SUPPLEMENTAL MATERIAL

Xu et al., http://dx.doi.org/10.1084/jem.20160528

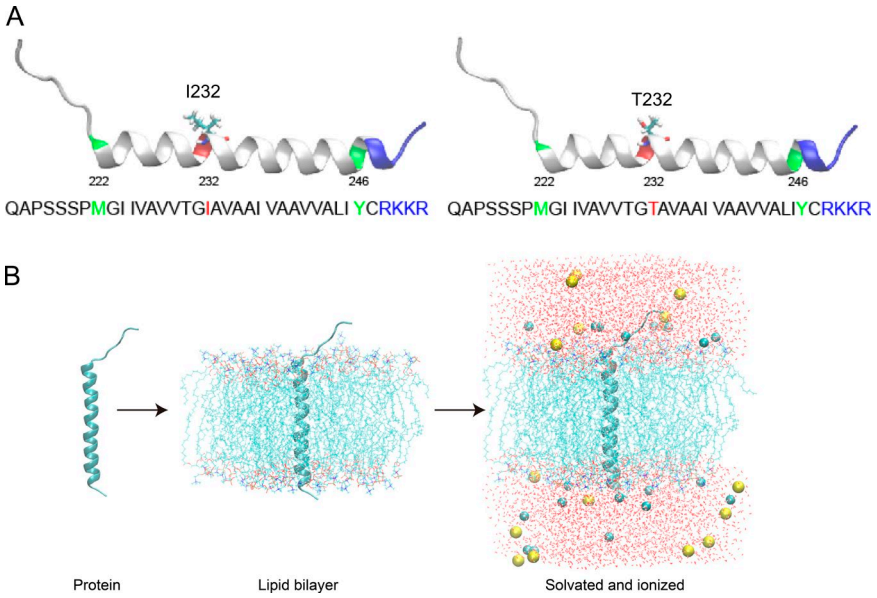


Figure S1. **The I232T polymorphism raises the degree of helix bending in MD simulations.** (A) The TM domain of FcγRIIB is modeled as a long helix (shown in the cartoon representation). The amino acid sequences of the TM domain of FcγRIIB-WT and FcγRIIB-I232T are shown beneath. The boundary residues of the TM region are colored in green, key residue 232 is colored in red (in Licorice representation), and the basic residues located at the C terminus are colored in blue. (B) In MD simulations, the TM domain of FcγRIIB was inserted into the lipid bilayer, solvated by water molecules, and neutralized by NaCl.

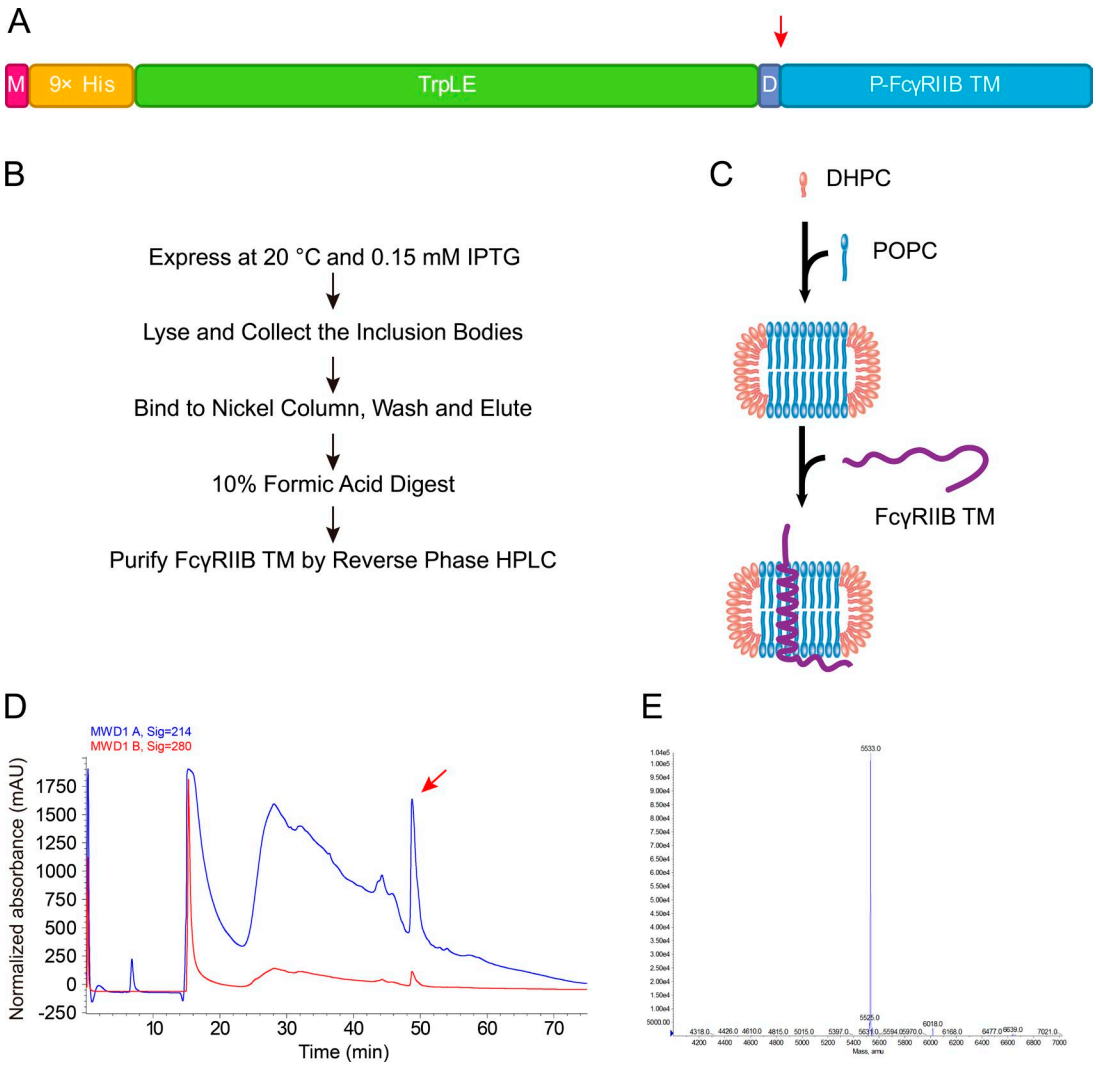
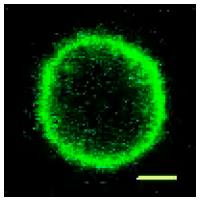
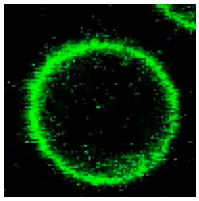


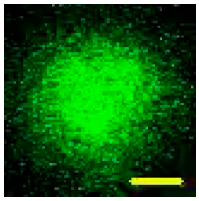
Figure S2. **Purification strategy and schematic diagram for the reconstitution of FcγRIIB TM into bicelles.** (A) The FcγRIIB TM peptide was expressed with a 9× His-TrpLE tag as the inclusion body in an *E. coli* BL21 strain. An additional Asp was added before the FcγRIIB sequence to generate a cleavage site (Asp-Pro) of formic acid. D, Asp; M, Met. (B) Flow chart for the purification of FcγRIIB peptide. HPLC, high-performance liquid chromatography; IPTG, isopropyl β-D-1-thiogalactopyranoside. (C) POPC bicelle was generated by mixing POPC powder with DHPC stock solution, followed by freezing and thawing until clear solution was obtained. The POPC-DHPC bicelle solution was then used to dissolve the FcγRIIB peptide. After several rounds of freezing and thawing, the FcγRIIB peptide can be successfully reconstituted into the bicelle. (D) Reverse-phase high-performance liquid chromatography purification profile for the FcγRIIB peptide. The fraction of FcγRIIB peptide is marked with a red arrow. mAU, milli–arbitrary units. (E) Mass spectroscopy spectrum of the FcγRIIB peptide demonstrated that the sample was with high purity. amu, atomic mass units.



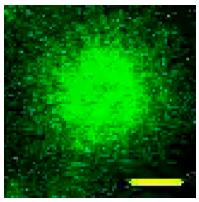
Video 1. **The dynamic recovery of FcγRIIB-WT within the photobleached area on the plasma membrane of A20II1.6 B cells.** Given are time-lapse images in a real-time time course of 30 s with a 93-ms interval at 25°C. The video is shown at 50 frames per second (FPS). Bar, 1.5 µm.



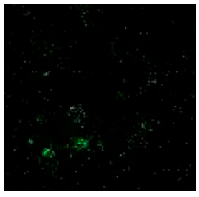
Video 2. **The dynamic recovery of FcγRIIB-I232T within the photobleached area on the plasma membrane of A20II1.6 B cells.** Given are time-lapse images in a real-time time course of 30 s with a 93-ms interval at 25°C. The video is shown at 50 FPS. Bar, 1.5 µm.



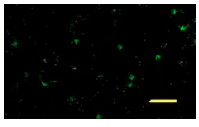
Video 3. **The dynamic recovery of FcγRIIB-WT within the photobleached area on the top flat area of the plasma membrane of ST486 human B cells.** Given are time-lapse images in a real-time time course of 40 s with a 93-ms interval at 37°C. The video is shown at 50 FPS. Bar, 1.5 µm.



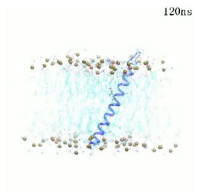
Video 4. **The dynamic recovery of FcγRIIB-I232T within the photobleached area on the top flat area of the plasma membrane of ST486 human B cells.** Given are time-lapse images in a real-time time course of 40 s with a 93-ms interval at 37°C. The video is shown at 50 FPS. Bar, 1.5 µm.



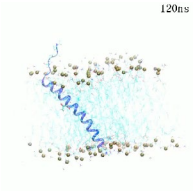
Video 5. **SPT of FcγRIIB-WT molecules on the plasma membrane of ST486 human B cells that were placed on PLBs tethering F(ab')$_2$ fragment anti–MHC-1 antibody.** Given are time-lapse images in a real-time time course of 4 s with a 20-ms interval at 37°C. The video is shown at 50 FPS. Bar, 3 µm.



Video 6. **SPT of FcγRIIB-I232T molecules on the plasma membrane of ST486 human B cells that were placed on PLBs tethering F(ab')$_2$ fragment anti–MHC-1 antibody.** Given are time-lapse images in a real-time time course of 4 s with a 20-ms interval at 37°C. The video is shown at 50 FPS. Bar, 3 µm.



Video 7. **The trajectory of the last 80 ns of FcγRIIB-WT TM helix in POPC.** The C terminus of the helix was moved to the center of the lower leaflet. The video is shown at 30 FPS.



Video 8. **The trajectory of the last 80 ns of FcγRIIB–I232T TM helix in POPC.** The C terminus of the helix was moved to the center of the lower leaflet. The video is shown at 30 FPS.