

**Supplementary Figure 1: Scrambled tissues do not undergo bulk morphogenetic movements.** Time-lapse sequence of representative stage 14 dorsal isolate (left) and scrambled tissue (right) cultured in DFA. Dorsal isolate undergoes convergence and extension however scrambled tissue does not undergo macroscopic shape change over two hours. Anterior-posterior length is plotted over time. Full sequence in Supplementary Video 2.



Supplementary Figure 2: Mesoderm cells in scrambled tissues undergo stereotypic protrusive activity. Lifeact-GFP expressing mesoderm cells mixed with unlabeled scrambled tissues cultured on fibronectin coated glass substrate. Yellow circles indicate lamellipodial protrusions at the substrate level (green), typical of the converging and extended mesoderm in native dorsal isolates (Shih and Keller 1992b; Shih and Keller 1992a). Scale bar 50µm.



**Supplementary Figure 3: Aged scrambled tissues stiffen, however not to the extent of native tissues.** Aged scrambled tissues stiffened by 45% over 5 hours (pval=0.001; 2-way ANOVA). Tissues were cultured in DFA and mechanically tested when stage-matched whole embryos reached stage 21. In each experiment, elastic modulus of aged scrambled tissue is normalized to stage 14 scrambled tissue. Each cluster represents one experiment and the value along x-axis for each cluster represents n tissues tested per group.



**Supplementary Figure 4: Cellular Solids Model (CSM) predictions for tissues with larger and smaller cells.** A) Plot of Relative Young's Modulus vs. Relative Density for a closed cell foam based on eq. 1 in main text. Predictions for cell size manipulation experiments in inset. B) CSM Predictions on the relationship between increasing cell density and modulus in embryonic tissues (assuming constant E<sub>s</sub>).



Supplementary Figure 5: Rock inhibition reduces modulus of stage 14 and 21 dorsal isolates. Stage 14 and 21 dorsal isolates treated with Y-27632 (50 µM) for at least one hour were tested in the nNFMD and compared with untreated DMSO controls. Y-27632 treatment significantly reduced modulus at stage 14 (\* p-val: 0.017; 2-way ANOVA) and stage 21 (\* p-val: 0.019; 2-way ANOVA). Experiment done in parallel with stage-wise comparison in Figure 1C. Each cluster represents one experiment and n value represents number of explants tested per group. Error bars represent 1 S.D.



Supplementary Figure 6: F-actin cross-linkers expressed during gastrulation and neurulation in X. laevis. Deep sequencing data compiled from (Session et al. 2016) revealing temporal pattern of whole embryo expression of abundant F-actin crosslinking proteins in transcripts per million (TPM). Notably, Fascin-1 (fscn1) is expressed at high levels during gastrulation but decreases as neurulation progresses and  $\alpha$ -actinin-4.L (actn4.L) ramps up expression over 2-fold after gastrulation and persists through neurulation



Supplementary Figure 7:  $\triangle$ ABD-actinin rescues blastopore closure delay caused by overexpression of  $\alpha$ -actinin-1. Embryos were injected with ACTN1-eGFP or ACTN1-eGFP + ACTN- $\triangle$ ABD at the 4-cell stage in the equatorial region of both dorsal blastomeres. Green signal in top row represents tissues overexpressing actinin constructs. Blastopore area was compared between embryos to assess degree of rescue (n=4 embryos in each bar).



**Supplementary Figure 8:** α-actinin-1 overexpression increases cortex stability in animal cap epithelium. Fluorescent recovery after photobleaching (FRAP) experiments were conducted on apical animal cap epithelial cells overexpressing a-actinin-1 and compared with wildtype controls. To compare groups, Mann-Whitney U test was performed between groups (Half life \*\* p-val: 0.002; Immobile fraction \* p-val: 0.012).



## Supplementary Figure 9: ACTN-ΔABD overexpression slightly delays

**gastrulation.** Embryos were injected with ACTN- $\Delta$ ABD at the 4-cell stage in the equatorial region of both dorsal blastomeres. Blastopore closure is slightly delayed at stage 11 however development progresses through stage 16.



**Movie 1.** Three-dimensional rendering of mesoderm cells in early, flat neural plate stage Xenopus (stage 14) and post neural tube closure (stage 21). Scattered cells within a whole embryo labeled with Lifeact-eGFP. Scale bar 20µm.



**Movie 2.** Convergent extension of a dorsal isolate (left) contrasted with stage-matched scrambled tissue (right). Scale bar 200µm.