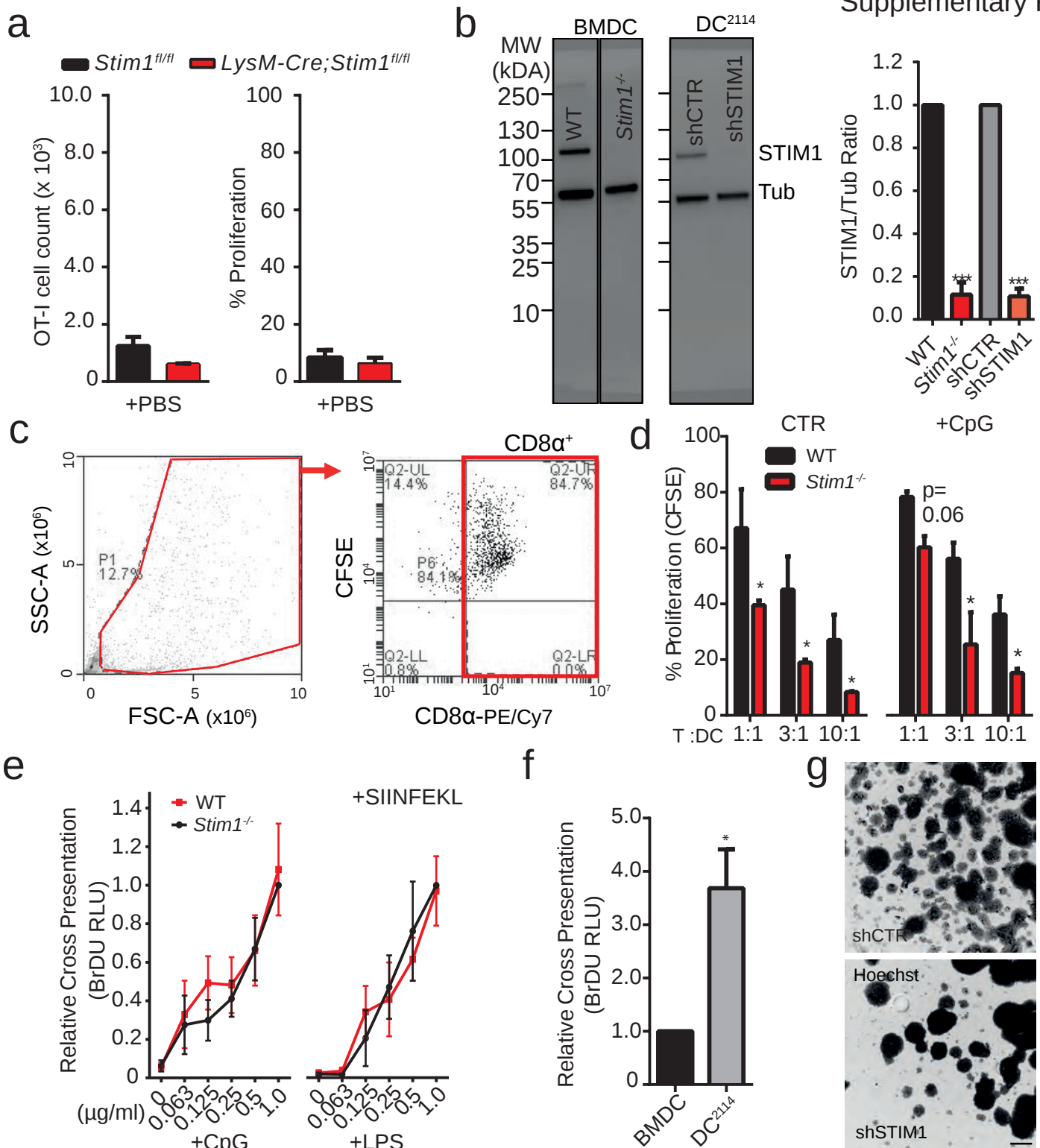
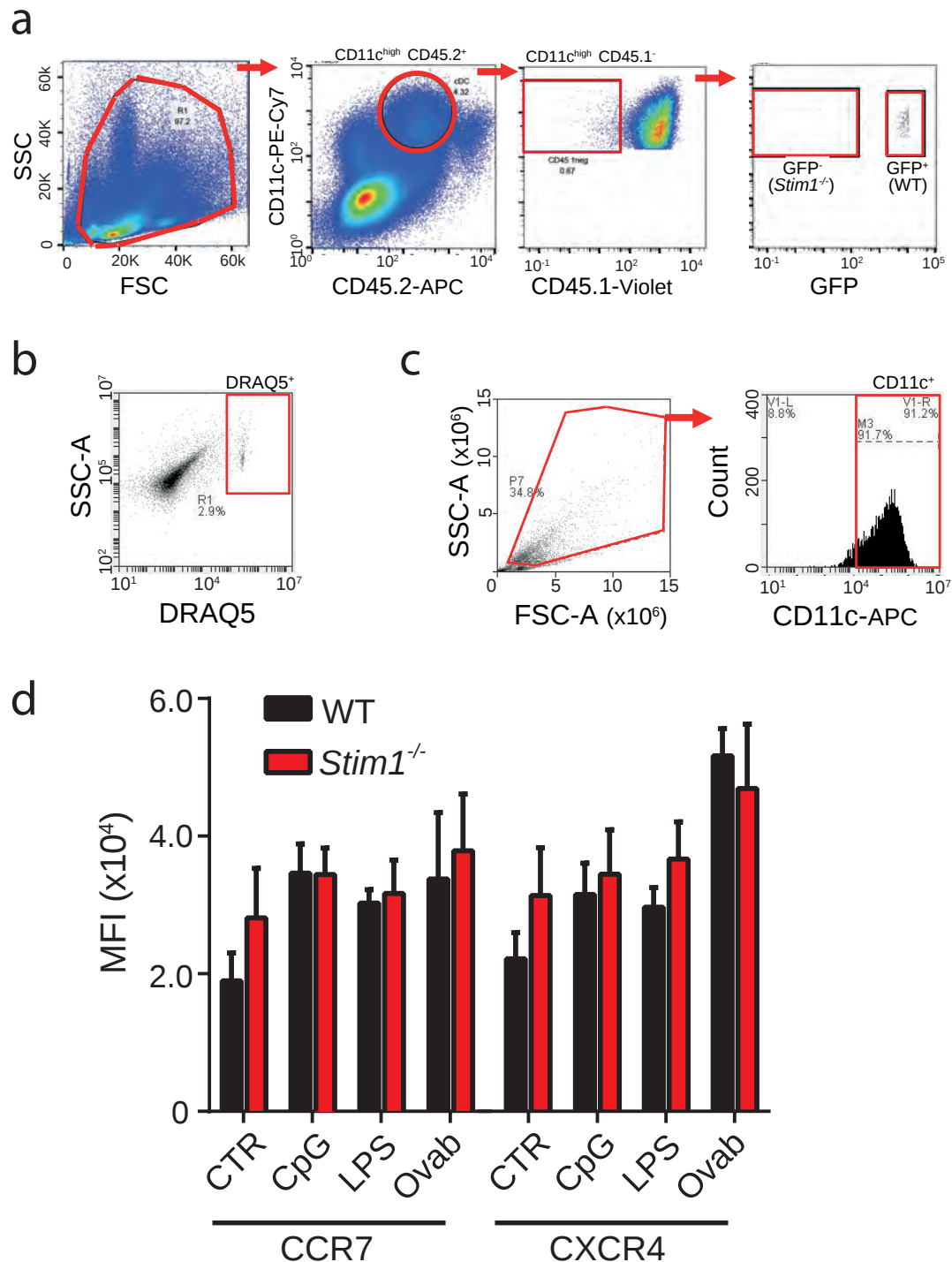
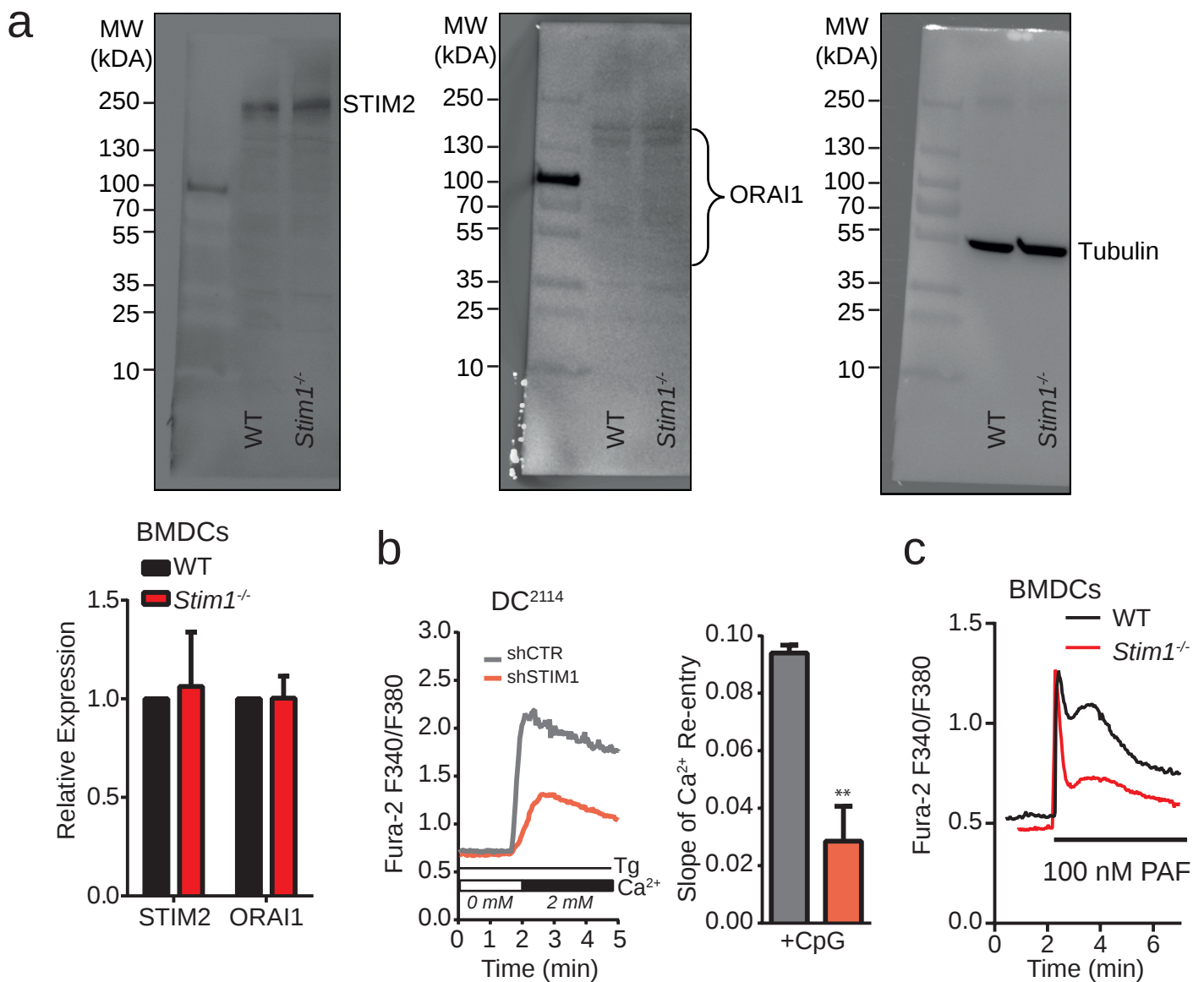




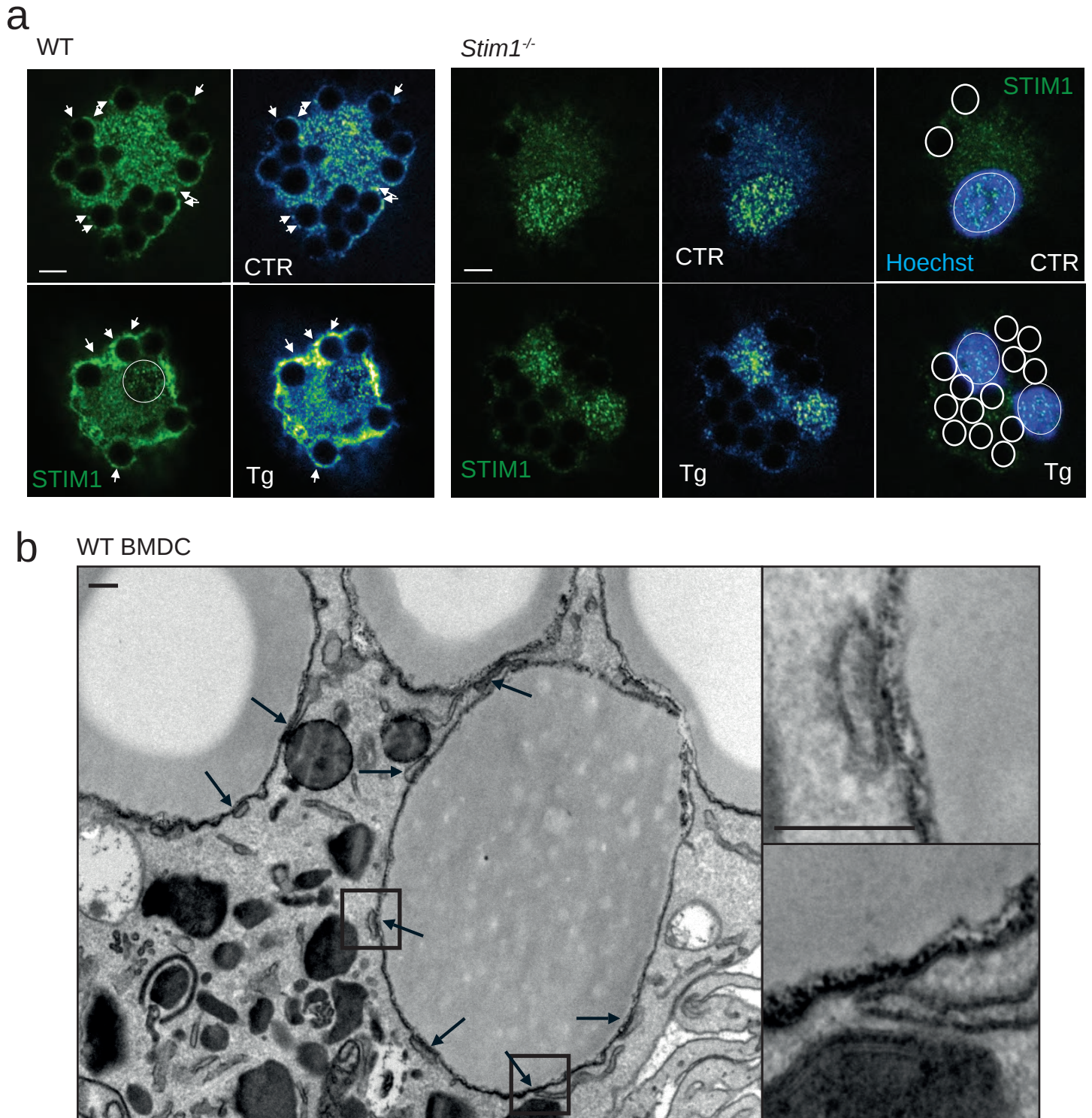
Supplementary Fig. 1. a) Isolating draining lymph nodes of *LysM-Cre; Stim1*^{fl/fl} mice or wild-type *Stim1*^{fl/fl} littermates injected with PBS only, and with 1x10⁶ CD45.1⁺ CFSE-labelled OT-I cells revealed no differences in the total number of CD45.1⁺ OT-I cells (left) nor in their proliferation (right). The gating strategy is shown in Fig. 1a. See also Fig. 1b. **b)** Western blots show a potent reduction in STIM1 expression both in *Stim1*^{-/-} BMDCs as compared to WT, and in DC²¹¹⁴ cells stably transduced with shSTIM1 as compared to shCTR. N=3. Values are STIM1 to Tubulin (Tub) ratios normalized to WT or shCTR. **c)** Full gating strategy for panel d. **d)** Co-culture of BMDCs and CFSE-labelled OT-I cells at varying ratios confirmed that STIM1 ablation reduces cross-presentation both in immature (CTR) and mature (CpG) cells. N=3, triplicate wells. **e)** Cross-presentation, assessed by BrDU, of WT BMDCs matured or not with CpG or LPS, exposed to varying concentration of SIINFEKL peptide and co-cultured 1:1 with OT-I cells. No significant differences between WT and *Stim1*^{-/-} BMDCs were detected. N = 3, triplicate wells. **f)** Cross-presentation, assessed by BrDU, of WT BMDCs (20,000 cells/well) or DC²¹¹⁴+shCTR (10,000 cells/well), exposed to OVA and co-cultured 1:1 with OT-I cells. Normalizing to WT BMDCs revealed the higher cross-presentation efficiency of DC²¹¹⁴ cells. N=3, triplicate wells. **g)** The strong OT-I proliferation induced by CpG-matured CD8α⁺ DC²¹¹⁴ exposed to OVA could be easily discerned by microscopy as large Hoechst⁺ colonies (black staining). Bar = 400 μm. Error bars are means ± SEM, p < 0.05, *** < 0.001 using a 2-way ANOVA and Sidak's post-test for panels d and e, and a Student's t-test for panels b and f.



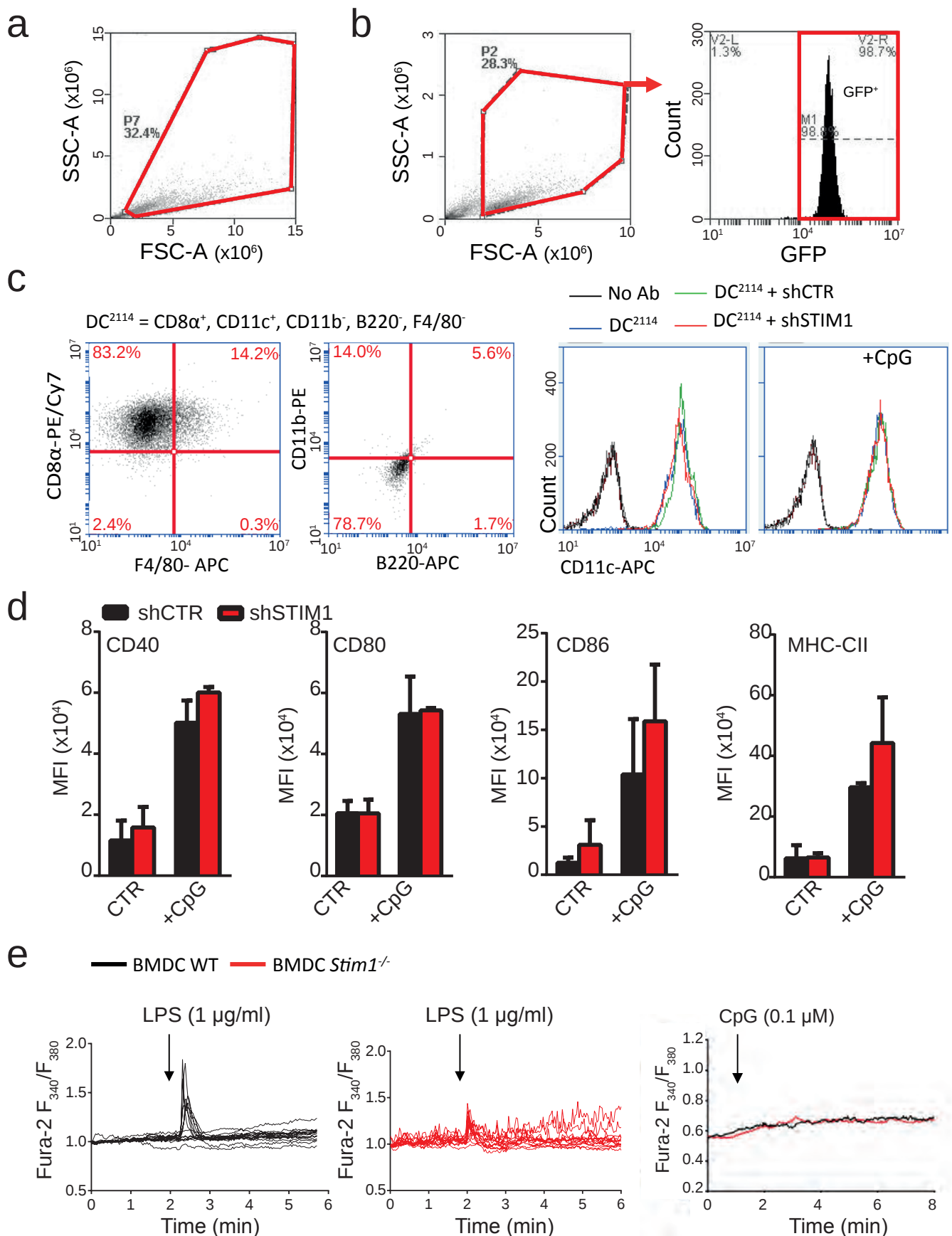
Supplementary Fig. 2. a) Full gating strategy for the *in vivo* migration assay in Fig. 2a. **b)** Full gating strategy for the *in vitro* migration assay in Fig. 2b. **c)** Full gating strategy for panel d, as well as for Figs 5b and 5d. **d)** Cell-surface immunostaining revealed no significant differences in CCR7 or CXCR4 receptors between WT and *Stim1*^{-/-} CD11c⁺ BMDCs in either controls or when cells were exposed to CpG, LPS (18 h) or OVA (4 h). N=3, in triplicate wells. Error bars are means + SEM.



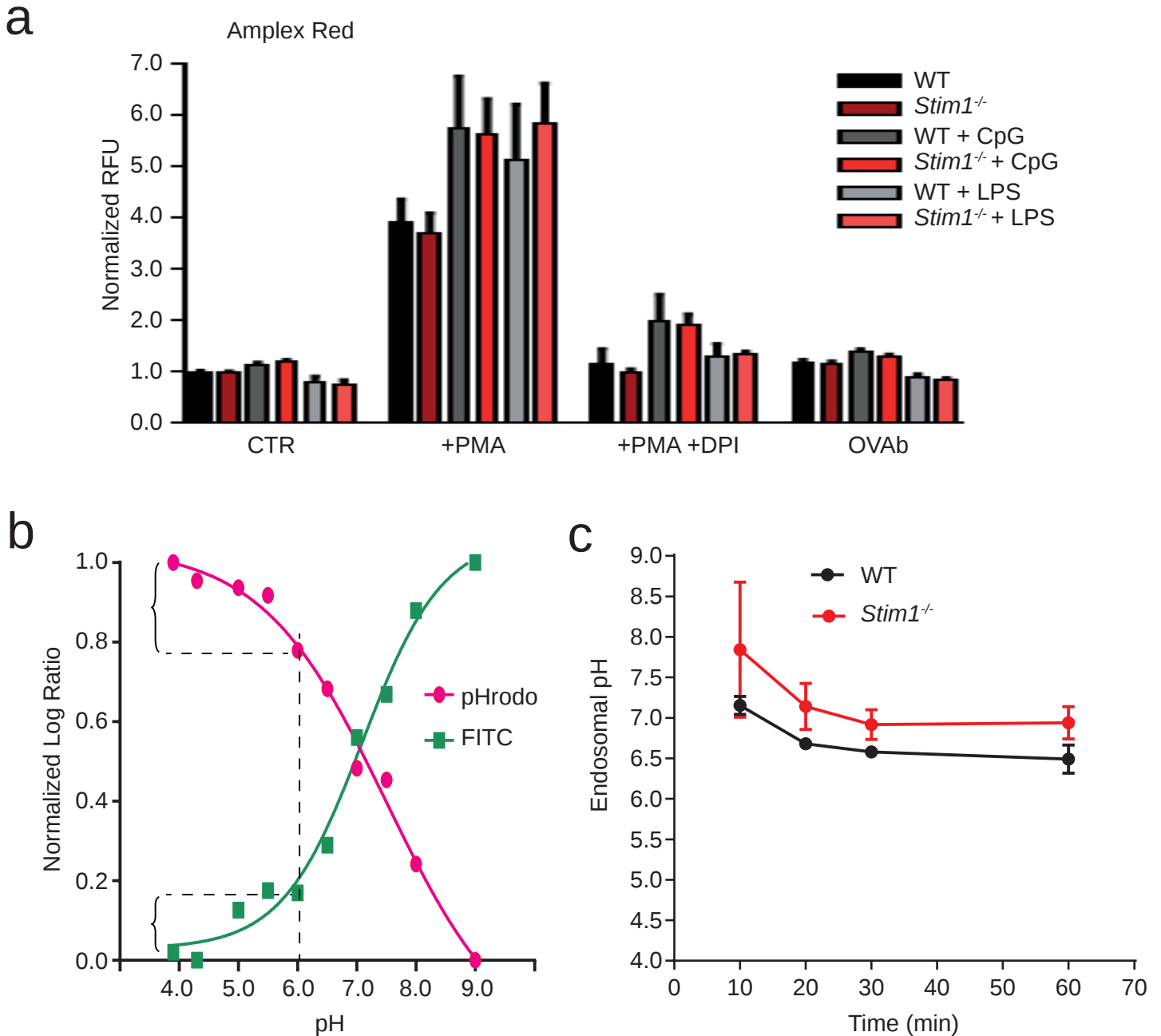
Supplementary Fig.3. **a)** Western blots showed no differences in STIM2 (left) or Orail1 (middle) expression in *Stim1*^{-/-} BMDCs as compared to WT. N=3. Values are normalized to tubulin (right) expression. **b)** STIM1 knockdown strongly decreased SOCE, measured in CpG matured DC²¹¹⁴ cells as the slope of Ca²⁺-re-entry after store depletion with 1 μ M Tg in Ca²⁺-free medium and 2 mM Ca²⁺ re-addition. N=3. **c)** Addition of 100 nM PAF produces similar Ca²⁺ responses as 2 μ M PAF (See Fig. 3b). Error bars are means + SEM. p **< 0.01.



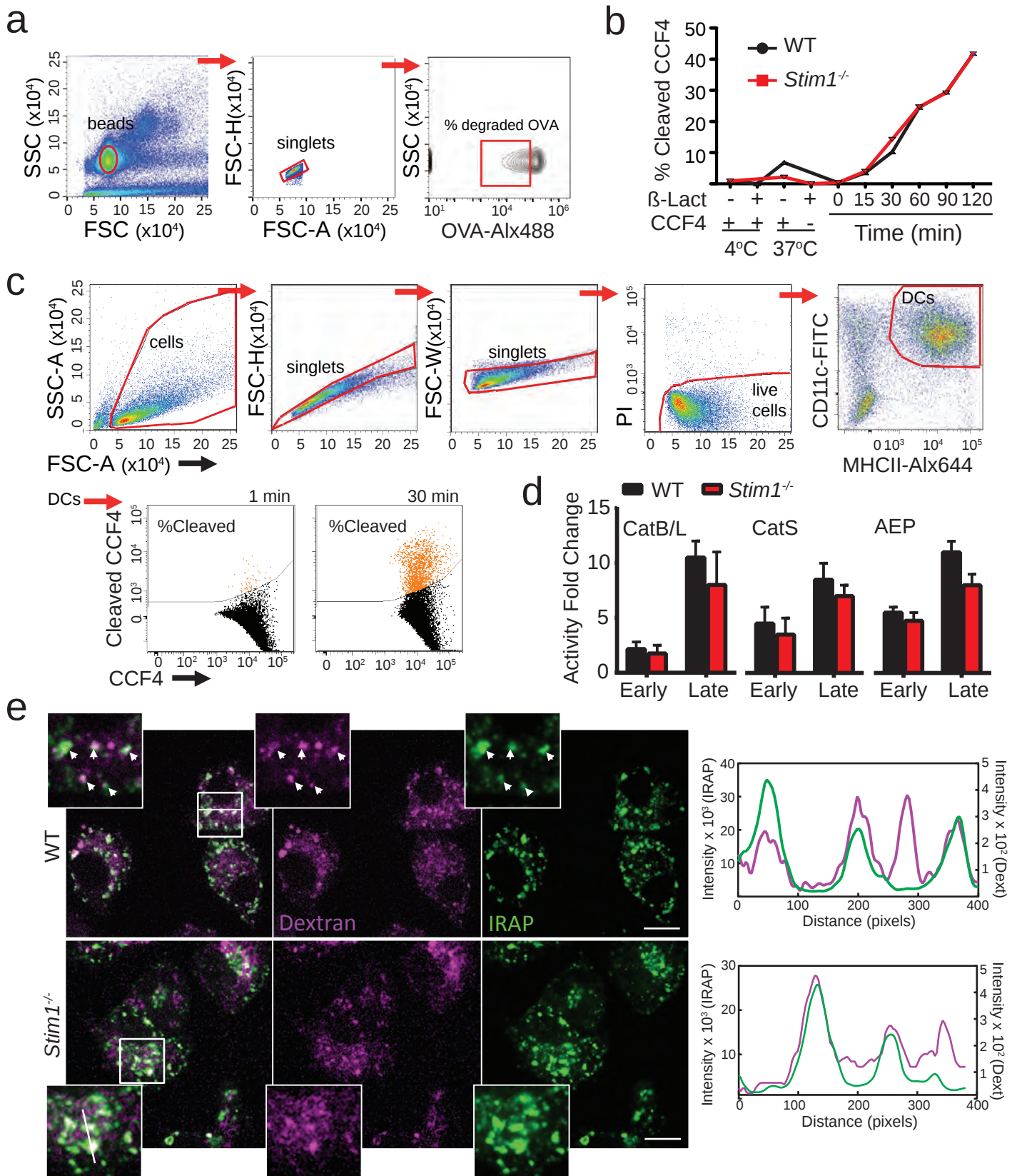
Supplementary Fig. 4. a) Immunofluorescent staining with mouse anti-STIM1 of WT (left) and *Stim1*^{-/-} (right) BMDCs exposed to OVA_B for 30 min and treated or not (CTR) with 1 μ M thapsigargin (Tg) for the last 10 min before fixation. Small periphagosomal STIM1 clusters (arrows) can be discerned that are more prominent when cells are treated with Tg (lower left-hand panels). Images are shown using identical contrast settings, and pseudocolored images are shown to the right of their respective images to highlight higher-intensity clusters. A small background cytosolic staining and a prominent, non-specific nuclear staining are observed in STIM1-deficient cells. Phagosomes and nuclei are outlined in white in the right-hand panels for STIM1-deficient cells. White bar = 3 μ m. **b)** Transmission electron micrograph of a WT BMDC exposed to OVA_B for 30 min. Arrows and insets illustrate multiple sites of contact between the ER and phagosomes. Bar = 100 nm.



Supplementary Fig. 5. a) Gating strategy for Fig 5a. **b)** Full gating strategy for panels c and d. **c)** Representative dot plot (left) showing that DC²¹¹⁴ cells are CD8 α ⁺, F4/80⁻, CD11b⁻ and B220⁻. Representative histograms (right) showing that CD11c levels are not affected by expression of either shCTR or shSTIM1 in either immature or CpG-matured DC²¹¹⁴ cells. **d)** Cell surface immunostainings showed no significant differences in maturation markers CD40, CD80, CD86 and MHC-II expression or upregulation upon pre-treatment with CpG. N=3. **e)** Representative single-cell traces from coverslips where a subset of WT and *Stim1*^{-/-} BMDCs show an increase in cytosolic Ca²⁺, detected using Fura-2 imaging, upon acute exposure to LPS (right, middle panels). No responses were detected in response to CpG (right panel, whole-coverslip averages are shown). Error bars are means + SEM.



Supplementary Fig. 6. a) Extracellular ROS levels measured with an Amplex Ultra Red assay in immature or CpG- or LPS-matured WT and *Stim1*^{-/-} BMDCs revealed no differences. Exposure to 100 nM PMA as a positive control increased extracellular ROS, an effect that was blocked by 10 μ M DPI. In contrast to intracellular ROS (see Fig. 6a) OVA did not induce detectable levels of extracellular ROS. N=3, triplicate wells. **b)** Representative *in situ* calibration curves for FITC- and pHrodo/Alexa568-coupled zymosan after 90 min of phagocytosis. The logs of the ratio values are normalized between 0 and 1 to allow comparison of the sensitivity of the two dyes on the same scale. pHrodo displays a higher sensitivity at lower (< 6.0) pH values than FITC. **c)** Endosomal pH was measured in BMDCs loaded with FITC- and Alexa-568-coupled dextran and analysed by flow cytometry. Although the endosomal pH of *Stim1*^{-/-} BMDCs showed a trend for a higher pH, the difference was not significant. N = 5/4/5/5 for WT (10/20/30/60 min) and N=3 for all *Stim1*^{-/-} timepoints. Error bars are means $\pm$ SEM.



Supplementary Fig. 7. a) Full gating strategy for Fig. 7b. **b)** Cytosol transfer was measured by loading cells with CCF4 (4 μ M) for 1h before incubating them with β -lactamase (β -lact) for the indicated time points. Negative controls cells incubated at 4°C or in the absence of either enzyme or substrate are shown on the left. N=3. **c)** Full gating strategy for panel b. **d)** Protease activity was measured in early (30 min) and late (60 min) phagosomes. The activity of cathepsins B/L (Cat B/L, left), cathepsin S (CatS, middle) and asparagine endopeptidase (AEP, right) were not significantly different in STIM1-deficient BMDCs as compared to WT. N=3. Error bars are means $\pm$ SEM. **e)** Dextran-containing endosomes (magenta) partially overlap with IRAP-positive endosomes (green) in both WT and STIM1-deficient cells. Line-scan profiles illustrating signal overlap are shown on the right. Bar = 10 μ m.