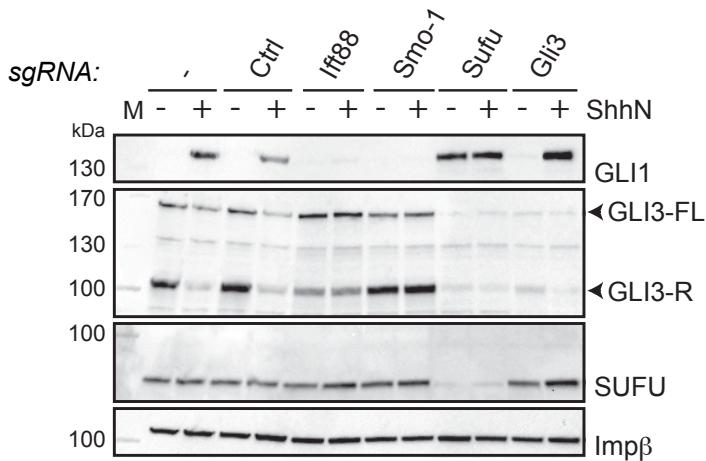


a

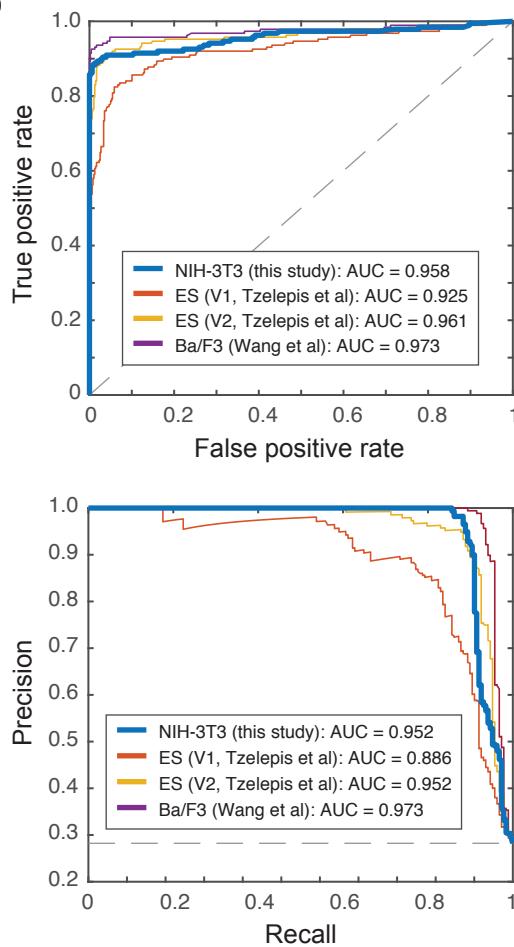
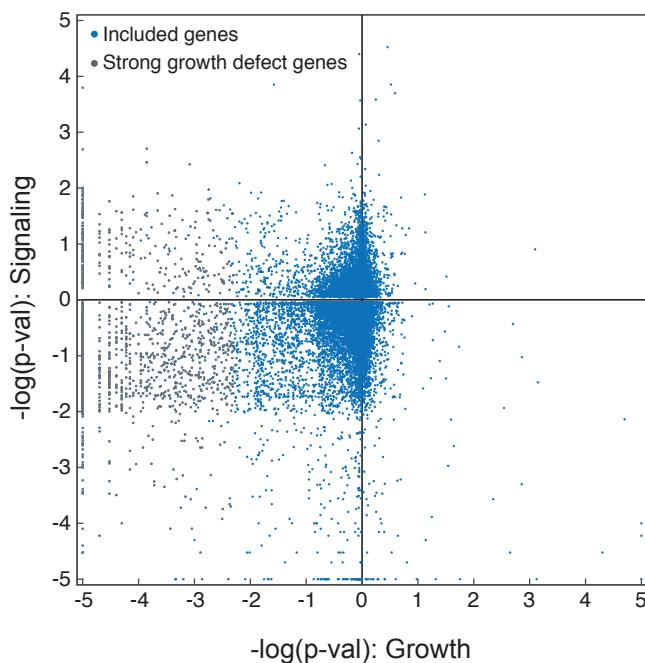


Supplementary Figure 1. Positive and negative control sgRNAs have expected effects on the Shh-blasticidin reporter.

a) Lysates from cells transduced with the indicated sgRNAs were analyzed by Western blot of the indicated proteins. GLI3-FL and GLI3-R indicate the positions of full-length and repressor forms of GLI3, respectively.

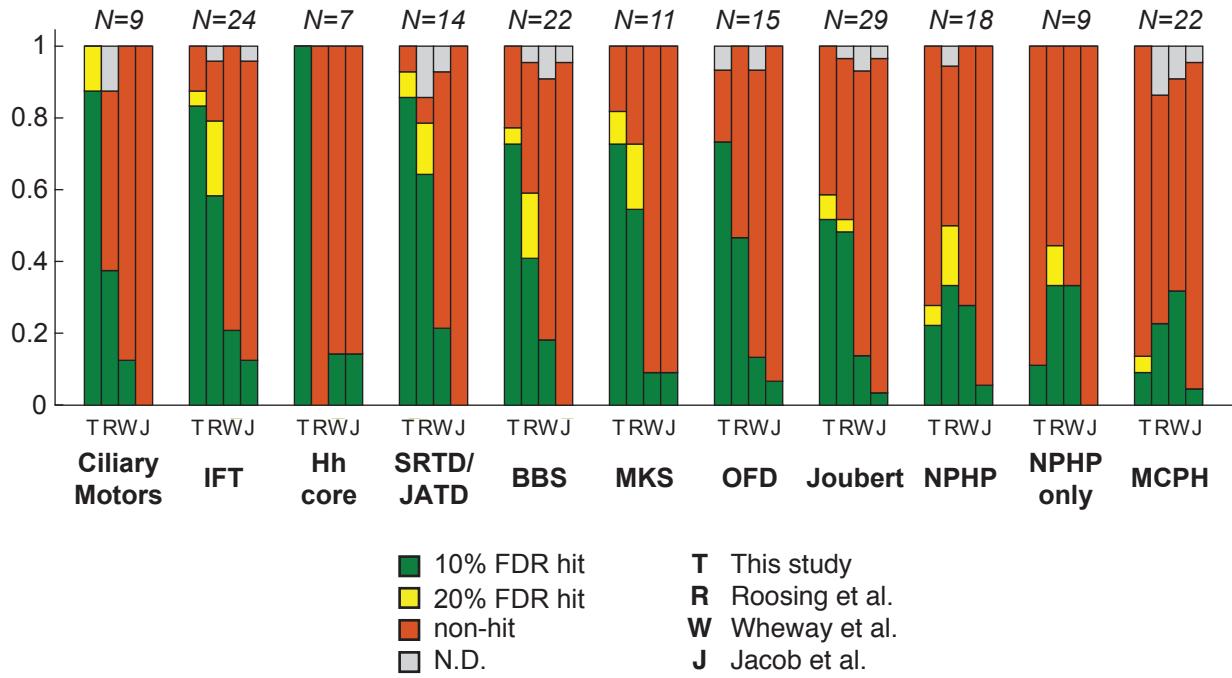
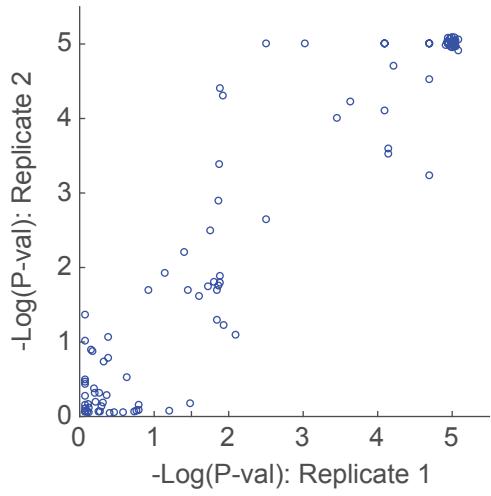
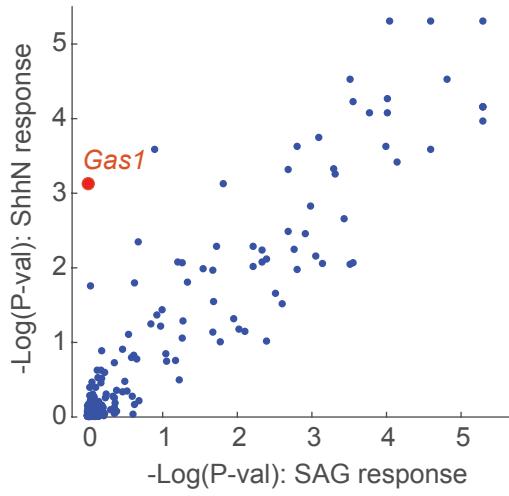
a

Sub-library	Num sgRNAs	Screen Batch
Apoptosis/cancer A	~16000	B
Apoptosis/cancer B	~16000	A
Drug targets/kinase/phosphatase A	~11000	B
Drug targets/kinase/phosphatase B	~11000	B
Gene expression A	~10000	C
Gene expression B	~10000	C
Membrane proteins A	~5500	A
Membrane proteins B	~5500	B
Mouse unique 1A	~15000	D
Mouse unique 1B	~15000	D
Mouse unique 2A	~16500	D
Mouse unique 2B	~16500	D
Stress/proteostasis A	~15000	B
Stress/proteostasis B	~15000	C
Trafficking/mito/motility A	~10000	C
Trafficking/mito/motility B	~10000	C
Unassigned A	~10500	A
Unassigned B	~10500	A
Unassigned 2A	~11000	C
Unassigned 2B	~11000	D
Cilia/Hh controls (replicate)	1000	D

b**c**

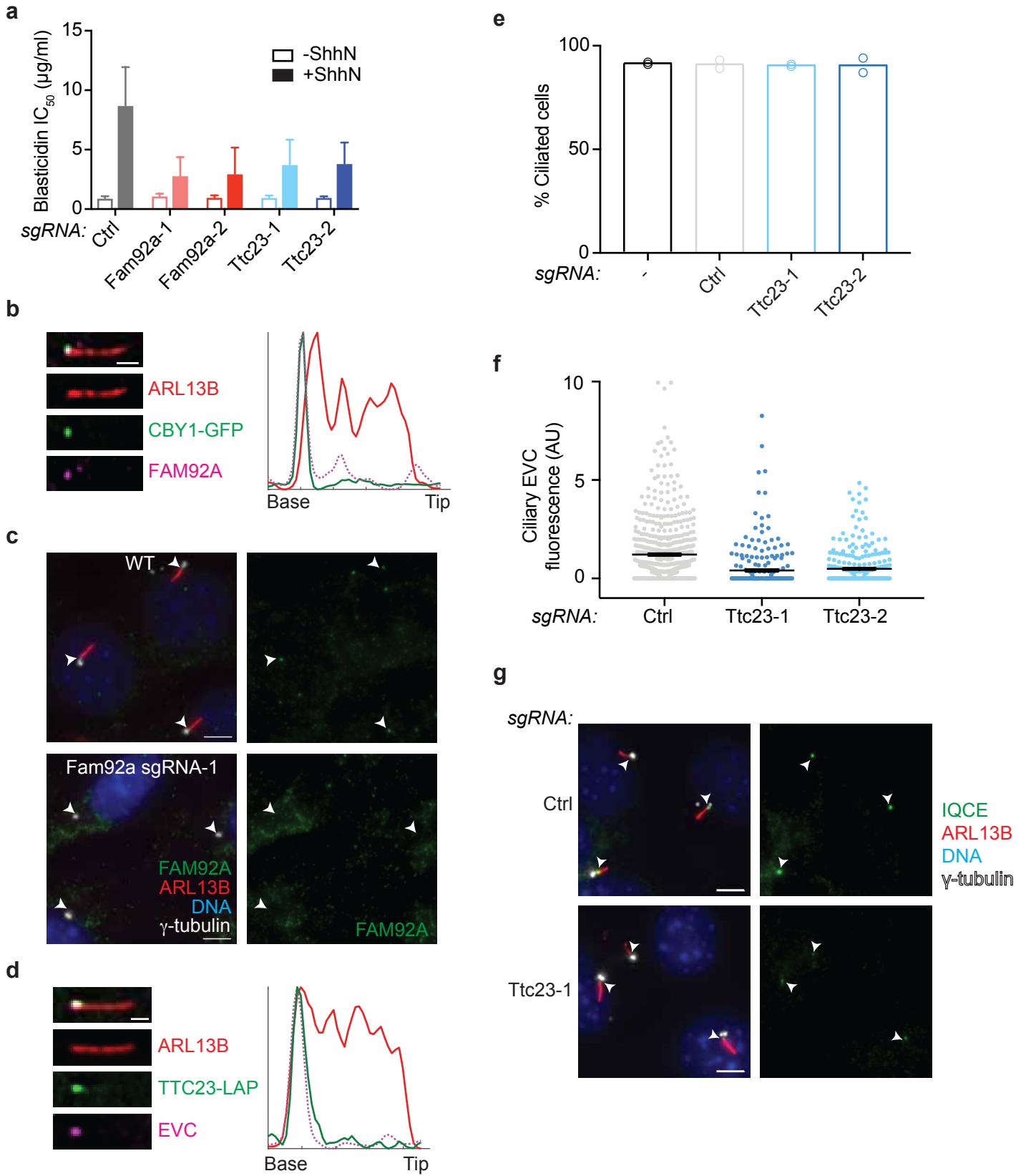
Supplementary Figure 2. Overview of sgRNA library and genome-wide screen results.

a) Description of sgRNA library used, indicating sub-libraries, number of sgRNAs per sub-library, and grouping of sub-libraries into four screen batches. **b)** Assessment of screen performance at detecting genes with growth phenotypes using positive and negative reference sets. Performance was determined by ROC curve (top) precision-recall analysis (bottom), with the area under each curve (AUC) shown. Performance for various screens using different libraries are plotted, with the cell type and data source indicated for each. Dashed lines indicate performance of a random classification model. **c)** Relationship between growth and signaling phenotypes for genome-wide screen data. Positive values indicate enrichment (increased growth or blasticidin resistance); negative values indicate depletion (reduced growth or blasticidin sensitivity). Genes shown in grey were filtered out of further analyses due to strong negative growth phenotypes.

a**b****c**

Supplementary Figure 3. Evaluation of screen performance.

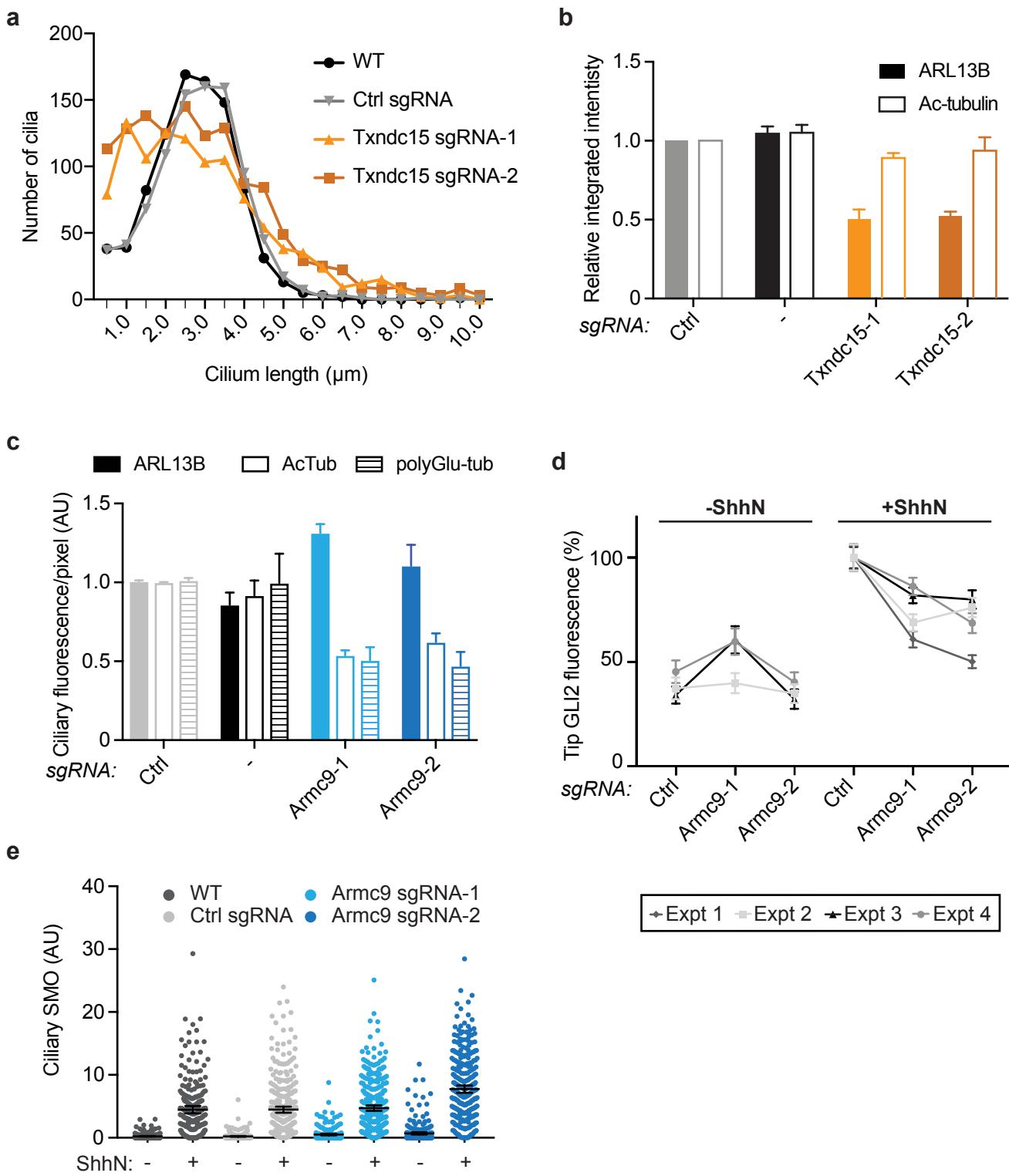
a) Comparison of hit gene detection rates across various screen datasets and functional classes of genes. The total number of genes in each class is indicated above each group of bars. See Supplementary Table 4 for additional details. **b)** Comparison of screen replicates for 95 genes measured in two different screen batches. Note: a small amount of jitter was added during plotting so that overlapping data points can be resolved. **c)** Comparison of screen replicates for 263 genes analyzed for roles in response to ShhN-induced versus SAG-induced Hh signaling. *Gas1* is highlighted in red as a prominent exception to the high level of concordance between screen results.



Supplementary Figure 4. Characterization of FAM92A and TTC23 as transition zone and EvC zone components.

a) Signaling-induced blastcidin resistance is shown for 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs. **b)** Transfected CBY-GFP co-localizes with endogenous FAM92A at the transition zone of IMCD3 cells. Line plot at right shows relative intensity from base to tip. Scale bar: 1 μ m. **c)** Validation of the anti-FAM92A antibody. Staining reveals prominent transition zone staining in wildtype (WT) cells but not cells transduced with a Fam92a-targeting sgRNA.

Scale bar: 5 μ m. **d**) TTC23-LAP stably expressed in IMCD3 cells co-localizes with EVC. Line scans at right shows relative intensity from base to tip. Scale bar: 1 μ m. **e**) The frequency of ciliogenesis was assessed in 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs. Bars show mean fraction of ciliated cells; dots show ciliated fraction in each independent experiment (>400 cells were analyzed from two independent experiments). **f**) Ciliary EVC fluorescence was measured for cells transduced with the indicated sgRNAs. The mean and standard error of the mean are plotted for N>277 cells measured in each condition for one representative experiment out of two replicates. **g**) Representative images showing ciliary IQCE staining in cells transduced with negative control (Ctrl) and Ttc23-targeting sgRNAs. Scale bars: 5 μ m.



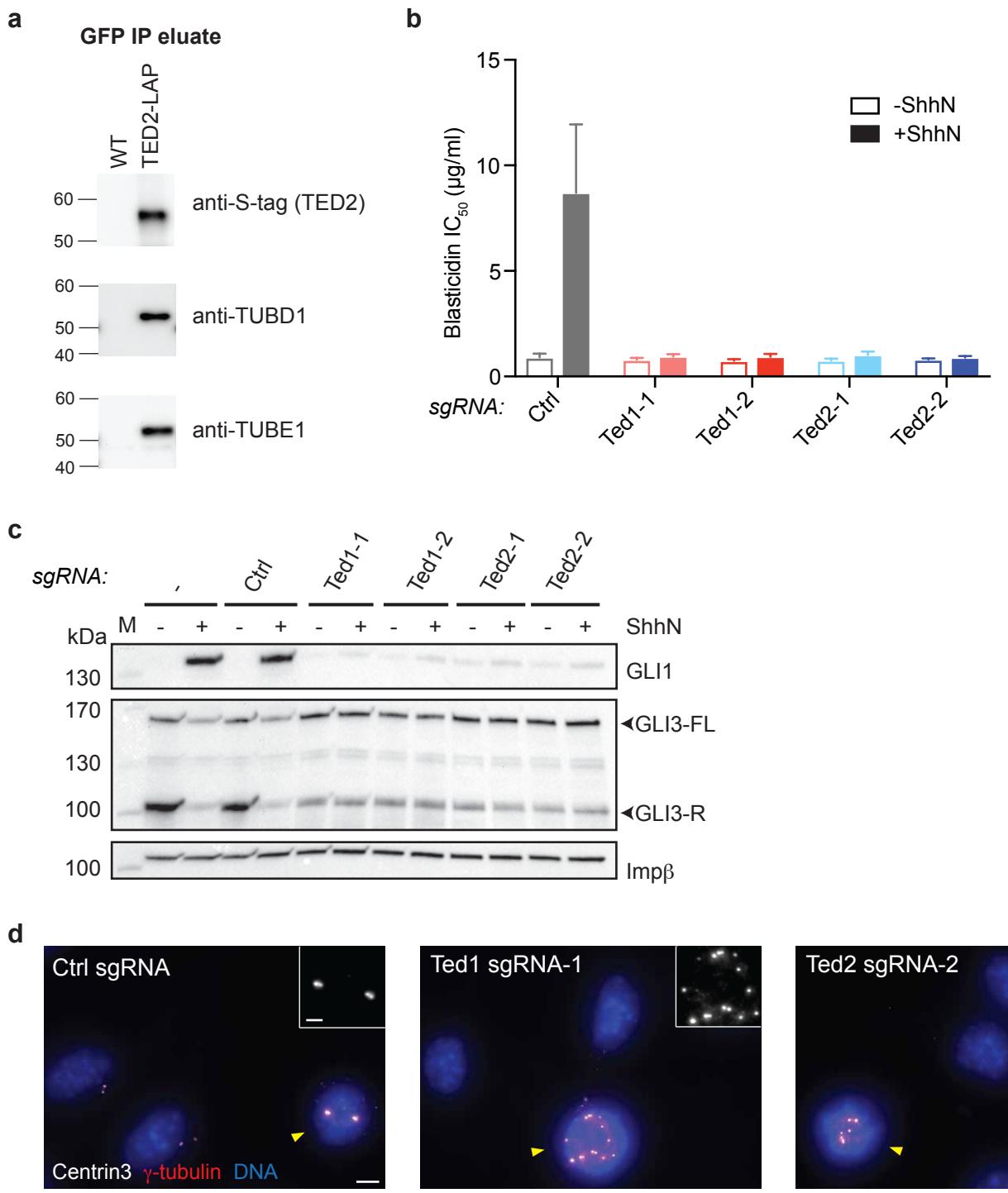
Supplementary Figure 5. Functional characterization of TXND15 and ARMC9.

a) Distribution of cilium lengths measured for 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs. Both sets of Txndc15 mutant cells were significantly different from WT or control (Ctrl) sgRNA cells ($P < 1 \times 10^{-8}$ for each comparison, made using the Kolmogorov-Smirnov test; $N > 800$ cilia from 3 independent experiments for each cell pool).

b) Staining for ciliary markers ARL13B and acetylated tubulin was performed for cells transduced with the indicated sgRNAs. Integrated ciliary fluorescence is shown relative to that of Ctrl sgRNA. Bars show mean and SEM for means of $N > 200$ cilia per experiment, three independent experiments.

c) Staining for ciliary markers ARL13B, acetylated tubulin, and polyglutamylated tubulin was performed for 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs. Ciliary pixel intensity is shown relative to Ctrl sgRNA = 1. Bars show mean and standard deviation for means of $N > 200$.

per experiment, three independent experiments. **d**) Fluorescence intensity of GLI2 at the ciliary tip was measured for 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs and treated with ShhN or left untreated. Mean intensity (relative to Ctrl sgRNA cells +ShhN) and standard error of the mean are shown for each of four experiments ($N > 250$ cilia in each experiment). **e**) Fluorescence intensity of ciliary SMO was measured for cells transduced with the indicated sgRNAs and treated with ShhN or left untreated. Mean and 95% confidence interval are shown ($N > 230$ cilia, one representative replicate out of two independent experiments).



Supplementary Figure 6. Functional characterization of TED1 and TED2.

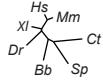
a) Proteins affinity purified from IMCD3 wildtype (WT) or TED2-LAP cells were analyzed by Western blotting with the indicated antibodies. **b)** Signaling-induced blasticidin resistance is shown for 3T3-[Shh-BlastR;Cas9] cells transduced with the indicated sgRNAs. Data shown is the mean and standard deviation of two independent experiments performed in duplicate. **c)** Signaling-induced regulation of GLI3 processing and expression of target gene GLI1 were analyzed by Western blot using cells transduced with the indicated sgRNAs. GLI3-FL and GLI3-R indicate the positions of full-length and repressor forms of GLI3, respectively. **d)** For cells transduced with the indicated sgRNAs, centrioles were visualized by staining with antibodies to centrin3 and γ -tubulin. Insets show centrin3 staining in mitotic cells, marked by yellow arrowheads. Scale bars: 5 μm (2 μm for insets).

a

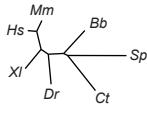
Species or other classification	Common name	Tubulins	Ted2 (C16orf59)	Ted1 (C14orf80)
<i>Homo sapiens</i>	man	a,b,g,e,d	NP_079384.2	NP_001128347
<i>Mus musculus</i>	mouse	a,b,g,e,d	NP_082332.1	NP_598802.2
<i>Monodelphis domestica</i>	opossum	a,b,g,e,d,z	XP_016279715	XP_007473397.1
<i>Anolis carolinensis</i>	lizard	a,b,g,e,d,z	XP_008122311	
<i>Alligator sinensis</i>	alligator	a,b,g,e,d,z	XP_006015645	
<i>Gallus gallus</i>	chicken	a,b,g,e,d		XP_422217
<i>Chelonia mydas</i>	turtle	a,b,g,e,d,z	XP_007054613	
<i>Xenopus tropicalis</i>	frog	a,b,g,e,d,z	XP_002932464	
<i>Xenopus laevis</i>	frog	a,b,g,e,d,z	XP_018091968.1	XP_018086758.1
<i>Danio rerio</i>	zebrafish	a,b,g,e,d	XP_001340891.5	XP_706561
<i>Ictalurus punctatus</i>	catfish	a,b,g,e,d,z		
<i>Oikopleura dioica</i>	tunicate	a,b,g,e,d		
<i>Ciona intestinalis</i>	sea squirt (tunicate)	a,b,g,e,d,z		XP_002129476
<i>Branchiostoma floridae</i>	lancelet (chordate)	a,b,g,e,d,z	XP_002611730	
<i>Branchiostoma belcheri</i>	lancelet (chordate)	a,b,g,e,d,z	XP_019625169.1	XP_019647986.1
<i>Sacoglossa kowalevskii</i>	acorn worm (hemichordate)	a,b,g,e,d,z	XP_006825593	XP_006813427
<i>Nematostella vectensis</i>	sea anemone (hemichordate)	a,b,g,e,d,z		
<i>Strongylocentrotus purpuratus</i>	sea urchin	a,b,g,e,d	XP_003730952	XP_003726479
<i>Trichoplax adhaerens</i>	placozoa	a,b,g,e,d,z		XP_002113523
<i>Amphimedon queenslandica</i>	sponge	a,b,g,e,d,z		
<i>Lottia gigantea</i>	limpet (mollusc)	a,b,g,e,d,z	XP_009060182	
<i>Echinococcus multilocularis</i>	tapeworm (platyhelminthe)	a,b,g,e,d		
<i>Capitella teleta</i>	polychaete worm (annelide)	a,b,g,e,d,z	ELU15716	ELT99332
<i>Helobdella robusta</i>	leech (annelide)	a,b,g,e,d,z		XP_009020484
<i>Caenorhabditis elegans</i>	nematode	a,b,g		
<i>Tribolium castaneum</i>	beetle (insect)	a,b,g,e,d		
<i>Apis mellifera</i>	honey bee (insect)	a,b,g,e,d		XP_016769925
<i>Drosophila melanogaster</i>	fruit fly (insect)	a,b,g		
<i>Giardia lamblia</i>		a,b,g,e,d		
<i>Naegleria gruberi</i>		a,b,g,e,d,z		
<i>Trypanosoma brucei</i>		a,b,g,e,d,z		
<i>Trichomonas vaginalis</i>		a,b,g,e,d		
<i>Porphyridium purpureum</i>		a,b,g		
<i>Galdieria sulphuraria</i>		a,b,g		
<i>Physcomitrella patens</i>	moss	a,b,g,d,e		XP_001751900
<i>Selaginella moellendorffii</i>	lycophyte (plant)	a,b,g,e,d		XP_002971306
Spermatophyta (all species)		a,b,g		
<i>Chlamydomonas reinhardtii</i>		a,b,g,e,d,z		
<i>Encephalitozoon cuniculi</i>		a,b,g		
<i>Rhizopus arrhizus</i>		a,b,g		
<i>Allomyces macrocygnus</i>		a,b,g,e,z		
<i>Batrachochytrium dendrobatidis</i>		a,b,g,e,z		
Non-ciliated fungi (all species)		a,b,g		
<i>Monosiga brevicollis</i>		a,b,g,e,d		
<i>Thecamonas trahens</i>		a,b,g,e,d,z		XP_013762418
<i>Dictyostelium discoideum</i>		a,b,g		
<i>Guillardia theta</i>		a,b,g,e,z		
<i>Phytophthora sojae</i>	oomycete (soybean parasite)	a,b,g,e,d,z		XP_009532968
<i>Fragilaropsis cylindrus</i>		a,b,g		
<i>Ectocarpus siliculosus</i>		a,b,g,e,d		
<i>Blastocystis hominis</i>		a,b,g		
<i>Bigelowiella natans</i>		a,b,g,e,z		
<i>Plasmodium falciparum</i>		a,b,g,e,d		
<i>Babesia bovis</i>		a,b,g,e,d		
<i>Paramecium tetraurelia</i>		a,b,g,e,d,z		XP_001439264
<i>Tetrahymena thermophila</i>		a,b,g,e,d,z		XP_001030296

b

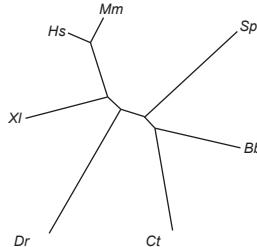
Tube1



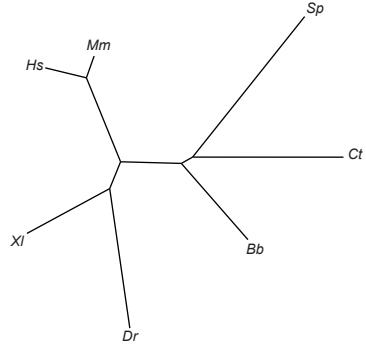
Tubd1



Ted1



Ted2

Hs: *Homo sapiens* (human)Mm: *Mus musculus* (mouse)XI: *Xenopus laevis* (clawed frog)Dr: *Danio rerio* (zebrafish)Bb: *Branchiostoma belcheri* (lancelet)Sp: *Strongylocentrotus purpuratus* (sea urchin)Ct: *Capitella teleta* (polychaete worm)

Supplementary Figure 7. Evolutionary analysis of TED complex components.

a) Table showing species with identified homologs for TED1, TED2 and tubulins alpha, beta, gamma, delta, epsilon, and zeta (a, b, g, d, e, z). **b)** Tree diagrams showing sequence relationships for select proteins, with branch lengths scaled equally for all proteins.

Supplementary Table 8. List of primary antibodies.

REAGENT	SOURCE	IDENTIFIER
Antibodies		
Mouse anti-acetylated tubulin (6-11B-1)	Sigma Aldrich	Cat#T6793; RRID: AB_477585
Mouse anti-gamma tubulin (GTU-88)	Sigma-Aldrich	Cat#T6557; RRID: AB_477584
Mouse anti-centrin2 (clone 20H5)	EMD Millipore	Cat#04-1624; RRID: AB_10563501
Mouse anti-centrin3 (clone 3E6)	Novus Biologicals	Cat#H00001070-M01; RRID: AB_537701
Mouse anti-Arl13b (clone N295B/66)	UC Davis/NIH NeuroMab	Cat#73-287; RRID: AB_11000053
Mouse anti-Cby1 (clone 8-2)	Santa Cruz Biotechnology	Cat#sc-101551; RRID: AB_1561972
Mouse anti-Gli1 (clone L42B10)	Cell Signaling Technology	Cat#2643S; RRID: AB_2294746
Mouse anti-polyglutamylated tubulin (clone GT335)	Carsten Janke	
Rabbit anti-GFP	Maxence Nachury	N/A
Chicken anti-GFP	Thermo Fisher	Cat#A10262; RRID: AB_2534023
Goat anti-Gli2	R&D Systems	Cat#AF3635; RRID: AB_2111902
Goat anti-Gli3	R&D Systems	Cat#AF3690; RRID: AB_2232499
Goat anti-Sufu	Santa Cruz Biotechnology	Cat#sc-10933; RRID: AB_671172
Rabbit anti-Smo	Ocbina, et al. ¹	N/A
Rabbit anti-Importin Beta	Santa Cruz Biotechnology	Cat#sc-11367; RRID: AB_2265549
Rabbit anti-Fam92a1	Proteintech	Cat#24803-1-AP
Rabbit anti-Ninein	Michel Bornens	N/A
Rabbit anti-Iqce	Pusapati, et al. ²	N/A
Rabbit anti-Evc	Dorn, et al. ³	N/A
Rabbit anti-Tube1	Sigma Aldrich	Cat#HPA032074; RRID: AB_10601216
Rabbit anti-Tubd1	Sigma Aldrich	Cat#HPA027090; RRID: AB_1858457
Mouse anti-Flag M2	Sigma Aldrich	Cat#F1804; RRID: AB_262044
Rabbit anti-Flag	Sigma Aldrich	Cat#F7425; RRID: AB_439687
Mouse anti-Myc (9E10)	ATCC for hybridoma	Cat#CRL-1729; RRID: CVCL_G671
Rabbit anti-Myc	Santa Cruz Biotechnology	Cat#sc-789; RRID: AB_631274

Supplementary Table 9. List of oligonucleotides and recombinant DNA.

REAGENT	SOURCE	IDENTIFIER
Oligonucleotides		
sgRNA library oligos (genome-wide and cilia/Hh pathway-focused libraries)	Agilent	N/A
siGenome Smartpool Iqce siRNA, targeting sequences: GAAAGAACGCCAUGGUGGA, GAAAGGAGUCGGCAGAUAGA, GCAUUGCACUGGAAACAUAA, ACCUAGCUCGUUCGAAG	Dharmacon, Pusapati, et al. ²	Cat#M-059692-01-0005
siGenome Non-targeting Smartpool #1, targeting sequences: UAGCGACUAACACAUCAA, UAAGGCCAUAGAAGAGAUAC, AUGAUUUGGCCUGUAUUAG, AU-GAACGUGAAUUGCUCUCAA	Dharmacon	Cat#D-001206-13-05
siRNA Evc2, targeting sequence: GAUGGAAUCCAGACUUUCA	Sigma Aldrich, Pusapati, et al. ²	Cat#SASI_Mm01_00106977
Mision siRNA universal negative control #1	Sigma Aldrich	Cat#SIC001
Primer: sgRNA_Amp_F1: AGGCTTGGATTCTATAACTTCGTATAGCATACATTATAC	Deans, et al. ⁴	N/A
Primer: sgRNA_Amp_R1: ACATGCATGGCGGTAAATACGGTTATC	Deans, et al. ⁴	N/A
Primer: sgRNA_Amp_F2: CAAGCAGAACGACGGCATACGAGATGCACAAAAGGAAAC TCACCCCT	Deans, et al. ⁴	N/A
Primer: sgRNA_Amp_R2: AATGATA CGGCGACCACCGAGATCTACACGATCGGAAG AGCACACGTCTGAACTCCAGTCACNNNNNCGACTCGG TGCCCACTTTTC	Deans, et al. ⁴	N/A
Primer: sgRNA_Seq: GCCACTTTTCAAGTTGATAACGGACTAGCCTTATTAA AACTTGCTATGCTGTTCCAGCTAGCTCTAAC	This paper	N/A
Recombinant DNA		
Plasmid: pGL-8xGli-Bsd-T2A-GFP-Hyg	This paper	N/A
Plasmid: pGL3-8xGli-Firefly-luciferase	Philip Beachy	N/A
Plasmid: pGL3-SV40-Renilla-luciferase	Philip Beachy	N/A
Plasmid: pHr-Pgk-Cas9-BFP	This paper	N/A
Plasmid: pMCB320-mU6-sgRNA-mCherry-Puro (see Supplementary Table 1 for sgRNA sequences)	Han, et al. ⁵	Addgene #89359
Plasmid: pMCB306-mU6-sgRNA-GFP-Puro	Han, et al. ⁵	Addgene #89360
Plasmid library: mouse CRISPR KO	Morgens, et al. ⁶	N/A
Plasmid library: mouse cilia/Hh sgRNAs	This paper	N/A
pDONR-221	Thermo Fisher	Cat#12536017
pENTR-4	Thermo Fisher	Cat#A10465
pMD2.G	Michael Bassik	Addgene #12259
pRSV-Rev	Michael Bassik	Addgene #12253
pMDLg/RRE	Michael Bassik	Addgene #12251
pCMV-ΔR-8.91	Bob Weinberg	N/A
pCMV-VSVG	Bob Weinberg	Addgene #8454
cDNA: Armc9 (mouse, IMAGE clone 6406321)	Dharmacon (GE Healthcare)	Cat# MMM1013-202859176

cDNA: Ted2 (mouse, IMAGE clone 6414405)	Dharmacon (GE Healthcare)	Cat#MMM1013-202859268
cDNA: Ttc23 (rat, IMAGE clone 7745883)	Dharmacon (GE Healthcare)	Cat#MRN1768-202784317
cDNA: Fam92a-sgResist (mouse)	This paper	N/A
cDNA: Txndc15-sgResist (human)	Dharmacon (GE Healthcare)	Cat#MHS6278-202801775
Plasmid: pENTR-Txndc15-mut	This paper	N/A
Plasmid: pENTR-Ted1(mouse)-sgResist	This paper	N/A
Plasmid: pENTR-Cby1 (human)	This paper	N/A
Plasmid: pEF5B-FRT-DEST-LAP	Liew, et al. ⁷	N/A
Plasmid: pEF5B-FRT-DEST-3xFlag	This paper	N/A
Plasmid: pEF5B-FRT-DEST-6xMyc	This paper	N/A

Supplementary References

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