SUPPLEMENTARY METHODS

Molecular Cloning of CD4:GFP and CD8:mCherry constructs

Mouse CD4 (NP_038516) fragment containing aa residues1-424 was amplified with oligo CD4- Not1F: ATGTGCCGAGCCATCTCTCTTAG and CD4 424 NheR: TAATGCTAGCTTGGTGCCGGCACCTGACACAGC. GFP was amplified with F oligo containing 5' NheI site: TAATGCTAGCGTGAGCAAGGGCGAGGAGC and R oligo with EcoRI site: TAATGAATTCTTACTTGTACAGCTCGTCCATGCC. Standard cloning methods utilized NheI site to combine the cloned CD4 and GFP fragments in frame.

Mouse CD8 (NP_001074579) fragment containing aa residues 1-247 were amplified with CD8 NF: TAATGCGGCCGCCATGGCCTCACCGTTGACC and
CD8 247 NheR: TAATGCTAGCCACAATTTTCTCTGAAGG. mCherry was amplified with F oligo containing 5' NheI site: mCh NheF:
TAATGCTAGCGTGAGCAAGGGCGAGGAGG and R oligo containing EcoRI site mCh ER: TAATGAATTCTTACTTGTACAGCTCGTCCATG. Standard cloning methods utilized NheI site to combine the cloned CD8 and mCherry fragments in frame.

Proteins expressed from these constructs were properly targeted to plasma membrane, as visualized by fluorescence microscopy.

Cell Sorting with Magnetic beads protocol

These experiments were performed in triplicate. *Ciona* eggs were electroporated with FoxC>CD4:GFP and ZicL>CD8:mCherry constructs, and embryos developed to neurula stage. Embryos were dissociated to single cells with trypsin in calcium- and magnesium-free seawater (CMF-ASW), and washed in CMF-ASW supplemented with 0.05% BSA, as previously described (Christiaen et al., 2009). Negative selection was performed by rotating cell suspension with 50 µl mouse CD8 (Lyt-2) Dynabeads (Invitrogen) for 30 min at 4°C. CD8-expressing cells were then collected with magnetic rack, and supernatant was transferred to fresh tube containing 25 µl mouse CD4 (L3T4) Dynabeads (Invitrogen) for positive selection, which was done for 20 min at 4°C. RNA was isolated from the enriched CD8+ population by adding 100 µl Lysis buffer from Ambion RNAqueous Micro kit directly to the collected beads in magnetic rack, followed by gentle vortexing, and recollection of beads with magnetic rack; the cell lysate in the supernatant was transferred to fresh tube. After positive selection of CD4-expressing cells, 3 washes were performed with CMF +BSA. After last wash, Lysis buffer was added as described for the CD8 population, and RNA was isolated and eluted in ~20µl. The RNA from both the CD4+ and CD8+ populations was DNAse-treated.

1-4 µl of RNA from each population was reverse transcribed with random hexamers and Superscript Reverse Transcriptase III (Invitrogen). cDNA was analyzed by q-PCR to assess enrichment of sorted cell populations, and integrity of RNA was assessed with Bioanalyzer (Agilent). Samples that passed this quality control step were labeled using

Ovation Pico WTA System and Encore Biotin Module (NuGen) according to the manufacturer's recommendation. The fragmented and labeled RNAs were hybridized to CINT06a520380F (Affymetrix GeneChip for *Ciona intestinalis*), washed and scanned according to the manufacture's recommendations. Microarray data were normalized using the GCRMA algorithm (Irizarry et al., 2003); ratios of normalized probe set intensity values were calculated for each sample pair (in which M value = $\log_2$[ICAM1(+)/ICAM1(−)]) and then averaged among the three replicate pairs.

Literature Cited

Christiaen, L., Wagner, E., Shi, W., and Levine, M. (2009). Isolation of individual cells and tissues from electroporated sea squirt (Ciona) embryos by fluorescence-activated cell sorting (FACS). *Cold Spring Harb Protoc*. **2009**, pdb prot5349.

Irizarry, R.A., Hobbs, B., Collin, F., Beazer-Barclay, Y.D., Antonellis, K.J., Scherf, U., Speed, T.P. (2003). Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics* **4,** 249–264.

GGCGCGCCGCCTCGCTTAATTGCGGTAAGTTTGTGGGTTGTTTAATAAAGTAGGGGGGT
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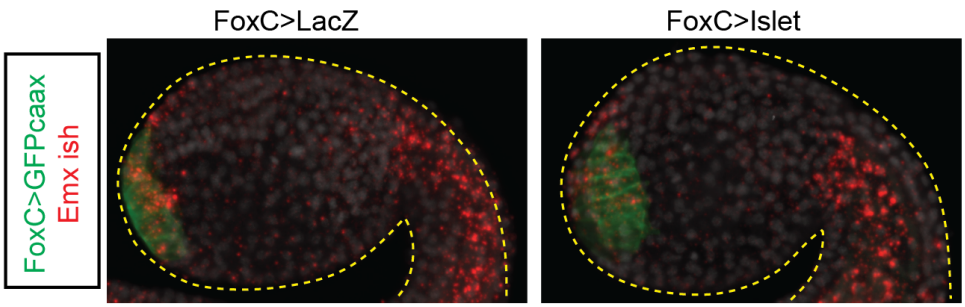
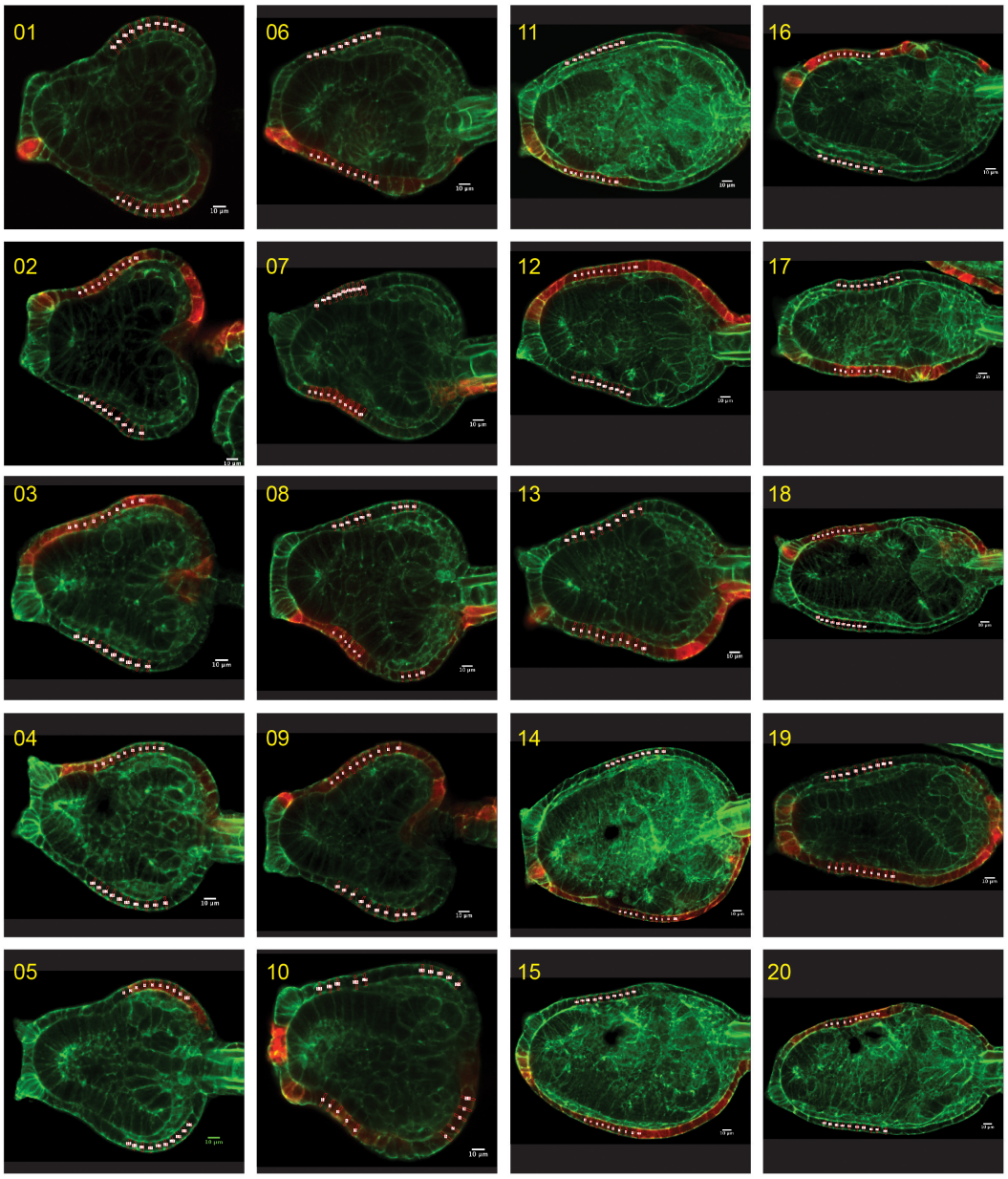
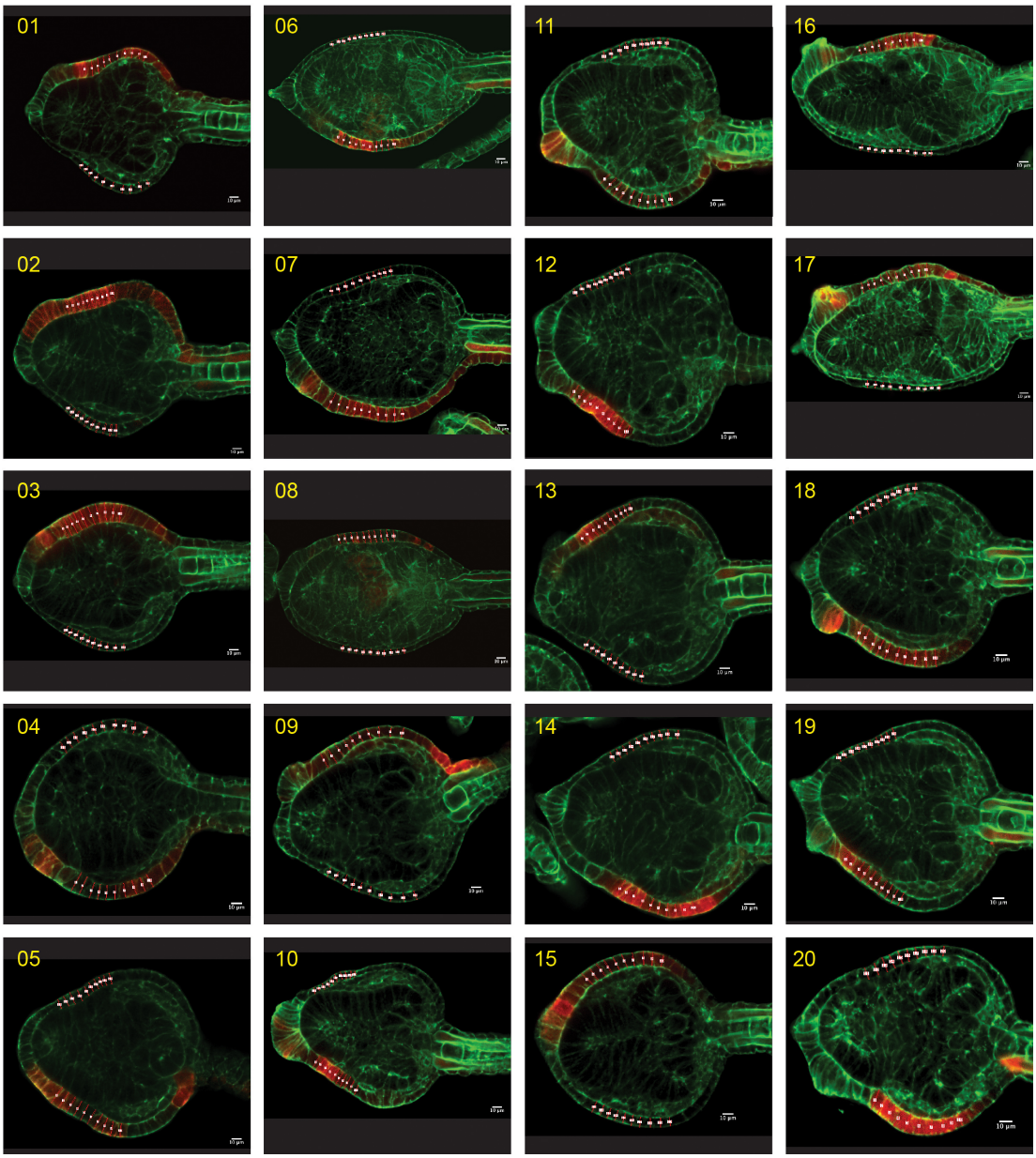
Supplemental Figure S1. Sequence of *Islet* cis-regulatory DNA. AscI, XhoI, and NotI restriction sites are highlighted in yellow. Black font (Xho/Not fragment) includes native promoter region; start codon was mutated (bold text). Blue font (Asc/Xho fragment) includes the enhancer and maps to the first intron of *Islet* gene.



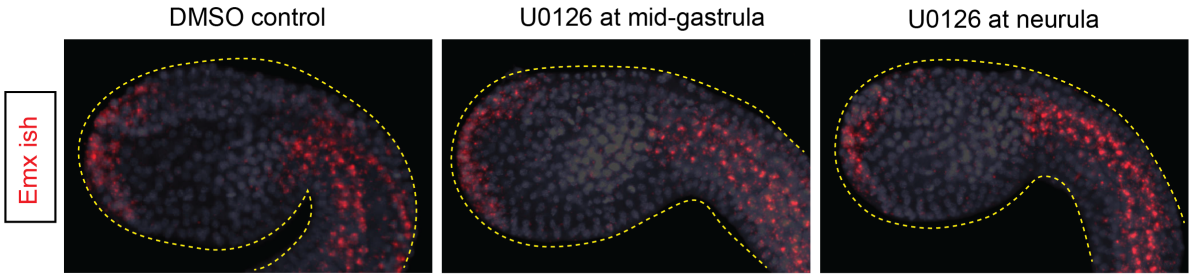
Supplemental Figure S2. Emx in situ hybridization (red) in the presence of FoxC>LacZ control, or FoxC>Islet. Perturbed cells express FoxC>GFPcaax reporter (green). Nuclei stained with Hoechst.



Supplemental Figure S3.
Set of 20 embryos expressing FoxF>LacZ. Images show a single confocal section and denote the measurements used to calculate normalized cell lengths.



Supplemental Figure S4.
Set of 20 embryos used to measure cell lengths in mosaic embryos expressing FoxF>Islet.



Supplemental Figure S5. Emx transcripts detected by in situ hybridization, in the presence of DMSO, or U0126 treatment at the mid-gastrula and neurula stages. Emx expression in the palps is unaffected with drug treatment (expression in the tail ectoderm is also unaffected). Embryos shown are at mid-tailbud stage; the ring-shaped pattern emerges slightly later, at the late tailbud stage.