# *Supplementary Material*

**Supplementary Figures and Tables**

**Table S1.** Antibodies used in the study

| Antigen | Source | Company | Application | Dilution |
|---|---|---|---|---|
| BMI1 | Rabbit | Proteintech | WB; IF; ChIP | 1:1000; 1:200; 10μg/ChIP |
| PLZF | Goat | R&D | IF | 1:1000 |
| Tubulin | Mouse | Beyotime | WB | 1:10000 |
| ub-Histone H2A(K119) | Rabbit | Cell Signal | ChIP | 10μg/ChIP |
| PH3 | Rabbit | Cell Signal | IF | 1:1000 |
| β-catenin | Mouse | BD | WB;IF | 1:1000;1:500 |
| LIN28 | Rabbit | Abcam | IF | 1:500 |
| Stra8 | Rabbit | Millipore | IF | 1:500 |
| EZH2 | Rabbit | Cell Signal | ChIP | 5μg/ChIP |
| H3K4me3 | Rabbit | Millipore | ChIP | 5μg/ChIP |
| c-KIT | Goat | R&D | IF | 1:500 |
| H3K27me3 | Rabbit | Millipore | ChIP | 5μg/ChIP |

**Table S2: The list of primer sequences for gene expression analysis.**

| Gene | Forward (5'->3') | Reverse (5'->3') | Product length (bp) |
|---|---|---|---|
| Axin2 | GGTCCTGGCAACTCAGTAACA | CTCATGTGAGCCTCCTCTCTTTT | 144 |
| Dlx5 | CCGTCTCAGGAATCGCCAAC | CTCCGCCACTTCTTTCTCTGG | 285 |
| Esrrb | TTGCATGGGGCTCTGTG | GGTTCAGCAGCATGGTTGG | 300 |
| Fgfr3 | GCGACAGGTGTCCTTGGAA | CTTGGCAGTACGGTCCTTGT | 244 |
| Fzd10 | TGTGACAACCCAGGCAAGTT | CGATGGGTCGATGAGGAAGG | 189 |
| Hesx1 | TTTCACCCAGAACCAGGTCG | TTGCTCGGCGATTTTGGAAC | 143 |
| Id3 | CATCTCCCGATCCAGACAGC | GTTCAGTCCTTCTCTCGGGC | 266 |
| Inhba | GGTGCTGCTCAAGTGCCAATA | CTGGCAGCAAAAGTCGTGTG | 208 |
| Isl1 | TCTGCAAATGGCAGCCGAA | GTCCTTGCACCGCTTGTTTT | 244 |
| Meis1 | ACTGCTGTCTTGGTGGAACC | TAGGTCGTCGTACCTTTGCG | 266 |
| Nodal | AGGGACCCTGGTGTTGTTTG | CCGGTCACGTCCACATCTTG | 251 |
| Otx1 | GGGAGGGCAGTAGAAAGGTG | GCCTGGCCTGCCCTC | 162 |
| Pax6 | CACCAGACTCACCTGACACC | TCACTCCGCTGTGACTGTTC | 193 |
| Smad9 | CACCTCTCGGACAGAGTAAGC | GCAAGTACCTGTCCACGGAT | 141 |
| Sox2 | TTTGTCCGAGACCGAGAAGC | ATCATGCTGTAGCTGCCGTT | 279 |
| Wnt3 | TGTTCCAACTATTGGGGGCG | GGCCAGGGACCACCAAAT | 163 |
| Wnt8b | TGACCGGTCCAAAGGCTTAC | CCCAGTTGTCCATTTCGGGA | 280 |
| Wnt10b | GAGTTAGGTCGAGCAGAGCC | GTCAGTCAGCGCCTCCAG | 286 |
| Wnt16 | GCAGGCAACATGACCGAATG | CATTAACTTGGCGACAGCCTGC | 231 |
| Zic3 | CGCGCTCTTGAGTAGAGGAG | CCGGAGCTGCAACTTTCCTA | 248 |
| Neurog1 | GCTGCCCTCGGTCTATTTCT | GCCGGCTCAAACCGAATTTT | 261 |
| 18s rRNA | AAACGGCTACCACATCCAAG | CCTCCAATGGATCCTCGTTA | 155 |

**Table S3: The list of primer sequences for ChIP-qPCR.**

| Gene | Forward (5'->3') | Reverse (5'->3') | Product length (bp) |
|---|---|---|---|
| Axin2 | CAGCCAAGCTCTCCGGTTTA | GGAACCTGGGTCTTCATGGG | 115 |
| Dlx5 | AGCCCTAGTGGTGTTTGCGT | GCGCTTCGCTGGCTAATCC | 99 |
| Esrrb | CTCCTACAGACTCCTCCCCC | CAAGTACTGTGGGAGCTGGG | 116 |
| Fgfr3 | TGCCCATACATGACACGTCC | CTTCCAACTCCACAGAGCCC | 78 |
| Fzd10 | CTTCCTCATCGACCCATCGC | ATAGCCCACCGAATAAACGCA | 88 |
| Hesx1 | GGAAGCAGACTCCGCTAGG | AGACAAGAGAGAGAACAGAGTTGC | 70 |
| Id3 | TGCTGCCTGTCGGAACG | GGCTAAGAGGCTCCTCGGT | 76 |
| Inhba | GGATGCACTTTGGATATTCAGGAC | CCAGTCGATATTCACCTGTGCT | 80 |
| Isl1 | TCCGGGTTTGTCCCCTAAC | CTTCACTGACTCCCGTGCT | 116 |
| Meis1 | CGTGCGAATTTGTGGAGTGT | ACAGGTCGCGTCTTACACAAT | 121 |
| Nodal | CCCTCTGGCGTACATGTTGA | AGGCTTGCAGCCTACCTTG | 92 |
| Otx1 | GCTCCTTAGGGCAAGCAAGA | AGGAGAAAGTTCTGCACCCG | 151 |
| Pax6 | AGGAAAGTTAGCGCCTGCC | GGTTCTACGCGAGGACCTG | 80 |
| Smad9 | TACCATTGGGTGCCAGCG | AGAAGTGCAGCGATTAGCCAC | 130 |
| Sox2 | TAAGTACACGCTTCCCGGAG | ATCATGCTGTAGCTGCCGTT | 153 |
| Wnt3 | TCCCGGAGAAAAGCGAGGT | CCGTCTTCCAAAAGCAACGAA | 123 |
| Wnt8b | TCGTGAATGAGCCTAGCGAT | AATCGGGAATGTTGCAACTCG | 104 |
| Wnt10b | AGTGGGGCTAATCGTGATCC | GACTTGACCAGGAGAACGCAG | 93 |
| Wnt16 | GCGCGTTCCTTCGATAGAGT | CACCTCGCACCAAATGTTCC | 83 |
| Zic3 | ACCGCAGGGTTCAGGTTATG | GCGCGTGGAGTTGAAAGC | 121 |
| Neurog1 | CTCCAAACCTCCTGTCCGTC | GCTGGAGCAGTCGAGATCAG | 90 |
| Gapdh | AACCCAAACTAACAGTTGTCCCAA | ACTCCTTGGAGGCCATGTAGG | 102 |

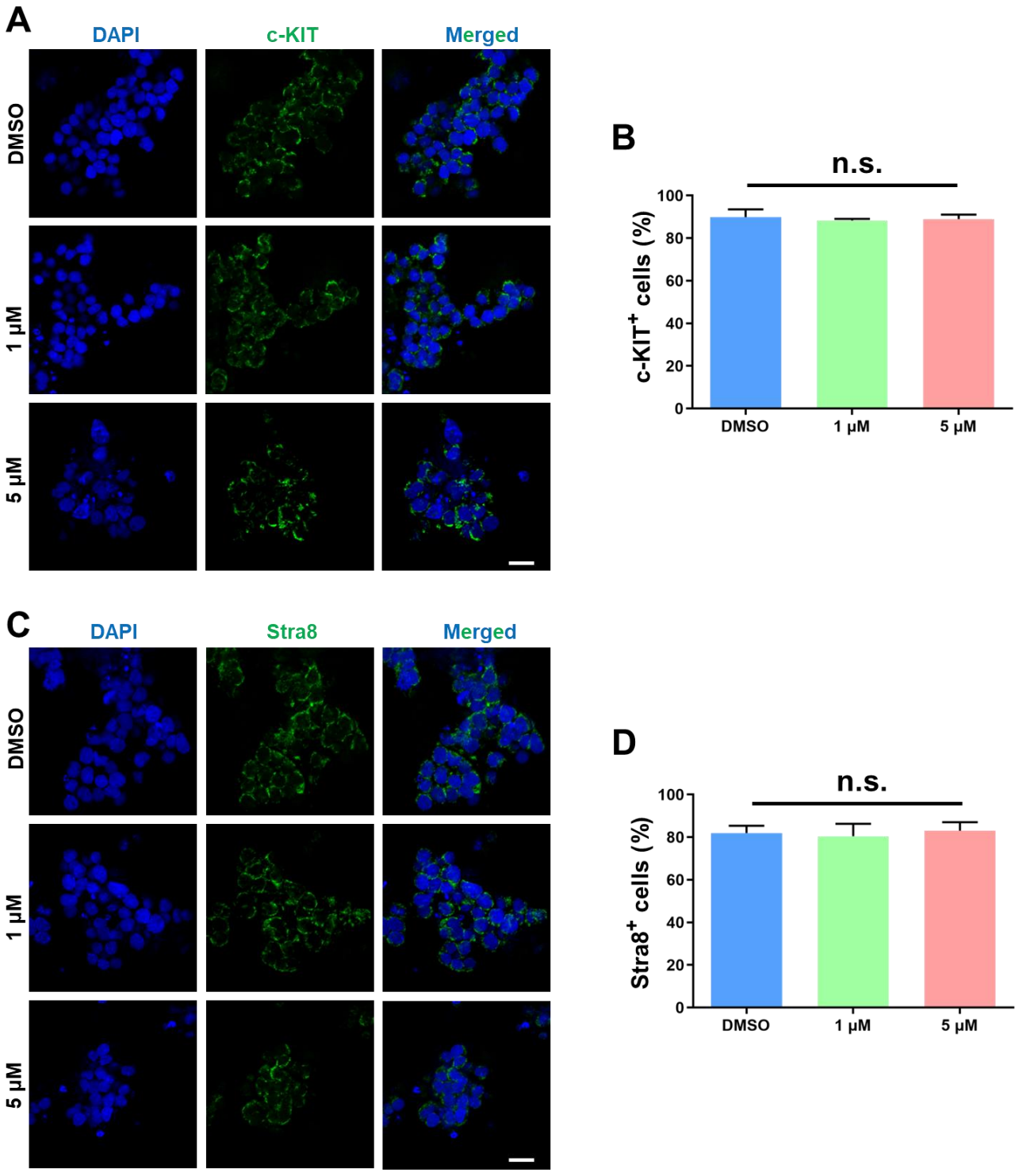


**Figure S1. PTC-209 has no effect on SSC differentiation upon retinoic acid treatment. (A)** Immunostaining for c-KIT in SSCs treated with DMSO, 1 μM, or 5 μM PTC-209 for 48 h. **(B)** Quantification of (A). **(C)** Immunostaining for Stra8 in SSCs treated with DMSO, 1 μM, or 5 μM

PTC-209 for 48 h. **(D)** Quantification of (C). For (A) to (D), SSCs were treated with 1 μM retinoic acid for 48h.Data represent mean ± SD, n = 3 independently differentiated groups. Statistical analysis was performed using One-way ANOVA with Dunnett post hoc test. not significant (n.s.), $P > 0.05$. Scale bar, 20 μm.

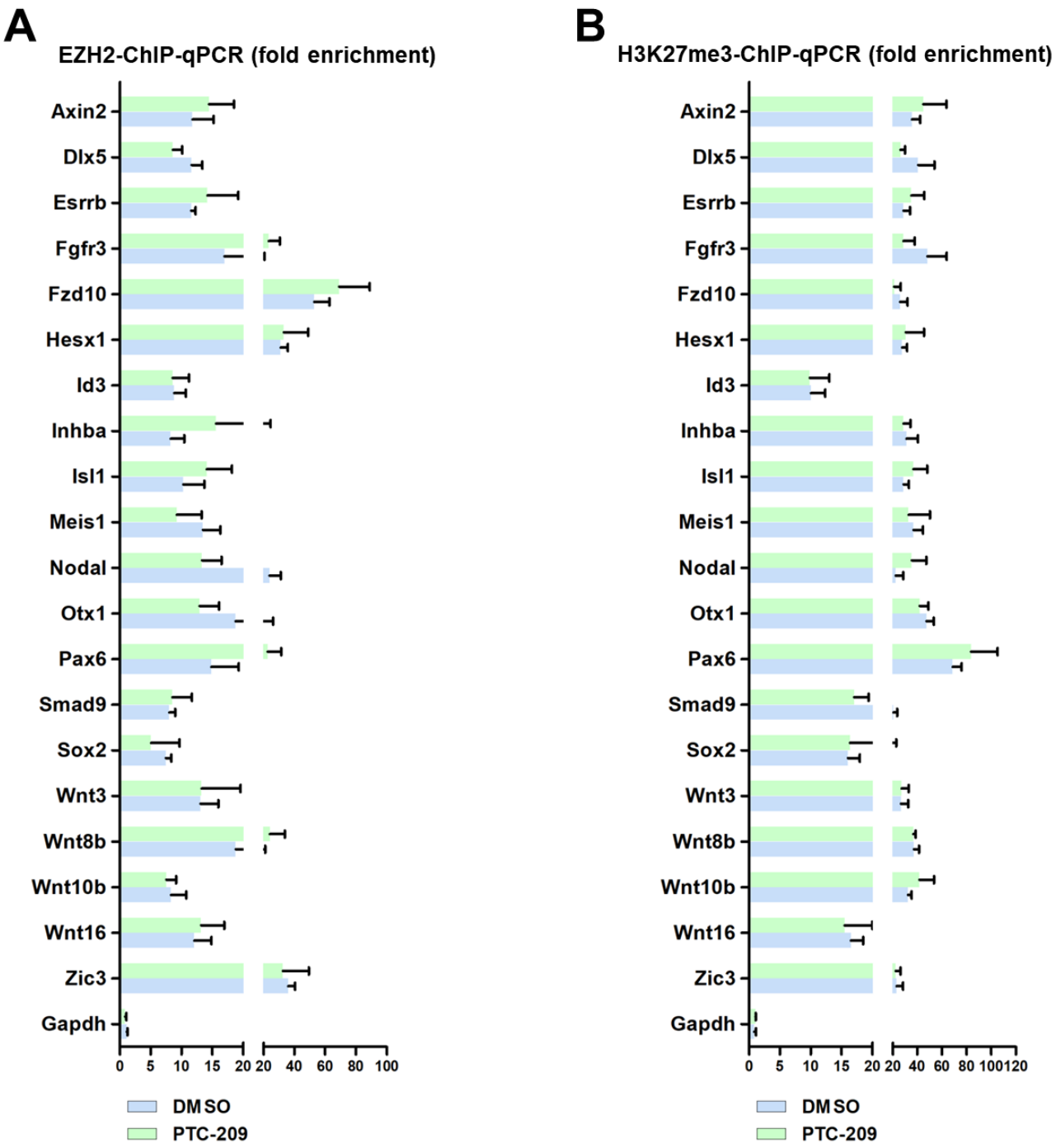


**Figure S2. Distribution of EZH2 and H3K27me3 at the BMI1 targets. (A-B)** ChIP-qPCR of BMI1 target genes in SSCs treated with DMSO or 5 μM PTC-209 for 48 h using anti-EZH2 (A) and anti-H3K27me3 (B) antibodies. Data represent mean ± SD, n = 3 independently differentiated groups. Statistical analysis was performed using Student's two tailed t test. For all tests, $P > 0.05$.

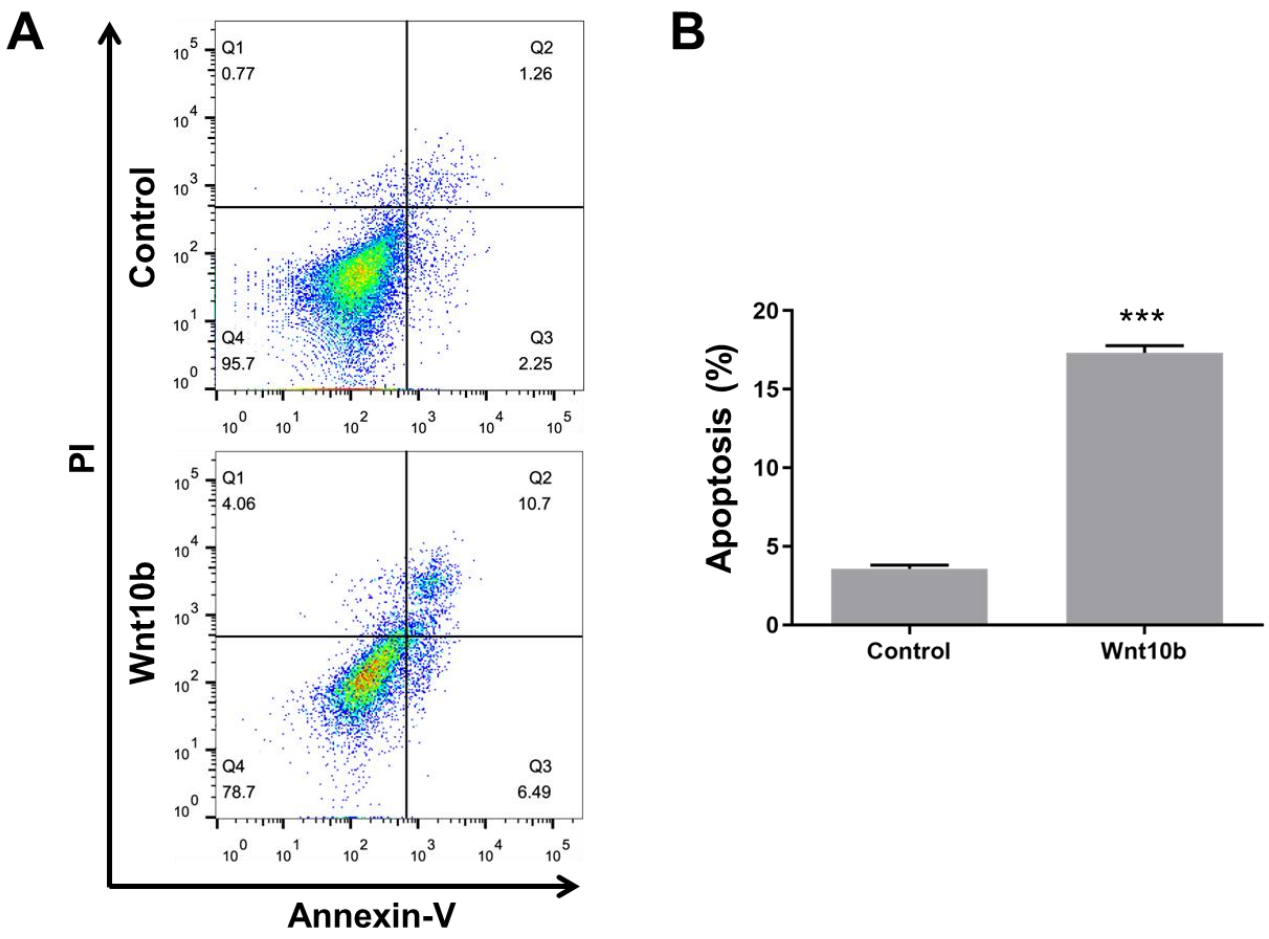


**Figure S3. The effect of Wnt10b on SSC apoptosis. (A)** Flow cytometry-based Annexin V-FITC/PI staining of SSCs treated with PBS (control) or 2000 ng/mL Wnt10b for 48 h. **(B)** Quantification of Q2 + Q3 in (A). Data represent mean ± SD, n = 3 independently differentiated groups. Statistical analysis was performed using Student's two tailed t test. ***$P < 0.001$.

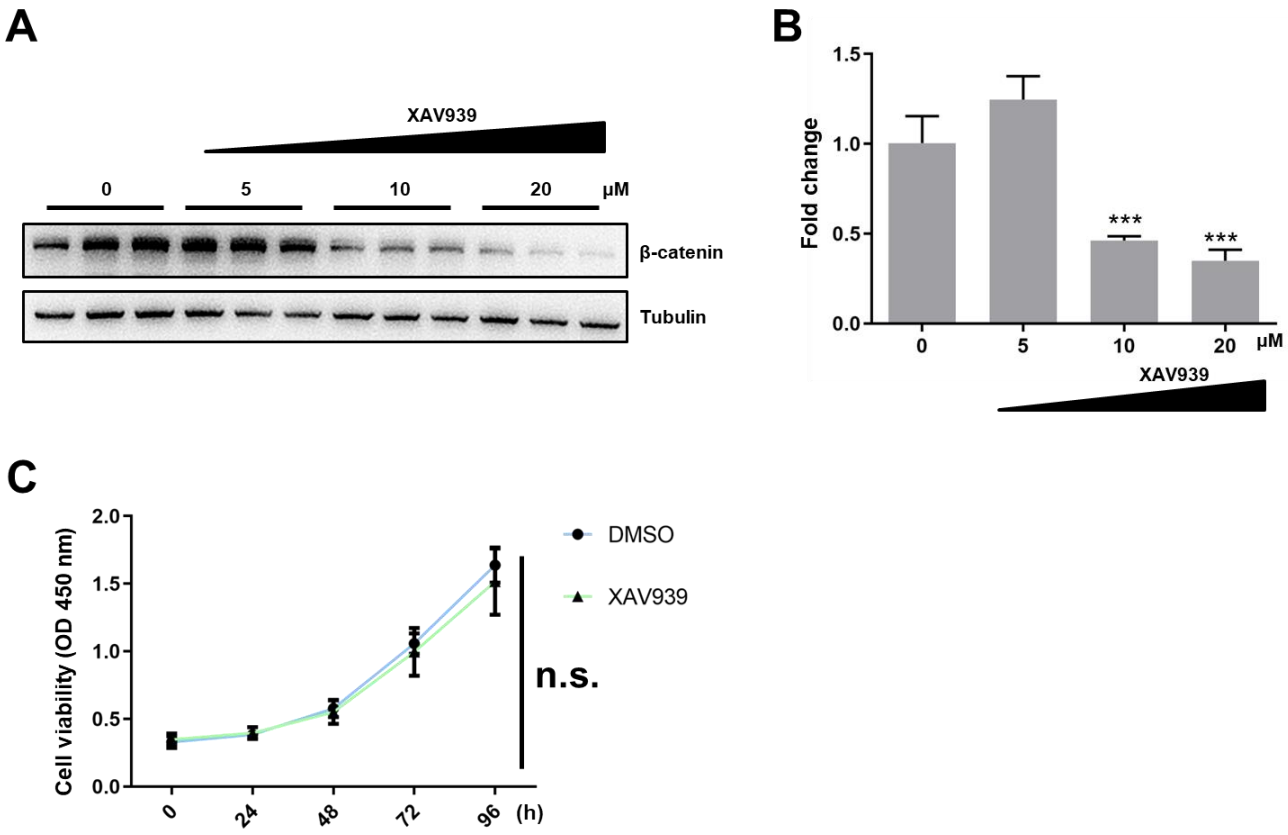


**Figure S4. XAV939 treatment alone has no effect on SSC viability. (A)** SSCs were cultured in feeder-free conditions with the indicated doses of XAV939 for 48 h. Cells were subsequently harvested and subjected for western blotting analysis. **(B)** Quantification of (A). Data are presented as mean ± SD, n = 3 independently differentiated groups. Statistical analysis was performed using One-way ANOVA with Dunnett post hoc test. **(C)** CCK-8 assay for SSCs treated with DMSO or 20 μM XAV939 under feeder-free conditions. The time point for XAV939 treatment was identified as 0 h. Data represent mean ± SD, n = 6 independently differentiated groups. Statistical analysis was performed using Student's two tailed t test. ***$P < 0.001$. not significant (n.s.), $P > 0.05$.

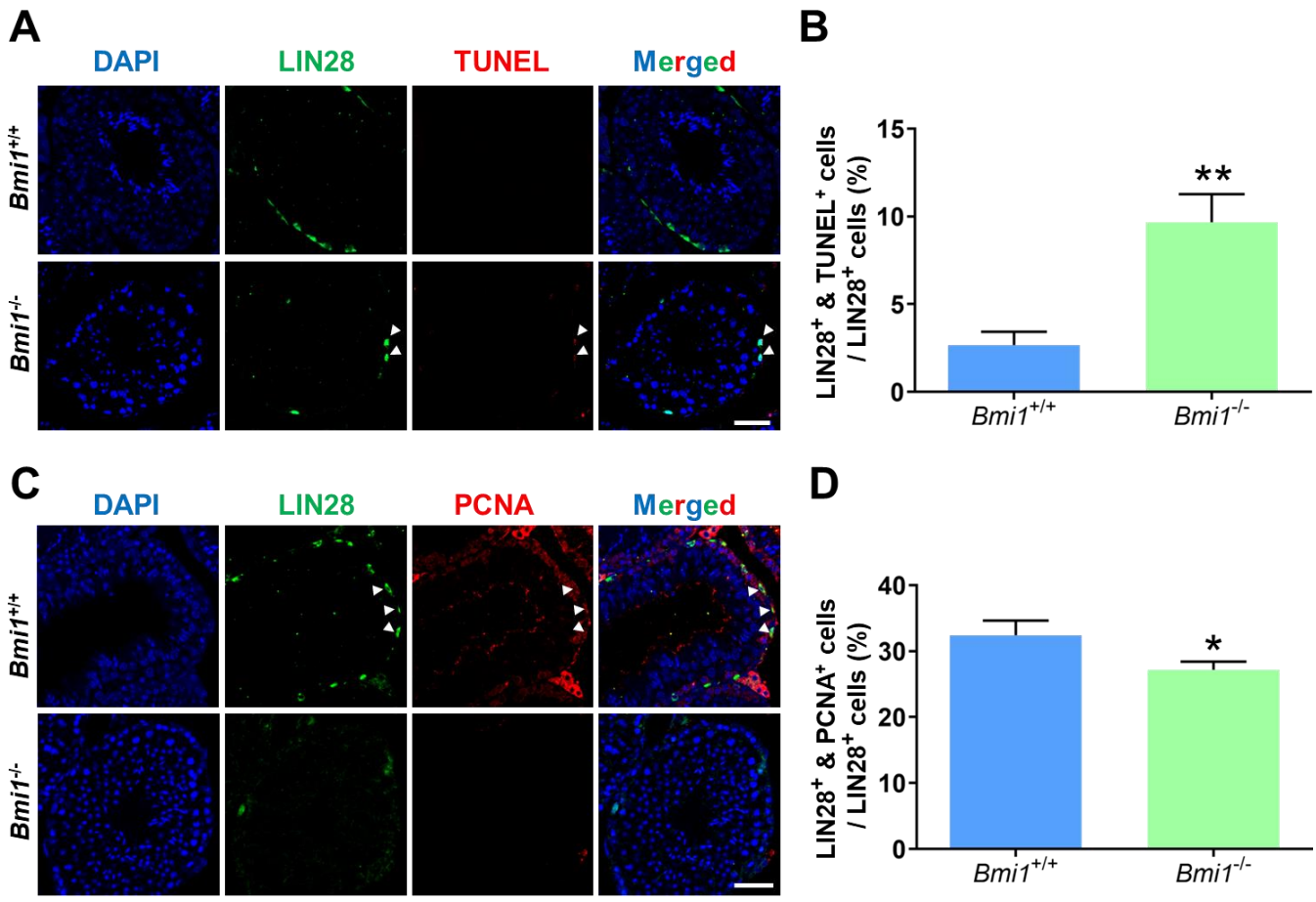


**Figure S5. Effects of *Bmi1* knock-out on cell proliferation and apoptosis of SSCs in murine testes. (A)** Co-immunostaining for LIN28 and TUNEL in *Bmi1*$^{+/+}$ and *Bmi1*$^{-/-}$ murine testes. **(B)** Quantification of (A). Apoptotic SSCs were indicated with arrowheads. **(C)** Co-immunostaining for LIN28 and PCNA, a marker of proliferative cells, in *Bmi1*$^{+/+}$ and *Bmi1*$^{-/-}$ murine testes. **(D)** Quantification of (C). Proliferative SSCs were indicated with arrowheads. For (A) to (D), data represent mean ± SD, n = 3 independently differentiated groups. Statistical analysis was performed using Student's two tailed t test. *$P < 0.05$; **$P < 0.01$. Scale bar, 50 μm.