## SUPPLEMENTAL INFORMATION






C ATGCAAGCGTGCGAGGGCAGCGCAGCCGGACGCCGGGCCTTCGACAGCATCTGCCCCAACAGGATGCTGG
 ACCTGTCGCGGCGGACCCTCGGCAAGCCCGGGAAGCCGGAGAGGAAGTTCGTTCCTTCGTGGAAGTCCTT
 TTCGGGATGCGGTGGCGGCAGCagagcccgccccactgccagctctcacaaccatagacctgcaggacct $H_{G} \underset{G}{G} \underset{G}{G}$ cgccgactgcacctcgctgctcggaaccgaagcgtctcctagtggtgattcgtccgcgtcgcagaacccc



Fig. S1. Generation of a deletion allele at the mouse Mci locus. (A) Partial genomic sequence of the mouse Mci gene, showing the gRNAs (pink arrows) and their target sites on the forward and reverse strands (highlighted in yellow) used to induce a 32 bp deletion within exon 2. Binding sites for genotyping primers (McidasGT1 and McidasGT2) are also indicated. (B) Electropherogram showing 32 bp deletion in Mci exon 2. Also shown below is the conceptual translation of the wild-type and mutant Mci coding sequence around the deletion site. (C) Conceptual translation of the predicted mutant Mci ORF shows a highly truncated MCI protein, retaining only 54 native amino acids at the N -terminus. Sequences highlighted in yellow indicate disruption of the reading frame before the premature STOP codon. (D) Gel image of DNA fragments amplified in wild-type, heterozygote and homozygous Mci mutants using primers flanking the 32 bp deletion. Size of the wild-type band is 290 bp and the mutant band is 258 bp . (E) Sequence analysis of Mci cDNA obtained from tracheal tissue of the homozygous mutants confirms a deletion of 32 bp .


Fig. S2. Gross phenotypes of Mci knockout mice. (A) Mci knockout mice are smaller in size compared to the wild-type. (B) The body weight comparison between wild-type and Mci mutant mice at post-natal day (P) 28. $n=9$ for each genotype. (C) Percentage of lethality of wild type and Mci knockout mice at P28. $n=22$ for each genotype. (D) Wildtype mouse brain coronal section stained with H\&E. (E) Mci mutant mouse brain coronal section stained with $H \& E$. Note hydrocephalus with dilation of the lateral
ventricles (LV) and the third ventricle (TV). Scale bar $=1 \mathrm{~mm}$. (F) Nuclear localized FOXJ1 expression in MCCs of wild-type brain ependyma. Multicilia are indicated by arrows and the cytoskeletal microtubule network by asterisks. (G) Nuclear localized FOXJ1 expression in monociliated cells of Mci mutant brain ependyma. Monocilia are indicated by arrows and the cytoskeletal microtubule network by asterisks. Scale bars, 5 $\mu \mathrm{m}$.


Fig. S3. Mci mutant MCCs precursors differentiate a single cilium that localizes motile cilia-specific proteins but are unable to make multiple basal bodies. (A) RSPH9 co-localization with acetylated tubulin to MCC cilia of wild-type trachea (arrows). (B) RSPH9 localization to MCC cilia of wild-type trachea (arrows; display of only RSPH9 staining from panel A). (C) RSPH9 co-localization with acetylated tubulin
to single cilium of Mci mutant trachea (arrow). (D) RSPH9 localization to single cilium of Mci mutant trachea (arrow; display of only RSPH9 staining from panel C). (E) CCDC40 co-localization with acetylated tubulin to MCC cilia of wild-type trachea (arrows). (F) CCDC40 localization to MCC cilia of wild-type trachea (arrows; display of only CCDC40 staining from panel A). (G) CCDC40 co-localization with acetylated tubulin to single cilium of Mci mutant trachea (arrow). (H) CCDC40 localization to single cilium of Mci mutant trachea (arrow; display of only CCDC40 staining from panel C). (I) Wild-type MCC differentiated in ALI culture with multiple basal bodies (stained with anti-CENTRIN antibodies) and multiple cilia. (J) Display of only CENTRIN staining from panel E. (K) Mci mutant cells differentiated in ALI culture with single basal body (expressing CENTRIN, arrow) and single cilium. (L) Display of only CENTRIN staining from panel G showing single basal body (arrow). In all preparations, cilia were stained with anti-acetylated tubulin antibodies (green) and nuclei with DAPI (blue). Scale bars A-D $=10 \mu \mathrm{~m} ; \mathrm{E}-\mathrm{L}=5 \mu \mathrm{~m}$.


Fig. S4. Interaction of MCI with E2F factors and transcriptional activity of the

GMNC-MCI chimeric protein in HEK293T cells. (A) Co-immunoprecipitation data
showing interaction of MCI with E2F4 as well as E2F5. Human proteins were used for this experiment. (B) Amino acid sequence alignment of human GMNC, MCI and GM proteins. The C-terminal TIRT domain from MCI used to generate GM is underlined in red, and the TIRT resides in MCI and GM proteins are highlighted in blue. (C) Unlike wild-type GMNC, the GM chimeric protein is unable to induce FOXJ1 expression by itself or together with the E2F factors. (D) The GM protein is not more efficient in inducing $C D C 20 B$ expression than wild-type GMNC either by itself or with the E2F factors. For C and D, relative expression levels have been plotted along the $y$-axis, and overexpression conditions indicated along the $x$-axis. Error bars: SEM. Immunoblot and qPCR data are representative of 2 independent biological replicates. $\mathrm{p}:{ }^{* *} \leq 0.01$.


Fig. S5. Overexpression of GMNC and MCI in wild-type airway cell ALI culture induces supernumerary MCCs. (A) Lentivirus mediated overexpression of GFP in wild-type airway cell ALI culture does not affect numbers of differentiating MCCs. (B) Overexpression of GMNC in wild-type airway cell ALI culture induces supernumerary MCCs. (C) Overexpression of MCI in wild-type airway cell ALI culture induces supernumerary MCCs. Scale bars, $5 \mu \mathrm{~m}$. (D) Quantification of MCC numbers per field of view upon overexpression of GFP, GMNC and MCI in wild-type airway cell ALI
cultures. (E,F) RT-qPCR analysis of GMNC and MCI expression levels on overexpression of GMNC and MCI in Mci mutant airway cells cultured under ALI conditions. Relative expression levels have been plotted along the $y$-axis, and overexpression conditions indicated along the $x$-axis. Lentivirus-mediated overexpression of GFP, MCI and GMNC in ALI cultures represent 2 independent biological replicates; qPCR analysis represents 2 independent technical replicates. Error bars: SEM. p: ${ }^{*} \leq 0.05,{ }^{* *} \leq 0.01,{ }^{* * *} \leq 0.001$.


Fig. S6. RT-qPCR analysis of ciliary transcription factor and DD pathway genes expression levels on overexpression of MCI and GMNC in Mci mutant airway cells
cultured under ALI conditions. (A-F) Relative expression levels have been plotted along the $y$-axis, and overexpression conditions indicated along the $x$-axis. Error bars represent SEM. Analysis was done on 3 independent biological replicates. p: ${ }^{*} \leq 0.05$.

Table S1. Primer seqences

| Name of gRNA/primer | Sequence (5'-3') | Remarks |
| :---: | :---: | :---: |
| gRNA-Mcidas1 | CAGCCCGGTGGCGGTGTACGGTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGCTAGTCCGTT ATCAACTTGAAAAAGTGGCACCGAGTCGGTGC TTT | gRNA sequences |
| gRNA-Mcidas2 | GGGTCCTCGTACACCGCCACGTTTTAGAGCTA GAAATAGCAAGTTAAAATAAGGCTAGTCCGTT ATCAACTTGAAAAAGTGGCACCGAGTCGGTGC TTT |  |
| McidasGT1(for ward) | TGGTCCTGGCTCTGGGAGAGTCTGCC | Primers for genotyping of Mci mutant mice |
| McidasGT2(rev erse) | ACCAGGACCCTCAGTGAGGACCTCGG |  |
| Mci-L | CGGAGCAGTACTGGAAGGAG | qPCR primers for mouse genes |
| Mci-R | TTCGTTGTTGCCTTGATCTG |  |
| Gmnc-L | TCTGGAAGAGAAGGCCAAGA |  |
| Gmnc-R | CCCAGGTTGTTCCTCACAGT |  |
| Foxj1-L | GAGCTGGAACCACTCAAAGG |  |
| Foxj1-R | GGTAGCAGGGCAGTTGATGT |  |
| Rfx2-L | TGTGAGCCGATCCTACAGTG |  |
| Rfx2-R | ACCTTGGTCTGGATGACCTG |  |
| Rfx3-L | CAGACAGTTCAGCAGGTCCA |  |
| Rfx3-R | CTGGGCAGAACTTCCTTGAG |  |
| Deup1-L | AGATGCGGGCTTTAGAGACA |  |
| Deup1-R | CGGTGAATTTGGTTTTGCTT |  |
| Ccno-L | GCTGAGCCTAACGGATTACG |  |
| Ccno-R | TGATGGACACTAGCGTCTGC |  |


| Cdc20b-L | GAAGGAAAATCTTGCCACCA |  |
| :---: | :---: | :---: |
| Ccdc20b-R | TTGGCATGTGGAATGGTAGA |  |
| Ccdc78-L | ACCAGGTGCCACCATTAGAG |  |
| Ccdc78-R | AAGCCAGTTGCTGACCAGTT |  |
| Gapdh-L | AACTTTGGCATTGTGGAAGG |  |
| Gapdh-R | ACACATTGGGGGTAGGAACA |  |
| Cep63-L | TCTGTGAGTGCAACATGCAA |  |
| Cep63-R | GAGGAACACTTGGCAGAAGC |  |
| Plk4-L | AAACCAAAAAGGCTGTGGTG |  |
| Plk4-R | GGAGGTCTGTCAGCAAGAGG |  |
| Cep152-L | GCTGTGGACACTGCTTTCAA |  |
| Cep152-R | CACCCTGCTGTTCTCСТСТС |  |
| Sas6-L | CCTGCAGCTTACAAACCAGG |  |
| Sas6-R | CTGGCTAATCCGCGTAAAG |  |
| MCI-L | GCCTGAGCAATACTGGAAGG | qPCR primers |
| MCI-R | AGTTCCTTCAGCTGCACGTT | for human |
| GMNC-L | CCCAAAAATGCCAAAAGAAA | genes |
| GMNC-R | AATGTGCTGGCGACTCTTCT |  |
| FOXJ1-L | CACGTGAAGCCTCCCTACTC |  |
| FOXJ1-R | GGATTGAATTCTGCCAGGTG |  |
| DEUP1-L | CACAAAGAAAGCTGCCCTTC |  |
| DEUP1-R | TCGGAGCCTTTCATTCTCAT |  |
| CCNO-L | TCTACAGACCTTCCGCGACT |  |
| CCNO-R | TCCAGAGTGTTCACCGTCAG |  |
| CDC20B-L | GAAGACACCGCCTGAGAAAG |  |
| CDC20B-R | CACAGAGCTGCATTTTTCCA |  |
| GAPDH-L | GAGTCAACGGATTTGGTCGT |  |
| GAPDH-R | TTGATTTTGGAGGGATCTCG |  |
| GM-N-N | AGTCAGTCAAGCTTATGAAC ACCATTCTGCCT | Primers to generate |
| GM-C-N | GGATGCGGGTGCTGAATGCCAT CTCTGTCTTG | GMNC Nterminus and |
| GM-N-C | CAAGACAGAGATGGCATTCAGC ACCCGCATCC | MCI C- <br> terminus |
| GM-C-C | AGTCAGTCGCGGCCGCACTGGGGA CCCAGCGGAAC | chimera |
| GMNC-HA- <br> XhoI-pLvx | GATCGATCCTCGAGGCCACCATGT ACCCATACGACGTGCCAGACTACG | Primers to clone HA- |

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\begin{array}{|l|l|l|}\hline & \text { CAATGAACACCATTCTGCC } & \text { tagged GMNC } \\
\text { into PLVX } \\
\text { GMNC-C-XbaI- } \\
\text { pLvx }\end{array}
$$ \begin{array}{l}GATCGATCTCTAGACTAAGACTGC <br>

TTAGGGAC\end{array}\right]\)| vector |
| :--- |

