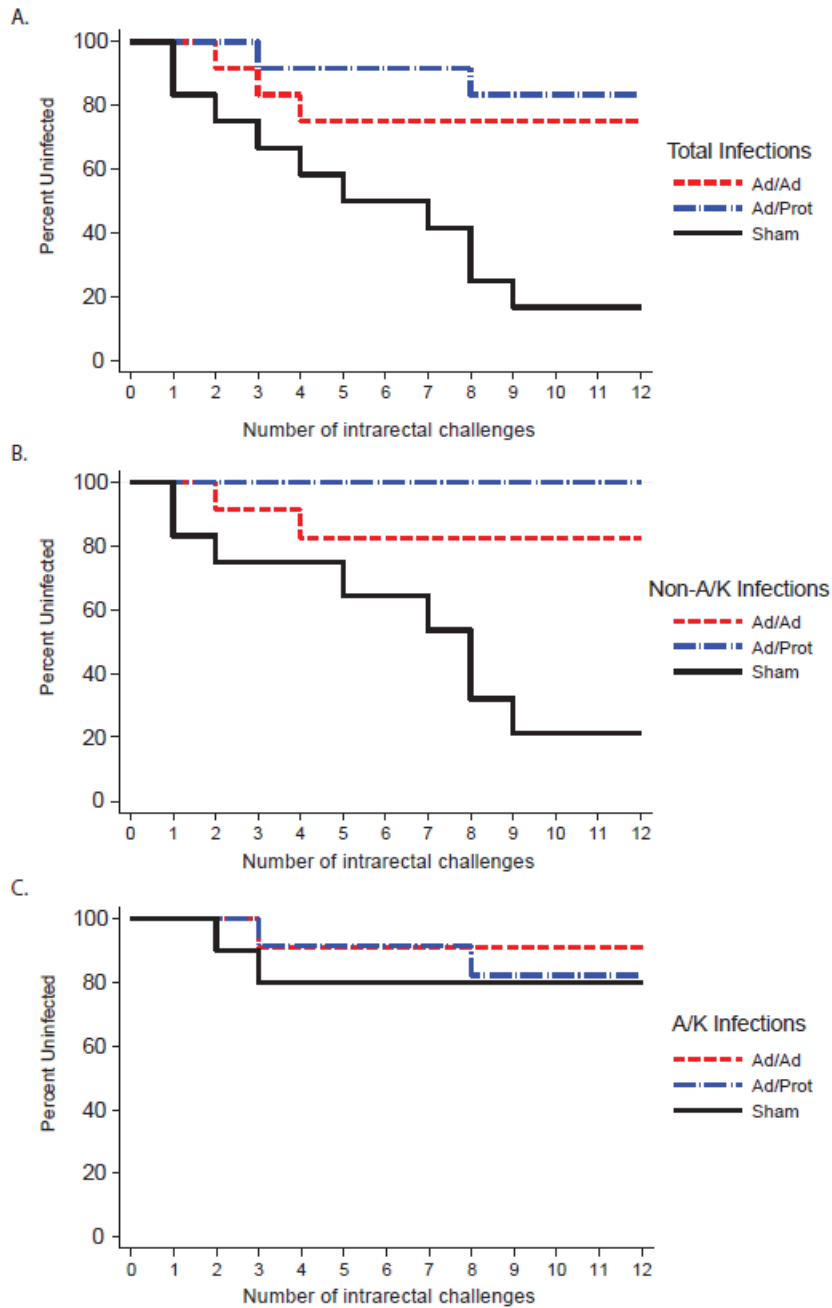
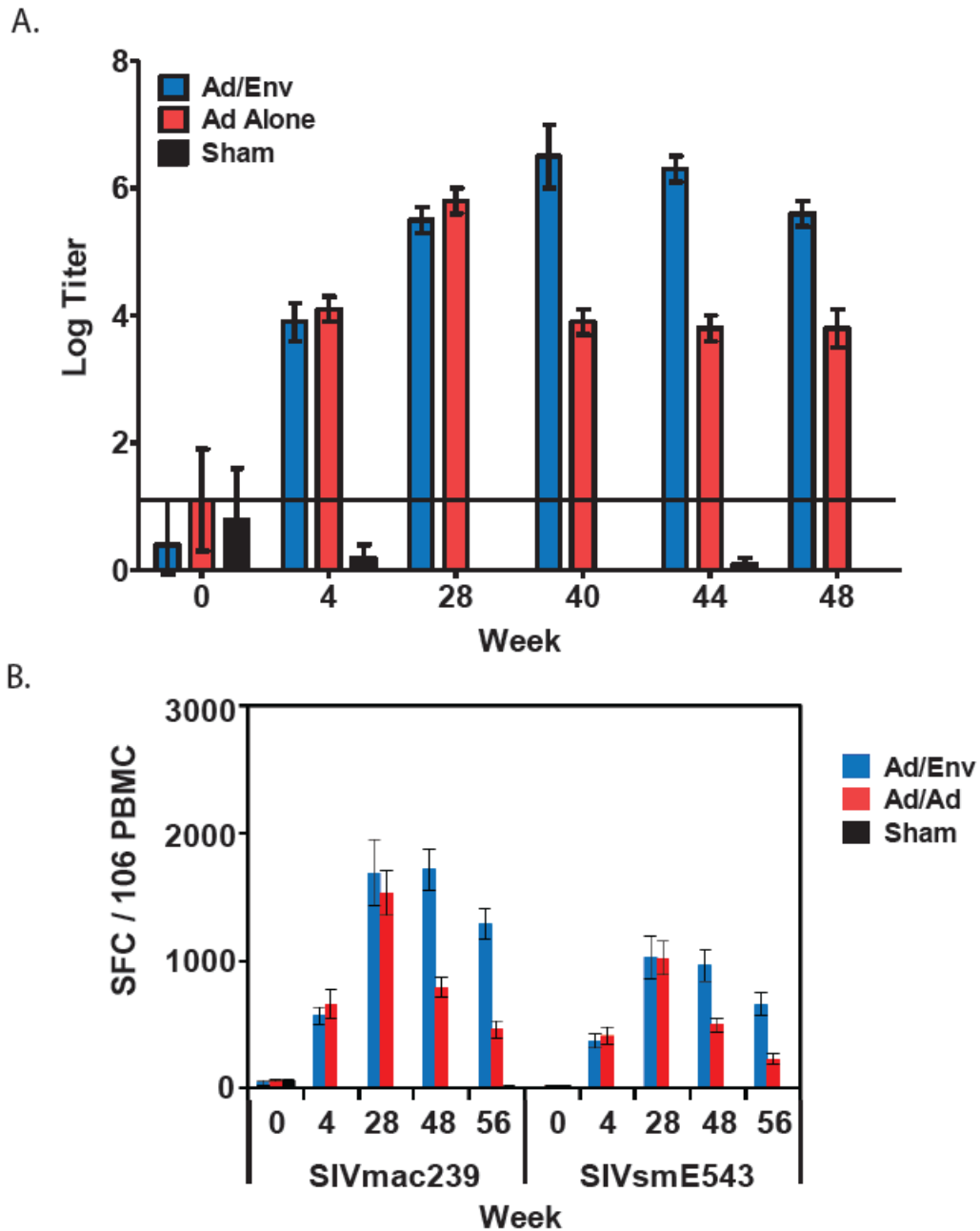


Supplementary Figure 1. Variant enumeration. Composite tree (A) and representative tree and highlighter alignments (B-D) demonstrate the ability to discriminate animals infected with a single T/F genomes (B), two TF variants (C) or three T/F variants (D). Each T/F lineage is color coded and mapped onto the composite tree. Recombinant genomes are identified phylogenetically and are shown in grey. Individual polymorphisms are shown as individual tick-marks in alignment.



Supplementary Figure 2. Protective efficacy stratified by neutralization resistance in the RLD challenge study. The number of challenges required for detection of productive infection for each vaccine group separated into all T/F genomes (A), non-A/K (B), and A/K (C). Kaplan-Meier plots denote the percent of uninfected animals after each challenge. Ad/Env (blue) indicates animals primed with Ad35/Ad26 and boosted with adjuvanted Env gp140 (Ad35/Ad26/Env). Ad/Ad (red) indicates animals vaccinated with Ad35/Ad26 only.



Supplementary Figure 3. Humoral and cellular immune responses in the SHD challenge study. Vaccine-induced binding antibodies titers (N=10/group) were determined by SIVmac251 Env ELISA at week 0, 4, 28, 40, 44, and 48 following vaccination (A). Cellular immune responses to SIVmac239 and SIVsmE543 Gag, Pol, and Env were determined by IFN- γ ELISPOT assay at weeks 0, 4, 28, 48, and 56 and reported as spot-forming cells (SFC) per million peripheral blood mononuclear cells (PBMC). Ad/Env (blue) indicates animals primed with Ad35/Ad26 and boosted with adjuvanted Env gp140 (Ad35/Ad26/Env). Ad/Ad (red) indicates animals vaccinated with Ad35/Ad26 only. Sham controls (black) were vaccinated with Ad35/Ad26 expressing no relevant antigens. Error bars represent standard error of mean (SEM).

Supplementary Table 1. Immunologic data

Log ELISA Titer (RLD Challenge Study)

Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad
Wk 0	Wk 0	Wk 4	Wk 4	Wk 28	Wk 28	Wk 40	Wk 40	Wk 44	Wk 44	Wk 48	Wk 48
0.0	0.0	4.3	4.8	5.8	5.3	6.3	4.8	6.3	5.3	5.8	4.8
0.0	0.0	4.3	4.3	5.8	5.3	6.3	5.3	5.8	5.3	6.3	5.3
0.0	0.0	4.3	4.8	5.8	5.3	6.7	4.8	5.8	5.3	6.3	4.8
0.0	0.0	4.3	4.3	5.8	5.8	6.7	4.8	5.8	5.3	6.7	5.3
0.0	0.0	3.8	3.8	5.8	5.3	6.7	4.8	5.8	4.8	6.3	4.8
0.0	0.0	4.3	3.8	5.8	5.3	6.3	4.8	5.8	5.3	5.8	4.8
0.0	0.0	3.8	4.3	5.8	5.8	6.3	5.3	6.3	5.3	6.3	5.3
0.0	0.0	4.3	4.3	5.8	5.3	6.3	4.8	5.8	4.8	6.7	4.8
0.0	0.0	4.3	4.3	5.3	5.8	5.8	4.8	5.8	5.3	5.3	5.3
0.0	0.0	4.3	4.3	6.3	5.8	6.3	5.3	5.8	5.8	5.3	5.3
0.0	0.0	3.8	3.8	5.3	5.3	5.8	5.3	5.8	5.3	5.8	5.3
0.0	0.0	4.3	4.3	6.7	5.3	6.3	5.3	5.8	4.8	6.3	5.3

Log ELISA Titer (SHD Challenge Study)

Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad	Ad/Env	Ad
Wk 0	Wk 0	Wk 4	Wk 4	Wk 28	Wk 28	Wk 40	Wk 40	Wk 44	Wk 44	Wk 48	Wk 48
0.0	1.4	3.8	3.8	5.3	5.8	6.7	3.8	6.7	3.8	5.8	3.8
1.4	1.4	3.8	3.8	5.8	6.3	6.7	3.8	6.3	3.8	5.8	3.8
0.0	1.4	3.8	4.8	5.8	5.3	6.3	3.8	6.3	3.8	5.3	3.8
0.0	0.0	4.3	4.3	5.3	5.3	6.7	3.8	6.3	3.8	6.3	3.8
1.4	1.4	4.3	3.8	5.8	5.8	5.8	3.8	5.8	4.3	5.3	3.8
0.0	1.4	3.8	4.3	5.8	5.8	6.3	4.8	6.3	4.3	5.8	4.8
0.0	1.4	3.8	4.3	5.3	5.3	6.3	3.8	6.3	3.8	5.3	3.8
0.0	0.0	3.8	3.8	5.8	5.8	5.8	3.8	5.8	3.8	5.3	3.8
0.0	1.4	3.8	3.8	5.3	5.3	6.7	4.3	6.3	3.8	5.8	4.3
1.4	1.4	3.8	4.3	5.3	6.3	6.7	3.8	6.7	3.8	5.8	3.8

**Raw ELISPOT Data (Spot-Forming Cells / 200,000 PBMC)
Week 0**

Ad/Ad

R10	Median										Data												
	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM
2	0	0	2	2	2	1	2	1	1	1	903	218-10	4	0	0	1	2	1	1	0	1	1	889
3	5	4	6	13	12	4	3	2	1	5	476	219-10	9	0	0	5	9	3	2	2	1	1	937
2	2	2	1	5	6	2	0	2	2	2	495	220-10	3	2	3	5	17	3	5	1	1	1	977
1	1	1	3	4	3	2	1	2	2	2	468	221-10	2	8	4	6	9	14	5	0	2	1	974
4	4	5	4	5	2	2	2	2	2	5	471	222-10	2	1	3	0	4	10	1	0	2	1	976
3	1	2	1	6	7	2	2	2	0	1	389	223-10	2	0	2	0	2	4	4	2	1	2	957
2	1	2	3	11	16	2	3	3	1	6	499	224-10	0	2	0	5	8	1	0	3	3	3	976
1	2	3	1	9	3	1	2	2	3	2	392	225-10	3	4	2	6	3	4	1	3	0	1	977
3	1	4	3	11	3	2	1	7	0	1	616	226-10	1	1	1	1	5	6	1	1	3	2	944
4	2	2	4	4	1	5	4	4	3	4	540	227-10	3	5	4	5	6	5	2	1	3	2	944
												228-10	4	1	2	1	8	2	2	1	0	1	934
												229-10	3	1	3	1	10	17	2	2	1	0	930
												230-10	0	0	1	1	10	14	1	4	4	2	978
												231-10	2	1	2	1	9	2	0	1	3	2	988
												232-10	0	0	3	0	8	3	1	2	1	3	996
												233-10	2	0	5	1	4	2	2	1	7	0	936
												234-10	3	2	2	5	17	3	2	6	0	1	936
												235-10	3	2	3	6	1	2	3	5	4	1	941
												236-10	4	1	2	6	1	0	7	4	1	5	938

Ad/Env

R10	Median										Data												
	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM
2	4	5	5	11	8	2	1	2	1	1	551	219-10	2	4	2	5	10	8	2	1	2	2	955
4	5	3	2	18	8	2	2	2	2	3	535	220-10	7	4	7	4	12	7	1	1	1	1	936
1	4	25	3	53	16	2	2	5	2	1	777	221-10	4	1	3	2	9	9	2	3	0	1	923
4	1	1	1	13	12	1	1	2	3	2	417	222-10	3	0	2	1	27	7	0	3	2	4	946
1	4	4	2	4	3	2	1	3	2	1	337	223-10	1	4	0	4	20	11	1	0	2	2	929
3	4	2	3	2	4	3	5	3	1	4	541	224-10	1	3	49	2	36	19	3	1	7	1	925
1	1	2	2	17	1	2	14	1	0	2	578	225-10	3	1	1	1	26	12	0	1	2	3	948
2	2	4	5	55	17	2	4	2	2	3	372	226-10	5	1	1	0	9	12	1	0	1	1	986
6	5	4	5	9	5	2	6	8	3	3	574	227-10	1	1	2	3	24	2	1	26	2	0	944
4	2	2	2	2	1	2	4	3	3	1	556	228-10	3	2	5	2	85	21	0	4	0	1	936
												229-10	0	2	2	7	66	19	3	3	2	2	938
												230-10	11	7	7	2	13	7	1	8	9	3	982
												231-10	1	3	1	7	5	2	2	4	2	4	989
												232-10	1	3	4	2	2	1	2	3	4	2	950
												233-10	3	1	0	2	1	1	1	4	2	3	952

Sham

R10	Median										Data												
	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM
2	1	2	1	4	1	0	1	0	1	0	261	217-10	2	1	2	0	6	1	0	1	0	1	909
6	4	2	4	48	47	2	4	6	3	2	498	218-10	1	1	1	1	2	1	0	0	0	0	972
2	1	1	1	2	3	3	0	3	1	2	482	219-10	3	5	1	6	54	47	3	3	11	3	926
8	5	7	3	20	20	4	4	2	5	9	599	220-10	3	1	1	0	42	46	0	4	1	0	969
1	4	1	0	1	3	3	9	1	2	0	490	221-10	1	1	1	0	2	5	1	0	3	0	900
4	2	2	2	1	2	2	2	2	2	2	526	222-10	2	0	1	1	5	1	4	0	2	1	962
2	0	0	2	2	2	2	2	1	2	2	470	223-10	6	2	5	4	29	19	4	3	5	5	959
2	2	1	1	1	1	1	1	3	2	1	470	224-10	0	2	0	0	2	4	3	57	0	0	983
2	1	2	1	0	1	3	0	2	3	3	479	225-10	2	1	2	0	10	20	3	5	1	4	991
2	3	4	1	6	3	3	4	3	3	3	605	226-10	2	2	0	2	2	4	3	3	37	0	983
												227-10	2	1	2	1	0	2	4	1	3	1	989
												228-10	5	3	2	3	1	2	2	1	1	6	997
												229-10	1	0	0	0	2	3	1	0	0	1	955
												230-10	1	0	0	1	0	0	2	2	2	2	937
												231-10	2	0	0	1	1	0	2	0	2	2	964
												232-10	1	1	1	1	1	2	0	1	3	2	976
												233-10	2	0	2	1	0	1	4	0	0	3	981
												234-10	1	2	2	1	0	1	2	0	3	2	977
												235-10	0	3	6	1	6	3	3	2	2	1	928
												236-10	3	1	1	1	6	3	2	1	3	2	981

Raw ELISPOT Data (Spot-Forming Cells / 200,000 PBMC)
Week 4

Ad/Ad

Median												Data												
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
0	146	17	14	12	7	131	6	4	20	5	713	218-10	0	155	16	12	14	6	128	6	15	3	693	
1	15	65	3	71	5	14	26	12	43	0	488	218-10	0	157	83	15	10	6	124	4	24	0	728	
1	30	165	10	46	93	27	14	11	44	21	456	220-10	0	35	25	1	61	1	18	35	13	42	0	488
1	129	13	17	5	3	89	4	9	2	1	448	220-10	0	35	67	5	81	9	9	17	11	41	0	487
0	13	9	7	1	1	17	5	2	3	1	203	222-10	1	25	124	0	42	26	37	15	6	15	0	509
1	71	22	11	5	7	69	12	13	1	4	389	222-10	1	37	175	9	49	87	27	15	13	38	1	442
1	10	13	15	8	14	9	8	18	4	4	548	223-10	0	121	13	17	6	3	88	4	11	3	1	454
1	23	13	6	11	5	10	7	8	7	4	383	223-10	0	117	13	16	3	3	79	4	7	1	1	441
0	13	8	8	7	1	8	2	3	8	2	382	226-10	0	11	10	1	1	1	20	4	2	2	1	147
14	76	46	32	28	34	63	34	28	15	36	448	226-10	0	25	7	9	0	1	13	3	1	3	1	238
												227-10	1	66	15	10	0	6	67	11	13	3	6	381
												227-10	1	76	29	11	4	8	71	13	12	0	2	385
												229-10	1	8	15	17	10	14	8	7	17	4	2	331
												229-10	1	12	11	13	5	13	10	8	18	4	4	548
												239-10	0	23	10	7	10	6	9	10	5	7	2	421
												244-10	0	22	15	5	11	4	11	10	6	9	1	345
												244-10	0	9	8	10	6	1	7	1	2	6	1	347
												245-10	0	17	8	6	8	1	9	9	3	10	9	417
												245-10	14	64	39	37	23	39	60	32	27	17	28	488
												245-10	14	68	52	26	33	29	66	26	29	13	43	428

Ad/Env

Median												Data												
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
1	23	16	11	5	8	11	5	19	7	13	549	219-10	0	16	19	8	6	7	10	5	15	9	11	534
1	42	63	41	32	12	24	31	34	14	5	557	219-10	1	29	16	13	4	9	11	5	22	7	7	513
2	39	55	12	6	5	47	20	4	8	6	511	221-10	0	41	63	17	37	17	34	41	10	3	1	576
1	73	16	17	44	15	73	2	13	45	8	555	221-10	1	49	63	45	28	6	21	28	28	18	0	537
1	13	19	31	9	4	10	14	16	9	12	507	225-10	2	37	65	13	4	1	48	20	6	7	8	528
2	143	35	58	13	47	129	7	29	4	33	601	230-10	2	40	43	11	3	4	46	20	4	8	4	494
2	7	16	30	6	5	4	4	6	5	4	468	230-10	1	84	28	18	45	13	82	9	12	10	7	576
0	14	15	11	28	21	12	6	13	22	13	367	230-10	0	61	17	15	47	16	64	0	13	40	0	512
6	32	21	18	24	10	19	5	3	15	2	552	233-10	3	14	11	23	3	2	5	9	14	14	11	496
3	6	11	4	4	5	4	3	4	6	2	449	234-10	0	12	26	38	14	6	15	19	17	4	13	517
												234-10	3	156	39	53	16	37	118	6	29	2	32	601
												235-10	1	139	30	63	10	56	140	7	29	6	34	601
												235-10	0	8	15	30	2	3	4	2	2	0	0	479
												235-10	3	8	17	29	2	3	4	6	9	9	9	479
												231-10	0	10	5	13	15	17	13	5	16	20	9	342
												240-10	0	28	26	9	60	24	13	7	9	21	17	392
												240-10	8	29	15	13	16	9	16	7	3	14	2	569
												246-10	3	25	27	22	32	10	21	2	15	2	2	524
												246-10	5	7	15	3	8	2	3	4	4	2	3	457
												246-10	1	4	7	4	0	7	4	9	3	9	1	440

Sham

Median												Data												
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
0	1	1	1	1	0	1	2	0	3	0	46	217-10	0	0	1	0	0	0	1	0	2	0	0	18
1	1	1	1	0	2	0	0	0	1	1	409	217-10	0	0	1	1	0	0	0	1	0	4	0	14
1	1	0	0	0	1	0	0	0	1	1	263	224-10	1	1	2	1	0	2	0	0	0	1	1	408
1	3	6	2	1	1	1	4	0	1	1	414	228-10	2	0	0	0	2	0	0	0	0	2	0	410
1	1	1	0	0	1	0	1	1	0	0	420	228-10	0	1	0	0	0	0	0	0	0	0	1	291
0	0	0	1	2	1	0	0	1	1	0	392	230-10	1	1	5	3	0	2	1	0	3	0	1	412
0	1	0	1	0	2	1	1	1	1	1	218	236-10	1	1	0	0	0	1	0	1	0	0	0	490
0	1	0	1	0	2	1	1	1	1	1	218	236-10	1	0	0	0	0	1	0	1	0	0	0	490
0	1	1	0	1	0	0	0	0	0	1	183	237-10	0	0	0	0	0	1	0	0	1	0	0	355
0	0	1	0	1	0	1	0	1	1	0	332	237-10	0	0	0	1	4	0	0	0	1	1	0	428
2	2	1	1	0	0	1	0	1	1	0	349	238-10	0	0	0	0	0	0	1	0	1	0	0	193
												241-10	0	0	1	0	0	0	0	0	1	0	0	187
												242-10	0	0	0	0	1	0	0	0	1	0	0	242
												241-10	0	1	0	0	0	0	0	0	0	0	1	179
												242-10	0	0	0	0	0	0	0	0	0	0	0	187
												242-10	0	0	1	0	0	0	0	0	0	0	0	275
												243-10	2	1	0	1	0	0	2	0	1	0	0	345
												243-10	3	0	1	1	0	0	0	0	0	1	0	353

Raw ELISPOT Data (Spot-Forming Cells / 200,000 PBMC)
Week 28

Ad/Ad

Median											Data													
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
1	198	38	26	48	17	178	4	22	32	11	*	218-10	1	192	37	28	52	11	167	4	25	36	5	*
0	66	278	2	230	9	17	148	13	201	1	**	218-10	0	263	283	23	22	189	4	22	24	17	**	
1	22	185	6	46	116	26	15	1	30	8	**	220-10	0	59	283	2	224	15	155	12	192	0	**	
1	156	30	32	43	16	137	12	23	41	10	**	220-10	0	53	273	2	236	10	139	13	209	1	*	
1	76	45	13	12	7	62	19	15	5	11	**	222-10	0	20	180	5	51	113	18	20	0	20	8	**
17	121	77	57	38	22	91	42	32	17	20	**	223-10	0	158	32	31	38	18	153	12	20	45	5	*
1	18	26	47	24	41	13	8	44	17	9	**	223-10	0	155	78	33	48	14	121	11	20	37	14	*
1	15	21	43	19	8	9	7	14	18	1	**	226-10	0	25	42	10	16	1	55	12	12	7	8	*
1	45	47	48	34	5	31	34	76	17	2	**	226-10	1	77	48	16	7	10	68	20	17	3	13	*
0	72	45	57	17	68	73	27	9	3	59	*	227-10	0	139	86	50	28	16	82	40	18	17	17	**
												227-10	28	131	69	64	36	28	89	42	26	16	26	*
												229-10	0	16	25	43	20	51	8	7	36	16	8	*
												229-10	1	20	26	53	27	30	18	8	52	18	0	*
												244-10	0	44	19	42	16	11	9	6	13	23	4	*
												244-10	1	36	23	24	21	5	27	37	24	12	1	*
												245-10	0	49	47	60	20	3	37	31	82	18	1	*
												245-10	0	49	47	56	37	7	34	35	76	16	2	*
												245-10	0	79	44	66	20	71	83	31	10	4	52	*
												245-10	0	64	45	47	13	65	82	23	7	1	66	*

Ad/Env

Median											Data														
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM		
1	15	25	21	8	15	11	6	23	11	9	*	219-10	0	14	27	13	7	16	12	4	20	10	13	**	
2	185	163	364	57	48	138	99	43	26	46	*	221-10	2	190	182	152	60	56	146	117	44	30	51	*	
0	70	84	16	33	51	84	50	17	23	40	**	225-10	0	119	143	175	54	39	129	80	27	22	43	**	
0	173	19	41	72	8	198	4	12	50	17	**	230-10	0	72	77	12	37	17	87	53	9	25	49	*	
1	16	7	23	18	12	5	5	2	8	4	**	232-10	0	193	16	48	48	31	8	192	4	12	53	29	**
0	336	59	145	77	308	320	22	43	42	201	*	234-10	0	331	57	163	79	310	135	22	44	37	195	*	
1	32	73	62	47	18	22	35	48	20	12	**	235-10	0	349	60	127	25	306	304	22	41	46	207	*	
1	24	28	35	88	70	30	19	15	14	52	**	235-10	1	27	86	56	40	26	14	24	45	21	12	*	
2	118	67	31	60	13	96	18	19	34	2	**	231-10	0	26	31	37	100	88	30	20	13	15	41	*	
0	51	222	26	35	41	27	17	6	30	8	*	240-10	2	124	64	34	55	14	103	38	23	37	0	*	
												246-10	0	111	26	27	86	11	88	18	14	80	6	**	
												246-10	0	54	213	25	40	42	32	19	6	35	6	*	
												246-10	0	47	230	27	29	39	21	15	5	25	10	*	

Sham

Median											Data													
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
1	1	1	1	1	0	1	0	0	1	0	*	217-10	1	0	1	1	1	0	1	0	0	1	1	*
1	1	0	1	2	1	1	1	0	1	1	*	224-10	1	1	1	0	0	0	0	0	0	0	0	*
1	0	1	1	1	1	1	0	0	0	0	**	228-10	0	1	0	1	2	1	1	0	0	2	0	*
1	1	3	1	5	2	2	3	0	0	2	**	230-10	2	0	1	0	1	1	0	0	0	0	0	*
1	4	2	2	2	4	3	1	3	0	0	**	236-10	1	1	2	1	5	2	2	4	0	0	3	*
1	2	1	1	4	1	1	0	0	1	1	**	237-10	1	2	1	0	1	1	0	0	0	1	1	*
0	1	1	1	3	1	1	0	1	0	0	*	238-10	0	1	0	1	5	0	1	0	0	0	1	*
1	1	1	0	1	1	2	1	2	0	1	*	241-10	1	1	1	1	1	2	1	1	2	0	1	*
0	1	1	0	1	0	0	1	1	1	1	*	242-10	0	0	0	0	1	0	0	0	0	1	0	*
1	2	0	0	1	1	0	0	0	0	0	**	243-10	0	1	1	0	1	0	0	1	1	0	0	*
												243-10	0	1	0	0	1	1	0	0	0	0	0	*

Raw ELISPOT Data (Spot-Forming Cells / 200,000 PBMC)
Week 48

Ad/Ad

R10	Median										Data														
	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM	R10	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM		
1	71	13	14	17	5	63	5	8	12	6	272	218-10	1	80	19	11	39	4	62	7	13	13	6	255	
0	24	114	5	100	4	9	64	4	65	2	191	218-10	2	52	12	17	34	4	64	5	10	5	229		
1	11	87	8	25	80	5	8	1	24	10	185	220-10	0	16	111	8	56	3	65	2	20	2	174		
4	40	8	8	12	4	31	7	7	8	3	264	222-10	0	10	82	11	39	26	4	6	2	15	11	194	
3	44	27	8	10	5	30	12	16	4	7	250	223-10	1	43	31	9	10	4	32	4	5	2	4	275	
2	68	57	37	13	18	75	15	27	9	7	183	226-10	2	27	6	4	13	4	30	8	9	14	1	271	
2	5	16	20	9	15	6	3	16	4	6	275	227-10	3	39	22	7	6	2	31	11	13	3	6	245	
0	12	11	7	16	4	6	2	4	6	1	195	227-10	2	67	48	15	11	16	64	15	24	11	9	180	
1	12	9	20	12	7	4	6	14	2	0	265	229-10	1	6	15	18	7	16	5	1	10	3	4	279	
2	61	22	38	24	58	42	18	3	6	33	291	230-10	2	3	16	22	31	13	7	4	22	4	4	284	
												234-10	1	16	9	6	11	1	6	1	4	5	0	214	
												234-10	0	16	12	25	21	6	6	3	3	6	1	175	
												244-10	1	14	10	15	14	6	4	6	14	3	0	284	
												245-10	2	62	19	31	26	64	36	22	4	7	33	307	
												245-10	1	60	25	45	22	52	48	33	1	4	4	33	274

Ad/Env

R10	Median										Data														
	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM	R10	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM		
1	14	94	58	10	11	5	14	83	6	6	333	219-10	2	11	87	48	11	19	4	14	9	5	334		
1	90	75	103	38	23	47	25	54	23	26	312	221-10	0	17	103	67	8	12	6	36	75	2	6	331	
0	10	56	32	4	7	13	36	25	6	3	262	225-10	0	80	78	83	28	23	18	31	62	23	26	316	
2	89	60	51	34	12	86	2	31	21	6	247	225-10	1	69	72	122	47	22	35	29	46	3	28	307	
0	6	15	113	11	5	5	4	17	3	2	122	230-10	0	13	62	33	6	9	9	25	32	7	4	254	
4	151	189	260	45	120	155	17	178	19	73	268	232-10	4	67	54	53	26	13	81	1	29	25	69	338	
1	10	59	65	16	13	10	10	46	7	7	295	232-10	0	81	65	49	41	11	32	3	17	2	235		
1	15	84	75	75	44	18	18	50	7	36	227	233-10	0	5	15	124	14	4	6	3	20	6	2	136	
5	55	125	156	43	6	51	13	147	18	3	293	234-10	2	6	14	100	8	5	14	0	6	0	1	137	
2	27	163	53	17	21	10	46	42	13	7	313	235-10	1	160	176	239	44	108	149	14	171	19	74	295	
												235-10	2	141	202	280	46	112	161	159	184	18	72	241	
												236-10	0	10	55	74	19	10	9	19	40	6	3	302	
												236-10	1	9	63	56	12	16	10	52	5	10	288		
												231-10	0	18	90	84	70	49	16	22	54	7	35	208	
												240-10	2	21	77	77	66	71	39	20	14	35	7	37	246
												240-10	4	55	141	154	49	7	50	146	12	1	1	289	
												246-10	2	46	109	147	37	5	54	148	24	4	4	297	
												246-10	1	30	164	155	36	22	11	49	17	14	9	335	
												246-10	1	23	162	50	18	19	8	43	36	12	11	291	

Sham

R10	Median										Data													
	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM	R10	SIvmac239 Gag	SIvmac239 Env1	SIvmac239 Env2	SIvmac239 Pol1	SIvmac239 Pol2	SIvm543 Gag	SIvm543 Env1	SIvm543 Env2	SIvm543 Pol1	SIvm543 Pol2	PHAM	
2	2	2	3	22	4	2	1	2	1	1	298	217-10	0	0	1	2	19	4	1	1	0	0	104	
5	1	2	1	21	6	1	1	1	1	1	417	224-10	3	3	3	3	25	3	2	0	3	2	1	292
0	0	2	6	0	1	2	0	1	2	1	301	224-10	4	1	0	0	8	0	1	0	1	0	1	424
1	1	4	4	3	1	1	6	0	2	1	323	229-10	0	0	0	5	0	0	2	0	0	1	1	420
1	1	0	4	1	3	6	1	0	2	0	247	229-10	0	0	3	6	0	2	1	0	1	3	1	304
1	1	1	1	4	1	0	2	0	0	0	289	230-10	1	1	3	2	3	0	2	4	0	3	1	297
1	1	2	1	3	2	1	0	1	0	0	285	236-10	0	0	0	4	0	0	7	0	0	3	0	313
1	1	2	3	2	2	0	3	1	1	1	247	236-10	1	1	1	1	2	0	5	1	0	3	0	288
1	2	2	3	2	2	0	3	1	1	1	247	237-10	1	1	1	1	2	0	2	0	0	0	0	160
1	2	1	2	9	34	1	1	1	1	0	338	237-10	1	1	0	1	4	1	1	0	0	0	0	289
1	1	0	2	8	5	1	1	1	1	0	320	238-10	1	1	1	1	1	1	0	0	0	0	0	307
												241-10	0	0	1	2	4	1	0	2	2	1	1	245
												242-10	0	2	1	1	10	0	1	1	0	0	0	152
												243-10	1	3	0	2	8	67	0	1	1	1	0	134
												243-10	0	0	0	0	0	3	4	0	1	1	0	114
												243-10	1	1	0	3	11	5	1	0	0	0	0	125

Raw ELISPOT Data (Spot-Forming Cells / 200,000 PBMC)
Week 56

Ad/Ad

Median											Data															
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM			
1	49	11	6	19	5	44	1	5	6	3	685	218-10	1	38	12	4	21	6	34	1	5	7	712			
2	32	113	5	120	8	5	68	2	67	0	450	218-10	2	39	12	6	16	3	34	1	5	7	638			
0	9	55	3	23	55	7	0	1	9	2	449	220-10	1	36	117	5	125	11	6	23	1	74	0	466		
2	39	7	4	9	6	32	4	9	9	2	526	222-10	0	0	0	4	20	0	0	0	1	11	2	465		
0	29	9	8	3	2	23	9	8	2	3	599	223-10	3	34	9	3	8	8	35	5	12	8	1	538		
0	38	12	16	6	4	24	7	5	0	3	393	226-10	0	25	9	4	10	4	2	25	0	10	0	4	605	
1	2	7	10	4	9	2	1	11	3	4	533	227-10	0	32	8	8	1	2	20	8	5	3	2	593		
0	5	9	6	6	0	4	2	3	5	1	274	227-10	0	41	14	16	3	1	23	3	6	0	0	3	393	
2	25	15	19	21	4	5	6	18	2	2	483	229-10	0	55	10	15	7	5	25	7	4	0	2	3	393	
1	36	13	19	9	28	26	7	8	2	27	515	229-10	0	1	6	13	3	10	1	0	9	2	3	499		
												230-10	1	2	4	7	4	8	3	2	12	2	4	2	6	567
												230-10	0	2	4	7	10	0	4	3	1	6	1	1	317	
												230-10	0	8	14	5	2	0	3	1	2	3	0	2	240	
												244-10	0	25	18	16	21	2	4	7	15	0	0	3	489	
												244-10	4	24	11	22	20	6	5	6	20	3	1	4	476	
												245-10	0	37	11	21	12	27	31	4	7	3	24	527		
												245-10	1	34	14	17	5	25	21	9	9	0	0	30	500	

Ad/Env

Median											Data													
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
0	9	82	36	5	7	7	10	78	4	2	584	219-10	0	12	32	23	7	7	9	85	4	0	0	563
0	126	68	107	42	22	51	31	47	16	20	472	219-10	0	6	71	42	2	6	31	75	3	3	0	605
2	20	145	110	7	15	20	53	59	3	4	510	221-10	0	110	84	108	48	23	54	43	44	12	25	465
1	66	46	37	41	8	63	4	18	23	4	374	221-10	0	111	81	105	39	21	47	39	49	19	18	479
1	5	30	93	11	5	4	12	20	7	3	295	225-10	1	35	112	102	4	15	54	46	64	1	1	530
2	129	192	194	22	85	119	17	194	22	40	559	230-10	1	25	152	118	10	10	26	59	54	4	4	490
2	5	35	52	14	8	3	7	36	3	3	514	230-10	1	46	54	40	40	9	71	4	22	23	6	381
0	13	59	44	39	37	10	12	22	11	28	484	230-10	0	65	37	13	34	6	55	4	9	24	3	361
3	26	71	162	14	4	33	8	95	13	2	534	233-10	0	6	26	92	8	1	4	12	15	10	2	318
2	29	144	77	13	13	18	28	51	17	6	587	234-10	1	4	14	34	14	4	11	24	4	4	3	273
												234-10	2	116	206	178	48	80	126	15	195	29	47	576
												235-10	2	121	178	209	25	89	111	29	193	14	33	542
												235-10	2	4	39	48	11	3	3	9	14	6	2	517
												235-10	0	12	51	33	47	32	10	33	21	11	33	497
												238-10	0	24	66	54	31	42	10	20	22	11	22	470
												240-10	0	29	83	160	17	5	37	7	78	9	1	507
												240-10	1	28	59	164	11	8	28	8	111	17	6	584
												246-10	1	29	152	184	34	11	12	37	150	18	6	585
												246-10	2	28	136	70	12	14	23	39	52	16	5	589

Sham

Median											Data													
R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	R10	SIVmac239 Gag	SIVmac239 Env1	SIVmac239 Env2	SIVmac239 Pol1	SIVmac239 Pol2	SIVsm543 Gag	SIVsm543 Env1	SIVsm543 Env2	SIVsm543 Pol1	SIVsm543 Pol2	PHAM	
1	1	1	2	6	2	0	2	1	0	2	558	217-10	0	1	1	0	1	4	0	0	1	0	3	152
3	1	1	3	5	5	1	1	1	0	1	518	217-10	2	1	0	3	3	8	0	0	0	0	1	563
1	2	0	0	1	2	1	0	0	1	0	504	224-10	0	1	0	5	4	4	1	1	1	0	0	518
2	1	5	3	2	1	1	2	0	1	1	541	228-10	0	1	0	0	1	1	1	0	0	1	0	495
1	2	0	1	0	1	0	0	0	0	0	528	230-10	2	2	5	2	2	2	0	0	0	0	0	513
1	1	0	1	1	1	0	2	1	2	1	557	230-10	1	0	0	0	0	0	0	0	0	0	0	532
1	1	0	1	1	1	1	0	0	1	1	550	236-10	1	0	0	1	0	0	0	0	0	0	0	555
0	0	0	1	3	2	1	0	2	0	0	527	237-10	1	1	0	1	1	1	0	2	0	0	1	577
0	0	1	0	1	0	0	0	0	0	0	340	237-10	0	0	0	1	8	1	0	1	1	3	0	587
1	0	0	0	1	1	1	0	0	1	1	550	238-10	1	0	0	0	0	0	0	0	0	0	1	538
0	0	0	0	1	3	2	1	0	2	0	527	241-10	0	0	0	0	2	1	1	0	3	0	0	572
0	0	0	0	1	0	0	0	0	0	0	340	241-10	0	0	0	0	1	2	0	0	0	0	0	482
1	0	0	0	1	1	1	0	1	0	1	413	242-10	0	0	1	0	0	0	0	0	0	0	0	331
												243-10	0	0	0	0	1	0	0	0	0	0	0	348
												243-10	1	0	0	0	0	1	1	0	0	0	0	422
												243-10	0	0	0	0	0	0	1	0	1	0	1	403

Supplementary Table 2. P values Following Fisher's Exact/Permutation Tests of All Informative Sites

Amino Acid Site	Sham vs. Stock	Ad35/Ad26 vs. Stock	Ad35/Ad26/Env vs. Stock	Ad35/Ad26 vs. Sham	Ad35/Ad26/Env vs. Sham	Ad35/Ad26 vs. Ad35/Ad26/Env	Ad35/Ad26 + Ad35/Ad26/Env vs. Stock	Ad35/Ad26 + Ad35/Ad26/Env vs. Sham
23	0.016	0.007	0.018	0.392	0.390	1.000	0.001	0.258
45	0.013	<0.001	0.002	0.032	0.090	1.000	<0.001	0.013
47	0.181	<0.001	<0.001	0.001	0.007	1.000	<0.001	<0.001
70	<0.001	<0.001	<0.001	0.001	0.008	1.000	<0.001	<0.001
130	0.670	0.653	1.000	0.582	1.000	1.000	1.000	0.642
132	1.000	0.768	0.449	0.771	0.451	1.000	0.427	0.433
133	0.574	0.345	0.170	0.650	0.103	0.070	1.000	0.679
134	0.831	0.569	0.448	0.773	0.448	1.000	0.297	0.432
137	0.670	0.380	1.000	0.159	1.000	0.541	0.688	0.377
138	0.132	0.160	0.198	1.000	0.034	0.040	0.603	0.371
139	1.000	0.784	0.448	0.787	0.286	0.647	0.447	0.331
140	0.481	0.652	0.055	1.000	0.015	0.035	0.680	0.237
141	0.481	0.652	0.055	1.000	0.015	0.035	0.680	0.237
142	0.595	0.652	0.055	1.000	0.016	0.035	0.680	0.322
143	0.598	0.821	0.032	1.000	0.015	0.035	0.540	0.237
144	0.821	1.000	0.679	0.771	0.451	1.000	0.583	0.433
154	0.118	0.539	0.338	0.672	1.000	1.000	0.567	0.480
161	0.038	0.784	0.105	0.060	0.776	0.137	0.644	0.222
185	0.841	0.464	1.000	0.326	0.740	1.000	0.519	0.387
186	0.005	0.539	0.019	0.102	0.702	0.095	0.157	0.399
208	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
355	0.479	0.661	0.578	1.000	1.000	1.000	0.398	1.000
380	0.306	0.436	1.000	1.000	0.480	0.445	0.501	1.000
424	0.271	0.613	0.723	0.164	1.000	0.460	1.000	0.318
428	0.284	0.330	0.726	0.064	1.000	0.285	0.823	0.161
433	0.644	0.763	0.679	1.000	0.451	0.647	1.000	0.794
486	0.211	1.000	0.019	0.319	0.136	0.030	0.304	1.000
721	0.592	1.000	0.356	1.000	0.427	0.375	0.546	1.000
726	0.497	0.320	1.000	1.000	1.000	1.000	0.411	1.000
760	1.000	1.000	0.338	1.000	0.437	0.326	1.000	1.000
762	0.606	1.000	0.042	1.000	0.015	0.030	0.400	0.298
763	0.536	0.303	0.035	0.099	0.091	0.008	0.715	1.000
770	1.000	0.512	0.382	0.519	0.386	0.128	1.000	1.000
780	0.621	0.539	1.000	1.000	1.000	1.000	1.000	1.000
805	0.494	0.391	0.019	0.830	0.044	0.099	0.079	0.246
808	0.490	0.375	0.038	0.660	0.080	0.285	0.073	0.232
820	0.864	0.271	0.250	0.382	0.386	1.000	0.120	0.181
830	1.000	0.549	1.000	0.552	1.000	1.000	0.267	0.280
881	0.233	0.813	1.000	0.601	0.502	1.000	0.832	0.495
885	0.540	1.000	1.000	0.601	0.502	1.000	0.828	0.495
888	0.160	0.813	0.759	0.427	0.290	0.730	1.000	0.255

Note: After Bonferroni corrections, p-values<0.00625 considered significant