Structural and functional basis for LILRB immune checkpoint receptor recognition of HLA-G isoforms

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Table S1 Residue list of HLA-G1-LILRB1 interactions

Hydrogen bonding (site 1)

chain D (LILRB1)	distance (Å)	chain B (β2m)
D:ALA 127 [N]	2.86	B:GLN 2 [O]
D:ILE 100 [N]	3.29	B:THR 86 [O]
D:TRP 67 [NE1]	3.25	B:ILE 92 [O]
D:GLN 125 [O]	3.46	B:GLN 2 [N]
D:ALA 98 [O]	3.06	B:SER 88 [N]
D:GLY 97 [O]	2.43	B:SER 88 [OG]
D:ALA 98 [O]	3.50	B:SER 88 [OG]
D:GLN 18 [OE1]	3.22	B:GLN 89 [NE2]
D:GLU 184 [OE1]	3.56	B:LYS 91 [NZ]

chain H (LILRB1)	distance (Å)	chain F (β2m)
H:ALA 127 [N]	2.92	F:GLN 2 [O]
H:ILE 100 [N]	3.51	F:THR 86 [O]
H:TRP 67 [NE1]	2.60	F:ILE 92 [O]
H:GLN 125 [O]	3.75	F:GLN 2 [N]
H:ALA 98 [O]	3.29	F:SER 88 [N]
H:ALA 98 [O]	3.49	F:SER 88 [OG]
H:GLY 97 [O]	3.57	F:SER 88 [OG]
H:GLN 18 [OE1]	3.79	F:GLN 89 [NE2]
H:GLN 18 [O]	3.04	F:GLN 89 [NE2]
H:GLU 184 [OE1]	2.72	F:LYS 91 [NZ]
H:TYR 99 [OH]	3.66	F:LYS 91 [NZ]
H:GLU 68 [OE2]	3.17	F:LYS 94 [NZ]

Hydrogen bonding (site 2)

chain H (LILRB1)	distance (Å)	chain E (HLA-G1)
H:THR 43 [OG1]	3.49	E:PRO 193 [O]

Hydrophobic interaction

chain D (LILRB1)	distance (Å)	chain B (β2m)
D:ILE 100 [CG2]	3.75	B:VAL 85 [O]
D:ILE 100 [CD1]	3.63	B:VAL 85 [O]
D:ALA 98 [O]	3.53	B:LEU 87 [CA]
D:ALA 98 [O]	3.79	B:LEU 87 [C]
D:ALA 98 [O]	3.86	B:LEU 87 [CD2]
D:TRP 67 [NE1]	3.25	B:ILE 92 [O]
D:TRP 67 [CE2]	3.91	B:ILE 92 [O]
D:TRP 67 [CZ2]	3.89	B:ILE 92 [O]
D:TRP 67 [CZ2]	3.91	B:VAL 93 [CG1]
D:TRP 67 [CZ2]	3.78	B:VAL 93 [CG2]

chain H (LILRB1)	distance (Å)	chain E (HLA-G1)
H: TYR 38 [CB]	3.85	E: PHE 195 [CD1]
H: TYR 38 [CB]	3.57	E: VAL 194 [O]
H: TYR 38 [CG]	3.97	E: PHE 195 [CG]
H: TYR 38 [CG]	3.45	E: PHE 195 [CD1]
H: TYR 38 [CG]	3.79	E: PHE 195 [CE1]
H: TYR 38 [CD1]	3.97	E: PHE 195 [CG]
H: TYR 38 [CD1]	3.88	E: PHE 195 [CD1]
H: TYR 38 [CD2]	3.47	E: PHE 195 [CD1]
H: TYR 38 [CD2]	3.32	E: PHE 195 [CE1]
H: TYR 38 [CE2]	3.92	E: PHE 195 [CD1]
H: TYR 38 [CE2]	3.33	E: PHE 195 [CE1]
H: TYR 38 [CE2]	3.66	E: PHE 195 [CZ]
H: TYR 38 [CZ]	3.79	E: PHE 195 [CE1]
H: TYR 38 [CZ]	3.71	E: PHE 195 [CZ]

chain H (LILRB1)	distance (Å)	chain F (β2m)
H: TRP 67 [CD1]	3.11	F: ILE 92 [O]
H: TRP 67 [NE1]	3.81	F: VAL 93 [CG2]
H: TRP 67 [NE1]	3.69	F: VAL 93 [CA]
H: TRP 67 [NE1]	3.66	F: ILE 92 [C]
H: TRP 67 [NE1]	2.60	F: ILE 92 [O]
H: TRP 67 [CE2]	3.86	F: ILE 92 [O]
H: TRP 67 [CZ2]	3.43	F: VAL 93 [CG1]
H: TRP 67 [CZ2]	3.97	F: VAL 93 [CA]
H: TRP 67 [CD1]	3.11	F: ILE 92 [O]
H: TRP 67 [NE1]	3.81	F: VAL 93 [CG2]
H: TRP 67 [NE1]	3.69	F: VAL 93 [CA]
H: TRP 67 [NE1]	3.66	F: ILE 92 [C]
H: TRP 67 [NE1]	2.60	F: ILE 92 [O]
H: TRP 67 [CE2]	3.86	F: ILE 92 [O]
H: TRP 67 [CZ2]	3.43	F: VAL 93 [CG1]
H: TRP 67 [CZ2]	3.97	F: VAL 93 [CA]
H: ALA 98 [O]	3.84	F: LEU 87 [CD2]
H: ALA 98 [O]	3.74	F: LEU 87 [CA]
H: TYR 99 [CD1]	3.57	F: LEU 87 [CD2]
H: TYR 99 [CE1]	3.59	F: LEU 87 [CD2]
H: ILE 100 [CG1]	3.89	F: VAL 85 [O]
H: ILE 100 [CD1]	3.89	F: VAL 85 [C]
H: ILE 100 [CD1]	2.80	F: VAL 85 [O]

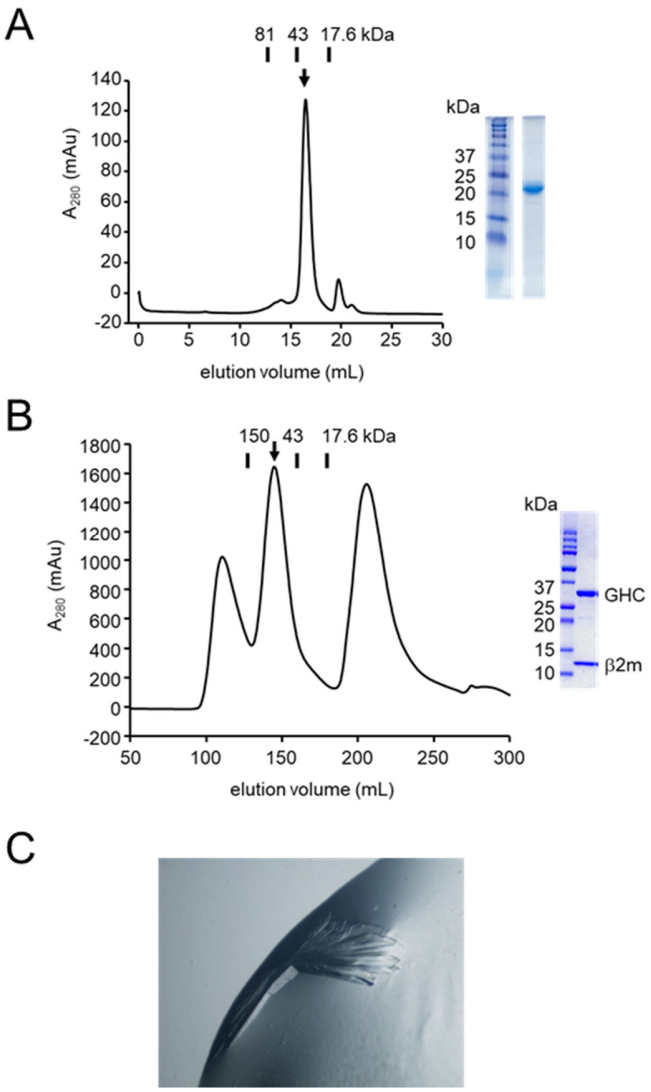

Figure S-1 Purification and crystallization of the LILRB1-HLA-G1 complex.

(A) Gel filtration chromatogram of the refolded LILRB1 protein using a Superdex-75 10/300 column and SDS-PAGE of the main peak fraction under reducing conditions. (B) Gel filtration chromatogram of the refolded HLA-G1 protein using a HiLoad 26/60 Superdex-75 column and SDS-PAGE of the peak fraction arrowed in black under reducing conditions. (C) The crystals of the LILRB1-HLA-G1 complex.

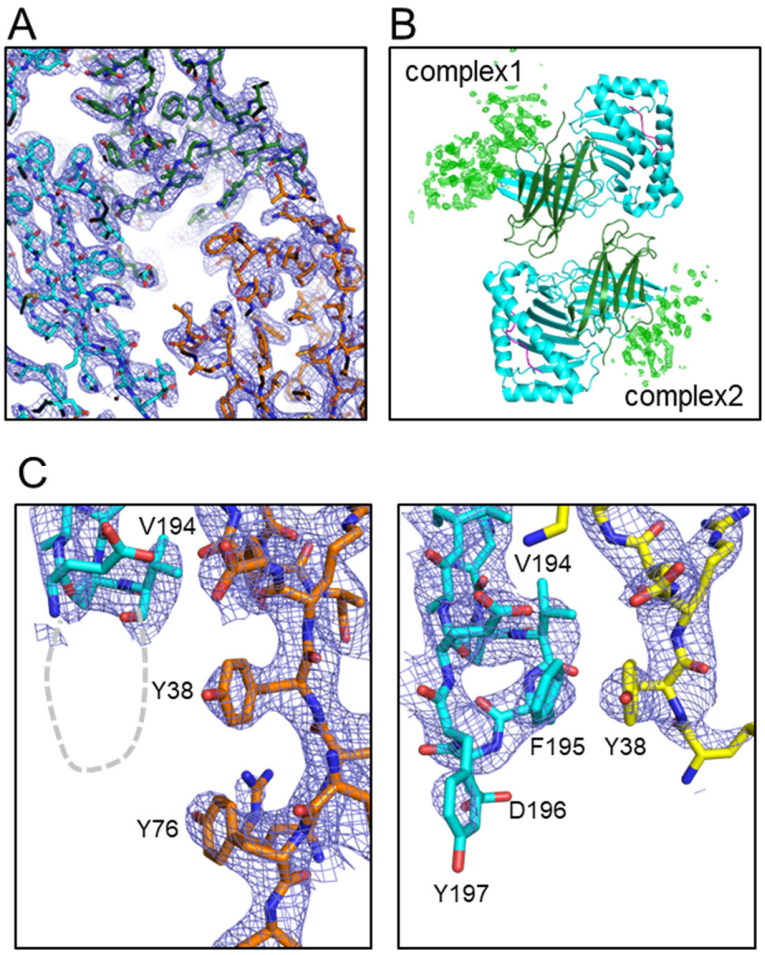

Figure S-2 Electron density map of LILRB1-HLA-G1.

(A) The 2*mF*o-*DF*c electron density map of the intersection among LILRB1 (orange), HLA-G1 α3 domain (cyan) and β2m (green) is shown as the blue mesh contoured at 1.0 σ. (B) The content of ASU in the LILRB1-HLA-G1 crystal. The LILRB1-HLA-G1 complex 1 (upper left) and 2 (lower right) are shown as a cartoon representation. The *mF*o-*DF*c electron density map is shown as a green mesh contoured at 3.0 σ. (C) The close-up view of the LILRB1-HLA-G1 interface in site 2. Protein interactions between the HLA-G1 α3 domain and LILRB1 (left; complex 1, right; complex 2) in site 2. The disordered region is shown as a dotted line with gray color (left, complex 1). The 2*mF*o-*DF*c electron density map is shown as a blue mesh contoured at 1.0 σ.

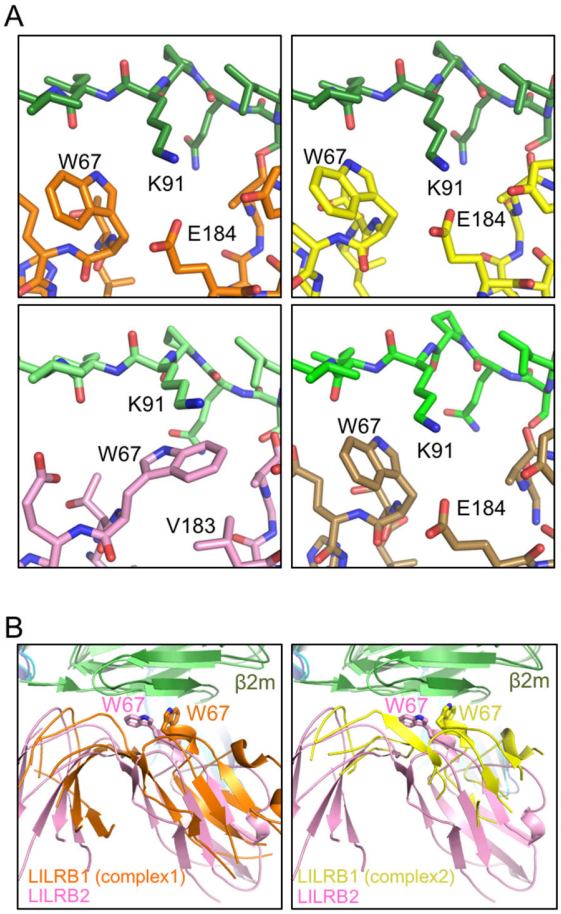

Figure S-3 The structural comparison of LILRB-β2m interfaces in LILRB-HLA complexes. (A) The close-up views of LILRB-β2m interfaces in LILRB1-HLA-G1 complex1 (upper panel, left), LILRB1-HLA-G1 complex2 (upper panel, right), LILRB2-HLA-G1 (lower panel, left) and LILRB1-HLA-A2 (lower panel, right) complexes. LILRB1 and β2m in LILRB1-HLA-G1 complex1 are colored orange and dark green, LILRB1 and β2m in LILRB1-HLA-G1 complex2 are colored yellow and dark green, LILRB2 and β2m in LILRB2-HLA-G1 are colored pink and lime green, and LILRB1 and β2m in LILRB1-HLA-A2 are colored sand yellow and green. The key residues involved with LILRB-β2m interactions are shown as sticks and are labeled. The oxygen atoms and nitrogen atoms are colored with red and blue, respectively. (B) The orientations of LILRB molecules correlating with Trp76 flips in LILRB1-HLA-G1 and LILRB2-HLA-G1 complexes. LILRB1-HLA-G1 complex1 (left) and complex2 (right) are superimposed onto LILRB2-HLA-G1. LILRB1, LILRB2 and HLA-G1 are shown as cartoon and Trp67 is shown as stick. All molecules are colored same as (A).