

Online Supplemental Data

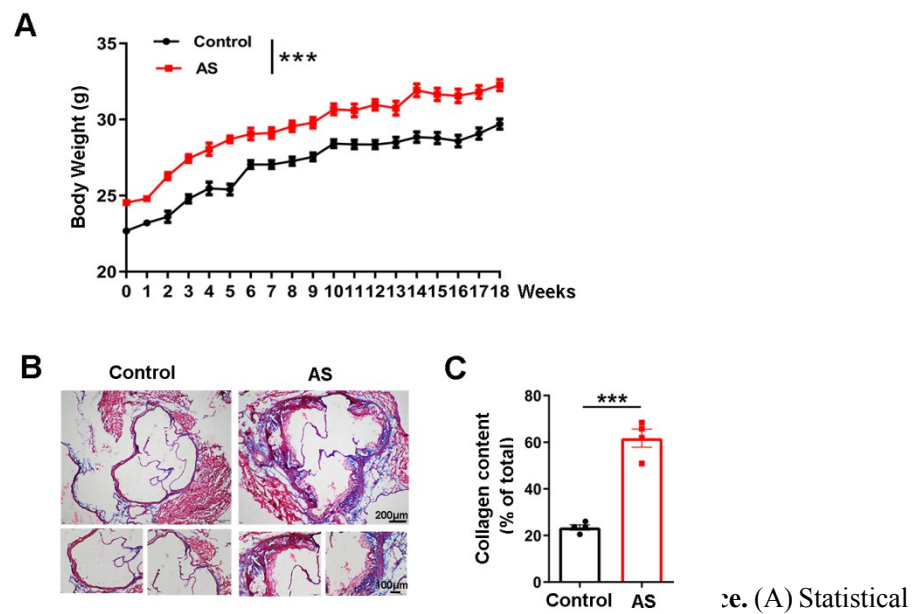


Fig. S1. The bod

data of body weight of control and AS mice. $n=7-10$, *** $P < 0.001$ vs. Control. (B, C) Representative masson staining of aorta root and quantification data. $n=4$, *** $P < 0.001$ vs. Control. All data were shown as mean \pm SEM.

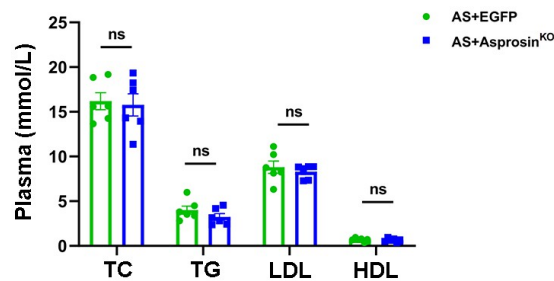


Fig. S2. Asprosin deficiency in adipose tissue has little effect on blood lipid levels in atherosclerosis mice. TC, TG, LDL and HDL levels in serum of mice. $n=6$. $^{ns}P > 0.05$ vs. (AS+EGFP). All data were shown as mean \pm SEM.

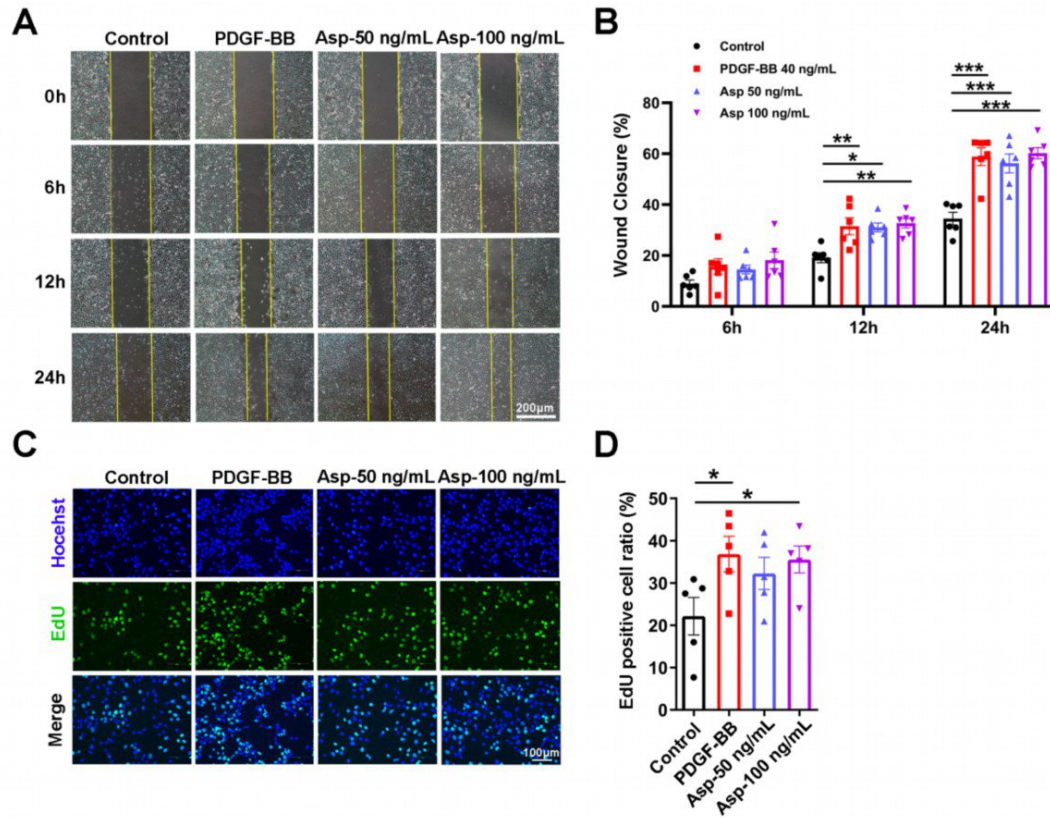


Fig. S3. Asprosin induces MOVAs migration and proliferation. (A, B) Representative images of wound scratch assay showed migration of MOVAs treated by PDGF-BB (40 ng/mL) and asprosin (50 ng/mL, 100 ng/mL) at the time 0 h, 6 h, 12 h and 24 h. $n=6$, $*P<0.05$ vs. Control. $**P<0.01$ vs. Control. $***P<0.001$ vs. Control. (C, D) Representative images and statistical data showed proliferation of MOVAs induced by PDGF-BB (40 ng/mL) and asprosin (50 ng/mL, 100 ng/mL), $n=5$ in each group. $*P<0.05$ vs. Control. All data were shown as mean \pm SEM.

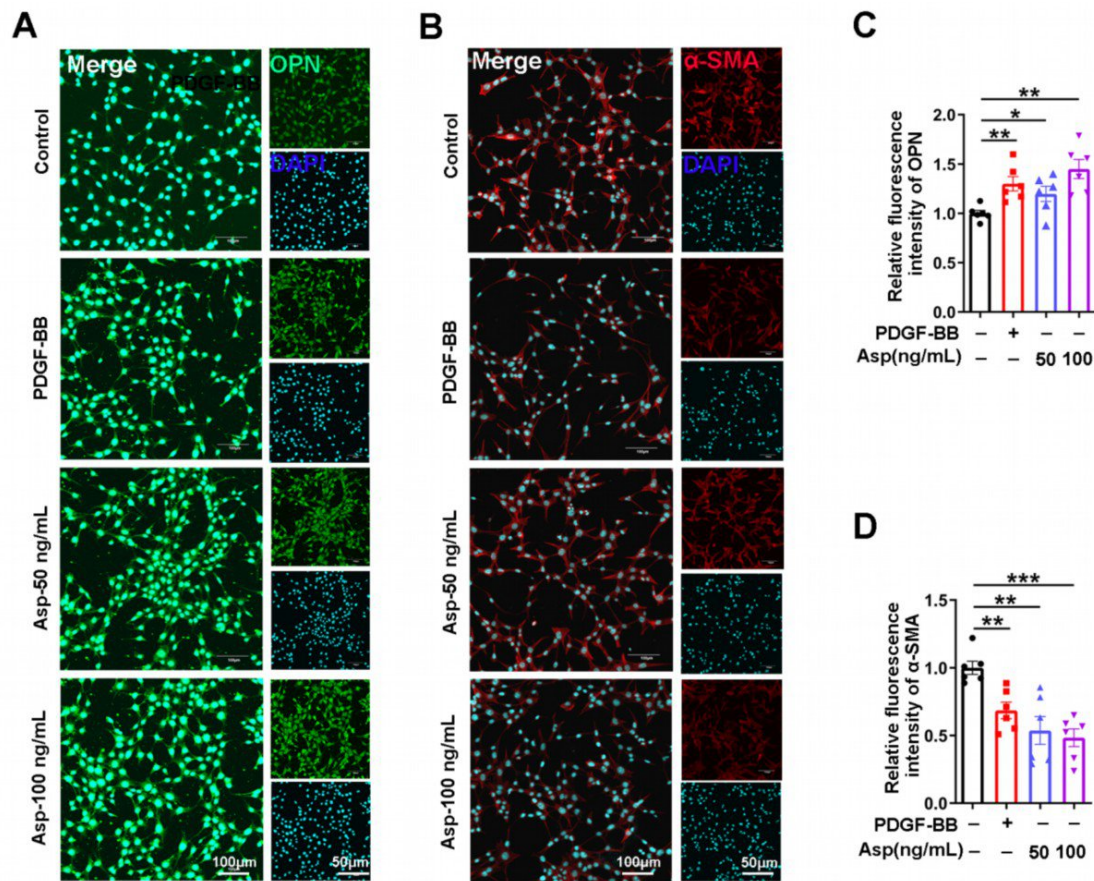


Fig. S4. Asprosin induces the transformation of MOVAs from contractile phenotype to synthetic phenotype. (A, C) Representative OPN immunofluorescence staining and statistical data, $n=6$. $*P<0.05$ vs. Control. $**P<0.01$ vs. Control. (B, D) Representative α -SMA immunofluorescence staining and statistical data, $n=6$. $**P<0.01$ vs. Control. $***P<0.001$ vs. Control. All data were shown as mean \pm SEM.

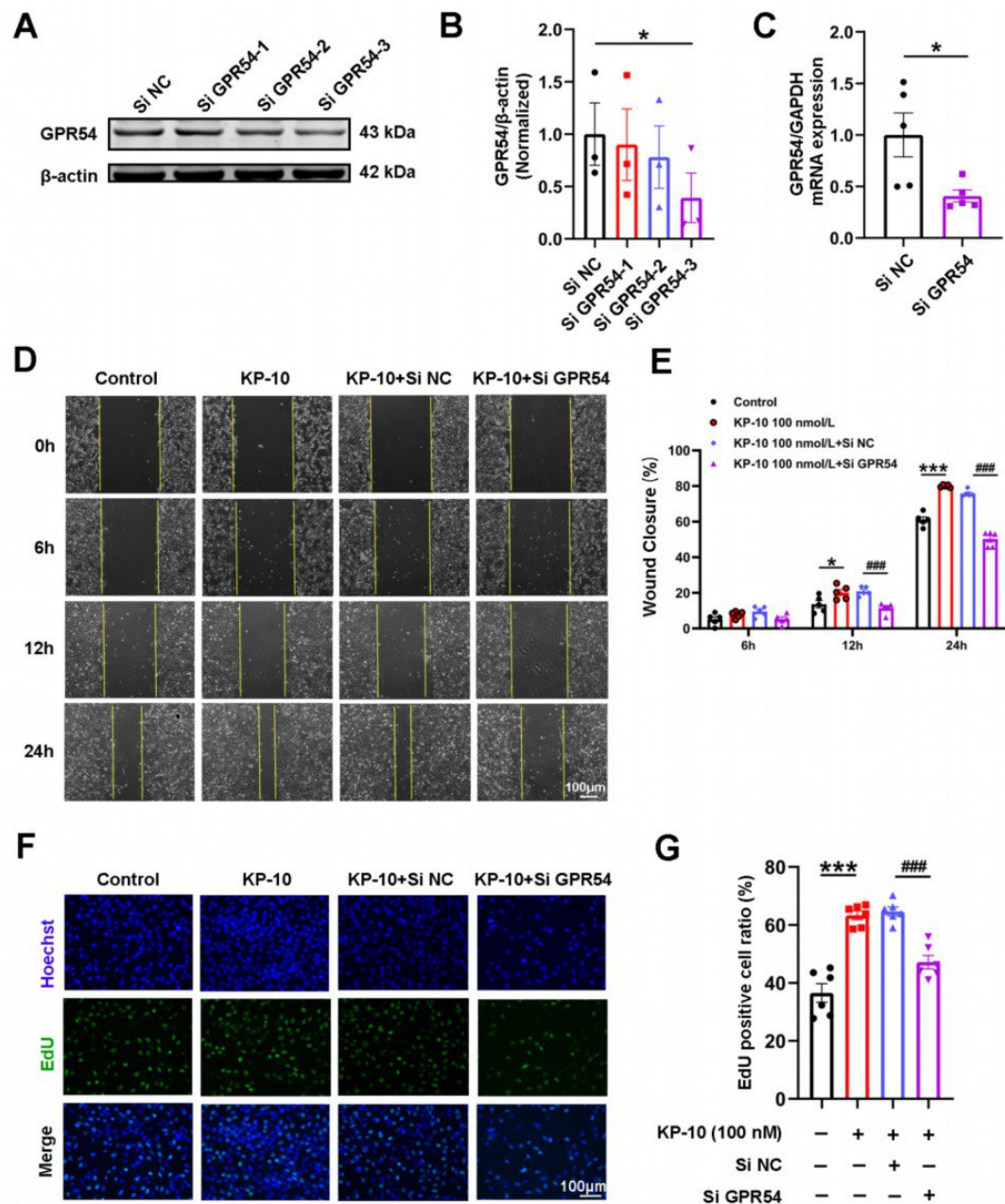


Fig. S5. Activation of GPR54 promotes MOVAs migration and proliferation. (A, B) Representative western blot and quantification data of GPR54 protein levels treated with 100 nM Si NC and Si GPR54. $n=3$, $*P < 0.05$ vs. Si NC. (C) GPR54 mRNA levels treated with 100 nM Si NC and Si GPR54-3. $n=5$, $*P < 0.05$ vs. Si NC. (D, E) Representative and statistical data showed migration of MOVAs treated by KP-10 (100 nM), KP-10+Si NC and KP-10+Si GPR54 at the time 0 h, 6 h, 12 h and 24 h measured by wound scratch assays. $n=5$, $*P < 0.05$ vs. Control. $***P < 0.001$ vs. Control. $###P < 0.001$ vs. (KP-10+Si NC). (F, G) Representative images and statistical data showed proliferation of MOVAs treated by KP-10 (100 nM), KP-10+Si NC and KP-10+Si GPR54,

n=6 in each group, *** $P<0.001$ vs. Control. ### $P<0.001$ vs. (KP-10+Si NC). All data were shown as mean \pm SEM.

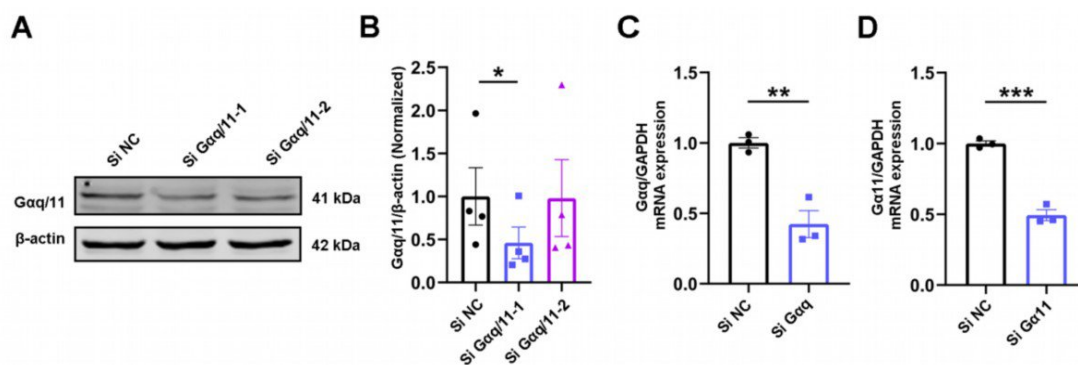
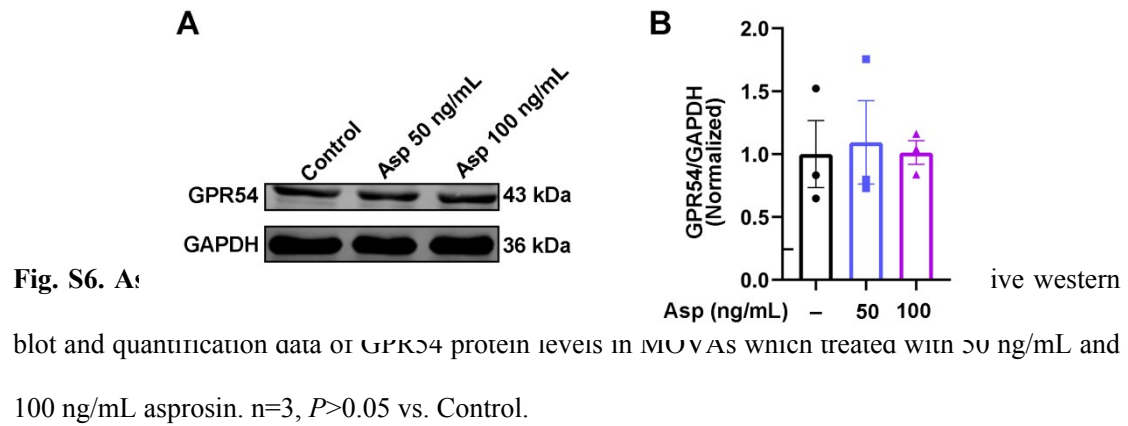


Fig. S7. Knock-down efficiency detection of Gaq/11 in MOVAs. (A, B) Representative western blot and quantification data of Gaq/11 protein levels which treated with 100 nM Si NC and Si Gaq/11. n=4, * $P<0.05$ vs. Si NC. (C, D) Gaq and Ga11 mRNA levels treated with 100 nM Si NC and Si Gaq/11-1. n=3, ** $P<0.01$ vs. Si NC. *** $P<0.001$ vs. Si NC. All data were shown as

mean \pm SEM.

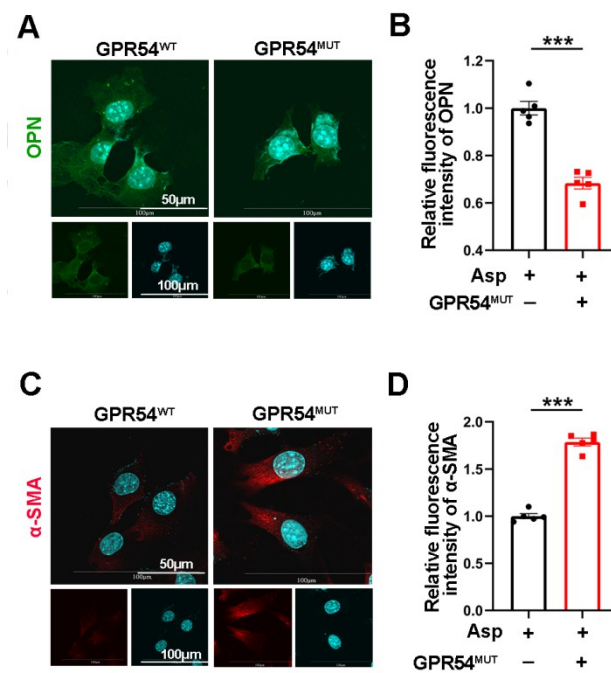


Fig. S8. The mutant GPR54-(267, 307) residue inhibits MOVAs phenotype transformation.

(A, B) Representative OPN immunofluorescence staining and statistical data, n=5. *** P <0.001 vs.

GPR54^{WT}. (C, D) Representative α -SMA immunofluorescence staining and statistical data, n=5.

*** P <0.001 vs. GPR54^{WT}.

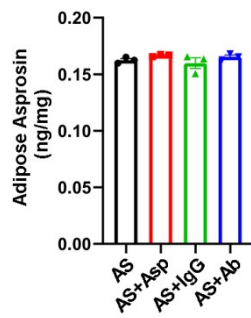


Fig. S9. Exogenous injection of a **dy fails to alter asprosin levels in** **adipose.** Asprosin level in adipose tissue of mice in four groups, including AS, (AS+Asp), (AS+IgG), (AS+Ab). $n=3$, $P>0.05$. All data were shown as mean \pm SEM.

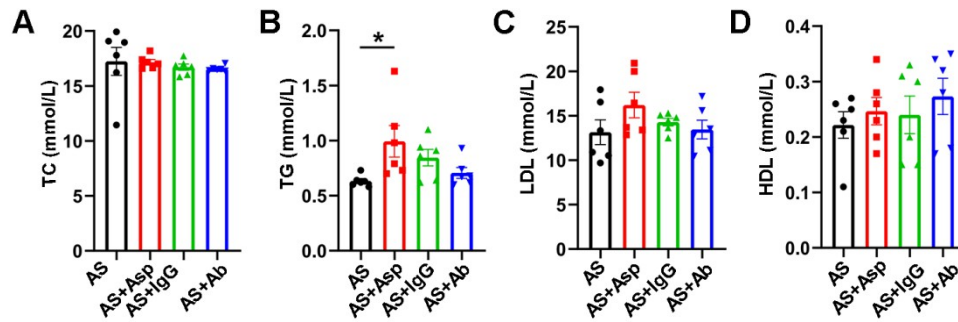


Fig. S10. Exogenous treatment of asprosin has little effect on blood lipid levels in atherosclerosis mice. (A) TC levels in serum of mice in four groups, including AS, (AS+Asp), (AS+IgG), (AS+Ab). $n=6$. (B) TG levels in serum of mice in four groups. $n=6$, $*P<0.05$ vs. AS. (C) LDL levels in serum of mice in four groups. $n=6$. (D) HDL levels in serum of mice in four groups. $n=6$. All data were shown as mean \pm SEM.

Table S1. Clinical characteristics of the study subjects

Features	Healthy controls	Coronary atherosclerosis patients
Sex (M/F)	18 (9/9)	27 (21/6)
Age	56.33 \pm 1.58	61.93 \pm 1.32
BMI (kg/m ²)	24.95 \pm 0.62	24.71 \pm 0.63
FBG	5.10 \pm 0.19	4.97 \pm 0.10

HbA1c	5.64±0.18	5.49±0.06
TC (mmol/L)	3.45±0.20	3.94±0.18
TG (mmol/L)	1.08±0.21	1.72±0.45
LDL-C (mmol/L)	2.80±0.30	2.20±0.15
HDL-C (mmol/L)	2.24±0.21	1.10±0.05

$P>0.05$, t-test.

Table S2. Arterial blood pressure of mice in each group

Groups	Systolic blood pressure	Diastolic blood pressure
AS	82.34±1.90	64.92±2.58
AS+Asp	79.58±1.90	60.42±2.86
AS+IgG	81.73±2.27	64.49±3.46
AS+Ab	83.46±3.04	67.98±2.25

$n=6$, $P>0.05$, t-test.

Table S3. Arterial blood pressure of mice in each group

Groups	Systolic blood pressure	Diastolic blood pressure
AS+EGFP	82.86±2.98	64.47±1.54
AS+Asprosin ^{KD}	80.04±3.34	65.45±3.82

$n=6$, $P>0.05$, t-test.

Table S4. Primary antibodies used for western blotting, immunoprecipitation, and immunofluorescence

Target antigen	Working dilutions	Catalog NO.	Vendor or Source
α -SMA	1/10000 (WB) 1/500 (IF)	67735-1-Ig	Proteintech, CHI, USA
OPN	1/1000 (WB) 1/100 (IF)	22952-1-AP	Proteintech, CHI, USA
Vimentin	1/2000 (WB)	10366-1-AP	Proteintech, CHI, USA

transgelin/SM22	1/5000 (WB)	10493-1-AP	Proteintech, CHI, USA
His-Tag Monoclonal antibody	1/5000 (WB) 1/500 (IF) 15µg (IP)	66005-1-Ig	Proteintech, CHI, USA
KISS1R	15µg (IP)	13776	CST, MA, USA
Gαq/11	1/1000 (WB)	sc-515689	Santa Cruz, CA, USA
KISS1R	1/1000 (WB) 1/200 (IF)	DF7123	Affinity, Jiangsu, China
OR4M1	1/1000 (WB)	ER62228	Huabio, Hangzhou, China
Mouse IgG	15µg (IP)	B900620	Proteintech, CHI, USA
Rabbit IgG	15µg (IP)	B900610	Proteintech, CHI, USA
Phospho-PKC	1/1000 (WB)	2060	CST, MA, USA
PKCα	1/1000 (WB)	2056	CST, MA, USA
Phospho-PLCβ3	1/10000 (WB)	ab76031	Abcam, Cambridge, UK
PLC	1/1000 (WB)	ab303940	Abcam, Cambridge, UK
phospho-P42/44 MAPK	1/2000 (WB)	4370	CST, MA, USA
P42/44 MAPK	1/1000 (WB)	4695	CST, MA, USA
phospho-MEK1/2	1/2000 (WB)	2338	CST, MA, USA
MEK1/2	1/1000 (WB)	8727	CST, MA, USA
phospho-stat3 (Tyr705)	1/2000 (WB)	9145	CST, MA, USA
STAT3	1/1000 (WB)	30835	CST, MA, USA
anti-Asprosin mAb	200 mg/mL	AG-20B-0073	AdipoGen, CA, USA
Mouse IgG1 Isotype Control	200 mg/mL	AG-35B-0003	AdipoGen, CA, USA
Asprosin antibody	1/1000 (WB)	FNab09797	Fine test, Wuhan, China
GAPDH	1/20000 (WB)	60004-1-Ig	Proteintech, CHI, USA
β-actin	1/4000 (WB)	bs-0061R	Bioss, Beijing, China
Histone-H3	1/2000 (WB)	17168-1-AP	Proteintech, CHI, USA

Table S5. Primer sequences for RT-PCR

Mouse	Forward 5' to 3'	Reverse 5' to 3'
Asprosin	CAAGAGACGGAGAAGCACG A	GCAGGAGCTCTAGGATTTCGG
kiss1r-1	ATGCTGGACGTTGGAGGTTA	CCAATCATGATGGCCTGGTG
kiss1r-2	CAGCAGTGTGGAATGAGAG C	CGTACCCATCTGCCTGTACT
kiss1r-3	AGTGACCAGCCTTCAGAGAC	CCAGGAACAAGCACCCCTAGA
Gnaq-1	TGTGGAGAAGGTGTCTGCTT	ATAGGAAGGGTCGGCTACAC
Gnaq-2	GGAATCCAGGAGTGCTACGA	CACGTCTTGTTGTGTAGGCA
Gnaq-3	GATGGACACGCTCAAGATCC	AAGCAGACACCTTCTCCACA
Gna11-1	TGTTTGTGGACCTGAACCCC T	ACACAAAGCGGATGTTCTCG
Gna11-2	CATCAAGACGCTGTGGAGTG	CTCCAGGTCAAACGGGTACT
Gna11-3	CCTGGTTCCAGAACTCGTCT	CTGTGGCCATCAAACCTCAGG
Spp1	CATGGACGACGATGATGACG	CGACTGTAGGACGATTGGA
ACTA2	ACCCAGCACCATGAAGATCA	TCTGCTGGAAGGTAGACAGC
OLFR73 4	CCGGATTGCTTGTGCCAATA	TGGATATAGCCCTGCTGGTG
OR4M1	CCATCCATCTACATTTATGC	ACTTCCTTGTTTCTCAATGT
GAPDH	ATGTGTCCGTCGTGGATCTG A	TTGCTGTTGAAGTCGCAGGA G