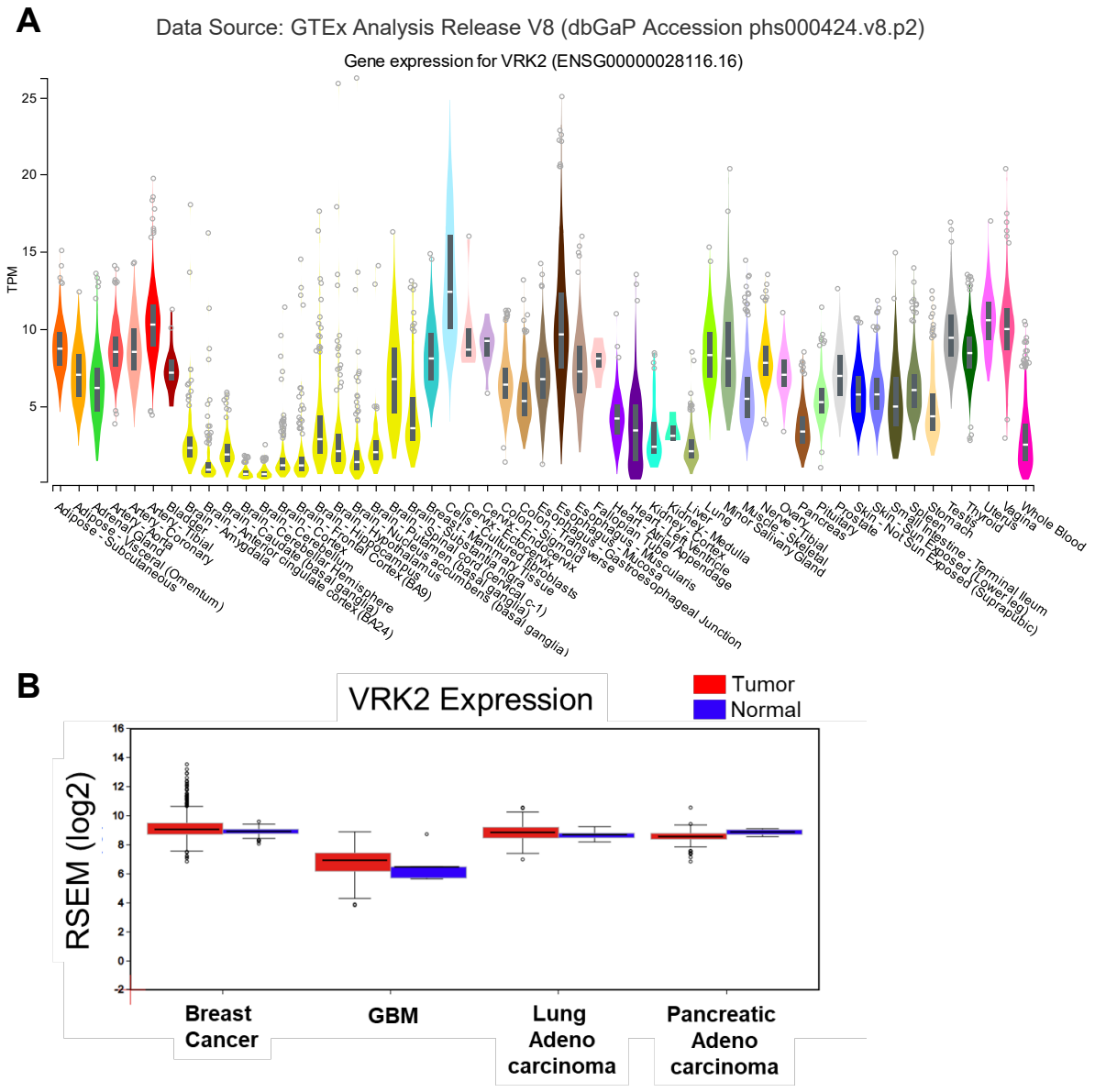
Supplemental Figure 1

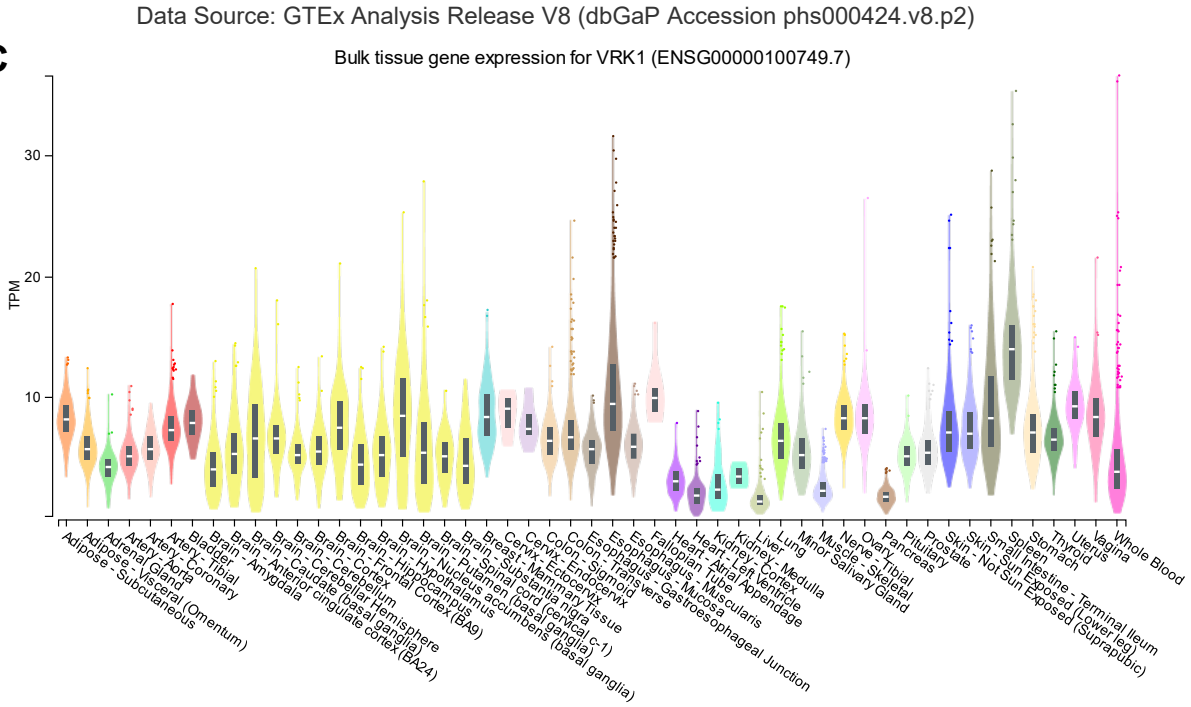


Supplemental Figure 1 (continued on next page)

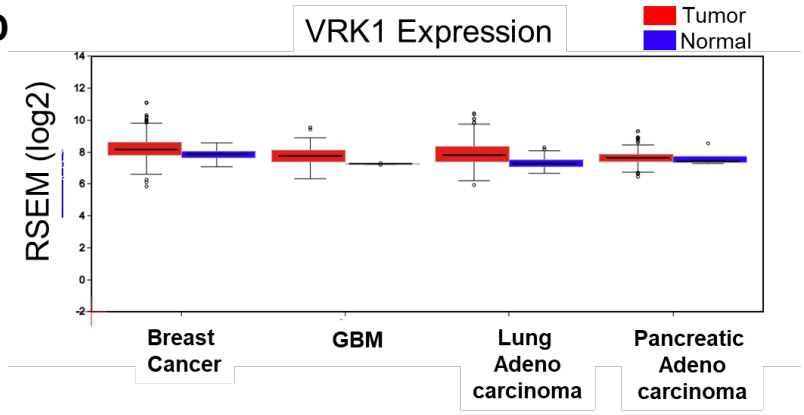
(A) VRK2 mRNA expression across all normal tissues (B) VRK2 mRNA expression in cancer and normal adjacent tissue (Source: Firebrowse.org) (C) VRK1 mRNA expression across all normal tissues (D) VRK1 mRNA expression in cancer and normal adjacent tissue (Source: Firebrowse.org) (E) Percentage of VRK2 alterations in cancer cell lines (left) and in human tumors (F) Percentage of VRK2 low cases in MGMT-methylated and IDH mutant tumors

Supplemental Figure 1 (continued)

C



D



E

Cell line data (CCLE)

Mutation type	Damaging	Other non-conserving	Deletion
Percentage	1.89%	1.75%	0.07%

Patient tumor data (TCGA PanCancer)

Alteration type	Truncating mutation	Splice mutation	Missense mutation	Deletion
Percentage	0.15%	0.03%	0.65%	0.10%

F

MGMT methylation

TCGA Cohort	Number of VRK2-low cases *	Total cases	Calculated percentage
LGG	289	530	54.53%
GBM	29	138	21.01%

*selection criteria: MGMT methylation >0.2 and VRK2 expression <0

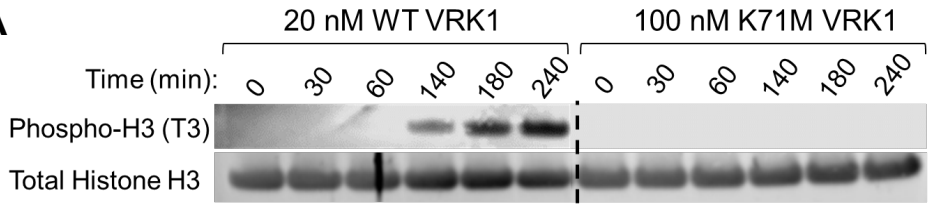
IDH1/2 mutation

TCGA Cohort	Number of VRK2-low cases*	Total cases	Calculated percentage
LGG	168	530	31.70%

*selection criteria: IDH1/IDH2 mutation and VRK2 expression <0

Supplemental Figure 2

A

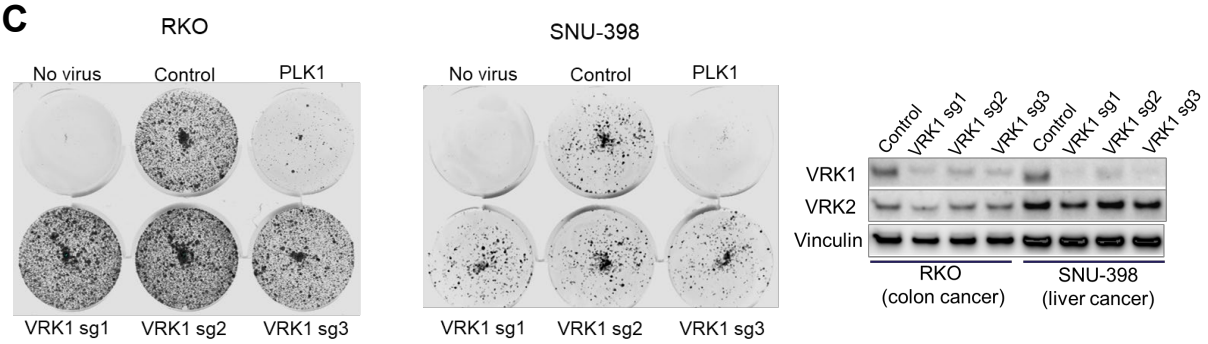


B

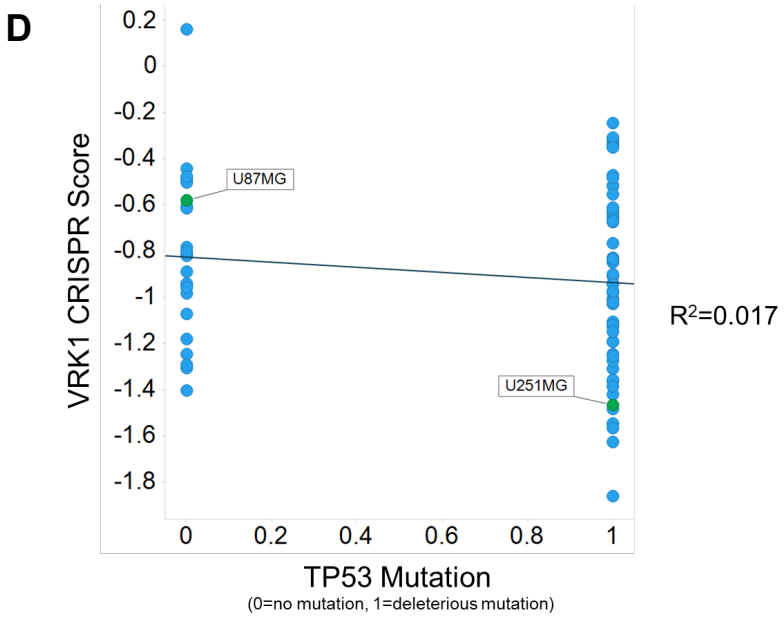
Cell Line Name	VRK1 CRISPR Score (CERES)	VRK2 Expression log2(TPM+1)	VRK2 Methylation Fraction	MGMT Methylation	TP53 Mutation	TP53 Variant Type/ Genotype/Impact
T98G	-0.72	4.26	N/A	N/A	M237I	SNV/hom/moderate
LN-229	-0.59	3.92	N/A	0.77	P98L	SNV/hom/moderate
YH13	-0.89	2.71	0.54	0.04	F113V	SNV/hom/moderate
KALS-1	-1.05	2.91	0.18	0.14	S241F	SNV/hom/moderate
YKG1	-0.30	4.12	0.01	0.05	WVDS146-149C	Del/het/moderate
KS-1	N/A	N/A	N/A	N/A	wildtype	
KNS60	-0.81	0.37	0.94	0.35	H193Y	SNV/hom/moderate
U-118 MG	-1.30	0.32	0.91	0.26	R213Q	SNV/hom/moderate
SW 1088	-1.35	0.26	0.85	0.62	R273C	SNV/hom/moderate
H4	-0.59	0.16	0.97	0.37	wildtype	
LN-18	-1.02	0.15	N/A	N/A	C238S	SNV/hom/moderate
U-251 MG	-1.24	0.16	0.97	0.30	R273H	SNV/hom/moderate
U87MG	-0.58	3.89	0.29	0.48	wildtype	
SNU-398	-0.08	6.14	0.00	0.07	wildtype	
RKO	-0.10	4.13	0.00	0.39	splice site	SNV/hom/moderate

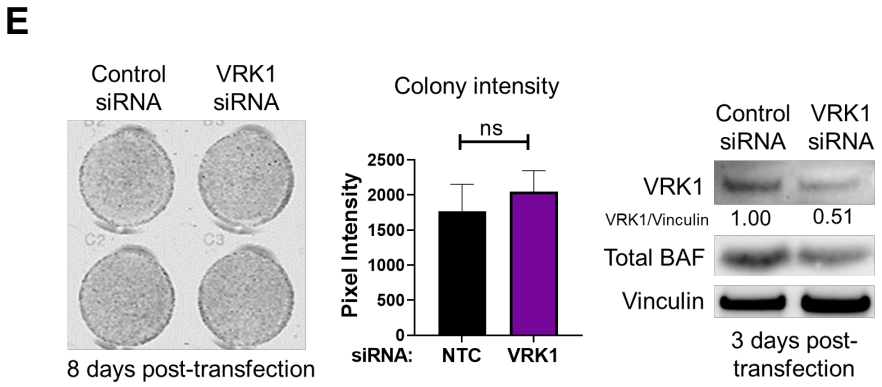
Data source: DepMap Expression 21Q2 Public, CERES Score 21Q2, Fraction Methylation (1kb upstream TSS), CCLE Mutation

C



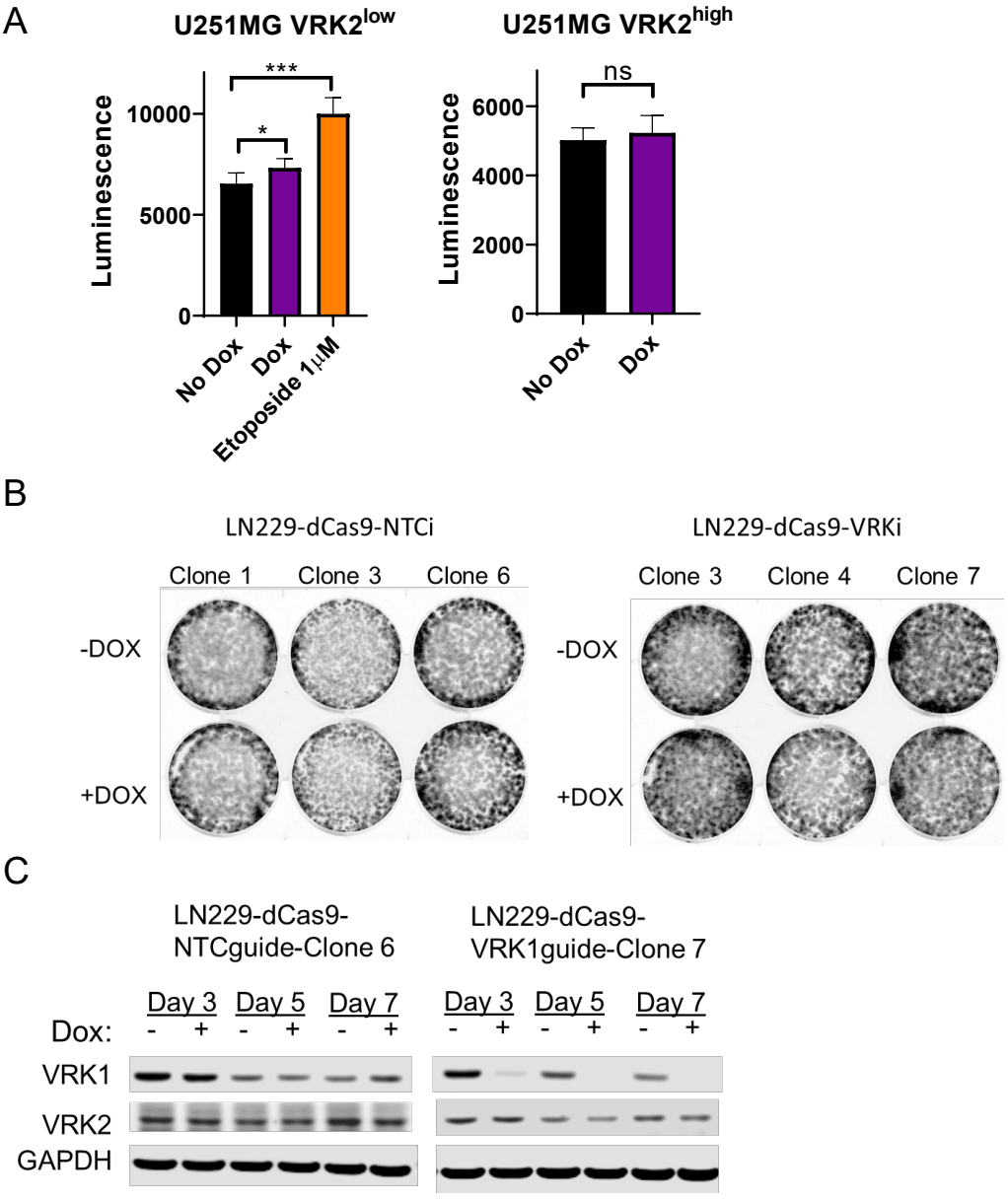
Supplemental Figure 2 (continued)





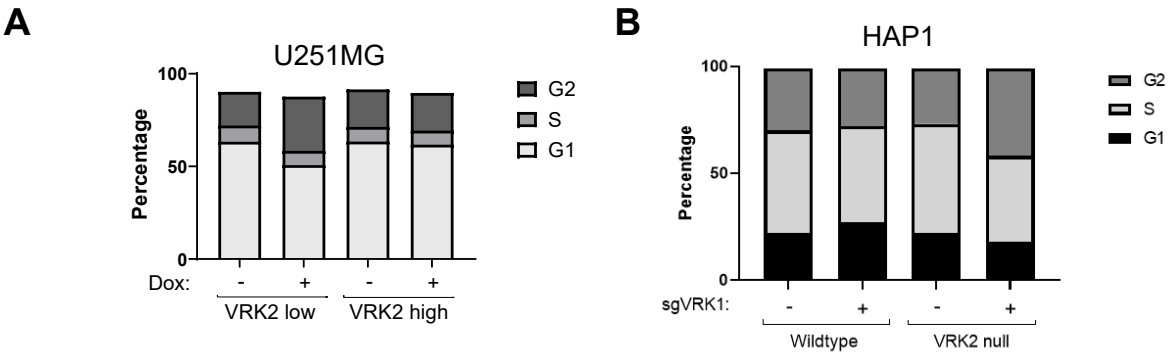
Supplemental Figure 2.
(A) Immunoblot depicting in vitro kinase activity of wildtype and mutant VRK1 on a histone H3 protein (B) Table showing CRISPR score, VRK2 expression, VRK2 methylation, MGMT methylation, and TP53 status of a panel of GBM cell lines (C) 14-day colony forming assay as in Figure 2A of VRK1 CRISPR knockdown in two non-GBM cell lines (D) Correlation between VRK1 sensitivity and TP53 mutation status in 79 cell lines of the Central Nervous System (E) Colony forming assay in primary astrocytes with control or VRK1 knockdown (left), colony quantification (middle), and immunoblots with VRK1 levels 3-days post-transfection (right).

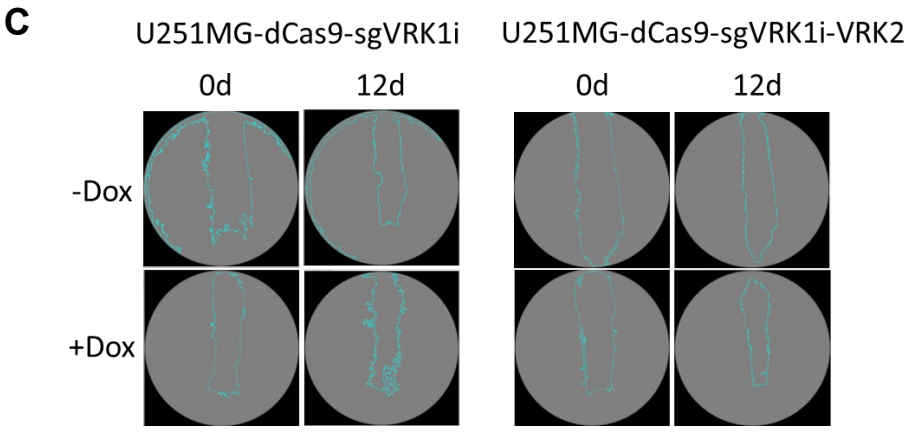
Supplemental Figure 3

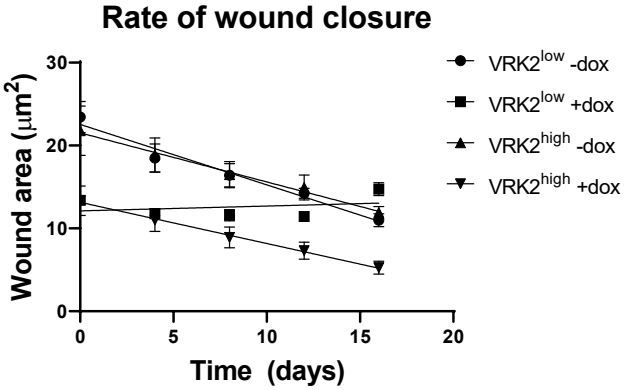


Supplemental Figure 3.
(A) Annexin V apoptosis assay in U251MG VRK2 low and VRK2 high cell lines after 7 days of doxycycline *p<0.05, ***p<0.001, two-tailed t-test (B) 14-day colony forming assay of CRISPR-dCas9-KRAB inducible VRK1 knockdown (as in Figure 3A) of VRK2-high expressing LN229 clonal cell lines (C) Immunoblots of cells from (B) demonstrating VRK1 knockdown.

Supplemental Figure 4

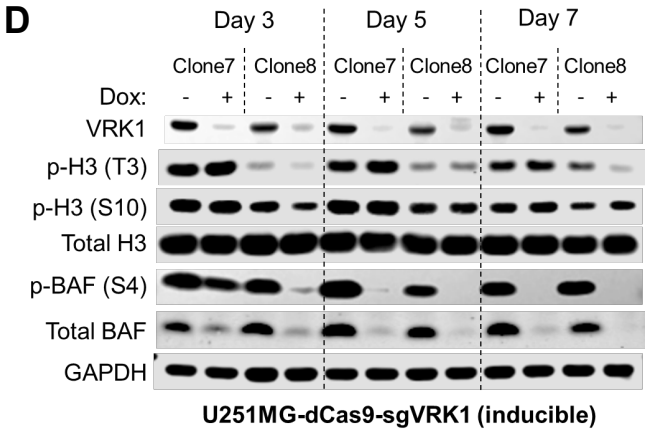


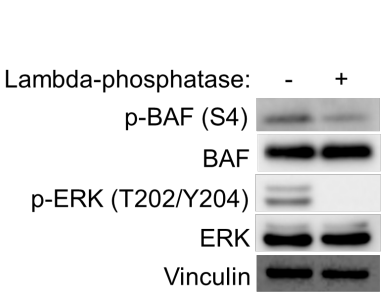


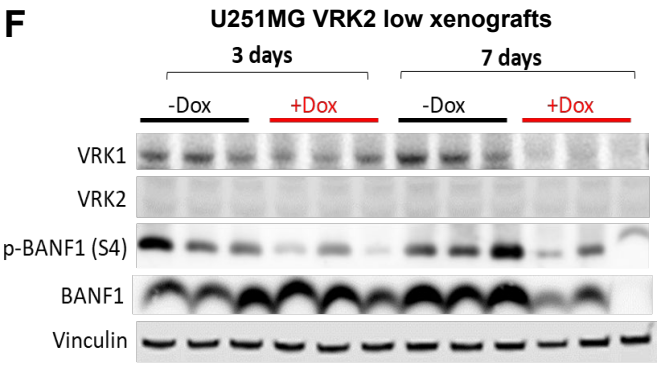


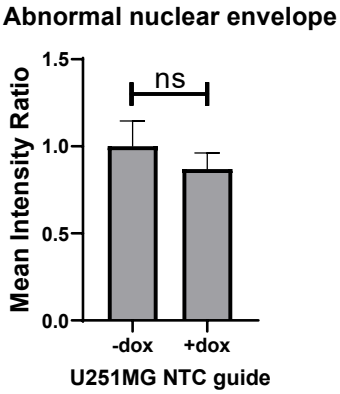
	VRK2^{low} -dox	VRK2^{low} +dox
Slope	-0.7289	0.05850

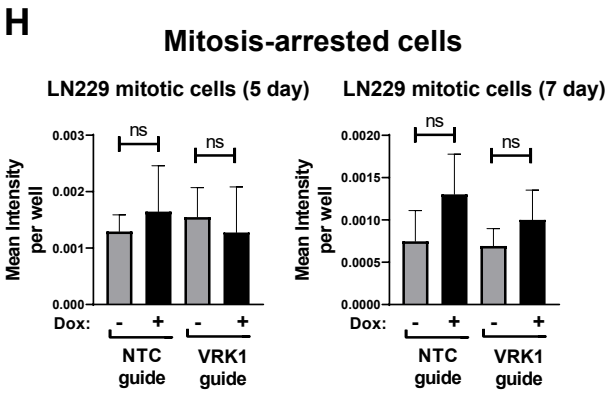
	VRK2^{high} -dox	VRK2^{high} +dox
Slope	-0.5921	-0.4971

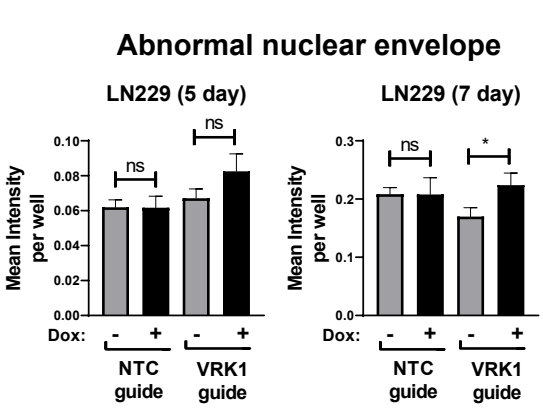






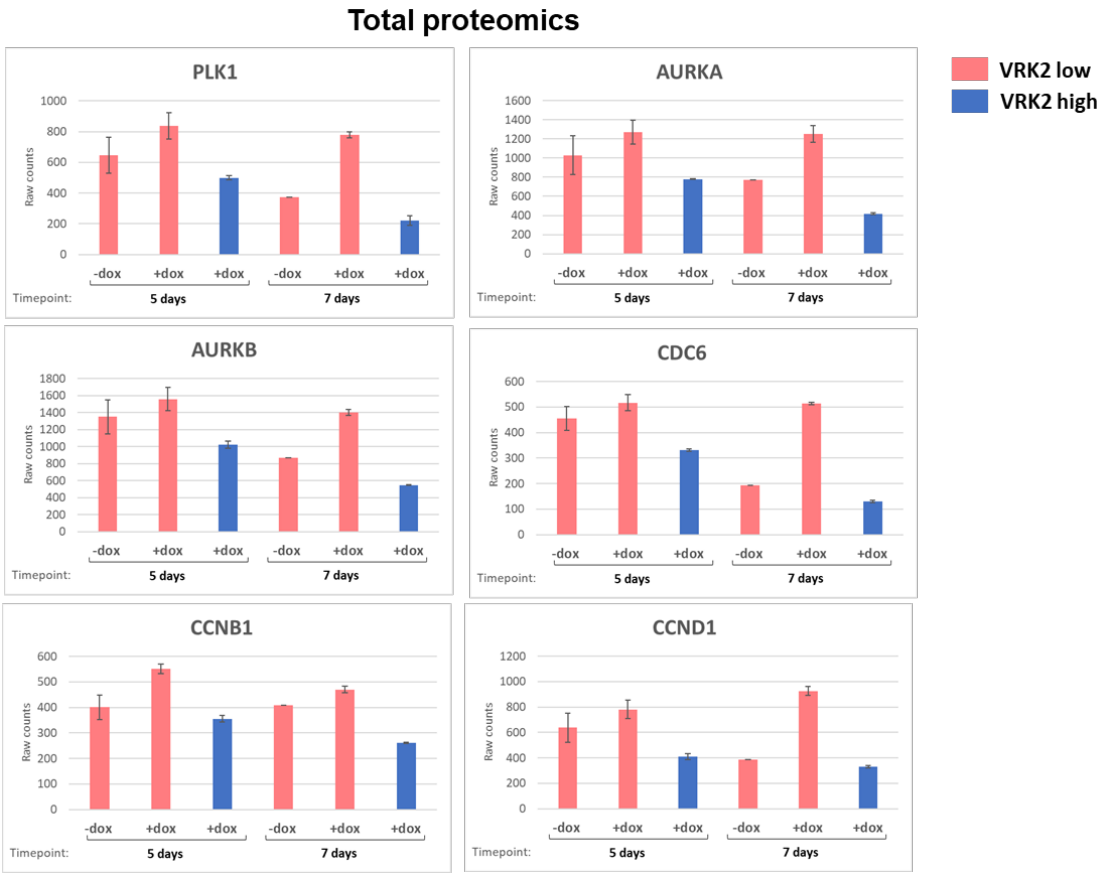






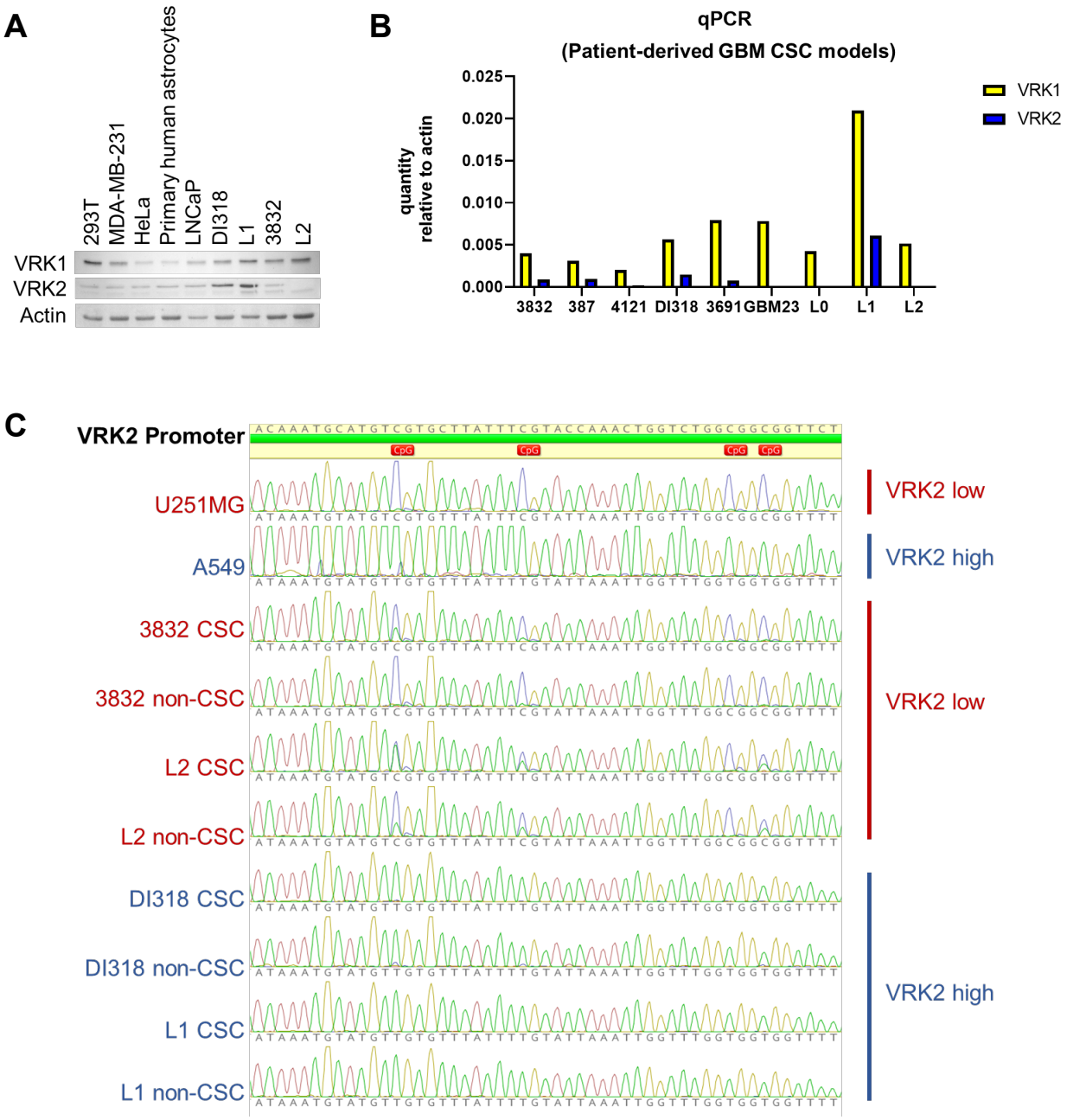
(A) Quantification of cell cycle distributions from Figure 4A (B) Quantification of cell cycle distributions from Figure 4B (C) Wound healing assay in U251MG VRK2 low and VRK2 high cell lines treated with doxycycline over the course of 16 days (D) Immunoblots of U251MG VRK2-low clonal cell lines at the indicated times (E) Immunoblots of HAP1 parental cell lysates treated with or without lambda phosphatase for 1 hour (F) Immunoblots of tumor lysates from U251MG VRK2 low xenografts treated with doxycycline for 3 and 7 days (G) Quantification of nuclear envelope phenotype in U251MG VRK2-low NTC control cell line treated with and without doxycycline (H) Quantification of LN229 VRK2 high cells arrested in mitosis upon VRK1 knockdown for 5 and 7 days (I) Quantification of abnormal nuclear envelope in LN229 VRK2 high cells upon VRK1 knockdown for 5 and 7 days

Supplemental Figure 5



Total proteomics at 5- and 7-days post-doxycycline for cell cycle proteins.

Supplemental Figure 6



(A) VRK1 and VRK2 immunoblots of PDX models alongside cancer cells and normal astrocytes (B) RT-qPCR for VRK1 and VRK2 from 9 CSC GBM models (C) Sanger sequencing traces from PCR products of bisulfite-treated genomic DNA for the indicated cell lines