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Supplemental Information

Opposing Roles of FANCJ and HLTF Protect Forks and Restrain Replication during Stress

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Supplemental Figures:

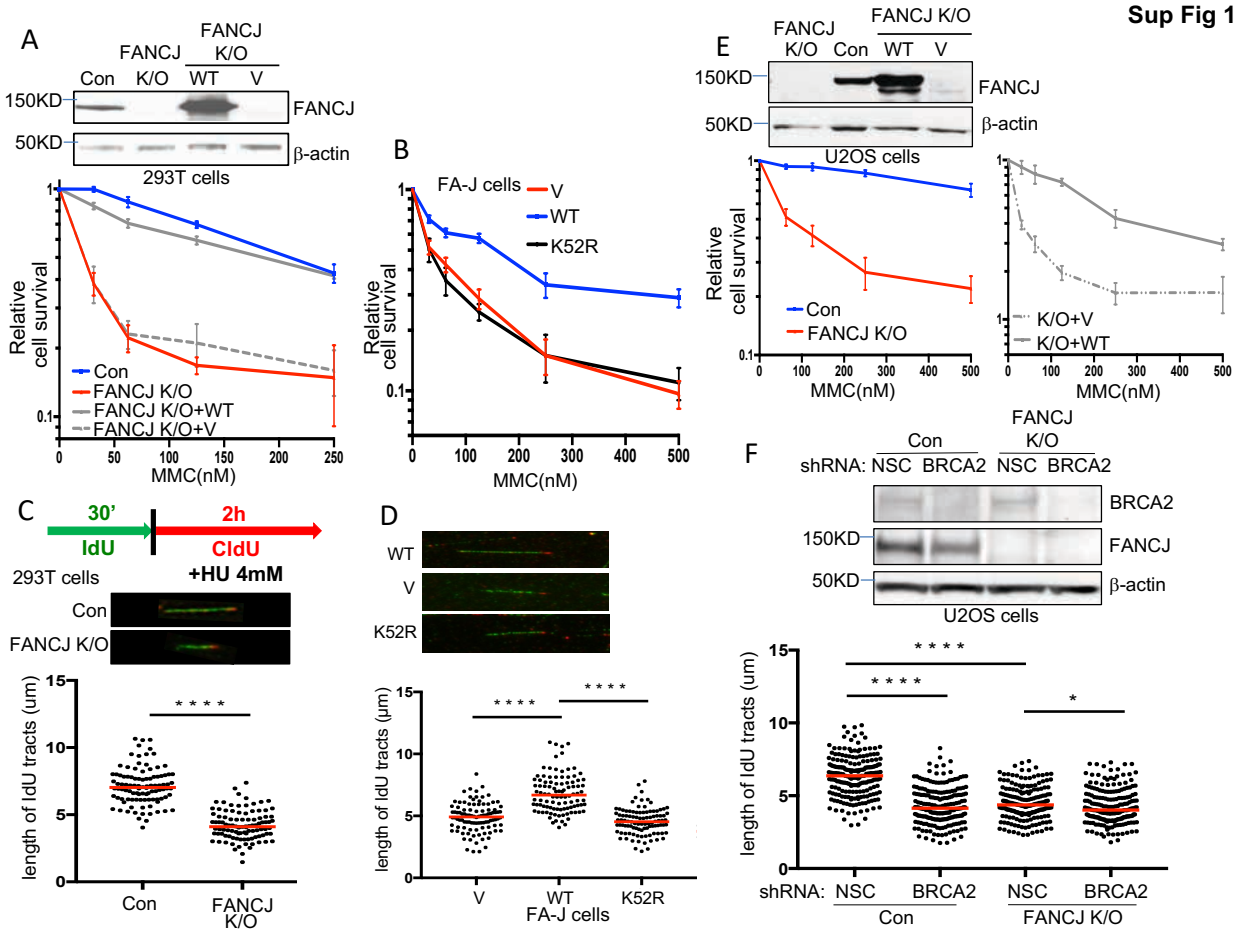

Figure S1: FANCJ and its helicase activity protect nascent DNA at replication forks. Related to Figure 1. (A) Western blot analysis with the indicated antibodies (Abs) of lysates from FANCJ K/O, control 293T cells, or FANCJ K/O cells transfected with vector (V) or wild-type FANCJ (WT). Cell survival assay with indicated cells under increasing concentrations of MMC. (B) Cell survival assay with FA-J cells complemented with V, WT or K52R under increasing concentrations of MMC. (C) Schematic, representative images, and quantification of IdU tract length in FANCJ K/O and control 293T cells. (D) Representative images, and quantification of IdU tract length in FANCJ null FA-J cells complemented as noted. For (C) and (D), at least 100 fibers are quantified for each. (E) Western blot analysis with the indicated Abs of lysates from FANCJ K/O, control U2OS cells, or FANCJ K/O cells transfected with V or WT. Cell survival assay with indicated cells under increasing concentrations of MMC. (F) Western blot analysis with the indicated Abs of lysates from FANCJ K/O and control U2OS cells expressing shRNA against BRCA2 or NSC. Quantification of IdU tract length in FANCJ K/O and control U2OS cells with NSC or BRCA2 shRNA. At least 200 fibers are quantified from two independent experiments. Red bars represent the median. Statistical analysis according to two-tailed Mann-Whitney test; ****, $P < 0.0001$; *, $P<0.05$.

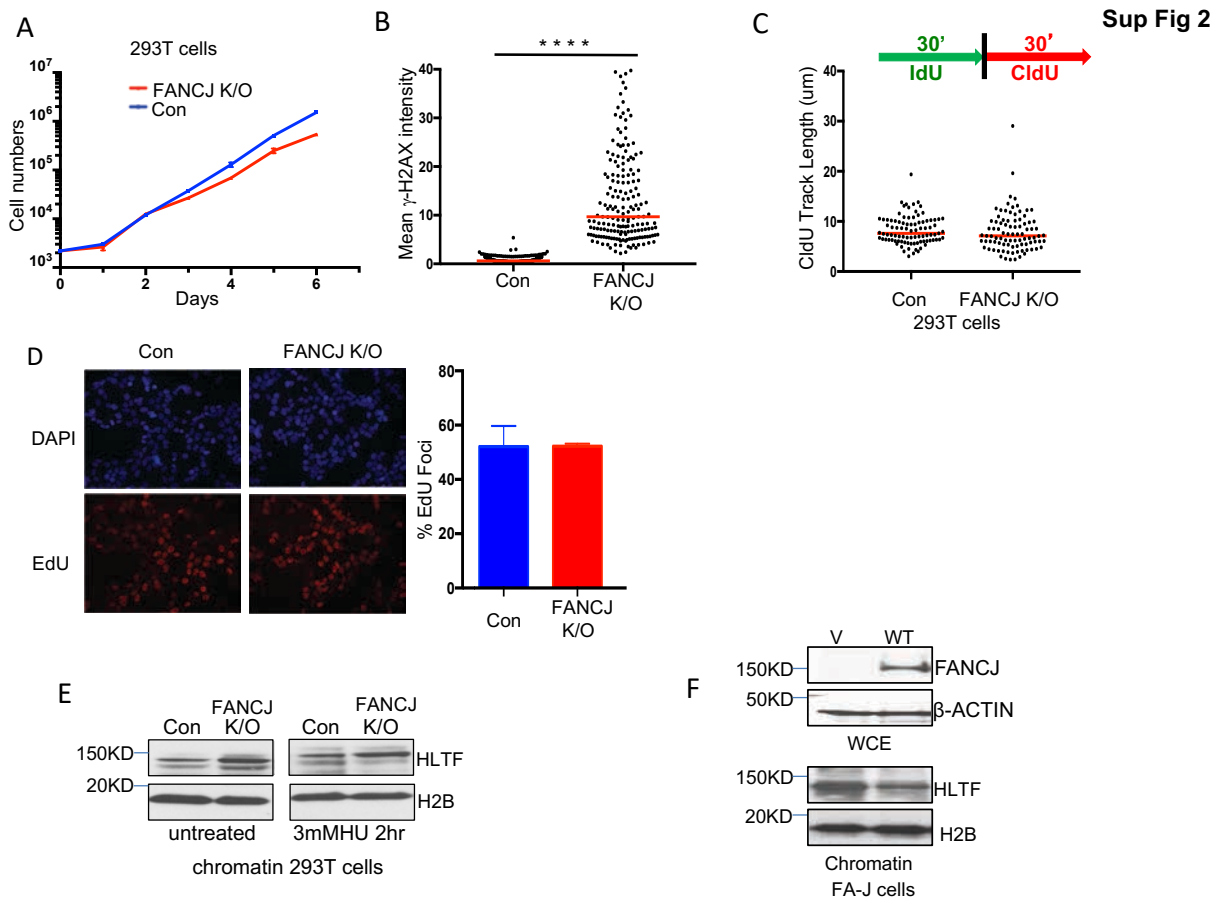

Figure S2. FANCJ loss in 293T cells does not measurably alter replication fork progression in unchallenged conditions but HLTF is enriched in FANCJ K/O cells with or without HU. Related to Figure 2. (A) Cell growth assay with FANCJ K/O or control 293T cells plated at low density and counted as indicated. (B) Quantification of γ-H2AX intensity in FANCJ K/O or control 293T cells. Statistical analysis according to two-tailed Mann-Whitney test; ****, $P < 0.0001$. (C) Schematic and quantification of CldU tract length in FANCJ K/O and control 293T cells. The intensity was measured in at least 150 cells for each. (D) Cells were labeled with EdU and carried out with Click-iT EdU imaging kit. A representative image is shown. The percent of cells with EdU foci was quantified and graphed. Data represent mean ± s.d. from three independent experiments. (E) 293T cells were treated with 3mM HU for 2hr or left untreated. Chromatin fractions were analyzed by immunoblotting for the indicated proteins. (F) FA-J cells complemented with WT or V were collected and fractionated, chromatin fractions were analyzed by immunoblotting for the indicated proteins.

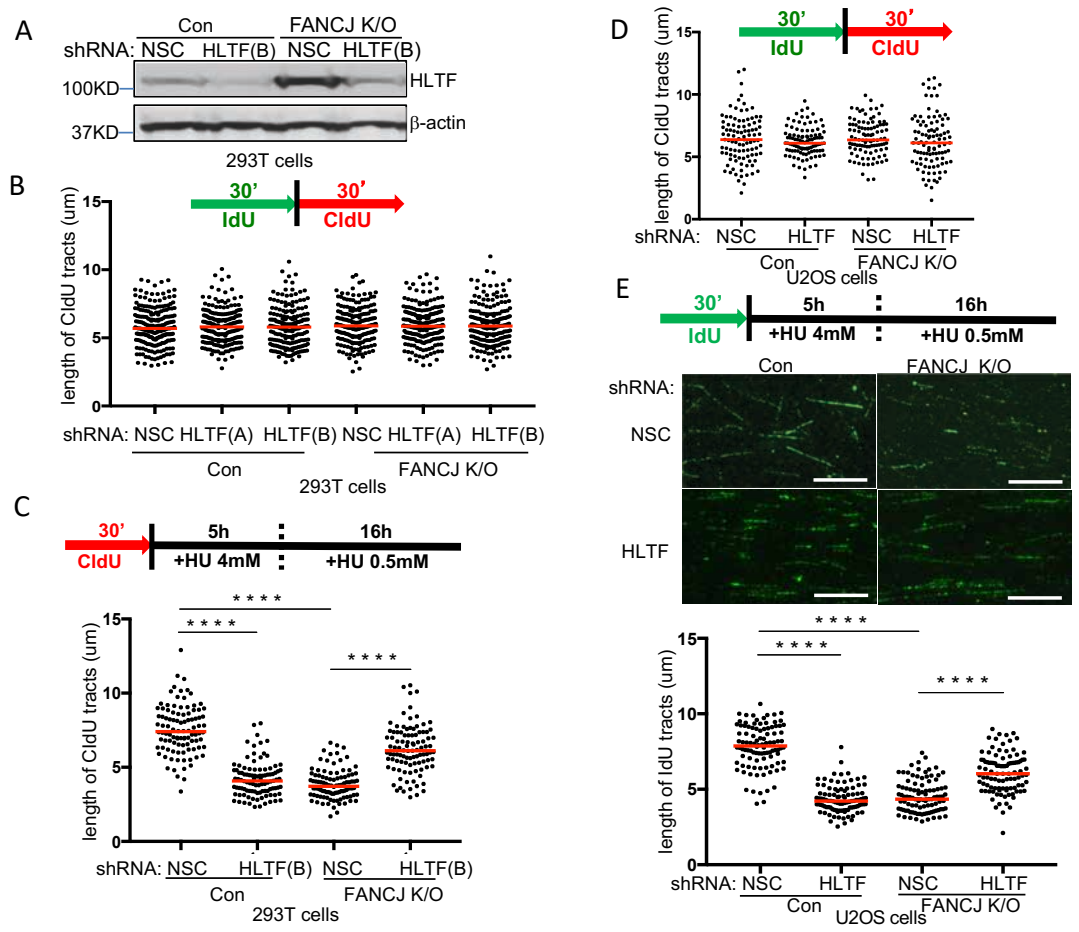

Figure S3. HLTF contributes to fork degradation in FANCJ K/O 293T cells and under prolonged stress, FANCJ contributes to fork degradation in HLTF depleted 293T or U2OS cells. Related to Figure 3. (A) Western blot analysis with the indicated Abs of lysates from control and FANCJ K/O 293T cells expressing shRNA against HLTF (reagent B) or non-silencing control (NSC). (B) Schematic and quantification of CldU tract length in 293T untreated cells with shRNA HLTF (reagent A or B). At least 200 fibers are quantified from two independent experiments. (C) Schematic and quantification of CldU tract length after prolonged HU treatment as indicated. (D) Schematic and quantification of CldU tract length in U2OS untreated cells with shRNA HLTF (E) Schematic, representative images and quantification of ldU tract length after prolonged HU treatment as indicated. For (C), (D) and (E), at least 100 fibers are quantified for each. Each dot represents one fiber; red bars represent the median. Statistical analysis according to two-tailed Mann-Whitney test; ****, $P < 0.0001$. Scale bar, 10μm.

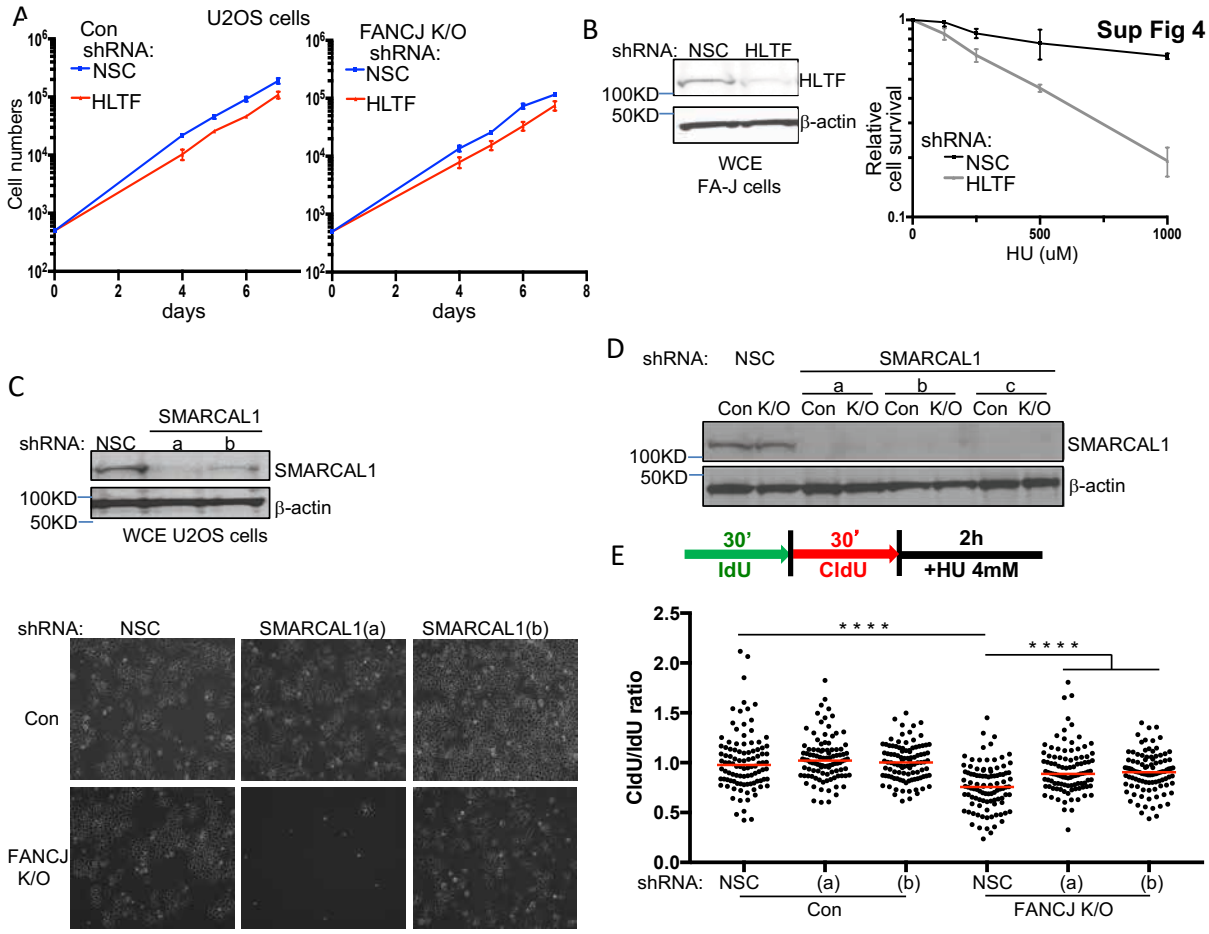

Figure S4. Growth dynamics and fork degradation in FANCJ K/O U2OS, FA-J, or 293T K/O cells upon HLTF or SMARCAL1 depletion. Related to Figure 4. (A) FANCJ K/O and control U2OS cells expressing shRNA against HLTF or NSC were plated at low density, then collected and counted at indicated time. (B) Western blot analysis with the indicated Abs of lysates from FA-J cells expressing shRNA against HLTF or NSC. Cell survival assays with FANCJ null FA-J cells expressing shRNA against HLTF or NSC under increasing concentrations of HU. Data represent the mean percent ± s.d. of survival from three independent experiments. (C) Western blot analysis with the indicated Abs of lysates from control U2OS cells. Live cell imaging of FANCJ K/O and control U2OS cells expressing shRNA (a) or (b) reagent to SMARCAL1 or NSC following puromycin selection. (D) Western blot analysis with the indicated Abs of lysates from FANCJ K/O and control 293T cells. (E) Schematic and quantification of CldU/IdU ratio after HU treatment. At least 100 fibers are quantified for each. Each dot represents one fiber; red bars represent the median. Statistical analysis according to two-tailed Mann-Whitney test; ****, $P < 0.0001$.

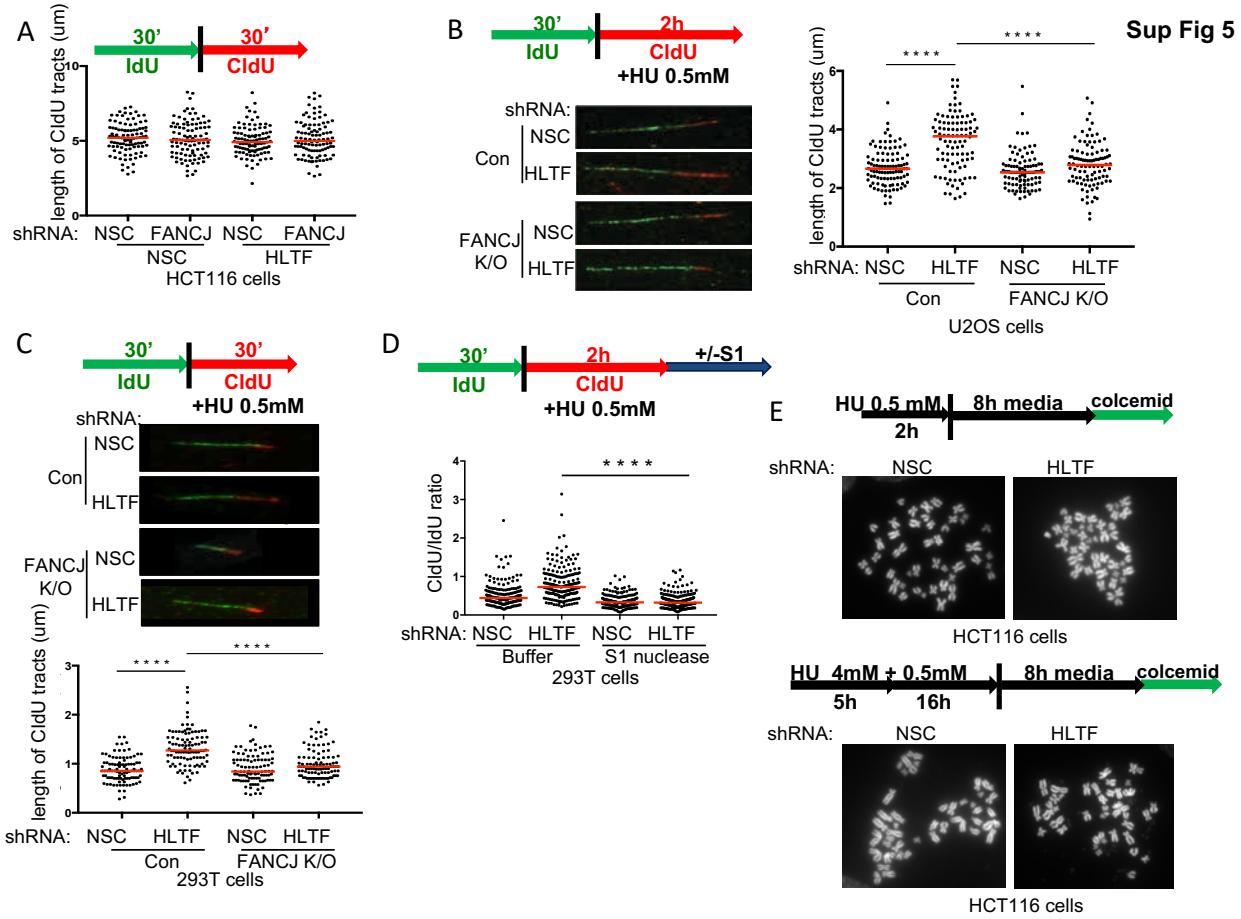

Figure S5. HLTF and FANCJ loss do not alter fork progression in unchallenged conditions and FANCJ contributes to unrestrained replication in HLTF depleted cells that leads to S1 nuclease sensitivity. Related to Figure 5. (A) Schematic and quantification of CldU tract length in HCT116 untreated cells with shRNA FANCJ. (B) Schematic, representative images and quantification of CldU tract length during HU treatment in the indicated shRNA expressing U2OS cells. (C) Schematic, representative images and quantification of CldU tract length during HU treatment in the indicated shRNA expressing 293T cells. (D) Schematic and quantification of CldU to IdU ratio in the indicated shRNA expressing 293T cells, with or without S1 nuclease incubation. Each dot represents one fiber. At least 100 fibers are quantified for each. Red bars represent the median. Statistical analysis according to two-tailed Mann-Whitney test; ****, $P < 0.0001$. (E) Schematic and representative images of chromosome spreads from HU-treated HCT116 cells expressing the indicated shRNAs.

Supplemental Table legends

Sup Table 1

HELLS	EEF1A1	CROCC	ZNF90	HNRNPAB	RAN	RPSA	HSPA1B	DDX5	HIST1H2AJ	SUMO3	HP1BP3
VIM	PRSS3	HIST1H2BI	HNRNPC	HMGB1	UBB	ILF2	NONO	H2AFV	PP1A	RCC1	RPL35A
HNRNPD	THAP6	HMGB3	GAPDH	DPYSL5	LMNB1	HNRNPA3	HNRNPU	KCTD12	HNRNPA1	ZNF236	TUBB
C2CD2L	PARP3	SMARCA5	ENO1	OR10J4	HIST1H1D	ACTG1	AHNAK	HIST1H2BL	RACGAP1	MYH14	PCCA
HSPA8	TMPO	KIF21B	NPM1	HIST1H1E	HIST1H1C	EIF4A1	ACACA	CALML5	HSP90AB1	EEF2	DNMT1
CFL1	IMPDH2	H2AFY	HIST2H2BE	TXN	HSPA6; HSPA7	HNRNPK	TRIM28	H2AFY2	PARP1	H2AFX	MYH9
LRMP	UHRF1BP1	PSIP1	H1F0	HSPD1	NCL	HIST1H4A	HIST2H3PS2	BAZ1B	HIST2H3A	HIST3H2BB	SHROOM3
TUBA1C	ALDOA	LDHA	HSP90AA1	TOP2A	HNRNPA2B1	RPS25	HDGFRP2	SUPT16H	GTF2I		

Table S1: Table of proteins found in Thymidine chase. Proteins in red were also found significant in iPOND (related to Figure 2D).