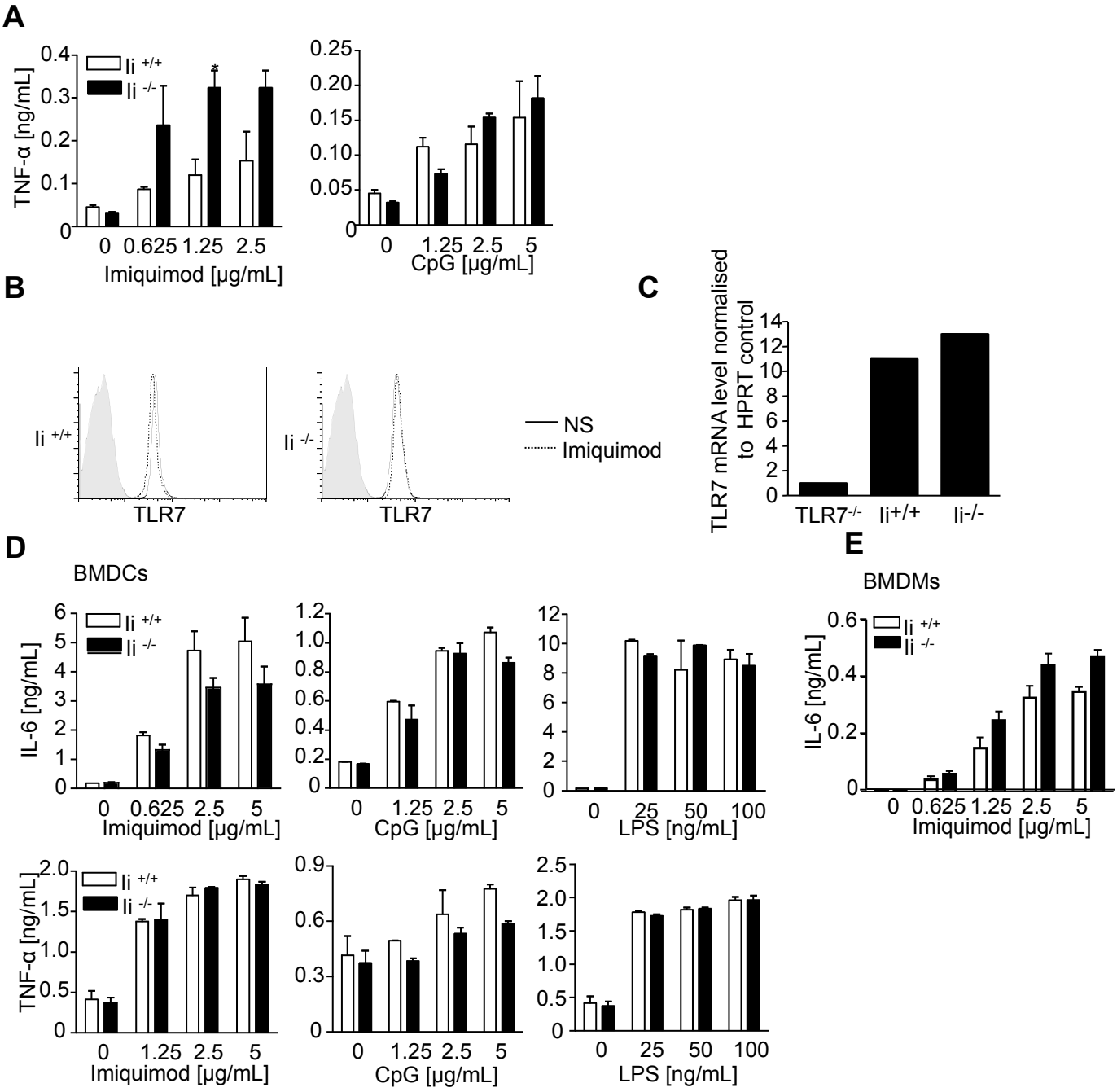

Fig S1. TLR7 stimulation increases TNFα production specifically in Ii chain deficient B cells. (A) $Ii^{+/+}$ or $Ii^{-/-}$ mature splenic B cells were stimulated with different TLRs ligands for 12h and secretion of TNF-α was measured by ELISA. (n=8, graphs show mean ± SEM, *p <0.05). (**B**) $Ii^{+/+}$ or $Ii^{-/-}$ mouse splenic B cells were treated without (black lines) or with (dashed lines) 5 μg/ml of imiquimod for 16h and stained for TLR7 expression using fluorescent antibody. Grey histograms represent staining of B cells with the antibody isotype control. One experiment representative out of two is shown (**C**) TLR7 mRNA level was monitored by quantitative real-time PCR from total RNA extracted from $Ii^{+/+}$, $Ii^{-/-}$ and $TLR7^{-/-}$ resting mature splenic B cells. One experiment representative out of two is shown. IL-6 and TNF-α production from Ii wt or Ii-deficient BMDCs (**D**) and IL-6 production from Ii wt or Ii-deficient BMDMs (**E**) incubated with different TLRs ligands. Cytokine production was measured by ELISA. (n=3, graphs show mean ± SEM).

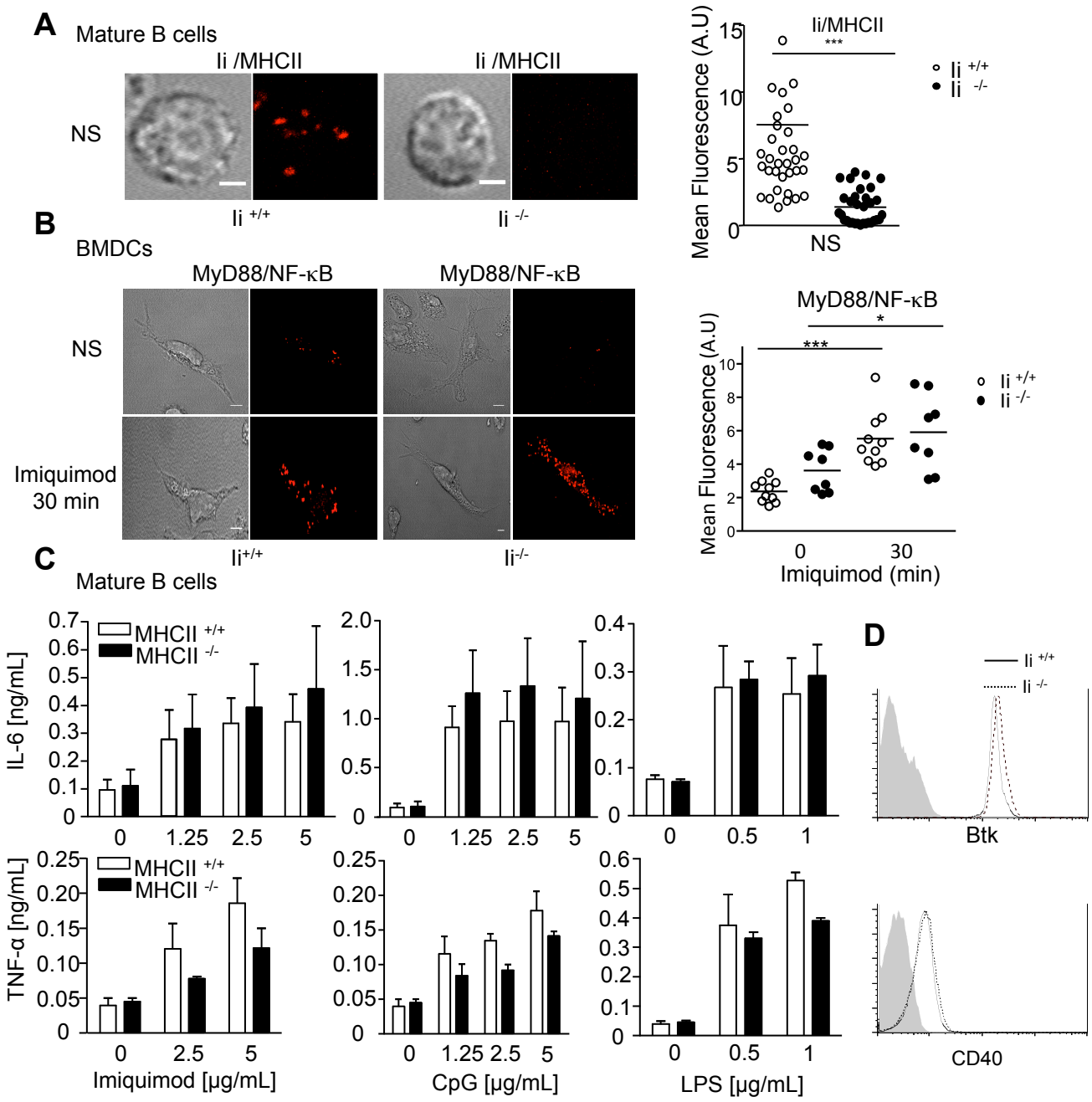

Fig S2. TLR7 stimulation leads to similar cytokine production in MHCII wt and deficient B cells. (**A**) Detection of Ii and MHCII interaction using PLA *in situ* with anti MHCII and anti Ii specific mAbs in resting splenic mature B cells. PLA signals are shown in red. One representative experiment out of three is shown. Quantification of mean fluorescence using Image J software (n=30-35 cells, ***p< 0.001). (**B**) Detection of Myd88 and NF-κB interaction using PLA *in situ* with specific mAbs in wt- and Ii- deficient BMDCs stimulated or not with imiquimod for 30 minutes. PLA signals are shown in red. One representative experiment out of three is shown. Quantification of mean fluorescence using Image J software (n=10 cells, *p< 0.05, ***p< 0.001). (**C**) IL-6 and TNF-α production by $MHCII^{+/+}$ or $MHCII^{-/-}$ mature splenic B cells incubated with different TLRs ligands. Cytokine production was measured by ELISA (n=3, graphs show mean ± SEM). (**D**) $Ii^{+/+}$ (black lines) or $Ii^{-/-}$ (dashed lines) mouse splenic B cells were stained for Btk or CD40 expression using fluorescent antibodies. Grey histograms represent staining of B cells with the antibody isotype control. NS: not stimulated.

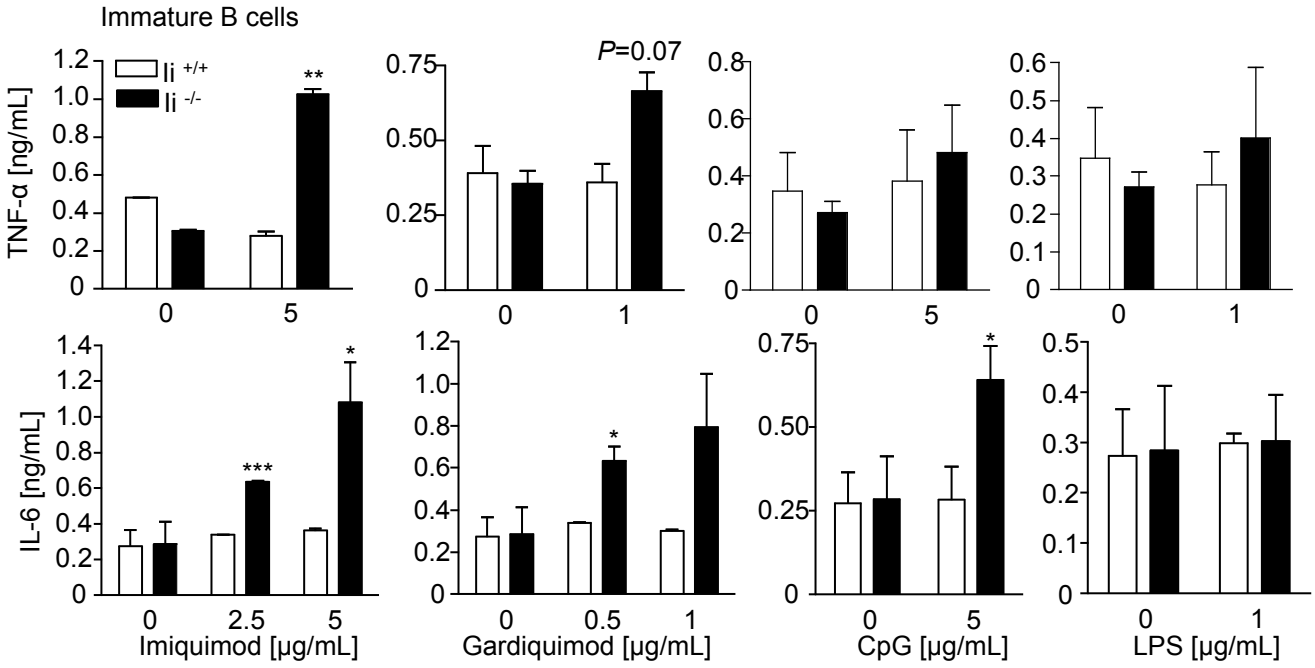

Fig S3. TLR7 signaling is increased in Ii chain deficient immature bone marrow B cells. Immature bone marrow B cells from $Ii^{-/-}$ or $Ii^{+/+}$ were activated with different TLRs ligands and cytokine secretion was measured by ELISA. (n=3, graphs show mean ± SEM, *p< 0.05,** p< 0.01).

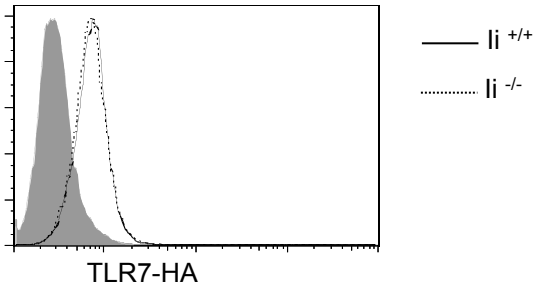

Fig S4. $Ii^{+/+}$ and $Ii^{-/-}$ B cells display similar transfected TLR7-HA expression. $Ii^{+/+}$ or $Ii^{-/-}$ mature splenic B cells were transfected with a cDNA coding for FL TLR7 tagged HA. TLR7 expression was assessed 48h after transfection by flow cytometry with an anti-HA specific antibody (black or dotted lines) or with the antibody isotype control (grey histograms). One experiment out of three is shown (mean of three experiments, expression of TLR7 in $Ii^{+/+}$: 27% and in $Ii^{-/-}$: 32%).

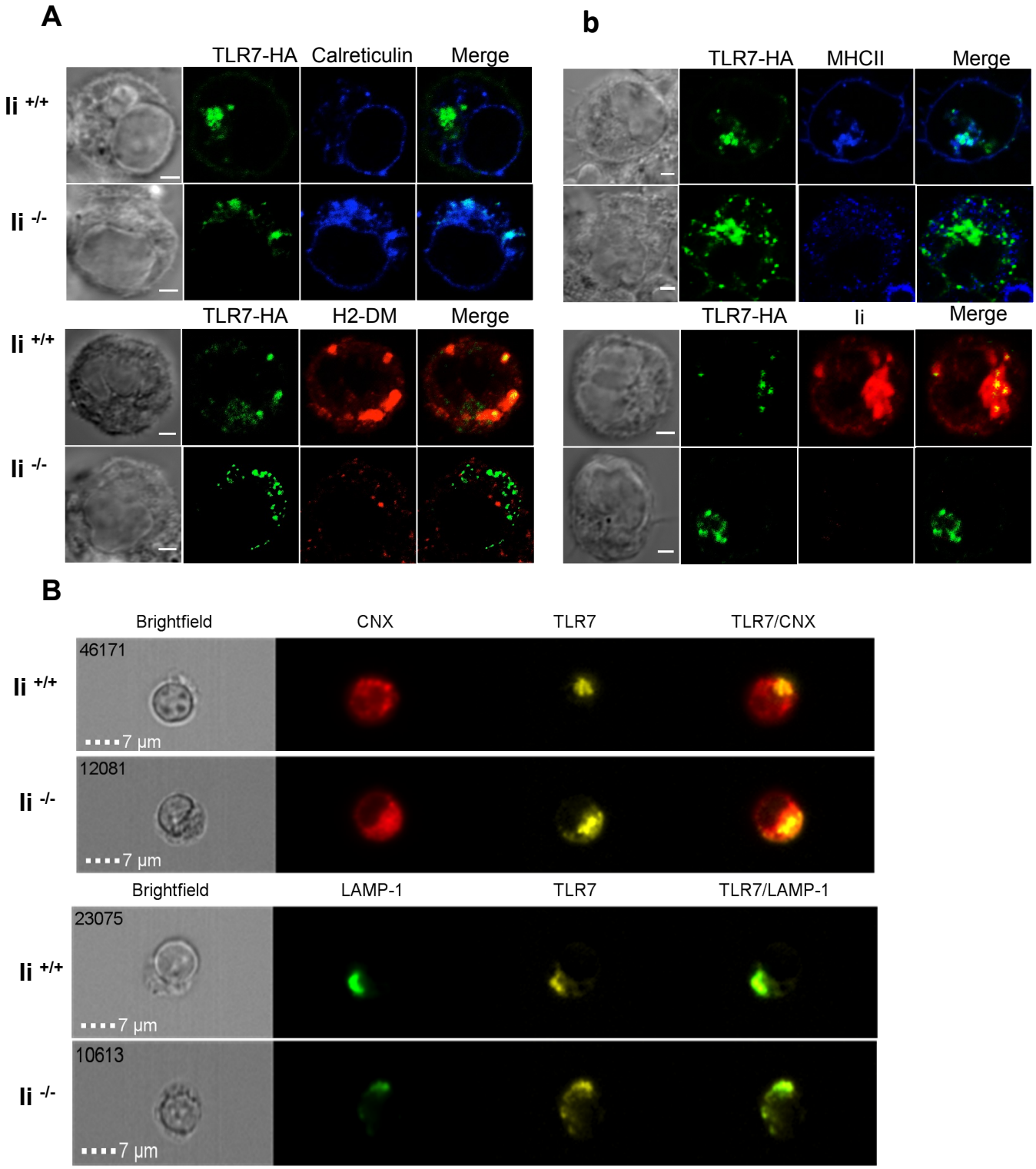

Fig S5. Ii regulates TLR7 trafficking in B cells. (**A**) Immunofluorescence microscopy of TLR7-stimulated $Ii^{+/+}$ or $Ii^{-/-}$ splenic B cells transfected with FL TLR7 tagged HA and stained for TLR7 (green), calreticulin (blue), H2-DM (red), MHCII (blue) and Ii (red). One experiment representative out of three is shown. (**B**) Representative images of calnexin (CNX), LAMP-1, and TLR7 intracellular staining in TLR7-stimulated B cells from Ii+/+ or $Ii^{-/-}$ acquired by imaging flow cytometry.One experiment representative out of three is shown.

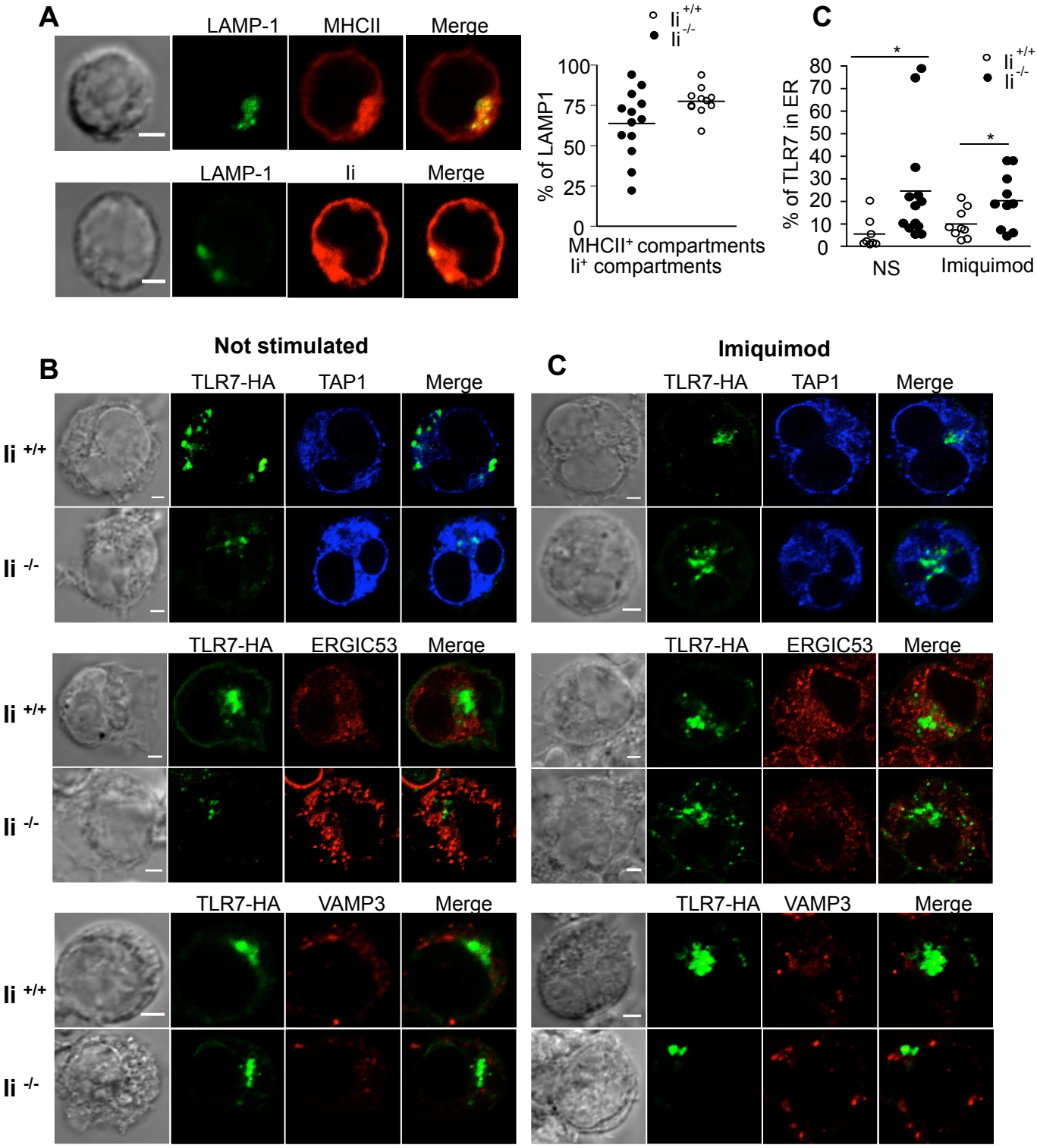

Fig S6. TLR7 is expressed in ER in Ii deficient B cells. (**A**) Immunofluorescence microscopy of resting $Ii^{+/+}$ splenic B cells stained for LAMP1 (green), MHCII (red) and Ii (red), left panel. One experiment representative out of three is shown (left panel). (**B**) Immunofluorescence microscopy of resting or TLR7-stimulated $Ii^{+/+}$ or $Ii^{-/-}$ splenic B cells transfected with FL TLR7 tagged HA and stained for TLR7 (green), TAP1 (blue), ERGIC 53 (red) and VAMP3 (red). One experiment representative out of three is shown. (**A, C**) Quantification of colocalization using Image J software (n=10 cells, *p< 0.05), right

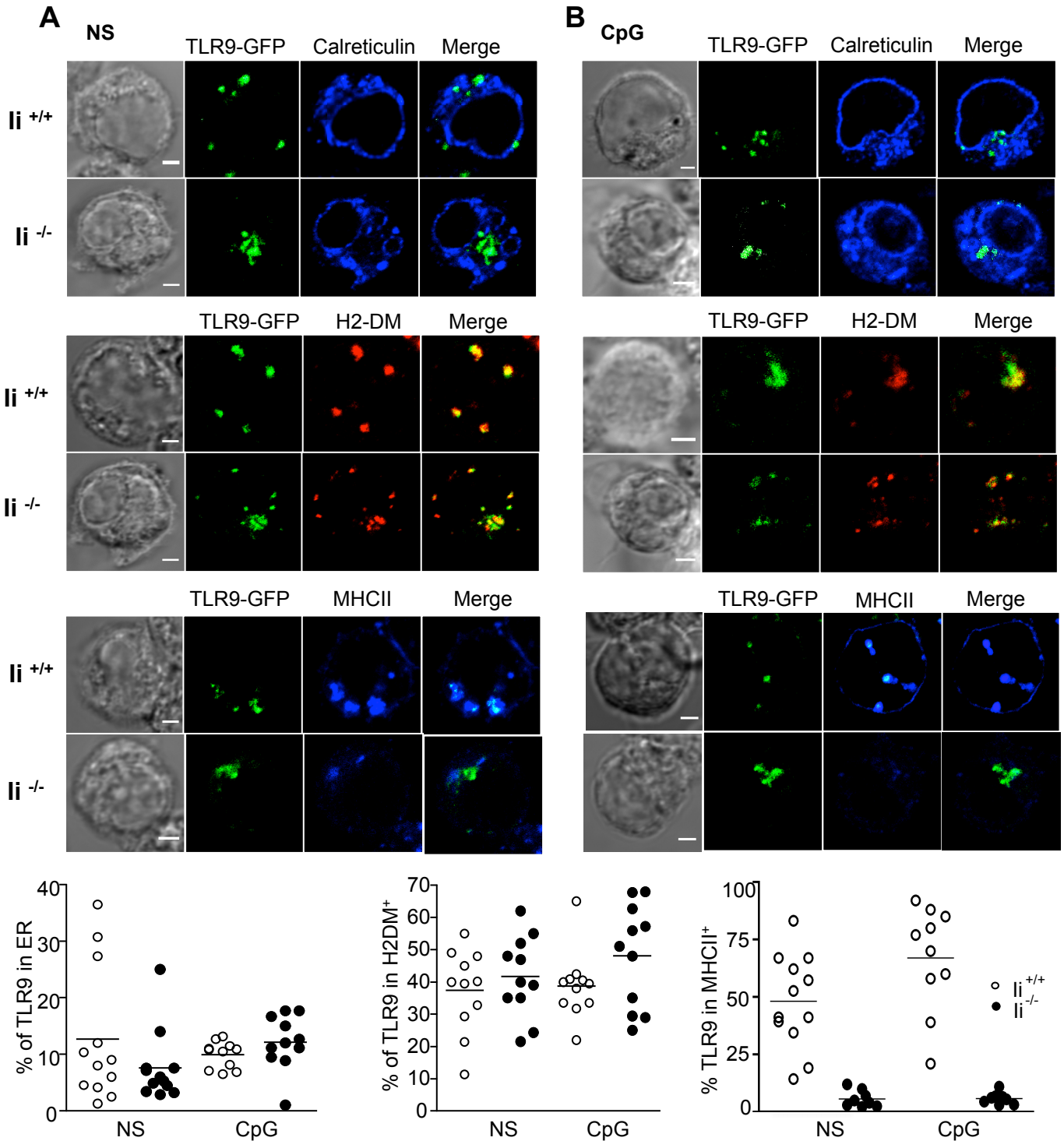

Fig S7. Ii does not regulate TLR9 trafficking in B cells. Immunofluorescence microscopy of resting (**A**) or TLR9-stimulated (**B**) $Ii^{+/+}$ or $Ii^{-/-}$ splenic B cells transfected with FL TLR9 tagged GFP and stained for TLR9 (green), calreticulin (blue), H2-DM (red) and MHCII (blue). One experiment representative out of three is shown. (**C**) Quantification of colocalization using Image J software (n=10), lower panel.

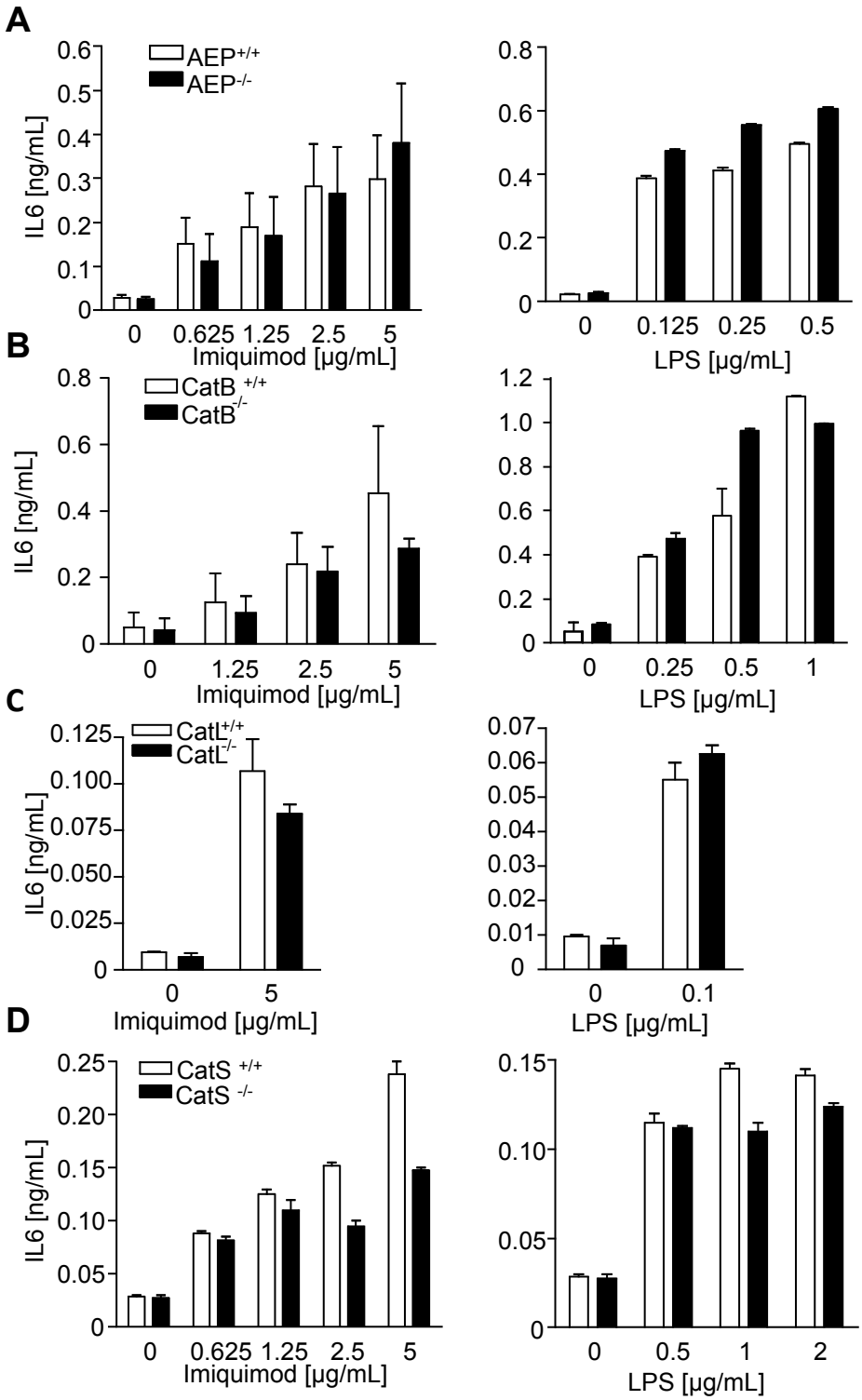

Fig S8. Cytokine production in wild type and different cysteine proteases deficient B cells upon TLR sensing. Wild type B cells or B cells deficient for AEP (**A**), cathepsin B (**B**), cathepsin L (**C**) and Cathepsin S (**D**) B cells were stimulated with imiquimod or LPS for 12h and secretion of IL-6 was measured by ELISA. (n=3, graphs show mean ± SEM).