|  |
| --- |
| Antibodies |
| Name | Supplier &Catalog No. | note |
| Anti-Smoothened antibody | Abcam, Cat. No. ab72130 |  |
| Anti-Gli1 antibody | Affinity Biosciences, Cat. No.DF7523 |  |
| Anti-Gli2 antibody | Affinity Biosciences, Cat. No. DF7541 |  |
| Anti-Hcn4 antibody | Millipore, Cat. No. AB5808 |  |
| Anti-Tbx5 antibody | Sigma, Cat. No. HPA008786 |  |
| Anti-Cdk6 antibody | Abclonal, Cat. No. A0705 |  |
| Anti-phospho Smad1/5/8 | Millipore, Cat. No. AB3848-I |  |
| Phospho-histone H3(Ser10) | Cell Signaling Technology, Cat. No.9701s |  |
| Chemicals, Recombinant Proteins and Reagents |
| BMP2 recombinant human protein | ThermoFisher, Cat. No. phc7145 |  |
| Rat tail collagen I | ThermoFisher, Cat. No.1048301 |  |
| Blocking reagent | Sigma, Cat. No. 11096176001 |  |
| BM purple AP substrate | Sigma, Cat. No. 11442074001 |  |
| Anti-digoxigenin-AP, Fab fragments from sheep | Sigma, Cat. No. 11093274910 |  |
| NTP Dig labelling mix | Sigma, Cat. No. 11277073910 |  |
| RevertAid Firs-Strand cDNA Synthesis Kit | ThermoFisher, Cat. No. K1622 |  |
| SYBR Green Powerup Master Mix | ThermoFisher, Cat. No. A25776 |  |
| Lipofectamine 3000 | ThermoFisher, Cat. No. L3000001 |  |
| TriPure | Roche, Cat. No. 11667165001 |  |
| EdU cell proliferation kit | RiboBio Co., Ltd., Cat.No.C10321 |  |
| p*CEFL3xHAmGli2* | Addgene, Cat. No. 37671 |  |
| *pTbx5-hrGFPII* | Homemade |  |
| *pcDNA3.1-Gli1* | YouBio |  |
| DNA Oligos (5’ to 3’) for RNA Probes |
| TATAGCAGCTGTACCGTCACCAC | CTGTCTCTGCTCTCCCTCCATTC | *Tbx5* |
| GGAACCACCTGCTGCACATAAG | TTCTAAGGTGTTGGCTGACAGGA | *Wnt2* |
| GCAGGAGAGGGTTAAGTCAGCA | AGATTCCCCACCATCAGCAACA | *Hcn4* |
| TATGAATTCGACCCAGCCAAAGACCCTCGG | TATGGATCCCAGGCTCCCGGTCCTAGTG | *Nkx2.5* |
| TATAAGCTTGTAGCCTGCAGCCGACAGCTC | TATGGATCCTCTCCGGACTAGCAGCCACCA | *Isl1* |
| TATGAATTCAAGCGGATAGAAGGCGGGAGC | TATGGATCCAGAGTGGGCTGTGGGTCACCT | *Myl7* |
| ATGCAGGAGAACCATCCGAAGT | GTTGTTGTTGCATGGGGACGAT | *Meis1* |
| AATCAACGGCAGGGAAGACAGA | AAAGTCTGGTGATCCTGGGGTG | *Arid3b* |
| CCTCTCAGTTGCCTTCTGTGGA | TACTGTTAGGTCGGTCGAAGCG | *Egln1* |
| GACGGACTGCGGTCTCCTAAAG | CATCTCTGGAAGTTCCTCCACGG | *Bmp2* |
| GCCGGAGACCTAGATGTCATTG | TTTGCAGGCCAGTTTGATCCCA | *Twist1* |
| DNA Oligos (5’ to 3’) for qPCR, \*: from PrimerBank, \*\*: self-designed |
| AGATCCGGTTATCCCTGGGAC | CAGCAGCCCCCACTAACTG | *Tbx3\** |
| CTTACGGCGTTCGTCTCCAAG | CTCTCTAGCACCTCCCGGTA | *Shox2\** |
| GTACCTGGCTTGGCACGAC | GCATTGCTGGAAACATGCG | *Tbx18\** |
| CTTCACCCAGCAGGGCATGG | CTAGGAAACATTCTCCTCCCTGCC | *Tbx5\*\** |
| GCAGGAGAGGGTTAAGTCAGCA | AGATTCCCCACCATCAGCAACA | *Hcn4\*\** |
| GCTCCAGGTCCCTCGGACAGA | GACCTGGGGAAGCAGCAACACT | *Bmp2\*\** |
| CAGAAGACTGTAACACAGGGGAAGAAG | CTTCCTTCTCTGCCCCTTCGAC | *Mybpc3\*\** |
| ACGGCGACCATCCACAAAG | AAAGTCTGTTGCCAAATCCTTCT | *RyR2\*\** |
| GGCTGTATTCCCCTCCATCG | CCAGTTGGTAACAATGCCATGT | *β-actin* |

**Table S1.** Materials and chemicals

**Table S2.** Survival rate of *Smo* mKO mutants

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| Embryonic stages | E7.5-8.5 | E9.0 | E9.5 | E10.5 | E11.5 | E14-15 |
| *Smo* mKO | 147 | 51 | 378 | 32 | 1 | 1 |
| Total | 551 | 190 | 1445 | 171 | 33 | 81 |
| *Smo* mKO/total (%) | 26.6 | 26.8 | 26.1 | 18.7 | 3.0 | 1.2 |



**Figure S1**Expression of Hh pathway components in normal developing embryos. **(A)** Expression of Gli1 in the mesoderm at E7.25. **(B** and **C)** Expression of Gli2 in the mesoderm at E7.25. (**D**-**D’’**) A very low level of Smo expression was detected in the primitive heart tube at ~E8.5. Scale bars: 100 µm.

**Figure S2.** Gross appearance of the *Smo* mKO mutant embryo. *Smo* mKO mutant embryo displayed normal turning at E8.75. The dashed circles indicate the heart. Scale bar: 200 µm.



**Figure S3.** Impaired outflow tract and right and left ventricles of *Smo* mKO mutants. (**A**-**C1**) A smaller outflow tract and reduced right and left ventricles were detected in the *Smo* mKO mutants at ~E8.75. (**D**-**F1**) A smaller outflow tract and reduced right and left ventricles were observed in the *Smo* mKO mutants at ~E9.0. Blue dashed lines: outflow tract; red dashed lines: right ventricle; black dashed lines: left ventricle. Scale bars: 200 µm.

**Figure S4.** Expression of *Nkx2.5* mRNA in the developing heart at E9.5. Whole-mount *in situ* hybridization indicated that the expression of *Nkx2.5* mRNA in the mutant heart returned to a normal level by E9.5. Scale bar: 200 µm.

**Figure S5.** Expression of MF20 in the developing heart tube. Immunostaining of serial sections indicated that the expression of MF20 in the mutant heart was identical to that in the control hearts at the E8.25-8.5 stages. Scale bar: 100 µm.

**Figure S6.** Altered expression of genes related to cardiac development in the atrium/IFT of the *Smo* mKO mutants at E9.5. **(A** and **B)** Reduced expression of *Meis1* mRNA in the inflow tract of the mutant (arrows). **(C** and **D)** Reduced expression of *Myl7* mRNA in the atrium/IFT of the mutant (arrows: IFT; arrowheads: atrium). **(E** and **F)** Reduced expression of *Arid3b* mRNA in the IFT of the mutant. Scale bars: 200 µm.



**Figure S7.** Proliferation ofcardiac progenitor cells in the developing heart. **(A)** Phospho-histone H3 immunostaining indicated that the proliferation of cardiac progenitor cells exhibited a decreasing trend in the *Smo* mKO mutants at E8.25. **(B)** EdU incorporation showed that the proliferation of cardiomyocytes was decreased in the mutants at E9.5.