



Figure 1- figure supplement 1. Seipin does not affect cell lipid synthesis or composition. **(A)** Typical efficiency of seipin knockdown with dsRNA in *Drosophila* S2 cells. Primers for dsRNA and qPCR are described in Supplementary file 1. n=3. ***, p<0.001. **(B)** Glycerolipid synthesis in the ER is not affected by seipin knockdown in *Drosophila* S2 cells. Metabolic labeling of cells was as described in Figure 1C. Microsomes were purified, and lipids were extracted, separated with TLC, exposed on an imaging screen, and quantified with FIJI software. n=3. No significant change was observed. **(C)** Sequence analysis of seipin knockout clone. Seipin knockout clone of SUM159 cell line was generated with CRISPR/Cas9-mediated genome editing. Genomic DNA was extracted and sequenced. The seipin knockout clones contain heterozygous mutations in exon 3, with an 8-nucleotide deletion on one allele and a 1-nucleotide deletion on the other, leading to frame shift in both alleles. **(D)** Expression of seipin protein in wildtype and seipin knockout SUM159 cells was examined by western blot with antibody against endogenous seipin. No detectable seipin protein was found in the seipin knockout clone. **(E)** Seipin does not affect lipid levels in SUM159 cells. Lipids were extracted from cell homogenate and microsomes of wildtype or seipin knockout SUM159 cells. Lipid classes and species were identified with LC-MS based lipidomics. n=3 biological replicates and 2 technical replicates. PA standard curve shows that PA measurement was linear at a concentration range similar to that in samples, as low as 0.75 pmol. **(F)** No apparent PA accumulation at LD formation sites in seipin-depleted cells. SUM159 cells co-transfected with cherry-LiveDrop and GFP-PASS were imaged 30 min after adding oleic acid. The majority of the cherry and GFP signal does not overlap at the LD formation site. As a positive control, addition of PMA to control cells induced GFP-PASS distribution to the plasma membrane (arrowhead).