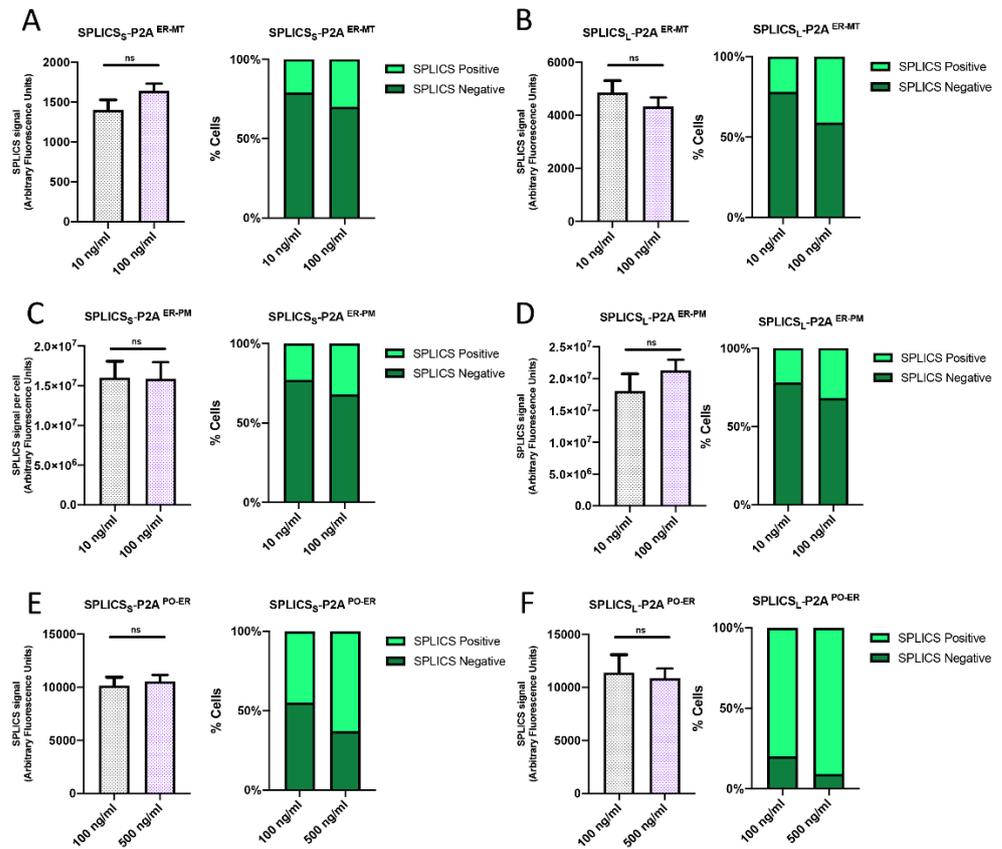
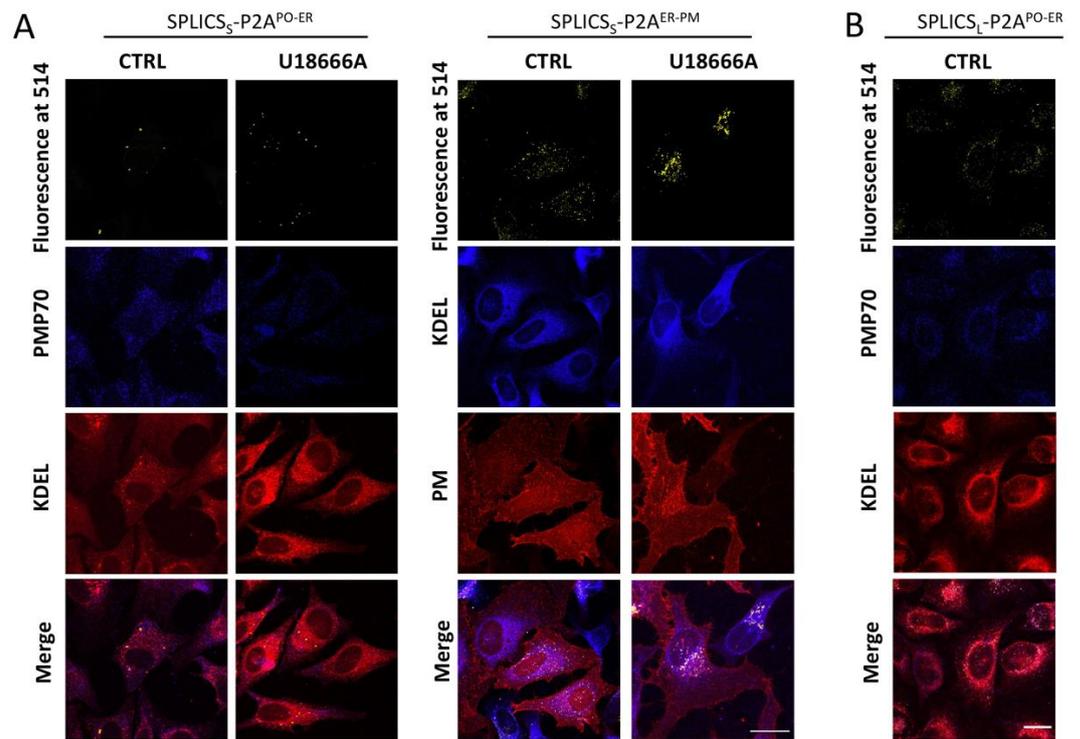


**Figure S1. Characterization of HeLa polyclonal stable cell lines expressing SPLICS<sub>S/L</sub>-P2A<sup>ER-PM</sup> and PO-ER, SPLICS<sub>S/L</sub>-P2A<sup>ER-MT</sup> and SPLICS<sub>S/L</sub>-P2A<sup>PO-MT</sup>.** (A) Representative full-scan image reporting the typically dot pattern of ER-mitochondria interaction detected in HeLa polyclonal stable cell lines expressing SPLICS<sub>S</sub>-P2A<sup>ER-MT</sup> 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<sub>S/L</sub>-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- $\beta$  Tubulin antibody.



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



**Figure S3. Immunofluorescence analysis in HeLa polyclonal stable cell lines expressing SPLICS<sub>5</sub>-P2A<sup>ER-PM</sup>, SPLICS<sub>5</sub>-P2A<sup>PO-ER</sup>.** (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<sub>5</sub>-P2A<sup>PO-ER</sup>, and the morphology of ER and plasma membrane (PM) in HeLa polyclonal stable cell lines expressing SPLICS<sub>5</sub>-P2A<sup>ER-PM</sup>. Scale bar: 20  $\mu$ m. (B) Immunofluorescence analysis in cells untreated showing the morphology of ER and peroxisomes in HeLa polyclonal stable cell lines expressing SPLICS<sub>5</sub>-P2A<sup>PO-ER</sup>. Scale bar: 20  $\mu$ m.