Sandu et al., http://www.jcb.org/cgi/content/full/jcb.201004086/DC1

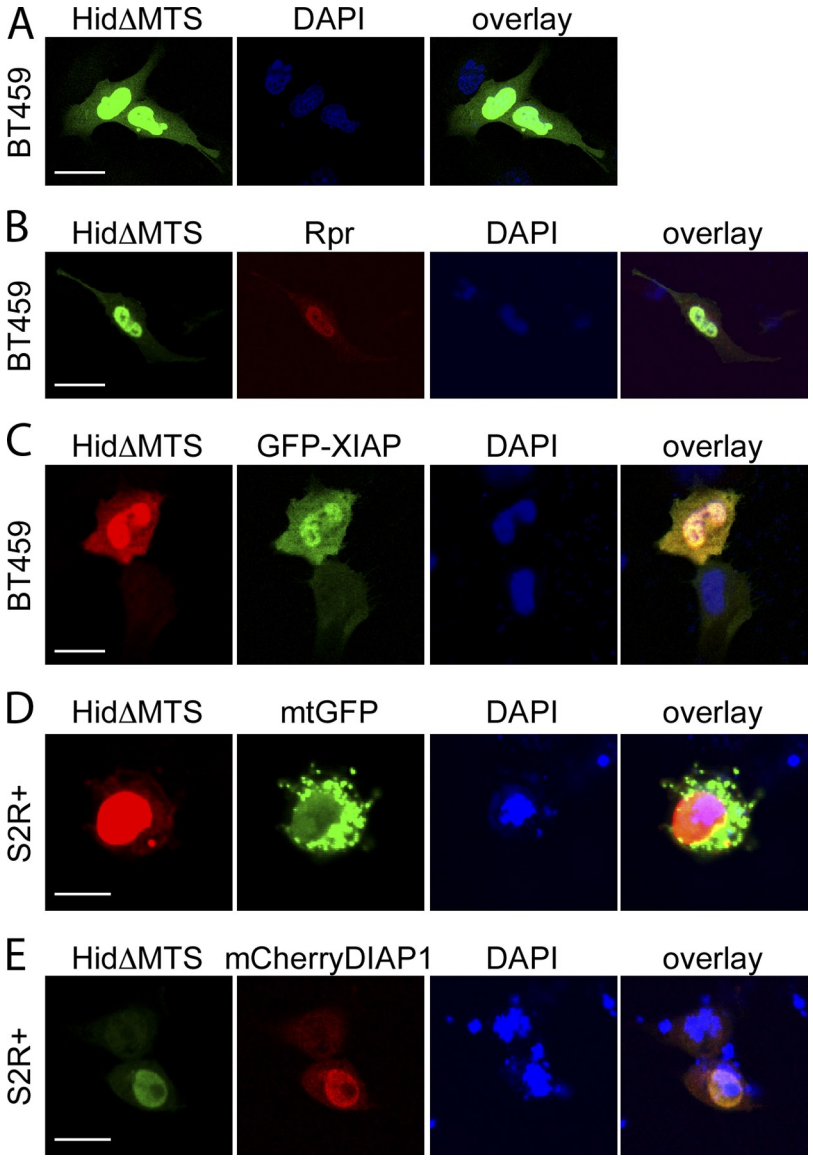

Figure S1. **Immunolocalization of HidΔMTS and its effects on Rpr, XIAP, and DIAP1 localization.** (A) HidΔMTS localizes to the nucleus in BT549 cells. The cells were transfected with a HidΔMTS-Myc construct, and immunostained with an anti-Myc antibody coupled to a FITC-labeled secondary antibody. HidΔMTS is shown in green, nuclei are shown in blue. Bar, 20 µm. (B) Rpr colocalizes with HidΔMTS in the nucleus of BT549 cells. The cells were cotransfected with HidΔMTS-Myc and Rpr-HA constructs, and immunostained with an anti-Myc antibody coupled to a FITC-labeled secondary and an anti-HA antibody coupled with an Alexa 546–labeled secondary antibody. HidΔMTS is shown in green, Rpr is shown red. Bar, 20 µm. (C) GST-XIAP is targeted to the nucleus after cotransfection with HidΔMTS. BT549 cells cotransfected with HidΔMTS-Myc and GFP-XIAP constructs. HidΔMTS-Myc was stained with an anti-Myc antibody coupled with a Cy3-labeled secondary antibody. GFP-XIAP cellular distribution was visualized by GFP fluorescence. Nuclei were labeled by DAPI. Bar, 20 µm. (D) HidΔMTS localizes to the nucleus in S2R+ cells. S2R+ cells were transfected with HidΔMTS-Myc and mtGFP constructs. HidΔMTS nuclear localization was revealed by immunostaining with an anti-Myc antibody coupled to a Cy3-labeled secondary antibody. Mitochondria were visualized by GFP fluorescence. Nuclei were labeled by DAPI. Bar, 5 µm. (E) mCherryDIAP1 colocalizes with HidΔMTS to the nucleus. S2R+ cells were cotransfected with HidΔMTS-Myc and mCherryDIAP1 constructs. HidΔMTS localization was revealed by immunostaining with an anti-Myc antibody coupled to a FITC-labeled secondary antibody; DIAP1 localization was revealed by mCherry fluorescence. DAPI was used to label nuclei. Bar, 5 µm.

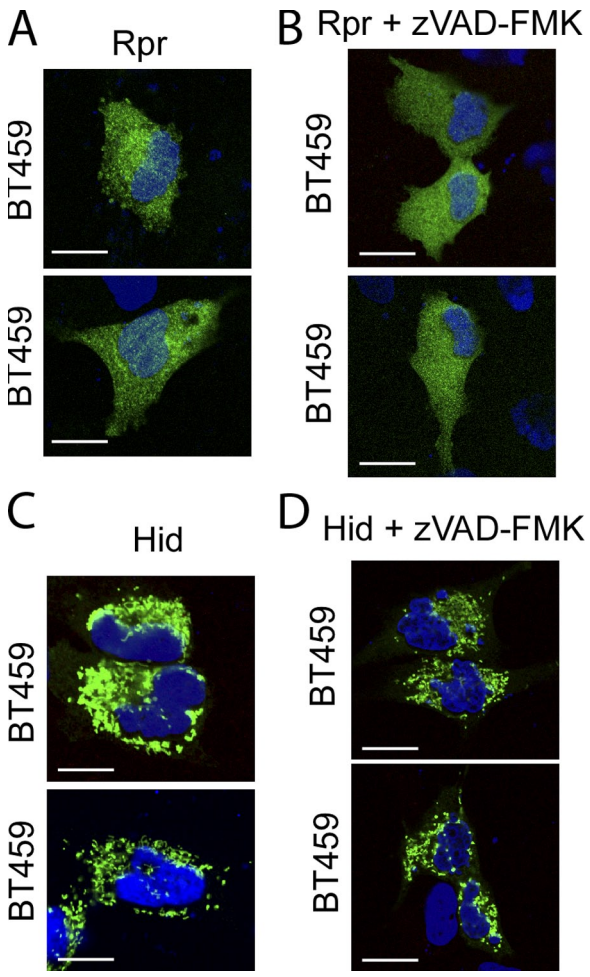

Figure S2. **Effect of zVAD-FMK on Rpr or Hid intracellular localization.** BT549 cells were transfected with a Rpr-HA construct in the absence (A) or presence (B) of 20 µM zVAD-FMK. 16 h after transfection, the cells were fixed and stained with an anti-HA antibody coupled with an Alexa 488–labeled secondary antibody. DAPI was used for nuclear staining. Rpr intracellular localization is not affected by addition of zVAD-FMK. In the following two panels, BT549 cells were transfected with a Hid-HA construct in the absence (C) or presence (D) of 20 µM zVAD-FMK. 16 h after transfection, the cells were fixed and stained with an anti-HA antibody coupled with an Alexa 488–labeled secondary antibody. DAPI was used for nuclear staining. Hid mitochondrial localization is not affected by addition of zVAD-FMK. Bar, 20 µm.

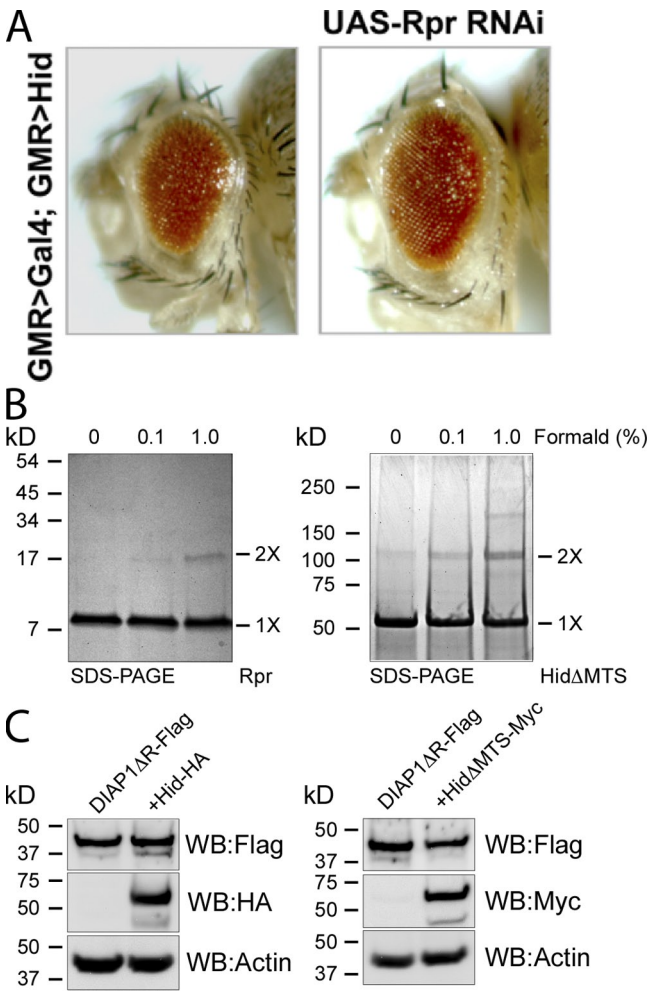

Figure S3. **Hid's ability to induce cell death, ubiquitinate DIAP1, and form oligomeric species.** (A) Rpr mRNA knockdown rescues to degree Hid-induced rough eye in *Drosophila*. (Left) A rough eye phenotype caused by overexpression of Hid in the eye. Genotype: GMR>Gal4/+;Sco/GMR>Hid;Sb/TM6B. (Right) Partial rescue of the Hid-induced rough eye phenotype by Rpr mRNA knockdown. Genotype: GMR>Gal4/+;Sco/GMR>Hid;Sb/UAS:Rpr RNAi. Crosses were incubated at 21°C. (B) Formaldehyde cross-linking reveals formation of oligomeric species by Rpr and HidΔMTS. (Left) Rpr forms oligomeric species after formaldehyde cross-linking. 5 µg purified Rpr protein was incubated for 10 min at room temperature in the absence or presence of 0.1% or 1.0% formaldehyde. The samples were neutralized with 3 M Tris, pH 8.0, and separated on SDS-PAGE. Appearance of dimeric species is visible after Coomassie staining. (Right) Formation of oligomeric species by HidΔMTS after formaldehyde cross-linking. 5 µg purified HidΔMTS was incubated at room temperature in the absence or presence of 0.1% or 1.0% formaldehyde. After incubation the reaction was neutralized by addition of 3 M Tris, pH 8.0, and samples prepared and separated by SDS-PAGE. Appearance of dimeric and oligomeric species is visible after Coomassie staining. (C) Hid full-length or HidΔMTS cannot effectively induce DIAP1ΔR degradation in HEK293 cells. (Left) Western blots on cell extracts derived from HEK293 cells, transfected with either DIAP1ΔR-Flag or DIAP1ΔR-Flag and Hid-HA constructs. Expression of Hid full-length does not induce degradation of DIAP1ΔR. Actin was used as a loading control. Expression level of DIAP1ΔR, Hid, and Actin was assessed with anti-Flag, anti-HA, and anti-Actin antibodies. (Right) Western blots on cell extracts derived from HEK293 cells, transfected with either DIAP1ΔR-Flag or DIAP1ΔR-Flag and HidΔMTS-Myc. Expression of HidΔMTS does not cause effective DIAP1ΔR degradation. Actin was used as a loading control. Expression level of DIAP1ΔR, HidΔMTS, and Actin was assessed with anti-Flag, anti-Myc, and anti-Actin antibodies.