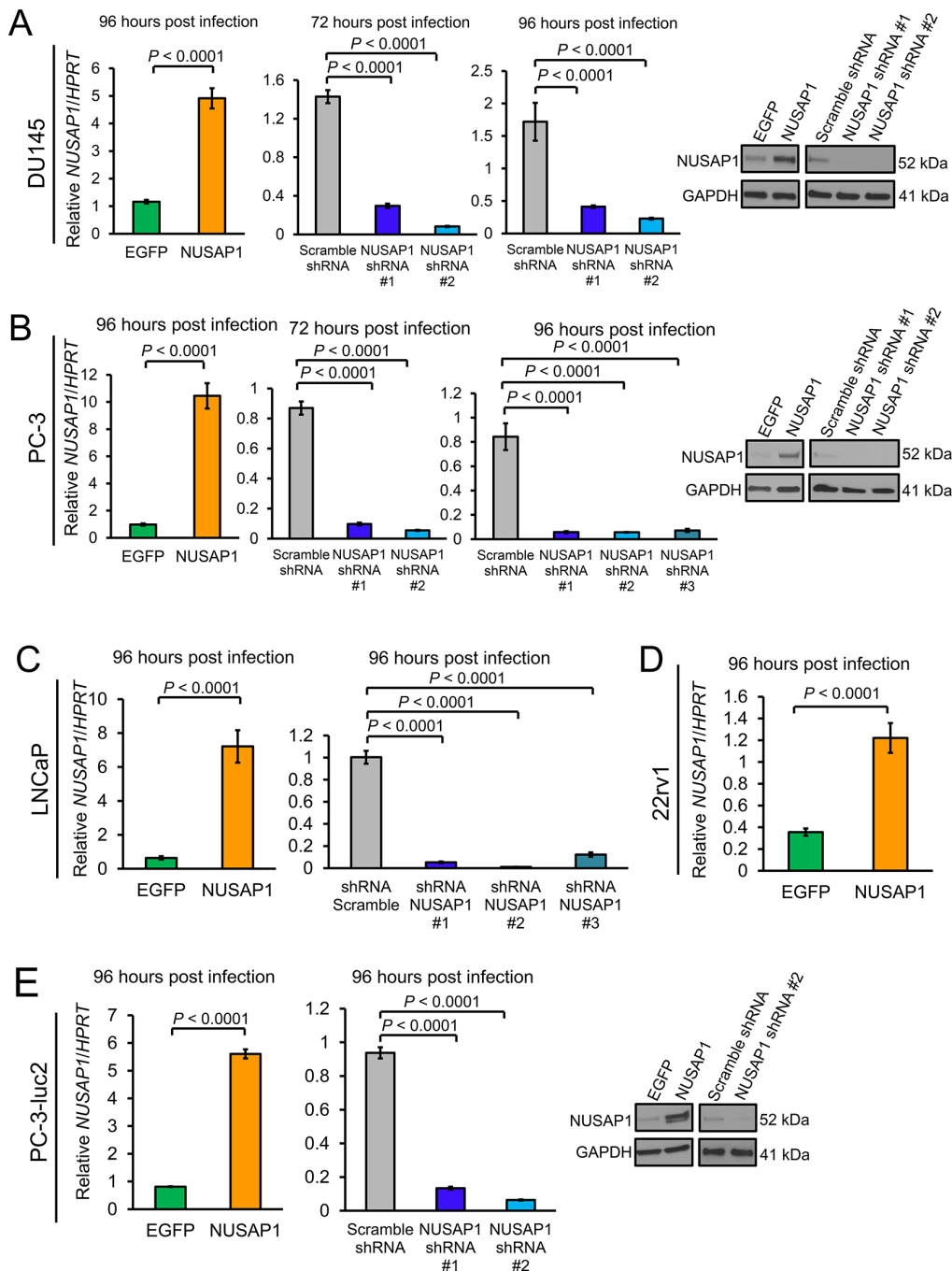
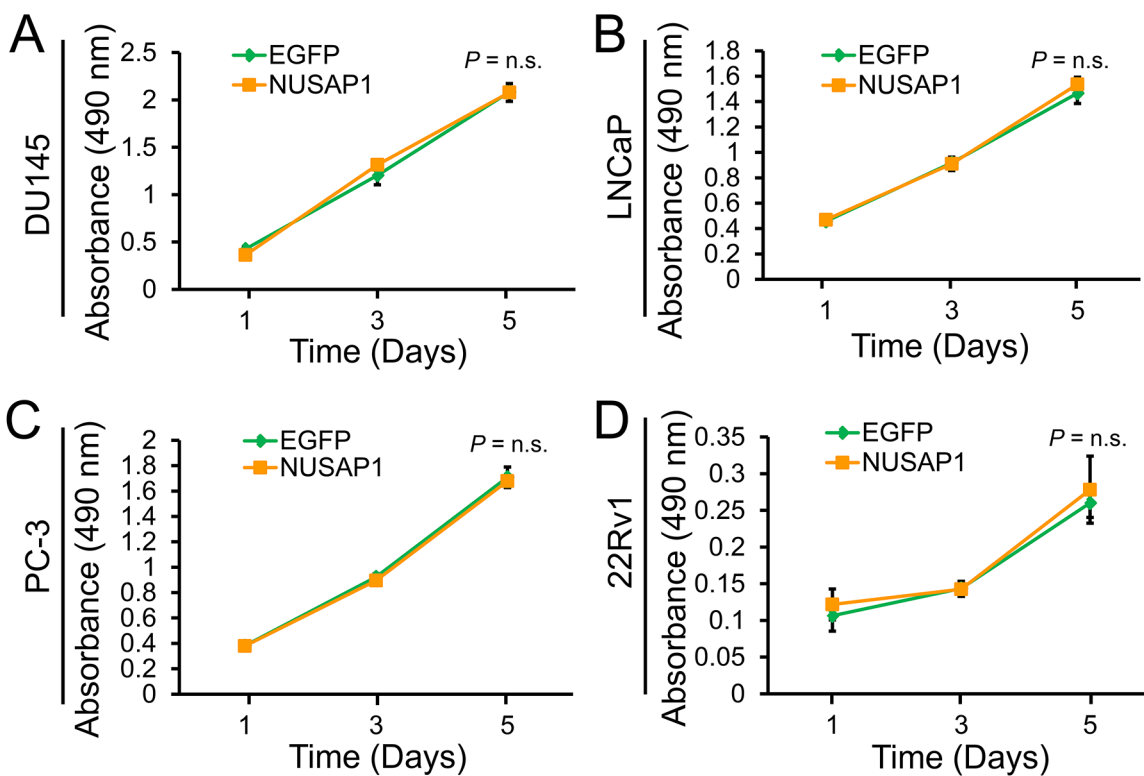


# NUSAP1 promotes invasion and metastasis of prostate cancer

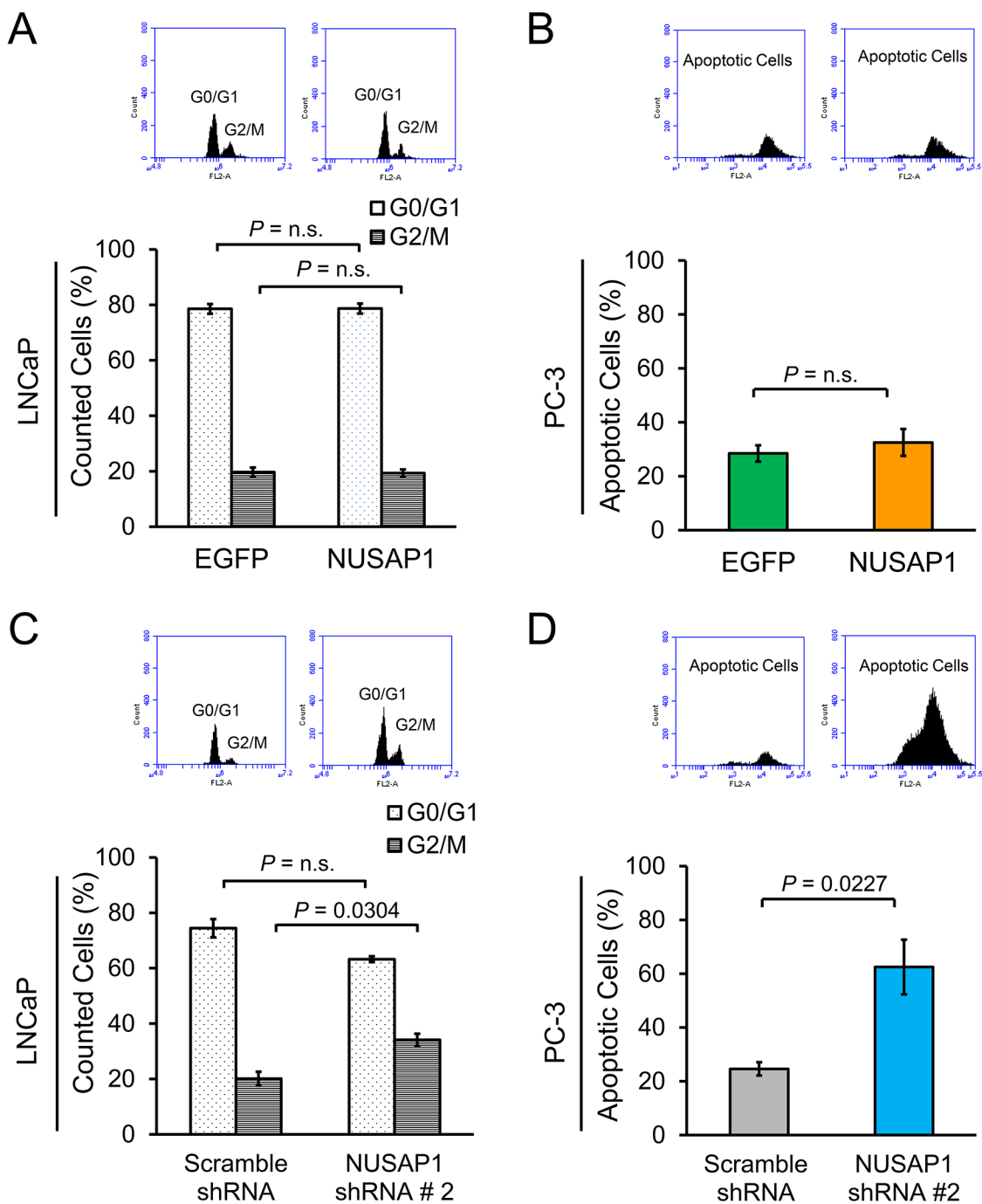
## SUPPLEMENTARY FIGURES AND TABLES



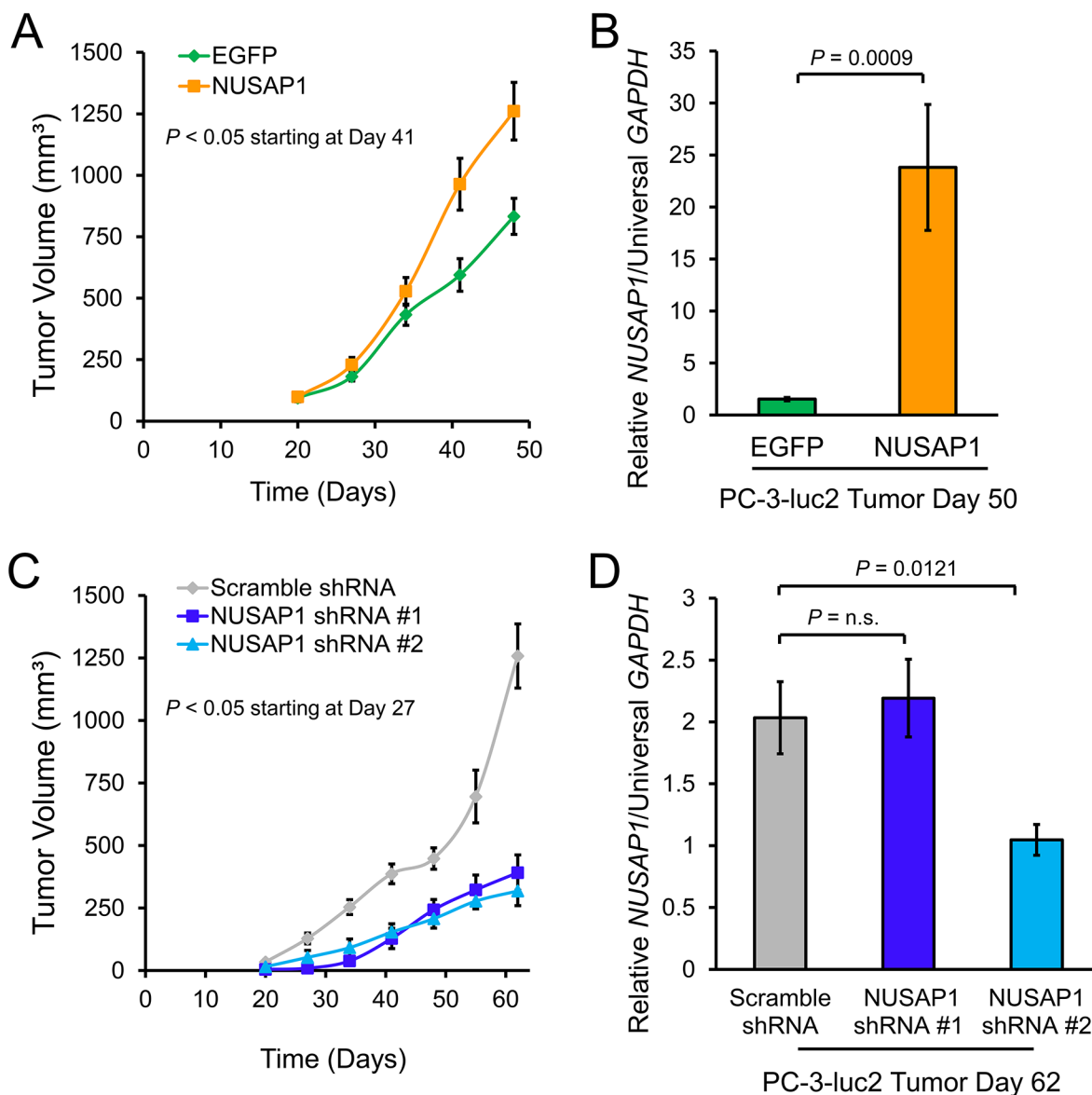
**Supplementary Figure 1: *NUSAP1* expression levels in cells overexpressing or underexpressing *NUSAP1*.** Lentiviral infections were used to overexpress or knockdown *NUSAP1* or controls in **A**. DU145, **B**. PC-3, **C**. LNCaP, **D**. 22Rv1 cells, or **E**. PC-3-luc2 cells. RNA was extracted from cells at indicated times post lentiviral infections and RT-qPCR was performed using SYBR green chemistry. Relative *NUSAP1* expression was determined by normalization to the *HPRT1* reference gene. Bars: mean  $\pm$  SD (standard deviation). *P*-values were calculated using the two-tailed Student's *t*-test. Proteins were extracted 96 hours post lentiviral infections and western blots were performed with antibodies recognizing *NUSAP1* and *GAPDH*.



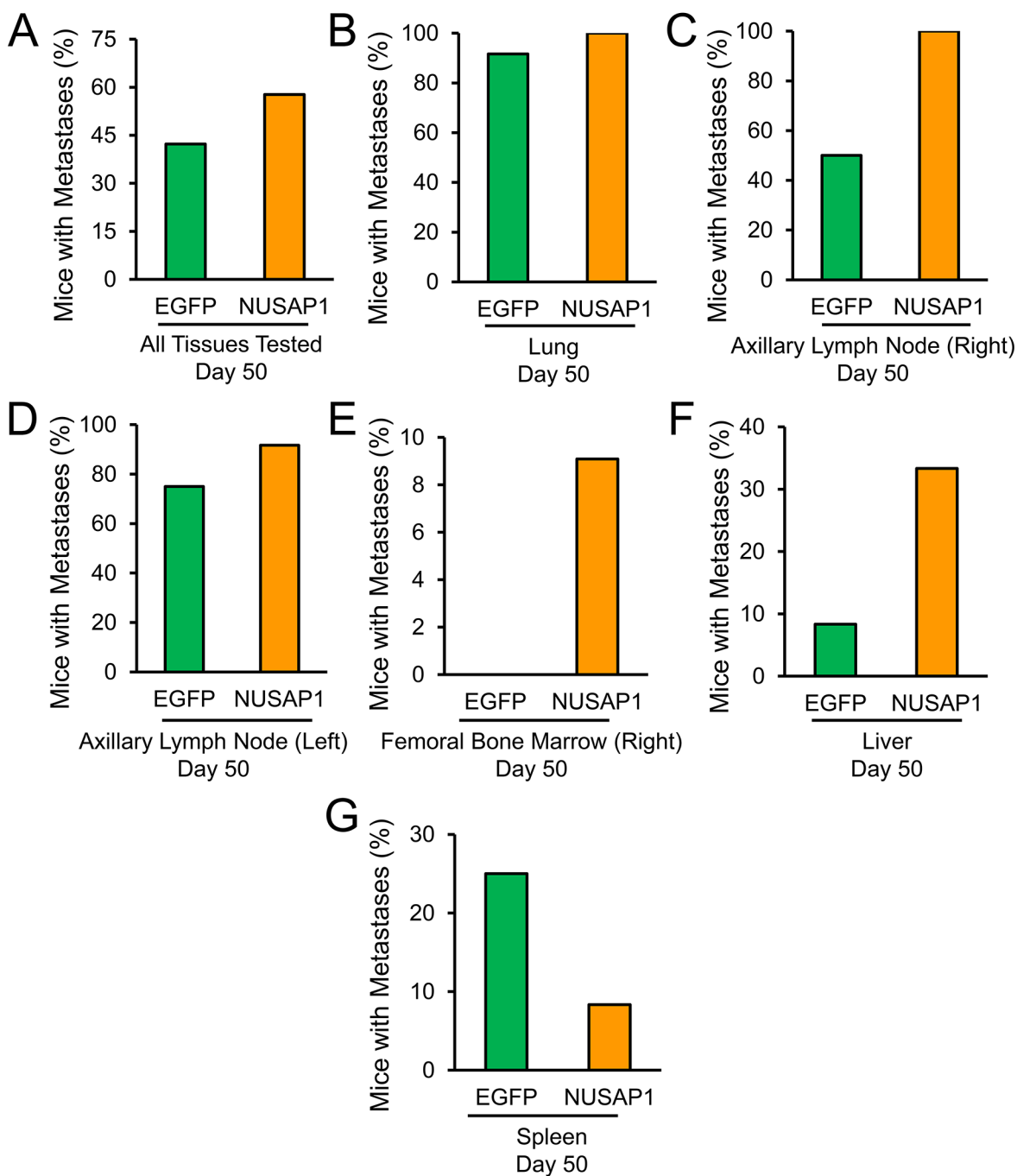
**Supplementary Figure 2: *NUSAP1* overexpression does not affect proliferation of prostate cancer cells grown in culture.** Lentiviral infections were used to stably overexpress *NUSAP1* or *EGFP* control in **A.** DU145, **B.** LNCaP, **C.** PC-3, or **D.** 22Rv1 prostate cancer cell lines. Cells were assessed for proliferation over 5 days using the CellTiter 96 Aqueous One Solution Cell Proliferation Assay. Points: mean ± SD. *P*-values were calculated using the two-tailed Student’s *t*-test.



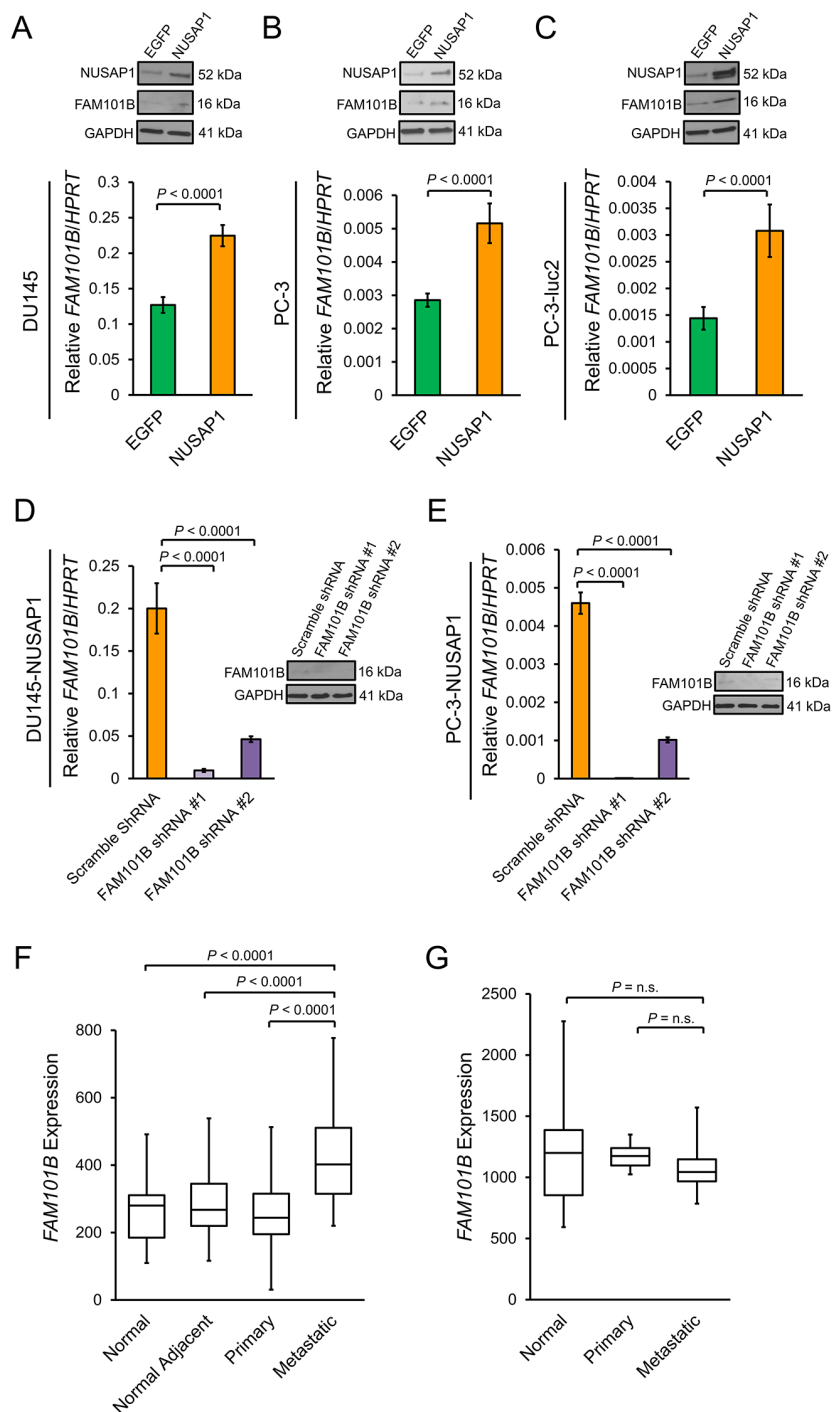
**Supplementary Figure 3: Analysis of G0/G1, G2/M, and apoptotic cells upon *NUSAP1* overexpression or knockdown.** Prostate cancer cells overexpressing or underexpressing *NUSAP1* or controls were stained with propidium iodide for flow cytometry analysis. **A.** G0/G1 and G2/M distribution analysis of LNCaP cells with *NUSAP1* overexpression. **B.** Apoptotic distribution analysis of PC-3 cells with *NUSAP1* overexpression. **C.** G0/G1 and G2/M distribution analysis of LNCaP cells with *NUSAP1* knockdown. **D.** Apoptotic distribution analysis of PC-3 cells with *NUSAP1* knockdown. All images represent original flow cytometry data. All bars: mean  $\pm$  SEM. All *P*-values were calculated using the two-tailed Student's *t*-test.



**Supplementary Figure 4: *NUSAP1* overexpression eventually increases tumor volume while *NUSAP1* knockdown decreases tumor volume.** PC-3-luc2 cells overexpressing or underexpressing *NUSAP1* or controls were subcutaneously injected into the flanks of male SCID-beige mice. **A.** Caliper measurements of tumor volume over time in mice with tumors overexpressing *NUSAP1* versus *EGFP*. Points: mean  $\pm$  SEM. EGFP:  $n = 21$ ; NUSAP:  $n = 21$ . **B.** RNA was harvested from tumors overexpressing *NUSAP1* or *EGFP* at day 50 and RT-qPCR was performed using SYBR green chemistry. Relative *NUSAP1* expression was determined by normalization to the universal *GAPDH* reference gene. Bars: mean  $\pm$  SD. EGFP:  $n = 12$ ; NUSAP1:  $n = 11$ . **C.** Caliper measurements of tumor volume over time in mice with tumors underexpressing *NUSAP1* versus control. Points: mean  $\pm$  SEM. Scramble shRNA:  $n = 10$ ; NUSAP1 shRNA #1:  $n = 11$ ; NUSAP1 shRNA #2:  $n = 7$ . **D.** RNA was harvested from tumors with knockdown of *NUSAP1* or control at day 62 and RT-qPCR was performed using SYBR green chemistry. Relative *NUSAP1* expression was determined by normalization to the universal *GAPDH* reference gene. Bars: mean  $\pm$  SD. Scramble shRNA:  $n = 12$ ; NUSAP1 shRNA # 1:  $n = 9$ ; NUSAP1 shRNA #2:  $n = 9$ . All  $P$ -values were calculated using the two-tailed Student's  $t$ -test.



**Supplementary Figure 5: Percentage of mice with metastases is generally higher when *NUSAP1* is overexpressed in the flank tumor.** PC-3-luc2 cells overexpressing *NUSAP1* or *EGFP* control were subcutaneously injected into the flanks of male SCID-beige mice. On day 50, mice were sacrificed and tissues (lungs, axillary lymph nodes [right and left], femoral bone marrow [right], livers, and spleens) were extracted to quantify metastases by RT-qPCR using SYBR green chemistry. Expression of human-specific *GAPDH* relative to universal *GAPDH* was used to quantify metastases, and percentage of mice with metastases was determined for **A**. all six tissue sites combined and the **B**. lung, **C**. right axillary lymph node, **D**. left axillary lymph node, **E**. right femoral bone marrow, **F**. liver, and **G**. spleen.



Yu YP *et al.*, 2004 and Chandran UR *et al.*, 2007

Varambally S *et al.*, 2005

**Supplementary Figure 6: *FAM101B* expression in prostate cancer cell lines and patient samples.** A-E. Lentiviral infections were used to stably overexpress *NUSAP1* or knockdown *FAM101B* or controls in (A) DU145, (B) PC-3, (C) PC-3-luc2 cells, (D) DU145-NUSAP1, or (E) PC-3-NUSAP1 cells. RNA and proteins were extracted from cells 96 hours post lentiviral infections. RT-qPCR was performed using SYBR green chemistry. Relative *FAM101B* expression was determined by normalization to the *HPRT1* reference gene. Bars: mean  $\pm$  SD. Western blots were performed with antibodies recognizing *NUSAP1*, *FAM101B*, and *GAPDH*. F. Box plots of *FAM101B* expression in the Yu YP *et al.*, 2004 [36] and Chandran UR *et al.*, 2007 [37] datasets (GEO Accession: GDS2546; GEO Profile: 34877214). Normal: n = 17; Normal Adjacent: n = 59; Primary: n = 66; Metastatic: n = 25. G. Box plots of *FAM101B* expression in the Varambally S *et al.*, 2005 [38] dataset (GEO Accession: GDS1439; GEO Profile: 14261532). Normal: n = 6; Primary: n = 7; Metastatic: n = 6. All *P*-values were calculated using the two-tailed Student's t-test.

**Supplementary Table 1: Metastases as seen by *ex vivo* bioluminescence imaging**

Day	Expression	Metastases					
		Lung		Right Axillary Lymph Node		Left Axillary Lymph Node	
		number	%	number	%	number	%
50	EGFP	0/5	0	1/5	20	0/5	0
50	NUSAP1	2/6	33.3	3/6	50	2/6	33.3
59	EGFP	3/6	60	2/6	33.3	2/6	33.3
59	NUSAP1	4/6	66.7	5/6	83.3	3/6	50
62	Scramble shRNA	3/6	50	2/6	33.3	2/6	33.3
62	NUSAP1 shRNA #1	1/6	16.7	0/6	0	1/6	16.7
62	NUSAP1 shRNA #2	0/7	0	2/7	28.6	0/7	0

**Supplementary Table 2: Differentially expressed genes and their fold-changes as determined by RNA-Seq when *NUSAP1* is overexpressed in PC-3 cells.**

See Supplementary File 1

**Supplementary Table 3: Differentially expressed genes and their fold-changes as determined by RNA-Seq when *NUSAP1* is knocked down in DU145 and PC-3 cells.**

See Supplementary File 2

**Supplementary Table 4: Primer sequences used for RT-qPCR**

Gene	Primer Sequence
<i>NUSAP1</i>	Forward: 5'-CCCTCAAGTACAGTGACCTGC-3' Reverse: 5'-TCATTTCCTTTTCTTGCCTCA-3'
<i>HPRT1</i>	Forward: 5'-TGACACTGGCAAACAATGCA-3' Reverse: 5'-GGTCCTTTTCACCAGCAAGCT-3'
Human-specific <i>GAPDH</i>	Forward: 5'-AGATCCCTCCAAAATCAAGTG-3' Reverse: used universal <i>GAPDH</i> reverse primer
Universal <i>GAPDH</i>	Forward: 5'-CCATGGAGAAGGCTGGGG-3' Reverse: 5'-CAAAGTTGTCATGGATGACC-3'
<i>FAM101B</i>	Forward: 5'-CTGAGGCTTTGTCCCCTGT-3' Reverse: 5'-AAGTGCCTCTCGGAGTCGTA-3'