Supporting Information

Wiedemeyer et al. 10.1073/pnas.1001613107

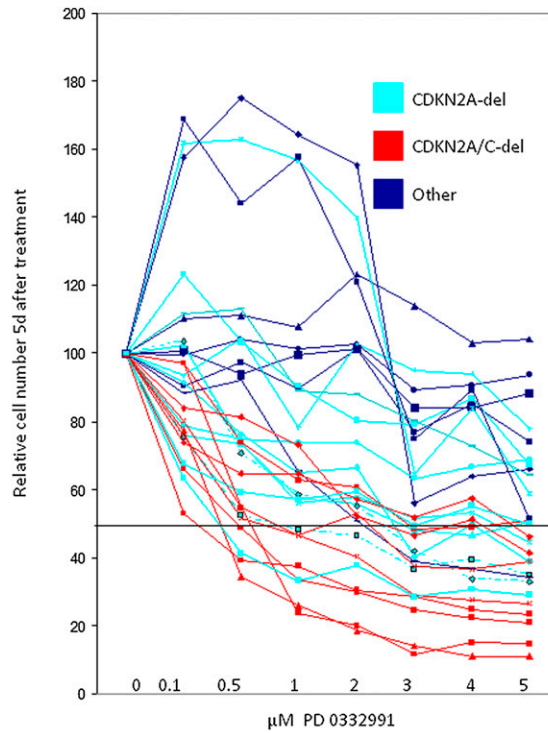


Fig. S1. Codeletion of *CDKN2A* and *CDKN2C* sensitizes glioma cells to pharmacological CDK4/6 inhibition. Glioma cell lines representing different RB alteration classes were incubated in various concentrations (0.1–5 μM) of the CDK4/6 inhibitor PD0332991 for 5 d (IC_{50} values for individual cell lines are listed in Dataset S2). Relative cell number was determined by a luminometric assay. *CDKN2A*/*CDKN2C*-codeleted cell lines (red) were most sensitive to PD0332991 (IC_{50} <1 μM).

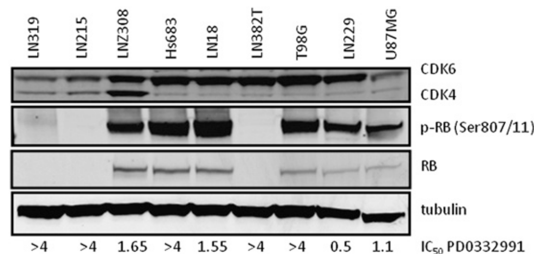
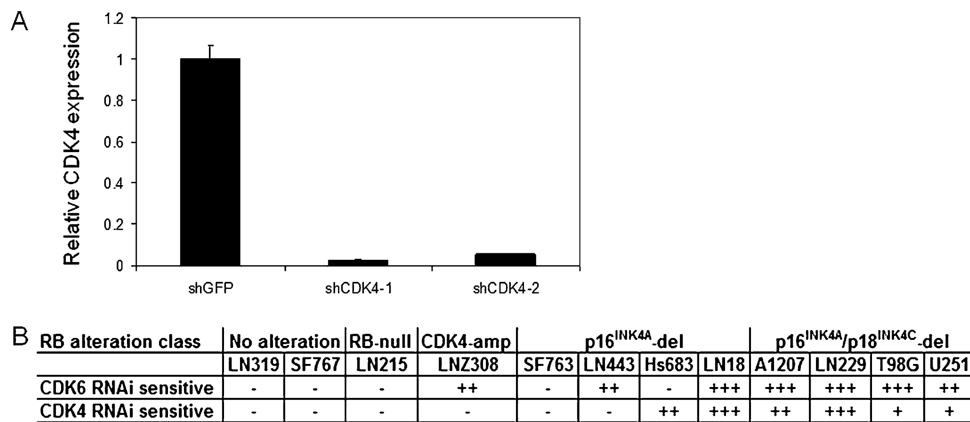


Fig. S2. Protein expression of RB pathway components in glioma cell lines. Western blot showing expression of CDK4, CDK6, phospho-RB(Ser807/811), and total RB in nine glioma cell lines with tubulin as loading control. CDK4 and CDK6 were expressed in all cell lines at varying levels. RB is absent in LN-215 and LN-382T and barely detectable in LN-319.



RB alteration class	No alteration		RB-null	CDK4-amp	$p16^{INK4A}$-del				$p16^{INK4A}/p18^{INK4C}$-del			
	LN319	SF767	LN215	LNZ308	SF763	LN443	Hs683	LN18	A1207	LN229	T98G	U251
CDK6 RNAi sensitive	-	-	-	++	-	++	-	+++	+++	+++	+++	++
CDK4 RNAi sensitive	-	-	-	-	-	-	++	+++	++	+++	+	+

Fig. S3. *CDKN2A/C*-codeleted cell lines are sensitive to RNAi-mediated depletion of CDK4 or CDK6. (*A*) LN-229 cells were infected with lentiviral shRNAs targeting GFP or CDK4. RNA was isolated from stable polyclonal cell populations and analyzed for CDK4 expression relative to RPL13A expression by quantitative RT PCR. shCDK4-1 and shCDK4-2 reduced CDK4 RNA levels significantly. (*B*) Glioma cells (*N* = 500,000) were seeded per 10-cm dish and infected with lentiviral shRNAs targeting GFP (shGFP), CDK4 (shCDK4-1, shCDK4-2, and shCDK4-4), or CDK6 (sh374, sh696, and sh720). Infected cells were selected with 2.5 μg/mL puromycin and stained with crystal violet 5 d after infection. Viability was calculated relative to shGFP-expressing cells (100%). Individual shRNAs reducing viability to no greater than 80% of shGFP are represented by a "+". "+++" indicates that all three shRNAs targeting a single gene reduced viability by at least 20%, whereas "–" indicates that either none of the three shRNAs reduced viability significantly or that at least one of the three shRNAs increased viability by at least 20% (for details see Dataset S3).

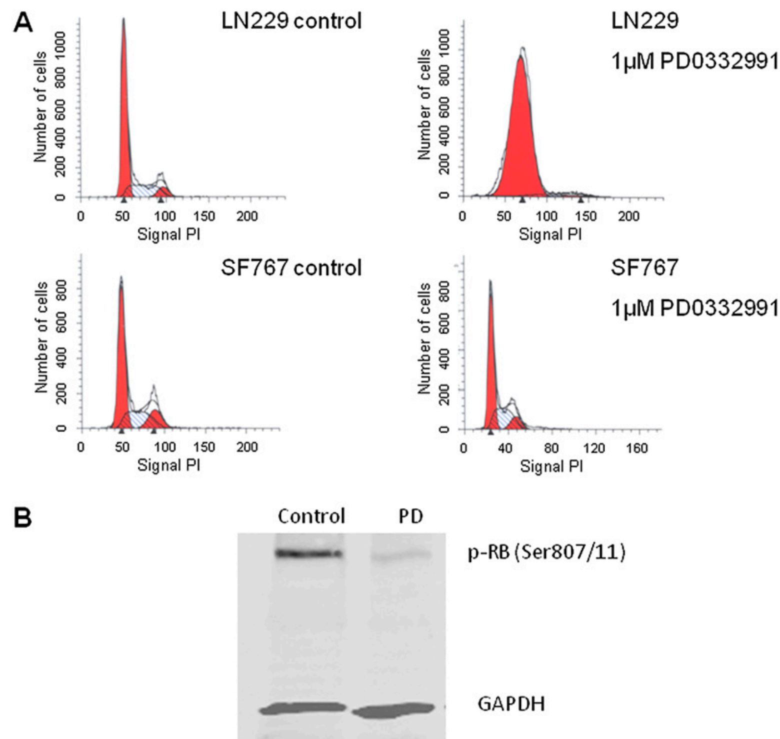


Fig. S4. PD0332991 causes G1 arrest. (*A*) Representative examples of PD0332991-treated and untreated glioma cell lines that were ethanol-fixed, stained with propidium iodide, and analyzed for cell cycle distribution by flow cytometry on a BD FACScan. SF767 cells (resistant) maintain their cell cycle profile in the presence of 1 μM PD0332991 whereas LN-229 cells (sensitive) arrest in the G1 phase. The proportion of cells in G1 was determined by the ModFit software (Dataset S4). (*B*) LN-229 cells were treated with 1 μM PD0332991 for 72 h. Protein was analyzed by Western blot using antibodies against phosphorylated RB protein and GAPDH. PD0332991 caused a drastic reduction of phosphorylated RB.

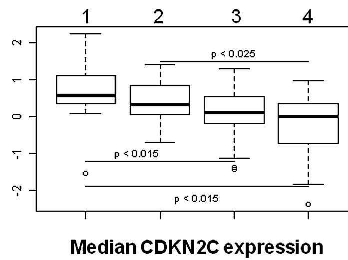


Fig. S5. Median CDKN2C expression in different RB alteration classes. In the TCGA dataset, the highest CDKN2C levels are present in *RB*-null and *RB*-mutant tumors (box 1), whereas levels are lowest in the "no alteration" class (box 4). *CDKN2A*–del/loss (box 3) and *CDK4*-amp classes (box 2) have intermediate levels.

Other Supporting Information Files

Dataset S1 (XLSX)
Dataset S2 (XLSX)
Dataset S3 (XLSX)
Dataset S4 (XLSX)