**Title Page** 

Title:

A novel cystathionine  $\gamma$ -lyase inhibitor, I194496, inhibits the growth and metastasis of human TNBC via downregulating multiple signaling pathways

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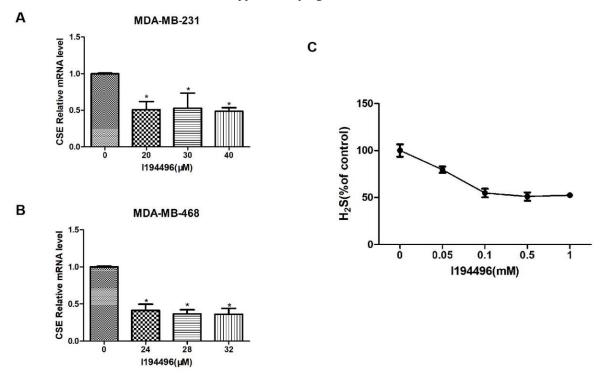
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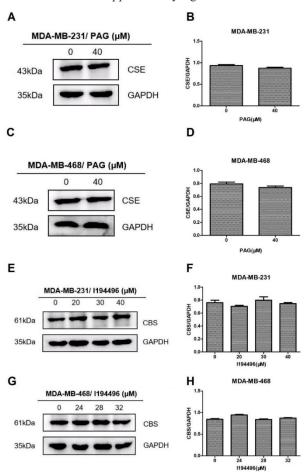
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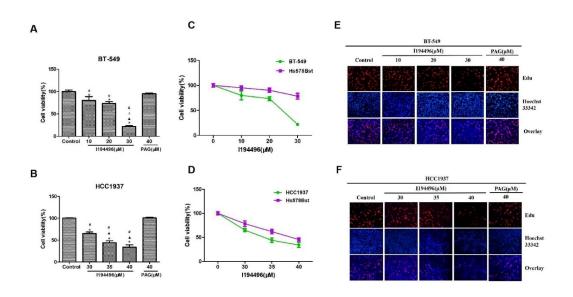
ORCID: 0000-0003-4038-2050Supplementary figure 1



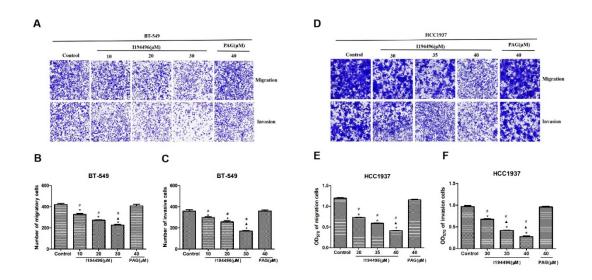
Supplementary figure 1. (A-B) The effect of I194496 on mRNA expression levels of CSE gene in MDA-MB-231 cells and MDA-MB-468 cells. The results showed that I194496 can effect the mRNA of CSE gene but not in a concentration - dependent manner. \* P<0.05 vs 0  $\mu$ M I194496 group.(C) The effect of I194496 on the recombinant CSE protein. The results showed that I194496 possessed significant inhibitory activity against CSE protein with IC50 of 0.79mM.



Supplementary figure 2. (A-B) The effect of PAG on CSE expression in MDA-MB-231 cells. (C-D) The effect of PAG on CSE expression in MDA-MB-468 cells. (E-F) The effect of 1194496 on CBS expression in MDA-MB-231 cells. (G-H) The effect of 1194496 on CBS expression in MDA-MB-468 cells. The results showed that 40  $\mu$ M PAG did not affect the expression of CSE protein. Meanwhile, the novel CSE inhibitor I194496 did not inhibit the expression of CBS, which suggested that I194496 was specific for CSE.



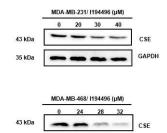
Supplementary figure 3. (A-B) I194496 inhibits the growth and proliferation of TNBC cells. (A) MTS assay was used to detect the effect of I194496 on the viability of BT-549 cells. \*P<0.05 vs Control group.  $\triangle P<0.05$  vs 10 µM I194496 group.  $\triangle P<0.05$  vs 20 µM I194496 group. "P<0.05 vs PAG group. (B) MTS assay was used to detect the effect of I194496 on the viability of HCC1937 cells. \*P<0.05 vs Control group.  $\triangle P<0.05$  vs 30 µM I194496 group. "P<0.05 vs PAG group. (C-D) Comparison of the inhibitory effect of I194496 in normal breast epithelial cells and breast cancer cells. (E-F) EdU assay was performed to detect the proliferation of BT-549 and HCC1937 cells. The results showed that I194496 decreased the EdU+ cell number in a dose-dependent manner. EdU, 5-ethynyl-2'-deoxyuridine. Image magnification, x200.



Supplementary figure 4. (A-B) I194496 inhibits the migration and invasion of TNBC cells. (A-C) The effect of I194496 on the migration and invasion of BT-549 cells. \*P<0.05 vs Control group.  $\triangleq$ P<0.05 vs 10  $\mu$ M I194496 group. #P<0.05 vs PAG group. (D-F) The effect of I194496 on the migration and invasion of HCC1937 cells. \*P<0.05 vs Control group.  $\triangleq$ P<0.05 vs 30  $\mu$ M I194496 group. #P<0.05 vs PAG group. Image magnification, x200.

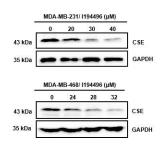
# Multiple exposure images

### Figure 2 repeat 1



35 kDa GAPDH

# Figure 2 repeat 2



### Figure 5 repeat 1

	MDA-MB-468/ 1194496 (µM)			MDA-MB-231/ 1194496 (µM)	MDA-MB-468/ 1194496 (µM)				MDA-MB-231/1194496			
	0 24 28 32			0 20 30 40		21 kDa	100	28 32	Ras		0 20 30 40	_
110 kDa	States and the second	PI3K 11	10 kDa		РІЗК			AND DOOR		21 kDa		Ras
60 kDa		Akt	60 kDa		Akt	80 kDa	Same react	Sec. 1. 1	Raf	80 kDa	and been seen of	Raf
			l		AN	42/44 kDa			ERK1/2	42/44 kDa		ERK1/2
60 kDa	24 35 M	pAkt (	60 kDa		pAkt	42/44 kDa		-	pERK1/2	42/44 kDa	20 20 20 20 20 20 20 20 20 20 20 20 20 2	pERK1/2
35 kDa		GAPDH 3	35 kDa		GAPDH	35 kDa			GAPDH	35 kDa		GAPDH

# Figure 5 repeat 2

	MDA-MB-231/ 1194496 (µM)			MDA-MB-468/ 1194496 (µM)	0		MDA-MB-231/ 1194496 (µM)			MDA-MB-468/ 1194496 (µM)	
	0 20 30 40		21 kDa	0 24 28 32	Ras		0 20 30 40			0 24 28 32	
110 kDa		PI3K				21 kDa		Ras	110 kDa		РІЗК
60 kDa		Akt	80 kDa 42/44 kDa	-	Raf	80 kDa			60 kDa		Akt
60 kDa	11 11	pAkt			ERK1/2	42/44 kDa		ERK1/2	60 kDa		
			42/44 kDa		pERK1/2	42/44 kDa		pERK1/2		a construction of the second second	pAkt
35 kDa		GAPDH	35 kDa		GAPDH	35 kDa		GAPDH	35 kDa		GAPDH

#### Figure 6 repeat 1

	MDA-MB-468/1194496 (µM)			MDA-MB-231/ 1194496 (µM)	MDA-MB-468/ 1194496 (µM)					MDA-MB-231/ 1194496 (µM)			
	0 24 28 32			0 20 30 40			0 24 28 32		37 kDa	0	20 30 40	1	
25 kDa		VEGF	25 kDa		VEGF	37 kDa		Anxa2		1.000	-	Anxa2	
110 kDa		FAK	440.60-			37 kDa		p-Anxa2	37 kDa	-	and the second	p-Anxa2	
TIORDa			110 kDa		FAK	86 kDa		STAT3	86 kDa			STAT3	
65 kDa		Paxillin	65 kDa		Paxillin	86 kDa		p-STAT3	86 kDa			p-STAT3	
35 kDa		GAPDH	35 kDa		GAPDH	35 kDa		GAPDH	35 kDa			GAPDH	

Figure 6 repeat 2

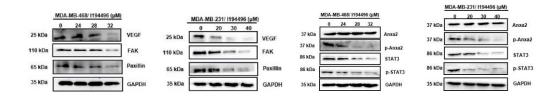


Figure 8 repeat 1

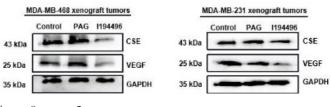
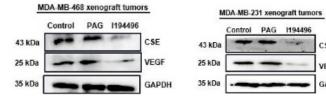
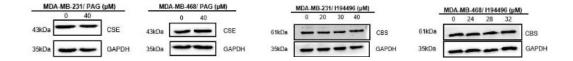


Figure 8 repeat 2



Supplementary figure 2 repeat 1



CSE

VEGF

GAPDH

#### Supplementary figure 2 repeat 2

