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Supplementary Materials for

CReP mediates selective translation initiation at the endoplasmic reticulum

Jonathan P. Kastan, Elena Y. Dobrikova, Jeffrey D. Bryant, Matthias Gromeier*

*Corresponding author. Email: grome001@mc.duke.edu

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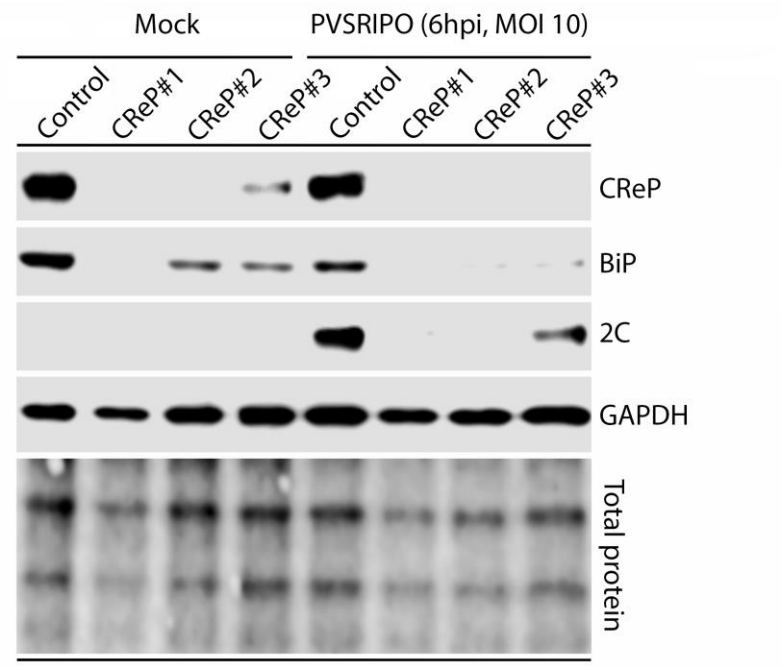
The PDF file includes:

Figs. S1 to S10

Other Supplementary Material for this manuscript includes the following:

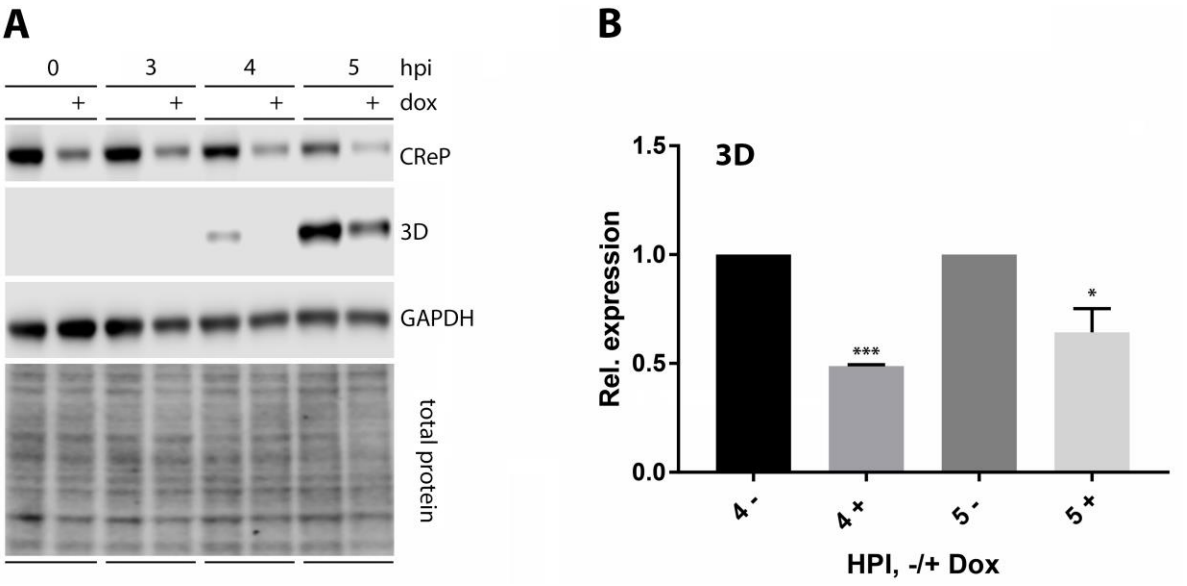
(available at advances.sciencemag.org/cgi/content/full/6/23/eaba0745/DC1)

Table S1



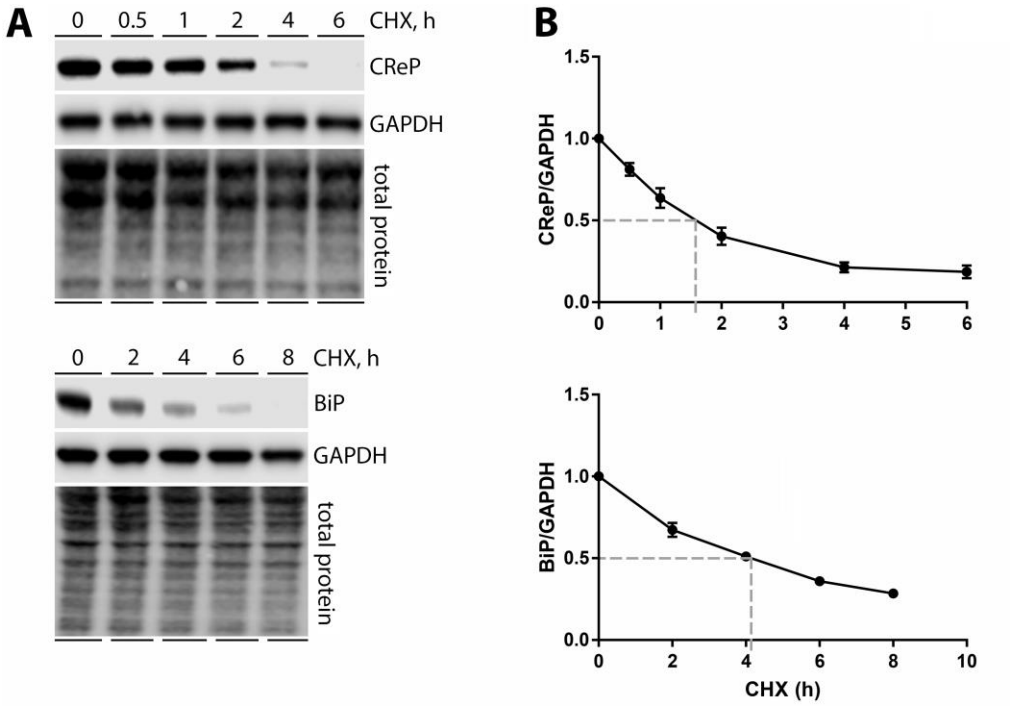
Supplemental Fig. 1 (related to Fig. 2A). siRNA-mediated CReP depletion reduces PVSRIPO translation in HeLa cells.

HeLa cells were transfected with a control siRNA or 1 of 3 siRNAs targeting CReP; 48h post transfection, the cells were infected with PVSRIPO (MOI 10) and lysed 6hpi to assess viral translation (viral protein 2C).



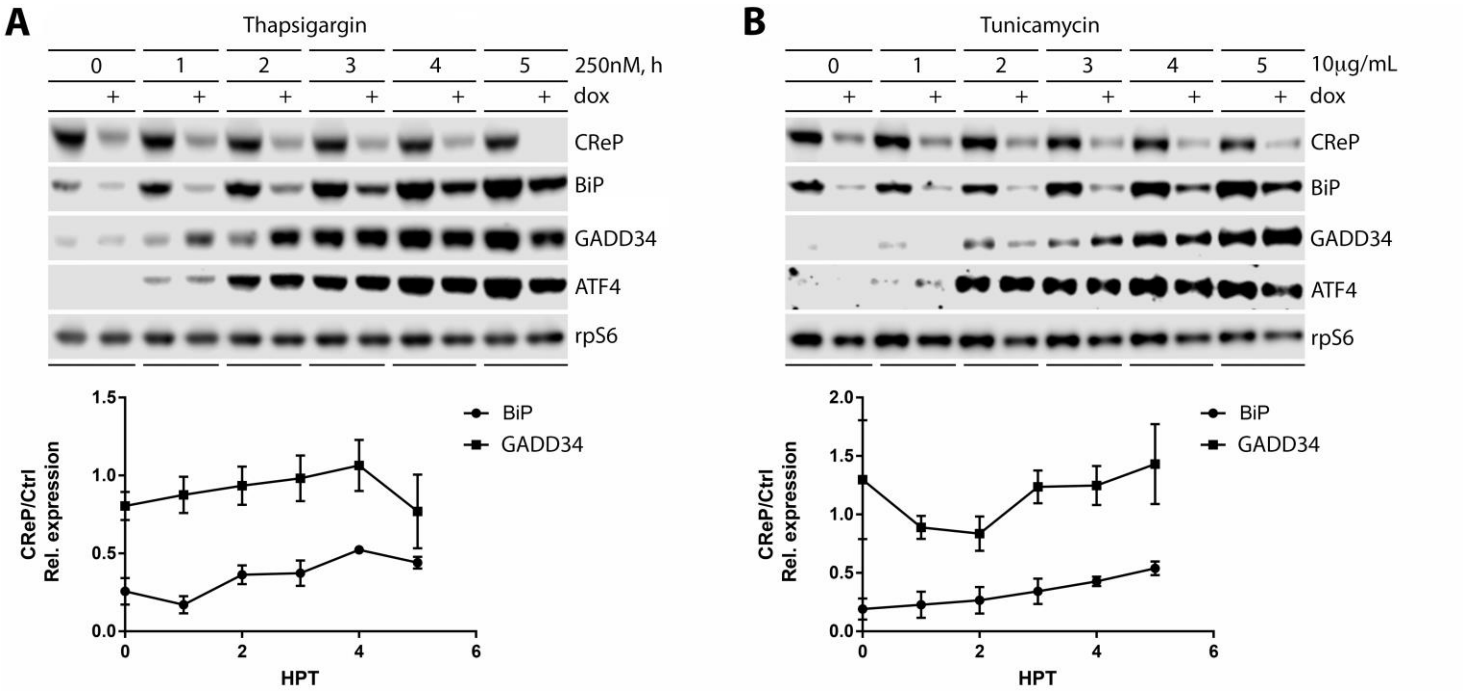
Supplemental Fig. 2 (related to Fig. 2A). Coxsackievirus B3 translation is inhibited by CReP depletion.

Cells with dox-inducible CReP depletion were mock- or dox-treated (4μg/mL; ~40h), then infected with Coxsackievirus B3 (MOI 10). Cells were harvested for quantitative analysis of the viral 3D polymerase by immunoblot at the indicated intervals. Statistics were done with a student's t-test comparison at the indicated time points, comparing -/+ dox (n=3). Bar graphs represent mean + SEM; *, **, *** corresponds to p <0.05, 0.005, 0.0005, respectively.



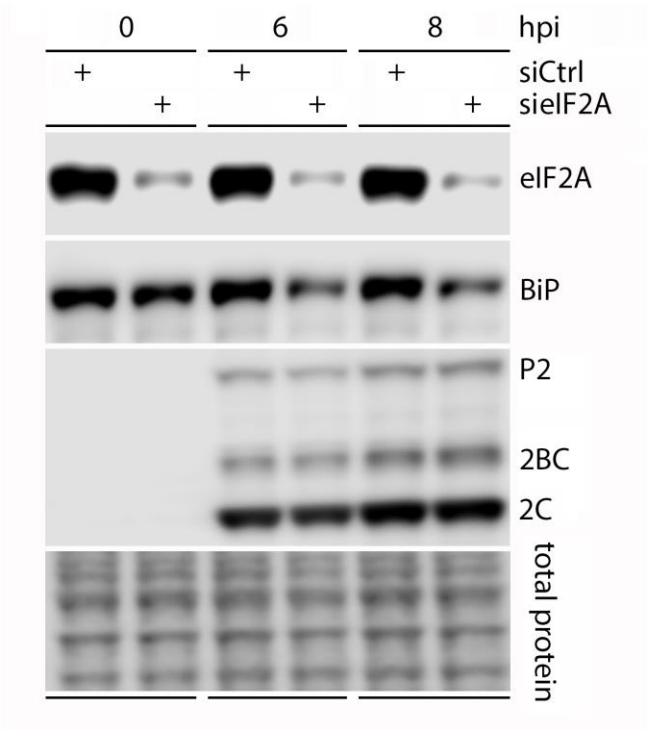
Supplemental Fig. 3 (related to Fig. 2). Half-lives of CReP and BiP determined in cycloheximide (CHX)-treated HeLa cells.

(**A**) HeLa cells were subjected to CHX (20µg/mL) as shown, lysed, and subjected to immunoblot analysis. (**B**) Expression of CReP and BiP relative to GAPDH is quantified over time (graphs represent mean -/+ SEM; n = 3).



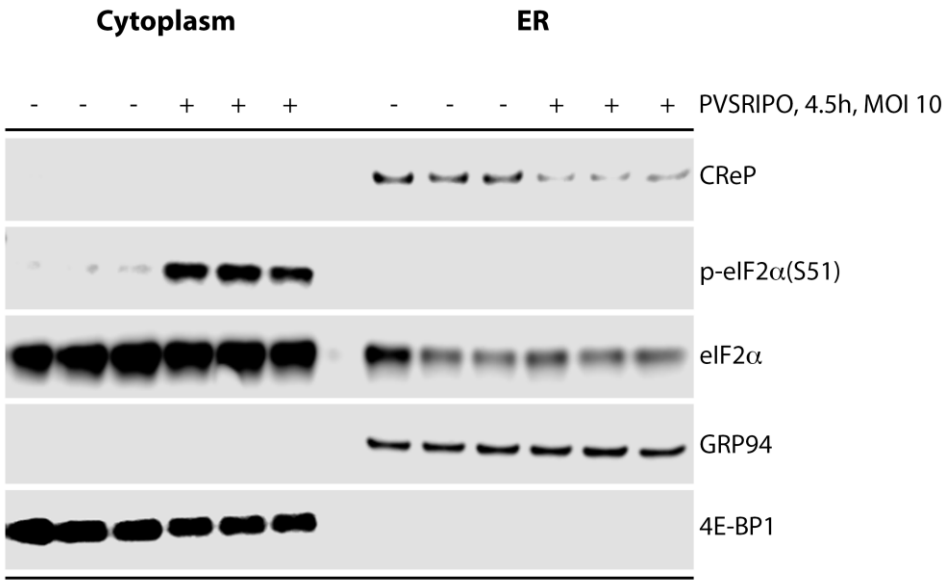
Supplemental Fig. 4 (related to Fig. 2E). CReP depletion specifically affects BiP upon ER stress.

(**A**, **B**) Dox-inducible CReP depletion cells were mock- or dox-treated (4μg/mL; ~40h), then treated with either Thapsigargin (250nM) (A) or Tunicamycin (10μg/mL) (B), and lysed at the indicated time points for immunoblot analysis and quantification (bar graphs represent mean +/- SEM; n=3).



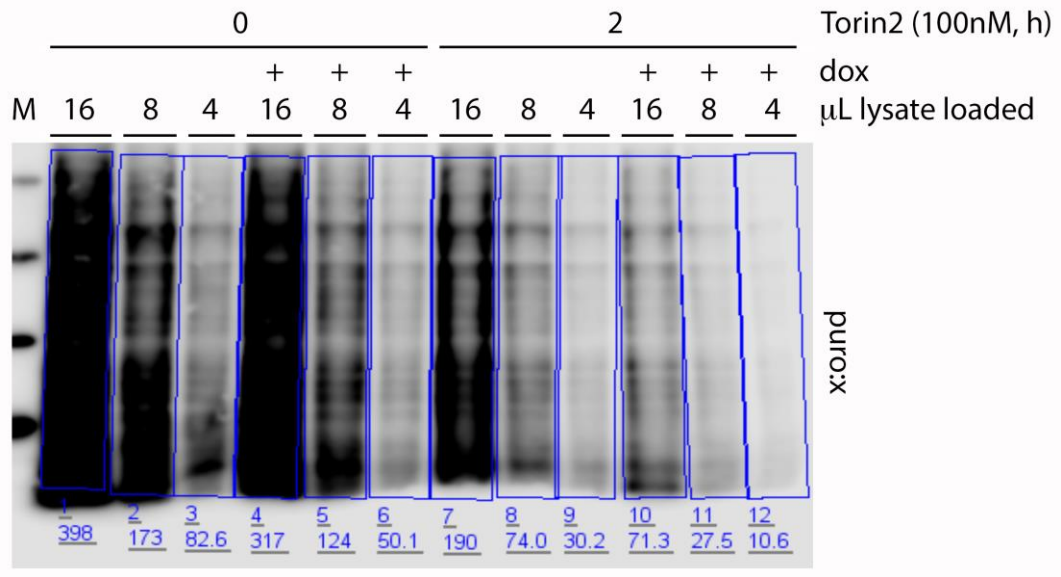
Supplemental Fig. 5 (related to Fig. 2). The effects of CReP depletion on PVSRIPO translation are eIF2A-independent.

Cells were transfected with either a control siRNA or an siRNA targeting eIF2A (48h), then infected with PVSRIPO (MOI 10), and harvested at the indicated time points for immunoblot analysis (n=2). BiP expression was modestly lower in PVSRIPO -infected cells with eIF2A depletion, consistent with a role for eIF2A in promoting BiP translation during stress.



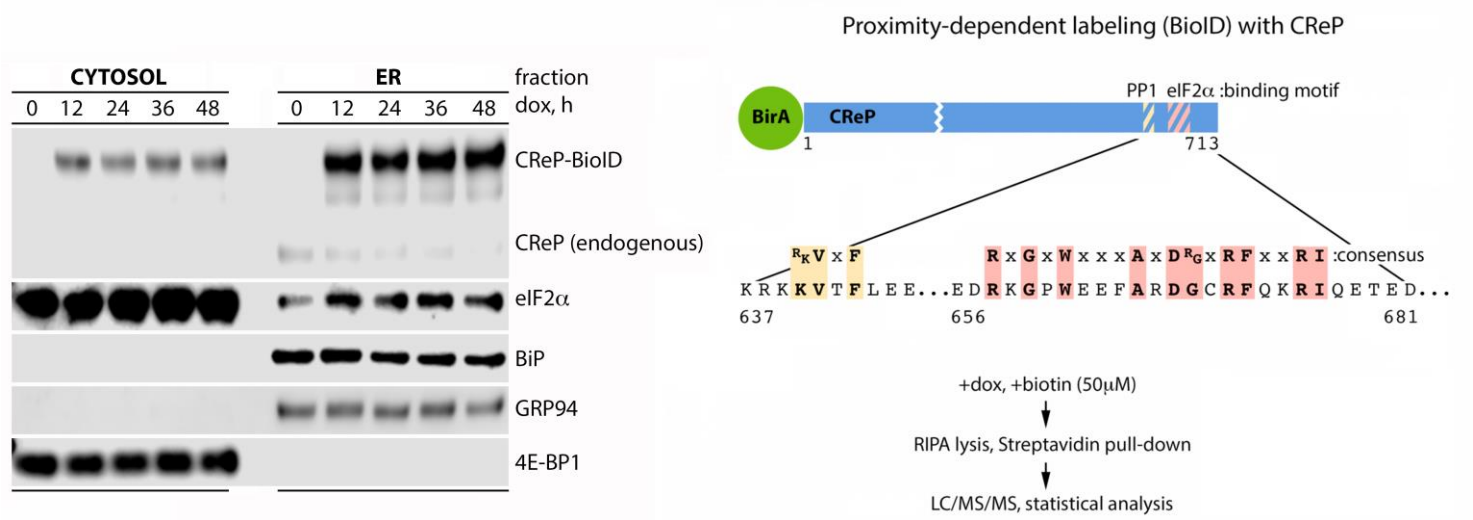
Supplemental Fig. 6 (related to Fig. 4E). The cytoplasm is the primary site of p-eIF2α(S51) induction in PVSRIPO infected cells.

Cells were fractionated/analyzed as in Fig. 4E at 4.5 hpi with PVSRIPO. At this timepoint, loss of eIF2α from the ER (Fig. 4E) had not set in. This assay demonstrates that profuse PVSRIPO-induced eIF2α(S51) phosphorylation occurs in the cytoplasm, but is restricted from the ER.



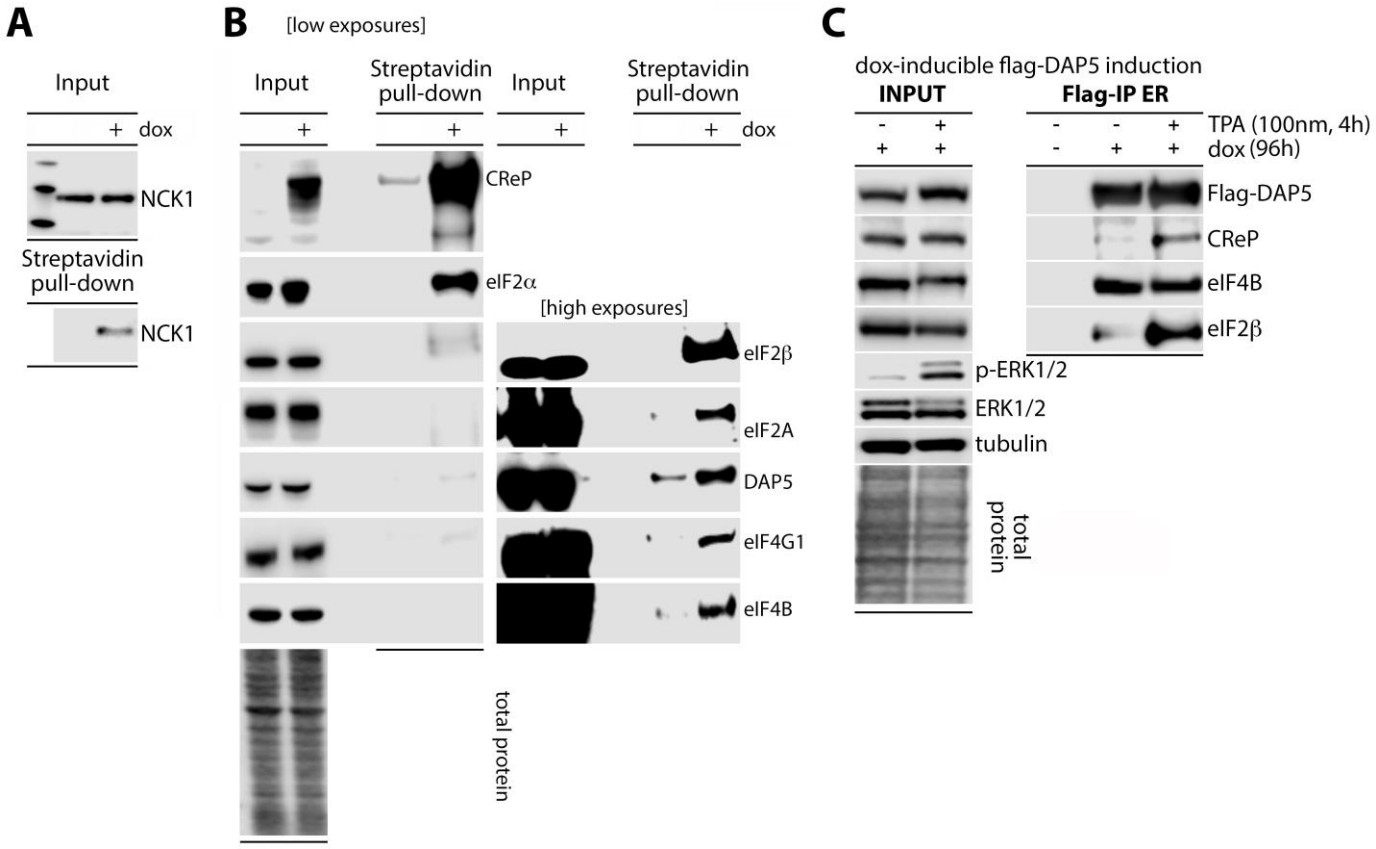
Supplemental Fig. 7 (related to Fig. 5A). The effects of Torin2 treatment on global protein synthesis in mock- and CReP-depleted cells are not due to effects of linear range.

Lysates from the experiment shown in Figure 5A, from cells treated -/+ dox, and -/+ Torin2 (2h; 100nM), were run at different volumes (16, 8, 4µL) for Li-COR Odyssey quantification (represented below the gel in blue letters) across 3 different sets of signal strength.



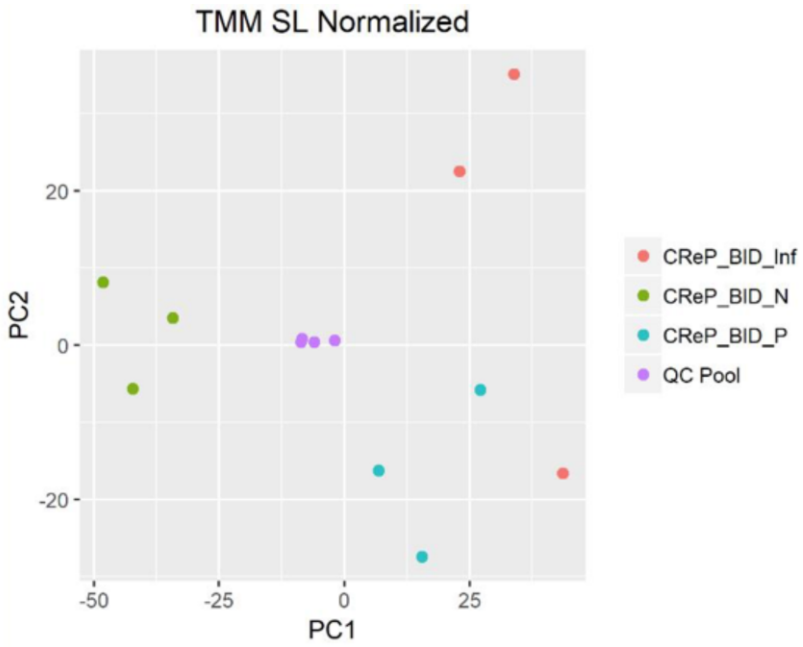
Supplemental Fig. 8 (related to Fig. 6). Proximity-dependent labeling of the CReP 'interactome'.

(**Left Panel**) Cells with dox-inducible CReP-BirA expression were treated with dox for the indicated intervals, fractionated as described for Figure 4A-C and analyzed by immunoblot with the indicated antibodies. GRP94 and 4EBP-1 were used as ER and cytosolic markers, respectively. (**Right Panel**) Proximity-dependent labeling of the CReP 'interactome'. (Top) Diagram of the BirA-CReP fusion construct with the BirA moiety fused to the CReP N-terminus. The sequence and location of the PP1 (yellow) and eIF2α binding domains (pink) are shown in detail; both domains are >aa600 distant from the BirA moiety. The BirA-CReP construct was inserted into the HeLa cell line with dox-inducible depletion of endogenous CReP. The cells were treated with dox and biotin (24h), left uninfected or infected by PVSRIPO (MOI 10; 6h), lysed, and subjected to Streptavidin bead pull-down and submitted for quantitative LC/MS/MS and statistical analysis (Table S1). A set of 372 proteins were significantly enriched in either the (-dox) negative control or the +dox sample. Fifty-three of these hits were significantly enriched in the -dox negative control, and we used this number as a cutoff benchmark, and eliminated the bottom 53 hits (by p-value) that were enriched in +dox sample, leaving 265 proteins (Table S1).



Supplemental Fig. 9 (related to Fig. 6). Targeted verification of putative CReP interactors.

(**A, B**) Cells with dox-inducible CReP-BirA expression were left untreated or treated with dox and 50µM biotin (24h) prior to lysis and Streptavidin pull-down, as done for proteomic analysis. The samples treated with Streptavidin pull-down were run alongside input samples for the previously reported CReP binder NCK1 (A) and for immunoblot detection of the identified translation initiation factors (B). (**C**) HEK293 cells with dox-inducible flag-DAP5 were dox-induced (96h) prior to treatment with vehicle or 12-*O*-tetradecanoyl-phorbol-13-acetate (TPA; 4h). Cell lysates were analyzed by Flag-IP and immunoblot. (n=3).



Supplemental Fig. S10 (related to Materials and Methods).

2D PCA of z-score transformed protein intensities across all 4 unique groups.