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PILRα Is a Herpes Simplex Virus-1 Entry Coreceptor That Associates with Glycoprotein B

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Supplemental Experimental Procedures

Construction of plasmids

A cDNA fragment of human PILRα (GenBank accession number: AF161080) was amplified by RT-PCR from human PBMC cDNA by using sense primer (5'-AAT GAA TTC AAC AAG GCC ATG GGT CGG-3') and antisense primer (5'- AAT AAT GCG GCC GCA GGG CTG TCC ATT GGT TAG G-3'). This fragment was inserted into Eco RI and Not I sites of the pMXs-IRES-DsRed vector. Mouse MHC class I H2-K^b cloned into pMXs-IRES-DsRed was used for control transfections.

For the generation of Ig fusion proteins, a cDNA fragment corresponding to the extracellular domain of human PILRα, human HVEM (GenBank accession number: NM_003820) or human Nectin-1 (GenBank accession number: NM_002855) was amplified by PCR using sense primer (5'-AAT CTC GAG CAG CCT AGT GGC TCC ACA GG-3') and anti-sense primer (5'-AAT AAT CTC GAG AAC CCT GAT GGC AGT GTC-3'), sense primer (5'- AAT CTC GAG CCA GCT CTG CCG TCC TGC AA-3') and anti-sense primer (5'- AAT CTC GAG CCA TAC CCA GTG GGA GCT GC-3') or sense primer (5'-AAT CTC GAG AAC GAC TCC ATG TAT GGC TT-3') and anti-sense primer (5'-AAT CTC GAG GGG CAC CGG CCC GGC GCG CC-3'), respectively. These fragments were inserted into the Xho I cloning site of a modified pME18S expression vector that contained a mouse CD150 leader segment at the N-terminus and the Fc segment of human IgG1 at the C-terminus (GenBank accession number: AAH14667; positions: 249-479), in which the leucines at position 266 and 267 of the Fc were mutated to alanine and glutamine,

respectively, in order to reduce the affinity of binding to cellular Fc receptors (Shiratori et al., 2004). Furthermore, histidine at position 467 was mutated to arginine in order to reduce the affinity of binding to HSV-1 Fc receptor (gE) (Chapman et al., 1999).

cDNA fragments corresponding to the HSV-1 gB that lacks cytoplasmic 40 amino acids (GenBank accession number: M14164), full length gD (GenBank accession number: X14112), gH, and gL were amplified by PCR using the following primers: gB, sense primer (5'-AAT GGA TCC GGC CCC CGT AGT CCC GCC AT -3') and anti-sense primer (5'-AAT AAT CTC GAG TCA CGC GCT CGT GCC CTT CTT CT-3'); gD, sense primer (5'- CAC CCG ATC ATC AGT TAT CCT TA -3') and anti-sense primer (5'- TGG GTG CGA CAG GCG GAA CAG -3'); gH, sense primer (5'-CAC CAC CCC AAC CCC CAA GTT CG-3') and anti-sense primer (5'-TTC GGT CGG GCG GAG AAA CGG-3'); gL, sense primer (5'-AAT GGA TCC TGG TTG TGT TAC TGG GCA AA-3') and anti-sense primer (5'-AAT AAT GCG GCC GCG ACG AGG AAA ACG TCA CAC C-3'). These fragments were cloned into pcDNA3.1 expression vector (Invitrogen).

Supplemental References

Chapman, T. L., You, I., Joseph, I. M., Bjorkman, P. J., Morrison, S. L., and Raghavan, M. (1999). Characterization of the interaction between the herpes simplex virus type I Fc receptor and immunoglobulin G. J. Biol. Chem. *274*, 6911-6919.

Shiratori, I., Ogasawara, K., Saito, T., Lanier, L. L., and Arase, H. (2004). Activation of natural killer cells and dendritic cells upon recognition of a novel CD99-like ligand by paired immunoglobulin-like type 2 receptor. J. Exp. Med. *199*, 525-533.

Supplemental Figures

Figure S1. Mass spectrometry analysis of 110 kDa protein precipitated by PILR-Ig

Amino acid sequences of HSV-1 gB (upper panel) and gH (lower panel) identified by mass spectrometry analysis. Identified peptides are shown in red. 29 peptide sequences identical to gB, and 2 peptide sequences identical to gH were obtained.

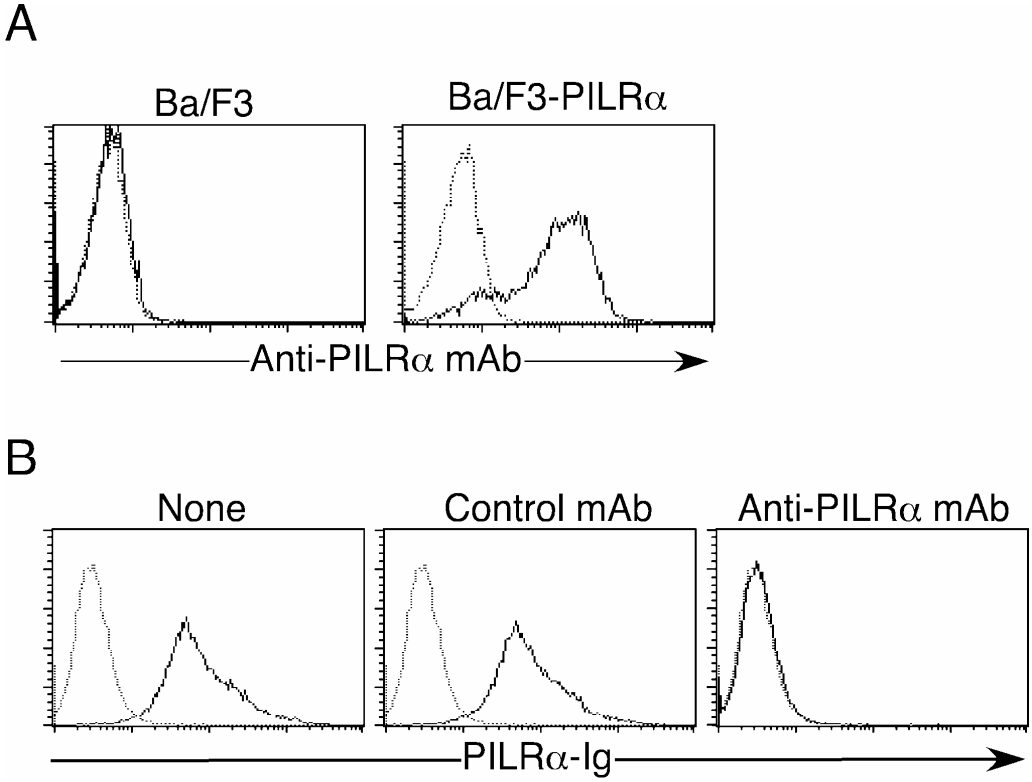

Figure S2. Generation of a blocking anti-human PILRα mAb

(A) Anti-human PILRα mAb (M4) specifically recognizes human PILRα-transduced Ba/F3 cells. Ba/F3 cells stably transduced with human PILRα were stained with anti-human PILRα mAb (M4) (solid line) or control mAb (dotted line), followed by PE-labeled anti-mouse IgG Ab.

(B) Anti-human PILRα mAb (M4) blocks the binding of PILRα-Ig to gB. PILRα-Ig was incubated with anti-PILRα mAb for 30 min and was mixed with 293T cells transiently transfected with gB that lacks the C-terminal 40 amino acids in its cytoplasmic tail, followed by incubation with PE-labeled anti-human IgG Ab (solid line). As a control, cells stained with PE-labeled anti-human IgG Ab alone are shown (dotted line)

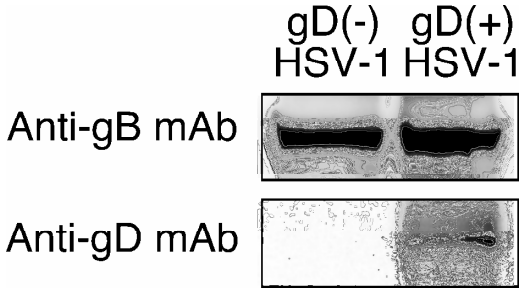

Figure S3 Western blot analysis of gD-deficient virus
gD (-) and gD (+) viruses were analyzed by SDS-PAGE and were blotted with anti-gB and anti-gD mAbs. Because gD-deficient virus is non-infectious, concentrations of virions were verified by the relative amounts of gB.

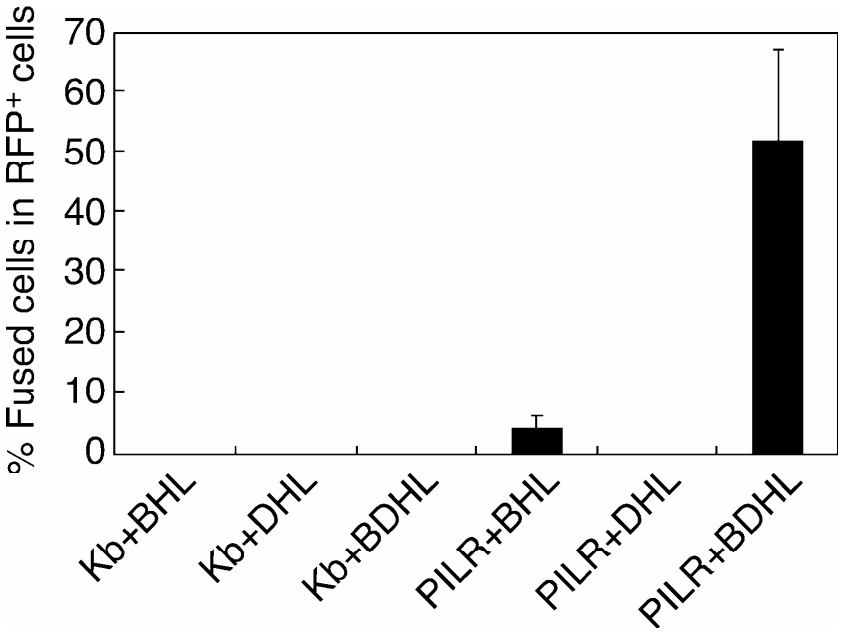

Figure S4. Frequency of fused cells in fusion assay
gB, gD, gH and gL (BDHL), gB, gH and gL (BHL), or gD, gH, and gL (DHL) were co-transfected into CHO-K1 cells with GFP. PILRα or control plasmid (Mock) was co-transfected into CHO-K1 cells with a RFP-containing vector. Numbers of cells expressing RFP alone or both RFP and GFP were counted and the frequency of cells expressing both RFP and GFP among RFP-positive cells was calculated. Mean ± SD of 3 independent fields are shown.

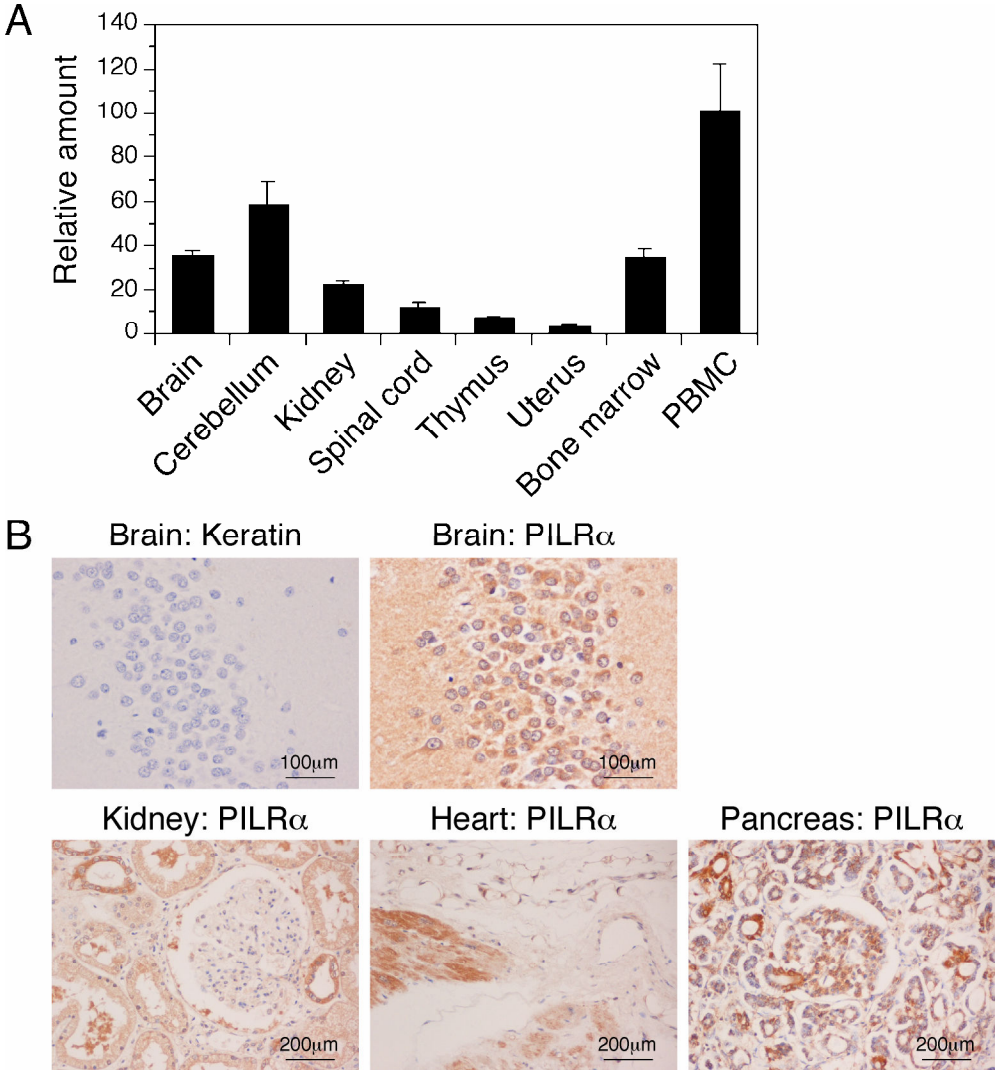

Figure S5. Expression of PILRα in various human tissues

(A) Real-time quantitative RT-PCR analysis of PILRα transcripts in various tissues. Expression levels of transcript for PILRα were analyzed by real-time RT-PCR by using the delta CT method. Data were normalized as the ratio to β-actin and relative amounts of PILRα expression in various tissues to PBMC were shown. Data are presented as mean ± SD of triplicates. Representative data from 4 independent analyses are shown.

(B) Immunohistochemical analysis of PILRα expression. Tissue sections from human brain (hippocampus), kidney, heart, and pancreas were stained with anti-PILRα or anti-Keratin antibodies. Granular cells of the hippocampus in the brain, urinary epithelial cells of both proximal and distal tubules in the kidney, cardiomyocytes of the heart, and epithelial cells of pancreatic duct and acinus, and neuroendocrine cells of Langerhans island in the pancreas were stained with anti-PILRα mAb. Anti-PILRα mAb did not react with glomerulus and interstitial mesenchymal cells of the kidney, fatty tissue, or vascular endothelial cells of the pericardium.

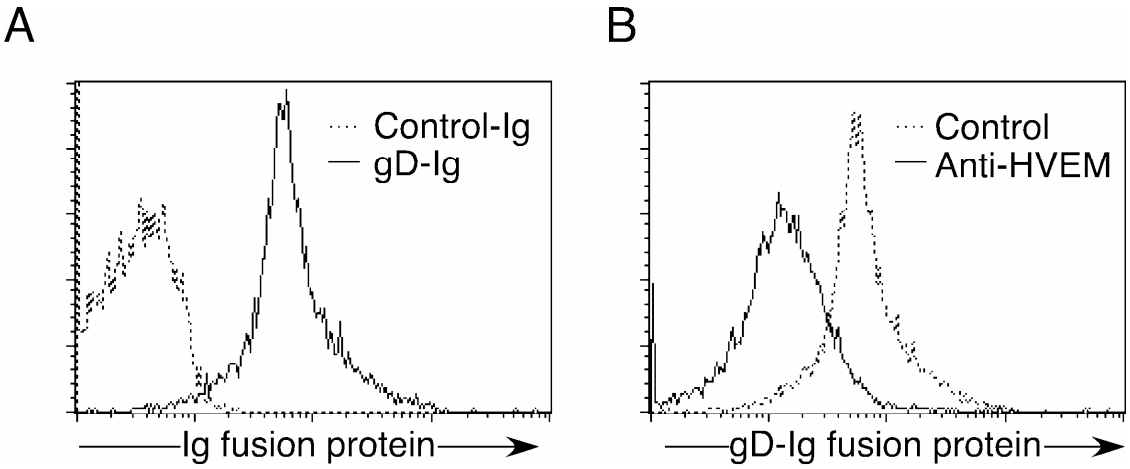

Figure S6. HVEM expressed on CD14-negative peripheral blood mononuclear cells binds gD.
(A) CD14-negative lymphocyte population was stained with gD-Ig (solid line) or control Ig (dotted line) fusion protein.
(B) CD14-negative lymphocyte population was stained with gD-Ig fusion protein in the presence of anti-HVEM serum (solid line) or control serum (dotted line). Binding of gD-Ig fusion protein to CD14-negative lymphocyte population was blocked by anti-HVEM serum but not control serum.