The nucleotide triphosphohydrolase HD82 maintains genome integrity and replication stability through dNTP homeostasis control in *Trypanosoma brucei*

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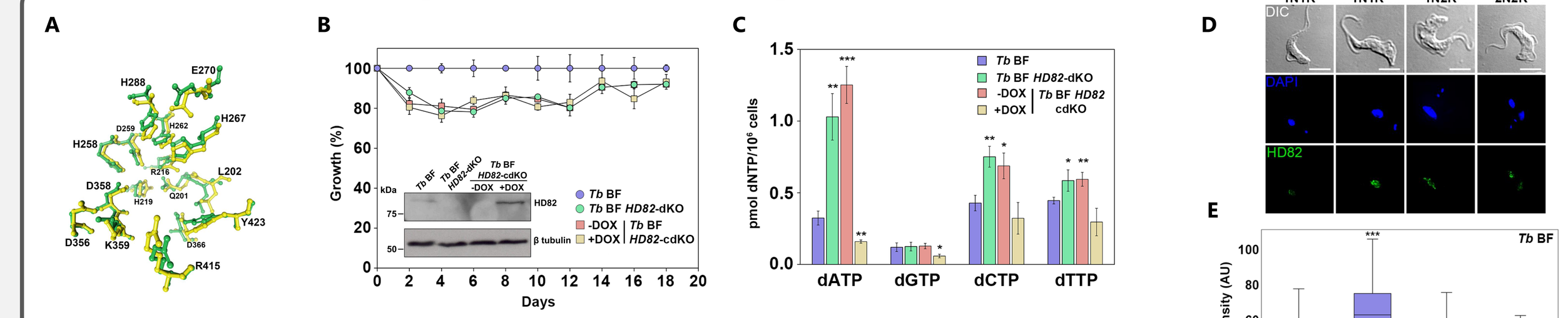
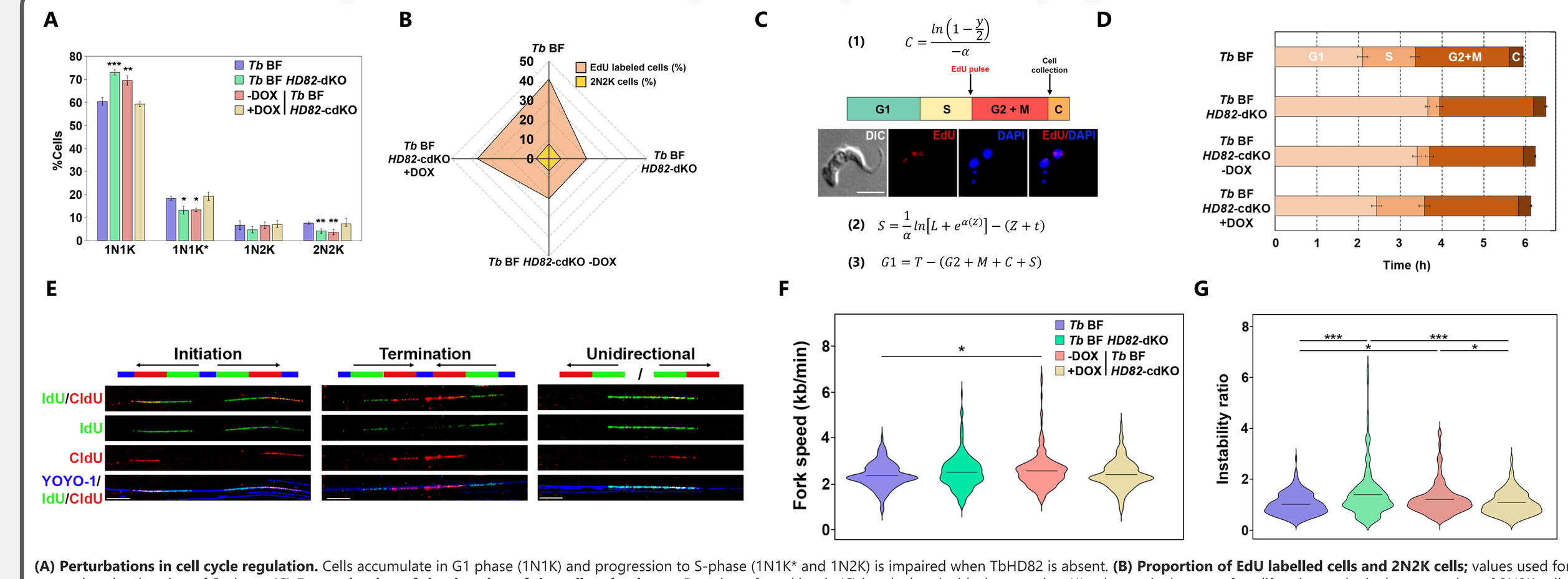
Agents modulating synthesis and incorporation of nucleotides in DNA are widely used as chemotherapeutics. Processes such as DNA replication and DNA repair depend on the accurate regulation of the synthesis and degradation of nucleotides. In humans, the main dNTPase is SAMHD1, which controls the homeostatic balance of dNTP pools. TbHD82 is a SAMHD1 ortholog identified in *Trypanosoma brucei* and sequence alignments show that the amino acids involved in substrate binding and catalysis are all conserved. While TbHD82 is not essential *in vitro* for proliferation of procyclic and bloodstream form parasites, its absence induces an accumulation of dATP, dCTP and dTTP, suggesting that the protein is a dNTPase. The expression of TbHD82 is cell cycle-dependent and HD82-deficient parasites exhibit a hypermutator phenotype, defects in cell cycle progression with a shortened S-phase and increased fork speed and instability. In addition, *TbHD82* null mutants exhibit enhanced activation of the DNA damage response and the enzyme is up-regulated upon genotoxic insult. All these features are in line with the consequences of dNTP imbalances. We suggest that TbHD82 contributes to the maintenance of genome integrity and replication stability by modulating the excessive or unbalanced accumulation of dNTPs.

TbHD82 is a nuclear enzyme with dNTPase activity



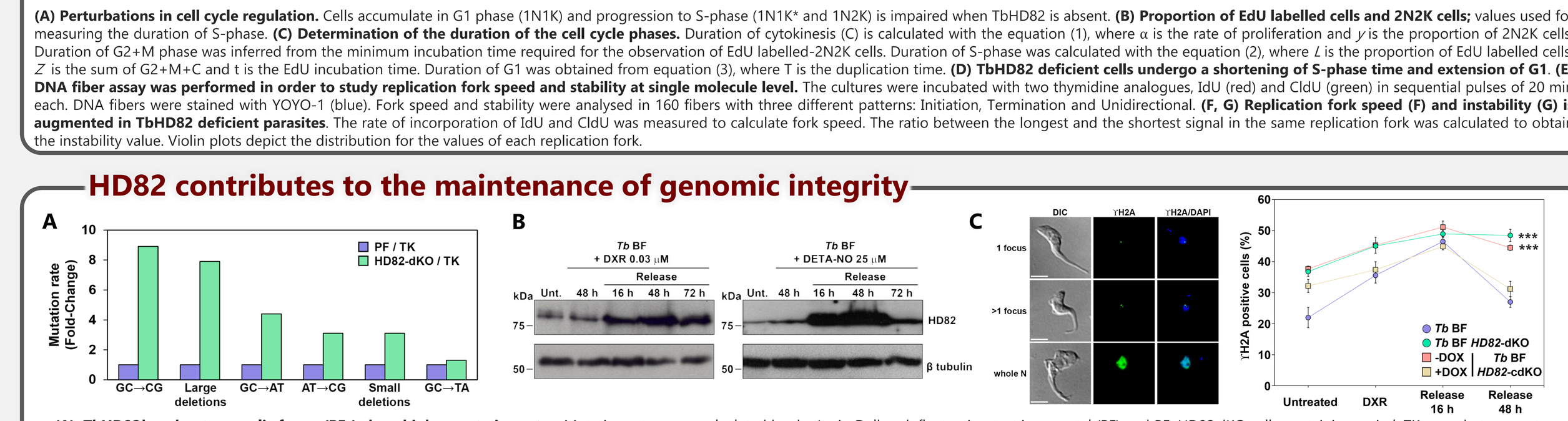
(A) Residues involved in dNTPase activity are conserved in TbHD82. Comparison of the spatial arrangement of catalytic residues from both human SAMHD1 (yellow) and TbHD82 (green). **(B) Growth of TbHD82 deficient cells.** Proliferation relative to parental cells (BF) was monitored for knock-out (dKO) and conditional knock-out cells (cdKO), which harbour an ectopic copy of *TbHD82* inducible by DOX. **(C) HD82 is involved in dNTP hydrolysis.** Quantification of dNTP pools in *HD82*-dKO and cdKO cells. **(D) TbHD82 is a nuclear protein.** Immunofluorescence study showing BF parasites marked with DAPI (blue) and anti-TbHD82 antibody (green). DAPI-stained cells were categorized according to the number of nuclei (N) and kinetoplasts (K) in 1N1K (G1-phase), 1N1K* (early S-phase), 1N2K (late S-phase) and 2N2K (G2/M-phase) populations; asterisks denote an enlarged K under segregation. **(E) TbHD82 expression is cell cycle regulated.** Quantification of signal intensity by immunofluorescence in parental BF parasites taking into account their N and K endowment as described in (D).

Absence of HD82 produces defects in cell cycle and replication fork progression



(A) Perturbations in cell cycle regulation. Cells accumulate in G1 phase (1N1K) and progression to S-phase (1N1K* and 1N2K) is impaired when TbHD82 is absent. **(B) Proportion of EdU labelled cells and 2N2K cells;** values used for measuring the duration of S-phase. **(C) Determination of the duration of the cell cycle phases.** Duration of cytokinesis (C) is calculated with the equation (1), where α is the rate of proliferation and *y* is the proportion of 2N2K cells. Duration of G2+M phase was inferred from the minimum incubation time required for the observation of EdU labelled-2N2K cells. Duration of S-phase was calculated with the equation (2), where *L* is the proportion of EdU labelled cells, *Z* is the sum of G2+M+C and t is the EdU incubation time. Duration of G1 was obtained from equation (3), where T is the duplication time. **(D) TbHD82 deficient cells undergo a shortening of S-phase time and extension of G1**. **(E) DNA fiber assay was performed in order to study replication fork speed and stability at single molecule level.** The cultures were incubated with two thymidine analogues, IdU (red) and CldU (green) in sequential pulses of 20 min each. DNA fibers were stained with YOYO-1 (blue). Fork speed and stability were analysed in 160 fibers with three different patterns: Initiation, Termination and Unidirectional. **(F, G) Replication fork speed (F) and instability (G) is augmented in TbHD82 deficient parasites**. The rate of incorporation of IdU and CldU was measured to calculate fork speed. The ratio between the longest and the shortest signal in the same replication fork was calculated to obtain the instability value. Violin plots depict the distribution for the values of each replication fork.

HD82 contributes to the maintenance of genomic integrity



(A) *TbHD82* knockout procyclic forms (PFs) show higher mutation rates. Mutation rates were calculated by the Luria-Delbruck fluctuation test in parental (PF) and PF *HD82*-dKO cells containing a viral *TK* gene that generates ganciclovir sensitivity, being 3.8 times higher in *HD82* null mutants. Mutation spectra were evaluated using the *TK* reporter gene. Differentially enriched mutations are shown in the column plot. **(B) TbHD82 is overexpressed upon genotoxic insult.** A release assay was performed by exposing BF parasites to doxorubicin (DXR) and DETA-NO. The treatments were performed for 48 h, followed by a wash-out to remove the compounds. TbHD82 expression was verified by western blot before and after treatment as well as during the release period. **(C) TbHD82 contributes to efficient DNA damage repair.** The DNA damage response marker γ-H2A was detected by IF. Knock-out cells exhibit higher γ-H2A levels compared to parental parasites in both treated and untreated cells and are more inefficiently repaired.

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