

## Supplementary Data

### Supplementary Table 1

Additional *PIK3R1* and *PIK3R3* genes identified by IPA pathway analysis validated as androgen regulated in LNCaP cells by real-time PCR

### Supplementary Table 2

Previously published known AR binding sites within 100 kb of the *PIK3R1* gene and four AR binding sites within 50 kb of the *PIK3R3* gene.

### Supplementary Table 3

Meta-analysis of *PIK3R1*, *PIK3R3*, *PIK3CA* and *CCND1* mRNA expression in 728 prostate cancer tumours using data from 10 previously published studies<sup>6,21-28</sup>. Data was generated using OncoPrint [30].

### Supplementary Table 4

Primer sequences used

### Supplementary Figure 1: IPA Pathway Analysis of genes associated with *PIK3R1*.

Gene expression changes in LNCaP cells cultured in the presence or absence of androgens for 24 hours. Gene lists from Rajan et al.<sup>10</sup> were uploaded to the web-based Ingenuity Pathway Analysis (IPA; Ingenuity Systems) software programme, and used to identify novel androgen-regulated genes associated with *PIK3R1*. Genes up-regulated by androgens are highlighted in red, and down-regulated genes are in green. Expression changes for 34 of these genes were validated by real-time PCR (Supplementary Table 1).

**Supplementary Figure 2: IPA Pathway Analysis of genes associated with *PIK3R3*.**

Gene expression changes in LNCaP cells cultured in the presence or absence of androgens for 24 hours. Gene lists from Rajan et al.<sup>10</sup> were uploaded to the web-based Ingenuity Pathway Analysis (IPA; Ingenuity Systems) software programme, and used to identify novel androgen-regulated genes associated with *PIK3R3*. Genes up-regulated by androgens are highlighted in red, and down-regulated genes are in green.

**Supplementary Figure 3: Over-expression of p85 $\alpha$  in HEK293 cells stabilises p110 $\alpha$ .**

**(A)** Over-expression of p85 $\alpha$ , p55 $\gamma$  and p110 $\alpha$  in Flp-In HEK293 cells was induced by addition of 1  $\mu$ g/ml tetracycline to the tissue culture medium for 72 hours and detected by western blotting using its corresponding antibody. This confirmed the specificity of our p85 $\alpha$ , p55 $\gamma$  and p110 $\alpha$  antibodies and showed that an increase in p85 $\alpha$  protein stabilises p110 $\alpha$ .  
**(B)** Depletion of *PIK3R3* / p55 $\gamma$  in LNCaP cells by esiRNA was confirmed by western blotting (upper panel) and by real-time PCR (lower panel).

**Supplementary Figure 4: *PIK3R3* and *PIK3CA* expression in clinical samples.**

**(A)** Real-time PCR analysis of *PIK3R1* mRNA expression in 32 BPH and 17 PCa tissue samples, and from 9 matched normal and tumour tissue pairs (normalised to 3 housekeeping genes). **(B)** Real-time PCR analysis of *PIK3R1* mRNA expression in 32 BPH and 17 PCa tissue samples, and from 9 matched normal and tumour pairs (normalised to 3 housekeeping genes).

## Supplementary Table 1

### PI3K pathway

### RT\_PCR

#### Up-regulated

#### *Taylor et al.*

	Fold change*	SE	P value	Fold change	P value
LDLR	6.19	0.87	3.98E-03		not sig.
RAB3B	5.02	0.45	8.40E-04	1.659	1.45E-04
HMGCR	3.28	0.73	0.03521		not sig.
VEGFA	4.23	0.34	6.40E-04		not sig.
NR4A1	13.33	3.95	0.03547	1.220	0.027
SOCS2	9.75	1.56	4.96E-03	1.147	8.00E-03
MTOR	4.25	0.51	3.07E-03	1.215	2.14E-04
ERRFI1	3.49	0.86	0.04492	1.290	2.00E-03
VLDLR	2.20	0.28	0.01313	1.258	3.00E-03
SCR	1.05	0.15	0.74037		not sig.
ANGPT2	31.82	2.80	3.90E-04		not sig.
CALU	7.55	0.85	1.54E-03		not sig.
ORM1	446.31	57.45	1.49E-03		not sig.
LIFR	13.95	3.40	0.01896	1.303	9.00E-03
GHR	2.65	1.01	0.17985	1.095	2.00E-03
MERTK	14.00	3.01	0.0125		not sig.
SEC14L2	8.46	0.74	5.50E-04		not sig.
CSK	1.96	0.42	0.08213	1.083	6.00E-03
ARFIP2	2.08	0.31	0.02542	1.216	9.94E-05
TBC1D4	4.64	0.41	9.30E-04	1.467	8.65E-06

#### Down-regulated

#### *Taylor et al.*

	Fold change*	SE	P value	Fold change	P value
TLL1	0.38	0.12	5.73E-03	-1.145	2.25E-06
F2R	0.31	0.09	1.80E-03		not sig.
SI	0.02	0.01	7.23E-09		not sig.
ADD3	0.21	0.09	8.30E-04	-1.284	0.002
SDC4	0.29	0.04	4.32E-05	-1.471	2.13E-09
KLF10	0.06	0.00	1.50E-08		not sig.
DEPDC6	0.16	0.02	1.14E-06	-1.475	9.00E-06
SORBS2	0.16	0.02	3.67E-06		not sig.
PIK3C2B	0.58	0.02	4.19E-05		not sig.
ASAP2	0.56	0.23	0.1304		not sig.
SDC2	0.31	0.07	4.70E-04	-1.272	1.33E-07
HSPG2	0.49	0.11	9.21E-03	-1.370	1.07E-06
ST5	0.38	0.11	4.90E-03	-1.704	1.93E-13
AMPK (PPL)	0.67	0.16	0.1088	-1.081	2.36E-04

\* Fold change = relative quantification of androgen treated cells normalised to steroid deplete (set as 1)

## Supplementary Table 2

<b>PIK3R1 gene</b>	start	end	
chr5:	67,511,584	67,597,649	
<b>PIK3R1 CHIP-seq peaks</b>	start	end	distance
	67419340	67419849	<b>92,244</b>
	67424772	67425507	<b>86,812</b>
<b>PIK3R3 gene</b>	start	end	
chr1:	46505812	46598708	
<b>PIK3R3 CHIP-seq peaks</b>	start	end	distance
	46634665	46635340	<b>35957</b>
	46634214	46634617	<b>35909</b>
	46634665	46635340	<b>36632</b>
	46640496	46640802	<b>42094</b>
<b>PIK3CA gene</b>	start	end	
chr3:	178,866,311	178,952,497	
<b>PIK3CA CHIP-seq peaks</b>	start	end	distance
	178,791,302	178791302	<b>75,009</b>

### Supplementary Table 3

Cancer v normal analysis fold changes  
747 samples

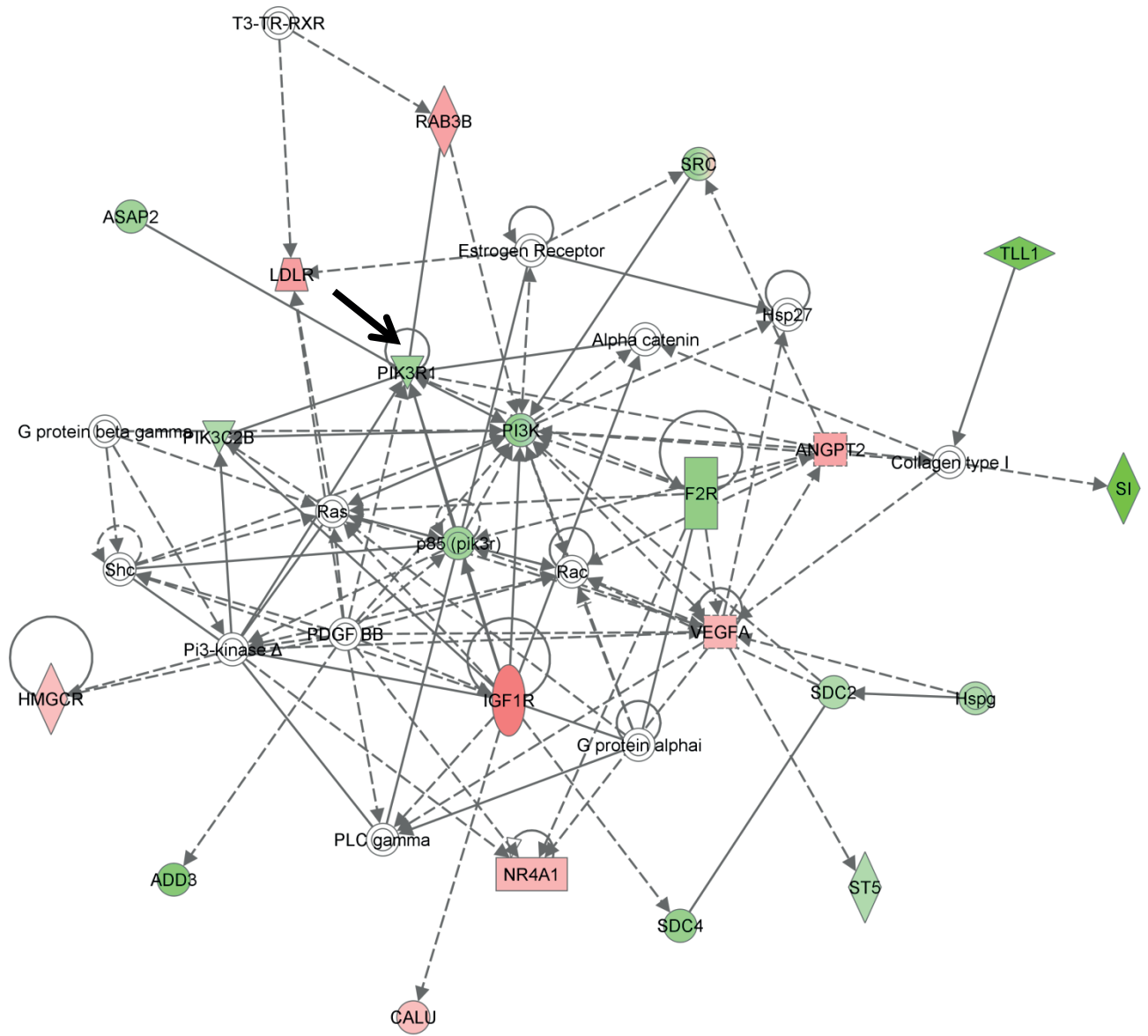
	<b>Tomlins et al. 2007</b> 101 samples Prostate carcinoma epithelia v normal	<b>Lapointe et al. 2004</b> 112 samples Prostate carcinoma v normal	<b>Grasso et al 2012</b> 122 samples Prostate carcinoma vs normal
<b>PIK3R1</b>	-3.266 p=5.87E-7	-1.561 p=1.89E-7	-1.428 p=4.99E-5.
<b>PIK3R3</b>	1.771 p=0.999	1.109 p=0.966	1.275 p=0.934
<b>PIK3CA</b>	1.581 p=0.063	1.058 p=0.878	-1.079. p=0.943.
<b>CCND1</b>	1.039 p=0.569.	-1.061 p=0.212.	-1.425 p=1.92E-7.
	<b>Luo et al. 2002</b> 30 samples Prostate carcinoma vs normal	<b>Arredouani et al. 2009</b> 21 samples Prostate carcinoma vs normal	<b>Vanaja et al. 2003</b> 40 samples Prostate adenocarcinoma vs normal
<b>PIK3R1</b>	-6.355 p=0.034.	p=0.002. -1.972.	p=0.253. -1.118
<b>PIK3R3</b>	1.156 p=0.406.	p=0.709. 1.070	-1.174. p=0.019.
<b>PIK3CA</b>	2.451 p=0.106	p=0.708.-1.049	-1.087. p=0.043
<b>CCND1</b>	-1.296 p=0.091.	-1.338 p=0.002.	-1.052 p=0.668.
	<b>Taylor et al 2010</b> 185 samples Prostate carcinoma vs normal	<b>Varmabally et al 2007</b> 19 samples Prostate carcinoma vs normal	
<b>PIK3R1</b>	-1.408 p=1.73E-6	-1.446 p=0.002	
<b>PIK3R3</b>	1.183 p=0.999	p=0.474.-1.040	
<b>PIK3CA</b>	-1.072 p=0.879.	p=0.937.-1.181	
<b>CCND1</b>	-1.375 p=3.08E-11.	-1.865 p=1.33E-4.	
	<b>Magee et al. 2001</b> 15 samples Prostate carcinoma vs normal	<b>Singh et al. 2002</b> 102 samples Prostate carcinoma vs normal	
<b>PIK3R1</b>	p=0.006. -1.657.	p=0.021. -1.537.	
<b>PIK3R3</b>	p=0.287. -2.676.	-2.924. p=5.91E-5	
<b>PIK3CA</b>	-1.895. p=0.883.	p=0.508. -1.003	
<b>CCND1</b>	-1.100 p=0.227.	-1.416 p=0.015.	

## Supplementary Table 4

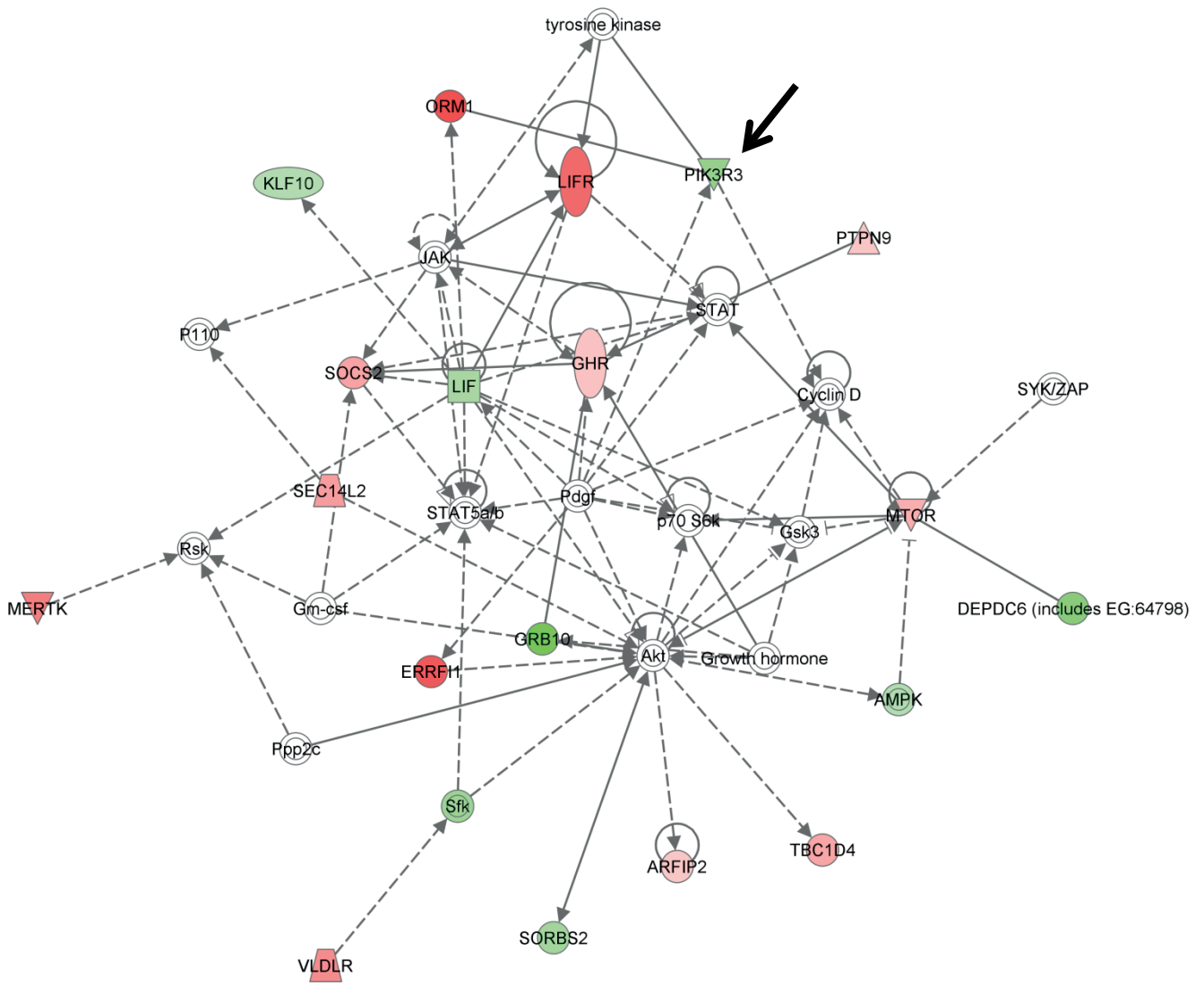
### Primer sequences used

Gene	Forward	Reverse
<i>PIK3R1</i>	GATTCTCAGCAGCCAGCTCTGAT	GCAGGCTGTCGTTTCATTCCAT
<i>PIK3R3</i>	CGTGTGCCATTTGTTTTGAC	ATTCCAGAGCCAAGCATCAT
<i>PIK3CA</i>	GAGAGGGGAATGAAAAGGAGA	ATCATGAATCTCACCCAGACG
<i>GAPDH</i>	AACAGCGACACCCATCCTC	CATACCAGGAAATGAGCTTGACAA
<i>Actin</i>	CATCGAGCACGGCATCGTCA	TAGCACAGCCTGGATAGCAAC
<i>B-tubulin</i>	CTTCGGCCAGATCTTCAGAC	AGAGAGTGGGTCAGCTGGAA
<i>CCND1</i>	CCGCTGGCCATGAACTACCT	ACGAAGGTCTGCGCGTGTT
<i>LDLR</i>	AAGGACACAGCACACAACCA	CAAAGGAAGACGAGGAGCAC
<i>RAB3B</i>	ACGAGAAGCGGGTGAAACT	CCAGTCTTGACAGCATTGA
<i>HMGCR</i>	GACCTTTCAGAGCAAGCAC	ACCAACTCCAATCACAAGACA
<i>VEGFA</i>	AAGGAGGAGGGCAGAATCAT	CATCAGGGGCACACAGGAT
<i>NR4A1</i>	GCTACCTTCAAACCCAAGC	CAGCATCTTCTTCCCAAAG
<i>SOCS2</i>	TGCCTTGCCCTTAGGTTT	GCTTGGTTCCTTCCCACTTC
<i>MTOR</i>	ACGCTGTCATCCCTTATCG	GCTCAAACACCTCCACCTTC
<i>ERRF1</i>	GGAGCAGTCGCAGTGAGTTT	AATAAGAAGAGGGGGCAAGC
<i>VLDLR</i>	GCACAAATGGTCGCTGTATT	GCACTGTTCTGGGCTTTCAT
<i>SCR</i>	TCCTGGTGGGAGAGAACCTG	CCGACTTGATGGTGAAGC
<i>ANGPT2</i>	GGGAAGGAATGAGGCTTAC	GGTTGGCTGATGCTGCTTAT
<i>CALU</i>	TGAGCAAACCCACAGAAAAG	AGCACCCAAGAAGGCATCAT
<i>ORM1</i>	CCAACAAGACAGAGGACACG	TGGACATTCAGGTAGGTGGTG
<i>LIFR</i>	CCACCTTCCAAAATAGCGAGT	GTTCCGACCGAGACGAGTTA
<i>GHR</i>	AAGTGAGATGGGAAGCACCA	CCAGAGTTTCGTTGTTTGA
<i>MERTK</i>	CTGAAAGTGGCAGTGAAGACC	GACATTTGGGTGGCTGAAGT
<i>SEC14L2</i>	ACCTCATCAAACCTTCTCTG	GTGCCCCATACTCCACA
<i>CSK</i>	GCCCAGGATGAGTTCTACCG	ACTTGACGGCGACTTTGTTC
<i>ARFIP2</i>	CCATCGGGACAAGTATGAGAA	AAAGTAGGCGGACACAGCAT
<i>TBC1D4</i>	GCAATGAGTCCCTAAGTGTGG	CGTTCCTGTCCAATCCATCT
<i>TLL1</i>	GGCAATCTCTATCGGCAAGA	CCTGGCTGGATGTTTTCTCT
<i>F2R</i>	GCCAGAATCAAAGCAACAA	CTCCTCATCCTCCCAAATG
<i>SI</i>	TTCTTGGTGCTTCTTCGTT	CGATTGGGTGCTGATTTTG
<i>ADD3</i>	ACCAGTCCCCGAACCAA	GGTCCTGCTGTTTTCAAGTCA
<i>SDC4</i>	CCACCGAACCCAAGAAACTA	ATGCCACCCACAATCAGAG
<i>KLF10</i>	CAATGAAAGCAGCCAGCAT	GAGGAAGGCACAGAAAAGTCA
<i>DEPDC6</i>	GTCCGTGCTGAAGAGACCTG	CCGTTGACAGAGACGACAAA
<i>SORBS2</i>	CAAATAAGCCACAGCGTCCT	CCACAAACCAGCCGTCAT
<i>PIK3C2B</i>	AATGCTGTCCCCCTCAAAC	GTCCTGGCGAAGGTCGTC
<i>ASAP2</i>	ATCCGAAAAGTGTGGCAGAA	TGGCAGGTTAGCAGGTTGAG
<i>SDC2</i>	CCAGCCGAAGAGGATACAAA	ATGCGATACACCAACAGCAG
<i>HSPG2</i>	GTGCTCATCCAGACCGTGTA	GACAAGCCAGAATAGCCAATG
<i>ST5</i>	TACCCCTCTTCTCCCACTGA	TCCTGCTCTTCGGCTCTTTA
<i>AMPK (PPLAB)</i>	AGGCACCAGAGAAGGAGGAA	CAGTTGTTGAAGGACCCAGA

# Supplementary Figure 1



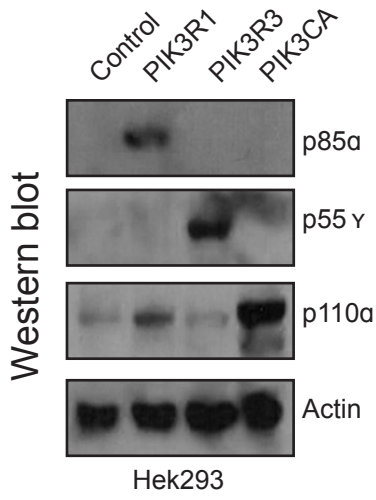
# Supplementary Figure 2



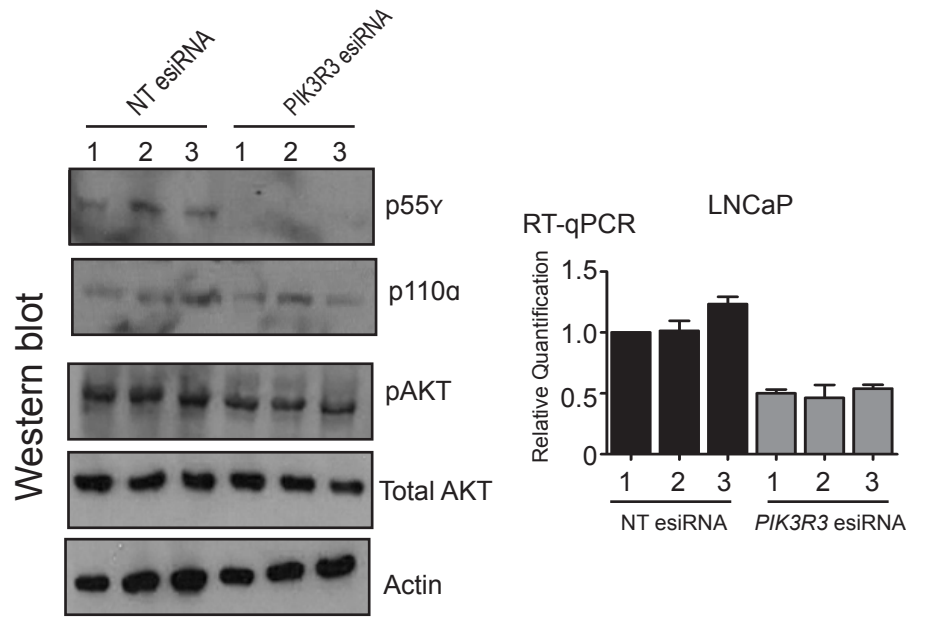


# Supplementary Figure 3

**A**

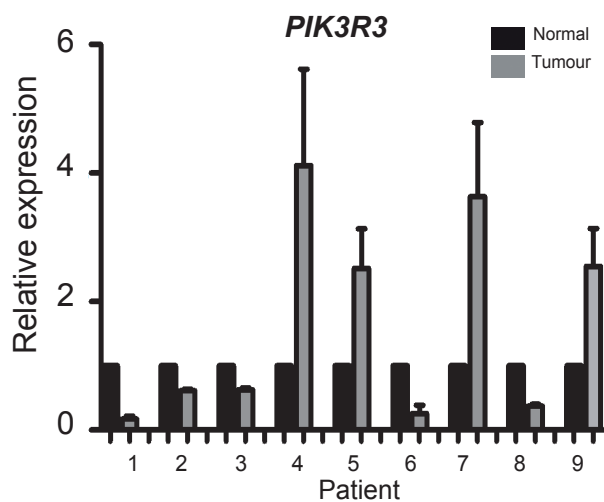


**B**



# Supplementary Figure 4

**A**



**B**

