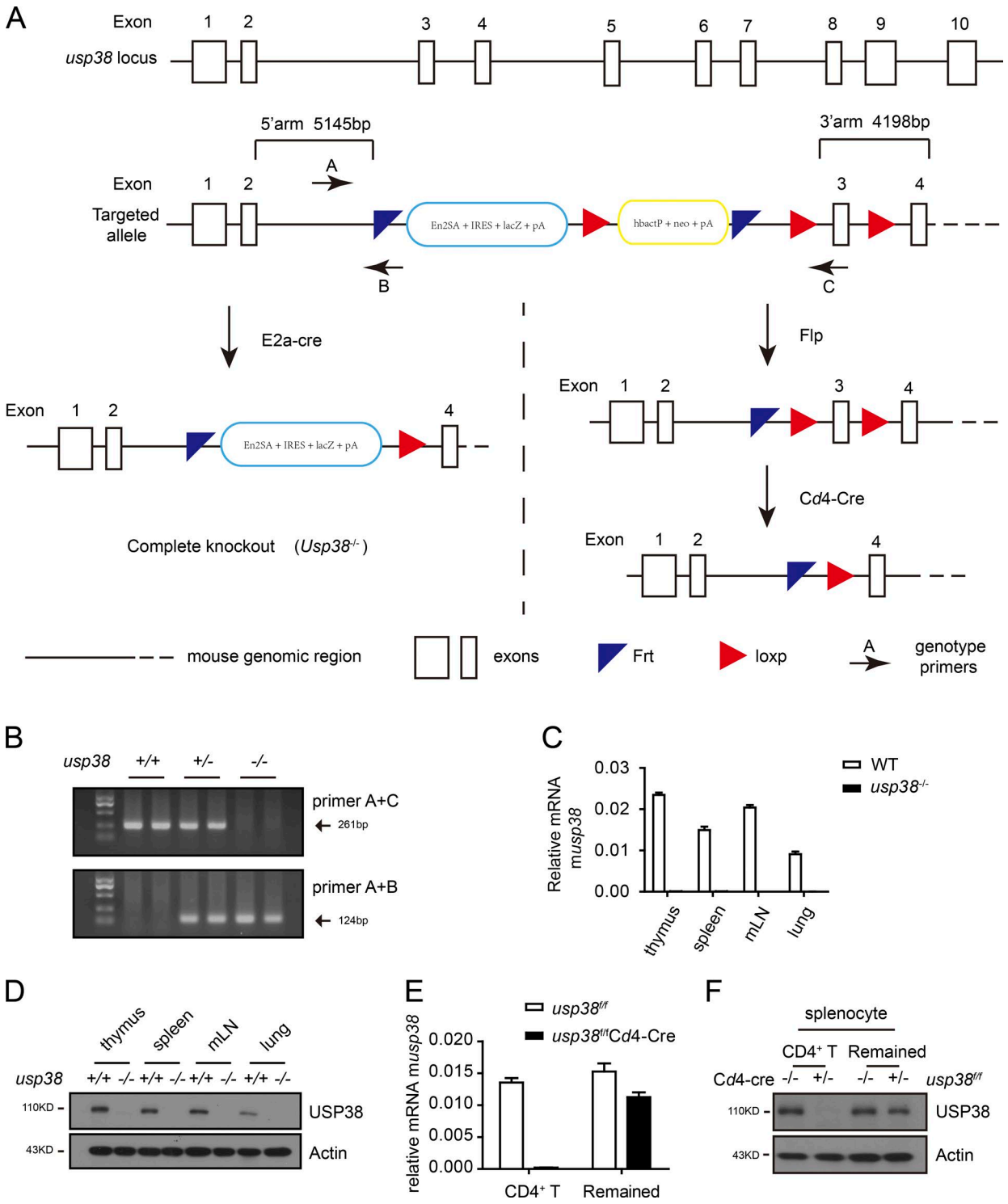

Figure S1. **Generation of *usp38* complete–deficient or T cell specific–deficient mice. (A)** Targeting strategy to generate complete or cell-type–specific deletion of *usp38* gene. **(B)** Genomic genotype of *usp38*-targeted allele by PCR. **(C and D)** The relative mRNA level (C) or the protein level (D) of *usp38* in the indicated tissues from WT and *usp38* complete knockout mice. **(E and F)** The relative mRNA level (E) or the protein level (F) of *usp38* in the isolated CD4$^+$ T cells or the remaining splenocytes after separation of CD4$^+$ T cells from *usp38*$^{f/f}$*Cd4*-Cre and control mice (*usp38*$^{f/f}$). All the data are representative of three independent experiments. Statistical significance was determined by Student's *t* test. Error bars indicate the mean ± SEM.

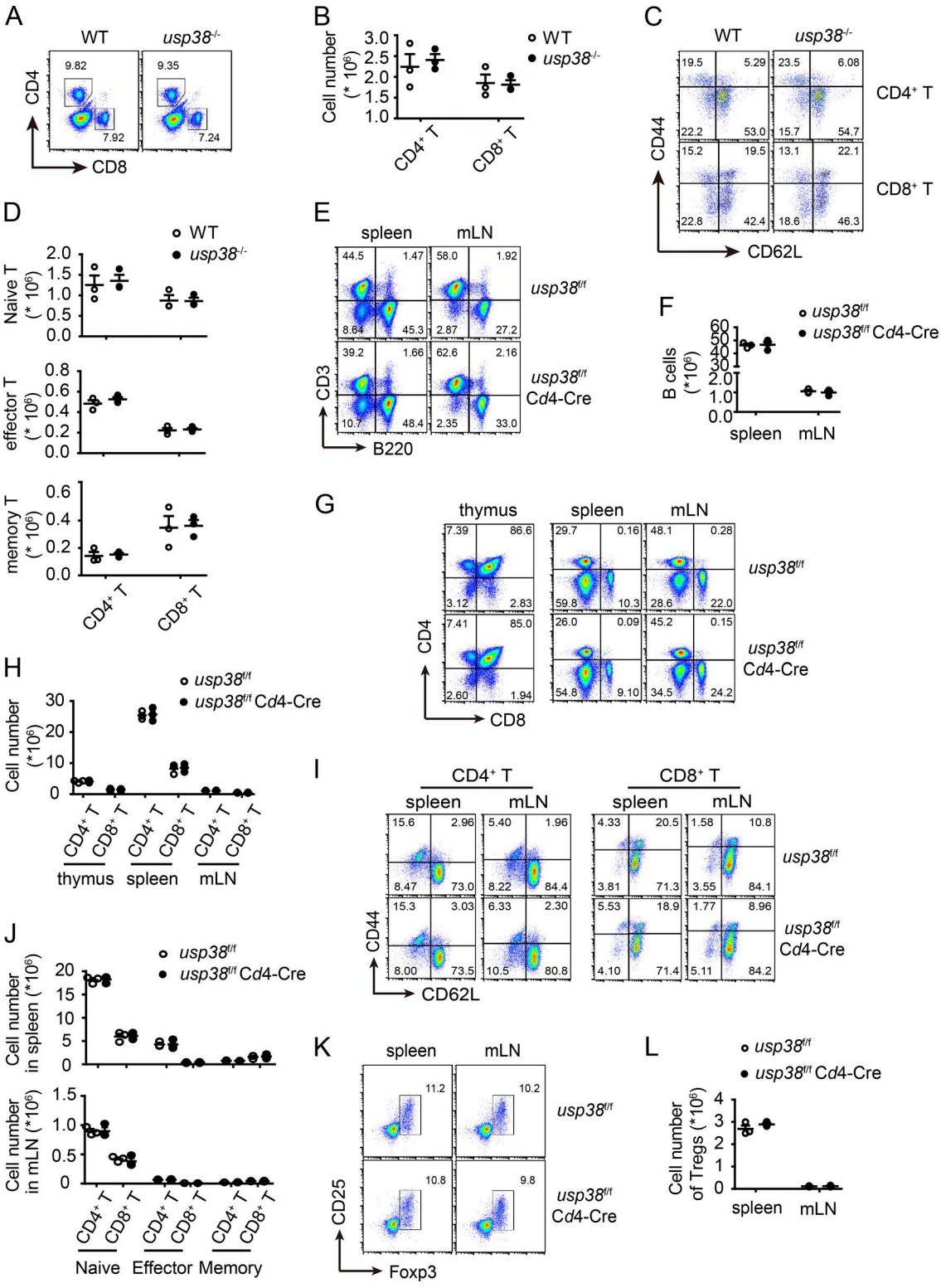

Figure S2. **USP38 deficiency neither affect homeostatic immune cells development nor alter their migration to lung.** All the experiments below were carried on 6–10-wk-old littermates. **(A and B)** Cytoflow analysis (A) and quantification (B) of CD4- or CD8-positive populations in lungs out of *usp38*$^{-/-}$ or WT mice. **(C and D)** Cytoflow analysis (C) and quantification (D) of naive, effector, and memory CD4$^+$ T cell populations, as well as CD8$^+$ T cells, in lungs out of *usp38*$^{-/-}$ or WT mice. **(E and F)** Cytoflow analysis (E) and quantification (F) of B cells in the indicated tissues out of *usp38*$^{f/f}$*Cd4*-Cre or *usp38*$^{f/f}$ mice. **(G and H)** Cytoflow analysis (G) and quantification (H) of CD4- or CD8-positive populations in the indicated tissues out of *usp38*$^{f/f}$*Cd4*-Cre or *usp38*$^{f/f}$ mice. **(I and J)** Cytoflow analysis (I) and quantification (J) of naive, effector, and memory CD4$^+$ T cell populations, as well as CD8$^+$ T cells, in the indicated tissues out of *usp38*$^{f/f}$*Cd4*-Cre or *usp38*$^{f/f}$ mice. **(K and L)** Cytoflow analysis (K) and quantification (L) of T reg cells in the indicated tissues out of *usp38*$^{f/f}$*Cd4*-Cre or *usp38*$^{f/f}$ mice. Data are representative of three (A–L) independent experiments. Statistical significance was determined by Student's *t* test. Error bars indicate the mean ± SEM.

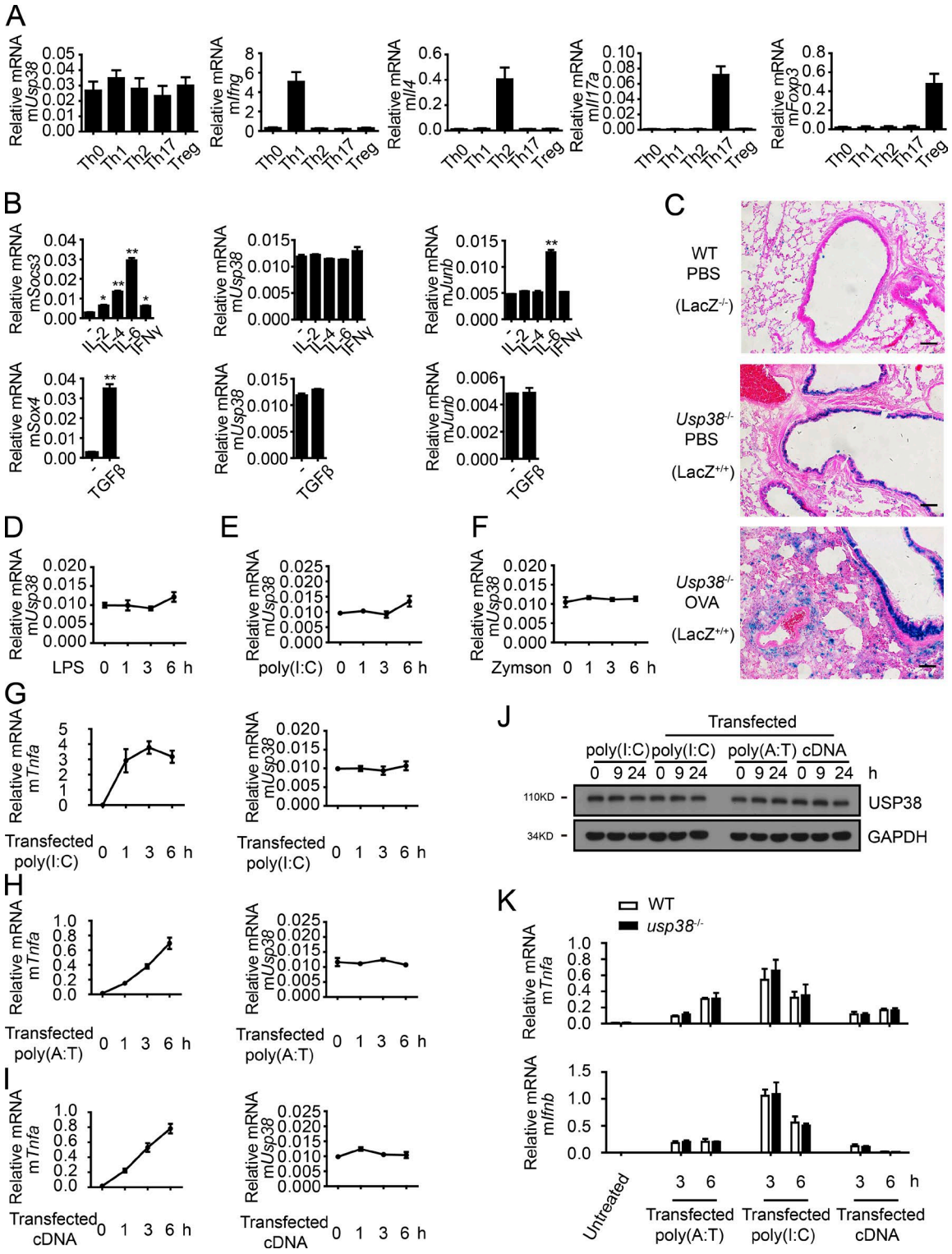

Figure S3. ***Usp38* is not induced by subset-specific cytokines in T cells nor by innate sensor triggers in BMDMs. (A)** mRNA levels of the indicated genes assessed by qPCR in different CD4+ T cell subpopulations. **(B)** mRNA levels of the indicated genes assessed by qPCR in CD4+ T cells treated by IL-2, IL-4, IL-6, IFN-γ, and TGF-β for 6 h. **(C)** β-Gal staining of lung tissues out of PBS-treated WT mice (without LacZ) and PBS or OVA-treated *usp38*$^{-/-}$ mice (with LacZ). **(D–F)** mRNA level of *usp38* assessed by qPCR in WT BMDMs that were stimulated with 100 ng/ml LPS (D), 10 µg/ml poly(I:C) (E), and 10 µg/ml zymson (F) for the indicated time points. **(G–I)** mRNA levels of *Tnfa* and *usp38* assessed by qPCR in WT BMDMs that were transfected with 2 µg/ml poly(I:C) (G), 2 µg/ml poly(A:T) (H), and 10 µg/ml carrier DNA (I) for the indicated time points. **(J)** Immunoblotting of cell lysates from WT BMDMs that were stimulated with 10 µg/ml poly(I:C), transfected 2 µg/ml poly(I:C), 2 µg/ml poly(A:T), or 10 µg/ml carrier DNA for the indicated time points. **(K)** mRNA levels of *Tnfa* and *Ifnb* assessed by qPCR in *usp38*$^{-/-}$ or WT BMDMs that were treated as indicated. Data are repeated for three (A–I) or two (J and K) independent times. Statistical significance was determined by Student's *t* test; *, P < 0.05; **, P < 0.01. Error bars indicate the mean ± SEM.

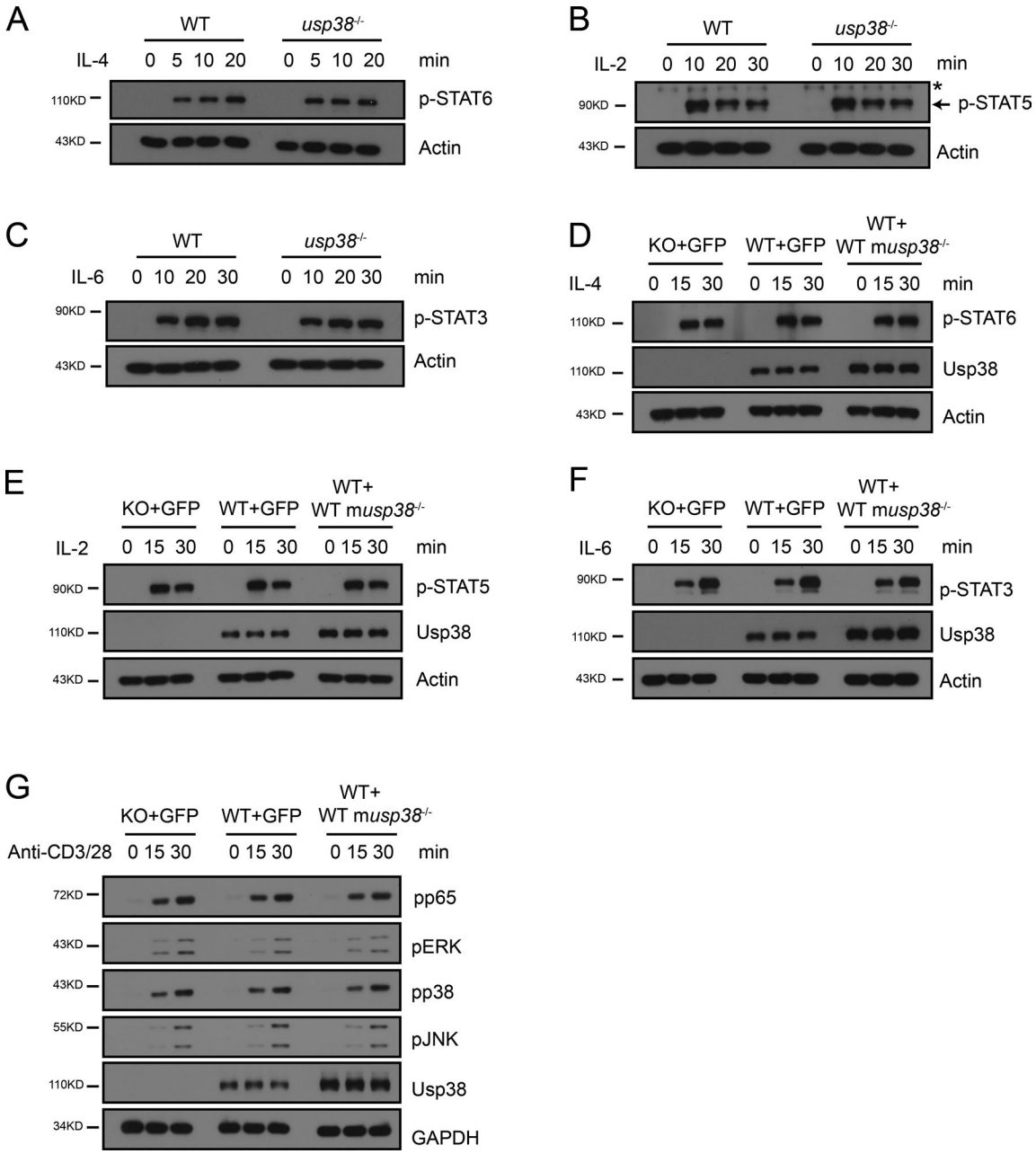

Figure S4. **USP38 does not affect the cytokines-induced signaling and TCR-induced activation of NF-κB and MAPKs. (A–C)** Immunoblotting of cell lysates of *usp38*$^{-/-}$ or WT CD4^{+} T cells that were deprived of serum for 2 h and followed by stimulation with the indicated cytokines for the indicated time points. **(D–F)** Immunoblotting of cell lysates of *usp38*$^{-/-}$ or WT CD4^{+} T cells that were infected with empty retrovirus (GFP) or retrovirus encoding WT m*usp38*. The cells were then deprived of serum for 2 h and followed by stimulation with the indicated cytokines for the indicated time points. **(G)** Immunoblotting of cell lysates of *usp38*$^{-/-}$ or WT CD4^{+}T cells that were infected with empty retrovirus (GFP) or retrovirus encoding WT m*usp38* and then treated by anti-CD3/28 for the indicated times. *, nonspecific band. Data are representative of three (A–C) independent and two (D–G) independent experiments.

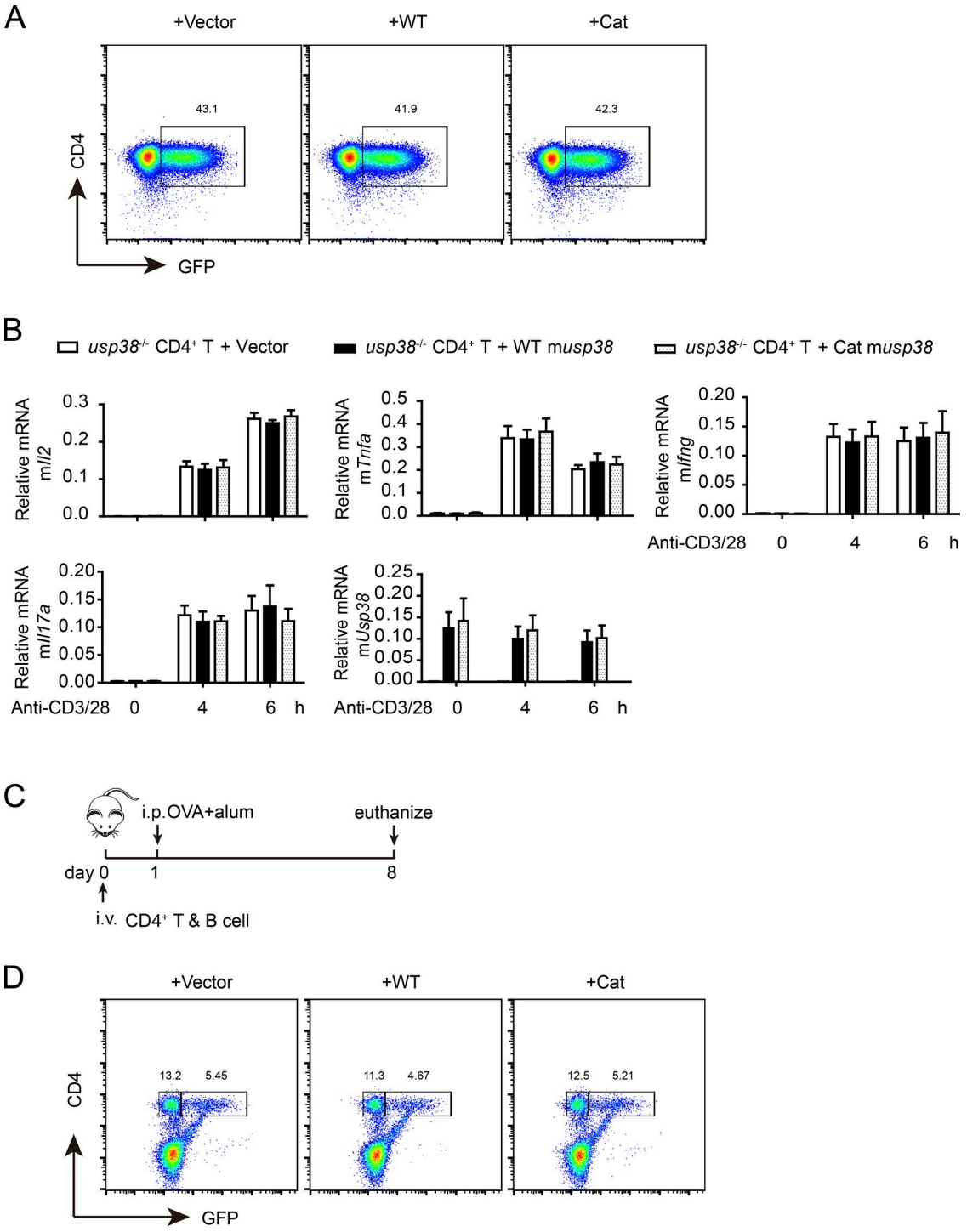

Figure S5. **The DUB activity of USP38 is essential for Th2 development. (A–D)** *Usp38*$^{-/-}$ CD4^{+} T cells were restored with empty retrovirus (GFP) or retrovirus encoding WT mouse *usp38* and its DUB activity mutant (Cat). **(A)** The percentages of GFP-positive population determined by cytoflow for restoration efficiency by the viruses. **(B)** mRNA levels of *Il2*, *Tnfa*, *Ifng*, *Il17a*, and *usp38* determined by qPCR in the virus restored cells that were stimulated with anti-CD3 and anti-CD28 for the indicated time points. **(C)** A schematic diagram of adoptive transfer experiments. **(D)** Cytoflow analyses of splenocytes from *rag2*$^{-/-}$ recipient mice after adoptive cell transfer and OVA immunization. *Usp38*$^{-/-}$ CD4^{+} T cells were infected with the empty virus or virus encoding *usp38* or its DUB mutant. The infected cells together with WT B cells were transferred into *rag2*$^{-/-}$ mice and the next day the mice were immunized with OVA in alum for 7 d. Data are repeated for three (A and B) or two (C and D) independent times. Statistical significance was determined by Student's *t* test. Error bars indicate the mean ± SEM.