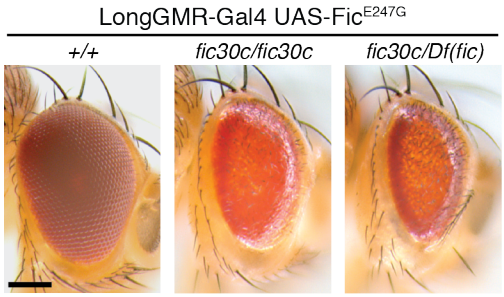
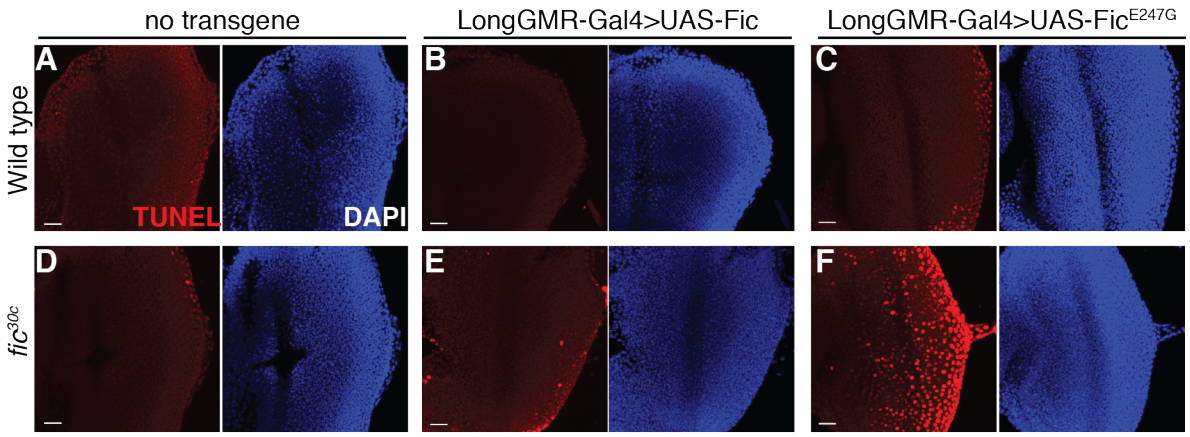
Supplemental Material

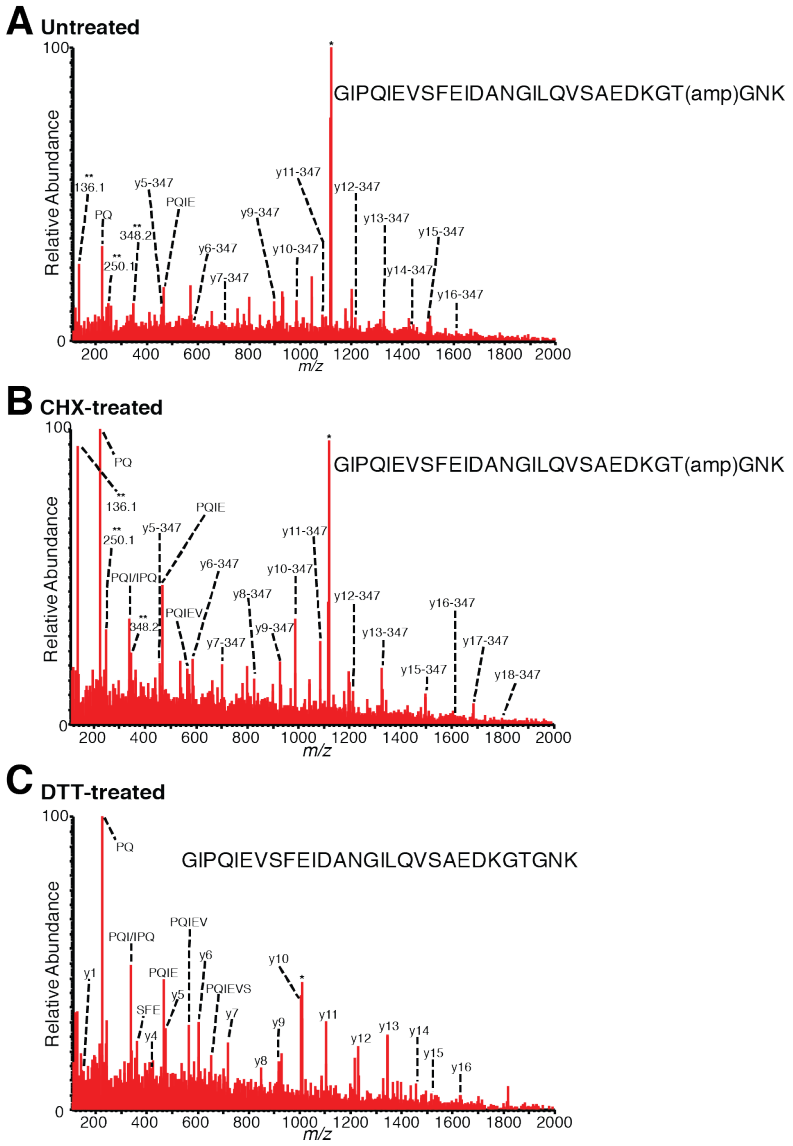
Fic-mediated deAMPylation is not dependent on homo-dimerization and rescues toxic AMPylation in flies



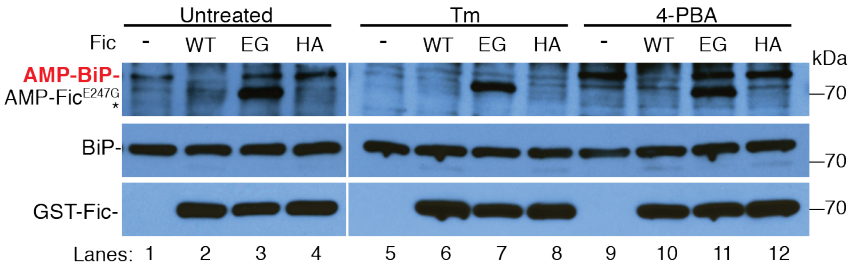
Supplemental Figure 1: The *fic^{30C}* mutation is a genetic null. Representative Stereoscope images of LongGMR-Gal4-driven expression of UAS-Fic^{E247G} in a wild-type *fic* background, a *fic^{30C}/fic^{30C}* homozygous mutant background, or a hemizygous *fic^{30C}/Df(2L)BSC296* background. Identical phenotypes in *fic^{30C}/fic^{30C}* homozygous versus *fic^{30C}/Df(2L)BSC296* hemizygous backgrounds indicate that *fic^{30}* genetically behaves as a *fic* null allele.



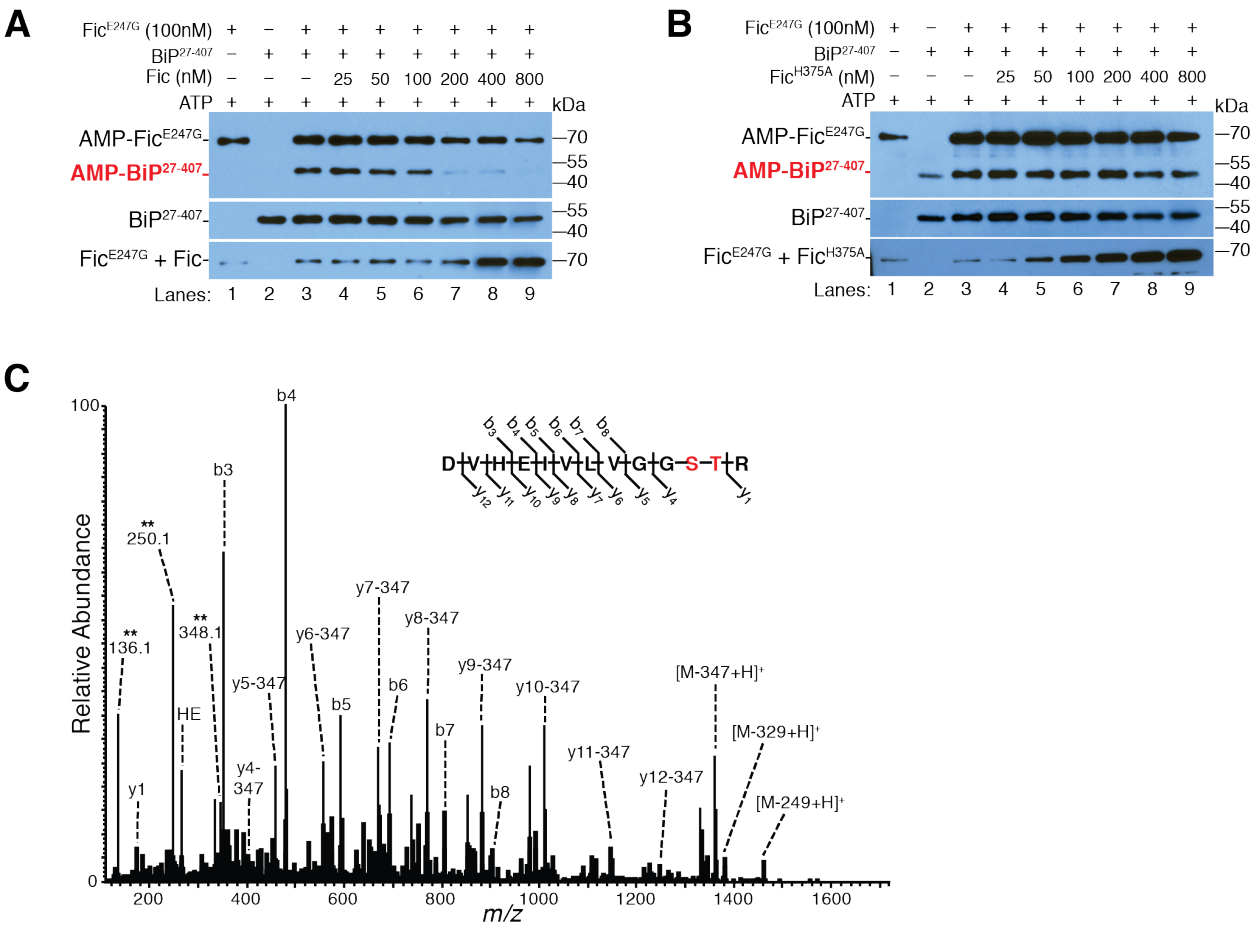
Supplemental Figure 2: LongGMR-Gal4 expression of Fic^{E247G} induces apoptosis in *fic*^{30C} eye imaginal discs. Cell death was detected with TUNEL staining in larval eye discs. **(A-C)** Wild-type eye discs expressing **(A)** no transgene, **(B)** UAS-Fic transgene, or **(C)** UAS-Fic^{E247G} transgene. **(D-F)** *fic*^{30C} mutant eye discs expressing **(D)** no transgene, **(E)** UAS-Fic transgene, or **(F)** UAS-Fic^{E247G} transgene. DAPI nuclear stain for eye disc morphology. Scale bar = 20μM.



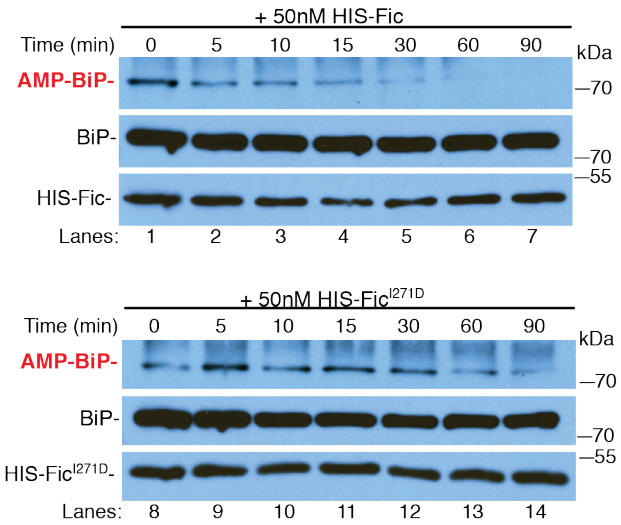
Supplemental Figure 3: MS/MS spectra of BiP T518 AMPylated peptide ions: (A-C) MS/MS spectra of **(A, B)** GIPQIEVSFEIDANGILQVSAEDKGT(amp)GNK and unmodified peptide ion: **(C)** GIPQIEVSFEIDANGILQVSAEDKGTGNK. Samples were collected from ATP washes of S2 cell lysates after **(A)** no drug treatment, **(B)** treatment with cyclohexamide, or **(C)** treatment with DTT. AMPylation of T518 was not observed after treatment with DTT. The precursor ions, **(A, B)** *m/z* 1120.20 (3+) and **(C)** *m/z* 1010.52 (3+) are labeled with a single asterisk and were subjected to HCD fragmentation to generate the spectra shown. The fragmentation spectra of the AMPylated peptides **(A, B)** include y-ions with characteristic mass shifts corresponding to loss of the AMP group (-347 Da). Unique ions corresponding to neutral loss of the AMP group (labeled with **) are also present at 136.1, 250.1, and 348.2 Da in **(A, B).**



Supplemental Figure 4: Fic-mediated deAMPylation of S2 lysates treated with ER stressors and stress inhibitors. Fic-mediated deAMPylation of endogenous BiP from S2 lysates was assayed by western blot. Representative western blot of S2 lysates untreated (lanes 1-4), treated with Tunicamycin (Tm) (lanes 5-8), or treated with 4-phenylbutyrate (4-PBA) (lanes 9-12) for 4 hours. S2 lysates were incubated with recombinant enzymes: GST-FicΔ70 (lanes 2,6,10), GST-FicΔ70^{E247G} (lanes 3,7,11), or GST-FicΔ70^{H375A} (lanes 4,8,12). The asterisk (*) indicates an unspecific band. Blots were probed with anti-AMP-Threonine, anti-BiP, and anti-dFic antibodies.



Supplemental Figure 5: Fic competition with Fic^{E247G} AMPylation *in vitro*. **(A-C)** Representative immunoblots of AMPylation assays in which 6xHis-BiP$^{27-407}$ was used as a substrate for GST-FicΔ70^{E247G}. Increasing concentrations of **(A)** GST-FicΔ70 or **(B)** GST-dFicΔ70^{H375A} were added to the AMPylation reaction. Blots were probed with anti-AMP-Threonine, anti-BiP, and anti-GST antibodies. **(C)** MS/MS spectrum of AMPylated BiP$^{27-407}$ peptide ion: DVHEIVLVGGSTR. The precursor ion, *m/z* 855.90 (2+), of the AMPylated peptide was subjected to HCD fragmentation to generate the fragmentation spectrum shown. Fragment ions containing the modified residue show characteristic mass shifts corresponding to loss of the AMP group (-347 Da). Unique ions corresponding to neutral loss of the AMP group (labeled with **) are present at 136.1, 250.1, and 348.1 Da. The modification site can be localized to either the serine-365 or threonine-366 residue highlighted in red.



Supplemental Figure 6: Time-dependent deAMPylation activity of 50nM 6xHIS-FicΔ70 (lanes 1-7) and 50nM 6xHIS-FicΔ70^{I271D} was assayed by western blot. Blots were probed with anti-AMP-Threonine, anti-BiP, and anti-dFic antibodies.

		Spectral Count
Modified Residue	**Peptide**	**BiP$^{27-407}$**
T70	ITPSYVAFTADGER	2
T87/T92	NQLTTNPENTVFDAK	3
T107	EWSDTNVQHDIK	2
S130/T133/S134	NSKPHISVDTSQGAK	1
T166/T171	VTHAVVTVPAYFNDAQR	2
T184	QATKDAGVIAGLQVMR	1
S319/T321/T323	IEIESFFEGDDFSETLTR	1
S365/T366	DVHEIVLVGGSTR	3
S365/T366	DVHEIVLVGGSTRIPK	6

Table S1: Mass spectrometry analysis of AMPylated BiP$^{27-407}$.
Mascot ion score cutoff of 15 was applied to determine MS2 spectral count.