

Fig. S1. PC2 represents lateral asymmetry of cell shape. PC2 does not show significant variation over elapsed time or between TF knockdowns. Time (t) is in minutes. Error bars are s.e.m.

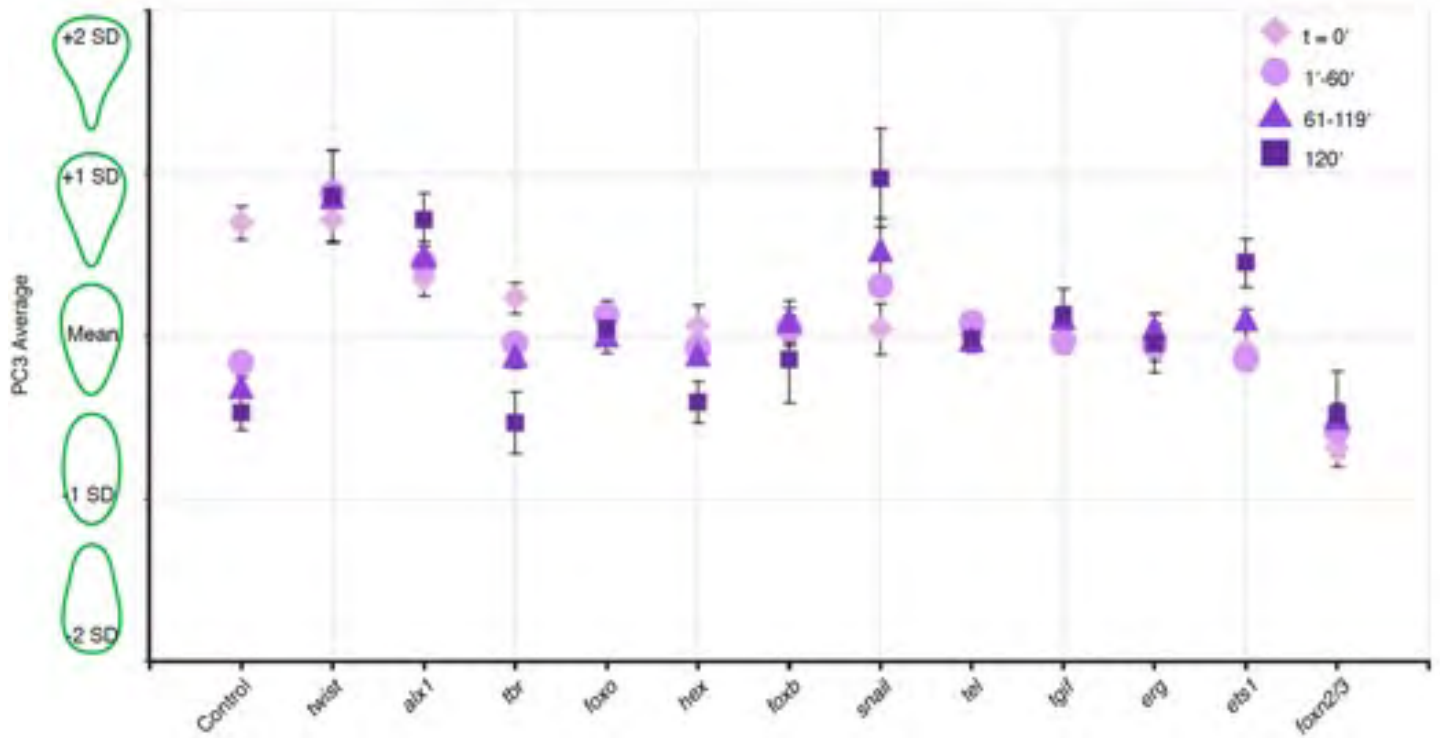


Fig. S2. Foxn2/3 and tbr are required for apical-basal polarity. PC3 represents cytoplasm distribution along the apical-basal axis from polarized apically, -2 SD, to polarized basally with minor apical constriction, $+2$ s.d. Foxn2/3 is the only knockdown to average more apical cytoplasm than the mean shape across all time points and tbr has progressively more apical cytoplasm over the time-course. Time is in minutes.

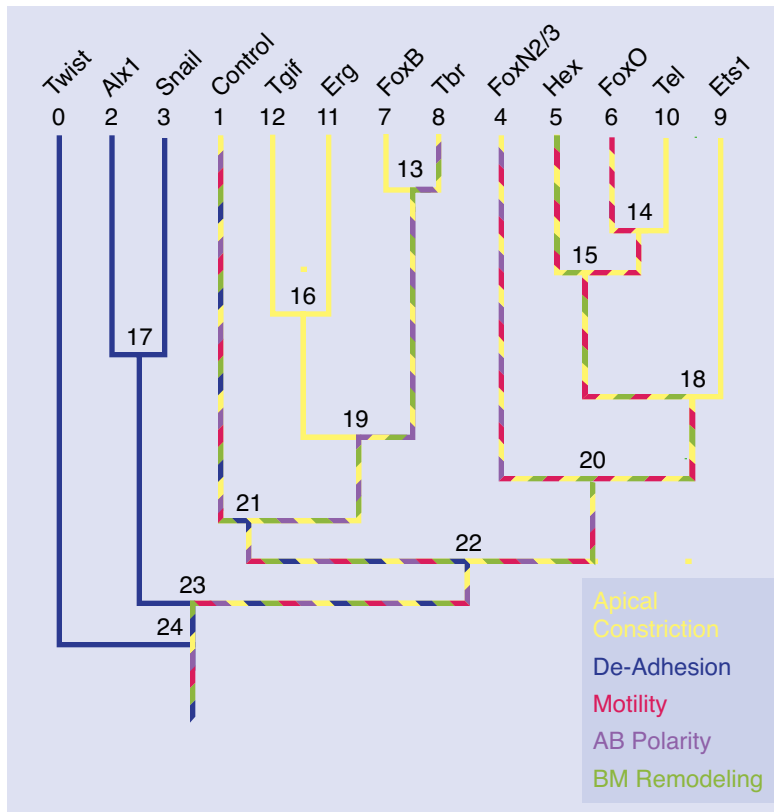
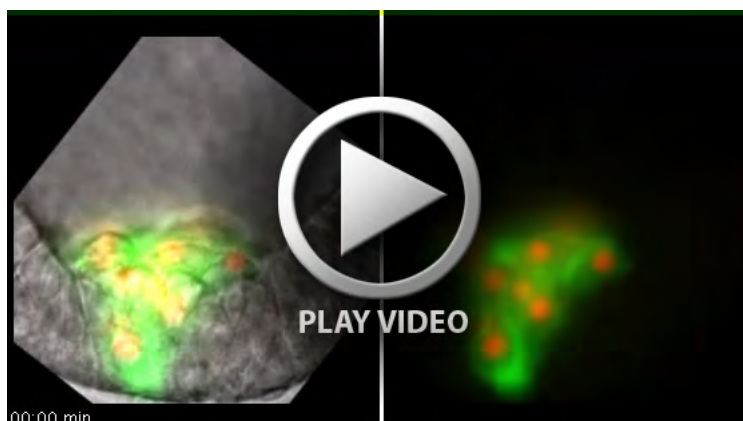
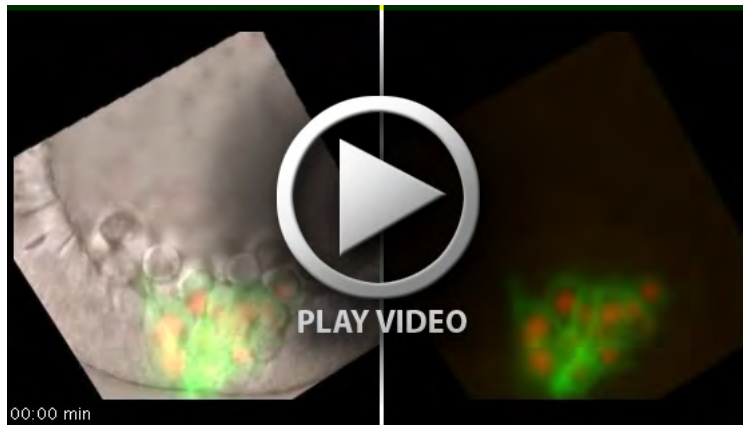


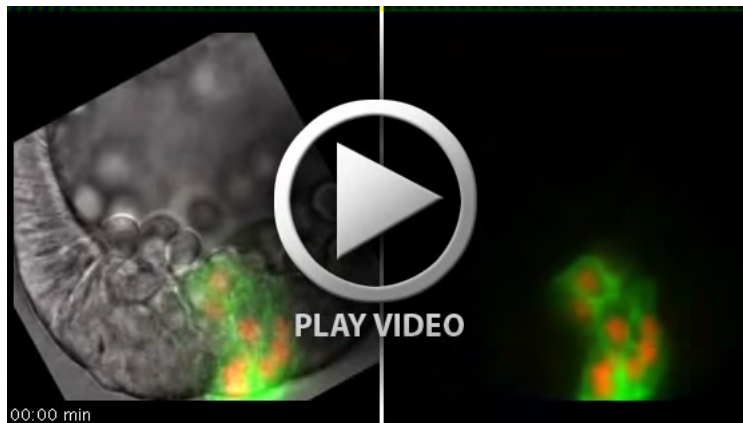
Fig. S3. Unsupervised analysis of TF knockdown phenotypes. K-means cluster analysis of displacement, principal component, and BM remodeling data. The hierarchical tree resulting from clustering shows the relationships between TFs based on total phenotypes. Five cellular behaviors of EMT are color coded; apical constriction (yellow), motility (red), apical-basal (AB) polarity (purple), de-adhesion (blue), and BM remodeling (green).



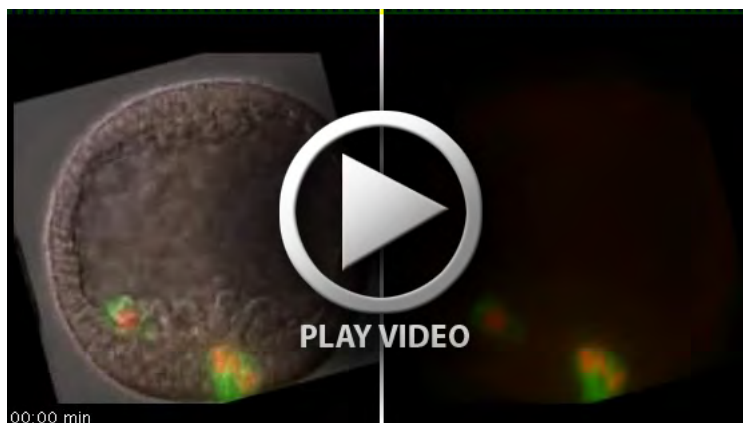
Movie 1. Time-lapse of Control-1. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



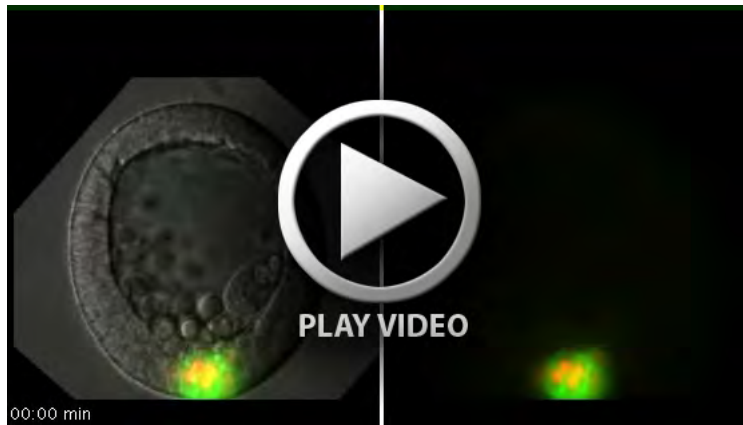
Movie 2. Time-lapse of Control-2. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



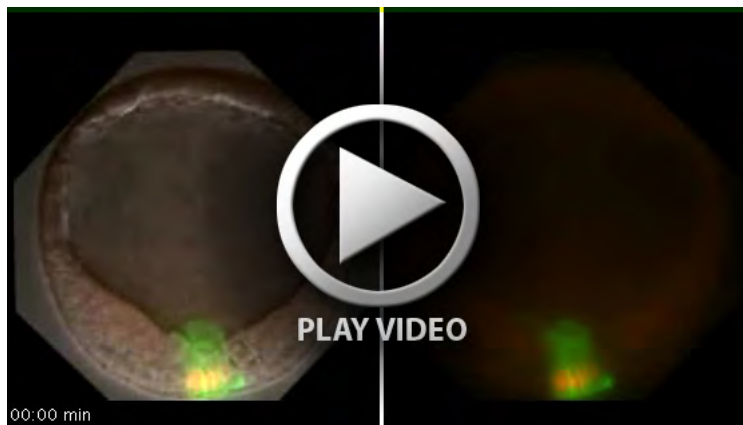
Movie 3. Time-lapse of alx1 morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



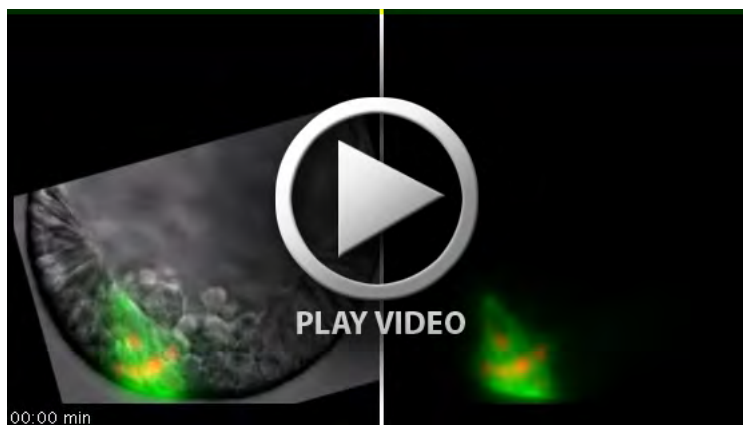
Movie 4. Time-lapse of erg morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



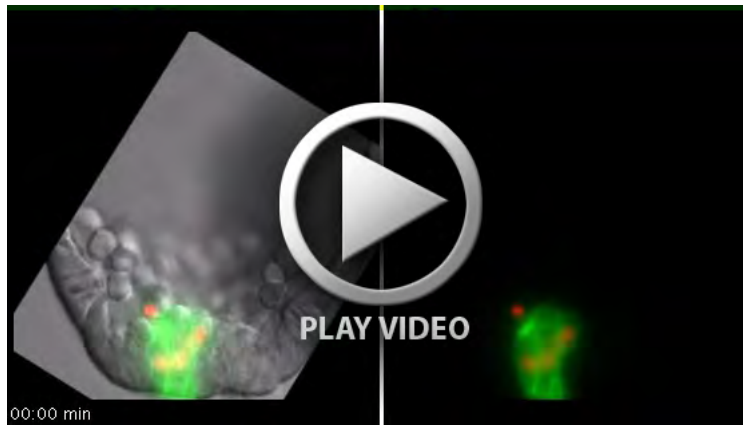
Movie 5. Time-lapse of *ets1* dominant-negative knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 6. Time-lapse of *foxb* morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 7. Time-lapse of *foxn2/3* morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



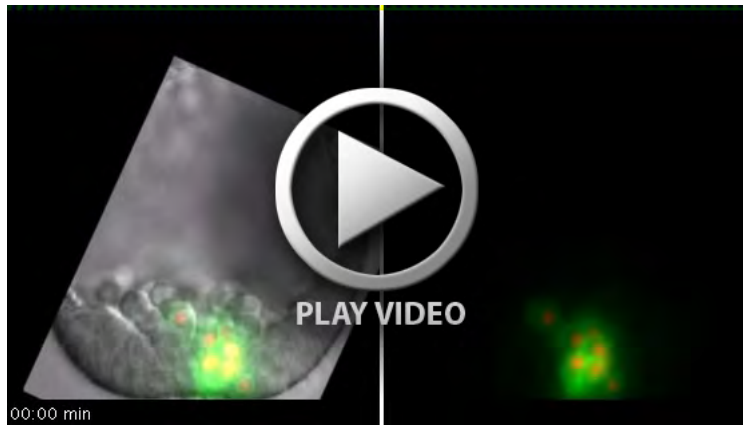
Movie 8. Time-lapse of foxo morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



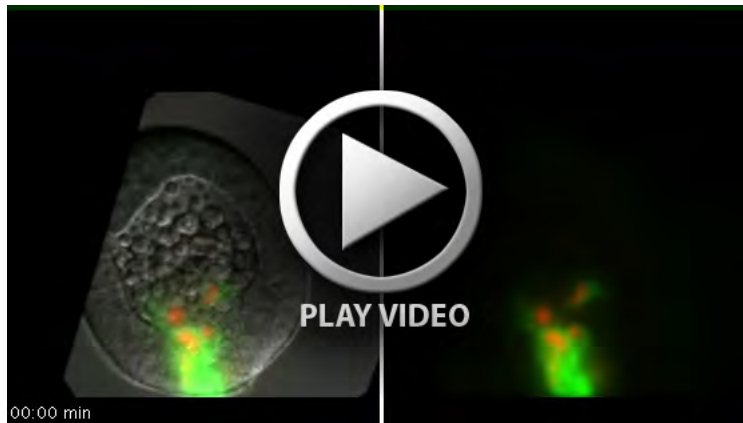
Movie 9. Time-lapse of hex morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



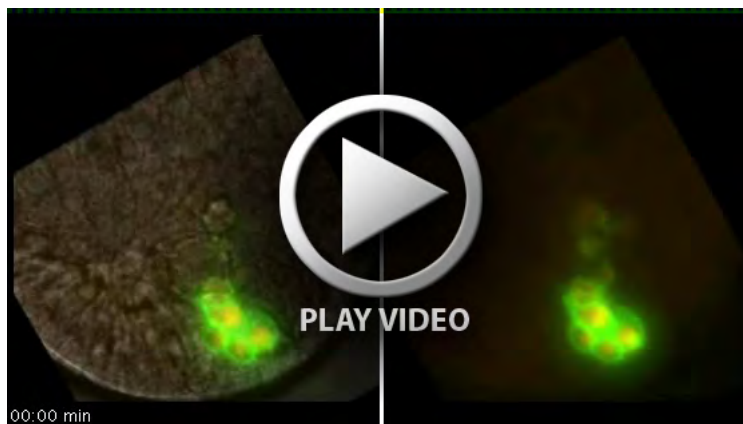
Movie 10. Time-lapse of snail morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 11. Time-lapse of *tbr* morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 12. Time-lapse of *tel* morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 13. Time-lapse of *tgif* morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.



Movie 14. Time-lapse of twist morpholino antisense oligonucleotide knockdown. Green label is cell membrane (mem-GFP). Red label is nuclei (H2B-RFP). Left panel: DIC, mem-GFP, H2B-RFP. Right panel: mem-GFP, H2B-RFP.

Table S1. Primer Sequences

Gene	Forward Primer	Reverse Primer
Dri	5' – GTAACACTGACTTTAGCGATCCTTCG – 3'	5' – GCTCAGATGGTGAACCTCAAACCTCTTC – 3'
Erg	5' – TCCGACTGCCTTTGAGT AAAGCAACGTCG – 3'	5' – GCACTTCGTTTACGAGTCT TTGTAATAGGAGTCC – 3'
FoxB	5' – GGACCTTTGCACTTGTGCGAGAATCG – 3'	5' – GAGTCTCTCTTCTCTTGAGTCAGTG – 3'
FoxO	5' – TAAGGATCCATGGTTGATAACG – 3'	5' – TAAGAATTCTTAATGAACC – 3'
Hex	5' – AAGGATCCATGTGCGACTATACC – 3'	5' – AAGAATTCTCAAGCATCTCG – 3'
Tel	5' – GCTGCCACCAAAAAGTCAGCT – 3'	5' – GATGCCACAAAACAGACATCTC – 3'
Tgif	5' – CACTTATTCTGGTTGTCTCCTGAGC – 3'	5' – TTACGGTAAGGCATGGAATTGCCTGG – 3'

Table S2. Morpholino Sequences and Concentrations

Morpholino	Concentration	Antisense Oligo Sequence
LvDri1	2.0mM	5' – GTAAAGTCTACAGACATTCGTTTGC – 3'
LvDri2	1.5mM	5' – CGCGGTGGTTCACCCGAAAACCGAA – 3'
LvErg1	0.75mM	5' – AATAATCACCAATCGTACACGACGT – 3'
LvErg2	0.5mM	5' – GCTTTACTCAAAGGCAGTCGGATA – 3'
LvFoxB1	0.3mM	5' – CTGGTATTTACAGAAAAGTCATGC – 3'
LvFoxB2	0.5mM	5' – CCCCTTCTAAATAGATAACAATGGTC – 3'
LvFoxO1	1.0mM	5' – GGGTCGTTATCAACCATTTTGATGA – 3'
LvFoxO2	0.75mM	5' – ATCCTAAATTGGGTCACAAGTACAC – 3'
LvHex1	2.0mM	5' – TGCACGAACAAGTATCCAGAAATGC – 3'
LvHex2	1.5mM	5' – GGTGCTGAACTTTACAAACAACCTCT – 3'
LvTel1	0.75mM	5' – ATCGTTCTTGGTCCTGGGCAGTTCC – 3'
LvTel2	1.0mM	5' – ATGGTTCCTGGTCTTGTGAACCTGA – 3'
LvTgif1	0.5mM	5' – ATCTTTCTTTTGATAAATCCGCATC – 3'
LvTgif2	1.0mM	5' – CGTATGTTGACTTTTTTCGCAGTGTT – 3'

Table S3. Sea urchin TFs required for EMT.

Sea Urchin EMT - TF	TF Family	Human Homologues TF Family Members	Vertebrate evidence of EMT relationship	Type of Evidence	Citation Examples
Alx1	Homeobox	ALX1 (CART1, FND3)	promotes EMT via SNAI1	direct	PMID: 23288509
		ALX3 (FND1)	upregulated in mesenchyme, craniofacial disorder	indirect	PMID: 23181698, 20534379, 9676189
		ALX4 (FND2)	gastric cancer metastasis, mammary cancer metastasis, craniofacial disorder	indirect	PMID: 22017425, 20145299, 11903336
Ets1	ETS (ETS subfamily)	ETS1 (ETS-1, EWSR2)	inhibits ECM production, required for neural crest EMT, breast carcinoma EMT	direct	PMID: 22829018, 17987123, 9247254
		ETS2	Upregulates MMP-2 in trophoblast invasion, breast cancer metastasis/invasion	indirect	PMID: 19939245, 9639404, 9500466, 16380248
Tel	ETS (TEL subfamily)	ETV6 (TEL, TEL/ABL)	myeloid malignancy, fusion protein in malignant leukemia	indirect	PMID: 22823977, 19287094
		ETV7 (TEL2, TELB, TEL-2)	B-cell malignancy, hematopoietic oncogenesis	indirect	PMID: 15743832, 16234363
Erg	ETS (ERG subfamily)	ERG (erg-3, p55)	overexpression induces EMT in prostate cancer	direct	PMID: 23027626, 21747944, 20713528
		FLI1 (EWSR2, SIC-1)	fusion protein in metastatic Ewing's sarcoma	indirect	PMID: 16204072, 9552022
		FEV (HSRNAFEV, PET-1)	fusion protein in metastatic Ewing's sarcoma	indirect	PMID: 17620387
Tbr	T-Box (TBR1 subfamily)	TBR1 (TBR-1, TES-56)	medulloblastoma	indirect	PMID: 22832583
		EOMES (TBR2)	loss blocks EMT	direct	PMID: 18171685
		TBX21 (T-PET, T-bet, TBET, TBLYM)	metastasis of gastric cancer	indirect	PMID: 22416188
Hex	Homeobox	HHEX (HEX, PRH, HMPH, PRHX)	mesenchymal cell expression after EMT induction, malignant haematopoiesis	indirect	PMID: 21490434, 20676125
Tgif	TALE Homeobox	TGIF1 (TGIF, HPE4)	overexpression progresses urothelial carcinoma	indirect	PMID: 22771156, 22728270
		TGIF2	required for gastrulation, overexpressed in ovarian cancer	indirect	PMID: 20040491, 11006116
Snail	Snail	SNAI1 (SNAIL, SNAIL1)	overexpression induces EMT, E-cadherin repressor	direct	Reviewed in PMID: 23076049, 22945800, 17587826
		SNAI2 (SLUG, SNAIL2)	overexpression induces EMT, E-cadherin repressor	direct	Reviewed in PMID: 19273255, 21665887
		SNAI3 (SMUC, SNAIL3)	thyroid carcinoma	indirect	PMID: 22641097
Twist	Twist	TWIST1 (TWIST, bHLHa38)	overexpression induces EMT, E-cadherin repressor	direct	Reviewed in PMID: 19272800, 15640618
		TWIST2 (DERMO1, bHLHa39)	overexpression induces EMT, E-cadherin repressor	direct	PMID: 21602879, 22018873, 22103974
FoxB	Forkhead	FOXB1 (FKH5)	expression pre-neural crest delamination	indirect	PMID: 8861101
		FOXB2 (FKH4)	expression in developing spleen and thymus	indirect	PMID: 7689224
FoxO	Forkhead	FOXO1 (FKH1, FKHR, FOXO1A)	colorectal cancer, malignant parathyroid carcinoma	indirect	PMID: 21901254, 23001705
		FOXO3 (FOXO2, FKHL1, FOXO3A)	promotes cancer cell invasion	indirect	PMID: 19564415, 21965295
		FOXO4 (AFX, AFX1, MLLT7)	required for neural crest migration	indirect	PMID: 19895805
FoxN2/3	Forkhead	FOXN2 (HTLF)	presomitic mesoderm	indirect	PMID: 12351180
		FOXN3 (CHES1)	neural crest development, craniofacial defects	indirect	PMID: 21050205, 17089409, 20691664
Dri	ARID	ARID3A (BRIGHT, DRIL1, DRIL3, E2FBP1),	oncogenic, promotes fibrosis, required for gastrulation	indirect	PMID: 11812999, 18583319, 15680369
		ARID3B (BDP, DRIL2),	malignant neuroblastoma, neural crest development	indirect	PMID: 22751132, 16951138, 16530748

Each TF in the sea urchin EMT GRN has at least one human homologue. 8 homologues have been directly implicated in vertebrate EMT. 22 homologues have not been assayed for EMT regulation but are expressed in a tissue where this is an EMT event. PMID = PubMed Identifier.