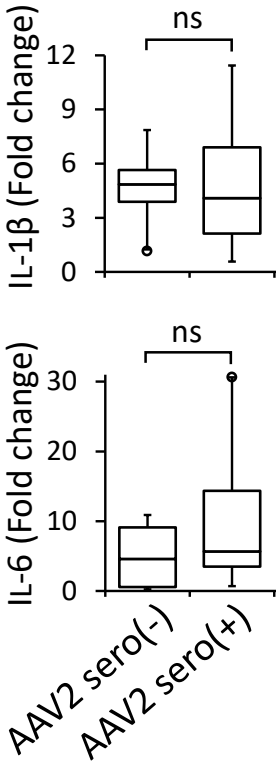
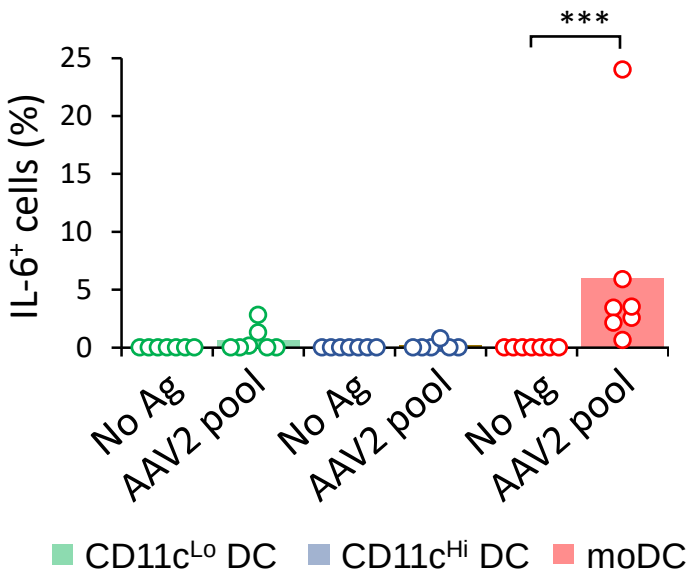
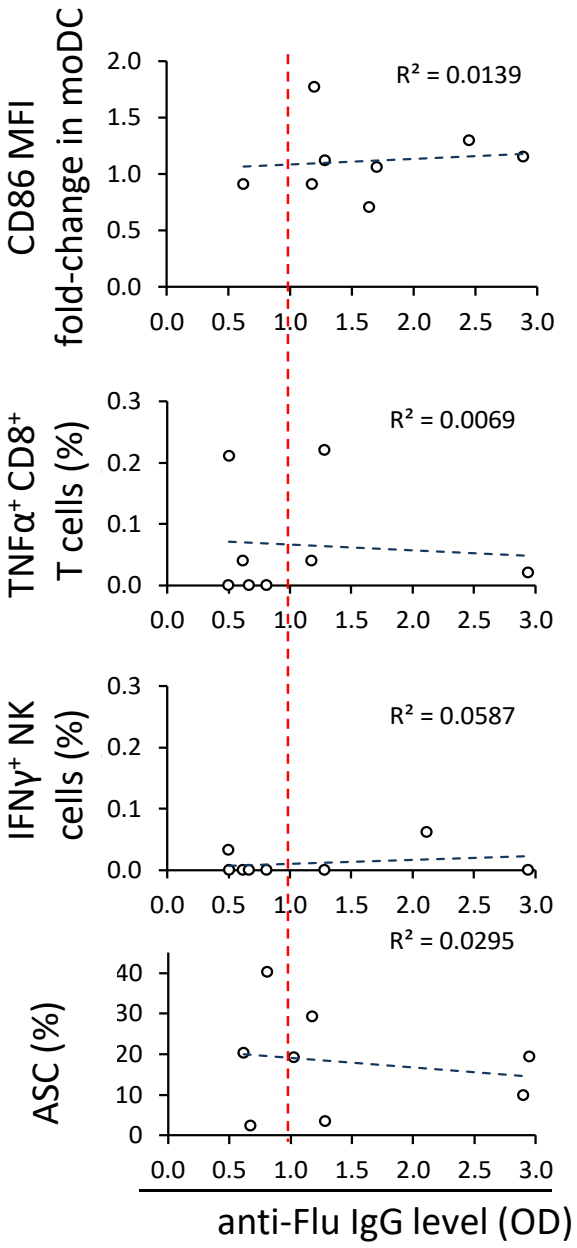


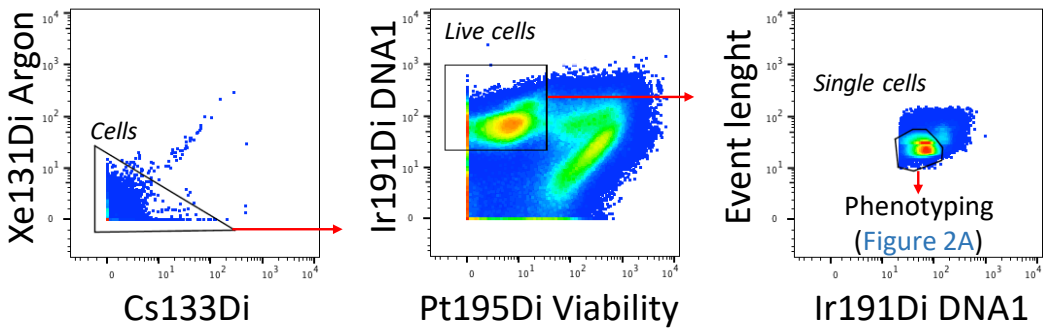
Supplemental Figure 1. IL-1β and IL-6 secretion is not correlated with the AAV-serology status of donors. Fold change of IL-1β and IL-6 concentration in PBMCs cultures restimulated with the AAV2 pool of peptides, obtained from seronegative (*n* = 4) or seropositive (*n* = 7) donors. Boxplots show median ±SD. ns- not significant by non-parametric, two-tailed Mann-Whithney test.



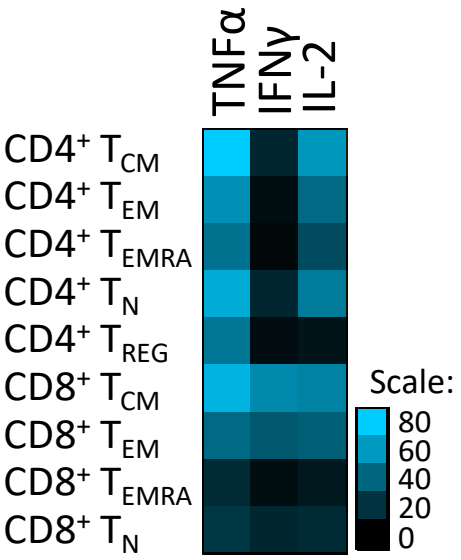
Supplemental Figure 2. IL-6 is predominantly secreted by moDCs. Percentage of IL-6-positive cells in a given DC subset measured by the ICS assay 24h after restimulation with indicated antigens (n = 7). Histograms represent means and open symbols individual sample values. Only samples in which IL-6 secretion was detected in any of the cellular subsets are shown. *** P = 0.0006. Statistical analysis was performed by non-parametric, two-tailed Mann-Whithney test.



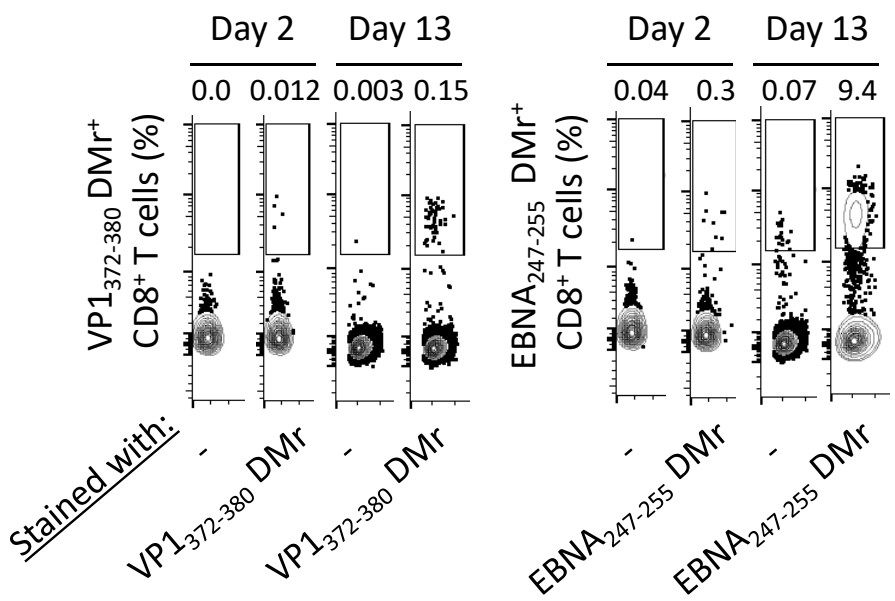
Supplemental Figure 3. Immune responses induced in PBMCs stimulated with Flu antigens (pool of peptides) are plotted against anti-Flu IgG levels in plasma of respective donors. Red dashed line indicates cut off for antibody positivity. Blue dashed line represents linear trendline. Based on R-squared values there is no correlation in the graphs.



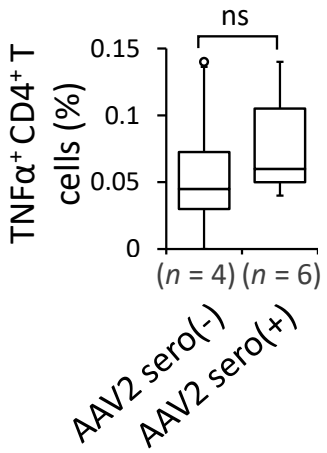
Supplemental Figure 4. CyTOF initial gating strategy. After exclusion of argon contamination, dead cells and cell aggregates were excluded from analysis based on Cell-ID Cisplatin and Intercalator-Ir staining (Fluidigm).



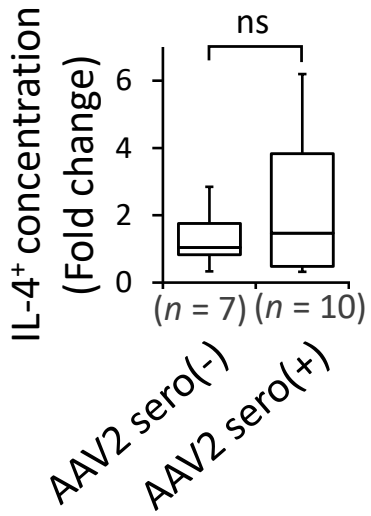
Supplemental Figure 5. Positive control of IFNγ secretion compared to TNFα and IL-2. Heatmap represents percentage of positive cells in a given cellular subset responding to 5h stimulation with PMA/Ionomycin, measured by CyTOF.



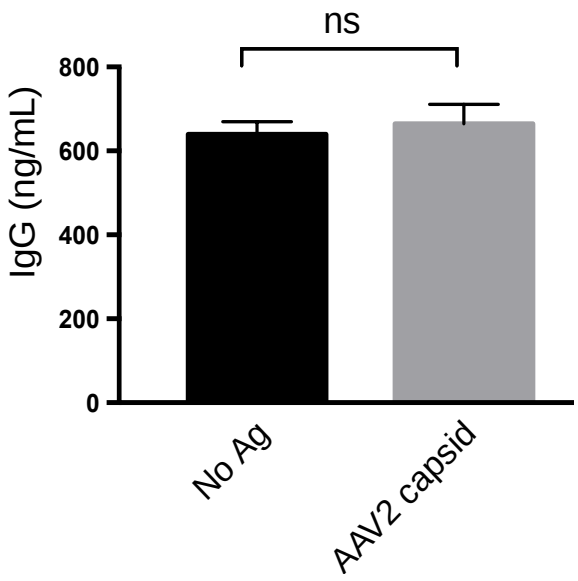
Supplemental Figure 6. Efficacy of the antigen-specific $CD8^+$ T-cell expansion *in vitro* measured with DMr staining. Flow cytometry staining represent the percentage of dextramer (DMr)-positive $CD8^+$ T cells two days after restimulation or after one cycle of expansion (day 13) with antigen-specific peptides. B*0702 restricted peptides were used for the stimulation of PBMC from a healthy donor with the B*0702 haplotype; AAV2 peptide, $VP1_{372-380}$ or control EBV peptide $EBNA_{247-255}$. EBV – Epstein–Barr virus.



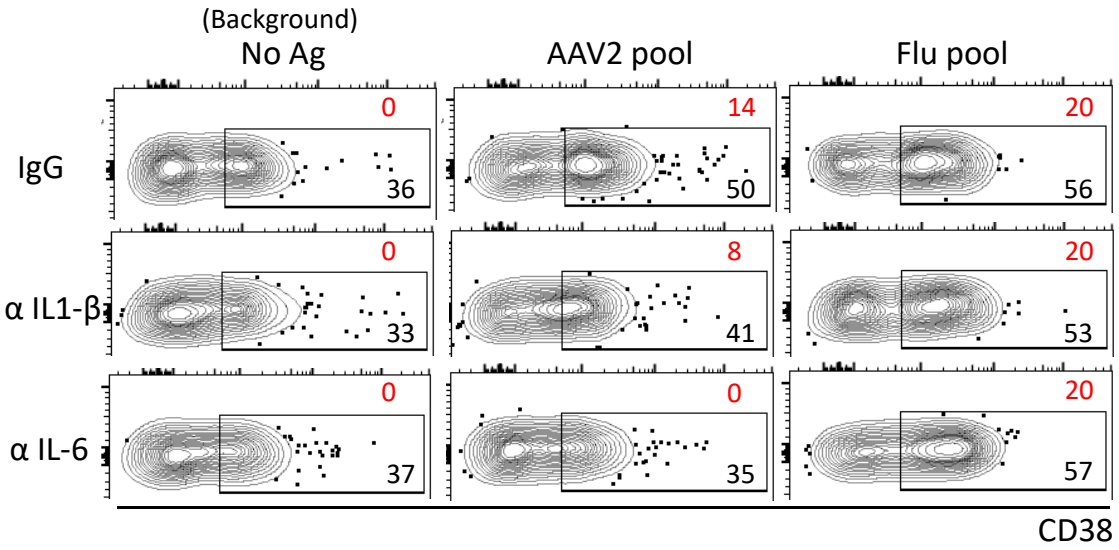
Supplemental Figure 7. Percentage of AAV2 capsid-specific TNFα$^+$ CD4$^+$ T cells measured *ex vivo* in PBMCs from seronegative vs. seropositive donors. Boxplots show median ±SD. ns- not significant by non-parametric, two-tailed Mann-Whithney test.



Supplemental Figure 8. Fold change of IL-4 concentration in PBMCs cultures restimulated with the AAV2 pool of peptides, obtained from seronegative or seropositive donors. Boxplots show median ±SD. ns- not significant by non-parametric, two-tailed Mann-Whithney test.



Supplemental Figure 9. Concentration of anti-AAV2 IgG secreted in PBMC cultures, stimulated or not (No Ag) with the AAV2 capsid particles. PBMCs obtained from AAV2-seropositive donors. ns- not significant by non-parametric, two-tailed Mann-Whithney test.



Supplemental Figure 10. AAV-induced B-cell differentiation depends on IL-1β and IL-6 secretion. Representative FACS staining shows the percentage of antigen-specific ASCs in PBMC cultures, 7 days after restimulation with the AAV2 or Flu pool of peptides. The $CD3^{-}CD19^{+}IgD^{-}CD24^{-}CD27^{+}$ cells are shown in plots. In red, percentage of ASCs after subtraction of the background response in the absence of antigen (No Ag).

Supplemental Table 1. Binding (BAb) and neutralizing (NAb) antibody titers to AAV2 and Flu in healthy donors. Anti-AAV2-IgG titers were used to define AAV2-seropositive and -seronegative donors in the study. Positivity cutoff for AAV2 NAb >1:3.16; Positivity cutoff for AAV2 BAb >1:3; Positivity cutoff for Flu BAb >1. Positive values are highlighted in red.

HD #	Age (Yr)	Sex	NAb AAV2 (Titer)	BAb anti-AAV2-IgG (Titer)	BAb anti Flu-IgG (OD)
37	21	M	<1:1	<1:1	ND
27	20	F	1:1	<1:1	1.65
28	20	M	<1:1	<1:1	1.38
18	50	M	<1:1	<1:1	0.67
26	38	F	1:3.16	<1:1	1.70
24	23	F	1:3.16	<1:1	2.45
15	18	M	<1:1	<1:1	0.81
19	22	F	1:3.16	<1:1	0.50
39	20	M	1:1	<1:1	ND
38	20	F	1:10	<1:1	ND
32	50	M	1:10	1:3	2.95
25	19	F	1:10	1:3	2.89
8	22	F	1:10	1:10	1.18
20	52	M	1:10	1:10	1.28
23	25	F	1:10	1:10	1.20
36	22	M	1:100	1:270	ND
30	32	M	1:316	1:270	2.12
34	32	M	1:316	1:270	0.87
35	39	M	1:100	1:810	ND
30	49	M	1:3160	1:810	1.03
31	42	M	1:1000	1:810	2.90
7	65	M	1:3160	1:2430	0.62
21	61	M	1:3160	1:2430	0.51
33	57	M	1:3160	1:2430	0.83

ND- not determined