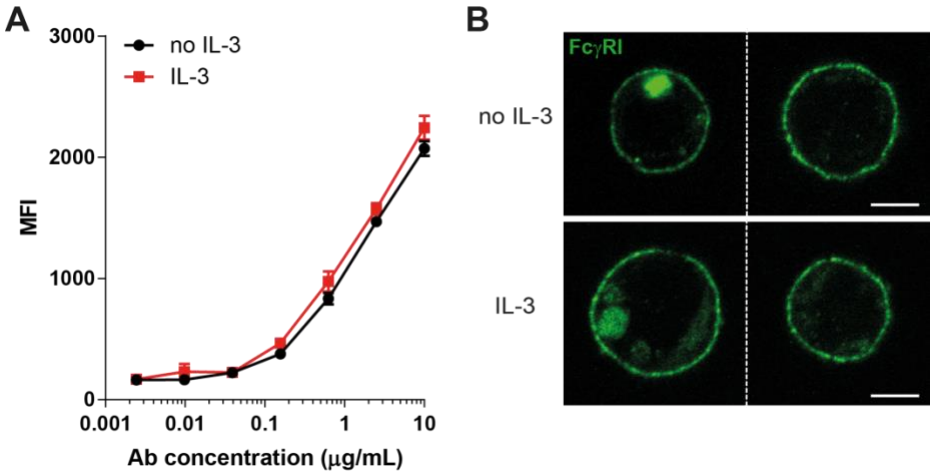
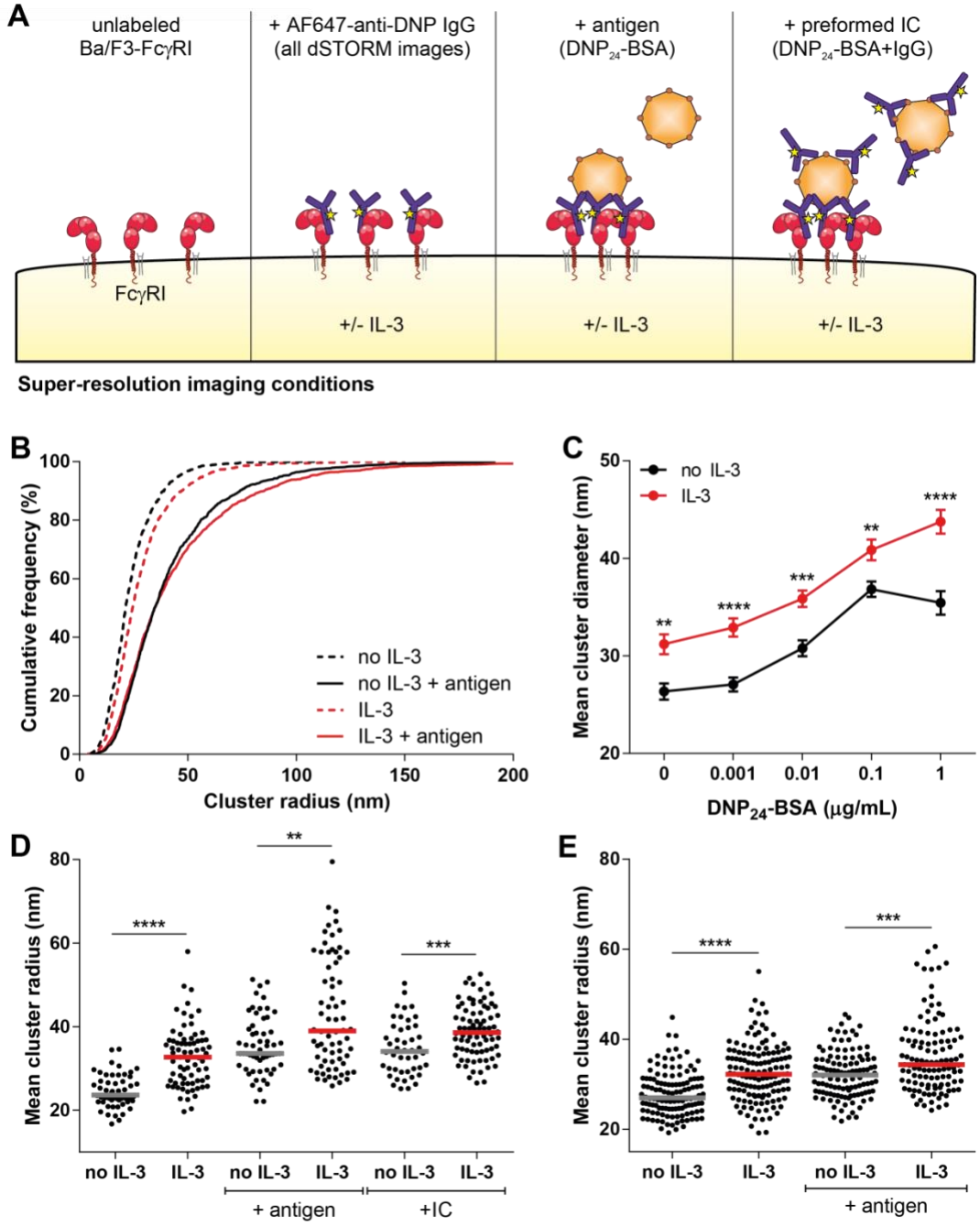
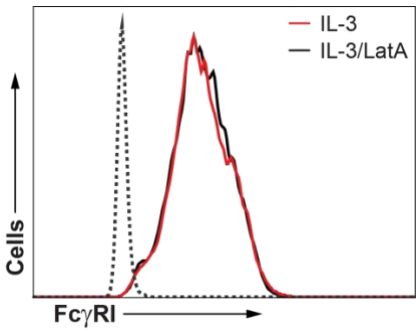


Supplementary Figure 1. **FcγRI surface expression does not change after IL-3 stimulation.** (**A**) Flow cytometry analysis of the binding of increasing concentrations of monomeric anti-DNP IgG to Ba/F3-FcγRI cells after IL-3 stimulation. Mean fluorescence intensity (MFI) data are representative of 3 independent experiments. (**B**) Confocal microscopy analysis of FcγRI distribution in labeling Ba/F3-FcγRI cells. Two images for each condition are representative of 2 independent experiments. Scale bar, 5 µm.

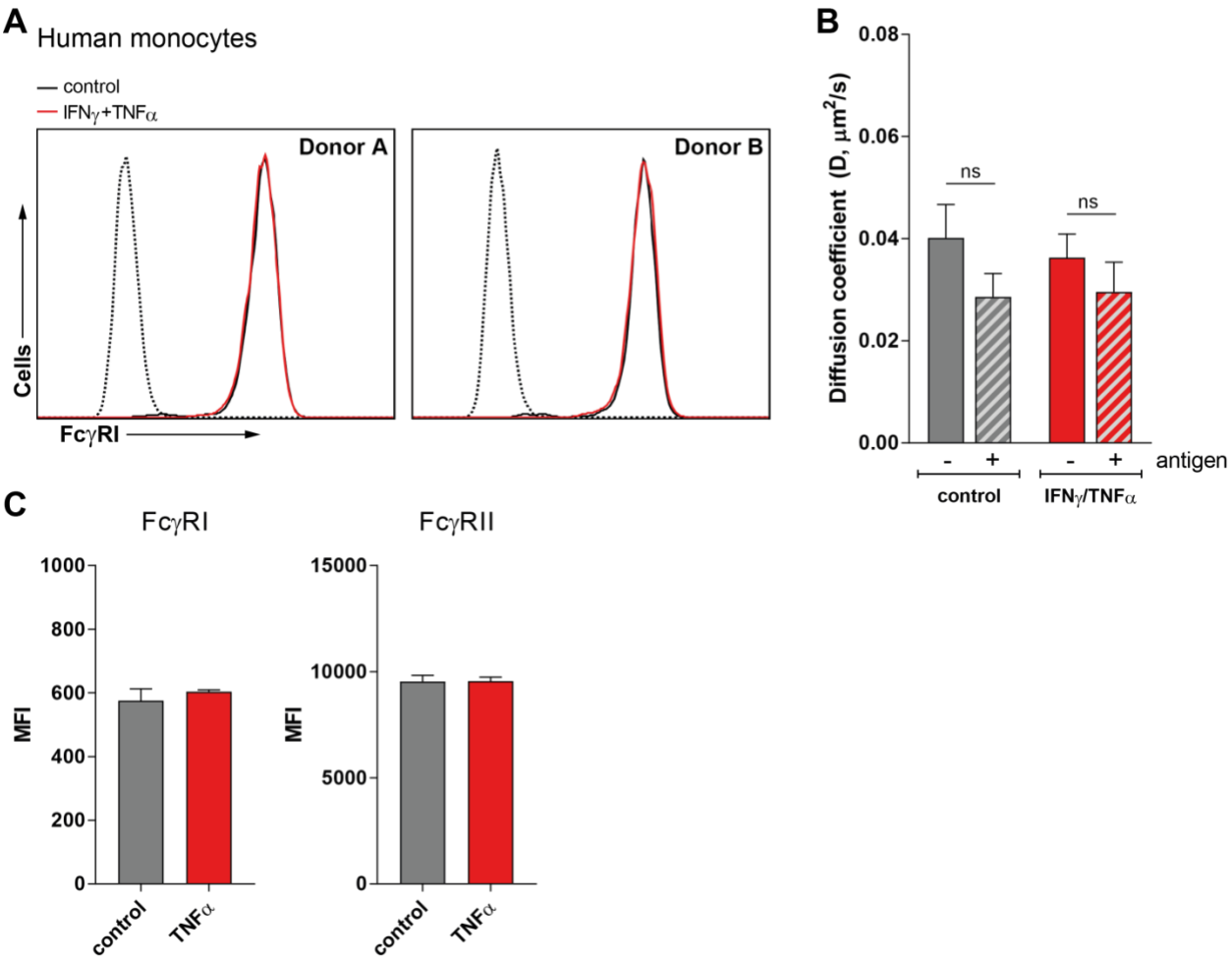


Supplementary Figure 2. FcγRI clustering increases after cytokine stimulation. (**A**) Schematic overview of the dSTORM super-resolution microscopy imaging conditions. (**B** and **C**) dSTORM analysis of AF647-labeled anti-DNP IgG on Ba/F3-FcγRI cells stimulated as indicated. FcγRI-bound IgG clusters were identified by DBSCAN analysis and the cumulative distribution of cluster sizes for all identified FcγRI clusters in (see Fig. 2C) were calculated (B). Data are representative of the analysis of 20-30 cells per condition analysed within several regions of interest (ROIs) per cell from 3 independent experiments, resulting in >1500 cluster sizes per condition. FcγRI cluster sizes at different DNP_{24}-BSA concentrations (C) data are means ± SEM of ROIs of 15-20 cells per condition pooled from 2 independent experiments. (**D**) dSTORM analysis of AF647-labeled anti-DNP IgG on Ba/F3-FcγRI cells stimulated with IL-3, DNP antigen or preformed IC, as indicated. Mean radius cluster and median data are from the analysis of 3-5

ROIs on 8-12 cells per condition from 2 independent experiments. (**E**) dSTORM analysis of AF647-labeled anti-TNP human IgG1 on Ba/F3-FcγRI cells stimulated as indicated. Mean cluster radius per ROI with medians are from the analysis of 8-11 cells per condition from 2 independent experiments. **P<0.01, ***P<0.001, ****P<0.0001 by Mann-Whitney U test.



Supplementary Figure 3. FcγRI surface expression does not change after latrunculin A pretreatment. Flow cytometry analysis of FcγRI expression on Ba/F3-FcγRI cells after 1 hour IL-3 stimulation, with (IL-3/LatA) and without (IL-3) pretreatment of 0.1 μg/mL latrunculin A. Dotted line represents the isotype control. Histograms are representative of 3 independent experiments.



Supplementary Figure 4. FcγRI expression does not change after cytokine stimulation of human myeloid cells. (**A**) Flow cytometry analysis of FcγRI expression on CD14^{high} monocytes within PBMCs from two healthy donors stimulated with cytokines (IFNγ+TNFα) as indicated. Dotted line represents the isotype control. Histograms are representative of 2 independent experiments. (**B**) Single particle tracking (SPT) by microcopy analysis of the movement of QD655-labeled anti-DNP IgG on live human monocytes stimulated with cytokines (IFNγ+TNFα) and with and without antigen (DNP_{24}-BSA) to induce IC as indicated. Diffusion coefficients for QD655-labeled IC mobility were calculated using mean square displacement (MSD) analysis of individual SPT trajectories. Data are representative of the analysis of >33 cells per condition from 2 independent experiments. (**C**) Flow cytometry analysis of FcγRI and FcγRII expression on isolated neutrophils after TNFα stimulation for 4 hours. Data are mean fluorescence intensity (MFI) ± SD representative of 3 independent experiments. ns: not significant by Student's t test.