Supplementary Information

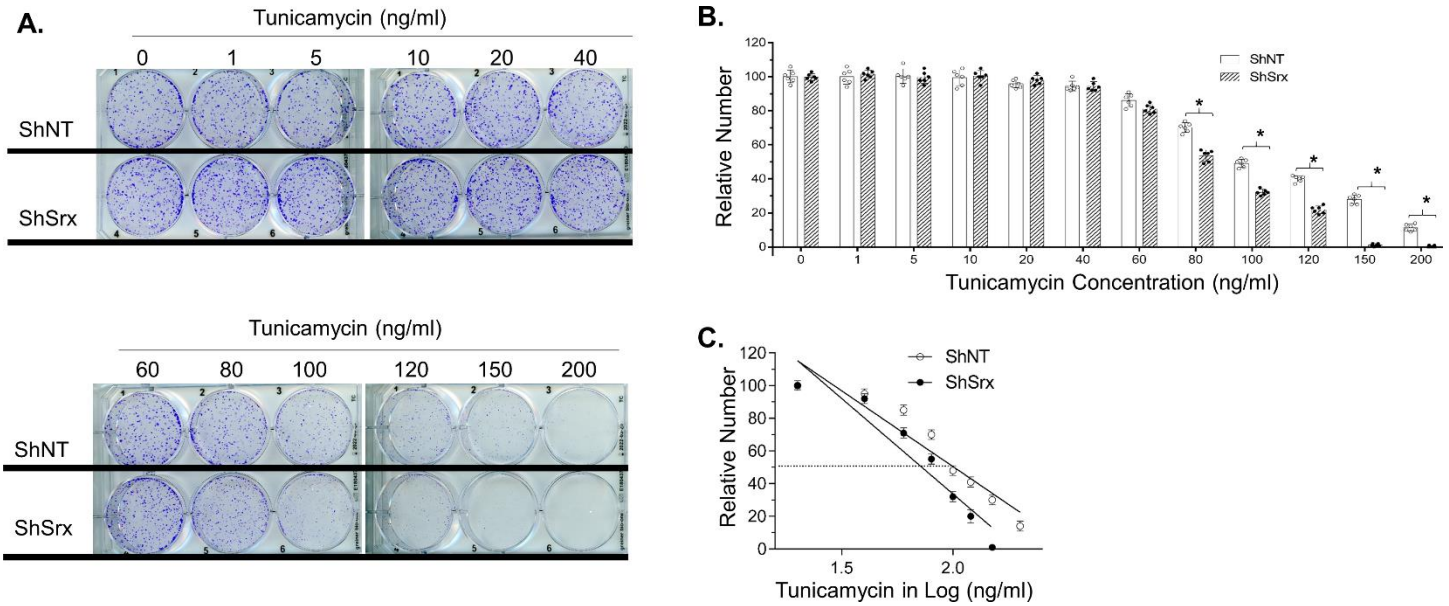


Fig. S1. Clonogenic assay of A549 control and Srx knockdown cells in the presence of increasing dose of tunicamycin. ShNT, control cells with non-target ShRNA. ShSrx, cells with knockdown of Srx. (A) Colonies stained by crystal violet and visualized by imaging. (B) Quantitative summary of colony numbers clonogenic assay (* $p < 0.05$, *t* test). (C) Determination of IC50 of tunicamycin to inhibit colony formation in control cells (IC50 = 101 ng/ml) and in Srx knockdown cells (72 ng/ml). Since concentration lower than 40 ng/ml has no effect on colony formation, only data with the treatment of tunicamycin from 40 to 200 ng/ml were used in the regression analysis to determine the IC50.

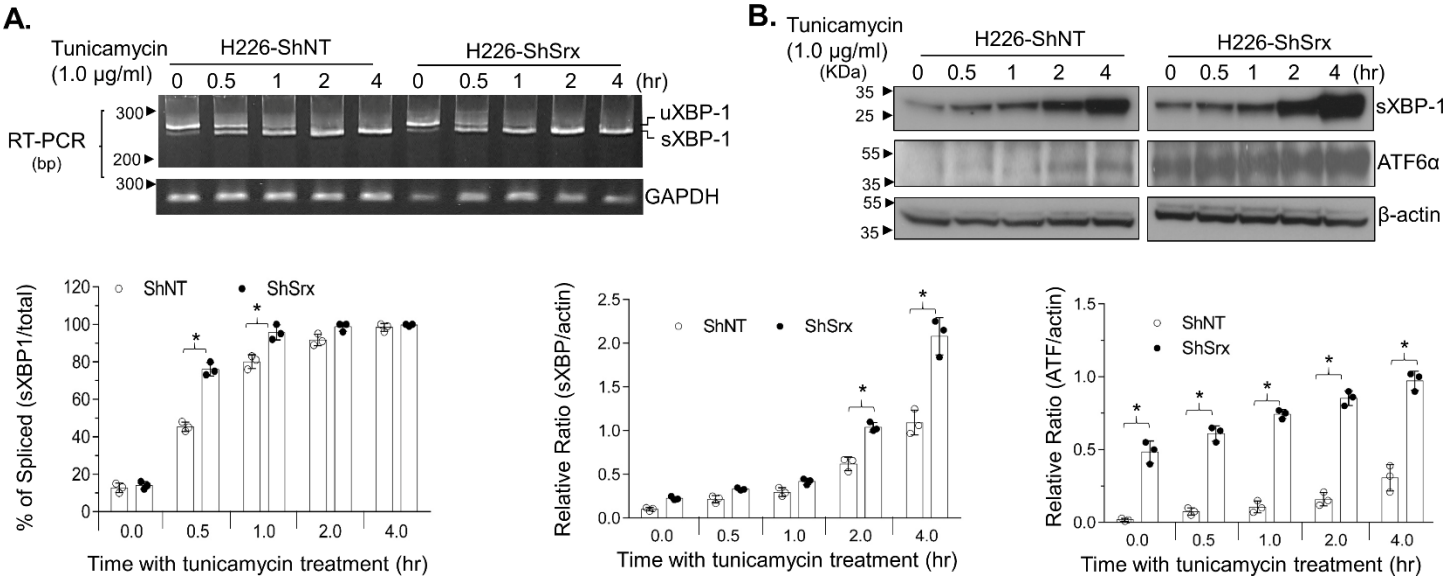
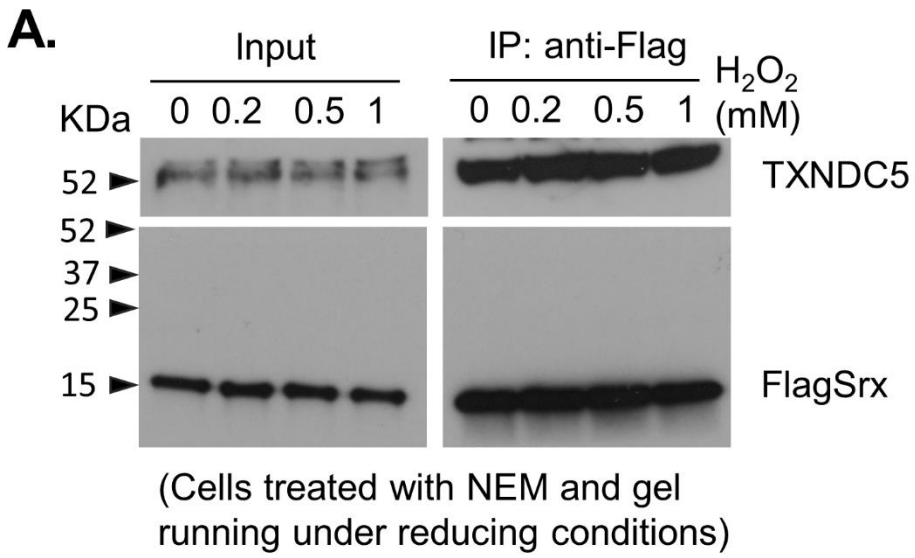


Fig. S2. Knockdown of Srx sensitizes human lung cancer H226 cells to ER-stress induced cell death. (A) Knockdown of Srx in H226 cells induces a rapid response to UPR as indicated by the accelerated splicing of XBP-1 mRNA. (B) Expression of spliced XBP protein and activation of ATF6 in the presence of tunicamycin in H226 control and Srx knockdown cells. Bar graph with dot plot indicates the quantitative results from three independent experiments (*$p < 0.05$, *t* test).



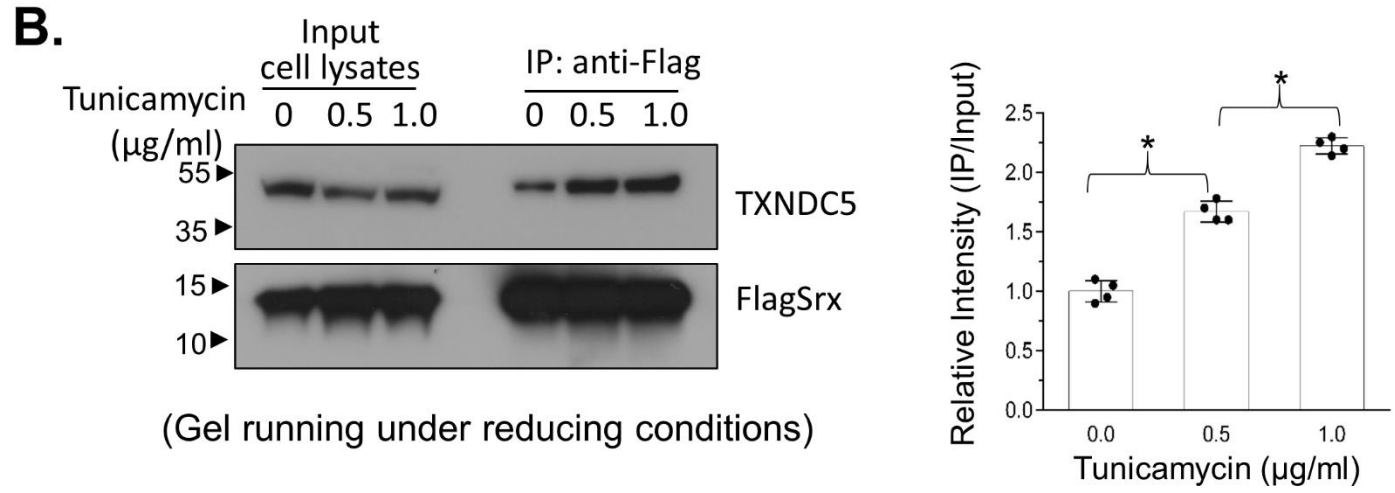


Fig. S3. The effect of exogenous H_2O_2 and tunicamycin on the formation of the Srx-TXNDC5 complex. (A) HEK293-FlagSrx cells were treated with vehicle or increasing concentrations of H_2O_2 for 10 min in the presence of thiol blocker N-ethylmaleimide (NEM). Cell lysates were collected and IPs were performed using anti-Flag antibody. IP eluates were separated by SDS-PAGE under reducing conditions. Western blot results indicate that treatment of cells with H_2O_2 does not affect the amount of endogenous TXNDC5 pull down by Flag-Srx in HEK293T cells. (B) HEK293-FlagSrx cells were treated with vehicle or increasing concentrations of tunicamycin for 16 hrs. Cell lysates were collected and IPs were performed using anti-Flag antibody. IP eluates were separated by SDS-PAGE under reducing conditions and western blot indicate increased complex formation. Bar graph with dot plot indicates the quantitative results from three independent IP experiments (*$p < 0.05$, *t* test).

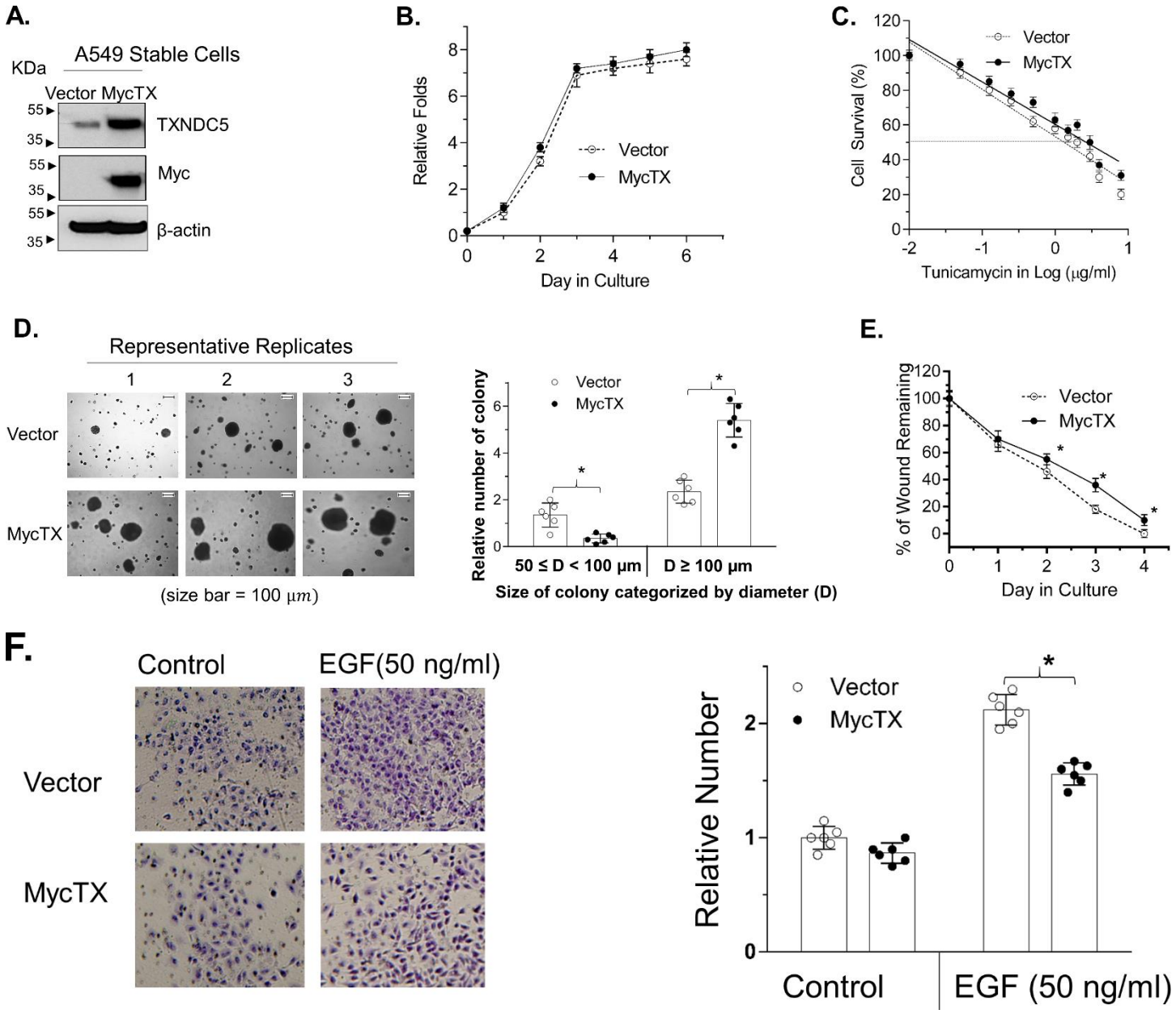


Fig. S4. Overexpression of Myc-TXNDC5 in A549 cells promotes cell invasion. (A) Overexpression of Myc-TXNDC5 in A549 cells. (B) Representative results of cell proliferation evaluated by the modified XTT assay. (C) Myc-TXNDC5 expressing cells are more resistant to tunicamycin-induced cell death. The IC50 for Myc-TXNDC5 cells and control vector cells are 2.6 and 1.33 μg/ml, respectively. (D) Overexpression of Myc-TXNDC5 stimulates anchorage-independent colony formation in soft agar, in particular, the growth of colonies with diameter larger than 100 μm. (E) Data from wound-healing assay indicate that overexpression of Myc-TXNDC5 enhances cell migration. (F) Matrigel invasion assay indicate that Myc-TXNDC5 cells are less invasive in the presence of EGF. Bar graph with dot plot indicates the quantitative results (*$p < 0.05$, *t* test).

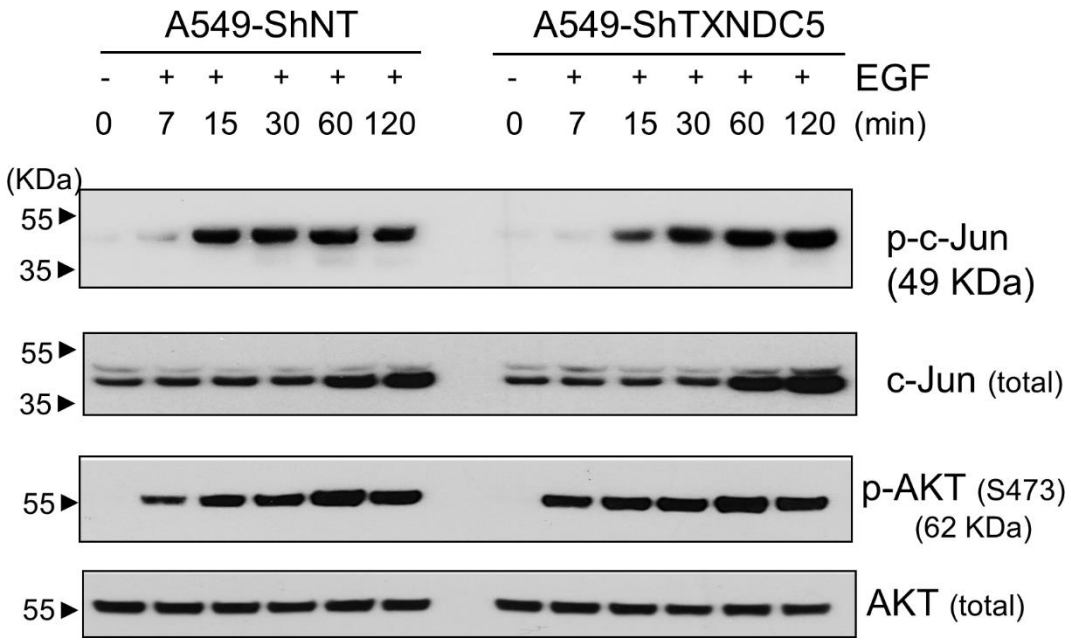


Fig. S5. Knockdown of TXNDC5 has no effect on the activation of c-Jun and AKT. A549 control and TXNDC5 knockdown cells were serum-starved for overnight and treated with fresh medium containing EGF for indicated period of time. Cell lysates were harvested for western blotting to examine the activation of c-Jun and AKT. After quantification of band intensity, no significant differences were found between control and TXNDC5 knockdown cells.