A

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B

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rattus norvegicus      GTGTGATCAC TAATGGGCAA AGGGTTTGGG CTCCTGAGGA AAACCTGCCA

                               ▼▼▼
human                  GTCCATCCTG GCTGAGTCCT CGCAGTCCCC GGCAGATCTT GAAGAAAAGA
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mus musculus           GTCAGTTGTG GCTGAGCCGC AGCAGTACTC AGC...GCTG GAGGAAGAGA
rattus norvegicus      GTCAGTTGTG GCTAATCCTC AGCAGCACTC AGC...TCTG GAGGAAGAGA

                                          ***
human                  AGGAAGAAGA CAGCAACATG AAGAGAGAGC AGCCCAGAGA GCGTCCCAGG
pongo abelii           AGGAAGAAGA CAGCAACATG AAGAGAGAGC AGCCCAGAGA GCGTCCCAGG
mus musculus           GGAC...... CATGAAGAGG AAGAGAGTGC TGTCTAGAGA CCTCTGCAGT
rattus norvegicus      AGAA...... CATGAAGAGG AAGAGAGTGC TGTCTAGAGA GCTCTGCAGT

human                  GCCTGGGACT ACCCTCATGG CCTGGTTGGT TTACACAACA TTGGACAGAC
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mus musculus           GCCTGGGACA GCCCTCATGG TCTGGTTGGT TTACACAACA TCGGACAGAC
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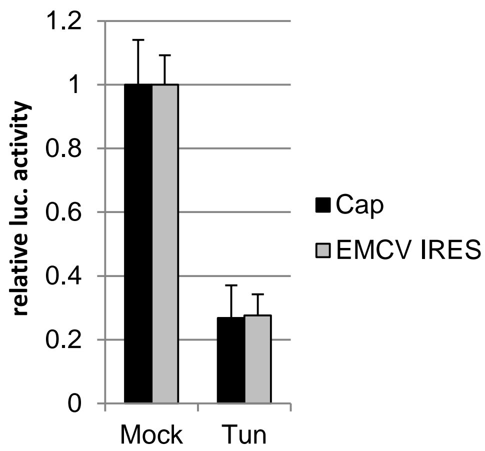
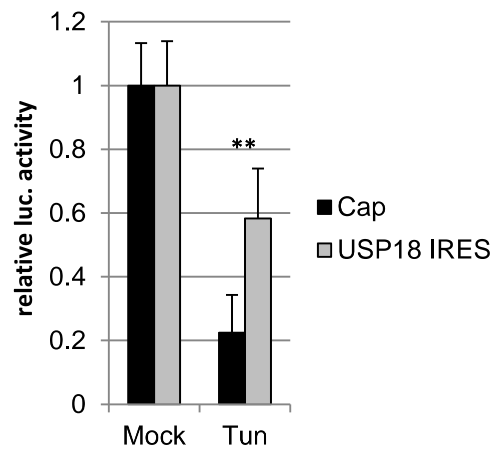
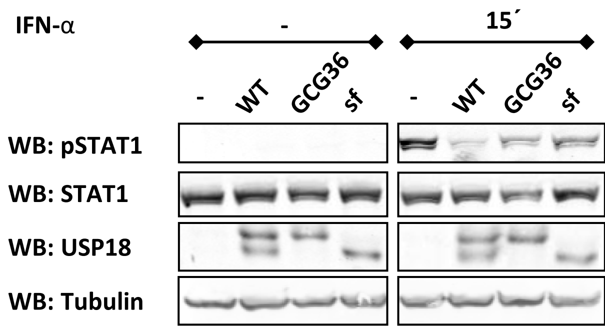
Supplementary Figure S1

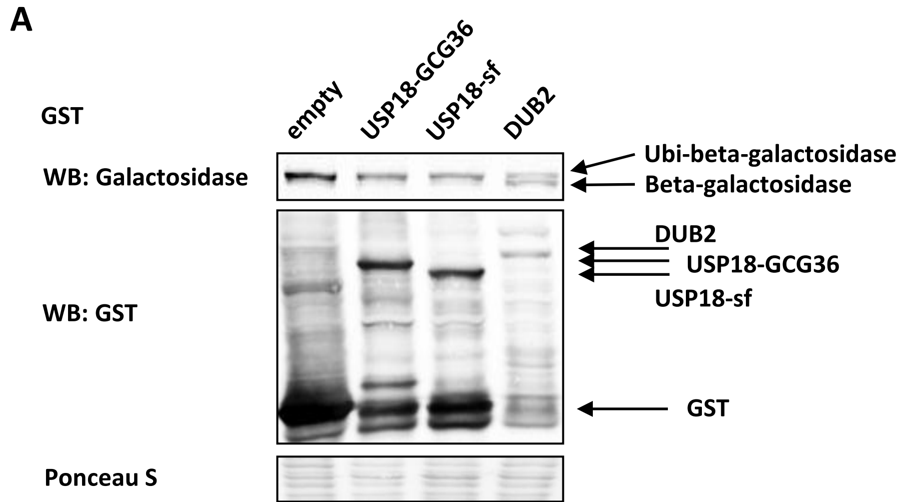


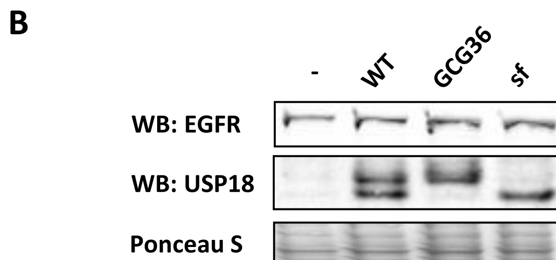


Supplementary Figure S2

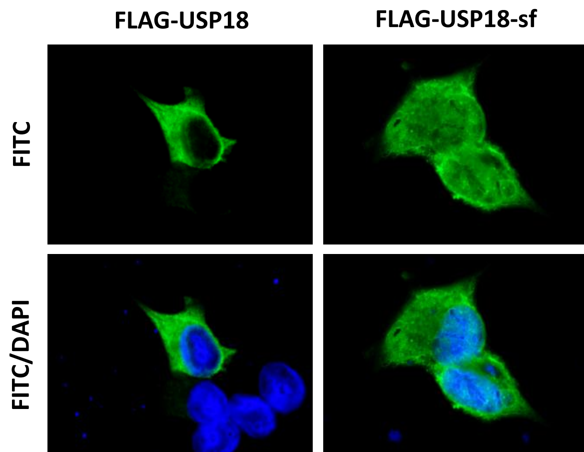


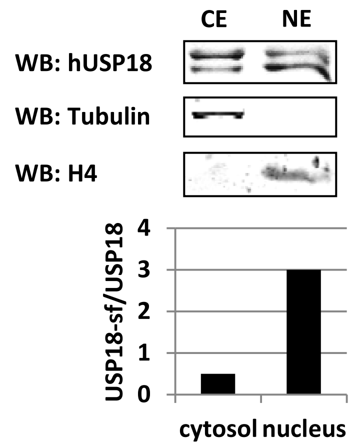
Supplementary Figure S3

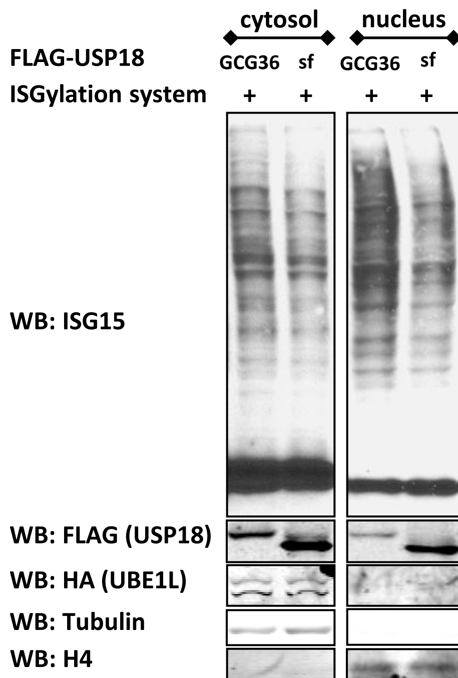




Supplementary Figure S4







Supplementary Figure S5

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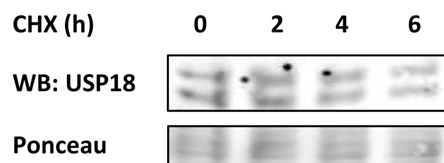

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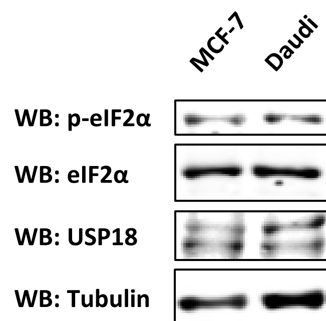

Supplementary Figure S6

Supplementary Figure S1: Deduced protein sequence of human *USP18* starting from CTG16 and cross species alignment of the 5' region of *USP18* coding sequence.

A) Predicted amino acid sequence of human *USP18*. USP18-sf translation initiation site (Met36) is marked with an asterisk and active site cysteine (Cys49) with a square. Conserved domains of UBP family members are indicated as follows: Cys box; QQDAQEF motif; LPQILVIHLKRF consensus; His box.
B) Human, orangutan, mouse and rat *USP18* sequences were aligned. In-frame ATGs and CTGs are highlighted gray. Kozak consensus is underlined. Primary translation initiation site of human *USP18* (CTG16) is marked with arrows and ATG36 marked with asterisks.

Supplementary Figure S2: USP18 IRES activity is relatively resistant to Tunicamycin induced translational stress

Dicistronic plasmids depicted in *Figure 3A* containing either EMCV or USP18 IRES were transiently transfected into HeLa cells. 24h post-transfection cells were treated with 2.5 μg/ml Tunicamycin (Tun) or carrier (Mock) for 14h before analysis. Luciferase activities corresponding to firefly luciferase (IRES) and renilla luciferase (Cap) were measured. Shown are relative luciferase activities of three independent experiments. Asterisks indicate statistical significance (t-test) of $P<0.05$.

Supplementary Figure S3: Both USP18 isoforms downregulate type I interferon induced JAK/STAT signaling in MCF-7 cells.

MCF-7 cells stably expressing USP18, USP18-GCG36 or USP18-sf were treated with 10 ng/ml hIFN-α for 15min. Cells were lysed and protein lysates subjected to USP18, STAT1 as well as phospho-STAT1 Western Blotting. Tubulin was used as loading control.

Supplementary Figure S4: USP18 isoforms do not show significant De-ubiquitin activity or upregulation of EGFR expression.

A) *E.Coli* (BL21) were cotransformed with GST-hUSP18 contructs and a construct coding for Ubiquitin-β-galactosidase. Bacterial cell lysates were subjected to GST and Galactosidase Western Blotting. Only the positive control DUB2 shows significant cleavage of ubiquitin. Ponceau S staining was used as loading control.
B) Whole cell lysates from HeLa cells stably expressing USP18, USP18-GCG36 or USP18-sf were probed for EGFR and USP18 by Western Blotting. Ponceau S staining was used as loading control.

Supplementary Figure S5: USP18-sf is the dominant isoform in the nucleus of MCF-7 cells and USP18-sf transfected cells show reduced protein ISGylation in the nucleus.

A) MCF-7 cells were transfected with FLAG-USP18 or FLAG-USP18-sf. Immunostaining of MeOH fixed cells shows that FLAG-USP18-sf is localized in the nucleus and the cytosol whereas FLAG-USP18 is mostly excluded from the nucleus.
B) MCF-7 cells were treated with 10 ng/ml IFN-α for 36h to induce expression of USP18. Cells were then lysed and nuclear/cytosolic fractions prepared. Each subcellular fraction was probed for USP18 as well as nuclear and cytosolic markers. USP18-sf/USP18 ratio was quantified with *Li-Cor* Odyssey system.
C) MCF-7 cells were transiently transfected with ISGylation system and either FLAG-USP18-GCG36 or FLAG-USP18-sf. 36h after transfection cytosolic and nuclear extracts were prepared and probed for ISG15, USP18, UBE1L as well as nuclear and cytosolic markers.

Supplementary Figure S6: USP18 isoforms do not show a difference in protein turnover and expression of USP18-sf does not correlate with phosphorylation of eIF2α.

A) MCF-7 cells stably expressing USP18 were treated with 20µg/ml of cycloheximide (CHX) and USP18 protein levels at indicated time points analyzed by Western Blotting. Ponceau S staining was used as loading control.

B) MCF-7 and Daudi cells stably expressing USP18 were analyzed for eIF2α phosphorylation and expression of the two USP18 isoforms by Western Blotting. Tubulin was used as loading control.