Fig. S1. Graded recruitment of Myosin II drives gastrulation
A) Schematic cross-section of an embryo initiating ventral furrow (VF) invagination during NC 14, after completion of cellularization. The nuclear gradient of Dorsal protein drives expression of twist in the mesoderm, and subdivides the embryo into three zones of gene expression. Recruitment of Myosin II (green shading) to the apical surface of the ventral cells drives their apical constriction and invagination. B) The apical wedge-shape of the gastrulating tissue is triggered by graded recruitment of Myosin II, peaking at the ventral midline.
**Fig. S2. Binding of Dorsal and Zelda**

CHIP seq data from (Sun et al., 2015) was plotted. Right-pointing arrows mark transcription start sites and the extent of the transcribed regions are shown by bold black lines. A) Binding of Dorsal to the *twi* regulatory region. A’) Binding of Zelda to the *twi* regulatory region. Prominent binding of both is detected immediately upstream to the TS. B,B’) Binding of Dorsal and Zelda to the *T48* regulatory the region. Prominent binding is detected upstream to the TS and in the first intron. C,C’) Binding of Dorsal and Zelda to the *mist* regulatory region. Prominent binding is detected upstream to the TS and for Zld also within the transcribed region.
**Fig. S3. Pol II Pausing**

Chip seq data of Pol II from (Saunders et al., 2013) was plotted. Right-pointing arrows mark transcription start sites and the extent of the transcribed regions are shown by bold black lines. Prominent stalling was observed. A) *twi*. B) *T48*. C) *mist*.
**Fig. S4. Modelling Dorsal-dependent mRNA accumulation**

Based on experimental results, Dorsal was assumed to determine both the promoter activation probability and Pol II loading rate of the T48 gene. Mature transcripts are assumed to be stable and accumulate during the course of the simulation (~15 minutes). Differential equations used to simulate the dynamical system - Activation probability \( p_{\text{activation}} \) and Pol II loading rate \( r_{\text{pol2}} \) depend linearly on Dorsal level and thus on the position along the DV axis. The dynamics of the activated promoter fraction \( F_{\text{activated}} \) and the mRNA level \( \text{mrna} \) is simulated for 15 minutes after model initiation (no mRNA or activated promoters at time 0). Model parameters were either arbitrary \( r_{\text{pol2}} \) at maximal Dorsal levels) or based on experimental results \( p_{\text{activation}} \) at maximal Dorsal=0.07 min\(^{-1}\) (Ambrosi et al., 2014).

**Fig. S5. Uniform activation of mist in Toll\(^{\text{DLRR}}\) embryos**

Uniform expression of the constitutive Toll\(^{\text{DLRR}}\) construct drives activation of the pathway and nuclear targeting of Dorsal along the entire embryo circumference. Induction of \( \text{twi} \) (green) and \( \text{mist} \) (red) TSs responds accordingly. In a younger embryo (A-B') one or two \( \text{twi} \) TSs are observed in most nuclei, while only a single or no \( \text{mist} \) TSs are observed in many nuclei. In an older embryo (C-D') the majority of nuclei exhibit two TSs for both genes. Scale bar 10 µm.
**Fig. S6. Determination of embryo age at NC 14**

The reported apical-basal lengths of DAPI-stained nuclei were calculated as the average length of 20 nuclei on both sides of the pole cells (PC). This value was used to estimate the embryo age in NC 14 at 20°C, according to (Lecuit and Wieschaus, 2000). The embryos shown correspond to the panels in Figure 7. A) 8 µm, B) 5.4 µm, C) 7.2 µm, D) 12.5 µm. Scale bar 10 µm.

**Table S1. smFISH probes.** Position of probes is marked from the first transcribed nucleotide.

| Gene   | Probe      | # Probes | Fluorofour        |
|--------|------------|----------|-------------------|
| twist  | 53-2,362   | 48       | Quasar 670        |
| T48    | 1-29,056   | 48       | Quasar 570        |
| T48 5’ | 1-2,491    | 48       | Quasar 570        |
| T48 intron | 13,800-16,474 | 48 | Cal-Fluor-Red 610 |
| T48 3’ | 27,796-20,056 | 48 | Quasar 670        |
| mist 5’| 6-2,524    | 48       | Quasar 570        |
Movie 1. Dynamics of Myosin II recruitment

The dynamic localization pattern of Myosin II during early NC 14, monitored using a GFP “protein-trap” in the Myosin II heavy-chain (zipper) gene locus, as visualized in cross section by Lightsheet microscopy. During the process of cellularization, Myosin II is initially recruited to the basal aspect of the future cell membranes as they extend from the surface. Upon completion of cellularization, Myosin II disappears from the basal position and is recruited to the apical side in the ventral-most cells, where it drives furrow formation. The graded pattern of Myosin II recruitment within the ventral domain shapes the pattern of cell invagination. The movie was obtained at a rate of 1 frame/min.
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