Supplemental Information

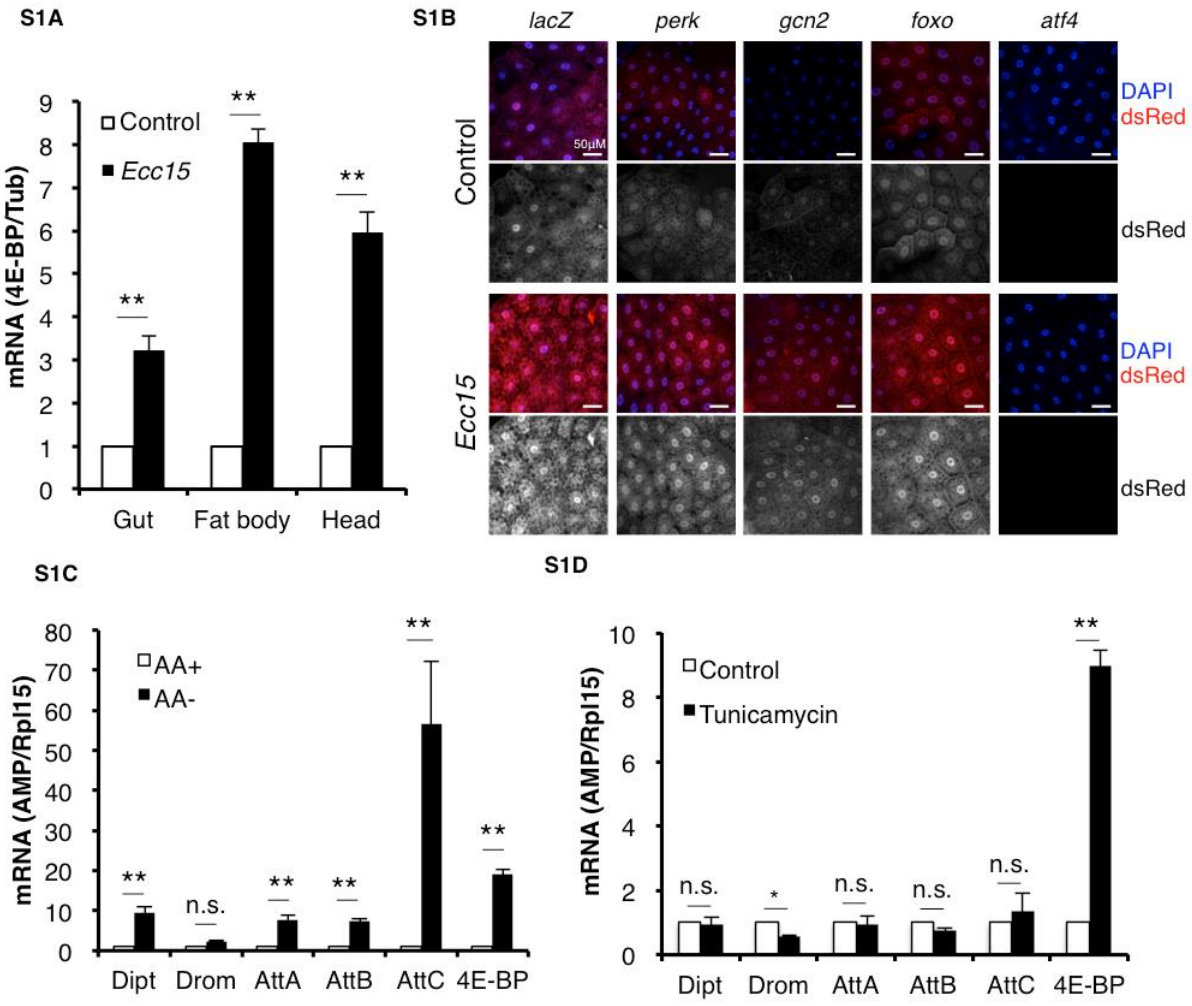

Fig. S1. GCN2 regulates *4e-bp* in the fat body during infection (Related to Figure 1)

S1A. qPCR analysis of *4E-BP* in tissues indicated on the X-axis from control or infected *yw* larvae.

S1B. 4E-BP^{intron}-dsRed expression in the uninfected control larva (top) and in larva infected with *Ecc15* (bottom) in various RNAi backgrounds driven by *Dcg-Gal4*.

S1C. qPCR analysis of AMP induction in response to amino acid deprivation in S2 cells.

S1D. qPCR analysis of AMP induction in response to tunicamycin treatment in S2 cells.

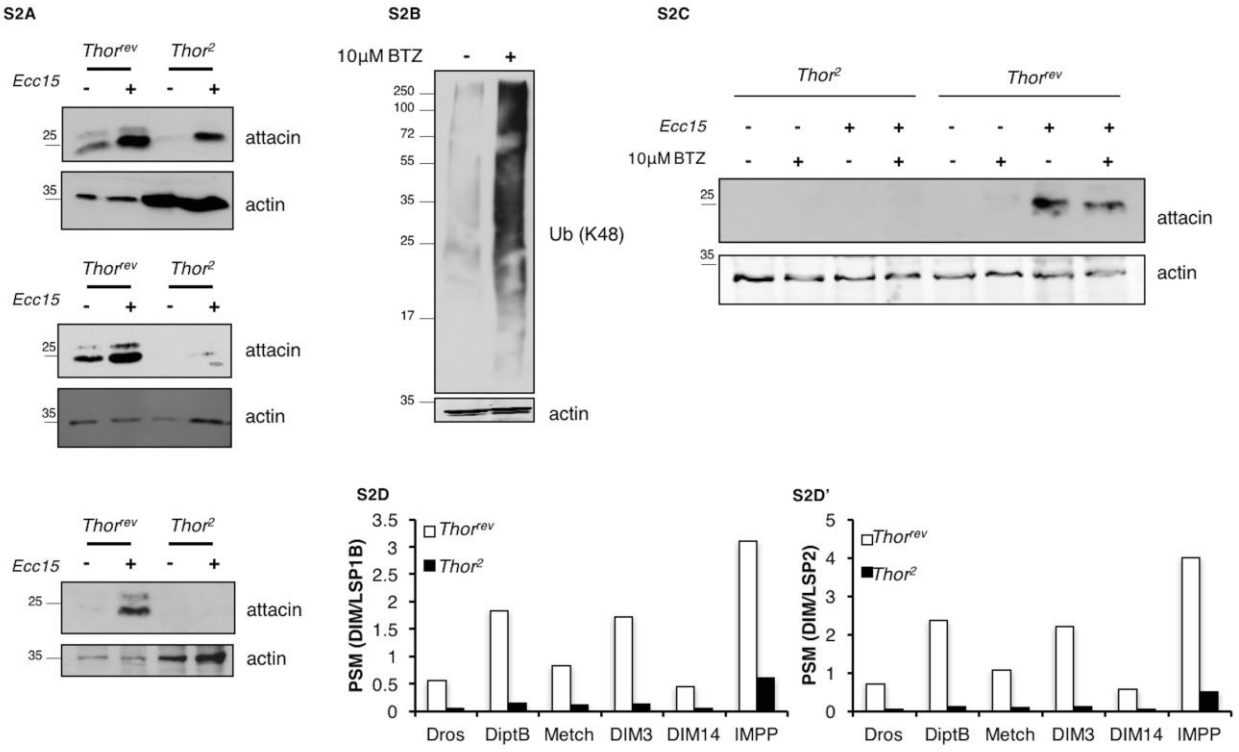

Fig. S2. 4E-BP regulates translation of AMPs (Related to Figure 2)

S2A. Individual blots used for quantification in 2B'.

S2B. Western blot analysis of whole larval lysates from larvae fed with 10µM bortezomib (BTZ) with anti-ubiquitin (Ub K48) and anti-actin.

S2C. Western blot analysis of hemolymph collected from homozygotic *Thor*^{2} and *Thor*^{rev} larvae infected with *Ecc15* and simultaneously fed with BTZ.

S2D, D'. Mass spectrometry analysis of hemolymph from *Thor*^{rev}(white) and *Thor*^{2}(blue) similar to Fig. 2C. Graph on top shows data normalized to LSP1B and on bottom to LSP2. (LSP= larval serum protein).

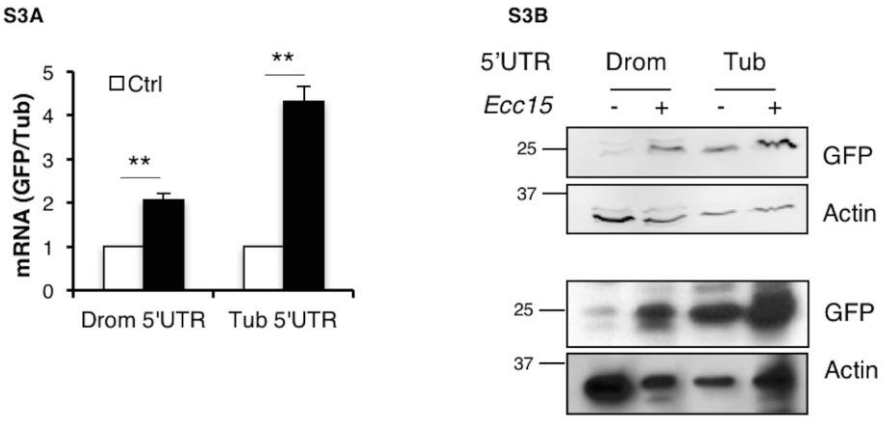

Fig. S3. 4E-BP enhances cap-independent translation mediated by Drosomycin 5'UTR during infection (Related to Figure 4)

S3A. qPCR analysis of transgenic larvae expressing monocistronic reporters (schematic on top) driven by the Drosomycin promoter (DromP) with indicated 5'UTRs (Drom= Drosomycin, Tub= Tubulin) infected with *Ecc15*.

S3B. Individual western blots used for quantitation in Figure 4D'.

Detailed methods

Antibodies

Mouse anti-GFP 1:500 for IF and 1:2000 for WB (A6455, Life Technologies), rabbit anti-dsRed 1:500 for IF and 1:2000 for WB (R10367, Thermo Fisher), mouse anti-actin 1:5000 WB (MAB1501, Millipore), mouse anti-attacin (Dr. Donggi Park) 1:500 WB.

Molecular cloning

Luciferase bicistronic and monocistronic reporter: The 5' UTR sequences for *Drosomycin*, *Attacin A* and *4E-BP*, were subcloned into previously described luciferase bicistronic and monocistronic reporter constructs (Kang et al., 2017; Marr et al., 2007).

Fluorescent bicistronic reporter: The reporter backbone was generated in a pUAST vector by sequentially cloning in a GFP (XbaI) and dsRed (EcoRI), with the NotI and SacII sites in between them available for insertion of various 5'UTRs. 5'UTRs of *Drosomycin* and *Attacin A* were assembled by extension PCR and inserted in the NotI/SacII sites in either the forward or reverse orientation. Expression was driven in S2 cells by co-transfecting *Act-Gal4*. All 5'UTR sequences used are indicated in Table S2.

Drosomycin-GFP monocistronic reporter: The Drosomycin promoter ($Drom^P$) was cloned into the pattB vector at the BglII/KpnI sites. The Drosomycin 5'UTR-Drosomycin GFP and Tub5'UTR-Drosomycin GFP sequences were generated by gene synthesis (General Biosystems) and cloned sequentially into the pattB-$Drom^P$ vector using the KpnI/XbaI sites. The reporters were inserted at the attP2 site on chromosome III (BestGene). Table S3 lists sequence and primer information.

Liquid chromatography and Mass spectrometry

To enrich for small molecular weight AMPs, the hemolymph was passed through a 10kDa filter (Millipore). The fractions were then reduced with dithiothreitol (2 µL of 0.2 M, pH 8) for 1 hr at 57°C and subsequently alkylated with iodoacetamide (2 µL of 0.5 M, pH 8) for 45 min in the dark at room temperature. Immediately following alkylation, peptides were desalted using a solid phase extraction (SPE) cartridge containing C18 resin. The resulting eluate was then concentrated to dryness and resuspended in 0.5% acetic acid for mass spectrometric analysis. Each sample was separated by reverse phase chromatography using an EASY-nanoLC 1000 system (Thermo Scientific) configured for preconcentration using an Acclaim PepMap trap column in line with an EASY-Spray 50 cm x 75 µm ID PepMap C18 analytical column (2 µm beads). Solvent A was 2% acetonitrile containing 0.5% acetic acid and solvent B was 80% acetonitrile with 0.5% acetic acid. Separation was carried out using a linear gradient from 5-35% solvent B over the first 60 min and then increased to 45% B over 10 min. The gradient was then ramped to 100% B over 10 min and held constant for an additional 10 min. The LC system was coupled via an EASY nano-electrospray ionization source (Thermo Scientific) maintained at 2.0 kV to a Q-Exactive Orbitrap mass spectrometer (Thermo Scientific). For all experiments, high-resolution MS1 spectra were acquired with a resolving power of 70K (at m/z 200), automatic gain control (AGC) target of 1e6, maximum ion time of 120 ms, and scan range of 400 to 1500 m/z.

Following each MS1 scan, data-dependent acquisition of high resolution HCD MS2spectra were acquired for the top 20 most abundant precursor ions in the preceding full scan. All MS2 spectra were collected using a single microscan at 17,500 (at m/z 200), AGC target of 5e4, maximum injection time of 120 ms, 2 m/z isolation window, and Normalized Collision Energy (NCE) of 27. All LC-MS/MS data was searched using a standalone version of the Byonic algorithm (Protein Metrics Inc.) against a *Drosophila* UniProt database modified to include known antimicrobial and immune-induced peptide/protein sequences. All data was analyzed using a no enzyme (non-specific) search with a precursor mass tolerance of ±10 ppm and fragment ion mass tolerance of ±10 ppm. Carbamidomethylation of Cys was added as a static modification and oxidation of methionine and deamidation of glutamine and asparagine were searched as a variable modification. The results were filtered to only include peptides identified with a Byonic score of 300 or better. Details for the peptides used in this analysis can be found in Table S4.

Luciferase assays buffers

FF assay buffer: 75 mM HEPES pH 8.0, 5 mM MgSO4, 20 mM DTT, 100 µM EDTA, 530 µM ATP, 0.5 mM coenzyme A, and 0.5 mM D-luciferin

Ren assay buffer: 25 mM Na4PPi, 10 mM NaOAc, 15 mM EDTA, 0.5 M Na2SO4, 1.0 M NaCl, and 0.1 mM Coelenterazine.

In vitro translation assays

Translation assays were performed in 10 µl reactions containing: 6 µl of rabbit reticulocyte Lysate (Green Hectares, McFarland, WI), 0.1 mM spermidine, 60 µm Amino Acids, 16.8 mM creatine phosphate, 800 ng of creatine kinase, 24 mM HEPES (pH 7.4), 0.4 mM Mg acetate, 30 mM K acetate, 1 µg of calf liver tRNA, 2 units SUPERase-In RNase Inhibitor (ThermoFisher, #AM2696), and 100 ng of template RNA. Luciferase activity was measured using 100 µl of FF assay buffer.

Supplementary tables

Genotype	Source
y^1 w; Thor^2*	BDSC #9559
Thor^{rev}	Generated by precise excision of a p-element insertion in the 4E-BP locus (y^1 w*; P{lacW}Thor^{k13517} , BDSC #9558)
cn^1 crc^1/SM5	BDSC #266
w^{1118}; foxo$^{\Delta 94}$/TM6B, Tb^1	BDSC #42220
4E-BP^{intron}-dsRed	Kang, M.J. *et al*, *Journal of Cell Biology*, 2017
Dcg-Gal4	Suh, J.M. *et al*, *Cell Metabolism*, 2007
UAS-ATF4^{RNAi}	VDRC #109014
UAS-FOXO^{RNAi}	VDRC #106097
UAS-PERK^{RNAi}	VDRC #110278
UAS-GCN2^{RNAi}	VDRC #103976

Table S1. List of fly stocks used

All stocks were ensured to be *Wolbacchia* free, and mutants were isogenized with the control genetic backgrounds wherever applicable.

5'UTR	Sequence	bps
HCV	CTCCCCTGTGAGGAACTACTGTCTTCACGCAGAAAGCGTCTAGCCATGGCGT TAGTATGAGTGTCGTGCAGCCTCCAGGACCCCCCCTCCCGGGAGAGCCATA GTGGTCTGCGGAACCGGTGAGTACACCGGAATTGCCAGGACGACCGGTCCT TTCTTGGATTAACCCGCTCAATGCCTGGAGATTTGGGCGTGCCCCCGCGAGA CTGCTAGCCGAGTAGTGTTGGGTCGCGAAAGGCCTTGTGGTACTGCCTGATA GGGTGCTTGCGAGTGCCCCGGGAGGTCTCGTAGACCGTGCATCATGAGCAC AAATCCTGAACCTCAAA	326
Drosomycin	CCACAAGTCGCTGATAATTCAAACAGAAATCATTTACCAAGCTCCGTGAGA ACCTTTTCCAAT	63
Attacin A	AGTCAGCTCCAGCAATCCAGTTCAGCAAC	29
4E-BP	AGCAATCGCCTAGCGAACAGCCAACGGTGAACACATAGCAGCCACACAAGC TCTATAGCTGATACAAGCAACGAAATACAAACAACGCAGTTTGTGTAAACA ATCAAATTGTCGTAGCCATATCGAGTGTGCTTACACGTCCAGCGGAAAGTTT TCGAAACCCATCCAATCAATCAGCTAAGATGTCCATG	191

Table S2. 5'UTR sequences tested in bicistronic and monocistronic assays

Drom^{P}-fwd	gaAGATCTaccagtgaagataacccgcc
Drom^{P}-rev	ggGGTACCcttgggaacttcgaggagaa
Drom5'UTR-Drom-GFP	CCACAAGTCGCTGATAATTCAAACAGAAATCATTTACCAAGCTCCGTGAGAACCTTT TCCAATATGATGCAGATCAAGTACTTGTTCGCCCTCTTCGCTGTCCTGATGCTGGTGG TCCTGGGAGCCAACGAGGCCGATGCCGACTGCCTGTCCGGAAGATACAAGGGTCCC TGTGCCGTCTGGGACAACGAGACCTGTCGTCGTGTGTGCAAGGAGGAGGGACGCTC CAGTGGCCACTGCAGCCCCAGTCTGAAGTGCTGGTGCGAAGGATGCATGGTGAGCA AGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCCTGGTCGAGCTGGACGGCGAC GTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACGG CAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCAC CCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACAT GAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGGCTACGTCCAGGAGCGCA CCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAG GGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGG CAACATCCTGGGGCACAAGCTGGAGTACAACTACAACAGCCACAACGTCTATATCA TGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCGCCACAACATC GAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGA CGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAA AGACCCCAACGAGAAGCGCGATCACATGGTCCTGCTGGAGTTCGTGACCGCCGCCG GGATCACTCTCGGCATGGACGAACTGTACAAGTAAATCCATGAGCAATTAGCATGA ACGTTCTGAAAAGCGCGTTTAGCTCTCCACTACTTACACATATTCTATGCTGCAATAT TGAAAATCTAATAAACAAAACTAATGTACATT
Tub5'UTR-Drom-GFP	TCATATTCGTTTTACGTTTGTCAAGCCTCATAGCCGGCAGTTCGAACGTATACGCTCT CTGAGTCAGACCTCGAAATCGTAGCTCTACACAATTCTGTGAATTTTCCTTGTCGCGT GTGAAACACTTCCAATAAAAACTCAATATGATGCAGATCAAGTACTTGTTCGCCCTC TTCGCTGTCCTGATGCTGGTGGTCCTGGGAGCCAACGAGGCCGATGCCGACTGCCTG TCCGGAAGATACAAGGGTCCCTGTGCCGTCTGGGACAACGAGACCTGTCGTCGTGTG TGCAAGGAGGAGGGACGCTCCAGTGGCCACTGCAGCCCCAGTCTGAAGTGCTGGTG CGAAGGATGCATGGTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCATCC TGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGC GAGGGCGATGCCACCTACGGCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAA GCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACCTACGGCGTGCAGTGCTT CAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGA AGGCTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCC GCGCCGAGGTGAAGTTCGAGGGCGACACCCTGGTGAACCGCATCGAGCTGAAGGGC ATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAGTACAACTACAA CAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACT TCAAGATCCGCCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAG CAGAACACCCCCATCGGCGACGGCCCCGTGCTGCTGCCCGACAACCACTACCTGAG CACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTCCTGC TGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAACTGTACAAGTAA ATCCATGAGCAATTAGCATGAACGTTCTGAAAAGCGCGTTTAGCTCTCCACTACTTA CACATATTCTATGCTGCAATATTGAAAATCTAATAAACAAAACTAATGTACATT

Table S3. Primer and sequence information for in vivo monocistronic reporters

Table S4. Mass spectrometric metrics for the peptides utilized in the analysis of AMP protein levels (Related to Figure 2)