

Description of Additional Supplementary Files

File Name: Supplementary Movie 1

Description: Differential Interference Contrast (DIC) time lapse of 5HT2A and 5HT2B null mutant embryos. WT (top panel), 5HT2A^{-/-} (middle panel) and 5HT2B^{-/-} (bottom panel). Images acquired every 1 min. A, Anterior; P, Posterior; D, Dorsal; V, Ventral.

File Name: Supplementary Movie 2

Description: MyoII dynamics in 5HT2A loss of function (5HT2A^{-/-}). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: 5HT2A null mutant, 5HT2A^{-/-}. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 3

Description: MyoII dynamics in 5HT2A gain of function (5HT2A⁺⁺). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: 5HT2A over-expression, 5HT2A⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 4

Description: MyoII dynamics in Trh loss of function (*Trh*⁰¹). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: Trh null mutant, *Trh*⁰¹. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 5

Description: MyoII dynamics in Trh gain of function (Trh⁺⁺). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: Trh over-expression, Trh⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 6

Description: MyoII dynamics in Trh knock-out and 5HT2A over-expression (Trh^{-/-} 5HT2A⁺⁺). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Middle panels: 5HT2A⁺⁺; Bottom panels: Trh^{-/-} 5HT2A⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 7

Description: MyoII dynamics in 5HT2A knock-out and Trh over-expression (5HT2A^{-/-} Trh⁺⁺). Live 100X imaging of MyoII (*sqh*::mCherry, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top to bottom panels: Control; Trh⁺⁺; 5HT2A^{-/-}; 5HT2A^{-/-}Trh⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 8

Description: MyoII dynamics in Toll triple knock-down following 5HT2A over-expression. Live 100X imaging of MyoII (*sqh::mCherry*, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top to bottom panels: Control; 5HT2A⁺⁺; Toll-2,6,8RNAi; 5HT2A⁺⁺ Toll-2,6,8RNAi. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 9

Description: MyoII dynamics in *Cirl* and 5HT2A genetic interaction. Live 100X imaging of MyoII (*sqh::mCherry*) in the ectoderm during germ-band extension. Top to bottom panels: Control; 5HT2A^{-/-}; *Cirl*^{-/-}; *Cirl*^{-/-} 5HT2A^{-/+}. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 10

Description: F-actin and MyoII dynamics in *Cirl* knock-out and 5HT2A over-expression. Live 100X imaging of actin (Lifeact::mCherry, left panels) and MyoII (*sqh::mCherry*, right panels) in the ectoderm during germ-band extension. Top panels: Control; Middle panels: *Cirl*^{-/-}; Bottom panels: *Cirl*^{-/-} 5HT2A⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 11

Description: Rho1-GTP biosensor dynamics in 5HT2A loss of function (5HT2A^{-/-}). Live 100X imaging of Rho1 biosensor (ANI-RBD::mEGFP) in the ectoderm during germ-band extension. Top panel: Control; Bottom panel: 5HT2A^{-/-}. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 1 min.

File Name: Supplementary Movie 12

Description: Rho1-GTP biosensor dynamics in 5HT2A gain of function (5HT2A⁺⁺). Live 100X imaging of Rho1 biosensor (ANI-RBD::mEGFP) in the ectoderm during germ-band extension. Top panel: Control; Bottom panel: 5HT2A⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 1 min.

File Name: Supplementary Movie 13

Description: Myosin phosphatase dynamics following 5HT2A⁺⁺. Live 100X imaging of myosin phosphatase (MBS::GFP) in the ectoderm during germ-band extension. Top panel: Control; Bottom panel: 5HT2A⁺⁺. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 30 sec.

File Name: Supplementary Movie 14

Description: MyoII dynamics in 5HT2B loss of function (5HT2B^{-/-}). Live 100X imaging of MyoII (*sqh::mCherry*, left panels) and Ecad::GFP (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: 5HT2B null mutant, 5HT2B^{-/-}. Video represents maximum projection of apical 10 planes spaced by 0.5 μ m acquired every 15 sec.

File Name: Supplementary Movie 15

Description: MyoII dynamics in 5HT2B knock-down. Live 100X imaging of MyoII

(*sqh::mCherry*, left panels) and *Ecad::GFP* (right panels) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: 5HT2B dsRNA. Video represents maximum projection of apical 10 planes spaced by 0.5 μm acquired every 15 sec.

File Name: Supplementary Movie 16

Description: MyoII dynamics in 5HT2A and 5HT2B double knock-down. Live 100X imaging of MyoII (*sqh::mCherry*, left panels) and *Ecad::GFP* (right panels) in the ectoderm during germ-band extension. Top to bottom panels: Control; 5HT2B dsRNA; *5HT2A*^{-/-}; *5HT2A*^{-/-} 5HT2B dsRNA. Video represents maximum projection of apical 10 planes spaced by 0.5 μm acquired every 15 sec.

File Name: Supplementary Movie 17

Description: Rho1-GTP biosensor and MyoII dynamics following Toll-2,6,8 triple knock-down. Live 100X imaging of MyoII (*sqh::mCherry*, left panels) and Rho1-GTP biosensor (ANI-RBD::mNeonGreen) in the ectoderm during germ-band extension. Top panels: Control; Bottom panels: Toll-2,6,8 RNAi. Video represents maximum projection of apical 10 planes spaced by 0.5 μm acquired every 1min.

File Name: Supplementary Movie 18

Description: Brightfield time lapse of chick embryo treated with DMSO; Control (left panel) and Ritanserin 200 μM (right panel). Images acquired every 6 min.

File Name: Supplementary Movie 19

Description: Particle Image Velocimetry (PIV) time lapse of the chick embryo in Video 18. Left panel: Control (DMSO) and right panel: Ritanserin 200 μM . PIV analysis on consecutive images acquired every 6 min.

File Name: Supplementary Movie 20

Description: Top panels: brightfield time lapse of chick embryo treated with DMSO; Control (left panel) and Ritanserin 50 μM (right panel). Images acquired every 6 min.

Bottom panels: Particle Image Velocimetry (PIV) time lapse of the above embryos. Left panel: Control (DMSO) and right panel: Ritanserin 50 μM .