Material and methods

1. RNA sequencing and analysis

RNA qualification, library preparation, sequencing, quality control, read mapping to the reference genome, and expression analysis were performed by BGI Genomics (Wuhan, China). In brief, the total RNAs from the mouse skin tissues were extracted with TRIzol reagent (Sigma-Aldrich, USA) according to the manufacturer' s instructions. The RNA samples were denatured at the appropriate temperature to reveal its secondary structure, and oligo(dT) magnetic beads were used to enrich the mRNA. Add fragmentation reagents to the mRNA to fragment the mRNA. Then one-strand and two-strand cDNA were synthesized, and the ends of the double-stranded cDNAs were repaired. and a single "A" nucleotide is added to the 3' end to connect the adapter to the cDNA, then the product is amplified. After denaturing the PCR product into a single-stranded product, prepare a cyclization reaction system to obtain a single-stranded circular product, and digest the uncirculated linear DNA molecules. Single-stranded circular DNA molecules replicate through rolling circles to form a DNA nanoball (DNB) containing multiple copies. DNBs were added to the mesh holes on the chip using high-density DNA nanochip technology and sequenced through combined probe-anchored polymerization technology (cPAS). Filter the raw data using SOAPnuke to obtain clean data. Use HISAT2 to align the clean data to the reference genome. Use Bowtie2 to align the clean data to the reference gene set. The expression levels of genes were calculated by RSEM. For immune infiltration analysis, relative levels of different immune cell types in WT mice and OPN KO mice were quantified by using the CIBERSORT algorithm (https://cibersort.stanford.edu).

Table S1

Target gene	Forward primers	Reverse primers
Human OPN	GCTGATTCTGGAAGTTCTGAGGA	GGACTTACTTGGAAGGGTCTCT
Human sOPN	atttccggtgaattcctcgagATGAGAATTGCAGTGATTTGCTTT	ggagggagaggggcgggatccTTAATTGACCTCAGAAGATGCACTATC
Human iOPN	atttccggtgaattcctcgagATGATACCAGTTAAACAGGCTGATTCTGG	ggagggagaggggcgggatccTTAATTGACCTCAGAAGATGCACTATC
Human IL6	ACTCACCTCTTCAGAACGAATTG	CCATCTTTGGAAGGTTCAGGTTG
Human Actin	CACCATTGGCAATGAGCGGTTC	AGGTCTTTGCGGATGTCCACGT
Human CCL2	AGAATCACCAGCAGCAAGTGTCC	TCCTGAACCCACTTCTGCTTGG
Human IL1B	ATGATGGCTTATTACAGTGGCAA	GTCGGAGATTCGTAGCTGGA
Human TNFa	CTCTTCTGCCTGCTGCACTTTG	ATGGGCTACAGGCTTGTCACTC
Mouse IL6	TACCACTTCACAAGTCGGAGGC	CTGCAAGTGCATCATCGTTGTTC
Mouse IL1B	TGGACCTTCCAGGATGAGGACA	GTTCATCTCGGAGCCTGTAGTG
Mouse TNFa	GGTGCCTATGTCTCAGCCTCTT	GCCATAGAACTGATGAGAGGGAG
Mouse NLRP3	TCACAACTCGCCCAAGGAGGAA	AAGAGACCACGGCAGAAGCTAG
Mouse Actin	CATTGCTGACAGGATGCAGAAGG	TGCTGGAAGGTGGACAGTGAGG
Mouse OPN	GCTTGGCTTATGGACTGAGGTC	CCTTAGACTCACCGCTCTTCATG

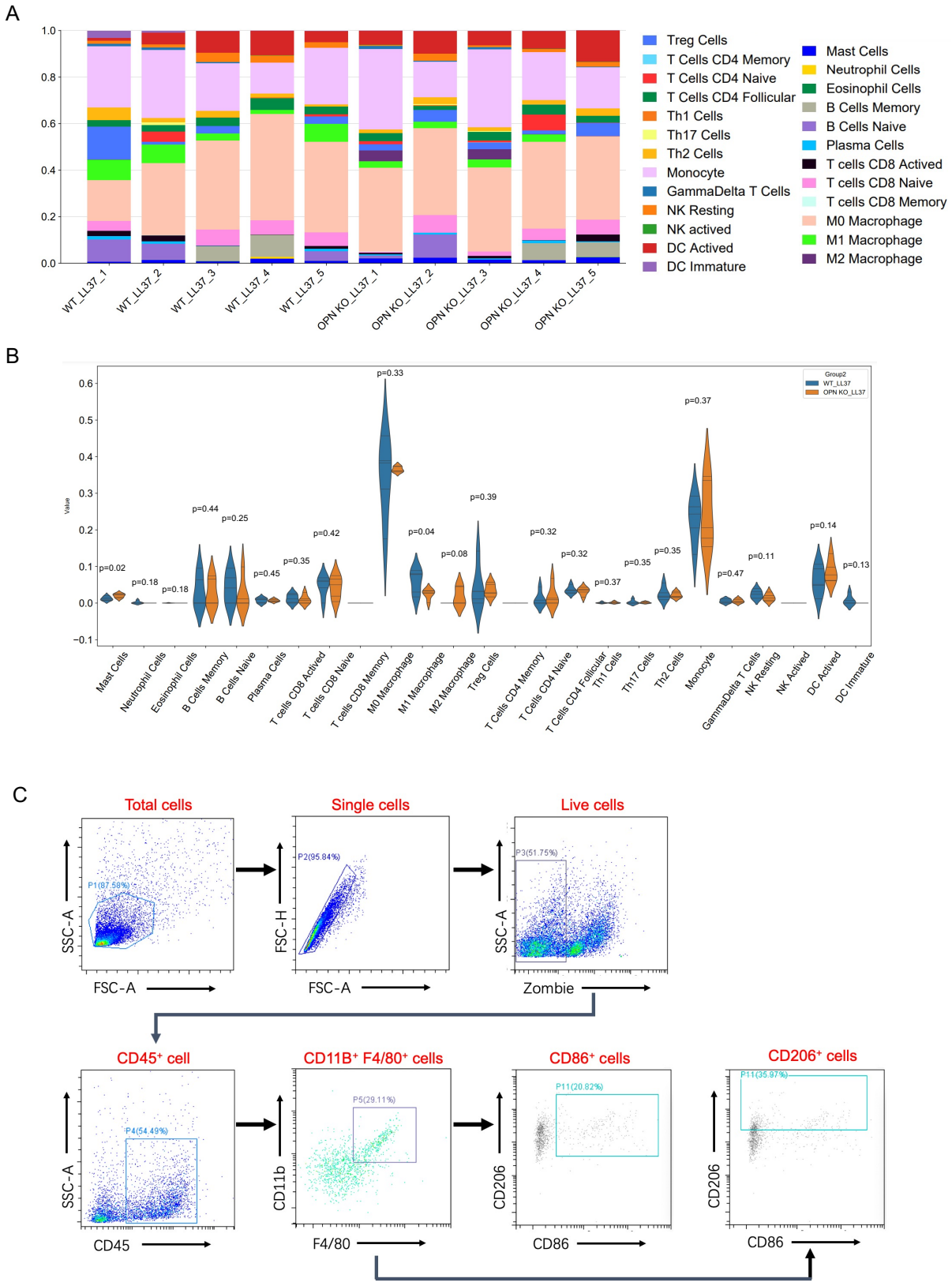

Figure S1

(A) Analysis of immune cells infiltration using CIBERSORT in skin lesions from WT mice and OPN KO mice injected with PBS or LL37 (n = 5 for each group). Numbers represent the degree of enrichment in cells. (B) Statistical analysis of immune cells infiltration in WT-LL37 group (blue bar) and OPN KO-LL37 group (yellow bar) was shown in the violin plot. T-test was used for statistical analyses. (C) The flow cytometry of macrophage gating strategy in LL37-induced rosacea lesions.