SUPPLEMENTAL INFORMATION

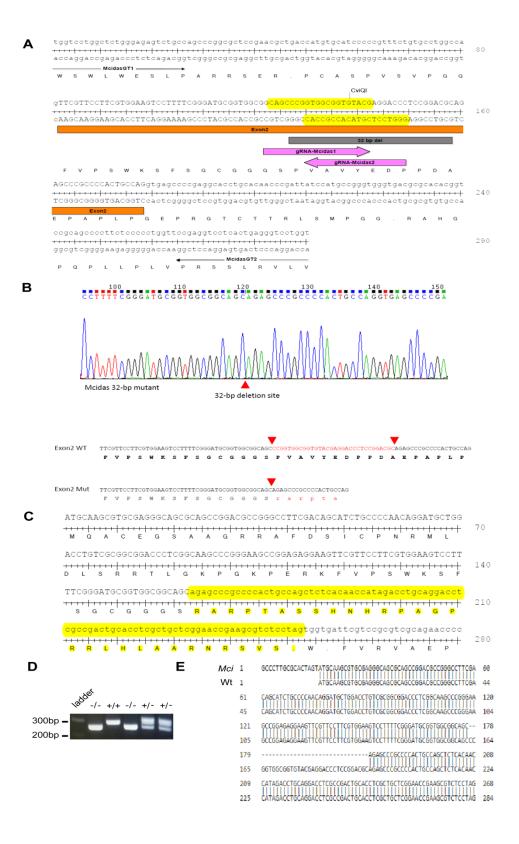


Fig. S1. Generation of a deletion allele at the mouse *Mci* locus. (A) Partial genomic sequence of the mouse *Mci* gene, showing the gRNAs (pink arrows) and their target sites on the forward and reverse strands (highlighted in yellow) used to induce a 32 bp deletion within exon 2. Binding sites for genotyping 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.

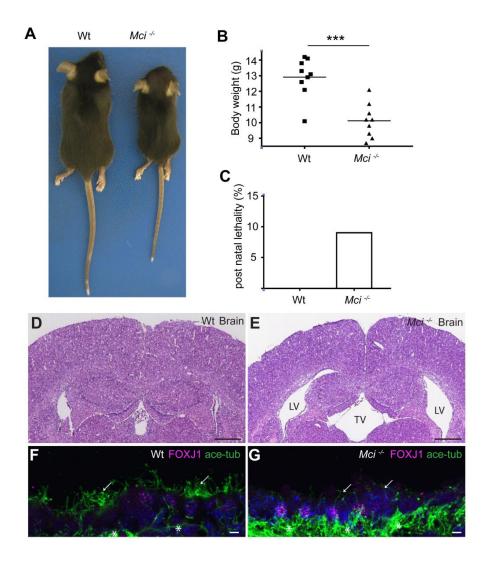


Fig. S2. Gross phenotypes of Mci knockout mice. (A) Mci knockout mice are smaller in size compared to the wild-type. (B) The body weight comparison between wild-type and Mci mutant mice at post-natal day (P) 28. n = 9 for each genotype. (C) Percentage of lethality of wild type and Mci knockout mice at P28. n = 22 for each genotype. (D) Wild-type 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 = 1mm. (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 µm.

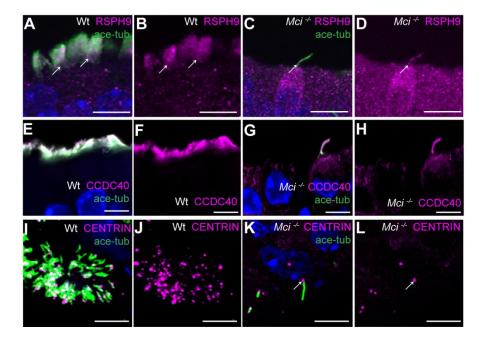


Fig. S3. *Mci* mutant MCCs precursors differentiate a single cilium that localizes motile cilia-specific proteins but are unable to make multiple basal bodies. (A)
RSPH9 co-localization with acetylated tubulin to MCC cilia of wild-type trachea (arrows). (B) RSPH9 localization to MCC cilia of wild-type 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 μ m; E-L = 5 μ m.

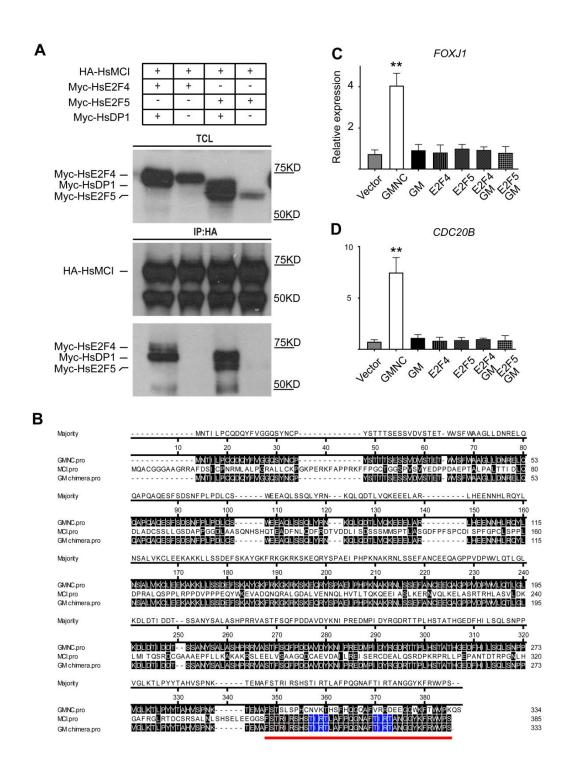


Fig. S4. Interaction of MCI with E2F factors and transcriptional activity of the GMNC-MCI chimeric protein in HEK293T cells. (A) Co-immunoprecipitation data

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 CDC20B 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. p: ** \leq 0.01.

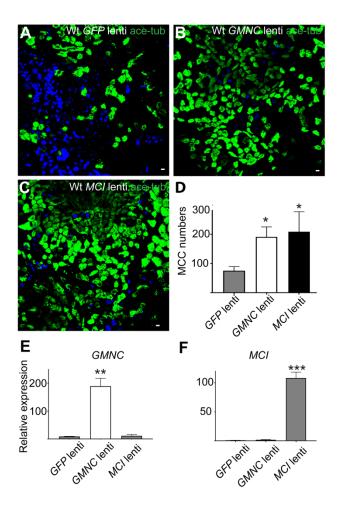


Fig. S5. Overexpression of GMNC and MCI in wild-type airway cell ALI culture induces supernumerary MCCs. (A) Lentivirus mediated overexpression of GFP in wild-type airway cell ALI culture does not affect numbers of differentiating MCCs. (B) Overexpression of GMNC in wild-type airway cell ALI culture induces supernumerary MCCs. (C) Overexpression of MCI in wild-type airway cell ALI culture induces supernumerary MCCs. Scale bars, 5 μ 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.

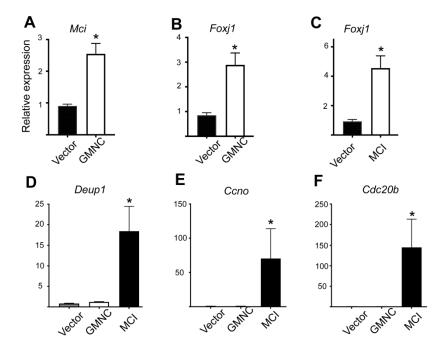


Fig. S6. RT-qPCR analysis of ciliary transcription factor and DD pathway genes expression levels on overexpression of MCI and GMNC in *Mci* mutant airway cells

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: $* \le 0.05$.

Table S1. Primer sequences

Name of	Sequence (5'-3')	Remarks
gRNA/primer		
gRNA-Mcidas1	CAGCCCGGTGGCGGTGTACGGTTTTAGAGCTA	gRNA
	GAAATAGCAAGTTAAAATAAGGCTAGTCCGTT	sequences
	ATCAACTTGAAAAAGTGGCACCGAGTCGGTGC	
	TTT	
gRNA-Mcidas2	GGGTCCTCGTACACCGCCACGTTTTAGAGCTA	
	GAAATAGCAAGTTAAAATAAGGCTAGTCCGTT	
	ATCAACTTGAAAAAGTGGCACCGAGTCGGTGC	
	TTT	
McidasGT1(for	TGGTCCTGGCTCTGGGAGAGTCTGCC	Primers for
ward)		genotyping of
McidasGT2(rev	ACCAGGACCCTCAGTGAGGACCTCGG	<i>Mci</i> mutant
erse)		mice
Mci-L	CGGAGCAGTACTGGAAGGAG	qPCR primers
Mci-R	TTCGTTGTTGCCTTGATCTG	for mouse
Gmnc-L	TCTGGAAGAGAGCCAAGA	genes
Gmnc-R	CCCAGGTTGTTCCTCACAGT	
Foxj1-L	GAGCTGGAACCACTCAAAGG	
Foxj1-R	GGTAGCAGGCAGTTGATGT	
Rfx2-L	TGTGAGCCGATCCTACAGTG	
Rfx2-R	ACCTTGGTCTGGATGACCTG	
Rfx3-L	CAGACAGTTCAGCAGGTCCA	
Rfx3-R	CTGGGCAGAACTTCCTTGAG	
Deup1-L	AGATGCGGCTTTAGAGACA	
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	CACCCTGCTGTTCTCCTCTC	
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	Primers to
	ACCATTCTGCCT	generate
GM-C-N	GGATGCGGGTGCTGAATGCCAT	GMNC N-
	CTCTGTCTTG	terminus and
GM-N-C	CAAGACAGAGATGGCATTCAGC	MCI C-
	ACCCGCATCC	terminus
GM-C-C	AGTCAGTCGCGGCCGCACTGGGGA	chimera
	CCCAGCGGAAC	
GMNC-HA-	GATCGATCCTCGAGGCCACCATGT	Primers to
XhoI-pLvx	ACCCATACGACGTGCCAGACTACG	clone HA-

	CAATGAACACCATTCTGCC	tagged GMNC
GMNC-C-XbaI-	GATCGATCTCTAGACTAAGACTGC	into PLVX
pLvx	TTAGGGAC	vector
MCI-HA-XhoI-	GATCGATCCTCGAGGCCACCATGT	Primers to
pLvx	ACCCATACGACGTGCCAGACTACG	clone HA-
	CAATGCAGGCGTGCGGGG	tagged MCI
MCI-C-XbaI-	GATCGATCTAGATCAACTGGG	into PLVX
pLvx	GACCCAGCG	vector