Multicilin promotes centriole assembly and ciliogenesis during multiciliate cell differentiation

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Multiciliate cells function prominently in the respiratory system, brain ependyma and female reproductive tract to produce vigorous fluid flow along epithelial surfaces. These specialized cells form during development when epithelial progenitors undergo an unusual form of ciliogenesis, in which they assemble and project hundreds of motile cilia. Notch inhibits multiciliate cell formation in diverse epithelia, but how progenitors overcome lateral inhibition and initiate multiciliate cell differentiation is unknown. Here we identify a coiled-coil protein, termed multicilin, which is regulated by Notch and highly expressed in developing epithelia where multicilies form. Inhibiting multicilin function specifically blocks multiciliate cell formation in *Xenopus* skin and kidney, whereas ectopic expression induces the differentiation of multiciliate cells in ectopic locations. Multicilin localizes to the nucleus, where it directly activates the expression of genes required for multiciliate cell formation, including *foxj1* and genes mediating centriole assembly. Multicilin is also necessary and sufficient to promote multiciliate cell differentiation in mouse airway epithelial cultures. These findings indicate that multicilin initiates multiciliate cell differentiation in diverse tissues, by coordinately promoting the transcriptional changes required for motile ciliogenesis and centriole assembly.

Cilia are microtubule-based organelles that project from the cell surface, and in vertebrates have diversified into structurally different subtypes. Primary cilia are generally short, immotile, and extended by a variety of cell types as a means to sense mechanical and chemical stimuli. Motile cilia are extended by specialized epithelial cells and used to generate fluid flow along their surfaces. Cilium diversity enables vertebrate cells to carry out specialized functions and is one factor underlying the spectrum of phenotypes observed in human ciliopathies. How cilium diversity is generated is an important problem in organelle biogenesis, and a poorly understood aspect of cell type differentiation.

Cilium diversity arises in part through the differential expression of components required for cilium subtype differentiation. *Foxj1*, a winged-helix transcription factor, exemplifies this mechanism by activating gene expression required for motile cilia formation. Motile cilia first appear in the embryo when node cells extend a single cilium to generate a flow that establishes left–right asymmetry. Motile cilium also form during organogenesis by multiciliate cells (MCCs) that differentiate within the respiratory airways, ependyma and oviduct. In *Foxj1* mutants, primary cilia are unaffected, but cilia formation is disrupted in both MCCs and the cells mediating left–right patterning. In gain-of-function experiments, *Foxj1* is sufficient to induce the formation of an ectopic motile monocilium. Thus, *Foxj1* activates gene expression required for motile ciliogenesis but acts downstream of the mechanism that determines cilium number.

Cilium number is determined by the centriole, a microtubule-based structure that anchors the cilium, and comprises the core of the centrosome. In G0/1, the mother centriole docks at the plasma membrane and serves as a basal body to nucleate the outgrowth of the ciliary axoneme. Typically, cells form a monocilium using a centriole pair that is duplicated only during S-phase. During MCC differentiation, however, hundreds of centrioles are generated in postmitotic progenitors, to serve as basal bodies required for multiple cilia. This form of centriole assembly still occurs in MCC precursors in *Foxj1*-mutant mice, indicating that *Foxj1* is not required for this process. The hallmark of MCC differentiation is the activation of a centrosome assembly pathway along with motile cilium outgrowth, but the underlying mechanism that triggers these events is unknown.

**RESULTS**

**Identification and expression of MCI in *Xenopus***

The formation of MCCs in various organ systems, including embryonic *Xenopus* skin, is known to be restricted by Notch signalling. We therefore compared RNA expression in isolated *Xenopus* skin after manipulating Notch activity (Methods), identifying genes that are

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repressed by Notch, and thus likely to promote MCC differentiation. The top gene in this comparison encodes a small coiled-coil protein that we have termed multicilin (MCI) for the reasons discussed below.

MCI is the *Xenopus* orthologue of human IDAS, a protein related to the cell-cycle regulators GMNN and GEMC1 (Supplementary Fig. S1a; refs 14–16). MCI is encoded in a region of the *X. tropicalis* genome that is syntenic to mammalian genomes containing MCI orthologues and flanked by CCNO and CDC20B. CCNO is functionally uncharacterized, but also strongly upregulated in our microarray analysis. Three members of the MIR449 family embedded in the CDC20B gene are expressed during, and required for, MCC differentiation17. Thus, MCI lies in a genomic region that seems to have evolved to play a major role in MCC formation.

MCI RNA expression in *Xenopus* embryos presages other markers of MCC differentiation, including *foxj1* and *α-tubulin* (ref. 7; Supplementary Fig. S1c–e, data not shown), occurring in a spotty pattern that correlates best with MCCs rather than other differentiated cell types18–20. MCI expression is lost by stage 26, when MCCs in the skin are fully differentiated, but is then detected in the developing nephrostomes of the kidneys where MCCs form at later stages (Supplementary Fig. S1f,g). Consistent with the microarray results, MCI RNA expression in the skin is inversely affected by the levels of Notch-regulated manner.

Knockdown of MCI activity inhibits the formation of MCCs in the skin

To examine MCI function in skin development, we injected embryos with a morpholino (MCI-MO<sup>sg</sup>*) designed to target and inhibit splicing of the *MCI* preRNA (ref. 21). Splicing of endogenous *MCI* RNA was inhibited in morphants, as predicted, resulting in *MCI* transcripts that should encode a truncated MCI protein (Supplementary Fig. S2a). Specificity of the morphant phenotype was verified by a rescue experiment (Supplementary Fig. S2d–f), and by identical phenotypes obtained with dominant-negative MCI mutants (see below). A second MCI morpholino targeting the *MCI* start site proved less effective, indicating that it only partially blocked MCI function (Supplementary Fig. S2b,c,g).

MCI-MO<sup>sg</sup>* morphants developed normally on the basis of external morphology, but completely lacked MCCs in the skin according to cilia staining (Fig. 1b versus Fig. 1a). MCI morphants also lacked other features of MCCs, such as multiple centrioles, indicating an early block in MCC differentiation, not just a block in basal-body docking as seen in *foxj1* morphants7 (Fig. 1c–f). MCCs first arise as precursors within the basal layer of the ectoderm, and differentiate by intercalating into the outer layer, along with an equal number of proton-secreting cells19,22 (PSCs). In *MCI*-MO<sup>sg</sup>* morphants, the total number of intercalating cells decreased by half, with only intercalating PSCs evident, consistent with a selective loss of MCC precursors (Fig. 1j). In addition, PSC numbers and subtypes were unchanged in MCC morphants compared to controls (Supplementary Fig. S3a–e). Thus, in the absence of MCI, epithelial progenitors fail selectively to give rise to MCCs, most likely remaining as undifferentiated basal cells.

**MCI induces ectopic MCC differentiation**

To determine whether MCI can promote MCC differentiation, myc-tagged MCI (MT-MCI) was mis-expressed in embryos by RNA injection (Fig. 2b). At high levels, injection of MT-MCI RNA was toxic, whereas at lower concentrations, injected embryos survived past gastrulation and showed two phenotypes (Fig. 2b versus Fig. 2a). In one phenotype, the outer epithelial cells were in some cases 2–4 times

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**Figure 1** MCI morphants lack MCCs. (a,b) Confocal micrographs of the skin of embryos that were injected with a control morpholino (MO<sup>Cont</sup>; a) or an MCI splicing morpholino (MCI-MO<sup>sg</sup>; b), fixed at stage 28 and stained for ZO-1 (red) and acetylated-tubulin (green) antibodies to label cell boundaries and cilia, respectively. (c–f) Confocal micrographs of the skin of embryos injected with MO<sup>Cont</sup> (c,e) or with MCI-MO<sup>sg</sup> (d,f), along with *Hyls*-GFP RNA to label centrioles (green; e,f). At stage 28, embryos were fixed, stained with rhodamine-phalloidin (red) to label actin and then imaged. (g) Diagram of a transplant assay where the outer epithelium (OL) was isolated at stage 10 from a donor embryo injected with *mRFP* RNA and transplanted onto the inner layer (IL) of a host embryo injected with *mGFP* RNA. (h,i) Host embryos were also injected with MO<sup>Cont</sup> (h) or with MCI-MO<sup>sg</sup> (i), fixed at stage 28 when intercalation and differentiation is complete and then imaged by confocal microscopy after staining for cilia (blue). (j) Quantification of outer cells (OC; red), ciliated cells (CC; green/blue) or PSCs (green) for transplants onto MO<sup>Cont</sup> and MCI-MO<sup>sg</sup>-injected embryos, showing the average (±s.d.) for 15 fields from 5 embryos. Ciliated cell formation is markedly reduced (t = 6 × 10<sup>−3</sup>), whereas PSC and outer cell numbers are statistically the same. Scale bars, 10 μm. Arrowheads (a,c,e,h) and arrows (h) denote MCCs and PSCs, respectively.
where all of the cells were multiciliated. The skin cell types that was broadly induced across the skin (Fig. 2d), resulting in areas (red) was transplanted onto host embryos injected with mGFP (MCI with markers (Supplementary Fig. S3f). Intercalation (Fig. 2h) and decrease the number of cells expressing PSCs ectopically in the outer epithelium. In addition, MCI can inhibit PSC differentiation (Fig. 3c,f), ectopic MCC differentiation was still induced by a high concentration of MCI-HGR RNA (Fig. 3b,f). At a lower concentration of MCI-HGR, however, ICD regained inhibitory activity, reducing the number of ectopic MCCs twofold (Fig. 3e,f). Significantly, under these conditions, a fraction of MCCs induced by MCI-HGR failed to complete differentiation, resulting in cells that extend a single cilium but do not assemble centrioles, whereas in others, centriole assembly occurred but axoneme elongation failed (Fig. 3e). Thus, MCCs induced downstream of notch to promote MCC formation, but notch inhibits MCI expression and its ability to promote different aspects of the differentiation process, including centriole assembly and motile cilia extension. MCI promotes both processes cell autonomously, according to the analysis of MCI-expressing clones (Supplementary Fig. S4b–e). Furthermore, when MCI-HGR is expressed along with a foxj1 morpholino, MCI still induces centriole formation, but cilogenesis

Figure 2 MCI induces MCC differentiation. (a,b) Confocal micrographs of the skin of embryos injected at the two-cell stage with MT-MCI and mRFP RNA (b) or mRFP RNA alone (a), fixed at stage 28 and stained for acetylated tubulin to label cilia (green). Asterisks in b denote unusually large cells. (c,d) Confocal micrographs of the skin of embryos injected with MCI-HGR RNA along with mRFP RNA as a tracer treated with DEX at stage 11.5 (d), and then fixed at stage 28 and stained for cilia (green). The outer epithelium from donor embryos injected with mRFP RNA (red) was transplanted onto host embryos injected with mGFP RNA (green), as illustrated in Fig. 1g. Host (f) and donor (g) embryos were also injected with MCI-HGR RNA. Embryos were treated with DEX at stage 11.5 and fixed at stage 28. Shown are confocal micrographs of a control transplant (e), a transplant onto a host injected with MCI-HGR RNA (f) and a transplant from a donor injected with MCI-HGR RNA (g), after staining for cilia (blue). (h) Cell types in the skin were scored as MCCs (green with blue cilia staining), PSCs (green with no cilia staining), outer cells (red) or outer cells with cilia (red with blue cilia). Data are presented as an average of 10–15 fields (±s.d.) obtained from at least three transplants and the asterisks denote experimental values significantly different from control values (P < .005). Scale bars, 20 μm.

larger than normal and when extreme in size, non-ciliated, and in the other phenotype, the number of MCCs increased markedly. As the apical size of the epithelial cells decreases in the early embryo through stage 10 without cell growth (Supplementary Fig. S4a), the apical size of the epithelial cells decreases in the early embryo.

The results above indicate that MCI may act as a cell fate switch that allows progenitor cells to overcome lateral inhibition and form an MCC. We examined this possibility further by expressing different concentrations of MCI-HGR RNA along with RNA encoding an activated form of notch, the intracellular domain (ICD). Under conditions where ICD efficiently represses endogenous MCC differentiation (Fig. 3c,f), ectopic MCC differentiation was still induced by a high concentration of MCI-HGR RNA (Fig. 3b,f). At a lower concentration of MCI-HGR, however, ICD regained inhibitory activity, reducing the number of ectopic MCCs twofold (Fig. 3e,f). Significantly, under these conditions, a fraction of MCCs induced by MCI-HGR failed to complete differentiation, resulting in cells that extend a single cilium but do not assemble centrioles, whereas in others, centriole assembly occurred but axoneme elongation failed (Fig. 3e). Thus, MCCs induced downstream of notch to promote MCC formation, but notch inhibits MCI expression and its ability to promote different aspects of the differentiation process, including centriole assembly and motile cilia extension. MCI promotes both processes cell autonomously, according to the analysis of MCI-expressing clones (Supplementary Fig. S4b–e). Furthermore, when MCI-HGR is expressed along with a foxj1 morpholino, MCI still induces centriole formation, but cilogenesis

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is severely disrupted, to the same extent as that seen in the MCCs of foxj1 morphants (Fig. 3g–i), indicating that MCI induces motile cilia outgrowth through foxj1.

MCI induces cell-cycle exit and centriole assembly

The molecular pathway that induces centriole assembly in dividing cells

1

[45x330]The molecular pathway that induces centriole assembly in dividing cells

1

[45x246]MCCs (Fig. 4a) lightly labels centrioles in outer layer cells as well as basal bodies in RNA, numerous, large, bright foci of sas6

[45x139]NATURE CELL BIOLOGY

[45x55]as deuterosomes are detected in outer cells (Fig. 4l). Centriole assembly

after DEX addition, centriole assembly sites with the same morphology

embryos are fixed and imaged by transmission electron microscopy 8 h

foci peaks 8 h after DEX addition, before dispersing into a large number

of sas6 foci of sas6

[45x186]GFP, a protein that seeds new centrioles when they

[45x184]MCI

[45x127]starting approximately 4 h after the addition of DEX, or slightly after

centriole assembly postmitotically. We therefore investigated whether

MCI activity drives cell-cycle exit, using ethynyl deoxyuridine (EdU)

labelling to score the proportion of cells in the outer layer that still

MCI-HGR caused a marked decrease in EdU labelling within

150 pg of MCI-HGR

but different at 37 pg of MCI-HGR RNA (P = 6 × 10⁻⁶). Scale bars, 20 μm. (f) The average percentage of MCCs per field (±s.d.) plotted on the basis of data obtained from three fields from four embryos (n = 12). Values marked with an asterisk are highly significant (P < .005). (g, h) Confocal micrographs of the skin of embryos injected with MCI-HGR, centrin4–RFP and mRFP RNAs, followed by a foxj1 (h; ref. 7) or a control morpholino (MOCnt; g). Embryos were treated with DEX at stage 11.5, fixed at stage 28 and stained for acetylated tubulin to label cilia (green). Scale bars, 20 μm. (i) Cilium number per cell was scored from two fields from five embryos (n = 10) for each condition, and plotted as the average fraction of cells (±s.d.) with different cilium number as indicated per field.

MCI is a direct regulator of MCC gene expression

MCI could mediate a cell fate switch by activating gene expression

required for MCC differentiation, a possibility consistent with the response of MCI-HGR to DEX treatment, and the nuclear localization of ectopically expressed, tagged forms of MCI (Supplementary Fig. S5a,b), similar to human IDAS (ref. 14). Indeed, the expression of foxj1 and α-tubulin was lost in embryos injected with MCI-MO95 (Fig. 5b,f) and conversely, markedly upregulated in embryos injected with MCI-HGR RNA (Fig. 5d,h). The response to MCI is likely to be mediated through the proximal promoters of these genes, because MCI-HGR also induced the expression of transgenes containing GFP driven by 1.5 kilobases (kb) of genomic DNA lying upstream of the translation start site in the foxj1 or α-tubulin genes22 (Supplementary Fig. S5c–j).

We next used quantitative RT-PCR to analyse MCI transcriptional activity in animal cap assays. MCI-HGR strongly induced foxj1 and α-tubulin expression in animal caps even in the presence of ICD, as described above (Fig. 5i). We then used microarrays to compare RNA expression in animal caps injected with ICD RNA alone, with that in animal caps injected with both ICD and MCI–HGR RNAs (Supplementary Table S2). As expected, genes upregulated by MCI-HGR included foxj1 and α-tubulin, as well as those previously shown in microarrays to be induced in animal caps by foxj1 (for

Figure 3 MCI acts downstream of notch to promote centriole assembly and motile cilia extension through foxj1. (a–e) Confocal micrographs of the skin of embryos injected with varying amounts of MCI-HGR RNA (a,d), ICD RNA (c) or both RNAs (b,e), along with mRFP (red) and Centrin4-GFP RNA (green) as a tracer and to mark centrioles, respectively. Embryos were treated with DEX at stage 11.5, fixed at stage 28 and stained for acetylated tubulin to visualize cilia (blue). In embryos injected with ICD and 37 pg of MCI-HGR RNA (e), MCC differentiation was sometimes incomplete, resulting in cells extending cilia but failing to undergo centriole assembly (arrowhead) or vice versa (arrow). Average number of centrioles (lower right, ±s.d.) is statistically the same with or without ICD at 150 pg of MCI-HGR RNA (d). To determine whether MCI-HGR caused a marked decrease in Edu labelling within 1–3 h after DEX induction, indicating that MCI activity rapidly induces centriole-cycle exit (Supplementary Fig. S4f–h).

Centriole assembly in MCCs is poorly characterized molecularly but is known to occur at deuterosomes24. To determine whether MCI induces centriole assembly through similar structures, we scored foci of sas6–GFP, a protein that seeds new centrioles when they multiply in MCCs (ref. 25). In control embryos, a sas6–GFP tracer lightly labels centrioles in outer layer cells as well as basal bodies in MCCs (Fig. 4a–e). In contrast, in embryos injected with MCI–HGR RNA, numerous, large, bright foci of sas6–GFP form in outer cells, starting approximately 4–6 h after the addition of DEX, or slightly after cell-cycle exit, as measured above (Fig. 4f–k). The formation of these foci peaks 8 h after DEX addition, before dispersing into a large number of centrioles/basal bodies at 10 h (Fig. 4j). When MCI-HGR-injected embryos are fixed and imaged by transmission electron microscopy 8 h after DEX addition, centriole assembly sites with the same morphology as deuterosomes are detected in outer cells (Fig. 4l). Centriole assembly
example, tektins, ccdc78; ref. 7). MCI also induced genes already known to be expressed in MCCs but poorly characterized functionally (for example, sox7; ref. 26), genes potentially involved in MCC differentiation on the basis of encoded structural motifs (for example, cdc45, cdc52) and genes encoding centriole components (for example, cep76). Genes identified in this microarray analysis need to be validated (as shown above for foxj1 and α-tubulin), but are consistent with the model that MCI selectively activates gene expression required for MCC differentiation, but not for other cell types, including PSCs (Supplementary Fig. S3f).

We next investigated whether MCI activates MCC gene expression directly. In embryos injected with MCI-HGR RNA treated with DEX at stage 10.5, induction of α-tubulin expression occurs rapidly, within 1 h (Fig. 5i, data not shown). Cycloheximide (CHX) treatment of control embryos between stage 11 and 12 blocks the onset of endogenous α-tubulin expression (Fig. 5k). In embryos injected with MCI-HGR, however, α-tubulin expression was still strongly activated in the presence of CHX, when DEX was added after the first hour of treatment (Fig. 5m). foxj1 expression was also induced by MCI-HGR following 1 h of DEX treatment (data not shown), indicating the MCI may directly regulate the genes required for MCC differentiation in parallel.

As MCI localizes to the nucleus and rapidly activates gene expression, we investigated whether MCI behaves as a transcriptional activator or repressor when recruited to DNA. MCI was fused to the DNA-binding domain of Gal4 and co-transfected into HEK293 cells along with a plasmid containing UAS-binding sites upstream of a luciferase reporter. In this assay, MCI strongly activated transcription of the reporter gene at least 10–100-fold over the basal level of transcription (Supplementary Fig. S5k), whereas gmnn, as a control, had no significant activity. Deletion mutants of MCI tested in this assay indicate that the transcriptional activation domain is not discrete, but distributed over several regions (Supplementary Fig. S5k).

**Structural features of MCI required for MCC differentiation**

To gain further insight into how MCI functions molecularly, we mapped the domains in MCI required for MCC differentiation using deletion constructs (Supplementary Fig. S6a). An MCI mutant lacking the coiled-coil domain (MCIΔ180–213) not only failed to induce MCC differentiation, but instead blocked both the appearance...
of MCCs in the skin and the expression of foxj1 and α-tubulin (Fig. 6c,g and Supplementary Fig. S6b,m). As in MCI morphants, MCIΔ280–213 blocked the formation of MCCs specifically, because the number of intercalating PSCs was unchanged (Supplementary Fig. S6c–e,r). Further deletion analysis (Fig. 6d,h and Supplementary Fig. S6b,n,s, data not shown) showed that a 40-amino-acid domain at the carboxy terminus, termed CT, is sufficient to generate this strong dominant-negative phenotype, and thus essential for MCI function.

In agreement with this interpretation, a form of MCI lacking CT (MCIΔ334–374) failed to induce ectopic MCC differentiation (Fig. 6i) but did induce the large-cell phenotype, as did a form containing just the coiled-coil domain (data not shown). MCIΔ334–374 did not qualitatively change the expression of the PSC marker, foxj1, or the MCC markers, foxj1 and α-tubulin (Fig. 6e and Supplementary Fig. S6o,t), but did cause weak ciliogenesis defects (Fig. 6i). In sum, both the coiled-coil and carboxy-terminal domain are required for MCI to function as a transcriptional regulator during MCC differentiation. The former domain can induce cell-cycle exit but not MCC gene expression and the latter is a potent specific inhibitor of MCC differentiation.

MCI is required for MCC differentiation in diverse epithelia

We next investigated whether MCI function is required for other cells to form motile cilia. In Xenopus, motile monociiliated cells that mediate left–right patterning arise on the gastrocoel roof plate (GRP) during gastrulation and extend cilia before the MCCs in the skin27. MCC RNA was not detectable in the superficial mesoderm that gives rise to the GRP (data not shown). Moreover, when MCI-MO94 or the potent MCI dominant-negative mutant was targeted to the mesoderm, motile cilia still appeared on the GRP (Supplementary Fig. S7a–i), indicating that the CHX treatment is effective, but does not block the activation of α-tubulin by MCI-HGR (m). Scale bar, 0.2 mm. Insets show a higher magnification.

We also examined the expression and function of MCI in primary cultures of mouse tracheal epithelial cells (MTECs), in which MCC differentiation is promoted by exposure to an air–liquid interface28 (ALI). On the basis of semi-quantitative RT-PCR, expression of mouse MCI (PubmedGene ID 622408) RNA markedly increases in MTEC cultures closely following the time course of MCC differentiation29 (Supplementary Fig. S8a). We next used lentiviral gene transfer to introduce either a myc-tagged form of full-length mouse MCI (MT-MCI) or a dominant-negative form that lacks the coiled-coil
One implication of our findings is that MCI determines cell fate not as a sequence-specific DNA-binding protein as in most cases, but rather as a scaffolding protein that is cell-type specific in expression and action. MCI presumably acts in a multi-protein complex that could conceivably function on or off DNA but is necessary and sufficient to activate gene expression associated with MCC differentiation. MCI may also function in part through its ability to heterodimerize with gmnn (ref. 14), a dual-function protein that acts broadly to inhibit embryonic gene expression associated with differentiation31. Indeed, overexpressing gmnn in embryos does not induce MCC formation but inhibits their differentiation (data not shown)31. However, MCI is unlikely to act solely as a gmnn inhibitor, because the coiled-coil domain of MCI, which is sufficient to bind gmnn (ref. 14; data not shown), does not promote MCC formation. Moreover, MCI activates only MCC gene expression, a small subset of the embryonic gene expression inhibited by gmnn (ref. 31). Thus, identification of additional binding partners for MCI is likely to be an essential step in revealing how MCI activates the genes required to acquire the MCC fate.

Centriole assembly in MCCs occurs through two pathways: approximately 10% of the centrioles form orthogonally to an existing centriole, much like in dividing cells; however, most assembly around deuterosomes24. The centriolar pathway could be driven by increased expression of factors that initiate centriole assembly at the G1/S transition, which can modestly amplify centriole number when overexpressed12–36. Our findings indicate that the acentriolar pathway may be driven by MCI, as evidenced by its ability to induce the appearance of ectopic deuterosomes (Fig. 4i), resulting in the appropriate centriole number (Fig. 4o). As this response correlates with the ability of MCI to activate transcription, we also suggest that MCI transcriptional targets are directly involved in initiating centriole assembly through the deuterosome-mediated, acentriolar pathway. Further characterization of these targets, therefore, will probably...
identify an alternative centriole assembly pathway that underlies this striking example of organelle biogenesis.

METHODS

Methods and any associated references are available in the online version of the paper at http://www.nature.com/naturecellbiology

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AUTHOR CONTRIBUTIONS

J.L.S., E.K.V. and C.K. carried out the experimental analysis. All authors participated in aspects of project planning, data analysis and manuscript preparation.

COMPETING FINANCIAL INTERESTS

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1. Sharma, N., Berbari, N. F. & Yoder, B. K. Ciliary dysfunction in developmental abnormalities and diseases. Curr. Top. Dev. Biol. 85, 371–427 (2008).
2. Thomas, J. et al. Transcriptional control of genes involved in cilogenesis: a first step in making cilia. Biol. Cell 102, 499–513 (2010).
3. Basu, B. & Brueckner, M. Cilia: multifunctional organelles at the center of vertebrate development. Curr. Top. Dev. Biol. 7, 151–174 (2008).
4. Satir, P. & Christensen, S. T. Overview of structure and function of mammalian cilia. Annu. Rev. Physiol. 69, 377–400 (2007).
5. Brody, S. L., Yan, X. H., Wuehrfel, M. K., Song, S. K. & Shapiro, S. D. Cilogenesis and left-right axis defects in forkhead factor HFH-4 null mice. Am. J. Respir. Cell Mol. Biol. 23, 45–51 (2000).
6. Chen, J., Knowles, H. Z., Hebert, J. L. & Hackett, B. P. Mutation of the mouse hepatocyte nuclear factor/forkhead homologue 4 gene results in an absence of cilia and random left-right asymmetry. J. Clin. Invest. 102, 1077–1082 (1998).
7. Stubbs, J. L., Oishi, I., Izpisua Belmonte, J. C. & Kintner, C. The forkhead protein Foxj1 specifies node-like cilia in Xenopus and zebrafish embryos. Nat. Genet. 40, 1454–1460 (2008).
8. Xu, Y., Ng, C. P., Habacher, H. & Roy, S. Foxj1 transcription factors are master regulators of the motile ciliogenic program. Nat. Genet. 40, 1445–1453 (2008).
9. Marshall, W. F. Basal bodies platforms for building cilia. Curr. Top. Dev. Biol. 85, 1–22 (2008).
10. Deblandre, G. A., Wettstein, D. A., Koyano-Nakagawa, N. & Nintert, C. A two-step mechanism generates the spacing pattern of the ciliated cells in the skin of Xenopus embryos. Development 126, 4715–4728 (1999).
11. Guseh, J. S. et al. Notch signaling promotes airway mucous metaplasia and inhibits alveolar development. Development 136, 1751–1759 (2009).
12. Morimoto, M. et al. Canonical Notch signaling in the developing lung is required for determination of arterial smooth muscle cells and selection of Clara versus ciliated cell fate. J. Cell Sci. 123, 213–224 (2010).
13. Tsao, P. N. et al. Notch signaling controls the balance of ciliated and secretory cell fates in developing airways. Development 136, 2297–2307 (2009).
14. Pefani, D. E. et al. Idas, a novel phylogenetically conserved geminin-related protein, binds to geminin and is required for cell cycle progression. J. Biol. Chem. 286, 23234–23246 (2011).
15. Catalano, A., Cosentino, C., Errico, A., Gardner, E. & Costanzo, V. GEMC1 is a TopBP1-interacting protein required for chromosomal DNA replication. Nat. Cell Biol. 12, 484–491 (2010).
16. Sea, S. & Kroll, K. L. Geminin’s double life: chromatin connections that regulate transcription at the transition from proliferation to differentiation. Cell Cycle 5, 374–379 (2006).
17. Marcet, B. et al. Control of vertebrate multiciliogenesis by miR-449 through direct repression of the Delta/Notch pathway. Nat. Cell Biol. 13, 693–699 (2011).
18. Hayes, J. M. et al. Identification of novel cilogenesis factors using a new in vivo model for mucociliary epithelial development. Dev. Biol. 312, 115–130 (2007).
19. Quigley, I. K., Stubbs, J. L. & Kintner, C. Specification of ion transport cells in the Xenopus larval skin. Development 138, 705–714 (2011).
20. Dubaissi, E. & Papalopulu, N. Embryonic frog epidermis: a model for the study of cell-cell interactions in the development of mucociliary disease. Dis. Model Mech. 4, 179–192 (2010).
21. Hessman, J. Morpholino oligos: making sense of antisense? Dev. Biol. 243, 209–214 (2002).
22. Stubbs, J. L., Davidson, L., Keller, R. & Kintner, C. Radial intercalation of ciliated cells during Xenopus skin development. Development 133, 2507–2515 (2006).
23. Kolm, P. J. & Sive, H. L. Efficient hormone-inducible protein function in Xenopus laevis. Dev. Biol. 171, 267–272 (1995).
24. Dirksen, E. R. Centriole and basal body formation during cilogenesis revisited. Biol. Cell 72, 31–38 (1991).
25. Vladi, E. K. & Stearns, T. Molecular characterization of centriole assembly in ciliated epithelial cells. J. Cell Biol. 178, 41–42 (2007).
26. Fawcett, S. R. & Klimkowski, M. W. Embryonic expression of Xenopus laevis SOX7, Gene Expr. Patterns, 29–33 (2004).
27. Schweickert, A. et al. Cilia-driven leftward flow determines laterality in Xenopus. Curr. Biol. 17, 60–66 (2007).
28. You, Y., Richer, E. J., Huang, T. & Brody, S. L. Growth and differentiation of mouse tracheal epithelial cells: selection of a proliferative population. Am. J. Physiol. Lung Cell Mol. Physiol. 283, L1315–L1321 (2002).
29. Liu, Y., Pathak, N., Kramer-Zucker, A. & Drummond, I. A. Notch signaling controls the differentiation of transporting epithelia and multiciliated cells in the zebrafish pronephros. Development 134, 1111–1122 (2007).
30. Ma, M. & Jiang, Y. J. Jagged2a-notch signaling mediates cell fate choice in the zebrafish pronephric duct. PLoS Genet. 3, 133–145 (2007).
31. Lim, J. W., Hummert, P., Mills, J. C. & Kroll, K. L. Geminin cooperates with Polycycl to restrain multi-lineage commitment in the early embryo. Development 138, 33–44 (2011).
32. Dzhindzhev, N. S. et al. Asterless is a scaffold for the onset of centriole assembly. Nature 467, 714–718 (2010).
33. Bettencourt-Dias, M. et al. SAK/PLK4 is required for centriole duplication and flagella development. Curr. Biol. 15, 2199–2207 (2005).
34. Hatch, E. M., Kulukian, A., Holland, A. J., Cleveland, D. W. & Stearns, T. Cep152 interacts with Plk4 and is required for centriole duplication. J. Cell Biol. 191, 721–729 (2010).
35. Cizmecioglu, D. et al. Cep152 acts as a scaffold for recruitment of Plk4 and CPAP to the centrosome. J. Cell Biol. 191, 731–739 (2010).
36. Habedanck, R., Stierhof, Y. D., Wilkinson, C. J. & Nigg, E. A. The Polo kinase Plk4 functions in centriole duplication. Nat. Cell Biol. 7, 1140–1146 (2005).
 METHODS

RNA isolation and microarray. Animal caps were isolated from embryos injected at the four-cell stage with RNA encoding iCD to activate Notch signalling or a dominant-negative form of suppressor of hairless (rpbj) or human mastermind like (HMM) to block Notch signalling. Total RNA was isolated using the proteinase K method. The cDNA was reverse transcribed and analyzed using the two-color analysis software using a paired t-test.

Xenopus transgenics. A 1.5-kb genomic fragment lying upstream of the translational start site in the X. tropicalis foxj1 gene was isolated by PCR, and cloned upstream of GFP using CS2 as the backbone. Foxj1–GFP transgenics were generated employing sperm nuclear transfer as previously described and modified8. The wild-type CS2 and F1 transgenics were generated using sperm nuclei derived from an F0 male as described previously22. Transgenic embryos were injected at the two-cell stage with MCI-HGR or foxj1 RNA, along with mRFP as a tracer or as a control, treated with DEX at stage 11, fixed at stage 26 and then imaged, after staining for cilia using an acetylated antibody.

MTEC cultures, lentiviral manipulation, immunofluorescence microscopy and RT-PCR. MTECs were cultured from wild-type C57BL/6J (The Jackson Laboratory) mice of six weeks of age or older, as previously described35. All procedures involving animals were approved by the Institutional Animal Care and Use Committee in accordance with established guidelines for animal care. In short, airway epithelial cells were isolated from trachea by protease digestion, contaminating fibroblasts were removed by prelabeling the suspension and the remaining unadhered epithelial cells were seeded onto collagen-coated Transwell- Clear permeable membranes (Corning). Cells were grown in MTEC complete medium22 until an Ali was created approximately 2 days after confluence by adding MTEC basal medium + 2% NuSerum22. Beating cilia were observed by phase microscopy 3 days after Ali creation. To block Notch activity, MTECs were treated with 1 μM DAPT in Ali media for 2 h from Ali + 1 day to day 4. Lentivirus production and infection of MTECs were carried out as previously described36. In short, lentivirus expressing GFP or MCI-DNA was produced by transient co-transfection of HEK293T/17 cells (ATCC) with the lentiviral transfer vector pRRL.sin-18.PPT.PGK.GFP.pre (ref. 43) and helper vectors (pCMVDR8.74 packaging vector and pMD2.GSVG-envelope vector)79 using the calcium phosphate co-precipitation method. The lentiviral supernatant was concentrated by centrifugation to achieve 107–108 infectious units per millilitre.

Quantitative RT-PCR. Embryos were injected at the two-cell stage with iCD and/or MCI-HGR RNA. Animal caps were dissected at stage 10, treated with DEX at stage 11 and then collected for total RNA extraction at stage 13. cDNA templates were generated from 3 μg of RNA using SuperScript III Reverse Transcriptase (Invitrogen). Quantitative RT-PCR reactions were carried out using the ABI Prism 7000HT Thermal Cycler, using primers for foxj1 and a-tubulin or for ornithine decarboxylase (odc) as a normalization control (Supplementary Table S1). Data were analysed using Applied Biosystems Sequence Detection System software.

EdU assays. Embryos were injected at the two-cell stage with MCI-HGR and mRFP RNA or mRFP RNA alone. DEX was added to embryos at mid-gastrula stages. At 1 h, 3 h or 7 h after the addition of DEX, embryos were injected four times (10 nl per injection) into the gastrocoel with 10 mM EdU (Invitrogen). Embryos were allowed to develop until stage 28, fixed for 1 h in 3.7% formaldehyde in 1× PBS and then dehydrated in ethanol. Embryos were stained using the Invitrogen Click-iT Alexa 488 kit (Invitrogen C10357). Briefly, embryos were rehydrated in PBT (1× PBS containing 10% Triton X-100). Embryos were permeabilized by washing with 1× PBT at room temperature and then incubated for 1 h in EdU reaction buffer made up as per the kit instructions. Embryos were washed several times in PBT, then mounted in PVA/DABCO and imaged as for other staining procedures.

Reporter assays. HEK293 cells were co-transfected with 140 ng of firefly luciferase reporter construct (Promega pGL4.23 #E4811) and 150 ng of expression constructs where MCI fragments were cloned in frame with the Gal4 DNA-binding domain in the pCMV–βD vector (Agilent #211342). Cells were transfected in 48-well plates using FuGENE 6 (Roche #1181443001) together with 10 ng of the Renilla luciferase.
pGL4.74 plasmid (Promega #E6921) as a transfection efficiency control. Firefly and Renilla luciferase activity was measured 40–48 h after transfection, using the Dual–Glo Luciferase Assay System (Promega #E2920). Values are mean ± standard deviation for three individual experiments.

**Transmission electron microscopy.** Embryos were fixed in 2% glutaraldehyde in 0.1 M sodium cacodylate buffer, rinsed, post-fixed in 1% osmium tetroxide and 1% potassium ferrocyanide, rinsed, en block stained in 1% uranyl acetate, dehydrated with glycol methacrylate and embedded in Epon. Thin sections (~60 nm) were cut on an ultramicrotome, collected onto formvar-coated slot grids and stained with 2% uranyl acetate and 0.2% lead citrate. The sections were examined at 80 kV in a Zeiss Libra 120 PLUS EF-TEM transmission electron microscope.

37. Fryer, C. J., Lamar, E., Turbachova, I., Kintner, C. & Jones, K. A. Mastermind mediates chromatin-specific transcription and turnover of the Notch enhancer complex. *Genes Dev.* **36**, 1397–1411 (2002).

38. Wettstein, D. A., Turner, D. L. & Kintner, C. The Xenopus homolog of Drosophila Suppressor of Hairless mediates Notch signaling during primary neurogenesis. *Development* **124**, 693–702 (1997).
Figure S1 MCI genes and expression in Xenopus embryos. (a) Xenopus MCI is a small protein most related to Geminin and GEMC1, based on homology within a central coiled-coil domain. (b) Shown is the region of chromosome 5 of the human genome with genomic synteny to the region where the MCI gene lies in mouse and *Xenopus*. (c-g) Expression of MCI in Xenopus embryos was detected at different developmental stages using whole-mount in situ hybridization. Shown in each case is both a low power view of the whole embryo and an inset at higher power. Expression is first detected during gastrulation (c, BPL=blastopore lip) in a spotty pattern within the prospective epidermis. Expression is excluded from the neural plate (d, NP=neural plate) at stage 13 (d) and covers the skin regions where MCCs are known to form by stage 16 (e). The pattern of MCI expression in the skin best correlates with MCCs, rather than the mucus-secreting cells that comprise most of the outer epithelium, or a second scattered population of proton secreting cells (PSCs)18-20. MCI expression is lost in most of the skin by stage 26 (f) when MCCs are differentiated, but is re-expressed in the developing nephrostomes of the kidney (g, arrowhead). (h,i) Shown is α-tubulin expression, a marker of MCCs in the skin, and in the nephrostomes of the kidney (i, arrowhead). (j-o) Embryos were injected twice in the animal pole of one blastomere at the two-cell stage with RNA encoding the intracellular domain of Notch (ICD) to activate Notch signaling (k,n), RNA encoding the DNA-binding mutant of Su(H) (dnSu(H)) to block Notch signaling (l,o), along with lacZ RNA as a tracer, or as a control (j,m). Embryos were fixed at stage 13, stained with Xgal (light blue reaction product) and then processed for whole mount in situ hybridization for the expression of α-tubulin (left panels) or MCI RNA (right panels). Shown in each case are the injected and uninjected (prime) sides of representative embryos with an inset at higher power. Similar results were obtained for at least twenty embryos in each case. Scale bars=0.5mm.
**Figure S2** MCI morpholino control. (a) RT-PCR of MCI transcript in embryos injected with MCI-MO^Spl^ (lanes 1-2) or control MO (lanes 3-4) using primers located in exon 3 and exon 6 of the MCI gene. The wildtype transcript gives a band of approximately 400bp (lane 3). The MCI-MO^Spl^ blocks splicing and results in a truncated transcript (asterisk). –RT controls show no amplification (lanes 2, 4). (b-c) Embryos were injected with a control morpholino (b) or MCI-MO^ATG^ (c), fixed at stage 28 and stained with antibodies against ZO-1 (red) and acetylated α-tubulin (green). MCI MO^ATG^ injections result in ciliogenesis defects (arrows in c). (d-f) Rescue experiment: a batch of embryos injected with MCI-MO^Spl^, were divided in two, half of which were also injected with RNA encoding MCI-HGR (f). Control embryos (d) and morphants (e,f) were also injected with RNA encoding mRFP (red) or Hysl-GFP (green), to label membranes and centrioles, respectively. Shown are confocal images of the skin after embryos were treated with DEX at stage 11.5, fixed at stage 26 and stained for cilia (blue). The average number of MCCs present per field is shown in the lower left corner (average of ten fields from 5 embryos ± s.d) and the average number of centrioles per MCC is shown in the right (average of ten cells from 5 embryos ± s.d). While the reduction of MCCs in response to the MCI morpholino is highly significant (p=9x10^-11), the number of centrioles following rescue by MCI is statistically the same. Scale bar (b) represent 20 microns in panels b-f. (g) Plot showing the average number (±s.d.) of different cell types per field in the skin of embryos injected with a control morpholino and MCI-MO^ATG^ Defective ciliated cells were scored based on a loss of cilia as shown in panel c.
Figure S3  MCI activity is not required for PSC differentiation.  (a-d) Embryos were injected with a control morpholino or the MCI splicing morpholino, fixed at stage 28 and then stained with antibodies against AE1 (a,c), or with antibodies to E-cadherin (green) and to ATP6V1B1 (red) (b,d). ATP6V1B1 staining identifies all PSCs in the skin, while AE1 identifies a subset analogous to alpha-intercalated cells in the mammalian kidney. Sib embryos stained with an acetylated tubulin antibody to mark cilia showed a predicted loss of MCCs in the MCI morphants (data not shown). Scale bars=20 microns.  (e) Average number of different PSCs subtypes in MCI and control morphants (±s.d.), based on data obtained from 3-5 fields from three different embryos (total n=12). Values are not significant different based on a two-tailed t-test. (f-k) Embryos were injected with MT-MCI RNA (f-h) along with nLacZ RNA as a tracer or as a control (i-k). At stage 24, embryos were fixed, stained with X-gal (light blue) and for the expression of Foxi1, a master regulator of PSC differentiation, pendrin-like, a marker of b-PSCs, and AE1, a marker of a-PSCs, using whole-mount in situ hybridization (dark purple). Note that MCI does not induce PSC marker gene expression, but may instead reduce PSC number. Scales bars=0.5mm.
Figure S4 MCI-HGR promotes different aspects of MCC differentiation. (a) The apical size of the surface epithelial cells at different developmental stages was measured in embryos injected with mRFP RNA, focusing on ectodermal regions that give rise to the developing skin. Shown is a plot of the average size (±s.d.) based on 30-120 cells from 6-10 embryos. Note that apical size decreases through stage 10, but remains relatively constant thereafter. (b-e) MCI activity was measured in clonal assay in which a small explant of ectodermal tissue was isolated from stage 10 donor embryos injected with MCI-HGR and mRFP RNA, and transplanted onto host embryos injected with ICD and mGFP RNA. Embryos were treated with DEX at stage 11.5, fixed at stage 28, and stained with an acetylated tubulin antibody (blue) to identify MCCs. Shown is a representative transplant at the boundary between donor (b, red), and host (c, green) tissue, showing that all of the MCCs (d, blue) are derived solely from donor cells (e, merge). Scale bars=40 microns. (f-h) EdU labeling of cycling cells was measured after inducing MCI-HGR activity. Embryos were injected with MCI-HGR RNA along with RNAs encoding mRFP and H2A-Cherry as a tracer or as a control, to stain membranes and nuclei, respectively. At stage 11, embryos were induced with DEX to induce MCI activity, and then pulsed with EdU at 1, 3 or 7 hrs later. At stage 28, embryos were fixed, stained for EdU incorporation (green) and then imaged by confocal microscopy. Shown is a representative image from a control (f) or MCI-HGR (g) injected embryo pulsed with EdU 1 hr after DEX treatment. Plot (h) showing the fraction nuclei labeled with EdU (±s.d.) at each condition based on data obtained from 16-20 fields from at least ten different embryos. All MCI-HGR values are significantly different from control values (p<.005) (i-l) Basal body orientation was measured in MCCs induced by MCI-HGR. Embryos were injected with CLAMP-GFP and Centrin4-RFP RNA alone (i) or with MCI-HGR RNA (k). At stage 11.5, the embryos were treated with DEX, and then fixed at stage 28 to visualize Centrin4-RFP (red), which localizes to the basal body, and Clamp-GFP (green), which localizes to the rootlet. Shown are representative images. Scale bars=10 microns. Basal body orientation was scored by drawing a line from Clamp-GFP to Centrin-RFP, as described previously (j,l)\(^2\). Polar plots (j,l) representing the orientation of individual cells in relation to embryonic axis, where different colors represent cells from two different embryos, (A: Anterior, P: Posterior, D: Dorsal) (see\(^2\) for details).
**Figure S5** MCI is a nuclear protein, regulates transgenic FoxJ1 and α-tubulin expression, and acts as a co-activator. (a–b) Embryos were injected with RNAs encoding a myc-tagged form of MCI (MT-MCI) and mGFP (green) as a tracer. At stage 10, embryos were fixed, stained with an anti-myc antibody (red), and imaged by confocal microscopy. Scale bar=20 microns. (c–j) Transgenic embryos were generated with the sperm nuclei transfer method, using proximal genomic sequences from α-tubulin (c–f) or FoxJ1 (g–j) to drive mGFP expression. At the two cell stage, embryos were also injected with RNA encoding MCI-HGR along with mRFP RNA as a tracer (e,f,i,j) or as a control (c,d,g,h) Embryos were treated with DEX at stage 11.5, fixed at stage 26, and then stained with antibodies to GFP (green) or to acetylated tubulin (blue) to label cilia. Shown are representative regions of skin labeled with the RFP tracer (not shown) imaged by confocal microscopy. Scale bar=10 microns. Note that both transgenic drivers express GFP only in MCCs and can be induced ectopically by MCI-HGR. In other experiments, neither transgene responds to FoxJ1 RNA injection (data not shown). (k) MCI domains were fused to the Gal4 DNA-binding domain and used in a Gal4 reporter assay using a construct containing five Gal4UAS binding sites fused to a minimal promoter located upstream of the luciferase gene. Fold activation is the ratio between the normalized luciferase activity induced by the Gal4BD-MCI domain fusion expression constructs and that induced by the empty Gal4BD expression vector. Constructs are as follows: MCI-FL is the full length MCI protein. MCIDCT lacks the C-terminal 40 amino acids also called D334-374. MCID180-213 lacks the coiled coil domain region from amino acids 180-213. MCID69-334 contains a large internal deletion of the coiled-coil domain but keeping the conserved 40 amino acids intact. MCI-CC encodes the coiled-coil domain from amino acids 141-236. MCI-5’CC includes the N-terminal half of the protein through amino acid 239 including the coiled-coil, 5’DCC lacks the coiled-coil domain and includes only the N-terminal 140 amino acids. MCI-3’CC encodes the C-terminal half of the protein starting at the amino acid 141 and includes the coiled-coil domain. MCI-3’DCC lacks the coiled-coil, beginning at amino acid 240. MCI-3’DCT encodes the C-terminal half of the protein beginning at amino acid 141 but lacks the conserved 40 amino acid domain. Error bars are mean ± S.D. (n=3).
Figure S6 MCI deletion mutants. (a) Diagram of MCI mutants analyzed. (b) Embryos were injected with RNAs encoding wildtype or MCI deletion mutants, fixed at stage 28 and stained with ZO-1 and acetylated tubulin antibodies to label cell boundaries and cilia, respectively (See Figure 6b-e in the main text). Outer cells (OCs), MCCs and PSCs were scored from two fields from five embryos (n=10) based on cilia staining and morphology, and plotted as the average number (±s.d.) per field. Data significantly different from controls are marked (*p<.005). (c,d) Outer layer tissue from mRFP injected embryos was transplanted onto hosts injected with just mGFP RNA (c) or with both mGFP and MCIΔ180-213 RNA (d) as shown in Fig. 1g of the main text. Shown are confocal images of the skin in embryos fixed at stage 26, and stained for cilia (blue). Scale bar (b)=10 microns. (e) Plot showing the average number (±s.d.) of OCs (red), MCCs (green/blue) and PSCs (green) per field based on three fields from five transplants (n=15). The reduction of MCCs is highly significant (asterisk: p<0.005) while PSCs are unchanged. (f) Embryos were injected with RNA encoding mRFP, along with different amounts of RNA encoding MCID334-374 as indicated, fixed at stage 28, and stained with an acetylated tubulin antibody (green) to label cilia. The number of outer cells (OC), ciliated cells (CC) and PSCs (small apical domain) was counted for each condition, collecting data from 2 fields from five embryos (n=10), and plotted as the average (± s.d.) cell types detected per field. After correcting for a decrease in cell number, the number of ciliated cells present in MCID334-374 expressing embryos is statistically different from control embryos (p<.005) but the number of PSCs cells is not. (g) Apical size of outer cells was measure under each condition, collecting data from 50 cells from 5 embryos. Error bars=±s.d. All values obtained with MCID334-374 injected embryos are statistically different from control values (t-test, p<.005). (h-j) Embryos were injected with mRFP and H2A-CHerry RNA alone or with RNA encoding MCID334-374. At stage 11, embryos were pulsed with EdU, fixed at stage 26 and processed for EdU labeling (green). Shown are confocal images from a control (i) or MCID334-374 RNA injected embryo (j). Scale bar (i)=20microns. (h) Plot showing the average number of EdU-labeled nuclei as a fraction of the total under each condition based on data derived from 16-20 fields taken from at least ten different embryos. Error bars=±s.d. X (k-t) Xenopus embryos were injected in the animal pole of each blastomere at the 4-cell stage with RNA encoding MCI, MCID180-213, MCID69-332, or MCID334-374, as indicated. At stage 13/14, embryos were fixed and stained for FoxJ1 RNA (k-o) or for Foxi1 RNA (p-t), a gene required for PSC differentiation19,20. Shown are representative embryos, with an insert taken at higher power. Note in the case of MCID334-374 that cell size is increased, consistent with a block in cell cycle progression. Scale bars=0.5mm.
Figure S7  MCI is not required for ciliogenesis on the GRP but is in the kidney. (a–d) *Xenopus* embryos were injected at the two-cell stage with a control or the MCI splicing morpholino, in the animal pole to target the skin (a,b) or in the marginal zone to target the GRP (c,d). At stage 12, the marginal zone injected embryos were fixed and stained for ZO-1 (red) and acetylated tubulin (green) in order to image cell boundaries and cilia, respectively in the GRP (c,d). At stage 26, the animal pole injected embryos were fixed and stained as above to image cell boundaries (red) and ciliated cell (green) in the skin (a,b). Note the number of ciliated cells in the GRP are unaffected by the MCI morpholino, while those in the skin are completely lost. Scale bars=10microns.

(e) Cilia length was measured on the GRP in control morphants (cilia, n=400), MCI morphants (n=260) and in embryos injected with a strong dominant-negative MCI mutant lacking the coiled-coil domain (See Fig.6c,g) (n=250). The MCI morpholino and mutant both cause a small decrease in cilia length that is statistically significant (p<3X10^-20). Thus, MCI is unlikely to have a major role in GRP cilia formation, but we cannot rule out a very minor contribution to cilia length, given the similar results obtained with the MCI morpholino and the dominant-negative mutant.

(f–i) One ventral vegetal blastomere of eight-cell stage embryos was injected to target the prospective marginal zone with MCI-HGR RNA along with lacZ RNA as a tracer (g), or with lacZ RNA alone as a control (f). Embryos were treated with DEX at stage 19/20, and then fixed at stage 30+, stained with X-gal (light blue) to reveal the injected side, and then for α-tubulin expression (dark purple), using whole-mount in situ hybridization. Shown is the injected side of a representative embryo with the region containing the nephrostomes (arrowhead) shown at higher power in the insert. One blastomere of two-cell stage embryos was injected with a MCI-MO^spl^ (i) or a control morpholino (h), followed by nLacZ RNA to mark the injected region. Embryos were processed for X-gal and α-tubulin staining at stage 30 as above. Scale bars=1mm.
**Figure S8** MCI acts downstream of Notch signaling in MTEC cultures. (a) RT-PCR was used to measure the levels of MCI RNA (M) expression in MTEC cultures, using GAPDH (C) levels as a control, at different days before or after switching to ALI culture conditions. PCR bands corresponding to MCI or GAPDH reaction products are indicated. (b-e) MTEC cultures were infected with a virus expressing GFP (b,c) or mouse MT-MCIDCC (d,e), switched to ALI conditions, and treated (c,e) or untreated (b,d) with DAPT to inhibit Notch signaling. After 4 days, the cultures were fixed, stained for GFP or the myc-tag (green), and with an antibody to FoxJ1 (red). Note that DAPT increases the number of FoxJ1 expressing cells by about 2-fold, while MT-MCIDCC completely blocks FoxJ1 expression in infected cells. Scale bars = 50 microns. (f) The number of infected and uninfected cells expressing FoxJ1 was measured per field under the indicated condition (n=20, ±s.e.). Values obtained for infected and uninfected cells in the GFP infected cultures are statistically the same, while those obtained for infected and uninfected cells in the MCI-ΔCC infected cultures are highly significant (p<0.005). (g) Shown is the infection rate for the experiment scored in panel f, indicating that neither DAPT nor MT-MCIDCC changes cell survival. (h) RT-PCR was used to measure the levels of MCI RNA expression in MTEC cultures either untreated (cont) or treated with DAPT, using GAPDH levels as a control. Values indicate relative expression levels after normalization.
Figure S9 Model for MCI action during ciliated cell differentiation. Our data suggests strongly that MCI is the critical target of the Notch pathway during lateral inhibition, when MCCs are selected out within ciliated epithelia. When MCI is expressed at high levels, it likely acts as a co-activator that targets, perhaps by direct binding, the proximal response elements in genes required to initiate centriole assembly based on the analysis of $\alpha$-tubulin. We propose that among the targets of MCI activation, will be those involved in deuterosome-mediated centriole assembly. Importantly MCI also appears to initiate cell cycle exit, thus ensuring that epithelial progenitors only initiate centriole assembly as postmitotic cells. MCI also activates the expression of FoxJ1, a critical regulator for the assembly of motile cilia. Thus, MCI is a pivotal factor that distinguishes multiciliate cells from monociliated cells.
| Primer Name | Primer Sequence | Notes |
|-------------|-----------------|-------|
| MCI-F1      | GGGATCCAAAAAAATGCAGAAC | for original cloning of MCI from cDNA library |
| MCI-R1      | GCTCGAGCGGTTGTCAATTCTT |  |
| MCI-F2      | GAAGGCCCTTGATGCAAGACAGAGG | to clone MCI in frame with Myc tag |
| MCI-R2      | CCGCTCGAGAATTGGGAACCCATC | to fuse MCI to HGR on 3’ end |
| MCI-F3      | CCTCGGGAAATCAAGCAAAGCATCTG | forward primer with Aval site to generate Δ180-213 construct |

| Primer Name | Primer Sequence | Notes |
|-------------|-----------------|-------|
| mMCI1 Stu F1 | GAAGGCCTAGGATGCAAGCGTGAGGGCAG | to clone mMCI into myc tagged CS2 vector |
| mMCI1 Xho R1 | CCGCTCGAGaGCTGGGGACCCAGCGGAATTTG |  |
| MT- mMCI Nhe F1 | CGGGATCCCCGGGTAGCATGGAGCAAAGGCTCATTTCGT | to clone mMCI into lentiviral vector |
| MT- mMCI Sal R1 | CGCTCGAGACGCGTCGACTGCTGGGGACCCAGCGG | to clone mMCI into lentiviral vector |
| MT- mMCI Bcl F2 | GCCTGATCACGCAGCTCCTGTCCGAGCCC | forward primer for making 3’ half of mMCI for Δ175-238 lentiviral vector |
| MT- mMCI Bcl R2 | CGGTGATCATCTCCGTTGGTGGCGGCGGCC | reverse primer for making 5’ half of mMCI for D175-238 lentiviral vector |
| mRT-PCR F | CCTAGTGTGGATTCGTCGCGTCG | forward primer for rt-pcr from MTEC cultures |
| mRT-PCR R | GGTATTTCTCTATGAGACAGTC | reverse primer for rt-pcr from MTEC cultures |
| mGAPDH F | GACTTCAACAGCAACTCCCAC | GAPDH forward primer for rt-pcr from MTEC cultures |
| mGAPDH R | TCCACCACCTCGTGCTGTA | GAPDH reverse primer for rt-pcr from MTEC cultures |

| Morpholino Name | Morpholino Sequence |
|-----------------|---------------------|
| MCI-MOsP        | GTGCCAGTCACTCACTGGTTT |  |
| MCI-MoATG       | AAAACGCTTCTCTGTCTGAT |  |
| Control-MO      | CCTCTTACCTCAGTTACATTATA |  |

Table 1 Legend: Shown are the nucleotide sequences used to generate constructs, or to design morpholinos used in this study.
| Column | Probeset ID | Gene Symbol | Gene Title | RefSeq   | p-value    | Fold-Change |
|--------|-------------|-------------|------------|----------|------------|-------------|
|        | XI2.51456.1.S1_at | --- | --- | --- | 0.0437432 | 39.4923 |
| 23300  | XI2.15616.1.S1_at | tekt2 | tektin 2 (testicular) | NM_001096211 | 0.0124408 | 25.9678 |
| 5038   | XI2.55428.1.S1_at | foxj1.2 | forkhead box J1, gene 2 | NM_001096421 | 0.0148935 | 20.4041 |
| 27237  | XI2.41899.1.S1_at | fam154b | family with sequence similarity 154 B | NM_001093710 | 0.00228547 | 19.3115 |
| 17604  | XI2.33942.1.S1_at | MGC131046 | Cyclin O | NM_001096396 | 0.017057 | 19.3037 |
| 15703  | XI2.14955.1.A1_at | --- | --- | --- | 0.029102 | 18.9438 |
| 4393   | XI2.23696.1.S1_at | tsga10 | testis specific, 10 | --- | 0.00377716 | 17.6298 |
| 10673  | XI2.2651.1.A1_at | --- | --- | --- | 0.0363674 | 17.6218 |
| 12822  | XI2.9077.1.S1_at | MGC78960 | hypothetical protein MGC78960 | NM_001091603 | 0.0160166 | 16.2694 |
| 31708  | XI2.50818.1.S1_at | ccno | cyclin O | NM_001096410 | 0.0179911 | 15.0963 |
| 22574  | XI2.55698.1.S1_at | MCI | hypothetical protein LOC100158359 | NM_001127804 | 0.0327364 | 14.7061 |
| 27520  | XI2.13768.1.A1_at | --- | --- | --- | 0.000172684 | 14.1659 |
| 3310   | XI2.50473.1.S1_at | MGC130946 | centrin2 | NM_001096664 | 0.0118188 | 14.1255 |
| 22222  | XI2.49880.1.S1_at | c11orf66 | chromosome 11 open reading frame 66 | NM_001095293 | 0.020659 | 13.9783 |
| 21769  | XI2.5987.1.S1_at | cav-3 | caveolin-3 | NM_001088827 | 0.0321827 | 13.7159 |
| 29223  | XI2.15612.1.A1_at | bbs12 | Bardet-Biedl syndrome 12 | NM_001099878 | 0.0171632 | 13.5396 |
| 5034   | XI2.21648.1.S1_at | kit | v-kit viral oncogene homolog | NM_001085708 | 0.0119181 | 13.5085 |
| 9600   | XI2.13265.1.S1_at | --- | tubulin tyrosine ligase | --- | 0.0084366 | 13.2239 |
| 2834   | XI2.4890.1.S1_at | ccdc78 | coiled-coil domain containing 78 | NM_001094136 | 0.0264737 | 11.1762 |
| 22541  | XI2.47013.1.S1_at | --- | --- | --- | 0.0409036 | 10.7431 |
| 19386  | XI2.52048.1.A1_at | --- | --- | --- | 0.0109775 | 10.7379 |
| 23893  | XI2.50448.1.S1_at | --- | --- | --- | 0.0059126 | 10.6394 |
| 22196  | XI2.46981.1.S1_at | c1orf158 | chromosome 1 open reading frame 158 | NM_001095298 | 0.0104278 | 10.2688 |
| 19346  | XI2.16202.1.S1_at | --- | --- | --- | 0.0103435 | 10.168 |
| 5003   | XI2.47124.1.S1_at | ankrd5 | ankyrin repeat domain 5 | NM_001091772 | 0.00762972 | 9.9975 |
| 17188  | XI2.15506.1.A1_at | fam166b | family with sequence similarity 166 B | NM_001112874 | 0.0146075 | 9.87313 |
| 4923   | XI2.49781.1.S1_at | dnal1 | dynein, axonemal, light chain 1 | NM_001094484 | 0.0319668 | 9.5289 |
| 21698  | XI2.51703.1.S1_at | c2orf62 | chromosome 2 open reading frame 62 | NM_001096840 | 0.0191736 | 9.3112 |
| 23541  | XI2.46873.1.S1_at | ttk4 | tektin 4 | NM_001095333 | 0.0276342 | 9.2703 |
| 19236  | XI2.16695.1.S1_at | ttc18 | tetratricopeptide repeat domain 18 | --- | 0.0449571 | 9.16799 |
| 6076   | XI2.16695.1.S1_at | elov2 | (FEN1/Elo2, SUR4/Elo3)-like 2 | NM_001094095 | 0.0382186 | 9.12043 |
| 30872  | XI2.7927.1.S1_at | wrd6 | WD repeat domain 8 | NM_001096522 | 0.0347115 | 8.91554 |
| 21923  | XI2.50146.1.A1_at | --- | --- | --- | 0.00767846 | 8.77527 |
| 26175  | XI2.54218.1.A1_at | --- | --- | --- | 0.016895 | 8.75101 |
| 21161  | XI2.48854.1.S1_a_at | --- | --- | --- | 0.0207428 | 8.51722 |
| 5624   | XI2.16226.1.A1_at | --- | --- | --- | 0.0312996 | 8.51029 |
| 2018   | XI2.12380.1.S1_at | ccdc19 | coiled-coil domain containing 19 | NM_001095394 | 0.00540275 | 8.49173 |
| 13882  | XI2.30.1.S1_at | kit | v-kit homolog | NM_001085579 | 0.00509678 | 8.40797 |
| 21843  | XI2.50027.1.S1_at | spef2 | sperm flagellar 2 | NM_001095399 | 0.0162176 | 8.33819 |
| 3365   | XI2.13831.2.A1_at | --- | --- | --- | 0.0444365 | 8.28793 |
| 2042   | XI2.1241.1.S1_at | sox7 | SRY (sex determining region Y)-box 7 | NM_001085868 | 0.0309793 | 8.22495 |
| 8809   | XI2.20259.1.S1_at | morn3 | MORN repeat containing 3 | NM_001095332 | 0.0145923 | 8.17571 |
| 30924  | XI2.7994.1.A1_at | --- | --- | --- | 0.0299608 | 8.1482 |
| 16067  | XI2.34517.1.A1_at | --- | --- | --- | 0.0364137 | 7.82168 |
| 13891  | XI2.30012.1.S1_at | tekt1 | tektin 1 | NM_001091757 | 0.0216221 | 7.44936 |
| 2177   | XI2.12567.1.A1_at | --- | --- | --- | 0.0142887 | 7.39464 |
| 8933   | XI2.20609.1.S1_at | tcctex1d1-a | tcctex1 domain-containing protein 1-A | NM_001096648 | 0.00298875 | 7.35295 |
| Gene Symbol | Description | Accession | Log2 Fold Change | p-Value |
|-------------|-------------|-----------|----------------|---------|
| XI2.50456.1.A1_at | --- | --- | --- | 0.0556426 | 7.28131 |
| XI2.23166.1.S1_at | tmem53-b transmembrane protein 53 | NM_001093021 | --- | 0.0160528 | 7.27875 |
| XI2.32096.1.S1_at | enkur enkuri, TRPC channel interacting protein | NM_001095267 | --- | 0.0181146 | 7.17614 |
| XI2.91621.1.S1_at | ttc21a tetratricopeptide repeat domain 21A | NM_001091636 | --- | 0.0388703 | 7.15609 |
| XI2.67881.1.S1_at | LOC496230 UPF0573 protein B | NM_001095416 | --- | 0.0175056 | 7.06311 |
| XI2.89121.1.S1_at | grasp GRP1-associated scaffold protein | NM_001085984 | --- | 0.0260989 | 7.07711 |
| XI2.16599.1.S1_at | MGC80975 MGC80975 protein | NM_001092408 | --- | 0.0423871 | 6.93597 |
| XI2.13352.1.S1_at | rshp1 radial spoke head 1 homolog | NM_001095320 | --- | 0.0156417 | 6.90444 |
| XI2.55482.1.S1_at | ccdc113 coiled-coil domain containing 113 | --- | --- | 0.0452484 | 8.68065 |
| XI2.2908.1.A1_at | --- | --- | --- | 0.0174418 | 8.6486 |
| XI2.16114.1.S1_at | --- | --- | --- | 0.0090755 | 8.61848 |
| XI2.3198.1.S1_at | c13orf26 chromosome 13 open reading frame 26 | NM_001095256 | --- | 0.0297405 | 7.68483 |
| XI2.7193.1.S1_at | --- | --- | --- | 0.0190573 | 6.6378 |
| XI2.11546.1.A1_at | --- | --- | --- | 0.0074031 | 6.63436 |
| XI2.33617.1.A1_at | --- | --- | --- | 0.0068934 | 6.62174 |
| XI2.19135.1.S1_at | --- | --- | --- | 0.0030149 | 6.53265 |
| XI2.12436.1.A1_at | --- | --- | --- | 0.0197631 | 6.50189 |
| XI2.1396.1.S1_at | foxj1 forkhead box J1 | NM_001090175 | --- | 0.0112107 | 6.46448 |
| XI2.7007.1.S1_at | poc1a POC1 centriolar protein homolog A | NM_001086414 | --- | 0.0024860 | 6.42153 |
| XI2.54538.1.S1_at | --- | --- | --- | 0.0305946 | 6.37087 |
| XI2.50128.1.S1_at | lirc67 leucine rich repeat containing 67 | NM_001095321 | --- | 0.012322 | 6.34559 |
| XI2.12902.1.A1_at | --- | --- | --- | 0.0017106 | 6.3427 |
| XI2.15603.1.A1_at | --- | --- | --- | 0.0417029 | 6.18644 |
| XI2.52195.1.S1_at | --- | --- | --- | 0.0070173 | 6.1654 |
| XI2.48614.1.S1_at | efcb1 EF-hand calcium binding domain 1 | NM_001096226 | --- | 0.0134406 | 6.04893 |
| XI2.25505.1.A1_at | MGC84778 MGC84778 protein | NM_001092848 | --- | 0.0083997 | 6.0201 |
| XI2.18686.1.A1_at | --- | --- | --- | 0.00042142 | 5.99943 |
| XI2.55424.1.S1_at | MGC130930 hypothetical protein MGC130930 | NM_001096367 | --- | 0.0362181 | 5.96315 |
| XI2.1504.1.A1_at | rshp3 radial spoke 3 homolog | NM_001127814 | --- | 0.0134057 | 5.8542 |
| XI2.48559.1.S1_at | c4orf47 chromosome 4 open reading frame 47 | NM_001094745 | --- | 0.0263804 | 5.8476 |
| XI2.21493.1.S1_at | hsp70 heat shock 70kDa protein | NM_001127675 | --- | 0.0259389 | 5.74419 |
| XI2.5593.1.S1_at | MGC131354 hypothetical protein MGC131354 | NM_001096328 | --- | 0.0164894 | 5.73295 |
| XI2.53906.1.S1_at | tbc1 tubulin folding cofactor E-like | NM_001096263 | --- | 0.00793291 | 5.71029 |
| XI2.16772.1.S1_at | dnal1 dynein, axonemal, light intermediate chain 1 | NM_001092504 | --- | 0.0166189 | 5.674 |
| XI2.32099.1.S1_at | --- | --- | --- | 0.0157209 | 5.51393 |
| XI2.9637.1.A1_at | --- | --- | --- | 0.0212796 | 5.49621 |
| XI2.8193.1.S1_at | fam161a family with sequence similarity 161A | NM_001094135 | --- | 0.0315916 | 5.42 |
| XI2.53803.1.S1_at | alphatub84b tubulin, alpha 1 | NM_001086587 | --- | 0.0163651 | 5.36767 |
| XI2.22601.1.S1_at | LOC496239 hypothetical LOC496239 | NM_001095424 | --- | 0.0099554 | 5.35343 |
| XI2.25762.1.A1_at | MGC154819 rieske domain protein (5K704) | NM_001096967 | --- | 0.0118107 | 5.33314 |
| XI2.3026.1.S1_at | ak7 adenylate kinase 7 | NM_001087577 | --- | 0.0483428 | 5.29683 |
| XI2.10830.1.S1_at | c8orf68 chromosome 8 open reading frame 68 | NM_001096282 | --- | 0.0081382 | 5.28235 |
| XI2.50244.1.A1_at | --- | --- | --- | 0.0320162 | 5.25955 |
| XI2.50116.1.S1_at | zmynd10 zinc finger, MYND-type containing 10 | NM_001096803 | --- | 0.00105248 | 5.22893 |
| XI2.46857.1.S1_at | c2orf73 chromosome 2 open reading frame 73 | --- | --- | 0.0102017 | 5.22128 |
| XI2.35387.1.S1_at | morn2 MORN repeat containing 2 | NM_001171791 | --- | 0.00378051 | 5.17837 |
| XI2.14916.1.A1_at | --- | --- | --- | 0.0451372 | 5.1507 |
| XI2.10706.1.A1_at | tcex1d1 Tcex1 domain containing 1 | NM_00113340 | --- | 0.0119346 | 4.99307 |
| XI2.50028.1.S1_at | lirc18 leucine rich repeat containing 18 | NM_001095397 | --- | 0.0319065 | 4.95714 |
| XI2.12748.1.S1_at | --- | --- | --- | 0.0465248 | 4.88995 |
| XI2.13320.1.A1_at | --- | --- | --- | 0.0107002 | 4.88707 |
| XI2.34888.1.S1_at | --- | --- | --- | 0.027676 | 4.87608 |
| XI2.15197.1.S1_at | --- | --- | --- | 0.0292191 | 4.87578 |
| XI2.15406.1.S1_at | --- | --- | --- | 0.0200187 | 4.82485 |
| Ensembl ID | Protein Name                                                   | Description                                                                 | EASE score | p-value  |
|------------|---------------------------------------------------------------|-----------------------------------------------------------------------------|------------|----------|
| X1L.53719.1.A1_at | ccdc61                                                        | coiled-coil domain containing 61                                            | NM_001096129 | 0.044177 3.65758 |
| X1L.14899.1.S1_at | c2orf59                                                       | chromosome 21 open reading frame 59                                          | NM_001093382 | 0.0198472 3.60673 |
| X1L.47916.1.S1_at | cep76                                                         | centrosomal protein 76kDa                                                    | NM_001093268 | 0.0206297 3.56949 |
| X1L.23570.4.S1_at | fam166b                                                       | family with sequence similarity 166B                                         | NM_001112874 | 0.0073435 3.55934 |
| X1L.14126.1.A1_at | LOC10013762                                                    | hypothetical protein LOC10013762                                            | ---         | 0.0473345 3.55656 |
| X1L.15295.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0147642 3.54979 |
| X1L.50709.1.S1_at | ---                                                           | ---                                                                         | ---         | 0.0134812 3.53433 |
| X1L.47812.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0085748 3.53098 |
| X1L.22899.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.00411775 3.50058 |
| X1L.13179.2.S1_at | ---                                                           | ---                                                                         | ---         | 0.0283086 3.49548 |
| X1L.11474.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0276951 3.48228 |
| X1L.15384.1.S1_at | rshph9                                                        | radial spoke head 9 homolog                                                  | NM_001096314 | 0.00117608 3.46226 |
| X1L.4896.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.00533392 3.45043 |
| X1L.33765.2.A1_at | ---                                                           | ---                                                                         | ---         | 0.00868042 3.44786 |
| X1L.5242.1.S1_at | fbxw9                                                         | F-box and WD repeat domain containing 9                                      | NM_001095104 | 0.00509669 3.43261 |
| X1L.15107.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.028586 3.38795 |
| X1L.47790.1.A1_at | map9                                                          | microtubule-associated protein 9                                            | NM_001135083 | 0.0443962 3.3631 |
| X1L.9586.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0118474 3.36106 |
| X1L.17997.1.S1_at | nme5                                                          | non-metastatic cells 5,                                                     | NM_001094325 | 0.000348802 3.35499 |
| X1L.9307.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0369663 3.31553 |
| X1L.34137.1.S1_at | tmem107                                                       | transmembrane protein 107                                                    | NM_001096639 | 0.0429918 3.28513 |
| X1L.50133.1.S1_at | cluap1                                                        | clusterin associated protein 1                                               | NM_001096997 | 0.0414825 3.28473 |
| X1L.41634.1.S1_at | ---                                                           | ---                                                                         | ---         | 0.0369187 3.27792 |
| X1L.14992.1.S1_at | adrb1                                                         | adrenergic, beta-1-, receptor                                               | NM_001090683 | 0.00402203 3.27067 |
| X1L.50660.1.S1_at | ---                                                           | ---                                                                         | ---         | 0.00754443 3.26597 |
| X1L.22500.2.S1_at | ---                                                           | ---                                                                         | ---         | 0.0271428 3.22273 |
| X1L.16544.1.S1_at | ropn1                                                         | ropparin 1-like                                                             | NM_001096022 | 0.0395763 3.21842 |
| X1L.18143.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.00412232 3.21254 |
| X1L.24665.1.S1_at | fam177a1                                                      | family with sequence similarity 177                                         | NM_001093442 | 0.0302837 3.20831 |
| X1L.18707.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0268119 3.20367 |
| X1L.49864.1.S1_at | cyb5d1                                                        | cytochrome b5 domain containing 1                                           | NM_001095358 | 0.0252493 3.20342 |
| X1L.7581.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0087326 3.1878 |
| X1L.50278.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.044593 3.17827 |
| X1L.598.2.S1_a_at | LOC10013761                                                  | 2 RadB-binding protein                                                       | NM_001114768 | 0.00532586 3.15692 |
| X1L.18865.1.S1_at | ankrd45                                                       | ankyrin repeat domain 45                                                     | NM_001095276 | 0.0236915 3.14263 |
| X1L.32093.1.S1_at | styxl1                                                        | serine/threonine/tyrosine interacting-like 1                                 | NM_001112823 | 0.0158346 3.13841 |
| X1L.11326.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0254898 3.13147 |
| X1L.50567.1.S1_at | MGC131269                                                    | hypothetical protein MGC131269                                              | NM_001096298 | 0.0370739 3.10646 |
| X1L.16441.1.S1_at | wrd16                                                        | WD repeat domain 16                                                          | NM_001095991 | 0.0205917 3.10544 |
| X1L.21906.1.S1_at | MGC53997                                                    | hypothetical protein MGC53997                                               | NM_001086046 | 0.0124281 3.1034 |
| X1L.18655.1.A1_at | ---                                                           | ---                                                                         | ---         | 0.0446853 3.06381 |
| X1L.23694.1.S1_at | paq8                                                         | progestin and adipoQ receptor                                                | NM_001085861 | 0.00983051 3.05876 |
| X1L.1507.1.S1_at | ttc25                                                        | tetra-tripeptide repeat domain 25                                            | NM_001091143 | 0.0250721 3.05198 |
| X1L.48341.1.S1_at | acyp1                                                        | acylphosphatase 1                                                            | NM_001093244 | 0.00350934 3.05172 |
| X1L.53987.1.S1_at | dna2                                                         | dynamin, axonal, intermediate chain 2                                       | NM_001096041 | 0.0267522 3.04577 |
| X1L.52384.2.S1_s_at | tbcel                                                        | tubulin folding cofactor E-like                                             | NM_001096263 | 0.00442192 3.04154 |
| X1L.49913.1.S1_at | ppi6                                                        | peptidylprolyl isomerase 6                                                  | NM_001095206 | 0.00408559 3.02166 |
| X1L.33489.1.S1_x_at | ---                                                           | ---                                                                         | ---         | 0.00302158 3.01153 |
| X1L.10381.1.S1_at | rage                                                         | renal tumor antigen                                                          | NM_001091332 | 0.0160438 3.00399 |
| Gene Symbol | Description                           | NM Accession | Log2 Fold Change |
|-------------|---------------------------------------|--------------|-----------------|
| Xt2.55518.1.A1_s_at | ift80, intraflagellar transport 80 homolog | ---          | 0.047283        |
| 19338       | ---                                    | ---          | 0.0139021       |
| 21971       | iqcd, IQ motif containing D             | ---          | 0.0496283       |
| 8517        | oscp1, organic solute carrier partner 1 | NM_001095812 | 0.000268336     |
| 13814       | ---                                    | ---          | 0.0346776       |
| 19293       | spag6, sperm associated antigen 6      | NM_001096217 | 0.0293709       |
| 447         | ---                                    | ---          | 0.0106676       |
| 14114       | arl2bp, ADP-ribosylation factor binding protein | NM_001092856 | 0.0278399       |
| 5233        | ---                                    | ---          | 0.0418996       |
| 24322       | ---                                    | ---          | 0.0321495       |
| 4326        | ---                                    | ---          | 0.0049692       |
| 15297       | xicl, Xicl gene                        | NM_001088319 | 0.0112724       |
| 25718       | ---                                    | ---          | 0.0308837       |
| 1324        | c16orf48, chromosome 16 open reading frame 48 | ---          | 0.0106164       |
| 31417       | giyd1, GIY-YIG domain containing 1     | NM_001096907 | 0.0127051       |
| 4557        | wrd78, WD repeat domain 78             | NM_001092231 | 0.0086076       |
| 4904        | ---                                    | ---          | 0.0469432       |
| 4715        | ---                                    | ---          | 0.0240036       |
| 5231        | ---                                    | ---          | 0.0341287       |
| 8229        | LOC398327, Leucine-rich protein         | NM_001088793 | 0.0037179       |
| 19392       | MGC78844, hypothetical protein MGC78844 | NM_001091575 | 0.0475398       |
| 20445       | tctn1, tectonic family member 1        | ---          | 0.0195683       |
| 1416        | ccdc45, coiled-coil domain containing 45 | NM_001094269 | 0.0262645       |
| 19343       | fam164a, family with sequence similarity 164A | NM_001095330 | 0.0376864       |
| 15641       | gmppaa, GDP-mannose pyrophosphorylase A a | NM_001092574 | 0.0197683       |
| 30095       | stil, SCL/TAL1 interrupting locus      | NM_001091352 | 0.0078397       |
| 17323       | ---                                    | ---          | 0.0160091       |
| 24372       | ---                                    | ---          | 0.0464603       |
| 11615       | bbs5, Bardet-Biedl syndrome 5          | NM_001094313 | 0.020913        |
| 3450        | ---                                    | ---          | 0.025805        |
| 4891        | cep70, centrosomal protein 70kDa      | NM_001095745 | 0.0063145       |
| 14270       | ---                                    | ---          | 0.0229752       |
| 2258        | ---                                    | ---          | 0.0054825       |
| 22026       | ---                                    | ---          | 0.0071458       |
| 31562       | ---                                    | ---          | 0.0196943       |
| 15474       | tsga14, testis specific, 14           | NM_001091191 | 0.0469745       |
| 29444       | six3, SIX homeobox 3                   | NM_001085702 | 0.045936        |
| 5801        | wdr92, WD repeat domain 92            | NM_001095400 | 0.0113546       |
| 24323       | ---                                    | ---          | 0.0017918       |
| 16319       | ---                                    | ---          | 0.0250045       |
| 412         | ---                                    | ---          | 0.0122942       |
| 22477       | ---                                    | ---          | 0.0270132       |
| 1327        | ---                                    | ---          | 0.0343579       |
| 21857       | hvcn1, hydrogen voltage-gated channel 1 | NM_001095406 | 0.022652        |
| 21783       | c9orf96, chromosome 9 open reading frame 96 | NM_001095209 | 0.0291666       |
| 3996        | lrcc1, leucine rich repeat and coiled-coil domains | NM_001091416 | 0.000267806     |
| 7838        | wdr54, D3Mm3e protein                  | NM_001096867 | 0.0460423       |
| 27374       | ---                                    | ---          | 0.0059904       |
| 646         | csppl, centrosome associated protein 1 | NM_001093709 | 0.0365307       |
| 28895       | ---                                    | ---          | 0.028690        |
| 12502       | ift52, intraflagellar transport 52 homolog | NM_001094130 | 0.0167209       |
| 27094       | ---                                    | ---          | 0.023339        |
| 6376        | ---                                    | ---          | 0.0279247       |
| Gene Symbol | Description | Mapped P-value | Mapped q-value |
|-------------|-------------|----------------|----------------|
| kif3b | hypothetically | 0.0207729 | 2.49753 |
| MGC154843 | MGC154843 | 0.0108184 | 2.49056 |
| dyx1c1 | dysexia susceptibility 1 candidate 1 | 0.00547077 | 2.48855 |
| MGC85335 | MGC85335 | 0.0146678 | 2.47833 |
| ribc2 | RIB43A domain containing 1 | 0.0294438 | 2.46685 |
| MGC53401 | MGC53401 | 0.00921702 | 2.43382 |
| cdk7 | cyclin-dependent kinase 7 | 0.00364861 | 2.42524 |
| MGC53601 | MGC53601 | 0.0214099 | 2.41435 |
| MGC53012 | MGC53012 | 0.006907 | 2.39497 |
| MGC154843 | MGC154843 | 0.0430293 | 2.3879 |
| MGC85335 | MGC85335 | 0.00352031 | 2.38686 |
| cdc52 | coiled-coil domain containing 52 | 0.0327136 | 2.38075 |
| MGC6951 | MGC6951 | 0.00711945 | 2.36706 |
| MGC154843 | MGC154843 | 0.0313371 | 2.36582 |
| MGC154843 | MGC154843 | 0.0245348 | 2.36354 |
| MGC85335 | MGC85335 | 0.0229635 | 2.35985 |
| phosphatase, orphan 2 | 0.0014433 | 2.3592 |
| katna2 | katanin p60 subunit A-like 2 | 0.00319868 | 2.34871 |
| MGC154843 | MGC154843 | 0.0011655 | 2.3416 |
| MGC154843 | MGC154843 | 0.0235788 | 2.34022 |
| heatr6 | HEAT repeat containing 6 | 0.0307357 | 2.33754 |
| MGC154843 | MGC154843 | 0.0301154 | 2.33473 |
| ankrd42 | ankyrin repeat domain 42 | 0.0217237 | 2.33276 |
| MGC154843 | MGC154843 | 0.00565958 | 2.32779 |
| nfi3 | nuclear factor, interleukin 3 regulated | 0.000322454 | 2.31563 |
| MGC154843 | MGC154843 | 0.0112065 | 2.31067 |
| heatr6 | HEAT repeat containing 6 | 0.0318565 | 2.30877 |
| heatr6 | HEAT repeat containing 6 | 0.04554 | 2.30209 |
| MGC154843 | MGC154843 | 0.00609102 | 2.29373 |
| D3Mm3e protein | 0.0244651 | 2.29143 |
| MGC154843 | MGC154843 | 0.00140921 | 2.29116 |
| MGC154843 | MGC154843 | 0.044514 | 2.2861 |
| MGC154843 | MGC154843 | 0.01252 | 2.28489 |
| transmembrane 4 L6 family member 18 | 0.036045 | 2.27364 |
| Rab interacting lysosomal protein-like 2 | 0.00987861 | 2.27269 |
| heatr6 | HEAT repeat containing 6 | 0.0310517 | 2.27113 |
| radial spoke 3 homolog | 0.0047759 | 2.27099 |
| MGC154843 | MGC154843 | 0.000188626 | 2.26974 |
| MGC154843 | MGC154843 | 0.0450235 | 2.26897 |
| chromosome 6 open reading frame 120 | 0.0298141 | 2.26466 |
| Ikaros family zinc finger 4 (Eos) | 0.00233767 | 2.26198 |
| RuvB-like 2 | 0.00937095 | 2.24147 |
| radial spoke 3 homolog | 0.0331739 | 2.23736 |
| trichoplein, keratin filament binding | 0.0333082 | 2.23486 |
| MGC154843 | MGC154843 | 0.0319668 | 2.23458 |
| MGC154843 | MGC154843 | 0.000102949 | 2.2337 |
| ADP-ribosylation factor-like 6 | 0.0345209 | 2.22841 |
| WD repeat domain 67 | 0.0113919 | 2.22783 |
| dynein, axonemal, light chain 4 | 0.00231503 | 2.22042 |
| MGC154843 | MGC154843 | 0.0391493 | 2.21875 |
| kinesin family member 3B | 0.0276089 | 2.20043 |
| hypothetical protein LOC10003723 | 0.00920296 | 2.19894 |
| Probe ID          | Description                                      | Accession Number | Log2 Ratio  | P-value       |
|------------------|--------------------------------------------------|------------------|-------------|---------------|
| X12.17143.1.S1_at| --                                               | ---              | ---         | 0.0089113     |
| X12.17266.2.A1_at| --                                               | ---              | ---         | 0.00841135    |
| X12.2054.1.A1_at | --                                               | ---              | ---         | 0.0106908     |
| X12.52635.1.S1_at| --                                               | ---              | ---         | 0.00854027    |
| X12.7809.1.S2_at | MGC81892                                        | NM_001092597     | 0.0339836   |
| X12.8721.1.S1_at | robbt2                                          | --               | 0.00931234  |
| X12.24168.1.A1_at| --                                               | ---              | 0.0203624   |
| X12.29662.1.S1_at| --                                               | ---              | 0.0329403   |
| X12.47125.1.S1_at| erffi1                                           | NM_001091771     | 0.0294676   |
| X12.53449.1.S1_at| wrd34                                           | NM_001096232     | 0.0274141   |
| X12.16223.1.A1_at| if5t7                                           | NM_001114796     | 0.0194608   |
| X12.16852.1.S1_at| --                                               | ---              | 0.030678    |
| X12.16013.1.A1_at| --                                               | ---              | 0.0145745   |
| X12.56196.1.A1_at| --                                               | ---              | 0.0117468   |
| X12.9867.1.A1_at | --                                               | ---              | 0.0488131   |
| X12.16666.1.A1_at| --                                               | ---              | 0.0457041   |
| X12.305.1.S1_at  | myb                                              | NM_001088299     | 0.0435306   |
| X12.6525.1.S1_at | --                                               | ---              | 0.0158054   |
| X12.11297.1.A1_at| --                                               | ---              | 0.017806    |
| X12.33183.3.A1_s_at| --                                           | ---              | 0.0155167   |
| X12.53938.1.A1_at| --                                               | ---              | 0.030971    |
| X12.9932.1.A1_at | c11orf60                                         | NM_001096924     | 0.0173787   |
| X12.18370.1.A1_at| --                                               | ---              | 0.0148488   |
| X12.52274.1.S1_at| --                                               | ---              | 0.00172095  |
| X12.14485.1.A1_at| --                                               | ---              | 0.00191374  |
| X12.25699.1.S1_at| gaint4                                           | NM_001091569     | 0.0354504   |
| X12.52847.1.A1_at| heat2                                            | NM_001097038     | 0.0347046   |
| X12.55004.1.A1_at| --                                               | ---              | 0.0244319   |
| X12.52662.1.S1_at| --                                               | ---              | 0.0392931   |
| X12.6491.1.S1_at | --                                               | ---              | 0.00472834  |
| X12.14491.1.A1_at| --                                               | ---              | 0.00451896  |
| X12.3143.2.S1_at | --                                               | ---              | 0.00175328  |
| X12.47133.1.S1_at| c14orf102                                         | NM_001091502     | 0.0401611   |
| X12.2079.1.S1_at | b3gn7                                            | NM_001092198     | 0.0257549   |
| X12.23066.1.S1_at| meis3-b                                          | NM_001086063     | 0.0267127   |
| X12.56882.1.S1_at| --                                               | ---              | 0.0262125   |
| X12.24254.1.A1_at| --                                               | ---              | 0.0288217   |
| X12.662.1.S2_x_at| rbm24                                            | NM_001087526     | 0.0265437   |
| X12.2800.1.A1_at | --                                               | ---              | 0.0258051   |
| X12.9041.1.A1_at | --                                               | ---              | 0.0128973   |
| X12.48328.1.S1_at| tuba4b                                           | NM_001094468     | 0.0368511   |
| X12.50631.1.S1_at| --                                               | ---              | 0.0364518   |
| X12.47781.1.A1_at| --                                               | ---              | 0.00292148  |
| X12.46776.1.S1_at| iqcb1                                            | NM_001091181     | 0.0213398   |
| X12.7406.1.S1_at | cep97                                            | NM_001092201     | 0.0314793   |
| X12.4467.1.S1_at | --                                               | ---              | 0.0285431   |
| X12.56109.1.S1_at| --                                               | ---              | 0.0349619   |
| X12.19456.1.S1_at| MGC82290                                         | NM_001091777     | 0.0265272   |
| X12.10624.1.S1_at| --                                               | ---              | 0.0365515   |
| X12.14489.3.A1_at| --                                               | ---              | 0.0204822   |
| X12.25454.1.S1_at| --                                               | ---              | 0.00622262  |
| X12.23201.1.A1_x_at| --                                           | ---              | 0.0139609   |
| X12.2148.2.A1_a_at| --                                               | ---              | 0.0275141   |
| X12.44987.2.A1_x_at| --                                           | ---              | 0.00898882  |
| X12.17481.1.S1_at| mthfd2                                           | NM_001093337     | 0.0297963   |

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| Gene Symbol | Description | Accession | Log2 Ratio |
|-------------|-------------|-----------|------------|
| XI2.11505.1.S1_at | ADP-ribosylation factor-like 2 | NM_001095515 | 0.0217797 |
| XI2.15912.1.A1_s_at | phosphatase, orphan 2 | NM_001096935 | 0.0227095 |
| XI2.51583.2.A1_at | --- | --- | 0.0497085 |
| XI2.47404.1.S1_at | endothelial PAS domain protein 1 | NM_001092249 | 0.0154864 |
| XI2.18887.1.A1_at | --- | --- | 0.0388404 |
| XI2.19559.1.S1_at | coiled-coil domain containing 15 | NM_001096685 | 0.0409453 |
| XI2.46928.2.A1_x_at | --- | --- | 0.0170737 |
| XI2.13994.1.A1_at | --- | --- | 0.0431144 |
| XI2.1383.1.A1_at | --- | --- | 0.0026359 |
| XI2.6615.1.S1_at | DNA-damage-inducible transcript 4 | NM_001086322 | 0.0235549 |
| XI2.1205.1.S1_a_at | H1 histone family, member 0 | NM_001089228 | 0.0077851 |
| XI2.51209.1.A1_at | --- | --- | 0.0306849 |
| XI2.18997.1.S1_at | NIMA-related kinase 4 | NM_001091106 | 0.0107314 |
| XI2.15408.1.A1_at | --- | --- | 0.0126523 |
| XI2.50686.1.S1_at | --- | --- | 0.0013887 |
| XI2.54115.1.S1_at | --- | --- | 0.0147367 |
| XI2.48479.1.S1_at | chromosome 11 open reading frame 65 | NM_001095354 | 0.0388083 |
| XI2.53251.1.S1_at | coiled-coil domain containing 13B | NM_001095943 | 0.0046565 |
| XI2.15781.1.S1_at | TBC1 domain family, member 16 | NM_001086481 | 0.0056517 |
| XI2.10911.1.A1_at | LOC100037203 | NM_001113429 | 0.040114 |
| XI2.15201.1.A1_at | --- | --- | 0.0372432 |
| XI2.4275.1.S2_at | NIMA-related kinase 2 | NM_001086021 | 0.0434318 |
| XI2.50648.1.A1_x_at | Wiskott-Aldrich syndrome | NM_001097903 | 0.0276232 |
| XI2.19380.2.S1_at | NOP2/Sun domain family, member 7 | NM_001127753 | 0.0441174 |
| XI2.18090.1.S1_at | PDX1 C-terminal inhibiting factor 1 | NM_001095735 | 0.0264631 |
| XI2.1242.1.S1_at | arg1 | NM_001086948 | 0.0058789 |
| XI2.53466.1.S1_at | --- | --- | 0.0052357 |
| XI2.925.1.S1_at | pericentriolar material 1 | NM_001087900 | 0.0173649 |
| XI2.24570.1.A1_at | --- | --- | 0.013027 |
| XI2.50157.1.S1_at | --- | --- | 0.0214899 |
| XI2.21947.1.A1_x_at | mycn | v-myc | NM_001171989 | 0.0412758 |
| XI2.4276.1.S1_at | LOC397885 | cyclin A1 | NM_001088046 | 0.0174732 |
| XI2.13942.2.S1_at | --- | --- | 0.0376487 |
| XI2.17269.1.A1_at | --- | --- | 0.0097198 |
| XI2.5764.1.S2_at | solute carrier family 7 | NM_001091766 | 0.0177278 |
| XI2.56540.1.S1_at | ATP2c2 | ATPase, Ca++ transporting | NM_001096502 | 0.0409726 |
| XI2.34138.1.A1_at | --- | --- | 0.0078136 |
| XI2.15912.2.S1_s_at | phosphatase, orphan 2 | NM_001096935 | 0.0407664 |
| XI2.45827.1.S1_at | --- | --- | 0.0159166 |
| XI2.4480.1.S1_at | DCP1 | DCP1 decapping enzyme homolog B | NM_001093497 | 0.0177252 |
| XI2.9562.1.S1_at | protein phosphatase 2 (formerly 2A) | NM_001089820 | 0.0062348 |
| XI2.34505.1.S1_at | fut3 | fucosyltransferase 3 | NM_001090197 | 0.0235245 |
| XI2.9793.1.A1_at | --- | --- | 0.0031668 |
| XI2.34567.1.A1_a_at | --- | --- | 0.0124017 |
| XI2.16229.1.S1_at | LOC494771 | Hypothetical LOC494771 | --- | 0.0269994 |
| XI2.16274.1.S1_at | gp120 | G patch domain containing 3 | NM_001093138 | 0.0240904 |
| XI2.3473.1.S1_at | --- | --- | 0.0142967 |
| XI2.17591.2.A1_at | art16 | ADP-ribosylation factor-like 16 | NM_001096935 | 0.0392404 |
| XI2.13831.1.S1_at | --- | --- | 0.0339391 |
| XI2.24108.1.A1_at | --- | --- | 0.0028748 |
| XI2.9175.1.S1_at | --- | --- | 0.0423111 |
| XI2.48549.1.S1_at | --- | --- | 0.0118618 |
| XI2.21133.1.S1_at | ugcg | UDP-glucose ceramide glucosyltransferase | NM_001095097 | 0.0103388 |
| XI2.25380.1.S1_at | --- | --- | 0.0033759 |

**Note:** The table represents gene expression data with log2 ratios indicating the fold change in expression. The accession numbers and log2 ratios are provided for each gene.
| Gene ID | Symbol | Description                                      | PMIDs                      | Log2 Fold Change |
|--------|--------|--------------------------------------------------|----------------------------|-----------------|
| Xl2.18427.1.A1_at | --- | ---                                             | ---                        | 0.0402823       |
| Xl2.50793.1.S1_at | --- | ---                                             | ---                        | 0.0169277       |
| Xl2.17300.1.A1_at | --- | ---                                             | ---                        | 0.0482853       |
| Xl2.50219.1.S1_at | zfand2b | zinc finger, AN1-type domain 2B                  | NM_001095372               | 0.047597        |
| Xl2.26415.1.S1_at | tepp | testis, prostate and placenta expressed          | NM_001172203               | 0.0354614       |
| Xl2.5330.2.S1_at | --- | ---                                             | ---                        | 0.0379947       |
| Xl2.51615.1.S1_a_at | --- | ---                                             | ---                        | 0.0178873       |
| Xl2.55012.1.A1_at | --- | ---                                             | ---                        | 0.0325429       |
| Xl2.18107.1.A1_at | --- | ---                                             | ---                        | 0.0175705       |
| Xl2.13570.1.S1_at | --- | ---                                             | ---                        | 0.00422703      |
| Xl2.56882.1.S1_a_at | --- | ---                                             | ---                        | 0.00746597      |
| Xl2.11681.1.S1_at | eirA | Ribonucleoprotein                               | NM_001090609               | 0.0205581       |
| Xl2.26303.1.S1_at | tmem110.2 | transmembrane protein 11                        | NM_001094770               | 0.00447563      |
| Xl2.13979.1.A1_at | --- | ---                                             | ---                        | 0.0330068       |
| Xl2.7087.1.S1_at | aida-a | axin interactor, dorsalization associated         | NM_001089723               | 0.0218335       |
| Xl2.53796.1.S2_at | cldn4 | claudin 4                                       | NM_001096114               | 0.0460746       |
| Xl2.162223.2.S1_at | ifit57 | intraflagellar transport 57 homolog             | NM_001114796               | 0.00952381      |
| Xl2.52119.1.S1_a_at | --- | ---                                             | ---                        | 0.00350505      |
| Xl2.11115.1.S1_at | MGC68847 | hypothetical protein MGC68847                  | NM_001086472               | 0.0311481       |
| Xl2.18065.1.S1_at | --- | ---                                             | ---                        | 0.0113684       |
| Xl2.21337.1.S1_at | --- | ---                                             | ---                        | 0.036548       |
| Xl2.15013.1.A1_at | --- | ---                                             | ---                        | 0.00267869      |
| Xl2.52154.1.A1_at | --- | ---                                             | ---                        | 0.0495246       |
| Xl2.51044.2.S1_s_at | --- | ---                                             | ---                        | 0.0383234       |
| Xl2.55425.1.S1_at | MGC131023 | hypothetical protein MGC131023                | NM_001096390               | 0.000994242     |
| Xl2.9817.1.A1_at | --- | ---                                             | ---                        | 0.00338675      |
| Xl2.5387.1.A1_at | --- | ---                                             | ---                        | 0.0081053       |
| Xl2.32266.1.A1_at | --- | ---                                             | ---                        | 0.035937        |
| Xl2.16443.1.A1_at | --- | ---                                             | ---                        | 6.95E-05        |
| Xl2.54741.1.A1_at | --- | ---                                             | ---                        | 0.0126945       |
| Xl2.26329.1.S1_at | fam98a | family with sequence similarity 98A              | NM_001093909               | 0.0187014       |
| Xl2.52489.1.S1_at | --- | ---                                             | ---                        | 0.00272243      |
| Xl2.3804.1.S1_at | rab25 | RAB25, member RAS oncogene family                | NM_001092754               | 0.0304989       |
| Xl2.13889.1.A1_at | --- | ---                                             | ---                        | 0.0133434       |
| Xl2.17162.1.S1_at |nbr1 | neighbor of BRCA1 gene 1                        | NM_001086498               | 0.0402227       |
| Xl2.46835.1.A1_at | --- | ---                                             | ---                        | 0.0169528       |
| Xl2.10421.1.A1_at | --- | ---                                             | ---                        | 0.00842378      |
| Xl2.50228.1.S1_at | LOC733401 | Hypothetical protein LOC733401              | ---                        | 0.0158585       |
| Xl2.15526.1.S1_at | MGC131013 | hypothetical protein MGC131013              | NM_001096386               | 0.0457184       |
| Xl2.53011.1.S1_at | --- | ---                                             | ---                        | 0.000316716     |
| Xl2.31053.1.A1_at | --- | ---                                             | ---                        | 0.0184217       |
| Xl2.6970.1.S1_at | ndufa8 | NADH dehydrogenase (ubiquinone)                | NM_001096329               | 0.0143902       |
| Xl2.12619.1.A1_at | --- | ---                                             | ---                        | 0.0403782       |
| Xl2.3583.1.A1_at | --- | ---                                             | ---                        | 0.0457662       |
| Xl2.48756.1.S1_at | parp11 | poly (ADP-ribose) polymerase family             | NM_001095214               | 0.00349853      |
| Xl2.14815.1.S1_a_at | ptpn4 | protein tyrosine phosphatase                    | NM_001091402               | 0.0378902       |
| Xl2.517.1.S1_at | rnd1 | Rho family GTPase 1                            | NM_001086916               | 0.0147073       |
| Xl2.55325.1.A1_at | --- | ---                                             | ---                        | 0.0394861       |
| Xl2.45447.1.S1_at | cab39l | calcium binding protein 39-like                | NM_001089927               | 0.0229577       |
| Xl2.8984.1.A1_at | --- | ---                                             | ---                        | 0.0336592       |
| Xl2.30259.1.S1_at | ccng2 | cyclin G2                                       | NM_001091414               | 0.0214231       |
| Xl2.13148.1.S1_x_at | hmgcs1 | hmgcs1                                          | NM_001086930               | 0.0097600       |
| Xl2.16687.1.S1_at | --- | ---                                             | ---                        | 0.0441968       |
| Xl2.21276.1.S1_at | --- | ---                                             | ---                        | 0.0116894       |
| Xl2.15405.1.S1_at | c2orf7 | chromosome 2 open reading frame 7              | ---                        | 0.0261782       |
| Gene          | Description                                  | Symbol   | Accession     | Log2 Fold Change | p-Value   |
|--------------|----------------------------------------------|----------|--------------|-----------------|-----------|
| XI2.24341.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0342072 | 1.43362 |
| XI2.1998.1.S1_at | ebi3, Epstein-Barr virus induced 3            | ---      | ---          | ---              | 0.00246739 | 1.43201 |
| XI2.54541.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0432921 | 1.42582 |
| XI2.19794.1.S1_at | rab37, RAB37, member RAS oncogene family      | ---      | ---          | ---              | 0.0127193 | 1.4256  |
| XI2.57009.2.S1_at | ---                                           | ---      | ---          | ---              | 0.00859164 | 1.42128 |
| XI2.48334.1.S1_at | pibf1, progesterone binding factor 1          | ---      | NM_001093473 | 0.0378279       | 1.41931  |
| XI2.56626.1.S1_at | ndufab1, NADH dehydrogenase (ubiquinone)     | ---      | NM_001096333 | 0.0342459       | 1.41228  |
| XI2.52459.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0264144 | 1.41128 |
| XI2.29269.1.S1_at | MGC81138, hypothetical protein MGC81138       | ---      | NM_001091403 | 0.0118032       | 1.41031  |
| XI2.4918.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.0245228 | 1.40707 |
| XI2.50372.1.S1_at | MGC84453, protein                           | ---      | NM_001094299 | 0.017179       | 1.40535  |
| XI2.13193.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0102991 | 1.40414 |
| XI2.9547.1.S1_at  | tmem106c, transmembrane protein 106C          | ---      | NM_001086160 | 0.0338772       | 1.40168  |
| XI2.10367.2.S1_at | bnxdc9, thioredoxin domain containing 9       | ---      | NM_001087441 | 0.0268988       | 1.39978  |
| XI2.5016.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.00543603 | 1.39812 |
| XI2.34606.1.S1_at | tuba8, tubulin, alpha 8                      | ---      | NM_001096986 | 0.0165895       | 1.3974  |
| XI2.47576.1.S1_at | fbxo8, F-box protein 8                       | ---      | NM_001092821 | 0.0311321       | 1.39414 |
| XI2.25809.1.S1_at | frs1, ferric-chelate reductase 1              | ---      | NM_001091659 | 0.000364852     | 1.39146  |
| XI2.22525.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0418729 | 1.38841 |
| XI2.49701.1.S1_x_at | LOC733253, Hypothetical protein LOC733253     | ---      | ---          | ---              | 0.0435899 | 1.37984 |
| XI2.34369.1.S1_at | LOC432287, hypothetical protein LOC432287     | ---      | ---          | ---              | 0.0111182 | 1.37778 |
| XI2.14597.2.A1_at | ---                                           | ---      | ---          | ---              | 0.0119299 | 1.37722 |
| XI2.1573.1.S1_at  | sfrs3, splicing factor, arginine serine-rich 3| ---      | NM_001086991 | 0.0201116       | 1.37203  |
| XI2.7735.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.0147425 | 1.37147 |
| XI2.14658.2.S1_at | ---                                           | ---      | ---          | ---              | 0.038711 | 1.36729 |
| XI2.15619.1.S1_at | MGC81201, hypothetical protein MGC81201      | ---      | NM_001091115 | 0.0284168       | 1.36587  |
| XI2.18948.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0174907 | 1.36467 |
| XI2.13357.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0155035 | 1.36409 |
| XI2.21384.1.S1_at | cat1, calcium transporter 1                   | ---      | NM_001088867 | 0.0177117       | 1.36341  |
| XI2.17269.2.S1_at | ---                                           | ---      | ---          | ---              | 0.0457999 | 1.35072 |
| XI2.48555.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0337596 | 1.34879 |
| XI2.53075.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0139442 | 1.34822 |
| XI2.6440.1.S1_at  | phc2, polyhomeotic homolog 2                 | ---      | NM_001096010 | 0.0377609       | 1.34686  |
| XI2.6698.1.S1_at  | ---                                           | ---      | ---          | ---              | 0.0415166 | 1.34347 |
| XI2.18450.1.S1_at | wrd37, WD repeat domain 37                   | ---      | NM_001093426 | 0.0229857       | 1.34183 |
| XI2.3518.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.0109055 | 1.33463 |
| XI2.20118.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0282849 | 1.33387 |
| XI2.18607.1.S1_at | tbpl2, TATA box binding protein like 2       | ---      | NM_001087452 | 0.0310028       | 1.33118  |
| XI2.3069.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.041939  | 1.33074 |
| XI2.1860.1.A1_at  | ---                                           | ---      | ---          | ---              | 0.0263271 | 1.33007 |
| XI2.16708.2.S1_at | ---                                           | ---      | ---          | ---              | 0.0444879 | 1.32757 |
| XI2.2125.1.S1_at  | dbia, diazepam binding inhibitor (dbi) a      | ---      | NM_001092478 | 0.0129649       | 1.32687  |
| XI2.26342.1.S1_at | gsr, glutathione reductase                    | ---      | NM_001095853 | 0.0166614       | 1.32639  |
| XI2.56890.1.S1_at | ---                                           | ---      | ---          | ---              | 0.00386361 | 1.32559 |
| XI2.19015.1.A1_at | ---                                           | ---      | ---          | ---              | 0.00132632 | 1.32363 |
| XI2.35201.1.S1_at | ---                                           | ---      | ---          | ---              | 0.0436287 | 1.32251 |
| XI2.14454.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0145266 | 1.32054 |
| XI2.36445.1.S1_at | ---                                           | ---      | ---          | ---              | 0.00704493 | 1.31368 |
| XI2.10958.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0404025 | 1.31212 |
| XI2.15698.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0434316 | 1.31122 |
| XI2.44683.2.S1_at | ---                                           | ---      | ---          | ---              | 0.0477823 | 1.30812 |
| XI2.32386.1.A1_at | ---                                           | ---      | ---          | ---              | 0.0291253 | 1.30793 |
| Gene ID | Description                  | Accession       | P-value       | FDR           |
|--------|------------------------------|-----------------|---------------|---------------|
| XI2.50126.1.S1_at | LOC495967 hypothetical LOC495967 | NM_001095234 | 0.0401229    | 1.30362       |
| XI2.6850.1.A1_at  | ---                           | ---             | 0.011087     | 1.30059       |
| XI2.9617.1.A1_at  | ---                           | ---             | 0.00886599   | 1.30018       |
| XI2.32146.1.S1_at | MGC81773 MGC81773 protein     | NM_001093298   | 0.037713     | 1.29752       |
| XI2.37003.1.S1_at | nccap1 NECAP endocytosis associated 1 | NM_001092025 | 0.0279955   | 1.29698       |
| XI2.16777.1.A1_a_at | ---                           | ---             | 0.0132112    | 1.29661       |
| XI2.4031.1.S1_at  | nsdh1 NAD(P) dependent steroid dehydrogenase | NM_001095421 | 0.0127558    | 1.29451       |
| XI2.6557.1.S1_at  | atp1b1 ATPase, Na+/K+ transporting | NM_001086759 | 0.0453075    | 1.29407       |
| XI2.56409.2.A1_at | ---                           | ---             | 0.0188269    | 1.2933        |
| XI2.55749.1.A1_at | ---                           | ---             | 0.016579     | 1.29129       |
| XI2.13063.1.S1_at | anks3 ankyrin repeat and sterile alpha motif | NM_001091170 | 0.0303508    | 1.29043       |
| XI2.53904.1.A1_s_at | LOC733261 hypothetical protein LOC733261 | NM_001099861 | 0.0496631    | 1.29015       |
| XI2.44203.1.S1_a_at | luc7i3 LUC7-like 3 | NM_001096916 | 0.0482926    | 1.28914       |
| XI2.2148.3.S1_at | ---                           | ---             | 0.0168607    | 1.28599       |
| XI2.15954.1.S1_at | ---                           | ---             | 0.0407998    | 1.28535       |
| XI2.11282.2.S1_at | ---                           | ---             | 0.0456687    | 1.28468       |
| XI2.2824.1.S1_at  | ---                           | ---             | 0.00521253   | 1.28434       |
| XI2.9285.1.A1_at  | ---                           | ---             | 0.0486068    | 1.28371       |
| XI2.10784.1.A1_at | ---                           | ---             | 0.00978073   | 1.28307       |
| XI2.48395.1.S1_at | cdkn2d p19, inhibits CDK4     | NM_001093415   | 0.0406928    | 1.28166       |
| XI2.45292.1.S1_at | cisd1a CDGSH iron sulfur domain 1 a | NM_001094218 | 0.0154703    | 1.27731       |
| XI2.53955.1.S1_at | MGC116491 hypothetical protein MGC116491 | NM_001096155 | 0.0309655    | 1.27492       |
| XI2.47170.1.S1_at | slc7a4 solute carrier family 7 | NM_001091323 | 0.03185     | 1.2733        |
| XI2.10068.2.S1_at | ---                           | ---             | 0.0342503    | 1.27154       |
| XI2.18883.1.A1_at | ---                           | ---             | 0.0119723    | 1.27104       |
| XI2.24238.1.A1_at | ---                           | ---             | 0.0435839    | 1.27001       |
| XI2.14709.1.A1_at | LOC100037124 hypothetical protein LOC100037124 | ---       | 0.016482    | 1.2687        |
| XI2.4351.1.S1_at  | LOC733432 hypothetical protein LOC733432 | ---       | 0.0302682    | 1.26524       |
| XI2.2037.1.S1_at  | ---                           | ---             | 0.0076415    | 1.2635        |
| XI2.48214.1.S1_at | pxk PX domain containing kinase | NM_001096762 | 0.0396156    | 1.25767       |
| XI2.46429.2.S1_at | ---                           | ---             | 0.0284683    | 1.25546       |
| XI2.48890.1.S1_at | rpe65 retinal pigment epithelium-specific protein | NM_001094320 | 0.0211928   | 1.25397       |
| XI2.52168.3.A1_at | ---                           | ---             | 0.0284352    | 1.25335       |
| XI2.13807.1.A1_at | ---                           | ---             | 0.0200973    | 1.25314       |
| XI2.801.1.A1_at  | goosecoid goosecoid protein   | ---             | 0.00665542   | 1.25017       |
| XI2.23386.1.S1_at | ---                           | ---             | 0.00778662   | 1.24919       |
| XI2.15490.3.A1_at | ---                           | ---             | 0.0436468    | 1.24896       |
| XI2.4028.1.S1_at  | ---                           | ---             | 0.0384269    | 1.24674       |
| XI2.33675.1.S1_at | rabeppk Rab9 effector protein with kelch motifs | NM_001093901 | 0.0153992    | 1.24288       |
| XI2.586.1.S1_at   | edf1 endothelial differentiation-related factor | NM_001092165 | 0.026594    | 1.24284       |
| XI2.9514.1.S1_at  | ---                           | ---             | 0.00177958   | 1.24257       |
| XI2.1381.1.A1_at  | ---                           | ---             | 0.00190368   | 1.24256       |
| XI2.876.1.S1_at   | lyn v-yes-1                    | NM_001085646   | 0.0384166    | 1.2402        |
| XI2.29353.2.S1_at | ---                           | ---             | 0.0411088    | 1.24017       |
| XI2.42186.1.S1_at | ---                           | ---             | 0.0281397    | 1.23945       |
| XI2.9424.1.A1_at  | ---                           | ---             | 0.0426276    | 1.23943       |
| XI2.12006.1.A1_at | XSSu(H)1 Suppressor of hairless protein 1 | NM_001090878 | 0.0218998    | 1.23838       |
| XI2.145.1.S1_at   | MGC154801 hypothetical protein MGC154801 | NM_001096926 | 0.0100067    | 1.23783       |
| XI2.859.1.S_s_at  | MGC78985 // neuronal calcium sensor 1 | NM_001090619 // NM_001091667 | 0.0291083 | 1.23736       |
| XI2.52075.1.A1_at | ---                           | ---             | 0.0289582    | 1.23731       |
| XI2.4929.1.S1_at  | aff6 activating transcription factor 6 | NM_001095322 | 0.0046506    | 1.23634       |
| XI2.7102.1.S1_at  | plk4 polo-like kinase 4       | NM_001089677   | 0.0414889    | 1.2354        |
| XI2.719.1.S1_at   | MGC81684 MGC81684 protein     | NM_001094170   | 0.0375208    | 1.2345        |
| XI2.7817.1.S2_at  | xcen centrin                  | NM_001087929   | 0.0110742    | 1.23392       |
| XM_9922.2.A1_at | --- | --- | --- | --- | 0.00418007 | 1.20606 |
| 1911 | XM_31021.2.S1_a_at | --- | --- | --- | --- | 0.00867573 | -1.50125 |
| 31971 | XM_9367.1.A1_at | --- | --- | --- | --- | 0.000436022 | -1.50695 |
| 25644 | XM_35675.1.S1_at | --- | --- | --- | --- | 0.0368767 | -1.50751 |
| 29476 | XM_6315.2.S1_at | c1orf124 | chromosome 1 open reading frame 124 | --- | --- | 0.0209863 | -1.50794 |
| 708 | XM_10681.1.A1_at | --- | --- | --- | --- | 0.0109568 | -1.50827 |
| 27152 | XM_55330.1.S1_at | --- | --- | --- | --- | 0.0334487 | -1.50921 |
| 22299 | XM_50544.1.S1_at | --- | --- | --- | --- | 0.0205217 | -1.5168 |
| 5746 | XM_16344.3.S1_a_at | --- | --- | --- | --- | 0.0304854 | -1.51979 |
| 22231 | XM_50484.1.A1_at | --- | --- | --- | --- | 0.0464604 | -1.52187 |
| 16516 | XM_35366.2.S1_at | --- | --- | --- | --- | 0.0133418 | -1.52226 |
| 11386 | XM_24487.1.S1_at | a2ld1 | AIG2-like domain 1 | --- | --- | 0.0112459 | -1.52841 |
| 24474 | XM_52580.1.S1_at | --- | --- | --- | --- | 0.0166239 | -1.52868 |
| 27608 | XM_55778.1.S1_at | --- | --- | --- | --- | 0.0415489 | -1.53771 |
| 25916 | XM_5928.1.S1_at | MGC116527 | hypothetical protein MGC116527 | --- | --- | 0.0360195 | -1.5415 |
| 20100 | XM_77342.2.S1_at | --- | --- | --- | --- | 0.00910342 | -1.54214 |
| 20931 | XM_48044.2.S1_at | --- | --- | --- | --- | 0.002331 | -1.54331 |
| 17844 | XM_42644.1.S1_at | --- | --- | --- | --- | 0.0224784 | -1.54358 |
| 23436 | XM_51600.1.S1_at | --- | --- | --- | --- | 0.0207494 | -1.55088 |
| 29794 | XM_6681.1.S1_at | limd2 | LIM domain containing 2 | --- | --- | 0.0151645 | -1.55538 |
| 2694 | XM_13093.2.A1_at | --- | --- | --- | --- | 0.0469415 | -1.5602 |
| 24851 | XM_52945.1.S1_at | --- | --- | --- | --- | 0.0052777 | -1.56977 |
| 13300 | XM_28980.2.S1_at | --- | --- | --- | --- | 0.0306863 | -1.5712 |
| 10131 | XM_22706.2.S1_at | --- | --- | --- | --- | 0.0469541 | -1.57335 |
| 25781 | XM_53808.1.A1_a_at | --- | --- | --- | --- | 0.0237275 | -1.57592 |
| 5931 | XM_16538.1.S1_at | sdc4-b | syndecan-4.2 | --- | --- | 0.0165505 | -1.57716 |
| 14225 | XM_31166.1.S1_s_at | zymm2 | zinc finger, MYM-type 2 | --- | --- | 0.0201738 | -1.5789 |
| 18866 | XM_48208.1.S1_at | MGC81667 | MGC81667 protein | --- | --- | 0.0179483 | -1.59322 |
| 10875 | XM_23934.1.S1_at | gins1 | GINS complex subunit 1 (Psf1 homolog) | --- | --- | 0.0115396 | -1.59632 |
| 4496 | XM_15066.2.S1_at | --- | --- | --- | --- | 0.0262745 | -1.59637 |
| 30173 | XM_712.1.S1_at | mcm10 | MCM 10 | --- | --- | 0.00264608 | -1.60001 |
| 30200 | XM_7149.1.S1_at | mcm6.2 | MCM 6 | --- | --- | 0.0121643 | -1.60182 |
| Gene ID | Gene Name | Description | Log2 Fold Change | FDR p-value |
|---------|-----------|-------------|-----------------|-------------|
| NM_001085885 | ICD | 7-dehydrocholesterol reductase | 0.0316756 | -1.6026 |
| NM_001085696 | ICD | forkhead box G1 | 0.00654142 | -1.61262 |
| NM_001087979 | MCM 4 | bromodomain containing 3 | 0.0459135 | -1.62783 |
| NM_001087841 | PPAR delta | --- | 0.0188613 | -1.66801 |
| NM_001091893 | MCM 4 | zinc finger protein 367 | 0.0266614 | -1.64549 |
| NM_001092778 | MCM 4 | vaccinia related kinase 3 | 0.016293 | -1.65683 |
| NM_001087797 | MCM 4 | MCM 4 | 0.0303446 | -1.65723 |
| NM_001095072 | GLI pathogenesis-related 2 | --- | 0.0374829 | -1.68464 |
| NM_001097140 | MCM 4 | --- | 0.0471837 | -1.68469 |
| NM_001096262 | MCM 4 | --- | 0.0220291 | -1.68574 |

Table 2 Legend: Embryos were injected at the two-cell stage with ICDRNA or with both MCI-HGR and ICD RNA. At stage 10, animal caps were dissected, incubated until the equivalent of stage 11.5, treated with DEX, and then harvested 5 hours later for total RNA. Two independent experiments produced two sets of RNAs for each condition that were analyzed by hybridization to Affymetrix arrays as described in the Material and Methods. Hybridization results can be found in the GEO repository GSE32452, and analyzed above by pairwise comparison, where fold changes greater than 1.2 fold are shown, with a p value<0.01.