In Pursuit of Stability Enhancement of a Prostate Cancer Targeting Antibody Derived from a Transgenic Animal Platform

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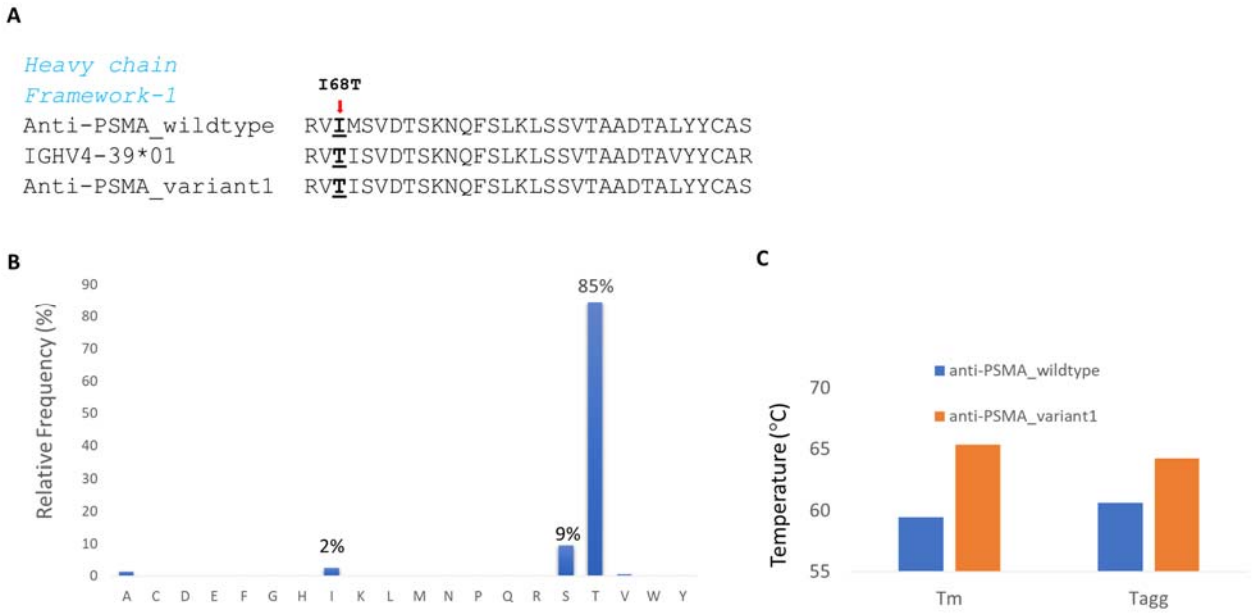
Supplementary information

Figure S1: (**a**) Sequence alignment of an anti-PSMA heavy chain (VH) with human germline IGHV4-39*01 and its engineered variant. An unusual framework residue (Ile) at position 68 was re-engineered to Thr. (**b**) Relative positional frequency of the SHM using the abYsis portal. (**c**) Intrinsic properties characterization of anti-PSMA using Differential Scanning Fluorimetry (DSF). An improvement in both Tm and Tagg was observed for the engineered variant.

Figure S2: (**a**) Sequence alignment of an anti-DLL3 heavy chain (VH) with human germline IGHV3-13*05 and the light chain (Vk) with human germline IGKV1-5*03 and a series of engineered variants. Two unusual framework residues at position 85 (in the VH) and at position 84 (in VL) were re-engineered. (**b**) The composition of mutations in VH and Vk for the three engineered variants (**c**) Intrinsic properties characterization of an anti-DLL3 using Differential Scanning Fluorimetry (DSF). An improvement in both Tm and Tagg was observed for the engineered variant. (**d**) Relative positional frequency of His85 (in VH) using the abYsis portal. (**e**) Relative positional frequency of Glu84 (in Vk) using the abYsis portal.

Supplementary Figure – S1



Supplementary Figure – S2

A

Heavy chain_Framework-3

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ANTI-DLL3_HC                    RFTISRENVKHSLYLQMNSLRVGDTAVYFCAR
IGHV3-13*05                     RFTISRENAKNSLYLQMNSLRAGDTAVYYCAR
ANTI-DLL3_variant-1_HC          RFTISRENVKNSLYLQMNSLRVGDTAVYFCAR
ANTI-DLL3_variant-2_HC          RFTISRENVKNSLYLQMNSLRVGDTAVYFCAR
ANTI-DLL3_variant-3_HC          RFTISRENVKHSLYLQMNSLRVGDTAVYFCAR
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Light chain_Framework-3

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ANTI-DLL3_LC                    GVPSRFSGSGSETEFTLTISSLQPDDFATYYC
IGKV1-5*03                      GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC
ANTI-DLL3_variant-1-LC          GVPSRFSGSGSETEFTLTISSLQPDDFATYYC
ANTI-DLL3_variant-2-LC          GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC
ANTI-DLL3_variant-3-LC          GVPSRFSGSGSGTEFTLTISSLQPDDFATYYC
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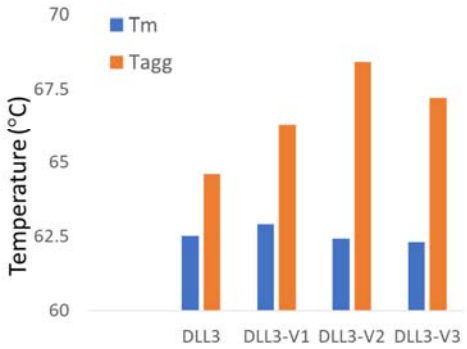
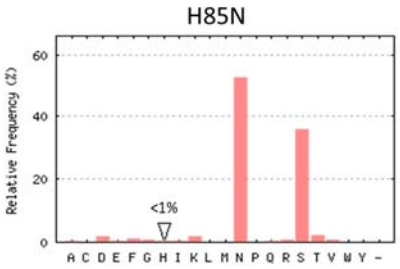
B

#	Name	Heavy chain	Light chain
1	Anti-DLL3-wildtype	DLL3_HC	DLL3_LC
2	Anti-DLL3-variant1 (V1)	DLL3_HC_H85N	DLL3_LC
3	Anti-DLL3-variant2 (V2)	DLL3_HC_H85N	DLL3_LC_E84G
4	Anti-DLL3-variant3 (V3)	DLL3_HC	DLL3_LC_E84G

C



D



E