

## Supplementary Materials

### Circular RNA hsa\_circ\_0062682 binds to YBX1 and promotes oncogenesis in hepatocellular carcinoma

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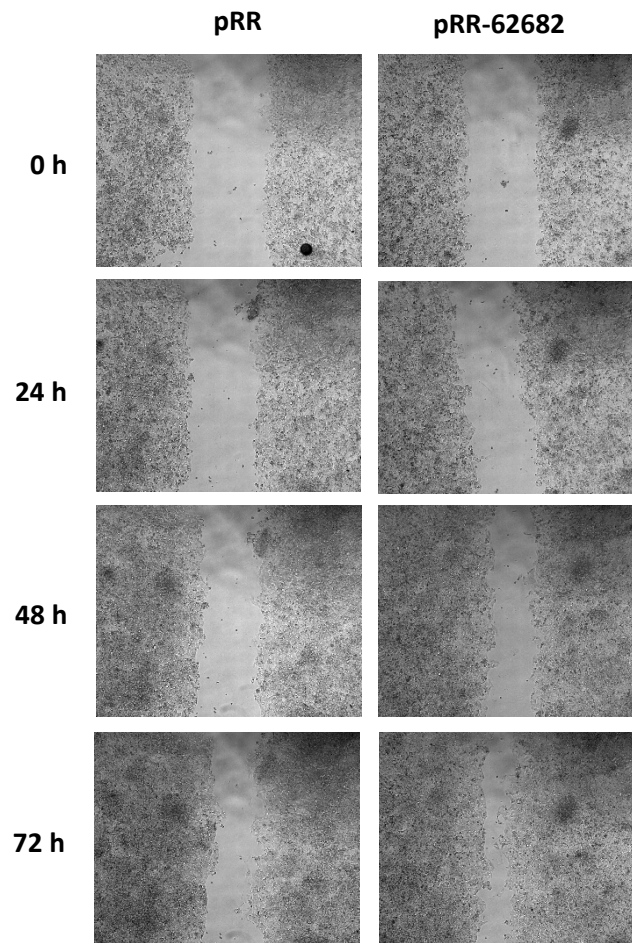
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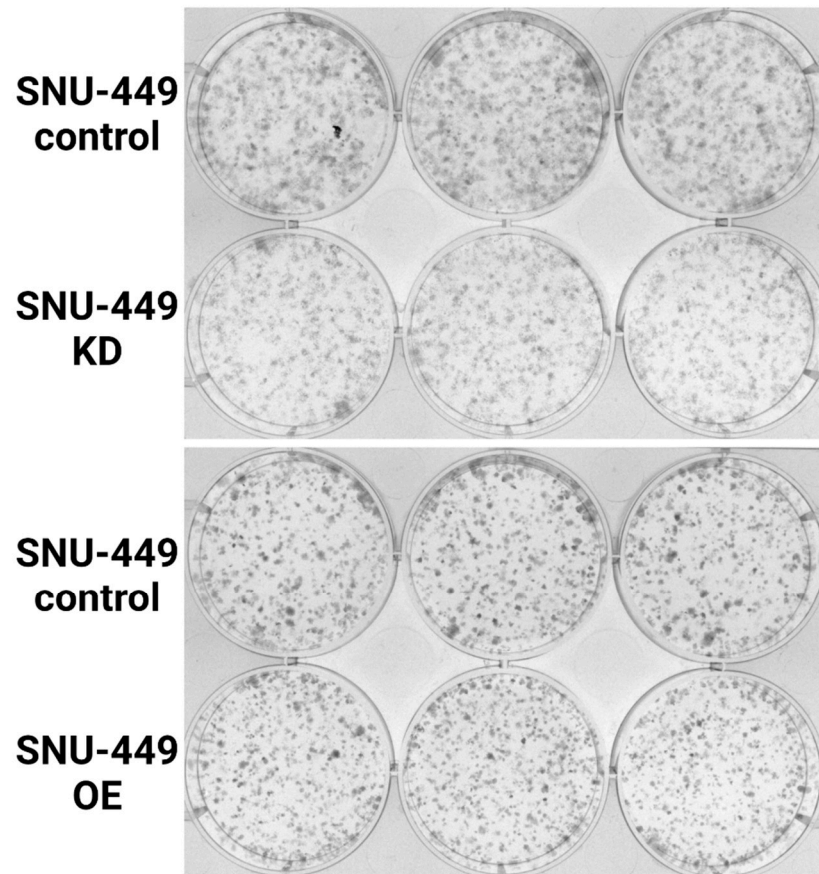
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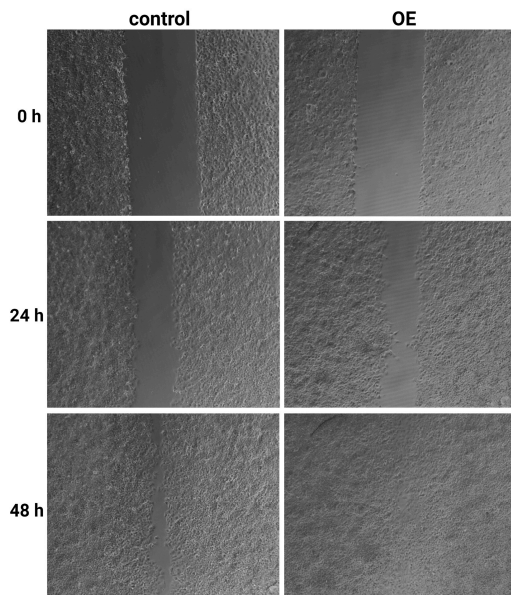
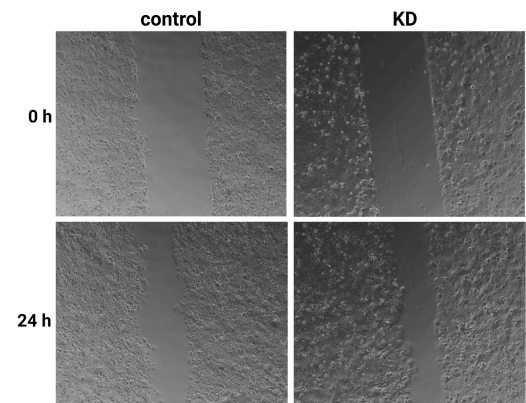
**Figure S1:** Schematic representation of the modification of a multiple cloning site in the mini-gene plasmid system pRR. Green colour indicates sites of introduced mutations. Red colour indicates splice sites.



**Figure S2:** Representative images of the wound healing assay of hsa\_circ\_0062682-overexpressing HepG2 cell line; pRR – empty plasmid, pRR-62682 – plasmid for hsa\_circ\_0062682 overexpression.

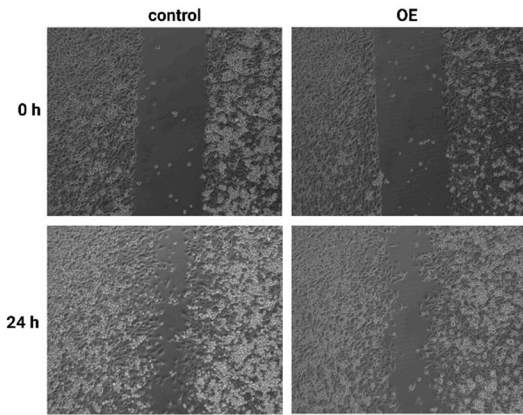


**Figure S3:** Colony forming assay in transduced SNU-449 cell lines; KD – knockdown (2.000 cells) ; OE – overexpression (1.000 cells).

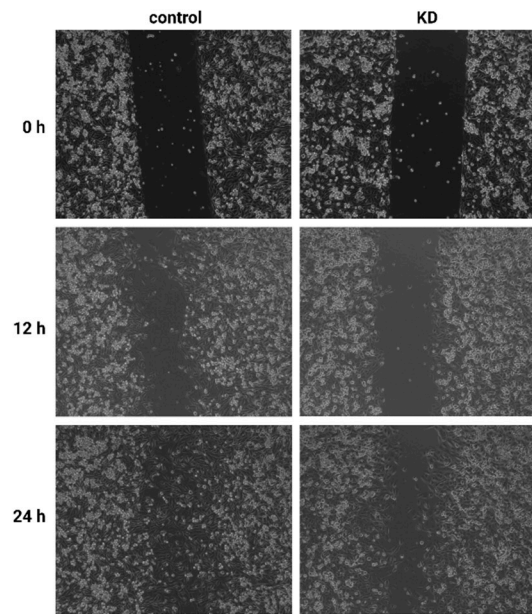
**A****B**

**Figure S4:** Representative images of a wound healing assay of overexpressing Huh-7 (A) and in a knockdown of Huh-7 (B); OE – overexpression, KD – knockdown.

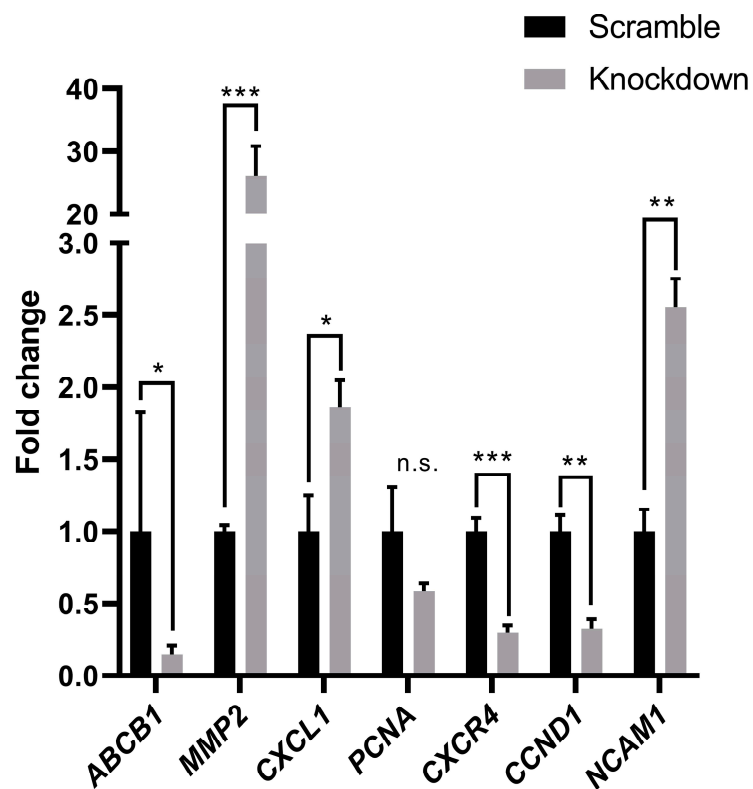
A



B

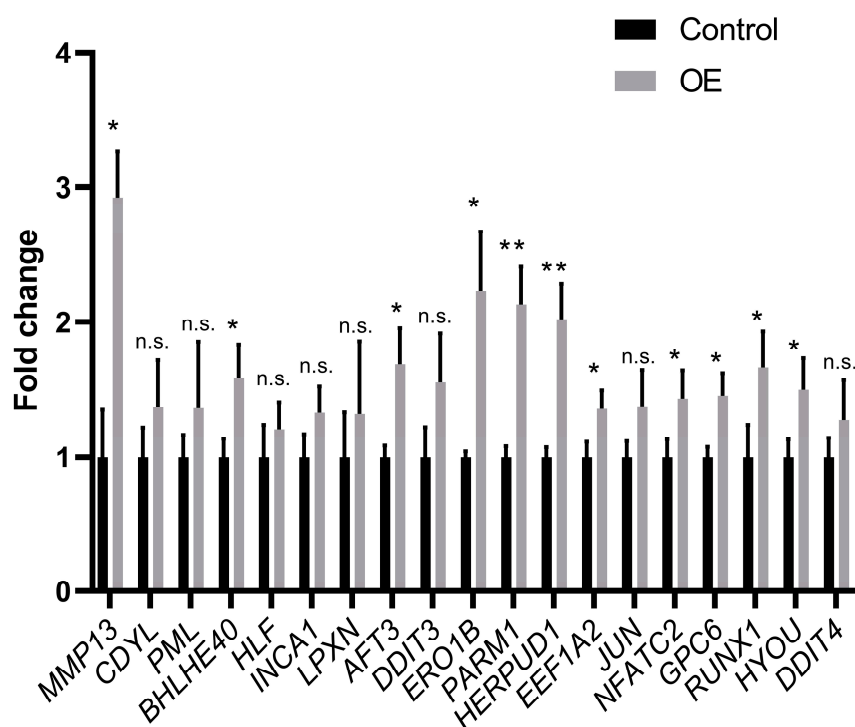


**Figure S5:** Representative images of a wound healing assay of overexpressing SNU-449 (A) and in a knockdown of SNU-449 (B); OE – overexpression, KD – knockdown.

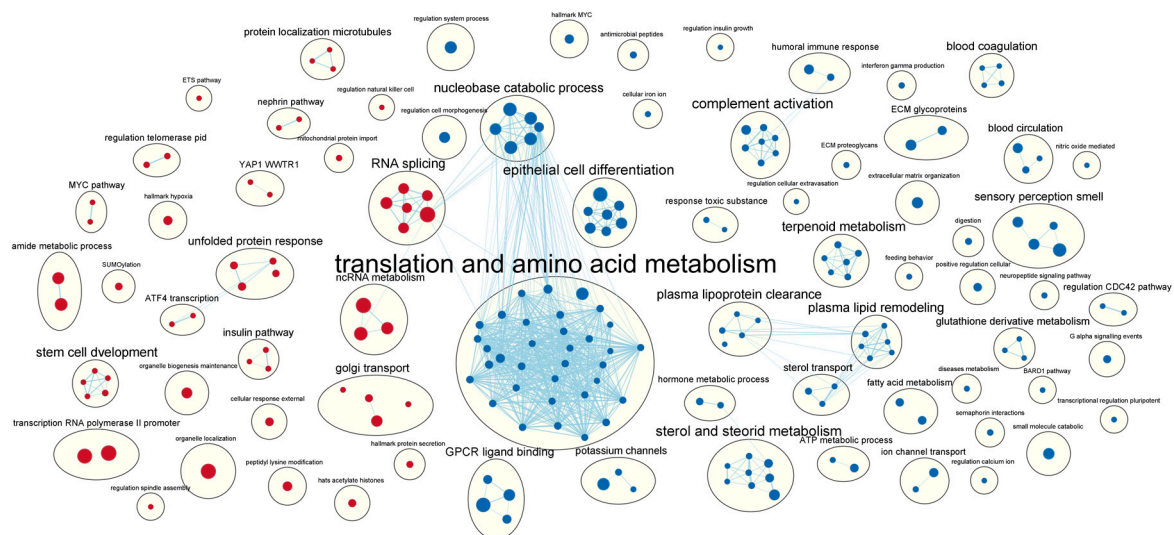


**Figure S6:** RT-qPCR validation of differentially expressed genes identified by microarray analysis in the knockdown cell line SNU-449. We analyzed the expression of target candidates of transcription factors E2F1

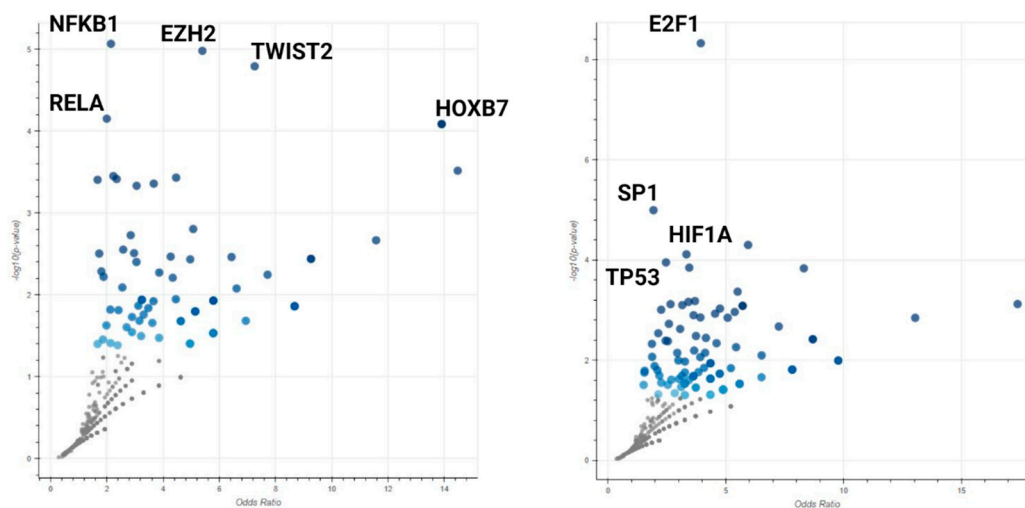
(*CCND1*, *PCNA*), *NFKB1* (*MMP2*, *CXCL1*, *NCAM1*) and *HIF-1A* (*ABCB1*, *CXCR4*). Results are represented as averages and error bars represent standard deviation. \* p-value  $\leq 0.05$ ; \*\* p-value  $\leq 0.01$ ; \*\*\* p-value  $\leq 0.001$ ; n.s. – not significant, Student's t-test.



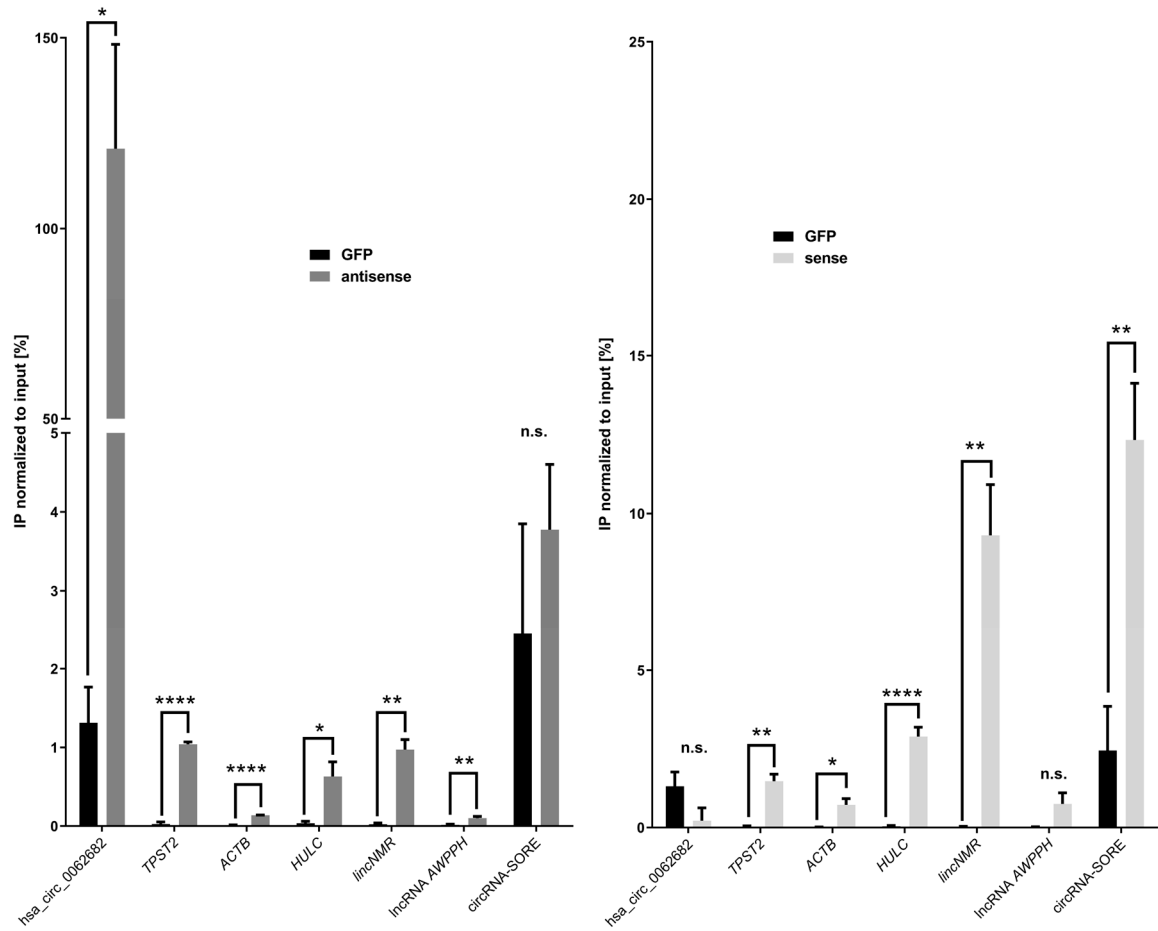
**Figure S7:** RT-qPCR validation of differentially expressed genes identified by microarray analysis in the transient overexpression of *hsa\_circ\_0062682* in Huh-7 cell line. Results are represented as averages and error bars represent standard deviation. \* p-value  $\leq 0.05$ ; \*\* p-value  $\leq 0.01$ ; n.s. – not significant, Student's t-test.



**Figure S8:** GSEA analysis of upregulated (red colour) and downregulated (blue colour) pathways of Huh-7 transient overexpression

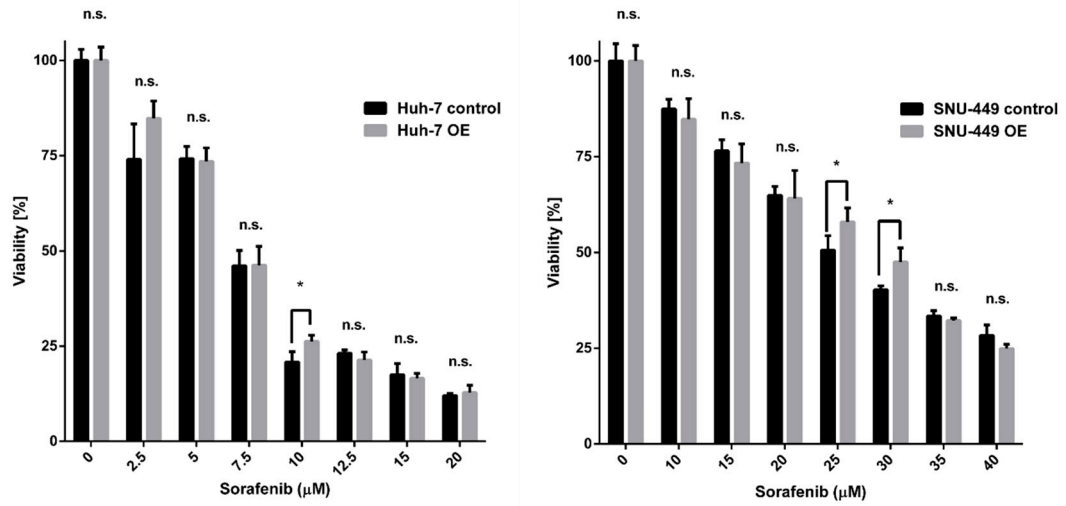


**Figure S9:** Volcano plots from Enrichr analysis of enriched gene sets in TRRUST transcription factors of upregulated (left) and downregulated (right) differentially expressed genes in SNU-449 knockdown cell line.

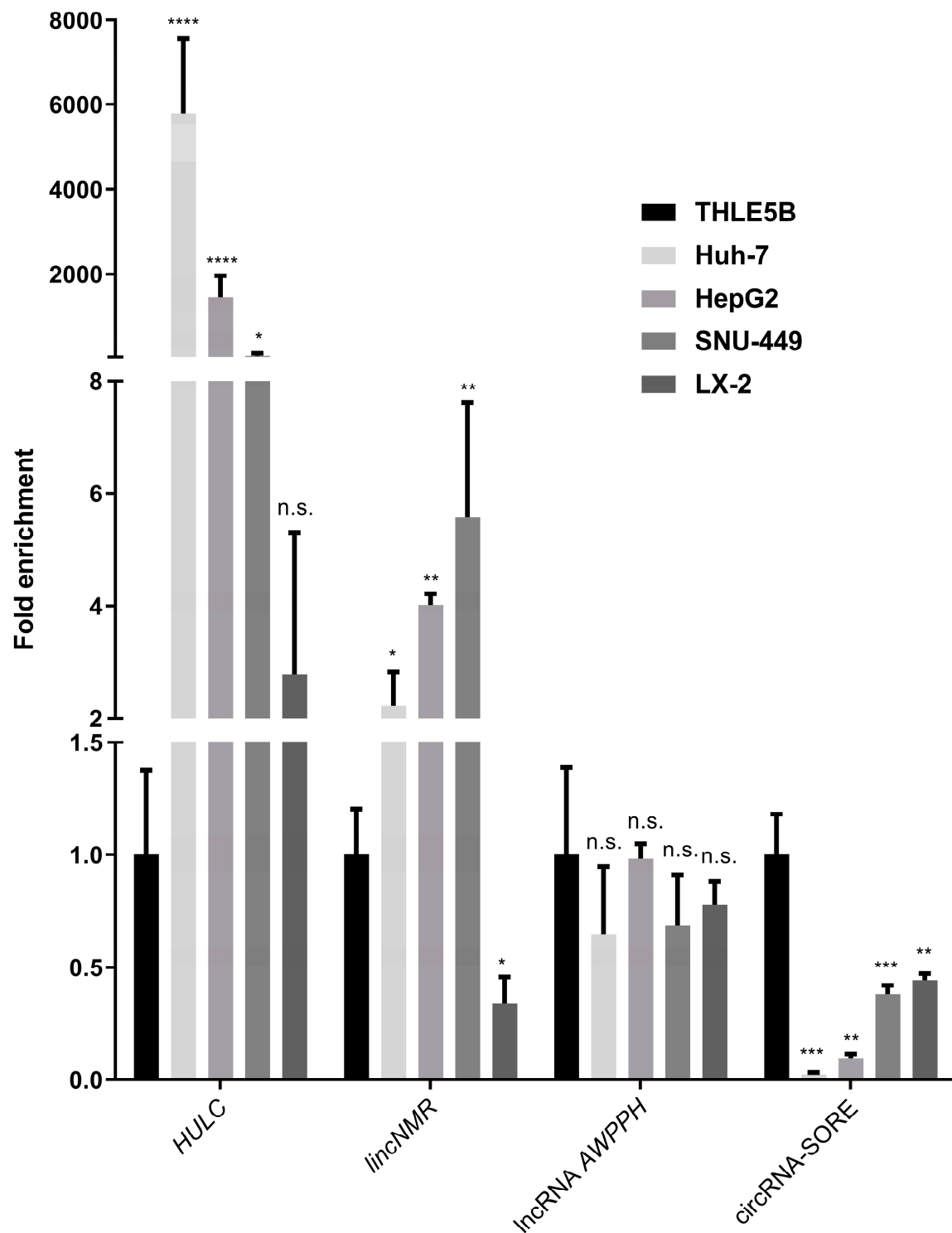


**Figure S10:** Enrichment of RNA species in biotinylated oligonucleotide pulldowns normalized the input; antisense (left), sense (right) (n = 3). Results are represented as averages and error bars represent standard deviation. \* p-value  $\leq 0.05$ ; \*\* p-value  $\leq 0.01$ ; \*\*\* p-value  $\leq 0.001$ ; \*\*\*\* p-value  $\leq 0.0001$ ; n.s. – not significant, Student's t-test.

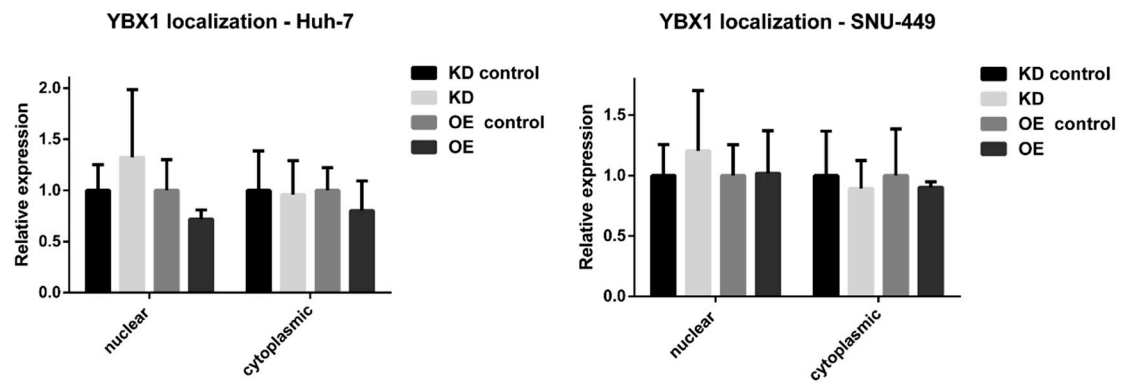




**Figure S11:** Viability of sorafenib treated OE cell lines measured by colorimetric CCK-8 assay (n = 4). Results are represented as averages and error bars represent standard deviation. \* p-value  $\leq 0.05$ ; n.s. – not significant, Student's t-test.



**Figure S12:** Relative expression of RNA species in human HCC model cell lines (Huh-7, HepG2, SNU-449) and human hepatic stellate cell line LX-2, normalized to the immortalized human hepatocyte cell line THLE5B (n = 3). Results are represented as averages and error bars represent standard deviation. \* p-value  $\leq 0.05$ ; \*\* p-value  $\leq 0.01$ ; \*\*\* p-value  $\leq 0.001$ ; \*\*\*\* p-value  $\leq 0.0001$ ; n.s. – not significant, Student's t-test.



**Figure S13:** YBX1 localization in hsa\_circ\_0062682 stable cell lines identified by western blotting (n = 3); OE – overexpression, KD – knockdown, cyto – cytoplasmic fraction, nuc – nuclear fraction. Results are represented as averages and error bars represent standard deviation.