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Article Title:	**The *UPF1* RNA Surveillance Gene is Commonly Mutated in Pancreatic Adenosquamous Carcinoma**
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SUPPLEMENTARY INFORMATION

The *UPF1* RNA Surveillance Gene is Commonly Mutated in Pancreatic Adenosquamous Carcinoma

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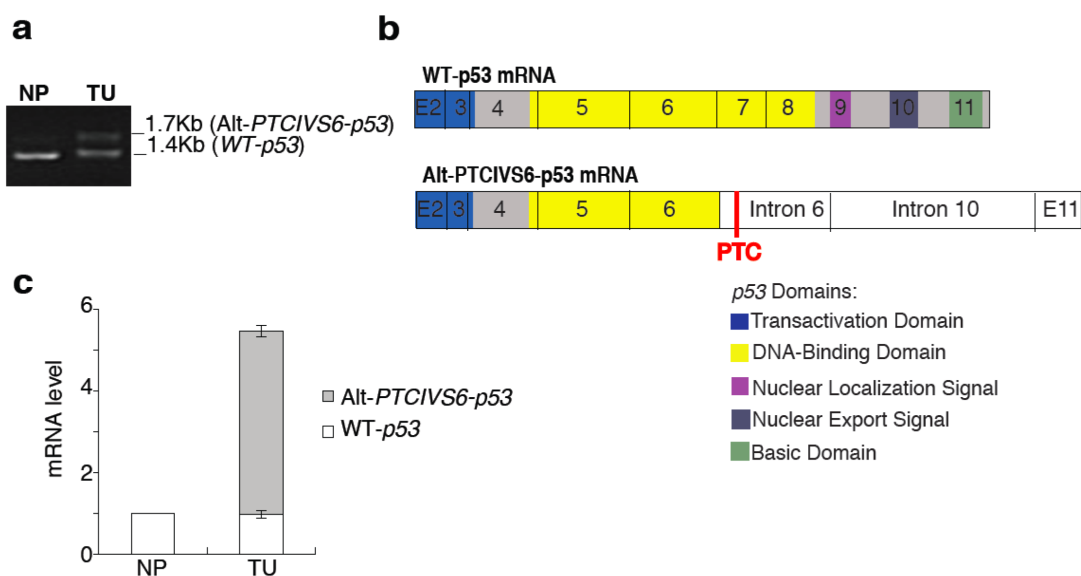

Supplementary Figure 1. An alternatively spliced p53 transcript is selectively expressed in an UPF1-deficient ASC tumor.

(**a**) RT-PCR analysis of p53 transcripts in ASC tumor (TU) tissue and the normal adjacent pancreatic tissue (NP) from patient 15 using the primer pair 5'-CTTTCCACGACGGTGACAC-3' / 5'-GCCAACTTGTTCAGTGGAGC-3'. (**b**) Schematic representation of *p53* transcripts detected in patient 15, based on sequence analysis of RT-PCR products: 1408 nt (WT-p53 mRNA) and 1692 nt (Alt-PTC-IVS6-p53). The alternative splicing event occurs at a region of sequence identity in introns 6 and 10 (tgggaggccaaggc), resulting in skipping of exons 7~10 and inclusion of parts of introns 6 and 10. The encoded p53 protein has a reading frame that ends more than 200 nucleotides into intron 6. While unusual, this type of alternative splicing has also been observed in other mRNAs, including *MDM2* transcripts[21]. (**c**) RT-qPCR analysis of p53 transcripts using the primer pair 5'-CTGCTCAGATAGCGATGGTCTGGC-3' / 5'-TTGTAGTGGATGGTGGTACAGTCAG-3' for normally spliced p53 (WT-p53) and the primer pair 5'-TGGCTCATGCCTGTAATCCCAGCAC-3' / 5'-CCTCAGCCTCCCGAGTAGGTAGCTG-3' for *alt-PTC-IVS6-p53* mRNA. Both primer pairs had comparable amplification efficiency, based on standard curve analysis. 18S mRNA was used as a normalization control. The values are mean RNA levels (± SEM) from three independent experiments relative to the level of normally spliced p53 mRNA in the NP, which was given a value of 1.

UPF1 EXON 10/intron 10/EXON 11

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GATGCAGAGCGCATTGAAAACGTTTGCCGTGGATGAGACCTCGGTGTCTGGCTACATCT
ACCACAAGCTGTTGGGCCACGAGGTGGAGGACGTAATCATCAAGTGCCAGCTGCCCAAG
CGCTTCACGGCGCAGGGCCTCCCCGACCTCAACCACTCCCAG/gtgcgcgccgtcctca
gcgcgcggggcctcgcccatgggccgggacgcaagcggaggctgcccctaacggccgct
tgtattgaag/GTTTATGCCGTGAAGACTGTGCTGCAAAGACCACTGAGCCTGATCCAG
GGCCCGCCAGGCACGGGGAAGACGGTGACGTCGGCCACCATCGTCTACCACCTGGCCCG
GCAAGGCAACGG
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UPF1 EXON 21/intron 21/EXON 22/intron 22/EXON 23

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GCCGGCCTTCCAGCATGTACTTCCAGACCCATGACCAGATTGGCATGATCAGTGCCGGC
CCTAGCCACGTGGCTGCCATGAACATTCCCATCCCCTTCAACCTGGTCATGCCACCCAT
GCCACCGCCTGGCTATTTTGGACAAGCCAACGGGCCTGCTGCAG/gtgagcatctgtgg
ctgcggctgggtgtggccctcctgagagctcttgagggtgtgcttgtctgcgaggccct
ggcctccttcggatcaccctggactgctgtctttcag/GGCGAGGCACCCCGAAAGGCA
AGACTGGTCGTGGGGACGCCAGAAGAACCGCTTTGGGCTTCCTGGACCCAGCCAGACT
AACCTCCCCAACAGCCAAGCCAGCCAGGATGTGGCGTCACAGCCCTTCTCTCAGGGCGC
CCTGACGCAGGGCTACATCTCCATGAGCCAGCCTTCCCAGATGAGCCAGCCCGGCCTCT
CCCAGCCGGAGCTGTCCCAG/gtgagcccgcccctgggacgggacttacctgagtgagg
gtggggctatgcacctgaaacattccctctgaagagccccagagagctggcctggccca
tgtccactgtctgaattacctgtccctgggctggggtcatcagagtgggtctcctgggt
cttagtttggggacgggttttccattcttttctctggggctgctgagggctgggtggat
gtgagcacccttggcctgtggcttgcttacctcctgaccttgtctttcag/GACAGTTA
CCTTGGTGACGAGTTTAAATCACAAATCGACGTGGCGCTCTCACAGGACTCCACGTACC
AGGGAGAGCGGGCTTACCAGCATGGCGGGGTGACGGGGCTGTCCCAGTATTAAAAG
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Legend:

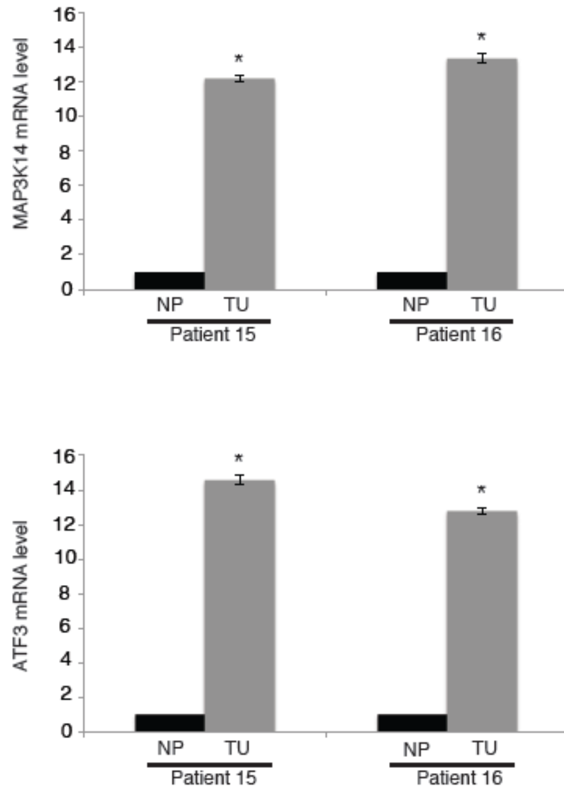
EXON / intron

Mutations

Exon/intron Splicing Enhancers (SE)

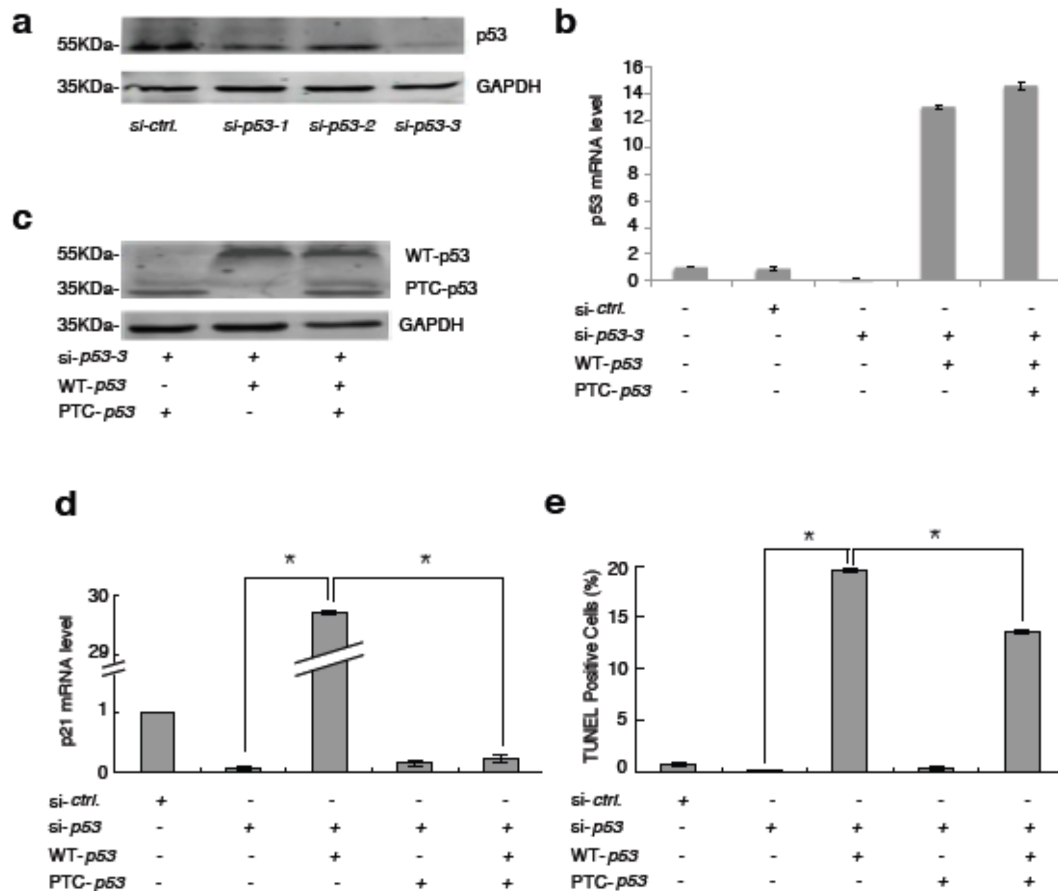
Supplementary Figure 2. Many *UPF1* mutations in ASC tumors are within or proximal to known splicing *cis* elements.

The program ESE Finder, which predicts binding sites for 4 of the >10 known ESE/ISE-binding proteins (SRSF1, SRSF2, SRSF5, SRSF6)[9,22], was used to locate several predicted ESEs and ISEs in the regions of the *UPF1* gene mutated in ASC tumors. Four of the 19 mutations in the exon 10-intron 10-exon 10 region were within predictable ESEs/ISEs. Several of the other mutations in the exon 10-intron 10-exon 10 region were closely adjacent to predicted ESEs and ISE core sequences (6 of the mutations were within 3 nt of these sequences). These ESE/ISE core-proximal mutations could disrupt intron 10 splicing, particularly since this intron is very small (85 nt) and has a high GC content (73%), which is likely to make its splicing particularly sensitive to modest alterations in pro-splicing signals in the adjacent exons[9,23].



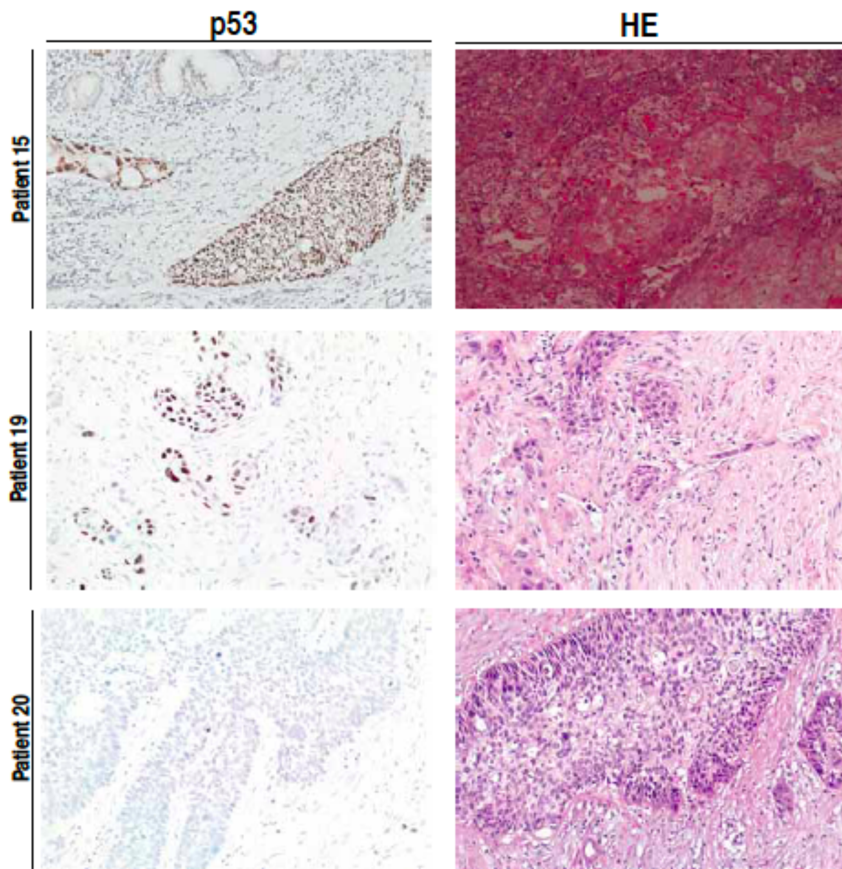
Supplementary Figure 3. Endogenous NMD substrates are dramatically upregulated in ASC tumors.

We and others have shown that one consequence of disruption of *UPF1* is the upregulation of dominant-negative forms of tumor suppressor genes[7,24]. UPF1 has also been shown to post-transcriptionally regulate, through NMD, several other mRNAs encoding proteins that can potentially influence tumor growth, including those involved in stress regulation[16] and cell differentiation[25]. Indeed, as shown in this figure, RT-qPCR expression-analysis of frozen tumor (TU) and normal adjacent pancreas (NP) from two ASC patients demonstrated that the NMD substrates, ATF3 and MAP3K14 mRNA, are dramatically upregulated in ASC tumors. The values shown are the average fold change (mean ± SEM) in TU tissue relative to NP (the former was given a value of 1) from three independent experiments. 18S mRNA was used as the endogenous control. Statistical analysis was performed using the Student's t test; $*P < 0.05$.



Supplementary Figure 4. Evidence that the *alt-PTC-IVS6-p53* mRNA encodes a dominant-negative p53 protein.

(**a**) Western blot analysis of PANC-1 cells transfected with 3 independent p53 siRNAs and a negative control siRNA (si-ctrl). 80 nmoles of siRNAs was transfected. See **Supplementary table 7** for siRNA sequences. (**b**) RT-qPCR analysis of *p53* mRNA levels in PANC-1 cells, treated as indicated in the panel. (**c**) Western blot analysis of p53 protein levels in PANC-1 cells, treated as indicated in the panel. (**d**) RT-qPCR analysis of *p21* mRNA expression in PANC-1 cells transfected as indicated in the panel. PTC-p53 is a plasmid expressing the protein encoded by the *Alt-PTC-IVS6-p53* mRNA. (**e**) TUNEL analysis of PANC-1 cells transfected as in panel d. Values in panels **b**, **d**, and **e** are the average fold change (mean ± SEM) from three independent experiments relative to the control, which was given a value of 1. 18S mRNA was used as the endogenous control. Statistical analysis was performed using the Student's t test; $*P < 0.05$.



Supplementary Figure 5. p53 immunohistochemical analysis.

Immunohistochemical analysis of two patient tumors exhibiting strong p53 nuclear staining (from patients 15 and 19) and a patient lacking detectable p53 (patient 20). **Supplementary table 1** provides a summary of all patient tumors evaluated for p53 status.

Supplementary Table 1. Summary of 23 ASC patients

Supplementary Table 1	UPF1 mutations	Confirmed UPF1 mutations	UPF1 IHC (Tu level compared to NT)	KRAS mutation	p53 IHC	UPF2	UPF3A	UPF3B	Sample: FFPE vs Frozen tissue	Sample origin	Clinical info: sex, age of diag.
Patient 1	IVS10+31G>A	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	41-year-old man
Patient 2	c.1401C>T IVS10+21G>A IVS10+32C>T IVS10-34C>T c.1463G>T	no residual sample	~5%	Not Tested	Not Tested	WT	WT	WT	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	73-year-old female
Patient 3	c.1378C>T IVS10-26G>A IVS10-22C>T	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	54-year-old female
Patient 4	c.1302G>A IVS10+31G>A IVS10-24C>A	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	57-year-old female
Patient 5	c.1401C>T IVS10+32C>T IVS10-34C>T c.1463G>T	c.1401C>T IVS10+32C>T IVS10-34C>T c.1463G>T	~5%	WT	P	WT	WT	WT	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	56-year-old female
Patient 6	IVS10+7G>A	IVS10+7G>A	~5%	G12D (G35A)	P	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	68-year-old man
Patient 7	c.1408G>T IVS10-25C>T	no residual sample	~5%	Not Tested	Not Tested	WT	WT	WT	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	75-year-old man
Patient 8	c.1401C>T IVS10+31G>A IVS10+32C>T IVS10-34C>T c.1463G>T	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	50-year-old man
Patient 9	IVS10-17G>A	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	72-year-old man
Patient 10	c.1411C>T IVS10-13G>A	c.1426 G>A	~5%	G12D (G35A)	P	WT	WT	WT	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	47-year-old female
Patient 11	IVS22+48C>T c.3300G >A	IVS22+48C>T	~5%	WT	P	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	54-year-old man
Patient 12	IVS22-83C>T c.3325C>T	no residual sample	~5%	G12D (G35A)	P	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	56-year-old man
Patient 13	c.2916C>T IVS21-44A>G	no residual sample	~5%	Not Tested	Not Tested	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	67-year-old female
Patient 14	c.2928G>A IVS22+128C>T	no residual sample	~5%	G12V (G35T)	P	Not Tested	Not Tested	Not Tested	FFPE	Zhongshan Hospital, Fudan University, Shanghai,China	61-year-old man
Patient 15	c.3239A>T	c.3239A>T	~5%	G12V (G35T)	P (Fig. S2)	WT	WT	WT	Frozen tissue	Changhai Hospital, Second Military Medical University, Shanghai China.	57-year-old man
Patient 16	IVS22+8 C>T	IVS22+8 C>T	~5%	G12D (G35A)	Not Tested	Not Tested	Not Tested	Not Tested	Frozen tissue	Changhai Hospital, Second Military Medical University, Shanghai China.	47-year-old female
Patient 17	WT	Not Tested	~85%	WT	Not Tested	Not Tested	Not Tested	Not Tested	Frozen tissue	Changhai Hospital, Second Military Medical University, Shanghai China.	70-year-old female
Patient 18	WT	Not Tested	~63%	G12V (G35T)	Not Tested	Not Tested	Not Tested	Not Tested	Frozen tissue	Changhai Hospital, Second Military Medical University, Shanghai China.	62-year-old man
Patient 19	c.3256G>A	Not Tested	Not Tested	WT	P (Fig. S2)	Not Tested	Not Tested	Not Tested	FFPE	MD Anderson Cancer Center, Houston, TX, U.S.A.	56-year-old man
Patient 20	WT	Not Tested	Not Tested	WT	Neg. (Fig. S2)	Not Tested	Not Tested	Not Tested	FFPE	MD Anderson Cancer Center, Houston, TX, U.S.A.	48-year-old female
Patient 21	WT	Not Tested	Not Tested	g.-9C>T	Neg.	Not Tested	Not Tested	Not Tested	FFPE	MD Anderson Cancer Center, Houston, TX, U.S.A.	59-year-old female
Patient 22	c.1413C>A IVS10-40C>T	c.1413C>A IVS10-40C>T	Not Tested	G12V (G35T)	Neg.	Not Tested	Not Tested	Not Tested	FFPE	MD Anderson Cancer Center, Houston, TX, U.S.A.	73-year-old female
Patient 23	WT	Not Tested	Not Tested	WT	Neg.	Not Tested	Not Tested	Not Tested	FFPE	MD Anderson Cancer Center, Houston, TX, U.S.A.	77-year-old female

Supplementary Table 2. Genomic *UPF1* mutations in pancreatic and lung tumors

Tumor	Mutations No. of patients/total
Pancreas	
Adenosquamous carcinoma	18/23 (78%)
Ductal adenocarcinoma	0/24
Solid pseudopapillary neoplasm	0/3
Neuroendocrine carcinoma	0/2
Lung	
Squamous carcinoma	0/21

Supplementary Table 3. Primers used for *UPF1* DNA sequencing					
Target	Sequence		Target	Sequence	
UPF1 Exon1	f	GCAGTTCCTGCTCTAGGCTG	UPF1 Exon12-13	f	AGCCCAGGATGTTGAGGCTG
	r	GATTGCAGGACCTGGGGCAG		r	TTCCAGGCAGCGAGATGTCC
	F	GCAGTTCCTGCTCTAGGCTG		F	TCACACCACTGCACTCCAGC
	R	TAAGCTCAGGCCGAGCCAGAG		R	ACAACTGAGCGCAGAGAGGC
UPF1 Exon2	f	GGCATGGACATGGCTCTGTG	UPF1 Exon14-15	f	TCACCACAGCCTGGACCATG
	r	TCAGGGTGTCGGAGGTTGCT		r	TGCCCTGACCTCAAGCAGTG
	F	GGTTAGACCAGCTGTGTGGG		F	TGTCTGGGAGGGACAGCTTG
	R	CACCTCCTGACCGGTAACAG		R	GAAGCTGATGGGCCAGTTCC
UPF1 Exon3	f	CAGCAGGACTCTCCTTGGAG	UPF1 Exon16-17	f	AGGACCTGCAGCACTGTAGC
	r	AGATCAGCGGGTGTGCCAAG		r	TCCTGGGCCATTCTGAGCTC
	F	TGCTCCTTATCCCCTCGGAG		F	TTGCCCTGTGTCTGAACTCA
	R	ACCCAGGTTTGTTAGCGTG		R	AGGAGACCAAGTGTGCCCGAG
UPF1 Exon4	f	GGATGAGGTGTGACTGCCTC	UPF1 Exon18	f	TCTAGCCTGGGTGACAGAGC
	r	AACGCTCTCATGCCTCACCC		r	CTGCTCAGAGTCCTCTGACC
	F	ATGGACCGTGAACGGTACCG		F	GCCTGCTGGCTGATAGTGAC
	R	TGGAGGACAACTCCCTGGAG		R	GGAAGTGAGGACCGATGAGC
UPF1 Exon5	f	CAGAGCTCAAGTGCACAGGG	UPF1 Exon19-20	f	GTGTGCAGGGTCAGTGGCTTG
	r	TCTCACTGGGTCAAGCCGTC		r	AAGCTGCAGCCATGGGAACG
	F	ATGCAGGGCATGCCCCTTTG		F	TCTTGGACCGTCCTGTGAGA
	R	TCCCCGCAACCAGAAACCAC		R	ATCCCGGTGTGAGGACAAGG

UPF1 Exon6	f	CCTGTGTGGCATGGAGTTCC	UPF1 Exon21-23	f	ATCTTCAGCCTGGGCAGAGC
	r	ACATCAGCTCCCACAGCCTG		r	ATACCACCCCTCCAGTGCAG
	F	AGGGTTCTCCTTGCAGGTGG		F	CAGGACAGATGTGCAGCTCC
	R	CTCTGGAAGCCTGAGGAGAG		R	CCCATCCTTCTCCTTGAGGC
UPF1 Exon7-8	f	ACGGCAGAGGCTTGCTGTAG	UPF1 Intron23	f	ATCGACGTGGCGCTCTCACAG
	r	AAGAGCAGTGGAGGGCGATG		r	CCTGTTGCTCACACAGTGGTG
	F	CTGTGCTGGAGGCTAACC		F	ACTCCACGTACCAGGGAGAG
	R	AGCATGAGATGCTGGCCCAG		R	CGTTGCTTAGCTCTTCCGCC
UPF1 Exon9	f	CGGCTCATGGTGAGGTAGAG	UPF1 Exon24	f	GTACCTGTGGGGCTCAGGTC
	r	GAACCCCACTCCACAGTGAC		r	GGGTTTGTCAAGGCTGCTGC
	F	AGCGTTTGGTGCAGAGCCAG		F	TGCCCTTCTCCCTCCTGACAG
	R	AGATGCCACAGGTGGCAACC		R	TACCCGGTGCATGCCTCTAC
UPF1 Exon10-11	f	GTCACTGTGGAGTGGGGTTC			
	r	AGAGAGCGGTAGGCACCATG			
	F	ACTCAGGATGTCGGAGAGGC			
	R	CACGTGCTCTCTTCGGTGTG			

Supplementary Table 4. RT-qPCR primers		
Target	Sequence	
18s (Endogenous Control)	REALF REALR	AGTCTAGAGCCACCGTCCAG CCTACCTAGAATGTGGCTG
WT p53 mRNAWT-p53	Realfex5 Realrex7	CTGCTCAGATAGCGATGGTCTGGC TTGTAGTGGATGGTGGTACA GTCA G
Alt-PTCIVS6-p53 mRNA	Realfin6 Realrin10	TGGCTCATGCCTGTAATCCCAGCA C CCTCAGCCTCCCGAGTA GGTA GCTG
p21	REALF	AGGTGGACCTGGAGACTCTCAGGGTC
	REALR	AAGGCAGAAGATGTAGAGCGGGCC

Supplementary Table 5. Primers used for *UPF1* mini-gene construction and analysis

Target	Sequence	
Minigene Construct E10-11	F	GGAATTCCATATGCGTCTTCTCCCATCACTGCC
	R	GGAATTCCATATGACACGGAACTCACGCTGCTG
Minigene Construct E21-23	F	GGAATTCCATATGTTCCCAGCAGACTCTCCTCG
	R	GGAATTCCATATGCCTCCCCGACTCTGATGAGG
Minigene Expression Analysis	F	CAACTTCAAGCTCCTAAGCCACTGC
	R	TAGGATCCGGTCACCAGGAAGTTGGTTAAATCA

Supplementary Table 6. Primers used for site-direct mutagenesis of the *UPF1* hybrid mini-gene

Mutation site		Sequence
IVS10+31G>A	F	CGCGGGGCCTCACCCATGGGCCG
	R	CGGCCCATGGGTGAGGCCCCGCG
c.1401C>T	F	ACGGCGCAGGGTCTCCCCGACCT
	R	AGGTCGGGGAGACCCTGCGCCGT
IVS10+21G>A	F	GCGAGGCCCCGTGCGCTGAGGAC
	R	GTCCTCAGCGCACGGGGCCTCGC
IVS10+32C>T	F	GCGGGGCCTCGTCCATGGGCCGG
	R	CCGGCCCATGGACGAGGCCCCGC
IVS10-34C>T	F	CGGGACGCAAGTGGAGGCTGCCC
	R	GGGCAGCCTCCACTTGCGTCCCG
c.1463G>T	F	CTGTGCTGCAAAGACCACTGATCCTGATCCAGG
	R	CCTGGATCAGGATCAGTGGTCTTTGCAGCACAG
c.1378C>T	F	TCAAGTGCCAGCTGTCCAAGCGCTTCACG
	R	CGTGAAGCGCTTGGACAGCTGGCACTTGA
IVS10-26G>A	F	GCAAGCGGAGGCTACCCTTAACGGCCGCTTG
IVS10-22C>T	R	CAAGCGGCCGTTAAGGGTAGCCTCCGCTTGC
c.1302G>A	F	GAAAACGTTTGCCGTGGATGAAACCTCGGTGTC
	R	GACACCGAGGTTTCATCCACGGCAAACGTTTTC
IVS10-24C>A	F	CGGCCGTTAGGTGCAGCCTCCGC

	R	GCGGAGGCTGCACCTAACGGCCG
IVS10+7G>A	F	GCTGAGGACGGTGCGCACCTGGG
	R	CCCAGGTGCGCACCGTCCTCAGC
c.1408G>T	F	GCAGGGCCTCCCCTACCTCAACCACTC
	R	GAGTGGTTGAGGTAGGGGAGGCCCTGC
IVS10-25C>T	F	AAGCGGAGGCTGTCCCTAACGGCCG
	R	CGGCCGTTAGGGACAGCCTCCGCTT
IVS10-17G>A	F	GAGGCTGCCCCTAACAGCCGCTTGTATTG
	R	CAATACAAGCGGCTGTTAGGGGCAGCCTC
c.1411C>T	F	GGCCTCCCCGACTTCAACCACTCCC
	R	GGGAGTGGTTGAAGTCGGGGAGGCC
IVS10-13G>A	F	GCTGCCCCTAACGGCCACTTGTATTGAAGGTTTA
	R	GCTGCCCCTAACGGCCACTTGTATTGAAGGTTTA
IVS22+48C>T	F	GAGTGAGGGTGGGGCTACGCACCTGAAACATTC
	R	GAGTGAGGGTGGGGCTACGCACCTGAAACATTC
c.3300G >A	F	TCACAGGACTCCACATACCAGGGAGAGCG
	R	CGCTCTCCCTGGTATGTGGAGTCCTGTGA
IVS22-83C>T	F	GGACGGGTTTTCCATTTTTTTCTCTGGGGCTGC
	R	GCAGCCCCAGAGAAAAAAATGGAAAACCCGTCC
c.3325C>T	F	GCGGGCTTACCAGTATGGCGGGGTGAC
	R	GTCACCCCGCCATACTGGTAAGCCCGC
c.2916C>T	F	TGATCAGTGCCGGTCCTAGCCACGTGG

	R	CCACGTGGCTAGGACCGGCACTGATCA
IVS21-44A>G	F	TGCTTGTCTGCGGGGCCCTGGCCTC
	R	GAGGCCAGGGCCCCGCAGACAAGCA
c.2928G>A	F	GGCCCTAGCCACGTAGCTGCCATGAAC
	R	GTTCATGGCAGCTACGTGGCTAGGGCC
IVS22+128C>T	F	TACCTGTCCCTGGGTTGGGGTCATCAGAG
	R	CTCTGATGACCCCAACCCAGGGACAGGTA
c.3239A>T	F	GGAGCTGTCCCAGGTCAGTTACCTTGGTG
	R	CACCAAGGTAACTGACCTGGGACAGCTCC

Supplementary Table 7. p53 siRNAs	
	Sequence
Si - h-*p53*_001	GGACAUACCAGCUUAGAUU dTdT
Si - h-*p53*_002	GCACAGAGGAAGAGAAUCU dTdT
Si - h-*p53*_003	GACUCCAGUGGUAAUCUAC dTdT

Methods

Subjects

We evaluated a set of tumors and corresponding normal tissues from 23 patients with Adenosquamous Carcinoma (ASC) of the pancreas (**Supplementary Table 1**), as well as tumors from 24 patients with ductal adenocarcinoma, 3 patients with solid pseudopapillary neoplasm, 2 patients with neuroendocrine carcinoma, and 21 lung squamous cell carcinoma (**Supplementary Table 2**). Of the 23 ASC patient specimens, 19 were formalin-fixed paraffin-embedded (FFPE) tissue sections provided by the Department of Pathology, Zhongshan Hospital, Shanghai, China and the University of Texas M. D. Anderson Cancer Center, Houston, TX, USA. The other 4 ASC patient specimens were frozen immediately after collection (during routine clinical protocols) from The Third General Surgery Department, Changhai Hospital, Shanghai. All patients were provided written informed consent, and the approval for the study was provided by the institutional ethics review board (IRB) of the University of Texas M. D. Anderson Cancer Center, the University of California San Diego, the Second Military Medical University, Fudan University, and Tongji University School of Medicine, Shanghai, China.

DNA/RNA sequencing and analysis

Genomic DNA from the frozen samples was extracted using the DNAeasy Blood & Tissue Kit (Qiagen). Genomic DNA from the FFPE samples were extracted using QIAamp DNA FFPE Tissue Kit (Qiagen). All *UPF1* exons and flanking intron sequences were amplified by nested PCR using the primers described in **Supplementary Table 3**.

Total RNA from the patient's samples and cell lines was extracted using Trizol (Invitrogen). To prevent contamination with genomic DNA, the samples were treated with RNase-free DNase I (Fermentas). The synthesis of complementary DNA (cDNA) was performed using the PrimeScriptTM RT

reagent Kit (Takara). Quantitative real-time reverse transcription PCR (RT-qPCR) analysis was performed using the relative quantification method ($\Delta\Delta C_T$) in a RotorGene RG-3000 thermal cycler systhem (Corbett Research). 18S mRNA was used as the endogenous controls. Primers are described in **Supplementary Table 4**. All samples were evaluated in triplicate.

Constructs, mutagenesis and analysis of UPF1 minigenes

Wild-type genomic sequences from human *UPF1* (nt 22,779-23,570 and nt 33,138-34,490 [RefSeq accession number NC_000019.9]) were amplified with the primers described in **Supplementary Table 5**. The PCR products were cloned into the *Nde*I site of the vector pTBNde(min), a gift from Dr. Francisco E. Baralle (International Centre for Genetic Engineering and Biotechnology, Italy). Point mutations were introduced by site-direct mutagenesis using the KOD-Plus-Neo kit (Toyobo Inc.) and the *Dpn*I enzyme (Takara Inc.). The primer sequences used for site-directed mutagenesis are listed in **Supplementary Table 6**. Mini-genes RT-PCR analysis was performed using the primers described in **Supplementary Table 5**.

Cell culture and transfections

Human PANC-1 (pancreatic ductal carcinoma) cells and human HEK293 (embryonic kidney) cells were maintained in DMEM supplemented with 10% FBS (Thermo) at 37°C under 5% CO2. The cells were transfected using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) and harvested for RNA extraction 48 h after transfection. The small interfering RNAs (siRNAs) transfected to deplete p53 are described in **Supplementary Figure 7**.

Protein analysis

Immunohistochemical analysis was performed on paraffin-embedded sections, as previously described[26]. Sections were deparaffinized two times in xylene, followed by serial dilutions of ethanol. After heat-induced antigen retrieval in

antigen unmasking solution (Vector Laboratory, Inc., Burlingame, USA), the internal peroxidase activity was quenched by incubation

with 3% hydroperoxide in methanol for 15 min. Sections were blocked in Avidin solution for 15 min, incubated in Biotin solution for 15 min (Vector Laboratory, Inc.), incubated in 5% bovine serum albumin and 20% goat serum in PBS for 1 h, all at room temperature. Sections were then incubated overnight at $4^{o}C$ with primary antibodies against UPF1 (Rabbit monoclonal, Abcam, ab109363) and p53 (Novocastra#NCL-L-p53-D07) at a 1:100 dilution.

TUNEL staining and analysis

The staining of apoptotic cells was carried out using the ApopTag Plus Fluorescein In Situ Apoptosis Detection Kit (Millipore, Inc.). TUNEL staining detection and quantification was performed by counting at least 1000 cells in five randomly selected high-power fields (magnification, ×200).

Statistical analysis

Pairwise comparisons were performed by two-tailed Student's *t*-test using Excel software (Microsoft). Data is expressed as mean ± SEM.

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