

Supplementary material for paper by Isakova-Sivak et al. “**Recombinant live attenuated influenza vaccine viruses carrying conserved T-cell epitopes of human adenoviruses induce functional cytotoxic T-cell responses and protect mice against both infections**”

Table S1. Experimentally established T- and B-cell epitopes of Human Adenoviruses deposited in the Immune Epitope Database (IEDB)

Viral protein	Total 320	T-cell epitopes					B-cell epitopes		
		Human		Mouse			B all	Human	C
		MHC I	MHC II	H2-b	H2-d	MHC II			
Human mastadenovirus E	1								
Hexon	1						1		
Human mastadenovirus B	69								
Fiber	24						24		2
Hexon	43	12	0				31		3
pVII	2		2						
Human mastadenovirus C	251								
Hexon	159	39	124		1		9	5	3
Fiber	40	1	2				38		3
DNA polymerase	3	1	2						
DNA-binding protein	2			1	2				
E1B 55 kDa protein	7			6		1			
E1B protein, small T-antigen	2		1				1		
Early E1A protein	10	1	2	7			4		
Hexon-interlacing protein	2		2						
I-leader protein	1	1							
23K endopeptidase	1						1		1
Packaging protein 3	1		1						
Penton protein	12	8	5				1		
Pre-core protein X	1						1		
Pre-early 3 receptor internalization and degradation alpha protein	1		1						
Pre-hexon-linking protein VIII	3		3						
Pre-histone-like nucleoprotein	2		1				1		
Pre-protein VI	2		2						
Adenovirus death protein	1		1						

Table S2. Experimentally established T-cell epitopes located within human adenovirus hexon region 855-935

IEDB ID	Epitope sequence	Location	Serotype ¹	Cross-reactive	Allele	Assay
68067	VDSITQKKFLCDRTLWRIPFSSNFMSMGAL	856-885	hAdV 5	C (B ⁴)	HLA-DR17 HLA class II	IFN- pat pro cyt deg IL- TN IL-
98568	VDSITQKKFLCDRTLWRIPFSSNFM	856-880	hAdV 5	C	HLA class II	IFN
98371	QKKFLCDRTLWRIPFSSNFMSMGAL	861-885	hAdV 5	C	HLA class II	IFN
134493	QKKFLCDRTLWRIPF	885-899	hAdV 5	C	HLA class II	IFN
134425	LCDRTLWRIPFSSNF	865-879	hAdV 5	C	HLA class II	IFN
78885	WRIPFSSNFMSMGALTDLGQNLLYANSAHA	871-900	hAdV 5	C	HLA class II	IFN
134535	SMGALTDLGQNLLYA	881-895	hAdV 5	C	HLA class II	IFN
134443	LTDLGQNLLYANSAH	885-899	hAdV 5	C	HLA-A*01:01	IFN
240899	LTDLGQNLLY	901-910	hAdV 2	C	HLA-A*01:01	cyt IFN IL- TN acti qua
63191	TDLGQNLLY	902-910	hAdV 5	C (B ⁴)	HLA-A1	cyt IFN qua pro
78847	TDLGQNLLYANSAHALDMTFEVDPMDEPTL	886-915	hAdV 5	C	HLA class II	IFN
144913	LLYANSAHAL	892-901	hAdV 5	C	HLA-A2 HLA-A*02:01	qua IFN IL- per TN cyt pro
189482	LYANSAHAL	893-901	hAdV 11	all	HLA-A*24:02	IFN
35228	LDMTFEVDPMDEPTLLYVLFEVFDVVRVHR	901-930	hAdV 5	5	HLA class II	IFN
8127	DEPTLLYVLFEVFDV	911-925	hAdV 5	C	HLA-DR HLA-DPw4 HLA-DPB1*04:01 HLA-DR7	cyt IFN pro

					HLA-DR15	pat
98027	EVDPMDEPTLLYVLFEVFDVVRVHRPHR	906-930	hAdV 5	5	HLA class II	IFN
68037	VDPMDEPTLLYVLFE	907-921	hAdV 5	C	HLA-DR HLA class II	IFN
						IL-
						IL-
						CC
						CC
						CC
						CX
						gra
						IL-
						IL-
						onc
						pro
						TN
						IL-
						IL-
						IL-
						IL-
						MI
						per
						TN
9683	DPMDEPTLLYVLFEV	908-922	hAdV 5	C	HLA-DR	IFN
134473	PMDEPTLLYVLFEVF	909-923	hAdV 5	C	HLA-DQB1*04:02 HLA-C*07:02 HLA-DQB1*04:02 HLA-C*07:02 HLA-DQB1*04:02 HLA-C*07:02	IFN cyt
134480	PTLLYVLFEVFDVVR	913-927	hAdV 5	C		IFN cyt
64950	TLLYVLFEV	914-922	hAdV 5	C	HLA-A*02:01 HLA-DPB1*04:01 HLA-DR	IFN qua cyt deg pro
64951	TLLYVLFEVFDVVRV	914-928	hAdV 5	C	HLA-DR	IFN
76342	YVLFEVFDVV	917-926	hAdV 5	C	HLA-A*02:01	IFN qua

78743	LYVLFVFDVVRVHRPHRGVIETVYL RTPF	916-945	hAdV 5	5	HLA class II	IFN
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- ¹ – AdV serotype used in the original study;
- ² – T-cell assays used in these studies;
- ³ – according to the IEDB data;
- ⁴ – cross-reactivity is shown based on sequence identity; experimental confirmation for cross-reactivity with this serotype is shown in parenthesis.

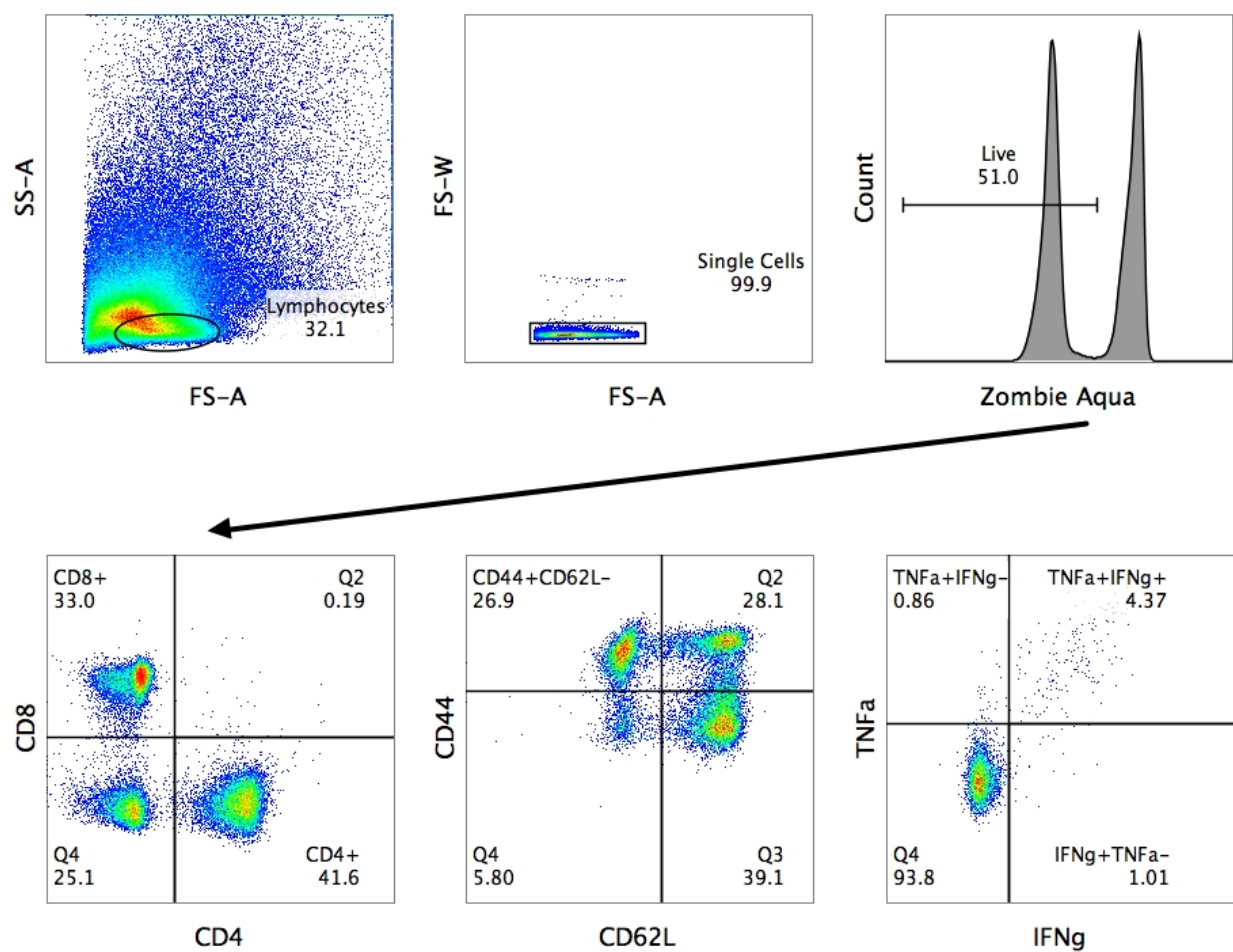


Figure S1. Gating strategy for the assessment of effector memory CD8 T-cell responses in mice using intracellular cytokine staining assay.

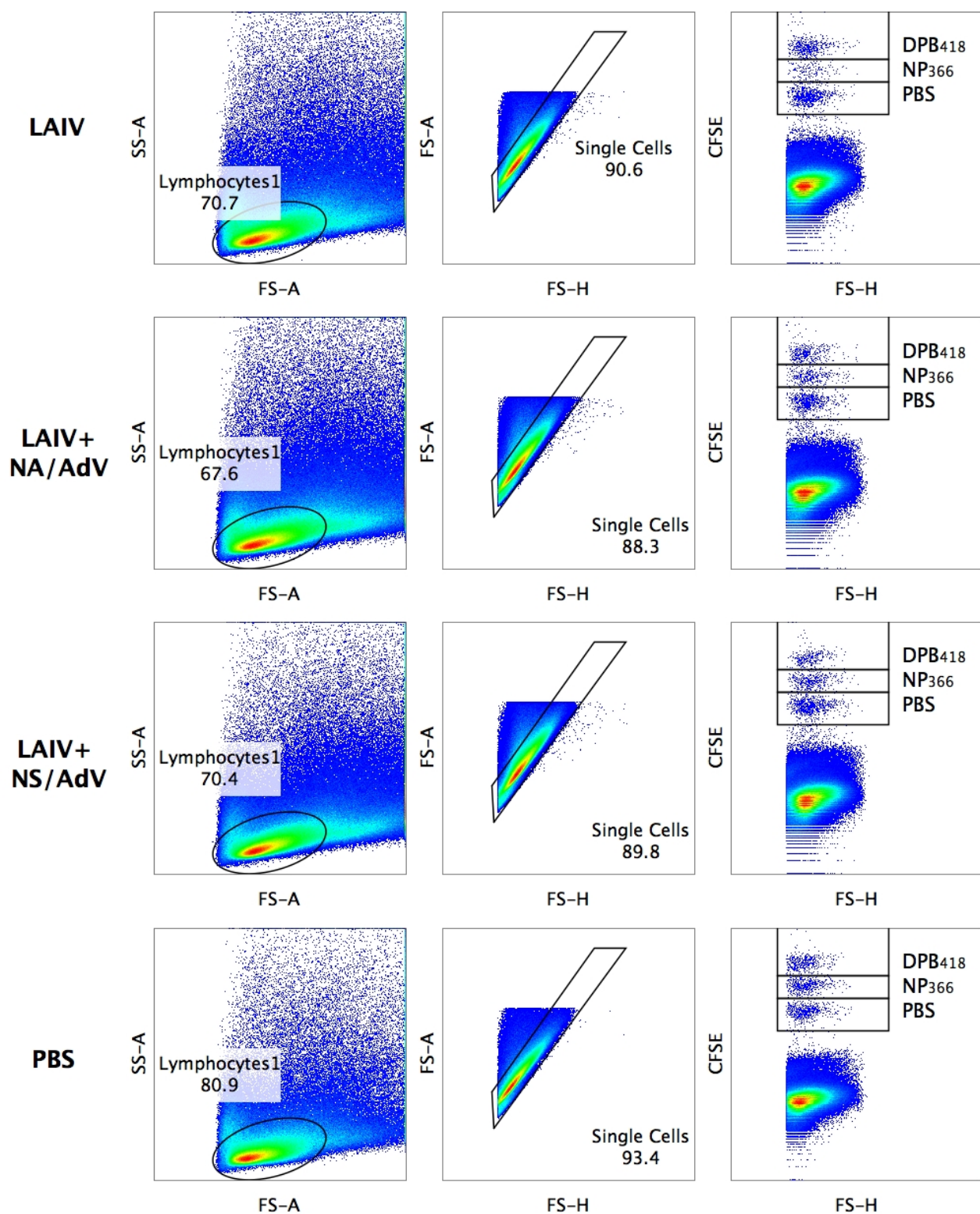


Figure S2. Gating strategy for CTL *in vivo* assay.

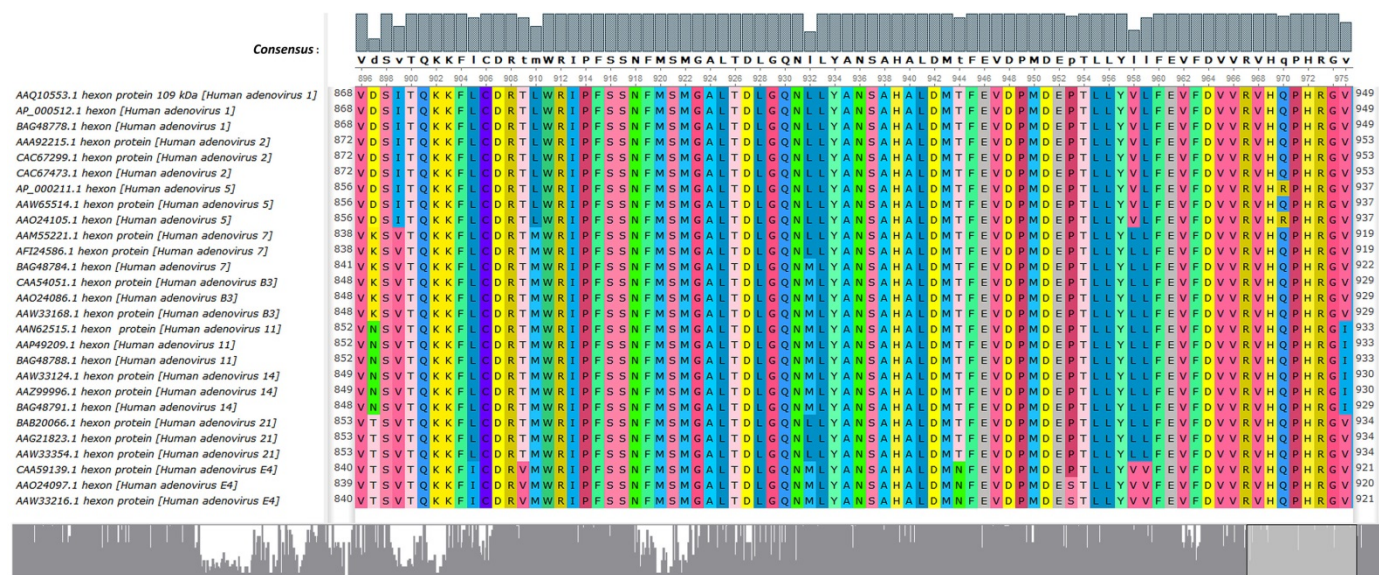


Figure S3. Alignment of a C-terminus fragment of AdV hexon (residues 855-935) among various AdV serotypes belonging to different AdV groups. Upper histogram shows conservancy of the amino acids within the selected fragment. Lower histogram shows the overall conservancy of the whole hexon protein, with the selected fragment depicted in the frame.

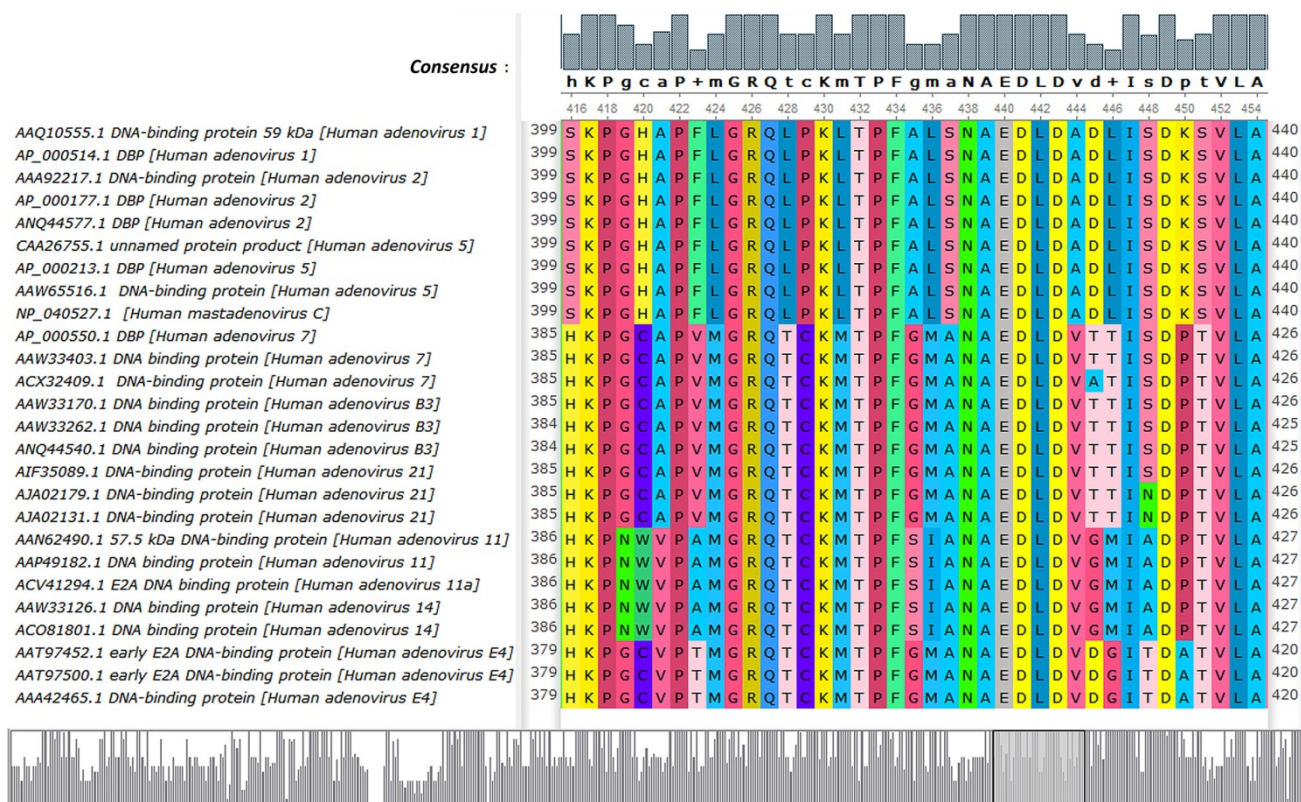


Figure S4. Alignment of a DBP region (residues 400-438) among various AdV serotypes belonging to different AdV groups. Upper histogram shows conservancy of the amino acids within the selected fragment. Lower histogram shows the overall conservancy of the whole DBP, with the selected fragment depicted in the frame.

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