## Supplementary Figure S1

AAV5/1-725 AAV12/1-743 AAV9/1-737 AAV9/1-737 AAV0/1-737 AAV0/1-737 AAV7/1-738 AAV13/1-734 AAV36/1-737 AAV0/03/1-737 AAV0/1-738 AAV2/1-738 AAV2/1-738 AAV5/1-725 AAV9/1-737	1 MSFVDHPPDWLEE-VGEGLREFLGLEAGPRKPKPNQCHQDARGLVLPGYNYLGFONGLDRGEPVNRADEVAREHDISYNEDLEAGDNPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPOPKANQCHDDNARGLVLPGYNYLGFFNGLDKGEPVNRADAALEHDKAYDKQLEGGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPOPKANQCHDDNARGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPOPKANQCHDDNARGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCKDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCKDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCKDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDNRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDNRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDNRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDNRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDRRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDRGCUVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKAGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIREWWALKPGAPKPKANQCHDDGRGLVLPGYNYLGFFNGLDKGEPVNAADAAALEHDKAYDQLKGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIRGWWLKKGPPPKPAEFKKDDSGLVLPGYNYLGFFNGLDKGEPVNEADAAALEHDKAYDQLKGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDNLSEGIRGWWLKKPGPPPKPAEFKKDDSGLVLPGYNYLGFFNGLDKGEPVNEADAAALEHDKAYDQLKGONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDTLSEGIRGWWLKKPGPPPKPAEFKKDDSGLVLPGYNYLGFFNGLDKGEPVNEADAALEHDKAYDQLKGSONPYLKYNHADAEFOGKL 1 MAADGYLPDWLEDTLSEGIRGWWLKKPGPPCFKPKGACKKFFLGFGCUNGG	103 104 104 104 104 104 104 104 104 104 104
AAV1/1-737 AAV6/1-737 AAV7/1-738 AAV13/1-734 AAV3b/1-737 AAV2b/1-737 AAV8/1-739 AAV10/1-739 AAV2/1-736 AAV2/1-738	105 GEDTSFGONLGRAVFGAKKRVLEPLGUVEGAKTAPOKKRPVEGSPG. EPDSSSGIGKT	198 198 199 197 197 198 198 199 199 199
AAUS/1-725 AAV12/1-743 AAUS/1-737 AAUS/1-737 AAUS/1-737 AAU7/1-738 AAU13/1-734 AAU3/1-737 AAULK03/1-737 AAU2/1-739 AAU2/1-738 AAU2/1-738	180 & AD TM S A G G G PL G D NN G A D C V G H A S G D WH C D S TWING D R V TK 'S TR TW U.P S'N NH O'F F [ K S G S V . D G S N A N Y F G Y S T PWG Y F D F N R H S H W S P R D W G L I 20 H D A E M A A P G G A P V A D N N E G A D C V G M A S G D WH C D S T W S G P V I T T S TR TW U.P TY N H L Y H U [ S N S T S G S S N D N A Y F G Y S T PWG Y F D F N R H C H F S P R D W G L I 190 & S L TM A S G G A P V A D N N E G A D C V G M S G N WH C D S T W L D R V I TT S TR TW A L P TY N N H L Y H U [ S S A S . TG A S N D N A Y F G Y S T P WG Y F D F N R H C H F S P R D W G L I 190 & G P T TM A S G G A P MAD N N E G A D C V G M S G N WH C D S TW L G D R V I TT S TR TW A L P TY N N H L Y H U I S S A S . TG A S N D N A Y F G Y S T P WG Y F D F N R H C H F S P R D WG R L I 190 & G P TT M A S G G A P MAD N N E G A D C V G M S G N WH C D S TW L G D R V I TT S TR TW A L P TY N N H L Y H U I S S A S . TG A S N D N H Y F G Y S T P WG Y F D F N R H C H F S P R D WG R L I 200 & S O TV A A S G G A P MAD N N E G A D C V G M S G N WH C D S TW L G D R V I TT S TR TW A L P TY N N H L Y H U I S S F . TG A S N D N H Y F G Y S T P WG Y F D F N R H C H F S P R D WG R L I 200 & S O TV A A S G G A P MAD N N E G A D C V G M S G N WH C D S TW L G D R V I TT S TR TW A L P TY N N H L Y H U I S S S G A T N D N H Y F G Y S T P WG Y F D F N R H C H F S P R D WG R L I 190 & S N TM A S G G A P MAD N N E G A D C V G N S S G N WH C D S N W L O D R V I TT S TR TWA L P TY N N H L Y H U I S S	291 300 302 301 302 299 300 300 303 303 303 303 300 302
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AAV5/1-725 AAV12/1-743 AAV9/1-737 AAV6/1-737 AAV6/1-737 AAV13/1-737 AAV13/1-737 AAV13/1-737 AAV26/1-737 AAV26/1-739 AAV2/1-736 AAV2/1-738	396 LR TONNE E TYNE E VPF HSS FAPSONLE KLANPL VDOYLYR VSTUTGG VOENKNLAG RYANTY (NWE POEMGET GOWNLOG SVN RAS VS 405 LR TONNE E VSNOF E VPF HSMYAHSOSLDRMMNPLLDOYLWR I OSTTTENSLOGTATTTYGKITTGDF AVYR (NWE POEMGET GOWNLOG SVN RAS VS 405 LR TONNE E VSNOF E VPF HSS VAHSOSLDRLMNPLLDOYLWY I SKTTINGSGONOT LKES VAGPS MAV GONYLPG FOYROGRVS TTYTONNNS EF 404 LR TONNE T FSVTF E VPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. GSAONKOLLF SRGS PAGMS VDP KNWE POEMGEV KITTDNNNS NF 404 LR TONNE T FSVTF E VPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. GSAONKOLLF SRGS PAGMS VDP KNWE POEMGEV KITTDNNNS NF 404 LR TONNE T FSVTF E VPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. GSAONKOLLF SRGS PAGMS VDP KNWE POEMGEV KITTDNNNS NF 404 LR TONNE T FSVTF E VPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. SAONKOLLF SRGS PAGMS VDP KNWE POEMGARVS KITTDNNNS NF 405 LR TONNE FSNS FE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. NG SAONKOLLF SRGS PAGMS VDP KNWE POEMGARVS KITTDNNNS NF 405 LR TONNE FSNS FE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TONGS. NO SAONKOLLF SRGS PAGMS VDP KNWE POEMGARVS KITTDNNNS NF 405 LR TONNE FSNS FE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TOTAL.SGTOGS NELLF SGAOPTSMSLDA KNWE POEVGARVE KITTDNNNS NF 405 LR TONNE OF SYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TOGT TSGT TNOSR LLF SGAOPTSMSLDA KNWE POEVGAR KITTDNNNS NF 406 LR TONNE OF SYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I NR TOGT TSGT TNOSR LLF SGAOPTSMSLDA KNWE POEVGAR KITADNNNS NF 406 LR TONNE OF SYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I STOTTO- OT ANTOT L GF SGGEN TMANDA KNWE POEVGAR KITADNNS NF 406 LR TONNE OF SYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I SR TOTTO- OT ANTOT L GF SGGEN TMANDA KNWE POEVGAR KITADNNS NF 406 LR TONNE OF TYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I SR TOTTO- OT ANTOT L GF SGGEN TMANDA KNWE POEVGAR KITADNNS NF 405 LR TONNE OF TYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I SR TOTTO- OT ANTOT L GF SGGEN TMANDA KNWE POEVGAR KITADNNS SF 405 LR TONNE OF TYTFE DVPF HSS VAHSOSLDRLMNPLIDOYLYY I SR TOTTO- TO ANTOT L GF SGGEN TMANDA KNWE POEVY	487 508 501 501 503 503 503 503 500 500 502
AAV5/1-725 AAV12/1-743 AAV9/1-737 AAV7/1-737 AAV7/1-737 AAV7/1-738 AAV13/1-737 AAV26/1-737 AAV26/1-737 AAV10/1-739 AAV10/1-738	488 A FATTNRME LE BASYQVP PO PNOMTNNLOG SN TYALENTMLE NSO PANPG TTATYLEG NMLITSE SE TOP VNR VAY UVG UCKVIII 500 LLKYD THTT NG RWS NMAPG PPMA TAGAGO SOF. SNSOLI FAG PNPSGNTTTSSNNLLFT SEEE IATTNPRDT MFG QIADNNNATTAPHIANLDA MG IV 500 LLKYD THTT NG RWS NMAPG PPMA TAGAGO SOF. SNSOLI FAG PNPSGNTTTSSNNLLFT SEEE IATTNPRDT MFG QIADNNNATTAPHIANLDA MG IV 500 LWF GASWA. NG RNS LWPG PAMASHKO ED KFF PM SO WII FOKESAGASNTALDNWII TNEEE IKTNPVATT RFOTVAVNFG SSTD PATO UN MA MAGAL 502 TWTGASKYN. NG RESI INPG TAMASHKO BCHKFF PM SO WII FOKESAGASNTALDNWII TDEEE IKATNPVATT RFOTVAVNFG SSTD PATO UN MA MAGAL 504 AWTGATKYH. NG RSLVNPG VAMATHKO DE DKFF PM SO WII FOKESAGASNTALDNWII TDEEE IKATNPVATT RFOTVAVNFG SSTD PATO UN MA GAL 504 AWTGATKYH. NG RSLVNPG VAMATHKO DE DKFF PM SO WII FOKESAGASNTALDNWII TDEEE IKATNPVATT RFOTVAVNFG SSTD PATO UN MO 504 AWTGATKYH. NG RSLVNPG VAMATHKO DE DKFF PM SO WII FOKESGAGASNTALDNWII TDEEE IRTTNPVATT RFOTVAVNFG SSTD PATO UN MO 504 AWTGATKYH. NG RSLVNPG VAMATHKO DE DKFF PM SO WII FOKESGTNAN NADLENVMI TD EEE IRTTNPVATT Q'Y SNNLONSNAO PTTG TNN HO GAL 504 AWTGATKYH. NG RSLVNPG VAMATHKO DE DKFF PM SO WII FOKEG TTASNALEDNWII TD EEE IRTTNPVATT Q'Y TVSNNLONSNAO PTTG TNN HO GAL 504 AWTGATKYH. NG RSLVNPG PAMASHKO DE KFF PMHO NLI FOKEG TTASNALEDNWII TD EEE IRTTNPVATT Q'Y TVSNNLONSNAO PTTG TNN HO GAL 504 AWTGATKYH. NG RSLVPG PAMASHKO DE KFF PMHO NLI FOKEG TTASNALEDNWII TD EEE IRTTNPVATT Q'Y TVSNNLONSNAO PTTG TNN HO GAL 504 AWTAG TKYH. NG RSLVPG VAMATHKO DE EKFF PMHO NLI FOKEG TTASNALEDNWII TD EEE IRTTNPVATT Q'Y TVSNNLONSNAO PTTG TNN HO GAL 504 AWTGATKYH. NG RSLVPG VAMASHKO DE EKFF PMHO NLI FOKEG TTASNALDNWII TD EEE IRTTNPVATT Q'Y TVSNNLONSNAO PTTG TNN D GAL 504 AWTGATKYH. NG RSLVPG VAMASHKO DE EKFF PMIO SLI FOKGGAGARDNO YSSVMLTSEEE IRTTNPVATT Q'Y TVSNNLONSNLONSNAO PTO INSOLAN 504 AWTAG TKYH. NG RSLVPG VAMASHKO DE EKFF PMIO SLI FOKGGAGARDNO YSSVMLTSEEE IRTTNPVATT Q'Y SVANNLO GAN TAP'I G'YNN D GAL 504 AWTAG TKYH. NG RSLVPG VAMASHKO DE EKFF PMIO SLI FOKGGAG ROM	591 602 602 602 603 599 602 604 604 604 604 604 601 603
AAV5/1-725 AAV12/1-743 AAV9/1-737 AAV6/1-737 AAV6/1-737 AAV7/1-738 AAV13/1-734 AAV32/1-737 AAV26/1-737 AAV26/1-738 AAV2/1-736 AAV20/1-738	592 PGSUWMERDVYLQGPIWAKIPETGAHFHPSPLMGGFGLKHPPPMMLIKNTPVPGNI.TSFSDVPVSSFITQYSTGQVTVEMEWELKKENSKRWNPEIQYTNYN 600 PGMVWQNRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPPQIFIKNTPVPANPNTFFSAKRINSFLTQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 603 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 604 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKNPPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 605 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKNPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 606 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKNPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 607 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKNPPQILIKNTPVPANPPAEFSATKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 608 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPPAFSAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 609 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQIMIKNTPVPANPPTNFSAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 605 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPPTNFSAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 605 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTNFSAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 806 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFSPAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 806 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFSAKFASFITQYSTGQVSVEIEWELQKENSKRWNPEIQYTSNY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFNYSTGAXVEIEWELQKENSKRWNPEIQYTSNY 806 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFNY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFNY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTPVPANPTTFNY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTYVPANPY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGGFGLKHPPQILIKNTYVPANPY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGFGLKHPPQILIKNTYVPANPY 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGFGLKHPPQILIKNTYVPANPX 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGFGLKHPPQILIKNTYVPANPX 807 PGMVWQDRDVYLQGPIWAKIPHTDGNFHPSPLMGFGLKHPPQILIKNTYVPANPX 807 PGMV	694 712 706 706 707 707 703 708 708 708 705 705 707
AAV5/1-725 AAV12/1-743 AAV9/1-737 AAV1/1-737 AAV7/1-738 AAV7/1-738 AAV13/1-734 AAV13/1-737 AAV2/60/1-737 AAV2/60/1-737 AAV2/10/1-739 AAV2/10/1-738	Multiple sequence alignment of wild-type and shuffled caps in this study. The alignments were generated using Clusta in this study. The alignments were generated using Clusta i.2.4, coloring is according to percentage identity and was generated jalview 2.11.3.3. Regions highlighted in light yellow, yellow, our red correspond to the variable regions I, IV, V ar respectively. The green line represents the peptide inserti AAV2, 7 and 9.	sids used al Omega nerated in range and and VIII, on site in

AAV2, 7 and 9.



**Correlation of AAV-specific nAb levels and age of the serum donors.** The nAb titers against the indicated AAVs as shown in Figure 3 and the age of the serum donors were subjected to a Spearman ranked correlation analysis. Each dot indicates an individual donor, the dashed line indicates the nAb detection limit.

Supplementary Figure S3



compared to the group of healthy controls were detected (Kruskal-Wallis one-way ANOVA on ranks and Dunn's multiple comparisons test.



**Correlation of AAV-specific bAb and nAb levels.** The bAb titers as shown in Figure 2 and the nAb titers as shown in Figure 3 were subjected to a Spearman ranked correlation analysis.

Each dot indicates an individual donor, the dashed line indicates the nAb detection limit.



**Correlation between bAb and nAb levels specific for different AAV types.** A Spearman ranked correlation analysis was performed of the bAb levels as shown in Figure 2 (bottom left half-square) and of the nAb levels against the different AAV types as shown in Figure 3 (upper right half-square).

The colours indicate the correlation coefficient, values within the cells indicate the correlation coefficient if *p*<0.001.