Online Methods

Drosophila Stocks and Genetics

The following fly stocks were used : *domeless-gal4 (*S. Noselli*)*, *hml$^{\Delta}$-gal4 UAS-2xeGFP* (S. Sinenko), *UAS-GTPx1* and *UAS-cat* (F. Missirlis), *puc^{e69}/TM3,Sb* (A. Martinez-Arias) the FLW-1 and LW-1 lines (for assessing polycomb downregulation are form R. Paro),

OregonR, *w; P{tubP-GAL80^{ts}}10; TM2/TM6B, Tb, y^{1} w^{67c23}; P{SUPor-P}Sod2^{KG06854}*, *UAS-2xeYFP* on the X chromosomes, *y^{1} w; P{lacW}Thor^{k13517}*, *w; P{UAS-bsk.K53R}20.1a* and *y^{1} w*; P{UAS-foxo.P}2* stocks from the Bloomington stock center. The RNAi stocks (*UAS-ND75RNAi, UAS-ND42RNAi, UAS-E(Pc)RNAi and UAS-ph-pRNAi*) were obtained from National Institute of Genetics Fly Stock Center (Japan). In all instances, crosses were set up at 25°C for a day. For the third instar RNAi experiments and all suppression experiments, the larvae were shifted to 18°C for 2 days, and then transferred to 29°C until dissection. For the second instar experiments, larvae were transferred to 29°C directly (without an intervening shift to 18°C). For the overexpression experiments (involving *UAS-foxo*, and *UAS-Gtpx-1*) the larvae were maintained at 25°C until dissection. In all instances, at least ten lymph glands were examined.

Gene Expression Analyses

Total RNA was extracted from third instar lymph glands using TRIZOL (Invitrogen), followed by RNA cleanup with the RNAeasy Kit (Qiagen). The RNA QuantiTect Reverse Transcription Kit (Qiagen) was used for first strand cDNA synthesis, and quantitative real-time PCR was performed with the QuantiTect SYBR Green PCR Kit (Qiagen) and analysed on an ABI Prism 7000 (Applied Biosystems) sequence detection system. The level of Actin transcripts were used to normalize total cDNA input. Relative quantitation of transcript levels was calculated using the comparative C_T method. Results shown represent the mean of three replicates. For semi-quantitative RT-PCR, transcripts

were analyzed after the twentieth cycle, and actin transcripts served as loading controls. Primers used are shown in supplementary table 1.