## **JEM**

## SUPPLEMENTAL MATERIAL

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

Α

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Wild type

Targeting

allele

С

Vector

transfected

4D10

C18

0

Bam H1

5' probe

construct Bam H1 Bam H1 Sca 1 Sca 1 Targeted allele a Bam H1 Ell Cre Sca 1 Sca Targeted a allele (- neo) b lox P D WT Lrrc8a+/- neo E Lrrc8a+/- neo Lrrc8a-/- neo kb 6.3 5.8 kb 9.4 F G I mc8a-/-W/T WB: LRRC8A WB: WIP 4.719 kb -2.500 kb ←0.656 kb Figure S1. Surface expression of LRRC8A and the generation of Lrrc8a<sup>-/-</sup> 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

LRRC8A

transfected

4D10

C18

isotype anti-LRRC8A

Sca 1

a

Sca 1

Vector

transfected

0 LRRC8A transfected

isotype
anti-FLAG

3' probe

Sca 1

в

0

0

Bam H1

Sca 1

3 Bam H

Sca 1

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<sup>-neo</sup>. The disrupted allele with the neo gene removed is designated Lrrc8a<sup>-</sup>. (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).