Supplementary Materials

Supplementary Materials and Methods

Acne lesion distribution on the face

To define whether the nose is a clinically relevant anatomic site for acne lesions, we asked two independent medical staff, who are trained in dermatology, to review 61 randomly selected cases of acne vulgaris on the face from an electronic dermatology database, where images were submitted only by expert dermatologists. While the two reviewers gave an average acne score on the nose lower than the surrounding face (1.6 *vs.* 3.4, respectively), 70.5% of the faces examined had obvious acne lesions on the nose. This finding demonstrates that the nose is a common skin site affected by acne and supports that the nose is a relevant sampling site for studying acne.

Vitamin B12 supplementation led to acne development in subject HL414

Several previous studies suggested that vitamin B12 induces acne (*21-26*). Their conclusions were based on the observations that the induced acne occurred within a short period of time after vitamin B12 supplementation and disappeared quickly after the discontinuation of vitamin B12 supplementation (*21-26*). We had the same clinical observations on subject HL414. This subject had clear skin prior to vitamin B12 supplementation. One week after vitamin B12 supplementation, multiple erythematous papules and comedones developed on the face. After discontinuation of the vitamin B12 supplementation, the symptoms disappeared quickly. The subject had clear skin when we resampled the face three months later. Throughout this process,

board certified dermatologists determined the facial skin conditions and diagnosis at each time point. Our study was also consistent with the previous studies in that vitamin B12 supplementation did not lead to acne development in all the subjects studied. We observed one out of ten subjects that had an acne breakout after vitamin B12 supplementation. Our observation of acne development in subject HL414 after vitamin B12 supplementation is highly consistent with the current literature.

Total RNA extraction

The follicular contents were individually picked from the pore strip using sterile forceps and placed in a 2 mL sterile microcentrifuge tube filled with ATL buffer (Qiagen) and glass beads (0.1mm diameter) (BioSpec Products, Inc.). Cells were lysed using a beadbeater for 3 minutes at 4,800 rpm at room temperature. To avoid overheating the samples, the beadbeating was paused every 1 minute and the samples were placed on ice for at least 1 minute. After centrifugation at 14,000 rpm for 5 minutes, the supernatant was retrieved and used for total RNA extraction. Twenty microliter proteinase K solution (Qiagen) was added, followed by incubation for 10 minutes at 56˚C. After incubation, 1 mL preheated QIAzol (Qiagen) at 65˚C was added and incubated for 5 minutes at room temperature. The supernatant was then separated into two phases by adding 200µL chloroform and centrifuging at 12,000 g for 5 minutes at 4˚C. The total RNA in the aqueous phase was then cleaned using RNA MinElute Clean-up kit (Qiagen) according to the manufacturer's instruction. After total RNA extraction, each sample was subjected to DNase digestion to remove residual genomic DNA contamination using TURBO DNA-free kit (Ambion).

Ribosomal RNA (rRNA) depletion, RNA amplification and cDNA synthesis

To enrich messenger RNA (mRNA), rRNA was depleted from the total RNA using MICROBExpress Kit (Ambion). The enriched mRNA was further polyadenylated and amplified by *in vitro* transcription-based mRNA linear amplification using MessageAmp II-Bacteria kit (Ambion). The amplified RNA was then converted to double-stranded cDNA using random hexamer (Promega) and SuperScript Double-Stranded cDNA synthesis kit (Life Technologies) (*74, 75*).

RNA-Seq

The cDNA libraries were prepared and sequenced using the Illumina sequencing platform (Illumina). The read length ranged from 82 – 101 bp, either single-end or paired-end (table S1).

Data cleaning

Sequence reads were trimmed from 3' end to remove low quality bases. Reads containing more than 90% of A/T within the entire sequence reads were removed. After trimming, cleaned sequence pairs, where both reads are longer than 40 bp and have fewer than three ambiguous base pairs, were used for further analysis.

Functional classification of OGUs

The representative protein sequence of each OGU was mapped against the COG database (*76*) and KEGG database (*77*) using BLASTP. The COG identifier or KEGG identifier of the best BLAST hit with an e-value < 1E-5 was assigned to the corresponding OGU. If multiple hits were

found from non-overlapping regions of a query sequence, all of the COG or KEGG identifiers were assigned to the OGU.

Rarefaction curve of the number of OGUs detected

For each sample, a random number of sequence reads aligned to *P. acnes* genomes was sampled. The number of expressed unique OGUs represented by the sampled sequence reads was calculated using the R package Vegan 1.17-0 (*78*). The number of sequence reads randomly sampled was increased from 0 to the sequencing depth of each sample. The rarefaction curve of each sample was plotted using the number of expressed unique OGUs as a function of the number of sampled sequence reads. For all nine samples, rarefaction curves reached plateaus of detecting expressed *P. acnes* OGUs after sampling 100 million base pairs. Rarefaction analysis showed that our sequencing depth of the non-ribosomal transcripts of *P. acnes* (ranging from 232 million base pairs to 2.4 billion base pairs) was sufficient for gene expression analysis (fig. S8).

Unsupervised hierarchical clustering analysis

For the clustering of the samples from the cross-sectional study, OGUs with detectable expression level (≥ 1 after normalization) in at least five samples and with a large variation across samples (standard deviation ≥ 150) were used in the hierarchical clustering analysis. The clustering was performed using centered Pearson correlation similarity metric and average linkage clustering method. A total of 562 OGUs passed filtering criteria and were used for clustering analysis of the nine cross-sectional samples.

For the clustering of the samples from the vitamin B12 longitudinal study and the cross-sectional study, OGUs with detectable expression level (≥ 1 after normalization) in all the 26 samples and with a large variation across samples (standard deviation ≥ 200) were used in the hierarchical clustering analysis. The clustering was performed using centered Pearson correlation similarity metric and average linkage clustering method. A total of 438 OGUs passed filtering criteria and were used for clustering analysis of the 26 RNA-Seq samples.

Analysis of differentially expressed OGUs

The expression levels of *P. acnes* OGUs in the RNA-Seq data were compared between samples from acne patients and samples from healthy individuals using the Student's t-test (unequal variance, two-sided test). Differentially expressed OGUs were identified using the criterion of $P < 0.05$. These differentially expressed OGUs were further confirmed by a Poisson model based comparison, ShotgunFunctionalizeR (*72*) in R package (http://www.r-project.org, http://shotgun.zool.gu.se), with a cutoff of Akaike's information criterion < 5,000 and adjusted $P < 0.05$. The heat map of differentially expressed OGUs was generated using R package gplots (*79*) heatmap.2 function based on z scores.

The expression levels of *P. acnes* OGUs in the samples from the vitamin B12 longitudinal study were compared between the Day0 samples before vitamin B12 supplementation and the Day14 samples after vitamin B12 supplementation. The statistical analysis was performed using the paired samples t-test (unequal variance, two-sided test). Differentially expressed OGUs were identified using the criterion of $P < 0.05$. We compared the expression levels of the OGUs only

if there were at least three pairs of samples with coverage of more than 10,000 bp (equivalent to ~100 reads) on the OGU to accurately quantify the gene expression changes (*80*).

The expression levels of *P. acnes* OGUs in sample HL414-Day14 were compared to the other Day14 samples using one sample Student's t-test (unequal variance, two sided test). The differential gene expression was determined with a false discovery rate < 5%. The same OGU coverage requirement as stated above was applied.

Analysis of differentially expressed pathways

An online functional annotation clustering analysis, DAVID (*19*) was used to identify the metabolic pathways and functional annotation clusters that were enriched in the differentially expressed gene set between acne patients and healthy individuals. The enriched metabolic pathways and functional annotation clusters had enrichment scores ranging from 0.1 to 0.8. Vitamin B12 biosynthesis and porphyrin metabolic process had an enrichment score of 0.41. Additionally, we used ShotgunFunctionalizeR pathway-centric analysis to identify differentially expressed metabolic pathways with a cutoff of Akaike's information criterion < 5,000 and adjusted $P < 0.05$. This package was used previously to compare metabolic pathway differences in metatranscriptomic data (*72*).

KEGG pathway mapping

The global KEGG metabolic pathways were mapped using iPath2 (*81*).

Determination of cobalt's effect on porphyrin biosynthesis in *P. acnes* cultures

The effect of cobalt on porphyrin biosynthesis in *P. acnes* cultures was determined using the method by Zaitseva *et al.* (*38*) with minor modifications. *P. acnes* was cultured in reinforce clostridium broth anaerobically to stationary phase. The cells were harvested by centrifugation, washed twice with sterile 0.1M sodium phosphate buffer (pH 7.0), and then re-suspended in a synthetic broth without carbon sources for a preliminary starvation. The composition of the synthetic broth is 0.1 M sodium phosphate buffer, 3 g/L $(NH_4)_2SO_4$, 0.48 g/L KH_2PO_4, 0.48 g/L K_2HPO_4, 0.2 g/L $MgSO_4$, 0.01 g/L NaCl, and 0.01 g/L $MnSO_4$. After being maintained in the synthetic medium for 24 hours at 30˚C anaerobically, the cells were centrifuged, washed, and re-suspended in a fresh synthetic broth with supplementation of 2% lactose as carbon sources and 5,6-dimethylbenzimidazole (5,6-DMB). Then the cell suspension was evenly distributed to two experimental conditions: without additional supplementation (control), and with 10 mg/L $CoCl_2 \cdot 6H_2O$ added. The cells were incubated at 30˚C for 48 hours anaerobically. The porphyrin production in each culture was then measured as described earlier. Since the bacterial cells were cultured in a synthetic medium, at a lower temperature, and in a shorter period of time, the porphyrins produced in this experiment were expected to be much less than in a regular culture condition.

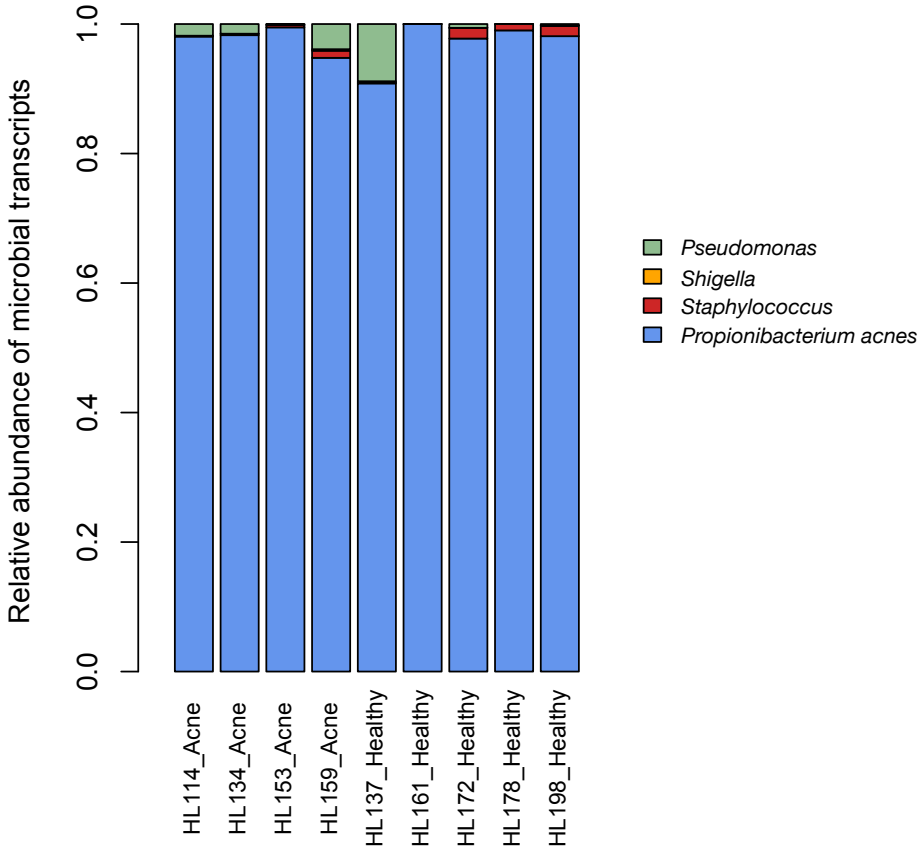

Fig. S1. The metatranscriptome composition of the skin microbiota in follicles.

P. acnes transcripts are the most abundant microbial transcripts in all samples (>90%). Additionally, we identified transcripts mapped to several other bacteria, including *Staphylococcus*, *Shigella,* and *Pseudomonas*.

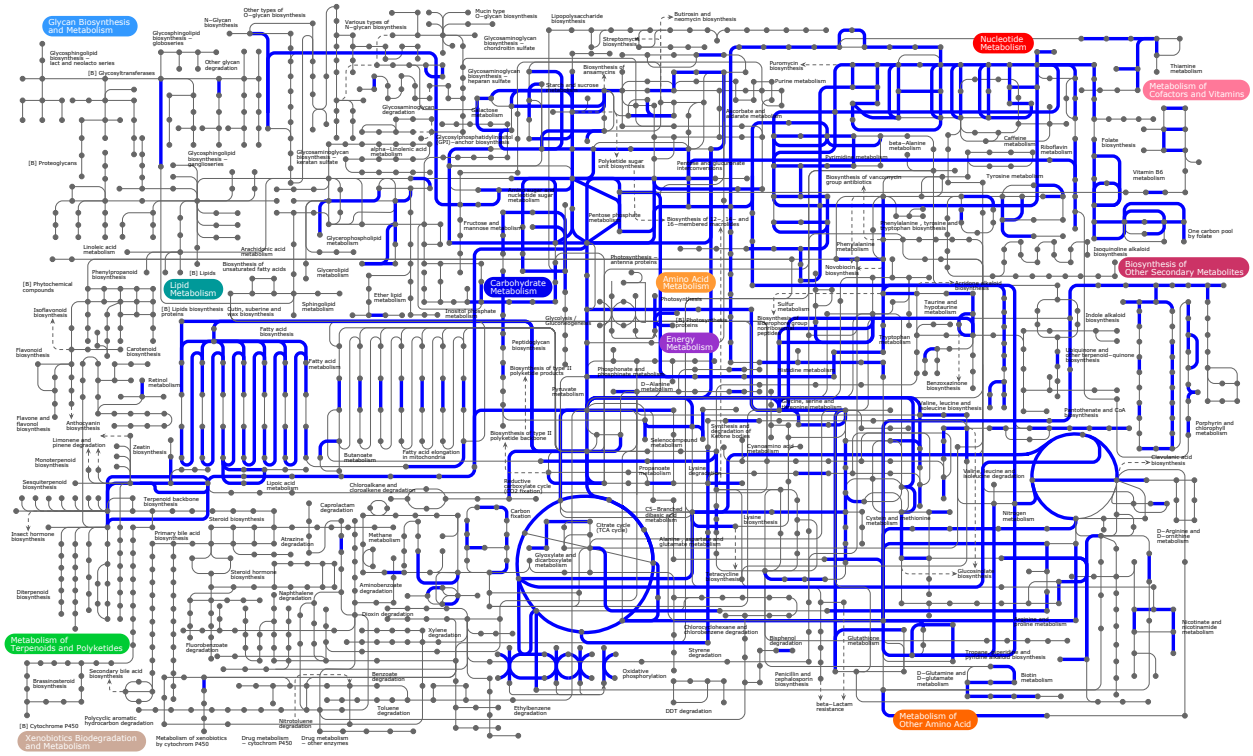

Fig. S2. The KEGG pathways expressed in all the samples.

The core *P. acnes* transcriptome shared by all the samples covered most of the metabolic pathways (blue lines), including sugar metabolism, nucleic acid metabolism, amino acid metabolism, lipid metabolism, and the metabolism of cofactors and vitamins.

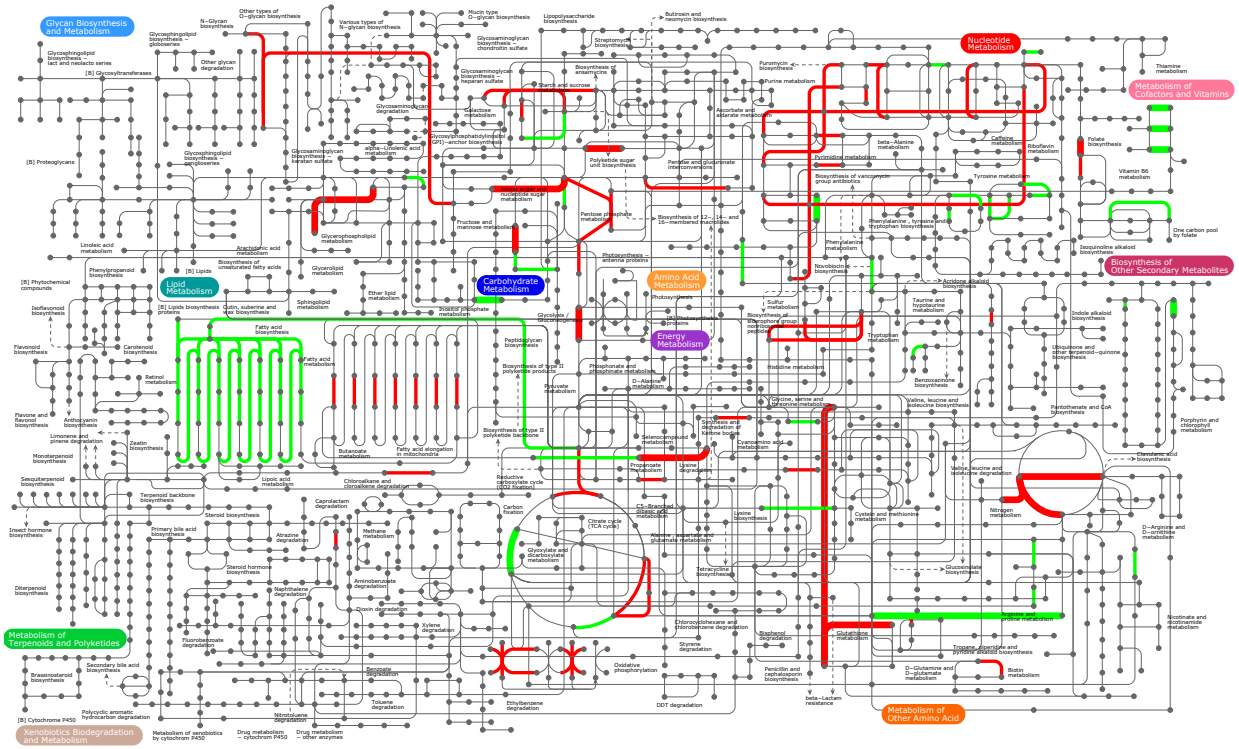

Fig. S3. The KEGG pathways with differentially expressed *P. acnes* OGUs.

Thick red lines indicate the up-regulated OGUs in the acne patients with $P < 0.05$. Thin red lines indicate the up-regulated OGUs in the acne patients with $0.05 \leq P < 0.1$. Thick green lines indicate the down-regulated OGUs with $P < 0.05$. Thin green lines indicate the down-regulated OGUs with $0.05 \leq P < 0.1$.

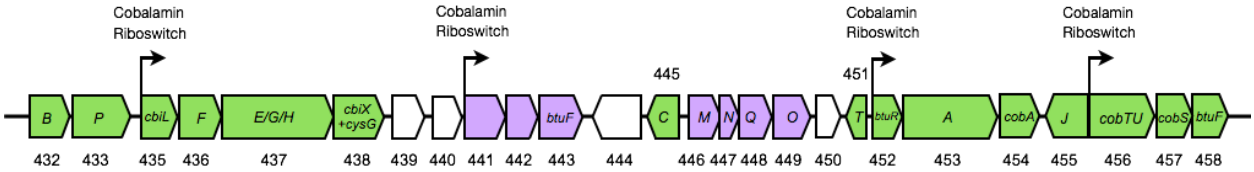

Fig. S4. *cob/cbi* operons in *P. acnes*.

The *cob/cbi* operons in *P. acnes* genome are shown. Green boxes represent the genes in vitamin B12 biosynthesis pathway. Purple boxes represent the genes encoding transporters. Among the transporter genes, PAGK_0441, PAGK_0442, and PAGK_0443 encode an iron complex transporter, and PAGK_0446, PAGK_0447, PAGK_0448, and PAGK_0449 encode a cobalt transporter. White boxes indicate genes with unclear functions in the vitamin B12 biosynthesis pathway. Among them, PAGK_0444 encodes AAA family ATPase, while PAGK_0439, PAGK_0440, and PAGK_0450 encode hypothetical proteins. The number below each box indicates the gene ID in HL096PA1 genome (*82*). The single letters in the boxes indicate *cbi* gene names. The black arrows indicate predicted cobalamin riboswitches. The gene IDs of *cbiL*, *cbiX+cysG*, and *btuR* are 435, 438, and 452, respectively. They are all located in the operons under the control of cobalamin riboswitches.

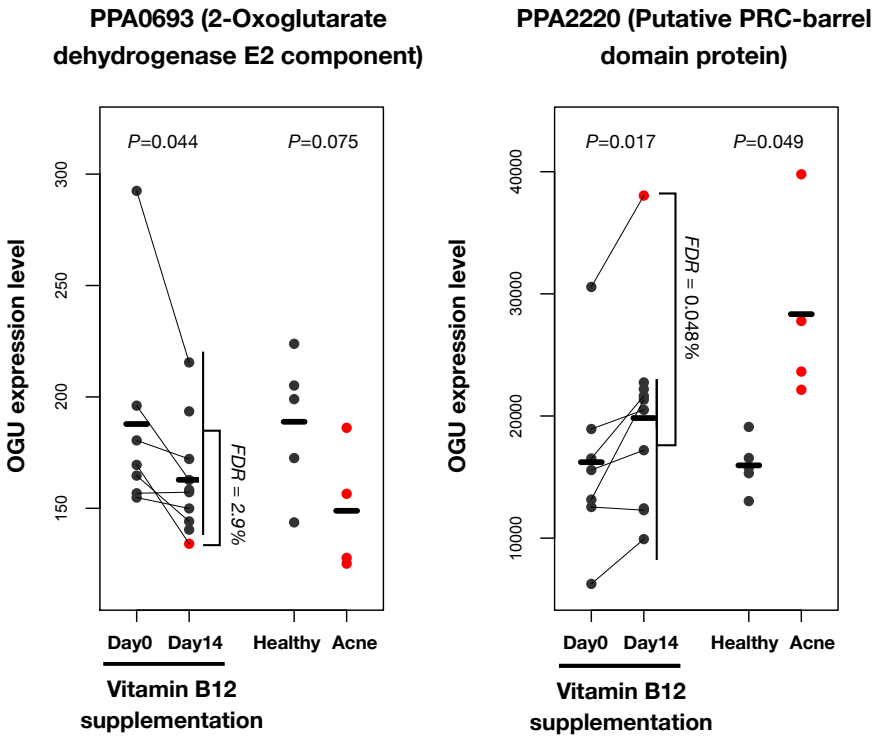

Fig. S5. Differentially expressed *P. acnes* OGUs in sample HL414-Day14 compared to the other Day14 samples.

The expression levels of the OGUs were plotted for four groups of samples: samples from the healthy subjects before vitamin B12 supplementation (Day0) (n=7), samples from the healthy subjects 14 days after vitamin B12 supplementation (Day14) (n=10), samples from the healthy individuals in the cross-sectional study (Healthy) (n=5), and samples from the acne patients (Acne) (n=4). The mean expression level of each OGU is indicated by a thick black bar. The Day0 sample and the Day14 sample from the same individual were connected by a line.

Significance was determined by Student's t-test. Red dots indicate the samples collected from the acne patients or subject HL414 after vitamin B12 supplementation (HL414-Day14). Black dots indicate the samples collected from the healthy subjects. FDR: false discovery rate.

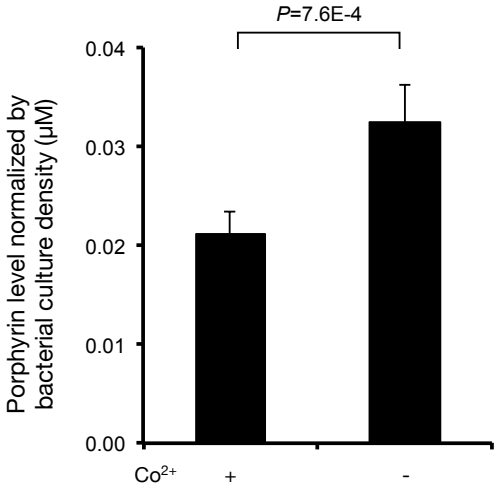

Fig. S6. The biosynthesis of porphyrins is inversely correlated with the biosynthesis of vitamin B12 in *P. acnes*.

Depletion of cobalt, which inhibits vitamin B12 biosynthesis, significantly increased porphyrin production in *P. acnes*. Significance was determined by Student's t-test. Data are presented as the mean ± standard deviation of five biological replicates (n=5).

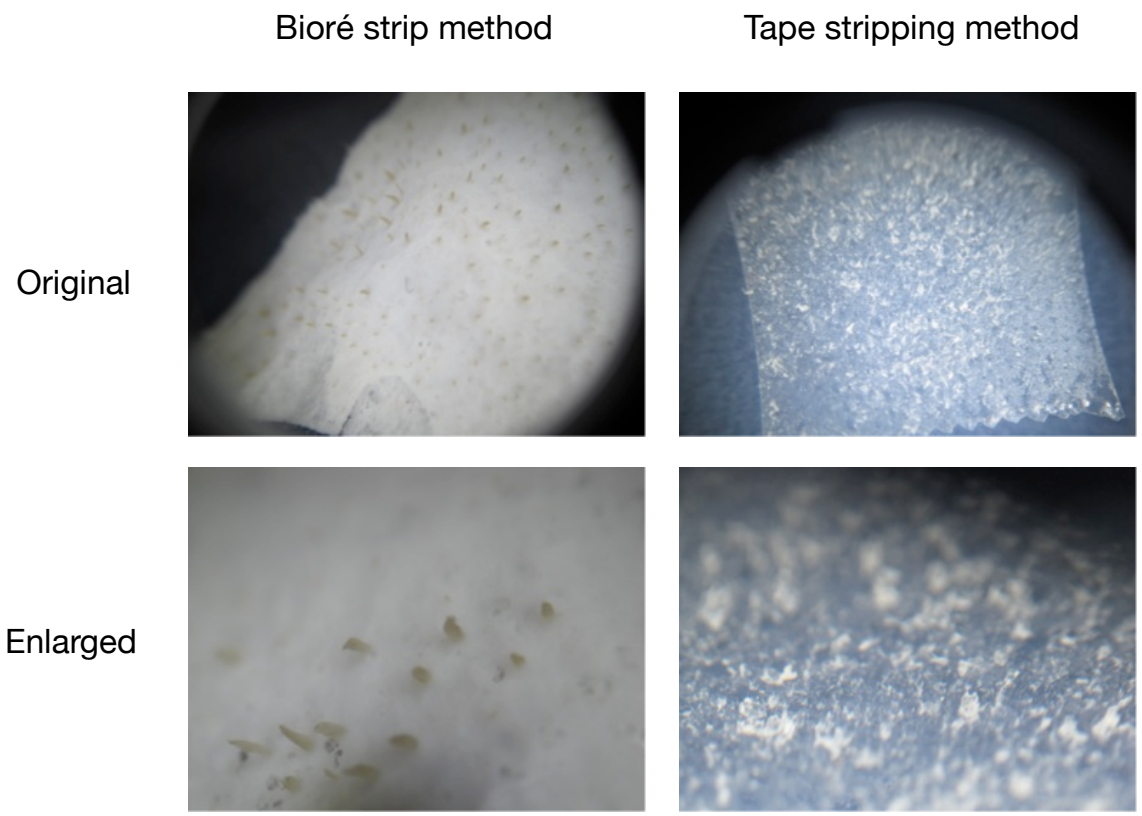

Fig. S7. A comparison of the samples collected from opposite sides of the nose of the same individual using a Biore strip method and a tape stripping method.

Samples from opposite sides of the nose of the same individual were obtained using the Biore strip method and the tape stripping method. As shown, the tape stripping method removes keratinocytes from the surface of the skin, sampling the stratum corneum of the epidermis. In contrast, the Biore strip method removes the contents of follicles to a depth of ~2 mm. It samples the microbiota residing inside follicles, including the anaerobic portion.

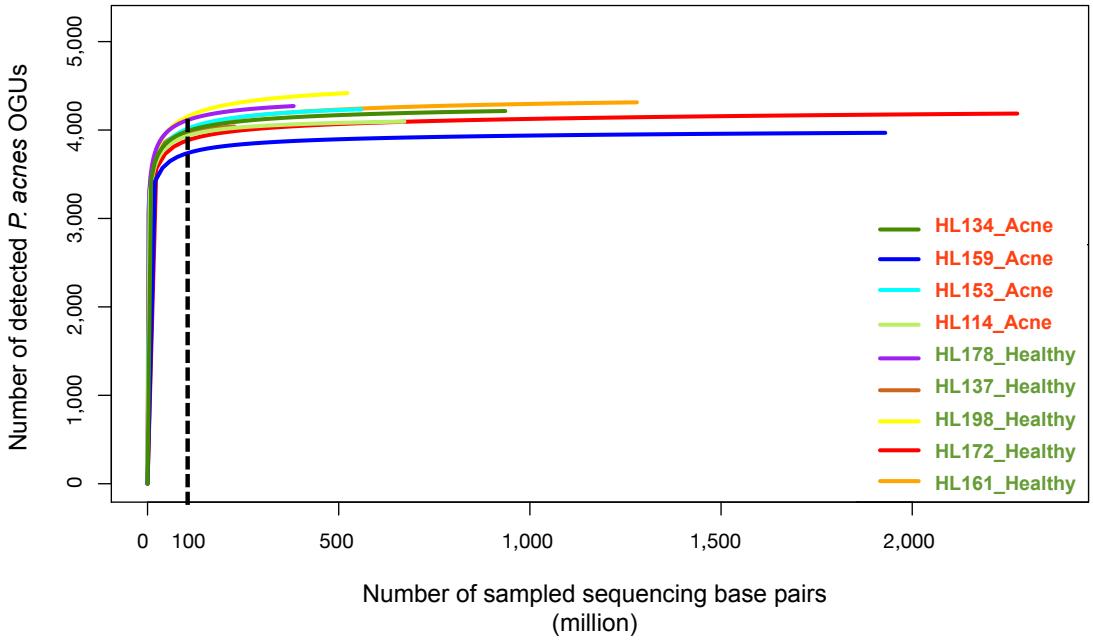

Fig. S8. Rarefaction curves indicate sufficient sequencing depths of the samples.

The rarefaction curves of all nine samples reached plateaus in detecting *P. acnes* OGUs with more than 100 million base pairs. This suggests that the sequencing depths of all these samples (with minimum 232 million base pairs) were sufficient for the gene expression analysis.

Table S1. High sequencing depths of the metatranscriptomic data.

Sample name	Subject type	Read length (bp)	Number of paired-end reads in raw data (million)	Number of paired-end reads in cleaned data (million)
HL114	acne	84	52	42
HL134	acne	100	170	96
HL153	acne	84	66	48
HL159	acne	100	160	136
HL137	healthy	84	44	32
HL161	healthy	84	72	50
HL178	healthy	82	58	46
HL172	healthy	100	182	148
HL198	healthy	84	52	44

Table S2. Differentially expressed OGUs shown in Fig. 1B.

Annotation (following the Figure 1B heatmap row order)	OGU expression change
hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
hypothetical protein PPA1279	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
RHS repeat-associated core domain protein	up-regulated in acne patients
30S ribosomal protein S13	up-regulated in acne patients
lipoprotein releasing system, ATP-binding protein	up-regulated in acne patients
transcription termination/antitermination factor NusG	up-regulated in acne patients
50S ribosomal protein L4	up-regulated in acne patients
hypothetical protein PPA2373	up-regulated in acne patients
putative ABC-type sugar transport system, permease component	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein HMPREF0675_4689	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein HMPREF0675_5344	up-regulated in acne patients
hypothetical protein PPA2385	up-regulated in acne patients
lipid A export permease/ATP-binding protein MsbA	up-regulated in acne patients
CobQ/CobB/MinD/ParA nucleotide binding domain protein	up-regulated in acne patients
ring hydroxylating dioxygenase, alpha subunit, rieske family	up-regulated in acne patients
ATP-dependent Clp protease, ATP-binding subunit ClpX	up-regulated in acne patients
hypothetical protein PPA2302	up-regulated in acne patients
conserved domain protein	up-regulated in acne patients
ATPase of the AAA family protein	up-regulated in acne patients
seryl-tRNA synthetase	up-regulated in acne patients
hypothetical protein PPA1082	up-regulated in acne patients
maltose phosphorylase	up-regulated in acne patients
putative amino acid permease	up-regulated in acne patients
PTS system, mannitol-specific IIABC component	up-regulated in acne patients
phosphoenolpyruvate-dependent sugar phosphotransferase system, EIIA	up-regulated in acne patients
putative hydrolase, NUDIX family	up-regulated in acne patients
HTH-type transcriptional regulator MalR (Maltose operon transcriptional repressor)	up-regulated in acne patients
oxoglutarate dehydrogenase (succinyl-transferring), E1 component	up-regulated in acne patients
ribosomal protein L22	up-regulated in acne patients

toxin-antitoxin system, toxin component, HicA family	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
dihydroneopterin aldolase	up-regulated in acne patients
aminopeptidase N	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
aerotolerance protein BatA	up-regulated in acne patients
excinuclease ABC subunit A	up-regulated in acne patients
hypothetical protein PPA1344	up-regulated in acne patients
mannose-6-phosphate isomerase, class I	up-regulated in acne patients
preprotein translocase subunit SecD	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
hemin import ATP-binding protein HmuV	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
sodium/alanine symporter family protein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
preprotein translocase subunit SecA	up-regulated in acne patients
phosphoglucosamine mutase	up-regulated in acne patients
hypothetical protein PPA1121	up-regulated in acne patients
ATP-dependent Clp protease proteolytic subunit	up-regulated in acne patients
hypothetical membrane-spanning protein	up-regulated in acne patients
putative PRC-barrel domain protein	up-regulated in acne patients
lysine-specific permease	up-regulated in acne patients
putative transport protein	up-regulated in acne patients
ion channel membrane protein	up-regulated in acne patients
preprotein translocase subunit SecY	up-regulated in acne patients
preprotein translocase subunit SecF	up-regulated in acne patients
ribosomal protein L23	up-regulated in acne patients
ABC transporter, permease/ATP-binding protein	up-regulated in acne patients
DNA-directed RNA polymerase, beta subunit	up-regulated in acne patients
ribosomal protein S4	up-regulated in acne patients
glycine betaine/L-proline ABC transporter, permease protein	up-regulated in acne patients
DNA-binding response regulator TrcR	up-regulated in acne patients

conserved hypothetical protein	up-regulated in acne patients
hypothetical protein PPA1391	up-regulated in acne patients
arginine deiminase	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
putative polysaccharide deacetylase	up-regulated in acne patients
protein associated to putative adhesion protein	up-regulated in acne patients
substrate binding component of glycine/betaine transport system	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
putative glucoamylase S1/S2 precursor	up-regulated in acne patients
hypothetical protein HMPREF0675_3620	up-regulated in acne patients
putative membrane protein	up-regulated in acne patients
sugar transport permease BglB	up-regulated in acne patients
conserved domain protein	up-regulated in acne patients
hypothetical protein PPA1052	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
putative lipoprotein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
hypothetical protein HMPREF0675_5050	up-regulated in acne patients
ATP-dependent Clp protease proteolytic subunit 2	up-regulated in acne patients
carbamate kinase	up-regulated in acne patients
putative outer membrane protein probably involved in nutrient binding	up-regulated in acne patients
glucose-6-phosphate dehydrogenase	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
glycogen debranching enzyme GlgX	up-regulated in acne patients
binding-protein-dependent transport system inner membrane component	up-regulated in acne patients
hypothetical protein	up-regulated in acne patients
conserved hypothetical protein	up-regulated in acne patients
ornithine carbamoyltransferase	up-regulated in acne patients
putative lipase	up-regulated in acne patients
transporter, small conductance mechanosensitive ion channel (MscS) family protein	up-regulated in acne patients
hypothetical protein PPA0695	up-regulated in acne patients
undecaprenyl-diphosphatase UppP	up-regulated in acne patients
tRNA adenylyltransferase	up-regulated in acne patients
drug resistance transporter, EmrB/QacA subfamily	up-regulated in acne patients
HTH domain protein	up-regulated in acne patients
hypothetical protein PPA0150	up-regulated in acne patients
methylmalonyl-CoA carboxyltransferase 1	down-regulated in acne patients

fumarate hydratase	down-regulated in acne patients
acetyltransferase, GNAT family	down-regulated in acne patients
conserved hypothetical protein	down-regulated in acne patients
oxidoreductase, aldo/keto reductase family	down-regulated in acne patients
iron chelate uptake ABC transporter, FeCT family, permease protein	down-regulated in acne patients
hypothetical protein PPA0496	down-regulated in acne patients
RNA methyltransferase, RsmD family	down-regulated in acne patients
toxin-antitoxin system, antitoxin component, MerR family	down-regulated in acne patients
pyridoxal kinase	down-regulated in acne patients
cob(I)yrinic acid a,c-diamide adenosyltransferase	down-regulated in acne patients
hypothetical protein PPA1537	down-regulated in acne patients
aspartyl-tRNA synthetase	down-regulated in acne patients
hypothetical protein PPA1660	down-regulated in acne patients
putative bacterial extracellular solute-binding protein	down-regulated in acne patients
putative bacteriochlorophyll 4-vinyl reductase	down-regulated in acne patients
ABC transporter, ATP-binding protein	down-regulated in acne patients
putative NADH-dependent dehydrogenase	down-regulated in acne patients
cobalt ABC transporter, permease protein CbiQ	down-regulated in acne patients
putative membrane protein	down-regulated in acne patients
hypothetical protein PPA0745	down-regulated in acne patients
ATPase related to phosphate starvation-inducible protein, PhoH family	down-regulated in acne patients
deoxycytidylate deaminase (dCMP deaminase)	down-regulated in acne patients
glutamate 5-kinase	down-regulated in acne patients
hypothetical protein PPA0478	down-regulated in acne patients
putative transcriptional regulator, LacI family	down-regulated in acne patients
HAD-superfamily hydrolase, subfamily IIB	down-regulated in acne patients

Table S3. Full names of the genes and substrates shown in Fig. 2.

Abbreviation	Full name
2-OG	2-Oxoglutarate
3P-D-gly	3-Phospho-D-glyceroyl phosphate
5-ALA	5-Aminolevulinate
Acetyl-acp	Acetyl-[acyl-carrier protein]
AMT	aminomethyltransferase [EC:2.1.2.10]
b-D-Fru-1,6P	beta-D-Fructose 1,6-bisphosphate
b-D-Fru-6P	beta-D-Fructose 6-phosphate
bmpA	basic membrane protein A
cbiB, cobD	adenosylcobinamide-phosphate synthase [EC:6.3.1.10]
cbiM	cobalt/nickel transport system permease protein
cbiN	cobalt transport protein
cbiO	cobalt/nickel transport system ATP-binding protein
cbiQ	cobalt/nickel transport system permease protein
clp1	ATP-dependent Clp protease proteolytic subunit [EC:3.4.21.92]
clp2	ATP-dependent Clp protease proteolytic subunit [EC:3.4.21.92]
clpX	ATP-dependent protease ATP-binding subunit ClpX
Co^{2+}	Cobalt ion
cobA-hemD	uroporphyrinogen III methyltransferase / synthase [EC:2.1.1.107 4.2.1.75]
cobB-cbiA	cobyrinic acid a,c-diamide synthase [EC:6.3.5.9 6.3.5.11]
cobH, cbiC	precorrin-8X methylmutase [EC:5.4.1.2]
cobI-cbiL	precorrin-2/cobalt-factor-2 C20-methyltransferase [EC:2.1.1.130 2.1.1.151]
cobK, cbiJ	precorrin-6X reductase [EC:1.3.1.54]
cobM, cbiF	precorrin-4 C11-methyltransferase [EC:2.1.1.133]
cobO, btuR	cob(I)alamin adenosyltransferase [EC:2.5.1.17]
cobQ, cbiP	adenosylcobyric acid synthase [EC:6.3.5.10]
cobS, cobV	adenosylcobinamide-GDP ribazoletransferase [EC:2.7.8.26]
COX15	cytochrome c oxidase assembly protein subunit 15
CS	citrate synthase [EC:2.3.3.1]
cyoE	protoheme IX farnesyltransferase [EC:2.5.1.-]
cysG	uroporphyrin-III C-methyltransferase [EC:2.1.1.107 1.3.1.76 4.99.1.4]
cysG+cbiX	fusion gene of cysG and cbiX, cobalamin synthesis protein
DLST	2-oxoglutarate dehydrogenase E2 component [EC:2.3.1.61]
E1.7.2.1	nitrite reductase (NO-forming) [EC:1.7.2.1]
FA	Fatty acid
fabD	[acyl-carrier-protein] S-malonyltransferase [EC:2.3.1.39]

fabF	3-oxoacyl-[acyl-carrier-protein] synthase II [EC:2.3.1.179]
fabG	3-oxoacyl-[acyl-carrier protein] reductase [EC:1.1.1.100]
fabZ	(3R)-hydroxymyristoyl-[acyl-carrier-protein] dehydratase [EC:4.2.1.59]
FhuB	ABC-type iron transporter, permease protein
FhuC	ABC-type iron transporter; ATP-binding protein [EC:3.6.3.34]
FhuD	ABC-type iron transporter, substrate-binding protein
ftsY	fused signal recognition particle receptor
fumC	fumarate hydratase, class II [EC:4.2.1.2]
GAPDH	glyceraldehyde 3-phosphate dehydrogenase [EC:1.2.1.12]
gdhA	glutamate dehydrogenase (NADP+) [EC:1.4.1.4]
glpK	glycerol kinase [EC:2.7.1.30]
Gly-3P	D-Glyceraldehyde 3-phosphate
gudB, rocG	glutamate dehydrogenase [EC:1.4.1.2]
hemA	glutamyl-tRNA reductase [EC:1.2.1.70]
hemB	porphobilinogen synthase [EC:4.2.1.24]
hemC	hydroxymethylbilane synthase [EC:2.5.1.61]
hemD	uroporphyrinogen-III synthase [EC:4.2.1.75]
hemE	uroporphyrinogen decarboxylase [EC:4.1.1.37]
hemH	ferrochelatase [EC:4.99.1.1]
hemL	glutamate-1-semialdehyde 2,1-aminomutase [EC:5.4.3.8]
hemN	oxygen-independent coproporphyrinogen III oxidase [EC:1.3.99.22]
hemY	oxygen-dependent protoporphyrinogen oxidase [EC:1.3.3.4]
HmuT	ABC-type iron transporter, substrate-binding protein
HmuU	ABC-type iron transporter, permease component
HmuV	ABC-type iron transporter; ATP-binding protein [EC:3.6.3.34]
HtaA	HtaA, for Fe transport, (PPA0779)
IDH1	isocitrate dehydrogenase [EC:1.1.1.42]
Malonyl-acp	Malonyl-[acyl-carrier protein]
manA	mannose-6-phosphate isomerase [EC:5.3.1.8]
MDT1	multidrug transporter
MDT2	multidrug transporter
MDT3	multidrug transporter
MDT4	multidrug transporter
MFS ST1	the major facilitator superfamily (MFS) sugar transporter
MFS ST2	the major facilitator superfamily (MFS) sugar transporter
N_2	Nitrogen
N_2O	Nitrous oxide
narG	nitrate reductase, alpha subunit [EC:1.7.99.4]

narH	nitrate reductase, beta subunit [EC:1.7.99.4]
narI	nitrate reductase, gamma subunit [EC:1.7.99.4]
narJ	nitrate reductase, delta subunit
NH_3	Ammonia
NO	Nitrogen monoxide
NO_2	Nitrite
NO_3	Nitrate
norB	nitric oxide reductase subunit B [EC:1.7.2.5]
OGDH	2-oxoglutarate dehydrogenase E1 component [EC:1.2.4.2]
PFK	6-phosphofructokinase [EC:2.7.1.11]
PP	Protoporphyrin; Protoporphyrin IX
PTS-Mtl-EIIA	PTS system, mannitol-specific IIA component
PTS-Mtl-EIIABC	PTS system, mannitol-specific IIABC component
PTS-Nag-EI	PTS system, N-acetylglucosamine-specific IIB component
secA	preprotein translocase subunit SecA
secD	preprotein translocase subunit SecD
secE	preprotein translocase subunit SecE
secF	preprotein translocase subunit SecF
secG	preprotein translocase subunit SecG
secY	preprotein translocase subunit SecY
SRP54, ffh	signal recognition particle subunit SRP54
ST1P	ABC-type sugar transporter, permease protein
ST1P1	ABC-type sugar transporter, permease protein
ST1S	ABC-type sugar transporter, substrate-binding protein
ST2A	ABC-type sugar transporter, ATP-binding protein
ST2P	ABC-type sugar transporter, permease protein
ST2P1	ABC-type sugar transporter, permease protein
ST2S	ABC-type sugar transporter, substrate-binding protein
ST3P	ABC-type sugar transporter, permease protein
ST3P1	ABC-type sugar transporter, permease protein
ST3S	ABC-type sugar transporter, substrate-binding protein
ST4P	ABC-type sugar transporter, permease protein
ST4P1	ABC-type sugar transporter, permease protein
ST4S	ABC-type sugar transporter, substrate-binding protein
ST5P	ABC-type sugar transporter, permease protein
ST5P1	ABC-type sugar transporter, permease protein
ST6P	ABC-type sugar transporter, permease protein

ST6P1	ABC-type sugar transporter, permease protein
ST6S	ABC-type sugar transporter, substrate-binding protein
ST7A	ABC-type sugar transporter, ATP-binding protein
ST7P	ABC-type sugar transporter, permease protein
T2SE	type II/IV secretion system protein (PPA0041)
T2SF1	type II secretion system F domain protein (PPA0042)
T2SF2	type II secretion system F domain protein (PPA0043)
TAG	Triacylglycerol
talAB	transaldolase [EC:2.2.1.2]
TGL	triacylglycerol lipase [EC:3.1.1.3]
UPGIII	Uroporphyrinogen III
yajC	preprotein translocase subunit YajC
yidC	preprotein translocase subunit YidC

Table S4. The relative expression levels (log_{10}) of the vitamin B12 biosynthesis genes in the skin microbiota of the healthy individuals and acne patients shown in Fig. 3.

Subject ID	Skin phenotype	Relative gene expression (log_{10})		
		cysG+cbiX	***cbiL***	***btuR***
HL138	Healthy	-4.23	-4.00	-4.66
HL402	Healthy	-4.40	-4.09	-5.05
HL403	Healthy	-4.25	-4.06	-5.23
HL407	Healthy	-4.05	-4.26	-5.23
HL413	Healthy	-3.82	-3.35	NA
HL414	Healthy	-3.93	-3.93	-5.04
HL415	Healthy	-4.17	-4.14	-4.86
HL417	Healthy	-4.15	-4.18	-5.10
HL418	Healthy	-3.55	-3.49	-4.93
HL421	Healthy	-4.67	-4.34	-5.45
HL422	Healthy	-4.01	-4.00	-4.94
HL423	Healthy	-4.21	-4.15	-5.31
HL424	Healthy	-3.07	-2.80	-4.39
HL430	Healthy	-4.08	-3.96	-4.94
HL431	Healthy	-4.03	-4.07	-4.78
HL126	Acne	-5.34	-5.06	-5.49
HL401	Acne	-4.53	-4.42	-5.48
HL404	Acne	-4.68	-4.34	-5.00
HL409	Acne	-4.60	-4.46	-5.17
HL426	Acne	-3.97	-3.87	-4.81
HL427	Acne	-4.34	-4.35	-4.88
HL428	Acne	-4.70	-4.31	-5.01
HL429	Acne	-4.66	-4.39	-5.44
HL437	Acne	-4.48	-4.24	-5.12

NA: the qRT-PCR data not available.

Table S5A. The relative expression levels (log_{10}) of the vitamin B12 biosynthesis genes in the skin microbiota of the healthy subjects shown in Fig. 4.

Gene name	Subject ID	Skin phenotype	Relative gene expression (log_{10})					
			With vitamin B12			Without vitamin B12		
			Day0	Day2	Day14	Day0	Day2	Day14
cysG+cbiX	HL413	Healthy	-3.82	-3.31	-4.42	-3.53	-3.27	-3.54
	HL414	Healthy	-3.93	-3.75	-4.59	-3.43	-3.16	-3.48
	HL415	Healthy	-4.17	-4.18	-4.56	-4.62	-3.42	-3.34
	HL417	Healthy	-4.15	-3.77	-4.53	-3.38	-3.53	-3.35
	HL418	Healthy	-3.55	-3.57	-4.57	-3.67	-4.72	-3.23
	HL422	Healthy	-4.01	-4.10	-4.59	-2.91	-2.95	-3.85
	HL423	Healthy	-4.21	-4.08	-4.79	-3.53	-3.20	NA
	HL424	Healthy	-3.07	-3.22	-4.45	NA	NA	NA
	HL430	Healthy	-4.08	-4.49	-4.49	-3.70	-4.12	-3.02
	HL431	Healthy	-4.03	-3.87	-4.62	-3.06	-3.04	-4.25
cbiL	HL413	Healthy	-3.35	-3.01	-4.31	-3.45	-3.27	NA
	HL414	Healthy	-3.93	-3.92	-4.62	-3.31	-2.65	-3.23
	HL415	Healthy	-4.14	-4.17	-4.55	-4.92	-3.15	-3.85
	HL417	Healthy	-4.18	-3.52	-4.65	-3.60	-3.72	-3.36
	HL418	Healthy	-3.49	-3.53	-4.46	-3.86	-4.81	-3.24
	HL422	Healthy	-4.00	-4.04	-4.56	-3.28	-3.15	-3.90
	HL423	Healthy	-4.15	-3.91	-4.61	-3.70	NA	-3.72
	HL424	Healthy	-2.80	-2.73	-4.37	NA	NA	NA
	HL430	Healthy	-3.96	-4.38	-4.34	-3.91	-4.33	-3.08
	HL431	Healthy	-4.07	-3.77	-4.55	-3.20	-3.31	-4.57
btuR	HL413	Healthy	NA	-3.67	-5.43	-4.50	-4.35	-3.91
	HL414	Healthy	-5.04	-4.68	-5.28	-4.67	-2.32	-5.55
	HL415	Healthy	-4.86	-5.35	-5.74	-5.85	-4.16	-4.65
	HL417	Healthy	-5.10	-4.81	-5.48	-4.21	-4.66	-4.73
	HL418	Healthy	-4.93	-4.69	-5.41	-4.62	NA	-4.59
	HL422	Healthy	-4.94	-5.06	-5.51	NA	-4.37	-4.98
	HL423	Healthy	-5.31	-5.01	-5.72	-4.52	NA	-4.09
	HL424	Healthy	-4.39	-4.71	-5.53	NA	NA	NA
	HL430	Healthy	-4.94	-5.42	-5.18	-4.64	-4.19	-4.28
	HL431	Healthy	-4.78	-4.94	-5.34	-4.39	-4.58	-4.33

NA: sample or data not available.

Table S5B. The relative expression levels (log_{10}) of the vitamin B12 biosynthesis genes in the skin microbiota of the acne patients shown in Fig. 4.

Gene name	Subject ID	Skin phenotype	Relative gene expression (log_{10})
cysG+cbiX	HL401	Acne	-4.53
	HL426	Acne	-3.97
	HL427	Acne	-4.34
	HL428	Acne	-4.70
	HL429	Acne	-4.66
	HL437	Acne	-4.48
	HL404	Acne	-4.68
	HL409	Acne	-4.60
	HL126	Acne	-5.34
cbiL	HL401	Acne	-4.42
	HL426	Acne	-3.87
	HL427	Acne	-4.35
	HL428	Acne	-4.31
	HL429	Acne	-4.39
	HL437	Acne	-4.24
	HL404	Acne	-4.34
	HL409	Acne	-4.46
	HL126	Acne	-5.06
btuR	HL401	Acne	-5.48
	HL426	Acne	-4.81
	HL427	Acne	-4.88
	HL428	Acne	-5.01
	HL429	Acne	-5.44
	HL437	Acne	-5.12
	HL404	Acne	-5.00
	HL409	Acne	-5.17
	HL126	Acne	-5.49

Table S6. The differentially expressed OGUs in both the comparison between sample HL414-Day14 and other Day14 samples and the comparison between Day0 and Day14 samples in the vitamin B12 supplementation study.

OGU[§]	Annotation	HL414-Day14 *vs.* other Day14 samples			Day0 *vs.* Day14 samples	
		P	**FDR**	**Log_2FC**	***P***	**Log_2FC**
PPA0693	2-oxoglutarate dehydrogenase, E2 component	5.0E-03	3.0E-02	-0.3	4.0E-02	-0.2
PPA2220	putative PRC-barrel domain protein	2.0E-06	5.0E-04	1.1	2.0E-02	0.3
PPA1377	putative ATP-dependent RNA helicase	1.0E-03	1.0E-02	0.5	4.0E-02	0.3
PPA0563	conserved hypothetical protein	2.0E-03	2.0E-02	0.3	3.0E-02	0.3
PPA0480	putative hypoxanthine/guanine permease	2.0E-03	2.0E-02	-0.4	4.0E-02	-0.1
PPA0329	RNA polymerase sigma factor, sigma-70 family	4.0E-03	3.0E-02	0.3	1.0E-02	0.2
PPA1174	conserved hypothetical protein	1.0E-02	5.0E-02	-0.2	7.0E-03	-0.5
PPA0054	ABC transporter, ATP-binding protein	5.0E-04	7.0E-03	0.6	3.0E-02	0.3
PPA2245	zinc-binding alcohol dehydrogenase family protein	1.0E-04	4.0E-03	0.4	3.0E-02	0.2
PPA0937	putative metalloprotease	7.0E-04	9.0E-03	-0.7	1.0E-02	-0.3
PPA1865	ribosomal protein S10	4.0E-03	3.0E-02	0.4	4.0E-02	0.3

FDR: false discovery rate; Log_2FC: log_2 transformed fold change.

[§]The gene from the *P. acnes* KPA171202 genome is used here to represent the OGU.

Table S7. The relative expression levels (log_{10}) of the vitamin B12 biosynthesis genes in the *P. acnes* cultures shown in Fig. 6A.

Gene name	Relative gene expression (log_{10})					
	Medium			Medium + 10 μg/mL vitamin B12		
	Day 2	Day 8	Day 14	Day 2	Day 8	Day 14
cysG+cbiX	-4.06	-4.50	-5.15	-4.33	-5.24	-5.96
	-3.85	-4.63	-5.15	-4.08	-5.07	-5.58
	-3.36	-4.45	-5.55	-3.85	-4.77	-6.43
cbiL	-4.23	-4.77	-5.19	-4.63	-5.40	-5.79
	-3.64	-3.99	-4.39	-4.18	-4.72	-5.18
	-3.43	-3.74	-4.95	-3.91	-3.97	-5.60
btuR	-4.51	-5.00	-5.42	-4.65	-5.46	-5.91
	-3.84	-4.74	-5.23	-4.03	-5.02	-5.90
	NA	-3.81	-5.08	NA	-4.14	-5.83

NA: qRT-PCR data not available.

Table S8. The porphyrin levels detected in the *P. acnes* cultures shown in Fig. 6B.

Replicate	Porphyrin level normalized by bacterial culture density (μM)					
	Medium			Medium + 10 μg/mL vitamin B12		
	Day 2	Day 8	Day 14	Day 2	Day 8	Day 14
1	-0.67	9.80	9.90	-0.30	11.97	11.18
2	0.03	9.31	9.14	-0.26	12.21	11.34
3	-0.44	8.44	9.55	-0.91	12.51	11.50
4	NA	7.64	8.97	NA	12.20	10.71

NA: data not available.