

Supplementary Materials: Cannabidiol (CBD) Protects Lung Endothelial Cells from Irradiation-Induced Oxidative Stress and Inflammation In Vitro and In Vivo

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Table S1. Individual data points of graphs shown in Figure 1.

Fig. 1A-C ROS in H5V		fold change, normalized to control at t0 (pre-irradiation)			
0Gy (control)	t0	1	1	1	1
	t1	2.492953	2.38	2.273682	2.350626
	t2	3.5097	3.43	3.228057	3.039537
0Gy NAC	t0	0.919382	1.543818	1.143865	0.724424
	t1	1.232334	2.361965	1.572503	0.905901
	t2	1.691546	2.96365	1.961111	0.98156
0Gy CBD	t0	1.09876	0.957137	0.871407	
	t1	1.954207	1.910328	1.201126	
	t2	2.523662	2.676485	1.291031	
0Gy NAC + CBD	t0	0.833077	1.390063	0.914651	0.915865
	t1	1.078934	1.834106	1.18768	1.18647
	t2	1.425491	2.214281	1.387208	1.257455
4Gy	t1	3.187219	3.613148	2.815378	3.0851
	t2	5.077068	5.874269	4.298343	4.805336
4Gy + CBD	t1	1.665962	1.876596	2.385724	
	t2	2.163079	3.034498	4.051357	
4Gy + NAC	t1	2.527459	2.764004	2.256409	
	t2	3.411879	3.380832	2.911377	
4Gy + NAC + CBD	t1	2.055129	2.398596	1.67418	2.089612
	t2	2.748396	2.661794	2.028834	2.244193
6Gy	t1	4.275709	3.952964	2.84084	3.5075
	t2	6.104782	6.3236	3.966026	5.417296
6Gy + CBD	t1	1.740059	2.426076	2.744465	1.959057
	t2	2.174294	3.578037	4.680119	2.558463
6Gy + NAC	t1	2.713097	2.081145	1.954796	
	t2	3.682199	2.46025	2.4989	
6Gy + NAC + CBD	t1	2.176869	1.778925	2.396032	
	t2	2.933634	1.845099	2.858667	
Fig. 1D ROS in primary luEC in vitro					
0Gy	t0	1	1	1	
	t1	2.86	1.29	1.85	
	t2	3.73	1.69	2.53	
0Gy + CBD	t0	0.81	0.72	0.64	
	t1	1.88	0.93	1.21	
	t2	2.59	1.07	1.35	
4Gy	t0	1	1	1	
	t1	3.26	1.61	3.23	
	t2	4.05	3.89	5.48	
4Gy + CBD	t0	0.78	0.96	0.91	
	t1	1.94	1.7	2.47	
	t2	2.26	2.3	3.96	

Table S2. Individual data points of graphs shown in figure 2.

Fig. 2A γH2AX in H5V				
normalized to β -actin	ctrl	CBD 10 μ M	4Gy	4Gy + CBD
	0.08696387	0.10616041	0.85395986	0.68732493
	0.56279	0.362684	0.974684	0.458273
	0.019507	0.016978	0.823102	0.733936
	0.66647	0.783243	1.076776	0.755359
Fig. 2B-C Apoptosis in H5V				
		Alive		
control	85.3	88.4	87.1	
CBD 10 μ M	87.5	88.1	87.8	
4Gy	71.6	71.7	72.6	
CBD 10 μ M + 4Gy	77.6	77.3	77.7	
		Necrotic		
control	4.51	4.39	4.21	
CBD 10 μ M	3.65	4.28	4.66	
4Gy	6.02	7.85	7.65	
CBD 10 μ M + 4Gy	6.25	6.69	6.22	
		Early Apoptosis		
control	3.41	2.69	3.15	
CBD 10 μ M	4.64	2.07	2.06	
4Gy	12.6	14.1	13.6	
CBD 10 μ M + 4Gy	10.4	8.02	8.11	
		Late Apoptosis		
control	6.77	4.51	5.54	
CBD 10 μ M	4.21	5.6	5.5	
4Gy	9.7	6.38	6.14	
CBD 10 μ M + 4Gy	5.76	8.02	7.94	
Fig. 2D Cell cycle distribution in H5V				
		G1-phase		
Ctrl		51.2	51.7	52.5
CBD		65.5	56.7	60.7
		S-phase		
ctrl		32.2	31.2	31.5
CBD		18.5	30.5	30.2
		G2-phase		
ctrl		14.1	16.7	16.1
CBD		14.7	12.8	9.13
Fig. 2E-F Apoptosis in luECs				
		Alive		
ctrl	40.3	43		
16Gy	36.1	37.1		
16Gy + CBD	47.2	46.9		
		Necrotic		
ctrl	2.95	2.13		
16Gy	2.36	2.96		
16Gy + CBD	1.33	3.7		
		Early apoptosis		
ctrl	30.6	36.8		
16Gy	42.8	39.7		
16Gy + CBD	33.7	29.2		
		Late apoptosis		
ctrl	26.2	18		
16Gy	18.7	20.3		
16Gy + CBD	17.7	20.2		

Table S3. Individual data points of graphs shown in Figure 3.

Fig. 3A HO-1 in H5V	ctrl	10μM CBD	4Gy	4Gy + CBD
normalized to β-actin, then to control	1	3.195032	1.722989	3.16774
	1	2.488107	1.544943	2.264293
	1	2.403741	1.188776	5.916877
	1	2.068048		
Fig. 3B HO-1 in lung	control	16Gy	16Gy + CBD 20mg/kg	
normalized to β-actin, then to control	1	1.05348	2.506101	
	1	0.99464	2.331856	
	1	0.997383	1.978858	
	1	1.198557	1.978327	

Table S4. Individual data points of graphs shown in Figure 4.

Fig. 4 A-D		2 weeks			10 weeks		
Inflammatory markers		ctrl	16Gy	16Gy + CBD	ctrl	16Gy	16Gy + CBD
ICAM-1	percentage	89.8	95.4	87.5	92.8	90.1	91.8
		92	93.2	90.5	93.8	92.5	88.3
		92.9	91.7	91.6	93.2	92.2	90.5
		91.8	93.8	90.3	93.4	90	
	MFI	20.4	27.8	10.6	24.1	19.2	21.8
		21.7	21.9	17.9	27.2	21	22.4
		19.6	27.9	21.4	22.8	26.4	12.5
		17.9	18.9	16.8	16.5		
	percentage	88.4	91.5	92.4	96.6	94.1	93.5
		92.8	95.3	93	91	96.8	87.1
		93	96.1	92.6	94.6	95.1	91.3
		95	94.8	92.9	95.4	99.5	
	MFI	8.82	13.7	12.5	13.6	19.1	14
		8.62	11.1	13.9	9.54	18	11.6
		10.2	15.1	15.9	14.4	18	8.06
		10.1	20.2	11.5	13.1	27	
VCAM-1	percentage	16.3	16.9	15.6	20.4	13.2	21.6
		20.5	17.5	15.2	17	12.9	21.6
		14.5	23.4	22.4	16.1	13.5	10.2
		11.3	9.23	13.6	16	12.3	
	MFI	2.35	2.73	2.65	3.13	2.73	2.8
		2.27	2.81	2.46	2.73	2.54	2.24
		2.38	2.45	2.88	2.73	2.5	2.65
		2.31	3.03	2.74	3.13	2.27	
	percentage	86.7	96.1	82.7	89.7	87	91.6
		89.8	93.9	85.6	91.7	90.1	89.8
		94.4	92	92.6	90.5	90.2	89.7
		92.9	94.2	90.3	92.2	89	
	MFI	12.4	21	6.79	11.1	9.6	11.4
		11.3	19.4	9.87	12.8	11.1	7.79
		12.1	15.9	11.8	12.2	12.3	13.9
		9.73	10.8	9.65	10.9	8.9	

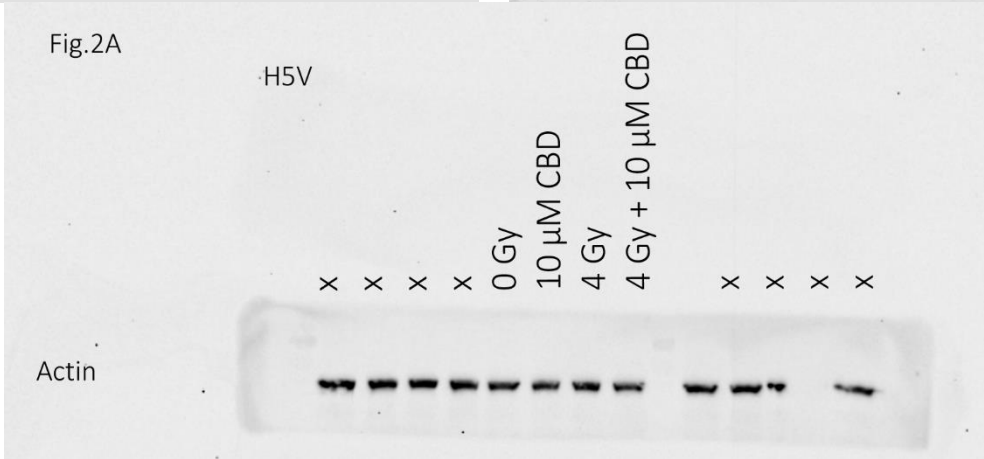
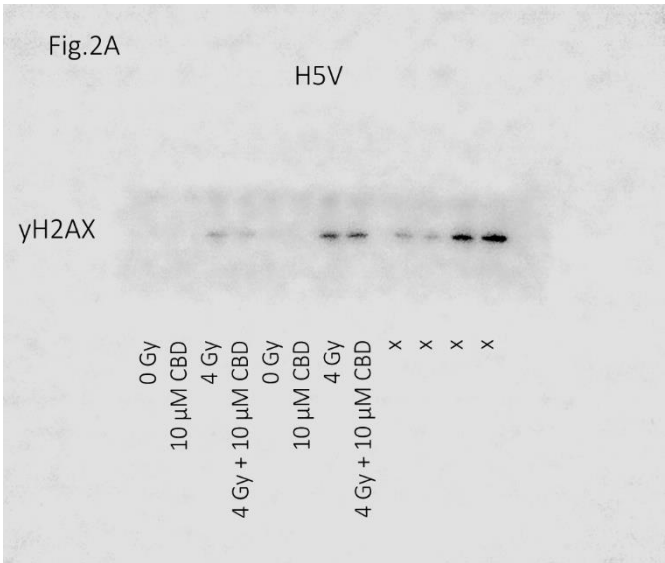
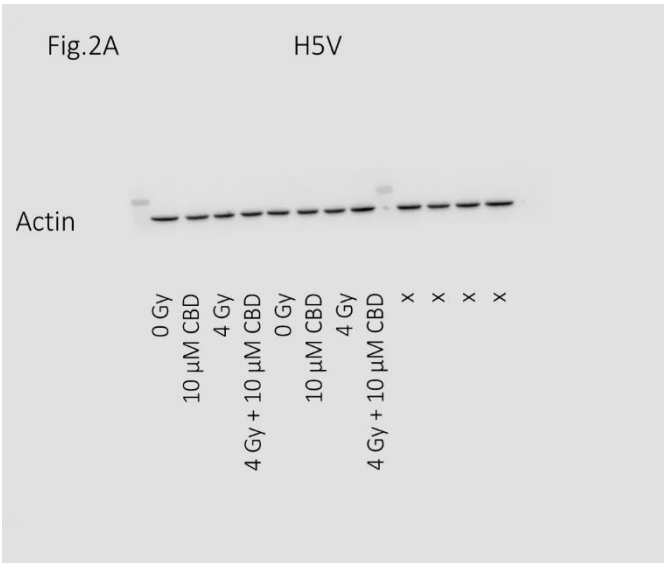
Table S5. Individual data points of graphs shown in Figure 5.

Fig. 5A-B		2 weeks			10 weeks		
Angiogenic markers		ctrl	16Gy	16Gy + CBD	ctrl	16Gy	16Gy + CBD
VE-Cadherin	percentage	76.7	90.9	71.6	90.1	82.4	86
		83.5	88.1	78.3	75.5	87.3	83.7
		81.8	88.6	80.4	89.3	87.5	81.9
		87.6	91.8	84.2	89.5	87.6	
	MFI	7.23	9.33	6.81	11.7	8.99	10.4
		5.76	9.93	5.44	5.51	10.4	8.95
		7.16	10.6	7.03	11.6	10.3	7.43
		7.36	12.5	7.04	10.8	10.7	
Endoglin	percentage	ctrl	16Gy	16Gy + CBD	ctrl	16Gy	16Gy + CBD
		69.6	82.6	85.1	88.4	85	87.4
		66	79.4	82.3	83.3	88.1	80.3
		80.8	89.1	87.2	86.4	86.7	62.2
	MFI	76.1	90.9	84.2	86.5	92.1	
		3.98	5.23	4.26	5.42	6.78	3.32
		3.39	4.67	5.33	5.33	7.27	5.53
		4.61	6.07	7.14	5.84	10.8	5.57
	4.49	5.33	4.48	5.46	14		

Table S6. Individual data points of graphs shown in Appendix.

Appendix Fig. A2 a		ROS in H5V after 24h					
		normalized to control					
control		CBD 10μM	4Gy	4Gy + CBD 10μM			
1		0.53956835	1.04316547	0.76258993			
1		0.80877193	1.35964912	0.99122807			
1		0.71816284	1.03966597	0.61169102			
1		0.89692102	1.99464525	1.2021419			
1		0.88888889	1.77777778	0.83492063			
Appendix Fig. A2 b		Clonogenic survival in H5V					
		normalized to control					
ctrl		CBD 10μM	4Gy	4Gy + CBD 10μM			
1		0.77777778	0.42735043	0.47863248			
1		1.09278351	0.28865979	0.40206186			
1		0.67826087	0.46086957	0.37391304			
1		0.95454545	0.22727273	0.81818182			
Appendix Fig. A4 b		Leukocyte adhesion to luECs					
		16 Gy	16 Gy + CBD 20 mg/kg				
		491	283				
		260	178				
		3074	167				
		2622	67				
Appendix Fig. A5 a-b		2 weeks			10 weeks		
CD34		ctrl	16Gy	16Gy + CBD	ctrl	16Gy	16Gy + CBD
percentage		29	35	37.4	67.2	43.9	73.8
		32.9	55.6	57	63.6	45.6	73.9
		34	53.5	51.6	63.8	53.7	69.1
		39	46.8	46.3	56.1	59.4	
MFI		2.82	2.75	3.22	5.41	3.79	7.33

Integrin-beta-3 percentage	2.45	2.8	3.28	4.98	3.7	5.67
	2.97	4.32	4.61	5.12	4.22	7.56
	2.8	4.29	3.82	4.44	4.39	
	ctrl	16Gy	16Gy + CBD	ctrl	16Gy	16Gy + CBD
MFI	31.2	32.5	22.4	32.8	27.9	35.5
	28.8	32	28.7	29.7	41.4	32.2
	26.6	22.9	16.8	37.3	31	29.5
	23	19.8	25.9	29.9	29	
	3.6	3.5	3.9	4.31	3.62	3.89
	3.8	4.48	3.43	4.87	6.33	3.92
	3.62	3.74	3.43	5.05	4.1	3.77
	3.58	4.31	3.74	5.14	3.99	



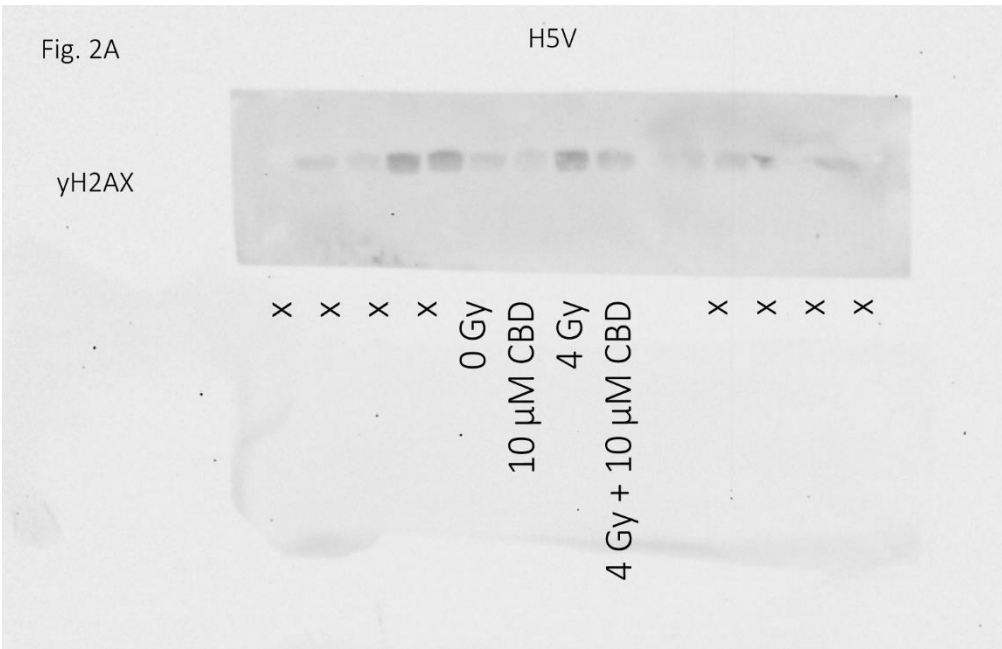
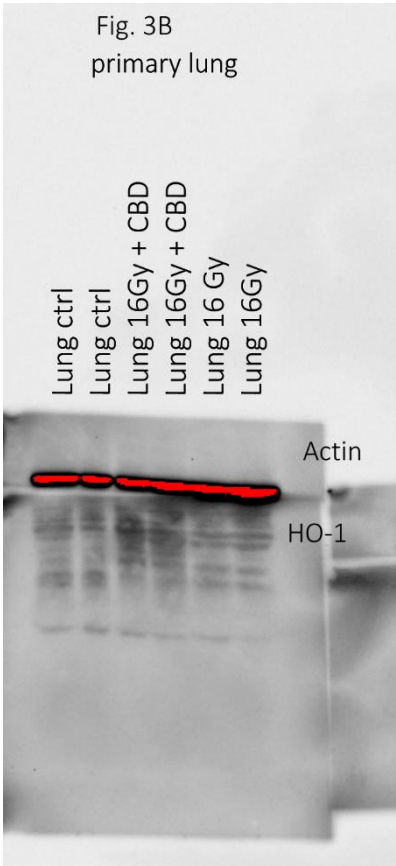
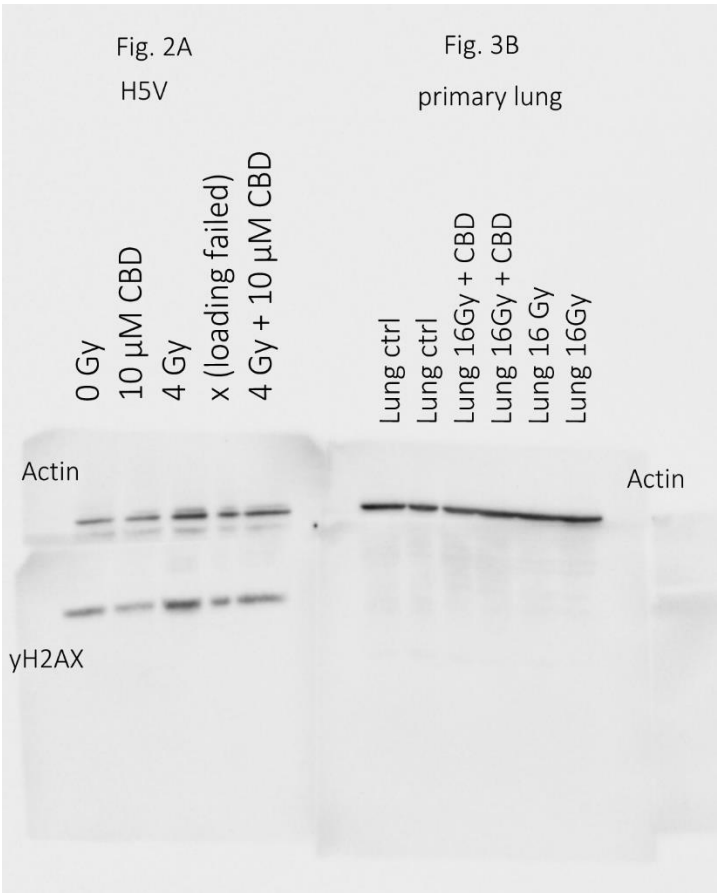


Figure S1. Annotated western blot images of figure 2A (n=1-3); intensity ratios (relative to β -actin) are given in supplementary table 2.



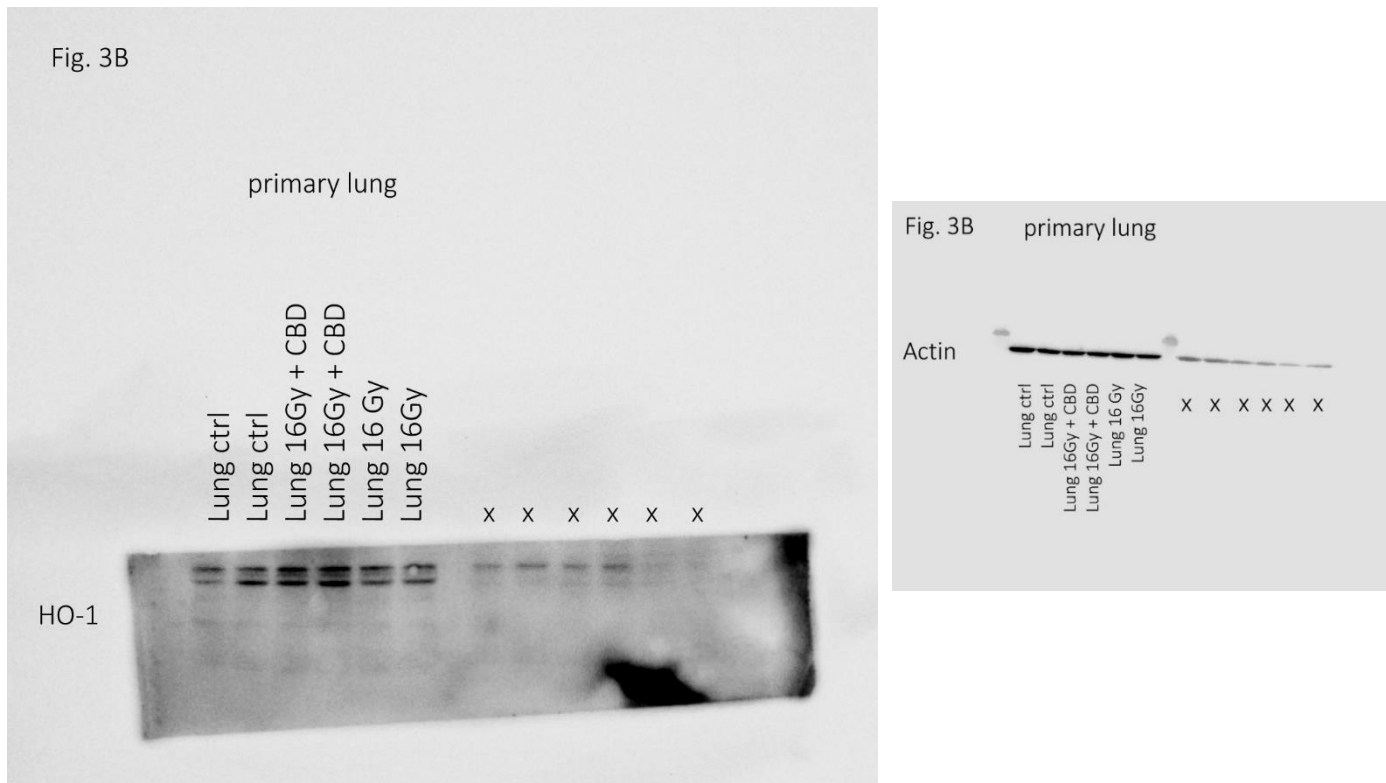
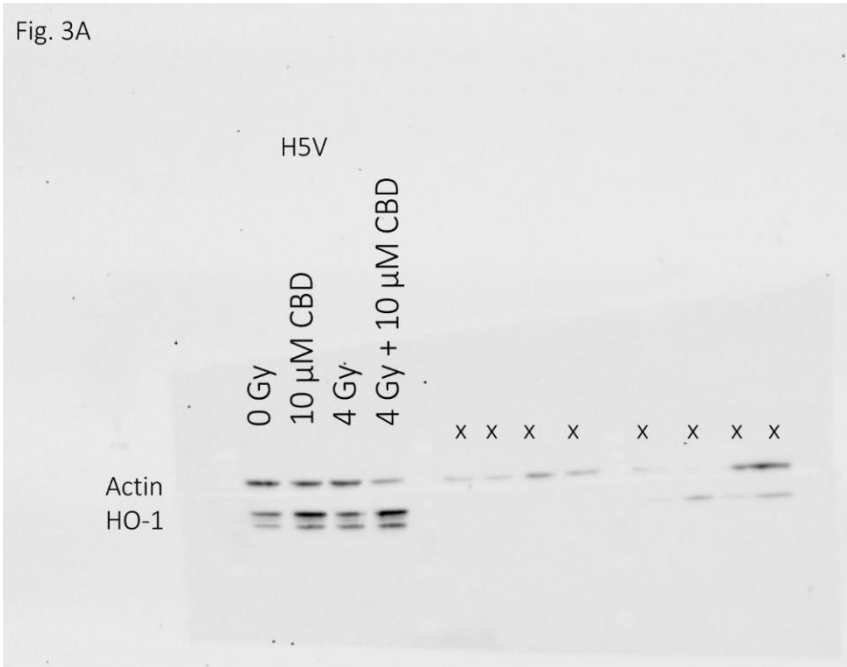


Figure S2. Annotated western blot images of figure 2A (n4) and figure 3B; HO-1 band intensity was measured at higher contrast due to weak bands; intensity ratios (relative to β-actin and normalized to control) are given in supplementary tables 2 and 3.



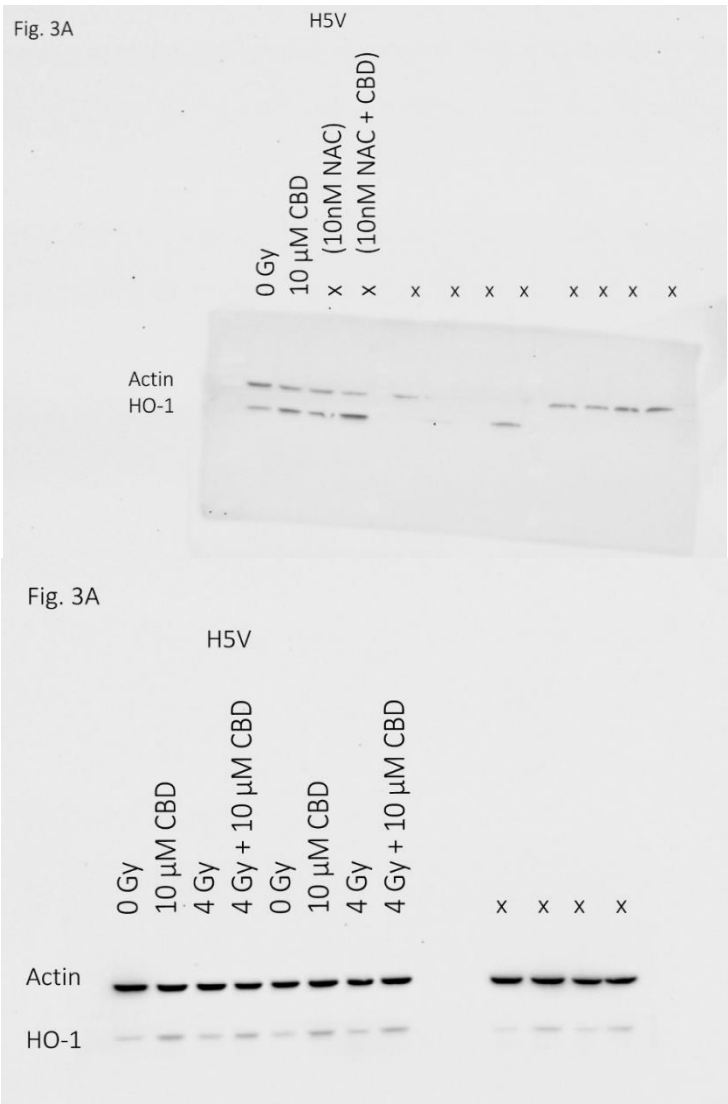


Figure S3. Annotated western blot images of figure 3A and appendix A3; intensity ratios (relative to β -actin and normalized to control) are given in supplementary table 3.