

Supplemental information

hsa_circ_0001018 promotes papillary thyroid cancer by facilitating cell survival, invasion, G₁/S cell cycle progression, and repressing cell apoptosis via crosstalk with miR-338-3p and SOX4

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Table S1 The baseline characteristics of PTC patients (n = 51)

Characteristics	No. of cases	Expression of circ_0001018		P
		High (n=26)	Low(n=25)	
Gender				
male	24	14	10	0.322
female	27	12	15	
Age				
<55	22	9	13	0.210
≥55	29	17	12	
TNM stage				
I-II	28	10	18	0.016 ^a
III-IV	23	16	7	
Tumor size				
<2	34	15	19	0.167
≥2	17	11	6	
Lymph node metastasis				
Present	38	23	15	0.020 ^a
Absent	13	3	10	
Distant metastasis				
Present	15	11	4	0.039 ^a
Absent	36	15	21	

The data were analyzed using the chi-square test. ^aP<0.05.

Table S2 the sequences of circ_0001018 OE, shRNA and miR-338-3p mimic

Name	Sequence (5'-3')
circ_0001018 OE-F	CGGATCCGATGGATAATCAAA
circ_0001018 OE-R	GGAATTCCGTTTGACGAGTTC
circ_0001018 shRNA1-F	GCTCAAGATGGATAATCAA
circ_0001018 shRNA1-R	TTGATTATCCATCTTGAGC
circ_0001018 shRNA2-F	GGTGATTAAGGATATTGAA
circ_0001018 shRNA2-R	TTCAATATCCTTAATCACC
miR-338-3p mimic	AACAATATCCTGGTGCTGAGTG
miR-338-3p inhibitor	UCCAGCAUCAGUGAUUUUGUUG
SOX4 OE-F	CGGATCCGGCTCTAAGCTGCA
SOX4 OE-R	GGAATTCCTGACTTGGGATTT

Table S3 Primers used in this study

Primer	Primer Sequence (5'-3')
circ0001018-F	CTGAGTTAGCTGAGGAGGTCA
circ0001018-R	TGGGCATAGTCAGAAACCACT
circ0000011-F	AGGTGGTGTTCGAGATGAG
circ0000011-R	GTAGCCAGCATAGGCCAAGA
circ0000326-F	CAAAGTCAGATCAGTTATGGGACA
circ0000326-R	TGCCAGTTACTCCAGCATAAA
circ0001199-F	CTACGCTTGTGACGATGAGC
circ0001199-R	GAAGGGGGTGTCAAACCTCA
circ0000937-F	GCACTATCTGCTGAGCCAAG
circ0000937-R	GAACTGGGGCTTGTCATCC
CCT4-F	GTATTACATCCAGCAGCCAGA
CCT4-R	CTGTGGCTGGGTCAATCACT
miR-338-3p-F	TGCGGTCCAGCATCAGTGAT
miR-338-3p-R	CCAGTGCAGGGTCCGAGGT
SOX4-F	GGTCTCTAGTTCTTGCACGCTC
SOX4-R	CGGAATCGGCACTAAGGAG

Figure S1 Expression levels of the four most significant circular RNAs in PTC and the detection of transfection efficiency of circ_0001018 shRNAs and overexpression vectors.

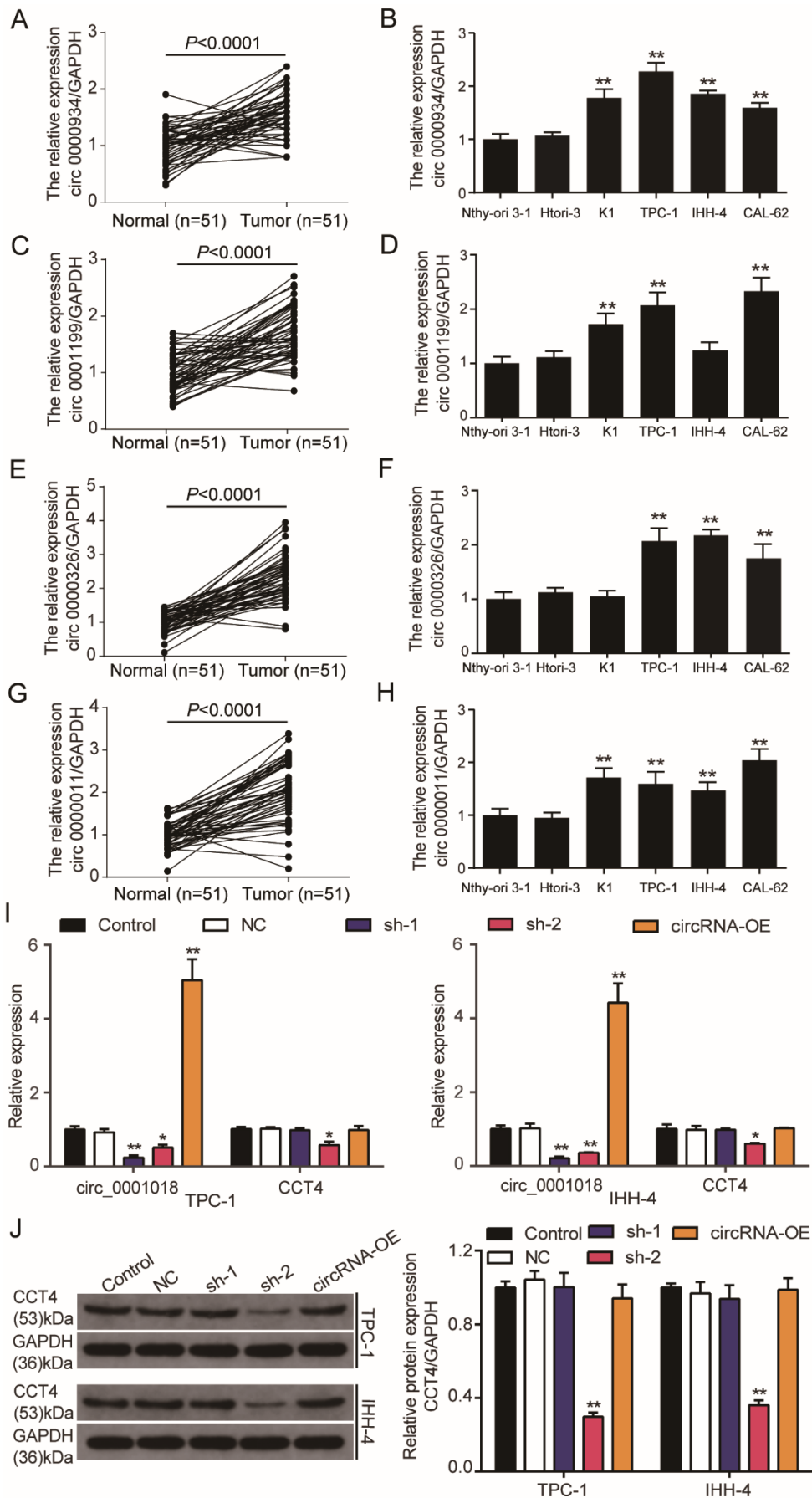


Figure S2 The enrichment of five most significant circular RNAs in PTC cells on miR-338-3p.

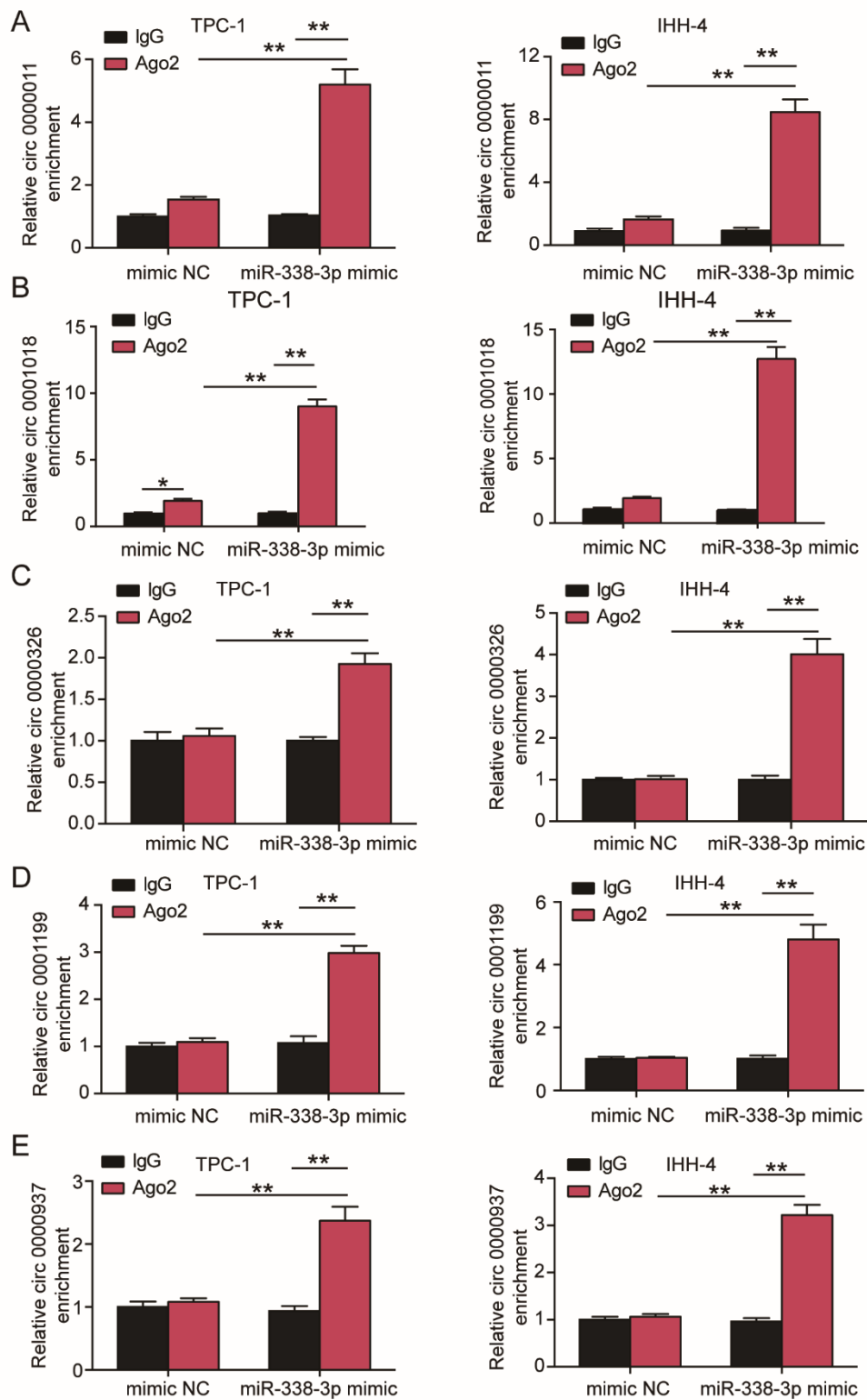


Figure S3 The structure of circ_0001018. CDS (coding sequences) are colored in green.

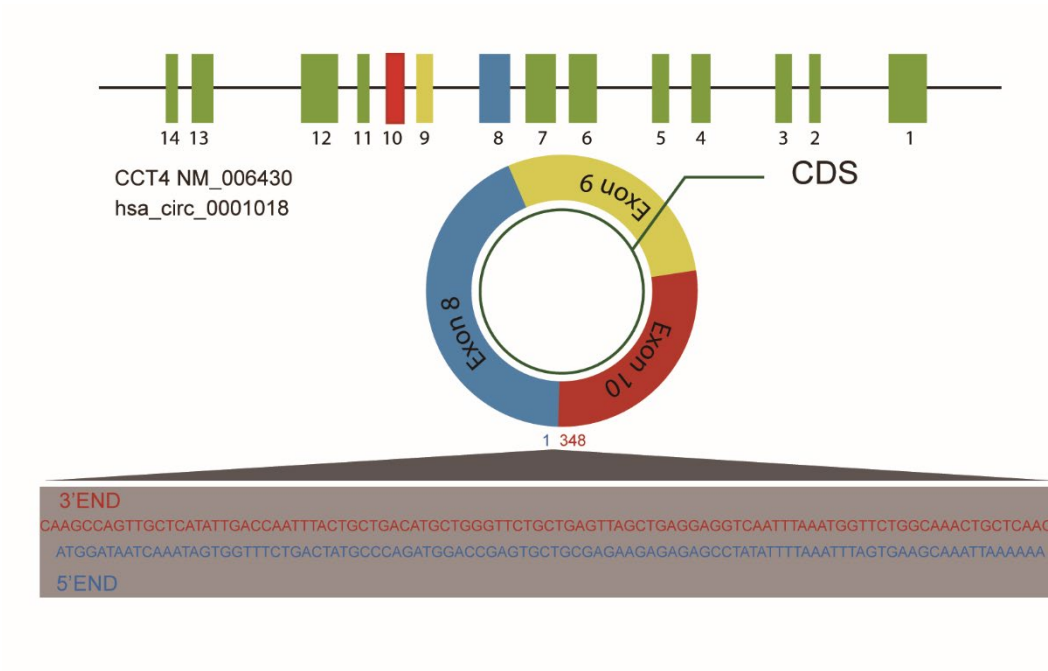


Figure S4 SOX4 was detected to express more in tumor tissues than the normal in TCGA database.

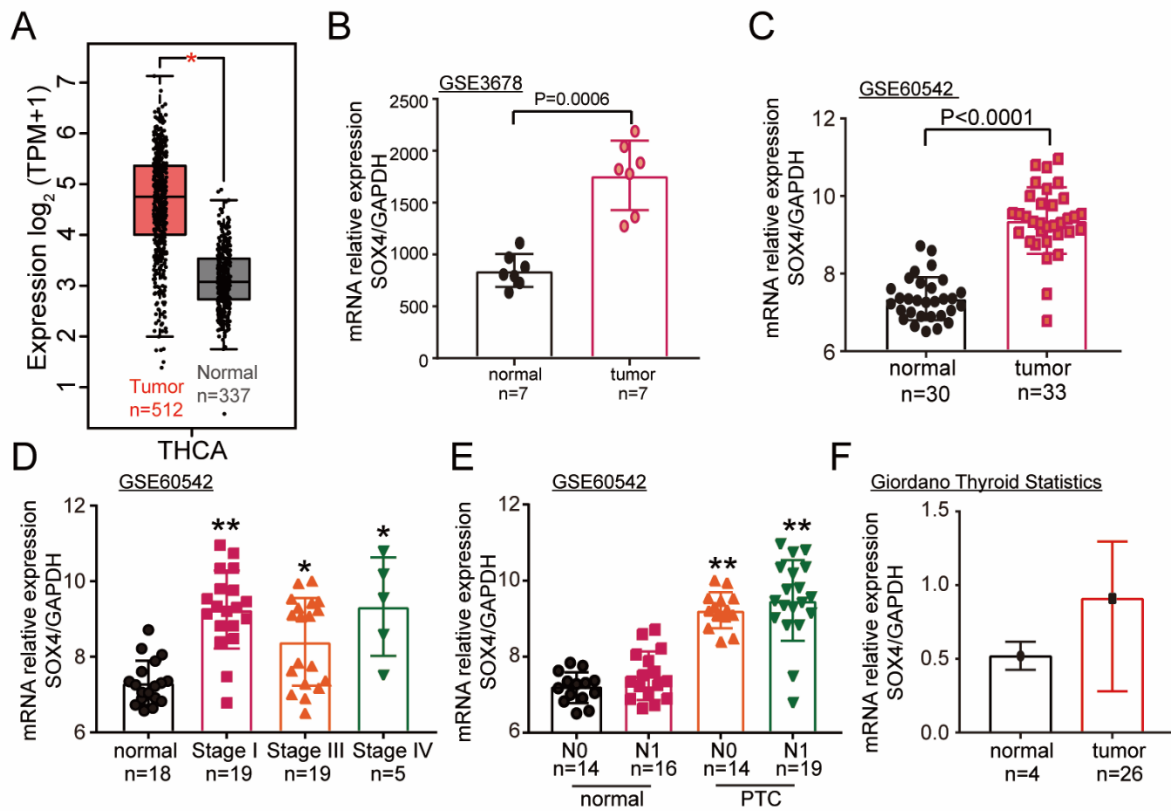
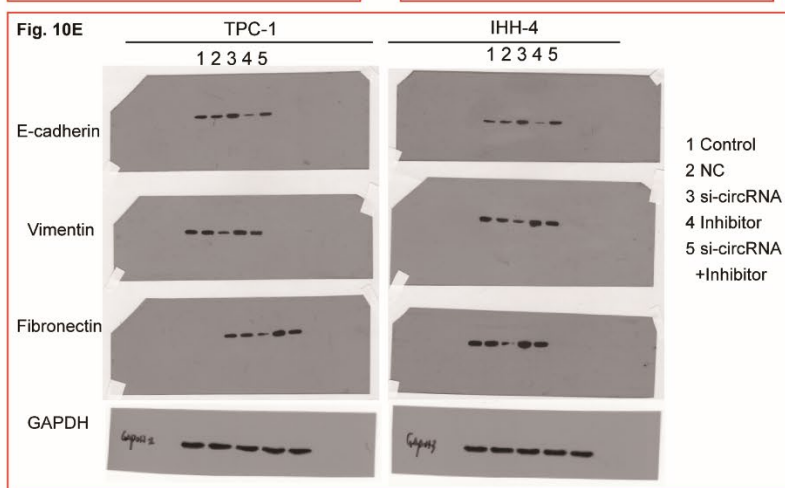
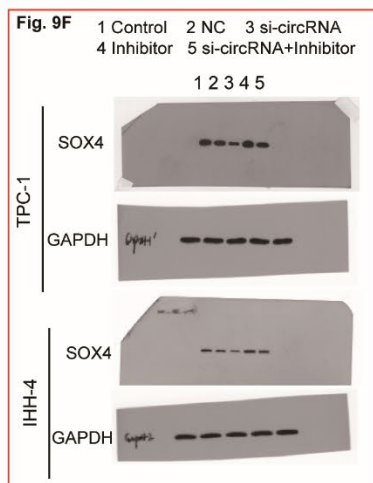
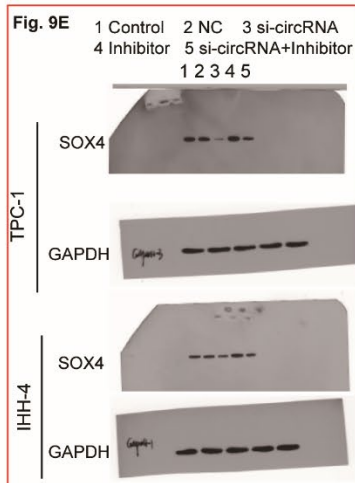
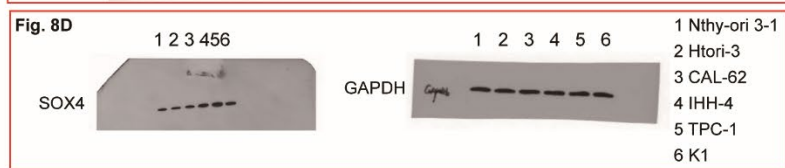
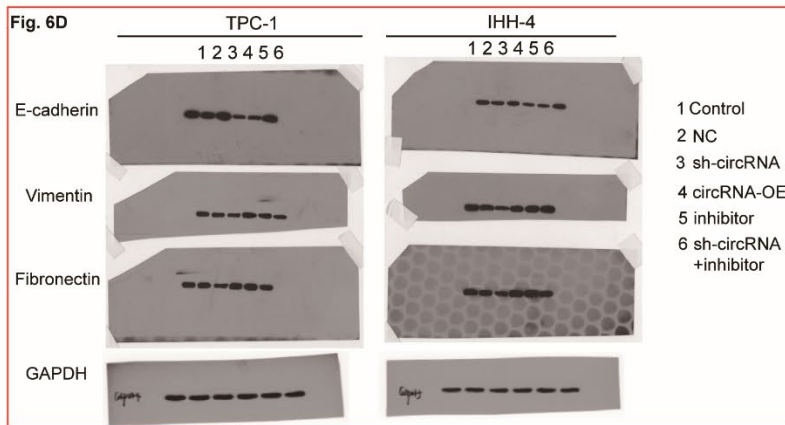


Figure S5 All images of western blot in this study.



References

1. Xing, M, Alzahrani, AS, Carson, KA, Viola, D, Elisei, R, Bendlova, B, et al. (2013). Association between BRAF V600E mutation and mortality in patients with papillary thyroid cancer. *Jama* 309: 1493-1501.
2. Siegel, RL, Miller, KD, and Jemal, A (2019). Cancer statistics, 2019. *CA: a cancer journal for clinicians* 69: 7-34.
3. Chen, W, Zheng, R, Baade, PD, Zhang, S, Zeng, H, Bray, F, et al. (2016). Cancer statistics in China, 2015. *CA: a cancer journal for clinicians* 66: 115-132.
4. Haugen, BR, Alexander, EK, Bible, KC, Doherty, GM, Mandel, SJ, Nikiforov, YE, et al. (2016). 2015 American Thyroid Association Management Guidelines for Adult Patients with Thyroid Nodules and Differentiated Thyroid Cancer: The American Thyroid Association Guidelines Task Force on Thyroid Nodules and Differentiated Thyroid Cancer. *Thyroid* 26: 1-133.
5. Hay, ID, Thompson, GB, Grant, CS, Bergstralh, EJ, Dvorak, CE, Gorman, CA, et al. (2002). Papillary thyroid carcinoma managed at the Mayo Clinic during six decades (1940-1999): temporal trends in initial therapy and long-term outcome in 2444 consecutively treated patients. *World J Surg* 26: 879-885.
6. Frohlich, E, and Wahl, R (2014). The current role of targeted therapies to induce radioiodine uptake in thyroid cancer. *Cancer Treat Rev* 40: 665-674.
7. Cai, X, Zhao, Z, Dong, J, Lv, Q, Yun, B, Liu, J, et al. (2019). Circular RNA circBACH2 plays a role in papillary thyroid carcinoma by sponging miR-139-5p and regulating LMO4 expression. *Cell death & disease* 10: 184.
8. Ashwal-Fluss, R, Meyer, M, Pamudurti, NR, Ivanov, A, Bartok, O, Hanan, M, et al. (2014). circRNA biogenesis competes with pre-mRNA splicing. *Mol Cell* 56: 55-66.
9. Alhasan, AA, Izuogu, OG, Al-Balool, HH, Steyn, JS, Evans, A, Colzani, M, et al. (2016). Circular RNA enrichment in platelets is a signature of transcriptome degradation. *Blood* 127: e1-e11.
10. Zlotorynski, E (2015). Non-coding RNA: Circular RNAs promote transcription. *Nature reviews Molecular cell biology* 16: 206.
11. Memczak, S, Jens, M, Elefsinioti, A, Torti, F, Krueger, J, Rybak, A, et al. (2013). Circular RNAs are a large class of animal RNAs with regulatory potency. *Nature* 495: 333-338.
12. Qu, S, Yang, X, Li, X, Wang, J, Gao, Y, Shang, R, et al. (2015). Circular RNA: A new star of noncoding RNAs. *Cancer letters* 365: 141-148.

13. Yang, C, Yuan, W, Yang, X, Li, P, Wang, J, Han, J, et al. (2018). Circular RNA circ-ITCH inhibits bladder cancer progression by sponging miR-17/miR-224 and regulating p21, PTEN expression. *Molecular cancer* 17: 19.
14. Li, Z, Huang, C, Bao, C, Chen, L, Lin, M, Wang, X, et al. (2015). Exon-intron circular RNAs regulate transcription in the nucleus. *Nature structural & molecular biology* 22: 256-264.
15. Granados-Riveron, JT, and Aquino-Jarquín, G (2016). The complexity of the translation ability of circRNAs. *Biochimica et biophysica acta* 1859: 1245-1251.
16. Nana-Sinkam, SP, and Croce, CM (2014). MicroRNA regulation of tumorigenesis, cancer progression and interpatient heterogeneity: towards clinical use. *Genome biology* 15: 445.
17. Wang, M, Chen, B, Ru, Z, and Cong, L (2018). CircRNA circ-ITCH suppresses papillary thyroid cancer progression through miR-22-3p/CBL/beta-catenin pathway. *Biochem Biophys Res Commun* 504: 283-288.
18. Li, Q, Pan, X, Zhu, D, Deng, Z, Jiang, R, and Wang, X (2019). Circular RNA MAT2B Promotes Glycolysis and Malignancy of Hepatocellular Carcinoma Through the miR-338-3p/PKM2 Axis Under Hypoxic Stress. *Hepatology (Baltimore, Md)* 70: 1298-1316.
19. Xu, Y, Yu, J, Huang, Z, Fu, B, Tao, Y, Qi, X, et al. (2020). Circular RNA hsa_circ_0000326 acts as a miR-338-3p sponge to facilitate lung adenocarcinoma progression. *J Exp Clin Cancer Res* 39: 57.
20. Qian, W, Huang, T, and Feng, W (2020). Circular RNA HIPK3 Promotes EMT of Cervical Cancer Through Sponging miR-338-3p to Up-Regulate HIF-1 α . *Cancer management and research* 12: 177-187.
21. Sui, GQ, Fei, D, Guo, F, Zhen, X, Luo, Q, Yin, S, et al. (2017). MicroRNA-338-3p inhibits thyroid cancer progression through targeting AKT3. *American journal of cancer research* 7: 1177-1187.
22. Dy, P, Penzo-Mendez, A, Wang, H, Pedraza, CE, Macklin, WB, and Lefebvre, V (2008). The three SoxC proteins--Sox4, Sox11 and Sox12--exhibit overlapping expression patterns and molecular properties. *Nucleic acids research* 36: 3101-3117.
23. Moreno, CS (2019). SOX4: The unappreciated oncogene. *Semin Cancer Biol.*
24. Hanieh, H, Ahmed, EA, Vishnubalaji, R, and Alajez, NM (2019). SOX4: Epigenetic regulation and role in tumorigenesis. *Semin Cancer Biol.*
25. Min, XS, Huang, P, Liu, X, Dong, C, Jiang, XL, Yuan, ZT, et al. (2015). Bioinformatics analyses

- of significant prognostic risk markers for thyroid papillary carcinoma. *Tumour Biol* 36: 7457-7463.
26. Guo, F, Fu, Q, Wang, Y, and Sui, G (2019). Long non-coding RNA NR2F1-AS1 promoted proliferation and migration yet suppressed apoptosis of thyroid cancer cells through regulating miRNA-338-3p/CCND1 axis. *J Cell Mol Med* 23: 5907-5919.
27. Huang, XH, Chen, JS, Wang, Q, Chen, XL, Wen, L, Chen, LZ, et al. (2011). miR-338-3p suppresses invasion of liver cancer cell by targeting smoothened. *The Journal of pathology* 225: 463-472.
28. Chen, X, Pan, M, Han, L, Lu, H, Hao, X, and Dong, Q (2013). miR-338-3p suppresses neuroblastoma proliferation, invasion and migration through targeting PREX2a. *FEBS letters* 587: 3729-3737.
29. Huang, N, Wu, Z, Lin, L, Zhou, M, Wang, L, Ma, H, et al. (2015). MiR-338-3p inhibits epithelial-mesenchymal transition in gastric cancer cells by targeting ZEB2 and MACC1/Met/Akt signaling. *Oncotarget* 6: 15222-15234.
30. Li, P, Chen, X, Su, L, Li, C, Zhi, Q, Yu, B, et al. (2013). Epigenetic silencing of miR-338-3p contributes to tumorigenicity in gastric cancer by targeting SSX2IP. *PLoS One* 8: e66782.
31. Liu, HS, Zheng, RN, Guo, LB, and Fu, XJ (2020). Circular RNA circ_0000615 knockdown suppresses the development of nasopharyngeal cancer through regulating the miR-338-3p/FGF2 axis. *Neoplasma*.
32. Qian, W, Huang, T, and Feng, W (2020). Circular RNA HIPK3 Promotes EMT of Cervical Cancer Through Sponging miR-338-3p to Up-Regulate HIF-1alpha. *Cancer Manag Res* 12: 177-187.
33. Pu, J, Wang, J, Li, W, Lu, Y, Wu, X, Long, X, et al. (2020). hsa_circ_0000092 promotes hepatocellular carcinoma progression through up-regulating HN1 expression by binding to microRNA-338-3p. *J Cell Mol Med*.
34. Wang, L, Peng, X, Lu, X, Wei, Q, Chen, M, and Liu, L (2019). Inhibition of hsa_circ_0001313 (circCCDC66) induction enhances the radio-sensitivity of colon cancer cells via tumor suppressor miR-338-3p: Effects of circ_0001313 on colon cancer radio-sensitivity. *Pathol Res Pract* 215: 689-696.
35. Li, S, Niu, X, Li, H, Liang, Y, Sun, Z, and Yan, Y (2019). Circ_0000003 promotes the proliferation and metastasis of non-small cell lung cancer cells via miR-338-3p/insulin receptor substrate 2. *Cell Cycle* 18: 3525-3539.
36. Zhang, L, and Ding, F (2019). Hsa_circ_0008945 promoted breast cancer progression by

targeting miR-338-3p. *Onco Targets Ther* 12: 6577-6589.

37. Li, Y, Chen, P, Zu, L, Liu, B, Wang, M, and Zhou, Q (2016). MicroRNA-338-3p suppresses metastasis of lung cancer cells by targeting the EMT regulator Sox4. *Am J Cancer Res* 6: 127-140.

38. Jin, Y, Zhao, M, Xie, Q, Zhang, H, Wang, Q, and Ma, Q (2015). MicroRNA-338-3p functions as tumor suppressor in breast cancer by targeting SOX4. *Int J Oncol* 47: 1594-1602.

39. Tong, Z, Meng, X, Wang, J, and Wang, L (2017). MicroRNA-338-3p targets SOX4 and inhibits cell proliferation and invasion of renal cell carcinoma. *Exp Ther Med* 14: 5200-5206.

40. Zhang, JX, Lu, J, Xie, H, Wang, DP, Ni, HE, Zhu, Y, et al. (2019). circHIPK3 regulates lung fibroblast-to-myofibroblast transition by functioning as a competing endogenous RNA. *Cell Death Dis* 10: 182.

41. Ren, H, Liu, Z, Liu, S, Zhou, X, Wang, H, Xu, J, et al. (2018). Profile and clinical implication of circular RNAs in human papillary thyroid carcinoma. *PeerJ* 6: e5363.

42. Lan, X, Cao, J, Xu, J, Chen, C, Zheng, C, Wang, J, et al. (2018). Decreased expression of hsa_circ_0137287 predicts aggressive clinicopathologic characteristics in papillary thyroid carcinoma. *Journal of clinical laboratory analysis* 32: e22573.

43. Wang, H, Yan, X, Zhang, H, and Zhan, X (2019). CircRNA circ_0067934 Overexpression Correlates with Poor Prognosis and Promotes Thyroid Carcinoma Progression. *Medical science monitor : international medical journal of experimental and clinical research* 25: 1342-1349.

44. Lan, X, Xu, J, Chen, C, Zheng, C, Wang, J, Cao, J, et al. (2018). The Landscape of Circular RNA Expression Profiles in Papillary Thyroid Carcinoma Based on RNA Sequencing. *Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology* 47: 1122-1132.

45. Peng, N, Shi, L, Zhang, Q, Hu, Y, Wang, N, and Ye, H (2017). Microarray profiling of circular RNAs in human papillary thyroid carcinoma. *PloS one* 12: e0170287.

46. Yang, C, Wei, Y, Yu, L, and Xiao, Y (2019). Identification of Altered Circular RNA Expression in Serum Exosomes from Patients with Papillary Thyroid Carcinoma by High-Throughput Sequencing. *Medical science monitor : international medical journal of experimental and clinical research* 25: 2785-2791.

47. Liu, Q, Pan, LZ, Hu, M, and Ma, JY (2019). Molecular Network-Based Identification of Circular RNA-Associated ceRNA Network in Papillary Thyroid Cancer. *Pathol Oncol Res*.

48. Dong, R, Zhang, XO, Zhang, Y, Ma, XK, Chen, LL, and Yang, L (2016). CircRNA-derived

pseudogenes. *Cell research* 26: 747-750.

49. Li, L, Song, Y, Shi, X, Liu, J, Xiong, S, Chen, W, et al. (2018). The landscape of miRNA editing in animals and its impact on miRNA biogenesis and targeting. *Genome Res* 28: 132-143.

50. Wang, M, Chen, B, Ru, Z, and Cong, L (2018). CircRNA circ-ITCH suppresses papillary thyroid cancer progression through miR-22-3p/CBL/ β -catenin pathway. *Biochemical and biophysical research communications* 504: 283-288.

51. Yao, Y, Chen, X, Yang, H, Chen, W, Qian, Y, Yan, Z, et al. (2019). Hsa_circ_0058124 promotes papillary thyroid cancer tumorigenesis and invasiveness through the NOTCH3/GATAD2A axis. *Journal of experimental & clinical cancer research : CR* 38: 318.

52. Pan, Y, Xu, T, Liu, Y, Li, W, and Zhang, W (2019). Upregulated circular RNA circ_0025033 promotes papillary thyroid cancer cell proliferation and invasion via sponging miR-1231 and miR-1304. *Biochemical and biophysical research communications* 510: 334-338.

53. Wei, H, Pan, L, Tao, D, and Li, R (2018). Circular RNA circZFR contributes to papillary thyroid cancer cell proliferation and invasion by sponging miR-1261 and facilitating C8orf4 expression. *Biochemical and biophysical research communications* 503: 56-61.

54. Chen, F, Feng, Z, Zhu, J, Liu, P, Yang, C, Huang, R, et al. (2018). Emerging roles of circRNA_NEK6 targeting miR-370-3p in the proliferation and invasion of thyroid cancer via Wnt signaling pathway. *Cancer biology & therapy* 19: 1139-1152.

55. Song, Z, Zhang, X, Lin, Y, Wei, Y, Liang, S, and Dong, C (2019). LINC01133 inhibits breast cancer invasion and metastasis by negatively regulating SOX4 expression through EZH2. *J Cell Mol Med* 23: 7554-7565.

56. Yang, J, Lin, X, Jiang, W, Wu, J, and Lin, L (2019). lncRNA LEF1-AS1 Promotes Malignancy in Non-Small-Cell Lung Cancer by Modulating the miR-489/SOX4 Axis. *DNA and cell biology* 38: 1013-1021.

57. Li, Y, Hao, J, Jiang, YM, Liu, Y, and Zhang, SH (2019). Long non-coding RNA DSCAM-AS1 indicates a poor prognosis and modulates cell proliferation, migration and invasion in ovarian cancer via upregulating SOX4. *European review for medical and pharmacological sciences* 23: 4143-4148.

58. Wei, D, Wang, W, Shen, B, Zhou, Y, Yang, X, Lu, G, et al. (2019). MicroRNA199a5p suppresses migration and invasion in oral squamous cell carcinoma through inhibiting the EMT-related transcription factor SOX4. *Int J Mol Med* 44: 185-195.

59. Chang, J, Gao, F, Chu, H, Lou, L, Wang, H, and Chen, Y (2019). miR-363-3p inhibits migration,

invasion, and epithelial-mesenchymal transition by targeting NEDD9 and SOX4 in non-small-cell lung cancer. *Journal of cellular physiology*.

60. Zhong, M, Wang, WL, and Yu, DJ (2019). Long non-coding RNA OR3A4 is associated with poor prognosis of human non-small cell lung cancer and regulates cell proliferation via up-regulating SOX4. *European review for medical and pharmacological sciences* 23: 6524-6530.

61. Ding, L, Zhao, Y, Dang, S, Wang, Y, Li, X, Yu, X, et al. (2019). Circular RNA circ-DONSON facilitates gastric cancer growth and invasion via NURF complex dependent activation of transcription factor SOX4. *Molecular cancer* 18: 45.

62. Ruan, M, Liu, M, Dong, Q, and Chen, L (2015). Iodide- and glucose-handling gene expression regulated by sorafenib or cabozantinib in papillary thyroid cancer. *The Journal of clinical endocrinology and metabolism* 100: 1771-1779.

63. Ruan, X, Shi, X, Dong, Q, Yu, Y, Hou, X, Song, X, et al. (2019). Antitumor effects of anlotinib in thyroid cancer. *Endocrine-related cancer* 26: 153-164.

64. Ribeiro, FR, Meireles, AM, Rocha, AS, and Teixeira, MR (2008). Conventional and molecular cytogenetics of human non-medullary thyroid carcinoma: characterization of eight cell line models and review of the literature on clinical samples. *BMC Cancer* 8: 371.

65. Saiselet, M, Floor, S, Tarabichi, M, Dom, G, Hebrant, A, van Staveren, WC, et al. (2012). Thyroid cancer cell lines: an overview. *Front Endocrinol (Lausanne)* 3: 133.

66. Meireles, AM, Preto, A, Rocha, AS, Rebocho, AP, Maximo, V, Pereira-Castro, I, et al. (2007). Molecular and genotypic characterization of human thyroid follicular cell carcinoma-derived cell lines. *Thyroid* 17: 707-715.

67. Schweppe, RE, Klopper, JP, Korch, C, Pugazhenthii, U, Benezra, M, Knauf, JA, et al. (2008). Deoxyribonucleic acid profiling analysis of 40 human thyroid cancer cell lines reveals cross-contamination resulting in cell line redundancy and misidentification. *J Clin Endocrinol Metab* 93: 4331-4341.

68. Sekiguchi, M, Shiroko, Y, Arai, T, Kishino, T, Sugawara, I, Kusakabe, T, et al. (2001). Biological characteristics and chemosensitivity profile of four human anaplastic thyroid carcinoma cell lines. *Biomed Pharmacother* 55: 466-474.

69. Song, B, Li, R, Zuo, Z, Tan, J, Liu, L, Ding, D, et al. (2019). LncRNA ENST00000539653 acts as an oncogenic factor via MAPK signalling in papillary thyroid cancer. *BMC Cancer* 19: 297.

70. Ge, MH, Jiang, LH, Wen, QL, Tan, Z, Chen, C, Zheng, CM, et al. (2018). Preliminary screening

and analysis of metastasis-related lncRNA and co-expressed papillary thyroid carcinoma mRNA. *Oncol Lett* 16: 3715-3725.

71. Wang, ZL, Wang, C, Liu, W, and Ai, ZL (2019). Emerging roles of the long non-coding RNA 01296/microRNA-143-3p/MSI2 axis in development of thyroid cancer. *Biosci Rep* 39.

72. He, Y, Cao, L, Wang, L, Liu, L, Huang, Y, and Gong, X (2020). Metformin Inhibits Proliferation of Human Thyroid Cancer TPC-1 Cells by Decreasing LRP2 to Suppress the JNK Pathway. *Oncotargets Ther* 13: 45-50.

73. Esposito, T, Lucariello, A, Hay, E, Contieri, M, Tammaro, P, Varriale, B, et al. (2019). Effects of curcumin and its adjuvant on TPC1 thyroid cell line. *Chem Biol Interact* 305: 112-118.

74. Yang, Y, Ding, L, Li, Y, and Xuan, C (2020). Hsa_circ_0039411 promotes tumorigenesis and progression of papillary thyroid cancer by miR-1179/ABCA9 and miR-1205/MTA1 signaling pathways. *J Cell Physiol* 235: 1321-1329.

75. Sui, H, Fan, S, Liu, W, Li, Y, Zhang, X, Du, Y, et al. (2020). LINC00028 regulates the development of TGF β 1-treated human tenon capsule fibroblasts by targeting miR-204-5p. *Biochem Biophys Res Commun*.