Supplementary material

Supplementary methods

Analysis of translation of DNA vaccines into vaccine proteins by transfection of HEK293E cells

HEK293E cells were transiently transfected with DNA (1µg) using opti-MEM (Life Technologies) and polyethylenimine (2µg PEI, Polysciences). Supernatants were harvested after 72 hours, centrifuged (13.000rpm, 5min) and stored at -20°C unless used directly. Secretion of vaccine proteins were analyzed by sandwich ELISA as previously described[1].

M315-specific antibodies elicited by immunization

Sera were harvested from the mice after immunization, prior to tumor challenge. M315-specific antibodies were measured by ELISA. Briefly, M315 protein (2µg/ml) was used as coat. Detection of M315-specific antibodies was performed using biotinylated anti-IgG1 or anti-IgG2a antibodies (1µg/ml, both BD Pharmingen). Biotinylated antibodies were detected by streptavidin-alkaline phosphatase (GE Healthcare Chicago, IL) and followed by phosphatase substrate (Sigma). Optical Density (OD) at 405nm was measured with a TECAN Sunrise Microplate using the Magellan v5.03 program. Affinity-purified anti-Id^{315} specific mAb (Ab2.1-4) served as control. The antibody titer was defined as the highest dilution of a serum sample with OD values > (2 x mean) of NaCl-vaccinated mice. Samples with titers <50 were not detected (ND) and assigned an endpoint titer of 1.

Generation of MOPC315.BM.GFP.λ2 and MOPC315.BM.dsRed.IgAλ2

MOPC315.BM.GFP.λ2 were generated by using the MSCV IRES GFP retroviral expression vector obtained from Dr. T Reya, Stanford University, via Addgene repository (Addgene; #20672). Ecotropic viral particles for transduction were generated by liposomal transfection of HEK293T cells with the expression vector and packaging plasmids pHIT60 (MLV gag/pol) and pHIT123 (MLV-E) as previously described[2]. GFP-positive cells were sorted on a FACS Aria II cell sorter (BD Biosciences. MOPC315.BM.dsRed.IgAλ2 were generated as described in[3].

In vitro cytotoxicity

Mice were immunized with 50µg DNA i.m. followed by electroporation (EP) as previously described[1] and boosted with MOPC315.BM.Luc.IgAλ2 ($4x10^4$ cells, 3 weeks), M315 protein (50µg, 9 weeks), and MOPC315.BM.Luc.IgAλ2 boost ($2x10^5$, 12 weeks) after immunization. Spleens were harvested 13 days after final boost. Single cells were obtained using gentleMACS™ Dissociators (Miltenyi Biotec). The cell suspension was treated with Tris-buffered ammonium chloride (ATC) for 7 min on ice to remove red blood cells, filtered through 70µm strainers, and washed in PBS. Target MOPC315.BM.dsRed.IgAλ2 and MOPC315.BM.GFP.λ2 cells were mixed in a 1:1 ratio ($1x10^5$ cells in total/500µl of medium) and added to the effector splenocytes at the indicated effector:target (E:T) ratios. After 24 hours of incubation, the ratio of dsRed to GFP cells was determined by flow cytometry.

Western blot

Cells were harvested, washed with ice-cold PBS and resuspended in lysis buffer. Aliquots of 15µl lysates were mixed with 5x SDS sample buffer without reducing agent, heated for 2 min at 98°C and separated on 4-12% Bis-Tris gels (Invitrogen). Proteins were transferred to a PVDF membrane using the iBlot Dry Blotting System (Invitrogen). Membranes were blocked with 5% BSA in Tris-buffered saline (TBS) with 0.1% Tween20 and incubated overnight at 4°C with rabbit anti-mouse IgA (1µg/ml, BD Pharmingen, San Diego, CA) or biotinylated anti-mouse λ2/3 mAb (2B6). Detection was performed with horseradish peroxidase-conjugated polyclonal goat anti-rabbit antibody (SouthernBiotech, Birmingham, LA, USA) or streptavidin-horseradish peroxidase (GE Healthcare Chicago, IL) and SuperSignal West Pico PLUS Chemiluminescent (Thermo Fischer Scientific, Rockford, IL, USA). Images were acquired using Syngene G:Box.

mRNA analysis

$5x10^6$ cells were washed with PBS. Total RNA was isolated using the RNeasy kit (Qiagen). The cDNA library was made using the First strand cDNA synthesis kit (Thermo Scientific). Heavy chain transcripts were amplified using a 5′primer that binds FR1 region of V_H^{315} (5′-GGTGTGCATTCCGATGTACAGCTTCAGGAGTCAG-3') in combination with 3′primer against IgA constant domain (5′-GATGGTGGGATTTCTCGCAGAC -3') that anneals to C_H1 domain. The λ genes were PCR amplified using the 5′primer mVλ1/2 (5'-CAGGCTGTTGTGACTCAGGAATC-3') together with the 3′primer mCλ (5'-

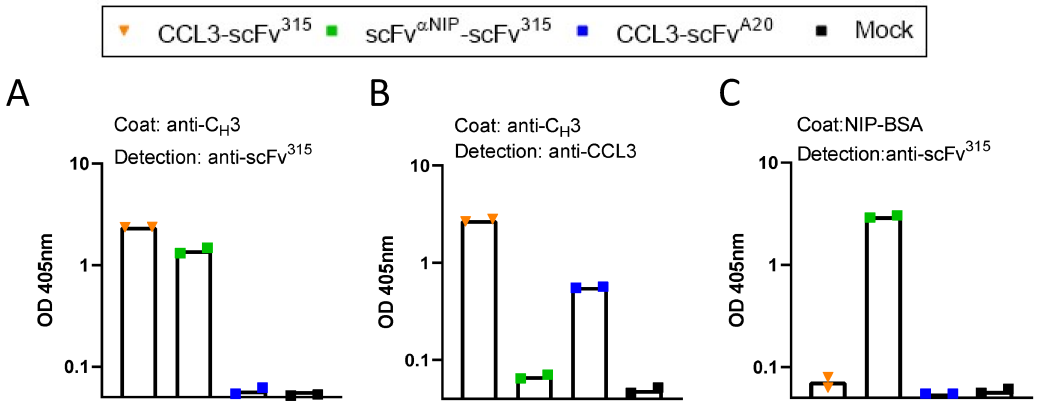
GTACCATYTGCCTTCCAGKCCACT-3') that anneal to Cλ1/2/3. GADPH was PCR amplified using 5′primer (5′- CAAGGTCATCCATGACAACTTTG-3') and 3′primer (5′- GTCCACCACCCTGTTGCTGTAG-3'). The amplicons were run on agarose gel and visualized on BioRad Gel Doc EZ imager (top). Bands were quantified by defining lanes with a rectangular tool to generate blot lane profiles, using gel analysis plugin in ImageJ version V2.0.0. Area under the curve values were determined for all bands and normalized to the control MOPC315.BM.luc.

Flow cytometry

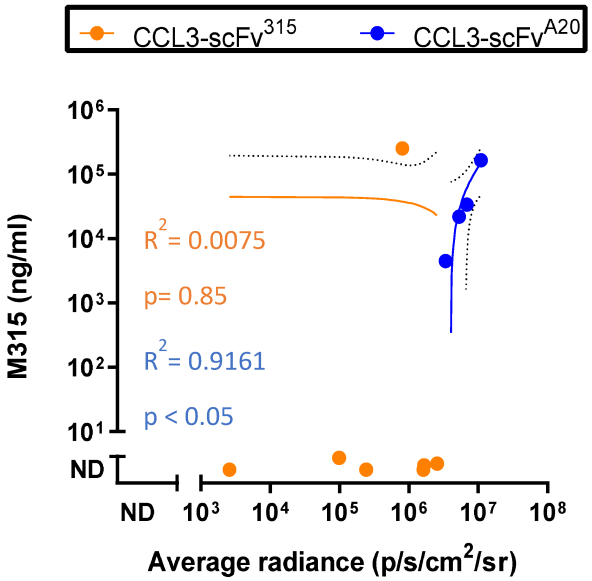
Cell lines as indicated were harvested, washed and stained with the following antibodies: Affinity purified biotinylated M315-specific mAb (Ab2.1-4) with streptavidin-PE for detection (Invitrogen). PE-conjugated anti-K^d (BD, clone SF1-1.1.1), PE-conjugated anti-H-$2L^d$ (Invitrogen, clone 30-5-7S) and BV421-conjugated and anti-D^d (BD, clone 34-2-12). PE-conjugated mouse IgG2a,κ (Invitrogen) and BV421-conjugated mouse IgG2a,κ (BD) were used as isotype controls. 30% rat serum and 100μg/ml 2.4G2 (anti-FcγRII/III Ab) were used for blocking. Data were acquired on an Attune NxT (ThermoFisher Scientific, Waltham, MA) and analyzed using FlowJo software (FlowJo, LLC, Ashland, OR).

References

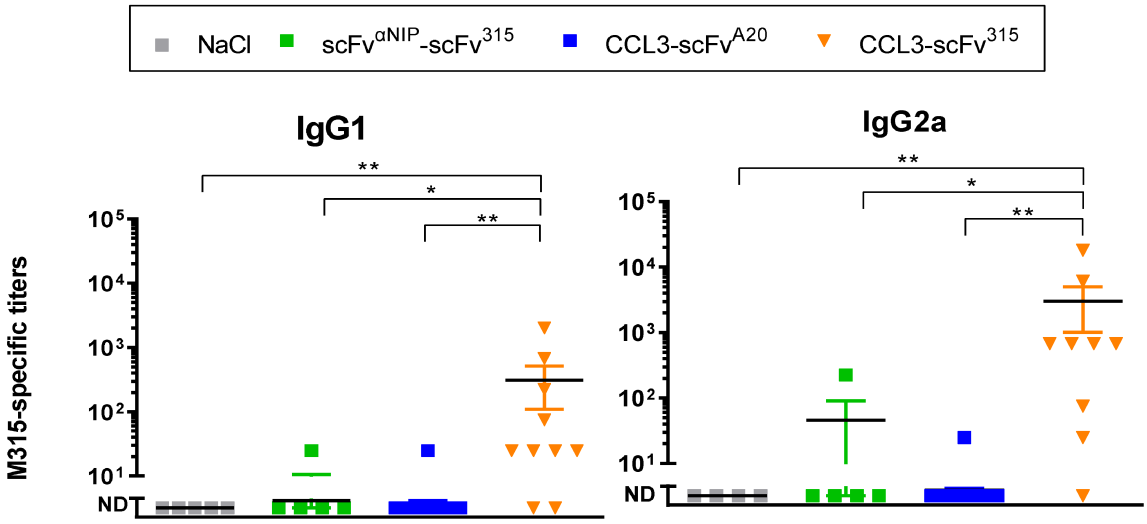
1. Braathen, R., H.C.L. Spång, M.M. Lindeberg, et al., *The Magnitude and IgG Subclass of Antibodies Elicited by Targeted DNA Vaccines Are Influenced by Specificity for APC Surface Molecules.* Immunohorizons, 2018. **2**(1): p. 38-53.
2. Pouw, N.M., E.J. Westerlaken, R.A. Willemsen, et al., *Gene transfer of human TCR in primary murine T cells is improved by pseudo-typing with amphotropic and ecotropic envelopes.* J Gene Med, 2007. **9**(7): p. 561-70.
3. Hofgaard, P.O., H.C. Jodal, K. Bommert, et al., *A Novel Mouse Model for Multiple Myeloma (MOPC315.BM) That Allows Noninvasive Spatiotemporal Detection of Osteolytic Disease.* PLOS ONE, 2012. **7**(12): p. e51892.



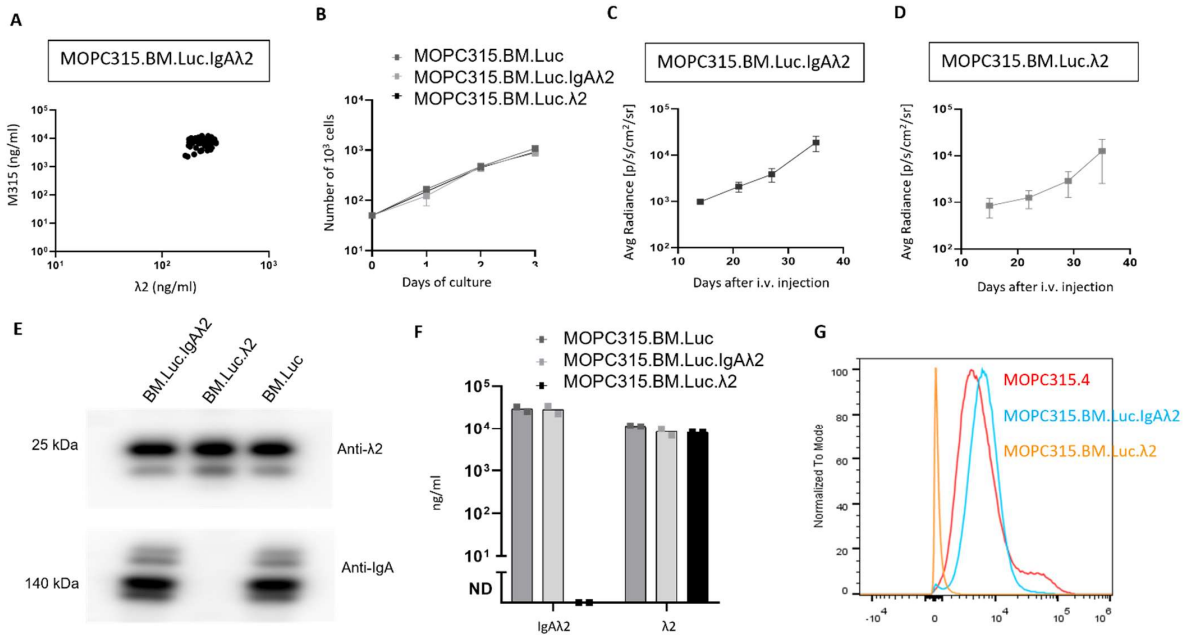
Supplementary Figure 1. Analysis of translation of DNA vaccines into vaccine proteins by transient transfection of HEK293E cells. A-C) HEK293E cells were transiently transfected with the indicated DNA plasmids (box). Supernatants were investigated by ELISA detecting C_H3 and $scFv^{315}$ (A), C_H3 and CCL3 (B), and $scFv^{\alpha NIP}$ and $scFv^{315}$ (C). Shown as technical duplicates and mean as bar.



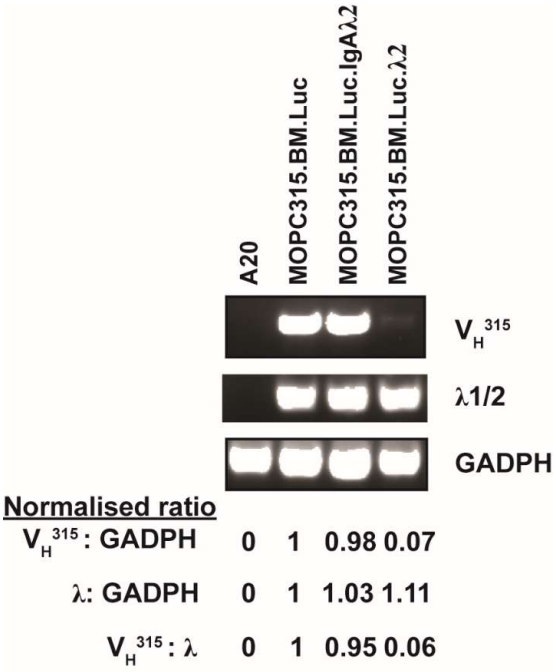
Supplementary Figure 2. Correlation of M315 serum levels and bioluminescence imaging (BLI) signal. BALB/c mice (n=4-7 mice /group) were immunized by i.m. injection of 50µg of the indicated plasmids/EP (box). MOPC315.BM.Luc ($2x10^5$ cells) were injected i.v. 14 days after vaccination**.** M315 levels in serum and BLI imaging data at day 35 after challenge is shown. Statistic: Pearson correlation, ND=not detected



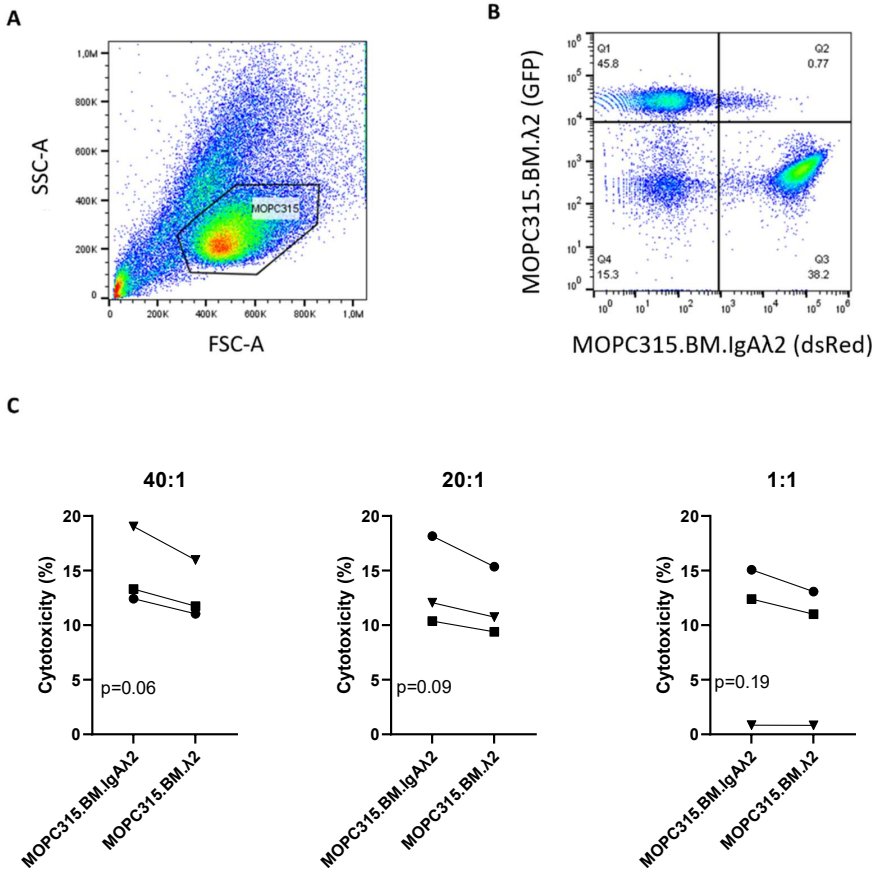
Supplementary Figure 3. M315-specific antibody levels after a single DNA vaccination. BALB/c mice were immunized by i.m. injection of 50µg of the indicated plasmids/EP (box). Sera was analysed on day 13 after immunization. M315-specific IgG1 (left) and IgG2a (right) were measured by ELISA, showing mean ± SEM. n=5-10 mice/group. Statistics: Kruskal Wallis with Dunns correction, *p<0.05, ** p<0.01. ND=not detected.



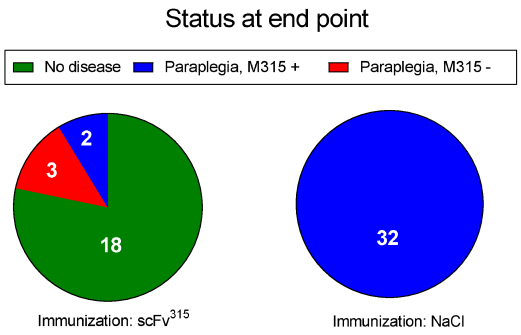
Supplementary Figure 4. Analysis of long-term stability and growth rate of M315-producing clones. A) A MOPC315.BM.Luc.IgAλ2 clone was *in vitro* passaged 24 times before recloned by limiting dilution. Complete M315 (IgAλ2) and λ2 were quantified in supernatants of single clones (dots) using ELISA **B)** Growth curves of MOPC315.BM.Luc.IgAλ2, MOPC315.BM.Luc.λ2 and MOPC315.BM.Luc *in vitro*. **C, D)** Growth of MOPC315.BM.Luc.IgAλ2 (C) and MOPC315.BM.Luc.λ2 (D) injected i.v. into naïve BALB/c mice. Growth was measured using bioluminescence imaging. **E, F)** Levels of M315 and λ2 in lysates from the MOPC315.BM.Luc.IgAλ2, MOPC315.BM.Luc.λ2, and MOPC315.BM.Luc cells measured by western blot (E) and ELISA (F). **G)** Expression of M315 on the cell surface of the indicated MOPC315 cell lines as measured by flow cytometry. Mean ± SD (B-D) and mean of technical duplicates (F) are indicated. ND=not detected.



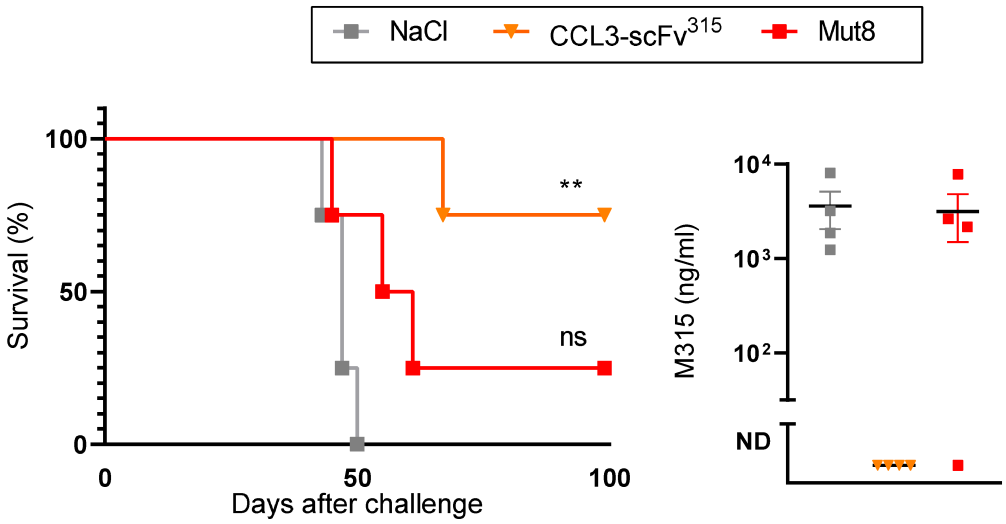
Supplementary Figure 5. mRNA analysis of MOPC315.BM.Luc.IgAλ2 and MOPC315.BM.Luc.λ2 cells. mRNA was isolated from MOPC315.BM.Luc.IgAλ2, MOPC315.BM.Luc.λ2 and MOPC315.BM.Luc cells, cDNA made, and V_H^{315}, λ2 and GADPH amplified by specific primers. The amplicons were run on agarose gel and visualized on BioRad Gel Doc EZ imager (top). The bands were quantified using gel analysis plugin in ImageJ version V2.0.0 and indicated ratios calculated (bottom).



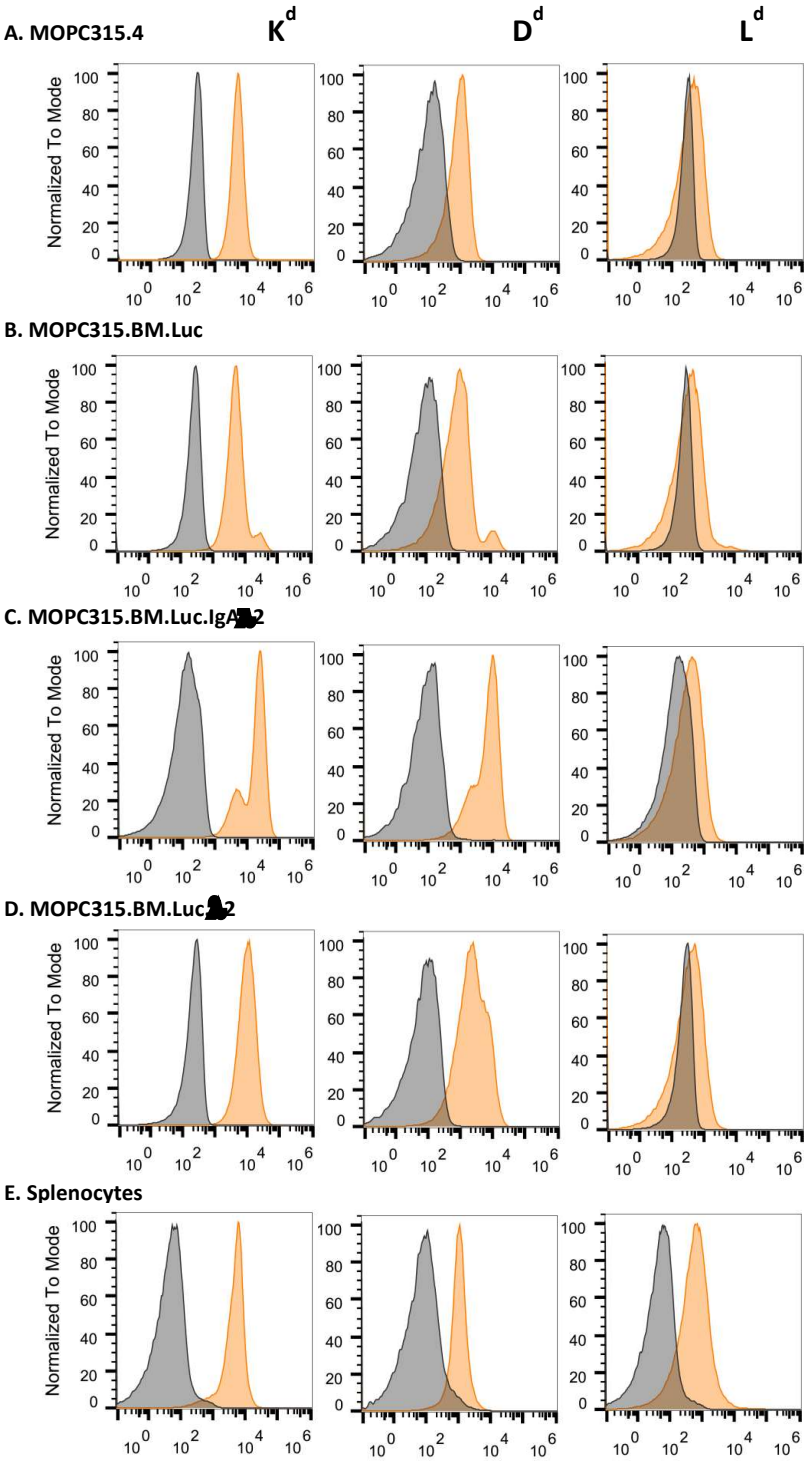
Supplementary Figure 6. Analysis of *in vitro* cytotoxicity induced by the Id-vaccine. A-C) BALB/c mice were immunized i.m./EP with 50µg CCL3-scFv^{315} and boosted with MOPC315.BM.Luc.IgAλ2 (4x10^4 cells, 3 weeks), M315 protein (50µg, 9 weeks), and MOPC315.BM.Luc.IgAλ2 boost (2x10^5, 12 weeks) after immunization. Spleens were harvested 13 days after final boost, and splenocytes were co-cultured for 24hours with a mixture of dsRed-labeled MOPC315.BM.IgAλ2 and GFP-labeled MOPC315.BM.λ2. (A) Scatter plot of the two labeled MOPC315 cells, (B) representative dot plot, and (C) cytotoxicity (%) of MOPC315.BM.IgAλ2 and MOPC315.BM.λ2. Three independent cultures are indicated by different symbols (downward triangle, square or circle). The effector:target cell ratio is indicated above the graphs. The data indicate that MOPC315.BM.IgAλ2 cells were killed to a larger extent than the H chain-loss variant MOPC315.BM.λ2 cells although the results are not statistically significant (two-tailed paired t-test).



Supplementary Figure 7. Status at end point of mice immunized with CCL3-scFv^{315} or carrier (NaCl). BALB/c mice were immunized with either 50µg CCL3-scFv^{315} DNA (n=23) or carrier (NaCl, n=32) i.m./EP. 14 days later, vaccinated mice were challenged with MOPC315.BM.Luc.IgAλ2 (2x10^5 cells). Status at end point (development of paraplegia with M315 in serum (blue) or without M315 in serum (red), or survival at day 100 without evidence of disease (green) is shown as pie charts. The number in each sector indicates number of mice. Summary of 5 independent experiments.

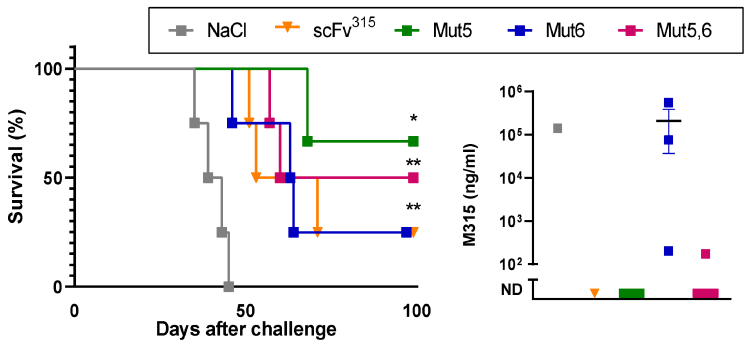


Supplementary Figure 8. Immunization with Mut8. BALB/c mice (n=4 mice/group) were immunized once with 50µg of DNA i.m./EP as indicated. MOPC315.BM.Luc.IgAλ2 ($2x10^5$ cells) were injected i.v. 14 days after vaccination**.** Survival (left) and M315 levels in the sera at endpoint (right). Statistic: Mantel Cox Log-Rank test compared to NaCl group, ** $p<0.01$, ns=not significant, ND=not detected.


A. MOPC315.4
K^d
D^d
L^d
Normalized To Mode
B. MOPC315.BM.Luc
C. MOPC315.BM.Luc.IgA[redacted]2
D. MOPC315.BM.Luc.[redacted]2
E. Splenocytes

Supplementary Figure 9. MHC class I molecule expression on MOPC315.BM.Luc variants. A-E) Expression of MHC class I molecules on MOPC315.4 (A) MOPC315.BM.Luc (B), MOPC315.BM.Luc.IgAλ2 (C), MOPC315.BM.Luc.λ2 (D), and spleenocytes (E) analysed by flow cytometry: K^d (left), D^d (middle), L^d (right). Isotype control is shown in gray.

A



B

	Survival (>CCL3-scFv^{315})	Serum M315^{+} (>1µg/ml)	Heavy chain loss/mice w/tumor
scFv^{315}		0/1‡	1/1‡
Mut5	+	0/3	1/1
Mut6	-	3/3	0/3
Mut5,6	+	1/4	1/2

Supplementary Figure 10. Immunization with Mut5, Mut6 and Mut5,6. A-B) BALB/c mice were immunized by i.m. injection of 50µg of plasmids/EP as indicated (box). MOPC315.BM.Luc (2x10^5 cells/mice) were injected i.v. 14 days after vaccination. (A) Survival (left) and M315 sera levels (right) at end point. (B) Table with a summary of the main findings at end point (either paraplegia or day 100). The serum M315 column shows the number of mice measured in the denominator, and the number of mice with serum M315 in the numerator. ‡ Due to technical difficulties only one mouse was bled at endpoint. Statistic: Mantel Cox Log Rank, *p<0.05, ** p<0.01, n=3-4 mice/group.

Supplementary Table 1. Predicted MHC class I epitopes in V_H^{315}

*Mutations in V_H^{315} are highlighted in red.

**SB=strong binder, WB=weak binder.

	Epitope*	aa position	Mutation	Bind level	SB/WB**
H2D^d restriction	AGDNDHLYYF	97-106	Mut8	0.4389	SB
	YSITSGYFW	27-35	Mut1	0.4571	SB
	QFPGNKLEW	40-48	-	0.9883	WB
	TGYSITSGYF	25-34	Mut1	1.1647	WB
	RQFPGNKLEW	39-48	-	1.3390	WB
	YSITSGYF	27-34	Mut1	1.3432	WB
	YNPSLKNRV	60-68	Mut6	1.4051	WB
	SGYFWNWI	31-38	Mut1	1.6537	WB
	VKPSQSLSL	12-20	-	1.6870	WB
	SENQFFLKL	75-83	Mut7	1.7547	WB
H2K^d restriction	DYWGQGTTL	107-115	-	0.1080	SB
	FDYWGQGTTL	106-115	-	0.1559	SB
	GYNPSLKNRV	59-68	Mut5,6	0.3417	SB
	YYCAGDNDHL	94-103	Mut8	0.3877	SB
	GYSITSGYF	26-34	Mut1	0.4287	SB
	YWGQGTTL	108-115	-	0.4639	SB
	QFFLKLNSV	78-86	-	0.7615	WB
	GYFWNWIRQF	32-41	Mut1	0.8312	WB
	YFWNWIRQF	33-41	Mut1	1.4935	WB
	GLVKPSQSL	10-18	-	1.8501	WB
	QFPGNKLEW	40-48	-	1.9726	WB
H2L^d restriction	FPGNKLEWL	41-49	Mut2	0.3388	SB
	SENQFFLKL	75-83	Mut7	0.4347	SB
	KPSQSLSL	13-20	-	0.4620	SB
	DYWGQGTTL	107-115	-	0.7101	WB
	YFWNWIRQF	33-41	Mut1	1.3903	WB
	NPSLKNRVSI	61-70	Mut6	1.6116	WB