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-REx[™]-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-REx[™]-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.

PageRuler ATGL_WT	PageRuler ATGL_G16K ATGL_F35E ATGL_F35A ATGL_F35A ATGL_C39E ATGL_C59L ATGL_C59L ATGL_C66A ATGL_L62K ATGL_L62K ATGL_C666L ATGL_C666L ATGL_C666L ATGL_C666L ATGL_C666L ATGL_P86K ATGL_P86K ATGL_P86K	PageRuler ATGL_S87A ATGL_S87A ATGL_R95L ATGL_R95K ATGL_R102K ATGL_P103K ATGL_P103K ATGL_A104E ATGL_A104E ATGL_A113K ATGL_R113K ATGL_R113L	ATGL_V127L
70 kDa	•		
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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 1

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1:1000

Ponceau S

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 (2µg/ml) for 24 hours and then treated with 200µM 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-ß 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).

20 µm



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.







Plin1 ATGL A104E





Plin1_ATGL_L178A



20 µm Plin1 ATGL F348E





Plin1 ATGL R351L



20 µm

Plin1_ATGL_F348E/R351L











20 µm



20 µ















20 µm

20 um

20 um

20 µm

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 (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.







Plin5_ATGL_L178A

20 µm







20 um





Plin5_ATGL_R351L

20 µm





Plin5_ATGL_F348E/R351L















Kohlmayr et al, **Suppl. Figure** 5:

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.







Plin1_ATGL_A104E





Plin1 ATGL L178A





Plin1 ATGL F348E









Plin1 ATGL F348E/R351L









Plin1_ATGL_R351L



















































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.





Plin5 ATGL A104E





Plin5 ATGL L178A





Plin5 ATGL F348E





Plin5 ATGL R351L







Plin5 ATGL F348E/R351L



























