

Supplemental Materials

Molecular Biology of the Cell

Rogne et al.

SUPPLEMENT TO

OPA1-anchored PKA phosphorylates perilipin 1 on S522 and S497 in human adipose stem cells

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Supplementary figures and legends:

Rogne et al., Suppl. Fig. 1

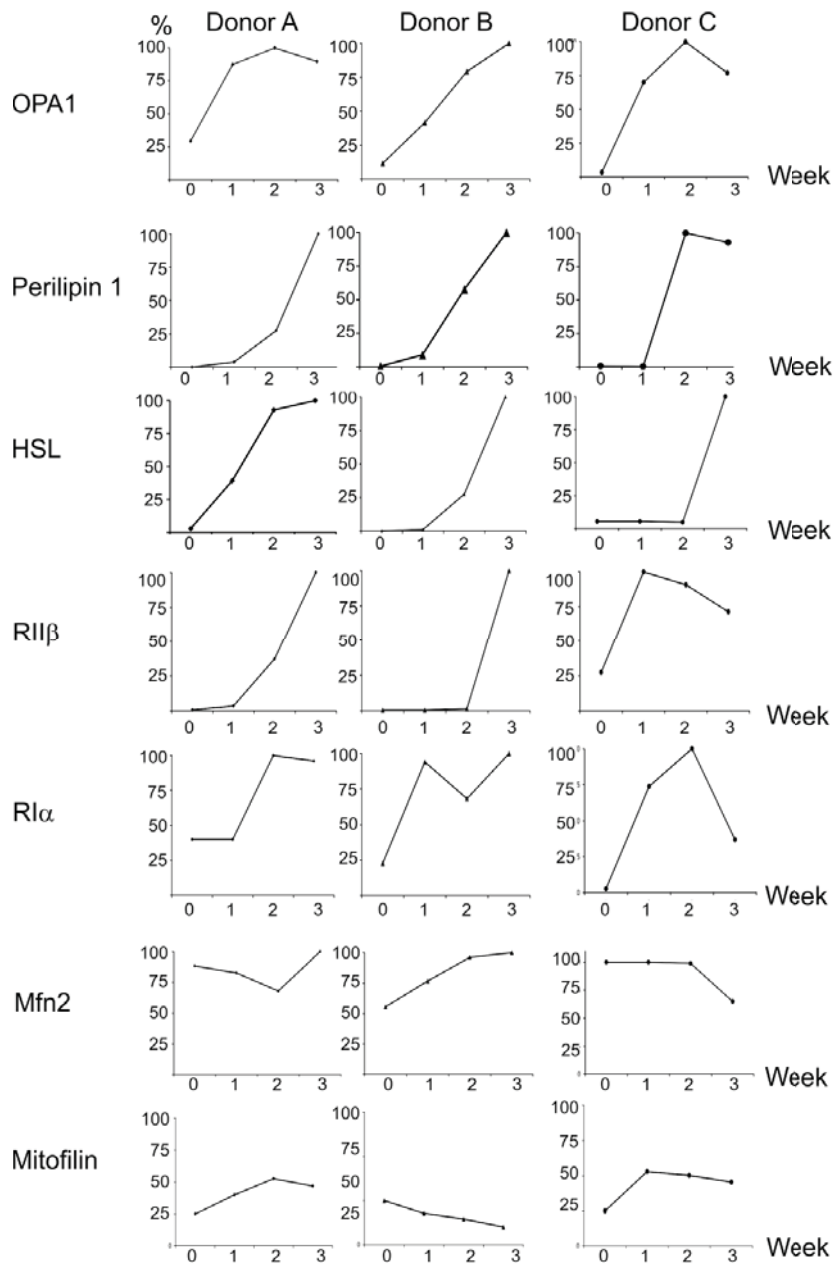


Figure S1. Regulation of OPA1 and adipocytic markers during adipocyte differentiation of hASCs. Levels of endogenous OPA1, perilipin 1, HSL, RII β , RI α , RII α , Mfn2, Mitofilin, OMA1, CGI-58, ATGL, CEBP α and Actin detected by western blot analysis in three donors during adipocyte differentiation (week 0, 1, 2, 3) (n=3).

Supplementary
figure 2

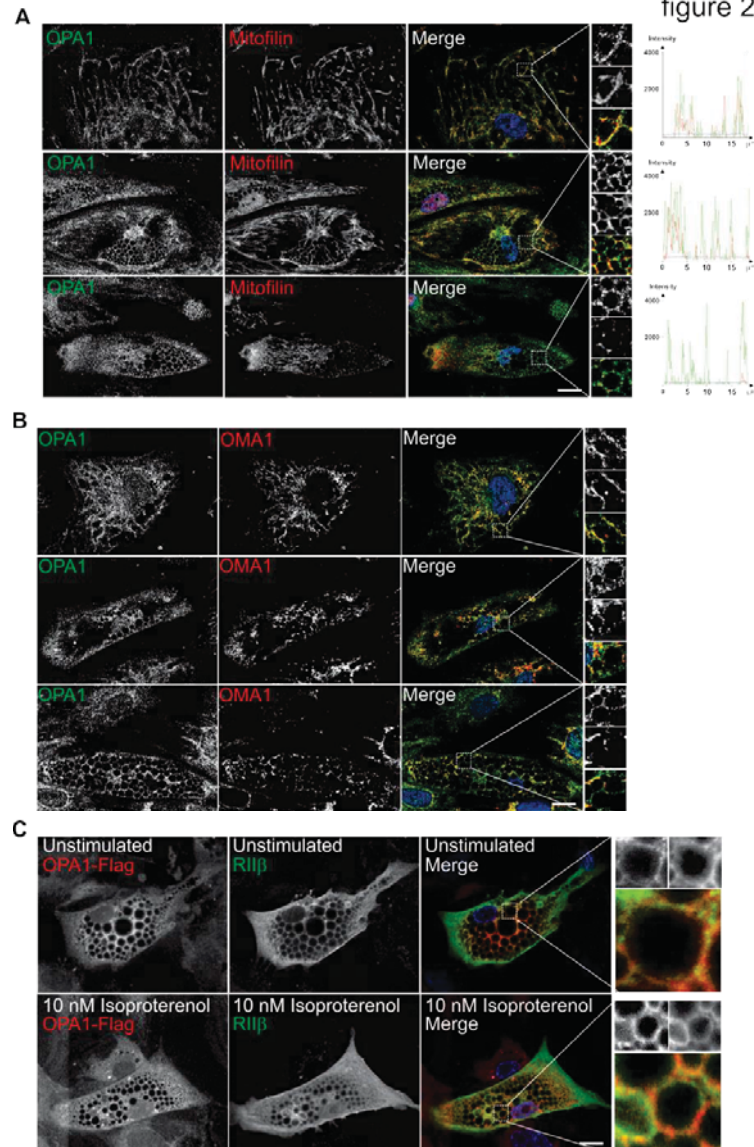


Figure S2. Subcellular localization of OPA1 during adipocyte differentiation of hASCs.

Representative images show hASC-derived adipocytes after 1, 2 and 3 weeks and immunostained for OPA1 (green) and Mitofilin (red) (A) or for OPA1 (green) and OMA1 (red) (B). Differentiated hASCs were transfected with OPA1-Flag and immunostained for OPA1-Flag (red) and RIIβ (green) (C). On the right-hand side enlarged pictures with details from the area indicated by dotted squares in the original image is shown. DAPI is included in merged pictures (n=3). Scale bars: 10 μm.

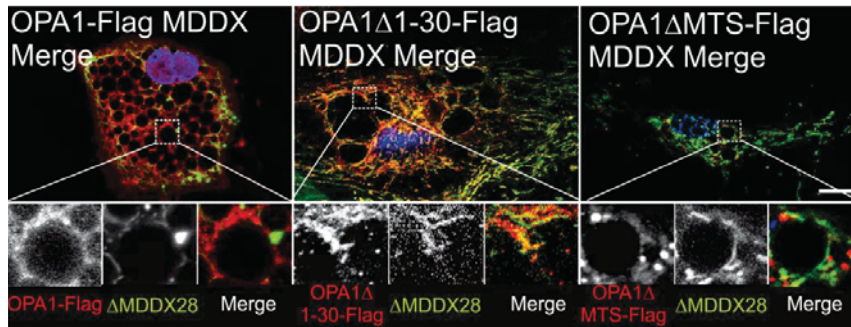


Figure S3. Effect of N-terminal truncations on localization of OPA1. hASC-derived adipocytes were transfected with OPA1-Flag, OPA1 Δ 1-30-Flag or OPA1 Δ MTS-Flag and MDDX-GFP before being immunostained for Flag (red) (A). Zoomed images with details from split channels. DAPI is included in merged pictures (n=3). Scale bars: 10 μ m.

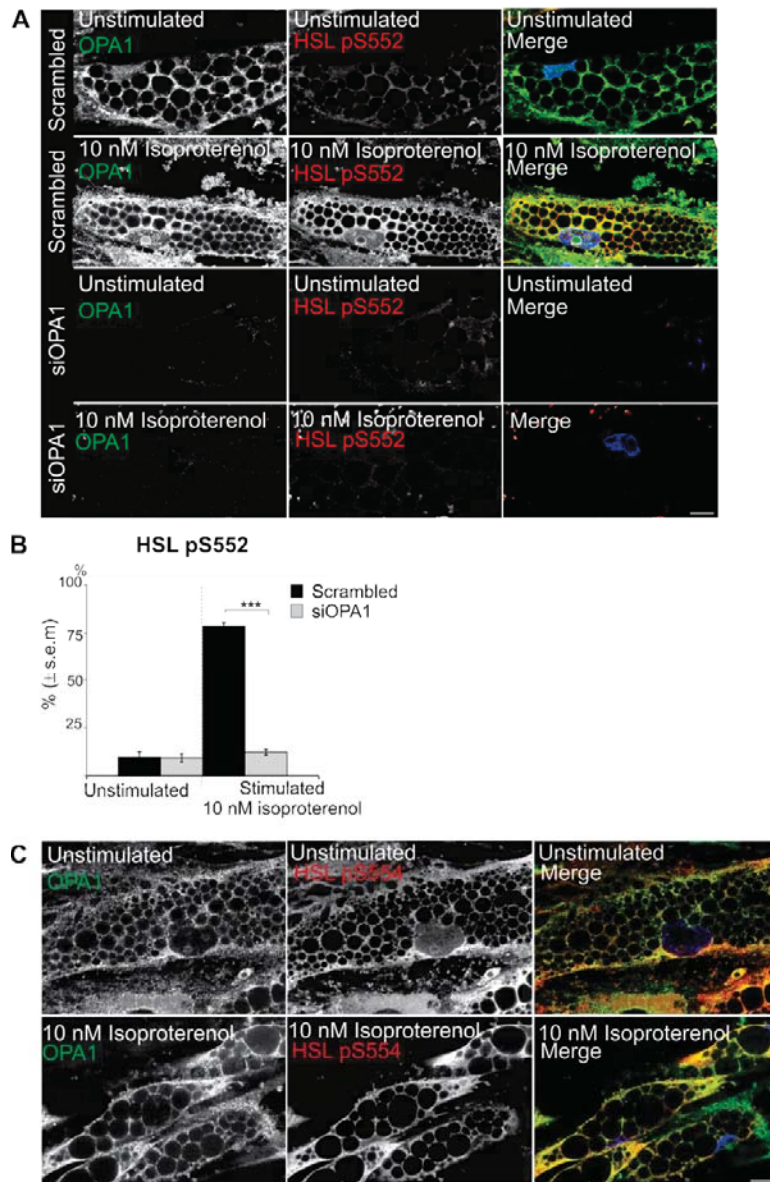


Figure S4. Requirement of OPA1 for HSL phosphorylation on S552 but not S554 upon adrenergic stimulation. Adipocyte-differentiated hASCs were transfected with OPA1 siRNA or scrambled control, stimulated with or without 10 nM of isoproterenol were analyzed by immunofluorescence for endogenous HSL pS552 (red) and OPA1 (green) (B). DAPI is included in merged pictures. Scale bars: 10 μ m. (C) Statistical analysis of experiments as in B (100 cells counted for each condition and donor, mean \pm s.e.m, n=3).