# Supporting Information

**Brown *et al*. 10.1073/pnas.0805589105**

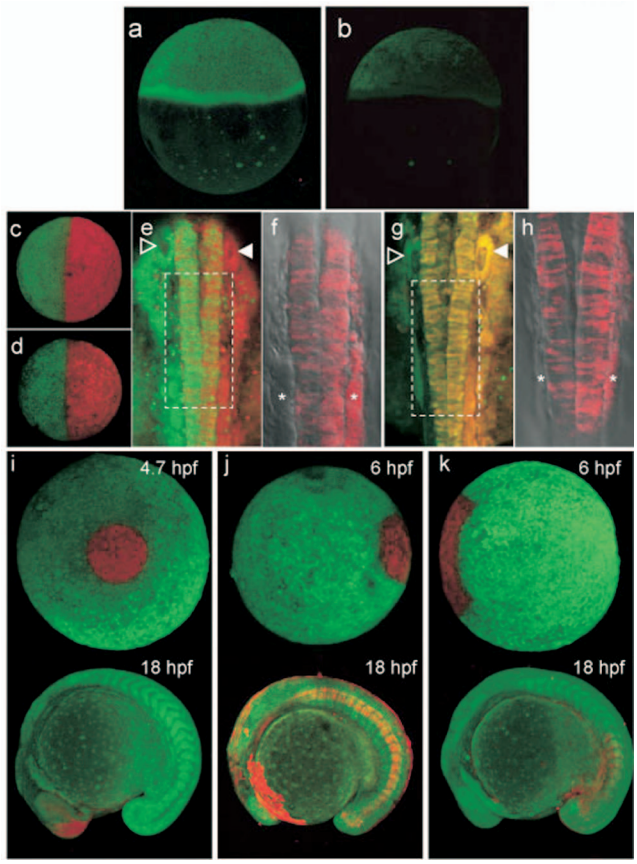


**Fig. S1.** Comparison of cell-lineage labeling by Kaede protein and *kaede* mRNA. (*A*) Late blastula-stage (40% epiboly) embryo that was injected with 3.5 ng of purified Kaede protein, lateral view. (*B*) Late-blastula-stage embryo that was injected with 300 pg of *kaede* mRNA, lateral view. (*C* and *D*) Kaede-protein-injected (*C*) and *kaede*-mRNA-injected (*D*) embryos immediately after photolabeling of right halves at shield stage (6 hpf), animal pole views, dorsal to the top. (*E* and *G*) Same Kaede-protein-injected (*E*) and *kaede*-mRNA-injected (*G*) embryos as in *C* and *D*, but now at the pharyngula stage (24 hpf). Dorsal views of trunk and posterior head are shown and the left (open arrowhead) and right (white arrowhead) ears are indicated. (*F* and *H*) Alternate and magnified views of the spinal cord regions marked by the dashed rectangles in *E* and *G*, highlighting the mixture of left and right cell lineages in the spinal cord, but not in the flanking paraxial mesoderm (asterisks), as previously documented (1). (*I–K*) Sample lineage tracing experiments with Kaede protein; two time points are shown for each embryo. (*I*) Labeled animal pole cells at the late blastula stage (4.7 hpf) contribute to the eye and forebrain in the anterior head region at 18 hpf. (*J*) Labeled dorsal gastrula organizer cells from the shield stage (6 hpf) contribute to the notochord and the floor plate in the trunk, the hatching gland in the anterior, and the fin mesenchyme and periderm along the surface. (*K*) Labeled ventral cells from the shield stage (6 hpf) contribute to somites, tail mesenchyme, and blood progenitors in the posterior. *A–E*, *G*, and *I–K* are overlays of red and green channels from multiple optical sections; *F* and *H* are overlays of red and DIC channels from single optical sections.

1. Kimmel CB, Warga RM, Kane DA (1994) Cell cycles and clonal strings during formation of the zebrafish central nervous system. *Development* 120:265–276.

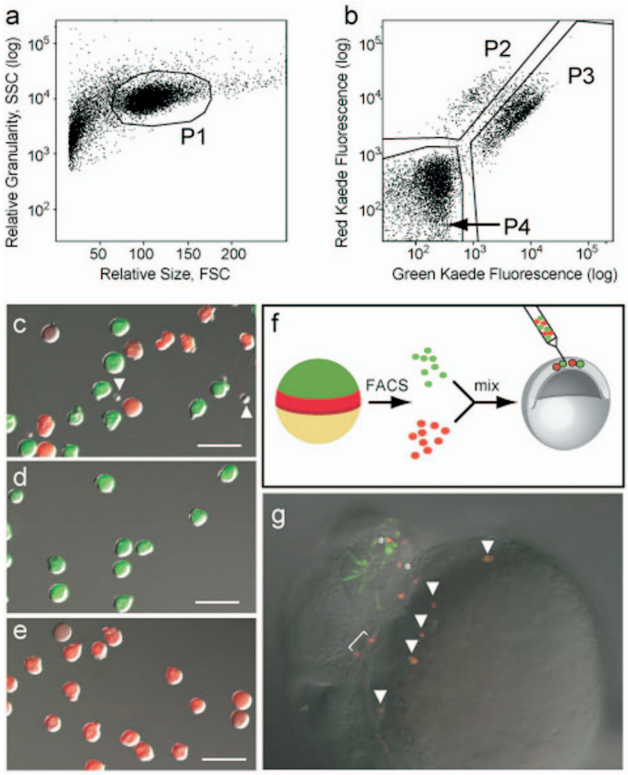


**Fig. S2.** Isolation and behavior of photolabeled cells. (*A* and *B*) Flow cytometry data from a mesendoderm and ectoderm precursor separation experiment. Nine Kaede-protein-injected embryos were photolabeled along the bottom (vegetal-most) six to eight cellular tiers, mixed with 21 uninjected (no Kaede) embryos, disaggregated, and sorted. (*A*) Plot showing relative cell size (forward scatter) vs. granularity (side scatter), which distinguishes embryonic cells from yolk and debris. The P1 gate corresponding to single embryonic cells is indicated. (*B*) Plot showing red Kaede vs. green Kaede fluorescence intensity of cells falling within the P1 gate in *A*. Gates P2 and P3 represent the sort criteria for red and green cells. Gate P4 represents nonfluorescent cells that were not collected. (*C*–*E*) Fluorescence microscopy images of red and green Kaede-labeled cells before and after sorting using the gates shown in panels *A* and *B*. (*C*) Presort image of cells from pooled and dissociated embryos. Half of these embryos had been injected with Kaede protein and photolabeled, and half had been injected with Kaede protein but not photolabeled. White arrowheads indicate yolk globules. (*D*) Image of post-sort green Kaede cells from *C*. (*E*) Image of post-sort red Kaede cells from *C*. (*F* and *G*) Germ layer precursors purified by FAM-P were repooled and 8–30 red mesendoderm precursors and green ectoderm precursors were cotransplanted to the animal poles of late blastula-stage (5 hpf) embryos, as schematized in *F*. (*G*) Sample outcome in a pharyngula-stage embryo (≈24 hpf). Almost all ectoderm-derived green cells and a few mesendoderm-derived red cells (asterisks) have contributed to the eye, normally an ectodermal derivative. Other mesendoderm-derived red cells have differentiated to cell types that are normally mesendoderm derived, namely pharyngeal endoderm (bracket) and blood (arrowheads). Results from this and other transplants are quantified in Table S1. Methods: Traditional embryo-to-embryo transplants were done as described (2). Donors for these transplants were injected with biotin-dextran ($M_r$ = 10,000, Molecular Probes, 3% solution) at the one to 2 cell stage. Cells were transplanted into host embryos at the late-blastula stage (5 hpf) and incubated overnight at 28°C. The transplant/host combinations were fixed in 4% paraformaldehyde at the pharyngula stage (24 hpf) and stained by using the Vectastain Elite ABC reagent and DAB Substrate Kit for Peroxidase (Vector Laboratories). For group cell transplants after FAM-P, sorted cell populations (donor cells) were concentrated by centrifugation and placed on a sterile plastic Petri dish lid on ice, where they were drawn as needed for single or group cell transplants. Donor embryos for group cell transplants were not injected with biotin-dextran and donor/host combinations were instead imaged *in vivo* at various time points.

2. Ho RK, Kimmel CB (1993) Commitment of cell fate in the early zebrafish embryo. *Science* 261:109–111.

**Table S1. Summary of transplantation assays and fate outcomes**

| | Epidermis | Eye | Forebrain | Midbrain | Neural crest | Blood | Endoderm | Muscle | EVL | Unclassified |
|---|---|---|---|---|---|---|---|---|---|---|
| Post FAM-P (Mes + Ect) transplants | | | | | | | | | | |
| Mes (red cell fates) N=8 | — | 3 | 4 | — | — | 6 | 6 | 1 | 1 | 1 |
| Ect (green cell fates) N=8 | — | 4 | 7 | — | — | — | — | — | 2 | 1 |
| Traditional single-cell transplants | | | | | | | | | | |
| Mes N=53 | 7 | 23 | 21 | — | 1 | 13 | 12 | — | 9 | 10 |
| Ect N=38 | 4 | 18 | 22 | — | — | — | — | — | 3 | 4 |

Two types of transplants were performed: (*i*) mixed (mesendoderm + ectoderm precursors) transplants of FAM-P-purified cells and (*ii*) single-cell transplants of mesendoderm (Mes) or ectoderm (Ect) precursors conventionally isolated by micropipette explantation. The number of observations of tissue-specific clones arising from mixed transplants of FAM-P-sorted cells and scored the next day are shown in the first two rows, with the outcomes of red and green cells scored separately. For these transplants, isolated red mesendoderm and green ectoderm precursors were pooled, and 8–30 cells were injected to the animal poles of host embryos. The day 2 outcomes from single-cell embryo-to-embryo transplants are shown in the bottom two rows. The mesendoderm precursor cells in these latter transplants were directly extracted from donor margins while the ectoderm precursor cells were directly removed from donor animal poles, and both were transplanted to host animal poles. Observed clones were assigned to one of 10 categories, with ectodermal outcomes shown in the first five columns, mesoderm and endoderm outcomes in the next three columns, extraembryonic outcomes in the next column, and outcomes we were unable to classify in the final column.

**Table S2. Identities and characteristics of the top 60 genes enriched among mesendoderm precursors, ectoderm precursors, and control cells (photoconverted vs. nonphotoconverted cells)**

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| **Mesendoderm precursors** | | | | |
| acsf2 | E | | 0.04 | 5.4 |
| aldh1a2 | E | RA (1) | 0.047 | 7.1 |
| bon | TF | N (2) | 0.032 | 7.1 |
| copeb | TF | | 0.048 | 4 |
| dkk1 | LAR | N (2), W (5) | 0.048 | 6.4 |
| efnb2b | LAR | N (2) | 0.04 | 17.5 |
| fgf24 | LAR | F (6) | 0.029 | 5 |
| fgf3 | LAR | N (2), F (7) | 0.03 | 10.2 |
| fgf8 | LAR | N (2), F (8) | 0.011 | 6.1 |
| foxa2 | TF | N (2) | 0.031 | 6.7 |
| foxa3 | TF | N (2), W (10) | 0.036 | 7.3 |
| foxc1a | TF | | 0.03 | 6 |
| frzb | LAR | W (12) | 0.048 | 5.7 |
| gata5 | TF | N (2) | 0.03 | 10 |
| gata6 | TF | | 0.039 | 8.6 |
| irx3a | TF | N (2) | 0.03 | 8.7 |
| ism1 | | | 0.036 | 7.3 |
| lft1 | LAR | N (2) | 0.032 | 7.1 |
| lhx1a | TF | N (2) | 0.04 | 17.5 |
| lhx1b | TF | | 0.031 | 4 |
| lmo1 | TF | | 0.035 | 21.6 |
| mespa | TF | RA (15) | 0.035 | 7.4 |
| mespb | TF | RA (15) | 0.03 | 5.9 |
| msgn1 | TF | | 0.036 | 7.2 |
| mta3 | TF | | 0.033 | 7.5 |
| myf5 | TF | | 0.04 | 8.1 |
| ndr1 | LAR | | 0.036 | 5.3 |
| ndr2 | LAR | | 0.032 | 5.3 |
| nr2f1 | TF | | 0.032 | 7.8 |
| og9x | TF | N (2) | 0.044 | 6.4 |
| osr1 | | | 0.048 | 8.5 |
| pcdh8 | | | 0.048 | 11.8 |
| pitx2a | TF | N (2) | 0.03 | 8.3 |
| pxk | E | | 0.049 | 5.2 |
| rag2 | | | 0.029 | 4.2 |
| scube2 | LAR | | 0.04 | 7.3 |
| shhb | LAR | | 0.035 | 5.2 |
| si:busm1–6a2.1 | LAR | | 0.031 | 4.2 |
| si:ch211–241e15.2 | | | 0.035 | 5.5 |
| six3b | TF | N (2), W (20) | 0.048 | 4.7 |
| six4.1 | TF | | 0.048 | 8.5 |
| sox21b | TF | | 0.039 | 10.7 |
| sox32 | TF | N (2) | 0.032 | 19.3 |
| sp5 | TF | W (22) | 0.03 | 8.8 |
| tagln2 | | | 0.043 | 4.3 |
| tbr1 | TF | | 0.04 | 11.5 |
| tph1b | E | N (2) | 0.03 | 6.7 |
| trim24 | TF | | 0.036 | 5.5 |
| wnt5b | LAR | W (23) | 0.029 | 4.4 |
| wu:fb71h11 | | | 0.031 | 5.4 |
| zgc:110248 | | | 0.04 | 21.8 |
| zgc:110712 | | | 0.044 | 5.4 |
| zgc:136605 | | | 0.049 | 5.7 |
| zgc:153377 | | | 0.03 | 6 |
| zgc:158459 | | | 0.035 | 4.6 |
| zgc:56201 | | | 0.03 | 8.1 |
| zgc:85811 | E | | 0.03 | 6 |
| zgc:86722 | E | | 0.029 | 4.5 |
| zgc:91960 | | | 0.046 | 4.2 |
| zic2a | TF | | 0.03 | 7.7 |
| **Ectoderm precursors** | | | | |

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| aldob | E | | 0.053 | 2.44 |
| atic | E | | 0.055 | 2.26 |
| ccna1 | | | 0.053 | 2.4 |
| cd9 | | | 0.053 | 4.04 |
| clasp2 | | | 0.053 | 2.45 |
| cobra1 | TF | | 0.053 | 2.28 |
| cs | E | | 0.053 | 2.61 |
| dbx1b | TF | | 0.053 | 2.61 |
| dio2 | E | | 0.053 | 2.74 |
| dullard | E | | 0.055 | 2.35 |
| eif4a1b | E | | 0.055 | 2.37 |
| f2rl1 | | | 0.053 | 2.29 |
| fbxl18 | | | 0.053 | 2.63 |
| foxh1 | TF | N (31) | 0.041 | 2.82 |
| foxi1 | TF | F (13) | 0.055 | 2.31 |
| gc3 | E | | 0.054 | 2.69 |
| gtpbp1l | E | | 0.052 | 2.91 |
| her4.2 | TF | | 0.055 | 3.23 |
| hoxd10a | TF | | 0.054 | 2.54 |
| hoxd13a | TF | | 0.044 | 2.87 |
| id1 | TF | | 0.055 | 2.92 |
| ilk | E | | 0.053 | 2.45 |
| itm1 | E | | 0.052 | 2.49 |
| ivns1abpb | | | 0.053 | 2.34 |
| lhx5 | TF | W (16) | 0.053 | 6.95 |
| mastl | E | | 0.055 | 2.32 |
| mcm2 | E | | 0.054 | 2.44 |
| mcm4 | E | | 0.052 | 2.51 |
| msxe | TF | | 0.053 | 2.86 |
| mtnr1a | LAR | | 0.053 | 2.78 |
| ndrg1 | | | 0.055 | 2.63 |
| ndrg3b | | | 0.054 | 2.3 |
| otx2 | TF | W (17, 18), F (17), RA (17) | 0.052 | 2.86 |
| piwil2 | | | 0.053 | 2.46 |
| pkm2 | E | | 0.048 | 3.76 |
| pole2 | E | | 0.054 | 2.51 |
| ppp2r1b | E | | 0.054 | 2.38 |
| prdm5 | TF | | 0.052 | 2.87 |
| racgap1 | | | 0.052 | 2.29 |
| rasl11b | E | | 0.053 | 2.5 |
| si:ch211–105d11.2 | | | 0.053 | 2.62 |
| si:ch211–238n5.5 | E | | 0.053 | 2.65 |
| slc25a12 | | | 0.053 | 2.44 |
| snai2 | | | 0.049 | 5.06 |
| sox19a | TF | | 0.053 | 4.75 |
| sox19b | TF | | 0.053 | 2.88 |
| sox3 | TF | | 0.049 | 7.23 |
| tfdp1l | TF | | 0.055 | 2.52 |
| th1l | TF | | 0.053 | 2.27 |
| tnpo3 | | | 0.055 | 2.27 |
| tsc22d3 | TF | | 0.052 | 2.34 |
| txnip | | | 0.053 | 2.36 |
| wnt4a | LAR | W (25) | 0.052 | 2.74 |
| wu:fj19d05 | | | 0.053 | 2.73 |
| yy1l | TF | | 0.036 | 2.92 |
| zgc:163117 | E | | 0.055 | 2.31 |
| zgc:171444 | | | 0.053 | 2.27 |
| zgc:85653 | | | 0.055 | 2.26 |
| zgc:86896 | | | 0.053 | 2.84 |
| zgc:92139 | E | | 0.055 | 2.36 |
| **Control** | | | | |
| amotl2 | | | 0.15 | 1.31 |
| arrdc2 | | | 0.16 | 1.25 |
| axin1 | | W (3) | 0.15 | 1.29 |

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| baiap2l1a | E | | 0.16 | 1.28 |
| cul3 | | | 0.15 | 1.27 |
| dedd1 | | | 0.15 | 1.53 |
| dusp7 | E | | 0.16 | 1.32 |
| dyrk2 | E | | 0.15 | 1.49 |
| ell | E | | 0.16 | 1.26 |
| foxp2 | TF | | 0.15 | 1.29 |
| ggps1 | E | | 0.15 | 1.36 |
| has2 | E | | 0.15 | 1.34 |
| hspa9 | E | | 0.15 | 1.25 |
| lgals3bp | | | 0.16 | 1.34 |
| mapk14b | E | | 0.16 | 1.35 |
| mark3 | E | | 0.16 | 1.23 |
| mpp7 | | | 0.15 | 1.33 |
| mtf1 | TF | | 0.16 | 1.56 |
| nr3c1 | TF | | 0.16 | 1.44 |
| pcf11 | | | 0.16 | 1.54 |
| pdap1 | | | 0.16 | 1.31 |
| per2 | | | 0.15 | 1.87 |
| ppan | | | 0.12 | 1.34 |
| prpf3 | | | 0.15 | 1.3 |
| rnuxa | | | 0.15 | 1.38 |
| robo3 | LAR | | 0.15 | 1.33 |
| sb:cb429 | | | 0.15 | 1.62 |
| si:ch211–266o5.1 | | | 0.15 | 1.29 |
| si:dkey-220f10.6 | | | 0.15 | 1.52 |
| smad1 | TF | | 0.15 | 1.27 |
| smad6 | TF | | 0.15 | 1.29 |
| supt6h | E | | 0.16 | 1.33 |
| tacc3 | | | 0.15 | 1.24 |
| top1l | E | | 0.15 | 1.24 |
| tpp1 | E | | 0.15 | 1.27 |
| wdhd1 | | | 0.15 | 1.25 |
| wu:fb39g08 | | | 0.16 | 1.54 |
| wu:fb39h11 | | | 0.16 | 1.48 |
| wu:fb51f10 | | | 0.16 | 1.43 |
| wu:fb61e06 | | | 0.16 | 1.36 |
| wu:fb68h05 | | | 0.15 | 1.26 |
| wu:fb74e01 | | | 0.15 | 1.39 |
| wu:fb81c07 | | | 0.15 | 1.81 |
| wu:fb92b11 | | | 0.16 | 2.55 |
| wu:fc32g11 | | | 0.16 | 1.27 |
| wu:fc37g06 | | | 0.16 | 1.29 |
| wu:fc50e08 | | | 0.16 | 1.26 |
| zgc:111944 | | | 0.15 | 1.28 |
| zgc:113220 | | | 0.15 | 1.42 |
| zgc:123046 | | | 0.16 | 1.33 |
| zgc:153456 | | | 0.14 | 1.43 |
| zgc:153646 | | | 0.12 | 1.36 |
| zgc:153708 | | | 0.15 | 1.41 |
| zgc:158450 | | | 0.15 | 1.24 |
| zgc:162648 | | | 0.15 | 1.4 |
| zgc:55664 | | | 0.15 | 1.39 |
| zgc:63523 | | | 0.16 | 1.29 |
| zgc:77817 | | | 0.16 | 1.24 |
| zgc:92542 | | | 0.15 | 1.5 |
| znf143 | TF | | 0.16 | 1.25 |
| **Link *et al*. 2006** | | | | |
| actcl | | | | |
| agtr1 | | | | |
| btg2 | | | | |
| calr | | N (4) | | |
| cDNA clone IMAGE:6898419, partial cds* | | | | |
| chd | | N (2), F (9) | | |

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| cldng | | | | |
| copa | | | | |
| cpn1 | | | | |
| csnk1d | | | | |
| cstlb | | N (2) | | |
| dact2 | | N (2), W (11) | | |
| dmd | | | | |
| ek3 | | | | |
| flh | | N (2) | | |
| foxa | | | | |
| foxa2 | | N (2) | | |
| foxa3 | | N (2), W (10) | | |
| foxc1a | | | | |
| fzd8a | | N (2) | | |
| fzd8b | | N (2),W (14) | | |
| gata5 | | N (2) | | |
| gpm6ab | | | | |
| gsc | | N (2), W (10) | | |
| hdlbp | | | | |
| hspa5 | | | | |
| hyou1 | | | | |
| id3 | | N (2) | | |
| insig1 | | | | |
| mbtps1 | | | | |
| mknk2 | | | | |
| msf | | | | |
| msgn1 | | | | |
| mylip | | W (19) | | |
| nog1 | | | | |
| nrp2b | | | | |
| ntd5 | | | | |
| otx1 | | N (2) | | |
| p4ha2 | | | | |
| pcdh10b | | | | |
| pitx2a | | N (2) | | |
| plod | | | | |
| ptpn12 | | | | |
| rpn1 | | | | |
| rrbp1 | | | | |
| sb:cb246 (epha4a) | | | | |
| sb:cb560* | | | | |
| sb:cb620 (si:ch211–284e13.2) | | | | |
| sec22l1b | | | | |
| sec23b | | | | |
| sec61b | | | | |
| shh | | N (26) | | |
| Si:dkey-239d21.1* | | | | |
| six3b | | N (2) | | |
| smox | | | | |
| sox32 | | N (2) | | |
| ssr2 | | | | |
| tbx1 | | N (2), RA (27) | | |
| tbx16 | | N (2), F (28) | | |
| tcf7l1a | | W (29) | | |
| tnfsf10l | | | | |
| tph1l | | N (2) | | |
| tra1 | | | | |
| tram | | | | |
| Transcribed locus* | | | | |
| Transcribed locus* | | | | |
| Transcribed locus* | | | | |
| Transcribed locus* | | | | |
| Transcribed locus, moderately similar to XP_216035.2* | | | | |
| ugdh | | | | |

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| wu:fa99h02 (zgc:92222) | | | | |
| wu:fb74c11* | | | | |
| wu:fb74c11* | | | | |
| wu:fb99h12* | | | | |
| wu:fc37b12* | | | | |
| wu:fd44f11* | | | | |
| wu:fj66a01* | | | | |
| wu:fj79f01 (zgc:136639) | | | | |
| xbp1 | | N (2) | | |
| za20d2 | | | | |
| zgc:55257 (cited3) | | | | |
| zgc:56419* | | | | |
| zgc:64098* | | | | |
| zgc:73265* | | | | |
| zgc:76908 (gapdhs) | | | | |
| zgc:77254 (rbm35b) | | | | |
| zgc:77327 (aga) | | | | |
| zgc:77731 (dnajc3) | | | | |
| zgc:77773 (pdia4) | | | | |
| zgc:85752* | | | | |
| zgc:86863 (cdkrap3) | | | | |
| zgc:86940 (ssr4) | | | | |
| zgc:92579 (arfip2a) | | | | |
| zgc:92668* | | | | |
| **Dickmeis *et al.* 2001** | | | | |
| 26s protease regulatory subunit 7* | | | | |
| 26s proteasome regulatory chain 12* | | | | |
| admp | | N (2) | | |
| beta-1 integrin* | | | | |
| bhik | | N (2) | | |
| cathepsin 1 related* | | | | |
| cathepsin 1* | | | | |
| cbs | | | | |
| cdh10 | | | | |
| cdh18 | | | | |
| chd | | N (2) | | |
| chn1 | | | | |
| ckb | | | | |
| d-3 phosphoglycerate dehydrogenase* | | | | |
| ddx54 | | | | |
| dhm1-like protein* | | | | |
| dipeptidyl-peptidase i* | | | | |
| eef1g | | | | |
| eif-3 eta | | | | |
| ell2 | | | | |
| enc1 | | | | |
| ephx1 | | | | |
| foxa2 | | N (2) | | |
| foxa3 | | N (2), W (10) | | |
| frizzled E3* | | | | |
| frzb | | W (10) | | |
| fscn1 | | | | |
| fzd8b | | N (2), W (14) | | |
| gata5 | | N (2) | | |
| gata6 | | | | |
| glutamine synthetase* | | | | |
| hdac1 | | W (21) | | |
| Heterogenous nuclear ribonucleoprotein h* | | | | |
| hnrnpu | | | | |
| id3 | | N (2) | | |
| intermediate filament protein on3* | | | | |
| itga5 | | | | |
| keratin* | | | | |
| khdrbs1 | | | | |

| Gene symbol | Cat. | Path | P value | Avg enrich. |
|---|---|---|---|---|
| klf4 | | | | |
| kpnb3 | | | | |
| kruppel-type zinc finger* | | | | |
| lft2 | | N (2) | | |
| malate dehydrogenase* | | | | |
| marcks | | | | |
| Max-like bHLH-Zip protein* | | | | |
| oep | | N (24) | | |
| otx1l | | N (2) | | |
| pdip5 | | N (2) | | |
| plasma membrane calcium-transporting ATPase, brain isoform* | | | | |
| pou5f1 | | | | |
| protein-tyrosine phosphatase (bm-008)* | | | | |
| prp8 protein* | | | | |
| ptb-associated splicing factor* | | | | |
| ranbp3-a* | | | | |
| retinal short-chain dehydrogenase/ reductase* | | | | |
| sec61a | | | | |
| sf3a3 | | | | |
| slc25a3 | | | | |
| sox32 | | N (2) | | |
| sp5 | | W (30) | | |
| srp72 | | | | |
| ssecks* | | | | |
| ssr2 | | | | |
| tbx16 | | N (2), F (28) | | |
| tfa | | | | |
| TFIIF-alpha* | | | | |
| tnpo2 | | | | |
| tra2a | | | | |
| translation initiation factor 4 gamma 2* | | | | |
| tristetraproline* | | | | |
| type II basic cytokeratin* | | | | |
| type-I cytokeratin (50% identity)* | | | | |
| ul snrnp 70kDa* | | | | |
| zgc:153632 | | | | |
| zp2 | | | | |

For each of these cohorts, the following information is given: gene symbol, functional category (cat.), pathway (path.), average enrichment (avg enrich), and the Benjamini-Hochberg corrected *P* value (representing the statistically predicted false positive rate). The cohorts were determined as follows: the standard 5% cutoff (Benjamini-Hochberg corrected $P < 0.05$) was applied to the mesendoderm-enriched cohort, but less stringent filters (5.5% and 16%, respectively) were applied to ectoderm-enriched and red control cell-enriched cohorts, so as to obtain sufficient numbers of relevant genes for parallel analyses. Duplicates and insufficiently annotated genes (i.e.,''LOC'' IDs and no RefSeq name) were also removed. Only selected molecular categories and pathways are shown. Molecular categories: E, enzyme; LAR, ligand (agonist), antagonistic ligand, or receptor; TF, transcription factor. Pathways: N, Nodal pathway; W, Wnt pathway; F, FGF pathway; RA, retinoic acid pathway. Pathway assignment was based on the referenced published data showing strong genetic or molecular links of a particular gene to a particular pathway. The same curation process for pathway assignment was also applied to data sets from Link *et al*. (33) and Dickmeis *et al*. (34), which we have relisted, with updated nomenclature where available.

*Definitive gene identity could not be determined.

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**Table S3. Numbers of genes enriched in the germ layer precursor populations (category) for various false positive rate cutoffs**

| category | False positive rate | number of oligo spots (number of unique genes) | number of hits from curated Nodal-regulated gene list* | estimate of additional Nodal-regulated genes |
|---|---|---|---|---|
| mesendoderm | <5% (Pcorr < 0.05%) | 240 (188) | 26/62 (41.9%) | 55 |
| mesendoderm | <5.3% (Pcorr < 0.053%) | 419 (325) | 28/62 (45.1%) | NA |
| mesendoderm | <5.5% (Pcorr < 0.055%) | 1075 (843) | 40/62 (64.5%) | NA |
| neurectoderm | <5% (Pcorr < 0.05%) | 114 (106) | 1/62 (1.6%) | |
| neurectoderm | <5.3% (Pcorr < 0.053%) | 388 (328) | 1/62 (1.6%) | |
| neurectoderm | <5.5% (Pcorr < 0.055%) | 1890 (1371) | 3/62 (4.8%) | |

See *Materials and Methods* for details. The number of unique genes was obtained by subtracting duplicates, ESTs, and predicted genes from the clone list. Ten of the 72 curated Nodal-regulated genes from Bennett *et al.* (1) are not represented on our oligo microarray, so the percentages shown in the last column are calculated from the 62 genes that could be compared. Twenty-two curated Nodal-regulated genes were not detected for the following reasons: 14 were mesendoderm-enriched but eliminated because of high *P* values; three (see table) were enriched in the neurectoderm, and five were not enriched in either population. The estimate of additional Nodal-regulated genes in our data set was calculated as follows: [188 (number of unique genes) − 26 (number of hits on curated Nodal-regulated list)] x 10/21 (our rate of validated margin expression ) x 22/31 [frequency of margin-enriched genes being Nodal-regulated (1)].

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