Supporting Information

Shear Stress Promotes Metastasis of Triple-negative Breast Cancer Cells Through Calcium Channel–ROS–FOS Axis

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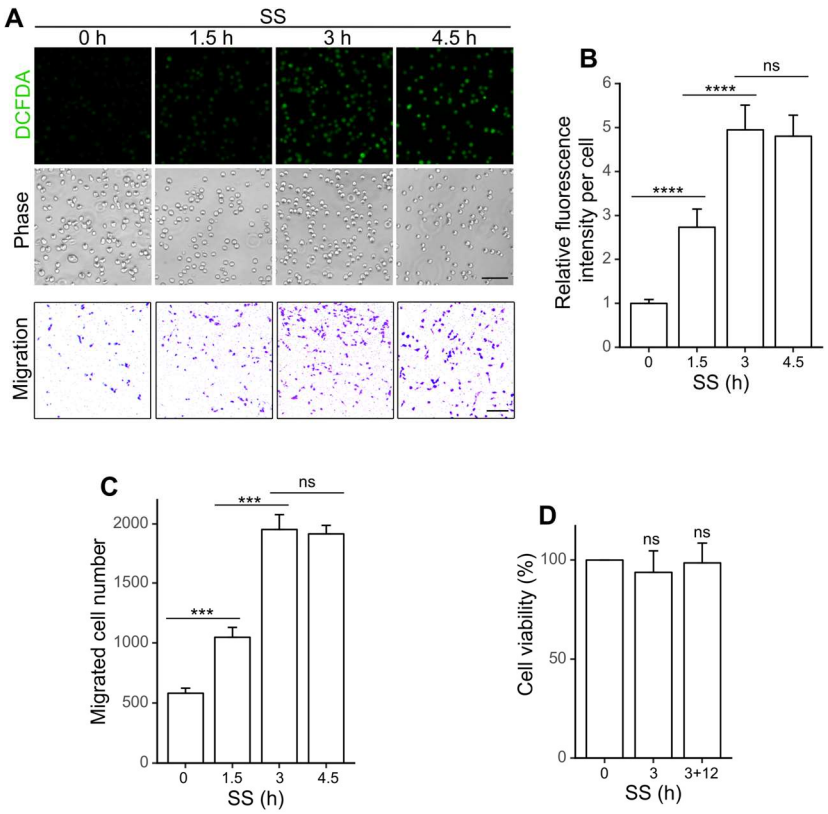

Figure S1. SS treatment induced ROS elevation and cell migration which reached a plateau at 3 h without affecting viability. (A-C) Representative images and quantifications of cellular ROS levels and migration assay in MDA-MB-231 cells under indicated conditions. For ROS detection, cells were stained with 5 μM CM-H_2-DCFDA for 15 min. For migration assay, 10^4 cells were seeded. Scale bar, 100 μm for ROS, 200 μm for migration. (D) Quantified results of cell viability before and after 3-h SS treatment, or after 12-h recovery post-SS treatment (SS 3+12 h, for observing delayed cell death) by MTT assay. The quantifications represent the means ± SD for three independent experiments. Significance was determined by t-test. *** $P < 0.001$, **** $P < 0.0001$, ns, not significant.

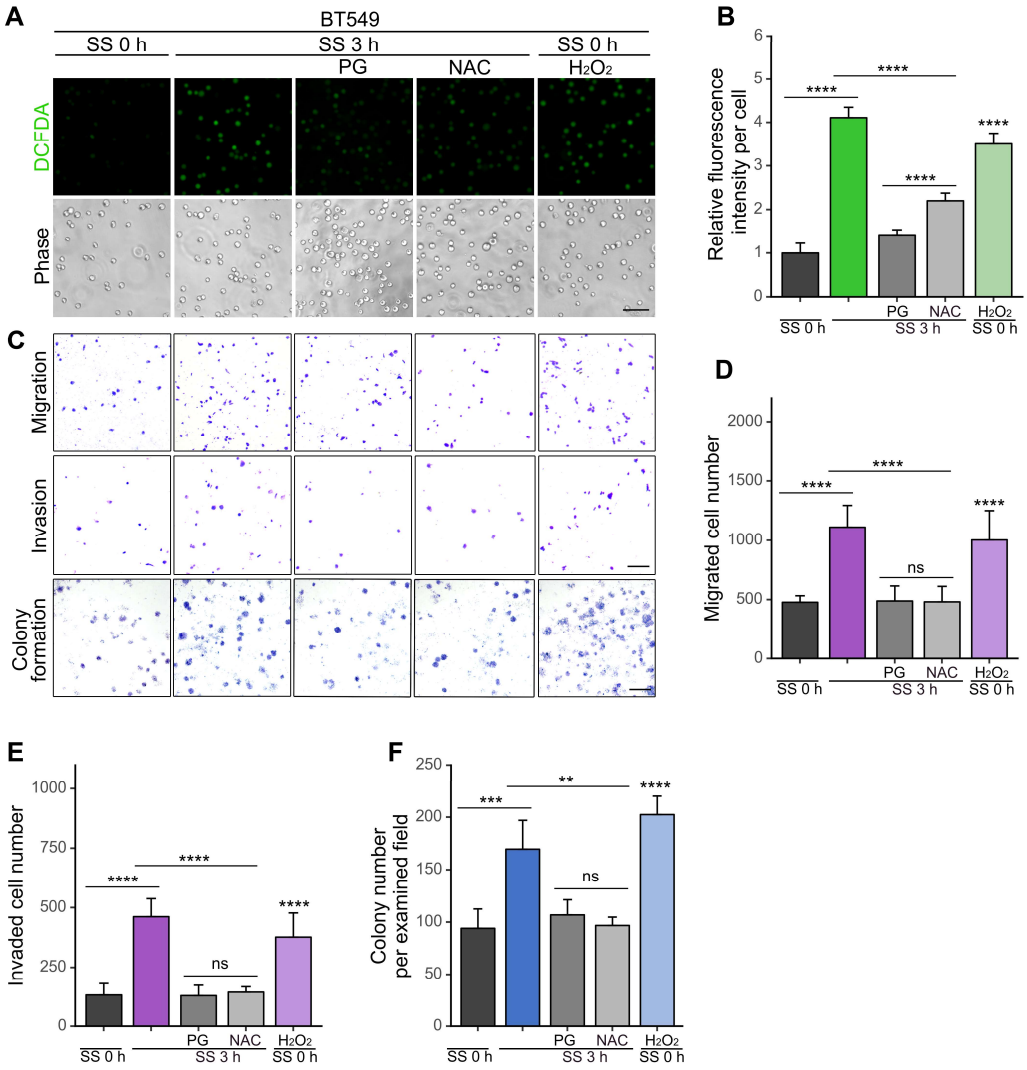

Figure S2. SS-induced ROS, migration, invasion and colony formation were also observed in BT549 cells. (A-B) Representative images and quantified results of cellular ROS levels in BT549 cells under indicated conditions: pre-treatment with 50 μM H_2O_2 for 3 h, other conditions were as described earlier. Scale bar, 100 μm. (C-F) Representative images and quantifications of migration, invasion and colony formation assays of BT549 cells under indicated conditions. For migration and invasion assays, 5,000 cells were seeded and allowed to migrate or invade for 18 h. For colony formation assay, 1,000 cells were seeded and allowed to grow for 10 days. Scale bar, 200 μm for migration and invasion, 2 mm for colony formation. The quantifications represent the means ± SD for three independent experiments. Significance was determined by one-way ANOVA with Tukey's test. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$, ns, not significant.

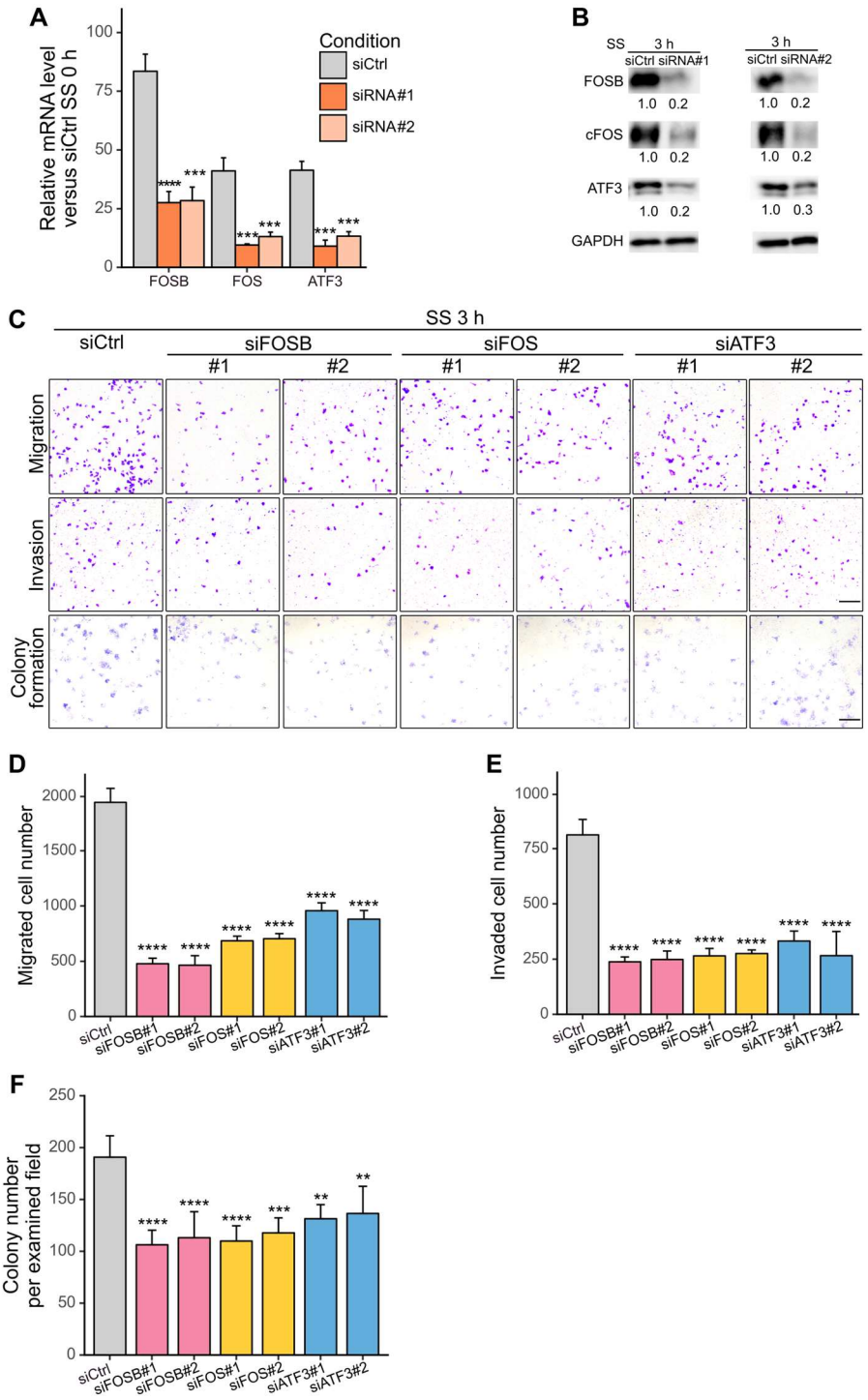

Figure S3. FOSB, FOS and ATF3 contributed to SS-induced metastatic abilities *in vitro*. (A-B) qPCR and Western blotting of knockdown efficiency in SS-treated MDA-MB-231 cells after knocking down FOSB, FOS or ATF3 using siRNAs. (C-F) Representative images and quantifications of migration, invasion and colony formation assays of siRNA-knocked-down cells. Scale bar, 200 μm for migration and invasion, 2 mm for colony formation. The quantifications represent the means (± SD) for three independent experiments. Significance was determined by one-way ANOVA with Tukey's test. ** $P < 0.01$, *** $P < 0.001$, **** $P < 0.0001$.

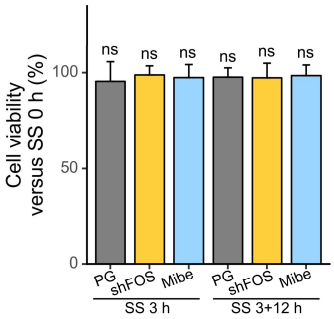

Figure S4. ROS, FOS and calcium channel activity were not responsible for cell survival under SS conditions. Quantified results of cell viability under SS treatment compared to no treatment by MTT assay, after scavenging ROS with PG, FOS knockdown or inhibiting calcium channels with Mibe. SS 3+12 h represented SS treatment for 3 h followed by 12-h culture for capturing delayed cell death events. The quantifications represent the means ± SD from three independent experiments. Significance was determined by t-test. ns, not significant.

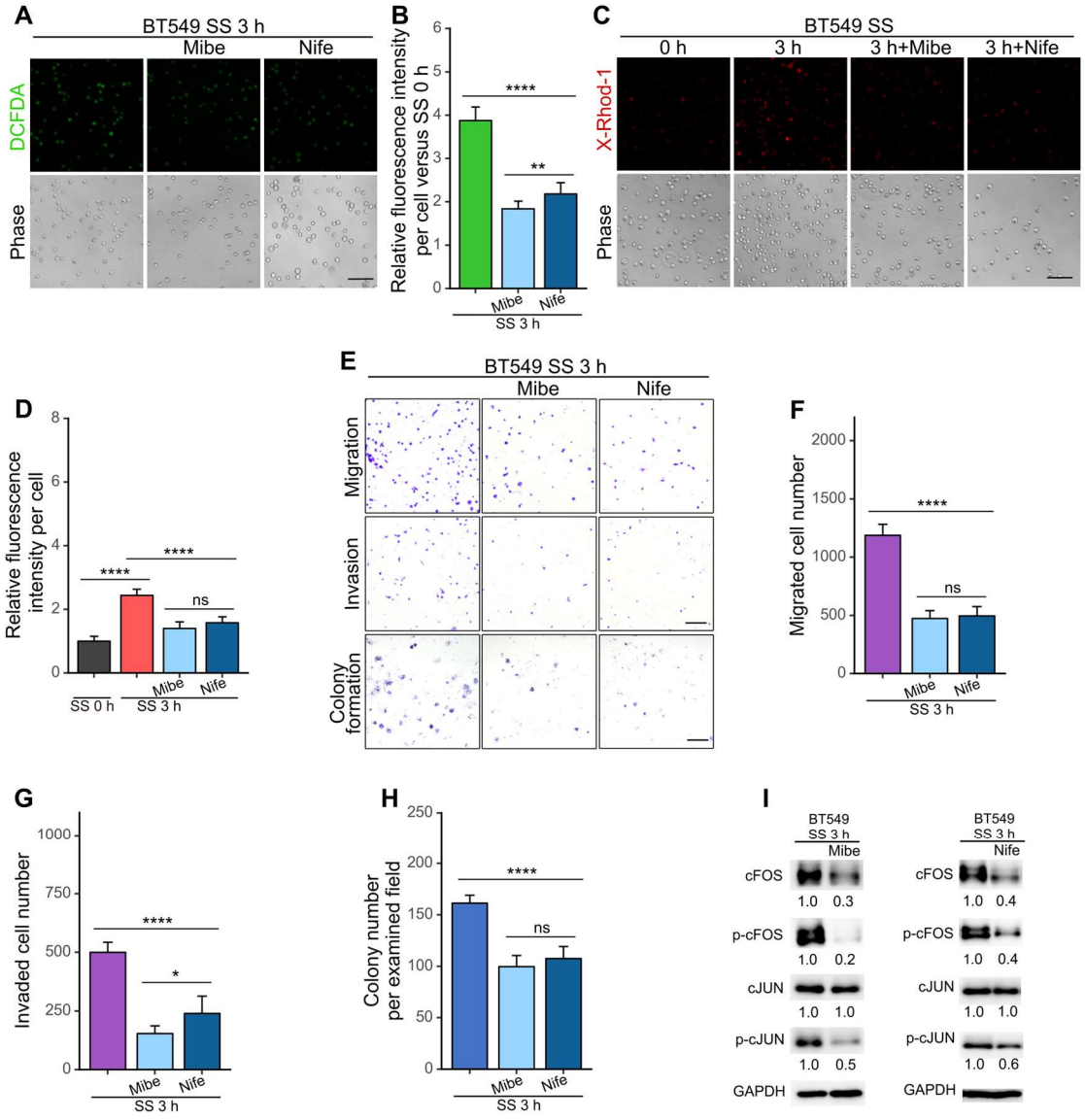

Figure S5. SS–calcium–ROS–metastasis axis was also functional in BT549 cells. (A-D) Representative images and quantified results of cellular ROS and Ca^{2+} levels under SS, with

or without pre-treatment and co-circulation with 20 μM Mibe or 100 μM Nife. Cells were stained with 5 μM CM-H_2-DCFDA (for ROS) or 2 μM X-Rhod-1 (for Ca^{2+}) for 15 min. Scale bar, 100 μm. (E-H) Representative images and quantifications of migration, invasion and colony formation assays under indicated conditions. Scale bar, 200 μm for migration and invasion, 2 mm for colony formation. (I) Western blotting showing the protein levels of cFOS, p-cFOS, cJUN and p-cJUN under indicated conditions. Relevant experiments were performed in BT549 cells. The quantifications represent the means (± SD) for three independent experiments. Significance was determined by one-way ANOVA with Tukey's test (B, D, F-H). * $P < 0.05$, ** $P < 0.01$, **** $P < 0.0001$, ns, not significant.

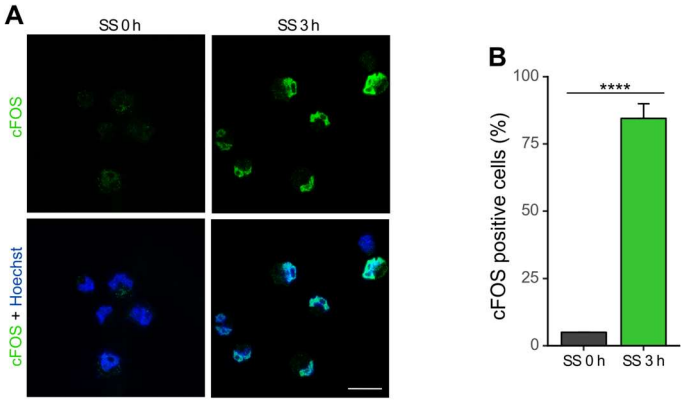

Figure S6. cFOS elevation was induced by SS treatment in a large proportion of cells. (A-B) Representative images and quantifications of immunofluorescence of cFOS in MDA-MB-231 cells before and after 3-h SS treatment. Scale bar, 20 μm. The 95th percentile of fluorescence intensity in untreated cells was set as the threshold for distinguishing cFOS positive and negative cells. The quantifications represent the means ± SD from three independent experiments (n ≥ 100 cells). Significance was determined by t-test. **** $P < 0.0001$.

Supplementary tables

Table S1. List of primers for qPCR

Gene name	Forward (5'-3')	Reverse (5'-3')
FOSB	GCTGCAAGATCCCCTACGAAG	ACGAAGAAGTGTACGAAGGGTT
RND1	TTAGCGAAGGATTGCTATCCAGA	GTATCCCAGAGACTAAGCTCCA
ATF3	CCTCTGCGCTGGAATCAGTC	TTCTTTCTCGTCGCCTCTTTTT
EGR2	TCAACATTGACATGACTGGAGAG	AGTGAAGGTCTGGTTTCTAGGT
GPR132	AAGGTGACCGCCTACATCTTC	GTCTTCCGTCTGGAACACCG
JUNB	ACGACTCATACACAGCTACGG	GCTCGGTTTCAGGAGTTTGTAGT
CYP1A1	TCGGCCACGGAGTTTCTTC	GGTCAGCATGTGCCCAATCA
SNAI1	TCGGAAGCCTAACTACAGCGA	AGATGAGCATTGGCAGCGAG
IL6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG

CXCL8	TTTTGCCAAGGAGTGCTAAAGA	TTTTGCCAAGGAGTGCTAAAGA
JUN	TCCAAGTGCCGAAAAAGGAAG	CGAGTTCTGAGCTTTCAAGGT
JUND	TCATCATCCAGTCCAACGGG	TTCTGCTTGTGTAAATCCTCCAG
FOS	GGGGCAAGGTGGAACAGTTAT	CCGCTTGGAGTGTATCAGTCA
FOSL1	CAGGCGGAGACTGACAAACTG	TCCTTCCGGGATTTTGCAGAT
FOSL2	CAGAAATTCCGGGTAGATATGCC	GGTATGGGTTGGACATGGAGG
ATF2	AATTGAGGAGCCTTCTGTTGTAG	CATCACTGGTAGTAGACTCTGGG
ATF4	ATGACCGAAATGAGCTTCCTG	GCTGGAGAACCCATGAGGT
ATF7	GAGACGACAGACCGTTTGTGT	AGGCGTTTGATCTGCAATGAT
GAPDH	CTGGGCTACACTGAGCACC	AAGTGGTCGTTGAGGGCAATG

Table S2. List of Antibodies

Antibody name	**Company**	**Catalog #**	**Application**
ATF3	CST*	18665	WB (1:1000), IF (1:100)
CCND1	CST	55506	WB (1:1000)
CCND3	CST	2936	WB (1:1000)
CD44	CST	3570	WB (1:1000)
ELK1	CST	9182	WB (1:1000)
p-ELK1 (Ser383)	CST	9181	WB (1:1000)
cFOS	CST	31254	WB (1:1000), IF (1:100), IHC (1:20)
p-cFOS (Ser32)	CST	5348	WB (1:1000), IF (1:100)
FOSB	CST	2251	WB (1:1000), IF (1:100)
GAPDH	CST	2118	WB (1:1000)
JNK	CST	9252	WB (1:1000)
p-JNK (Thr183/Tyr185)	CST	9251	WB (1:1000)
cJUN	CST	9165	WB (1:1000), IF (1:100)
p-cJUN (Ser73)	CST	3270	WB (1:1000), IF (1:100)
MMP-1	CST	54376	WB (1:1000)
MMP-2	CST	87809	WB (1:1000)
MMP-3	Abcam	Ab52915	WB (1:1000)
MMP-9	CST	13667	WB (1:1000)
N-cadherin	CST	13116	WB (1:1000)
p38	CST	8690	WB (1:1000)
p-p38	CST	4511	WB (1:1000)
Slug	CST	9585	WB (1:1000)
Snail	CST	3879	WB (1:1000)
Vimentin	CST	5741	WB (1:1000)
ZEB1	Sigma	90510	WB (1:1000)

Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor Plus 488	Invitrogen	A11034	IF (1:100)
Goat anti-Rabbit IgG (H+L) Secondary Antibody, Alexa Fluor Plus 594	Invitrogen	A11037	IF (1:100)
Goat anti-Rabbit IgG (H+L)-HRP Secondary Antibody	Bio-Rad	1706515	WB (1:5000)
Goat anti-Mouse IgG (H+L)-HRP Secondary Antibody	Bio-Rad	1706516	WB (1:5000)

*CST: Cell Signaling Technology

Table S3. List of siRNAs

siRNA name	**Target sequence (5'-3')**
siFOSB#1	UGUUAACCCUUCGUACACUUCTT
siFOSB#2	UGCAAGAUCCCCUACGAAGAGTT
siFOS#1	UCCAGAAGAAGAAGAGAAAAGTT
siFOS#2	UUCAUUAUUGGAAUUAACCUGTT
siATF3#1	AUAUUACUUAUUUAUCCUAGUTT
si ATF3#2	CUGUCAGAAUAAUAAAUAUUGTT
shCtrl	UUCUCCGAACGUGUCACGUTT

Table S4. List of shRNAs

shRNA name	**TRC Number**	**Target sequence (5'-3')**
shFOSB		UGUUAACCCUUCGUACACUUCTT
shFOS		UCCAGAAGAAGAAGAGAAAAGTT
shATF3		AUAUUACUUAUUUAUCCUAGUTT
shNC	SHC002	CAACAAGATGAAGAGCACCAA

Table S5. List of overexpression vectors

Vector name	**Transcript ID**	**Sequence length**
FOSB	NM_006732.3	1017 bp
FOS	NM_005252.4	1188 bp
ATF3	NM_001674.4	1917 bp