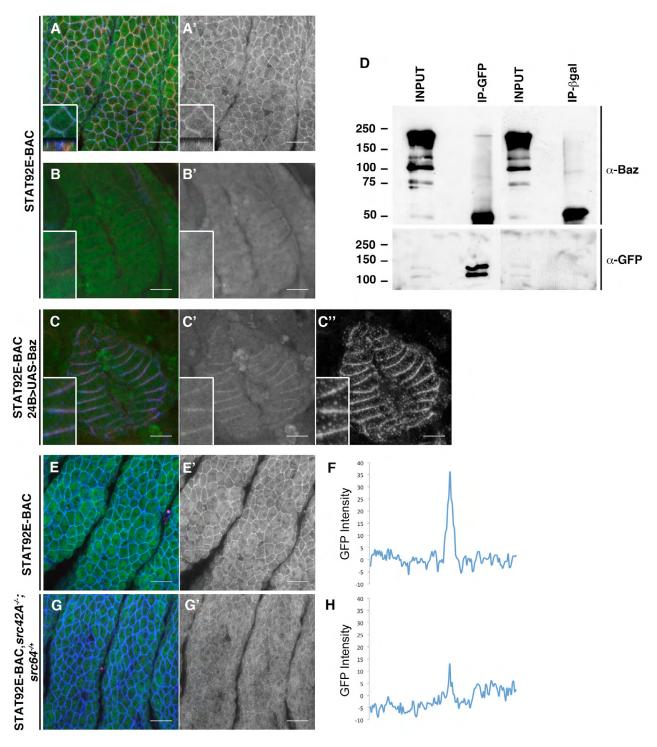


Fig. S1. Expression levels of the STAT92E transgenic lines used. Western blot analysis of the STAT92E-GFP variants in 12- to 20-hour old embryos. All forms, with the exception of the STAT92E BAC insertion, were expressed using the 69B-Gal4 line. 30  $\mu$ g of total protein was loaded and western blots with anti-GFP (upper panel) or anti- $\alpha$ -Tubulin (lower panel) were performed.



**Fig. S2. Baz interaction with endogenous STAT92E-GFP driven from a STAT92E-GFP BAC element.** Subcellular localization of a STAT92E-GFP fusion protein under the control of the regulatory genomic region of STAT92E. This BAC line completely rescues *stat92E* mutant alleles. (**A,A**′) Ectoderm of STAT92E-GFP BAC stage 15 embryos focusing on the subapical membrane. (**B,B**′) Mesodermal pharynx cells of STAT92E-GFP BAC stage 17 embryos. STAT92E signal can be detected both cytoplasmic and nuclear regions but not in the membrane. (**C-C**″) STAT92E-GFP protein relocalizes to the membrane of mesodermal pharynx cells (**C**, green; **C**′) when co-expressed with Baz (**C**, blue; **C**″). (**D**) STAT92E-GFP protein co-precipitates with Baz from embryonic extracts (upper panel). Control of GFP precipitation is shown below. (**E,E**′,**G,G**′) STAT92E-BAC membrane localization (green in **E,E**′) is strongly reduced in a *Src42A*<sup>26.1</sup>/*Src42A*<sup>26.1</sup>/*Src42A*<sup>26.1</sup>; *Src64B*<sup>KO</sup>/+ mutant background (green in **G,G**′). (**F,H**) Quantification of STAT92E-BAC apical membrane localization using Image J software in wild-type epithelia (**F**), or in *Src42A*<sup>26.1</sup>/*Src42A*<sup>26.1</sup>; *Src64B*<sup>KO</sup>/+ (**H**) mutant embryos. Graphs show fluorescence levels for STAT92E-GFP across the cell. Ten confocal images of 0.16 μm comprising the STAT92E-BAC expression domain were projected using the average intensity algorithm. Three cells were selected randomly and the fluorescence levels (pixel grey intensity in arbitrary units) for STAT92E-GFP (blue) were measured along a line of 10 μm that included the cell boundary at the centre. GFP is shown in green in A-C,E,G and in grey in A'-C', and E'-G'. Baz is shown in blue in A-C and in grey in C". Discs large is used as a basolateral marker and is shown in red in A-C and blue in E and G. Insets show higher magnifications. Scale bars: 10μm.

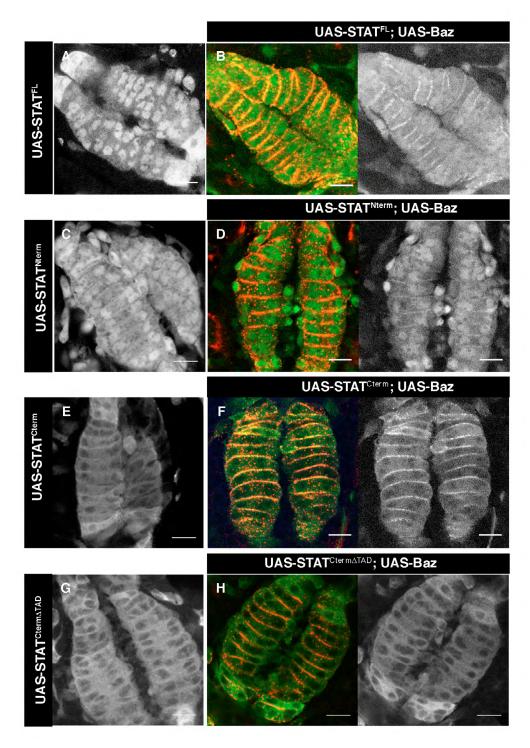
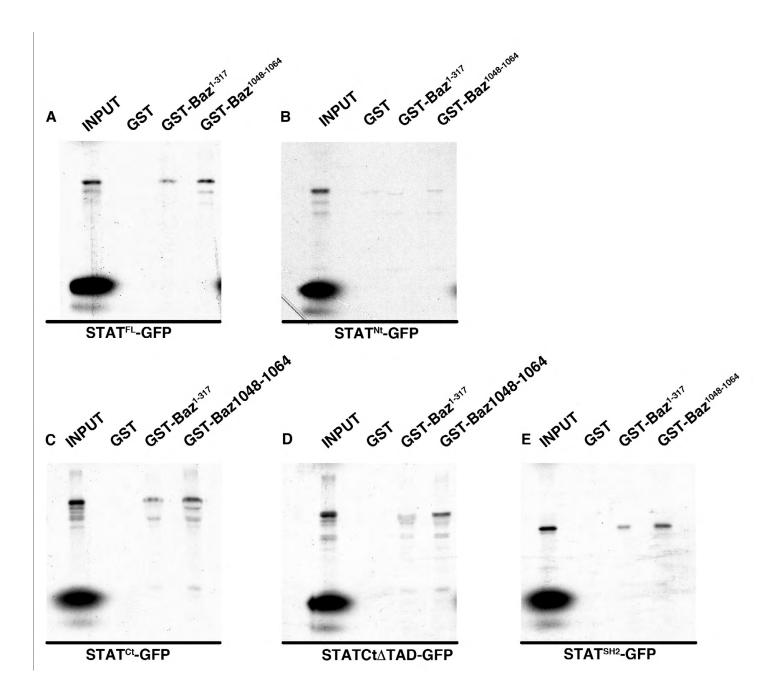
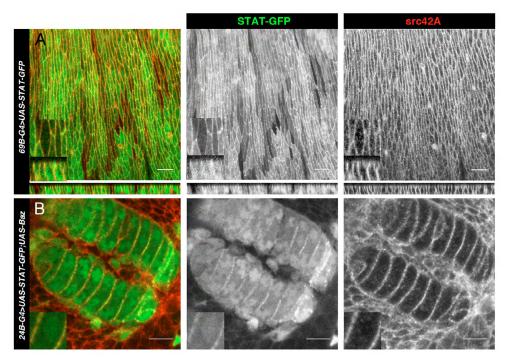


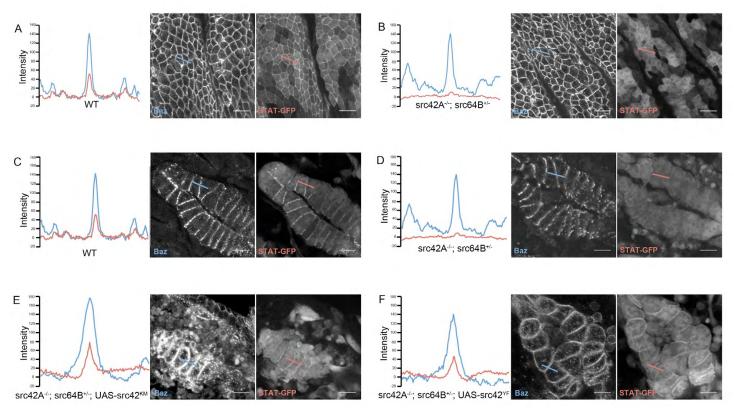
Fig. S3. Colocalization of STAT92E and Baz in mesodermal cells. (A-H) Stage 17 embryos expressing in the pharynx full-length STAT92E-GFP (A), the N-terminal half (C), the C-terminal half (E) or the C-terminal half without the TA domain (G) alone (A,C,E,G), or in combination with Baz (B,D,F,H, respectively), under the control of the 24B-Gal4 line. Both the C and the N-terminal regions can be detected colocalizing with Baz. This localization is lost when the TA domain is removed. (B,D,F,H) Left panels show double staining with anti-Baz in red. Anterior is upwards. Scale bars:  $10 \mu m$ .



**Fig. S4. STAT92E domains required for interaction with Baz.** GST-Baz fragments or control GST were incubated with STAT92E full-length (A), the STAT92E N-terminal region (B), the C-terminal region (C), the C-terminal region without the TA domain (D) or the SH2 domain (E) transcribed *in vitro* in the presence of Met-S35. (A) Full-length STAT92E binds to both domains but with higher affinity for the C-terminal region of Baz. (B) There is no interaction between the N-terminal region of STAT92E and the fragments of Baz. (C) The C-terminal region of STAT92E binds as the full-length protein to both Baz domains but with higher affinity for the C-terminal part. (D) The C-terminal region without the TA domain of STAT92E loses affinity for the N-terminal region of Baz but conserves the affinity for the C-terminal part. (E) The SH2 domain of STAT92E binds both the N- and C-terminal regions of Baz. However, considering we are expressing only the SH2 domain, without a proper protein environment these interactions may be nonspecific.



**Fig. S5. STAT92E and Src42A colocalization**. Subcellular localization of STAT92E-GFP (green) and Src42A (red) in epidermis (A) and in the pharynx (B). (**A**) The ectoderm of a stage 15 embryo expressing *STAT92E-GFP* under the control of *69B-Gal4*. Cross-sections shown below. (**B**) The pharynx of a stage 17 embryo expressing *STAT92E-GFP* (green) and *baz* under the control of *24B-Gal4*. Insets show higher magnifications. Anterior is leftwards. Dorsal is upwards in A; B shows a dorsal view. Scale bars: 10 μm.



**Fig. S6. Quantification of STAT92E membrane localization in** *Src* **mutant backgrounds**. (**A-F**) Quantification of STAT92E-GFP and Baz staining using Image J software, in epithelia (A,B) or pharynx (C-F) of wild-type (A,C), *Src42A*<sup>26.1</sup>/*Src42A*<sup>26.1</sup>; *Src64B*<sup>KO</sup>/+ (B,D) mutant embryos alone or co-expressing either *UAS-Src42A*<sup>KM</sup> (E) or *UAS-Src42A*<sup>KY</sup> (F). Graphs show fluorescence levels for Baz (blue) and STAT92E-GFP (red) across the cell. Ten confocal images of 0.16 μm containing the Baz expression domain were projected using the average intensity algorithm. Three cells were selected randomly and the fluorescence level (pixel grey intensity in arbitrary units) for Baz (blue) and STAT92E-GFP (red) were measured along a line of 10 μm that included the cell boundary at the centre. Representative images for each genotype are shown in the right-hand panels. Three different samples were measured for each genotype. Scale bars: 10μm.