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Human Nucleosome Assembly Protein 1-Like 5 (NAP1L5) Promotes The Proliferation of 293T Cells

Dear Editor,

The nucleosome assembly protein 1 (NAP-1), being a histone chaperone [1], primarily participates in the processes of nucleosome assembly and disassembly, histone transport, and histone eviction. Other functions were also endowed with this highly conserved protein. In budding yeast, NAP-1 specifically interacts with mitotic cyclins [2-3], and the absence of NAP-1 causes prolonged mitotic delays[2].

During evolution higher-ordered plants and mammals have acquired several paralogues of NAP-1 [4]. At least, five paralogous Nap-1 genes were identified in both mice and humans[5]. Mouse NAP1L2 protein could bind to condensed chromatin during the S phase, regulate neuronal cell proliferation, and play an essential role in neural tube development and neuronal differentiation[6]. The mouse Nap1l5 gene was first identified in mouse parthenogenetic embryos[7], as a paternally imprinted gene, its expression was also detected in the brains and adrenals of adult mice，as well as day-13 foetuses [7]. Human NAP1L1 can be detected in most tissues and cell lines, and its expression was frequently found in rapidly proliferating cells [4]. This protein promotes cell proliferation through the regulation of $p57^{Kip2}$ promoter methylation [8]. The predicted C-terminal protein sequence of the human Nap1l3 gene shares conserved motifs with yeast NAP-1, and its N-terminus shows little conservation[9]. Human NAP1L4 is also known as human NAP-2, acting as a histone chaperone throughout the cell cycle, and its expression level increases with cell cycle progression from the G0/G1 boundary to M-phase [10]. Until recently, the functions of the human NAP1L3 and NAP1L5 have not been elucidated.

The human Nap1l5 gene is imprinted and paternally expressed [7]. The gene is also known as DRLM (down-regulated in liver malignancy), located at 4q22.1. This region is a target of allelic loss in liver malignancies, such as hepatocarcinoma and hepatoblastoma, suggesting that candidate tumour suppressor genes were harboured there [11], and human NAP1L5 might play a negative role in cell cycle progression, since other validated tumour suppressors, such as p53, typically induce cell cycle arrest and inhibit cell growth [12]. This postulation about human NAP1L5 is inconsistent with the reported function of its homologous yeast NAP-1, mouse NAP1L2, as well as human NAP1L1 and NAP1L4, which promotes cell cycle progression [2-3]. Until recently, whether human NAP1L5 promotes or inhibits cell proliferation has not been determined.

To explore the role of human NAP1L5 in the regulation of cell proliferation, we established Nap1l5-knockdown- and Nap1l5-overexpressed-293T cells with lentiviral shRNA pLKO.1 vector and retroviral pQCXIG vector (modified pQCXIP vector with the puromycin gene replaced with GFP gene), respectively. The empty pLKO.1 and pQCXIG vectors were also transfected into 293T cells as negative controls. The qRT-PCR results confirmed that the level of NAP1L5 mRNA was significantly decreased in Nap1l5-knockdown-293T cells and dramatically increased in Nap1l5-overexpressed-293T cells compared with their corresponding negative controls, respectively (Figure 1a, b). We also attempted to validate the expression change of NAP1L5 protein by western blot analysis; however, the specificity of several commercially available NAP1L5 antibodies was poor.

To evaluate the effect of NAP1L5 on cell proliferation, stably transfected 293T cells were

cultured in 96-well plates at 1.8×10^4 cells/cm^2. Cell numbers were measured by CCK-8 assay at 48 h as previously reported[13]. Nap1l5-knockdown-293T cells showed a decreased growth rate and Nap1l5-overexpressed-293T cells displayed an increased growth rate compared to their control cells, respectively(Figure 1c, d). We also used crystal violet staining to verify the above results(Figure 1e, f), which was consistent with the CCK-8 results. The results indicated that NAP1L5 promotes the proliferation of 293T cells. We also overexpressed rat NAP1L5 in INS-1 cells, and the same result was obtained, indicating that rat NAP1L5 promoted cell proliferation (Figure S1).

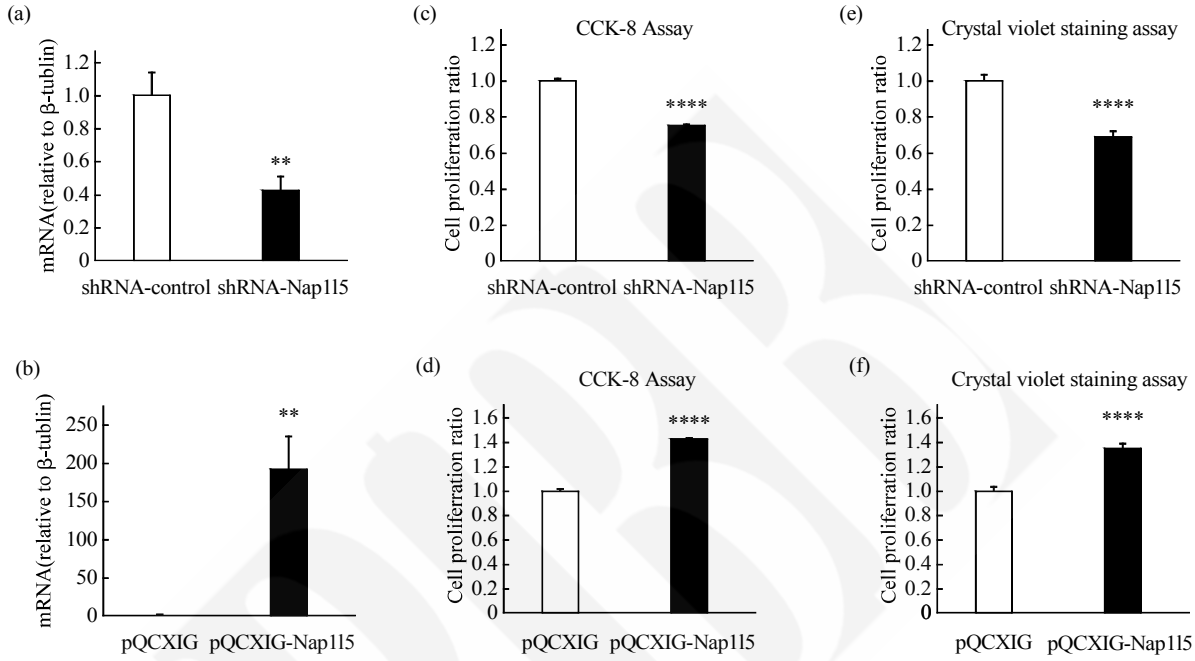

Fig. 1 NAP1L5 promotes the proliferation of 293T cells

The mRNA expression of NAP1L5 was detected by qRT-PCR in Nap1l5- knockdown (a), Nap1l5-overexpressed (b) and their corresponding control cells. The Nap1l5- knockdown and Nap1l5-overexpressed 293T cells were seeded onto 96-well plates at a density of 1.8×10^4 cells/cm^2. Cell proliferation was measured by CCK-8 assay at 48 h, and the normalized cell proliferation rate in Nap1l5-knockdown (c), Nap1l5-overexpressed (d) and their corresponding control cells was calculated. The cell proliferation rate was also determined by crystal violet staining assay with an initial seeding density of 3.0×10^4 cells/cm^2 in Nap1l5- knockdown (e), Nap1l5-overexpressed (f) and their corresponding control cells. The values are presented as the means± S.E.M; $^{**}P<0.01$; $^{****}P<0.0001$ (shRNA-Nap1l5 *vs*. shRNA-control; Nap1l5 *vs*. pQCXIG).

As NAP1L5 promotes cell proliferation, we analysed the cell cycle of Nap1l5-knockdown-293T and Nap1l5-overexpressed-293T cells and their controls by analyzing the DNA content with propidium iodide (PI) staining and flow cytometry (Figure 2a, b, c, d). Compared with the corresponding control groups, the percentage of Nap1l5-knockdown cells in G1 phase was higher than that in control cells at 12 h, and there was a lower percentage of cells in the G2 phase (Figure 2e), while in Nap1l5-overexpressed-293T cells, a lower percentage of cells was detected in the G1 phase, and a higher percentage of cells in the G2 phase (Figure 2f). These results indicated that the overexpression of NAP1L5 accelerated cell cycle progression, while the knockdown of NAP1L5 expression inhibited this process.

In the present study, we first showed that the overexpression of NAP1L5 dramatically promoted the proliferation of 293T cells, while Nap1l5-knockdown-293T cells showed a decrease in growth rate. Furthermore, we demonstrated that NAP1L5 accelerated the cell cycle process. These findings suggested that similar to homologous yeast NAP-1, mouse NAP1L2, as well as human NAP1L1 and

NAP1L4, human NAP1L5 promoted cell cycle progression, but did not inhibit cell proliferation. The findings of the present study excluded the possibility that human Nap1l5/DRLM served as tumour suppressor gene by playing a negative role in cell cycle progression and helped to elucidate the function of this unknown protein.

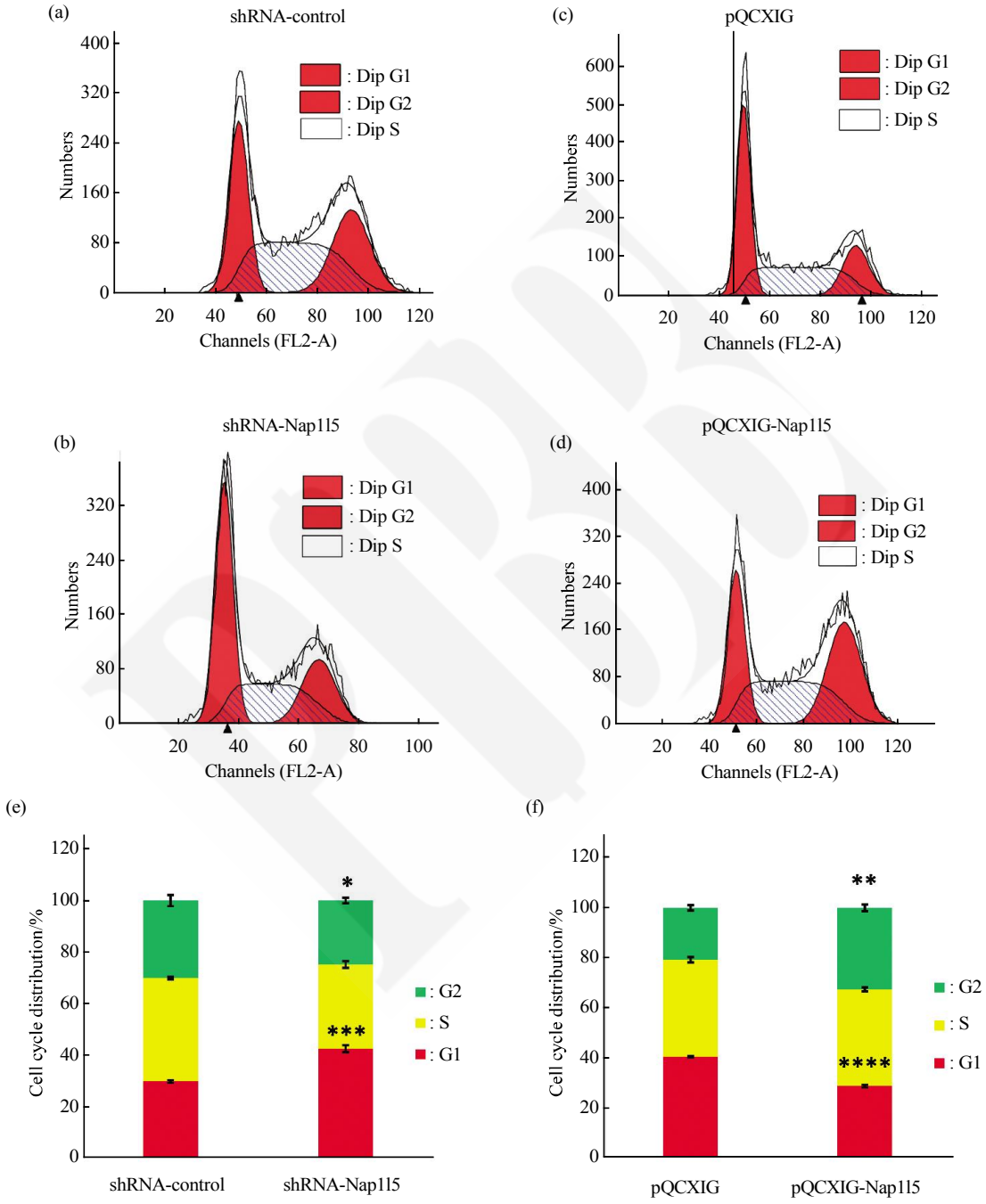

Fig. 2 Effect of NAP1L5 on the progression of the 293T cell cycle

Cell cycle distribution was monitored by flow cytometric analysis with PI staining in cells stably transfected with (a) shRNA-control. (b) shRNA-Nap1l5. (c) pQCXIG; and (d) pQCXIG-Nap1l5. The bar graph displayed the percentage of Nap1l5-knockdown-293T and control cells (e), Nap1l5-overexpressed-293T cells and their controls (f) in the G1, G2 and S phases. The values are presented as the means± S.E.M; $n = 3$; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ****$P < 0.0001$.

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Supplementary material Figure S1 is available at paper online(http://www.pibb.ac.cn).

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Supplements

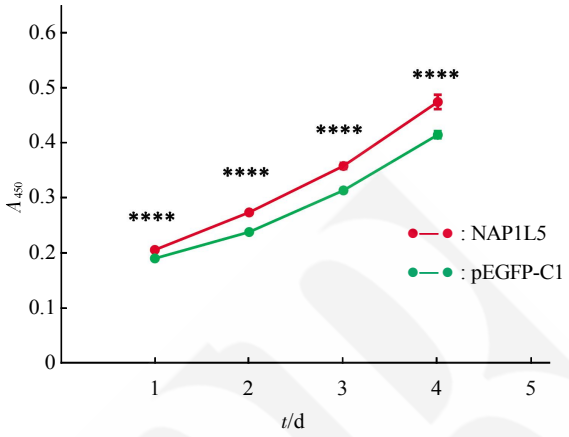

Fig. S1 NAP1L5 promotes the proliferation of INS-1 cells

Nap1l5-overexpressed and control cells were seeded onto 96-well plates at a density of 1.2×10^4 cells/cm^2. Cell proliferation was measured by CCK-8 assay. The values are presented as the means± S.E.M; ****$P < 0.0001$ (NAP1L5 *vs*. pEGFP-C1).