Supplementary material

Pten mRNA alternative 3'-UTRs confer stability and resistance to microRNAs

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Inventory of supplementary data

Supplementary Tables (7)

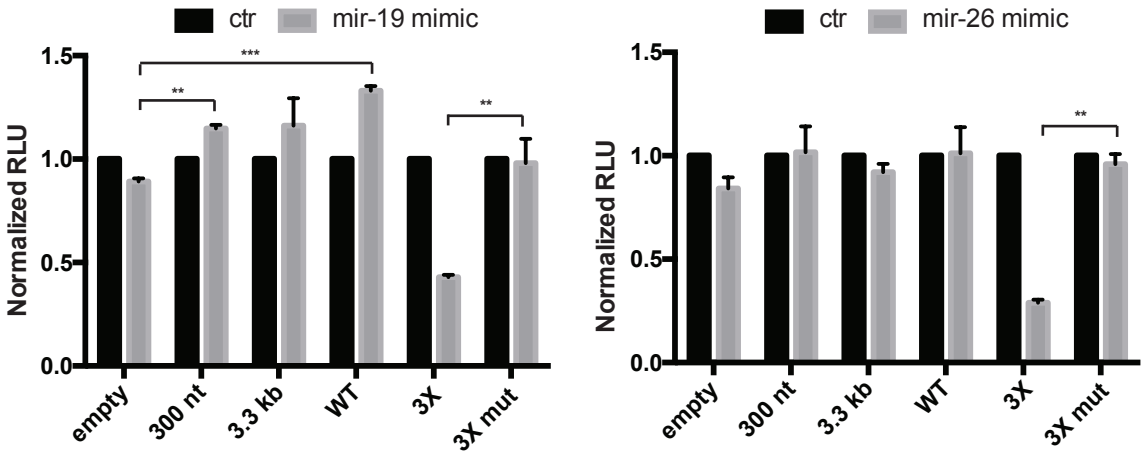
- **Supplementary Table S1 relates to Figure 1**
- **Supplementary Table S2 relates to Figure 1**
- **Supplementary Table S3 relates to Figure 1**
- **Supplementary Table S4 relates to Figure 1**
- **Supplementary Table S5 relates to Figure 1**
- **Supplementary Table S6 relates to Figure 4,5 and 6**
- **Supplementary Table S7 relates to Figure 5 and 6**
- **Supplementary Table S8 relates to Figure 3**

Supplementary Figures and legends (7)

- **Supplementary Figure S1 relates to Figure 3**
- **Supplementary Figure S2 relates to Figure 3**
- **Supplementary Figure S3 relates to Figure 4, 5 and 6**
- **Supplementary Figure S4 relates to Figure 4, 5 and 6**
- **Supplementary Figure S5 relates to Figure 4**
- **Supplementary Figure S6 relates to Figure 4**
- **Supplementary Figure S7 relates to Discussion**

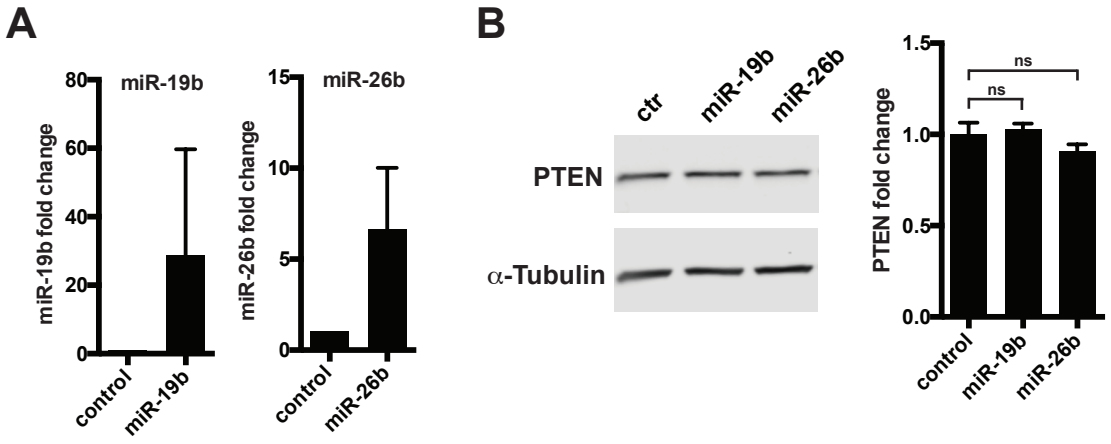
Supplementary Figure S1:

Luciferase assays in prostate cells (CaP2)

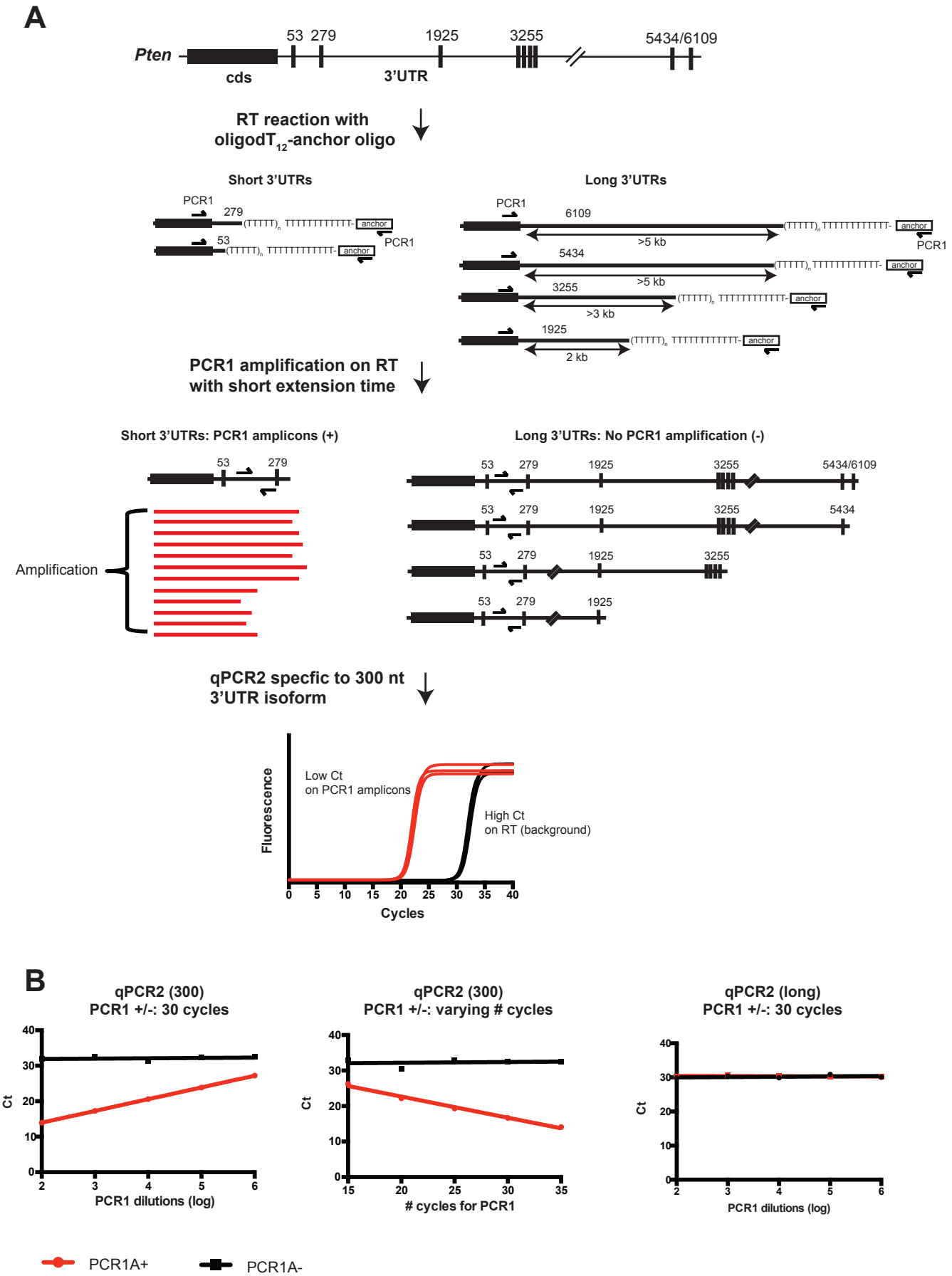


Supplementary Figure S2

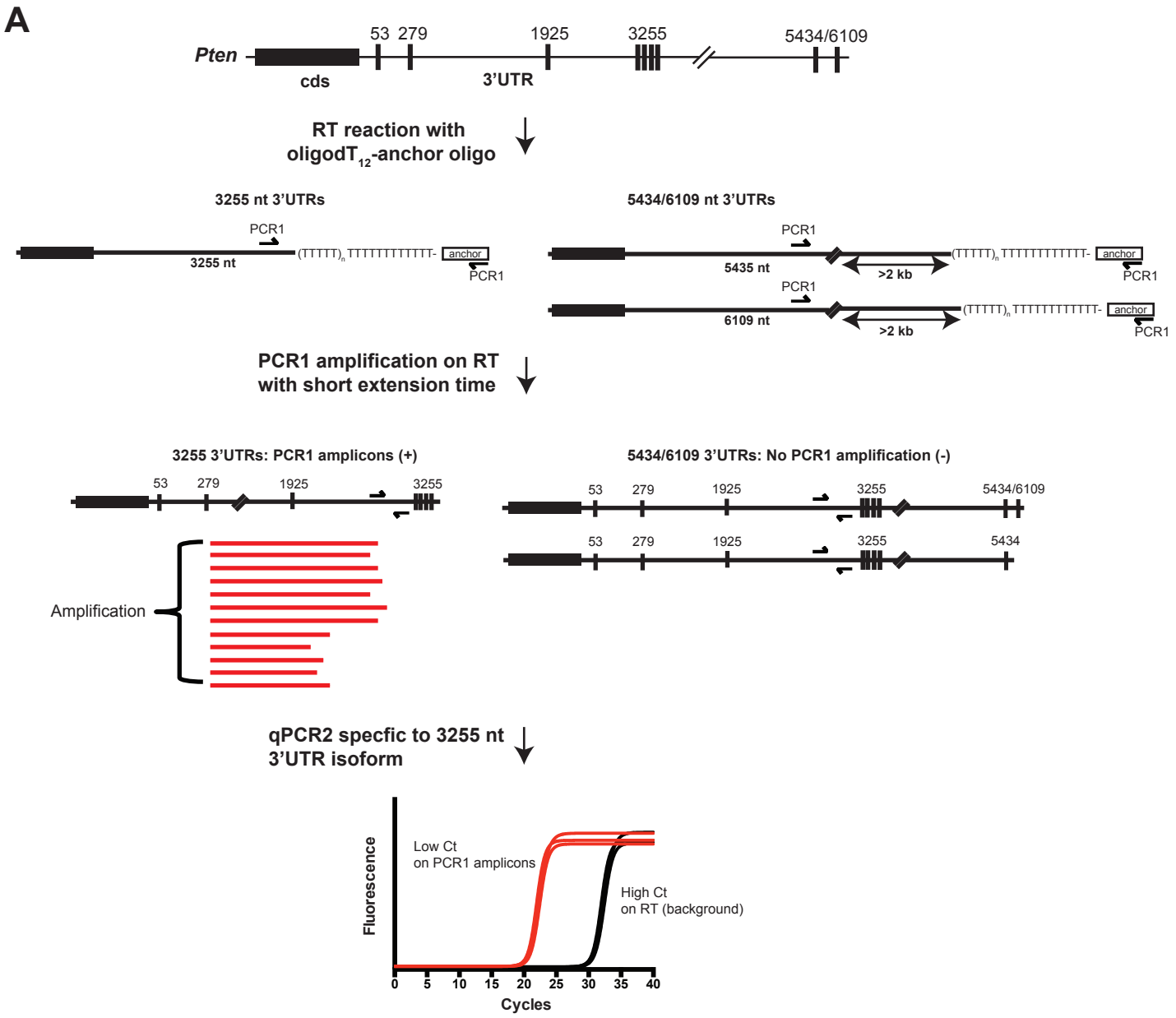
miRNA overexpression in NMuMG cells



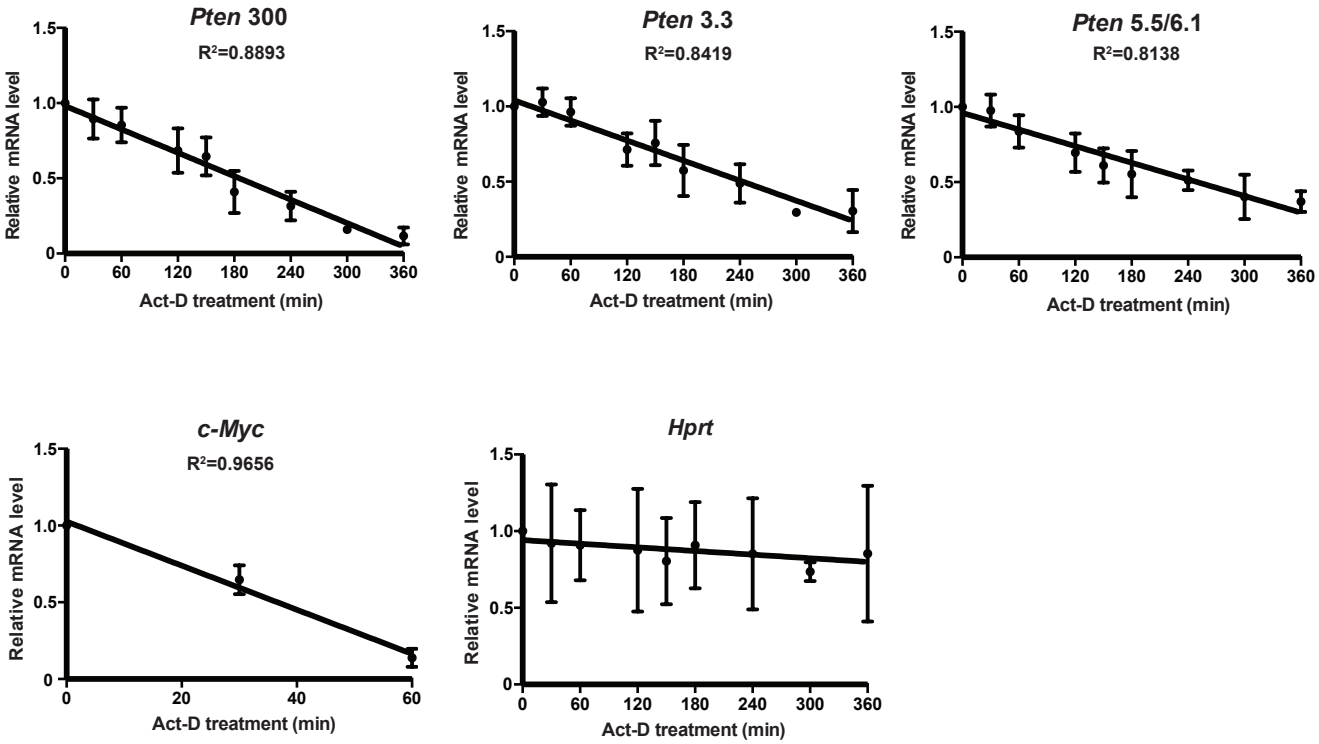
Supplementary Figure S3:
3'UTR isoform specifc qPCR strategy (300 nt 3'UTR isoform)



Supplementary Figure S4:
3'UTR isoform specifc qPCR strategy (3.3 kb 3'UTR isoform)



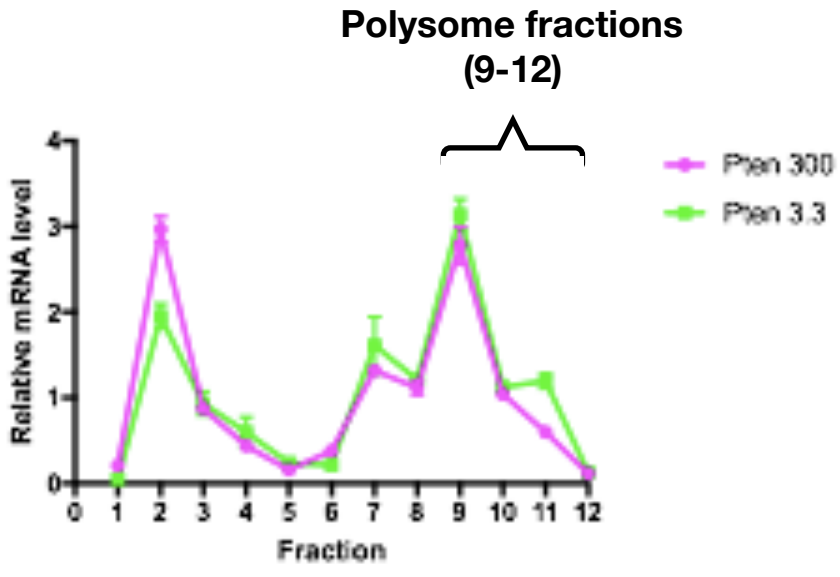
Supplementary Figure S5:
mRNA stability assay



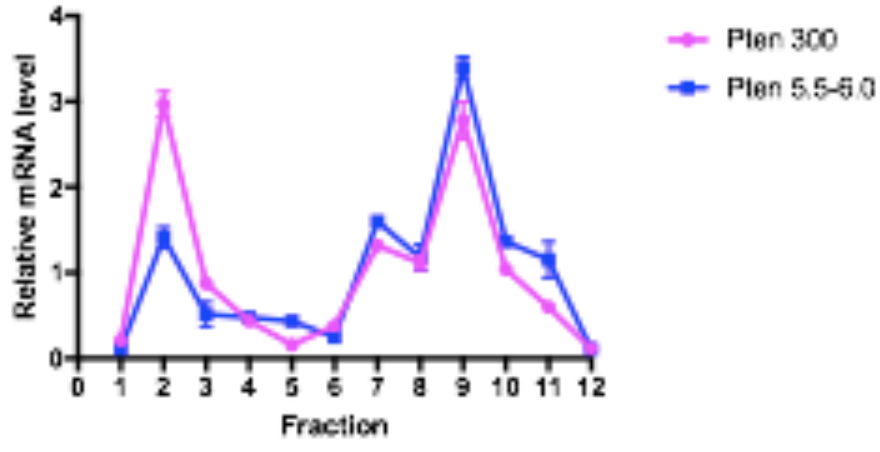
Supplementary Figure S6

NIH3T3 Pten mRNA isoform polysome analysis

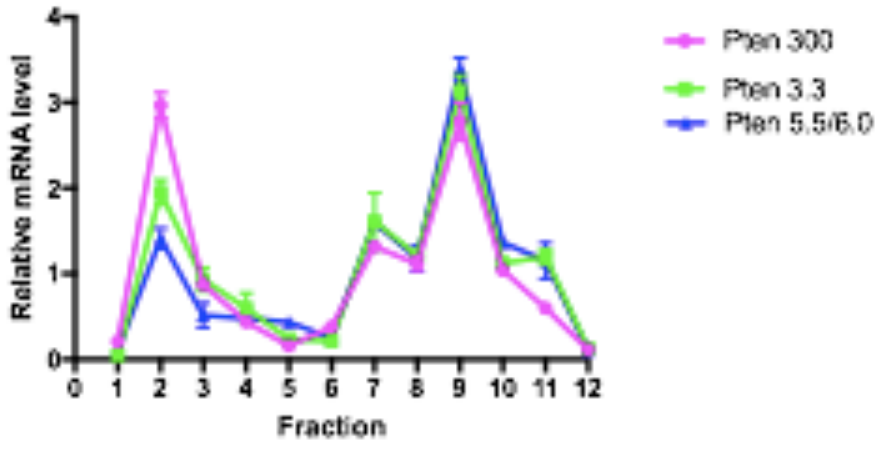
Pten 300 vs 3.3kb 3'UTR mRNAs



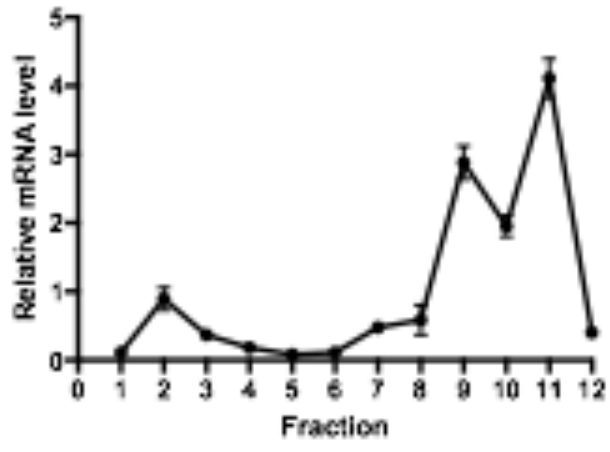
Pten 300 vs 5.5/6.1kb



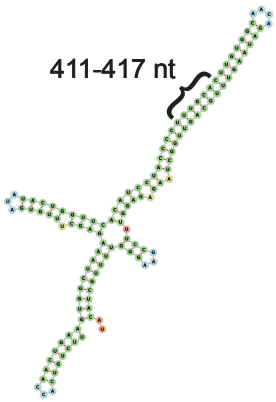
All isoforms



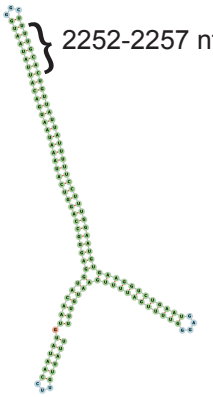
Hprt mRNA



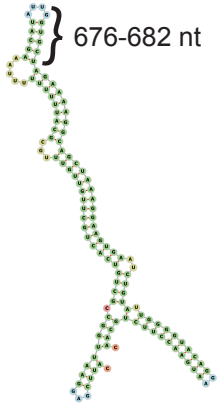
Supplementary Figure S7:



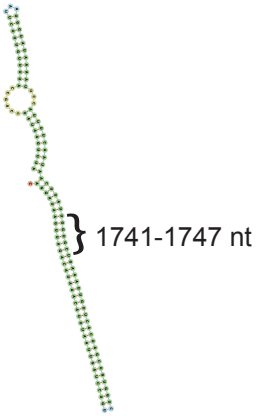
miR-19b site 1
-106.17 kcal/mol



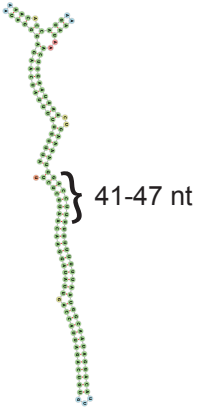
miR-19b site 2
-110.96 kcal/mol



miR-29a site 1
-113.75 kcal/mol

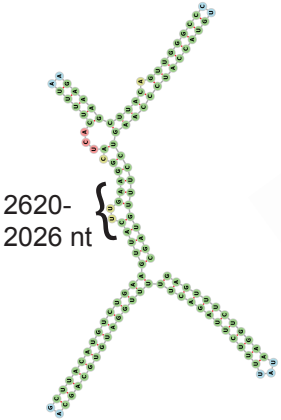


miR-29a site 2
-110.83 kcal/mol



miR-26a site 1
-110.72 kcal/mol

miR-26a site 2
-110.37 kcal/mol



miR-26a site 3
-106.49 kcal/mol

miR-17/20a
-110.34 kcal/mol

Supplementary Figure legends:

Supplementary Figure S1

Luciferase assay in prostate CaP2 cells co-transfected with isoform specific *Pten* 3'UTR reporter constructs and miRNA mimic control, miR-19 or miR-26. Control 3X-Bulge (3X) and 3X-Bulge mutated (3X mut) miR-19 and miR-26 were used as positive controls. Data shown are from three independent biological experiments.

Supplementary Figure S2

(A) Quantification of mature miRNA miR-19 and miR-26 by qRT-PCR after infection of NMuMG cells with control, miR-26b or miR-19b lentivirus. (B) PTEN analysis by western blot in NMuMG stable infected cells. α-Tubulin was used as a loading control. Bar graph shows PTEN fold change in miR-19 and miR-26 overexpressing cells compare to control. Data shown are from three independent biological experiments.

Supplementary Figure S3

(A) (i) A reverse transcriptase reaction was performed with an oligo dT-anchor primer. (ii) For PCR1 amplification, a forward primer close to the 300 nt isoform polyA tail and a reverse primer matching the anchor sequence were used. The specificity of qPCR2 for the 300 nt isoform relies on the short extension time of 20 seconds used for PCR1, which favor the amplification of the two short isoforms (53 and 273 nt) over the longer ones. (iii) A quantitative real-time PCR (qPCR2) is performed on PCR1 with primers localized in between the 53 nt and

273 nt 3'UTR isoforms, eliminating the possibility to amplify the 53 nt 3'UTR isoform. A difference of approximately 10 Cts is obtained between the qPCR2 performed on the PCR1 amplicons (PCR1+) compared to the RT background (PCR1-). (B) Linear regression analysis to confirm that PCR1 dilution and the number of cycles used are in the linear range. (C) qPCR2 amplification with primers designed to amplify 3'UTR isoforms longer than 300 nt confirm the specificity of the qPCR assay.

Supplementary Figure S4

(A) The same strategy was used to quantify specifically the 3.3 kb isoform with a forward oligo for PCR1 close to the polyA tail of the 3.3 kb isoform. The short extension time of PCR1 did not allowed the amplifications of longer isoforms and qPCR2 on the PCR1 amplicons (PCR1+) were 10 Ct lower than on the RT background (PCR1-). (B) Linear regression analysis to confirm that PCR1 dilution and the number of cycles used are in the linear range. (C) qPCR2 amplification with primers designed to amplify the 5.5/6.0 kb isoforms confirm the specificity of the qPCR assay.

Supplementary Figure S5

Linear regression analysis on NMuMG actinomycin treated cells for each *Pten* 3'UTR isoforms (Pten 300, Pten 3.3 and Pten 5.5/6.1), *c-Myc* positive control and the normalizing gene *Hprt*. Data shown are from four independent biological experiments.

Supplementary Figure S6.

Polysome gradient mRNA profiling was performed as in (52) on NIH3T3 cell lysates, and Pten mRNA isoforms were quantified by qRT-PCR as in Supplementary Figures S3, S4. Because they are expressed at different levels, the 300 nt, 3.3, and 5.5/6.1 kb 3'UTR isoforms were represented relatively to the total signal for comparison of their relative distribution. Hprt mRNA was quantified as a highly translatable control.

Supplementary Figure S7.

Mouse *Pten* 3' UTR structure prediction using MC-Fold. 60 nts on either side of seed-complementary sequences were used as input. Loweset minimum free energy (MFE) structures predicted by MC-fold are indicated for each site. Brackets indicate the positioning of seed-complementary sequences in *Pten* 3' UTR. Note that MC-Fold considers both canonical and non-canonical base-pairing of nucleotides.

Supplementary Table S1:

Summary of 3'RACE sequencing data in mouse cell lines

PAS-1 (53 nt) **Primers:** PCR1 Tdo886, PCR2 Tdo910	
NIH3T3:	
Clone-7:	TG**AATAAA**CTGAAATGGACCTTT**(A)$_{16}$**
NMuMG:	
Clone-5:	TG**AATAAA**CTG**(A)$_{19}$**
Clone-8:	TG**AATAAA**CTGAAATGGACCTTT**(A)$_{19}$**
PAS-2 (279 nt) **Primers:** PCR1 Tdo886, PCR2 Tdo2187	
NIH3T3:	
Clone-2:	ATA**AATAAA**AGATGGCACTTTCCCATT**(A)$_{59}$**
Clone-5:	ATA**AATAAA**AGATGGCACTTTCCCATTTTATTCCAGTTTTAT**(A)$_{36}$**
Clone-7:	ATA**AATAAA**AGATGGCACTTTCCCATTTT**(A)$_{54}$**
Clone-10:	ATA**AATAAA**AGATGGCACTTTCCC**(A)$_{45}$**
Clone-13:	ATA**AATAAA**AGATGGCACTTT**(A)$_{38}$**
NMuMG:	
Clone-5:	ATA**AATAAA**AGATGGCACTTTCC**(A)$_{44}$**
Clone-8:	ATA**AATAAA**AGATGGCACTTTCCCATTTT**(A)$_{67}$**
Clone-13:	ATA**AATAAA** AGATGGCACTTTCCCATTTT**(A)$_{63}$**
Clone-14:	ATA**AATAAA** AGATGGCACTTTCCCATTTT**(A)$_{67}$**
Clone-15:	ATA**AATAAA** AGATGGCACTTTCCC**(A)$_{63}$**
Clone-16:	ATA**AATAAA**AGATGGCACTTTCCCATTTTATTCCAGTTTTAT**(A)$_{14}$**
Clone-17:	ATA**AATAAA**AGATGGCACTTTCCCATTTTATTCCAGTTT **TATAAA**AAGTGG**(A)$_{42}$**
PAS-3: (1309 and 1444 nt) **Primers:** PCR1 Tdo1092, PCR2 Tdo1091 and PCR1:Tdo3162, PCR2:Tdo3163/Tdo3164 **No PCR amplification in both cell lines**	
PAS-4: (1925 nt) **Primers:** PCR1: Tdo946, PCR2: Tdo2296	
NIH3T3:	
Clone-5:	TCAACC**AATAAA**GTTTTTAAAATTGTAAC**(A)$_{13}$**
Clone-6:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{15}$**
Clone-9:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{15}$**
Clone-10:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{14}$**
NMuMG:	
Clone-4:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{16}$**
Clone-5:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{19}$**
Clone-9:	TCAACC**AATAAA**GTTTTTAAAATTGTAAC**(A)$_{16}$**

Clone-10:	TCAACC**AATAAA**GTTTTTAAAATTGT**(A)$_{14}$**
PAS-5 (3255, 3276, 3312 and 3351 nt) **Primers:** PCR1: Tdo3108, PCR2: Tdo3110 **NIH3T3:**	
Site 5-1: polyadenylation signal at 3255 in UTR 1/10	
Clone-4 :	GTGT**TATAAA**CTCCACTT**(A)$_{23}$**
Site 5-2: polyadenylation signal at 3275 in UTR 8/10	
Clone-1:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{15}$**
Clone-5:	AACTG**ATTAAA**GTCTCATTCTTGTCATTGTGTGGGTGTTTT **ATTAAA (A)$_{14}$**
Clone-6:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{15}$**
Clone-7:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{12}$**
Clone-8:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{13}$**
Clone-9:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{26}$**
Clone-10:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{23}$**
Clone-12:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{19}$**
Site 5-3: polyadenylation signal at 3302 in UTR 0/10	
Site 5-4: polyadenylation signal at 3351 in UTR 1/10	
Clone-11 :	ATCC**ATTAAA**ATGTTCAGTAATGGGCAGCCACAT**(A)$_{58}$**
NMuMG:	
Site 5-1: polyadenylation signal at 3255 in UTR 0/12	
Site 5-2: polyadenylation signal at 3275 in UTR 12/12	
Clone-1:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{43}$**
Clone-2:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{11}$**
Clone-3:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{34}$**
Clone-4:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{17}$**
Clone-5:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{20}$**
Clone-6:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{19}$**
Clone-7:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{11}$**
Clone-8:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{19}$**
Clone-9:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{17}$**
Clone-10:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{36}$**
Clone-11:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{22}$**
Clone-12:	AACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{40}$**
Site 5-3: polyadenylation signal at 3302 in UTR 0/12	
Site 5-4: polyadenylation signal at 3351 in UTR 0/12	
PAS-6 (5435, 5478, 5582, 5710, 5876, 5913, 6001 and 6109 nt) **Primers:** PCR1 Tdo3354, PCR2 Tdo3355 and PCR1 Tdo3356, PCR2 Tdo3357	
NMuMG:	
Site 6-1 polyadenylation signal at 5435 in UTR 5/5	
Clone-1:	TTTAA**AATAAA**GCTGCATATTTTTAAATG**(A)$_{18}$**
Clone-3:	TTTAA**AATAAA**GCTGCATATTTTTAAATG**(A)$_{24}$**
Clone-4:	TTTAA**AATAAA**GCTGCATATTTTT**(A)$_{24}$**
Clone-5:	TTTAA**AATAAA**GCTGCATATTTTTAAATG**(A)$_{15}$**

Clone-7:	TTTAA**AATAAA**GCTGCATATTTTTAAATG**(A)$_{35}$**
Site 6-2 polyadenylation signal at 5478 in UTR 0/5	
Site 6-3 polyadenylation signal at 5582 in UTR 0/5	
Site 6-4 polyadenylation signal at 5710 in UTR 0/5	
Site 6-5 polyadenylation signal at 5876 in UTR 0/7	
Site 6-6 polyadenylation signal at 5913 in UTR 0/7	
Site 6-7 polyadenylation signal at 6001 in UTR 0/7	
Site 6-8 polyadenylation signal at 6109 in UTR 7/7	
Clone-2:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{18}$**
Clone-3:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{32}$**
Clone-4:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{13}$**
Clone-5:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{18}$**
Clone-8:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{26}$**
Clone-12:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{13}$**
Clone-15:	AAACA**ATTAAA**AAGGTTCATTATTTTTG**(A)$_{19}$**

Supplementary Table S2:

Summary of 3'RACE sequencing data in human cell lines

PAS-1 (46 nt) **Primers:** PCR1 Tdo909, PCR2 Tdo911 **No PCR amplification in both cell lines**	
PAS-2 (265, and 297 nt) **Primers:** PCR1: Tdo909, PCR2: Tdo2199	
22RV1:	
Site 2-1: polyadenylation signal at 265 in UTR 4/6	
Clone-1:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTT TTAT$\mathbf{(A)_{31}}$
Clone-4:	GATT**AATAAA**GATGGCACTTTCCCGTTTT$\mathbf{(A)_{45}}$
Clone-6:	GATT**AATAAA**GATGGCACTTTCCCGTTTT$\mathbf{(A)_{40}}$
Clone-11:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTT TTAT$\mathbf{(A)_{16}}$
Site 2-2: polyadenylation signal at 265 in UTR 2/6	
Clone-3:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**AAGTGGAAACAG$\mathbf{(A)_{39}}$
Clone-5:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**AAGTGGAGACAG$\mathbf{(A)_{25}}$
T47D:	
Site 2-1: polyadenylation signal at 265 in UTR 8/8	
Clone-3:	GATT**AATAAA**GATGGCACTTTCCCGTTTT$\mathbf{(A)_{35}}$
Clone-4:	GATT**AATAAA**GATGGCACTTTCCCGTTTT$\mathbf{(A)_{32}}$
Clone-5:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTTT$\mathbf{(A)_{35}}$
Clone-6:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**$\mathbf{(A)_{18}}$
Clone-7:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**$\mathbf{(A)_{15}}$
Clone-8:	AAAAGGACATTTAAAATTCAATTAGGATT**AATAAA**$\mathbf{(A)_{16}}$
Clone-9:	GATT**AATAAA**GATGGCACTTTCCCGTTTT$\mathbf{(A)_{32}}$
Clone-11:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**$\mathbf{(A)_{17}}$
Clone-12:	GATT**AATAAA**GATGGCACTTTCCCGTTTTATTCCAGTTT **TATAAA**$\mathbf{(A)_{32}}$
Site 2-2: polyadenylation signal at 265 in UTR 0/8	
PAS-3 (880 nt) **Primers:** PCR1: Tdo2199, PCR2: Tdo1141	
22RV1:	

Clone-1:	ATTT**ATTAAA**TATGTTTTTTCAATTGT**(A)$_{36}$**
Clone-2:	ATTT**ATTAAA**TATGTTTTCTCAATTGTAACG**(A)$_{35}$**
Clone-3:	ATTT**ATTAAA**TATGTTTTCTCAATTGTAACG**(A)$_{30}$**
Clone-4:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{16}$**
Clone-5:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{22}$**
Clone-6:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{13}$**
Clone-7:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{17}$**
Clone-9:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{13}$**
Clone-10:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{35}$**
T47D:	
Clone-1:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{28}$**
Clone-2:	ATTT**ATTAAA**TATGTTTTCTC**(A)$_{17}$**
Clone-3:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{30}$**
Clone-4:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{31}$**
Clone-5:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{14}$**
Clone-6:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{17}$**
Clone-7:	ATTT**ATTAAA**TATGTTTTCTC**(A)$_{22}$**
Clone-8:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{28}$**
Clone-10:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{14}$**
Clone-11:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{21}$**
Clone-12:	ATTT**ATTAAA**TATGTTTTCTCAATTGT**(A)$_{34}$**
PAS-4 (1060 nt) **Primers:** PCR1: Tdo2199, PCR2: Tdo1141	
22RV1:	
Clone-11 :	TTCTC**ATTAAA**TATAAAATATTTTGTAATGCTGCACAGAAA TTTTC**(A)$_{21}$**
T47D: **Not detected**	
PAS-5 (1292 nt) **Primers:** PCR1: Tdo2201, PCR2: Tdo826 **No PCR amplification in both cell lines**	
PAS-6 (1622 nt) **Primers:** PCR1: Tdo1142, PCR2: Tdo2209 **No PCR amplification in both cell lines**	
PAS-7 (2178, 2226 nt) **Primers:** PCR1: Tdo828, PCR2: Tdo1147 **No PCR amplification in both cell lines**	
PAS-8 (3262, 3283 and 3319 nt) **Primers:** PCR1: Tdo3104, PCR2: Tdo3106	
22RV1:	

Site 8-1: polyadenylation signal at 3262 in UTR 3/11	
Clone-3:	TAGTGT**TATAAA**CTCCACTTAAAACTG**ATTAAA**GTCTC**(A)$_{24}$**
Clone-5:	TAGTGT**TATAAA**CTCCACTTAAAACTGATT**(A)$_{11}$**
Clone-12:	TAGTGT**TATAAA**CTCCACTTAAAACTGATT**(A)$_{14}$**
Site 8-2: polyadenylation signal at 3283 in UTR 8/11	
Clone-2:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{17}$**
Clone-4:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{16}$**
Clone-7:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{18}$**
Clone-9:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{17}$**
Clone-10:	ACTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{41}$**
Clone-13:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{18}$**
Clone-16:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{19}$**
Clone-17:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{12}$**
Site 8-3: polyadenylation signal at 3319 in UTR 0/11	
T47D:	
Site 8-1: polyadenylation signal at 3262 in UTR 5/10	
Clone-4:	GTGT**TATAAA**CTCCACTTAAAACTG**(A)$_{20}$**
Clone-10:	GTGT**TATAAA**CTCCACTTAAAACTG**(A)$_{17}$**
Clone-12:	GTGT**TATAAA**CTCCACTTAAAACTG**(A)$_{13}$**
Clone-16:	GTGT**TATAAA**CTCCACTTAAAACTG**(A)$_{17}$**
Site 8-2: polyadenylation signal at 3283 in UTR 4/10	
Clone-2:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{12}$**
Clone-14:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{13}$**
Clone-15:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{14}$**
Clone-18:	CTG**ATTAAA**GTCTCATTCTTGTC**(A)$_{21}$**
Site 8-3: polyadenylation signal at 3319 in UTR 1/10	
Clone-13:	CTG**ATTAAA**GTCTCATTCTTGTCATTGTGTGGGTGTTTT**ATTAA A**TGAGAGTTTATAATTC**(A)$_{16}$**

Supplementary Table S3:

List of primers used for PTEN 3'RACE (mouse)

Primer	**Sequence 5'-3'**	**Information**
Tdo679	GCGAGCTCCGCGGCCGCGTTTTTTTTTTTT	oligo dT anchor used for all RT
Tdo680	GCGAGCTCCGCGGCCGCG	Reverse primer used for all 3'RACE PCR
Tdo886	GCGTGCAGATAATGACAAGG	PAS-1 PCR1 PAS-2 PCR1
Tdo910	GAGGAGCCATCAAATCCAGA	PAS-1 PCR2
Tdo2187	CCACAGGGTTTTGACACTTG	PAS-2 PCR2
Tdo1092	TCCTACCCCTTTGCACTTGT	PAS-3 PCR1-1
Tdo1091	GGCTGCCAGTTTGTTTTTCT	PAS-3 PCR2-1
Tdo3162	TCTCAGTTGTAATGACTGCTCC	PAS-3 PCR1-2
Tdo3163	ACCTACTGTGGATGCTTCATGTG	PAS-3 PCR2-2
Tdo3164	CTTGAGATTCAACAGTAAGCAGG	PAS-3 PCR2-3
Tdo946	CAGCACTGCACGAATAATAAGG	PAS-4 PCR1
Tdo2296	GGGAATTTGGTGTCTTTCAA	PAS-4 PCR2
Tdo3108	TATGACAGTATTCACGATTAGCC	PAS-5 PCR1
Tdo3110	TCATAACGATGGCTGTGGTTG	PAS-5 PCR2
Tdo3354	TGCTCAGCAAATGCGTACCTACC	PAS-6 PCR1
Tdo3355	GCTCTGAATGTTGAGTATTCTGG	PAS-6 PCR2
Tdo3356	GAATGGAGCAAGGCTTGTAGTGG	PAS-6 PCR1-2
Tdo3357	CTATTGAGTTTGGAAGTGTGCAC	PAS-6 PCR2-2

Supplementary Table S4:

List of primers used for *PTEN* 3'RACE (human)

Primer	**Sequence 5'-3'**	**Information**
Tdo679	GCGAGCTCCGCGGCCGCGTTTTTTTTTTTT	oligo dT anchor used for all RT
Tdo680	GCGAGCTCCGCGGCCGCG	Reverse primer used for all 3'RACE PCR
Tdo909	TAGAGGAGCCGTCAAATCCA	PAS-1 PCR1 PAS-2 PCR1
Tdo911	CTGACACCACTGACTCTGATCC	PAS-1 PCR2 PAS-2 PCR1
Tdo2199	TGGCAATAGGACATTGTGTCA	PAS-2 PCR2 PAS-3 PCR1
Tdo1141	TTCACATCCTACCCCTTTGC	PAS-3 PCR2 PAS-4 PCR1
Tdo2200	TCAACAAAGAATGGGCTTGA	PAS-4 PCR2
Tdo2201	TCCATCTCCTGTGTAATCAAGG	PAS-5 PCR1
Tdo826	CTGTGGATGCTTCATGTGCT	PAS-5 PCR2
Tdo1142	ATAGCTGTCAGCCGTTCCAC	PAS-6 PCR1 PAS-7 PCR2
Tdo2209	TTGATTTGCTATTGAAAGAATAGGG	PAS-6 PCR2
Tdo828	TTGGTGCTGAAATTGTTCACT	PAS-7 PCR1
Tdo3104	GGCAGTATTCATAATTAGCCTG	PAS-8 PCR1
Tdo3106	CCACAAAGTGCCTCGTTTACC	PAS-8 PCR2

Supplementary Table S5

APA sites identified from GenXPro RNAseq databases

Human PTEN
position: Chr10, 89623195~89728532, CDS ends at 89725229

ALL (Acute Lymphoblastic Lymphoma)
http://tools.genxpro.net/apadb/browse/human/all/PTEN/

Sites	Position in 3'UTR (from GenXPro)	Position in Chr10	Reads
1	46	89725275	15
2	286	89725515	6
3	883	89726112	11
8	3282	89728511	5

HLF (Human Lung Fibroblasts)
http://tools.genxpro.net/apadb/browse/human/hlf/PTEN/?sort=location

Sites	Position in 3'UTR (from GenXPro)	Position in Chr10	Reads
1	49	89725278	288
2	282	89725511	59
3	902	89726131	38
6	1642	89726871	5
8	3275	89728504	62

Mouse Pten
position: Chr19, 32757577~32826160, CDS ends at 32820028

Muscle
http://tools.genxpro.net/apadb/browse/mouse/muscle/Pten/

Sites	Position in 3'UTR (from GenXPro)	Position in Chr19	Reads
1	61	32820089	16
2	297	32820325	21
5	3299	32823327	9
6-1	5432	32825460	32
new	5637	32825701	7
6-8	6127	32826155	50

Brain
http://tools.genxpro.net/apadb/browse/mouse/brain/Pten/

Sites	Position in 3'UTR (from GenXPro)	Position in Chr19	Reads
5	3300	32823328	15
6-1	5433	32825461	39
new	5674	32825702	8
new	6008	32826036	8
6-8	6131	32826159	19

Supplementary Table S6:

List of primers used for gene quantification

Primer and gene name	Sequence (5'-3')	Type of PCR
***Pten* 300 3'UTR isoform**		
Tdo886	GCGTGCAGATAATGACAAGG	PCR1
Tdo680	GCGAGCTCCGCGGCCGCG	
Tdo2197	TGGCAATAGGACATTGTGTCA	qPCR2
Tdo3734	CAAGTGTCAAAACCCTGTGG	
***Pten* 3.3 3'UTR isoform**		
Tdo3108	TATGACAGTATTCACGATTAGCC	PCR1
Tdo680	GCGAGCTCCGCGGCCGCG	
Tdo3764	ACCTGCCAGCTCAAAAGTTC	qPCR2
Tdo3765	TGCTGCACAGCACAAGAGTA	
***Pten* 5.5/6.0 3'UTR isoforms**		
Tdo3768	TGCTGCACAGCACAAGAGTA	qPCR
Tdo3769	ACAAGTCACAGAAGCACACA	
Hprt		
Tdo1610	ACAAGTCACAGAAGCACACA	qPCR
Tdo1611	AAGCTTGCTGGTGAAAAGGA	
***Pten* long 3'UTR isoforms**		
Tdo3130	TTGCGCTCATCTTAGGCTTT	qPCR
Tdo3131	TGCTGCCGGTAAACTCCACT	
***Pten* total**		
Tdo886	GCGTGCAGATAATGACAAGG	qPCR
Tdo887	TCTGGATTTGATGGCTCCTC	
c-myc		
Tdo1399	AGTGCTGCATGAGGAGACAC	qPCR
Tdo1400	GGTTTGCCTCTTCTCCACAG	

Supplementary Table S7:

Sequence of 3'UTR shRNAs

shRNA	Sequence 5'-3'
sh3	AATCTGGACATCCGAGAGATT
sh6	AATGGAGGGAATGCTCAGAAA
sh7	AAGAGCATATTGGTGCTAGAC
sh8	AATCCACAAAGGAAGGGATAT
sh9	AACCATACAAATGTGGAGGCT
sh10	AATGACTGCTCCATCTCCTAT
sh12	AATCATATACCTACTGTGGAT
sh13	AATTTACAGCACTGCACGAAT
sh15	AATGGTAATGTGAAGATGCTA
sh16	AAGTACAGATTGCATAGGACC
sh17	AAGTAGATTAGGCAGAACGCC

Supplementary Table S8:

Sequence of primers used to amplify miRNA precursors

Tdo #	**Sequence 5'-3'**
Mmu-miR-19b	
Tdo2337	TTGGATCCCCCAGCCAACTGTCCTGTTA
Tdo2338	TTGAATTCCCTGTGTAGAAAGGGGTTTGA
Mmu-miR-26b	
Tdo1102	CCGGGACCCAGTTCAAGTA
Tdo1103	GCCCCCGAGCCAAGTAAT