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Supplementary Information for

Excessive endosomal TLR signaling causes inflammatory disease in mice with defective SMCR8-WDR41-C9ORF72 complex function

William McAlpine, Lei Sun, Kuan-wen Wang, Aijie Liu, Ruchi Jain, Miguel San Miguel, Jianhui Wang, Zhao Zhang, Braden Hayse, Sarah Grace McAlpine, Jin Huk Choi, Xue Zhong, Sara Ludwig, Jamie Russell, Xiaoming Zhan, Mihwah Choi, Xiaohong Li, Miao Tang, Eva Marie Y. Moresco, Bruce Beutler, Emre Turer

Bruce Beutler
Email: Bruce.Beutler@UTSouthwestern.edu

Emre Turer
Email: Emre.Turer@UTSouthwestern.edu

This PDF file includes:

Figs. S1 to S4

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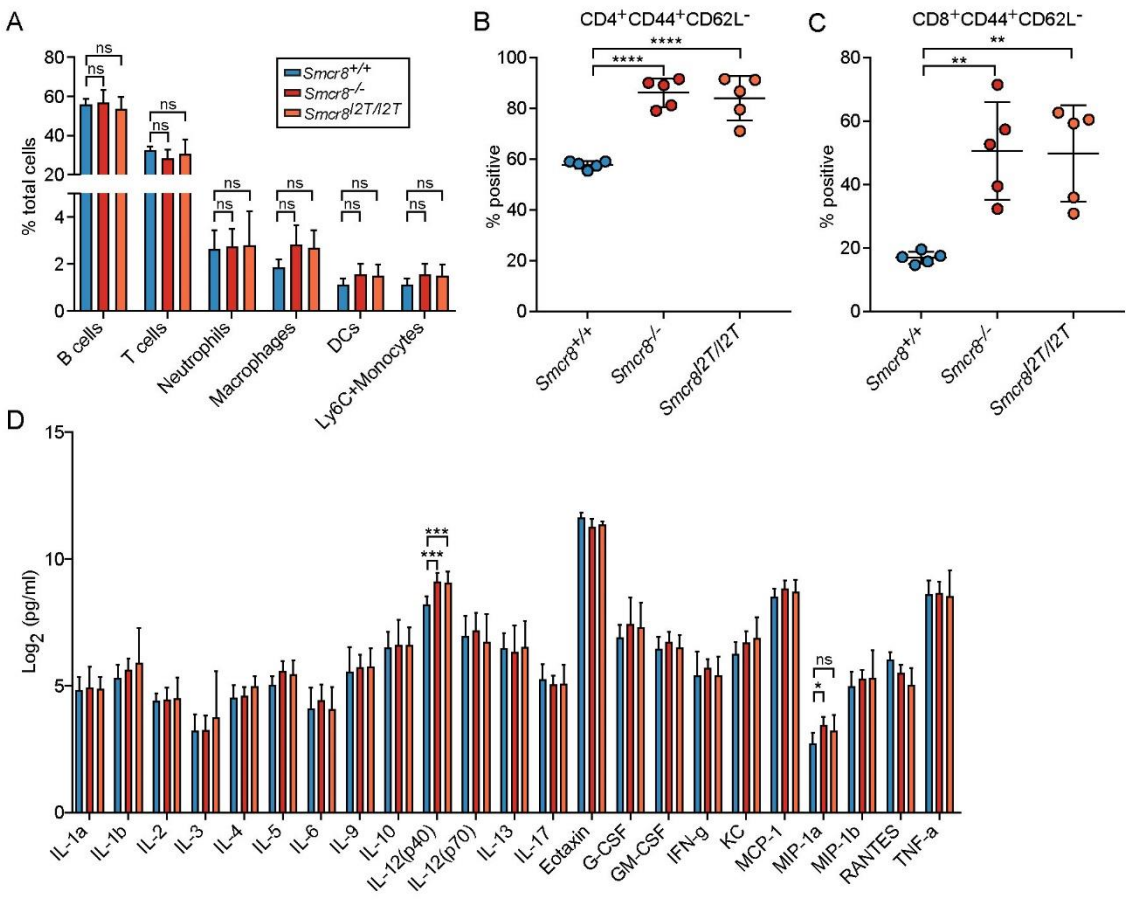

Fig. S1. Spontaneous inflammation in *Smcr8* mutant mice. (A-C) Spleens were harvested from *Smcr8*$^{+/+}$ (*n*=5), *Smcr8*$^{-/-}$ (*n*=5), and *Smcr8*$^{I2T/I2T}$ (*n*=5) mice at 12 months of age. (A) Percent of total spleen cells for indicated cell populations. (B,C) Frequency of CD44^{+}CD62L^{-} CD4^{+} (B) and CD8^{+} (C) T cells in spleen. (D) 23plex cytokine analysis of plasma harvested from *Smcr8*$^{+/+}$ (*n*=10), *Smcr8*$^{-/-}$ (*n*=10), and *Smcr8*$^{I2T/I2T}$ (*n*=10) mice at 6 months of age. In B and C, data points represent individual mice. Data are expressed as means ± s.d. and significance was determined by one-way ANOVA with Dunnett's multiple comparisons (ns, not significant, $P \geq 0.05$; *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001).

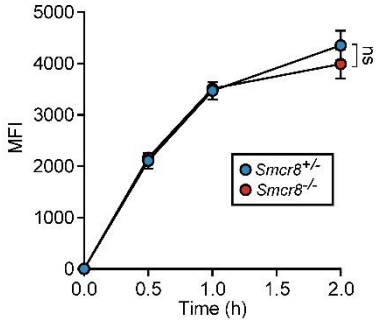

Fig. S2. Normal uptake of zymosan BioParticles. (A) Mean fluorescence intensity (MFI) of BMDMs (*n*=3 mice per genotype) incubated for the indicated times with Alexa Fluor 488 zymosan. Data are expressed as means ± SD, and the significance of differences between genotypes was determined by unpaired Student's *t* test (ns, not significant, $P \geq 0.05$).

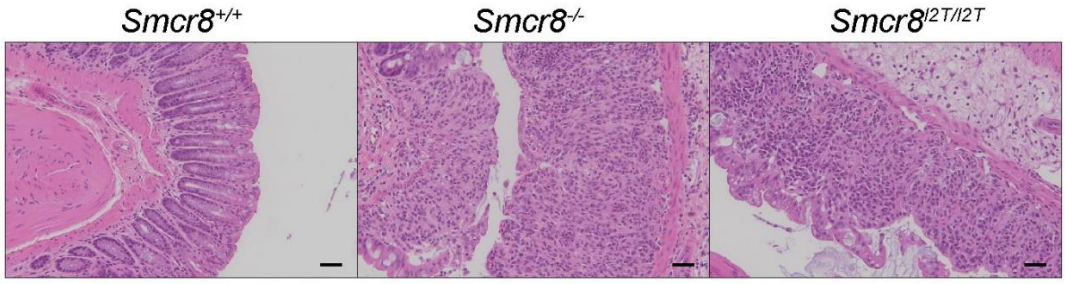

Fig. S3. Histopathological alterations in *Smcr8*$^{-/-}$ and *Smcr8*$^{I2T/I2T}$ mice treated with DSS. Representative H&E staining of *Smcr8*$^{-/-}$ and *Smcr8*$^{I2T/I2T}$ colons after 7 days of DSS treatment. Scale bar, 100 μm.

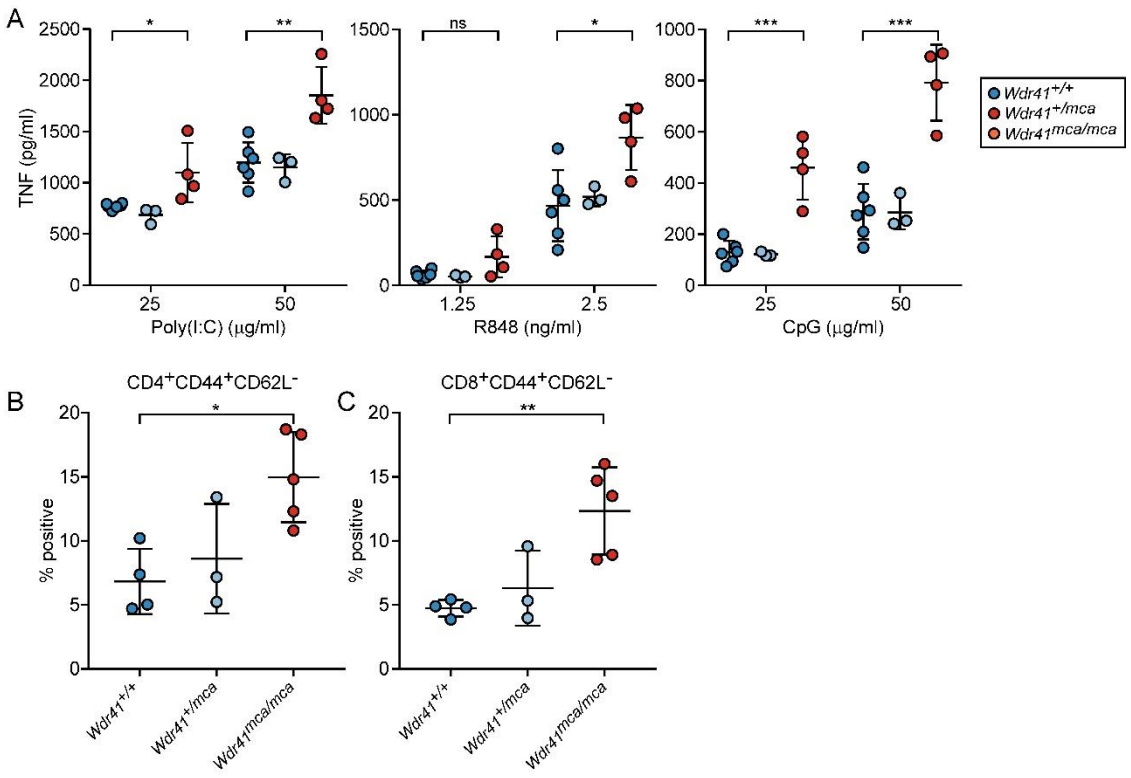

Fig. S4. *Wdr41*$^{mca/mca}$ mice phenocopy *Smcr8*$^{-/-}$ mice. (A) ELISA analysis of TNF secretion by peritoneal macrophages (*Wdr41*$^{+/+}$ (*n*=6), *Wdr41*$^{+/mca}$ (*n*=3), and *Wdr41*$^{mca/mca}$ (*n*=4)) stimulated for 6 hours with indicated concentrations of poly(I:C), R848, and CpG. (B,C) Frequency of $CD44^{+}CD62L^{-}$ $CD4^{+}$ (B) and $CD8^{+}$ (C) T cells in peripheral blood from *Wdr41*$^{+/+}$ (*n*=4), *Wdr41*$^{+/mca}$ (*n*=3), and *Wdr41*$^{mca/mca}$ (*n*=5) mice at 6 months of age. Data points represent individual mice. Experiments were performed one time. Data are expressed as means ± s.d. and the significance of differences between genotypes was determined by one-way ANOVA with Dunnett's multiple comparisons (A-C) (ns, not significant, $P \geq 0.05$; *$P<0.05$, **$P<0.01$, ***$P<0.001$).