

## Supplemental Figure S1

### Test of the specific NK-CHAT reactivity of anti-HLA-A2 DSAs toward two distinct HLA-2 EBV-B cell lines or untransformed B cell targets

(A) Comparative analysis of rituximab and anti-HLA-A2 DSA<sup>+</sup> sera reactivity toward B cell targets. The NK-CHAT assay of anti-HLA-A2 DSA<sup>+</sup> serum obtained from a KTR patient prior to the initiation of ABMR therapy was performed using two distinct HLA-A2 EBV-transformed B cell lines. The NK-CHAT of DSA reactivity toward B cell targets expressing CD20 and HLA-A2 antigens was evaluated by the flow cytometric analysis of the serum-induced modulation of CD16 and CD107a/Lamp1 expression in NK cells exposed to target cells in the presence of either control DSA<sup>-</sup> serum (CTL Serum), CTL serum + 10 µg/mL rituximab, DSA<sup>+</sup> ABMR serum containing anti-HLA-A2 Abs, DSA<sup>+</sup> serum containing anti-HLA-A2 DSA or platelets with previously absorbed HLA-A2.

(B) Untransformed human leukocyte HLA-A2 cell targets were prepared using PBMCs freshly isolated from a healthy control blood donor following the depletion of NK cells.

(C) FCMX of anti-HLA-A2 and anti-HLA-A3 was tested on NK cell-depleted HLA-A2 PBMC targets. The specific binding of ABMR DSA<sup>+</sup> alloserum to NK cell-depleted HLA-A2 PBMC targets was analyzed by flow cytometry using an anti-human Fc PE secondary antibody (right panel).

(D) CD16 (MFI, upper panel) and CD107a/Lamp1 (% , lower panel) expression in NK cells was analyzed after incubation with targets including DSA<sup>+</sup> alloserum containing DSA with matched or unmatched specificities toward HLA-A antigens expressed on target PBMCs. Platelet absorption of serum HLA specificities partially reverted the alloserum-induced modification of CD16 and CD107a/Lamp1 expression in NK cells resulting from cognate HLA-class I antigen recognition by target allogeneic PBMCs.

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