SUPPLEMENTARY FIGURES
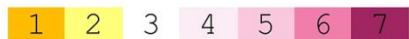
```
1                    11                   21                   31                   41
MQIPQAPWPV  VWAVLQLGWR  PGWFLDSPDR  PWNPPTFSPA  LLVVTEGDNA
51                   61                   71                   81                   91
TFTCSFSNTS  ESFVLNWYRM  SPSNQTDKLA  AFPEDRSQPG  QDCRFRVTQL
101                  111                  121                  131                  141
PNGRDFHMSV  VRARRNDSGT  YLCGAISLAP  KAQIKESLRA  ELRVTERRAE
151                  161                  171                  181                  191
VPTAHPSPSP  RPAGQFQTLV  VGVVGGLLGS  LVLLVWVLAV  ICSRAARGTI
201                  211                  221                  231                  241
GARRTGQPLK  EDPSAVPVFS  VDYGELDFQW  REKTPEPPVP  CVPEQTEYAT
251                  261                  271                  281
IVFPSGMGTS  SPARRGSADG  PRSAQPLRPE  DGHCSWPL
```

Legend:

The selection scale:

1	2	3	4	5	6	7

Positive selection   Purifying selection

Supplementary Figure 1. Detection of positive/purifying selection in PD1 homologous sequences from vertebrates. Color-coded results of Selecton analyses of human PD1, compared to sequences from 52 aligned nucleotide coding sequences. Selection pressure was measured on sequences using mechanistic-empirical combination (MEC) model of Selecton version 2.2. Yellow and brown highlights represent positive selection, grey and white highlights represent a neutral selection and purple highlight represent purifying selection on codons.

```
1               11              21              31              41
MRIFAVFIFM      TYWHLLNAFT      VTVPKDLYVV      EYGSNMTIEC      KFPVEKQLDL

51              61              71              81              91
AALIVYWEME      DKNIIQFVHG      EEDLKVQHSS      YRQRARLLKD      QLSLGNAALQ

101             111             121             131             141
ITDVKLQDAG      VYRCMISYGG      ADYKRITVKV      NAPYNKINQR      ILVVDPVTSE

151             161             171             181             191
HELTCQAEGY      PKAEVIWTSS      DHQVLSGKTT      TTNSKREEKL      FNVTSTLRIN

201             211             221             231             241
TTTNEIFYCT      FRRLDPEENH      TAELVIPELP      LAHPPNERTH      LVILGAILLC

251             261             271             281
LGVALTFIFR      LRKGRMMDVK      KCGIQDTNSK      KQSDTHLEET
```

Legend:

The selection scale:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 |

Positive selection   Purifying selection

Supplementary Figure 2. Detection of positive/purifying selection in PD-L1 homologous sequences from vertebrates. Color-coded results of Selecton analyses of human PD-L1, compared to sequences from 58 aligned nucleotide coding sequences. Selection pressure was measured on sequences using mechanistic-empirical combination (MEC) model of Selecton version 2.2. Yellow and brown highlights represent positive selection, grey and white highlights represent a neutral selection and purple highlight represent purifying selection on codons.

```
1               11              21              31              41
M I F L L L M L S L   E L Q L H Q I A A L   F T V T V P K E L Y   I I E H G S N V T L   E C N F D T G S H V
51              61              71              81              91
N L G A I T A S L Q   K V E N D T S P H R   E R A T L L E E Q L   P L G K A S F H I P   Q V Q V R D E G Q Y
101             111             121             131             141
Q C I I I Y G V A W   D Y K Y L T L K V K   A S Y R K I N T H I   L K V P E T D E V E   L T C Q A T G Y P L
151             161             171             181             191
A E V S W P N V S V   P A N T S H S R T P   E G L Y Q V T S V L   R L K P P P G R N F   S C V F W N T H V R
201             211             221             231             241
E L T L A S I D L Q   S Q M E P R T H P T   W L L H I F I P F C   I I A F I F I A T V   I A L R K Q L C Q K
251             261             271
L Y S S K D T T K R   P V T T T K R E V N   S A I
```

Legend:

The selection scale:

1	2	3	4	5	6	7

Positive selection   Purifying selection

Supplementary Figure 3. Detection of positive/purifying selection in PD-L2 homologous sequences from vertebrates. Color-coded results of Selecton analyses of human PD-L2, compared to sequences from 56 aligned nucleotide coding sequences. Selection pressure was measured on sequences using mechanistic-empirical combination (MEC) model of Selecton version 2.2. Yellow and brown highlights represent positive selection, grey and white highlights represent a neutral selection and purple highlight represent purifying selection on codons.

ConSurf Results

1	11	21	31	41
MQIPQAPWPV	VWAVLQLGWR	PGWFLDSPDR	PWNPPTFSPA	LLVVTEGDNA
eeeeebebeb	bbbbbebbbe	eebbbebeee	eeeebebeeb	ebebeeeeeb
f	sfs		f	s ff

51	61	71	81	91
TFTCSFSNTS	ESFVLNWYRM	SPSNQTDKLA	AFPEDRSQPG	QDCRFRVTQL
ebebebeeee	eebbbbbbeb	eeeeeeeebb	ebeeeeeeee	eeeebebbeb
fs sf	ss sf	ff ff f s	f	f

101	111	121	131	141
PNGRDFHMSV	VRARRNDSGT	YLCGAISLAP	KAQIKESLRA	ELRVTERRAE
eeeeebebbb	bbbeeeeeeb	bbbbbbebee	eeebeeeeee	ebebeeebee
	ffff	s s s	s ff f	s sff

151	161	171	181	191
VPTAHPSPSP	RPAGQFQTLV	VGVVGGLLGS	LVLLVWVLAV	ICSRAARGTI
eeeeeeeeee	eeeeebebbb	bbbbbbbbbb	bbbbbbbbbb	bbeebeeeee
f ff f			s	

201	211	221	231	241
GARRTGQPLK	EDPSAVPVFS	VDYGELDFQW	REKTPEPPVP	CVPEQTEYAT
eeeeeeeeee	eeeeebebbb	bebeebebee	eeeeeeeeee	eeeeeeebbb
f		fsffsfsf	f ff	ffsss

251	261	271	281
IVFPSGMGTS	SPARRGSADG	PRSAQPLRPE	DGHCSWPL
bbbeeeeeee	eeeeebeeee	eeeeeeeeee	eeebeeee
s s	f ff	f	f f ffff

The conservation scale:

1	2	3	4	5	6	7	8	9

Variable   Average   Conserved

e – An exposed residue according to the neural-network algorithm.
b – A buried residue according to the neural-network algorithm.
f – A predicted functional residue (highly conserved and exposed).
s – A predicted structural residue (highly conserved and buried).

Supplementary Figure 4. ConSurf output of PD1, using the UniRef90 protein database. Colors of the ConSurf output indicate the level of sequence conservation. Purple indicates conservation and blue indicates variability. Residues are predicted to be exposed (e), buried (b), highly conserved and exposed (f), or highly conserved and buried, (s).

ConSurf Results
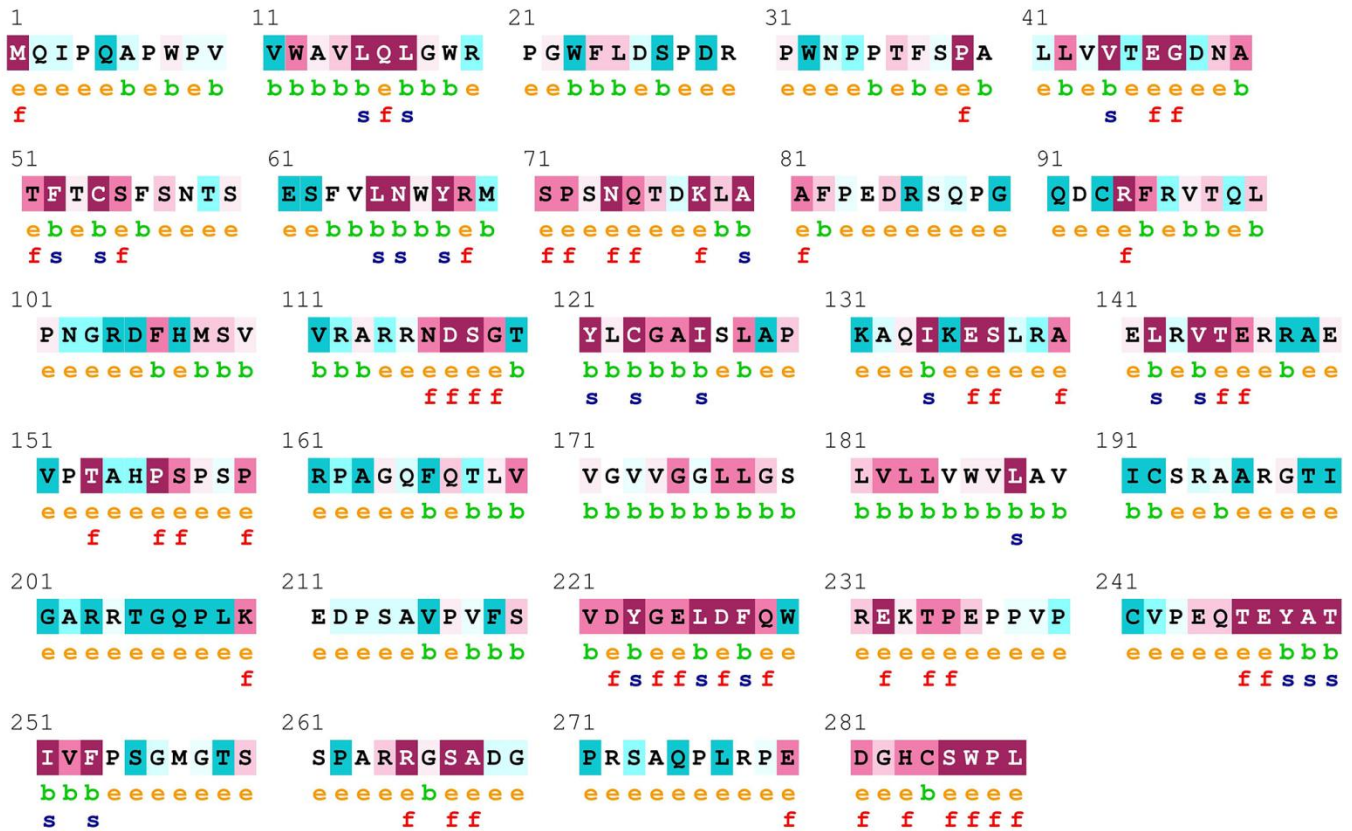

The conservation scale:

1 2 3 4 5 6 7 8 9

Variable   Average   Conserved

e – An exposed residue according to the neural-network algorithm.
b – A buried residue according to the neural-network algorithm.
f – A predicted functional residue (highly conserved and exposed).
s – A predicted structural residue (highly conserved and buried).

Supplementary Figure 5. ConSurf output of PD-L1, using the UniRef90 protein database. Colors of the ConSurf output indicate the level of sequence conservation. Purple indicates conservation and blue indicates variability. Residues are predicted to be exposed (e), buried (b), highly conserved and exposed (f), or highly conserved and buried, (s).
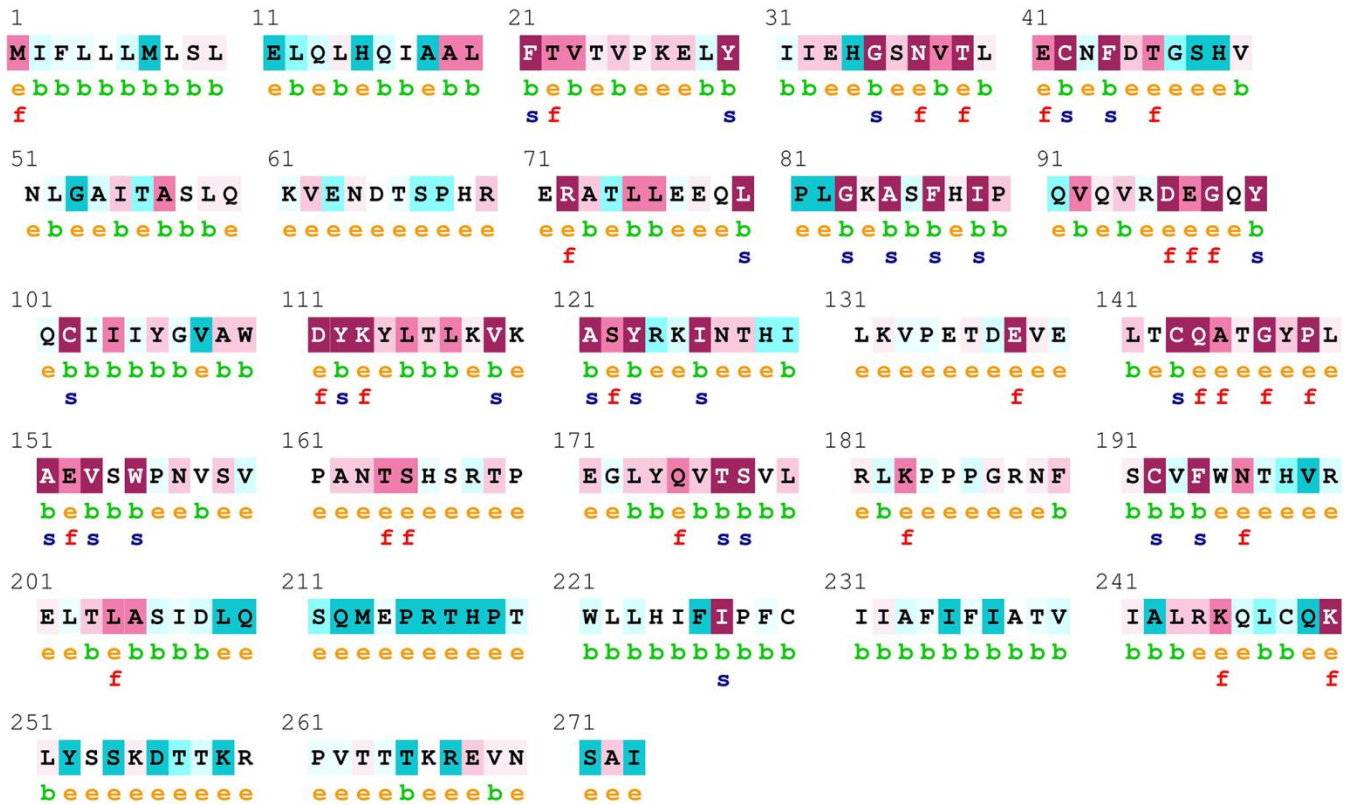
ConSurf Results

The conservation scale:

| 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 |

Variable   Average   Conserved

e – An exposed residue according to the neural-network algorithm.
b – A buried residue according to the neural-network algorithm.
f – A predicted functional residue (highly conserved and exposed).
s – A predicted structural residue (highly conserved and buried).

Supplementary Figure 6. ConSurf output of PD-L2, using the UniRef90 protein database. Colors of the ConSurf output indicate the level of sequence conservation. Purple indicates conservation and blue indicates variability. Residues are predicted to be exposed (e), buried (b), highly conserved and exposed (f), or highly conserved and buried, (s).

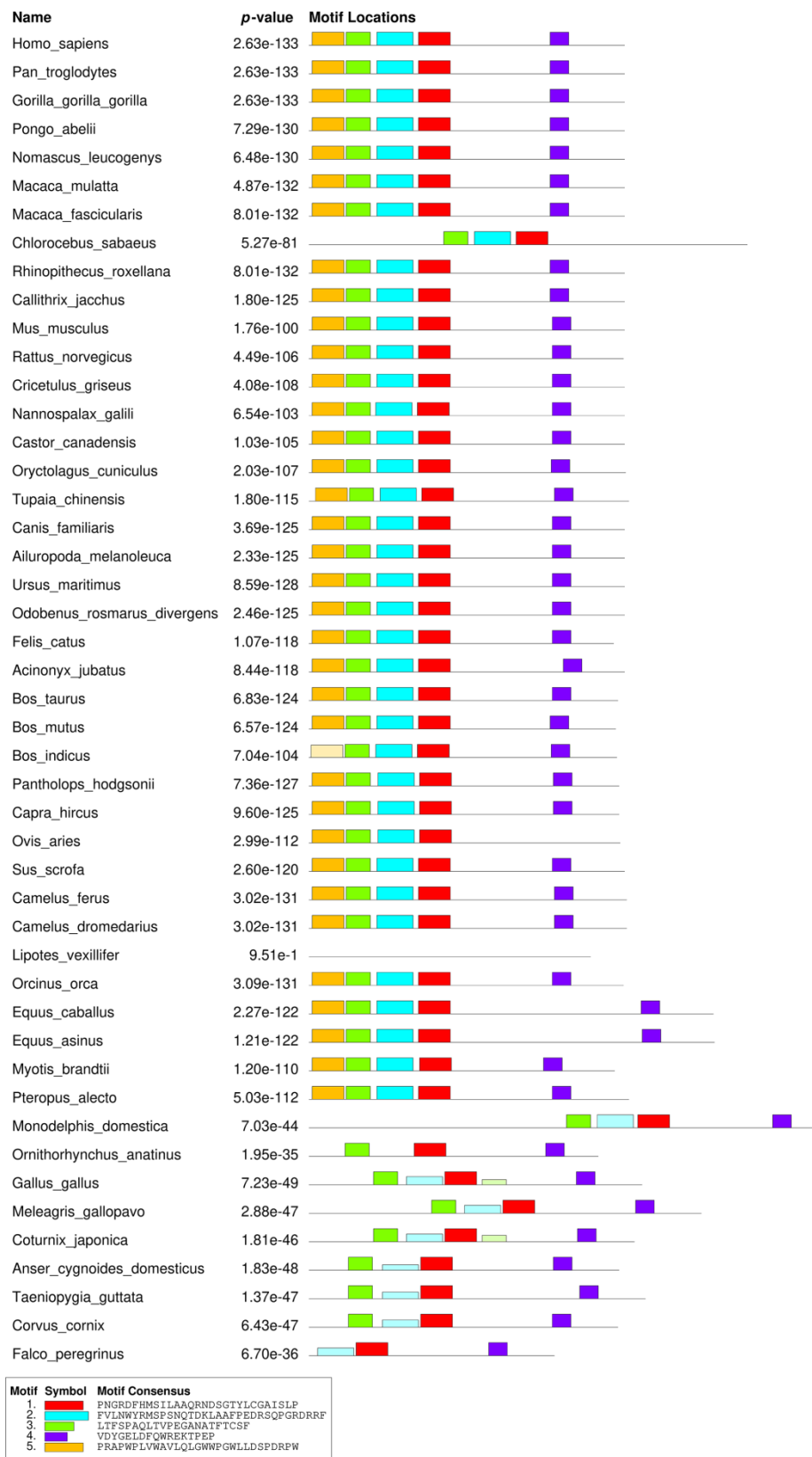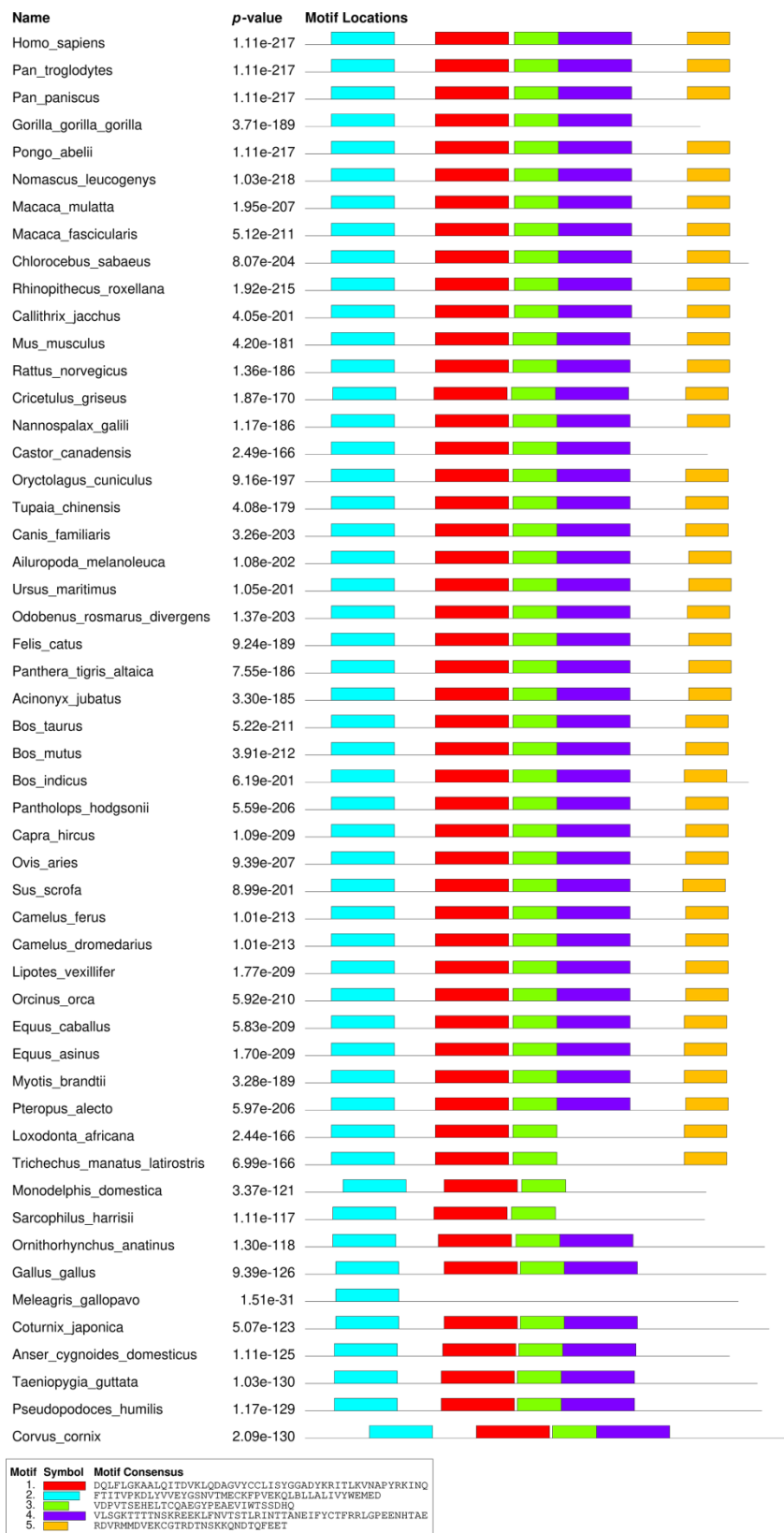| Name	p-value	Motif Locations
Homo_sapiens	2.63e-133	
Pan_troglodytes	2.63e-133	
Gorilla_gorilla_gorilla	2.63e-133	
Pongo_abelii	7.29e-130	
Nomascus_leucogenys	6.48e-130	
Macaca_mulatta	4.87e-132	
Macaca_fascicularis	8.01e-132	
Chlorocebus_sabaeus	5.27e-81	
Rhinopithecus_roxellana	8.01e-132	
Callithrix_jacchus	1.80e-125	
Mus_musculus	1.76e-100	
Rattus_norvegicus	4.49e-106	
Cricetulus_griseus	4.08e-108	
Nannospalax_galili	6.54e-103	
Castor_canadensis	1.03e-105	
Oryctolagus_cuniculus	2.03e-107	
Tupaia_chinensis	1.80e-115	
Canis_familiaris	3.69e-125	
Ailuropoda_melanoleuca	2.33e-125	
Ursus_maritimus	8.59e-128	
Odobenus_rosmarus_divergens	2.46e-125	
Felis_catus	1.07e-118	
Acinonyx_jubatus	8.44e-118	
Bos_taurus	6.83e-124	
Bos_mutus	6.57e-124	
Bos_indicus	7.04e-104	
Pantholops_hodgsonii	7.36e-127	
Capra_hircus	9.60e-125	
Ovis_aries	2.99e-112	
Sus_scrofa	2.60e-120	
Camelus_ferus	3.02e-131	
Camelus_dromedarius	3.02e-131	
Lipotes_vexillifer	9.51e-1	
Orcinus_orca	3.09e-131	
Equus_caballus	2.27e-122	
Equus_asinus	1.21e-122	
Myotis_brandtii	1.20e-110	
Pteropus_alecto	5.03e-112	
Monodelphis_domestica	7.03e-44	
Ornithorhynchus_anatinus	1.95e-35	
Gallus_gallus	7.23e-49	
Meleagris_gallopavo	2.88e-47	
Coturnix_japonica	1.81e-46	
Anser_cygnoides_domesticus	1.83e-48	
Taeniopygia_guttata	1.37e-47	
Corvus_cornix	6.43e-47	
Falco_peregrinus	6.70e-36	

| Motif	Symbol	Motif Consensus
1.		PNGRDFHMSILAAQRNDSGTYLCGAISLP
2.		FVLNWYRMSPSNQTDKLAAFPEDRSQPGRDRRF
3.		LTFSPAQLTVPEGANATFTCSF
4.		VDYGELDFQWREKTPEP
5.		PRAPWFLVWAVLQLGWWPGWLLDSPDRPW

Supplementary Figure 7. Motif distribution of PD1 gene in the vertebrate species. Motifs of PD1 gene from 52 species are predicted using MEME suite (http://meme.nbcr.net/meme/) based on amino acids sequences. All sequences are separated by 5 conservative motifs with colors, including motif 1 (red), motif 2 (cyan), motif 3 (green), motif 4 (purple) and motif 5 (brown).

Name | ***p*-value** | **Motif Locations**

Name	*p*-value
Homo_sapiens	1.11e-217
Pan_troglodytes	1.11e-217
Pan_paniscus	1.11e-217
Gorilla_gorilla_gorilla	3.71e-189
Pongo_abelii	1.11e-217
Nomascus_leucogenys	1.03e-218
Macaca_mulatta	1.95e-207
Macaca_fascicularis	5.12e-211
Chlorocebus_sabaeus	8.07e-204
Rhinopithecus_roxellana	1.92e-215
Callithrix_jacchus	4.05e-201
Mus_musculus	4.20e-181
Rattus_norvegicus	1.36e-186
Cricetulus_griseus	1.87e-170
Nannospalax_galili	1.17e-186
Castor_canadensis	2.49e-166
Oryctolagus_cuniculus	9.16e-197
Tupaia_chinensis	4.08e-179
Canis_familiaris	3.26e-203
Ailuropoda_melanoleuca	1.08e-202
Ursus_maritimus	1.05e-201
Odobenus_rosmarus_divergens	1.37e-203
Felis_catus	9.24e-189
Panthera_tigris_altaica	7.55e-186
Acinonyx_jubatus	3.30e-185
Bos_taurus	5.22e-211
Bos_mutus	3.91e-212
Bos_indicus	6.19e-201
Pantholops_hodgsonii	5.59e-206
Capra_hircus	1.09e-209
Ovis_aries	9.39e-207
Sus_scrofa	8.99e-201
Camelus_ferus	1.01e-213
Camelus_dromedarius	1.01e-213
Lipotes_vexillifer	1.77e-209
Orcinus_orca	5.92e-210
Equus_caballus	5.83e-209
Equus_asinus	1.70e-209
Myotis_brandtii	3.28e-189
Pteropus_alecto	5.97e-206
Loxodonta_africana	2.44e-166
Trichechus_manatus_latirostris	6.99e-166
Monodelphis_domestica	3.37e-121
Sarcophilus_harrisii	1.11e-117
Ornithorhynchus_anatinus	1.30e-118
Gallus_gallus	9.39e-126
Meleagris_gallopavo	1.51e-31
Coturnix_japonica	5.07e-123
Anser_cygnoides_domesticus	1.11e-125
Taeniopygia_guttata	1.03e-130
Pseudopodoces_humilis	1.17e-129
Corvus_cornix	2.09e-130

Motif | Symbol | Motif Consensus

1. DQLFLGKAALQITDVKLQDAGVYCCLISYGGADYKRITLKVNAPYRKINQ
2. FTITVPKDLYVVEYGSNVTMECKFPVEKQLBLLALIVYWEMED
3. VDPVTSEHELTCQAEGYPEAEVIWTSSDHQ
4. VLSGKTTTTNSKREEKLFNVTSTLRINTTANEIFYCTFRRLGPEENHTAE
5. RDVRMMDVEKCGTRDTNSKKQNDTQFEET

Supplementary Figure 8. Motif distribution of PD-L1 gene in the vertebrate species. Motifs of PD-L1 gene from 58 species are predicted using MEME suite (http://meme.nbcr.net/meme/) based on amino acids sequences. All sequences are separated by 5 conservative motifs with colors, including motif 1 (red), motif 2 (cyan), motif 3 (green), motif 4 (purple) and motif 5 (brown).
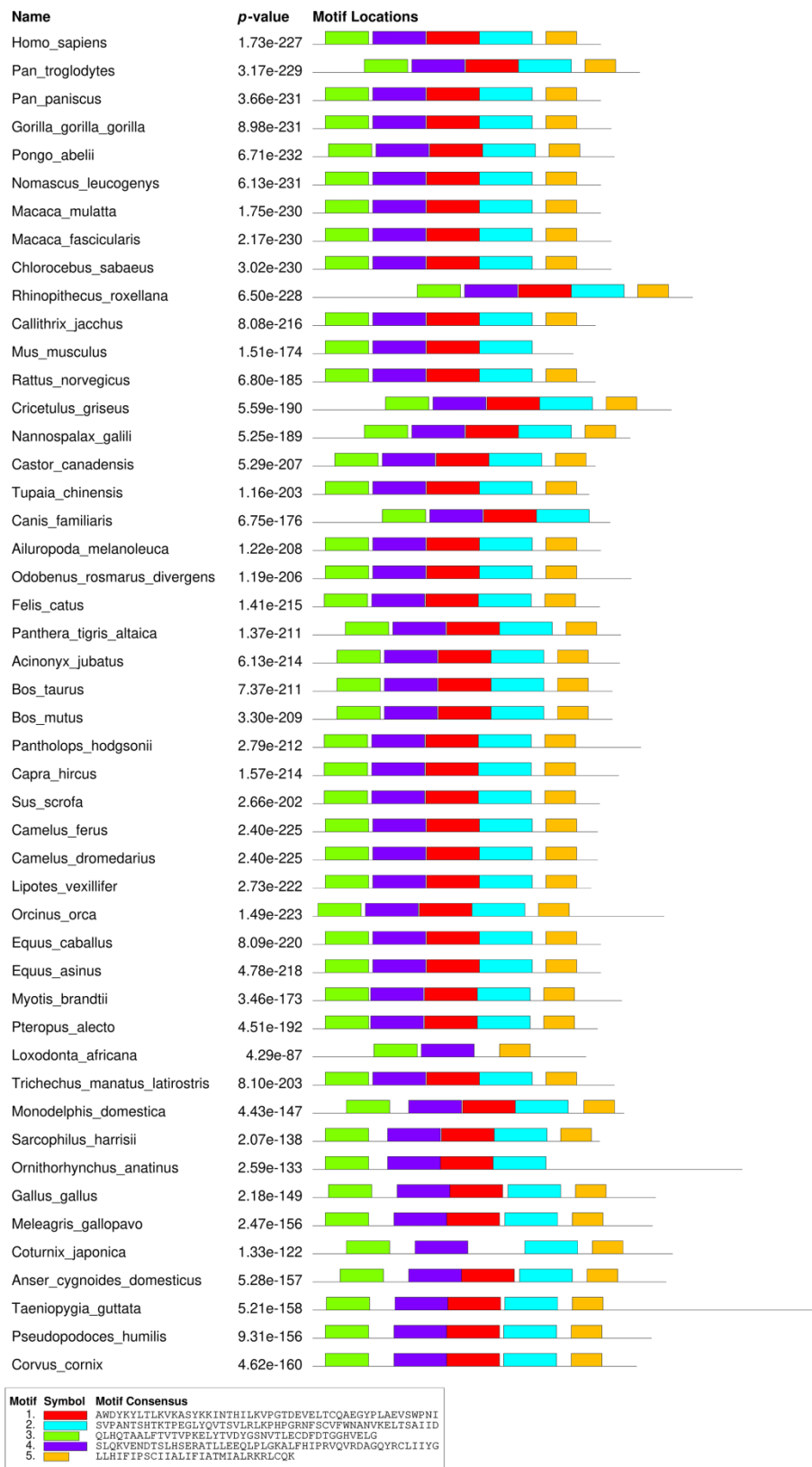
Supplementary Figure 9. Motif distribution of PD-L2 gene in the vertebrate species. Motifs of PD-L2 gene from 56 species are predicted using MEME suite (http://meme.nbcr.net/meme/) based on amino acids sequences. All sequences are separated by 5 conservative motifs with colors, including motif 1 (red), motif 2 (cyan), motif 3 (green), motif 4 (purple) and motif 5 (brown).
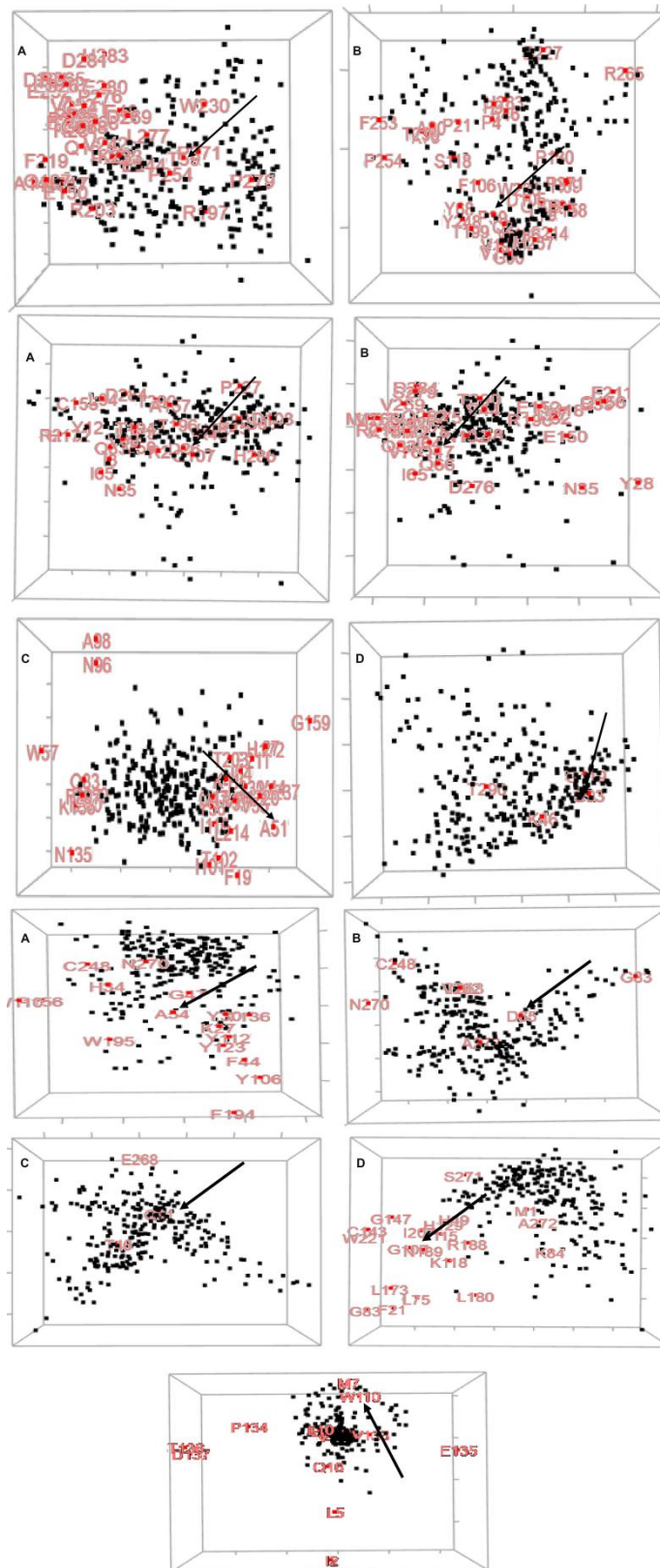
Supplementary Figure 10. 3D multidimensional scaling (MDS) scatterplots of co-varying residues in human PD1, PD-L1, and PD-L2. (**A**) Highlighted red are the residues corresponding to the positively selected residue. Both black and red dots can be optionally labeled with the residue identifiers.
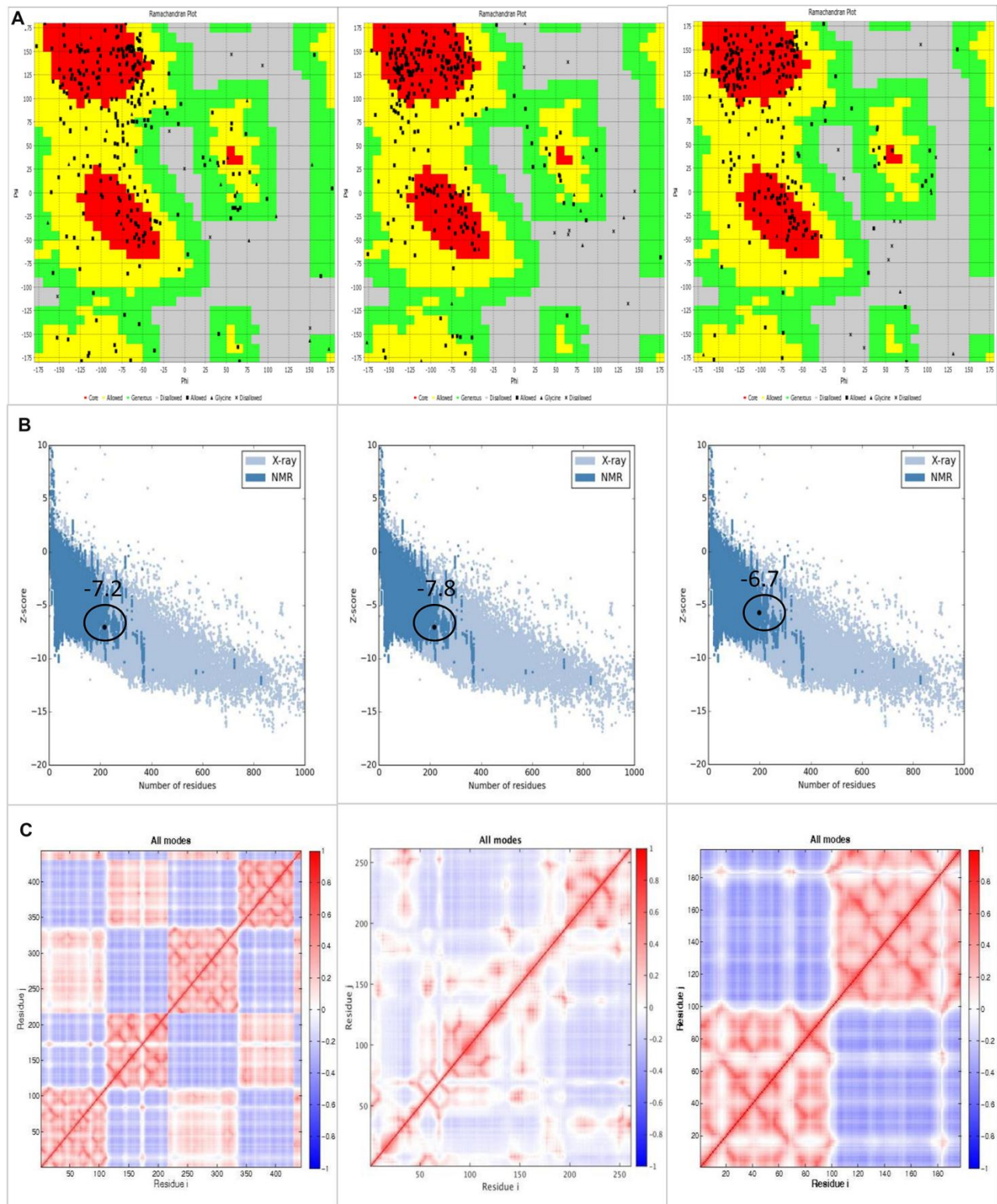
Supplementary Figure 11. (**A**) Ramachandran plot analysis of PD1, PDL1 and PDL2 proteins. The backbone conformation angles for respective residue in the modeled protein crystal structures. Red color indicates the core region, yellow indicates allowed region, green is allowed region, and grey is disallowed region. (**B**) Z-score displays the quality analysis of predicted structures. (**C**) The cross-correlation analysis of all predicted structures. Positive correlations are plotted in the upper left triangle and negative correlations in the lower right triangle. Open squares: functional hydrophobic and negative residues.