

Supplementary Information Appendix

Supplementary Materials and Methods

The Ig variable sequences of the new anti-PD1 antibodies that were used in the study:

The sequences encode the Ig variable regions of the new anti-PD1 antibodies that were used in the experiments in Figure 3-4, Figure S2 and S3. In the expression constructs, the human V_H3-33 leader sequence and the human V_κ3-11 leader sequence were appended to the 5' end of the variable region sequences of the HCs and LCs, respectively; human V_H3-33 and V_κ3-11 segments are germline components of the 17D8 antibody heavy and light chains, respectively. The IgH variable regions were expressed in association with the human IgG4 constant region, and the IgL variable regions were expressed in association with the human kappa constant region. The complete cDNAs for the HCs and LCs were cloned into the pcDNA expression vector and expressed in 293F or Expi293 cells.

M1-1HC

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ATGGAGACTCCGTGAAGGGCCGATTCACCGTCTCCAGAGACAATTCCAAGAACATGTTGTA
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M1-2HC

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M1-3HC

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M1-4HC

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M1-5HC

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M1-6HC

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M1-7HC

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M2-1HC

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TCTCATCAG

M2-2HC

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M2-3HC

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M2-4HC

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M2-5HC

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M1-1LC

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M1-2LC

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M1-3LC

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M1-4LC

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M1-5LC

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M1-6LC

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M1-7LC

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M2-1LC

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M2-2LC

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M2-3LC

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M2-4LC

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M2-5LC

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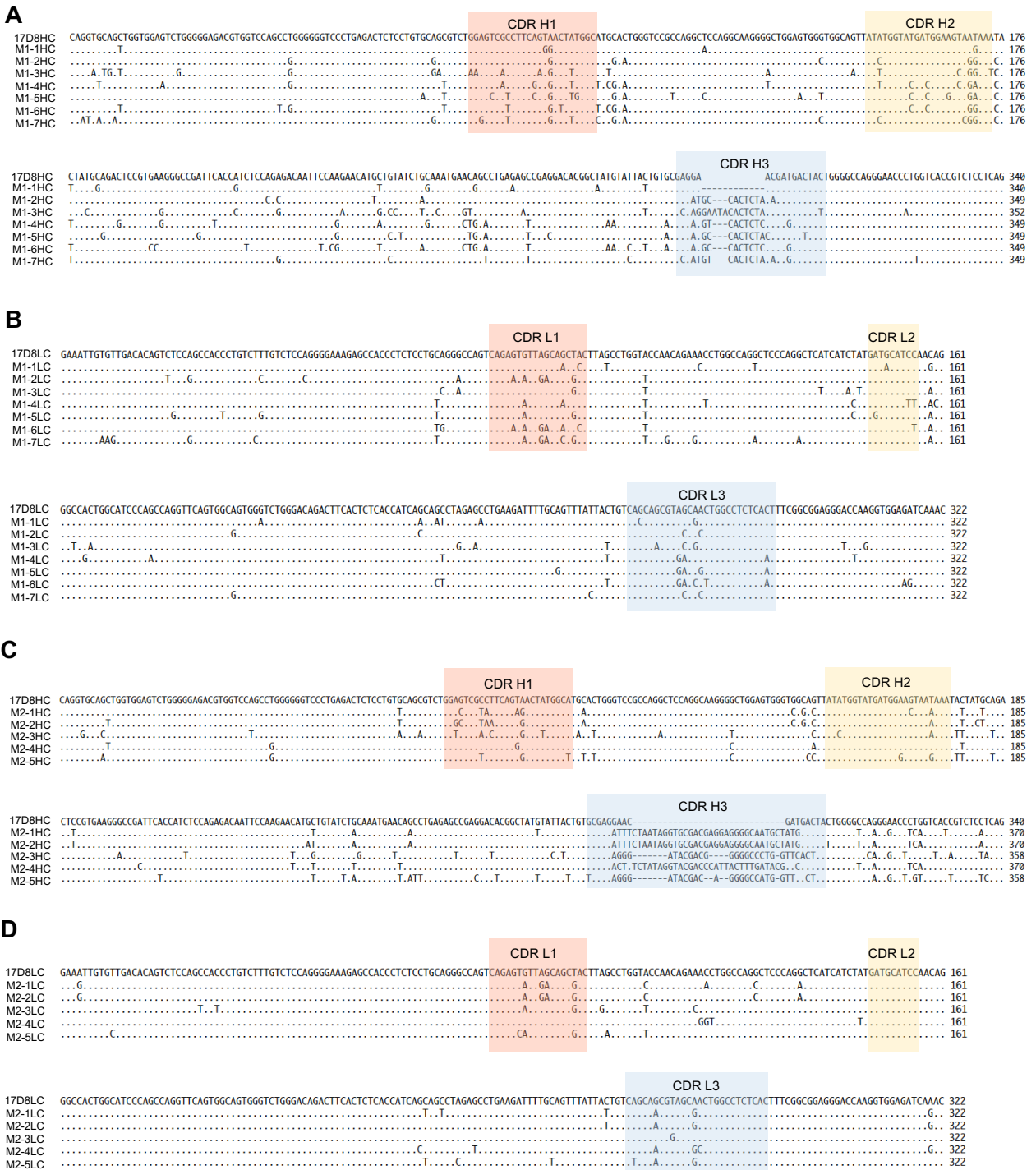
Fig. S1

Fig. S1. DNA Sequence alignments of the Ig variable regions of new anti-PD1 antibodies with the 17D8HC. (A) Alignment of M1-1 to M1-7HCs with the 17D8HC. The sequence of the 17D8HC serves as the template for alignment; only SHMs in new anti-PD1 antibodies are shown below, “.” Indicates identity, “-” indicates gap in alignment. The CDRs are shaded with color. Sections B-D are organized in the same way as this section. (B) Alignment of M1-1 to M1-7LCs with the 17D8LC. (C) Alignment of M2-1 to M2-5HCs with the 17D8HC. (D) Alignment of M2-1 to M2-5LCs with the 17D8LC.

Fig. S2. Analysis of PD1-binding activities of new anti-PD1 antibodies. This figure supplements Fig. 3 by showing the analysis of additional antibodies isolated from Mouse Models 1 and 2. Sections A-C in this figure correspond to A-C in Figure 3, Section D-F in this figure relate to E-G in Fig. 3.

Fig. S3

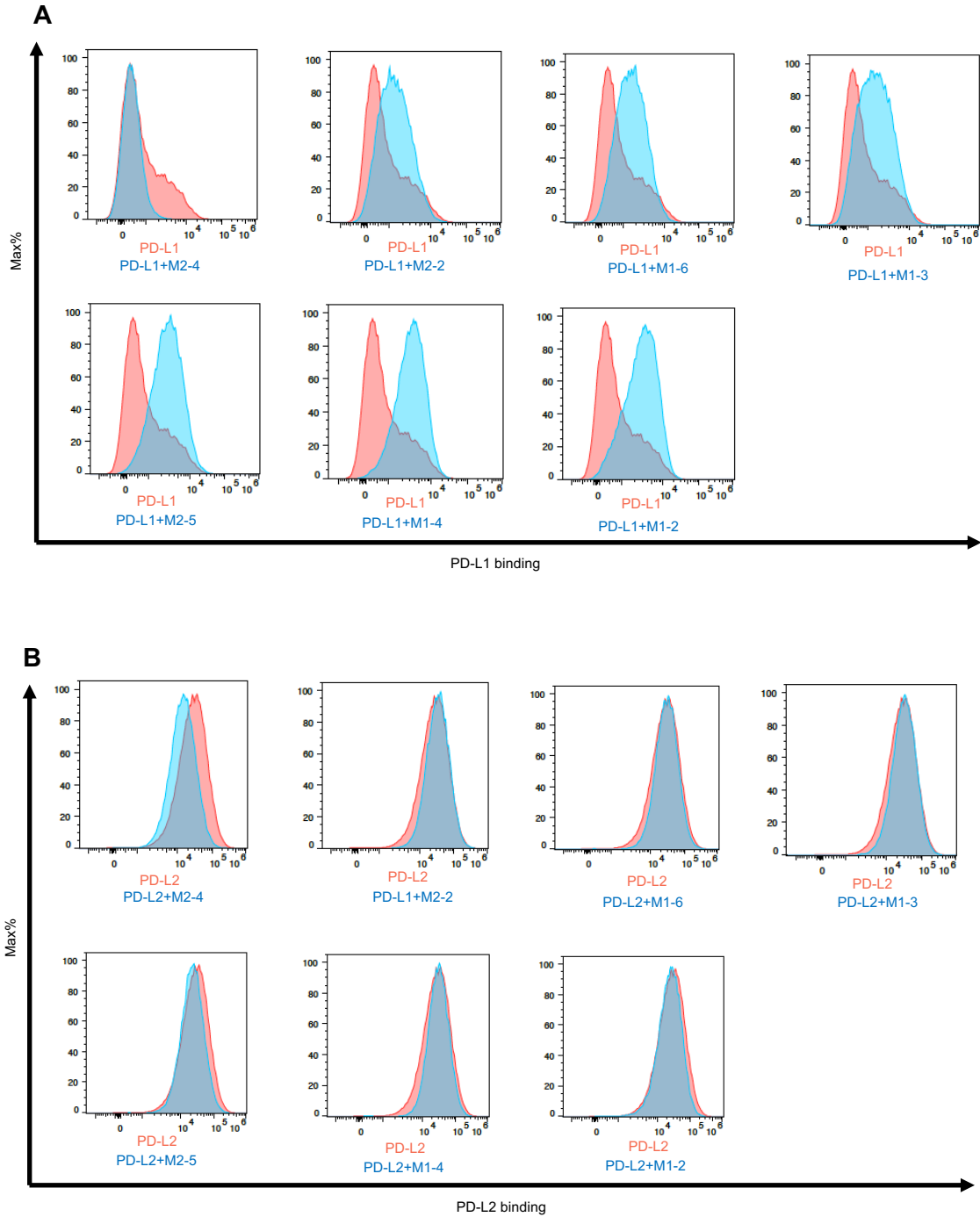


Fig. S3. The effects of new anti-PD1 antibodies on PD1/ligand interaction. This figure supplements Fig. 4 by presenting the characterization of additional antibodies isolated from Mouse Model 1 and 2. Sections A and B in this figure relate to (A) and (B) in Fig. 4.