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SHORT REPORT

Figures and figure supplements

Degradation of *Gadd45* mRNA by nonsense-mediated decay is essential for viability

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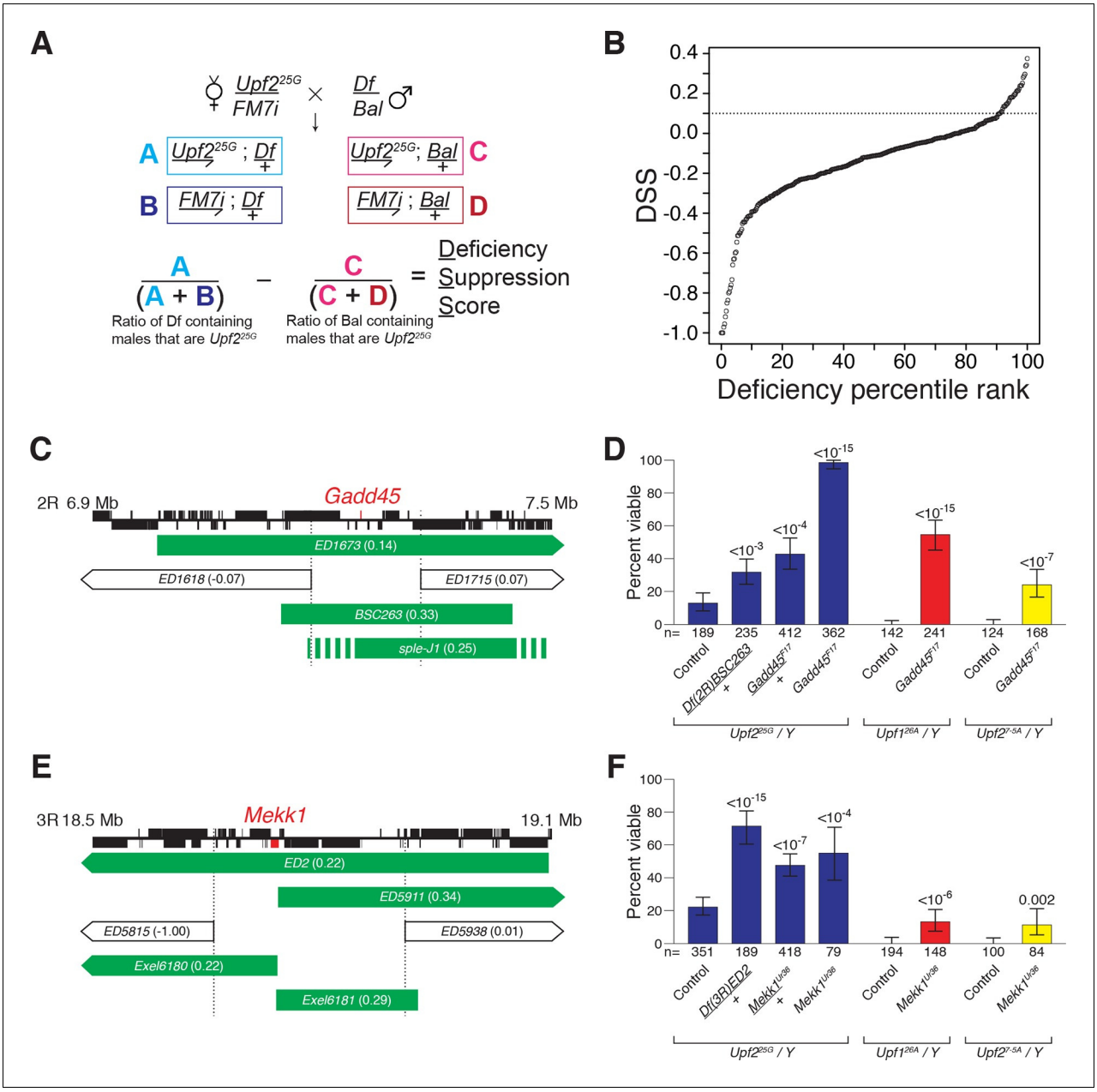

Figure 1. *Drosophila* suppressor screen identifies the *Gadd45* pathway as the inducer of NMD-mutant lethality. (**A**) Scheme to screen deficiencies for the suppression of $Upf2^{25G}$ partial lethality. The Deficiency Suppression Score (DSS) represents the relative difference in $Upf2^{25G}$ viability when crossed to a heterozygous deficiency (*Df*) compared to when crossed to a balancer (*Bal*) (See Methods). (**B**) DSS from 376 screened deficiencies ranked by score. A DSS greater than 0.1 (dotted line) indicates that deficiency suppresses $Upf2^{25G}$ lethality. (**C** and **E**) Candidate suppressing regions uncovering *Gadd45* (**C**) and *Mekk1* (**E**). DSSs are shown in parenthesis. Dotted lines denote extent of regions deleted by suppressing deficiencies but not non-suppressing deficiencies. Filled blocks on chromosomes indicate predicted gene spans, *Gadd45* pathway genes are indicated in red; suppressing deficiencies indicated in green, *sple-J1* has undefined breakpoints located within hashed regions. (**D** and **F**) NMD mutant adult viability in combination with $Gadd45^{F17}$ (**D**) or $Mekk1^{Ur36}$ (**F**) mutants. $Upf1^{26A}$ and $Upf2^{7-5A}$ are null alleles (***Frizzell et al., 2012***; ***Metzstein and Krasnow, 2006***). p-value compared to controls determined by the test of equal or given proportions indicated. Error bars represent 95% confidence interval of the binomial distribution. n equals total number of animals scored in each cross.

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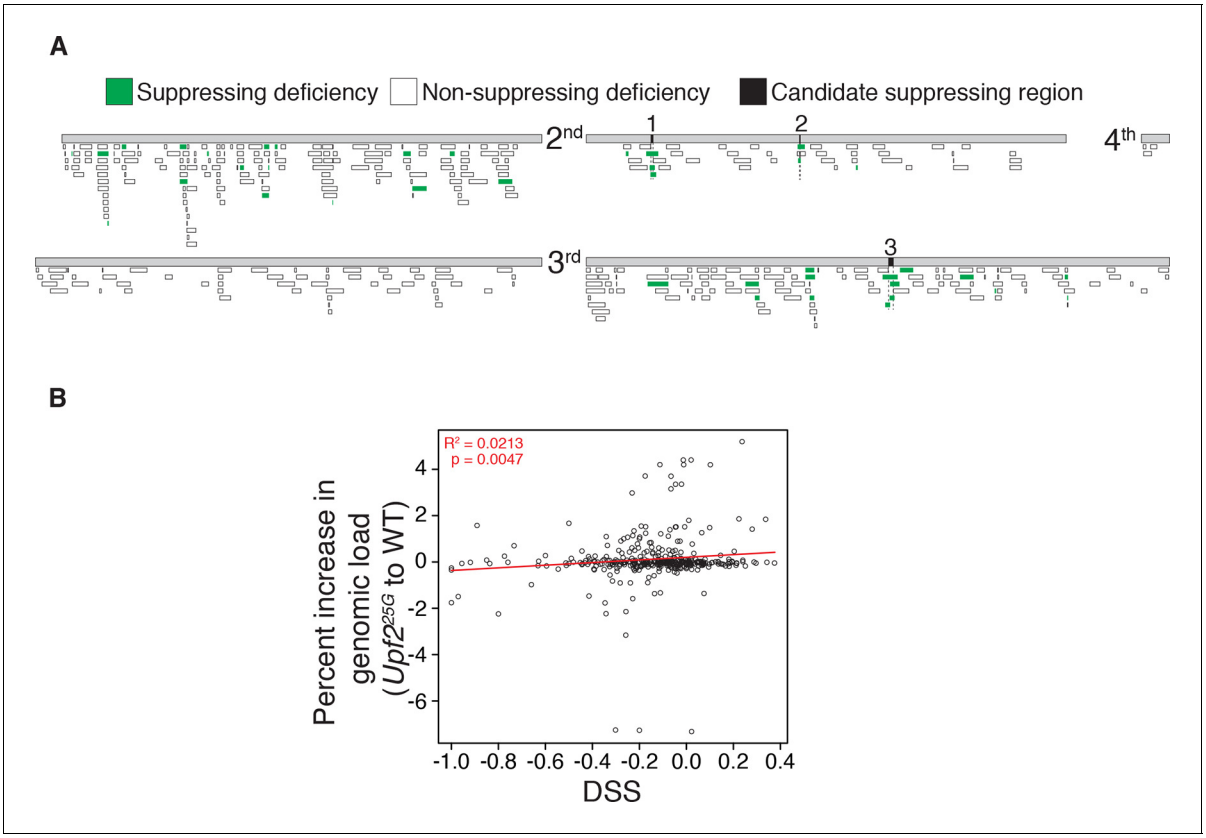

Figure 1—figure supplement 1. Reduced expression of specific loci, not overall mRNA abundance, produces NMD mutant suppression by deficiencies. (**A**) Map of 376 autosomal DrosDel deficiencies with an isogenic background and molecularly defined breakpoints (***Ryder et al., 2007***) and eight other deficiencies used to further test candidate suppressing regions without overlapping DrosDel deficiencies. 39 total deficiencies suppress *Upf2*^{25G} lethality, shown in green. Regions deleted by any non-suppressing deficiencies were eliminated as candidate suppressing regions, removing false positives and reducing the size of the candidate intervals. The three candidate suppressing regions that are deleted only by suppressing deficiencies are indicated in black and labeled 1–3. (**B**) Each deficiency's Deficiency Suppression Score (DSS) compared to percent increase in RNA abundance in *Upf2*^{25G} compared to wild-type from loci removed by that deficiency according to RNA-seq from ***Chapin et al. (2014)***. Trend line in red; statistics calculated using Pearson correlation test.

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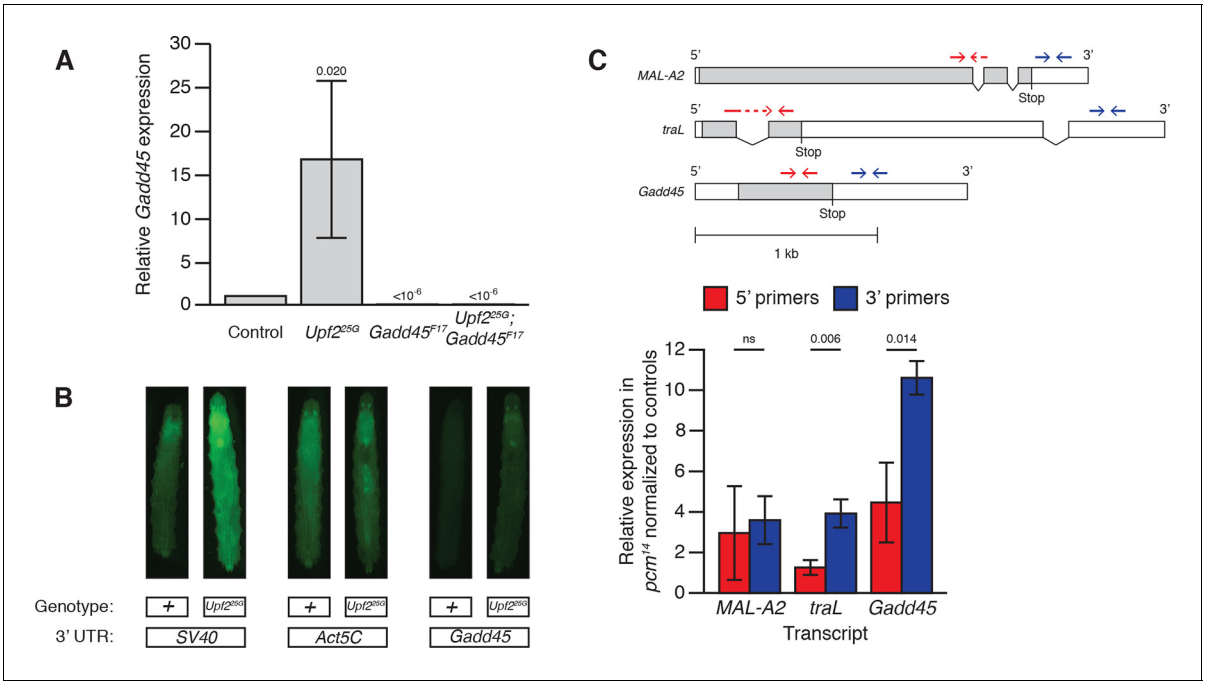

Figure 1—figure supplement 2. Drosophila Gadd45 is an endogenous direct NMD target. (**A**) *Gadd45* mRNA expression in adults of the given genotypes measured by qRT-PCR. *Gadd45* mRNA expression is increased 16.7-fold in $Upf2^{25G}$ mutants, and is eliminated by $Gadd45^{F17}$ mutants. p-values display one-sided Student's t-test of indicated condition compared to control. Error bars represent 2 SEM. (**B**) Fluorescence of GFP transgenes with *SV40, Act5C*, or *Gadd45* 3′ UTRs expressed by UAS driven by *Actin:GAL4* in $Upf2^{+}$ or $Upf2^{25G}$ third instar larvae. *SV40* and *Gadd45* 3′ UTR constructs show significantly increased fluorescence in $Upf2^{25G}$ animals compared to $Upf2^{+}$, indicating NMD-dependent post-transcriptional degradation of mRNAs containing these UTRs. The *Act5C* 3′ UTR construct has similar fluorescence in both backgrounds, indicating NMD does not regulate the post-transcriptional stability of this UTR. Micrographs show dorsal views with anterior at top. (**C**) *MAL-A2, traL*, and *Gadd45* 5′ and 3′ fragment mRNA expression measured by qRT-PCR in pcm^{14} null mutants (***Waldron et al., 2015***) normalized to controls. Transcript structures of a non-NMD-target, *maltase A2 (MAL-A2)*; a known NMD-targeted transcript, the non-sex specific isoform of *transformer (traL)* (***Rehwinkel, 2005***; ***Metzstein and Krasnow, 2006***); and the *Gadd45* transcript (note *Gadd45* has no introns). Open boxes indicate UTRs; grey boxes indicate coding regions. NMD targeting initiates endonucleolytic cleavage near the stop codon (***Gatfield and Izaurralde, 2004***), producing 5′ and 3′ fragments with unprotected ends, which are then subjected to degradation by cytoplasmic 3′-to-5′ and 5′-to-3′ exonucleases, respectively. qRT-PCR primer pairs 5′ (red) and 3′ (blue) to the cleavage site can be used to differentially measure the quantity of these fragments. The *Drosophila* 5′-to-3′ exonuclease is encoded by the *XRN1* homologue *pacman (pcm)*, and fragments 3′ to an endonucleolytic NMD cleavage accumulate in *Drosophila* cells with reduced XRN1 activity (***Gatfield and Izaurralde, 2004***). The *MAL-A2* 3′ primers show no difference in relative expression in pcm^{14} mutants compared to the 5′ primers, while *tra* and *Gadd45* have relatively increased levels of a 3′ fragment in pcm^{14} mutants, revealing endonucleolytic cleavage has occurred between the primer pairs, probably near the stop codon, indicative of NMD-initiated degradation. p-value between indicated samples using a two-sided Student's t-test are displayed. ns indicates a p-value greater than 0.05. Error bars represent 2 SEM.

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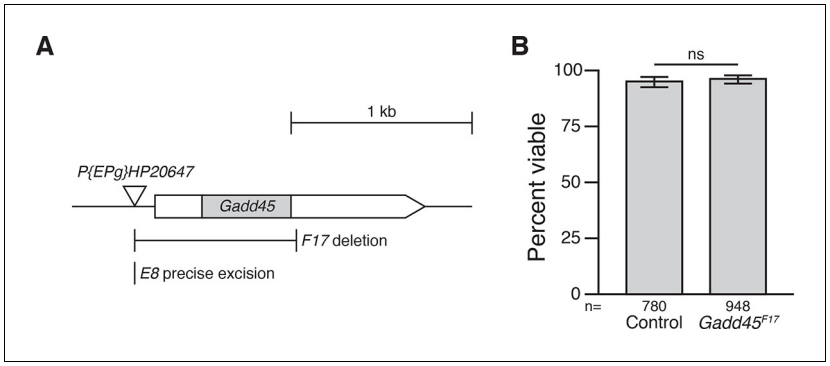

Figure 1—figure supplement 3. F17 is a null allele of Gadd45. (**A**) *Gadd45*^{F17} is an imprecise excision of the P-element *P{EPg}HP20647*, deleting a 894 bp region that includes the entire *Gadd45* coding region. *Gadd45*^{E8} is a precise excision of the same P-element, leaving *Gadd45* intact. Coding region in grey; untranslated regions in white. Arrowhead indicates direction of transcription. (**B**) Adult viability of control and *Gadd45*^{F17}mutants. p = 0.4463 between *Gadd45*^{F17} and controls, using the test of equal or given proportions. Error bars represent 95% confidence interval of the binomial distribution. n equals total number of animals scored in each cross.
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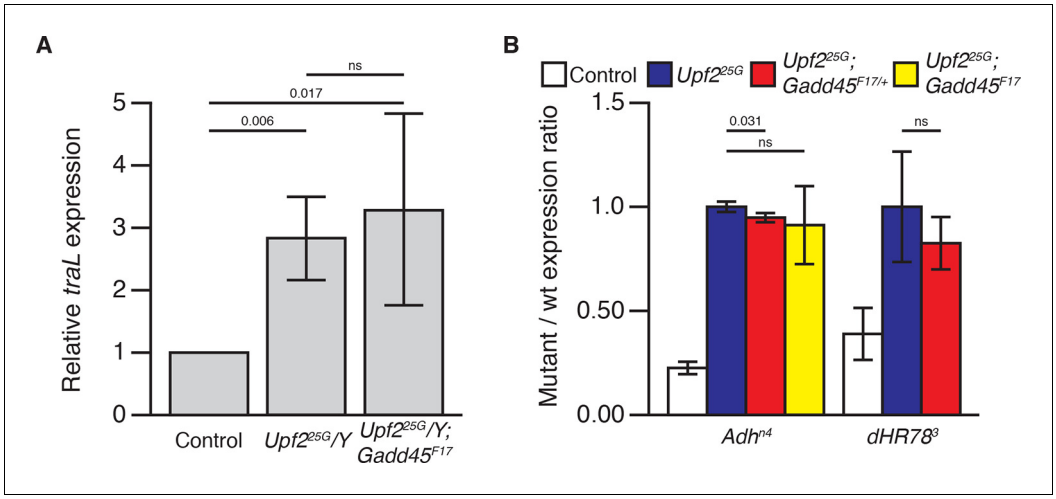

Figure 1—figure supplement 4. Loss of Gadd45 does not restore NMD activity in NMD mutants. (**A**) Expression of the endogenous NMD target transcript *traL* (***Rehwinkel, 2005***; ***Metzstein and Krasnow, 2006***) in control male, $Upf2^{25G}/Y$, and $Upf2^{25G}/Y$; $Gadd45^{F17}$animals, measured by qRT-PCR. There is no significant difference in *traL* expression between $Upf2^{25G}/Y$ and $Upf2^{25G}/Y$; $Gadd45^{F17}$animals. p-values determined by one-sided Student's t-test between indicated conditions are displayed. ns indicates a p-value greater than 0.05. (**B**) Relative abundance of PTC-containing Adh^{n4} (***Chia et al., 1987***) and $dHR78^{3}$ (***Fisk and Thummel, 1998***) allele mRNAs compared to wild-type allele mRNA abundance in animals heterozygous for Adh^{n4}or $dHR78^{3}$in each indicated genotype (stabilization of $dHR78^{3}$ was not determined in $Upf2^{25G}/Y$; $Gadd45^{F17}$ animals). Neither reduction nor elimination of *Gadd45* restored destabilization of these alleles. p-values determined by two-sided Student's t-test between indicated conditions are displayed. ns indicates a p-value greater than 0.05. Error represents 2 SEM.
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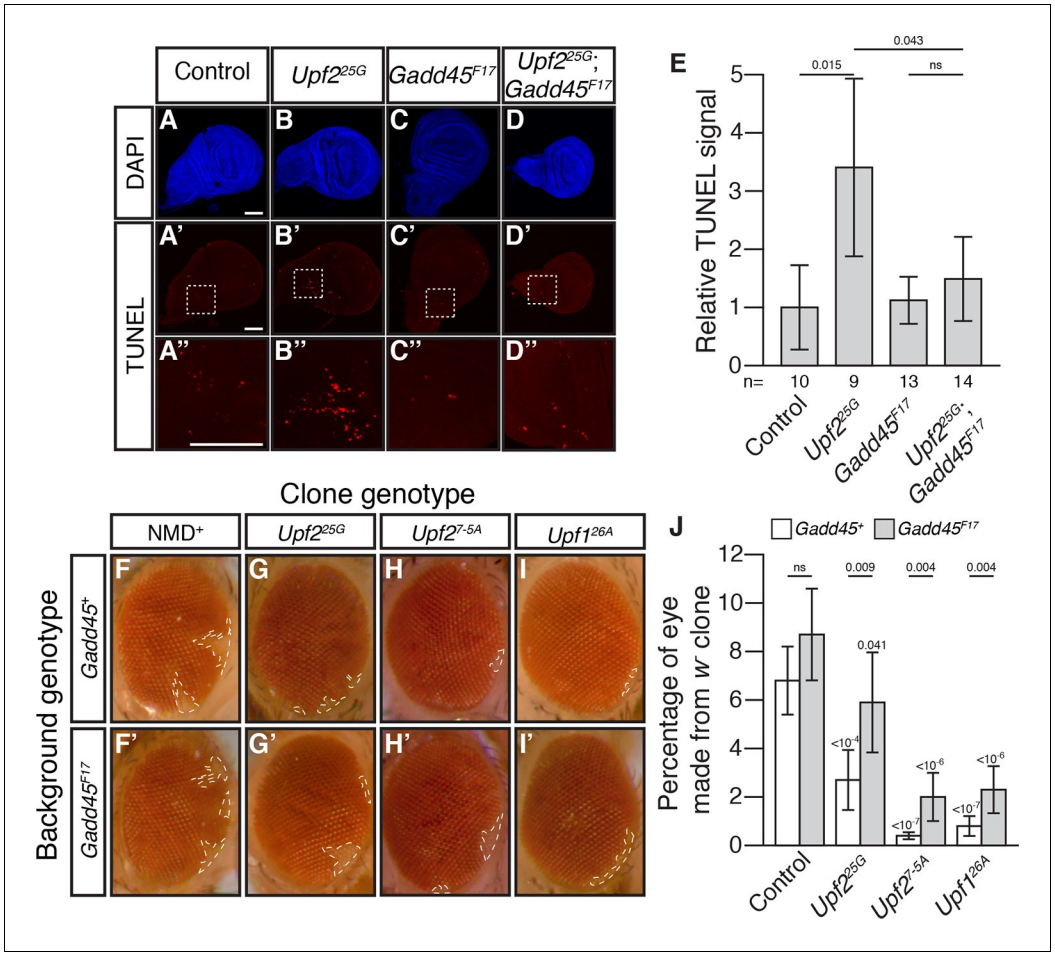

Figure 2. Loss of Gadd45 suppresses NMD-mutant cell death. (**A** to **D**) DAPI (blue) and (**A'**to **D'**) TUNEL (red) staining in late 3rd instar larval wing discs from control (**A**); *Upf2*^{25G} (**B**); *Gadd45*^{F17} (**C**); and *Upf2*^{25G}; *Gadd45*^{F17} (**D**) animals. (**A''** to **D''**) are 4x view of outlined section at the base of the blade of the wing disc from **A'-D'**, respectively. Scale bar represents 100 μm. (**E**) Relative TUNEL signal in control and mutant wing discs, normalized to control. p-value between indicated samples using a two-sided Student's t-test are displayed. ns indicates a p-value greater than 0.05. Error bars represent 2 SEM. n equals total number of discs scored. (**F** to **I**) *w*$^-$ eye clones in *Gadd45*$^+$ and *Gadd45*^{F17} backgrounds. Dashed lines indicate clone boundaries. (**J**) Quantification of the fraction of the eye composed of *w*$^-$ cells in control and mutant eyes. p-values indicate differences between *Gadd45* mutant and control in the same NMD background (indicated by horizontal bars) or NMD mutant and control in the same *Gadd45* background (indicated by value above each individual bar), using a two-sided Student's t-test. ns indicates a p-value greater than 0.05. Error bars represent 2 SEM. n = 20 eyes for all conditions.
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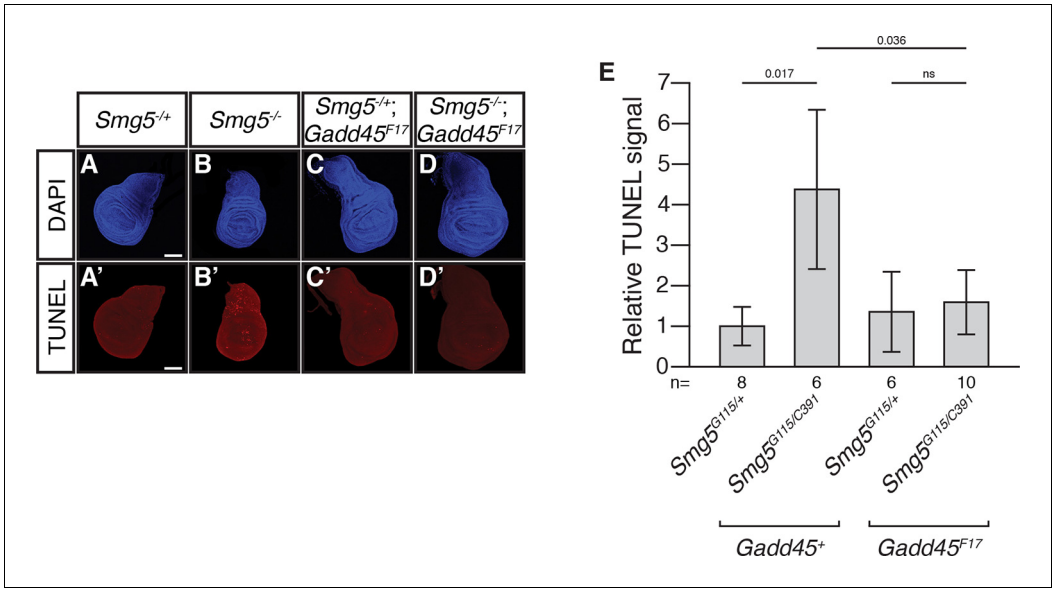

Figure 2—figure supplement 1. Loss of *Gadd45* suppresses ectopic cell death in *Smg5* mutant wing discs. (**A** to **D**) DAPI (blue) and (**A'** to **D'**) TUNEL (red) staining in late 3rd instar larval wing discs from $Smg5^{G115/+}$ (**A**); $Smg5^{G115/C391}$ (**B**); $Smg5^{G115/+}$ $Gadd45^{F17}$ (**C**); and $Smg5^{G115/C391}$ $Gadd45^{F17}$ (**D**) animals. $Smg5^{G115}$ and $Smg5^{C391}$ are null *Smg5* alleles (J.O.N. and M.M.M., unpublished). Scale bar represents 100 μm. (**E**) Relative TUNEL signal in wing discs, normalized to $Smg5^{G115/+}$. p-values determined by two-sided Student's t-test between indicated conditions are displayed. ns indicates a p-value greater than 0.05. Error bars represent 2 SEM.
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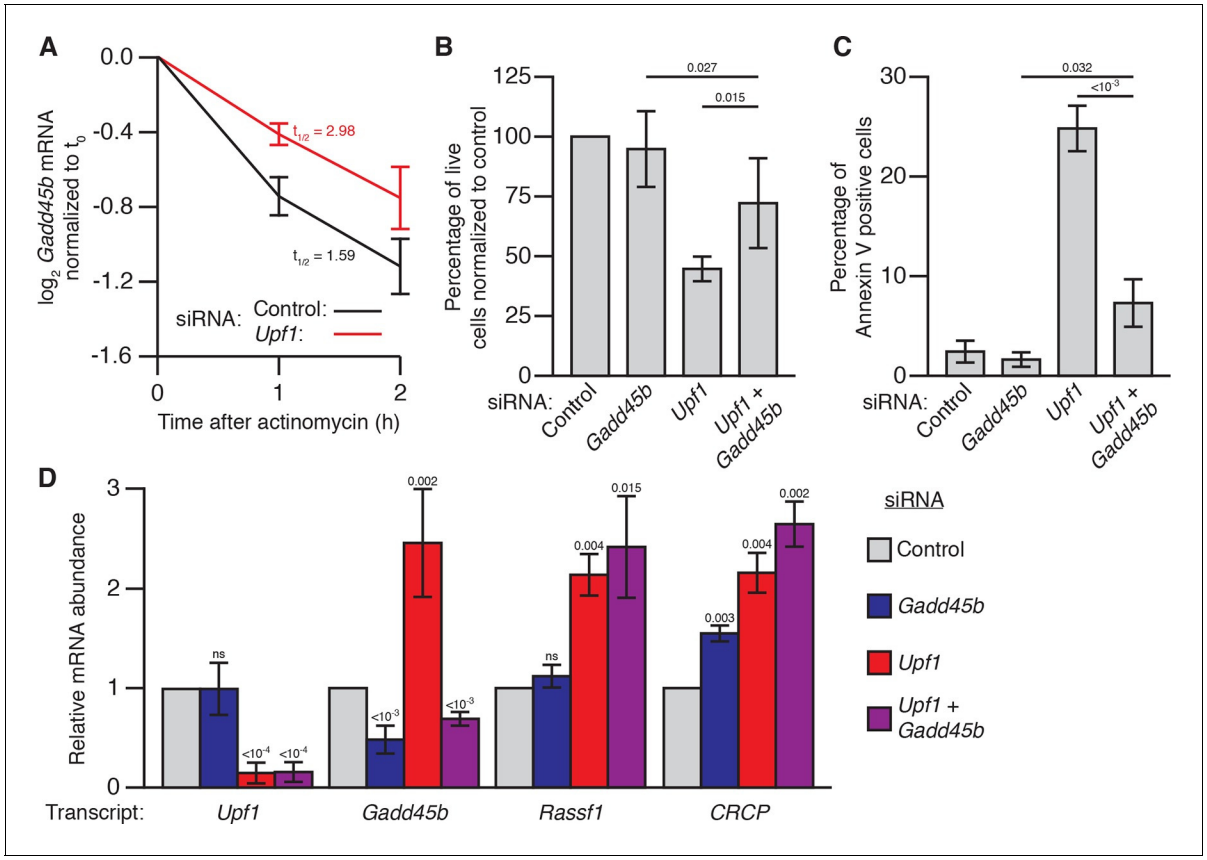

Figure 3. Gadd45b mediates cell lethality in Upf1 siRNA knockdown 3T3 mouse embryonic fibroblasts. (**A**) Relative *Gadd45b* mRNA expression measured by qRT-PCR in NIH-3T3 cells after 48 hr of control (black) or *Upf1* (red) siRNA treatment and 0 to 2 hr of actinomycin D treatment, normalized to expression prior to actinomycin treatment. The half-life calculated for each decay curve is indicated. (**B**) Relative viable cell count of *Upf1* and *Gadd45b* single and double siRNA treatment normalized to control siRNA. p-values display two-sided Student's t-test between indicated conditions. (**C**) Quantification of apoptosis as measured by annexin V staining. p-values display two-sided Student's t-test between indicated conditions. (**D**) Relative mRNA expression of *Upf1*, *Gadd45b*, and two mammalian endogenous NMD targets, *Rassf1* and *CRCP* (***Tani et al., 2012***) measured by qRT-PCR in *Gadd45b* and *Upf1* single and double siRNA knockdown cells, normalized to expression in the control siRNA condition. p-values display one-sided Student's t-test for each condition compared to control. Error bars represent 2 SEM.

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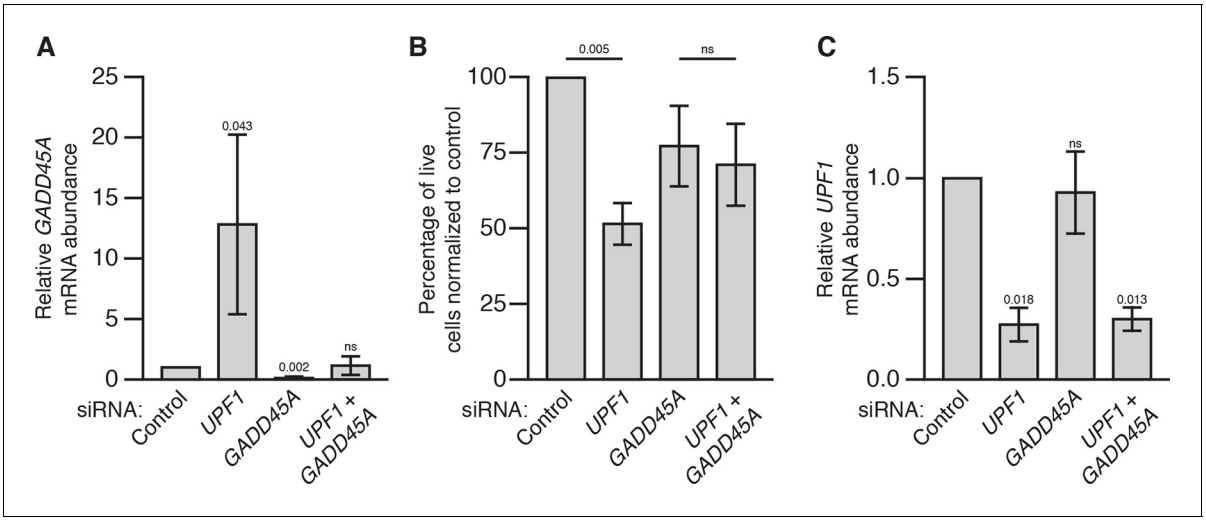

Figure 3—figure supplement 1. *GADD45A* mediates cell lethality in *Upf1* knockdown HEK293 cells. (**A**) Relative *GADD45A* mRNA expression measured by qRT-PCR in *UPF1* and *GADD45A* single and double siRNA knockdown 72 hr after siRNA transfection in HEK293 cells, normalized to expression in the control siRNA condition. p-values display one-sided Student's t-test for each condition compared to control. (**B**) Viable cell count of *UPF1* and *GADD45A* single and double siRNA-treated cells normalized to control siRNA -treated cells. p-values display two-sided Student's t-test between indicated conditions. (**C**) Relative *UPF1* mRNA expression measured by qRT-PCR in *UPF1* and *GADD45A* single and double siRNA knockdown, normalized to expression in control siRNA condition. p-values display one-sided Student's t-test for each condition compared to control. Error bars represent 2 SEM.

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