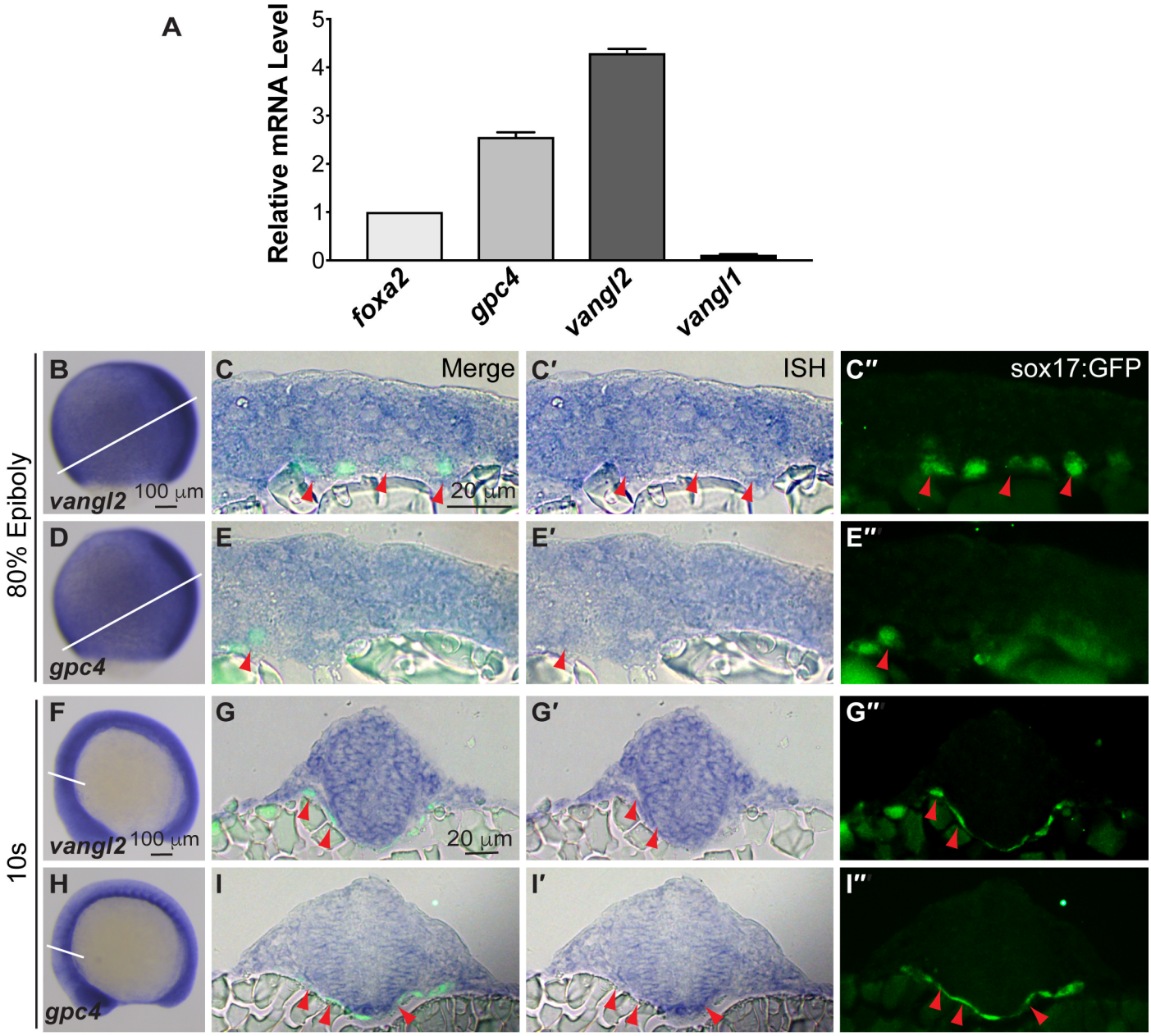



**Figure S1. The expression of *gpc4* and *vangl2* during gastrulation and early segmentation**.

(**A**) Expression of *gpc4*, *vangl2* and *vangl1* relative to that of *foxa2,* and endoderm marker, as determined by qRT-PCR, in GFP$^{+}$ cells sorted from *Tg(sox17:EGFP)* embryos at 18s. Bars represent the mean±s.e.m. (**B-I″**) The expression of *gpc4* and *vangl2* transcripts in *Tg(sox17:EGFP)* embryos at 80%E and 10 s, as detected by WISH. (B, D, F, H) Images of the whole embryo. White lines indicate the cross-sectional plane. (C-C″, E-E″, G-G″, I-I″) Transverse sections of the embryos. (C, E, G, I) Overlays of anti-GFP immunofluorescence staining (*sox17:EGFP* panels) and ISH for *vangl2* and *gpc4* (ISH panels), in endodermal cells (red arrowheads).

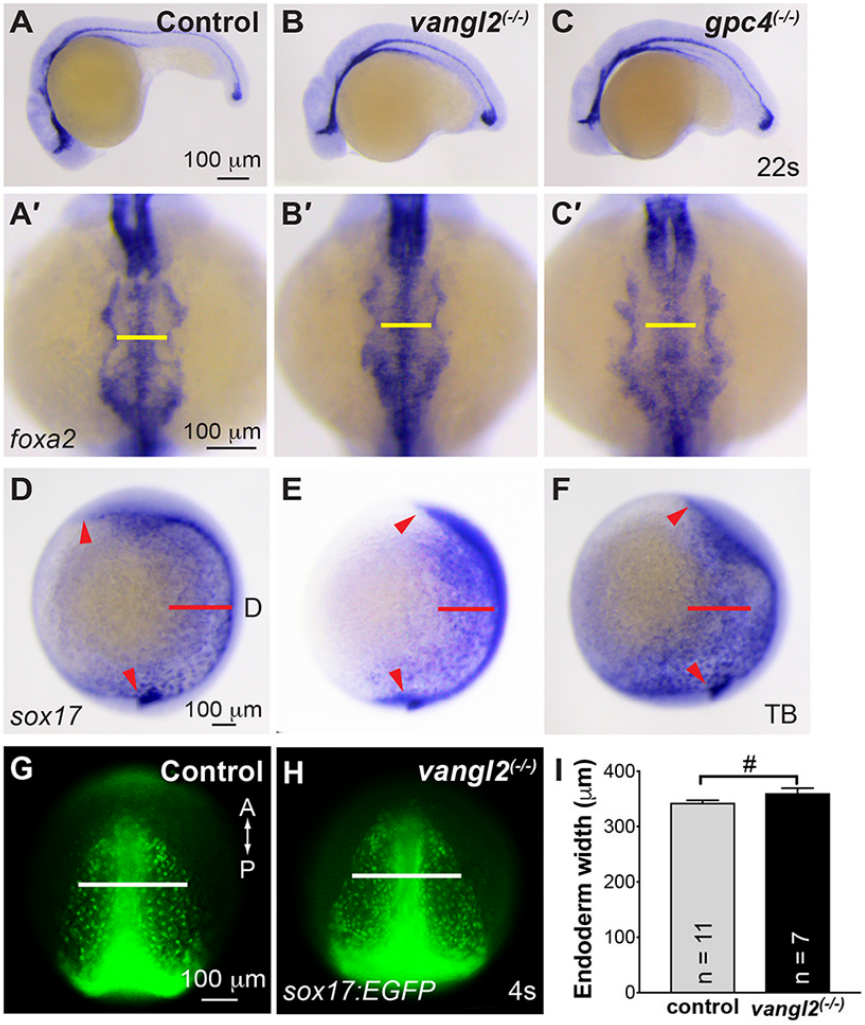
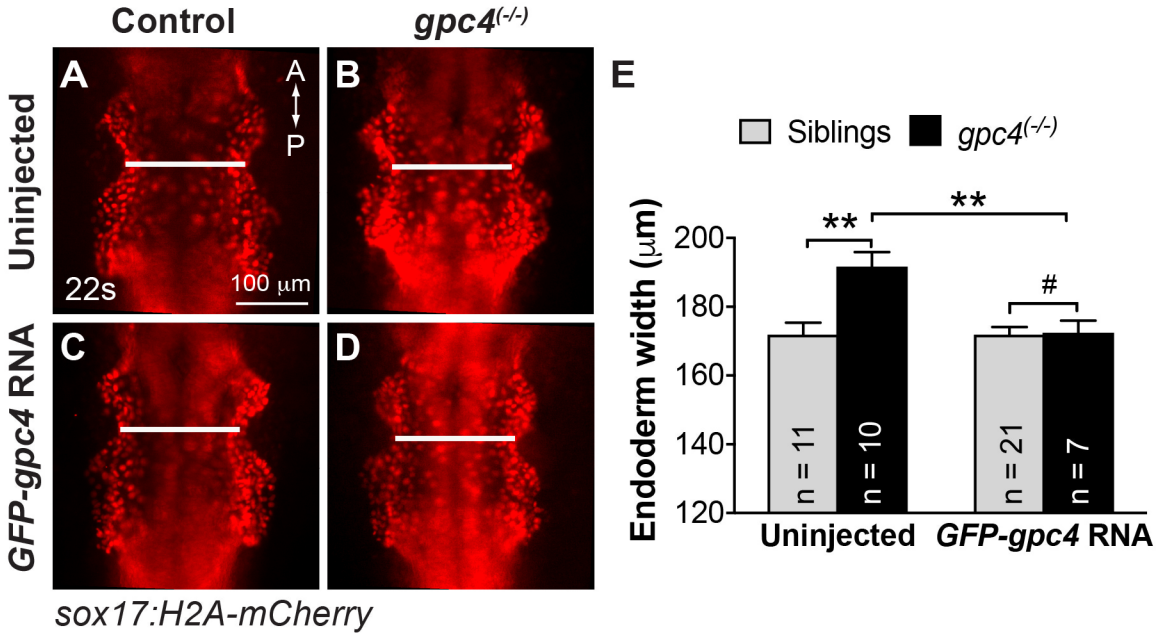



**Figure S2. Gpc4, but not Vangl2, is required for convergence of the anterior endoderm.**

(**A-F**) Expression of *foxa2* (A-C′) and *sox17* (D-F) in the indicated embryos, as detected by WISH. Lateral (A-C, D-F) and anterior-dorsal (A′-C′) views. Yellow lines of equivalent length indicate width of the anterior endodermal sheets. Red lines of equivalent length indicate the distance between the lateral-most endodermal cells and the dorsal site of embryo. Red arrowheads indicate the end of anterior and posterior body axes. D, dorsal. (**G,H**) Epifluorescence images of anterior endoderm in control, *vangl2* mutant *Tg(sox17:EGFP)* embryos at 4s. Anterior-dorsal view. White lines of equivalent length indicate width of the anterior endodermal sheets. A, anterior; P, posterior. (**I**) Quantification of endoderm width in each group of embryos shown in (G,H). Number of embryos for each group is indicated. Bars represent the mean±s.e.m. #, p>0.05; student's *t*-test.



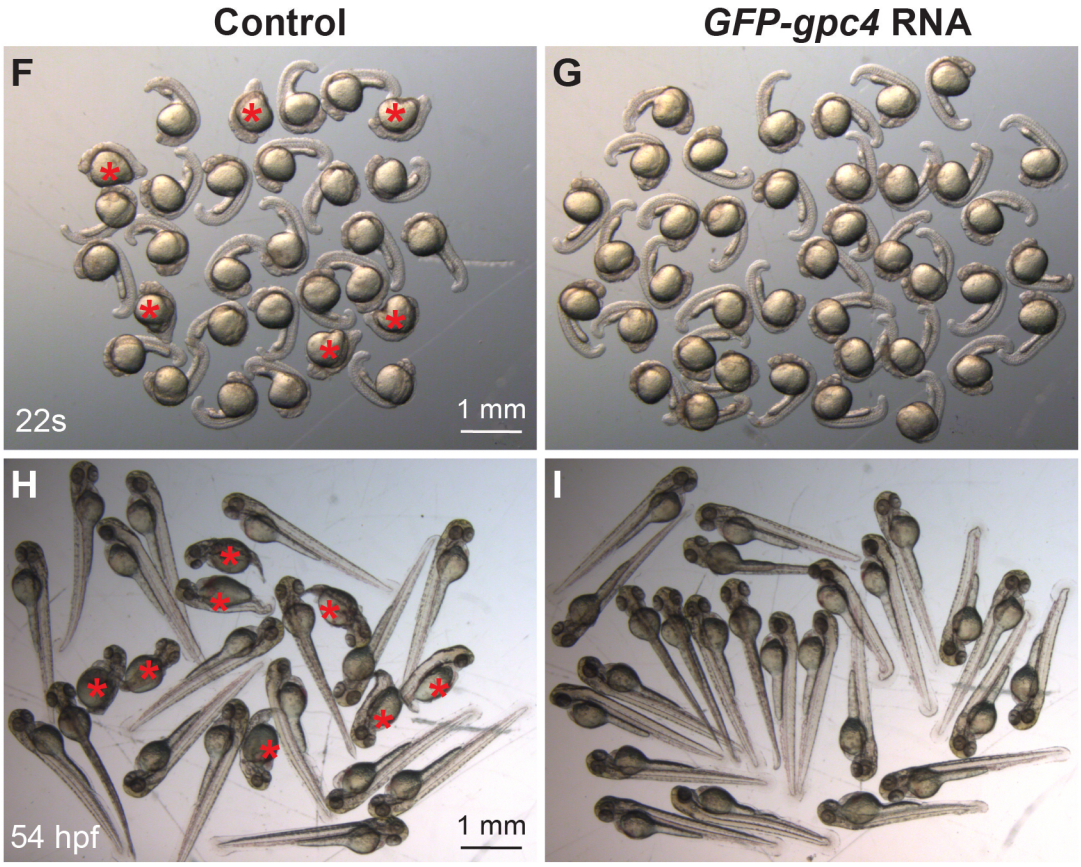



**Figure S3. Overexpression of GFP-Gpc4 rescues defects in length of body axis and convergence of anterior endoderm in *gpc4* mutant embryos**.

(**A-D**) Representative images of indicated *Tg(sox17:H2A-mCherry)* embryos injected with or without *GPF-gpc4* RNA at 22s. Anterior-dorsal view; white lines of equivalent length indicate width of the anterior endodermal sheets. A, anterior; P, posterior. (**E**) Quantification of the width of the anterior endodermal sheet in each group of embryos shown in (A-D). Data represent mean±s.e.m. The number of embryos is indicated. #, P>0.05; **, P<0.01, student's t-test.(**F-I**) Bright-field images of groups of embryos derived from crosses of *gpc4*$^{(+/-)}$ injected with or without *GPF-gpc4* RNA at 22s and 54 hpf. Red asterisks indicate *gpc4* homozygous embryos with a short anterior-posterior body axis.

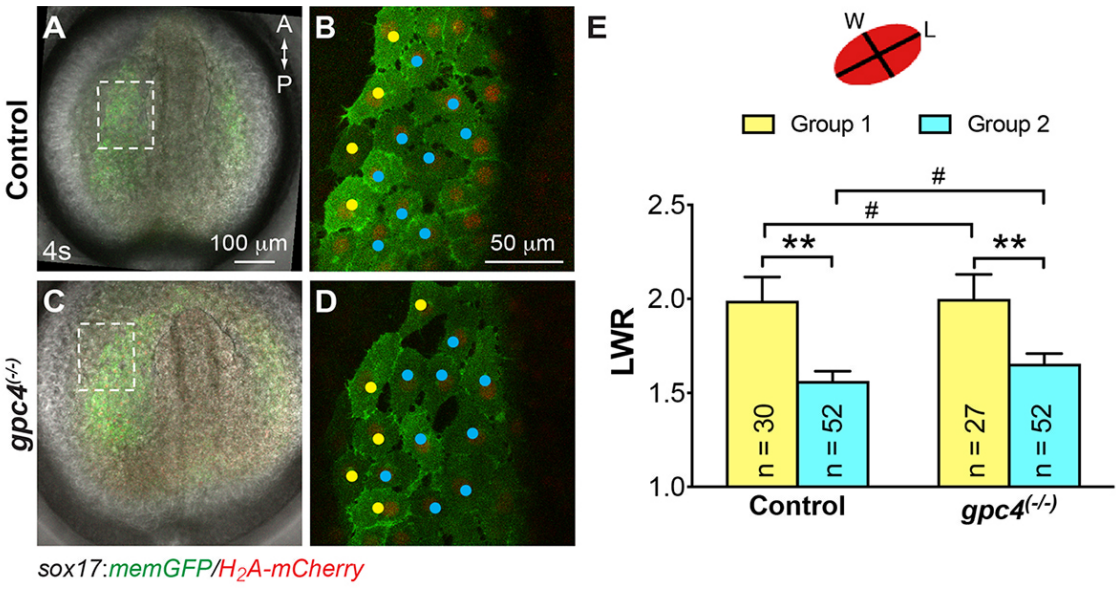



**Figure S4. Morphology of anterior endodermal cells is not affected in *gpc4* mutants.** (**A,C**) Overlay of bright-field and epifluorescence images of *Tg(sox17:memGFP/H$_2$A-mcherry)* embryos at 4s. Dashed boxes are regions in which cells were imaged for analysis of shape. (**B, D**) Confocal images of the endoderm at the region indicated in the dashed boxes in A, C. Endodermal cells at the lateral region and near the dorsal midline are labeled with yellow and cyan dots, respectively. A, anterior; P, posterior. (**E**) Schematic representation of the method used to measure cell shape (LWR, length-to-width ratio). Quantification of LWR of endodermal cells in seven control and six *gpc4* mutant embryos. Bars represent the mean±s.e.m. The number of cells analyzed is indicated. #, p>0.05; **, P<0.01, student's t-test.

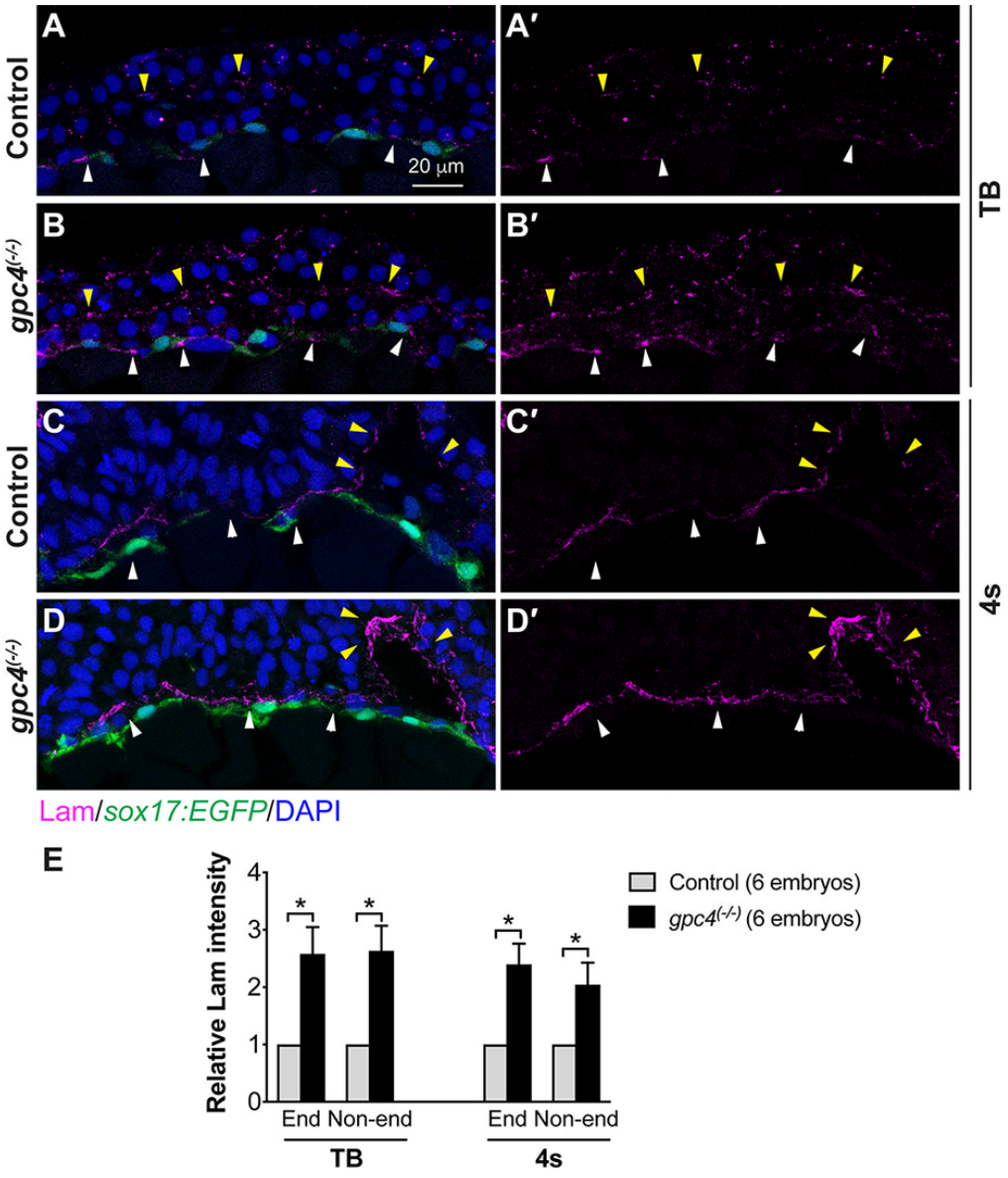



**Figure S5. Lam deposition is increased in *gpc4* mutant embryos.**

Transverse cryosections from *Tg(sox17:EGFP)* control and *gpc4* mutant embryos immunostained for Lam (magenta) and nuclei (DAPI, blue). (**A-D′**) Confocal z-stack images of embryos at tailbud (TB) (A-B′) and 4-somite (4s) (C-D′) stages. Lam assembly between the ectoderm and mesoderm (yellow arrowheads) and around the endodermal layer (white arrowheads). (**E**) Relative Lam intensity in non-endodermal (Non-end) tissue and around the endodermal layer (End) in control and *gpc4* mutant embryos at TB and 4s. The number of embryo analyzed is shown in the graph. Bars represent the mean±s.e.m. *, P<0.05, student's t-test.

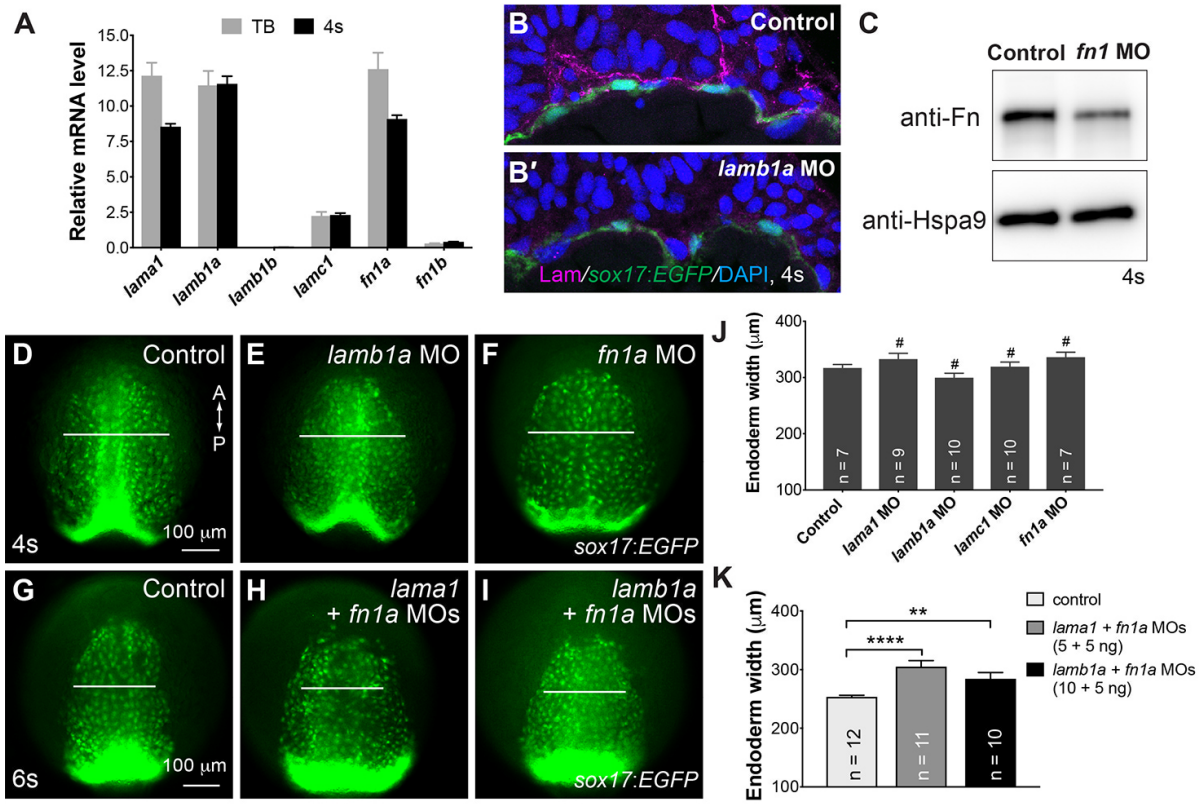



**Figure S6. Effects of suppressing Fn or/and Lam expression on endoderm C&E.**

(**A**) Expression of *lam1* (*a1, b1a, b1b, c1*) and *fn* (*1a and 1b*) relative to that of the housekeeping gene *eukaryotic translation elongation factor 1 alpha 1a (eef1a)* in WT embryos at TB and 4s, as determined by qRT-PCR. (**B-B′**) Confocal z-stack images of transverse cryosections immunostained for Lam (magenta) and nuclei (DAPI, blue) from the indicated embryos. (**C**) Western blot showing expression levels of Fn and Hspa9 (internal control) in embryos indicated. (**D-I**) Epifluorescence still images of the anterior endodermal sheet in embryos indicated. Anterior-dorsal view. A, anterior; P, posterior. White lines of equivalent length indicate width of anterior endodermal sheet of the embryos at the same stage. (**J,K**) Average width of anterior endoderm. (J) Embryos injected with the indicated MO (10 ng), shown in (D-F). (K) Embryos treated as indicated and shown in (G-I). Number of embryos analyzed is indicated for each group. #, $p>0.05$, **, $p<0.01$, ****, $p<0.0001$, student's t-test.

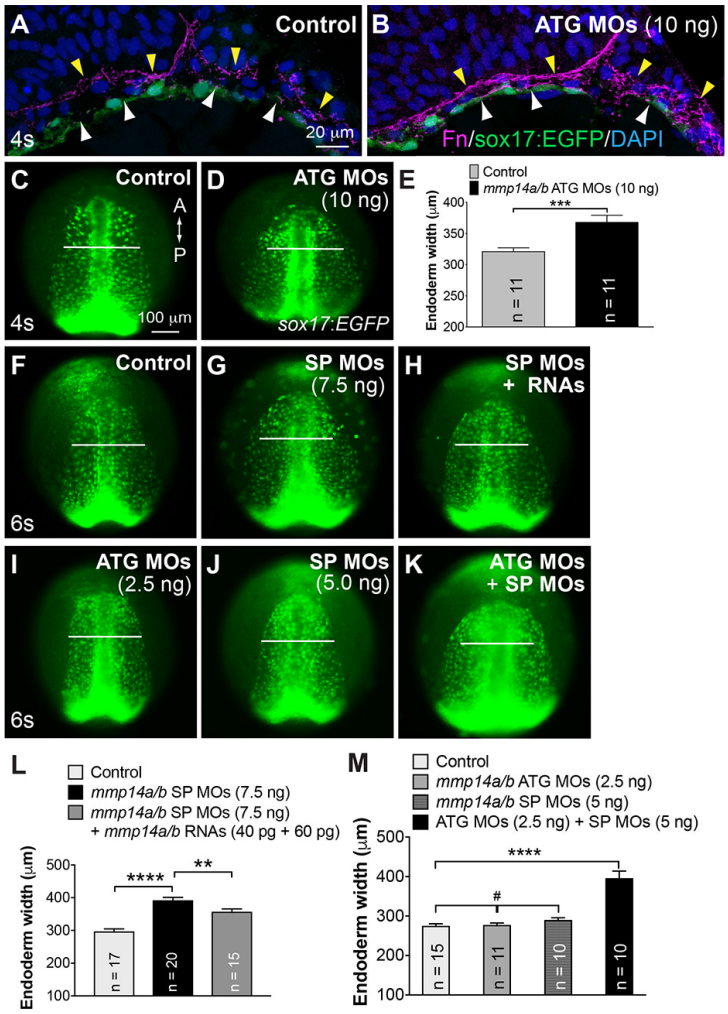



**Figure S7. Mmp14a/b are required for C&E movements of the anterior endodermal cells.**
(**A-B**) Confocal z-stack images of transverse cryosections from *Tg(sox17:EGFP)* control embryos and embryos injected with *mmp14a/b* ATG MOs (10ng, suppression of translation) immunostained for Fn (magenta) and nuclei (DAPI, blue). Fn assembly at mes/end (white arrowheads) and ect/mes (yellow arrowheads) boundaries. (**C-M)** Embryos injected with indicated MOs targeting *mmp14a/b* (ATG MOs target the translation; SP MOs target the splicing). (C-D, F-K) Epifluorescence still images of the anterior region of the endodermal sheet in the indicated embryos. Anterior-dorsal view. A, anterior; P, posterior. White lines of equivalent length indicate the width of anterior endodermal sheets of the embryos at the same stage. (E) Average endodermal width at the anterior region of embryos shown in (C,D). (L) Average width of anterior endoderm in embryos shown in (F-H). (M) Average width of anterior endoderm in embryos shown in (I-K). The number of embryos analyzed in each group is indicated. **, $p<0.01$; ***, $p<0.001$; ****, $p<0.0001$; #, $p>0.05$, student's *t*-test.

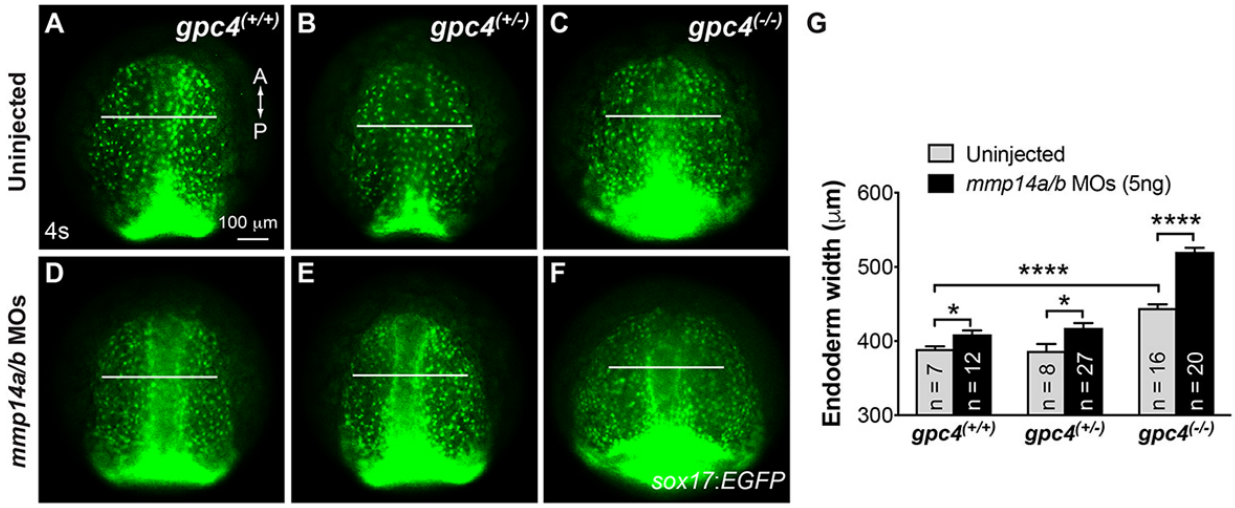
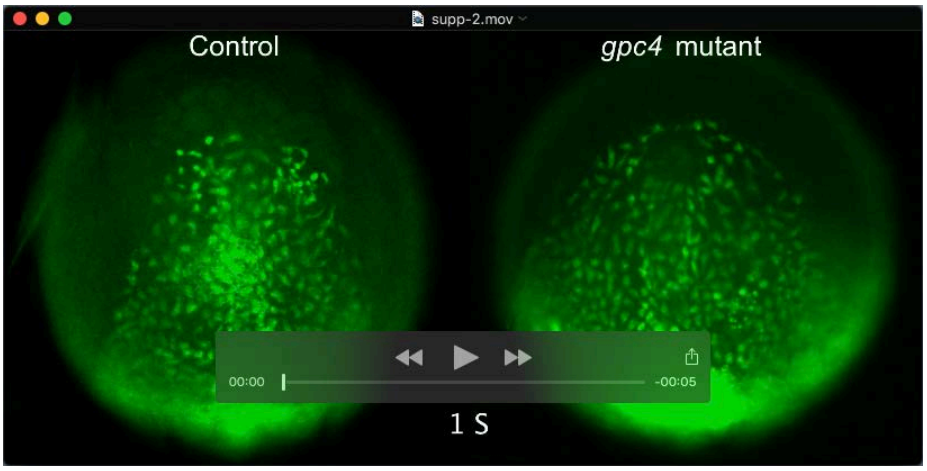
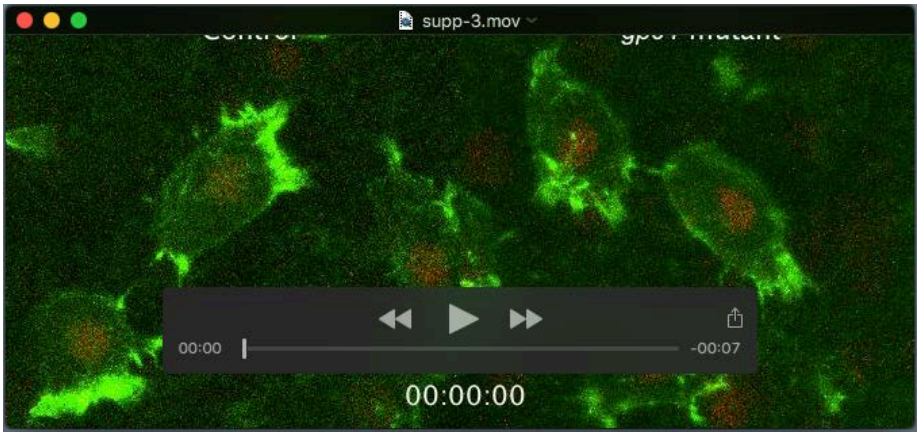


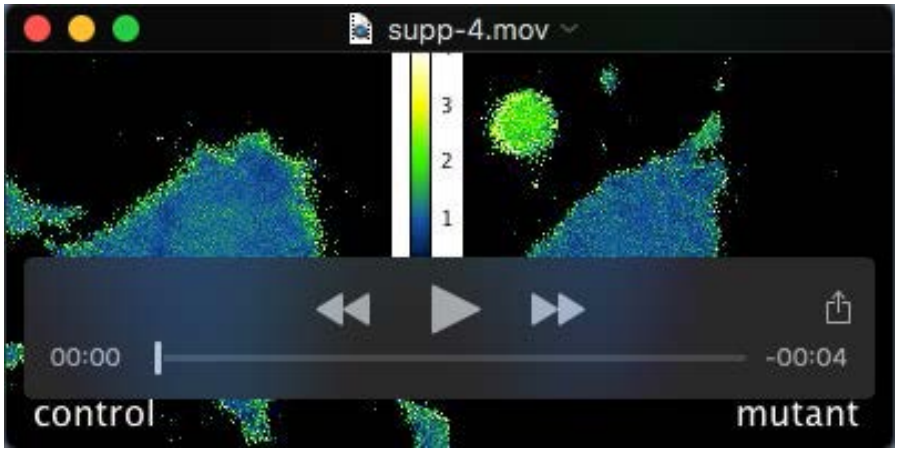

**Figure S8. Mmp14a/b and Gpc4 act synergistically in regulating endodermal migration**.
(**A-F**) Epifluorescence still images of the anterior endoderm at 4s in embryos derived from crosses of *gpc4*/*Tg(sox17:EGFP)* heterozygous zebrafish injected with or without a subdose of *mmp14a/b* ATG MOs (5 ng). White lines of equivalent length indicate the width of anterior endodermal sheets. A, anterior; P, posterior. (**G**) Average endoderm width in the anterior region. Numbers of embryos analyzed are indicated for each group. *, $p<0.05$; ****, $p<0.0001$; student's t-test.



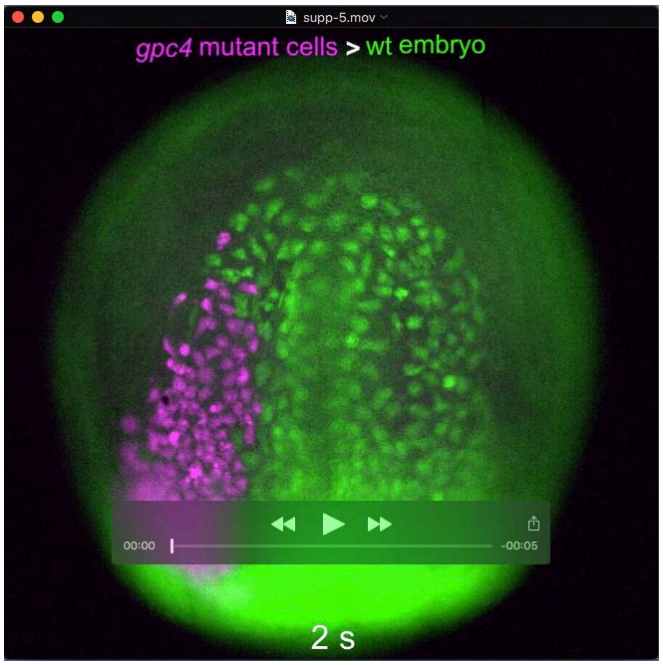

**Movie 1. Gpc4 is required for efficient endoderm C&E during early segmentation.**
Time-lapse experiments were performed on *Tg(sox17:EGFP)* control or *gpc4* mutant embryos from 1-6s, using an epifluorescence microscope (DMI 6000, Leica) with a 5x/NA 0.15 objective. Images were acquired at 5-min intervals and movie plays at 5 frames/sec.



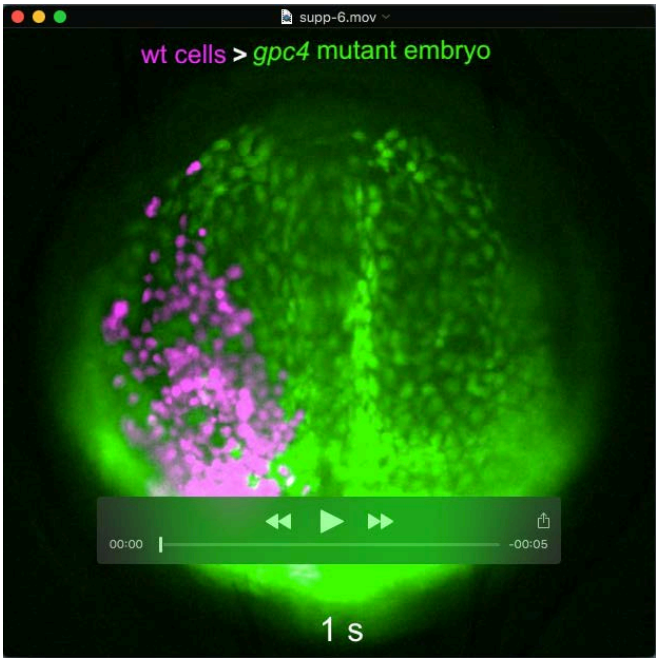

**Movie 2. Polarized actin-rich protrusions of migrating endodermal cells in control and *gpc4* mutant embryos.**
Confocal time-lapse experiments were performed on *Tg(sox17:GFP-UTRN)* control or *gpc4* mutant embryos at TB, using a Ziess LSM700 confocal microscope with a LD C-Apo 40×/NA 1.1 water objective. Images were acquired at 10-sec intervals and movie plays at 5 frames/sec.



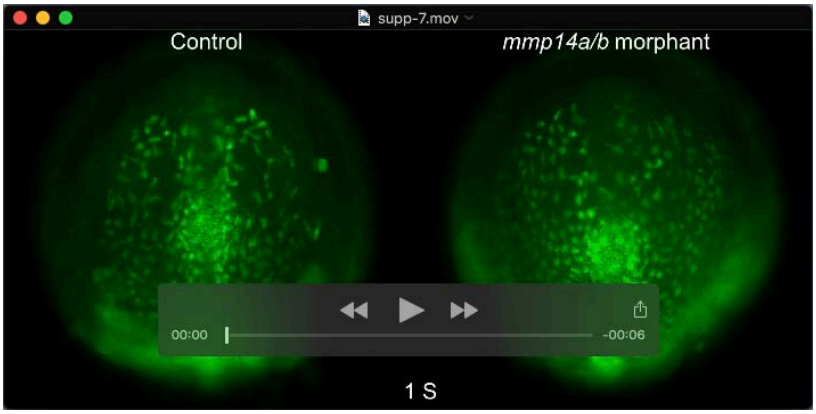

**Movie 3. Rac1 activity in migrating endodermal cells in control and *gpc4* mutant embryos.**
Representative time-lapse movies of anterior endoderm in control (left panel) or *gpc4* mutant (right panel) host embryo transplanted with *sox32,* PDB-GFP-expressing and rhodamine-labeled control (left panel) or *gpc4* mutant (right panel) cells. Images were acquired at 10-sec intervals using a Ziess LSM700 confocal microscope with a LD C-Apo 40×/NA 1.1 water objective and movie plays at 5 frames/sec.



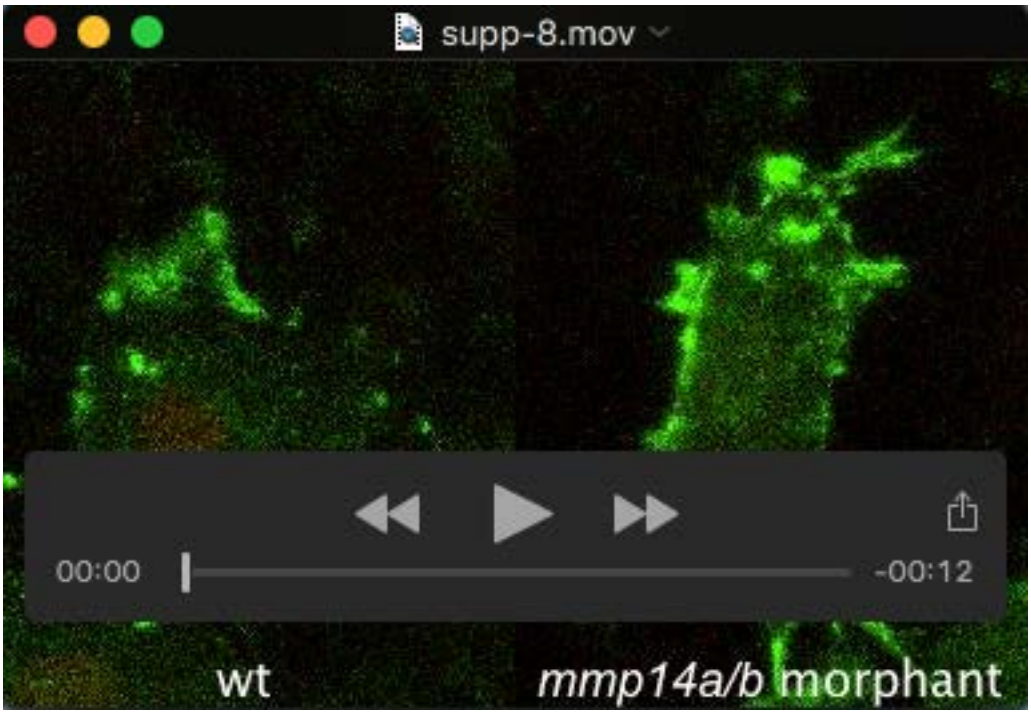

**Movie 4. C&E of *gpc4*-deficient donor cells in wild-type host embryo.**
Representative time-lapse movie of anterior endoderm of a *Tg(sox17:EGFP)* embryo transplanted with *sox32*-expressing, rhodamine-labeled *gpc4*-deficient cells (magenta), from 2-5s. Images were captured at 5-min intervals using an epifluorescence microscope (DMI 6000, Leica) with a 5x/NA 0.15 objective. The movie plays at 5 frames/sec.




**Movie 5. C&E of wild-type donor cells in *gpc4* mutant.**
Representative time-lapse movie of anterior endoderm of a *Tg(sox17:EGFP) gpc4* mutant transplanted with *sox32*-expressing, rhodamine-labeled wild-type cells (magenta), from 1-5s. Images were captured at 5-min intervals using an epifluorescence microscope (DMI 6000, Leica) with a 5x/NA 0.15 objective. The movie plays at 5 frames/sec.




**Movie 6. Mmp14a/b is required for efficient endoderm C&E during early segmentation.**
Time-lapse experiments were performed on control or *mmp14a/b* MO-injected *Tg(sox17:EGFP)* embryos from 1-6s, using an epifluorescence microscope (DMI 6000, Leica) with a 5x/NA 0.15 objective. Images were acquired at 5-min intervals. The movie plays at 5 frames/sec.




**Movie 7. Polarized actin-rich protrusions of migrating endodermal cells in control and *mmp14a/b* MO-injected embryos.**

Confocal time-lapse experiments were performed on *Tg(sox17:GFP-UTRN)* control or *mmp14a/b* MO-injected embryos at TB, using a Zeiss LSM700 confocal microscope with a LD C-Apo 40×/NA 1.1 water objective. Images were acquired at 10-sec intervals, and movie plays at 5 frames/sec.

**Supplemental table 1: The sequences of primers used for qRT-PCR of the indicated genes**

| Genes | Forward sequence (5'-3') | Reverse sequence (5'-3') |
|---|---|---|
| *vangl1* | AACTCACCACTATAACATGGGACAA | CACTTCCAGCACCATCCACA |
| *vangl2* | TTCCCAAATCCATCCTGTCCAA | GGTCCATCTCAGCCTCCTCGTAG |
| *gpc4* | CAGCTCAAACCCTTCGGAGAC | CGCTACAGTACGGGCAGTATAACAT |
| *foxa2* | CAGACTGGAGCACTTACTACGG | AGGACATGTTCATGGTGTTAGC |
| *eef1a1a* | GAGAAGTTCGAGAAGGAAGC | CGTAGTATTTGCTGGTCTCG |
| *fn1a* | GTGTATGCCGAAAGGAACG | CCCGGTAGGAACGAGAATT |
| *fn1b* | GTTTAGCCATCCACGAAAGT | AGTCCCATATCATGTTATCCTTT |
| *lama1* | CTGCCCCTGGGACCCTGTTA | TCCGCCACCGTCTGGTTGTA |
| *lamb1a* | CGCACCAAGTAACCAGCCACA | GCCGAACGCTCGATCACCA |
| *lamb1b* | GTGACAACCTTCGCTCCCA | GCCAGGTCCTCCCATAATCT |
| *lamc1* | TAGCGACATCTCGCCACTC | ACTTGCACCTTCCTCCCAC |
| *mmp14a* | GTGTTTCTGGTGCAGAGCG | CCGAGATAGCGGAGTTGATAG |
| *mmp14b* | CTGGAGCGGGTTTACGAGG | CATGGCAGCAATGGCAGAG |