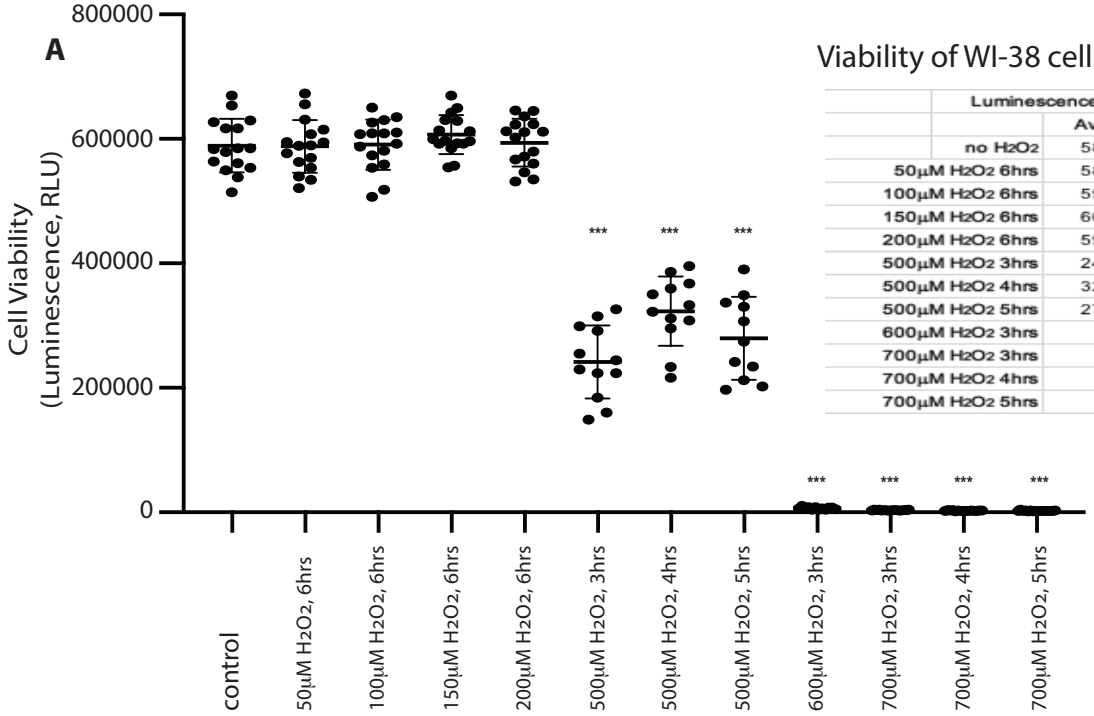
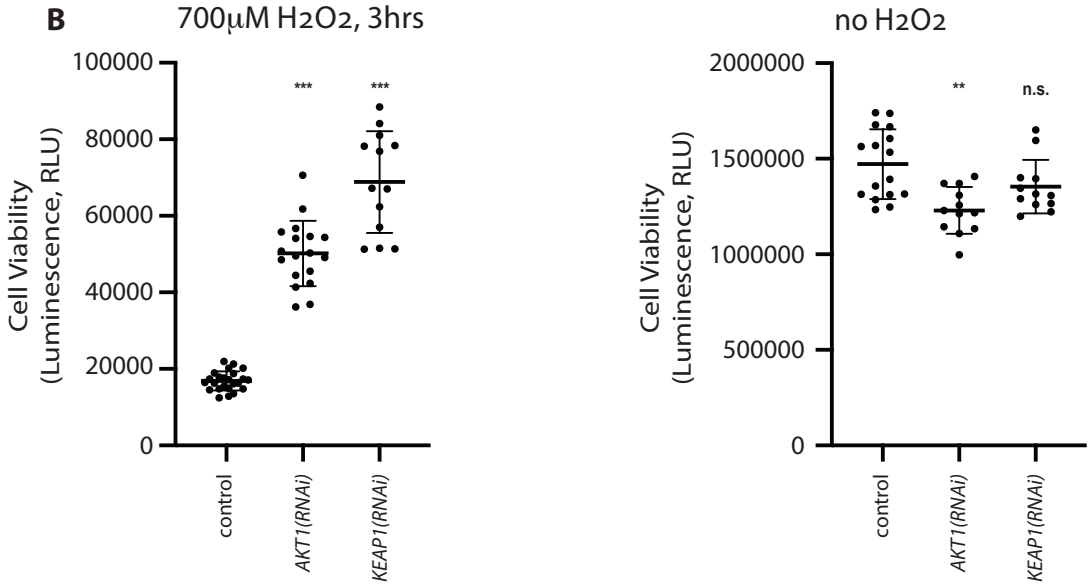
**Figure S1. Increased oxidative-stress resistance upon *AKT1* or *KEAP1* knockdown (related to Figure 1)**




Viability of WI-38 cells in response to $H_2O_2$

| | Luminescence, RLU | | |
|---|---|---|---|
| | Average | SD | norm. % |
| no $H_2O_2$ | 589277 | 43067 | 100.0% |
| 50μM $H_2O_2$ 6hrs | 588036 | 42302 | 99.8% |
| 100μM $H_2O_2$ 6hrs | 590679 | 40578 | 100.2% |
| 150μM $H_2O_2$ 6hrs | 607253 | 31326 | 103.1% |
| 200μM $H_2O_2$ 6hrs | 593893 | 38213 | 100.8% |
| 500μM $H_2O_2$ 3hrs | 241821 | 58606 | 41.0% |
| 500μM $H_2O_2$ 4hrs | 323332 | 55601 | 54.9% |
| 500μM $H_2O_2$ 5hrs | 279486 | 66610 | 47.4% |
| 600μM $H_2O_2$ 3hrs | 6708 | 1964 | 1.1% |
| 700μM $H_2O_2$ 3hrs | 3526 | 508 | 0.6% |
| 700μM $H_2O_2$ 4hrs | 2760 | 548 | 0.5% |
| 700μM $H_2O_2$ 5hrs | 2481 | 550 | 0.4% |



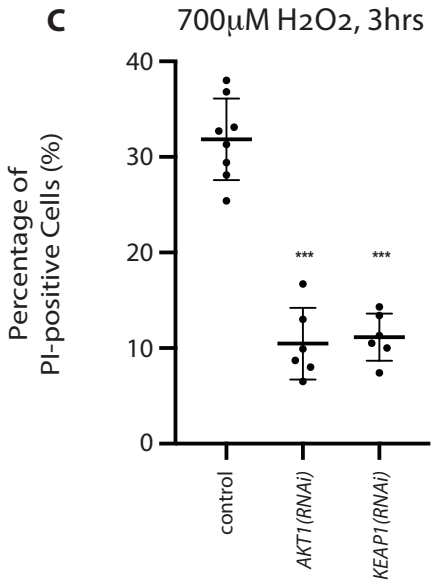



**Figure S2. Z-prime scores across the screen (related to Figure 1)**

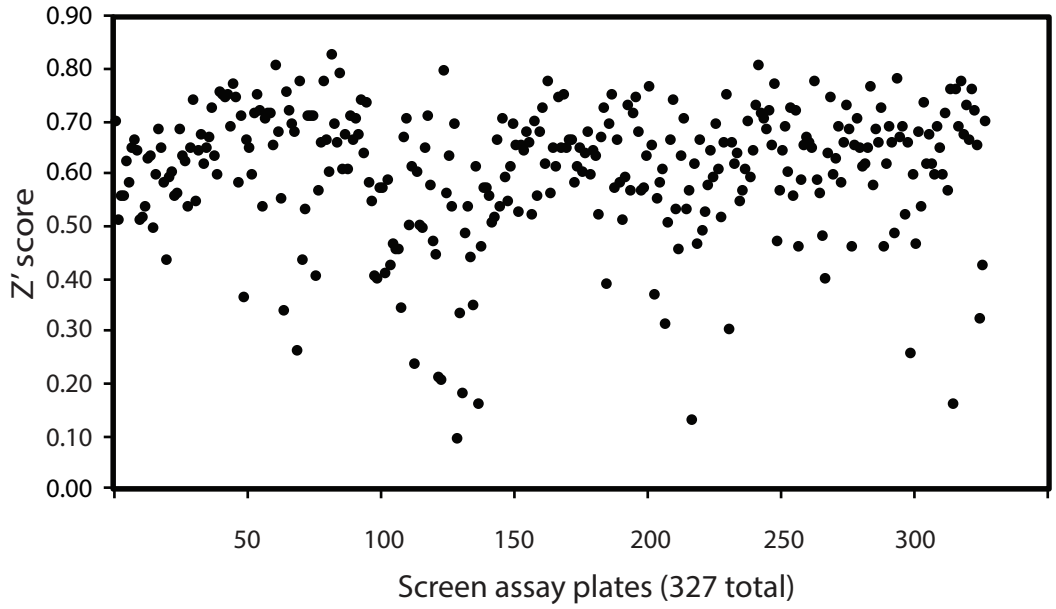



**Figure S3. ROS-scavenging capacity of certain small molecules in the absence of cells (related to Figure 1)**

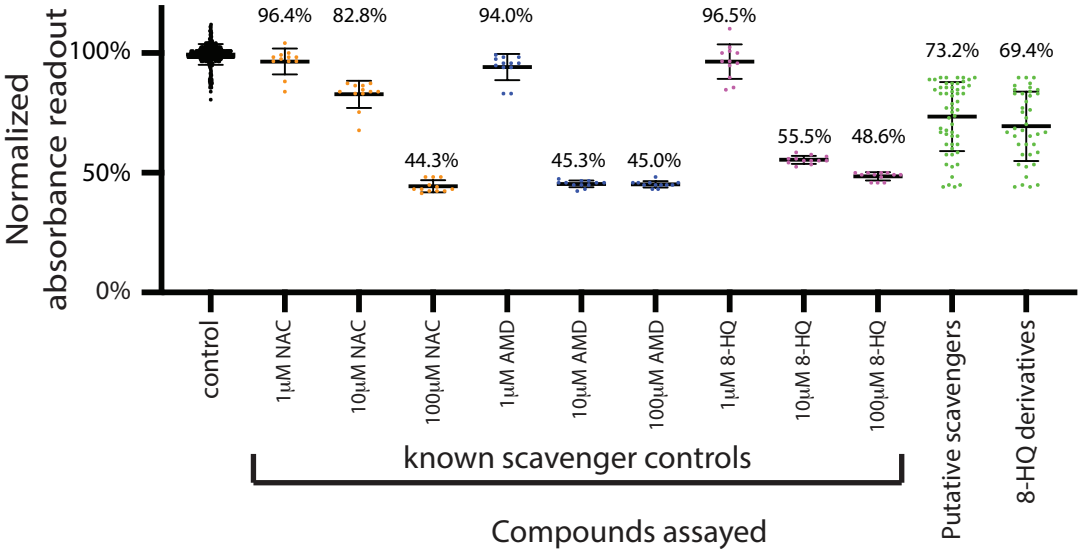



**Figure S4. No $H_2O_2$-quenching effects by small molecules in the absence of cells (related to Figure 2)**

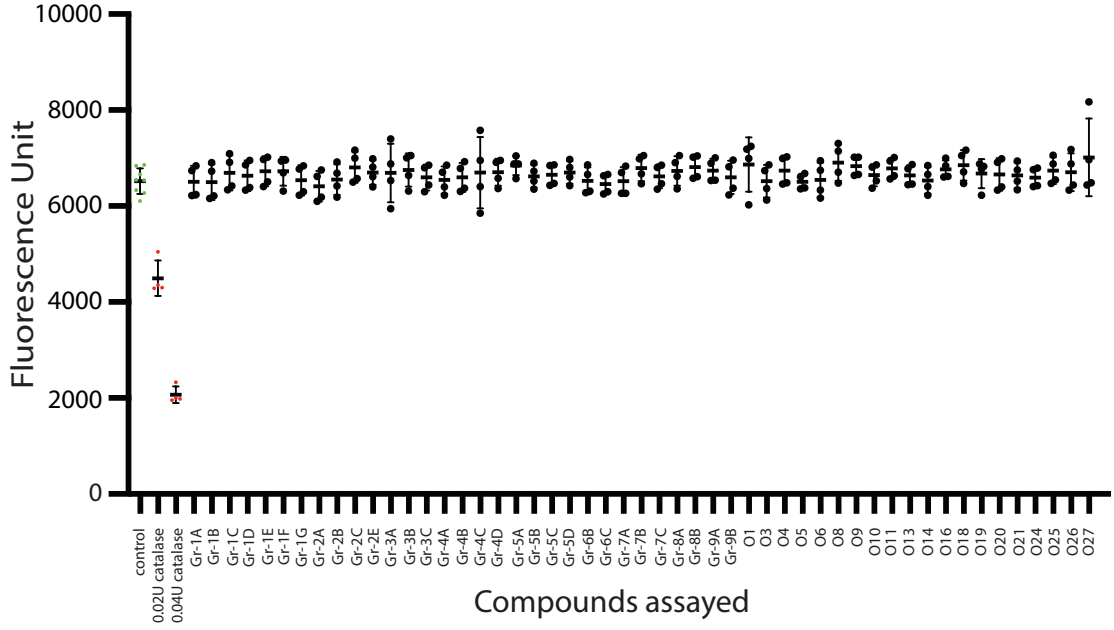
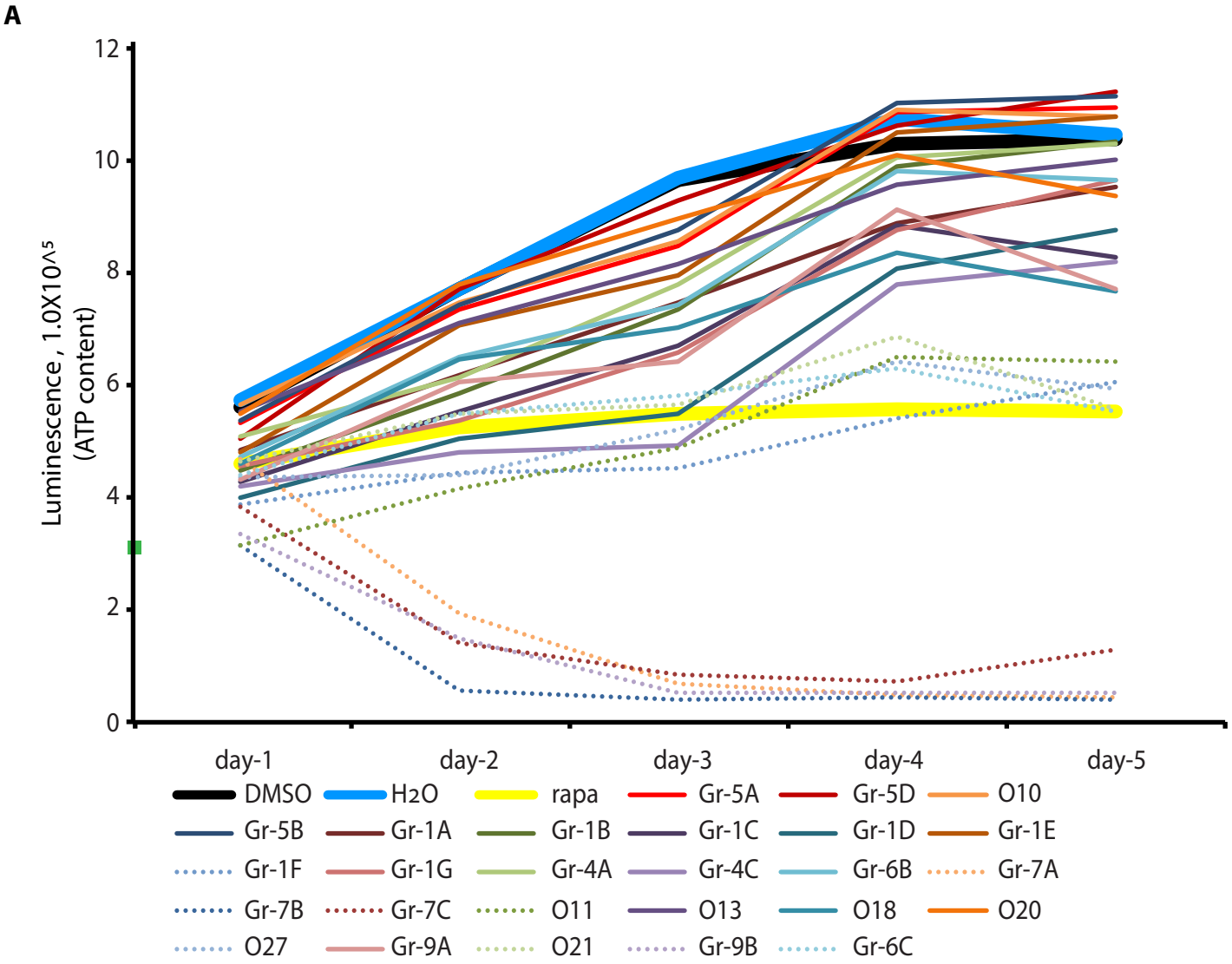


**Figure S5-1. Long-term effects of small molecules on ATP levels (related to Figure 3)**



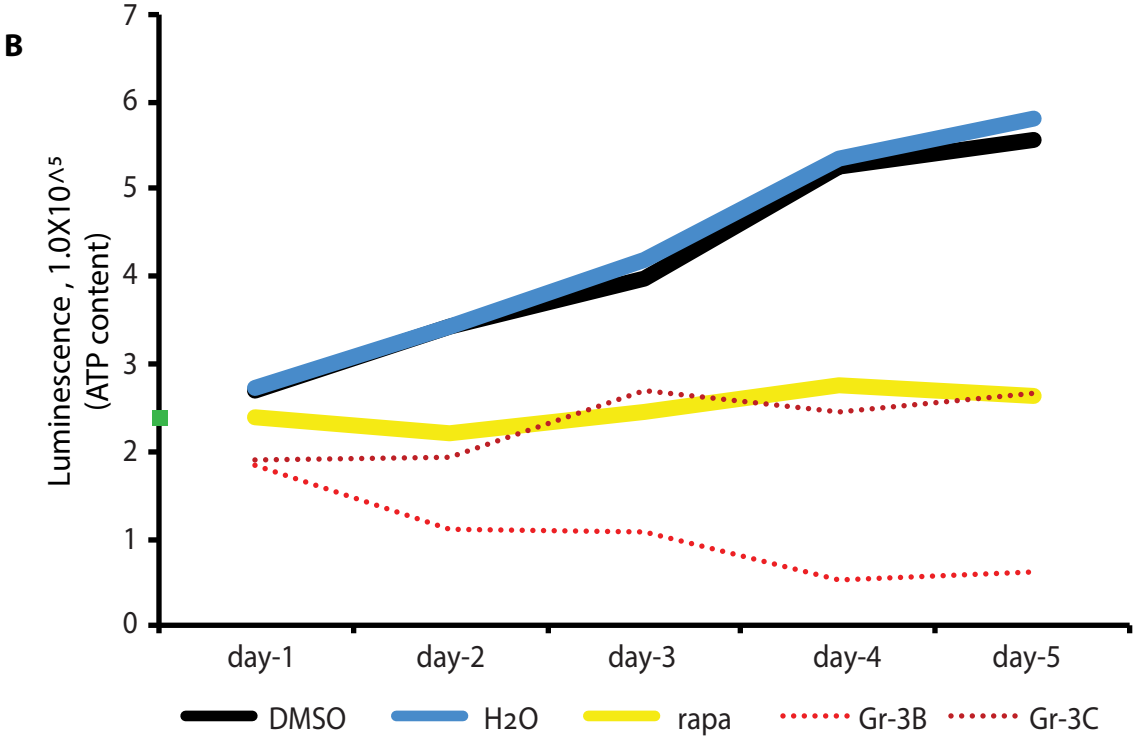
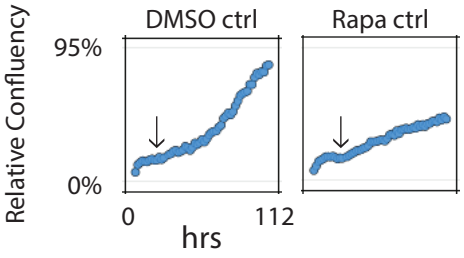
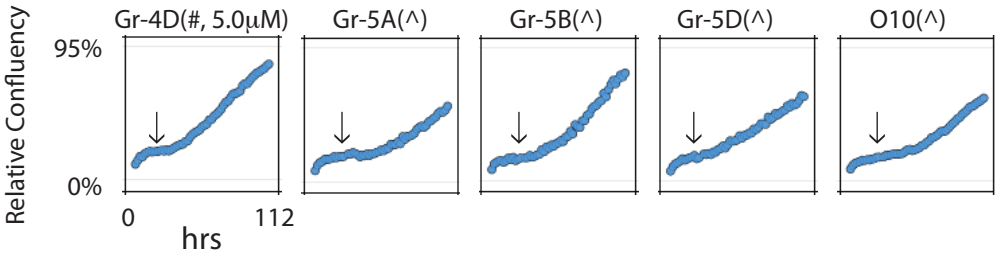



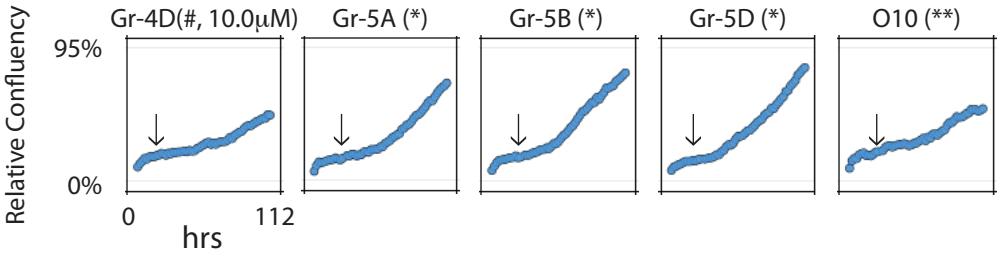
**Figure S5-2. Effects of prolonged small-molecule incubation on WI-38 cell confluency (related to Figure 3)**

**A** **Controls:**

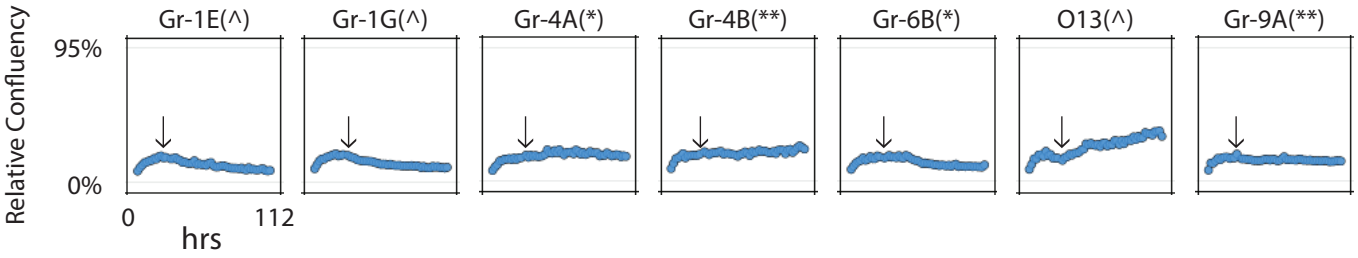


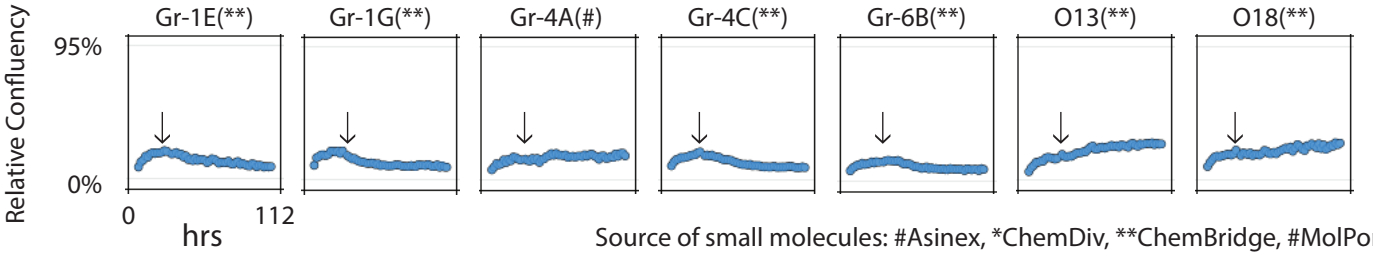

**B** **Small molecules that do NOT show obvious inhibitory effects on cell confluency:**




**C** **Small molecules that show obvious inhibitory effects on cell confluency:**




Source of small molecules: #Asinex, *ChemDiv, **ChemBridge, #MolPort, ^Vitascreen

**Figure S6. Increased cells viability upon $H_2O_2$ by small molecules (related to Figure 3)**

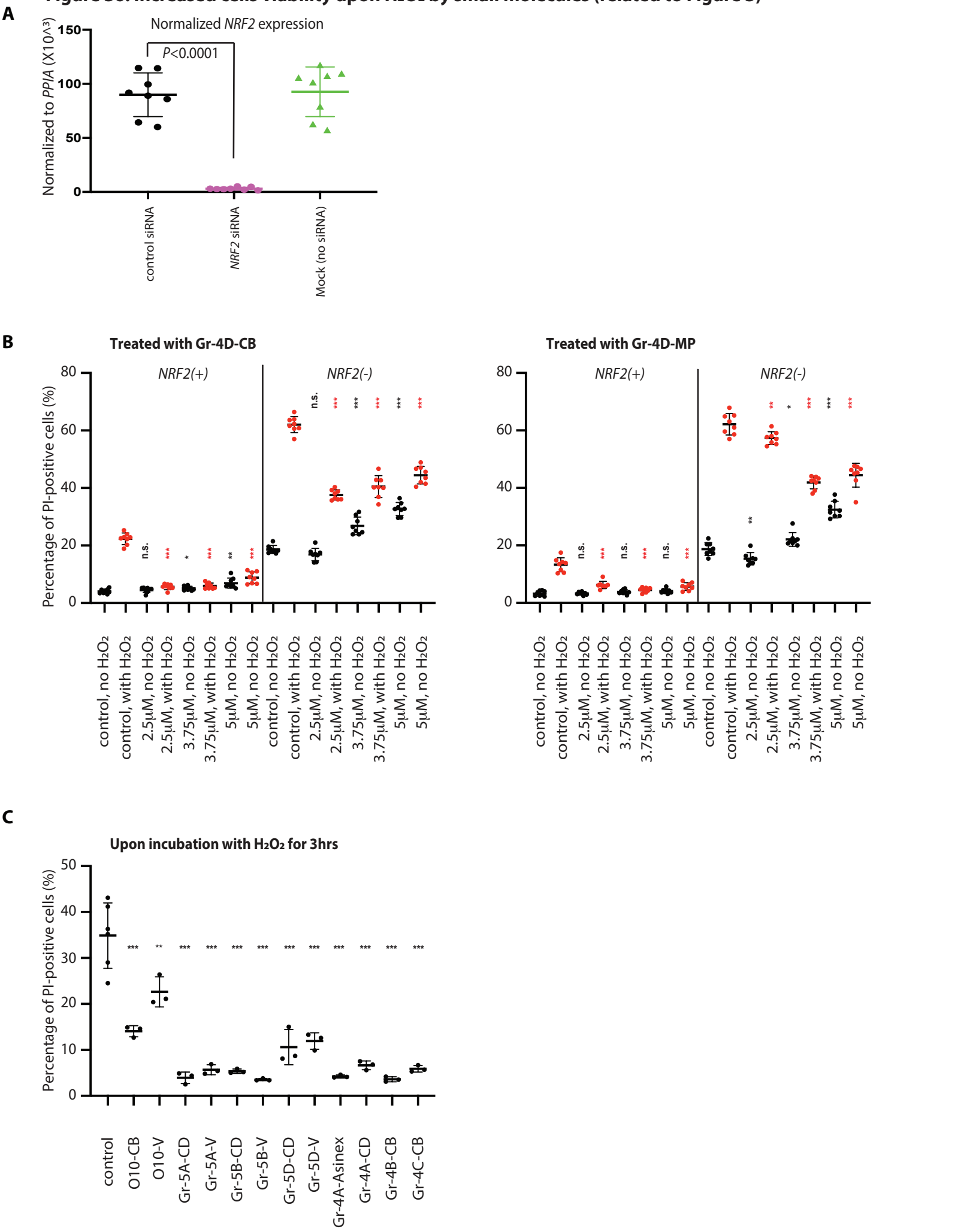


**Figure S7. Effects of small molecules on gene expression of WI-38 cells (related to Figure 3)**

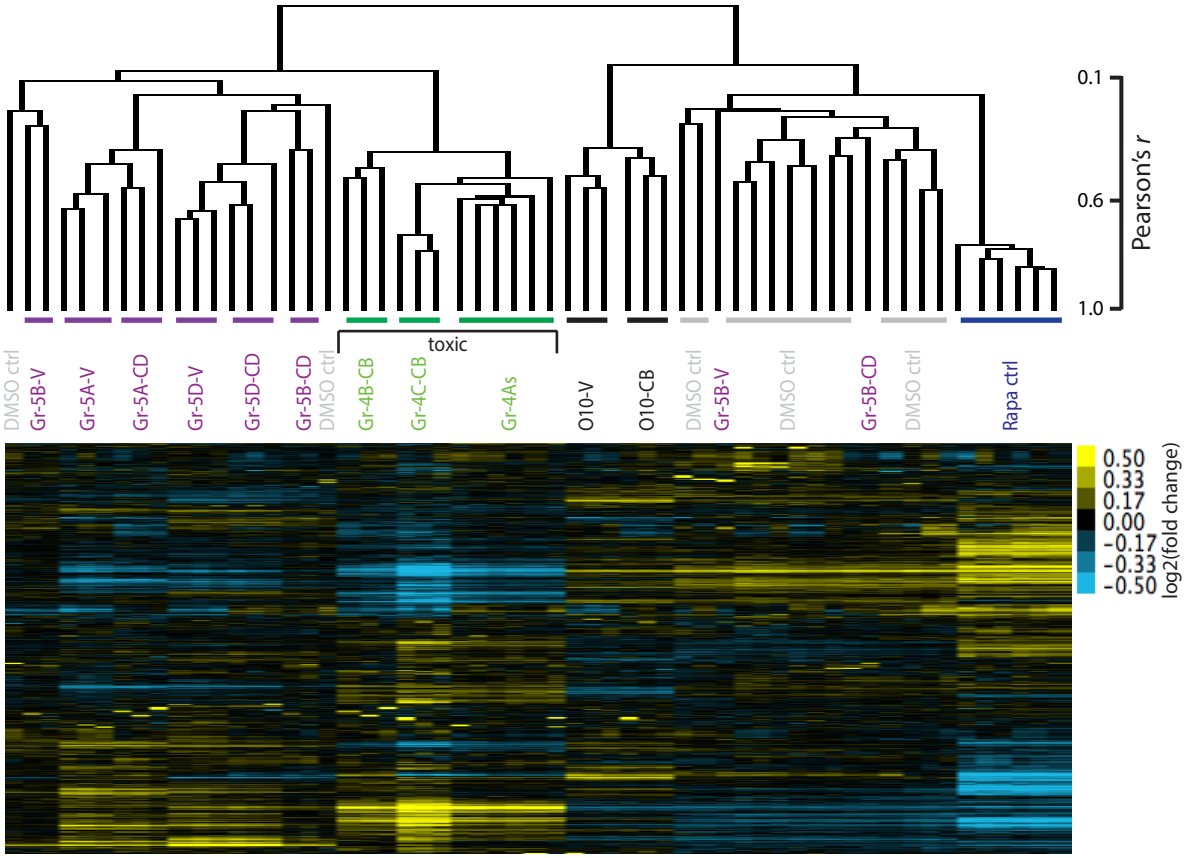


**Figure S8. Inhibitory effects of certain small molecules on PARP (related to Figure 2)**

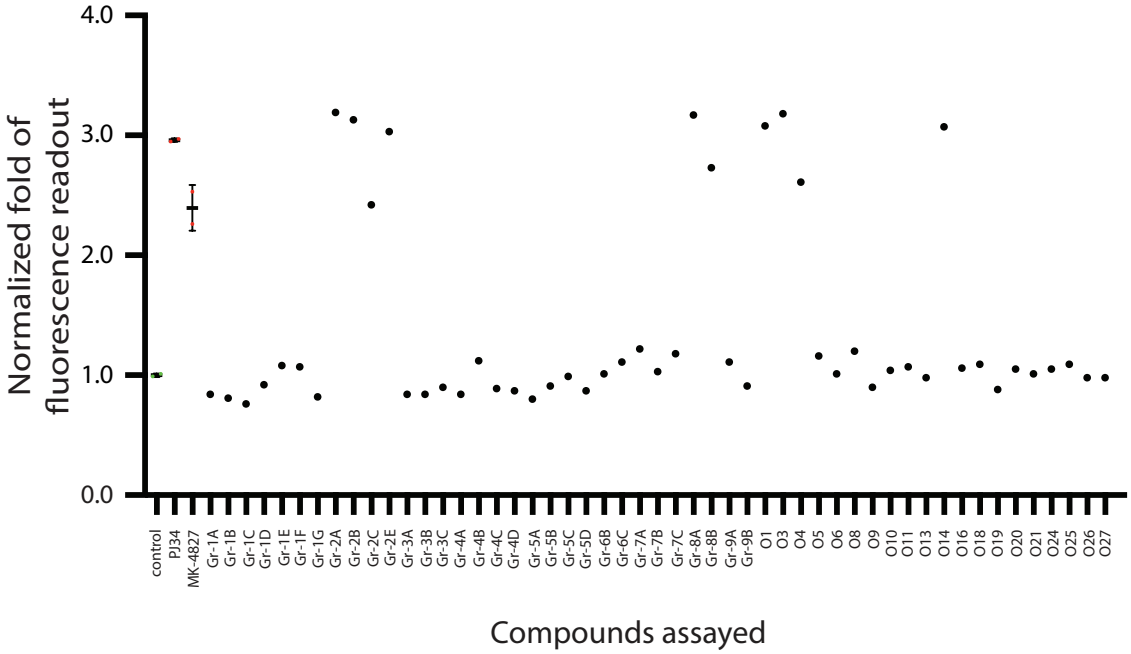


**Figure S9. Protective effects of certain small molecules against poly-Q toxicity (related to Table 1)**

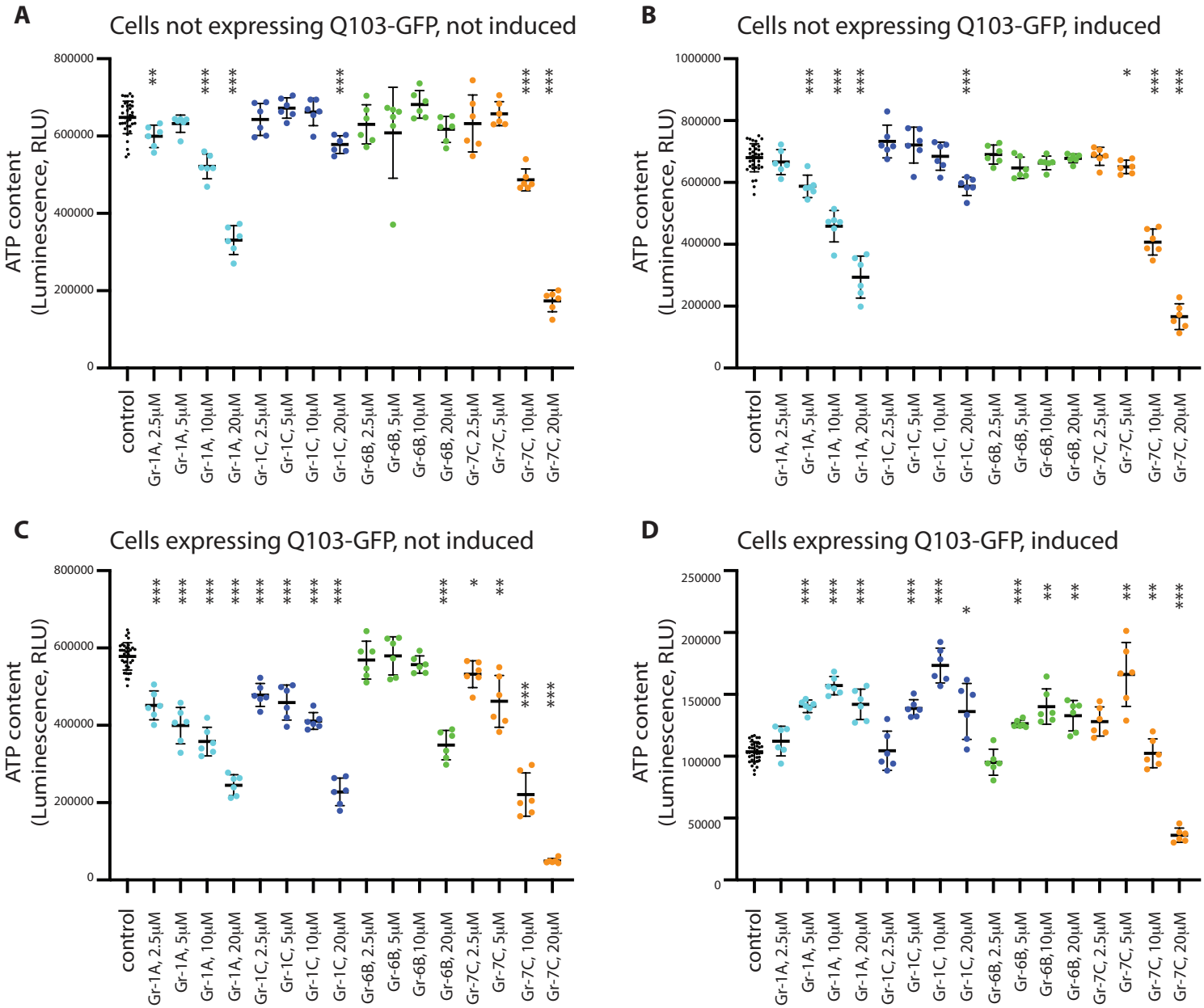
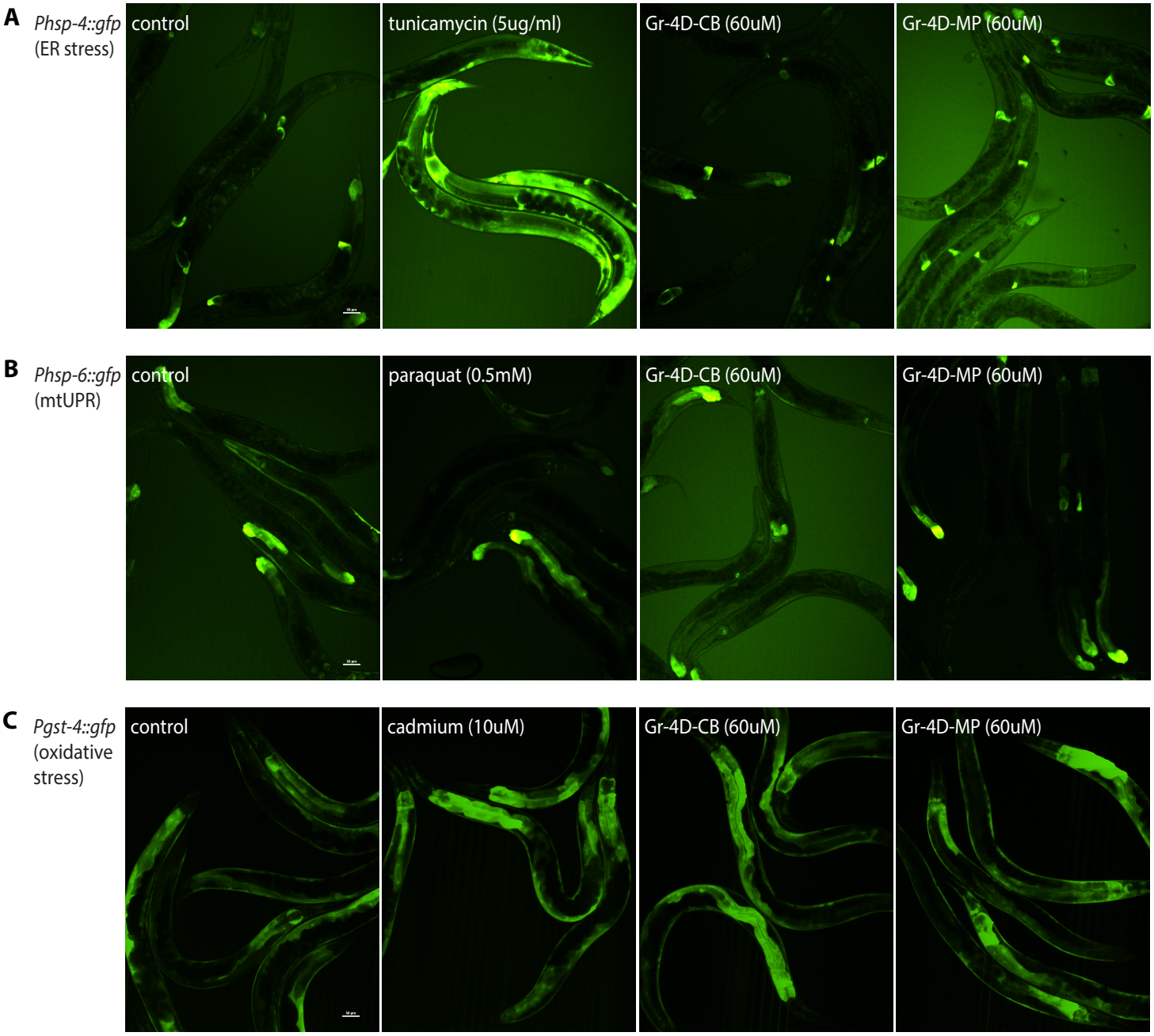
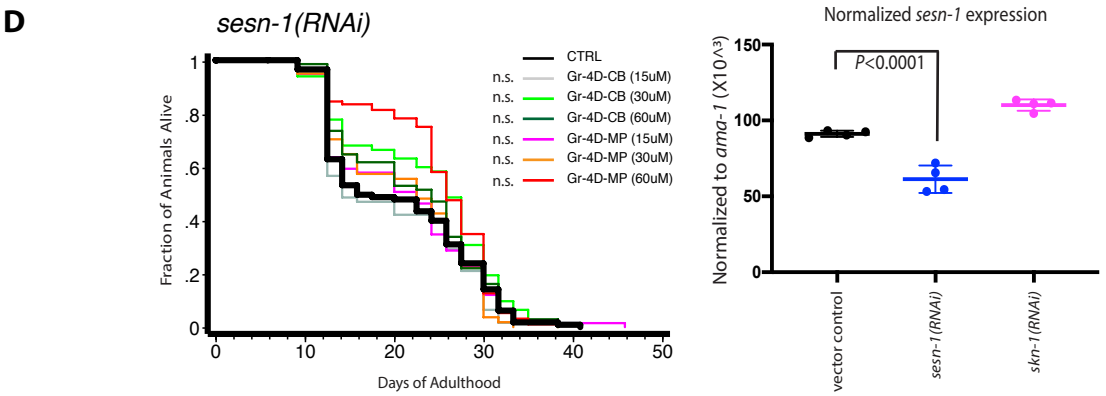


**Figure S10. Effects of Gr-4D on *C. elegans* expressing several pathway reporters & on lifespan of worms treated with *sesn-1(RNAi)* (related to Figure 4).**



**Supplemental Figure Legends**

Supplemental Figure 1 (related to Figure 1). Increased oxidative-stress resistance upon *AKT1* or *KEAP1* knockdown.

A) To define the optimal assay conditions for our screen, WI-38 cells were incubated with different doses of $H_2O_2$ for different periods of time in 384-well plates and then assayed for viability by measuring the ATP content. Shown here are representative data, indicating that high doses (above 600 μM) of $H_2O_2$ substantially depleted the ATP content after 3 hours of incubation (luminescence values are shown by the side). Control, n = 16; for all other $H_2O_2$ test samples, n = 11-16 (***, $P$ <0.0001 for 500 μM $H_2O_2$ and above, Student's t-test). Shown in all the figures, unless otherwise specified, were mean ± SD.

B) WI-38 cells were transfected with 20 nM AllStars negative control or *AKT1* or *KEAP1* siRNA oligos (Qiagen) and then assayed for viability 72 hours post-transfection upon $H_2O_2$. Representative data indicated that *AKT1* or *KEAP1* knockdown significantly increased cell viability, as measured by ATP content following 3 hours incubation in 700 μM $H_2O_2$. One-way ANOVA was performed for statistics ($p<0.001$), plus post-comparison with control (***, $p<0.001$). Control, n = 24; *AKT1* knockdown, n = 18; *KEAP1* knockdown, n = 13. Viability was also measured in the same experiment for cells not subjected to $H_2O_2$. One-way ANOVA ($p<0.001$), plus post-comparison with control (**, $p<0.01$; n.s., not significant). Control, n = 16; *AKT1* knockdown, n = 12; *KEAP1* knockdown, n = 12.

C) Propidium iodide staining was performed in parallel to analyze the fraction of dead/dying cells upon $H_2O_2$. Consistent with the ATP assay results, *AKT1* or *KEAP1*

knockdown significantly reduced the fraction of PI-positive cells. One-way ANOVA ($p<0.001$), plus post-comparison with control (***, $p<0.001$). Control, n = 8; *AKT1* knockdown, n = 6; *KEAP1* knockdown, n = 6.

Supplemental Figure 2 (related to Figure 1). Z' scores for the ATP assay across the screen. Shown is the Z' score for each of the 327 plates carrying a total of 104,121 library compounds screened. Z' score, defined as 1 – [(3X standard deviation for positive controls + 3X standard deviation for negative controls)/(mean value for positive controls - mean value for negative controls)], is typically used to access the assay quality in a high-throughput screen, and assay robustness is indicated by a Z' score greater than 0.5. The positive control calyculin (EMD Biosciences), a potent serine/threonine protein phosphatase inhibitor, significantly increased the levels of ATP in $H_2O_2$-stressed WI-38 cells, relative to the DMSO negative control. Average Z' score is 0.61±0.13 (mean ± SD) in our primary screen.

Supplemental Figure 3 (related to Figure 1). Cell-free ROS-scavenging assay of known ROS scavengers and our small molecules. The screen hits were analyzed together with several known ROS scavengers, including N-acetyl cysteine (NAC), amodiaquine dihydrochloride (AmD) and 8-hydroxyquinoline quinoline (8-HQ), in the absence of cells. Of 209 screen-hits assayed (at 10 μM), 56 molecules (including 40 that share a core structure of 8-HQ) were found to reduce the absorbance by 10% or more 24 hour-post incubation and were classified as putative ROS scavengers. The majority of these reduced the absorbance by ~30%. Across 3 assay plates: 0.4%

DMSO negative control, n = 417; each positive control of each dose, n = 12; each library compound, n = 3. Mean of absorbance normalized to DMSO control for each group was shown above.

Supplemental Figure 4 (related to Figure 2). No *in vitro* $H_2O_2$-quenching effects by small molecules. Catalase (0.02 units and 0.04 units), the positive control, substantially reduced the fluorescence in the Amplex Red assay that measures $H_2O_2$ concentration. By contrast, none of the 51 repurchased molecules appeared to reduce the absorbance. Across 4 assay plates: 0.2% DMSO negative control, n = 8; catalase positive control or each repurchased small molecule, n = 4.

Supplemental Figure 5 (related to Figure 3). 5-1) Long-term effects of small molecules on ATP levels of cultured WI-38 cells. Of 32 core-set hits, 28 molecules were analyzed in multiple 384-well plates in replicates in two batches, (A) 26 and (B) 2, to assess their effects on ATP levels upon prolonged incubation in the absence of $H_2O_2$ for up to 5 days (10 μM, n = 6 for each molecule, average standard deviation across the whole assay is ~5.9%). Four molecules (Gr-3A, Gr-4B, Gr-4D and O6) were not included in this experiment, since likely due to compromised stability, these 4 of this specific batch did not retest for $H_2O_2$-resistance on day 2 of treatment. However, Gr-4B and Gr-4D from a new batch purchased were analyzed in the IncuCyte analysis of cell confluency (see Supplemental Figure 5-2). Green mark on the y-axis indicates the start-point ATP level measured for 1,000 cells 24 hours post-seeding, before adding any small molecules. Note that compared with 0.1% DMSO control (black thick line) and

$H_2O$ control (aqua thick line), 5 μM rapamycin (yellow thick line), which is known to reduce cell proliferation and cell size, reduced ATP level by ~50% on day 5 of treatment. Dashed lines: of 28 analyzed, at least 11 small molecules (Gr-1F, Gr-3B, Gr-3C, Gr-6C, Gr-7A, Gr-7B, Gr-7C, Gr-9B, O11, O21 and O27) reduced the ATP level by more than 30% by day 5 (see Supplemental Table 4 for details). These molecules also were examined in the same experiment for their effects on cell morphology and cell death by PI-imaging on day 2 and day 5 of treatment. Unlike rapamycin, which also reduced ATP levels substantially, 9 of these 11 molecules (Gr-3B, Gr-6C, Gr-7A, Gr-7B, Gr-7C, Gr-9B, O11, O21 & O27) also produced cell toxicity (by the examination of cell morphology, data not shown). The other two, Gr-1F and Gr-3C, caused DNA damages, like the others (see Supplemental Table 4).

5-2) Effects of prolonged small-molecule incubation on WI-38 cell confluency. Shown is a representative plot of cell confluency for each treatment condition. Confluency of WI-38 cells was monitored in an IncuCyte Zoom Live-Cell Analysis System for 112 hours. Small molecules (10 μM final, n = 3 each) were introduced at 24 hours (indicated by arrows) following the start point. Fourteen different small molecules that did not produce obvious long-term cell toxicity (see Supplemental Figure 5-1) were retested (vendors are listed at the bottom). A) As a positive control, the TOR inhibitor rapamycin (2.5 μM) reduced cell confluency significantly. B) 5 molecules did not show strong inhibitory effects on cell confluency, and C) 9 molecules significantly reduced cell confluency.

Supplemental Figure 6 (related to Figure 3). Increased cell viability upon $H_2O_2$ by small molecules in the RNA-seq and microarray experiment, as well as *NRF2* non-dependency for Gr-4D. A) RT-qPCR analysis showing knockdown of *NRF2* expression by more than 95% in WI-38 cells transfected with siRNA oligos (normalized to *PPIA*, n = 8 each; one-way ANOVA, followed by Dunnett's multiple comparison, $P < 0.0001$). B) WI-38 cells were transfected with control or *NRF2* siRNA and then treated with Gr-4D (from two different vendors) at 3 different doses (2.5/3.75/5 μM), before subject to $H_2O_2$ or no $H_2O_2$ treatment, and scored for propidium iodide staining. Note that Gr-4D (from two different vendors) significantly increased the percentage of PI-positive cells, particularly, in *NRF2*-deficient WI-38 cells at higher doses in the absence of $H_2O_2$. Hence, we treated cells with a lower dose of $H_2O_2$ (500uM instead of 700uM) to prevent ceiling of death for *NRF2*-deficent cells. Gr-4D also protected *NRF2(-)* cells from $H_2O_2$, just like what it did in *NRF2(+)* cells. B & C) In parallel to our RNA-seq or microarray analysis of WI-38 treated with small molecules (in the absence of $H_2O_2$), cells were incubated with these molecules (Gr-4D, 2.5/3.75/5 μM, n = 8 for RNA-seq; or others, 10 μM, n = 3 for microarrays) or DMSO control (0.1%, n = 8 for RNA-seq or 6-8 for microarray) for 24 hours in 96-well plates and then analyzed for cell viability upon 3 hours of $H_2O_2$ treatment. Shown is the percentage of cells that scored positively for propidium iodide staining following treatment with DMSO control or small molecules (named according to the source of vendors: CB, ChemBridge; CD, ChemDiv; MP, MolPort; and V, Vitascreen). Student's t-test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; n.s. not significant; normalized to respective controls (color-coded).

Supplemental Figure 7 (related to Figure 3). Pearson's correlation between global transcriptional profiles for cells treated with small molecules. Shown is the normalized expression of the 18,683 probe sets detected in every array, grouped by unsupervised clustering using Pearson correlation coefficient as a distance metric. Note that most DMSO (0.1%) controls were clustered together on the tree, as were the six rapamycin (2.5 μM)-treated samples (highlighted in blue). The molecules were named according to the source of vendors (CB, ChemBridge; CD, ChemDiv; V, Vitascreen), and Gr-4A molecules were obtained from ChemDiv and Asinex.

Supplemental Figure 8 (related to Figure 2). Inhibitory effects of certain small molecules on PARP. Two known PARP inhibitors, PJ-34 (Tocris) ($IC_{50}$: ~20 nM) and MK-4827 ($IC_{50}$: ~3.8 nM), were shown to inhibit human PARP1 (at 200 nM and 40 nM, respectively), as indicated by a substantial increase of normalized fluorescence (n = 2). 10 of the 51 repurchased molecules also inhibited PARP at 10 μM. Note that all the molecules from group 2 assayed (one was not available for repurchasing), plus two group-8 molecules (analogs of the 4-amino-1,8-naphthalimide PARP inhibitor), were confirmed to be PARP inhibitors in this assay.

Supplemental Figure 9 (related to Table 1). Protective effects of certain small molecules against poly-Q toxicity. 51 repurchased molecules were introduced initially at 10 μM to neuron-like PC12 cells that express poly(Q)-tagged GFP (Q103-Htt-EGFP), and candidates showing protective effects were further retested at multiple doses (2.5 μM, 5 μM, 10 μM and 20 μM) to analyze their effects on ATP content upon the induction

of toxic poly(Q)103-Htt-EGFP aggregates. The parental PC12 cells (WT) that do not express poly(Q) were used as the control to demonstrate the specificity of protective effects. Note that 48 hours induction of poly(Q)103-Htt-EGFP reduced ATP content substantially (right, bottom panel), and several small molecules produced modest yet significant effects to enhance ATP content. However, except for Gr-6B, under non-induced conditions, these small molecules actually exerted cell toxicity and reduced ATP content in 72 hours (n = 6. Student's t-test, * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$).

Supplemental Figure 10 (related to Figure 4). Effects of Gr-4D on the expression of reporters for several pathways known to influence longevity in *C. elegans*. A to C) L4 animals expressing different *gfp* fusions were raised on plates containing DMSO (as the control) or different compounds as indicated for ~30 hours and then imaged for analysis. At least 12-15 worms were analyzed and shown are representative images. Note that Gr-4D (from two different suppliers), at a dose that extended lifespan, caused modest induction of the *gst-4* reporter, but not the others. No obvious effects of Gr-4D were observed on the number of LGG-1::GFP puncta, which was usually used as the readout of perturbed autophagy (data not shown). D) In an experiment to address *sesn-1* dependency, day-1 RNAi-sensitive mutant adults were fed with vector-control or RNAi bacteria expressing double-stranded RNA of *sesn-1* and treated with the chalcone Gr-4D (from two different vendors) at multiple doses. [Note: the *sesn-1* mutant and *skn-1(RNAi)* data were presented in Figure 4]. RT-qPCR analysis indicated that *sesn-1* mRNA level was reduced by ~30% (normalized to *ama-1*, n = 4 each; one-way ANOVA, followed by Dunnett's multiple comparison, $P < 0.0001$). Here, Gr-4D did not extend

lifespan of *sesn-1(RNAi)*-treated animals, though a high dose of this molecule (60 μM, Gr-4D-MP) produced a non-significant life-extending trend (n.s., log-rank test). See Supplemental Table 8 for details of lifespan data.