

1 **LncRNA Snhg1-driven self-reinforcing regulatory network promotes cardiomyocyte**  
2 **regeneration and repair after myocardial infarction**

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20  
21 **Running title:** LncRNA Snhg1 promotes cardiac regeneration

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## Detailed Methods

### 3 **Human tissue samples**

4 Human left ventricular tissue samples were obtained as described previously.<sup>1</sup> Briefly,  
5 adult myocardial tissue samples were obtained by endomyocardial biopsy from patients  
6 suffering from myocardial deposition disease based on arrhythmia and echocardiographic  
7 changes. The pathological findings showed no evidence of myocardial disease or  
8 functional abnormalities. Embryonic human myocardial samples were obtained after  
9 elective termination of pregnancy for nonmedical reasons. The present study conformed to  
10 the principles of the Declaration of Helsinki. The study protocol was approved by the  
11 Nanfang Hospital ethics committee, and written informed consent was obtained from all  
12 subjects.

13

### 14 **Human iPSC-CM cultures**

15 CMs derived from iPSCs were purchased from Cellapy (Beijing, China) and were cultured  
16 according to the manufacturer's instructions. The cells were allowed to adhere for 48 h  
17 before maintenance medium exchange and fresh medium was replaced every other day and  
18 then for transduction experiments.

19

### 20 **Isolation and culture of neonatal FBs**

21 FBs were isolated from mice as previously described.<sup>2</sup> Excised hearts were rinsed in cold  
22 Hank's balanced salt solution (HBSS), minced, and digested with type II collagenase and

1 pancreatin at 37 °C for 15 min. The first digestion was discarded. A second digestion was  
2 performed, and the collagenase medium containing FBs was collected, centrifuged, and  
3 resuspended in DMEM with 10% FBS, 100 U/mL penicillin, and 100 g/L streptomycin.  
4 Digestion was repeated 5-6 times until the digestion fluid became clear. Cells were plated  
5 in 60-mm dishes and allowed to attach for 60 min, and the media was then changed to  
6 remove CMs and endothelial cells. Isolated FBs were washed twice with PBS and cultured  
7 for transduction experiments.<sup>1</sup>

8

### 9 **Myocardial Cas9 knockin transgenic mouse model**

10 The Cre-dependent Cas9 knockin mouse model was obtained from Shanghai Model  
11 organisms Center, Inc. This model was generated by homologous recombination in JM8A3  
12 embryonic stem (ES) cells and implanted in C57BL/6J blastocysts as standard procedures.  
13 Briefly, the targeting vector was designed to contain a ubiquitously expressed CAG  
14 promoter, a loxP-flanked PGK-Neo-polyA sequence followed by a Cas9 protein inserted  
15 into intron 1 of the Rosa26 locus. The construct was linearized and electroporated into  
16 JM8A3 embryonic stem cells. Targeted single-ES cell colonies were screened by PCR with  
17 primers amplifying both recombinant arms. PCR products were sequenced to further  
18 validate correct insertion. Correctly targeted colonies were injected into blastocysts to  
19 obtain the chimeric mice. The resulting high-percentage chimeric male was crossed to  
20 female C57BL/6J mice to obtain the heterozygous of Cre-dependent Cas9 mice (Rosa26-  
21 LSL-Cas9-tdTomato/+). Rosa26-LSL-Cas9-tdTomato/+ mice intercross to obtain the  
22 homozygous of Rosa26- LSL-Cas9-tdTomato mice. The homozygous Rosa26-LSL-Cas9-

1 tdTomato mice were crossed with  $\alpha$ -MHC-Cre transgenic mice, generating myocardial  
2 Rosa26-Cas9-tdTomato mice and used in later experiments.

3

#### 4 **EdU administration *in vitro* and *in vivo***

5 In the *in vitro* experiment, for the 1-day-old and 7-day-old CMs, after transfection for 24  
6 h, the culture medium was replaced by fresh medium for 24 h. That is, 48 h after the  
7 cultured cells were seeded, 10  $\mu$ mol/L EdU was added for 24 h. Cells were fixed at 72 h  
8 after seeding and processed for immunofluorescence. Adult CMs were isolated from adult  
9 mice that were transduced AAV9-mediated Snhg1 or NC. EdU was administered  
10 intraperitoneally (50 mg/kg) at 12, 13 days after transduction. CMs were isolated and  
11 processed for immunofluorescence 14 days after transduction.

12 For the *in vivo* experiment, the P1 mice received EdU (50 mg/kg) by intraperitoneal daily  
13 injections at P5 and P6. The hearts of the injected mice were collected 7 days after  
14 transduction. In P7 and adult mice, EdU was administered intraperitoneally (50 mg/kg) at  
15 12, 13 days after transduction, and the hearts were collected 14 days after transduction. In  
16 adult mice, EdU was administered intraperitoneally (50 mg/kg) at 12, 13 days after  
17 transduction, and the hearts were collected 14 days after transduction.

18

#### 19 **Immunofluorescence analysis**

20 For *in vitro* cultured CMs, the culture medium was washed with PBS. The cells were fixed  
21 with 4% paraformaldehyde (Leagene), permeabilized with 0.2% Triton X-100 PBS, and  
22 blocked with PBS containing 1% BSA. The cells or slides were incubated with primary

1 antibodies, including cardiac troponin T (Abcam, ab33589), PCMI (Abcam, ab72443),  
2 vimentin (Abcam, ab8978 ), Ki67(Abcam, ab15580), pH3 (Abcam, ab170904 ), Aurora B  
3 (Abcam, ab2254 ), N-cadherin (Abcam, ab18203) and Anillin (Santa Cruz Biotechnology,  
4 sc-271814) for 2 h at room temperature, followed by incubation with goat anti-mouse  
5 IgG/Alexa Fluor 488 or goat anti-rabbit IgG/Alexa Fluor 555 secondary antibodies  
6 (Biosynthesis, bs-0296GA488, bs-0295G-AF555) for 1 h at room temperature. The cells  
7 or slides were washed and incubated with DAPI (BioWorld, St. Louis Park, MN, USA).  
8 CM borders were defined by staining of tissue with WGA conjugated to Alexa Fluor 555  
9 (Invitrogen) in PBS. Image acquisition was performed with an LSM 880 confocal  
10 microscope (Zeiss, Oberkochen, Germany). The Click-iT® EdU Imaging Kits (Life  
11 Technologies, USA) to detect EdU incorporation were used according to the  
12 manufacturer's instructions. Finally, cells were stained with DAPI.<sup>3-5</sup>  
13 For the *in vivo* experiments, formalin-fixed tissue slides were deparaffinized, and antigen  
14 retrieval was performed by microwaving the slides in citrate buffer (0.1 mol/L, pH 6.0) for  
15 14 min. When indicated, the cells or slides immunostained with EdU were further  
16 processed using a Click-iT EdU Alexa Fluor 555 Imaging Kit (Invitrogen) to reveal EdU  
17 incorporation according to the manufacturer's instructions. Slides were processed for  
18 immunofluorescence as described above for the cultured cells.

### 19 **Cardiomyocytes binucleation assay**

20 Isolated P7 CMs transduced Adv-Snhg1 were cultured with the medium containing 20  
21  $\mu\text{mol/L}$  EdU for 24 h. CMs were washed twice to remove the EdU and cultured for another  
22 24 h in neonatal CM medium with 10% FBS. CMs were harvested at 24 h and 48 h

1 respectively. the harvested CMs were marked with N-cadherin (Abcam, ab18203) ,  
2 cardiac troponin T (Abcam, ab33589) and The Click-iT® EdU Imaging Kits (Life  
3 Technologies, USA), and then detected the number of EdU-positive binuclear CMs.  
4 Binucleation index were calculated from EdU-positive binuclear CMs in 24 h and 48 h.

5

6 **Cardiomyocytes, fibroblasts and endothelial cells co-culture**

7 Cell co-culture was performed using a double-chamber co-culture system culture in which  
8 adenovirus infected fibroblasts or endothelial cells were cultured in the upper chamber, and  
9 CMs were cultured in the lower chamber. To assaying proliferation of the lower chamber  
10 CMs, the same amount of CMs were plated in the lower chamber with a 0.4 µm pore size  
11 membrane (12-well insert, BD Biosciences) separating the upper and lower chamber cells.  
12 After 24 h co-culture, CMs were taken out to assay by Immunofluorescence.

13

14 ***In situ* hybridization (ISH)**

15 ISH was performed utilizing the Panomics QuantiGene ViewRNA ISH tissue assay  
16 (Affymetrix, Santa Clara, CA, USA) as previously described.<sup>1</sup> Mouse hearts were fixed in  
17 10% formaldehyde and embedded in paraffin. Five-micron sections were cut,  
18 deparaffinized, boiled in pretreatment solution, and digested with proteinase K. Heart  
19 sections were hybridized for 3 h at 37 °C with a custom designed probe against *Snhg1*  
20 (Affymetrix). Bound probes were amplified according to the protocol from Panomics using  
21 PreAmp and Amp molecules. Working label probe oligonucleotides conjugated to 6-AP  
22 were added. AP-Enhancer solution was added to each tissue section after washing.

1 Working label probe oligonucleotides conjugated to 1-AP were added. Slides were  
2 counterstained with hematoxylin. Images were acquired with a Nikon Eclipse TE2000-S  
3 microscope (Nikon, Tokyo, Japan).

4

#### 5 **RNA Fluorescent in Situ Hybridization (RNA-FISH)**

6 Isolated CMs grown on coverslips were fixed in 4% paraformaldehyde, and frozen sections  
7 (5  $\mu$ m) of hearts were fixed with 95% ethanol. Then, the cells or the tissue slides were  
8 washed 3 times with PBS. The samples were permeabilized in 0.2% Triton X-100 PBS and  
9 washed with PBS for 3 times. Then the samples were refixed with 4% paraformaldehyde  
10 for 10 min and dehydrated through sequential 5-minute incubations in ethanol (70%, 80%,  
11 95%, 100%). The samples were incubated with prehybridization solution for 30 min and  
12 hybridized with hybridization solution and a labeled Snhg1 probe overnight at 42 °C. The  
13 sequences of Snhg1 probe were: 5'-  
14 AAAACGTGTTATTTGTA AAAATTGAACAGGCCTGGCTCCAAAGTGTA AAA-3'.

15 Next, the samples were washed with 50% formamide/2 $\times$ SSC, 0.1% NP40/1 $\times$ SSC,  
16 0.5 $\times$ SSC and 0.2 $\times$ SSC and blocked with PBS containing 1% BSA. The samples were  
17 incubated with primary antibodies, including cardiac troponin T (Abcam),  $\alpha$ -SMA  
18 (Abcam), CD31 (Abcam), and vimentin (Abcam) for 2 h at room temperature, followed by  
19 incubation with goat anti-rabbit IgG/Alexa Fluor 594 secondary antibodies (Biosynthesis)  
20 for 1 h at room temperature. The cells or slides were washed and incubated with DAPI.  
21 Image acquisition was performed with an LSM 880 confocal microscope.

22

1 **Stereological analysis**

2 Left ventricles (including the septum) were sampled as previously described.<sup>6, 7</sup> Briefly,  
3 heart tissues were embedded in 8% gelatin, and isectors were used to obtain an isotropic,  
4 uniform, random alignment of the samples with a maximum diameter of 4 mm. These  
5 isectors were used for stereological analysis. An anti-PCM1 antibody was applied to label  
6 CM nuclei. WGA was added to identify the cell borders. A minimum of 3-4 isectors were  
7 stained, and a minimum of 200 nuclei per animal were counted (nearly 2% of the area of  
8 the region of interest). CMs were cut along their longitudinal axis to determine the number  
9 of nuclei per cell. The two-step NVX vancomycin-resistant *Enterococcus faecium* (VREF)  
10 method was utilized to estimate the total numbers of nuclei in the heart, as previously  
11 described. NV was an estimate of the numerical CM density, and VREF is the reference  
12 left ventricle volume. The total number of CMs was calculated based on the number of CM  
13 nuclei and the multinucleation level. The analysis was performed by confocal laser  
14 scanning microscopy (Carl Zeiss).

15

16 **Estimation of total number of CMs *in vivo***

17 As previously described, 8 tissue pieces (1-2 mm diameter) from the left ventricle were  
18 sampled. CM nuclei were stained with antibodies against PCM-1, and nuclei were stained  
19 with DAPI. The cytoplasm of CMs was stained with cTnT. To facilitate the identification  
20 of the cell borders, WGA was added. Using the CAST software, the serial sections could  
21 be analyzed all together. In each block, the number of CMs was calculated, and serial

1 sections were counted to estimate the total number of CMs. For each animal, 4 different  
2 tissue blocks were analyzed.

3

#### 4 **Flow cytometry**

5 After transfected with siRNAs or adenovirus, isolated CMs were cultured for 48 h and then  
6 collected and fixed with cold 70% ethanol. Samples were centrifuged for 15 min at  $\times 1200$   
7 g. Cell pellets were re-suspended in FxCycle PI/RNase Staining Solution (Thermo Fisher  
8 Scientific) and analyzed on MoFlo XDP (Cell Sorter).

9

#### 10 **Time-lapse videos**

11 After the P7 CMs were transduced with Adv-Snhg1 or Adv-NC for 48 h, the CMs were  
12 labeled with tetramethylrhodamine ethyl ester (TMRE), a fluorescent dye that labels  
13 mitochondria. Then, the images were imaged for 48 h at 10 min intervals. Live-cell imaging  
14 was performed using a Delta Vision Elite system (Applied Precision) on an Olympus IX71  
15 in verted microscope, running Soft WorX6.0. Time-lapse imaging was carried out for 48 h  
16 at 10 min intervals, and acquired at a 10x magnification (10/0.3 NA objective) with a Cool  
17 Snap HQ2CCD (charge-coupled device) camera (Roper Scientific).

18 For the P7 CMs isolated from the MYH6-mCherry transgenic mice, after transduction with  
19 Adv-Snhg1 for 48 h cells were imaged for 48 h at 10 min intervals. Live-cell imaging was  
20 performed using a Delta Vision Elite System (Applied Precision), on an Olympus IX71 in  
21 verted microscope, running Soft WorX6.0. Time-lapse imaging was carried out for 48 h at  
22 10 min intervals, and acquired at 10 magnifications (10/0.3 NA objective) with a Cool Snap

1 HQ2CCD (charge-coupled device) camera (Roper Scientific).

2

### 3 **RNA-seq analyses**

4 RNA-seq and genome-wide transcriptome analyses. RNA was collected from the hearts of  
5 mice injected with AAV9-Snhg1 or AAV9-NC. RNA-seq experiments were performed by  
6 GENE DENOVE (Beijing, China). Briefly, the total RNA was isolated from fresh  
7 ventricular tissue using TRIzol (Invitrogen). The RefSeq and Ensembl transcript databases  
8 were used as the annotation references for mRNA analyses. The clustering of the index-  
9 coded samples was performed on a cBot Cluster Generation System using the TruSeq PE  
10 Cluster Kit v3-cBot- HS (Illumina) according to the manufacturer's instructions. Analysis  
11 of differential expression was performed using the edgeR R package (3.12.1). The P values  
12 were adjusted using the Benjamini and Hochberg method. GO and KEGG pathway  
13 analyses were implemented using the cluster Profiler R package. The hierarchical  
14 clustering heat map was generated with the ggplot library.

15

### 16 **Pull-down assay**

17 The probes of Snhg1 and its antisense RNA for the RNA pull-down assay were designed  
18 by Gzscbio Co., Ltd. (Guangzhou, China). The probes for the DNA pull-down assay were  
19 synthesized by Gzscbio Co., Ltd. Isolated CMs were washed in ice-cold PBS, lysed in 0.5  
20 mL co-IP buffer, and incubated with 3 µg biotinylated DNA oligo probes against the Snhg1  
21 back-splice sequence at room temperature for 4 h. Next, the CMs were incubated in 50 µL  
22 washed streptavidin-coated magnetic beads (Invitrogen) at room temperature for another

1 hour. RNase-free BSA and yeast tRNA (Sigma) were used to prevent the nonspecific  
2 binding of RNA and protein complexes. RNA bound to beads was extracted with TRIzol,  
3 while the bound protein was analyzed by Western blotting. The specific bands were  
4 extracted and then analyzed by mass spectrometry or Western blotting.

5

## 6 **RIP**

7 A biotin-labeled anti-PTEN antibody (1:1000, #4370, cell signaling technology,) and IgG  
8 antibodies were added to cell extracts and incubated overnight at 4 °C. Streptavidin-coated  
9 magnetic beads were then added and incubated for 4 h at 4 °C. Magnetic beads were  
10 pelleted, washed, and resuspended in 1 mL TRIzol. Isolated RNA was reverse transcribed  
11 to cDNA and then analyzed by RT-qPCR. The PCR cycling parameters were as follows:  
12 an initial denaturation of 95°C for 5 min followed by 40 cycles of 94 °C for 10 s and 65 °C  
13 for 40 s, with a final extension at 72 °C for 10 min.

14

## 15 **Whole Genome Resequencing**

16 Genome DNA was collected from the hearts of cas9 mice injected with Adv-sgRNA.  
17 30×DNA-seq experiments were performed by BGI Genomics (Shenzhen, China). Briefly,  
18 200 mg fresh ventricular tissue was incubated with lysis buffer containing proteinase K,  
19 then was centrifuged to obtain supernatant. The supernatant was extracted vigorously with  
20 phenol/chloroform/isopentanol followed by centrifuging for 10 min. The supernatant was  
21 gently mixed with isopropanol and sodium acetate trihydrate. The mix was put at -20 °C  
22 for two hours and then was centrifuged for 20 min and the pellet was washed by 75%

1 ethanol. Remove the ethanol by centrifuging and air-dry the pellet for several minutes. The  
2 pellet was dissolved by 30-200uL TE for further study.

3 After DNA extraction, genomic DNA was randomly fragmented to an average size of 200-  
4 400 bp. Selected fragments were end repaired and 3' adenylated, then the adaptors were  
5 ligated to the ends of these 3' adenylated fragments. The products were amplified by PCR  
6 and purified by the Agencourt AMPure XP-Medium kit. The purified double stranded PCR  
7 products were heat denatured to single strand, and then circularized by the splint oligo  
8 sequence. The single strand circle DNA (ssCirDNA) were formatted as the final library  
9 and qualified by QC. The final qualified libraries were sequenced by BGISEQ-500.  
10 ssCirDNA molecule formed a DNA nanoball (DNB) containing more than 300 copies  
11 through rolling-cycle replication. The DNBs were loaded into the patterned nanoarray by  
12 using high density DNA nanochip technology. Finally, pair-end 100 bp reads were  
13 obtained by combinatorial Probe-Anchor Syntpsis (cPAS).

14 The sequencing data was aligned to mouse reference sequences using Burrows-Wheeler  
15 Aligner (BWA) tool.<sup>8</sup> The region included 100 nucleotides upstream and downstream of  
16 the sgRNA binding site was analyzed to detected nucleotides deletion. Accordingly, the  
17 frequencies of nucleotides deletion is calculated to obtain the knockout efficiency of the  
18 sgRNA.

### 19 **Echocardiography**

20 To evaluate cardiac function and dimensions, transthoracic two-dimensional  
21 echocardiography was performed on mice anesthetized with 2% isoflurane using a Vevo  
22 2100 Imaging System (Visual Sonics, Ontario, Canada) equipped with a 40-MHz probe.

1 M-mode tracings in the parasternal short axis view were used to measure the left ventricular  
2 internal diameter at end-diastole (LVEDd) and end-systole (LVESd), which were used to  
3 calculate left ventricular fractional shortening (LVFS) and the left ventricular ejection  
4 fraction (LVEF).

5

## 6 **Tissue collection**

7 Mice were anesthetized with 2% isoflurane and then sacrificed by injection of 10% KCl.  
8 The hearts and lungs were excised, briefly washed with 0.9% NaCl, weighed, and fixed in  
9 10% formalin at room temperature. The hearts were embedded in paraffin and further  
10 processed for histology or immunofluorescence analysis.

11

## 12 **Masson's trichrome staining**

13 Formalin-fixed, paraffin-embedded heart tissue slides were deparaffinized via xylene and  
14 rehydrated through sequential incubations in ethanol (100%, 100%, 90%, 80%, and 70%)  
15 and water. The slices were incubated in Weigert hematoxylin iron for 5 min, differentiated  
16 in hydrochloric acid (HCl)-ethanol, incubated in ponceau acid fuchs for 5 min,  
17 phosphomolybdic acid for 5 min, and aniline blue or light green (Leagene, Beijing, China)  
18 for 5 min. The fibrotic area was measured as the percentage of the total left ventricular area  
19 showing fibrosis and quantified with ImageJ software (NIH, Bethesda, MD, USA).

20

## 21 **Immunohistochemistry**

22 The MI heart sections were incubated with antibodies against CD31 (Abcam, Cambridge,

1 UK) at 4 °C overnight, and then with secondary antibodies at 4 °C for 1 h and detected with  
2 3, 3'-diaminobenzidine. The sections were counterstained with hematoxylin.  
3 Immunohistochemistry images were captured with an Olympus BX51 microscope (Tokyo,  
4 Japan).

5

### 6 **RNA isolation and RT-qPCR**

7 Total RNA from isolated CMs or dissected ventricular heart tissue samples was extracted  
8 using the E.Z.N.A. ® Total RNA Kit II (Omega Biotek, Norcross, GA, USA) according to  
9 the manufacturer's instructions. Cytoplasmic and nuclear RNAs were separated with an  
10 RNeasy Midi Kit (Qiagen, Hilden, Germany). The RNAs were treated with DNase I  
11 (Invitrogen) to prevent DNA contamination. cDNA was reverse-transcribed from 1 µg of  
12 total RNA using the PrimeScript™ RT Master Mix (TaKaRa, Shiga, Japan). For reverse  
13 transcription of Snhg1, strand-specific primers and a reverse primer of Snhg1 were used as  
14 reverse transcription primers. RT-qPCR was performed with SYBRs Premix Ex Taq™ Kit  
15 (TaKaRa) on a Lightcycler 480 (Roche, Basel, Switzerland). Briefly, the 20µl reaction  
16 mixtures were incubated at 95 °C for 30 s for the initial 3 denaturation, followed by 40  
17 cycles at 95 °C for 5 s and 60 °C for 34 s. β-actin was used as a housekeeping control gene  
18 to normalize gene expression using the  $\Delta\Delta$ Ct method. All primers were designed by Sangon  
19 Biotech (Shanghai, China). Primer sequences are shown in Table S1.

20

### 21 **Western blotting**

22 Isolated mouse CMs or dissected mouse ventricular heart tissue samples were lysed in ice-

1 cold radio immunoprecipitation assay buffer (Ding guo Chang sheng, Beijing, China) with  
2 protease inhibitors and phosphatase inhibitors. Protein concentrations were determined  
3 with the BCA Protein Quantitative Analysis Kit (Fudebio-tech, Hangzhou, China).  
4 Standard Western blotting were performed.<sup>9</sup> Briefly, protein samples were separated by 8-  
5 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and  
6 transferred onto polyvinylidenedifluoride membranes (Millipore, Billerica, MA, USA).  
7 The membranes were incubated at room temperature for 2 h in blocking buffer (5% BSA  
8 in Tris-buffered saline and Tween 20 [TBST] buffer). After blocking, the membranes were  
9 incubated with primary antibodies overnight at 4 °C. Primary antibodies against the  
10 following proteins were used: PTEN (Cell Signaling Technology, #9188), p-Akt (Cell  
11 Signaling Technology, #4060), Akt (Cell Signaling Technology, #9272), pGsk3 $\beta$  (Abcam,  
12 ab131097), c-Myc (Cell Signaling Technology, #18583, Proteintech, 10828-1-AP), Vegfa  
13 (Proteintech, 66828-1-Ig ), Gapdh (Biosynthesis, bs-2188R) and  $\beta$ -actin (Biosynthesis, bs-  
14 0061R). After the membranes were washed three times with TBST, they were incubated  
15 with donkey anti-rabbit IgG (Abcam, ab16284) for 1 h at room temperature. The  
16 membranes were developed with the electrochemiluminescence method according to the  
17 manufacturer's instructions (Millipore) and detected on a chemiluminescence imaging  
18 GeneGnome XRQ System (Syngene, Bangalore, India). To calculate the relative density,  
19 ImageJ software was used, and the intensity of each band was normalized to that of  $\beta$ -actin.

20

## 21 **Luciferase assay**

22 Luciferase assays were conducted with the Dual-luciferase Reporter Kit (Promega). The

1 pRL vector constitutively expressing Renilla luciferase was used to normalize for  
2 transfection efficiency. A total of  $2 \times 10^5$  CMs were plated in 12-well dishes 24 h before  
3 transfections. On the day of transfection, each well was transfected with pRL, pGL3 Basic  
4 (to assess basal reporter activity) or Myc promoter-pGL3 and the indicated plasmids.  
5 Twenty-four hours later, luciferase activity was measured using the Wallac 1450  
6 MicroBeta TriLux System (Perkin Elmer). Experiments were carried out 3 times in  
7 triplicates, and error bars represent SD.

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12

## Supplemental figure legends

**Figure S1. Snhg1 is highly expressed in neonatal hearts and conserved across mammals.**

(A) RT-qPCR analysis of Snhg1, 3, 4, 5, 7, 8, 9, 12, 15, 17, 18, and 20 in P1 and adult mouse hearts as detected by RT-qPCR, n=5. (B) Conservation analysis of Snhg1. (C) RT-qPCR analysis of Snhg1 levels in P1 mouse heart, liver, muscle, skin, kidney, and brain tissues, n=5. (D) Detection of Snhg1 expression in adult heart tissue after MI by *ISH*, 1-3 respectively indicated remote zone, border zone and infarcted zone, n=5. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using t-tests in A, one-way ANOVA test followed by LSD post hoc test in C and D.

**Figure S2. Adenovirus-mediated Snhg1 overexpression promotes P7 CM proliferation *in vitro*.**

(A) The purity of isolated P7 CMs is approximately 80%, and isolated CMs were stained with cTnT, PCM-1, and DAPI, 751 cells from 5 mice. (B) GFP/cTnT double-staining in P7 CMs after transduction with PBS or Adv-Snhg1-GFP, 638 CMs from 5 mice in Adv-Snhg1-GFP group. (C) Quantification of Snhg1 expression by RT-qPCR in isolated P7 CMs transduced with Adv-NC or Adv-Snhg1, n=5. (D) Immunostaining of Anilin for P7 CMs transduced with Adv-Snhg1, PCM1 represent cardiomyocyte nuclei, 433 CMs from 5 mice in Ad-NC group, 503 CMs from 5 mice in Ad-Snhg1 group. (E) Western blotting analysis and quantification of pH3 and Aurora B protein levels in isolated P7 CMs transduced with Adv-NC or Adv-Snhg1.  $\beta$ -actin was used as a loading control, n=5. (F) the proportion of GFP+ cell in the EdU+ cardiomyocytes. 657 CMs from 5 mice, Arrow, GFP+ CMs, Triangle, GPP- CMs. (G) the proportion of GFP+ cell in the pH3+ cardiomyocytes. 574 CMs from 5 mice, Arrow, GFP+ CMs. (H) Quantification of the CM number after injected with Adv-NC or Adv-Snhg1, 545 CMs from 5 mice in Ad-NC group, 623 CMs from 5 mice in Ad-Snhg1 group. (I) Schematic of the CM binucleation assay after Adv-Snhg1 infection. Isolated CMs are treated with EdU for 24 h. EdU is then washed

1 out and CMs are cultured for another 24 h to complete one cell cycle. All data are expressed  
2 as the mean  $\pm$  SD, \* $P$ <0.05 using t-tests in A-H.

3

4 **Figure S3. Snhg1 overexpression promotes P7 CM proliferation *in vivo*.**

5 (A) The transduction efficiency of AAV9-Snhg1 was approximately 80%, as revealed by  
6 GFP and CM specific marker staining, n=5. (B) Quantification of Snhg1 expression by RT-  
7 qPCR in P7 mouse hearts injected with AAV9-NC or AAV9-Snhg1, n=5. (C) ISH assays  
8 confirmed that Snhg1 was significantly increased in P7 mouse hearts injected with AAV9-  
9 Snhg1 or AAV9-NC. the brown dot cluster indicates Snhg1, n=3. (D) Immunostaining and  
10 quantification of Aurora B in P7 mouse hearts injected with AAV9-NC or AAV9-Snhg1,  
11 n=5. (E) Immunostaining and quantification of pH3-positive CMs in P7 Myh6-mCherry  
12 transgenic mice injected with AAV9-NC or AAV9-Snhg1, n=5. All data are expressed as  
13 the mean  $\pm$  SD, \* $P$ <0.05 using t-tests in A-E.

14

15 **Figure S4. The effect of Endothelial cells, Fibroblasts and CMs co-culture on CMs**  
16 **proliferation.**

17 (A) Diagrammatic representation of endothelial cells (ECs)/fibroblasts (FBs) and CMs co-  
18 culture system. (B) Detection of Ki67+ CMs after co-cultured with FBs infected with Adv-  
19 NC or Adv-Snhg1, 473 CMs from 5 mice in CM group, 501 CMs from 5 mice in CM+FB  
20 group, 493 CMs from 5 mice in CM+ FB-Adv-NC group, 529 CMs from 5 mice in CM+  
21 FB-Adv-Snhg1 group. (C) Detection of pH3+ CMs after co-cultured with FBs infected  
22 with Adv-NC or Adv-Snhg1, 423 CMs from 5 mice in CM group, 478 CMs from 5 mice  
23 in CM+FB group, 514 CMs from 5 mice in CM+ FB-Adv-NC group, 539 CMs from 5  
24 mice in CM+ FB-Adv-Snhg1 group. (D) Detection of Ki67+ CMs after co-cultured with  
25 ECs infected with Adv-NC or Adv-Snhg1, 457 CMs from 5 mice in CM group, 523 CMs  
26 from 5 mice in CM+EC group, 534 CMs from 5 mice in CM+ EC-Adv-NC group, 507  
27 CMs from 5 mice in CM+ EC-Adv-Snhg1 group. (E) Detection of pH3+ CMs after co-  
28 cultured with ECs infected with Adv-NC or Adv-Snhg1. All data are expressed as the mean  
29  $\pm$  SD, \* $P$ <0.05 using one-way ANOVA test followed by LSD post hoc test in B-E.

30

1 **Figure S5. Snhg1 regulates human iPS-derived CM and macrophage proliferation,**  
2 **but not fibroblasts proliferation.**

3 (A) EdU immunofluorescence staining in fibroblasts (FBs) transduced with Adv-NC or  
4 Adv-Snhg1 and quantification of EdU-positive FBs, n=5. (B) EdU immunofluorescence  
5 staining in FBs transfected with si-NC or si-Snhg1 and quantification of EdU-positive FBs,  
6 n=5. (C) Representative FACS plots showing infection efficiency of GFP adenovirus in  
7 Thy1<sup>+</sup> cardiac fibroblasts infected with 10 or 100 MOI, compared to CMs infected with  
8 10 MOI of the adenovirus. (D) Immunostaining of Ki67 for macrophages transduced with  
9 Adv-sh-NC or Adv-sh-Snhg1, 785 macrophages from 5 independent experiments in Ad-  
10 sh-NC group, 638 macrophages from 5 independent experiments in Ad-sh-Snhg1 group.  
11 (E) Immunostaining of pH3 for macrophages transduced with Adv-sh-NC or Adv-sh-  
12 Snhg1, 785 macrophages from 5 independent experiments in Ad-sh-NC group, 836  
13 macrophages from 5 independent experiments in Ad-sh-Snhg1 group. (F)  
14 Immunocytochemistry of 60-day-old human iPS-derived CMs infected with Adv-NC or  
15 Adv-Snhg1 and immunostained 48 h later with antibodies against pH3, cardiac troponin T,  
16 and DAPI to mark nuclei, n=5. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using t-  
17 tests in A, B, D, E and F.

18

19 **Figure S6. The transduction efficiency of AAV9-Snhg1.**

20 (A) The transduction efficiency of AAV9-Snhg1 in adult CMs was approximately 80% as  
21 revealed by GFP and CM specific marker staining, n=5. (B) Representative In vivo  
22 bioluminescent images and Bright field captured on day 14 after injection with GFP-  
23 labelled AAV9-Snhg1 virus. Square indicates thoracic incision, arrow indicates the heart  
24 with GFP fluorescence. (C) GFP/cTnT double-staining in mouse adult hearts injected with  
25 PBS or AAV9-Snhg1-GFP, n=5. (D) Quantification of Snhg1 expression by RT-qPCR in  
26 adult mouse hearts injected with AAV9-NC or AAV9-Snhg1, n=5. (E) The transduction  
27 efficiency of AAV9-Snhg1-GFP in main types of cells in adult heart injected with AAV9-  
28 Snhg1-GFP, n=5. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using t-tests in A, C  
29 and D.

30

1 **Figure S7. Snhg1 induces adult CM proliferation**

2 (A) Stereological analysis revealed the number of CMs in adult mice injected with AAV9-  
3 NC or AAV9-Snhg1. Sections 1-8 out of 8 serial sections are shown, n=5. (B) CM size  
4 evaluation by WGA staining in adult mouse hearts after transduction with AAV9-NC or  
5 AAV9-Snhg1. (337 CMs from 5 mice in AAV9-NC group, 342 CMs from 5 mice in  
6 AAV9-Snhg1). (C) Heart weight (HW) to body weight (BW) ratio of adult hearts injected  
7 with AAV9-NC or AAV9-Snhg1, n=5. Morphology (upper panel) and Masson trichrome  
8 staining (lower panel) of hearts. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using t-  
9 tests in B.

10

11 **Figure S8. Snhg1 improves adult cardiac function post-MI.**

12 (A) The adult mouse MI model was confirmed using an electrocardiogram ST-segment  
13 elevation. (B) GFP/cTnT double-staining in remote zone, border zone and infarcted zone  
14 of MI mouse adult hearts injected with AAV9-Snhg1-GFP, n=5. (C) Immunostaining and  
15 quantification of pH3-positive CMs in P7 Myh6-mCherry transgenic mice injected with  
16 AAV9-NC or AAV9-Snhg1, n=5. (D) CM size evaluation by WGA staining in adult  
17 infarcted mouse hearts after transduction with AAV9-NC or AAV9-Snhg1. (357 CMs from  
18 5 mice in AAV9-NC group, 362 CMs from 5 mice in AAV9-Snhg1 group). (E) Heart  
19 weight (HW) to body weight (BW) ratio of adult infarcted hearts injected with AAV9-NC  
20 or AAV9-Snhg1. Morphology (upper panel) and Masson trichrome staining (lower panel)  
21 of hearts. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using t-tests in C-D.

22

23 **Figure S9. Snhg1 improves adult cardiac function in the I/R mouse model.**

24 (A-B) Immunofluorescence and quantification of EdU and pH3 for AAV9-NC and AAV9-  
25 Snhg1-injected adult mouse hearts 14 days after I/R, positive CMs are indicated by arrows,  
26 n=6. (C) Immunofluorescence staining and quantification of TUNEL-positive CMs in the  
27 AAV9-NC and AAV9-Snhg1-injected adult mouse hearts 14 days after I/R. TUNEL-  
28 positive CMs are indicated by arrows, n=6. (D) Immunofluorescence and quantification of  
29 IB4-positive cells for AAV9-NC and AAV9-Snhg1-injected adult mouse hearts 14 days  
30 after I/R. IB4-positive cells are indicated by arrows, n=5. (E) Representative images of

1 Masson's trichrome staining and immunohistochemistry for CD31 in AAV9-NC or AAV9-  
2 Snhg1-injected adult mice 28 days after I/R and quantification of CD31-positive cells, n=5.  
3 (F) Representative images of Masson's trichrome-stained heart sections in Sham or AAV9-  
4 NC or AAV9-Snhg1-injected adult mice 28 days after I/R and quantification of infarct size,  
5 n=6. (G) Representative images of echocardiography analysis on Sham or AAV9-NC or  
6 AAV9-Snhg1-injected adult mouse hearts at 28 days after I/R and quantification of LVESd,  
7 LVEDd, LVEF, and LVFS, n=10. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05 using  
8 t-tests in A-E, one-way ANOVA test followed by LSD post hoc test in F-G.

9

10 **Figure S10. Snhg1 depletion decreased neonatal CM proliferation *in vitro*.**

11 (A) RT-qPCR of Snhg1 in isolated P1 CMs transfected with si-NC or si-Snhg1, n=5. (B)  
12 Western blotting analysis of pH3 and Aurora B protein levels in isolated P1 CMs  
13 transfected with si-NC or si-Snhg1, n=5. All data are expressed as the mean  $\pm$  SD, \* $P$ <0.05  
14 using t-tests in A-B.

15

16 **Figure S11. Snhg1 depletion decreased neonatal CM proliferation *in vivo*.**

17 (A) Schematic of the experiments in 1day mouse hearts injected with adenovirus. IF,  
18 immunofluorescence. (B) RT-qPCR of Snhg1 expression in neonatal mouse hearts injected  
19 with Adv-sh-NC or Adv-sh-Snhg1, n=5. (C) *ISH* assays of Snhg1 expression in neonatal  
20 mouse hearts injected with Adv-sh-NC or Adv-sh-Snhg1, n=5. (D) Immunofluorescence  
21 of EdU in neonatal hearts injected with Adv-sh-NC or Adv-sh-Snhg1, n=5. Arrows,  
22 positive CMs. (E) Immunofluorescence of Ki67 and pH3 in neonatal hearts injected with  
23 Adv-sh-NC or Adv-sh-Snhg1, n=5. Arrows, positive CMs. (F-G) Immunofluorescence of  
24 Aurora B-positive CMs in neonatal hearts injected with Adv-sh-NC or Adv-sh-Snhg1, n=5.  
25 All data are expressed as the mean  $\pm$  SD, \* $P$ < 0.05 using t-tests in B-G.

26

27 **Figure S12. Generation of myocardial Snhg1-deficient mice using CRISPR-Cas9**  
28 **technology.**

29 (A) Schematic of sgRNA targeting site at snhg1 locus. The red color represents the sgRNA  
30 targeting sequence, and the green color represents the PAM sequence. (B) Immunostaining

1 of Cas9 in transgenic mouse hearts,  $\alpha$ -MHC-Cre- and  $\alpha$ -MHC-Cre+ mouse were littermates  
2 of generation of  $\alpha$ -MHC-Cre transgenic mice and Rosa26-LSL-Cas9-tdTomato. (C)  
3 Immunostaining of GFP in cas9-tdTomato mouse hearts at 7 days after injection with Adv  
4 -sgRNA (Snhg1)-GFP, n=5. (D) Base percentage composition along reads of the DNA-seq  
5 data from the heart of cas9 mouse injected Adv-sgRNA. The A curve overlap with the T  
6 curve, and the G curve overlap with the C curve, indicating a balanced base composition  
7 of raw reads. (E) Distribution of qualities along reads from the heart of cas9 mouse injected  
8 Adv-sgRNA. Each dot in the image represents the quality value of the corresponding  
9 position along reads. If the percentage of the bases with low quality (<20) was considered  
10 low, then the sequencing quality of this lane was considered good. (F) The knockout  
11 efficiency of Snhg1 using sgRNAs in heart of cas9 mouse, knockout efficiency was  
12 calculated as the frequencies of nucleotides deletion in sgRNA binding site, n=2. (G) RT-  
13 qPCR assays detecting Snhg1 expression in cas9-tdTomato mouse hearts at 7 days after  
14 injection with Adv-sgRNA (Snhg1)-GFP or Adv-NC, n=5. (H) Detection of Snhg1  
15 expression in sham operated and infarcted neonatal mouse hearts by using ISH analysis,  
16 n=5. All data are expressed as the mean  $\pm$  SD, \* $P$ < 0.05 using t-tests in C, G and H.

17

18 **Figure S13. Next-generation RNA sequencing (RNA-seq) of Snhg1 overexpression**  
19 **and control group from P7 mice**

20 (A) Hierarchical clustering of differentially expressed genes between AAV9-Snhg1 and  
21 control mimic-injected P7 hearts. Red and blue colors indicate up-regulated or down-  
22 regulated genes. (B) Scatter plot showing top 20 enriched GO terms of biological process  
23 for differentially expressed genes between AAV9-Snhg1 and control mimic-injected P7  
24 hearts. (C) RT-qPCR analysis of the expression of differentially expressed genes related to  
25 the cell cycle, PI3K-Akt, and Hippo pathways, n=5. All data are expressed as the mean  $\pm$   
26 SD, \* $P$ < 0.05 using t-tests in C.

27

28 **Figure S14. Snhg1 regulates CM proliferation through the PTEN/PI3K-Akt/c-Myc**  
29 **pathway.**

1 (A) NanoLC-MS/MS spectrum of PTEN peptides. (B) Western blotting analysis and  
2 quantification of PTEN protein levels in isolated CMs transduced with Adv-NC, Adv-  
3 Snhg1, si-NC, or si-Snhg1.  $\beta$ -Actin was used as a loading control, n=5. (C) Western  
4 blotting analysis and quantification of PI3K, p-Akt, and Akt protein levels in P7 mice  
5 injected with AAV9-NC, AAV9-Snhg1, AAV9-PTEN, and AAV9-Snhg1+AAV9-PTEN.  
6  $\beta$ -Actin was used as a loading control, n=4. (D-E) Immunofluorescence and quantification  
7 of Ki67-positive CMs in P7 mice injected with AAV9-NC, AAV9-Snhg1, AAV9-PTEN,  
8 or AAV9-Snhg1+AAV9-PTEN, n=5. (F) Western blotting comparing p-AKT, p-GSK3 $\beta$ ,  
9 and c-Myc levels in P7 CMs treated with si-NC, si-Snhg1, NC or LY294002.  $\beta$ -Actin was  
10 used as a loading control, n=4. (G) Western blotting comparing c-Myc levels in P7 CMs  
11 treated with NC, si-GSK3 $\beta$ , LY294002, or LY294002+si-GSK3 $\beta$ .  $\beta$ -Actin was used as a  
12 loading control, n=4. All data are expressed as the mean  $\pm$  SD, \* $P$ < 0.05 using t-tests in B,  
13 one-way ANOVA test followed by LSD post hoc test in C and E-G.

14

15 **Figure S15. Snhg1 promotes angiogenesis by Akt/ GSK3  $\beta$  /VEGF pathway.**

16 (A) Western blotting analysis and quantification of VEGFA and p-GSK3  $\beta$  protein levels  
17 in endothelial cells treated with Adv-NC, Adv-Snhg1, Adv-NC+LY294002, and Adv-  
18 Snhg1+LY294002. n= 5. (B) Western blotting analysis and quantification of VEGFA  
19 protein levels in endothelial cells treated with si-NC, si-GSK3 $\beta$ , si-NC+LY294002, and si-  
20 GSK3 $\beta$ +LY294002. n= 5. All data are expressed as the mean  $\pm$  SD, \* $P$ < 0.05 using one-  
21 way ANOVA test followed by LSD post hoc test in A and B.

22

23 **Figure S16. c-Myc binds to its promoter region of Snhg1.**

24 (A) Construction of Snhg1-WT and Snhg1-MU promoter sequences. (B) Luciferase  
25 activity in CMs transfected with luciferase-Snhg1-WT or luciferase-Snhg1-MU after Adv-  
26 c-Myc interference. (C) Western blotting analysis and quantification of c-Myc protein  
27 levels in cardiomyocyte at different age. n= 5. All data are expressed as the mean  $\pm$  SD,  
28 \* $P$ < 0.05 using one-way ANOVA test followed by LSD post hoc test in B and C.

29

30 **Figure S17. Entire unedited gel for all representative cropped gels in the manuscript.**

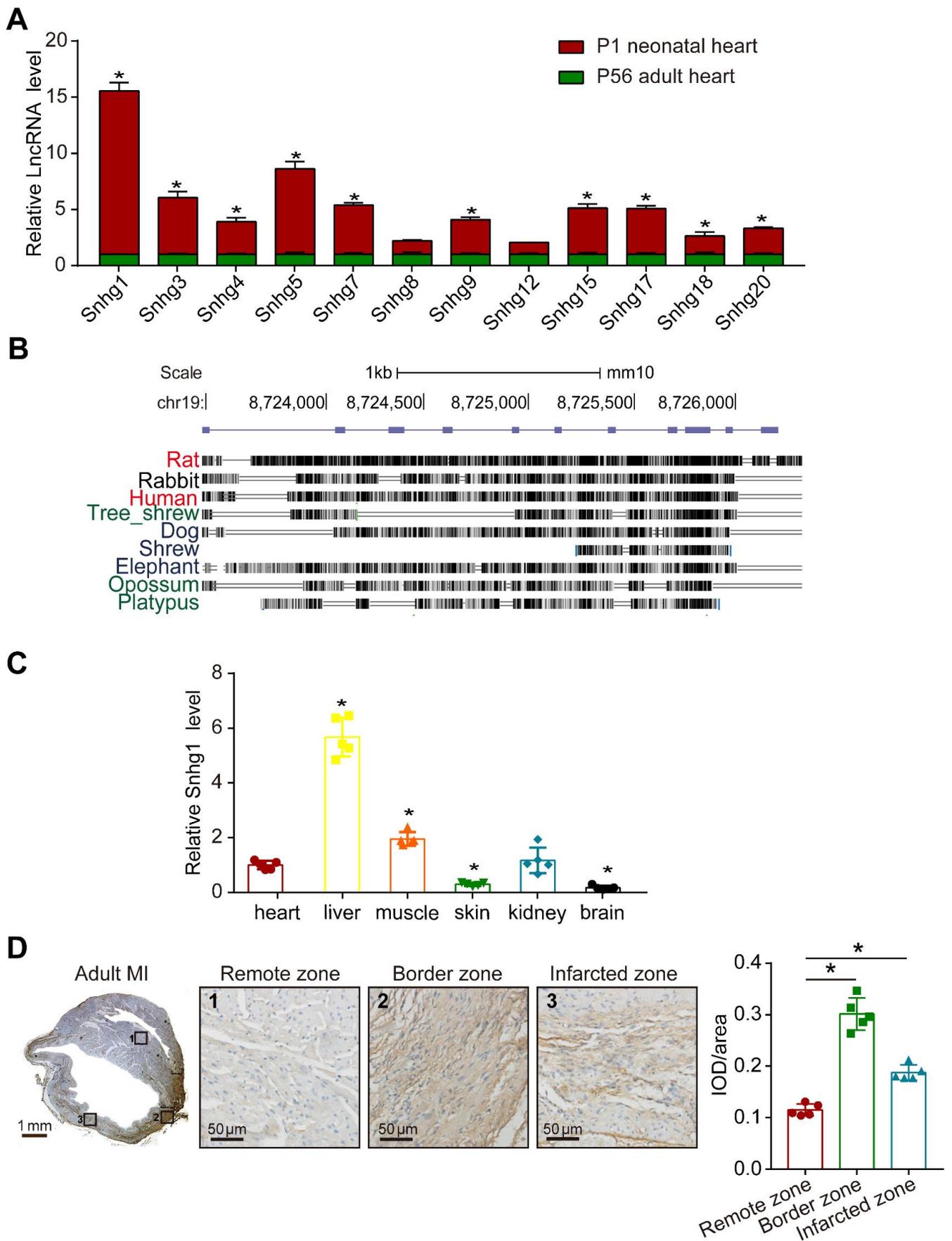


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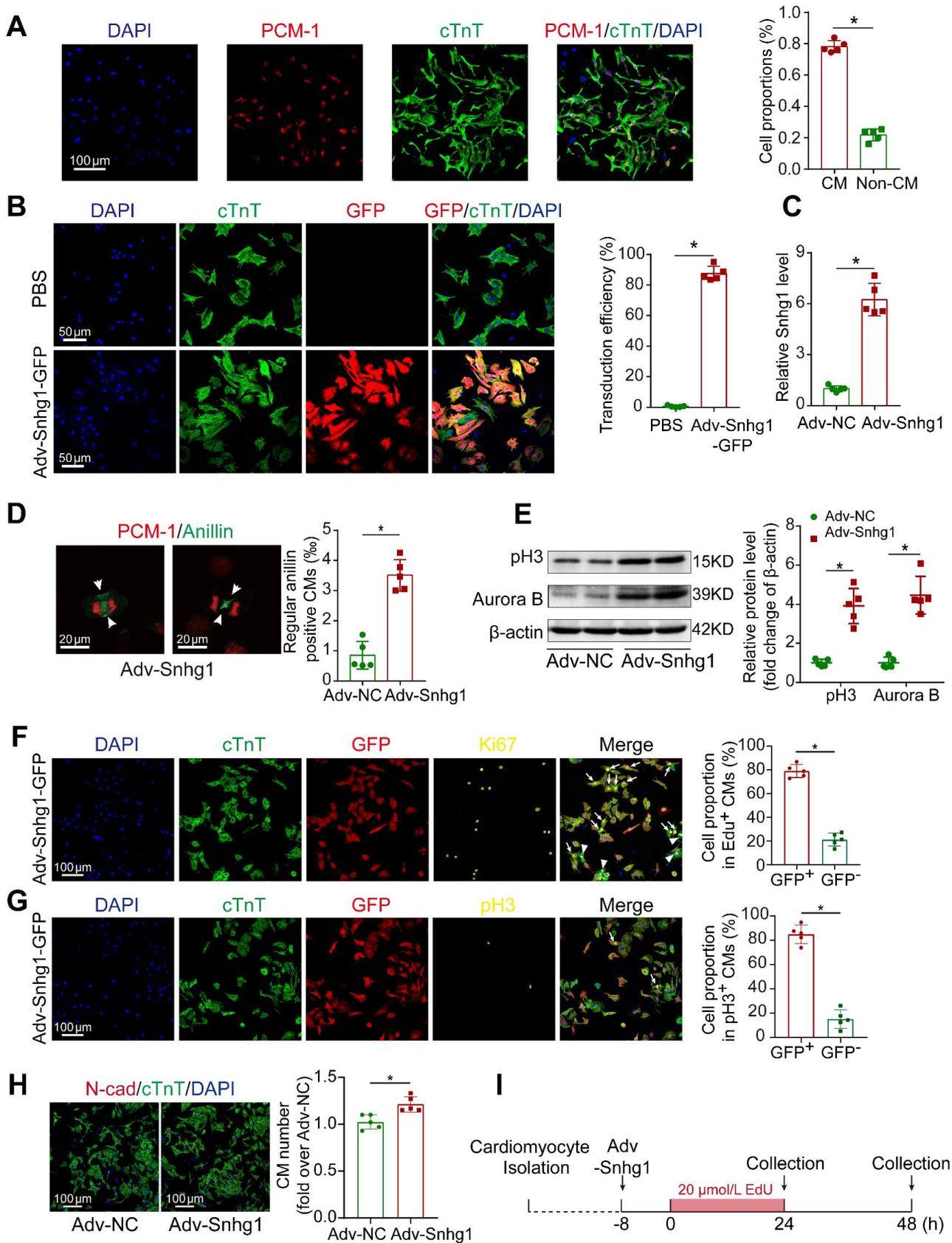


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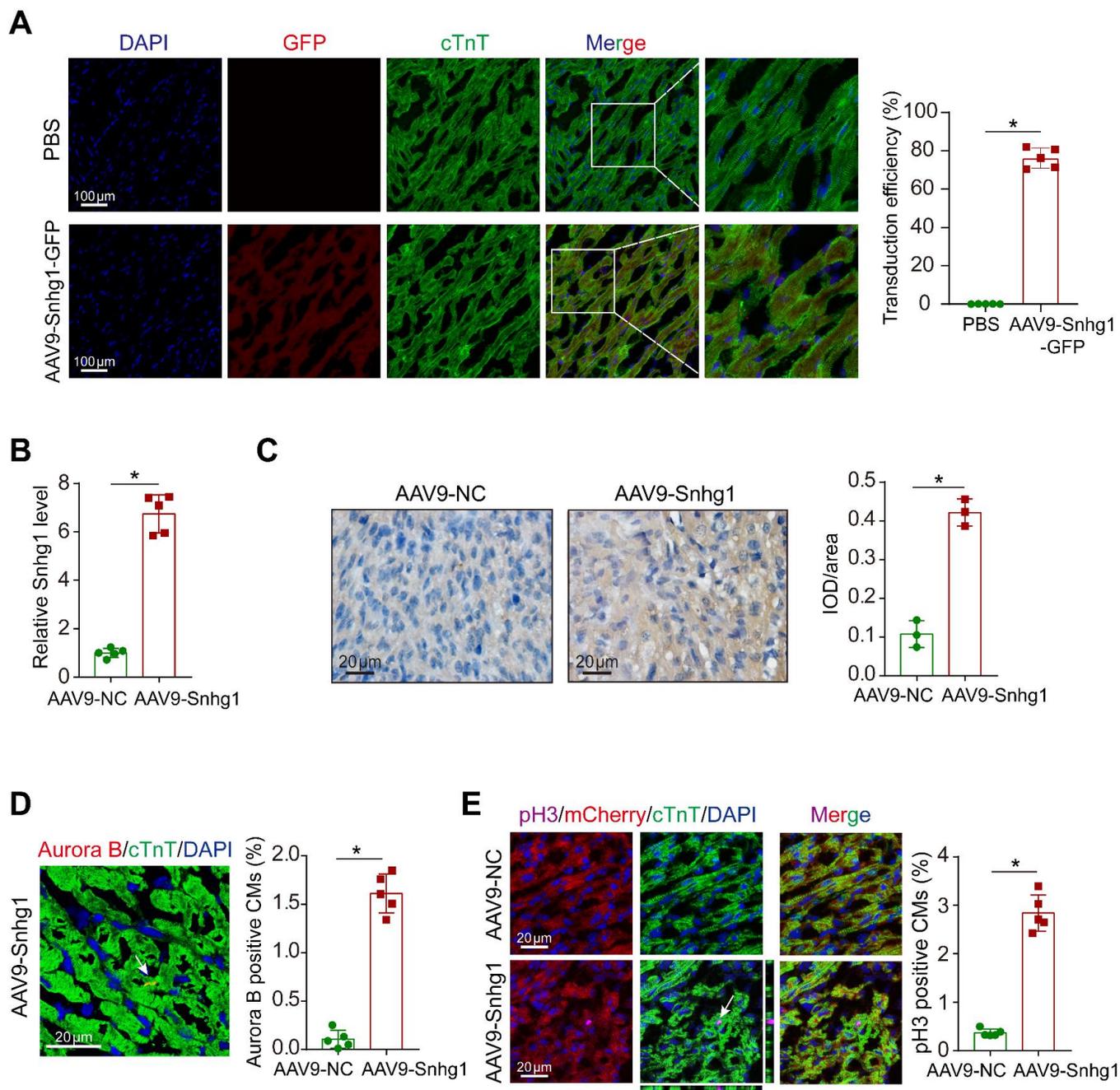


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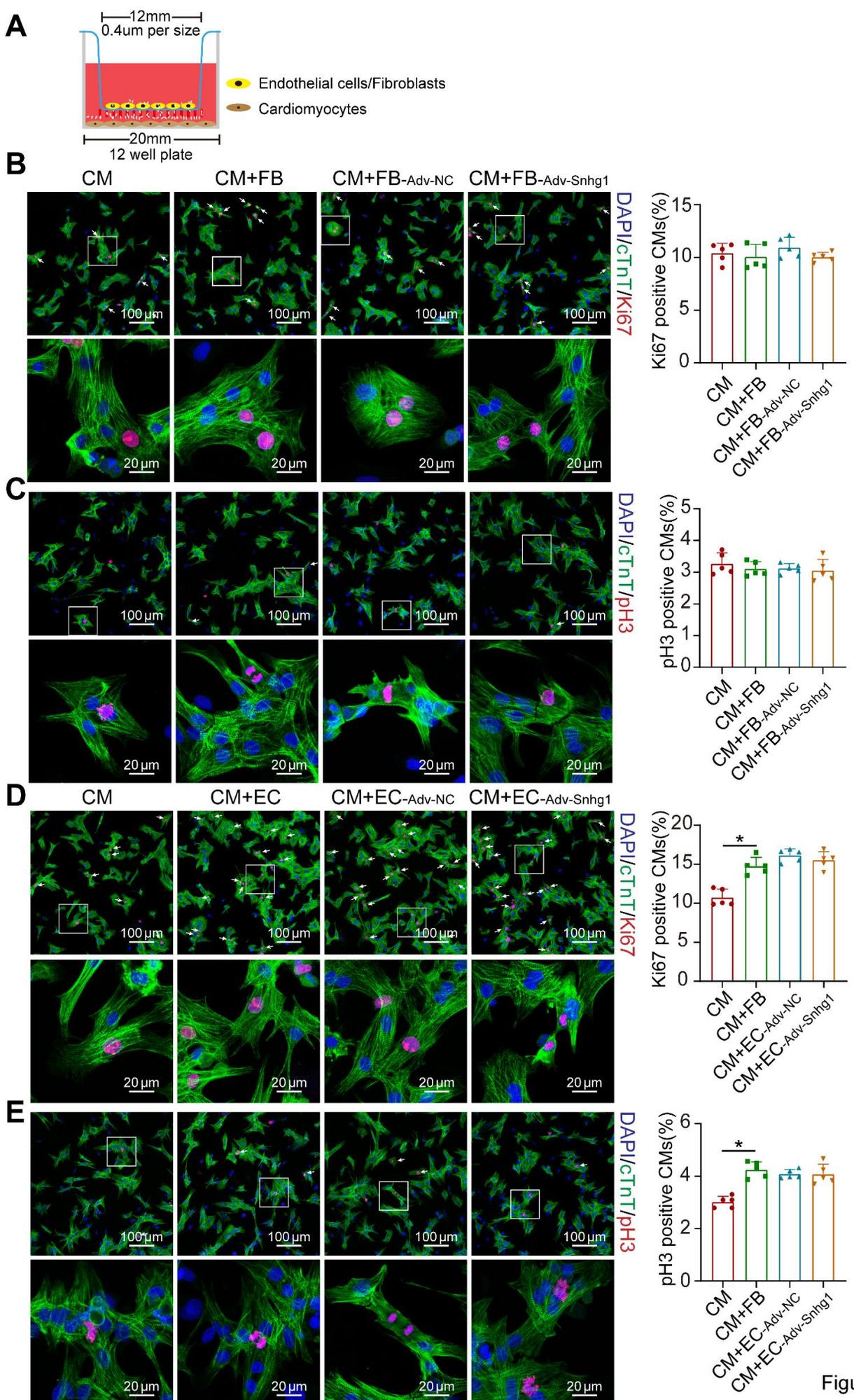


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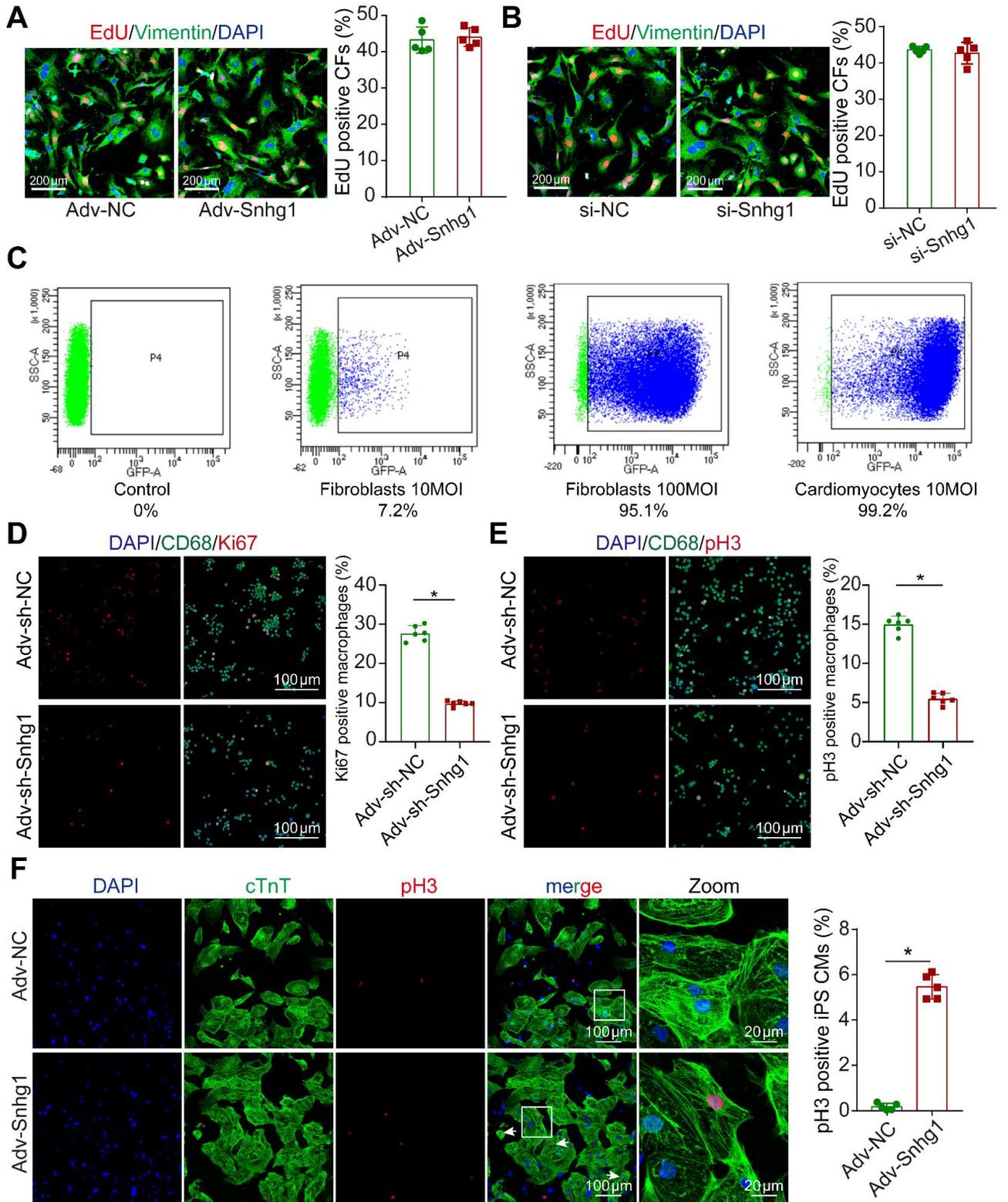


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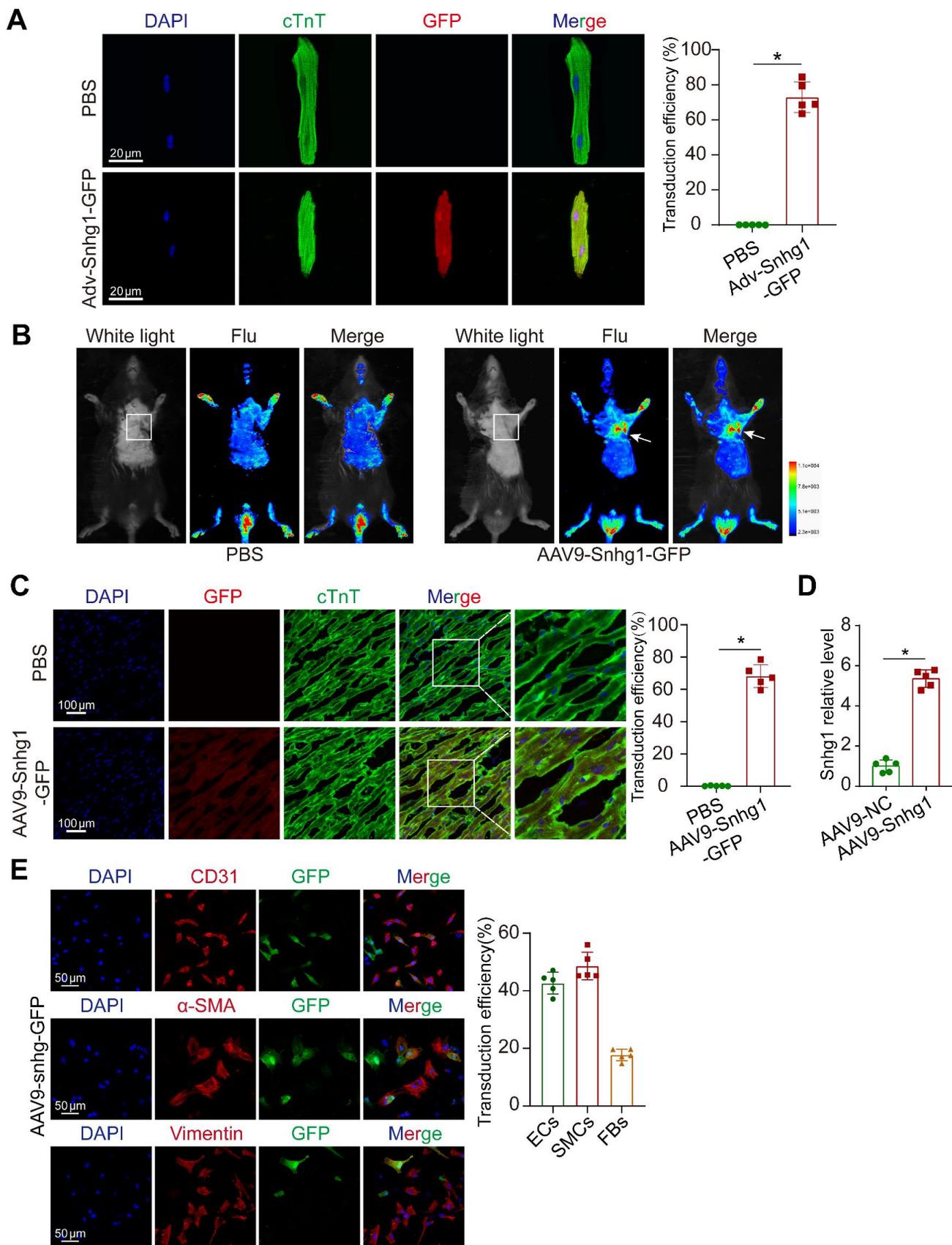


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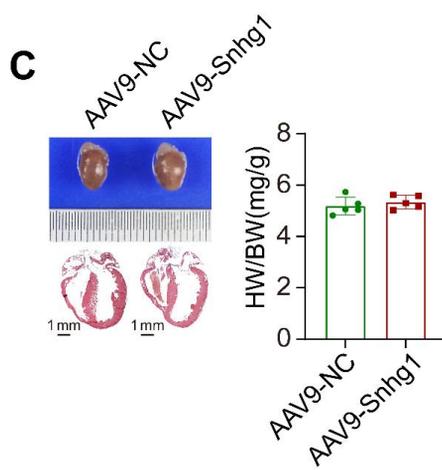
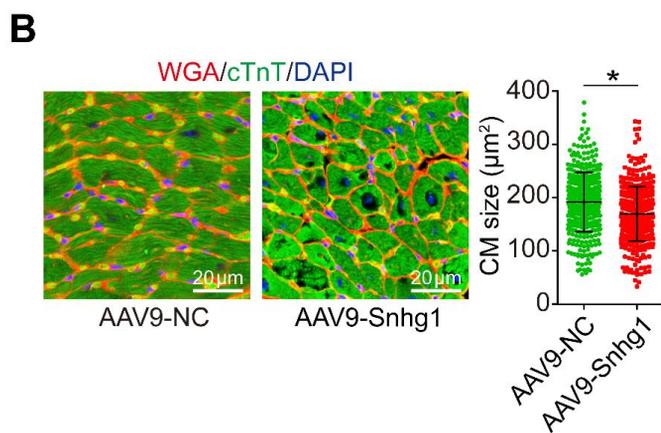
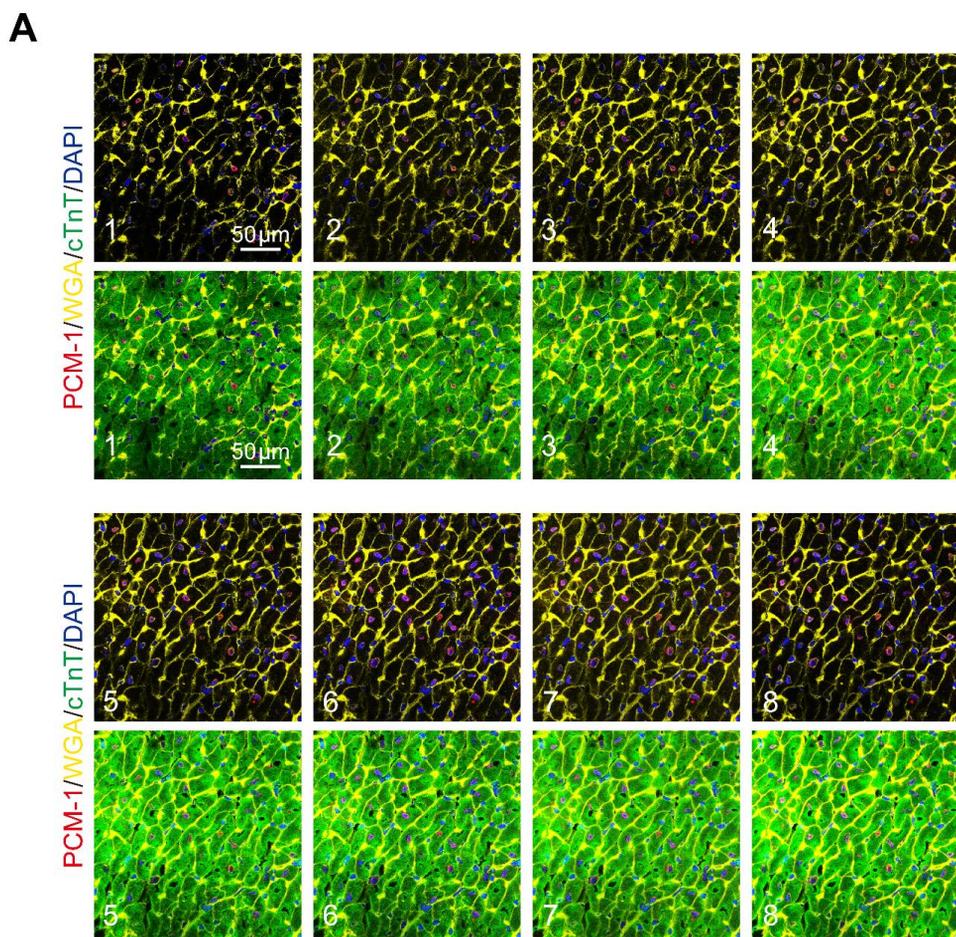


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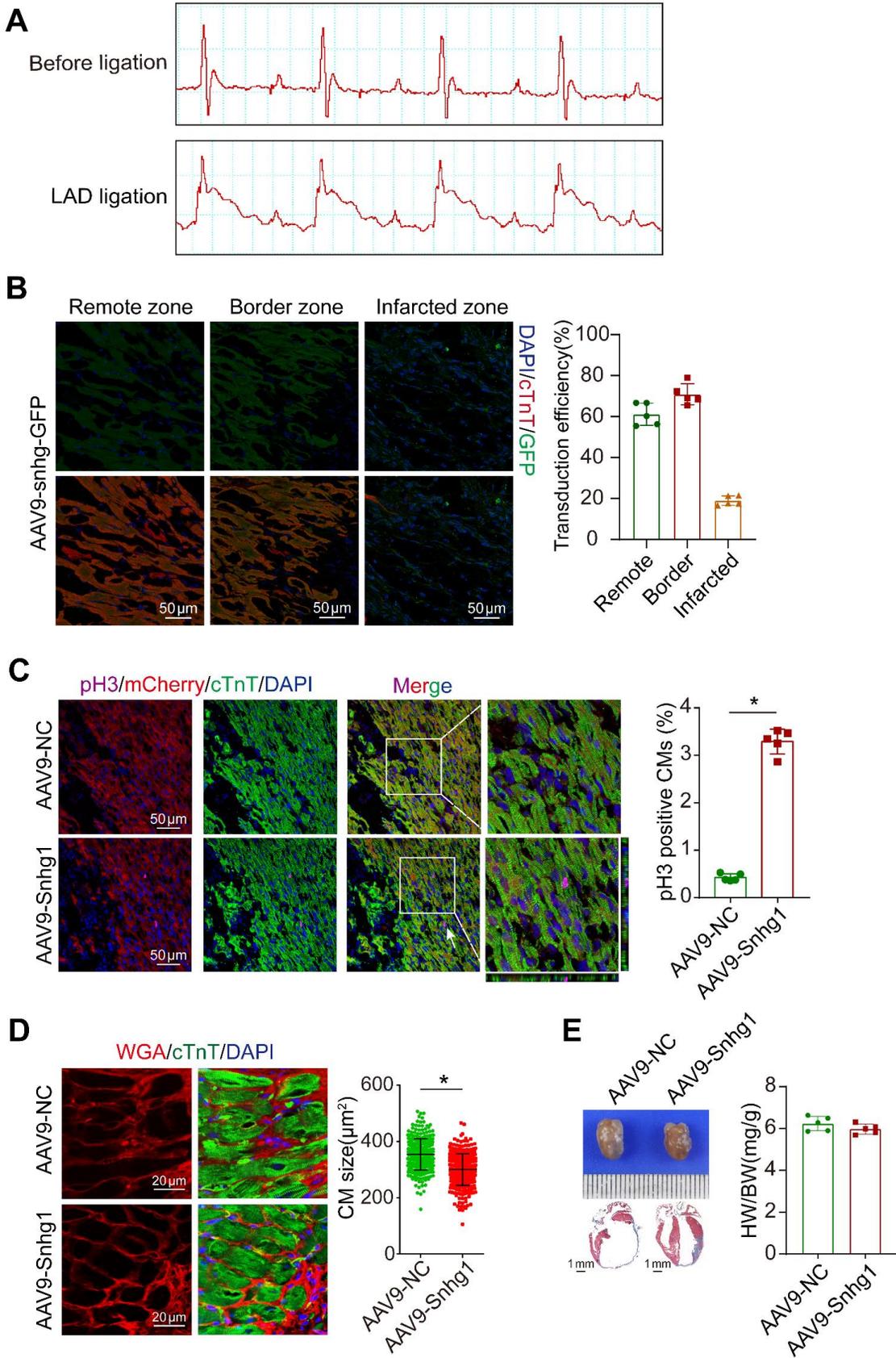


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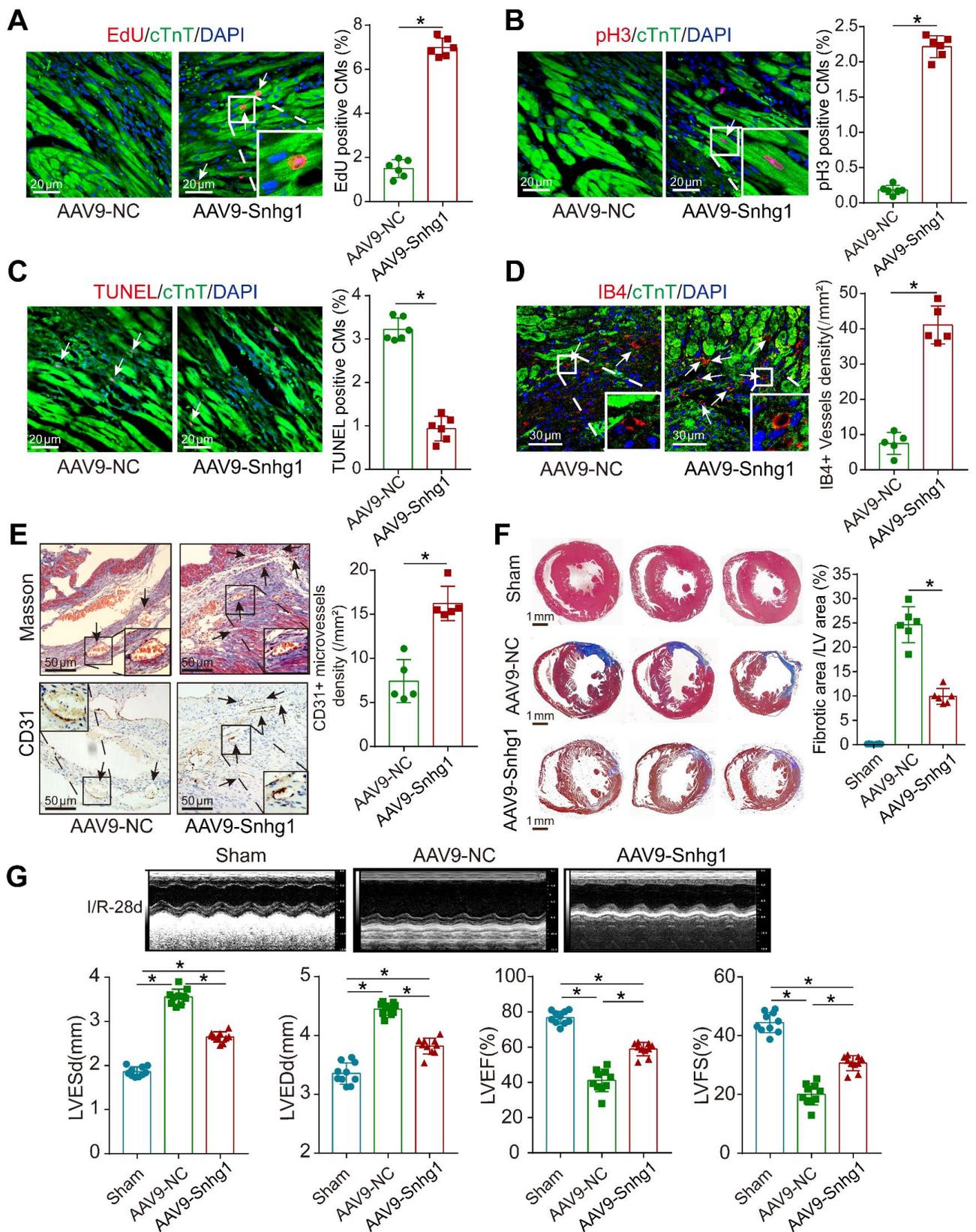
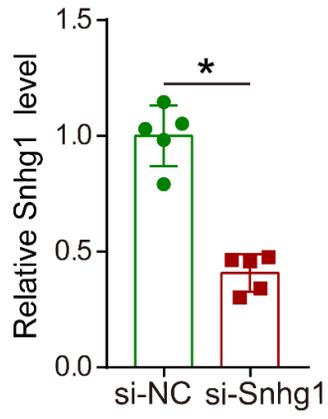
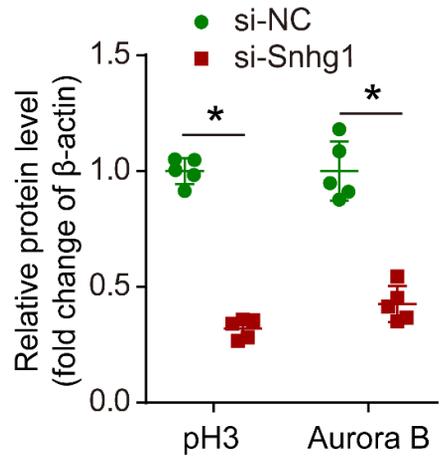
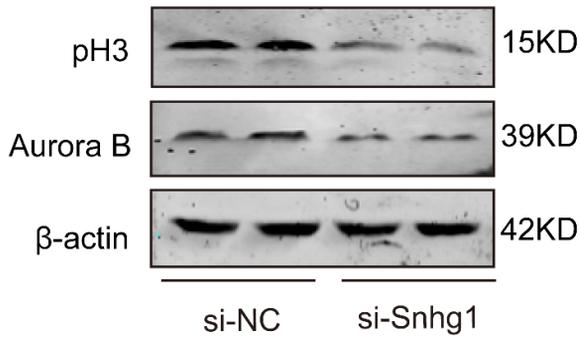


Figure S9

**A****B**

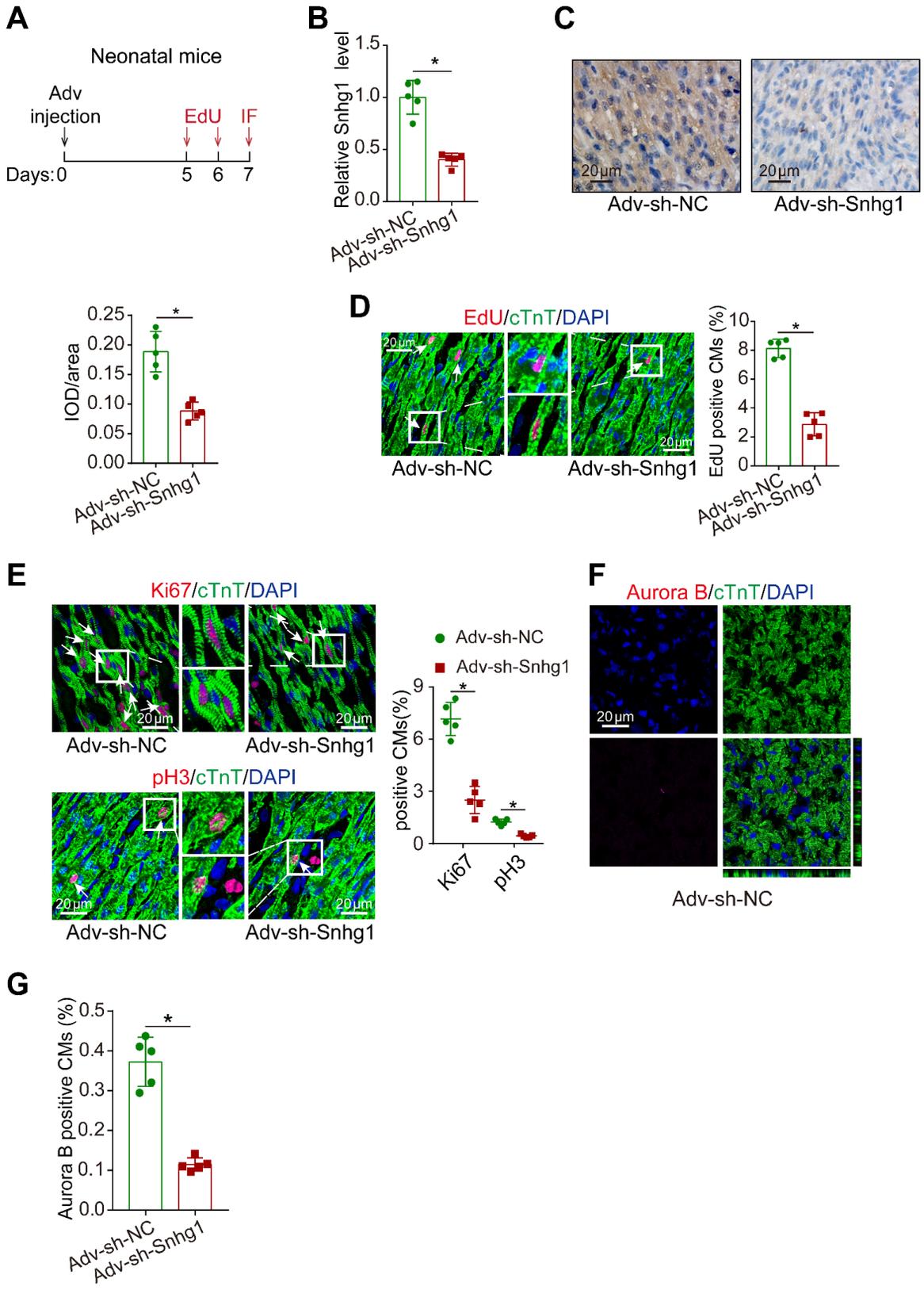
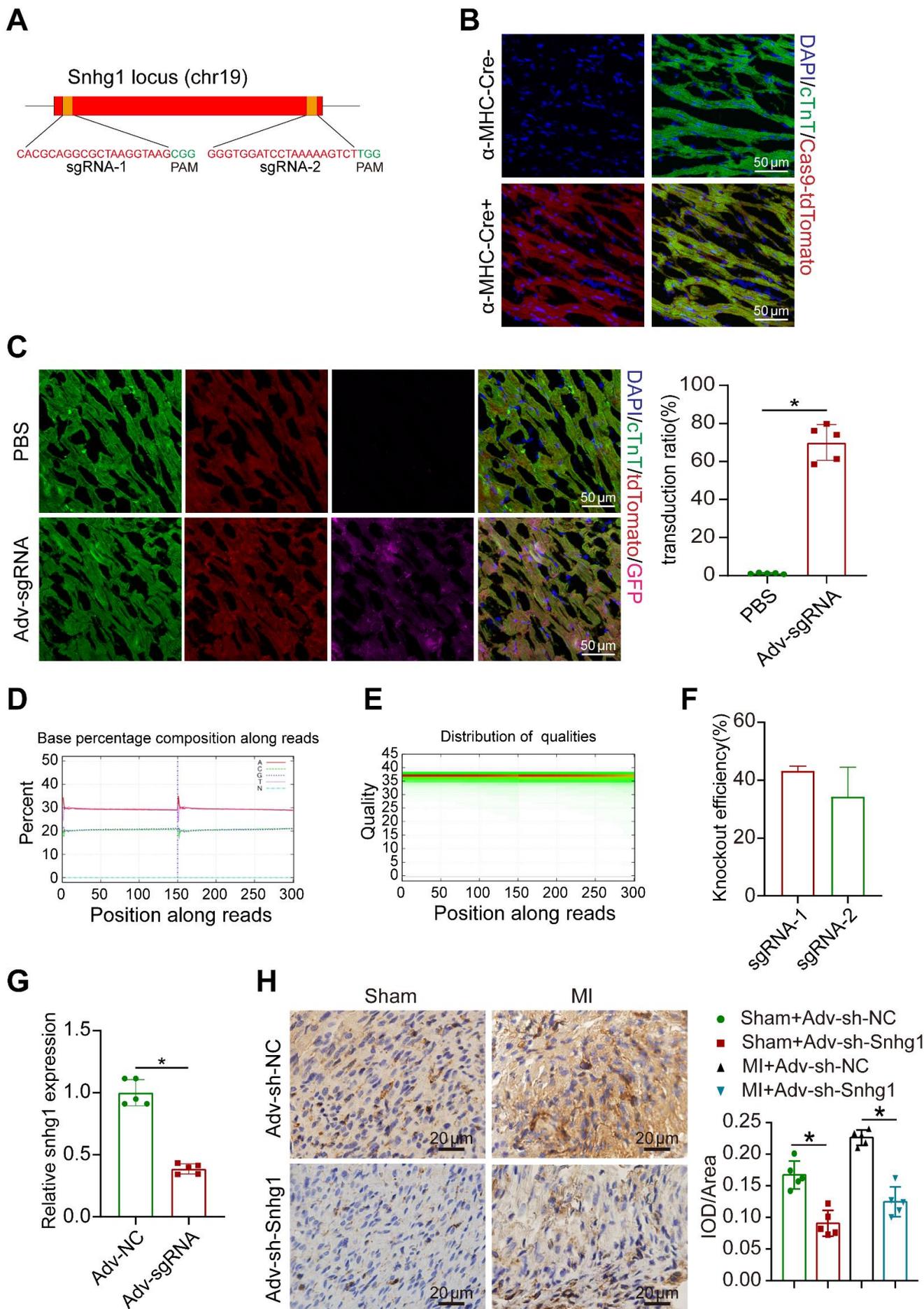


Figure S11



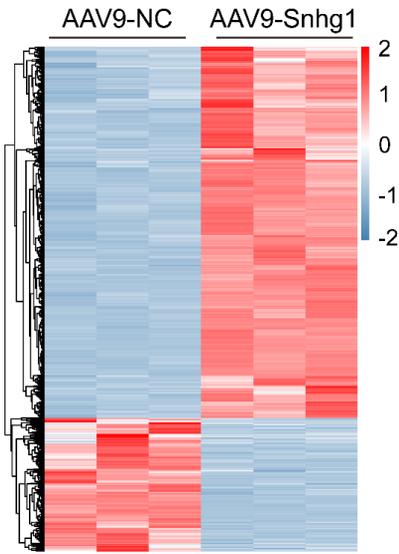
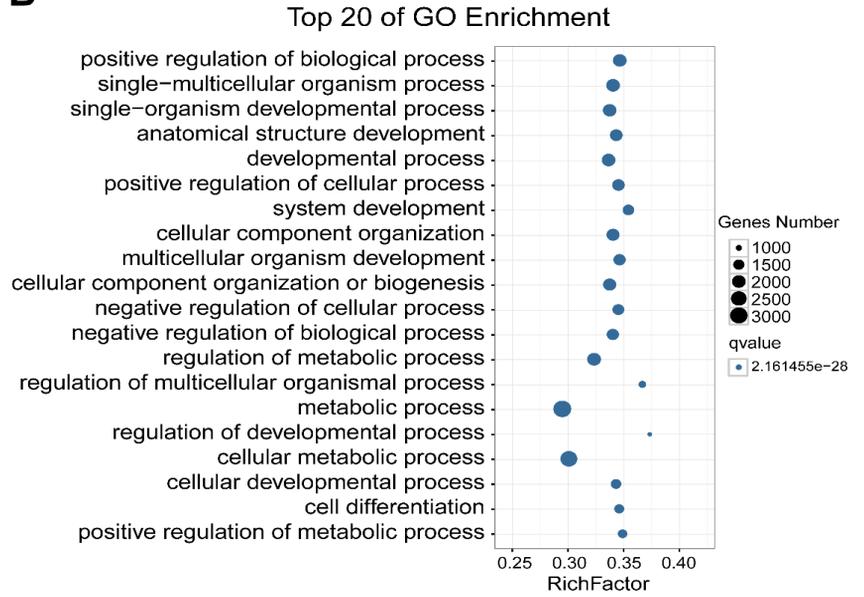
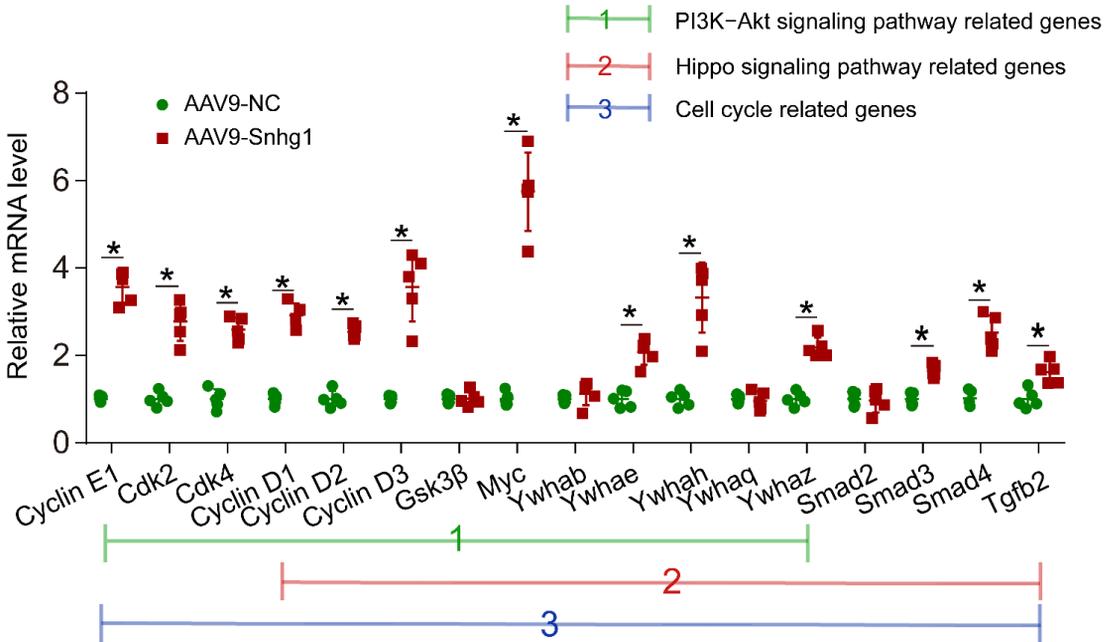
**A****B****C**

Figure S13

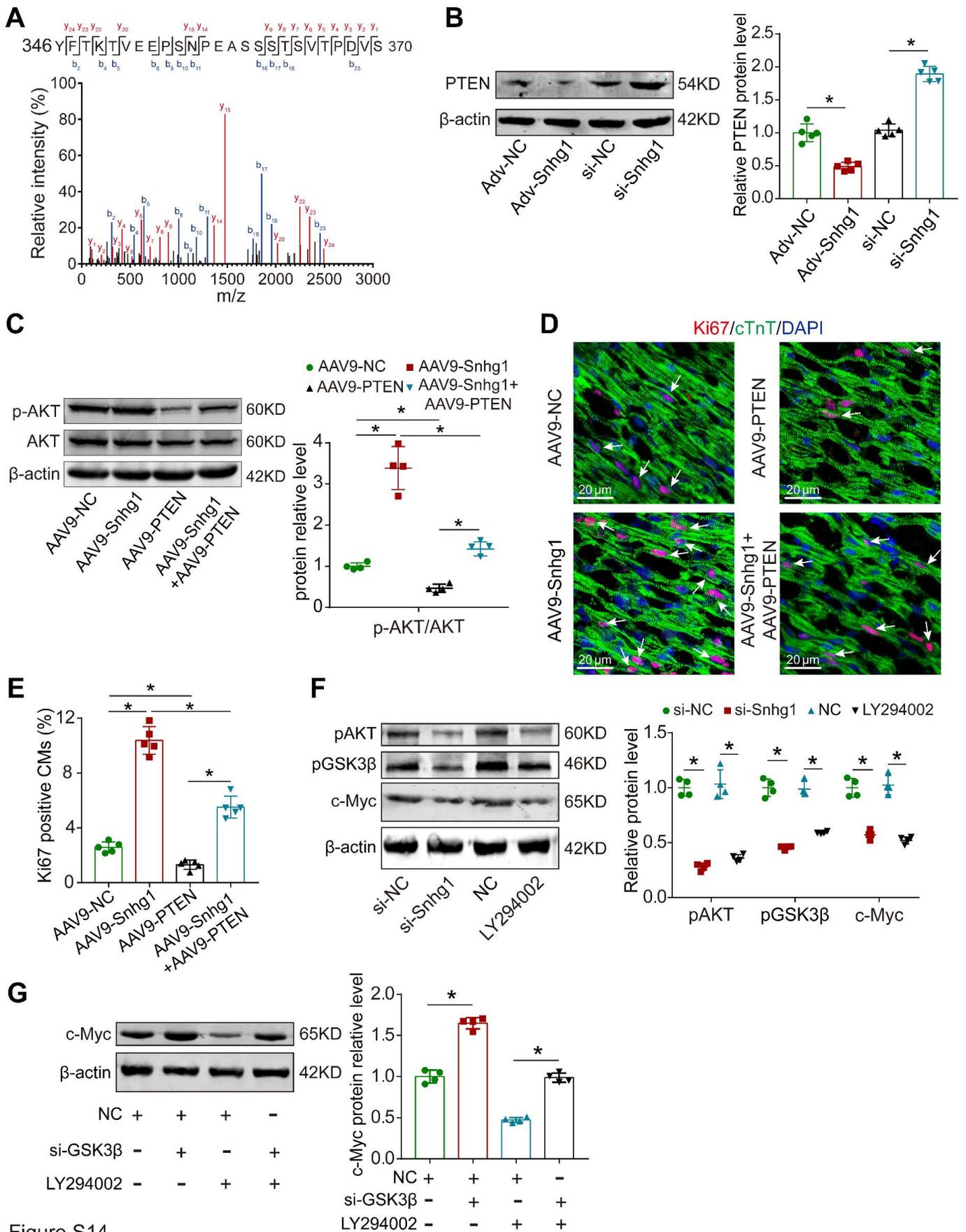
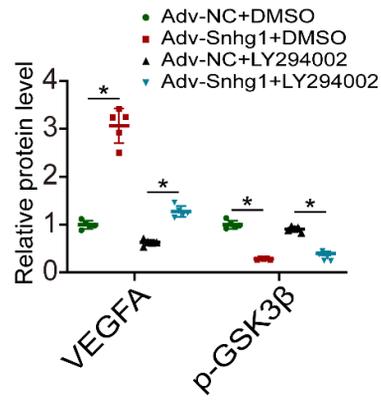
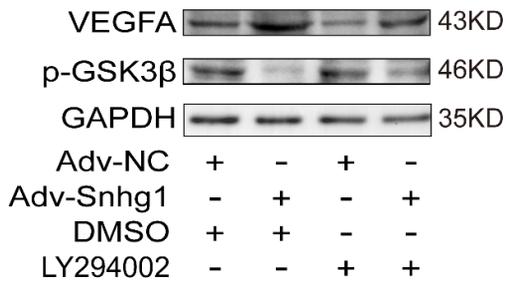
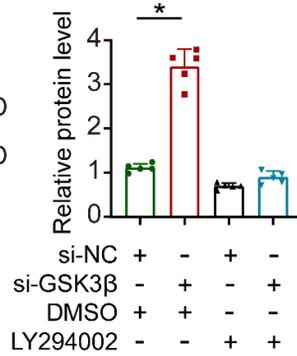
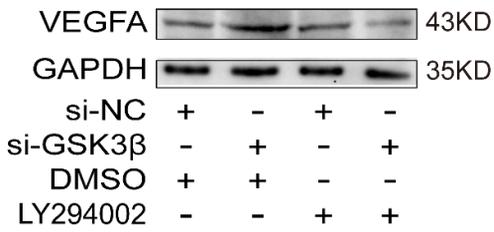


Figure S14

**A****B**

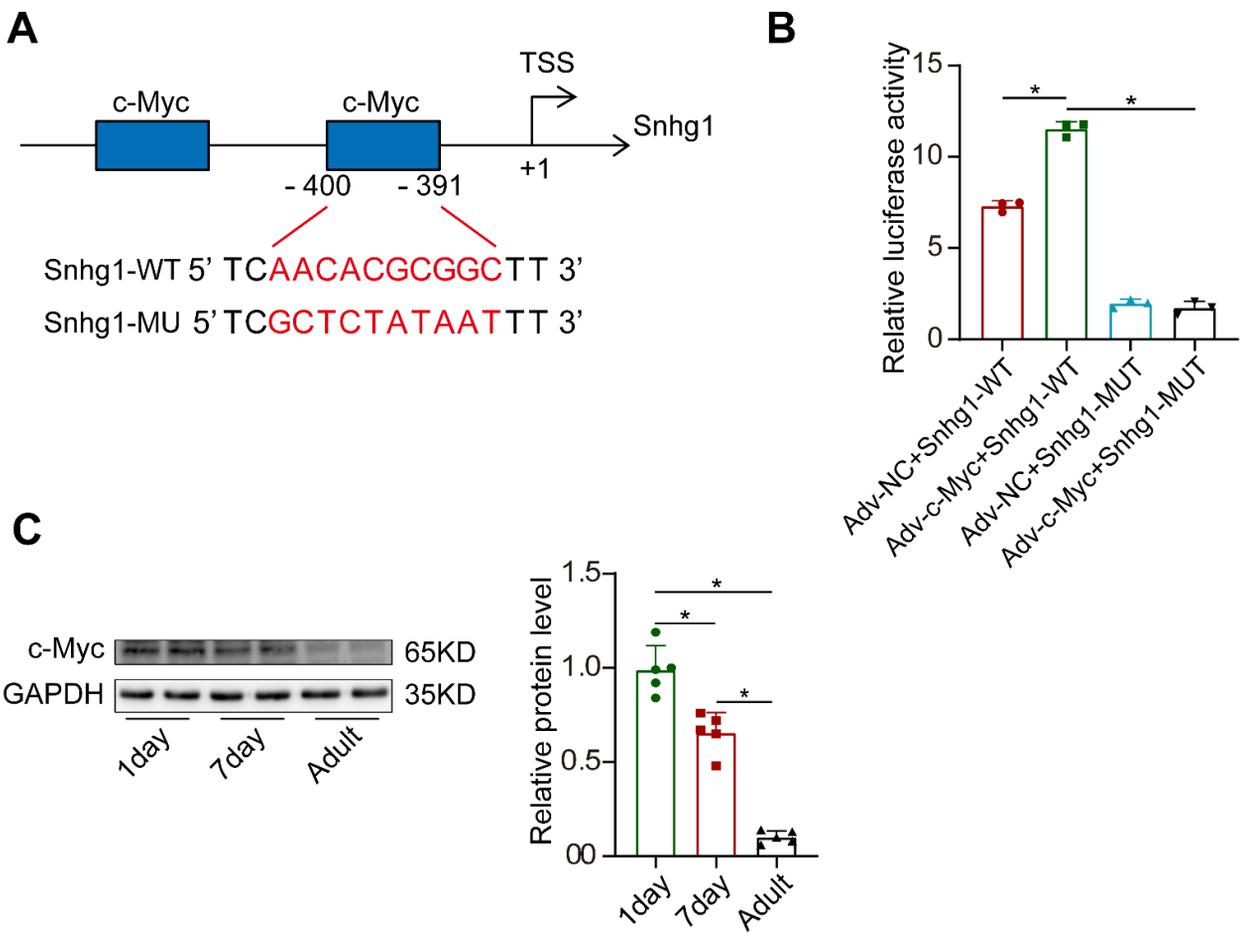
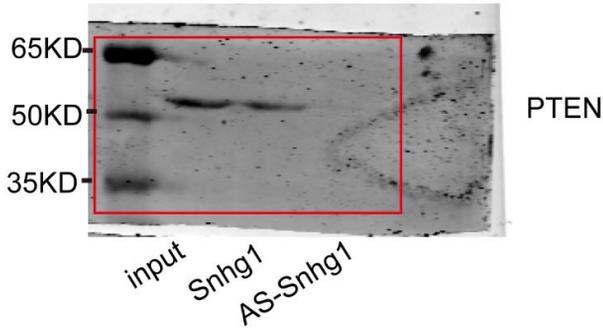


Figure S16

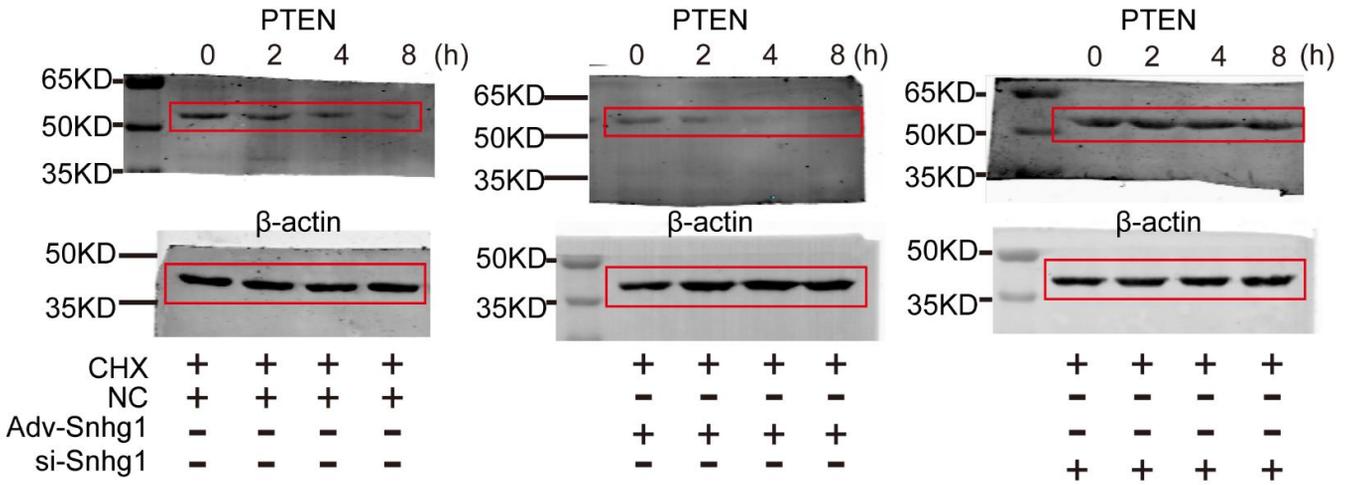
**Supplementary Table 1: Sequences of primers for RT-qPCR**

	Forward	Reverse
Snhg1	TTTGCTTGTAGTCAGGGTGCT	AGCCAGACACACCATCTCTG
CCNA2	GCCTTCACCATTCATGTGGAT	TTGCTCCGGGTAAAGAGACAG
CCNB1	CTCAGGGTCACTAGGAACACG	GCTCTTCGCTGACTTTATTACC
CCND2	CCAGACTGTGCCTTGGGAAT	GACACAGGGACAAGTGTGGT
CCNE1	CAGAGCAGCGAGCAGGAGA	GCAGCTGCTTCCACACCACT
CDK2	AGTTGACGGGAGAAGTTGTGG	TGACGATATTAGGGTGATTAAG
CDK4	CCCTCTTCTCACTCTGCGTC	TGCCAGAGATGGAGGAGTCT
CDK6	AGCTGTCTCCACCACCCAC	GGCCATCTGTCGTTAGCCAG
p21	CACAGCTCAGTGGACTGGAA	CCACCACCACACACCATAGA
$\beta$ -actin	TGCTGTCCCTGTATGCCTCTG	TTGATGTCACGCACGATTTC

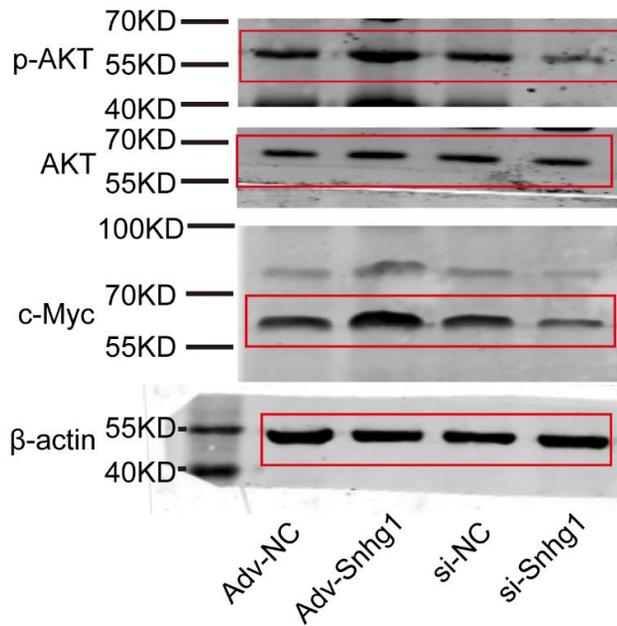
Full unedited gel for Figure 6F



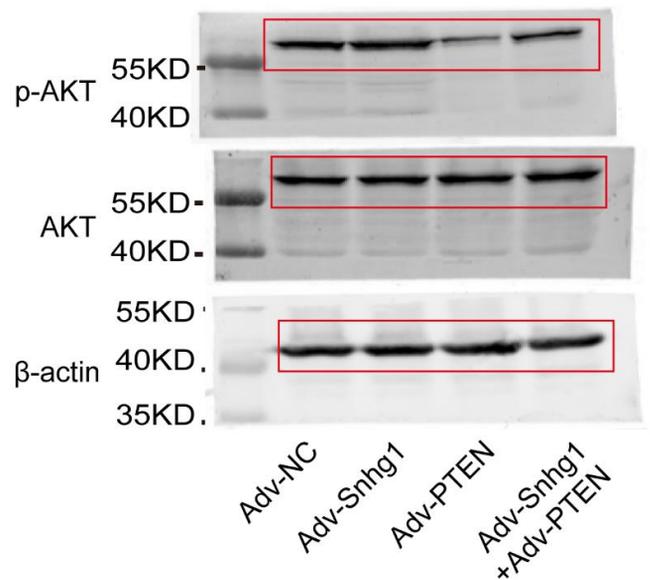
Full unedited gel for Figure 6I



Full unedited gel for Figure 6J

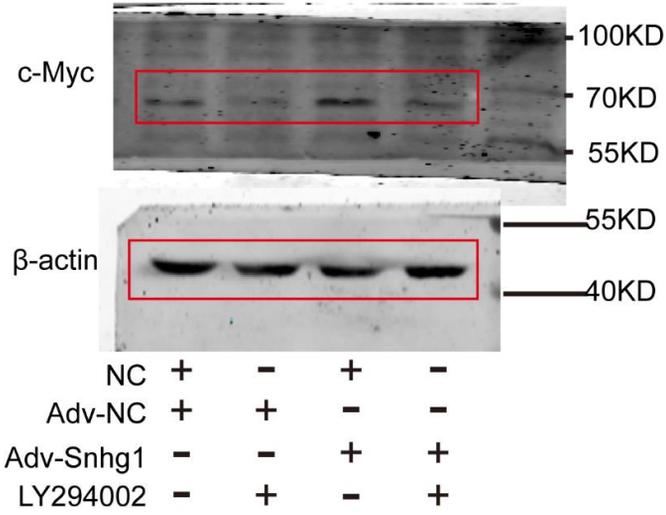


Full unedited gel for Figure 6K

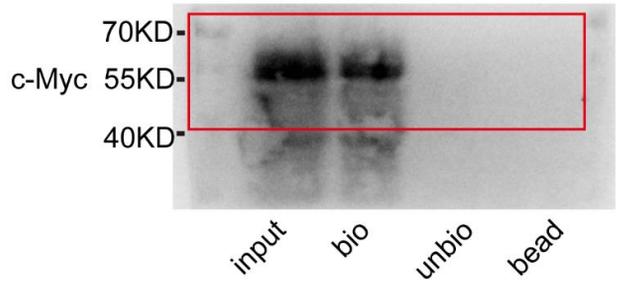


Entire unedited gel for representative cropped gels of Fig.6F,6I,6J,6K. Representative cropped gels are marked by red box.

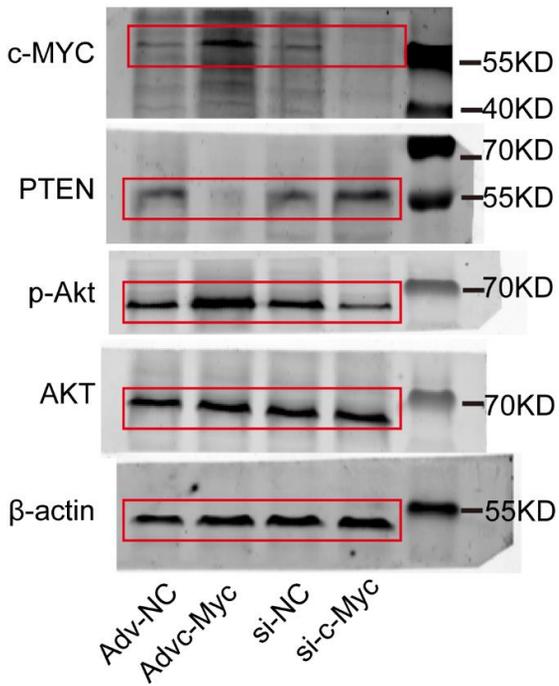
Full unedited gel for Figure 6M



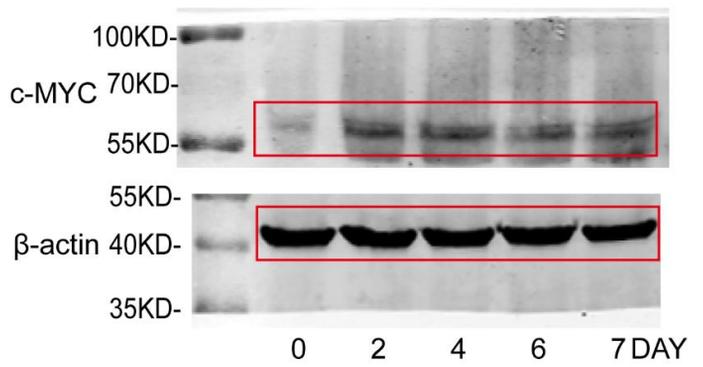
Full unedited gel for Figure 7B



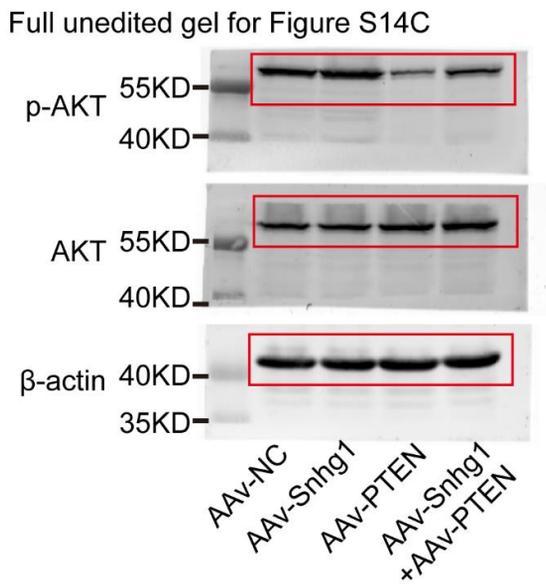
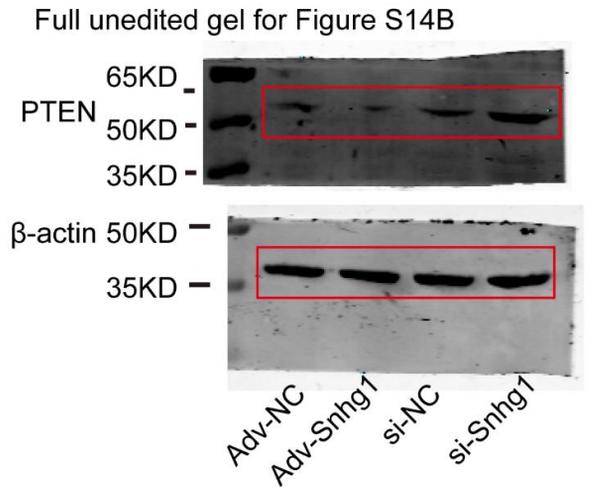
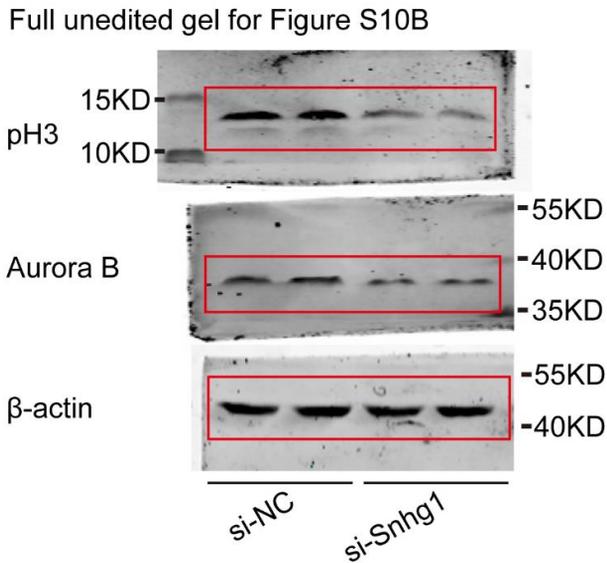
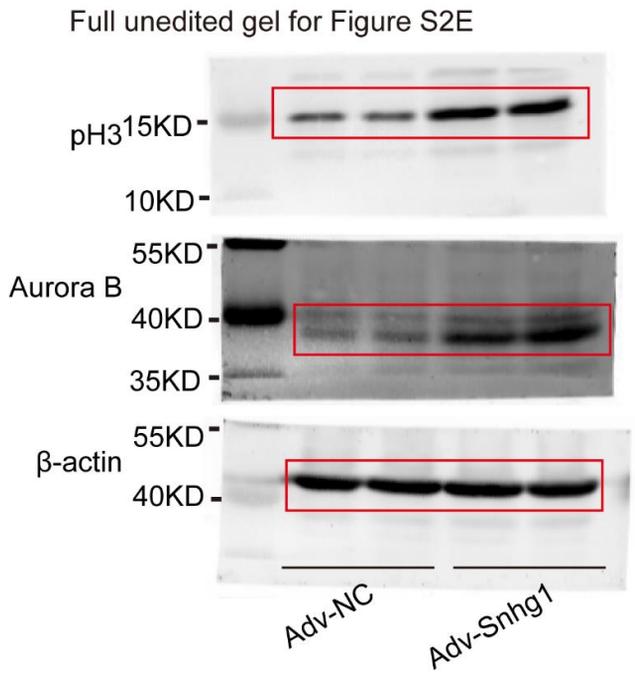
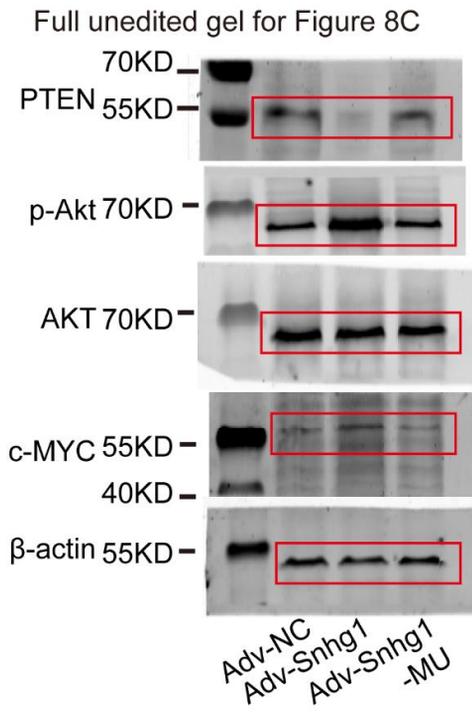
Full unedited gel for Figure 7G



Full unedited gel for Figure 7I

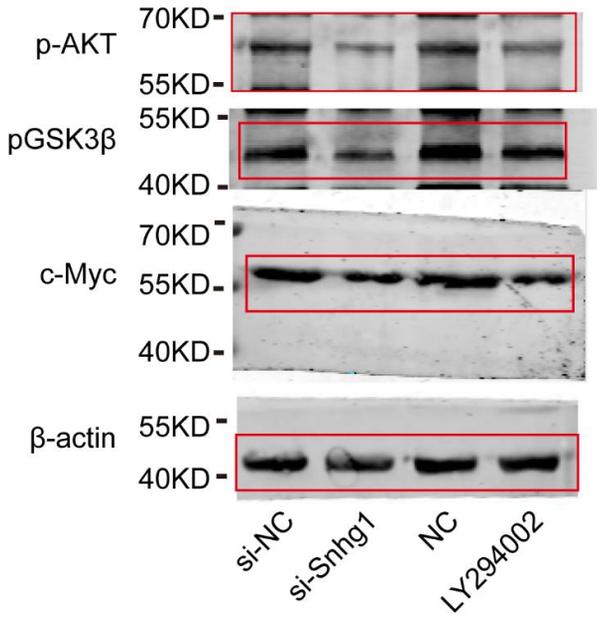


Entire unedited gel for representative cropped gels of Fig.6M,7B,7G,7I. Representative cropped gels are marked by red box.

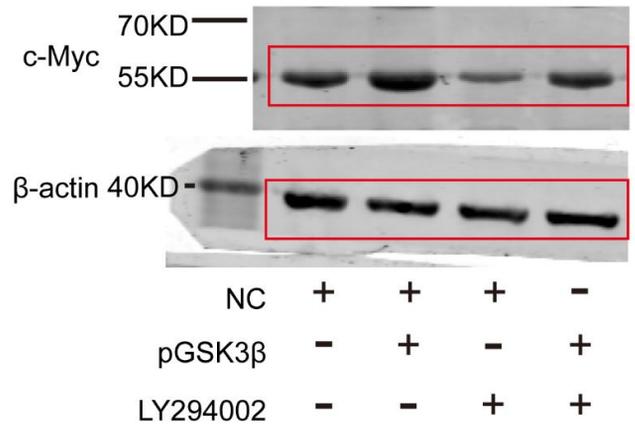


Entire unedited gel for representative cropped gels of Fig.8C,S2E,S10B,S14B,S14C.  
Representative cropped gels are marked by red box.

Full unedited gel for Figure S14F



Full unedited gel for Figure S14G



Entire unedited gel for representative cropped gels of Fig.S14F,S14G. Representative cropped gels are marked by red box.