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Supporting Material

Distribution and dynamics of RBL IgE receptors (FcεRI) on planar ligand-presenting surfaces

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Supporting Material

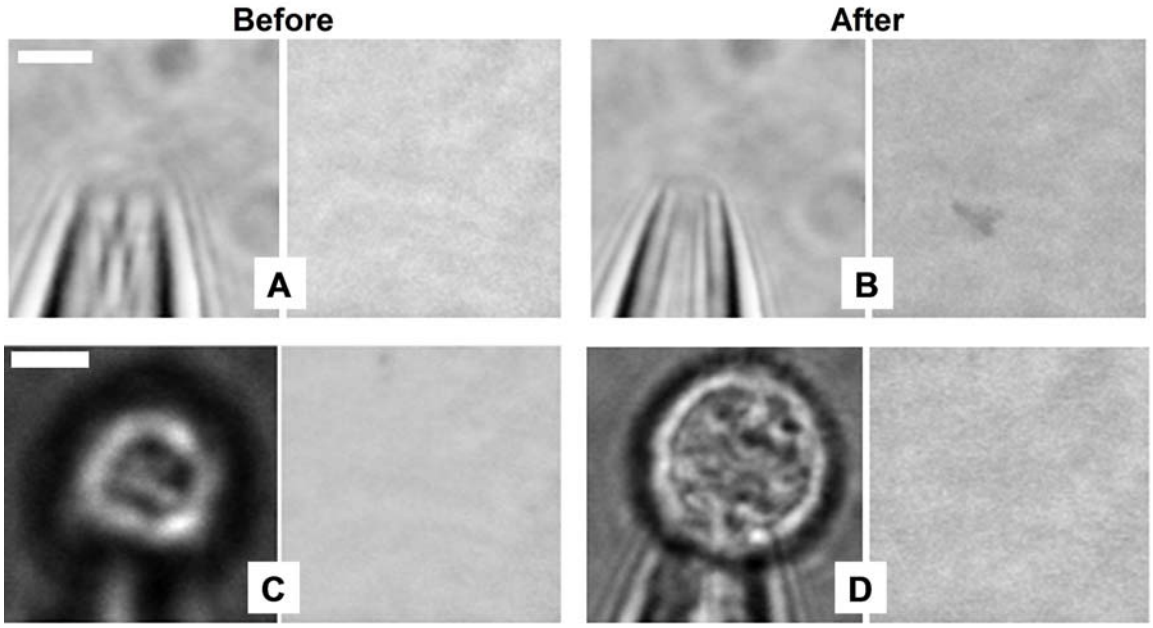

Figure S1: Membrane integrity before (left column) and after (right column) micromanipulation. To visualize the bilayer integrity, we took TIRF images of a POPC bilayer that in addition to 25 mol % DNP-lipid included a fluorescent lipid (BODIPY-DHPE) at 1 mol %. Images shown here are focused on the substrate surface. TIRF images are corrected for the illumination profile.
Top row: (A) Micrograph of out-of-focus micropipette (left panel) and TIRF image of uniform fluorescent bilayer (right panel). Here we show that there are no apparent defects in the bilayer before micromanipulation. (B) Micrograph of in-focus micropipette in *contact with bilayer* (left panel) and corresponding TIRF image of fluorescent bilayer (right panel). Here we purposely scratched a hole into the bilayer to produce a bilayer defect. We note that the field of view was not changed between (A) and (B). TIRF images in (A) and (B) used the same brightness scaling. Scale bar = 5 μm.
Bottom row: (C) Micrograph of out-of-focus micropipette with anti-DNP-IgE loaded RBL cell caught out of suspension (left panel) and TIRF image of uniform fluorescent bilayer (right panel) before micromanipulation. (D) Same cell as in (C) pipette-pressed onto the fluorescent bilayer. We note that *after initial contact* with the fluorescent bilayer no apparent bilayer defects were observed (left panel). TIRF images in (C) and (D) use the same brightness scaling. The field of view was not changed between (C) and (D); the slight overall decrease in fluorescence from (C) to (D) is due to bleaching. Scale bar = 5 μm.

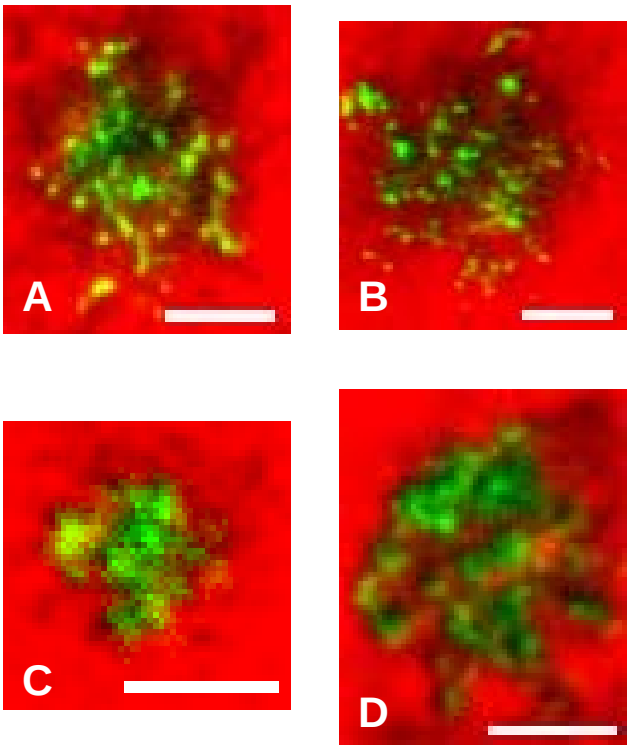

Figure S2: Two-color TIRF images of FcεRI receptors (green) and a soluble buffer marker (red, 20 nM Alexa Fluor 647-R-phycoerythrin streptavidin, Invitrogen, Carlsbad, CA). Close contacts between the cell and the coverslip exclude the soluble fluorophore, and essentially every close contact contains IgE. TIRF images were taken after about 5 s of initial contact of pipette-pressed cells on (A) crosslinked (EGS) DNP-BSA, (B) crosslinked (Glut) DNP-BSA, (C) POPC with 0 mol % DNP-Cap PE, and (D) bare glass. Scale bar = 5 μm.

Movie S1: Time-lapse TIRF images of Alexa-488 IgE-primed RBL-2H3 cell pipette-pressed onto a 0 mol% DNP-Cap PE bilayer. This movie is ten times faster than normal speed.