

## Supplementary Information

### Recombinant antibody production using a dual-promoter single plasmid system

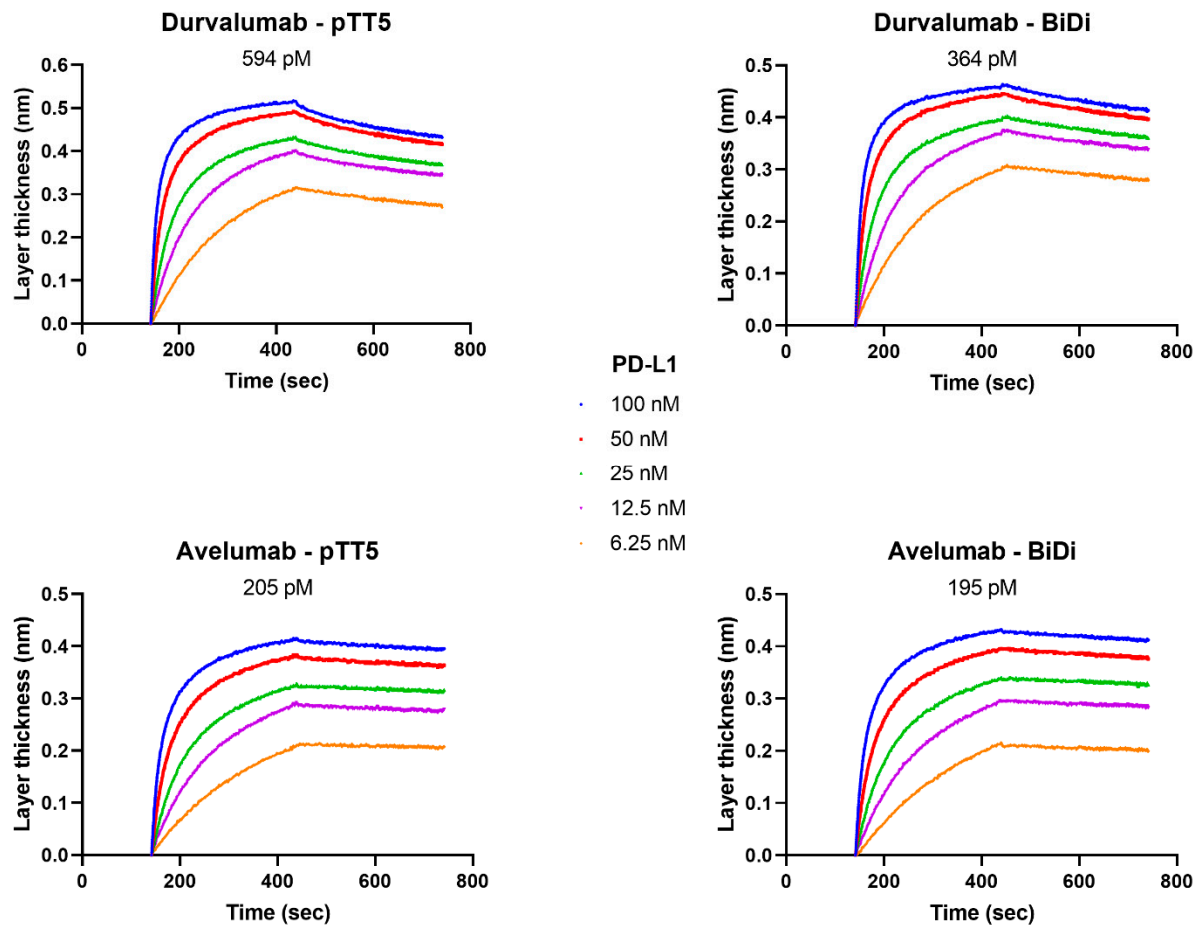
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**Table S1: Primers used for cloning of bidirectional constructs**

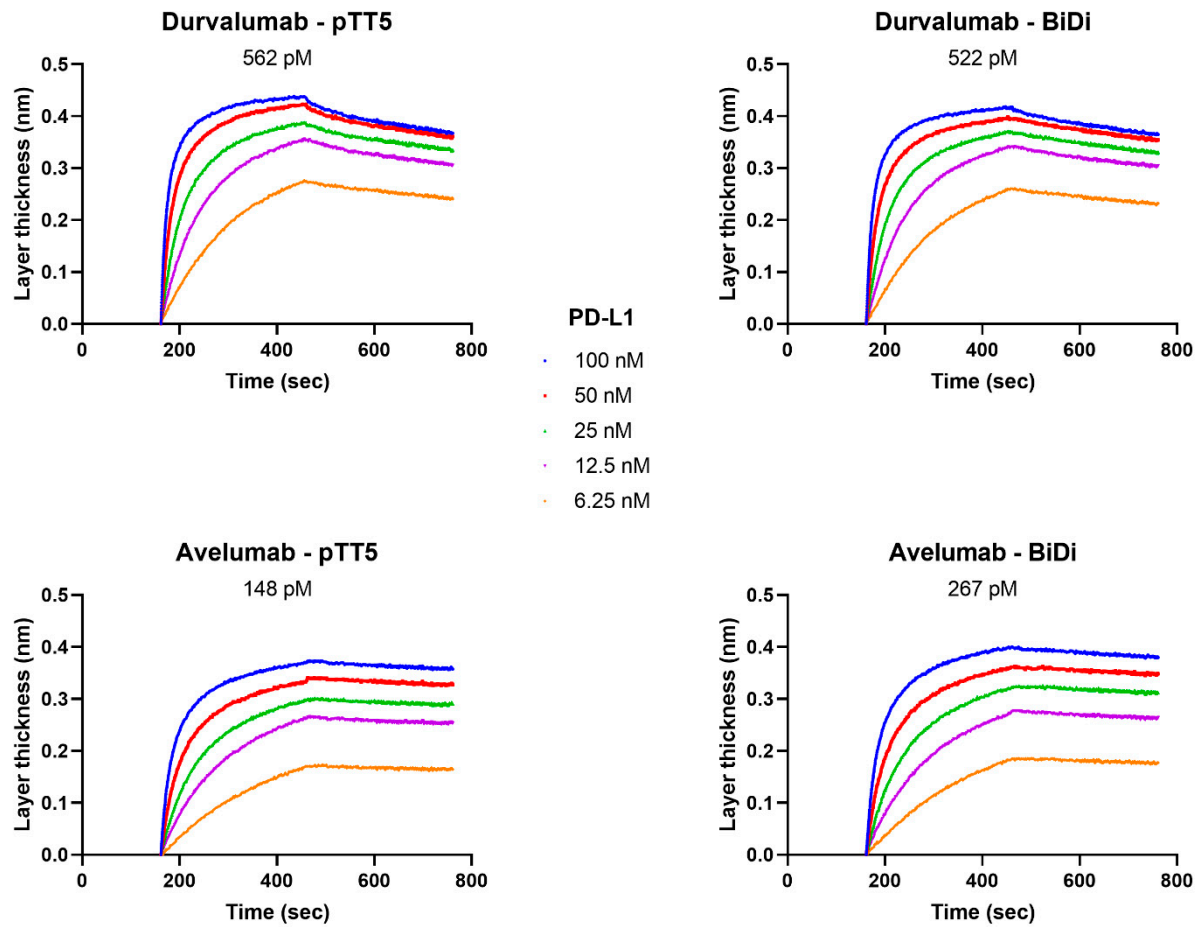
AvelumabVH_SapI_for	AAAAAGCTCTTCAAGTGAAGTTCAGCTGTTGG
AvelumabVH_SapI_rev	TTTTTTGCTCTTCTGGCAGAGGAGACAGTAACAAGAG
AvelumabVL_SapI_for	AAAAAGCTCTTCAAGTCAATCCGCCTTGACTC
AvelumabVL_SapI_rev	TTTTTTTGTCTTTCACCCGAGAACTGTGACCTTTG
DurvalumabVH_SapI_for	AAAAAGCTCTTCAAGTGAGGTTCAACTTGTTGAAAGCGG
DurvalumabVH_SapI_rev	TTTTTTGCTCTTCTGGCGCTTGAGACTGTAACGAGGG
DurvalumabVL_SapI_for	AAAAAGCTCTTCAAGTGAAATAGTGCTTACCCAAAGTCC
DurvalumabVL_SapI_rev	TTTTTTGCTCTTCATCGTTTAATTTGACCTTAGTAC
MD-Leader-VH_BbsI_for	ATATAGAAGACATCGCTTGCCACCATGAC
CH1_MD_Esp3I_rev	ATATATCGTCTCGGTATGGGTCTTGTCGCAGCTCTTGG
MD-Leader-VL_BbsI_for	ATATAGAAGACCGTCGCAGCCACCATGAC
Lam-CL_Esp3I_rev	ATATACGTCTCGAGATCTATTAGCTGCACTCGGTGGGGGCCACGGTTTT CTCCACGGTGCTGCCCTC
Kap-CL_Esp3I_rev	ATATACGTCTCGAGATCTATTAACACTCTCCCCTGTTGAAGCTC
eCMV-HC_BbsI_rev	GCGCGGAAGACATAGCGCGCTAGAGATCCGTTTAACTTGG
eCMV-LC_BbsI_rev	GCGCGGAAGACATGCGACGCTAGAGATCCGTTTAACTTGG
eCMV_LC-Stuffer-Con_BbsI_for	GCGCGGAAGACATGATGGTACATTTATATTGGCTCATGTCCAATATGAC CGC
eCMV_HC-Stuffer-Con_BbsI_for	GCGCGGAAGACATGGCTGTACATTTATATTGGCTCATGTCCAATATGAC CGC
CMV-HC_BbsI_rev	GCGCGGAAGACATAGCGGATCTGACGGTTCATAAACCAG
CMV-HC-Stuffer-Con_BbsI_for	GCGCGGAAGACATGGCTCCGCGTTACATAACTTACGGTAAATG
CMV-LC-BbsI_rev	GCGCGGAAGACATGCGAGATCTGACGGTTCATAAACCAGC
CMV-LC-Stuffer-Con_BbsI_for	GCGCGGAAGACATGATGCCGCGTTACATAACTTACGGTAAATG
EF1a-LC-Stuffer-Con_BbsI_for	GCGCGGAAGACATGATGGGC
EF1a-LC_BbsI_rev	ATATAGAAGACATGCGAAGCCTGCTTTTTTGTACAACTTGTCAC
HC-minCMV-CMV-LC_BbsI_rev	GCGCGGAAGACTAAGCGTCTGACGGTTCATAACGAGCTCTGC
HC-minCMV-CMV-LC_BbsI_for	GCGCGGAAGACATGCGAGATCTGACGGTTCATAAACCAGC

**Table S2: RT-qPCR primers for HC and LC constant regions**

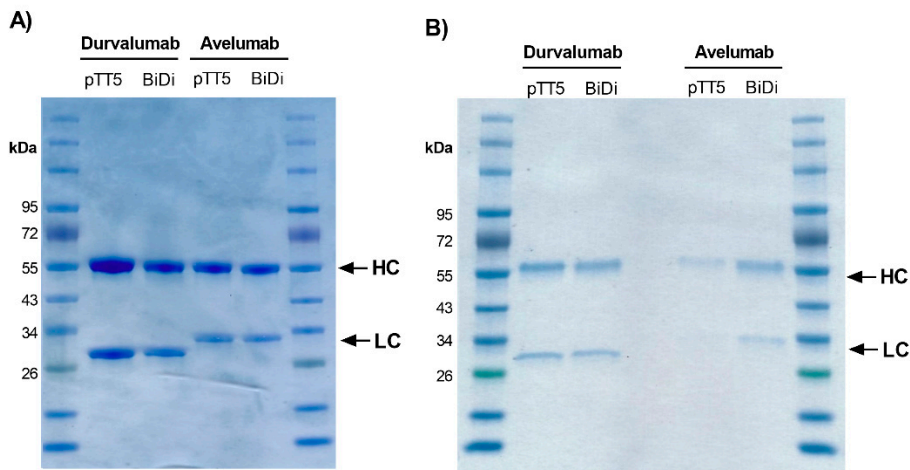
HC constant_fwd	TTCCAGAACCAGTCACCGTT
HC constant_rev	CCAAAGAAGAAGAGGGAACA
LC kappa constant_fwd	TGTAGGTGCTGTCCTTGCTG
LC kappa constant_rev	CTGTTGTGTGCCTGCTGAAT
LC lambda constant_fwd	GTAGCTCCTGTGGCTTTTC
LC lambda constant_rev	TGATCAGCGACTTCTACCC



**Figure S1: Affinity determination by BLI of antibodies produced in Expi293-F.** Binding of Durvalumab and Avelumab produced in Expi293-F cells to PD-L1. Antibodies were either produced by co-transfection (denoted as pTT5) or transfection using 2xeCMV BiDi construct (denoted as BiDi). The curves represent binding of 10 µg/mL antibody to different concentrations of PD-L1.



**Figure S2: Affinity determination by BLI of antibodies produced in ExpiCHO-S.** Binding of Durvalumab and Avelumab produced in ExpiCHO-s cells to PD-L1. Antibodies were either produced by co-transfection (denoted as pTT5) or transfection using 2xeCMV BiDi construct (denoted as BiDi). The curves represent binding of 10  $\mu\text{g/mL}$  antibody to different concentrations of PD-L1.



**Figure S3: SDS-PAGE analysis of purified antibodies.** Heavy and light chain bands at their expected molecular weights. A) represents purified antibodies from an Expi293-F production, while B) represents those produced in ExpiCHO-S cells. After purification, 3 µg or 0.5 µg antibodies were loaded onto an SDS-PAGE gel after Expi293-F and ExpiCHO-S production and purification, respectively.

**Sequence S1: DNA sequence of the designed Durvalumab-2xeCMV insert.** The colour codes represent the following regions: SV40 polyA signal (grey), **CL Kappa** (cyan), **VL Durvalumab** (magenta), **Signal peptide** (brown), **Enh. MLP** (dark green), **TLP** (red), **CMV promoter** (yellow), **CMV Enhancer** (green), **Stuffer** (blue), **VH Durvalumab** (dark blue), **CH1-CH2-CH3** (black), **Stop codon** (dark yellow).

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ACCTCTACAAATGTGGTATGGCTGATTATGAGCTAGAGATCTA**TTA**ACACTCTCCCCTGTTGAAGCTCTTTGTGACGGG  
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