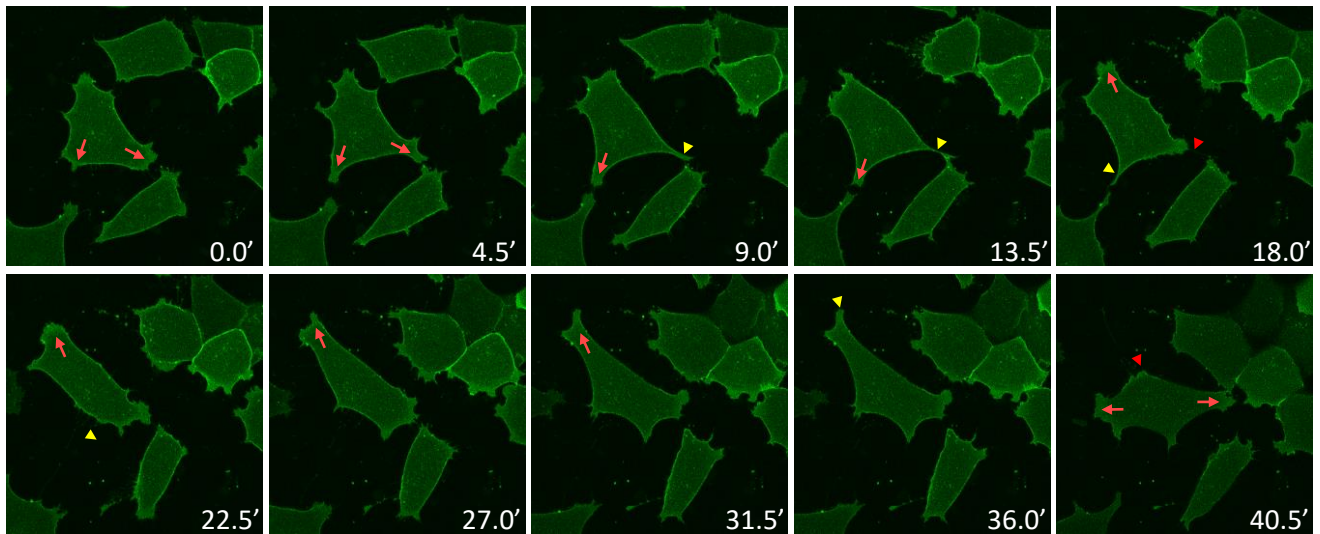


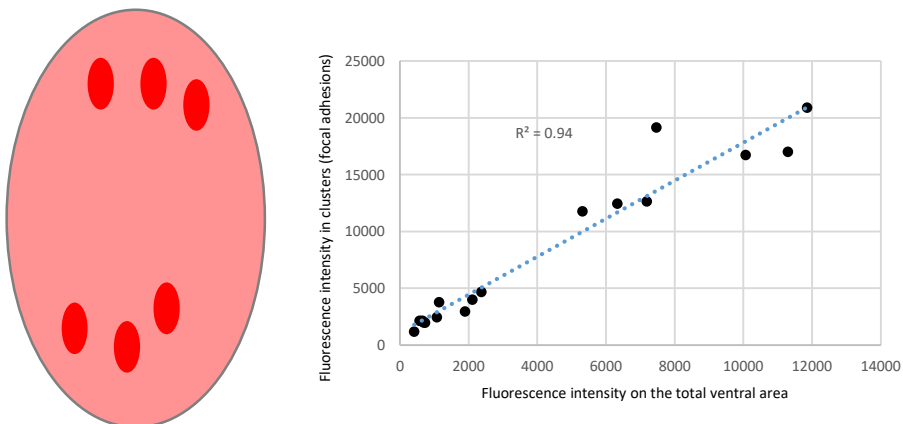
Figure S1

A



A) Mode of mesoderm locomotion (Related to figure 1) Consecutive frames from time lapse of mYFP labelled mesoderm cells migrating on FN. The behaviour of the central cell is highlighted: The cell emits one or multiple protrusions (red arrows). One of the protrusions becomes a tail (yellow arrowhead) as the cell stretches toward another direction, and eventually retracts (red arrowheads).

B



B) Quantification of accumulation of Vinculin-Cherry in focal adhesions: Linearity between fluorescence levels in focal adhesion and total intensity (Related to figure 1A-D) Because Vinculin-Cherry expression levels vary from cell to cell, quantification was performed for individual cells by measuring fluorescence in bright clusters (corresponding to focal adhesions) and in the total ventral cell surface (pink on the diagram). The plot shows the average intensity of the ventral surface versus the average intensity in focal adhesions for control mesoderm cells in one experiment, each dot corresponding to a single cell. It shows that accumulation at focal adhesions is proportional to total expression levels over a wide range. Linearity was similarly verified for each experiment.

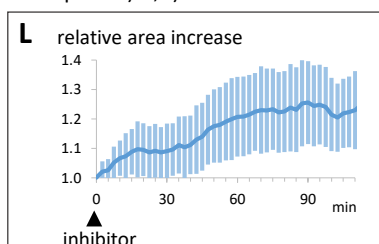
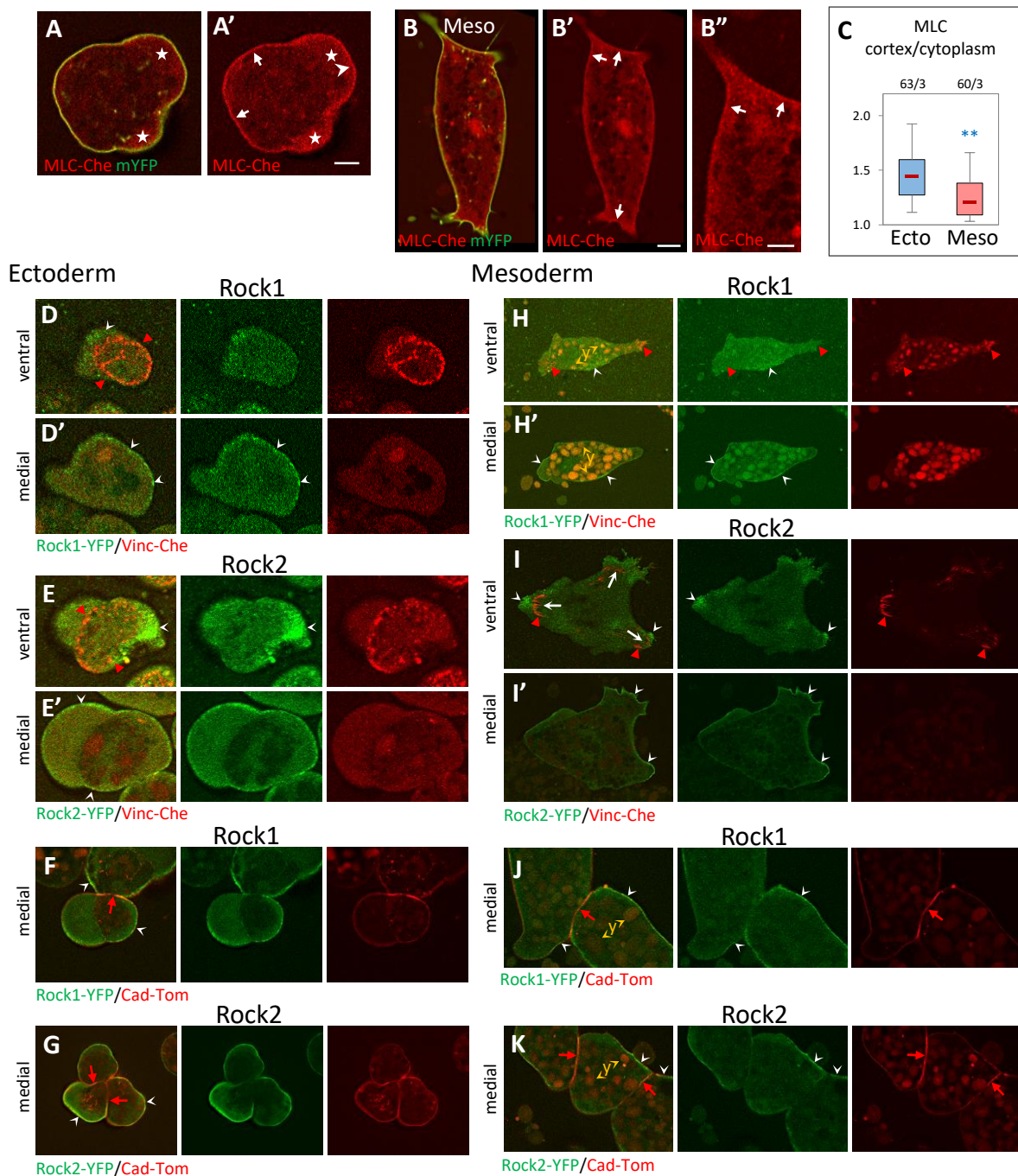
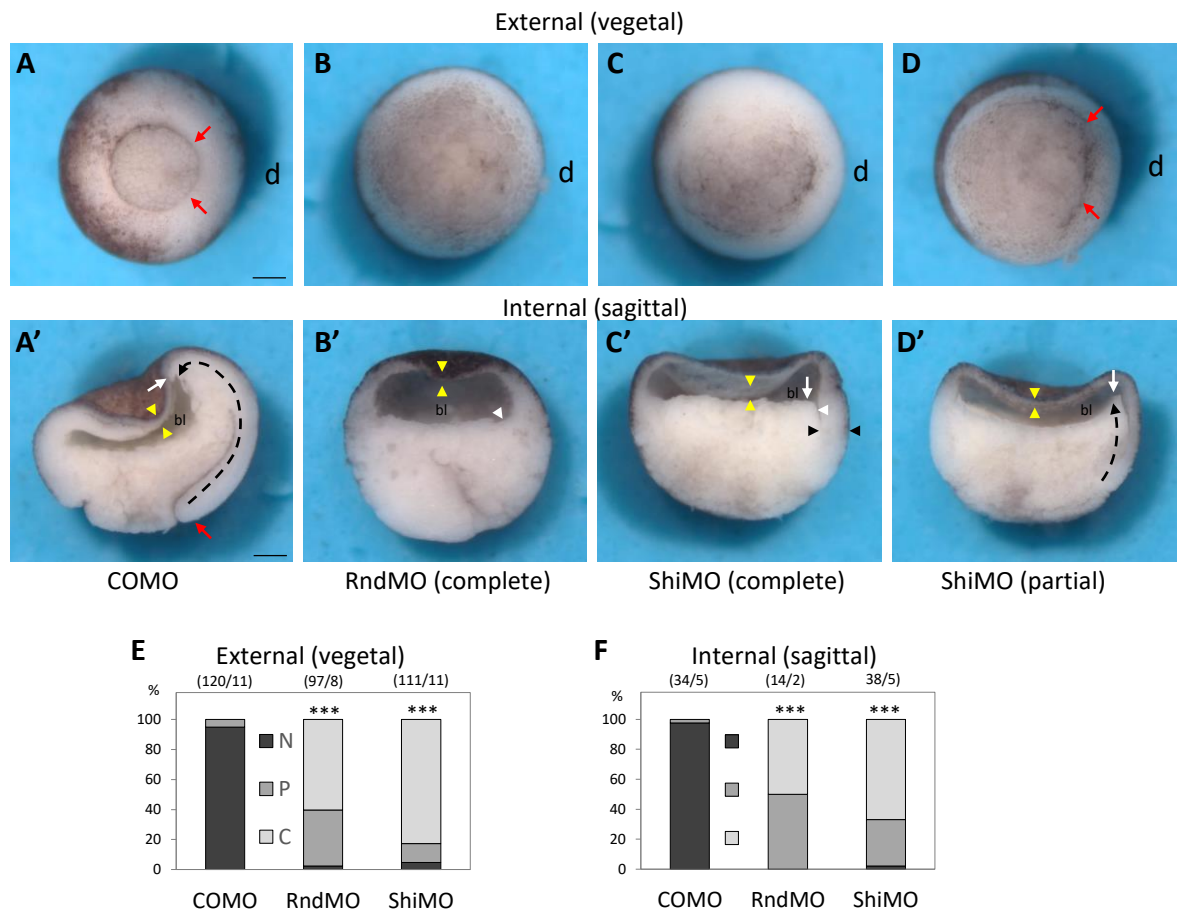
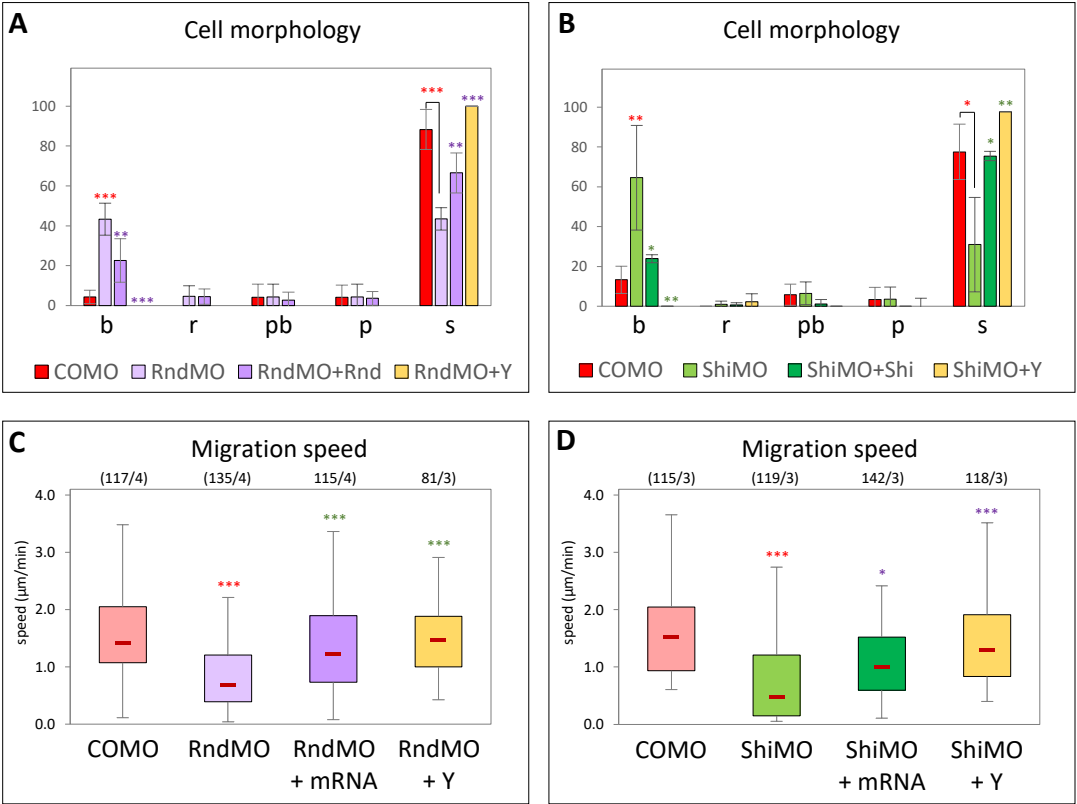
Figure S2**L) Related to Figure 2: Quantification of single ectoderm cell spreading upon H1125 treatment. Average and SD of 34 cells.**

Figure S3

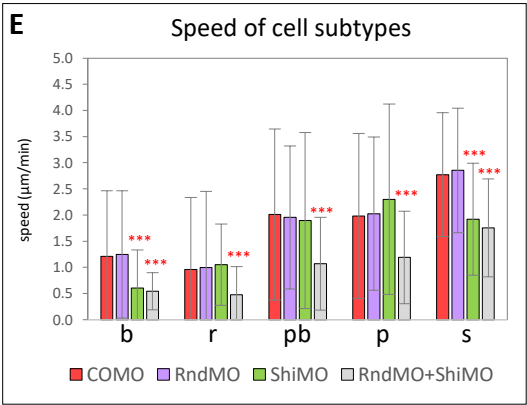


Loss-of-function whole embryo phenotypes (Related to Figure 3) 4-cell stage embryos were injected in the dorsal side (d) with a control (COMO), Rnd1 (RndMO) or Shirin (ShiMO) morpholinos. Embryos were fixed and imaged at stage 11. A-C) Examples of typical control, RndMO and ShiMO phenotypes. D) Example of a “partial” phenotype (here ShiMO). A-D) External views from the vegetal pole. Red arrows point to the position of the dorsal blastopore lip of a control embryo, absent in RndMO (B) and ShiMO (C) embryos. D) Partial phenotype, the lip is present but the blastopore has remained widely open compared to control. Note that in many embryos the ventral blastopore is also affected, due to the diffusion of the morpholinos to the ventral blastomeres before complete separation after the 2nd cleavage. A'-D') Sagittally bisected embryos. In a control embryo (A'), the extent of involution (dashed black arrow) can be seen by the position of the tip of the mesendoderm (white arrow) that has moved far away from the blastopore lip (red arrow). B') RndMO embryo lacking any sign of involution. The white arrowhead points to the dorsal edge of the blastocoel cavity (bl), resembling that of a pregastrula embryo. C') Characteristic ShiMO phenotype, with flat blastocoel floor (white arrow) and thicker non-involuting dorsal marginal zone (black arrowheads), both indicative of failed involution. D') Partial involution (white arrow). Yellow arrowheads: thin blastocoel roof, indicative of ectoderm epiboly in all conditions. Scale bars: 200µm. E and F) Score of the penetrance of the gastrulation external and internal phenotype: N, normal embryo; P, partial inhibition, C, complete inhibition. Comparison by one-way ANOVA followed by Tukey's HSD post hoc test.

Figure S4

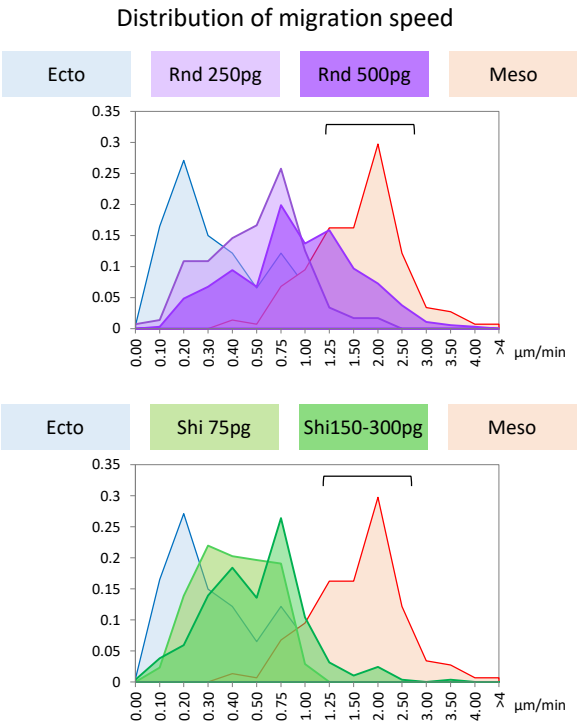


A-D) Rescue of Rnd1MO and ShiMO spreading and migration phenotypes. 4-cell stage embryos were injected in the dorsal side with COMO, RndMO, RndMO + YFP-Rnd1 mRNA (rescue), ShiMO, or ShiMO + YFP-Shirin mRNA (rescue). Dissociated mesoderm cells were plated on FN and time lapse movies were recorded. The fourth condition represents RndMO or ShiMO cells treated with 50μM Y27632 Rock inhibitor (Y). Red, purple and green asterisks: Comparison to COMO, RndMO or ShiMO, respectively. One-way ANOVA followed by Tukey's HSD post hoc test.



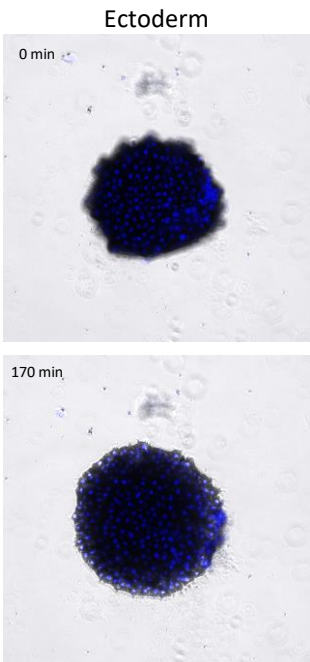
E) Migration speed for different cell morphology categories (Related to Figure 3) Analysis of data from figure 3K. Red asterisks: Comparison to COMO. one-way ANOVA followed by Tukey's HSD post hoc test.

Figure S5



Related to Figure 4: Histograms showing speed distribution for ectoderm cells expressing Rnd1 or Shirin, compared to wild type ectoderm and mesoderm. Brackets: Range of high speed, comparable to mesoderm, achieved mainly by Rnd1-expressing cells.

Figure S6



Related to figure 7: Example of ectoderm explant showing late partial spreading, which is only observed beyond the 120min.