Supplementary Information

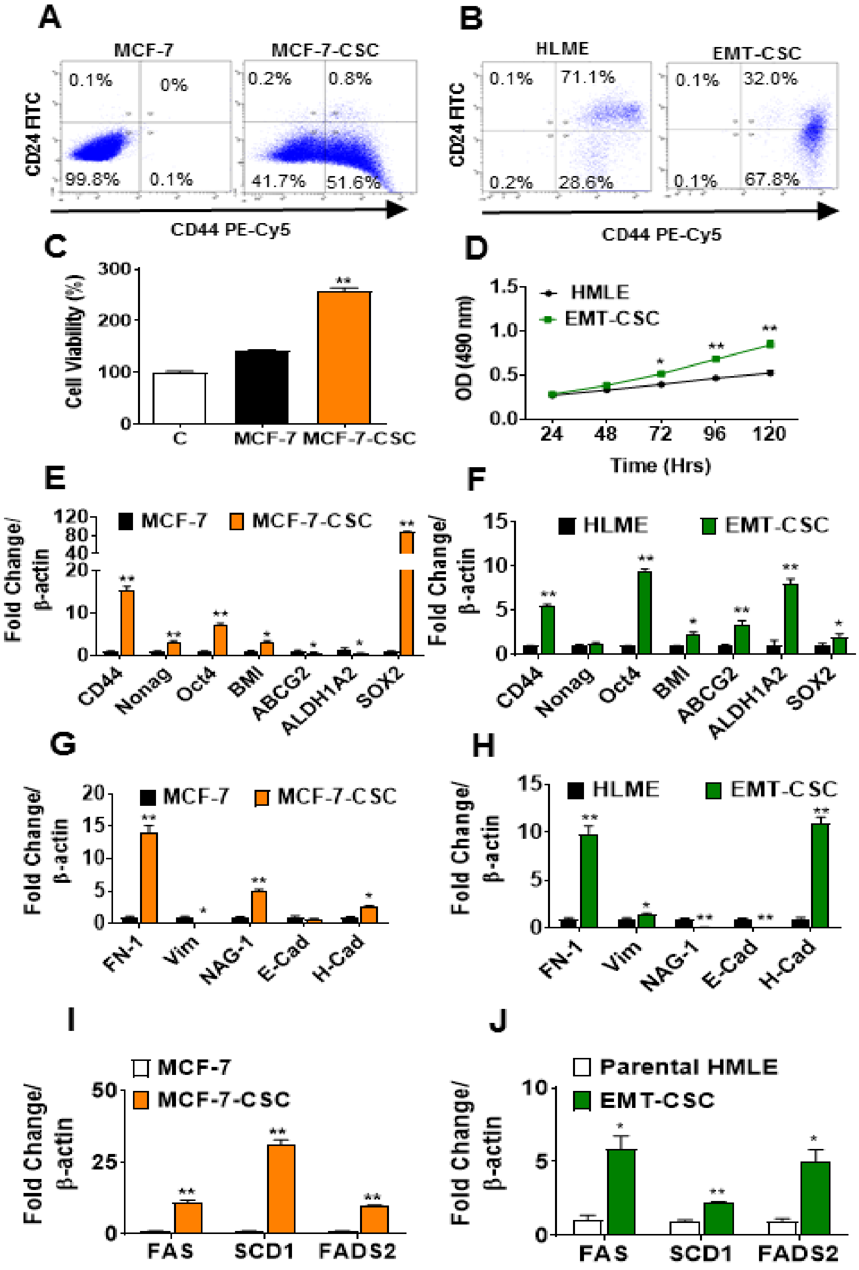

Figure. S1 The characteristics of breast cancer stem cells.

The breast CSC were identified by CD24^{low} and CD44^{+} using flow cytometry. The representative image of flow cytometry analysis in MCF-CSC (A) and EMT-CSC (B). (C) Comparison of cell viability between MCF-7 mammosphere cells (MCF-7-CSC) and MCF-7 cells after 72 h. The relative cell viability was based on the initial cell number of MCF-7 cells (100%). (D) Comparison of cell proliferation of parental HLME cells and EMT-CSC. mRNA expression of stem cell markers genes in MCF-7-CSC (E) and

EMT-CSC (F). mRNA expressions of EMT-related genes in MCF-7-CSC (G) and EMT-CSC (H). (I-J) Relative densiometric bar graphs of FAS, SCD1, and FADS2 analyzed from Fig. 1E&F. β-actin was used as a loading control. * $P<0.05$; ** $P<0.01$ vs. their control cells.

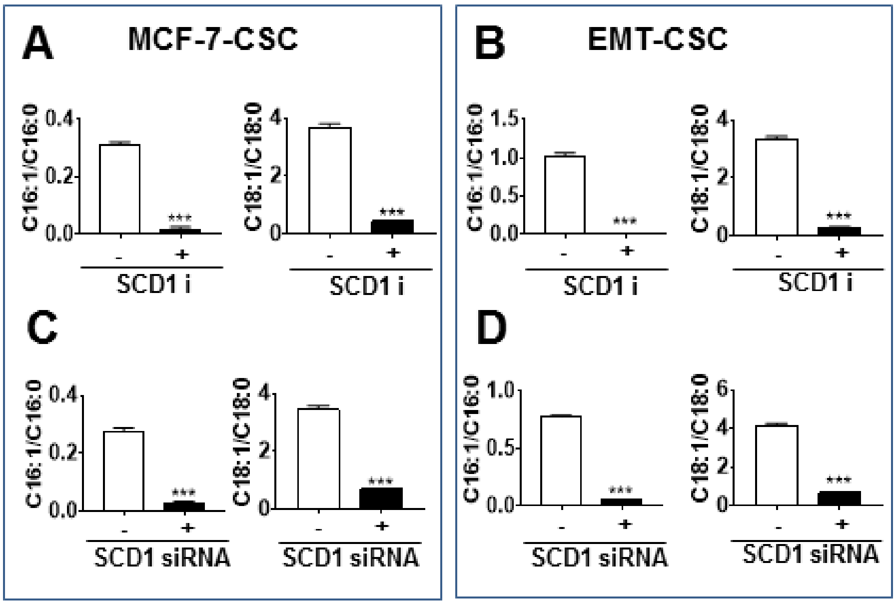

Figure. S2 The distinct fatty acid profile before and after SCD1 inhibitor treatment or siRNA knockdown.

Prior to measuring the fatty acid profiles of MCF-7-CSC and EMT-CSC, the cells were treated with SCD1 inhibitor for 48 h. The ratios of C16:1/C16:0 and C18:1/C18:0 in MCF-7-CSC were shown in (A). The ratios of C16:1/C16:0 and C18:1/C18:0 in EMT-CSC were shown in (B). After successful knockdown in SCD1, the fatty acid profiles were measured using GC. The changes of C16:1/C16:0 and C18:1/C18:0 in MCF-7-CSC were shown in (C). The changes of C16:1/C16:0 and C18:1/C18:0 in EMT-CSC were shown in (D).

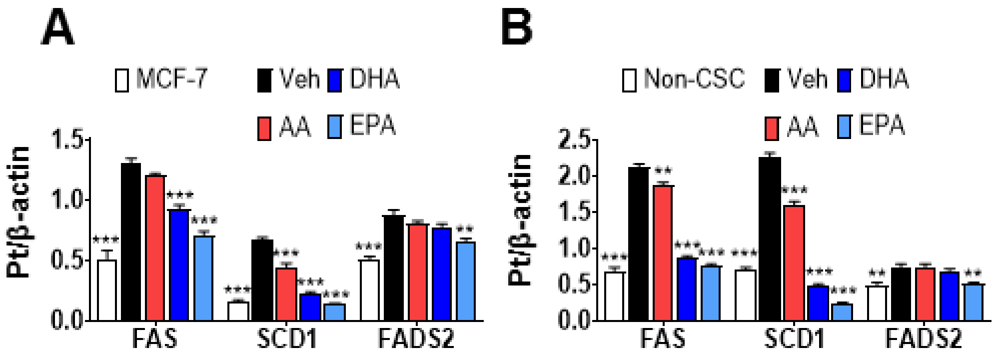

Figure. S3 n-3 PUFA suppress the growth of breast cancer stem cells by blocking lipid metabolism *in vitro*.

Representative Western blot for protein expressions were shown in Fig. 4E&F. Relative densiometric bar graphs of FAS, SCD1, and FADS2 were plotted in (A-B). β-actin was used as a loading control. ** *P*<0.01; *** *P*<0.001 vs. their parental cells.

Table. S1 Comparison of the fatty acid profile (%) of CSC and non-CSC

	HMLE		**MCF-7**	
	Parental	**EMT-CSC**	**MCF-7**	**MCF-7-CSC**
C10:0	0.1±0.05	0.11±0.06	1.33±0.15	1.63±0.34
C12:0	3.54±0.32	3.56±0.02	5.74±0.21	5.67±0.24
C16:0	21.83±0.61	18.79±0.62*	34.02±0.17	30.07±1.53*
C16:1	17.56±0.48	23.96±0.33**	4.89±0.53	13.09±0.07**
C18:0	13.1±0.82	9.23±0.59*	21.48±0.58	15.13±1.06**
C18:1	35.72±0.7	38.06±0.86*	23.38±0.66	25.17±0.14**
C18:2 n-6	0.9±0.02	0.43±0.03**	8.72±0.38	1.95±0.37**
C20:4 n-6	0.19±0.05	0.92±0.04**	ND	3.92±0.42**
C20:5 n-3	ND	ND	ND	ND
C22:5 n-3	ND	ND	ND	1.05±0.14**
C22:6 n-3	ND	ND	ND	0.95±0.14**
SFA	42.82±1.34	37.27±0.57**	63.01±1.16	52.79±2.05**
MUFA	56.09±1.32	61.7±0.56**	28.27±1.17	40.02±1.9**
PUFA	1.09±0.05	1.03±0.03	8.72±0.38	7.2±1.04
n-6 PUFA	1.09±0.05	1.03±0.03	8.72±0.38	5.87±0.17**
n-3 PUFA	ND	ND	ND	2±0.28**

The abbreviation was listed as follows, PUFA: Polyunsaturated fatty acid; MUFA: Monounsaturated fatty acid; SFA: Saturated fatty acid. C10:0: Capric acid; C12:0: Lauric acid; C16:0: Palmitic acid; C16:1: Hypogenic acid; C18:0: Stearic acid; C18:1: Oleic acid; C18:2 n-6: Linoleic acid; C20:4 n-6: Arachidonic acid; C20:5 n-3: Eicosapentaenoic acid; C22:5 n-3: Docosapentaenoic acid; C22:6 n-3: Docosahexaenoic acid. SFA: Saturated fatty acids;

MUFA: Monounsaturated fatty acids; PUFA: Polyunsaturated fatty acids; ND: not detectable; CSC vs Non-CSC or control, *$P < 0.05$; **$P < 0.01$.

Table. S2 Comparison of MUFA in CSC treated SCD1 inhibitor or siRNA

	EMT-CSC				**MCF-7-CSC**			
	siRNA control	SCD1 siRNA	Inhibitor control	SCD1 inhibitor	siRNA control	SCD1 siRNA	Inhibitor control	SCD1 inhibitor
C16:0	20.44±0.38	34.33±0.76	21.7±0.57	39.92±0.27	28.27±1.05	37.94±0.65	28.5±0.57	42.38±0.71
C16:1	15.95±0.25	1.84±0.12	22.46±0.41	ND	7.82±0.39	1.1±0.41	8.91±0.64	1.05±0.22
C18:0	10.55±0.16	31.14±0.36**	10.48±0.22	34.82±0.47**	11.92±0.69	28.23±0.45**	11.4±0.41	27.61±0.63**
C18:1	44.15±0.44	21.47±0.45**	35.2±0.42	10.45±0.18**	41.09±0.17	20.29±0.37**	41.65±1.5	12.83±0.23**

SCD1: Stearoyl-CoA desaturase 1; SCD1 siRNA or SCD1 inhibitor vs control, **$P<0.01$.

Table. S3 Comparison of fatty acid profiles (%) with different PUFA treatments in MCF-CSC

Fatty Acids$^{\#}$	MCF-7-CSC	AA	EPA	DHA
C10:0	1.66±0.37	1.49±0.40	1.26±0.38	0.91±0.12
C12:0	5.49±0.44	5.06±0.43	4.44±0.46	4.29±0.36*
C16:0	30.43±1.34	28.57±0.06	33.00±1.44	30.63±1.03
C16:1	13.13±0.33	12.27±0.21	10.00±0.75*	11.30±0.44*
C18:0	15.31±0.12	22.93±0.51**	31.67±1.22**	28.17±0.55***
C18:1	24.98±0.40	20.27±0.57**	13.33±1.15**	15.67±0.40***
C18:2 n-6	2.02±0.15	0.96±0.08**	0.81±0.05**	1.61±0.24
C18:3 n-3	ND	ND	ND	ND
C20:4 n-6	4.13±0.13	5.1±0.20**	2.06±0.13***	2.62±0.15***
C20:5 n-3	ND	ND	0.65±0.10***	ND
C22:5 n-3	0.55±0.07	0.58±0.07	0.63±0.07	0.94±0.06***
C22:6 n-3	0.54±0.08	0.43±0.05	0.76±0.10	1.53±0.17**
SFA	53.18±1.09	58.97±0.33*	71.76±0.33***	66.37±0.63***
MUFA	39.58±1.35	33.78±0.17*	23.33±0.4*	26.97±0.78**
PUFA	7.24±0.27	7.07±0.18	4.91±0.11**	6.70±0.22
C16:1/C16:0	0.43±0.02	0.43±0.01	0.30±0.02**	0.37±0.02*
C18:1/C18:0	1.63±0.03	0.88±0.01***	0.42±0.03***	0.56±0.01**
n-6 PUFA	6.15±0.20	6.06±0.26	2.87±0.08***	4.23±0.38**
n-3 PUFA	1.09±0.13	1.01±0.08	2.04±0.19**	2.47±0.23***

n-6/n-3	5.70±0.69	6.06±0.74	1.41±0.18*	1.73±0.30**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$ vs. MCF-7-CSC.

Table. S4 The basic information of 80 breast cancer patients for immunohistochemistry analysis

		Cases (#)	**Percentage (%)**
Histologic	IDC	49	61.2
	DSIC	2	2.5
	IDC+DSIC	21	26.2
	DSIC+IDC	3	3.7
	ILC	1	1.2
	IMC	2	2.5
	MA	2	2.5
Age	<60	29	36.3
	≥60	51	63.7
Grade	G1	5	6.2
	G2	33	41.3
	G3	38	47.5
	N/A	4	5.0
Stage	Ⅰ	4	5.0
	Ⅱ	34	42.5
	Ⅲ	32	40.0
	N/A	10	12.5
T classification	T1	36	45.0

	T2	38	47.5
	T3	5	6.3
	T4	1	1.2
N classification	N0	41	51.3
	N1	25	31.2
	N2	8	10.0
	N3	6	7.5

IDC: infiltrating ductal carcinoma; DCIS: ductal carcinoma in situ; ILC: infiltrating lobular carcinoma; IMC: invasive micropapillary carcinoma; MA: medullary/mucinous adenocarcinoma. N/A: not available.

Table S5. Fatty acid profile in tumor tissues (μg/mg of tissue)

Fatty Acid[1]	Control	n-3 PUFA
C12:0	0.19±0.09	0.04±0.03
C14:1	0.03±0.02	0.02±0.01
C16:0	2.20±0.98	1.75±0.43
C16:1	0.41±0.21	0.23±0.05
C17:0	0.22±0.06	0.48±0.13
C17:1	0.02±0.02	0.01±0.01
C18:0	1.36±0.56	1.25±0.33
C18:1	1.88±1.02	1.04±0.23
C18:2	1.90±1.04	1.19±0.28
C18:3	0.03±0.02	0.01±0.01
C20:0	0.06±0.02	0.05±0.01
C20:1	0.06±0.03	0.02±0.01
C20:2	0.07±0.03	0.03±0.01
C20:3	0.06±0.02	0.03±0.01
C20:4	0.60±0.26	0.41±0.17
C20:5	0.01±0.01	0.05±0.03
C22:0	0.08±0.02	0.06±0.01
C22:1	0.02±0.02	ND
C22:4	0.03±0.03	ND

C22:5	ND	0.04±0.04
C22:6	0.05±0.04	0.14±0.06
SFA	4.10±1.71	3.63±0.89
MUFA	2.42±1.31	1.32±0.28
PUFA	2.76±1.40	1.90±0.49
n-3 PUFA	0.09±0.04	0.24±0.10
n-6 PUFA	2.60±1.35	1.63±0.41
n-6/n-3	46.95±4.63	8.2 ±1.87

1 The abbreviation was listed as follows, C14:1 n-7: Myristoleate; C17:0: Heptadecanoate; C17:1: 10-Heptadecenoate; C20:0: Arachidate; C20:1: 11-Eicosenoate; C20:2: 11, 14-Eicosadienoate; C20:3 n-6: 11, 14, 17-Eicosatrienoate; C22:0: Behenate; C22:1: Erucate; C22:4 n-6: Docosatetraenoate. Others have been mentioned in Table S1.

Table S6. Primer sequences of gene used for real-time PCR

Gene	Forward (5’-3’)	Reverse (5’-3’)
Nanog	GAGACGGGGTTTCACTGTGT	CCTCCCAATCCCAAACAATA
COX2	GGATCTGTGGATGCTTCGTT	CCCAGGTTTTGTCAGCAGAT
Oct4	AGTGAGAGGCAACCTGGAGA	CAAAAACCCTGGCACAAACT
Sox2	AGTCTCCAAGCGACGAAAAA	GGAAAGTTGGGATCGAACAA
BMI	GTCCCCAGTCTGCAAAAGAA	AATGGGCTTTCAAGCAATTTT
ABCG2	GCCCTGACACTGGTCATCTT	GGGACAGGTATGTGAAAAGCA
ALDH1A2	TGAATTGCTATGGCGTGGTA	ACTTGGGGGTCACATTTCAG
NAG-1	CAGTCGGACCAACTGCTGGCA	TGAGCACCATGGGATTGTAGC
VIM	CCCTCACCTGTGAAGTGGAT	TCCAGCAGCTTCCTGTAGGT
CD44	GCCTGGTAGAATTGGCTTTTC	AGAGGAAGGGTGTGCTCTGA
FN-1	GCCCGCAATACTGTAGGAAC	AATTATTTCCCCCGAAGGTG
E-cadherin	TGCCCAGAAAATGAAAAAGG	GTGTATGTGGCAATGCGTTC
N-cadherin	ACAGTGGCCACCTACAAAGG	CCGAGATGGGGTTGATAATG
FAS	CGCTTGGTCTTTCTGTGCTT	TAAAAATGAAACGGGGTCCA
SCD1	GTGCCACTGACTTGCTGTGT	TGGCAATGCGTTGTTTATGT
D6D	CAGGGTGGAGCTGAGAAGAG	AAAATGTAGGGGTGGGGAAG
GAPDH	GTCAGTGGTGGACCTGACCT	AGGGGTCTACATGGCAACTG

Nanog: Nanog homeobox; Sox2: Sex-determining region Y-box2; Oct4: Octamer-binding protein; BMI: B lymphoma Mo-MLV insertion region 1 homolog; ABCG2: ATP-binding cassette sub-family G member 2; ALDH1A2: Aldehyde dehydrogenase 1 family; member A2, NAG-1:

Anti-inflammatory drug activated gene-1; VIM: Vimentin; FN-1: Fibronectin 1; FAS: Fatty acid synthase; SCD-1: Stearoyl-CoA desaturase enzyme 1;

D6D: Delta 6-Desaturase; GAPDH: Glyceraldehyde-3-phosphate dehydrogenase