**Supplementary Files for**

**Molecular Features Underlying Differential SHP1/SHP2 Binding of Immune Checkpoint Receptors**

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**Supplementary File 1.** *K*d values of individual SH2 of SHP1/SHP2 and phosphorylated ITIM/ITSM of PD-1/BTLA; mean ± s.d. (n = 3).

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| *K*d (M) | PD-1-pITIM | PD-1-pITSM | BTLA-pITIM | BTLA-pITSM |
| SHP1-nSH2 | 0.27 ± 0.1 | 0.083 ± 0.020 | 0.064 ± 0.012 | n.d. |
| SHP1-cSH2 | n.d. | 1.7 ± 1.4 | 1.8 ± 0.20 | 0.86 ± 0.31 |
| SHP2-nSH2 | 0.38 ± 0.10 | 0.14 ± 0.049 | 0.34 ± 0.082 | 2.1 ± 1.1 |
| SHP2-cSH2 | 1.4 ± 0.57 | 0.10 ± 0.029 | 1.1 ± 0.54 | 1.1 ± 0.19 |

n.d.: not detected

**Supplementary File 2.** ∆G of PD-1/BTLA interactions with SHP1/SHP2-tSH2 in a parallel or an anti-parallel mode.

|  |  |  |
| --- | --- | --- |
| ∆G (kJ/mol)a | Parallel | Anti-Parallel |
| PD-1:SHP1-tSH2 | -70.4 | -40.4 |
| PD-1:SHP2-tSH2 | -76.6 | -72.5 |
| BTLA:SHP1-tSH2 | -75.7 | -32.8 |
| BTLA:SHP2-tSH2 | -70.9 | -66.4 |

a: ∆G = RT\*ln (Kd), showing the change of Gibbs free energy;

T = 298.15 K, R is molar gas constant, R = 8.314 × 10-3 kJ/K/M

**Supplementary File 3.** ∆G of ITIM/ITSM and SH2 bindings.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| ∆G (kJ/mol)a | SHP1-nSH2 | SHP1-cSH2 | SHP2-nSH2 | SHP2-cSH2 |
| PD-1-pITIM | -37.5 | 0.0 | -36.6 | -33.4 |
| PD-1-pITSM | -40.4 | -32.9 | -39.1 | -40.0 |
| BTLA-pITIM | -41.1 | -32.8 | -36.9 | -34.0 |
| BTLA-pITSM | 0.0 | -34.6 | -32.4 | -34.0 |

a: ∆G = RT\*ln (Kd), showing the change of Gibbs free energy;

T = 298.15 K, R is molar gas constant, R = 8.314 × 10-3 kJ/K/M

**Supplementary File 4.** Immune receptors harboring both ITIM and ITSM

|  |  |  |  |
| --- | --- | --- | --- |
| Receptor | ITIM Y position  (AA) | ITSM Y position  (AA) | ITIM-ITSM distance (AA)a |
| PD-1 | 223 | 248 | 25 |
| BTLA | 257 | 282 | 25 |
| Siglec-3 | 340 | 358 | 18 |
| Siglec-5 | 520 | 544 | 24 |
| Siglec-6 | 426 | 446 | 20 |
| Siglec-9 | 433 | 456 | 23 |
| Siglec-11 | 644 | 668 | 24 |
| CD300LF | 249 | 284 | 35 |
| VSTM4 | 289 | 310 | 21 |
| SIRPα | 429  470  496 | 453 | 24  -16  -43 |
| SLAMF5 | 341 | 279  316 | -62  -25 |
| SLAMF6 | 274 | 285  309 | 11  35 |
| PECAM1 | 690 | 713 | 23 |
|  |  |  |  |
| MPIG6B | 211 | 237 | 26 |
| AA: amino acids  ITIM: immunoreceptor tyrosine-based inhibition motif  ITSM: immunoreceptor tyrosine-based switch motif  a: Calculated as (ITSM Y position) - (ITIM Y position) | | | |