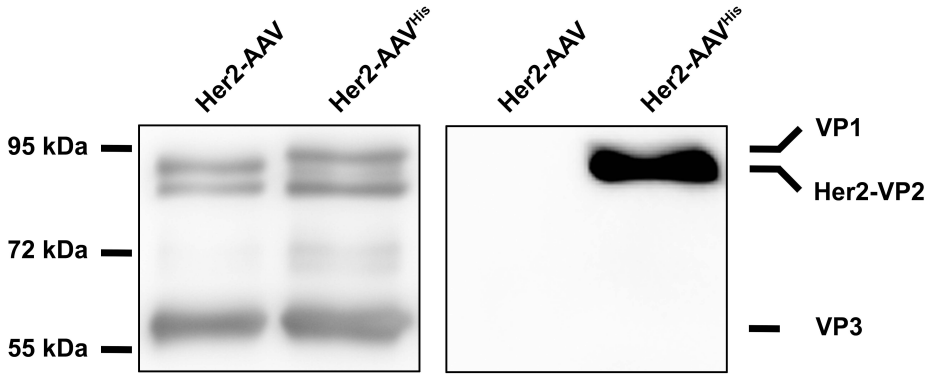
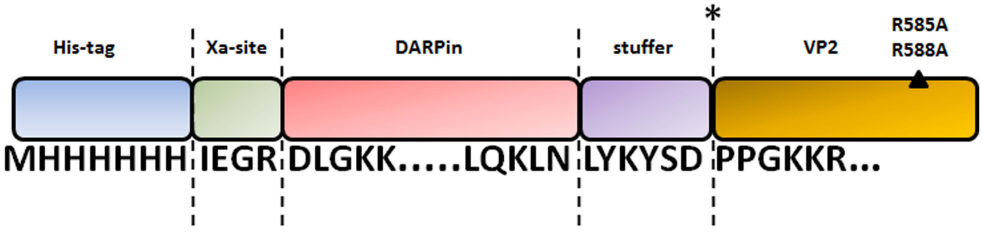
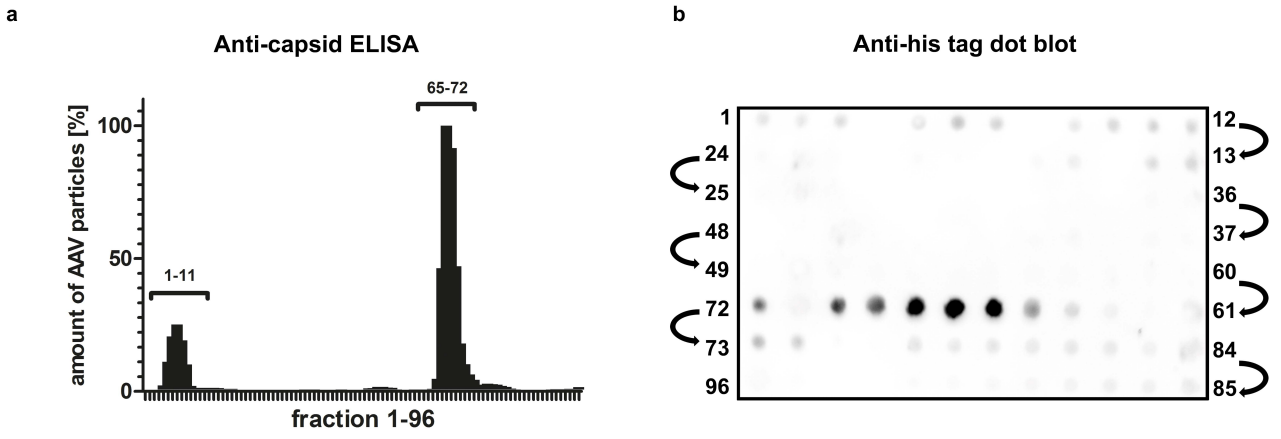


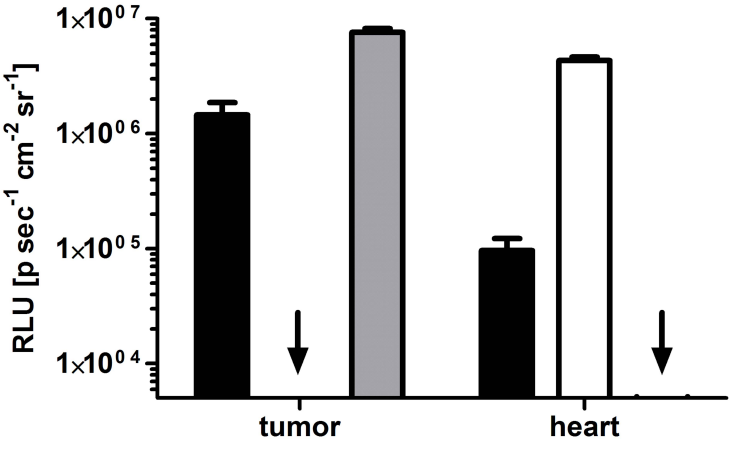
Supplementary Figure 1: Western Blot analysis of His-tagged Her2-AAV. Her2-AAV (2×10^{10} vector genomes), containing the DARPin-9.29-VP2 fusion protein, with the two point mutations R585A and R588A but no hexa-histidine motif, and Her2-AAV^{His} (2×10^{10} vector genomes), additionally containing a hexa-histidine motif and named "Her2-AAV" in the main text, were analyzed side by side on an 8% SDS-polyacrylamide gel. Proteins blotted on a nitrocellulose membrane (Amersham Biosciences, Freiburg, Germany) were detected using either the capsid protein-specific antibody B1 recognizing VP1, VP2 and VP3 because of their identical C-terminal region (Progen, Heidelberg, Germany) (left) or a hexa-histidine motif-specific antibody (Cell Signaling, Danvers, US) (right). Signals were visualized by enhanced chemiluminescence using the ECL Plus Western Blotting Detection System (GE Healthcare, Munich, Germany). Representative blots are shown.



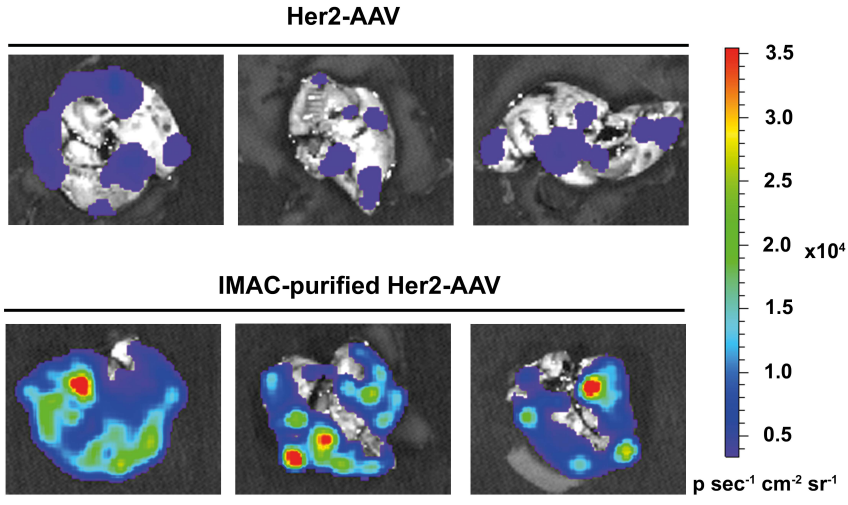
Supplementary Figure 2: Schematic representation of the DARPin-VP2 fusion protein. The VP2-start codon was mutated (labeled by an asterisk) to prevent expression of unmodified VP2. To abolish HSPG binding, R585 and R588 were substituted by alanine (labeled by a triangle). The DARPin coding sequence was fused to the N-terminus of VP2 via a stuffer sequence. The His-tag was added to the N-terminal end of the DARPin along with a factor Xa-site. Amino acid sequences present at the borders are indicated below.



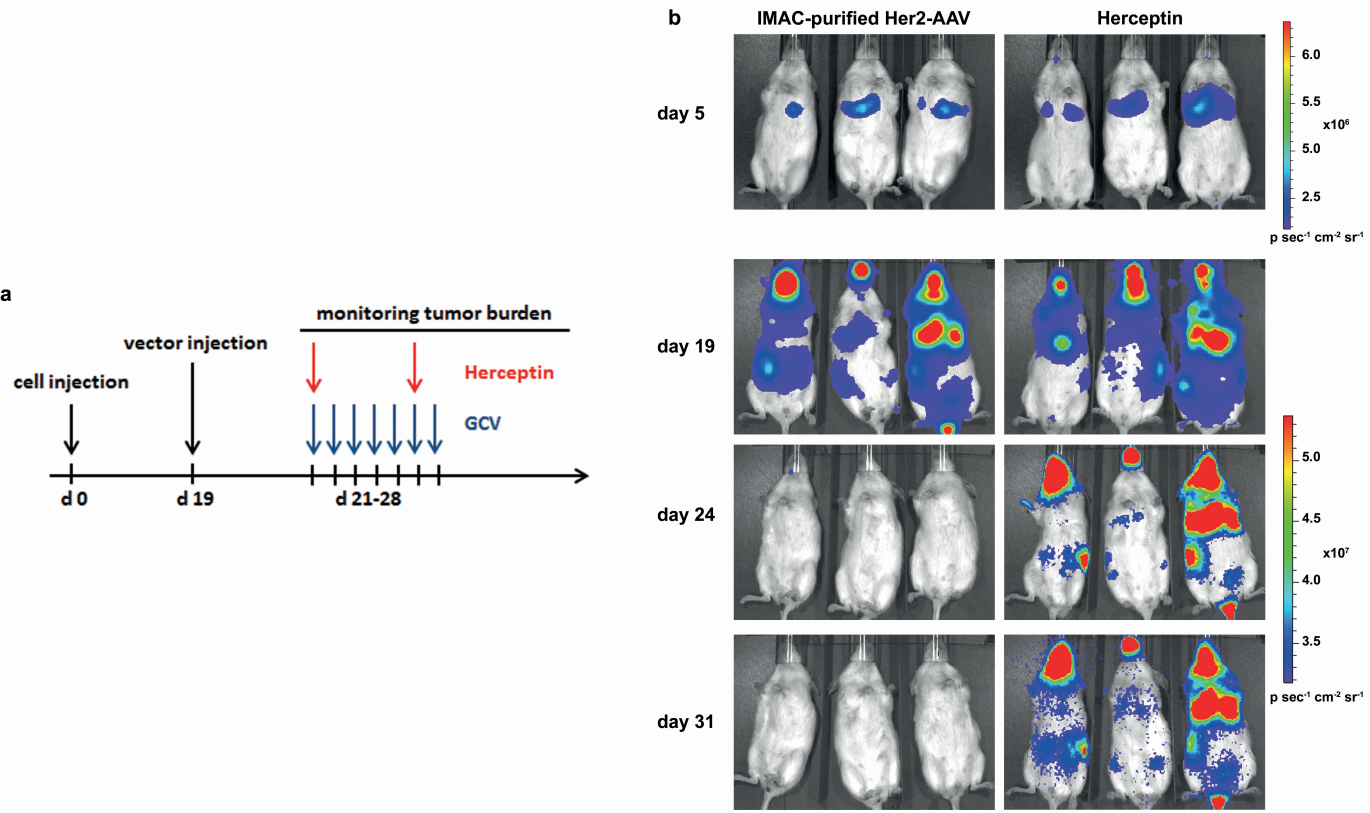
Supplementary Figure 3: Analysis of fractions from IMAC-purification of Her2-AAV shown in Fig. 1. a) 96 fractions collected during IMAC of Her2-AAV were analyzed by capsid ELISA to determine the AAV content in each fraction. AAV fractions were coated on Maxisorp immunoplates (Nunc, Wiesbaden, Germany) and detected with the anti-AAV-2-capsid antibody A20 (1:4 diluted in phosphate-buffered saline (PBS) containing 3% bovine serum albumin, 5% sucrose, 0.05% Tween20; Progen, Heidelberg, Germany). Subsequently, plates were incubated with a donkey anti-mouse biotin-conjugated antibody (1:25,000 in PBS containing 3% bovine serum albumin, 5% sucrose, 0.05% Tween20; Jackson ImmunoResearch, Suffolk, UK) and HRP-conjugated streptavidin (1:500 in PBS containing 3% bovine serum albumin, 5% sucrose, 0.05% Tween20; Dianova, Hamburg, Germany). Upon addition of TMB liquid substrate (Sigma-Aldrich, Hamburg, Germany) according to the manufacturer's instructions, the reaction product was quantified at a wavelength of 450 nm. Signals are expressed relative to the highest signal intensity. Fractions 1-11 representing the column flow-through and fractions 65-72 representing the column elution are indicated. A representative analysis is shown. b) 100 µl of collected fractions were denatured and transferred to nitrocellulose membrane (Amersham Biosciences) using a dot blot apparatus (BioRad) according to the manufacturer's instructions. Fractions were spotted in alternating order from top-left to bottom-left. Fractions 1-11 represent the column flow-through; fractions 65-72 the column elution. Vector particles were detected using a hexa-histidine tag-specific antibody



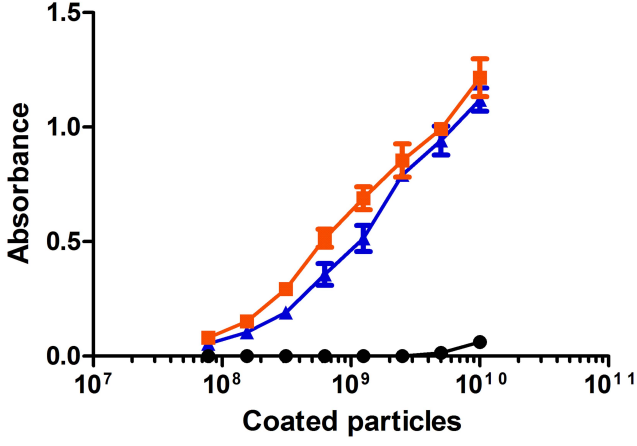
Supplementary Figure 4: Luciferase activities present in heart and tumor tissue of mice shown in Fig. 2a. One week after AAV vector administration mice were injected with luciferin and heart and tumor tissues were explanted immediately. Luciferase signals were quantified using the IVIS Spectrum *in vivo* imaging system. Each organ was analyzed separately and photons emitted from each organ were recorded. Black bars: iodixanol gradient-purified; grey bars: IMAC elution; white bars: IMAC flow-through. Intensity is expressed as photons/second/square centimeter/steradian (p sec^{-1} cm^{-2} sr^{-1}). N = 3; mean ± SEM.



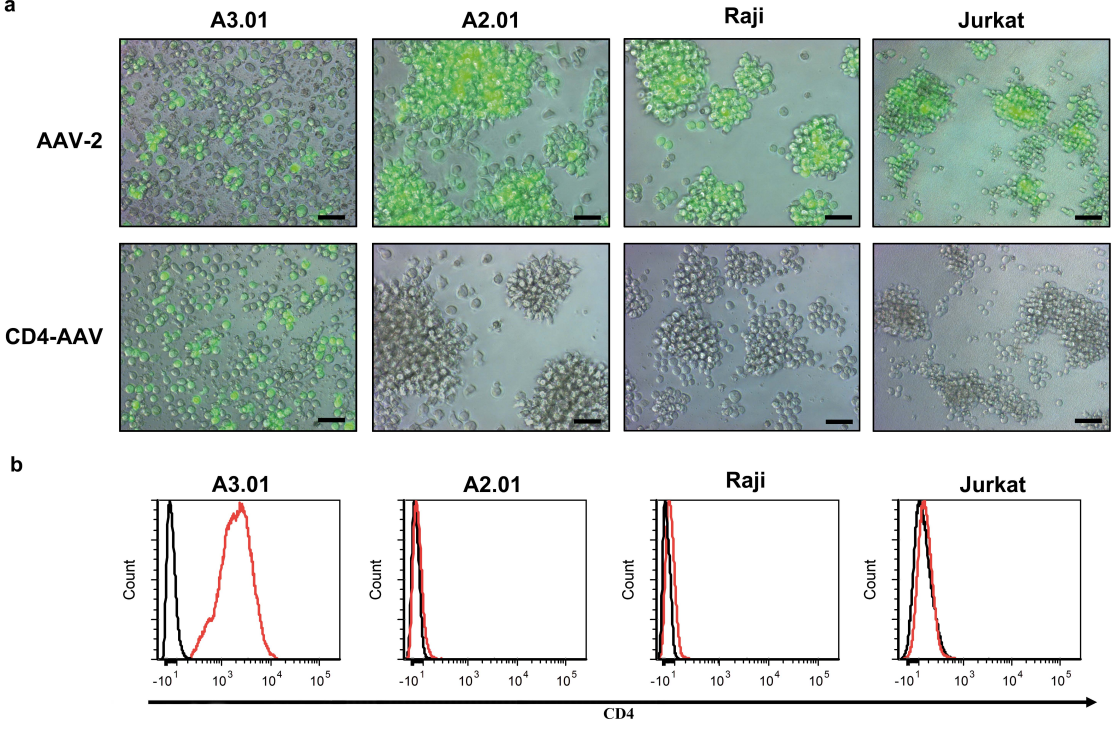
Supplementary Figure 5: Detection of tumor foci in lung. Photographs showing the luciferase activity present in lung tissue of mice shown in Fig. 2b. Luciferase signal intensity is expressed as photons/second/square centimeter/steradian (p sec^{-1} cm^{-2} sr^{-1}) (N=3).



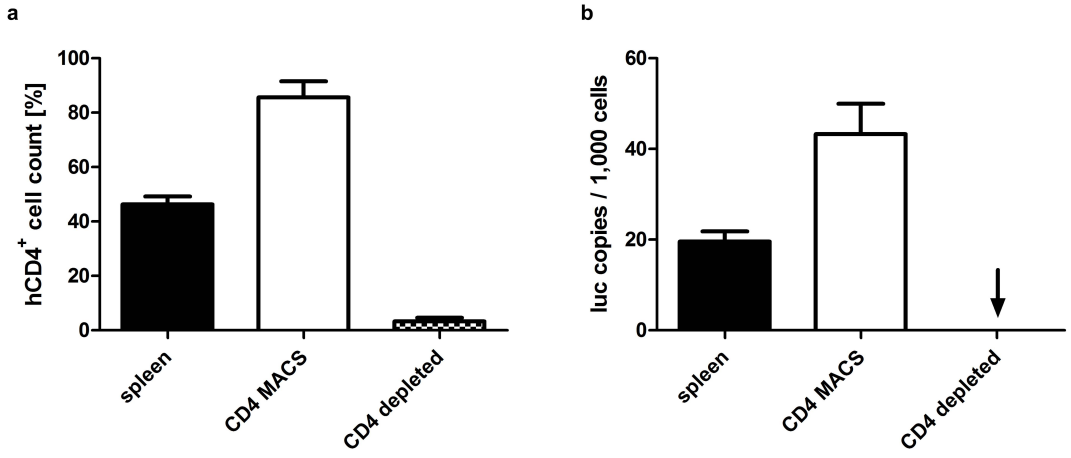
Supplementary Figure 6: Tumor burden of mice shown in Fig. 2c/d. a) Treatment regime of mice injected with MDA-MB-453-luc cells. b) *In vivo* imaging photographs taken at indicated time points. Mice were treated from day 21 – 28. Representative mice are shown. Her2-AAV: N = 5, Herceptin: N = 4.



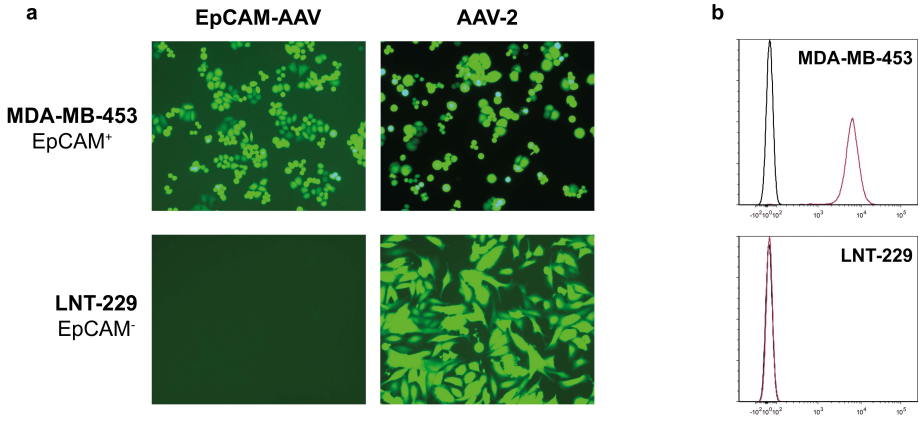
Supplementary Figure 7: DARPins displayed on the surface of AAV vector particles are accessible. DARPin-displaying vector particles carrying instead of the His-tag a Myc-tag at the N-terminus of the DARPin were generated. CD4-AAV^{Myc} (orange squares) or EpCAM-AAV^{Myc} (blue triangles) vector preparations were bound to ELISA plates pre-coated with a Myc-tag-specific antibody. AAV-2 particles (not displaying a DARPin) (black circles) were used as control. Bound vector particles were quantified using the AAV-2 capsid-specific antibody A20. N = 3 experiments; mean ± SD.



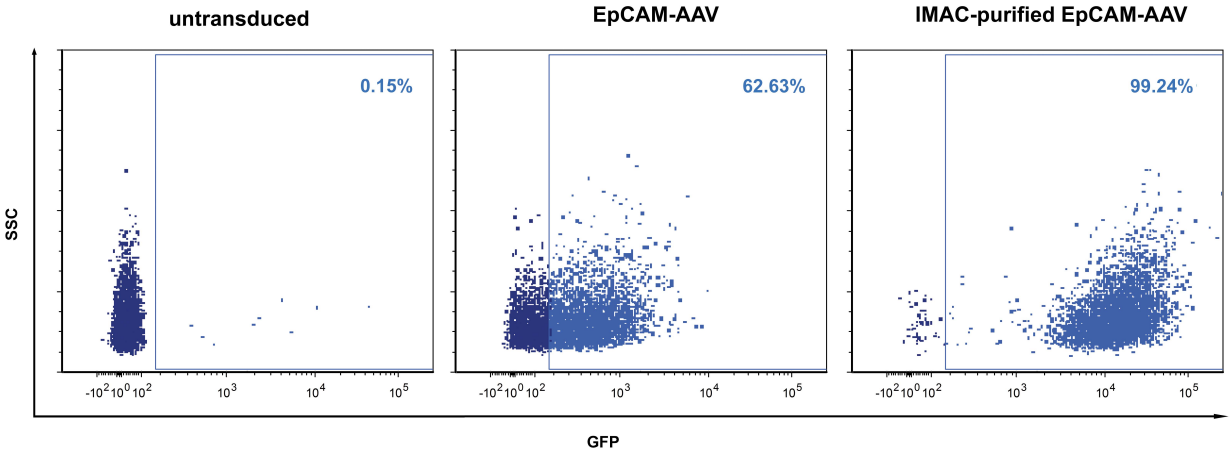
Supplementary Figure 8: CD4-AAV specifically transduces CD4-positive cells. a) CD4-positive (A3.01) and CD4-negative (A2.01, Jurkat, Raji) lymphocyte cell lines were transduced with 150,000 vector genomes per cell CD4-AAV or AAV-2 (GOI = 10,000) coding for GFP. The CD4-AAV stocks applied here had been iodixanol- but not IMAC-purified. 48 h after transduction, cells were analyzed by fluorescence microscopy for GFP expression. Scale bar represents 200 µm. Representative results are shown. b) CD4 expression levels of the cell lines determined by flow cytometry using a CD4-specific antibody (AB 3.20, BD Biosciences, Heidelberg, Germany).



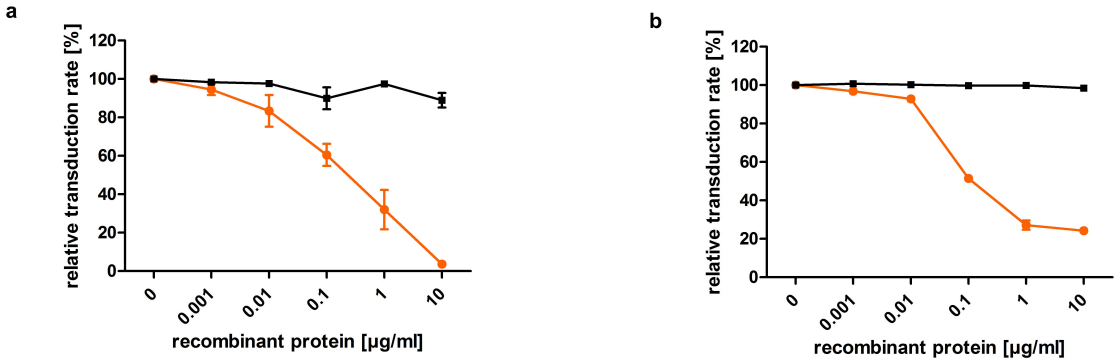
Supplementary Figure 9: CD4-AAV selectively transduces CD4 positive cells in mouse spleen. Spleens of the CD4-AAV (IMAC-purified, transferring the luciferase gene) injected mice shown in Fig. 3 were explanted and cell suspensions were prepared. CD4-positive cells were separated from CD4–negative cells using human anti-CD4 MicroBeads (Miltenyi Biotec, Bergisch Gladbach, Germany) and analyzed for the amount of CD4-positive cells by flow cytometry (left) and for the quantity of luciferase gene copies by qPCR (right). 428±68 luciferase copies were detected in 9747±256 cells of the CD4-enriched samples, while in CD4-depleted samples no luciferase gene copies were detected. N = 3, mean ± SEM.



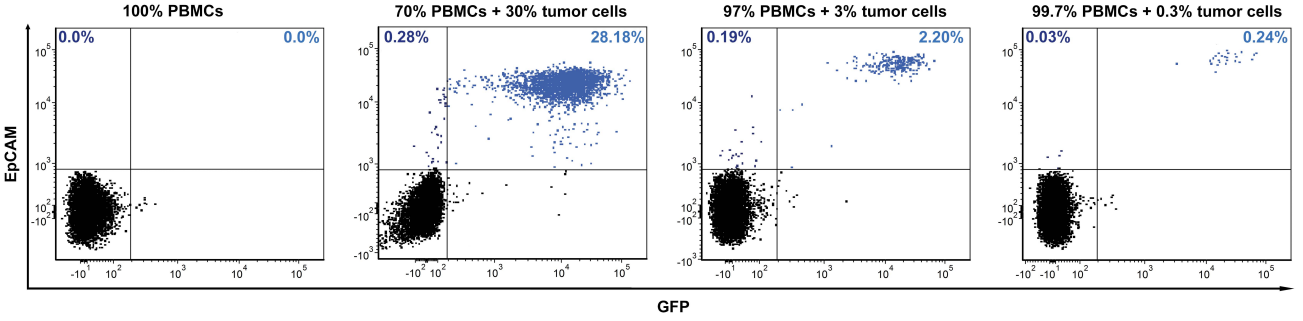
Supplementary Figure 10: EpCAM-AAV specifically transduces EpCAM positive cells. a) EpCAM-positive (MDA-MB-453) and EpCAM-negative cells (LNT-229) were transduced with EpCAM-AAV or AAV-2 (GOI = 70,000). 48 h after transduction cells were analyzed by fluorescence microscopy. b) EpCAM expression level of the indicated cell lines was determined by flow cytometry using the EpCAM-specific antibody anti-CD326-PE (Miltenyi Biotec, Bergisch Gladbach, Germany). The EpCAM-AAV stocks applied were iodixanol-, but not IMAC-purified.



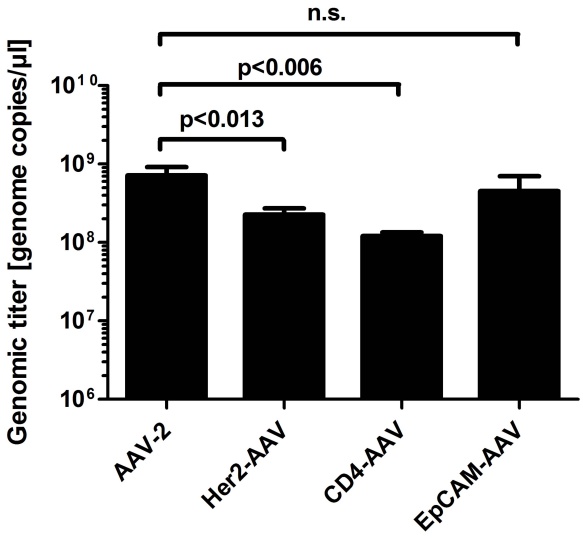
Supplementary Figure 11: IMAC-purification enhances EpCAM-AAV transduction. MDA-MB-453 cells were transduced with conventionally purified EpCAM-AAV or IMAC-purified EpCAM-AAV (GOI 60,000, each) coding for GFP and transgene expression was analyzed by flow cytometry 72 h post transduction.



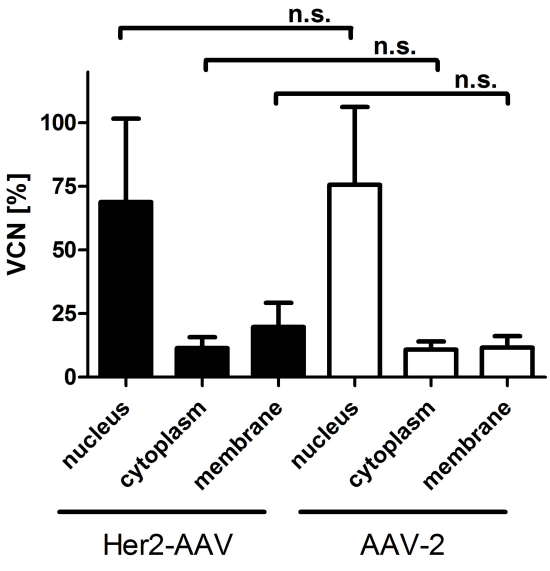
Supplementary Figure 12: Transduction mediated by CD4-AAV and EpCAM-AAV is target receptor dependent. a) CD4-AAV (GOI 90,000; orange circles) or b) EpCAM-AAV (GOI=10,000; orange circles) were incubated for 1 h at 4°C with increasing amounts of the entire extracellular domain of human CD4 or human EpCAM (Sino Biological, Beijing, China) as competitor. Following incubation, A3.01 (a) or MDA-MB-453 cells (b) were transduced and analyzed for GFP expression by flow cytometry 72 h post transduction. AAV-2 (not displaying a DARPin) (GOI 3,000 or 350; black square symbols) served as control. Data are normalized to the transduction efficiency measured without preincubation with recombinant protein. N = 3 experiments; mean ± SD.



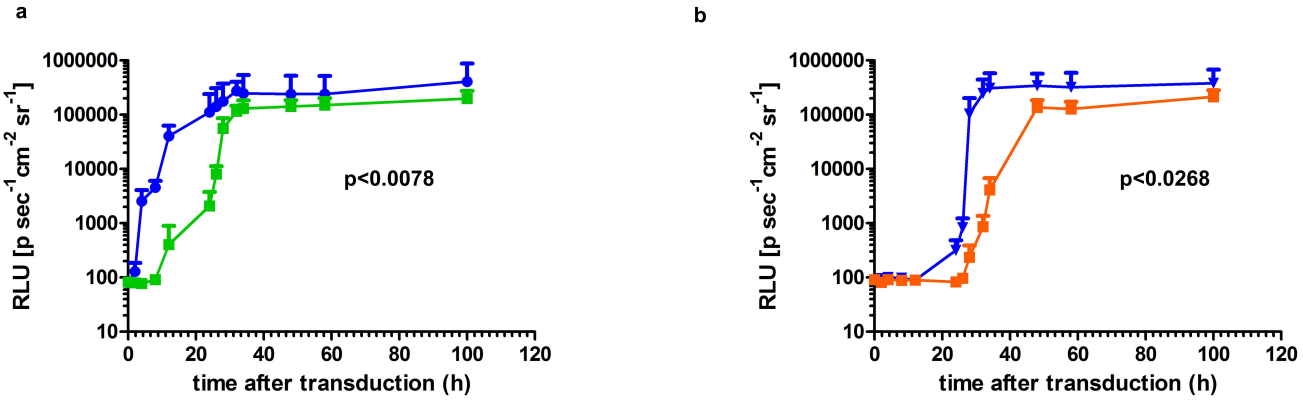
Supplementary Figure 13: EpCAM-AAV specifically detects strongly underrepresented tumor cells mixed with human PBMC. Human PBMCs were purified by density gradient centrifugation (30 min, 1800 rpm) using Histopaque (Sigma Aldrich, Hamburg, Germany). Remaining erythrocytes were subsequently lysed (20 min, 37°C, 0.86% ammonium chloride) and cells were washed with PBS. PBMCs alone or mixed in the indicated ratios with MDA-MB-453 cells were transduced with EpCAM-AAV (GOI=1,500). After 48 h, cells were analyzed by flow cytometry for EpCAM and GFP expression (as a measure of transduction). Representative cytometry data are shown.



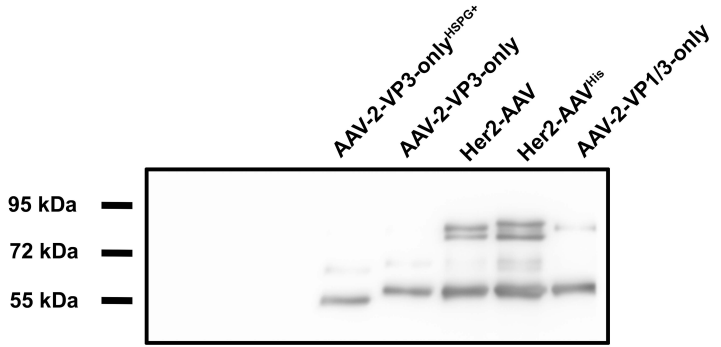
Supplementary Figure 14: Genomic titers of receptor-targeted AAVs. Vector preparations were quantified through isolation of vector genomes present in the indicated iodixanol gradient-purified vector stocks as described previously[44] using transgene (GFP) specific primers (Supplementary Table 1); N=3; mean ± SD; statistics by two-tailed t-test; $p < 0.05$ is considered to be statistically significant; n.s.=not significant.

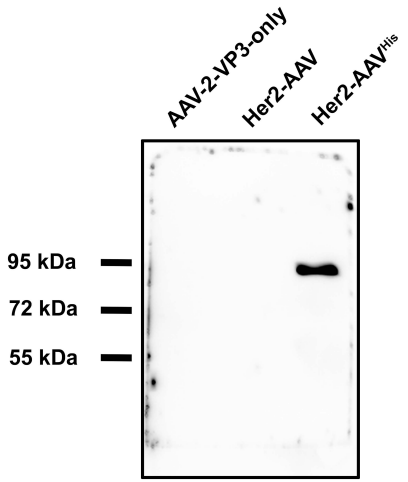


Supplementary Figure 15: Intracellular distribution of DARPin-AAV. 1x10^6 SK-OV-3 cells were transduced with Her2-AAV or AAV-2 at a dose of 30,000 vector genomes per cell transferring the GFP gene. Cells were kept on ice for 45 minutes prior to incubation at 37°C for 8 h. After harvesting of cells by extensive trypsin treatment and subsequent washing in PBS, cell fractionation was performed (subcellular protein fractionation kit, Thermo Scientific, Schwerte Germany). Cytoplasmic, membrane and nuclear fractions were collected and used in total for determining vector copy numbers (VCN) per cell fraction. Specifically, DNA was isolated from the indicated fractions using DNeasy Blood and tissue kit (Qiagen, Hilden Germany) and was eluted in Tris/HCl. VCN were quantified by LightCycler 480 qPCR (Roche) using SYBR Green and transgene (GFP)-specific primers (eGFPfw, eGFPrev) (Supplementary Tab. 2). N=3 experiments; mean ± SD; statistics by two-tailed t-test; $p< 0.05$ is considered to be statistically significant; n.s.=not significant.



Supplementary Figure 16: Kinetics of gene expression. CHO-Her2-k6 (a) or A3.01 (b) cells were transduced with 60,000 vector genomes per cell of Her2-AAV (green), CD4-AAV (orange), or AAV-2 (blue) transferring the luciferase gene, respectively. At the indicated time points after transduction, cells were lysed and luciferase expression was analyzed using the Dual-Luciferase Reporter Assay System. N=3 experiments; mean±SD; statistics by repeated-measures ANOVA; $p < 0.05$ is considered to be statistically significant.





Supplementary Figure 17: Uncropped Western Blot from Supplementary Figure 1.

Supplementary Table 1: Raw data from Figure 2c

	mock group					Herceptin group				AAV group				
days post cell injection	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5	mouse 1	mouse 2	mouse 3	mouse 4	mouse 1	mouse 2	mouse 3	mouse 4	mouse 5
5	9.60E+05	1.40E+05	1.40E+04	2.64E+05	3.97E+05	3.26E+05	1.44E+05	3.65E+06	2.97E+05	3.17E+05	1.48E+05	3.76E+05	3.61E+06	3.18E+05
10	5.90E+06	6.40E+06	9.74E+06	1.97E+06	5.14E+04	1.17E+06	4.55E+06	5.96E+06	1.07E+07	1.53E+06	2.41E+06	3.10E+07	5.85E+06	5.74E+06
19	1.90E+07	6.12E+07	7.53E+06	3.47E+07	5.36E+07	7.36E+06	1.35E+07	2.71E+07	1.77E+07	9.27E+06	4.96E+05	5.67E+06	4.33E+07	1.58E+07
24	9.00E+08	6.14E+07	9.34E+07	3.49E+07	6.47E+08	4.66E+07	6.09E+06	7.76E+06	9.84E+06	9.84E+05	2.39E+04	8.19E+05	2.08E+04	1.98E+05
31	5.00E+09					5.10E+06	5.33E+07	2.00E+07	1.64E+06	1.43E+05	1.56E+06	1.19E+04	2.01E+05	2.57E+05
41						1.00E+09	9.53E+07	1.34E+09	7.83E+07	9.50E+05	5.37E+04	5.65E+05	2.27E+05	1.70E+06
51						7.88E+09	9.45E+08	1.14E+09	2.43E+08	4.55E+05	4.10E+04	3.41E+06	6.78E+06	5.38E+07
64									1.94E+10	2.11E+07	6.48E+05	8.94E+07	4.93E+08	
93										1.51E+09	1.21E+07	7.43E+08		

Supplementary Table 2: Primer sequences

Primer	Sequence
DARPin-for	5'- GGAAGGACCGGTATGGACCTGGGTAAGAAACTG-3'
DARPin-rev	5'-CCCGGCCCTGTACAGATTAAGCTTTTGCAGGATTTC-3'
DARPinEC1-rev	5'-GTCCGAGCACTTGTACAGATTAAGCTTCGCCGCTTT-3'
His-forw:	5'-GGAAGGACCGGTATGCATCACCATCACCATCACATCGAGGGAAGGGACCTGGGTAAGAAACTGCTG-3'
Myc-forw:	5'-GGAAGGACCGGTATGGAACAAAAACTTATTTCTGAAGAAGATCTGATCGAGGGAAGG-GACCTGGGTAAGAAACTGCTG-3'
GFP-for	5'-GCTACCCCGACCACATGAAG-3'
GFP-rev	5'-GTCCATGCCGAGAGTGATCC-3'
eGFPfw	5'-CACAACGTCTATATCATGGC-3'
eGFPrev	5'-TGTGATCGCGCTTCTC-3'
luc-for	5'-TTCGGCTGGCAGAAGCTATG-3'
luc-rev	5'-GCTCGCGCTCGTTGTAGATG-3'
HSV-TK-for	5'-GCAGCAAGAAGCCACGGAAG-3'
HSV-TK-rev	5'-CCAGCAGTTGCGTGGTGGTG-3'