

Figure S1. Characterization of HeLa polyclonal stable cell lines expressing SPLICS_{S/L}-P2A^{ER-PM} and PO-ER, SPLICS_{S/L}-P2A^{ER-MT} and SPLICS_{S/L}-P2A^{PO-MT}. (A) Representative full-scan image reporting the typically dot pattern of ER-mitochondria interaction detected in HeLa polyclonal stable cell lines expressing SPLICS_S-P2A^{ER-MT} treated with increasing concentration of doxycycline after 8 hours induction. (B-H) Representative membrane for western blotting analysis of HeLa polyclonal stable cell lines lysates expressing SPLICS_{S/L}-P2A probes and stimulated with increasing concentration of doxycycline for 24h. GFP portions have been detected with an anti-GFP antibodies and an equal amount of total loaded lysate was verified by incubation with anti- β Tubulin antibody.

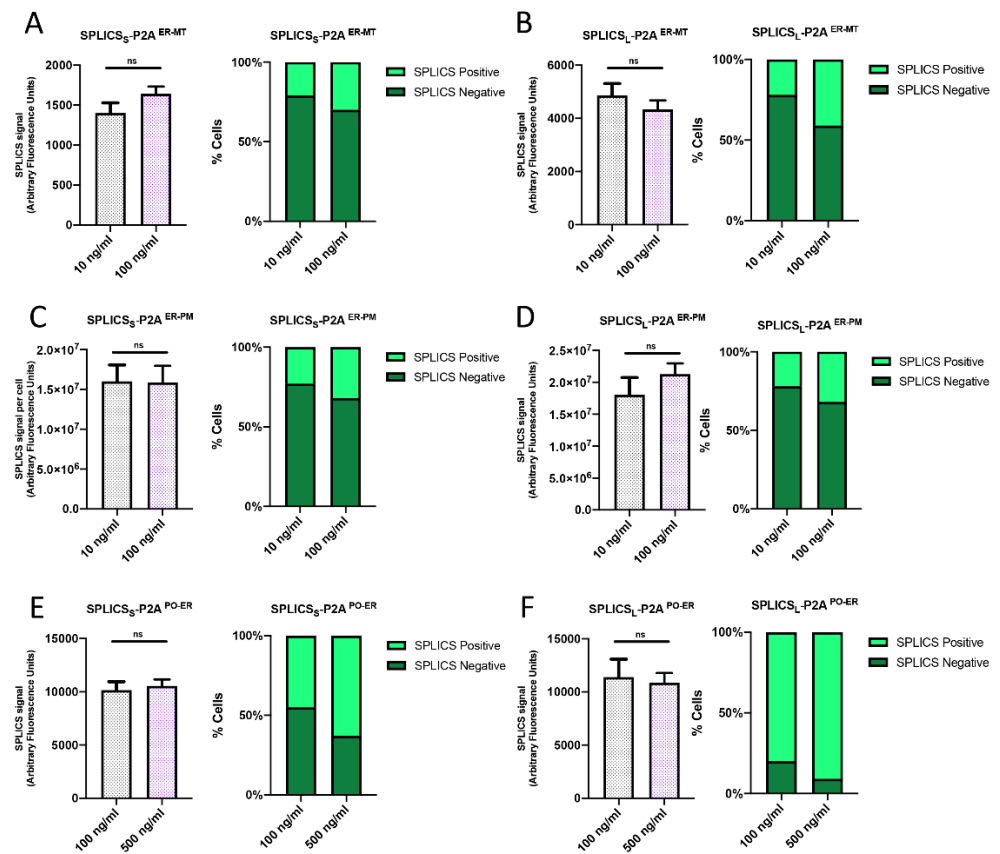


Figure S2. Characterization of HeLa polyclonal stable cell lines expressing SPLICS_{S/L}^{ER-MT}, SPLICS_{S/L}-P2A^{ER-PM} and SPLICS_{S/L}-P2A^{PO-ER}. (A-B) Quantification of the SPLICS fluorescence intensity and % of SPLICS expressing cells at 10 and 100 ng/ml of doxycycline for SPLICS_{S/L}-P2A^{ER-MT}. (C-D) Quantification of the SPLICS fluorescence intensity and % of SPLICS expressing cells at 10 and 100 ng/ml of doxycycline for SPLICS_{S/L}-P2A^{ER-PM}. (E-F) Quantification of the SPLICS fluorescence intensity and % of SPLICS expressing cells at 100 and 500 ng/ml of doxycycline for SPLICS_{S/L}-P2A^{PO-ER}.

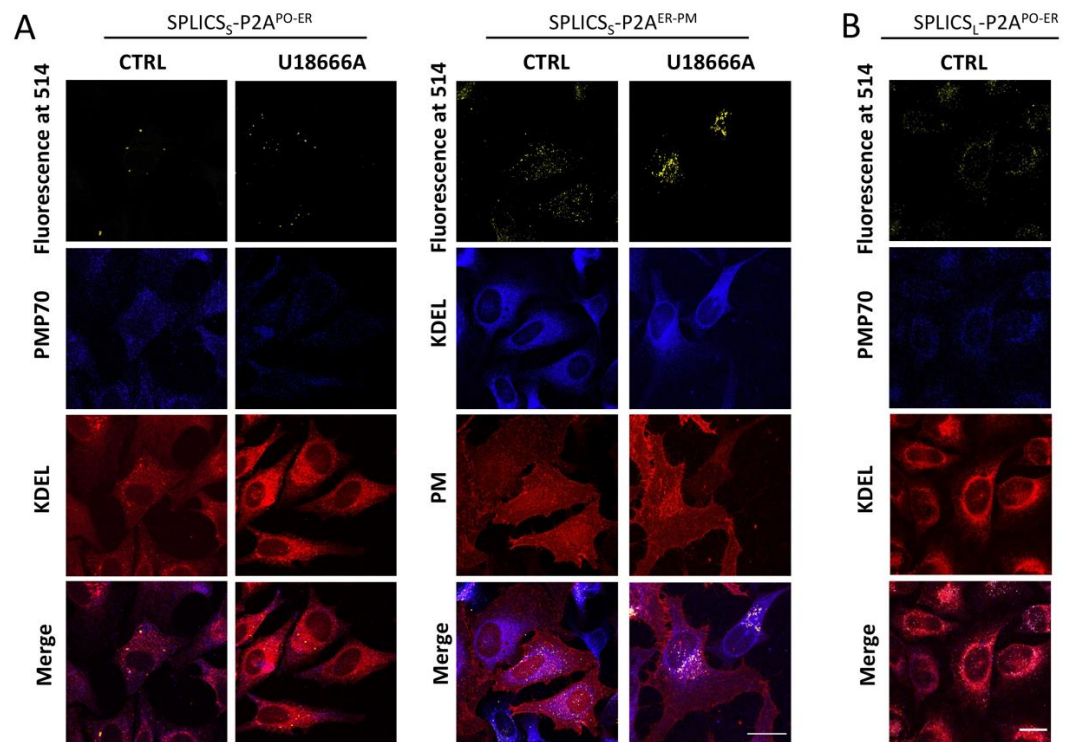


Figure S3. Immunofluorescence analysis in HeLa polyclonal stable cell lines expressing SPLICS_S-P2A^{ER-PM}, SPLICS_S-P2A^{PO-ER}. (A) Immunofluorescence analysis in cells untreated and treated with U18666A showing the morphology of ER and peroxisomes in HeLa polyclonal stable cell lines expressing SPLICS_S-P2A^{PO-ER}, and the morphology of ER and plasma membrane (PM) in HeLa polyclonal stable cell lines expressing SPLICS_S-P2A^{ER-PM}. Scale bar: 20 μm. (B) Immunofluorescence analysis in cells untreated showing the morphology of ER and peroxisomes in HeLa polyclonal stable cell lines expressing SPLICS_L-P2A^{PO-ER}. Scale bar: 20 μm.