SUPPLEMENTAL TABLES

Table S1. RNA-Seq of whole cell (Input) and spindle preparations (MS) isolated from WT (HCT) and STAU1-KO (CR1_3) cells

Click here to Download Table S1

Table S2. List of genes that are misregulated in STAU1-KO compared to wild-type HCT116 cells (Input)

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Table S3. Spindle-enriched RNAs in WT and STAU1-KO HCT116 cells

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Table S4. Number of individual transcripts and of transcripts per million (TPM) across RNA biotypes of spindle-enriched RNAs. Enrichment of TPM in mitotic spindle compared to input is shown.

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Table S5. Gene ontology (GO) of spindle-enriched RNAs in HCT116 cells (Metascape Gene Annotation & Analysis Resource).

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Table S6. Downregulated RNAs in spindle preparations of STAU1-KO cells compared to WT cells.

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Table S7. Gene ontology (GO) of downregulated RNAs in spindle preparations of STAU1-KO cells compared to WT cells (Metascape Gene Annotation & Analysis Resource).

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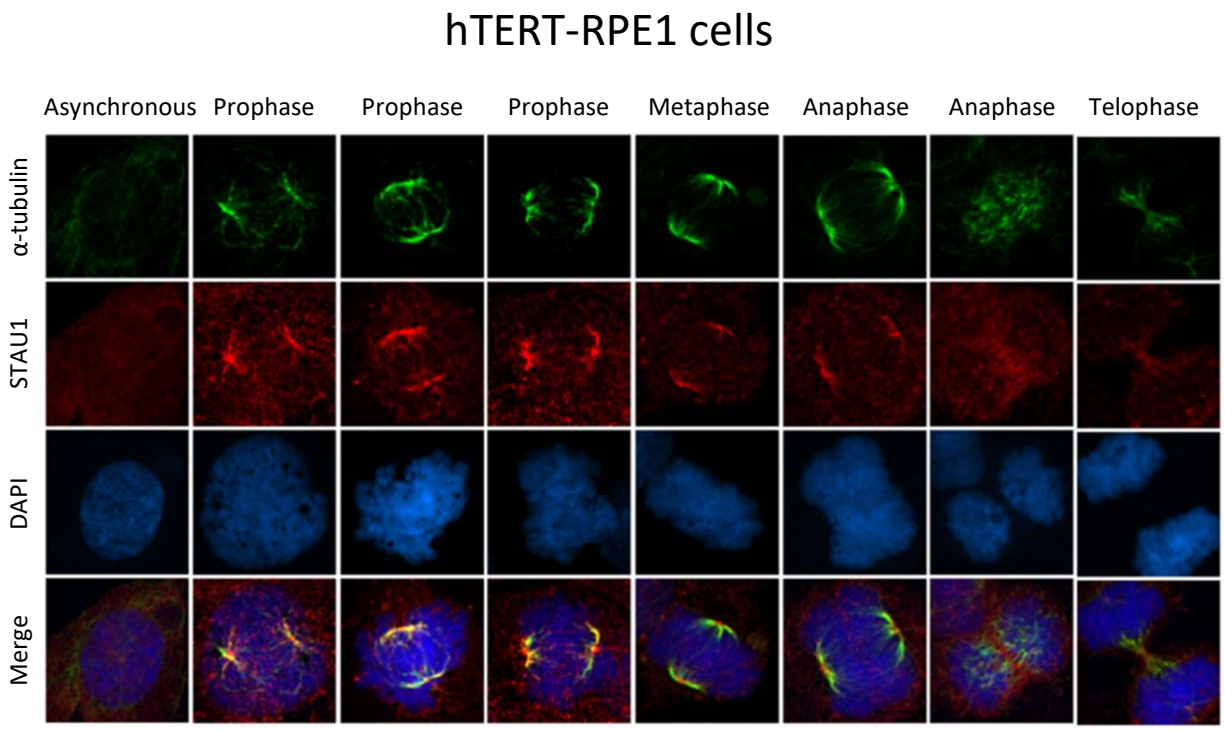


Figure S1. Co-localisation of STAU1 and α-tubulin on mitotic spindle in the non-transformed hTERT-RPE1 cells. hTERT-RPE1 cells were synchronized in late G_2 with RO-3306 and released from the block with fresh medium to reach mitosis. Cells were treated with Triton X-100 and then fixed. Proteins were stained with specific antibodies to detect Stau1 and α-tubulin. DNA was stained with DAPI. Cells at different steps of mitosis are shown.

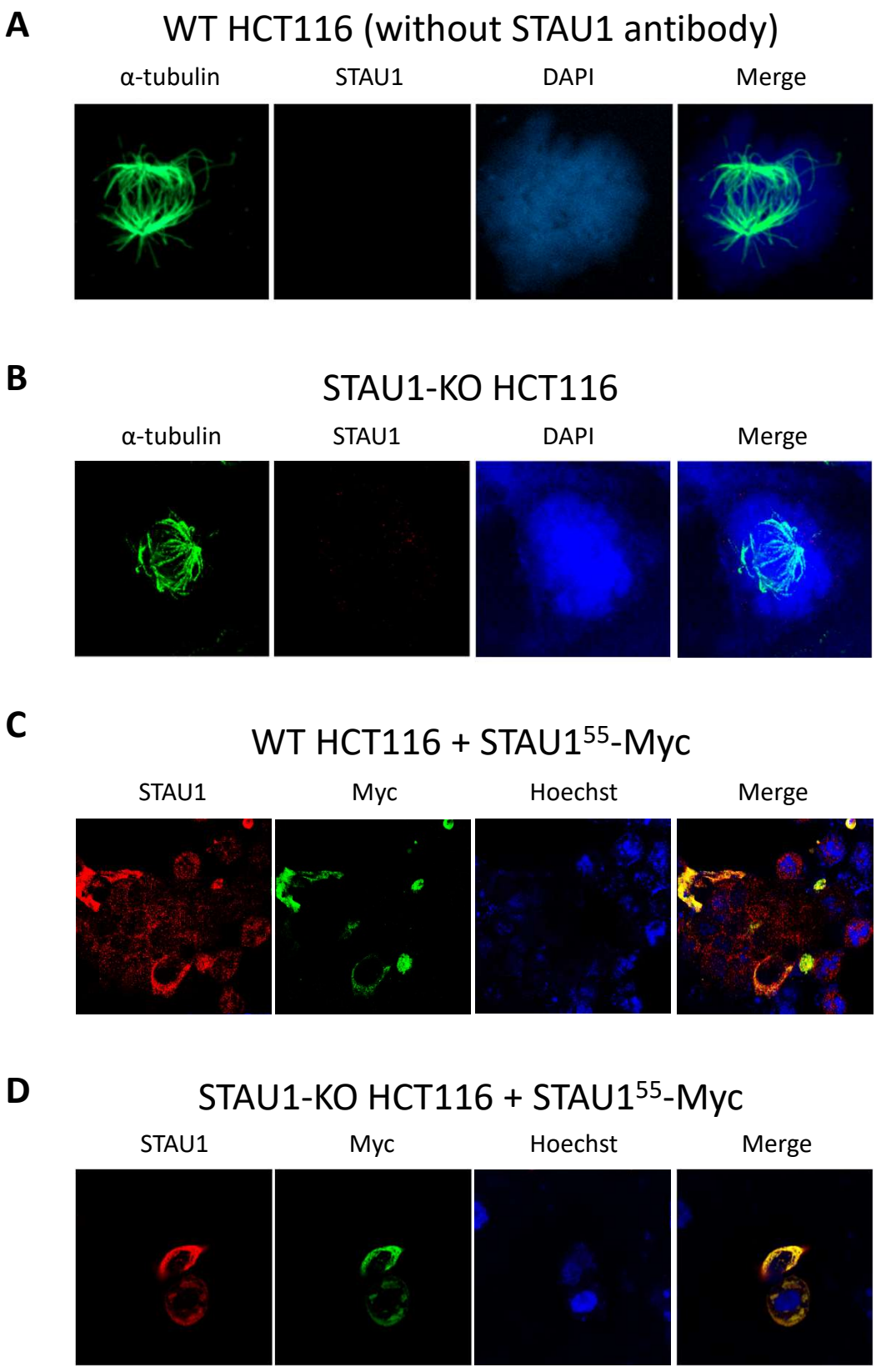
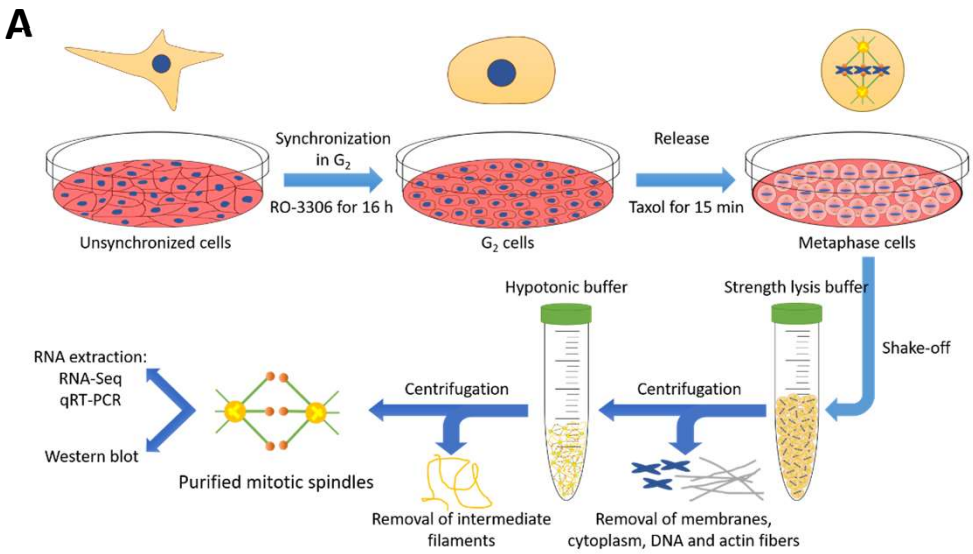
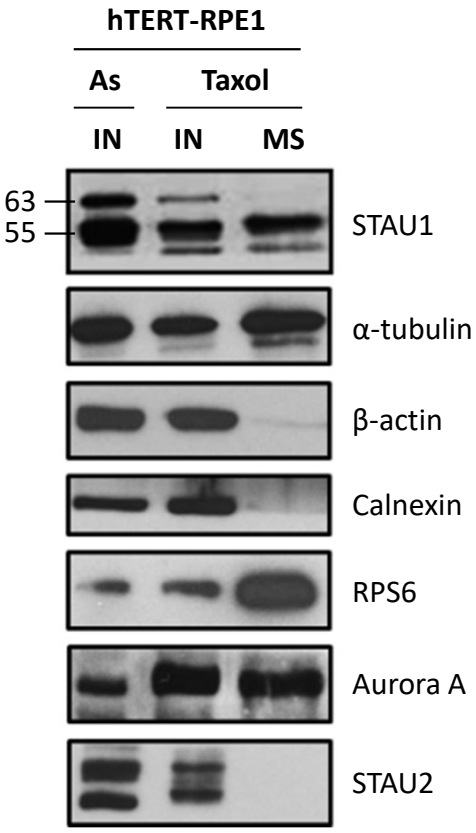


Figure S2. Control of antibody specificity. (A) To control for antibody specificity, mitotic HCT116 cells were stained with rabbit anti-tubulin antibody and anti-mouse (usually used to detect STAU1 expression) and anti-rabbit secondary antibodies. In the absence of anti-STAU1 antibody, no signal was detected. **(B)** STAU1-KO HCT116 cells (clone CR1.3) were stained with anti-STAU1 and anti-tubulin antibodies. No signal was detected with anti-STAU1 antibody. **(C,D)** HCT116 (C) and HCT116-KO (clone CR1.3) (D) cells were transfected with a plasmid coding for STAU1^{55}-myc and stained with anti-STAU1 and anti-myc antibodies. Perfect colocalization of STAU1 and myc signals are observed. These experiments are representative of at least three independently performed experiments.





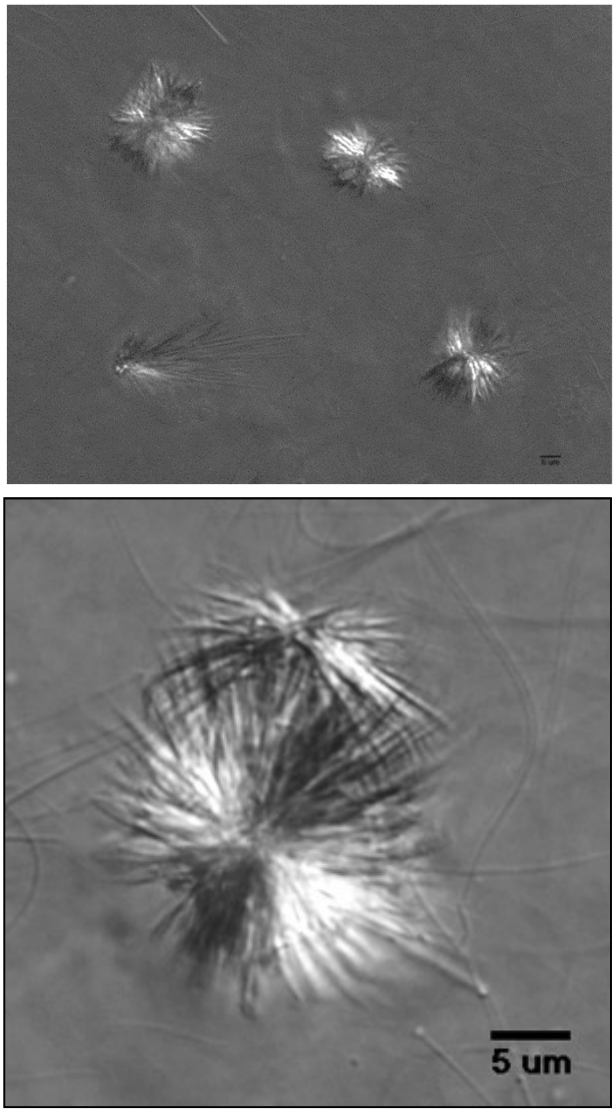
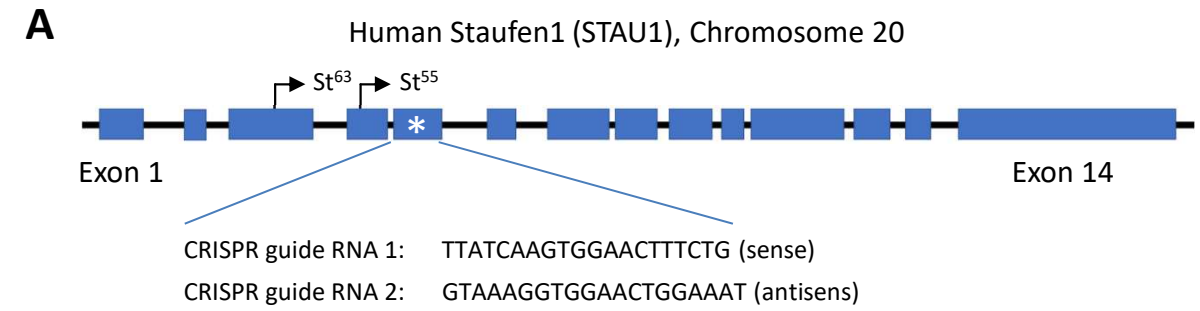
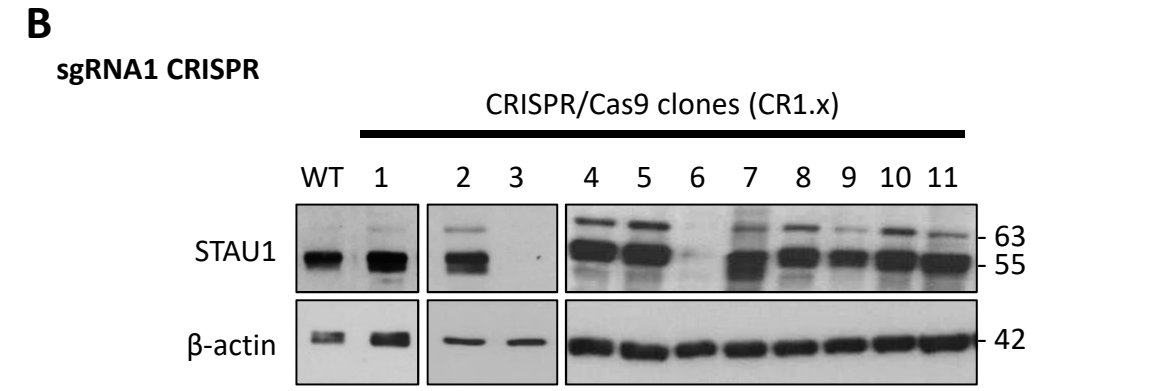
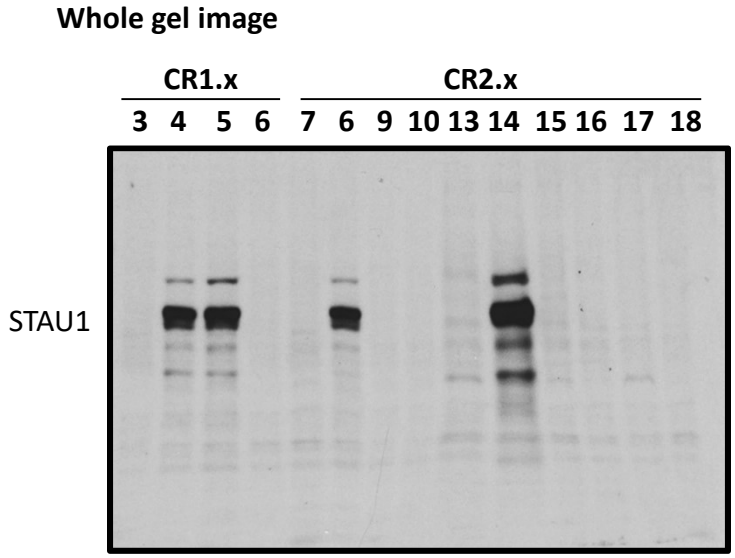
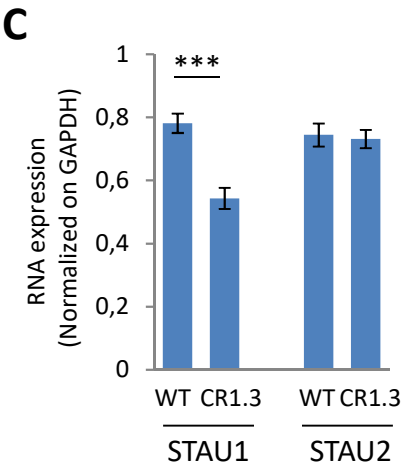


Figure S3. Validation of spindle preparations by microscopy. A) Schematic illustration of the protocol used to purify mitotic spindles. **B)** hTERT-RPE1 cells were synchronized in prometaphase and treated with taxol to stabilize microtubules. Mitotic spindles were purified and protein contents analyzed by western blotting. As, asynchronous cells; Taxol, mitotic cells; IN, input total cell extracts. MS: mitotic spindle. (n=3). **C)** Aliquots of spindle preparations were spread on microscopic slides and visualized by microscopy.









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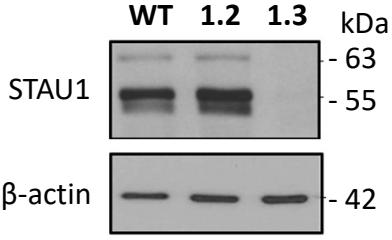

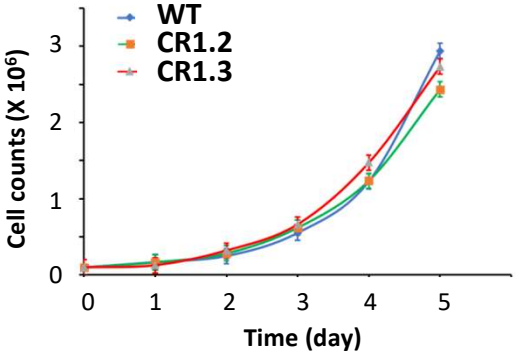

E

WT sequence:

sgRNA1 target sequence

```
TACTTTTACCCATTTCCAGTTCCACCTTTACTTTATCAAGTGGAACTTTCTGTGGGAGGACAGCAATTTAA
 Y  F  Y  P  F  P  V  P  P  L  L  Y  Q  V  E  L  S  V  G  G  Q  Q  F  N
```

CR1.3 - Allele 1:

```
TACTTTTACCCATTTCCAGTTCCACCTTTACTTTATCAAGTGGAACTTT--------GGACAGCAATTTAA
 Y  F  Y  P  F  P  V  P  P  L  L  Y  Q  V  E  L           W  T  A  I  Stop
```

CR1.3 - Allele 2:

```
TACTTTTACCCATTTCCAGTTCCACCTTTACTTTATCAAGTGGAAC---AGAAAGGCGGACAGGTATCCGG
 Y  F  Y  P  F  P  V  P  P  L  L  Y  Q  V  E     Q  K  G  G  Q  V  S  G

TAAGCGGCAGGGTCGGAACAGGAGAGCGCACGAGGGAGCTTCCAGGGGGAAACGCCTGGTATCTTTATAGT
  K  R  Q  G  R  N  R  R  A  H  E  G  A  S  R  G  K  R  L  V  S  L  Stop

CCTGTCGGGTTTCGCCACCTCTGACTTGAGCGTCGATTTTTGTGATGCTCGTCAGGGGGGCGGAGCCTATG
GAAAAACGCCAGCTGTGGGAGGACAGCAATTTAA
```

F

WT sequence:

sgRNA2 target sequence

```
TACTTTTACCCATTTCCAGTTCCACCTTTACTTTATCAAGTGGAACTTTCTGTGGGAGGACAGCAATTTAA
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```

CR2.9 - Allele 1:

```
TACTTTTACCCATTCTCCAGTTCCACCTTTACTTTATCAAGTGGAACTTTCTGTGGGAGGACAGCAATTTAA
 Y  F  Y  P  F  S  S  S  T  F  T  L  S  S  G  T  F  C  G  R  T  A  I  Stop
```

CR2.9 - Allele 2:

```
TACTTTTACCCATTTTCCAGTTCCACCTTTACTTTATCAAGTGGAACTTTCTGTGGGAGGACAGCAATTTAA
 Y  F  Y  P  F  S  S  S  T  F  T  L  S  S  G  T  F  C  G  R  T  A  I  Stop
```

Figure S4. Knockout of STAU1 in the colorectal HCT116 cancer cell line by the CRISPR/Cas9 complex system. (A) Schematic representation of the *STAU1* gene. Position of ATG initiation codons for STAU1 55 kDa and 63 kDa is indicated. * : site of CRISPR editing. Two different CRISPR guide RNAs were used. **(B)** HCT116 cells were transfected with plasmids expressing Cas9/sgRNA1 or Cas9/sgRNA2 complex targeting exon 6 of the *STAU1* gene. Colonies grown from single cell were screened for STAU1 expression by western blotting. 18% and 44% of the clones that were transfected with CRISPR sgRNA1 and CRISPR sgRNA2, respectively, were negative for STAU1 expression. Below, whole gel image of CR2.13 to CR2.18 cell extracts showing that STAU1-truncated products are not produced in CRISPR STAU1-KO cells. **(C)** Wild type (WT) and STAU1-KO (clone CR1.3) HCT116 cells were analyzed by RT-qPCR for STAU1 and STAU2 expression. **D)** Cells were plated at the same density and counted every day for five days. The graph shows the means and standard deviation of cell counts of three independently performed experiments**. E,F)** Biallelic sequencing of exon 6 of *STAU1* genomic DNA isolated from STAU1-KO CR1.3 (D) and CR2.9 (E) cells. Sequences of wild type (WT) and CRISPR alleles are shown as well as the corresponding protein sequence. The WT protein sequence is underlined. Red: Target sequences of the CRISPR sgRNA1. Green: Inserted nucleotides. Dash line: Deleted nucleotides

Supplementary Figure S5

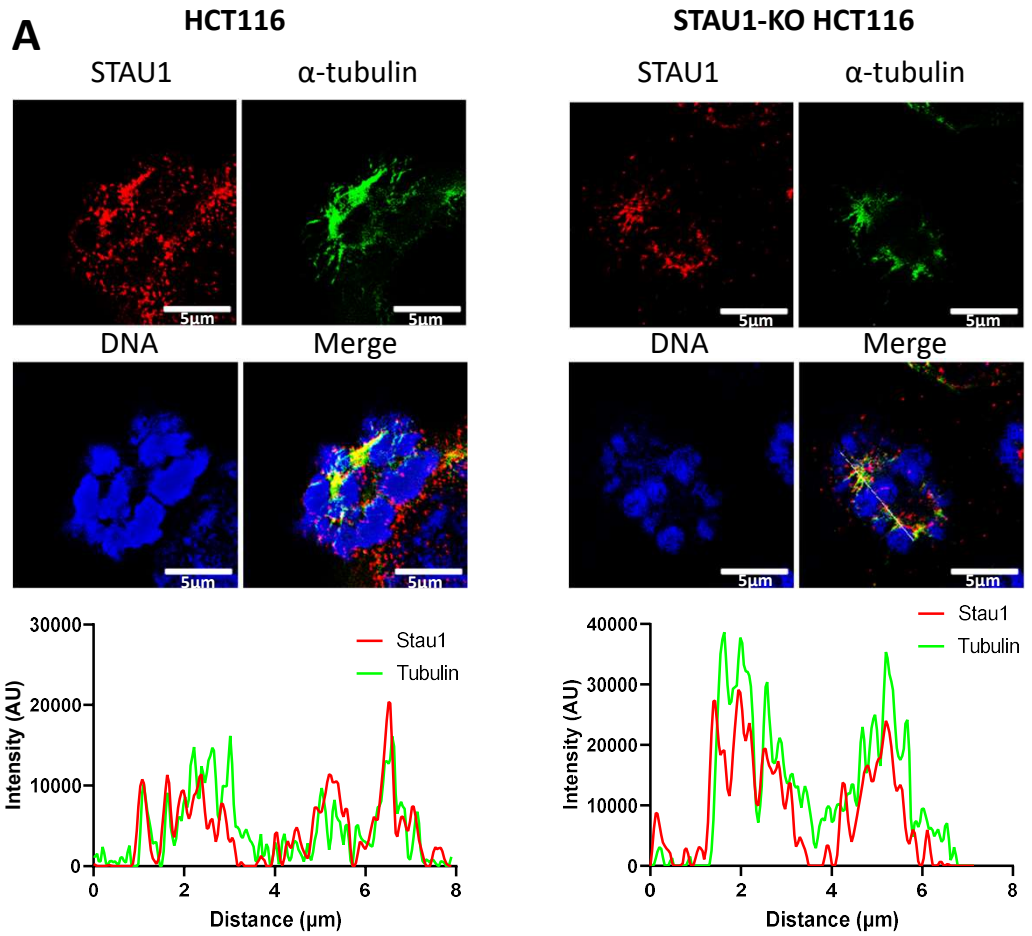


Figure S5. Characterization of exogenously expressed STAU1^{55}. A) HCT116 WT (left) and STAU1-KO CR1.3 (right) cells were transfected with a plasmid coding for STAU1^{55}-myc. STAU1 was detected with anti-STAU1 and anti-myc antibodies. Both signals co-localized as expected. **B)** Exogenously transfected STAU1^{55}-HA$_3$ purifies in mitotic spindle preparations, as does the endogenous protein. Western blot experiment showing endogenous STAU1 in cell extracts (IN) and spindle preparations (MS) of HCT116 cells (WT) and STAU1-HA$_3$ in cell extracts (IN) and spindle preparations (MS) of STAU1-KO HCT116 cells.

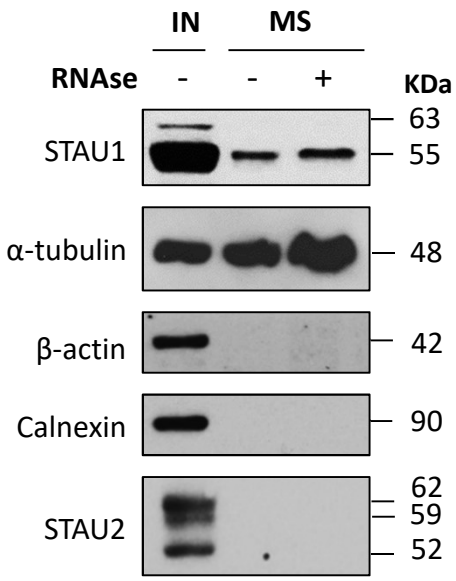


Figure S6. STAU1 association with spindle is resistant to RNase treatment. Western blot experiment of cell extracts (IN) and mitotic spindle preparations (MS) purified in the presence (+) or absence (-) of RNase.

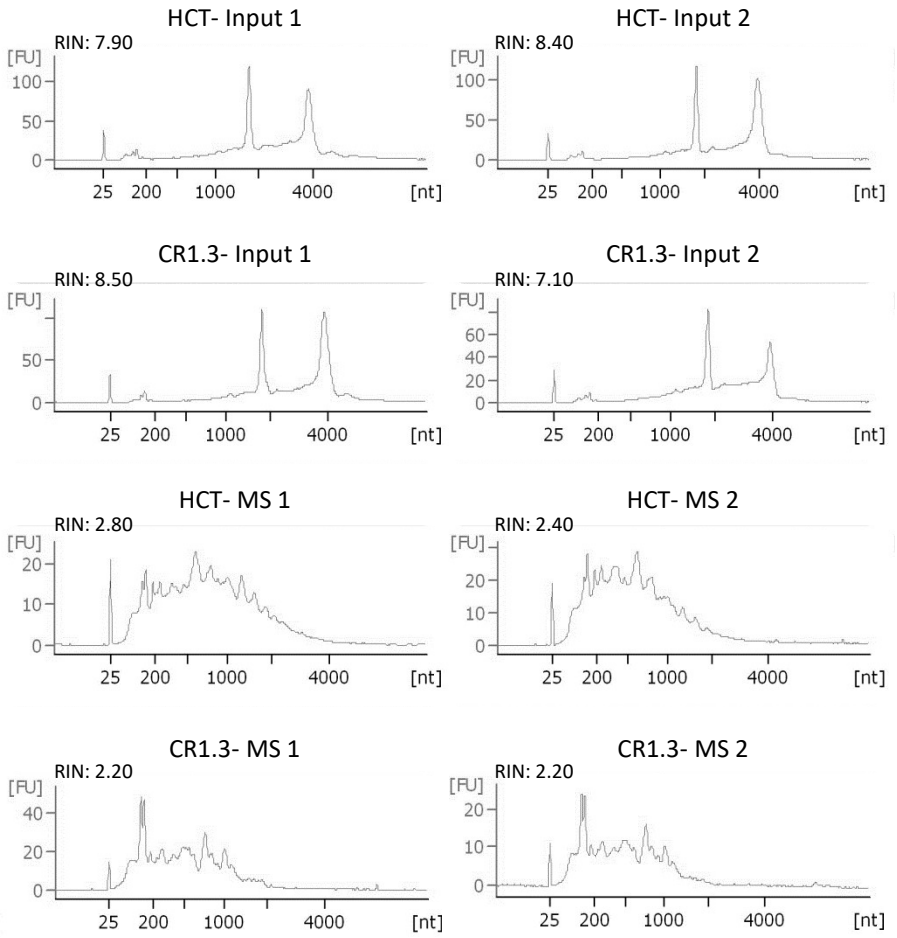
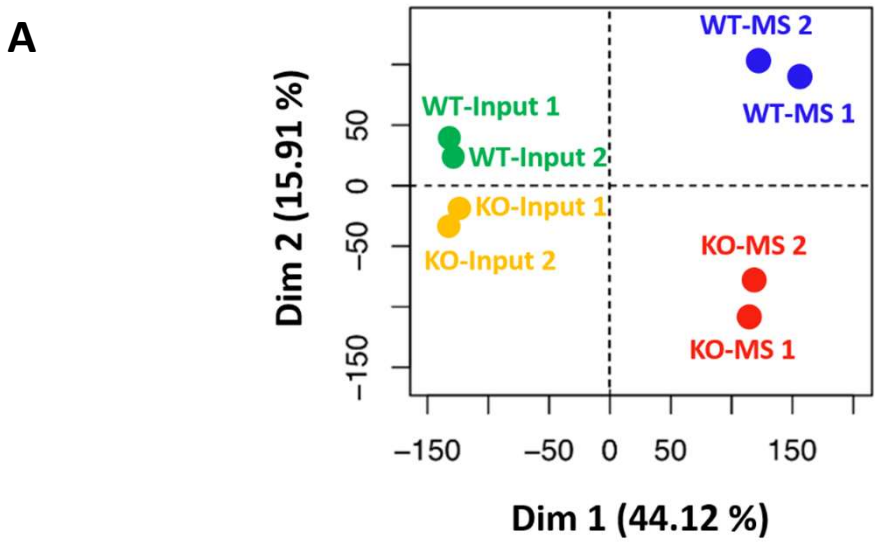
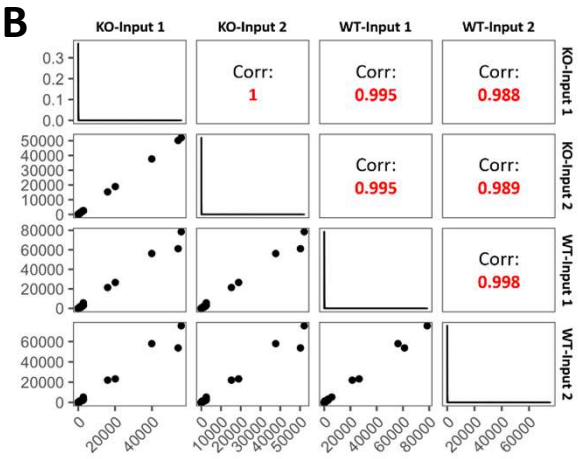
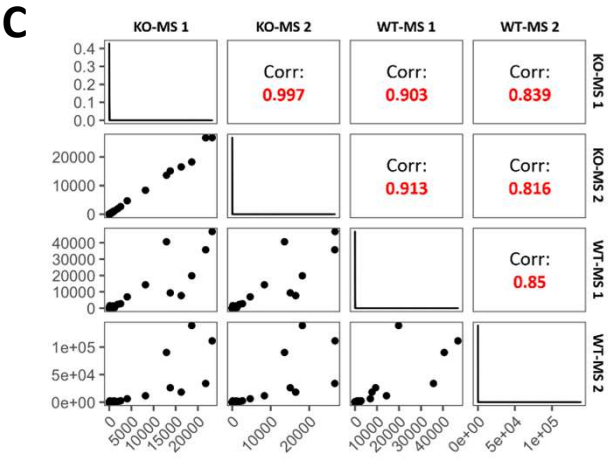


Figure S7. Quality controls of RNA-Seq data. RNAs were isolated from WT (HCT) and STAU1-KO (CR1.3) HCT116 cells and analyzed by Agilent RNA Pico. RNAs from total cell extracts (Input) and mitotic spindle preparations (MS) are shown.

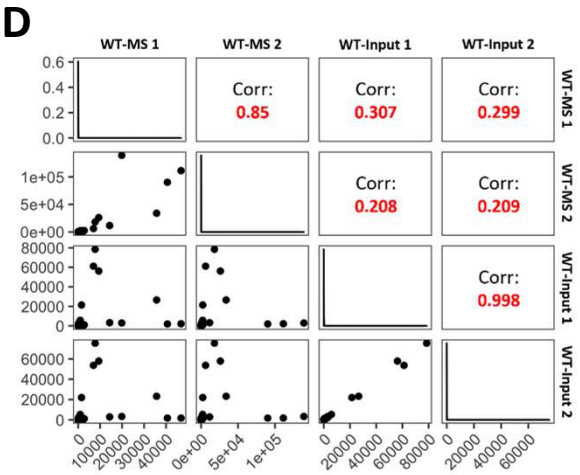




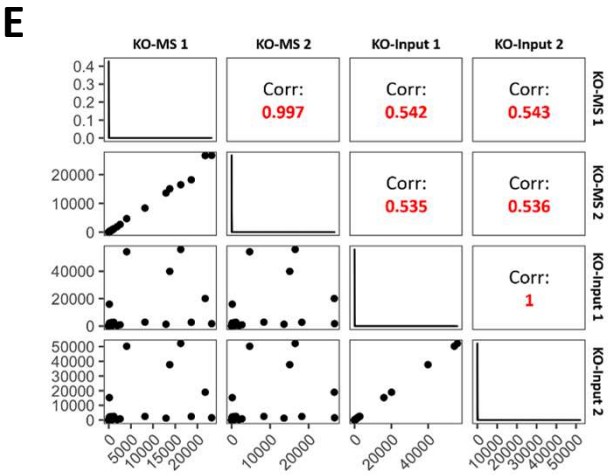
Correlations Input (WT vs STAU1-KO)



Correlations MS (WT vs STAU1-KO)



Correlations WT (Input vs MS)



Correlations STAU1-KO (Input vs MS)

Figure S8. Validation of the reproducibility of RNA-Seq data. **A)** RNA-Seq data from replicates prepared from mitotic cell extracts (Input) and mitotic spindle preparations (MS) from WT and STAU1-KO (KO) cells were compared on PCA plot. **B-E)** High degree of correlation was observed between duplicates of cell extracts (Input) and of spindle preparations (MS), respectively, and weak correlation between Input vs MS preparations. Correlations between Input (A) and mitotic spindle replicates (B) in WT vs STAU1-KO HCT116 cells. Correlations between WT (C) and STAU1-KO (D) input vs MS.