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Supporting Information

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A novel long non-coding RNA lnc030 maintains breast cancer stem cell stemness by stabilizing SQLE mRNA and increasing cholesterol synthesis

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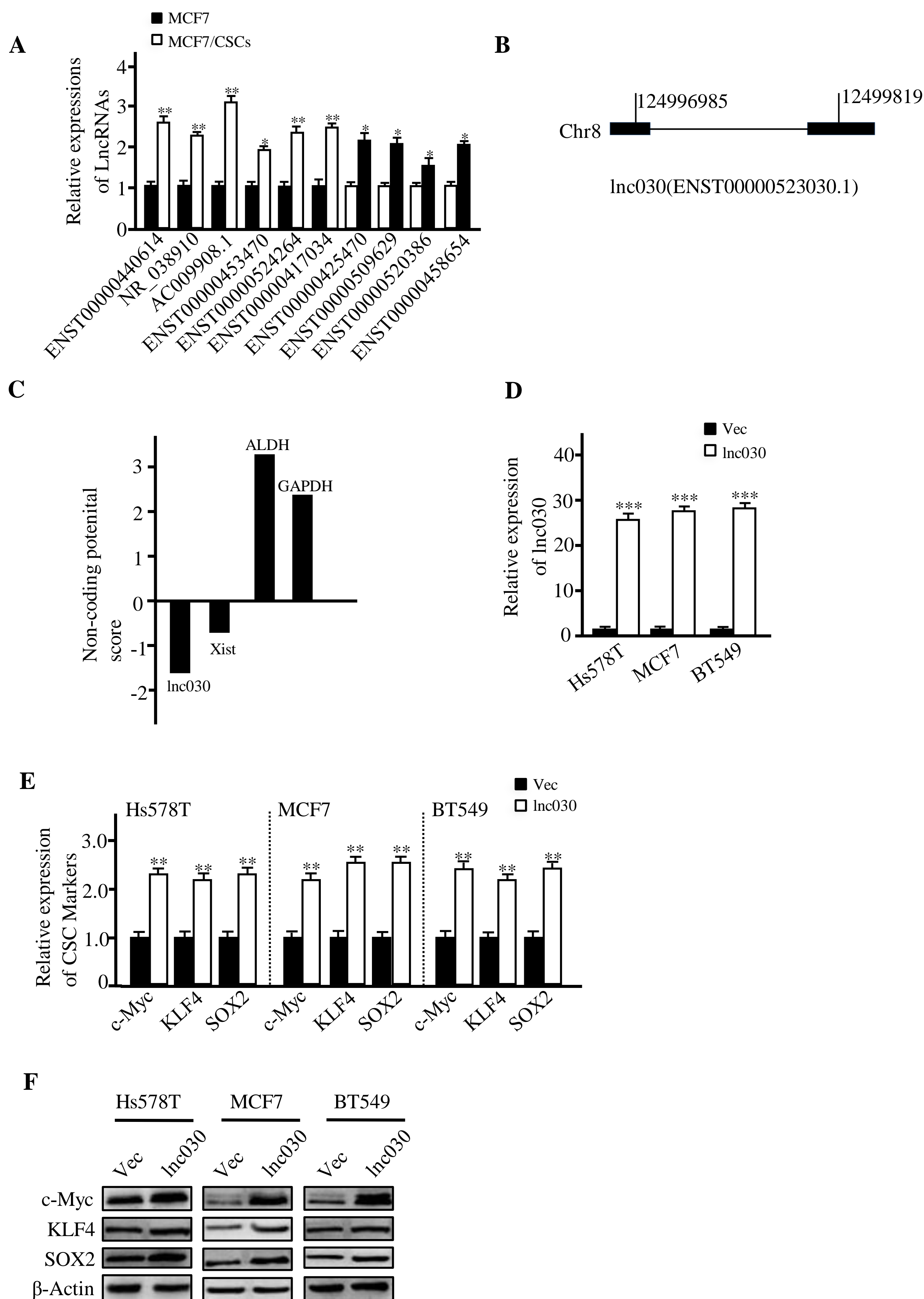
Fig. S1



Supplementary Figure legends

Figure S1. Lnc030 is highly expressed in BCSCs

(A). The expressions of LncRNAs were detected by qRT-PCR in MCF7 and MCF7/CSCs. qRT-PCR data are shown as means ± SD (*p<0.05, **p<0.01). (B). Annotated map of lnc030 on chromosome 8. (C). Coding potency of Lnc030 sequence was analyzed by Coding Protential Calculation. X inactivation-specific transcript (XIST) served as a control non-coding gene. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and β-actin serve as control coding genes. Scores more than 0 suggest a coding potential of lncRNA, whereas scores less than 0 represent no coding potential of the evaluated LncRNA. (D). Lnc030 was transfected into BCs by lentivirus. Lnc030-overexpressing stable cells were established. Data are shown as means ± SD (***p<0.001). (E, F). The expression of pluripotent transcription factors c-Myc, KLF4, and SOX2 was determined in the indicated breast cells with ectopic lnc030 and vector control by qRT-PCR (E) and Western blotting (F). qRT-PCR data are shown as means ± SD (**p<0.01).

Fig. S2

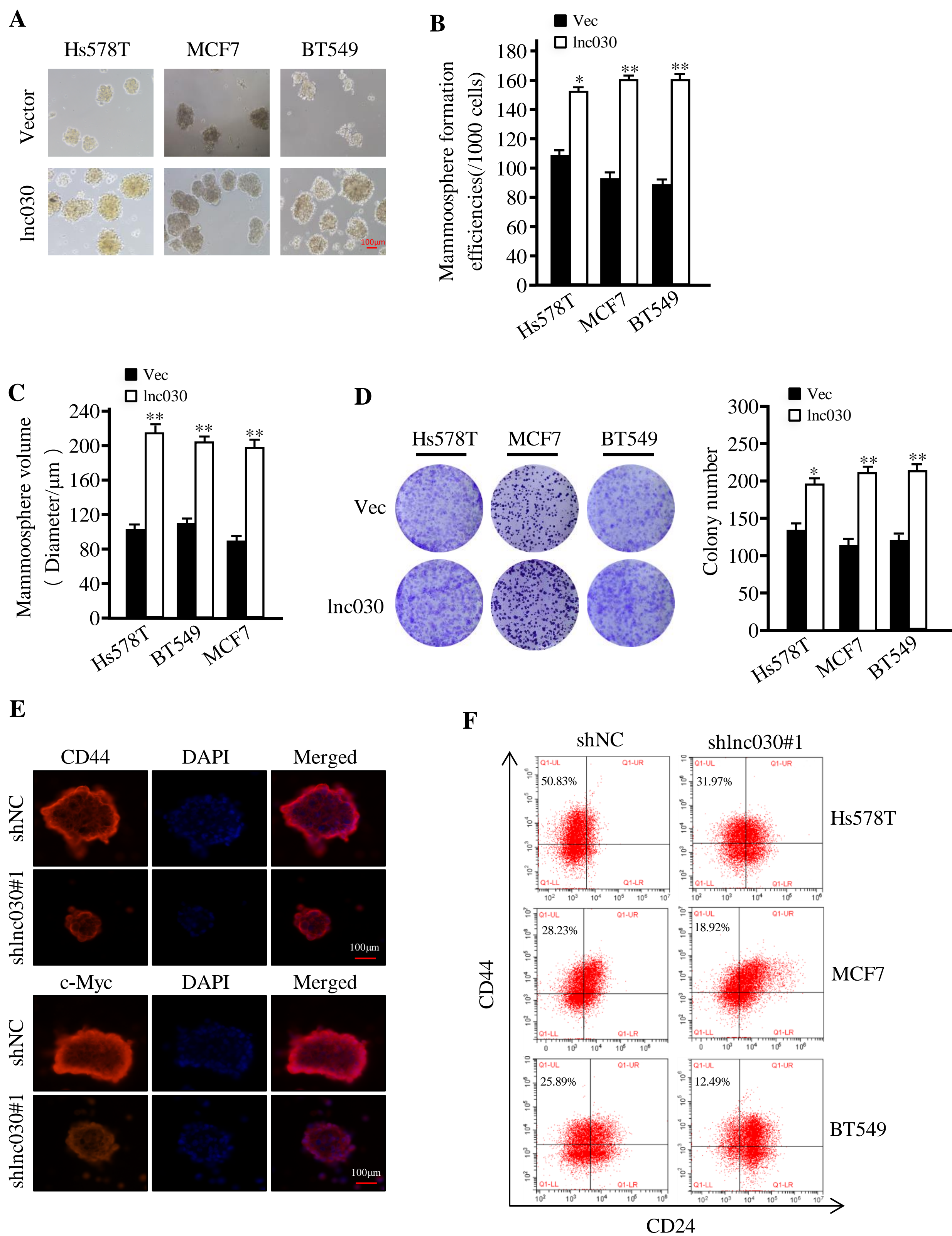


Figure S2. Lnc030 is required for maintaining BCSCs stemness

(A). Ectopic lnc030 increased sphere formation abilities in breast cancer cells. The representative mammopheres are shown (Scale bar, 100μm). (B, C). The sphere-formation efficiencies (left panel) and spheroid sizes (right panel) were quantified for the indicated engineered breast cancer cells and their control cells in (A). The data represent means ± SD (n=3; *$p<0.05$; **$p<0.01$). (D). Colonies of the indicated breast cancer cells were stained with crystal violet and photographed after 14 days of incubation. The numbers of colonies were counted and plotted in columns (right panel). The data represent means ± SD (*$p<0.05$; **$p<0.01$). (E). Immunofluorescence staining of stemness biomarkers (CD44 and c-MYC) in lnc030-silenced BCSCs and control BCSCs (Scale bar, 100 μm). (F). Flow cytometry was used to determine the percentage of $CD44^{+}/CD24^{-}$ cell population in secondary generation spheres derived from Hs578T, MCF7 and BT549 with shNC or shlnc030.

Fig. S3

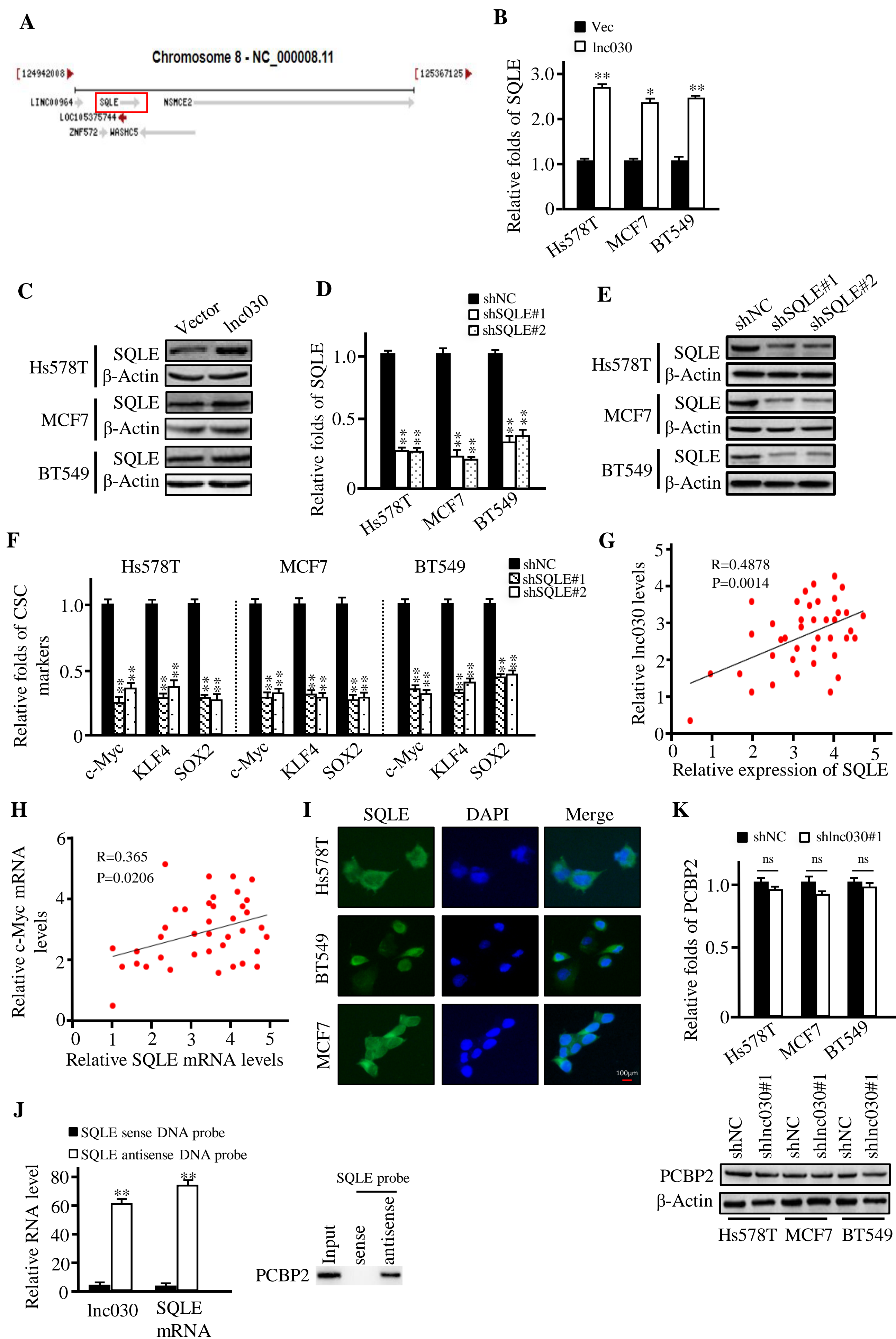


Figure S3. Lnc030 promotes SQLE expression to maintain BCSCs stemness

(A). The location of lnc030 and SQLE in human chromosome 8. (B, C). mRNA (B) and protein levels (C) of SQLE in lnc030-overexpressing breast cancer cells and control cells were determined by qRT-PCR and Western blotting. The data represent means ± SD (*p<0.05; **p<0.01). (D, E). Knockdown efficiencies of SQLE using two independent shRNAs were evaluated by qRT-PCR (D) and Western blotting (E) in BCSCs. Data are shown as means ± SD (**p<0.01). (F). The mRNA levels of CSC markers c-Myc, Klf4, and Sox2 mRNA levels were detected in SQLE-knockdown and control BCSCs. The data represent means ± SD (**p<0.01). (G, H). The positive correlation between lnc030 and SQLE (G), or c-Myc and SQLE (H) are shown in breast cancer tissues. The data represent means ± SD (n=40). (I). SQLE is mainly located in the cytoplasm of breast cancer cells checked by immunofluorescence (Scale bar, 100 μm). (J). Lysates from BT549 spheres were incubated with either sense or antisense biotin-labeled probe against SQLE mRNA. The pull-down precipitates were analyzed by qRT-PCR or Western blotting to detect the indicated RNAs and protein PCBP2, respectively. Data shown are the mean ± SD (n=3; **p<0.01). (K).The mRNA and protein levels of PCBP2 in spheres form Hs578T, BT549 and MCF7 transfected shNC or shlnc030#1 are shown. Data represent means ± SD (n = 3).

Fig. S4

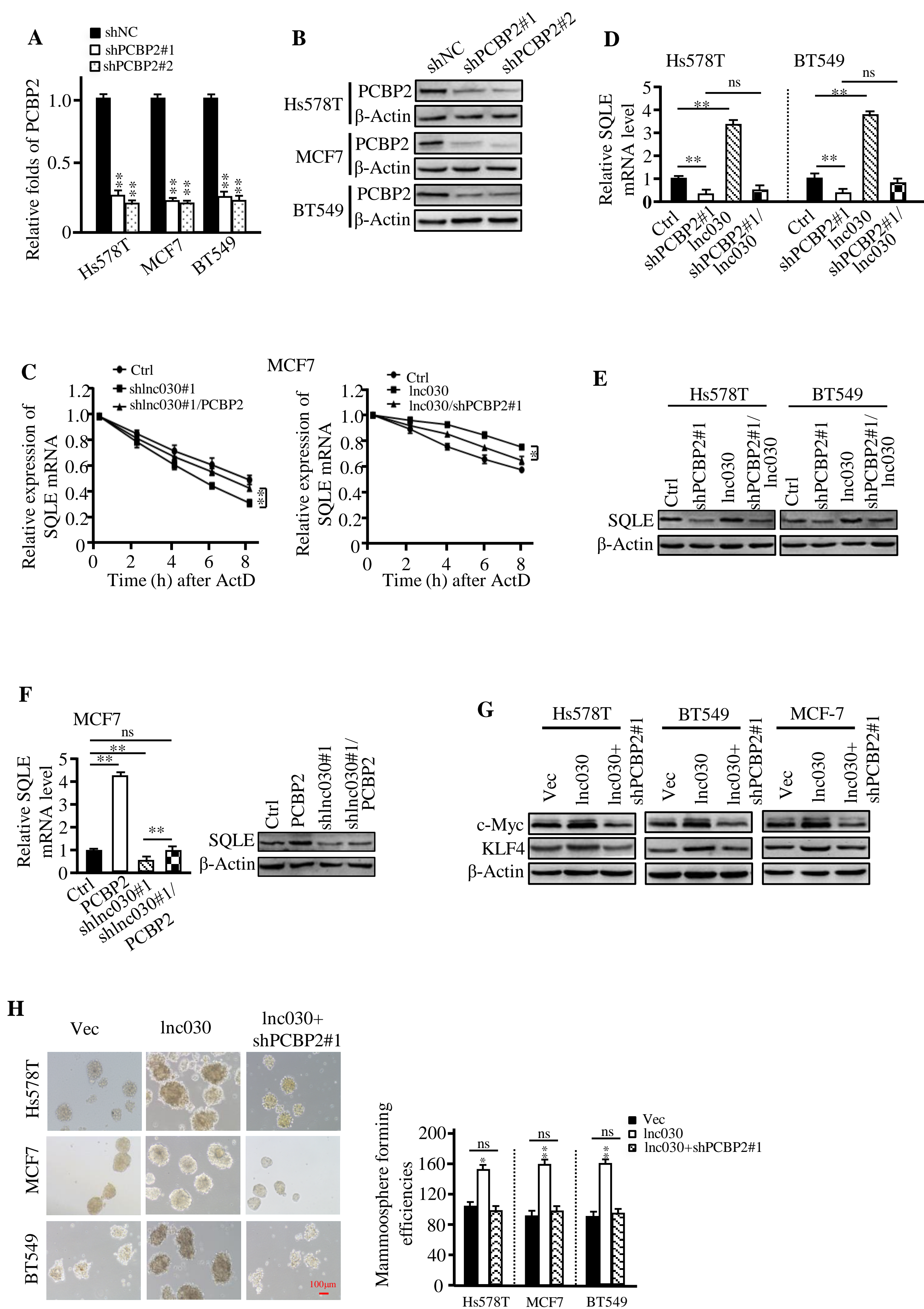


Figure S4. Lnc030 cooperates with PCBP2 to increase SQLE mRNA stability

(A, B). Knockdown efficiencies of PCBP2 using two independent shRNAs were evaluated by qRT-PCR (A) and Western blotting (B) in BCSCs. Data are shown as means ± SD (**p<0.01). (C). The degradation rates of SQLE mRNA in the engineered MCF7 cells and their controls were evaluated by qRT-PCR. The data represent means ± SD (*p<0.05; **p<0.01). (D-F). SQLE mRNA or protein was detected by qRT-PCR and Western blotting in the indicated mammospheres from Hs578T, BT549 cells (D, E), and MCF-7 cells (F) (**p<0.01). (G). Protein levels of CSC markers c-Myc, KLF4 were assessed in BC cells with control vector, lnc030, or lnc030 combined with shPCBP2#1. The data represent means ± SD. (H). The mammosphere-formation efficiency was evaluated for the breast cancer cells infected with control vector, lnc030, or lnc030 combined with shPCBP2#1. The data represent means ± SD (n=3; *p<0.05; **p<0.01, Scale bar, 100 μm).

Fig. S5

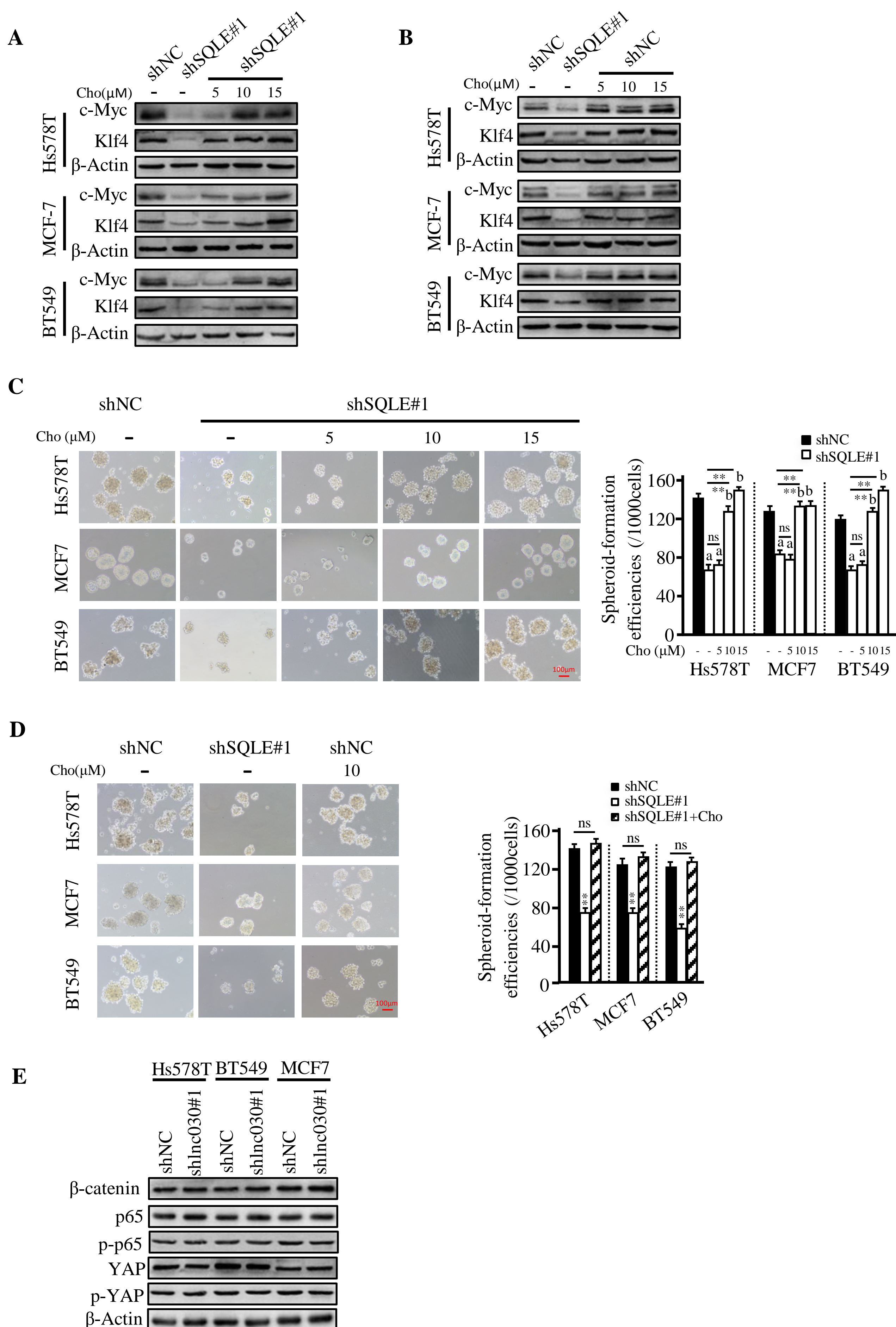


Figure S5. Intracellular cholesterol involves in maintenance of BCSC stemness

(A, B). c-Myc and KLF4 proteins were detected by Western blotting in SQLE-knockdown BCSCs (A) and control BCSCs (B) under treatment with or without cholesterol (labeled Cho) at 5μM, 10μM, or 15μM for 48 hours. (C). The sphere-forming capacities were assessed in control BC cells and SQLE-knockdown BC cells treated with cholesterol at 5μM, 10μM or 15μM for every 3 days. Data are shown as means ± SD (a, $p<0.01$, vs shNC + vehicle; b, $p>0.05$, vs shNC + vehicle; ns, $p>0.05$, vs shSQLE#1 + vehicle; **$p<0.01$, vs shSQLE#1 + vehicle,Scale bar, 100 μm). (D). The spheroid formation abilities were measured in SQLE-knockdown BC cells and control BC cells treated with or without cholesterol at 10μM. Data are shown as means ± SD (**$p<0.01$, Scale bar, 100 μm). (E). The expressions of β-catenin, p65, p-p65, YAP ,p-YAP were detected by Western blotting in lnc030-knockdown BCSCs and their control BCSC.

Fig. S6

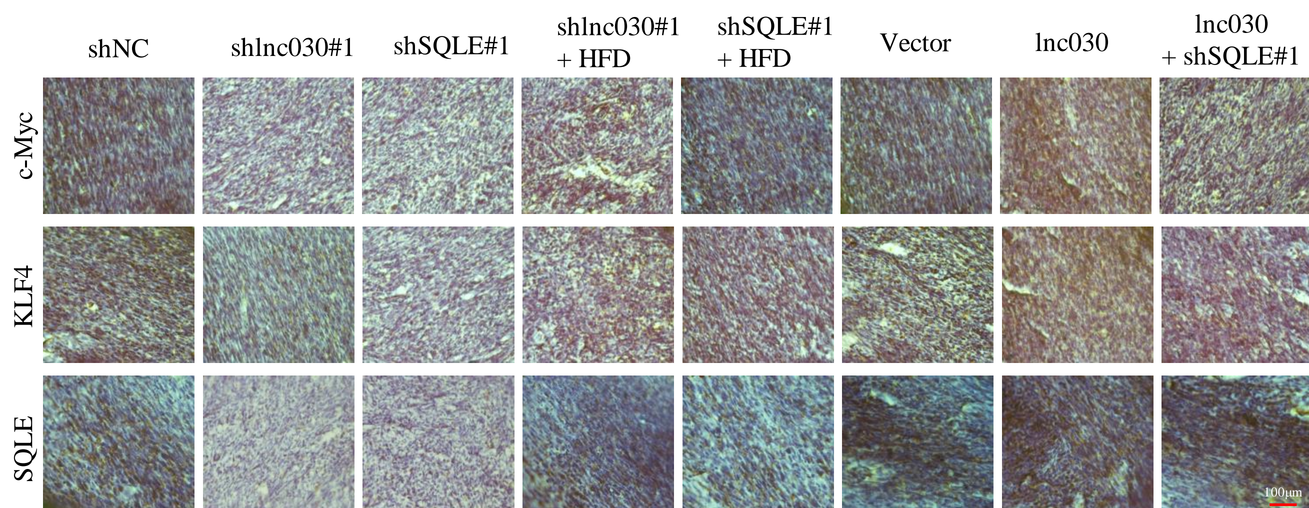


Figure S6. Lnc030 Promotes Breast Cancer Initiation and Progression

(A). Representative immunohistochemical staining of SQLE, c-Myc and KLF4 protein expression in tumor of xenografts (Scale bar, 100 μm).

Supplementary Table 1. The correlation between lnc030 expression and clinicopathological features of breast cancer patients (n=40)

Variables	Expression of lnc030		*p*-value
	Low(20)	High(20)	
Age			0.351
＜50	12	9	
⩾50	8	11	
Tumor size			0.109
⩽2	10	5	
2-5	7	7	
＞5	3	8	
Lymph node metastasis			0.023*
Yes	4	15	
No	16	5	
Distant metastasis			0.018*
Yes	2	17	
No	18	3	
Grade			0.026*
Ⅰ	11	3	
Ⅱ	5	8	
Ⅲ	4	11	

*p＜0.05 was considered to be statistically significant.

Supplementary Table 2. Sequences that target specific genes

Gene name	Sequence
lnc030	FW 5'-ACGTTTCCATTCATTCCGCTA-3'
	REV 5'-TCTGATTCCTCTTTCGGACT-3'
c-Myc	FW 5'-AAAGGCCCCCAAGGTAGTTA-3'
	REV 5'-GCACAAGAGTTCCGTAGCTG-3'
KLF4	FW 5'-GAACTGACCAGGCACTACCG-3'
	REV 5'-TTCTGGCAGTGTGGGTCTTA-3'
SOX2	FW 5'-GCACATGAACGGCTGGAGCAACG-3'
	REV 5'-TGCTGCGAGTAGGACATGCTGTA-3'
SQLE	FW 5'-CAGATAATCTCCGCCTTGACC-3'
	REV 5'-AAAGTGATGAAGTCCCCGAAC-3'
PCBP2	FW 5'-GAACACTGCTCGACATGGAC-3'
	REV 5'-R:CTCGCGCATCTTCTTAACTGA-3'
β-actin	FW 5'-TGACGTGGACATCCGCAAAG-3'
	REV 5'-CTGGAAGGTGGACAGCGAGG-3'
shlnc030#1	5'-ATGCTAGCGGAATGAATGGTT-3'
shlnc030#2	5'-TTGTAACATACAGGGCAGGTT-3'
shSQLE#1	5'-GCACCACAGTTTAAAGCAAAT-3'
shSQLE#2	5'-GCTCAGGCTCTTTATGAATTA-3'
shPCBP2#1	5'-CCTGAGAGAATTATCACTT-3'
shPCBP2#2	5'-CCATCATTGAGTGTGTCAATT-3'
Sequences of primers used for pcDNA3.1-lnc030 construction	
Full-length	F:GGCGCTCTGGGGCCGGGATC
	R:TGTTTTACCAAATAATTATT
1-150	F:GGCGCTCTGGGGCCGGGATC
	R:ATGGAAACGTTCCGACCCGA
151-300	F:TCATTCCGCTAGCATTTATT
	R:AAAGGATGTTCGAGTTCATG
301-412	F:GCAATTCTGTATCAGATACT
	R:TGTTTTACCAAATAATTATTT
Sequences of primers used for pcDNA3.1-SQLE mRNA construction	
5'UTR	F:GAGACGCGTGGAGCCTGGCG
	R:GGTTCCAAGGCTTCTTTGGTG
CDS	F:ATGTGGACTTTTCTGGGCAT
	R:TTAATGAACCATATACTTCAT
3'UTR	F:GCTTAAAGGGGAACCATTTG
	R:TTCATTCTTCATTTTATTTG
Sequences of primers used for pcDNA3.1-PCBP2 construction	
Full-length	F:ATGGACACCGGTGTGATTGA
	R:CTAGCTGCTCCCCATGCCAC

1-288	F:ATGGACACCGGTGTGATTGA
	R:GGGTCTACTGGCAGCTGTGC
226-858	F: ATTGACAAACTGGAAGAGGA
	R: TGAGCAGATGCATCCAAACC
487-1095	F: TGCGTGGTCATGTTGGAGAC
	R: GCTGCTCCCCATGCCACCCGT

Primers for in vitro transcription

The T7 RNA polymerase sequence(T7) was 5'taatacgactcactataggg'3

lnc030(sense)	**F:** (T7)GGCGCTCTGGGGCCGGGATCGGCGTCGGGG
	R:TGTTTTACCAAATAATTATTTTATTAGCAGATAAA
(antisense)	**F:**(T7) TGTTTTACCAAATAATTATTTTATTAGCAGATAAA
	R:GGCGCTCTGGGGCCGGGATCGGCGTCGGGG

Primers for antisense oligomer affinity pull-down assays

Lnc030 sense oligo DNA	5' (biotin-)AAAGCAAGGAAGTGGGACGC'3
Lnc030 antisense oligo DNA	5' (biotin-)TTCGAGTTCATGAAGAAGTTGC'3
SQLE sense oligo DNA	5' (biotin-) AACACGCGCAGACCTTCGTTG'3
SQLE antisense oligo DNA	5' (biotin-)ATCCAAATCTTGGTCCAAGAGTT'3