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Supplemental Information

Engineered DNA Vaccination against Follicle-Stimulating Hormone Receptor Delays Ovarian Cancer Progression in Animal Models

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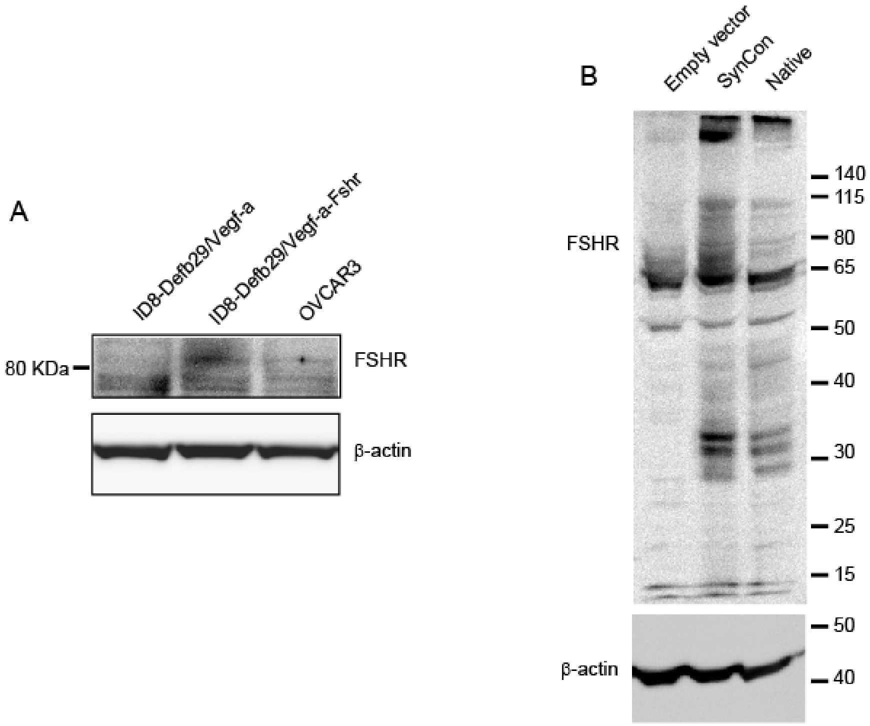
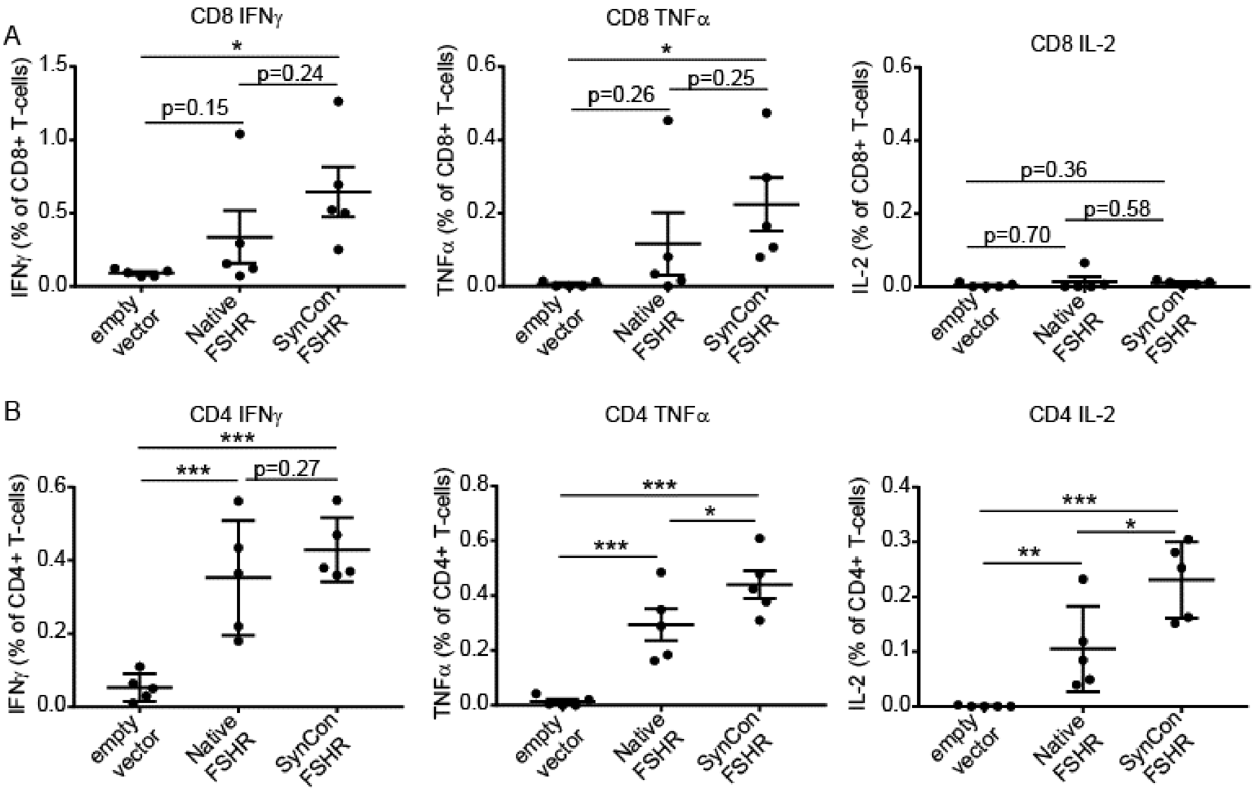
SUPPLEMENTAL FIGURES

SUPPLEMENTAL FIGURE 1



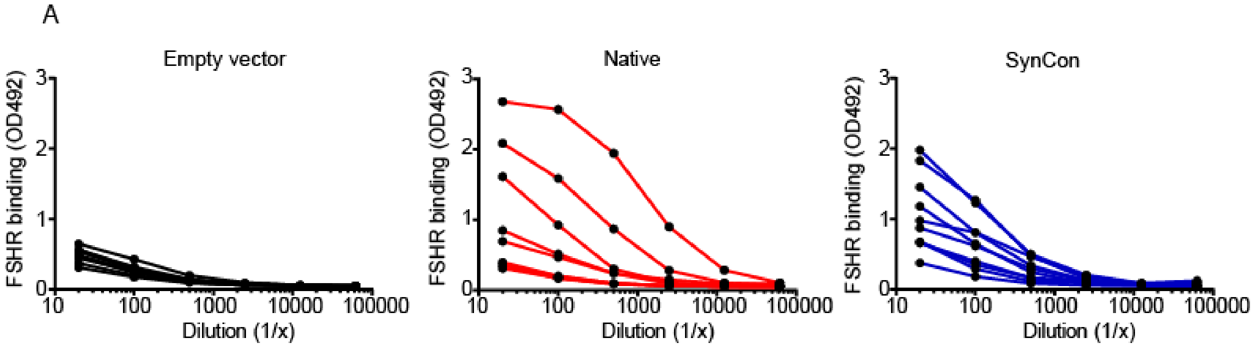
Supplemental Figure 1. Expression of SynCon and native FSHR vaccines. Western blot of protein derived from (**A**) OVCAR3, ID8-*Defb29/Vegf-a-Fshr* and ID8-*Defb29/Vegf-a* and (**B**) murine FSHR SynCon vaccine, murine native FSHR vaccine or empty vector transfected 293T cells (10% gel MOPS buffer) blotted for FSHR and β-actin (representative of 2 experiments)

SUPPLEMENTAL FIGURE 2



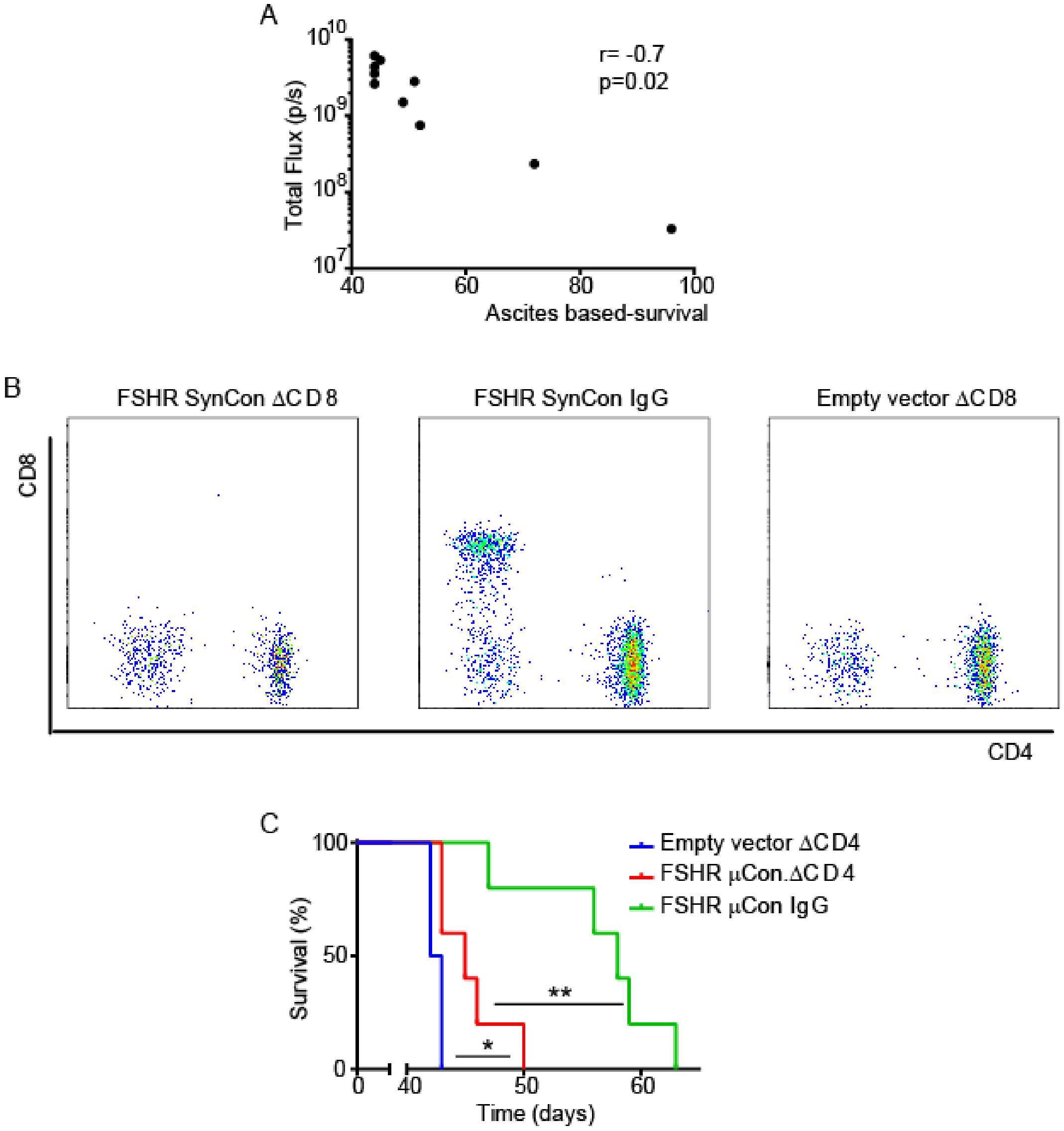
Supplemental Figure 2. Mouse FSHR DNA vaccine generates strong CD8 and CD4 responses when pulsed with vaccine matched peptides. Percentage of IFNγ, TNFα and IL-2 produced by (**A**) CD8+ or (**B**) CD4+ T cells from the spleen of mice immunized with FSHR SynCon vaccine, FSHR native vaccine or empty vector stimulated with consensus vaccine matched peptides. ANOVA. *p<0.05, **p<0.01, ***p<0.001.

SUPPLEMENTAL FIGURE 3



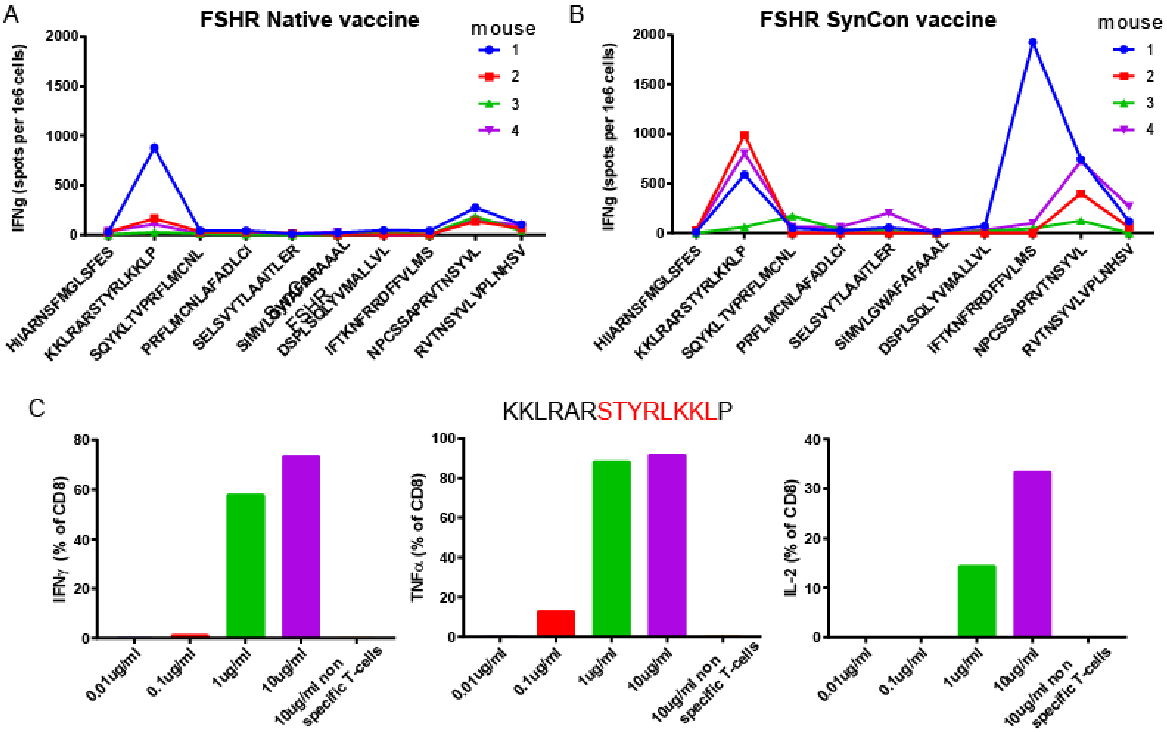
Supplemental Figure 3. Mouse FSHR DNA vaccine generates antibodies against FSHR. (**A**) Binding of sera from mice vaccinated with FSHR vaccine, native FSHR or pVAX empty vector to the extracellular domain of native murine FSHR in a binding ELISA. Each curve represents one mouse.

SUPPLEMENTAL FIGURE 4



Supplemental Figure 4. CD8 and CD8 depletion experiment. (A) Correlation between luciferase expression and ascites-based survival in luciferase transfected ID8-*Defb29/Vegf-a-Fshr* tumor challenge. **(B)**We vaccinated mice and challenged with ID8-*Defb29/Vegf-a-Fshr* one week after the last immunization. One day prior to the tumor challenge and twice weekly thereafter we administered either anti-mouse CD8 or rat polyclonal IgG. Flow plots showing CD4^{+} and CD8^{+} T cells in the tumor microenvironment of the different groups at day 21 after tumor inoculation. (**B**) Survival plot of the FSHR SynCon vaccine or pVAX empty vector with our without CD8 depletion (n=5 mice per group). Pearson correlation, Log-rank. *p<0.05, **p<0.01

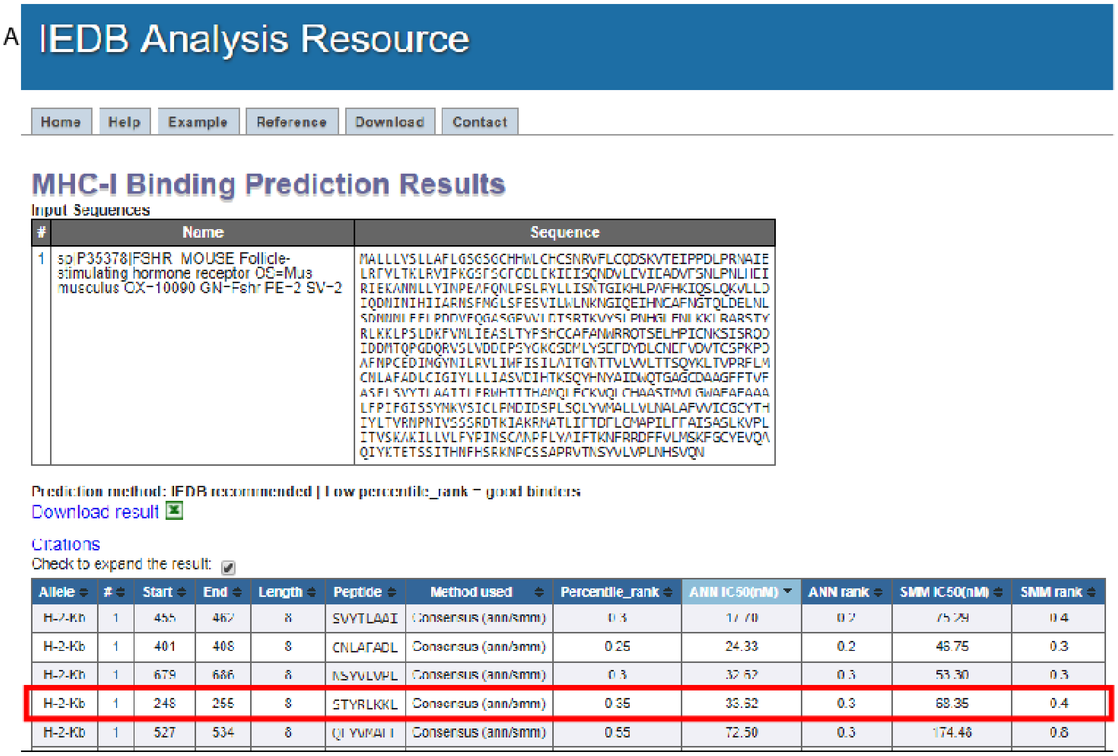
SUPPLEMENTAL FIGURE 5



Supplemental Figure 5. Murine FSHR SynCon vaccine increases the number and intensity of immunogenic sequences of FSHR. (**A&B**) IFNγ ELISpot of splenocytes from mice immunized with murine FSHR SynCon vaccine, native FSHR vaccine or empty vector pulsed with FSHR derived peptides predicted to bind with high affinitiy to H2-K(b) or H2-D (b) (n=4 mice per group). (**C**) Percentage of IFNγ, TNFα and IL-2 produced by CD8$^+$ T cells expanded from mice immunized with murine FSHR SynCon vaccine expanded with KKLRARSTYRLKKLP peptides and stimulated with the same peptides at different doses.

SUPPLEMENTAL FIGURE 6

A



MHC-I Binding Prediction Results

Input Sequences

#	Name	Sequence
1	sp|P35378|FSHR_MOUSE Follicle-stimulating hormone receptor OS=Mus musculus OX=10090 GN=Fshr PE=2 SV=2	MALLLVSLLAFLGSGSGCHHWLCHCSNRVFLCQDSKVTEIPPDLPRNAIE LRFVLTKLRVIPKGSFSGFGDLEKIEISQNDVLEVIEADVFSNLPNLHEI RIEKANNLLYINPEAFQNLPSLRYLLISNTGIKHLPAFHKIQSLQKVLLD IQDNINIHIIARNSFMGLSFESVILWLNKNGIQEIHNCAFNGTQLDELNL SDNNNLEELPDDVFQGASGPVVLDISRTKVYSLPNHGLENLKKLRARSTY RLKKLPSLDKFVMLIEASLTYPSHCCAFANWRRQTSELHPICNKSISRQD IDDMTQPGDQRVSLVDDEPSYGKGSDMLYSEFDYDLCNEFVDVTCSPKPD AFNPCEDIMGYNILRVLIWFISILAITGNTTVLVVLTTSQYKLTVPRFLM CNLAFADLCIGIYLLLIASVDIHTKSQYHNYAIDWQTGAGCDAAGFFTVF ASELSVYTLAAITLERWHTITHAMQLECKVQLCHAASVMVLGWAFAFAAA LFPIFGISSYMKVSICLPMDIDSPLSQLYVMALLVLNALAFVVICGCYTH IYLTVRNPNIVSSSRDTKIAKRMATLIFTDFLCMAPILFFAISASLKVPL ITVSKAKILLVLFYPINSCANPFLYAIFTKNFRRDFFVLMSKFGCYEVQA QIYKTETSSITHNFHSRKNPCSSAPRVTNSYVLVPLNHSVQN

Prediction method: IEDB recommended | Low percentile_rank = good binders

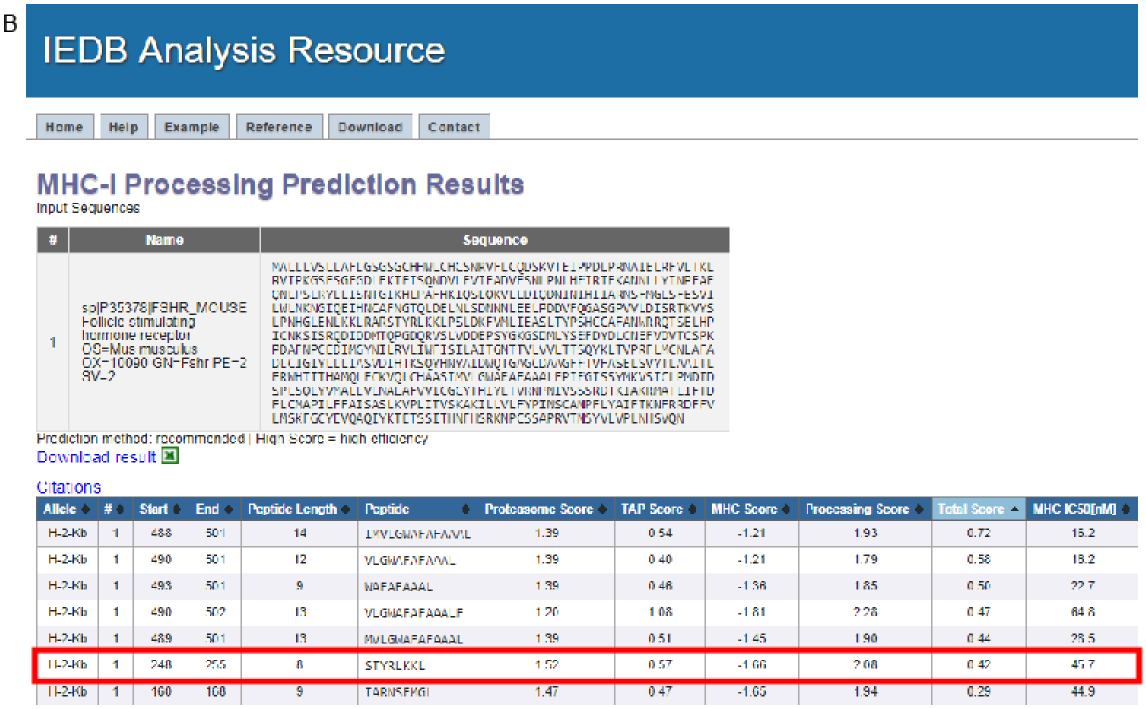
Download result

Citations

Check to expand the result: ☑

Allele	#	Start	End	Length	Peptide	Method used	Percentile_rank	ANN IC50(nM)	ANN rank	SMM IC50(nM)	SMM rank
H-2-Kb	1	455	462	8	SVYTLAAI	Consensus (ann/smm)	0.3	17.70	0.2	75.29	0.4
H-2-Kb	1	401	408	8	CNLAFADL	Consensus (ann/smm)	0.25	24.33	0.2	46.75	0.3
H-2-Kb	1	679	686	8	NSYVLVPL	Consensus (ann/smm)	0.3	32.52	0.3	53.30	0.3
H-2-Kb	1	248	255	8	STYRLKKL	Consensus (ann/smm)	0.35	33.52	0.3	68.35	0.4
H-2-Kb	1	527	534	8	QLYVMALL	Consensus (ann/smm)	0.55	72.50	0.3	174.46	0.8

B



MHC-I Processing Prediction Results

Input Sequences

#	Name	Sequence
1	sp|P35378|FSHR_MOUSE Follicle stimulating hormone receptor OS=Mus musculus OX=10090 GN=Fshr PE=2 SV=2	MALLLVSLLAFLGSGSGCHHWLCHCSNRVFLCQDSKVTEIPPDLPRNAIELRFVLTKL RVIPKGSFSGFGDLEKIEISQNDVLEVIEADVFSNLPNLHEIRIEKANNLLYINPEAF QNLPSLRYLLISNTGIKHLPAFHKIQSLQKVLLDIQDNINIHIIARNSFMGLSFESVI LWLNKNGIQEIHNCAFNGTQLDELNLSDNNNLEELPDDVFQGASGPVVLDISRTKVYS LPNHGLENLKKLRARSTYRLKKLPSLDKFVMLIEASLTYPSHCCAFANWRRQTSELHP ICNKSISRQDIDDMTQPGDQRVSLVDDEPSYGKGSDMLYSEFDYDLCNEFVDVTCSPK PDAFNPCEDIMGYNILRVLIWFISILAITGNTTVLVVLTTSQYKLTVPRFLMCNLAFA DLCIGIYLLLIASVDIHTKSQYHNYAIDWQTGAGCDAAGFFTVFASELSVYTLAAITL ERWHTITHAMQLECKVQLCHAASVMVLGWAFAFAAALFPIFGISSYMKVSICLPMDID SPLSQLYVMALLVLNALAFVVICGCYTHIYLTVRNPNIVSSSRDTKIAKRMATLIFTD FLCMAPILFFAISASLKVPLITVSKAKILLVLFYPINSCANPFLYAIFTKNFRRDFFV LMSKFGCYEVQAQIYKTETSSITHNFHSRKNPCSSAPRVTNSYVLVPLNHSVQN

Prediction method: recommended | High Score = high efficiency

Download result

Citations

Allele	#	Start	End	Peptide Length	Peptide	Proteasome Score	TAP Score	MHC Score	Processing Score	Total Score	MHC IC50[nM]
H-2-Kb	1	488	501	14	VMVLGWAFAFAAAL	1.39	0.54	-1.21	1.93	0.72	16.2
H-2-Kb	1	490	501	12	VLGWAFAFAAAL	1.39	0.40	-1.21	1.79	0.58	16.2
H-2-Kb	1	493	501	9	WAFAFAAAL	1.39	0.46	-1.36	1.85	0.50	22.7
H-2-Kb	1	490	502	13	VLGWAFAFAAALF	1.20	1.08	-1.81	2.28	0.47	64.8
H-2-Kb	1	489	501	13	MVLGWAFAFAAAL	1.39	0.51	-1.45	1.90	0.44	28.5
H-2-Kb	1	248	255	8	STYRLKKL	1.52	0.57	-1.66	2.08	0.42	45.7
H-2-Kb	1	160	168	9	TARNSFMGL	1.47	0.47	-1.65	1.94	0.29	44.9

Supplemental Figure 6. STYRLKKL is an FSHR derived octamer predicted to be efficiently processed and bind H2-K(b) with high affinity. (**A**) Screenshot showing the binding prediction to MCH-I of 8-mers derived from murine FHSR to H2-K(b) and H2-D(b) using the immunoepitope database (http://tools.iedb.org/mhci/). (**B**) Screenshot showing the proteasome and TAP processing prediction of all potential FSHR-derived peptides and binding to H2-K(b) and H2-D(b) using the immunoepitope database (http://tools.iedb.org/processing/).

SUPPLEMENTAL METHODS

Mouse SynCon FSHR DNA sequence

ATGGACTGGACCTGGATTCTGTTCCTGGTGGCCGCTGCCACAAGGGTGCACTCCTGC
CACCACTGGCTGTGCCACTGTTCTAACAGGGTGTTCCTGTGCCAGGACAGCAAGGTG
ACCGAGATCCCTCCCGATCTGCCCCGGAACGCCATCGAGCTGCGCTTCGTGCTGACA
AAGCTGAGAGTGATCCCTAAGGGCTCCTTCTCTGGCTTTGGAGATCTGGAGAAGATC
GAGATCTCCCAGAACGACGTGCTGGAAGTGATCGAGGCCGACGTGTTCAGCAACCT
GCCTAAGCTGCACGAGATCCGGATCGAGAAGGCCAACAACCTGCTGTACATCAACC
CCGAGGCTTTCCAGAACCTGCCTAGCCTGCGCTACCTGCTGATCTCCAACACCGGCA
TCAAGCACCTGCCAGCCGTGCACAAGATCCAGAGCCTGCAGAAGGTGCTGCTGGAC
ATCCAGGATAACATCAACATCCACATCATCGCTAGAAACTCCTTCATGGGACTGTCT
TTTGAGAGCGTGATCCTGTGGCTGAACAAGAACGGCATCCAGGAGATCCACAACTG
TGCCTTTAACGGAACACAGCTGGACGAGCTGAACCTGTCTGATAACAACAACCTGG
AGGAGCTGCCTAACGACGTGTTCCAGGGCGCCAGCGGACCAGTGATCCTGGATATC
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GAGGGCCAGATCCACATACAGACTGAAGAAGCTGCCTTCTCTGGACAAGTTCGTGA
CCCTGATGGAGGCTTCTCTGACATACCCAAGCCACTGCTGTGCCTTTGCTAACTGGA
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ATCGACGATATGACCCAGATCGGAGATCAGCGCGTGAGCCTGATCGACGATGAGCC
CTCCTACGGCAAGGGATCTGACATGATGTACAGCGAGTTCGACTTTGATCTGTGCAA
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ATATCATGGGCTACAACATCCTGCGGGTGCTGATCTGGTTTATCTCCATCCTGGCTAT
CACCGGAAACACCACAGTGCTGGTGGTGCTGACCACATCTCAGTACAAGCTGACAG
TGCCTCGCTTCCTGATGTGCAACCTGGCCTTTGCTGACCTGTGCATCGGCATCTACCT
GCTGCTGATCGCCTCTGTGGATATCCACACCAAGAGCCAGTACCACAACTACGCCAT
CGACTGGCAGACCGGCGCTGGATGTGATGCTGCCGGATTCTTTACAGTGTTCGCCTC
CGAGCTGAGCGTGTACACCCTGACAGCTATCACCCTGGCCAGGGCTCACACCATCAC
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CTACATGAAGGTGTCCATCTGTCTGCCTATGGACATCGATAGCCCACTGTCCCAGCT
GTACGTGATGGCCCTGCTGGTGCTGAACGTGCTGGCCTTCGTGGTCATCTGCGGCTG
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CACCAAGATCGCCAAGCGCATGGCTACCCTGATCTTCACAGATTTTCTGTGCATGGC
CCCAATCAGCTTCTTTGCCATCAGCGCCTCCCTGAAGGTGCCCCTGATCACCGTGAG
CAAGGCTAAGATCCTGCTGGTGCTGTTCTACCCAATCAACTCCTGCGCCAACCCCTT
TCTGTACGCTATCTTCACAAAGAACTTTCGGCGCGACTTCTTTATCCTGATGAGCAA
GTTCGGATGTTACGAGATGCAGGCCCAGATCTACCGGACCGAGACAAGCTCCGCCA
CCCACAACTTTCACGCTAGGAAGTCCCACTGCAGCAGCGCCCCCAGGGTGACAAAC
TCTTACGTGCTGGTGCCTCTGAACCACAGCGTGCAGAACTGATAA