Figure S1

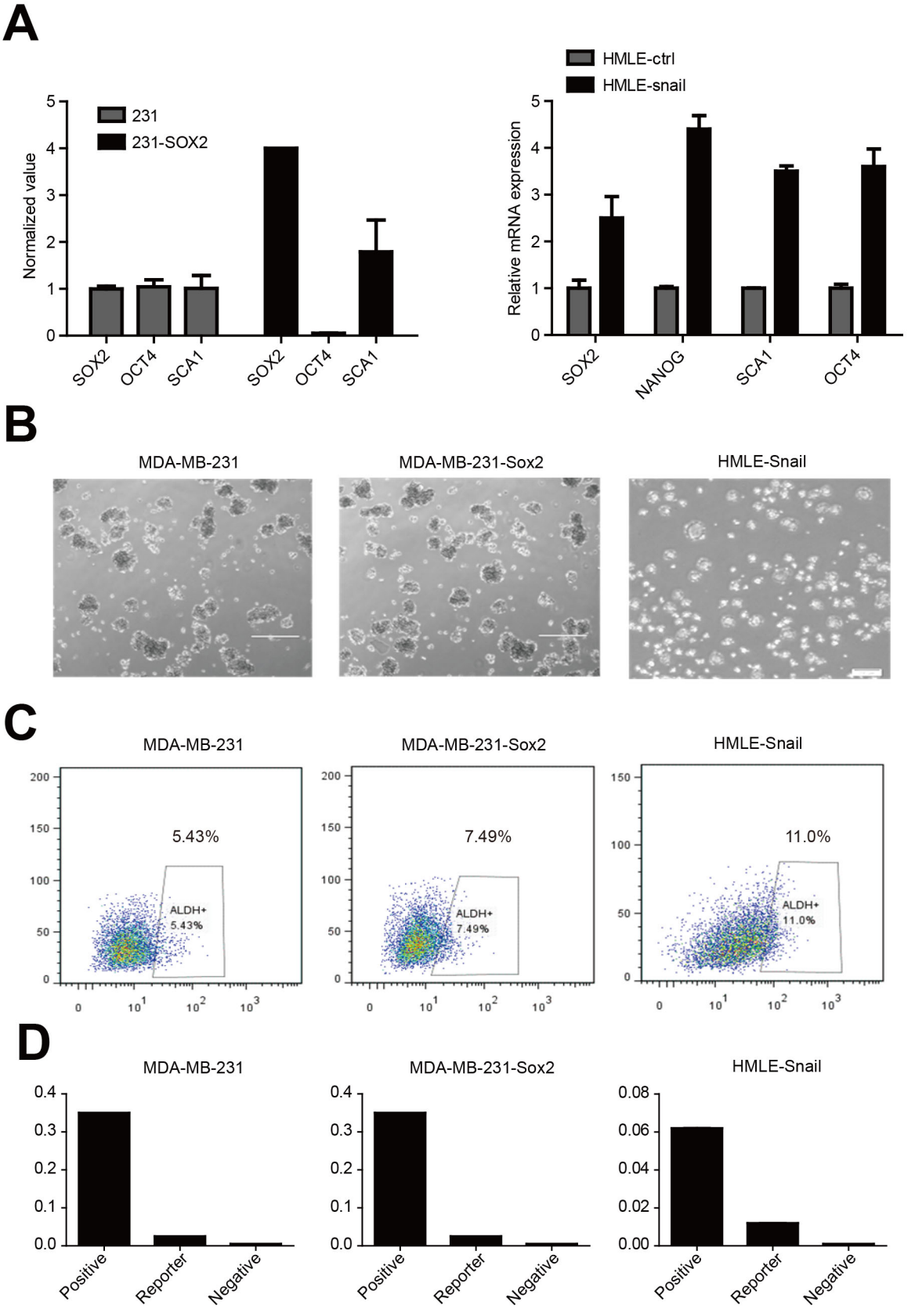
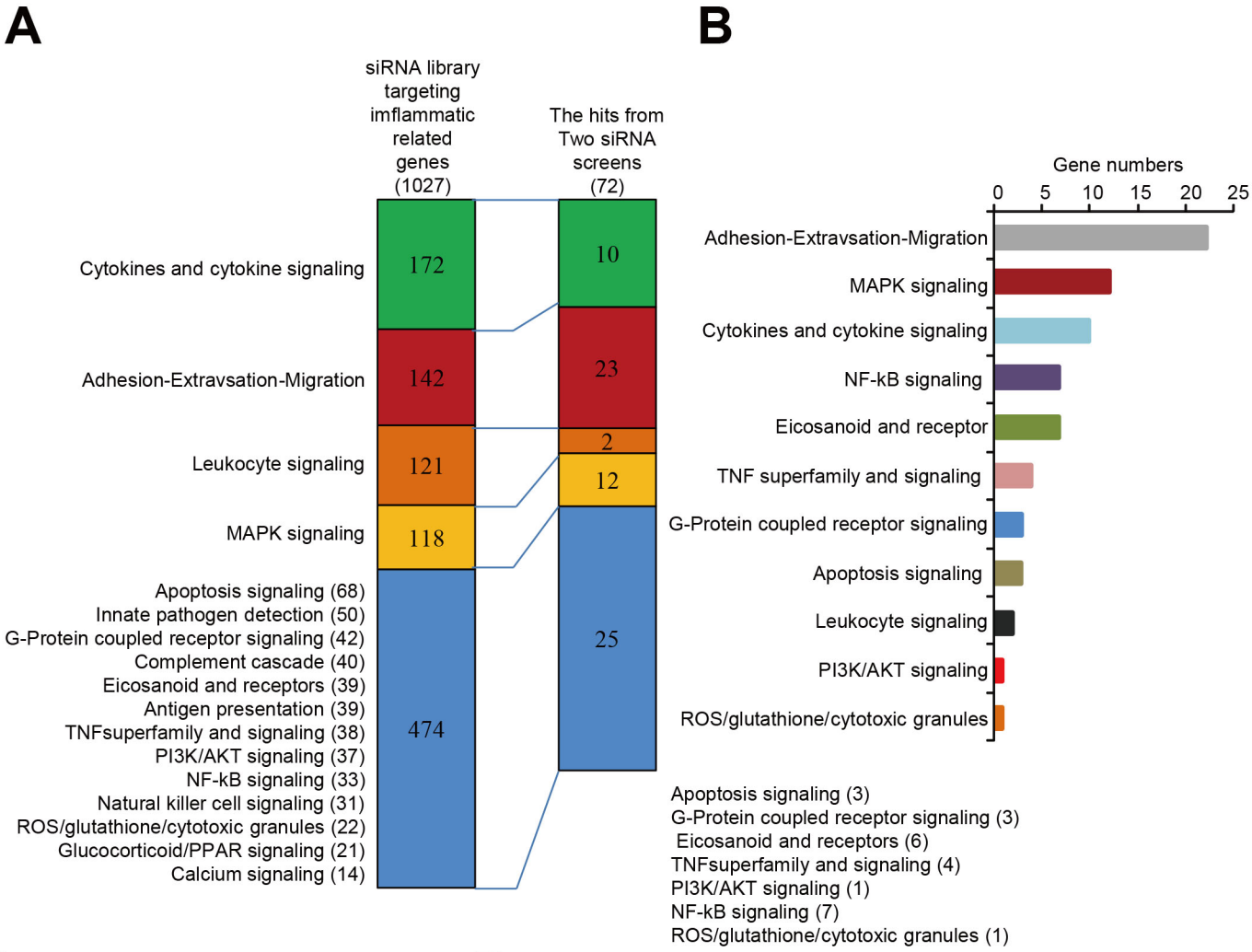
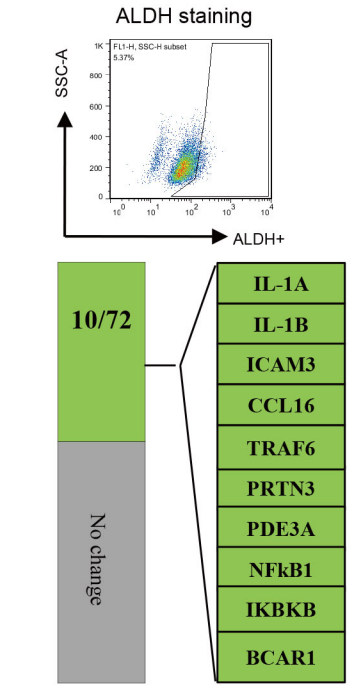

A
231
231-SOX2
Normalized value
SOX2
OCT4
SCA1
SOX2
OCT4
SCA1
HMLE-ctrl
HMLE-snail
Relative mRNA expression
SOX2
NANOG
SCA1
OCT4
B
MDA-MB-231
MDA-MB-231-Sox2
HMLE-Snail
C
MDA-MB-231
MDA-MB-231-Sox2
HMLE-Snail
5.43%
ALDH+
5.43%
7.49%
ALDH+
7.49%
11.0%
ALDH+
11.0%
D
MDA-MB-231
MDA-MB-231-Sox2
HMLE-Snail
Positive
Reporter
Negative
Positive
Reporter
Negative
Positive
Reporter
Negative

Figure S1. HMLE-snail cell is the suitable CSCs model for high-throughput siRNA screen.

(A) Stemness factors OCT4, SOX2, Nanog, and sca-1 were detected in MDA-MB-231, MDA-MB-231-SOX2 and HMLE-Snail cells by RT-PCR. (B) The sphere formation ability of three cell models were tested. (C) ALDH+ staining assay was performed in three candidate cell models. (D) The sensitivity to dual-luciferase OCT4 reporter was studied in three cell models.

Figure S2


A
siRNA library targeting imflammatic related genes (1027)
The hits from Two siRNA screens (72)
Cytokines and cytokine signaling
Adhesion-Extravsation-Migration
Leukocyte signaling
MAPK signaling
Apoptosis signaling (68)
Innate pathogen detection (50)
G-Protein coupled receptor signaling (42)
Complement cascade (40)
Eicosanoid and receptors (39)
Antigen presentation (39)
TNFsuperfamily and signaling (38)
PI3K/AKT signaling (37)
NF-kB signaling (33)
Natural killer cell signaling (31)
ROS/glutathione/cytotoxic granules (22)
Glucocorticoid/PPAR signaling (21)
Calcium signaling (14)
172
142
121
118
474
10
23
2
12
25
B
Gene numbers
0 5 10 15 20 25
Adhesion-Extravsation-Migration
MAPK signaling
Cytokines and cytokine signaling
NF-kB signaling
Eicosanoid and receptor
TNF superfamily and signaling
G-Protein coupled receptor signaling
Apoptosis signaling
Leukocyte signaling
PI3K/AKT signaling
ROS/glutathione/cytotoxic granules
Apoptosis signaling (3)
G-Protein coupled receptor signaling (3)
Eicosanoid and receptors (6)
TNFsuperfamily and signaling (4)
PI3K/AKT signaling (1)
NF-kB signaling (7)
ROS/glutathione/cytotoxic granules (1)


C
ALDH staining
SSC-A
ALDH+
10/72
No change
IL-1A
IL-1B
ICAM3
CCL16
TRAF6
PRTN3
PDE3A
NFkB1
IKBKB
BCAR1

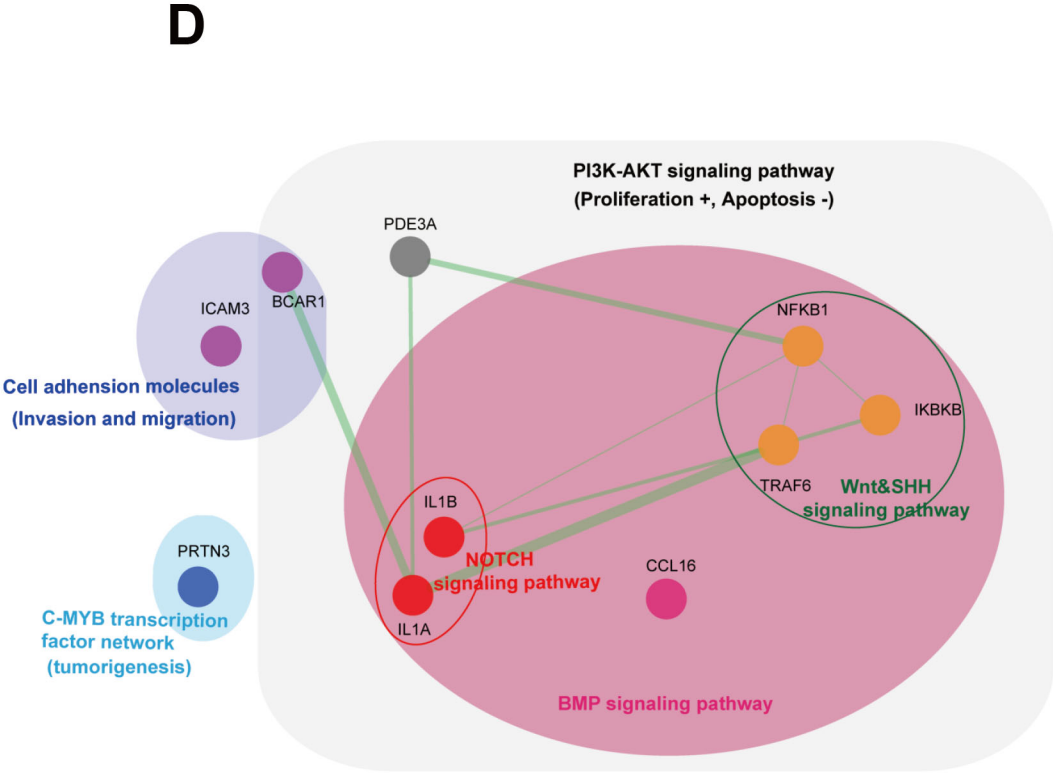

D
PI3K-AKT signaling pathway
(Proliferation +, Apoptosis -)
PDE3A
ICAM3
BCAR1
Cell adhesion molecules
(Invasion and migration)
NFKB1
IKBKB
TRAF6
Wnt&SHH
signaling pathway
IL1B
NOTCH
signaling pathway
IL1A
CCL16
PRTN3
C-MYB transcription
factor network
(tumorigenesis)
BMP signaling pathway

Figure S2. Various approaches to further identify important candidates.

(A) The pathway distribution of candidate genes. The siRNA library target 1027 inflammation genes involved in 17 functional sub-pathways of various phases of inflammation response. (B) The hits numbers in various inflammation pathways. (C) **ALDH assay was performed to screen genes which down-regulation could decrease ALDH+ subpopulation in HMLE-snail cells. 10 candidates were screened out from 72 genes.** (D) The correlation of the 10 identified genes with CSC related signaling pathways including Wnt & SHH, Notch, BMP, PI3K-AKT, and C-MYB transcription factor network were analyzed.

Supplemental Figure 3

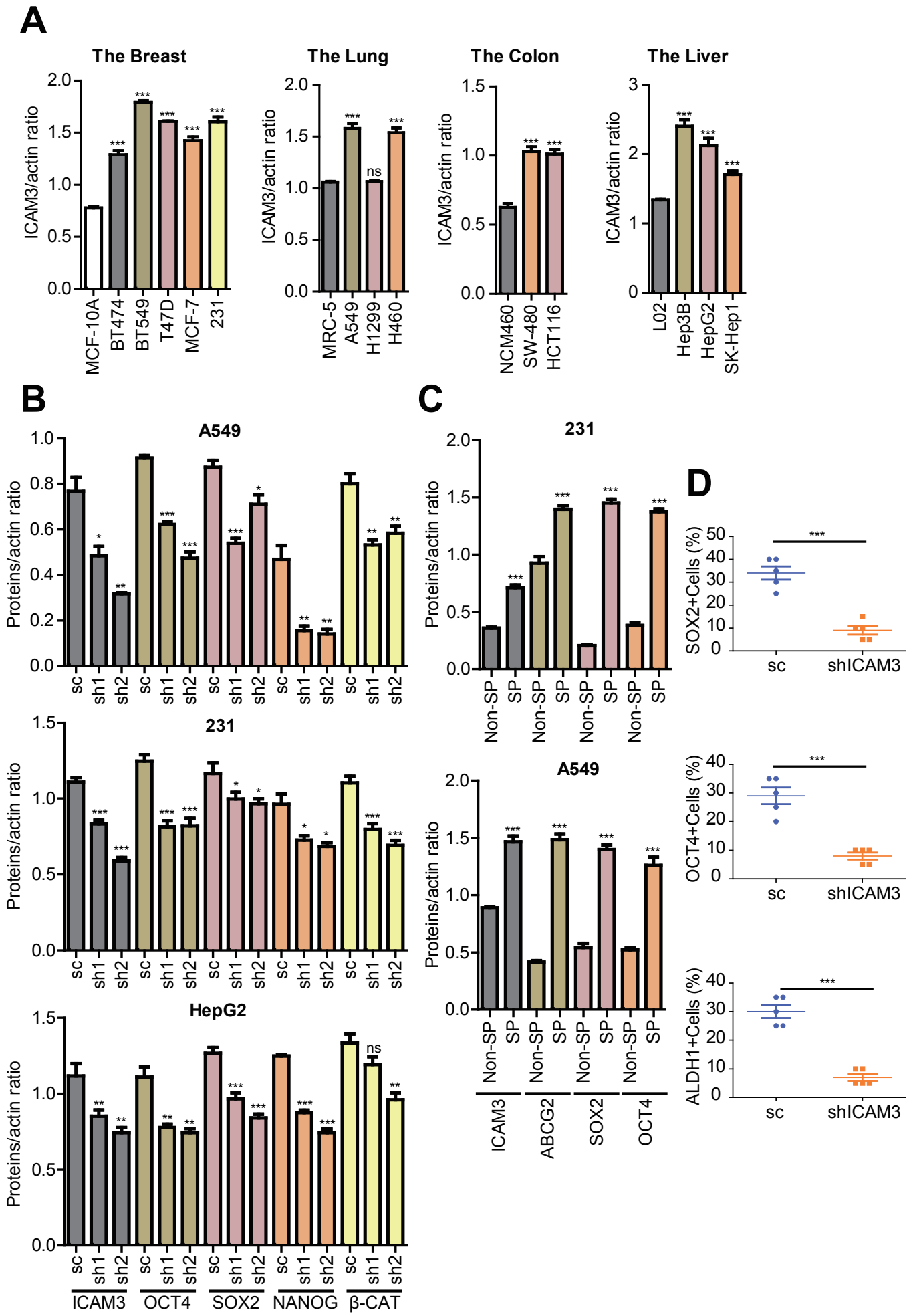


Figure S3. Quantification results of the western blot data by three biological repeats.

(A) Quantification results of figure 1E. (B) Quantification results of figure 2A. (C) Quantification results of figure 2E. (D) Quantification results of figure 2J. **The bar graph shows the statistical result of three separate experiments.** *Indicates significant difference with $P<0.05$, **indicates significant difference with $P<0.01$, ***indicates significant difference with $P<0.001$.

Supplemental Figure 4

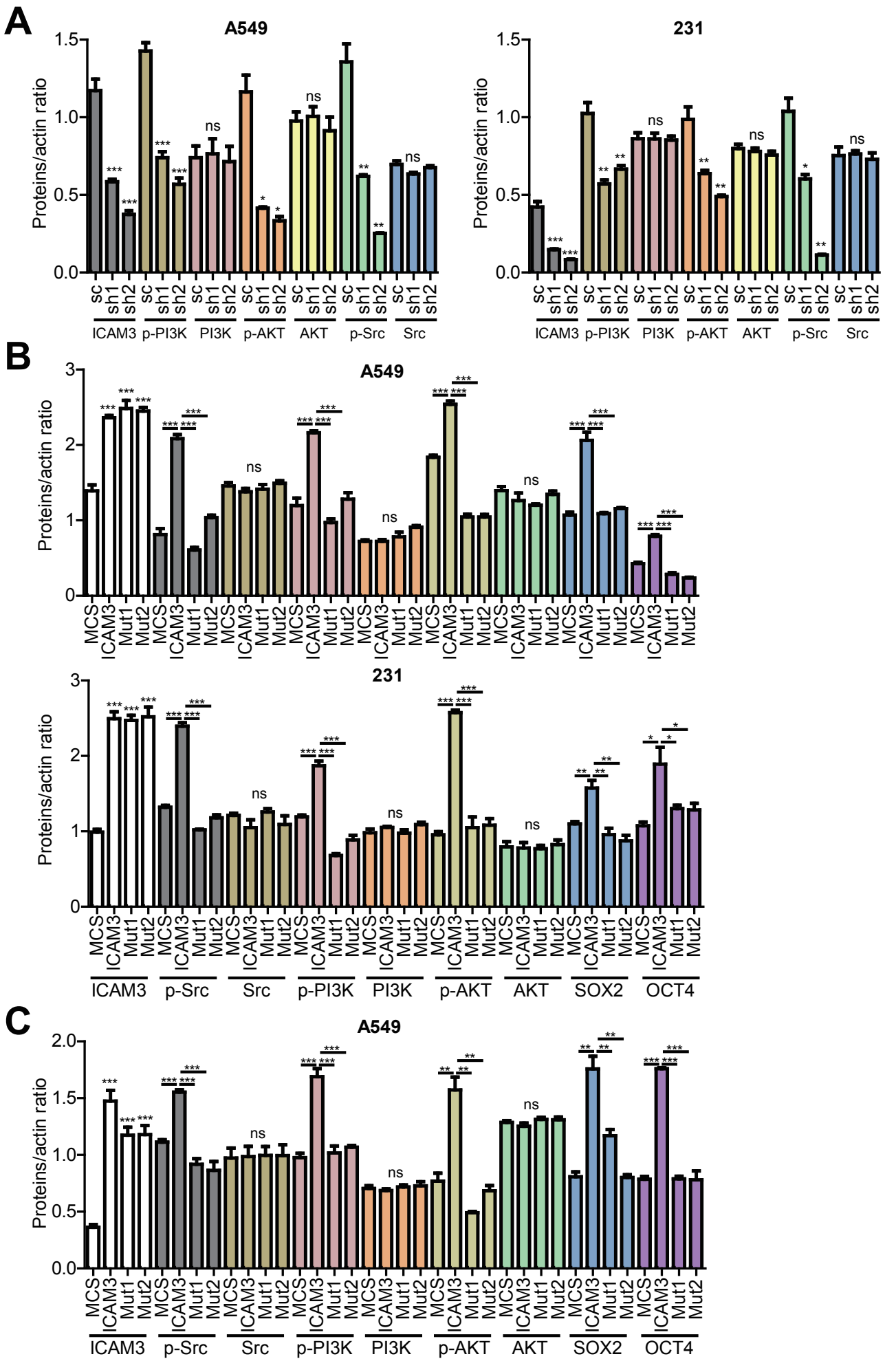


Figure S4. Quantification results of the western blot data by three biological repeats.

(A) Quantification results of figure 3A. (B) Quantification results of figure 3G. (C) Quantification results of figure 4B. **The bar graph shows the statistical result of three separate experiments.** *Indicates significant difference with $P<0.05$, **indicates significant difference with $P<0.01$, ***indicates significant difference with $P<0.001$.

Supplemental Figure 5

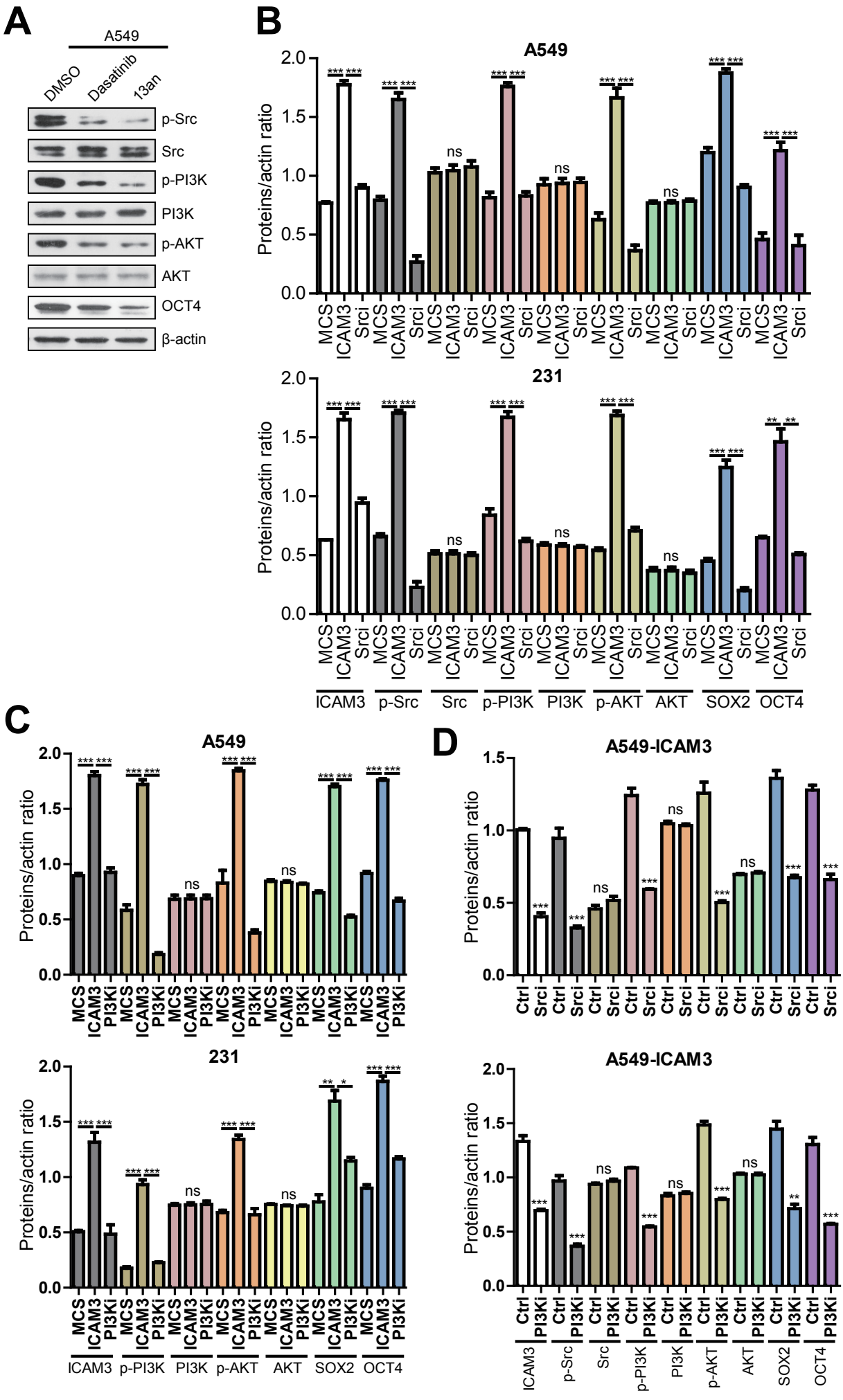


Figure S5. Quantification results of the western blot data by three biological repeats.

(A) Western was performed to detect the inhibition efficiency of different Src inhibitors to Src and downstream signaling activation. (B) Quantification results of figure 5A. (C) Quantification results of figure 5B. (D) Quantification results of figure 5C. **The bar graph shows the statistical result of three separate experiments.** *Indicates significant difference with $P<0.05$, **indicates significant difference with $P<0.01$, ***indicates significant difference with $P<0.001$.

Supplemental Figure 6

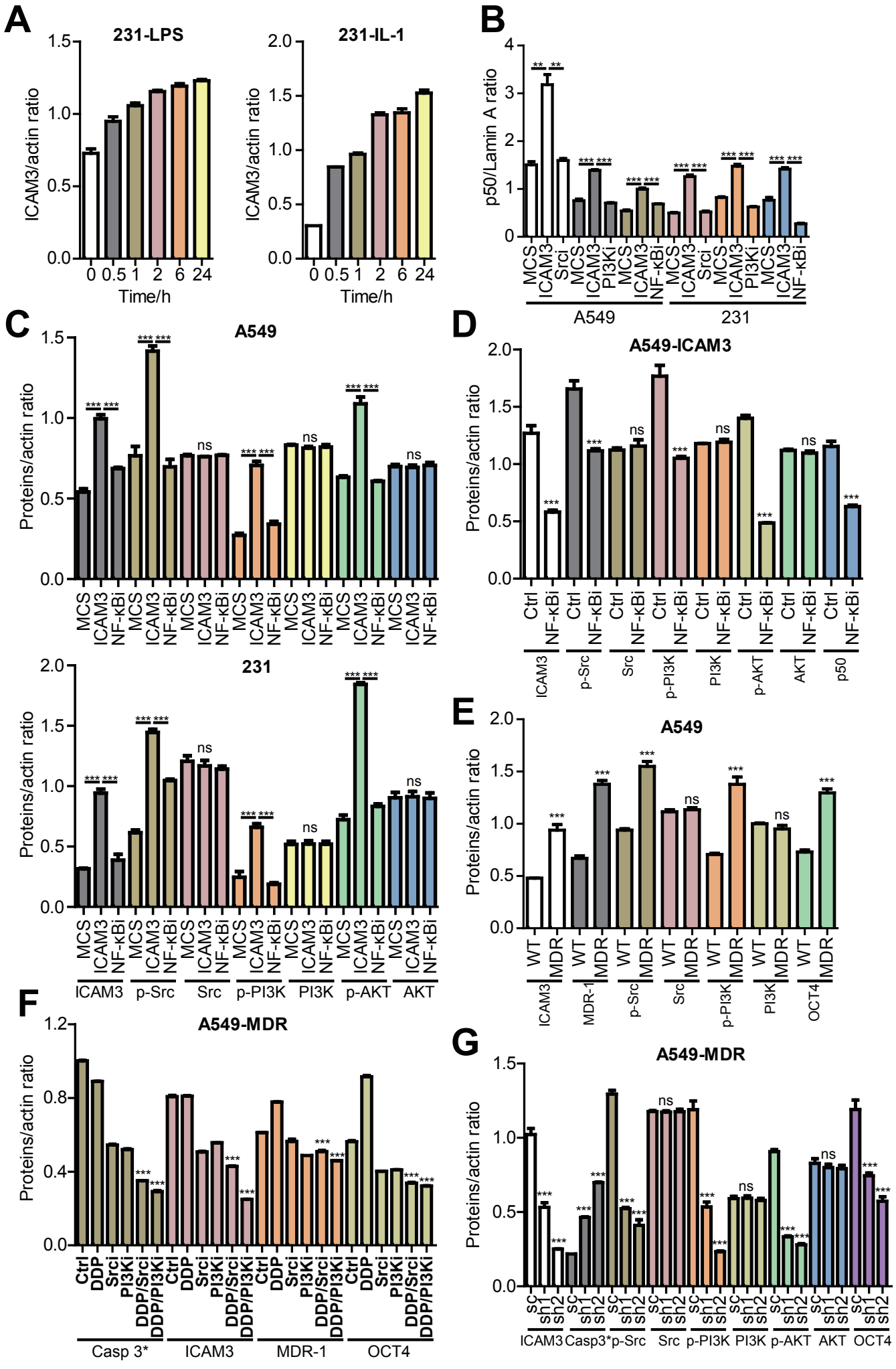


Figure S6. Quantification results of the western blot data by three biological repeats.

(A) Quantification results of figure 6A. (B) Quantification results of figure 6C. (C) Quantification results of figure 6D. (D) Quantification results of figure 6I. (E) Quantification results of figure 7A. (F) Quantification results of figure 7C. (G) Quantification results of figure 7G. **The bar graph shows the statistical result of three separate experiments.** *Indicates significant difference with $P<0.05$, **indicates significant difference with $P<0.01$, ***indicates significant difference with $P<0.001$.

Supplemental Table 1. Primer sequences

Name	Sequence
ICAM3-sh1	AAAAGCAGTACTGATTGTCCCAGCTTTGGATCCAAAGCTGGGACAATCAGTACTGC
ICAM3-sh2	AAAAGCAATGGCTCTCAGATAACAGTTGGATCCAACTGTTATCTGAGAGCCATTGC
SC	AAAAGCTACACTATCGAGCAATTTTGGATCCAAAATTGCTCGATAGTGTAGC
Flag-ICAM3-F	GCTCTAGAGCCACCATGGATTACAAGGATGACGACGATAAGAGCCCGATGGCCACCATGGTACC
Flag-ICAM3-R	CGACGCGTTCACTCAGCTCTGGACGG
ICAM3-mut1-R	CGACGCGTTCACTCAGCTCTGGACGGTTCTTCCCCCATTGCTTCTGTCGGCTGCATAGACGTGAGGGGCAGAGCGGTGCTCTCC
ICAM3-mut2-R	CGACGCGTCTAGGTGCTCTCCTCCCTAAC
ICAM3-CHIP-1-F	ATTGACTTAGCGCTTTCTCTGC
ICAM3-CHIP-1-R	TTAGGGAGTTTGAAGGCTTTATT
ICAM3-CHIP-2-F	GTGGGGATCCCGTTCTTC
ICAM3-CHIP-2-R	TCCCTGAAGACGTACATTAAGG
ICAM3-CHIP-3-F	CTTGCACAGGAACAGTAGCG
ICAM3-CHIP-3-R	ACGAAGAACGGGATCCC

ICAM3-CHIP-4-F	GAGGAAAGGGGAGGGC
ICAM3-CHIP-4-R	CGCTACTGTTCCTGTGCAAG

Supplemental Table 2. Antibodies

Antibody		Clone, Cat #	Vendor	City, State, Country
ICAM3	Rabbit monoclonal	EPR3994, ab109405	Abcam	Hong Kong, China
OCT4	Rabbit polyclonal	ab19857	Abcam	Hong Kong, China
Nanog	Rabbit polyclonal	ab80892	Abcam	Hong Kong, China
Akt1/2/3	Rabbit monoclonal	ab32505	Abcam	Hong Kong, China
SOX2	Rabbit polyclonal	H-65, sc-20088X	Santa Cruz Biotechnology	Santa Cruz, CA, USA
β-actin	Mouse monoclonal	sc-47778	Santa Cruz Biotechnology	Santa Cruz, CA, USA
ABCG2	Mouse monoclonal	sc-377176	Santa Cruz Biotechnology	Santa Cruz, CA, USA
MDR-1	Mouse monoclonal	sc-55510	Santa Cruz Biotechnology	Santa Cruz, CA, USA
β-Catenin	Rabbit monoclonal	#8480	Cell Signal Technology	Danvers, MA, USA
Src	Rabbit monoclonal	36D10, 2109s	Cell Signal Technology	Danvers, MA, USA
p-Src(Tyr416)	Rabbit monoclonal	D49G4, 6943t	Cell Signal Technology	Danvers, MA, USA
p-AKT(Ser473)	Rabbit monoclonal	D9E, 4060s	Cell Signal Technology	Danvers, MA, USA
PI3K p85	Rabbit monoclonal	4292s	Cell Signal Technology	Danvers, MA, USA
p-PI3K	Rabbit monoclonal	4228s	Cell Signal Technology	Danvers, MA, USA
Caspase3	Rabbit monoclonal	9662s	Cell Signal Technology	Danvers, MA, USA
Lamin A/C	Rabbit polyclonal	A01455-40	GenScript	Piscataway, NJ, USA
P50	Rabbit polyclonal	14220-1-AP	Proteintech	Rosemont, CHI, USA

Supplemental table 3

Cytokines & cytokine signaling （**10**）	**Adhesion Extravasation Migration** （**23**）	**Leukocyte signaling** （**2**）	**MAPK signaling** （**12**）	**Others** (**25**)
STAT2	CTTN	SLAMF7	RAPGEF3	PLA2G2A
SOCS3	MMP7	LAT	EEF2K	ALOX5AP
IL1A	MMP10		BCAR1	MGST2
IL1B	SELPLG		MAP2K4	PTGER4
TGIF2	PXN		KSR1	TBXAS1
IL17RA	ITGAX		PRKCA	PTGS1
PDGFB	MYH10		MEF2B	RGS1
IL12A	CCL8		MEF2C	PDE3A
CSF2	CCL13		YWHAG	PDE4C
IFNB1	CCL1		DUSP4	HSP90B1
	CCL5		ELK1	KIAA1271
	CCL16		DUSP9	RELA
	CCR7			NFRKB
	ITGA3			BCL3
	ICAM2			EIF2AK2
	PECAM1			NFKB1
	ICAM1			IKBKB
	ICAM5			AKT2
	ICAM3			PRTN3
	CEACA5			TNFRSF9
	CCR2			LTBR
	CCRL2			TNFRSF12A
	ALCAM			BBC3
	ICAM1			BCL2L12
	ICAM2			CASP10
	ICAM3			
	ICAM5			
	ALCAM			
	PECAM1			
	CEACAM5			
	ITGA3			
	ITGAX			
	SELPLG			
	PXN			

Supplemental table 3. 72 hit genes from twice high throughput siRNA screen.

The 72 hit genes from twice high throughput siRNA screen were listed in the table and they were divided into 5 groups including cytokines & cytokine signaling molecules, adhesion-extravasation-migration pathway molecules, leukocyte signaling molecules, MAPK signaling molecules and others.