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Supplementary Tables

Upstream	Target	Function	Timepoint [h]		Domain	Evidence[a]	Ref.
			start	end			
Blimp1	*brn1/2/4*	Activation	27		veg2 endoderm	MASO[b]	
Blimp1	*krl*	Repression	27		veg2 endoderm	MASO[b]	
Blimp1	*otxb*	Activation	18	23	veg2 endoderm	MASO,CR	1
Blimp1	*tgif*	Activation	24		veg2 endoderm	MASO[b]	
Bra	*foxa*	Activation	21	23	veg2 endoderm	CR	2
Bra	*hnf1*	Activation	24		veg1 endoderm	MASO[b]	
Bra	*otxb*	Activation	24		veg1 endoderm	MASO[b]	
Bra	*otxb*	Activation	19	23	veg2 endoderm	MASO[b]	
Bra	*tgif*	Activation	21	23	veg2 endoderm	MASO[b]	
Bra	*unc4.1*	Activation	24		veg1 endoderm	MASO[b]	
Brn1/2/4	*endo16*	Activation	27		veg2 endoderm	MASO,CR	3
Dac	*dac*	Repression	27		veg2 endoderm	MASO[b]	
Dac	*hh*	Activation	27		veg2 endoderm	MASO[b]	
Dac	*krl*	Repression	27		veg2 endoderm	MASO[b]	
Eve	*hnf1*	Activation	24		veg1 endoderm	MASO[b]	
Eve	*hox11/13b*	Activation	24		veg1 endoderm	MASO[b]	
Eve	*unc4.1*	Activation	24		veg1 endoderm	MASO[b]	
FoxA	*foxa*	Repression	20	24	veg2 endoderm	CR	2
FoxA	*gcm*	Repression	27		veg2 endoderm	MASO	4
FoxA	*hh*	Activation	24		veg2 endoderm	MASO	4
FoxA	*krl*	Repression	27		veg2 endoderm	MASO[b]	
GataE	*bra*	Activation	20	23	veg2 endoderm	CR[c]	
GataE	*brn1/2/4*	Activation	27		veg2 endoderm	MASO[b]	
GataE	*gatac*	Repression	27		veg2	MASO[b]	
GataE	*krl*	Repression	24		veg2 endoderm	MASO[b]	
GataE	*otxb*	Activation	24		veg2 endoderm	MASO[b] /CR	1
Hnf1	*hnf1*	Repression	27		veg1 endoderm	MASO[b]	
Hox11/13b	*blimp1b*	Activation	19	23	veg2 endoderm	MASO[b]	
Hox11/13b	*bra*	Activation	24		veg1 endoderm	MASO[b]	
Hox11/13b	*bra*	Activation	19	23	veg2 endoderm	MASO[b]	
Hox11/13b	*foxa*	Activation	19	23	veg2 endoderm	MASO[b] /CR	2
Hox11/13b	*gatae*	Activation	19	23	veg2 endoderm	MASO[b]	
Hox11/13b	*hox11/13b*	Repression	24		veg2 endoderm	MASO[b]	
Krl	*brn1/2/4*	Activation	27		veg2 endoderm	MASO[b]	
Krl	*hh*	Activation	27		veg2 endoderm	MASO[b]	
Myc	*hox11/13b*	Repression	27		veg2 endoderm	MASO[b]	
Myc	*krl*	Repression	27		veg2 endoderm	MASO[b]	

Myc	*myc*	Repression	24		veg2 endoderm	MASO [b]	
Myc	*tgif*	Activation	24		veg2 endoderm	MASO [b]	
Otx	*blimp1b*	Activation	19		veg2 endoderm	CR	5
Otx	*bra*	Activation	19	23	veg2 endoderm	CR[c]	
Otx	*endo16*	Activation	24		veg2 endoderm	MASO [b] /CR	6
Otx	*foxa*	Activation	24		veg2 endoderm	MASO	2
Otx	*gatae*	Activation			veg2 endoderm	CR[d]	
Otx	*hh*	Activation	27		veg2 endoderm	MASO [b]	
Otx	*otx*	Activation			veg2 endoderm	CR	1
Otxa	*brn1/2/4*	Activation	27		veg2 endoderm	MASO [b]	
SoxC	*soxc*	Repression	24		veg2 endoderm	MASO [b]	
Tcf/β-catenin	*blimp1b*	Activation	19		veg2 endoderm	CR	5,7
Tcf/β-catenin	*bra*	Activation	24		veg1 endoderm	CR [c]	7
Tcf/β-catenin	*bra*	Activation	19	23	veg2 endoderm	CR [c]	7
Tcf/β-catenin	*eve*	Activation	19		veg1 endoderm	CR	5,7
Tcf/β-catenin	*foxa*	Activation	19		veg2 endoderm	CR	2,7
Tcf/β-catenin	*hox11/13b*	Activation	19	23	veg2 endoderm	CR [e]	7
Tcf/β-catenin	*hox11/13b*	Activation	24		veg1 endoderm	CR [e]	7
Tcf/β-catenin	*krl*	Activation	19		veg2 endoderm	LiCl	8
Tcf/β-catenin	*wnt8*	Activation	19		veg1 endoderm	CR	9
Tgif	*hh*	Activation	27		veg2 endoderm	MASO [b]	

[a] Experimental evidence for interaction; MASO: perturbation of upstream factor by injection of gene-specific morpholinos; CR: *cis*-regulatory analysis of target gene; LiCl: perturbation of Wnt signaling pathway by injection of LiCl

[b] present study

[c] R.A. Cameron and E.H. Davidson, unpublished results

[d] P.Y. Lee and E.H. Davidson, unpublished results

[e] C. Theodoris, J. Smith and E.H. Davidson, unpublished results

Supplementary Table 1. **Proposed direct interactions in endoderm GRNs [19-30 h].** For each transcription factor with specific expression in endodermal domains ("Upstream"), all likely direct target genes ("Target") are listed along with the evidence supporting the regulatory interaction which causes activation or repression ("Function") of the target gene. Data for interactions identified in the present study are shown in Supplementary Fig. 5 (marked in yellow). Proposed direct interactions are incorporated in the endoderm GRN models shown in Fig. 4.

Upstream	Target	Function	Time-point	Domain upstream factor	Domain target gene	Argument
Blimp1	*endo16*	Activation	24-27	veg2 endod.	veg2 endod.	possibly indirect via Otx or Brn1/2/4
Blimp1	*foxa*	Activation	27	veg2 endod.	veg2 endod.	possibly indirect via Otx
Blimp1	*gatac*	Activation	24	veg2 endod.	veg2 mesod.	not in same domain
Blimp1	*blimp1b*	Repression	19-30	veg2 endod.	veg2 mesod.	direct interaction in mesoderm
Bra	*foxg*	Activation	24-27	Oral ectod.	Oral ectod.	proposed direct interaction in oral ectoderm
Bra	*foxb*	Activation	24	veg1 endod.	skeletog. mesod.	not in same domain
Bra	*hh*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via FoxA
Bra	*krl*	Repression	27	veg1 endod.	veg2 endod.	possibly indirect via FoxA
Dac	*foxb*	Activation	24	veg2 endod.	skeletog. mesod.	not in same domain
Dac	*unc4.1*	Activation	24	veg2 endod.	veg1	not in same domain
Eve	*brn1/2/4*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
Eve	*endo16*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1, Brn1/2/4
Eve	*gelsolin*	Activation	24	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
Eve	*hnf6*	Activation	27	veg1	veg1	possibly direct interaction in veg1 ectoderm
Eve	*orCT*	Activation	24-27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
Eve	*tgif*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
GataE	*hh*	Activation	24-27	veg2 endod.	veg2 endod.	possibly indirect via FoxA
Hox11/13b	*apobec*	Activation	24-27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
Hox11/13b	*foxn2/3*	Activation	27	veg1 endod.	veg2 mesod.	not in same domain
Hox11/13b	*hh*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via FoxA
Hox11/13b	*hnf1*	Activation	24-27	veg1 endod.	veg1 endod.	possibly indirect via Brachyury
Hox11/13b	*orCT*	Activation	27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1
Hox11/13b	*tgif*	Activation	24-27	veg1 endod.	veg2 endod.	possibly indirect via Blimp1 or Brachyury

Myc	*hh*	Activation	27		veg2 endod.	possibly indirect via Tgif
Notch	*apobec*	Repression	24	veg2 mesod.	veg2 endod.	indirect repression probably in mesoderm
Notch	*gatac*	Activation	24-27	veg2 mesod.	veg2 mesod.	proposed direct interaction in mesoderm
Notch	*gcm*	Activation	24-27	veg2 mesod.	veg2 mesod.	direct interaction in mesoderm
Otx	*foxg*	Activation	24-27	several	ectoderm	proposed direct interaction in ectoderm

Supplementary Table 2. Arguments for not including significant perturbation effects in endoderm GRN model. Incorporated in this list are all genes ("Target") showing significant changes of expression upon perturbation of a regulatory gene ("Upstream") which are unlikely to be the consequence of direct regulatory interactions for reasons stated ("Argument"). Data are shown in Supplementary Fig. 5 (marked in orange). Briefly, direct regulatory interactions require the co-expression of upstream factor and target gene and are only predicted if no other (e.g. indirect) regulatory interactions explain the observed effects.

Upstream	Target	Function	Time	Signal Origin	Signal Target	Predicted Signal
Eve	*blimp1b*	Activation	>24	veg1	veg2 endoderm	Signal V2
Eve	*gatae*	Activation	>27	veg1	veg2 endoderm	Signal V2
Hox11/13b	*hox11/13b*	Activation	>24	veg2	veg1 endoderm	Signal V1

Supplementary Table 3. **Proposed interactions involving signaling between two adjacent domains.** Target genes which are significantly affected by a given perturbation ("Upstream") but are expressed in cells adjacent to the upstream factor might be regulated by a signaling interaction. The corresponding signaling ligand is predicted to be expressed in one domain ("Signal Origin") under the control of the upstream regulatory factor and to affect target gene expression in the adjacent domain ("Signal Target"). Data are shown in Supplementary Fig. 5 (marked in green).

Gene	Gene identifier	primer seq (Forward)	primer seq (Reverse)
alx1	SPU_025302	CAGTGCAGCTTTACGTGGAC	TTAAGTCTCGGCACGACAAA
apobec	SPU_011837	ACCCAGTTTCACCCTCCTCT	AGGCACTCAGCTGCAAAGTT
blimp1b	SPU_027235	TCGCTATGCGGGATCTCTAC	GGGGTCCTTGACCTCGTAA
bra	SPU_013015	ACACATCGACCCATCATCAA	CATGGTGTCGTATCTTGGAAAG
bra2	SPU_013015	CGCCGTACAGTCAGAGATGA	AAGTCGAGCAGGACGGAGTA
brn1/2/4	SPU_016443	GTCGCATTAAGCTCGGCTAC	CAGCGGCTTCAGTTTACACA
dac	SPU_028061	GGATGCGAACCTGTTCTACG	CAATTCAAAAGCTTGTGGCA
delta	SPU_016128	ACGGAGCTACATGCCTGAAC	TCACAATGGACCGAATCAGA
elk	SPU_012469	ATCATGGTCGCTAGTCCTCTCCT	TGACAAGAGAACAGTCGGTGTGA
endo16	SPU_011038	GACCGAACGCCGATATAAGA	GCCATCGTCCCTTTAGTTCA
eve	SPU_012253	CACAGACCCTGGACTTTCGT	GACAAACGGTCATCCCACTT
foxa	SPU_006676	CCAACCGACTCCGTATCATC	CGTAGCTGCTCATGCTGTGT
foxb	SPU_004551	AAGCCATCCACAACCAAATC	CATATCCGTCGAACGAGTCA
foxg	SPU_009771	CGCTCGAGTCCAGAGAAAAG	TGTCGAGGGACTTTCACAAA
foxn2/3	SPU_015243	TTCATGTCGATAGAGGACTGC	TTCGGAAGCACTTGTTGAGA
foxp	SPU_009876	TGTGGCAGCATTTCTTCTGA	TACGTATGCTGCCCTCATCA
gatac	SPU_027015	CAGGGACATCATGTGCAAAC	CCGTGTTTGAATGCCTTCTT
gatae	SPU_010635	CTGGCTCAAGACGAGAAGGA	CCTCTTCCGAGTCTGAATGC
gcm	SPU_006462	CGACTGATAACCACGCTCAA	TTAACGACGTCGGTCGATTC
gelsolin	SPU_020607	CTCCATCGACGAGAGGAGAA	CCTTCTGCTACGACCGAAAC
hh	SPU_021608	GGCTTCGATTGGGTCAACTA	GTTGACCACGGCTACCTCAT
hesc	SPU_012238	CCAGAACAGGGCGAATCTAA	CGAAGACGGGTTTCAATGTC
hnf1	SPU_008196	CGTGCCCTTATTCAAATGCT	CCATGGCAAGTAGCGAAGAT
hnf6	SPU_016449	TGCAGCTTCTCTGCATACCA	ACTCCAACATGCCTCCAAAC
hox11/13b	SPU_002631	CACAGGCTCTCGACCTAACC	GGTGGATGAGGTGGTAGATGA
kakapo	SPU_003256	GTGGCATTTATGAGCGGTCT	CGGCCCAGTACTTCAAGGAGA
krl	SPU_004148	CACGAACTCTTCGCAATCAA	CCAAGGGACAGGAGTGAAGA
msx	SPU_022049	AGCACAAGACAAACCGGAAG	CGTTCGGCTATCGAGAGGTA
myc	SPU_003166	CCCGCCATCCTCACATAAT	GGAACAGCGCTTTACCACTT
notch	SPU_014131	ACGGAGCCAAGCCTAAGAA	TCGTCACAGGCAACGAATAA
orct	SPU_012646	GACTTCAGACGCGTTGGTCT	TTATCACTGTCGGGGAGTTTG
otx-alpha	SPU_010424	CCTTACCAGCACCTGATCG	CTGGTCCTGCTGAACAAGGT
otxb	SPU_010424	GTTTAGGAACGTCGCTGGAA	TGAAGGTGGTGGTGATGTTG
soxb1	SPU_022820	ATTCTGTGAACGTCATGGCA	TTGTCCTCTTGACCACACCA
soxc	SPU_002603	CATGGTTTGGTCACAAATCG	TACGGAGATTTCGCCACTTC
tgif	SPU_018126	GCTCTACCTATCTCGCTTGGC	TGGTGAACTTGTCAGGGTCT
ubq	SPU_021496	CACAGGCAAGACCATCACAC	GAGAGAGTGCGACCATCCTC
unc4.1	SPU_013704	TGAACACGTGACTCAACAAGG	CAATGGTTGGCAGCTGGAG
wnt8	SPU_020371	TGTCGTTCATTCAAGCCATC	TATCACTCGCCATTCGTTCA
z188	SPU_023727	GGCATTGCTAAGTCCATCGT	GCTCTTTGTTCATGGTGGCT

Supplementary Table 4. QPCR primers used in perturbation experiments

Gene	MASO sequence	MASO interfering with
blimp1b	CTCCCTTTCGCTTGAAAAACACCGC	Translation[10]
bra	CGCTCATTGCAGGCATAGTGGCG	Translation
dac	CCTGAGTAGGCGGTGGGCTTCCCAT	Translation
eve	CAGAAACCACTCGATCAATGTTTGC	Translation
foxa	TGGGTTCCTCTTTGAAATCCACGAT	Translation[4]
gatae	GACTTACACCGACCTGATGTGGCAT	Translation
hnf1	CTAGTTCGTCACCCGAATGCAGCAT	Translation
hox11/13b	AAGCCTGTTCCATGCCGATCTGCAT	Translation[11]
krl	CGCCGCGTGTAGACGGTTCATGTCG	Translation[8]
myc	ACTGCCAGTGCCATTTTGCAGGTGA	Translation
notch	CCTGGATGGGTAGTCCGCCTCATCT	Translation
otx HD	TGGATCTAGACAGAGGAGGAGAAAC	Splicing
otx-alpha	CATGTACCACTGCTTGACCGGTGCT	Translation
soxc	GAACCATCTTGAAGTCAGCATTCAC	Translation[12]
tgif	ATCTTTCTTGTGGTAAATCCGCATC	Translation[12]

Supplementary Table 5. Morpholino antisense oligonucleotide (MASO) sequences. For evidence of morpholino activity and specificity see Supplementary Fig. 5

Gene	WMISH probe source	Sequence origin	Sequence fragment [bp]	WMISH primer fwd	WMISH primer rev
blimp1b	PCR	DQ177152.1	765-1398	AGAGAAACCAGTTGTCGCGT	ATCTTGCTTCATGCTGGCTT
bra	[13]		600bp cDNA		
dac	cDNA clone[a]	GLEAN3_28061	1-398		
		GLEAN3_18581	43-384; 429-491		
eve	cDNA clone[7]	GLEAN3_12253	57-756		
foxa	[4]		1659 bp cDNA		
gcm	[7]				
hnf1	PCR	XM_001179097.1	436-1426	GCCGTTACAACCTCACCTGT	TAGCATGCCCGATAAGATCC
hox11/13b	PCR	GLEAN3_02631 CDS Sequence	380-1001	GCCCCTACGCCAATACATTC	TGACCAACTGAGGGATGTGA
krl	PCR	AF314167	1-1216 plus 60bp 3'UTR sequence	ATTCTGAACATTTGTCTTCTCTAGAGCA	TATAGCAAAGGTTTTAACGTCAATAACC
myc	PCR	NM_214579.2|	6-1515	GCCTTGTCAAACTCTAATCGG	GTGGTCCAAATCCAATACGC
soxc	[12]				
tgif	[12]				
wnt8	[9]				

[a] see Supplementary Fig. 8

Supplementary Table 6. cDNA fragment sequences used to generate probes for whole mount in situ hybridizations (WMISH). Primer sequences, where given, were used to amplify probe templates from cDNA, which was generated from total RNA (extracted from 27h embryos). Indicated sequence positions ("sequence fragments") refer to the sequences given in "Sequence origin". Sequences for GLEAN models to be found on SpBase (http://www.spbase.org).

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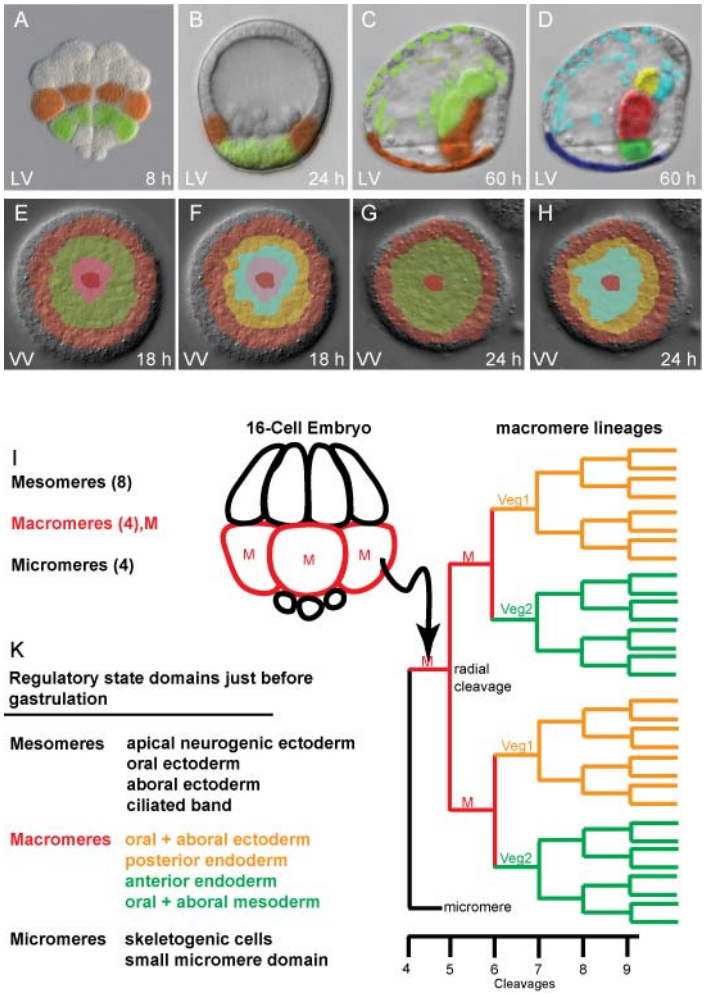
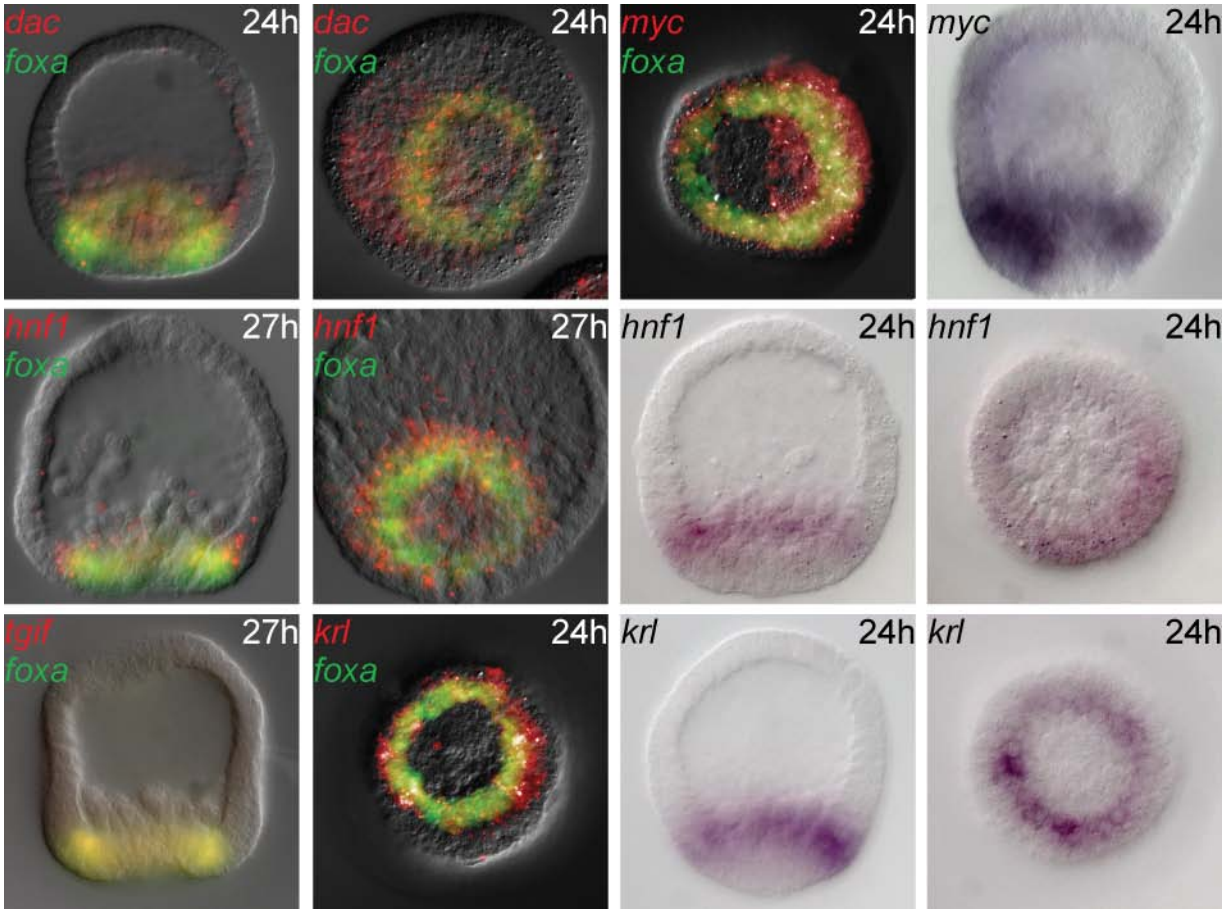
Supplementary Figures

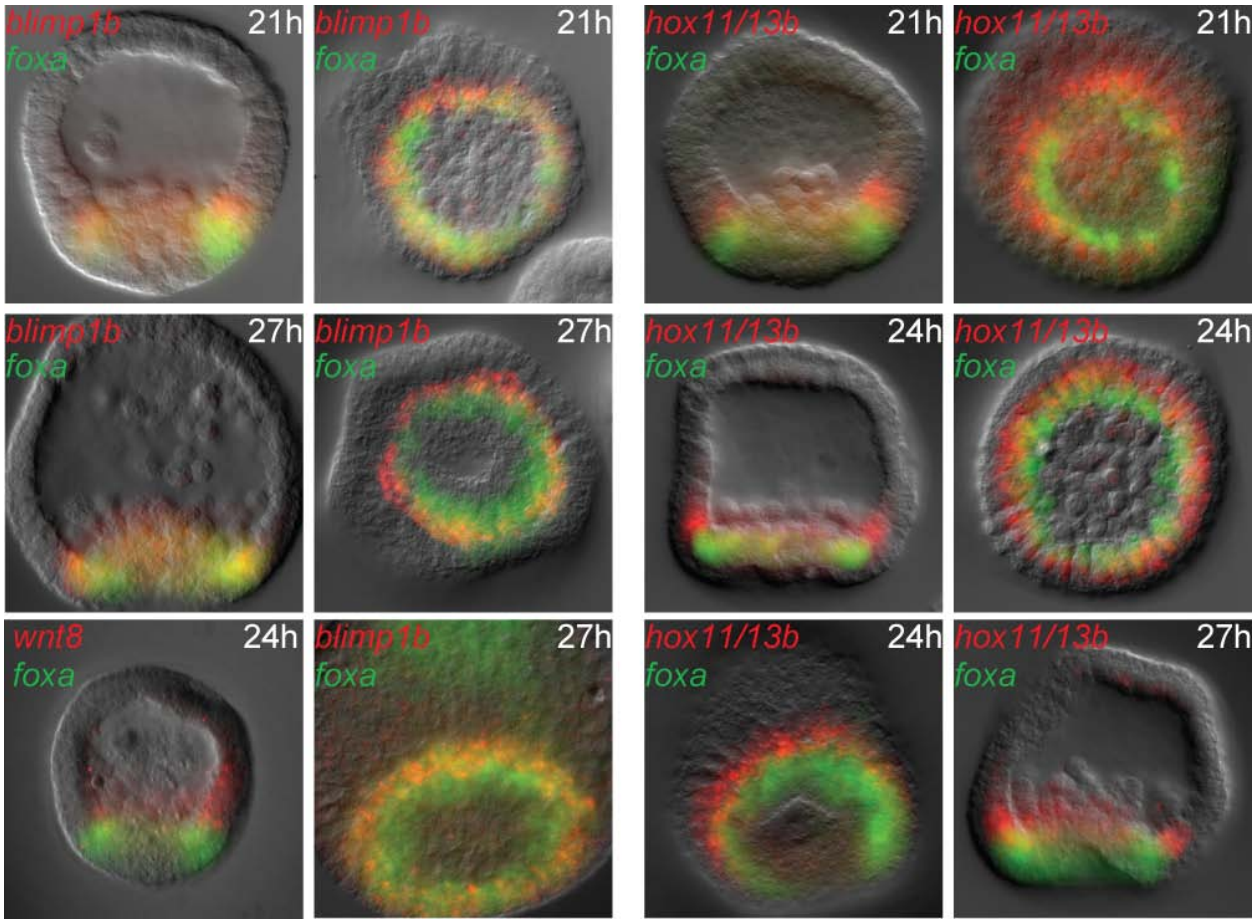


Supplementary Figure 1. Veg1 and veg2 lineages and cell fates in the sea urchin embryo. (a-c), Veg1 (orange) and veg2 (green) lineages, displayed in color superimposed on lateral DIC images of *Strongylocentrotus purpuratus* embryos. (a), At 6th cleavage (8h), two rings of 8 cells each, here shown highlighted in color, are formed by a horizontal cleavage, thus founding the veg1 and veg2 lineages. The endoderm of the later embryo derives entirely from these lineages. Four cells of each lineage can be seen in the lateral view shown. Above, toward the animal pole, are cells that will form the neurogenic apical plate, the animal portion of the oral ectoderm and its structures including the parts of the ciliated band and the mouth, and the aboral ectoderm, except for the peri-anal portion. Below veg2 are the 4 skeletogenic micromeres. Two of the 4 small micromeres, the descendants of which will be incorporated in the set-aside cells of the coelomic pouches, can be seen protruding from the vegetal pole of the embryo. (b), Mesenchyme blastula stage (24h); the 16 progeny of the skeletogenic cells can be seen to have ingressed into the blastocoel, and the vegetal plate, a disc of cells composing the bottom wall of the embryo, now consists entirely of veg2 lineage cells, except for 8 small micromere descendants in the center (here invisible). Immediately abutting the veg2 cells is a ring of veg1 descendants also seen here in optical section from the side. (c), At late gastrula stage (60h), the tripartite archenteron, or embryonic gut, has formed. Gastrulation begins at 30h, with the

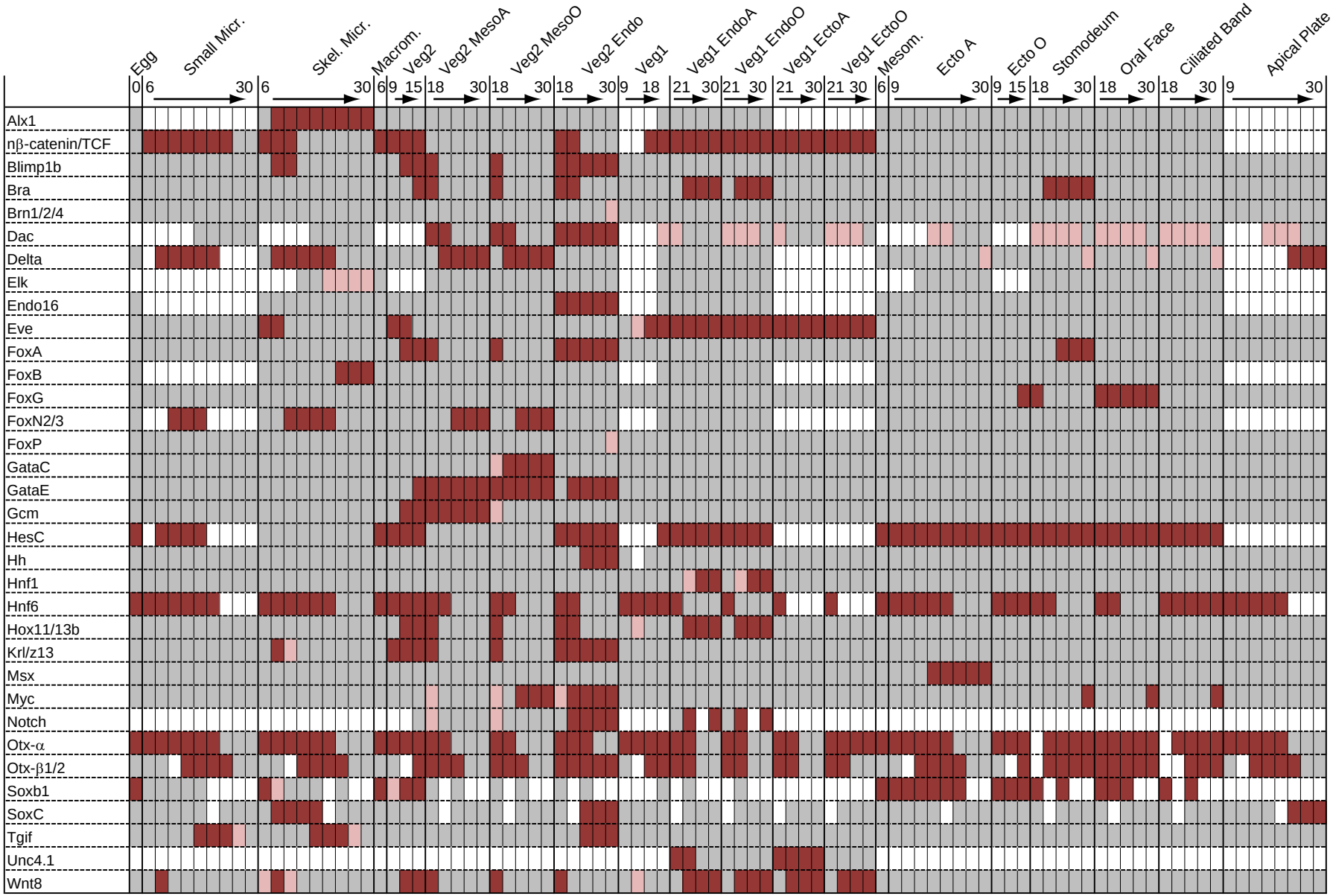
invagination of veg2 cells, and proceeds by a classic process of convergent extension. Veg2 descendants constitute the foregut endoderm and some veg2 mesodermal cells also migrate inward at the anterior end of the foregut. Veg1 descendants many hours later move inward to form the hindgut, and the midgut consists of veg2 descendants on the aboral side and veg1 descendants on the oral side. This asymmetry is due to the much larger amount of late veg1 invagination on the oral side. (d), Endomesodermal cell types deriving from the veg1 and veg2 lineages; same embryo as shown in (c): hindgut, green; posterior and peri-anal ectoderm, dark blue; midgut, pink; foregut, yellow; non-skeletogenic mesoderm cells, light blue. The skeletogenic, apical plate and oral and aboral ectoderm cells not of veg1 or veg2 origin are left uncolored, aboral ectoderm facing left, oral right. The mouth or stomodeum will form where the archenteron approaches the oral ectoderm. (e,g), Schematic displays of the disposition of veg1 and veg2 cell lineages superimposed on vegetal views of 18h (e) and 24h (g) embryos. Radially, from center, small micromeres, dark red; skeletogenic micromeres, pink; veg2 descendants, green; veg1 descendants, orange. (f,h), Cell fate maps from vegetal views; the embryo in (f) is the same 18h embryo as in (e), and that in (h) the same 24h embryo as in (g). In (f) and (h) the small micromeres are shown in dark red; skeletogenic micromeres in pink; veg2 progenitor cells of mesodermal cell types in cyan; veg2 progenitor cells of anterior endoderm, in yellow; veg1 progenitor cells of posterior endoderm in orange. Note that between 18 and 24h the skeletogenic cells have disappeared from the vegetal plate here portrayed, having by now ingressed as seen in (c). (i), Exact origin of the veg1 and veg2 lineages. The diagram is a lateral view of the 16-cell, 4^{th} cleavage embryo, the macromere grandparents of veg1 and veg2 outlined in red. The lineage diagram displays the macromere descendants throughout the remainder of cleavage. These form a ring of 8 cells at 5^{th} cleavage, and then the veg1 (orange) and veg2 (green) lineages are founded at 6^{th} cleavage as in (a). One of four identical macromere lineages is shown. (k), chart indicating cell types arising from the 4th cleavage mesomeres, macromeres, and micromeres (see key in (i)).



Supplementary Figure 2. Whole mount in situ hybridization (WMISH) of the endoderm genes *dac*, *myc*, *hnf1*, *tgif*, *krl*, and *foxa* at 24 and 27h. Double fluorescent WMISH shows that *dac* expression is coincident with *foxa* expression; i.e., *dac* is expressed in veg2 endoderm (side view left, vegetal view right). The *myc* gene is expressed in veg2 endoderm and part of veg2 mesoderm precursors, as seen in vegetal view in the double WMISH, and from the side in the single WMISH; also expressed in veg2 endoderm are *tgif*, lateral view, and *krl*, vegetal view, again as demonstrated by coincidence with the *foxa* expression domains by double WMISH. Expression of *krl* in a typical veg2 pattern is also seen in the single WMISH displays from lateral and vegetal views. In contrast, *hnf1* is expressed outside the veg2 domain marked by *foxa* expression, i.e., it is a veg1 endoderm gene. This is seen in the lateral and vegetal view double WMISH, left, and in the lateral and vegetal single WMISH, right.



Supplementary Figure 3. WMISH of the endoderm genes *blimp1b*, *hox11/13b*, *wnt8* and *foxa* at 21, 24, and 27 h. The progression of *blimp1b* expression is shown at 21 and 27h from lateral and vegetal views (left columns) by double WMISH with *foxa*, which serves as a marker for veg2 endoderm up to 30h. Note that at 27h the inner ring of *foxa* expressing cells has turned off *blimp1b* expression. *Blimp1b* is now restricted to peripheral veg2 descendants, mostly overlapping with *foxa* expression, but possibly asymmetrical with respect to the oral/aboral axis. This could presage the differential contribution of veg2 and veg1 across this axis in the future midgut (Supplementary Fig.1c). At lower left a double WMISH shows that by 24h *wnt8* is expressed exclusively in veg1 as there is no overlap with the *foxa* expression domain. Over the whole 21-27h period *hox/11/13b* is also expressed only in veg1 as seen in lateral and vegetal views by double WMISH with *foxa*.

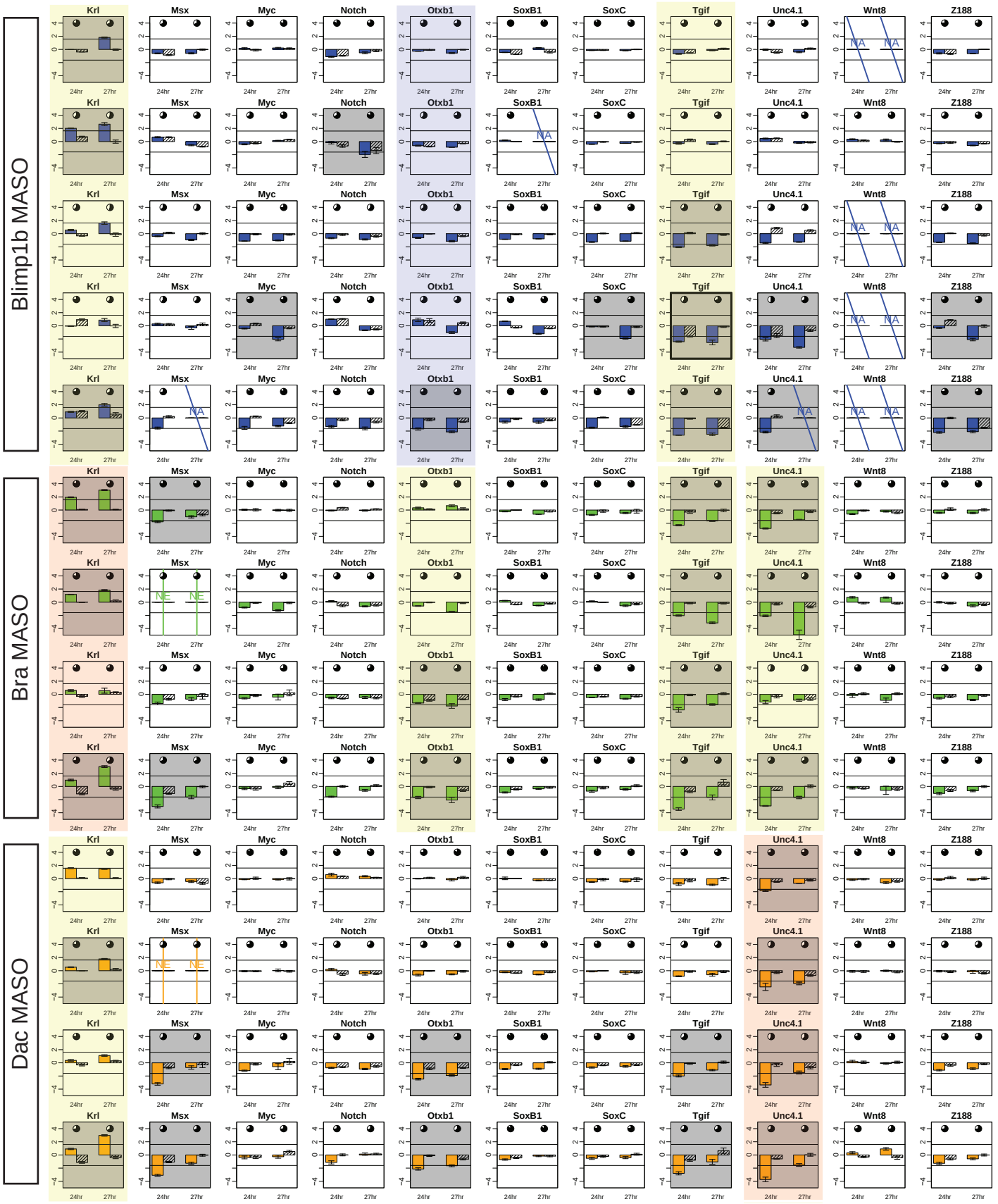

Egg
Small Micr.
Skel. Micr.
Macrom.
Vege2
Vege2 MesoA
Vege2 MesoO
Vege2 Endo
Vege1
Vege1 EndoA
Vege1 EndoO
Vege1 EctoA
Vege1 EctoO
Mesom.
Ecto A
Ecto O
Stomodeum
Oral Face
Ciliated Band
Apical Plate
0 6 30 6 30 6 9 15 18 30 18 30 18 30 9 18 21 30 21 30 21 30 21 30 6 9 30 9 15 18 30 18 30 18 30 9 30
Alx1
nβ-catenin/TCF
Blimp1b
Bra
Brn1/2/4
Dac
Delta
Elk
Endo16
Eve
FoxA
FoxB
FoxG
FoxN2/3
FoxP
GataC
GataE
Gcm
HesC
Hh
Hnf1
Hnf6
Hox11/13b
Krl/z13
Msx
Myc
Notch
Otx-α
Otx-β1/2
Soxb1
SoxC
Tgif
Unc4.1
Wnt8

Supplementary Figure 4. Digital spatial and temporal expression chart for all genes included in this study. Genes are listed alphabetically in left column. Each cell in the vertical columns of the chart represents a 3h interval, and the spatial domains of the embryo are indicated for the periods (h post fertilization) at which they obtain, across the top of the chart: Micr., micromeres; Skel., skeletogenic; MesoA., aboral non-skeletogenic mesoderm; MesoO., oral non-skeletogenic mesoderm; Endo., endoderm; EndoA., aboral endoderm; EndoO., oral endoderm; EctoA., aboral ectoderm; EctoO, oral ectoderm; Mesom., mesomeres.

Figure S5

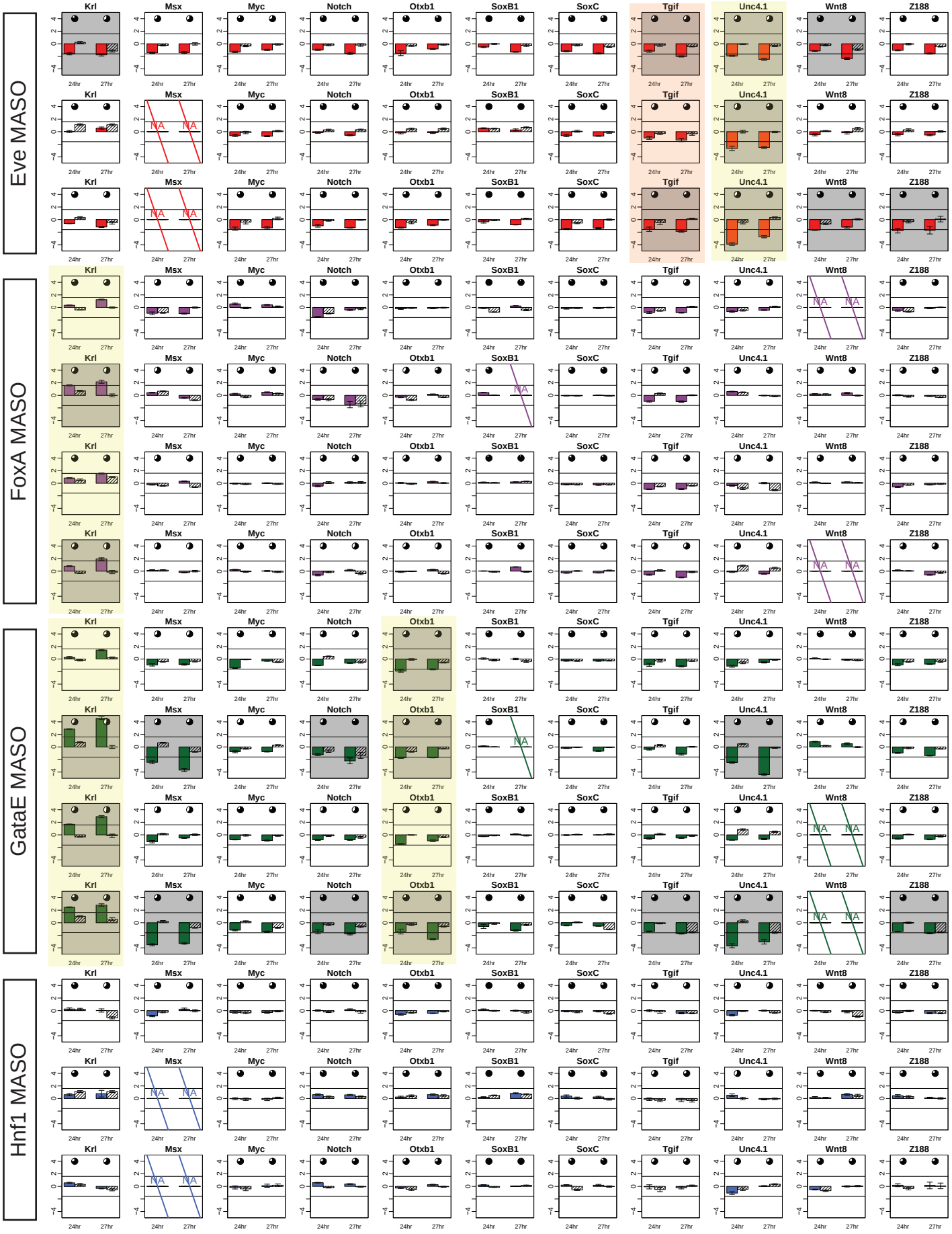

Blimp1b MASO
Bra MASO
Dac MASO
Alx1
Blimp1b
Bra
Brn1_2_4
Dac
Delta
Elk
Endo16
Eve
FoxA
FoxB
24hr
27hr
NA
NE


Blimp1b MASO
Bra MASO
Dac MASO
FoxG
FoxN2_3
FoxP
GataC
GataE
Gcm
HesC
Hh
Hnf1
Hnf6
Hox11_13b
24hr
27hr
NA
NE


Blimp1b MASO
Bra MASO
Dac MASO
Krl
Msx
Myc
Notch
Otxb1
SoxB1
SoxC
Tgif
Unc4.1
Wnt8
Z188
24hr
27hr
NA
NE

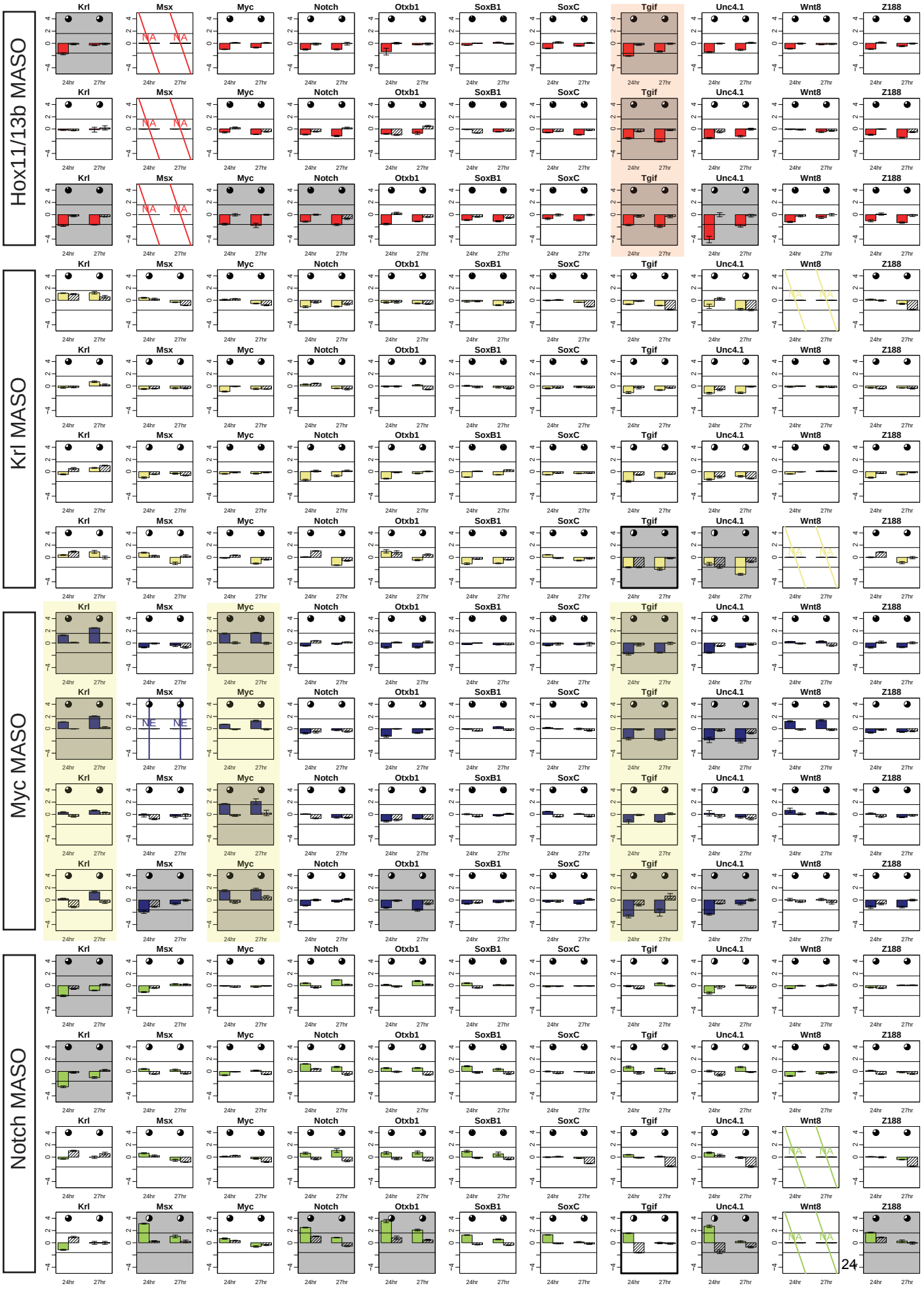

Eve MASO
FoxA MASO
GataE MASO
Hnf1 MASO
Alx1
Blimp1b
Bra
Brn1_2_4
Dac
Delta
Elk
Endo16
Eve
FoxA
FoxB
24hr
27hr
NE
NA


Eve MASO
FoxA MASO
GataE MASO
Hnf1 MASO
FoxG
FoxN2_3
FoxP
GataC
GataE
Gcm
HesC
Hh
Hnf1
Hnf6
Hox11_13b
24hr
27hr
NA
NE

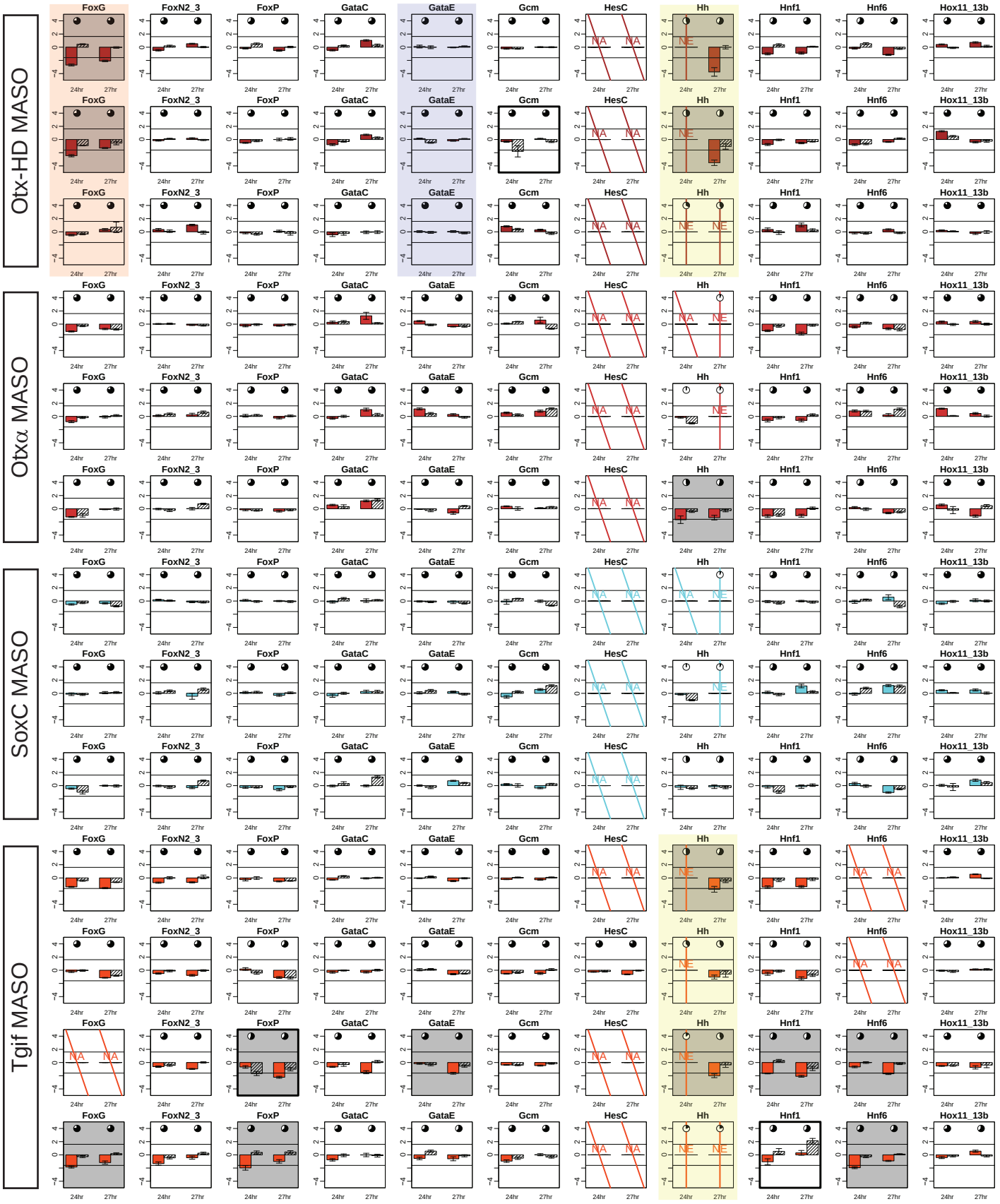

Eve MASO
FoxA MASO
GataE MASO
Hnf1 MASO
Krl
Msx
Myc
Notch
Otxb1
SoxB1
SoxC
Tgif
Unc4.1
Wnt8
Z188
24hr
27hr
NA

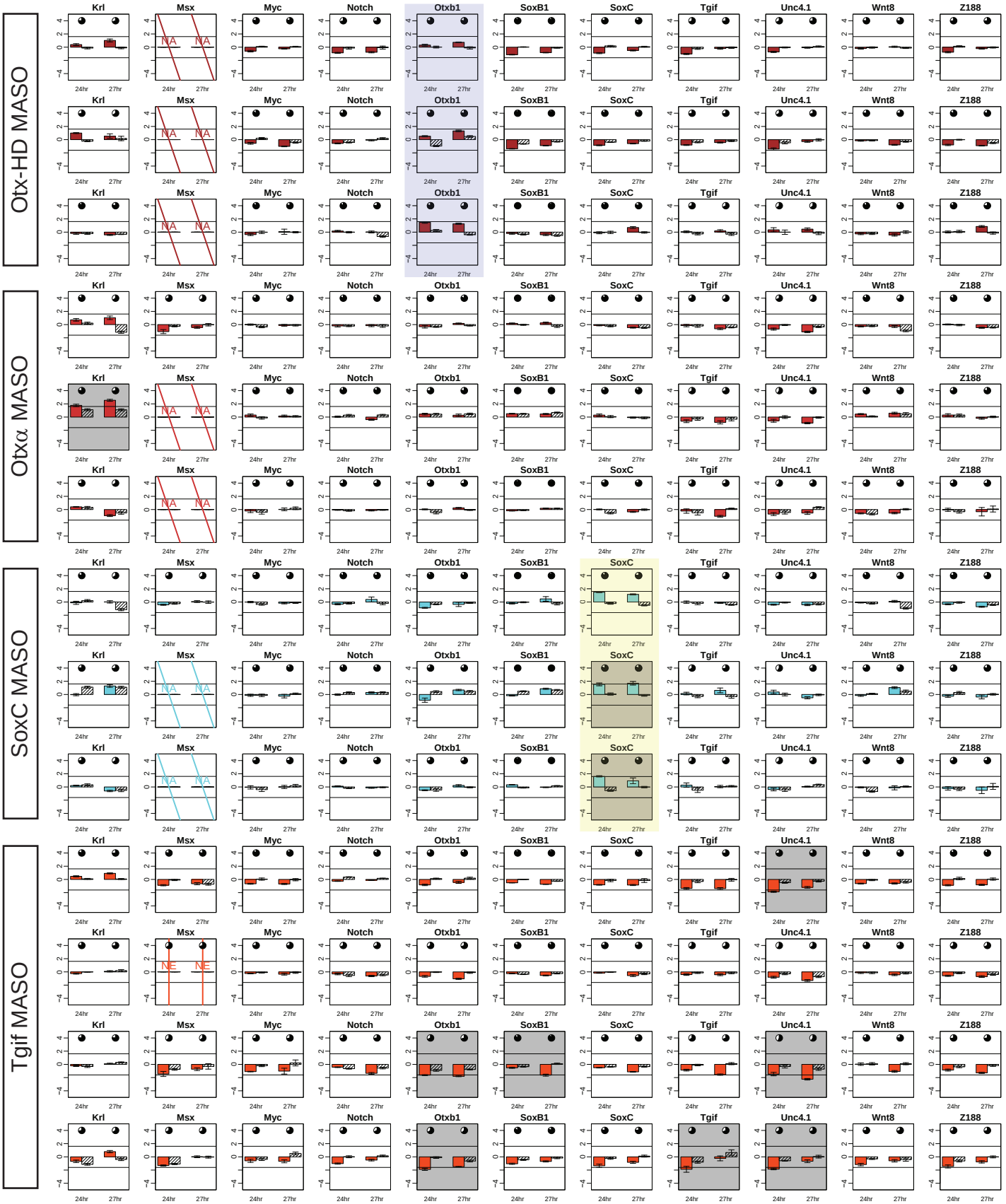

Hox11/13b MASO
Krl MASO
Myc MASO
Notch MASO
Alx1
Blimp1b
Bra
Brn1_2_4
Dac
Delta
Elk
Endo16
Eve
FoxA
FoxB
24hr
27hr
NE
NA


Hox11/13b MASO
Krl MASO
Myc MASO
Notch MASO
FoxG
FoxN2_3
FoxP
GataC
GataE
Gcm
HesC
Hh
Hnf1
Hnf6
Hox11_13b
24hr
27hr
NA
NE

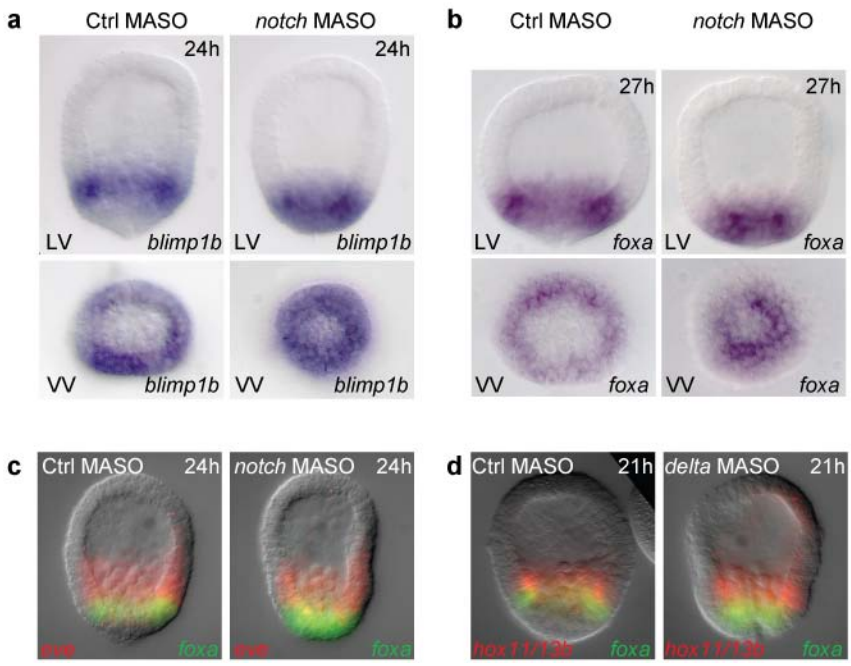

Hox11/13b MASO
Krl MASO
Myc MASO
Notch MASO
Krl
Msx
Myc
Notch
Otxb1
SoxB1
SoxC
Tgif
Unc4.1
Wnt8
Z188
24hr
27hr
NA
NE


Otx-HD MASO
Otxα MASO
SoxC MASO
Tgif MASO
Alx1
Blimp1b
Bra
Brn1_2_4
Dac
Delta
Elk
Endo16
Eve
FoxA
FoxB
24hr
27hr
NE
NA


Otx-HD MASO
Otxα MASO
SoxC MASO
Tgif MASO
FoxG
FoxN2_3
FoxP
GataC
GataE
Gcm
HesC
Hh
Hnf1
Hnf6
Hox11_13b
24hr
27hr
NA
NE


Otx-HD MASO
Otxα MASO
SoxC MASO
Tgif MASO
Krl
Msx
Myc
Notch
Otxb1
SoxB1
SoxC
Tgif
Unc4.1
Wnt8
Z188
NA
NE
24hr
27hr

Supplementary Figure 5. Complete gene expression perturbation data. Changes in gene expression levels were measured by QPCR and plotted as ddCT (in cycle number), the difference between normalized gene expression levels (compared to *ubiquitin*, see Methods) in embryos treated with morpholino (MASO) and levels in uninjected embryos, at 24 and 27h post fertilization. A similar data set for 18h embryos was reported earlier[14]. The data presentation in this computational platform is as in ref [12]. The gene specific MASO used for each series of repeats is given in the row headings at left. Each repeat was carried out using a separate batch of embryos, which were injected immediately after fertilization with gene-specific MASO or control MASO or left untreated and cultured for 24 or 27h. Within the individual data sets response of embryos to a control MASO is shown by gray rectangles and the response to the gene specific MASO by colored boxes. Colors used are changed for each gene specific MASO series for ease of visualization. Each data point reflects the average of three technical replicates. Error bars are standard errors. Grey plot backgrounds indicate ddCTs for embryos injected with gene specific MASO of >1.6 (increased expression levels compared to uninjected embryos) or < -1.6 (decreased expression levels). Thick plot border indicates ddCTs >1.6 or < -1.6 for embryos injected with control MASO. Each time point has a pie plot, which indicates the average CT in the control MASO injection. Full pie means average CT is 20, blank pie means average CT is 40 (i.e. not expressed). In general, average CTs of >30 (half blank pie) are not reliable. Changes in gene expression levels of >1.6 cycles (3-fold) were considered significant if expression levels of target genes decreased, indicating an activating input from the perturbed gene. If target gene expression levels increased upon perturbation, changes of >1 cycle (2-fold) were considered significant, since the underlying repression mechanism might occur in an embryonic domain different from the target gene expression domain and the resulting expansion of target gene expression might not exceed a 2- fold change in overall expression levels. Each perturbation was analyzed in at least three independent experiments, as shown. Genes showing significant changes of expression levels upon a given perturbation in the majority (i.e. >50%) of experiments were considered as potential target genes. Data points highlighted in yellow reflect potential direct interactions, and these are listed in Supplementary Table 1. Data points highlighted in orange show significant changes in expression levels but are not considered as direct interactions for the individual reasons given in Supplementary Table 2. Data points were highlighted in blue where genes were not significantly affected by the perturbation of a given gene, even though other evidence (e.g., experimental *cis*-regulatory evidence) indicates a direct regulatory interaction. Data points highlighted in green reflect the result of predicted signaling interactions. The relevant signaling interactions are listed in Supplementary Table 3.



Supplementary Figure 6. Repression of veg2 but not veg1 endodermal regulatory genes downstream of Delta/Notch signaling. The expression of *blimp1b* **(a)** and *foxa* **(b)** is detected in the veg2 endodermal precursor cells in embryos injected with control morpholino (Ctrl MASO), but expands inward to cells which usually give rise to mesodermal cell types in embryos injected with *notch* morpholino (MASO). On the other hand, the same perturbation does not affect veg1 endodermal regulatory gene expression, as shown in **(c)** and **(d)**: **(c)** *eve* expression is detected in cells peripheral to *foxa* expressing cells in control morpholino and in *notch* morpholino injected embryos. **(d)** expression of *hox11/13b* in control morpholino and *delta* morpholino injected embryos is detected outside of the *foxa* expression domain. In both, **(c)** and **(d)**, the perturbation affects *foxa* expression, which expands towards the vegetal pole.

a Number of GFP transcripts/embryo:

	7.5 hpf	9 hpf	10.5 hpf	12 hpf	15 hpf	19 hpf	24 hpf
Wnt8-GFP.BAC	15909	14487	11615	15486	17038	6218	8044
Wnt8-GFP.BACdelBlimp	6960	15008	21535	14491	18913	7856	6846

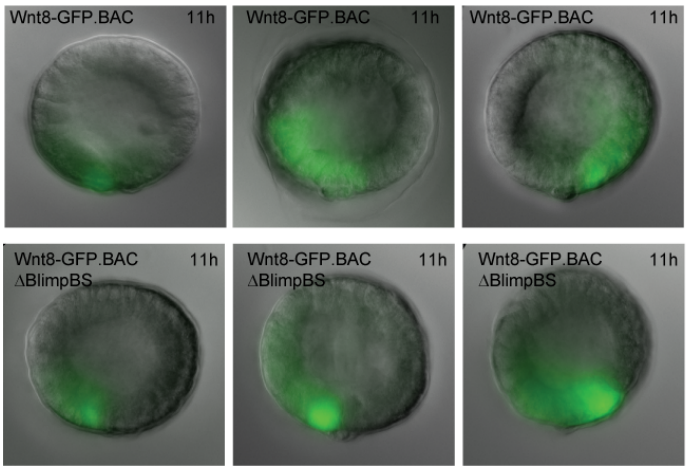
Number of BAC copies/embryo:

	7.5 hpf	9 hpf	10.5 hpf	12 hpf	15 hpf	19 hpf	24 hpf
Wnt8-GFP.BAC	24.44	15.07	13.73	15.47	14.90	8.81	7.83
Wnt8-GFP.BACdelBlimp	11.19	12.02	11.95	15.20	11.81	4.99	5.14

Number of GFP transcripts/BAC

	7.5 hpf	9 hpf	10.5 hpf	12 hpf	15 hpf	19 hpf	24 hpf
Wnt8-GFP.BAC	651	961	846	1001	1144	706	1027
Wnt8-GFP.BACdelBlimp	622	1248	1803	953	1601	1576	1333

b



Supplementary Figure 7. Expression of Wnt8:GFP-BAC does not require activation by Blimp1. A recent model from this laboratory proposed that the expression of *wnt8*, a crucial input into the endoderm GRN, requires transcriptional activation by Blimp1[5,9,15]. In turn, expression of *blimp1b* is activated by Tcf/β-catenin in cells receiving Wnt signaling and repressed by its own gene product Blimp1[5,10]. This circuitry was proposed to result in a moving torus expression of *blimp1b*, *wnt8* and presumably other Tcf dependent regulatory genes: gene expression would be activated in a ring of cells due to Tcf/β-catenin accumulation in cells receiving Wnt signaling and would be turned off due to the auto-repression of Blimp1, which would lead to absence of Blimp1-driven *wnt8* expression. The evidence supporting the requirement of Blimp1 for *wnt8* expression derived from *cis*-regulatory analyses of *wnt8*. The non-conserved Module C located 2.9 kb upstream of exon 1 required for its activity binding sites for both Tcf and Blimp1[9]. Here we tested whether the previously identified Blimp1 binding sites are required for expression of a recombinant Wnt8-GFP BAC (Sp_wnt8_BAC, 041A08, 78 kb; for sequence see http://www.spbase.org/SpBase/resources/bac_sequences.php). The Blimp1 binding site was deleted in Wnt8-GFP.BACdelBlimp (original sequence: TACAAAG*TTCCCTTTC*AC; after deletion: TACAAAGAC). Injection of Wnt8-

GFP.BACdelBlimp resulted in similar quantitative **(a)** and spatial **(b)** expression of GFP like the control Wnt8-GFP.BAC. These Blimp1 binding sites are therefore not required to activate *wnt8* expression.

>SP_Dachshund_partial_cDNA

ATGGGAAGCCCACCGCCTACTCAGGTTCCCCTGAGCAGTGTTCTGTACAAGCTGGATCGGGTAGCCTACA
GTACCCCACCGCCTGTCAGCAACGACCCCGCTAACAACGAGTGCAAACTCATCGAATATCGAGGGGCAAA
AATTGCCAGTTTCAACATTAATGGAGATTGTATGATATGCTTGCCACAAGCTTTTGAATTGTTTCTGAAA
CATCTTGTGGGCGGACTACACACGGTTTACACAAAACTGAAGAGACTGAGCATCACTCCGGTGGTTTGTA
ACGTAGAACAGGTTCGCATCCTGCGGGGACTTGGGGCGATCCAGCCGGGAGTCAACCGCTGTAAACTGCT
CACCAGGAAACAGTTTGATATTCTCTACGAAGATTGCACCACTGCTAGTCGACCAGGTAGACCTCCGAAA
CGAAACATTCCTCTCTCACTCACACCAACCATCCATGGACTCTCACGTATGGGACCCACCCTCGACCTCC
TCAAAAGGCCCAAACTGGAAAATGGTCACCACCGTCTGCCGTTCAATGGATACGAGCACTTGGCTGCAGC
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ACCGCATCACCCGCTAAGGCTGCCGAGATGCACCACCTTCACCATGCCGACCTGGGCGCCGCCCACCTTC
ACAACCTGCACAAGCTCTCCGTAGGCATCCCCAGATCGTCGAGCCAGCCCAGCTCTCACAGCCACGCATC
GGTCGACATCGACGACCACGAGGAAGACCT

GLEAN3_28061 bp 1-398

GLEAN3_18581 bp 43-384

GLEAN3_18581 bp 429-491

Supplementary Figure 8. Partial cDNA sequence of dachshund mapping to two different GLEAN3 gene models. Sequences for GLEAN models to be found on SpBase (http://www.spbase.org)