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Supplemental Material to:

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The role of GABARAPL1/GEC1 in autophagic flux and mitochondrial quality control in MDA-MB-436 breast cancer cells

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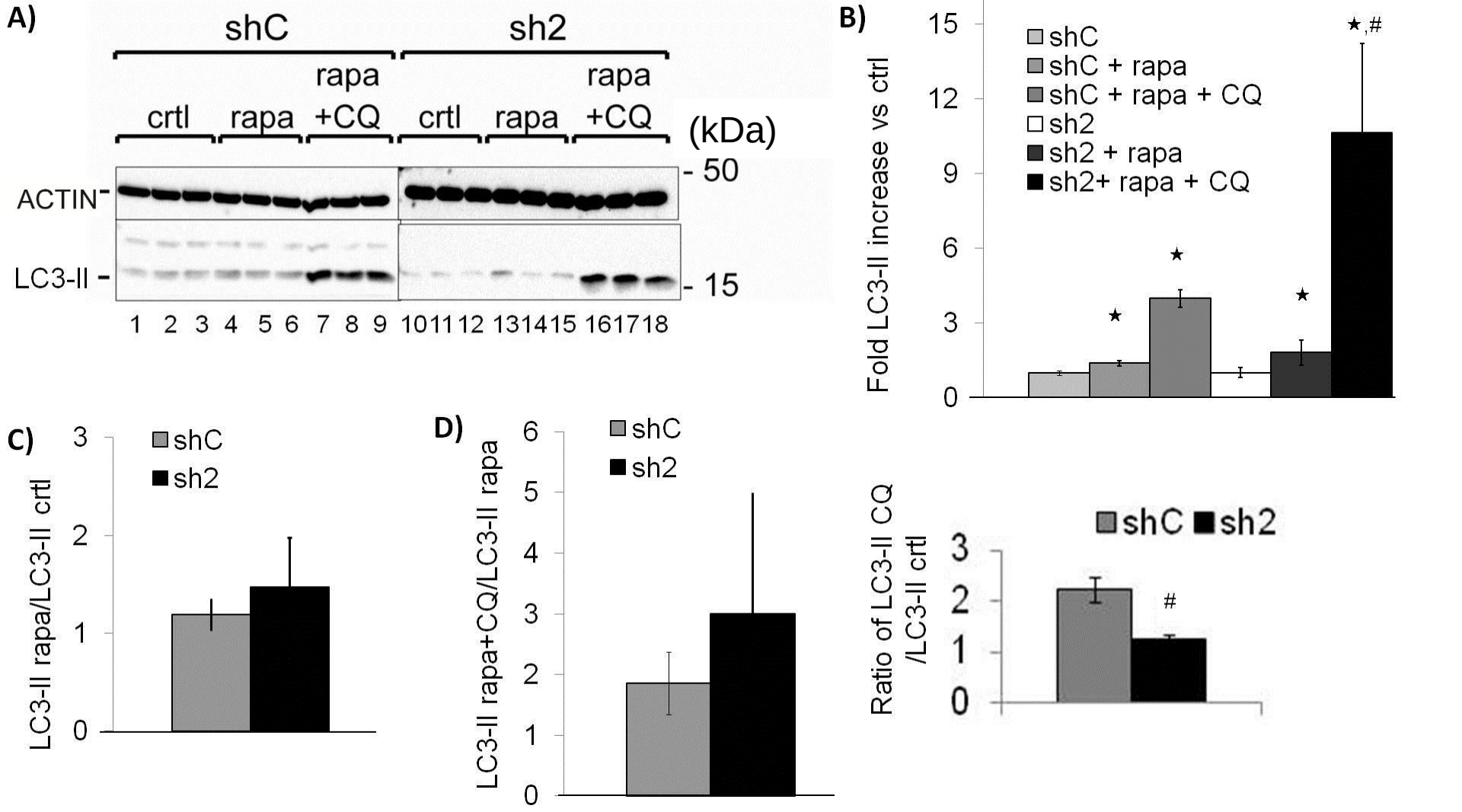

Figure S1. Both shC and sh2 cells exhibit increased autophagic flux in response to rapamycin (rapa). (**A**) MDA-MB436-shC and sh2 cells were cultured for 5 h (crtl), or in the presence of the autophagy inducer rapamycin (rapa) (1 μM) (lanes 4 to 6 and 13 to 15), or the combination of the autophagy inducer rapamycin and the lysosome inhibitor chloroquine (CQ) (40 μM) (lanes 7 to 9 and 16 to 18). Total proteins (25 μg) were separated on 12.5% SDS-PAGE gels followed by immunoblotting with anti-LC3 and anti-ACTIN antibodies and the ECL Plus reagent. A representative experiment of 3 performed is shown. (**B**) Quantification of the signals observed on the western blot in (**A**). ★: $P<0.05$ vs crtl (n=5), #: $P<0.05$, vs shC cells (n=5). (**C**) The autophagy induction was determined as the levels of LC3-II rapa divided by the levels of LC3-II crtl (n=5). (**D**) The autophagic flux was determined as the levels of LC3-II rapamycin in the presence of CQ divided by the levels of LC3-II rapamycin in the absence of CQ (n=5).

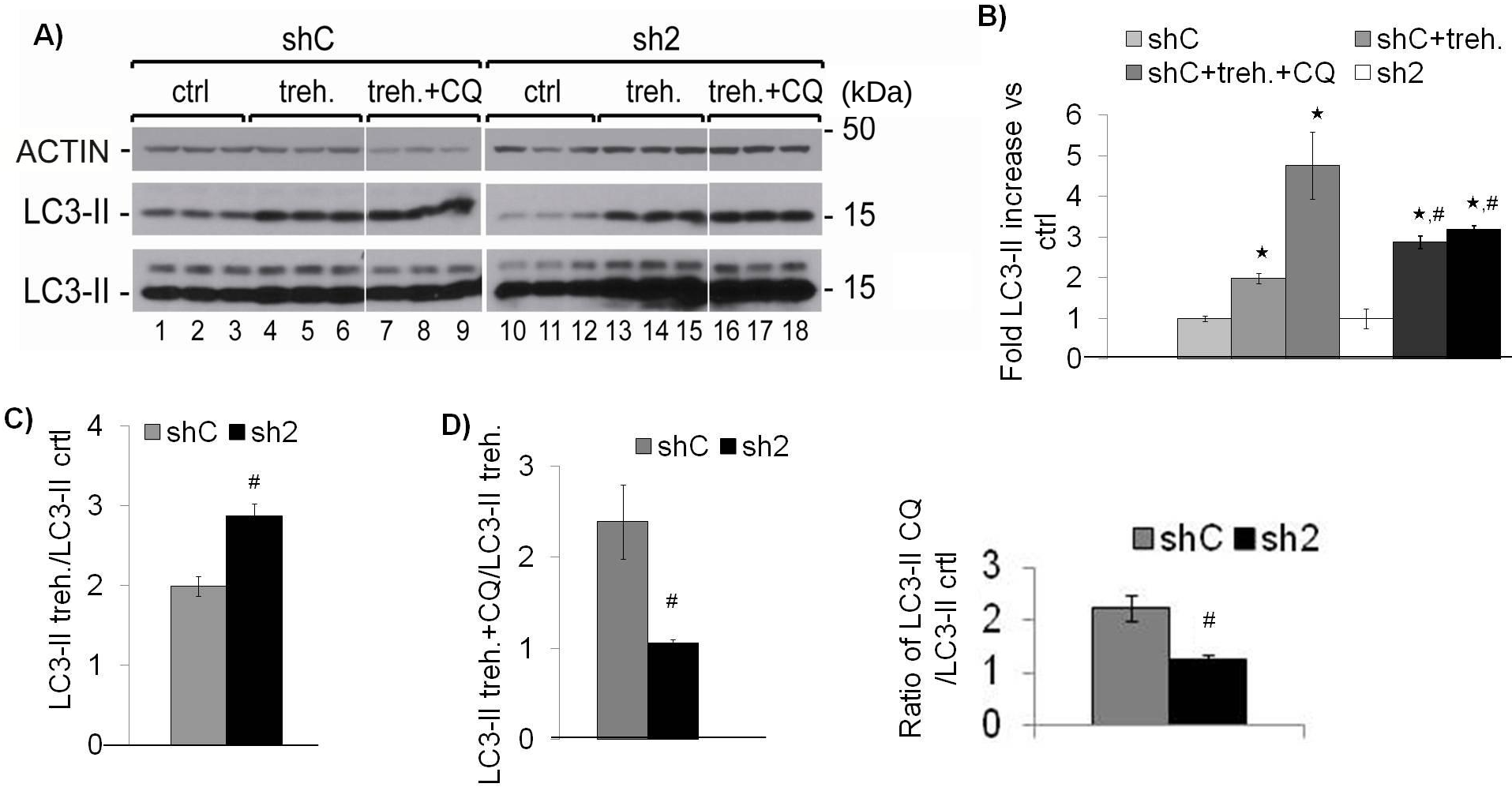

Figure S2. sh2 cells do not increase autophagic flux in response to trehalose (treh.). (**A**) MDA-MB436-shC and sh2 cells were cultured for 5 h in the absence (crtl) or presence of the autophagy inducer trehalose (treh.) (100 mM) (lanes 4 to 6 and 10 to 12), or the combination of trehalose and the lysosome inhibitor chloroquine (CQ) (40 µM) (lanes 7 to 9 and 13 to 15). Total proteins (25 µg) were separated on 12.5% SDS-PAGE followed by immunoblotting with anti-LC3 and anti-ACTIN antibodies and the ECL Plus reagent. A representative experiment of 3 performed is shown.(**B**) Quantification of the signals observed on the western blot in (**A**). (**C**) The autophagy induction was determined as the levels of LC3-II treh. divided by the levels of LC3-II crtl. (**D**) The autophagic flux was determined as the levels of LC3-II trehalose+CQ divided by the levels of LC3-II trehalose. ★: $P<0.05$, vs crtl. #: $P<0.05$, vs shC cells (n=3). #: $P<0.05$, vs shC cells.

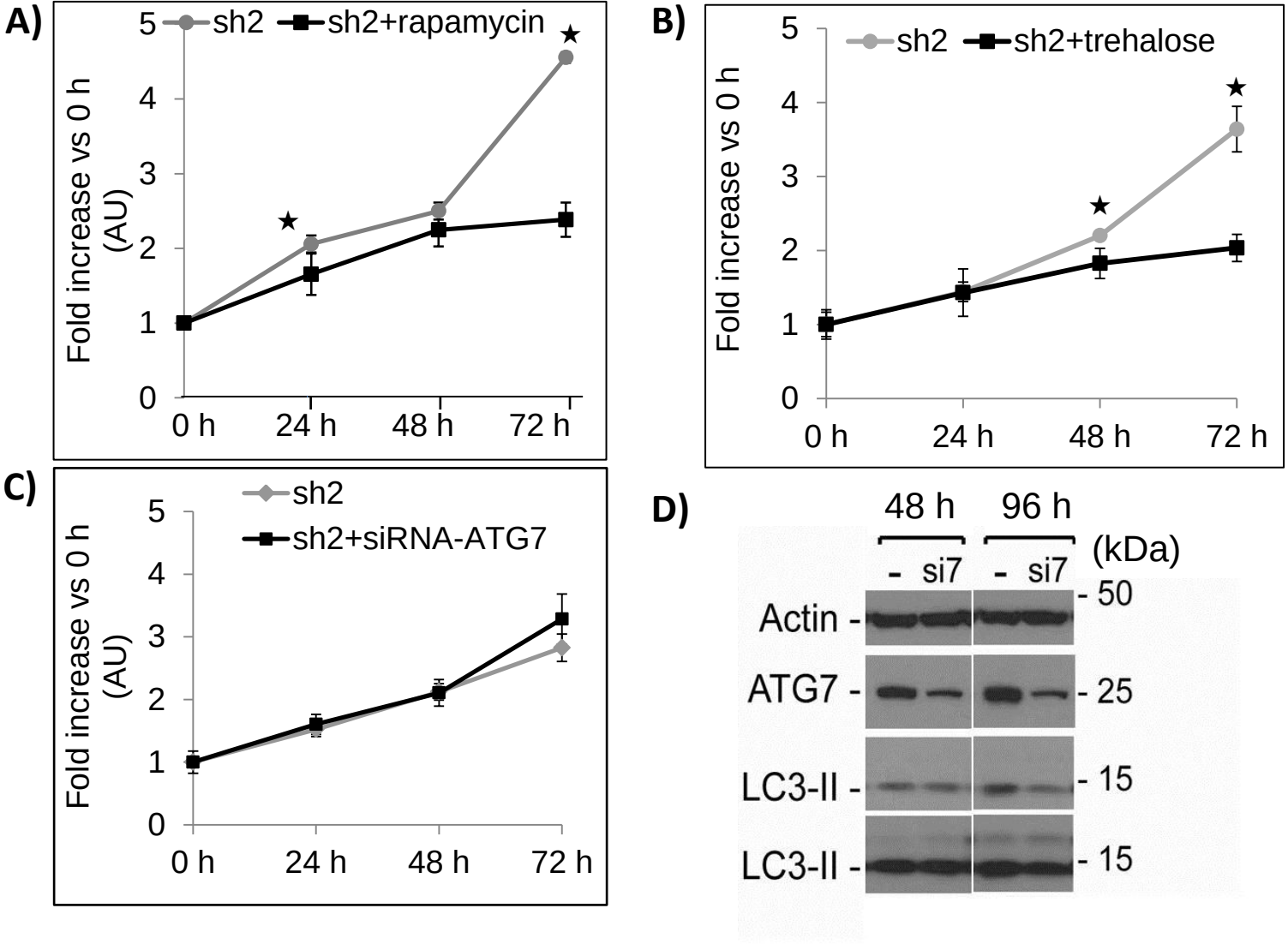

Figure S3. Both rapamycin and trehalose attenuate, whereas knockdown of ATG7 did not change, proliferation of the sh2 cells. MDA-MB436-sh2 cells were cultured in the absence or the presence of an MTOR-dependent autophagy inducer (10 nM rapamycin) (**A**), or an MTOR-independent autophagy inducer (100 mM trehalose) (**B**). Cells were counted everyday over a 72 h period and the number of live cells was determined by the trypan blue exclusion method, and expressed as the fold increase over 0 h. ★: $P<0.05$, vs untreated cells (n=3). (**C**)MDA-MB436-sh2 cells were electroporated with either the control nontargeted siRNA (-) or the *ATG7* siRNA (si7), then cells were counted everyday over a 72 h period by the trypan blue exclusion method, and expressed as the fold increase over 0 h (n=3). (**D**)MDA-MB436-sh2 cells electroporated with the siRNA-*ATG7* were analyzed at 48 h and 96 h post transfection. Total proteins (25 µg) were separated on 12% SDS-PAGE gels followed by immunoblotting with anti-LC3B, anti-ATG7 and anti-ACTIN antibodies and the ECL Plus reagent. A representative experiment of 3 performed is shown.

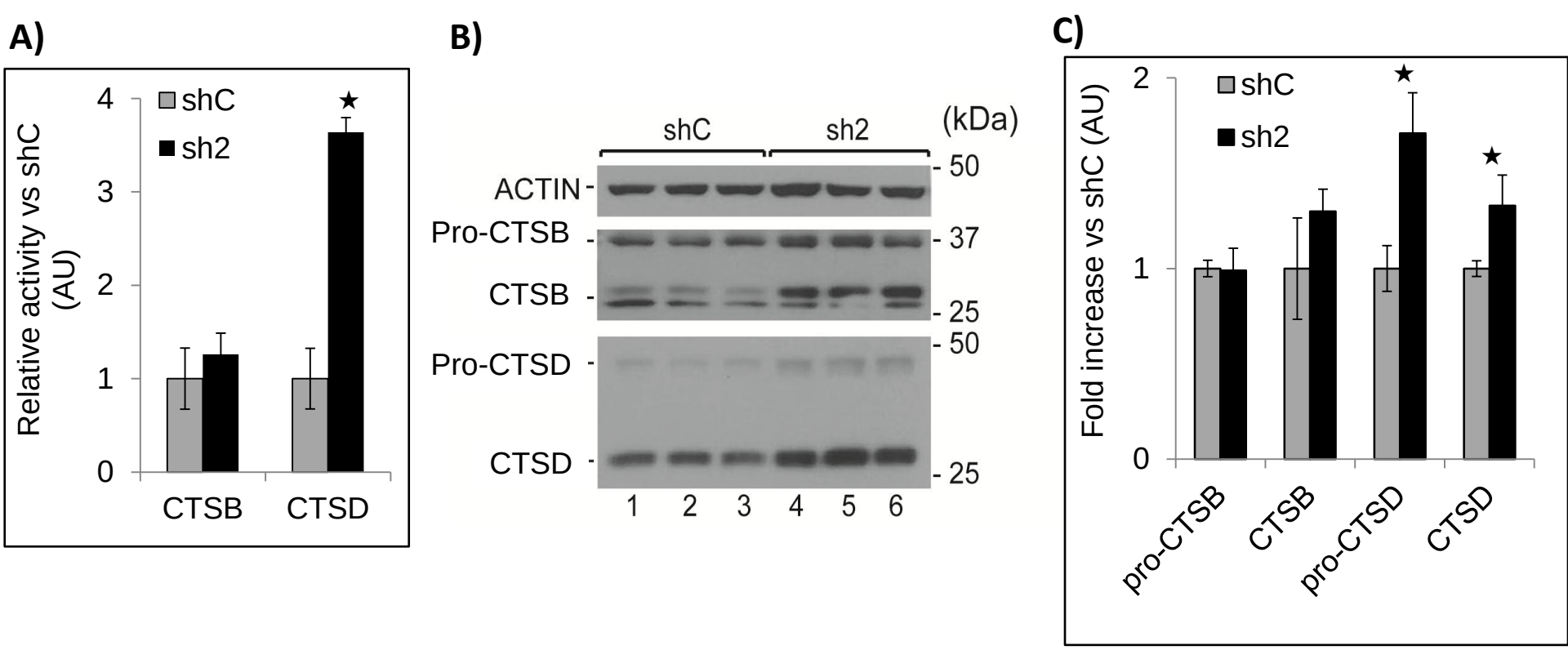

Figure S4.GABARAPL1 knockdown increases CTSD levels and activity. (**A**) MDA-MB436-shC and sh2 cells were cultured for 24 h at 37℃ and then CTSB and CTSD activities were quantified using the CTSB and CTSD assay kits from AbNova and Sigma according to the manufacturer's instructions. ★: *P*<0.05, vs shC (n=3). (**B**) MDA-MB436-shC and sh2 cells were cultured for 24 h at 37℃ and then total proteins (25 µg) were separated on 12% SDS-PAGE followed by immunoblotting with anti-CTSB, anti-CTSD and anti-ACTIN antibodies and the ECL Plus reagent. A representative experiment of 3 performed is shown. (**C**) Quantification of the signals observed on the western blot in (**C**). ★: *P*<0.05, vs shC (n=3).

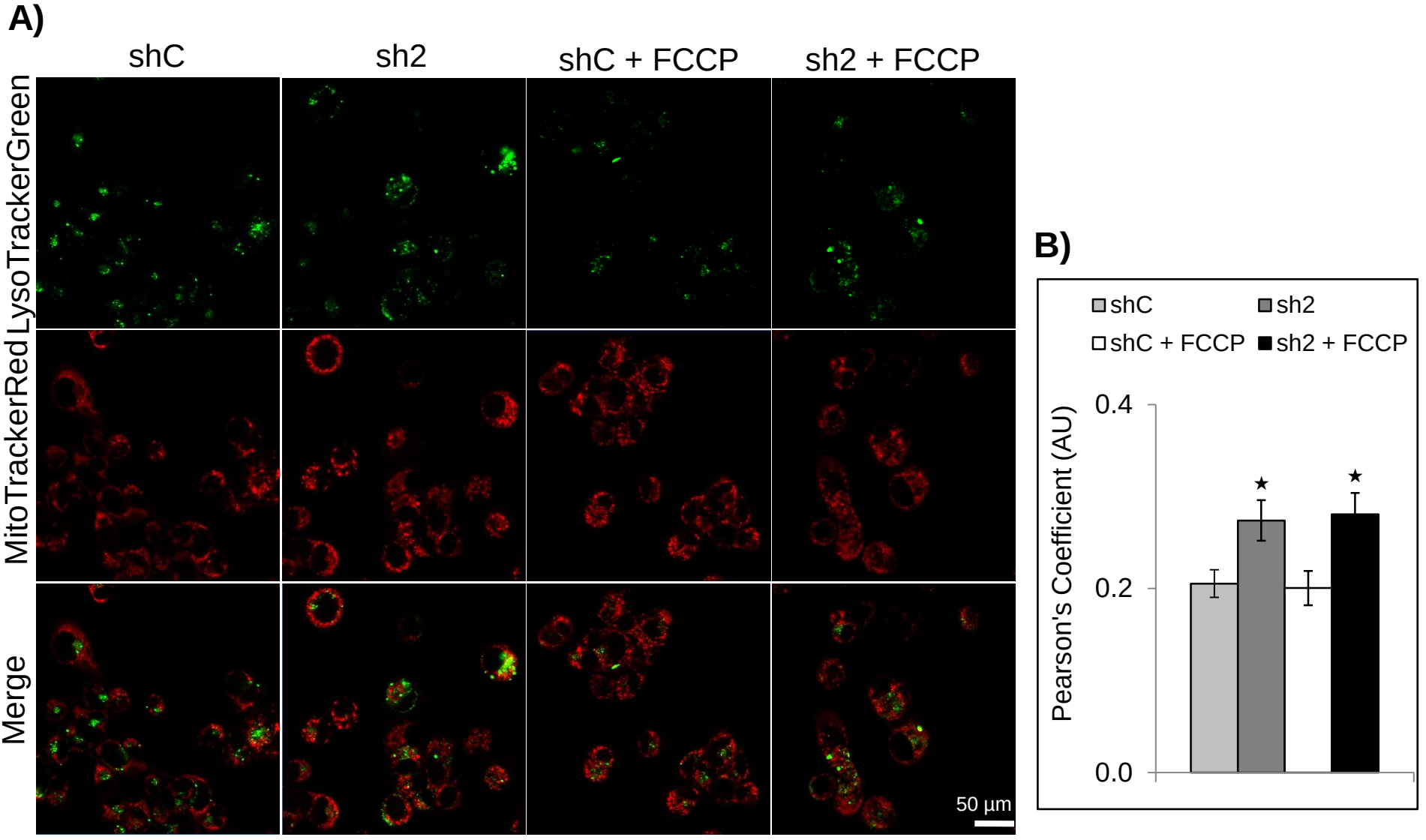

Figure S5. Knockdown of GABARAPL1 does not regulate FCCP-induced MitoTracker and LysoTracker colocalization. (**A**) MDA-MB436-shC and sh2 cells (280,000) were cultured in Lab-TekTMII chambers for 24 h at 37°C and 5% CO_2. Cells were then stained with 50 nM MitoTrackerRed and 100 nM LysoTrackerGreen for 45 min at 37°C in the presence or absence of 10 µM FCCP and washed with PBS before being observed using the Zeiss LSM 710 confocal microscope and the Zen 2008 software. The pictures show representative staining of shC and sh2 cells. Scale bar: 50 µm. (**B**) LysoTrackerGreen and MitoTrackerRed colocalization was analyzed using the ImageJ software (Coloc 2) and the Pearson coefficient. For each cell line, 45 cells were randomly selected. ★: $P<0.05$, vs shC cells.