



**Figure S3**

**Figure S3: SLC7A11 knockdown in PDAC CAFs does not affect glutamate secretion and is maintained in the presence of oxidative stress.** A) Gating strategy, representative histogram and quantification of the geometric mean of fluorescence peaks for CAFs treated with or without 40 $\mu$ M tBHP. Circles indicate replicates (n=3). B) Bars show average secreted glutamate in cell supernatants from CAFs transfected with ns-siRNA or SLC7A11-siRNA pool (as a fraction of control-siRNA), as assessed by colorimetric assay. Circles indicate replicates (n=6). C) Representative Western blot and densitometry of SLC7A11 in total protein extracts from CAFs 72h after transfection with ns-siRNA or SLC7A11-siRNA pool, and 48h post treatment with tBHP.  $\alpha$ -tubulin = loading control. Circles indicate replicates (n=3). D) Gating strategy and representative AnnexinV-PE/DAPI flow cytometry pseudocolour plots for CAFs treated with ns-siRNA or SLC7A11-siRNA, with or without 40 $\mu$ M tBHP. E) Representative Western blot for LC3BI/II and densitometry for LC3BII (increases during autophagy) in total protein extracts from CAFs transfected with ns-siRNA or SLC7A11-siRNA. GAPDH = loading control. Circles indicate replicates (n=3). F) Bars represent fraction of total live cells in each cell cycle phase, as determined by DAPI stain and flow cytometry 72h after transfection with ns-siRNA or SLC7A11-siRNA pool (n=6). Bars/lines in all graphs = mean $\pm$ s.e.m. Student t-test used for (A-B, E-F), one-way ANOVA used for (C). Asterisks in all graphs indicate significance (ns=not significant, \* $p\leq 0.05$ , \*\*\* $p\leq 0.001$ , \*\*\*\* $p\leq 0.0001$ ). Replicate numbers for all CAF experiments refer to independent transfections/treatments using CAF cells isolated from different PDAC patients.