### Supplemental Table 1. Kinetics parameters for the interactions between CD160 or BTLA and HVEM compared with those of other protein-protein interactions.

<table>
<thead>
<tr>
<th>Analyte</th>
<th>Immobilized</th>
<th>$k_{on}$ x10^5 (M⁻¹s⁻¹)</th>
<th>$k_{off}$ (s⁻¹)</th>
<th>$K_{d,kin}$ (µM)</th>
<th>$K_{d,eq}$ (µM)</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD160 (I27-S159)</td>
<td>HVEM</td>
<td>3.4</td>
<td>0.044</td>
<td>0.13</td>
<td>0.34 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>*0.72</td>
<td>*0.012</td>
<td>*0.17</td>
<td>*ND</td>
<td></td>
</tr>
<tr>
<td>BTLA (S33-D135)</td>
<td>HVEM</td>
<td>6.2</td>
<td>0.074</td>
<td>0.12</td>
<td>0.29 ± 0.01</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>*ND</td>
<td>*ND</td>
<td>*ND</td>
<td>*0.25</td>
<td></td>
</tr>
<tr>
<td><strong>Other protein-protein interactions</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>E-selectin</td>
<td>ESL</td>
<td>0.48</td>
<td>2.7</td>
<td>56</td>
<td></td>
<td>1</td>
</tr>
<tr>
<td>L-selectin</td>
<td>GlyCAM-1</td>
<td>&gt;1</td>
<td>&gt;10</td>
<td>108</td>
<td></td>
<td>2</td>
</tr>
<tr>
<td>P-selectin</td>
<td>PSGL-1</td>
<td>44</td>
<td>1.4</td>
<td>0.32</td>
<td></td>
<td>3</td>
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<tr>
<td>LILRB1D1D2</td>
<td>UL18</td>
<td>1.4</td>
<td>0.0028</td>
<td>0.0021</td>
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<td>4</td>
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<tr>
<td>LILRB1D1D2</td>
<td>HLA-B35</td>
<td>5.0</td>
<td>3.7</td>
<td>7.4</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>LILRB1D1D2</td>
<td>HLA-Cw4</td>
<td>6.3</td>
<td>5.0</td>
<td>8.1</td>
<td></td>
<td>5</td>
</tr>
<tr>
<td>KIR2DL3</td>
<td>HLA-Cw7/DS11</td>
<td>2.1</td>
<td>1.1</td>
<td>5.2</td>
<td></td>
<td>6</td>
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<tr>
<td>CD8αα</td>
<td>MHC class I</td>
<td>≥1.0</td>
<td>≥18</td>
<td>~200</td>
<td></td>
<td>7</td>
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<tr>
<td>CD22</td>
<td>CD45</td>
<td>≥1.5</td>
<td>≥18</td>
<td>117</td>
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<td>8</td>
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<tr>
<td>CD80</td>
<td>CTLA-4</td>
<td>9.4</td>
<td>0.43</td>
<td>0.46</td>
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<td>9</td>
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<tr>
<td>CD80</td>
<td>CD28</td>
<td>6.6</td>
<td>1.6</td>
<td>2.4</td>
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<td></td>
</tr>
<tr>
<td>IgE-Fc</td>
<td>sFcεRIα</td>
<td>2.5</td>
<td>6.5</td>
<td>0.0054</td>
<td></td>
<td>10</td>
</tr>
<tr>
<td>FeyRIIa,IIb,III</td>
<td>hFcε1</td>
<td>3.8 − 4.4</td>
<td>0.31 − 0.69</td>
<td>0.72 − 1.9</td>
<td></td>
<td>11</td>
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<tr>
<td>TCR</td>
<td>Peptide-MHC</td>
<td>0.009 − 0.2</td>
<td>0.01 − 0.1</td>
<td>1 − 90</td>
<td></td>
<td>12, 13</td>
</tr>
</tbody>
</table>

The $K_{d,eq}$ values were obtained from the equilibrium analysis.

The $K_{d,kin}$ values were calculated from the simple 1:1 Langmuir binding model.

* Values were reported previously and are shown here for comparison. ND, not determined.

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**References**


Supplemental Figure 1. The MALDI-TOF mass spectrometric analysis of the extracellular Ig-V set domain of CD160h.

(A) MALDI-TOF mass spectrometric analysis of the purified CD160h Ig-V set domain expressed in HEK293 GNTI cells (Figure 1A, mixture of arrowheads 3-6) shows a molecular mass consistent with a monomer. The molecular mass of 18145.7 was detected. This molecular mass indicates the monomer Ig-V set domain of CD160h (estimated molecular weight; 15904.8) with two sugar modifications (Man5GlcNAc2) derived from HEK293S GNTI cells.

(B) The extracellular Ig-V set domain of CD160 expressed in \textit{E. coli} and refolded \textit{in vitro} (Supplemental Figure 2A, arrowhead) also showed the monomer molecular mass. A molecular mass of 14819.7, similar to the estimated molecular weight 14795.7, was detected.

Supplemental Figure 2. The extracellular Ig-V set domain of CD160, expressed in \textit{E. coli} and refolded \textit{in vitro}, is also monomeric.

(A) The inclusion bodies of the extracellular Ig-V set domain of CD160 expressed in BL21(DE3)plysS cells was refolded and purified by gel filtration chromatography (Superdex 75 26/60). The CD160 Ig-V set domain eluted in the 15 kDa range (Arrowhead).

(B) The CD160 Ig-V set domain purified in (A) was denatured with SDS sample buffer, with or without DTT, and resolved in a 15% SDS-PAGE gel followed by Coomassie Brilliant Blue (CBB) staining.

Supplemental Figure 3. DNTB assay of the refolded CD160.

DNTB assay of the extracellular Ig-V set domain of CD160, expressed in \textit{E. coli} and refolded \textit{in vitro} (Supplemental Figure 2A, arrowhead). A standard SH (reduced glutathione) calibration
curve with error bars is shown. Several concentrations of reduced glutathione were mixed with DNTB, and the OD$_{412}$ values were measured. The curve-fitting equation is $Y = -5.2 \times 10^{-3} + 0.4 \times 10^{-3}X$; the correlation coefficient obtained is $R^2 = 0.998$. The OD$_{412}$ values for 150 µM and 200 µM CD160 were measured three times at each concentration, and the estimated free SH concentrations are indicated. From these data, we conclude that refolded CD160 contains one non-paired thiol group.

**Supplemental Figure 4. Binding responses of CD160 and BTLA to HVEM.**
The purified, biotinylated form of either HVEM or BSA (negative control) was immobilized on a streptavidin-coupled CM5 sensor chip, and different concentrations of purified (A) CD160 and (B) BTLA were injected onto the chip. Analyte concentrations were the same in (A) and (B) (2-fold serial dilutions, 3 µM highest concentration). The solid lines indicate HVEM responses, and the dotted lines indicate BSA responses.

**Supplemental Figure 5. Purification of BTLA Ig-V set domain.**
(A) The inclusion bodies containing the BTLA Ig-V set domain (S33-D135) expressed in BL21(DE3)plysS cells were refolded and purified by gel filtration chromatography (Superdex 75 26/60). The eluted BTLA (Arrowheads 1-6) was concentrated and used for SPR analysis. Arrowheads 1-6 indicate the fraction numbers.

(B) BTLA purified in (A) was denatured with SDS sample buffer, mixed with DTT, and resolved on a 15% SDS-PAGE gel followed by CBB staining. The numbers 1-6 on the top of the membrane are the fractions 1-6 shown in (A). M indicates the protein size marker.
Supplemental Figure 1

A

B

Intensity (a.u.)

Mass (m/z)

14819.7

18145.7

Intensity (a.u.)

Mass (m/z)

14819.7
Supplemental Figure 2

A

B

Volume (ml)

A_{280} (mAU)

kDa

DTT

+ -

100

75

50

37

25

20

15

10
Supplemental Figure 3

\[ Y = -5.2 \times 10^{-3} + 0.4 \times 10^{-3} X \]
\[ R^2 = 0.998 \]

<table>
<thead>
<tr>
<th>CD160 conc. (μM)</th>
<th>OD_{412}</th>
<th>estimated free SH conc. (μM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>150</td>
<td>0.050 / 0.048 / 0.045</td>
<td>132 ± 6.3</td>
</tr>
<tr>
<td>200</td>
<td>0.067 / 0.073 / 0.069</td>
<td>187 ± 7.6</td>
</tr>
</tbody>
</table>
Supplemental Figure 4

A

CD160

- HVE M immobilized
- BSA immobilized

B

BTLA

- HVE M immobilized
- BSA immobilized