Cell Research

www.nature.com/cr
www.cell-research.com

LETTER TO THE EDITOR

Loss of YTHDF2-mediated m^{6}A-dependent mRNA clearance facilitates hematopoietic stem cell regeneration

Cell Research (2018) 28:1035–1038;
https://doi.org/10.1038/s41422-018-0082-y

Dear Editor,

Hematopoietic stem cells (HSCs) have unique self-renewal capacity to replenish the entire blood system, which is essential for various stress conditions, such as serial transplantation, ionizing radiation, anti-cancer medication, hemorrhage or infection.[1] *N*^{6}-methyl-adenosine (m^{6}A), the most prevalent reversible nucleotide modification in cellular mRNA in eukaryotes,[2] is associated with the maturation, translation, and eventual decay of protein-coding transcripts.[3,4] "Reader" proteins of m^{6}A modification mediate many of the properties of m^{6}A-methylated transcripts through methyl-specific RNA binding.[5,6] Although the function of m^{6}A modification in cell fate decision of embryonic stem cells and hematopoietic stem and progenitor cells (HSPCs) has been recently reported,[7,8] the role of "Reader" proteins in HSC function has not been reported yet, especially under hematological stresses. It is of great importance to understand how HSCs recognize and respond to such modifications. Here, we investigated the "Reader" protein of m^{6}A modification in HSC homeostasis and regeneration upon various stresses.

The differentiation and self-renewal of HSCs involve a large amount of mRNA regeneration and degradation. Within the cytoplasm, the m^{6}A reader protein YTHDF2 facilitates m^{6}A-dependent mRNA decay under normal and stress conditions.[5,9] We hence hypothesize that YTHDF2-dependent post-transcriptional m^{6}A-mRNA silencing plays an important role in HSC homeostatic and hematological stress.

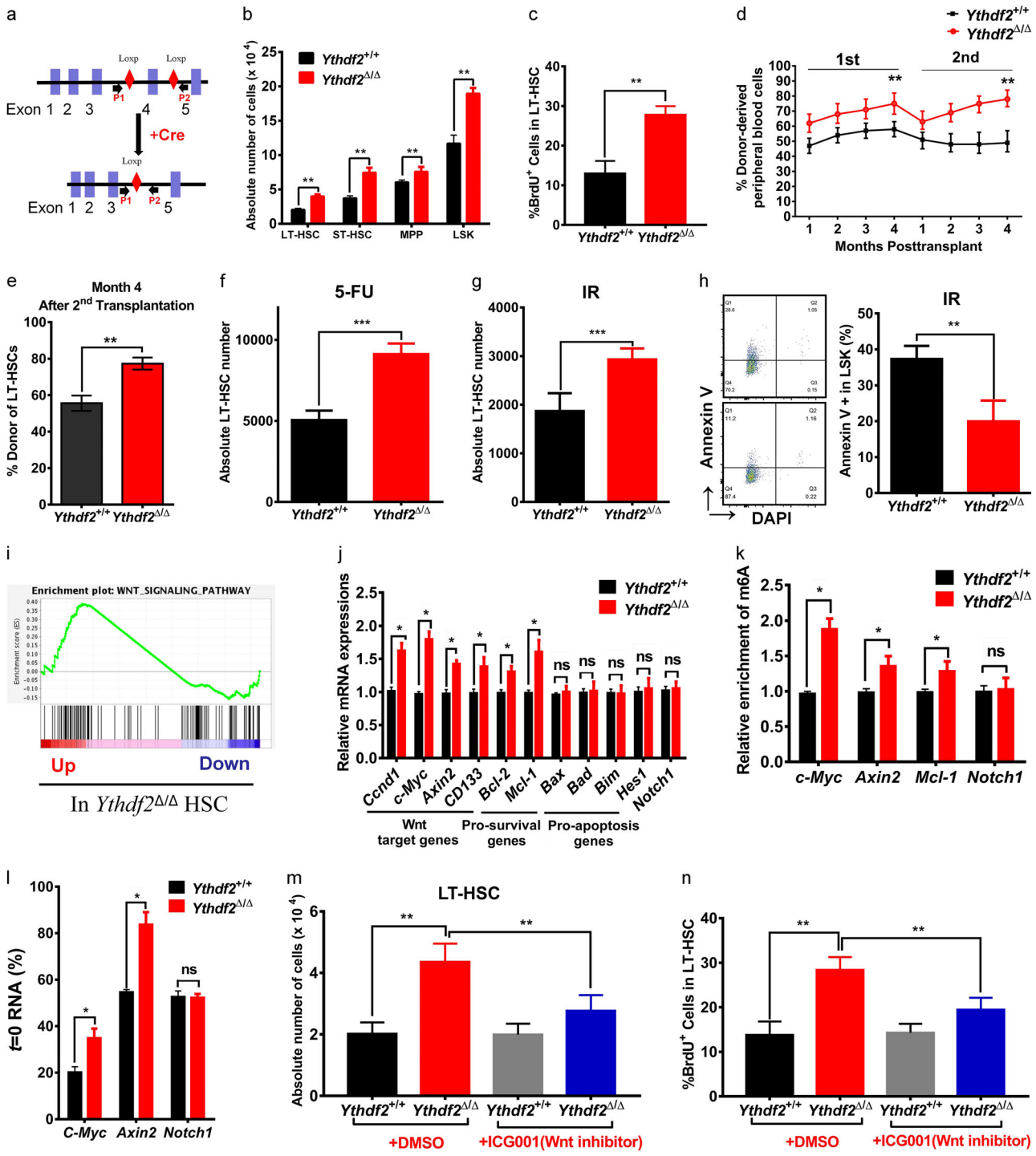
We utilized three Cre transgenic mouse lines (Mx1-Cre, Ert-Cre and Vav-Cre) to cross with *Ythdf2*$^{fl/fl}$ mice to deplete the expression of YTHDF2 specifically in adult HSCs of mouse bone marrow (BM), respectively (Fig. 1a). The knockout efficiency of YTHDF2 in BM HSCs of *Ythdf2*$^{fl/fl}$ Vav-Cre, *Ythdf2*$^{fl/fl}$ Mx1-Cre and *Ythdf2*$^{fl/fl}$ Ert-Cre was validated by multiple approaches (hereafter, the pIpC-treated *Ythdf2*$^{fl/fl}$ Mx1-Cre mice are designated as *Ythdf2*$^{\Delta/\Delta}$ and the pIpC-treated *Ythdf2*$^{+/+}$ Mx1-Cre mice as *Ythdf2*$^{+/+}$) (Supplementary information, Fig. S1a-d). Flow cytometry analysis indicated that the proportion and absolute number of long-term HSC (LT-HSC), short-term HSC (ST-HSC), multipotent progenitor (MPP) and CD150$^+$CD48$^-$Lin$^-$Sca1$^+$c-Kit$^+$ (SLAM-LSK) in the *Ythdf2*$^{\Delta/\Delta}$ mice were significantly higher than that in the *Ythdf2*$^{+/+}$ control mice (Fig. 1b; Supplementary information, Fig. S1e, f). The percentage and differentiation capacity of B cells, T cells, myeloid cells, granulocyte/monocyte progenitors (GMP), common myeloid progenitor (CMP), megakaryocyte/erythrocyte progenitor (MEP), common lymphoid progenitor (CLP) and peripheral blood (PB) cells were comparable between the *Ythdf2*$^{\Delta/\Delta}$ mice and control mice (Supplementary information, Fig. S1g-j). These data suggest that YTHDF2 deletion uniquely affects the homeostasis of HSPCs.

To acquire further insight into the expansion of HSC pools after *Ythdf2* deletion, we conducted cell cycle and cell proliferation analysis on LT-HSCs. Ki67 staining revealed a significantly higher frequency of cycling cells (Ki67$^+$) in *Ythdf2*$^{\Delta/\Delta}$ LT-HSCs than in *Ythdf2*$^{+/+}$ cells (~30% in *Ythdf2*$^{\Delta/\Delta}$ vs. ~18% in *Ythdf2*$^{+/+}$; Supplementary information, Fig. S2a). Three-day bromodeoxyuridine (BrdU) labeling further identified a higher frequency of proliferating cells in *Ythdf2*$^{\Delta/\Delta}$ LT-HSCs (BrdU$^+$; ~27% in *Ythdf2*$^{\Delta/\Delta}$ vs. ~14% in *Ythdf2*$^{+/+}$) (Fig. 1c). Similarly, *Ythdf2*$^{fl/fl}$ Vav-Cre mice also exhibited a larger HSC pool than *Ythdf2*$^{+/+}$ Vav-Cre mice (Supplementary information, Fig. S2b). Together, these data suggest that YTHDF2 plays an important role in maintaining adult HSC quiescence. The loss of YTHDF2 drives proliferation of HSCs.

We hypothesize that although there are a higher number of *Ythdf2*-null HSCs, their functionality might be impaired upon transplantation or stresses. To this end, we conducted competitive transplantation experiments. Surprisingly, in comparison with *Ythdf2*$^{+/+}$ HSCs, *Ythdf2*$^{\Delta/\Delta}$ HSCs exhibited a 50% increase in their long-term repopulating ability as revealed by PB and LT-HSC chimerism analysis and secondary transplantation (Fig. 1d, e; Supplementary information, Fig. S2c, d). The analysis of recipient mice further revealed no remarkable defects in the homing ability of *Ythdf2*$^{\Delta/\Delta}$ HSC and multi-lineage reconstitution of *Ythdf2*$^{\Delta/\Delta}$ donor-derived cells after the secondary transplantation (Supplementary information, Fig. S2e, f). Limiting-dilution transplantation using four dose concentrations of BM cells revealed a higher frequency of multi-lineage repopulating cells at 12 weeks in *Ythdf2*$^{\Delta/\Delta}$ donor bone marrow (Supplementary information, Fig. S2g). To further strengthen the above results, we used the Ert-Cre line to induce YTHDF2 depletion in adult *Ythdf2*$^{fl/fl}$ Ert-Cre mice and tested the function of *Ythdf2*-null HSCs with competitive repopulation assays. Consistent with the findings in *Ythdf2*$^{fl/fl}$ Mx1-Cre HSCs, *Ythdf2*$^{fl/fl}$ Ert-Cre HSCs exhibited expansion and a robust long-term repopulation activity (Supplementary information, Fig. S2h-j).

To examine the regenerative capacity of YTHDF2-deficient HSCs after hematopoietic injury, *Ythdf2*$^{+/+}$ and *Ythdf2*$^{\Delta/\Delta}$ mice were treated with 5-FU to ablate the proliferating hematopoietic cells. A dramatic increase in the HSC number and function was observed in *Ythdf2*$^{\Delta/\Delta}$ mice compared with the *Ythdf2*$^{+/+}$ controls (Fig. 1f; Supplementary information, Fig. S3a-c). We also observed a significant delay in 5-FU-induced hematopoietic failure in *Ythdf2*$^{\Delta/\Delta}$ mice (Supplementary information, Fig. S3d). Further, to test the regenerative capacity of YTHDF2-deficient HSCs *in vivo* after ionizing radiation, we examined the recovery of HSCs in mice following sequential 4.5 Gy exposure on day 0 and day 15. The results showed that *Ythdf2*$^{\Delta/\Delta}$ mice displayed a significant increase in HSCs compared with the *Ythdf2*$^{+/+}$ controls (Fig. 1g and

Received: 9 April 2018 Revised: 9 July 2018 Accepted: 27 July 2018
Published online: 27 August 2018



Supplementary information, Fig. S3e). In addition, Annexin V analysis showed reduction of apoptosis rate in *Ythdf2*$^{\Delta/\Delta}$ HSCs compared with the *Ythdf2*$^{+/+}$ controls (Fig. 1h), suggesting that the increased HSC number was related to cell viability. These data indicate that YTHDF2 deficiency resulted in elevated regeneration capacity and protection in HSCs under transplantation and hematopoietic stresses.

To further explore the underlying molecular mechanisms of YTHDF2-mediated HSC homeostasis and regeneration, we performed RNA-seq analysis on freshly isolated *Ythdf2*$^{+/+}$ and *Ythdf2*$^{\Delta/\Delta}$ HSCs. The gene-set enrichment analysis (GSEA) revealed a marked upregulation of genes in Wnt signaling pathway (Fig. 1i and Supplementary information, Fig. S4a). Expression of a list of Wnt signaling downstream targets (including *Myc*, *Ccnd1*, *Axin2*) and pro-survival genes (including *Mcl-1*, *Bcl2*) was significantly increased in the *Ythdf2*$^{\Delta/\Delta}$ HSCs as detected by qPCR (Fig. 1j). In addition, m^{6}A-RIP-qPCR assay showed that the enrichment of m^{6}A on *Myc* and *Axin2* mRNA was notably increased in *Ythdf2*$^{\Delta/\Delta}$ LSK cells (Fig. 1k). Furthermore, YTHDF2-RIP-qPCR assay showed the enrichment of *Myc* and *Axin2* mRNAs in LSK cells (Supplementary information, Fig. S4b). Next, we performed RNA decay assays and found that *Myc* and *Axin2* mRNA levels were both increased in *Ythdf2*$^{\Delta/\Delta}$ HSCs in comparison to *Ythdf2*$^{+/+}$ HSCs 2 h after actinomycin-D treatment (Fig. 1l).

Fig. 1 Deletion of *Ythdf2* improved HSC regeneration via Wnt signaling and pro-survival genes during hematopoietic stress. **a** Experimental schematic for generation of mice with HSC-specific deletion of *Ythdf2*. *Ythdf2*$^{flox/flox}$ mice were crossed with the interferon-inducible transgenic Mx1-Cre to generate *Ythdf2*$^{+/+}$ Mx1-Cre mice and *Ythdf2*$^{fl/fl}$ Mx1-Cre mice. Animals were then treated with intraperitoneal (i.p.) injections of 300 μg of pIpC every other day for seven times to delete *Ythdf2* alleles. *Ythdf2*$^{Δ/Δ}$ denotes mice with HSC-specific deletion of *Ythdf2*, whereas *Ythdf2*$^{+/+}$ denotes mice without *Ythdf2* deletion as control. **b** FACS analysis of LSK (Lin$^-$c-Kit$^+$Sca-1$^+$), LT-HSCs (CD34$^-$FIK2$^-$LSK), ST-HSCs (CD34$^+$FIK2$^-$LSK), and MPPs (CD34$^+$FIK2$^+$LSK) in *Ythdf2*$^{+/+}$ and *Ythdf2*$^{Δ/Δ}$ BM cells. The absolute cell numbers are shown ($n = 5$ mice per genotype). **c** Proliferation analysis of LT-HSC from *Ythdf2*$^{+/+}$ and *Ythdf2*$^{Δ/Δ}$ mice. The percentage of BrdU-positive cells is shown. $n = 5$ mice per group. **d** Percentage of donor-derived PB cells at the indicated time points in serial competitive transplantation assay (1st, the first competitive transplantation, $n = 6$ mice per genotype; 2nd, the second competitive transplantation, $n = 6$ mice per group). **e** Percentage of donor-derived LT-HSCs 16 weeks after secondary transplantation ($n = 6$ mice per group). **f** Absolute number of LT-HSCs from mice treated with 150 mg/kg 5-FU on day 12 ($n = 7$ per genotype). **g** Absolute number of LT-HSCs from mice irradiated with 4.5 Gy on day 15 ($n = 7$ per genotype). **h** Representative FACS plots (left panel) and frequency (right panel) of apoptotic cells (ANNEXIN V$^+$ DAPI$^-$) in the LSK from mice after IR ($n = 7$ mice per genotype). **i** GSEA analysis of the Wnt signaling transcriptional signature of HSCs in *Ythdf2*$^{Δ/Δ}$ relative to *Ythdf2*$^{+/+}$ group. NES normalized enrichment score. **j** qPCR validation of expression of Wnt signaling target genes and survival-related genes in *Ythdf2*$^{Δ/Δ}$ and *Ythdf2*$^{+/+}$ LT-HSCs ($n = 7$ mice per genotype). **k** m^{6}A-RIP-qPCR assay showing the enrichment levels of m^{6}A on *c-Myc*, *Axin2*, *Mcl-1*, *Notch1* mRNAs in *Ythdf2*$^{Δ/Δ}$ and *Ythdf2*$^{+/+}$ LSK cells. **l** RNA degradation assay shows that *c-Myc* and *Axin2* mRNAs decay slower in *Ythdf2*$^{Δ/Δ}$ LSK cells than in *Ythdf2*$^{+/+}$ LSK cells 2 h after actinomycin D treatment. The residual RNAs were normalized to $t = 0$. Three independent experiments were performed for all of the blots and qPCR assays. **m** Number of LT-HSCs in *Ythdf2*$^{+/+}$ and *Ythdf2*$^{Δ/Δ}$ mice after Wnt inhibitor ICG001 treatment or DMSO in vivo ($n = 5$ mice per group). **n** Cell cycle analysis of LT-HSCs in *Ythdf2*$^{+/+}$ and *Ythdf2*$^{Δ/Δ}$ mice after Wnt inhibitor ICG001 treatment or DMSO in vivo ($n = 5$ mice per group). Data represent the mean ± SD from three independent experiments. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; ns, not significant

To determine whether the activation of Wnt signaling in *Ythdf2*$^{Δ/Δ}$ LT-HSC contributes to HSC proliferation and subsequent functional enhancement, we administered the small-molecule Wnt/β-catenin inhibitor ICG-001 to *Ythdf2*$^{+/+}$ and *Ythdf2*$^{Δ/Δ}$ mice and examined the HSC numbers and cell cycle status. The small molecule ICG-001 targets cAMP-responsive element binding (CREB)-binding protein (CBP) to inhibit β-catenin/LEF1-mediated transcription. ICG-001 treatment partially abolished the proliferation and phenotypic expansion of HSCs in *Ythdf2*$^{Δ/Δ}$ mice under normal condition and 5-FU stress (Fig. 1m, n; Supplementary information, Fig. S4c, d). Taken together, these data suggest that both the phenotypic expansion and proliferation of YTHDF2-deficient HSCs are partially due to the activation of Wnt signaling.

In summary, we utilized three Cre/LoxP systems to generate the hematopoietic-specific knockout mice of *Ythdf2*, and identified a unique function of YTHDF2-mediated m^{6}A recognition in mouse adult HSC. Mechanistically, the expression of *Ythdf2* in HSCs facilitates decay of the m^{6}A-modified mRNAs of Wnt target genes, contributing to the repression of Wnt signaling at steady state (Supplementary information, Fig. S4e). Usually, this repression is protective, since the abnormal activation of Wnt signaling results in expansion of HSC number but exhaustion of its functionality upon hematopoietic stresses.[10] Interestingly, upon deletion of YTHDF2, not only the number of HSCs was expanded but also the regenerative capacity of HSCs was increased under stress conditions. Our data suggested that YTHDF2 deficiency simultaneously prevented the degradation of mRNAs of both Wnt target genes and survival-related genes during hematopoietic stresses, and this combined effect synergistically enhanced the regenerative capacity of HSC.

A therapeutic challenge of hematopoietic regeneration is to stimulate stem cell proliferation for immediate tissue repair while avoiding stem cell depletion under stress.[1] Our current findings revealed the functional importance of YTHDF2-mediated reading and processing of RNA m^{6}A modification in HSCs, by integrating the proliferation-related genes and the pro-survival genes. YTHDF2 deficiency boosted HSC expansion without losing its viability under stress conditions. These findings established the crucial role of YTHDF2 in regulating HSC protection, self-renewal and regeneration, and thereby provided preliminary results supporting the possibility that these effects could also be translated into effective clinical applications.

Materials and Methods are available in Supplementary Information.

ACKNOWLEDGEMENTS

This work is supported by the National Key R&D Program of China (2017YFA0103302, 2016YFA0100602, 2018YFA0109300) and the National Natural Science Foundation of China (91749203, 91749117, 81525010, 81420108017 and 81770155). This work was also supported by the National Science Fund for Excellent Young Scholars (31622039), the Science Foundation for Distinguished Young Scholars of Jiangsu Province (BK20160045).

AUTHOR CONTRIBUTIONS

H.W. and Z.J. initiated the study and developed the concept of the paper. H.W.and H. Z. performed all experiments. H.W. and H.Z. analyzed and interpreted the data. H.W., H.Z., and Z.J. wrote the manuscript, with help from J.L., F.W., Y.G., X.Z., B.L., F.X., W.W., G.H., and B.S.

ADDITIONAL INFORMATION

Supplementary information accompanies this paper at https://doi.org/10.1038/s41422-018-0082-y.

Competing interests: The authors declare no competing interests.

Hu Wang[1,2], Hongna Zuo[2], Jin Liu[1], Fei Wen[2], Yawei Gao[3], Xudong Zhu[2], Bo Liu[1], Feng Xiao[1], Wengong Wang[4], Gang Huang[5], Bin Shen[6] and Zhenyu Ju[1,2]

[1]*Key Laboratory of Regenerative Medicine of Ministry of Education, Institute of Aging and Regenerative Medicine, Jinan University, Guangzhou, Guangdong, China;* [2]*Institute of Aging Research, Hangzhou Normal University School of Medicine, Hangzhou, Zhejiang 311121, China;* [3]*Clinical and Translational Research Center of Shanghai First Maternity and Infant Hospital, School of Life Sciences and Technology, Tongji University, Shanghai 200092, China;* [4]*Department of Biochemistry and Molecular Biology, Beijing Key Laboratory of Protein Posttranslational Modifications and Cell Function, School of Basic Medical Sciences, Peking University Health Science Center, 38 Xueyuan Road, Beijing 100191, China;* [5]*Division of Pathology and Experimental Hematology and Cancer Biology, Cincinnati Children's Hospital Medical Center, 3333 Burnet Avenue, Room S7.224, Cincinnati, OH, USA and* [6]*Department of Histology and Embryology, State Key Laboratory of Reproductive Medicine, Nanjing Medical University, Nanjing, Jiangsu, China*

These authors contributed equally: Hu Wang, Hongna Zuo.

Correspondence: Hu Wang (wanghu19860315@163.com) or Bin Shen (binshen@njmu.edu.cn) or Zhenyu Ju (zhenyuju@163.com)

REFERENCES

1. Orford, K. W. & Scadden, D. T. *Nat. Rev. Genet.* **9**, 115–128 (2008).
2. Zhao, B. S., Roundtree, I. A. & He, C. *Nat. Rev. Mol. Cell Biol.* **18**, 31–42 (2017).
3. Lin, Z. et al. *Cell Res.* **27**, 1216–1230 (2017).
4. Xu, K. et al. *Cell Res.* **27**, 1100–1114 (2017).
5. Wang, X. et al. *Nature* **505**, 117–120 (2014).
6. Hsu, P. J. et al. *Cell Res.* **27**, 1115–1127 (2017).
7. Geula, S. et al. *Science* **347**, 1002–1006 (2015).
8. Zhang, C. et al. *Nature* **549**, 273–276 (2017).
9. Ivanova, I. et al. *Mol. Cell* **67**, 1059–1067 (2017).
10. Wang, H. et al. *Cell. Stem. Cell.* **18**, 495–507 (2016).