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

Cell Research

LETTER TO THE EDITOR OPEN

Pharmacological inhibition of SRC-1 phase separation suppresses YAP oncogenic transcription activity

Cell Research (2021) 31:1028–1031; https://doi.org/10.1038/s41422-021-00504-x

Dear Editor,

Yes-associated protein (YAP) is a transcriptional coactivator that plays an essential role in promoting cell proliferation, development and stem cell fate.[1] Aberrant YAP activation is prevalent in diverse types of human solid cancers.[2] In mammals, a kinase cascade including MST1/2 and LATS1/2 phosphorylates YAP to prevent its nuclear translocation and subsequent interaction with the TEA domain transcription factors TEAD1–4 in the canonical Hippo pathway. Previous studies have intensively emphasized the upstream signals from the Hippo kinase cascade that regulates YAP;[1,3] however, the epigenetic regulatory mechanism of YAP transcriptional activity is far less understood. Given the importance of YAP in cancer progression and limited druggability of YAP/TEAD as transcriptional factors, identification of YAP proximal regulators is of great importance to develop new therapeutic strategies for the treatment of cancer.

To gain insights into the epigenetic regulatory mechanism of YAP activity, we first explored whether histone acetyltransferases (HATs) might be responsible for YAP target gene expression. A focused genetic screen using an siRNA library containing three independent siRNAs for 15 reported HATs encoded by the human genome was initially performed in a YAP-amplified SF268 cell line (Supplementary information, Fig. S1a, b) to circumvent YAP-irrelevant effects. We found that knockdown of SRC-1 (KAT13A) by all three siRNAs consistently reduced the expression of YAP-targeted *CTGF* (Supplementary information, Fig. S1c). It was further confirmed that SRC-1 knockdown inhibited the expression of another YAP target gene *ANKRD1*, but not that of *YAP* itself in additional two cancer cell lines (Supplementary information, Fig. S1d). Global gene expression profiling (Supplementary information, Fig. S1e) further corroborates the regulation on YAP transcriptome by SRC-1. These data demonstrate that SRC-1 facilitates YAP transcriptional activity.

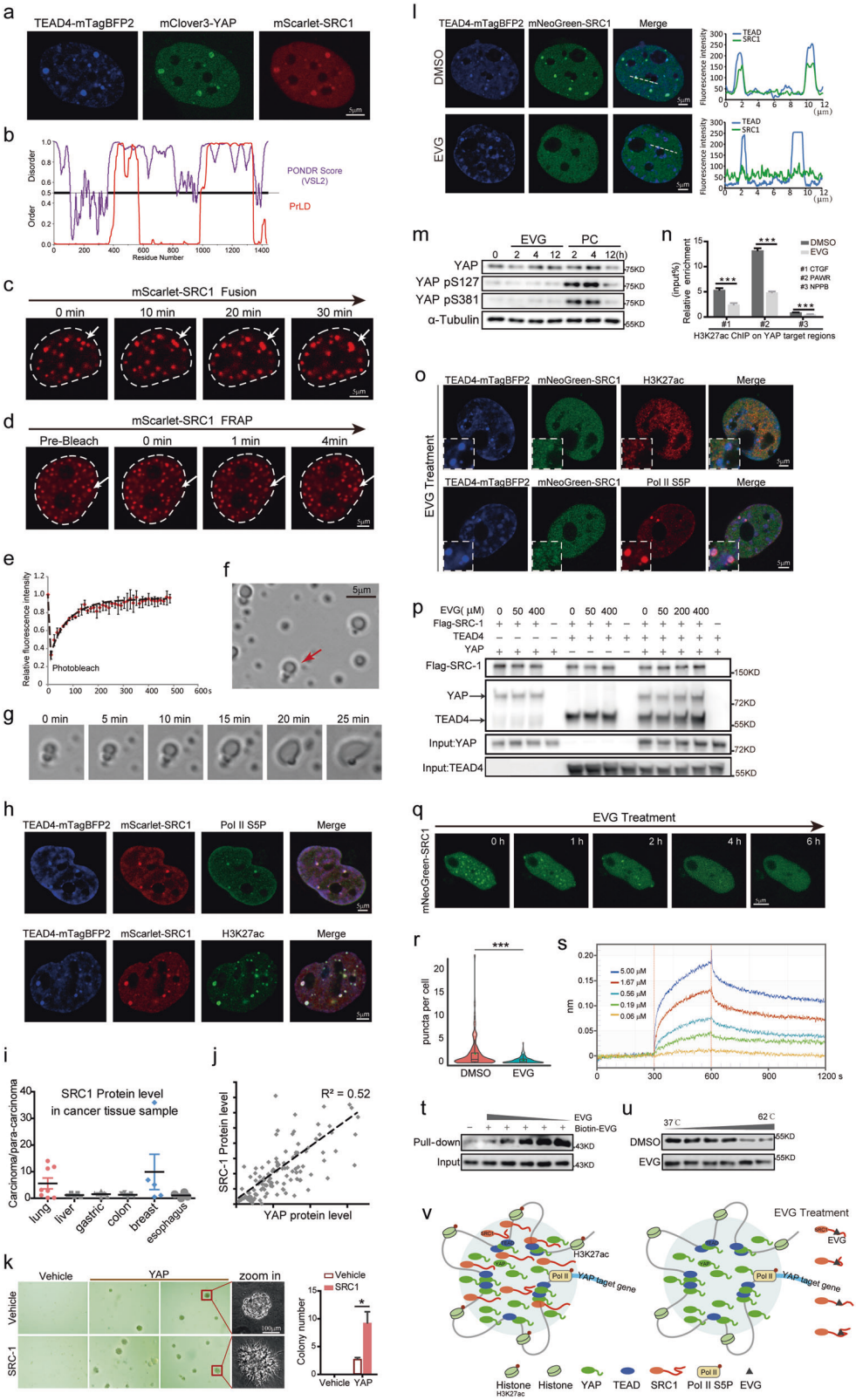
Mounting evidence revealed that gene regulation occurs in transcriptional condensates, which concentrate transcription factors, coactivators, the transcription and elongation machinery for spatial and temporal transcription control.[4–6] Two recent articles report that Hippo pathway transcriptional coactivators YAP and TAZ could phase separate to regulate downstream transcription.[7,8] To investigate whether SRC-1 co-exists in YAP/TEAD phase-separated transcriptional condensates, we ectopically expressed mClover3-YAP, TEAD4-mTagBFP2 and mScarlet-SRC1 in SF268 cells. Microscopic imaging revealed that SRC-1 distributes to the YAP/TEAD condensates (Fig. 1a) and exhibits rapid recovery upon photobleaching (Supplementary information, Fig. S2). Given that SRC-1 protein is highly disordered and contains two prion-like domains (PrLDs) (Fig. 1b), we investigated whether SRC-1 bears intrinsic capability of phase separation. Fusion events and fluorescence recovery after photobleaching (FRAP) experiments demonstrated the liquid-like properties of SRC-1 puncta in cells (Fig. 1c–e). Microscopic imaging investigating the distribution of truncated SRC-1 fluorescent proteins in cells revealed that the second intrinsic disordered region (IDR-2) is responsible for SRC-1 phase separation and other domains participate in maintaining SRC-1 condensates (Supplementary information, Fig. S3). Purified IDR-2 could undergo phase separation in physiological buffer without crowding agent and exhibited liquid-like features (Fig. 1f, g). Importantly, we detected enrichment of both CTD-phosphorylated RNA polymerase II (Pol II) and H3K27ac in the YAP/TEAD/SRC-1 condensates (Fig. 1h), suggesting that the SRC-1-co-occupied YAP/TEAD liquid–liquid phase separation (LLPS) puncta are the sites of active transcription. These results support that ectopically expressed SRC-1 forms LLPS condensates compartmented with YAP/TEAD to promote gene expression.

Next we performed coimmunoprecipitation experiments under endogenous conditions (Supplementary information, Fig. S4a, b) to verify that SRC-1 is a component of YAP/TEAD complex. To investigate the interaction between SRC-1 and YAP/TEAD complex, we conducted in vitro and in cell pull-down assays with truncated SRC-1, YAP and TEAD4 proteins, and found that YAP-C interacts with both SRC-1-N and SRC-1-C, while both TEAD-N and TEAD-C bind to SRC-1-N (Supplementary information, Fig. S4c–g). Then we analyzed the previously reported ChIP-seq datasets[9] to investigate the genome-wide localization of SRC-1 on YAP/TEAD transcriptome. Significant genomic co-occupancy of SRC-1 with both YAP and TEAD2 was observed (Supplementary information, Fig. S5a–d). Consistently, ChIP-qPCR data in SF268 cells confirmed the enrichment of SRC-1 and YAP/TEAD in the loci of YAP target genes including *ANKRD1*, *NBBP* and *PAWR* (Supplementary information, Fig. S5e).

As described above, we uncovered that SRC-1, a previously known transcriptional coactivator for nuclear hormone receptors,[10] plays a critical role in YAP regulation. Sophisticated transcription factor crosstalk has been a longstanding puzzle due to the limitations of current research techniques; the emergence of high-resolution imaging coupled with phase separation model on transcription control offers novel approaches to address this question. In our study, microscopic images revealed that SRC-1 could form transcriptional condensates interplaying between the ERα signaling and Hippo pathway under different cell contexts (Supplementary information, Fig. S6a). SRC-1 preferentially co-phase separated with the activated transcription factor (E2-stimulated ERα or constitutively active YAP mutant) and the SRC-1/TEAD condensates did not coalesce with SRC-1/ERα condensates (Supplementary information, Fig. S6b). These observations highlight the specificity of SRC-1 regulation on cell-specific and pathway-specific transcriptional activation.

While SRC-1 has been associated with breast cancer previously,[11] we found elevated expression of SRC-1 in non-small cell lung cancer (NSCLC) which was correlated with malignant features and poor prognosis (Fig. 1i; Supplementary information, Fig. S7a, b). A series of functional assays

Received: 21 December 2020 Accepted: 25 March 2021
Published online: 13 April 2021



(Supplementary information, Fig. S8a–f) conducted using SRC-1-knockdown H1299 cells revealed that SRC-1 is essential for lung cancer proliferation, migration and invasion. These results support the noncanonical but critical oncogenic function of SRC-1 in NSCLCs. Because our data show that SRC-1 can form transcription complex with YAP/TEAD, we next explored whether SRC-1 functionally interacts with YAP in NSCLCs. After analyzing SRC-1 and YAP expression in 120 NSCLC samples by

Fig. 1 Pharmacological inhibition of SRC-1 phase separation suppresses YAP oncogenic transcription activity. a Live-cell images showing the co-localization of TEAD4-mTagBFP2, mClover3-YAP, and mScarlet-SRC1 condensates in SF268 cells. Scale bar, 5 μm. **b** Predictions of PrLDs and disordered regions by PLAAC and PONDER algorithms, respectively. **c** Fusion event of mScarlet-SRC1 puncta in SF268 cells. Scale bar, 5 μm. **d** Representative images of FRAP experiments of mScarlet-SRC1 puncta in SF268 cells. Scale bar, 5 μm. **e** Quantification of FRAP data (means ± SEM, $n = 3$ experiments) from **d**. **f** Microscopic image of in vitro phase-separated SRC-1 IDR-2 (967–1362) protein in physiological buffer. Scale bar, 5 μm. **g** Time-lapse images recording the fusion event of SRC-1 IDR-2 droplets. **h** H1299 cells co-expressing YAP^{5SA}, TEAD4-mTagBFP2, and mScarlet-SRC1 were stained with anti-RNA Pol II-S5P (top) and anti-H3K27ac (bottom). **i** Representative SRC-1 protein levels in lung, liver, gastric, colon, breast, and esophagus samples determined by IHC. **j** Representative SRC-1 and YAP protein levels in 120 NSCLC samples by IHC. The correlation of two protein levels was analyzed. **k** Colony-formation assay in BEAS-2B cells transfected with vehicle, YAP, and/or SRC-1 plasmids. Quantification result of colony number in image is shown on the right. Error bars show means ± SEM ($n = 4$), $^{*}P < 0.05$. **l** Live-cell images showing the distribution of TEAD4-mTagBFP2 and mNeoGreen-SRC1 in nucleus of H1299 cells co-transfected with YAP^{5SA} plasmids that were treated with or without 20 μM EVG. Quantification of fluorescence intensity of TEAD4-mTagBFP2 and mNeoGreen-SRC1 along the dashed line indicated in the merged image was shown on the right. Scale bar, 5 μm. **m** SF268 cells were treated with 20 μM EVG or 20 μM PC (positive compound, fedratinib) for the indicated times and subjected to western blotting analysis. **n** Analysis of H3K27ac at YAP target regions from SF268 cells treated with DMSO or EVG by ChIP-qPCR. Error bars show means ± SEM ($n = 3$), $^{***}P < 0.001$. **o** Immunofluorescence staining with anti-H3K27ac (top) and anti-RNA Pol II-S5P (bottom) in H1299 cells co-expressing YAP^{5SA}, TEAD4-mTagBFP2, and mNeoGreen-SRC1 treated with 20 μM EVG. Scale bar, 5 μm. **p** Purified Flag-SRC-1 (1–807) protein was mixed with YAP and/or TEAD4 in the presence of increasing concentrations of EVG and was subjected to three independent pull-downs using anti-Flag beads. **q** Time course of live-cell imaging of mNeoGreen-SRC1 condensates upon EVG treatment in H1299 cells. Scale bar, 5 μm. **r** Quantification result of high-content image data for mScarlet-SRC1 puncta in H1299 cells treated with or without 20 μM EVG. $^{***}P < 0.001$. **s** Bio-layer interferometry (BLI) assays were performed with purified SRC-1 protein and EVG. Biotin-labeled EVG was immobilized on the streptavidin biosensors and dipped into wells containing increasing concentrations of SRC-1 protein. **t** Purified Flag-SRC-1 (500–807) protein was incubated with EVG-Biotin in the presence of increasing concentrations of EVG and further subjected to streptavidin bead pull-down assays. **u** Purified Flag-SRC-1 (500–807) protein was subjected to thermal stability assay (37 °C, 42 °C, 47 °C, 52 °C, 57 °C, and 62 °C) in the presence of 20 μM EVG or DMSO. **v** Schematic representation of the SRC-1-co-occupied YAP/TEAD transcriptional condensates. (Left) SRC-1 interacts with YAP and TEAD to facilitate YAP target gene expression. The co-localization with H3K27ac and RNA Pol II-S5P indicates that SRC-1-co-occupied YAP/TEAD puncta are actively transcribed. (Right) EVG could antagonize YAP activity through disrupting the SRC-1 phase separation in SRC-1/YAP/TEAD transcription condensates, but had no effect on LLPS of YAP/TEAD. The H3K27ac was not enriched in TEAD condensates upon EVG treatment, whereas Pol II-S5P remained unchanged.

immunohistochemistry (IHC), we found that SRC-1 and YAP were co-upregulated, with a strong correlation between the protein levels of SRC-1 and YAP ($R^2 = 0.52$) (Fig. 1j; Supplementary information, Fig. S9a). Importantly, SRC-1 and YAP exhibited a similar distribution pattern (Supplementary information, Fig. S9b). To explore whether SRC-1 and YAP cooperatively drive tumorigenesis, we transformed normal human lung bronchial epithelium cells (BEAS-2B) with YAP and/or SRC-1. Microscopic observations revealed that both the number and migratory features of colony formation were more pronounced in BEAS-2B cells co-transfected with SRC-1 and YAP compared to that with YAP alone, whereas no colonies were formed with SRC-1 expression alone (Fig. 1k; Supplementary information, Fig. S10). These results demonstrated that SRC-1 facilitated YAP oncogenic function to promote lung cancer progression.

Despite the significance of SRC-1 and YAP/TEAD in cancer progression, their limited druggability hinders the development of targeted therapy. The emergence of LLPS provides a novel approach to target intractable and undruggable proteins,[5,6,12] for example, it was recently reported that LLPS of disease-associated SHP2 mutants could be specifically attenuated by SHP2 allosteric inhibitors.[13] Motivated by the actively-transcribed SRC-1/YAP/TEAD LLPS puncta, we explored whether specific disruption of SRC-1 phase separation could be a feasible approach to inhibit YAP oncogenic activity. Interestingly, we found that the phase-separated SRC-1 condensates, but not the YAP/TEAD4 condensates, were selectively disrupted by the treatment of an anti-HIV drug elvitegravir (EVG) (Fig. 1l) that we newly identified from a YAP reporter cell-based screening in a library of FDA-approved drugs to suppress YAP transcriptional activity (Supplementary information, Fig. S11a–c). Global gene expression profiling verified that EVG downregulates expression of YAP target genes (Supplementary information, Fig. S11d). Particularly, EVG regulated YAP activity independent of canonical Hippo kinase cascade as revealed by the unaffected nuclear translocation and phosphorylation pattern of YAP (Fig. 1m; Supplementary information, Fig. S12a–c). After excluding the possibility that EVG may block the access of YAP to target genes (Supplementary information, Fig. S12d), we found that EVG epigenetically regulates YAP transcriptional activity by reducing H3K27ac mark levels at YAP target genes (Fig. 1n; Supplementary information, Fig. S12e). Importantly, microscopic images revealed that EVG inhibited the enrichment of H3K27ac at TEAD puncta, but exhibited no effect on that of RNA Pol II phosphorylated at Ser 5 (CTD) (Fig. 1o). Next we conducted in vitro pull-down assays using recombinant proteins and found that EVG did not affect SRC-1's binding with YAP and TEAD even at concentrations up to 400 μM, indicating that the exclusion of SRC-1 from YAP/TEAD condensates was not due to interrupted binding with YAP or TEAD (Fig. 1p). Indeed, EVG suppressed the nuclear SRC-1 puncta formation in H1299 cells expressing either mNeoGreen- or mScarlet-labeled SRC-1 (Fig. 1q; Supplementary information, Fig. S13a–c, Movie S1). High-content image results corroborated the inhibitory effects of EVG on SRC-1 phase separation (Fig. 1r). We next asked whether EVG disrupts SRC-1 LLPS by directly binding to SRC-1 using the biotinylated EVG as a probe (Supplementary information, Fig. S13d). Biophysical studies confirmed that EVG directly binds to SRC-1 (Fig. 1s). Further competitive pull-down and thermal shift assays (Fig. 1t, u; Supplementary information, Fig. S13e–f) indicated that the binding was both direct and specific. We found that EVG effectively inhibited the proliferation of a variety of lung cancer cell lines and inducible knockdown of SRC-1 rendered A549 cells partially resistant to EVG's anti-proliferative effects (Supplementary information, Fig. S14a, b), suggesting that such effects are SRC-1 dependent. Moreover, treatment of SRC-1 and YAP co-expressing BEAS-2B cell colonies with EVG dramatically inhibited the migratory activity at the outer colony border (Supplementary information, Fig. S14c). Taken together, EVG antagonized YAP oncogenic transcription activity by disturbing SRC-1 LLPS in SRC-1/YAP/TEAD condensates (Fig. 1v).

In this study, we report an uncanonical but critical role of SRC-1 in regulating Hippo/YAP signaling. SRC-1 interacts with YAP/TEAD to enhance YAP transcription activity through compartmentalized SRC-1/YAP/TEAD condensates. SRC-1 interplays

between ERα and YAP transcriptional condensates under diverse cell contexts. SRC-1 co-expresses with YAP in NSCLC and is crucial for lung cancer growth. Importantly, EVG that disrupts SRC-1 phase separation in actively-transcribed SRC-1/YAP/TEAD condensates could efficiently inhibit YAP oncogenic transcription activity. Our data show that EVG does not interfere with YAP/TEAD/SRC-1 complex formation in vitro; however, EVG specifically disrupts SRC-1 condensate formation in cells. These results suggest that co-phase separation of SRC-1 with YAP/TEAD depends on intrinsic LLPS capability of SRC-1. EVG can directly bind to SRC-1 and may reshape its conformation or alter its intermolecular multivalency required for its phase separation capability. This work provides a phase separation-based pharmacological strategy to target the undruggable SRC-1/YAP/TEAD complex for constraining YAP-dependent cancer cell growth, demonstrating the potential of LLPS-targeted therapeutics as a powerful and novel approach to address undruggable targets and intractable diseases.

ACKNOWLEDGEMENTS

We thank Drs. Junying Yuan and Nan Liu for critical reading of the manuscript. We thank J. Hu Lab and Y. Geng for help with live-cell imaging. We thank Z. Zhang Lab for the protein purification platform. We thank National Center for Protein Science Shanghai for the BLI assays. This work was supported by the National Key R&D Program of China (2016YFA0501900), the National Natural Science Foundation of China (21532002 and 21877123), the Shanghai Municipal Science and Technology Major Project (2019SHZDZX02), and the joint postdoc program with Janssen R&D.

AUTHOR CONTRIBUTIONS

G.Z., J.X., X.G., and J.Z. designed the study. G.Z., J.X., Z.F., and Z.C. performed the experiments. M.W. synthesized the compounds. G.Z., J.X., Q.Z., H.H., and J.Z. analyzed the data. G.Z., J.X., and J.Z. wrote the manuscript.

ADDITIONAL INFORMATION

Supplementary information The online version contains supplementary material available at https://doi.org/10.1038/s41422-021-00504-x.

Competing interests: J.Z. is a co-founder of Etern Biopharma Co., Ltd. and a member of its scientific advisory board. H.H., Z.C., and X.G. are employees of Etern Biopharma Co., Ltd.

Guangya Zhu[1,2], Jingjing Xie[1,2], Zhenzhen Fu[1], Mingliang Wang[3], Qichen Zhang[4], Hao He[5], Zijun Chen[5], Xin Guo[5] and Jidong Zhu[1,5]

[1]Interdisciplinary Research Center on Biology and Chemistry, Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 201203, China; [2]University of Chinese Academy of Sciences, Beijing 100049, China; [3]Department of Natural Products Chemistry, Fudan University, Shanghai 201203, China; [4]Department of Gastroenterology, Changhai Hospital, Navy Medical University, Shanghai 201203, China and [5]Etern Biopharma Co., Ltd., Shanghai 201203, China

These authors contributed equally: Guangya Zhu, Jingjing Xie

Correspondence: Jidong Zhu (zhujd@sioc.ac.cn)

REFERENCES

1. Meng, Z., Moroishi, T. & Guan, K. L. *Genes Dev.* **30**, 1–17 (2016).
2. Harvey, K. F., Zhang, X. & Thomas, D. M. *Nat. Rev. Cancer* **13**, 246–257 (2013).
3. Halder, G., Dupont, S. & Piccolo, S. *Nat. Rev. Mol. Cell Biol.* **13**, 591–600 (2012).
4. Hnisz, D., Shrinivas, K., Young, R. A., Chakraborty, A. K. & Sharp, P. A. *Cell* **169**, 13–23 (2017).
5. Alberti, S., Gladfelter, A. & Mittag, T. *Cell* **176**, 419–434 (2019).
6. Lee, T. I. & Young, R. A. *Cell* **152**, 1237–1251 (2013).
7. Cai, D. et al. *Nat. Cell Biol.* **21**, 1578–1589 (2019).
8. Lu, Y. et al. *Nat. Cell Biol.* **22**, 453–464 (2020).
9. Davis, C. A. et al. *Nucleic Acids Res.* **46**, D794–D801 (2018).
10. Onate, S. A., Tsai, S. Y., Tsai, M. J. & O'Malley, B. W. *Science* **270**, 1354–1357 (1995).
11. Redmond, A. M. et al. *Clin. Cancer Res.* **15**, 2098–2106 (2009).
12. Viny, A. D. & Levine, R. L. *Science* **368**, 1314–1315 (2020).
13. Zhu, G. et al. *Cell* **183**, 490–502 (2020).