

## Supplementary Information

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

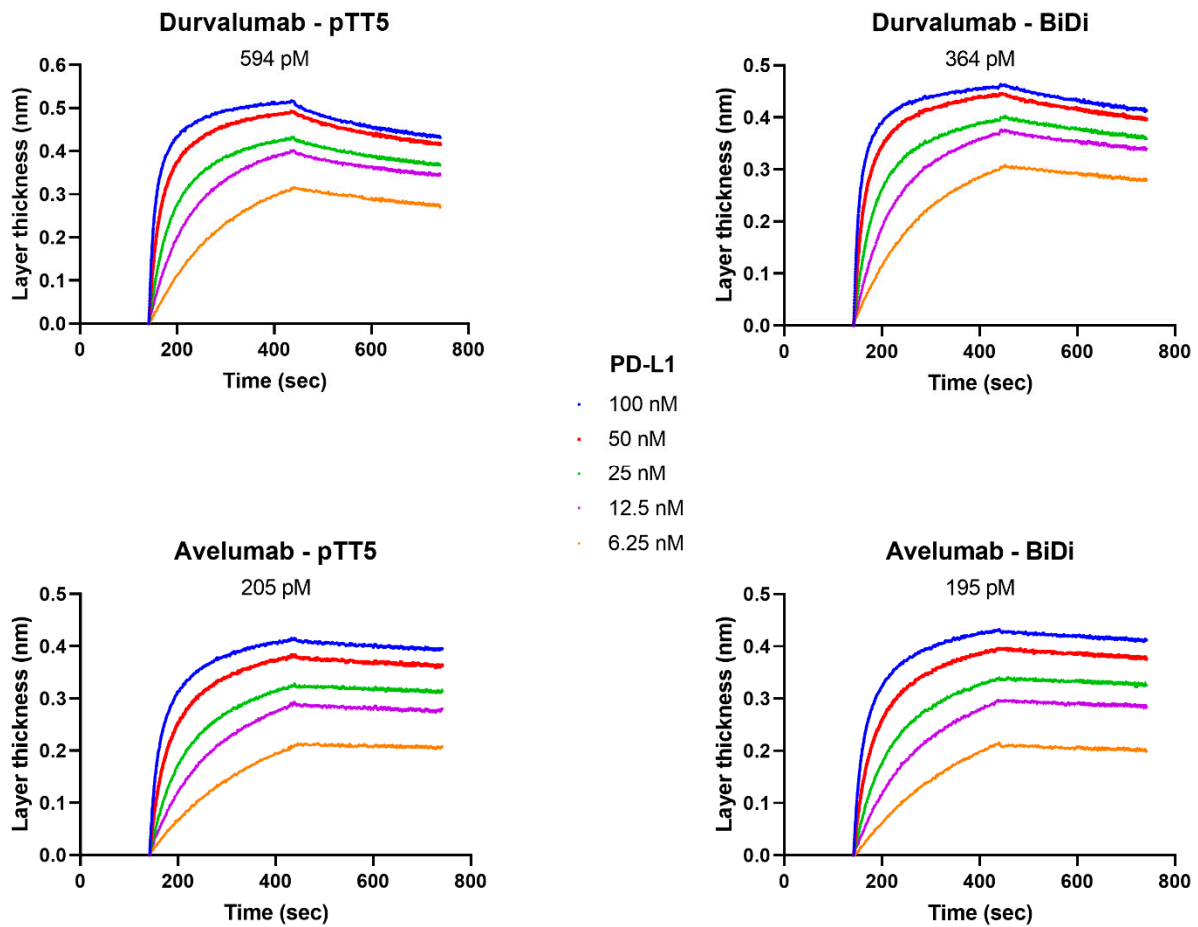
Stefania C. Carrara<sup>+</sup>, David Fiebig<sup>+</sup>, Jan P. Bogen<sup>+</sup>, Julius Grzeschik, Björn Hock, and Harald Kolmar

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

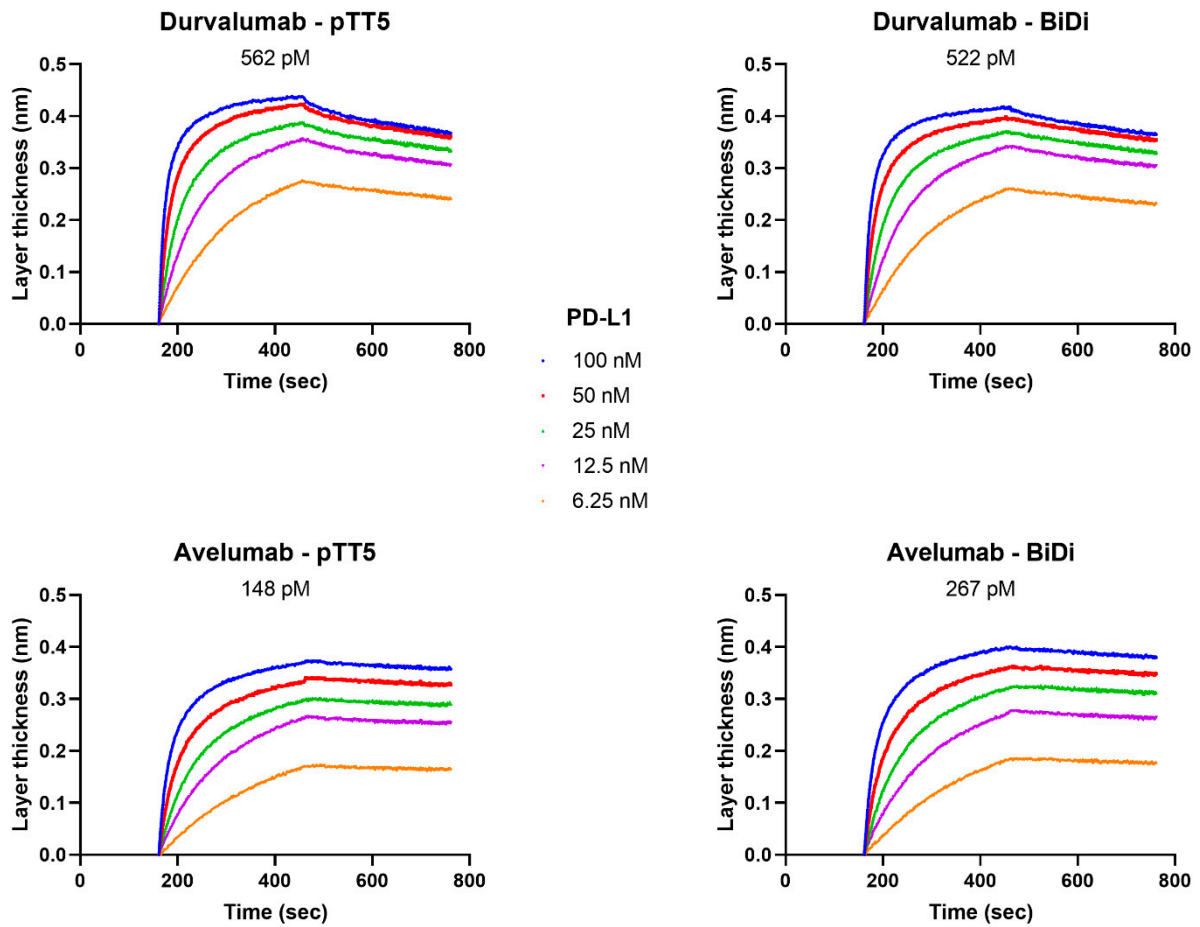
AvelumabVH_Sapl_for	AAAAAGCTCTTCAAGTGAAGTTCAGCTGTTGG
AvelumabVH_Sapl_rev	TTTTTTGCTCTTCTGGCAGAGGAGACAGTAACAAGAG
AvelumabVL_Sapl_for	AAAAAGCTCTTCAAGTCAATCCGCCTTGACTC
AvelumabVL_Sapl_rev	TTTTTTTTGCTCTTCAACCCGAGAACTGTGACCTTTG
DurvalumabVH_Sapl_for	AAAAAGCTCTTCAAGTGAGGTTCAACTTGTTGAAAGCGG
DurvalumabVH_Sapl_rev	TTTTTTGCTCTTCTGGCGCTTGAGACTGTAACGAGGG
DurvalumabVL_Sapl_for	AAAAAGCTCTTCAAGTGAAATAGTGCTTACCCAAAGTCC
DurvalumabVL_Sapl_rev	TTTTTTGCTCTTCATCGTTTAATTTGACCTTAGTAC
MD-Leader-VH_Bbsl_for	ATATAGAAGACATCGCTTGCCACCATGAC
CH1_MD_Esp3I_rev	ATATATCGTCTCGGTATGGGTCTTGTCGCAGCTCTTGG
MD-Leader-VL_Bbsl_for	ATATAGAAGACCGTCGCAGCCACCATGAC
Lam-CL_Esp3I_rev	ATATACGTCTCGAGATCTATTAGCTGCACTCGGTGGGGGCCACGGTTTT CTCCACGGTGCTGCCCTC
Kap-CL_Esp3I_rev	ATATACGTCTCGAGATCTATTAACACTCTCCCCTGTTGAAGCTC
eCMV-HC_Bbsl_rev	GCGCGGAAGACATAGCGCGCTAGAGATCCGTTTTAACTTGG
eCMV-LC_Bbsl_rev	GCGCGGAAGACATGCGACGCTAGAGATCCGTTTTAACTTGG
eCMV_LC-Stuffer-Con_Bbsl_for	GCGCGGAAGACATGATGGTACATTTATATTGGCTCATGTCCAATATGAC CGC
eCMV_HC-Stuffer-Con_Bbsl_for	GCGCGGAAGACATGGCTGTACATTTATATTGGCTCATGTCCAATATGAC CGC
CMV-HC_Bbsl_rev	GCGCGGAAGACATAGCGGATCTGACGGTTCATAAACCAG
CMV-HC-Stuffer-Con_Bbsl_for	GCGCGGAAGACATGGCTCCGCGTTACATAACTTACGGTAAATG
CMV-LC-Bbsl_rev	GCGCGGAAGACATGCGAGATCTGACGGTTCATAAACCAGC
CMV-LC-Stuffer-Con_Bbsl_for	GCGCGGAAGACATGATGCCGCTTACATAACTTACGGTAAATG
EF1a-LC-Stuffer-Con_Bbsl_for	GCGCGGAAGACATGATGGGC
EF1a-LC_Bbsl_rev	ATATAGAAGACATGCGAAGCCTGCTTTTTTGTACAACTTGTAC
HC-minCMV-CMV-LC_Bbsl_rev	GCGCGGAAGACTAAGCGTCTGACGGTTCATAAACCAGCTCTGC
HC-minCMV-CMV-LC_Bbsl_for	GCGCGGAAGACATGCGAGATCTGACGGTTCATAAACCAGC

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

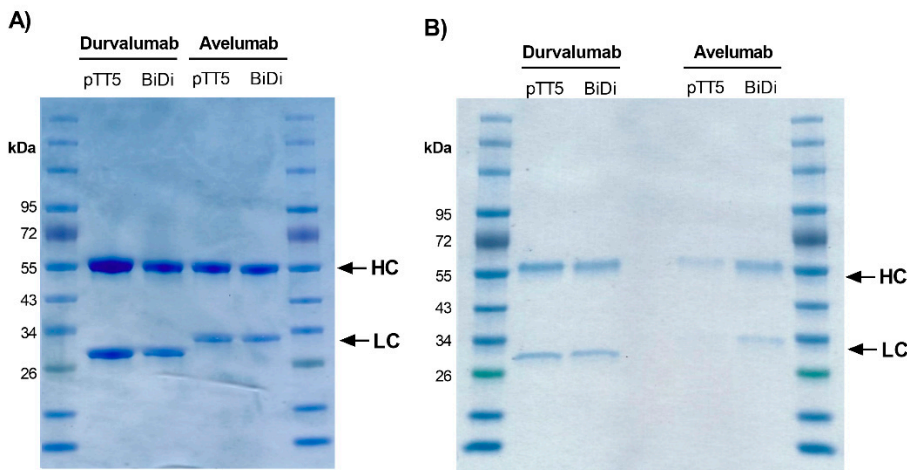
HC constant_fwd	TTCCAGAACCAGTCACCGTT
HC constant_rev	CCAAAGAAGAAGAGGGAACA
LC kappa constant_fwd	TGTAGGTGCTGTCCTTGCTG
LC kappa constant_rev	CTGTTGTGTGCCTGCTGAAT
LC lambda constant_fwd	GTAGCTCCTGTGGCTTTTC
LC lambda constant_rev	TGATCAGCGACTTCTACCC



**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  $\mu\text{g}/\text{mL}$  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  $\mu\text{g}/\text{mL}$  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  $\mu$ g or 0.5  $\mu$ g 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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GCTGCTGAGGCTGTAGGTGCTGTCTTGTCTGCTGTGACTCTCTGGGAGTTACCCGATTGGAGGGCGTT  
ATCCACCTTCCACTGTA**CTTTGGCCTCTCTGGGATAGAAGTTATTCAGCAGGCACACAACAGAGGCAGTTCAGATTTCC**  
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CCGAATGTCCAAGGGAGACTACCATATTGTTGACAGTAGTAGACTGCAAAATCTTCCGGTTCAGCCGGGAAATTGTA  
AGTGTA**AAATCTGTGCCGAACCGCTTCTGAAAACCTATCAGGTATGCCAGTAGCTCTAGAGGATGCGTCATAGATC**  
AACAGACGCGGTGCTT**GCCCAGGCTTTTGTGATACCATGCAAGATATGAACTGGATACCTTTGTGAAGCTCTGCAA**  
GACAGAGTTGCTCTTT**CACCCGGGAGAGACTCAGGGTGCCGGGACTTTGGGTAAGCACTATTTCA**AGCCAAGGCAAT  
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