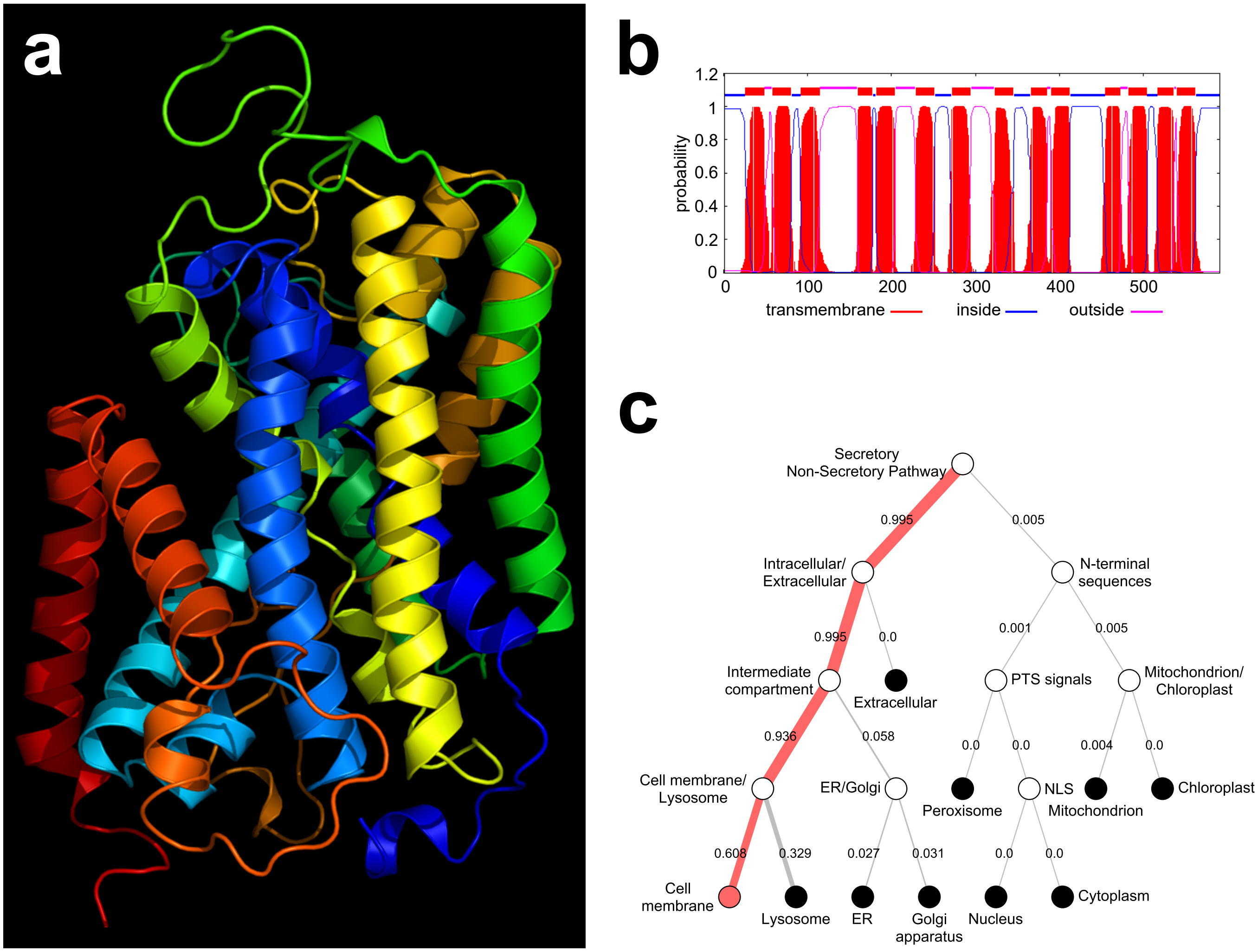


Figure S1. ***Ae. aegypti*** **AaCAT1 is a transmembrane protein that localizes to the plasma membrane.** **a.** Tertiary structure of the AaCAT1 protein was visualized using PyMOL (https://pymol.org/2/). **b.** The TMHMM transmembrane prediction tool, Krogh et al. [39] was used to predict the transmembrane domains, and intracellular and extracellular regions of AaCAT1 to design the bait fragment for yeast two-hybrid analysis. **c.** AaCAT1 is predicted to localize to the plasma membrane as determined by the DeepLoc-1.0 subcellular localization tool, Almagro Armenteros et al. [52].

Table S1. Annotations of all CAT entries used for CAT annotation tree.

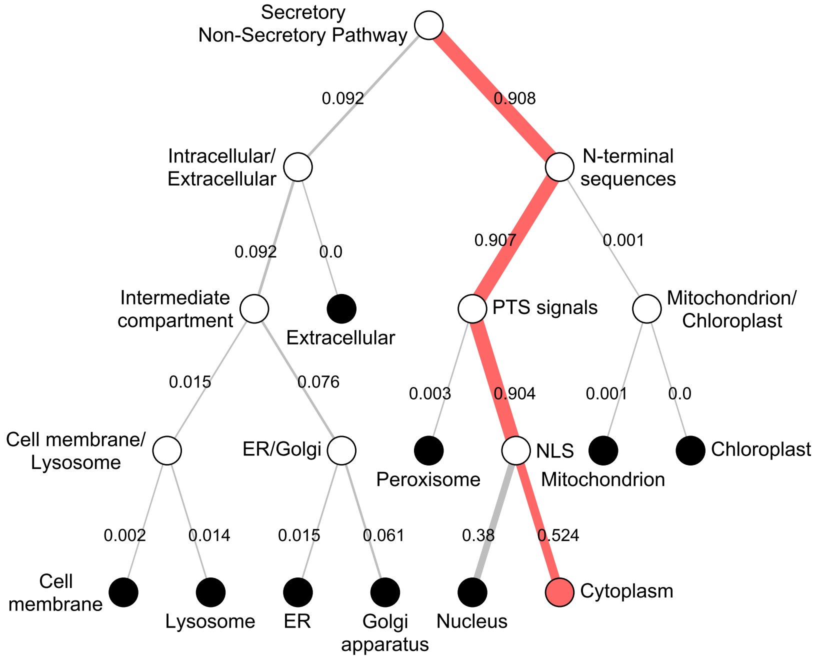
Entrez ID	Annotation	Organism	Proposed re-annotation	Source
XP_001662273.1	AaCAT1	*Ae. aegypti*		Hansen et al. 2011, Carpenter et al. 2012
XP_001659995.1	AaCAT2	*Ae. aegypti*		Carpenter et al. 2012
XP_001662274.1	AaCAT3	*Ae. aegypti*		Carpenter et al. 2012
XP_001659990.1	AaCAT4	*Ae. aegypti*		Carpenter et al. 2012
XP_001661832.1	AaCAT5	*Ae. aegypti*		Carpenter et al. 2012
5575859	Cationic amino acid transporter 2	*Ae. aegypti*	Cationic amino acid transporter 1	
5571884	Cationic amino acid transporter 2	*Ae. aegypti*		
5575863	Cationic amino acid transporter 3	*Ae. aegypti*		
5575110	Cationic amino acid transporter 3	*Ae. aegypti*	Cationic Amino acid transporter 5	
5575860	Cationic amino acid transporter 4	*Ae. aegypti*		
AAT73699.1	Cationic amino acid transporter slimfast	*Ae. aegypti*		Attardo et al. 2006
AKN80692.1	Slimfast	*Ae. aegypti*		Boudko et al. 2015
AAZ07714.1	iCAT2	*Ae. aegypti*		Attardo et al. 2006
5568181	Probable cationic amino acid transporter	*Ae. aegypti*		
35370	Torn and diminished rhabdomeres	*D. melanogaster*		
40510	Slimfast	*D. melanogaster*		
39622	CG7255	*D. melanogaster*		
39990	CG5535	*D. melanogaster*		
40254	CG13248	*D. melanogaster*		
32986	CG12531	*D. melanogaster*		

Entrez gene IDs are provided for all current gene annotations, and protein IDs are provided for previous annotations. Proposed re-annotations are based on MEGA, Tamura et al. [38] multiple sequence alignment and neighbor-joining tree analysis presented in Fig. 1a.

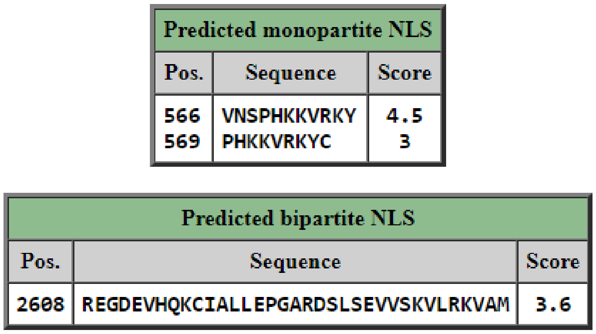
Table S2. Potential protein interactors identified from AaCAT1 yeast two-hybrid screen.

Identified potential prey interactors	Number of hits	NCBI Accession number	Location in the cell
eIF-2-alpha kinase activator GCN1	3	XP_001651826.2	Cytoplasm/ Ribosome
Sodium/potassium-transporting ATPase subunit beta-2	3	XP_001654893.1	Plasma membrane
PREDICTED: Aedes aegypti long-chain-fatty-acid--CoA ligase 4 (LOC5574220), transcript variant X4, mRNA	2	XM_021849348.1	Peroxisome/ Mitochondria/ Plasma membrane/ Endoplasmic reticulum
Serine protease SP24D	2	XP_001659969.2	Cytoplasm/ Extracellular region
Protein dj-1beta	2	XP_001648396.2	Cytoplasm/ Mitochondria/ Nucleus
Serine/threonine-protein phosphatase 6 catalytic subunit	1	XP_001648846.1	Cytoplasm/ Mitochondria
14-3-3 protein zeta isoform X1	1	XP_001652301.1	Cytoplasm/ Endosome
PREDICTED: Aedes aegypti prostatic acid phosphatase (LOC5563717), transcript variant X3, mRNA	1	XM_021837530.1	Cytoplasm
Chymotrypsin-like protein	1	AAL85585.1	Cytoplasm
trypsin 3A1 isoform X1	1	XP_001652943.1	Cytoplasm
PREDICTED: Aedes aegypti serine protease easter (LOC5569890), mRNA	1	XM_001658734.3	Cytoplasm
Annexin B9 isoform X5	1	XP_021708898.1	Cytoplasm/ Endomembrane system
Guanine nucleotide-binding protein subunit beta-like protein	1	XP_001663282.1	Cytoplasm

a



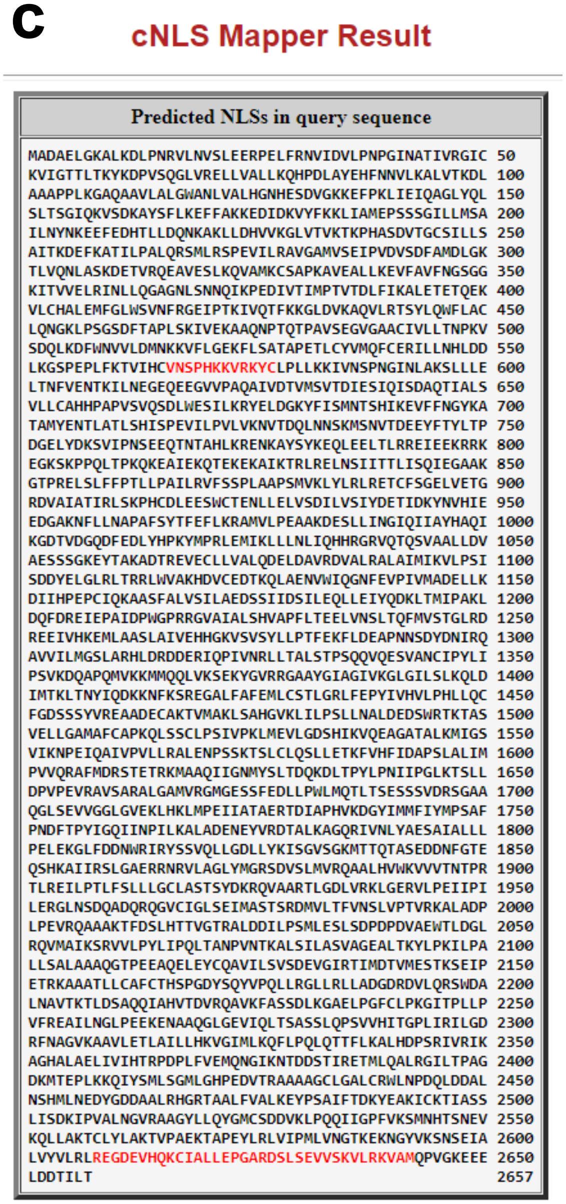
b



Predicted monopartite NLS		
Pos.	**Sequence**	**Score**
566	VNSPHKKVRKY	4.5
569	PHKKVRKYC	3

Predicted bipartite NLS		
Pos.	**Sequence**	**Score**
2608	REGDEVHQKCIALLEPGARDSLSEVVSKVLRKVAM	3.6

c



cNLS Mapper Result

Predicted NLSs in query sequence

```
MADAELGKALKDLPNRVLNVSLEERPELFRNVIDVLPNPGINATIVRGIC  50
KVIGTTLTKYKDPVSQGLVRELLVALLKQHPDLAYEHFNNVLKALVTKDL  100
AAAPPLKGAQAAVLALGWANLVALHGNHESDVGKKEFPKLIEIQAGLYQL  150
SLTSGIQKVSDKAYSFLKEFFAKKEDIDKVYFKKLIAMEPSSSGILLMSA  200
ILNYNKEEFEDHTLLDQNKAKLLDHVVKGLVTVKTKPHASDVTGCSILLS  250
AITKDEFKATILPALQRSMLRSPEVILRAVGAMVSEIPVDVSDFAMDLGK  300
TLVQNLASKDETVRQEAVESLKQVAMKCSAPKAVEALLKEVFAVFNGSGG  350
KITVVELRINLLQGAGNLSNNQIKPEDIVTIMPTVTDLFIKALETETQEK  400
VLCHALEMFGLWSVNFRGEIPTKIVQTFKKGLDVKAQVLRTSYLQWFLAC  450
LQNGKLPSGSDFTAPLSKIVEKAAQNPTQTPAVSEGVGAACIVLLTNPKV  500
SDQLKDFWNVVLDMNKKVFLGEKFLSATAPETLCYVMQFCERILLNHLDD  550
LKGSPEPLFKTVIHCVNSPHKKVRKYCLPLLKKIVNSPNGINLAKSLLLE  600
LTNFVENTKILNEGEQEEGVVPAQAIVDTVMSVTDIESIQISDAQTIALS  650
VLLCAHHPAPVSVQSDLWESILKRYELDGKYFISMNTSHIKEVFFNGYKA  700
TAMYENTLATLSHISPEVILPVLVKNVTDQLNNSKMSNVTDEEYFTYLTP  750
DGELYDKSVIPNSEEQTNTAHLKRENKAYSYKEQLEELTLRREIEEKRRK  800
EGKSKPPQLTPKQKEAIEKQTEKEKAIKTRLRELNSIITTLISQIEGAAK  850
GTPRELSLFFPTLLPAILRVFSSPLAAPSMVKLYLRLRETCFSGELVETG  900
RDVAIATIRLSKPHCDLEESWCTENLLELVSDILVSIYDETIDKYNVHIE  950
EDGAKNFLLNAPAFSYTFEFLKRAMVLPEAAKDESLLINGIQIIAYHAQI  1000
KGDTVDGQDFEDLYHPKYMPRLEMIKLLLNLIQHHRGRVQTQSVAALLDV  1050
AESSSGKEYTAKADTREVECLLVALQDELDAVRDVALRALAIMIKVLPSI  1100
SDDYELGLRLTRRLWVAKHDVCEDTKQLAENVWIQGNFEVPIVMADELLK  1150
DIIHPEPCIQKAASFALVSILAEDSSIIDSILEQLLEIYQDKLTMIPAKL  1200
DQFDREIEPAIDPWGPRRGVAIALSHVAPFLTEELVNSLTQFMVSTGLRD  1250
REEIVHKEMLAASLAIVEHHGKVSVSYLLPTFEKFLDEAPNNSDYDNIRQ  1300
AVVILMGSLARHLDRDDERIQPIVNRLLTALSTPSQQVQESVANCIPYLI  1350
PSVKDQAPQMVKKMMQQLVKSEKYGVRRGAAYGIAGIVKGLGILSLKQLD  1400
IMTKLTNYIQDKKNFKSREGALFAFEMLCSTLGRLFEPYIVHVLPHLLQC  1450
FGDSSSYVREAADECAKTVMAKLSAHGVKLILPSLLNALDEDSWRTKTAS  1500
VELLGAMAFCAPKQLSSCLPSIVPKLMEVLGDSHIKVQEAGATALKMIGS  1550
VIKNPEIQAIVPVLLRALENPSSKTSLCLQSLLETKFVHFIDAPSLALIM  1600
PVVQRAFMDRSTETRKMAAQIIGNMYSLTDQKDLTPYLPNIIPGLKTSLL  1650
DPVPEVRAVSARALGAMVRGMGESSFEDLLPWLMQTLTSESSSVDRSGAA  1700
QGLSEVVGGLGVEKLHKLMPEIIATAERTDIAPHVKDGYIMMFIYMPSAF  1750
PNDFTPYIGQIINPILKALADENEYVRDTALKAGQRIVNLYAESAIALLL  1800
PELEKGLFDDNWRIRYSSVQLLGDLLYKISGVSGKMTTQTASEDDNFGTE  1850
QSHKAIIRSLGAERRNRVLAGLYMGRSDVSLMVRQAALHVWKVVVTNTPR  1900
TLREILPTLFSLLLGCLASTSYDKRQVAARTLGDLVRKLGERVLPEIIPI  1950
LERGLNSDQADQRQGVCIGLSEIMASTSRDMVLTFVNSLVPTVRKALADP  2000
LPEVRQAAAKTFDSLHTTVGTRALDDILPSMLESLSDPDPDVAEWTLDGL  2050
RQVMAIKSRVVLPYLIPQLTANPVNTKALSILASVAGEALTKYLPKILPA  2100
LLSALAAAQGTPEEAQELEYCQAVILSVSDEVGIRTIMDTVMESTKSEIP  2150
ETRKAAATLLCAFCTHSPGDYSQYVPQLLRGLLRLLADGDRDVLQRSWDA  2200
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VFREAILNGLPEEKENAAQGLGEVIQLTSASSLQPSVVHITGPLIRILGD  2300
RFNAGVKAAVLETLAILLHKVGIMLKQFLPQLQTTFLKALHDPSRIVRIK  2350
AGHALAELIVIHTRPDPLFVEMQNGIKNTDDSTIRETMLQALRGILTPAG  2400
DKMTEPLKKQIYSMLSGMLGHPEDVTRAAAAGCLGALCRWLNPDQLDDAL  2450
NSHMLNEDYGDDAALRHGRTAALFVALKEYPSAIFTDKYEAKICKTIASS  2500
LISDKIPVALNGVRAAGYLLQYGMCSDDVKLPQQIIGPFVKSMNHTSNEV  2550
KQLLAKTCLYLAKTVPAEKTAPEYLRLVIPMLVNGTKEKNGYVKSNSEIA  2600
LVYVLRLREGDEVHQKCIALLEPGARDSLSEVVSKVLRKVAMQPVGKEEE  2650
LDDTILT                                            2657
```

Figure S2. ***In silico*** **predictions of GCN1 subcellular localization.** **a.** GCN1 is predicted to localize to the cytoplasm, but also may localize to the nucleus. **b.** Predicted nuclear localization sequences (NLS) found in GCN1 reveal several NLS with scores indicating a mixed nuclear-cytoplasmic localization pattern. **c.** Result from part b showing location of predicted NLS within the context of the GCN1 amino acid sequence.

Subcellular localization prediction was performed using the DeepLoc-1.0 subcellular localization tool, Almagro Armenteros et al. [52]. NLS prediction was performed using the cNLS Mapper prediction tool, Kosugi et al. [54].

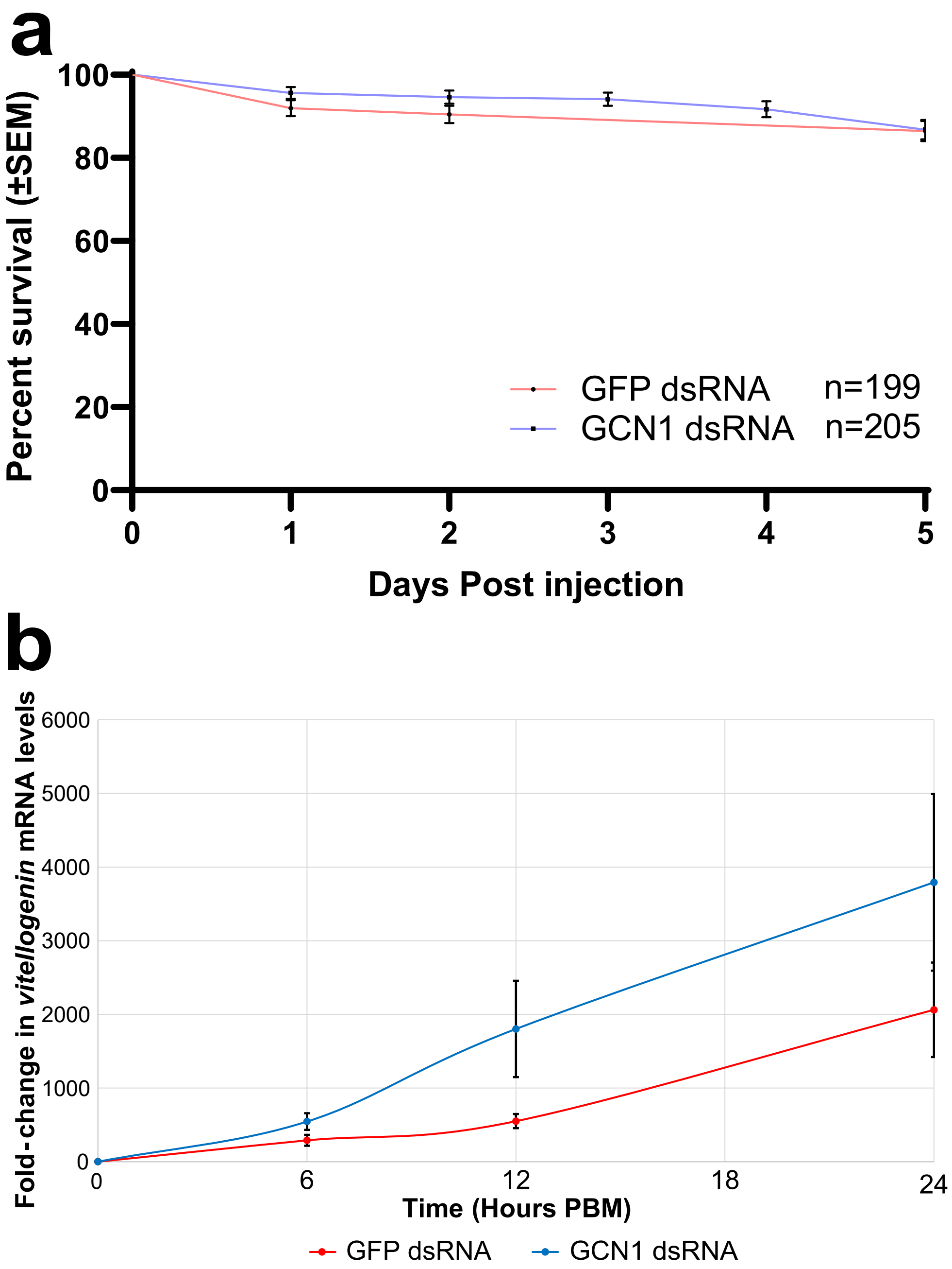

Figure S3. GCN1 knockdown is not lethal, and does not affect YPP transcription. a. Survival of GCN1 dsRNA-injected and GFP dsRNA-injected control mosquitoes was not significantly different up to five days post-injection. A log-rank Mantel-Cox test was used to test for significant differences between the two treatments. **b.** qRT-PCR analysis of *vg* transcription in GCN1 dsRNA-injected and GFP dsRNA-injected control mosquitoes. Three biological replicates were analyzed per treatment at each time point and *vg* mRNA levels were normalized to β-*actin* mRNA prior to calculating fold-change in *vg* expression through the first 24 hr PBM. Paired t-tests were used to test for significant differences between the two treatments at each time point.

Figure S4. The following pages contain Western blot and Coomassie blue images used in main figures without cropping, color removal, and brightness/ contrast correction. The label above each image contains a reference to the main text figure where the image is included.

Figure S4a. Cross-link Coomassie stain gel (Fig. 1e).

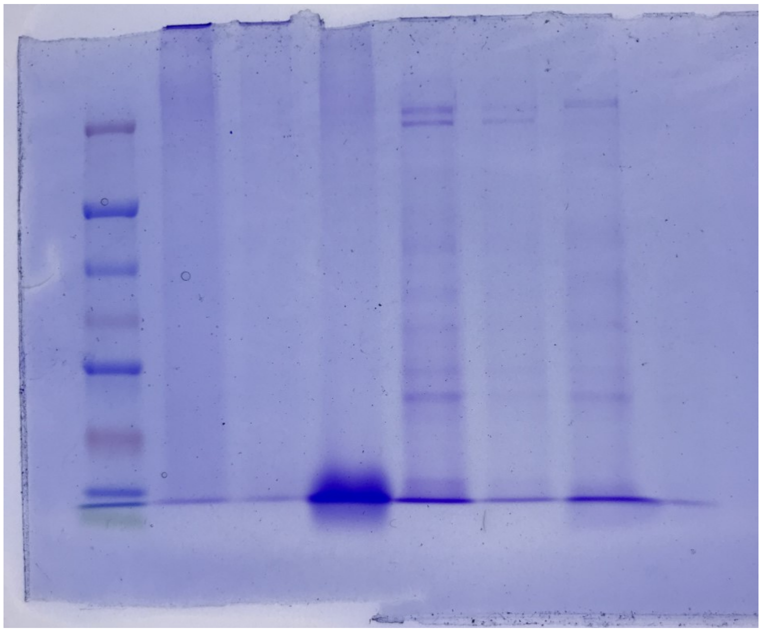

Figure S4b. Pulldown Western Blot (Fig. 1f).

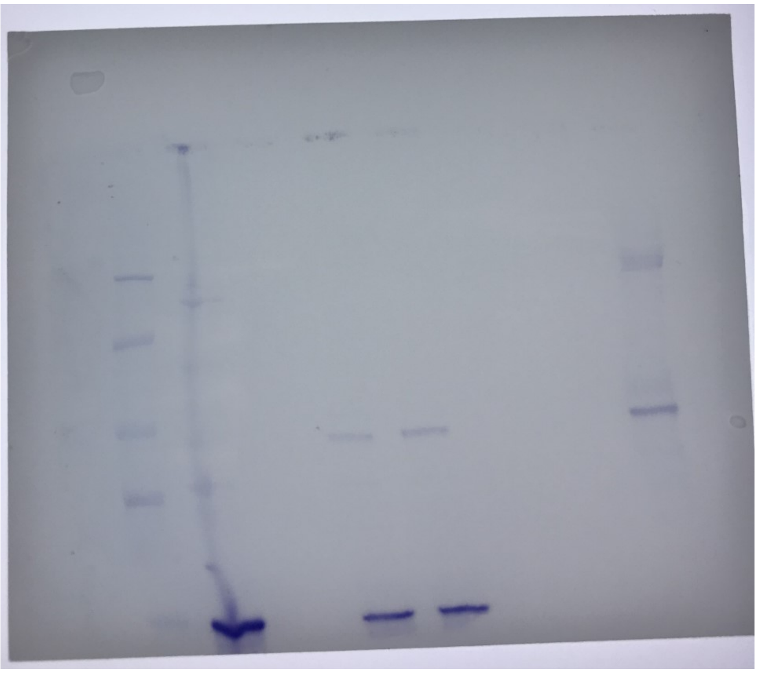

Figure S4c. GCN1 expression in adult tissues (Fig. 2a bottom).

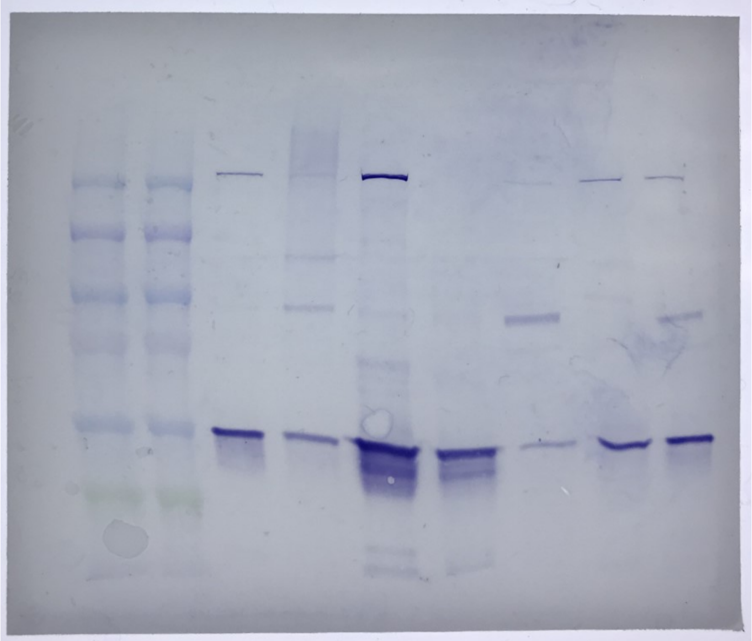

Figure S4d. Confirmation of GCN1 dsRNA knockdown (Fig. 2b).

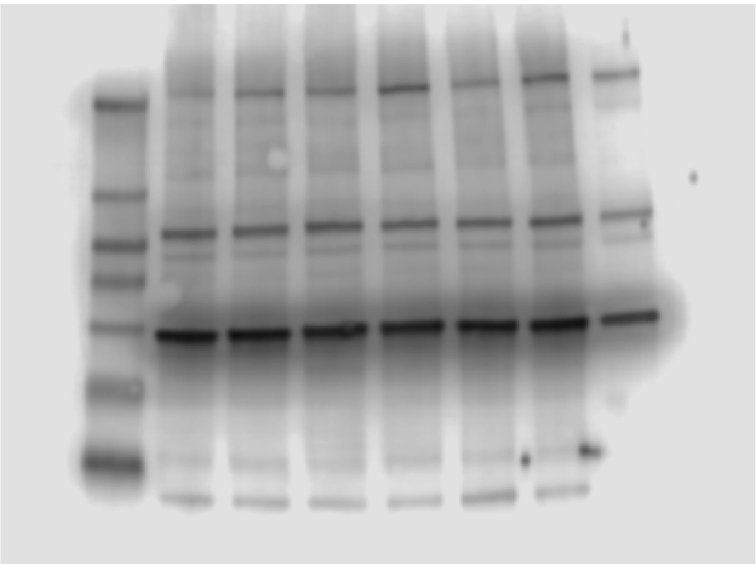

Figure S4e. Phospho-eIF2α Western blots (Fig. 2d top).

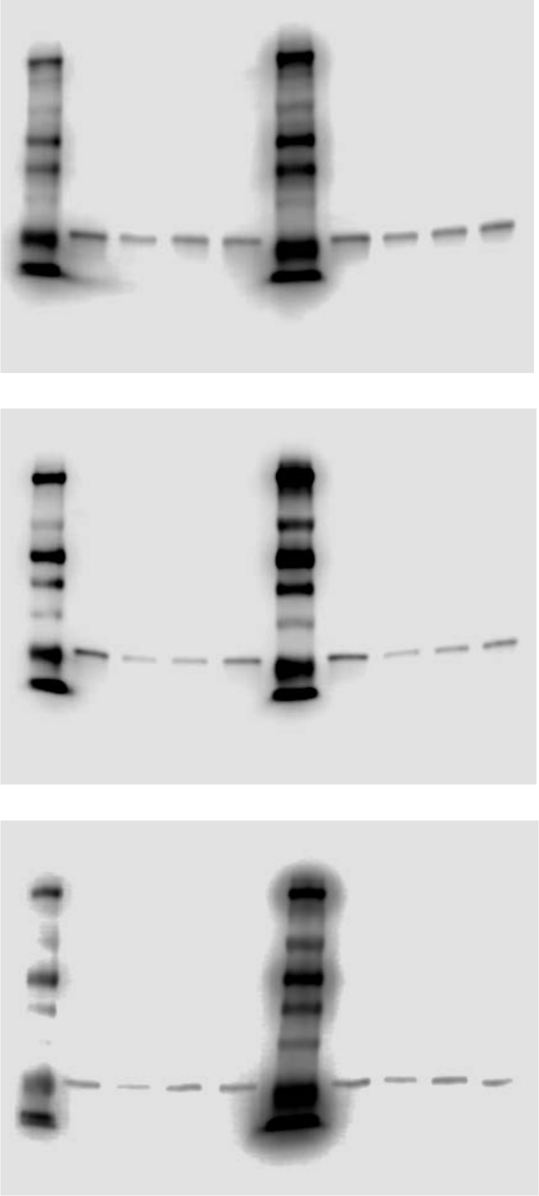

Figure S4f . eIF2α Western blots
(Fig. 2d middle).

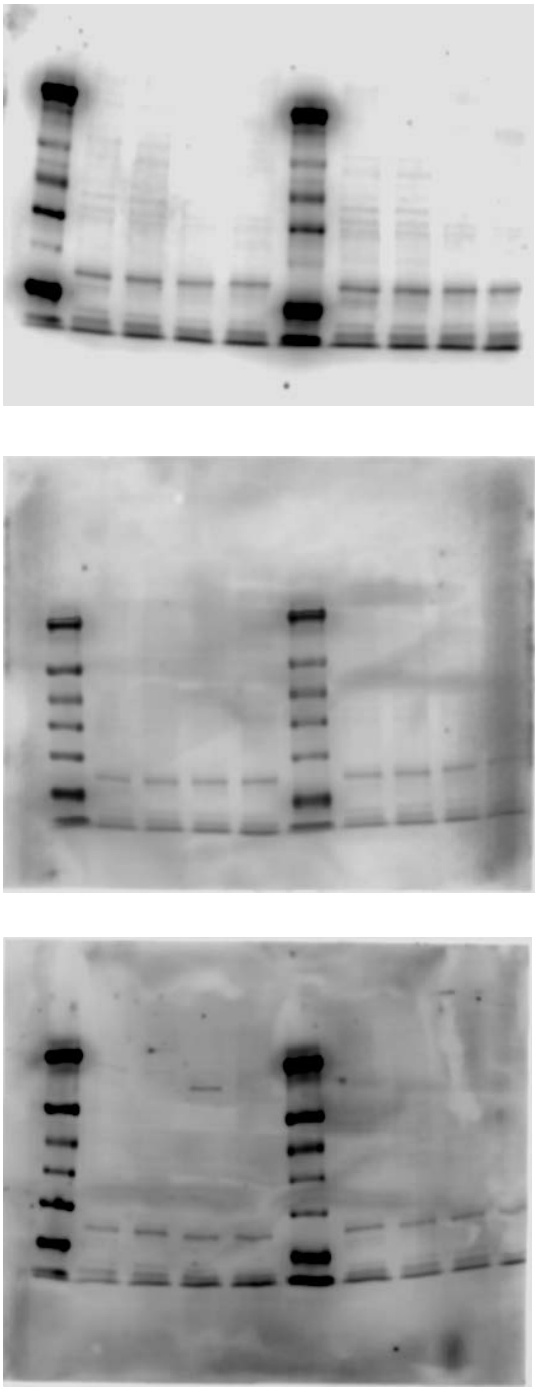

Figure S4g. Actin Western Blots
(Fig. 2d bottom).