SUPPLEMENTARY INFORMATION

Fig S1

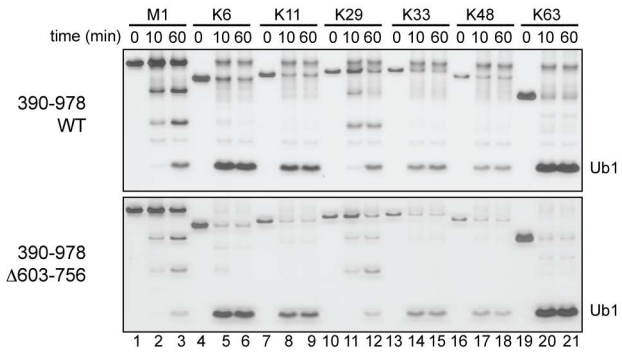

Figure S1. USP35 insertion loop is dispensable for the catalytic activity.
Wild-type and Δ603-756 human USP35 fragment spanning residues 390-978 that includes the catalytic domain was expressed in *E. coli* and purified by chromatographic means. Purified DUB fragments (375 nM) were incubated with tetraubiquitins of defined linkage types (733 nM) at 37°C for the indicated times. Reactions were resolved by SDS-PAGE and results visualised by silver staining of the gel.

Fig S2

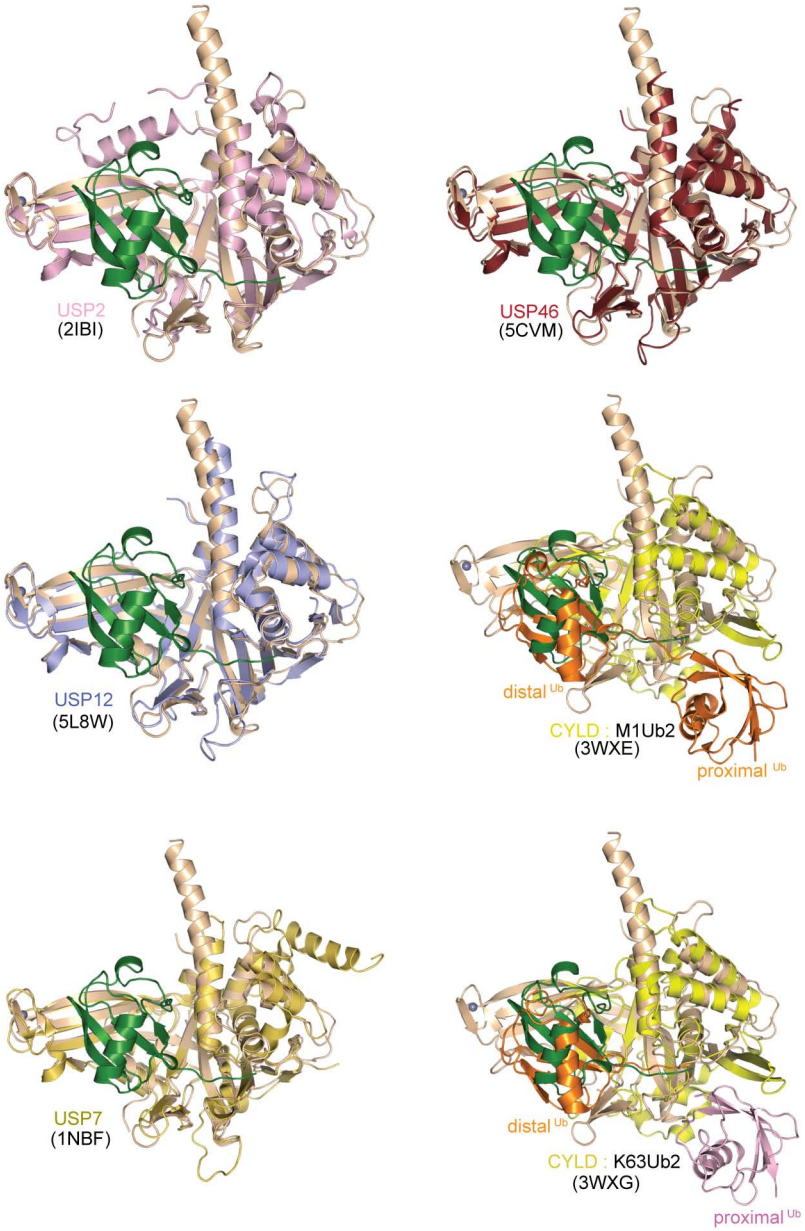

Figure S2. USP35 catalytic domain closely resembles other USP domains.
Structural alignment of USP35 with other USP family DUBs was performed with USP35 fragment shown in wheat and ubiquitin in green. High degree of similarity between USP35 and other DUBs is apparent with the most pronounced difference for CYLD alignment in the region of central helix and finger subdomain. USP2 2IBI (RMSD 1.2 Å), USP7 1NBF (RMSD 2.2 Å), USP12 5L8W (RMSD 1.9 Å), USP46 5CVM (RMSD 1.8 Å), CYLD in complex with linear (3WXE, RMSD 2.41 Å) or Lys63-linked (3WXG, RMSD 2.37 Å) diubiquitin. Distal and proximal ubiquitins of CYLD-diubiquitin complex are shown.

Fig S3

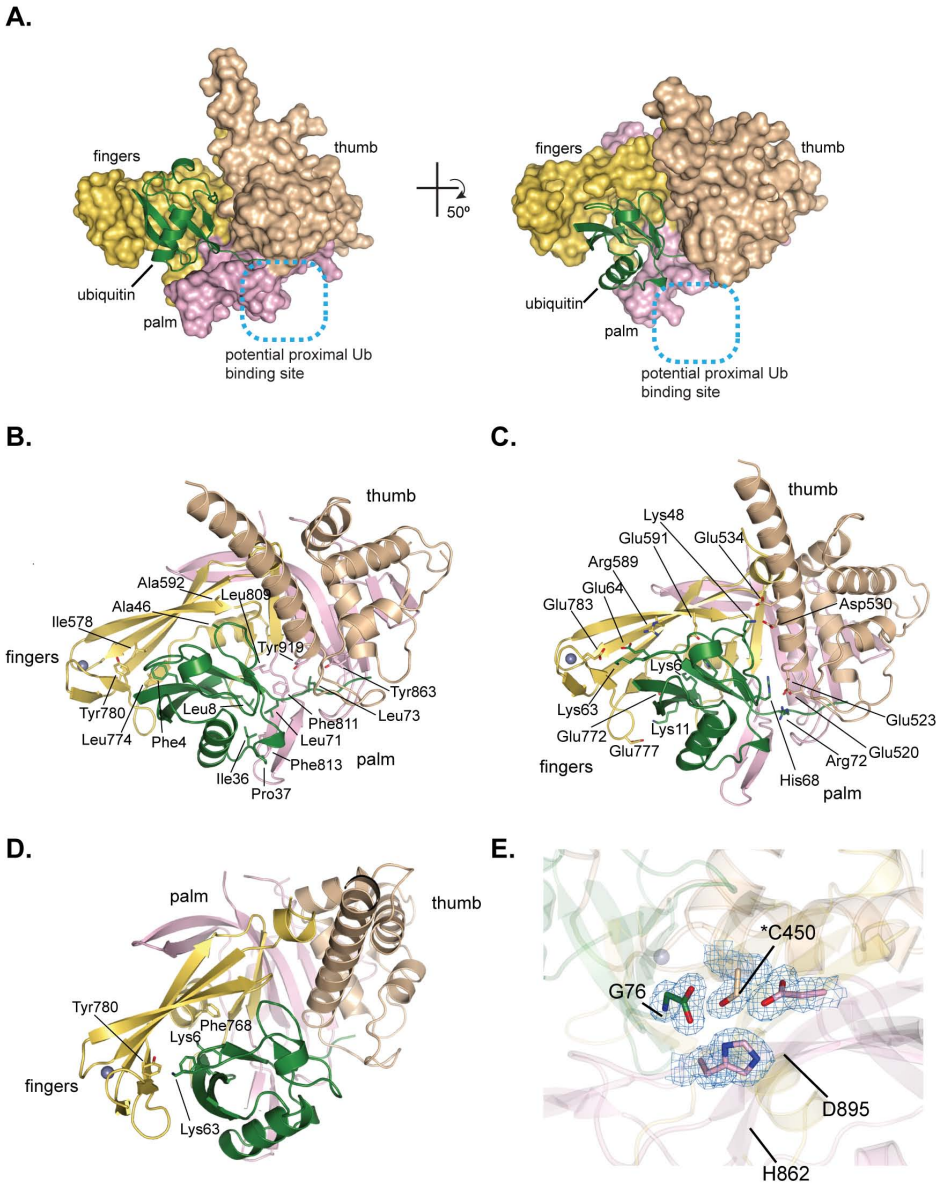

Figure S3. Polyubiquitin recognition by USP35 catalytic domain.

A. Surface representation of USP35 is shown with the distal ubiquitin in cartoon representation (green). Position of the proximal ubiquitin binding site was determined by superposition with the CYLD K63-diubiquitin complex (PDB: 3WXG, RMSD 2.37 Å) and superposed on the distal ubiquitin of USP35 complex.

B-D. Residues involved in USP35 hydrophobic (B), ionic (C) and cation-pi (D) interactions with ubiquitin are shown.

E. USP35 catalytic residues and Gly76 of ubiquitin are shown in stick with 2Fo-Fc electron density map contoured at 1.0 σ.

Fig S4

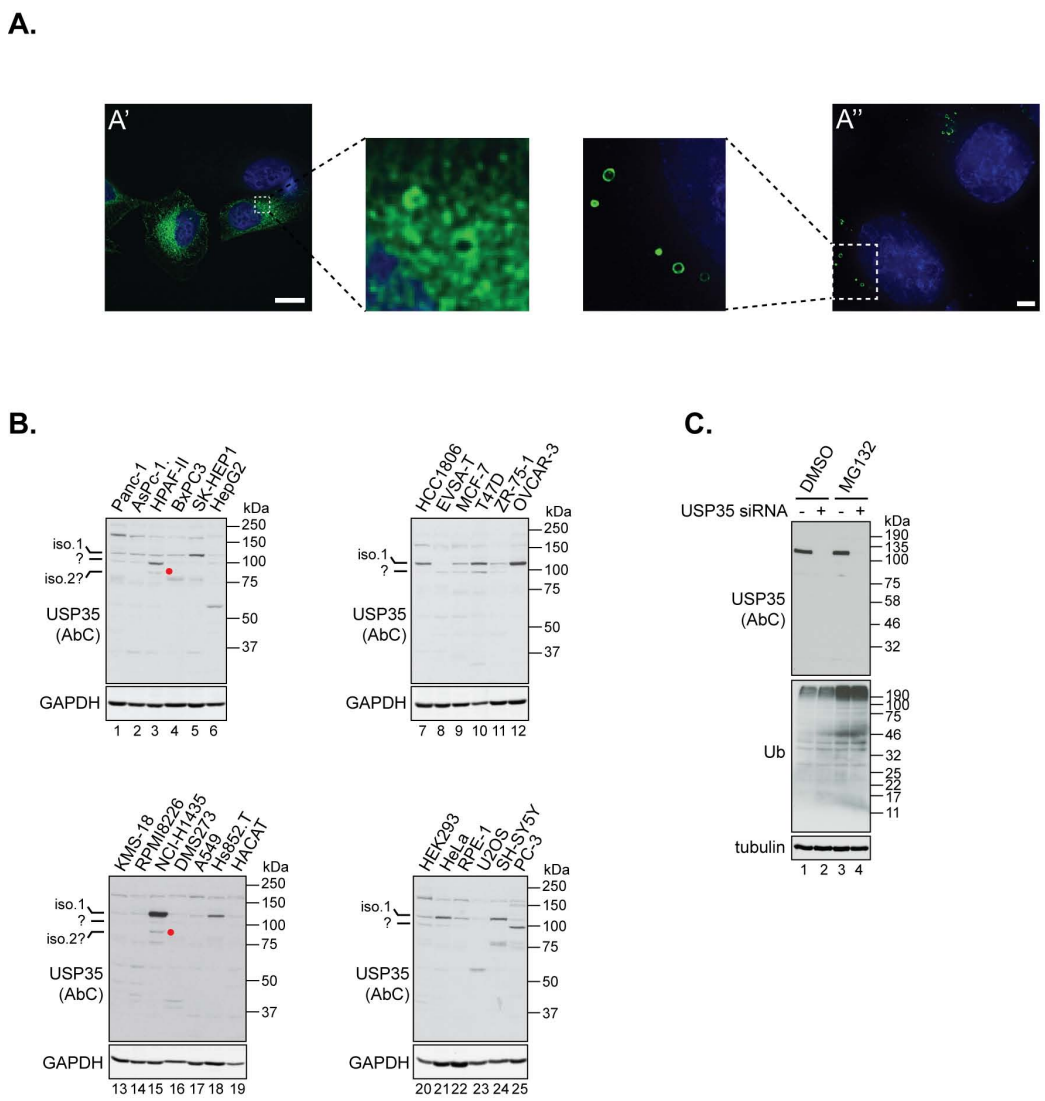

Figure S4. USP35 expression profile across cell lines.

A. Expression of USP35^{iso2} tagged with GFP at the C-terminus was induced in U2OS FlpIn cells for 8h, cells were fixed with PFA and intrinsic GFP fluorescence analysed by fluorescence microscopy. Scale bar, 15 µm (panel A') and 5 µm (panel A'').

B. Indicated cell lines were lysed, samples resolved by SDS-PAGE and immunoblotted with anti-USP35 antibody raised against its C-terminal region. Isoform 1 has been assigned based on the migration of the ectopically expressed untagged protein (not shown). Protein species potentially corresponding to USP35 isoform 2 is indicated with red, filled circle.

C. U2OS FlpIn cells were transfected with USP35 ON-TARGET plus siRNA pool (Dharmacon), incubated for 72h and lysed. Prior to lysis cells were incubated for 6h with 20 µM MG132 or DMSO as a control. Samples were resolved by SDS-PAGE and immunoblotted using indicated antibodies.

Fig S5

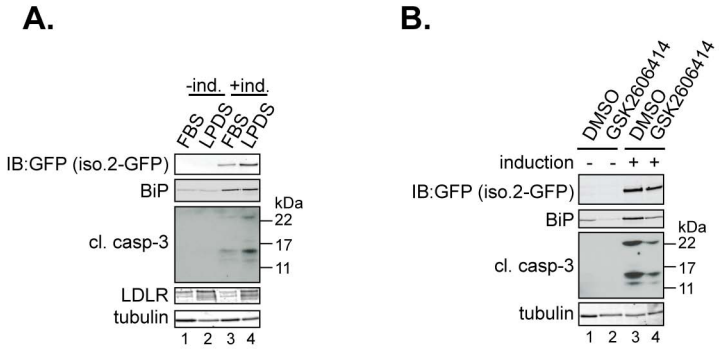

Figure S5. USP35^{iso2} causes ER stress and apoptosis.

A. U2OS FlpIn cells were grown in media supplemented either with standard foetal bovine serum (FBS) or lipoprotein-deficient serum (LPDS) supplemented with 0.5 mM mevalonolactone. Expression of USP35^{iso2} was induced for 48h, cells were lysed and samples immunoblotted with the indicated antibodies. Upregulation of LDLR was used to assess the efficiency of LPDS treatment.

B. U2OS FlpIn cells treated with 5 μM GSK2606414 or DMSO control were induced to express USP35^{iso2} for 48h. Cells were lysed and samples immunoblotted with the indicated antibodies.

Fig S6

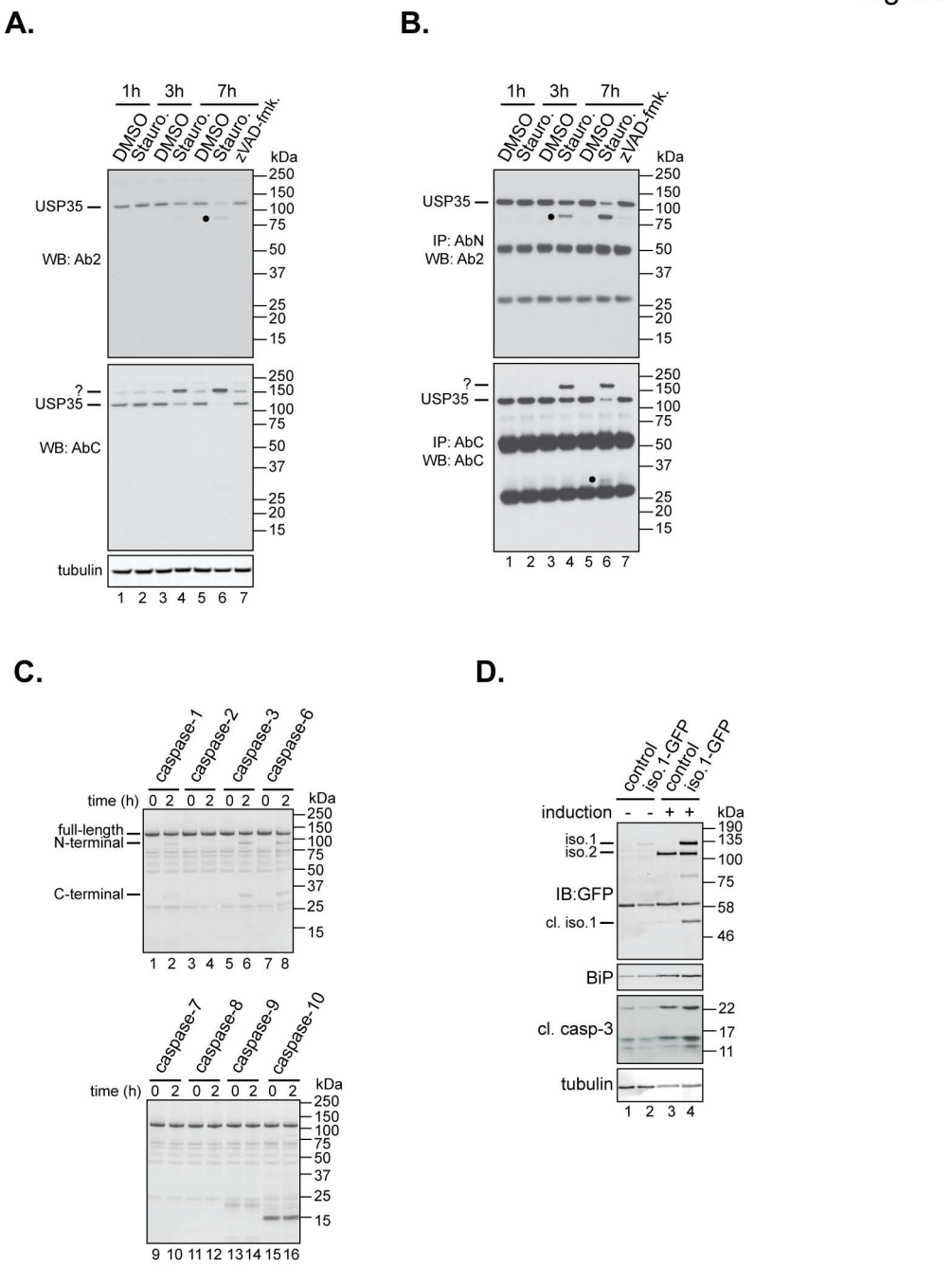

Figure S6. USP35 is cleaved by caspases during apoptosis.

A, B. HeLa cells were treated with 1 μM staurosporine or DMSO for the indicated time. Where shown 20 μM zVAD-fmk inhibitor was added together with staurosporine. Cells were lysed and total cell lysates (A) and material immunoprecipitated with antibodies raised against either the N-terminal (B, top panel, AbN) or C-terminal (B, bottom panel, AbC) portions of USP35 were resolved by SDS-PAGE. Samples were immunoblotted with anti-USP35 antibodies raised against the middle (Ab2) or C-terminal (AbC) portion of USP35. Filled dots indicate cleaved fragments of USP35.

C. Recombinant, purified $USP35^{C450A}$ (4 μg) was incubated with the indicated purified caspases (2 U) for 2h at 37°C. Samples were resolved by SDS-PAGE and stained with Coomassie Brilliant Blue.

D. U2OS FlpIn cells were transfected with an empty vector or a plasmid encoding $USP35^{iso1}$ with a C-terminal GFP tag. Expression of $USP35^{iso2}$ was induced at the same time for 48h, at which point the cells were lysed and samples immunoblotted with the indicated antibodies. cl. iso.1 indicates caspase-cleaved C-terminal fragment of $USP35^{iso1}$.

Fig S7

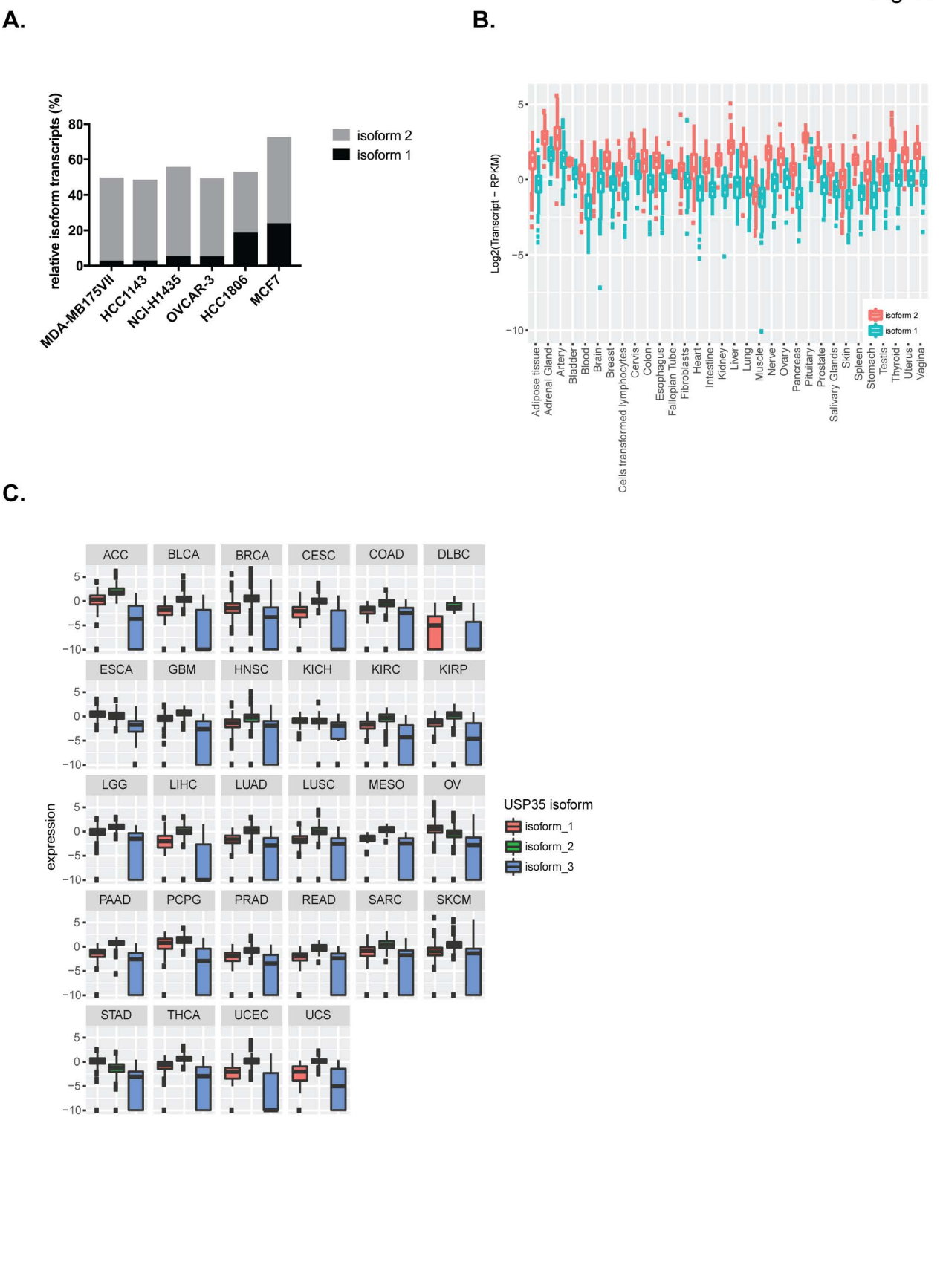

Figure S7. Transcriptomic analysis of USP35 mRNA levels.

A. Indicated cell lines were subjected to RNAseq sequencing and USP35 transcripts analysed. Relative transcription of isoforms 1 and 2 is shown as percentage of total USP35 transcripts detected (including non-translated transcripts).

B. Distributions of expression (in binary log of Reads Per Kilobase of transcript per Million mapped reads (RPKM)) of each of the two human USP35 transcript isoforms of interest across samples from the Genotype-Tissue Expression (GTEx) project (Mele et al., 2015) grouped by organ/tissue.

C. USP35 isoform transcripts in primary tumours were analysed based on the data available from UCSC Xena version 2016-04-12. Values on the Y axes correspond to transcripts per million (TPM) expression values after transformation to log2(tpm+0.001) values. ACC – adrenocortical cancer, BLCA – bladder cancer, BRCA – breast cancer, CESC – cervical cancer, COAD – colon cancer, DLBC – diffuse large B-cell lymphoma, ESCA – Esophageal Cancer, GBM – glioblastoma, HNSC – head and neck cancer, KICH – kidney chromophobe, KIRC – kidney clear cell carcinoma, KIRP – kidney papillary cell carcinoma, LGG – lower grade glioma, LIHC – liver cancer, LUAD – lung adenocarcinoma, LUSC – lung squamous cell carcinoma, MESO – mesothelioma, OV – ovarian cancer, PAAD – pancreatic cancer, PCPG – pheochromocytoma and paraganglioma, PRAD – prostate cancer, READ – rectal cancer, SARC – sarcoma, SKCM – skin cutaneous melanoma, STAD – stomach cancer, THCA – thyroid cancer, UCEC – uterine corpus endometrial carcinoma, UCS – uterine carcinosarcoma.

Supplementary tables

Table S1. Alternative isoforms are extensively utilised to expand the functionality of DUBs. Total number of transcripts and the ones predicted to be protein-coding that were identified as associated with translating ribosomes in HEK293 cells (Floor and Doudna, 2016) are listed.

Click here to Download Table S1

	USP35-Ubiquitin
Data collection	
Space group	P4(3)2(1)2
Cell dimensions	
a, *b*, *c* (Å)	104.24, 104.24, 106.38
α, β, γ (°)	90, 90, 90
Resolution (Å)	104.2 - 1.84(2.01 – 1.84) *
R_{merge}	11.7 (91.8)
CC/(1/2)	0.999 (0.890)
$I / \sigma I$	37 (4.3)
Completeness (%)	100.0 (99.9)
Redundancy	37.4 (20.6)
Anomalous completeness (%)	100 (99.8)
Anomalous redundancy	19.5 (10.6)
Refinement**	
Resolution (Å)	26.9 - 1.84
No. reflections	51309
R_{work} / R_{free}	17.2 / 19.6
No. atoms	
Protein	3168
Ligand/ion	28
Water	683
B-factors (Å^2)	
Protein	21.3
Ligand/ion	37.5
Water	42.3
R.m.s deviations	
Bond lengths (Å)	0.009
Bond angles (°)	0.94

* values in parentheses are for highest-resolution shell

**values as output by autoBUSTER (Bricogne et al., 2016)

Table S2. Data collection, phasing and refinement statistics

Table S3. Interacting partners of USP35 isoforms.
Interacting partners of USP35 isoform 1 (sheet 1) and USP35 isoform 2 (sheet 2) identified using BioID methodology are shown.

Click here to Download Table S3

Cell line	Tumour type	USP35 total copy number
22Rv1	prostate	2
BHY	head and neck	7
BT-474	breast	2
CAMA-1	breast	2
DU 145	prostate	3
DU4475	breast	2
EVSA-T	breast	3.37688
HCC1143	breast	5
HCC1419	breast	2
HCC1806	breast	4
HCC1937	breast	2
HCT 116	colon	2
HPAF-II	pancreas	3
HT-29	colon	4
Hep G2	liver	2
MCF7	breast	4
MDA-MB-175-VII	breast	6.5
MDA-MB-231	breast	3
MDA-MB-468	breast	4.5
NCI-H1435	non-small cell lung	11
NCI-H460	non-small cell lung	2
OVCAR-3	ovarian	13
PC-3	prostate	4
SK-OV-3	ovarian	2
SUM190PT	breast	4
T-47D	breast	4
U-2 OS	bone	4
ZR-75-1	breast	3

Table S4. Copy number of USP35 gene in selected cancer cell lines.