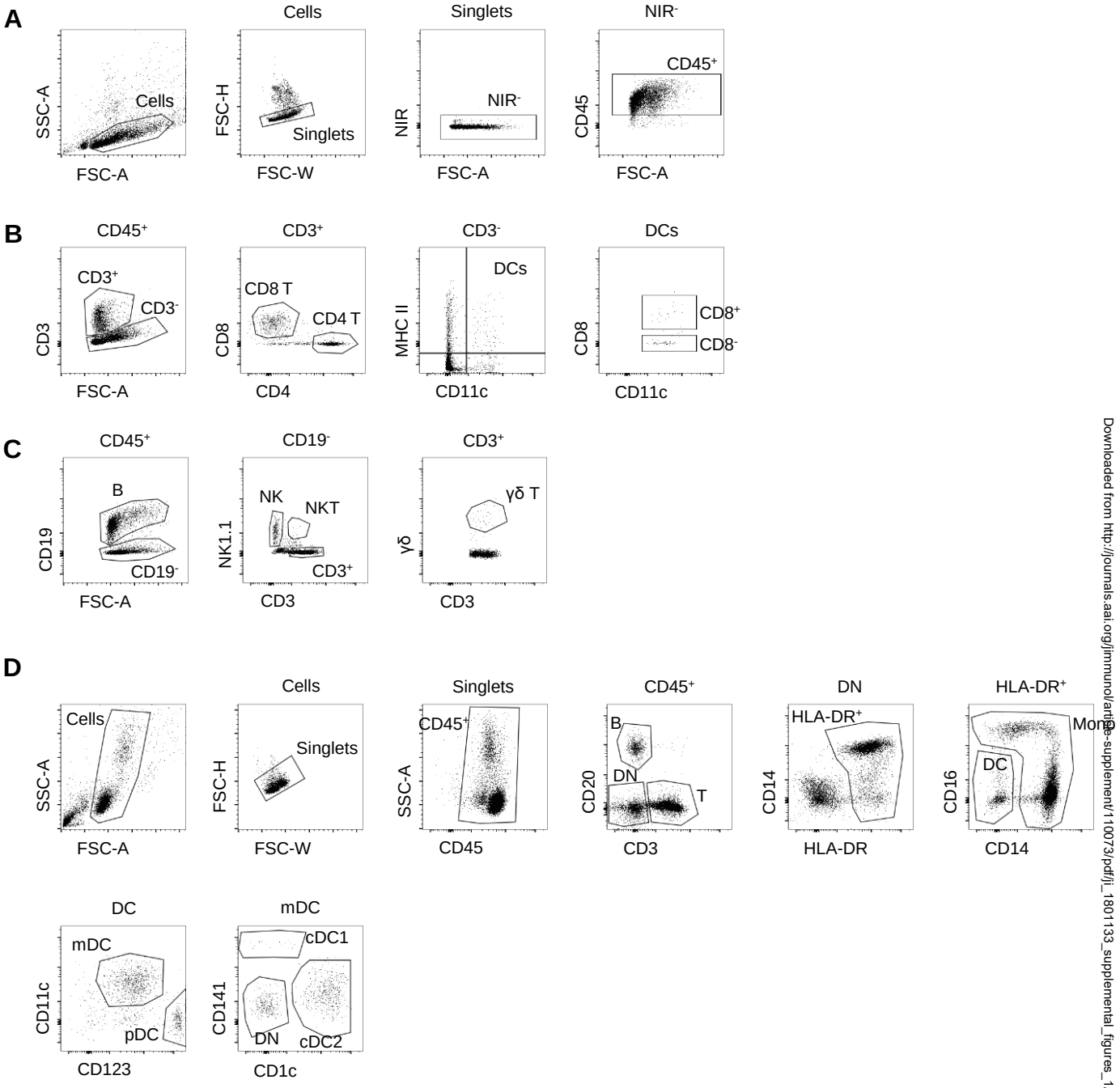
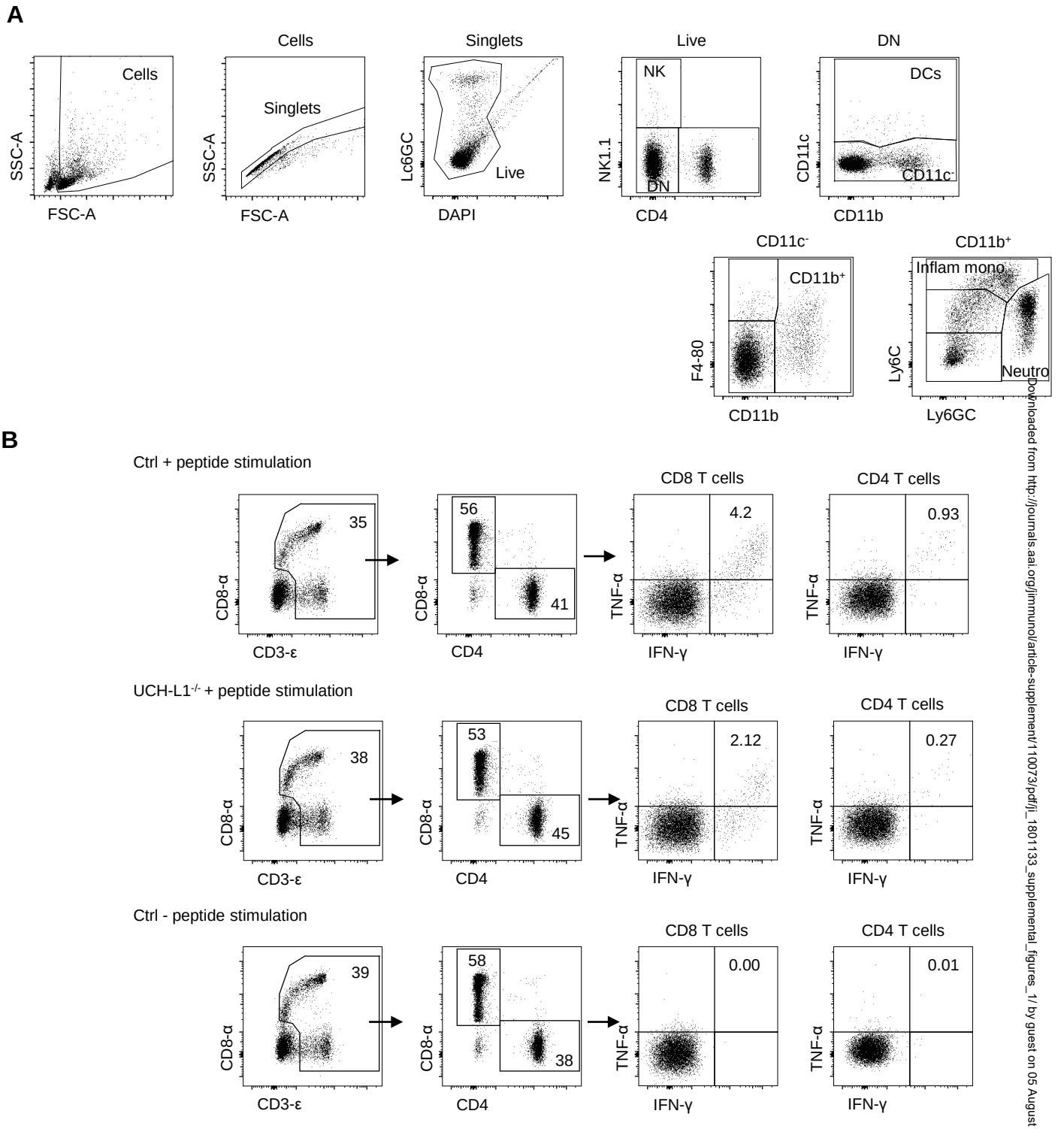
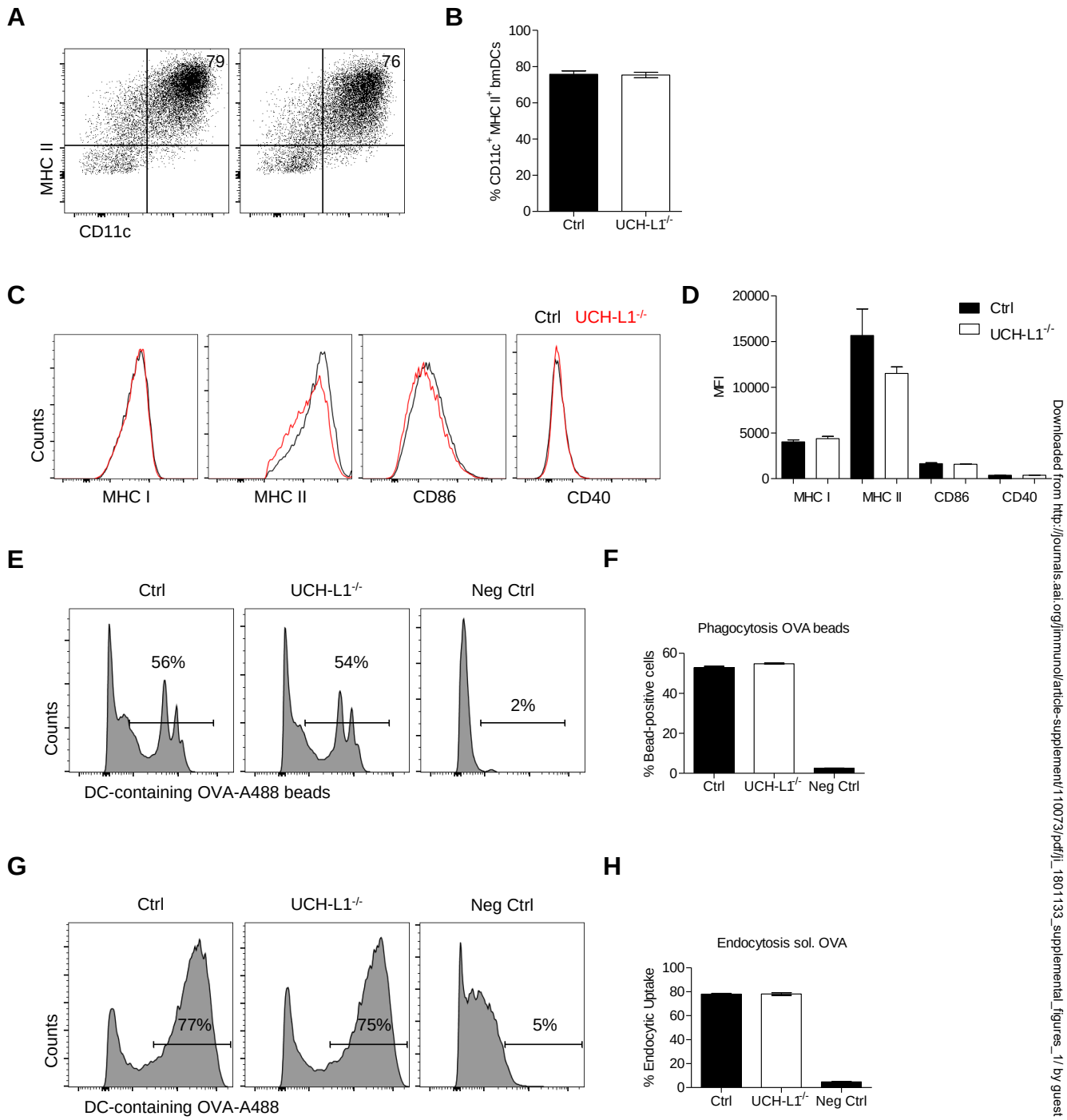


Supplemental figure 1: Immune cell population sorting from mouse spleen and human PBMCs for UCH-L1 mRNA expression analysis.

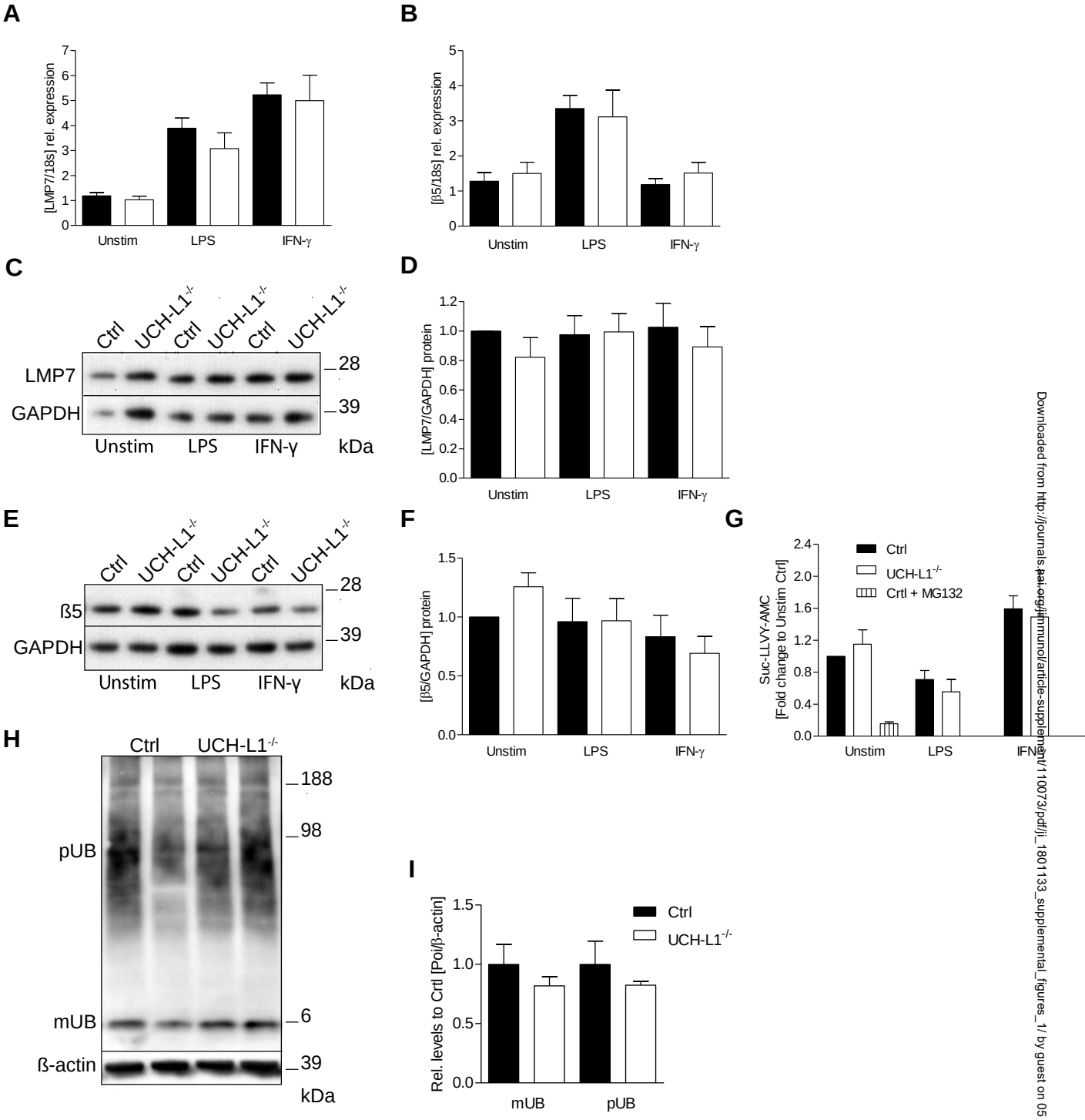
A: Mouse spleen cells are gated on forward scatter area (FSC-A)/ side scatter area (SSC-A) to gate cells, FSC-W (width) /FSC-H (height) to gate singlets, NIR$^-$ to gate live cells, and FSC-A/CD45$^+$ to gate haematopoetic cells. **B:** CD8$^+$ and CD4$^+$ T cells are gated from CD45$^+$/CD3$^+$ gates, while CD3$^-$ cells are further gated with CD11c and MHC II to define DCs with gated CD8$^+$ and CD8$^-$ subsets. **C:** B cells are gated from CD45$^+$/CD19$^+$ gates. CD19$^-$ cells are further gated to define CD3$^-$/NK1.1$^+$ NK cells, and CD3$^+$/NK1.1$^+$ NKT cells. CD3$^+$ cells are further gated γδ$^+$ to define γδ T cells. **D:** Human PBMCs are gated on FSC-A/SSC-A to gate cells, FSC-W/FSC-H to gate singlets, CD45+/SSC-A to gate haematopoetic cells. T and B cells are gated from CD3$^+$/CD20$^-$ and CD3$^-$/CD20$^+$ respectively. Double negative (DN) cells are gated as HLA-DR$^+$ cells and DCs and monocytes are further gated as CD14$^+$/CD16$^-$ and CD14$^+$/CD16^{hi}, respectively. DC subsets are defined as CD123^{hi}/CD11c$^-$ pDCs, CD123$^+$/CD11c$^+$ mDCs. mDCs are further defined as CD1c$^+$/CD141^{înter} cDC2 subset, CD1c$^-$/CD141^{hi} cDC1 subset, and CD1c$^-$/CD141$^-$ DN subset.



Supplemental figure 2: Gating strategies to identify cell populations and intracellular cytokine staining in spleen post listeria infection. A: Sample data from control spleen following 3 day infection with LmEGD. Cells are gated on forward scatter area (FSC-A)/ side scatter area (SSC-A), FSC-A (area) /FSC-H (height) to gate singlets, and DAPI negative to identify live cells. $CD4^-$, $NK1.1^+$ cells are gated as NK cells. $CD4^-$, $NK1.1^-$ double negative (DN) cells are further gated as $CD11c^+$, $CD11b^{+/-}$ DCs. $CD11b^+$ cells are further gated as $Ly6GC^{hi}$, $Ly6C^+$ neutrophils and $Ly6GC^+$, $Ly6C^{hi}$ inflammatory monocytes. **B:** Intracellular staining of IFN-γ and TNF-α cytokine secreting CD8 and CD4 T cells on day 9 post listeria infection. Example dotplots of intracellular staining of splenic T cells from control (Ctrl) and UCH-L1-deficient ($UCH\text{-}L1^{-/-}$) littermate mice following stimulation with MHC I- and MHC II-restricted peptides (+ peptide stimulation) or unstimulated (Ctrl – peptide stimulation) are shown. T cells are gated on CD3-ε and CD8-α and further gated based on CD4 and CD8-α markers. The percentage IFN-γ and TNF-α double positive CD8-α and CD4 T cell subsets are indicated.



Supplemental figure 3: Phenotype of control and UCH-L1-deficient bmDCs. A: Surface expression of CD11c and MHC II on bmDCs from control (Ctrl) and UCH-L1-deficient (UCH-L1$^{-/-}$) littermates **B:** Quantification of frequency of CD11c^{+}MHCII^{+} DCs, mean +/- SEM, n= 10. **C:** Surface staining of MHC I, MHC II and co stimulatory markers on naïve bmDCs. **D:** Mean fluorescence intensity (MFI) of surface staining is quantified, mean +/- SEM, n=3 animal bmDC cultures per group. **E:** Phagocytic uptake of fluorescent OVA coated 3 µM beads is measured in control (Ctrl) and UCH-L1-deficient (UCH-L1$^{-/-}$) littermate bmDCs by flow cytometry following a 5 hour incubation with OVA-A488 adsorbed beads at 37^{o}C. Cells incubated with OVA-A488 adsorbed beads at 4^{o}C serve as negative controls. Plots are representative of similar results obtained in 2 separate experiments **F:** Quantification of frequencies of uptake, mean +/- SEM, n= 2. **G:** Receptor-mediated endocytosis is measured in control and UCH-L1-deficient littermate bmDCs by flow cytometry following 20 min incubation with fluorescent soluble OVA-A488 at 37^{o}C. Cells incubated with OVA-A488 at 4^{o}C serve as negative controls. Plots are representative of similar results obtained in 2 separate experiments. **H:** Quantification of frequencies of uptake, mean +/- SEM, n= 3 animal bmDC cultures per group.



Supplemental figure 4: Ubiquitin Proteasome System is equivalent in control and UCH-L1-deficient littermate bmDCs. A, B: qPCR for LMP7 and β5 subunit transcripts in unstimulated (Unstim), LPS or IFN-γ 24h stimulated control (Ctrl) and UCH-L1-deficient (UCH-L1$^{-/-}$) bmDCs, n=6-9 bmDC cultures per group. **C:** Western blot of LMP7 in unstimulated, LPS or IFN-γ 24h stimulated control and UCH-L1-deficient bmDCs. Blot is representative of similar data obtained in 3 separate experiments **D:** Quantification of LMP7 protein with GAPDH as housekeeper control. Values are calculated relative to unstimulated controls +/- SEM, n= 4 bmDC cultures per group. **E, F:** As in C, D for β5. **G:** Chymotrypsin-like activity as measured by cleavage of the fluorogenic substrate Suc-LLVY-AMC in unstimulated, LPS or IFN-γ 24h stimulated control and UCH-L1$^{-/-}$ bmDCs. Control plus the proteasome inhibitor MG132 (Ctrl + MG132) serves as negative control for the assay. Values are calculated relative to unstimulated controls +/- SEM, n= 4 animal bmDC cultures per group. **H:** Western blot of polyubiquitin (pUb) and monoubiquitin (mUb) levels in control and UCH-L1-deficient littermate bmDCs. Blot is representative of similar data obtained in 2 separate experiments **I:** Quantification of pUb or mUb protein of interest (Poi) with β-actin as housekeeper control. Values are calculated relative to unstimulated controls +/- SEM, n= 4 animal bmDC cultures per group.