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## Supplemental Information

# Computational Redesign of PD-1 Interface for PD-L1 Ligand Selectivity

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Supplementary Table 1. (Related to Fig. 3) The first three columns list hPD-L1, hPD-1 residues in the interface and the corresponding rs-pharmacophore preferences for PD-L1. The last three columns show, in a similar fashion, the rs-pharmacophores for mPD-L2, and the structurally corresponding interface residues of mPD-1 and mPD-L2. The central column lists single residue mutants of hPD1 that were selected to induce PD-L1 specificity. Amino acids in red color represent matched rs-pharmacophores with wild type residues, whereas blue colored amino acids in rs-pharmacophore refer to previously studied mutants, not explored experimentally in the current study. In column with star (*), positions are shown for mouse PD1, while in the brackets numbers refer to the equivalent position in human PD1 (4ZQK.B). # indicates structurally aligned positions between mouse and human PD-L2 when these are different residues.

| **human PD-L1/PD1 (4ZQK)** | | | designs | **mouse PD-L2/PD1 (3BP5)** | | |
|---|---|---|---|---|---|---|
| Interface of hPD-L1 (4ZQK.A) | Interface of hPD1 (4ZQK.B) | rs-pharmacophore | | rs-pharmacophore | Interface of mPD1 (3BP5.A) * | Interface of mPD-L2 (3BP5.B) |
| F19 | K78 | HKNQRTW | K78R | HKNQW | K45(78) | F21 |
| D26 | Q75, S73 | HKNQRWY | Q75N, S73RK | HKNQRWY | Q42(75), S40(73) | E28 |
| Y56 | A132, I134 | HIKLMNPQRVWY | I134QEDTNF | HNPQRSTWY | A99(132), I101(134) | Q60 |
| Q66 | A132, K131 | HKNPQRTWY | K131H | KNQRTWY | K100(131) | S67 |
| R113 | E136 | DEPQSY | E136QN | | | |
| M115 | I126, L128 | FIMVWY | I126V, L128VMW | DFHIKLMNPQRSTVWY | I93(126), A92(125), N33(66), K45(78), G91 (124), M31(64)# | W110 |
| G120 | E84 | HPY | E84Y | | | |
| A121 | N66, K78 | KNQRSTWY | N66QE | | | |
| D122 | K78, Y68 | HKNQRTWY | Y68KRNQ | HKNQRSWY | K45(78) | D111 |
| Y123 | E136, G124, I126, I134, T76, Y68 | DEFHIKLMNPQRTVWY | I126V, T76DQENSY, G124VMY | DEFHLMNPQRSTVWY | E103(136), I101(134), T43(76), N35 (68)# | Y112 |
| K124 | D77, T76 | DENPQSTY | D77E | DENQST | T43(76) | K113 |
| R125 | Q75 | HNPQSTW | | HIKLMNPQRSTVWY | N41(74), Q42(75), E103(136), T43(76) | Y114 |

Supplementary Table 2. (Related to Fig 3) List of tested mutations of PD-1 (first column), five technical replicates of cell-assay-based experiments. The second and third column show average binding, normalized to wild type to PD-L1 and PD-L2. The fourth and fifth columns are the corresponding standard deviations. The sixth and seventh columns are the t-statistics and corresponding two-tailed p-values for differences in PD-1 binding affinity to PD-L1 and PD-L2.

| Mutation | AVE-hPD-L1 | AVE-hPD-L2 | STD-hPD-L1 | STD-hPD-L2 | t - statistics | Two-tailed p-value |
|---|---|---|---|---|---|---|
| N66Q | 3.62E-01 | 1.05E-02 | 1.14E-01 | 4.80E-03 | 6.91E+00 | 2.27E-03 |
| N66E | 6.10E-03 | 4.38E-03 | 4.03E-03 | 3.19E-03 | 7.49E-01 | 4.76E-01 |
| Y68N | 1.06E+00 | 1.08E-01 | 9.96E-02 | 9.14E-02 | 1.58E+01 | 2.84E-07 |
| Y68K | 7.75E-01 | 2.23E-02 | 8.98E-03 | 1.88E-02 | 8.07E+01 | 5.54E-10 |
| Y68R | 8.76E-01 | 3.34E-02 | 8.44E-03 | 1.03E-02 | 1.42E+02 | 1.89E-14 |
| Y68Q | 9.47E-01 | 2.02E-01 | 2.52E-02 | 8.28E-02 | 1.92E+01 | 1.12E-05 |
| S73R | 9.71E-01 | 1.11E+00 | 4.50E-02 | 8.98E-02 | -3.17E+00 | 1.98E-02 |
| S73K | 9.68E-01 | 1.00E+00 | 2.71E-02 | 1.26E-01 | -6.38E-01 | 5.55E-01 |
| Q75N | 9.70E-01 | 1.13E+00 | 2.23E-02 | 5.07E-02 | -6.44E+00 | 9.38E-04 |
| T76D | 1.07E+00 | 2.05E-01 | 1.02E-01 | 1.11E-01 | 1.28E+01 | 1.40E-06 |
| T76Q | 9.57E-01 | 9.80E-01 | 5.88E-02 | 9.46E-02 | -4.65E-01 | 6.56E-01 |
| T76E | 9.64E-01 | 6.42E-01 | 4.14E-02 | 7.93E-02 | 8.06E+00 | 1.91E-04 |
| T76N | 9.28E-01 | 9.98E-01 | 8.56E-02 | 9.13E-02 | -1.26E+00 | 2.43E-01 |
| T76S | 8.85E-01 | 9.54E-01 | 2.92E-02 | 9.69E-02 | -1.51E+00 | 1.95E-01 |
| T76Y | 1.08E+00 | 1.11E+00 | 7.99E-02 | 8.81E-02 | -7.18E-01 | 4.93E-01 |
| D77E | 9.57E-01 | 1.13E+00 | 3.87E-02 | 4.01E-02 | -6.79E+00 | 1.40E-04 |
| K78R | 7.17E-01 | 1.25E-01 | 2.38E-02 | 1.02E-01 | 1.26E+01 | 1.23E-04 |
| E84Y | 9.87E-01 | 9.99E-01 | 3.43E-02 | 5.26E-02 | -4.10E-01 | 6.94E-01 |
| G124V | 9.61E-01 | 1.16E+00 | 2.95E-02 | 9.93E-02 | -4.26E+00 | 9.21E-03 |
| G124M | 8.45E-01 | 7.05E-01 | 4.30E-02 | 1.28E-01 | 2.32E+00 | 6.92E-02 |
| G124Y | 6.51E-01 | 5.57E-01 | 1.28E-01 | 1.87E-01 | 9.21E-01 | 3.88E-01 |
| I126V | 8.63E-01 | 7.11E-01 | 5.95E-02 | 1.47E-01 | 2.15E+00 | 8.13E-02 |
| L128V | 8.82E-01 | 1.03E+00 | 2.45E-02 | 1.12E-01 | -2.82E+00 | 4.29E-02 |
| L128W | 8.97E-01 | 1.03E+00 | 4.68E-02 | 1.12E-01 | -2.50E+00 | 5.12E-02 |
| K131H | 9.94E-01 | 1.58E+00 | 9.26E-02 | 1.55E-01 | -7.24E+00 | 2.38E-04 |
| I134F | 7.54E-01 | 1.11E+00 | 1.53E-01 | 2.68E-01 | -2.57E+00 | 4.01E-02 |
| I134Q | 9.79E-02 | 2.52E-02 | 6.55E-02 | 2.86E-02 | 2.27E+00 | 6.76E-02 |

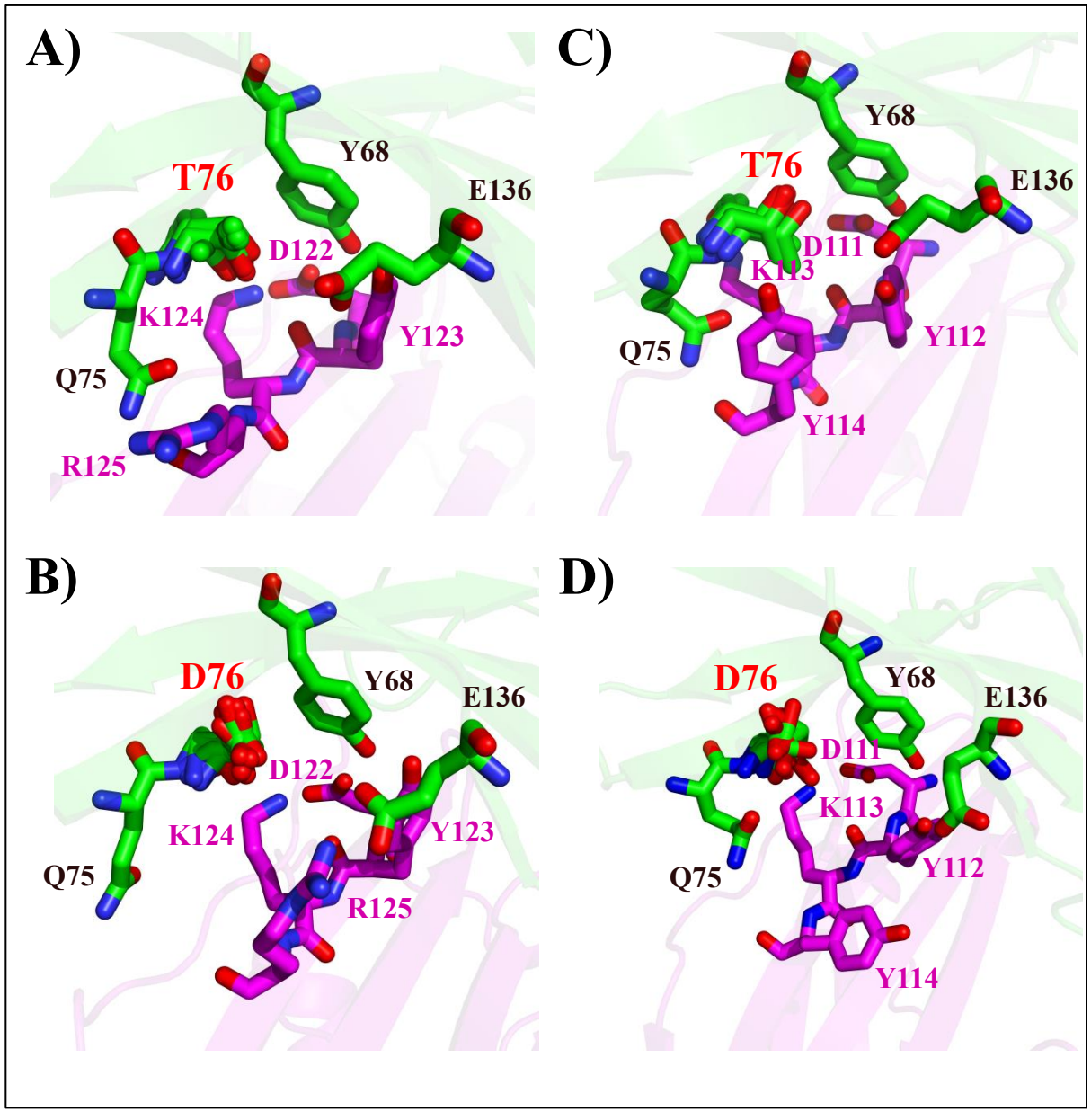
| | | | | | | |
|---|---|---|---|---|---|---|
| I134D | 1.30E-02 | 1.05E-02 | 8.74E-03 | 6.40E-03 | 5.06E-01 | 6.28E-01 |
| I134T | 3.79E-01 | 3.07E-01 | 1.17E-01 | 1.48E-01 | 8.56E-01 | 4.18E-01 |
| I134N | 4.24E-01 | 5.34E-02 | 1.40E-01 | 5.08E-02 | 5.58E+00 | 2.49E-03 |
| E136Q | 8.61E-01 | 9.67E-01 | 7.45E-02 | 7.95E-02 | -2.17E+00 | 6.17E-02 |
| E136N | 8.89E-01 | 8.20E-01 | 4.31E-02 | 5.45E-02 | 2.23E+00 | 5.84E-02 |
| WT | 1 | 1 | 0 | 0 | | |

Supplemental Table 3 (related to Figure 3)

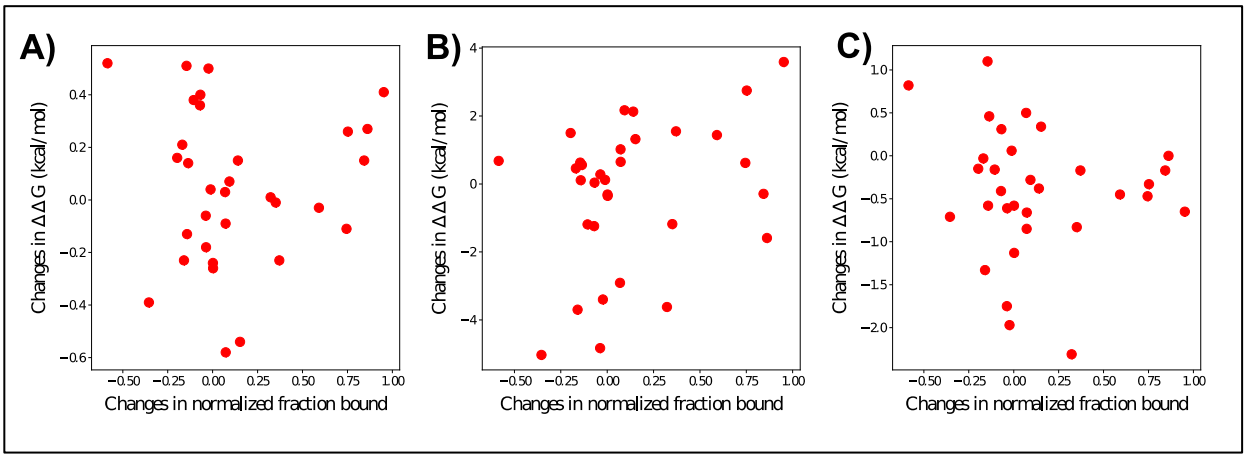
| Mutation | Forward PRIMER | Reverse PRIMER |
|---|---|---|
| N66Q | GAGCTTCGTGCTACAGTGGTACCGCATGAG | CTCATGCGGTACCACTGTAGCACGAAGCTC |
| N66E | GAGCTTCGTGCTAGAGTGGTACCGCATGAG | CTCATGCGGTACCACTCTAGCACGAAGCTC |
| Y68K | CGTGCTAAACTGGAAACGCATGAGCCCCAG | CTGGGGCTCATGCGTTTCCAGTTTAGCACG |
| Y68R | CGTGCTAAACTGGAGACGCATGAGCCCCAG | CTGGGGCTCATGCGTCTCCAGTTTAGCACG |
| Y68N | GTGCTAAACTGGAACCGCATGAGCC | GGCTCATGCGGTTCCAGTTTAGCAC |
| Y68Q | CGTGCTAAACTGGCAACGCATGAGCCCCAG | CTGGGGCTCATGCGTTGCCAGTTTAGCACG |
| S73R | CATGAGCCCCAGAAACCAGACGGAC | GTCCGTCTGGTTTCTGGGGCTCATG |
| S73K | CGCATGAGCCCCAAAAACCAGACGGAC | GTCCGTCTGGTTTTTGGGGCTCATGCG |
| Q75N | GAGCCCCAGCAACAATACGGACAAGCTGG | CCAGCTTGTCCGTATTGTTGCTGGGGCTC |
| T76D | CCCAGCAACCAGGACGACAAGCTGGCC | GGCCAGCTTGTCGTCCTGGTTGCTGGG |
| T76Q | CCCAGCAACCAGCAGGACAAGCTGG | CCAGCTTGTCCTGCTGGTTGCTGGG |
| T76E | CCCAGCAACCAGGAGGACAAGCTGG | CCAGCTTGTCCTCCTGGTTGCTGGG |
| T76N | CCAGCAACCAGAACGACAAGCTGGCC | GGCCAGCTTGTCGTTCTGGTTGCTGG |
| T76S | CAGCAACCAGAGCGACAAGCTGGCC | GGCCAGCTTGTCGCTCTGGTTGCTG |
| T76Y | CCCAGCAACCAGTACGACAAGCTGGCCG | CGGCCAGCTTGTCGTACTGGTTGCTGGG |
| D77E | CAACCAGACGGAAAAGCTGGCCGCT | AGCGGCCAGCTTTTCCGTCTGGTTG |
| K78R | AACCAGACGGACCGGCTGGCCGCTT | AAGCGGCCAGCCGGTCCGTCTGGTT |
| E84Y | GCCGCTTTCCCCTATGACCGCAGCCAG | CTGGCTGCGGTCATAGGGGAAAGCGGC |
| G124V | CCTACCTCTGTGTCGCCATCTCCCTG | CAGGGAGATGGCGACACAGAGGTAGG |
| G124M | CACCTACCTCTGTATGGCCATCTCCCTG | CAGGGAGATGGCCATACAGAGGTAGGTG |
| G124Y | CACCTACCTCTGTTACGCCATCTCCCTGG | CCAGGGAGATGGCGTAACAGAGGTAGGTG |
| I126V | ACCTCTGTGGGGCCGTATCCCTGGCCCCCA | TGGGGGCCAGGGATACGGCCCCACAGAGGT |
| L128V | TGGGGCCATCTCCGTAGCCCCCAAGGCGCA | TGCGCCTTGGGGGCTACGGAGATGGCCCCA |
| L128M | TGGGGCCATCTCCATGGCCCCCAAGG | CCTTGGGGGCCATGGAGATGGCCCCA |
| L128W | TGGGGCCATCTCCTGGGCCCCCAAGGC | GCCTTGGGGGCCCAGGAGATGGCCCCA |
| K131H | CTCCCTGGCCCCCCATGCGCAGATCAAAG | CTTTGATCTGCGCATGGGGGGCCAGGGAG |
| I134Q | CCCAAGGCGCAGCAGAAAGAGAGCCTGC | GCAGGCTCTCTTTCTGCTGCGCCTTGGG |
| I134E | CCCAAGGCGCAGGAGAAAGAGAGCCTGC | GCAGGCTCTCTTTCTCCTGCGCCTTGGG |
| I134D | CCCAAGGCGCAGGACAAAGAGAGCC | GGCTCTCTTTGTCCTGCGCCTTGGG |
| I134T | CCAAGGCGCAGACAAAAGAGAGCCTG | CAGGCTCTCTTTTGTCTGCGCCTTGG |
| I134N | CCAAGGCGCAGAATAAAGAGAGCCTGC | GCAGGCTCTCTTTATTCTGCGCCTTGG |
| I134F | CCCAAGGCGCAGTTCAAAGAGAGCC | GGCTCTCTTTGAACTGCGCCTTGGG |
| E136Q | GCGCAGATCAAACAGAGCCTGCGGG | CCCGCAGGCTCTGTTTGATCTGCGC |
| E136N | GGCGCAGATCAAAAACAGCCTGCGGGCAG | CTGCCCGCAGGCTGTTTTTGATCTGCGCC |
| Y68K | GTGCTAAACTGGAAACGCATGAGCC | GGCTCATGCGTTTCCAGTTTAGCAC |
| Y68R | GTGCTAAACTGGAGACGCATGAGCC | GGCTCATGCGTCTCCAGTTTAGCAC |

| | | |
|---|---|---|
| Y68Q | GTGCTAAACTGGCAACGCATGAGCC | GGCTCATGCGTTGCCAGTTTAGCAC |
| K78R | CAGACGGACCGGCTGGCCGCTTTC | GAAAGCGGCCAGCCGGTCCGTCTG |
| L128M | GGGCCATCTCCATGGCCCCCAAGG | CCTTGGGGGCCATGGAGATGGCCC |
| K131H | CCTGGCCCCCCATGCGCAGATCAAAG | CTTTGATCTGCGCATGGGGGGCCAGG |
| I134E | CCCAAGGCGCAGGAGAAAGAGAGCC | GGCTCTCTTTCTCCTGCGCCTTGGG |
| I134F | CCAAGGCGCAGTTCAAAGAGAGCCTG | CAGGCTCTCTTTGAACTGCGCCTTGG |
| I134F | CCCAAGGCGCAGTTCAAAGAGAGCCTGC | GCAGGCTCTCTTTGAACTGCGCCTTGGG |

Supplementary Figure 1. (Related to Fig. 4.) **Structural models of mutant PD-1:L1:L2 interfaces**. (A) Wild-type hPD-1 (green) : hPD-L1 (magenta) complex, (B) mutant (T76D) hPD-1 (green) : hPD-L1 (magenta) complex, (C) wild-type hPD-1 (green) : hPD-L2 (magenta) complex, and (D) mutant (T76D) hPD-1 (green) : hPD-L2 (magenta) complex. For PD1 five conformations taken every 4ns during the MD simulation are shown.



Supplementary Figure 2. (Related to Fig. 4.) Comparison between experimentally observed and computationally calculated binding preferences of PDL1 and PDL2 to various mutants of PD1. X-axis shows the difference of the normalized fraction of PDL1 and PDL2 bound to 32 mutant variants of PD1. Y-axis shows the computationally calculated ΔΔG Kcal/mol for the same cases as obtained from BeAtMusic (A), FoldX (B), and MutaBind (C), respectively.



Supplementary Figure 3. (Related to Fig. 3.) Averaged mCherry expression data for all of the PD-1 mutants. The percent binding in Fig. 3 is calculated as a percentage of mCherry expressing cells (direct C-term fusion with PD-1) therefore this calculation corrects for any minor deviations in protein expression.