Supporting Information

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SI Materials and Methods

Mice and Cells. BALB/c mice were purchased from the National Cancer Institute and maintained under specific pathogen-free conditions. Human PBMCs were isolated by Ficoll-Hypaque gradient centrifugation. All protocols were reviewed and approved by the Albert Einstein College of Medicine Institutional Animal Care and Use Committee and Institutional Review Board. Cell lines were cultured in complete DMEM or RPMI1640 media.

Antibodies and Flow Cytometry. Cells were incubated with Fc blocking reagents and then stained with combinations of the following antihuman antibodies: CD152-PE, CD28-PE, B7-1-PE, B7-2-PE, PD-1-PE, PD-L1-PE, PD-L2-PE, ICOS-PE, ICOSL-PE, B7H4-PE, CD14-FITC, CD19-FITC, CD8a-PerCP-Cy5.5, CD4-APC, CD83-APC, streptavidin, and isotype controls (eBioscience). Biotinylated anti-hB7-H3 was purchased from R&D. For receptor binding, cells were incubated with HHLA2-Ig, B7x-Ig, or control Ig for 45 min on ice and then stained with PE-antihuman IgG Fc (Jackson Immunoresearch). Samples were acquired on a FACS-Calibur, LSRII or LSRII yellow (BD Biosciences), and analyzed with FlowJo (Treestar).

Cytokine Analysis. Aliquots of supernatants were collected at 70 h after initiation of T cell cultures. Th1/Th2/Th9/Th17/Th22 13plex FlowCytomix Multiplex (eBioscience) was used for the measurement of human IFN-γ, IL-1β, IL-2, IL-4, IL-5, IL-6, IL-9, IL-10, IL-12 p70, IL-13, IL-17A, IL-22, and TNF-α according to the manufacturer's instructions.

Confocal Microscopy. Cells were seeded on glass-bottom microwell dishes (MatTek) for 48 h and then were observed by using Leica SP2 confocal microscopy.

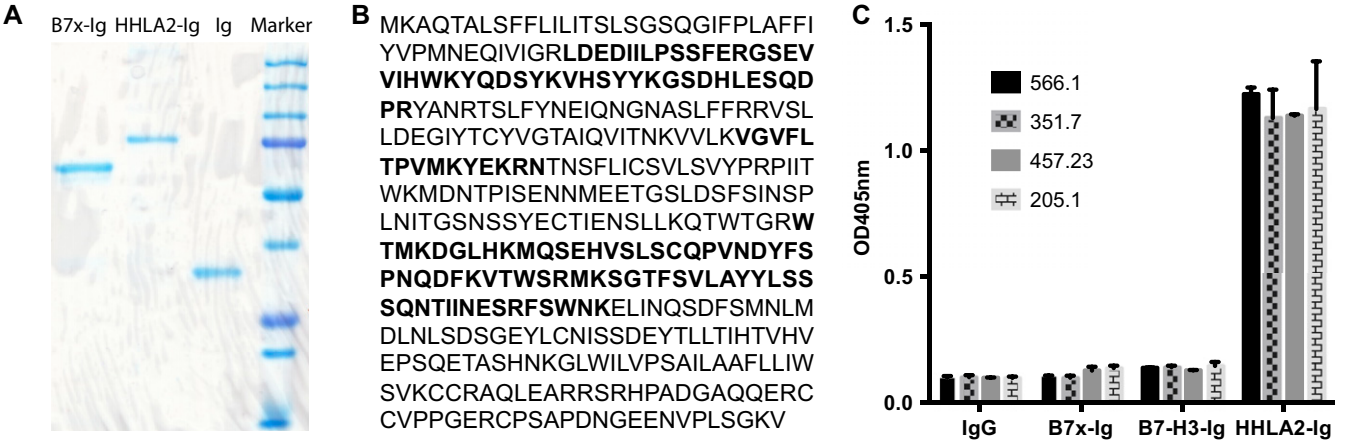

Fig. S1. Purification of fusion proteins and screening of anti-HERVHLTR-associating 2 (HHLA2) monoclonal antibodies. (*A*) HHLA2-Ig, B7x-Ig, and control Ig were purified and run on SDS/PAGE gel. (*B*) Purified HHLA2-Ig protein was sequenced with MALDI-TOF-MS/MS and identified amino acid sequences are in bold. (*C*) Anti-HHLA2 monoclonal antibodies 566.1 (IgG1), 351.7 (IgG1), 457.23 (IgG1), and 205.1 (IgG1) were screened by ELISA with human IgG, B7x-Ig, B7-H3-Ig, and HHLA2-Ig as antigens.