Table 1. A list of downregulated or upregulated genes in Dyrk2$^{-/-}$ MEFs

Down-regulated genes in Dyrk2$^{-/-}$

ID	GeneSymbol	Description	Ratio of Dyrk2$^{-/-}$ per wild-type in the presence of SAG	Ratio of Dyrk2$^{-/-}$ per wild-type in the absence of SAG
ENSMUSG00000028630	Dyrk2	Dual-specificity tyrosine-(Y)-phosphorylation regulated kinase 2	0.02	0.03
ENSMUSG00000035683	Melk	Maternal embryonic leucine zipper kinase	0.23	0.22
ENSMUSG00000074476	Spc24	NDC80 kinetochore complex component%2C homolog (*S. cerevisiae*)	0.25	0.21
ENSMUSG00000020808	Pimreg	PICALM interacting mitotic regulator	0.28	0.28
ENSMUSG00000033952	Aspm	Abnormal spindle microtubule assembly	0.31	0.25
ENSMUSG00000026683	Nuf2	NDC80 kinetochore complex component	0.31	0.30
ENSMUSG00000037466	Tedc1	Tubulin epsilon and delta complex 1	0.31	0.26
ENSMUSG00000030867	Plk1	Polo-like kinase 1	0.31	0.17
ENSMUSG00000022033	Pbk	PDZ binding kinase	0.33	0.29
ENSMUSG00000027326	Knl1	Kinetochore scaffold 1	0.33	0.20
ENSMUSG00000041431	Ccnb1	Cyclin B1	0.33	0.26
ENSMUSG00000036777	Anln	Anillin actin binding protein	0.33	0.26
ENSMUSG00000001403	Ube2c	Ubiquitin-conjugating enzyme E2C	0.33	0.25
ENSMUSG00000027496	Aurka	Aurora kinase A	0.34	0.26
ENSMUSG00000001349	Cnn1	Calponin 1	0.34	0.31
ENSMUSG00000032218	Ccnb2	Cyclin B2	0.34	0.28
ENSMUSG00000026039	Sgo2a	Shugoshin 2A	0.34	0.25
ENSMUSG00000015880	Ncapg	Non-SMC condensin I complex subunit G	0.34	0.34
ENSMUSG00000027379	Bub1	BUB1 mitotic checkpoint serine/threonine kinase	0.36	0.23
ENSMUSG00000040084	Bub1b	BUB1B mitotic checkpoint serine/threonine kinase	0.36	0.29
ENSMUSG00000045328	Cenpe	Centromere protein E	0.36	0.22
ENSMUSG00000032254	Kif23	Kinesin family member 23	0.37	0.25
ENSMUSG00000028873	Cdca8	Cell division cycle associated 8	0.37	0.30
ENSMUSG00000032135	Mcam	Melanoma cell adhesion molecule	0.37	0.29
ENSMUSG00000027469	Tpx2	TPX2microtubule-associated	0.37	0.33
ENSMUSG00000028678	Kif2c	Kinesin family member 2C	0.37	0.24
ENSMUSG00000027715	Ccna2	Cyclin A2	0.38	0.23
ENSMUSG00000048327	Ckap2l	Cytoskeleton associated protein 2-like	0.39	0.23
ENSMUSG00000040204	Pclaf	PCNA clamp associated factor	0.40	0.19
ENSMUSG00000029414	Kntc1	Kinetochore associated 1	0.42	0.24
ENSMUSG00000034311	Kif4	Kinesin family member 4	0.42	0.24

Table 1 continued on next page

Table 1 continued

Down-regulated genes in Dyrk2$^{-/-}$

ID	GeneSymbol	Description	Ratio of Dyrk2$^{-/-}$ per wild-type in the presence of SAG	Ratio of Dyrk2$^{-/-}$ per wild-type in the absence of SAG
ENSMUSG00000031004	Mki67	Antigen identified by monoclonal antibody Ki 67	0.42	0.21
ENSMUSG00000020914	Top2a	Topoisomerase (DNA) II alpha	0.42	0.21
ENSMUSG00000033031	Cip2a	Cell proliferation regulating inhibitor of protein phosphatase 2A	0.42	0.32
ENSMUSG00000035783	Acta2	Actin alpha two smooth muscle aorta	0.43	0.48
ENSMUSG00000024795	Kif20b	Kinesin family member 20B	0.43	0.30
ENSMUSG00000038943	Prc1	Protein regulator of cytokinesis 1	0.43	0.26
ENSMUSG00000026494	Kif26b	Kinesin family member 26B	0.43	0.25
ENSMUSG00000023015	Racgap1	Rac GTPase-activating protein 1	0.43	0.26
ENSMUSG00000026605	Cenpf	Centromere protein F	0.44	0.25
ENSMUSG00000027306	Nusap1	Nucleolar and spindle associated protein 1	0.45	0.28
ENSMUSG00000028068	Iqgap3	IQ motif containing GTPase activating protein 3	0.46	0.21
ENSMUSG00000003779	Kif20a	Kinesin family member 20A	0.47	0.25
ENSMUSG00000005410	Mcm5	Minichromosome maintenance complex component 5	0.47	0.26
ENSMUSG00000034906	Ncaph	Non-SMC condensin I complex subunit H	0.47	0.27
ENSMUSG00000006398	Cdc20	Cell division cycle 20	0.48	0.29
ENSMUSG00000037313	Tacc3	Transforming acidic coiled-coil containing protein 3	0.48	0.36
ENSMUSG00000027699	Ect2	ect2 oncogene	0.48	0.26
ENSMUSG00000020330	Hmmr	Hyaluronan-mediated motility receptor (RHAMM)	0.50	0.28
ENSMUSG00000020649	Rrm2	Ribonucleotide reductase M2	0.50	0.26
ENSMUSG00000019942	Cdk1	Cyclin-dependent kinase 1	0.50	0.34
ENSMUSG00000024590	Lmnb1	Lamin B1	0.51	0.33
ENSMUSG00000037725	Ckap2	Cytoskeleton associated protein 2	0.55	0.42

Upregulated genes in Dyrk2$^{-/-}$

ID	GeneSymbol	Description	Ratio of Dyrk2$^{-/-}$ per wild-type in the presence of SAG	Ratio of Dyrk2$^{-/-}$ per wild-type in the absence of SAG
ENSMUSG00000056673	Kdm5d	Lysine (K)-specific demethylase 5D	Inf	Inf
ENSMUSG00000068457	Uty	Ubiquitously transcribed tetratricopeptide repeat gene Y chromosome	Inf	Inf
ENSMUSG00000069049	Ddx3y	DEAD (Asp-Glu-Ala-Asp) box polypeptide 3 Y-linked	Inf	8278
ENSMUSG00000069045	Eif2s3y	Eukaryotic translation initiation factor 2 subunit three structural gene Y-linked	Inf	Inf
ENSMUSG00000112616	Gm47434	Predicted gene 47434	719	Inf

Table 1 continued on next page

Table 1 continued

Down-regulated genes in Dyrk2$^{-/-}$

ID	GeneSymbol	Description	Ratio of Dyrk2$^{-/-}$ per wild-type in the presence of SAG	Ratio of Dyrk2$^{-/-}$ per wild-type in the absence of SAG
ENSMUSG00000025582	Nptx1	Neuronal pentraxin 1	4.74	11.91
ENSMUSG00000024164	C3	Complement component 3	4.47	11.59
ENSMUSG00000039457	Ppl	Periplakin	4.30	11.11
ENSMUSG00000025784	Clec3b	C-type lectin domain family three member b	3.99	8.60
ENSMUSG00000002944	Cd36	CD36 molecule	3.20	3.45
ENSMUSG00000035385	Ccl2	Chemokine (C-C motif) ligand 2	2.86	2.84
ENSMUSG00000095478	Gm9824	Predicted pseudogene 9824	2.60	4.14
ENSMUSG00000038642	Ctss	Cathepsin S	2.58	3.19
ENSMUSG00000043719	Col6a6	Collagen type VI alpha 6	2.44	4.64
ENSMUSG00000033327	Tnxb	Tenascin XB	2.37	3.61
ENSMUSG00000069516	Lyz2	Lysozyme 2	2.30	3.08
ENSMUSG00000016494	Cd34	CD34 antigen	2.29	2.26
ENSMUSG00000042129	Rassf4	Ras association (RalGDS/AF-6) domain family member 4	2.29	3.43
ENSMUSG00000004730	Adgre1	Adhesion G-protein-coupled receptor E1	2.27	2.49
ENSMUSG00000030144	Clec4d	C-type lectin domain family member d	2.26	3.74
ENSMUSG00000029816	Gpnmb	Glycoprotein (transmembrane) nmb	2.22	2.66
ENSMUSG00000042286	Stab1	Stabilin 1	2.18	2.70
ENSMUSG00000020120	Plek	Pleckstrin	2.18	2.99
ENSMUSG00000040254	Sema3d	Sema domain immunoglobulin domain (Ig) short basic domain secreted (semaphorin) 3D	2.17	2.89
ENSMUSG00000005268	Prlr	Prolactin receptor	2.17	4.44
ENSMUSG00000024621	Csf1r	Colony-stimulating factor one receptor	2.10	2.74
ENSMUSG00000074896	Ifit3	Interferon-induced protein with tetratricopeptide repeats 3	2.04	3.96
ENSMUSG00000002985	Apoe	Apolipoprotein E	2.03	2.51
ENSMUSG00000057137	Tmem140	Transmembrane protein 140	2.02	3.18
ENSMUSG00000002289	Angptl4	Angiopoietin-like 4	2.02	5.94
ENSMUSG00000050335	Lgals3	Lectin galactose binding soluble 3	1.99	2.66
ENSMUSG00000090877	Hspa1b	Heat-shock protein 1B	1.98	2.13
ENSMUSG00000054404	Slfn5	Schlafen 5	1.96	3.77
ENSMUSG00000031209	Heph	Hephaestin	1.92	2.48
ENSMUSG00000027996	Sfrp2	Secreted frizzled-related protein 2	1.91	5.68
ENSMUSG00000050953	Gja1	Gap junction protein alpha 1	1.90	2.45
ENSMUSG00000005413	Hmox1	Heme oxygenase 1	1.90	1.97
ENSMUSG00000046805	Mpeg1	Macrophage expressed gene 1	1.85	2.57
ENSMUSG00000022037	Clu	Clusterin	1.83	3.06

Table 1 continued on next page

Table 1 continued

Down-regulated genes in Dyrk2$^{-/-}$

ID	GeneSymbol	Description	Ratio of Dyrk2$^{-/-}$ per wild-type in the presence of SAG	Ratio of Dyrk2$^{-/-}$ per wild-type in the absence of SAG
ENSMUSG00000026389	Steap3	STEAP family member 3	1.81	2.24
ENSMUSG00000041577	Prelp	Proline arginine-rich end leucine-rich repeat	1.81	2.01
ENSMUSG00000027339	Rassf2	Ras association (RalGDS/AF-6) domain family member 2	1.80	2.72

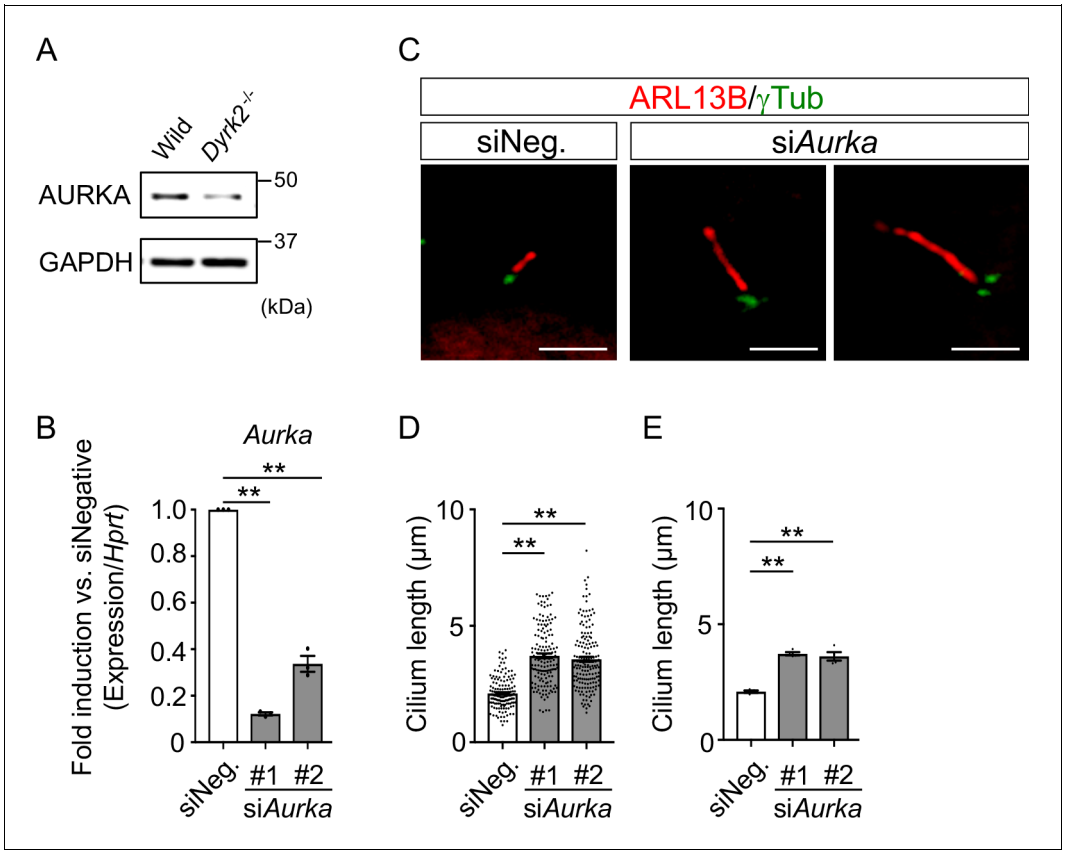

Figure 8. Elongation of primary cilia in wild-type MEFs treated with si*Aurka*. (**A**) Immunoblotting of AURKA in wild-type and *Dyrk2*$^{-/-}$ MEFs. GAPDH serves as a loading control. (**B**) Knockdown efficiency of *Aurka*-expression in wild-type MEFs treated with two independent si*Aurka* for 48 hr was measured by qPCR. *Hprt* was used as an internal standard, and fold change was calculated by comparing expression levels relative to those of siNegative (siNeg.). Data are presented as the means ± SEM (n = 3 biological replicates per condition). (**C**) Primary cilia in wild-type cells treated with siNegative (siNeg.) or two independent si*Aurka* were immuno-stained with ARL13B and gamma-tubulin antibodies. Scale bars, 5 μm. (**D, E**) Measurements of cilia length in wild-type MEFs treated with siNeg. or two independent si*Aurka* using ARL13B and acetylated-tubulin as a cilia axoneme marker. Cilia lengths are presented as pooled from four MEFs derived from independent wild-type embryos (**D**) and represent an average of each MEF (**E**). Data are presented as the means ± SEM (n = 4 biological replicates per condition). The statistical significance was determined by one-way ANOVA followed by Tukey's multiple comparison test. (**) p<0.01.
The online version of this article includes the following source data for figure 8:

Source data 1. Source data for ***Figure 8B and D–E***.

Table 2. List of primer sets.

For genotyping			
Gene	**Sequence (5′→3′)**		**Accession number**
Dyrk2 tm1b-WT	Forward	TGGGTCCAAATGCAAAGAAACGCCA	NC_000076.6
	Reverse	GCTTCTCGTTCCGCACCATCTTCAG	
Dyrk2 tm1b-KO	Forward	CCTTCTCCCTCCTCCACTCTGACCCA	NC_000076.6
	Reverse	CCACACCTCCCCCTGAACCTGAAAC	
For amplification of the probes for in situ hybridization or Southern blotting			
Gene	**Sequence (5′→3′)**		**Accession number**
Mouse *Foxf2*	Forward	GAGATTAACCCTCACTAAAGG GAGGTTATGGTGGCCTCGACAT	NM_010225.2
	Reverse	GAGTAATACGACTCACTATAG GGACACACACACCTCCCTTTTCA	
Mouse *Gli1*	Forward	GAGTATTTAGGTGACACTATAGA AGCAGGGAAGAGAGCAGACTG	NM_010296.2
	Reverse	GAGTAATACGACTCACTATAGGG GCTGAGTGTTGTCCAGGTC	
Mouse *Ptch1*	Forward	GAGATTAACCCTCACTAAAGGGA CATGGCCTCGGCTGGTAAC	NM_008957.3
	Reverse	GAGTAATACGACTCACTATAGGG TGTACCCATGGCCAACTTCG	
Southern for *Dyrk2*	Forward	CTTCGAATCCTTTTATCCTTCAGGC	NC_000076.6
	Reverse	ACATCATGTTCATTGGTTTTGCTCT	
For cloning			
Gene	**Sequence (5′→3′)**		**Accession number**
Mouse *Aurka CDS*	Forward	GGACTCAGATCTCGAGAC ATGGCTGTTGAGGGCG	NM_011497.4
	Reverse	GTCGACTGCAGAATTCC TAAGATGATTTGCTGGTTG	
Mouse *Dyrk2 CDS*	Forward	GTGCGCGATCGCCATGT TAACCAGGAAACCTTCGGC	NM_001014390.2
	Reverse	CTCCGTTTAAACGCTAA CGAGTTTCGGCAACAC	
For real-time PCR			
Gene	**Sequence (5′→3′)**		**Accession number**
Human *DYRK2*	Forward	GGGGAGAAAACGTCAGTGAA	NM_006482.3
	Reverse	TCTGCGCCAAATTAGTCCTC	
Human *HPRT1*	Forward	GGACTAATTATGGACAGGACTG	NM_000194.3
	Reverse	GCTCTTCAGTCTGATAAAATCTAC	
Mouse *Aurka*	Forward	CACACGTACCAGGAGACTTACAGA	NM_011497.4
	Reverse	AGTCTTGAAATGAGGTCCCTGGCT	
Mouse *Cdc20*	Forward	GAGCTCAAAGGACACACAGC	NM_023223.2
	Reverse	GCCACAACCGTAGAGTCTCA	
Mouse *Dyrk2*	Forward	CTACCACTACAGCCCACACG	NM_001014390.2
	Reverse	TCTGTCCGTGGCTGTTGA	
Mouse *Foxf2*	Forward	AGCATGTCTTCCTACTCGTTG	NM_010225.2
	Reverse	TCTTTCCTGTCGCACACT	
Mouse *Gli1*	Forward	GCACCACATCAACAGTGAGC	NM_010296.2
	Reverse	GCGTCTTGAGGTTTTCAAGG	
Mouse *Hprt*	Forward	CTCATGGACTGATTATGGACAGGAC	NM_013556.2
	Reverse	GCAGGTCAGCAAAGAACTTATAGCC	

Table 2 continued on next page

Table 2 continued

For genotyping			
Gene	**Sequence (5′→3′)**		**Accession number**
Mouse *Kif2c*	Forward	GAGAGCAAGCTGACCCAGG	NM_134471.4
	Reverse	CCTGGTGAGATCATGGCGATC	
Mouse *Plk1*	Forward	CCAAGCACATCAACCCAGTG	NM_011121.4
	Reverse	TGAGGCAGGTAATAGGGAGACG	
Mouse *Ptch1*	Forward	CTCTGGAGCAGATTTCCAAGG	NM_008957.3
	Reverse	TGCCGCAGTTCTTTTGAATG	
Mouse *Shh*	Forward	GTGAAGCTGCGAGTGACCG	NM_009170.3
	Reverse	CCTGGTCGTCAGCCGCCAGCACGC	
Mouse *Tpx2*	Forward	GCGAGGTTGTCAGGTGTGTA	NM_001141977.1
	Reverse	TTGATAAAGTCGGTGGGGGC	
Mouse *Ube2c*	Forward	CTGCTAGGAGAACCCAACATC	NM_026785.2
	Reverse	GCTGGAGACCTGCTTTGAATA	

temperature in darkness, and then immersed in 1% OsO_4 solution for 2 hr at room temperature. After dehydration in graded ethanol, the samples were transferred into isoamyl acetate and dried at the critical point in liquid CO_2, and this was followed by a metal coating procedure (Hitachi, Tokyo, Japan). The surfaces of tissues were then observed using scanning electron microscopy (Hitachi).

Plasmid constructs

Full-length cDNA fragments of mouse *Dyrk2* and *Aurka* were amplified by PCR and cloned in frame into the pFN22K-HaloTag-CMVd1-Flexi-vector (Promega, Madison, WI) and pEGFP-C1 (TaKaRa Bio, Otsu, Japan), respectively. The nucleotide sequences of the primers used are listed in ***Table 2***.

Cell culture and transfection

Primary mouse embryonic fibroblast (MEFs) were generated from wild-type and *Dyrk2*$^{-/-}$ embryos at E13.5. MEFs and immortalized human retinal pigment epithelia cells (hTERT-RPE1; Cat# CRL-4000, RRID:CVCL_4388, ATCC, Manassas, VA) were cultured in DMEM (nacalai tesque, Kyoto, Japan) with 10% FBS (biowest, Nuaille, France), 1% GultaMAX (Gibco, Gaithersburg, MD), and 1% Penicillin-streptomycin (nacalai tesque) at 37˚C under 5% CO_2. hTERT-RPE1 cells were authenticated by the STR profiling and negative for mycoplasma contamination. To induce ciliogenesis, cells were grown to 80–90% confluency and serum-starved (0.5% FBS) for 24 hr. For SAG-stimulation, cells were treated with 100 nM SAG (Merck) for 24 hr after serum-starvation. For rapamycin-stimulation, cells were treated with 0.5 µM rapamycin (LC Laboratories, Woburn, MA) for 24 hr after serum-starvation. Transient knockdown was achieved using the Lipofectamine RNAiMAX transfection regent (Thermo-Fisher Scientific, Waltham, MA) for 48 hr under serum-starvation conditions according to the manufacturer's instructions with a final concentration of 6–20 nM siRNA (Key resources table). For over-expression of DYRK2-HaloTag, transfection was performed using X-tremeGENE9 (Merck) for hTERT-RPE1 cells according to the manufacturer's instructions and the cells were cultured for 24 hr under serum-starvation condition for ciliogenesis. For over-expression of AURKA-EGFP or EGFP, transfection was performed using Xfect (TaKaRa Bio) for MEFs according to the manufacturer's instructions, and the cells were cultured for 24 hr under serum-starvation condition for ciliogenesis.

Adenovirus infection

Adenovirus construction and infections were performed according to a previous report (***Maekawa et al., 2013***; ***Yokoyama-Mashima et al., 2019***). Briefly, Flag-DYRK2 and Flag-DYRK2-K251R (***Taira et al., 2010***; ***Mimoto et al., 2013***) were expressed depending upon *Cre*-expression. Following infection at a MOI (multiplicity of infection) of 100, MEFs were extracted for gene-