

SUPPLEMENTARY DATA

Nicotinic acid suppresses sebaceous lipogenesis of human sebocytes via activating hydroxycarboxylic acid receptor 2 (HCA₂)

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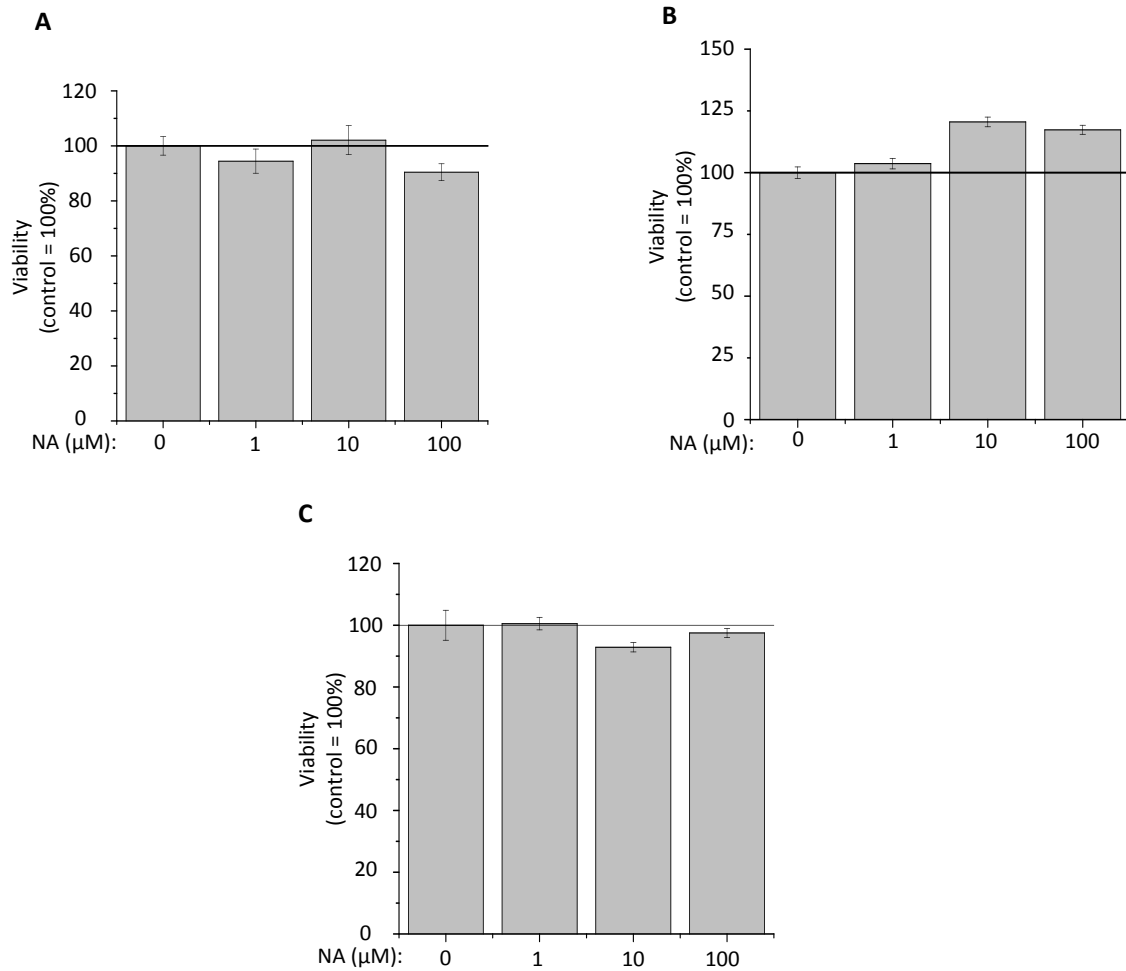
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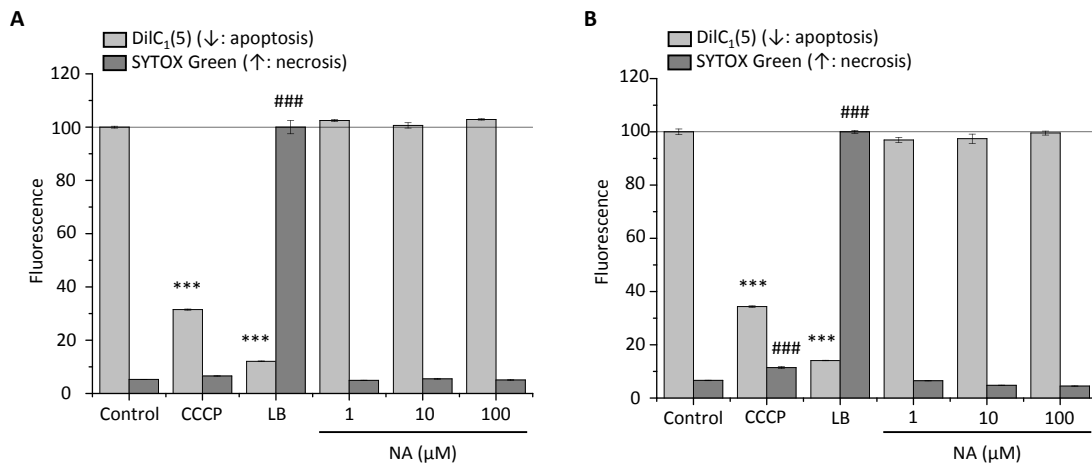
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SUPPLEMENTARY FIGURES



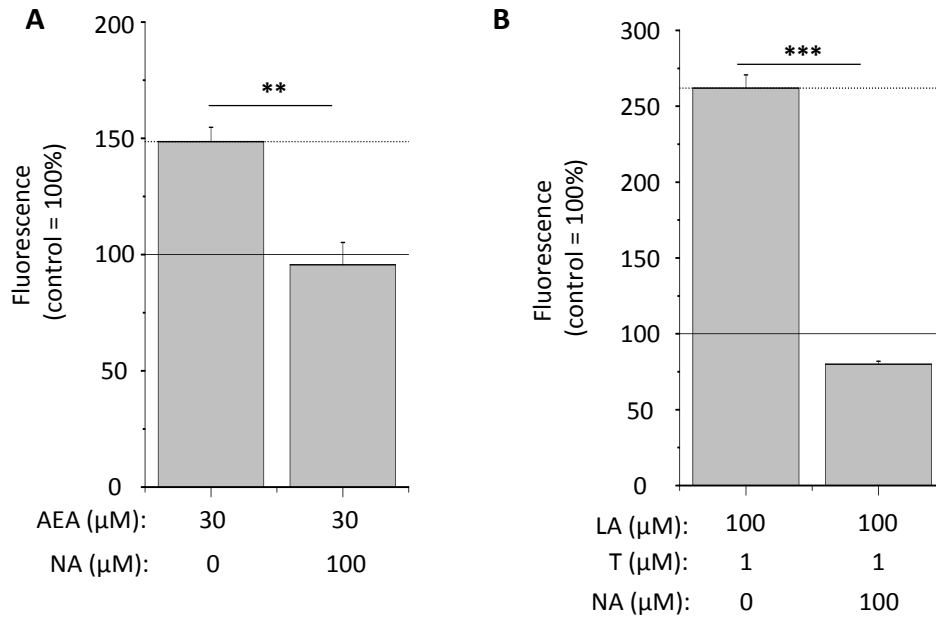
Supplementary Figure S1 *Up to 100 μM , NA does not influence viability of human sebocytes*

MTT-assays. Viability of SZ95 sebocytes was monitored following 24- (**A**), 48- (**B**), and 72-hr (**C**) treatments. Results are expressed in the percentage of the vehicle control (100%, solid line) as mean \pm SEM of four independent determinations. Two additional experiments yielded similar results.



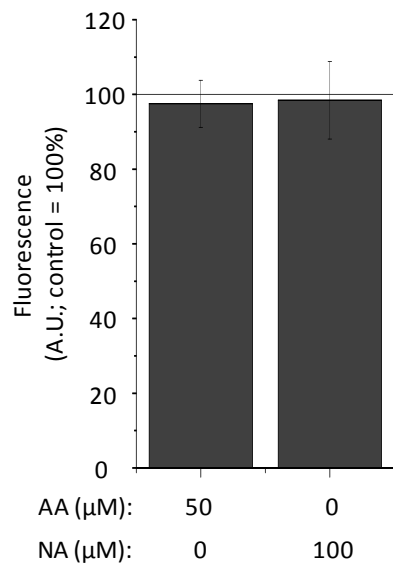
Supplementary Figure S2 *Up to 100 μM, NA can be applied without the risk of cytotoxicity*

Combined fluorescent DiIC₁(5)-SYTOX Green labeling. To monitor apoptotic and necrotic cell death, SZ95 sebocytes were treated as indicated for 24 (**A**) or 48 (**B**) hours. Results are expressed in the percentage of the vehicle control (100%, solid line; DiIC₁(5) apoptosis data) or in the percentage of the positive control (100%, solid line; SYTOX Green necrosis data) as mean±SEM of four independent determinations. Two additional experiments yielded similar results. *** and ### mark significant ($P < 0.001$ in both cases) differences compared to the vehicle control group. **CCCP**: carbonyl cyanide m-chlorophenyl hydrazone (1:200; apoptosis positive control); **LB**: lysis buffer (1:100; positive control for necrosis); **NA**: nicotinic acid.



Supplementary Figure S3 *NA exerts universal lipostatic effects*

Nile Red assay. Lipostatic efficiency of NA was assessed following 48-hr treatments in the presence of AEA (**A**) or LA+T combination (**B**). Results are expressed in the percentage of the vehicle control (100%, solid line) as mean±SEM of four independent determinations. One additional experiment yielded similar results. ** and *** mark significant ($P<0.01$ and 0.001, respectively) differences, as indicated. **AEA:** anandamide; **LA:** linoleic acid; **NA:** nicotinic acid; **T:** testosterone.



Supplementary Figure S4 *Neither AA, nor NA influence HCA₂ expression in human sebocytes*

Immunofluorescent labeling. HCA₂ expression was assessed following the indicated 24-hr treatments. Following appropriate background subtraction (for details, see the **Materials and methods** section) data of the green channel were expressed in the percentage of the vehicle control, and presented as mean±SEM of N=15-16 cells in each group. **A.U.:** arbitrary units.