Title: Maternal MAPK/Erk signaling regulates embryonic dorsal organizer formation in zebrafish

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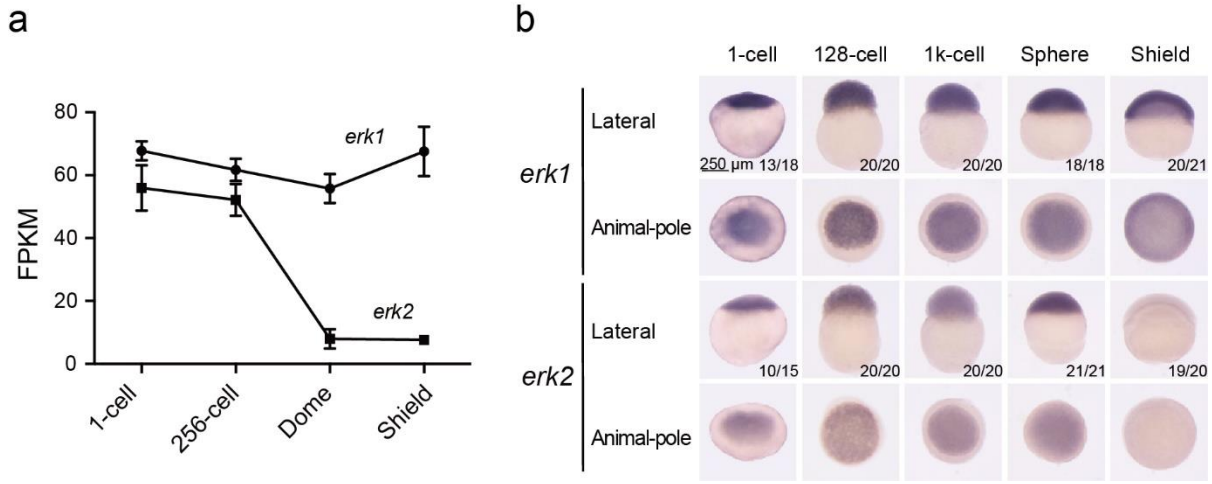
Supplementary Material file includes:

Supplementary Figures S1-S5

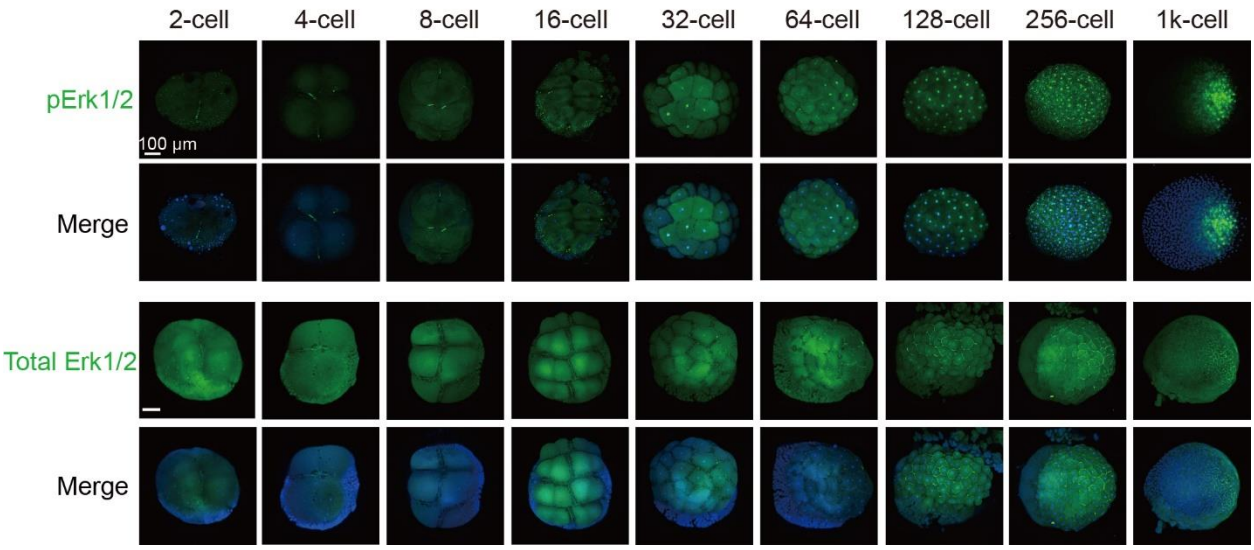
Supplementary Tables S1-S3

Supplementary Figure 1



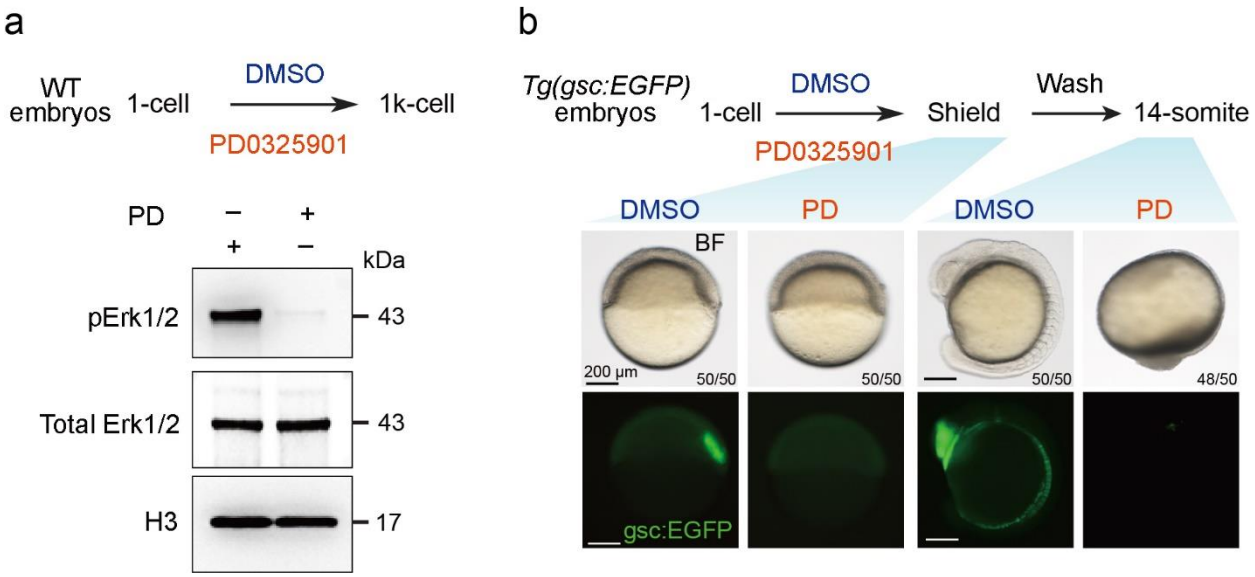
Supplementary Fig. 1. | Expression of *erk1* and *erk2* during early embryogenesis. a Expression levels of *erk1* and *erk2* at the indicated stages of embryonic development, extracted from RNA-seq data [46]. **b** Expression patterns of *erk1* and *erk2* during early embryonic development, examined by WISH. Note that *erk1* and *erk2* mRNAs are ubiquitously distributed in the blastoderm.

Supplementary Figure 2



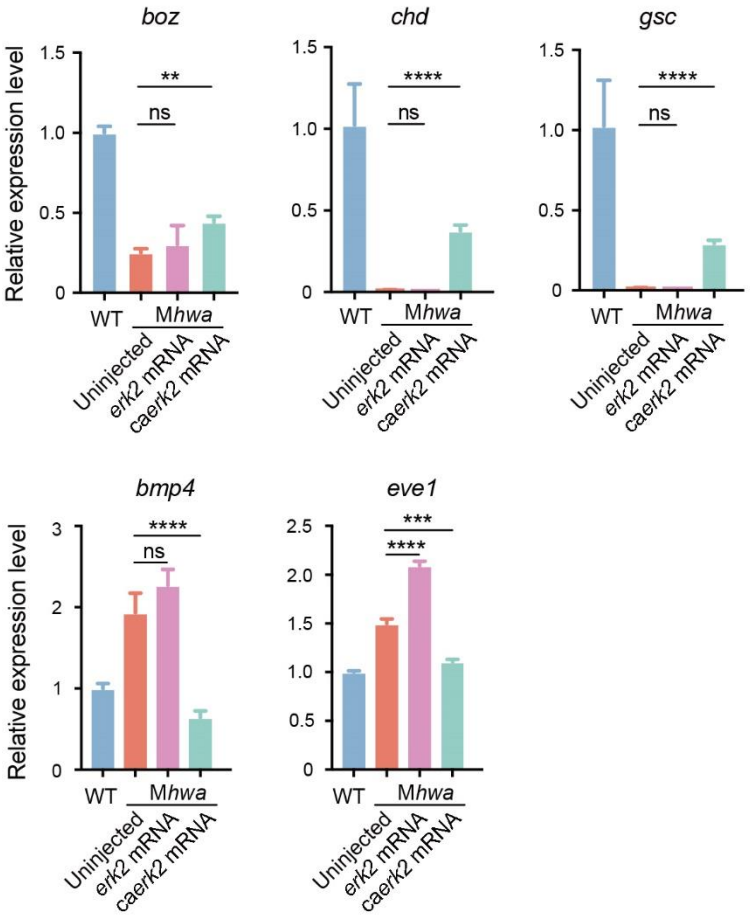
Supplementary Fig. 2. | Distribution of pErk1/2 and total Erk1/2 proteins during early embryogenesis. pErk1/2 and total Erk1/2 were detected by immunostaining with specific antibodies (green) along with nuclei stained by DAPI (blue). Immunostained embryos were observed by confocal microscopy. Embryos were viewed from the animal pole.

Supplementary Figure 3



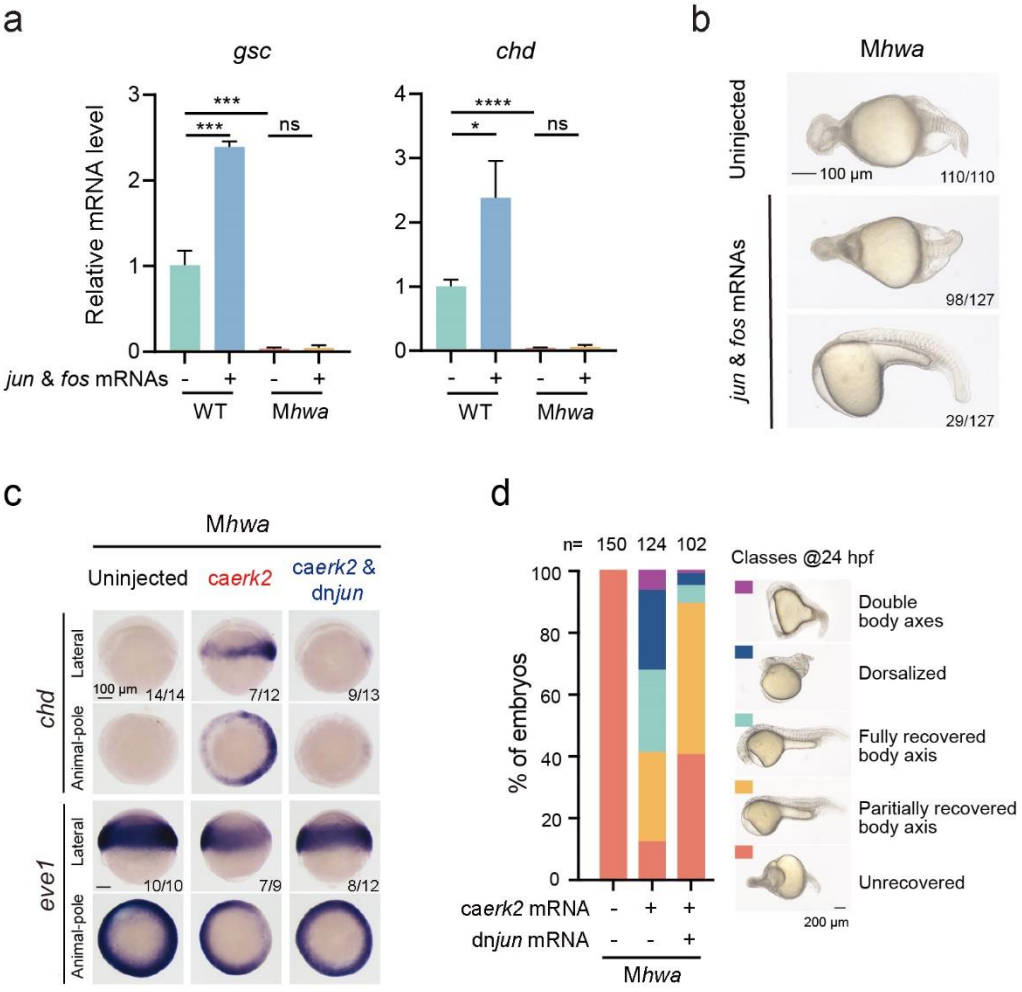
Supplementary Fig. 3. | Inhibition of MAPK/Erk signaling suppresses Erk1/2 activation and dorsal organizer formation during early embryonic development. a Erk1/2 activation in WT embryos is effectively suppressed by treatment with 20 μM of the MAPK/Erk inhibitor PD, while total Erk1/2 level remains unaltered. The proteins shown were detected by Western blotting. **b** Postfertilization inhibition of Erk1/2 activation impairs organizer formation. *Tg(gsc:EGFP)* transgenic embryos were treated with DMSO or 20 μM PD at the 1c stage and washed at the shield stage, followed by incubation in fresh culture water until the desired stages. Embryos were observed under a fluorescent dissection microscope. The ratio of embryos with the representative morphology was indicated. Note that EGFP expressed in the organizer in PD-treated embryos at the shield stage was completely abolished and most of the treated embryos died during the midsegmentation period.

Supplementary Figure 4



Supplementary Fig. 4. | Effects of *erk2* or ca*erk2* overexpression on dorsal and ventral markers in M*hwa* mutants. a Expression levels of the dorsal markers (*boz*, *chd*, and *gsc*) and ventral markers (*bmp4* and *eve1*) in WT, M*hwa*, and *erk2*- or ca*erk2*-injected M*hwa* embryos at the shield stages. 1c stage embryos were injected with 200 pg (per embryo) *erk2* or *caerk2* mRNA and collected at the shield stage for qRT-PCR analysis. Data were mean ± s.d. from three replicates; two-tailed unpaired Student's *t*-test was performed with ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, and ns with $P > 0.05$.

Supplementary Figure 5



Supplementary Fig. 5. | Erk1/2 signaling functions in dorsal organizer and body axis formation through Jun and Fos. a Effects of *jun/fos* overexpression on expression levels of the organizer markers *gsc* and *chd* in WT and M*hwa* embryos at the shield stage. Embryos were injected with *jun* and *fos* mRNAs (each 200 pg per embryo) at the 1c stage and collected at the shield stage for qRT-PCR analysis. Data were mean ± s.d. from three replicates; two-tailed unpaired Student's *t*-test was performed with * $P < 0.05$, *** $P < 0.001$, **** $P < 0.0001$, and ns with $P > 0.05$. **b** Overexpression of *jun/fos* in M*hwa* mutant embryos is unable to restore the body axis. M*hwa* mutant embryos were injected with *jun* and *fos* mRNAs (each 200 pg per embryo) at the 1c stage and observed at 24 hpf. The ratio of embryos with the representative morphology was indicated. **c** WISH-detected expression patterns of *chd* and *evel* in shield stage M*hwa* embryos co-injected with ca*erk2* and dn*jun* mRNAs. 1c stage embryos were injected with 200 pg (per embryo) *caerk2* mRNA and 200 pg dn*jun* mRNA (same for **d**). **d** Morphological changes of 24-hpf M*hwa* embryos injected with ca*erk2* mRNA or ca*erk2* mRNA plus dn*jun* mRNAs. n, number of observed embryos.

Supplementary Table S1.

Sequences of primers for genotyping *erk1*$^{-/-}$ and *erk2*$^{-/-}$ mutants.

Table S1 Sequences of primers for genotyping

Mutants	Sequences (5'-3')
erk1-M1 (-47 bp)	Forward: GATCTGAAAGCAAATACGAGGC
erk1-M2 (-8 bp)	Reverse: TACTGGAGATCAGTGTATCGGG
erk2-M3 (-5 bp)	Forward: ACCGAGTCTTCGGTTCAGTTTA
	Reverse: GTAGGAGAGGTTGCTGTAACGC
erk2-M4 (-2 bp)	Forward: GTACAAGCTGTTGAAGACGCAG
	Reverse: TAACGTCCAAGATGAGACCCTT

Supplementary Table S2.

Sequences of probe antisense primers for whole-mount in situ hybridization.

Table S2 Sequences of probe primers for whole-mount in situ hybridization

Genes	Sequences (5'-3')
erk1	Sense: GCCAGGAACTACCTCCAG
	Antisense: TAATACGACTCACTATAGGGGCCCAAACTAGCGCACAGC
erk2	Sense: CATGACCACACCGGCTTCC
	Antisense: TAATACGACTCACTATAGGGCGAAAGAGGAGCACAGCGC

Supplementary Table S3.

Sequences of primers for quantitative RT-PCR analysis of gene expression.

Table S3 Sequences of primers for quantitative RT-PCR

Genes	Sequences (5'-3')
erk1	Forward: GGATGGTCTGCTCAGCTTTTG
	Reverse: CCTGTACGATATAGACATCCCTC
erk2	Forward: GATGAAAGACGTTTACATCGTACAGG
	Reverse: GCCCAAAATCACAGATCTTGAGGTC
pla2g4aa	Forward: CGGCAAGACAACCAAAGTG
	Reverse: GTTGCACAGTCAAACACGCC
crtc3	Forward: CGGAACGCCAGCATGAATG
	Reverse: GCTCAGATTGGGATACCCAGC
elk1	Forward: GCATGAGATGCGAGTTTGTCAG
	Reverse: CCAACAGACGGAGGCTTTATG
hwa	Forward: GCATCATCCCACAGGAGAAC
	Reverse: GTGACGTAACTTGGGTCGTA
boz	Forward: ACACCAGCAGGCAAACAGCA
	Reverse: CAACCGGCTACGGCATAAGG
chd	Forward: AGACTGCTGTAAGGAGTGTCCTC
	Reverse: CCATGAAGTCCTCTATGCATTCCG
gsc	Forward: TCCAGCGCCGAACTTACAAT
	Reverse: GCGCTGTCATAACCTGTAGGAA
bmp4	Forward: CCAACACCGTGAGAGGATTCC
	Reverse: TCCACAGCAAGGCCATGATTAG
eve1	Forward: ATGCCAGAGGACAGGGAGTT
	Reverse: GAGTCAGCTGTTCCCTCGTG
β-actin	Forward: ATGGATGATGAAATTGCCGCAC
	Reverse: ACCATCACCAGAGTCCATCACG