

**YMTHE, Volume 28**

## **Supplemental Information**

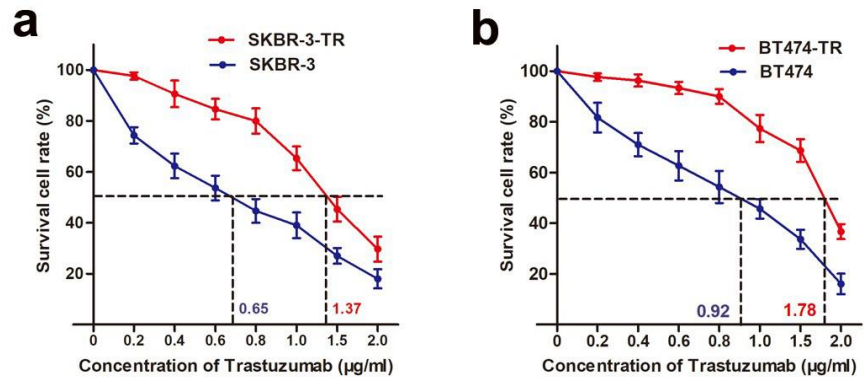
**lncRNA ZNF649-AS1 Induces**

**Trastuzumab Resistance by Promoting**

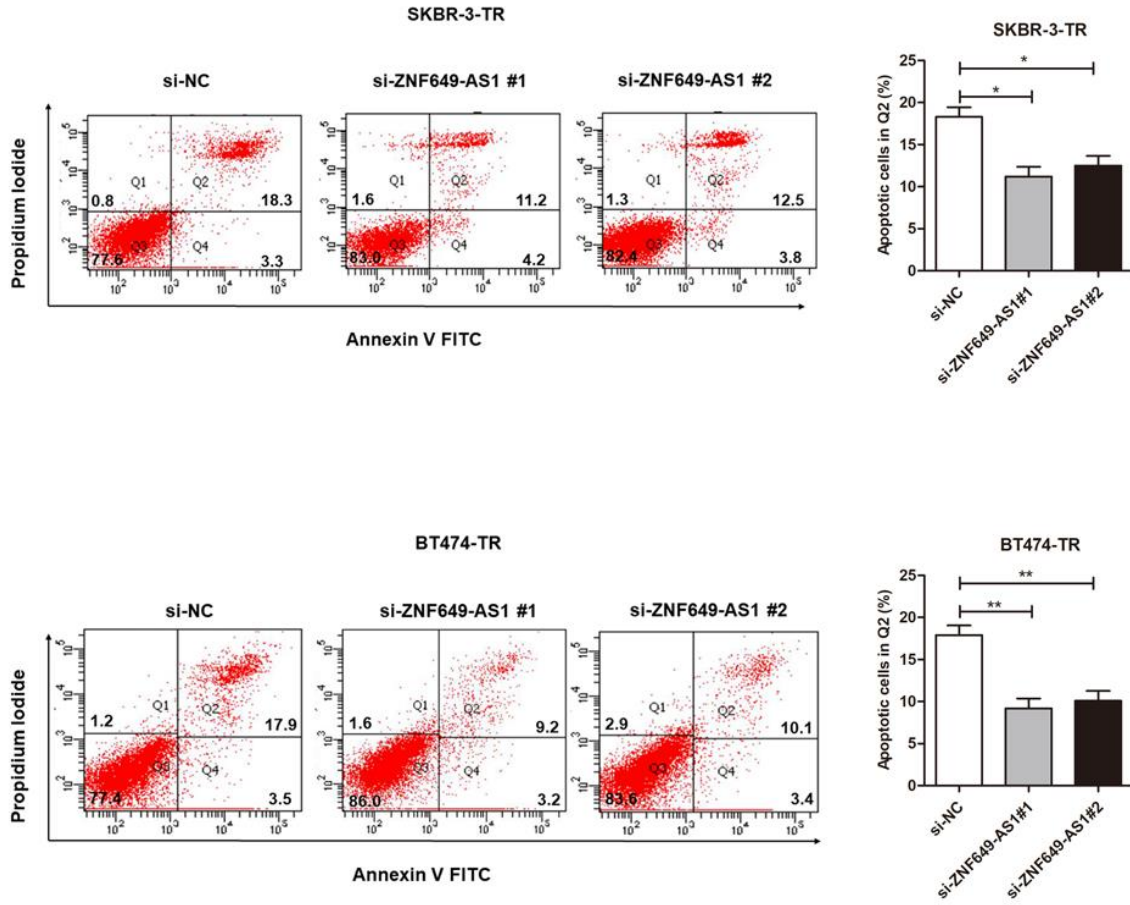
**ATG5 Expression and Autophagy**

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Supplemental Figures



**Supplementary Figure S1** (a-b) The IC50 values of breast cancer parental cells (SKBR-3 and BT474) and established trastuzumab-resistant cells (SKBR-3-TR and BT474-TR) were determined via CCK8 assay.



**Supplementary Figure S2** Flow cytometry analysis showed that knockdown of ZNF649-AS1 significantly suppressed apoptosis of SKBR-3-TR and BT474-TR cells. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .

## Supplemental Tables

**Table S1.** Information of the qPCR primer sequences and silencing RNA sequences

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<b>qPCR primer name</b>	<b>Sequence (5'-3')</b>
ZNF649-AS1 (Forward)	TGACTACCTTGTCTGAGAAA
ZNF649-AS1 (Reverse)	CCAGATTCCTGTATGTCCATT
ATG5 (Forward)	TTCTCAAATATACTGTTTC
ATG5 (Reverse)	TATTATGTATCACAAATGG
PTBP1 (Forward)	ACCAGGCCTTCATCGAGAT
PTBP1 (Reverse)	GTTGGGAGAGCTGTCGGTCTT
GAPDH (Forward)	GCACCGTCAAGGCTGAGAAC
GAPDH (Reverse)	ATGGTGGTGAAGACGCCAGT
U6 (Forward)	GGAACGATACAGAGAAGATTAGC
U6 (Reverse)	TGGAACGCTTCACGAATTTGCG
U1 (Forward)	GGGAGATACCATGATCACGAAGGT
U1 (Reverse)	CCACAAATTATGCAGTCGAGTTTCCC
<b>ChIP qPCR primer</b>	<b>Sequence (5'-3')</b>
ZNF649-AS1 promoter-F	TGTTCATACCAAATAAGGG
ZNF649-AS1 promoter-R	AGTGTCATTAATAAATAAG
<b>RIP qPCR primer</b>	<b>Sequence (5'-3')</b>
ZNF649-AS1 Forward	AGTACCTCTAATGTAAGATG
ZNF649-AS1 Reverse	AACTCACCACGATTGACAAC
<b>si-RNA name</b>	<b>Sequence (5'-3')</b>
si-ZNF649-AS1#1	CGACCAACTGACTTTGAACCT
si-ZNF649-AS1#2	AGACAGAAGTGTAACGTTTCTC
si-PTBP1	CTCTTCGGCACCCCTCGAGTGAC
si-ATG5	AAGATGTGCTTCGAGATGTGTGG
Negative control	UUCUCCGAACGUGUCACGUTT
<b>shRNA name</b>	<b>Sequence (5'-3')</b>
ZNF649-AS1 shRNA	CCGGAGCGGTCTCAGCCGAATGACTCTCGAGAGTCA TTCGGCTGAGACCGCTTTTTTG
Scrambled control	CCGGTTTCTCCGAACGTGTACGTCTCGAGA CGTGACACGTTCCGAGAATTTTTG

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**Table S2.** Candidate lncRNAs selected on a basis of the Hiseq analysis

LncRNAs	Location	Regulation (Res vs Par)	Fold change	<i>P</i> value
ZNF649-AS1	Chr19q13.41	Up	58.3681	0.00002486
LINC02474	Chr1q41.2	Up	35.6529	0.00008746
AL590483.1	Chr1q11.1	Up	29.1744	0.00022134
AC010789.1	Chr10q11.21	Down	45.9873	0.00005691
FOXD3-AS1	ChrXp22.33	Down	24.8693	0.00050972

Res: SKBR-3-TR cells; Par: SKBR-3 parental cells.

**Table S3.** The multivariate analysis and univariate analysis for clinical and biological characteristics of overall survival and progression-free survival in breast cancer patients

Parameters	OS			PFS		
	Univariate analysis <i>p</i> value	Multivariate analysis OR (95% CI)	<i>p</i> value	Univariate analysis <i>p</i> value	Multivariate analysis OR (95% CI)	<i>p</i> value
ZNF649-AS1 expression (high vs. low)	0.005	3.634 (1.023–12.912)	0.046	0.001	3.018 (1.275-7.143)	0.012
Age (years) (> 60 vs. ≤ 60)	0.518			0.998		
Gender (male vs. female)	0.180			0.661		
TNM stage (III/IV vs. I/II)	0.003	4.398 (1.305–10.357)	0.011	0.001	3.548 (1.192–8.320)	0.005
Lymphoma node (involved vs. not involved)	0.303			0.136		

**Table S4: Identification of ZNF649-AS1 binding proteins by MS.**

<b>Protein</b>	<b>Beads</b>	<b>ZNF649-AS1</b>	<b>Ratio (ZNF649-AS1/Beads)</b>
PTBP1	0	3	NA
PTPN13	0	3	NA
IGF2BP1	0	3	NA
DPM1	1	3	3
SMAD4	0	2	NA
SUZ12	1	3	3
MEIS1	1	3	3
GSTO1	0	2	NA
EWS-FLI1	1	3	3
PCH2	1	3	3

MS: mass spectrometry;

Beads: spectral counts of proteins in beads only group;

ZNF649-AS1: spectral counts of proteins in ZNF649-AS1 group;

Ratio (ZNF649-AS1/Beads): spectral count ratio of proteins comparing ZNF649-AS1 group to beads only group;

NA: not available.