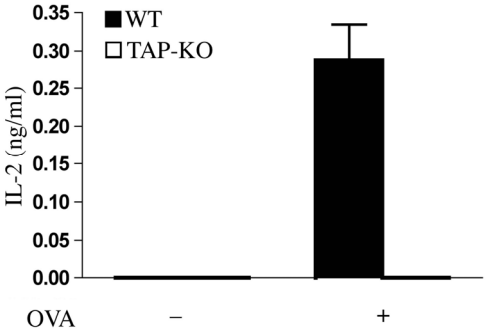
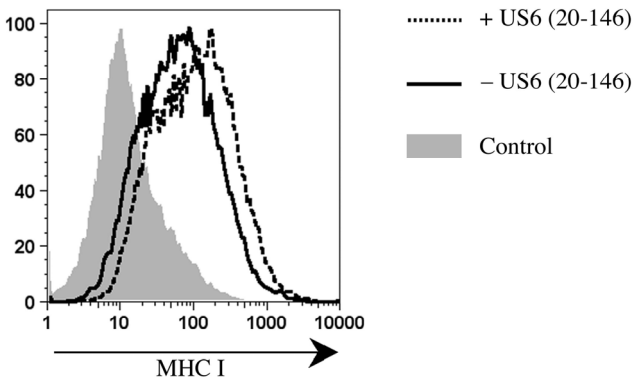
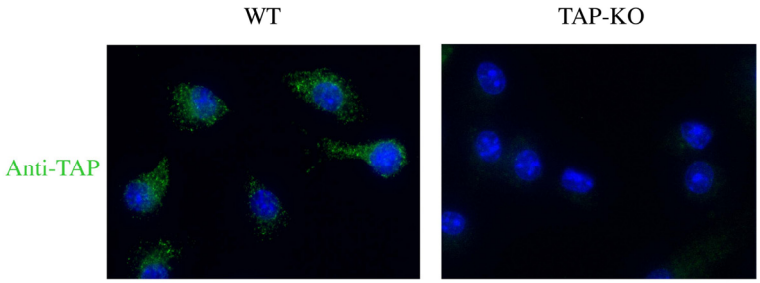
Supplementary Figures



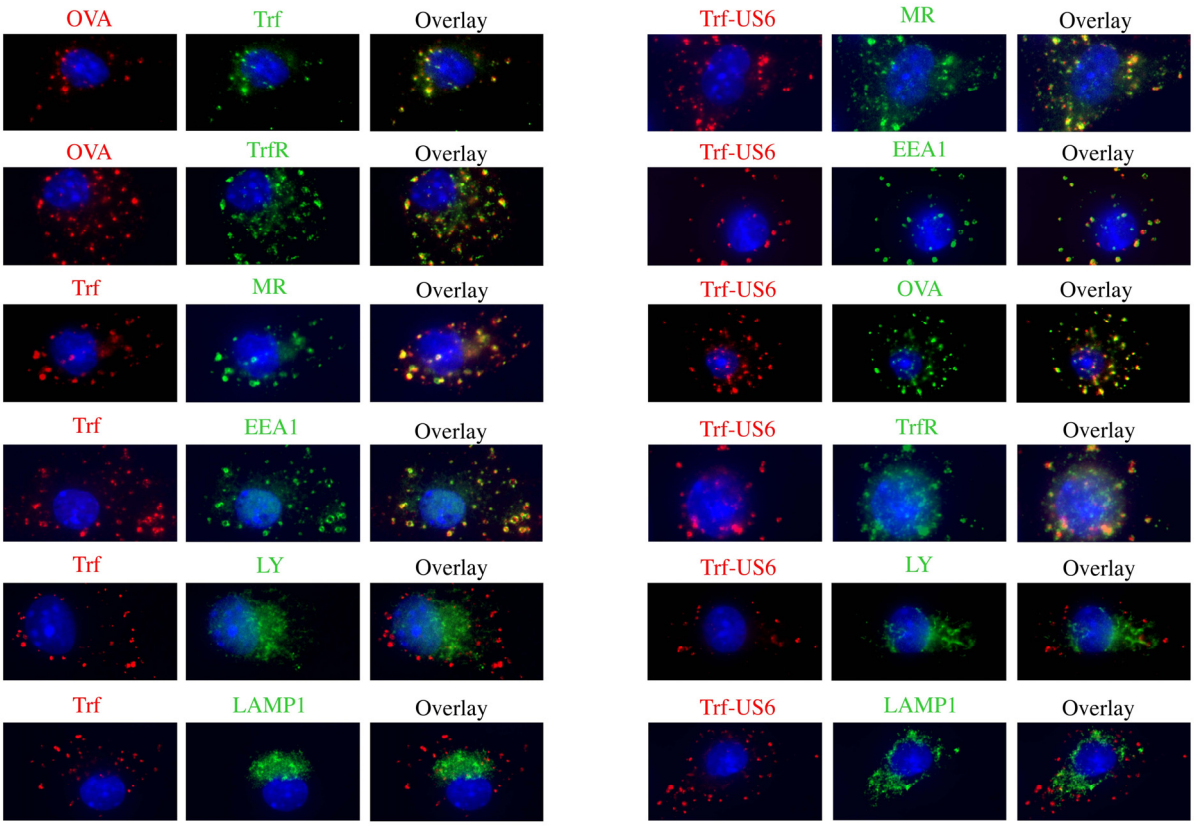
Supplementary Figure 1. Cross-presentation of OVA is abolished in DCs from TAP-deficient mice. IL-2 ELISA on the supernatant of OT-I cells cultured with OVA-treated DC from wildtype or TAP-deficient mice. WT: wildtype; error bars, mean + s.e.m.



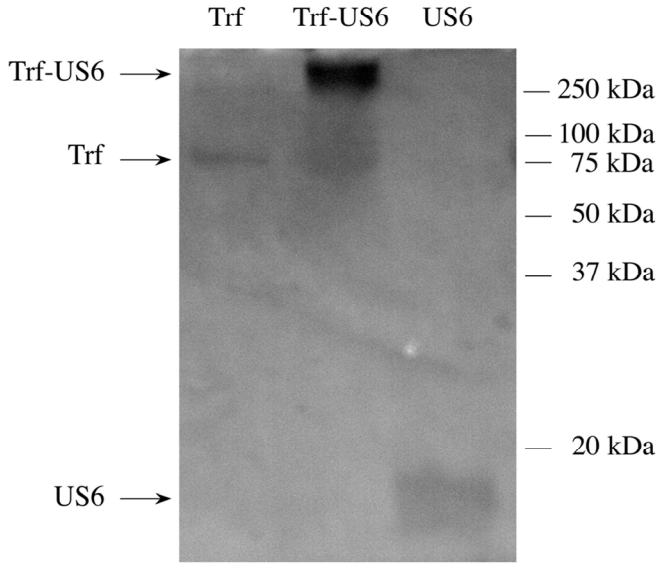
Supplementary Figure 2. MHC I expression is not downregulated after incubation with US6 for 2 h. DCs were incubated with 1 mg/ml US6 (20-146). After 2h, surface expression of MHC I was determined by flow cytometry.



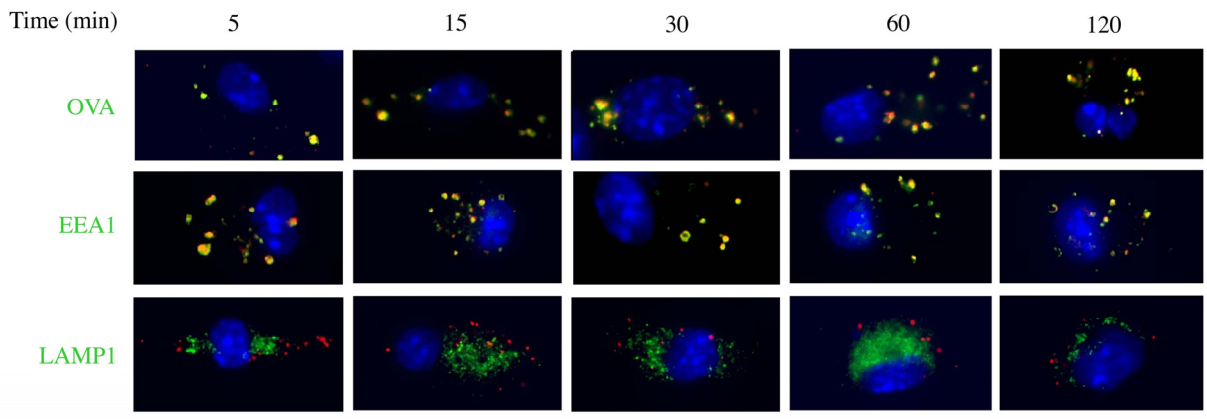
Supplementary Figure 3. Specificity control of TAP-staining. DCs from WT and TAP-KO mice were stained with anti-TAP antibody and analyzed by fluorescence microscopy. Nuclei were counterstained with DAPI (blue).



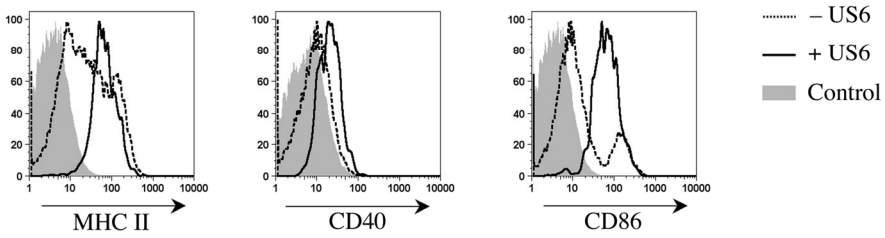
Supplementary Figure 4. Trf-US6 is routed into an early endosomal compartment. This supplemental figure adds the separate channel images to **Fig. 2a, b**, which contained the overlay channel pictures only. Nuclei were counterstained with DAPI (blue).



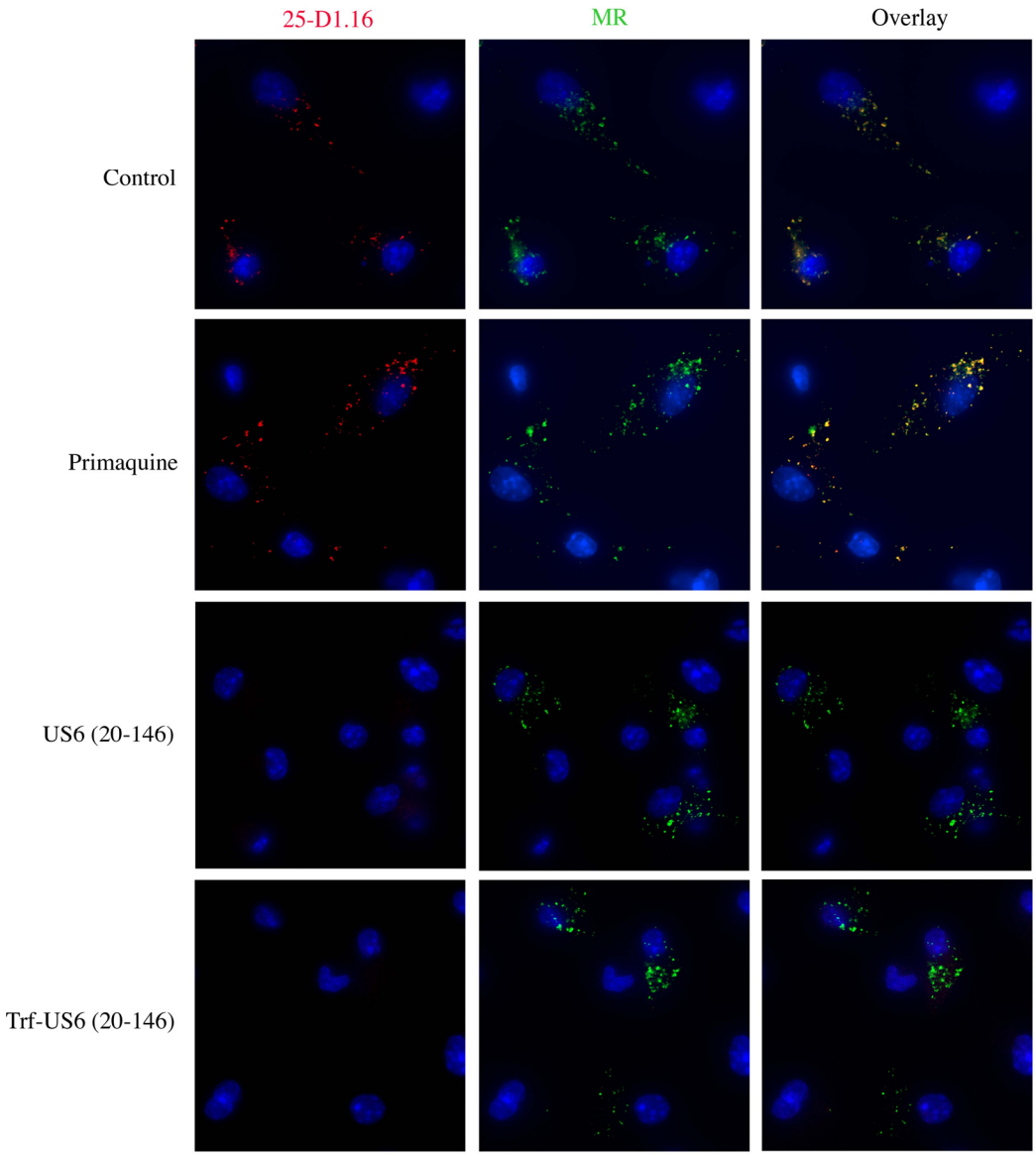
Supplementary Figure 5. Coupling of Trf to US6. SDS-PAGE of Trf, Trf-bound US6 and free US6, followed by Coomassie Blue staining.



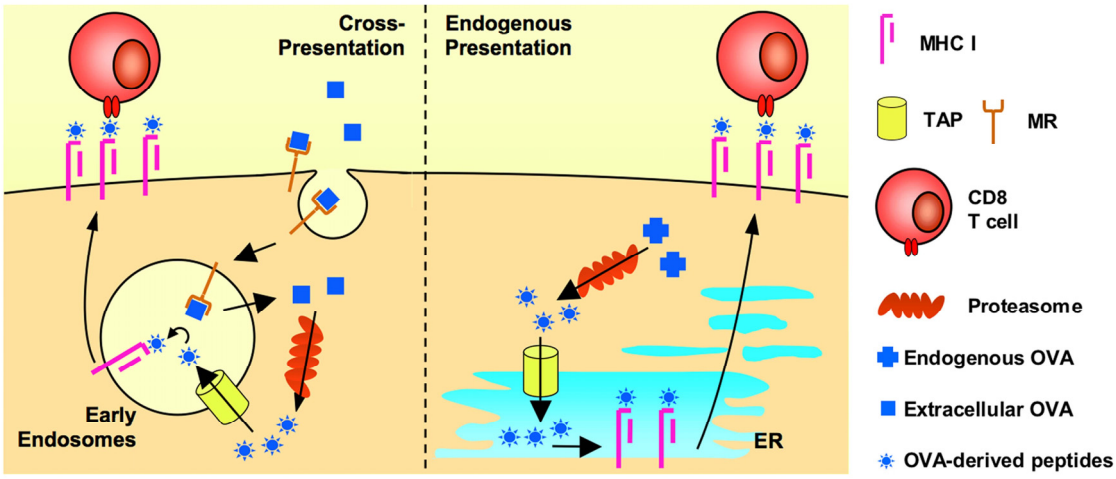
Supplementary Figure 6. Localization of Trf-US6 in early endosomes is stable. After incubation of DCs with fluorochrome-labeled Trf-US6 (red) and, in the top row, with OVA (green), cells were chased with medium for the indicated intervals. The middle row shows co-staining with the early endosomal marker EEA1 (green), the bottom row with the late endosomal-lysosomal marker LAMP1 (green). Nuclei were counterstained with DAPI (blue).



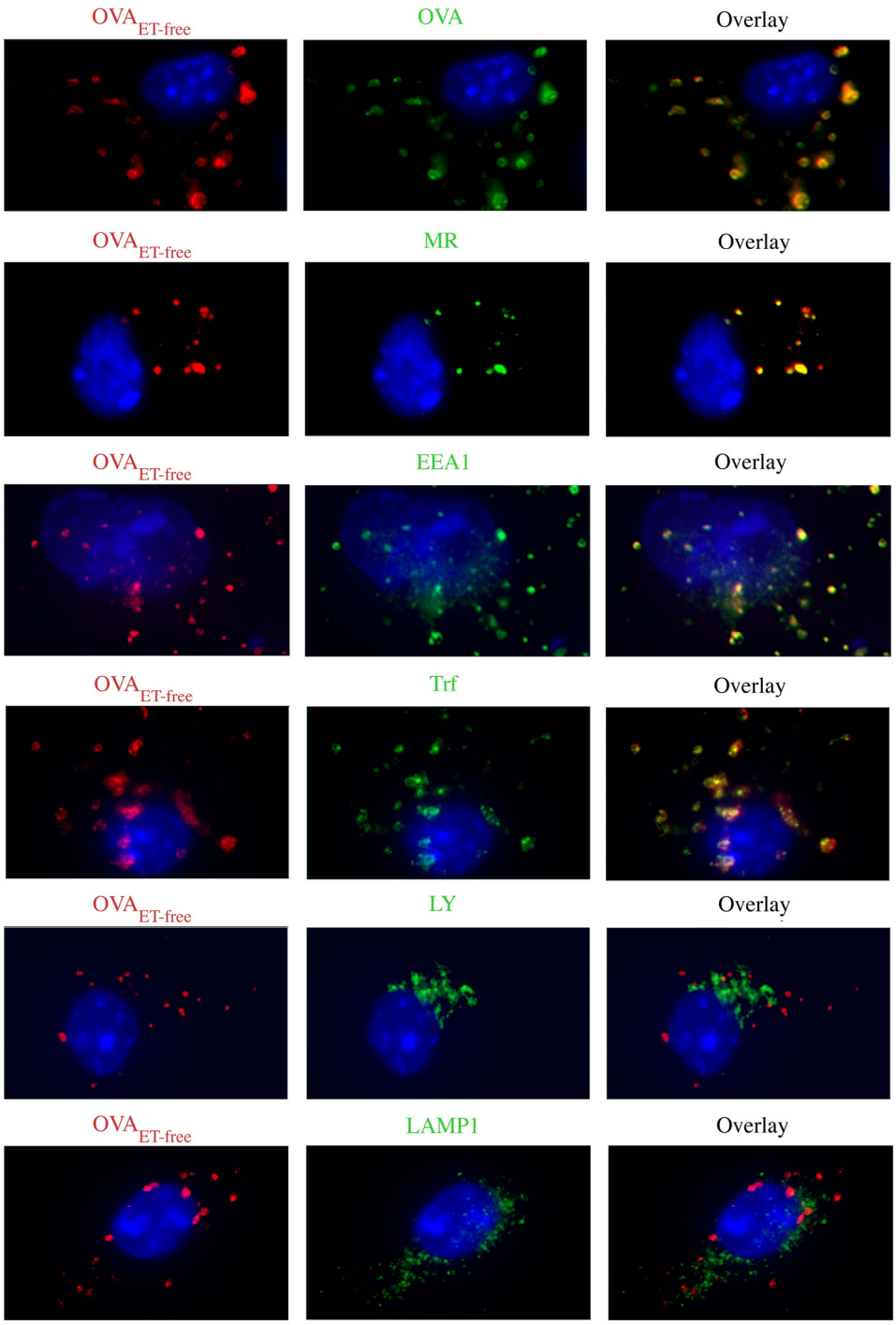
Supplementary Figure 7. Incubation with recombinant US6 upregulates co-stimulatory molecules. Flow-cytometry of DCs incubated with 1 mg/ml US6 (20-125). After 24 h, surface expression of MHC II, CD40, and CD86 were determined.



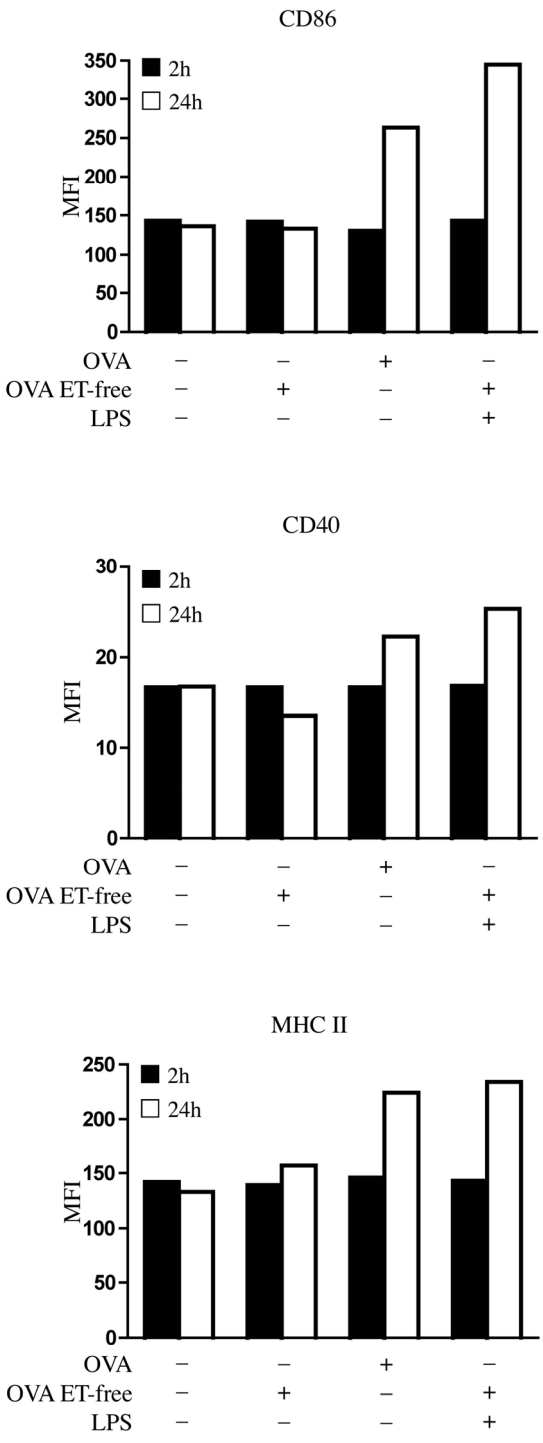
Supplementary Figure 8. TAP inhibition, but not primaquine treatment, prevents the presence of cross-presented OVA in early endosomes. This supplemental figure adds co-staining with the MR (green) to **Fig. 4e**.



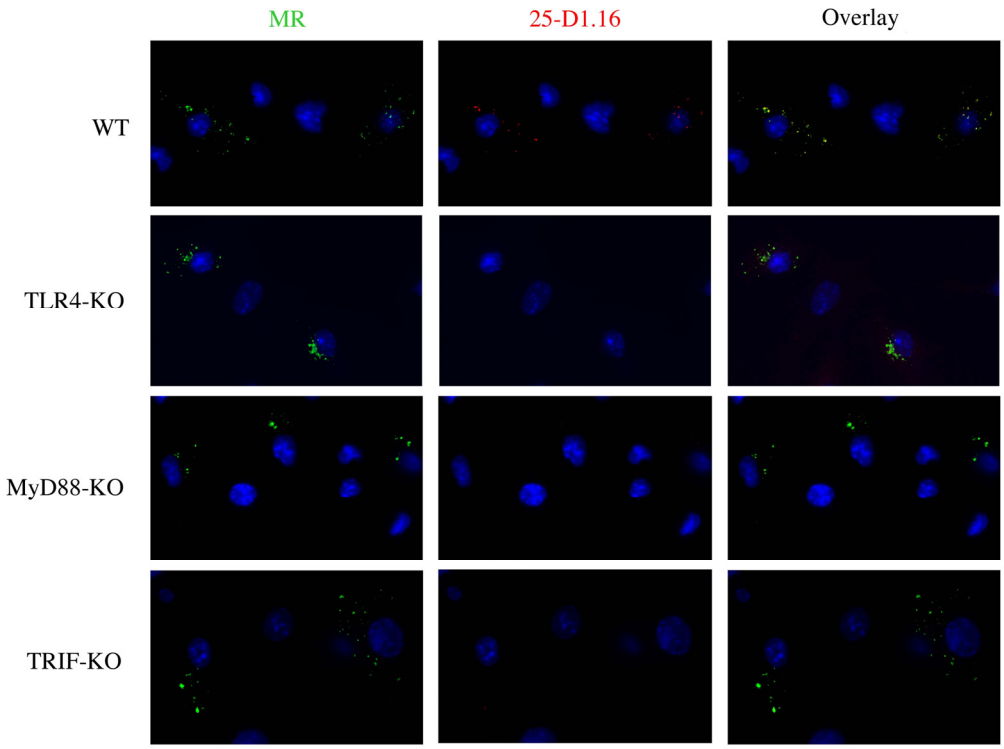
Supplementary Figure 9. Spatial separation of MHC I-restricted presentation of endogenous and extracellular antigens.



Supplementary Figure 10. Endotoxin-free and ordinary OVA are routed into the same early endosomal compartments. DCs were cultured with endotoxin-free OVA labeled with alexa$_{647}$ (red). In the top row, they were simultaneously offered ordinary OVA labeled with alexa$_{488}$ (green). The rows below show co-stainings with markers for early endosomes (MR, EEA1, Trf, all green) and late endosomes-lysosomes (LY, LAMP1, all green). Nuclei were counterstained with DAPI (blue). ET: endotoxin



Supplementary Figure 11. Cell surface expression of CD86, CD40 and MHC II is unaltered after OVA treatment for 2 h. DCs were incubated for 2 h or 24 h with ordinary OVA, endotoxin-free OVA and LPS. Cell surface expression was determined by flow cytometry and is indicated as mean florescence intensity (MFI). ET: endotoxin.



Supplementary Figure 12. Cross-presented OVA was detected in early endosomal compartments of wildtype DCs, but not of TLR4-, MyD88- or TRIF-deficient DCs. Cross-presented OVA was revealed by the 25-D1.16 antibody (red). This supplemental figure adds co-staining with the MR (green) to **Fig. 7a**.