SUPPLIMENTAL FIGURE AND MOVIE LEGENDS:

Movies:

Movie S1 illustrates the defective shock response of Actβ escaper females compared to heterozygous controls.

Movie S2 shows a close up view of Actβ mutant females exhibiting poor locomotion and a held out wing phenotype compared to heterozygous controls.

Movie S3 demonstrates a defective shock response of adults in which Actβ was knocked down in motoneurons using RNAi (Ok371>Gal4, UAS Actβ RNAi).

Movie S4 shows a close up view of Ok371>Gal4, UAS Actβ RNAi Actβ knockdown adults exhibiting poor locomotion and a held out wing phenotype similar to that exhibited by Actβ null escaper flies (Movie 1).

Figure S1 *Actβ* does not affect muscle nuclei number

3rd instar larvae were filleted and stained with DAPI (blue) and phalloidin (red). (A) Quantification of the number of nuclei in muscle #6 and #7 for w^{1118} controls and $Act\beta^{80}$mutants shows no significant difference. (B) Representative image of muscle fiber (red) with stained nuclei (blue) for w^{1118} controls and $Act\beta^{80}$mutant. Each image has muscle #6 (left) and muscle #7 (right) outlined with dashed line. N is shown for each group in A, ns = not significant

Figure S2 Size of various polyploid nuclei in *Actβ* mutants.

Proventriculus, salivary gland, and the prothoracic gland of 3rd instar wandering larvae were dissected, stained with DAPI (green, A, B, D, E; grey, G, H) and phalloidin (magenta, A, B, D,

E) to quantify nuclei size. (A-C) The nuclei of the cells of the proventriculus in *Actβ80*mutants are 10% larger. (D-F) The nuclei of the salivary gland cells in *Actβ80*mutants are 48% larger. (G-I) The nuclei of the prothoracic gland cells are -37% smaller. Size of scale bar indicated on the image. For each group n = 60-170.

Figure S3 Different Actβ-GAL4 lines vary in strength of expression

Two different *Actβ-GAL4* lines, 27A3 on 3rd chromosome (A), and 2A2 on 2nd chromosome (B) were crossed to *UAS-GFP*. 3rd instar larval brains from each cross were dissected and imaged for GFP fluorescence intensity with different exposure times. (A) 27A3 line results in higher expression of GFP which can be seen easily with short exposure time, and long exposure leads to image saturation (A'). The 2A2 line results in weak GFP expression, which can be barely detected at short exposure time equivalent to that used in panel A. Long exposure, equivalent to that of A', reveals the expression pattern. Estimation of overall intensity indicates that the 27A3 line is expressed ~7 fold high level than the 2A2 line, Images were captured with the same gain settings.

Figure S4 RNA *in situ* hybridization of *Actβ* shows expression in various tissues

(A-I) *Actβ* is expressed in cell body of motoneurons (A) and neuroendocrine cells (B) in the ventral ganglion of 3rd instar larval CNS, (C) eye disc, (D) CC cells, (F) trachea, (G) maturing follicle cells, (H) enteroendocrine cells in the midgut, (I) two PNS neurons.

Figure S5 Overexpression of *Actβ* in motoneurons or neuroendocrine cells increases adult body size. Relative to controls (UAS-*Actβ*, left) overexpression of *Actβ* (line 3B2 or 4BA) in

motoneurons (OK6-Gal4, right) or neuroendocrine cell (C929-Gal4, middle) results in larger animals. The body length for the indicated genotype shown (bottom panel) with representative images of adult flies (top panel). N= 30-40 individuals, bars indicate mean ± SEM.

Figure S6 Hyperactivation of Activin signaling in muscles but not fatbody is larval lethal

Images of pupae resulting from overexpression of constitutively activated Babo ($Babo^{CA}$) or *Actβ* in the fat-body (*Cg-Gal4,* left) or muscles (*MHC-Gal4*, right). Note that the left most pupa in which activated Babo was expressed in the fatbody produces a normal pupa that gives rise to a viable adult. However expression of the ligand (Actβ) in the fatbody produces a 100% larval lethal phenotype (similar to that produced by either overexpression of Actβ or activated receptor in muscle. We conclude that overexpression of Actβ in the fatbody produces a lethal phenotype by non-autonomous activity in the muscles.

Figure S7 Knockdown of *Actβ* in neuroendocrine cells does not affect body size.

Knockdown of *Actβ* in neuroendocrine cells using *929-GAL4* (triangles) does not affect pupal volume (body size) relative to RNAi controls (squares).

Figure S8 OK372-Gal4 is not expressed in Dimm + neuroendocrine cells

Third instar larvae brains of *OK371-Gal4>UAS-GFP* (green) were dissected and stained with α-Dimm (red). We find no overlap in signals demonstrating that OK371-Gal4 is not expressed in Dimm+ cells (merge panel, right).

Figure S9 Muscle specific activation of Activin signaling increases body size but not wing size

(A-B) Overexpression of activated Babo ($Babo_{CA}$) in muscles results in larger pupae (A) and adult larger adult flies (B). (C-D) Overexpression of activated dSmad2 ($dSmad2_{CA}$) in muscles also results in larger adult flies. (D) adult wings of *MHC>dSmad2*$_{CA}$ are smaller than *MHC-Gal4* control.