Supporting Information

An engineered antibody fragment targeting mutant β-catenin via HLA neoantigen presentation

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Running title: *Targeting mutant β-catenin*

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Fig. S1. 2mFo-DFc map of β-catenin S45F mutant peptide. A.

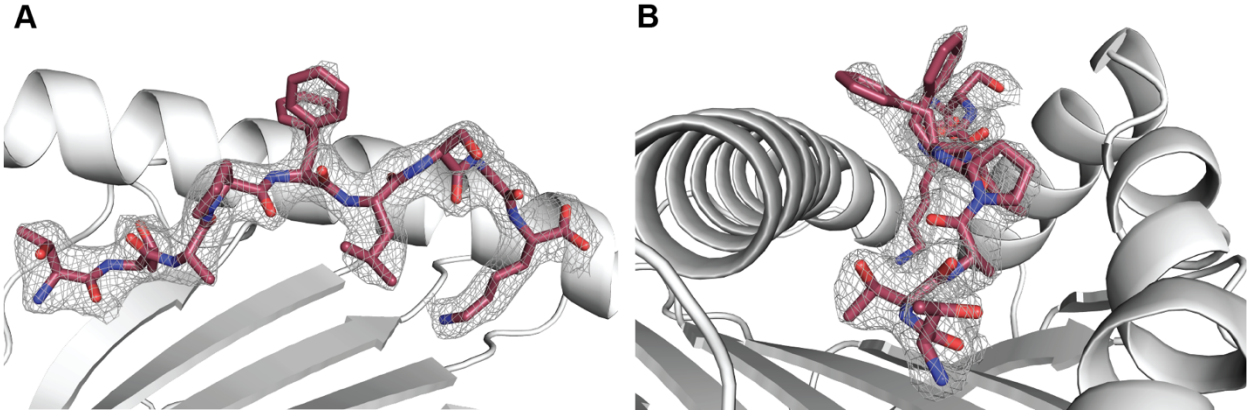

Fig. S2. Amino acid sequences of S45F mutant β-catenin$_{41-49}$pHLA-A*03:01 specific scFvs.

E10

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTL
TISSLQPEDFATYYCQQSYYSPPTFGQGTKVEIKRTGGGSGGGASEVQLVESGGGLVQPGGSLRLSCAASGFN
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YSYYSAMDVWGQGTLVTVSS

cl. 3

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSAYFLYSGVPSRFSGSRSGTDFTL
TISSLQPEDFATYYCQQIYTSPITFGQGTKVEIKRTGGGSGGGGSGGGASEVQLVESGGGLVQPGGSLRLSCA
ASGFNFITTGMHWVRQAPGKGLEWVARIGPGSDYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRYYYASALDYWGQGTLVTVSS

cl. 4

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTL
TISSLQPEDFATYYCQQRAYFPITFGQGTKVEIKRTGGGSGGGGSGGGASEVQLVESGGGLVQPGGSLRLSC
AASGFNFSDYGMHWVRQAPGKGLEWVAMLIPASGYTNYADSVKGRFTISADTSKNTAYLQMNSLRAEDTA
VYYCSRGWSYYMDYWGQGTLVTVSS

cl. 7

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSASFLYSGVPSRFSGSRSGTDFTL
TISSLQPEDFATYYCQQQYAYTPITFGQGTKVEIKRTGGGSGGGGSGGGASEVQLVESGGGLVQPGGSLRLS
CAASGFNVWSYGIHWVRQAPGKGLEWVAGVTPDGSYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDT
AVYYCSRSYGWAMDYWGQGTLVTVSS

cl. 9

DIQMTQSPSSLSASVGDRVTITCRASQDVNTAVAWYQQKPGKAPKLLIYSAYFLYSGVPSRFSGSRSGTDFTL
TISSLQPEDFATYYCQQIHYKPLTFGQGTKVEIKRTGGGSGGGGSGGGASEVQLVESGGGLVQPGGSLRLSCA
ASGFNVAWYSIHWVRQAPGKGLEWVAQVYGGSSYTYYADSVKGRFTISADTSKNTAYLQMNSLRAEDTAV
YYCSRDFYSSGMDYWGQGTLVTVSS

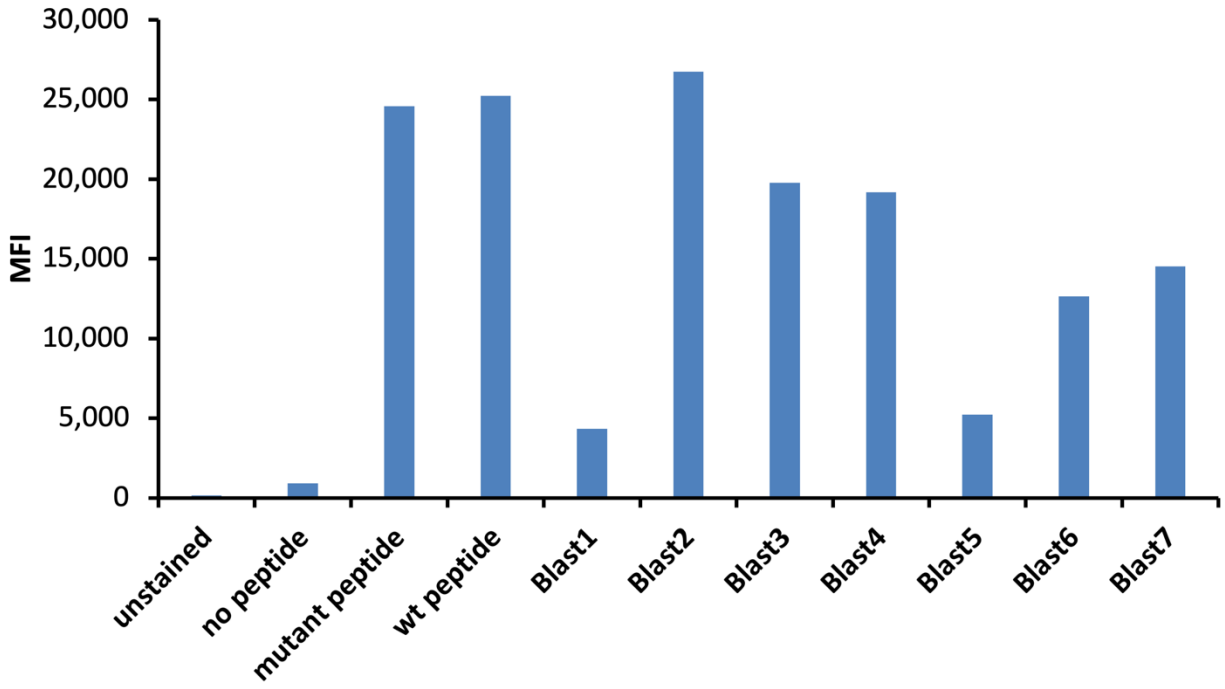

Fig. S3. Anti-HLA-A3 staining to assess peptide pulsing of CTNNB peptides and BLAST peptides. T2A3 cells were peptide-pulsed overnight at 37°C in serum-free media with either the CTNNB mutant S45F peptide (TTAPFLSGK) and β2M, CTNNB wildtype peptide (TTAPSLSGK) and β2M, or β2M only. Cells were incubated with anti-HLA-A3 antibody clone GAP.A3 PE conjugate. Cells were stained with a live/dead Near-IR dye, washed, and analyzed by an LSRII flow cytometer.

Table S1. Blast Peptides. BLAST peptides were identified by a BLASTp search of the CTNNB S45F mutant peptide (TTAPFLSGK) against the normal human proteome. Hits were then analyzed by netMHCv4.0 for binding to HLA-A*03:01.

Peptide Name	Peptide	Predicted Affinity (nM)	Protein
Blast1	QLLDFLSGK	91.7	PCNA-interacting partner isoform 1 [Homo sapiens]
Blast2	SLNPKFLSGK	16.9	ephrin-B1 precursor [Homo sapiens]
Blast3	IIYNFLSGK	7.3	orexin receptor type 2 isoform X1 [Homo sapiens]
Blast4	RTVTFLSGK	13.4	nuclear receptor subfamily 1, group D, member 2 [Homo sapiens]
Blast5	TAFDPFLGGK	178.5	heat shock protein 105 kDa isoform 3 [Homo sapiens]
Blast6	RIIPFLPGK	18.0	SH3 and PX domain-containing protein 2A isoform 1 [Homo sapiens]
Blast7	IQNPFLSSK	60.4	SCAN domain-containing protein 3 isoform 1 [Homo sapiens]