Supplemental materials

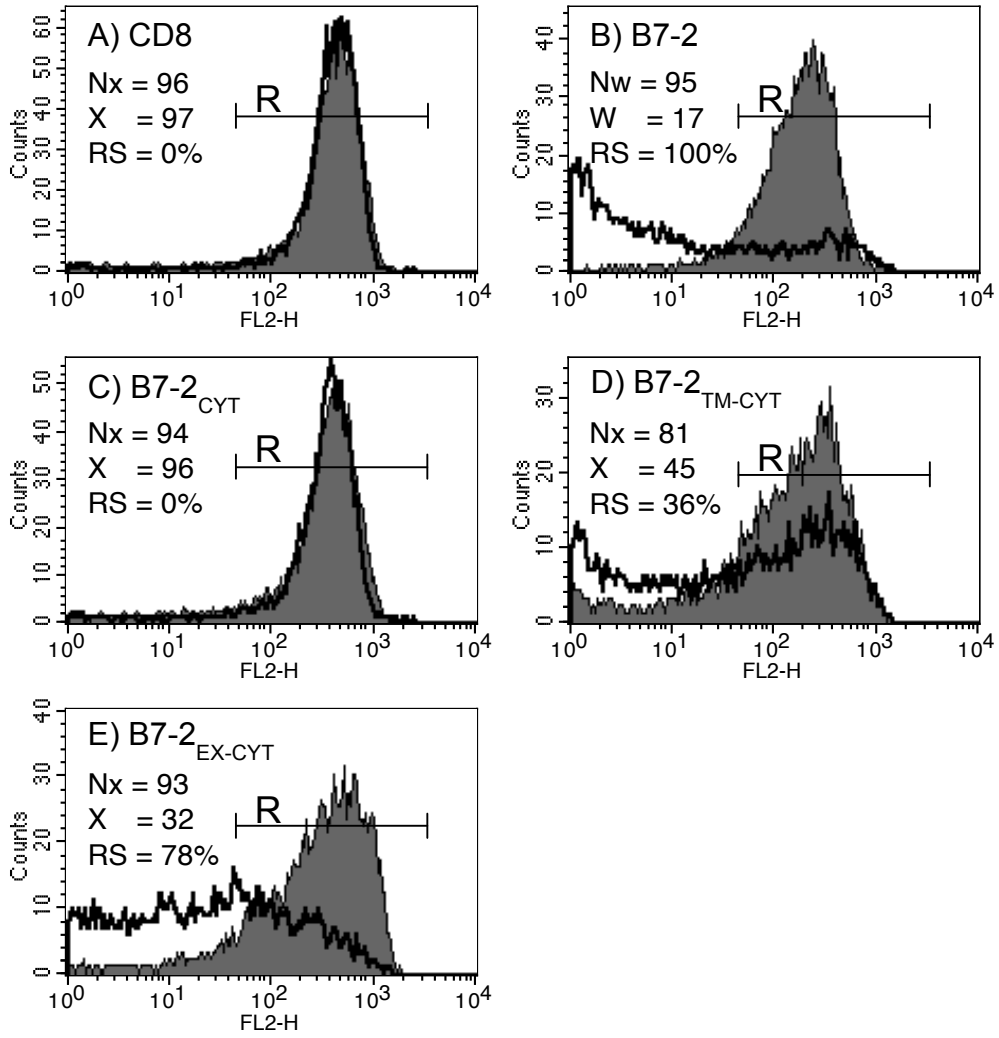

FIG. S1. Histogram plots of chimera expression used for the calculation of relative sensitivities in Figure 1B. FLAG-tagged chimera-expressed cells were stained with phycoerythrin (PE)-conjugated anti-DDDDK tag antibody. The cell surface expression of CD8 (A), B7-2 (B), B7-2_{CYT} (C), B7-$2_{TM\text{-}CYT}$ (D), and B7-$2_{EX\text{-}CYT}$ (E) on the cells transfected with plasmid which expresses only GFP (shaded histogram) or with plasmid which expresses both MIR2 and GFP (open histogram) was examined by flow cytometry, and expression level of each surface moleucle on GFP positive cells was determined as shown in single parameter histograms. The cell population included in the region "R" was defined as PE-positive cell and the percentage of PE-positive cells was used for calculation of relative sensitivity as described in the material and methods. Each value was demonstrated in each panel. Data are representative of three independent experiments. Other calculations of relative sensitivity or activity were performed similar to these examples. RS: relative sensitivity

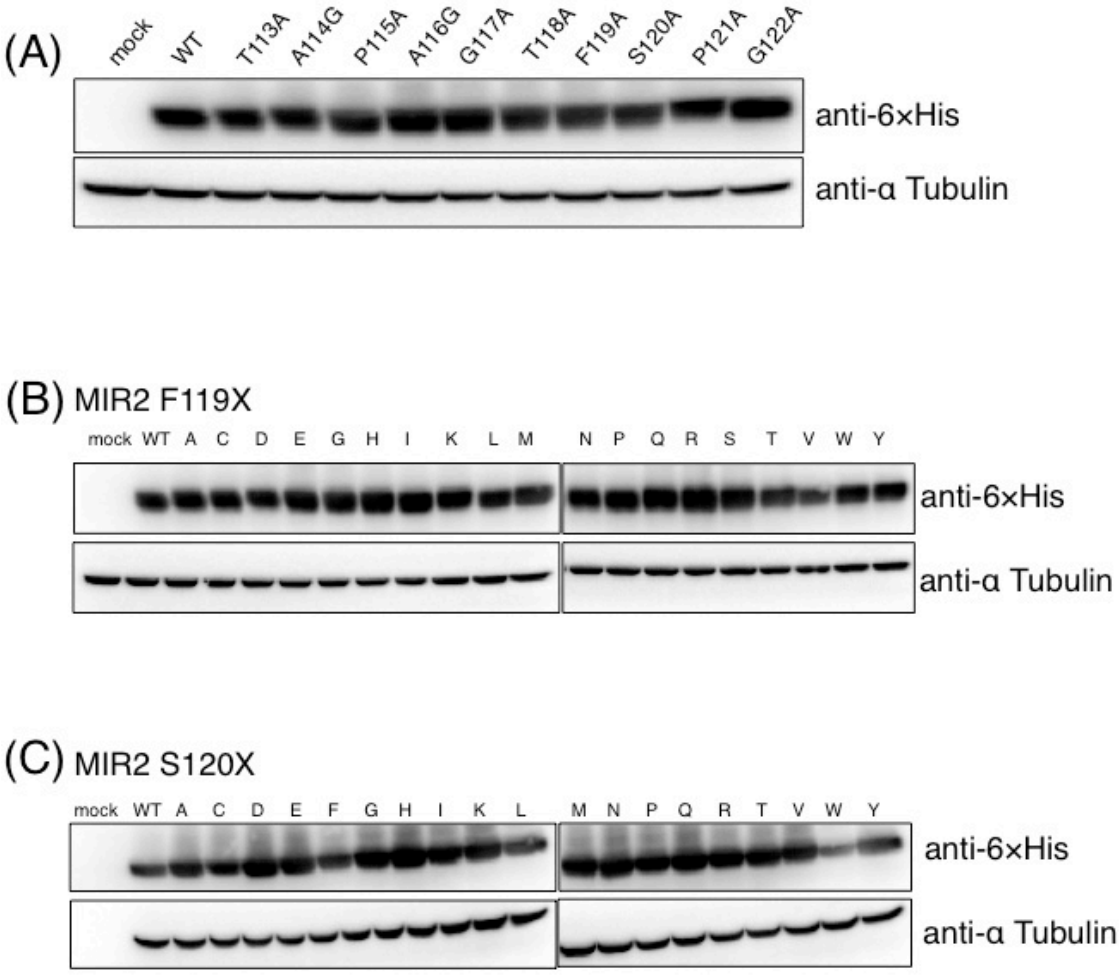

FIG. S2. Expression levels of wild type and mutant MIR2. (A) Alanine (or glycine when the WT residue is alanine) mutants of the MIR2 ITM. (B) Site-saturated mutants of Phe119 in the MIR2 ITM. (C) Site-saturated mutants of Ser120 in the MIR2 ITM.

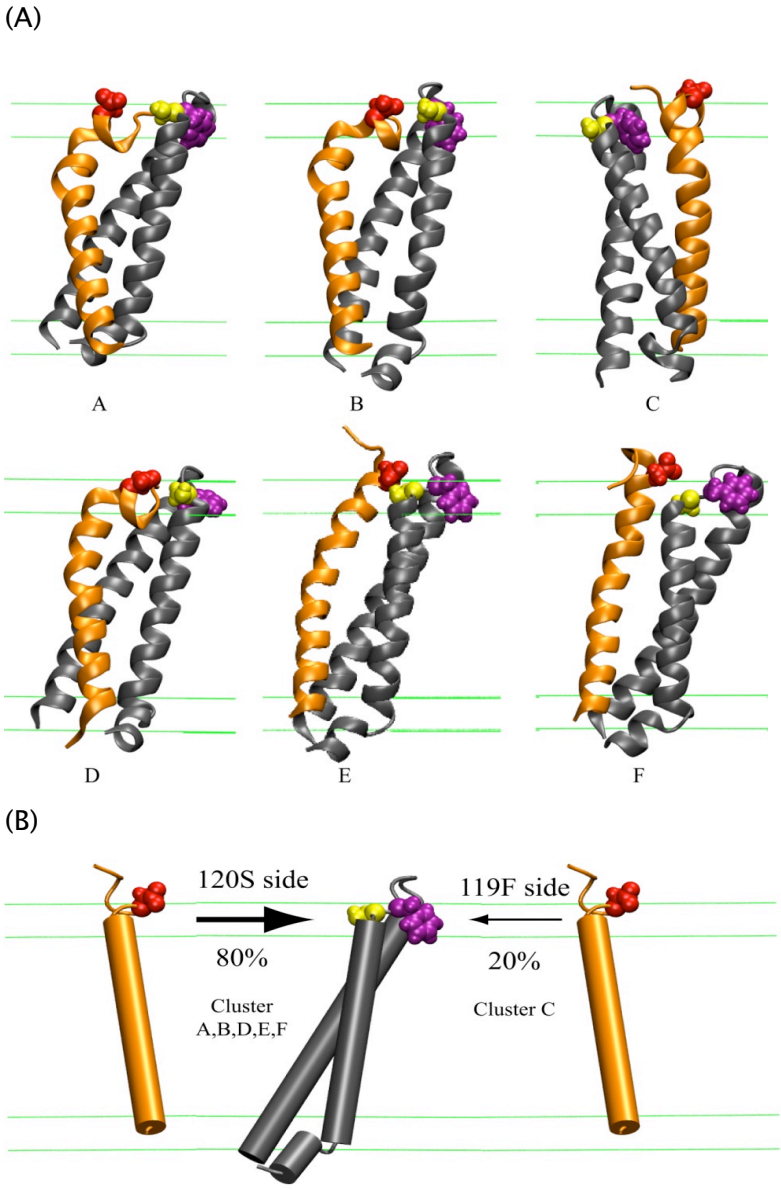

FIG. S3. (A) Structures for the top 6 populated clusters (A-F) for the MIR2-B7-2 complex. (The population of each cluster is shown in Table S1.) (B) Cartoon showing how B7-2 binds to different MIR2 surfaces for the top 6 populated clusters: B7-2 binds to MIR2 from the Ser120 side for clusters A, B, D, E, and F, while it binds to MIR2 from the Phe119 side for cluster C.

Table S1. Analysis of the centroid structure of top 6 clustered MIR2-B7-2 complexs.

Cluster	Population (2-30ns)	Shortest residue-residue contact distance	Shortest residue-residue contact pairs
A	26%	3.0 Å	(120S, 239P)
B	23%	2.7 Å	(118T, 239P)
C	18%	2.8 Å	(149S, 269K)
D	8%	2.8 Å	(120S, 239P)
E	6%	2.6 Å	(120S, 244D)
F	4%	3.1 Å	(146C, 268W)