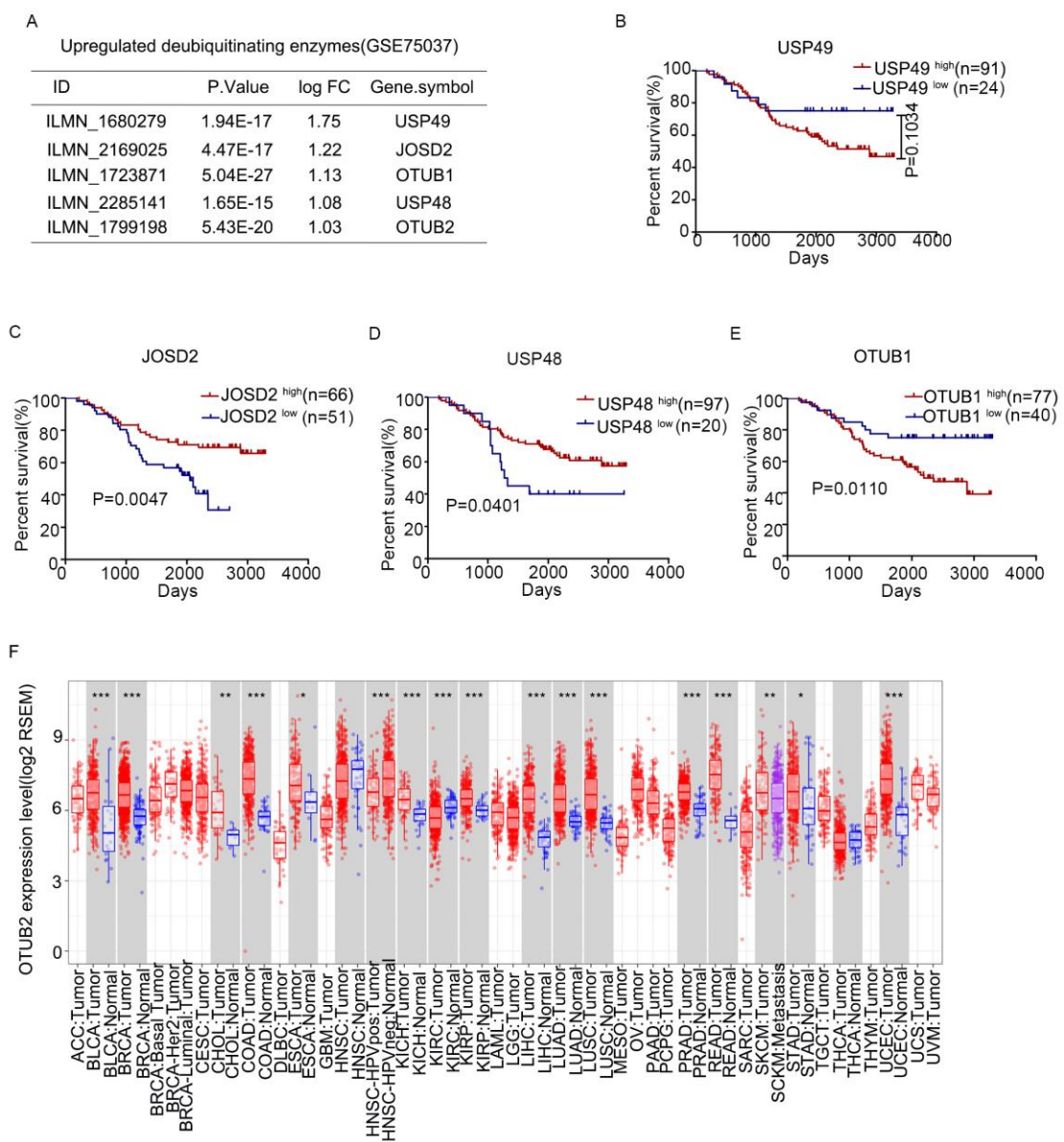
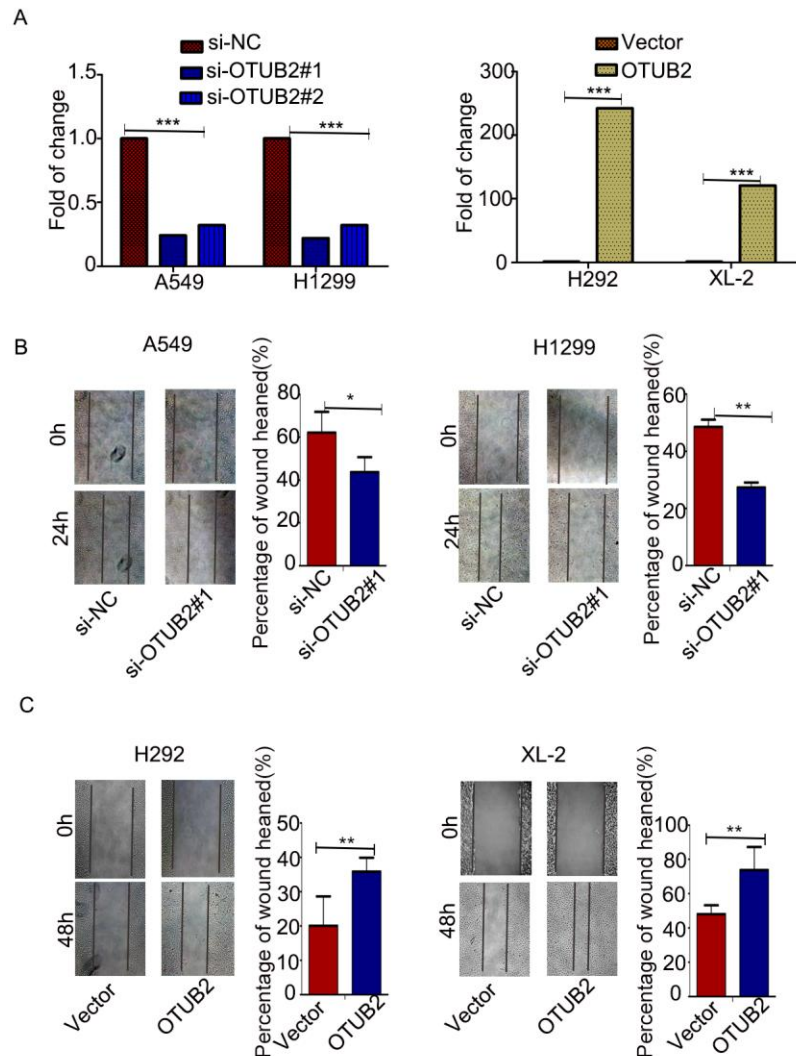


Supplementary Figure 1. Deubiquitinating enzymes expression signatures in GSE75037 and GSE13213



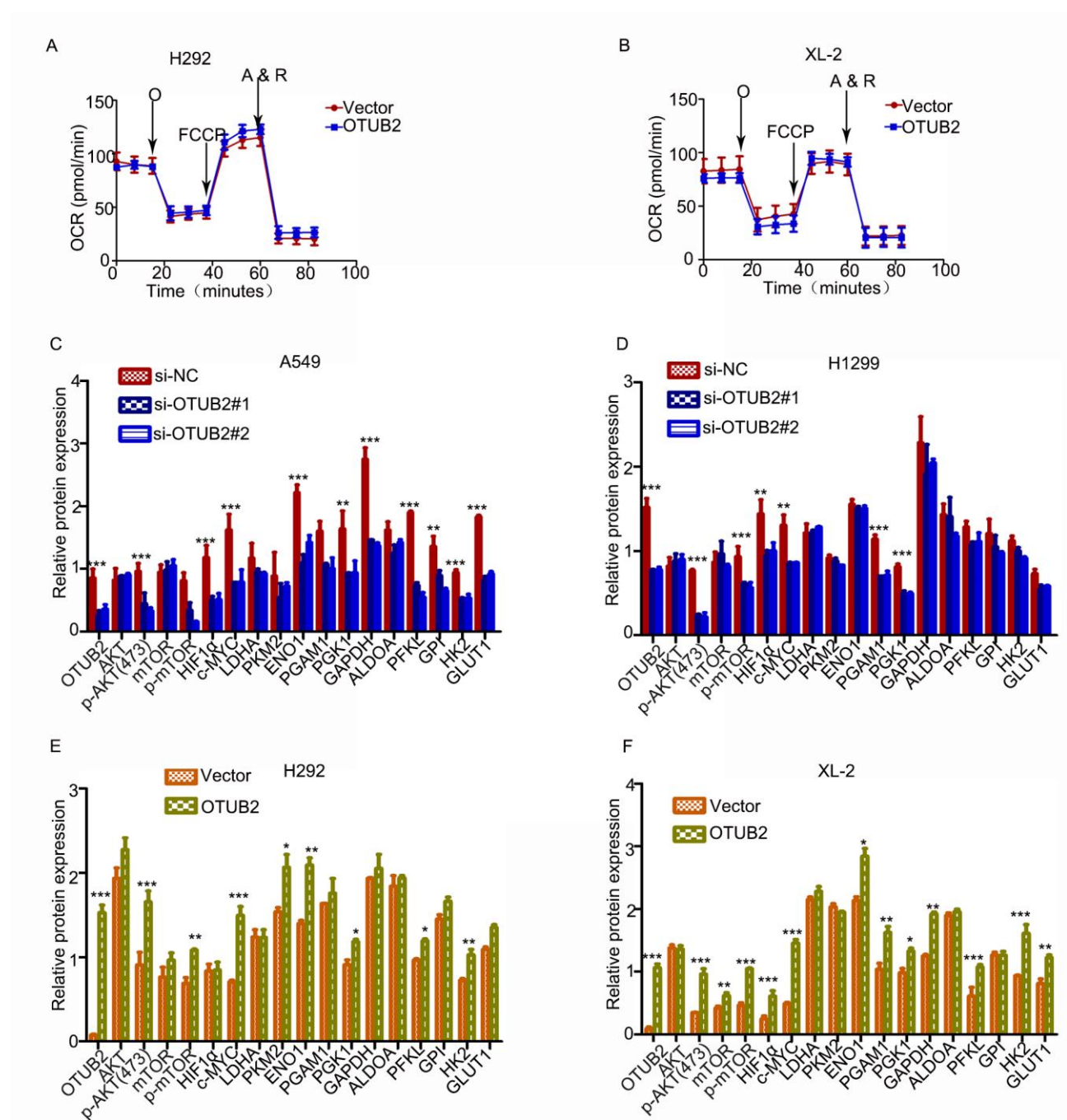
(A)The mRNA expression signature of five deubiquitinating enzymes in 83 paired of NSCLC samples with adjacent noncancerous lung tissues (GEO Submission: GSE75037). (B-E) Kaplan-Meier’s survival analysis the correlation between the expression levels of four deubiquitinating enzymes and overall survival in 117 NSCLC patients (GEO Submission: GSE13213). (F) OTUB2 mRNA expression in various human cancers as revealed by the TIMER database.

Supplementary Figure 2. OTUB2 promoted of NSCLC cell migration rates.



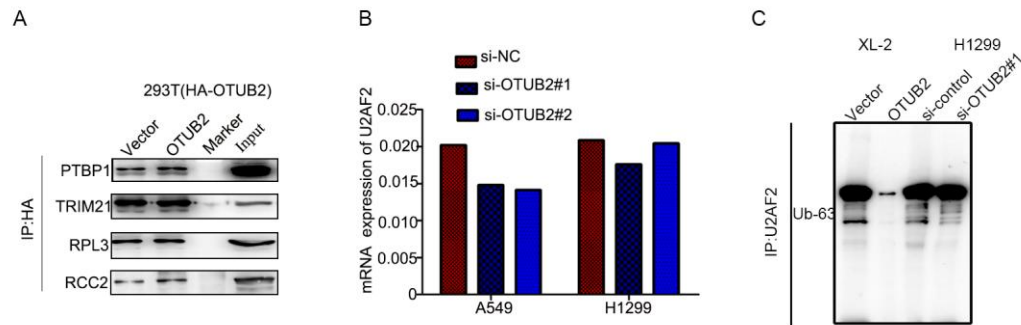
(A) Real-time PCR analyses of OTUB2 expression in H292 and XL-2 cells stable expressed HA-OTUB2, and A549 and H1299 cells transfected with two independent OTUB2 siRNAs. (B) Wound-healing assays in A549 and H1299 cells transfected with OTUB2 siRNAs. (C) Wound-healing assays in H292 and XL-2 cells stable expressed HA-OTUB2. Statistical analysis was performed using student's t-test. Error bars represent the S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Figure 3.



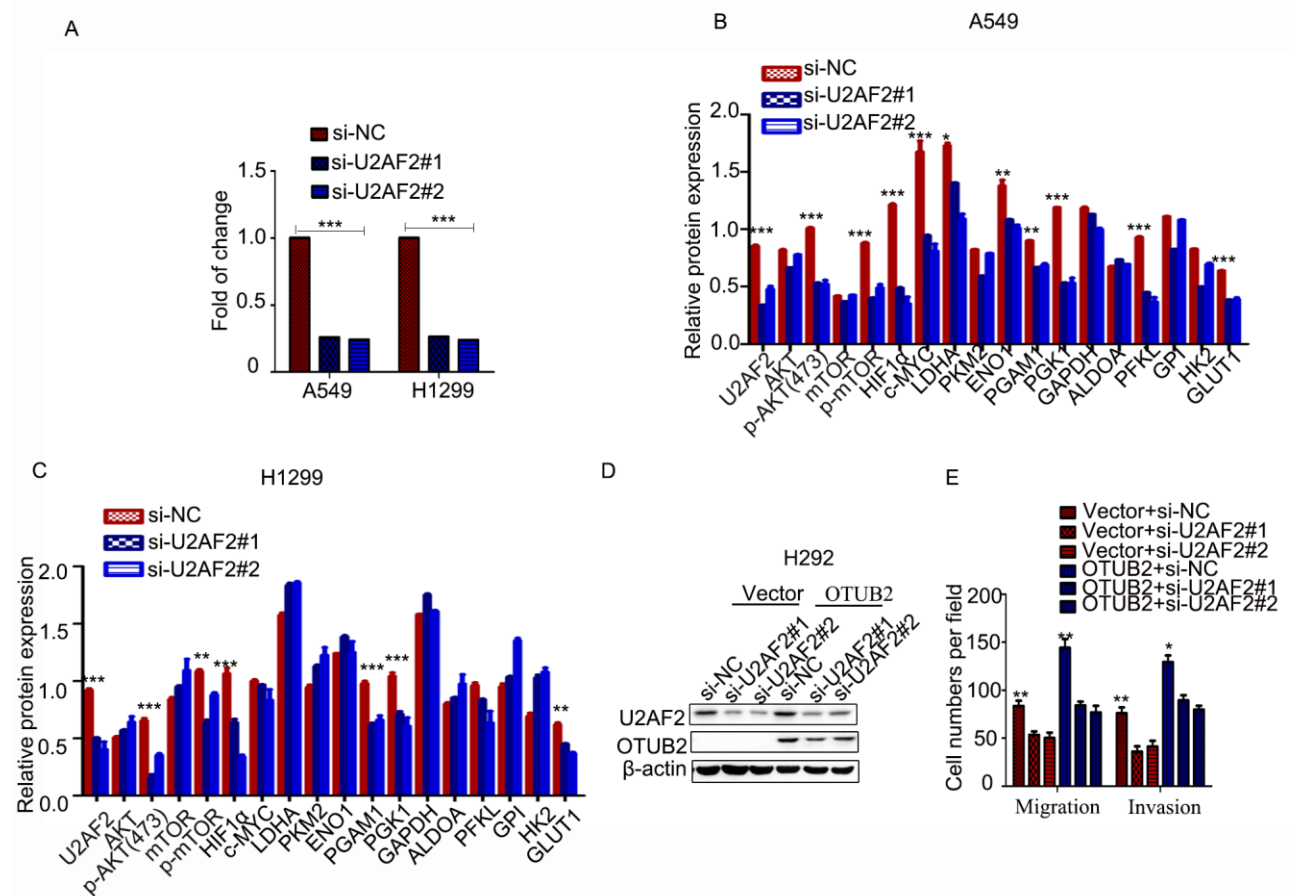
(A-B) Oxygen consumption ratio (OCR) in H292 and XL-2 cells stable expressed HA-OTUB2. O, oligomycin; F,FCCP (carbonyl cyanide 4-[trifluoromethoxy] phenylhydrazine); A & R, antimycin A and rotenone. (C-F) Intensity histogram of figure 3H.

Supplementary Figure 4. OTUB2 not change the mRNA expression of U2AF2 and K63-linked polyubiquitination levels of U2AF2.



(A) Immunoprecipitation was carried out using anti-HA antibody and then analyzed specific associations of PTBP1,TRIM21,RPL3,RCC2 with OTUB2 by western blotting in HEK293T transfected with plasmids HA-OTUB2. (B) U2AF2 mRNA levels were quantified by Real-time PCR in A549 and H1299 cells transfected with OTUB2 siRNAs. (C) A549 cells knockdown of OTUB2 and XL-2 cells overexpressed HA-OTUB2 were treated with MG132 (10 μ M) for 20h. Cell lysates were immunoprecipitated using control IgG or U2AF2 antibody, and immunoblotted for K63-ubiquitin. Statistical analysis was performed using student's t-test. Error bars represent the S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Figure 5.



(A) Real-time PCR analyses of U2AF2 mRNA expression. (B-C) Intensity histogram of Figure 5H. (D) Western blot analyses of U2AF2 and OTUB2 protein expressions. (E) Rescue assays for transwell migration and invasion assays were performed after U2AF2 silencing in H292 cells stably over-expressing OTUB2. Statistical analysis was performed using student's t-test. Error bars represent the S.E.M. * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Supplementary Figure 6. U2AF2 expression signature in GSE13213

PGK1	ATGTCGCTTTCTAACAAGCTGA	GCGGAGGTTCTCCAGCA
PGAM1	GGAAACGTGTACTGATTGCAGCCC	TTCCATGGCTTTGCGCACCCTCT
ENO1	GACTTGGCTGGCAACTCTG	GGTCATCGGGAGACTTGAA
ENO2	TCATGGTGAGTCATCGCTCAGGAG	ATGTCCGGCAAAGCGAGCTTCATC
PKM2	GCCCGTGAGGCAGAGGCTGC	TGGTGAGGACGATTATGGCCC
LDHA	ATGGCAACTCTAAAGGATCA	GCAACTTGCAGTTCGGGC
β-actin	GTCATTCCAAATATGAGATGCGT	GCATTACATAATTTACACGAAAGCA

SiRNA used in this study

OTUB2-siRNA1	ACCACATCGTGCAGTTCCT
OTUB2-siRNA2	TGTGACATTCTATCCATTC
U2AF2-siRNA1	GTGAGTACGTGGACATCAA
U2AF2-siRNA2	CCAACTACCTGAACGATGA

Supplementary Table 2 Antibodies for western blot used in this study

Name	Company	Catalog Number	Assay
OTUB2	Sangon Biotech	#D199590	WB
OTUB2	Novus	#H00078990-M14	IP
U2AF2	Proteintech	#15624	WB
U2AF2	Abcam	#ab37530	IP
PFKM	Proteintech	#55028	WB
LDHA	Proteintech	#19987	WB
HIF1A	Proteintech	#20960	WB
GLTU1	Proteintech	#55031	WB
HK2	Proteintech	#22029	WB
PGK1	Proteintech	#17811	WB
PGAM1	Proteintech	#16126	WB
PKM2	Proteintech	#15822	WB
ENO1	Proteintech	#11204	WB
GPI	Proteintech	#15171	WB
c-MYC	Proteintech	#10828	WB
ALDOA	Proteintech	#11217	WB
GAPDH	Proteintech	#60004	WB
mTOR	Cell Signaling Technology	#2983	WB
P-mTOR(ser2481)	Cell Signaling Technology	#2971	WB
AKT	Cell Signaling Technology	#4691	WB
P-AKT(ser473)	Cell Signaling Technology	#4060	WB
β-actin	Sigma-aldrich	#A3854	WB
Ubiquitin	Abcam	#EPR8589	WB
Ubiquitin,Lys48-Specific	Millipore	#05-1307	WB
Ubiquitin,Lys63-Specific	Millipore	#05-1313	WB
HA	Santa Cruz	#sc-7392	WB

Supplementary Table 3 List of possible OTUB2-interacting proteins identified by Mass Spectrometry

Protein Name	Organism Species	Gene Name	Unique Peptides	Peptides
60S ribosomal protein L3	Homo sapiens	RPL3	11	62
E3 ubiquitin-protein ligase TRIM21	Homo sapiens	TRIM21	7	27
Elongation factor 1-alpha 1	Homo sapiens	EEF1A1	7	24
tRNA-splicing ligase RtcB	Homo sapiens	RTCB	6	21
Vimentin	Homo sapiens	Vim	6	20
Polymerase delta-interacting protein 3	Homo sapiens	POLDIP3	6	10
Plasminogen activator inhibitor 1 RNA-binding protein	Homo sapiens	SERBP1	3	11
Heterogeneous nuclear ribonucleoprotein F	Homo sapiens	HNRNPF	3	8
Probable ATP-dependent RNA helicase DDX28	Homo sapiens	DDX28	3	7
Polypyrimidine tract-binding protein 1	Homo sapiens	PTBP1	3	6
Eukaryotic initiation factor 4A-III	Homo sapiens	EIF4A3	3	5
RCC2 protein	Homo sapiens	RCC2	2	5
Splicing factor U2AF 65 kDa	Homo sapiens	U2AF2	2	4
Pre-mRNA-processing factor 19	Homo sapiens	PRPF19	2	3
Pyruvate kinase PKM	Homo sapiens	PKM	2	3
Zinc finger protein 211	Homo sapiens	ZNF211	1	6
Transcription factor SOX-10	Homo sapiens	SOX10	1	4
Glyceraldehyde-3-phosphate dehydrogenase	Homo sapiens	GAPDH	1	3
Nuclear receptor coactivator 5	Homo sapiens	NCOA5	1	3

Supplementary Table 4 Chi square test of the correlation between OTUB2 and U2AF2

	OTUB2-high	OTUB2-low	P
U2AF2-high	15	12	0.043
U2AF2-low	10	23	

Supplementary Table 5 Variables predictive of survival by COX proportional hazards model in NSCLC patients

Parametes	Wald χ^2	Risk Ratio	P
OTUB2	2.911	1.362	0.088
U2AF2	1.601	1.319	0.206
PGK1	1.668	1.551	0.197