

## Legends to Supplementary Material

**Fig. S1.** Group mean titer ( $\pm$ SEM) of rabbit HIV-1 CN54gp140 and HSP70 specific IgG and IgA concentrations after intravaginal immunizations with the HIV-HSP70 vaccine (n=4) or saline control (n=4) before and 16 weeks after immunization (Im.). No significant differences were found between baselines and 16 weeks post-immunization or saline and vaccine injection.

**Fig. S2.** Correlation between (A) MIP-1 $\beta$  (pg/ml) and APOBEC3G (%), between CCR5 in CD4<sup>+</sup> T cells (%) and proliferation (%) of (B) unstimulated, (C) HIVgp140 stimulated and (D) HSP70 PBMC cultures.

**Table S1.** (A) Consort Checklist of subjects enrolled. (B) Severity of adverse events and relationship to vaccine.

**Table S2.** Sequential plasma RANTES (CCL-5) concentration (ng/ml) of each vaccinated female at 0, 4 and 12 weeks, sampled at 0 (pre-), 4, 8, 16 and 20 weeks after immunization. The mean ( $\pm$ sem) is presented and the p value was calculated using the Wilcoxon rank sum test. (n=8).

**Table S3.** Sequential concentrations in pg/ml of (A) MIP-1 $\alpha$  (CCL-3), (B) MIP-1 $\beta$  (CCL-4) of the culture supernatant from CD4<sup>+</sup> T cells in unstimulated (Medium), HIVgp140 and HSP70 stimulated cells from each vaccinated female at 0, 4 and 12 weeks, sampled at 0 (pre-), 4, 8, 16 and 20 weeks. The mean ( $\pm$ sem) are presented and the p value was calculated using the Wilcoxon rank sum test: (n=7 as one culture supernatant was lost).

**Table S4.** Sequential percentage of CCR5 expression in CD4<sup>+</sup> T cells of each vaccinated female at 0, 4 and 12 weeks, sampled at 0 (pre-), 4, 8 and 20 weeks. The mean ( $\pm$ sem) are presented and the p value was calculated using the Wilcoxon rank sum test (n=7).

**Table S5.** Sequential percentage of APOBEC3G of each vaccinated female at 0, 4 and 12 weeks, sampled at 0 (pre-), 4, 8, 16 and 20 weeks. The mean ( $\pm$ sem) are presented and the p value was calculated using the Wilcoxon rank sum test. (n=8).

**Table S6.** Sequential percentage of (A) PBMC, (B) CD4<sup>+</sup> T cells and (C) CD8<sup>+</sup> T cell proliferation of unstimulated, HIVgp140 and HSP70 stimulated cell cultures in each vaccinated female at 0, 4 and 12 weeks, sampled at 0 (pre-), 4, 8, 16 and 20 weeks. The mean ( $\pm$ sem) are presented and the p value was calculated using the Wilcoxon rank sum test. (n=8).

**Table S7.** The cytokines IL-12 and IL-6 were assayed by intracellular staining with the corresponding monoclonal antibodies of CD4<sup>+</sup> T cells before (0) and 20 weeks (8 weeks after the 3<sup>rd</sup> immunization). The data are presented in each of the immunized females (n=6, available cells) as % and as the net change; pre-immunization % subtracted from the post-immunization %. The corresponding viral loads (VL) are given pre- and post-immunization as area under the curve (AUC) and the net change as above.

Fig. S1

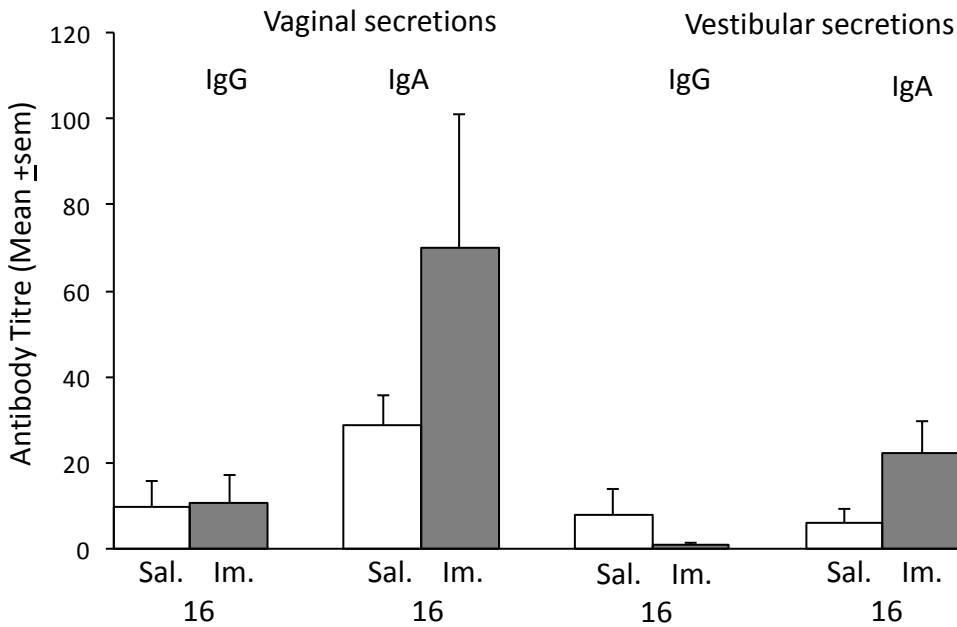
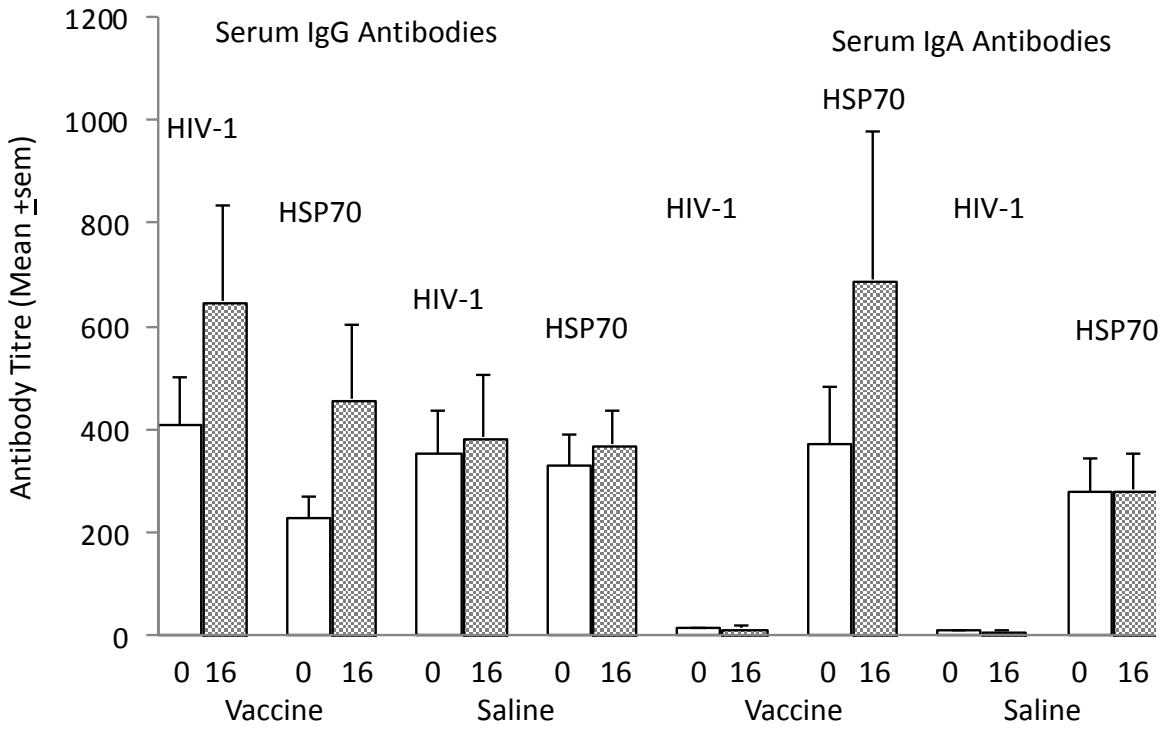
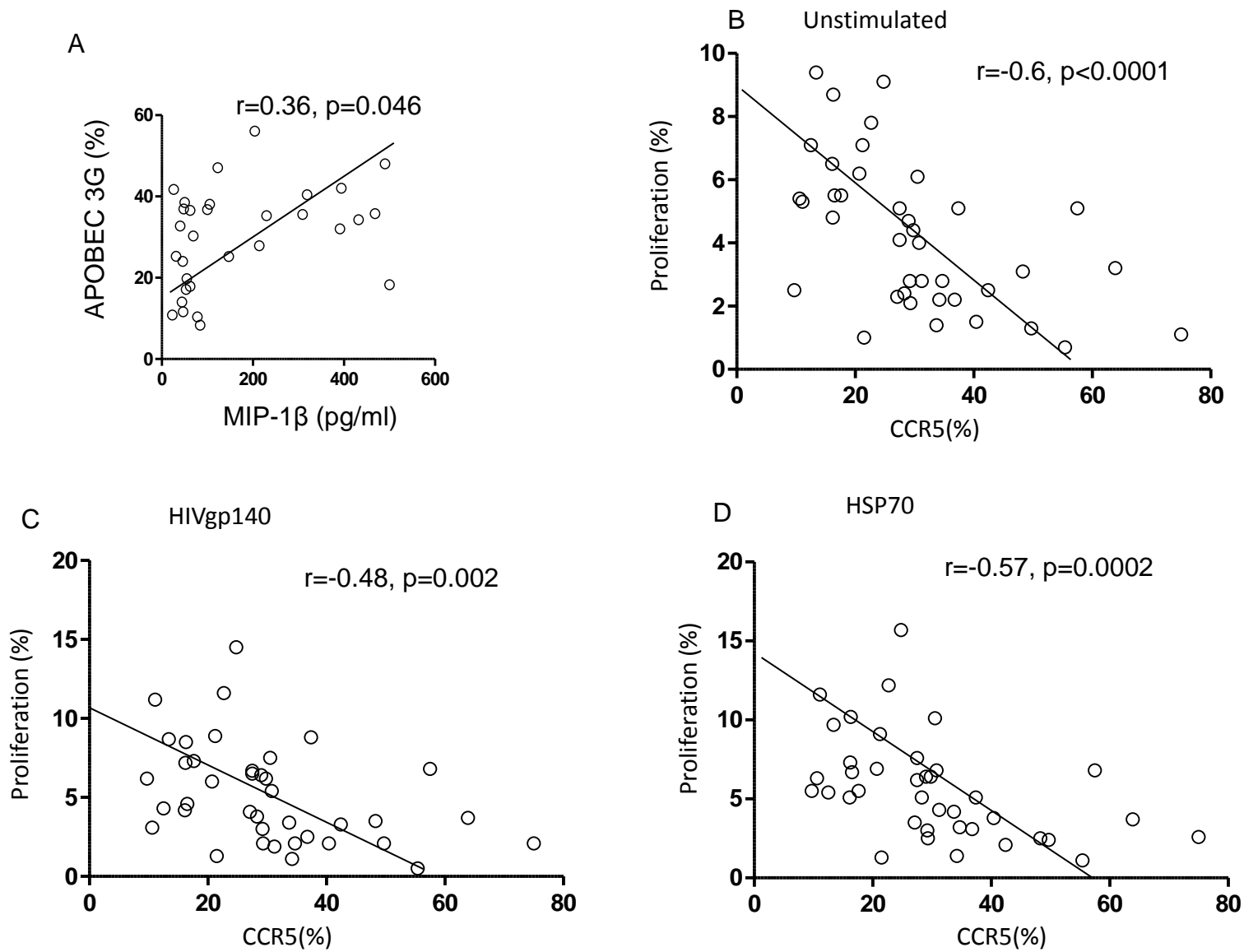


Fig. S2



**Table S1A Consort Checklist of subjects enrolled**

<b>Enrolment</b>	Assessed for eligibility (n=14)
<b>Allocation</b>	Allocated to intervention (n=9) ◇ Received allocated intervention (n=8) ◇ Did not received allocated intervention (n=1) (No longer meeting inclusion criteria n=1)
<b>Follow-Up</b>	◇ Lost to follow-up (n=0) ◇ Discontinued intervention (n=1) (Unrelated SAE n=1)
<b>Analysis</b>	Analysed (n=8) ◇ Excluded from analysis (n=0)
	*Excluded (n=5) ◇ Not meeting inclusion criteria (n=2) ◇ Withdrew consent (n=3)

**Table S1B Severity of adverse Events and relationship to vaccine**

<b>Organ System</b>	<b>Severity</b>			<b>Relationship to vaccine</b>	
	<b>Mild</b>	<b>Moderate</b>	<b>Severe</b>	<b>Unrelated</b>	<b>Possible</b>
Bone and Joints			1	1	
Dental		1	1	2	
Gastrointestinal		1		1	
Hepatobiliary	3			3	
Immune System		1		1	
Headache	6	5		11	
Renal			1	1	
Vulvitis	10	4	1	12	3
Respiratory	5	1		6	
Skin and Soft Tissue	3	1		4	
<b>Relationship to vaccine</b>					
Unrelated	27	11	4		
Possible		3			
<b>Total number of AEs</b>	<b>27</b>	<b>14</b>	<b>4</b>	<b>42</b>	<b>3</b>

**Table S2****Plasma CCL-5 (RANTES) ng/ml**

	Pre- <b>0</b>	Post-Immunization (weeks)			
		4	8	16	20
301	51.1	71.1	76.9	73.8	107.1
302	141	159	223	170	261
303	191	180	184	291	231
304	124	217	275	180	139
305	40	35	102	210	51
308	88	65	57		66
310	100	170	175	124	239
313	55	48	171	94	211
mean	98.8	118.1	158	161.1	163.1
sem	18.2	24.9	26.4	24.1	29.3
p'		0.46	0.055	0.0156	0.039

**Table S3 (A) Culture supernatant MIP-1 $\alpha$** 

	Post-Immunization (weeks)			
	Pre-0	4	8	16
<b>Medium</b>				
302	2.6	19.7	43.4	356
303	103.4	107	80.1	327
304	2.2	84.8	265	234
305	2.2	12.4	149.3	231.5
308	34.6	147	161	246
310	1.5	7.3	123	231
313	101	56	121	124
mean	35.36	62.03	134.7	249.9
sem	17.83	20.15	26.51	28.46
p'		0.156	0.047	0.0156

**HIVgp140**

302	32.4	37.5	38.3	873.7
303	207.5	168		1772.4
304	4	81.6	287	987
305	67	10.2	343.7	495.8
308	85	229.4	231	1234
310	22	45	786	987
313	121	109	212.6	654
mean	76.99	97.24	316.4	1001
sem	26.49	29.57	102.9	157.5
'p'		0.69	0.03	0.0156

**HSP70**

302	13.8	326.2	348.7	1245
303	489.6	465		1345
304	7.3	1000.3	435	1234
305	2.9	83	1015	805.9
308	58	569	213	2122
310	2.2	51.7	1125	1044
313	112	90	321	432
mean	97.97	369.3	576.3	1175
sem	67.03	130	159.4	197.6
p'		0.078	0.03	0.0156

**(B) Culture supernatant MIP-1 $\beta$** 

	Post-Immunization (weeks)			
	Pre-0	4	8	16
<b>Medium</b>				
25	32	23		1037.3
139	634	95		3770.9
65	195	264		2356
61	80	170		211
26	50	115		212.3
80	30	67		324
142	92	718		1158
76.86	159	226		1296
18.12	81.98	104		503.1
	0.45	0.22		0.0156

**HIVgp140**

44	23	26		1191.4
161	599	93		4874.3
56	260	371		5495.3
25	67	367		432
50	228	234		1077.2
50	34	98		1116
167	638	1112		3245
79	264.1	368.2		2490
22.26	97.98	139.3		772.9
	0.078	0.11		0.0156

**HSP70**

63	245	312		8031.5
264	745	56		7654
86	718	469		7709.9
12	400	407		510.3
70	798	323		5369.3
50	31	166		1923.9
285	432	1876		4321
118.6	481.3	592.2		5074
41.24	108.4	260.1		1131
	0.03	0.047		0.0156

**Table S4**

	<b>CCR5 expression in CD4+ T cells</b>										
	301	302	304	303	305	308	310	313	Mean	sem	p
Pre-	55.4	48.3	34.7	49.7	29.3	75	63.9	40.4	49.6	5.4	
4 wks	21.5	16.5	31.2	33.7	30.5	28.3	30.8	34.2	28.3	2.2	0.015
8 wks	36.8	9.7	10.6	27.1	11.1		16.2	29.8	20.2	4.1	0.0156
16 wks	29.2	13.4	12.5	27.5	24.8	57.5	21.2	37.4	27.9	5.1	0.0142
20 wks	42.4	16.3	16.1	20.7	29	17.6	27.5	22.7	24	3.1	0.0078

**Table S5**

	<b>APOBEC3G expression in CD4+ T cells (%)</b>										
	301	302	304	303	305	308	310	313	Mean	sem	p
Pre-	11.6	10.8	17.9	27.9	36.6	53.3	32	25.3	24.7	3.6	
4 wks	10.4	14	36.8	40.4	38.5	35.6	42	41.7	32.4	4.5	0.195
8 wks	8.3	24	25.2	34.3	47.1		18.3	36.9	27.7	4.9	0.375
16 wks	19.8	17.1	38.1	35.8	30.3	56.1	48	32.7	34.7	4.6	0.023
20 wks	23.1	15	36	32.6	42.5	32.7	26	36.8	30.6	3.1	0.202



**Table S6**

**(A) PBMC Proliferation**

	<b>Medium</b>										
	301	302	304	303	305	308	310	313	mean	sem	p
Pre-	0.7	3.1	2.8	1.3	2.1	1.1	3.2	1.5	1.98	0.34	
Post-4	1	5.5	2.8	1.4	6.1	2.4	4	2.2	3.17	0.66	0.25
Post-8	2.2	2.5	5.4	2.3	5.3		4.8	4.4	3.84	0.55	0.03
Post-16	2.8	9.4	7.1	4.1	9.1	5.1	7.1	5.1	6.23	0.83	0.002
Post-20	2.5	8.7	6.5	6.2	4.7	5.5	5.1	7.8	5.88	0.68	0.001
	<b>HIVgp140</b>										
Pre-	0.5	3.5	2.1	2.1	2.1	2.1	3.7	2.1	2.28	0.35	
Post-4	1.3	4.6	1.9	3.4	7.5	3.8	5.4	1.1	3.63	0.78	0.36
Post-8	2.5	6.2	3.1	4.1	11.2		7.2	6.2	5.79	1.12	0.005
Post-16	3	8.7	4.3	6.5	14.5	6.8	8.9	8.8	7.69	1.24	0.002
Post-20	3.3	8.5	4.2	6	6.4	7.3	6.7	11.6	6.75	0.91	0.002
	<b>HSP70</b>										
Pre-	1.1	2.5	3.2	2.4	2.5	2.6	3.7	3.8	2.73	0.3	
Post-4	1.3	6.7	4.3	4.2	10.1	5.1	6.8	1.4	4.99	1.03	0.066
Post-8	3.1	5.5	6.3	3.5	11.6		7.3	6.4	6.24	1.07	0.009
Post-16	3	9.7	5.4	7.6	15.7	6.8	9.1	5.1	7.8	1.38	0.003
Post-20	2.1	10.2	5.1	6.9	6.4	5.5	6.2	12.2	6.38	1.1	0.01

**(B) CD4+ T cell Proliferation**

	<b>Medium</b>										
	301	302	304	303	305	308	310	313	mean	sem	p
Pre-	0.5	3.4	3.2	2.3	2.4	2.6	2.9	0.4	2.21	0.41	
Post-4	0.9	4.5	3	4.1	3.9	4.8	3.6	1.3	3.26	0.51	0.06
Post-8	3.3	4.4	3.4	3.1	3.5		3.6	2.1	3.34	0.26	0.04
Post-16	2.2	5.6	1.2	4.1	5.8	7.4	6.8	1.9	4.38	0.84	0.2
Post-20	1.9	6.2	1.1	5.3	5.2	7.3	5.7	4.4	4.64	0.75	0.04
	<b>HIVgp140</b>										
Pre-	0.3	2.7	3.4	4.1	2.2	1.5	2.1	0.6	2.11	0.71	
Post-4	3.2	1.6	2.9	5.5	4.3	7.1	3.9	0.9	3.68	0.46	0.1
Post-8	4.2	8.8	3.1	6.5	10.7		8.3	4.3	6.55	1.07	0.001
Post-16	3.5	7.4	3.6	6.4	9.1	7.4	4.5	2.8	5.59	0.81	0.004

**Table S7**Cytokine and Viral Load (VL) in CD4<sup>+</sup> T cells

		IL-12		IL-6		VL (AUC)	
		%	Net	%	Net	%	Net
No. 301	0	6.2		13.5		11.7	
			-5.1		+2.9		+0.8
	20	1.1		10.6		12.5	
303	0	8.4		11.5		10.5	
			+1.0		-1		-0.7
	20	9.4		10.5		9.8	
304	0	2.3		13.4		4.7	
			+3.2		-4.4		-3.1
	20	5.5		9		1.6	
305	0	2.6		20.9		8.5	
			+5.1		+8.7		-6.9
	20	7.7		29.6		1.0	
310	0	3.1		8		8.8	
			-0.3		+7.8		0
	20	2.8		15.8		8.8	
313	0	3.3		13.2		12.4	
			+2.4		+6.5		-3.4
	20	5.7		19.7		9.0	

Post-20	3.2	7.7	3	7.8	4.7	8.2	5.5	7.4	5.94	0.75	0.002
<b>HSP70</b>											
Pre-	1.4	3.7	2.3	3.6	2.1	1.9	2.8	4.2	2.75	0.35	
Post-4	2	5.2	4.9	5	4.6	8.8	4	1.2	4.46	0.81	0.08
Post-8	4.3	3.8	5.3	5.5	10.5		6.5	3.3	5.6	0.91	0.004
Post-16	2.3	8.6	8.3	5.8	12.5	6.9	7.1	3	6.81	1.15	0.01
Post-20	3.8	7.1	6.3	7.9	6.5	6.1	6.8	2	5.81	0.59	0.007
<b>(C) CD8+ T cells Proliferation</b>											
	301	302	304	303	305	308	310	313	mean	sem	p
<b>Medium</b>											
Pre-	0.3	2.2	2.7	2.4	1.1	1.9	2	0.6	1.65	0.52	
Post-4	1.9	1.3	2.3	4.5	4.6	2.6	2.7	0.3	2.53	0.31	0.24
Post-8	5	2.9	1.7	3.3	5		3.7	1.8	3.34	0.51	0.04
Post-16	4.8	5.2	2.5	3.2	3.3	3.1	3.9	5.2	3.9	0.37	0.0003
Post-20	5.2	1.8	1.6	5.3	1.7	3.1	3.7	3.8	3.28	0.53	0.08
<b>HIVgp140</b>											
Pre-	0.2	3.6	3.6	2.1	1.9	2.1	2.3	1	2.1	0.41	
Post-4	3	1.7	3.8	5.2	6.6	4.1	4.3	1.1	3.73	0.63	0.065
Post-8	5.3	2.1	6.1	3.8	8.9		5.8	4.1	5.16	0.81	0.006
Post-16	4	7.8	7.3	4.5	6.1	5.9	5.3	3.9	5.6	0.51	0.0009
Post-20	4	6.3	6.3	6.3	6	4.9	6.4	8.7	6.11	0.48	0.0009
<b>HSP70</b>											
Pre-	0	3.2	3.8	1.8	2.1	2.5	2.3	5.1	2.6	0.53	
Post-4	2.8	3.5	3.6	3.5	6	3.1	4.4	0.9	3.48	0.51	0.23
Post-8	4.9	1	3.9	6.2	10.5		4.5	7.6	5.51	1.1	0.04
Post-16	3.5	4.4	4.6	4.6	6.4	6.7	6.3	2.6	4.89	0.52	0.01
Post-20	5.6	3.5	5.2	5.3	3.6	4.8	7.8	5.7	5.19	0.48	0.003