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Supplemental Information

A Novel DNA Vaccine Platform Enhances Neo-antigen-like T Cell Responses against WT1 to Break Tolerance and Induce Anti-tumor Immunity

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Figure S1

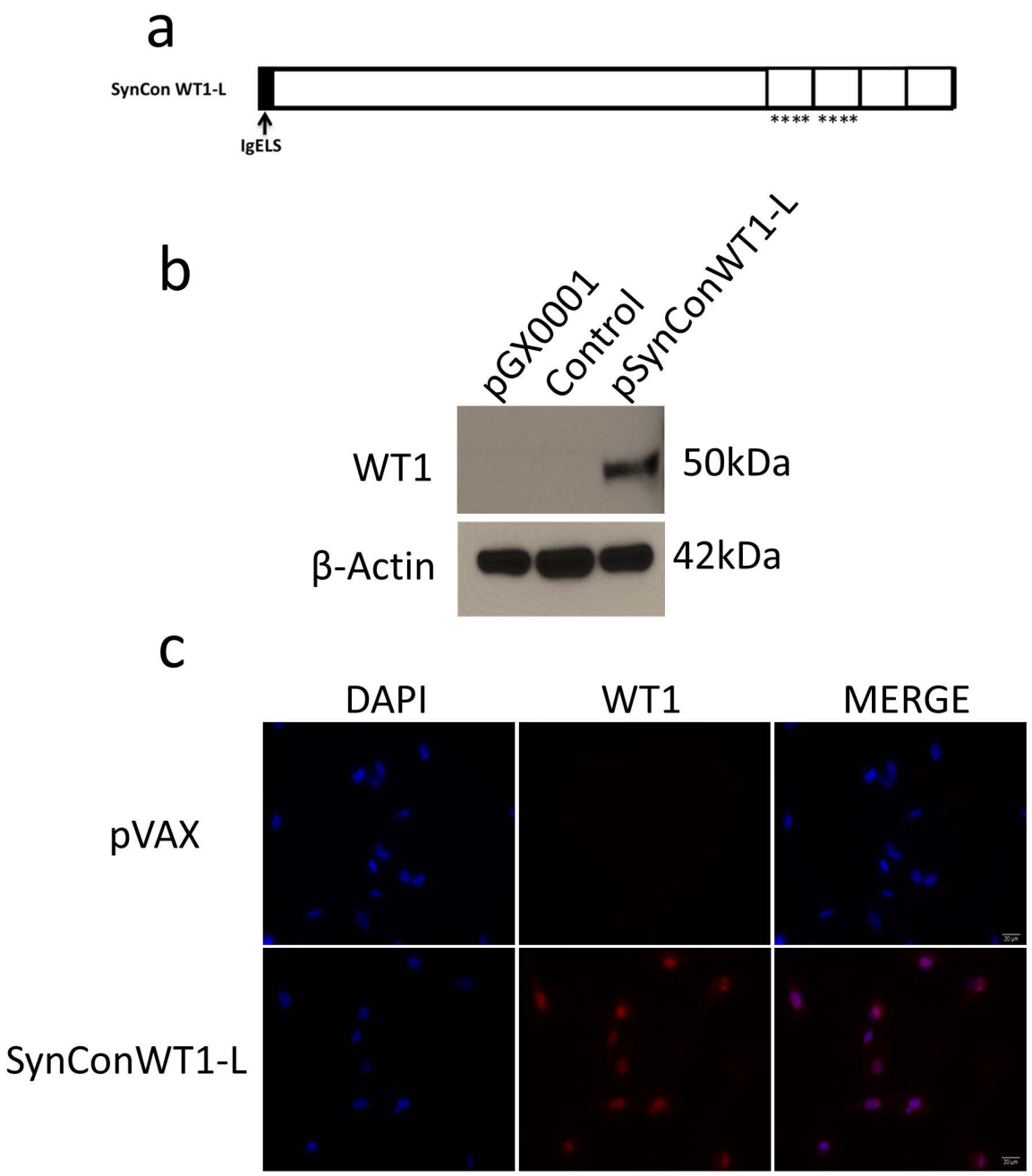
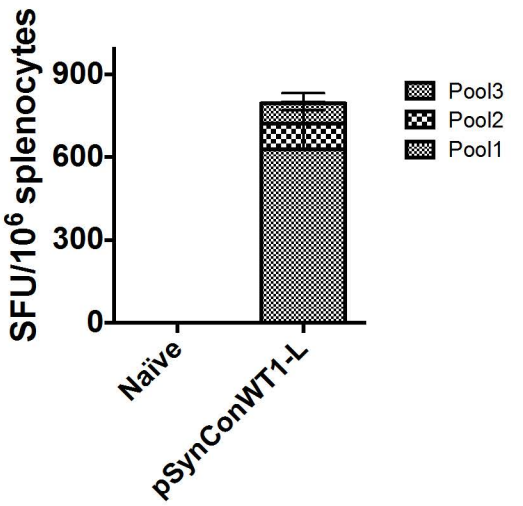
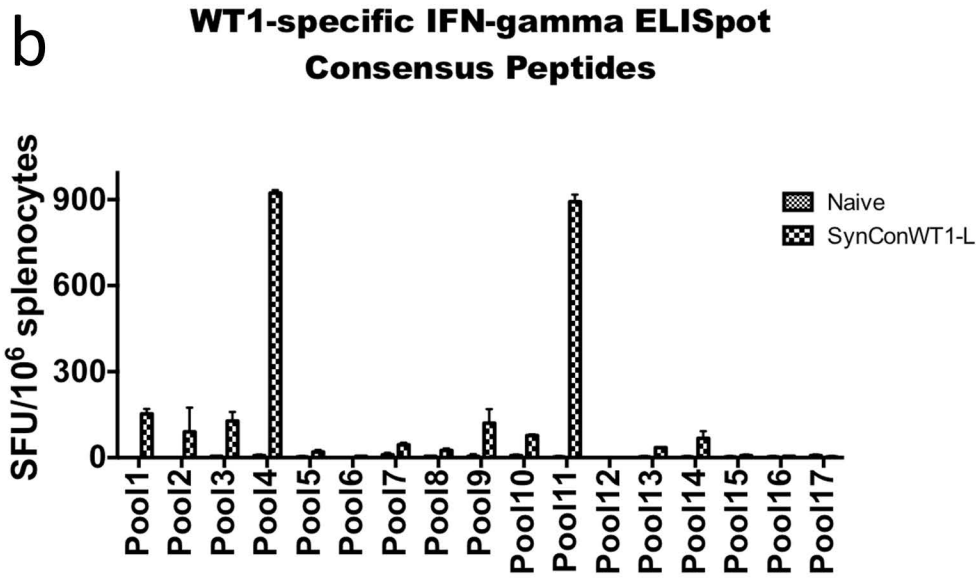

a
SynCon WT1-L
IgELS
**** ****
b
pGX0001
Control
pSynConWT1-L
WT1
50kDa
β-Actin
42kDa
c
DAPI
WT1
MERGE
pVAX
SynConWT1-L

Figure S2

a



b

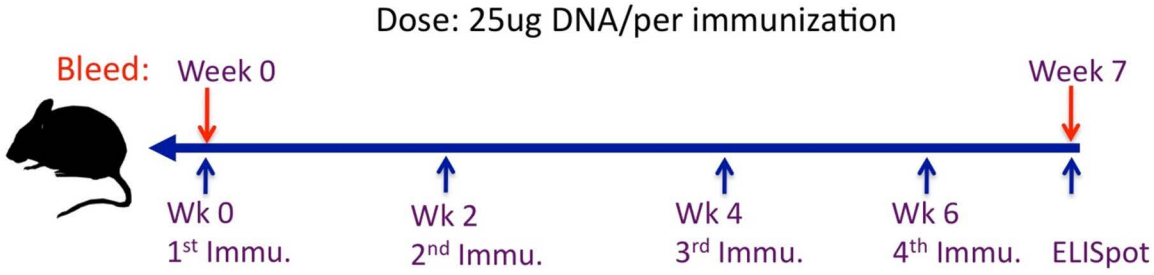


c

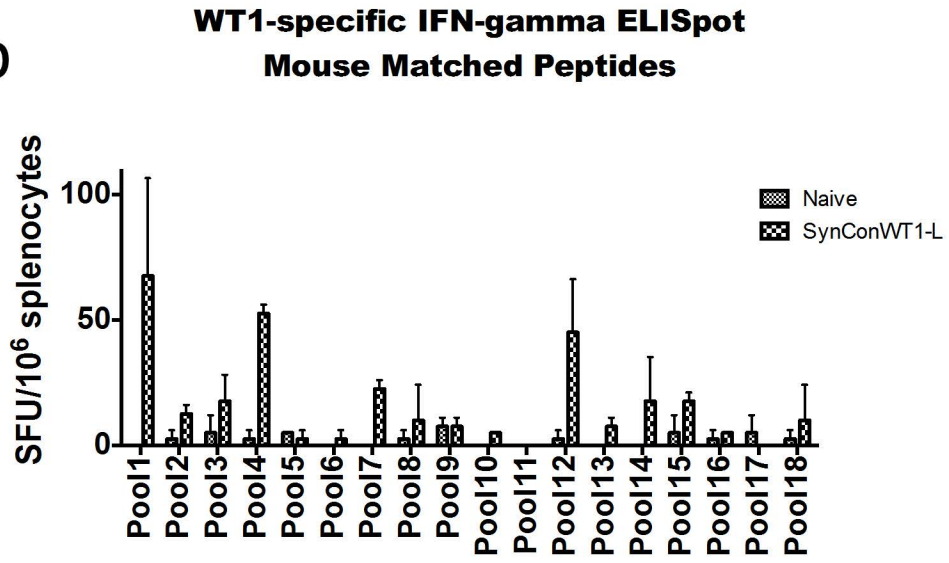
Plasmid	Pool #	No. of Epitope-Comprising Peptides	Sequence of Epitope-Comprising Peptides	Corresponding mouse sequence
WT1-L (Consensus Peptides)	Pool 1 aa 1 - 171	18 Peptides	ATRVHSGSDVRDLNA - GSDVRDLNALLPAVP - LNALLPAVPSLPGGG - AVPSLPGGGGCALPV - AQWAP*VLDFAPP*AAP - *LDFAPP*AAPYGSLGG - AAPYGSLGGPHSFIK – LGGPHSFIKQEPSWG – FIKQEPSWGGADPHE - SWGGADPHEEQCLSA - HFSGQFTGTAGACRY - TGTAGACRYGPFGAP - CRYGPFGAPPPSQAP - GAPPPSQAPSGQARM - QAPSGQA*RMFPNAPY* - A*RMFPNAPYL*PNCLE - AIRNQGYSTVAFDGT – YSTVAFDGTPSYGHT	ATRVHSGSDVRDLNA- GSDVRDLNALLPAVS- LNALLPAVSSLGGGG- AVSSLGGGGGGCGLPV- AQWAPVLDFAPPGAS- LDFAPPGASAYGSLGG- ASAYGSLGGPAPPPAPPPPPPPPHSFIK- LGGPAPPPAPPPPPPPHSFIKQEPSWG- FIKQEPSWGGAEPHE- SWGGAEPHEEQCLSA- HFSGQFTGTAGACRY- TGTAGACRYGPFGPP- CRYGPFGPPPPSQAS- GPPPPSQASSGQARM- QASSGQARMFPNAPY- ARMFPNAPYLPSCLE- TIRNQGYSTVTFDGA- YSTVTFDGAPSYGHT
	Pool 2 aa 163-321	12 Peptides	HTPTDSCTGSQALLL - CTGSQALLLRTPYNS - LLLRTPYNSDNLYQM - YNSDNLYQMTSQLEC - *NQMNL*GSTLKGHATG – STLKGHATGYESDNH – DNHTTPMLYSCGAQY - ATGYESDNHTTPMLY - MLYSCGAQYRIHTHG - AQYRIHTHGVFRGIQ - RVPGVAPTIVRSASE – PTIVRSASETNEKRP	HTPTDSCTGSQALLL- CTGSQALLLRTPYSS- LLLRTPYSSDNLYQM- YSSDNLYQMTSQLEC- NQMNLGATLKGMAAG- ATLKGMAAGSSSSVKWTEGQSNHGTGYESENH- ENHTAPILCGAQY- GTGYESENHTAPIL- LCGAQYRIHTHG- AQYRIHTHGVFRGIQ- RVSGVAPTLVRSASE- PTLVRSASETSEKRP
	Pool 3 aa 313 - 436	None		

Figure S3

a



b

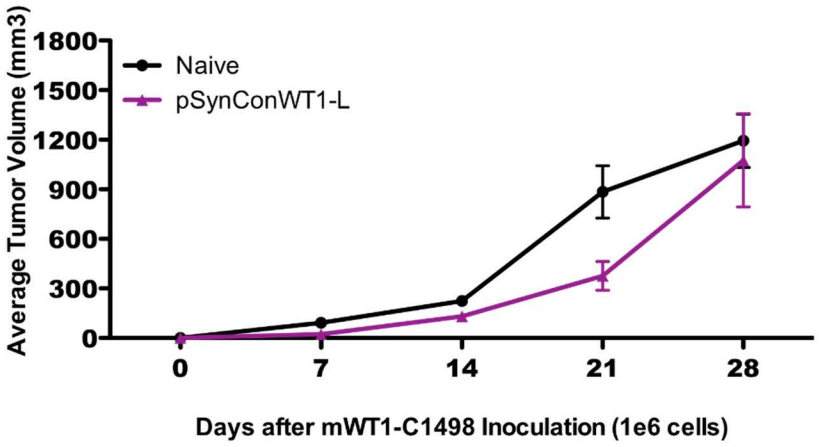


c

Plasmid/Peptide	Pool Number	No. of Epitope-Comprising Peptides	Sequence of Epitope-Comprising Peptides	Corresponding SynCon sequence
WT1-L (Mouse Peptides)	Pool 1 aa 1 - 154	3 Peptides	**AGACRYGPFGPPPPS SGQA**RMFPNAPYLPS **SQPTIRNQGYSTVTF**	**AGACRYGPFGAPPPS-SGQARMFPNAPYLPN-SQPAIRNQGYSTVAF**
	Pool 2 aa 146 - 304	None		
	Pool 3 aa 296 - 449	None		

Figure S4

a


Tumor Therapeutic Study
Average Tumor Volume (mm3)
1800
1500
1200
900
600
300
0
Naive
pSynConWT1-L
0
7
14
21
28
Days after mWT1-C1498 Inoculation (1e6 cells)

b

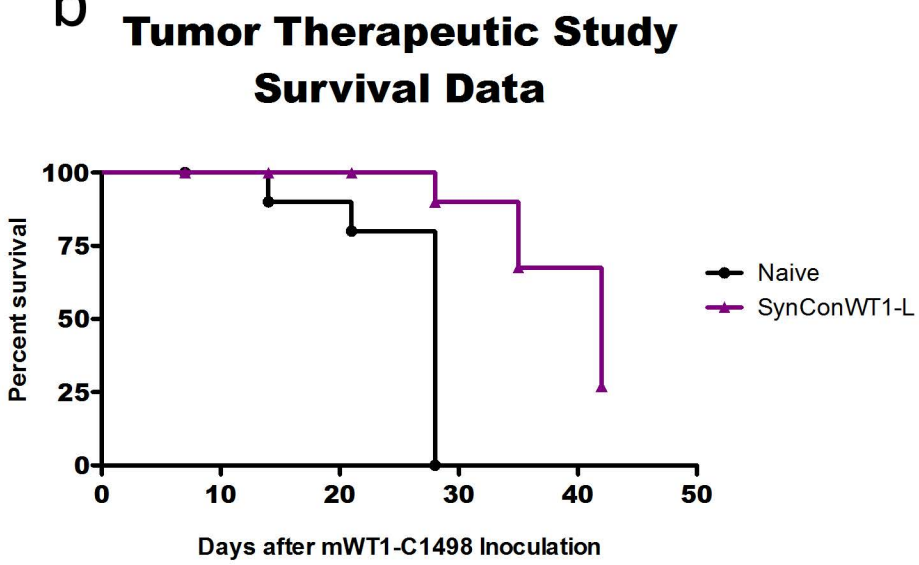
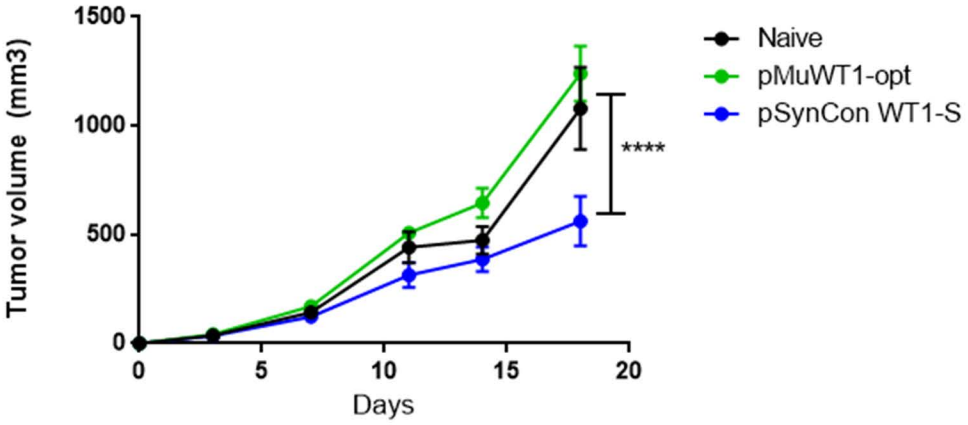

Tumor Therapeutic Study
Survival Data
Percent survival
100
75
50
25
0
0
10
20
30
40
50
Days after mWT1-C1498 Inoculation
Naive
SynConWT1-L

Figure S5

a


Tumor volume (mm3)
1500
1000
500
0
0
5
10
15
20
Days
****
Naive
pMuWT1-opt
pSynCon WT1-S

b

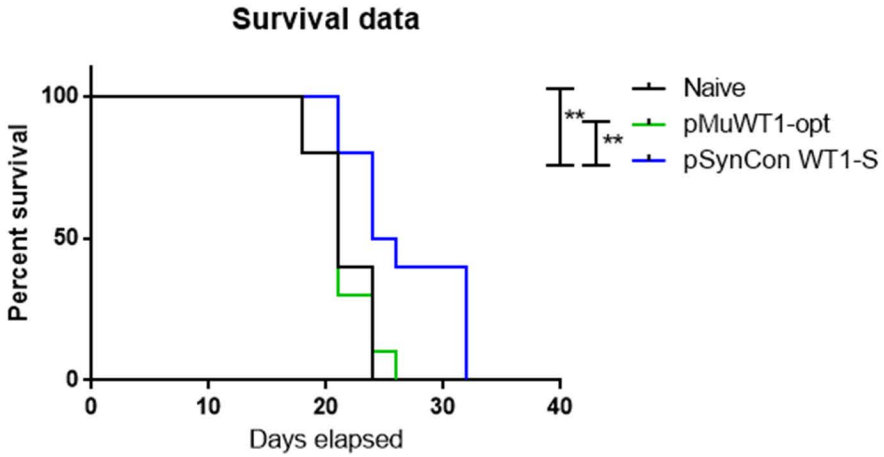

Survival data
Percent survival
100
50
0
0
10
20
30
40
Days elapsed
**
**
Naive
pMuWT1-opt
pSynCon WT1-S

Table S1

Assessment of physiological parameters in rhesus macaques immunized with WT1

	Pre-Vaccination	Post-Vaccination		Normal Range
	Week 0	Week 6	Week 14	
WBC Count (#/10^3/ml)	5-9.6	3.8-12.7	3.5-9.1	**4.0-15.0**
Creatinine (mg/dL)	0.7-1.0	0.8-1.2	0.8-1.2	**0.3-1.4**
BUN (mg/dL)	12-21	10-14	13-19	**9-29**
CPK	251-482	200-451	287-1146	**63-3878**
ALK P (U/L)	83-312	80-267	96-213	**65-641**
AST (U/L)	23-32	21-26	28-38	**23-175**
ALT (U/L)	18-40	20-64	20-65	**18-204**
TBIL (mg/dL)	0.1-0.2	0.1-0.2	0.1-0.2	**0.1-0.6**

Out of Upper Range Animal ID -

Supplemental Figure Legends

Figure S1. In vitro expression of pSynConWT1-L vaccine. (a) Plasmid map of pSynConWT1-L. The stars indicate point mutations to inactivate WT1 function. **(b)** Detection of pSynConWT1-L expression by immunoblotting in transfected RD cells. **(c)** Immunofluorescence assay of pSynConWT1-L in transfected RD cells.

Figure S2. Characterization of consensus WT1-specific IFN-γ responses and immunodominant epitopes for pSynConWT1-L vaccine. (a) Frequency of WT1-specific IFN-γ spot-forming units (SFU) per million splenocytes isolated from vaccinated mice, determined by IFN-γ ELISpot assay using consensus peptides. Mice were vaccinated with the same dose and schedule shown in Figure 2. **(b)** Matrix mapping to determine the WT1 consensus-specific immunodominant epitopes, comparing naïve and pSynConWT1-L vaccinated mice. **(c)** List of immunodominant epitopes identified in the matrix mapping in **(b)**, and comparison to the corresponding native mouse sequence. Error bars represent the average +/- SEM.

Figure S3. Characterization of mouse WT1-specific IFN-γ responses and immunodominant epitopes for pSynConWT1-L vaccine. (a) DNA vaccine immunization schedule showing the dosage of vaccine. C57BL/6 (B6) mice (n =5 per group) were immunized at weeks 0, 2, 4 and 6 with pSynConWT1-L via IM/electroporation. **(b)** Matrix mapping to determine the WT1 mouse-specific immunodominant epitopes, comparing naïve and pSynConWT1-L vaccinated mice, measured by IFN-γ ELISpot. **(c)** List of immunodominant epitopes identified in the matrix mapping in (b), and comparison to the corresponding SynCon sequence. Error bars represent the average +/- SEM.

Figure S4. Anti-tumor immunity elicited by pSynConWT1-L. (a) Mice were challenged with 10^6 mWT1-C1498 tumor cells injected subcutaneously, and were vaccinated weekly starting 3 days post-tumor implant. Tumor measurements are reported in terms of tumor volume, only for surviving mice until day 28. **(b)** Survival data from the tumor therapeutic challenge in (a). Vaccination with pSynConWT1-L extended survival in tumor-bearing mice. Error bars represent the average +/- SEM.

Figure S5. Anti-tumor immunity elicited by pSynCon WT1-S compared to pMuWT1-opt. (a) Mice were challenged with 10^6 mWT1-C1498 tumor cells injected subcutaneously, and were vaccinated weekly starting 3 days post-tumor implant. Tumor measurements are reported in terms of tumor volume until day 18. (b) Survivial data from the tumor therapeutic challenge in (a). Error bars represent the average +/- SEM.

Table S1. Assessment of physiological parameters in rhesus macaques immunized with pSynConWT1-L. No significant weight loss was observed and WBC counts remained within normal range. No elevation of alkaline phosphatase (ALK P), alanine aminotransferase (ALT), aspartate aminotransferase (AST) and total bilirubin (TBIL) indicated that induction of WT1-specific immune responses did not cause significant damage to the liver. No evidence of impaired kidney function was seen, as Creatinine and Blood Urea Nitrogen (BUN) remained within normal limits. Creatine phosphokinase (CPK) was evaluated to determine if EP or induction of immune responses negatively influenced skeletal or cardiac muscle. Elevation of CPK was not detected. Overall, we did not observe any vaccine-induced adverse effects in NHP despite evidence of strong *WT1*-specific CTLs *in vivo*.