Overexpression of Suprabasin is Associated with Proliferation and Tumorigenicity of Esophageal Squamous Cell Carcinoma

Jinrong Zhu[1, 2], Geyan Wu[3], Qingyuan Li[4], Hui Gong[5], Junwei Song[3], Lixue Cao[3], Shu Wu[2], Libing Song[2], Lili Jiang[1]

Department of Pathophysiology, Guangzhou Medical University, Guangzhou, Guangdong 511436, China;

[2] Department of Experimental Research, State Key Laboratory of Oncology in Southern China, Sun Yat-sen University Cancer Centre, Guangzhou, 510060, China;

[3] Department of Biochemistry, Zhongshan School of Medicine, Sun Yat-sen University, Guangzhou, Guangdong 510080, China;

[4] Guangdong Country Garden School, Shunde, Foshan, Guangdong 528312, China;

[5] Central Laboratory, Nanshan Hospital, Guangdong Medical University, Shenzhen, Guangdong, 518052, China.

Correspondence: Lili Jiang, MD. Department of Pathophysiology, Guangzhou Medical University, Guangzhou 511436; P.R. China. Phone: +86(20)37104123;

E-mail: jianglili126@163.com

Supplementary figure legends

Supplementary Figure 1. Suprabasin expression is increased in ESCC. (a) IHC examination of suprabasin protein expression in paired ESCC and adjacent non-tumor tissue specimens. (b) Statistical analysis of the mean optical density (MOD) of suprabasin staining in paired ESCC and adjacent non-tumor tissue specimens. Bars represent the mean ± SD of three independent experiments. * P <0.05.

Supplementary Figure 2. Kaplan–Meier analysis of the correlation between suprabasin protein levels and survival in ESCC. (a) Statistical significance of suprabasin expression in patients at clinical stages I and II (left) or III and IV (right). (b) Statistical significance of suprabasin expression in patients in T1–T2 (left) or T3–T4 (right) subgroups. (c) Statistical significance of suprabasin expression in patients in the lymph node–negative (left) or -positive (right) subgroups. (d) Kaplan–Meier curves of univariate analysis (log-rank) of overall survival for all patients with low versus high suprabasin expression, determined by MOD. *P*-values were calculated using the log-rank test.

Supplementary Figure 3. Downregulation of suprabasin suppresses the tumorigenicity of ESCC cells *in vitro* and *in vivo*. (a) Western blotting analysis of suprabasin expression in ESCC cell lines stably silencing suprabasin. (b) Downregulation of Suprabasin inhibits growth rate of ESCC cells, determined by MTT assay. (c) Quantification of colonies formed by the indicated cells as determined by colony formation assays. (d) Quantification of the BrdUrd incorporation assay in the indicated cells. (e) Quantification of colonies formed by the indicated cells, determined by anchorage-independent growth ability assays. (f) Representative images of the tumors from all mice in each group. (g) Tumor volumes were measured

on the indicated days. (h) Mean tumor weights of all mice in each group. Bars represent the mean ± SD of three independent experiments. * $P < 0.05$.

Supplementary Figure 4. Suprabasin promotes cell proliferation of NEEC cells *in vitro*. (a) Western blotting analysis of suprabasin expression in NEEC1 cell stably overexpressing suprabasin. (b) The growth rate of indicated cells, determined by MTT assay. (c) Quantification of colonies formed by the indicated cells as determined by colony formation assays. All experiments were performed with NEEC1 cells stably overexpressing suprabasin or vector control. Bars represent the mean ± SD of three independent experiments. Bars represent the mean ± SD of three independent experiments. * $P <0.05$.

Supplementary Figure 5. GSK-3β is essential for the oncogenic active effect of suprabasin on Wnt/β-catenin activity in ESCC. (a) Western blotting analysis of GSK-3β and phospho-GSK-3β in indicated cells. (b) Wnt/β-catenin signaling activity determined by luciferase assay of TCF/LEF transcriptional activity in the indicated cells, treated with BIO-A (GSK-3β inhibitor, 1μM) or vehicle control. (c) Cell viability determined by MTT assay in indicated cells, treated with BIO-A (1μM) or vehicle control. (d) Quantification of colonies formed by the indicated cells treated with BIO-A (1μM) or vehicle control, determined by anchorage-independent growth ability assays. Bars represent the mean ± SD of three independent experiments. * $P < 0.05$.

Supplementary Figure 6. Suprabasin promotes angiogenesis of ESCC. The IHC staining displaying that overexpression of suprabasin induced, whereas downregulation of suprabasin inhibited angiogenesis of ESCC *in vivo*, as indicated by the percentages CD31 (right). Bars represent the mean ± SD of three independent

experiments. * P <0.05.

Supplemental Tables

Table 1. Clinicopathological characteristics of patient samples and suprabasin expression in ESCC

	Number of cases (%)
Gender	
Male	128(75.3)
Female	42(24.7)
Age (years)	
≦57	88(51.8)
>57	82(48.2)
Smoking history	
Negative	69(40.6)
Positive	101(59.4)
Clinical stage	
I	10(5.9)
IIa	74(43.5)
IIb	15(8.8)
III	62(36.5)
IV	9(5.3)
T classification	
T1	12(7.1)
T2	40(23.5)
T3	104(61.2)
T4	14(8.2)
N classification	
N0	93(54.7)
N1	77(45.3)
M classification	
M0	161(94.7)
M1	9(5.3)
Differentiation	
Well	76(44.7)
Moderate	61(35.9)
Poor	33(19.4)
Treatment methods*	
Resection	45(26.5)
CT/RT/CRT	55(32.4)

Resection+CT/RT/CRT	70(41.2)
Vital status (at follow-up)	
Alive	59(34.7)
Death	111(65.3)
Expression of suprabasin	
Low expression	85(50.0)
High expression	85(40.0)

* Resection: Endoscopic resection or esophagectomy; CT: Chemotherapy; RT: Radiotherapy; CRT: Chemoradiotherapy;

Table 2. Correlation between suprabasin expression and clinicopathologic characteristics of ESCC

Characteristic		Suprabasin		Chi-square test *P*-value
		Low No. cases (%)	High No. cases (%)	
Gender	Male	60(70.6)	68(80.0)	0.155
	Female	25(29.4)	17(20.0)	
Age (years)	≦57	44(51.8)	44(51.8)	1.000
	>57	41(48.2)	41(48.2)	
Smoking history	Negative	33	36	0.338
	Positive	45	66	
Clinical stage	I	8(9.4)	2(2.4)	<0.0001
	IIa	60(70.6)	14(16.5)	
	IIb	3(3.5)	12(14.1)	
	III	13(15.3)	49(57.6)	
	IV	1(1.2)	8(9.4)	
T classification	T1	10(11.8)	2(2.4)	<0.0001
	T2	26(30.6)	14(16.5)	
	T3	48(56.5)	56(65.9)	
	T4	1(1.2)	13(15.3)	
N classification	N0	69(81.2)	24(28.2)	<0.0001
	N1	16(18.8)	61(71.8)	
M classification	M0	84(98.8)	77(90.6)	0.016
	M1	1(1.2)	8(9.4)	
Differentiation	Well	59(69.4)	17(20.0)	<0.0001
	Moderate	21(24.7)	40(47.1)	
	Poor	5(5.9)	28(32.9)	
Vital status	Alive	46(54.1)	13(15.3)	<0.0001
	Death	39(45.9)	72(84.7)	

Table 3. Spearman correlation analysis between suprabasin expression and clinical pathologic factors

Variables	Suprabasin expression level	
	Spearman correlation	*P*-value
Gender	0.001	0.988
Age	-0.109	0.157
Clinical stage	0.575	<0.0001
T classification	0.325	<0.0001
N classification	0.532	<0.0001
M classification	0.068	0.374
Differentiation	0.512	<0.0001
Tumor size	0.183	0.017
Survival	-0.619	<0.0001
Vital status	0.412	<0.0001

Table 4. Univariate and multivariate analyses of various prognostic parameters in patients with ESCC Cox-regression analysis

	Univariate analysis		*Multivariate analysis*		
	P	**Relative risk (SE)**	***P***	**Relative risk**	**95% confidence interval**
Gender	0.009	0.523(0.247)	0.014	0.537	0.327-0.881
Age	0.002	1.029(0.009)	0.002	1.031	1.012-1.052
Clinical stage	0.003	1.272(0.082)	0.001	0.609	0.456-0.814
T classification	0.002	1.601(0.150)	0.194	1.237	0.897-1.706
N classification	<0.001	2.109(0.193)	0.053	1.828	0.991-3.373
M classification	0.603	0.788(0.458)	0.616	0.707	0.182-2.741
Differentiation	<0.001	2.723(0.131)	<0.001	2.114	1.568-2.849
Tumor size	0.029	1.005(0.002)	0.4	1.033	1.013-1.054
Expression of suprabasin	<0.001	4.295(0.204)	<0.001	4.709	2.658-8.342

Table 5. Spearman correlation analysis between suprabasin expression (determined by MOD) and clinical pathologic factors

Variables	Suprabasin expression level	
	Spearman correlation	*P*-value
Gender	0.082	0.289
Age	-0.067	0.386
Clinical stage	0.459	<0.001
T classification	0.362	<0.001
N classification	0.461	<0.001
M classification	0.079	0.307
Differentiation	0.292	<0.001
Tumor size	0.434	<0.001
Survival	-0.335	<0.001
Vital status	0.259	0.001

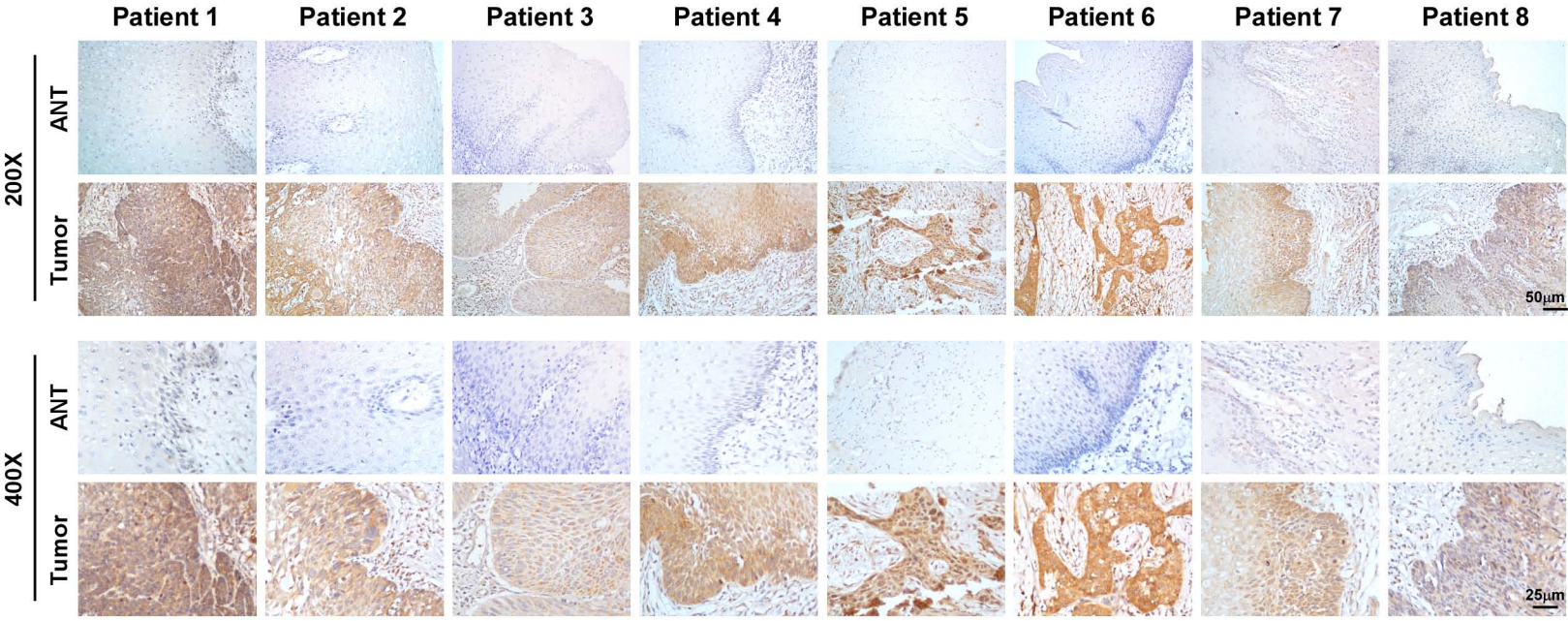
Table 6. Univariate and multivariate analyses of various prognostic parameters in patients with ESCC Cox-regression analysis

(Expression of suprabasin determined by MOD)

	Univariate analysis		*Multivariate analysis*		
	P	**Relative risk (SE)**	***P***	**Relative risk**	**95% confidence interval**
Gender	0.009	0.523(0.247)	0.015	0.660	0.399-1.091
Age	0.002	1.029(0.009)	0.006	1.028	1.008-1.049
Clinical stage	0.003	1.272(0.082)	0.032	0.789	0.489-1.273
T classification	0.002	1.601(0.150)	0.350	1.199	0.820-1.753
N classification	<0.001	2.109(0.193)	0.140	1.974	0.800-4.870
M classification	0.603	0.788(0.458)	0.662	0.749	0.206-2.730
Differentiation	<0.001	2.723(0.131)	<0.001	2.555	1.942-3.362
Tumor size	0.029	1.005(0.002)	0.382	1.003	0.997-1.009
Expression of suprabasin (MOD)	<0.001	2.200(0.195)	0.030	1.633	1.048-2.545

Supplementary Figure 1

a



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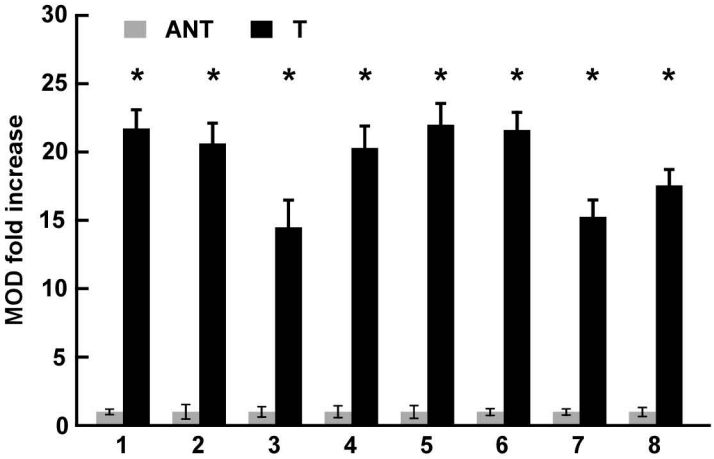

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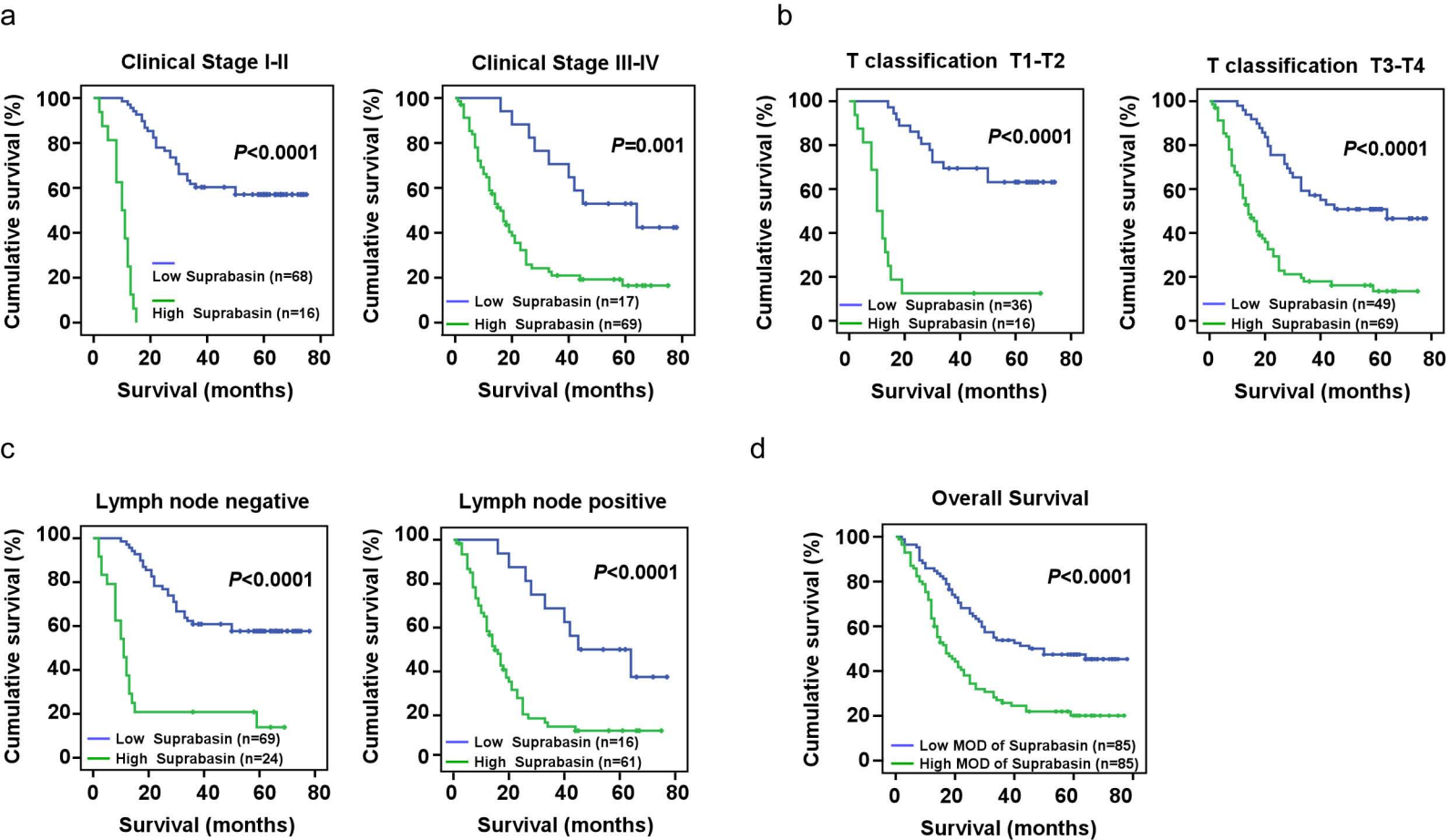

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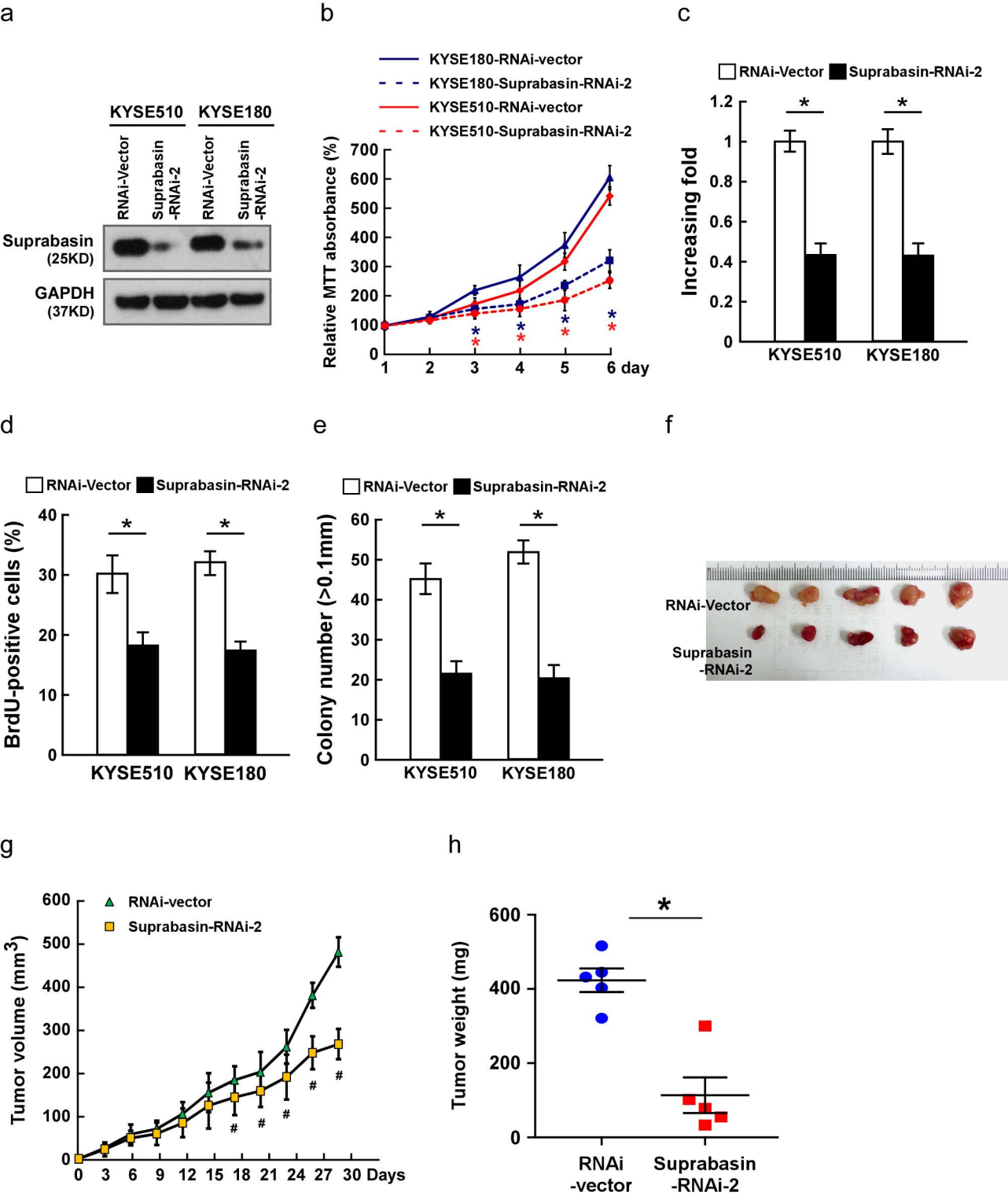

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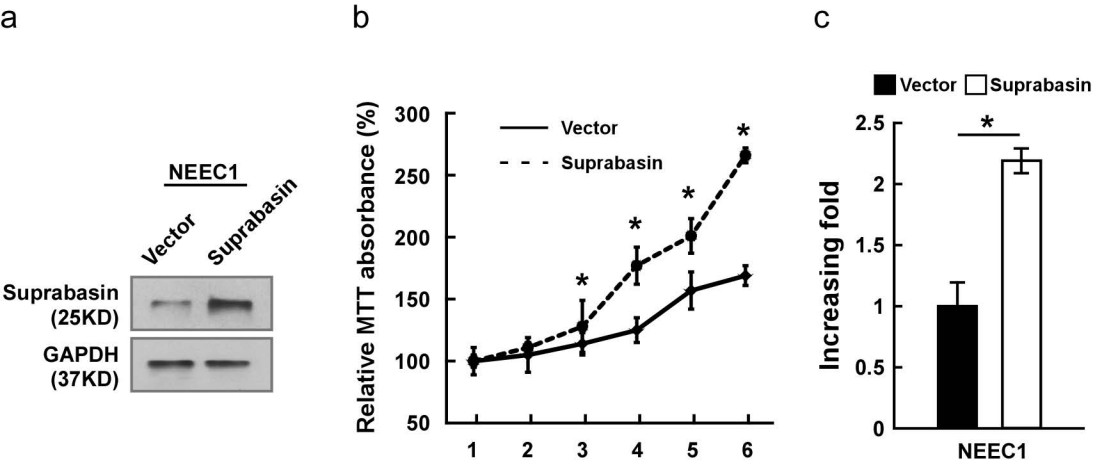
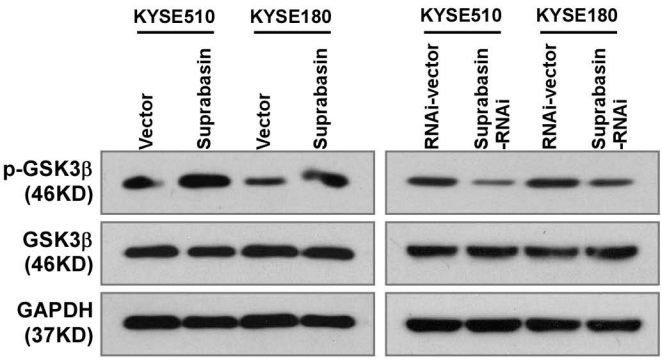


Supplementary Figure 5

a



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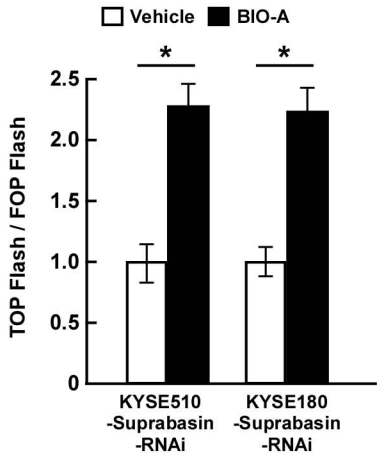

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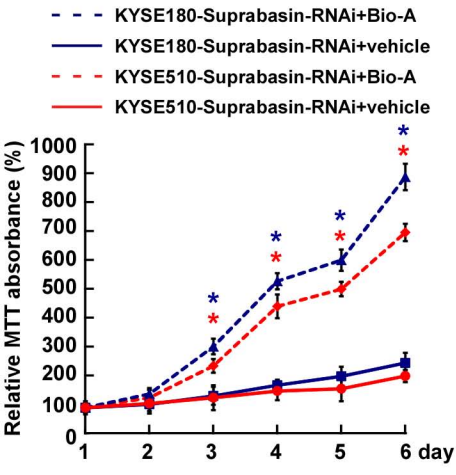

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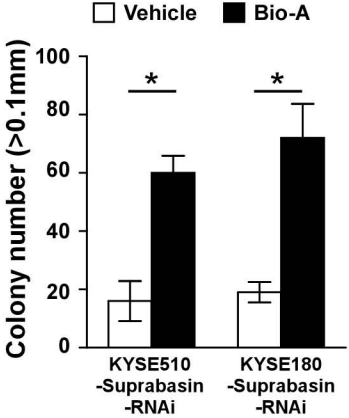

Supplementary Figure 6