YMTHE, Volume *29*

Supplemental Information

Self-Replicating RNAs Drive Protective Anti-tumor T Cell Responses to Neoantigen Vaccine Targets in a Combinatorial Approach

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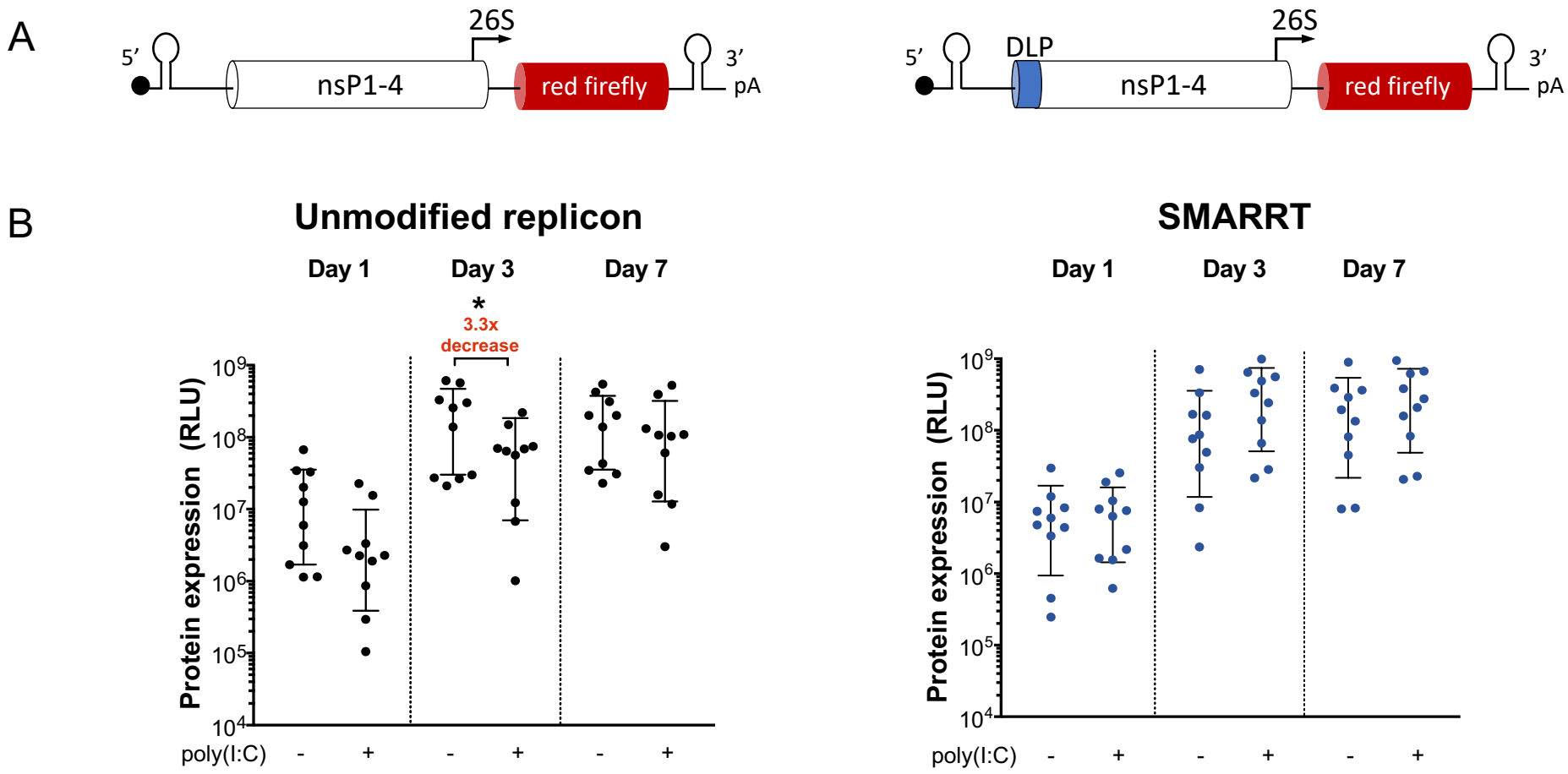


Figure S1: SMAART technology allows for enhanced protein expression *in vivo*. A) Schematic of unmodified replicon and SMARRT molecular structures showing placement of DLP. B) *In vivo expression* of luciferase plotted as the geometric mean of the total flux was measured as a correlate of protein expression at Day 1, 3 and 7 in mice that received 15 mg of unmodified a replicon (left) or SMARRT replicon (right) in the presence (with) or absence (w/o) of poly(I:C) pretreatment at Day-1. Statistical testing was carried out using a two-tailed unpaired Student's T test. *$p<0.05$

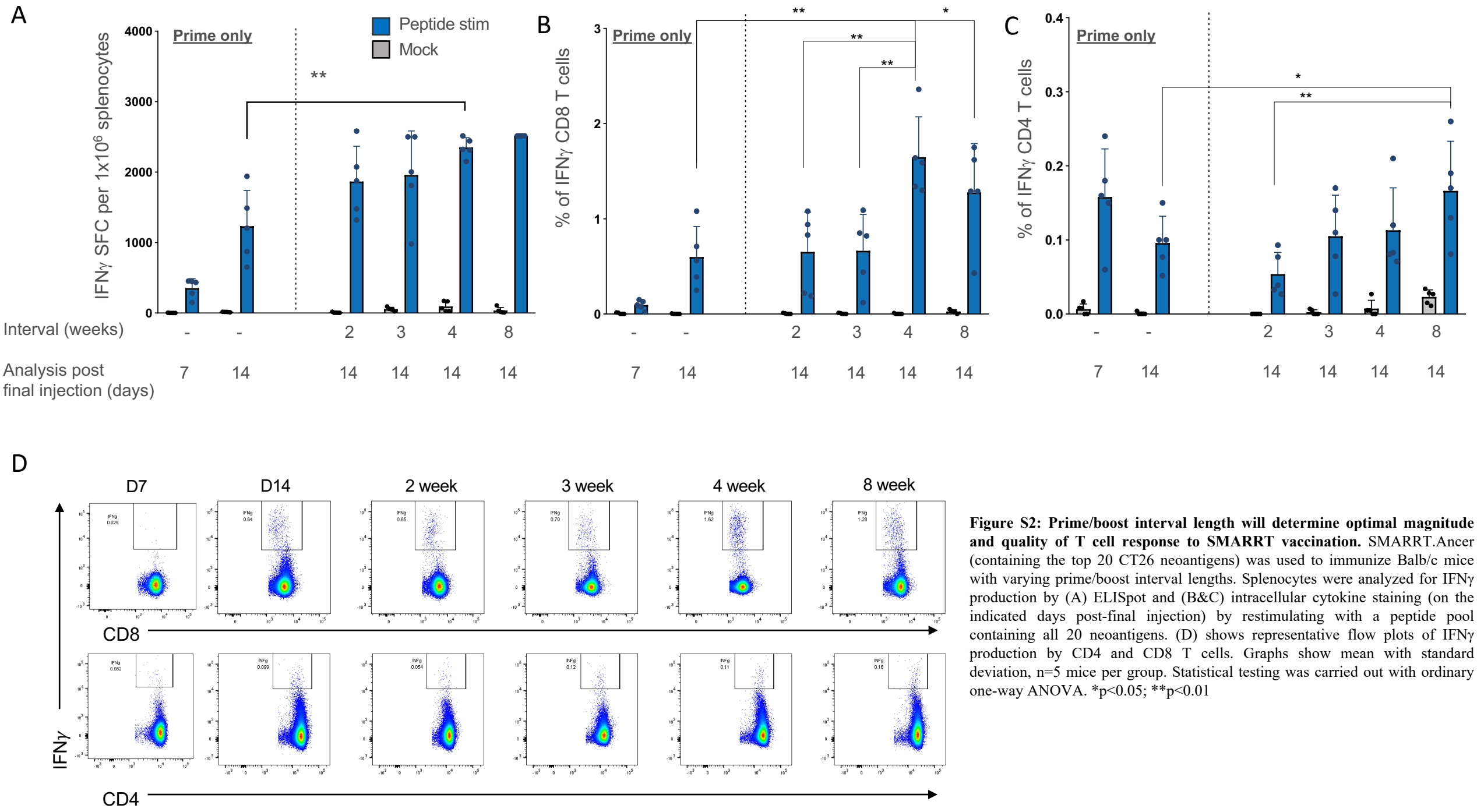
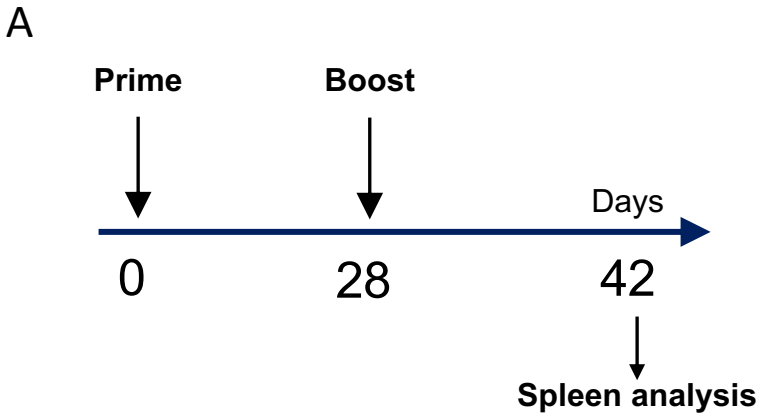


Figure S2: Prime/boost interval length will determine optimal magnitude and quality of T cell response to SMARRT vaccination. SMARRT.Ancer (containing the top 20 CT26 neoantigens) was used to immunize Balb/c mice with varying prime/boost interval lengths. Splenocytes were analyzed for IFNγ production by (A) ELISpot and (B&C) intracellular cytokine staining (on the indicated days post-final injection) by restimulating with a peptide pool containing all 20 neoantigens. (D) shows representative flow plots of IFNγ production by CD4 and CD8 T cells. Graphs show mean with standard deviation, n=5 mice per group. Statistical testing was carried out with ordinary one-way ANOVA. *p<0.05; **p<0.01



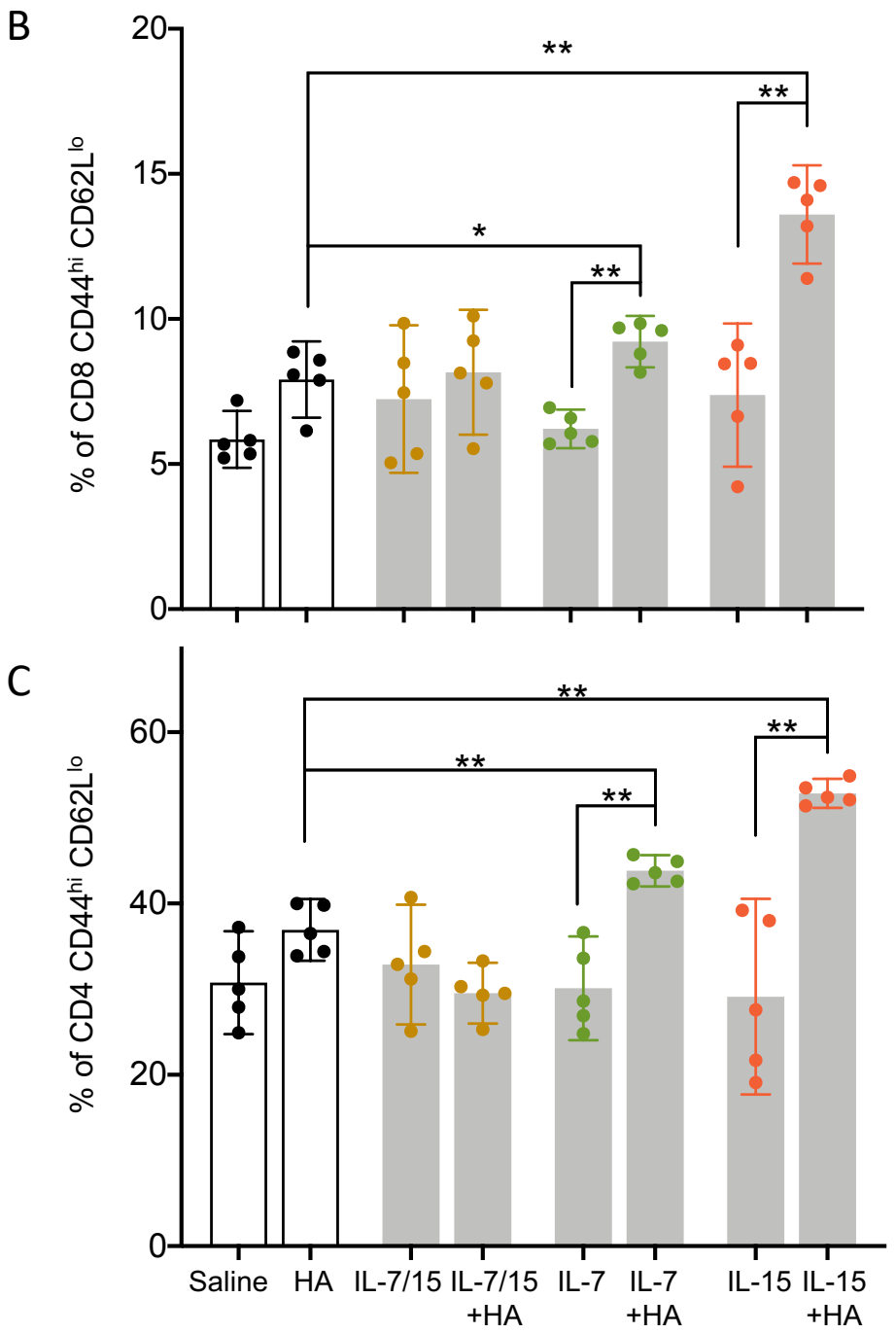


Figure S3: Expansion of CD8 and CD4 effector memory T cell responses when combining SMARRT-expressed IL-7 or IL-15 cytokines to SMARRT influenza vaccine. (A) Schematic of *in vivo* study depicting the prime-boost strategy to hemagglutinin H5 from influenza. Frequency of CD8 (B) and CD4 (C) T effector memory cells in the spleens of immunized mice. Graphs show mean with standard deviation, n=5 mice per group. Statistical testing for B and C was done using Mann-Whitney U test. Statistical testing in D was done using Ordinary one-way ANOVA to compare the total IFNγ+ CD8 T cells, *p<0.05; **p<0.01.

Table S1: Neoantigen sequences identified from CT26 cell line

Pep ID	Mutated Sequence	CHR	Postion mm10	Gene	Ref	AA Pos	Alt
EO_CT26_01	LQARLTSYETLK	CHR4	86583172	Haus6	Ala	821	Thr
EO_CT26_02	ETPEACRQARNYLEFSE	CHR11	69649178	Fxr2	Ser	287	Asn
EO_CT26_03	SSRVQYVVNPAVKIVF	CHR2	128676212	Anapc1	Asp	241	Asn
EO_CT26_04	TSKYYMRDVIAIESA	CHR2	158851764	Dhx35	Thr	646	Ile
EO_CT26_05	PALLIKHMYNKLIS	CHR6	3377051	Samd9l	Arg	70	His
EO_CT26_06	LSWDTSKKNLTEYLSRF	CHR5	100037938	Hnrnpdl	Asp	163	Asn
EO_CT26_07	NNVHYLNDGDAIIYHTAS	CHR12	98815985	Eml5	Asp	1396	Ala
EO_CT26_08	PQPDLYRFVRRISI	CHRX	60293650	Atp11c	Gly	223	Arg
EO_CT26_09	DTKCTKADCLFTHMSR	CHR12	98785005	Zc3h14	Pro	653	Leu
EO_CT26_10	EEDGIAVWTLLNGN	CHR15	3275728	Sepp1	Asp	122	Ala
EO_CT26_11	ATVHSSMNKMLEE	CHR7	55873449	Cyfip1	Glu	71	Lys
EO_CT26_12	ILGYRYWTGIGVLQSC	CHR12	91825363	Sel1l	Ala	299	Thr
EO_CT26_13	FCYVTYKGEIRGAS	CHR6	52729334	Tax1bp1	His	107	Tyr
EO_CT26_14	VKICNMQKAAIL	CHR2	109298148	Kif18a	Glu	383	Ala
EO_CT26_15	RQFPVVEANWTMLHDE	CHR10	122089020	Tmem5	Ser	259	Asn
EO_CT26_16	MSYAEKSDEITKD	CHR2	180713221	Gid8	Pro	7	Ser
EO_CT26_17	RIQEFVRSHFY	CHR7	45442527	Gys1	Gly	310	Ser
EO_CT26_18	KVGLTVKTYEFLERNIP	CHR5	129697821	Sept14	Leu	97	Phe
EO_CT26_19	NSSTYWKGNPEMETLQ	CHR7	65663891	Tarsl2	Glu	353	Lys
EO_CT26_20	RKSYYMQKYFLDTV	CHR11	58188928	Gm12250	Asn	390	Lys
EO_CT26_21	AKNLSLNFQAVKEN	CHR12	51365554	G2e3	Ser	459	Phe
EO_CT26_22	AQAQHSKDSLY	CHR5	106983158	Cdc7	Glu	500	Lys
EO_CT26_23	LDFQNGRNTLPSS	CHR9	96687178	Zbtb38	Asp	618	Asn
EO_CT26_24	DLESQQKFYGLNLA	CHR5	49960399	Adgra2	Ser	1269	Phe
EO_CT26_25	DGGLEITGYVVKHQKVGD	CHR2	76753053	Ttn	Glu	20753	Lys
EO_CT26_26	CIQARWKYDGDDDCLDGSD	CHR2	41449239	Lrp1b	Cys	864	Tyr
EO_CT26_27	SNPRAMQVLLQIQ	CHR13	58179616	Ubqln1	Ala	456	Val
EO_CT26_28	NIGQMLQTHFT	CHR4	52484165	Smc2	Arg	1132	Gln
EO_CT26_29	DLNSEIDTNQTSLREN	CHR15	6429351	Dab2	Asn	248	Thr
EO_CT26_30	HDNKVIWLVSWTENI	CHR2	160705245	Top1	Thr	413	Ile
EO_CT26_31	NALYNMIKICLNP	CHR2	66201193	Ttc21b	Glu	1064	Lys
EO_CT26_33	PGPGNYFWKCLFMS	CHR10	82642084	Tdg	His	169	Tyr
EO_CT26_34	EQIRQNCQNMIKTY	CHR19	56801905	Ccdc186	Asp	455	Asn
EO_CT26_35	VNFSMRDGIDDES	CHR10	36993650	Hdac2	Pro	228	Ser
EO_CT26_37	ELLNVGVESNLILKG	CHR7	111079320	Eif4g2	Lys	108	Asn
EO_CT26_38	NTSFASDGFPSPLG	CHR2	147038212	Xrn2	Ser	485	Phe
EO_CT26_39	AARGINVQGLSAEEI	CHR10	109883641	Nav3	Val	154	Ile
EO_CT26_40	LRELERYVLACLR	CHR17	34113210	Brd2	Ser	703	Ala
EO_CT26_41	KNGAKGEPGACGER	CHR1	45332015	Col3a1	Arg	445	Cys
EO_CT26_42	DDDVIIGKVFMQEFK	CHR1	74256028	Arpc2	Val	176	Ile
EO_CT26_43	SVAIMPQLFMVSKT	CHR7	45881542	Kdelr1	Leu	132	Met
EO_CT26_45	LLDFLAVNQQTG	CHR2	126822502	Trpm7	Ala	986	Thr
EO_CT26_46	PKMQNAAKPSRKK	CHR11	78226939	Supt6	Ala	484	Pro
EO_CT26_47	KESQVNLQDSQLSS	CHR1	189683824	Cenpf	Glu	101	Asp
EO_CT26_48	NVQSYWIWLELMKPIIRQV	CHR1	93561157	Farp2	Pro	101	Leu
EO_CT26_49	LCVYGFKEETIRD	CHR19	12587394	Fam111a	Gly	213	Glu
EO_CT26_50	VMLSENRSLFFLRDIVE	CHR1	195117595	Cr1l	Ser	257	Phe

Table S2: Polytope insert design

Construct	Neoantigen Composition
C1	EO_CT26_01, EO_CT26_06, EO_CT26_12, EO_CT26_02, EO_CT26_08, EO_CT26_15, EO_CT26_07, EO_CT26_17, EO_CT26_09, EO_CT26_03, EO_CT26_18, EO_CT26_16, EO_CT26_11, EO_CT26_20, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_19, EO_CT26_05
C3	EO_CT26_026, EO_CT26_012, EO_CT26_016, EO_CT26_027, EO_CT26_015, EO_CT26_019, EO_CT26_03, EO_CT26_02, EO_CT26_022, EO_CT26_04, EO_CT26_028, EO_CT26_018, EO_CT26_030, EO_CT26_029, EO_CT26_031, EO_CT26_020, EO_CT26_013, EO_CT26_014, EO_CT26_010, EO_CT26_025
C4	EO_CT26_49, EO_CT26_16, EO_CT26_15, EO_CT26_39, EO_CT26_41, EO_CT26_35, EO_CT26_29, EO_CT26_37, EO_CT26_03, EO_CT26_18, EO_CT26_30, EO_CT26_28, EO_CT26_50, EO_CT26_34, EO_CT26_02, EO_CT26_22, EO_CT26_04, EO_CT26_12, EO_CT26_27, EO_CT26_48, EO_CT26_24, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_23, EO_CT26_42, EO_CT26_40, EO_CT26_33, EO_CT26_17, EO_CT26_09
C5	EO_CT26_11, EO_CT26_39, EO_CT26_29, EO_CT26_16, EO_CT26_34, EO_CT26_41, EO_CT26_35, EO_CT26_49, EO_CT26_21, EO_CT26_26, EO_CT26_43, EO_CT26_06, EO_CT26_45, EO_CT26_19, EO_CT26_15, EO_CT26_37, EO_CT26_03, EO_CT26_18, EO_CT26_30, EO_CT26_12, EO_CT26_27, EO_CT26_48, EO_CT26_33, EO_CT26_17, EO_CT26_02, EO_CT26_22, EO_CT26_04, EO_CT26_28, EO_CT26_50, EO_CT26_47, EO_CT26_23, EO_CT26_10, EO_CT26_24, EO_CT26_42, EO_CT26_40, EO_CT26_46, EO_CT26_13, EO_CT26_14, EO_CT26_25, EO_CT26_38
C6	EO_CT26_02, EO_CT26_10, EO_CT26_03, EO_CT26_04, EO_CT26_26, EO_CT26_31, EO_CT26_28, EO_CT26_20, EO_CT26_30, EO_CT26_27, EO_CT26_25, EO_CT26_14, EO_CT26_12, EO_CT26_29, EO_CT26_13, EO_CT26_15, EO_CT26_22, EO_CT26_16, EO_CT26_18, EO_CT26_19
C7	EO_CT26_06, EO_CT26_15, EO_CT26_19, EO_CT26_03, EO_CT26_18, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_12, EO_CT26_16, EO_CT26_02
C8[1]	EO_CT26_01, EO_CT26_06, EO_CT26_12, EO_CT26_02, EO_CT26_08, EO_CT26_15, EO_CT26_07, EO_CT26_17, EO_CT26_09, EO_CT26_03
C8[2]	EO_CT26_18, EO_CT26_16, EO_CT26_11, EO_CT26_20, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_19, EO_CT26_05
C9	EO_CT26_01, EO_CT26_06, EO_CT26_12, EO_CT26_02, EO_CT26_08, EO_CT26_15, EO_CT26_07, EO_CT26_17, EO_CT26_09, EO_CT26_03, EO_CT26_18, EO_CT26_16, EO_CT26_11, EO_CT26_20, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_19, EO_CT26_05
C10	EO_CT26_01, EO_CT26_06, EO_CT26_12, EO_CT26_02, EO_CT26_08, EO_CT26_15, EO_CT26_07, EO_CT26_17, EO_CT26_09, EO_CT26_03, EO_CT26_18, EO_CT26_16, EO_CT26_11, EO_CT26_20, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_19, EO_CT26_05
C11	EO_CT26_01, EO_CT26_06, EO_CT26_12, EO_CT26_02, EO_CT26_08, EO_CT26_15, EO_CT26_07, EO_CT26_17, EO_CT26_09, EO_CT26_03, EO_CT26_18, EO_CT26_16, EO_CT26_11, EO_CT26_20, EO_CT26_13, EO_CT26_14, EO_CT26_10, EO_CT26_04, EO_CT26_19, EO_CT26_05
C12[1]	EO_CT26_01, EO_CT26_06, EO_CT26_15, EO_CT26_19, EO_CT26_08, EO_CT26_18, EO_CT26_13, EO_CT26_10, EO_CT26_04, EO_CT26_12, EO_CT26_16, EO_CT26_07, EO_CT26_17, EO_CT26_09
C12[2]	EO_CT26_01, EO_CT26_08, EO_CT26_02, EO_CT26_09, EO_CT26_03, EO_CT26_18, EO_CT26_04, EO_CT26_05