A Chemical Switch for Controlling Viral Infectivity

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Electronic Supplementary Information

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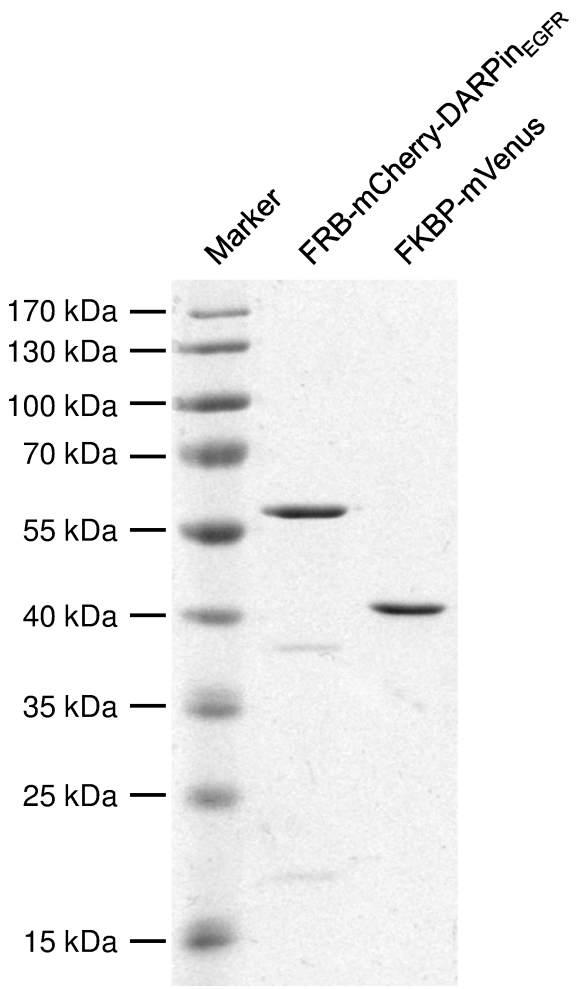

Fig. S1 SDS-PAGE of adapter protein FRB-mCherry-DARPin$_{EGFR}$ and adapter-binding protein FKBP-mVenus. The proteins were purified by immobilized metal ion affinity chromatography (IMAC) and separated on 12 % SDS-PAGE. The gel was subsequently stained by Coomassie. Calculated molecular weight: FRB-mCherry-DARPin$_{EGFR}$, 56.9 kDa; FKBP-mVenus, 39.7 kDa.

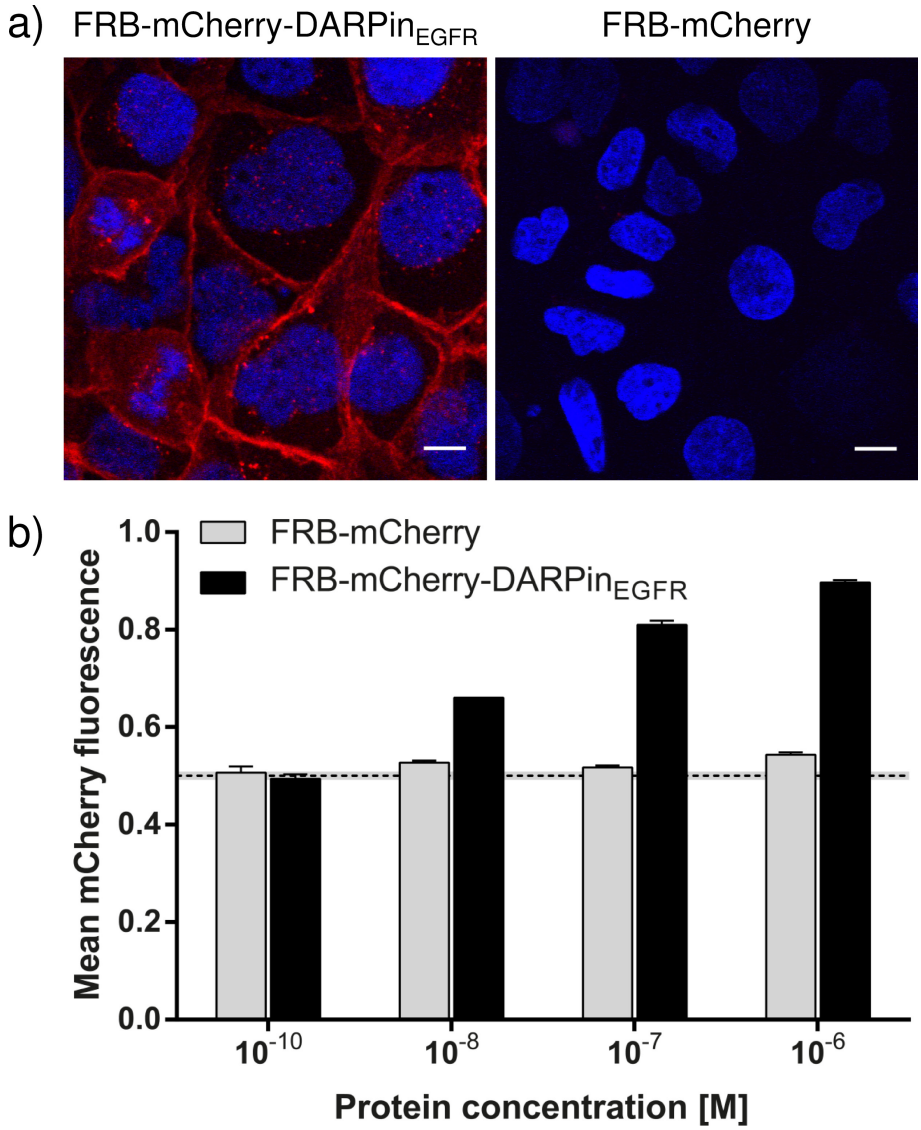

Fig. S2 Interaction of the adapter FRB-mCherry-DARPin$_{EGFR}$ with A-431 human epidermoid carcinoma cells. **(a)** Microscopical analysis of adapter binding. FRB-mCherry-DARPin$_{EGFR}$ (10^{-7} M) was added to $1.4x10^5$ A-431 cells in 0.5 ml cultivation medium. After 1 h, cells were fixed and analyzed for mCherry fluorescence (red) by confocal imaging. Cell nuclei were DAPI-stained (blue). As negative control, FRB-mCherry was used. Scalebar, 10 µm. **(b)** Quantitative analysis of FRB-mCherry-DARPin$_{EGFR}$-binding to A-431 cells. Increasing concentrations of FRB-mCherry-DARPin$_{EGFR}$ were added to $1.4x10^5$ A-431 cells in 0.5 ml cultivation medium for 1 h prior to quantifying cell-associated mCherry fluorescence by flow cytometry. A minimum of 40,000 cells per condition were analyzed. Data represent the mean (n = 3) ± 1 standard deviation. The dashed line represents background fluorescence of non-treated A-431 cells (grey area: 1 standard deviation).

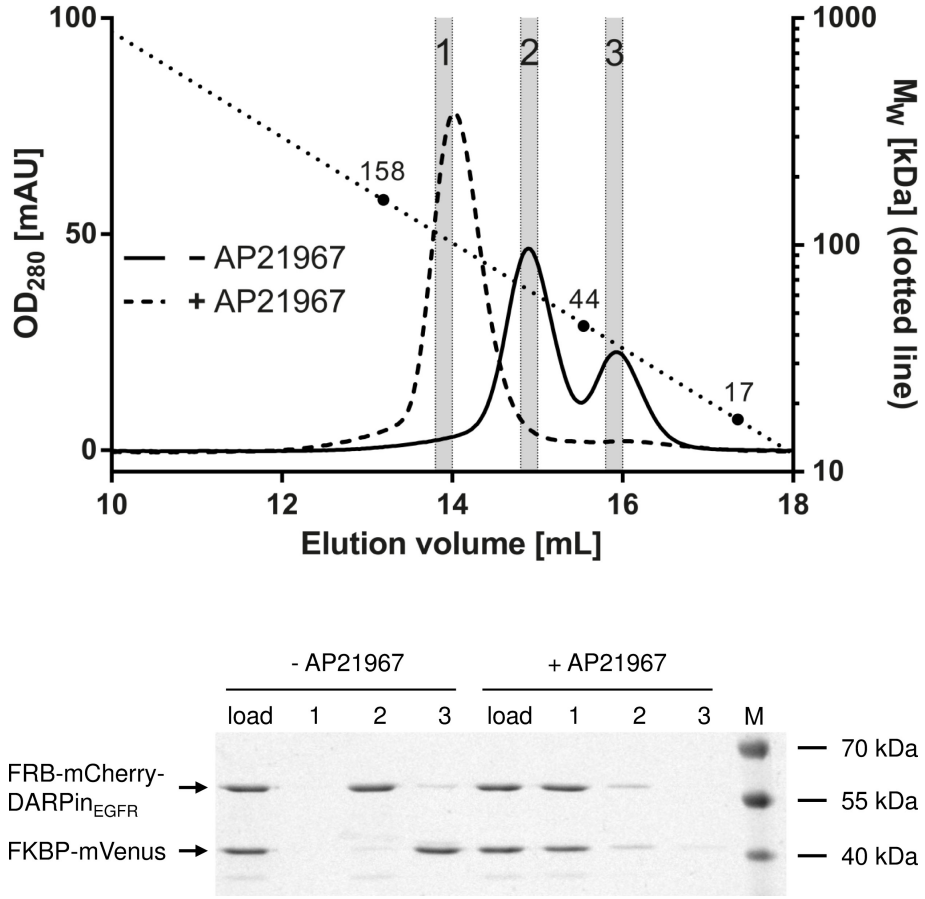

Fig. S3 Chemically switchable interaction of the adapter FRB-mCherry-DARPin$_{EGFR}$ with FKBP. 0.5 nmol FRB-mCherry-DARPin$_{EGFR}$ (56.9 kDa) were mixed with 0.5 nmol FKBP fused to the fluorescent protein mVenus (FKBP-mVenus, 39.7 kDa) and incubated for 1 h at RT in the absence or presence of 2.5 nmol AP21967 in a total volume of 100 µl PBS (pH 7.4). The reaction mix was resolved by gel permeation chromatography (upper panel) and the indicated fractions (1-3) were further analyzed by 12 % SDS-PAGE (lower panel) and subsequent Coomassie staining. M, molecular weight marker; mAU, milli absorbance units; OD_{280}, optical density at 280 nm; PBS, phosphate-buffered saline.

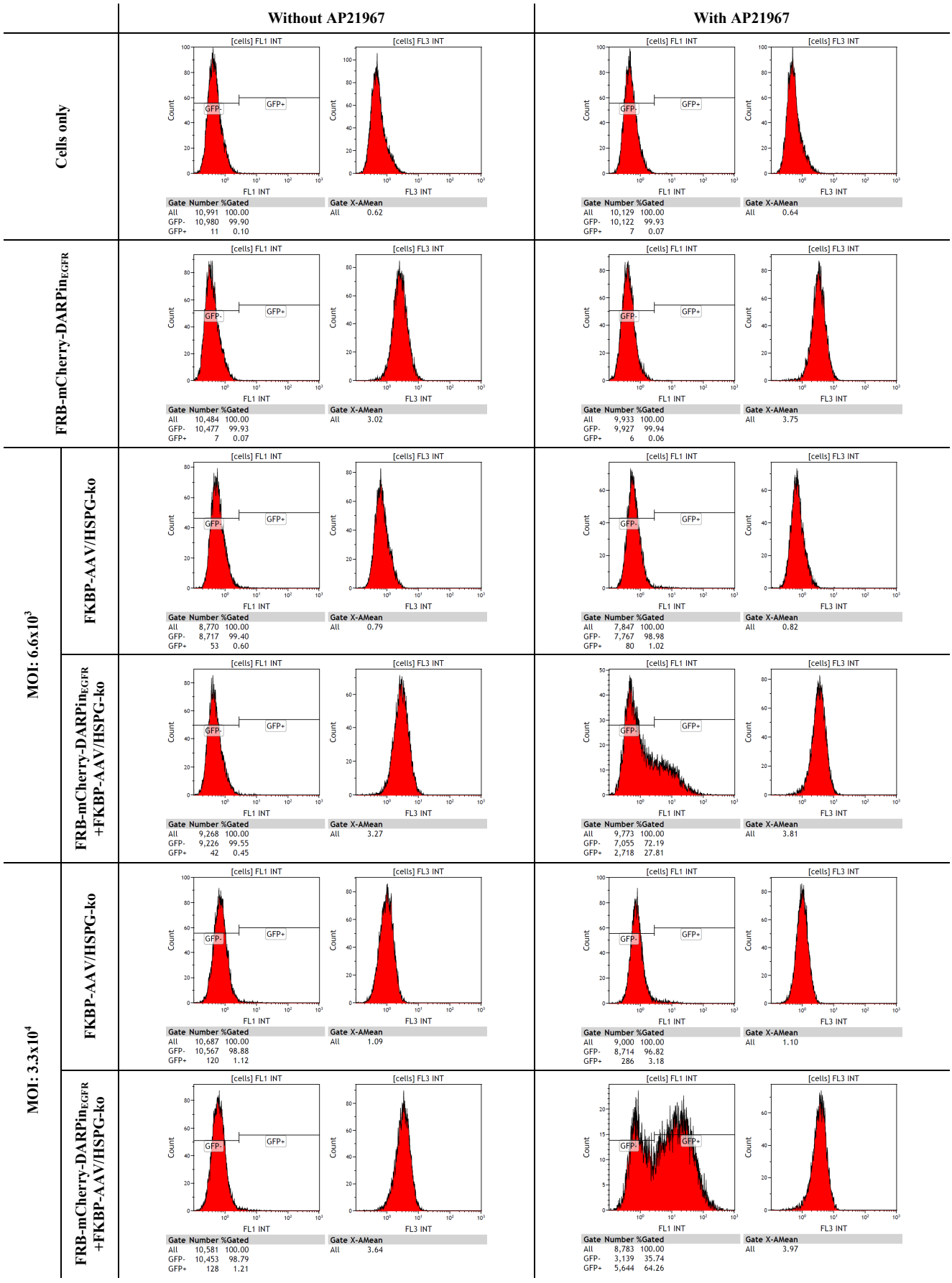

Fig. S4 Representative histograms of flow cytometry data shown in Fig. 2b depicting respective GFP (FL1) and mCherry (FL3) fluorescence. The gates for GFP-negative and -positive cells were defined to obtain 0.1 % GFP-positive cells within non-treated cells. MOI, genomic particles per cell.

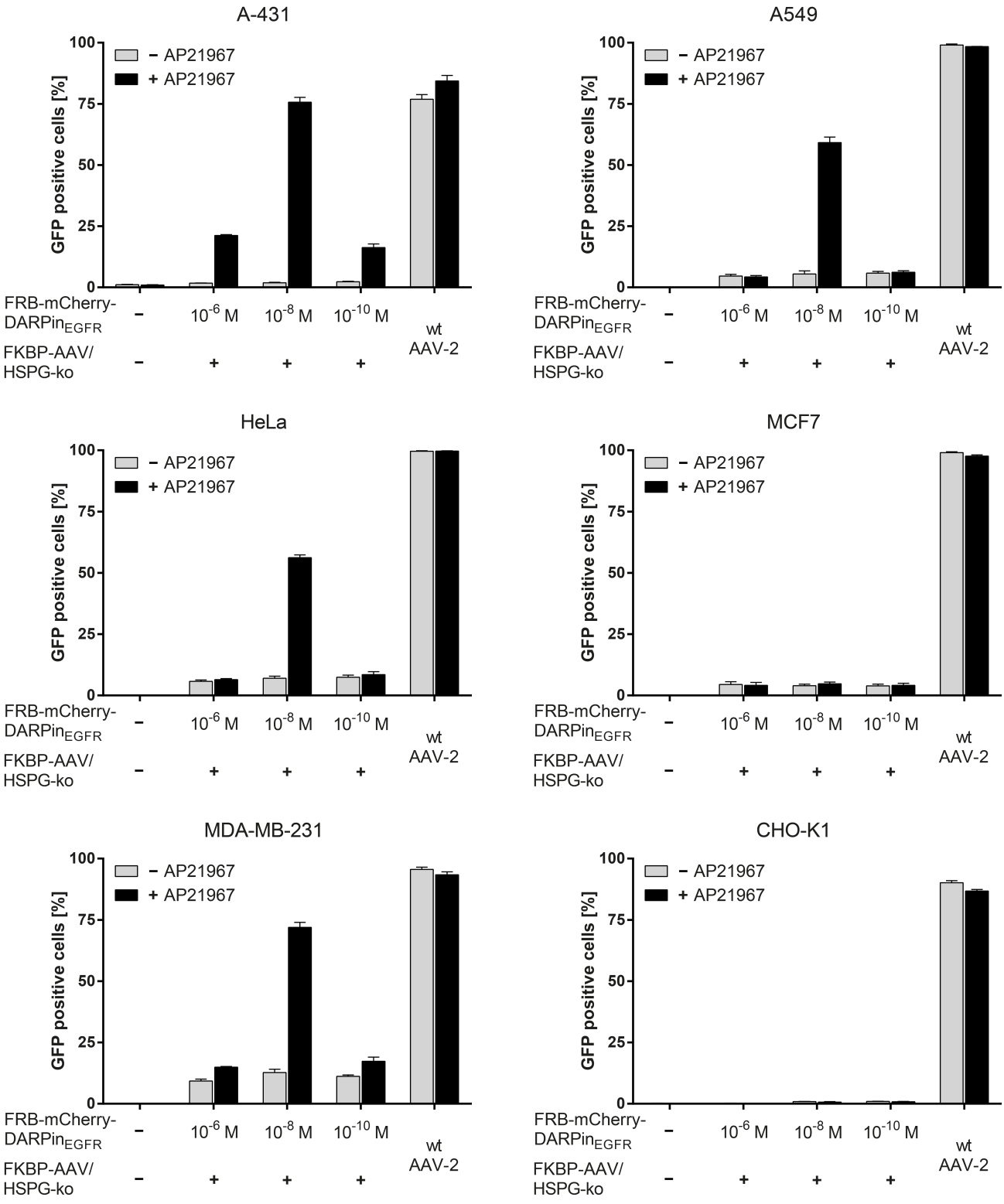

Fig. S5 Chemically switchable viral infection of mammalian cell lines with different EGFR expression levels (Fig. S6). $1.6x10^4$ cells cultivated in 100 µl medium were supplemented with different concentrations of FRB-mCherry-DARPin$_{EGFR}$ and $5.3x10^8$ FKBP-AAV/HSPG-ko particles (MOI: $3.3x10^4$). To show that the cell lines can in principle be infected by AAV-2, cells were treated with GFP-encoding AAV-2 vectors with wildtype capsids (wt AAV-2, MOI: $3.3x10^4$). The samples were cultivated for 48 h in the absence or presence of 1 µM AP21967 prior to flow cytometry analysis for quantifying GFP fluorescence. A minimum of 5000 cells were analyzed per condition. Data are means ± standard deviation (n = 3). MOI, genomic particles per cell.

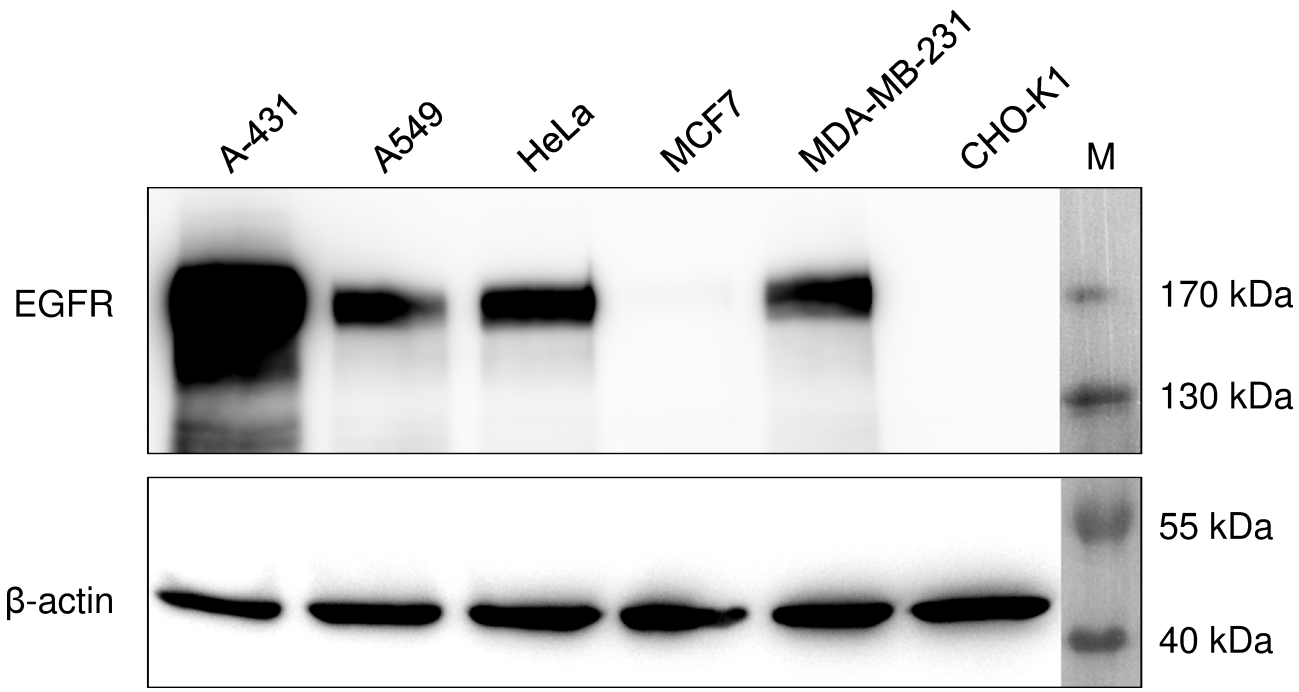

Fig. S6 Western Blot analysis of EGFR expression levels for cell lines utilized in Fig. S5. Per lane, 2 µg protein of whole cell lysate was loaded on a 9 % SDS-PAGE gel. As housekeeping protein, β-actin was detected as loading control. M, molecular weight marker.

Materials and methods

Construction of plasmids

The bacterial and mammalian expression vectors were generated either by classical restriction enzyme treatment and ligation[1] or by an isothermal, enzymatic DNA assembly reaction as described previously.[2] The *vp2*-coding sequence carrying the mutations R585A and R588A for the heparin sulfate proteoglycan (HSPG) knockout was PCR-amplified from pRCVP2koA.[3] The coding sequences for *fkbp*, *frb*[4] and $darpin_{EGFR}$[5] were generated by DNA synthesis. All necessary linkers, restriction sites and tags were included in PCR primers. The nucleic acid sequences of the expression vectors and the thereby encoded amino acid sequences are provided in Table S1 and S2, respectively.

Table S1 Nucleic acid sequences of plasmids constructed in this study. The individual functional domains are highlighted in color. The mutations R585A and R588A in VP2 are highlighted in black.

Construct	Nucleic acid sequence (5' → 3')
Mammalian expression vectors	
pMH156 (P_{CMV}-*fkbp*-*vp2*/R585A,R588A) Backbone: pEGFP-C3 (Clontech)	···GGTCGCCACCATGGGCGTTCAGGTTGAAACCATTAGCCCGG GAGATGGCCGTACATTTCCGAAACGTGGCCAGACATGTGTTG TTCACTATACCGGCATGCTGGAGGATGGTAAAAAATTTGATA GCAGCCGTGACCGTAATAAACCGTTCAAATTCATGCTGGGCA AACAGGAAGTCATTCGCGGTTGGGAAGAAGGTGTCGCACAG ATGAGTGTGGGTCAACGTGCCAAACTGACGATTAGTCCTGAC TATGCCTATGGAGCAACAGGTCATCCAGGGATTATTCCGCCT CACGCTACTCTGGTCTTTGATGTGGAACTGCTGAAACTGGAAT ACTCAGATCCTCCGGGAAAAAAGAGGCCGGTAGAGCACTCTC CTGTGGAGCCAGACTCCTCCTCGGGAACCGGAAAGGCGGGCC AGCAGCCTGCAAGAAAAAGATTGAATTTTGGTCAGACTGGAG ACGCAGACTCAGTACCTGACCCCCAGCCTCTCGGACAGCCAC CAGCAGCCCCCTCTGGTCTGGGAACTAATACGATGGCTACAG GCAGTGGCGCACCAATGGCAGACAATAACGAGGGCGCCGAC GGAGTGGGTAATTCCTCGGGAAATTGGCATTGCGATTCCACA TGGATGGGCGACAGAGTCATCACCACCAGCACCCGAACCTGG GCCCTGCCCACCTACAACAACCACCTCTACAAACAAATTTCC AGCCAATCAGGAGCCTCGAACGACAATCACTACTTTGGCTAC AGCACCCCTTGGGGGTATTTTGACTTCAACAGATTCCACTGCC ACTTTTCACCACGTGACTGGCAAAGACTCATCAACAACAACT GGGGATTCCGACCCAAGAGACTCAACTTCAAGCTCTTTAACA TTCAAGTCAAAGAGGTCACGCAGAATGACGGTACGACGACG ATTGCCAATAACCTTACCAGCACGGTTCAGGTGTTTACTGACT CGGAGTACCAGCTCCCGTACGTCCTCGGCTCGGCGCATCAAG GATGCCTCCCGCCGTTCCCAGCAGACGTCTTCATGGTGCCAC AGTATGGATACCTCACCCTGAACAACGGGAGTCAGGCAGTAG GACGCTCTTCATTTTACTGCCTGGAGTACTTTCCTTCTCAGAT GCTGCGTACCGGAAACAACTTTACCTTCAGCTACACTTTTGAG

	GACGTTCCTTTCCACAGCAGCTACGCTCACAGCCAGAGTCTG GACCGTCTCATGAATCCTCTCATCGACCAGTACCTGTATTACT TGAGCAGAACAAACACTCCAAGTGGAACCACCACGCAGTCA AGGCTTCAGTTTTCTCAGGCCGGAGCGAGTGACATTCGGGAC CAGTCTAGGAACTGGCTTCCTGGACCCTGTTACCGCCAGCAG CGAGTATCAAAGACATCTGCGGATAACAACAACAGTGAATAC TCGTGGACTGGAGCTACCAAGTACCACCTCAATGGCAGAGAC TCTCTGGTGAATCCGGGCCCGGCCATGGCAAGCCACAAGGAC GATGAAGAAAAGTTTTTTCCTCAGAGCGGGGTTCTCATCTTTG GGAAGCAAGGCTCAGAGAAAACAAATGTGGACATTGAAAAG GTCATGATTACAGACGAAGAGGAAATCAGGACAACCAATCCC GTGGCTACGGAGCAGTATGGTTCTGTATCTACCAACCTCCAG GCAGGCAACGCACAAGCAGCTACCGCAGATGTCAACACACA AGGCGTTCTTCCAGGCATGGTCTGGCAGGACAGAGATGTGTA CCTTCAGGGGCCCATCTGGGCAAAGATTCCACACACGGACGG ACATTTTCACCCCTCTCCCCTCATGGGTGGATTCGGACTTAAA CACCCTCCTCCACAGATTCTCATCAAGAACACCCCGGTACCT GCGAATCCTTCGACCACCTTCAGTGCGGCAAAGTTTGCTTCCT TCATCACACAGTACTCCACGGGACAGGTCAGCGTGGAGATCG AGTGGGAGCTGCAGAAGGAAAACAGCAAACGCTGGAATCCC GAAATTCAGTACACTTCCAACTACAACAAGTCTGTTAATGTG GACTTTACTGTGGACACTAATGGCGTGTATTCAGAGCCTCGCC CCATTGGCACCAGATACCTGACTCGTAATCTGTAAGATCTCG AGC···
Bacterial expression vectors	
pMH212 (P_{T7}-*frb*-*mcherry*-*darpin*$_{EGFR}$-H_6) Backbone: pRSET (Invitrogen)	···AGATATACATATGGCCTCTCGCATCCTGTGGCATGAAATGT GGCACGAAGGTCTGGAAGAAGCATCTCGCCTGTATTTTGGCG AGCGTAACGTGAAAGGCATGTTTGAAGTGCTGGAGCCTCTGC ACGCAATGATGGAGCGTGGACCTCAAACTCTGAAAGAGACAT CCTTCAATCAGGCTTATGGCCGTGATCTGATGGAGGCACAGG AATGGTGTCGTAAATATATGAAAAGCGGGAATGTGAAAGACC TGCTGCAAGCATGGGATCTGTATTATCATGTGTTCCGCCGTAT CAGCAAAGGCAGCGGTGGTGGCAGCGGTGGCACTAGTATGGT GAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGT TCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCC ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTAC GAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGG CCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCC GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAG CGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACC CAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTG AAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATG CAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGAT GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGA GGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTC AAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGG CGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAA

	CGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGG GCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGGCA GCGGTGGCGCTAGCGCCGGCGACCTGGGCAAGAAGCTGCTGG AAGCCGCCAGAGCCGGACAGGACGACGAAGTGCGGATCCTG ATGGCCAACGGCGCCGACGTGAACGCCGACGATACCTGGGG CTGGACCCCTCTGCACCTGGCCGCCTATCAGGGCCACCTGGA AATCGTGGAAGTGCTGCTGAAGAACGGGGCCGATGTGAATGC CTACGACTACATCGGCTGGACACCCCTGCATCTCGCTGCCGA CGGCCACCTCGAGATTGTCGAGGTCCTGCTGAAAAATGGCGC TGATGTGAACGCCAGCGATTATATCGGCGACACCCCCCTCCA TCTGGCCGCCCACAACGGCCATCTGGAAATTGTCGAAGTCCT CCTCAAGCACGGCGCAGATGTCAACGCCCAGGACAAGTTCGG CAAGACCGCCTTCGACATCAGCATCGACAACGGCAACGAGG ACCTGGCCGAGATCCTCCAGCATCATCACCATCACCATTAAG CGGCCGCGAGATCAAGCTTGATC···
pMH228 (P_{T7}-*frb*-*mcherry*-H_6) Backbone: pRSET (Invitrogen)	···AGATATACATATGGCCTCTCGCATCCTGTGGCATGAAATGT GGCACGAAGGTCTGGAAGAAGCATCTCGCCTGTATTTTGGCG AGCGTAACGTGAAAGGCATGTTTGAAGTGCTGGAGCCTCTGC ACGCAATGATGGAGCGTGGACCTCAAACTCTGAAAGAGACAT CCTTCAATCAGGCTTATGGCCGTGATCTGATGGAGGCACAGG AATGGTGTCGTAAATATATGAAAAGCGGGAATGTGAAAGACC TGCTGCAAGCATGGGATCTGTATTATCATGTGTTCCGCCGTAT CAGCAAAGGCAGCGGTGGTGGCAGCGGTGGCACTAGTATGGT GAGCAAGGGCGAGGAGGATAACATGGCCATCATCAAGGAGT TCATGCGCTTCAAGGTGCACATGGAGGGCTCCGTGAACGGCC ACGAGTTCGAGATCGAGGGCGAGGGCGAGGGCCGCCCCTAC GAGGGCACCCAGACCGCCAAGCTGAAGGTGACCAAGGGTGG CCCCCTGCCCTTCGCCTGGGACATCCTGTCCCCTCAGTTCATG TACGGCTCCAAGGCCTACGTGAAGCACCCCGCCGACATCCCC GACTACTTGAAGCTGTCCTTCCCCGAGGGCTTCAAGTGGGAG CGCGTGATGAACTTCGAGGACGGCGGCGTGGTGACCGTGACC CAGGACTCCTCCCTGCAGGACGGCGAGTTCATCTACAAGGTG AAGCTGCGCGGCACCAACTTCCCCTCCGACGGCCCCGTAATG CAGAAGAAGACCATGGGCTGGGAGGCCTCCTCCGAGCGGAT GTACCCCGAGGACGGCGCCCTGAAGGGCGAGATCAAGCAGA GGCTGAAGCTGAAGGACGGCGGCCACTACGACGCTGAGGTC AAGACCACCTACAAGGCCAAGAAGCCCGTGCAGCTGCCCGG CGCCTACAACGTCAACATCAAGTTGGACATCACCTCCCACAA CGAGGACTACACCATCGTGGAACAGTACGAACGCGCCGAGG GCCGCCACTCCACCGGCGGCATGGACGAGCTGTACAAGGGCA GCGGTGGCGCTAGCCATCATCACCATCACCATTAAGCGGCCG CGAGATCAAGCTTGATC···
pMH229 (P_{T7}-*fkbp*-*mvenus*-H_6) Backbone: pRSET (Invitrogen)	···AGATATACATATGGGCGTTCAGGTTGAAACCATTAGCCCGG GAGATGGCCGTACATTTCCGAAACGTGGCCAGACATGTGTTG TTCACTATACCGGCATGCTGGAGGATGGTAAAAAATTTGATA GCAGCCGTGACCGTAATAAACCGTTCAAATTCATGCTGGGCA AACAGGAAGTCATTCGCGGTTGGGAAGAAGGTGTCGCACAG ATGAGTGTGGGTCAACGTGCCAAACTGACGATTAGTCCTGAC TATGCCTATGGAGCAACAGGTCATCCAGGGATTATTCCGCCT CACGCTACTCTGGTCTTTGATGTGGAACTGCTGAAACTGGAA

	GAATTCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTG GTGCCCATCCTGGTCGAGCTGGACGGCGACGTAAACGGCCAC AAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTAC GGCAAGCTGACCCTGAAGCTGATCTGCACCACCGGCAAGCTG CCCGTGCCCTGGCCCACCCTCGTGACCACCCTGGGCTACGGC CTGCAGTGCTTCGCCCGCTACCCCGACCACATGAAGCAGCAC GACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAG CGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGC GCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATC GAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTG GGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTAT ATCACCGCCGACAAGCAGAAGAACGGCATCAAGGCCAACTT CAAGATCCGCCACAACATCGAGGACGGCGGCGTGCAGCTCGC CGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGT GCTGCTGCCCGACAACCACTACCTGAGCTACCAGTCCGCCCT GAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGCT GGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGA GCTGTACAAGCATCATCACCATCACCATTAAAAGCTTGATC···

Table S2 Amino acid sequences encoded by plasmids constructed in this study. The individual functional domains are highlighted in color. The mutations R585A and R588A in VP2 are highlighted in black.

Construct	Amino acid sequence (amino terminus → carboxy terminus)
Mammalian expression vectors	
pMH156 (P_{CMV}-*fkbp*-*vp2*/R585A,R588A) Backbone: pEGFP-C3 (Clontech)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDR NKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGAT GHPGIIPPHATLVFDVELLKLEYSDPPGKKRPVEHSPVEPDSSSGT GKAGQQPARKRLNFGQTGDADSVPDPQPLGQPPAAPSGLGTNT MATGSGAPMADNNEGADGVGNSSGNWHCDSTWMGDRVITTST RTWALPTYNNHLYKQISSQSGASNDNHYFGYSTPWGYFDFNRF HCHFSPRDWQRLINNNWGFRPKRLNFKLFNIQVKEVTQNDGTTT IANNLTSTVQVFTDSEYQLPYVLGSAHQGCLPPFPADVFMVPQY GYLTLNNGSQAVGRSSFYCLEYFPSQMLRTGNNFTFSYTFEDVP FHSSYAHSQSLDRLMNPLIDQYLYYLSRTNTPSGTTTQSRLQFSQ AGASDIRDQSRNWLPGPCYRQQRVSKTSADNNNSEYSWTGATK YHLNGRDSLVNPGPAMASHKDDEEKFFPQSGVLIFGKQGSEKTN VDIEKVMITDEEEIRTTNPVATEQYGSVSTNLQAGNAQAATADV NTQGVLPGMVWQDRDVYLQGPIWAKIPHTDGHFHPSPLMGGFG LKHPPPQILIKNTPVPANPSTTFSAAKFASFITQYSTGQVSVEIEW ELQKENSKRWNPEIQYTSNYNKSVNVDFTVDTNGVYSEPRPIGT RYLTRNL

Bacterial expression vectors	
pMH212 (P_{T7}-*frb*-*mcherry*-*darpin*$_{EGFR}$-H_6) Backbone: pRSET (Invitrogen)	MASRILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMM ERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQA WDLYYHVFRRISKGSGGGSGGTSMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDG GVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQ LPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKG SGGASAGDLGKKLLEAARAGQDDEVRILMANGADVNADDTWG WTPLHLAAYQGHLEIVEVLLKNGADVNAYDYIGWTPLHLAAD GHLEIVEVLLKNGADVNASDYIGDTPLHLAAHNGHLEIVEVLLK HGADVNAQDKFGKTAFDISIDNGNEDLAEILQHHHHHH
pMH228 (P_{T7}-*frb*-*mcherry*-H_6) Backbone: pRSET (Invitrogen)	MASRILWHEMWHEGLEEASRLYFGERNVKGMFEVLEPLHAMM ERGPQTLKETSFNQAYGRDLMEAQEWCRKYMKSGNVKDLLQA WDLYYHVFRRISKGSGGGSGGTSMVSKGEEDNMAIIKEFMRFK VHMEGSVNGHEFEIEGEGEGRPYEGTQTAKLKVTKGGPLPFAW DILSPQFMYGSKAYVKHPADIPDYLKLSFPEGFKWERVMNFEDG GVVTVTQDSSLQDGEFIYKVKLRGTNFPSDGPVMQKKTMGWEA SSERMYPEDGALKGEIKQRLKLKDGGHYDAEVKTTYKAKKPVQ LPGAYNVNIKLDITSHNEDYTIVEQYERAEGRHSTGGMDELYKG SGGASHHHHHH
pMH229 (P_{T7}-*fkbp*-*mvenus*-H_6) Backbone: pRSET (Invitrogen)	MGVQVETISPGDGRTFPKRGQTCVVHYTGMLEDGKKFDSSRDR NKPFKFMLGKQEVIRGWEEGVAQMSVGQRAKLTISPDYAYGAT GHPGIIPPHATLVFDVELLKLEEFMVSKGEELFTGVVPILVELDG DVNGHKFSVSGEGEGDATYGKLTLKLICTTGKLPVPWPTLVTTL GYGLQCFARYPDHMKQHDFFKSAMPEGYVQERTIFFKDDGNYK TRAEVKFEGDTLVNRIELKGIDFKEDGNILGHKLEYNYNSHNVYI TADKQKNGIKANFKIRHNIEDGGVQLADHYQQNTPIGDGPVLLP DNHYLSYQSALSKDPNEKRDHMVLLEFVTAAGITLGMDELYKH HHHHH

Protein production and purification

For production of FRB-mCherry-DARPin$_{EGFR}$, plasmid pMH212 was transferred into *E. coli* BL21 Star^{TM} (DE3)pLysS (Invitrogen, Carlsbad, CA, cat. no. C602003) and selected with 100 µg ml^{-1} ampicillin and 36 µg ml^{-1} chloramphenicol. Plasmids encoding FRB-mCherry (pMH228) and FKBP-mVenus (pMH229) were transferred into *E. coli* BL21 STAR^{TM} (DE3) (Invitrogen, cat. no. C601003) and selected with 100 µg ml^{-1} ampicillin. Cells were grown in LB medium supplemented with the indicated antibiotics at 37 °C until an OD_{600} of 0.8 was reached. At this point, protein production was induced by addition of 1 mM IPTG and the cells were incubated for another 3 h at 37 °C. The cells were then harvested by centrifugation at 4 °C and 8000 *g* for 10 min, resuspended

in Ni-lysis buffer (50 mM NaH_2PO_4, 300 mM NaCl, 10 mM imidazole, pH 8.0), shock-frozen in liquid nitrogen and stored at -80 °C. Afterwards, the cells were disrupted using a French Press (APV 2000, APV Manufacturing, Bydgoszcz, Poland) at 1000 bar and centrifuged at 30000 *g* for 30 min to remove cell debris. The supernatant was loaded onto a column containing Ni-NTA agarose (Qiagen, Hilden, Germany, cat. no. 30210), washed with 20 column volumes of Ni-wash buffer (50 mM NaH_2PO_4, 300 mM NaCl, 20 mM imidazole, pH 8.0) and eluted in 3 column volumes of Ni-elution buffer (50 mM NaH_2PO_4, 300 mM NaCl, 250 mM imidazole, pH 8.0).

Size exclusion chromatography

To verify the AP21967-dependent interaction between the proteins FRB-mCherry-$DARPin_{EGFR}$ and FKBP-mVenus, 0.5 nmol of each protein were mixed and incubated for 1 h at RT in the absence or presence of 2.5 nmol AP21967 (Clontech, Mountain View, CA, cat. no. 635057) in a total volume of 100 µl PBS (2.68 mM KCl, 1.47 mM KH_2PO_4, 8.03 mM Na_2HPO_4, 137 mM NaCl, pH 7.4). Afterwards, the protein mixture was separated by size exclusion chromatography using a Superdex 200 10/300 GL (GE Healthcare, Freiburg, Germany, cat. no. 17-5175-01) column with PBS at 0.5 ml/min on an ÄKTA Explorer (GE Healthcare) fast protein liquid chromatography system. Fractions of interest were analyzed on a 12 % (w/v) sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and proteins were visualized by Coomassie staining. As protein size standard, a gel filtration standard (Bio-Rad, Hercules, CA, cat. no. 151-1901) or a PageRuler prestained protein ladder (Thermo Scientific, Rockford, IL, cat. no. 26616) was used for size exclusion chromatography or SDS-PAGE, respectively.

Cell culture

A-431 (human epidermoid carcinoma), A549 (human lung carcinoma), HEK-293T (human embryonic kidney), HeLa (human cervix adenocarcinoma), MCF7 (human breast adenocarcinoma) and MDA-MB-231 (human breast adenocarcinoma) cell lines were maintained in Dulbecco's Modified Eagle Medium (DMEM, PAN Biotech, Aidenbach, Germany, cat. no. P03-0710) supplemented with 10 % (v/v) fetal calf serum (FCS, PAN Biotech, cat. no. P30-3602, batch no. P101003TC) and 1 % (v/v) penicillin/streptomycin solution (PAN Biotech, cat. no. P06-07100) (DMEM complete) at 37 °C in a humidified atmosphere containing 5 % CO_2. Chinese hamster ovary cells (CHO-K1) were cultivated at the same conditions in HTS medium (Cell Culture Technologies, Gravesano, Switzerland, cat. no. CHTS) supplemented with 10% fetal calf serum,

2 mM L-glutamine (Sigma-Aldrich, St. Louis, MO, cat. no. 49419) and 1 % (v/v) penicillin/streptomycin solution (HTS complete).

Confocal microscopy of adapter binding

A-431 cells were seeded on coverslips in a 24-well tissue culture plate at a density of $7x10^4$ cells per well 24 h prior to addition of adapter proteins. Adapter FRB-mCherry-DARPin$_{EGFR}$ and negative control FRB-mCherry were added in DMEM complete to the wells at final concentrations of 10^{-7} M and incubated for 1 h at 37 °C in a humidified atmosphere containing 5 % CO_2. The samples were fixed in 4 % paraformaldehyde solution, and the nuclei of A-431 cells were DAPI stained. Images were acquired using a 40x Plan-Neofluar oil objective (NA 1.3) on a Zeiss (Jena, Germany) LSM 510 META confocal scanning system attached to an Axiovert 200 inverted microscope. Fluorescence of mCherry was detected using a 543 nm laser and a BP 565 - 615 nm emission filter. DAPI was visualized using a 2-photon laser at 750 nm and a BP 435 - 485 nm emission filter.

Sample preparation for flow cytometry analysis of adapter binding

To evaluate the binding of the adapter protein to the cellular surface, A-431 cells were seeded in a 24-well tissue culture plate at a density of $7x10^4$ cells per well. After 24 h, the adapter FRB-mCherry-DARPin$_{EGFR}$ or the negative control FRB-mCherry were added to the cells at final concentrations of 10^{-10} – 10^{-6} M in DMEM complete and incubated for 1 h at 37 °C in a humidified atmosphere containing 5 % CO_2. Subsequently, cells were washed with PBS, trypsinized, resuspended in PBS supplemented with 1 % FCS and analyzed by flow cytometry.

AAV vector particle production

Viral vector production was performed using the adenovirus helper-free packaging system.[6] To produce FKBP-modified adeno-associated viral (AAV) vectors of serotype 2 carrying the R585A and R588A mutations in the viral capsid genes (FKBP-AAV/HSPG-ko), HEK-293T cells were transfected with the plasmids pHelper (Cell Biolabs, San Diego, CA, cat. no. VPK-402), the self-complementary vector plasmid pCMVgfp, pRCVP2koA2[3] and pMH156 in an equimolar ratio. In order to produce control AAV-2 vectors with unmodified capsids (wt AAV-2) the plasmids pHelper, pCMVgfp and pAAV-RC2 (Cell Biolabs, cat. no. VPK-402) were transfected into HEK-293T cells in an equimolar ratio. Per 15 cm cell culture dish, 60 µg total plasmid DNA were mixed

with 200 µg PEI in 3 ml OptiMEM (Life Technologies, Carlsbad, CA, cat. no. 22600-134) and incubated for 15 min at RT prior to addition to the cells. After 5 hours medium was exchanged and the viral vectors were harvested 72 h post-transfection. To this aim, cells were detached from the cell culture plates, sedimented by centrifugation for 15 min at 400 *g* and resuspended in PBS (500 µl PBS per 15 cm dish). Cell pellets were lysed by five freeze / thaw cycles (liquid nitrogen / 37 °C), supplemented with Benzonase (50 U/ml, Merck Millipore, Darmstadt, Germany, cat. no. 70664-3) and incubated for 1 h at 37 °C, before cell debris was eliminated by centrifugation for 15 min at 4000 *g*. The supernatant was subjected to discontinuous iodixanol density gradient centrifugation as described elsewhere.[7] Centrifugation was performed at 4 °C for 2 h at 171000 *g* using the Optima L-90 K ultracentrifuge equipped with a Type 70.1 Ti fixed-angle rotor (Beckman Coulter, Krefeld, Germany).

Viral vector titration

Genomic titers of viral vectors were determined by quantitative real-time PCR (qPCR) as described previously.[8] The SensiMix™ II Probe Kit (Bioline, Luckenwalde, Germany, cat. no. BIO-91002) and the following primer and probe sequences that specifically bind to the *gfp* sequence of the vector plasmid pCMVgfp were used for amplification: 5‘-GAGCGCACCATCTTCTTCAAG-3‘ (GFP_fw), 5‘-TGTCGCCCTCGAACTTCAC-3‘ (GFP_rev), 5’-FAM-ACGACGGCAACTACA-BHQ1-3’ (GFP_probe). Genomic particles were calculated using the *gfp*-containing vector plasmid as a standard.

Western Blot analysis of viral vectors

Iodixanol-purified viral vector particles were mixed with SDS loading buffer and boiled at 95 °C for 5 min. After separation on an 8 % (w/v) SDS-PAGE gel, proteins were transferred onto a PVDF membrane. The membrane was blocked with blocking buffer (PBS containing 3 % (w/v) milk powder) overnight at 4 °C and then incubated for 2 h with a hybridoma cell supernatant containing AAV-2 specific B1 antibody[9] diluted 1:10 in blocking buffer. After washing the membrane three times with PBS-T (PBS containing 0.05 % (v/v) Tween 20), the membrane was incubated for 1 h at RT with a secondary anti-mouse HRP-conjugated antibody (GE Healthcare, cat. no. NA931) diluted 1:2000 in blocking buffer and washed again with PBS-T. Detection was performed by addition of the chemiluminescence substrate ECL Prime Western Blotting Detection Reagent (GE Healthcare, cat. no. RPN2232) on an ImageQuant LAS 4000mini system (GE Healthcare).

Western Blot analysis of EGFR expression level

To compare the EGFR expression level of different cell lines, cells were seeded in a 6-well tissue culture plate at a density of $8x10^5$ cells per well. After 24 h, the cells were washed with PBS and 250 µl lysis buffer (20 mM Tris/-HCl, 100 mM NaCl, 1 mM EDTA, 1 % (v/v) Triton X-100, 0.2 % (w/v) SDS, pH 7.5, supplemented with protease inhibitor (Roche, Mannheim, Germany, cat. no. 04693132001)) was added to each well. After incubation for 30 min on ice, lysates were sonified and centrifuged at 15000 *g* and 4 °C for 5 min. The protein concentration of the supernatant was determined using a biuret test based assay (Roti-Quant, Carl Roth, Karlsruhe, Germany, cat. no. 0120.1). The lysates were mixed with SDS loading buffer, boiled at 95 °C for 5 min and afterwards 2 µg of total protein were loaded per lane on a 9 % (w/v) SDS-PAGE gel. After separation and transfer of the proteins onto a PVDF membrane, the membrane was blocked with blocking buffer (TBS (50 mM Tris/-HCl, 150 mM NaCl, pH 7.6) containing 0.1 % (v/v) Tween 20 (TBS-T) with 5 % (w/v) BSA (Sigma-Aldrich, cat. no. 05479)) for 1 h at RT and divided at the colored 70 kDa band of the protein ladder (Thermo Scientific, cat. no. 26616) into two pieces. The membranes were incubated overnight at 4 °C with an anti-EGFR antibody (Cell Signaling, Danvers, MA, cat. no. 4267) diluted 1:2500 in blocking buffer (upper part) or an anti-β-actin antibody (Santa Cruz Biotechnology, Dallas, TX, cat no. sc-47778) diluted 1:1000 in blocking buffer (lower part). After washing the membranes three times with TBS-T, the membranes were incubated for 1 h at RT with a secondary anti-rabbit HRP-conjugated antibody (Santa Cruz Biotechnology, cat. no. sc-2004; upper part) or a secondary anti-mouse HRP-conjugated antibody (GE Healthcare, cat. no. NA931, lower part) both diluted 1:5000 in blocking buffer and washed again with TBS-T. Detection was performed as described above.

Microscopy analysis of chemically controlled infection

For the analysis of the chemically controlled AAV-2 infection by confocal microscopy, A-431 cells were seeded on coverslips in a 24-well tissue culture plate at a density of $4.8x10^4$ cells per well. After 24 h, the medium was removed and FRB-mCherry-DARPin$_{EGFR}$ (final concentration: 10^{-7} M) and $3.2x10^9$ FKBP-AAV/HSPG-ko viral vector particles (MOI: $3.3x10^4$ gc/cell) were added in DMEM complete (final volume: 600 µl) with or without 1 µM AP21967 to each well. After further 48 h of incubation, the samples were fixed in 4 % paraformaldehyde solution and the nuclei of A-431 cells were stained with DAPI. Images were acquired on a Leica SP8 laser scanning confocal microscope using a 63x HC PL APO CS2 oil objective (NA 1.4). DAPI was excited by a UV laser at 405 nm and detected between 420 and 480 nm. GFP and mCherry were excited with a tunable

white light laser at either 488 or 587 nm and detected with HyD detectors between 500 and 550 nm or 595 and 650 nm, respectively.

Sample preparation for flow cytometry analysis of chemically controlled infection

For the analysis of the chemically controlled AAV-2 infection by flow cytometry, cells were seeded in a 96-well tissue culture plate at a density of $0.8x10^4$ cells per well. After 24 h, the medium was removed and indicated amounts of FRB-mCherry-DARPin$_{EGFR}$ and of FKBP-AAV/HSPG-ko viral vector particles were added to the cells in DMEM complete (final volume: 100 µl; for CHO-K1 cells HTS complete was used instead) with or without 1 µM AP21967. After further 48 h incubation, cells were trypsinized, resuspended in PBS supplemented with 1 % FCS, and the percentage of GFP-positive cells and the mean mCherry intensity were quantified by flow cytometry. All controls (without FRB-mCherry-DARPin$_{EGFR}$ and FKBP-AAV/HSPG-ko, only FRB-mCherry-DARPin$_{EGFR}$ or only FKBP-AAV/HSPG-ko) with or without 1 µM AP21967 were treated as described above.

Flow cytometry analysis of adapter binding and chemically controlled infection

A-431 cells were treated and prepared for flow cytometry analysis as described above. Data acquisition was performed on a Gallios™ flow cytometer (Beckman Coulter). For GFP and mCherry detection, samples were excited using a 488 nm laser and emission was detected with 525/40 nm or 620/30 nm bandpass filters, respectively. Flow cytometry parameters were set as follows: Forward side scatter (FSC) was used as discriminator. Two-dimensional dot plots (FSC/SSC) were used to define the population of living cells, whereas one-dimensional histograms were used to visualize fluorescence of the samples. Flow cytometry data were analyzed using Kaluza® analysis software v1.2 (Beckman Coulter). The GFP and mCherry channel were compensated for correction of fluorescence spillover after data acquisition using the "Positives with Autofluorescence Subtraction" algorithm implemented in the Kaluza® analysis software.

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