# Supplementary Material

## Cancer-associated fibroblast-derived periostin promotes papillary thyroid tumor growth through integrin-FAK-STAT3 signaling

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### Supplementary methods

#### Western blot

Total proteins were collected from tumor tissues or cells using RIPA lysis buffer, augmented with protease and phosphatase inhibitors (Roche). All samples were subjected to electrophoresis on 8%-14% SDS-PAGE gels and transferred onto PVDF membranes (Millipore). Membranes were blocked in 5% BSA at room temperature for 2 hours and incubated with primary antibodies at 4°C overnight. Subsequently, the membranes were washed with TBST for 30 minutes and incubated with HRP-conjugated secondary antibody at room temperature for 1 hour. After washing again, proteins were visualized through X-ray films. The primary antibodies used are listed in Table S1. The primary antibodies against: GAPDH (Cell Signaling Technology, 92310, 1:5000), POSTN (Adipogen, AG-20B-0033, 1:3000), Cyclin D1 (Cell Signaling Technology, 2978, 1:2000), β-actin (Cell Signaling Technology, 8457, 1:5000), IL-4 (Abcam, ab62351, 1:2000), p-FAK (Cell Signaling Technology, 3281, 1:2000), FAK (Abcam, ab40794, 1:2000), p-STAT3 (Cell Signaling Technology, 9145, 1:2000), STAT3 (Cell Signaling Technology, 9139, 1:2000), p-ERK1/2 (Cell Signaling Technology, 9101, 1:5000), ERK1/2 (Cell Signaling Technology, 9102, 1:5000), p-STAT6 (Abcam, ab188080, 1:2000) and STAT6 (Abclonal, A19120, 1:2000) were used.

#### qRT-PCR analysis

Total RNA was extracted from tumor cells or tissue samples using TriPure Isolation

Reagent (Roche) following the manufacturer's instructions. RNA was reverse transcribed into cDNA by using the ReverTra Ace qPCR RT kit (TOYOBO, FSQ-101) according to the manufacturer's instructions. qRT-PCR analysis was conducted on CFX Connect Real-Time PCR machine (Bio-Rad) using the BrightCycle Universal SYBR Green qPCR Mix with UDG (Abclonal, RK21219) in line with kit protocol. mRNA expression of target genes was normalized by the expression of *GAPDH*. Primer sequences are listed in the Table S5.

**Generation of stable cell lines for POSTN overexpression**

To generate stable cell lines for POSTN overexpression, the pCDH-CMV-POSTN plasmids were transfected along with lentiviral packaging plasmids (pCMV-VSVG, pRSV-REV and pMDL) into HEK 293T cells with polyethyleneimine to produce viruses. 48 hours after transfection, the viral supernatants were collected and filtered with 0.22 μm filters. Lentiviruses were concentrated by ultracentrifugation. Target cells were infected with the lentiviruses, and selected with puromycin to generate stable cell lines. After puromycin selection, the cell lines of POSTN overexpression were validated by western blot analysis.

**ELISA assay**

Cytokines in the orthotopic mouse tumor tissues and cells were detected with mouse POSTN ELISA kits (R&D systems, AF2955-SP) or human IL-4 ELISA kit (Abclonal, RK00003) according to the manufacturer's instructions.

**Multiplex immunofluorescence staining**

Multiplex immunofluorescence staining assay was performed on human papillary thyroid tumors to explore the distributions of POSTN and IL-4 expression using the TSA Fluorescence Triple Staining Kit (Abclonal, RK05903) according to the manufacturer's instructions. Antibodies against POSTN (Adipoge, AG-20B-0033, 1:400), EpCAM (Abcam, ab213500, 1:200) and IL-4 (ABclonal, A5649, 1:200) were used.

## Supplementary figures

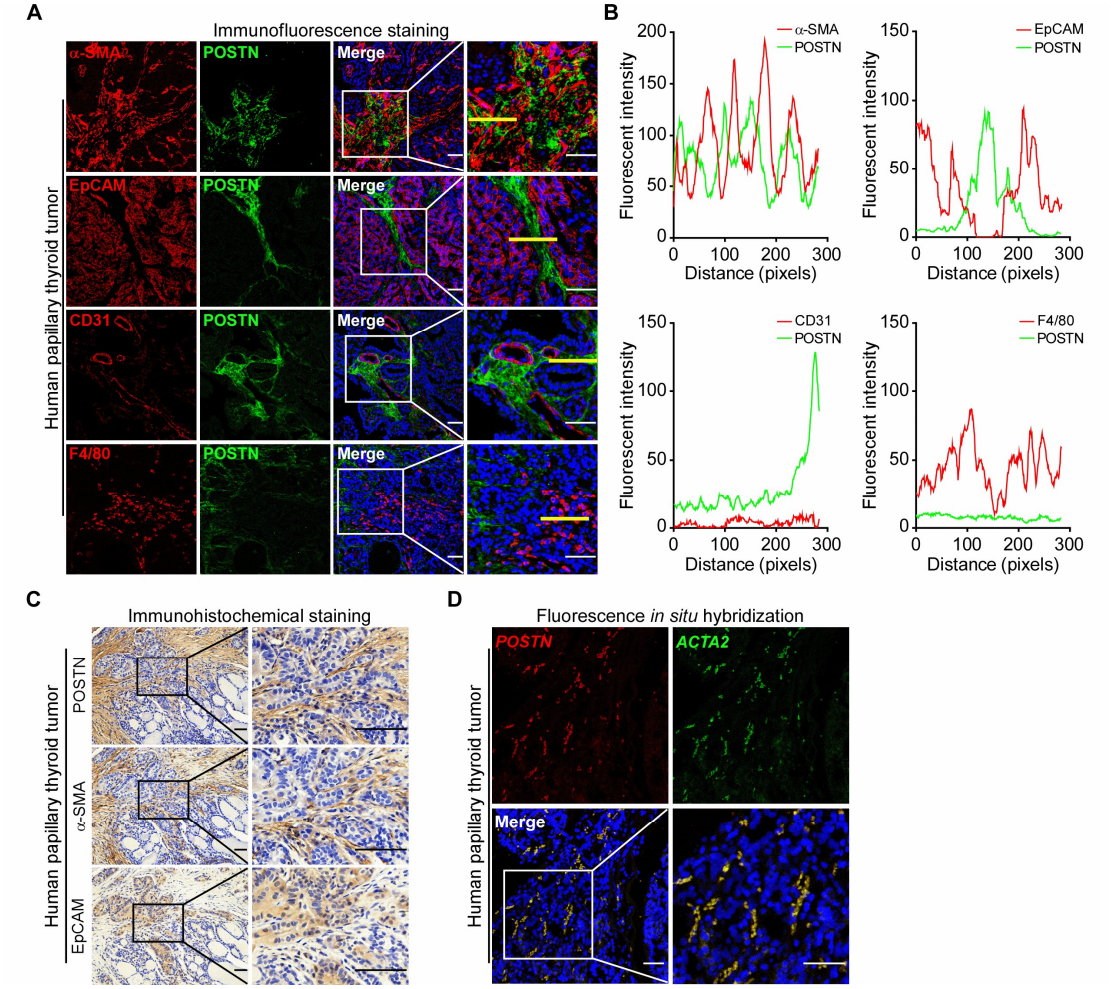


**Figure S1. POSTN is mainly derived from CAFs in human papillary thyroid tumors.** (**A**) Representative immunofluorescence co-staining of POSTN with α-SMA, EpCAM, CD31 or F4/80 in human papillary thyroid tumors. Nuclei were counterstained with DAPI. Scale bars, 50 μm. (**B**) Plots of fluorescent intensity along the yellow line of images in (**A**). (**C**) Representative immunohistochemical staining of POSTN, α-SMA and EpCAM in the human papillary thyroid tumors. Scale bars, 50 μm. (**D**) Fluorescence *in situ* hybridization of *POSTN* and *ACTA2* in human papillary thyroid tumors. Nuclei were counterstained with DAPI. Scale bars, 50 μm.

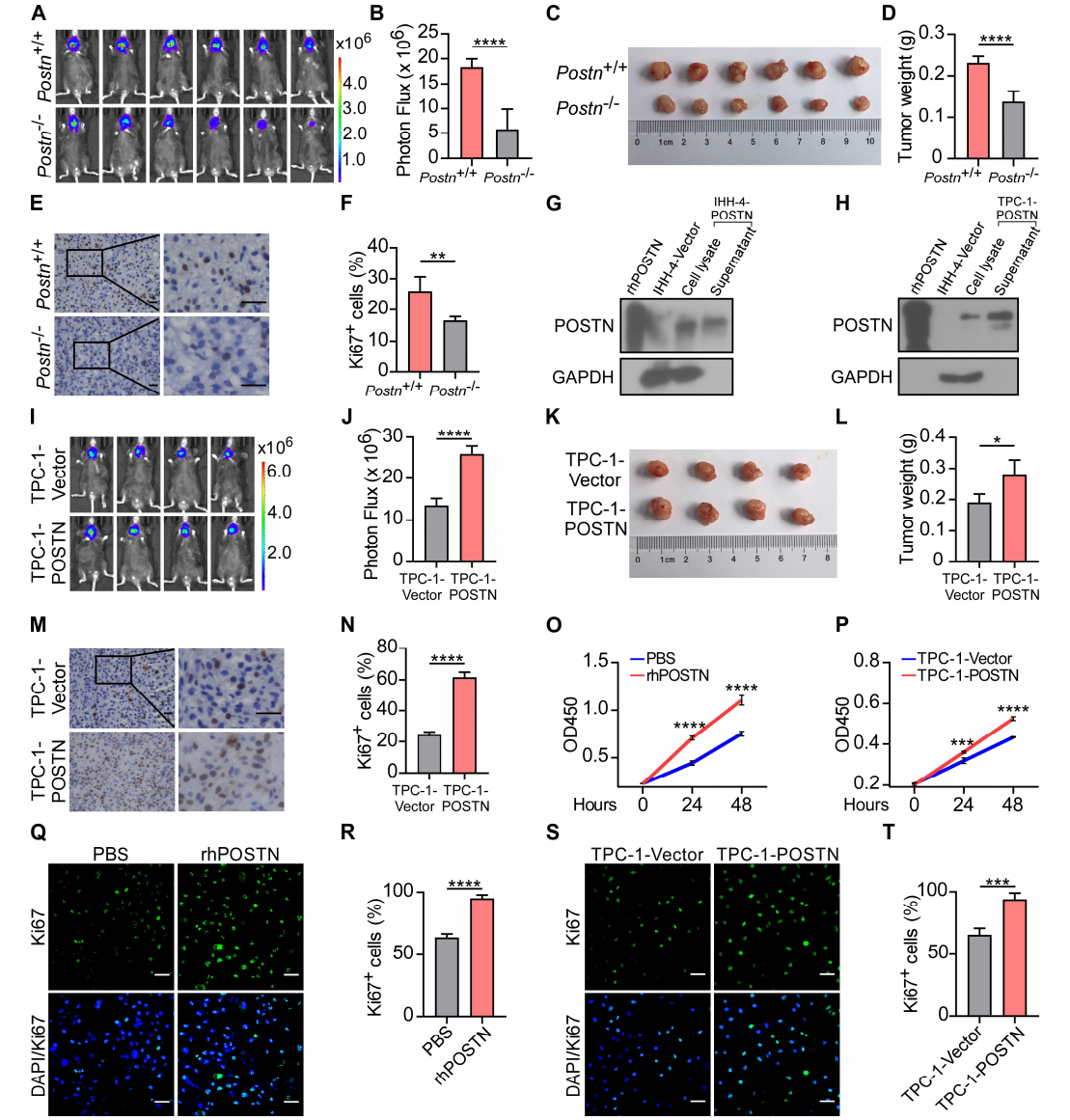


**Figure S2. POSTN deficiency inhibits tumor growth *in vivo*, whereas POSTN promotes TPC-1 cell growth *in vivo* and *in vitro*.** (**A** and **B**) Bioluminescence imaging (**A**) and quantification of tumor burden (**B**) in *Postn*$^{+/+}$*Rag1*$^{-/-}$ (*Postn*$^{+/+}$) and *Postn*$^{-/-}$*Rag1*$^{-/-}$ (*Postn*$^{-/-}$) mice after orthotopic injection with TPC-1 cells (n = 6). (**C** and **D**) Images (**C**) and quantification of weights (**D**) of isolated orthotopic tumors from mice in each group in (**A**) (n = 6). (**E** and **F**) Immunohistochemical staining for Ki67 (**E**) and quantitative analysis of Ki67$^{+}$ cells (**F**) in tumor tissues from each group in (**A**). Scale bars, 25 μm (n = 4). (**G** and **H**) Western blot analysis of POSTN protein level in total cell lysates and culture supernatants of IHH-4 (**G**) or TPC-1 (**H**) cells with either vector or POSTN stably expressed. rhPOSTN was used as a positive control. (**I** and **J**) Bioluminescence imaging (**I**) and quantification of tumor burden (**J**)

in *Postn*$^{-/-}$*Rag1*$^{-/-}$ mice after orthotopic injection with TPC-1 cells transfected with POSTN or control vector (n = 4). (**K** and **L**) Images (**K**) and quantification of weights (**L**) of isolated orthotopic tumors from mice in each group in (**I**) (n = 4). (**M** and **N**) Immunohistochemical staining for Ki67 (**M**) and quantitative analysis of Ki67$^{+}$ cells (**N**) in tumor tissues from mice in each group in (**I**). Scale bars, 25 μm (n = 6). (**O**) Analysis of the proliferation of TPC-1 cells treated with or without rhPOSTN for the indicated time by CCK8 assay (n = 3). (**P**) Analysis of the proliferation of TPC-1 cells transfected with POSTN or control vector (n = 6). (**Q** and **R**) Representative images of the immunofluorescence staining of Ki67 (**Q**) and quantification of Ki67$^{+}$ cells (**R**) in TPC-1 cells treated with or without rhPOSTN. Scale bars, 50 μm (n = 5). (**S** and **T**) Representative images of the immunofluorescence staining of Ki67 (**S**) and quantification of Ki67$^{+}$ cells (**T**) in TPC-1 cells transfected with POSTN or control vector. Scale bars, 50 μm (n = 4). Data are shown as means ± SEM. Student's t test (B, D, F, J, L, N, R and T). Two-way ANOVA (O and P). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

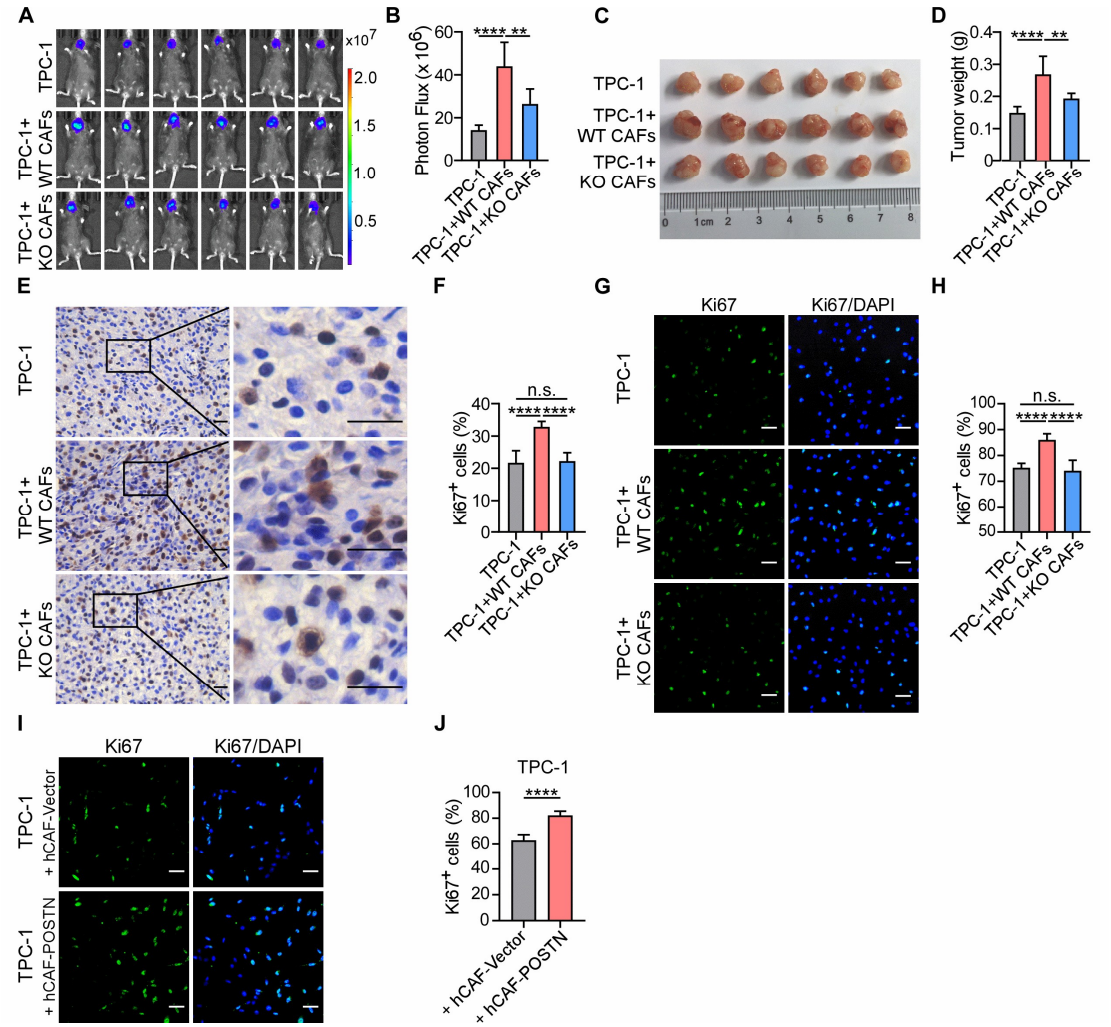


**Figure S3. Deficiency of POSTN in CAFs impairs CAF-promoted papillary thyroid tumor growth *in vivo* and TPC-1 cell proliferation *in vitro*.** (**A** and **B**) Bioluminescence imaging (**A**) and quantification of tumor burden (**B**) of *Postn*$^{-/-}$*Rag1*$^{-/-}$ mice after orthotopic injection with TPC-1 cells alone or together with WT or KO CAFs (n = 6). (**C** and **D**) Images (**C**) and quantification of weights (**D**) of isolated orthotopic tumors from mice in each group in (**A**) (n = 6). (**E** and **F**) Immunohistochemical staining for Ki67 (**E**) and quantitation of Ki67$^{+}$ cells (**F**) in tumor tissues from each group in (**A**). Scale bars, 25 μm (n = 3). (**G** and **H**) Immunofluorescence images (**G**) and quantitation (**H**) of Ki67$^{+}$ TPC-1 cells cultured alone or co-cultured with WT or KO CAFs (n = 6). (**I** and **J**) Immunofluorescence images (**I**) and quantitation (**J**) of Ki67$^{+}$ TPC-1 cells co-cultured with the vector control or POSTN-overexpressing human CAFs (n = 5). Data are shown as means ± SEM. One-way ANOVA (B, D, F and H). Student's t test (J). **, $P < 0.01$; ****, $P < 0.0001$; n.s., no significant difference.

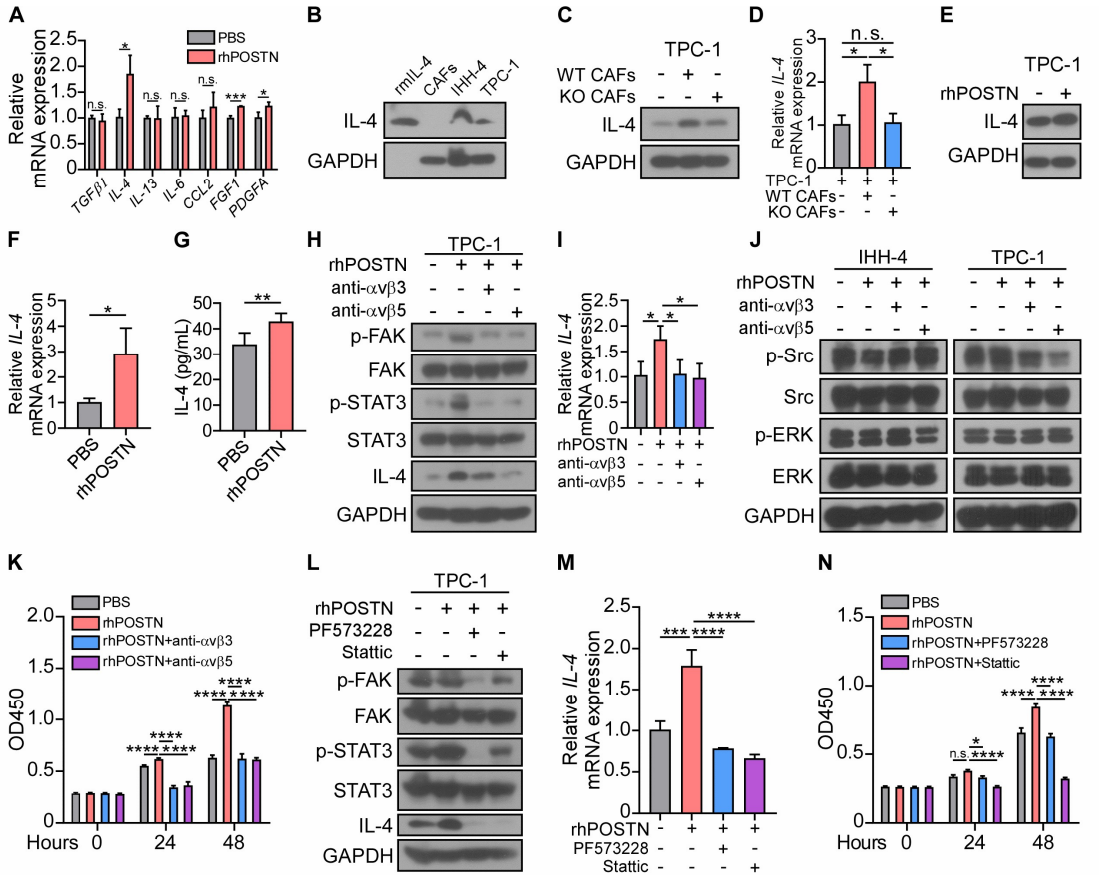


**Figure S4. POSTN promotes the proliferation and IL-4 expression in TPC-1 cells by activating the integrin-FAK-STAT3 signaling.** (**A**) qRT-PCR analysis of the indicated genes in IHH-4 cells treated with or without rhPOSTN (n = 3). (**B**) Western blot analysis of IL-4 in CAFs, IHH-4 and TPC-1 cells. rmIL-4 was used as a positive control. (**C** and **D**) Western blot (**C**) and qRT-PCR (**D**) analyses of IL-4 expression in TPC-1 cells cultured alone or co-cultured indirectly with WT or KO CAFs (n = 3). (**E-G**) Western blot (**E**), qRT-PCR (**F**) and ELISA (**G**) analyses of IL-4 expression in TPC-1 cells treated with or without rhPOSTN (n = 3). (**H** and **I**) Western blot (**H**) and qRT-PCR (**I**) analyses of the indicated proteins and genes in TPC-1 cells treated with rhPOSTN in combination with antibodies against integrins αvβ3 or αvβ5 (n = 3). (**J**) Western blot analyses of the indicated proteins in IHH-4 or TPC-1 cells treated with rhPOSTN in combination with antibodies against integrins αvβ3 or αvβ5. (**K**) Analysis of the proliferation of TPC-1 cells treated as in (**H**) for the indicated time by CCK8 assay (n = 5). (**L** and **M**) Western blot (**L**) and qRT-PCR (**M**) analyses of the indicated proteins and genes in TPC-1 cells treated with rhPOSTN alone or together with the indicated inhibitors (n = 3). (**N**)

Analysis of the proliferation of TPC-1 cells treated as in (**L**) for the indicated time by CCK8 assay (n = 4). Data are shown as means ± SEM. Two-way ANOVA (A, K and N). One-way ANOVA (D, I and M). Student's t test (F and G). *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$; n.s., no significant difference.

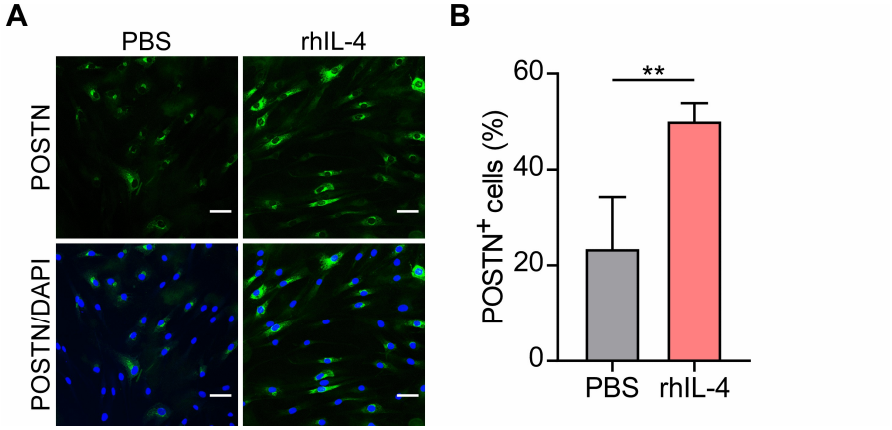


**Figure S5. IL-4 promotes POSTN expression in isolated CAFs of human papillary thyroid tumors.** (**A** and **B**) Immunofluorescence images (**A**) and quantitation (**B**) of $POSTN^+$ cells in isolated CAFs of human papillary thyroid treated with or without rhIL-4. Scale bars, 50 μm (Student's t test, n = 4). Data are shown as means ± SEM. **, $P < 0.01$.

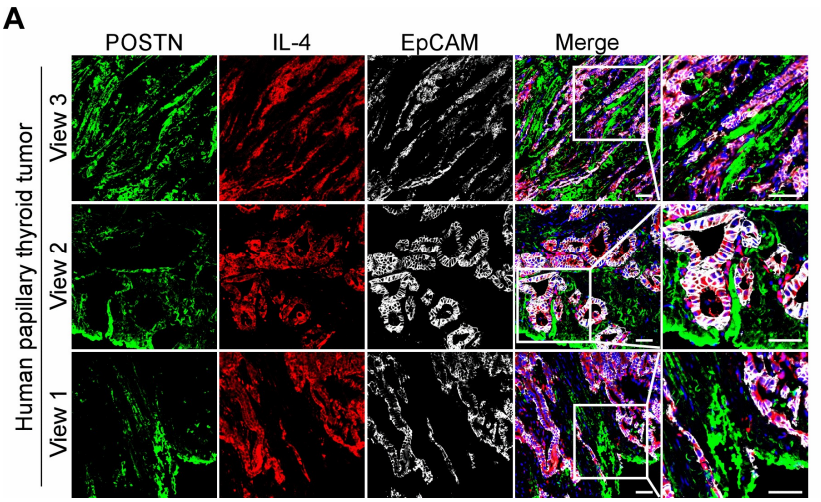


**Figure S6. The correlation between POSTN and IL-4 expression in human papillary thyroid tumors.** (**A**) Representative images of POSTN, IL-4 and EpCAM in clinical papillary thyroid tumors by multiplex immunofluorescence staining. Scale bars, 50 μm.

## Supplementary tables

### Table S1. Reagents or Resources used in the experiments

| Antibodies | Source | Identifier |
|---|---|---|
| Mouse monoclonal anti-Periostin | Adipogen | Cat# AG-20B-0033; RRID: AB_2490231 |
| Rabbit polyclonal anti-α-SMA | Cell Signaling Technology | Cat# 19245; RRID: AB_2734735 |
| Rabbit monoclonal anti-EpCAM | Abcam | Cat# ab213500; RRID: AB_2884975 |
| Rabbit polyclonal anti-Ki67 | Abcam | Cat# ab15580; RRID: AB_443209 |
| Rabbit monoclonal anti-IL-4 | Abcam | Cat# ab300138; RRID: N/A |
| Rabbit monoclonal anti-Phospho-STAT3 | Cell Signaling Technology | Cat# 9145; RRID: AB_2491009 |
| Rabbit monoclonal anti-GAPDH | Cell Signaling Technology | Cat# 92310; RRID: N/A |
| Rabbit monoclonal anti-Cyclin D1 | Cell Signaling Technology | Cat# 2978; RRID: AB_2259616 |
| Rabbit monoclonal anti-β-actin | Cell Signaling Technology | Cat# 8457; RRID: AB_10950489 |
| Rabbit monoclonal anti-IL-4 | Abcam | Cat# ab62351; RRID: N/A |
| Rabbit polyclonal anti-IL-4 | ABclonal | Cat# A5649; RRID: AB_2766409 |
| Rabbit polyclonal anti-Phospho-FAK | Cell Signaling Technology | Cat# 3283; RRID: AB_2173659 |
| Rabbit monoclonal anti-FAK | Abcam | Cat# ab40794; RRID: AB_732300 |
| Mouse monoclonal anti-STAT3 | Cell Signaling Technology | Cat# 9139; RRID: AB_331757 |
| Rabbit monoclonal anti-Phospho-ERK1/2 | Cell Signaling Technology | Cat# 9101; RRID: AB_331646 |
| Rabbit monoclonal anti-ERK1/2 | Cell Signaling Technology | Cat# 9102; RRID: AB_330744 |
| Rabbit monoclonal anti-Phospho-SRC | Cell Signaling Technology | Cat# 2101; RRID: AB_331697 |
| Rabbit monoclonal anti-SRC | Cell Signaling Technology | Cat# 2109; RRID: AB_2106059 |
| Rabbit monoclonal anti-Phospho-STAT6 | Abcam | Cat# ab188080; RRID: AB_2862613 |
| Rabbit monoclonal anti-STAT6 | ABclonal | Cat# A19120; RRID: N/A |
| Rabbit monoclonal anti-CD31 | Abcam | Cat# ab76533; RRID: AB_1523298 |
| Anti-Integrin αvβ3 antibody | Millipore/Merck | Cat# MAB1876-Z; RRID: AB_569486 |
| Anti-Integrin αvβ5 antibody | Millipore/Merck | Cat# MAB1961; RRID: AB_94466 |
| Rabbit monoclonal anti-F4/80 | Abcam | Cat# ab300421; RRID: AB_2936298 |
| Rabbit monoclonal anti-IL-4R | Abcam | Cat# ab271041; RRID: N/A |
| Rabbit monoclonal anti-Phospho-STAT6 | Abcam | Cat# ab235591; RRID: N/A |
| Rabbit IgG antibody | Sigma-Aldrich | Cat# I5006; RRID: AB_1163659 |
| **Chemicals, peptides, and recombinant proteins** | | |
| FAK inhibitor PF573228 | Selleck Chemicals | Cat# S2013 |
| STAT3 inhibitor Stattic | Selleck Chemicals | Cat# S7024 |
| Recombinant Human Periostin Protein | R&D Systems | Cat# 3548-F2-050 |
| Recombinant Mouse IL-4 Protein | ABclonal | Cat# RP01161 |
| Recombinant Human IL-4 Protein | ABclonal | Cat# RP00995 |

| | | |
|---|---|---|
| AS1517499 | MedChemExpress | Cat# HY-100614 |
| Collagenase II | Gibco | Cat# 17101015 |
| DNase I | Roche | Cat# 04716728001 |
| **Critical Commercial Assays** | | |
| Mouse Periostin/OSF-2 DuoSet ELISA | R&D | Cat# DY2955 |
| Human IL-4 ELISA Kit | ABclonal | Cat# RK00003 |
| Cell Counting Kit-8 | Beyotime | Cat# C0038 |
| TriPure Isolation Reagent | Roche | Cat# 11667165001 |
| ReverTra Ace qPCR RT kit | TOYOBO | Cat# FSQ-101 |
| BrightCycle Universal SYBR Green qPCR Mix with UDG | Abclonal | Cat# RK21219 |
| **Experimental Models: Organisms** | | |
| Mouse: B6.129-*Postn*$^{tm1Jmol}$/J | The Jackson Laboratory | JAX: 009067 |
| Mouse: C57BL/6 | The Animal Laboratory Center of Xiamen University | N/A |
| Mouse: B6.129S7-*Rag1*$^{tm1Mom}$/J | The Jackson Laboratory | JAX: 002216 |

**Table S2. Primer sequences for FISH**

| **FISH probe** | Sequence (5'-3') |
|---|---|
| FAM-*Postn* Mouse | GCGTTGGTCCATGCTCAGAGTGTC |
| TAMRA-*Acta2* Mouse | AGAGCTACGAACTGCCTGAC |
| TAMRA-*POSTN* Human | GCTACCACGAACAAACTTAATTTGGATG |
| FAM-*ACTA2* Human | GAGACAGCACCGCCTGGATAGCC |

**Table S3. Primers of plasmids for luciferase reporting assay**

| **Gene** | Vector | Primer sequence 5' | Primer sequence 3' |
|---|---|---|---|
| *Postn* promotor Mouse | pGL3-basic | TCTTACGCGTGCTAGCCCT CATCAATAAACATTCTAAC | GATCGCAGATCTCGAGAGC AGACTGCTCTGAGAACT |
| *Stat6* Mouse | pCMV5 | CCGGAATTCATGTCTCTGT GGGGCCTAATTTC | CTAGTCTAGATCACCAGC TGGGGTTGGTC |
| *Postn* promotor Mut1 Mouse | pGL3-basic | CCCGGGCTCGAGTTCCAAC TGTAAAACAGCCACAGT | CGCAGATCTCGAGAGCAGA CTGCTCTGAGAACTCAGA |
| *Postn* promotor Mut2 Mouse | pGL3-basic | CTAGCCCGGGCTCGAGAC CCTGCAAATGCCAACAGT | CGCAGATCTCGAGAGCAGA CTGCTCTGAGAACTCAGA |

**Table S4. Primers of *Postn* promoter segments for ChIP-qPCR assay**

| **Promoter region** | | Sequence (5'-3') |
|---|---|---|
| *Postn* (-1643~-1439bp) | Forward | CCTCATCAATAAACATTCTAACAGCC |

| | | |
|---|---|---|
| | Reverse | TCTCTTATTTATATTTATCTAAATGAAA |
| *Postn* (-1417~-1164bp) | Forward | GCCCTGGAAGGAACATAGTTTACT |
| | Reverse | TTAGTCCAGAGAGAATTCCTTATCTGG |
| *Postn* (-1144~-892bp) | Forward | TTCCAACTGTAAAACAGCCACAGT |
| | Reverse | GGTCTTCACACATGACCAGATATGA |
| *Postn* (-867~-617bp) | Forward | AAACAAAAAAAACAAAGTGTCTTCCC |
| | Reverse | ATCTATCTATCTATCTATCTATCTTTC |
| *Postn* (-563~-272bp) | Forward | AGATCTTTGTTACTTAACCCTCTATAC |
| | Reverse | ATTCTGAGTTTAGGGATCTCTCTGCC |
| *Postn* (-133~+123bp) | Forward | AGGCCATCGCAAGCTTCAG |
| | Reverse | CCTGATCCCGACCCCTGA |
| *Postn* (+157~+444bp) | Forward | CACTTAGCTTGTTCTAGAAATATGCGT |
| | Reverse | AAAATGCCAAGTAAACTGAATTAGTAA |
| *Postn* (+517~+750bp) | Forward | GTTTTAAGATGTCCTGCAAGATATCAG |
| | Reverse | CTGCTCTGAGAACTCAGAAAAATGA |

**Table S5. Sequences of primers for qRT-PCR analysis**

| **Gene** | | Sequence (5'-3') |
|---|---|---|
| *POSTN* | Forward | GCACTCTGGGCATCGTGGGA |
| Human | Reverse | AATCCAAGTTGTCCCAAGCC |
| *GADPH* | Forward | AGGTCGGAGTCAACGGATTTG |
| Human | Reverse | GGGGTCATTGATGGCAACA |
| *IL-4* | Forward | CGGCAACTTTGTCCACGGA |
| Human | Reverse | TCTGTTACGGTCAACTCGGTG |
| *TGFβ1* | Forward | CGCCGAGCCCTGGACACCAACT |
| Human | Reverse | TGCGTGTCCAGGCTCCAAATGTAGG |
| *IL-13* | Forward | CCTCATGGCGCTTTTGTTGAC |
| Human | Reverse | TCTGGTTCTGGGTGATGTTGA |
| *IL-6* | Forward | AGACAGCCACTCACCTCTTCAG |
| Human | Reverse | TTCTGCCAGTGCCTCTTTGCTG |
| *CCL2* | Forward | AGAATCACCAGCAGCAAGTGTCC |
| Human | Reverse | TCCTGAACCCACTTCTGCTTGG |
| *FGF1* | Forward | ACACCGACGGGCTTTTATACG |
| Human | Reverse | CCCATTCTTCTTGAGGCCAAC |
| *PDGFA* | Forward | GCAAGACCAGGACGGTCATTT |
| Human | Reverse | GGCACTTGACACTGCTCGT |
| *Postn* | Forward | TAGCCCAATTAGGCTTGGCATCC |

| | | |
|---|---|---|
| Mouse | Reverse | TAAGAAGGCGTTGGTCCATGCT |
| *Gadph* | Forward | TGTGTCCGTCGTGGATCTGA |
| Mouse | Reverse | TTGCTGTTGAAGTCGCAGGAG |
| *Il-4* | Forward | ATCATCGGCATTTTGAACGAGGTC |
| Mouse | Reverse | ACCTTGGAAGCCCTACAGACGA |
| *Ccnd1* | Forward | GCAGAAGGAGATTGTGCCATCC |
| Mouse | Reverse | AGGAAGCGGTCCAGGTAGTTCA |
| *Acta2* | Forward | ATGCTCCCAGGGCTGTTTTCCCAT |
| Mouse | Reverse | GTGGTGCCAGATCTTTTCCATGTCG |