

**Additional information for**

***LOXL1-AS1* contributes to metastasis in sonic-hedgehog medulloblastoma by promoting cancer stem-like phenotypes**

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This file includes additional tables and figures:

Supplementary Table S1. shRNA targeting sequences used in the study

Supplementary Table S2. Gene-specific primer pairs used in qRT-PCR

Supplementary Figure S1

Supplementary Figure S2

Supplementary Figure S3

Supplementary Figure S4

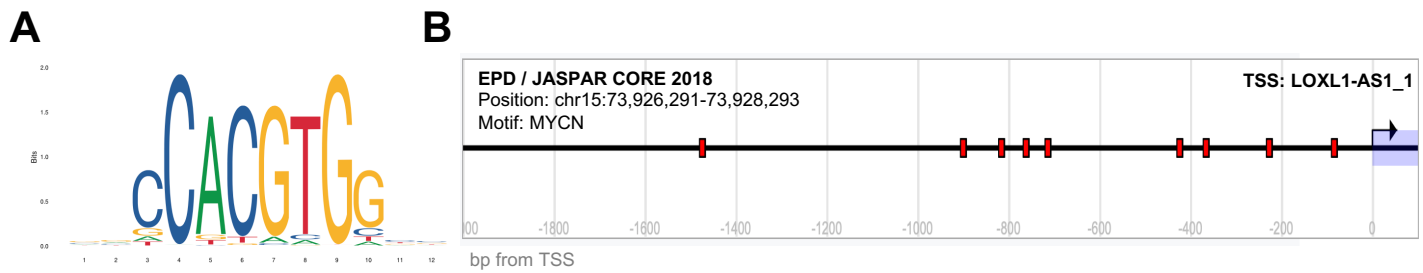
Supplementary Figure S5

**Supplementary Table S1.** shRNA targeting sequences used in the study.

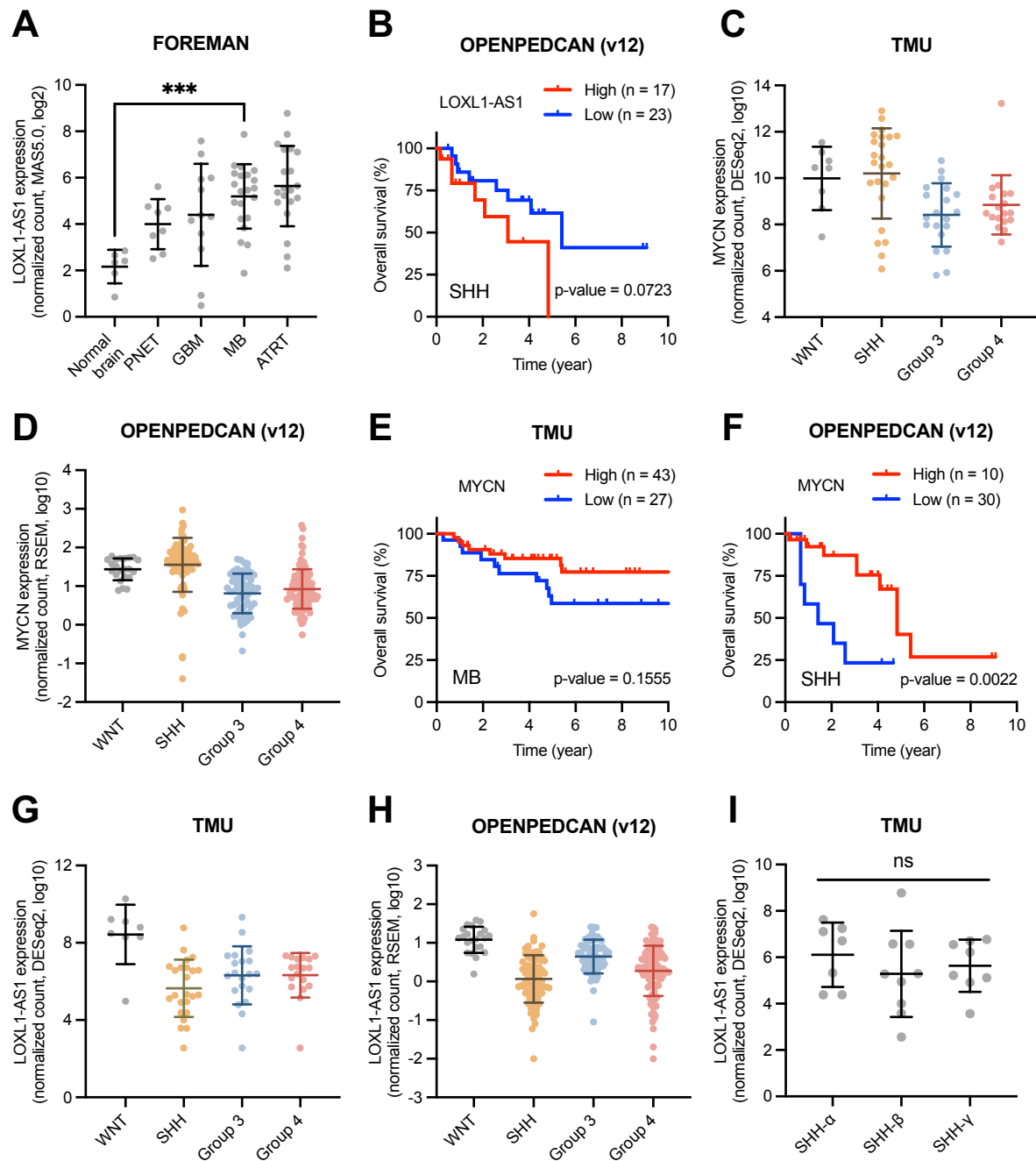
| Target gene      | Gene ID            | shRNA name        | Target sequence       |
|------------------|--------------------|-------------------|-----------------------|
| <i>LOXL1-AS1</i> | <i>NR_040066.1</i> | shLOXL1-AS1 #1196 | GGTGCATACAAGCACTCAA   |
| <i>LOXL1-AS1</i> | <i>NR_040066.1</i> | shLOXL1-AS1 #2278 | GGTCTTGGACTTTATTGTT   |
| <i>TGFB2</i>     | <i>NM_003238</i>   | shTGFB2           | CGGATTGAGCTATATCAGATT |
| Negative control | -                  | shNC              | TTCTCCGAACGTGTCACGT   |

**Supplementary Table S2.** Gene-specific primer pairs used in qRT-PCR.

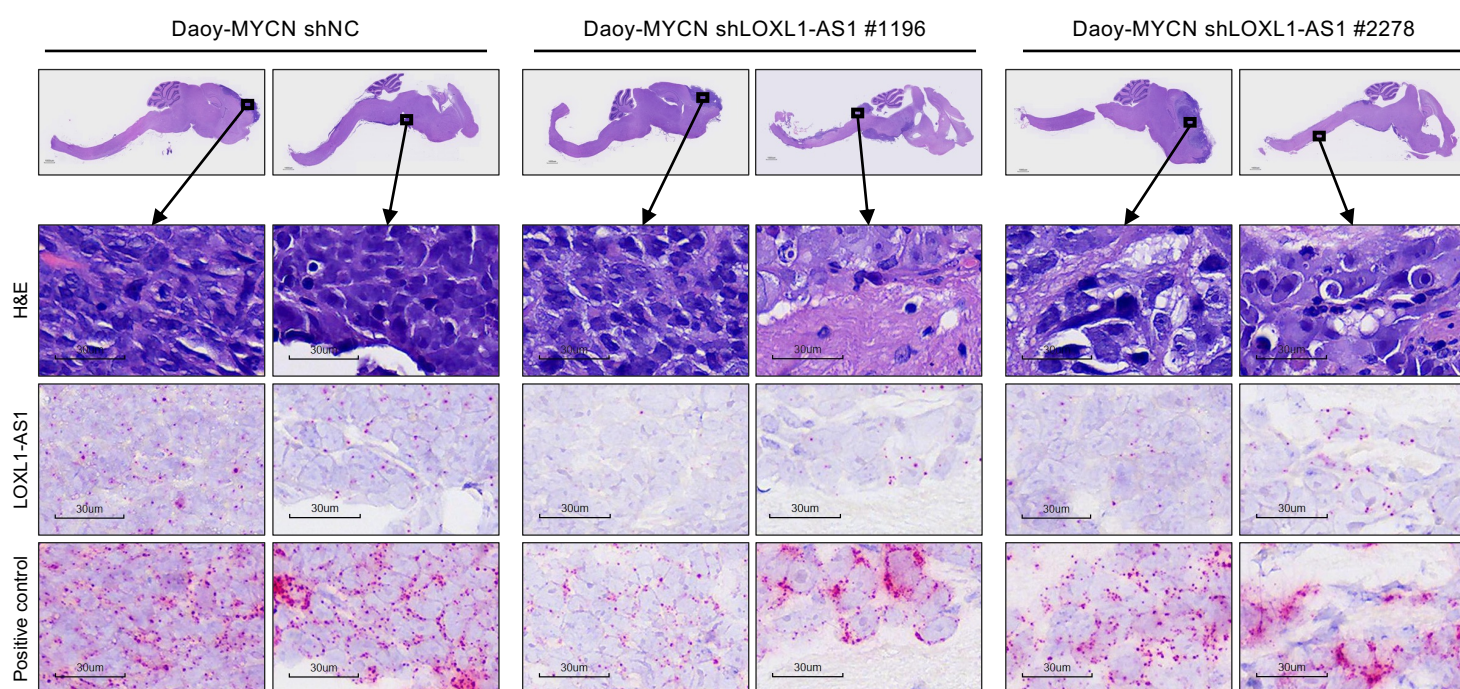
| Gene name        | Forward primer          | Reverse primer           |
|------------------|-------------------------|--------------------------|
| <i>MYCN</i>      | CCACAAGGCCCTCAGTACC     | TCTTCCTCTTCATCATCTTCATCA |
| <i>LOXL1-AS1</i> | AGTCCACAAATCCTAGGTGTA   | CTCGTTTCCGATCCAGCCAGG    |
| <i>SOX2</i>      | TTGCTGCCTCTTTAAGACTAGGA | TAAGCCTGGGGCTCAAACCT     |
| <i>OCT4</i>      | ATCGAGAACCGAGTGAGAGG    | CACTCGGACCACATCCTTCT     |
| <i>NANOG</i>     | CTCCAACATCCTGAACCTCAGC  | CGTCACACCATTGCTATTCTTCG  |
| <i>BMI1</i>      | AATCCCCACCTGATGTGTGT    | GCTGGTCTCCAGGTAACGAA     |
| <i>TGFB2</i>     | ACACTCAGCACAGCAGGGTCCT  | TTGGGACACGCAGCAAGGAGAAG  |
| <i>HOTAIR</i>    | CCAGAGAACGCTGGAAAAACCTG | GGAGATGATAAGAAGAGCAAGGAA |
| <i>RAMP2-AS1</i> | GAAGTCAGGCCAGATTTACAAG  | TTGGGTCCTACAGCAACCAT     |
| <i>LINC01606</i> | GCTGGACATTTCTCCCTTCA    | GAGTCCTCTCGCTTCCTCCT     |
| <i>TRHDE-AS1</i> | GGGTGTAGAGAGGGAAGTTAGG  | TTCTCTCCAGCTGCAGGGTGTA   |
| <i>CLMN</i>      | AGGCTTTCAGCATCGCACAGGA  | CTAGAAACTGTGCCACGTAAGTC  |
| <i>KCNQ3</i>     | CGTCTGATTGCCGCCACCTTTT  | TTCTGACGGTGTTGCTCCTGCA   |
| <i>USP2</i>      | GAGATACGCACCGCGCTTTGTT  | GGTTGGACTTAGGTCTCAGTGTC  |
| <i>LOXL1</i>     | ACAGCACCTGTGACTTCGGCAA  | CGGTTATGTCGATCCACTGGCA   |
| <i>SAMD11</i>    | GAGCATCAGAGCCGCTGTGAAT  | AAAGCAGTCGCTGCTGATACGG   |
| <i>HSPCB</i>     | AGCCTACGTTGCTCACTATTACG | GAAAGGCAAAAGTCTCCACCT    |



**Supplementary Figure S1.** A genomic region of 2-kilo base pairs upstream of the transcription starting site (TSS) of *LOXL1-AS1* transcript 1 (NR\_040066) was used as input for screening of transcription factor binding sites using EPD (Eukaryotic Promoter Database, <https://epd.expasy.org/>) and JASPAR 2018 (<https://jaspar.genereg.net>) databases. **(A)** Illustration of MYCN binding motif. **(B)** Detection of MYCN binding motif within the genomic region of interest.

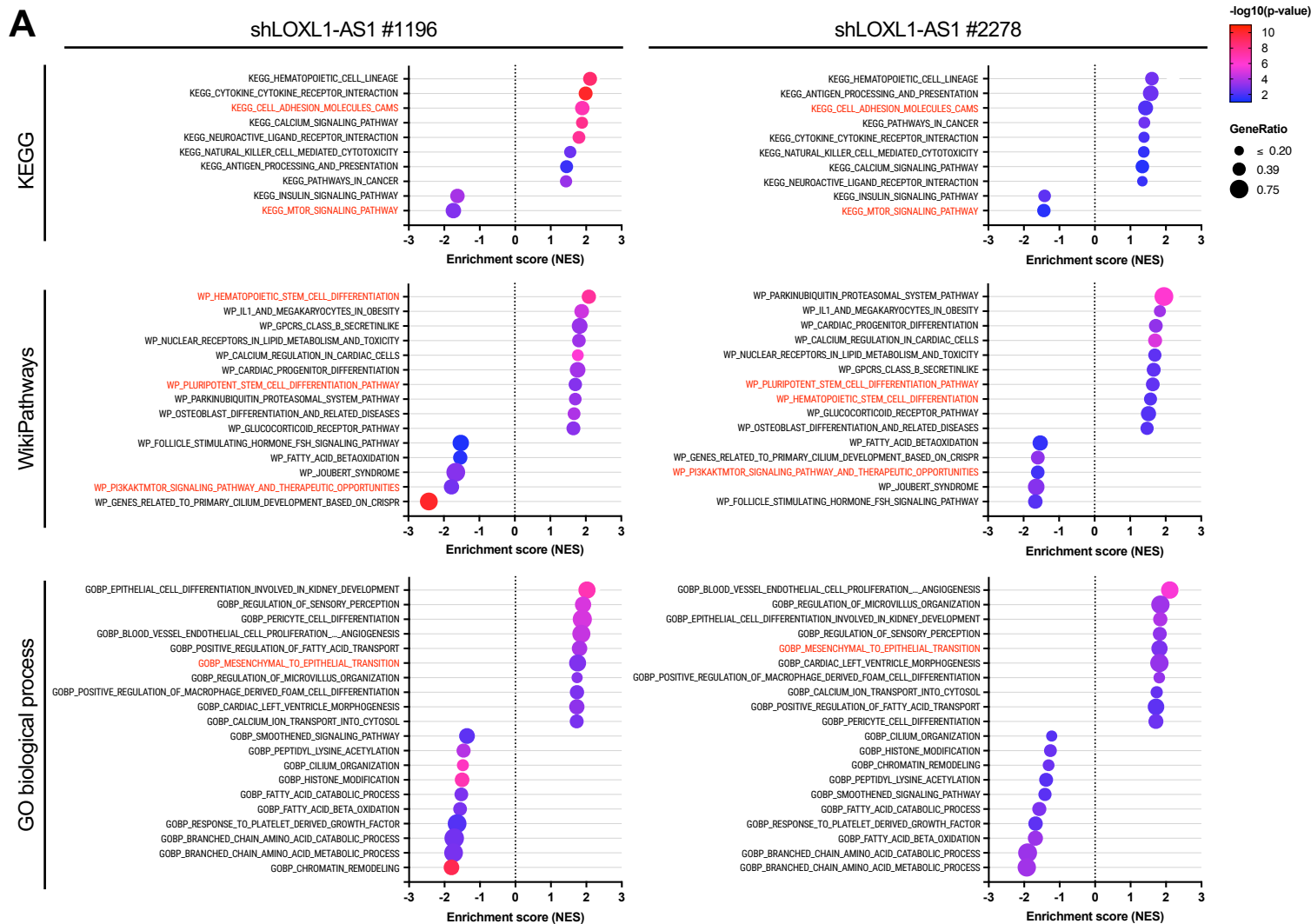


**Supplementary Figure S2.** (A) *LOXL1-AS1* expression in the normal brain and different types of brain tumor in a public dataset (Foreman, R2 platform). PNET, primitive neuroectodermal tumor; GBM, glioblastoma; MB, medulloblastoma; ATRT, atypical teratoid/rhabdoid tumor. (B) Survival analyses of *LOXL1-AS1* expression in SHH-MB in the OpenPedCan cohort. (C-D) MYCN expression level across four molecular subgroups in the TMU and OpenPedCan cohorts. (E-F) Survival analyses of MYCN expression in the TMU and OpenPedCan cohorts. (G-I) *LOXL1-AS1* expression in the TMU and OpenPedCan cohorts, including (G-H) expression level across four molecular subgroups of MB and (I) expression level across three molecular subtypes of SHH-MB. ns, non-significant; \*\*\*  $p < 0.001$

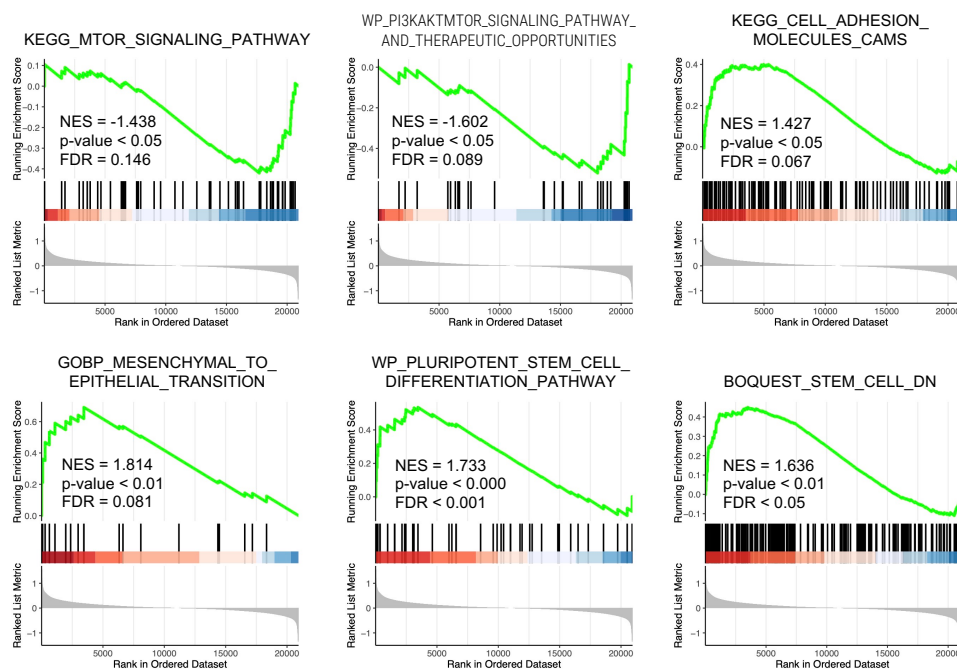


**Supplementary Figure S3. (A)** H&E staining and *in situ* hybridization of *LOXL1-AS1* and positive control RNA in the brain and spinal cord tissue of representative mice from each group. Scale bars 30 μm.

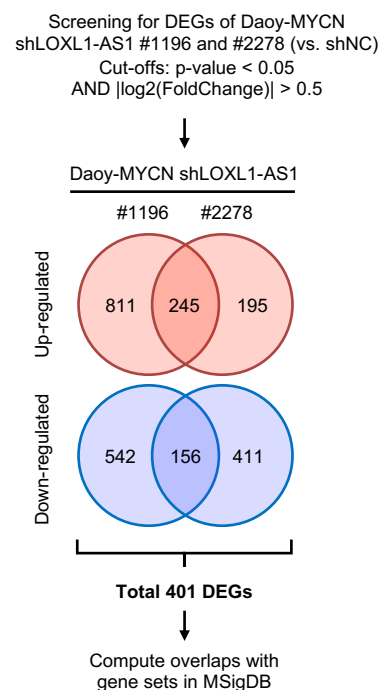
**A**



**B**

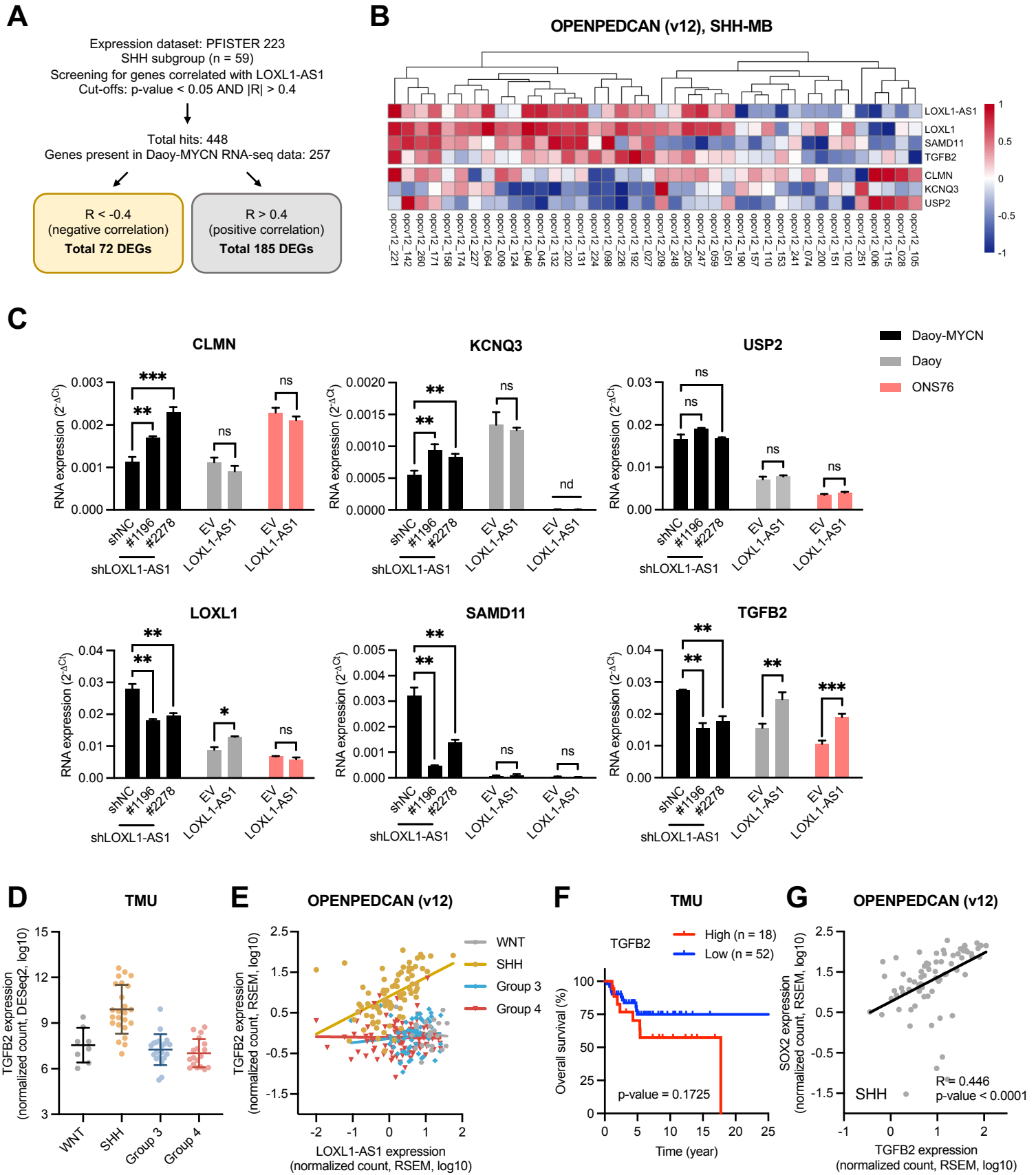


**C**



Supplementary Figure S4. (Legend next page)

**Supplementary Figure S4.** (A) Gene set enrichment analysis (GSEA) of Daoy-MYCN-shLOXL1-AS1 #1196 (left) and #2278 (right) in terms of normalized enrichment score (NES), p-value and gene ratio. Three gene set collections of KEGG, WikiPathways, and GO biological process are presented with top 10 up-regulated and top 10 down-regulated gene sets in each collection. Gene sets of interest are highlighted in red. (B) GSEA plots of Daoy-MYCN-shLOXL1-AS1 #2278 versus shNC samples in six gene sets of interest. (C) Schematic outline describing the workflow of selecting significant DEGs from two shLOXL1-AS1 knockdown samples. A total of 401 DEGs were input for computing overlaps with other gene sets using the Molecular Signature Database (MSigDB, [www.gsea-msigdb.org](http://www.gsea-msigdb.org)) in Figure 6C.



Supplementary Figure S5. (Legend next page)

**Supplementary Figure S5.** (A) Schematic outline describing the workflow of screening for genes correlated with *LOXLI-ASI* in a public dataset (Pfister-223, SHH-MB, n=59). (B) *LOXLI-ASI* expression of 6 candidate genes in OpenPedCan cohort (SHH-MB, n=40). (C) qRT-PCR validation of 6 candidate genes in SHH-MB cell lines. (D) *TGFB2* expression across four molecular subgroups in TMU cohort. (E) Correlation between *TGFB2* and *LOXLI-ASI* across four molecular subgroups in OpenPedCan cohort. (F) Survival analysis of *TGFB2* in TMU cohort. (G) Correlation between *TGFB2* and *SOX2* in SHH-MB in OpenPedCan cohort.