Supplementary figures



Supplementary Fig. 1

Expression of GPR109A in adipose tissue (A) and spleen (B). Shown are sections of both tissues stained with DAPI and analyzed for mRFP expression. Macrophages in spleen sections were visualized using FITC-labeled F4/80 antibodies. Bar length: 75 μ m (A), 25 μ m (B).



Hanson et al., Suppl. Fig. 2

Supplementary Fig. 2

Epidermal sections from wild-type mice were stained with an anti-keratin antibody which was then visualized using a secondary Cy2-labelled antibody (upper row). Cell nuclei were stained with DAPI. To test for the specificity of the antibody and to rule out unspecific signals due to the secondary antibody, the same procedure was performed in the absence of the primary anti-keratin antibody (lower row).



Supplementary Fig. 3

RT-PCR on mouse and human keratinocyte preparations using primers specific for langerin. Genomic DNA was used as a positive control. The cDNA synthesis reaction was performed in the absence (-RT) or presence of reverse transcriptase (+RT).



Hanson et al., Suppl. Fig 4

Supplementary Fig. 4

Effect of nicotinic acid (100 μ M) on the intracellular free Ca²⁺ concentration in Fura-2loaded CHO-K1 cells heterogously expressing wild-type GPR109A and GPR109A fused to mRFP (GPR109A-mRFP). The time point of nicotinic acid application is indicated by an arrow. R.F.U., relative fluorescence units.



Hanson et al., Suppl. Fig 5

Supplementary Fig. 5

A) Effects of 100 μ M nicotinic acid (NA) and 10% fetal bovine serum (FBS) on ERK1/2 phosphorylation in keratinocytes prepared from GPR109A^{-/-};Krt5^{Gpr109a-mRFP} mice were analyzed with an antibody-recognizing phosphorylated ERK1/2 (pERK). In parallel, the total amount of ERK1/2 was determined (ERK1/2). B) Effect of 100 μ M nicotinic acid (NA) on the intracellular free Ca²⁺ concentration in Fura-2-loaded keratinocytes from GPR109A^{-/-};Krt5^{Gpr109a-mRFP} mice; ATP (100 μ M) was applied as a positive control.



Hanson et al., Suppl. Fig. 6

Supplementary Fig. 6

Epidermal sections from wild-type mice (WT) and COX-2-deficient mice ($Ptgs2^{-/-}$) were stained with anti-COX-2 antibodies. Cell nuclei were visualized by staining with DAPI. Bar length: 10 µm.



Supplementary Fig. 7

Effect of 500 μ M of nicotinic acid (NA) and mono-Methyl fumarate (MF) on the release of PGD₂ from mouse keratinocytes (A) and human keratinocytes (B). Shown are mean values ± S.E.M. (n = 4).