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# CCRK depletion inhibits glioblastoma cell proliferation in a cilium-dependent manner

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**Loss of primary cilia is frequently observed in tumour cells, including glioblastoma cells, and proposed to benefit tumour growth, but a causal link has not been established. Here, we show that CCRK (cell cycle-related kinase) and its substrate ICK (intestinal cell kinase) inhibit ciliogenesis. Depletion of CCRK leads to accumulation of ICK at ciliary tips, altered ciliary transport and inhibition of cell cycle re-entry in NIH3T3 fibroblasts. In glioblastoma cells with deregulated high levels of CCRK, its depletion restores cilia through ICK and an ICK-related kinase MAK, thereby inhibiting glioblastoma cell proliferation. These results indicate that inhibition of ciliogenesis might be a mechanism used by cancer cells to provide a growth advantage.**

Keywords: CCRK; cilia; glioblastoma; ICK; MAK
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## INTRODUCTION

Primary cilia are microtubule-based organelles assembled from mother centrioles by intraflagellar transport (IFT) [1] and act as sensors of environmental conditions [2]. Incomplete ciliogenesis has been noticed in human glioblastoma cells [3], but whether this defect contributes to their malignancy remains to be established. The recent observation that cilia inhibit cell cycle re-entry [4,5] suggests that loss of cilia might provide tumour cells a growth advantage. Cell cycle-related kinase (CCRK) is overexpressed in glioblastomas and implicated in cell cycle regulation, but the molecular mechanisms remain controversial [6–10]. Links between ciliogenesis and CCRK homologues in *Chlamydomonas* [11] and in zebrafish [12] led us to explore whether CCRK regulates ciliogenesis in mammalian cells and whether this function contributes to its oncogenic properties.

## RESULTS

### CCRK inhibits ciliogenesis and Hedgehog signalling

To study the ability of mammalian CCRK to regulate cilia, we depleted CCRK in NIH3T3 cells followed by a serum starvation to promote ciliogenesis. An 80% reduction in CCRK messenger RNA level (supplementary Fig S1A online) did not lead to detectable alterations in the cell cycle profile after a 24-h serum starvation (supplementary Fig S1B online). The cilia in CCRK-depleted cells, however, were elongated (Fig 1A,B) with two independent short interfering RNAs (siRNAs) (Fig 1B; supplementary Fig S1C online). The role of CCRK in ciliary length control is consistent with previous observations in *Chlamydomonas*, where a CCRK homologue (LF2) is involved in flagella length regulation [11]. The elongated primary cilia in CCRK-depleted NIH3T3 cells were not detectably curled as reported for motile cilia in CCRK-deficient zebrafish kidney tubules [12], suggesting that CCRK regulation of cilia formation is context and possibly dose dependent as for LF2 [11]. Notably in NIH3T3 cells, knockdown of Broad-minded (Bromi) also did not lead to curled cilia (unpublished observations) unlike in zebrafish kidney tubules [12].

After serum starved for 24 h, almost all NIH3T3 cells were ciliated and cilia frequency was not significantly altered despite the cilia elongation in siCCRK cells (Fig 1C, serum starvation 24 h). However, in cells starved for only 8 h, 54.4 ± 6.3% of siNT cells were ciliated whereas this fraction was significantly higher in siCCRK cells (74.2 ± 2.4%; Fig 1C, serum starvation 8 h), indicating that CCRK knockdown promotes cilia formation. After serum restimulation, disassembly of cilia was attenuated in siCCRK cells (Fig 1C, serum restimulation), indicating that CCRK knockdown increases cilia stability.

To investigate whether the regulation of ciliogenesis by CCRK requires its kinase activity, siRNA-resistant constructs expressing green fluorescent protein (GFP)-tagged wild-type (WT) or kinase-deficient (K33M) CCRK were transfected together with siNT or siCCRK to NIH3T3 cells followed by a 24-h serum starvation. The results indicate that overexpression of GFP–CCRK–WT, but not GFP–CCRK–K33M, inhibits ciliogenesis as evidenced both by decreased cilia frequency (Fig 1D, black bars) and length (Fig 1E, black bars) in siNT cells. Furthermore, only the WT CCRK rescues the cilia elongation in siCCRK cells (Fig 1E and supplementary Fig S1D online). Together, these results establish mammalian CCRK as a kinase negatively regulating ciliogenesis.

We next asked whether the elongated cilia in CCRK-deficient cells are functionally altered by investigating transcriptional responses of Hedgehog (Hh) signalling following ligand (ShhN) stimulation by measuring either Gli-luciferase reporter activity or endogenous mRNA levels of two Hh target genes (*Gli1* and *Ptch*). A significant elevation of Hh pathway activity was noted

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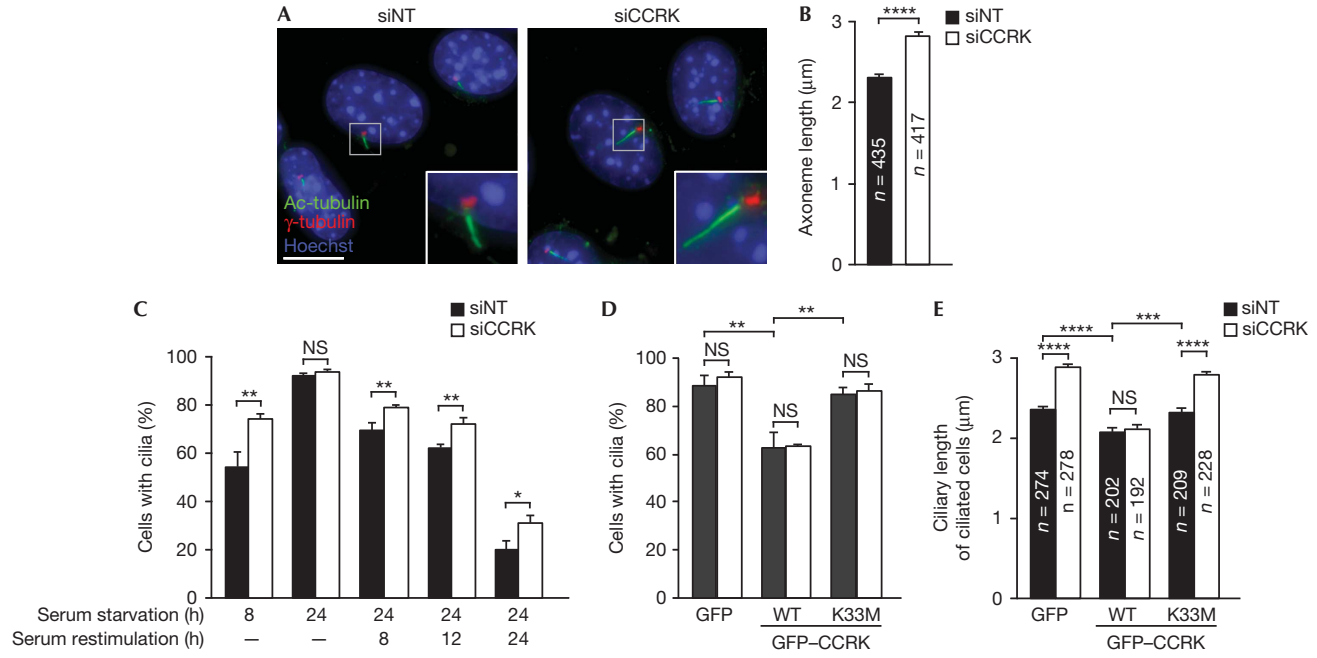


**Fig 1** | Knockdown of CCRK increases ciliogenesis in NIH3T3 cells. (**A**) Representative immunofluorescence images of serum-starved NIH3T3 cells transfected with indicated siRNAs and stained with antibodies against acetylated α-tubulin (Ac-tubulin; green), γ-tubulin (red) and counterstained with Hoechst (blue). Scale bar, 10 μm. Insets are higher magnifications of boxed regions. (**B**) Average ciliary axoneme length from three experiments in siNT or siCCRK transfected NIH3T3 cells serum starved for 24 h. (**C**) Percentage of ciliated cells following transfection with siNT or siCCRK, serum starvation and restimulation for indicated hours (h). (**D**) Frequency of cilia in GFP-positive NIH3T3 cells expressing GFP, GFP–CCRK–WT or –K33M transfected with siNT (black) or siCCRK (white) and serum starved for 24 h before fixation. (**E**) Average of ciliary length in ciliated NIH3T3 cells from three experiments described in **D**. In **B** and **E**, the numbers of cilia measured are indicated (*n*) and error bars represent the s.e.m. In **C** and **D** about 100 cells were analysed for each condition per experiment and columns indicate means from three separate experiments with s.d. shown as error bars. Significances of difference between indicated samples were determined by Student's *t*-test (****$P<0.0001$; ***$P<0.001$; **$P<0.01$; *$P<0.05$; NS, $P>0.05$). CCRK, cell cycle-related kinase; GFP, green fluorescent protein; NS, not significant; siRNAs, short interfering RNAs; WT, wild type.

in siCCRK cells by both assays (supplementary Fig S1F–G online) in conditions where cilia frequency is comparable to siNT cells owing to a long serum starvation. We subsequently analysed the translocation of Gli3 to ciliary tips representing an early event of Hh signalling activation [13]. Here, cells serum-starved for 24 h and subsequently stimulated with ShhN for 1 h were used. Interestingly, the percentage of cells with Gli3 at ciliary tips was significantly higher in siCCRK cells than in siNT cells (supplementary Fig S1H–I online), suggesting that CCRK knockdown leads to increased ciliary transport of Gli3 in conjunction with increased Hh signalling.

## CCRK is required for ciliogenesis inhibition by ICK

Despite the full competence to rescue, GFP–CCRK is not enriched in cilia or basal bodies (supplementary Fig S1E online), suggesting an indirect role of CCRK in regulating ciliogenesis. In this regard, two CCRK substrates—the intestinal cell kinase (ICK) [14] and MAK (male germ cell-associated kinase) kinases [14,15]—are interesting candidate effectors because their homologues in lower species have been implicated in length control of cilia/flagella [16–19]. Moreover, *Mak*-null mouse photoreceptor cells demonstrate longer cilia, and overexpression of MAK in NIH3T3 cells reduces ciliary length in a kinase-dependent manner [20]. As MAK is not expressed at detectable level in NIH3T3 cells (supplementary Fig 2A online), we therefore investigated ICK as a potential effector of CCRK in regulating ciliogenesis in this system.

Expression of glutathione *S*-transferase (GST)-tagged ICK reduced cilia frequency dramatically (Fig 2A, panels a,c and Fig 2B). In cells lacking cilia, GST–ICK was occasionally accumulated at the microtubule-organizing centre (Fig 2A, panel c inset). In the rare GST–ICK-positive cells with detectable cilia, ICK was enriched at the distal tips of cilia (supplementary Fig S2B online), suggesting a more direct role of ICK in regulating ciliogenesis. Consistent with a suppressive role of ICK in regulating ciliogenesis, depletion of ICK in NIH3T3 cells led to increased ciliary length comparable to CCRK knockdown (supplementary Fig S3A online, top panel). Interestingly, the ability of exogenous ICK to inhibit cilia formation was severely blunted when CCRK level is reduced (Fig 2A, panels c,d and Fig 2B), demonstrating that CCRK is required for ICK-mediated inhibition of ciliogenesis. Cells expressing GST–ICK with reduced CCRK level demonstrated prominent cilia, and again ICK was enriched at distal tips (Fig 2A, panel d inset).

To further investigate ICK as an effector of CCRK in inhibiting ciliogenesis, we mutated the CCRK phosphorylation site on ICK [14] to a nonphosphorylatable alanine (T157A) expected to significantly reduce ICK activity [21]. In NIH3T3 cells, GST–ICK–T157A also localized to distal tips (Fig 2A, panels e,f) but displayed a severely blunted ability to suppress cilia formation

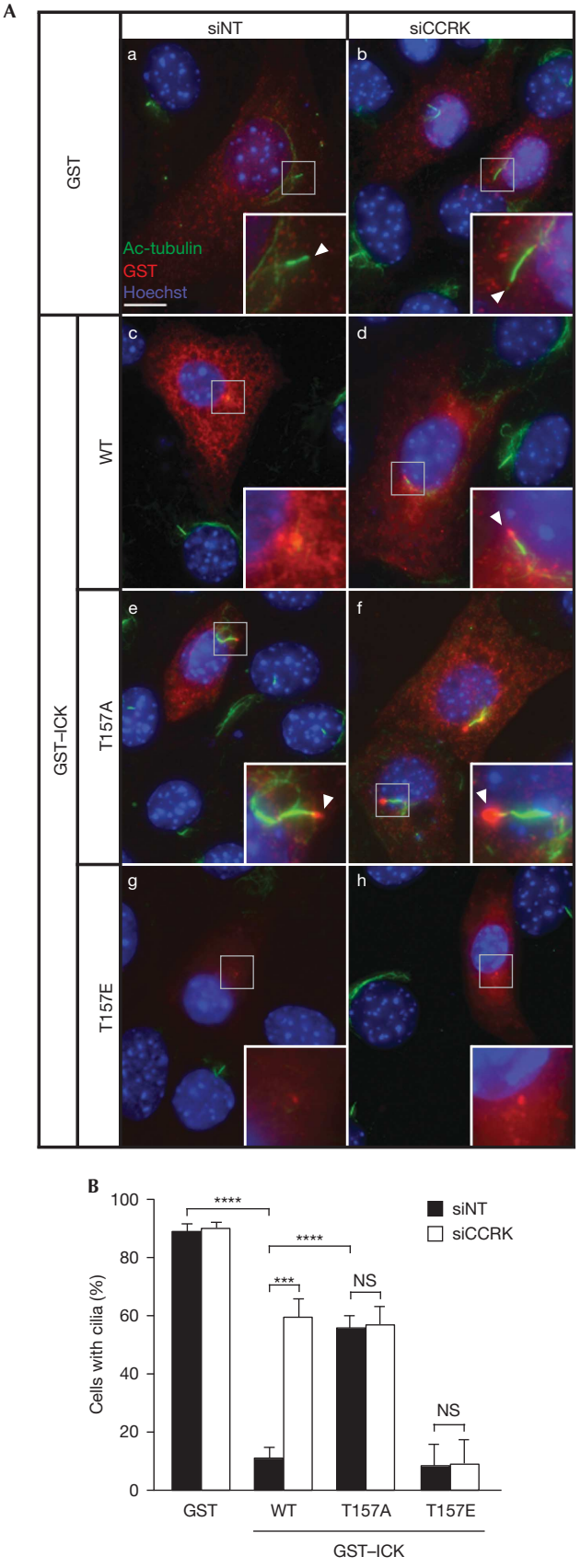


**Fig 2** | Inhibition of ciliogenesis by ICK requires both T157 and CCRK. (**A**) Micrographs of NIH3T3 cells initially transfected with indicated siRNAs and subsequently with GST (a,b), GST–ICK (c,d), GST–ICK–T157A (e,f) or GST–ICK–T157E (g,h) expression plasmids and serum starved for 24 h before staining with antibodies to acetylated α-tubulin (Ac-tubulin; green) for cilia, GST (red) for plasmid transfected cells and Hoechst (blue) for nuclei. Scale bar, 10 μm. Insets are higher magnifications of boxed regions. Arrowheads mark the distal tips of cilia. (**B**) Columns indicate frequency of cilia in GST-positive cells shown in **A**. Fifty to sixty GST-positive cells were scored for each condition from three separate experiments. Error bars indicate s.d. Significances of differences by Student's *t*-test are indicated (****$P<0.0001$; ***$P<0.001$; NS, $P>0.05$). CCRK, cell cycle-related kinase; GST, glutathione *S*-transferase; ICK, intestinal cell kinase; NS, not significant; siRNAs, short interfering RNAs; WT, wild type.

compared with GST–ICK (Fig 2B). The remaining suppression is likely owing to the residual activity of ICK–T157A [21]. Consistent with these observations on ciliation, GST–ICK suppressed Hh signalling whereas GST–ICK–T157A did not (supplementary Fig S2C online).

In addition, overexpression of an ICK phosphomimetic mutant (ICK–T157E) reduced cilia frequency comparably to WT ICK (Fig 2A, panel g and Fig 2B) and GST–ICK–T157E in rare ciliated cells was enriched at the ciliary tips (supplementary Fig S2D online). In contrast to WT ICK, the ability of the phosphomimetic mutant to inhibit cilia formation was resistant to CCRK depletion (Fig 2A, panels g,h and Fig 2B). These data together indicate that the ciliary function of CCRK is mediated through ICK–T157 phosphorylation.

## CCRK and ICK regulate cell cycle through cilia

The findings that cilia inhibit S-phase entry [4,5] and that CCRK and ICK promote G1 progression [6,7,9,10,22] prompted us to investigate whether the cell cycle effects of CCRK and ICK are mediated through cilia. For this, we generated pools of NIH3T3 cells stably expressing non-targeting short-hairpin RNA (shRNA) (shNT) or shRNA-targeting Kif3a (shKif3a), a subunit of Kinesin II anterograde IFT motor required for ciliogenesis [23]. In Kif3a-depleted cells, ciliogenesis was suppressed as evidenced by an increase in the percentage of cells with absent or short cilia (supplementary Fig S3A online, 0–1 μm). Importantly, knockdown of CCRK or ICK did not increase the length of cilia in Kif3a-depleted cells (supplementary Fig S3A online, lower panel).

We then examined the effect of CCRK and ICK depletion on G1/S transition induced by serum restimulation in shNT or shKif3a cells by labelling newly synthesized DNA with ethynyl deoxyuridine (EdU). Subsequent detection of the incorporated EdU using click chemistry allowed efficient identification of replicating cells (Fig 3A). When CCRK or ICK was depleted in control (shNT) cells, a significantly decreased EdU incorporation was noted both 8 and 12 h after restimulation (Fig 3B, shNT), indicating decreased G1/S transition. This inhibition was rescued in shKif3a cells (Fig 3B, shKif3a), despite comparable knockdown efficiencies of CCRK and ICK in shNT and shKif3a cells (supplementary Fig S3B online). A similar rescue was observed in pools of NIH3T3 cells stably expressing shIft172 depleting another IFT component

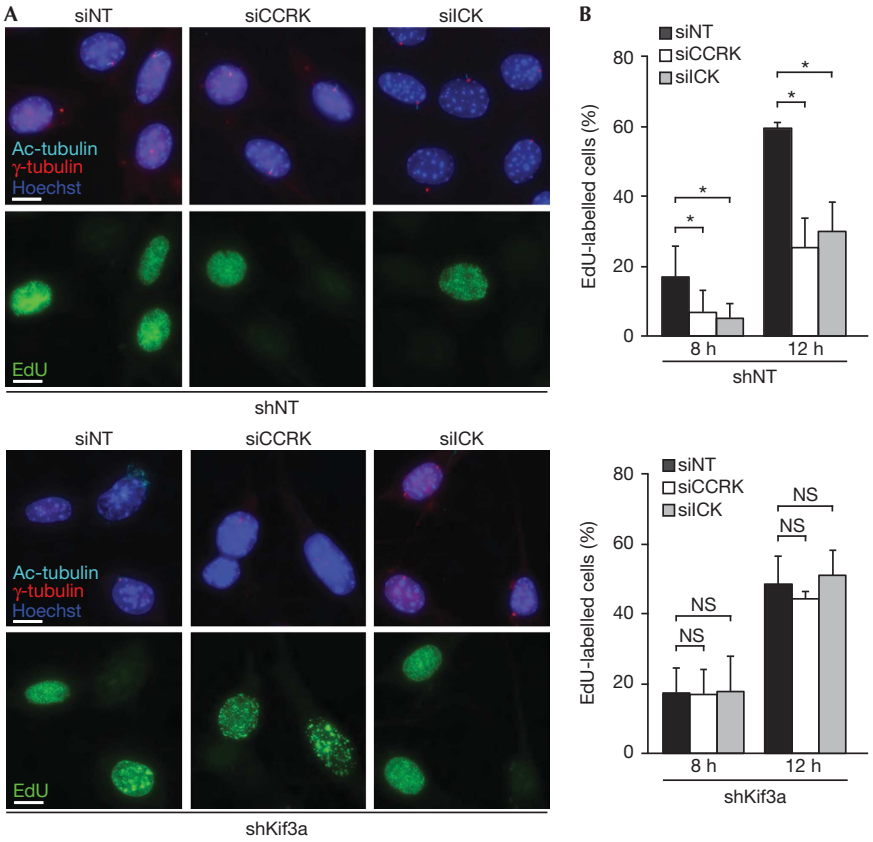


**Fig 3** | Inhibition of cell cycle re-entry following CCRK and ICK depletion requires Kif3a. (**A**) Fluorescence micrographs of NIH3T3 cells stably expressing non-targeting shRNA (shNT; top panels) or shRNA-targeting Kif3a (shKif3a; bottom panels) and transfected with indicated siRNAs. Following a 24 h starvation, cells were restimulated with serum for 12 h and labelled with EdU during the final 1.5 h. The merged panels demonstrate cilia (Ac-tubulin; cyan), centrioles (γ-tubulin; red) and nuclei (Hoechst; blue). Nuclei that have incorporated EdU are shown in green (EdU). Scale bar, 10 μm. (**B**) Percentage of EdU-labelled cells in cells expressing shNT or shKif3a, transfected with indicated siRNAs and collected 8 or 12 h after serum restimulation. More than 150 cells were checked for each group in each experiment. Bars represent means ± s.d. from three separate experiments. Significances of differences by Student's paired *t*-test are indicated (*$P<0.05$; NS, $P>0.05$). CCRK, cell cycle-related kinase; EdU, ethynyl deoxyuridine; ICK, intestinal cell kinase; NS, not significant; shRNA, short-hairpin RNA; siRNAs, short interfering RNAs.

Ift172 [24]. These results indicate that the inhibition of cell cycle progression by depletion of CCRK and ICK is dependent on cilia in NIH3T3 cells.

## High CCRK in glioblastoma cells inhibits ciliogenesis

As cilia have been implicated in proliferation in the context of cancer [25,26] and CCRK has been identified as an overexpressed oncogene promoting cell proliferation in glioblastoma [7], we next investigated whether loss of cilia in glioblastoma cells is owing to CCRK overexpression and whether this is associated with increased proliferation. To this end, we depleted CCRK in U251MG glioblastoma cells overexpressing CCRK similarly to most glioblastoma cell lines [7]. Interestingly, CCRK knockdown lead to formation of robust cilia in a fraction of glioblastoma cells (Fig 4A–D) indicating that CCRK depletion is sufficient to restore cilia. A comparable restoration was noted following a double knockdown of ICK and MAK (Fig 4E), whereas alone ICK or MAK did not restore cilia. Triple knockdown of CCRK, ICK and MAK did not increase cilia restoration compared with CCRK knockdown alone (Fig 4B,E), despite comparable knockdown efficiencies (supplementary Fig S4C online). The data together with previous results on ICK (this study and Fu *et al* [14]) and MAK [15] indicate that overexpression of CCRK inhibits ciliogenesis in glioblastoma cells through ICK and MAK. Interestingly, cells with restored cilia are severely compromised in their ability to incorporate EdU indicating a block or severe delay in cell cycle progression (supplementary Fig S4A–B online).

To explore possible upstream regulators of CCRK overexpression, we treated U251MG cells with a panel of inhibitors implicated in ciliogenesis or cell growth followed by analysis of cilia frequency and CCRK mRNA levels. The phosphoinositide 3-kinase inhibitor LY294002 was the only agent that significantly increased frequency of cilia (supplementary Fig S4D online, black bars). Remarkably, LY294002 also decreased CCRK mRNA levels unlike any of the other tested inhibitors (supplementary Fig S4D online, white bars), suggesting that the aberrant activation of phosphoinositide 3-kinase pathway in glioblastoma [27] contributes to overexpression of CCRK. Consistently, the ability of LY294002 to restore cilia was less efficient in siCCRK cells (supplementary Fig S4E–F online).

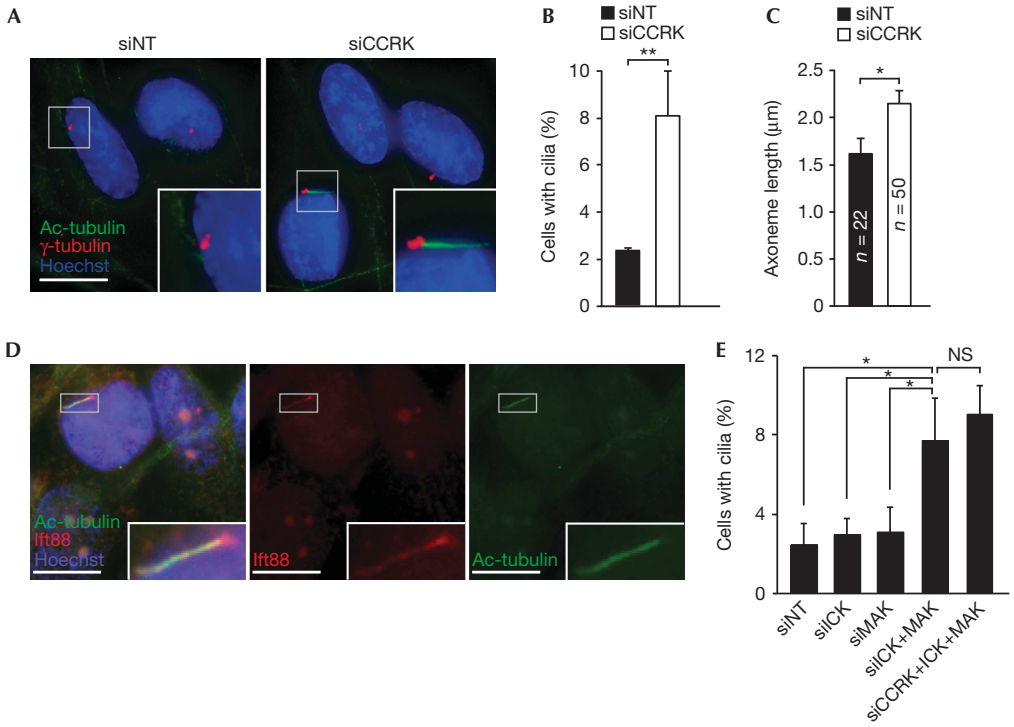


**Fig 4** | Depletion of CCRK in glioblastoma cells restores cilia through its substrate kinases ICK and MAK. (**A**) Immunofluorescence micrographs of serum-starved U251MG cells transfected with siNT or siCCRK, and stained for primary cilia (Ac-tubulin; green), centrioles (γ-tubulin; red) and nuclei (Hoechst; blue). (**B**) Percentage of ciliated cells in **A**. (**C**) Average length of restored cilia in U21MG cells transfected with siNT or siCCRK. The numbers of cilia measured are indicated (*n*). Error bars represent s.e.m. (**D**) Immunofluorescence micrographs of siCCRK-treated U251MG cells with restored cilia stained for primary cilia (Ift88; red and Ac-tubulin; green) and nuclei (Hoechst; blue). (**E**) Percentage of ciliated U251MG cells transfected with indicated siRNA(s). In **A** and **D** scale bar indicates 10 μm and insets are higher magnifications of boxed regions. In **B** and **E** columns represent means ± s.d. from three separate experiments, where more than 200 cells were analysed for each sample per experiment. Significances of differences by Student's *t*-test are indicated (**$P<0.01$; *$P<0.05$; NS, $P>0.05$). CCRK, cell cycle-related kinase; ICK, intestinal cell kinase; MAK, male germ cell-associated kinase; NS, not significant; siRNAs, short interfering RNAs.

## CCRK promotes cell proliferation by inhibiting ciliogenesis

To investigate the potential effects of the restored cilia on glioblastoma cell proliferation, we generated pools of U251MG cells stably expressing shNT or shKIF3A after puromycin selection. Restoration of cilia was readily detected following CCRK knockdown in shNT cells but not in shKIF3A cells (supplementary Fig S5A online). Concomitantly, a small albeit nonsignificant increase in the total G1 population was noted in CCRK-depleted cells (supplementary Fig S5B online); this increase was not noted in shKIF3A cells. These results indicated that the shNT and shKIF3a cells can be used to identify the cilium-dependent functions of CCRK. Subsequently, we investigated possible effects of CCRK knockdown on proliferation of shNT and shKIF3A cells by transduction of hygromycin-resistant shNT or shCCRK expressing lentiviruses. Depletion of CCRK decreased cell propagation to 22.4 ± 4.1% in control cells (Fig 5A, left panels and Fig 5B). By contrast, in shKIF3A cells knockdown of CCRK reduced cell propagation only to 60.1 ± 9.3% (Fig 5A, right panels and Fig 5B; knockdown efficiency in supplementary Fig S5C online). Considering the duration of the experiment (8 days) and doubling time of the U251MG cells (24 h; [28]), this 37.7% difference is close to a calculated percentage (39.1%) if the increased ciliated cells following CCRK depletion would not proliferate as supported by the marked reduction in EdU incorporation (supplementary Fig S4A–B online). A similar effect of CCRK knockdown was observed in U251MG cells depleted of another IFT component IFT20 (supplementary Fig S5D–E online). Together, these results demonstrate that the inhibition of ciliogenesis by overexpressed CCRK promotes proliferation of glioblastoma cells.

## DISCUSSION

This study demonstrates that CCRK inhibits ciliogenesis through ICK and MAK, and further that this mechanism is used to increase proliferative capacity of glioblastoma cells. MAK also inhibits ciliary length in photoreceptor cells, where two microtubule-associated ciliary proteins—Retinitis pigmentosa 1 (RP1) and Rp1-like protein—have been suggested to mediate the ciliary function of MAK [20]. However, the exclusive expression of RP1 and Rp1-like protein in photoreceptor cells [29] suggests that they do not mediate the effects of ICK/MAK on ciliogenesis in fibroblasts or glioblastoma cells observed here. The observed increase in cilial translocation of Gli3 (an IFT cargo) in CCRK-depleted NIH3T3 cells (supplementary Fig S1H–I online), together with reported abnormal accumulation of certain IFT components in CCRK or ICK/MAK homologue mutants [19,20,30], suggests

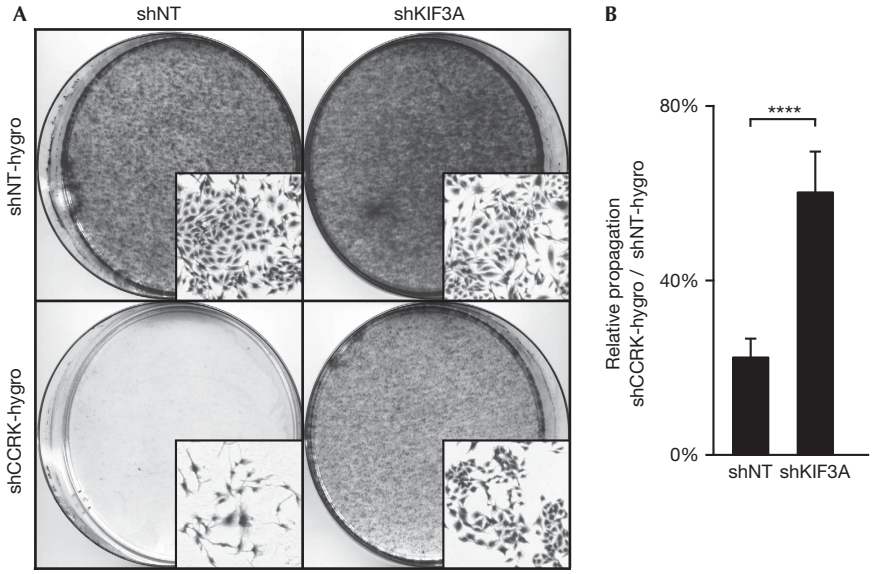


**Fig 5** | Depletion of CCRK in glioblastoma cells inhibits cell growth in part dependent on cilia. (**A**) Crystal violet-stained plates of U251MG cells cultured for 8 days after transduction with indicated shRNA lentiviruses and double selection. Insets show micrographs from the plates. (**B**) Propagation of CCRK-depleted glioblastoma cells compared with controls in shNT (shNT, $n = 4$ from three separate experiments) or shKif3a (shKif3a, $n = 7$ from three separate experiments) samples measured as ratios (shCCRK-hygro versus shNT-hygro) of crystal violet-stained areas. Bars represent means ± s.d. Significance of differences by Student's *t*-test is indicated (****$P < 0.0001$). CCRK, cell cycle-related kinase; hygro, hygromycin B; shRNA, short-hairpin RNA.

that CCRK and ICK/MAK inhibit ciliogenesis through regulating IFT. The lack of cilia elongation following knockdown of CCRK or ICK in cells with a reduced level of Kif3a (supplementary Fig S3A online) further suggests that the ciliogenesis regulation by CCRK and ICK is IFT dependent. It is important to note that in contrast to other known ciliogenesis-regulating kinases that localize to the basal body or the proximal region (for example, CDKL5 [31] and Aurora A [32]), ICK and MAK are enriched in the tips (Fig 2 and [20]) where IFT particles switch from anterograde to retrograde transport and microtubules are constantly turning over [1]. Identified mediators of these processes [1] are interesting candidate ICK/MAK substrates.

Our results indicate that CCRK overexpression is a mechanism by which glioblastoma cells deregulate a pathway operational in normal cells to eliminate cilia and promote growth. When considering the potential involvement of other ciliogenesis regulators in cancer, the HEF1-Aurora A-HDAC6 pathway [32] is particularly interesting because its components are overexpressed in cancers [32]. Although two inhibitors of this pathway (MLN8327 and tubacin) did not restore cilia in glioblastoma cells (supplementary Fig S4D online), inhibition of ciliogenesis by deregulation of this pathway could be involved in other types of cancer. Indeed, recent reports indicate that depletion or inhibition of HEF1, Aurora A or HDAC6 could restore cilia in VHL-defective renal cancer cells [33] and ovarian adenocarcinoma cells [34]. It remains to be established whether the negative effects of HEF1, Aurora A and HDAC6 on ciliogenesis contribute to their oncogenic properties. Mutations in VHL tumour suppressor cause renal cysts that lack cilia and precede renal carcinogenesis [35]. Although restoration of WT VHL is able to reverse the arrest of ciliogenesis [35], it is not clear whether preservation of cilia forms part of the tumour-suppressive function of VHL. The link established here between cilia restoration and growth inhibition together with the identified potential of cancer cells to restore cilia (this study and [refs 33–36]) should stimulate exploring cilia restoration as a therapeutic opportunity in cancer.

## METHODS

**siRNA transfections.** All siRNA oligos were obtained from Dharmacon and transfected alone or with plasmids as described in Supplementary Methods.

**shRNA lentivirus transductions and selections.** shRNA lentiviruses were produced and used in transduction as described in Supplementary Methods. At 48 h after transduction, cells were split and selected for 2–3 days with 3 μg/ml puromycin and subsequently used for EdU labelling (NIH3T3) or transduced with shCCRK-hygro or shNT-hygro lentivirus (U251MG). At 48 h after the second infection, each well of U251MG cells was split into two 10 cm plates in media containing 3 μg/ml puromycin and 300 μg/ml hygromycin B for double selection. After 8 days, one plate was used to analyse mRNA and another for analysis of cell confluence by fixing with 4% paraformaldehyde for 15 min and staining with 1.5% crystal violet for 5 min followed by PBS washes. Confluency was determined from scanned images by quantifying stained area following background subtraction using ImageJ 1.45S.

**Ciliary length quantification.** The ciliary axoneme was selected from the stained MIP images (Supplementary Methods) using the Trace tool and the length of traced features was measured in pixels (1 pixel = 0.1 μm) using the Measurement toolbox of Image-Pro Plus 5.1.

**Supplementary information** is available at EMBO *reports* online (http://www.emboreports.org).

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*Author contributions:* Y.Y. and T.P.M. designed the experiments, interpreted data and prepared the manuscript. Y.Y. performed most of the experiments. N.R. performed and analysed some of the experiments.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

1. Ishikawa H, Marshall WF (2011) Ciliogenesis: building the cell's antenna. *Nat Rev Mol Cell Biol* **12:** 222–234
2. Berbari NF, O'Connor AK, Haycraft CJ, Yoder BK (2009) The primary cilium as a complex signaling center. *Curr Biol* **19:** R526–R535
3. Moser JJ, Fritzler MJ, Rattner JB (2009) Primary ciliogenesis defects are associated with human astrocytoma/glioblastoma cells. *BMC Cancer* **9:** 448
4. Kim S, Zaghloul NA, Bubenshchikova E, Oh EC, Rankin S, Katsanis N, Obara T, Tsiokas L (2011) Nde1-mediated inhibition of ciliogenesis affects cell cycle re-entry. *Nat Cell Biol* **13:** 351–360
5. Li A, Saito M, Chuang JZ, Tseng YY, Dedesma C, Tomizawa K, Kaitsuka T, Sung CH (2011) Ciliary transition zone activation of phosphorylated Tctex-1 controls ciliary resorption, S-phase entry and fate of neural progenitors. *Nat Cell Biol* **13:** 402–411
6. Liu Y, Wu C, Galaktionov K (2004) p42, a novel cyclin-dependent kinase-activating kinase in mammalian cells. *J Biol Chem* **279:** 4507–4514
7. Ng SS *et al* (2007) Cell cycle-related kinase: a novel candidate oncogene in human glioblastoma. *J Natl Cancer Inst* **99:** 936–948
8. Wohlbold L, Larochelle S, Liao JC, Livshits G, Singer J, Shokat KM, Fisher RP (2006) The cyclin-dependent kinase (CDK) family member PNQALRE/CCRK supports cell proliferation but has no intrinsic CDK-activating kinase (CAK) activity. *Cell Cycle* **5:** 546–554
9. An X *et al* (2010) Functional characterisation of cell cycle-related kinase (CCRK) in colorectal cancer carcinogenesis. *Eur J Cancer* **46:** 1752–1761
10. Feng H *et al* (2011) Cell cycle-related kinase is a direct androgen receptor-regulated gene that drives beta-catenin/T cell factor-dependent hepatocarcinogenesis. *J Clin Invest* **121:** 3159–3175
11. Tam LW, Wilson NF, Lefebvre PA (2007) A CDK-related kinase regulates the length and assembly of flagella in Chlamydomonas. *J Cell Biol* **176:** 819–829
12. Ko HW, Norman RX, Tran J, Fuller KP, Fukuda M, Eggenschwiler JT (2010) Broad-minded links cell cycle-related kinase to cilia assembly and hedgehog signal transduction. *Dev Cell* **18:** 237–247
13. Tukachinsky H, Lopez LV, Salic A (2010) A mechanism for vertebrate Hedgehog signaling: recruitment to cilia and dissociation of SuFu-Gli protein complexes. *J Cell Biol* **191:** 415–428
14. Fu Z *et al* (2006) Identification of yin-yang regulators and a phosphorylation consensus for male germ cell-associated kinase (MAK)-related kinase. *Mol Cell Biol* **26:** 8639–8654
15. Wang LY, Kung HJ (2011) Male germ cell-associated kinase is overexpressed in prostate cancer cells and causes mitotic defects via deregulation of APC/C(CDH1). *Oncogene* **31:** 2907–2918
16. Asleson CM, Lefebvre PA (1998) Genetic analysis of flagellar length control in *Chlamydomonas reinhardtii*: a new long-flagella locus and extragenic suppressor mutations. *Genetics* **148:** 693–702
17. Berman SA, Wilson NF, Haas NA, Lefebvre PA (2003) A novel MAP kinase regulates flagellar length in Chlamydomonas. *Curr Biol* **13:** 1145–1149
18. Bengs F, Scholz A, Kuhn D, Wiese M (2005) LmxMPK9, a mitogen-activated protein kinase homologue affects flagellar length in *Leishmania mexicana*. *Mol Microbiol* **55:** 1606–1615
19. Burghoorn J, Dekkers MP, Rademakers S, de Jong T, Willemsen R, Jansen G (2007) Mutation of the MAP kinase DYF-5 affects docking and undocking of kinesin-2 motors and reduces their speed in the cilia of *Caenorhabditis elegans*. *Proc Natl Acad Sci USA* **104:** 7157–7162
20. Omori Y *et al* (2010) Negative regulation of ciliary length by ciliary male germ cell-associated kinase (Mak) is required for retinal photoreceptor survival. *Proc Natl Acad Sci USA* **107:** 22671–22676
21. Togawa K, Yan YX, Inomoto T, Slaugenhaupt S, Rustgi AK (2000) Intestinal cell kinase (ICK) localizes to the crypt region and requires a dual phosphorylation site found in map kinases. *J Cell Physiol* **183:** 129–139
22. Fu Z, Kim J, Vidrich A, Sturgill TW, Cohn SM (2009) Intestinal cell kinase, a MAP kinase-related kinase, regulates proliferation and G1 cell cycle progression of intestinal epithelial cells. *Am J Physiol Gastrointest Liver Physiol* **297:** G632–G640
23. Marszalek JR, Ruiz-Lozano P, Roberts E, Chien KR, Goldstein LS (1999) Situs inversus and embryonic ciliary morphogenesis defects in mouse mutants lacking the KIF3A subunit of kinesin-II. *Proc Natl Acad Sci USA* **96:** 5043–5048
24. Ezratty EJ, Stokes N, Chai S, Shah AS, Williams SE, Fuchs E (2011) A role for the primary cilium in Notch signaling and epidermal differentiation during skin development. *Cell* **145:** 1129–1141
25. Han YG, Kim HJ, Dlugosz AA, Ellison DW, Gilbertson RJ, Alvarez-Buylla A (2009) Dual and opposing roles of primary cilia in medulloblastoma development. *Nat Med* **15:** 1062–1065
26. Wong SY, Seol AD, So PL, Ermilov AN, Bichakjian CK, Epstein EH Jr., Dlugosz AA, Reiter JF (2009) Primary cilia can both mediate and suppress Hedgehog pathway-dependent tumorigenesis. *Nat Med* **15:** 1055–1061
27. Chakravarti A, Zhai G, Suzuki Y, Sarkesh S, Black PM, Muzikansky A, Loeffler JS (2004) The prognostic significance of phosphatidylinositol 3-kinase pathway activation in human gliomas. *J Clin Oncol* **22:** 1926–1933
28. Wang J, Hu L, Gupta N, Shamseldin T, Ozawa T, Klem J, Cardell M, Deen DF (1999) Induction and characterization of human glioma clones with different radiosensitivities. *Neoplasia* **1:** 138–144
29. Yamashita T *et al* (2009) Essential and synergistic roles of RP1 and RP1L1 in rod photoreceptor axoneme and retinitis pigmentosa. *J Neurosci* **29:** 9748–9760
30. Phirke P *et al* (2011) Transcriptional profiling of *C. elegans* DAF-19 uncovers a ciliary base-associated protein and a CDK/CCRK/LF2p-related kinase required for intraflagellar transport. *Dev Biol* **357:** 235–247
31. Tam LW, Ranum PT, Lefebvre PA (2013) CDKL5 regulates flagellar length and localizes to the base of the flagella in Chlamydomonas. *Mol Biol Cell* **24:** 588–600
32. Pugacheva EN, Jablonski SA, Hartman TR, Henske EP, Golemis EA (2007) HEF1-dependent Aurora A activation induces disassembly of the primary cilium. *Cell* **129:** 1351–1363
33. Xu J *et al* (2010) VHL inactivation induces HEF1 and Aurora kinase A. *J Am Soc Nephrol* **21:** 2041–2046
34. Egeberg DL, Lethan M, Manguso R, Schneider L, Awan A, Jorgensen TS, Byskov AG, Pedersen LB, Christensen ST (2012) Primary cilia and aberrant cell signaling in epithelial ovarian cancer. *Cilia* **1:** 15
35. Lutz MS, Burk RD (2006) Primary cilium formation requires von hippel-lindau gene function in renal-derived cells. *Cancer Res* **66:** 6903–6907
36. Seeley ES, Carriere C, Goetze T, Longnecker DS, Korc M (2009) Pancreatic cancer and precursor pancreatic intraepithelial neoplasia lesions are devoid of primary cilia. *Cancer Res* **69:** 422–430