Supplementary Materials

Supplementary Table S1. List of qPCR primers

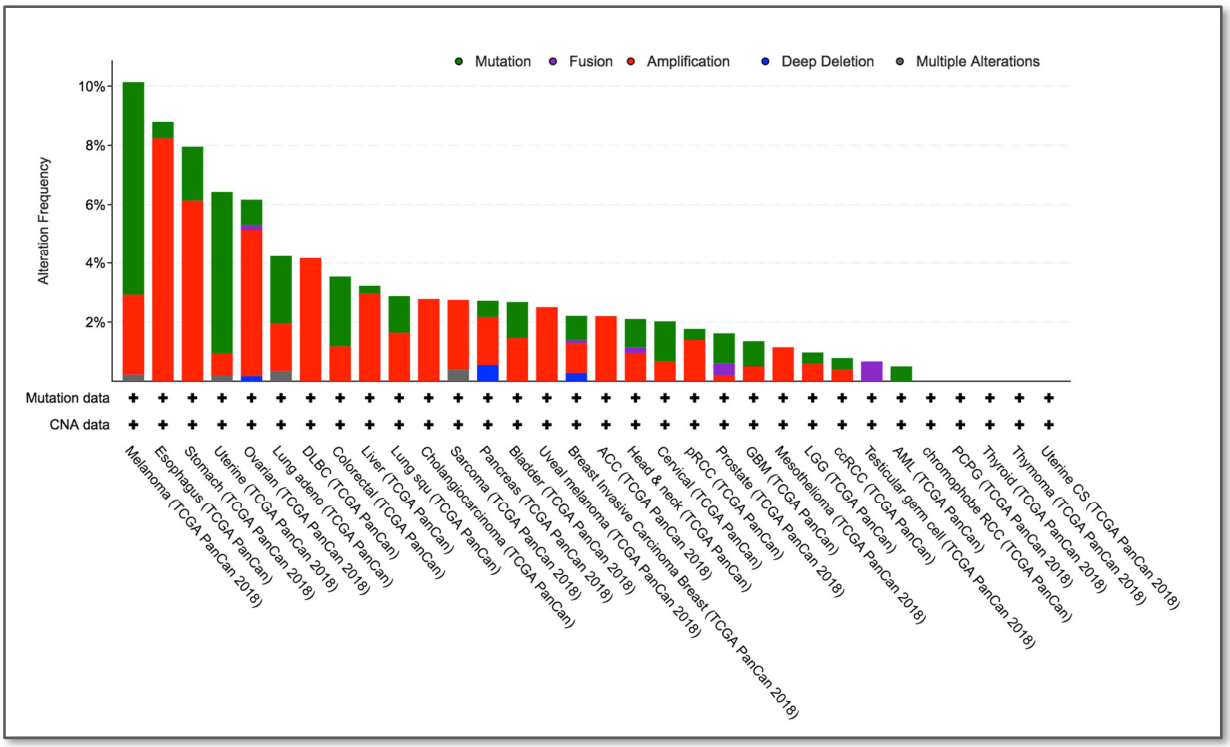
Gene	Forward primer sequence	Reverse primer sequence
CREB	Quantitect primer assay purchased from Qiagen	
ERK	ACACCAACCTCTCGTACATCGG	TGGCAGTAGGTCTGGTGCTCAA
HPRT	Quantitect primer assay purchased from Qiagen	
p53	Quantitect primer assay purchased from Qiagen	
PTK7	GAGATGACGCTGGCAACTACAC	CGCTCTGGTTCCACTTTGAAGG
P21	Quantitect primer assay purchased from Qiagen	

Supplementary Table S2. List of primary and secondary antibodies.

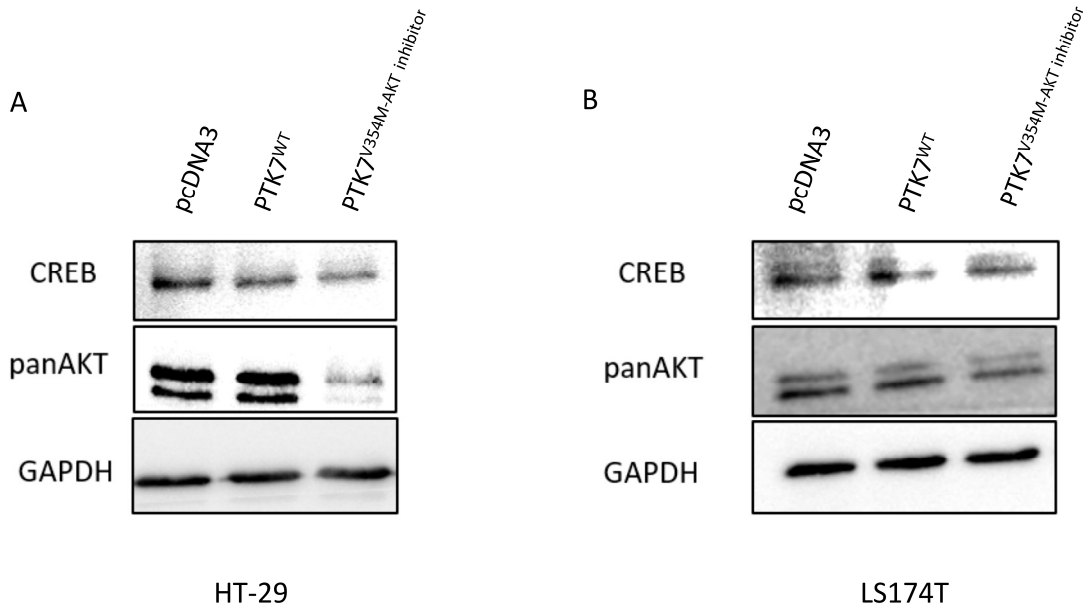
PRIMARY ANTIBODIES

Protein target	Host	Cat Number	Company	Dilution buffer	Dilution factor
pAKT (Ser473)	Rabbit	4060	Cell Signaling Technology	5% BSA in TBST	1:2000
PanAKT	Rabbit	4691s	Cell Signaling Technology	5% milk in TBST	1:1000
P21	Rabbit	Ab109199	Abcam	5% milk BSA in TBST	1:1000
CREB	Mouse	86B10	Cell Signaling Technology	5% milk in TBST	1:1000
ERK	Rabbit	9102	Cell Signaling Technology	5% milk in TBST	1:1000
GAPDH	Mouse	CB1001	Millipore	5% milk in TBST	1:3000
Anti-alpha Tubulin	Rabbit	ab4074	Abcam	5% milk in TBST	1:1000

p53	Mouse	1C12	Cell Signaling Technology	5% milk in TBST	1:1000
PTK7	Goat	AF4499	Novus biologicals	5% BSA in TBST	1:200
SECONDARY ANTIBODIES					
Species reactivity	**Host**	**Catalog number**	**Company**	**Dilution buffer**	**Dilution factor**
Goat	Donkey	Sc-2020	Santa Cruz Biotechnology	5% milk in TBST	1:5000
Mouse	Horse	7076s	Cell Signaling Technology	5% milk in TBST	1:5000
Rabbit	Goat	7074s	Cell Signaling Technology	5% milk in TBST	1:5000



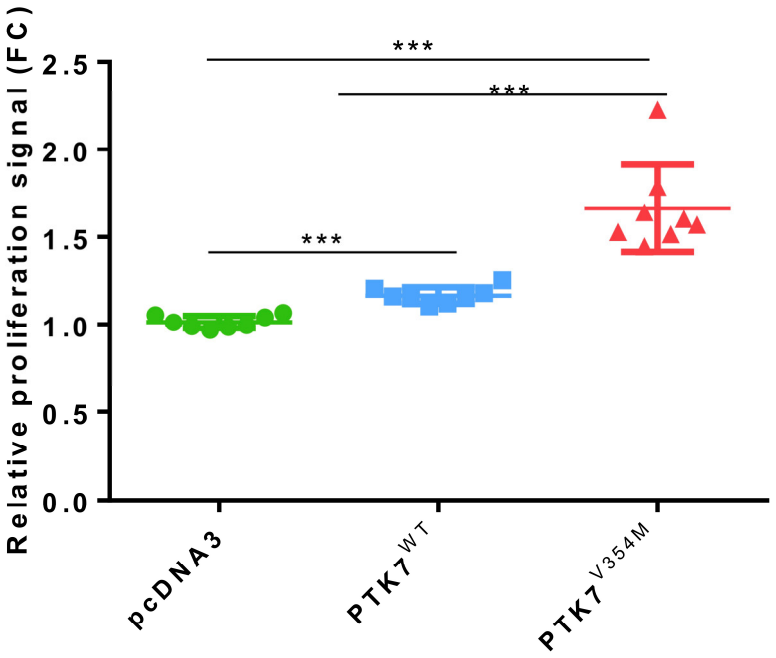
Supplementary Figure S1. PTK7 alterations in cancers. Histogram of alteration frequencies according to TCGA PanCancer data was extracted from cBioPortal (www.cbioportal.org) on 15th of March 2020.



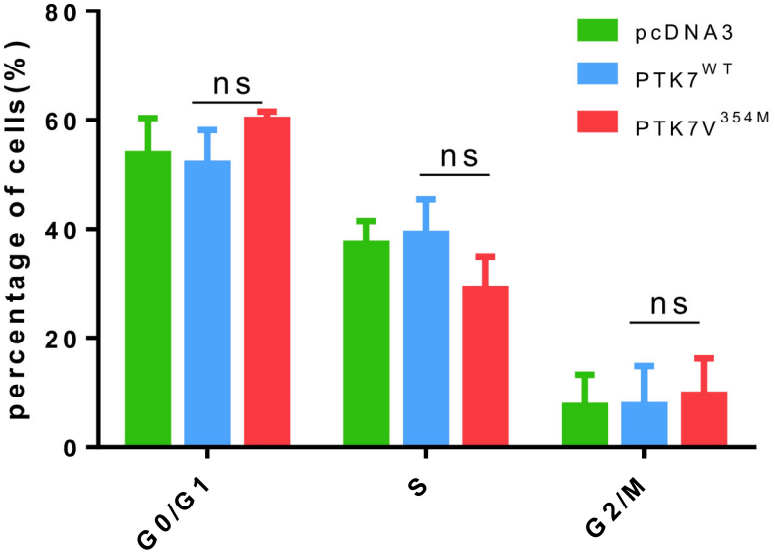
Supplementary Figure S2. Effect of AKT inhibition on CREB expression in *PTK7V*^{354M} variant

Inhibition of AKT expression by using Perifosine did not rescue the CREB expression in (A) HT-29 and (B) LS174T cells transfected with *PTK7*^{V354M}. AKT was inhibited successfully in both cell lines, but there was only a trend towards restoration of CREB in LS174T cells.

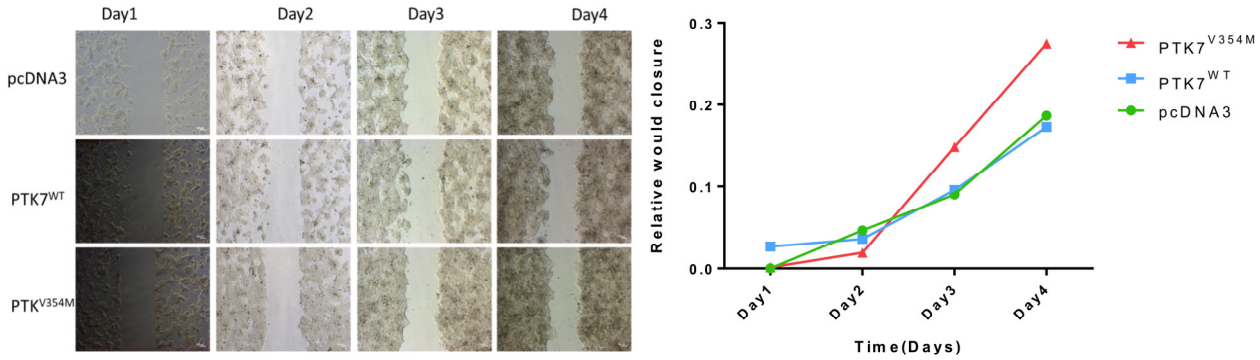
A


Relative proliferation signal (FC)
2.5
2.0
1.5
1.0
0.5
0.0
***
***
***
pcDNA3
PTK7 WT
PTK7 V354M

B


percentage of cells(%)
80
60
40
20
0
ns
ns
ns
pcDNA3
PTK7 WT
PTK7V 354M
G0/G1
S
G2/M

C



Supplementary Figure S3. *PTK7*^{V354M} variant increases cell proliferation, cell cycle progression and migration. (**A**) Proliferation assay showed significantly increased viable cell numbers of LS174T-*PTK7*^{V354M} compared to LS174T-*PTK7*^{WT} cells. Significance levels are included for each time point. *** - $p \leq 0.001$. (**B**) LS174T ^{V354M} transfected cells resulted in significant cell cycle progression. (**C**) Representative image of migrated LS174T cells after overexpression of *PTK7*^{WT} *and* LS174T-*PTK7*^{V354M}.