Supplemental Materials

Molecular Biology of the Cell

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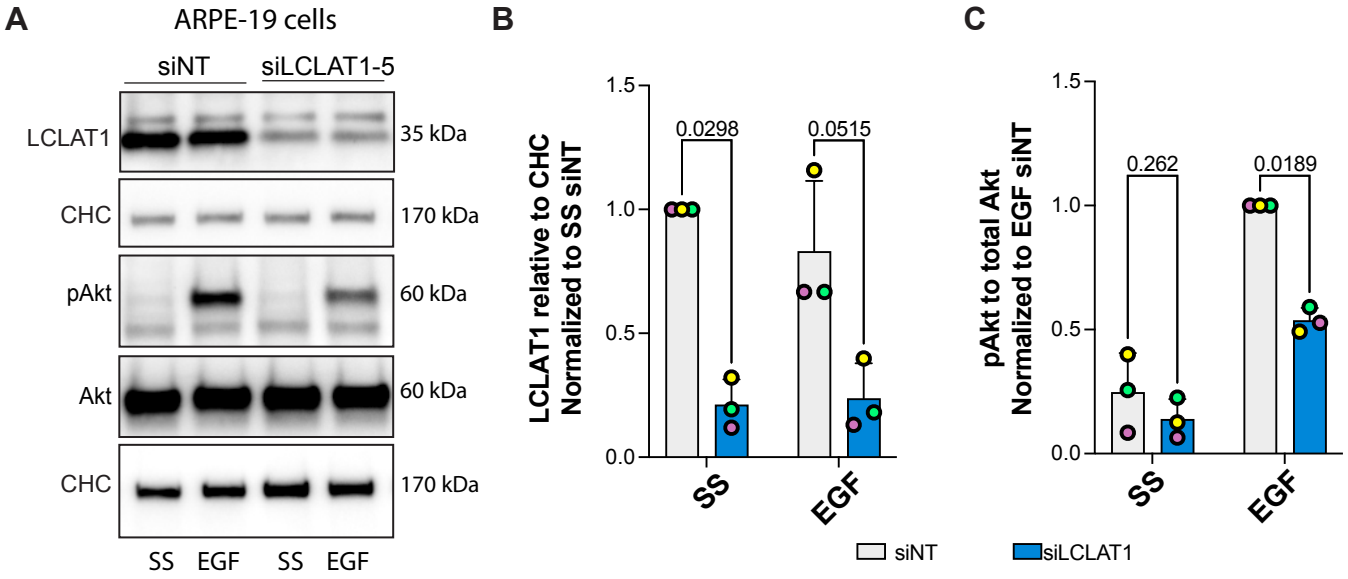
Supplemental Figure S1: LCLAT1 silencing in ARPE-19 cells with an independent siRNA oligonucleotide disrupts Akt signalling. ARPE-19 cells were transfected with oligonucleotide siLCLAT1-5 or non-targeting control. Cells were then serum-starved (SS), followed by 5 ng/mL EGF stimulation for 5 min. **A.** Lysates were probed for LCLAT1 expression, p-Akt, Akt, and clathrin heavy chain (CHC), which was used as a loading control. **B.** Quantification of LCLAT1 silencing by normalizing LCLAT1 to CHC signal in ARPE-19 cells. **C.** Quantification of p-Akt levels relative to total Akt. Data are shown as mean ±STD are shown from n=3 independent experiments. Data points from matching independent experiments are colour coded. Data was analysed by a repeated measures two-way ANOVA and Sidak's post-hoc test. p values are disclosed.

Supplemental Figure S2: LCLAT1 silencing in MDA-MB-231 cells with independent siRNA oligonucleotides disrupts Akt signalling. **A.** Western blotting showing LCLAT1 silencing in MDA-MB-231 cells transfected with non-targeting siRNA, siLCLAT1-1, siLCLAT1-2, or siLCLAT1-3 oligonucleotides. Cells were then serum-starved (SS) or stimulated with 5 ng/mL EGF for 5 min. Lysates were probed for LCLAT1 expression, p-Akt, and clathrin heavy chain (CHC), which was used as a loading control. **B.** Quantification of LCLAT1 silencing by normalizing LCLAT1 to CHC signal in MDA-MB-231 cells transfected as in D. **C.** Quantification of p-Akt levels relative to clathrin in MDA-MB-231 cells mock-silenced or LCLAT1-silenced with one of three siRNA oligonucleotides and either serum-starved or stimulated with 5 ng/mL EGF for 5 min. Data points from matching independent experiments are colour coded. Data in B were analysed with a repeated measures one-way ANOVA and Dunnett's post-hoc test. Data in C were analysed with a repeated measures two-way ANOVA and Tukey's post-hoc test. p values are indicated.

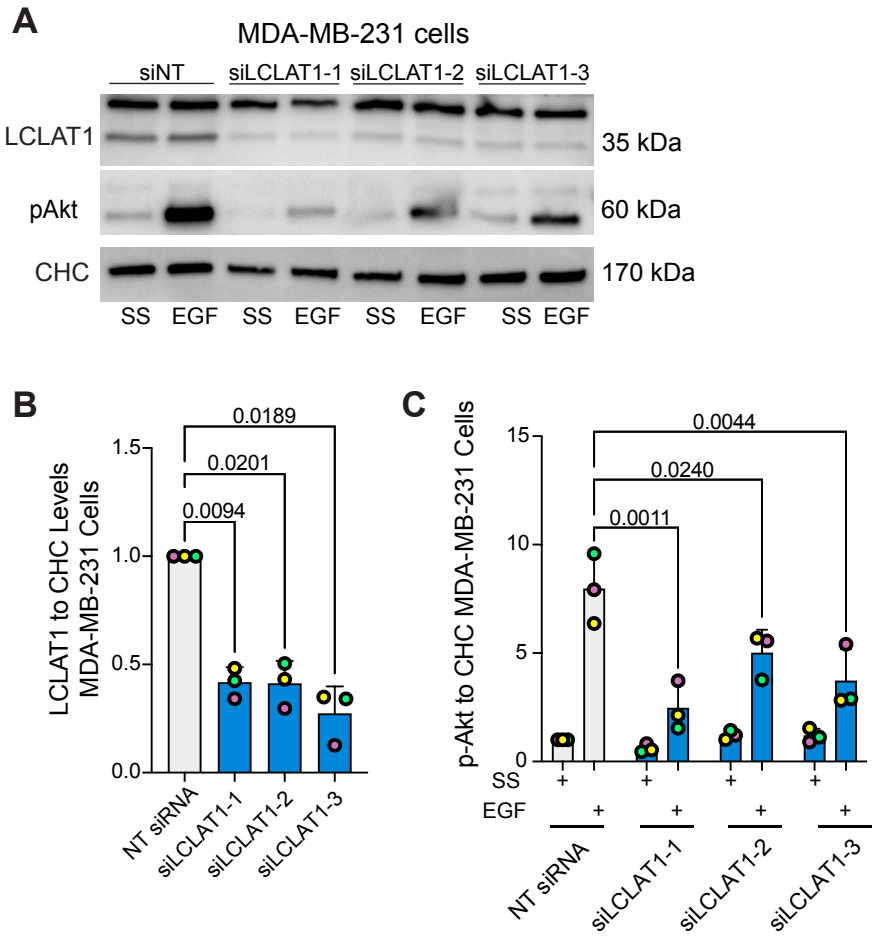
Supplemental Figure S3. Relative levels of 38:4-PtdInsPs to 36:x-PtdInsPs in ARPE-19 and

MDA-MB-231 cells silenced for LCLAT1. ARPE-19 cells (**A-B**) and MBA-MB-231 cells (**C-D**) were mock silenced (siCon) or LCLAT1-silenced. Cells were then grown in regular medium (control), serum-starved (ss), and stimulated with 5 ng/mL EGF for 5 min (EGF). Reactions were quenched and lipid extracted after addition of internal standards to primary cell extracts. PtdInsPs were measured by mass spectrometry (HPLC-MS). Shown is the ratio of standardized 38:4-mono-PtdIns (A, C) and 38:4-bis-PtdInsP2 (B, D) to the respective standardized sum of 36:1 and 36:2- (referred to as 36:x-PtdIns) mono-PtdInsP and bis-PtdInsP2. Lipid analysis was repeated four independent times. Data points from matching independent experiments are colour coded. Shown are the mean ±STD. A repeated measures two-way ANOVA and Sidak's post-hoc test was used to test data. p values are indicated.

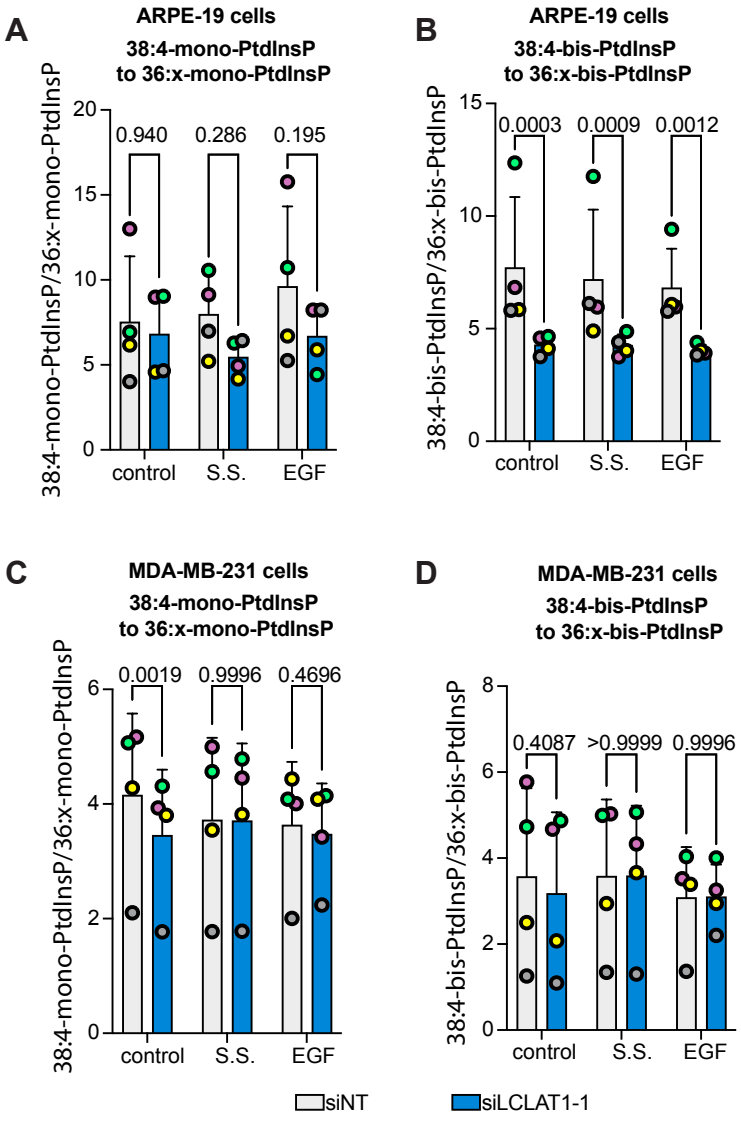
Supplemental Figure S4: LCLAT1 silencing effect on cell cycle checkpoint proteins, Mdm2, p21, and p53. A, B. Western blotting showing LCLAT1 silencing in ARPE-19 (A) and MDA-MB-231 (B) cells transfected with non-targeting siRNA, siLCLAT1-1, or siLCLAT1-5 oligonucleotides. Cells were serum-starved or stimulated with 5 ng/mL EGF for 5 min. Lysates were probed for LCLAT1, Mdm2, p53, and p21 expression. Clathrin heavy chain (CHC) was probed as a loading control. **B, C, D, F, G, H.** Quantification of Mdm2, p21, and p53 expression relative to CHC expression in ARPE-19 (B-D) and MDA-MB-231 (F-H) cells. Data are the mean ± STD from n=4 independent experiments. Data points from matching independent experiments are colour coded. Data was analysed with a repeated measures two-way ANOVA and Tukey's post-hoc test with p values indicated.



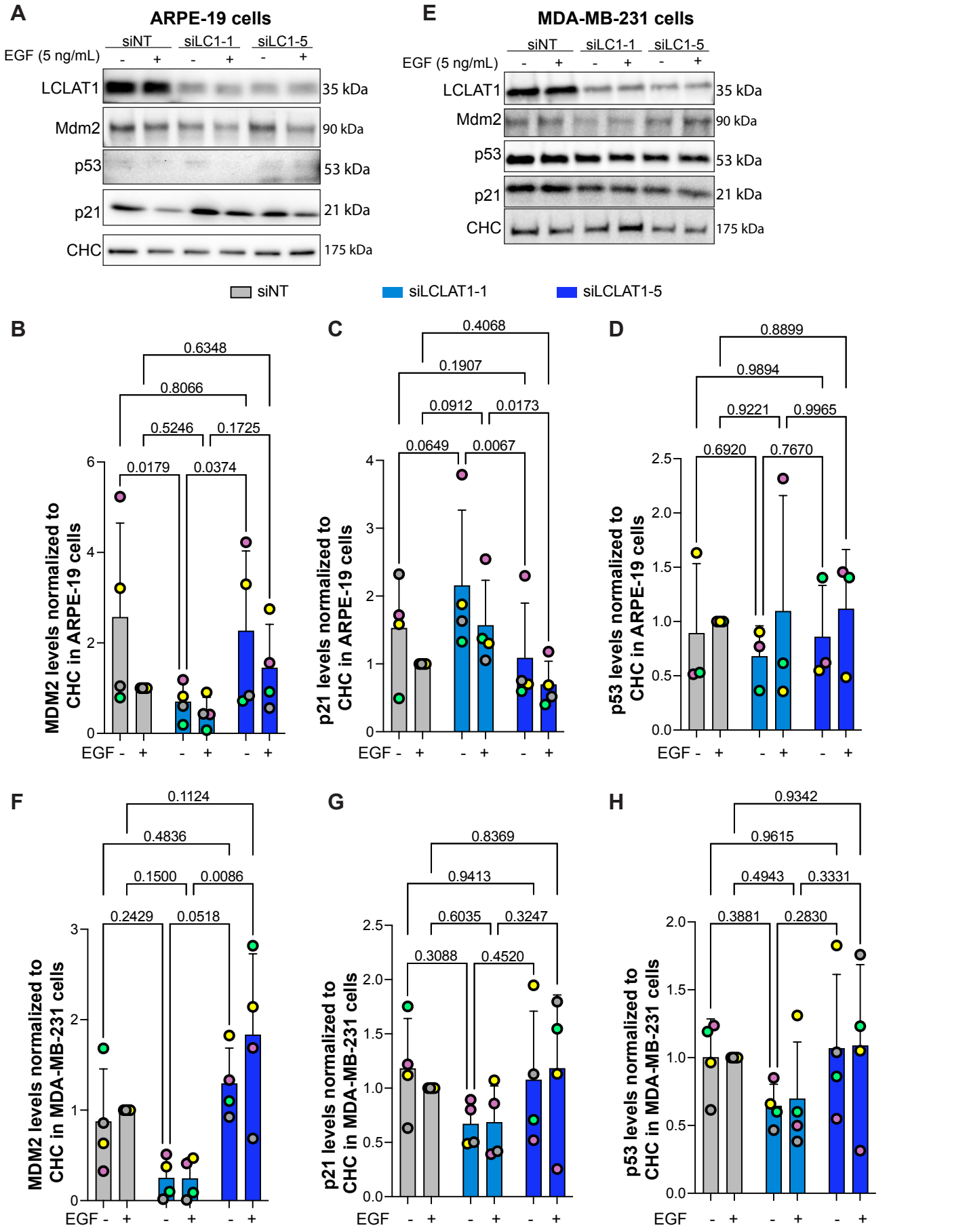
Supplemental Figure S1



Supplemental Figure S2



Supplemental Figure S3



Supplemental Figure S4