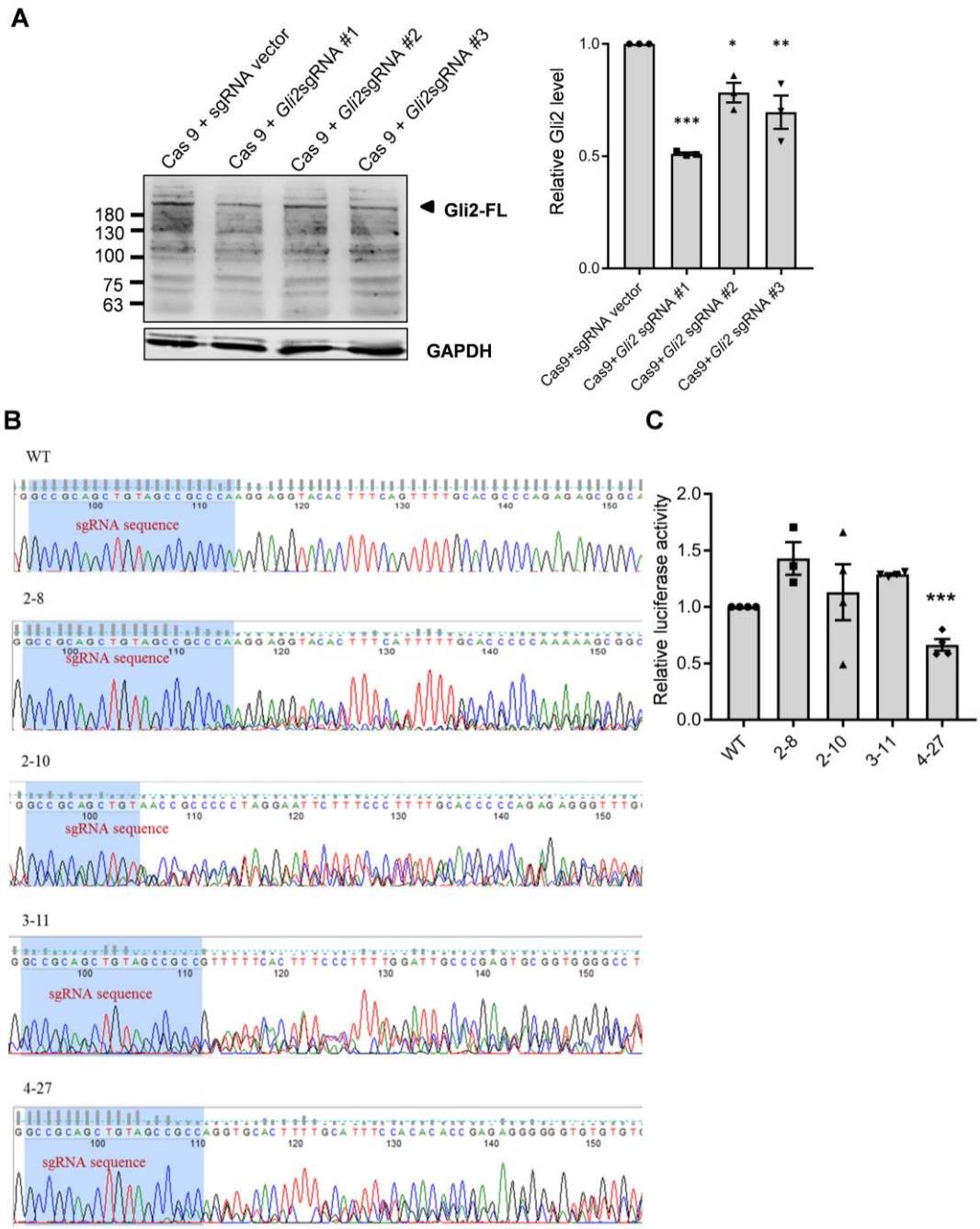

Figure S1. Selection and validation of ideal NIH3T3 clone for Gli2 knockout

(A) Protein lysates from NIH3T3 cells, transfected with Cas9 along with either sgRNA vector, Gli2sgRNA#1, Gli2sgRNA#2 or Gli2sgRNA#3, were immunoblotted for Gli2 or GAPDH (loading control). Bar graph along with individual data points represents the relative Gli2 protein level in each group (n = 3 trials). One-way ANOVA, post-hoc: Bonferroni test, *: $p<0.05$, **: $p<0.01$, ***: $p<0.001$.

(B) NIH3T3 cells were transfected with Gli2sgRNA#1 and Cas9, followed by expansion of 104 single cells for genomic DNA sequencing. Among them, clone #2-8, #2-10, #3-11, and #4-27 showed disruptions in the exon 2 portion of *Gli2* gene next to the sgRNA targeting sequence (blue).

(C) Gli-luciferase assay of the clone #2-8, #2-10, #3-11, and #4-27. Bar graph along with individual data points shows that the luciferase activity in clone #4-27 was significantly decreased compared to the wild type (n = 3 trials).

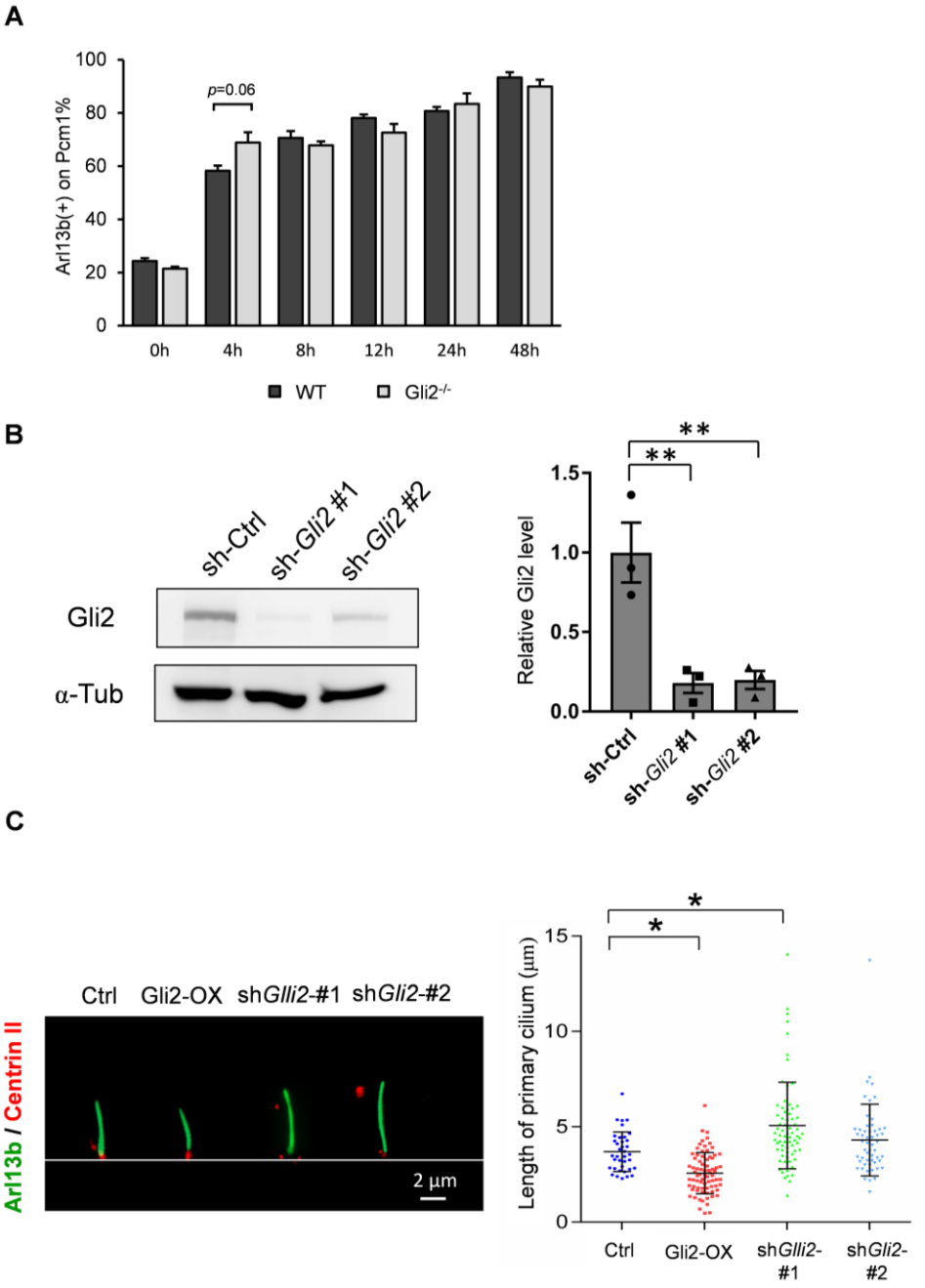

Figure S2. Percentage of ciliated cells and ciliary length in wild type and Gli2 depleted cells

(A) Statistics for the percentage of ciliated cells in NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells after serum starvation for indicated times. Bar graph exhibited that there is no difference between NIH3T3^{WT} cells and NIH3T3$^{Gli2-/-}$ cells possessing the primary cilium labelled by Arl13b. Cell were counted from 3 trials and cell numbers are listed as (WT, Gli2$^{-/-}$): 0hr (225, 299); 4hr (170, 179); 8hr (257, 333); 12hr (252, 281); 24hr (320, 313); 48hr (90, 93). Student's *t* test: $p>0.05$.

(B) Gli2 expression in NIH3T3 cells transfected with shRNAs. Protein lysates from NIH3T3 cells transfected with sh-Ctrl, sh-*Gli2* #1, and sh-*Gli2* #2 were immunoblotted with antibody against Gli2 and α-tubulin (α-Tub, loading control). Bar graph along with individual data points shows decreased Gli2 expression by both shRNAs (n = 3). One-way ANOVA, post-hoc: Bonferroni test, **: $p<0.01$.

(C) Examples of primary cilia in live NIH3T3 cells transfected with Gli2 overexpression or shRNA constructs after serum starvation for 24 hrs. PC2-Arl13bGFP (Green) and pmCherry-Centrin II (Red) were co-transfected to mark the primary cilia and basal bodies. Scatter plots shows that Gli2 knockdown by sh-*Gli2* #1 (n = 71 cells) or sh-*Gli2* #2 (n = 54 cells) increased the length of primary cilia compared to control cells (n = 37 cells), while Gli2-OX (n = 76 cells) slightly reduced the length of primary cilia. Kruskal Wallis test: $p<0.001$. Post-hoc: Mann-Whitney U test, *: $p<0.05$.

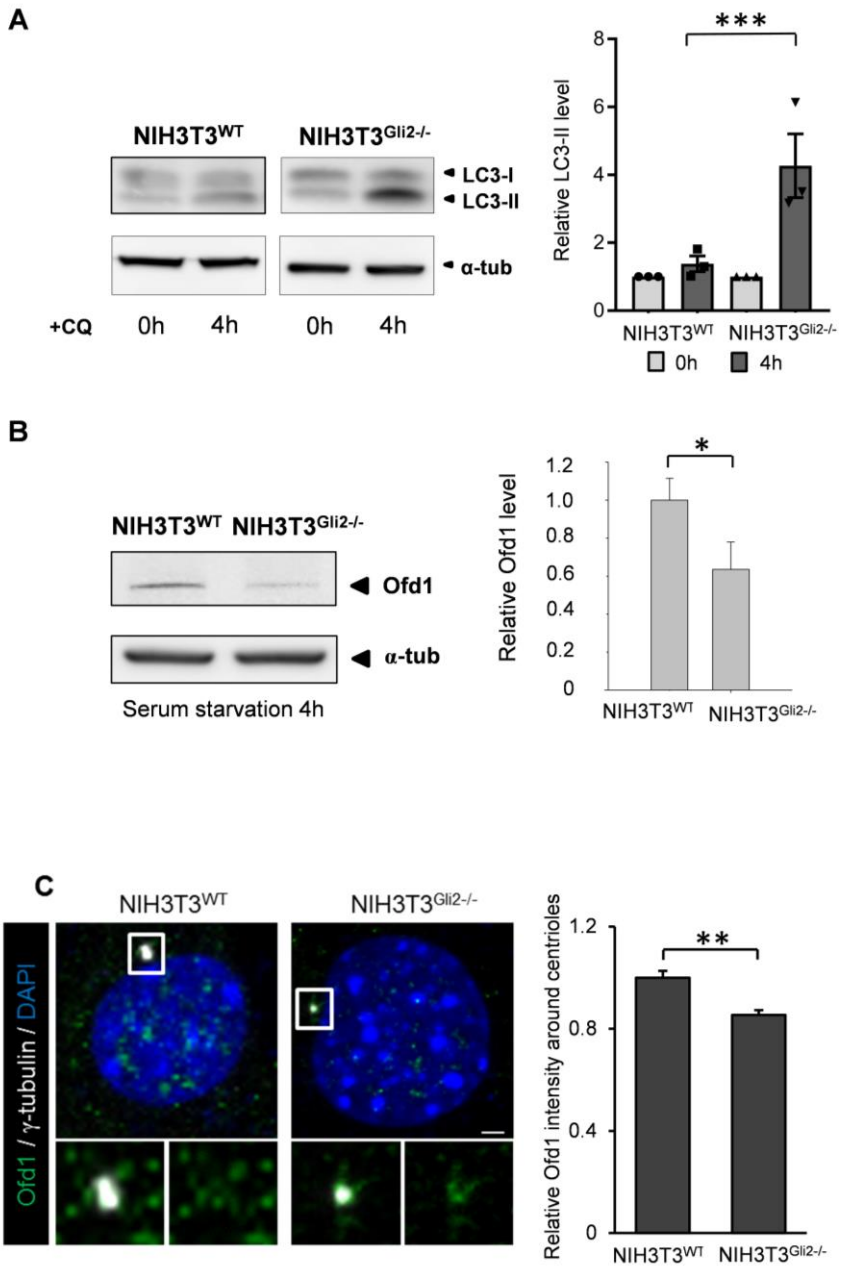

Figure S3. The induction of LC3-II and the reduction of Ofd1 in Gli2 knockout cells

(A) Protein lysates from NIH3T3^{WT} cells and NIH3T3$^{Gli2-/-}$ cells were treated with CQ for 0h and 4h under serum free condition, and then immunoblotted with antibody against LC3, and α-tubulin (α-tub, loading control). Bar graph along with individual data points exhibits the relative protein level of LC3-II at 4h compared to the basal condition (0h) in each group. N = 3 trials, Mann-Whitney *U* test, ***: $p<0.001$.

(B) Protein lysates were collected from NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells after serum starvation for 4h, and immunoblotted for Ofd1, and α-tub (loading control). Bar graph shows the relative protein levels of Ofd1 in NIH3T3$^{Gli2-/-}$ cells compared to WT cells; n = 6 trials, *: $p< 0.05$, student's *t* test.

(C) Immunostaining of Ofd1 satellites (Green) around the centrioles (γ-tubulin, white) in NIH3T3^{WT} cells and NIH3T3$^{Gli2-/-}$ cells after serum starvation for 24h. Boxed regions are enlarged in the bottom. Scale bar: 2μm. Bar graph shows the relative Ofd1 intensity surrounding the centrioles. NIH3T3^{WT}: n = 169 cells, NIH3T3$^{Gli2-/-}$: n = 127 cells. Mann-Whitney *U* test, **: $p<0.01$.

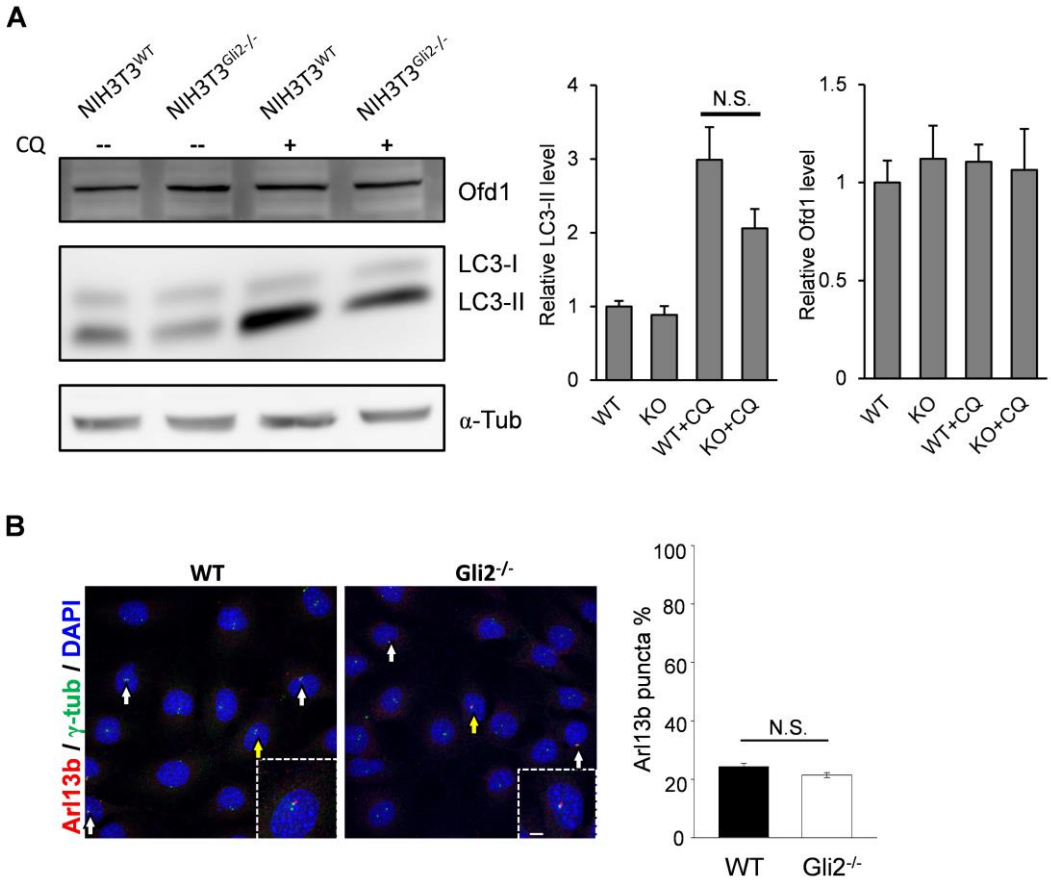

Figure S4. Comparable autophagy activity, Ofd1 protein level and percentage of primary cilium in Gli2 knockout cells under basal condition

(A) Protein lysates from NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells were treated with or without CQ for 4h under serum-rich condition, following by immunoblotting with antibody against Ofd1, LC3, and α-tubulin (α-tub, loading control). Bar graph displayed the relative protein level in each condition. n = 6 trials; ANOVA test, post-hoc: Bonferroni test.

(B) Immunostaining of primary cilia (Arl13b, red), centrioles (γ-tubulin, green) and DAPI (blue) under serum-rich condition. Arl13b+ puncta (arrows) were seldom detectable in both NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells. Centrioles pointed by the yellow arrows were magnified in the inlets. Scale bar: 5μm. Bar graph represents the percentage of centrioles possessing the Arl13b+ puncta. N = 225 in NIH3T3^{WT} cells, N = 299 in NIH3T3$^{Gli2-/-}$ cells; student's *t* test, $p>0.05$.

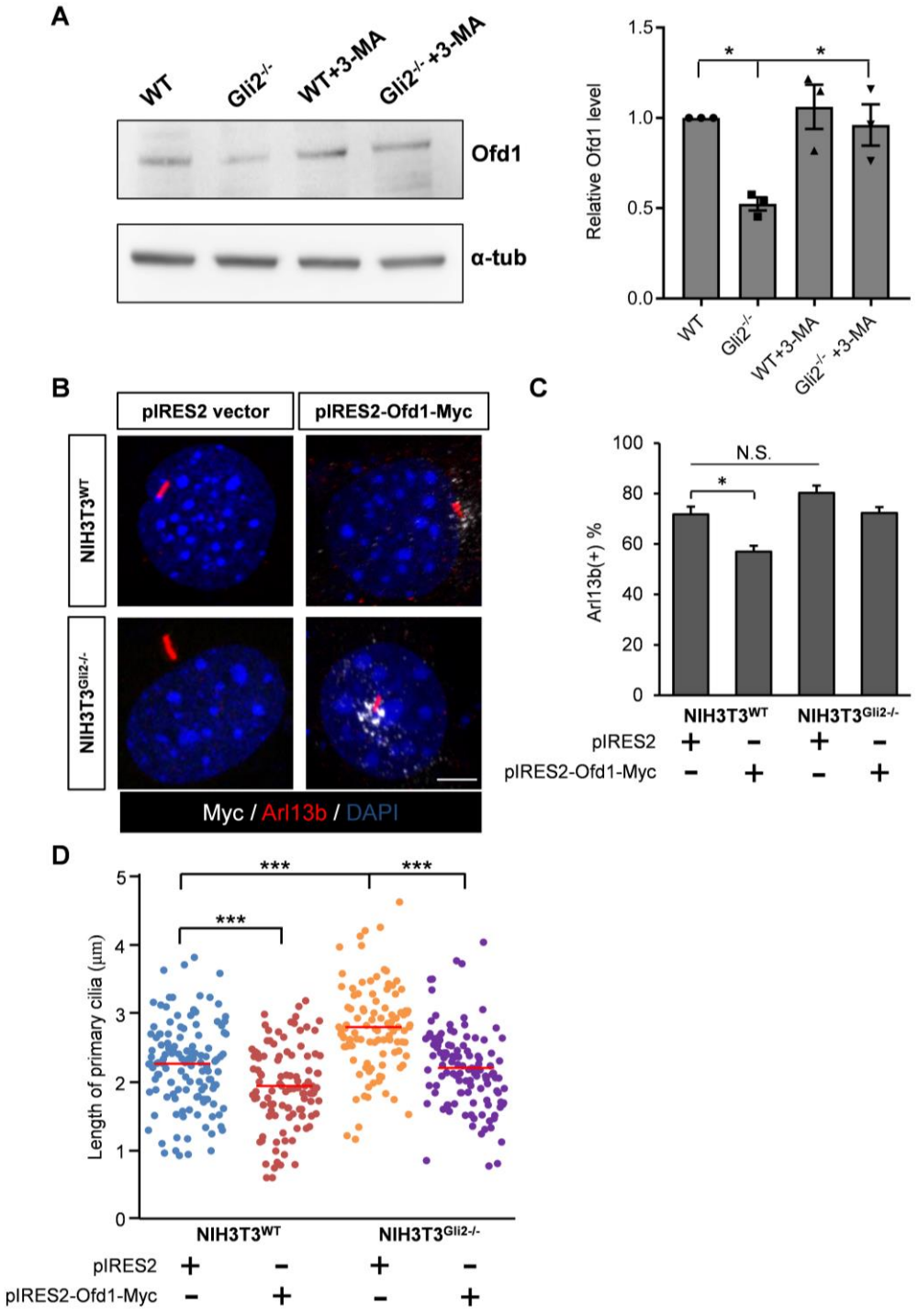

Figure S5. Rescue effect of Ofd1 reduction and ciliary elongation in Gli2 knockout cells treated with autophagy inhibitors and Ofd1 overexpression

(A) Protein lysates from NIH3T3^{WT} cells and NIH3T3$^{Gli2-/-}$ cells treated with or without 3-MA during serum starvation for 4h, were immunoblotted with antibodies against Ofd1 and α-tubulin. Bar graph along with individual data points represents the relative Ofd1 protein level in each condition. $n = 3$, ANOVA: $p = 0.008$; post-hoc: Bonferroni test, *: $p<0.05$.

(B) Immunostaining of Arl13b (red), myc-tag (Gray) and DAPI (blue) in NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells transfected with either vector (pIRES2) or myc-tagged Ofd1 (pIRES2-Ofd1-Myc) and

serum starved for 24h. Expression of Ofd1 shortened the length of primary cilia. Scale bar: 5µm.

(C) Bar graph represents the percentage of ciliated cells in each group. number of cells (WT, Gli2$^{-/-}$) transfected with pIRES2 = (291, 182); with pIRES2-Ofd1-myc = (208, 214). ANOVA test, post-hoc: Bonferroni test, *: $p<0.05$.

(D) Scatter plot exhibits the length of individual primary cilium in each group. Red lines mark the mean. Expression of Ofd1-myc significantly reversed the lengthening of primary cilia in NIH3T3$^{Gli2-/-}$ cells. In NIH3T3^{WT} cells, n = 126 and 104 cilia for pIRES2 and pIRES2-Ofd1-myc respectively; meanwhile, n = 104 and 118 cilia for NIH3T3$^{Gli2-/-}$ cells transfected with pIRES2 and pIRES2-Ofd1-myc respectively. ANOVA test: $p<0.001$. Post-hoc: Bonferroni test, ***: $p<0.001$.

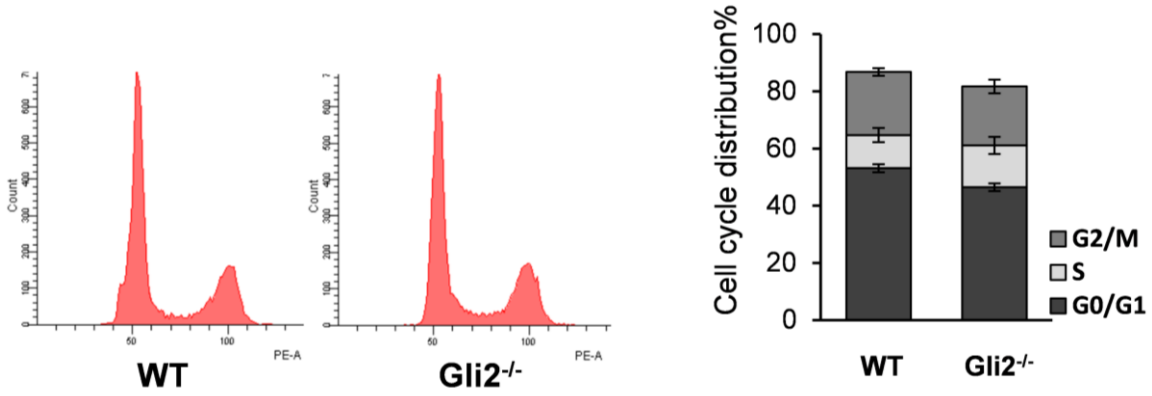

Figure S6. Gli2 knockout cells exhibit similar cell cycle distribution as wild type cells

Cell cycle analysis by flow cytometry in NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells for 24 hours after seeding. Bar graph indicates that the percentages of G0/G1, S and G2/M phases were not significant different between NIH3T3^{WT} and NIH3T3$^{Gli2-/-}$ cells (n = 3 trials). Student's *t* test, $p>0.05$.

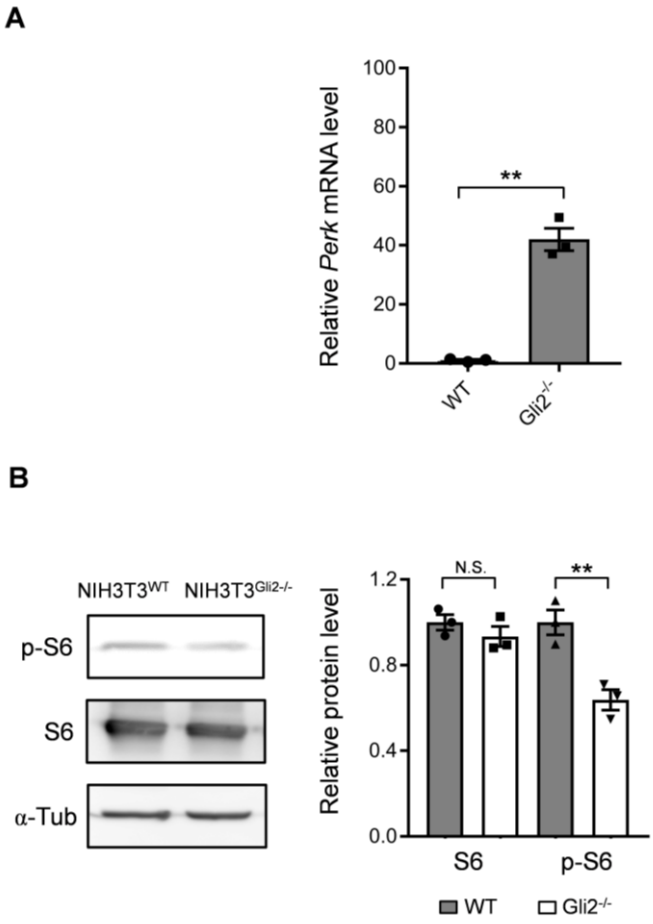

Figure S7. Potential mechanism in downstream of Gli2 for autophagy activation

(A) RT-qPCR for the expression of *Perk* mRNA upon serum starvation for 24h. Bar graph along with individual data points shows a significant increase in *Perk* mRNA in NIH3T3$^{Gli2-/-}$ cells compared to NIH3T3^{WT}. $n = 3$ trials. Student's t test, **: $p<0.01$.

(B) Protein lysates from NIH3T3^{WT} cells and NIH3T3$^{Gli2-/-}$ cells after serum starvation for 4h, and then immunoblotted with antibody against phospho-ribosomal protein S6 (p-S6), S6 and α-tubulin. Bar graph along with individual data points represents a significant decrease of the phosphorylation in S6 in NIH3T3$^{Gli2-/-}$ cells compared to NIH3T3^{WT} cells. $n = 3$ trials. Student's t test, **: $p<0.01$.

Table S1. Sequences for Gli2 guide RNA used in this study

No.	Direction	Sequence
*Gli2*sgRNA#1	Forward	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACA CCGCCGCAGCTGTAGCCGCCCA
	Reverse	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAA CTGGGCGGCTACAGCTGCGGC
*Gli2*sgRNA#2	Forward	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACA CCGTGCGGCCACCGCCAGAGGAC
	Reverse	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAA CGTCCTCTGGCGGTGGCCGCAC
*Gli2*sgRNA#3	Forward	TTTCTTGGCTTTATATATCTTGTGGAAAGGACGAAACA CCGACAGCAGCTTCCCCGACCC
	Reverse	GACTAGCCTTATTTTAACTTGCTATTTCTAGCTCTAAAA CGGGTCGGGGAAGCTGCTGTC

Table S2. Query coverage of candidate Gli2 KO lines.

NIH3T3 cell number	Query cover (%)
2-8	90%
2-10	99%
3-11	22%
4-27	10%