Supporting Online Material for

PNUTS functions as a proto-oncogene by sequestering PTEN

Sridhar Kavela¹, Swapnil R Shinde¹, Raman Ratheesh², Kotapalli Viswakalyan², Murali D. Bashyam², Swarnalata Gowardhankar³, Mohana Vamsy⁴, Sujit Pattnaik⁴, Subramanyeshwar Rao⁴, Regulagadda A Sastry⁶, Mukta Srinivasulu⁷, Junjie Chen⁸ & Subbareddy Maddika¹*

¹Laboratory of Cell Death & Cell Survival and ²Laboratory of Molecular Oncology, Centre for DNA Fingerprinting and Diagnostics (CDFD), Nampally, Hyderabad 500001, INDIA

³Apollo Hospitals, Hyderabad 500033, INDIA

⁴Indo-American Cancer Institute and Research centre, Hyderabad 500034, INDIA

⁶Nizam’s Institute of Medical Sciences, Hyderabad 500082, INDIA

⁷MNJ Institute of Oncology and regional cancer centre, Hyderabad 500004, INDIA

⁸Department of Experimental Radiation Oncology, The University of Texas M. D. Anderson Cancer Center, 1515 Holcombe Boulevard, Houston, Texas 77030, USA

⁵Present address: Omega Hospitals, Hyderabad 500034, INDIA

*To whom correspondence should be addressed.

Dr. Subbareddy Maddika

Laboratory of Cell Death & Cell Survival, Centre for DNA Fingerprinting and Diagnostics (CDFD), Nampally, Hyderabad 500001, INDIA

Tel: +91-40-24749353
Fax: +91-40-24749448
E-mail: msreddy@cdfd.org.in
Supplementary Figure S1- S5

Figure S1: Effect of PNUTS interaction on PTEN localization and stability.

Figure S2: PNUTS is overexpressed in cancers.

Figure S3: PNUTS control Akt activation and apoptosis.

Figure S4: Effect of PNUTS knock down on Akt signaling in MDA-MB-231 and BPH1 cells.

Figure S5: PNUTS promotes cell survival signaling in PTEN dependent manner.

Supplementary Figure legends

Supplementary Figure 1: Effect of PNUTS interaction on PTEN localization and stability.

(a) 293T cells transfected with SFB-tagged PTEN and Myc-PNUTS were immunoprecipitated with either control IgG or Flag antibody. The interaction of PNUTS with PTEN was detected by immunoblotting with anti-Myc antibody. (b) GST pull down assay was performed using immobilized control GST or GST-PTEN fusion proteins on agarose beads followed by incubation with extracts prepared from 293T cells. The in vitro interaction of PNUTS with PTEN was assessed by immunoblotting with PNUTS specific antibodies. (c) SFB-tagged PTEN Full Length and PTEN C2 domain alone were expressed in HEK 293T cells along with Full Length Myc-PNUTS, the cell lysates were pulled down with Streptavidin beads and the interaction of PNUTS was detected with anti-Myc antibody. (d) HeLa cells were transfected with GFP-PNUTS and the localization of endogenous PTEN was detected by immunofluorescence staining using anti-PTEN antibody. PTEN is differentially localized in the presence and absence of PNUTS. (e) HeLa cells transfected with either control siRNA or PNUTS siRNAs were treated with cycloheximide for the indicated times and the expression of PTEN and PNUTS was
detected by immunoblotting with specific antibodies. (f) HeLa cells transfected with the indicated constructs were used for extraction of cytoplasmic and nuclear extracts and the localization of PTEN was detected by immunoblotting. GAPDH and HDAC2 were used as controls.

**Supplementary Figure 2: PNUTS is overexpressed in cancers.** (a) The gene expression data for PNUTS derived from publicly available Oncomine database was shown.

**Supplementary Figure 3: PNUTS control Akt activation and apoptosis.** (a) K562 myeloid leukemia cells were transfected with the indicated siRNAs and cells were analyzed by flow cytometry after Annexin-V staining. M2 represents the Annexin-V positive cells. (b) K562 cells transfected with the indicated siRNAs were lysed and the expression of PTEN and PNUTS was detected by immunoblotting. (c) K562 cells were transfected with the indicated siRNAs and the percentage of apoptotic cells was measured by using Annexin-V staining. Error bar indicates standard deviation (n=3), P<0.01; students *t*-test.

**Supplementary Figure 4: Effect of PNUTS knock down on Akt signaling in MDA-MB-231 and BPH1 cells.** (a) PTEN positive MDA-MB231 breast cancer cells were transfected with control siRNA, PNUTS siRNA, PTEN siRNA, or a combination of PTEN siRNA and PNUTS siRNA, and the cell lysates were immunoblotted with indicated antibodies. (b) Normalized pAkt/Akt data from MDA-MB231 cells in supplementary figure 5a was shown. (c) MDA-MB231 cells were transfected with the indicated siRNAs and the percentage of apoptosis was measured by using Annexin-V staining. Error bar indicates standard deviation (n=3), P<0.01;
students t-test. (d) BPH1 prostate epithelial cells were transfected with control siRNA, PNUTS siRNA, PTEN siRNA, or a combination of PTEN siRNA and PNUTS siRNA, and the cell lysates were immunoblotted with indicated antibodies. (e) Normalized pAkt/Akt data from BPH1 cells in supplementary figure 5d was shown. (f) BPH1 cells were transfected with the indicated siRNAs and the percentage of apoptosis was measured by using Annexin-V staining. Error bar indicates standard deviation (n=3), P<0.01; students t-test.

Supplementary Figure 5: PNUTS promotes cell survival signaling in PTEN dependent manner. (a) DU-145 cells and (c) PC-3 cells were stably transfected with either retroviral based control shRNA or three different PNUTS shRNAs. The expression levels of various proteins were analysed by immunoblotting with their respective antibodies. (b) Normalized pAkt/Akt data from DU145 cells (d) and from PC-3 cells stably expressing PNUTS shRNA was shown. (e) A prostate epithelial BPH1 parental cell line along with BPH1-PNUTS wt or PNUTS W401A mutant or Delta TF2S PNUTS stable cells were tested for the expression of the indicated proteins.