Supplementary Information

Identification of PP1-Gadd34 substrates involved in the unfolded protein response using K-BIPS, a method for phosphatase substrate identification

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I. Supplemental Data

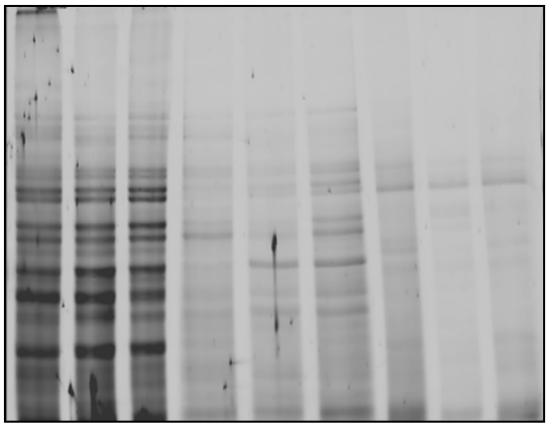

Figure S1: Full gel image from K-BIPS with OA treatment. Biotinylation was carried out with ATP-biotin and HeLa lysates either untreated (ethanol only) or treated with OA (1 or 0.01 μM, as indicated in the figure). Biotinylated proteins were purified using Avidin resin after reaction. The input before avidin purification (lanes 1-3), flow through (lanes 4-6), and elution (lanes 7-9) were separated by SDS-PAGE and visualized by SYPRO® Ruby stain. A control reaction was carried out with HeLa cell lysates not treated with OA (lanes 1, 4, and 7). The image is a representative of two replicates. A portion of Figure S1 is shown in Figure 2A of the manuscript.

Table S1: The 71 K-BIPS hits with OA treatment[a]

Gene names	T1-OA	T1+OA	T2-OA	T2+OA	T1 Fold change	T2 Fold change	Known Interactions
SMC1A[1]	2.2E+03	0.0E+00	5.3E+02	0.0E+00	∞	∞	
STAR	1.1E+02	0.0E+00	2.0E+02	0.0E+00	∞	∞	
C1QBP	2.5E+03	3.7E+02	6.7E+01	0.0E+00	6.8	∞	PPP2R1A,RRP1B,PPP2R2A
EIF2S1[2]	7.2E+02	2.1E+02	3.8E+02	0.0E+00	3.4	∞	PPP1CC,PPP1R7,PPP1R15A
TRMT10C	2.2E+02	0.0E+00	3.2E+02	1.6E+02	∞	2.1	
EPB41	6.4E+02	3.5E+02	3.4E+02	0.0E+00	1.8	∞	
DDX60L	4.0E+03	0.0E+00	3.9E+02	2.3E+02	∞	1.7	
HSPD1[3]	1.7E+03	1.1E+03	6.8E+01	0.0E+00	1.5	∞	PPP2R1B,PPP2R1A,PPP2R2C
BBS1	3.8E+02	2.6E+02	2.0E+02	0.0E+00	1.5	∞	
DAP3	1.3E+04	1.6E+02	8.6E+02	2.4E+02	80.7	3.6	
MRPL44	1.2E+04	2.1E+02	4.5E+02	1.8E+02	58.3	2.5	
TUBA1B	5.7E+05	1.0E+04	3.8E+04	2.3E+04	57.4	1.6	
TUBB[4]	8.3E+04	1.7E+03	6.6E+03	2.4E+03	48.2	2.7	PPP1CC,PPP2R2A
LRPPRC	1.6E+04	4.5E+02	4.2E+03	8.2E+02	35.9	5.1	
EIF3A	1.1E+04	3.6E+02	9.6E+02	4.2E+02	29.3	2.3	
NCL[5]	2.9E+04	1.3E+03	2.9E+03	8.0E+02	22.6	3.6	PPP1CB,RRP1B
PHGDH	5.6E+04	3.5E+03	2.8E+04	2.9E+03	15.8	9.5	
GCN1L1	2.0E+04	1.0E+03	1.3E+03	8.6E+02	19.8	1.5	PPP6R1
HSP90AB1	1.7E+05	9.6E+03	2.0E+04	1.1E+04	17.7	1.8	PPP5C,PPP6R3
TUBA4A	1.2E+04	7.3E+02	9.3E+02	5.3E+02	16.3	1.8	
EIF4A3	1.7E+04	1.2E+03	1.1E+03	6.3E+02	14.4	1.7	PPP6R3
EZR[6]	2.9E+03	2.1E+02	6.9E+02	3.9E+02	13.6	1.8	
DDX1	7.1E+03	5.4E+02	7.7E+02	3.8E+02	13.2	2.0	PPP1CA,PPP1R8
HLA-B	1.1E+03	4.8E+02	2.3E+03	1.9E+02	2.4	12.2	PPP2CB,PPP1R16A
DHX9	1.5E+04	1.3E+03	1.0E+03	4.7E+02	11.8	2.1	PPP1CB
PCNA	2.6E+03	2.2E+02	3.7E+02	1.9E+02	11.9	1.9	PPP1CC,PP2A[7]
ZNF83	7.8E+03	6.8E+02	4.7E+02	2.7E+02	11.5	1.7	
KATNAL2	1.5E+04	1.4E+03	2.0E+03	1.2E+03	11.0	1.7	
ACADVL	3.0E+03	1.3E+03	2.8E+03	2.8E+02	2.3	10.0	
TOM1L2	5.8E+03	9.4E+02	7.2E+02	1.4E+02	6.1	5.1	
EXTL3	3.1E+03	4.1E+02	8.3E+02	3.0E+02	7.6	2.8	PPP6R2
HSP90B1	1.7E+03	2.8E+02	7.9E+02	2.0E+02	6.2	4.0	PPP5C[8]
VDAC2	2.9E+04	7.6E+03	5.8E+04	9.6E+03	3.8	6.0	
CANX	3.4E+04	7.3E+03	2.2E+04	5.1E+03	4.7	4.4	PPP2R1A
VDAC1[9]	5.3E+04	1.9E+04	1.3E+05	2.1E+04	2.8	6.0	
GTF2H4	1.3E+03	2.2E+02	2.5E+02	9.1E+01	5.8	2.7	
KIAA1755	5.3E+02	1.5E+02	4.9E+02	1.0E+02	3.6	4.7	
MCM3[10]	4.4E+04	7.6E+03	1.5E+04	5.8E+03	5.8	2.5	PPP2R1B,PPP2R1A

Gene names	T1-OA	T1+OA	T2-OA	T2+OA	T1 Fold change	T2 Fold change	Known Interactions
SERPINB6	7.4E+04	1.7E+04	1.2E+04	3.6E+03	4.4	3.3	
RALGAPA1	1.8E+03	9.7E+02	3.4E+03	6.0E+02	1.9	5.6	RRP1B
TNFRSF21	3.4E+03	1.5E+03	6.5E+03	1.3E+03	2.4	5.0	
HORMAD1[11]	1.5E+03	9.2E+02	9.5E+02	1.7E+02	1.6	5.5	
KPNB1	3.7E+04	8.2E+03	1.8E+04	7.0E+03	4.5	2.6	PPP2CA[12],PPP2R2A[12]
HADHA	1.8E+04	6.3E+03	1.4E+04	3.6E+03	2.8	3.9	PPP6R1
CAD	1.4E+03	6.1E+02	2.0E+03	4.8E+02	2.3	4.1	PPP1CA,PPP2CA
C3orf17	1.1E+03	4.0E+02	8.4E+02	2.5E+02	2.9	3.4	
HADHB	5.0E+04	1.9E+04	1.3E+04	4.3E+03	2.7	3.1	PPP6R1
WDR87	1.2E+03	4.6E+02	1.1E+03	3.6E+02	2.7	3.1	
RNF207	4.9E+02	1.4E+02	3.4E+02	1.7E+02	3.5	2.0	
IMMT	7.0E+02	2.6E+02	1.0E+03	3.8E+02	2.7	2.7	
ARFGEF2[13]	1.2E+03	3.4E+02	4.7E+02	3.1E+02	3.6	1.5	
SERPINB1	1.1E+05	3.2E+04	1.8E+04	1.1E+04	3.3	1.7	
OR5H2	2.2E+03	9.4E+02	8.6E+02	3.3E+02	2.4	2.6	
C2orf78	4.8E+02	2.8E+02	4.9E+02	1.5E+02	1.7	3.2	
RYR2[14]	4.3E+03	1.4E+03	2.3E+03	1.3E+03	3.0	1.8	PPP1CA,PPP1CB,PPP1CC,PPP2CA
RDH13	3.1E+03	9.9E+02	6.8E+02	4.4E+02	3.2	1.5	
RPL27	7.4E+03	2.6E+03	1.2E+03	7.4E+02	2.9	1.6	PPP1CC,Repo-Man[15]
BDP1	7.0E+02	4.7E+02	1.2E+03	3.9E+02	1.5	3.0	
LDHA	1.8E+04	9.3E+03	1.6E+04	6.4E+03	1.9	2.5	
APMAP	3.8E+03	2.5E+03	1.8E+03	6.5E+02	1.5	2.8	
EEF2[16]	3.6E+04	1.3E+04	1.4E+04	8.7E+03	2.7	1.6	PPP2CA,PPP2R1B,PPP2R1A,PPP2R2B
MSN[17]	3.4E+05	1.3E+05	2.4E+05	1.5E+05	2.6	1.5	PPP1R2
KIF20B	2.1E+03	1.4E+03	5.6E+02	2.4E+02	1.5	2.3	
TPP1	3.1E+03	1.7E+03	6.8E+02	3.4E+02	1.8	2.0	
PML	1.4E+02	7.0E+01	1.2E+02	7.5E+01	2.0	1.6	PPP1CA[18]
CALR[19]	7.1E+02	3.7E+02	6.1E+02	3.7E+02	1.9	1.7	
ZNF618	1.8E+03	1.2E+03	2.6E+03	1.3E+03	1.5	2.0	
PPIA	1.0E+03	6.5E+02	7.4E+02	4.3E+02	1.6	1.7	
PKM[20]	4.5E+04	3.0E+04	3.7E+04	2.1E+04	1.5	1.7	PPP1CA
TCF20	2.0E+03	1.2E+03	2.2E+03	1.5E+03	1.6	1.5	
HSPA6	3.4E+04	2.2E+04	4.5E+03	2.9E+03	1.6	1.5	

[a] Protein hits observed in the K-BIPS study with OA mediated phosphatase inactivation. The TMT reporter intensity observed for each sample (-OA: Without okadaic acid, +OA: With okadaic acid) for the two trials (T1: trial 1, T2: trial 2) is shown. Fold change for each trial was calculated by dividing the TMT reporter intensity observed in the okadaic acid untreated sample (phosphatase active) by the TMT reporter intensity observed for the okadaic acid treated sample (phosphatase inactive). Infinity (∞) signifies that no TMT reporters were observed in the okadaic acid treated sample, making a numeric ratio calculation impossible. The known substrates of the inhibited phosphatases are green colored. The K-BIPS hits that are not previously known substrates, but known to interact with the inhibited phosphatases, are blue colored. Proteins are ordered according to the fold change value. The primary literature demonstrating that K-BIPS hits are known phosphatase substrates is provided with the gene name. When no citation is provided, interactions information was obtained from Uniprot or BioGrid databases.

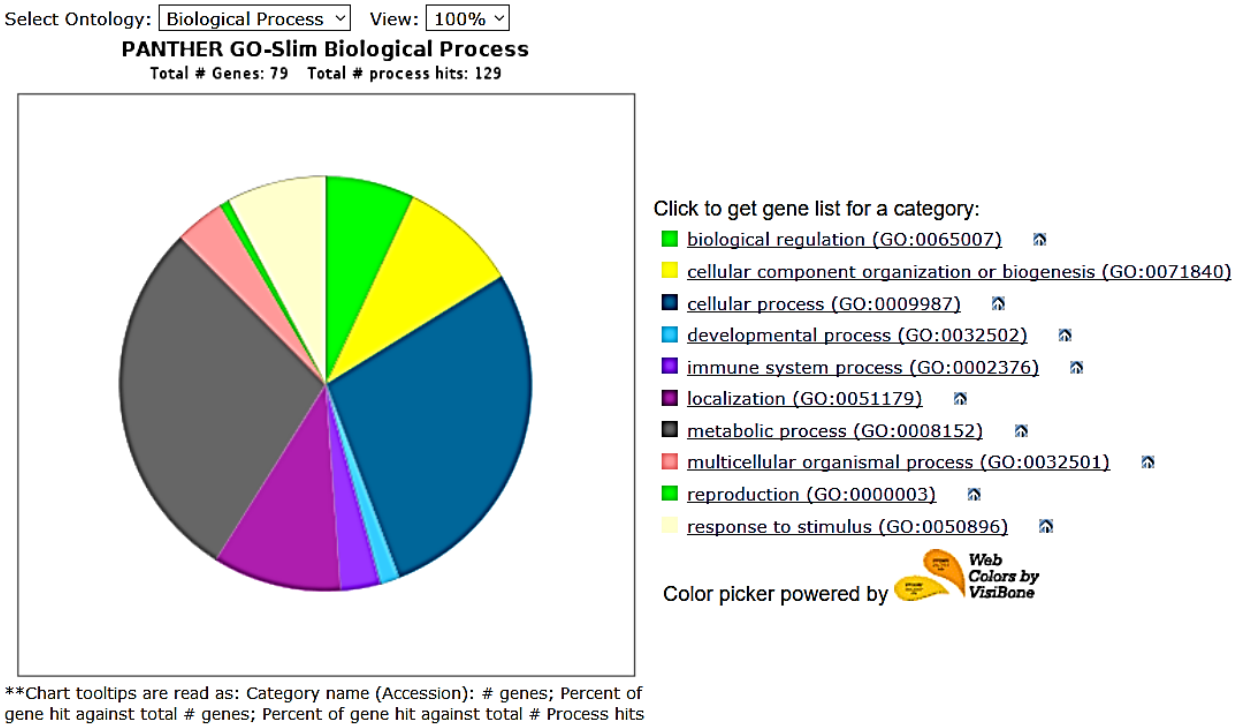

Figure S2: Functional classification of K-BIPS hits with OA treatment. The proteins were classified based on their biological processes by the enrichment analysis tool available through the Gene Ontology Consortium.

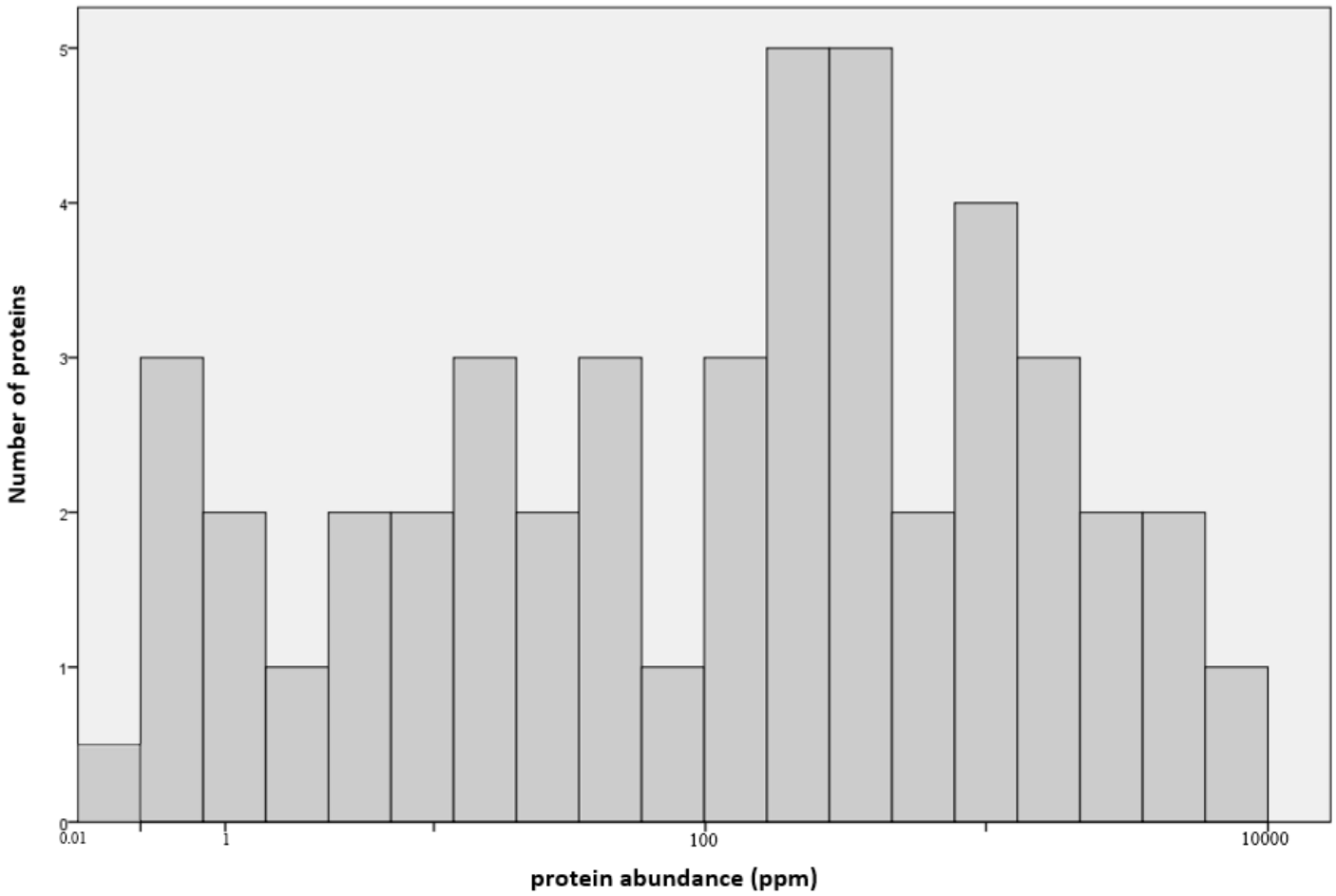

Figure S3: Cellular abundance of the K-BIPS hits with OA treatment. The previously reported abundance values[21] of the K-BIPS hits available on Pax database[22] were plotted with Excel. Proteins with a range of abundance values (0.01 to 8,785 ppm) were identified by K-BIPS. The full range of protein abundances in HeLa cells is 0.01 to 10,000, which indicates that the K-BIPS hits are found throughout the range of abundances.

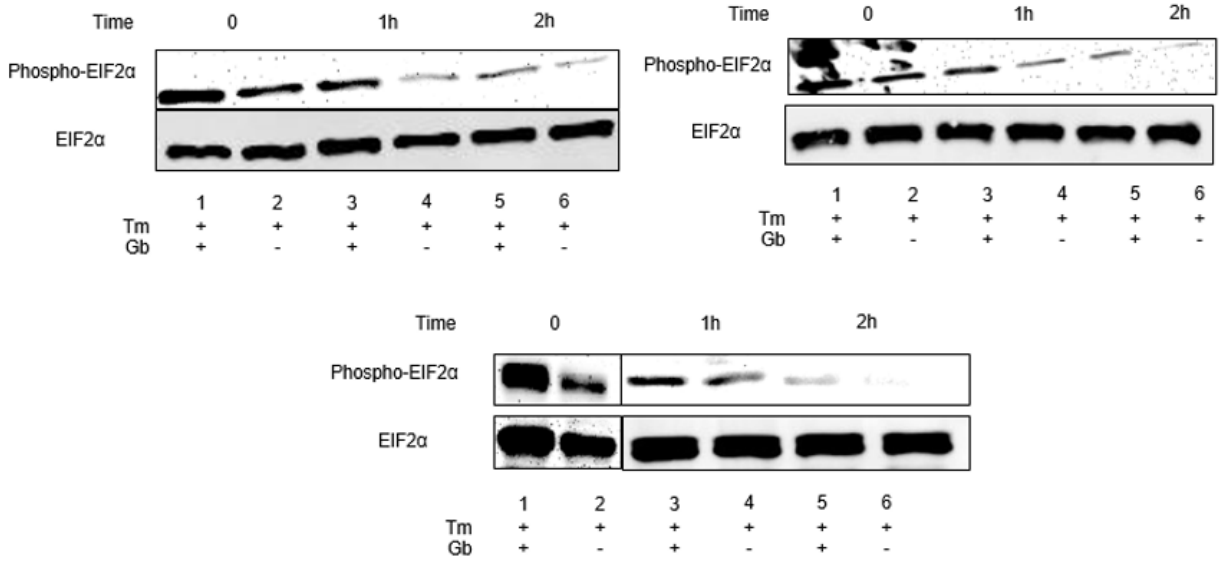

Figure S4: Analysis of eIF2α phosphorylation levels. Lysates from Tm and Gb treated HeLa cells or Gb only treated cells were pre-incubated with Gb for 15 minutes at room temperature. A portion of lysates was saved (time = 0). The rest of the lysates were incubated at 31°C for 2 hours and portions of lysates were removed at 1 hour and 2 hours. The removed lysates were separated by SDS-PAGE, transferred to a PDVF membrane and total eIF2α levels and eIF2α phosphorylation were assessed by Western blot. Three independent trials are shown.

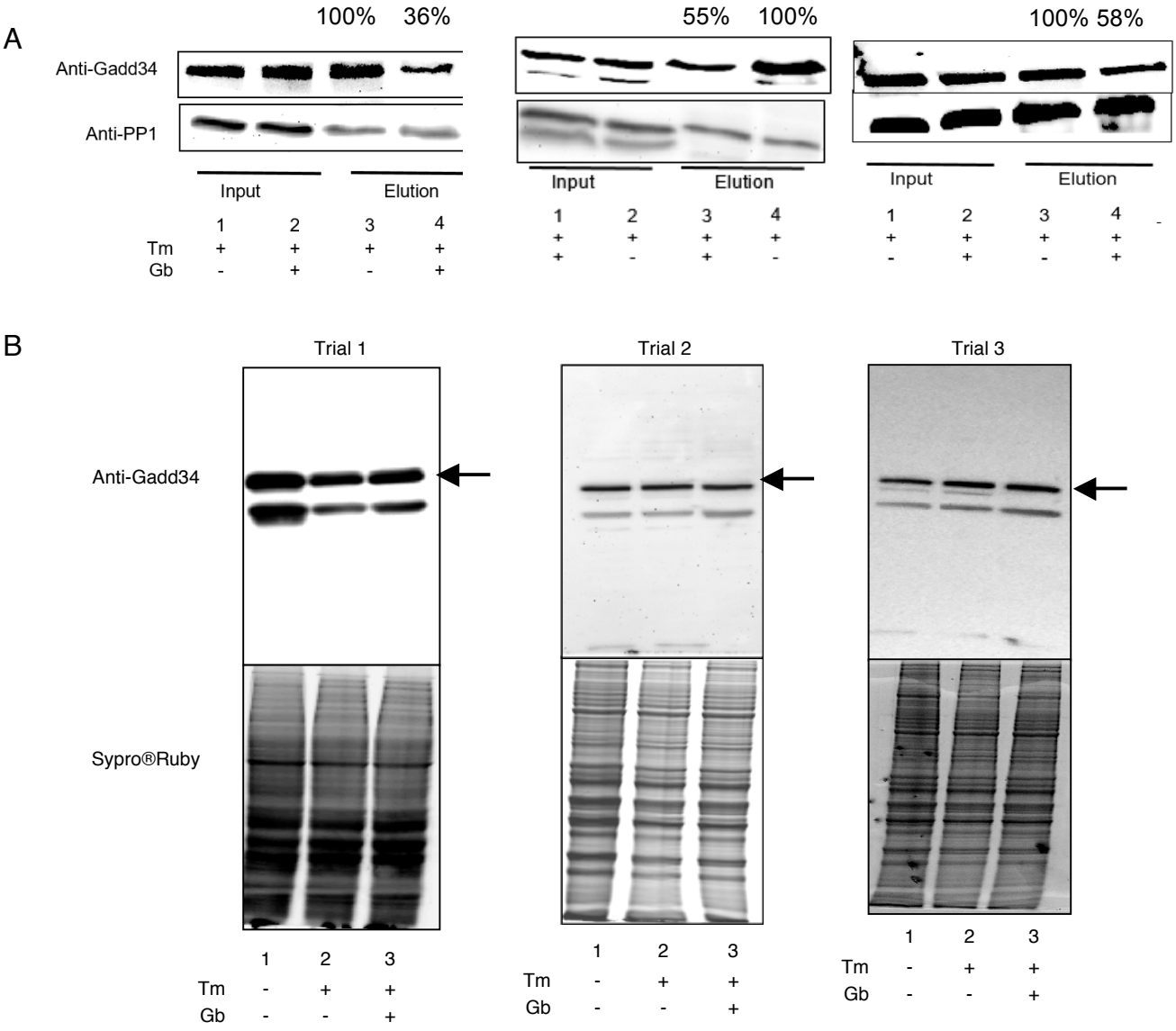

Figure S5: Control experiments with Gb-treated cells. A) Co-immunoprecipation of Gadd34-PP1 as a function of Gb. PP1 was immunoprecipitated from lysates treated with Tm alone or treated with both Tm and Gb. The input and the elution from the immunoprecipitation were separated on a 10% SDS-PAGE gel and levels of PP1 and Gadd34 were assessed by Western blot. Three independent trials are shown with the relative intensity of the Gadd34-reactive band in the elution indicated above each lane. B) Gadd34 expression as a function of Gb. Gadd34 expression was probed by Western blot (top gel) in untreated cells (lane 1), cells treated with only Tm (lane 2), or cells treated with Tm and Gb (lane 3). All samples showed relatively equal expression of Gadd34 (top gel- arrow indicates Gadd34 band). Sypro®Ruby staining (bottom gel) of the lysates showed equal protein loading. Three independent trials are shown.

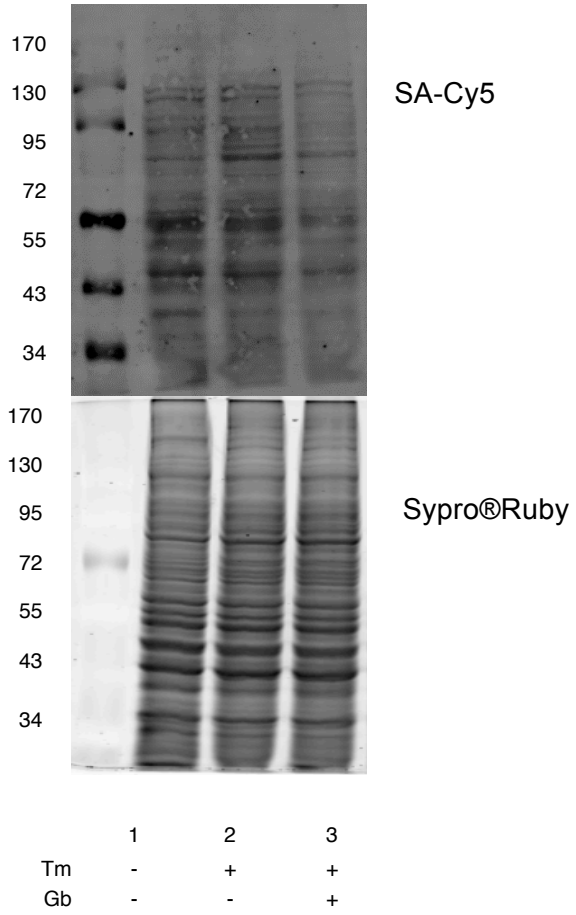

Figure S6. Full gel image from biotinylation reactions after Gb treatment. Biotinylation was carried out with HeLa lysates treated with Tm in the presence or absence of Gb. Biotinylated proteins were separated by SDS-PAGE and visualized by Streptavdin-Cy5 (top) and SYPRO® Ruby stain (bottom). Control reactions were carried out with untreated HeLa lysates (lane 1) and HeLa lysates treated only with Tm (lane 2). A cropped image of Figure S6 is shown in Figure 3A in the manuscript.

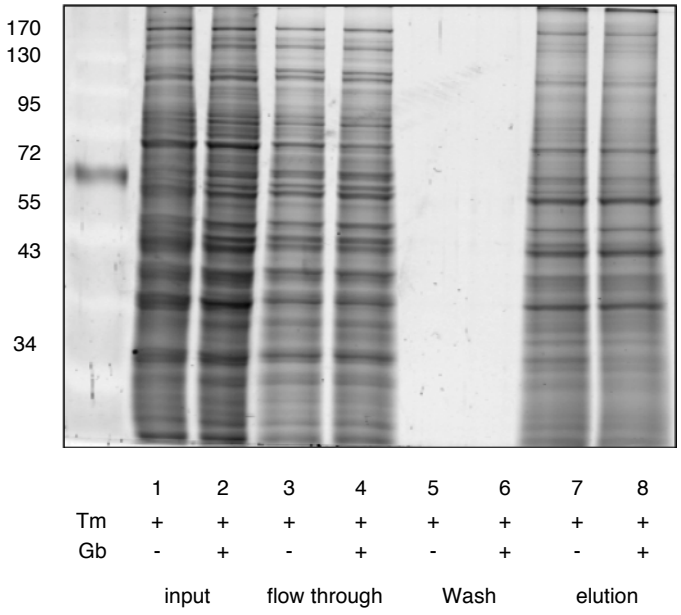

Figure S7: K-BIPS with Gb treatment. Biotinylation was carried out with lysates treated with Tm and Gb (lanes 2, 4, 6, and 8) and with lysates treated only with Tm (lanes 1, 3, 5, and 7). After reaction, biotin-tagged proteins were purified with streptavidin resin and separated by SDS-PAGE. The input before streptavidin purification (lanes 1 and 2), flow through (lanes 3 and 4), and last wash (lanes 5 and 6) were also loaded on gel, along with the eluted proteins (lanes 7 and 8). Total proteins were visualized with Sypro®Ruby stain. The image is a representative of the two trials used for LC-MS/MS analysis.

Table S2: Full listing of the 130 hits from K-BIPS with Gb treatment [a]

Gene names	T1- Gb	T1 + Gb	T2 -Gb	T2 +Gb	T1 ratio	T2 ratio	Associated with
RPS12	5.5E+07	0.0E+00	6.5E+06	0.0E+00	∞	∞	Ribosome
FHOD1	5.5E+06	0.0E+00	1.7E+07	0.0E+00	∞	∞	
ARF1	2.1E+07	0.0E+00	1.7E+06	0.0E+00	∞	∞	
STK3	5.6E+06	0.0E+00	1.5E+07	0.0E+00	∞	∞	
IST1	1.2E+07	0.0E+00	6.4E+06	0.0E+00	∞	∞	
SNRPF	1.9E+06	0.0E+00	6.4E+06	0.0E+00	∞	∞	
EIF4G3	4.7E+06	0.0E+00	2.5E+06	0.0E+00	∞	∞	Translation initiation
SLC9A3R1	5.5E+07	1.7E+06	3.1E+06	0.0E+00	31.3	∞	
RELA	1.1E+08	5.7E+06	4.4E+06	0.0E+00	20.0	∞	ER stress[23]
TIMM50	2.6E+07	1.8E+06	2.0E+07	0.0E+00	14.5	∞	
FAM120A	3.3E+07	2.3E+06	2.7E+07	0.0E+00	14.0	∞	
FDPS	4.8E+08	3.8E+07	2.3E+07	0.0E+00	12.8	∞	
PTMA	1.6E+07	1.7E+06	4.7E+06	0.0E+00	9.0	∞	
GPI	3.2E+07	3.9E+06	4.2E+06	0.0E+00	8.1	∞	
PRDX4	5.7E+07	1.0E+07	1.3E+07	0.0E+00	5.7	∞	Oxidative stress
AIMP2	1.9E+07	0.0E+00	8.4E+07	1.5E+07	∞	5.6	
CTBP2	3.0E+07	6.2E+06	4.8E+06	0.0E+00	4.9	∞	
FMR1	1.7E+07	3.6E+06	6.9E+06	0.0E+00	4.7	∞	Stress granules[24]
TIAL1	1.9E+07	4.7E+06	1.0E+07	0.0E+00	4.1	∞	Stress granules[24]
HMGB1P1	1.4E+06	4.4E+05	4.6E+06	0.0E+00	3.1	∞	
MAPK1	2.0E+08	6.9E+07	1.1E+07	0.0E+00	2.9	∞	ER stress
ITPR1	6.2E+06	0.0E+00	3.9E+06	1.5E+06	∞	2.5	ER stress
PPP2R4	1.0E+08	0.0E+00	8.3E+06	3.3E+06	∞	2.5	
STARD7	1.7E+07	0.0E+00	7.4E+06	3.1E+06	∞	2.4	
VPS26B	1.1E+07	0.0E+00	1.2E+07	5.1E+06	∞	2.4	
CAPN2	1.6E+06	0.0E+00	2.6E+08	1.1E+08	∞	2.3	ER stress
VPS13A	3.5E+05	0.0E+00	9.9E+06	4.4E+06	∞	2.3	
SNX2	3.0E+07	0.0E+00	1.5E+07	6.5E+06	∞	2.2	
NARS	7.4E+05	0.0E+00	2.0E+07	9.2E+06	∞	2.2	
ERLIN1	8.3E+06	0.0E+00	6.2E+06	2.9E+06	∞	2.1	ER stress
CKAP4	2.4E+06	0.0E+00	9.2E+07	4.4E+07	∞	2.1	
PUF60	4.8E+07	0.0E+00	4.0E+07	2.0E+07	∞	2.0	
TNPO2	1.5E+07	0.0E+00	3.9E+06	2.0E+06	∞	2.0	
CAPRIN1	1.3E+08	0.0E+00	1.7E+08	8.7E+07	∞	2.0	Stress granules[25]
MYO18A	2.2E+06	1.1E+06	1.9E+07	0.0E+00	2.0	∞	
IGF2BP3	1.1E+07	5.7E+06	2.2E+06	0.0E+00	1.9	∞	Stress granules[26]

Gene names	T1- Gb	T1 + Gb	T2 -Gb	T2 +Gb	T1 ratio	T2 ratio	Associated with
SYNCRIP	9.4E+06	0.0E+00	2.5E+08	1.4E+08	∞	1.8	
DAP3	7.2E+06	0.0E+00	1.0E+07	5.9E+06	∞	1.7	
PITPNA	5.5E+05	0.0E+00	2.0E+08	1.2E+08	∞	1.7	
DNAJB11	4.8E+07	0.0E+00	1.5E+07	8.9E+06	∞	1.7	UPR[27]
TIMM44	3.3E+07	1.9E+07	1.7E+06	0.0E+00	1.7	∞	
RRM2	7.3E+07	0.0E+00	1.1E+08	6.6E+07	∞	1.6	
ATP6V0D1	3.4E+07	0.0E+00	1.1E+08	7.0E+07	∞	1.5	UPR
THUMPD1	1.3E+06	0.0E+00	4.6E+06	3.0E+06	∞	1.5	
CSNK2A3	1.5E+07	0.0E+00	1.3E+07	9.0E+06	∞	1.5	UPR
RNF213	2.1E+05	0.0E+00	6.0E+07	4.3E+07	∞	1.4	Protein ubiquitination
MRPS9	5.6E+08	0.0E+00	1.3E+07	9.5E+06	∞	1.4	
ANP32E	1.1E+07	0.0E+00	6.7E+07	4.9E+07	∞	1.4	
WDR5	5.9E+07	0.0E+00	2.2E+07	1.6E+07	∞	1.4	UPR
PSMC3	7.8E+07	6.2E+07	1.3E+07	0.0E+00	1.3	∞	ER stress
KHDRBS1	5.4E+07	0.0E+00	3.5E+07	2.7E+07	∞	1.3	
SMG9	5.9E+06	0.0E+00	7.5E+06	5.8E+06	∞	1.3	
NUP37	5.8E+07	0.0E+00	2.7E+07	2.1E+07	∞	1.3	
COPS5	9.0E+07	0.0E+00	1.4E+08	1.1E+08	∞	1.3	UPR
STRAP	5.7E+08	9.6E+06	1.9E+08	6.7E+07	59.1	2.8	
GOT2	3.0E+08	6.2E+06	1.4E+08	1.0E+08	48.4	1.4	
ARHGDIA	8.2E+07	2.3E+06	4.6E+06	3.6E+06	35.2	1.3	
GAPVD1	4.8E+07	1.4E+06	1.2E+07	9.0E+06	34.7	1.3	
EEF1D	1.4E+09	4.1E+07	6.3E+08	3.8E+08	33.9	1.7	
DYNC1LI2	2.6E+07	7.7E+05	1.4E+07	1.1E+07	34.1	1.3	
G3BP1	1.6E+08	6.3E+06	1.6E+08	1.1E+08	25.7	1.5	Stress granules[25]
ACOT7	9.0E+08	4.3E+07	4.9E+07	2.6E+07	20.8	1.9	
UBE2Z	5.0E+08	2.7E+07	1.6E+07	5.1E+06	18.4	3.0	Protein ubiquitination
PARVA	1.5E+08	8.4E+06	2.6E+08	1.1E+08	17.4	2.3	
PSAT1	6.0E+08	3.5E+07	9.3E+07	5.9E+07	17.3	1.6	
RPL10A	6.9E+07	4.2E+06	1.5E+08	1.1E+08	16.5	1.5	Ribosome
PPA1	3.7E+08	2.4E+07	6.6E+07	4.2E+07	15.2	1.6	
PDHB	2.4E+08	1.6E+07	6.7E+07	3.6E+07	14.7	1.8	
RBBP7	9.8E+07	8.7E+06	6.3E+07	2.6E+07	11.3	2.5	
FXR1	1.0E+07	1.1E+06	1.1E+07	4.3E+06	9.8	2.6	Stress granules[24]
ALDOC	4.7E+08	4.9E+07	2.3E+07	8.4E+06	9.4	2.7	
ST13	9.5E+07	1.1E+07	5.6E+07	1.8E+07	8.6	3.1	
NACA	3.2E+08	3.2E+07	1.0E+09	7.8E+08	10.1	1.3	
CAPG	5.4E+08	1.5E+08	4.8E+06	6.4E+05	3.7	7.5	
ACTR2	2.7E+08	2.9E+07	3.6E+07	2.4E+07	9.6	1.5	
PLXNB2	5.4E+07	5.6E+06	2.2E+07	1.8E+07	9.6	1.3	

Gene names	T1- Gb	T1 + Gb	T2 -Gb	T2 +Gb	T1 ratio	T2 ratio	Associated with
NAP1L4	2.2E+07	3.1E+06	1.1E+07	2.8E+06	6.9	3.8	
PICALM	5.3E+08	1.4E+08	1.5E+07	2.2E+06	3.9	6.8	
TALDO1	5.3E+08	5.8E+07	1.8E+08	1.2E+08	9.2	1.5	
GNB2	8.4E+07	9.2E+06	1.5E+08	1.2E+08	9.1	1.3	
VPS26A	3.6E+08	4.1E+07	2.1E+08	1.3E+08	8.7	1.6	
SH3GL1	4.5E+07	5.5E+06	1.6E+07	8.6E+06	8.1	1.8	
ALDOA	5.5E+09	1.2E+09	1.9E+08	4.3E+07	4.5	4.5	
VIM	6.2E+08	8.9E+07	5.2E+09	2.6E+09	7.0	2.0	
SEH1L	5.9E+07	8.6E+06	1.9E+07	9.1E+06	6.8	2.1	
PTPN12	1.9E+07	2.8E+06	1.2E+07	5.7E+06	6.6	2.1	
TWF1	2.3E+08	3.2E+07	8.6E+07	6.5E+07	7.1	1.3	
NDRG1	8.6E+08	1.3E+08	5.4E+07	3.2E+07	6.6	1.7	
HNRNPAB	8.7E+06	1.3E+06	5.4E+07	4.1E+07	6.6	1.3	
EIF3M	7.6E+08	1.4E+08	2.0E+07	8.3E+06	5.4	2.5	Translation initiation
HSD17B4	6.4E+08	1.1E+08	1.4E+08	6.5E+07	5.6	2.1	
TUBA4A	1.4E+08	3.9E+07	2.0E+07	4.8E+06	3.6	4.1	
NDC1	5.4E+06	8.9E+05	4.5E+06	3.1E+06	6.1	1.5	
HM13	1.9E+07	3.3E+06	5.9E+06	3.9E+06	5.8	1.5	ER stress
PRKAR2A	5.1E+07	8.4E+06	3.4E+07	2.7E+07	6.0	1.3	
PSMD13	4.2E+08	9.1E+07	4.5E+06	1.7E+06	4.6	2.6	
RAN	1.2E+09	6.8E+08	3.7E+07	7.2E+06	1.8	5.1	
MAP2K3	7.4E+07	1.6E+07	3.8E+07	2.7E+07	4.7	1.4	
SEPHS1	6.5E+07	2.4E+07	1.4E+07	4.3E+06	2.7	3.3	
NAP1L1	1.6E+08	3.6E+07	1.7E+08	1.1E+08	4.6	1.5	
VAT1	8.5E+07	2.2E+07	2.0E+08	9.2E+07	3.8	2.2	
GALE	1.8E+08	4.1E+07	3.6E+07	2.4E+07	4.5	1.5	
CALU	2.2E+07	7.0E+06	3.9E+08	1.3E+08	3.1	2.9	
ANXA2	1.4E+10	3.0E+09	6.4E+09	5.0E+09	4.5	1.3	
RPL26L1	1.5E+07	3.8E+06	1.3E+07	8.0E+06	4.0	1.6	Ribosome
SMARCC1	2.5E+07	1.3E+07	6.5E+06	2.0E+06	1.9	3.3	
ENO1	9.2E+08	3.0E+08	3.7E+08	1.7E+08	3.0	2.2	
GMPS	1.2E+08	3.2E+07	1.9E+08	1.2E+08	3.6	1.6	
DNAJA1	1.3E+08	3.7E+07	4.9E+07	2.8E+07	3.4	1.7	ER stress[28]
SFN	1.3E+08	3.8E+07	1.4E+07	8.5E+06	3.5	1.6	
PSMC2	1.3E+08	5.9E+07	6.5E+07	2.3E+07	2.3	2.8	ER stress
EIF3G	5.6E+07	1.9E+07	5.8E+07	3.1E+07	3.0	1.8	Translation initiation
RPLP0	3.9E+09	1.1E+09	7.8E+09	5.7E+09	3.4	1.4	Ribosome
PPP2R1B	1.7E+07	6.8E+06	6.5E+06	2.9E+06	2.5	2.2	
MDH1	2.1E+08	7.0E+07	3.7E+08	2.1E+08	3.0	1.7	
EIF2S1	1.2E+09	5.1E+08	5.4E+08	2.3E+08	2.4	2.3	UPR
UBE3A	2.9E+06	8.7E+05	2.3E+07	1.7E+07	3.3	1.3	Protein ubiquitination

Gene names	T1- Gb	T1 + Gb	T2 -Gb	T2 +Gb	T1 ratio	T2 ratio	Associated with
UBQLN2	2.0E+06	1.6E+06	1.1E+07	3.3E+06	1.3	3.3	ER stress[29]
BZW1	6.8E+07	2.0E+07	3.3E+07	2.6E+07	3.3	1.3	
SFPQ	6.0E+08	1.8E+08	4.0E+07	3.1E+07	3.3	1.3	
PRKAR1A	2.4E+07	7.4E+06	5.1E+07	3.9E+07	3.2	1.3	
PGAM1	6.0E+07	3.2E+07	8.9E+06	3.6E+06	1.9	2.5	Oxidative stress
PDIA6	2.0E+08	1.1E+08	2.8E+08	1.1E+08	1.8	2.5	ER stress[30]
SF3A1	2.3E+07	1.1E+07	2.2E+07	9.7E+06	2.1	2.2	
NUDC	2.7E+08	1.1E+08	2.5E+07	1.5E+07	2.5	1.7	
SEPT7	9.4E+05	4.4E+05	1.5E+08	7.2E+07	2.1	2.0	
LAP3	2.6E+07	1.1E+07	3.3E+07	1.9E+07	2.3	1.7	
CFL1	1.9E+08	9.7E+07	3.3E+07	1.6E+07	2.0	2.0	
SUCLG2	4.1E+06	2.0E+06	1.4E+07	7.3E+06	2.1	1.9	
FKBP10	2.7E+07	1.0E+07	3.2E+07	2.4E+07	2.6	1.3	ER stress[31]

[a] Protein hits observed in the K-BIPS study with PP1-Gadd34 inactivation. The peptide intensity observed for each sample (-Gb: Without guanabenz, +Gb: With guanabenz) for the two trials (T1: trial 1, T2: trial 2) is shown. Fold change for each trial was calculated by dividing the peptide intensity observed in the guanabenz untreated sample (PP1-Gadd34 active) sample by the peptide intensity observed for the guanabenz treated sample (PP1-Gadd34 inactive). Infinity (∞) signifies that no peptides were observed in the guanabenz treated sample, making a numeric ratio calculation impossible. EIF2S1 (eIF2α), the known substrate of PP1-Gadd34 is green colored. Except when cited, information on functional association was obtained from Uniprot. Proteins are ordered according to the fold change.

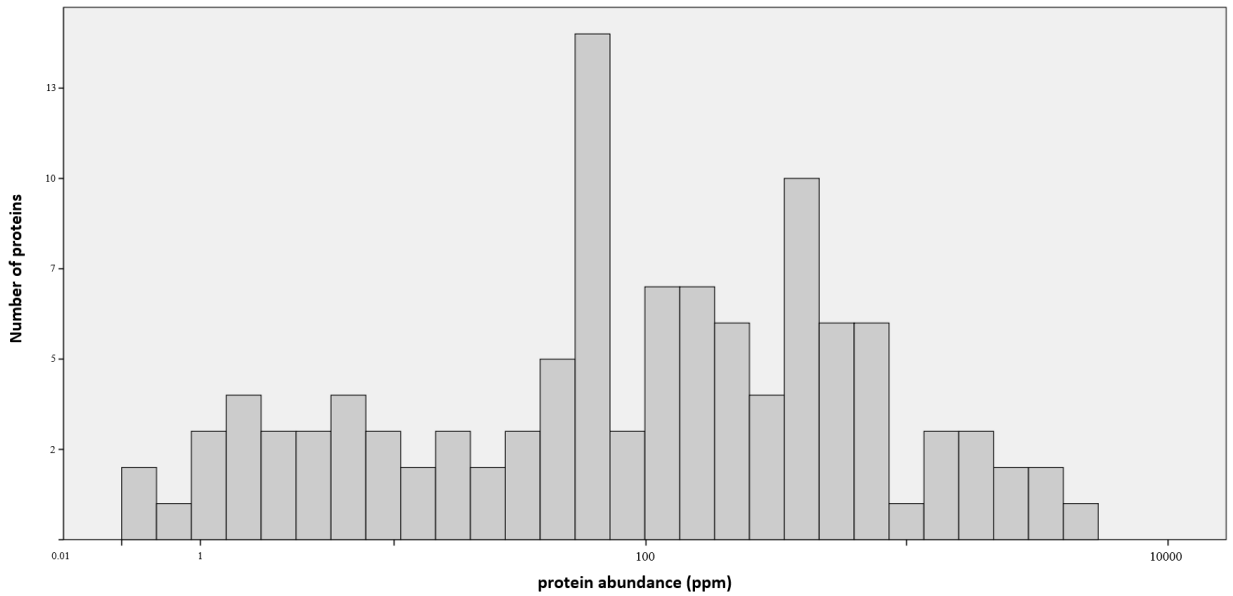

Figure S8: The abundance of proteins identified from the K-BIPS study with Gb treatment. Abundance values were taken from Pax database[22] available from previously published reports[21] and plotted. K-BIPS with Gb identified proteins with a range of abundance of 0.02 to 5,188. The reported range of abundance in HeLa cells is from 0.01 to 10,000.

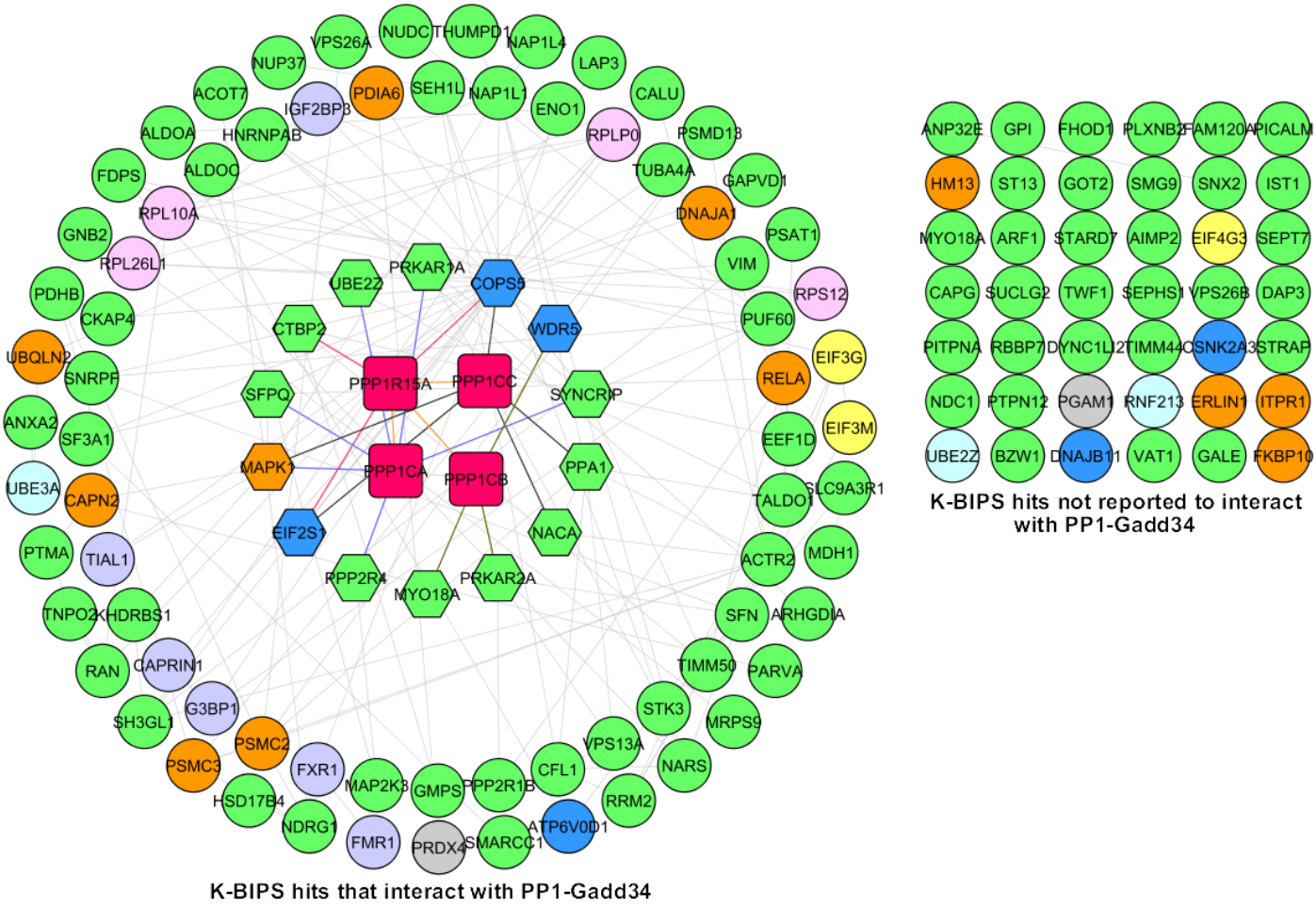

Figure S9: Enlarged Cytoscape map. Known physical protein-protein interactions among the 130 K-BIPS hits and PP1-Gadd34 were mapped using GeneMANIA in Cytoscape. The inner circles of hexagons include known direct interacting proteins of PP1 catalytic subunits (PPP1CA, PPP1CB, PPP1CC, red rectangles) or Gadd34 (PPP1R15A, red rectangle). The red star indicates the known PP1-Gadd34 substrate, EIF2S1 (eIF2α). The outer circles represent indirect interacting proteins of PP1-Gadd34 Protein colors indicate the biological function of the proteins: yellow - translation initiation; pink – ribosome; dark blue – UPR; orange - ER stress; light blue – protein ubiquitination; purple - stress granules; grey - oxidative stress. Except when cited (Table S2), the functional information was obtained from Uniprot database. Grey lines indicate interactions among the enriched proteins. The other line colors indicate the proteins that directly interact with PPP1CA (blue lines), PPP1CB (green lines), PPP1CC (black lines) or Gadd34 (PPP1R15A, red lines). Orange lines indicate the interaction of Gadd34 with PPP1CA, PPP1CB and PPP1CC. Line thickness and length were adjusted arbitrary to improve clarity. The K-BIPS hits not previously known to interact with PP1-Gadd34 are also shown in the top right corner.

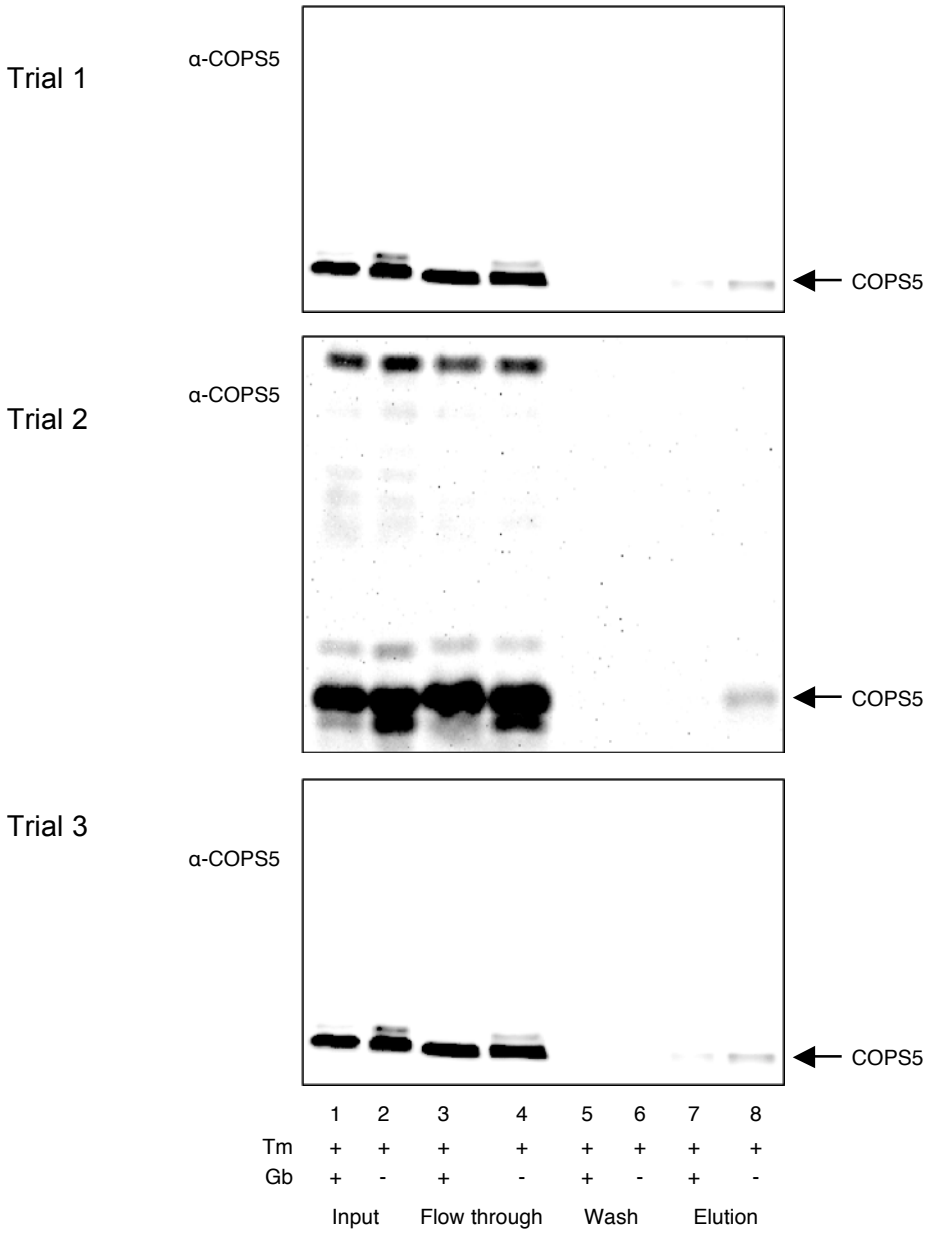

Figure S10: Validation of COPS5 using Gb treatment and ATP-biotin labeling. Biotinylation was carried out with HeLa lysates treated with Tm and Gb, or Tm alone. Biotinylated proteins were then enriched with streptavidin resin and the input, flow through, wash, and elution were separated by SDS-PAGE and visualized by antibodies specific to COPS5 by Western blot. The arrows indicate the band corresponding to COPS5. Three independent trials are shown. Trial 1 is shown in Figure 4A of the manuscript.

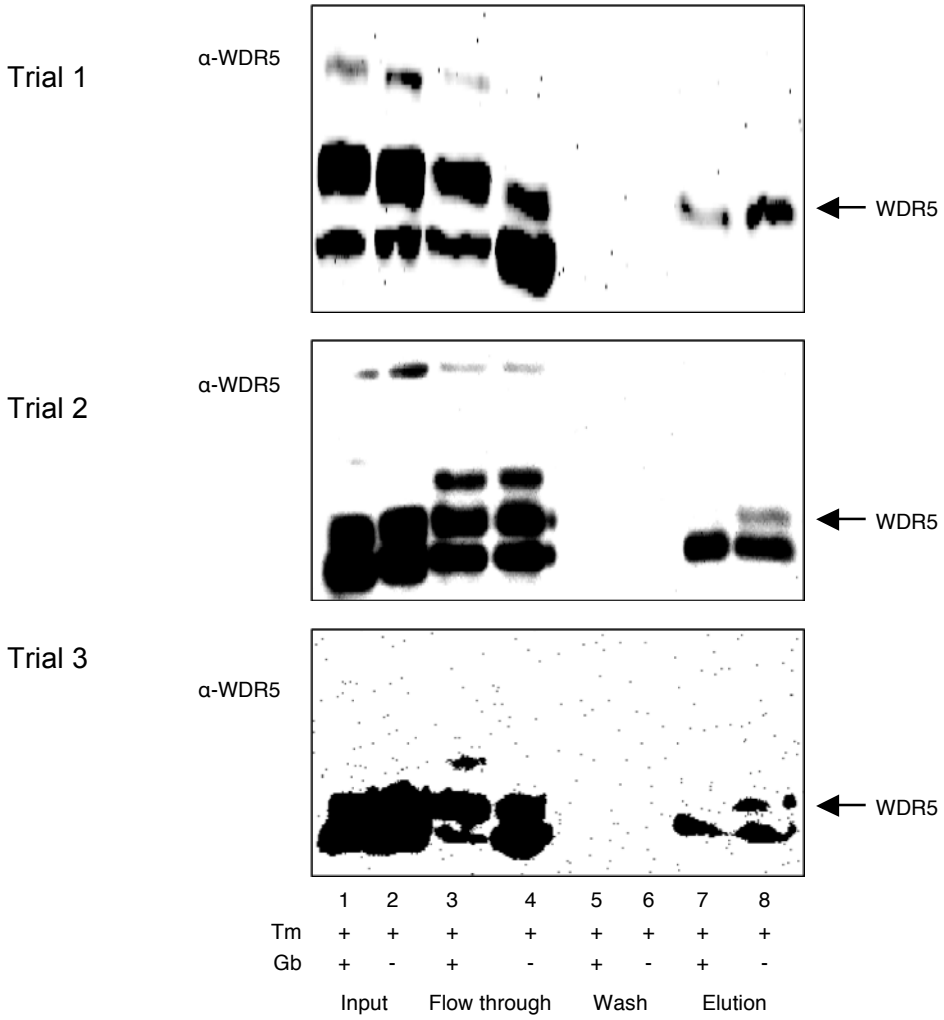

Figure S11: Validation of WDR5 using Gb treatment and ATP-biotin labeling. Biotinylation was carried out with HeLa lysates treated with Tm and Gb, or Tm alone. Biotinylated proteins were then enriched with streptavidin resin and the input, flow through, wash, and elution were separated by SDS-PAGE and visualized by antibodies specific to WDR5 by Western blot. The arrows indicate the band corresponding to WDR5. Three independent trials are shown. Trial 1 is shown in Figure 4A of the manuscript.

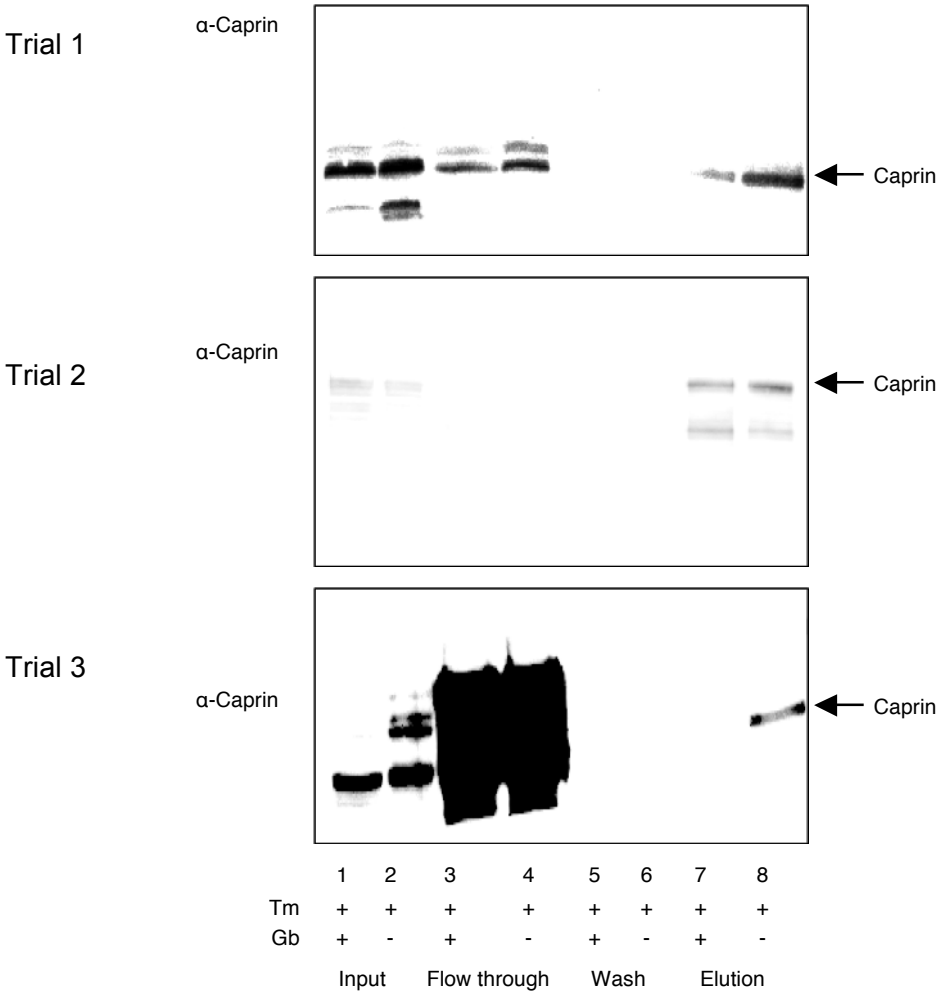

Figure S12: Validation of Caprin using Gb treatment and ATP-biotin labeling. Biotinylation was carried out with HeLa lysates treated with Tm and Gb, or Tm alone. Biotinylated proteins were then enriched with streptavidin resin and the input, flow through, wash, and elution were separated by SDS-PAGE and visualized by antibodies specific to Caprin by Western blot. The arrows indicate the band corresponding to Caprin. Three independent trials are shown. Trial 1 is shown in Figure 4A of the manuscript.

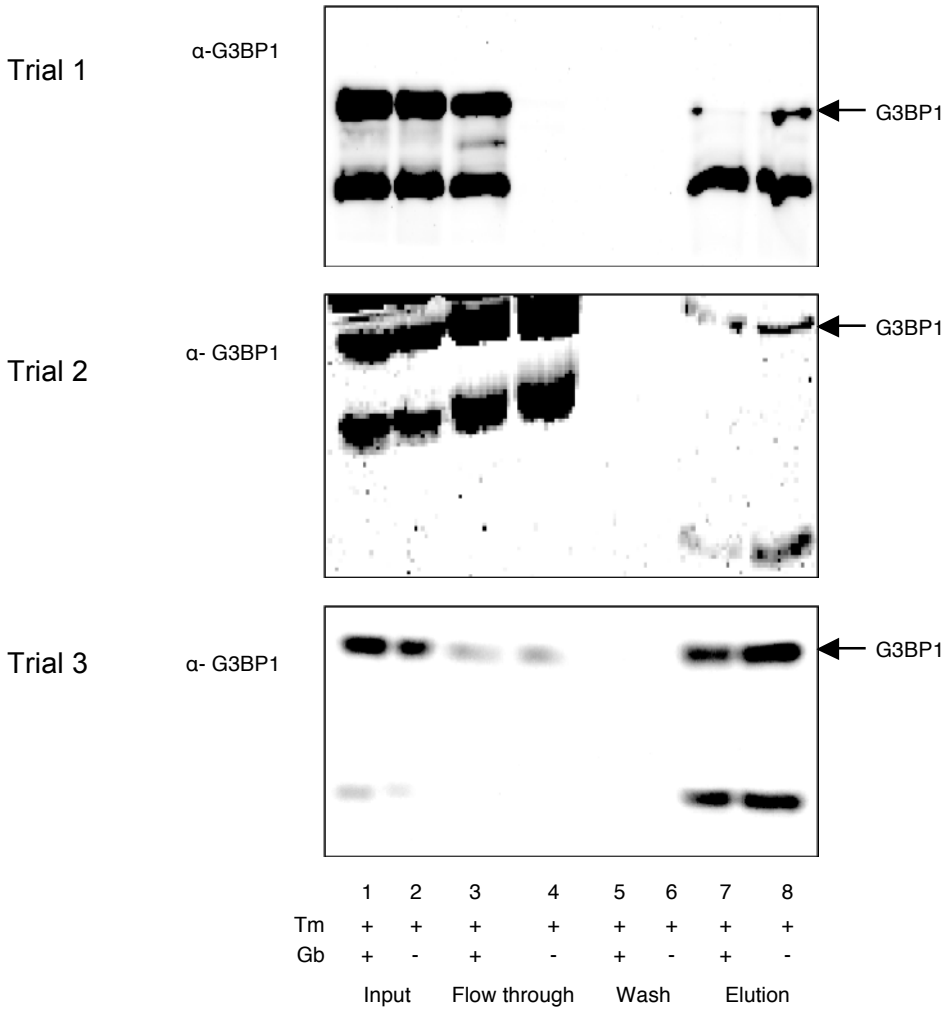

Figure S13: Validation of G3BP1 using Gb treatment and ATP-biotin labeling. Biotinylation was carried out with HeLa lysates treated with Tm and Gb, or Tm alone. Biotinylated proteins were then enriched with streptavidin resin and the input, flow through, wash, and elution were separated by SDS-PAGE and visualized by antibodies specific to G3BP1 by Western blot. The arrows indicate the band corresponding to G3BP1. Three independent trials are shown. Trial 1 is shown in Figure 4A of the manuscript.

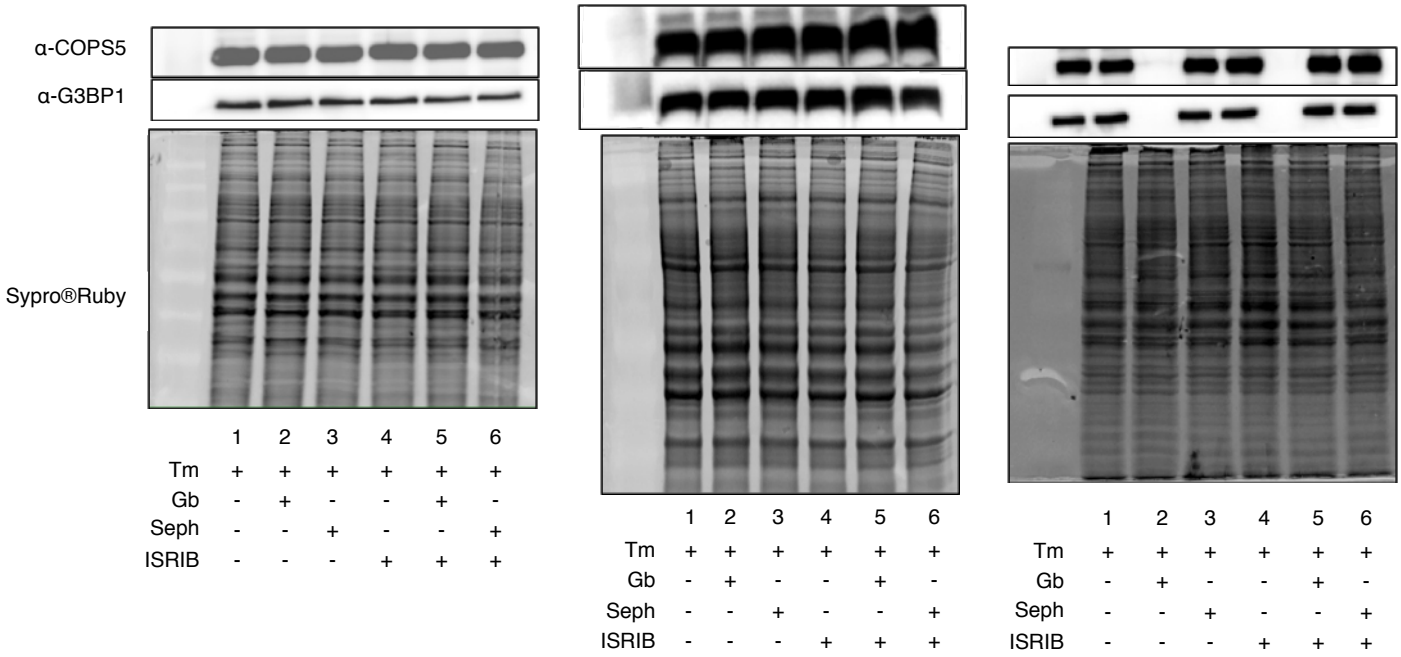

Figure S14: COPS5 and G3BP1 protein levels as a function of Guanabenz treatment. HeLa cells were treated with combination of Tunicamycin (Tm), Guanabenz (Gb), Sephin1 (Seph), and/or ISRIB at 37°C for 6 hours. The treated cells were lysed before the proteins in the lysates were separated by 10% SDS-PAGE. Levels of G3BP1 and COPS5 were probed by Western blot using primary antibodies (anti-COPS5 and anti-G3BP1) and HRP conjugated secondary antibody. Sypro®Ruby staining (bottom gel) of the lysates showed equal protein loading. Three independent trials are shown.

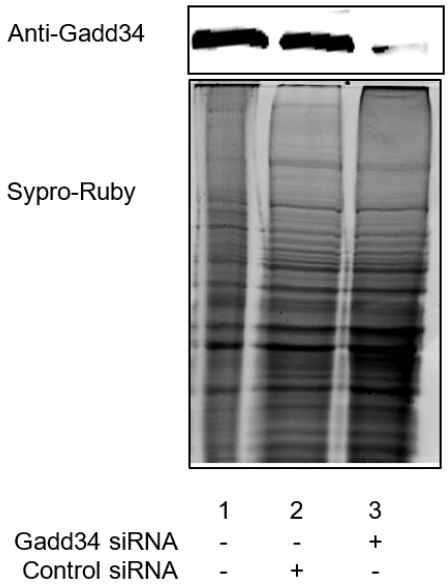

Figure S15: Gadd34 knock down in HeLa cells. HeLa cells were treated with a pool of siRNA targeting Gadd34 (lane 3) or a pool of control siRNA (lane 2), or with the transfection reagent alone (lane 1). The cells were also treated with (Tm) (all lanes). Then, cells were lysed and Gadd34 expression was monitored by Western blot (top gel image). The lower SyproRuby blot shows equal protein loading.

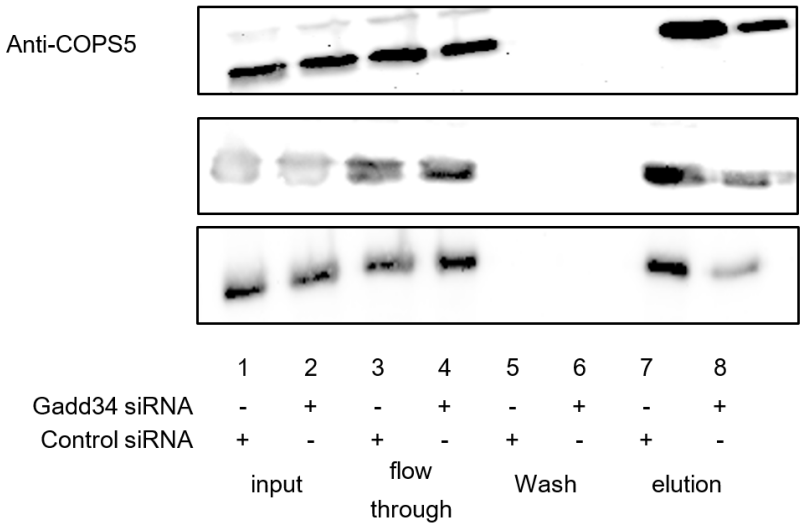

Figure S16: Validation of COPS5 using Gadd34 knockdown. Biotinylation was carried out with HeLa lysates treated with a pool of siRNA targeting Gadd34 (lanes 2, 4, 6, and 8) or a pool of control siRNA (lanes 1, 3, 5, and 7). Biotinylated proteins were enriched using streptavidin resin and the input, flow through, wash, and the elution were run on a SDS-PAGE gel. COPS5 levels were monitored by Western blot. Three trials are shown in the three gel images. The top trial is shown in Figure 4B.

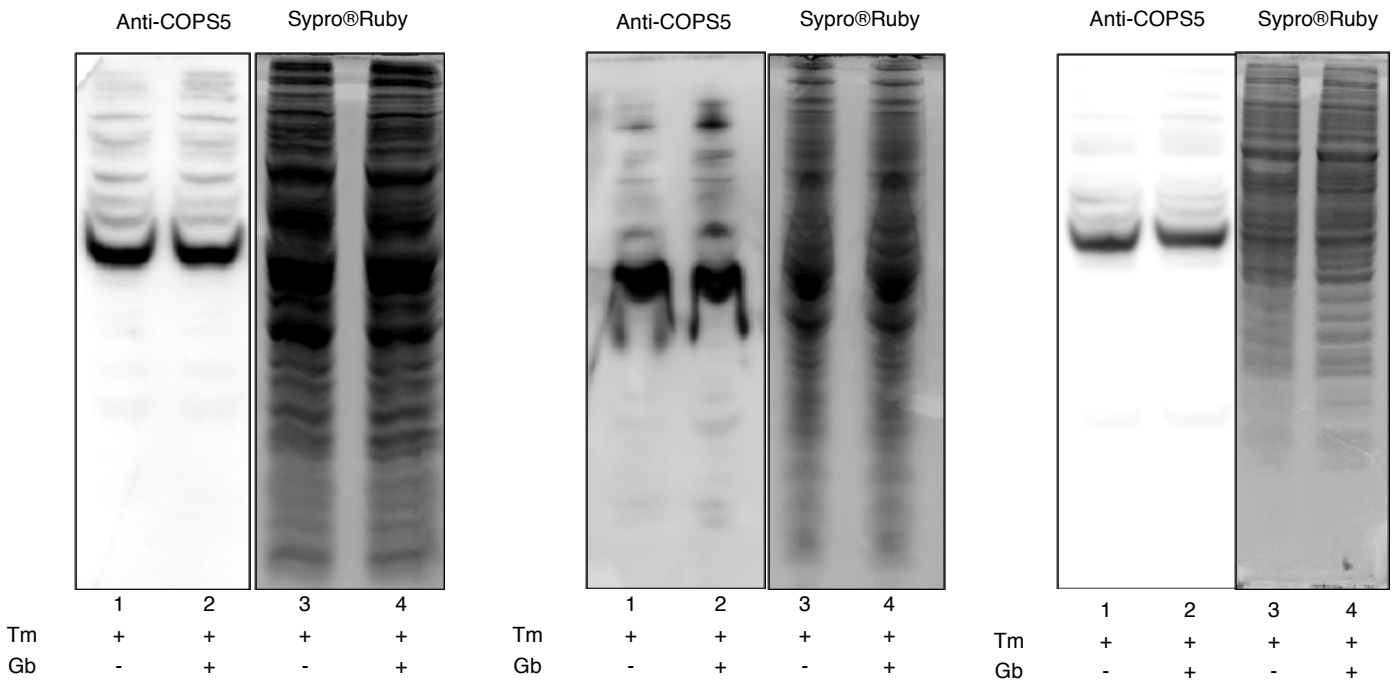

Figure S17: Validation of COPS5 using Phos-tag™ SDS-PAGE. HeLa cells were treated with Tm and Gb, or Tm alone. After lysis, proteins were separated using Phos-tag™ SDS-PAGE. COPS5 was visualized with COPS5 primary antibody and HRP-conjugated secondary antibody. Total proteins were visualized as a load control by Sypro®Ruby total protein stain. Three independent trials are shown. Trial 1 is shown in Figure 4C of the manuscript.

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