SUPPLEMENTARY FOR

Palmitate and glucose increase extracellular vesicle amyloid precursor protein: Missing link between metabolic syndrome and Alzheimer's disease

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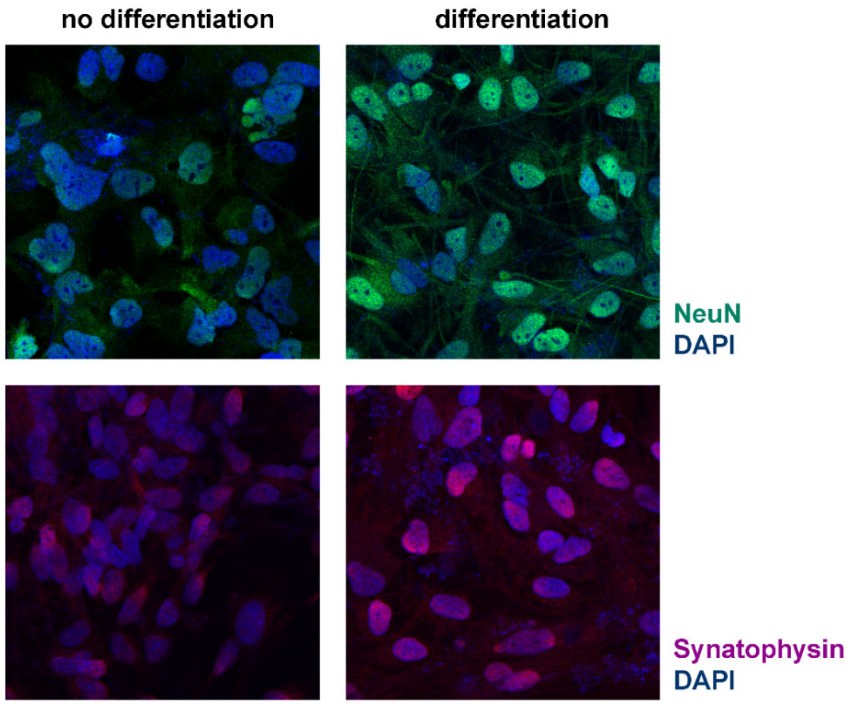
Supplementary Data 1. Differentiated HK-532 express neuronal markers.

We examined NeuN (Wolf, Buslei et al. 1996, Gusel'nikova and Korzhevskiy 2015) and synaptophysin (Wiedenmann, Franke et al. 1986, Smith, Nikulasson et al. 1993) expression as the differentiated neuronal markers by immunohistochemistry. Differentiating HK-532 cells for 1 wk in NSDM (differentiation media) greatly increased NeuN and synaptophysin expression, suggesting fully differentiated neurons (**Supplementary Figure S1; below**).

Method: Differentiated and undifferentiated HK-532 cells were plated onto glass cover slides coated with poly-L-lysine (catalog # P4832, Sigma, St Louis, MO) and fixed in 4% paraformaldehyde for 15 min. Cells were rinsed 3 times with wash buffer (PBS with 0.1 % Triton X-100). Cells were then incubated with anti-NeuN (1:500; catalog # 104225, Abcam, Cambridge, UK) or anti-synaptophysin (1:200; catalog # 101006, Synaptic System, Goettingen, Germany) antibodies for 16 h. Cells were then rinsed and incubated in AlexaFluor 405 anti-rabbit (for NeuN; catalog # A48258, Invitrogen, Thermo Fisher Scientific, Waltham, MA) or AlexaFluor 594 anti-chicken (for synaptophysin; catalog # A11042, Invitrogen) secondary antibodies for 2 h. Cells were mounted on slide glass with ProLong gold with DAPI (Invitrogen) and images were taken on a Leica Stellaris 8 Lightning confocal using a 1.4 numerical aperture Plan-APO 63x oil objective. Images were rendered with the lightning deconvolution feature and were exported as tiffs (Elzinga, Henn et al. 2022).

Supplementary Figure S1. **Differentiated HK-532 express neuronal markers.** HK-532 cells were stained for NeuN (green channel) or synaptophysin (purple channel) along with DAPI (blue channel).

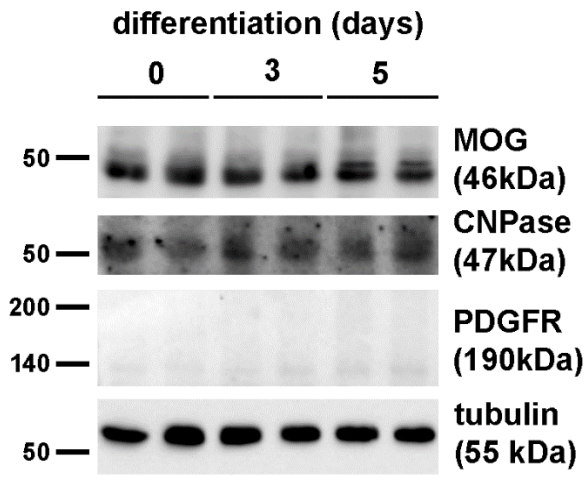


Supplementary Data 2. HOG cells express oligodendrocyte markers.

We examined myelin oligodendrocyte glycoprotein (MOG) and 2',3'-cyclic-nucleotide 3'-phosphodiesterase (CNPase) as mature human oligodendrocyte (HOG) markers and platelet-derived growth factor receptor (PDGFR) as an undifferentiated oligodendrocyte progenitor cell (OPC) marker (Kuhn, Gritti et al. 2019). Cells were differentiated with a combination of T3, selenium, insulin, and transferrin for 3 and 5 days. HOG cells already expressed MOG and CNPase before the differentiation protocol, which did not further alter protein levels (**Supplementary Figure S2; below**). PDGFR was not detected in any condition. These results suggest that HOG cells already represent mature oligodendrocytes characteristics.

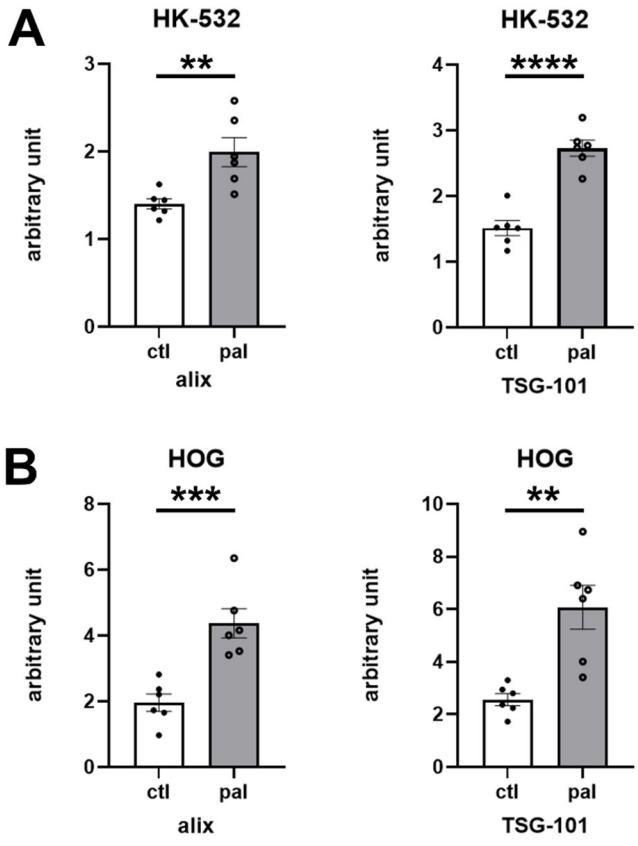
Method: HOG cells were differentiated in DMEM with 30 nM 3,3',5-triiodo-L-thyronine sodium salt (T3, catalog # T6397, Sigma), 30 nM selenium (catalog # S5261, Sigma), 50 µg/ml transferrin (catalog # 3188-AT-001G, R&D Systems, Minneapolis, MN), 0.5 µg/ml insulin (catalog # I0516, Sigma) and 0.05% fetal bovine serum. Western blotting was performed as in Materials and Methods using anti-MOG (catalog # MAB2439, R&D Systems), anti-CNPase (catalog # 66729-1-Ig, Proteintech, Rosemont, IL) or anti-PDGFR (catalog # ab134123, Abcam) antibodies.

Supplementary Figure S2. **HOG cells express oligodendrocyte markers.** HOG cells were differentiated for 0-5 days and cell lysates were analyzed for MOG, CNPase, PDGFR, and tubulin. HOG cells expressed MOG and CNPase prior to differentiation, which did not further alter protein levels.



Supplementary Figure S3. Palmitate increases EV secretion from neurons and oligodendrocytes.

HK-532 cells and human oligodendrocytes (HOG) were treated with control (ctl, white; bovine serum albumin vehicle) and palmitate (pal, grey; 150 μM) conditions for 48 h. EVs were obtained and the same amount of protein in all conditions (control-treated HK-532, palmitate treated HK-532, control-treated HOG, palmitate treated HOG) were resolved by gel electrophoresis and blotted for EV proteins Alix and TSG101 (main text Fig 4D-E). Intensity of Alix and TSG101 bands were quantified directly from the blots; since equal amounts of protein were loaded, band intensity is internally normalized and relative to total loaded protein. Palmitate increased EVs, i.e., levels of Alix and TSG101, in both (**A**) HK-532 and (**B**) HOG cells. Data are presented as mean ± SEM from 3 separate experiments with duplicate treatments. **P<0.01, ***P<0.001, ****P<0.0001, by Student's t-test.



Supplementary Table S1. List of antibodies.

Antibodies	Company	Catalog #	Description	RRID
pAPP	CST	6986	Rabbit monoclonal (D90B8)	AB_10831197
APP	Millipore	MAB348	Mouse monoclonal (22c11)	AB_94882
APP-CTF	BioLegend	825001	Rabbit polyclonal	AB_2564886
pTau, pSer199/Ser202	Invitrogen	44-768G	Rabbit polyclonal	AB_1502103
pTau, pThr231	Invitrogen	44-746G	Rabbit polyclonal	AB_1502124
pTau, pSer396	Abcam	ab109390	Rabbit monoclonal (EPR2731)	AB_10860822
Tau5	Invitrogen	AHB0042	Mouse monoclonal (TAU-5)	AB_1502093
BACE	Abcam	ab108394	Rabbit monoclonal (EPR3956)	AB_10861218
pIRS-1, pSer612	CST	3203	Rabbit monoclonal (C15H5)	AB_1031167
pIRS-1, pSer636/Ser639	CST	2388	Rabbit polyclonal	AB_330339
pAkt, pSer473	CST	4060	Rabbit monoclonal (D9E)	AB_2315049
Akt, pan	CST	4691	Rabbit monoclonal (C67E7)	AB_915783
pERK, pThr202/Tyr204	CST	9101	Rabbit polyclonal	AB_331646
ERK	CST	9102	Rabbit polyclonal	AB_330744
pJNK, pThr183/Tyr185	CST	4668	Rabbit monoclonal (81E11)	AB_823588
TSG101	Abcam	ab125011	Rabbit monoclonal [EPR7130(B)]	AB_10974262
Flotillin-1	CST	18634	Rabbit monoclonal (D2V7J)	AB_2773040
Alix	CST	2171	Mouse monoclonal (3A9)	AB_2299455
VDAC	CST	4661	Rabbit monoclonal (D73D12)	AB_10557420
Calnexin	CST	2679	Rabbit monoclonal (C5C9)	AB_2228381
β-Actin	Abcam	ab8227	Rabbit polyclonal	AB_2305186
Tubulin	CST	3873	Mouse monoclonal (DM1A)	AB_1904178

Abcam, Cambridge, UK; BioLegend, San Diego, CA; CST, Cell Signaling Technology, Danvers, MA; Invitrogen, Thermo Fisher Scientific, Waltham, MA; Millipore, Merck, Burlington, MA.

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