Supplemental Information

Supplemental Dataset S1. Peptide counts from the TTP IP LC/MS-MS analysis. Listed are peptide spectral counts for all proteins identified in the LC/MS-MS analysis.

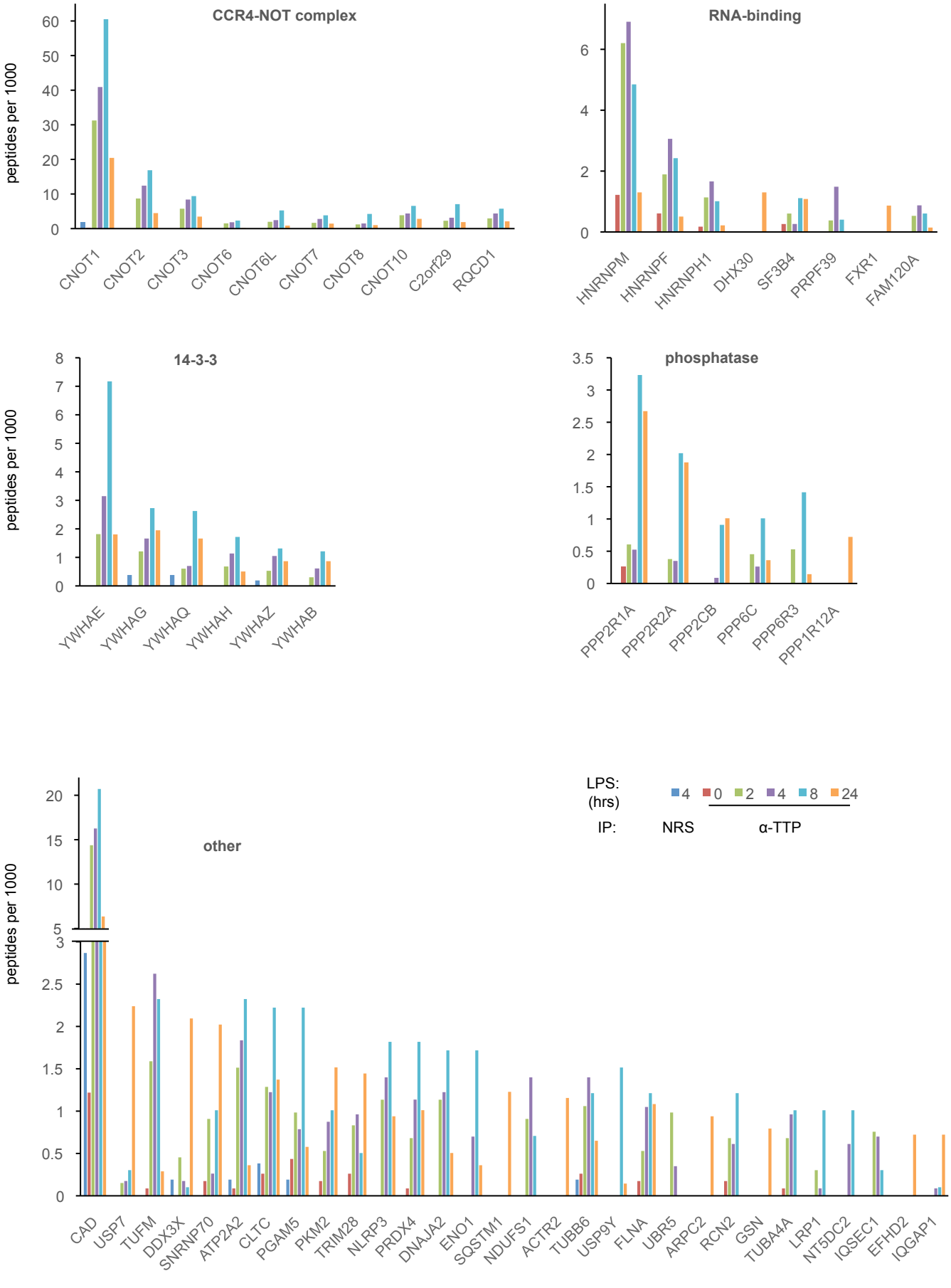
Supplemental Figure S1. Proteins enriched in the TTP IP LC/MS-MS analysis. Graphs showing the fraction of peptides (per 1000 total peptides) for all proteins that are at least 4-fold enriched in the anti-TTP IP at the 2, 4, 8, and/or 24 hr time-point over each of the negative control samples (NRS and 0 hr LPS anti-TTP samples) and whose maximum number of peptides counted in anti-TTP samples is at least 10. Bars in the graphs are color coded according to the time-points they represent as indicated in the legend.

Supplemental Figure S2. The interaction between 4EHP and TTP is greatly enhanced by GYF2. *In vitro* pulldown of His_6-4EHP and/or His_6-GYF2 after GST or GST-TTP were incubated with glutathione beads similar to the experiment in Figure 1C.

Supplemental Figure S3. Amino acids 61-93 of TTP are sufficient for association with 4EHP and GYF2. Western blots of input and anti-FLAG IP samples from RNase A-treated extracts of HEK293T cells transiently expressing indicated mouse TTP variants. FL-WT: Full-length wild-type TTP. TTP_{41-93}, TTP_{61-93}, TTP_{81-93}: fragments of TTP fused at their N-terminus with a FLAG-tag and MS2 coat protein.

Supplemental Figure S4. Evidence that TTP-mediated repression of the luciferase ARE-reporter mRNA occurs at the level of translation. (*A*) Levels of firefly luciferase mRNA normalized to *Renilla* luciferase mRNA quantified by qRT-PCR from samples in Figure 4A. Error bars represent SEM (*n* = 4). **(*B*)** Translation efficiency calculated as ratios of luciferase luminescence over mRNA levels as listed in the shown formula. Error bars represent SEM (*n* = 4).

Supplemental Figure S5. 4EHP and GYF2 protein levels do not dramatically change during LPS activation of RAW264.7 cells and okadaic acid inhibits TTP association with the CCR4-NOT deadenylase complex but not with 4EHP-GYF2. ***(A)*** Western blots of RAW264.7 cells treated with LPS for indicated lengths of time. ***(B)*** Western blots of total and anti-FLAG IP samples from RNase A-treated extracts of HEK293T cells transiently expressing indicated FLAG-tagged TTP variants, with or without a 2 hour 1 μM okadaic acid (Calbiochem) treatment.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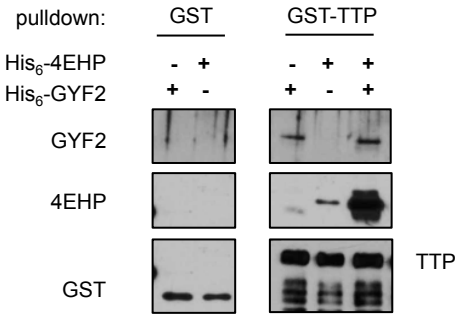
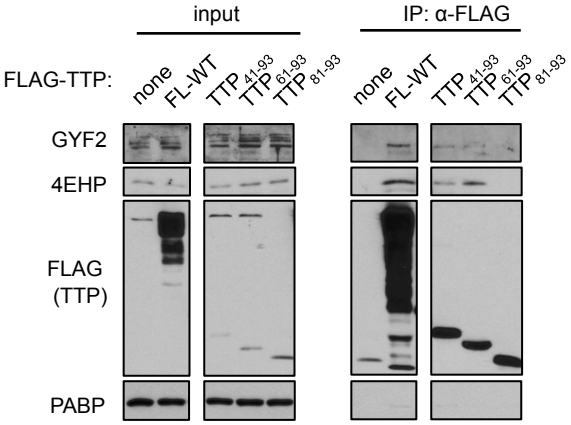
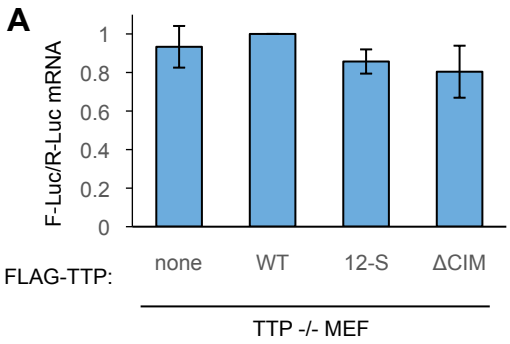
Supplemental Fig. S2

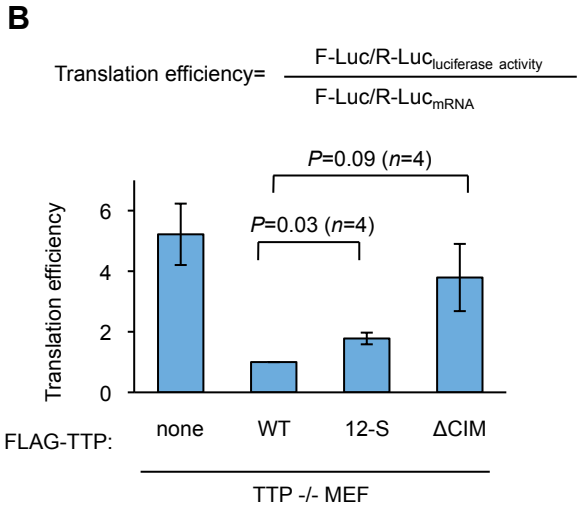


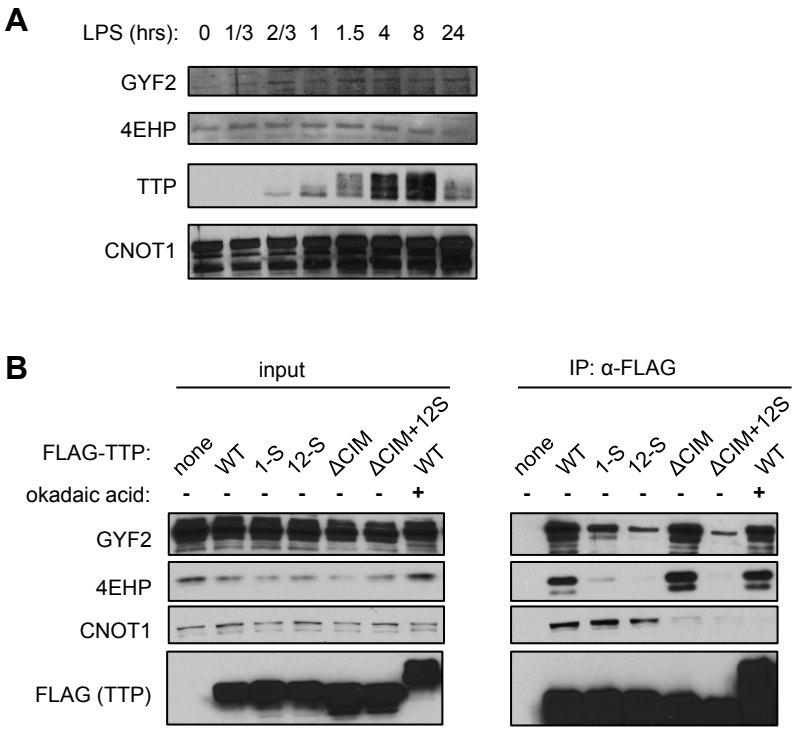
Supplemental Fig. S3



Supplemental Fig. S4



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TTP
CNOT1
B
input
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FLAG-TTP:
none
WT
1-S
12-S
ΔCIM
ΔCIM+12S
WT
okadaic acid:
- - - - - - +
GYF2
4EHP
CNOT1
FLAG (TTP)