

Supplemental Information

Mutational scanning pinpoints distinct binding sites of key ATGL regulators in lipolysis

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Suppl. Figure 1: Expression of ATGL constructs in HEK293T cells for Co-IP analysis.

Suppl. Figure 2: Protein expression and phenotype of T-RExTM-293 cells stably expressing PLIN1 or PLIN5.

Suppl. Figure 3:

Single cell confocal images of stained, live T-RExTM-293 cells stably expressing doxycycline induced PLIN1.

Suppl. Figure 4:

Single cell confocal images of stained, live T-RExTM-293 cells stably expressing doxycycline induced PLIN5.

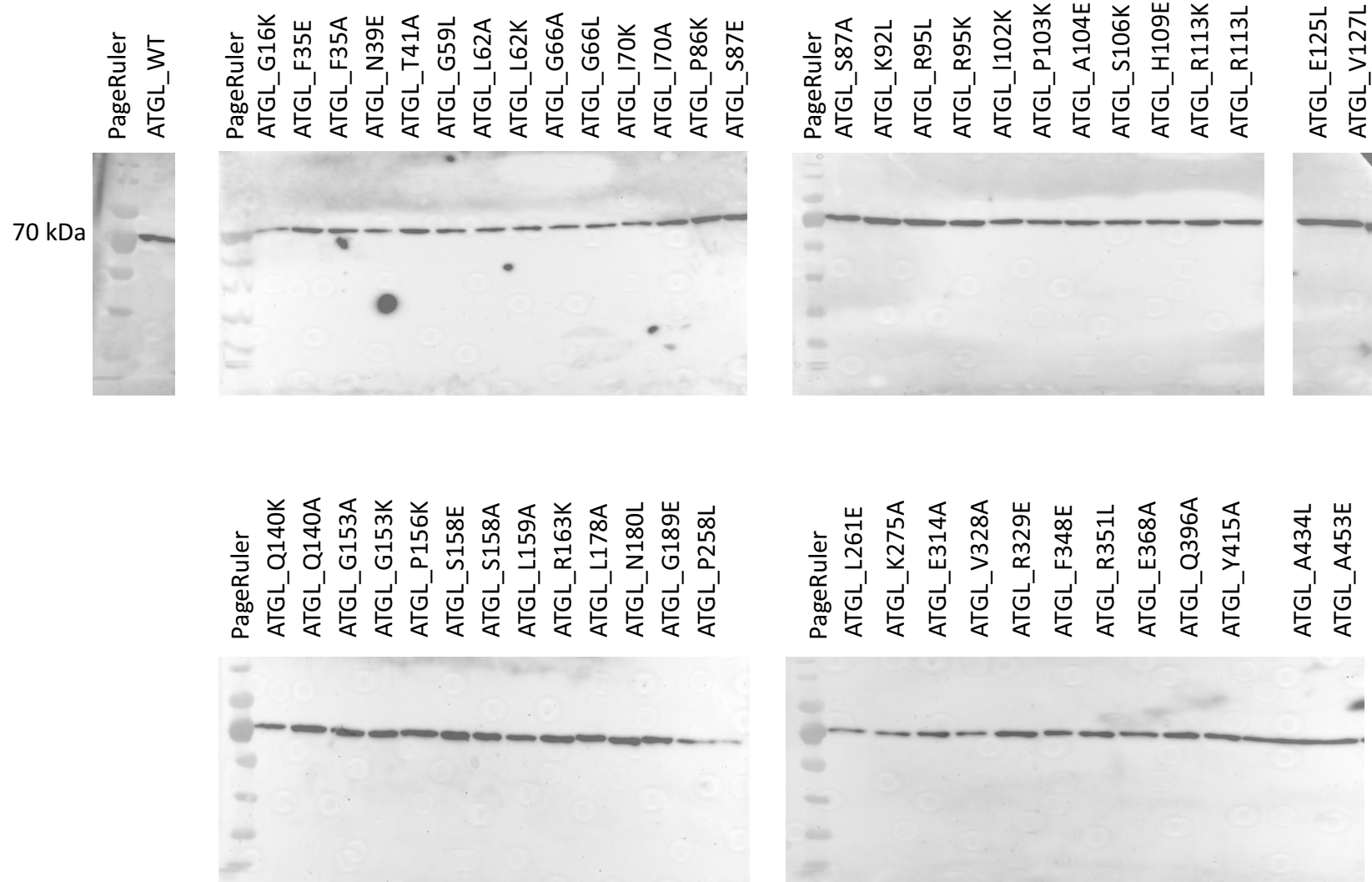
Suppl. Figure 5:

Confocal images of stained, live T-RExTM-293 cells stably expressing doxycycline induced PLIN1.

Suppl. Figure 6:

Confocal images of stained, live T-RExTM-293 cells stably expressing doxycycline induced PLIN5.

Suppl. Figure 1

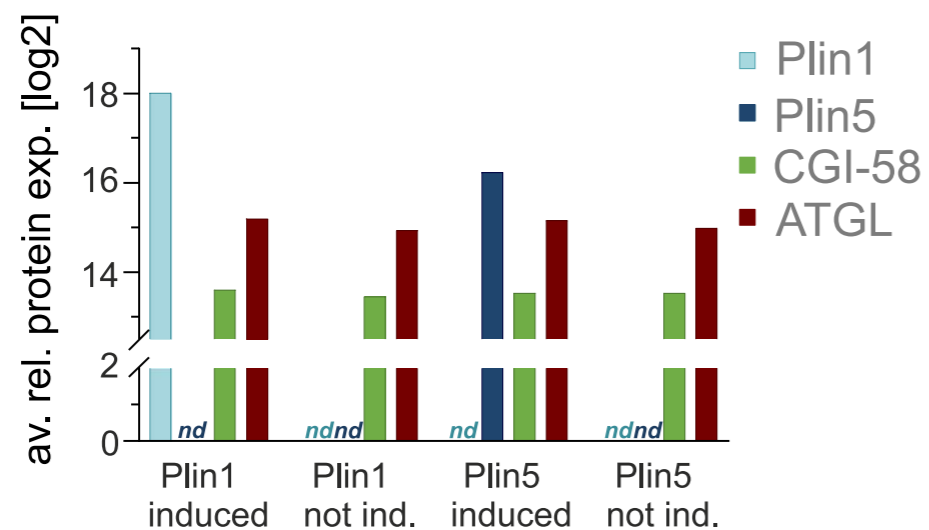
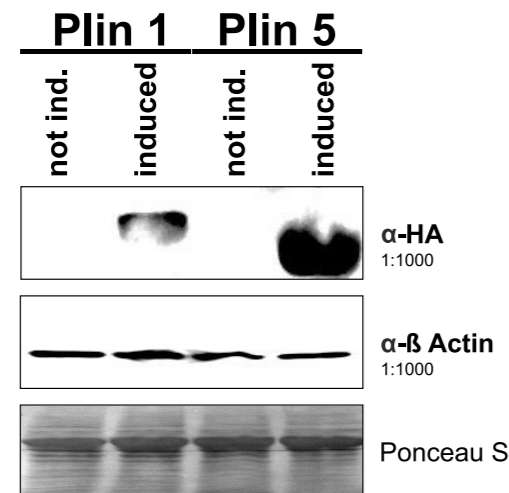
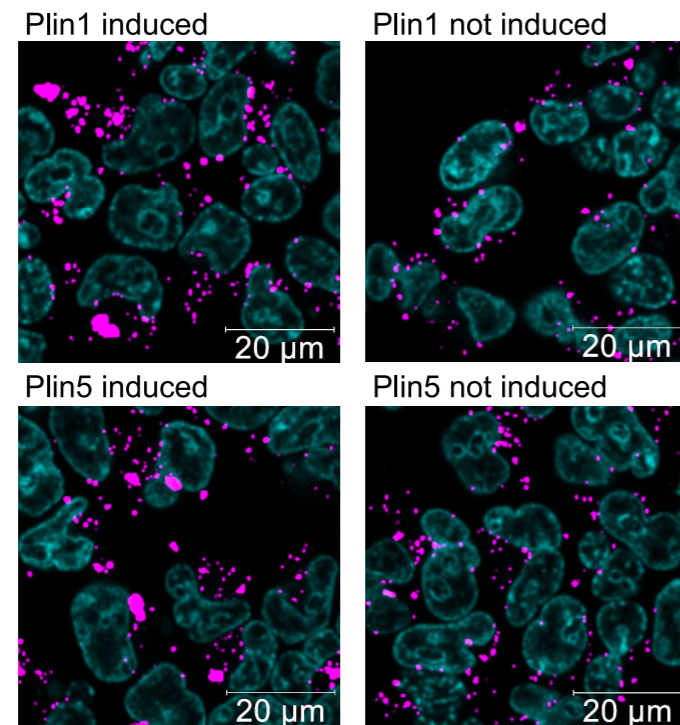


Kohlmayr et al, **Suppl. Figure 1:**

Expression of ATGL constructs in HEK293T cells for Co-IP analysis. Cells were lysed in SDS-free RIPA2 buffer, and cleared samples were loaded on a 10% SDS-gel and blotted on a nitrocellulose membrane. Immunodetection was performed using an Ab against Protein A (1:3000, anti-rabbit-IgG-HRP linked, NA934, GE Healthcare/Amersham).

Suppl. Figure 2

A



Kohlmayr et al, **Suppl. Figure 2:**

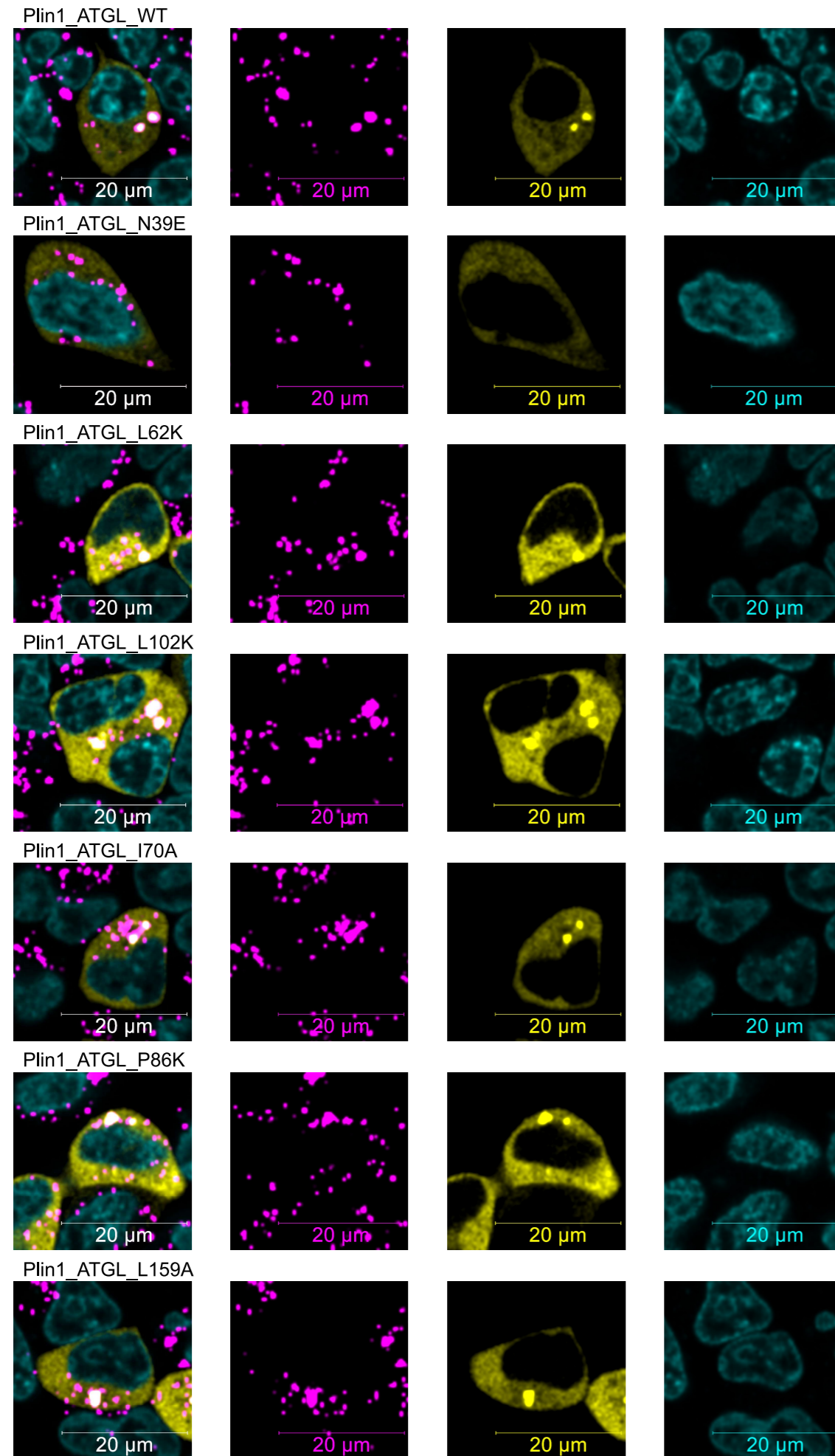
Protein expression and phenotype of T-REx™-293 cells stably expressing PLIN1 or PLIN5.

Top: Confocal images of T-REx™-293 cells stably expressing PLIN1 or PLIN5 (induced) or not induced. Plin1/5 expression was induced with doxycycline (2 μ g/ml) for 24 hours and then treated with 200 μ M oleic acid for 6h. Pictures are merged fluorescent images of live cells stained with Hoechst in cyan and Bodipy 558/568 C12 in magenta.

Middle: Western blot of whole cell extracts of T-REx™-293 cells expressing PLIN1 or PLIN5 (induced) or not induced. Cells were treated as in A. Top: PVDF membrane developed with anti-HA.11 Epitope Tag antibody (1:10000, 901501, Biolegend); Middle PVDF membrane developed with anti- β Actin (clone AC-15) antibody (1:5000, A5441, Sigma); Bottom: Ponceau stained PVDF membrane.

Bottom: Mass spectrometry based relative protein quantification of PLIN1, PLIN5, CGI-58 and ATGL from total cell extract of T-REx™-293 cells stably expressing doxycycline inducible PLIN1 or PLIN5 (induced and not induced).

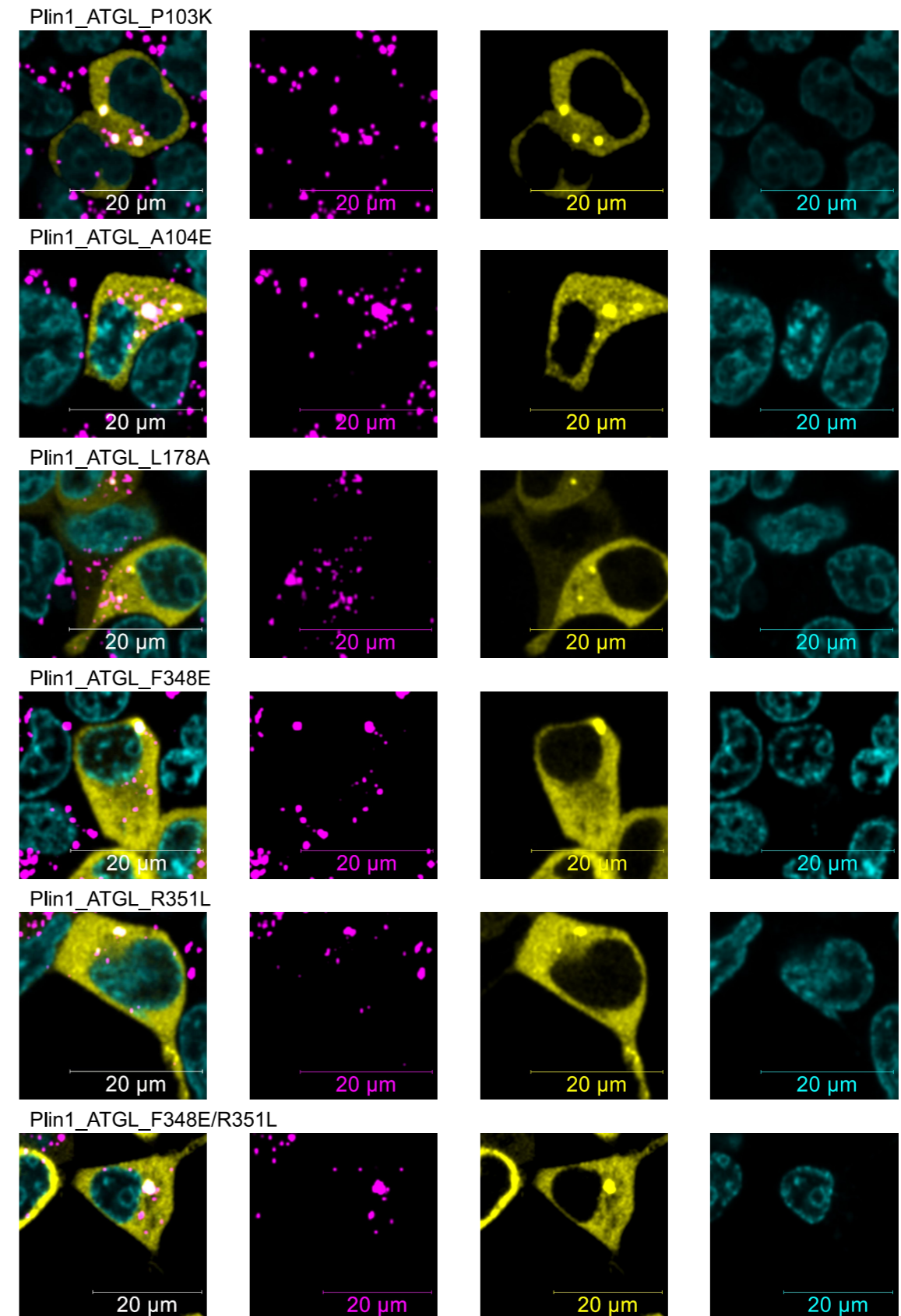
Suppl. Figure 3



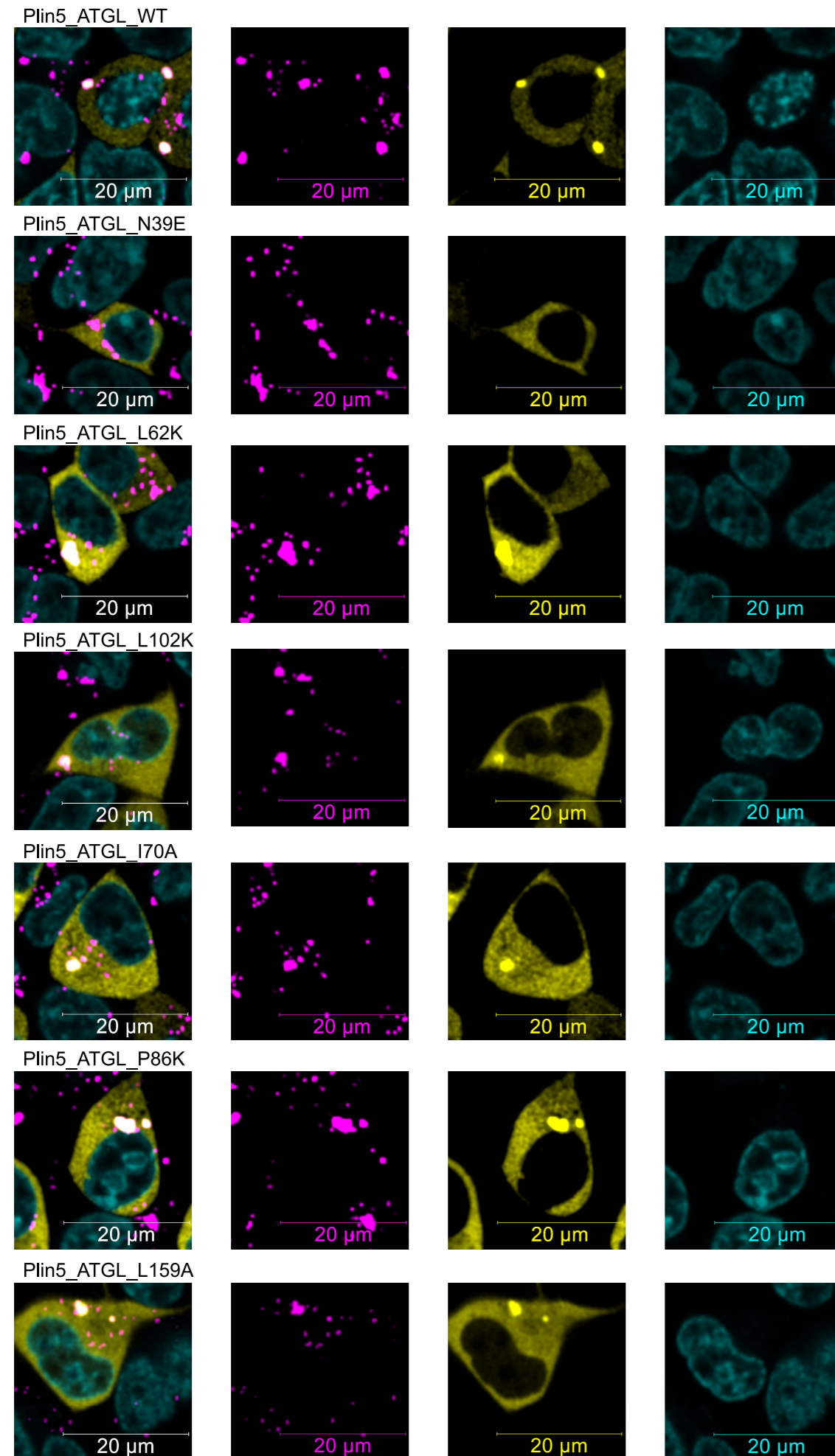
Kohlmayr et al, Suppl. Figure 3:

Single cell confocal images of stained, live T-REx™-293 cells stably expressing doxycycline induced PLIN1.

Cells were transiently transfected with YFP-ATGL variants and PLIN1/5 expression simultaneously induced with doxycycline (2μg/ml). After 24 hours cells were treated with 200μM oleic acid for 6h. Pictures are merged fluorescent images with Hoechst in cyan, YFP-ATGL in yellow and Bodipy 558/568 C12 (D3835, FisherScientific) in magenta, next to the three separated channels. The experiments were repeated three times with similar outcome.



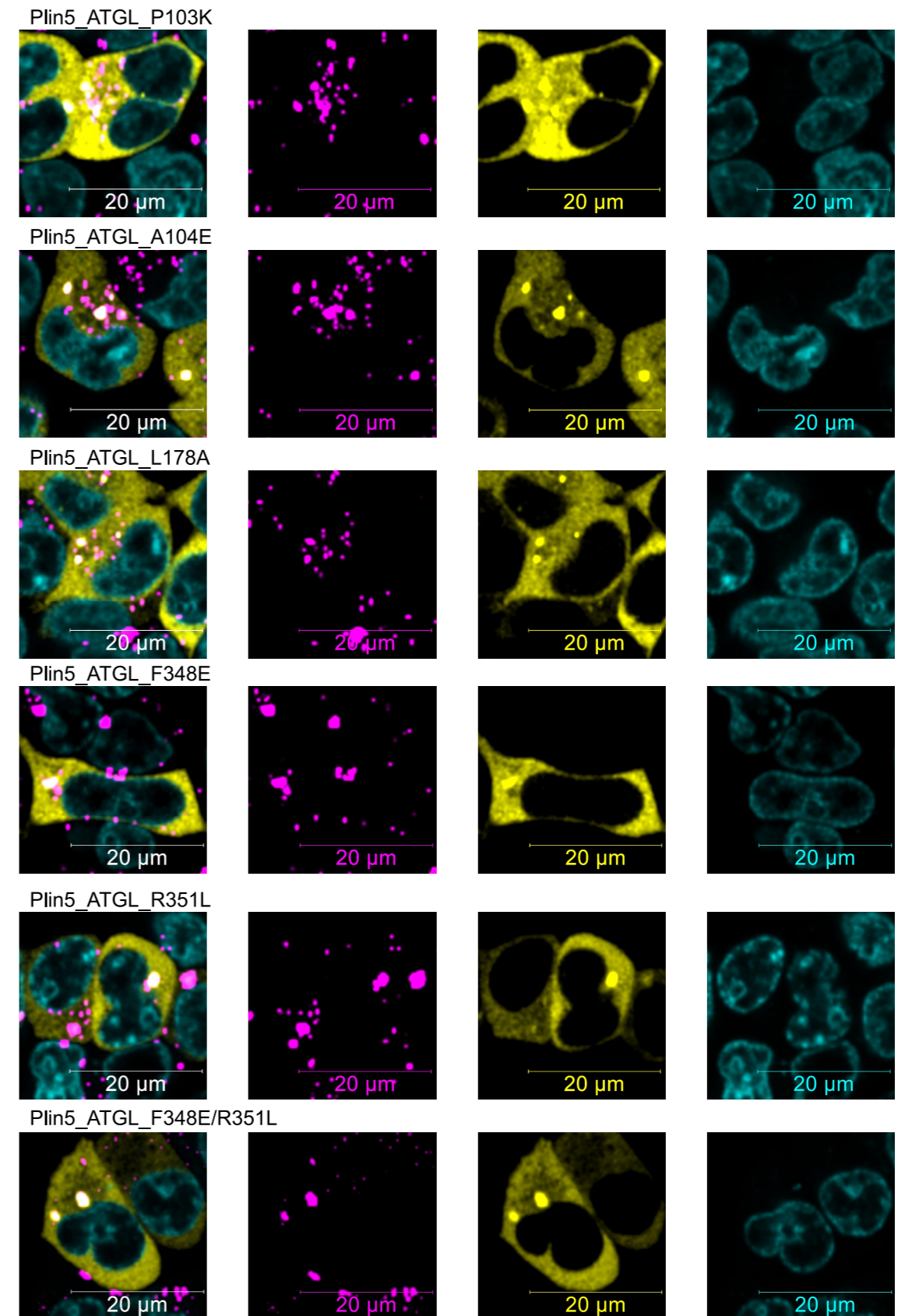
Suppl. Figure 4



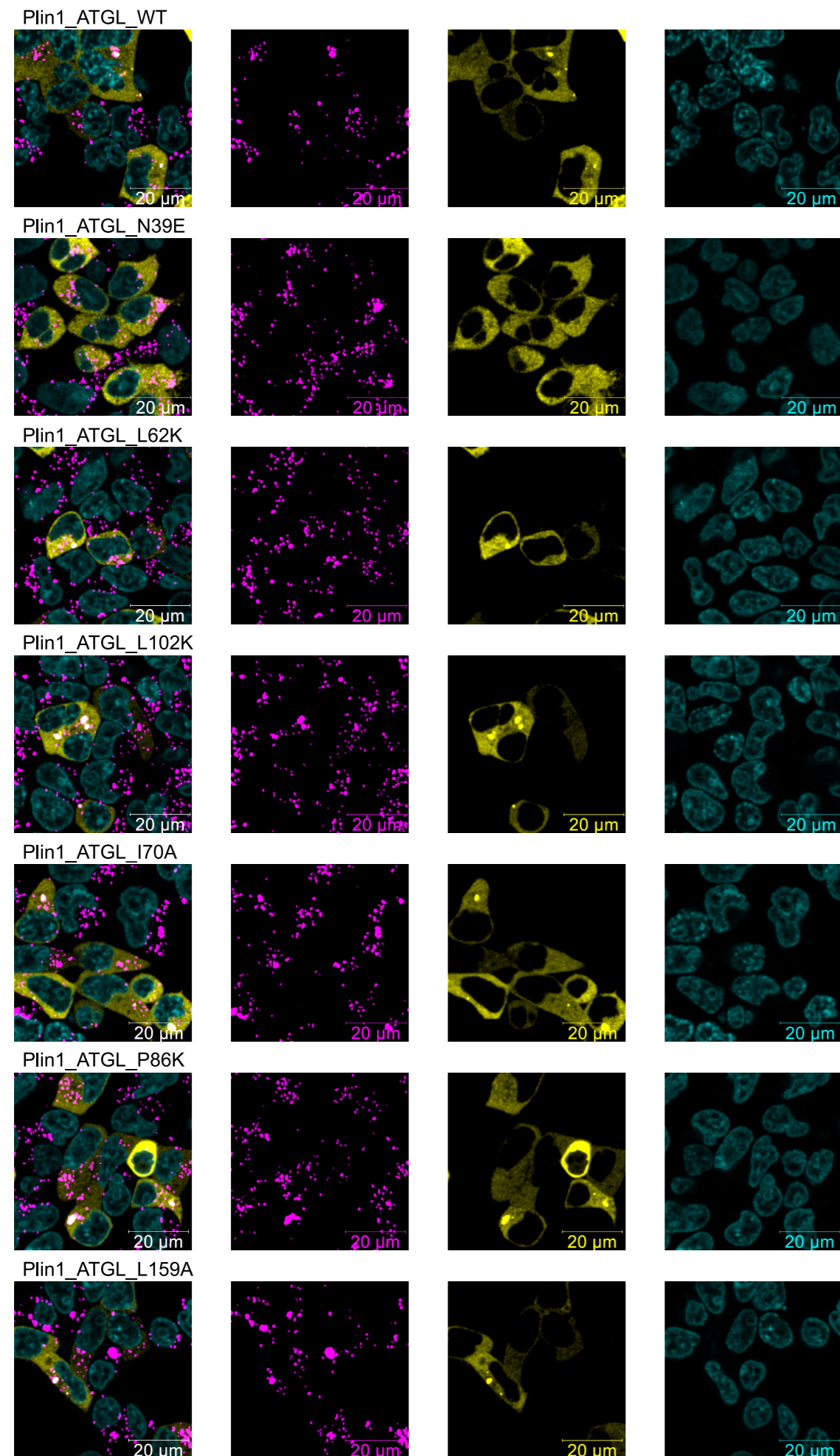
Kohlmayr et al, Suppl. Figure 4:

Single cell confocal images of stained, live T-REx™-293 cells stably expressing doxycycline induced PLIN5.

Cells were transiently transfected with YFP-ATGL variants and PLIN1/5 expression simultaneously induced with doxycycline (2 μg/ml). After 24 hours cells were treated with 200 μM oleic acid for 6h. Pictures are merged fluorescent images with Hoechst in cyan, YFP-ATGL in yellow and Bodipy 558/568 C12 (D3835, FisherScientific) in magenta, next to the three separated channels. The experiments were repeated three times with similar outcome.



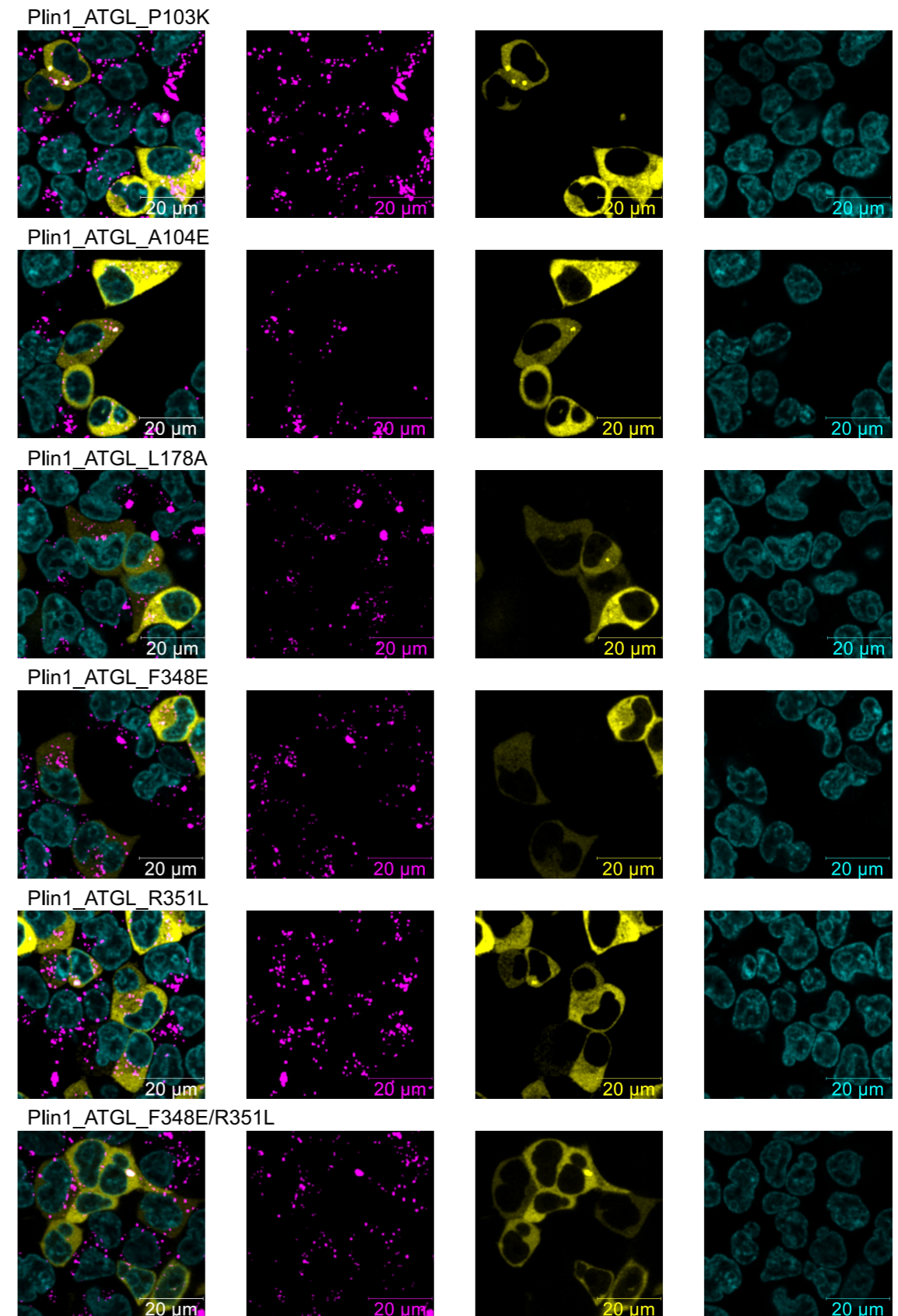
Suppl. Figure 5



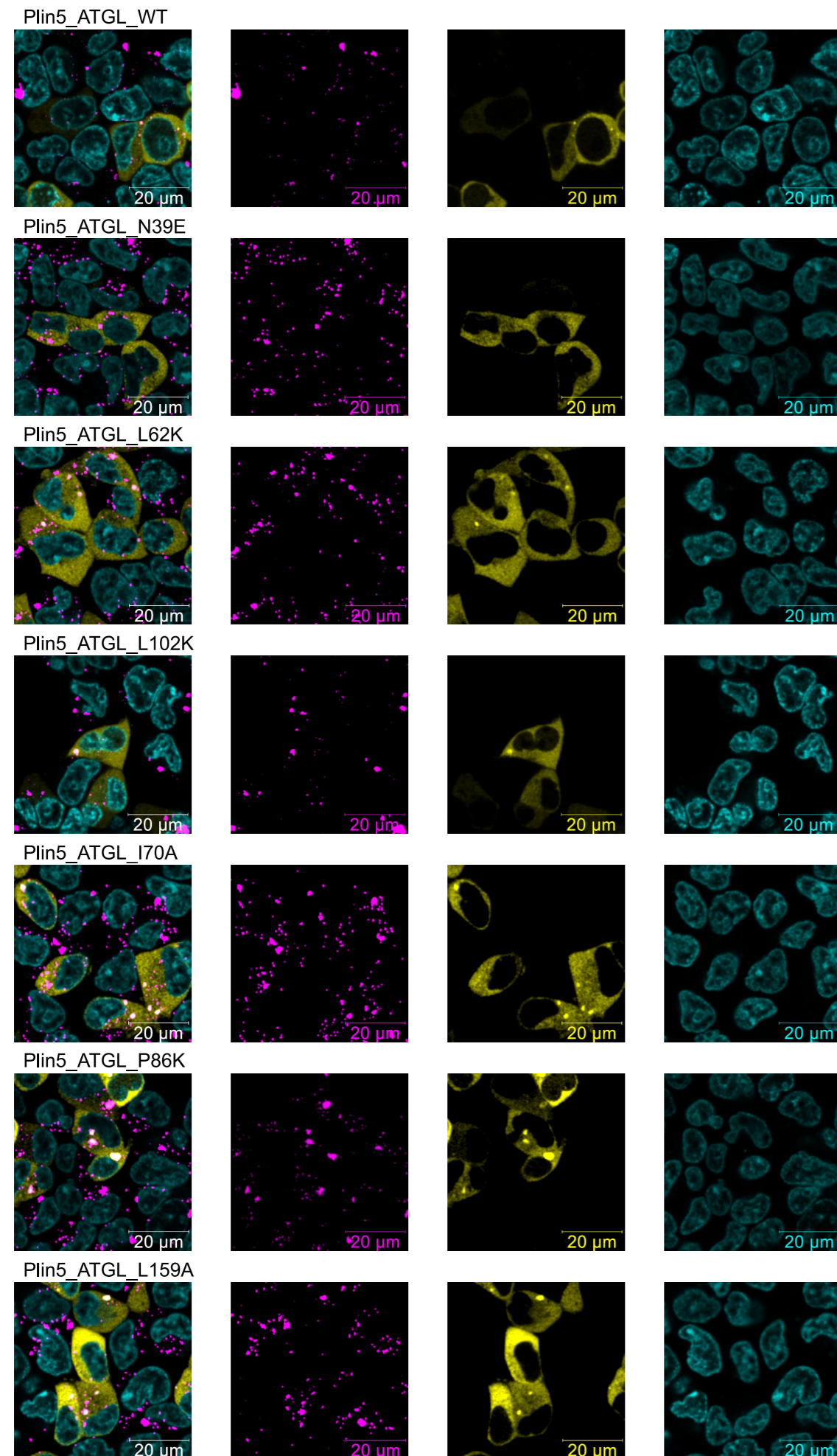
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Suppl. Figure 6



Kohlmayr et al, Suppl. Figure 6:

Confocal images of stained, live T-REx™-293 cells stably expressing doxycycline induced PLIN5.

Cells were transiently transfected with YFP-ATGL variants and PLIN1/5 expression simultaneously induced with doxycycline (2μg/ml). After 24 hours cells were treated with 200μM oleic acid for 6h. Pictures are merged fluorescent images with Hoechst in cyan, YFP-ATGL in yellow and Bodipy 558/568 C12 (D3835, FisherScientific) in magenta, next to the three separated channels. The experiments were repeated three times with similar outcome.

