SUPPLEMENTAL MATERIAL

Kumar et al., http://www.jem.org/cgi/content/full/jem.20131379/DC1

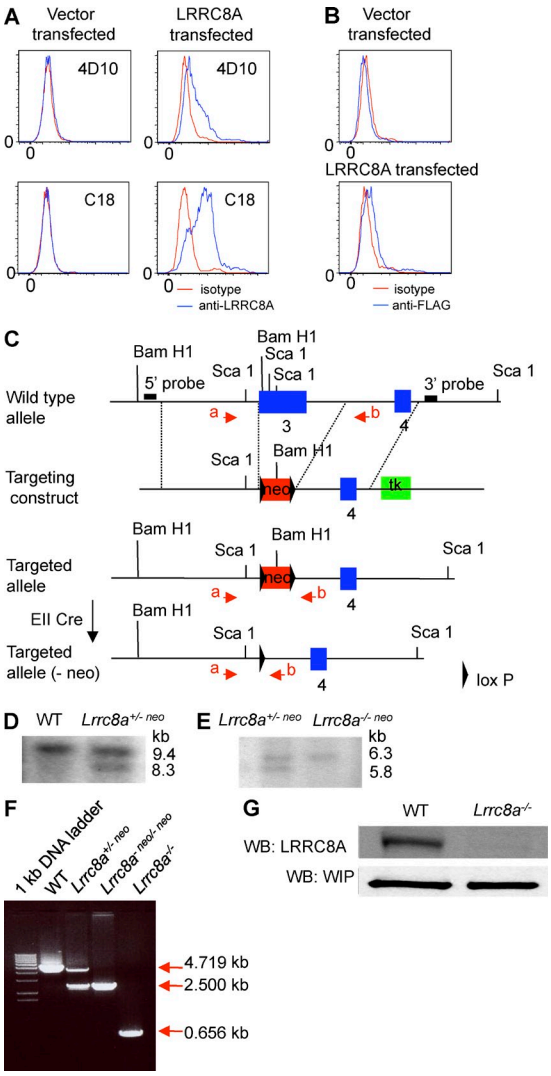

Figure S1. Surface expression of LRRC8A and the generation of *Lrrc8a*$^{-/-}$ mice. (A) FACS analysis of LRRC8A surface expression by 293T cells transfected with empty vector or vector encoding LRRC8A, using 4D10 mAb and C18 rabbit polyclonal antibody. (B) FACS analysis of LRRC8A surface expression by 293T cells transfected with empty vector or vector encoding LRRC8A-FLAG using anti-FLAG mAb. (C) Structure of the WT *Lrrc8a* allele, the targeting construct, the targeted allele that has undergone homologous recombination before and after Cre-mediated removal of the *neo* gene. *Lrrc8a* exons are represented by blue boxes. *neo* = neomycin resistance gene, *tk* = thymidine kinase gene. The external 5′ and 3′ probes are indicated by the bars. The PCR primers used are indicated by the arrows. (D) Homologous recombination in ES cells detected by Southern blotting. Sca 1–digested genomic DNA was probed using the 3′ external probe. (E) Genotyping of mice before removal of the *neo* gene by Southern blotting. Bam H1–digested tail genomic DNA was probed with the 5′ external probe. (F) Genotyping by PCR analysis of tail DNA using the primers a and b to identify the *Lrrc8a* targeted allele before and after removal of the *neo* gene. The disrupted allele with the *neo* gene retained is designated *Lrrc8a*$^{-neo}$. The disrupted allele with the *neo* gene removed is designated *Lrrc8a*$^{-}$. (G) Immunoblot analysis of LRRC8A in thymocyte lysates. Wiskott-Aldrich Interacting Protein (WIP) was used as a loading control. Data are representative of three independent experiments with one sample per group (A and B), and three independent experiments with one sample (D) and one mouse per group (E–G).