Click here to download Supplemental Text and Figures
DocumentS1.pdf

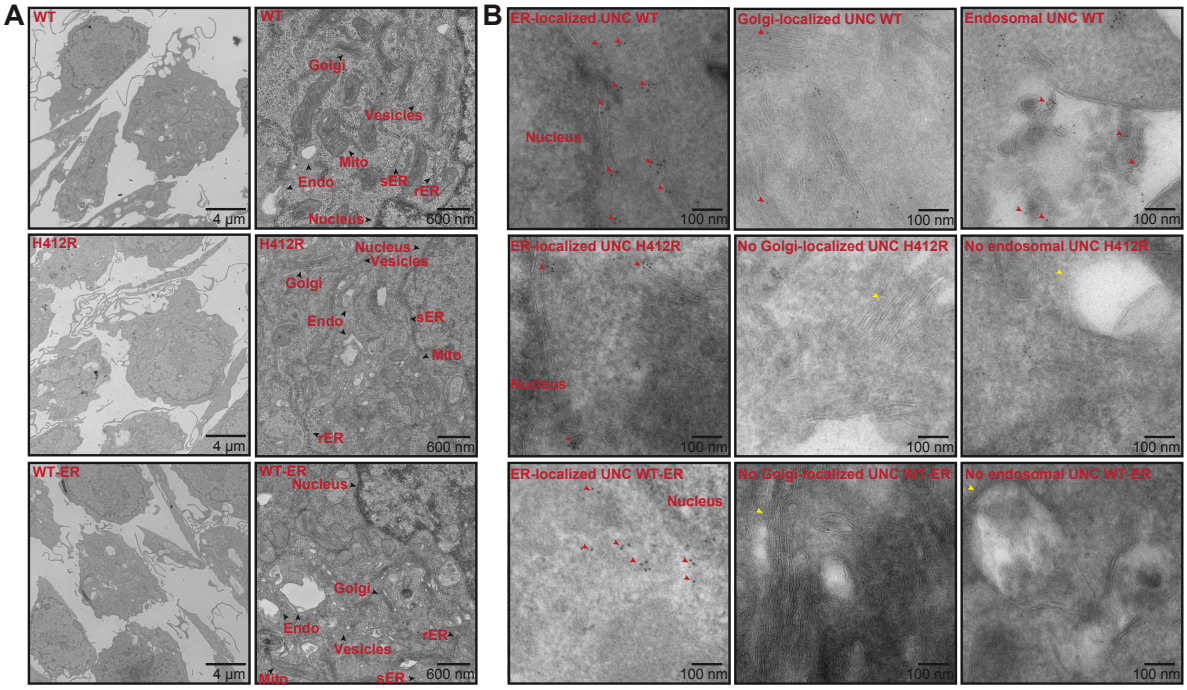

Figure S1. UNC93B1-mCitrine WT, but not UNC93B1-mCitrine H412R or WT-ER populate Golgi and endosomal compartments, related to Figure 1. (A) Morphology of *Unc93b1*$^{-/-}$ iMOs reconstituted with UNC93B1-mCitrine WT, H412R or WT-ER as determined by electron microscopy. Subcellular compartments are indicated. (B) Cryo-EM staining detects wt UNC93B1-mCitrine at ER, Golgi and endosomal membranes, and UNC93B1-mCitrine H412R and WT-ER at ER membranes only. Red arrows mark labeled compartments, yellow arrows mark unlabeled compartments.

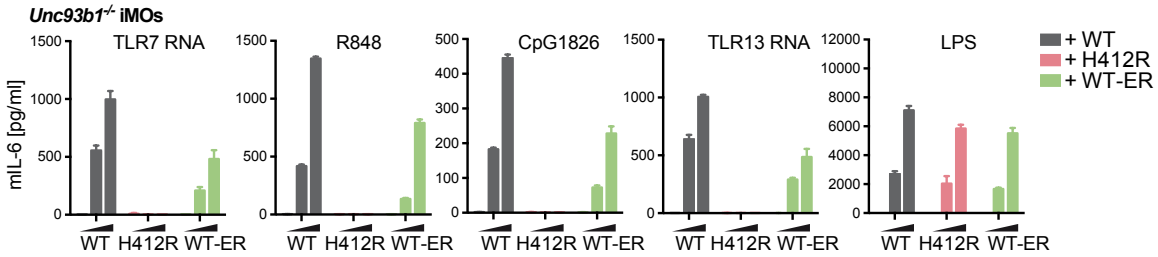

Figure S2. ER-retained UNC93B1 is sufficient to restore TLR-induced proinflammatory cytokine secretion in *Unc93b1*$^{-/-}$ macrophages sorted for physiologic expression levels of UNC93B1, related to Figure 3. *Unc93b1*$^{-/-}$ iMOs reconstituted with the indicated versions of UNC93B1-mCitrine were sorted for expression levels comparable to endogenous UNC93B1, stimulated for 14 h with increasing concentrations of ligands for TLR7 (TLR7-stimulatory RNA and R848), TLR9 (CpG1826), TLR13 (TLR13-stimulatory RNA) and TLR4 (LPS) and analyzed for IL-6 secretion. Shown are combined data from three independent experiments (mean + SEM).

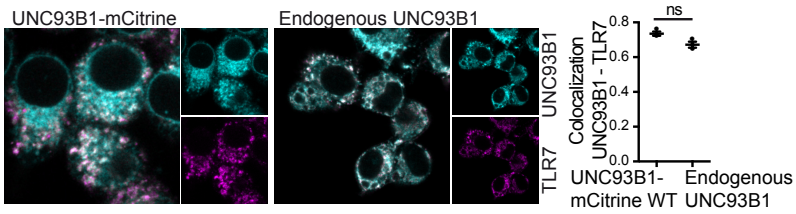

Figure S3. Endogenous UNC93B1 expression pattern and partial colocalization with TLR7 is resembled by UNC93B1-mCitrine, related to Figure 5. *Unc93b1*$^{-/-}$ iMOs expressing UNC93B1-mCitrine WT and WT iMOs expressing endogenous UNC93B1 were fixed, permeabilized and stained for UNC93B1 using the purified UNC-C antibody as described in Brinkmann et al., 2007 and TLR7 using the A94B10 anti-TLR7 antibody. The graph represents the overall correlation between UNC93B1 and TLR7 with each data point representing the mean of one independent experiment. Shown are data from three independent experiments (mean + SEM). Shapiro-Wilk normality test showed normal distribution of data, paired t-test revealed no significance (p=0.145).

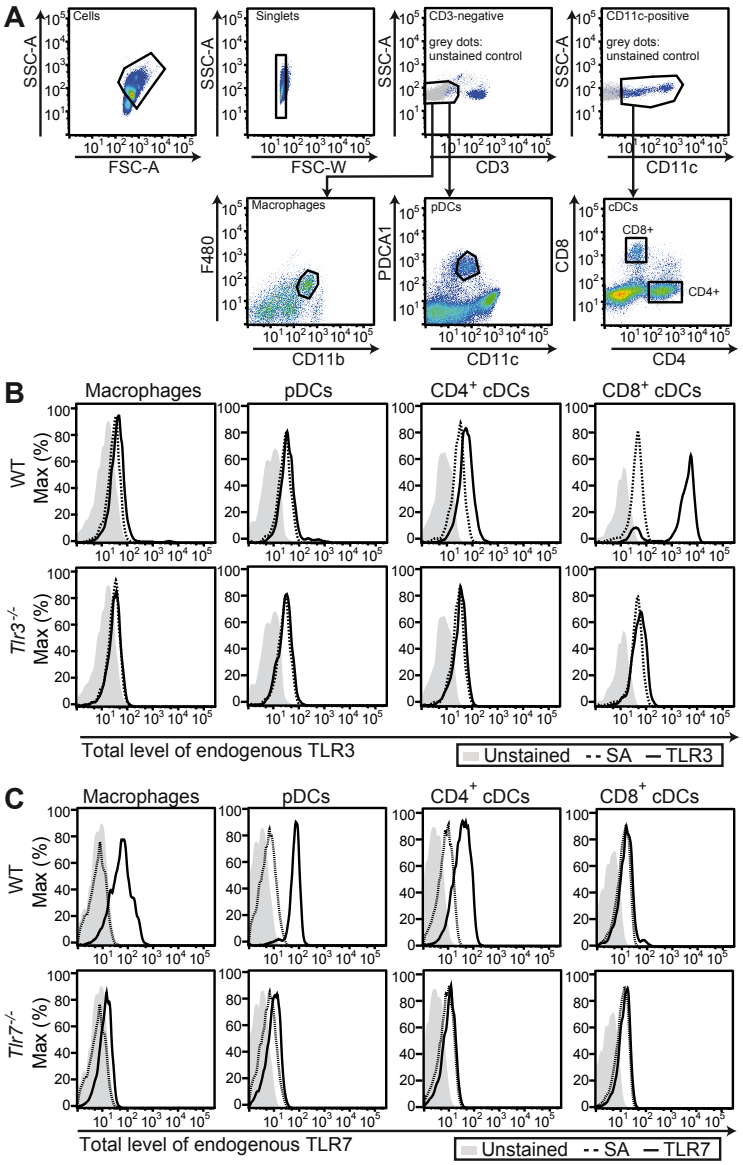

Figure S4. Gating strategy to dissect different splenic immune cell types, related to Figure 7. (A) Splenocytes were isolated from C57BL/6 WT, *Unc93b1*$^{-/-}$, *Tlr3*$^{-/-}$ and *Tlr7*$^{-/-}$ mice, selected for CD19-negative cells using magnetic bead separation and stained for various cell surface markers. From the CD3-negative cell fraction, macrophages were identified as CD11b-positive, F4/80-positive cells, pDCs as CD11c-positive, PDCA1-positive cells, and cDCs as CD11c-positive cells. cDCs were further subdivided into CD4-positive and CD8-positive cDCs. (B) Upon permeabilization, cells were stained with biotinyated anti-TLR3 antibody (clone PAT3) (Murakami et al., 2014) or (C) biotinylated anti-TLR7 antibody (clone A94B10) (Kanno et al., 2013) coupled to fluorescently labeled streptavidin (SA) and analyzed by flow cytometry. Dotted histograms represent negative controls stained with fluorescently labeled SA alone. Data are representative of two independent experiments.

Click here to access/download

Supplemental Videos and Spreadsheets

TableS1.xlsx

Supplemental Information

Document S1. Figures S1-S4

Table S1. Quantitative proteomics of mouse UNC93B1 WT, H412R and WT-ER immunoprecipitates, Related to Figure 2

Inventory of Supplemental Information

Main text Figure 1 is supported by Supplemental Figure S1.

Main text Figure 2 is supported by Supplemental Table S1.

Main text Figure 3 is supported by Supplemental Figure S2.

Main text Figure 5 is supported by Supplemental Figure S3.

Main text Figure 7 is supported by Supplemental Figure S4.