

Supplementary Table S1

Supplementary Table S1. The sequences of primers.

| Gene                  | Primer sequence (5'-3')  | Product size (bp) | Application |
|-----------------------|--|-------------------|-------------|
| mU6                   | F: TTTGGCGCCGGCTCGAGTGTACA   | 375               | Vector      |
|                       | R: AAACAAGGCTTTTCTCCAAGGG  |                   |             |
| <i>Tubb4b</i> -SgRNA1 | F: TGGAGAAAAGCCTTGTTTgCTACAACGAAG<br>CCACCGGTAGTTTTAGAGCTAGAAATAGC | 163               | Vector      |
| <i>Tubb4b</i> -SgRNA2 | F: TGGAGAAAAGCCTTGTTTgATGGGGTAGAG<br>GAGCCTTACGTTTTAGAGCTAGAAATAGC |                   | Vector      |
| <i>Tubb4b</i> -SgRNA3 | F: TGGAGAAAAGCCTTGTTTgGGGTAGAGGAG<br>CCTTACCGGGTTTTAGAGCTAGAAATAGC |                   | Vector      |
| SgRNA                 | R: CACCGGTTAGCGCTAGCTAATGCC  |                   | Vector      |
| <i>Cas9</i>           | F: GGTATCCACGGAGTCCCAGCAGCC  | 4176              | Genome      |
|                       | R: TTA CTTTTTCTTTTTTGCCTGGCCGG                                     |                   |             |
| <i>Tubb4b</i>         | F: GGGAGGTAATCAGCGACGAG  | 464               | Genome      |
|                       | R: CCGCAGTCAGTTGGACCTTC  |                   |             |
| <i>P21</i>            | F: CCAAGATAGCCGAGTTCA  | 115               | qRT-PCR     |
|                       | R: ACAACCAGGAAGACGACA  |                   |             |

Note: F means forward primer, and R means reverse primer. The gene sequence of *Gapdh*, *C/ebp α*, *C/ebp β*, *G-csf*, *Cdk 2*, *Cdk 4*, *Cyclin D1*, *Cyclin E* and *Skp2*, were the same as previously described (Feng, M.; Bai, Y.; Chen, Y.; Wang, K. Knockout of the Transducin-Like Enhancer of Split 6 Gene Affects the Proliferation and Cell Cycle Process of Mouse Spermatogonia. *Int. J. Mol. Sci.* **2020**, *21*, doi: 10.3390/ijms21165827.).

Supplementary Table S2

Supplementary Table S2. GO enrichment of DEGs in cell proliferation and cell cycle.

| Name  | ID         | enriched genes  |
|---|------------|---|
| negative regulation of cell population proliferation    | GO:0008285 | Cdkn1a, Ackr3, Ctsl, Esr1, Fas, Fosl1, Gdf11, Klf4, Lif, Meis1, Ppp1r15a, Nos3, Nppb, Etv4, Pmp22, Ptpm, Rbp4, Rps6ka2, Ccl2, Sfrp4, Slc16a2, Slfn2, Snai2, Sox11, Tgfbr3, Tgif1, Tnfaip3, Tnfrsf9, Vdr, Ceacam1, Ndr2, Nupr1, Slurp1, Cd274, Trp53inp1, Trim35, Dhcr24, Il33, Atf5, Cth, Trib1, Kctd11, Fbxo2, Sulfl, Chd5 |
| negative regulation of cell cycle G2/M phase transition | GO:1902750 | Ccng1, Cdkn1a, Fhl1, Hmga2, Ier3, Orc1, Cdc6, Atf5, Clspn, Brsk1  |
| positive regulation of cell death                       | GO:0010942 | Atf3, Bnip3, Cdkn1a, Clu, Gadd45a, Ddit3, Fas, Fos, Fosl1, Fyn, Hmga2, Hp, Mmp3, COX2, Ppp1r15a, Nqo1, Mycn, Nos2, Nos3, Pik3cd, Rps6ka2, Ccl2, Sfrp4, Plscr1, Unc13b, Unc5c, Vdr, Ceacam1, Irf5, Htra1, Nupr1, Cd274, Trp53inp1, Trim35, Ecscr, Fam162a, Akap12, Acox2, Bmf, Zc3h12a, Nfkbid                               |