Supplementary Materials

Supplementary Methods

Publicly available data analysis

To identify breast cancer associated lncRNAs from publicly available microarray datasets, Affymetrix Human Genome U133 Plus 2.0 Array (platform GPL570) CDF files were downloaded from NCBI GEO website (https://www.ncbi.nlm.nih.gov/geo). LncRNA probe sets were selected based on Refseq and Ensembl annotation. A total of 3989 probes on the GPL570 platform were identified as lncRNA probes. Raw data of five breast cancer datasets (GSE10780, GSE42568 GSE29431, GSE7904, GSE21422) [1-4] were downloaded and globally normalized with the RMA (Robust Multichip Average) method using the affy package in R 3.1.2 program. LncRNA probes were sorted out and differential expressed lncRNAs were called with the eBayes method using limma package. LncRNAs which were differentially expressed in at least 4 of 5 datasets (Fold change >1.5, $p<0.01$) were selected.

APAL (ST8SIA6-AS1) expression in TCGA BRCA with clinical information was downloaded from TCGA portal (www.tcgaportal.org). APAL expression (probe 242350_s_at) and related clinical information of other breast cancer datasets GSE20685 [5] and E-MTAB-365) [6] were downloaded from GEO and ArrayExpress (https://www.ebi.ac.uk/arrayexpress/), respectively. Online tool Kaplan-Meier Plotter (http://kmplot.com/analysis/) was used to check the association of APAL (242350_s_at) expression with patient outcomes of breast cancer [7], lung cancer [8]

and pan-cancers, with auto-selected best cut-off for splitting groups. Affymetrix U133A and U133 plus2 microarray data were used in the KM Plotter integrative tool, and the raw data were MAS5 normalized in the R statistical environment using the affy Bioconductor library, as previously described [7].

For CNA analysis, TCGA BRCA segmented copy number data were downloaded from UCSC Xena Functional Genomics Explorer (https://xenabrowser.net/heatmap/). The copy number segments were measured using the Affymetrix Genome-Wide Human SNP Array 6.0 platform. The gene level of CNA value was defined as the average log2 (tumor/normal) value of all the segments' CNA values corresponding to the gene. The CNA burden of TCGA BRCA patients, defined by the percentage of genome that has been affected by copy number gains or losses, was downloaded from cBioPortal (http://www.cbioportal.org/study?id=brca_ tcga&tab=clinicalData) [9]. APAL gene CNA data in METABRIC breast cancer dataset with clinical information was downloaded from https://www.synapse.org/#! Synapse:syn1688369/wiki/27311 [10].

Cell lines, reagents and antibodies. All cancer cell lines were obtained from American Type Culture Collection (ATCC) and cultured in DMEM medium supplemented with 10% fetal bovine serum (Gibco). MCF-10A cells were cultured in DMEM/F12 with supplements. Pan-caspase inhibitor Z-VAD-FMK was purchased from Selleckchem (S7023) and used at a concentration of 20 μM. Nocodazole was purchased from Sigma (M1404).

The following primary antibodies were used in western blotting assays: PLK1

(CST, #4513, 1:1000), phosphor-PLK1 (Thr210) (CST, #9062, 1:1000), Aurora A (CST, #14475, 1:1000), phosphor-Aurora A (Thr288) (CST, #3079, 1:1000), HA (CST, #3724, 1:1000), Flag (Sigma, #F1804,1:5000), β -actin (Proteintech, #66009, 1:1000).

RNA extraction, quantitative RT-PCR and Northern blot. Total RNA was extracted from cultured cells using Trizol (15596-026, Invitrogen, Carlsbad, CA) according to the manufacturer's instructions. 1 μg total RNA was reverse transcribed into cDNAs using Superscript First-Strand cDNA Synthesis Kit (18080-051, Invitrogen, Carlsbad, CA). Quantitative RT-PCR was performed using SYBR Premix Ex Taq II kit (DRR081A, TAKARA,Otsu, Shiga, Japan) on LightCycler 480 System (Roche, Basel, Switzerland), with primers listed in Supplementary Table 3. Northern blot assays was performed using DIG Luminescent Detection Kit for Nucleic Acids (11363514910, Roche, Basel, Switzerland) according to the manufacturer's instructions.

Locked nucleic acids (LNAs), siRNAs and plasmids. For knockdown experiments, cells were transiently transfected with LNAs antisense oligonucleotides (Exiqon) or siRNAs using Lipofectamine 2000 (Invitrogen, Carlsbad, CA) according to the manufacturer's protocol. The LNA sequences are as following: APAL LNA-1 GACATGACAATGGTA, APAL LNA-2 TTTGGCCATGATGGAT, LNA-NC AACACGTCTATACGC. The siRNAs sequences were listed in Supplementary Table 4.

Snovector was a gift from Lingling Chen lab [11]. For stable expression in the nucleus, full-length of APAL cDNA sequences were inserted into the snovector.

Full-length or mutants of APAL were cloned into pcDNA3.1 vector for RNA pulldown assay. PLK1 full-length, PLK1 (210A), PLK1 (210D), PLK1 (aa 1-344) and PLK1 (aa 345-603) sequences were cloned into pcDNA3.1 with HA tag. Aurora A full-length, Aurora A (aa 1-128) and Aurora A (aa 129-403) sequences were cloned into p3xFLAG-CMV7.1 vector. The cDNAs coding for PLK1 were subcloned into a pGEX-4T-1 vector for in vitro expression of GST-PLK1. The sequences of APAL hairpins and mutants were listed in Supplementary Table 5.

Rapid amplification of cDNA ends (RACE) assay. RACE assay was performed using SMARTer RACE cDNA Amplification Kit (634923, Clontech, Mountain View, CA) according to manufacturer's instructions.

The primers were used as following: (5'-race: CATTCTCTGGCACAGATATCA GGTGAAGGACGA, 5'-race-nest: TCATCTTTGTAGGCGCCGTGA, 3'-race: TCAT GCCCAAACATTTGCATGCACCTT, 3'-race-nest: AATGAGGCAAAGGCCTTGT CA)

Apoptosis and cell cycle assays. To detect apoptosis, cells were stained with annexin V and PI for 20 min using the FITC Annexin V Apoptosis Detection Kit II (BD Biosciences) according to the manufacturer's instructions. To study the effect of APAL expression on mitosis progression, MDA-MB-231 cells stably overexpressing sno-APAL or control vector were synchronized to G2/M phases via exposure to nocodazole (200 nM) for 16 h in a 37℃ humidified incubator. Cells arrested in M phase were shaked off and collected, washed with ice-cold PBS, and then released to nocodazole-free medium. At 0, 2, 4 and 6 h after release, cells were harvested and

stained with PI for 30 min using the Cell Cycle Detection Kit (BD Biosciences) according to the manufacturer's instructions.

RNA pulldown. The 3'-end biotin-labeled APAL was transcribed using MEGAscript T7 High Yield Transcription Kit (AM1334, Ambion, Carlsbad, CA) and purified with MEGAclear™Kit (AM1908, Ambion, Carlsbad, CA) according to manufacturer's instructions. 5 pmol of purified RNA in RNA structure buffer was heated to 95°C for 5 min, then put on ice for 3 min, and left at room temperature for 20 minutes to allow proper secondary structure formation. The cell lysates were freshly prepared with Anti-RNase, Protease/ Phosphatase Inhibitor Cocktail supplemented in the lysis buffer, and 5 pmol folded RNA were incubated with 10mg cell lysates for 1h at room temperature. The Dynabeads™ Streptavidin magnetic beads (65801D,Invitrogen, Carlsbad, CA) were first rinsed with IP lysis buffer and then subjected to RNA-binding protein complexes for 30 minutes at room temperature with agitation. Then samples were collected for mass spectrometry analysis or western blots.

RNA Immunoprecipitation. Lysates of MCF7 cells expressing HA-PLK1 and Flag-Aurora A were immunoprecipitated using anti-HA and anti-Flag primary antibody. RNA immunoprecipitation were performed using Magna RIP RNA-Binding Protein Immunoprecipitation Kit (17-700, Millipore, Billerica, MA) according to manufacturer's instructions.

***In-vitro* kinase assay.** 2μg of purified GST-PLK1 protein was mixed with 50 ng recombinant human Aurora A protein (Millipore, 14-511) and 5 pmol purified RNA. Kinase reactions were performed in 40 μL of kinase buffer containing 200 μM ATP at

30℃ for 30 min, as described [12]. Phosphorylation of PLK1 (T210) was detected by western blots.

Animal Experiment. Mice studies were carried out according to the ethical regulations approved by Sun Yat-sen University Animal Care and Use Committee. 5~6 weeks old female Balb/c nude mice were purchased from Center of Experimental Animal of Sun Yat-sen University and housed under standard conditions at the animal care facility. 2×10^6 of A548 or MCF7 cells were injected directly into the mammary fat pad of mice orthotopically in 100 μl of sterile PBS. Once tumors reached 100 mm^3, 10 mg/kg of LNA-2 or LNA-NC were injected I.P. every 3 days for up to three weeks. All animal studies were carried out according to the ethical regulations approved by Sun Yat-sen University Animal Care and Use Committee. Tumor volume was calculated every 3 days according to the formula V (mm^3) =0.5 × (length × $width^2$). Xenografts were harvested, weighed, and snap-frozen for cryosectioning when LNA treatments ended.

Immunohistochemistry and TUNEL assay. Paraffin-embedded slides were deparaffinized with xylene and and dehydrated with graded alcohols. Then immunohistochemistry was performed using mouse anti-Ki-67 Nuclear Antigen Monoclonal Antibody (ZSGB-BIO,China) in conjunction with goat anti-mouse horseradish peroxidase (HRP)-conjugated antibodies and visualized by DAB reaction. TUNEL assays were performed using DeadEnd™ Fluorometric TUNEL System (G3250,Promega,USA) according to manufacturer's instructions. To evaluate the stainings, positive cells in the blue channel were counted in four different fields using

ImageJ.

In situ hybridization and data analysis. The probe targeting APAL gene (TTTATCATTCTCTGGCACA) was designed by Exiqon, and the In Situ Hybridization (ISH) was performed as described [13]. Briefly, after dewaxing and rehydration, the samples were digested with 0.05% trysin, fixed in 4% paraformaldehyde, and hybridized with the 5'digoxin-labeled APAL probe (Exiqon, Vedbaek, Danmark) at 55°C overnight, then subsequently incubated with anti-digoxingenin antibody (Abcam, ab419) overnight at 4°C. Positive expression of APAL was primarily detected in the nuclear. The staining scores were determined based on both the intensity and proportion of positive cells in 10 random fields under a 20× objective, as described [13]. The cells at each staining intensity were recorded on a scale of 0 (no staining), 1 (light blue), 2 (blue) and 3 (dark blue). The proportion of positively stained tumor cells in sections was graded as follows: 0, no positive cells; 1, <20%; 2, 10% -50%; and 3, >50%. The staining index (SI) was calculated as follows: SI = staining intensity×proportion of positively stained cells. Using this method, the expression was evaluated using SI and scored as 0, 1, 2, 3, 4, 6 or 9. A SI score of 3 was used as a cut-off value based on the distribution of frequency of SI score for APAL expression and a measurement of heterogeneity with the log-rank test statistic with respect to overall survival, and the expression levels of APAL were defined as high (SI>3) or low (SI<3).

Patient-derived organoid culture and analysis. Tumors were from surgical resections of three patients with breast carcinomas. Breast cancer tissues were

mechanically disaggregated and digested with 200 U/ml collagenase (Sigma) and 100 U/ml hyaluronidase (Sigma), followed by further digestion in TrypLE and Dispase/DNaseI as described [14]. Organoids were mixed with growth factor-reduced matrigel and plated as 80-μL domes in 24-well plates with DMEM/F12 medium supplemented with 10 μg/ml of insulin, 0.5 μg/ml of hydrocortisone, 50 ng/ml of EGF, 50 ng/ml of bFGF and B27, as described [15].

Organoids were transiently transfected with LNAs (Exiqon) using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). 48 h after transfection, organoids in matrigel were harvested using Gentle Cell Dissociation Reagent (Stemcell technologies) for RNA isolation and qRT-PCR analysis. Live/dead discrimination of cells in organoids was measured by calcein AM/ EthD-1 staining using LIVE/DEAD Viability/Cytotoxicity Kit (Thermo) according to manufacturer's instructions.

References

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Supplementary Tables

Supplementary Table 1. Association of APAL expression with breast cancer clinicopathological parameters

Characteristics	All breast cancer (n=199)				TNBC (n=39)				Non-TNBC (n=160)			
	Total No. (%)	Expression, No. (%)		P-value*	Total No. (%)	Expression, No. (%)		P-value*	Total No. (%)	Expression, No. (%)		P-value*
		Low	High			Low	High			Low	High	
Age				0.75				0.68				0.36
<35 y	13(6.53)	8(4.02)	5(2.51)		5(12.82)	2(5.13)	3(7.69)		8(5.00)	6(3.75)	2(1.25)	
≥35 y	186(93.67)	106(53.27)	80(40.20)		34(87.18)	17(43.59)	17(43.59)		152(95.00)	89(55.63)	63(39.38)	
Grade				<0.001				0.04				0.007
0	12(7.19)	11(6.59)	1(0.60)		5(12.82)	5(12.82)	0(0.00)		7(5.47)	6(4.69)	1(0.78)	
1	29(17.37)	23(13.77)	6(3.59)		6(15.38)	4(10.26)	2(5.13)		23(17.97)	19(14.84)	4(3.13)	
2	64(38.32)	33(19.76)	31(18.56)		9(23.08)	5(12.82)	4(10.26)		55(42.97)	28(21.88)	27(21.09)	
3	62(37.13)	25(14.97)	37(22.16)		19(48.72)	6(15.38)	13(33.33)		43(33.57)	19(14.84)	24(18.75)	
Stage				<0.001				0.11				0.003
0	14(7.10)	12(6.09)	2(1.02)		6(14.63)	5(12.20)	1(2.44)		8(5.13)	7(4.49)	1(0.64)	
1	75(38.07)	53(26.90)	22(11.17)		14(34.15)	8(19.51)	6(14.63)		61(39.10)	45(28.85)	16(10.26)	
2	68(34.52)	35(17.77)	33(16.75)		13(31.71)	7(17.07)	6(14.63)		55(35.26)	28(17.95)	27(17.31)	
3	35(17.77)	12(6.09)	23(11.68)		7(17.07)	1(2.44)	6(14.63)		28(17.95)	11(7.05)	17(10.90)	
4	5(2.54)	1(0.51)	4(2.03)		1(2.44)	0(0.00)	1(2.44)		4(2.56)	1(0.64)	3(1.92)	
ER				0.07				--				--
negative	55(27.23)	26(12.87)	29(14.36)		--	--	--		--	--	--	
Positive	147(72.77)	90(44.55)	57(28.22)		--	--	--		--	--	--	
PR				0.27				--				--
negative	62(30.69)	32(15.84)	84(41.58)		--	--	--		--	--	--	
positive	140(69.31)	30(14.85)	56(27.72)		--	--	--		--	--	--	

HER2				0.82			--			--
negative	159(82.81)	90(46.88)	69(35.94)	--	--	--	--	--	--	
positive	33(17.19)	18(9.38)	15(7.81)	--	--	--	--	--	--	

* P values were based on the chi-squared test. Abbreviation: TNBC, triple negative breast cancer. Dash (--) indicates analysis was not done.

Supplementary Table 2. Patient information of breast cancer in organoid cultures

No	Age	Pathoolgy/ Diagnosis	Stage	Grade	pTNM	ER	PR	HER-2
1	45y	invasive ductal carcinoma	IIA	II	pT2N0M0	+	+	-
2	44y	ductal carcinoma in situ	0	0	0	+	+	-
3	47y	invasive lobular carcinoma	IIIC	not available	pTxN3M0	+	-	-

Supplementary Table 3. Primers for RT-PCR

Genes	Forward (5'→3')	Reverse (5'→3')
APAL-1	TTCAGTGGCATGGTTCAGTC	CTCAGAAGCGACAGGGTTTC
APAL-2	TCAGAAATCATGCCCAAACA	ATGGGGAGATTCTTGTGTGC
APAL-3	CCATCCATCATGGCCAAAGC	CAGGGTTTCTTCGGTCGTCA
APAL-4	TCTGGCCAACAGAGCAAGAA	ATCATTTCCCTGGGGCTTCC
ST8SIA6	CCATCAGATGCCCAAAGAAT	CCTCCTTTGGTGTTTGGAGA
NEAT1	TTCTCTAGTGTTCCTCATGGC	TCCTGCAATGCTAGGACTC
HOTAIR	GGTAGAAAAAGCAACCACGAAGC	ACATAAACCTCTGTCTGTGAGTGCC
NKILA	AACCAAACCTACCCACAACG	ACCACTAAGTCAATCCCAGGTG
ACTB	TGAAGTGTGACGTGGACATC	GGAGGAGCAATGATCTTGAT
PTER	TATCATATCTGGAGCCGGG	ATAAGGACATCGGTAAGCTG
C1QL3	CTTCACCTACCACGTCCTG	CTTGGGCAATTGCACTAGC
CUBN	CTTGCAGCAGACTGTTGAC	GAATCATGCAGATTGAGGCAG
RSU1	AAAGTTGCAGATACTCAGCC	TCTTTAAGCTGGGTAAGCTC
TRDMT1	TCTTGGCATTCCAAATTCAAGG	CATCAGTACCTGACCAGGG
VIM	TCCACGAAGAGGAAATCCA	CAGGCTTGGAAACATCCAC
PTPLA	GCCTTGCTTGAGATAGTTCAC	TCTTGAACTCACTTGGACCC
STAM	ACTTTGAAGCTGCTGAAGAC	TCCACCAGTTAGGATCACTG
TMEM236	TCTACTGATCTGGGTTCCTG	CAGAACAGGTCTCCATCCT
MRC1-1	GATGAAAGGCAAGGATGGG	CTTCGTGATTTCATCTTGCAG
MRC1-2	CCAACAACAGAACGCTGAG	GTCAAGGAACTGGTTAATCCTG
SLC39A12	TTCCTTTCACAGAATGAGACAG	TTCCATACACTGGCTTTGAG
CACNB2	AAAGCAAAGACAAAGCCCG	CACTGGAACATCATCTTCATGG
Aurora A	GTCCCTGAGTGTCCTTGGC	CCTGGGCAATGGAGTGAGAC
HNRNPL	TTTTCGATCCGGGACGGC	TGATGGGTCCAAACTCCTGC
EIF4A3	CATCAGCAATCCAGCAACGA	CTCTTGTGGGAGCCAAGATCA
RBM47	AGAAGGCGGACGAGAAGATG	TTTCCAAGGCCTCCGTTACC
SFPQ	CGACAACTCCTCGTCCAGTC	CGTACTCAAACGTGCCATGC
PLK1	TGTTAGTGGGCAAACCACCT	CAGCTCGTTAATGGTTGGGC
RBMX	GCCCTCGTTGCGCAGT	GGGCGATCTGCTTCAACCAT
YBX1	AAGGTCATCGCAACGAAGGT	ACTGCGAAGGTACTTCCTGG
NONO	GAGCTCCGTCGTCTCGTTTC	TACTGGGCCTCTCAACTTCG
PTBP1	GGTCGGTTCCTGCTATTCCG	TCAGATCCCCGCTTTGTACC

Supplementary Table 4. siRNA sequences

Genes	**siRNA#1 (5'→3')**	**siRNA#2 (5'→3')**
Aurora A	GAAGAGAGUUAUUCAUAGA	UCUGGCUCUUAAAGUGUUA
EIF4A3	CGAGCAAUCAAGCAGAUCA	CUCUCGGUGACUACAUGAA
HNRNPL	GACGGGUCUUGCAGUUACA	GUCCAUACCCUUACACUCU
NONO	CAGGCGAAGUCUUCAUUCA	AGGUCAUGCUAAUGAGACA
PLK1	CAACCAAAGUCGAAUAUGA	CCUCACAGUCCUCAAUAAA
PTBP1	GCACAGUGUUGAAGAUCAU	GCCUCAACGUCAAGUACAA
RBM47	GCCUCAUGAUGGACUUUGA	GCUACGCCUUCGUCAUGUA
RBMX	AAGUUCUCGUGAUACUAGA	CUGUAUCACGUGGAAGAGA
SFPQ	CCAGAAAUUUCCACCUCUA	GGAGGAACGACGUAGAAGA
YBX1	GGAUAUGGUUUCAUCAACA	CGUAACCAUUAUAGACGCU

Supplementary Table 5. Mutant sequences of APAL used in RNA pulldown

Name	Sequence
Hairpin1 (nt 521-639)	CCAGGTGTGGTGGGTCATGCCTGTAATCTCAGCAATTTGGGAGGCCAAGGCAG GATCTCAGCAATTTGGGAGGCGAAGGCAGGCAGATCACTTGAGCCTAGGAGTT CAAGACCAGCTTGG
Hairpin2 (nt 427-520)	GGTTCAATACCTAGGAACTCCTGTAGAGGGTGTTGTGGAATCTTCTTTAAAAG AACAAAACAAGGCAAAACAAAGTTTAATAGGGTAGAGCAGCC
Mutant1	5’-AGGGAGGAGTTATTCAGGCCTCCGCCAGCTTCAAGGCCCTGGGGATGGTCTT TCACCTCCCTCTTTCTGATCTCTTTTTCATGCTCCTCCTTGCTCCAAAGAAAAGC CGGATGGCAAAAGAGCCCAGAACCTATTGGAACTGACAAAATCAAGTCACGGC GCCTACAAAGATGAGGGGCAGATTCTGGCTGCCTTTTAATTTCGTCCTTCACCT GATATCTGTGCCAGAGAATGTCTTCCAGGAGTTCTGCTACAGAGAAGAGAGTA ACCCCCATCCATCATGGCCAAAGCACCCAGTCAGGCTCCGCTCTGGATCCAGCC CGACAAATGCAACCCTTGAATAGGGTTTGTGCAAGCAAACTGGATGACGACCG AAGAAACCCTGTCGCTTCTGAGAAGACACCCAATCCAAGAATGAAAGCATCAG GTTCAATACCTAGGAACTCCTGTAGAGGGTGTTGTGGAATCTTCTTTAAAAGAA CAAAACAAGGCAAAACAAAGTTTAATAGGGTAGAGCAGCCAGGTGGGTCATGC CTGTAATCTCAGCAATTTGGGcGGCCAAGGCcGGATCTCAGCAATTTGGGAGGC GAAGGCAGGCAGATCACTTGAGCCTAGGAGTTCAAGACCAGCTTGGGCAACAT AGCAAGACCCTGCCTATACCAAAAAAAAAAAGTGAACCTAGGAGTATGAGGCT GCAGTAAGCTGTGGTTGTACCAGTGCACTTTGGGAGGCCAAGGTAGGCGGGTC TTCTGAGATCGAGAGTTTGAGACCAGCCTGACCAACATGGAGAAACCCCGTCT CTACTAAAAGTACAAAATTAGCCGGCATGGTGGTGCATGCCTGTAATCCCAGCT ACTCGGGAGGCTGAGGCGGGAGAATTGCTTGAACTCAGGAGGCAGAGGTTGT GGTGAGCCAAGATCGCGCCATTGCACTCCAGCCTGGACAACAAGAGCGAAACT CCATCACAAACAAACAAACAATAAACAAAAACCATAGAGTGATTTCTGGCCAA CAGAGCAAGAAAGAAGTGAACCAATGAAAACCGGTCCAAGTCTGGGAGTCAA TATGGAAAAGCTGCCTAATTAATGTGGAAGCCCCAGGGAAATGATATACCATGA AAACCTAACACTACAAAAACTAGTTGTCAGACACATGTGAGCAGTGAACAGAA TCCTAATATGCTGGTTTAACATTCAAAACTGGAGAGTGTTTGTGTATCTTCTAAA GACTGTTTGGGGTTTATTTGTGGAGTTCAGCTGAGTGTCATCAGAACAAAGATT AGTGCAAAAAATCTTTCAGAAATCATGCCCAAACATTTGCATGCACCTTAAATA AGAAATGAAATAGCAAGAAGAGTATGTCACAATAAAGTATAAATAGCAAGTGAT ATGTCTCATCGTACTGAGACAGTTTTATAAATGAGGCAAAGGCCTTGTCAAAAA ATAATGATGATTTTCAAAATTCTGAAACGCAAAGGAGGAAAACTACTTCATTCA GTTAACAGGAAGCACACAAGAATCTCCCCATCAACCAAGATTTTTTTTTTCTATA ACCCACGCTTTGTAAGACAATAATAGCAAAAACTATAAACTAGAATTTGTTGAA AGAACAACTTCTAGAATTTGCCCTTTATGTGCTGTTTTACCATTGTCATGTCTGT CTCTGGTATGACTTCCTCATCTATTAGTAGCCTCC-3’
Mutant2	5’-AGGGAGGAGTTATTCAGGCCTCCGCCAGCTTCAAGGCCCTGGGGATGGTCTT TCACCTCCCTCTTTCTGATCTCTTTTTCATGCTCCTCCTTGCTCCAAAGAAAAGC CGGATGGCAAAAGAGCCCAGAACCTATTGGAACTGACAAAATCAAGTCACGGC GCCTACAAAGATGAGGGGCAGATTCTGGCTGCCTTTTAATTTCGTCCTTCACCT

	GATATCTGTGCCAGAGAATGTCTTCCAGGAGTTCTGCTACAGAGAAGAGAGTA ACCCCCATCCATCATGGCCAAAGCACCCAGTCAGGCTCCGCTCTGGATCCAGCC CGACAAATGCAACCCTTGAATAGGGTTTGTGCAAGCAAACTGGATGACGACCG AAGAAACCCTGTCGCTTCTGAGAAGACACCCAATCCAAGAATGAAAGCATCAG GTTCccggCCTAGGAACTCCTGTAGAGGGTGTTGTGGAATCccCTTTAAAAGAACA AAACAAGGCAAAACAAAGTTTAATAGGGTAGAGCAGCCAGGTGTGGTGGGTCA TGCCTGTAATCTCAGCAATTTGGGAGGCCAAGGCAGGATCTCAGCAATTTGGGA GGCGAAGGCAGGCAGATCACTTGAGCCTAGGAGTTCAAGACCAGCTTGGGCA ACATAGCAAGACCCTGCCTATACCAAAAAAAAAAAGTGAACCTAGGAGTATGA GGCTGCAGTAAGCTGTGGTTGTACCAGTGCACTTTGGGAGGCCAAGGTAGGCG GGTCTTCTGAGATCGAGAGTTTGAGACCAGCCTGACCAACATGGAGAAACCCC GTCTCTACTAAAAGTACAAAATTAGCCGGCATGGTGGTGCATGCCTGTAATCCC AGCTACTCGGGAGGCTGAGGCGGGAGAATTGCTTGAACTCAGGAGGCAGAGG TTGTGGTGAGCCAAGATCGCGCCATTGCACTCCAGCCTGGACAACAAGAGCGA AACTCCATCACAAACAAACAAACAATAAACAAAAACCATAGAGTGATTTCTGG CCAACAGAGCAAGAAAGAAGTGAACCAATGAAAACCGGTCCAAGTCTGGGAG TCAATATGGAAAAGCTGCCTAATTAATGTGGAAGCCCCAGGGAAATGATATACC ATGAAAACCTAACACTACAAAAACTAGTTGTCAGACACATGTGAGCAGTGAAC AGAATCCTAATATGCTGGTTTAACATTCAAAACTGGAGAGTGTTTGTGTATCTTC TAAAGACTGTTTGGGGTTTATTTGTGGAGTTCAGCTGAGTGTCATCAGAACAAA GATTAGTGCAAAAAATCTTTCAGAAATCATGCCCAAACATTTGCATGCACCTTA AATAAGAAATGAAATAGCAAGAAGAGTATGTCACAATAAAGTATAAATAGCAAG TGATATGTCTCATCGTACTGAGACAGTTTTATAAATGAGGCAAAGGCCTTGTCAA AAAATAATGATGATTTTCAAAATTCTGAAACGCAAAGGAGGAAAACTACTTCAT TCAGTTAACAGGAAGCACACAAGAATCTCCCCATCAACCAAGATTTTTTTTTTC TATAACCCACGCTTTGTAAGACAATAATAGCAAAAACTATAAACTAGAATTTGTT GAAAGAACAACTTCTAGAATTTGCCCTTTATGTGCTGTTTTACCATTGTCATGTC TGTCTCTGGTATGACTTCCTCATCTATTAGTAGCCTCC-3’
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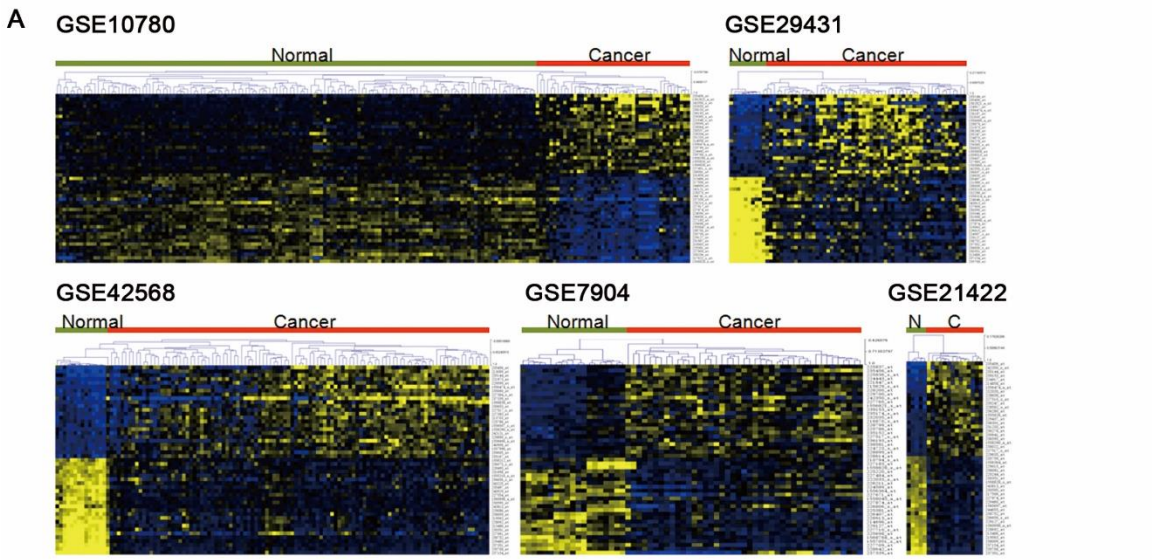
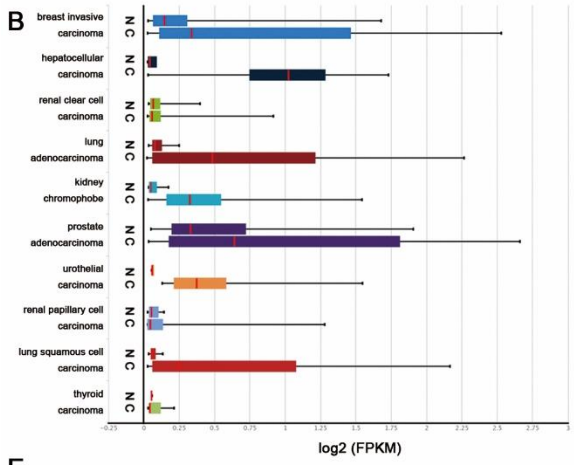
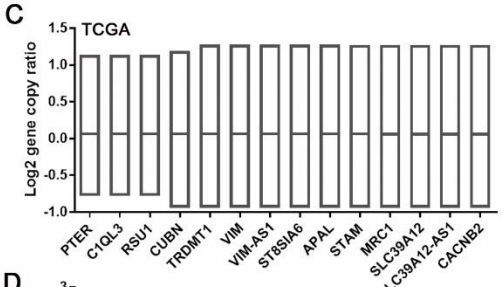
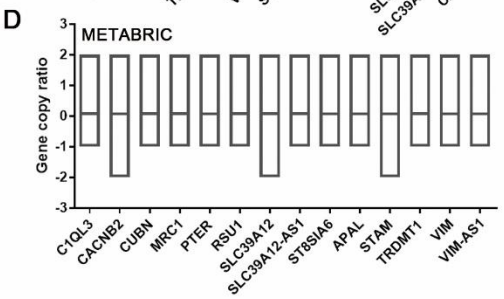
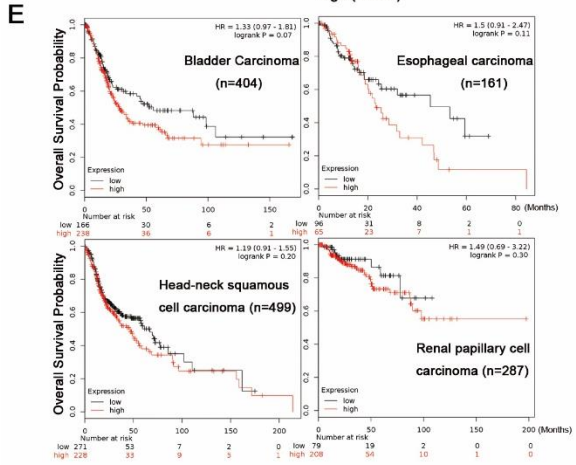
Supplementary Figures







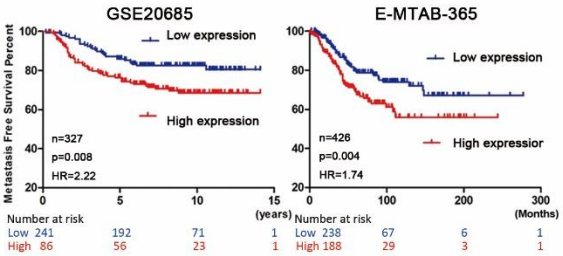




F

Cox regression analysis of APAL as a continuous variable in breast cancer datasets

Dataset	P-value	HR (95% CI)
TCGA	0.65	1.007 (0.977, 1.038)
GSE20685	0.005	1.121 (1.034, 1.215)
E-MTAB-365	0.004	1.146 (1.044, 1.257)



G

Multivariate Cox regression analysis in TCGA breast cancer dataset

Covariates	P-value	HR (95% CI)
APAL (categorical)	0.02	1.632 (1.099, 2.425)
CNA burden	0.20	1.833 (0.727, 4.620)

Supplementary Fig. 1. APAL expression in cancer datasets, related to Fig. 1 and 2.

(A) Heatmaps of differentially expressed lncRNAs in five GEO breast cancer datasets using Afymatrix U133 plus 2.0 microarray.

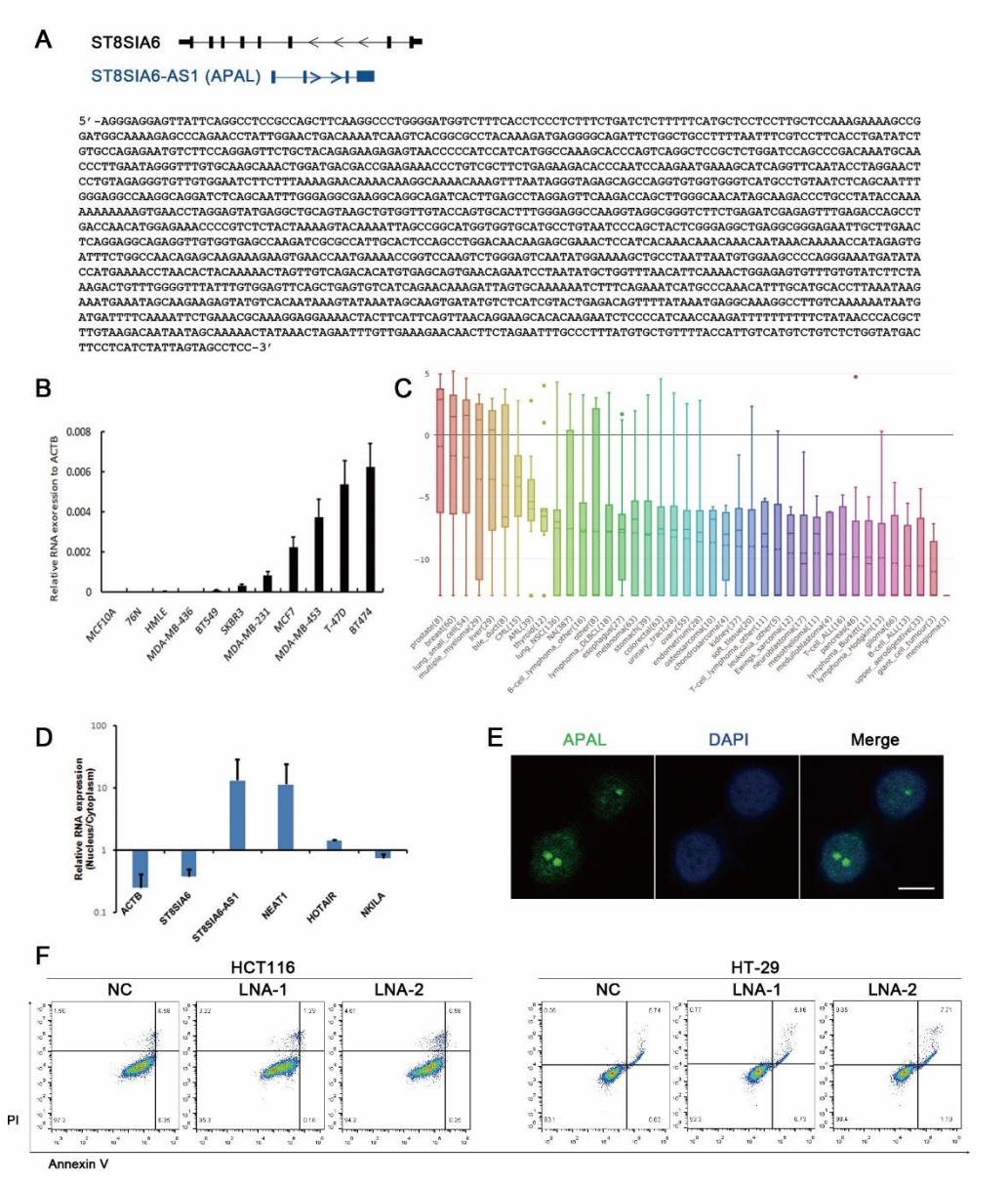
(B) The expression of APAL in various types of tumors from LncRNAtor.

(C, D) CNA of APAL and its neighboring genes in TCGA and METABRIC breast cancer datasets.

(E) Association of APAL with the overall survival of bladder carcinoma, esophageal carcinoma, head-neck squamous cell carcinoma and renal papillary cell carcinoma.

(F) Univariate Cox regression analysis of APAL as a continuous variable in the breast cancer datasets TCGA BRCA (n=957), GSE20685 (n=327), E-MTAB-365 (n=426).

(G) Multivariate Cox regression analysis of APAL and CNA burden in TCGA breast cancer dataset (n=957).



Supplementary Fig. 2. APAL expression in cancer cells, related to Fig. 3.

(A) The schematic diagram of human APAL gene (ST8SIA6-AS1) and its antisense gene. Arrows represent the direction of transcription. 5’ and 3’ RACE identify APAL sequence in MCF7 cells.

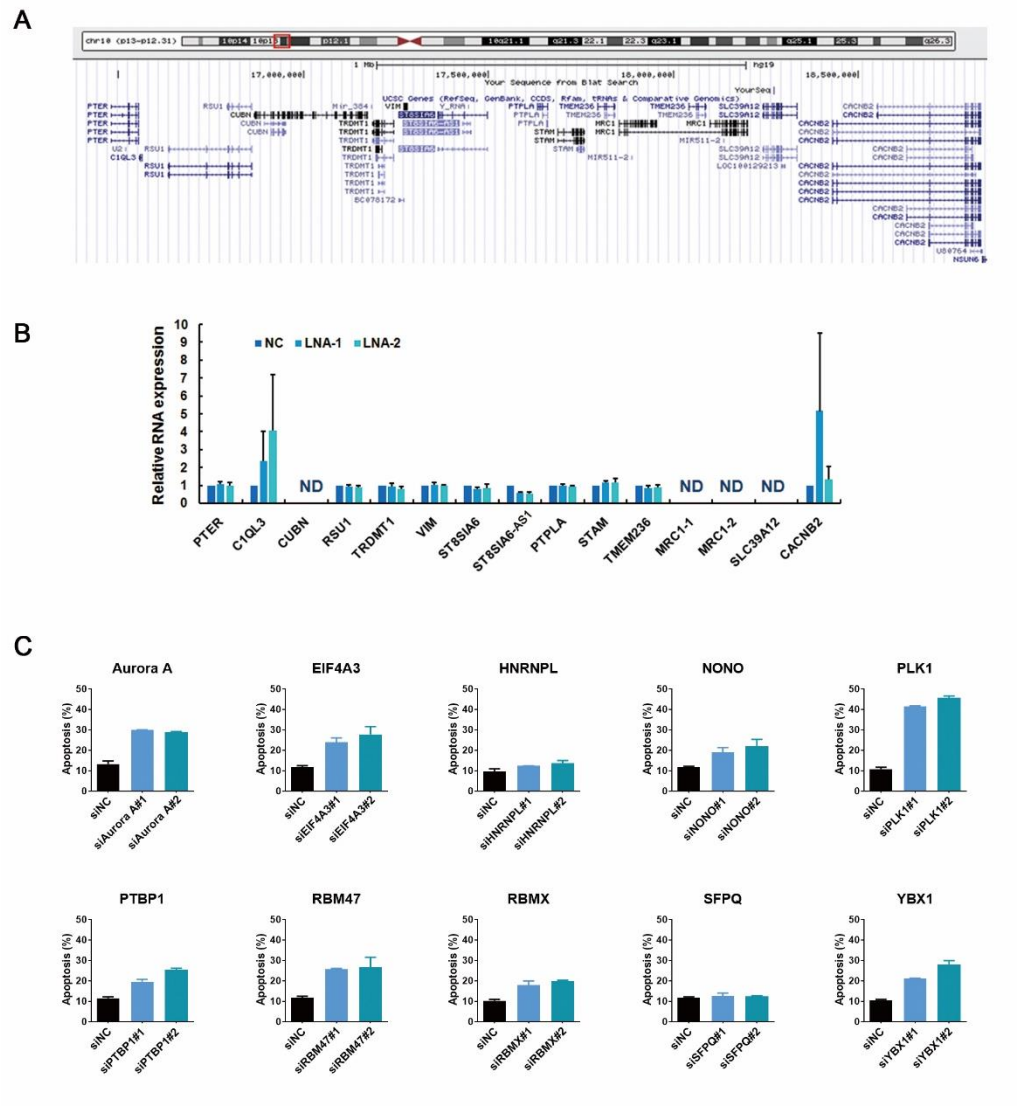
(B) Relative expression of APAL in 3 normal or immortalized breast cell lines and 8 breast cancer cell lines, as detected by qRT-PCR.

(C) APAL levels in different cancer cell lines, as shown in Broad Institute Cancer Cell Line Encyclopedia (CCLE).

(D) Subcellular localization of APAL (ST8SIA6-AS1). Nuclear and cytoplasmic fractions of MCF7 cells were subjected to RNA isolation and qRT-PCR detection.

(E) FISH of APAL in MCF7 cells. APAL signaling was shown in green. Scale bar, 10μm.

(F) Apoptosis analysis in HCT116 and HT-29 colon cancer cells.

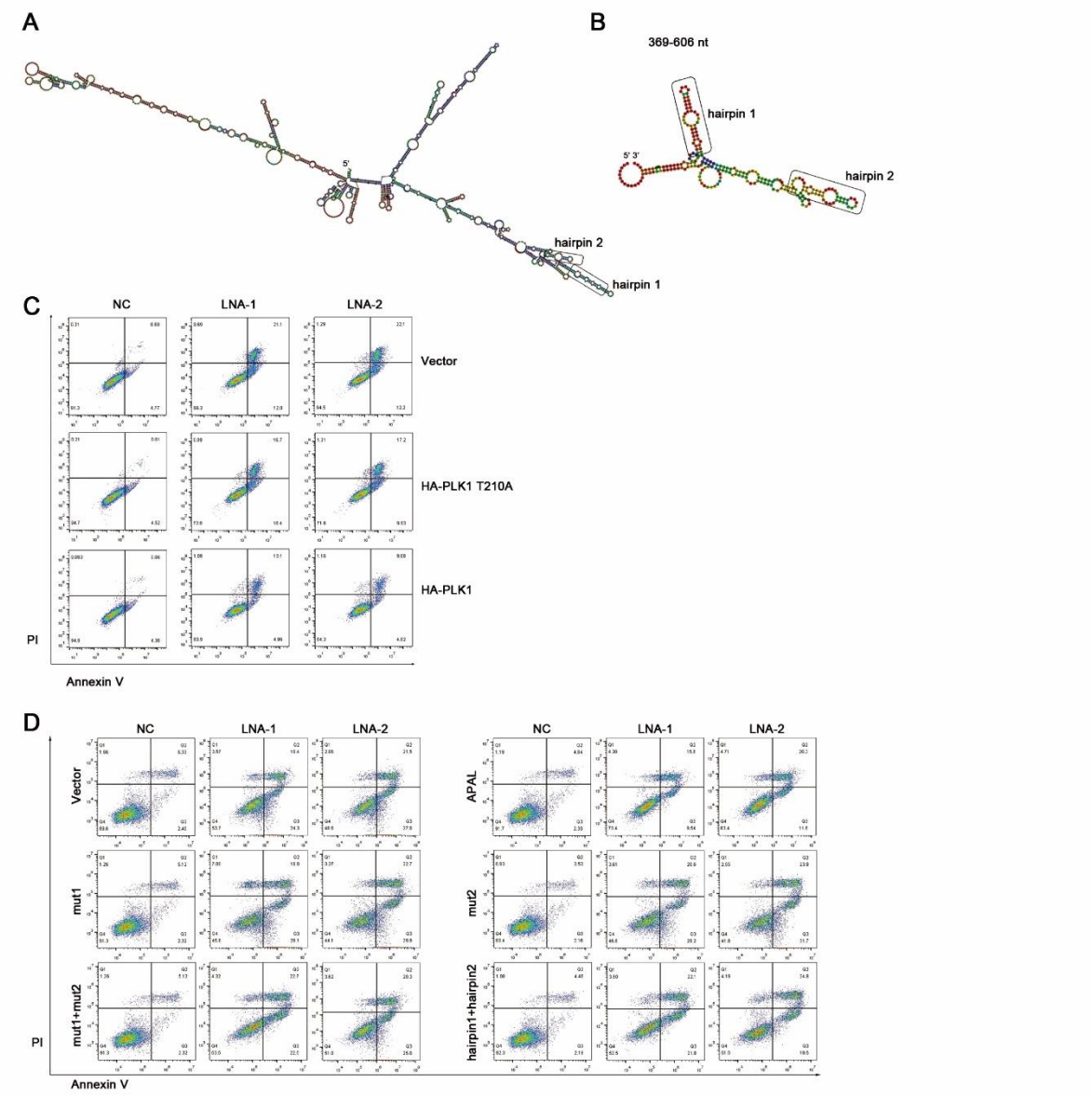


Supplementary Fig. 3. Analysis of APAL neighbor genes and candidate proteins that bind to APAL, related to Fig. 6.

(A) The genomic region of human APAL gene and its nearby genes.

(B) Effects of APAL knockdown on the expression of its nearby genes in MCF7 cells.

(C) Apoptosis analysis of silencing candidate genes in MDA-MB-231 cells. 10 candidate proteins were knocked down by siRNAs. Apoptosis was measured 48 h after siRNA transfection. Graphs represent means±SD and two-sided independent sample t-test was used for comparison.



Supplementary Fig. 4. Predicted APAL structure, related to Fig. 7.

(A) The structure of full-length APAL, as predicted by RNA fold.

(B) The structure of 369-606nt of APAL, as predicted by RNA fold.

(C) Representative flow cytometry analysis of apoptosis in PLK1 rescue experiments.

(D) Representative flow cytometry analysis of apoptosis in APAL rescue experiments.