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Supplemental information

Fat body Ire1 regulates lipid homeostasis through the Xbp1s-FoxO axis in *Drosophila*

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Supplementary information

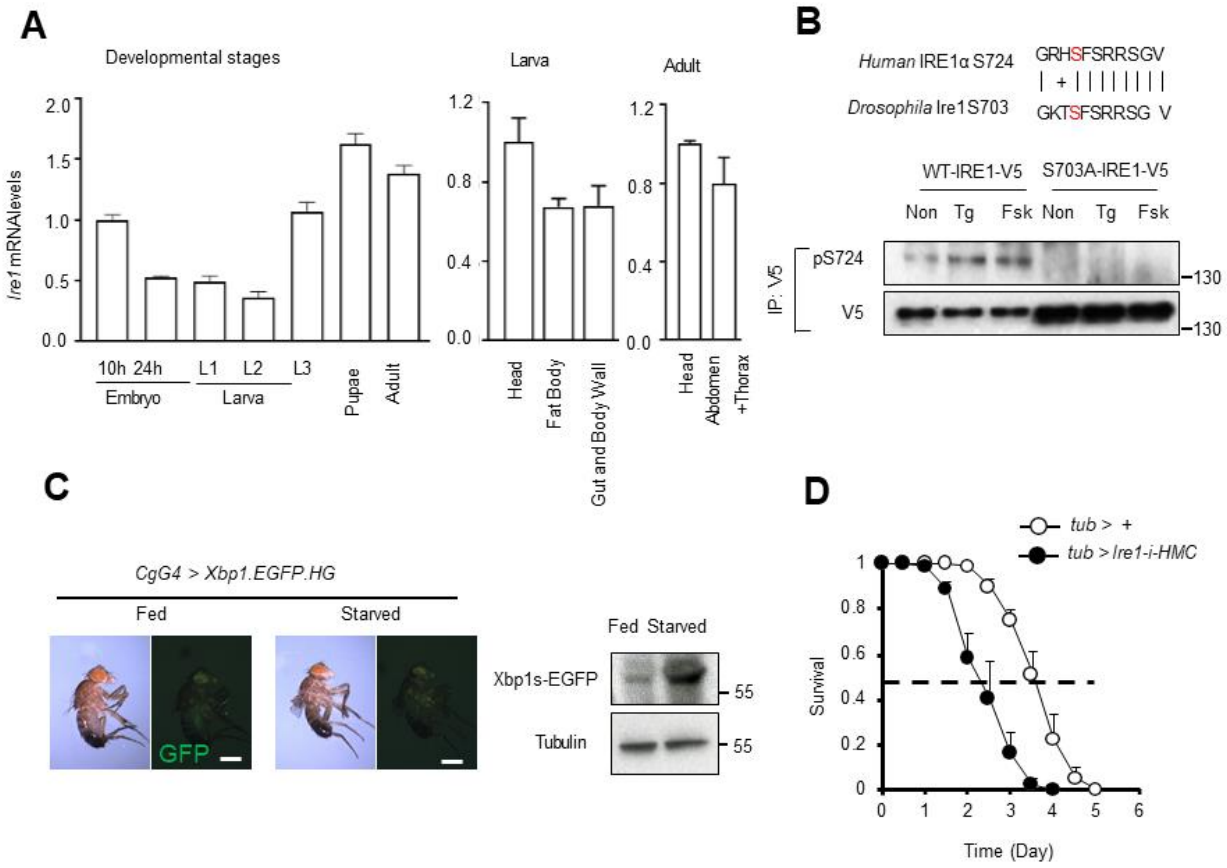

Figure S1. **Physiological analysis of Ire1 expression and activation. Related to Figure 1.**

(**A**) Relative expression levels of Ire1 mRNA at different developmental stages (left) and multiple larval (middle) and adult (right) tissues of w^{1118} flies (n=3). (**B**) A commercial antibody that specifically recognizes phosphorylation of *Drosophila* Ire1 at S703, equivalent to S724 of human IRE1α. S2 cells were transfected with plasmids expressing a wild-type or S703A-mutant Ire1 fused to a V5 tag for 48 hrs and treated with 500 nM Thapsigargin (Tg) or 10 μM Forskolin (Fsk) for 30 min prior to RIPA lysis. The lysates were pulled down using beads conjugated with V5 antibodies and subsequently used for immunoblot analysis. (**C**) Representative images (up) and immunoblots (down, against GFP) of flies overexpressing Xbp1.EGFP.HG, a reporter monitoring Xbp1 splicing, in the fat body under either 48-hrs fed or starved condition, scare bars=1 mm. (**D**) Survival rates of flies with ubiquitous *Ire1* knockdown, induced by the RNAi line HMC05163, under starvation (5 days of age; n=160 flies/group). $\chi 2$=195.6, P<0.0001 by log-rank test. Data are presented as the mean ± SEM. *P<0.05.

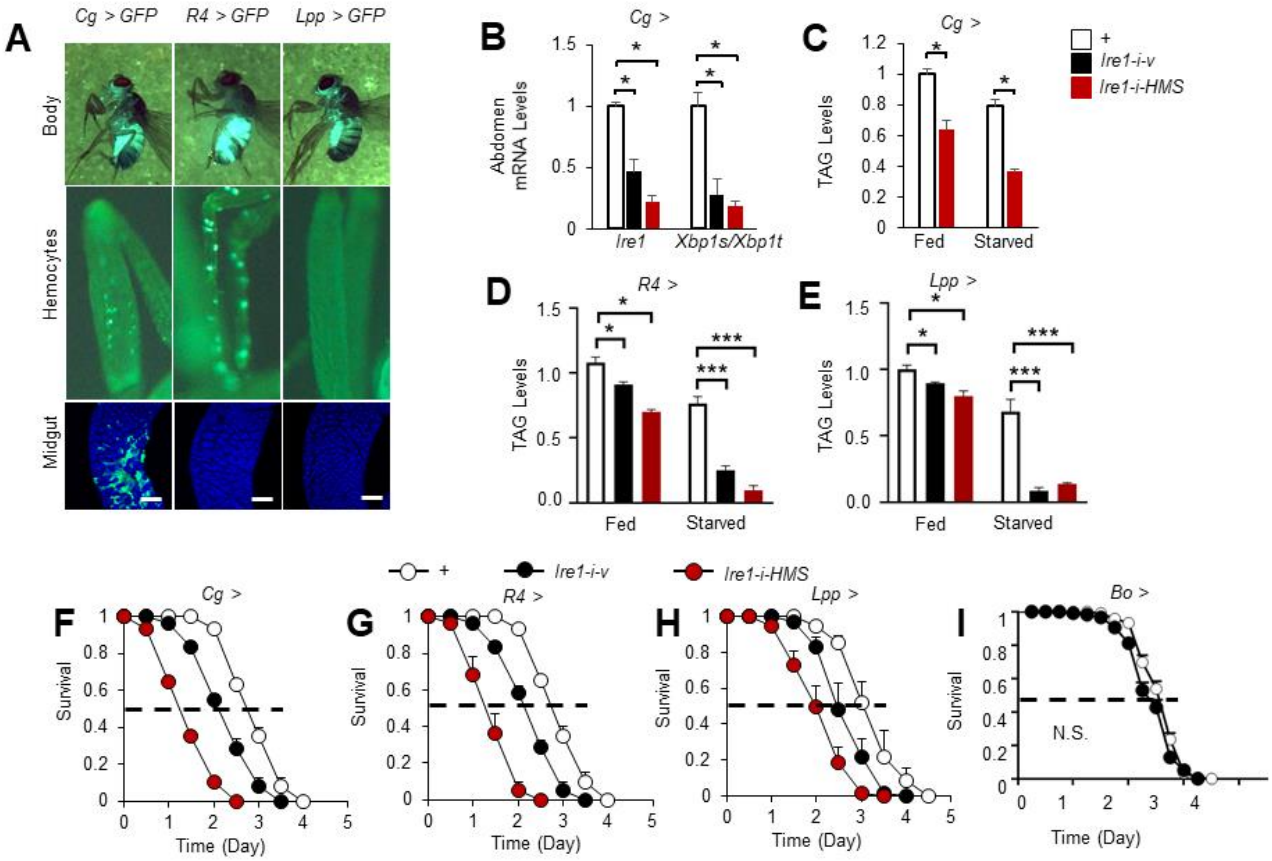

Figure S2. Ire1 knockdown in the fat body increases lipid mobilization and starvation sensitivity. Related to Figure 2. (**A)** Representative images of GFP expression in the adult flies driven by different fat-body *GAL4* lines, scare bars=100 μm. (**B**) mRNA levels of *Ire1* and *Xbp1s/Xbp1t* in the abdomens of flies with Ire1 RNAi (*Ire1-i-v*, *v39561*; *Ire1-i-HMS*, *HMS03003*) in the fat body (40 flies/group, 10 flies pooled per sample). (**C-E**) Whole body TAG levels of male flies with indicated genotypes under either 24-h fed or starved condition (80 flies/group, 20 flies pooled per sample). (**F-I**) Survival rates of starved male adult flies of the indicated lines (n=120 flies/group). Difference between + versus *Ire1-i-v* (**F**, χ^2=194.9; **G**, χ^2=147.4; **H**, χ^2=186.4; **I**, χ^2=3.572) and + versus *Ire1-i-HMS* (**F**, χ^2=165.1; **G**, χ^2=199.5; **H**, χ^2=186.7) in fat-body groups are all statistically significant. P<0.001 by log-rank test. Data are presented as the mean ± SEM. *P<0.05 by two-way ANOVA.

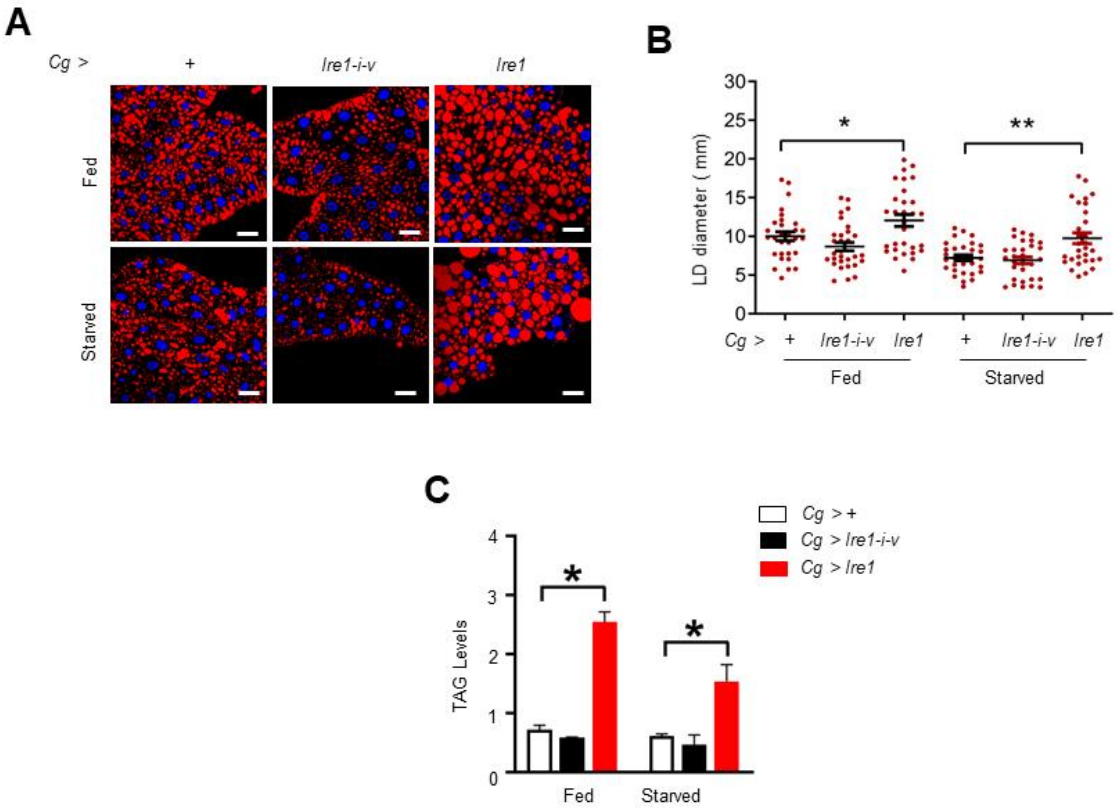

Figure S3. Fat body Ire1 regulates lipid content in the larvae. Related to Figure 3. *Cg*>+, *Cg>Ire1-i-v* and *Cg>Ire1* flies were ad libitum fed or starved for 24 hrs. (**A**) LDs of isolated fat bodies from the 3rd-instar larvae were indicated by Nile Red staining. Representative images are shown, scare bars=50 μm. (**B**) Quantification of LD diameter on the right (12 images/group). Each point represents a single LD. (**C**) TAG contents in the whole larvae were measured (40 larvae/group, 10 larvae per pooled sample). Data are presented as the mean ± SEM. **P*<0.05, ***P*<0.001 by two-way ANOVA.

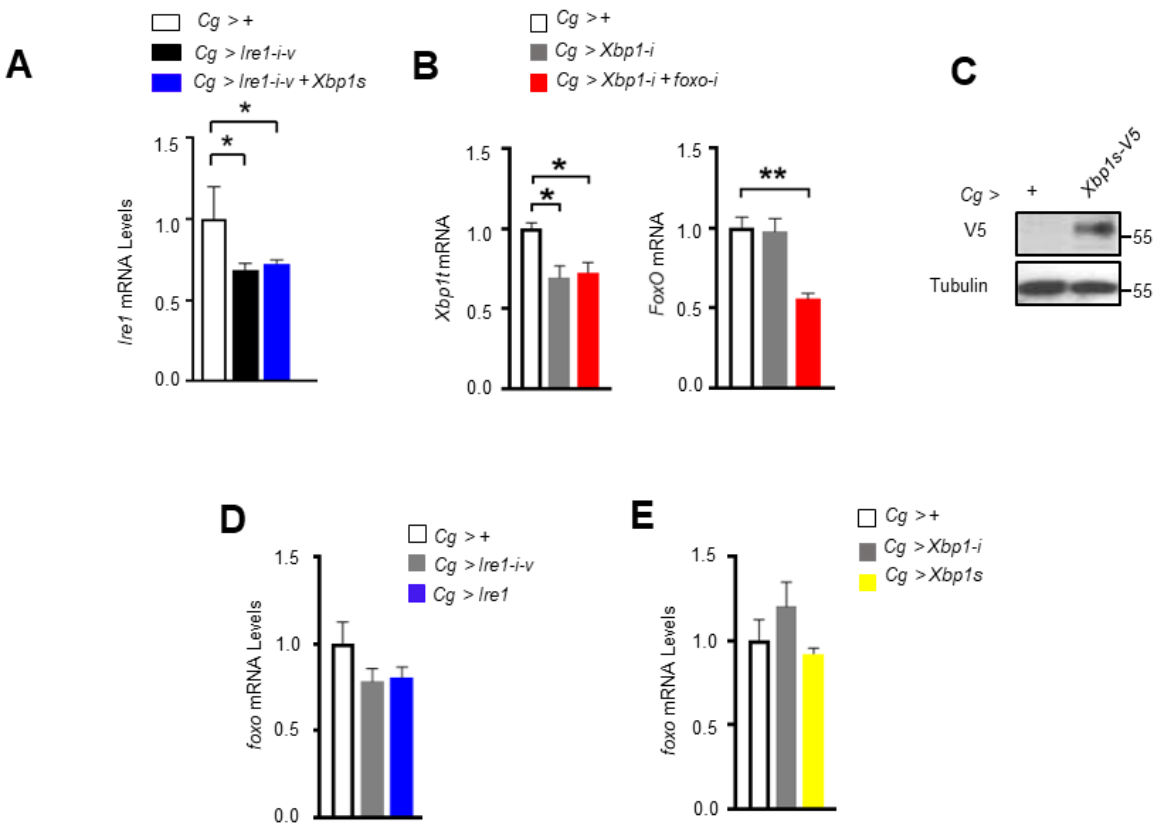

Figure S4. Ire1 and Xbp1 knockdown or overexpression in the fat body. Related to Figure 4. *Ire1*, *Xbp1* and *foxo* mRNA levels (**A**, **B**, **D** and **E**) and V5-tagged Xbp1s protein levels (**C**) in the abdomen of indicated 3-day old adult flies (40 flies/genotype, 10 flies pooled per replicate). Data are presented as the mean ± SEM. **P*<0.05, ***P*<0.001 by one-way ANOVA.

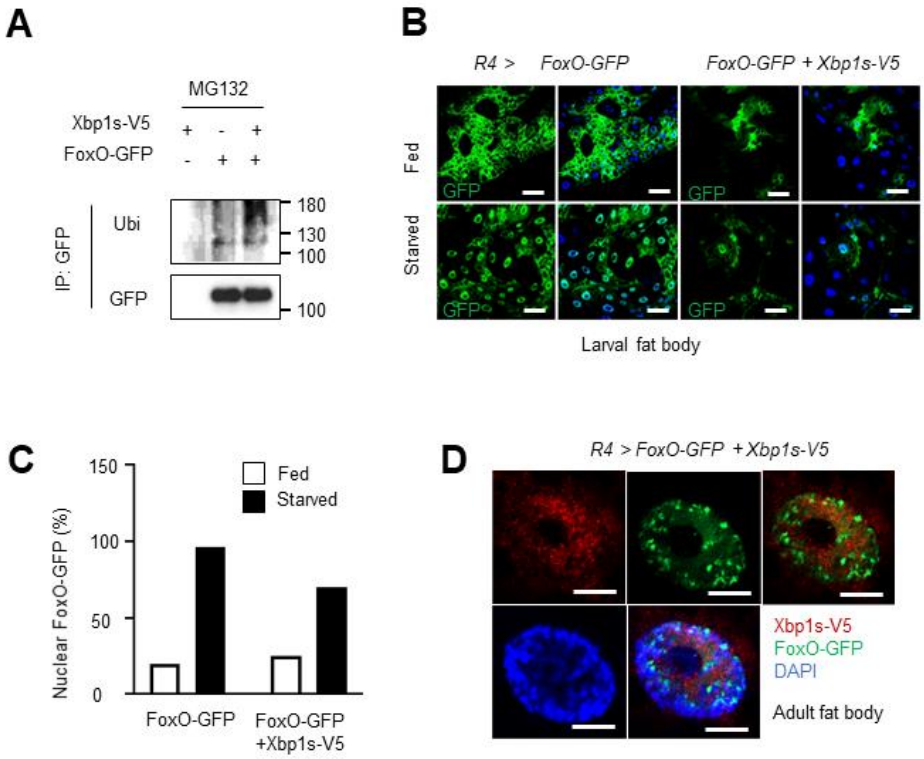

Figure 5. The Xbp1s effects on FoxO protein. Related to Figure 5. (**A**) S2 cells were transiently transfected to express indicated proteins for 48 hours prior to immunoprecipitation and immune blot analysis of ubiquitinated FoxO-GFP. (**B-C**) Cellular localization of FoxO-GFP in fat body from indicated 3rd instar larvae fed or 5-hrs starved condition. Shown are representative images of fat body cells that express FoxO-GFP (10 larvae per genotype), scare bar=50 μm (**B**) and quantification of nuclear/total FoxO-GFP (**C**). (**D**) Representative images of the intranuclear localizations of FoxO-GFP and Xbp1-V5 in the fat body of 5d-old adult flies after 24-hrs starvation, scare bar=10 μm.

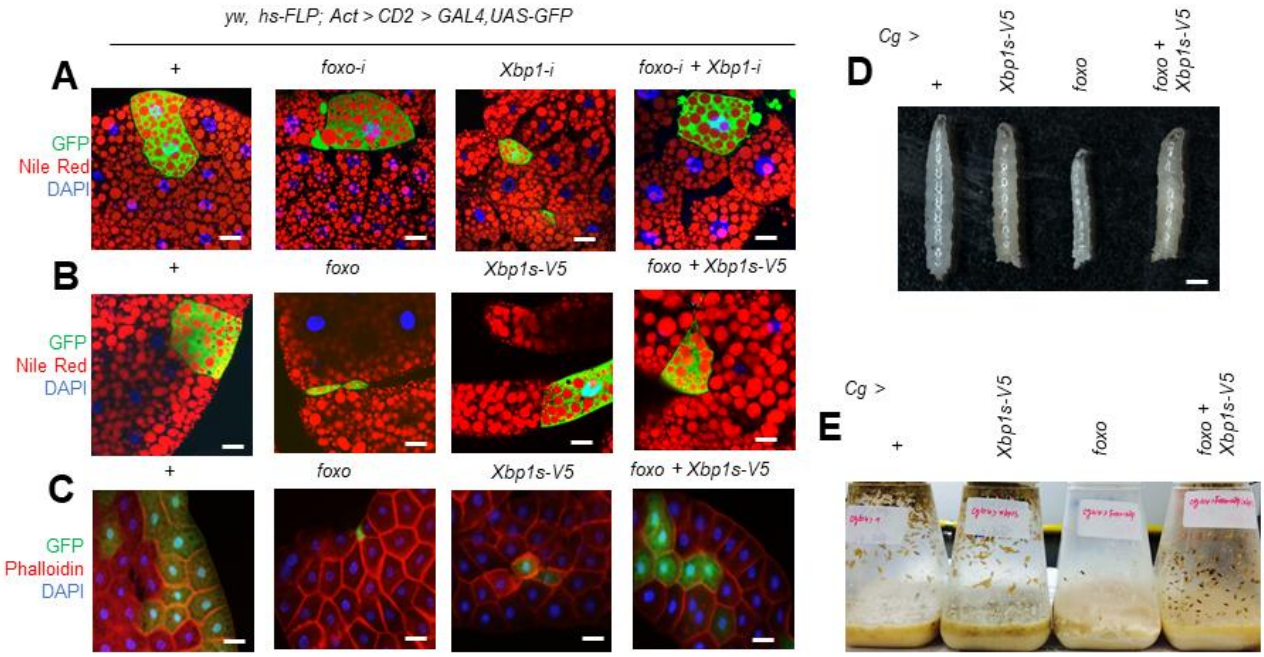

Figure S6. FoxO is required for Ire1/Xbp1 regulation of growth and lipid metabolism. Related to Figure 6. (**A-C**) Representative images of intracellular LDs, scare bars=20 μm (**A-B**) or cell sizes, scare bars=40 μm (**C**) of 3rd-instar larval fat-body cell clones with indicated gene expression. (**D-E**) Representative images of 3rd-instar larval growth , scare bars=1 mm (**D**) and pupation (**E**) of indicated flies.