Supplemental Materials

Molecular Biology of the Cell

Baumann et al.

Supplemental Figure S1: Effect of BFA, GCA or MON on protein secretion. A549 cells stably expressing *Gaussia* Luciferase-flag (GLuc) were treated for 8 h or 20 h with the indicated compound concentrations before measurement of secreted GLuc into the supernatant (see also Materials and Methods). Shown is one example of two independent experiments using three wells per condition and time point. Asterisk symbols represent significant differences between compound treatment and DMSO (vehicle); * = $p < 0.05$, ** = $p < 0.01$.

Supplemental Figure S2: Gene expression profiling of A549 cells treated for 8 h with Golgi stress-inducing compounds. (A) Heat map representation of significantly regulated genes (FDR p-value cut-off < 0.05) by BFA, GCA or MON. (B) Venn diagram indicating shared genes between the three treatments. 52 genes that are upregulated by one compound and downregulated by another are not captured by this representation. (C) Transcription factor binding motif enrichment analysis of significantly regulated genes by BFA, GCA and MON. Some p-values are zero since the decimal value exceeds the number of digits in the 64-bit space. (D) GO-term enrichment analysis of significantly regulated genes by BFA, GCA and MON. (E) KEGG pathway enrichment analysis of significantly regulated genes by BFA, GCA and MON.

Supplemental Figure S3: Knockdown validation of ELK1, ETS1 and GABPA and effects on cell viability in response to secretory stressors. (A-F) Knockdown validation of stable A549 and HeLa single and double knockdown cells via qPCR or western blot (GABPA or ETS1 levels were normalized to β-actin, numbers below the Western blots indicate the remaining protein expression relative to shLUC). Protein lysates were run on the same gel, and dashed lines in blots indicate where unrelated samples were cropped out. (G-J) Survival ratios of A549 (G) or HeLa (H) ELK1 knockdown cells treated with ER stressors tunicamycin (TUN) or thapsigargin (THA). (I, J) Survival ratios of A549 (I) or HeLa (J) ETS1 knockdown cells treated with ER stressors tunicamycin (TUN) or thapsigargin (THA). (G-J) Data are shown as mean and standard deviation

of one representative example of three independent experiments measuring six wells per genotype and condition; * = $p \leq 0.05$, two-way ANOVA with Bonferroni post-test. (K-L) qPCR analysis of A549 ELK1/ETS1 or ELK1/GABPA double knockdown cells. (M, N) Survival ratios and expression validation (qPCR) of HeLa cells stably expressing shRNAs against ELK1 and GABPA; a = significant compared to Avg. Ctrl, b = significant compared to the corresponding single knockdown; *, a, b = $p \leq 0.05$, two-way ANOVA with Bonferroni post-test.

Supplemental Figure S4: (Co-) overexpression of ETS factors and impact on cell viability following stress treatments. Survival ratios of A549 cells with stable overexpression of multiple FLAG-tagged transcription factors or control proteins treated with Torin2 (A), Doxorubicin (B) or thapsigargin (C). (A-C) Data are shown as mean and standard deviation of one representative example of three independent experiments measuring six wells per genotype and condition; a = significant compared to Avg. Ctrl, b = significant compared to the corresponding single knockdown; *, a, b = $p \leq 0.05$, two-way ANOVA with Bonferroni post-test.

Supplemental Figure S5: Golgi stress causes MEK/ERK activation. Time course analyses of 786-0 (A) or HeLa (B) cells treated with 40 nM BFA. Expression levels of the indicated proteins were determined via western blot at the indicated time points following treatment. (C-F) 786-0 (C, D) and HeLa (E, F) were treated for the indicated duration with either 1.75 μM GCA or 500 nM MON before cell lysis and immunoblotting for the indicated antibodies. (C-I) Experiment was performed once. (G-I) HeLa cells were treated with 40 nM BFA (G), 1.75 μM GCA (H) or 500 nM MON (I) in the absence or presence of 10 μM U0126 for the indicated duration before cell lysis and immunoblotting with the indicated antibodies (n=1).

Supplemental Figure S6: Regulation of *FOSB* by ELK1, survival of ERK1/2 knockdown

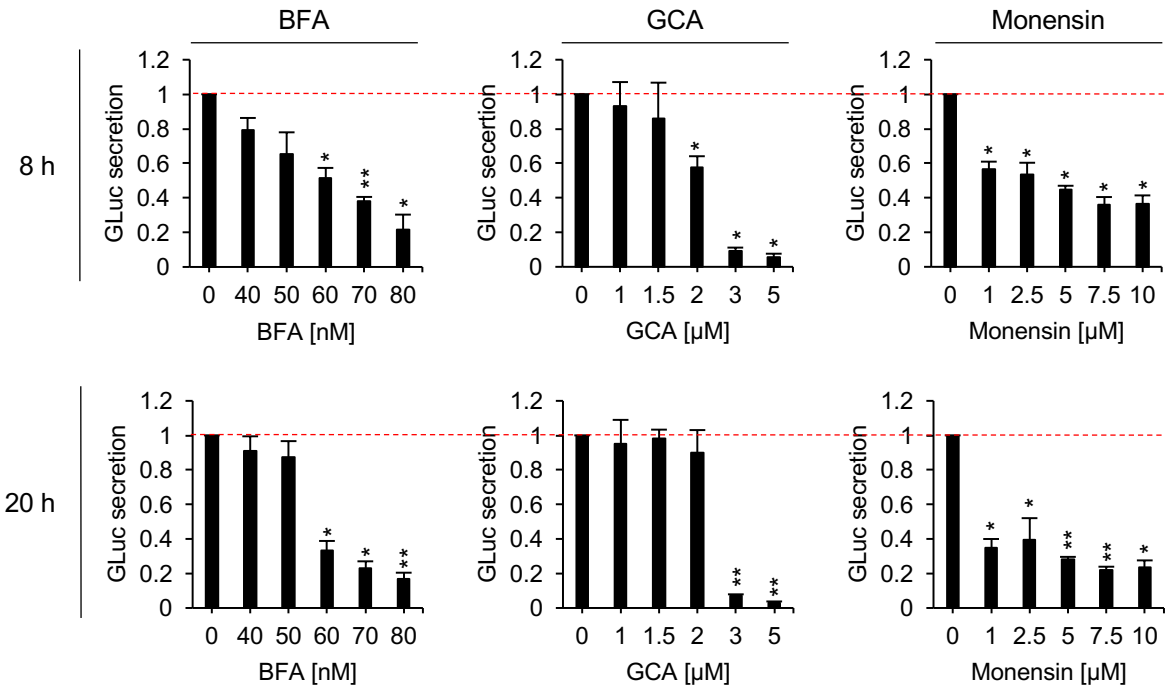
cells and effect of ARF1 overexpression on phospho-ERK1/2 in response to BFA. (A, B) A549 (A) and HeLa (B) control and ELK1 knockdown cells were treated for 24 h with 60 nM BFA (A549) or 30 nM BFA (HeLa) before RNA isolation, and *FOSB* levels were quantified by qPCR analysis. *FOSB* levels were normalized to *36B4* mRNA levels. Data are shown as mean and standard deviation of two independent experiments; * = $p \leq 0.05$, Student's t-test. (C, D) Cell counts and knockdown validation of ERK1/2 HeLa knockdown cells treated with 40 nM BFA (E) or ERK1/2 A549 knockdown cells treated with 60 nM BFA (F). Data are shown as mean and standard deviation of two independent experiments measuring three wells per genotype and condition; * = $p \leq 0.05$, two-way ANOVA with Bonferroni post-test. (E) HeLa cells stably expressing epitope-tagged ARF1 or a control protein (RAP2A) were treated for 24 h with the indicated concentrations of BFA. Expression levels of the indicated proteins were determined via western blot and signals were quantified for total ERK1/2 and phospho-ERK1/2 levels.

Supplemental Figure S7: Model summarizing the effects of Golgi stress on MEK-ERK signaling, ETS transcription factors and MCL1 splicing. Golgi stress induced by BFA, GCA or MON leads to dispersal of the Golgi complex. If cellular repair mechanisms are sufficient to cope with the inflicted stress, Golgi reassembly occurs and cell death is averted. Upon prolonged treatment with BFA, GCA or MON, MEK1/2 and ERK1/2 are activated coinciding with the induction of targets of the three ETS transcription factors ELK1, GABPA and ETS1, the activity and stability of which is enhanced via ERK1/2-mediated phosphorylation. Among the ELK1-regulated factors following BFA/GCA treatment are several components of the spliceosome, which may contribute to the enhanced production of the pro-apoptotic MCL1-S isoform. Increased levels of MCL1-S, which can dimerize with MCL1-L, may tilt the balance towards a pro-apoptotic cell fate in response to Golgi stress.

Supplemental Tables

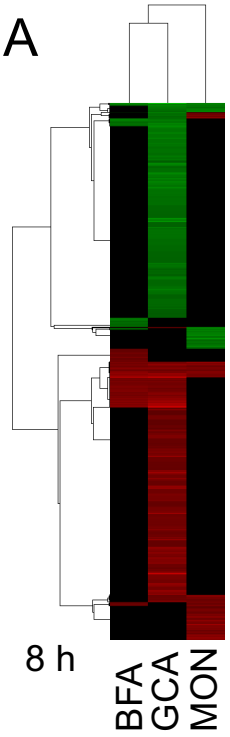
Supplemental Table 1: List of significantly regulated genes (FDR $p < 0.05$) and the associated mRNA fold changes in A549 cells after 20 h of treatment with either 71 nM (20 ng/mL) BFA, 5 μM GCA or 10 μM MON.

Supplemental Table 2: List of significantly regulated genes (FDR $p < 0.05$) and the associated mRNA fold changes in A549 cells after 8 h of treatment with either 71 nM (20 ng/mL) BFA, 5 μM GCA or 10 μM MON.



Supplemental Figure S1

A



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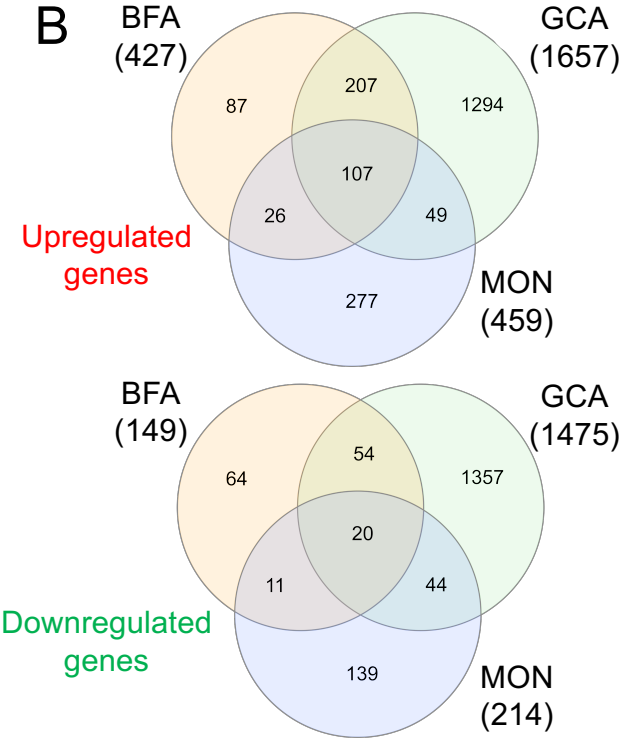


C

BFA

	Motif	FWER p-val
	No significant enrichment	

GCA

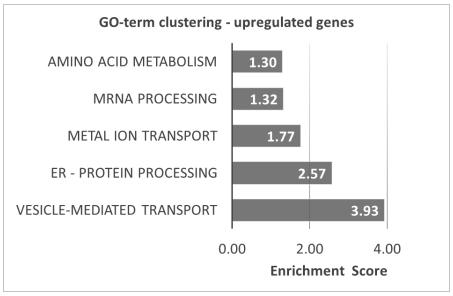
	Motif	FWER p-val
1	SCGGAAGY_V$ELK1_02	0.000
2	V$ELK1_02	0.000
3	V$NRF2_01	0.001
4	V$GABP_B	0.001
5	MGGAAGTG_V$GABP_B	0.001
6	GGAANCGGAANY_UNKNOWN	0.001
7	V$ATF6_01	0.011
8	V$CETS1P54_01	0.000

MON

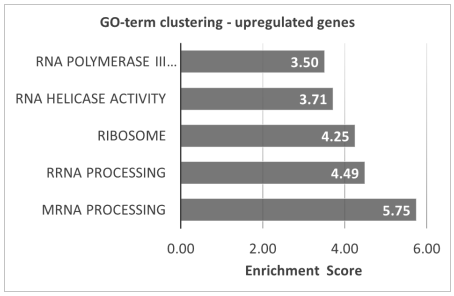
	Motif	FWER p-val
1	TCANNTGAY_V$SREBP1_01	0.000
2	CACGTG_V$MYC_Q2	0.021

D

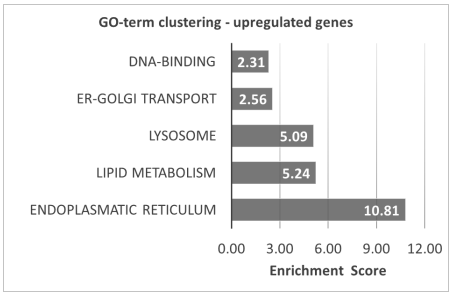
BFA



GCA



MON



E

BFA

	KEGG-Pathway	Nom. p-val
hsa04141	Protein processing in ER	4.90E-10
hsa00520	Amino and nucleotide sugar metabolism	3.11E-04
hsa04710	Circadian rhythm	2.91E-03
hsa00250	Ala, asp and glutamate metabolism	4.56E-03
hsa00220	Arginine biosynthesis	6.52E-03

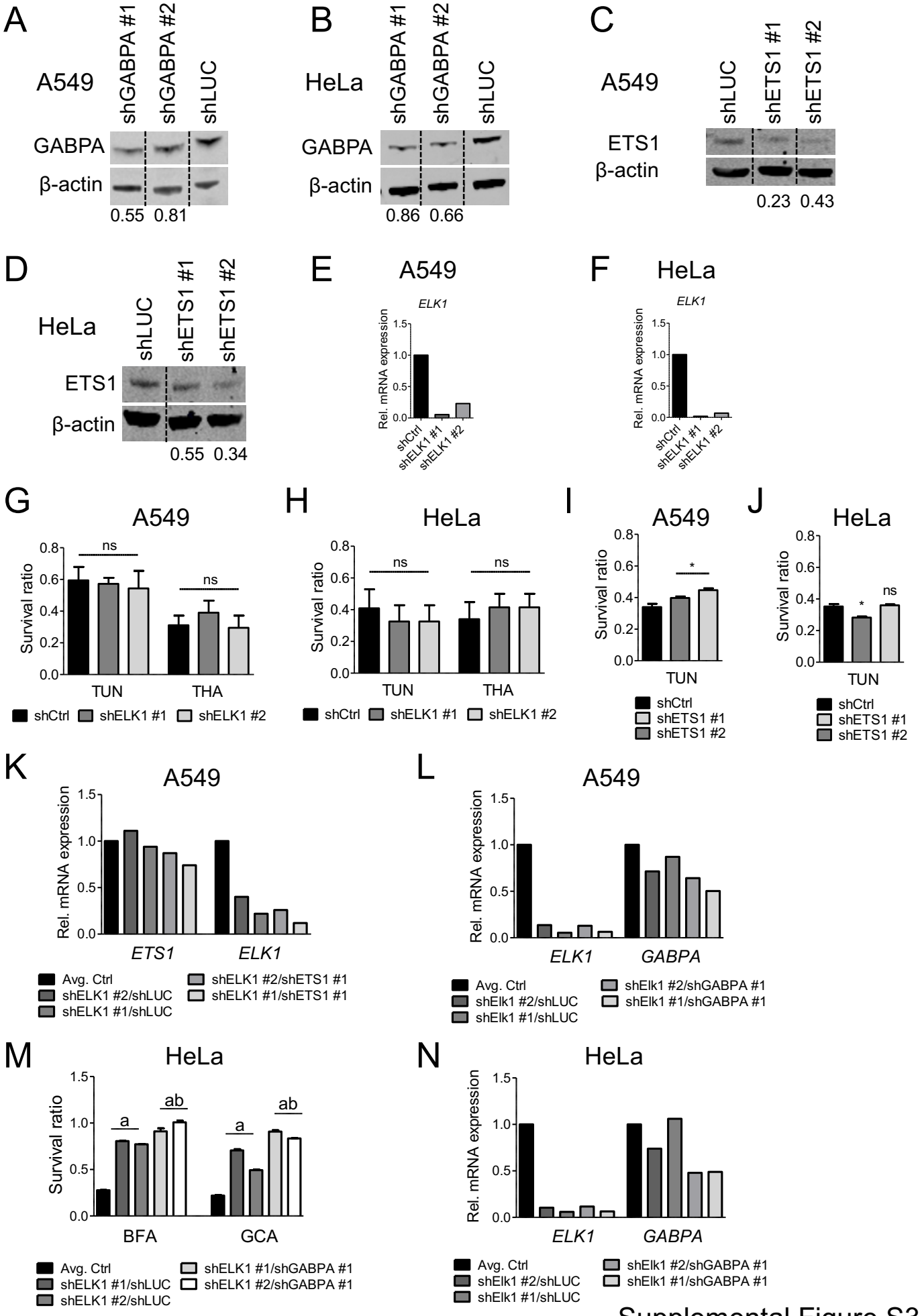
GCA

	KEGG-Pathway	Nom. p-val
hsa04141	Protein processing in ER	2.56E-13
hsa03040	Spliceosome	8.88E-09
hsa03008	Ribosome biogenesis	2.13E-08
hsa03020	RNA polymerase	9.00E-07
hsa00230	Purine metabolism	4.18E-05

MON

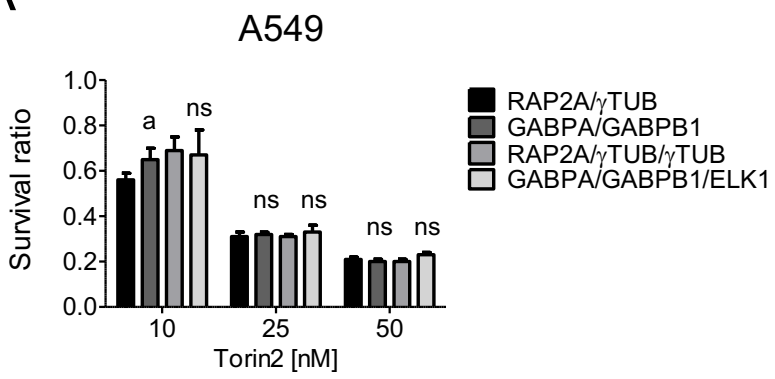
	KEGG-Pathway	Nom. p-val
hsa04141	Protein processing in ER	2.02E-08
hsa04142	Lysosome	3.91E-06
hsa00100	Steroid biosynthesis	9.93E-06
hsa05110	Vibrio cholerae infection	4.94E-04
hsa04120	Ubiquitin mediated proteolysis	3.73E-03

Supplemental Figure S2



Supplemental Figure S3

A



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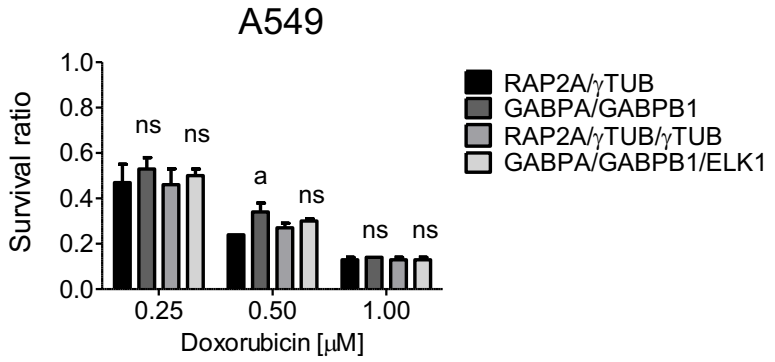

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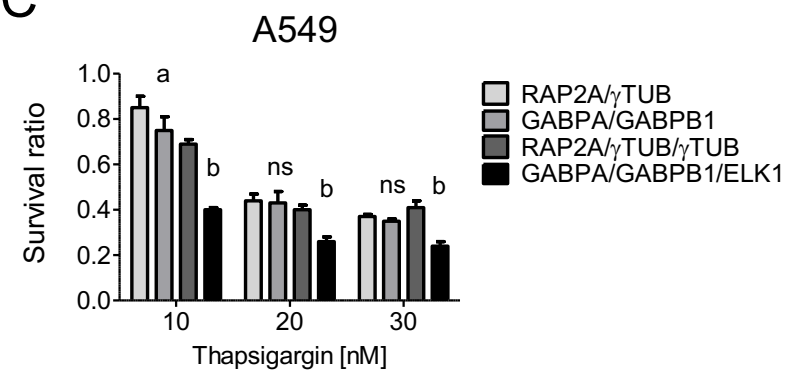
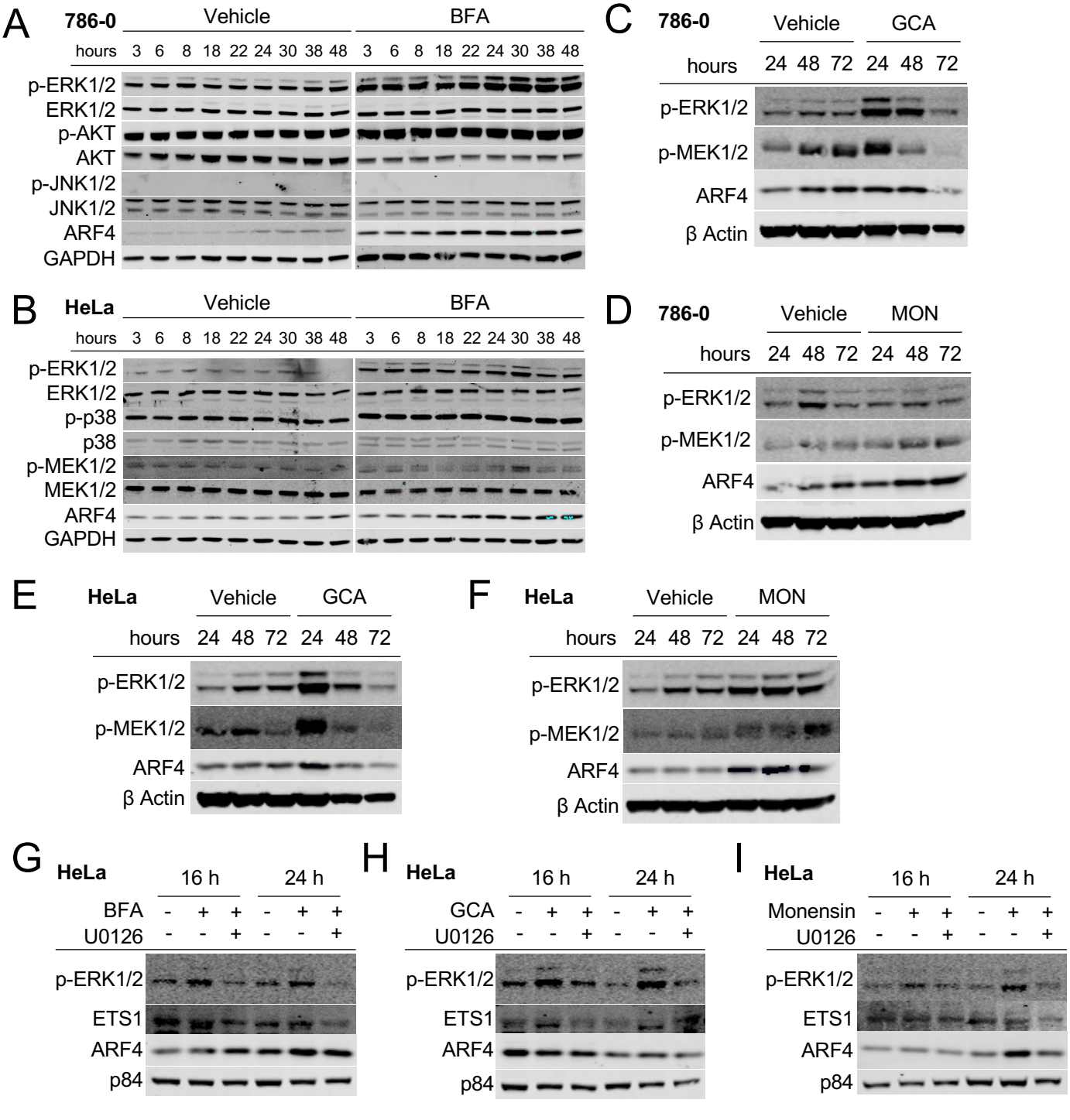
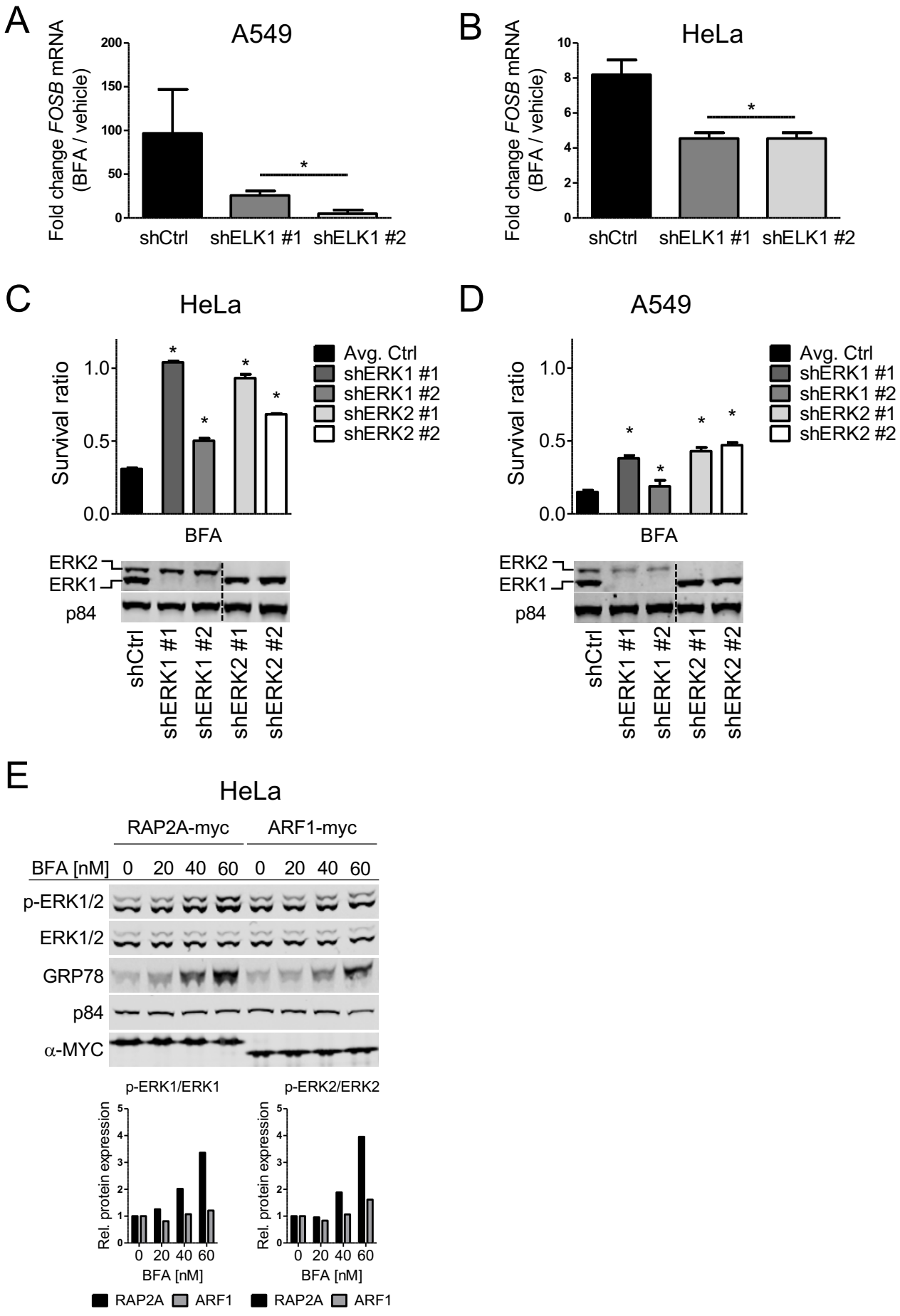


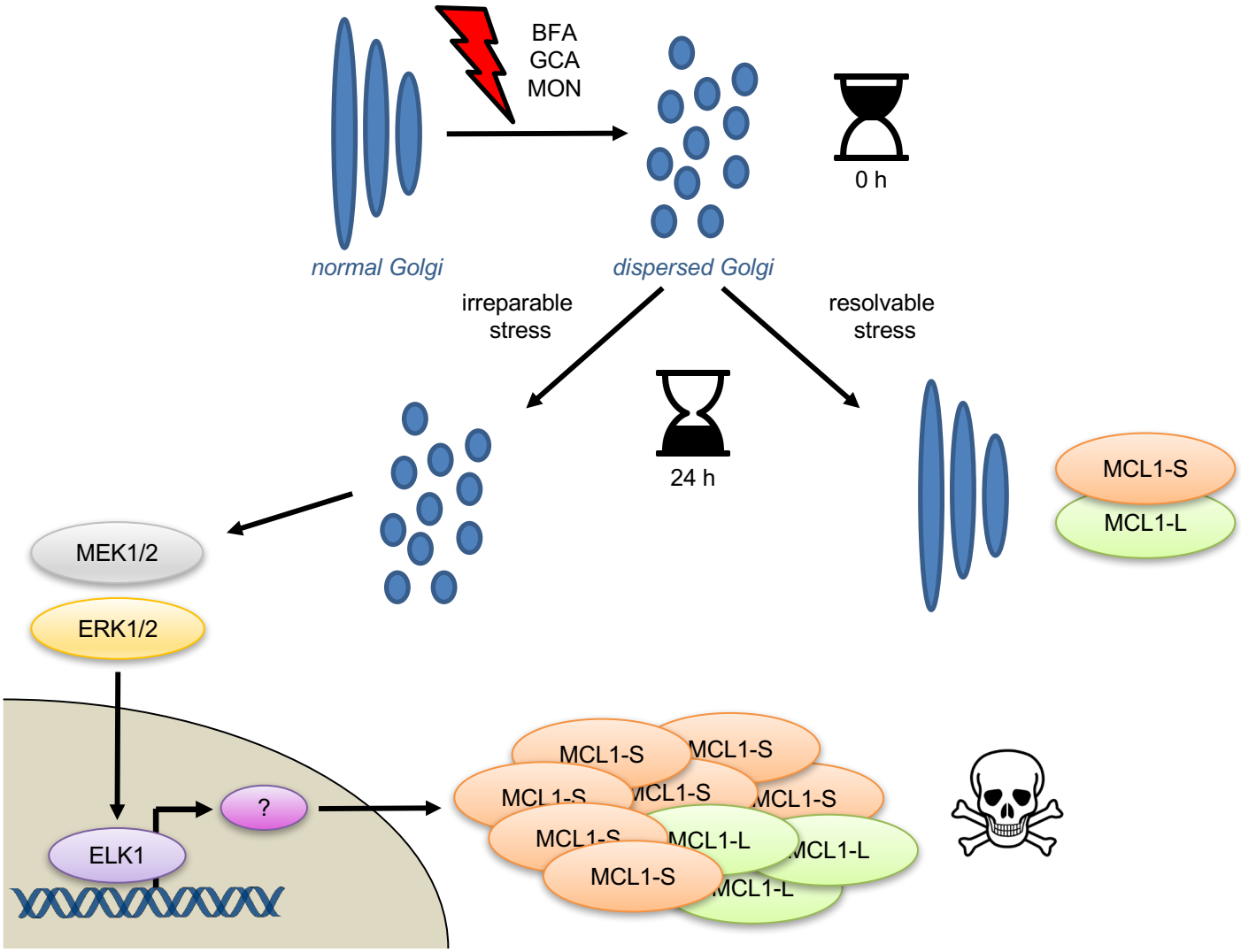
Supplemental Figure S4



Supplemental Figure S5



Supplemental Figure S6



Supplemental Figure S7