OMTN, Volume *28*

Supplemental information

The circular RNA circNlgn mediates doxorubicin-induced cardiac remodeling and fibrosis

Jindong Xu, William W. Du, Nan Wu, Feiya Li, Xiangmin Li, Yizhen Xie, Sheng Wang, and Burton B. Yang

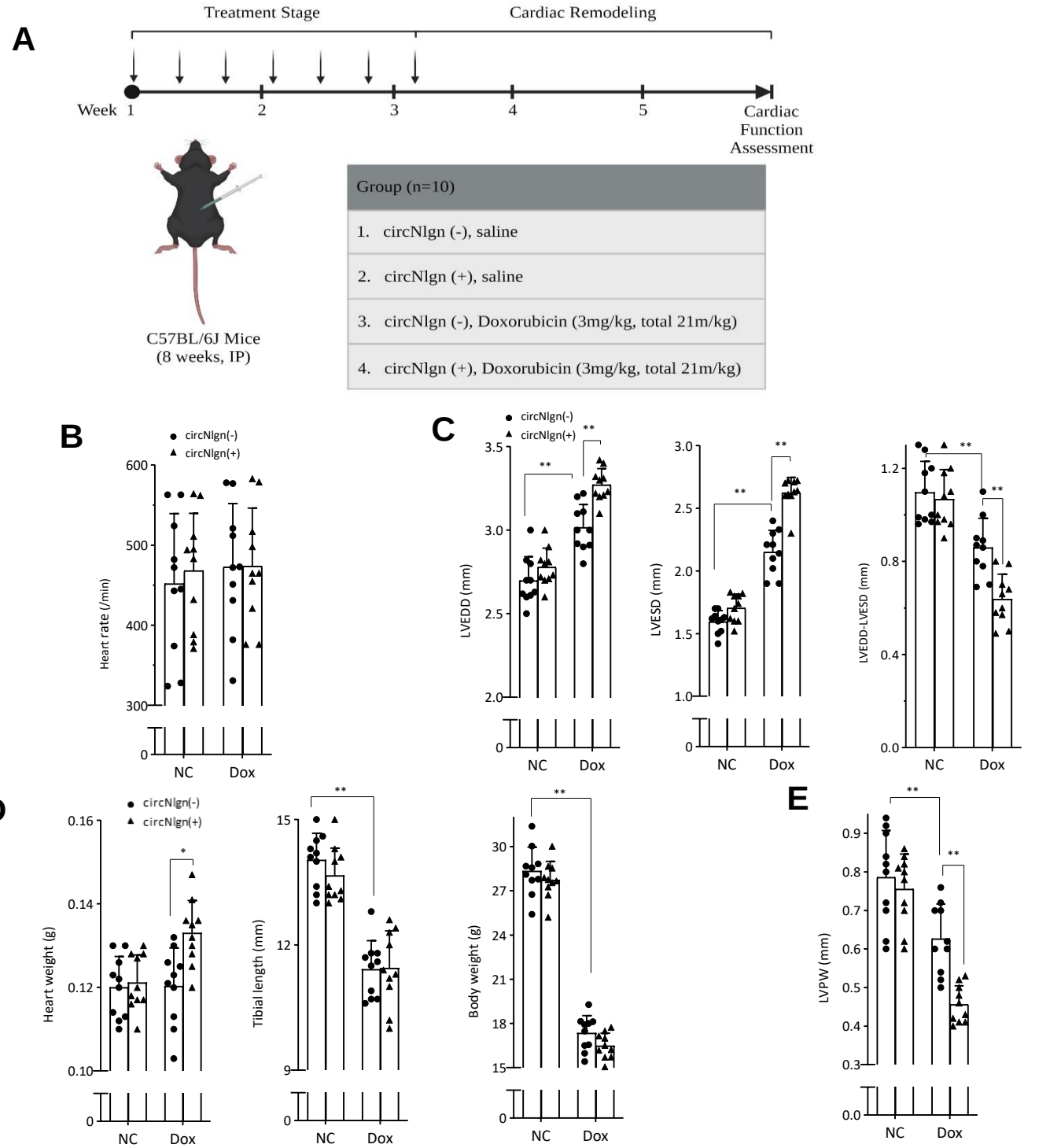

Fig S1. Heart functions affected by Doxorubicin-treatment.

A. Scheme of the *in vivo* experiment.

B. Both circNlgn transgenic mice circNlgn(+) and the litter-matched negative mice circNlgn(-) showed no significant difference in heart rates with or without Doxorubicin treatment. (*n=10*).

C. circNlgn transgenic mice circNlgn(+) showed increased left ventricular end-diastolic diameter (LVEDD) and left ventricular end-systolic diameter (LVESD), but decrease in LVEDD-LVESD relative to the litter-matched negative mice circNlgn(-) after Doxorubicin treatment. ***p<0.01* versus circNlgn(-) *(n=10)*

D. Left, circNlgn transgenic mice circNlgn(+) showed increased heart weight relative to the litter-matched negative mice circNlgn(-) after Doxorubicin treatment. There were significant difference in Tibial length and body weight between circNlgn(+) and circNlgn(-) with or without Doxorubicin treatment. **p<0.05*, ***p<0.01* versus circNlgn(-) *(n=10).*

E. circNlgn transgenic mice circNlgn(+) showed decreased left ventricular posterior wall thickness (LVPW) relative to the litter-matched negative mice circNlgn(-) after Doxorubicin treatment. ***p<0.01* versus circNlgn(-) *(n=10).*

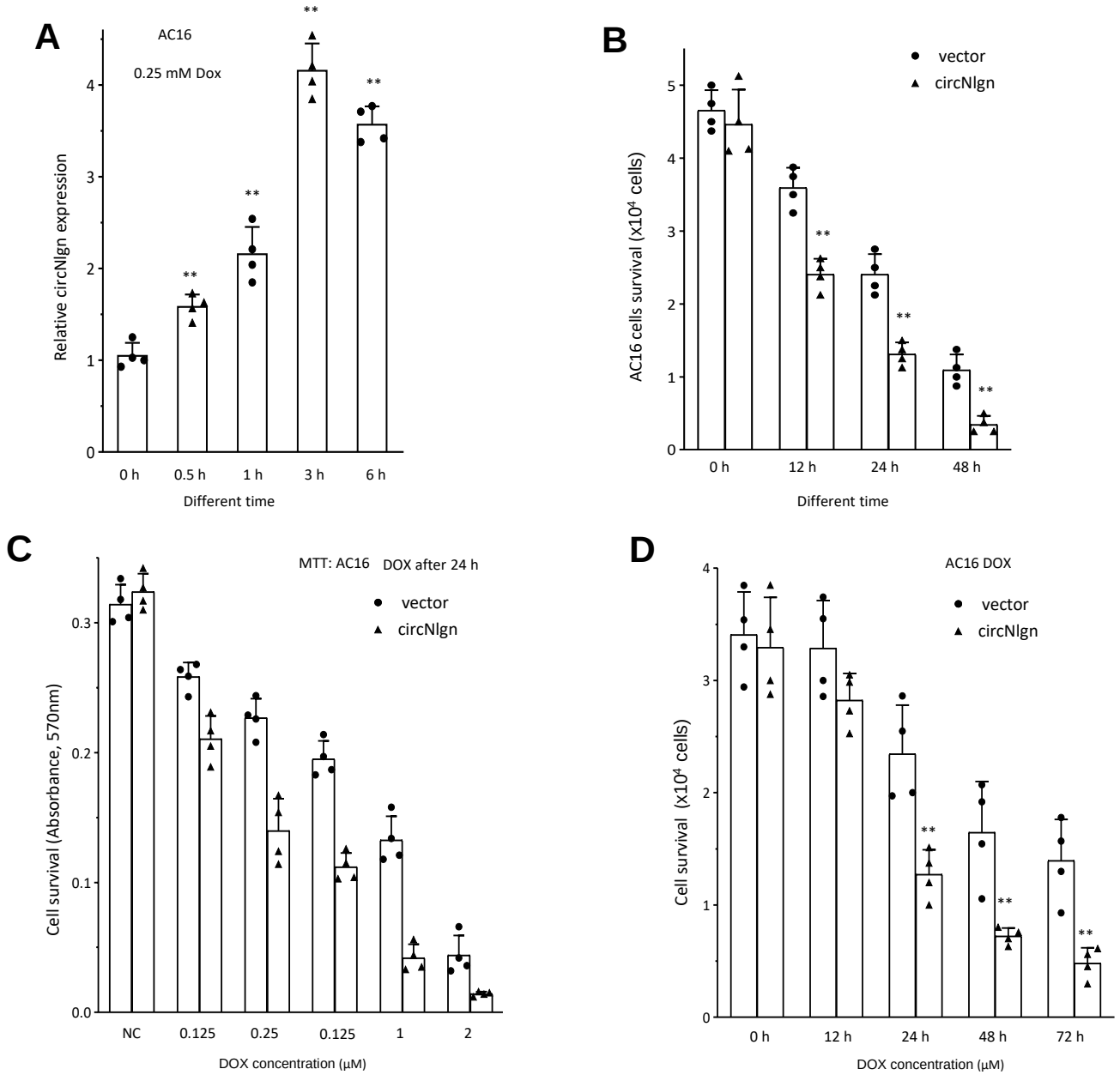

Fig S2. AC16 cell survival affected by circNlgn expression.

A. AC16 cells were cultured in basal medium treated with 0.25 μM Doxorubicin for indicated time points, and processed to RT-PCR. Doxorubicin treatment increased circNlgn levels. *$p<0.05$, **$p<0.01$ versus 0 μM Doxorubicin ($n=4$).

B. Control vector- and circNlgn-transfected AC16 cells were cultured in basal medium treated with 0.25 μM Doxorubicin for indicated time points, and the cells were harvested and counted by Coulter Counter under an inverted microscope. Expression circNlgn suppressed cell survival when treated with 0.25 μM Doxorubicin for indicated time points. *$p<0.05$, **$p<0.01$ versus control ($n=4$).

C. AC16 cells were transfected with control vector and circNlgn, cultured with basal medium and treated with Doxorubicin at indicated concentrations for 24 h. MTT assays showed that expression circNlgn repressed AC16 cell survival. *$p<0.05$, **$p<0.01$ versus control ($n=4$).

D. AC16 cells were transfected with control vector and circNlgn, and treated with 0.25 μM Doxorubicin for indicated time points. Expression circNlgn repressed cell survival. *$p<0.05$, **$p<0.01$ versus control ($n=4$).

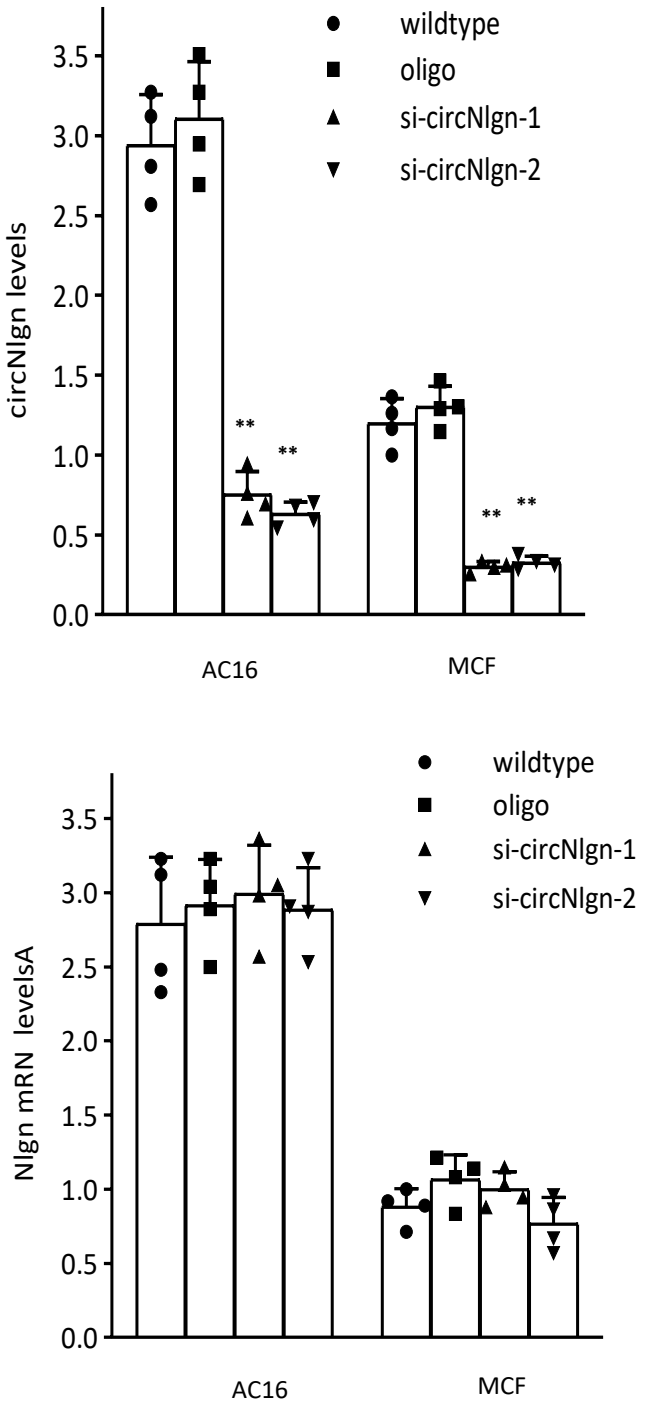

Fig S3. Silencing circNlgn expression.

AC16 and MCF cell cultures were transfected with siRNAs targeting circNlgn, followed by real-time PCR measurement of circNlgn (upper) and Nlgn mRNA (lower) levels. Transfection with the siRNAs significantly decreased circNlgn levels relative to the controls. ***p<0.01.* (*n=4*).

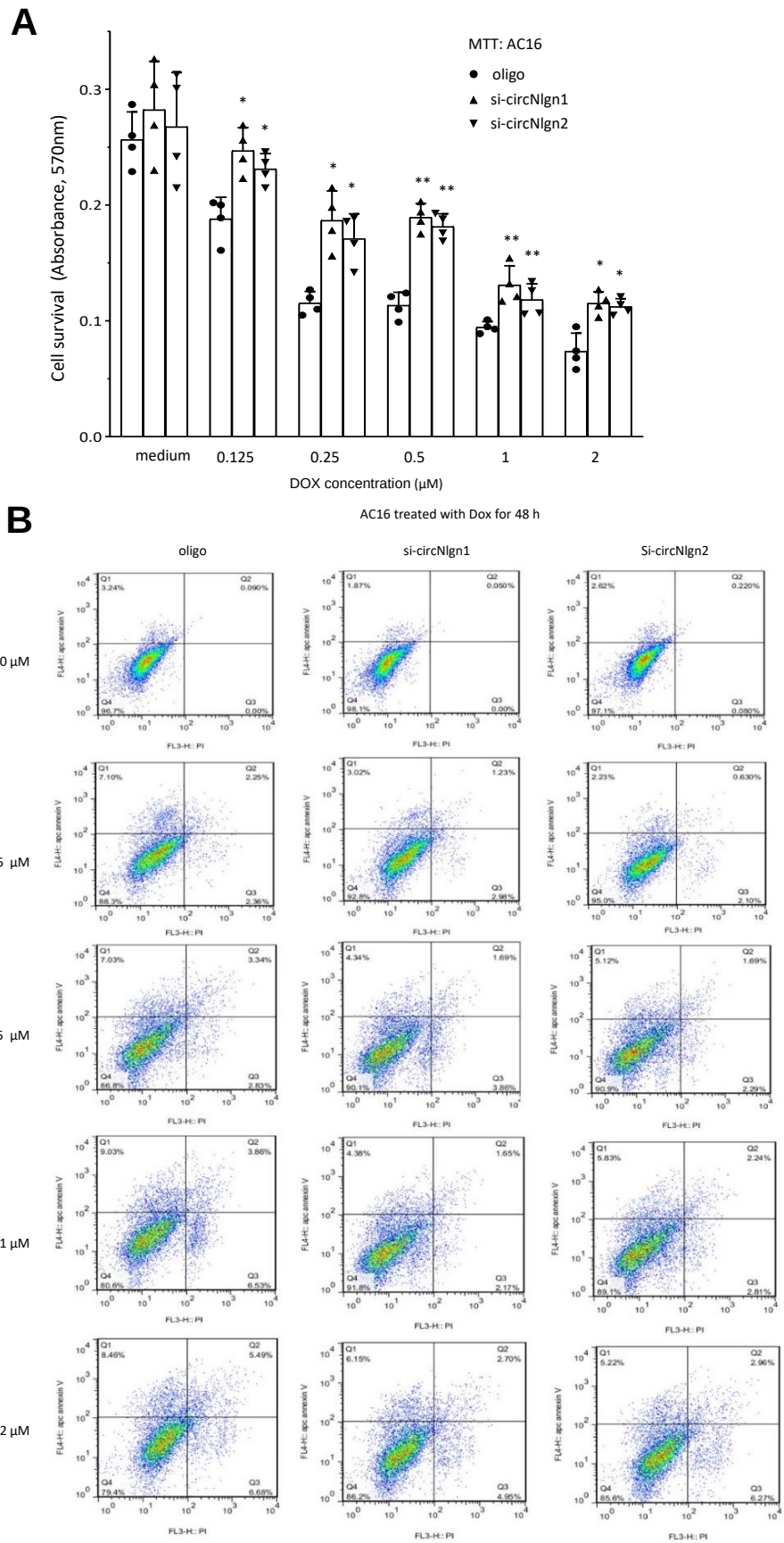

Fig S4. Silencing circNlgn increased AC16 cell viability.

A. AC16 cells were transfected with control oligo and circNlgn siRNAs, cultured in basal medium, and treated with Doxorubicin at indicated concentrations for 24 h. MTT assays showed that silencing circNlgn enhanced AC16 cell survival. *$p<0.05$, **$p<0.01$ versus control ($n=6$).

B. Lower, typical profiles of cell apoptosis are shown.

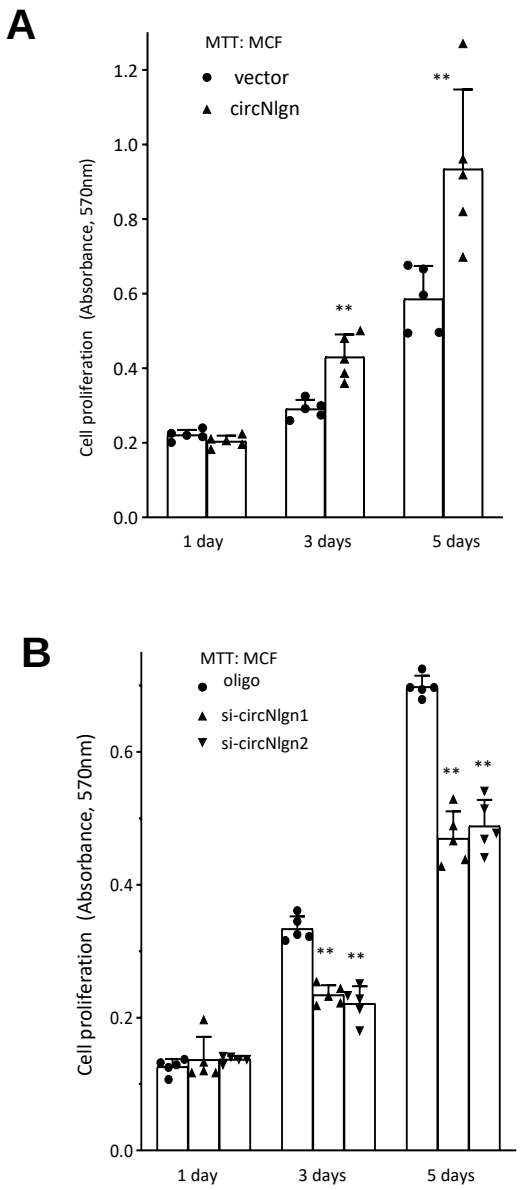

Fig S5. MCF cell activities affected by circNlgn expression.

A. Control vector- and circNlgn-transfected MCF cells were cultured in basal medium for indicated time points. Cell proliferation was measured by MTT assays. Expression of circNlgn promoted cell proliferation. *$p<0.05$, **$p<0.01$ versus control ($n=5$).

B. MCF cells were transfected with control oligo and circNlgn siRNAs, and cultured in basal medium for indicated time points. MTT assays showed that silencing circNlgn with siRNAs repressed MCF cell proliferation. *$p<0.05$, **$p<0.01$ versus control ($n=5$).

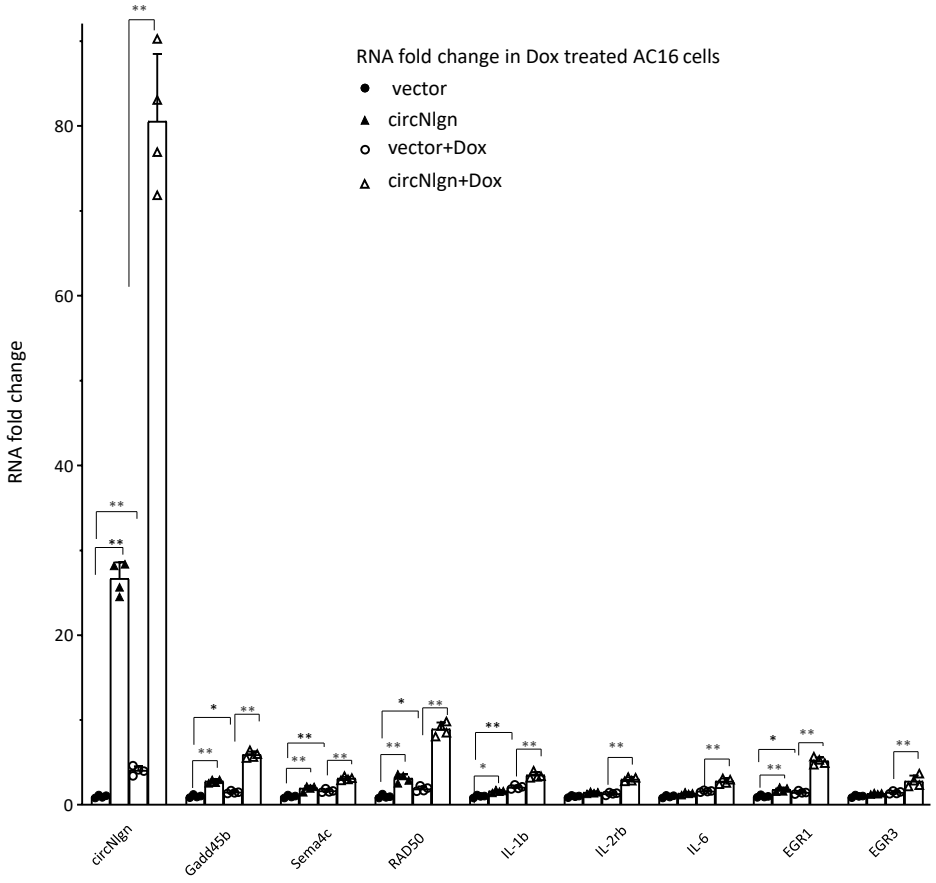

Fig S6. Effect of Doxorubicin on circNlgn-promoted expression of molecules associated with fibrosis.

Control vector- and circNlgn-transfected AC16 cells were cultured in basal medium treated with 0.25 μM Doxorubicin for 24 h, harvested, and subjected to RT-PCR. Expression circNlgn increased Gadd45b, Sema4C, RAD50, IL-1b, IL-2Rb, IL-6, EGR1, and EGR3 levels, which were further promoted by Doxorubicin treatment. *$*p<0.05$, **$*p<0.01$ ($n=4$).