

## Original Article

# NudCD1 affects renal cell carcinoma through regulating LIS1/Dynein signaling pathway

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**Abstract:** Renal cell carcinoma (RCC) is one of the most common malignant tumors in urogenital system with an incidence accounting for about 3% of the whole body malignant tumor. NudC domain containing 1 (NudCD1), a new member of NudC family distributed in nucleus, is found to be upregulated in multiple tumors. However, its expression and role in RCC tissue has not been elucidated. NudCD1 expression in RCC tissue was measured by western blot and immunohistochemistry (IHC). NudCD1 level was elevated by overexpression vector to investigate its regulatory role on LIS1/Dynein signaling pathway. Cell morphology, intracellular localization, and cell division were observed by immunofluorescence together with delayed microscope photograph. The impact of NudCD1 overexpression on cell migration was assessed by Transwell assay. NudCD1 expression was significantly increased in RCC tissue compared with that in adjacent normal control. NudCD1/LIS1/Dynein signaling pathway was obviously upregulated in RCC tissue. Overexpression of NudCD1 level in A498 cell line markedly elevated NudCD1/LIS1/Dynein signaling pathway, suggesting they might be involved in RCC process. NudCD1 upregulation also caused abnormal microtubule fasciculus structure with multinuclear morphology, and promoted cell migration. NudCD1 expression was obviously increased in RCC and affected RCC cell division and migration possibly through activating NudCD1/LIS1/Dynein signaling pathway, indicating therapeutic targeting NudCD1 might be a new approach to inhibit RCC cell migration.

**Keywords:** Renal cell carcinoma, NudCD1, LIS1/Dynein signaling pathway, microtubule fasciculus, migration

## Introduction

The incidence and death rate of renal cell carcinoma (RCC) are increased year by year. It is urgent to strengthen the basic research on the early occurrence of RCC. Exploration of the molecular markers that are closely related to the early occurrence and progression of RCC is of great significance [1].

It was found that NudC domain containing 1 (NudCD1), a new member of NudC family distributed in nucleus, is a kind of broad-spectrum tumor antigen or cancer gene [2]. It plays an important role in the process of cell mitosis and affects cell movement through acting on tubulin [3]. It was showed that NudCD1 is not only highly expressed in chronic myelogenous leukemia cells [4, 5], but also expressed in lung cancer, melanoma, and prostate cancer tissue and cell lines [6-8]. Moreover, it is likely to be involved in cell mitosis, invasion, and migration.

However, the role of NudCD1 in RCC and the possible signaling pathway, as well as its influence on cell division and migration remain to be further studied. Cell biology proposes that disorders of cell mitosis, intracellular transportation, cell cycle, and cell migration are the earliest cytological changes in tumor-acquired malignant biological behaviors [9]. Nuclear distribution gene C (NudC)/Lissencephaly 1 (LIS1)/Dynein signaling pathway and related family members that can regulate abovementioned cell function and are related to tubulin play an important role in the development of tumor [10, 11]. However, it is still unclear whether overexpressed NudCD1 can activate the signaling pathway to affect the behavior of cancer cells.

Therefore, this study aimed to detect NudCD1 protein expression and NudCD1/LIS1/Dynein signaling pathway activation in RCC tissues and adjacent normal tissues by IHC. Then, we overexpressed NudCD1 to explore its influence on

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NudCD1/LIS1/Dynein signaling pathway. Cell morphology, intracellular localization, and cell division were observed by immunofluorescence and delayed microscope photograph. The impact of NudCD1 overexpression on cell migration was detected by Transwell assay. This study may provide a theoretical basis for the discovery of new biomarker for the diagnosis of RCC.

### Materials and methods

#### *Main reagents*

Total protein extraction kit was purchased from Keygen (Nanjing, China). Western blot RIPA and BCA protein quantification kits were obtained from Beyotime (Suzhou, China). NudCD1 antibody was bought from Proteintech (Wuhan, China). HRP labeled goat anti mouse IgG (H+L) and HRP labeled goat anti rabbit IgG (H+L) were got from ZSbio (Beijing, China). FBS and cell medium were provided by Gibco (New York, NY). Other reagents were supplied by Sigma (CA, USA).

#### *Main instruments*

Benchtop was obtained from Boxun (Shanghai, China). Gel imaging system was provided by UVP Multispectral Imaging System (CA, USA). PS-9 semi-dry electrophoresis apparatus was bought from Jingmai company (Dalian, China). BD FACS Calibur flow cytometry was purchased from BD Biosciences (San Diego, CA). Carbon dioxide incubator and Thermo-354 microplate reader were got from Thermo Fisher (New York, NY). Leica SR GSD inverted fluorescence microscope was derived from Leica (Shanghai, China).

#### *Sample source*

A total of 145 cases of human RCC tissues and adjacent normal tissues which were located at least 3 cm away from the tumor margin were collected from the Department of Surgery between Jan 2016 and Jan 2017. The sample was stored at -80°C. All the subjects had signed informed consents.

#### *Immunohistochemistry*

The tissue was embedded by wax and cut into slices. After dewaxing, the sample was incubated with 3% hydrogen peroxide for 20 min to

eliminate endogenous peroxidase activity. Then the sample was incubated with citrate buffer at 95°C for 10 min to repair the antigen and further blocked in goat serum for 20 min. Next, the sample was incubated with NudCD1 primary antibody (1:100 dilution) at 4°C overnight and further incubated with HRP labeled secondary antibody. The sample was further developed by DAB and redyed by hematoxylin. The positive result was scored according to the cell number and staining intensity. Score 0, positive cell number < 5%; score 1, positive cell number between 5% and 25%; score 2, positive cell number between 26% and 50%; score 3, positive cell number between 51% and 75%; and score 4, positive cell number between 76% and 100%. For staining intensity evaluation, score 0, colourless; score 1, faint yellow; score 2, claybank; score 3, sepia. The positive degree was assessed by the product of the two scores. Negative (-), score 0; weak positive (+), score 1-4; positive (++), score 5-8; strong positive (+++), score 9-12.

#### *Cell culture and transfection*

RCC cell line A498 was purchased from the cell bank of Chinese academy of sciences (Shanghai, China). All the cells were maintained in DMEM medium containing 10% FBS, 100 U/ml penicillin, and 100 µg/ml streptomycin. The cells were cultured at 37°C with 5% CO<sub>2</sub>.

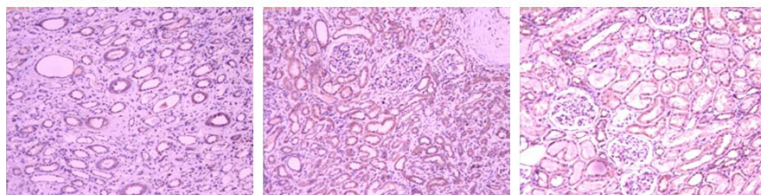
GFP labeled NudCD1 overexpression vector was designed and synthesized by Genepharma. The cells were seeded into 24-well plate on the previous day before transfection. A total of 1.25 µl siRNA or overexpression vector at 20 µM was solved in 100 µl Opti-MEM medium, while 1 µl Lipofectamine 2000 or Lipofectamine™ RNAiMAX was solved in Opti-MEM medium. After 5 min incubation, the two types of solutions were mixed for 20 min. After 4 h incubation, the fluid was changed back to DMEM medium containing 10% FBS.

#### *Western blot*

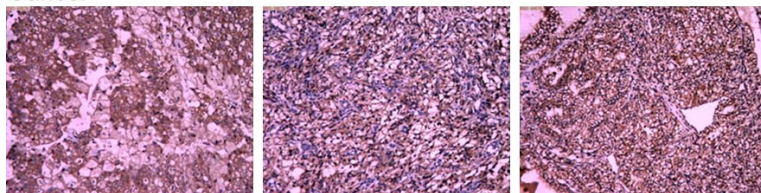
The lysis buffer was added with PMSF to make a final concentration of 1 mM. A total of 100 mg tissue was lysed in the buffer at 4°C for 15 min and then centrifuged at 14000 rpm for 15 min. The protein was moved to a new EP tube and quantified by BCA method. After boiled for 5 min, the protein was stored at -20°C. The pro-

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### Paracancer



### Cancer



**Figure 1.** NudCD1 expression and cellular localization in RCC tissue and adjacent normal tissue (20 $\times$ ).

tein was separated by 8-12% SDS-PAGE and transferred to PVDF membrane at 300 mA for 1 h. Next, the membrane was incubated with NudCD1 primary antibody (1:1000) at 4 $^{\circ}$ C overnight and washed by TTBS for three times. Then the membrane was further incubated with goat anti rabbit secondary antibody (1:1000) at 37 $^{\circ}$ C for 2 h. At last, the membrane was developed by chemiluminescence and captured by Bio-rad system containing Chemi HR camera. The image was analyzed by Gel-Pro Analyzer Version 4.0 software.

### Immunofluorescence

NudCD1 overexpressed cells were moved to six-well plate and fixed in 4% paraformaldehyde. Then 0.2% Triton X100-PBS was added into the cells for blockage. Next, the sample was incubated with NudCD1 (1:50) and  $\beta$ -tubulin (1:100) primary antibodies, and further incubated with fluorescence secondary antibody. At last, the cells were incubated in DAPI and observed under inverted fluorescence microscope for intracellular localization, chromosomal aggregation, cytoplasmic division, and nuclear abnormalities. pGFP-NudCD1 was green,  $\beta$  tubulin was red, and DNA was blue.

### Cell migration

The cells in logarithmic phase were seeded in the upper chamber, while cell medium containing 20% FBS was added to the lower chamber. After 18 h cultivation, the cells on the membrane was fixed and counted under the microscope.

### Statistical analysis

All data analyses were performed on SPSS 19.0 software. The measurement data were presented as mean  $\pm$  standard deviation (SD) and compared by t test or one-way ANOVA.  $P < 0.05$  was depicted as statistical significance.

### Results

#### *NudCD1 expression and cellular localization in RCC tissue and adjacent normal tissue*

As shown in **Figure 1**, NudCD1 presented positive (++) or strong positive (+++) expression in RCC tissue, while negative or weak positive expression in adjacent normal control. It was mainly located in the cytoplasm of renal parenchyma, including renal tubule and collecting tube, but not glomerulus. NudCD1 protein expression demonstrated certain tendency with differentiation degree.

#### *NudCD1, LIS1, and Dynein expressions in RCC tissue and paracancer tissue*

NudCD1, LIS1, and Dynein levels were significantly upregulated in RCC tissue compared with those in paracancer tissue ( $P < 0.05$ ) (**Figure 2**).

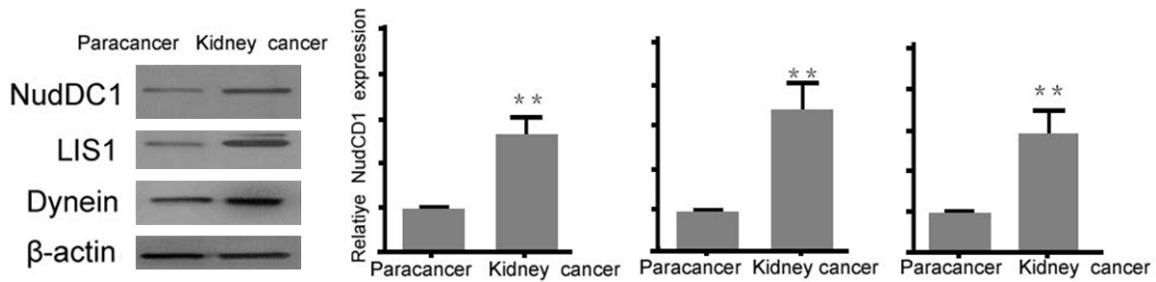
#### *LIS1 and Dynein expressions changes in A498 cells after lentivirus transfection*

To investigate the impact of NudCD1 on signaling pathway, we applied transfection to overexpress NudCD1. NudCD1 level was obviously increased after transfection compared with empty vector group (**Figure 3**). LIS1 and Dynein expressions were markedly upregulated after NudCD1 overexpression compared with GFP empty vector group ( $P < 0.05$ ) (**Figure 3**).

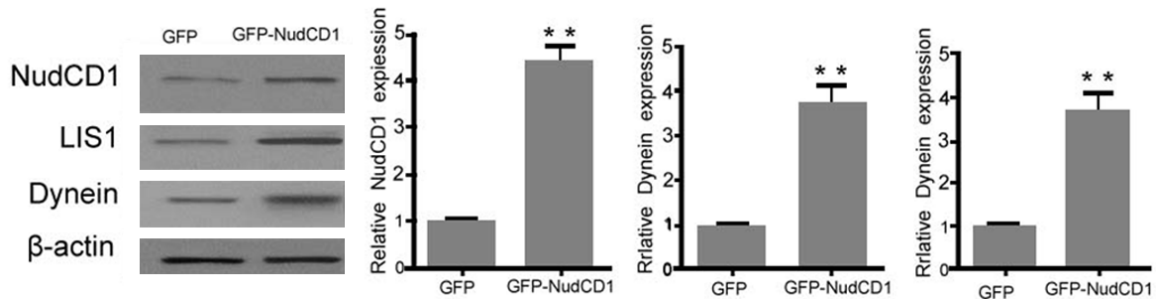
#### *The influence of NudCD1 on cancer cell nucleus*

The cells were transfected with GFP-NudCD1 overexpression vector and stained by DAPI. Immunofluorescence revealed that cells transfected with GFP-NudCD1 displayed multinucleate phenomenon compared with blank vector group (**Figure 4**).

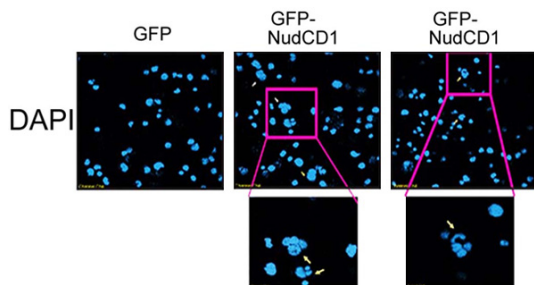
## NudCD1 affects RCC migration



**Figure 2.** NudCD1, LIS1, and Dynein expressions in RCC tissue and paracancer tissue. \*\*P < 0.05, compared with adjacent normal tissue.



**Figure 3.** NudCD1, LIS1, and Dynein expressions after NudCD1 overexpression. \*\*P < 0.05, compared with GFP empty vector group.



**Figure 4.** The influence of NudCD1 on cancer cell nucleus.

### *The impact of NudCD1 on cell division*

To explore the mechanism of multinucleate phenomenon, we recorded the cell division process through delay video microscope. As shown in **Figure 5**, the division furrow exhibited abnormal microtubule fasciculus structure. The annulus disappeared in some cells, while the daughter cell junction was steady which cannot be clipped. Thus, we speculated that NudCD1 caused microtubule fasciculus abnormality, which may be the reason of multinucleation.

### *The effect of NudCD1 on cell migration*

Transwell assay was performed to test the effect of NudCD1 on cell migration. As shown in

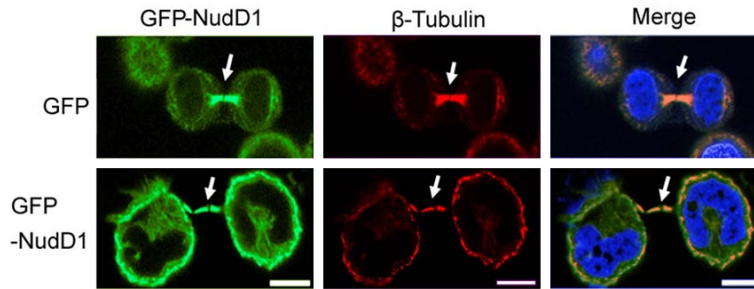
**Figure 6**, NudCD1 overexpression markedly elevated the cell amount penetrated the membrane and thus enhanced cell migration compared with GFP empty vector group (P < 0.05).

## Discussion

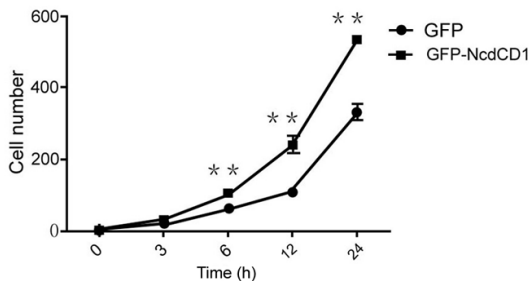
The incidence and mortality of RCC are increased year by year, whereas the treatment is still imperfection. Strengthening the basic research related to the early occurrence of RCC and screening early markers are extremely urgent [1]. This study was to investigate the effect of NudCD1 on the pathogenesis of RCC by testing NudCD1 expression in RCC and adjacent tissues and found that NudCD1 was highly expressed in RCC and can activate NudCD1/LIS1/Dynein.

NudCD1, a new member of the NudC family, is a broad-spectrum tumor antigen or oncogene [12, 13]. It may be involved in NudC/LIS1/Dynein signaling pathway that was associated with cell migration and division [14]. Previous studies found that the NudC/LIS1/Dynein pathway had a variety of members that were mostly localized or distributed along the microtubules [15, 16]. Dynein, the downstream effector protein, also known as a motor protein, is a microtubule-associated protein that converts the

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**Figure 5.** The impact of NudCD1 on cell division.



**Figure 6.** The effect of NudCD1 on cell migration. \*\* $P < 0.05$ , compared with GFP empty vector group.

chemical energy in ATP high-energy phosphate bonds into mechanical energy [17]. It moves along microtubules to the negative terminal to transport intracellular materials, thus plays a vital role in the process of cell division. In the mammalian cell mitosis, Dynein not only promotes cell centrosome separation, nuclear membrane disintegration, and spindle assembly checkpoint inactivation, but also binds with Dynactin protein through its intermediate chain to drive chromosomal separation movement and membranous organelle transport [18]. Subsequently, multiple genes were found to be involved in the regulation of Dynein function [19]. LIS, the pathogenicity of human I-type Lissencephalia, is co-localized on chromosome kinetochore, centrosome, and anterior membrane with Dynein in chromosome, which regulates Dynein-mediated cell mitosis and cell migration [20]. RNAi-mediated downregulation of NudC expression can lead to abnormal cell division with various morphologies, such as abnormal chromosomal arrangement in the metaphase of cell division, the presence of multiple division terminal, and the appearance of multinucleated cells [21]. NudC abnormal expression induced cell chromosomal abnormalities, cytokinesis disorders, polystic cells, and multinucleated cells were the early stage

of structural changes in tumor cells [22]. However, whether NudCD1 also has a similar role in RCC remains poorly understood.

It was found that NudCD1 is immunogenic and can be detected in the serum of patients with related tumors. Thus, it can be used as an indicator of immune diagnosis and treatment of related tumors [23,

24]. Wang Q *et al.* adopted siRNA to knockdown NudCD1, leading to HeLa cell loss of ability in proliferation, invasion, and metastasis, suggesting that NudCD1 may be involved in the direct regulation of tumor progression. Our study observed that NudCD1 expression was elevated in RCC.

This study firstly analyzed the impact of NudCD1 on the nuclear heterogeneity, cell proliferative activity, and metastasis ability of RCC, and further explored the role and molecular mechanism of NudCD1 in the development and progression of RCC. It provided the experimental basis for targeted therapy constructed by NudC family members and NudC/LIS1/Dynein signaling pathways. However, the exact mechanism by how NudCD1 affects RCC still needs further verification in animal experiments.

### Conclusion

NudCD1 expression was obviously increased in RCC and affected RCC cell division and migration, which might be through activating NudCD1/LIS1/Dynein signaling pathway, indicating that NudCD1 might be a new therapeutic target for inhibiting RCC cell migration.

### Disclosure of conflict of interest

None.

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### References

- [1] Basappa N and Pouliot F. New research in kidney cancer, ASCO-GU 2017. *Can Urol Assoc J* 2017; 11 Suppl 2: S163-s165.

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- [2] Banks JD and Heald R. Chromosome movement: dynein-out at the kinetochore. *Curr Biol* 2001; 11: R128-131.
- [3] Faulkner NE, Dujardin DL, Tai CY, Vaughan KT, O'Connell CB, Wang Y and Vallee RB. A role for the lissencephaly gene LIS1 in mitosis and cytoplasmic dynein function. *Nat Cell Biol* 2000; 2: 784-791.
- [4] Aumais JP, Williams SN, Luo W, Nishino M, Caldwell KA, Caldwell GA, Lin SH and Yu-Lee LY. Role for NudC, a dynein-associated nuclear movement protein, in mitosis and cytokinesis. *J Cell Sci* 2003; 116: 1991-2003.
- [5] Zhou T, Aumais JP, Liu X, Yu-Lee LY and Erikson RL. A role for Plk1 phosphorylation of NudC in cytokinesis. *Dev Cell* 2003; 5: 127-138.
- [6] Zhu XJ, Liu X, Jin Q, Cai Y, Yang Y and Zhou T. The L279P mutation of nuclear distribution gene C (NudC) influences its chaperone activity and lissencephaly protein 1 (LIS1) stability. *J Biol Chem* 2010; 285: 29903-29910.
- [7] Sun W, Zhang K, Zhang X, Lei W, Xiao T, Ma J, Guo S, Shao S, Zhang H, Liu Y, Yuan J, Hu Z, Ma Y, Feng X, Hu S, Zhou J, Cheng S and Gao Y. Identification of differentially expressed genes in human lung squamous cell carcinoma using suppression subtractive hybridization. *Cancer Lett* 2004; 212: 83-93.
- [8] Wraage M, Ruosaari S, Eijk PP, Kaifi JT, Hollmen J, Yekebas EF, Izbicki JR, Brakenhoff RH, Streichert T, Riethdorf S, Glatzel M, Ylstra B, Pantel K and Wikman H. Genomic profiles associated with early micrometastasis in lung cancer: relevance of 4q deletion. *Clin Cancer Res* 2009; 15: 1566-1574.
- [9] Dinarvand P and Lai J. Solid pseudopapillary neoplasm of the pancreas: a rare entity with unique features. *Arch Pathol Lab Med* 2017; 141: 990-995.
- [10] Gutierrez PA, Ackermann BE, Vershinin M and McKenney RJ. Differential effects of the dynein-regulatory factor lissencephaly-1 on processive dynein-dynactin motility. *J Biol Chem* 2017; 292: 12245-12255.
- [11] Blagden SP and Glover DM. Polar expeditions-provisioning the centrosome for mitosis. *Nat Cell Biol* 2003; 5: 505-511.
- [12] Suemori K, Fujiwara H, Ochi T, Azuma T, Yamnouchi J, Narumi H, Yakushijin Y, Hato T and Yasukawa M. Identification of a novel epitope derived from CML66 that is recognized by anti-leukaemia cytotoxic T lymphocytes. *Br J Haematol* 2009; 146: 115-118.
- [13] Suemori K, Fujiwara H, Ochi T, Azuma T, Yamnouchi J, Narumi H, Yakushijin Y, Hato T, Hasegawa H and Yasukawa M. Identification of an epitope derived from CML66, a novel tumor-associated antigen expressed broadly in human leukemia, recognized by human leukocyte antigen-A\*2402-restricted cytotoxic T lymphocytes. *Cancer Sci* 2008; 99: 1414-1419.
- [14] Wang Q, Li M, Wang Y, Zhang Y, Jin S, Xie G, Liu Z, Wang S, Zhang H, Shen L and Ge H. RNA interference targeting CML66, a novel tumor antigen, inhibits proliferation, invasion and metastasis of HeLa cells. *Cancer Lett* 2008; 269: 127-138.
- [15] Fu Q, Wang W, Zhou T and Yang Y. Emerging roles of NudC family: from molecular regulation to clinical implications. *Sci China Life Sci* 2016; 59: 455-462.
- [16] Yang Y, Yan X, Cai Y, Lu Y, Si J and Zhou T. NudC-like protein 2 regulates the LIS1/dynein pathway by stabilizing LIS1 with Hsp90. *Proc Natl Acad Sci U S A* 2010; 107: 3499-3504.
- [17] Kubo T and Oda T. Electrostatic interaction between polyglutamylated tubulin and the nexin-dynein regulatory complex regulates flagellar motility. *Mol Biol Cell* 2017; 28: 2260-2266.
- [18] Ali A, Veeranki SN, Chinchole A and Tyagi S. MLL/WDR5 complex regulates Kif2A localization to ensure chromosome congression and proper spindle assembly during mitosis. *Dev Cell* 2017; 41: 605-622, e607.
- [19] Fang F, Yang W, Florio JB, Rockenstein E, Spencer B, Orain XM, Dong SX, Li H, Chen X, Sung K, Rissman RA, Masliah E, Ding J, Wu C. Synuclein impairs trafficking and signaling of BDNF in a mouse model of Parkinson's disease. *Sci Rep* 2017; 7: 3868.
- [20] Baumbach J, Murthy A, McClintock MA, Dix CI, Zalyte R, Hoang HT and Bullock SL. Lissencephaly-1 is a context-dependent regulator of the human dynein complex. *Elife* 2017; 6.
- [21] Höfer K, Li S, Abele F, Frindert J, Schlotthauer J, Grawenhoff J, Du J, Patel DJ, Jäschke A. Structure and function of the bacterial decapping enzyme NudC. *Nat Chem Biol* 2016; 12: 730-4.
- [22] Zhang C, Zhang W, Lu Y, Yan X, Yan X, Zhu X, Liu W, Yang Y and Zhou T. NudC regulates actin dynamics and ciliogenesis by stabilizing cofilin 1. *Cell Res* 2016; 26: 239-253.
- [23] Yang XF, Wu CJ, McLaughlin S, Chillemi A, Wang KS, Canning C, Alyea EP, Kantoff P, Soiffer RJ, Dranoff G and Ritz J. CML66, a broadly immunogenic tumor antigen, elicits a humoral immune response associated with remission of chronic myelogenous leukemia. *Proc Natl Acad Sci U S A* 2001; 98: 7492-7497.
- [24] Zhang W, Choi J, Zeng W, Rogers SA, Alyea EP, Rheinwald JG, Canning CM, Brusci V, Sasada T, Reinherz EL, Ritz J, Soiffer RJ and Wu CJ. Graft-versus-leukemia antigen CML66 elicits coordinated B-cell and T-cell immunity after donor lymphocyte infusion. *Clin Cancer Res* 2010; 16: 2729-2739.