Supplemental Information

Identification of broadly applicable AAV vectors by systematic comparison of commonly used capsid variants in vitro

Jonas Weinmann, Julia Söllner, Gudrun Zimmermann, Kai Zuckschwerdt, Sarah Abele, Christine Mayer, Jenny Danner-Liskus, Alexander Peltzer, Michael Schuler, Thorsten Lamla, Benjamin Strobel

Supplementary Figure 1

Suppl. Fig. 1: Exemplary output of web app. The web app allows a detailed insight into all data obtained with the AAV panel on several cell types across organs and tissues. Upon selection of a cell type of interest (here: MioM1), experimental parameters are displayed in the upper panel, while a representative GFP fluorescence micrograph is shown in the middle panel. The lower panel provides quantitative results, i.e., the percentage of GFP-positive cells, mean GFP intensity and the composite "Activity score". The data can either be sorted by (**a**) the location of the respective AAV variant on the AAV panel plate or (**b**) the experimental values in an ascending or descending fashion, enabling the rapid identification of the most or least efficient capsids. Mouse-over features have been implemented to display the actual value of each bar. The interactive bar plots also allow exporting the data.

Supplementary Methods

1) Sequence of AAV expression cassette: pFB-scAAV2-CMV-eGFP-SV40

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2) Cell culture methods

Tissue	Name/ Abbreviation as in Fig. 5	Species/Description	Seeding density per 96-well	Growth time before transd. [d]	vg/well	Incubation time before analysis [d]	Source/Cat.no/Donor/Protocol	Ref
Adipose Tissue	3T3-L1	Murine fibroblast-like cell line	7.5E+03	1	2.0E+09	2	3T3-L1 cells were obtained from ATCC (#CL-173) and cultured in DMEM + 10% FBS + 1% P/S, using standard culture techniques.	
	3T3-L1 Diff	Murine 3T3-L1, differentiated into adipocyte-like phenotype	7.5E+03	1	2.0E+09	2	For differentiation, 3T3-L1 cells were cultured in the regular culture medium, supplemented with 0.5 mM IBMX + 1 µM Dexamethasone + 1 µg/mL Insulin for 2 days, followed by culturing in culture medium supplemented with Insulin only for another 4 days. Cells were then transferred back to regular culture medium and seeded for transduction.	
	iBA	Murine immortalized brown adipocyte cell line	1.0E+04	2	2.0E+09	3	IBAs were a gift from Christian Wolfrum at ETH Zürich and cultured in DMEM + 10% FBS + 1% P/S, using standard techniques, with medium exchange every other day.	1
	huDFAT	Human primary de-differentiated fat cells	1.0E+04	2	2.0E+09	3	Primary DFAT cells were isolated from subcutaneous adipose tissue samples obtained from Hepacult (#A458544). Briefly, the tissue was dissociated by incubation with 1 mg Collagenase-II solution per mg tissue for 5-10 min at 37°C under gentle agitation followed by filtration through gauze and washing of the cells with medium (see below) to stop the Collagenase reaction. The solution then sat for 1-2 h. Floating cells were then seeded into a culture flask filled with DMEM/F12 + 15% FBS + 1% Anti-Anti (#BE12-719F, Lonza; #15240062, Thermo Fisher) and incubated upside down for one week to facilitate attachment of the floating cells to the bottom of the flask. For differentiation, cells were cultured in Omental Adipocyte Differentiation Medium (#OM-DM-500, Zenbio) for one week, followed by Omental Adipocyte Maintenance Medium (#OM-AM-500).	2
	huDFAT Diff	Human primary DFAT cells, differentiated into mature adipocytes	1.5E+04	16	2.0E+09	3		
	huDFAT Spheroid	Human primary DFAT cells, differentiated into mature adipocytes, grown as spheroids	2.5E+03	12	2.5E+08	3	For spheroid formation, DFAT cells were prepared as described above and seeded at 2500 cells/384-well into spheroid microplates (#3830, Corning). Differentiation was then initiated by changing the medium to Omental Adipocyte Differentiation Medium (#OM-DM-500, Zenbio) for one week, followed by Omental Adipocyte Maintenance Medium (#OM-AM-500).	
	huFPad	Human primary fat pad emigrated cells (FPEC)	1.0E+04	2	2.0E+09	3	Subcutaneous adipose tissue samples obtained from Hepacult (#A458544) was dissected into approx. 5 mm pieces, which were washed in PBS and then cultured in DMEM/F12 + 15% FBS + 1% Anti-Anti for 3 weeks (2 x medium exchange per week). After removal of the tissue pieces, the remaining emigrated cells (FPEC) were cultured further. For differentiated FPEC cells, tissue pieces were cultured in browning medium, instead: DMEM (high glucose) + GlutaMax + Pyruvate + 10% FCS, 1% P/S, 20 mM HEPES, 44,45 µg/mL Vitamin C, 1 µM Insulin, 1 µM Dexamethasone, 500 µM IBMX, 50 µM Indomethacin, 1 µM Rosiglitazone, 1 µM CL316243, 250 nM triiodothyronine, 25 ng/mL hVEGF.	
	huFPad Diff	Human primary FPEC, differentiated into brown adipocytes	1.5E+04	16	2.0E+09	3		3
	huSVF Diff	Human primary pre-adipocytes from the stromal vascular fraction, differentiated into adipocytes (white phenotype)	1.0E+04	15	2.0E+09	3	Preadipocytes were isolated by following the initial steps described for DFAT cells. Following isolation of the floating cells (DFAT), the remaining buffer was removed, filtered through a 100 µm cell strainer and centrifuged (400 g, 10 min). The supernatant was removed and the SVF pellet was resuspended in 10 mL erythrocyte-lysis buffer for 10 min at RT. After centrifuging, the pre-adipocyte containing pellet was resuspended in cultivation medium. Cells were differentiated by cultivation in Adipocyte Differentiation Medium (see DFAT protocol).	

Brain	iPSC-Neuron	Human iPSC-derived neurons	8.0E+04	1	2.0E+09	2	iPSC line AD0202 (re-programmed from adult fibroblasts) was obtained from the StemBancc Consortium and cultured using mTeSR Plus cGMP Pluripotent Stem Cell Maintenance Medium (#100-0276, Stemcell Technologies) on Matrigel. The cells were then differentiated into midbrain neurons or NPCs, following a previously described protocol (see reference).	4
	iPSC-NPC iPSC-NPC 2	Human iPSC-derived neural progenitor cells	2.0E+04 4.0E+04	1 3	2.0E+09 2.0E+09	3 3		
	mNeuron	Murine primary cortical neurons	8.0E+04	7	2.0E+09	1.2	Primary cortical neurons were prepared using standard procedures. Briefly, embryonic cortices were mechanically and chemically dissociated by applying the procedure and buffers previously described (see reference). Following cell separation using a 70 µm cell strainer, neurons were seeded in MEM supplemented with FCS, GlutaMax and P/S. Three hours after seeding, medium was changed to Neurobasal medium (#21103, Gibco) supplemented with SM1 (#05711, Stemcell Technologies), L-Glutamine (#25030, Gibco) and P/S. Medium was changed every 4 days.	5
	ratNeuron	Rat primary cortical neurons	8.0E+04	7	2.0E+09	1.2		
Endo-thel-ium	HUVEC	Human primary umbilical vein endothelial cells	1.0E+04	1	2.0E+09	2	HUVEC were obtained from Thermo Fisher (#C0035C) and cultured in Endothelial Cell Growth Medium (#CC-3162, Lonza) + 10% FBS, using standard culture techniques.	
Eye	MIO-M1	Human Müller glia-like cell line	1.0E+04	1	2.0E+09	2	MIO-M1 cells were obtained from the UCL Institute of Ophthalmology and cultured in DMEM high glucose with GlutaMax (#61965-026, Thermo Fisher) + 10% FBS, using standard culture techniques.	6
	iPSC-RPE	Human iPSC-derived retinal pigment epithelial cells	1.0E+05	73	2.0E+09	2	Cellartis Human iPS Cell Line 22 (ChiPSC22) was obtained from Takara Bio (#Y00325) and cultured using the Cellartis DEF-CS 500 Culture System (#Y30010) according to the manufacturer's instructions. Following the transfer of single cells to colonies in mTeSR Plus cGMP Pluripotent Stem Cell Maintenance Medium (#100-0276, Stemcell Technologies), differentiation to neuroepithelial cysts and ultimately RPE cells was induced through a series of different media, following an adapted version of a previously published protocol (see reference).	7
	hufRPE	Human primary fetal retina pigment epithelial cells	1.0E+05	7	2.0E+09	2	Primary fetal RPE cells were obtained from Lonza (#CC-5034) and cultured in RtEGM Medium (#00195409, Lonza), using standard techniques.	
GI Tract	STC1	Murine intestinal entero-endocrine-like cell line	1.0E+04	1	2.0E+09	2	STC-1 cells were obtained from ATCC (#CRL-3254) and cultured in DMEM + 10% FBS, using standard culture techniques.	
	huSI-Fibrobl.	Human primary small intestinal fibroblasts (HSIF)	8.0E+03	1	2.0E+09	2	Small intestinal fibroblasts were isolated from a Crohn's disease patient surgical explant, obtained from Conversant Bio. Crypts were isolated, following a published protocol (see reference). The remaining (crypt-free) tissue was then cultured for one week to continuously harvest lamina propria mononuclear cells from the supernatant. The remaining lamina propria was then cultured in DMEM + 10% FBS, L-glutamine, P/S to allow for fibroblast emigration over 9-14 days. Following removal of the tissue piece, adherently growing fibroblasts were expanded in ScienCell Fibroblast Media #2301 and seeded for transduction.	8
Immune cells	THP1	Human monocyte cell line	6.0E+04	1	2.0E+09	3	THP1 cells were obtained from ATCC (#TIB-202) and cultured in RPMI 1640 + 10% FBS + 1 % P/S. For differentiation, cells were stimulated with 20 ng/mL PMA (#P1585, Sigma) for 8 h and subsequently cultured in regular medium.	
	THP1 +PMA	Human THP1 cells, PMA-differentiated into macrophage-like phenotype	6.0E+04	1	2.0E+09	3		
	huMdM	Human primary monocyte-derived macrophages	4.0E+04	0	2.0E+09	4	Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood samples of healthy donors (Boehringer Ingelheim) by standard density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare). Monocytes were isolated using the MACS Pan Monocyte Isolation Kit (#130-096-537, Miltenyi Biotec). Monocytes were differentiated to macrophages by culturing in RPMI-1640 GlutaMAX (#61870, Gibco) + 10% Spezial-HI FCS (#16140) + 1% NEAA (#11140) + 1% P/S, supplemented	

							with 100 ng/mL rhM-CSF (#216-MCC/CF, R&D Systems) for 1 week. Macrophages were then seeded in medium containing 10 ng/mL M-CSF and simultaneously transduced.	
	huMo-DC	Human primary monocyte-derived dendritic cells	2.0E+04	1	2.0E+09	5	Peripheral blood mononuclear cells (PBMCs) were prepared from heparinized blood samples of healthy donors (Boehringer Ingelheim) by standard density gradient centrifugation with Ficoll-Paque Plus (GE Healthcare). CD14+ monocytes were isolated from PBMCs using CD14 antibody-coated magnetic MicroBeads (#130-050-201, Miltenyi Biotec). Cells were kept at a density of $1x10^6$ cells/mL in RPMI1640 supplemented with 2mM L-Glutamine and 10% FCS. For differentiation to monocyte-derived dendritic cells (huMo-DC), medium was supplemented with 800 IU/mL GM-CSF (#130-093-866, Miltenyi Biotec) and 250 IU/mL IL-4 (#130-093-922, Miltenyi Biotec). After 2 days, fresh medium containing the double concentration of cytokines was added and immature Mo-DCs were harvested at day 6.	
	huMonocyte	Human primary monocytes	5.0E+04	1	2.0E+09	5		
	huTCell	Human primary T-cells	2.0E+04	1	2.0E+09	5	The Pan T cell isolation kit (#130-096-535, Miltenyi Biotec) was used to isolate T cells from PBMCs (see above). Cells were cultured in RPMI1640 supplemented with 2mM L-Glutamine, 10% FCS, and 50 IU/mL IL-2, (#130-097-744, Miltenyi Biotec) at a density of $0.5x10^6$ cells/mL.	
Joint	huSynoviocytes	Human primary synoviocytes	7.5E+03	1	2.0E+09	3	Primary human synoviocytes were obtained from Articular Engineering (#CDD-H-2910, healthy donor) and cultured in M2700 + Sup1 + Sup2 + 10% FBS (Articular Engineering), using standard techniques.	
	ratSynoviocytes	Rat primary synoviocytes	7.5E+03	1	2.0E+09	3	Primary rat synovial cells were obtained from AcceGen (#ABC-H0047X) and cultured in DMEM + 10% FBS + P/S, using standard techniques.	
Kidney	HEK293H	Human embryonic kidney cell line	1.0E+04	1	2.0E+09	3	HEK293H were obtained from Thermo Fisher Scientific (#11631017) and cultured in DMEM + 10% FBS, using standard techniques.	
Liver	FL83B	Murine hepatocyte cell line	4.0E+03	1	2.0E+09	3	FL83B cells were obtained from ATCC (CRL-2390) and cultured in Ham's F12K + 10% FBS, using standard culture techniques.	
	HepG2	Human hepatocellular carcinoma cell line	5.0E+04	1	2.0E+09	2	HepG2 cells were obtained from ATCC (#HB-8065) and cultured in MEM with 1% NEAA + 10 % FBS (#41090-028, #11140-050, Thermo Fisher), using standard culture techniques.	
	HepRG	Human hepatocyte cell line (HepaRG)	4.0E+04	1	2.0E+09	3	HepaRG cells were obtained from Thermo Fisher (#HPRGC10) and cultured in William's E (#RNBH1215, Sigma) supplemented with 1% GlutaMax and 1x General Purpose Medium Supplement (#HPRG770, Gibco), using standard techniques.	
	LX2	Human hepatic stellate cell line	1.0E+04	1	2.0E+09	3	LX-2 cells were obtained from Sigma-Aldrich (#SCC064) and cultured in DMEM + 10% FCS, 2 mM GlutaMax, using standard techniques.	
	huHepato-Spheroid	Human primary hepatocytes, cultured as spheroids	5.0E+04	0	2.0E+09	13	Primary hepatocytes were obtained from Thermo Fisher (#HMCPIL, HU8200) and thawed in William's E (w/o Glutamine), supplemented with 10% FCS, 2mM L-Glutamine, 1% ITS, 100 nM Dexamethasone, 1x P/S. After pelleting (100 xg), cells were taken up in medium again and seeded at 50,000 cells/well of a 96-ultra low attachment plate. The cells were transduced the next day.	
	huHSC	Human primary hepatic stellate cells	1.1E+04	1	2.0E+09	3	Primary hepatic stellate cells were obtained from Zenbio (#HP-F-S, HSC061218) and cultured in Stellate Cell Medium basal SteCM-b (#5301-b ScienCell/Innoprot) + 1x SteCGS (#5352, ScienCell/Innoprot) + 2% FBS + GA-1000 in Collagen-I coated flasks.	
	mHepatocyte	Murine primary hepatocytes	8.0E+03	1	2.0E+09	3	Primary murine hepatocytes were obtained from Thermo Fisher (#MSCP10) and thawed in 39% HBSS-dissolved Percoll (#P4937, Sigma) + CHRM (#CM7000, Thermo). Following centrifugation, cells were seeded in William's E (w/o Glutamine, Phenol red) (#A1217601, Thermo) supplemented with 10% FCS, 3% Thawing/Plating cocktail (#CM3000, Thermo) and 1 µM Dexamethasone. Cells were cultured using standard techniques.	

Lung	huALI	Human primary small airway epithelial cells (huSAEC) cultured at air liquid interface for epithelial polarization	2.5E+04	38	2.0E+09	3	huSAEC cells were obtained from Lonza (#CC-2547). Following expansion, cells were seeded on Collagen-I coated transwells, cultured submerged for 4 days and then lifted to initiate polarization. The detailed protocol for both, conventional expansion and ALI cultures is described in detail in the referenced manuscript. Transduction was conducted by adding AAVs to the basal compartment.	9
	huNHLF	Human primary lung fibroblasts	1.0E+04	4	2.0E+09	2	Primary normal human lung fibroblasts were obtained from Lonza (#CC-2512, 543644) and cultured in Fibroblast basal medium (#CC-3131, Lonza), using standard techniques. 5 ng/mL TGFβ1 were conditionally added to the culture medium for 72 h, prior to transduction.	
	huNHLF +TGFβ	Human NHLF, stimulated with TGFβ1	1.0E+04	4	2.0E+09	2		
	huSAEC	Human primary small airway epithelial cells	7.5E+03	1	2.0E+09	3	huSAEC cells were obtained from Lonza (#CC-2547) and cultured in Pneumacult EX Plus complete (#05041 + 05042, Stemcell Technologies). 5 ng/mL TGFβ1 were conditionally added to the culture medium for 48 h, prior to transduction.	
	huSAEC +TGFβ	Human SAEC, stimulated with TGFβ1	2.5E+03	3	2.0E+09	3		
Skin	HaCaT	Human epidermal keratinocyte cell line	4.0E+04	1	2.0E+09	2	HaCaT cells were obtained from AddexBio (#T0020001) and cultured in Ham's F-12 + 10% FCS, using standard techniques.	
Stem cells	iPSC	Human induced pluripotent stem cells	1.0E+04	1	2.0E+09	3	Cellartis Human iPS Cell Line 22 (ChiPSC22) was obtained from Takara Bio (#Y00325) and cultured using the Cellartis DEF-CS 500 Culture System (#Y30010) according to the manufacturer's instructions.	
	iPSC 2	Human induced pluripotent stem cells	n/a	1	6.6E+08	3	iPSC line AD0202 (re-programmed from adult fibroblasts) were obtained from the StemBancc Consortium and cultured using mTeSR Plus cGMP Pluripotent Stem Cell Maintenance Medium (#100-0276, Stemcell Technologies) on Matrigel.	
	mHemSC	Murine primary bone marrow-derived hematopoietic multipotent stem cells	5E+04	28	2.0E+09	3	Murine multipotent stem cells were purified from bone marrow following the procedure described in steps 1-18 of the referenced published protocol. Key steps follow a two-step process, i.e., MACS to enrich c-Kit+ cells, followed by FACS-sorting of c-Kit+ Sca1+ Lin- cells. Cells were expanded for 28 days and seeded in fibronectin-coated plates for transduction.	10

Supplementary References

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