SUPPLEMENTARY INFORMATION

The scavenger receptor SCARF1 mediates apoptotic cell clearance and prevents autoimmunity

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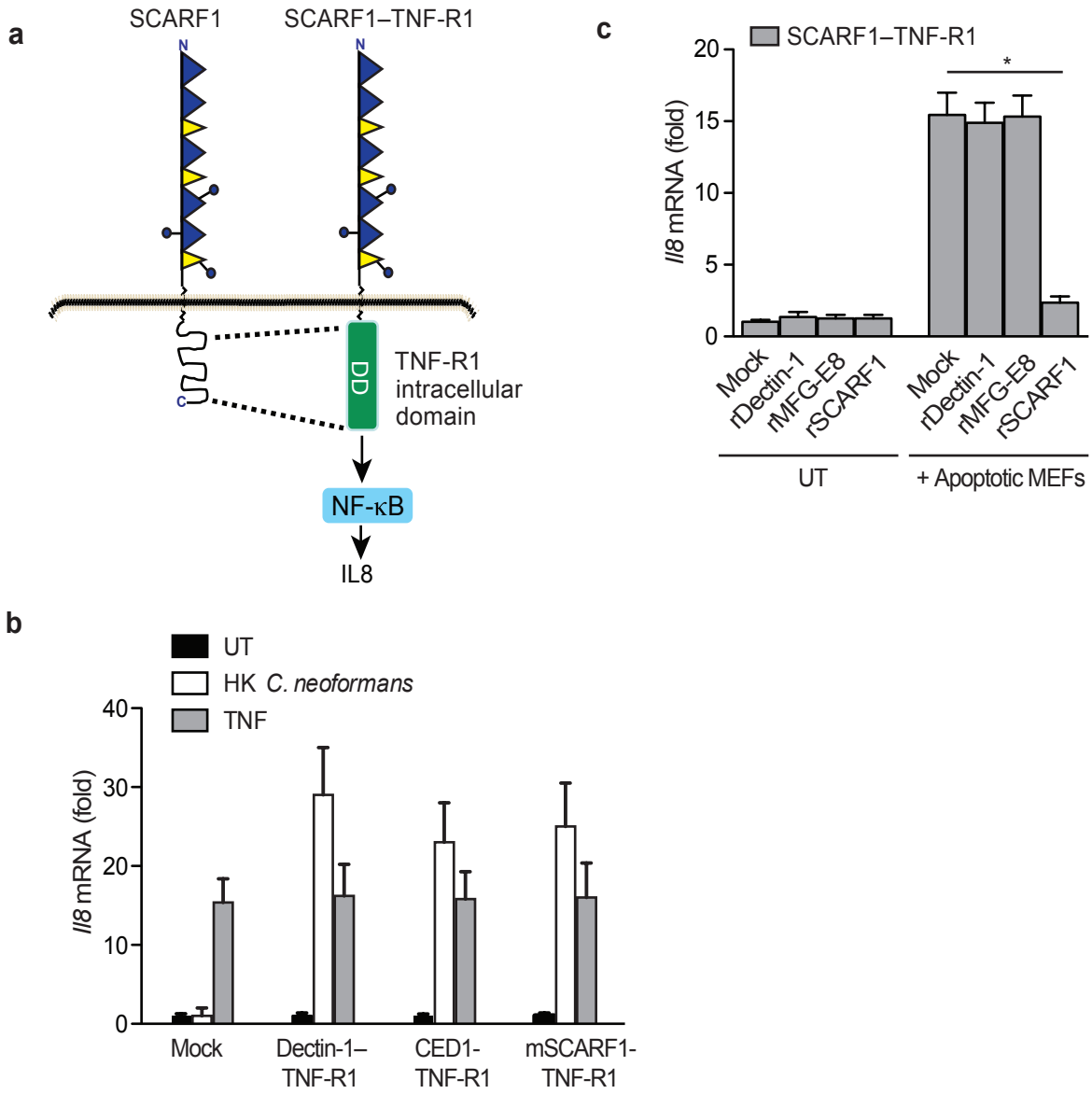
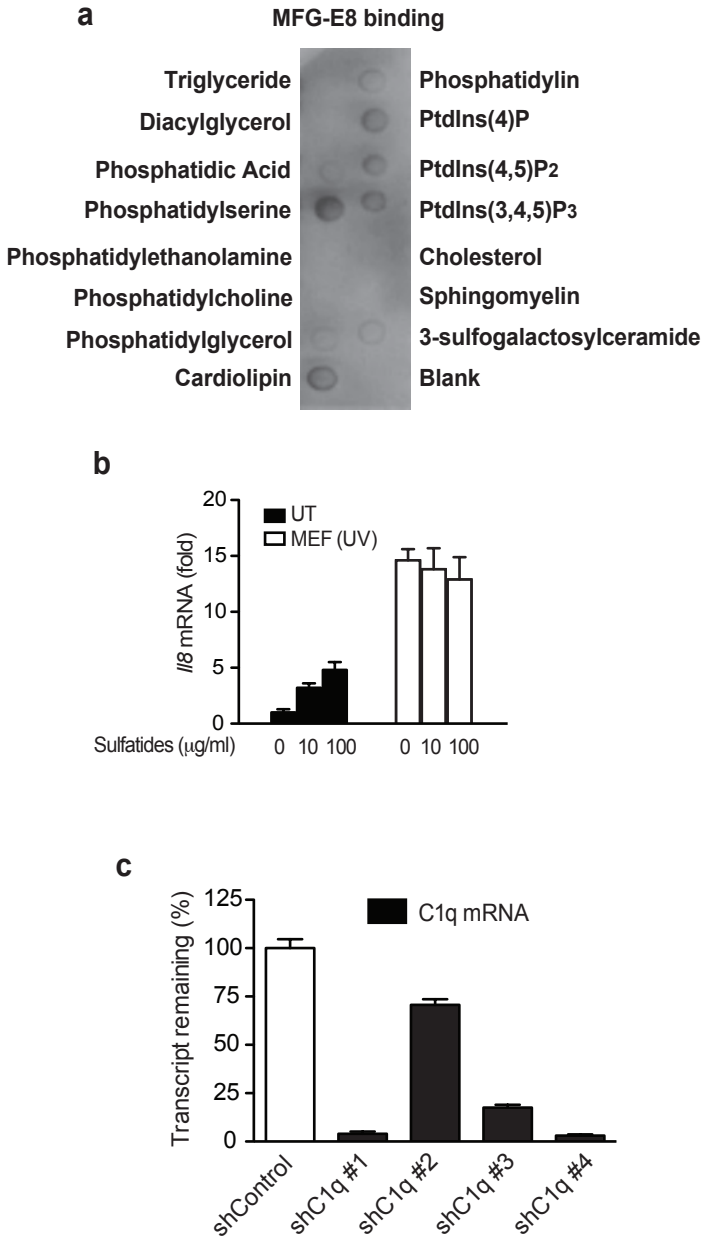
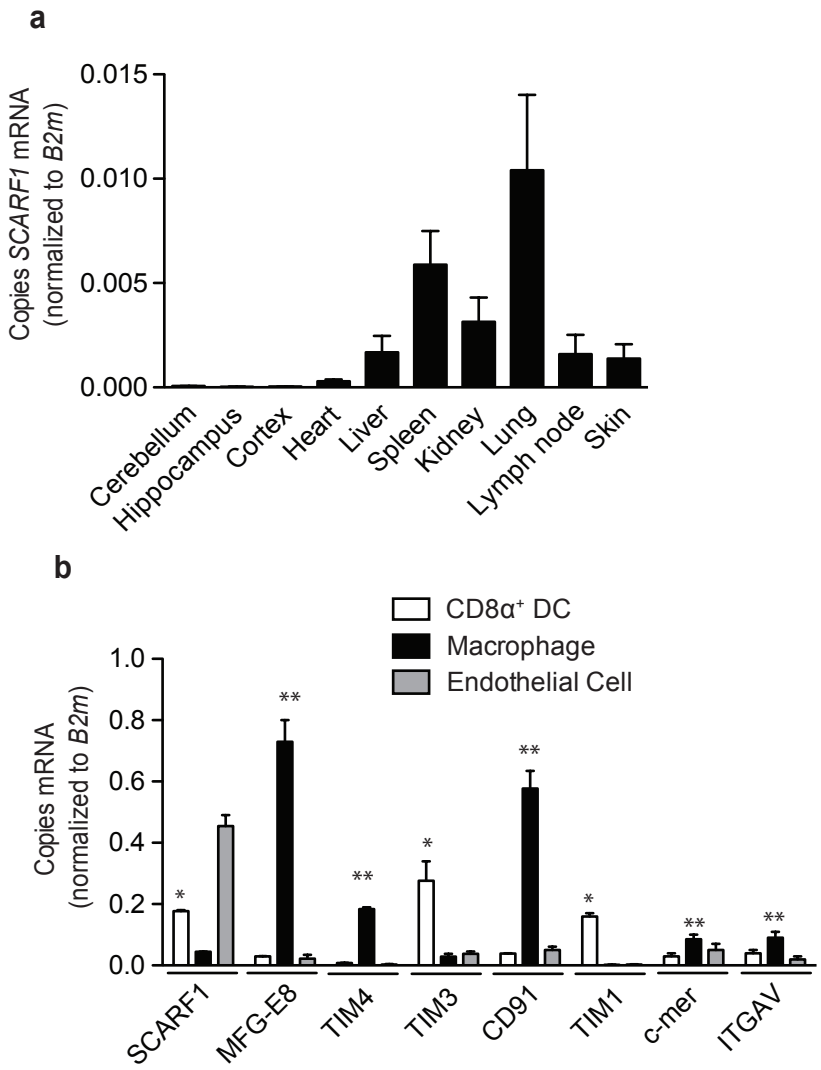


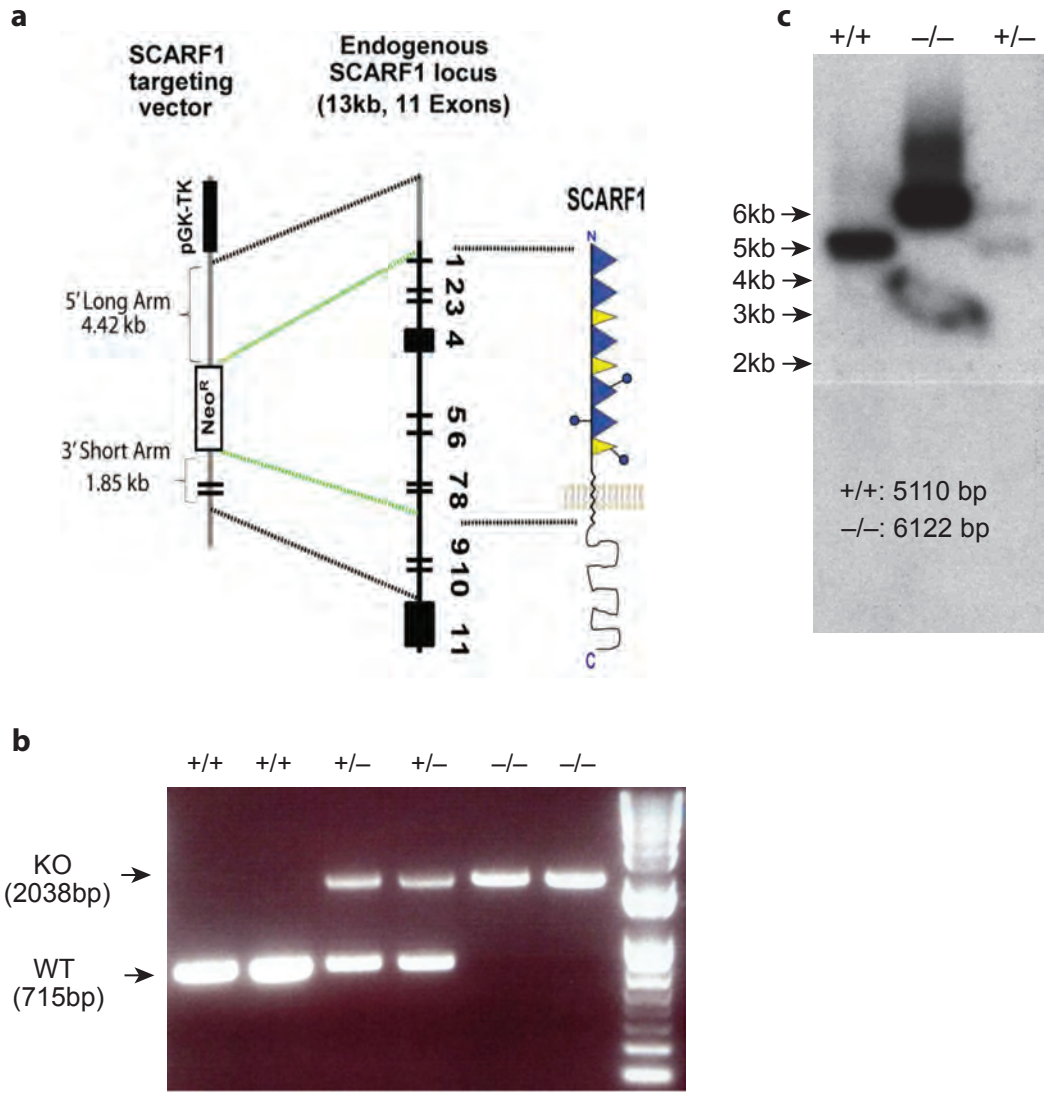
Supplementary Figure 1. SCARF1 signaling induced by apoptotic cells.
a, The extracellular and transmembrane domains of mouse and human SCARF1 (accession: NM_003693 and NM_001004157), human SCARF2 (NM_153334), *C. elegans* CED1 (NM_170927), and human Dectin-1 (NM_197947) were fused in-frame to the intracellular domain (amino acids 272-455) of the human TNF-R1 (NM_001065) and placed in mammalian expression vectors. Reporter cells were generated by transfection of plasmids encoding these chimeric receptors in HEK293T cells. Ligand recognition by these chimeric receptors induced NFkB signaling and IL8 expression. **b**, Transfected HEK293T cells (5×10^5) expressing mouse SCARF1–TNF-R1, human Dectin-1–TNF-R1, or CED-1–TNF-R1 were treated with heat-killed (HK) *Cryptococcus neoformans* (2.5×10^6) or TNF (10 ng/mL) for 3 hours. RNA was extracted and *Il8* mRNA levels were measured by qPCR. c, Mouse SCARF1–TNF-R1 reporter cells were treated with a 1:1 ratio of apoptotic UV MEFs in the presence of recombinant Dectin-1, MFG-E8, or SCARF1 protein. All data shown are from one representative experiment of at least three performed and error bars denote the mean and s.d. of triplicate measurements. *P<0.001; Student's t-test.



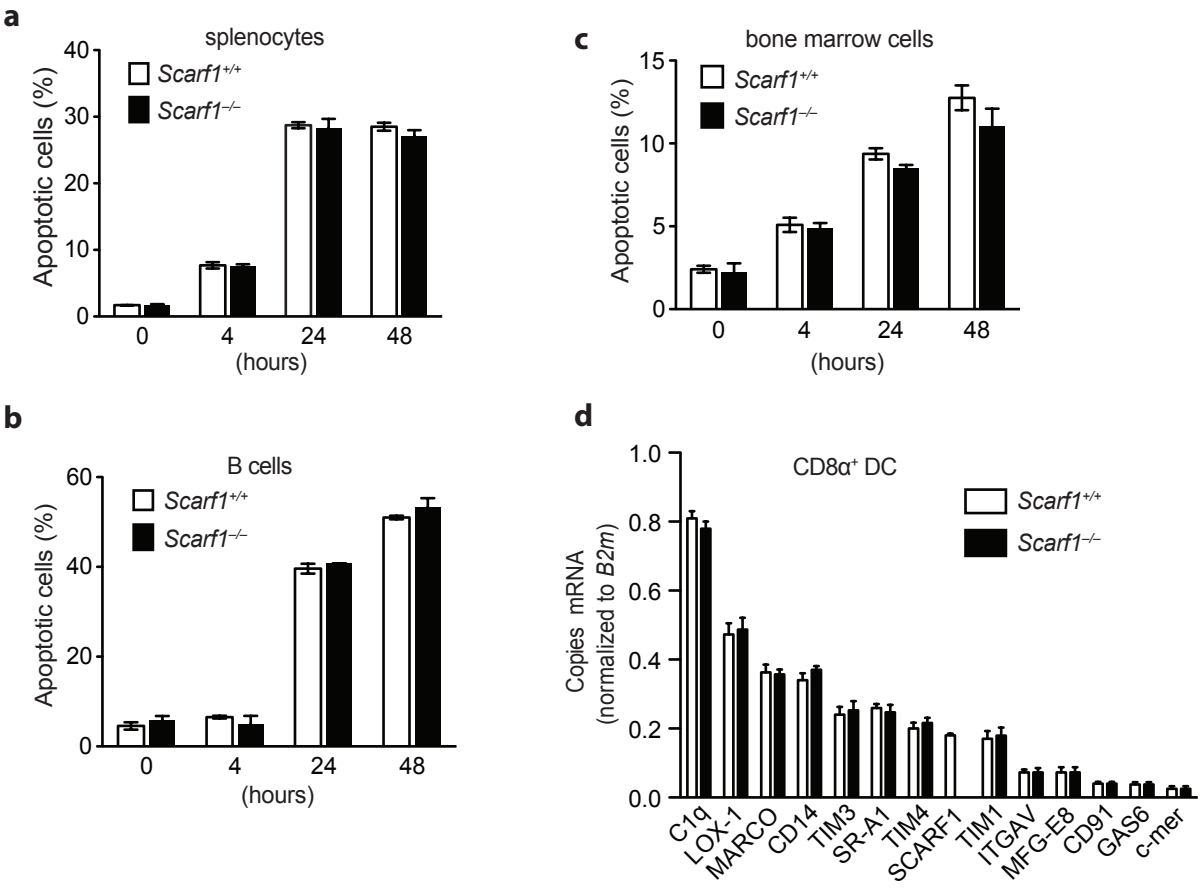
Supplementary Figure 2. Specificity of SCARF1 binding to C1q
a, Dot blot representing the binding of MFG-E8 to triglycerides, diacyl-glycerol, phosphatidic acid, phosphatidylserine, phosphatidylethanolamine, phosphatidlycholine, phosphatidylglycerol, cardiolipin, phosphatidylin, ptdIns(4)P, ptdIns(4,5)P_2, ptdIns(3,4,5)P_3, cholesterol, sphingomyelin or 3-sulfogalactosylceramide. **b**, HEK293T cells expressing mouse SCARF1–TNF-R1 were pretreated with 0, 10 or 100 μg/mL of sulfatides for 1 hour. Following pretreatment, UV-irradiated apoptotic MEFs (1:1 ratio) were co-incubated with the HEK293T mSCARF1–TNF-R1 cells for 3 hours. RNA was extracted and the levels of *Il8* mRNA were measured by qPCR. Data represent the average of 3 independent experiments and error bars denote the means and s.d. of at least triplicate measurements. **c**, qPCR analysis of *C1q* mRNA abundance in MEFs infected with viruses targeting the gene encoding *C1q*, data presented relative to the abundance of *C1q* mRNA in negative control infected MEFs (shControl).



Supplementary Figure 3. Expression of apoptotic cell clearance pathways in CD8α$^+$ DCs, macrophages, and endothelial cells.
a, RNA was obtained from indicated organs of *Scarf1*$^{+/+}$ mice and analyzed for expression of *Scarf1* by qPCR. **b**, RNA was obtained from CD8α$^+$ DCs, macrophages, and endothelial cells isolated from the spleens of *Scarf1*$^{+/+}$ mice and analyzed for expression of genes involved in apoptotic cell clearance by qPCR. Bars denote means and s.d. of three independent experiments performed with triplicate measurements. *P<0.001 CD8α$^+$ DCs vs. Macrophage; **P<0.001 Macrophage vs. CD8α$^+$ DCs and Endothelial cells; Student's t-test.



Supplementary Figure 4. Generation of *Scarf1*$^{-/-}$ mice.
a, The targeting vector used to generate *Scarf1*$^{-/-}$ mice contained 4.42-kb of 5' sequence upstream of the transcriptional start site, a neomycin resistance cassette replacing exons 1-8 (which encode the entire extracellular and transmembrane domains), and 1.85-kb of 3' sequence spanning exons 9-10. The linearized vector was electroporated into C57BL/6 ES cells, and targeted clones that were selected in the presence of the antibiotics neomycin and ganciclovir were identified by PCR and Southern blot analysis (inGenious Targeting Laboratory). Targeted ES clones were injected into C57BL/6 mice blastocysts, yielding several lines of chimeric mice that transmitted the disrupted allele through the germline DNA. **b**, **c**, Genomic DNA was isolated from *Scarf1*$^{+/+}$, *Scarf1*$^{+/-}$, and *Scarf1*$^{-/-}$ mice and targeted disruption of the *Scarf1* gene confirmed by PCR and Southern blot analysis.



Supplementary Figure 5. Rate of cell death and expression of apoptotic cell recognition receptors in *Scarf1*$^{-/-}$ cells.

a, **b**, **c**, Splenocytes, B cells, and bone marrow cells were isolated from *Scarf1*$^{+/+}$ and *Scarf1*$^{-/-}$ mice. Cells were incubated at 37°C for 0, 4, 24 or 48 hours. Rate of cell death was determined using live/dead fixable cell stain and analyzed by flow cytometry. Percentage of apoptotic cells was obtained by FlowJo analysis. Bars denote means and s.d. of three independent experiments (n=6 mice). **d**, RNA was obtained from splenic CD8α^{+} DCs isolated from *Scarf1*$^{+/+}$ and *Scarf1*$^{-/-}$ mice and analyzed for expression of apoptotic cell receptors by qPCR. Bars denote means and s.d. of three independent experiments performed with triplicate measurements.