**Sup Fig. 1. Specification of RNAscope probes in zebrafish.** (A-F) (B-C) *bmp2b* mRNA (red), (E-F) *Venus* mRNA (gray), DAPI (blue) and (D) Venus-Bmp2b protein (green) expression in zebrafish cryosections at 4 hpf, into which *venus-bmp2b* fusion mRNA was injected at the 1 cell stage. Scale bar: 10 μm. (C, F) The white box in B and E with higher magnification. (H-I) *bmp2b* (green), *chd* (red) and DAPI in zebrafish whole-mount embryos at 2.5 hpf. (I) The white box in H with higher magnification. (J, K) *tld* mRNA imaged on a wide-field microscope (J) and confocal 20x objective (K). Scale bar: 5μm

**Sup Fig. 2. Transcription inhibition of *bmp2b* and *tld* mRNA in zebrafish at 6 hpf.** (A, B) *bmp2b* mRNA (green) in control (A) and embryos injected with alpha-amanitin (B). Scale bar: 10 μm. (C-F) *tld* mRNA (green) in the control (C, D) and embryos injected with alpha-amanitin (E, F). (D, F) The white box in C and E at higher magnification.

**Sup Fig. 3. mRNA quantification related to Figure 2**. (A-D) Intensity drop-off correction in whole-mount embryos (n=5). (A) The nuclear intensity at the 647 channel decreased from the animal pole to the marginal region, and quantification of the averaged intensity at each slice is shown in (C). (B) Nuclear intensity after correction, and the corrected scatter points are shown in D. (E, F) Averaged *bmp2b* mRNA intensity distribution at 4.7 hpf (E, n=3) and at 5.7 hpf (F, n=4). (G-H) Statistical distribution of individual mRNA numbers at different volume matrix pixels (G) and intensities (H) of individual mRNAs.

**Sup Fig. 4. mRNA and protein expression from the blastula to gastrula stage, related to Figure 3**. (A-B’) Simultaneous expression of pSmad (red), *bmp2b* (A, A’) or szl (B, B’) (green) and *chd* (orange) single molecular mRNA in lateral view at 5.7 hpf. (C, C’) *szl* (red) and *chd* (orange) single molecular mRNA in lateral view at 8 hpf with nuclei stained by DAPI (blue).