YAP-Induced Endothelial-Mesenchymal Transition in Oral Submucous Fibrosis

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Appendix

Supplementary Materials and Methods

Immunohistochemical analysis

For immunofluorescence staining, normal or OSF tissue sections were deparaffinized, rehydrated, and incubated with sodium citrate buffer (pH 6.0) for antigen retrieval. The sections were blocked with 3% hydrogen peroxide, and then incubated overnight at 4°C with the primary antibodies (Supplementary Table 2) to observe protein expression and localization. An anaplastic lymphoma kinase (ALK) staining kit (ZK-9600, Zhongshan Goldenbridge Biotechnology, Beijing, China) was used to visualize the sections. Hematoxylin was used to counterstain the sections, and photomicrographs were obtained for further analysis.

Protein extraction and analyses

Total protein lysates were obtained using lysis buffer [radioimmunoprecipitation assay buffer (200 μL; 89901, Thermo Fisher Scientific), protease inhibitor cocktail (2 μL; B14001, Bimake), phosphatase inhibitor cocktail (2 μL; B15001, Bimake)]. Protein concentrations were detected using the bicinchoninic acid assay. Total protein lysates were resolved by 10% (w/v) SDS-PAGE, and transferred onto polyvinylidene fluoride membranes (Millipore). The membranes were blocked in 5% fresh skim milk and incubated overnight at 4°C in Tris-buffered saline with Tween 20 (TBST) + 5% bovine serum albumin (BSA) containing primary antibodies (Supplementary Table 2). The membranes were incubated with anti-rabbit, anti-mouse, or anti-goat peroxidase-conjugated secondary antibody at 37°C for 1 h according to the source of the primary antibody species. Immobilon

Western Chemiluminescent HRP Substrate (Millipore) was used to visualize the protein bands.

RNA extraction, cDNA synthesis, and RT-PCR

RNA was extracted from cells or tissues without epithelium using TRIzol (Thermo Fisher Scientific). RNA samples were reverse-transcribed to cDNA using a PrimeScript™ RT reagent Kit (RR074A, Takara). We performed real-time PCR on a C1000 Touch™ thermal cycler (Bio-Rad) with SYBR Green mix (Bio-Rad). The relative transcript levels were calculated using the comparative threshold cycle (ΔΔCT) method; the results were normalized to the expression of GAPDH. Supplementary Table 4 lists the PCR primer sequences.

Supplementary Table 1. Patients' clinical characteristics

Number	Sex	Age	Part	Smoking history	Areca nut chewing history	Systemic disease history	Pathophysiology number	Disease stage	Purpose
NOR1	female	21	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR2	male	15	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR3	female	23	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR4	female	26	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR5	female	39	Left lower lip mucosa	none	none	Hepatitis B (HBsAg, HBeAb, and HBcAb test positive)	-	-	IHC
NOR6	female	19	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR7	female	50	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR8	female	23	The buccal mucosa around 18 tooth	none	none	none	-	-	IHC
NOR9	female	23	The buccal mucosa around 48 tooth	none	none	none	-	-	IHC
NOR10	female	20	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR11	female	40	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR12	male	25	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR13	male	30	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR14	male	31	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR15	male	32	The buccal mucosa around 38 tooth	none	none	none	-	-	IHC
NOR16	female	21	The buccal mucosa around 48 tooth	none	none	none	-	-	fresh-frozen
NOR17	female	19	The buccal mucosa around 48 tooth	none	none	none	-	-	fresh-frozen
NOR18	female	24	The buccal mucosa around 48 tooth	none	none	none	-	-	fresh-frozen
NOR19	male	25	The buccal mucosa around 38 tooth	none	none	none	-	-	fresh-frozen
E-OSF1	male	24	right buccal mucosa	10/day, 5-6 years	2-3/day, 5 years	none	20786	early phase	IHC
E-OSF2	male	28	left buccal mucosa	20/day, 3 years	5/day, 5 years	none	20634	early phase	IHC
E-OSF3	male	27	right buccal mucosa	10/day, 2 years	3-4/day, 10 years	none	20978	early phase	IHC
E-OSF4	male	36	right buccal mucosa	20/day, 10+ years	10/day, 5-6 years	none	21388	early phase	IHC
E-OSF5	male	63	right buccal mucosa	20/day, 30-40 years	10/day, 10+ years	hypertension	20393	early phase	IHC
E-OSF6	male	43	left buccal mucosa	20/day, 20 years	5/day, 10 years	none	22107	early phase	IHC
E-OSF7	male	51	right buccal mucosa	20/day, 20 years	10/day, 5 years	none	979579	early phase	IHC
E-OSF8	male	60	right tongue mucosa	20/day, 30 years	10/day, 30 years	none	1182350	early phase	IHC
E-OSF9	male	26	right buccal mucosa	none	1-2/day, 1 year	none	982804	early phase	IHC
E-OSF10	male	30	right buccal mucosa	10/day, 2 years	10/day, 7 years	none	981141	early phase	IHC
E-OSF11	male	25	right buccal mucosa	none	5/day, 10 years	none	959031	early phase	IHC
E-OSF12	male	31	left buccal mucosa	10/day, 5 years	10/day, 5 years	none	993090	early phase	IHC
E-OSF13	male	41	right buccal mucosa	20/day, 10 years	10-20/day, 10+ years	none	1070601	early phase	IHC
E-OSF14	male	54	left buccal mucosa	40/day, 20+ years	10/day, 10 years	hypertension	937611	early phase	IHC
E-OSF15	male	41	left buccal mucosa	20/day, 20 years	10/day, 10 years	none	945379	early phase	IHC
E-OSF16	male	27	left buccal mucosa	5/day, 1 years	5/day, 10 years	none	949331	early phase	fresh-frozen
E-OSF17	male	36	left buccal mucosa	20/day, 7 years	10/day, 10+ years	none	962412	early phase	fresh-frozen
E-OSF18	male	36	right buccal mucosa	20/day, 10 years	5-6/day, 5 years	none	9978443	early phase	fresh-frozen

E-OSF19	male	35	left buccal mucosa	10/day, 5 years	10/day, 10 years	none	21175	early phase	fresh-frozen
E-OSF20	male	43	left buccal mucosa	20/day, 10 years	10/day, 20 years	none	21092	early phase	fresh-frozen
E-OSF21	male	36	left buccal mucosa	20/day, 6 years	10/day, 5 years	none	21689	early phase	fresh-frozen
ML-OSF1	male	27	right buccal mucosa	none	10/day, 10+ years	none	21417	middle-late phase	IHC
ML-OSF2	male	50	right buccal mucosa	40/day, 30 years	20/day, 30 years	none	20781	middle-late phase	IHC
ML-OSF3	male	39	left buccal mucosa	20/day, 10 years	10-20/day, 10+ years	none	20460	middle-late phase	IHC
ML-OSF4	male	43	right buccal mucosa	none	20-30/day, 20+ years	none	20679	middle-late phase	IHC
ML-OSF5	male	38	left buccal mucosa	10/day, 10 years	10/day, 20 years	none	20380	middle-late phase	IHC
ML-OSF6	male	26	right buccal mucosa	none	20/day, 7 years	none	20694	middle-late phase	IHC
ML-OSF7	male	57	right buccal mucosa	40/day, 20+ years	30/day, 30 years	hypertension	21262	middle-late phase	IHC
ML-OSF8	male	25	right buccal mucosa	5/day, 1 years	20/day, 5 years	none	21654	middle-late phase	IHC
ML-OSF9	male	36	left buccal mucosa	20/day, 10 years	20-30/day, 10 years	none	20915	middle-late phase	IHC
ML-OSF10	male	49	left buccal mucosa	20/day, 20 years	10/day, 20 years	none	20557	middle-late phase	IHC
ML-OSF11	male	29	left buccal mucosa	none	10/day, 10 years	none	20782	middle-late phase	IHC
ML-OSF12	male	22	right buccal mucosa	none	3-4/day, 7years	none	20788	middle-late phase	IHC
ML-OSF13	male	20	right buccal mucosa	10/day, 3 years	10/day, 3 years	none	991144	middle-late phase	IHC
ML-OSF14	male	39	right buccal mucosa	20/day, 10 years	20/day, 10 years	none	936727	middle-late phase	IHC
ML-OSF15	male	32	right buccal mucosa	20/day, 6 years	10/day, 6 years	none	992609	middle-late phase	IHC
ML-OSF16	male	32	right buccal mucosa	none	10/day, 10 years	none	980303	middle-late phase	fresh-frozen
ML-OSF17	male	20	right buccal mucosa	none	3-4/day, 3 years	none	958907	middle-late phase	fresh-frozen
ML-OSF18	male	34	right buccal mucosa	10/day, 5 years	10/day, 5 years	none	958305	middle-late phase	fresh-frozen
ML-OSF19	male	39	right buccal mucosa	20/day, 7 years	20/day, 10+ years	none	962930	middle-late phase	fresh-frozen
ML-OSF20	male	30	right buccal mucosa	20/day, 5 years	20/day, 5 years	none	23840	middle-late phase	fresh-frozen
ML-OSF21	male	39	right buccal mucosa	20/day, 10 years	10/day, 10 years	none	23838	middle-late phase	fresh-frozen
ML-OSF22	male	30	right buccal mucosa	10/day, 4 years	10/day, 4 years	none	23852	middle-late phase	fresh-frozen

Supplementary Table 2. List of primary antibodies

For immunofluorescence staining

Antibody	Product information	Dilution
N-cadherin	ab18203, Abcam, Cambridge, UK	1:1000
Vimentin	ab8978, Abcam, Cambridge, UK	1:1000
α-SMA	ab5694, Abcam, Cambridge, UK	1:200
YAP	14074S, Cell Signaling Technology, Boston, MA, USA	1:200

For western blotting

Antibody	Product information	Dilution
N-cadherin	ab18203, Abcam, Cambridge, UK	1:1000
E-cadherin	3195, Cell Signaling Technology, Boston, MA, USA	1:1000
Vimentin	ab8978, Abcam, Cambridge, UK	1:1000
α-SMA	ab5694, Abcam, Cambridge, UK	1:500
PERK	sc-9481, Santa Cruz, TX, USA	1:1000
p-PERK	sc-32577, Santa Cruz, TX, USA	1:1000
p-eIF2α	3398, Cell Signaling Technology, Boston, MA, USA	1:1000
ATF4	11815S, Cell Signaling Technology, Boston, MA, USA	1:1000
YAP	14074S, Cell Signaling Technology, Boston, MA, USA	1:1000
IRE1	27528-1-AP, Proteintech, Rosemont, USA	1:500
ATF6	24169-1-AP, Proteintech, Rosemont, USA	1:1000
MST1	#14946, Cell Signaling Technology, Boston, MA, USA	1:1000
SAV1	#13301, Cell Signaling Technology, Boston, MA, USA	1:1000
LATS1	#3477, Cell Signaling Technology, Boston, MA, USA	1:400
p-LATS1	#8654, Cell Signaling Technology, Boston, MA, USA	1:300
MOB1	#13730, Cell Signaling Technology, Boston, MA, USA	1:1000
p-YAP	#13008, Cell Signaling Technology, Boston, MA, USA	1:1000
α-tubulin	sc-23948, Santa Cruz, TX, USA	1:4000
GAPDH	sc-137179, Santa Cruz, TX, USA	1:2000

For immunocytochemistry

Antibody	Product information	Dilutions
YAP	14074S, Cell Signaling Technology, Boston, MA, USA	1:100
TOMM20	ab186734, Abcam, Cambridge, UK	1:250

Supplementary Table 3. The YAP siRNA constructs used

Number	Sequence
#1	GGUGAUACUAUCAACCAAATT
#2	GACAUCUUCUGGUCAGAGATT

Supplementary Table 4. Primers used for RT-PCR

For human

Target	Forward primer	Reverse primer
CTGF	CAGCATGGACGTTCGTCTG	AACCACGGTTTGGTCCTTGG
CYR61	CCCATTCCAGGGTTTGAGC	TGCACGAAGAGGTGTTTGTTG
COL1A2	GTTGCTGCTTGCAGTAACCTT	AGGGCCAAGTCCAACTCCTT
COL3A1	GGAGCTGGCTACTTCTCGC	GGGAACATCCTCCTTCAACAG
CDH2	TCAGGCGTCTGTAGAGGCTT	ATGCACATCCTTCGATAAGACTG
CDH1	CGAGAGCTACACGTTCACGG	GGGTGTCGAGGGAAAAATAGG
SNAI1	TCGGAAGCCTAACTACAGCGA	AGATGAGCATTGGCAGCGAG
MMP1	AAAATTACACGCCAGATTTGCC	GGTGTGACATTACTCCAGAGTTG
MMP9	TGTACCGCTATGGTTACACTCG	GGCAGGGACAGTTGCTTCT
GAPDH	GGAGCGAGATCCCTCCAAAAT	GGCTGTTGTCATACTTCTCATGG

For mice

Target	Forward primer	Reverse primer
Col1α2	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA
Col3α1	CTGTAACATGGAAACTGGGGAAA	CCATAGCTGAACTGAAAACCACC
Cdn1	CAGTTCCGAGGTCTACACCTT	TGAATCGGGAGTCTTCCGAAAA
Snai1	CACACGCTGCCTTGTGTCT	GGTCAGCAAAAGCACGGTT
Mmp9	GCAGAGGCATACTTGTACCG	TGATGTTATGATGGTCCCACTTG
Gapdh	AGGTCGGTGTGAACGGATTTG	GGGGTCGTTGATGGCAACA

Supplementary Table 5. Mouse grouping information and weights (g)

		PRE-TREAT	1st WEEK	2nd WEEK	3rd WEEK	4th WEEK	5th WEEK	6th WEEK	7th WEEK	8th WEEK	9th WEEK	10th WEEK	11th WEEK	12th WEEK
Blank	MALE	24.7	26.3	26	26.2	27.5	28.9	30.1	30.5	31.1	32	32.5	32.8	32.5
	FEMALE	17.5	18.4	19.4	19.1	20	21.5	21.1	21.6	22	21.8	21.3	22.2	22.6
PBS	MALE	25.1	23.4	23.5	24.8	25.1	25.6	26	26.6	27.1	27.6	27.9	28.8	29.5
		23.9	23.8	24.1	24.6	25.5	26	27.3	27.7	27.9	28.4	29	29.5	30.4
		24.5	23.2	24.6	26.3	26.1	26.4	27.1	27.7	28	28.2	29.1	29.7	30.9
		25.3	23.5	24.7	25.3	25.7	26.1	27.2	27.9	28.4	28.7	29.2	29.6	31.1
	FEMALE	18.6	19.3	19.2	21.9	20.9	20.5	20.4	20.9	21.1	21.4	22	21.8	22.5
		16.1	15.8	17	17.6	17.9	18.1	18	18.6	18.8	19.1	18.7	19.9	19.5
		16.7	16.2	17.4	18.7	18.2	19.1	20	20.8	21	21.2	21.9	21.8	22.6
		17	16.7	17.6	18.2	18.6	18.7	19.2	20.7	21.5	21.8	22.3	22.1	22.5
VP	MALE	23.7	24.3	24.7	26.6	26.7	28.2	27.9	28.6	28.8	29	29.5	29.7	30
		23.7	24.1	23.9	25.6	26.4	26.4	27.3	27.6	27.8	28.3	28.9	30	31.1
		24.9	24.5	24.8	25.5	26.4	27.2	27.7	28.4	28.9	29.1	29.6	30.3	31
		25.2	24.7	25	26	26.7	27.4	27.4	28	28.3	28.7	29.2	30.5	31.4
	FEMALE	17.9	19.1	18.3	20.6	19.6	20.6	20.2	20.5	20.6	21.1	20.6	21.7	22.3
		17.1	16.6	17	19.1	18.3	18.5	18.3	19.4	19.7	19.1	19	19.5	19.7
		16.5	16.1	16.4	19.2	19.8	20.2	19.4	20.3	21	21.2	20.8	21.4	22.1
		17.6	17.2	17.6	20	19.6	20.6	20.5	20.9	21.2	20.9	20.7	21	22.3
AREC.	MALE	24.3	23.8	25.5	25.1	26.8	26.7	26.4	27	28.6	29.3	29.1	30.3	30.8
		23.8	24	26.5	25.2	28.3	27.2	27.3	28.7	30.3	30.5	31.1	31.4	31.4
		24.3	23.8	25.6	25.5	26.1	26.2	26.3	27.2	27.7	28.6	29.3	29.6	29.3
		23.8	25.4	26.3	25.7	26.7	27.1	27.6	28.3	29.4	29.3	29.7	30.3	30.7
	FEMALE	18.9	19.8	20.1	20.1	22.3	21.5	21.9	20	20.3	20.4	20.5	21.8	22.4
		18.4	19.2	19.5	19.6	22.2	19.8	20.1	20.4	20.7	21.3	21.2	22.1	21.8
		17.6	19.4	18.9	18.7	19.8	20.4	20.4	21.1	21.8	21.4	21.7	22.3	22.2
		18.5	19.4	19.8	19.5	21	20.5	21.2	21.7	22	21.8	21.6	22.2	23.1
AREC.+VP	MALE	23	23	25.6	24.6	25.4	24.5	25.1	26.4	27.2	27.6	27.7	27.3	30
		23.4	23.4	25.9	24.5	26.1	25.4	25.9	26.5	27	27.3	27.6	27.2	29.1
		23.7	23.4	24.4	25	26.2	26.1	26.8	27.5	28.2	28.8	29.7	31	31.7
		25.2	26.3	26.2	27.4	28.1	28.7	29.3	29.3	30	30.5	31.1	31.3	31.8
	FEMALE	17.5	19.2	22.3	19.6	20.2	18.7	19.5	19.4	19.9	19.7	20.7	21.3	21.7
		18.5	20	21.3	20.1	21.5	19.1	19.7	19.9	20.2	20.4	20.9	21.5	21.9
		18.1	19.4	20.7	19.7	20.7	19.3	20.9	21.1	21.4	21	21.2	21.7	22.1
		17	16.4	17	16.8	17.3	17.4	18.2	18.3	18.8	18.1	18.3	18.4	18.8

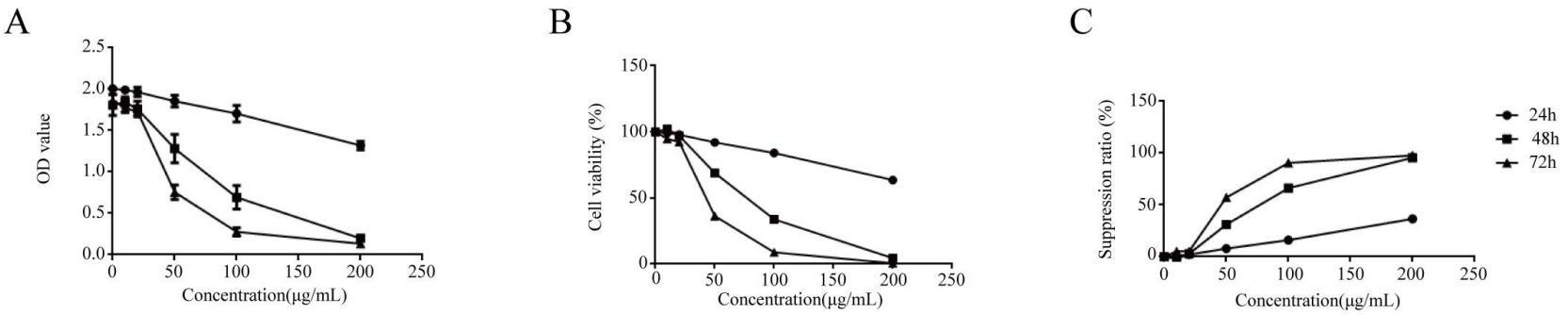

Fig. M1. Arecoline affects HUVEC proliferation. Cell Counting Kit-8 (CCK-8) was used to the effects of arecoline on the HUVEC proliferation, (A) optical density (OD) value, (B) cell viability, and (C) suppression ratio. The results indicated that high concentrations of arecoline inhibited cell proliferation, and that the optimal concentration was 10 μg/mL.

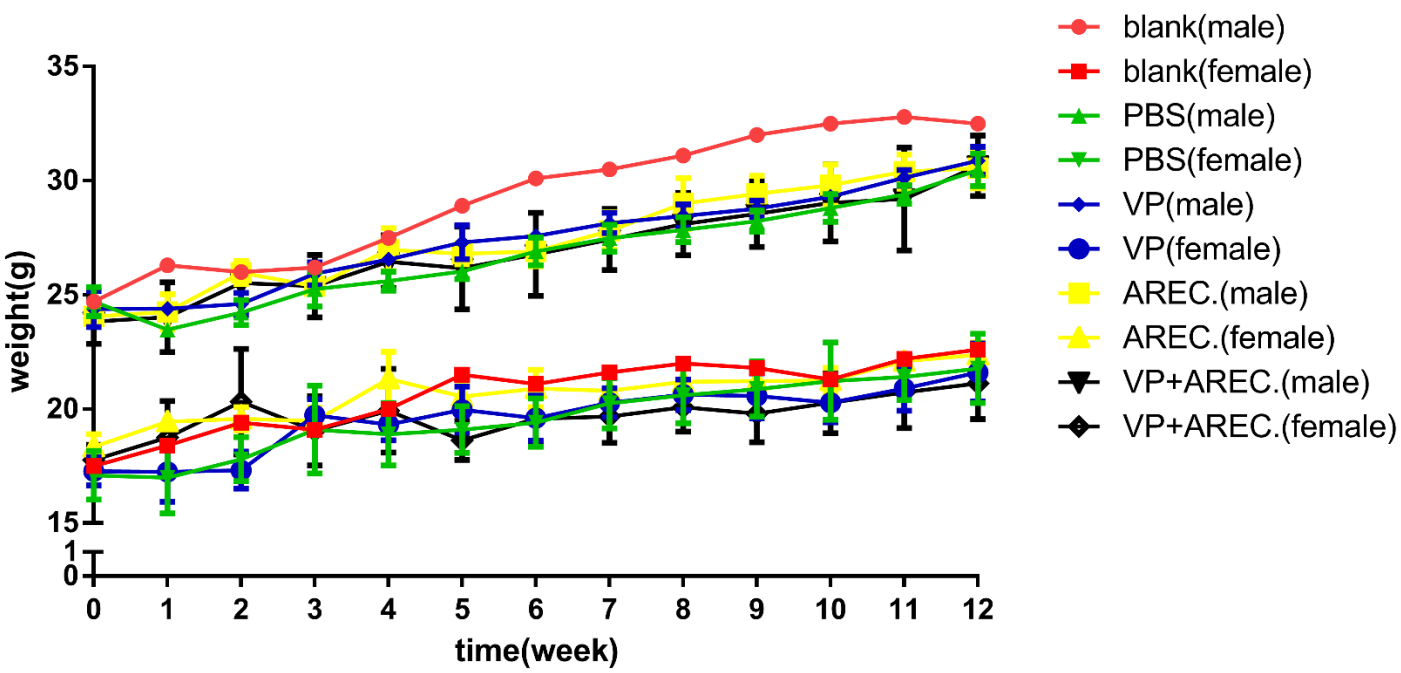

Fig. M2. The growth curves of mice in each group.

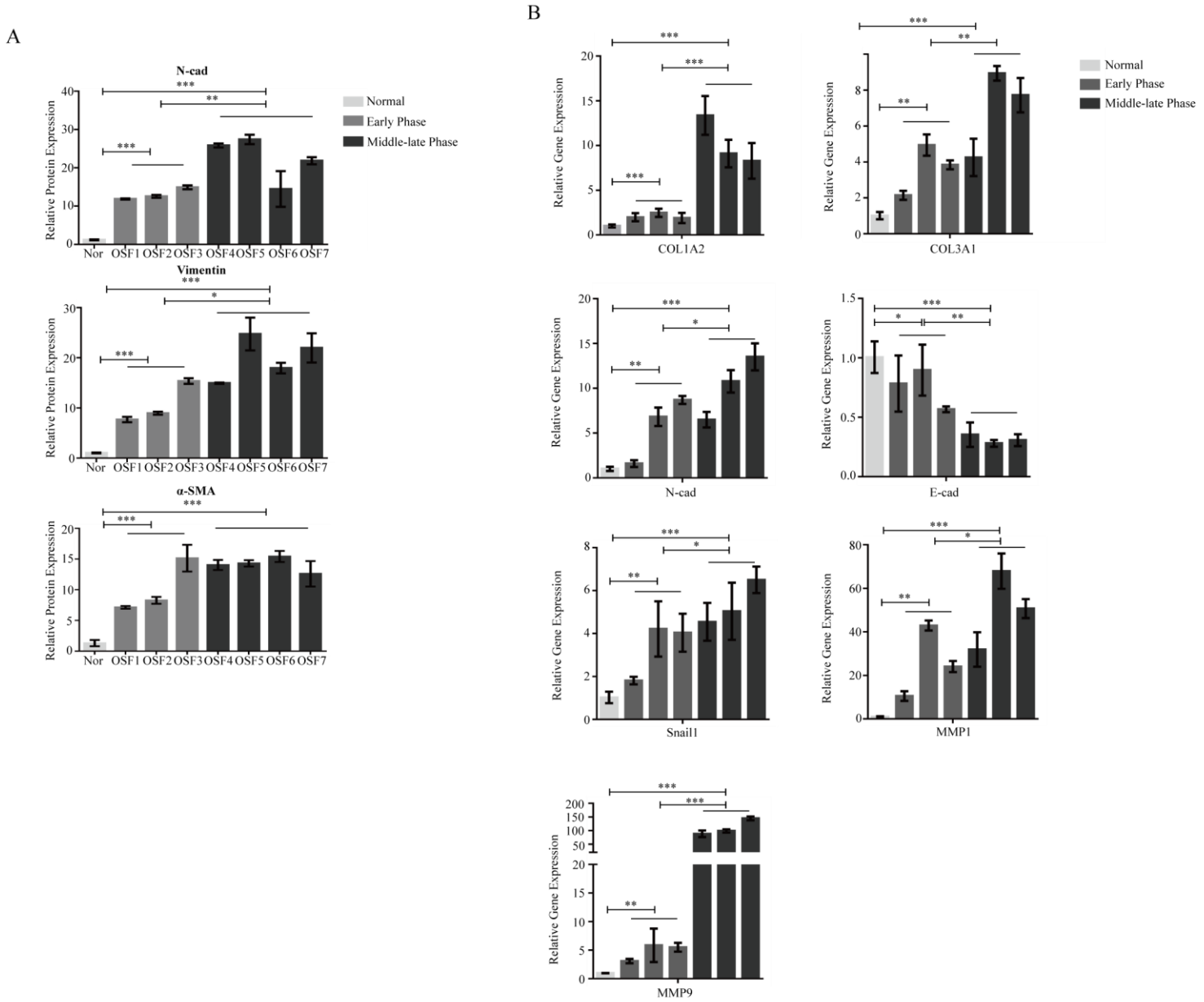
A
N-cad
Vimentin
α-SMA
Relative Protein Expression
Nor OSF1 OSF2 OSF3 OSF4 OSF5 OSF6 OSF7
Normal
Early Phase
Middle-late Phase
B
COL1A2
COL3A1
N-cad
E-cad
Snail1
MMP1
MMP9
Relative Gene Expression

Fig. S1. Microvascular changes and EndMT were present in human OSF tissues, Related to Fig. 1. (A) Densitometric analysis for Fig. 1B with ImageJ. The experiment was repeated 3 times independently. Results shown are the mean ± SD. ANOVA, n = 8. *P < 0.05. **P < 0.01. ***P < 0.001. (B) RT-qPCR for COL1A2, COL3A1, N-cadherin, E-cadherin, Snail, MMP1, and MMP9 in OSF tissues vs. normal tissues. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA, n = 7. *P < 0.05. **P < 0.01. ***P < 0.001. ANOVA, analysis of variance; COL1A2, type I collagen; COL3A1, type III collagen; EndMT, endothelial-mesenchymal transformation; MMP1, matrix metalloproteinase 1; MMP9, matrix metalloproteinase 9; OSF, oral submucous fibrosis; RT-qPCR, reverse transcription quantitative polymerase chain reaction.

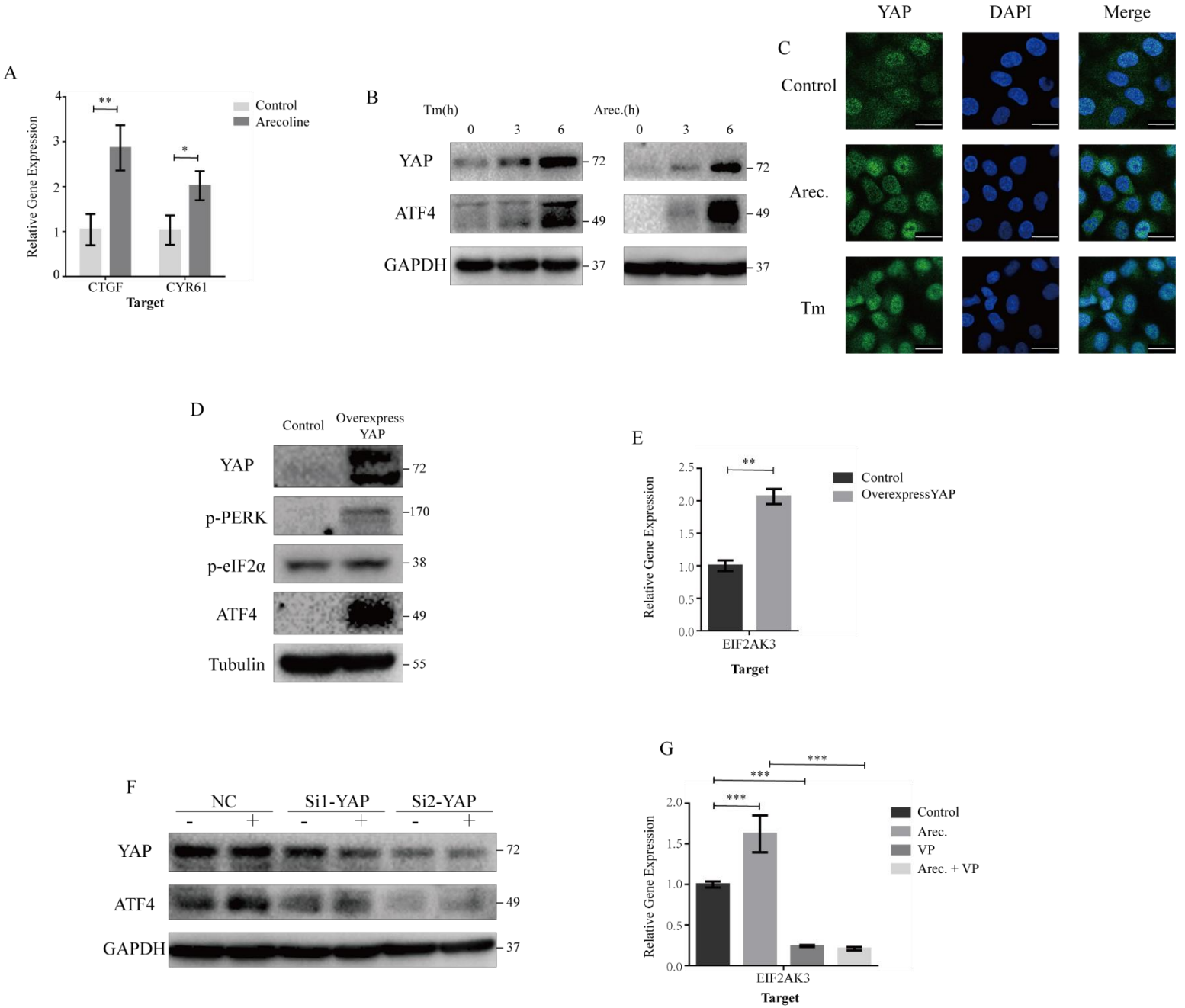

Fig. S2 Arecoline activated YAP via the PERK pathway in endothelial cells, Related to Fig. 2. (A) RT-qPCR for CTGF and CYR61 in HUVECs

treated with arecoline for 6 h. The experiment was repeated 3 times independently. Results are shown as mean ± SD. t test. *P < 0.05. **P < 0.01. (B) Western blots of YAP and ATF4 in HUVECs treated with Tm or arecoline for 0, 3, or 6 h. (C) Fluorescent staining for YAP in Tm- vs. arecoline-treated HUVECs (FCFM, ×630 magnification, scale bar = 25 μm). (D, E) Western blots of PERK, p-PERK, p-eIF2α, ATF4, and YAP and RT-qPCR of *EIF2AK3* in YAP overexpression HUVECs vs. control. The experiment was repeated 3 times independently. Results are shown as mean ± SD. *t* test. ***P* < 0.01. (F) Western blots of YAP and ATF4 following YAP RNA interference in arecoline-treated HUVECs. (G) RT-qPCR of *EIF2AK3* following VP pretreatment in HUVECs treated with arecoline for 6 h vs. untreated HUVECs. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA. ****P* < 0.001. ER, endoplasmic reticulum; FCFM, fibered confocal fluorescence microscopy; HUVEC, human umbilical vein endothelial cell; RT-qPCR, reverse transcription quantitative polymerase chain reaction; Tm, tunicamycin; VP, verteporfin; YAP, Yes-associated protein;

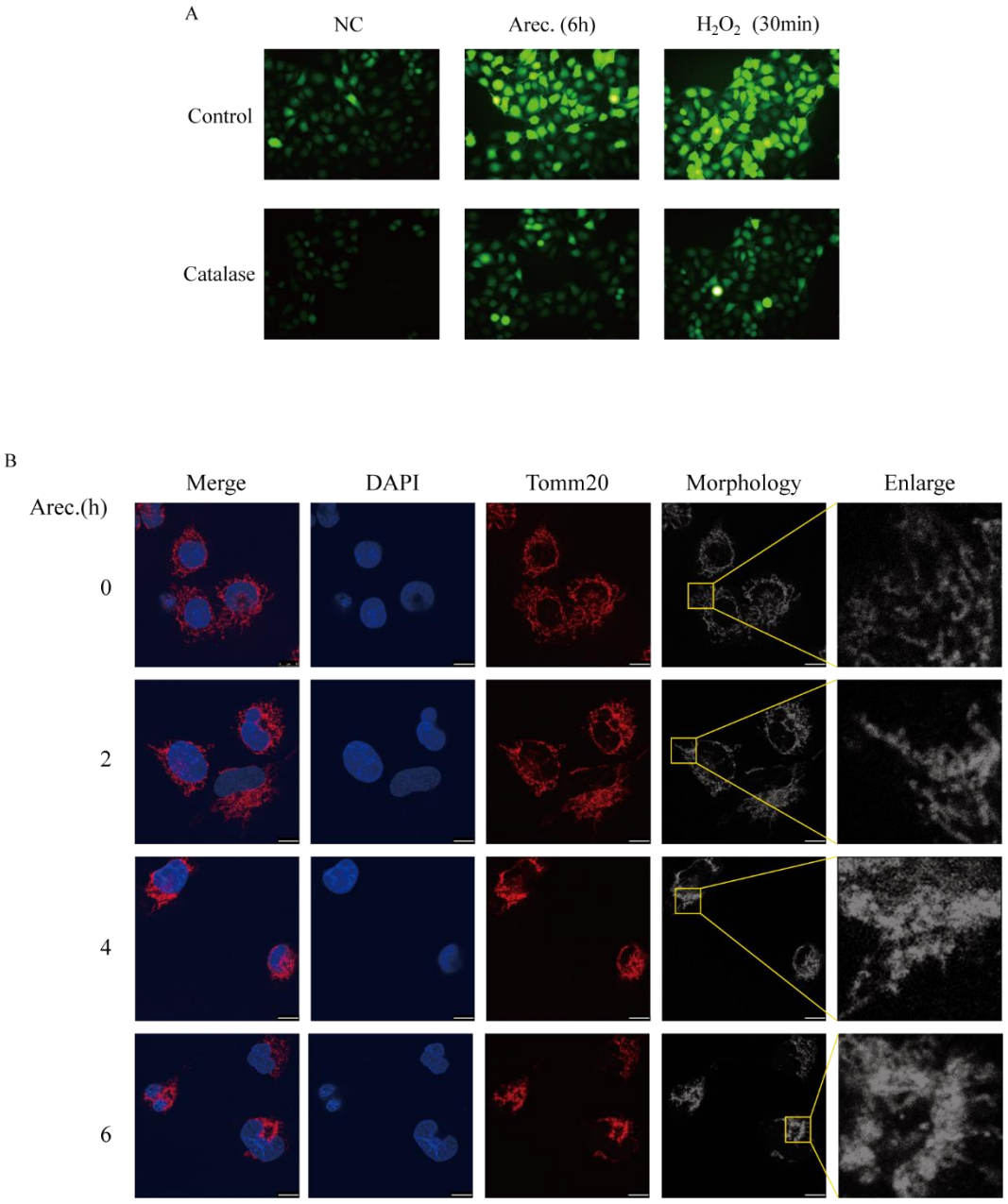

Fig. S3 ROS was required for PERK-induced YAP activation, Related to Fig 3. (A) Fluorescent staining for ROS after catalase pretreatment in

HUVECs treated with arecoline and H2O2 vs. the control (×400 magnification). (B) Fluorescent staining for TOMM20 in HUVECs treated with arecoline for 2, 4, or 6 h (FCFM, ×1,500 magnification; scale bar = 10 μm). FCFM, fibered confocal fluorescence microscopy; HUVEC, human umbilical vein endothelial cell; ROS, reactive oxygen species; YAP, Yes-associated protein.

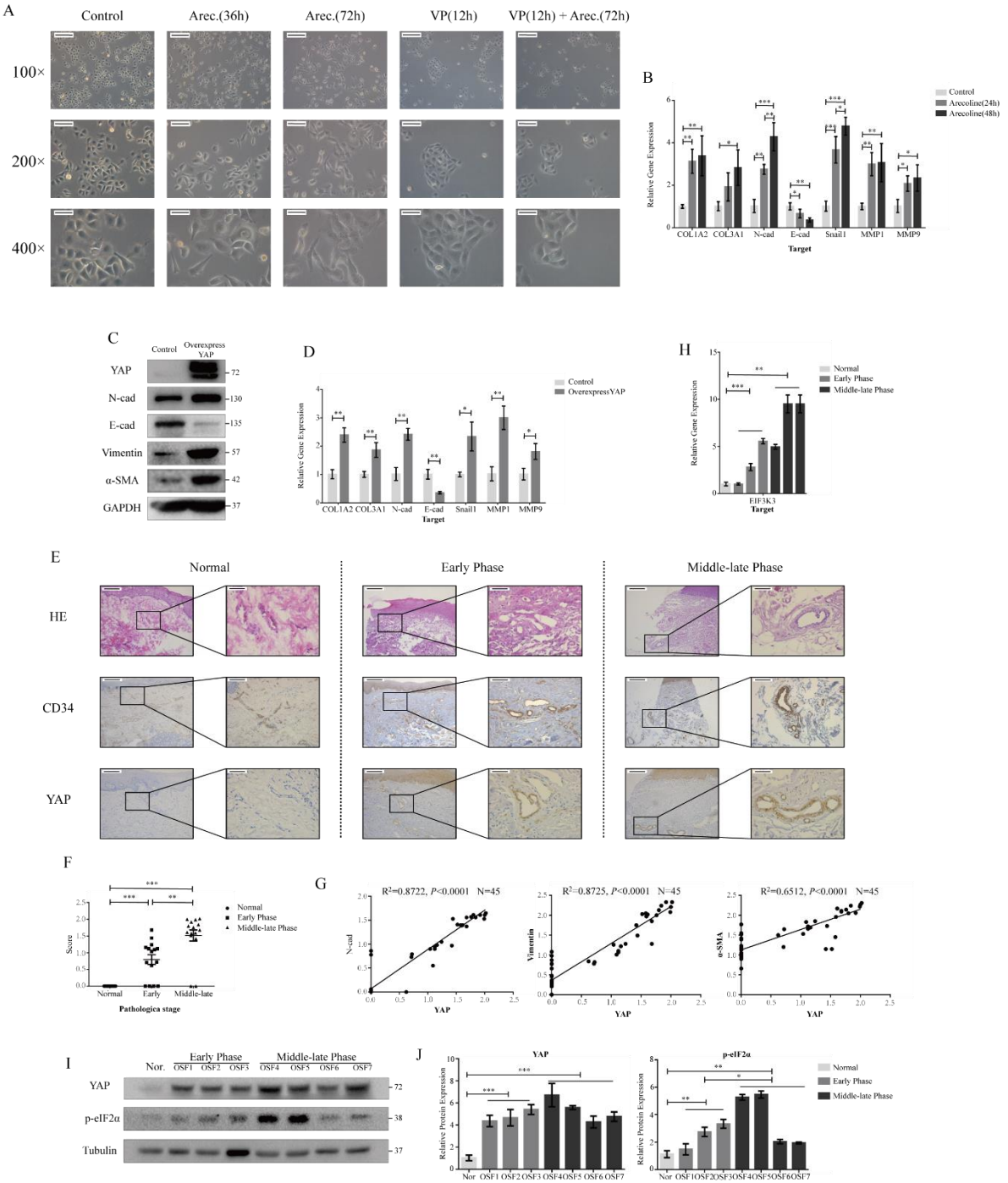

Fig S4 YAP mediated arecoline-induced EndMT, Related to Fig. 4. (A) Cellular morphology changes of HUVECs treated with arecoline for 0, 48,

or 72 h following VP pretreatment (top, × 100 magnification [scale bar = 200 μm]; middle, ×200 magnification [scale bar = 100 μm]; bottom, ×400 magnification [scale bar = 50 μm]). (B) RT-qPCR for COL1A2, COL3A1, N-cadherin, E-cadherin, Snail, MMP1, and MMP9 in HUVECs treated with arecoline for 0, 24, or 48 h. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA. *P < 0.05. **P < 0.01. ***P < 0.001. (C, D) Western blot of N-cadherin, E-cadherin, vimentin, and α-SMA, and RT-qPCR for COL1A2, COL3A1, N-cadherin, E-cadherin, Snail, MMP1, and MMP9 in YAP overexpression HUVECs compared with control. The experiment was repeated 3 times independently. Results are shown as mean ± SD. t test. *P < 0.05. **P < 0.01. (E) HE and immunohistochemical staining for CD34 and YAP in OSF tissues vs. normal tissues (left, ×100 magnification [scale bar = 200 μm]; right, ×400 magnification [scale bar = 50 μm]). (F) The immunoreactive score. (G) Correlation analysis between YAP and N-cadherin, vimentin, and α-SMA. (H) RT-qPCR for EIF2AK3 in OSF tissues vs. normal tissues. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA, n = 7. *P < 0.05. **P < 0.01. ***P < 0.001. (I) Western blots of YAP and p-eIF2α in OSF tissues vs. normal tissues. (J) densitometric analysis using ImageJ. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA, n = 8. *P < 0.05. **P < 0.01. ***P < 0.001. α-SMA, α–smooth muscle actin; ANOVA, analysis of variance; COL1A2, type I collagen; COL3A1, type III collagen; EndMT, endothelial-mesenchymal transformation; HUVEC, human umbilical vein endothelial cell; MMP1, matrix metalloproteinase 1; MMP9, matrix metalloproteinase 9; OSF, oral submucous fibrosis; RT-qPCR, reverse transcription quantitative polymerase chain reaction; VP, verteporfin; YAP, Yes-associated protein.

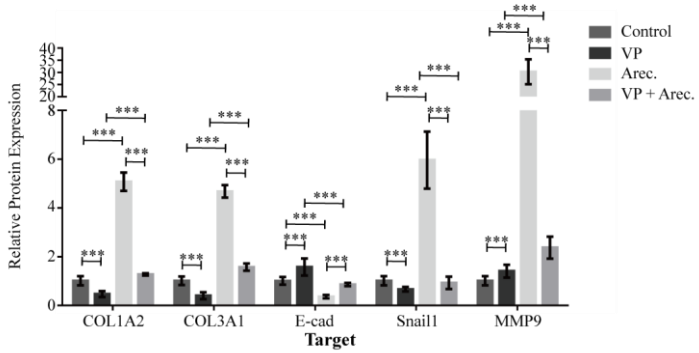

Fig. S5 VP treated arecoline-induced OSF in mice, Related to Fig. 5. RT-qPCR for Col1a2, Col3a1, E-cadherin, Snail1, and Mmp9 in mouse tissues. The experiment was repeated 3 times independently. Results are shown as mean ± SD. ANOVA, n = 32. ***P < 0.001. α-SMA, α–smooth muscle actin; ANOVA, analysis of variance; Col1α2, type I collagen; Col3α1, type III collagen; Mmp9, matrix metalloproteinase 9; RT-qPCR, reverse transcription quantitative polymerase chain reaction; VP, verteporfin.