Xbp1-independent Ire1 signaling is required for photoreceptor differentiation and rhabdomere morphogenesis in *Drosophila* by Dina S. Coelho, Fatima Cairrão, Xiaomei Zeng, Elisabete Pires, Ana V. Coelho, David Ron, Hyung Don Ryoo and Pedro M. Domingos

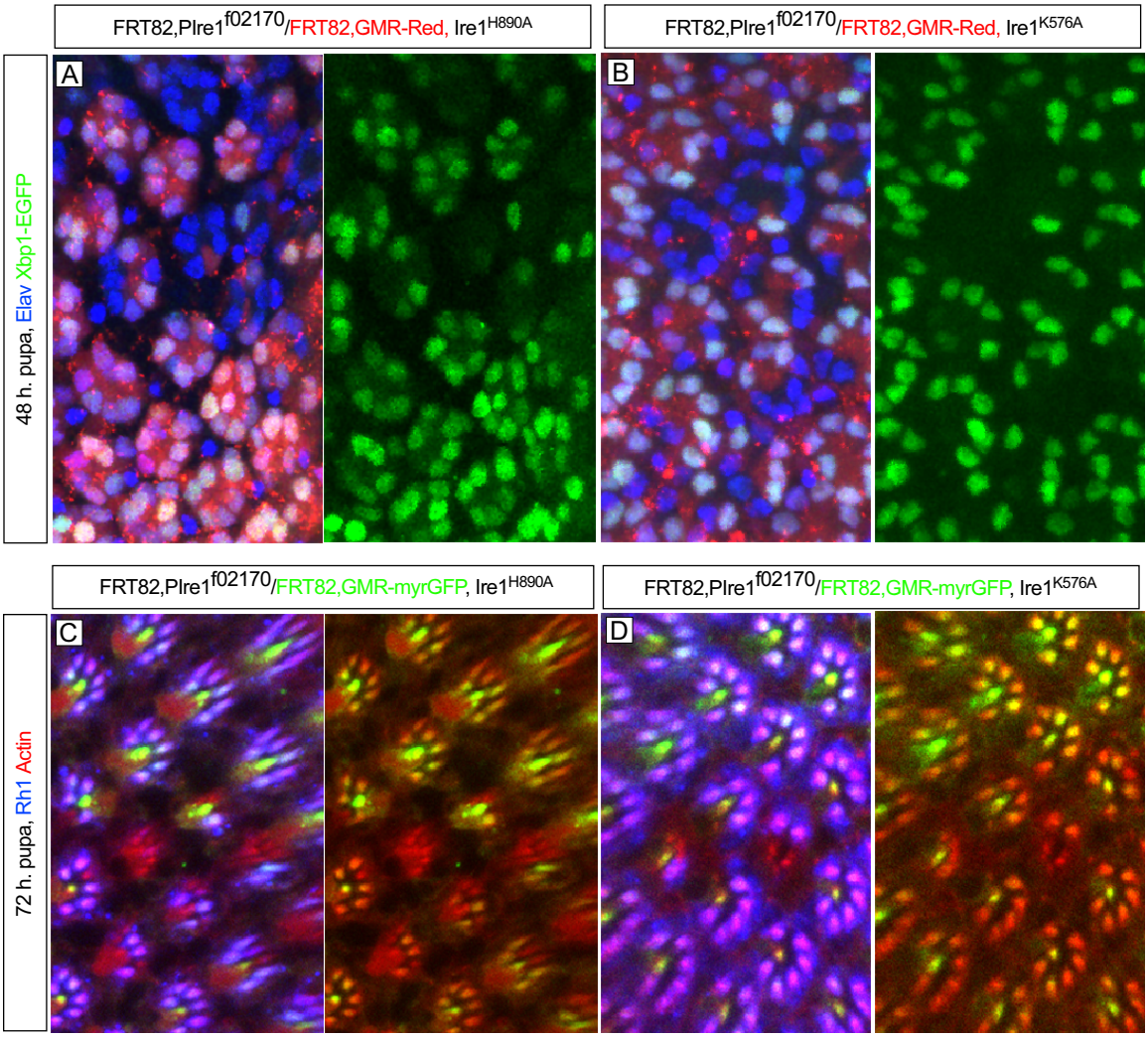


**Figure S1. Ire1 RNase (Ire1$^{H890A}$) and kinase (Ire1$^{K576A}$) mutants fail to rescue Xbp1-EGFP expression and Rh1 localization in the rhabdomeres of *PBac{WH}Ire1$^{f02170}$* homozygous photoreceptors, Related to Figures 2 and 4.**

(A) Ire1$^{H890A}$ and (B) Ire1$^{K576A}$ fail to rescue expression of Xbp1-EGFP (green) in clones of with *PBac{WH}Ire1$^{f02170}$* homozygous cells, labeled by the absence of DsRed. Elav is in blue.

(C) Ire1$^{H890A}$ and (D) Ire1$^{K576A}$ fail to rescue rhabdomeric localization of Rh1 (blue) in clones of *PBac{WH}Ire1$^{f02170}$* homozygous cells, labeled by the absence of myrGFP (green). Actin is in red.

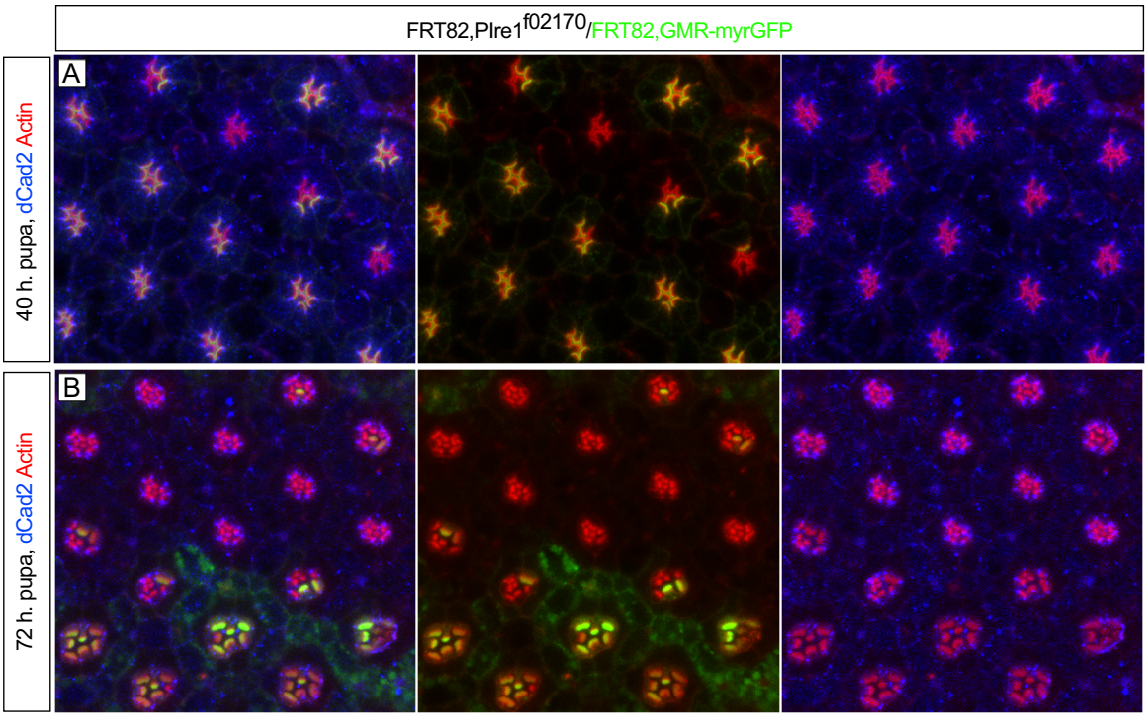


**Figure S2. Cadherin is normally localized in *PBac{WH}Ire1$^{f02170}$* homozygous photoreceptors, Related to Figure 3**

(A) 40 h pupal eye with *PBac{WH}Ire1$^{f02170}$* homozygous cells, labeled by the absence of myrGFP (green), show normal localization of Cadherin (blue) and Actin (red).

(B) 72 h pupal eye with *PBac{WH}Ire1$^{f02170}$* homozygous photoreceptors show normal localization of Cadherin (blue).

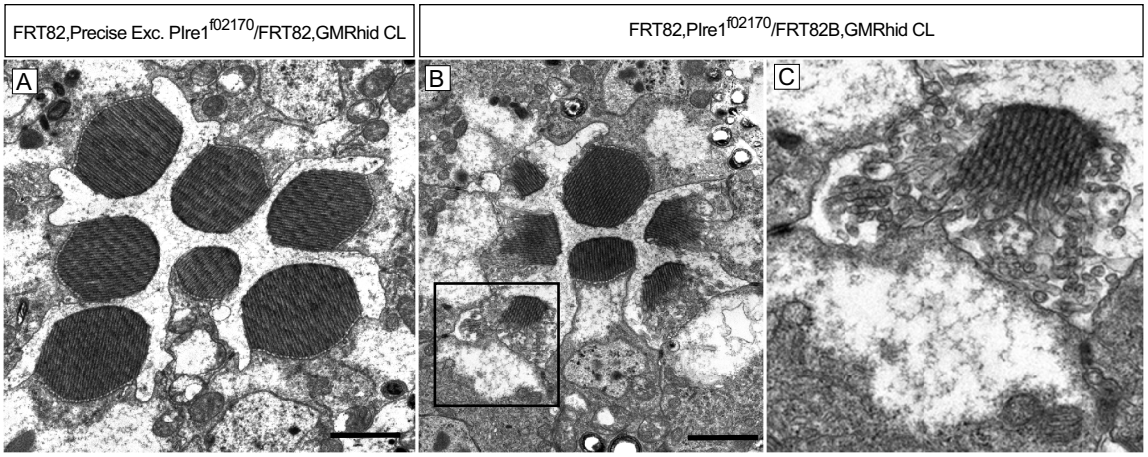


**Figure S3. Electron microscopy analysis of adult *PBac{WH}Ire1$^{f02170}$* homozygous photoreceptors, Related to Figure 4**

(A) Control ommatidium homozygous for a precise excision of *PBac{WH}Ire1$^{f02170}$* presents normal rhabdomeres.

(B) Ommatidium homozygous for *PBac{WH}Ire1$^{f02170}$* presents rhabdomeres of reduce size with disorganized vesicles at the base of each rhabdomere

(C) Magnification of inset in (B). Scale bars, 1 μm.

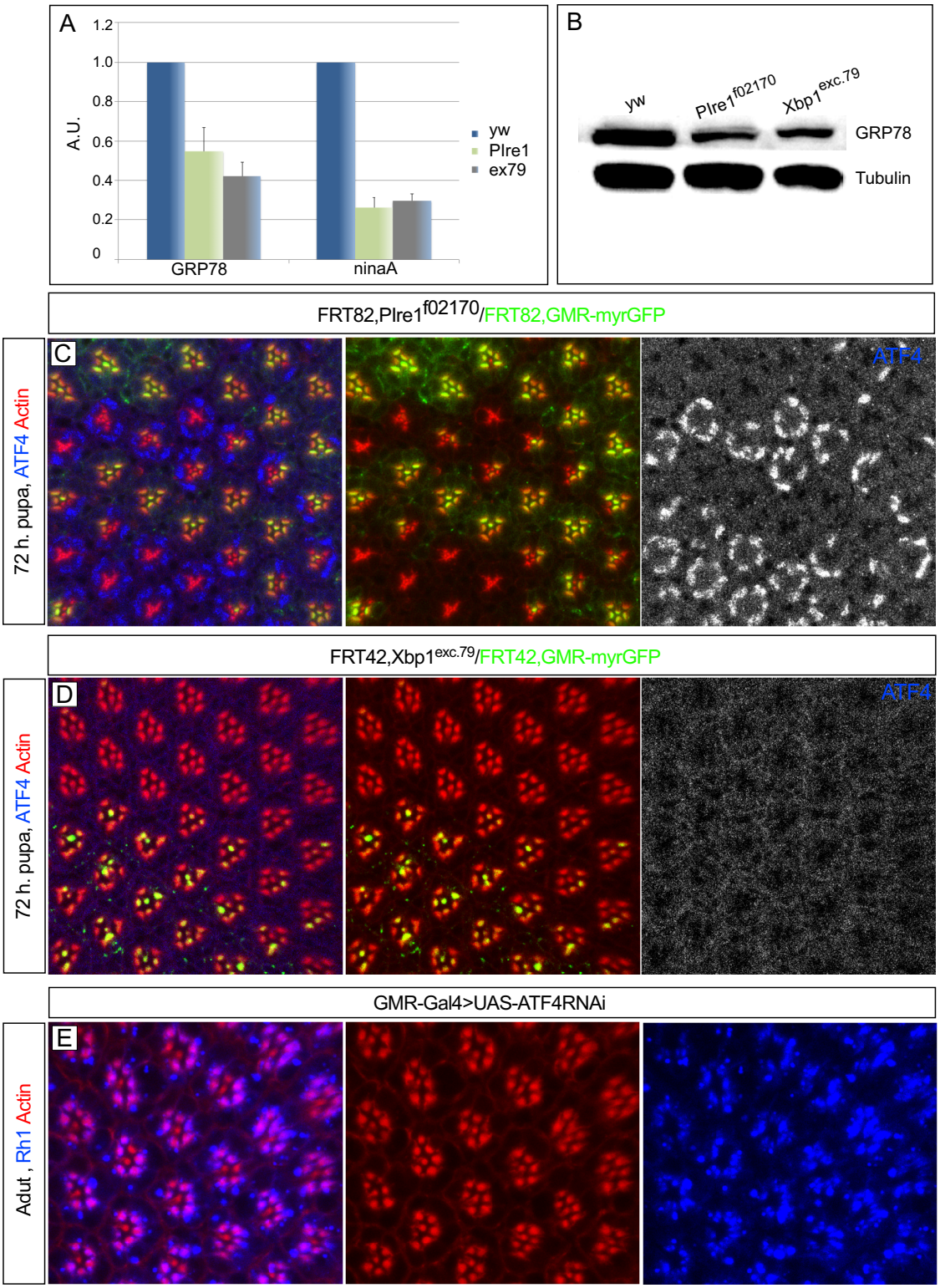


**Figure S4. Analysis of ER stress markers in Ire1 and Xbp1 mutant photoreceptors, Related to Figures 4 and 5**

(A) qRT-PCR analysis of GRP78 and ninaA in yw (control), *PBac{WH}Ire1*$^{f02170}$ and Excision 79 homozygous adult eyes.

(B) Western blot analysis of GRP78 in yw (control), *PBac{WH}Ire1*$^{f02170}$ and Excision 79 homozygous adult eyes.
(C) ATF4 (blue and monochrome) is upregulated in *PBac{WH}Ire1*$^{f02170}$ homozygous clones.
(D) ATF4 (blue and monochrome) is not upregulated in Excision 79 homozygous clones.
(E) GMRGal4>UAS-ATF4-RNAi (VDRC #2935) presents normal loading of Rh1 (blue) into the rhabdomere (actin, red).

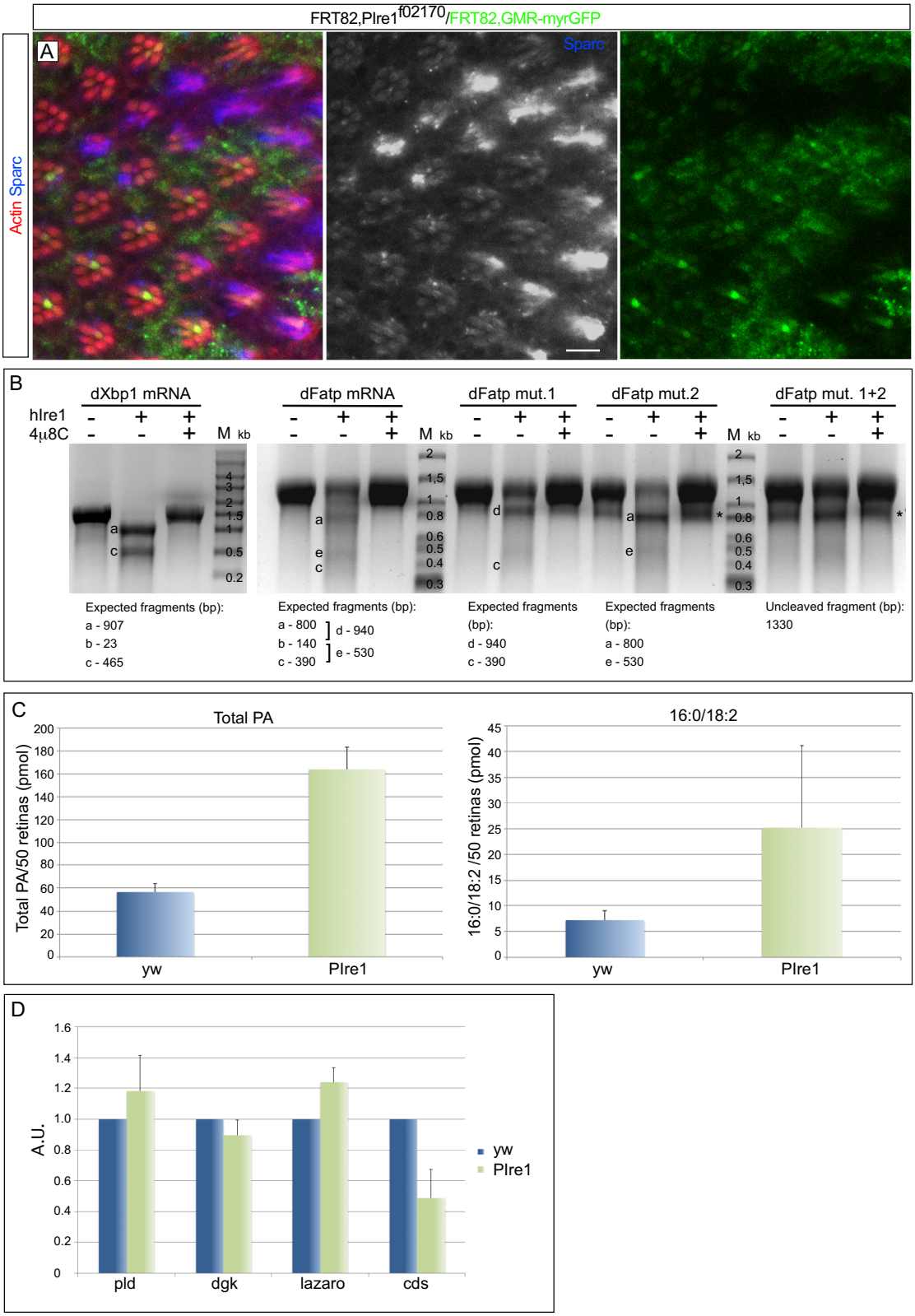


**Figure S5. RIDD in the *Drosophila* eye, Related to Figure 6**

(A) Clones of *PBac{WH}Ire1*$^{f02170}$ homozygous ommatidia (absence of myrGFP, green) have higher levels of Sparc (blue and monochrome). Actin is in red.

(B) *Drosophila* Xbp1 and Fatp mRNAs are cleaved by purified human Ire1. The size (in base pairs, bp) of the expected fragments is indicated below each panel. An unexpected band (*) in the Fatp Mut2 panels probably results from an mRNA conformation that was resistant to the denaturing conditions used.
(C) Quantification of phosphatidic acid (PA) species in yw (control) and *PBac{WH}Ire1*$^{f02170}$ homozygous adult eyes by LC-MS. Total PA (left) includes the quantification of 16:0/16:1, 16:0/18:0, 16:0/18:2, 16:1/18:2, 18:1/18:2 and 16:0/20:4 species.
(D) qRT-PCR analysis of Pld, dgk, lazaro and cds in yw (control) and *PBac{WH}Ire1*$^{f02170}$ homozygous adult eyes.