SUPPLEMENTARY MATERIAL

	Microarray				qPCR	
Gene name	Gene symbol	Probeset ID	Fold Change	Pseudo P-value	Fold Change	P-value
Cyclin dependent kinase inhibitor 1A	*CDKN1A*	202284_s_at	2.94	**	3.02 ± 0.48	**
Cyclin dependent kinase 4	*CDK4*	202246_s_at	-1.14	*	-2.09 ± 0.15	*
BCL2 associated X, apoptosis regulator	*BAX*	211833_s_at	1.29	*	1.75 ± 0.217	*
BCL2 like 11	*BCL2L11*	1558143_a_at	1.17	*	2.32 ± 0.166	*
BCL2 binding component 3	*BBC3*	211692_s_at	-1.04	ns	3.98 ± 1.02	*
Phorbol-12-myristate-13-acetate-induced protein 1	*PMAIP1*	204285_s_at	1.36	ns	5.01 ± 1.90	*
Fas ligand	*FASLG*	211333_s_at	1.04	ns	5.04 ± 1.11	**

*p< 0.05; **p<0.01

Table S1: Representative set of p53 pathway genes in MCF-7 cells altered by SPCA2KD**.** Comparison of a subset of p53 pathway genes determined to be differentially expressed in the microarray (n = 2) to expression measured by qPCR (n = 3). For genes that contain multiple probe sets in the Affymetrix Human Genome U133 Plus 2.0 Array, a representative value was selected, typically the value of highest significance.

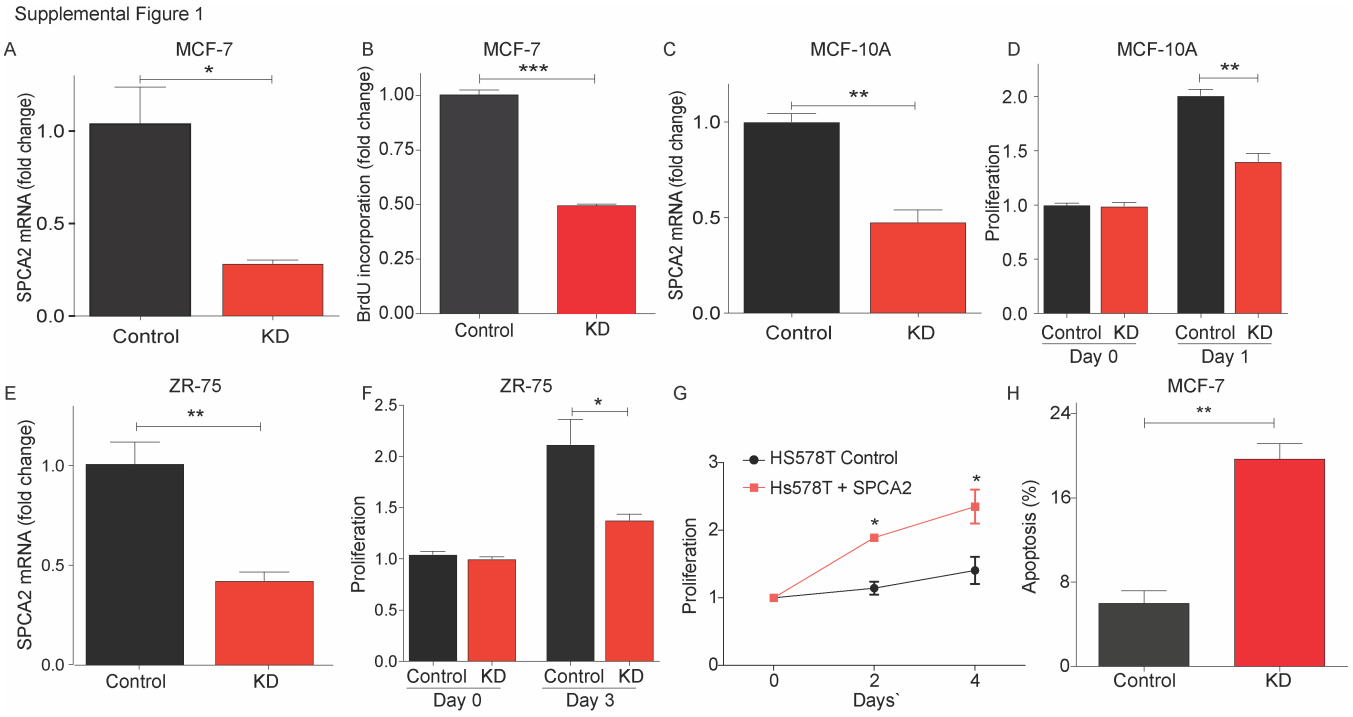

Figure S1: SPCA2 drives cell cycle progression and survival

(A) Knockdown of SPCA2 (SPCA2 KD) in MCF-7 cells was confirmed by qPCR, n=3. (B) SPCA2 KD in MCF-7 significantly decreased BrdU incorporation compared to control (n=6 for control, n=5 for KD. (C) Knockdown of SPCA2 in MCF-10A cells was confirmed by qPCR, n=3. (D) SPCA2 KD in MCF-10A significantly decreased cell proliferation compared to control, n=3. (E) Knockdown of SPCA2 in ZR-75 cells was confirmed by qPCR, n=3. (F) SPCA2 KD in ZR-75 significantly decreased cell proliferation compared to control, n=3. (G) SPCA2 overexpression (using silencing resistant construct SPCA2R) in HS578T increased cell proliferation compared to vector control, n=3. (H) SPCA2 KD significantly increased percentage of apoptotic cells compared to control, n=3. Significance: *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

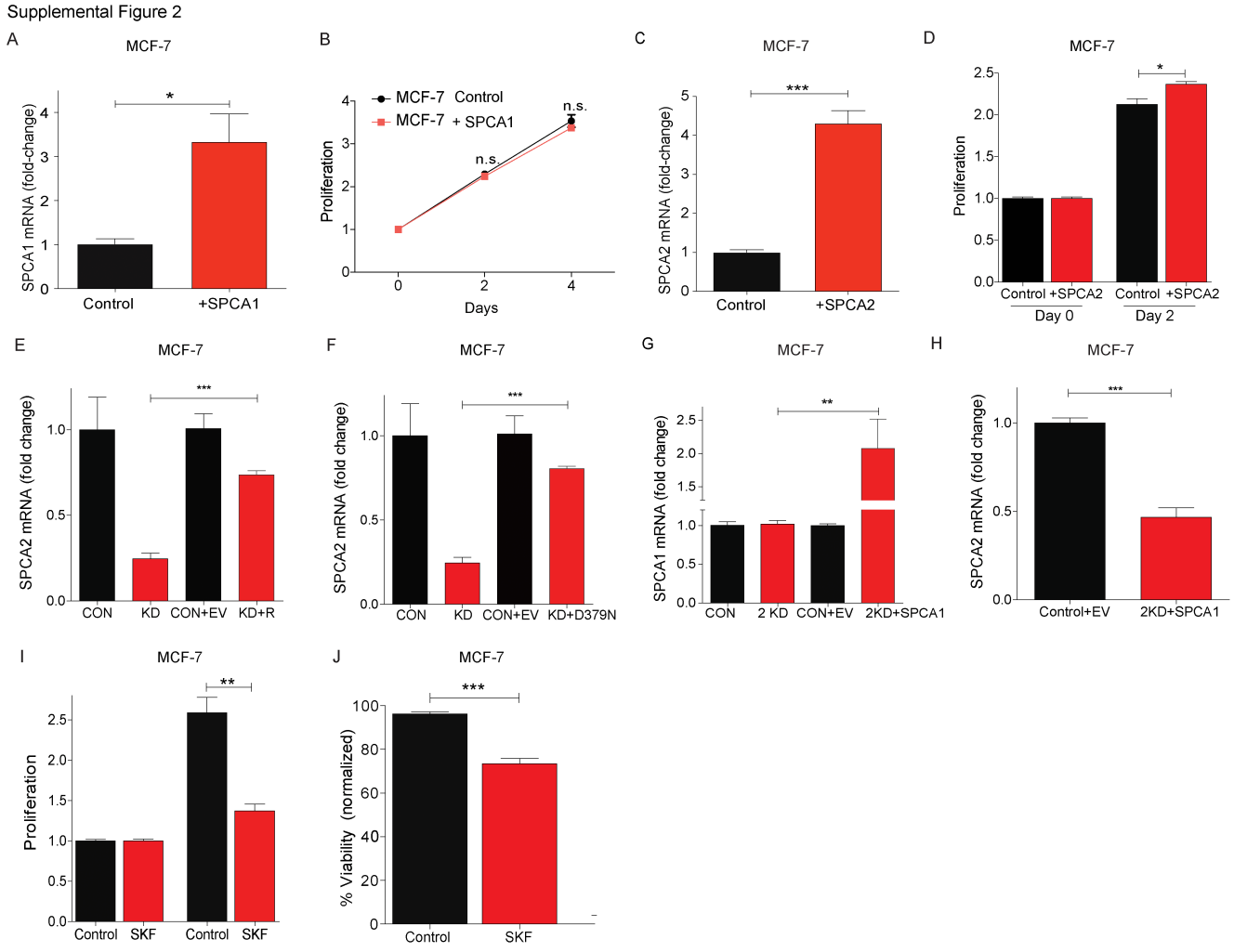

Figure S2: **ATPase deficient mutation in SPCA2 does not block cell proliferation or Ca^{2+} entry**

(A) Overexpression of SPCA1 in MCF-7 cells was confirmed by qPCR, n=3. (B) SPCA1 overexpression in MCF-7 did not significantly increase cell proliferation compared to vector control, n=3. (C) Overexpression of SPCA2 in MCF-7 cells was confirmed by qPCR, n=3. (D) SPCA2 overexpression in MCF-7 significantly increased cell proliferation compared to vector control, n=3. (E-F) SPCA2 transcript levels measured by qPCR in MCF-7 control, SPCA2 knockdown (KD), and after transfection with empty vector (EV), recombinant, silencing-resistant SPCA2R construct (R) or mutant D379N, as indicated n=3. (G) SPCA1 transcript was significantly increased in MCF-7 SPCA2 KD (2KD) cells as confirmed by qPCR, n=3. (H) SPCA2 transcript remains significantly decreased in MCF-7 SPCA2 KD cells which overexpress SPCA1 (2KD+SPCA1), as confirmed by qPCR, n=3. (I-J) Treatment of MCF-7 cells with SKF-96365 (10 μM, 65 hours) reduces cell proliferation and viability. n=3. Significance: ^{ns} $P > 0.05$, *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

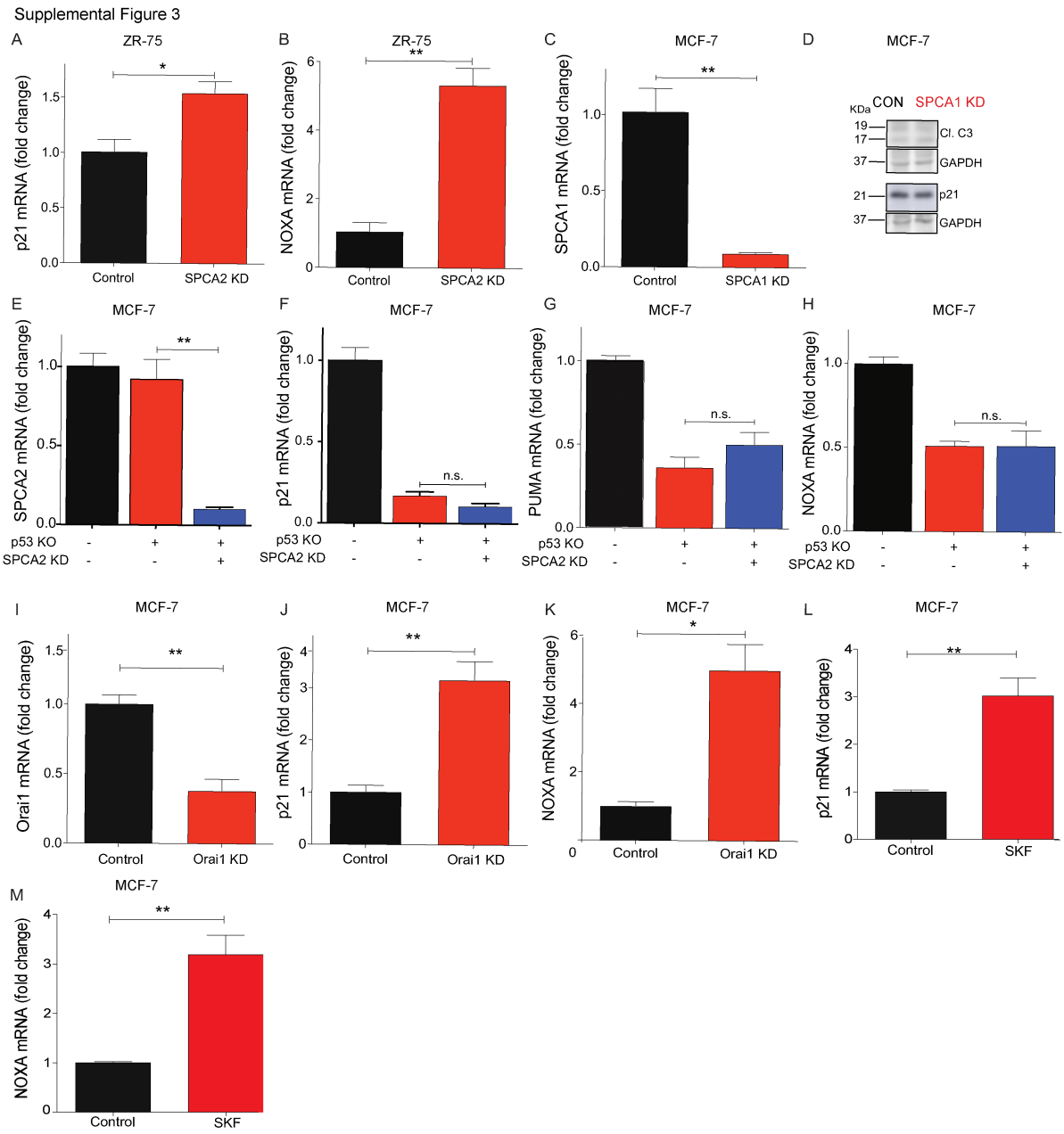

Figure S3: **Store-independent Ca^{2+} entry inhibits p53 signaling**

(A-B) SPCA2 KD in ZR-75 cells significantly increased p21 and NOXA, n=3. (C) Knockdown of SPCA1 in MCF-7 cells was confirmed by qPCR, n=3. (D) Representative Western blot of cleaved caspase-3 and p21 in MCF-7 control and SPCA1 KD cells; GAPDH was used as a loading control. (E) SPCA2 KD in p53 KO MCF-7 cell line was confirmed by qPCR, n=3. (F-H) SPCA2 KD did not increase p21, PUMA, and NOXA mRNA in p53 KO MCF-7 cell line, measured by qPCR, n=3. (I) Orai1 knockdown in MCF-7 was confirmed by qPCR, n=3. (J-K) Orai1 knockdown in MCF-7 cells increased p21 and NOXA, n=3. (L-M) Treatment of MCF-7 cells with SKF-96365 (20 μM, 24 hours) increased p21 and NOXA, n=3. Significance: ^{ns} $P > 0.05$, $^{*}P < 0.05$, ** $P < 0.01$.

Supplemental Figure 4

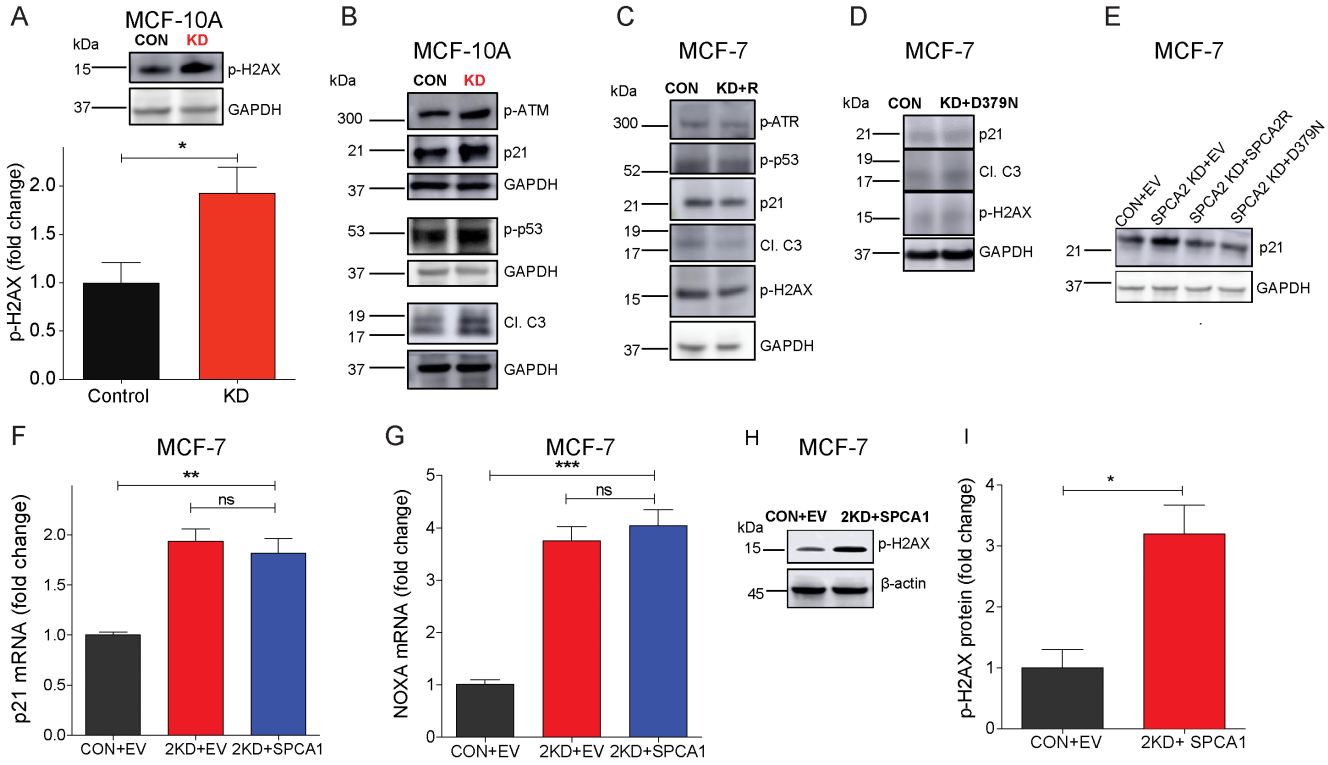

Figure S4: **Loss of SPCA2 activates the ATM/ATR-p53 pathway**

(A) Representative Western blot of p-H2AX in MCF-10A control and SPCA2 KD cells; GAPDH was used as a loading control. SPCA2 KD in MCF-10A showed a significant increase in p-H2AX compared to control, n=3. (B) Representative Western blots of p-ATM, p21, p-p53, and cleaved caspase-3 (Cl. C3) in MCF-10A control and SPCA2 KD cells; GAPDH was used as a loading control. (C) Western blots of p-ATR, p-p53, p21, cleaved caspase-3 (Cl. C3), p-H2AX in MCF-7 control and SPCA2 KD cells transfected with SPCA2R (KD+R); GAPDH was used as a loading control. (D) Western blots of p21, cleaved caspase-3 (Cl. C3), and p-H2AX in MCF-7 control and SPCA2 KD cells transfected with silencing-resistant SPCA2 mutant D379N (KD+D379N), GAPDH was used as a loading control. (E) Western blot of p21 in MCF-7 control + Empty vector (EV), SPCA2 KD +EV, and SPCA2 rescued cells (KD+SPCA2R, and KD+D379N); GAPDH was used as a loading control. (F-G) SPCA1 overexpression in MCF-7 SPCA2 KD cells (2KD+SPCA1) fails to reverse the increase in p21 and NOXA seen in SPCA2 KD cells compared to control. n=3. (H-I) MCF-7 SPCA2 KD cells, which overexpress SPCA1 (2KD+SPCA1), show an increase in p-H2AX, n=3; β-actin was used as a loading control**.** Significance: ^{ns} $P > 0.05$, *$P < 0.05$, ** $P < 0.01$, *** $P < 0.001$.

Supplemental Figure 5

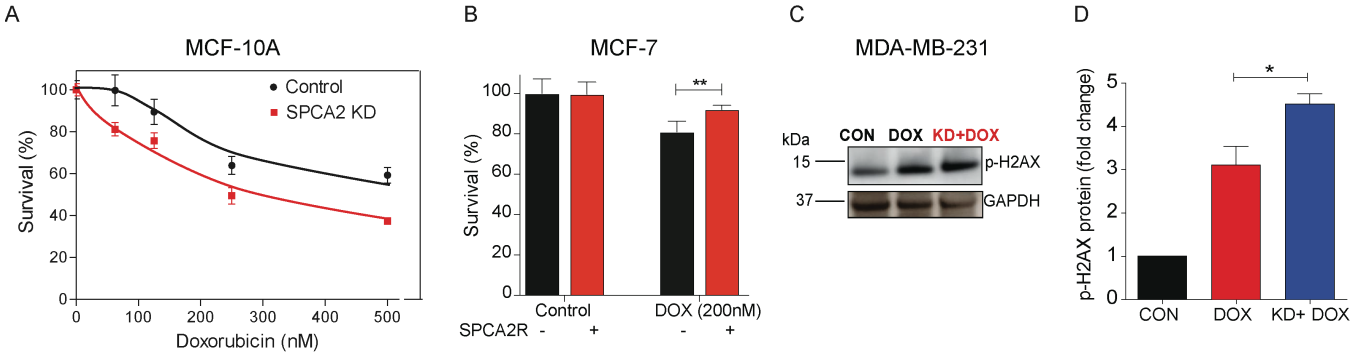

Figure S5: **Loss of SPCA2 expression sensitizes cells to DNA damaging agents**

(A) SPCA2 KD in MCF-10A cells increased chemosensitivity to doxorubicin (0-500 nM) compared to control (n=5 or 6, time=120 hours). (B). Approximately 10,000 cells were plated and incubated overnight. Cells were then treated with doxorubicin (DOX; 200 nM) (time=72 hours). Chemosensitivity to doxorubicin is significantly reversed by forced overexpression of SPCA2R, n=5. (C) Representative western blot of p-H2AX in MDA-MB-231 control and SPCA2 KD cells treated with doxorubicin (DOX) (250 nM, 24 hours); GAPDH was used as a loading control. (D) MDA-MB-231 SPCA2 KD cells treated with doxorubicin showed a significant increase in p-H2AX compared to control cells treated with doxorubicin, n=3. Significance: *P < 0.05, ** P < 0.01.

Supplemental Figure 6

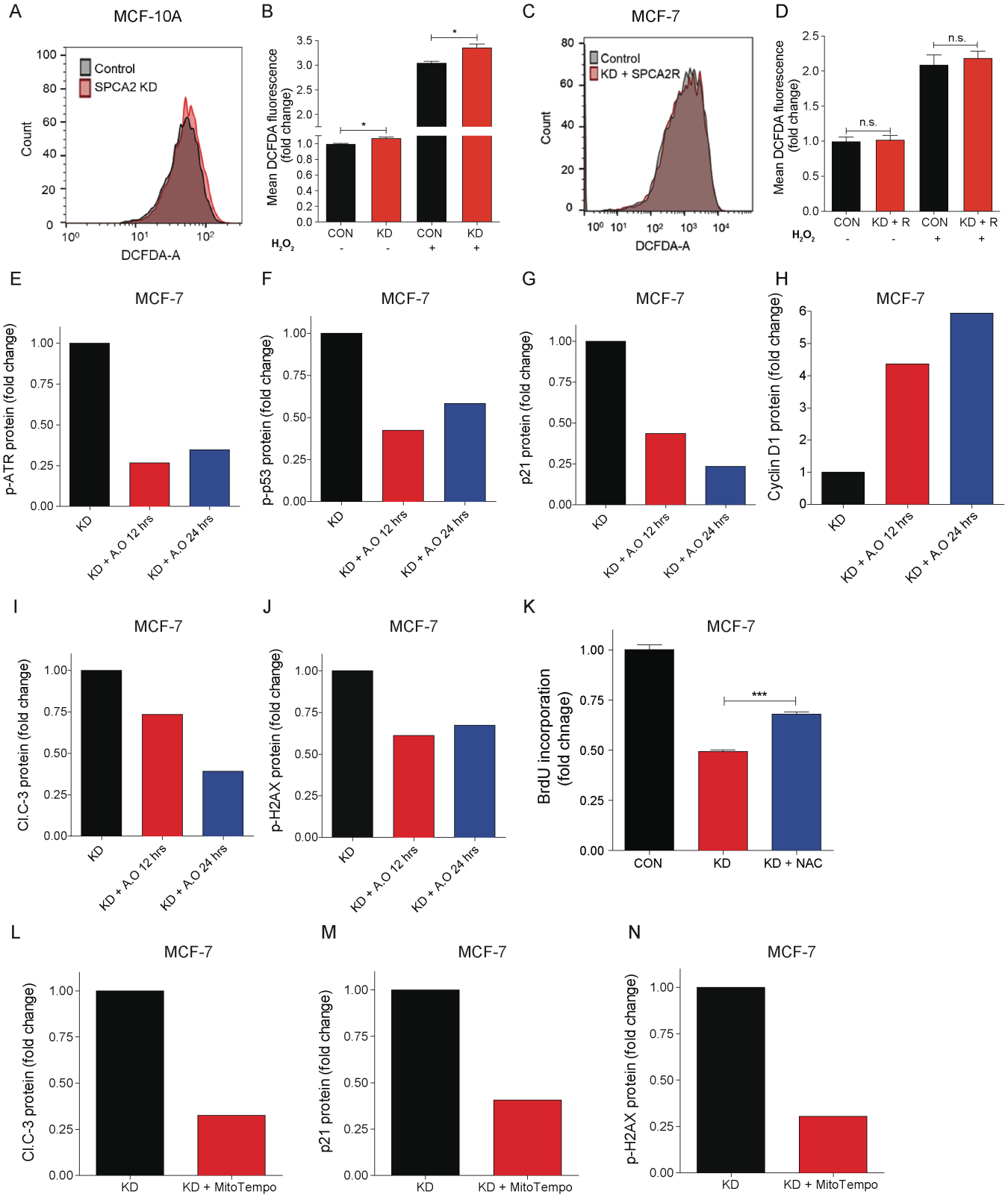

Figure S6: **SPCA2 protects against ROS-mediated DNA damage**

(A) Representative flow cytometry showing ROS production in MCF-10A control and SPCA2 KD cells. (B) ROS production is significantly increased in SPCA2 KD compared to control. H_2O_2 (500 μM for 20 minutes) was used as positive control. n=3 for SPCA2 KD and control, n=2 for H_2O_2 experiment. (C) Representative flow cytometry showing ROS production in MCF-7 control and SPCA2 KD rescued (KD + SPCA2R) cells. (D) There was no significant difference in ROS production between control and SPCA2 KD rescued cells, n=3. (E-J) Anti-oxidants (Vitamin C, 500 μM + N-acetyl cysteine, 500 μM) were added to MCF-7 SPCA2 KD cells for 12 hours and 24 hours and immunoblotting is performed using GAPDH as loading control. Quantification of p-ATM, p-p53, p21, cleaved caspase-3, p-H2AX, and Cyclin D1. (K) MCF-7 SPCA2 KD cells were treated with NAC (500 μM) for 24 hours. Cell proliferation was measured using BrdU proliferation assay. NAC treatment significantly rescued the cell proliferation compared to MCF-7 SPCA2 KD cells. n=6 for control, n=5 for KD and n=4 for KD+ NAC (500 μM). (L-N) Mitochondrial anti-oxidant (MitoTempo, 5 μM) was added to MCF-7 SPCA2 KD cells for 24 hours and immunoblotting was performed using GAPDH as loading control. Quantification of cleaved caspase-3, p21 and p-H2AX. Significance: ^{ns} $P > 0.05$, $^{*}P < 0.05$.

Supplemental Figure 7

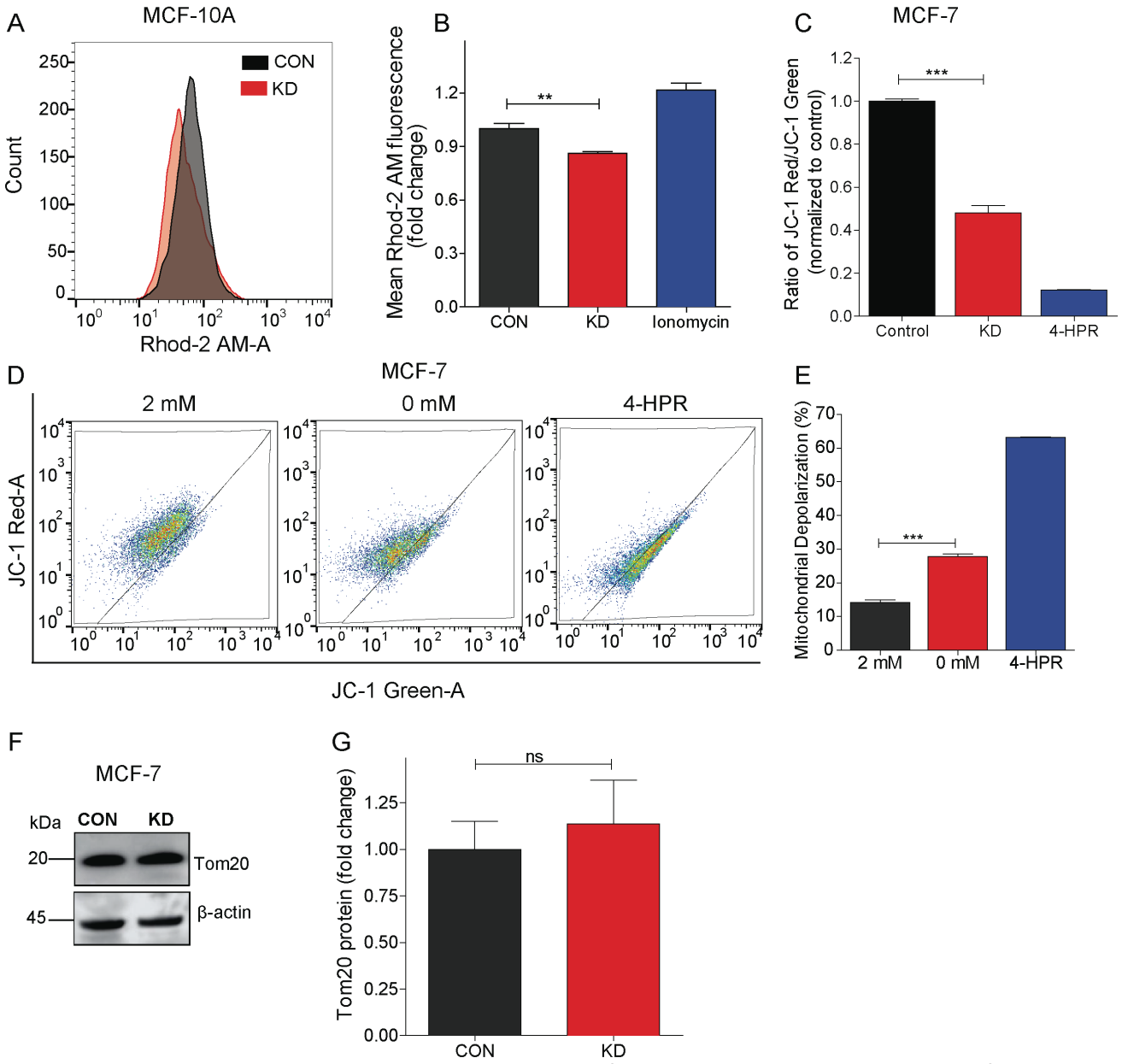

Figure S7: **Loss of SPCA2 and extracellular Ca^{2+} decrease mitochondrial Ca^{2+} levels and membrane potential**

(A) Representative flow cytometry images showing mitochondrial calcium (Rhod-2 AM fluorescence) in MCF-10A control and SPCA2 KD cells. (B) Quantification of flow cytometry in MCF-10A. Mitochondrial calcium was significantly decreased in SPCA2 knockdown compared to control. Ionomycin (10 μM for 15 seconds) was used as positive control; n=3. (C) SPCA2 KD in MCF-7 showing significant decrease in ratio of JC-1 Red/ JC-1 green compared to control. n=3 for control and n=4 for SPCA2 KD. (D) Representative flow cytometry showing mitochondrial membrane depolarization in MCF-7 cells exposed to Ca^{2+}-free media for 15 min compared to cells grown in media with 2 mM Ca^{2+}. Fenretinide (4-HPR) (10 μM for 20 hours) was used as

positive control. (E) Mitochondrial membrane depolarization was significantly increased in in MCF-7 cells exposed to Ca^{2+}-free media compared to cells grown in media with 2 mM Ca^{2+}. n=3. (F-G) Representative Western blot of Tom20 in MCF-7 control and SPCA2 KD cells; β-actin was used as a loading control. SPCA2 KD in MCF-7 did not significantly increase Tom20 protein expression compared to control, n=3. Significance: ^{ns} $P > 0.05$, $^{*}P < 0.05$, $^{***}P < 0.001$.