Supplemental Materials

Molecular Biology of the Cell

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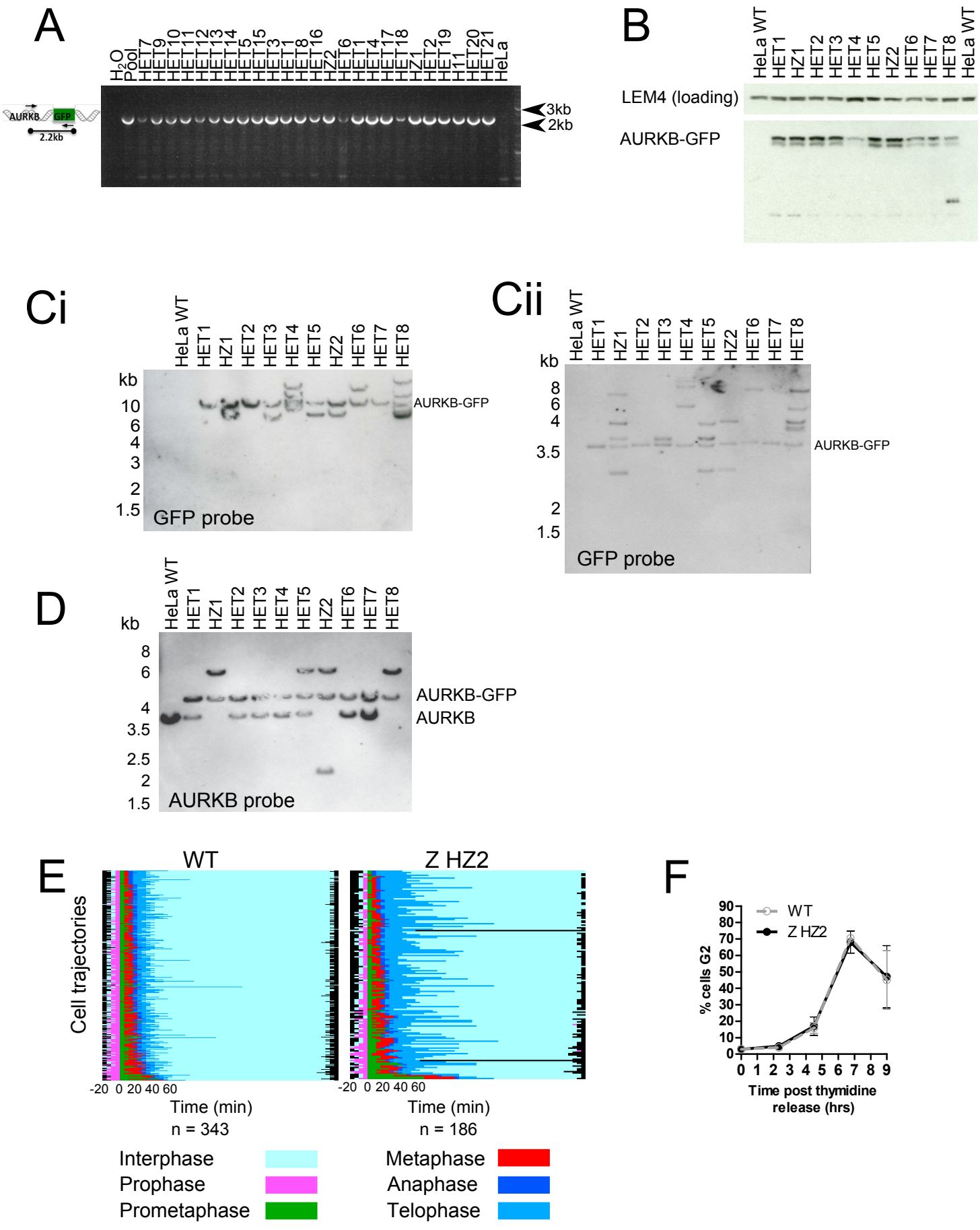
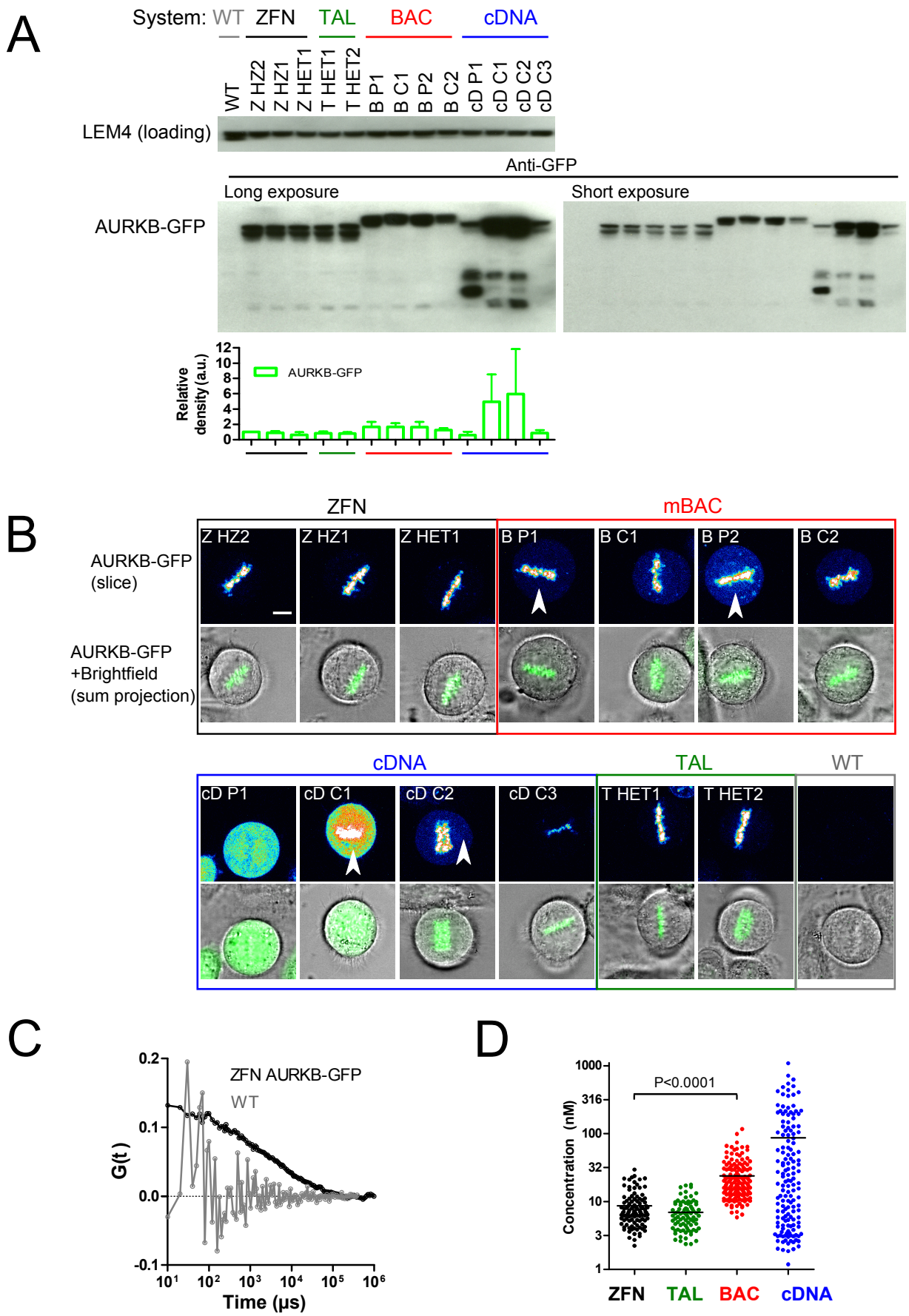
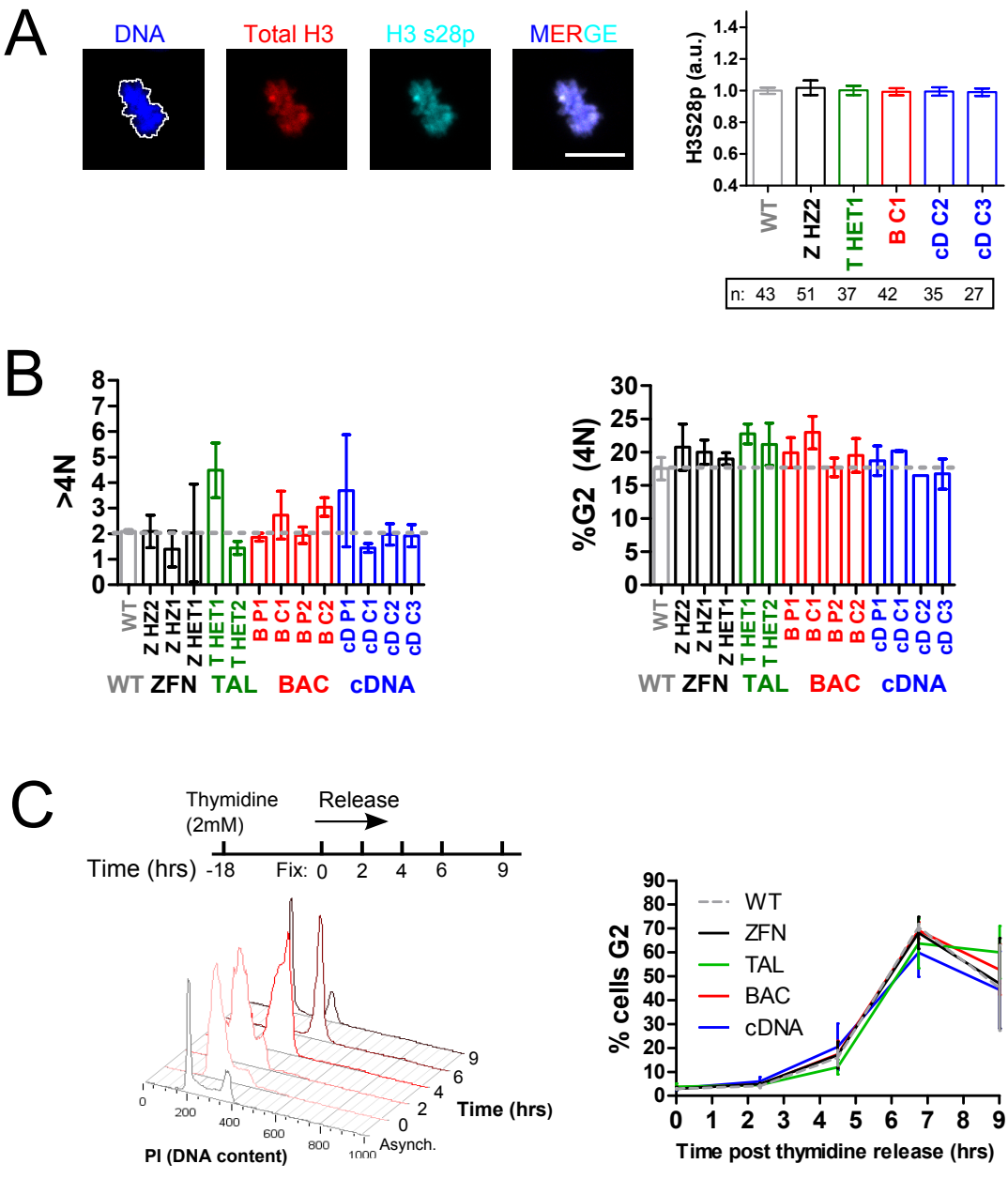


Supplemental Figure 1: Construction and validation of clonal ZFN cell lines expressing AURKB-GFP (A) Additional junction PCR screening of EGFP incorporation at AURKB loci in ZFN cells, using a primer pair with one primer annealing inside of GFP. (B) Western blot screening of nocodazole arrested AURKB-GFP ZFN cells using anti-GFP antibody. (C) Southern blot screening of EGFP genomic incorporation in ZFN clones using an EGFP DNA probe, after digestion with either (i) Kpn1 and Nsi1 or (ii) Xba1. (D) Southern blot screening of AURKB genomic incorporation using an AURKB 3' probe, after Xba1 digestion. (E) Cell trajectories from live cell imaging data calculated by automated cell cycle stage classification in CellCognition (Held et al., 2010). (F) Cell cycle progression after release from thymidine arrest, measured by flow cytometry analysis of propidium iodide staining. The graph shows the mean +/- SD from three experiments.



Supplemental Figure 2: Plasmid based AURKB-GFP gene tagging causes cytosolic overexpression. (A) Western blot of AURKB-GFP as described in Figure 1F but using anti-GFP antibody. The graph displays densitometry of only the bands corresponding in size to AURKB-GFP. (B) Confocal images of AURKB-GFP metaphase cells taken using the same imaging conditions throughout and represented as a sum intensity z-stack projection, false coloured relative to fluorescence intensity. Arrows denote high cytoplasmic levels. Scale bar 7µm. (C) Single example FCS autocorrelation curves from ZFN genome edited AURKB-GFP (Z 1HZ1) and wild type cells (negative control). (D) Cytoplasmic concentrations of AURKB measured as in Figure 2D except pooling all clones of one expression type together. Horizontal lines represent the mean. Statistical test with Mann-Whitney test.



Supplemental Figure 3: Overexpression does not increase AURKB kinase activity or alter cell cycle progression. (A) Immunofluorescent staining of the AURKB substrate Histone H3 S28p, scale bar 15µm. Chromatin was automatically located by thresholding of Hoechst (white line in DNA panel). The graph denotes the ratio of the intensity of H3 S28p relative to total H3, normalised to wild type, from three experiments, mean -/+ SD. (B) Flow cytometry enumeration of aneuploidy and G2% based on propidium iodide staining of DNA content. Mean and SD are plotted. (C) Flow cytometry analysis of cell cycle progression after release from arrest. The example raw histograms show propidium iodide staining in wild type cells. The graph plots the mean +/- SD from three experiments, measuring G2% by fitting the propidium iodide histograms using the cell cycle algorithm Watson (pragmatic). Clones are grouped according to tagging system.