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Supplementary Materials for

Commensal *Cutibacterium acnes* induce epidermal lipid synthesis important for skin barrier function

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This PDF file includes:

Other Supplementary Materials for this manuscript includes the following:

SUPPLEMENTAL FIGURE 1

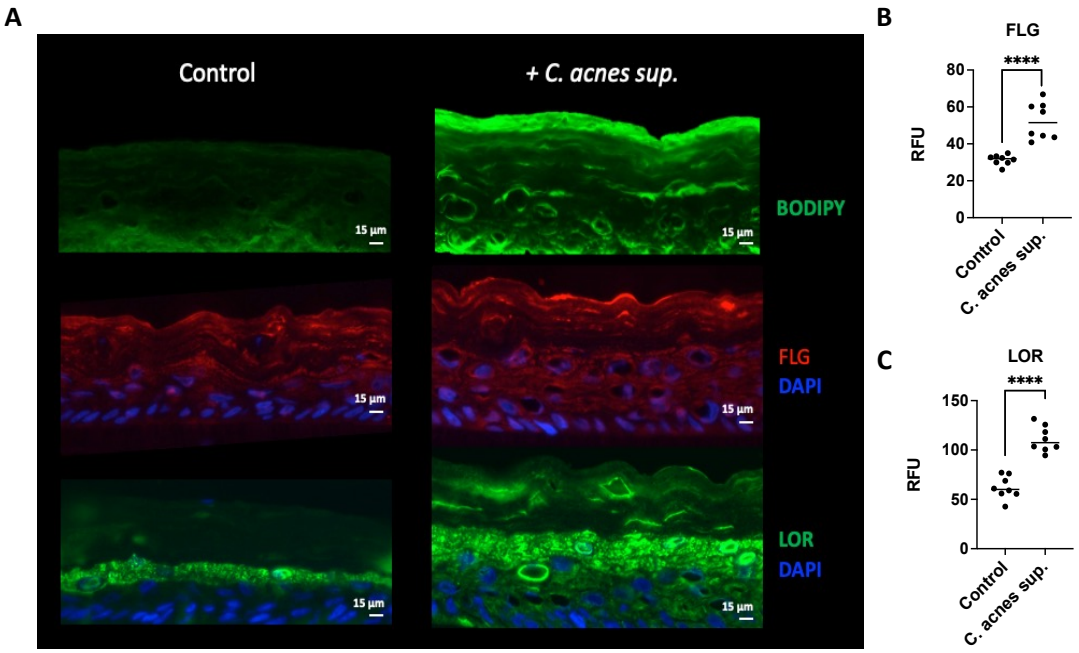

Figure S1: *C. acnes* increase the level of loricrin and filaggrin in Human reconstructed epidermis.

(A) Fluorescence and immunofluorescence images of reconstructed human epidermis after treatment with or without sterile supernatant from *C. acnes* conditioned culture media. Fluorescence quantification of filaggrin (FLG) **(B)** and loricrin (LOR) **(C)** in reconstructed human epidermis. RFU: Relative Fluorescence Units.

SUPPLEMENTAL FIGURE 2

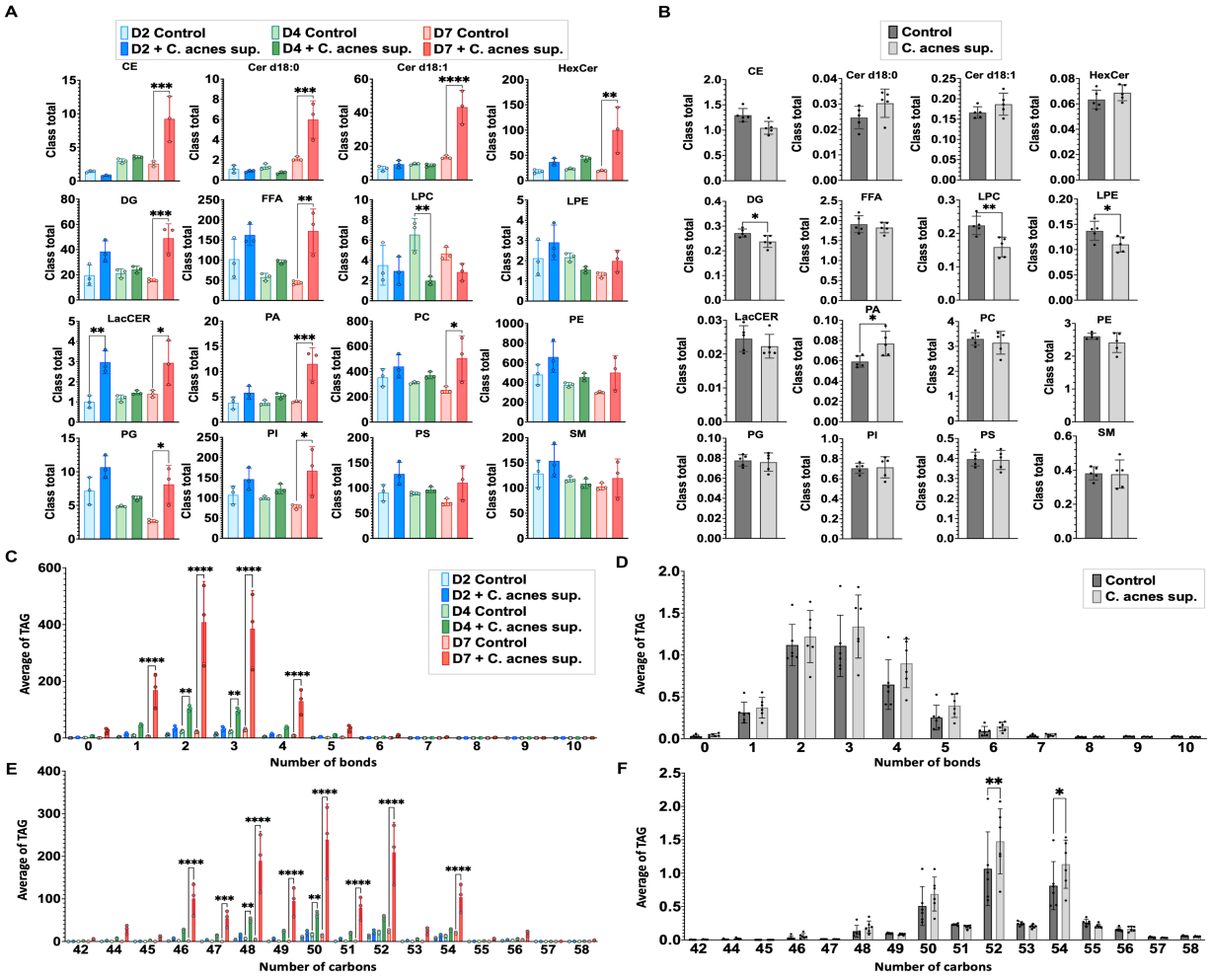

Figure S2: Analysis of lipid composition showing increases in total triacylglycerol, ceramides, cholesterol esters, and free fatty acids in NHEKs and in mice epidermis.
(A) Differential mobility spectrometry-based shotgun lipidomic analysis of extracted lipids from NHEK after exposure to15% sterile supernatant from *C. acnes* for 2,4 and 7 days (n=3) and **(B)** from mice epidermis 4 days after application of live *C. acnes* (n=6). Number bonds and number of carbons analysis in triacylglycerols from NHEK treated with 15% of *C. acnes* supernatant **(C and E)** and from mice after exposure to *C. acnes* topically **(D and F).** Cholesterol esters (CE), Ceramides (Cer d18:1), Diacylglycerols (DG), Dihydroceramides (Cer d18:0), Free Fatty Acids (FFA), Hexosyl ceramides (HexCER), Lactosyl ceramides (LacCER), Lysophosphatidylcholine (LPC), Lysophosphatidylethanolamine (LPE), Phosphatidic acid (PA), Phosphatidylcholine (PC), Phosphatidylethanolamine (PE), Phosphatidylglycerol (PG), Phosphatidylinositol (PI), Phosphatidylserine (PS), Sphingomyelin (SM). *: $p<0.05$ **: $p<0.01$, ***: $p<0.001$, ****: $p<0.0001$.

SUPPLEMENTAL FIGURE 3

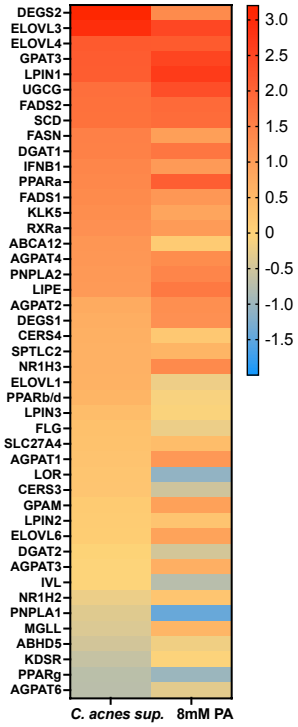

Figure S3: Global upregulation of lipid synthesis-related genes and other select genes after exposure to *C. acnes* CM or propionic acid validated by qPCR

Heat map display of qPCR measurements of the expression of the log2 fold change of genes involved in the lipid synthesis by NHEKs in response to exposure to 15% of sterile *C. acnes* supernatant or 8mM propionic acid for four days in comparison to control.

SUPPLEMENTAL FIGURE 4

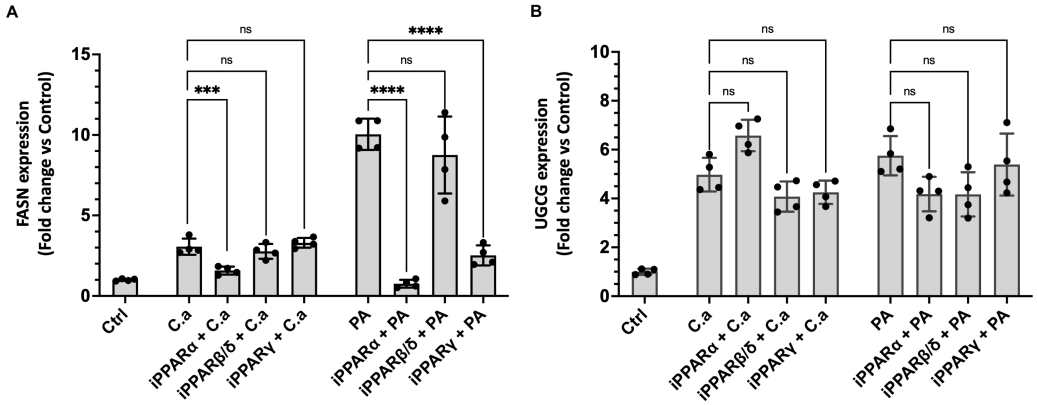

Figure S4: Inhibition of PPARα also inhibited expression of *FASN* but not *UGCG*.
Expression of FASN **(A)** and UGCG **(B)** genes as measured by qPCR in NHEKs after adding PPARα inhibitor at 3µM, PPARβ/δ and PPARγ inhibitors at 10µM, 15% *C. acnes* supernatant or 8mM PA. ***: $p<0.001$, ****: $p<0.0001$.

SUPPLEMENTAL FIGURE 5

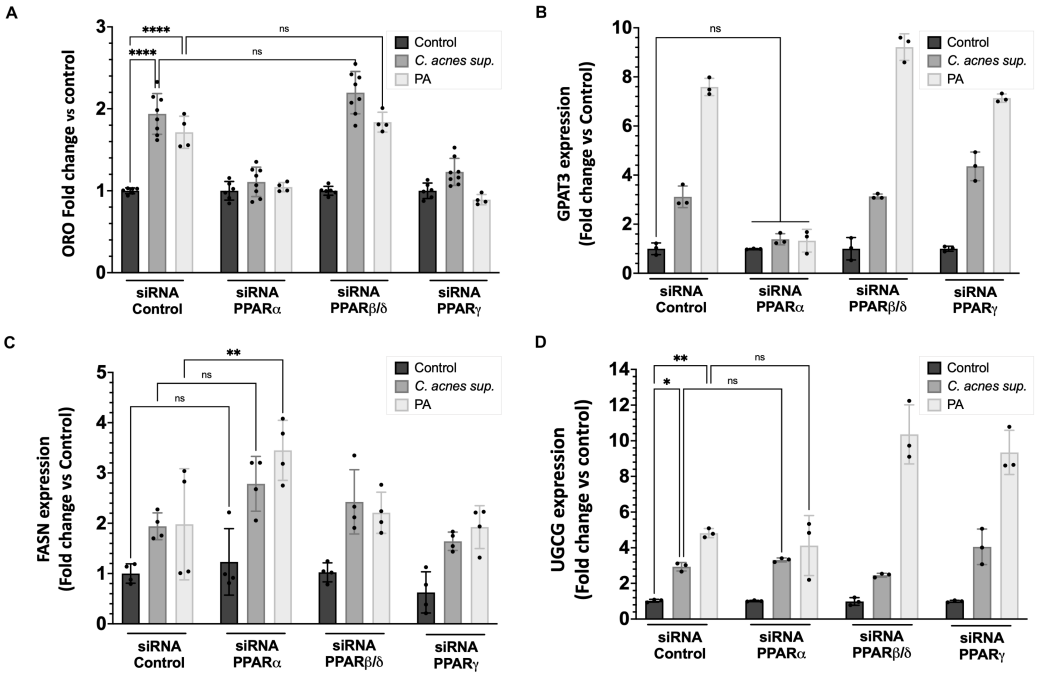

Figure S5: Confirmation of the activation of PPARα by *C. acnes* CM or propionic acid to induce lipid accumulation and expression of the lipid synthesis genes *GPAT3*.

Oil Red O quantification in NHEKs exposed to 15% *C. acnes* supernatant or 8mM of Propionic acid after silencing PPARα, PPARβ/δ and PPARγ receptors with siRNA **(A)** and expression of GPAT3, FASN and UGCG genes by qPCR **(B-D)**. Experiments conducted were performed at least in triplicate. *: $p<0.05$ **: $p<0.01$, ****: $p<0.0001$.

SUPPLEMENTAL FIGURE 6

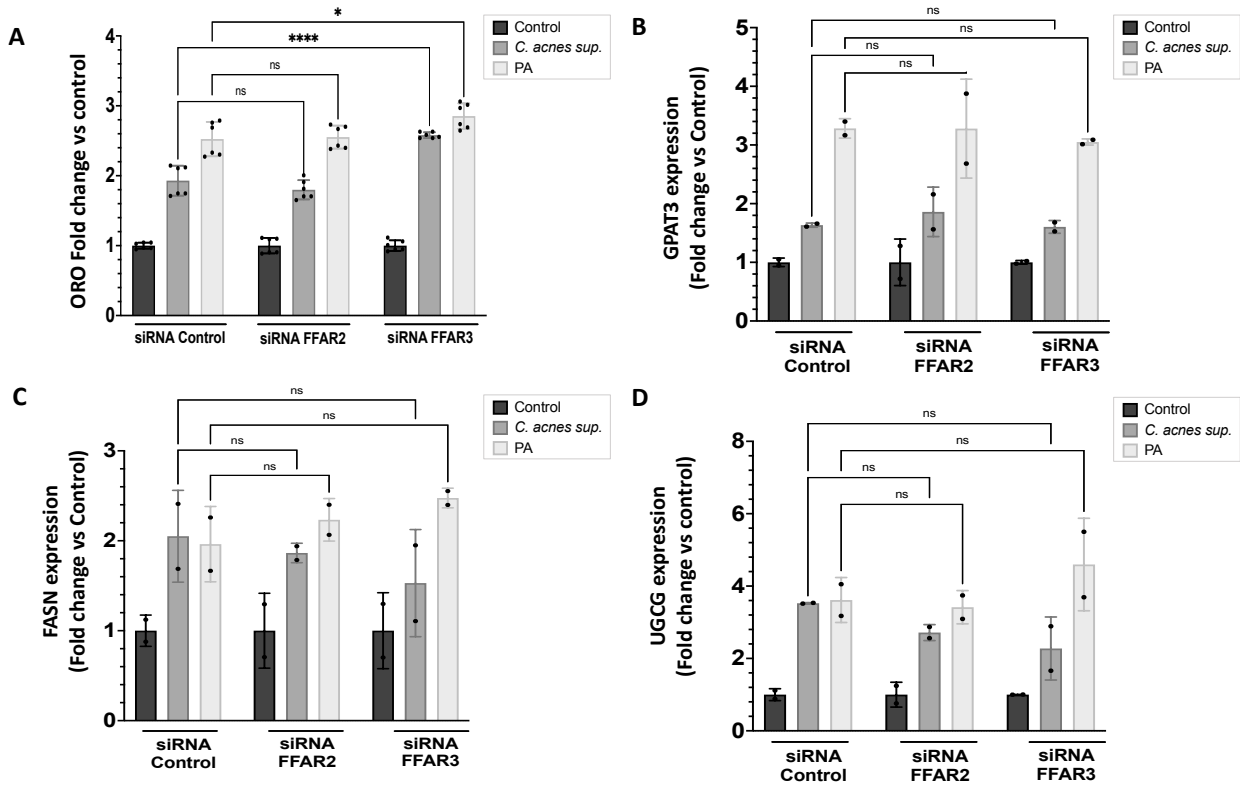

Figure S6: *FFAR2* or *FFAR3* did not play a role in the capacity of *C. acnes* CM or PA to induce lipid production in NHEKs and expression of *GPAT3, FASN* and *UGCG*.

Oil Red O quantification in NHEKs exposed to 15% *C. acnes* supernatant or 8mM of Propionic acid after silencing FFAR2 and FFAR3 receptors with siRNA **(A)** and expression of GPAT3, FASN and UGCG genes by qPCR **(B-D)**. Experiments conducted were performed at least in duplicate. *: $p<0.05$, ****: $p<0.0001$.

SUPPLEMENTAL FIGURE 7

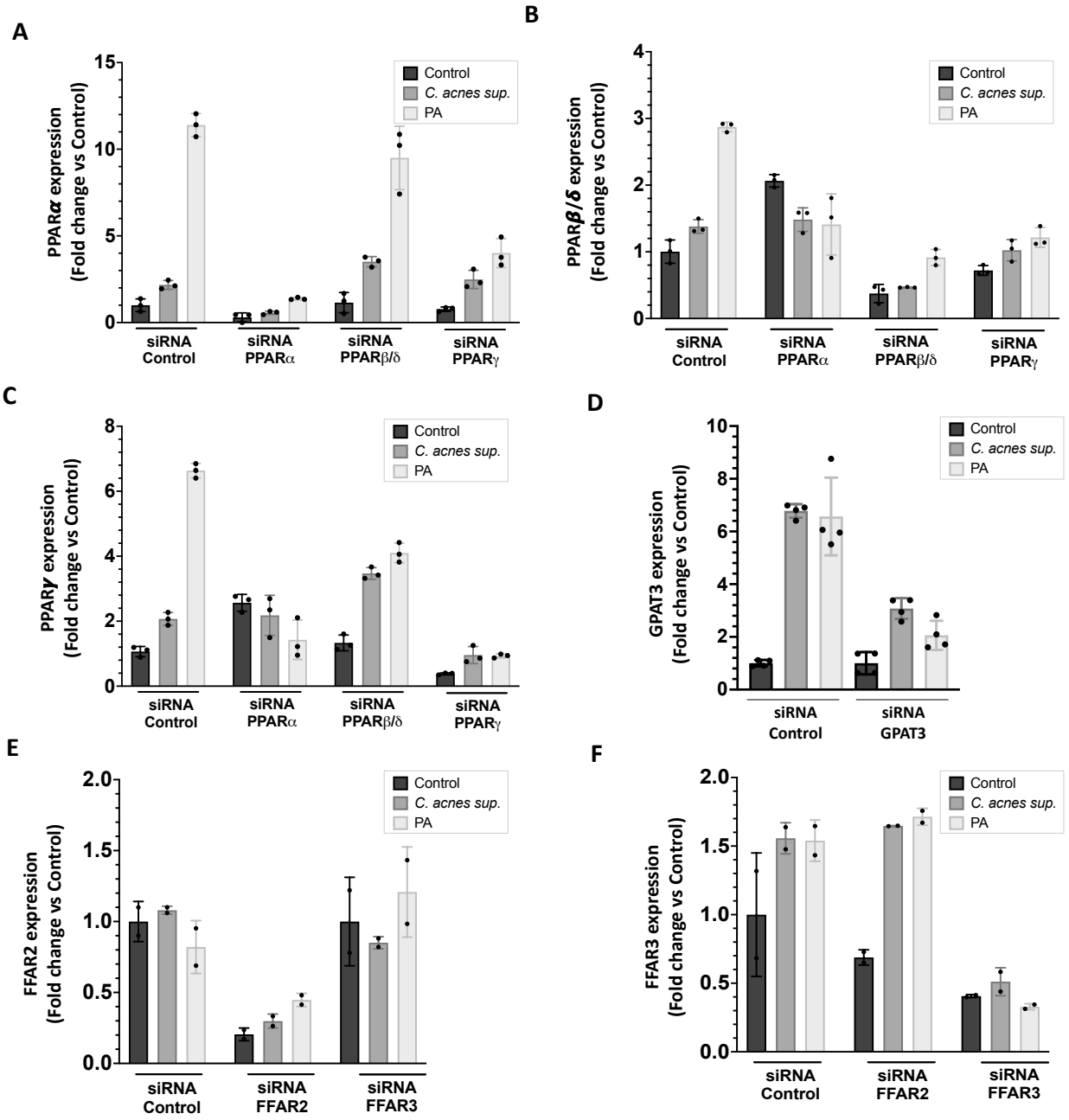

Figure S7: Silencing efficiency of PPARs receptors, GPAT3 and FFARs receptors
Measurement of silencing efficiency of PPARs receptors **(A-C)**, GPAT3 **(D)** and FFARs receptors **(E and F)** by qPCR in NHEKs after treatment with 15% of *C. acnes* supernatant or 8mM propionic acid. Experiments conducted were performed at least in duplicate.

Table S1: (DMS)-based shotgun lipidomic data from NHEKs and mice.