Supplementary material and methods

Cell isolation and culture

Primary mouse and human lung fibroblast isolation were performed as previously described [1, 2]. Fresh lungs from 12-weeks old normal mice or normal human lung tissues were perfused, isolated under sterile conditions and excised into nearly 1-mm³ fragments, which were then digested in DMEM supplemented with 10 mg/ml dispase (Sigma-Aldrich, D4693) and 20 mg/ml Collagenase type I (Gibco, Thermo Fisher Scientific, 9001-12-1) at 37°C for 2 hours and then sequentially filtered through 100-μm, 40-μm and 15-μm pore filters to remove undigested tissues. Single cells were washed to remove the enzyme and maintained for 1 week in DMEM supplemented with 10% fetal bovine serum (FBS, Gibco) and penicillin-streptomycin. The cells were then cultured in a humidified atmosphere of 5% CO₂ at 37°C as described previously to allow fibroblasts to grow and become the dominant cell type [3, 4]. Outgrowing fibroblasts were reached confluence and passaged by trypsinization. Cultured fibroblasts were used for experiments at passages 3 to 6. Identification of fibroblasts was based on the expression of vimentin, collagen I, and α-SMA. Human fetal pulmonary fibroblasts (MRC5 cells) and HEK293T cells were purchased from American Type Culture Collection (ATCC).

Vectors and reagents
For loss of Nestin function, retrovirus vectors (pSM2) encoding Nestin shRNA were used as previously described [5]. Scramble shRNA served as a control. All shRNAs were constructed in our laboratory. Details on the plasmids are provided in Supplemental Table 3. ShRNA transfections were performed using the MegaTran 1.0 Transfection Reagent (OriGene) according to the manufacturer’s instructions. For Nestin overexpression, Full-length Nestin were cloned into pcDNA3.1-Myc vector (Invitrogen). Myc-Nestin vector was constructed using Invitrogen’s Gateway System. The pcDNA3.1-Myc served as the empty control vector. Flag-tagged TβRI, Nestin and Rab11 were constructed using Invitrogen’s Gateway System. TGF-β, CHX and Chloroquine (Chlq) were purchased from Sigma-Aldrich.

Generation of murine 3D-LTCs and AAV6 treatment

3D-LTCs from C57BL/6 mice and bleomycin-induced pulmonary fibrosis mice model were performed as previously described [6, 7]. The procedure was performed under sterile conditions. Control and bleomycin-induced pulmonary fibrosis mice were anaesthetised with isoflurane. After intubation and dissection of the diaphragm, lungs were flushed via the heart with sterile sodium chloride solution. Using a syringe pump, lungs were infiltrated with warm, low gelling temperature agarose (2%, A9414; Sigma; kept at 40°C) in sterile cultivation medium (DMEM/Ham’s F12; Gibco, supplemented with 100 U·mL⁻¹ penicillin, 100 μg·mL⁻¹ streptomycin and 2.5 μg·mL⁻¹ amphotericin B; Sigma). The
trachea was ligated with thread to retain the agarose inside the lung. The lung was excised, transferred into a tube with cultivation medium and cooled on ice for 10 min to allow gelling of the agarose. The lobes were separated and cut with a vibratome (VT1200s, Leica, Germany) to a thickness of 300 μm using a speed of 10-12 μm·s⁻¹, a frequency of 150 Hz and an amplitude of 1.2 mm. The 3D-LTCs were cultivated in medium supplemented with 0.1% fetal calf serum (FCS, Gibco). Individual 3D-LTCs were cultivated at 37°C in humidified conditions containing 5% (volume/ volume) CO₂ in 24-well plates under submerged conditions with changes of medium every other day. 3D-LTCs obtained from mice subjected to PBS or bleomycin were stimulated either with AAV6-Scramble or AAV6-ShNES (1×10¹²vg/slice) for 72h.

**Preparation of pulmospheres**

Lung biopsies (approximately 6 mm × 6 mm) from IPF patients or control subjects were perfused, isolated under sterile conditions and excised into nearly 1-mm³ fragments, washed with cold PBS solution. The tissue was then digested in DMEM supplemented with 20 mg/ml Collagenase type I (Gibco, Thermo Fisher Scientific, 9001-12-1) at 37°C for 1 hour. The tube containing tissue was then vigorously shaken for 1 min and the resulting suspension was filtered through a 100-μm strainer. The filtered cell suspension was centrifuged for 5 minutes at 300 g. The cell pellet was resuspended in DMEM and then seeded into HEMA-coated (5 mg/ml made in 95% ethanol) 96-well U-bottom
plates in complete culture medium consisting of DMEM supplemented with 10% fetal bovine serum (FBS, Gibco) and penicillin-streptomycin. The cells were cultured in a humidified atmosphere of 5% CO₂ at 37°C as described previously.

*Real-time quantitative PCR*

Total RNA was extracted from lung tissues or cells using the TRIzol reagent (Molecular Research Center, Inc.) following the manufacturer's protocol. Quantification was performed with a NanoDrop 8000 spectrophotometer and 1 µg of total RNA was used to reverse transcription with a RevertAid First Strand cDNA Synthesis Kit (Thermo Fisher Scientific, K1622). The obtained cDNAs were used as the template for real-time quantitative PCR (qPCR) reactions with the FastStart Essential DNA Green Master Mix (Roche, 06924204001). All samples were run in triplicate and the results were normalized to those obtained for the 18S rRNA or GAPDH. The primers designed and used for qPCR are described in Supplemental Table 4.

*Histopathological evaluation*

After sacrifice, mouse lung tissues were perfused with 4% paraformaldehyde and then subjected to paraffin embedding or saturation in 30% sucrose for 24 hours for frozen sections. Paraffin-embedded human IPF and mouse lung
tissue sections were exposed to H&E or Masson trichrome staining to analyze collagens and inflammation, respectively, in the lung tissue sections.

**Immunohistochemistry (IHC) and Immunofluorescent staining (IF)**

Paraffin-embedded human IPF and mouse lung tissue sections were subjected to immunostaining using an UltraSensitiveTM SP (Mouse/Rabbit) IHC Kit (MXB, KIT-9710). Following deparaffinization and antigen retrieval, the IPF lung tissue specimens were incubated overnight with corresponding antibodies listed in Supplemental Table 5. Signal amplification and detection were performed using a DAB system according to the manufacturer's instructions (MXB, MAX-001).

Immunofluorescent staining of human IPF and mouse lung tissue sections was performed using primary antibodies listed in Supplemental Table 5. Semi-quantitative analyses of Nestin staining were performed in mouse lungs using five non-overlapping tissue fields evaluated under ×20 magnification. Stained sections were imaged using a two-photon fluorescence microscopy (FVMPE-RS), a Zeiss 800 Laser Scanning Confocal Microscope, a Zeiss 880 Laser Scanning Confocal Microscope with Airyscan and Dragonfly CR-DFLY-202 2540 (Andor, UK). All mean fluorescence intensity of immunofluorescent staining results were analyzed with the Image J software (NIH).

*Lung Tissue Dissociations*
Single-cell suspensions of lung cells were prepared by mincing and enzymatic digestion. Briefly, lungs were rinsed in sterile phosphate-buffered saline (PBS) following removal of tracheas, and were finely minced with sterile scissors and incubated in 3 mg/ml collagenase type I (#17018029, ThermoFisher) (Diluted with HBSS, in supplement with 0.1% BSA) in a volume of 2 ml per lung for 60 minutes at 37°C in a shaking incubator. The resulting cell suspension was further filtered through a 40 µm sieve successively to avoid the cell aggregates, and washed twice in PBS supplemented with 1% FBS, 1 mM EDTA (#A100105, Sangon Biotech) by centrifugation (1,100 rpm, 5 minutes, room temperature). Then, prior to the next step, all the tissues were washed (a 5-min centrifugation at 1100rpm, room temperature) twice with FACS buffer, which was PBS in supplemented with 1% FBS, 1 mM EDTA (#A100105, Sangon Biotech).

Flow Cytometric Assay

Single-cell suspensions of lung cells where stained using corresponding antibodies listed in Supplemental Table 5, thereafter fixed with 4% PFA, permeabilized using PBS buffer (0.1% saponin in PBS with 0.1% BSA) for 90min at room temperature. Cells were then stained with secondary antibody conjugated with Alexa Flour 647 in the dark for 45min on ice. Cells were then washed twice with FACS buffer and run on CytoFLEX (Beckman Coulter, USA). Data were analyzed using the Flow Jo software (Tree Star Inc., Ashland, Oregon).
**Quantification of cell surface TβRI using FACS analysis**

The cells were seeded at 2\( \times 10^5 \) cells per well of six-well plates, harvested from plates and washed with FACS buffer (1% bovine serum albumin in PBS containing 0.5 mM EDTA) twice. Cells were stained with anti-mouse TβRI-APC antibody or rat IgG_{2A} APC isotype control and then incubated in the dark for 30 min (on ice). Cells were then washed twice with FACS buffer and run on CytoFLEX (Beckman Coulter, USA). Data were analyzed using the Flow Jo software (Tree Star Inc., Ashland, Oregon).

**Adeno-associated virus (AAV) delivery**

Two versions of adeno-associated virus vector serotype 6 expressing ShNES under the control of the human CMV promoter were purchased from the Hanbio Biotechnology Co., Ltd (Shanghai, China). C57BL/6 mice (8-week-old, male) were deeply anesthetized and intratracheally administered with 1.5×10^{12} viral genomes of a pseudotyped AAV6 GFP vector or 1.5×10^{12} viral genomes of a pseudotyped AAV6 Nestin vector.

**Cell fractionation**

Cytoplasmic and nuclear fractions were separated using a Nucleoprotein Extraction Kit (Sangon Biotech, C510001) according to the manufacturer’s instructions. The nuclear pellet was resuspended in 20 mM Hepes (pH 7.9), 1
mM EGTA, 1 mM EDTA, 0.4 M NaCl, 1 mM dithiothreitol, and 1 mM phenylmethylsulfonyl fluoride. Purity of the fractions was assessed by western blot analysis of the obtained fractions. β-actin and Lamin B were marker proteins for the cytosolic extract and the nuclear extract respectively.

**Immunoprecipitation and immunoblotting**

p-Smad2, Collagen I, Nestin, Smad2, β-Actin, Flag, TβRI, TβRII, Rab11, Rab4, Lamin B, p-Akt, Akt, Cav1 protein expression in lung tissue samples and cells were measured by immunoblotting performed as previously described [5]. Antibodies are listed in Supplemental Table 5. Immunoprecipitation was assessed in cells using a previously described method [5]. Bands from at least three independent blots were quantified using Image J software.

**Gene targeting by the CRISPR/Cas9 system**

The plasmids used for CRISPR/Cas9 KO in MRC5 was constructed in our previous study according to the established cloning protocol [8, 9], and T7E1 assay was also performed for sgRNA screening as previously described [5]. MRC5 Transfection Kit (Altogen Biosystems, Catalog #2175) was used for introducing the control (Cas9-GFP) and Nestin-knockout (G2-Cas9-GFP) plasmids into the MRC5 cells. Three days after transfection, MRC5 cells were single cell sorted using an Influx Cell Sorter (BD, USA) into a plastic flat-bottomed 96 well plate, and allowed to expand. Approximately 27.56% of these
clones survived and were initially screened for Nestin gene deletion by PCR amplicons from selected genomic region on a duplicated plate and subjected to 2% agarose gel electrophoresis. Clones with negative results were further validated for Nestin KO by a western blot and were then cultured and harvested for further experiments.

**Luciferase reporter assays**

The pGL3-SRE luciferase reporter (Cat. #11545ES03) and pGL3-ELK-1 luciferase reporter (Cat. #11567ES03) were purchased from Yeasen Biotechnology (Shanghai). The pGL3-FOXO luciferase reporter, pGL3-AP-1 luciferase reporter were generous gifts from Junchao Cai. The pGL3-(SBE)9-reporter and Renilla luciferase vectors were purchased from Promega and the assays were performed as described by the provided manual (Cat.# E1960). Briefly, cells were transiently co-transfected with the Renilla luciferase vector and the respective reporters. At 24 h post-transfection, the cells were starved for 20 h in culture medium with 1% FBS and then treated with TGF-β (5 ng/ml) or vehicle (0.1% BSA; 4 mM HCl) for 24 h. The luciferase activity was analyzed using a Dual Luciferase Assay System (Promega). The luciferase data of the cells transfected with pGL3 Basic vectors were calculated and normalized with respect to the Renilla luciferase activity. All transfection was carried out in triplicate. All data were plotted as mean values obtained from triplicate determinations, with SDs.
Biotinylation assay for plasma membrane TβRI, TβRI endocytosis and TβRI recycling

All cells were first treated with chloroquine (Chlq) (100μM) for 4h and then were performed the following experiments.

To quantitate plasma membrane TβRI, cells were placed on ice, washed twice with ice-cold PBS, incubated with 0.2 mM biotin (Thermo Fisher Scientific) in PBS at 4°C for 30 min, and washed twice with 0.1 M glycine. Biotinylated proteins were pulled down with streptavidin agarose (S1638, Sigma-Aldrich) and immunoblotted for plasma membrane TβRI.

To quantitate endogenous TβRI endocytosis, cells were moved to 4°C and labeled with cleavable biotin. Two washes were performed with ice-cold PBS, and then the cells were resuspended in pre-warmed culture medium and incubated at 37°C to allow for TβRI endocytosis. Thirty minutes later, the cells were then returned to 4°C and washed once with ice-cold PBS to stop membrane trafficking. To strip the remaining biotin from the cell surface, cells were treated twice with stripping buffer (50 mM glutathione, 75 mM NaCl, 10 mM EDTA, 1% BSA, 0.075 N NaOH) at 4°C for 15 min. Cell lysates were then subjected to streptavidin agarose pull down and immunoblotted for TβRI.

To quantitate TβRI recycling at 37°C, cells were moved to 4°C, labeled with cleavable biotin and incubated for 30 or 60 min in a 37°C incubator. Cells were treated without stripping buffer (50 mM glutathione, 75 mM NaCl, 10 mM EDTA,
1% BSA, 0.075 N NaOH) and harvested at the end of incubation or subjected to two additional washes at 4°C for 15 min with stripping buffer (50 mM glutathione, 75 mM NaCl, 10 mM EDTA, 1% BSA, 0.075 N NaOH) to ensure the complete de-biotinylation of recycled TβRI. Cell lysates were subjected to streptavidin agarose pull down and analyzed by immunoblotting. All immunoblotting results were analyzed with the Image J software (NIH). In the lysates of cell incubated for 60 min without glutathione treatment, the biotinylated TβRI included the internalized and recycled TβRI. While in the lysates of cells incubated for 60 min with glutathione treatment, all of the biotinylated TβRI was remained internalized TβRI. The recycling rate of TβRI after 60 min of incubation was calculated by the following formula: TβRI recycling rate = (internalized and recycled TβRI at 60 min – internalized TβRI at 60 min) / total internalized TβRI at 30 min × 100% [10, 11].

*Rab11 activity assay*

Rab11 activity was determined using a Rab11 activity assay kit (NewEast Biosciences, King of Prussia, PA) according to the manufacturer’s instructions. Cell lysates containing Rab11-GTP were incubated with anti-active Rab11 mouse monoclonal antibody (Catalog No. 26919). The bound active Rab11 was pulled down with protein A/G agarose (Catalog No. 30301) and detected by immunoblotting using anti-Rab11 rabbit polyclonal antibody (Catalog No. 21157).
**Hydroxyproline assay**

Frozen lung tissue samples from mice were incubated in 250 μl PBS, after which 250 μl of 12 N HCl was added into samples for pellet hydrolysis at 110°C were overnight. Samples were neutralized by the addition of 10 N NaOH. 100 μl of each sample was mixed with 400 μl oxidizing solution that contained 1.4% chloramine-T, 10% N-propanol, and 80% citrate-acetate buffer in PBS. Lung samples were then incubated for 20 minutes. Samples were finally incubated for 30 minutes at 65°C shortly after adding Ehrlich's solution (Sigma-Aldrich). The reaction absorbance was measured at 550 nm. Sample concentrations were determined from the standard curve generated using trans-4-hydroxy-l-proline (Sigma-Aldrich). A standard curve was generated using trans-4-hydroxy-l-proline (Sigma-Aldrich). Hydroxyproline levels were expressed as micrograms hydroxyproline per microgram of lung tissue samples.

**Public bulk and single-cell RNA-sequencing dataset acquisition**

The gene expression and cell type annotation of bulk and single-cell RNA-sequencing data from lungs of normal and IPF human (GSE124685, GSE132771), of control and bleomycin-treated mice (GSE110533, GSE132771) were downloaded from the Gene Expression Omnibus.

**scRNA-seq data processing**
DESeq2[12], Seurat (version 2.3.2) and Scanpy (1.4.4.post1[13, 14]) were used to perform the bulk and single-cell RNA-seq data processing following the standard procedure (mentioned at https://scanpy.tutorials.readthedocs.io/en/latest/pbm3k.html and https://satijalab.org/seurat/v3.1/pbm3k_tutorial.html).
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**Table S1. Clinicopathological variables and quantification for Nestin expression by staining scores in freshly IPF clinical samples.**

| Patient No. | Gender | Age(y) | Smoking status | FVC% pred | FVC absolute L | FEV1% pred | FEV1 absolute L | DLCO% pred | TLC% pred | Nestin staining score |
|-------------|--------|--------|----------------|-----------|----------------|------------|----------------|------------|-----------|---------------------|
| #1          | M      | 62     | Y              | 73.8      | 2.45           | 69.725     | 1.84           | 22.6       | 54.77     | 3                   |
| #2          | M      | 73     | N              | 54.22     | 1.72           | 64.71      | 1.57           | 42.5       | 46.4      | 6                   |
| #3          | F      | 31     | Y              | 45.87     | 1.43           | 45.65      | 1.24           | 33.6       | 62.9      | 9                   |
| #4          | M      | 60     | Y              | 62.33     | 2.32           | 70         | 2.05           | 76         | 61        | 4                   |
| #5          | M      | 51     | N              | 48.33     | 2.32           | 50         | 1.74           | 36.4       | 44.3      | 12                  |
| #6          | M      | 68     | N              | 65.63     | 1.81           | 79.77      | 1.72           | 49.7       | 59.4      | 1                   |
| #7          | M      | 68     | Y              | 49        | 1.71           | 53         | 1.44           | 29         | 42        | 8                   |
| #8          | M      | 69     | Y              | 90.33     | 2.42           | 98         | 2.06           | 66         | 74        | 2                   |
| #9          | M      | 59     | N              | 81.33     | 2.6            | 93         | 2.4            | 79         | 70        | 2                   |
| #10         | M      | 62     | N              | 84        | 3.08           | 91.3       | 2.63           | 82         | 75        | 1                   |
| #11         | M      | 62     | Y              | 83.33     | 3.09           | 91.48      | 2.52           | 60.84      | 72.36     | 2                   |
| #12         | M      | 52     | Y              | 54.94     | 2.11           | 61.95      | 1.93           | 29.8       | 52.5      | 16                  |
| #13         | M      | 65     | N              | 70.83     | 2.62           | 72.74      | 2.1            | 44.5       | 58.8      | 4                   |
| #14         | M      | 54     | N              | 80        | 3.64           | 85.6       | 3.09           | 73         | 77        | 6                   |
| #15         | M      | 51     | Y              | 62.29     | 2.28           | 65.54      | 1.96           | 41.8       | 58.6      | 16                  |
| #16         | M      | 65     | N              | 82.36     | 3.12           | 86.85      | 2.57           | 71         | 74.3      | 2                   |
| #17         | M      | 57     | Y              | 90.62     | 3.65           | 93.1       | 2.99           | 47.2       | 73.8      | 1                   |
| #18         | F      | 48     | N              | 77.37     | 2.09           | 78.99      | 1.82           | 49.78      | 74.28     | 4                   |
| #19         | M      | 56     | Y              | 100.89    | 3.8            | 106.55     | 3.22           | 47.18      | 73.37     | 1                   |
| #20         | M      | 66     | Y              | 58.28     | 1.98           | 66.3       | 1.76           | 38.74      | 53.86     | 2                   |
| #21         | F      | 66     | N              | 92.2      | 2.37           | 94.43      | 2.03           | 52.72      | 69.42     | 4                   |
| #22         | M      | 62     | Y              | 68.6      | 2.83           | 74         | 2.39           | 50.2       | 64.2      | 2                   |
| #23         | M      | 49     | Y              | 72.03     | 3.11           | 74.28      | 2.6            | 36.89      | 56.48     | 6                   |
| #24         | M      | 53     | Y              | 47.18     | 1.73           | 53.95      | 1.61           | 53.5       | 68.1      | 12                  |
| #25         | M      | 70     | N              | 49.27     | 1.66           | 51.16      | 1.32           | 27.12      | 43.62     | 12                  |
| #26         | M      | 60     | Y              | 104.63    | 4.25           | 87.06      | 2.79           | 42.3       | 76.6      | 4                   |
| #27         | F      | 49     | N              | 44.42     | 1.22           | 49.35      | 1.15           | 48.4       | 25.6      | 12                  |
| #28         | M      | 59     | Y              | 48.65     | 1.84           | 54.11      | 1.62           | 14.98      | 45.05     | 12                  |
| #29         | M      | 67     | Y              | 74.22     | 2.12           | 86.29      | 1.98           | 47.53      | 57.06     | 2                   |
| #30         | M      | 63     | Y              | 68.33     | 2.74           | 71.95      | 2.26           | 47.16      | 62.99     | 3                   |
| #31         | M      | 61     | Y              | 38.1      | 1.47           | 45.78      | 1.4            | 19.2       | 24.6      | 12                  |
| #32         | M      | 70     | Y              | 55        | 1.85           | 66         | 1.71           | 36         | 54        | 3                   |
| #33         | F      | 28     | N              | 67.5      | 2.33           | 72.3       | 2.17           | 51.15      | 75.13     | 1                   |
| #34         | M      | 66     | Y              | 104.34    | 3.78           | 111.66     | 3.15           | 84.9       | 86.4      | 1                   |
| #35         | F      | 61     | N              | 66.23     | 1.6            | 69.17      | 1.4            | 55.4       | 62.3      | 4                   |
IPF: idiopathic pulmonary fibrosis; % pred: % predicted; FVC: forced vital capacity; FEV1: forced expiratory volume in 1 s; DLCO: diffusing capacity of the lung for carbon monoxide; TLC: total lung capacity.
Table S2. Summary of clinicopathological characteristics of study subjects.

| Variable         | IPF       | Controls  |
|------------------|-----------|-----------|
| Subjects         | 35        | 13        |
| Male %           | 82.86     | 53.85     |
| Age years        | 58.94 ± 9.94 | 49.91 ± 8.30 |
| Ever-smoker %    | 60.00     | 15.38     |
| FVC% pred        | 69.04 ± 18.03 | 100.62 ± 12.79 |
| FVC absolute L   | 2.43 ± 0.77 | 3.43 ± 1.03  |
| FEV₁% pred       | 73.88 ± 17.59 | 94.79 ± 12.03 |
| FEV₁ absolute L  | 2.06 ± 0.57  | 2.76 ± 0.82  |
| DLCO% pred       | 48.23 ± 17.60 |           |
| TLC% pred        | 60.86 ± 14.44 |          |

Data are presented as mean ± SD. IPF: idiopathic pulmonary fibrosis; % pred: % predicted; FVC: forced vital capacity; FEV₁: forced expiratory volume in 1 s; DLCO: diffusing capacity of the lung for carbon monoxide; TLC: total lung capacity.
Table S3: Target sequences of shRNAs. Related to Experimental Procedures.

| Name                     | Sequences (5′ to 3′)                                      |
|--------------------------|---------------------------------------------------------|
| **Human:**               |                                                         |
| NESTIN shRNA#1           | 5′-GCTAGTCCCTGCTGAATA-3′                                  |
| NESTIN shRNA#2           | 5′-GCAGACATCATTTGCTTAAT-3′                                |
| **Mouse:**               |                                                         |
| NESTIN shRNA1            | 5′-GGAAGAAGTTCCAGGCTTCT-3′                                |
| NESTIN shRNA2            | 5′-GCTGAAGCTGCATTCTTGG-3′                                 |
| NESTIN shRNA (AAV-6)     | 5′-GTGAGACTGTGAAATGCAA-3′                                 |
| Scramble shRNA (AAV-6)   | 5′-TTCTCCGAACGTGTCACGTAA-3′                               |
Table S4: Primer used to amplify the human transcripts or genome DNA during PCR. Related to Experimental Procedures.

| Gene      | Sequences (5’ to 3’)                  | application |
|-----------|---------------------------------------|-------------|
| hNESTIN   | Forward: 5’-CTGCTACCCTTGAGACACCTG-3’  | qPCR        |
|           | Reverse: 5’-GGGCTCTGATCTCTGCATCTAC-3’|             |
| hGAPDH    | Forward: 5’-GTCGGAGTGCAACGGATTT-3’    | qPCR        |
|           | Reverse: 5’-GGAATCATATTGGAACATGTAACC-3’|             |
| mNESTIN   | Forward: 5’-GCAGGAGAGACAGGGTCTAC-3’   | qPCR        |
|           | Reverse: 5’-GGGGTCAGGAAAGCCAA-3’      |             |
| mActa2    | Forward: 5’-TGAGACCTTCAATGC CCCGC-3’  | qPCR        |
|           | Reverse: 5’-TCACACCATCTCCAGAGTCAGC-3’|             |
| mCollagen I | Forward: 5’-CCCAGAGTGGAACACGATT-3’  | qPCR        |
|           | Reverse: 5’-ATGAGGTCTTCGCTGGGAT-3’    |             |
| mTβRI     | Forward: 5’-TGGGACTTGCTGTGAGACAT-3’   | qPCR        |
|           | Reverse: 5’-ATGTCAGCGCGTTGGAAGGA-3’   |             |
| mTβRII    | Forward: 5’-AACGACTTGACCTGTTGCT-3’    | qPCR        |
|           | Reverse: 5’-TCCGTCTGCTTGAACGACTC-3’   |             |
| m18S      | Forward: 5’-GTGACGGTACATCCGTAAAGA-3’  | qPCR        |
|           | Reverse: 5’-GCCGGACTCATCGTACTCC-3’    |             |
Table S5: Primary and secondary antibodies.

| Product                          | Catalogue Number | Supplier                  |
|----------------------------------|------------------|---------------------------|
| **Primary antibody:**            |                  |                           |
| **WB:**                          |                  |                           |
| mouse anti-Nestin                | 611658           | BD Biosciences            |
| mouse anti-Nestin                | MAB353           | Millipore                 |
| rabbit anti-DYKDDDDK (Flag Tag)  | 14793            | Cell Signaling Technology |
| mouse anti-DYKDDDDK (Flag Tag)   | 8146             | Cell Signaling Technology |
| mouse Anti-β-actin               | 600081           | Proteintech               |
| rabbit anti-Collagen I           | ab34710          | Abcam                     |
| rabbit anti-TβRI                 | ab31013          | Abcam                     |
| rabbit anti-TβRII                | ab186838         | Abcam                     |
| rabbit Anti-p-Smad2              | 18338            | Cell Signaling Technology |
| rabbit Anti-Smad2                | 5339             | Cell Signaling Technology |
| mouse anti-α-SMA                 | ab7817           | Abcam                     |
| rabbit anti-Rab4                 | ab109009         | Abcam                     |
| rabbit anti-Rab11                | 5589             | Cell Signaling Technology |
| mouse anti-Rab11                 | 610656           | BD Biosciences            |
| rabbit anti-Lamin B              | 12586            | Cell Signaling Technology |
| rabbit anti-Caveolin-1           | ab2910           | Abcam                     |
| rabbit anti-Phospho-Akt          | 4060             | Cell Signaling Technology |
| rabbit anti-Akt                  | 4691             | Cell Signaling Technology |
**IP:**

- mouse anti-DYKDDDDK (Flag Tag) 8146  Cell Signaling Technology
- rabbit anti-TβRI ab31013  Abcam
- rabbit anti-Rab11 5589  Cell Signaling Technology

**IF:**

- rabbit anti-Nestin ABD69  Millipore
- mouse anti-Nestin MAB5326  Millipore
- mouse anti-Nestin MAB353  Millipore
- rabbit anti-CD31 ab28364  Abcam
- rabbit anti-CD31 ab76533  Abcam
- mouse anti-α-SMA ab7817  Abcam
- rabbit anti-NG2 AB5320  Millipore
- rabbit anti-NG2 ab183929  Abcam
- rabbit anti-Prosurfactant Protein C AB3786  Millipore
- rabbit anti-Rab11 5589  Cell Signaling Technology
- mouse-anti-Rab11 05-853  Millipore
- mouse anti-Aquaporin 5 sc-514022  Santa Cruz Biotechnology
- rabbit anti-Aquaporin 5 ab92320  Abcam
- mouse anti-TβRI sc-518086  santa crauz
- rabbit anti-Rab4 ab109009  Abcam
- rabbit anti-Calponin 1 ab46794  Abcam
- rabbit Anti-p-Smad2 18338  Cell Signaling Technology
rat Anti-LAMP1  MABC39  Millipore
rat Anti-LAMP2  428019  Millipore
rabbit anti-β-catenin  ab16051  Abcam

IHC:
rabbit anti-Nestin  ABD69  Millipore
mouse anti-Nestin  MAB353  Millipore
mouse anti-α-SMA  ab7817  Abcam
mouse anti mouse IgG1  5415  Cell Signaling Technology
mouse anti mouse IgG2a  61656  Cell Signaling Technology
mouse anti human IgG2a  ab200699  Abcam
rabbit anti human IgG  ab195574  Abcam

Flow:
Mouse TGF-β RI/ALK-5 APC-conjugated Antibody  FAB5871A-100  R&D Systems
Rat IgG2A Allophycocyanin Isotype  IC006A  R&D Systems

Secondary antibody:

WB:
anti-mouse IgG HRP-linked Ab  7076  Cell Signaling Technology
anti-rabbit IgG HRP-linked Ab  7074  Cell Signaling Technology
| Antibody                        | Code   | Manufacturer |
|--------------------------------|--------|--------------|
| goat anti-mouse IgG Alexa 488  | A11001 | Invitrogen   |
| goat anti-rabbit IgG Alexa 488 | A11008 | Invitrogen   |
| goat anti-rabbit IgG Alexa 555 | A21428 | Invitrogen   |
| goat anti-mouse IgG Alexa 555  | A21422 | Invitrogen   |
| goat anti-rat IgG Alexa 488    | A11006 | Invitrogen   |
| goat anti-rat IgG Alexa 555    | A21434 | Invitrogen   |