Table S1: Primers used in this study.

Underline; restriction enzyme sites, lowercase letters; plasmid backbone sequence.

Gene	Purpose	Primer Name	Primer Sequence	Linker
TbPOLIC (927.7.3990)	3' UTR RNAi construct	MK622	5'-TCA ATT ACG CGT GCG GAG GTG AGG AGT AGC GTC G-3'	MluI
		MK623	5'-TCA ATT AAG CTT GCG GAG GTG AGG AGT AGC GTC G-3'	HindIII
		MK624	5'-TCT ATT TCT AGA GTG TAG TAA TCA GGG CGA CG-3'	XbaI
	Subcloning	MK653	5'-ATC ATC AAG CTT ATG CGA CGT ACT TTC AGT C-3'	HindIII
		MK654	5'-ATC ATC TCT AGA CCT GA CAA CTC CCC TAG TG-3'	XbaI
	Gibson Assembly	MK840	5'-cac caa aaa gta aaa ttc aca agc ttA TGC GAC GTA CTT TCA G-3'	-
		MK842	5'- GC GCG TAC CGA GTT GGG AAG CAG GC-3'	-
		MK843	5'-GC GCG TAC CGA GTT GGG AAG CAG GC -3'	-
		MK844	5'-cca cct gat ctt cca gcg gtc tag aCC TGG ACA ACT CCC CTA G-3'	-
		MK841	5'-ACG TTA TCT AGA CAA CAC ATT GAT CCG C-3'	-
	Site directed mutagenesis	MK663	5'-TCG TAT GAT TGA GGC GGC CTA TAG TCA GTT GGA AG-3'	
		MK664	5'-GTT AAT ACC GTT CAC GCT TGC GTT TGG ATT GAT GCC CAC GAA TC-3'	
	qPCR	MK561	5'-ATG CTC TTT GTC CCA ACC CTC TCA-3'	-
		MK562	5'-ATG ATC CGG TTC CTC CCA CTG TTT-3'	-
TbPOLIC 3' UTR	qPCR	MK651	5'-TGG CGG ACC GAA TGT TAG TGG AAA-3'	-
		MK652	5'-TGT GTT ACG GGC AAC TCC TCG GA-3'	-
TbPOLID (927.11.3260)	qPCR	MK559	5'-TGG CGA AAT CAT ACG GTG GTC AGA-3'	-
		MK560	5'-TGC GCG AGT GCT CGT TCT ATA TGT-3'	-
TbPOLIB (927.11.4690)	qPCR	MK563	5'-GTG ATT GCA TTC ATG GCG ACG GAA-3'	-
		MK564	5'-AGT ACT TGG TCC ATG GCT CCA CAA-3'	-
TERT (927.11.10190)	qPCR	MK804	5'-GAG CGT GTG ACT TCC GAA GG-3'	-
		MK805	5'-AGG AAC TGT CAC GGA GTT TGC-3'	-
Glu Synth (927.7.4000)	qPCR	MK665	TCT GTT GTA TGC AGC CGC TAT GGA	-
		MK666	AGG AGC TTC CCA GTG AAG TTG ACA	-
PTP	PTP tag amplification	MK681	5'-TTA TTA TCT AGA CCG CTG GAA GAT CAG GTG G-3'	XbaI
		MK713	5'-TTG TTG TGA TCA TCA GGT TGA CTT CCC CGC-3'	BclI

Miller JC et al Supplemental Figure 1

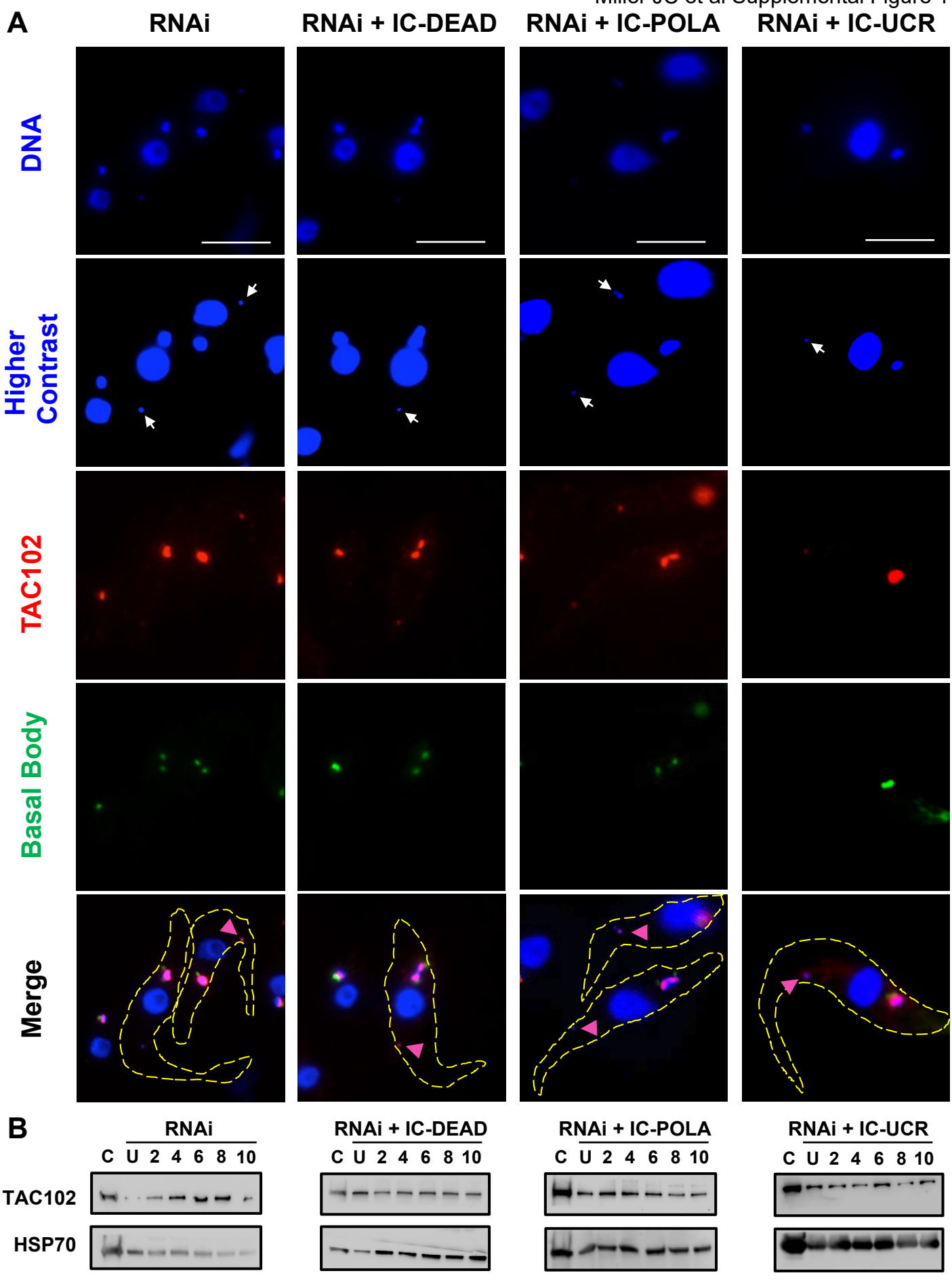

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Fig S1. TAC102 colocalizes with ancillary kDNA. (A) Representative images of TAC102 and ancillary kDNA co-localization in the IC-UTR, IC-DEAD RNAi + OE, IC-POLA RNAi + OE, and IC-UCR RNAi + OE cell lines. DAPI-staining (blue); anti-TAC102 (red); YL1/2 (green). The posterior end of the cell is the rounded end and the expected position of normal kDNA. White arrows indicate ancillary kDNA and purple arrowheads show TAC102 colocalized with ancillary kDNA. Scale bar, 5 µm. **(B)** Western blot detection of TAC102 and HSP70 protein levels following 10 days of induction for each of the listed cell lines. $1x10^6$ cell equivalents was loaded into each well except for C where $5x10^6$ cell equivalents was loaded. +C, single expresser control cell line.

Miller JC et al Supplemental Figure 2

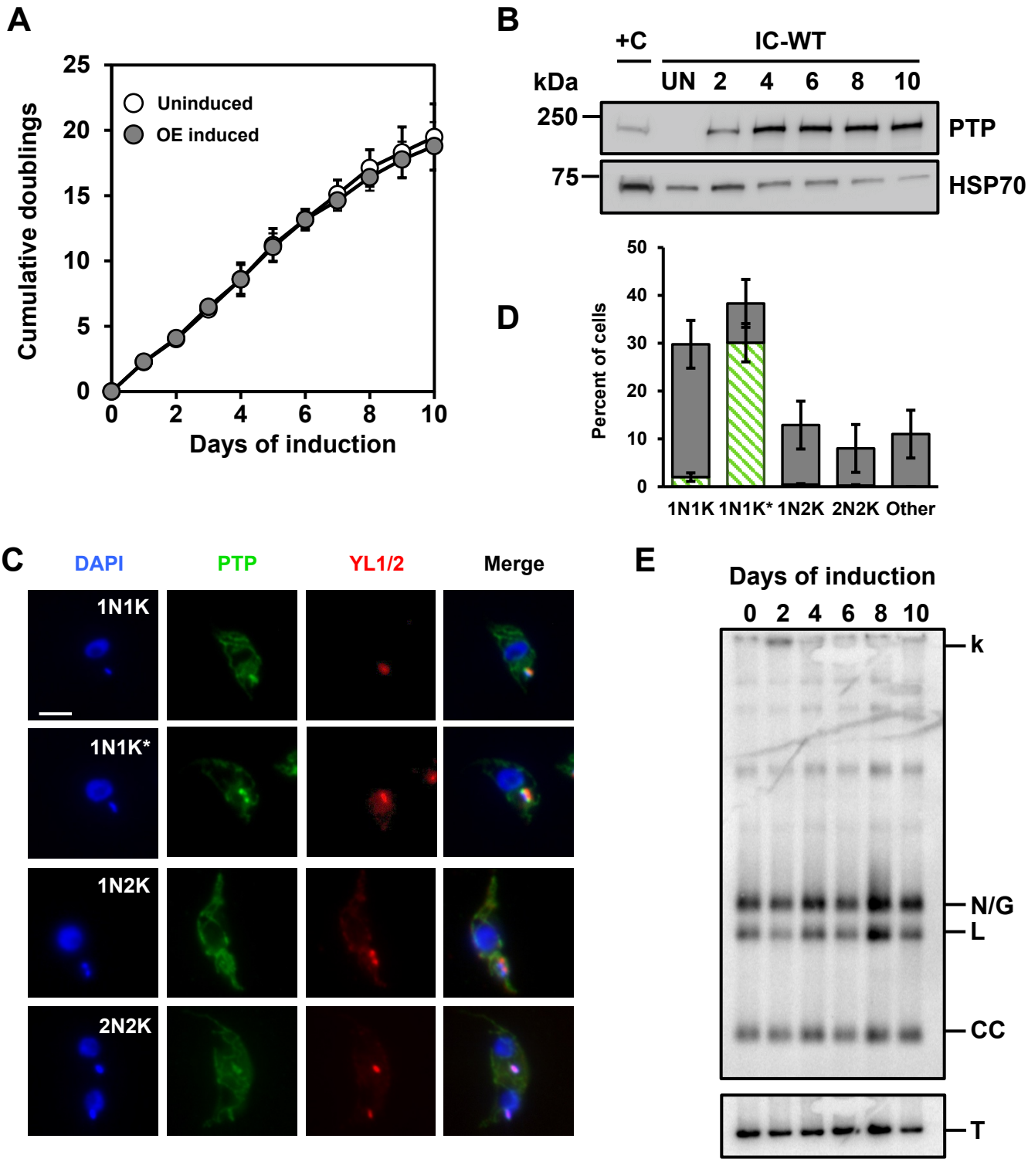

Fig S2. Overexpression of ectopically expressed IC-WT in the absence of *POLIC* RNAi. (A) Growth curves of uninduced and tetracycline-induced procyclic cells. Error bars represent the standard deviation from the mean of three biological replicates. **(B)** Western blot detection of the PTP tag and HSP70 protein levels following 10 days of induced overexpression. $1x10^6$ cell equivalents loaded except for +C where $5x10^6$ cell equivalents was loaded. +C, POLIC-PTP single expresser cell line. **(C)** Representative images of OE IC-WT-PTP throughout the cell cycle. Scale bar, 5 µm. **(D)** Quantification of PTP foci formation at each cell cycle stage. Percentages refer to the fraction of total cells in the population. Green/white hashed, POLIC-PTP foci positive; gray, foci negative. **(E)** Representative Southern blot showing the effect on free minicircle population. k, kDNA network; N/G, nicked/gapped; L, linearized; CC, covalently closed; T, tubulin.

Miller JC et al Supplemental Figure 3

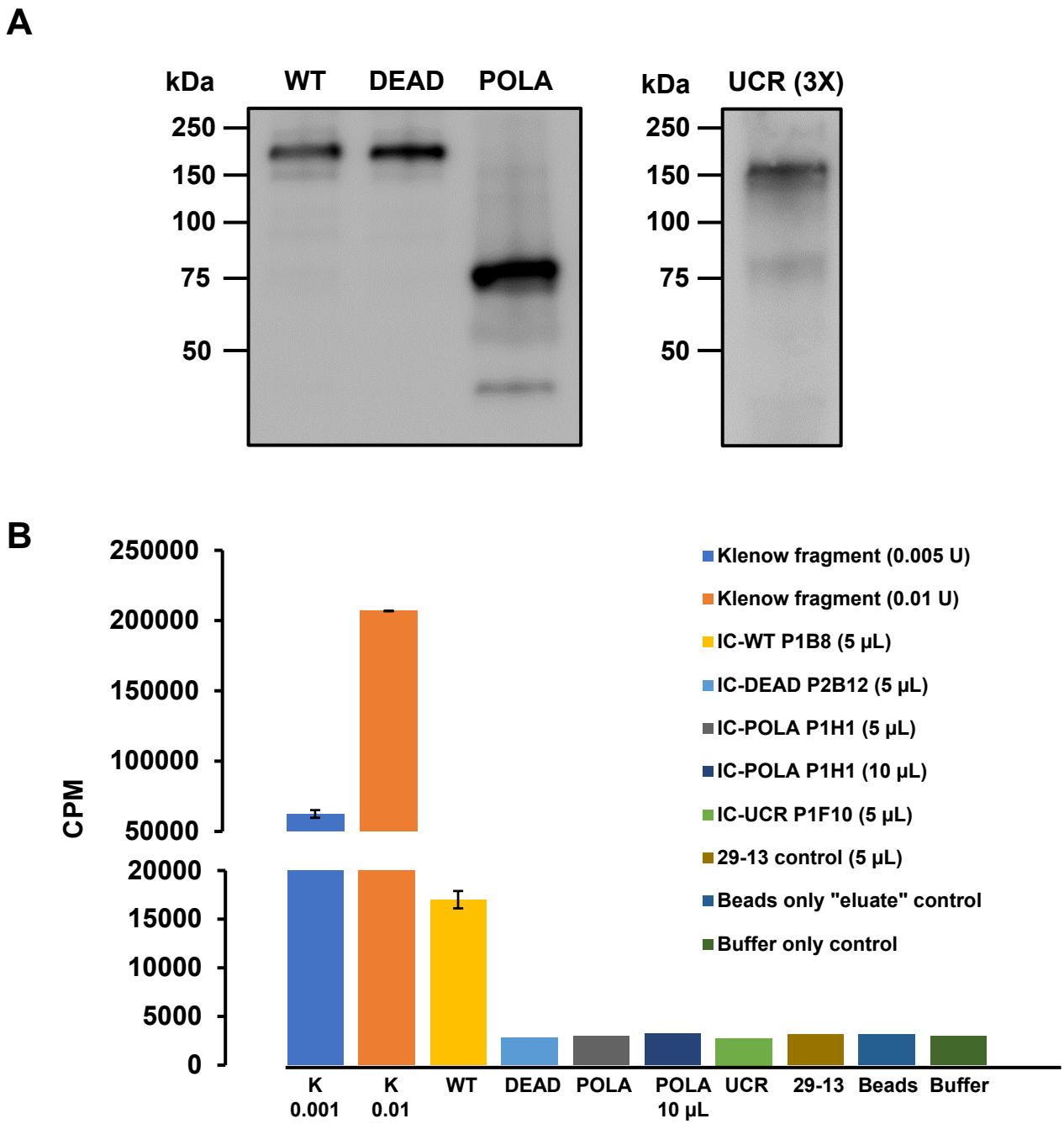

Fig S3. **IC-WT displays nucleotide incorporation activity. (A)** Representative immunoblot analyses of 5 µL eluate from POLIC variant immunoprecipitation from three separate experiments. IP products were run on an 8% SDS-PAGE gel, blotted, and detected with PAP reagent (Sigma). 3X value indicates the number of total cells that were isolated for IP. Protein marker masses are indicated in kilodaltons (kDa). **(B)** Representative assay for DNA incorporation activity of Klenow fragment (Invitrogen) and POLIC variants *in vitro* as measured by counts per minute (CPM) in a scintillation counter from three separate experiments. K, Klenow, numbers underneath indicate enzyme units (U) input; WT, IC-WT; DEAD, IC-DEAD; POLA, IC-POLA, UCR, IC-UCR; numbers indicate µL of eluate input; 29-13, 29-13 cell lysate; Beads, beads only; Buffer, buffer only. Error bars represent standard deviation from two technical replicates.

Miller JC et al Supplemental Figure 4

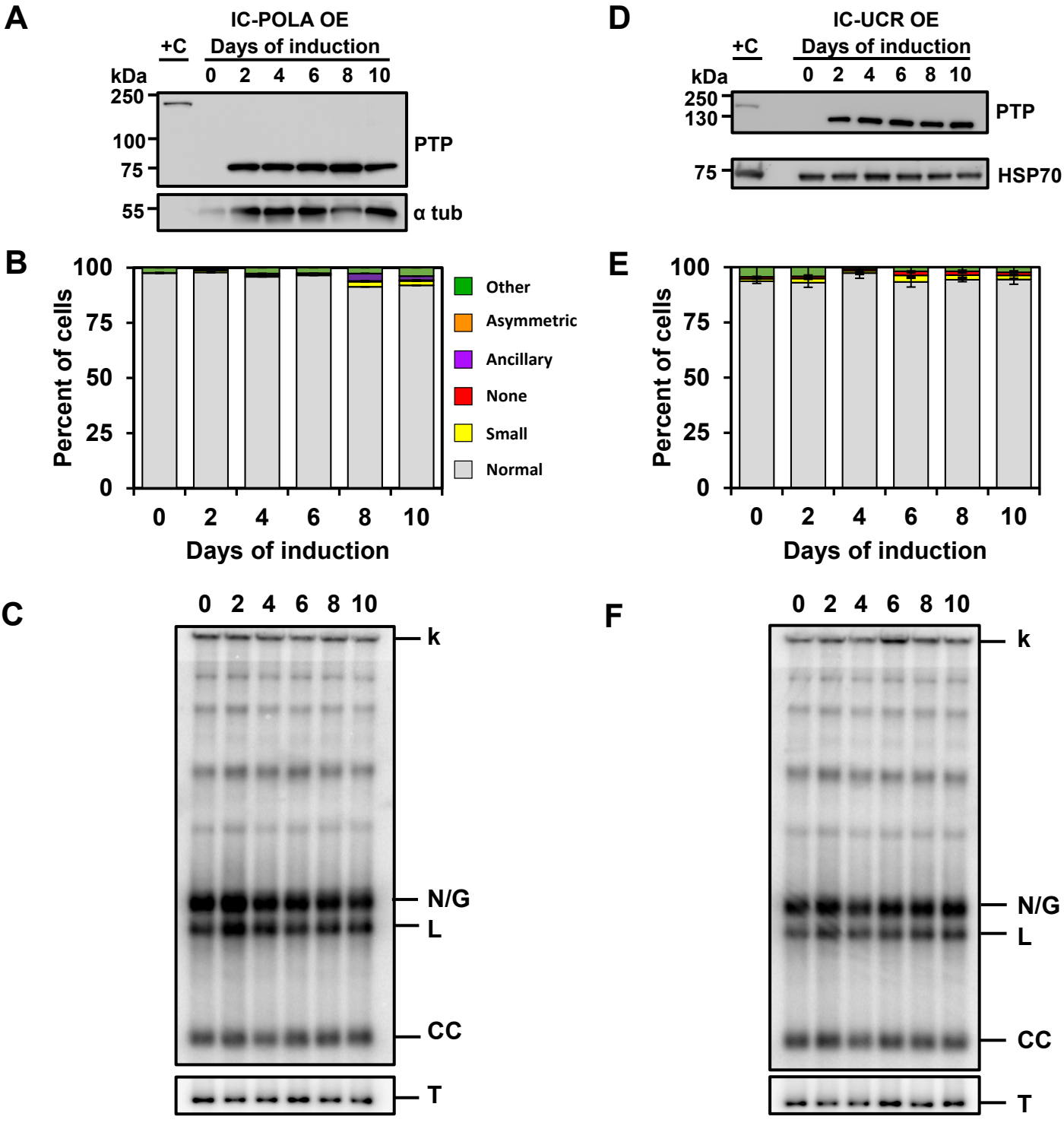

Fig S4. Characterization of IC-POLA and IC-UCR overexpression cell lines. (A) Western blot detection of the PTP tag and HSP70 or alpha tubulin protein levels following 10 days of induction in the OE IC-POLA cell line. **(B)** Quantification of kDNA morphology over the course of the induction in the OE IC-POLA cell line. Over 300 cells were scored at each time point and error bars represent standard deviation from the mean of three biological replicates. Gray, normal kDNA; yellow, small kDNA; red, no kDNA; purple, ancillary kDNA; orange, asymmetric size; green, other. **(C)** Representative Southern blot showing the effect on free minicircle population in the OE IC-POLA cell line. k, kDNA network; L, linearized; N/G, nicked/gapped; CC, covalently closed; T, tubulin. **(D-F)** Same as A-C but for the OE IC-UCR cell line.