A facile approach to enhance antigen response for personalized cancer vaccination

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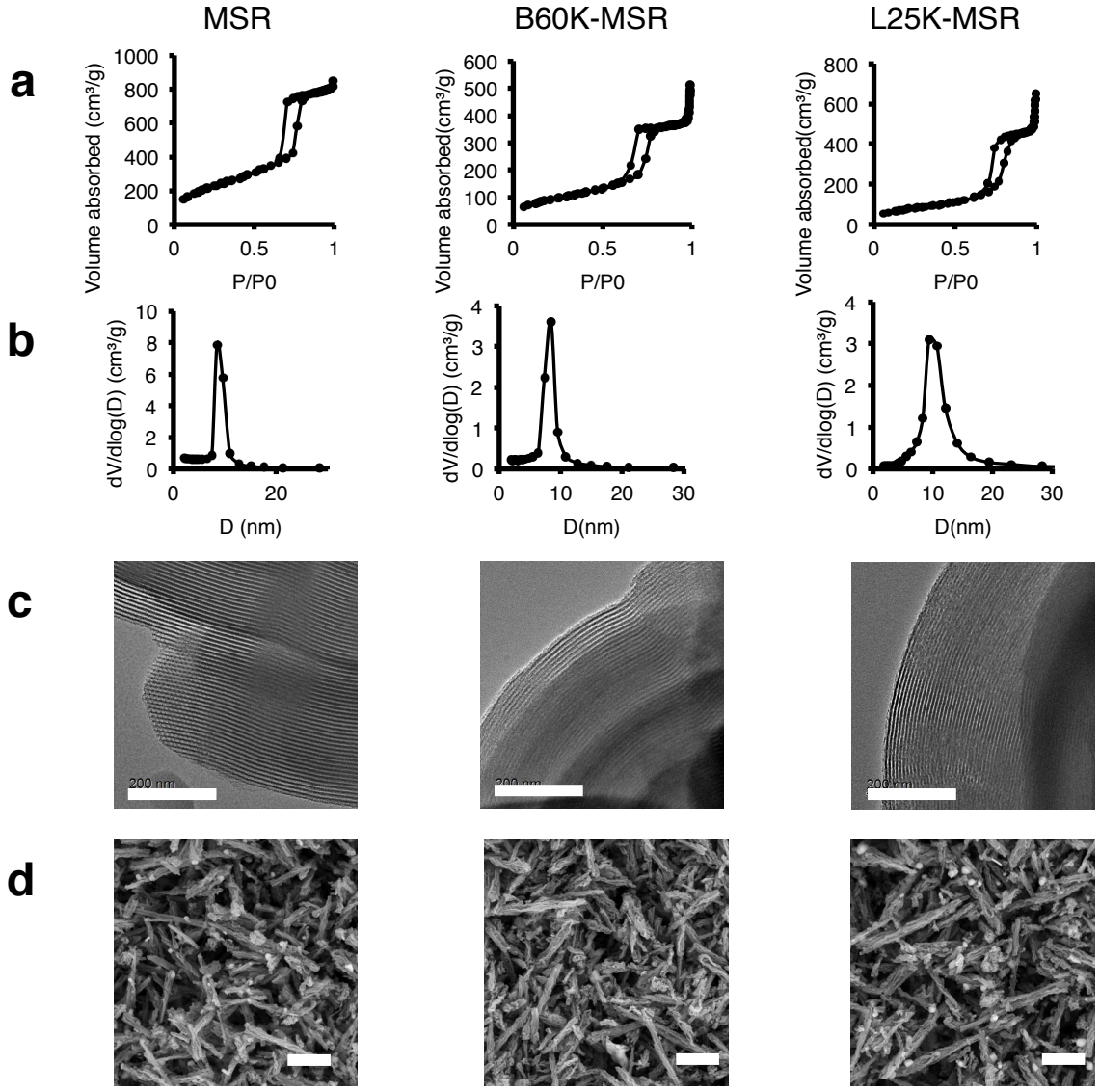
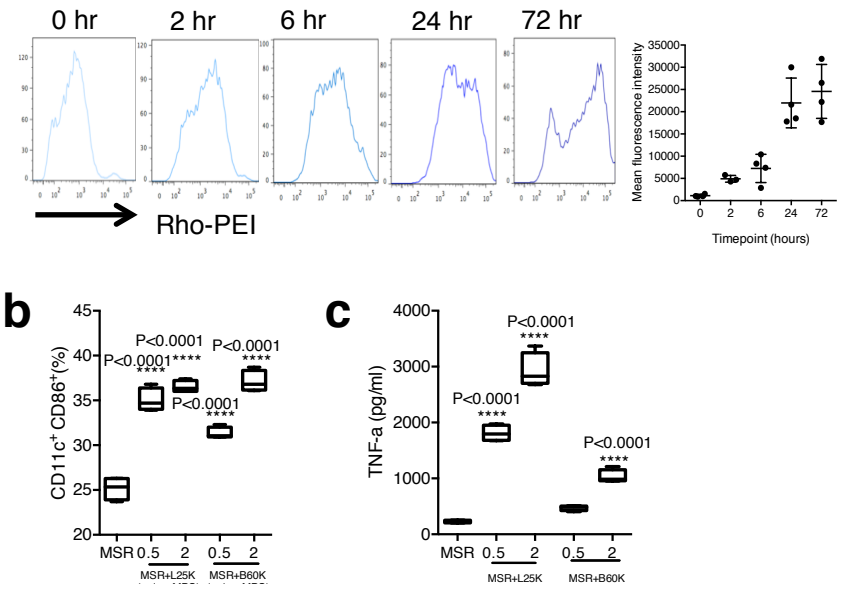
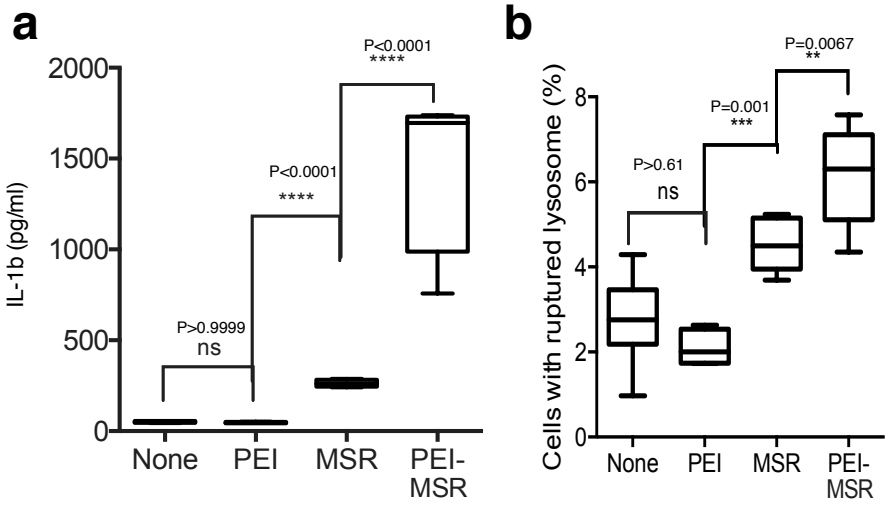
Supplementary information:



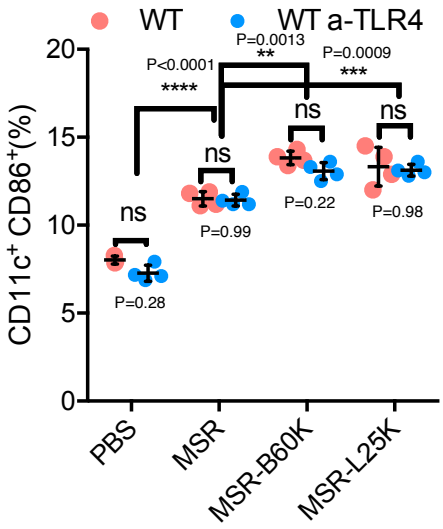
Supplementary Figure 1: Characterization of MSRs with and without PEI adsorption. (a) Nitrogen sorption isotherms, (b) BET pore size, (c) TEM images (scale bar = 200nm) and (d) SEM images (scale bar = 20um) of various MSRs or MSR-PEIs



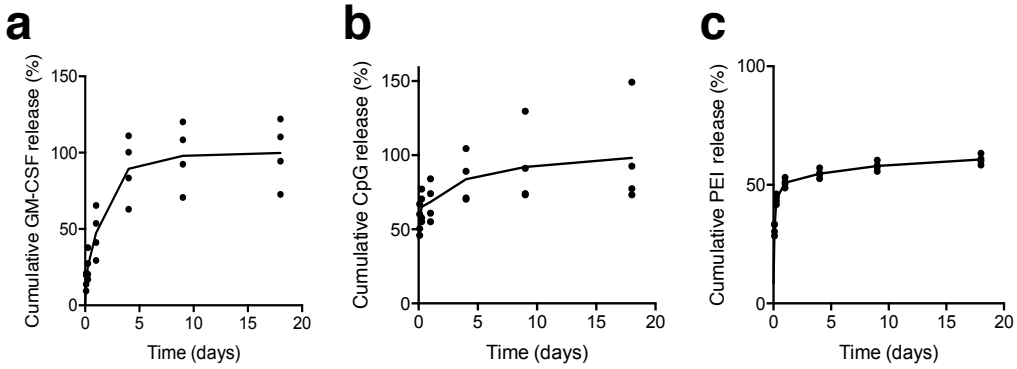
Supplementary Figure 2: BMDC uptake kinetics of PEI and stimulation by MSR-PEI. (a) Flow cytometry analysis of rhodamine+ murine BMDCs after stimulating with soluble Rhodamine-PEI for 0, 2, 6, 24 and 72 hours (n=4). (b). Flow cytometry analysis of CD86 expression on murine BMDCs after 24 hours of stimulation with 40ug MSR particles (MSR) or 40ug MSR-PEI particles containing 0.5ug or 2ug of B60K or L25K PEI (n=4, compared to MSR by two-way ANOVA). (c) ELISA analysis of TNF-a concentration in murine BMDC supernatant after 24 hours of stimulation with 40ug MSR particles (MSR) or 40ug MSR-PEI particles containing 0.5ug or 2ug of B60K or L25K PEI (n=4, compared to MSR by two-way ANOVA).



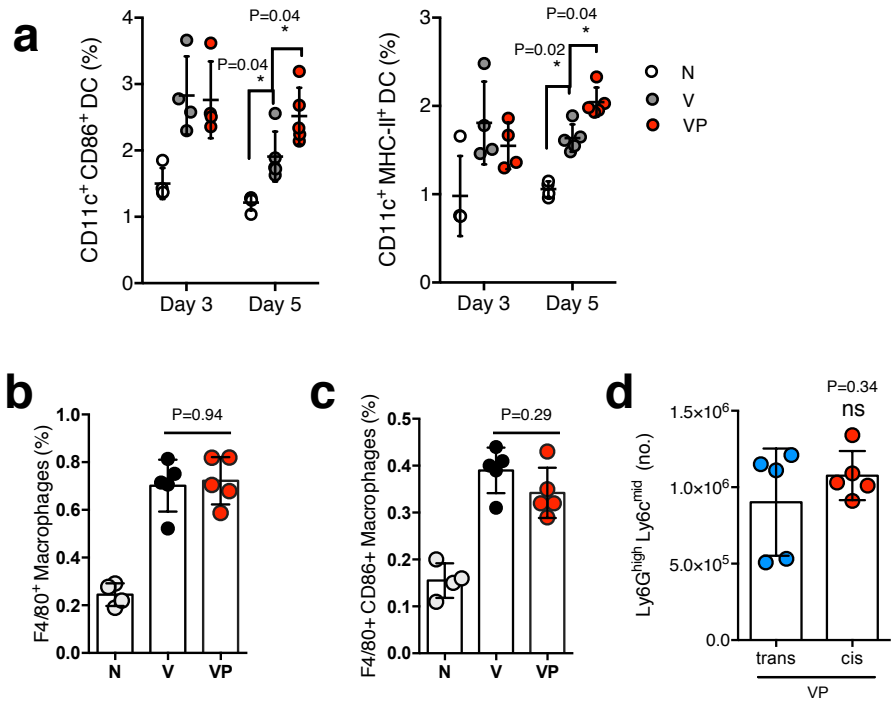
Supplementary Figure 3: MSR-PEI particles activate the inflammasome through lysosomal disruption. (a) ELISA analysis of IL-1b concentration in murine BMDC supernatant after 18 hours of stimulation with 0.8 ug free L25K PEI, 40 ug MSRs or 40ug of MSR-PEIs (20ug L25K/mg MSR), or left untreated (n=4, one-way ANOVA). (b) Flow cytometry analysis of BMDCs stained with acridine orange after 18 hours of stimulation with 0.8 ug free L25K PEI, 40 ug bare MSRs or 40ug of MSR-PEIs (20ug L25K/mg MSR), or left untreated (n=4 for PEI, n=8 for all else, one-way ANOVA). Cells with ruptured lysosome indicates the loss of lysosomal staining with acridine orange.



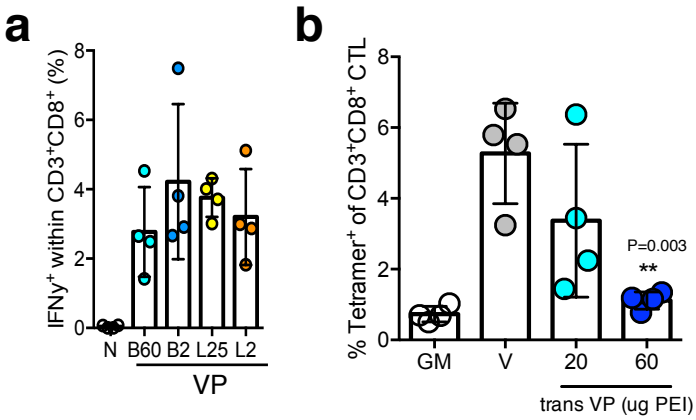
Supplementary Figure 4: Effect of TLR-4 neutralization on BMDC activation. (a) Flow cytometry analysis of CD11c and CD86 expression in BMDCs pretreated with a-TLR4 (WT a-TLR4) or nothing (WT) and stimulated with PBS, MSRs, MSRs adsorbed with B60K PEI (MSR-B60K) or MSRs adsorbed with L25K PEI (MSR-L25K). n=4, * indicates comparison within a-TLR4 groups, ns between WT and a-TLR4 groups by two-way ANOVA. Data depicts mean +/- sd



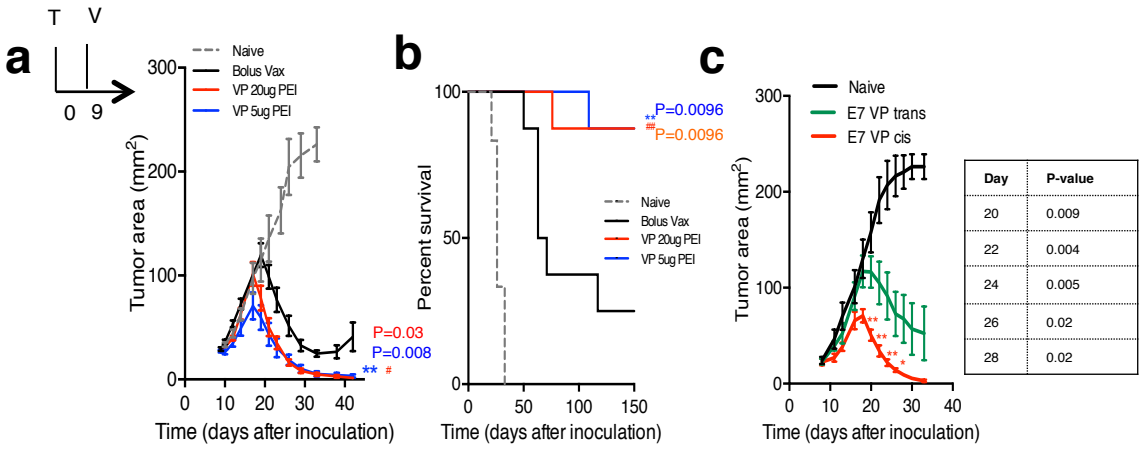
Supplementary Figure 5: In vitro release of (a) GM-CSF, (b) CpG-ODN and (c) L25K PEI in complete media. n=4



Supplementary Figure 6: Lymph node analysis after immunization. Percentage of (a) CD11c$^+$ CD86$^+$ or CD11c$^+$ MHC-II$^+$ activated DCs (n=4 for day 3, n=5 for day 5, two-way ANOVA), (b) F4/80$^+$ macrophages and (c) F4/80$^+$ CD86$^+$ activated macrophages in the dLN on day 5 post immunization with the MSR vaccine (V) or the MSR-PEI vaccine (VP) or left unimmunized (N) (n=5, n=4 for N, one-way ANOVA). (d) Total number of Ly6G$^+$ Ly6C^{mid} myeloid cells/neutrophils at the vaccine site explanted on day 3 post immunization with the trans MSR-PEI vaccine (trans, VP) or the cis MSR-PEI vaccine (cis, VP) (n=5, student T test). Data depicts mean +/- sd

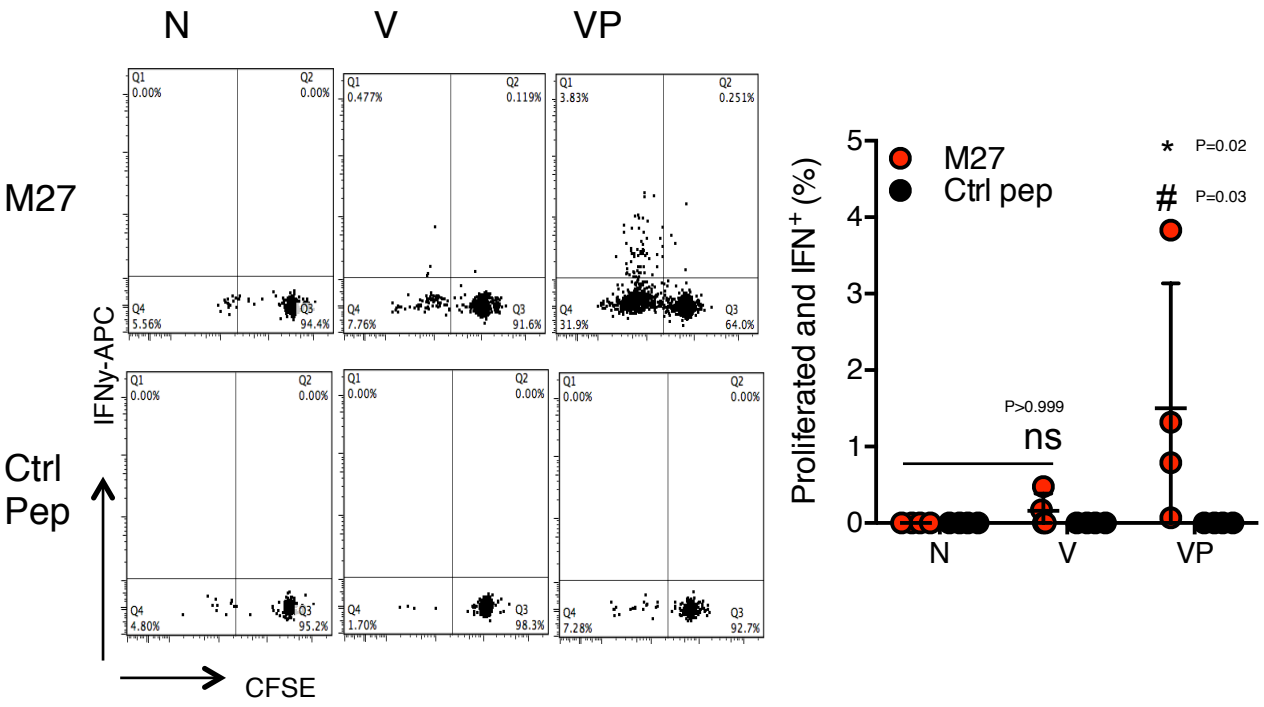


Supplementary Figure 7: CTL response induced by MSR PEI vaccines. (a) Percentage of IFNγ⁺ CD8⁺ T cells isolated from peripheral blood on day 7 after immunization with the MSR-PEI vaccine containing Branched 60K (B60), Branched 2K (B2), Linear 25K (L25) or Linear 2K (L2) PEI or left unimmunized, and subsequently stimulated with SIINFEKL (n=4). (b) Percentage of tetramer⁺ CD8⁺ T cells in peripheral blood on day 7 after immunization with MSRs delivering only GM-CSF (GM), the MSR vaccine (V), trans MSR-PEI vaccines containing 20ug (trans VP 20) or 60ug (trans VP 60) of B60K PEI (n=4, one-way ANOVA). Data depicts mean +/- sd

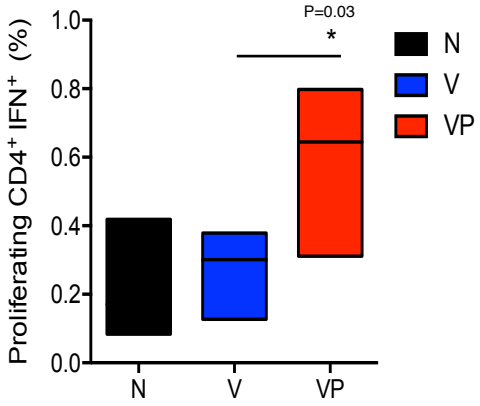


Day	P-value
20	0.009
22	0.004
24	0.005
26	0.02
28	0.02

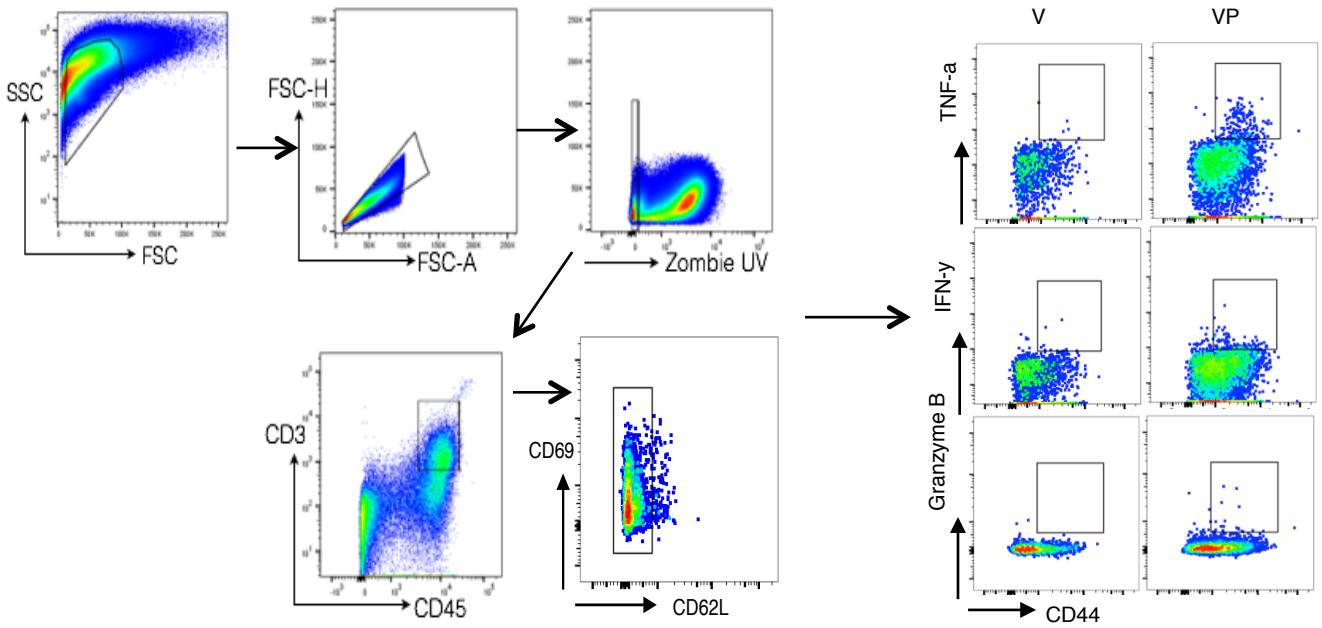
Supplementary Figure 8: Therapeutic E7 expressing tumor study. (a) Tumor growth and (b) overall survival of mice bearing established E7 expressing TC-1 tumors (allowed to develop for 9 days) and treated with a bolus vaccine (Bolus Vax; 1ug GM-CSF, 50ug E7, 100ug CpG), or the MSR-PEI vaccine (VP; 5mg MSR, 1ug GM-CSF, 100ug CpG, 50ug E7) with either 5ug or 20ug of L25K PEI (n=6 for Naive, n=8 for Bolus, VP 5ug and VP 20ug, *denotes between VP 5ug and Bolus Vax, # denotes between VP 20ug and Bolus Vax by one-way ANOVA (a) and by Log-rank Test (b)). (c) Tumor growth in mice bearing established E7 expressing TC-1 tumors (allowed to develop for 8 days) and treated with the trans MSR- PEI vaccine (E7 VP trans) or the cis MSR-PEI vaccine (E7 VP cis), or left untreated (N) (n=5, between E7 VP trans Vax and E7 VP cis Vax by two-way ANOVA) . In (a and c), data depicts mean +/- sem



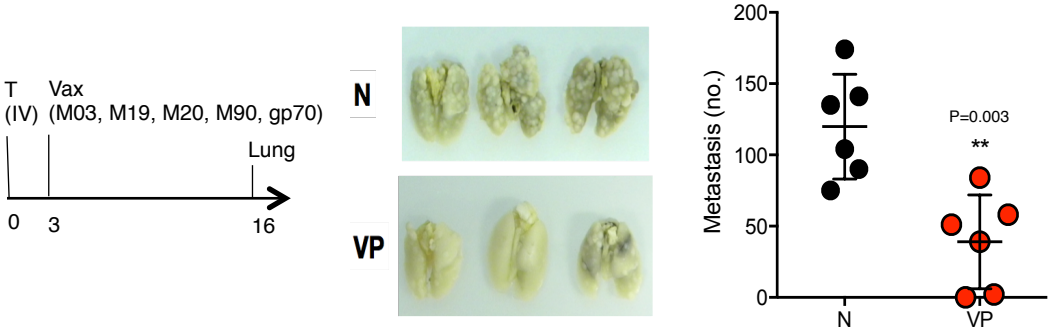
Supplementary Figure 9: MSR-PEI vaccine enhances CD8 T cell response towards B16 neoantigen. Percentage of proliferating and IFNγ + splenic CD8 T cells in response to M27 or a control peptide 8 days after vaccination with the MSR vaccine (V), the MSR-PEI vaccine (VP) or left untreated (Naive). Data depicts mean +/- sd, n=4, * denotes between VP and N, # denotes between VP and V, ns between V and N by two-way ANOVA



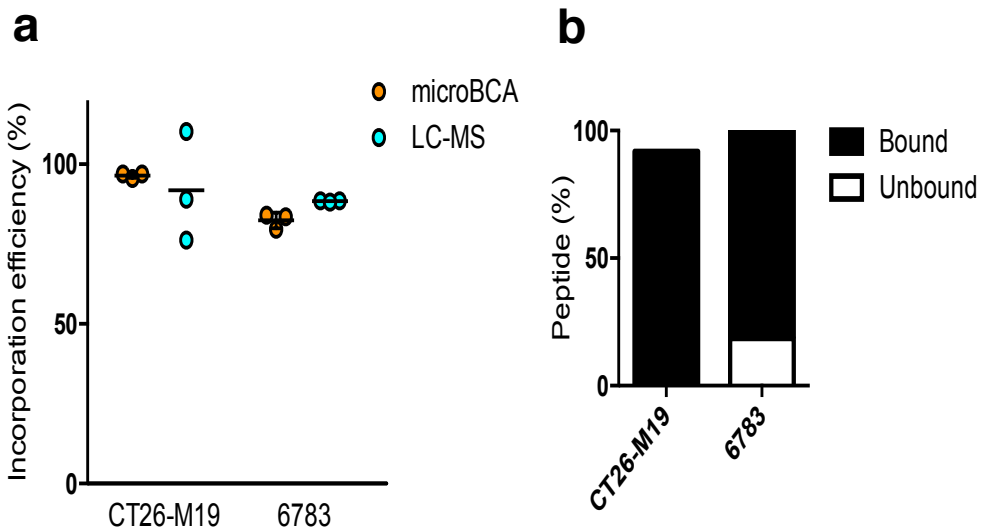
Supplementary Figure 10: MSR-PEI vaccine enhances CD4 T cell response towards B16 neoantigen in tumor bearing animals. Mice were vaccinated with the MSR vaccine (V), the MSR-PEI vaccine (VP) using L25K PEI and 50ug of the B16 neoantigens (M27 and M30), or left untreated (Naive). On day 14 after vaccination, mice were inoculated with B16F10 tumors. On day 10 after tumor inoculation, T cells were isolated and assessed for percentage of proliferating and IFNγ^+ CD4$^+$ T cells in response to M30. Data depicts mean +/- sd, n=5, one-way ANOVA



Supplementary Figure 11: Primary gating strategy of TIL analysis. Singlet cells were selected from the cell population and dead cells were then excluded. $CD3^{+}CD45^{+}$ T cells were selected from the live cell population. Subsequently, CD62L low effector T cells were selected from the T cell population. Finally, $CD44^{+}TNF\alpha^{+}$, $CD44^{+}IFN\gamma^{+}$, and $CD44^{+}$Granzyme B^{+} cells were analyzed.



Supplementary Figure 12: Therapeutic CT26 Lung metastasis. Number of lung metastases formed after 16 days in mice that received IV inoculation of CT26 colorectal carcinomas ($2x10^5$ cells and allowed to develop for 3 days) and treated with the MSR-PEI vaccine (VP) using L25K PEI and 50ug of CT26 neoantigens or left untreated (N). Primary representative photographs of excised lungs are shown in the figure. Data depicts mean +/- sd and n=6. two-tailed T test.



Supplementary Figure 13: Measurement of peptide incorporation. (a) Incorporation efficiency of neoantigens onto bare MSR determined using micro-BCA or HPLC LC-MS (mean +/- sd, n=3). (b) Quantification of unbound and bound peptides after loading onto bare MSRs.

Supplementary Table 1: BET pore volume and surface area of MSR-PEI particles

	Surface area (m^2/g)	**Pore Volume (cm^3/g)**
MSR	778	1.26
B60K-MSR	341	0.64
L25K-MSR	285	0.79

Supplementary Table 2: Endotoxin level of MSR-PEI vaccine components

Sample	EU/vax
MSR	<0.05
PEI L2K	<0.05
PEI L25K	<0.05
PEI B2K	<0.05
PEI B60K	<0.05
CpG-ODN	<0.05
GM-CSF*	<0.1
E7	<0.05
B16-M30	<0.05
B16-M67	<0.05
B16-M98	<0.05
CT26-M03	<0.05
CT26-M19	<0.05
CT26-M20	<0.05
CT26-M90	<0.05
CT26-GP70	<0.05

* Endotoxin level obtained from Manufacturer (Peprotech)

Supplementary Table 3: Loading efficiency of vaccine components in the cis MSR-PEI (cis VP) and trans MSR-PEI (trans VP) vaccines

	cis VP (%, mean +/- sd)	**trans VP (%, mean +/- sd)**
GM-CSF	99.6 +/- 0.1	99.7 +/- 0.02
CpG	56.5 +/- 8.9	62.8 +/- 0.99
E7	98.2 +/- 0.5	98.2 +/- 0.9
OVA	86.3 +/- 0.6	81.5 +/- 0.5

Supplementary Table 4: Peptide information

Name	Sequence	Origin
$E7_{43-77}$	GQAEPDRAHYNIVTFCCKCDSTLRLCVQSTHVDIR	HPV E6/E7
$E7_{49-57}$	RAHYNIVTF	HPV E7
B16-M27	REGVELCPGNKYEMRRHGTTHSLVIHD	B16 neoantigen
B16-M30	PSKPSFQEFVDWENVSPELNSTDQPFL	B16 neoantigen
B16-M47	GRGHLLGRLAAIVGKQVLLGRKVVVVR	B16 neoantigen
B16-M48	SHCHWNDLAVIPAGVVHNWDFEPRKVS	B16 neoantigen
CT26-M03	DKPLRRNNSYTSYIMAICGMPLDSFRA	CT26 neoantigen
CT26-M19	QAIVRGCSMPGPWRSGRLLVSRRWSVE	CT26 neoantigen
CT26-M20	PLLPFYPPDEALEIGLELNSSALPPTE	CT26 neoantigen
CT26-M90	LHSGQNHLKEMAISVLEARACAAAGQS	CT26 neoantigen
GP70	SPSYAYHQF	CT26 antigen
6941	AHRQGEKQHLLPVFSRLALRLPWRHSVQL	Human neoantigen
6942	VSWGKKVQPIDSILADWNEDIEAFEMMEKD	Human neoantigen
6943	DMAWRRNSRLYWLIKMVEQWQEQHLPSLSS	Human neoantigen
6783	LLTDRNTSGTTFTLLGVSDYPELQVPLFLVFLA	Human neoantigen
7412	LRVFIGNIAVNHAPVSLRPGLGLPPGAPPGTVP	Human neoantigen

Supplementary Table 5: Flow cytometry antibody information

Antibody	Manufacturer	Clone
Anti-mouse CD11c	eBioscience 17-0114	N418
Anti-mouse CD86	eBioscience 12-0862	GL1
Anti-mouse MHC-II	eBioscience 11-5322	NIMR-4
Anti-mouse CCR7	eBioscience 12-1971	4B12
Anti-mouse SIINFEKL-H2K^{b}	eBioscience 25-5743	25-D1.16
Anti-mouse IFNγ	eBioscience 17-7311	XMG1.2
Anti-mouse TNFα	BD Bioscience 554420	MP6-XP22
Anti-mouse CD3e	Biolegend 100241	17A2
Anti-mouse CD4	eBioscience 12-0042	RM4-5
Anti-mouse CD4	eBioscience 11-0042	RM4-5
Anti-mouse CD8a	eBioscience 53-0081	53-6.7
Anti-mouse CD8a	Biolegend 100730	53-6.7
Anti-mouse CD45	Biolegend 103107	30-F11
Anti-mouse CD44	Biolegend 103049	IM7
Anti-mouse CD69	Biolegend 104521	H1.2F3
Anti-mouse CD62L	Biolegend 104441	MEL-14
Anti-mouse Granzyme B	eBioscience 25-8898	NGZB
Anti-mouse F4/80	eBioscience 2-4801	BM8
Anti-mouse Ly6G	eBioscience 11-5931	RB6-8C5
Anti-mouse Ly6C	Biolegend 128013	HK1.4

Supplementary Table 6: Details for statistical analyses. F indicates F-values for ANOVA tests, T indicates t-values for t-tests, and df indicates degrees of freedom.

Figure	Analysis	One-tail or two-tailed	F or T, df
Fig 1e (m30)	Student T test	Two-tailed	T=5.327 df=4
Fig 1e (m20)	Student T test	Two-tailed	T=4.173 df=7
Fig 1f (6783)	Student T test	Two-tailed	T=10.42, df=4
Fig 1f (6942)	Student T test	Two-tailed	T=6.663, df=4
Fig 1g	One-way ANOVA	Two-tailed	F(4,15)=45.8
Fig 1h	One-way ANOVA	Two-tailed	F(4,15)=43.83
Fig 1i	One-way ANOVA	Two-tailed	F(4,15)=8.62
Fig 1j	One-way ANOVA	Two-tailed	F (3, 8) = 117.0
Fig 2b	Student T test	Two-tailed	T=0.5084 df=6
Fig 2c	Student T test	Two-tailed	T=5.148 df=6
Fig 2d	Student T test	Two-tailed	T=3.742 df=6
Fig 2e	Student T test	Two-tailed	T=3.254 df=6
Fig 2f	Two-way ANOVA	Two-tailed	F (2, 20) = 49.32
Fig 2g (CD86)	Two-way ANOVA	Two-tailed	F (2, 20) = 24.33
Fig 2g (MHC-II)	Two-way ANOVA	Two-tailed	F (2, 20) = 33.69
Fig 2h	Two-way ANOVA	Two-tailed	F (2, 20) = 7.463
Fig 2j	Student T test	Two-tailed	T=2.874 df=8
Fig 2k	Student T test	Two-tailed	T=3.584 df=8
Fig 2l	Student T test	Two-tailed	T=3.234 df=8
Fig 3a	One-way ANOVA	Two-tailed	F (2, 11) = 16.54
Fig 3b	One-way ANOVA	Two-tailed	F (2, 10) = 16.07
Fig 3c	Student T test	Two-tailed	T=4.925 df=6
Fig 3d	One-way ANOVA	Two-tailed	F (4, 15) = 7.699
Fig 3e	One-way ANOVA	Two-tailed	F (2, 9) = 14.55
Fig 3f	One-way ANOVA	Two-tailed	F (3, 35) = 12.51
Fig 4a	One-way ANOVA	Two-tailed	F (3, 12) = 9.234
Fig 4b	One-way ANOVA	Two-tailed	F (3, 12) = 7.965

Supplementary Table 6 continued:

Figure	Analysis	One-tail or two-tailed	F or T, df
Fig 4c	One-way ANOVA	Two-tailed	F (2, 11) = 7.977
Fig 4d	Two-way ANOVA	Two-tailed	F (2, 250) = 371.3
Fig 4e	Log-rank (Mantel-Cox) test	N/A	N/A
Fig 4f	Log-rank (Mantel-Cox) test	N/A	N/A
Fig 4h	Two-way ANOVA	Two-tailed	F (3, 278) = 311.7
Fig 5a (TNFa)	One-way ANOVA	Two-tailed	F (2, 8) = 9.267
Fig 5a (IFNy)	One-way ANOVA	Two-tailed	F (2, 8) = 4.477
Fig 5a (Granzyme B)	One-way ANOVA	Two-tailed	F (2, 8) = 7.587
Fig 5b	Student T test	Two-tailed	T=11.73 df=10
Fig 5c	Student T test (AUC analyzed)	Two-tailed	T=2.585 df=14
Fig 5d	Student T test	Two-tailed	T=4.621 df=14
Fig 5e	One-way ANOVA (AUC analyzed)	Two-tailed	F (2, 22) = 7.624
Supplementary Fig 2b	Two-way ANOVA	Two-tailed	F (4, 12) = 79.42
Supplementary Fig 2c	Two-way ANOVA	Two-tailed	F (4, 12) = 339.6
Supplementary Fig 3a	One-way ANOVA	Two-tailed	F (3, 12) = 32.77
Supplementary Fig 3b	One-way ANOVA	Two-tailed	F (3, 24) = 27.05
Supplementary Fig 4	Two-way ANOVA	Two-tailed	F (3, 23) = 179.6
Supplementary Fig 6a (CD86)	Two-way ANOVA	Two-tailed	F (2, 20) = 21.40
Supplementary Fig 6a (MHC-II)	Two-way ANOVA	Two-tailed	F (2, 20) = 17.75
Supplementary Fig 6b	One-way ANOVA	Two-tailed	F (2, 11) = 36.69
Supplementary Fig 6c	One-way ANOVA	Two-tailed	F (2, 11) = 29.28

Supplementary Table 6 continued:

Figure	Analysis	One-tail or two-tailed	F or T, df
Supplementary Fig 6d	Student T test	Two-tailed	T=1.010 df=8
Supplementary Fig 7b	One-way ANOVA	Two-tailed	F (3, 12) = 10.56
Supplementary Fig 8a	One-way ANOVA (AUC analyzed)	Two-tailed	F (3, 26) = 25.49
Supplementary Fig 8b	Log-rank (Mantel-Cox) test	N/A	N/A
Supplementary Fig 8c	Two-way ANOVA	Two-tailed	F (11, 132) = 42.78
Supplementary Fig 9	One-way ANOVA	Two-tailed	F (2, 18) = 2.996
Supplementary Fig 10	One-way ANOVA	Two-tailed	F (2, 12) = 6.675
Supplementary Fig 12	Student T test	Two-tailed	T=4.011 df=10