

TABLE S1. Mutant frequency of the *cII* transgene in various organs of Big Blue mice chronically treated with 4-ABP or control (DMSO).

Treatment	Mouse ID	Total number of plaques (<i>pfu</i> [*])	Mutant plaques	Mutant frequency ($\times 10^{-5}$)	Average mutant frequency ($\times 10^{-5}$) [†]
Bladder					
Control	238	321,250	6	1.87	2.06 \pm 0.20
	239	985,000	23	2.34	
	240	850,000	16	1.88	
	241	436,250	9	2.06	
	251	1,080,000	25	2.31	
4-ABP (6 Weeks)	229	1,030,000	149	14.47	18.86 \pm 4.77
	230	1,050,000	198	18.86	
	231	1,005,000	184	18.31	
	232	710,000	205	28.87	
	220	880,000	199	22.61	
4-ABP (6 Weeks) + Recovery (6 Weeks)	221	995,000	259	26.03	17.20 \pm 4.88
	222	1,255,000	202	16.10	
	223	1,030,000	249	24.17	
	247	1,330,000	172	12.93	
	248	1,070,000	184	17.20	
Liver					
Control	238	540,000	11	2.04	2.04 \pm 0.56
	239	1,940,000	40	2.06	
	240	455,000	15	3.30	
	241	2,095,000	42	2.00	

	251	930,000	15	1.61	
4-ABP (6 Weeks)	229	1,170,000	28	2.39	4.54 ± 1.08
	230	722,500	19	2.63	
	231	1,095,000	52	4.75	
	232	545,000	27	4.95	
	220	858,750	39	4.54	
4-ABP (6 Weeks) + Recovery (6 Weeks)	221	995,000	66	6.63	4.62 ± 0.98
	222	1,215,000	55	4.53	
	223	1,000,000	49	4.90	
	247	1,170,000	54	4.62	
	248	1,100,000	39	3.55	
Kidney					
Control	238	840,000	40	4.76	2.59 ± 1.22
	239	1,080,000	28	2.59	
	240	1,045,000	41	3.92	
	241	1,280,000	15	1.17	
	251	566,250	14	2.47	
4-ABP (6 Weeks)	229	1,330,000	33	2.48	3.64 ± 0.93
	230	880,000	34	3.86	
	231	1,565,000	57	3.64	
	232	785,000	41	5.22	
	220	1,095,000	31	2.83	
4-ABP (6 Weeks) + Recovery (6 Weeks)	221	1,195,000	62	5.19	6.57 ± 1.94
	222	1,195,000	31	2.59	
	223	820,000	59	7.20	
	247	1,150,000	96	8.35	
	248	700,000	46	6.57	

Lung					
Control	238	1,200,000	43	3.58	3.58 ± 0.33
	239	1,045,000	37	3.54	
	240	1,035,000	29	2.80	
	241	820,000	30	3.66	
	251	1,155,000	43	3.72	
4-ABP ^(6 Weeks)	229	855,000	38	4.44	3.07 ± 0.69
	230	1,030,000	35	3.40	
	231	1,555,000	37	2.38	
	232	1,165,000	32	2.75	
	220	1,270,000	39	3.07	
4-ABP ^(6 Weeks) + Recovery ^(6 Weeks)	221	1,720,000	31	1.80	2.90 ± 4.18
	222	1,740,000	32	1.84	
	223	1,310,000	38	2.90	
	247	935,000	122	13.05	
	248	483,750	17	3.51	
Stomach					
Control	238	287,500	5	1.74	2.44 ± 0.84
	239	302,500	12	3.97	
	240	615,000	15	2.44	
	241	895,000	20	2.23	
	251	410,000	15	3.66	
4-ABP ^(6 Weeks)	229	180,000	11	6.11	3.96 ± 1.68
	230	675,000	11	1.63	
	231	530,000	21	3.96	
	232	485,000	14	2.89	

	220	323,750	19	5.87	
4-ABP ^(6 Weeks) + Recovery ^(6 Weeks)	221	455,000	18	3.96	3.39 ± 1.30
	222	945,000	31	3.28	
	223	805,000	23	2.86	
	247	885,000	30	3.39	
	248	805,000	53	6.58	

Male adult Big Blue transgenic mice received weekly intraperitoneal injection of 4-ABP or control DMSO for a duration of 6 consecutive weeks, after which they were sacrificed either immediately, or after a 'Recovery' period of 6 weeks.

* Plaque forming unit

† Results are expressed as median ± 95% CIs.

TABLE S2. Types of mutations in the *cII* transgene in bladder of Big Blue mice chronically treated with 4-ABP or DMSO control.

Mutation type		4-ABP	Control	4-ABP*	Control*	
Single mutation		143 (99.3%) [†]	138 (98.6%)	130 (92.2%)	100 (98.0)	
Multiple mutations		1 (0.7%)	2 (1.4%)	1 (0.8%)	2 (2.0)	
Base substitution	Single	128 (88.9%)	105 (75.0%)	116 (88.5%)	80 (78.4%)	
	Tandem	AG→CT	0 (0%)	1 (0.7%)	0 (0%)	1 (1.0%)
		TG→AT	1 (0.7%)	0 (0%)	1 (0.8%)	0 (0%)
		CT→TG	1 (0.7%)	0 (0%)	1 (0.8%)	0 (0%)
	Multiple	G→T and (T) Ins.	1 (0.7%)	0 (0%)	1 (0.8%)	0 (0%)
		G→A and G→A	0 (0%)	1 (0.7%)	0 (0%)	1 (1.0%)
		T→A and C→T	0 (0%)	1 (0.7%)	0 (0%)	1 (1.0%)
Deletion	Single	11 (7.6%)	16 (11.4%)	10 (7.6%)	13 (12.7%)	
	Multiple	0 (0%)	1 (0.7%)	0 (0%)	1 (1.0%)	
Insertion	Single	1 (0.7%)	15 (10.7%)	1 (0.8%)	5 (4.9%)	
	Multiple	1 (0.7%)	0 (0%)	1 (0.8%)	0 (0%)	

Male adult Big Blue transgenic mice received weekly intraperitoneal injection of 4-ABP or control DMSO for a duration of 6 consecutive weeks, after which they were sacrificed either immediately, or after a 'Recovery' period of 6 weeks.

* Adjusted after exclusion of sibling mutations that occurred more than once at the same nucleotide position in the same animal.

† Percentage values are indicated in the brackets.

Ins. = Insertion

Del. = Deletion

TABLE S3. Comparative mutation spectra of the *cII* transgene in bladder of Big Blue mice chronically treated with 4-ABP or DMSO control.

Mutation type	Number of Mutations				% Mutations				Absolute Mutant Frequency ($\times 10^{-5}$)			
	4-ABP	Control	4-ABP*	Control*	4-ABP	Control	4-ABP*	Control*	4-ABP	Control	4-ABP*	Control*
G:C→C:G	17	3	16 (8)	3	11.6	2.1	11.9 (6.0)	2.9	2.18	0.04	2.05 (1.03)	0.04
G:C→T:A	62	13	59 (14)	12 (4)	42.2	9.1	44.0 (10.4)	11.4 (3.8)	7.95	0.19	7.56 (1.79)	0.17 (0.06)
G:C→A:T	36	65	31 (14)	44 (35)	24.5	45.5	23.1 (10.4)	41.9 (33.3)	4.62	0.94	3.97 (1.79)	0.63 (0.50)
A:T→T:A	7	10	5	10	4.8	7.0	3.7	9.5	0.90	0.14	0.64	0.14
A:T→G:C	7	8	7	8	4.8	5.6	5.2	7.6	0.90	0.12	0.90	0.11
A:T→C:G	5	12	4	9	3.4	8.4	3.0	8.6	0.64	0.17	0.51	0.13
Del.	11	17	10 (2)	14	7.5	11.9	7.5 (1.5)	13.3	1.41	0.24	1.28 (0.26)	0.20
Ins.	2	15	2	5	1.4	10.5	1.5	4.8	0.26	0.22	0.26	0.07

Male adult Big Blue transgenic mice received weekly intraperitoneal injection of 4-ABP or control DMSO for a duration of 6 consecutive weeks, after which they were sacrificed either immediately, or after a 'Recovery' period of 6 weeks.

* Adjusted after exclusion of sibling mutations that occurred more than once at the same nucleotide position in the same animal.

† Mutations occurring specifically at CpG dinucleotides are indicated in the brackets.

‡

Ins. = Insertion

Del. = Deletion

Figure S1

Distribution of mutations in the *cII* transgene in bladder of 4-ABP-treated mice and controls.

The spectra of mutations in the *cII* transgene in 4-ABP-treated mice and controls were established by DNA sequencing of mutants from bladder DNA of carcinogen-treated mice and controls, as described in 'Materials and Methods'. The 4-ABP-induced mutations are indicated above the reference *cII* sequence, whereas the control mutations are shown below the reference *cII* sequence.

5'-CpG-containing sequences are indicated by asterisks in control.

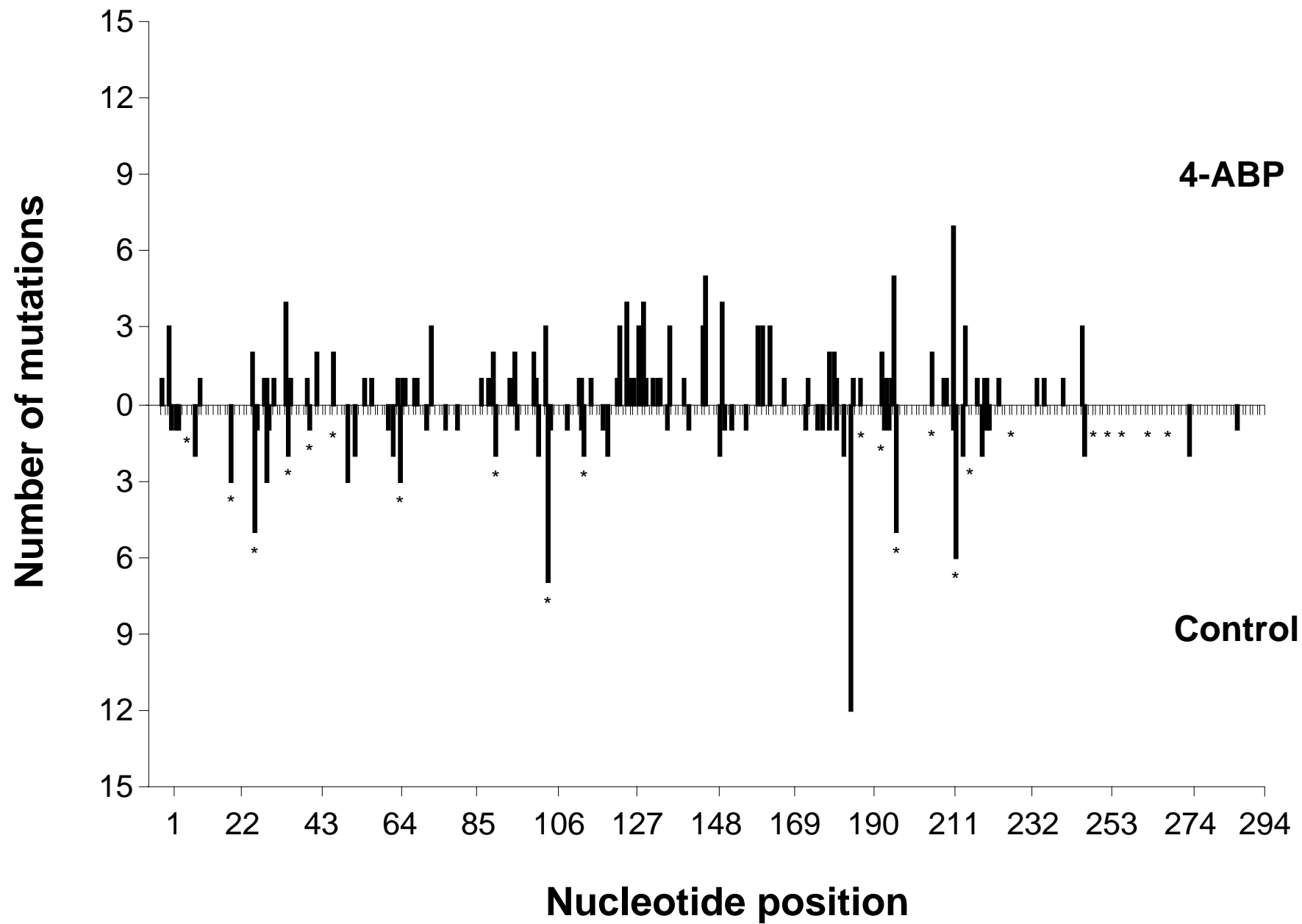


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