Supplementary Information

Figure S1. The expression of *efemp2a* in paraxial mesoderm is regulated by Stat5.1.

(A) The expression pattern of *efemp2a* in paraxial mesoderm. (B) The expression pattern of *stat5.1* in paraxial mesoderm. (C, D) Knockdown *stat5.1* induced reduction of *efemp2a* expression. Ctl-MO injected embryo (C), Stat5.1-MO injected embryo (D). Embryos were the tail-bud stage and dorsal views are shown with anterior on the top (A-D).

Figure S2. Disruption of Efemp2a function blocks the anterior migration of prechordal plate progenitors during gastrulation.

(A) Quantification of the phenotypes induced by micro-injection experiments. (B) Anti-human Efemp2 antibody was able to detect the zebrafish Efemp2a-GFP fused protein. (C) Endogenous Efemp2a expression was identified through immunoblot using Anti-human Efemp2 antibody in Ctl-MO and Efemp2a-MO -injected embryos at bud stage. (D) Efemp2a-MO inhibited the expression of the zebrafish Efemp2a-GFP fusion protein in zebrafish embryos at the 75%-epiboly stage.

Figure S3. Monitoring the tracks of the signed cell clusters during anterior migration or convergent movement.

(A, B) Three clusters including six cells were randomly highlighted in different colors among the prechordal plate progenitors (A) or lateral mesendodermal cells (B) in control, Stat3-depleted, Efemp2a-deficient or recovered embryos. Cell tracks are displayed in figure 2A and S4A. Animal-pole views are shown with dorsal on the top (A), lateral views are shown with anterior on the top and dorsal to the right (B). Embryos were the 75%-epiboly stage (A, B).

Figure S4. Stat3-Efemp2a signaling does not control the coherence of lateral mesendodermal cells during convergent migration.

(A) Tracks of the monitored lateral mesendodermal cells in the embryo injected with Ctl-MO, Stat3-MO, Efemp2a-MO, Stat3-MO combined with Efemp2a mRNA or Efemp2a-MO

combined with Efemp2a mRNA. Three clusters including six neighboring cells highlighted in different colors. (B) Analyzed parameters: speed, coherence, persistence and distance. * P < 0.05, ** P<0.01 versus control, ns: no significant, t-test.

Figure S5. Defective ECM induces abnormal morphology of prechordal plate progenitors during gastrulation.

(A-D) The morphology of prechordal plate progenitors in Ctl-MO (A), Efemp2a-MO (B), Stat3-MO (C) or 70 kD-FN (D) -injected embryos. The magnified views of the regions marked by dashed rectangles in A-D (a-d). (E) *gsc* marked the region of prechordal plate in Ctl-MO (a), Stat3-MO (b), Efemp2a-MO (c) or 70 kD-FN (d) -injected embryos. w: the width of *gsc* expression. Dash line indicated the width of *gsc* expression. Quantification of the width of *gsc* expression (e). Embryos were the 75%-epiboly stage (A-E). ** P<0.01 versus control, t-test.

Figure S6. Efemp2a is an extracellular protein and plays a non-cell autonomous role.

(A, C) Prechordal mesendodermal (PCME) cells from wild-type (green) or Efemp2a-deficient donors (red) were transplanted into the inner domain of the shield of a wild-type or Efemp2a-deficient host. Images were taken at the end of gastrulation. (B, D) Lateral mesendodermal (LME) cells from wild-type (green) or Efemp2a-deficient (red) donors were transplanted into the lateral side of a wild-type or Efemp2a-deficient host. Images were taken at the end of gastrulation. Lateral views are shown with anterior on the top and dorsal on the right (A, C); dorsal views are shown anterior on the top (B, D). (E) Adenoviruses overexpressing GFP or Efemp2-GFP were used to infect AD293 cells, and the culture medium was collected. Through immunoprecipitation, GFP-tagged Efemp2a rather than β-actin was detected in the culture medium. Embryos were the tail-bud stage (A-D).

Figure S7. FN and LM rather than Elastin are expressed at gasstrulation in zebrafish embryo.

(A) The expression of elastin was detected by RT-PCR at early gastrulation and 10 dpf. (B, C) *fn1* and *lamb1* expression were examined by ISH at bud stage. Dashed indicated the region of

prechordal plate. Embryos were the tail-bud stage and animal views are shown with ventral on the top (B, C).

Figure S8. Alteration of Efemp2a activity does not affect the expression of FN and LM.

RT-PCR was used to detect expression of fibronectin and three subunits of laminin-1 at shield, 75%-epiboly and bud stages. Expression of *gapdh* was used as RNA loading control.

Figure S9. The recombinant Efemp2 promotes the assembly of FN and LM mediated by cultured cell *in vitro*.

(A) MDCK cells were cultured on coverglass slips. Adding recombinant Efemp2 (1ug) and FN (10ug) (a, e), recombinant Efemp2 (0.5ug) and FN (10ug) (b), recombinant Efemp2 (0.1ug) and FN (10ug) (c) or FN (10ug) (d, f) into culture medium. Cells were infected by adenovirus expressing C350 (AD-C350) in e, f. g is blank control. (B) HeLa cells were cultured on coverglass slips. Adding recombinant Efemp2 (1ug) and FN (10ug) (a, e), recombinant Efemp2 (0.5ug) and FN (10ug) (b), recombinant Efemp2 (0.1ug) and FN (10ug) (c) or FN (10ug) (d, f) into culture medium. Cells were infected by adenovirus expressing C350 (AD-C350) in e, f. g is blank control.

Figure S10. FN matrix influences gastrula movements.

(A-D) 70 kD-FN interfered the assembly of WT FN *in vitro*. Adding FN (10ug) (A), FN (10ug) combined with 70 kD-FN (5ug) (B), 70 kD-FN (5ug) (C) into the medium of cultured MDCK cells. D is blank control. (E, F) Whole-mount immunohistochemistry staining for BSA (0.8ug/ul) or 70 kD-FN (0.8ug/ul) -injected embryos. Dashed indicated the region of prechordal plate. (G) Tracks of the monitored prechordal plate progenitors in the embryo injected with BSA or 70 kD-FN. Three clusters including six neighboring cells highlighted in different colors. (B) Analyzed parameters: speed, persistence and coherence. Embryos were the tail-bud stage (E, F). * $P < 0.05$, ** $P<0.01$ versus control, t-test.

Figure S11. Alteration of Efemp2a activity does not induce EMT.

(A) The expression of EMT marker genes, including *e-cadherin* (a-c), *tjp1a* (d-e), *n-cadherin* (g-i) and *snai1b* (j-l) were detected by ISH in zebrafish embryos injected with Ctl-MO, Efemp2a or Efemp2a mRNA. (B) HeLa cells overexpressing GFP, Efemp2a or C350 by

adenovirus infection were assessed for expression levels of the EMT marker proteins by immunoblot. E-cadherin, Zo1 were epithelial markers; N-cadherin and Zeb1 were used as mesenchymal markers. Embryos were the tail-bud stage (A). Animal-pole views are shown with ventral on the top (Aa-Ac, Aj-Al), lateral views are shown with anterior on the top and dorsal to the right (Ad-Ai).

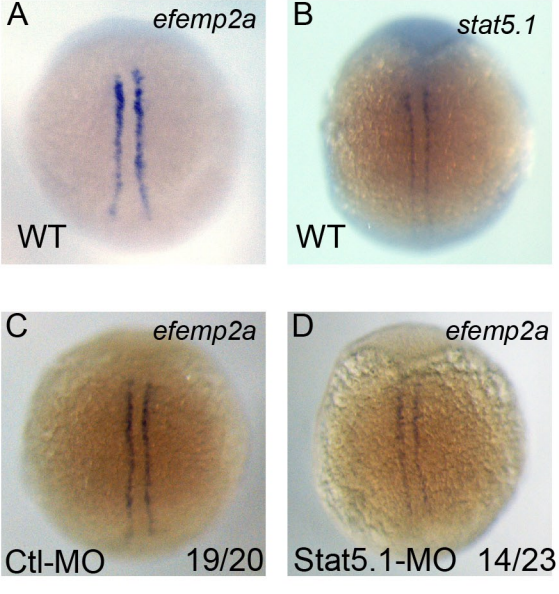
Figure S12. The changes of the focal adhesion receptors expression in Efemp2a-deficient embryos.

(A-F) Detection of the expression of *itgaV* (A, B), *itgb1* (C, D) and *sdc4* (E, F) via ISH at bud stage. Animal-pole views are shown with ventral on the top (A-D), lateral views are shown with anterior on the top and dorsal to the right (E, F).

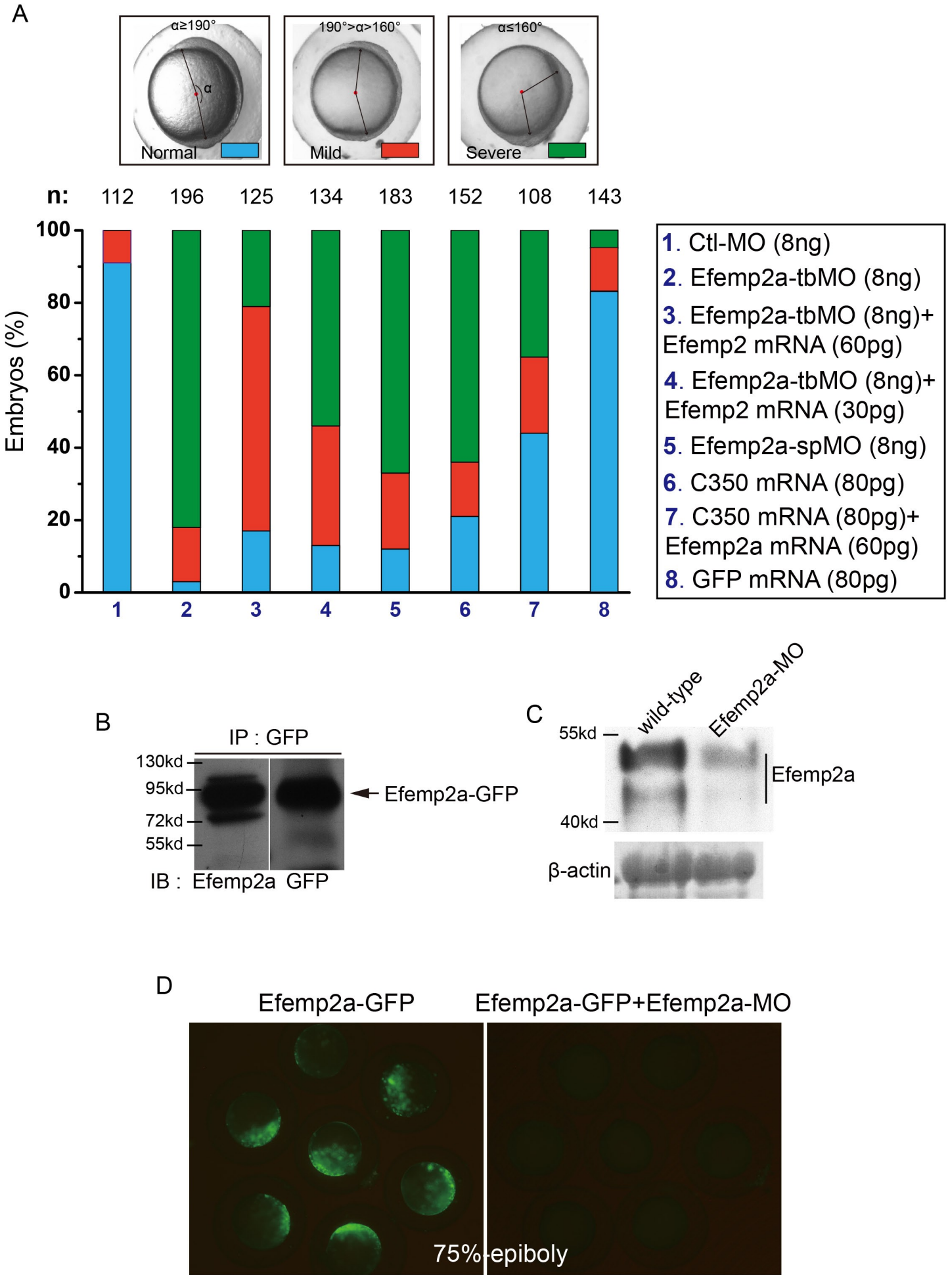
Figure S13. A proposed model for the role of Stat3-Efemp2a signaling during the embryonic cells collective migration.

Stat3 regulates the expression of Efemp2a playing non cell-autonomous role. Efemp2a promotes FN and LM assembly into correct matrix to control coherence of multi-cell migration. The other Stat3 downstream targets, X proteins function as cell-autonomous role and mainly regulate the cellular persistence and mobility during cell movements.

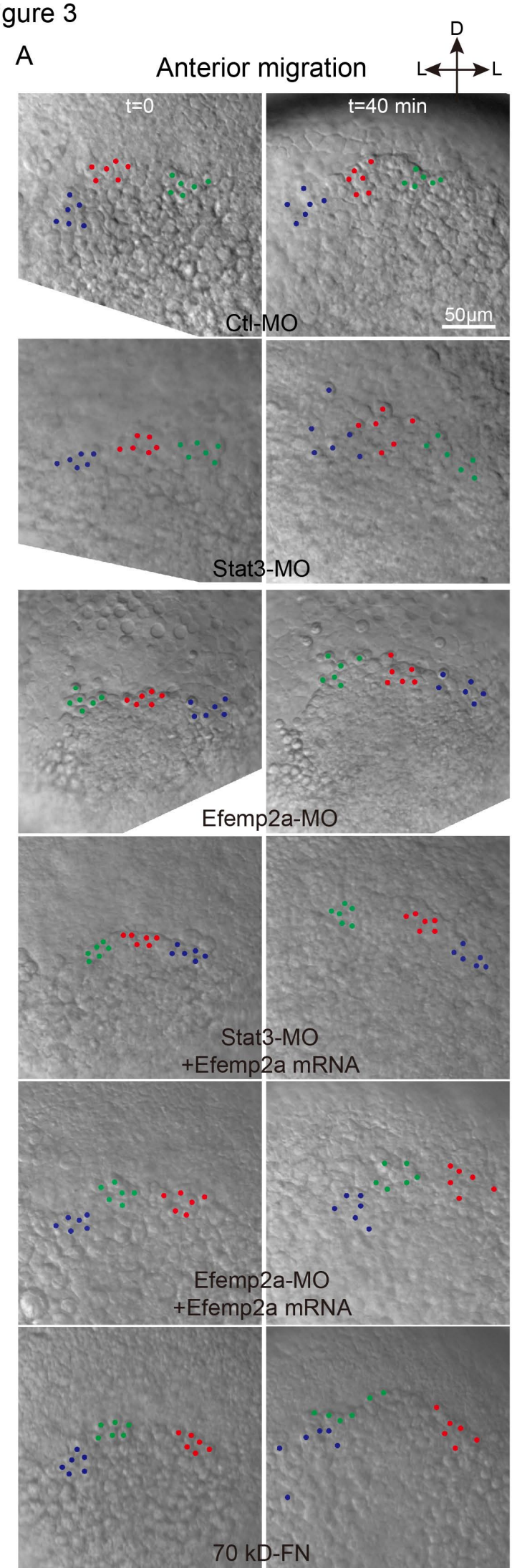
Sfigure 1


A
efemp2a
WT
B
stat5.1
WT
C
efemp2a
Ctl-MO
19/20
D
efemp2a
Stat5.1-MO
14/23

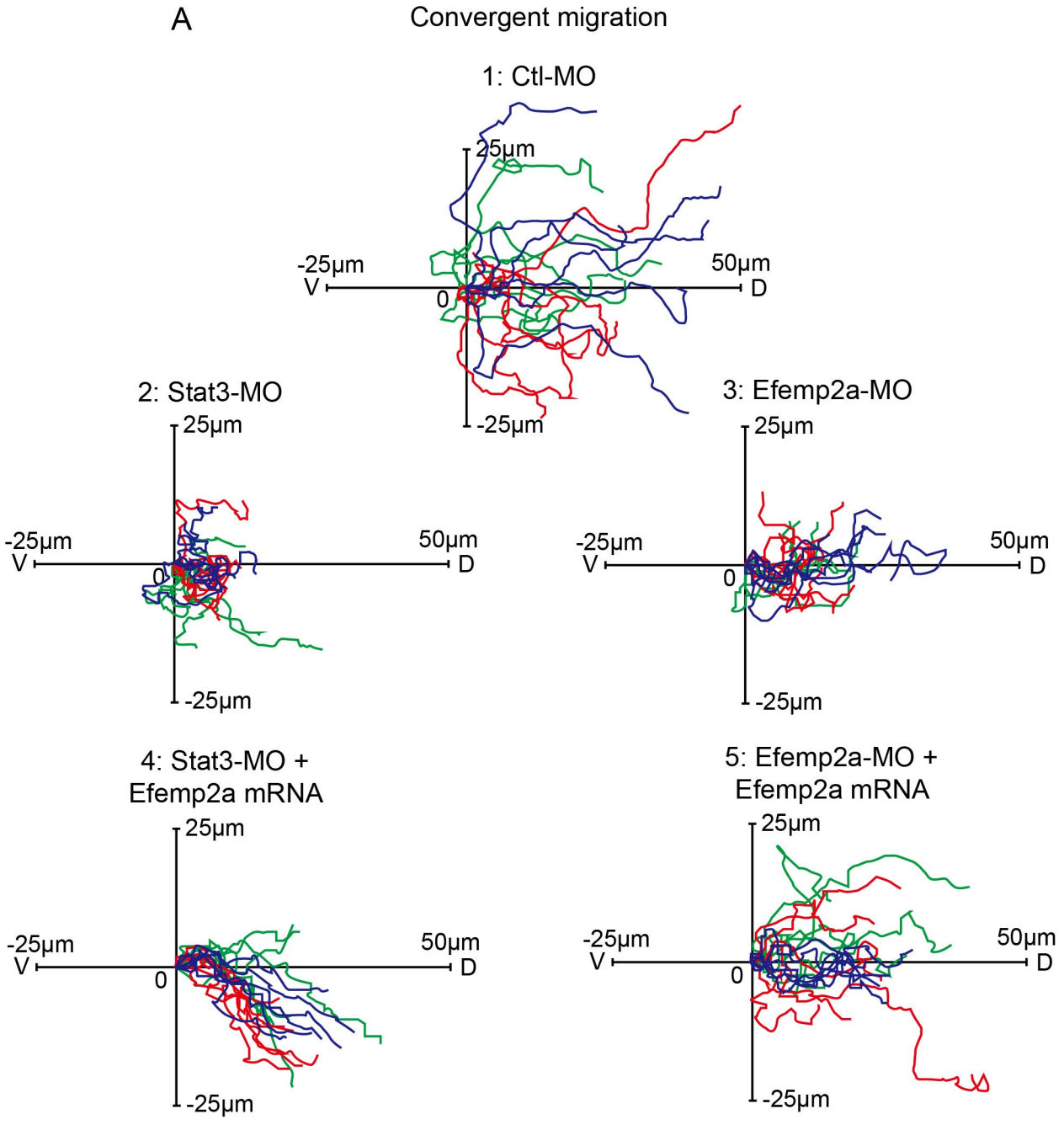
Sfigure 2

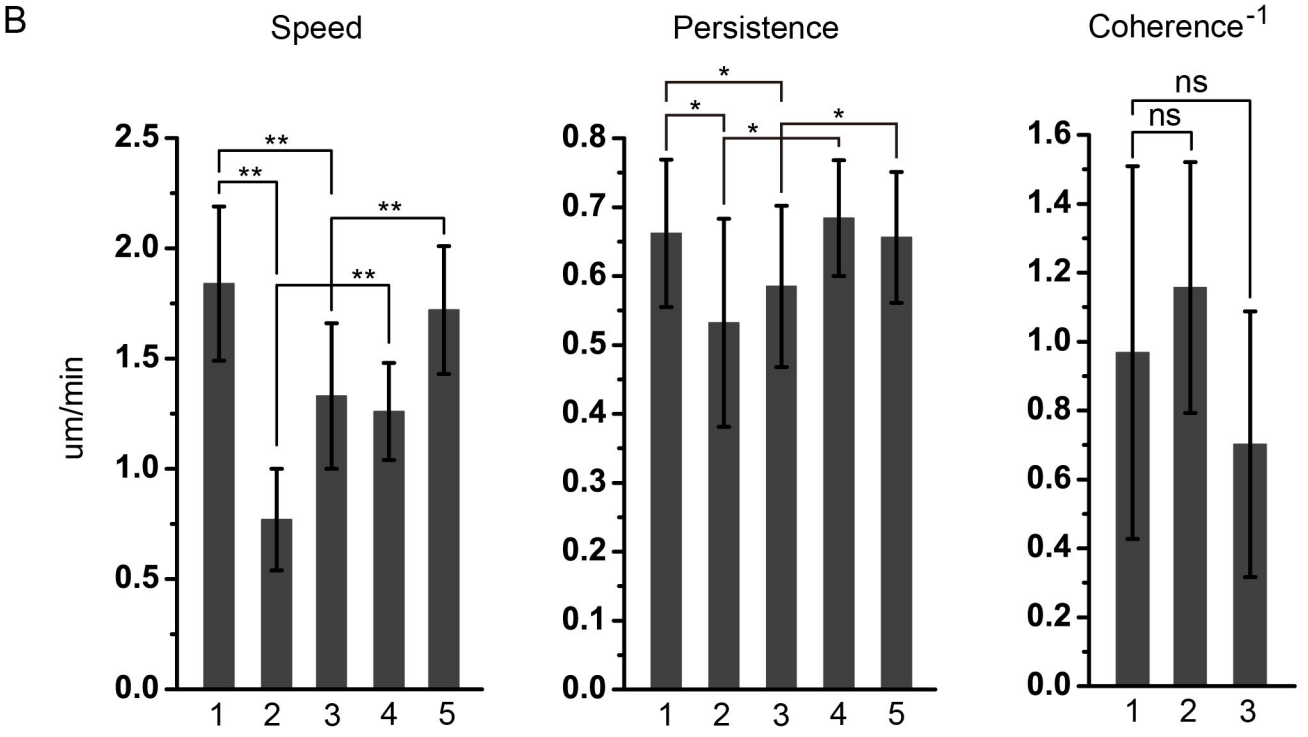

A
α≥190°
190°>α>160°
α≤160°
α
Normal
Mild
Severe
n: 112 196 125 134 183 152 108 143
Embryos (%)
100
80
60
40
20
0
1 2 3 4 5 6 7 8
1. Ctl-MO (8ng)
2. Efemp2a-tbMO (8ng)
3. Efemp2a-tbMO (8ng)+ Efemp2 mRNA (60pg)
4. Efemp2a-tbMO (8ng)+ Efemp2 mRNA (30pg)
5. Efemp2a-spMO (8ng)
6. C350 mRNA (80pg)
7. C350 mRNA (80pg)+ Efemp2a mRNA (60pg)
8. GFP mRNA (80pg)
B
IP : GFP
130kd
95kd
72kd
55kd
Efemp2a-GFP
IB : Efemp2a GFP
C
wild-type
Efemp2a-MO
55kd
40kd
Efemp2a
β-actin
D
Efemp2a-GFP
Efemp2a-GFP+Efemp2a-MO
75%-epiboly

Sfigure 3

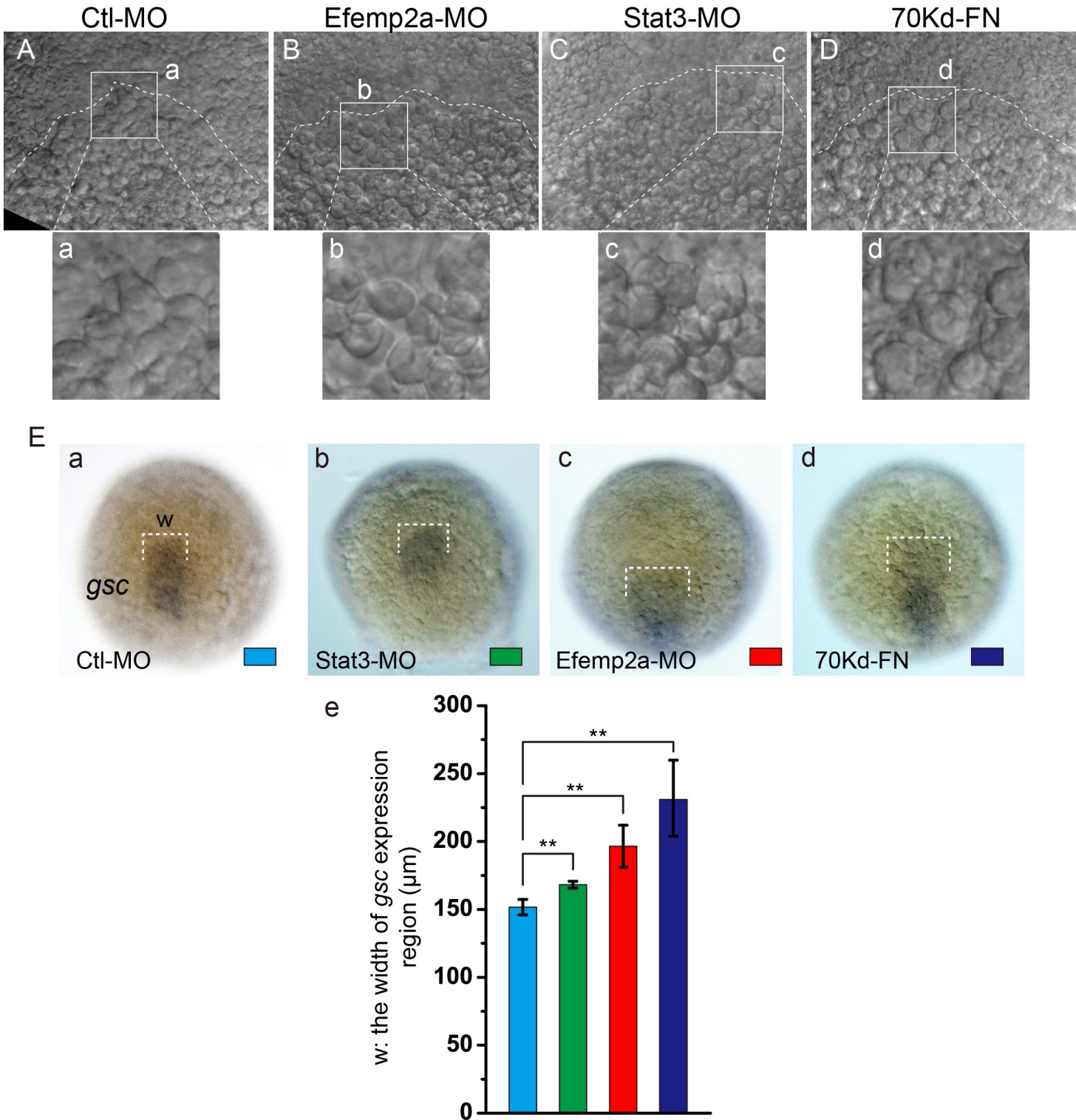

A
Anterior migration
D
L
L
t=0
t=40 min
50μm
Ctl-MO
Stat3-MO
Efemp2a-MO
Stat3-MO
+Efemp2a mRNA
Efemp2a-MO
+Efemp2a mRNA
70 kD-FN
B
Convergent migration
A
D
t=0
t=20 min
25μm
Ctl-MO
Stat3-MO
Efemp2a-MO
Stat3-MO
+Efemp2a mRNA
Efemp2a-MO
+Efemp2a mRNA

Sfigure 4

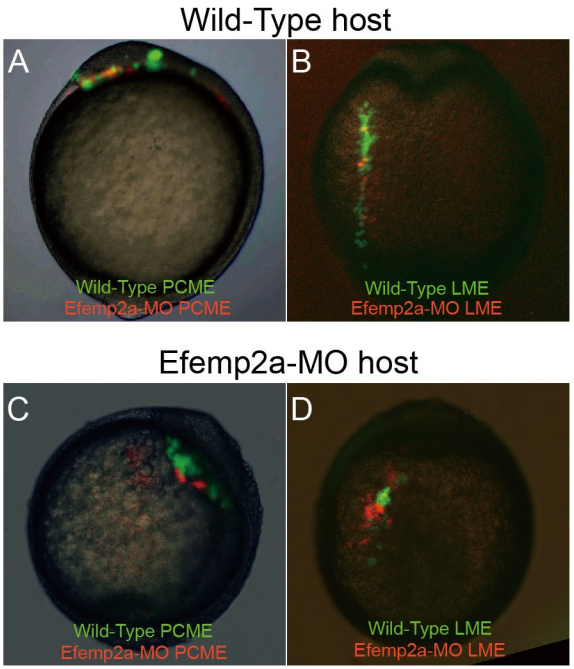

A
Convergent migration
1: Ctl-MO
2: Stat3-MO
3: Efemp2a-MO
4: Stat3-MO +
Efemp2a mRNA
5: Efemp2a-MO +
Efemp2a mRNA
25μm
-25μm
50μm
V
D
0

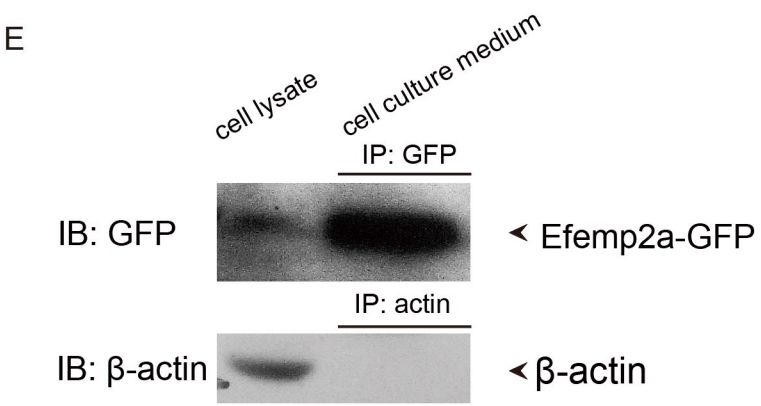

B
Speed
Persistence
Coherence-1
um/min
**
*
ns

Sfigure 5

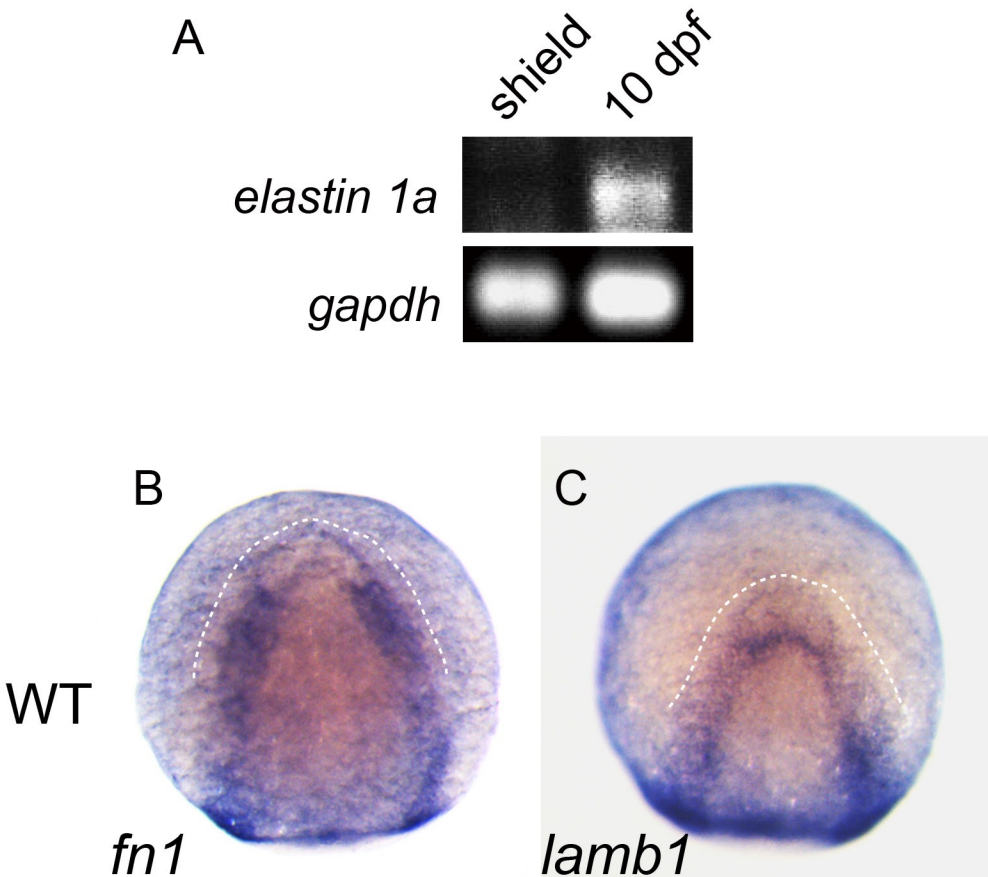

Ctl-MO
Efemp2a-MO
Stat3-MO
70Kd-FN
A
B
C
D
a
b
c
d
E
w
gsc
Ctl-MO
Stat3-MO
Efemp2a-MO
70Kd-FN
e
w: the width of gsc expression region (μm)
300
250
200
150
100
50
0
**
**
**

Sfigure 6

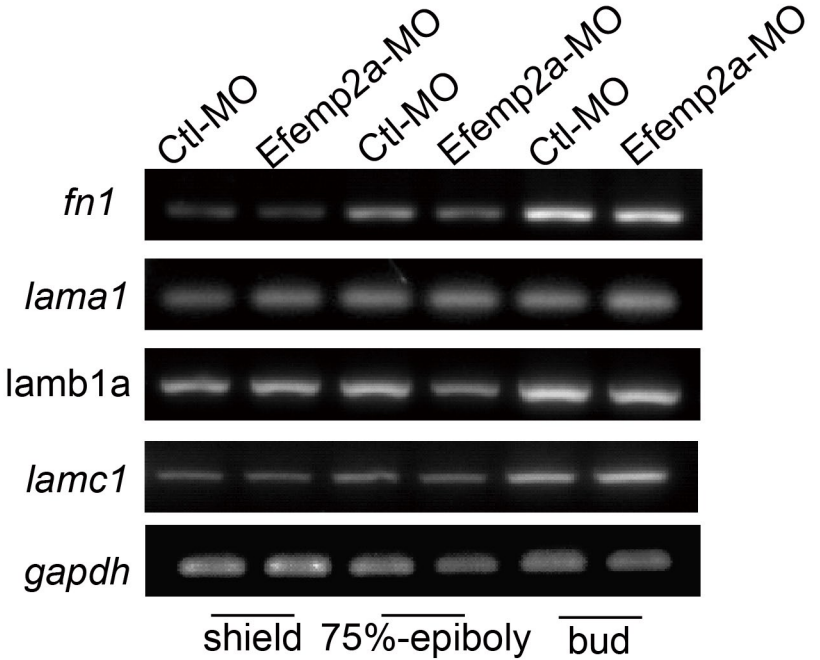

Wild-Type host
A
Wild-Type PCME
Efemp2a-MO PCME
B
Wild-Type LME
Efemp2a-MO LME
Efemp2a-MO host
C
Wild-Type PCME
Efemp2a-MO PCME
D
Wild-Type LME
Efemp2a-MO LME


E
cell lysate
cell culture medium
IP: GFP
IB: GFP
Efemp2a-GFP
IP: actin
IB: β-actin
β-actin

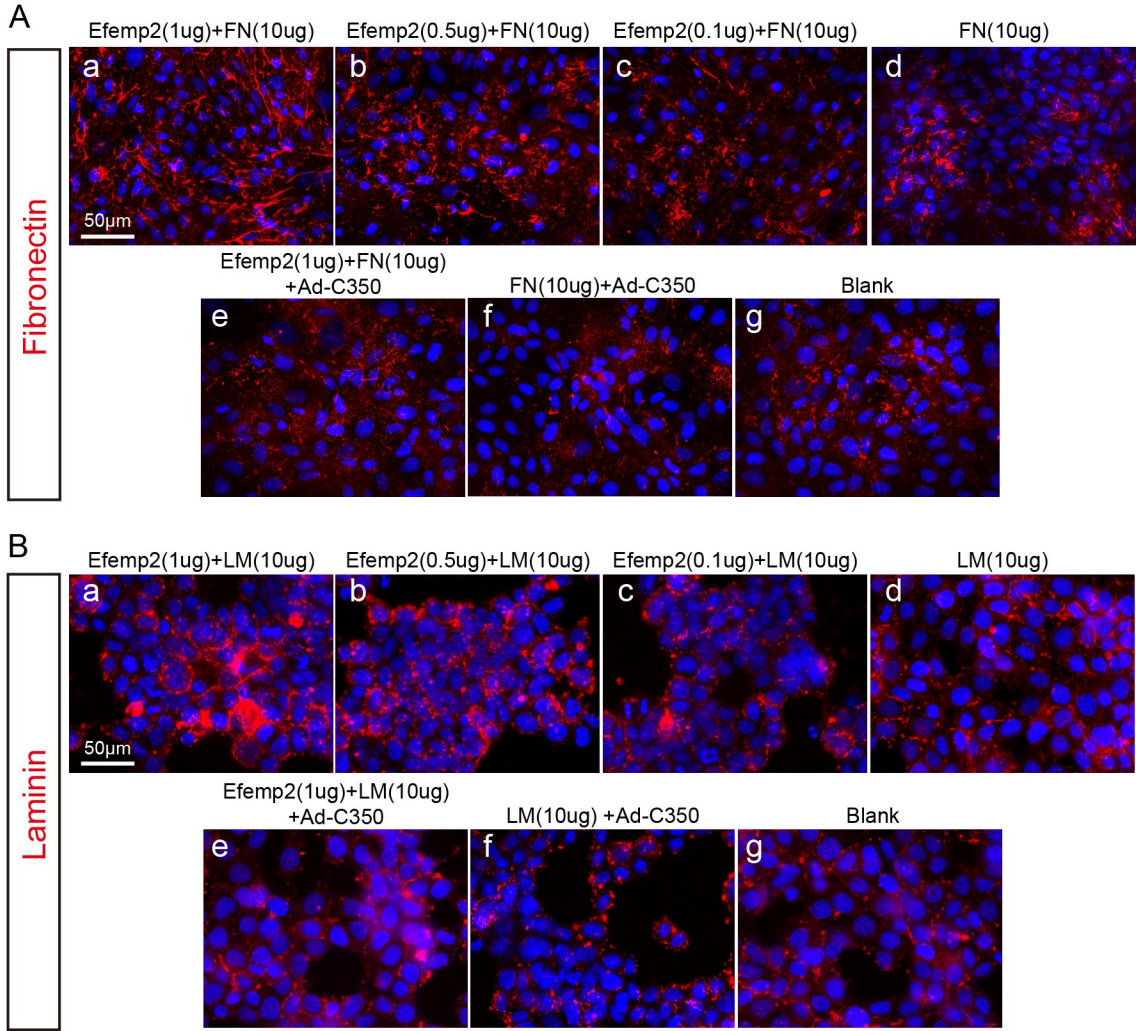
Sfigure 7



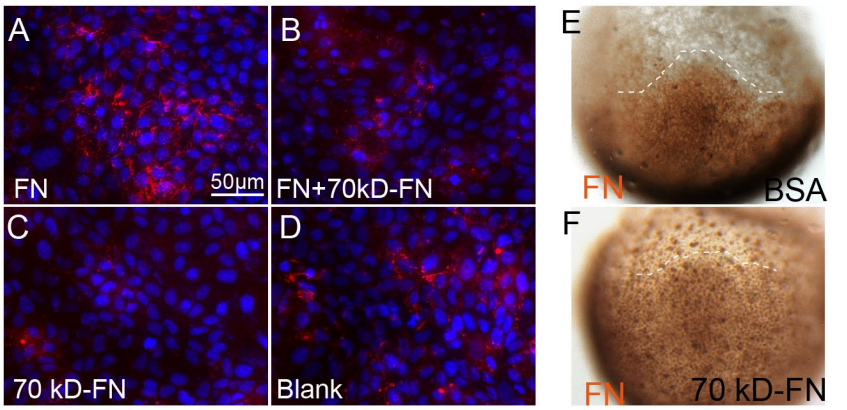
Sfigure 8

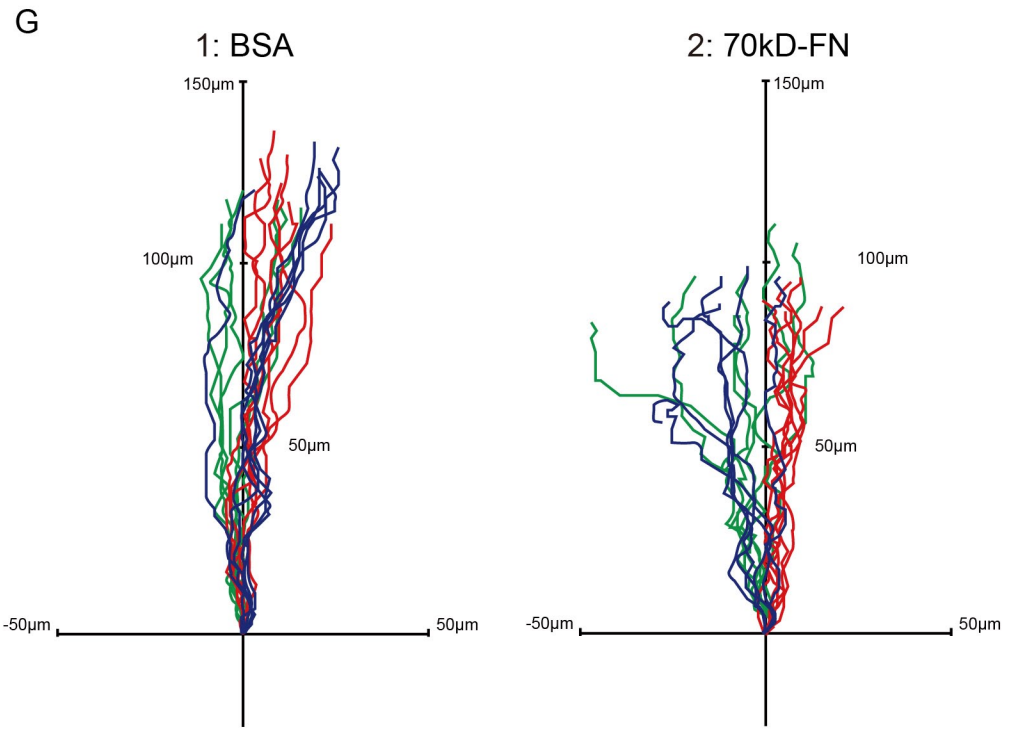

Ctl-MO
Efemp2a-MO
Ctl-MO
Efemp2a-MO
Ctl-MO
Efemp2a-MO
fn1
lama1
lamb1a
lamc1
gapdh
shield
75%-epiboly
bud

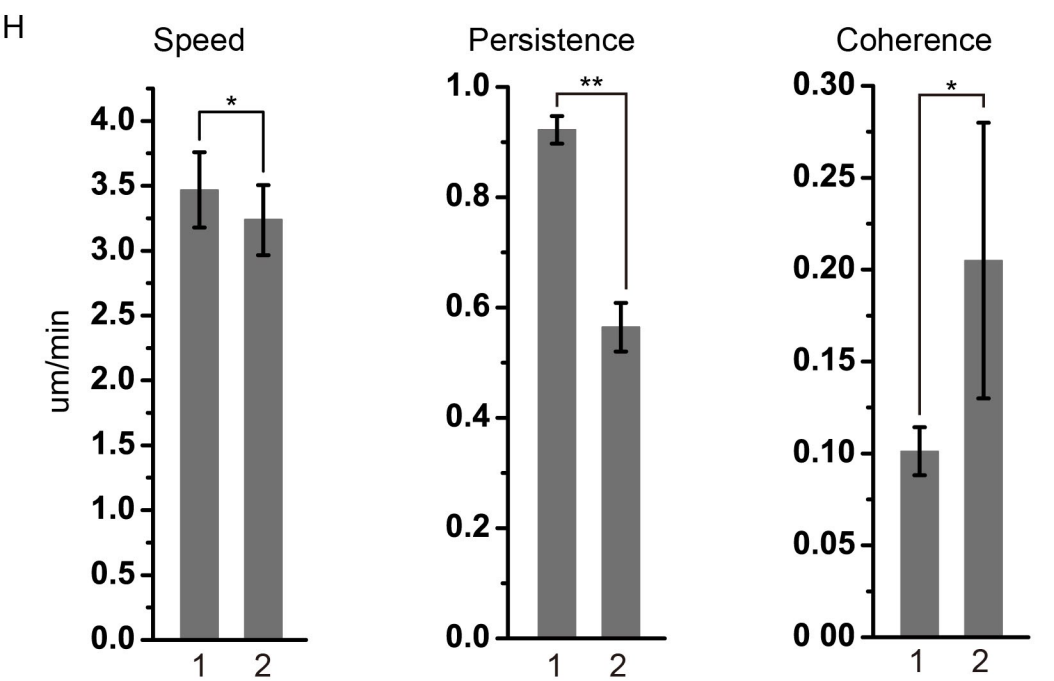
Sfigure 9


A
Efemp2(1ug)+FN(10ug)
Efemp2(0.5ug)+FN(10ug)
Efemp2(0.1ug)+FN(10ug)
FN(10ug)
Fibronectin
a
b
c
d
50μm
Efemp2(1ug)+FN(10ug)
+Ad-C350
FN(10ug)+Ad-C350
Blank
e
f
g
B
Efemp2(1ug)+LM(10ug)
Efemp2(0.5ug)+LM(10ug)
Efemp2(0.1ug)+LM(10ug)
LM(10ug)
Laminin
a
b
c
d
50μm
Efemp2(1ug)+LM(10ug)
+Ad-C350
LM(10ug) +Ad-C350
Blank
e
f
g

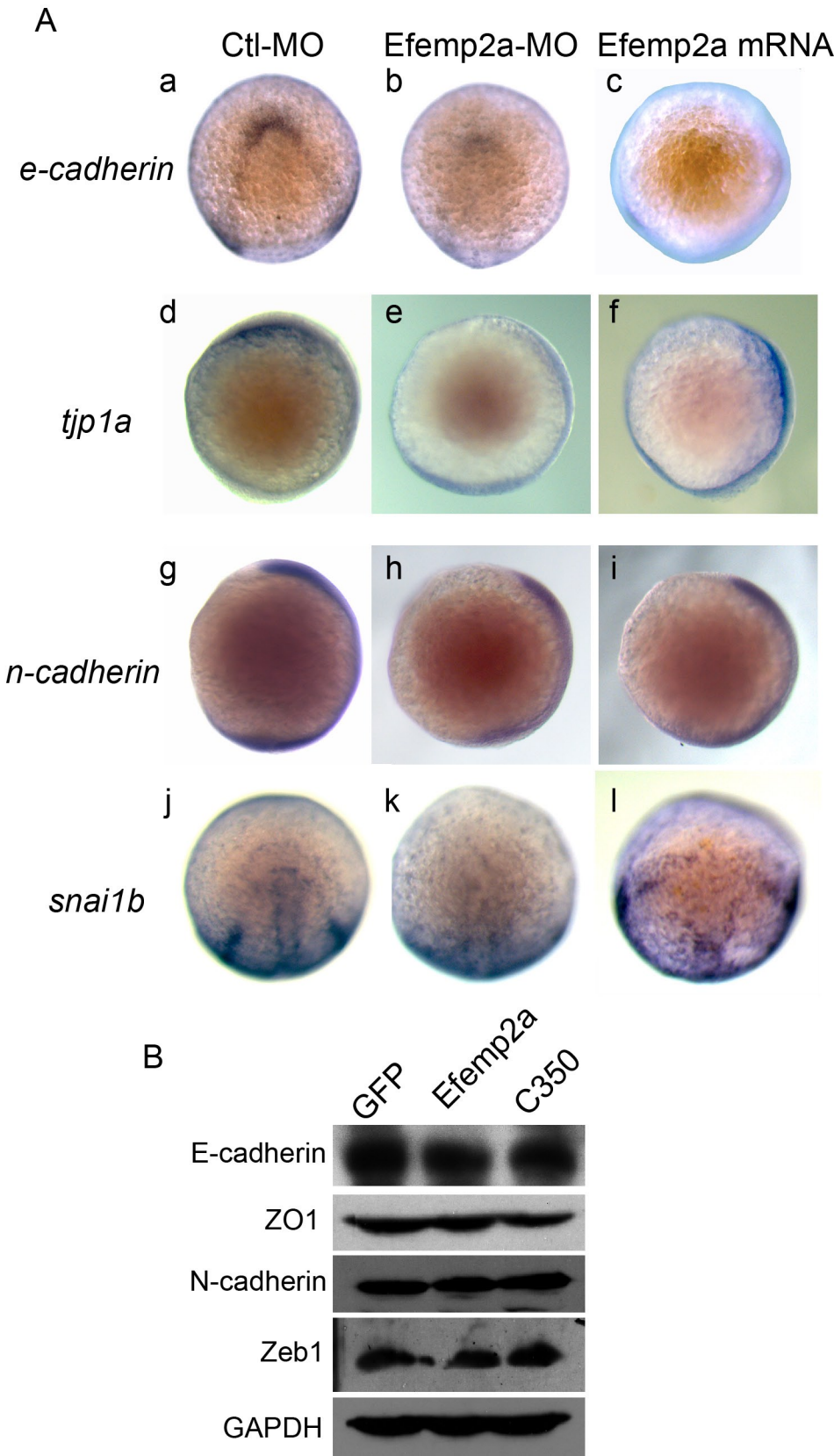
Sfigure 10


A
FN
50μm
B
FN+70kD-FN
C
70 kD-FN
D
Blank
E
FN
BSA
F
FN
70 kD-FN

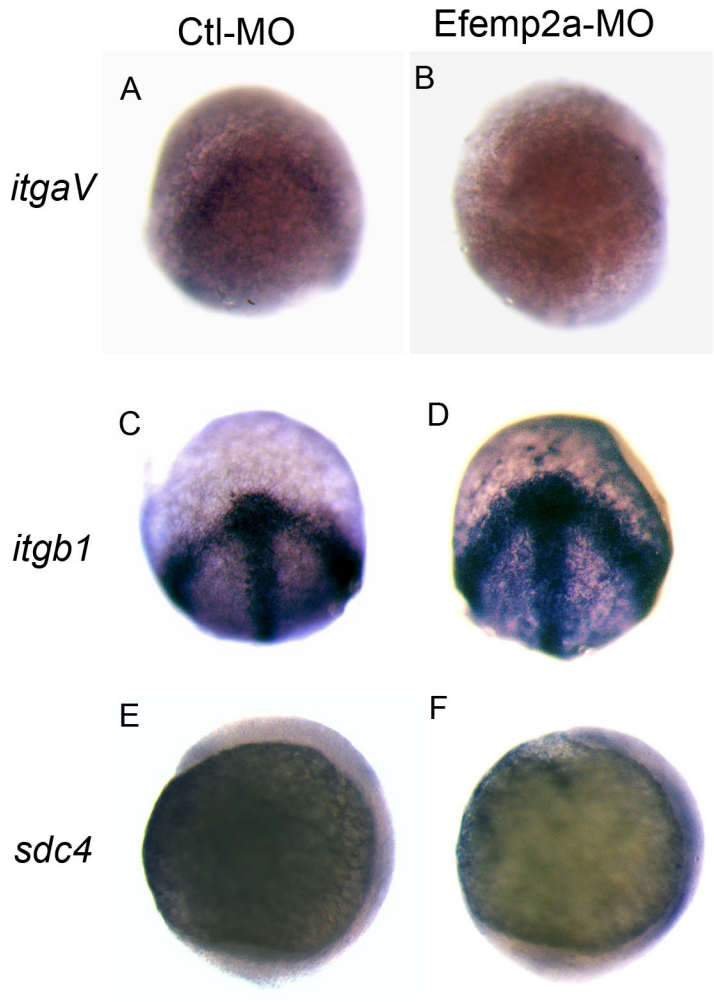

G
1: BSA
2: 70kD-FN
150μm
100μm
50μm
-50μm
50μm


H
Speed
Persistence
Coherence
um/min
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
0.0
1.0
0.8
0.6
0.4
0.2
0.0
0.30
0.25
0.20
0.15
0.10
0.05
0 00
1
2
*
**

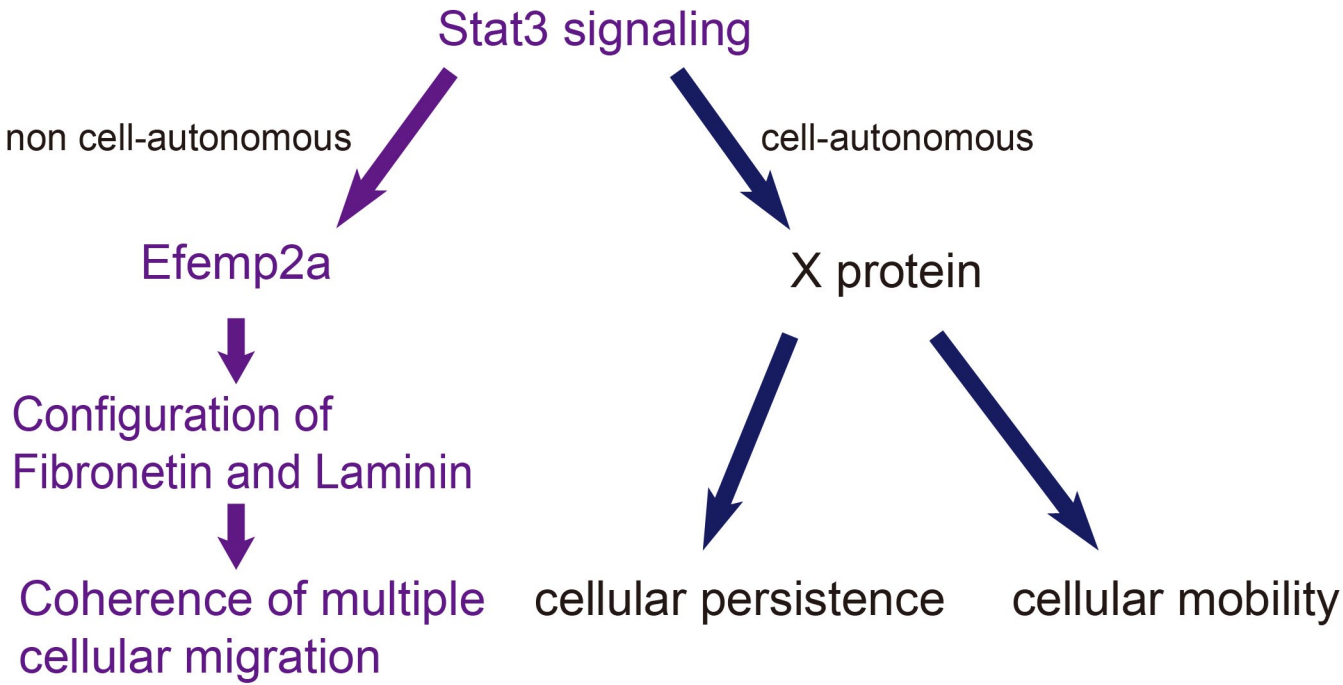
Sfigure 11


A
Ctl-MO
Efemp2a-MO
Efemp2a mRNA
a
b
c
e-cadherin
d
e
f
tjp1a
g
h
i
n-cadherin
j
k
l
snai1b
B
GFP
Efemp2a
C350
E-cadherin
ZO1
N-cadherin
Zeb1
GAPDH

Sfigure 12



Sfigure 13


Stat3 signaling
non cell-autonomous
cell-autonomous
Efemp2a
X protein
Configuration of
Fibronetin and Laminin
Coherence of multiple
cellular migration
cellular persistence
cellular mobility