Supporting Information

Guen et al. 10.1073/pnas.1711534114

SI Methods

Cell Culture. Primary mammary epithelial cells were cultured in EpiCult-B mouse medium (STEMCELL Technologies) supplemented with proliferation supplements, 10 ng/mL recombinant human epidermal growth factor, 10 ng/mL recombinant human basic fibroblast growth factor, 4 μg/mL heparin, and penicillin/streptomycin. HMLE cells were cultured in a 1:1 mixture of DMEM/F-12 supplemented with 10% FBS, 0.01 mg/mL insulin, 0.48 μg/mL hydrocortisone, and complete MEGM medium (Lonza) supplemented with bovine pituitary hormone (Lonza). Primary ciliogenesis was analyzed at high cell confluence. DMEM/F12 was used to serum starve the cells. For activating tetracycline-inducible Snail and Zeb1 expression, cells were grown in medium with 1 μg/mL doxycycline hyclate (Sigma-Aldrich). For erismodegib and ciliobrevin A treatments, cells were grown until confluence and were treated overnight in DMEM/F12.

Western Blot Experiments. Western blot experiments were conducted using standard procedures as described previously (1, 2). Western blots were performed using the primary antibodies against Snail (Cell Signaling 3879; 1:500), Twist (Abcam ab50887; 1:50), Zeb1 (Santa Cruz Biotechnology sc-25388; 1:50), GLI1 (Cell Signaling 2534; 1:500), GLI2 (R&D Systems AF3635; 1:500), E-cadherin (Cell Signaling 3195; 1:1,000), vimentin (Dako M0725; 1:1,000), fibronectin (BD Biosciences 610077; 1:1,000), *N*-cadherin (BD Biosciences 610920; 1:1,000), KIF3A (Proteintech 13930-1-AP; 1:1,000), IFT20 (Proteintech 13615-1-AP; 1:100), GAPDH (Santa Cruz Biotechnology sc-365062; 1:5,000), HSP90 (BD Biosciences 610418; 1:2,000), and secondary antibodies HRP-coupled anti-mouse (GE Healthcare NA931V; 1:4,000) or anti-rabbit (GE Healthcare NA934V; 1:4,000).

Cell-Cycle Analysis. The cell cycle analysis was performed using subconfluent HMLE cells that were fixed in 70% ethanol for 1 h on ice. DNA was stained with propidium iodide/RNase staining buffer (BD Biosciences) for 15 min. DNA content of 30,000 cells for each condition was determined using a BD FACS Canto II flow cytometer and the ModFit software.

Real-Time qPCR. Total RNA was isolated using the PicoPure RNA Isolation Kit (Thermo Fisher Scientific) for mouse cells or the RNeasy kit (Qiagen) for human cells, and cDNAs were generated with random primers and SuperScript III Reverse Transcriptase (Life Technologies). Real-time qPCR reactions were performed with a StepOnePlus Real-Time PCR system (Life Technologies) using Fast SYBR Green PCR Master Mix (Life Technologies) and gene-specific primers. Relative expression levels were normalized to GAPDH. Primers used for qPCR analysis are listed in Table S1.

RNA-Sequencing Analysis. Raw sequencing data for GSE60450 and GSE63310 were downloaded from the Gene Expression Omnibus/Sequence Read Archive repository (https://ftp-trace.ncbi.nlm.nih.gov/sra), and reads were mapped to the University of California Santa Cruz mm9 mouse genome build (https://genome.ucsc.edu) using RSEM (3). Targeted pairwise differential expression analyses between basal and luminal samples were conducted using EBSeq v1.4.0 (4) with median normalization. All RNA-sequencing analyses were conducted in the R Statistical Programming language (https://www.r-project.org/).

Statistical Analysis. Prism was used to analyze data, draw graphs, and perform statistical analyses. Data are presented as the mean ± SEM. Statistical analyses were carried out by Student's *t* test unless otherwise specified. $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, $^{***}P \leq 0.001$ were considered significant.

1. Guen VJ, et al. (2013) CDK10/cyclin M is a protein kinase that controls ETS2 degradation and is deficient in STAR syndrome. *Proc Natl Acad Sci USA* 110:19525–19530.
2. Guen VJ, et al. (2016) STAR syndrome-associated CDK10/Cyclin M regulates actin network architecture and ciliogenesis. *Cell Cycle* 15:678–688.
3. Li B, Dewey CN (2011) RSEM: Accurate transcript quantification from RNA-Seq data with or without a reference genome. *BMC Bioinformatics* 12:323.
4. Leng N, et al. (2013) EBSeq: An empirical Bayes hierarchical model for inference in RNA-seq experiments. *Bioinformatics* 29:1035–1043.

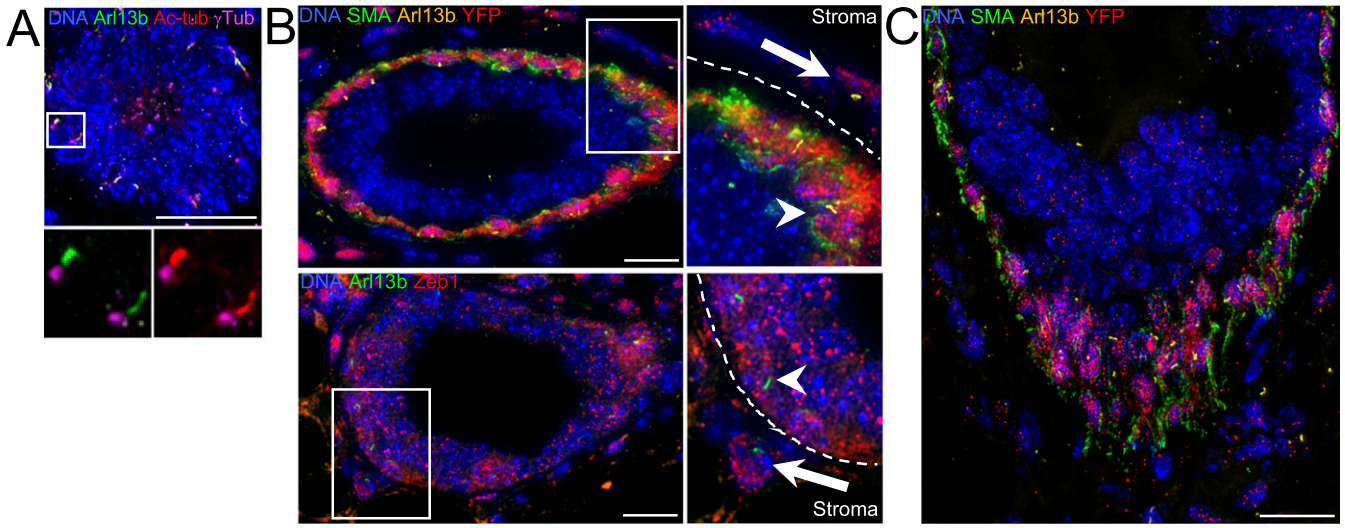

Fig. S1. Primary cilia are associated with EMT-TF–expressing cells. (*A*–*C*) Normal mammary gland sections from Slug-IRES-YFP or nontransgenic adult females were stained for the indicated proteins to analyze ciliogenesis. (Scale bars, 15 μm.) *Insets*: 3× magnification in A; 2× magnification in *B*. Arrowheads indicate basal ciliated cells, and arrows indicate stromal ciliated cells.

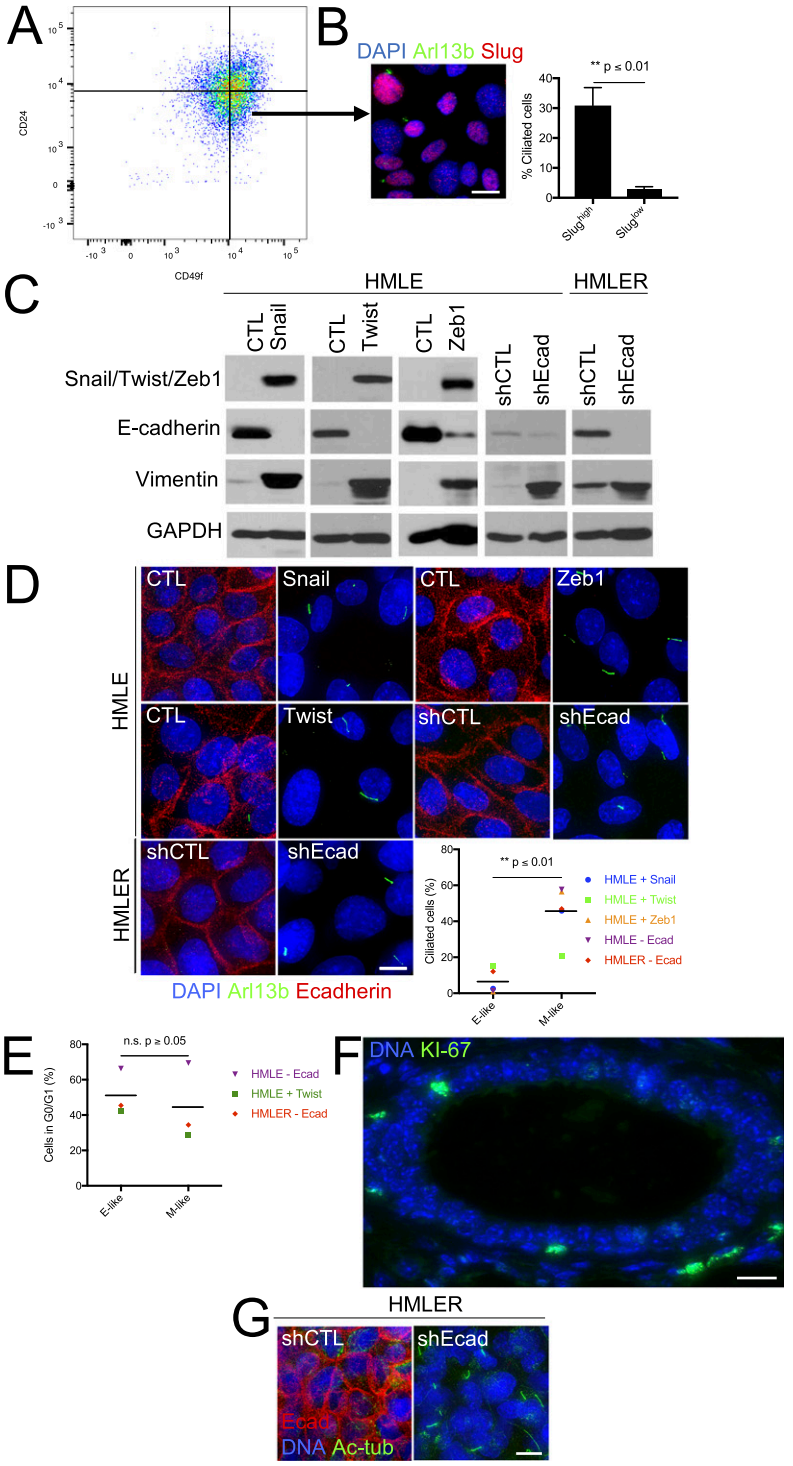

Fig. S2. EMT programs induce primary ciliogenesis independently from cell-cycle arrest. (*A* and *B*) CD24^{Lo};CD49f^{Hi} HMLE cells were isolated by FACS and examined by immunofluorescence for the indicated proteins (n = 3, mean ± SEM). (Scale bar, 15 μm.) (*C*) Western blot analysis of EMT-TFs, E-cadherin, and vimentin protein expression levels in E-like [CTL, sh (short hairpin)CTL] and M-like (Snail, Twist, Zeb1, shEcad) HMLE and HMLER cells. (*D* and *E*) Indicated HMLE and HMLER variants in E-like and M-like states were cultured in the presence of growth factors. (*D*) Cells were stained for the indicated proteins to determine the percentage of ciliated cells. (Scale bar, 15 μm.) Representative results from three independent experiments are shown. (*E*) Cells were harvested at sub-confluence, and the percentage of cells in G_0/G_1 was determined by FACS analysis of their DNA content. (*F*) Normal mammary gland sections from non-transgenic adult females were stained for KI-67 to detect proliferating cells. (Scale bar, 15 μm.) (*G*) E-like (shCTL) and M-like (shECad) HMLER cells were stained for the indicated proteins to assess the representation of ciliated cells. (Scale bar, 15 μm.)

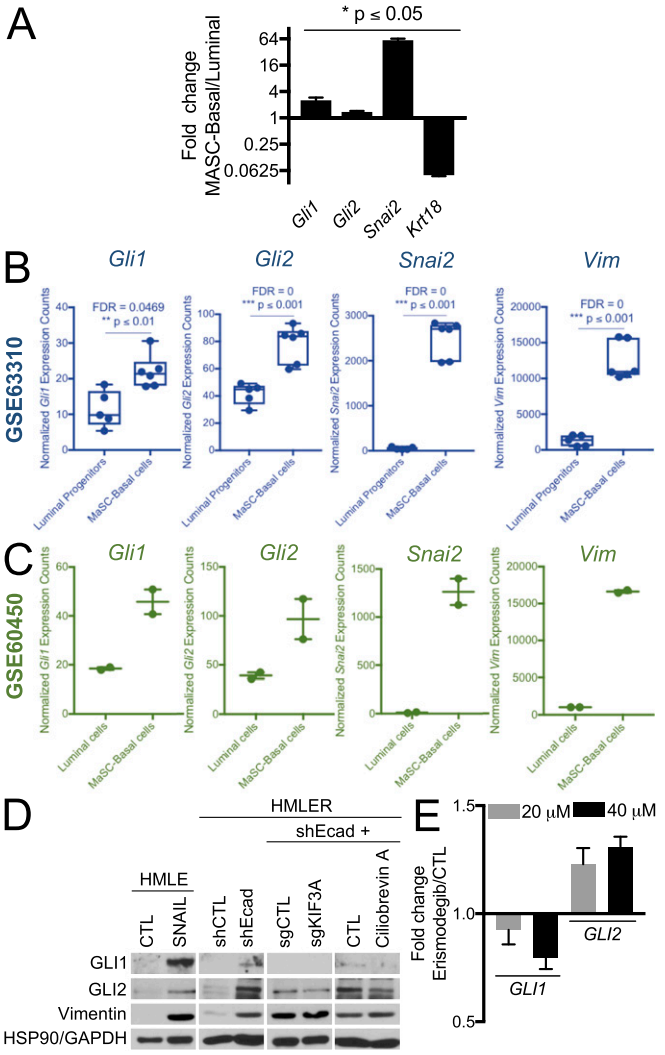

Fig. S3. EMT programs are associated with higher expression levels of GLI-TFs that depend on primary cilia. (*A*) Relative levels of the indicated gene transcripts were determined by real-time qPCR analysis ($n = 3$, mean ± SEM). (*B* and *C*) Box and whisker plots showing *Gli1*, *Gli2*, *Snai2* (*Slug*), and *Vim* expression in adult female mice. (*B*) MaSC-enriched basal cells versus luminal progenitors (GSE63310) (1). (*C*) MaSC-enriched basal cells versus luminal cells (GSE60450) (2). (*D*) Western blot analysis of GLI1, GLI2, and vimentin protein expression levels in E-like [CTL, sh (short hairpin)CTL], M-like (Snail, shEcad) HMLE, and HMLER cells. The impact of ciliogenesis inhibition on protein levels was determined in KIF3A-knockout (sgKIF3A) versus control cells (sgCTL) and in ciliobrevin A- versus vehicle-treated (CTL) M-like HMLER cells. (*E*) Relative *GLI1* and *GLI2* RNA levels ($n = 3$, mean ± SEM) in vehicle-treated control (CTL) versus erismodegib-treated M-like HMLER (shEcad) cells were analyzed by real-time qPCR.

1. Sheridan JM, et al. (2015) A pooled shRNA screen for regulators of primary mammary stem and progenitor cells identifies roles for Asap1 and Prox1. *BMC Cancer* 15:221.
2. Fu NY, et al. (2015) EGF-mediated induction of Mcl-1 at the switch to lactation is essential for alveolar cell survival. *Nat Cell Biol* 17:365–375.

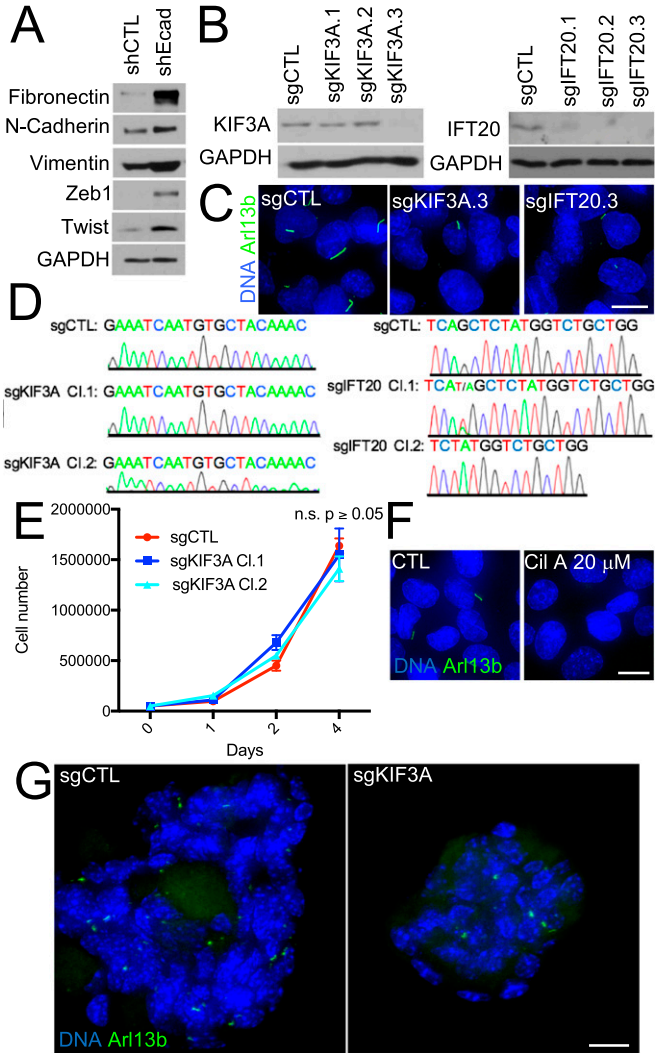

Fig. S4. Frameshift mutations within the *KIF3A* and *IFT20* genes and ciliobrevin A treatment result in inhibition of primary ciliogenesis. (*A*) Western blot analysis of the protein expression levels of EMT markers in E-like [sh (short hairpin)CTL] and M-like (shEcad) HMLER cells. (*B*) Western blot analysis of KIF3A and IFT20 expression levels in M-like (shEcad) HMLER cells following CRISPR mutations of their coding genes, using three different guide RNAs per gene. (*C*) HMLER control (sgCTL), and *KIF3A* (sgKIF3A.3) or *IFT20* (sgIFT20.3) mutant cell populations were stained for the indicated proteins to assess primary ciliogenesis. (Scale bar, 15 μm.) (*D*) Sanger sequencing of the genomic DNA from the indicated cells was used to compare the sequence of the targeted *KIF3A* and *IFT20* loci for the sgKIF3A and sgIFT20 clones versus the sgCTL population. (*E*) The proliferation rate of the indicated cells was analyzed by daily counting the total cell number per well (three wells per line) for 4 d. Data are shown as mean ± SEM. (*F*) The impact of ciliobrevin A treatment on M-like HMLER cells (shEcad) was assessed by immunofluorescence for the indicated protein. (Scale bar, 15 μm.) (*G*) Organoids arising from sgCTL- and sgKIF3A-transduced MaSC-enriched basal cell populations were cut and stained for the indicated protein. (Scale bar, 15 μm.)

Table S1. Primers used in qPCR for measuring gene expression

Gene	Forward	Reverse
mGli1	CCAAGCCAACTTTATGTCAGGG	AGCCCGCTTCTTTGTTAATTTGA
mGli2	GTGCACAGCAGCCCCACACTCTC	GGTAATAGTCTGAAGGGTTGGTGCCTGG
mZeb1	CACAGTGGAGAGAAGCCATAC	CACTGAGATGTCTTGAGTCCTG
mSnai2	CTCACCTCGGGAGCATACAG	GACTTACACGCCCCAAGGATG
mKrt14	CAGCAAGACAGAGGAGCTGAACC	CCAGGGATGCTTTCATGCTGAG
mKrt18	GGTCTCAGCAGATTGAGGAGAG	CCAAGTCAATCTCCAAGGTCTGG
hGLI1	CACAAGTGCACGTTTGAAGGG	CATGTATGGCTTCTCACCCG
hGLI2	GAGCAGCTGGTGCATCACATC	CACCACCAGCATGTACTGCG
VIM	GGAAATGGCTCGTCACCTTC	GAAATCCTGCTCTCCTCGCC
OCLN	CTATAAATCCACGCCGGTTC	GTTACCACCGCTGCTGTAAC
GAPDH	TGCACCACCAACTGCTTAGC	GGCATGGACTGTGGTCATGAG

Table S2. Oligonucleotides used for CRISPR mutations and amplification of the targeted loci

Target	Forward	Reverse
Single-guide RNAs		
sgCTL	GCTGATCTATCGCGGTCGTC	GACGACCGCGATAGATCAGC
sghKIF3A.1	CGAGGTGTTTCGGTAGTCTC	GAGACTACCGAAACACCTCG
sghKIF3A.2	CCCGGGCGGGGACGTCCGCC	GGCGGACGTCCCCGCCCGGG
sghKIF3A.3	GAAATCAATGTGCTACAAAC	GTTTGTAGCACATTGATTTC
sghIFT20.1	ACTGAACAAGCTGAGGGTGT	ACACCCTCAGCTTGTTCAGT
sghIFT20.2	AGGACATCCTGGGTGAAGCA	TGCTTCACCCAGGATGTCCT
sghIFT20.3	CCAGCAGACCATAGAGCTGA	TCAGCTCTATGGTCTGCTGG
sgmKIF3A	GAAGTCAATGTGCTATAGAC	GTCTATAGCACATTGACTTC
sgmIFT20	CCAGCAGACTGTGGAGCTCA	TGAGCTCCACAGTCTGCTGG
Gene		
KIF3A	GTGTGCAAGGCACTGAGTTGG	CACTCATCAAACTGCACAGCTGC
IFT20	CTGGGAAGTGATGAGGAATTG	GGACACATGAGAGCACAAGAG