SOD2 deregulation enhances migration, invasion and has poor prognosis in salivary adenoid cystic carcinoma

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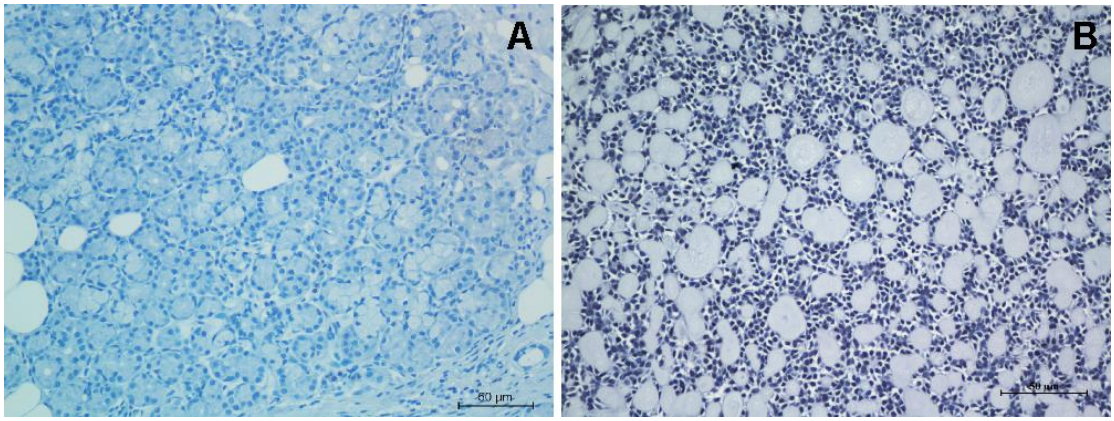
S1 Table. Clinicopathologic characteristics of SACC patients

		No. of cases (%)
Gender	Male	19(38%)
	Female	31(62%)
Age(years)	≤40	18(36%)
	＞40	32(64%)
Tumor stage	T 1	4(8%)
	T 2	27(54%)
	T 3	6(12%)
	T 4	13(26%)
Distant metastasis	Negative	8(16%)
	Positive	42(84%)
Recurrence	Negative	39(78%)
	Positive	11(22%)
Vital status	Alive	42(84%)
	Death	8(16%)
Expression of SOD2	Low expression	28(56%)
	High expression	22(44%)
Tumor site	Parotid	23(46%)
	Submandibular	23(46%)
	Sublingual	4(8%)

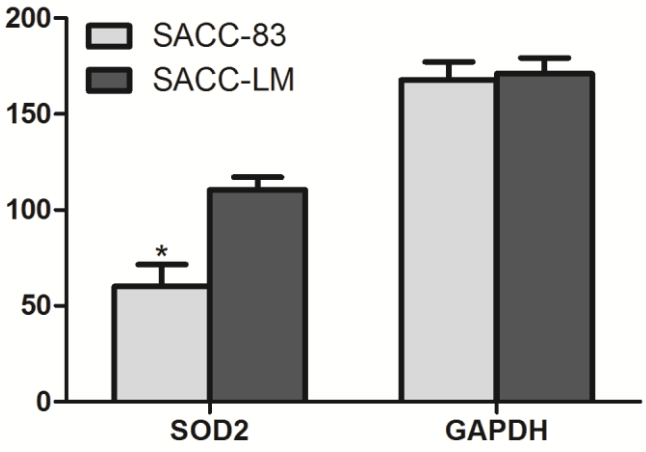
S2 Table. The histological normal salivary gland tissues used in this study

	No. of cases	Tissues from
normal sublingual glands	**7**	from sublingual gland cyst
	1	from radical neck dissection with tongue squamous cell carcinoma
normal parotid glands	**2**	from radical neck dissection with tongue squamous cell carcinoma
	2	from parotid benign hypertrophy
normal submandibular glands	8	from radical neck dissection with tongue squamous cell carcinoma



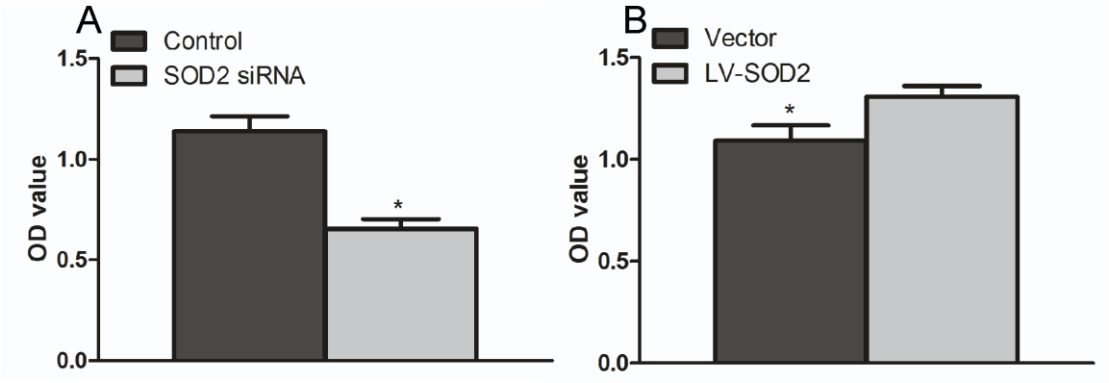
S1 Figure: Isotype control for immunohistochemistry staining of SOD2 expression in SACC and normal salivary gland tissue

Isotype control immunohistochemistry staining for SOD2 expression in A: normal salivary gland; B: salivary gland adenoid cystic carcinoma



S2 Figure. The quantification of SOD2 expression in SACC cell lines

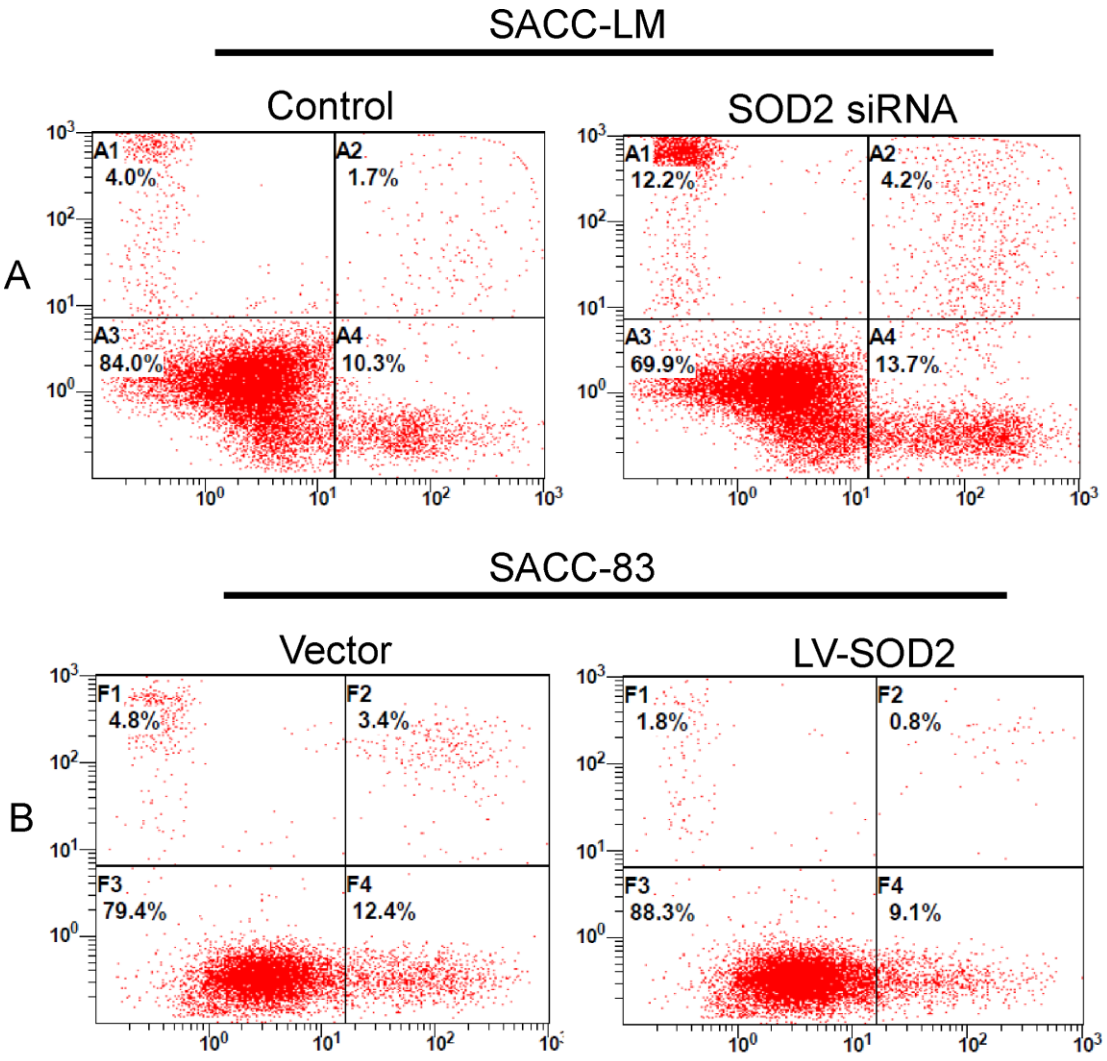
SOD2 protein expression was quantified by Image J software. SOD2 expression was significantly higher in SACC-LM cells than in SACC-83 cells. *P =0.018



S3 Figure. Cell proliferation of SACC cells was measured using a CCK-8 assay

(A) The cell proliferation rate of SACC-LM line was significantly inhibited after transfection with the SOD2 siRNA. **P* = 0.000. (B) The cell proliferation rate of SACC-83 line was significantly enhanced after SOD2 overexpression. **P* = 0.007. Student's T test was used to compare the difference between groups.

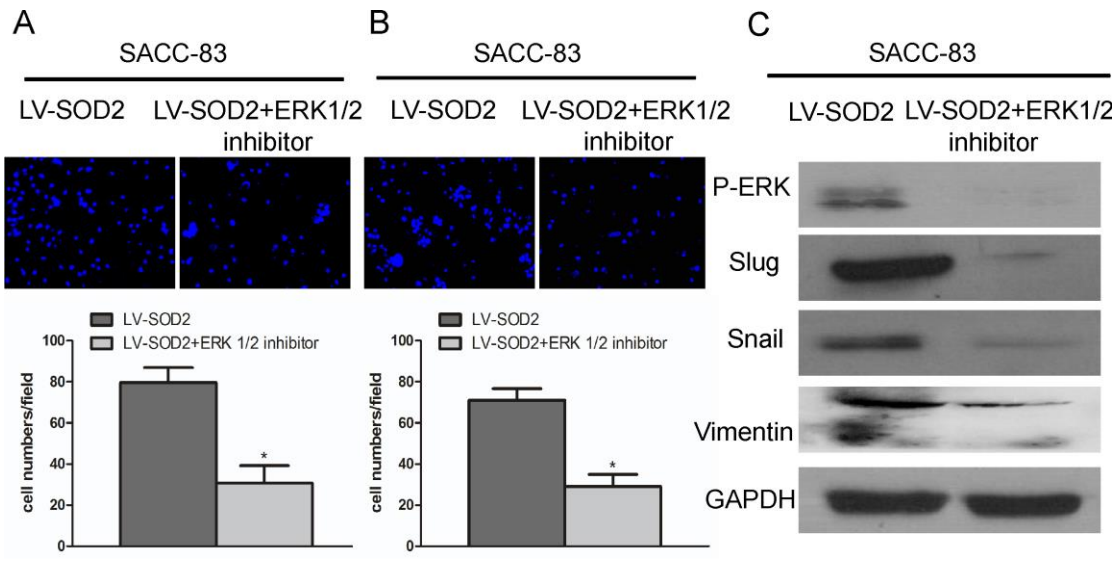
Cell proliferation was measured by Cell Counting Kit-8 assay according to the manufacturer's instructions. In brief, cells were seeded in 96-well plates at the density of 5 × 10^3 cells per well after incubation for 24 h. Then added 10ul of the CCK-8 solution to each well of the plate and incubate the plate for 2 h in the incubator. The absorbance value of each well was assayed using a plate reader at a wavelength of 450 nm. Experiments were performed in triplicate.



S4 Figure. Apoptosis of SACC cells was detected by Annexin V-FITC-PI flow cytometry assay

(A) The apoptosis rates was 10.3+1.7% in control siRNA compare to 13.7+4.2% in SOD2 siRNA transfected group. (*P*= 0.176). (B) The apoptosis rates was 12.4+3.4% in control lentivirus compare to 9.1+0.8% in SOD2 overexpression group. (*P* = 0.098). Student's T test was used to compare the difference between groups.

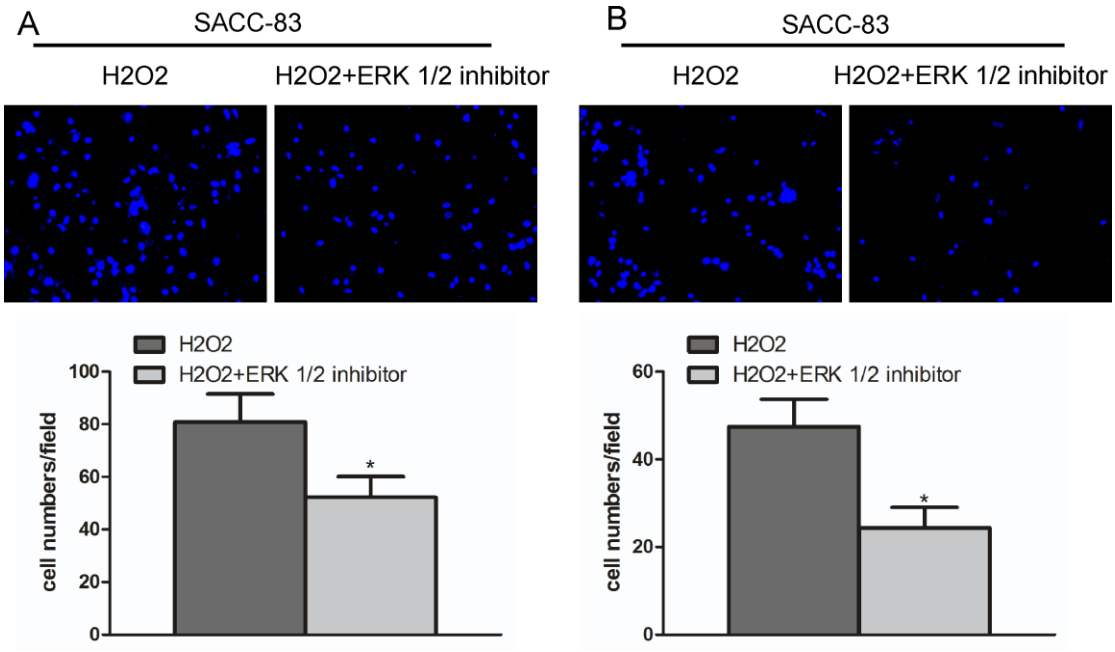
Annexin V-FITC-PI flow cytometry assay was used to detect apoptosis. Briefly, the SACC cells were transfection with the SOD2 siRNA for 24h or lentivirus containing SOD2 for 48h. Following collection, the cells were stained with Annexin V-FITC and PI. The apoptotic cells were estimated using a Beckman-Gallios flow cytometer (Beckman-Gallios, Miami, FL, USA). Experiments were performed in triplicate.



S5 Figure. ERK 1/2 inhibitor blocks SOD2-induced metastasis and decrease its related protein expression in SACC.

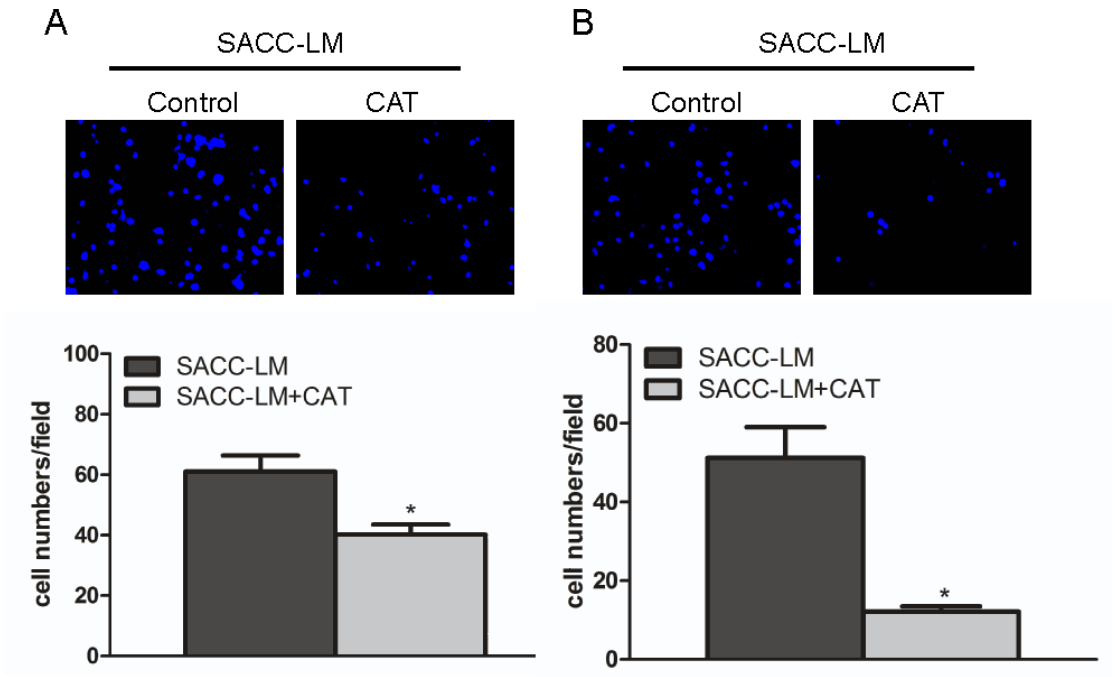
ERK 1/2 inhibitor blocked SOD2-induced migration (A, *P* = 0.000) and invasion (B, *P* = 0.000) in SACC-83 cells after overexpression of SOD2. **P* <0.001. (C) Vimentin, Slug, Snail, p-ERK were decreased in SACC-83 cells with SOD2 overexpression after treated with ERK 1/2 inhibitor.

We purchase the ERK 1/2 inhibitor (SCH772984) which was dissolved in DMSO for in vitro studies from the Selleck chemicals. The SACC-83 cells were harvested for following experiments after added SCH772984 for 48h.



S6 Figure. ERK 1/2 inhibitor blocks H2O2-induced metastasis in SACC.

ERK 1/2 inhibitor blocked H2O2-induced migration (A, *P* = 0.0013) and invasion (B, *P* = 0.002) in SACC-83 cells after treated with H2O2. **P* <0.05.



S7 Figure: Catalase inhibits both the migration and invasion of SACC

To gain more insight into the function of H_2O_2, SACC-LM cells were treated with catalase,

which can remove of H_2O_2. The migration (A, *P*=0.000) and invasion (B, *P*=0.000) of SACC-LM were significantly inhibited after treatment with 600 U catalase. *P <0.001.