Supplementary information: "Src tyrosine kinase signaling antagonizes nuclear localization of FOXO and inhibits its transcription factor activity"

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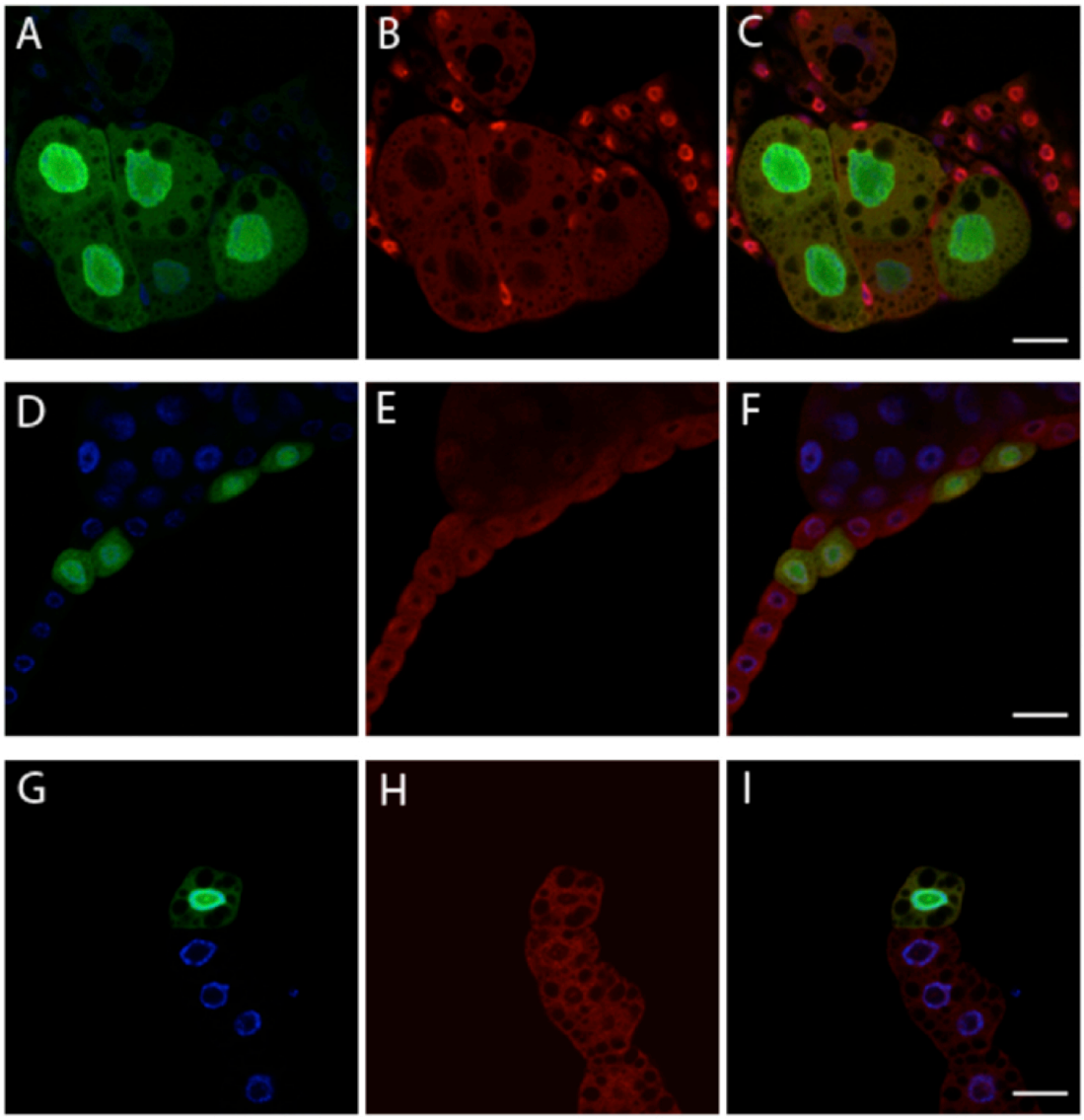
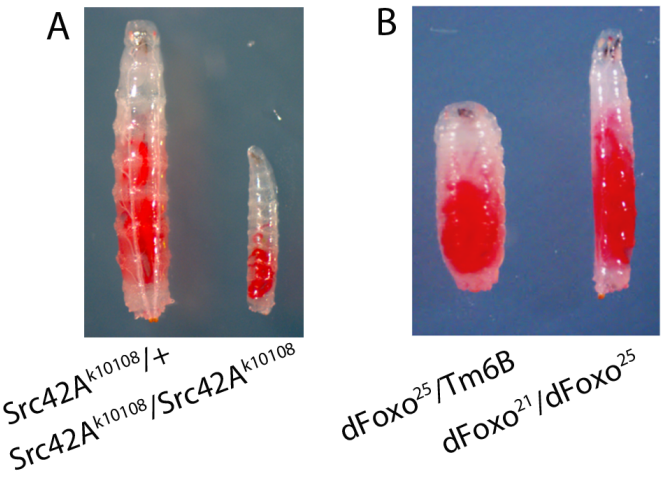
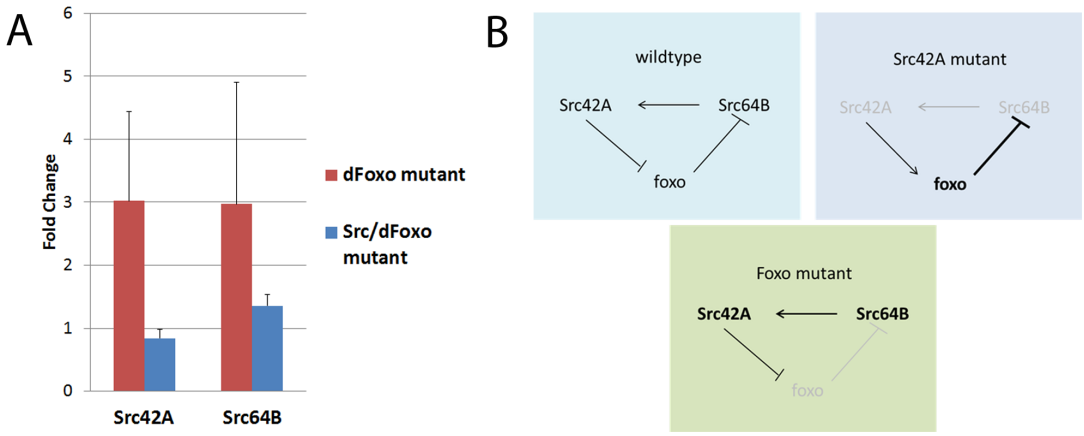


Supplementary Figure 1: Effect of insulin receptor and CSK overexpression on fatbody cell size and dFOXO localization. Fatbody clones from late 2nd to early 3rd instar larvae are shown. Immunostaining was performed with mouse α-GFP (green),

rabbit α-dFOXO (red) antibodies and a nuclear DAPI counterstain (blue). (**A-C**) Larvae were raised on yeast for 48 h and starved on PBS for 24 h. GFP-positive, transgene-expressing cells are large despite nutrient deprivation and display cytoplasmic dFOXO localization, while wildtype cells are small and contain mostly nuclear dFOXO. Genotype is *y w hs-flp;; Act>CD2>Gal4 UAS-GFP/UAS dInR*. (**D-F**) Larvae were raised on yeast for 48 h and starved on PBS for 24 h. Genotype is *y w hs-flp;; Act>CD2>Gal4 UAS-GFP/UAS dCSK-IR*. (**G-I**): Larvae were fed on yeast for 48 h. CSK (C-terminal Src-kinase) is a negative Src regulator, but does not have any effect on the size of fatbody cells or on dFOXO localization in our experiments in either starved or fed conditions. Genotype is *y w hs-flp;; Act>CD2>Gal4 UAS-GFP/UAS dCSK*. Scale bars represent 20 µM



Supplementary Figure 2: Food ingestion in Src42A (**A**) and dFOXO (**B**) mutants is normal. 3rd instar hetero- and homozygous *dFOXO*^{25} mutant larvae ingest food normally, as shown by the red Carmine dye in the yeast paste.



Supplementary Figure 3: A putative feedback loop from Src to Src via dFOXO. (A) In dFOXO mutants, the transcription of both Src genes is increased. In the Src-dFOXO double mutants, this increase is absent. It may therefore be speculated that dFOXO is involved in the transcriptional regulation of Src genes (B). Such a "double negative" feedback mechanism (Src inhibits dFOXO inhibits Src) would result in a net positive effect of Src activity on Src transcription, providing a working hypothesis that may explain the observation shown in Figure 3A and 3B, in which both Src genes are transcriptionally repressed in both Src mutants.