Cancer Cell, Volume *33*

Supplemental Information

Cancer Cells Co-opt the Neuronal Redox-Sensing Channel TRPA1 to Promote Oxidative-Stress Tolerance

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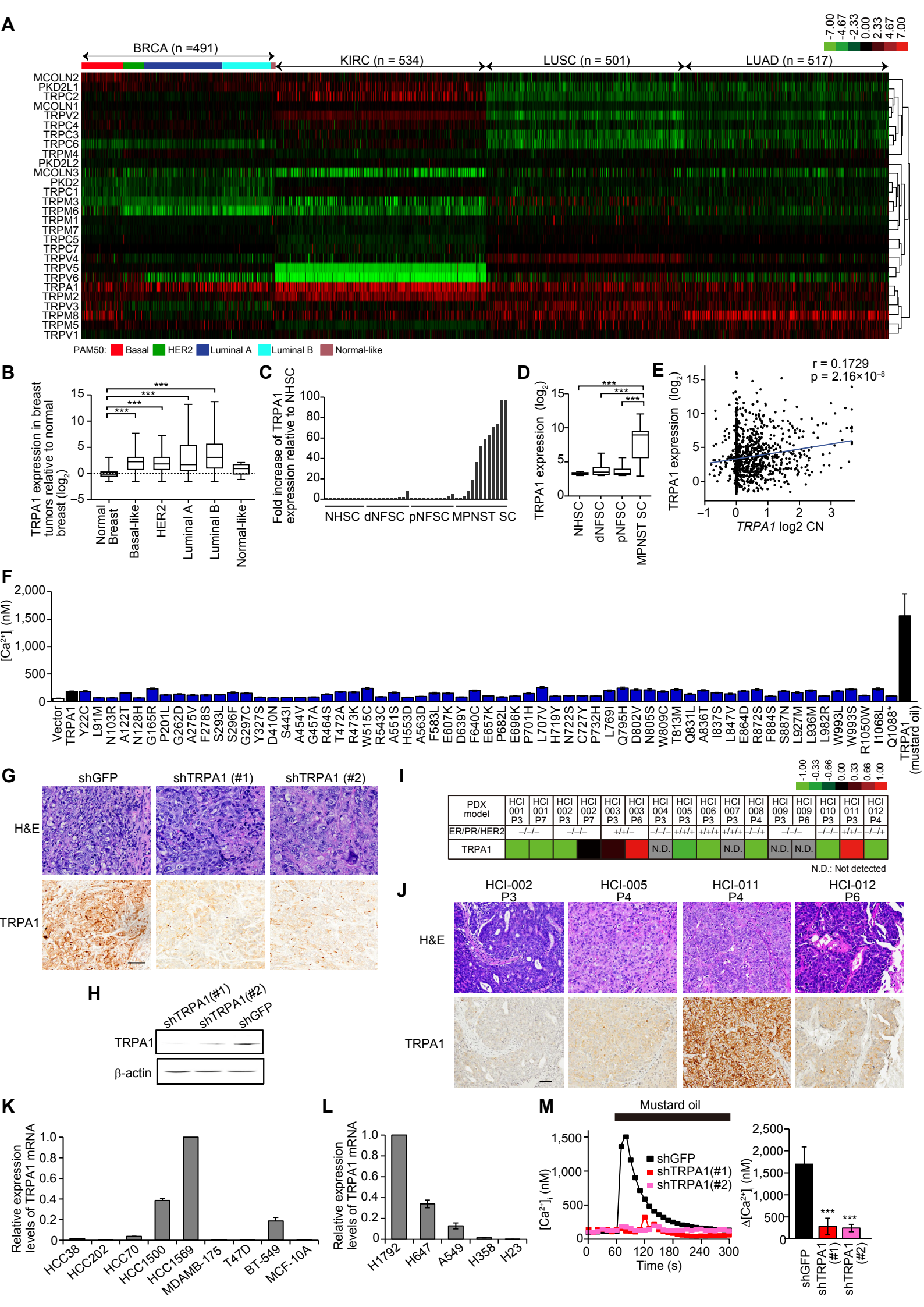
A
BRCA (n =491)
KIRC (n = 534)
LUSC (n = 501)
LUAD (n = 517)
MCOLN2
PKD2L1
TRPC2
MCOLN1
TRPV2
TRPC4
TRPC3
TRPC6
TRPM4
PKD2L2
MCOLN3
PKD2
TRPC1
TRPM3
TRPM6
TRPM1
TRPM7
TRPC5
TRPC7
TRPV4
TRPV5
TRPV6
TRPA1
TRPM2
TRPV3
TRPM8
TRPM5
TRPV1
PAM50: Basal HER2 Luminal A Luminal B Normal-like
B
TRPA1 expression in breast tumors relative to normal breast (log2)
Normal Breast
Basal-like
HER2
Luminal A
Luminal B
Normal-like
C
Fold increase of TRPA1 expression relative to NHSC
NHSC dNFSC pNFSC MPNST SC
D
TRPA1 expression (log2)
NHSC
dNFSC
pNFSC
MPNST SC
E
r = 0.1729
p = 2.16×10−8
TRPA1 expression (log2)
TRPA1 log2 CN
F
[Ca2+]i (nM)
Vector
TRPA1
Y22C
L91M
N103R
A122T
N128H
G165R
P201L
G262D
A275V
F278S
S293L
S296F
G297C
Y327S
D410N
S443I
A454V
G457A
R464S
T472A
R473K
W515C
R543C
A551S
H553D
A563D
F583L
E607K
D639Y
F640C
E657K
P682L
E696K
P701H
L707V
H719Y
N722S
C727Y
P732H
L769I
Q795H
D802V
N805S
W809C
T813M
Q831L
A836T
I837S
L847V
E864D
R872S
F884S
S887N
L927M
L936M
L982R
W993L
W993S
R1050W
I1068L
Q1088*
TRPA1 (mustard oil)
G
shGFP
shTRPA1 (#1)
shTRPA1 (#2)
H&E
TRPA1
H
shTRPA1(#1)
shTRPA1(#2)
shGFP
TRPA1
β-actin
I
PDX model
ER/PR/HER2
TRPA1
HCI 001 P3
HCI 001 P7
HCI 002 P3
HCI 002 P7
HCI 003 P3
HCI 003 P6
HCI 004 P3
HCI 005 P3
HCI 006 P3
HCI 007 P3
HCI 008 P4
HCI 009 P3
HCI 009 P6
HCI 010 P3
HCI 011 P3
HCI 012 P4
N.D.
N.D.: Not detected
J
HCI-002 P3
HCI-005 P4
HCI-011 P4
HCI-012 P6
H&E
TRPA1
K
Relative expression levels of TRPA1 mRNA
HCC38
HCC202
HCC70
HCC1500
HCC1569
MDAMB-175
T47D
BT-549
MCF-10A
L
Relative expression levels of TRPA1 mRNA
H1792
H647
A549
H358
H23
M
Mustard oil
[Ca2+]i (nM)
shGFP
shTRPA1(#1)
shTRPA1(#2)
Time (s)
Δ[Ca2+]i (nM)
shGFP
shTRPA1 (#1)
shTRPA1 (#2)

Figure S1. TRPA1 Is Upregulated in Diverse Tumor Types, Related to Figure 1.

(**A**) Heatmap (red, increased expression; green, decreased expression) of TRP channel mRNA levels in TCGA BRCA, KIRC, LUSC, and LUAD relative to corresponding normal tissues (n = 121 for BRCA, n = 72 for KIRC, n = 51 for LUSC, and n = 59 for LUAD). The samples, whose PAM50 has not been identified, are not included for BRCA. Averaged mRNA level of each TRP channel relative to corresponding normal tissues are listed in Table S1.

(**B**) Box plots displaying TRPA1 mRNA levels in Basal-like (n = 107), HER2 (n = 51), Luminal A (n = 201), Luminal B (n = 123), and Normal-like (n = 9) subtypes of BRCA relative to normal breast tissues (n = 121). $^{***}p < 0.001$ (one-way ANOVA).

(**C**) Waterfall plot displaying fold increases of TRPA1 mRNA expression in dermal and plexiform NF1-derived primary benign neurofibroma Schwann (dNFSCs and pNFSCs, respectively) and MPNST Schwann cells (MPNST SC) relative to normal human Schwann cells (NHSC), using a published microarray dataset (Miller et al., 2009). The vertical axis of the graph is cut off at 100.

(**D**) Box plots displaying TRPA1 mRNA levels in NHSC (n = 10), dNFSC (n = 11), pNFSC (n = 11), and MPNST SC (n = 13). $^{***}p < 0.001$ (one-way ANOVA).

(**E**) Correlation of *TRPA1* copy number (CN) alterations with TRPA1 mRNA expression in BRCA (n = 1036).

(**F**) Averaged intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) upon treatment with or without 30 μM mustard oil in HEK293 cells transfected with TRPA1 mutant construct. Data shown as mean ± SEM (n = 37–208). Cancer types where each mutation was found are listed in Table S2.

(**G**) Representative H&E (upper panels) and TRPA1 IHC staining (lower panels) of the indicated HCC1569 xenograft tumor sections from three independent experiments. TRPA1 antibody from Sigma. Scale bar 50 μm.

(**H**) Immunoblot analysis of TRPA1 in HCC1569 cells transduced with shRNA against TRPA1 or GFP (control) from two independent experiments. TRPA1 antibody from Novus.

(**I**) Heatmap representing relative levels of TRPA1 mRNA in PDX models (DeRose et al., 2011). The data are expressed as log2 median-centered ratio. P represents passage number.

(**J**) H&E (upper panels) and TRPA1 IHC staining (lower panels) of the indicated PDX tumor sections. Scale bar 50 μm.

(**K**) TRPA1 mRNA levels in the indicated breast cancer cell lines as determined by quantitative PCR. Data are normalized to HCC1569 cells. Data shown as the mean ± SEM of three independent experiments.

(**L**) TRPA1 mRNA levels in the indicated lung cancer cell lines as determined by quantitative PCR. Data are normalized to H1792 cells. Data shown as the mean ± SEM of three independent experiments.

(**M**) Averaged time courses and the maximal $[Ca^{2+}]_i$ rises ($\Delta[Ca^{2+}]_i$) evoked by 30 μM mustard oil in HCC1569 cells transduced with shTRPA1 or shGFP. Data shown as mean ± SEM (n = 9–59). $^{***}p < 0.001$ compared to shGFP (Student's t test).

In (B) and (D), box and whiskers graphs indicate the median and the 25th and 75th percentiles, with minimum and maximum values at the extremes of the whiskers.

Table S1. Averaged TRP channel mRNA levels in TCGA BRCA, KIRC, LUSC, and LUAD relative to corresponding normal tissues (n = 121 for BRCA, n = 72 for KIRC, n = 51 for LUSC, and n = 59 for LUAD), related to Figure 1.

BRCA (n = 491)

Ranking	TRP subtype	mRNA level relative to normal breast tissues (log_2)	p value (vs normal breast)	p value (vs TRPA1)
1	*TRPA1	2.87	5.03×10^{-22}	
2	MCOLN2	1.23	4.87×10^{-20}	2.23×10^{-27}
3	PKD2L1	1.17	1.26×10^{-13}	4.71×10^{-31}
4	*TRPM2	1.10	1.39×10^{-19}	2.57×10^{-32}
5	TRPM5	0.924	6.53×10^{-9}	1.72×10^{-37}
6	TRPC4	0.505	4.19×10^{-7}	1.16×10^{-55}
7	TRPM4	0.310	2.22×10^{-6}	2.50×10^{-63}
8	TRPM8	0.102	0.758	3.15×10^{-55}
9	TRPC7	0.0581	0.0892	1.13×10^{-79}
10	MCOLN1	0.0269	0.895	1.07×10^{-79}
11	*TRPV2	0.0103	0.788	4.39×10^{-76}
12	TRPV5	−0.0186	0.0198	1.63×10^{-80}
13	TRPC3	−0.0945	0.721	2.08×10^{-80}
14	*TRPC5	−0.269	6.74×10^{-12}	2.96×10^{-94}
15	*TRPV1	−0.330	2.18×10^{-5}	1.95×10^{-93}
16	PKD2L2	−0.404	2.65×10^{-10}	1.66×10^{-99}
17	TRPC2	−0.448	1.49×10^{-6}	2.18×10^{-98}
18	*TRPM7	−0.456	2.33×10^{-9}	1.35×10^{-101}
19	*TRPV3	−0.577	0.00621	7.96×10^{-87}
20	TRPM1	−0.628	1.11×10^{-34}	4.41×10^{-111}
21	*TRPV4	−0.773	0.00496	5.21×10^{-106}
22	MCOLN3	−1.23	1.00×10^{-12}	1.58×10^{-114}
23	TRPV6	−1.56	0.00710	3.32×10^{-103}
24	TRPC1	−1.59	1.12×10^{-48}	2.88×10^{-150}
25	PKD2	−1.69	1.11×10^{-71}	8.06×10^{-159}
26	TRPC6	−1.87	1.09×10^{-57}	2.19×10^{-163}
27	TRPM3	−2.55	4.16×10^{-117}	7.71×10^{-201}
28	TRPM6	−3.06	1.68×10^{-53}	1.62×10^{-192}

KIRC (n = 534)

Ranking	TRP subtype	mRNA level relative to normal kidney tissues (log_2)	p value (vs normal lung)	p value (vs TRPA1)
1	*TRPA1	4.18	1.19×10^{-51}	
2	*TRPM2	3.15	4.96×10^{-76}	1.78×10^{-24}
3	*TRPV2	2.13	4.31×10^{-80}	3.27×10^{-92}
4	TRPC2	2.13	2.65×10^{-12}	1.38×10^{-48}
5	PKD2L1	1.62	3.47×10^{-16}	1.07×10^{-98}
6	TRPM8	1.27	5.77×10^{-6}	1.00×10^{-113}
7	TRPC4	0.841	2.03×10^{-7}	4.15×10^{-165}
8	MCOLN2	0.644	1.39×10^{-5}	1.46×10^{-176}
9	TRPC1	0.515	3.38×10^{-10}	4.44×10^{-219}
10	TRPC6	0.458	0.000599	1.06×10^{-193}
11	MCOLN1	0.342	1.81×10^{-9}	2.36×10^{-241}
12	PKD2L2	0.181	0.0443	1.72×10^{-245}
13	*TRPV1	0.154	0.0223	2.78×10^{-221}
14	*TRPV3	0.116	0.346	3.74×10^{-214}
15	PKD2	−0.114	0.000892	5.16×10^{-272}
16	TRPC3	−0.200	0.302	1.83×10^{-249}
17	*TRPV4	−0.534	0.00209	2.86×10^{-258}
18	TRPM1	−0.683	4.64×10^{-9}	1.24×10^{-291}
19	*TRPM7	−0.743	3.14×10^{-23}	0
20	TRPC7	−0.858	2.09×10^{-12}	5.99×10^{-291}
21	TRPM4	−0.936	4.38×10^{-22}	0
22	*TRPC5	−1.20	1.64×10^{-29}	0
23	TRPM5	−1.37	1.94×10^{-58}	0
24	TRPM3	−2.41	1.96×10^{-15}	1.10×10^{-289}
25	TRPM6	−2.82	1.30×10^{-40}	0
26	MCOLN3	−3.30	8.53×10^{-35}	0
27	TRPV5	−4.76	4.85×10^{-133}	0
28	TRPV6	−6.18	5.63×10^{-158}	0

LUSC (n = 501)

Ranking	TRP subtype	mRNA level relative to normal lung tissues (log_2)	p value (vs normal lung)	p value (vs TRPA1)
1	*TRPA1	2.27	1.25×10^{-13}	
2	*TRPV3	2.07	2.99×10^{-14}	0.106
3	TRPM8	2.01	5.31×10^{-14}	0.0380
4	*TRPV4	1.93	7.33×10^{-23}	0.00249
5	*TRPM2	1.09	1.28×10^{-20}	2.05×10^{-26}
6	TRPM5	0.96	0.00212	2.35×10^{-24}
7	*TRPV1	0.37	0.0192	2.61×10^{-61}
8	TRPV5	0.272	0.138	4.46×10^{-76}
9	*TRPM7	0.243	0.00302	3.36×10^{-76}
10	TRPC7	0.101	0.0643	1.51×10^{-88}
11	*TRPC5	0.0929	0.655	4.09×10^{-85}
12	TRPV6	0.0498	0.513	2.70×10^{-54}
13	TRPM3	−0.0600	0.896	9.21×10^{-62}
14	TRPM1	−0.128	0.0220	7.27×10^{-84}
15	PKD2L2	−0.207	0.0245	9.80×10^{-107}
16	TRPM4	−0.282	0.135	8.33×10^{-104}
17	MCOLN3	−0.635	0.0471	1.53×10^{-93}
18	TRPC1	−0.705	6.79×10^{-5}	2.24×10^{-120}
19	TRPM6	−0.721	0.000210	4.65×10^{-110}
20	PKD2	−0.772	7.38×10^{-21}	3.32×10^{-144}
21	MCOLN2	−0.798	4.80×10^{-5}	5.53×10^{-124}
22	TRPC4	−1.00	2.78×10^{-9}	9.68×10^{-140}
23	MCOLN1	−1.11	7.12×10^{-40}	2.55×10^{-169}
24	PKD2L1	−2.12	2.54×10^{-17}	2.64×10^{-192}
25	TRPC2	−2.29	1.57×10^{-59}	4.56×10^{-235}
26	TRPC3	−2.50	4.24×10^{-48}	2.36×10^{-237}
27	TRPC6	−2.67	4.36×10^{-40}	5.59×10^{-235}
28	*TRPV2	−2.67	7.90×10^{-56}	4.75×10^{-247}

LUAD (n = 517)

Ranking	TRP subtype	mRNA level relative to normal lung tissues (log_2)	p value (vs normal lung)	p value (vs TRPA1)
1	TRPM8	3.90	1.54×10^{-22}	1.69×10^{-62}
2	*TRPA1	1.27	9.08×10^{-6}	
3	*TRPM2	1.20	1.19×10^{-14}	0.49
4	*TRPV1	1.07	5.82×10^{-16}	0.0359
5	TRPM5	1.05	3.44×10^{-5}	0.0554
6	*TRPV3	0.891	1.27×10^{-6}	0.000385
7	*TRPC5	0.325	0.465	2.54×10^{-27}
8	TRPV5	0.184	0.404	1.40×10^{-36}
9	*TRPV4	0.0781	0.562	1.01×10^{-30}
10	TRPC7	0.0195	0.197	2.23×10^{-48}
11	PKD2L2	−0.0170	0.425	1.21×10^{-46}
12	TRPM4	−0.0950	0.912	3.35×10^{-44}
13	*TRPM7	−0.228	0.00336	3.15×10^{-62}
14	MCOLN2	−0.379	0.0375	1.28×10^{-56}
15	TRPC1	−0.608	0.000134	2.97×10^{-77}
16	TRPM3	−0.742	1.17×10^{-6}	3.64×10^{-83}
17	PKD2	−0.790	7.06×10^{-13}	1.19×10^{-99}
18	TRPM1	−0.821	1.86×10^{-23}	6.83×10^{-104}
19	MCOLN1	−0.956	1.97×10^{-31}	2.22×10^{-117}
20	TRPC4	−0.986	1.06×10^{-12}	1.27×10^{-104}
21	TRPV6	−1.04	2.32×10^{-5}	3.60×10^{-69}
22	TRPM6	−1.09	9.44×10^{-8}	1.94×10^{-90}
23	TRPC6	−1.40	9.71×10^{-11}	4.62×10^{-109}
24	TRPC2	−1.48	1.33×10^{-15}	1.09×10^{-125}
25	PKD2L1	−1.54	2.08×10^{-13}	2.22×10^{-123}
26	MCOLN3	−1.68	2.55×10^{-10}	1.19×10^{-112}
27	TRPC3	−1.88	5.57×10^{-24}	8.20×10^{-162}
28	*TRPV2	−1.97	3.44×10^{-47}	7.00×10^{-181}

Asterisk (*) denotes redox-senstive TRP channels

Table S2. Primer sequences used for the construction of TRPA1 mutants, related to Figure 1.

		Mutation primer sequence (5' → 3')	
Mutants	Cancer types	Forward primer	Reverse primer
Y22C	LUSC	agggcgttgtctGtgaggatgtgcc	ggcacatcctcacagaCaacgccct
L91M	Colorectal	cctctttggaagtgAtgcatgaaatggatg	catccatttcatgcaTcacttccaaagagg
H103R	Stomach	gaaataccctctgcGttgtgctgtagaaa	tttctacagcacaaCgcagaggggtatttc
A122T	Uterine	ttctcagcagaggaAcaaacccaaacctcc	ggaggtttgggtttgTtcctctgctgagaa
N128H	Melanoma	acccaaacctccgaCacttcaacatgatg	catcatgttgaagtGtcggaggtttgggt
G165R	LUSC	tggaaggagaaaatCgaaacacagctgtga	tcacagctgtgtttcGattttctccttcca
P201L	Melanoma	aatggggatgtttccTtattcaccaagctg	cagcttggtgaataAggaaacatccccatt
G262D	Uterine	tgtgcctggacaatgAtgcacaaatagacc	ggtctatttgtgcaTcattgtccaggcaca
A275V	GBM	agggaaggtgcacagTcattcattttgctg	cagcaaaatgaatgActgtgcaccttccct
F278S	Ovarian	cagccattcattCtgctgccaccca	tgggtggcagcaGaatgaatggctg
S293L	Colorectal	gttaaactgatgatatTgtcctattctggtagcgtg	cacgctaccagaataggacAatatcatcagtttaac
S296F	BRCA	gttaaactgatgatatcgtcctattTtggtagcgtg	cacgctaccaAaataggacgatatcatcagtttaac
G297C	LUAD	gatatcgtcctattctTgtagcgtggatattgtt	aacaatatccacgctacAagaataggacgatatc
Y327S	Ovarian	catgagctagcagactCtttaatttcagtgggag	ctcccactgaaattaaaGagtctgctagctcatg
D410N	Colorecta & Glioma	tggtaatggatgaaAacaacgatgggtgta	tacacccatcgttgtTttcatccattacca
S443I	Colorectal	cattccaaaATCaaagataagaaatcacctctgc	gcagaggtgatttcttatctttGATtttggaatg
A454V	Stomach	gcattttgcaGTCagttatgggcgtatcaa	ttgatacgcccataactGACtgcaaaatgc
G457A	LUAD	gcagccagttatGCGcgtatcaatacctgt	acaggtattgatacgCGCataactggctgc
R464S	LUSC	caatacctgtcagAGCctcctacaagacataagtg	cacttatgtcttgtaggagGCTctgacaggtattg
T472A	Stomach	gacataagtgatGCGaggcttctgaatgaagg	ccttcattcagaagcctCGCatcacttatgtc
R473K	Uterine	gacataagtgatacgAAGcttctgaatgaaggtgac	gtcaccttcattcagaagCTTcgtatcacttatgtc
W515C	LUAD	accacaatggcTGCacagctttgcat	atgcaaagctgtGCAgccattgtggt
R543C	Melanoma	tgaagtgcacagatTGCttggatgaagacg	cgtcttcatccaaGCAatctgtgcacttca
A551S	Colorectal	aagacgggaacactTCActtcactttgctgc	gcagcaaagtgaagTGAagtgttcccgtctt
H553D	LUSC	ggaacactgcacttGACtttgctgcaagg	ccttgcagcaaaGTCaagtgcagtgttcc
A563D	LUSC	ccacgccaaaGACgttgcgcttctt	aagaagcgcaacGTCtttggcgtgg
F583L	Stomach	agcaggcctccCTTttgcaccttgc	gcaaggtgcaaAAGgaggcctgct
E607K	Melanoma	caggagcaaaagatgggatAAAtgtcttaagattttc	gaaaatcttaagacaTTTatcccatcttttgctcctg
D639Y	Uterine	gcatgaaggtacttttaTATttctgcatgttgcattc	gaatgcaacatgcagaaATAtaaaagtaccttcatgc
F640C	Uterine	atgaaggtacttttagatTGCtgcatgttgcattc	gaatgcaacatgcaGCAatctaaaagtaccttcat
E657K	Colorectal	gacaagtcctgccgagactattatatcAAGtataatttc	gaaattataCTTgatataatagtctcggcaggacttgtc
P682L	Colorectal	caggatgttatatatgaaCTGcttacagccctcaacg	cgttgagggctgtaagCAGttcatatataacatcctg
E696K	Melanoma	caaaataaccgcataAAGcttctcaatcatcctg	caggatgattgagaagCTTtatgcggttattttg
P701H	Stomach & Uterine	cgcatagagcttctcaatcatCATgtgtgtaaagaata	tattctttacacacATGatgattgagaagctctatgcg
L707V	Uterine	gtgtgtaaagaatatCTActcatgaaatggttggc	gccaaccatttcatgagTAGatattctttacacac
H719Y	LUAD	ggcttatggatttagagctTATatgatgaatttaggatc	gatcctaaattcatcatATAagctctaaatccataagcc
N722S	LUSC	gagctcatatgatgAGTttaggatcttactgtcttgg	ccaagacagtaagatcctaaACTcatcatatgagctc
C727Y	Uterine	gaatttaggatcttacTATcttggtctcatacctatgacc	ggtcataggtatgagaccaagATAgtaagatcctaaattc
P732H	Uterine	gtcttggtctcataCATatgaccattctcgttgtc	gacaacgagaatggtcatATGtatgagaccaagac
L769I	Uterine	ctagataccacgaattcatatATAataaaaacttgtatg	catacaagtttttatTATatatgaattcgtggtatctag
Q795H	Uterine	cggggcaaattttccaaCACaaaaggaattatt	aataattccttttGTGttggaaaatttgccccg
D802V	LUAD	ggaattattttatgGTTataagcaatgttcttgaatgg	ccattcaagaacattgcttatAACcataaaataattcc
N805S	LUAD	tatggatataagcAGTgttcttgaatggattatctacac	gtgtagataatccattcaagaacACTgcttatatccata
W809C	GBM	atataagcaatgttcttgaaTGCattatctacacgacg	cgtcgtgtagataatGCAttcaagaacattgcttatat
T813M	GBM	gaatggattatctacATGacgggcatcattttgt	acaaaaatgatgcccgtCATgtagataatccattc
Q831L	GBM	agctcatctgCTGtggcaatgtgga	tccacattgccaCAGcagatgagct
A836T	LUAD & Pancreas	tggcaatgtggaACAattgctgtttacttct	agaagtaaacagcaatTGTtccacattgcca
I837S	Colorectal	tggcaatgtggagcaAGTgctgtttacttct	agaagtaaacagcACTtgctccacattgcca
L847V	Uterine	cttctattggatgaatttcGTAttgtatcttcaaagatt	aatctttgaagatacaaTACgaaattcatccaatagaag
E864D	Uterine	ggaatttttattgttatgttgGACgtaattttgaaaac	gttttcaaaattacGTCcaacataacaataaaaattcc
R872S	Glioma	tgaaaactttgttgAGCtctacagttgtatttatcttcc	ggaagataaatacaactgtagaGCTcaacaaagttttca
F884S	Stomach	ccttcttctggctTCTggactcagctttaca	tgtaaaagctgagtccAGAagccagaagaagg
S887N	Colorectal	ttctggcttttggactcAACttttacatcctcct	aggaggatgtaaaaGTTgagtccaaaagccagaa
L927M	Uterine & Colorectal	tcctagaaccatatATGagaaatgaattggcac	gtgccaattcatttctCATatatggttctagga
L936M	Colorectal	ggcacatccagttATGtcctttgcacaac	gttgtgcaaaggaCATaactggatgtgcc
L982R	Stomach	ctatgcaggtggaaCGTcataccagctta	taagctggtatgACGttccacctgcatag
W993L	GBM & NSCLC	gaagctgccacttTTGtttctacgcaaagt	actttgcgtagaaaCAAaagtggcagcttc
W993S	LUSC	gaagctgccacttTCGtttctacgcaaagt	actttgcgtagaaaCGAaagtggcagcttc
R1050W	Uterine	taaagcagaaatacTGGctgaaggatcttacttttc	gaaaagtaagatccttcagCCAgtatttctgcttta
I1068L	LUAD	gagctcattaaactgCTCattcagaagatgg	ccatcttctgaatGAGcagtttaatgagctc
Q1088*	LUSC	gccattgttcttttTAAgacaggtttaagaaagagc	gctctttcttaaacctgtcTTAaaaagaacaatggc

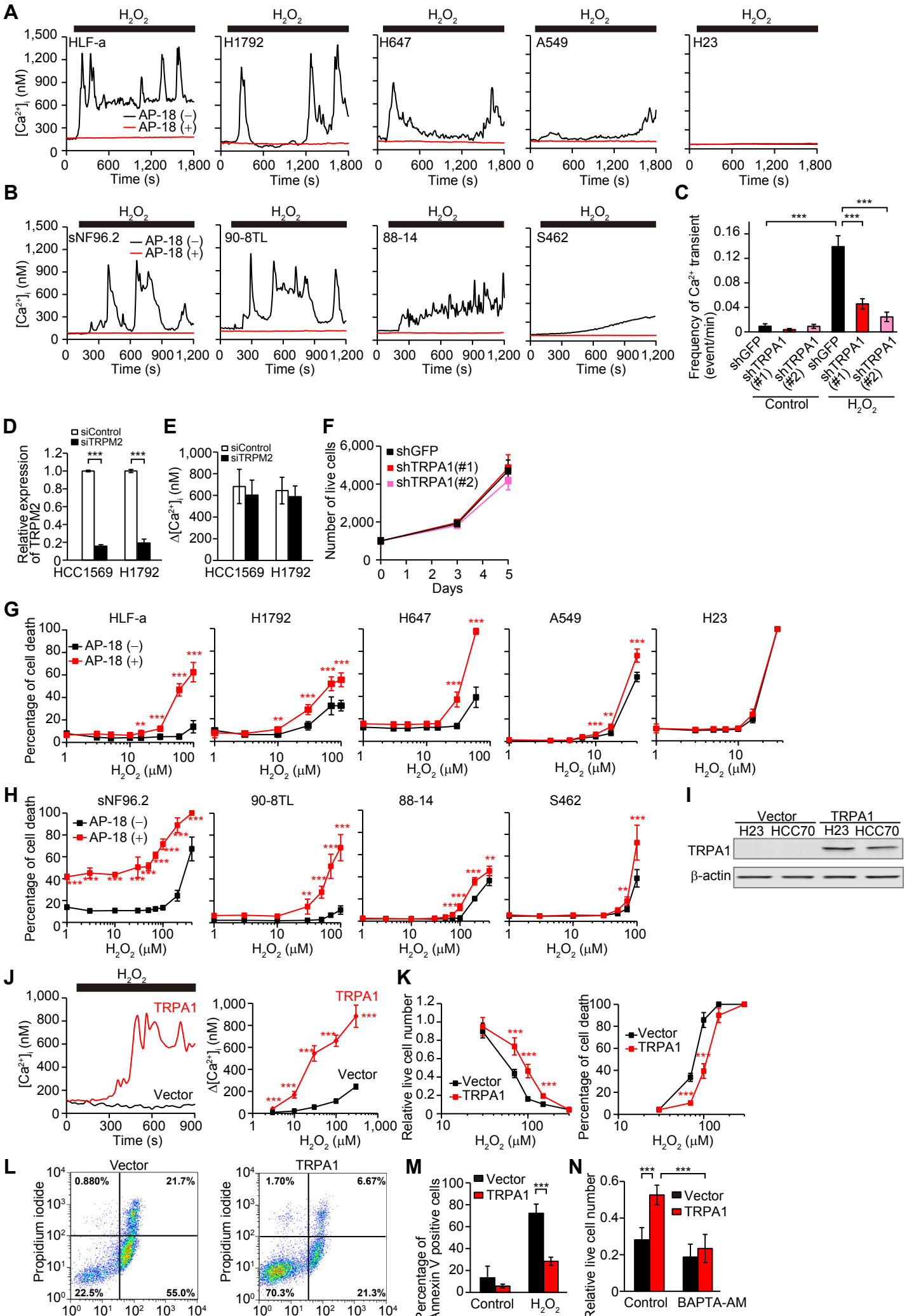
A
B
C
D
E
F
G
H
I
J
K
L
M
N
H2O2
HLF-a
H1792
H647
A549
H23
sNF96.2
90-8TL
88-14
S462
AP-18 (–)
AP-18 (+)
[Ca2+]i (nM)
Time (s)
Frequency of Ca2+ transient (event/min)
shGFP
shTRPA1 (#1)
shTRPA1 (#2)
Control
siControl
siTRPM2
Relative expression of TRPM2
HCC1569
Δ[Ca2+]i (nM)
Number of live cells
Days
Percentage of cell death
Vector
TRPA1
β-actin
Relative live cell number
Propidium iodide
Annexin V-Alexa Fluor 488
0.880%
21.7%
22.5%
55.0%
1.70%
6.67%
70.3%
21.3%
Percentage of Annexin V positive cells
BAPTA-AM

Figure S2. Ca^{2+} Influx through TRPA1 Suppresses Cell Death under Oxidative Stress, Related to Figure 2.

(**A and B**) Representative time courses of $[Ca^{2+}]_i$ changes in the indicated lung cancer (A) or MPNST cells (B) upon treatment with 10 μM H_2O_2 (A) or 30 μM H_2O_2 (B) in the presence or absence of 10 μM AP-18.

(**C**) Frequency of Ca^{2+} transient ($\Delta[Ca^{2+}]_i$ > 200 nM) in HCC1569 cells transduced with shTRPA1 or shGFP and treated with or without 10 μM H_2O_2. Data shown as mean ± SEM (n = 35–150). $^{***}p < 0.001$ (one-way ANOVA).

(**D**) mRNA expression of TRPM2 in HCC1569 and H1792 cells transfected with siControl or siTRPM2. Data were normalized to siControl cells. Data shown as mean ± SD of three independent experiments. $^{***}p < 0.001$ (Student's t test).

(**E**) $\Delta[Ca^{2+}]_i$ evoked by 10 μM H_2O_2 in the indicated cell lines. Data shown as mean ± SEM (n = 28–34).

(**F**) Live cell numbers in HCC1569 cells transduced with shTRPA1 or shGFP for the indicated times. Data shown as mean ± SD from the sum of three independent experiments performed in duplicate.

(**G and H**) Percentage of cell death in the indicated lung cancer (G) and MPNST cells (H) upon treatment with H_2O_2 for 72 hr in the presence or absence of 10 μM AP-18. Data shown as mean ± SD from the sum of three independent experiments performed in duplicate. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to vehicle control for AP-18 [AP-18 (–)] (Student's t test).

(**I**) Immunoblot of lysates from H23 or HCC70 cells transduced with an empty lentiviral vector or TRPA1 from two independent experiments.

(**J**) Representative time course of $[Ca^{2+}]_i$ rises evoked by 100 μM H_2O_2 and dose-response of $\Delta[Ca^{2+}]_i$ elicited upon treatment with increasing concentrations of H_2O_2 in MCF-10A cells transduced with an empty lentiviral vector or TRPA1. Data shown as mean ± SEM (n = 58–167). $^{***}p < 0.001$ compared to vector (Student's t test).

(**K**) Live cell numbers relative to H_2O_2-untreated cells and percentage of cell death in the indicated MCF-10A cells upon treatment with H_2O_2 for 72 hr in EGF-free conditions. Data shown as mean ± SD from the sum of three independent experiments performed in duplicate. $^{***}p < 0.001$ compared to vector (Student's t test).

(**L**) Representative flow cytometric plots of the indicated MCF-10A cells treated with 70 μM H_2O_2 for 72 hr.

(**M**) Percentage of annexin V-positive cells from experiment in (L). Data shown as mean ± SD of three independent experiments. $^{***}p < 0.001$ (Student's t test).

(**N**) Live cell numbers relative to H_2O_2-untreated cells in the indicated MCF-10A cells treated with 100 μM H_2O_2 in the presence or absence of 10 μM BAPTA-AM. Data shown as mean ± SD from the sum of two independent experiments performed in triplicate. $^{***}p < 0.001$ (one-way ANOVA).

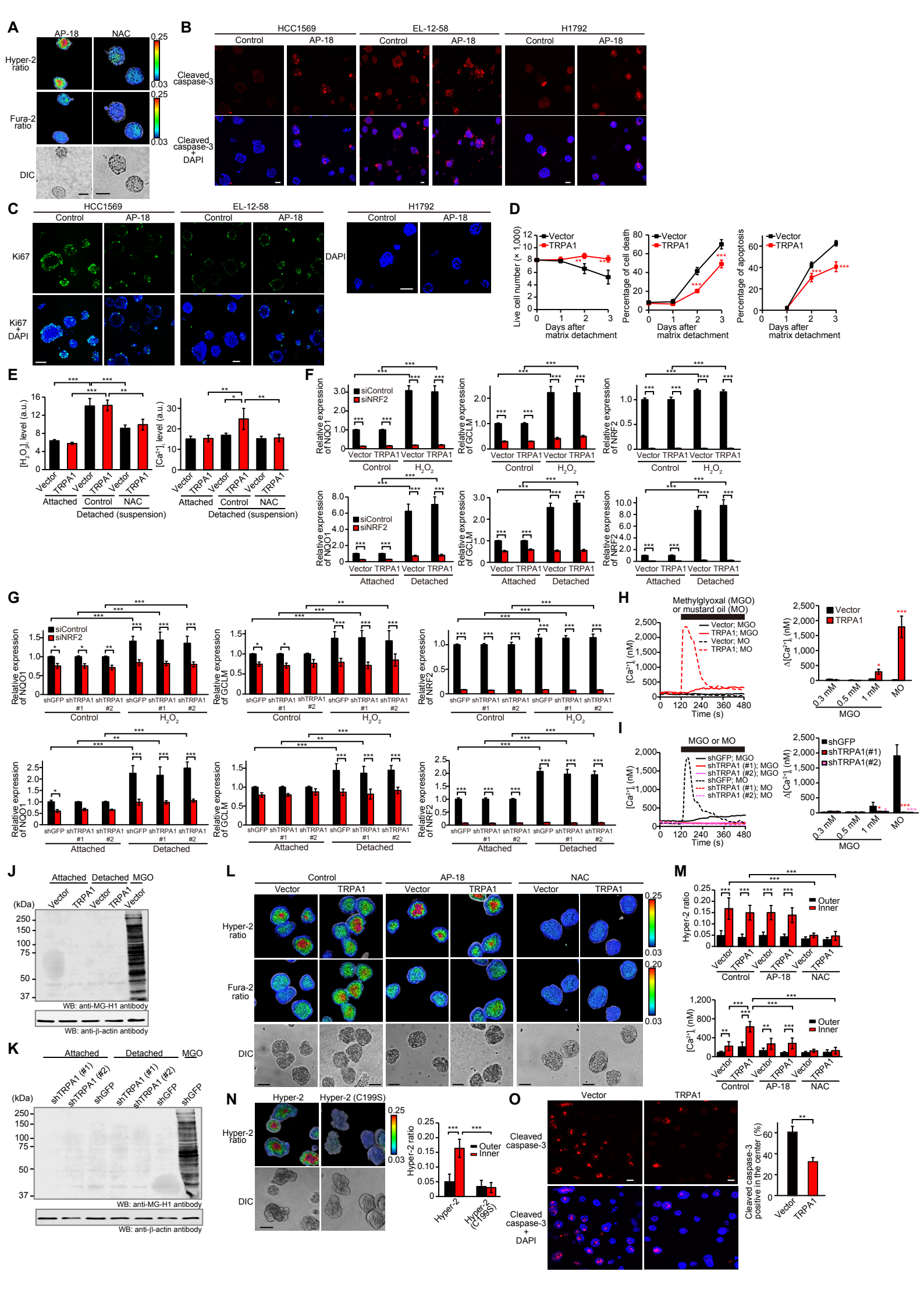
A
AP-18
NAC
Hyper-2 ratio
Fura-2 ratio
DIC
0.25
0.03
B
HCC1569
EL-12-58
H1792
Control
AP-18
Cleaved caspase-3
Cleaved caspase-3 + DAPI
C
Ki67
Ki67 + DAPI
DAPI
D
Vector
TRPA1
Live cell number (× 1,000)
Percentage of cell death
Percentage of apoptosis
Days after matrix detachment
E
[H2O2]i level (a.u.)
[Ca2+]i level (a.u.)
Attached
Control
NAC
Detached (suspension)
F
siControl
siNRF2
Relative expression of NQO1
Relative expression of GCLM
Relative expression of NRF2
Control
H2O2
Attached
Detached
G
shGFP
shTRPA1 #1
shTRPA1 #2
H
Methylglyoxal (MGO) or mustard oil (MO)
Vector; MGO
TRPA1; MGO
Vector; MO
TRPA1; MO
[Ca2+]i (nM)
Time (s)
Δ[Ca2+]i (nM)
0.3 mM
0.5 mM
1 mM
MO
MGO
I
MGO or MO
shGFP; MGO
shTRPA1 (#1); MGO
shTRPA1 (#2); MGO
shGFP; MO
shTRPA1 (#1); MO
shTRPA1 (#2); MO
shGFP
shTRPA1(#1)
shTRPA1(#2)
J
Attached
Detached
MGO
(kDa)
250
150
100
75
50
37
WB: anti-MG-H1 antibody
WB: anti-β-actin antibody
K
shTRPA1 (#1)
shTRPA1 (#2)
shGFP
L
Control
AP-18
NAC
Hyper-2 ratio
Fura-2 ratio
DIC
M
Hyper-2 ratio
Outer
Inner
N
Hyper-2
Hyper-2 (C199S)
O
Cleaved caspase-3
Cleaved caspase-3 + DAPI
Cleaved caspase-3 positive in the center (%)

Figure S3. TRPA1 Induces Ca^{2+} Influx in Response to ROS Produced upon Matrix Dissociation and Suppresses Anoikis, Related to Figure 3.

(**A**) Representative Hyper-2 and fura-2 ratio images of day-5 HCC1569 spheroids transduced with shGFP upon treatment with 10 μM AP-18 or 5 mM NAC (pH was adjusted to 7.4). Scale bar 50 μm.

(**B**) Representative images of day-10 HCC1569, day-10 EL-12-58, or day-6 H1792 spheroids treated with or without 10 μM AP-18. The spheroids were stained with cleaved caspase-3 and DAPI. Scale bar 50 μm.

(**C**) Representative images of day-15 HCC1569, day-15 EL-12-58, or day-10 H1792 spheroids treated with or without 10 μM AP-18. The spheroids were stained with Ki67 and/or DAPI. Scale bar 100 μm.

(**D**) Live cell number, percentage of cell death, and percentage of apoptosis in the indicated MCF-10A cells after matrix detachment for the indicated time. Data shown as mean ± SD of four independent experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to vector (Student's t test).

(**E**) Intracellular H_2O_2 and Ca^{2+} levels as determined by peroxy green-1 and Fluo-4 staining, respectively, in MCF-10A cells with an empty lentiviral vector or TRPA1 24 hr after plating in adherent or non-adherent plates. Cells were treated with or without 1 mM NAC. Data shown as mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA).

(**F and G**) mRNA expression of NQO1, GCLM, or NRF2 in the indicated MCF-10A (F) or HCC1569 cells (G) treated with or without 20 μM H_2O_2 or cultured in adherent (attached) or non-adherent plates (detached) for 24 hr. Data were normalized to siControl cells treated with vehicle control for H_2O_2. Data shown as mean ± SD of four independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ (one-way ANOVA).

(**H and I**) Representative time courses of $[Ca^{2+}]_i$ rises evoked by 1 mM methylglyoxal (MGO) or 30 μM mustard oil (MO), dose-response of $\Delta[Ca^{2+}]_i$ elicited upon treatment with increasing concentrations of MGO, and $\Delta[Ca^{2+}]_i$ evoked by 30 μM MO in the indicated MCF-10A (H) or HCC1569 cells (I). Data shown as mean ± SEM (n = 8–39). $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to vector in (H) or shGFP in (I) (Student's t test).

(**J and K**) Methylglyoxal adducts detected by immunoblotting using anti-MG-H1 antibody in the indicated MCF-10A (J) or HCC1569 cells (K) 24 hr after plating in adherent (attached) or non-adherent plates (detached) or treated with 1 mM MGO for 12 hr. Data from two independent experiments.

(**L**) Representative Hyper-2 and fura-2 ratio images of day-6 MCF-10A acini transduced with vector or TRPA1 and treated with 10 μM AP-18 or 5 mM NAC (pH was adjusted to 7.4). Scale bar 50 μm.

(**M**) Averaged Hyper-2 ratio and $[Ca^{2+}]_i$ in the inner and outer region of the indicated day-6 MCF-10A acini. The acini were treated with or without 10 μM AP-18 or 5 mM NAC (pH was adjusted to 7.4). Data shown as mean ± SD (n = 5–13). $^{**}p < 0.01$ and $^{***}p < 0.001$ (one-way ANOVA).

(**N**) Representative Hyper-2 and Hyper-2 (C199S) ratio images of day-6 MCF-10A acini and averaged ratio in the inner and outer region of the acini. Scale bar 50 μm. Data shown as mean ± SD (n = 7–11). $^{***}p < 0.001$ (one-way ANOVA).

(**O**) Representative images of day-8 TRPA1- or vector-transduced MCF-10A acini stained with cleaved caspase-3 and percentage of acini with cleaved caspase-3-positive staining in the center. Scale bar 50 μm. Data shown as mean ± SEM of five independent experiments. $^{**}p < 0.01$ (Student's t test).

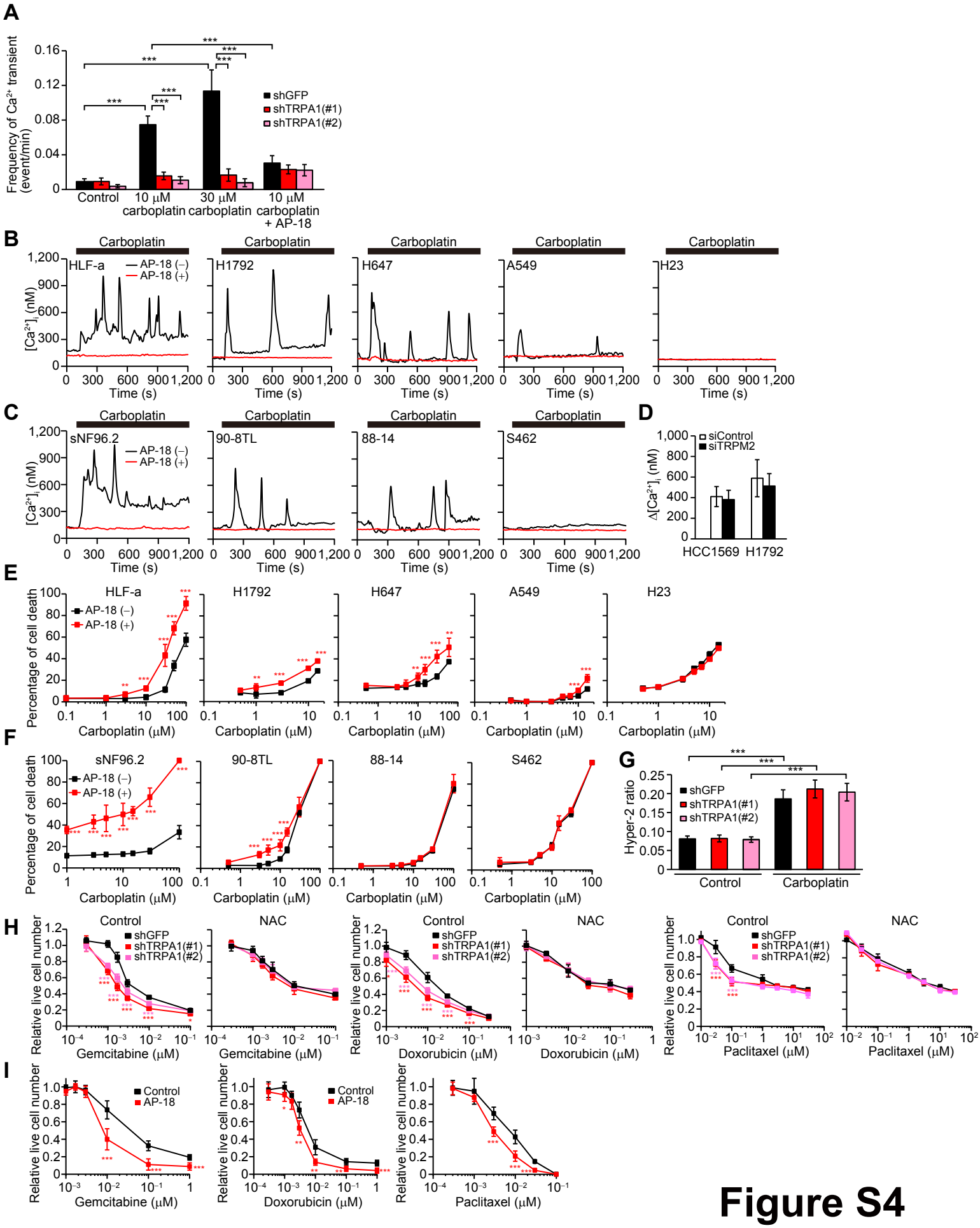
A
Frequency of Ca2+ transient (event/min)
shGFP
shTRPA1(#1)
shTRPA1(#2)
Control
10 μM carboplatin
30 μM carboplatin
10 μM carboplatin + AP-18
B
Carboplatin
HLF-a
H1792
H647
A549
H23
AP-18 (–)
AP-18 (+)
[Ca2+]i (nM)
Time (s)
C
sNF96.2
90-8TL
88-14
S462
D
siControl
siTRPM2
Δ[Ca2+]i (nM)
HCC1569
H1792
E
Percentage of cell death
Carboplatin (μM)
F
G
Hyper-2 ratio
H
Control
NAC
Relative live cell number
Gemcitabine (μM)
Doxorubicin (μM)
Paclitaxel (μM)
I
Control
AP-18

Figure S4

Figure S4. Ca^{2+} Influx through TRPA1 Suppresses Cell Death under Chemotherapy Treatment without Affecting Cellular Redox Status, Related to Figure 4.

(**A**) Frequency of Ca^{2+} transient ($\Delta[Ca^{2+}]_i$ > 200 nM) in HCC1569 cells transduced with shRNA against TRPA1 or GFP and treated with or without carboplatin in the presence or absence of 10 μM AP-18. Data shown as mean ± SEM (n = 34–151). $^{***}p < 0.001$ (one-way ANOVA).

(**B and C**) Representative time courses of $[Ca^{2+}]_i$ changes in the indicated lung cancer (B) and MPNST cells (C) upon treatment with 10 μM carboplatin in the presence or absence of 10 μM AP-18.

(**D**) $\Delta[Ca^{2+}]_i$evoked by 10 μM carboplatin in HCC1569 or H1792 cells transfected with siControl or siTRPM2. Data shown as mean ± SEM (n = 24–31). The effect of siTRPM2 on TRPM2 mRNA expression was shown in Figure S2D.

(**E and F**) Percentage of cell death in the indicated lung cancer (E) and MPNST cells (F) upon treatment with carboplatin for 72 hr in the presence or absence of 10 μM AP-18. Data shown as mean ± SD from the sum of three independent experiments performed in duplicate. $^{**}p < 0.01$ and $^{***}p < 0.001$ compared to AP-18 (–) (Student' s t test).

(**G**) Averaged Hyper-2 ratio in the indicated HCC1569 cells treated with or without 30 μM carboplatin for 48 hr. Data shown as mean ± SEM (n = 106–233). $^{***}p < 0.001$ (one-way ANOVA).

(**H**) Live cell numbers relative to chemotherapy-untreated cells in the indicated HCC1569 cells treated with gemcitabine, doxorubicin, or paclitaxel in the presence or absence of 5 mM NAC (pH was adjusted to 7.4) for 72 hr. Data shown as mean ± SD from the sum of two or three independent experiments performed in duplicate. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to shGFP (Student' s t test).

(**I**) Live cell numbers relative to chemotherapy-untreated cells in H1792 cells treated with gemcitabine, doxorubicin, or paclitaxel in the presence or absence of 10 μM AP-18 for 72 hr. Data shown as mean ± SD from the sum of three independent experiments performed in duplicate. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to vehicle control for AP-18 (Student' s t test).

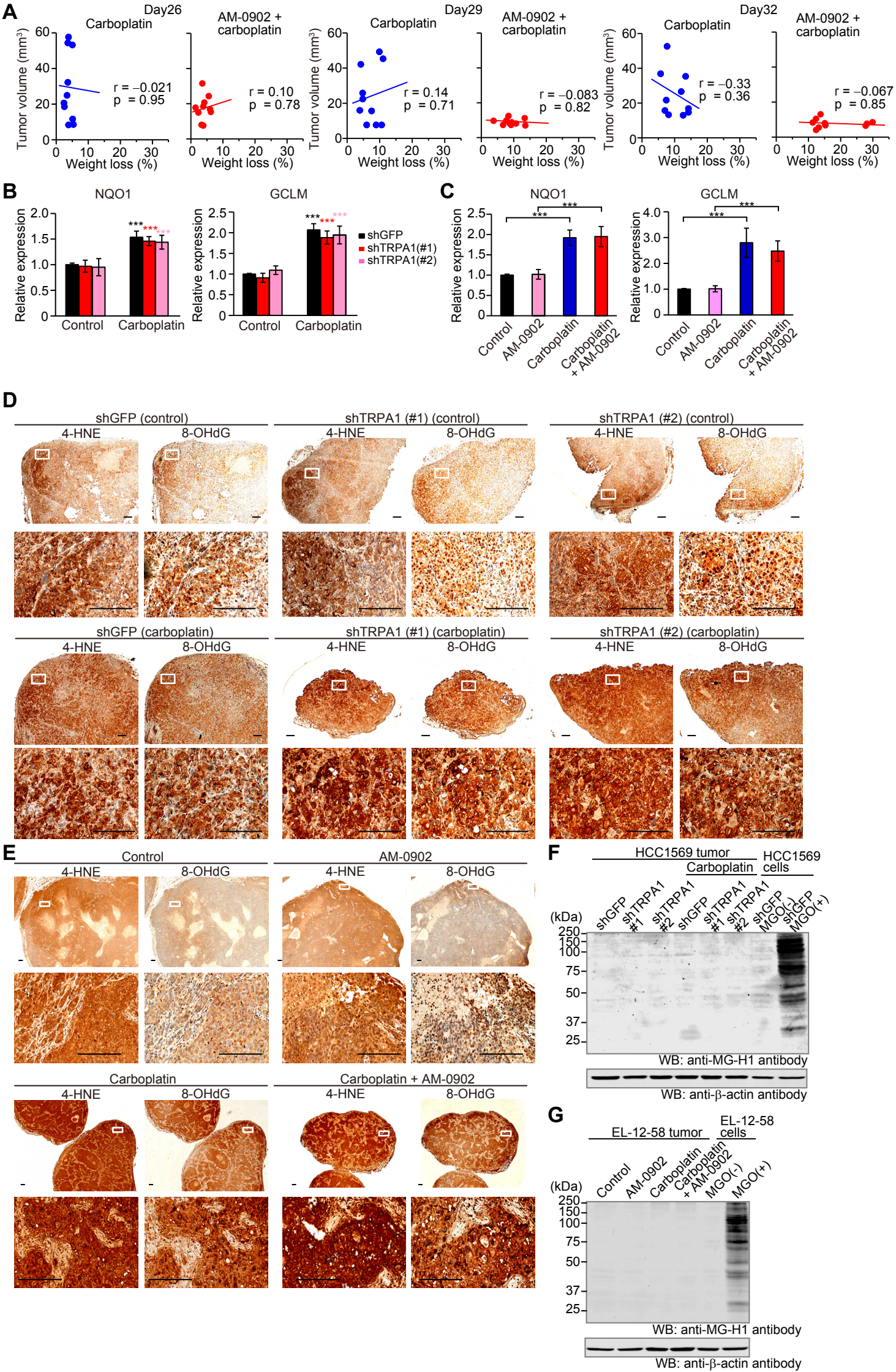
A
Day26
Carboplatin
AM-0902 + carboplatin
Day29
Day32
Tumor volume (mm3)
Weight loss (%)
r = –0.021
p = 0.95
r = 0.10
p = 0.78
r = 0.14
p = 0.71
r = –0.083
p = 0.82
r = –0.33
p = 0.36
r = –0.067
p = 0.85
B
NQO1
GCLM
Relative expression
shGFP
shTRPA1(#1)
shTRPA1(#2)
Control
Carboplatin
C
AM-0902
Carboplatin + AM-0902
D
shGFP (control)
shTRPA1 (#1) (control)
shTRPA1 (#2) (control)
shGFP (carboplatin)
shTRPA1 (#1) (carboplatin)
shTRPA1 (#2) (carboplatin)
4-HNE
8-OHdG
E
Control
AM-0902
Carboplatin
Carboplatin + AM-0902
F
HCC1569 tumor
Carboplatin
HCC1569 cells
(kDa)
shGFP
shTRPA1 #1
shTRPA1 #2
MGO(-)
MGO(+)
250
150
100
75
50
37
25
WB: anti-MG-H1 antibody
WB: anti-β-actin antibody
G
EL-12-58 tumor
EL-12-58 cells
Control
AM-0902
Carboplatin
Carboplatin + AM-0902

Figure S5. HCC1569 and EL-12-58 Tumors Are Exposed to Oxidative Stress but Not Dicarbonyl Stress, Related to Figure 5.
(**A**) Correlation of weight loss (%) with tumor volume (mm^3) in EL-12-58 xenograft tumors treated with carboplatin (n = 10) or AM-0902 + carboplatin (n = 10) at the indicated time points. Averaged weight loss: day 26, 3.94 ± 1.02% for carboplatin and 4.20 ± 1.52% for carboplatin + AM-0902; day 29, 7.01 ± 2.80% for carboplatin and 9.07 ± 2.94% for carboplatin + AM-0902; day 32, 10.3 ± 3.36% for carboplatin and 17.6 ± 7.45% for carboplatin + AM-0902. Data shown as mean ± SD.
(**B**) mRNA expression of NQO1 or GCLM in the indicated HCC1569 tumors. Tumors were resected from mice in Figure 5A at the end point. Data were normalized to vehicle control. Data shown as mean ± SD of four independent experiments. $^{***}p < 0.001$ compared to vehicle control (one-way ANOVA).
(**C**) mRNA expression of NQO1 or GCLM in the indicated EL-12-58 tumors. Tumors were resected from mice in Figure 5D at the end point. Data were normalized to vehicle control. Data shown as mean ± SD of four independent experiments. $^{***}p < 0.001$ (one-way ANOVA).
(**D and E**) Representative images of 4-HNE or 8-OHdG IHC in HCC1569 (D) or EL-12-58 tumor sections (E). Tumors were resected from mice in Figure 5A for HCC1569 tumors or Figure 5D for EL-12-58 tumors at the end point. Enlarged views of the boxed regions shown in lower panel. Scale bar 200 μm.
(**F**) Methylglyoxal adducts detected by immunoblotting using anti-MG-H1 antibody in the indicated HCC1569 tumors or cells. Tumors were resected from mice in Figure 5A at the end point. Cells were treated with or without 1 mM MGO for 12 hr. Data from two independent experiments.
(**G**) Methylglyoxal adducts detected by immunoblotting using anti-MG-H1 antibody in the indicated EL-12-58 tumors or cells. Tumors were resected from mice in Figure 5D at the end point. Cells were treated with or without 1 mM MGO for 12 hr. Data from two independent experiments.

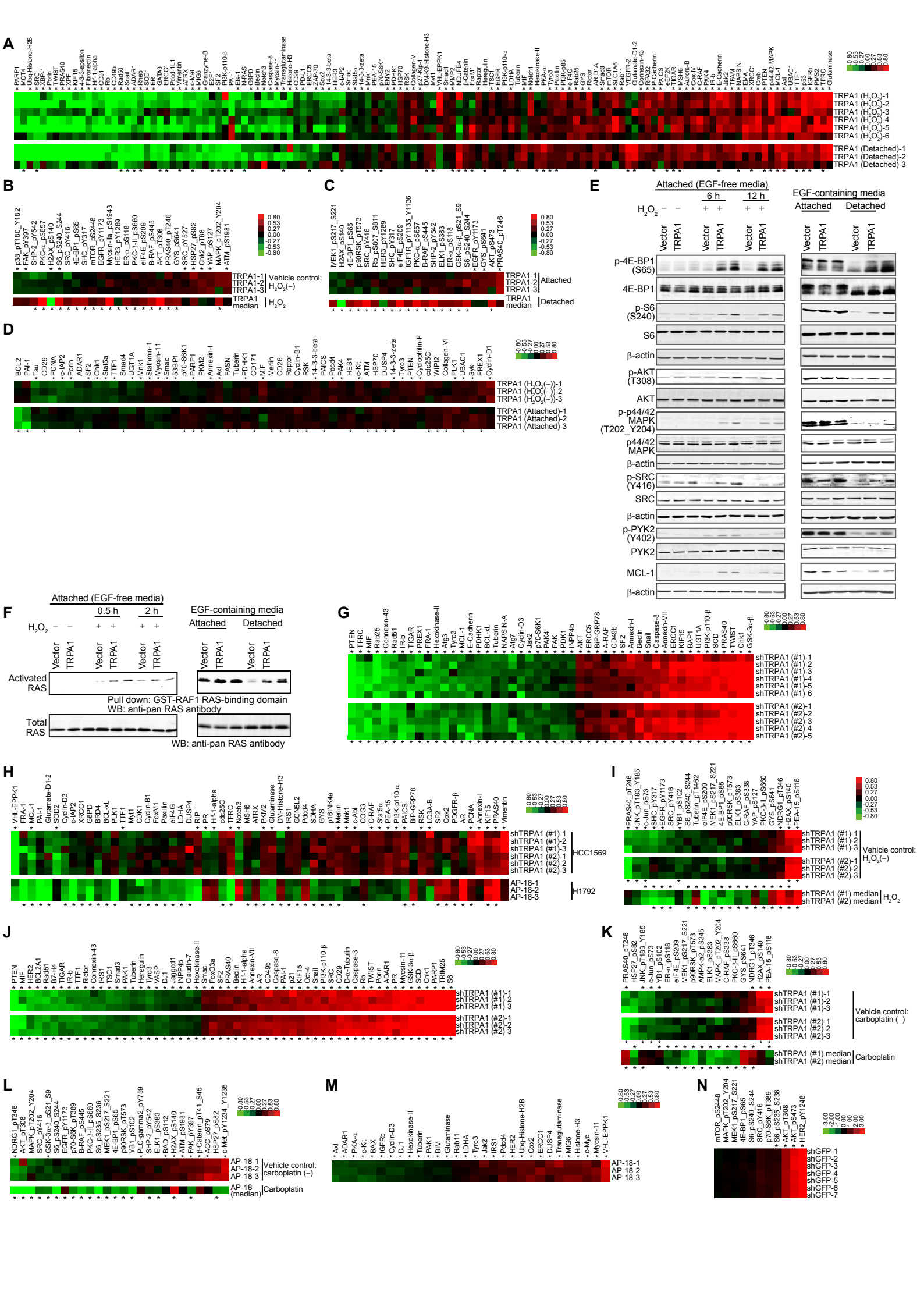
A
TRPA1 (H2O2)-1
TRPA1 (H2O2)-2
TRPA1 (H2O2)-3
TRPA1 (H2O2)-4
TRPA1 (H2O2)-5
TRPA1 (H2O2)-6
TRPA1 (Detached)-1
TRPA1 (Detached)-2
TRPA1 (Detached)-3
B
Vehicle control: H2O2(–)
TRPA1 median
H2O2
C
Attached
Detached
D
TRPA1 (H2O2(–))-1
TRPA1 (H2O2(–))-2
TRPA1 (H2O2(–))-3
TRPA1 (Attached)-1
TRPA1 (Attached)-2
TRPA1 (Attached)-3
E
Attached (EGF-free media)
6 h
12 h
H2O2
EGF-containing media
Attached
Detached
Vector
TRPA1
p-4E-BP1 (S65)
4E-BP1
p-S6 (S240)
S6
β-actin
p-AKT (T308)
AKT
p-p44/42 MAPK (T202_Y204)
p44/42 MAPK
p-SRC (Y416)
SRC
p-PYK2 (Y402)
PYK2
MCL-1
F
Attached (EGF-free media)
0.5 h
2 h
H2O2
EGF-containing media
Attached
Detached
Activated RAS
Pull down: GST-RAF1 RAS-binding domain
WB: anti-pan RAS antibody
Total RAS
WB: anti-pan RAS antibody
G
shTRPA1 (#1)-1
shTRPA1 (#1)-2
shTRPA1 (#1)-3
shTRPA1 (#1)-4
shTRPA1 (#1)-5
shTRPA1 (#1)-6
shTRPA1 (#2)-1
shTRPA1 (#2)-2
shTRPA1 (#2)-3
shTRPA1 (#2)-4
shTRPA1 (#2)-5
H
shTRPA1 (#1)-1
shTRPA1 (#1)-2
shTRPA1 (#1)-3
shTRPA1 (#2)-1
shTRPA1 (#2)-2
shTRPA1 (#2)-3
HCC1569
AP-18-1
AP-18-2
AP-18-3
H1792
I
shTRPA1 (#1)-1
shTRPA1 (#1)-2
shTRPA1 (#1)-3
Vehicle control: H2O2(–)
shTRPA1 (#2)-1
shTRPA1 (#2)-2
shTRPA1 (#2)-3
shTRPA1 (#1) median
shTRPA1 (#2) median
H2O2
J
shTRPA1 (#1)-1
shTRPA1 (#1)-2
shTRPA1 (#1)-3
shTRPA1 (#2)-1
shTRPA1 (#2)-2
shTRPA1 (#2)-3
K
shTRPA1 (#1)-1
shTRPA1 (#1)-2
shTRPA1 (#1)-3
Vehicle control: carboplatin (–)
shTRPA1 (#2)-1
shTRPA1 (#2)-2
shTRPA1 (#2)-3
shTRPA1 (#1) median
shTRPA1 (#2) median
Carboplatin
L
AP-18-1
AP-18-2
AP-18-3
Vehicle control: carboplatin (–)
AP-18 (median)
Carboplatin
M
AP-18-1
AP-18-2
AP-18-3
N
shGFP-1
shGFP-2
shGFP-3
shGFP-4
shGFP-5
shGFP-6
shGFP-7

Figure S6. RPPA Analysis Reveals Signaling Pathway Alterations Induced by TRPA1 under Oxidative Stress or Carboplatin Treatment, Related to Figure 6.

(**A**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$) between TRPA1- and vector-transduced MCF-10A cells, either in H_2O_2 treatment or in suspension (detached). Lysates of cells treated as in Figure 6A were analyzed by RPPA. Samples normalized to vector control.

(**B**) Heatmap of statistically significantly changed phosphorylated proteins ($^{*}p < 0.05$) between TRPA1- and vector-transduced MCF-10A cells, either in H_2O_2 treatment or in its vehicle treatment. Cells were treated with 100 μM H_2O_2 or its vehicle for 24 hr. Samples normalized to vector.

(**C**) Heatmap of statistically significantly changed phosphorylated proteins ($^{*}p < 0.05$) between TRPA1- and vector-transduced MCF-10A cells, either in attachment or in detachment. Cells were cultured in adherent or non-adherent plates for 24 hr. Samples normalized to vector.

(**D**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$) between TRPA1- and vector-transduced MCF-10A cells, either in vehicle control for H_2O_2 treatment or in attachment (control for detachment). Samples normalized to vector.

(**E and F**) The uncropped blots for Figure 6C (E) and 6D (F).

(**G**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$) between shTRPA1 (#1 and #2)- and shGFP-transduced HCC1569 cells treated with 10 μM H_2O_2 for 24 hr. Samples normalized to shGFP.

(**H**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$), either between shTRPA1(#1 and #2)- and shGFP-transduced HCC1569 cells or between AP-18-treated and AP-18-untreated H1792 cells. Cells were treated with 10 μM carboplatin for 24 hr in the presence or absence of 10 μM AP-18. Samples normalized to shGFP for HCC1569 cells and AP-18 (–) for H1792 cells.

(**I**) Heatmap of statistically significantly changed phosphorylated proteins ($^{*}p < 0.05$) between shTRPA1 (#1 and #2)- and shGFP-transduced HCC1569 cells, either in H_2O_2 treatment or in its vehicle treatment. Cells were treated with 10 μM H_2O_2 or its vehicle for 24 hr. Samples normalized to shGFP.

(**J**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$) between shTRPA1 (#1 and #2)- and shGFP-transduced HCC1569 cells treated with vehicle for H_2O_2 treatment. Samples normalized to shGFP.

(**K**) Heatmap of statistically significantly changed phosphorylated proteins ($^{*}p < 0.05$) between shTRPA1(#1 and #2)- and shGFP-transduced HCC1569 cells, either in carboplatin treatment or in its vehicle treatment. Cells were treated with 10 μM carboplatin or its vehicle for 24 hr. Samples normalized to shGFP.

(**L**) Heatmap of statistically significantly changed phosphorylated proteins ($^{*}p < 0.05$) between AP-18-treated and AP-18-untreated H1792 cells, either in carboplatin treatment or in its vehicle treatment. Cells were treated with 10 μM carboplatin or its vehicle for 24 hr in the presence or absence of 10 μM AP-18. Samples normalized to AP-18 (–).

(**M**) Heatmap of proteins whose expression was statistically significantly changed ($^{*}p < 0.05$) between AP-18-treated and AP-18-untreated H1792 cells. Cells were treated with vehicle for carboplatin for 24 hr in the presence or absence of 10 μM AP-18. Samples normalized to AP-18 (–).

(**N**) Heatmap of RAS-ERK, AKT, and mTOR signaling proteins in shGFP-transduced HCC1569 cells treated with 10 μM H_2O_2 for 24 hr. Samples normalized to TRPA1-expressing MCF-10A cells treated with 100 μM H_2O_2 for 24 hr. $^{*}p < 0.05$ compared between HCC1569 and MCF-10A cells (Student' s t test).

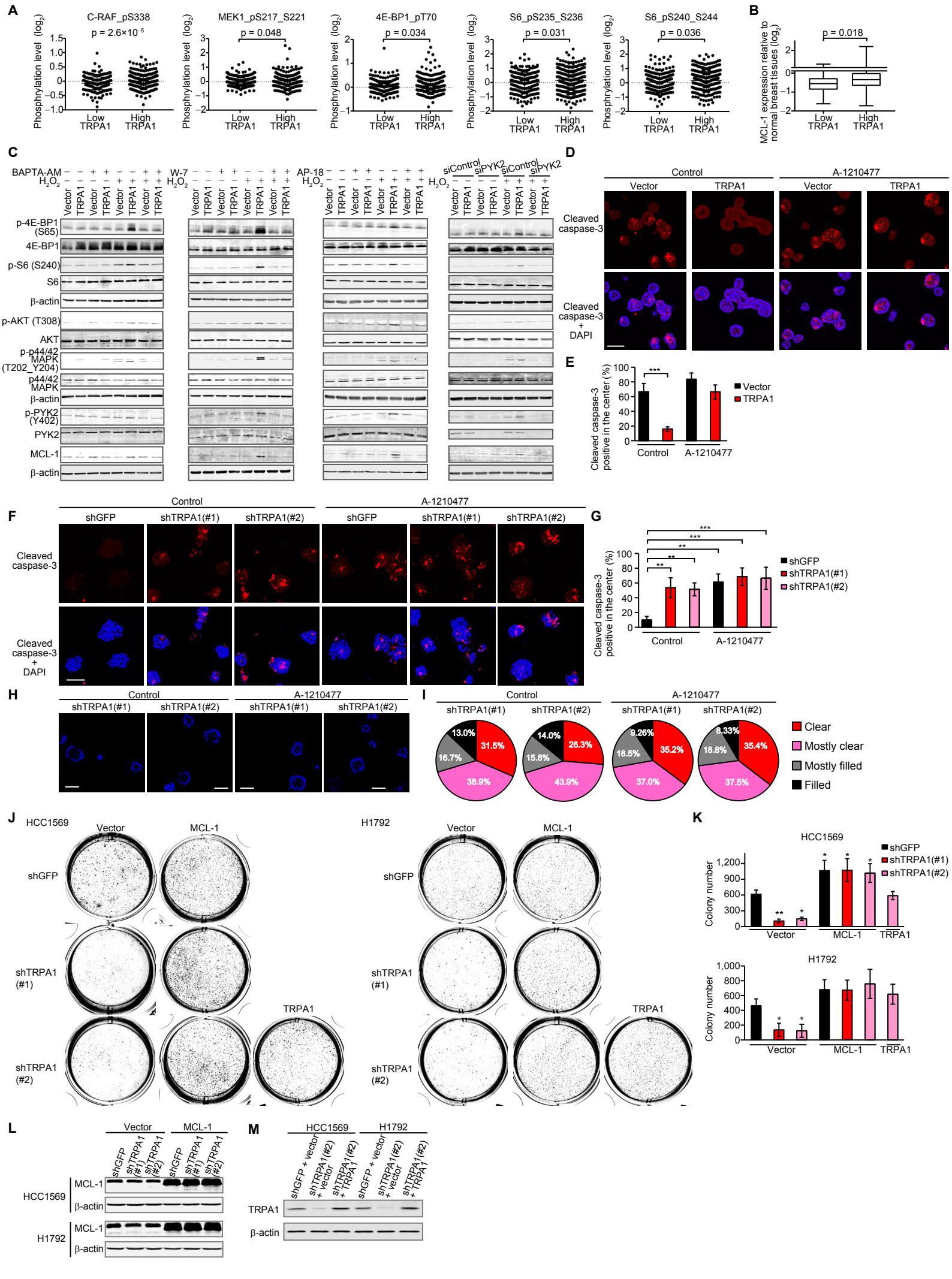
A
C-RAF_pS338
p = 2.6×10⁻⁵
MEK1_pS217_S221
p = 0.048
4E-BP1_pT70
p = 0.034
S6_pS235_S236
p = 0.031
S6_pS240_S244
p = 0.036
Phosphrylation level (log2)
Low TRPA1
High TRPA1
B
p = 0.018
MCL-1 expression relative to normal breast tissues (log2)
C
BAPTA-AM
W-7
AP-18
H2O2
siControl
siPYK2
Vector
TRPA1
p-4E-BP1 (S65)
4E-BP1
p-S6 (S240)
S6
β-actin
p-AKT (T308)
AKT
p-p44/42 MAPK (T202_Y204)
p44/42 MAPK
p-PYK2 (Y402)
PYK2
MCL-1
D
Control
A-1210477
Cleaved caspase-3
Cleaved caspase-3 + DAPI
E
Cleaved caspase-3 positive in the center (%)
F
shGFP
shTRPA1(#1)
shTRPA1(#2)
G
shGFP
shTRPA1(#1)
shTRPA1(#2)
H
I
Clear
Mostly clear
Mostly filled
Filled
13.0%
31.5%
16.7%
38.9%
14.0%
26.3%
15.8%
43.9%
9.26%
35.2%
18.5%
37.0%
8.33%
35.4%
18.8%
37.5%
J
HCC1569
H1792
Vector
MCL-1
TRPA1
shGFP
shTRPA1 (#1)
shTRPA1 (#2)
K
Colony number
L
M
HCC1569
H1792
shGFP + vector
shTRPA1(#2) + vector
shTRPA1(#2) + TRPA1

Figure S7. TRPA1 Mediates Oxidative-Stress Defense through Upregulation of Anti-Apoptotic Programs Involving MCL-1, Related to Figure 6.

(**A**) Phosphorylation levels of the indicated proteins in TCGA BRCA with low (percentile: < 0.4, n = 256) or high TRPA1 mRNA levels (percentile: > 0.4, n = 384). Statistical significance was determined by the Student's t test.

(**B**) Box plots displaying MCL-1 mRNA levels relative to normal breast tissues in TCGA BRCA with low (percentile: < 0.18, n = 104) or high TRPA1 mRNA levels (percentile: > 0.18, n = 474). Statistical significance was determined by the Student's t test.

(**C**) Immunoblot analysis of the effect of 10 μM BAPTA-AM, 1 μM W-7, 10 μM AP-18, and siPYK2 on the indicated proteins in TRPA1- and vector-transduced MCF-10A cells treated with or without 100 μM H_2O_2 for 12 hr in the absence of EGF in monolayer culture.

(**D**) Representative images of day-8 TRPA1- or vector-transduced MCF-10A acini treated with or without 3 μM A-1210477. The spheroids were stained with cleaved caspase-3 and DAPI. Scale bar 100 μm.

(**E**) Percentage of day-8 TRPA1- or vector-transduced MCF-10A acini with cleaved caspase-3 staining in the center. The spheroids were treated with or without 3 μM A-1210477. Data shown as mean ± SD from three independent experiments. $^{***}p < 0.001$ (one-way ANOVA).

(**F**) Representative images of day-10 HCC1569 spheroids treated with or without 3 μM A-1210477. The spheroids were stained with cleaved caspase-3 and DAPI. Scale bar 100 μm.

(**G**) Percentage of day-10 HCC1569 spheroids with cleaved caspase-3 staining in the center. The spheroids were treated with or without 3 μM A-1210477. Data shown as mean ± SD from three independent experiments. $^{**}p < 0.01$ and $^{***}p < 0.001$ (one-way ANOVA).

(**H**) Representative images of day-13 HCC1569 transduced with shTRPA1 (#1) or shTRPA1 (#2). The spheroids were treated with or without 3 μM A-1210477 and stained with DAPI. Scale bar 100 μm.

(**I**) Pie chart of clearance levels of Day-13 HCC1569 transduced with shTRPA1 (#1) (n = 54 for control and n = 54 for A-1210477: the sum of two independent experiments) or shTRPA1 (#2) (n = 57 for control and n = 48 for A-1210477: the sum of two independent experiments). The spheroids treated with or without 3 μM A-1210477.

(**J and K**) The indicated HCC1569 or H1792 cells were grown in soft agar, and after 18 days, images were taken after iodonitrotetrazolium chloride staining (J). Colony numbers are also shown (K). Data represent mean ± SD of three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to cells transduced with both shGFP and an empty lentiviral vector (one-way ANOVA).

(**L and M**) Immunoblot of lysates from the indicated HCC1569 or H1792 cells transduced with an empty lentiviral vector, TRPA1, or MCL-1.

In (A) and (B), box and whiskers graphs indicate the median and the 25th and 75th percentiles, with minimum and maximum values at the extremes of the whiskers. In (C), (L), and (M), blots are representative of at least two independent experiments.

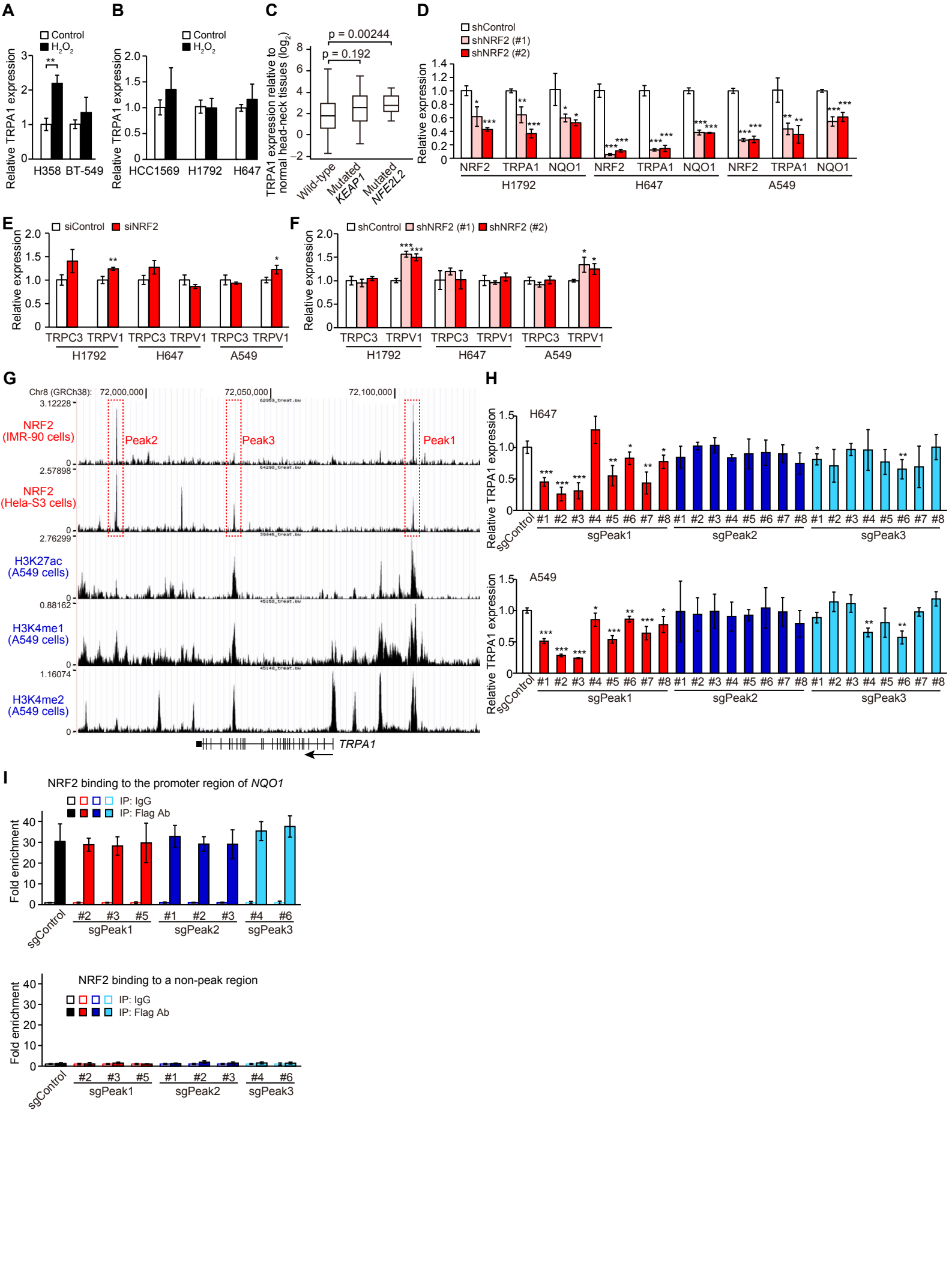
A
Control
H2O2
Relative TRPA1 expression
H358
BT-549
B
Control
H2O2
Relative TRPA1 expression
HCC1569
H1792
H647
C
p = 0.00244
p = 0.192
TRPA1 expression relative to normal head-neck tissues (log2)
Wild-type
Mutated KEAP1
Mutated NFE2L2
D
shControl
shNRF2 (#1)
shNRF2 (#2)
Relative expression
NRF2
TRPA1
NQO1
H1792
H647
A549
E
siControl
siNRF2
Relative expression
TRPC3
TRPV1
H1792
H647
A549
F
shControl
shNRF2 (#1)
shNRF2 (#2)
Relative expression
TRPC3
TRPV1
H1792
H647
A549
G
Chr8 (GRCh38):
72,000,000
72,050,000
72,100,000
NRF2 (IMR-90 cells)
Peak2
Peak3
Peak1
NRF2 (Hela-S3 cells)
H3K27ac (A549 cells)
H3K4me1 (A549 cells)
H3K4me2 (A549 cells)
TRPA1
H
H647
A549
Relative TRPA1 expression
sgControl
sgPeak1
sgPeak2
sgPeak3
I
NRF2 binding to the promoter region of NQO1
IP: IgG
IP: Flag Ab
Fold enrichment
sgControl
sgPeak1
sgPeak2
sgPeak3
NRF2 binding to a non-peak region
IP: IgG
IP: Flag Ab
Fold enrichment
sgControl
sgPeak1
sgPeak2
sgPeak3

Figure S8. NRF2 Directly Induces TRPA1 Expression in Cancer Cells, Related to Figure 7.

(**A**) mRNA expression of TRPA1 in H358 or BT-549 cells treated with or without 10 μM H_2O_2 for 48 hr in serum-containing complete media (i.e. 10% FBS-containing media). Data were normalized to vehicle control. Data shown as mean ± SD of three independent experiments. $^{**}p < 0.01$ (Student's t test).

(**B**) mRNA expression of TRPA1 in HCC1569, H1792, or H647 cells treated with or without 10 μM H_2O_2 for 48 hr in serum-starved conditions (0.1% FBS). Data were normalized to vehicle control. Data shown as mean ± SD of three independent experiments.

(**C**) Box plots displaying TRPA1 mRNA levels relative to normal head-neck tissues in wild-type (n = 249) and *KEAP1*- (n = 13) or *NFE2L2*-mutated HNSC (n = 17) based on TCGA dataset. Box and whiskers graphs indicate the median and the 25th and 75th percentiles, with minimum and maximum values at the extremes of the whiskers. Statistical significance was determined by the Student's t test.

(**D**) mRNA expression of NRF2, TRPA1, and NQO1 in the indicated cancer cells transduced with doxycycline-inducible shControl or shNRF2. Cells were treated with 1 μg/ml doxycycline for 48 hr. Data were normalized to shControl. Data shown as mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to shControl (Student's t test).

(**E**) mRNA expression of TRPC3 and TRPV1 in the indicated cancer cells transfected with siControl or siNRF2. Data were normalized to siControl. Data shown as mean ± SD of three independent experiments. $^{*}p < 0.05$ and $^{**}p < 0.01$ compared to siControl (Student's t test).

(**F**) mRNA expression of TRPC3 and TRPV1 in the indicated cancer cells transduced with doxycycline-inducible shControl or shNRF2. Cells were treated with 1 μg/ml doxycycline for 48 hr. Data were normalized to shControl. Data shown as mean ± SD of three independent experiments. $^{*}p < 0.05$ and $^{***}p < 0.001$ compared to shControl (Student's t test).

(**G**) Cistrome ChIP-seq data (http://cistrome.org) for the distribution of NRF2, histone H3K27ac, H3K4me1, and H3K36me2 are plotted around the *TRPA1* locus. The Peak1 [Chr8: 72107522–72107682 (GRCh38)], Peak2 [Chr8: 71987814–71987948 (GRCh38)], Peak3 [Chr8: 72035191–72035330 (GRCh38)].

(**H**) mRNA expression of TRPA1 in H647 or A549 cells transduced with Cas9 plus sgControl, sgPeak1, sgPeak2, or sgPeak3. Data were normalized to sgControl. Data shown as mean ± SD of three independent experiments. $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$ compared to sgControl (Student's t test).

(**I**) Chromatin immunoprecipitation with Flag antibody of the fragmented chromatin containing the promoter of *NQO1* [Chr16: 69727000–69727111 (GRCh38)] or non-Peak region [Chr8: 72086158–72086422 (GRCh38)] in Flag-NRF2-overexpressing H1792 cells transduced with Cas9 plus sgControl, sgPeak1, sgPeak2, or sgPeak3. Binding was normalized to that in immunoprecipitation with IgG control. Data shown as mean ± SD of three independent experiments.

Table S3. Expression levels of classical NRF2-induced genes in tumors with low (below median) or high TRPA1 mRNA (above median) from TCGA BRCA, LUSC, or LUAD, related to Figure 7.

	BRCA (n = 578)			LUSC (n = 501)			LUAD (n = 517)		
	mRNA level relative to normal breast tissues (log_2)		p value (low vs high TRPA1)	mRNA level relative to normal lung tissues (log_2)		p value (low vs high TRPA1)	mRNA level relative to normal lung tissues (log_2)		p value (low vs high TRPA1)
	In tumors with low TRPA1	In tumors with highTRPA1		In tumors with low TRPA1	In tumors with highTRPA1		In tumors with low TRPA1	In tumors with highTRPA1	
ABCC3	−0.715	−0.448	0.0347	−0.706	−0.374	0.0371	2.47	1.90	1.08×10^{-5}
GCLC	−0.323	−0.352	0.613	2.69	2.77	0.553	0.884	1.62	1.21×10^{-5}
GCLM	−0.406	−0.256	0.0122	1.43	2.02	3.55×10^{-5}	0.620	0.976	0.000292
GLS	−0.469	−0.295	0.0164	−0.997	−0.790	0.00204	−0.398	−0.155	0.0243
GSR	0.541	0.599	0.511	0.755	1.05	0.00660	0.652	0.676	0.801
HMOX1	0.568	0.794	0.00544	−1.08	−0.95	0.259	−0.814	−0.627	0.0286
KEAP1	0.325	0.235	0.0520	0.690	0.864	0.00624	0.114	−0.00171	0.0308
MAFG	−0.502	−0.378	0.0263	0.181	0.431	0.00105	−0.150	−0.0201	0.0257
NRF2	−0.586	−0.448	0.000414	0.685	0.511	0.0438	−0.473	−0.537	0.126
NQO1	0.422	0.461	0.738	1.41	1.89	0.0132	2.48	2.34	0.347
SLC7A11	1.239	1.69	0.000191	2.93	3.45	0.0100	2.11	2.65	0.00296
TXN	0.794	0.965	0.00270	1.26	1.52	0.0105	0.875	0.912	0.631
TXNRD1	0.130	0.290	0.0113	0.649	1.12	0.000718	0.575	1.18	3.91×10^{-5}

Table S4. Primer sequences used for the construction of lentiCRISPR plasmids, related to STAR Methods.

	Mutation primer sequence (5' → 3')	
Plasmid ID	Forward primer	Reverse primer
sgPeak1 #1	caccgTGAGTCATCATTGCGGAAGT	aaacACTTCCGCAATGATGACTCAc
sgPeak1 #2	caccgACTTCCGCAATGATGACTCA	aaacTGAGTCATCATTGCGGAAGTc
sgPeak1 #3	caccgAACTTCCGCAATGATGACTC	aaacGAGTCATCATTGCGGAAGTTc
sgPeak1 #4	caccgGAAGCCCTGAGTCATCATTG	aaacCAATGATGACTCAGGGCTTCc
sgPeak1 #5	caccgAGGACCTCTATTTATTCAGC	aaacGCTGAATAAATAGAGGTCCTc
sgPeak1 #6	caccgATGGTGGAATTTGTGTTACT	aaacAGTAACACAAATTCCACCATc
sgPeak1 #7	caccgTACCATAAAGGTTTACTAAT	aaacATTAGTAAACCTTTATGGTAc
sgPeak1 #8	caccgCCACTGTATTTCAAAAGCTT	aaacAAGCTTTTGAAATACAGTGGc
sgPeak2 #1	caccgTAAGAGCTGAATGGTAGCAC	aaacGTGCTACCATTCAGCTCTTAc
sgPeak2 #2	caccgTTGAACTCAGGCATAGTGCT	aaacAGCACTATGCCTGAGTTCAAc
sgPeak2 #3	caccgAAGAGCTGAATGGTAGCACA	aaacTGTGCTACCATTCAGCTCTTc
sgPeak2 #4	caccgAGTGGGGCAGAAGTTTGGAC	aaacGTCCAAACTTCTGCCCCACTc
sgPeak2 #5	caccgGAGAGAATGACTCAGCAGAT	aaacATCTGCTGAGTCATTCTCTCc
sgPeak2 #6	caccgTTGGACTGGTAAGAGCTGAA	aaacTTCAGCTCTTACCAGTCCAAc
sgPeak2 #7	caccgGAATGGTAGCACAGGGCAGC	aaacGCTGCCCTGTGCTACCATTCc
sgPeak2 #8	caccgAGAGAGAATGACTCAGCAGA	aaacTCTGCTGAGTCATTCTCTCTc
sgPeak3 #1	caccgTTCAGGCACTGTCACACCCT	aaacAGGGTGTGACAGTGCCTGAAc
sgPeak3 #2	caccgATAGGATTTAAGACAGGTTC	aaacGAACCTGTCTTAAATCCTATc
sgPeak3 #3	caccgCTTGGCTCTGTCATTATTAG	aaacCTAATAATGACAGAGCCAAGc
sgPeak3 #4	caccgACATATATAGTGACTCAGCA	aaacTGCTGAGTCACTATATATGTc
sgPeak3 #5	caccgCACTAATAATGACAGAGCCA	aaacTGGCTCTGTCATTATTAGTGc
sgPeak3 #6	caccgACTAATAATGACAGAGCCAA	aaacTTGGCTCTGTCATTATTAGTc
sgPeak3 #7	caccgTACAAGATTCTAAAGCTGAA	aaacTTCAGCTTTAGAATCTTGTAc
sgPeak3 #8	caccgTAATAAGTCATCTTTGTCAA	aaacTTGACAAAGATGACTTATTAc

Table S5. Primer sequences used for quantitative PCR, related to STAR Methods.

Quantitative RT-PCR primers

	Primer sequence (5' → 3')	
	Forward primer	Reverse primer
TRPA1	ATCAGAAATCCACCATCGTG	TTGACTGCTCTCAACACAGTATTC
TRPC3	TTCCTGGCCATTGGCTACT	AAAAGGGCTTCGCAGAATTT
TRPV1	AGAGTCACGCTGGCAACC	CGGCAGAGACTCTCCATCAC
NRF2	GAGAGCCCAGTCTTCATTGC	TTGGCTTCTGGACTTGGAAC
NQO1	TGAAGAAGAAAGGATGGGAGGT	GGCCTTCTTTATAAGCCAGAACA
GCLM	CTCCTGCTGTGTGATGCCA	CTCGTGCGCTTGAATGTCAG
β-Actin	GACAGGATGCAGAAGGAGATC	TGCTGATCCACATCTGCTG

ChIP primers

	Primer sequence (5' → 3')	
	Forward primer	Reverse primer
Peak1 region	ATGTTTATTGCTGTGGTTTGAG	CTTCCGCAATGATGACTCAG
Peak2 region	AAGTGAGAGCCAGACAGTCC	GGAATTGAGAAGAGAAGACAGATTG
Peak3 region	CCCAGAAATAGGATTTAAGACAGG	AGGAACTTACAAGATTCTAAAGCTG
non-Peak region	GTGTACAGAATGCGGAAAAC	CCCAAAGTGCTAGGATTACAG
NQO1 promoter	AAGTGCAGAATCTGAATCTTG	AGATTCTGCTGAGTCACTGTG