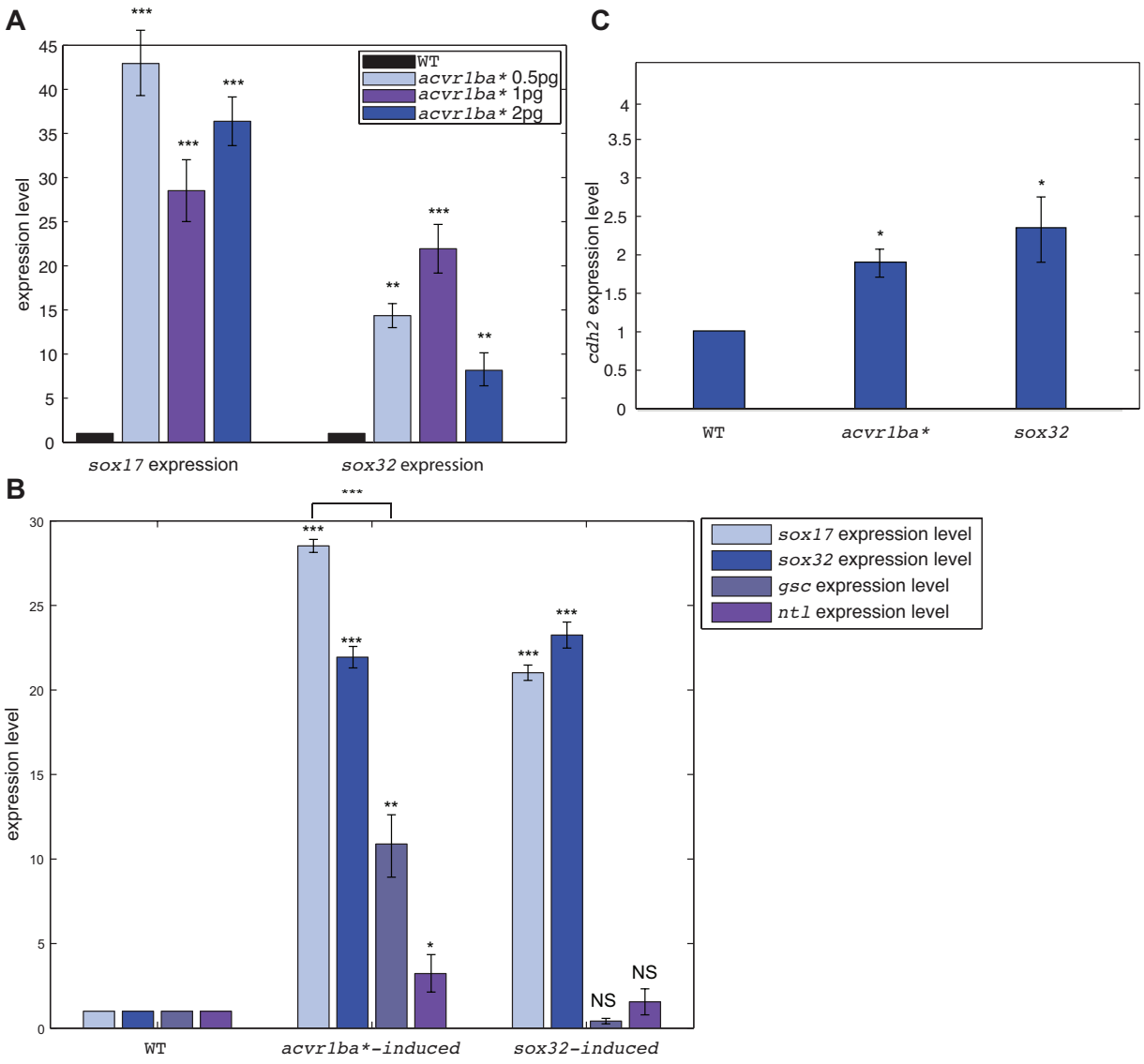


Figure S1: ***acvr1ba**** **induces expression of *sox17* and *sox32*. Related to Figure 1.**

(A) Expression of *sox17* and *sox32* endodermal markers was measured by real-time quantitative PCR. Constitutive activation of the Nodal pathway by expression of *acvr1ba** upregulated *sox17* and *sox32* expression (normalized to uninjected controls). **p<0.01, ***p<0.001.

(B) Expression of *sox17*, *sox32*, *gsc* and *ntl* was measured by real-time quantitative PCR in *acvr1ba**-expressing cells and *sox32*-expressing cells in wildtype background. Both acvrb1a* and sox32 more potently induce endodermal markers (*sox17* and *sox32*) than mesodermal markers (*gsc* and *ntl*). *p<0.05, **p<0.01, ***p<0.001, NS, not significant.

(C) Expression of *cdh2* at 6hpf was measured by real-time quantitative PCR. Both *acvr1ba** and *sox32*-induced endodermal cells have elevated expression comparing to wild type uninjected controls. *p<0.05.

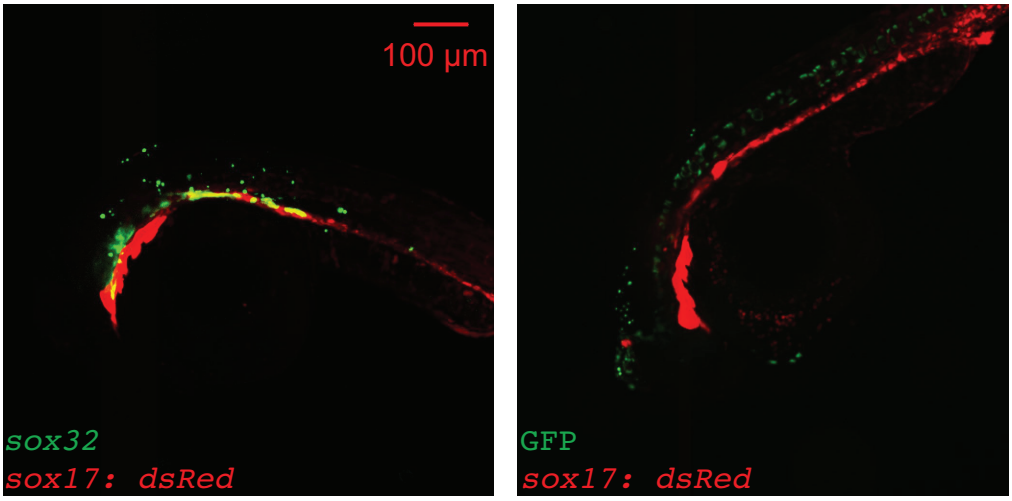


Figure S2: *sox32*-expressing cells preferentially segregate to endoderm-derived tissues when placed near the dorsal margin. Related to Figure 2.

Representative images showing distribution of *sox32*-overexpressing cells or GFP-expressing cells that were transplanted to the margin of wild-type host embryos. At 21-somite stage, transplanted *sox32*-overexpressing cells primarily localized to endodermal tissues while GFP-expressing cells localized to mesodermal tissues.

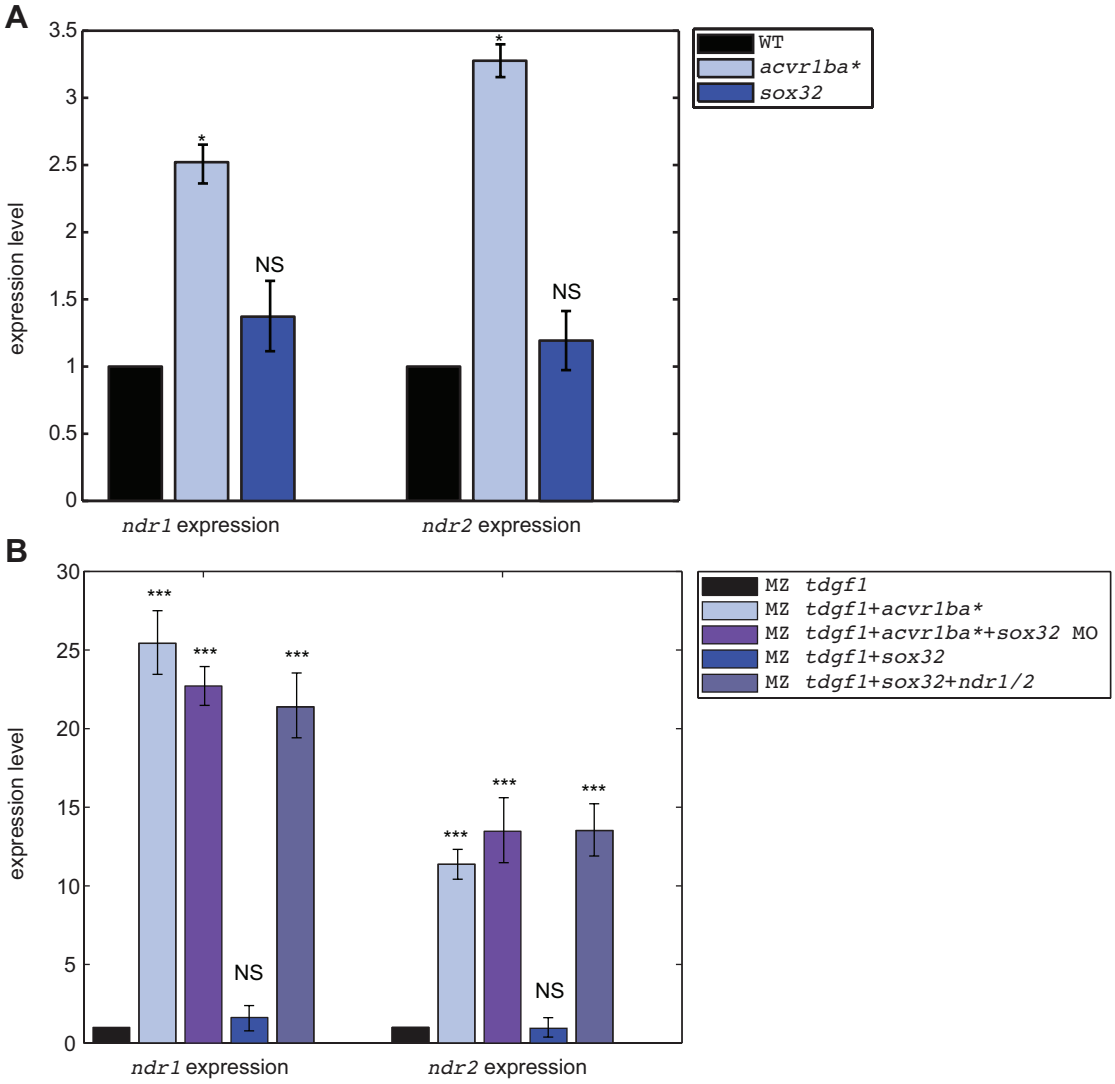


Figure S3: ***ndr1/2* is upregulated by *acvr1ba**, and *sox32* is neither necessary or sufficient for this upregulation. Related to Figure 2.**

(A) *ndr1/2* expression in *acvr1ba**-expressing cells and *sox32*-overexpressing cells in wildtype background measured by real-time quantitative PCR. *p<0.05, NS, not significant.

(B) *ndr1/2* expression under all experimental conditions in MZ *tdgf1* background, which removes the confounding effects of maternally deposited Ndr1/2 on driving nodal signaling. ***p<0.001, NS, not significant.

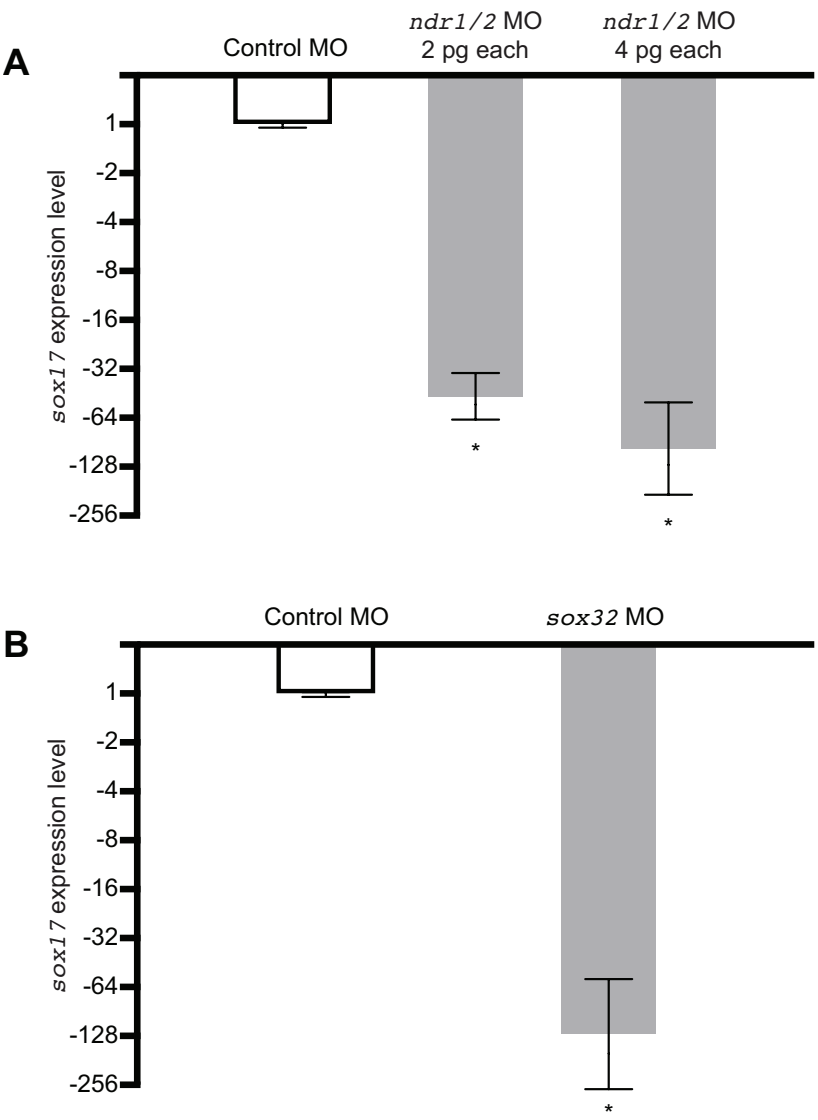


Figure S4: Validation of *ndr1*, *ndr2* and *sox32* morpholinos. Related to Figure 2.

(A) Validation of *ndr1/2* knockdown. Embryos were injected at the 1-cell stage with standard control (Gene Tools) or *ndr1* and *ndr2* MO. Total RNA was collected at 70% epiboly (7 hpf), and *sox17* expression was quantified by qPCR. ndr1/2 knockdown reduced *sox17* expression by 50-fold when 2pg each was injected and 80-fold when 4pg each was injected. Data represents averages of 3 biological replicates. Error bars, S.E.M. *p=0.01.

(B) Validation of *sox32* knockdown. Embryos were injected at the 1-cell stage with 2ng of standard control (Gene Tools) or *sox32* MO. Total RNA was collected at 70% epiboly (7 hpf), and *sox17* expression was quantified by qPCR. *sox32* knockdown reduced *sox17* expression by 125-fold. Data represents averages of 3 biological replicates. Error bars, S.E.M. *p=0.01.

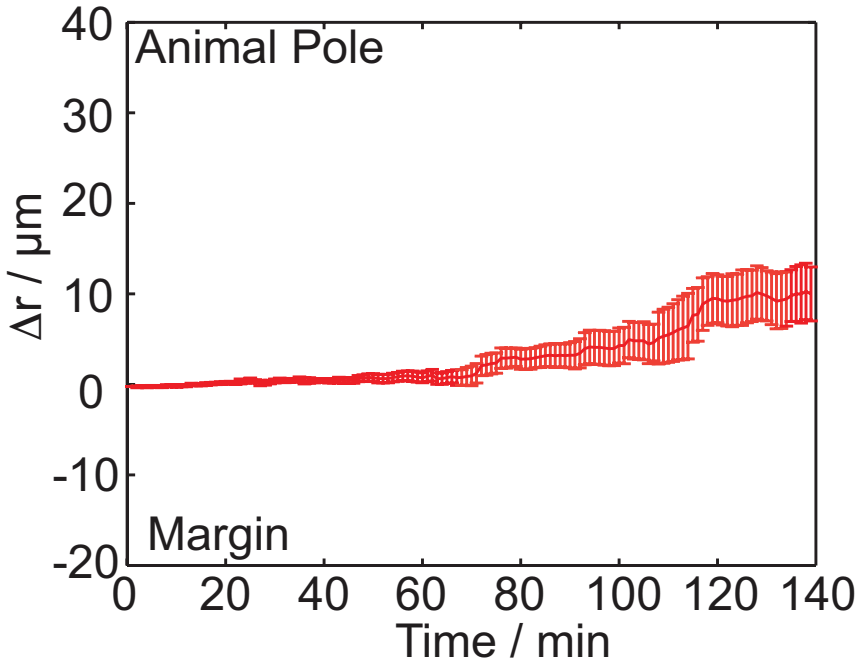


Figure S5: Single-cell tracking analysis of ingression of *acvr1ba-expressing cells with *sox32* MO. Related to Figure 2.**

Average relative distance with standard error plotted against time. Relative distance was calculated as in Fig. 2I. Unlike cells expressing *acvr1ba** only, cells also containing *sox32* MO move toward the surface of the embryo with their ectodermal neighbors.

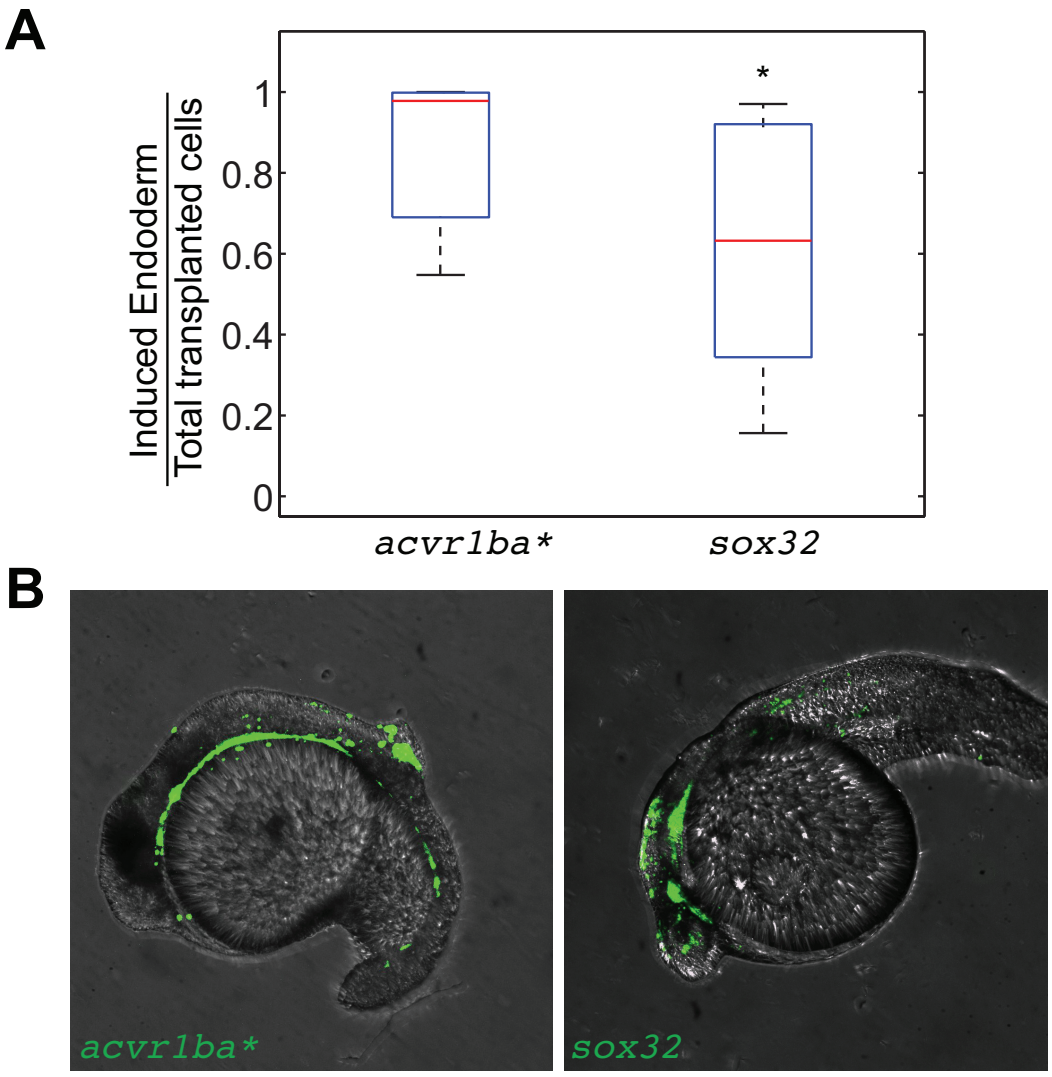


Figure S6: Induced endodermal cells internalize following transplantation to the margin.
(A) Boxplot quantification of endoderm contribution of transplanted cells at 18 hpf. Data is shown as mean ± SEM of independent transplantation experiments with 14 embryos per condition. Student's t-test was performed. * $p<0.05$.
(B) Representative image showing distribution of transplanted cells depicted in (A) at 18 hpf. *acvr1ba**-expressing cells localized to the endoderm-derived tissue (green). Cells overexpressing *sox32* localize to both endoderm and ectoderm-derived tissue. Lateral view, anterior to the left.

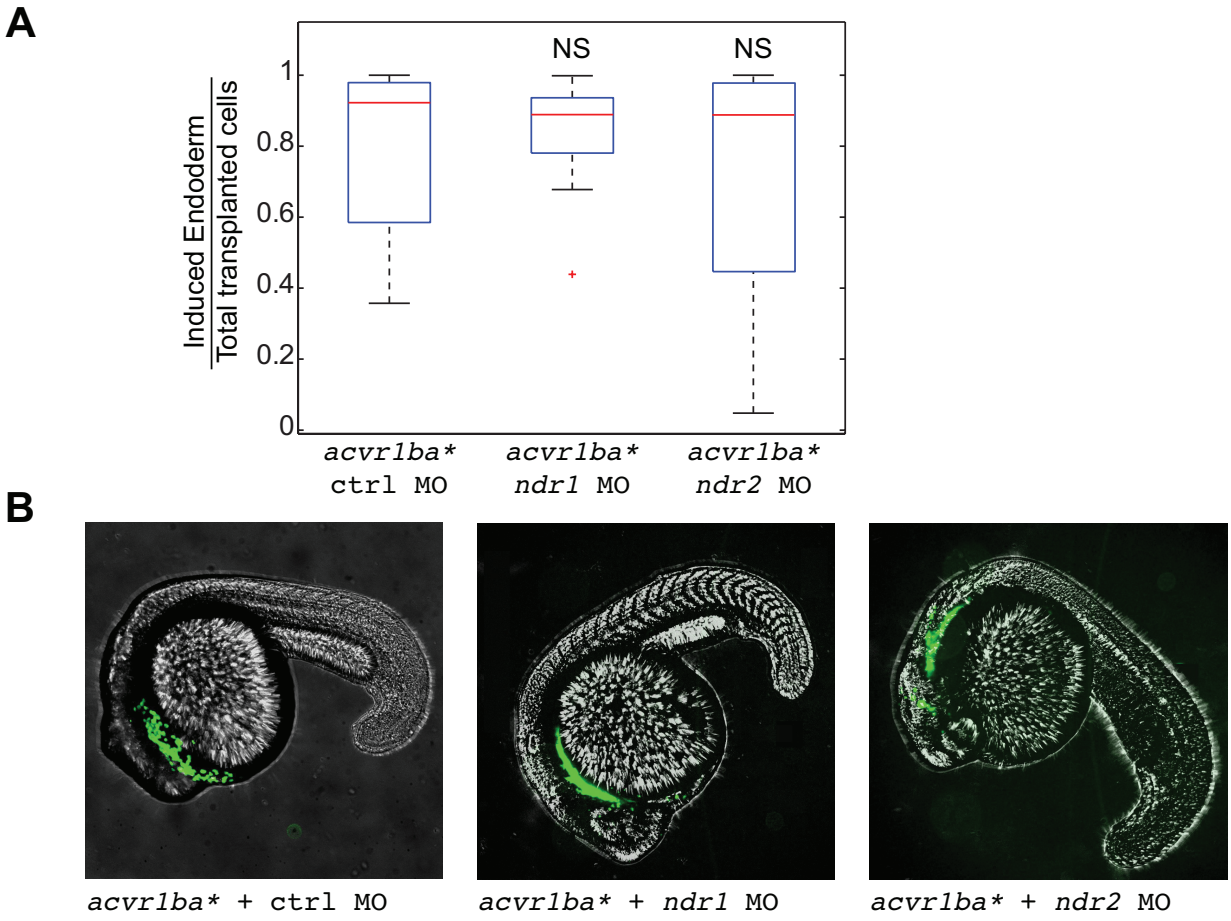


Figure S7: Ndr1 and Ndr2 act redundantly to support the ability of *acvr1ba cells to internalize.**

(A) Boxplot quantification of endoderm contribution of transplanted cells at 20 hpf. Data is shown as mean ± SEM of independent transplantation experiments with 16 embryos per condition. Student's t-test was performed. NS, not significant.

(B) Representative image showing distribution of transplanted cells depicted in (A) at 18 hpf. *acvr1ba**-expressing cells localized to the endoderm-derived tissue (green)in all three conditions, in contrast to the block of internalization when both Ndr1 and Ndr2 MO are combined in acvr1ba* cells (**Fig. 2H**). Lateral view, anterior to the left.

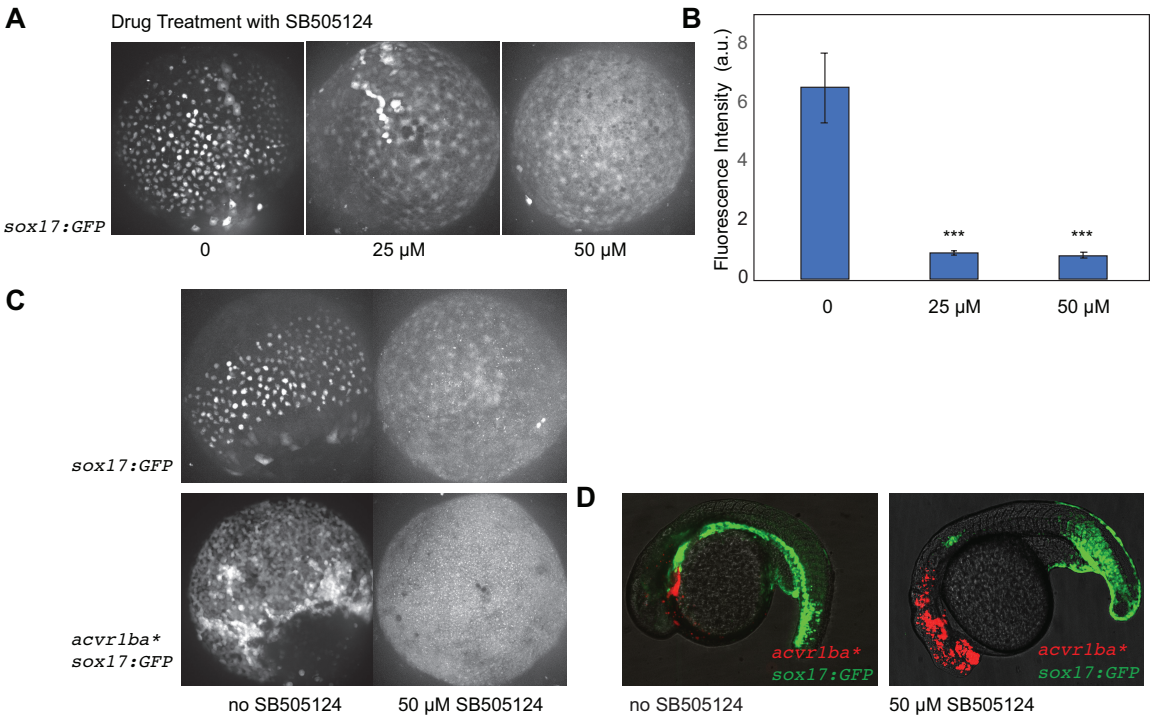


Figure S8: Nodal signaling inhibitor SB505124 blocks *acvr1ba-expressing cells from sorting.**

(A) Representative images of *sox17:GFP* expression under 0, 25 μM or 50 μM SB505124. Drug treatment began at 6 hpf, images were taken at 10 hpf. Animal pole view.

(B) Quantification of *sox17:GFP* fluorescence intensity under 0, 25 μM or 50 μM SB505124. *** p<0.001. n=3.

(C) *sox17:GFP* expression for embryos with or without injection of acvr1ba* and under no drug treatment or treated 50 μM drug SB505124 treatment.

(D) Transplant of *acvr1ba**-expressing cells into *sox17:GFP* background under DMSO control and 50 μM drug SB505124 treatment at 18hfp.

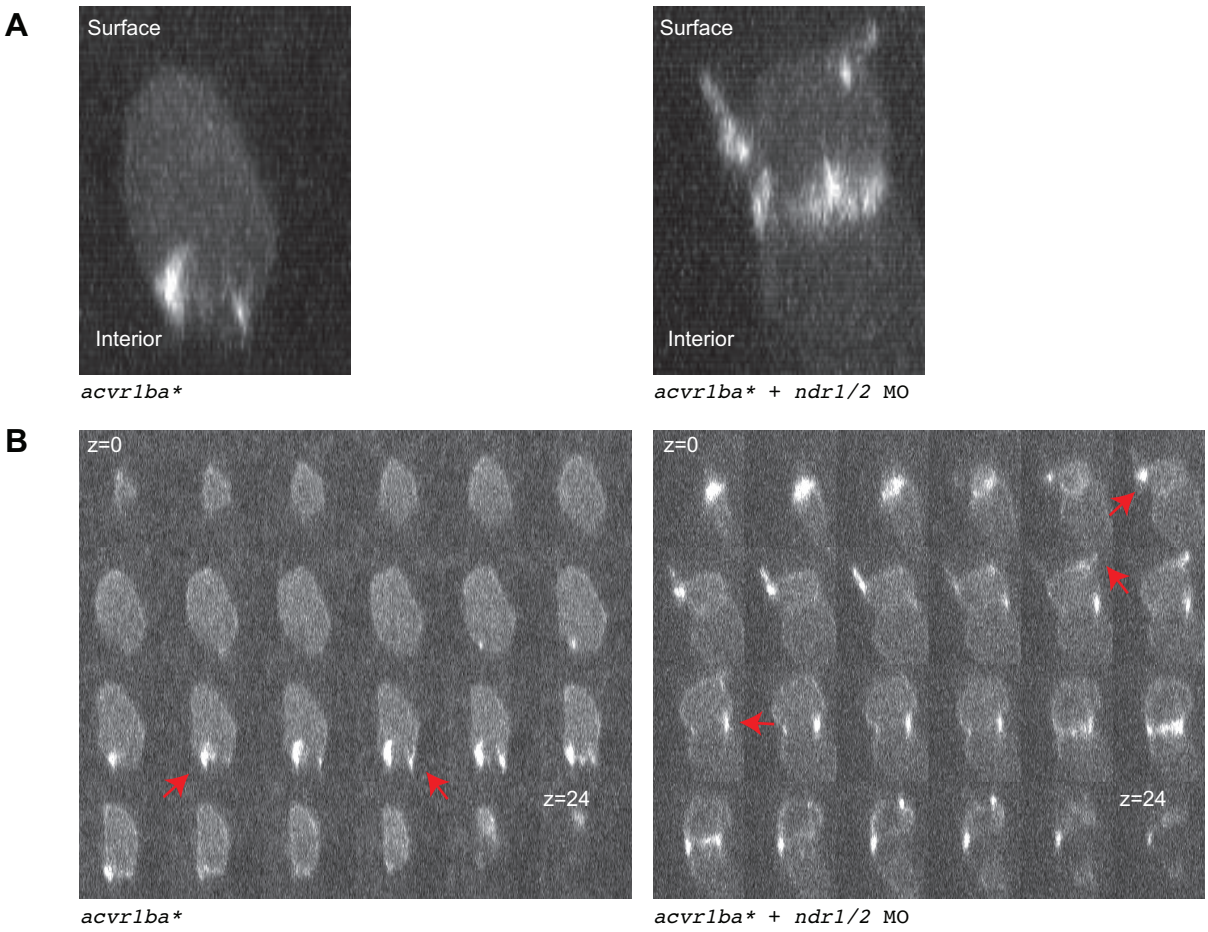


Figure S9: Blocking autocrine production of *ndr1/2* interferes with polarity of actin-based protrusions in *acvr1ba cells. Related to Figure 5.**

(A) Maximum? Z projection of individual transplanted cells injected with either *acvr1ba** alone or *acvr1ba** with *ndr1/2* MOs.

(B) Montage of Z stack of cells shown in (A). Red arrows indicate actin enrichment. Numbers indicate μm?

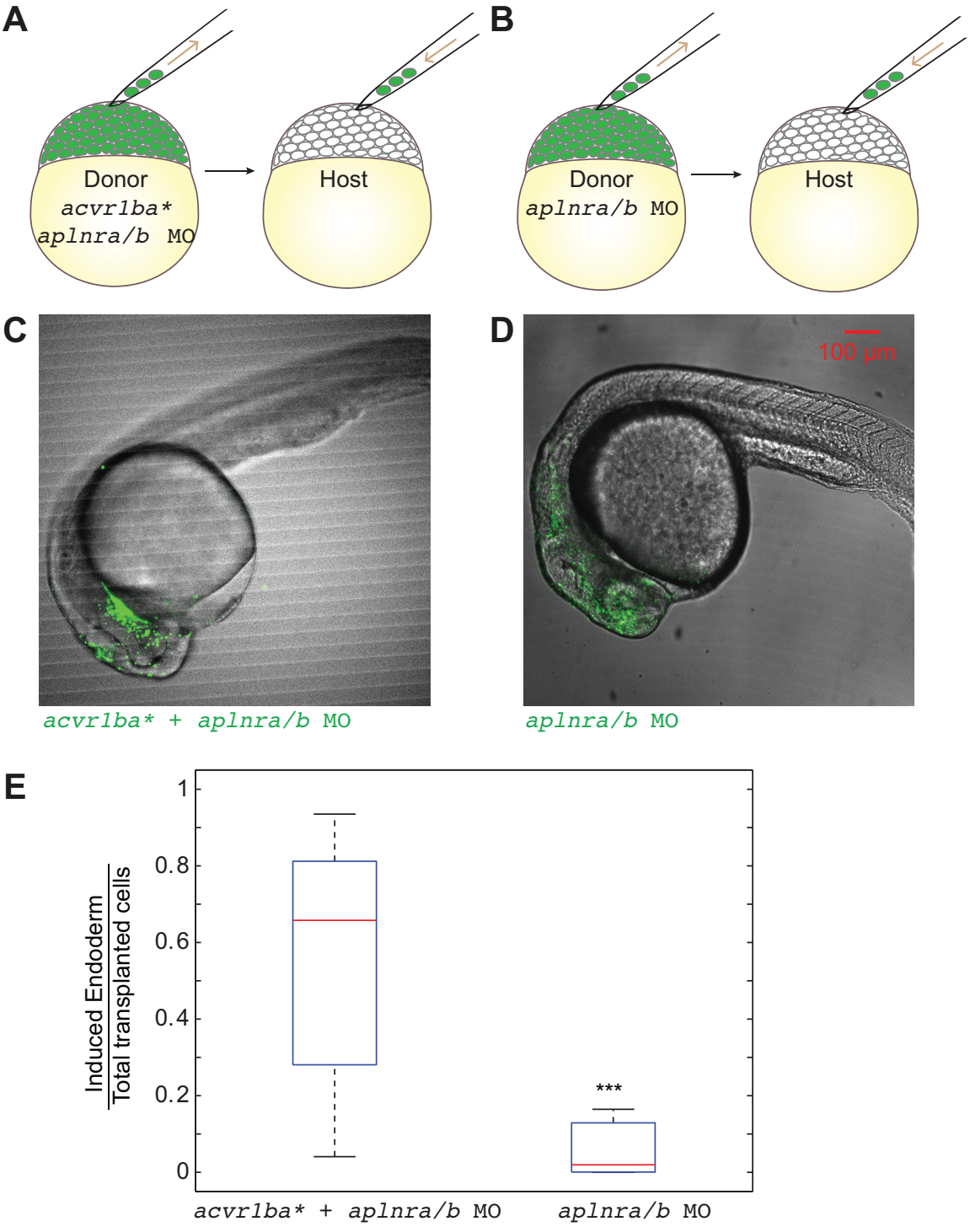


Figure S10: **Apelin receptor signaling is not essential for ectopic endoderm ingression.** **(A-B)** Schematic diagrams depicting single donor transplant assay to test the role of apelin receptor signaling. (A) *acvr1ba**-expressing cells with *aplnra* and *aplnrb* MOs were transplanted

to the animal pole of a wild-type host embryo. (B) Cells with *aplnra* and *aplnrb* MOs alone were transplanted to the animal pole of a wild-type host embryo.
(C-D) Representative images showing distribution of induced endodermal cells in a wild-type host. Donor cells in (A) (green) mainly localized to endoderm-derived tissue (C), while donor cells in (B) mainly localized to ectoderm-derived tissue (D). Lateral view, anterior to the right.
(E) Boxplot quantification of endoderm contribution at 21 hpf of transplanted cells depicted in (A-B). *acvr1ba**-expressing cells with *aplnra* and *aplnrb* MOs contributed to endoderm significantly more than cells with *aplnra* and *aplnrb* MOs alone. Data is shown as mean ± SEM of 3 independent transplantation experiments with 18 embryos per condition. Student's t-test was performed. *** $p<0.001$.

Table S1. List of Oligonucleotides

Oligonucleotide Name	Sequence
ef1a_forward	5′-CAAGAAGAGTAGTACCGCTAGCAT-3′
ef1a_reverse	5′-CACGGTGACAACATGCTGGAG-3′
sox17_forward	5'-CACAATGCGGAGCTGAGTAA-3'
sox17_reverse	5'-GCCTCCTCAACGAATGGAC-3'
sox32_forward	5'-CGGACCTGGAGAACACTGAC-3'
sox32_reverse	5'-GCATGTACGGACGCTTATCTG-3'
cdh2_forward	5'-CATCCCGGAGACATAGGAGA-3'
cdh2_reverse	5'-GCCCTCGTAGTCAAACACCA-3'
Oep5	5'-GAGATGGAGATGTTCTAATG-3'
Oep3m	5'-GAACAGTTGACTCGTCAC-3'
Oep3w	5'-GAACAGTTGACTCGTCAT-3'
Sox32 MO	5'-GCATCCGGTCGACATACATGCTGTT-3'
Sqt MO	5'-ATGTCAAATCAAGGTAATAATCCAC-3'
Cyc MO	5'-GCGACTCCCGAGCGTGTGCATGATG-3'
Aplnr a MO	5'-CGGTGTATTCCGGCGTTGGCTCCAT-3'
Aplnr b MO	5'-CAGAGAAGTTGTTTGTCATGTGCTC-3'
Control MO	5'-CCTCTTAACCTCAGTTACAATTTATA-3'

Table S2. Key Resource Table

Reagent or Resource	Source	Identifier
Chemicals, Peptides, and Recombinant Proteins		
Dextran, Alexa Fluor™ 647	Invitrogen	Cat#D22914

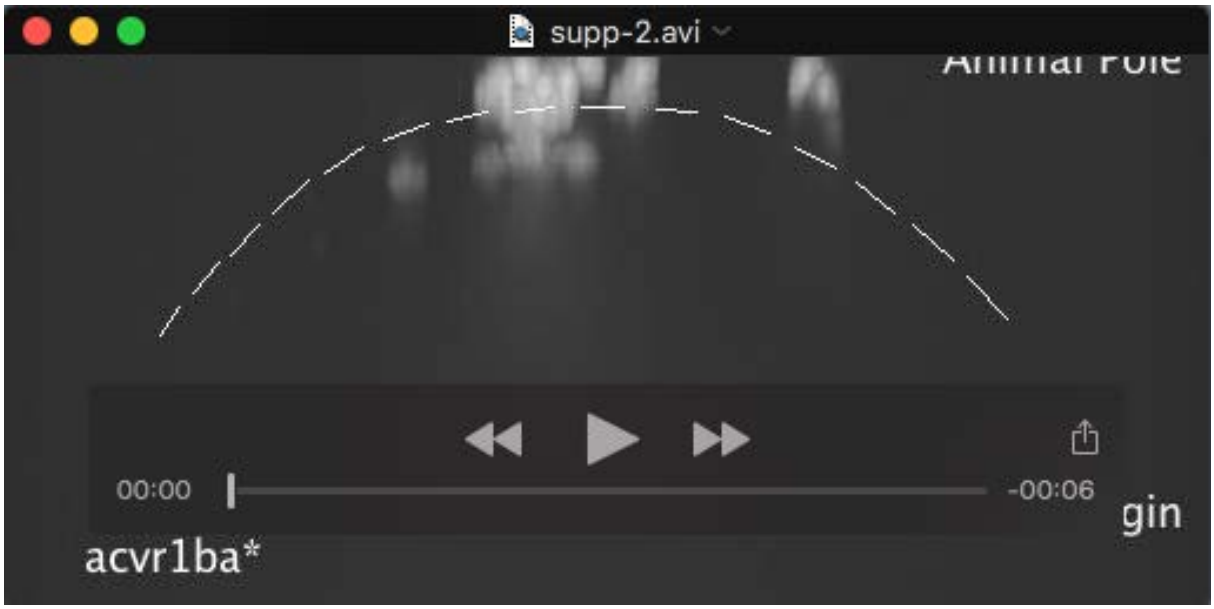
Dextran, Tetramethylrhodamine	Invitrogen	Cat#D1868
Dextran, Fluorescein	Invitrogen	Cat#D1821
Dextran, Alexa Fluor™ 680	Invitrogen	Cat#D34680
Histone H1 From Calf Thymus, Alexa Fluor™ 488 Conjugate	Invitrogen	Cat#H13188
Critical Commercial Assays		
mMESSAGE mMACHINE SP6 Transcription Kit	Ambion	Cat#AM1340
SuperScript VILO cDNA Synthesis Kit	Invitrogen	Cat#11754050
SYBR green PCR master mix	Applied Biosciences	Cat#4309155
Experimental Models: Organisms/Strains		
Zebrafish: AB/TL	This study	ZFIN: ZDB-GENO-960809-7
Zebrafish: EKW	This study	ZFIN: ZDB-GENO-031202-1
Zebrafish: Tg(sox17:GFP)	This study	ZFIN: ZDB-GENO-061228-1
Zebrafish: Tg(sox17:DsRed)	This study	ZFIN: ZDB-GENO-080812-1
Zebrafish: Tg(h2afva:h2afva-mCherry)	This study	ZFIN: ZDB-GENO-100923-1
Zebrafish: Tg(ubb:GFP-Smad2)	This study	N/A

Zebrafish: *tdgf1*$^{tz57/+}$	Lilianna Solnica-Krezel lab	ZFIN: ZDB-GENO-080708-1
Zebrafish: *tdgf1*$^{tz57/tz57}$	This study	ZFIN: ZDB-GENO-980202-989
Oligonucleotides		
List of oligonucleotides	See Table S1	N/A
Recombinant DNA		
pCS2-acbr1ba*	This study	N/A
pCS2-acbr1ba*-p2a-tBFP	This study	N/A
pCS2-sox32	This study	N/A
pCS2-sox32-p2a-tBFP	This study	N/A
pCS2-ndr1	This study	N/A
pCS2-ndr1-GFP	This study	N/A
pCS2-ndr2	This study	N/A
pCS2-ndr2-tBFP	This study	N/A
pCS2-GFP-UTRN	This study	N/A
pCS2-GFP	This study	N/A
pCS2-h2a-mCherry	This study	N/A
pCS2-tdgf1	This study	N/A
pmTol2-ef1a:Venus-Smad2	Steve Harvey	N/A
Software and Algorithms		

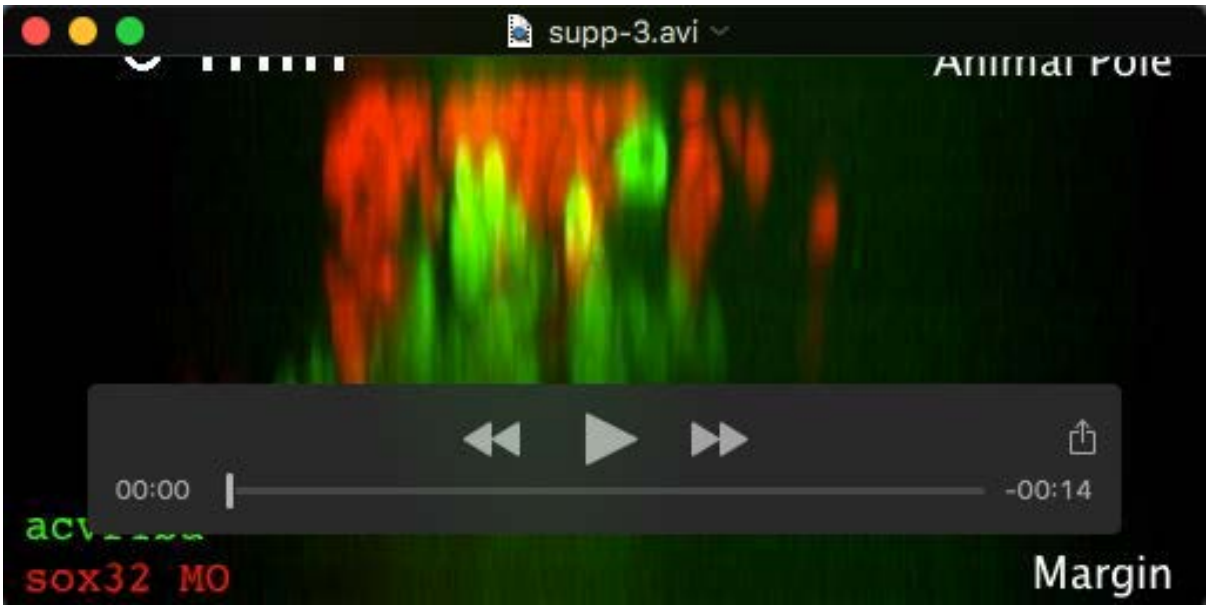
Fiji	NIH	https://fiji.sc
Matlab2013a	MathWorks Inc.	http://mathworks.com
TGMM	Philipp Keller lab	https://www.janelia.org/lab/keller-lab/software/fast-accurate-reconstruction-cell-lineages-large-scale-fluorescence

Contact for Reagent and Resource Sharing

Further information and requests for resources and reagents should be directed to Orion Weiner (orion.weiner@ucsf.edu).



Movie S1: *acvr1ba**-induced endodermal cells ingress into the inner layer when transplanted to the animal pole. Related to Figure 1. Frames were acquired every 5 min for 195 min. Playback is 7 frames/s.



Movie S2: *acvr1ba**-induced endodermal cells and *sox32* MO induced ectodermal cells segregate into two separate layers. *sox32* MO-injected donor cells (red) remain on the outer layer of the embryo, while *acvr1ba**-injected donor cells (green) migrate into the inner layer of the embryo. Related to Figure 1. Frames were acquired every 3 min for 288 min. Playback is 7 frames/s.