Supplementary Information for

Structural insight into T cell co-inhibition by PD-1H (VISTA)

Benjamin T. Slater[a,1], Xue Han[a,1], Lieping Chen[a,2], Yong Xiong[b,2]

[a]Department of Immunobiology, Yale University, New Haven, Connecticut 06511; [b]Department of Molecular Biophysics and Biochemistry, Yale University, New Haven, Connecticut 06520

[1]These authors contributed equally to this work
[2]To whom correspondence may be addressed. Email: yong.xiong@yale.edu or lieping.chen@yale.edu

This PDF file includes:

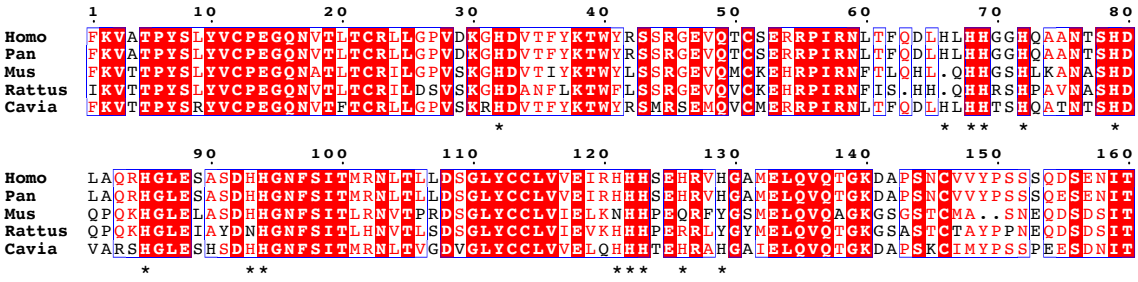

Fig. S1. Multiple sequence alignment of PD-1H orthologs. Similar residues are colored red, and invariant residues have red backgrounds. Conserved histidines are marked with an asterisk.

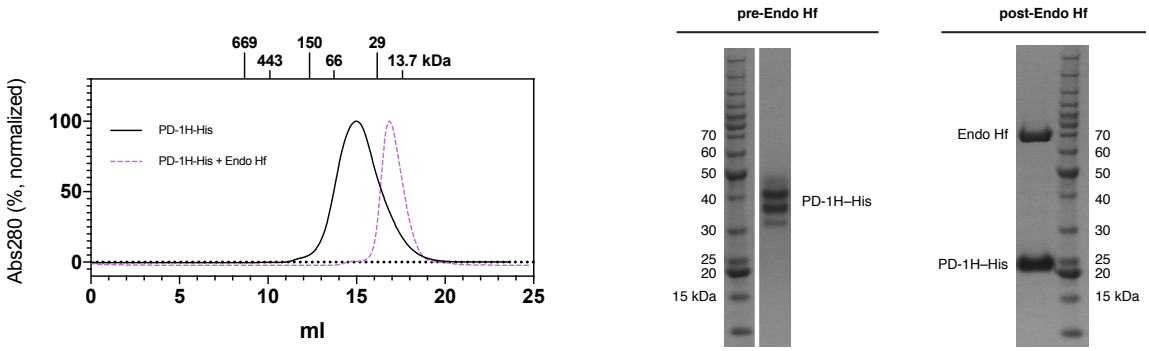

Fig. S2. Size-exclusion chromatography (SEC) and SDS-PAGE analysis of glycosylated and deglycosylated PD-1H–His. Left: purified PD-1H–His was analyzed by SEC before (black trace) and after (purple dotted trace) deglycosylation with Endoglycosidase Hf. Absorbance at 280nm was normalized so that 100% represents maximum absorbance. Molecular weight standard elution volumes are plotted above. Right: SDS-PAGE analysis of purified PD-1H–His before and after digestion with Endoglycosidase Hf.

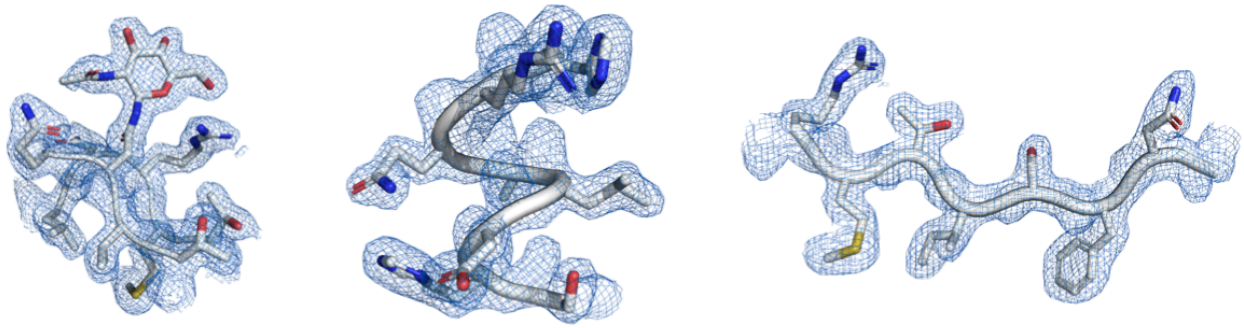

Fig. S3. Electron density map quality. 2Fo–Fc electron density maps (blue mesh) are contoured at 1.0σ. Left: N-acetylglucosamine residue attached to Asn17. Center: α helix between C″ and D β strands. Right: E β strand.

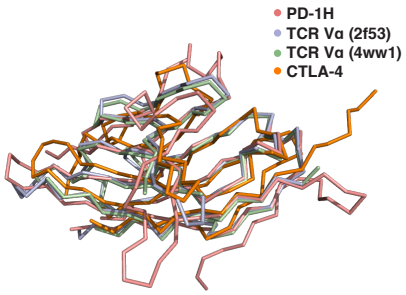

Fig. S4. Structural alignment of similar proteins from the structural comparison server DALI. TCR Vα domains (blue, PDB ID 2f53; green, PDB ID 4ww1), CTLA-4 (orange, PDB ID 3osk) and PD-1H (red) are largely similar, except for the additional C-terminal β-strand and the unique CC′ loop of PD-1H.

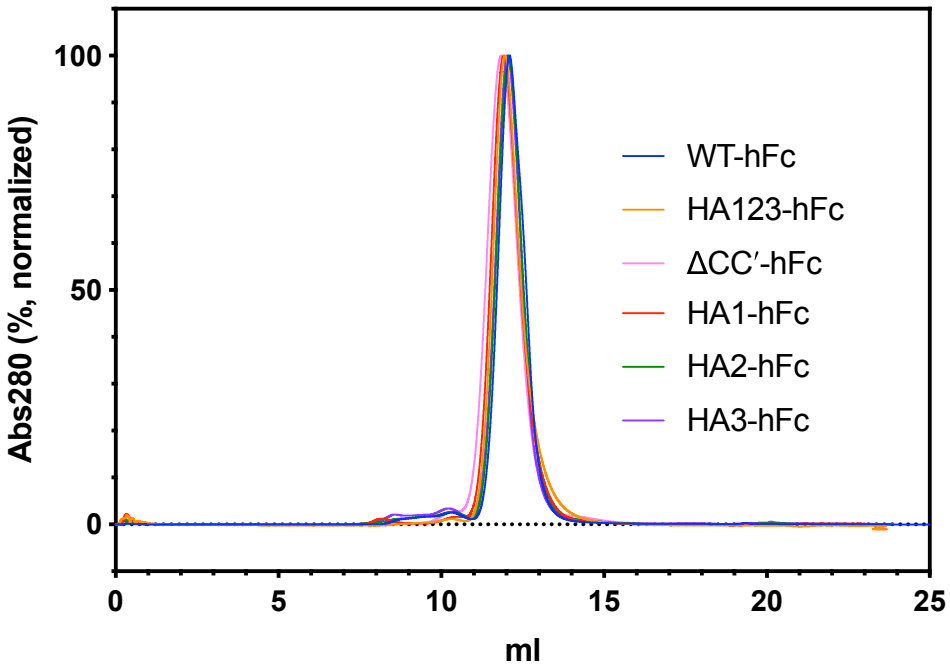

Fig. S5. Size-exclusion chromatography (SEC) analysis of PD-1H–hFc variants.
Purified PD-1H–hFc variants were compared by SEC. Absorbance at 280nm was normalized so that 100% represents maximum absorbance.

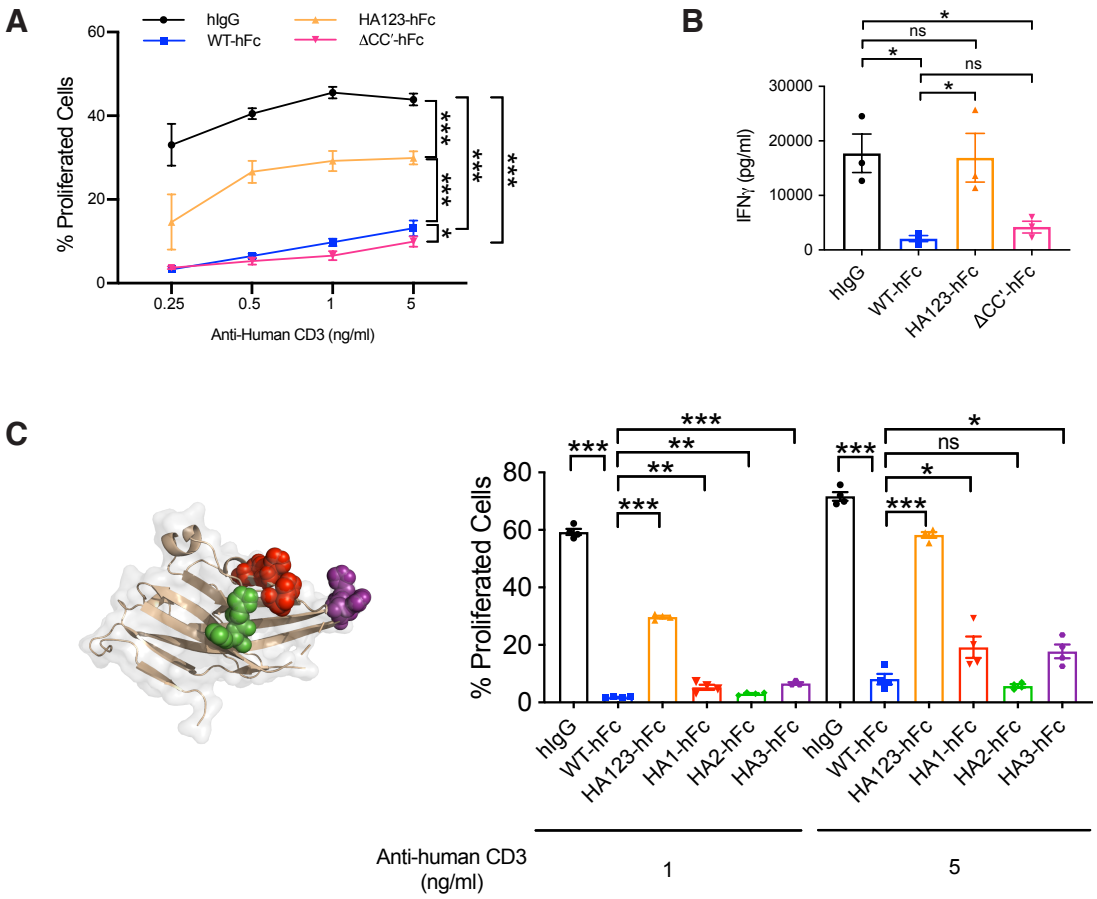

Fig. S6. Replicates from additional PBMC donors used for *in vitro* experiments.
(*A*) T cell proliferation after activation with α-CD3 in the presence of control hIgG, wildtype PD-1H (WT-hFc), or mutant PD-1H where either all three histidine clusters were mutated to alanine (HA123-hFc) or the CC′ loop was deleted (ΔCC′-hFc). (*B*) Cytometric bead array staining of IFN-γ in supernatants from T cells activated in the presence of control or PD-1H protein variants. (*C*) Similar experiment as (*A*) but with histidine clusters (in different colors) mutated individually. Representative results of at least three independent experiments were shown. Statistical analyses were carried out with two-tailed Student's t-test, and all error bars reflect SEM.

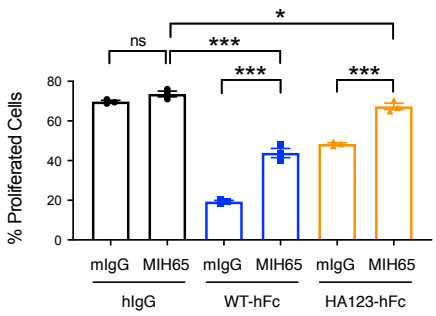

Fig. S7. T cell inhibition by PD-1H in the presence of antagonistic antibody MIH65. T cell proliferation after activation with α-CD3 on plates coated with control hIgG, wildtype PD-1H (WT-hFc), or mutant PD-1H where either all three histidine clusters were mutated to alanine (HA123-hFc) and in the presence of either antagonistic antibody MIH65 or isotype control mouse IgG (mIgG). Statistical analyses were carried out with two-tailed Student's t-test, and all error bars reflect SEM.

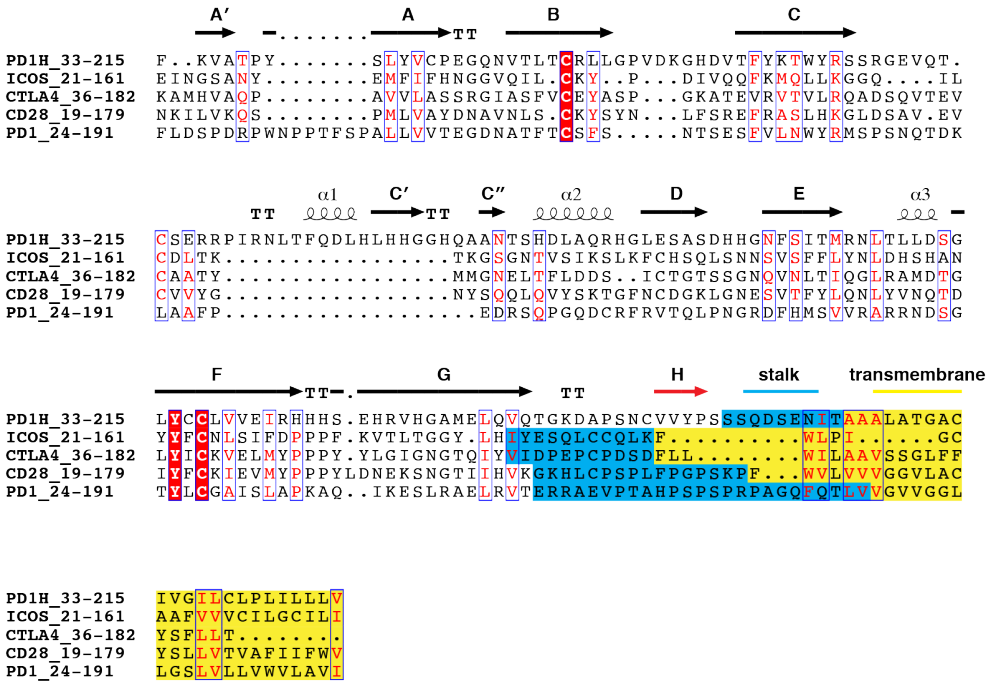

Fig. S8. Multiple sequence alignment of CD28 family members. Extracellular domains and transmembrane regions as annotated in UniProt were aligned using Clustal Omega(1) and processed with ESPript(2). Similar residues are colored red, and invariant residues have red backgrounds. Transmembrane (yellow background) residues are based on sequence annotation from UniProt(3). Stalk regions (blue background) for CD28, CTLA-4, and PD-1 are based on crystal structures (PDB IDs 1yjd, 3osk, and 3rrq, respectively), and for ICOS based on homology modeling on CTLA-4 using 3osk as a template with the SWISS-MODEL server(4). The novel H β strand of PD-1H (red arrow) is located in a region that corresponds to where the stalk or transmembrane region would be in other CD28 family members.

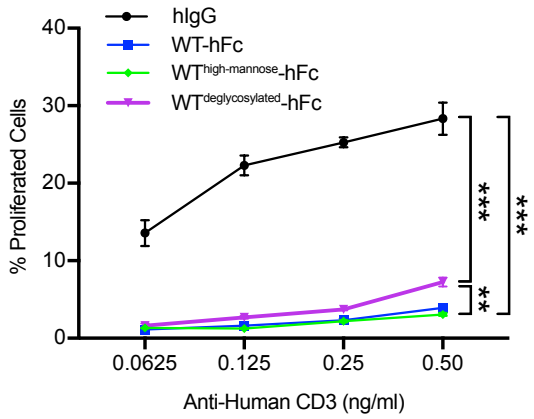

Fig. S9. T cell inhibition by PD-1H glycosylation variants. T cell proliferation after activation with α-CD3 on plates coated with control hIgG, wildtype PD-1H (WT-hFc), PD-1H in which glycans were arrested in high-mannose form by addition of 20 μM swainsonine during protein expression ($WT^{high\text{-}mannose}$-hFc), or PD-1H in which glycans were removed by enzymatic processing of $WT^{high\text{-}mannose}$-hFc by Endoglycosidase Hf ($WT^{deglycosylated}$-hFc). Statistical analyses were carried out with two-tailed Student's t-test, and all error bars reflect SEM.

References

1. Chojnacki S, Cowley A, Lee J, Foix A, & Lopez R (2017) Programmatic access to bioinformatics tools from EMBL-EBI update: 2017. *Nucleic Acids Res* 45(W1):W550-w553.
2. Robert X & Gouet P (2014) Deciphering key features in protein structures with the new ENDscript server. *Nucleic Acids Res* 42(Web Server issue):W320-324.
3. Consortium TU (2019) UniProt: a worldwide hub of protein knowledge. *Nucleic Acids Res* 47(D1):D506-d515.
4. Waterhouse A*, et al.* (2018) SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res* 46(W1):W296-w303.