Supplementary Material

Myeloid cell-derived LL-37 promotes lung cancer growth by activating Wnt/β-catenin signaling

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Supplementary Table

Table S1 Primer used for qRT-PCR

qRT-PCR primers	forward (5'-3')	reverse (5'-3')
Gapdh	AAGAAGGTGGTGAAGCAG	TCATACCAGGAAATGAGC
Cdkn1a	AAAACGGAGGCAGACCAG	GCTAAGGCCGAAGATGGG
Axin2	GCTCCAGAAGATCACAAAGAGC	AGCTTTGAGCCTTCAGCATC
c-myc	GCTGTTTGAAGGCTGGATTTC	GATGAAATAGGGCTGTACGGAG
Ccnd1	GCCATCCAAACTGAGGAAAA	GATCCTGGGAGTCATCGGTA
Gsk3b	TCCGAGGAGAGCCCAATGTTTCAT	TGGACGTGTAATCAGTGGCTCCAA
Wnt3	GCCGACTTCGGGGTGCTGGT	CTTGAAGAGCGCGTACTTAG
AKT	TAAAGAAGGAGGTCATCGTGG	CGGGACAGGTGGAAGAAAA
LEF1	GCCACCGATGAGATGATCCC	TTGATGTCGGCTAAGTCGCC
TCF1	GACCTGACCGAGTTGCCTAAT	GCG-AAGTCTTCCCCATCGTC
TLR2	GCCTACTGGGTGGAGAACCTT	CCAGTTCATACTTGCACCACTC
TLR4	GATTAGCATACTTAGACTACTACC TCCATG	GATCAACTTCTGAAAAAGCATTCCC AC
TTP	TCCACAACCCTAGCGAAGAC	GAGAAGGCAGAGGGTGACAG
mouse β-actin	AGCCTCGCCTTTGCCGA	CTGGTGCCTGGGGCG
mouse CRAMP	AATTTTCTTGAACCGAAAGGGC	TGTTTTCTCAGATCCTTGGGAGC

Supplementary Figure

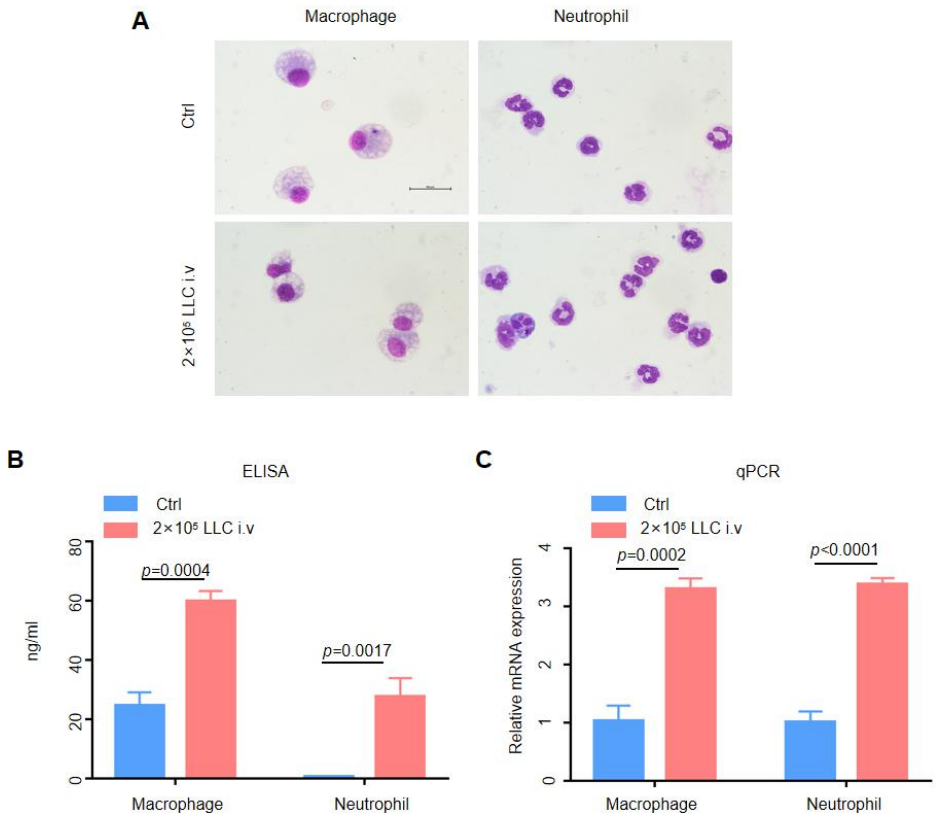

Figure S1. (A) Wright stain of the macrophages and neutrophils isolated from lung tissues of mice with or without 2×10^5 LLC cells inoculation. Scale bar, 500 μm. (B) Freshly isolated macrophages or neutrophils (1×10^6) from lung tissues of mice with or without 2×10^5 LLC cells inoculation were incubated in serum-free DMEM for 24h. After incubation, LL-37 was measured by ELISA Supernatants were collected and assessed for LL-37 production by ELISA. (C) Cells were pelleted, lysed, and mRNA measured by quantitative qRT-PCR.

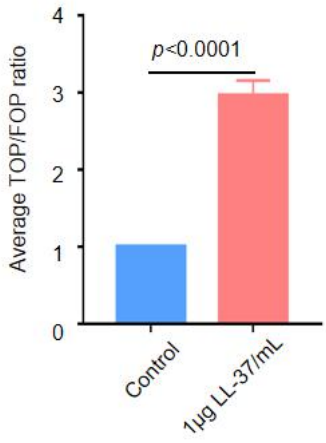

Figure S2. LL-37 increased β-catenin-mediated transcriptional activity. A549 cells were co-transfected with a TCF/LEF reporter (TOPFlash) or flash reporter or Renilla luciferase (pRL) plasmids. At 24 h post-transfection, A549 cells were serum starved (2%) for 12 h; subsequently, Wnt/β-catenin signaling was stimulated with LL-37 (1 ug/ml) for a 12 h period. After incubation, cells were washed and lysed, and luciferase activity was assayed. The activity was normalized with respect to the activity of the Renilla luciferase.

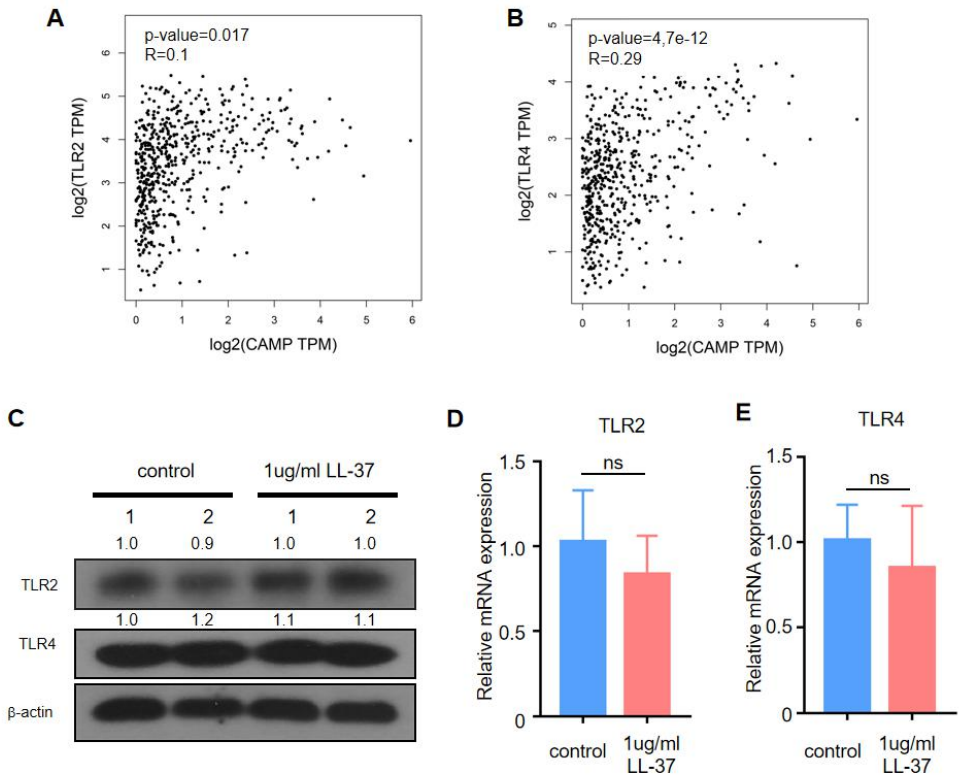

Figure S3. Correlation of mRNA level of CAMP (LL-37) with TLR2 (A) or TLR4 (B) in NSCLC in the TCGA and CGGA databases (Pearson correlation analysis). (C) Western blot analysis of TLR2, TLR4, and β-actin in A549 cells after indicated treatments. (D) qRT-PCR analysis of TLR2 and TLR4 in A549 cells after indicated treatments.

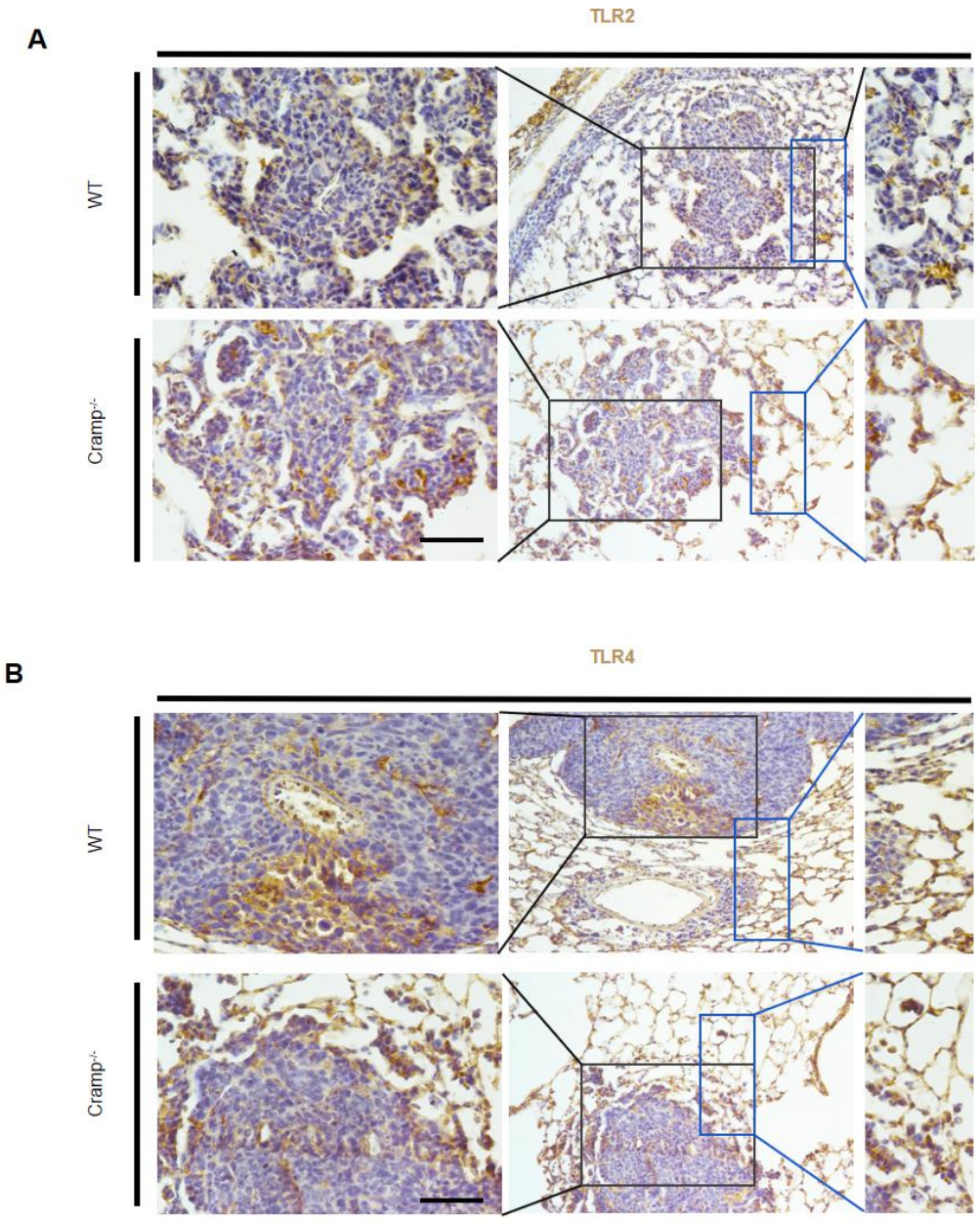

Figure S4. Immunohistochemical analysis of TLR2 (A) and TLR4 (B) expression in wild-type (WT) and Camp$^{-/-}$ mice. Scale bar, 50 μm.

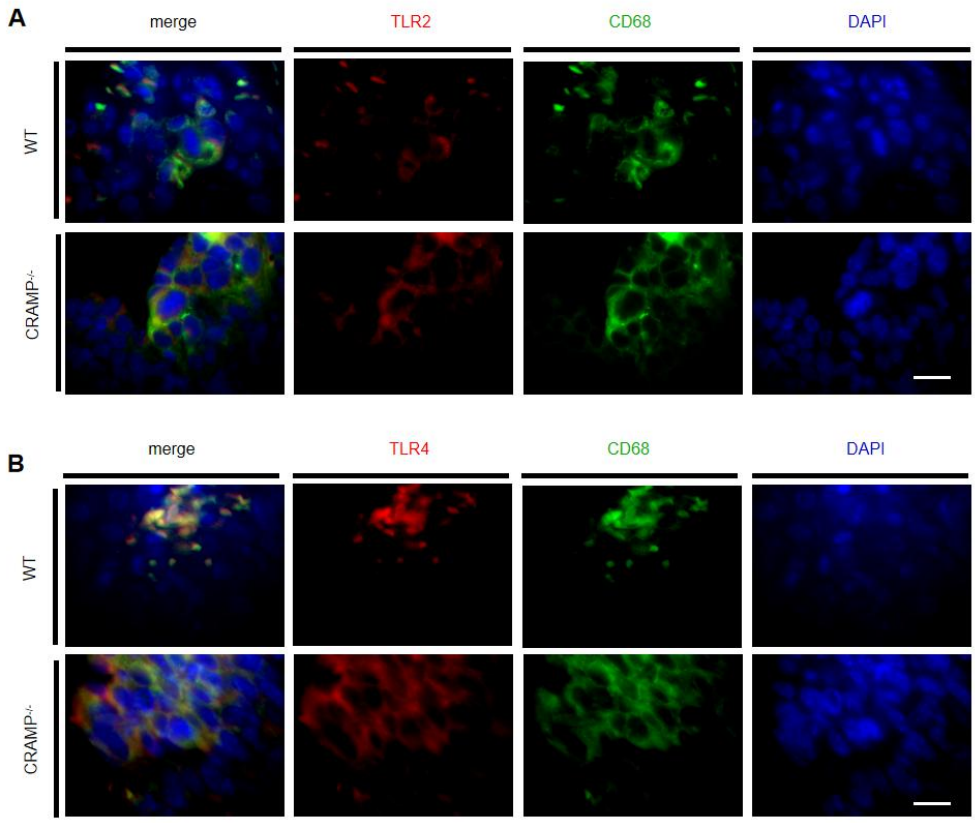

Figure S5. (A) Immunofluorescence analysis of TLR2 and CD68 (macrophage marker) expression in wild-type (WT) and Camp$^{-/-}$ mice. Scale bar, 50 μm. (B) Immunofluorescence analysis of TLR4 and CD68 (macrophage marker) expression in wild-type (WT) and Camp$^{-/-}$ mice. Scale bar, 50 μm.

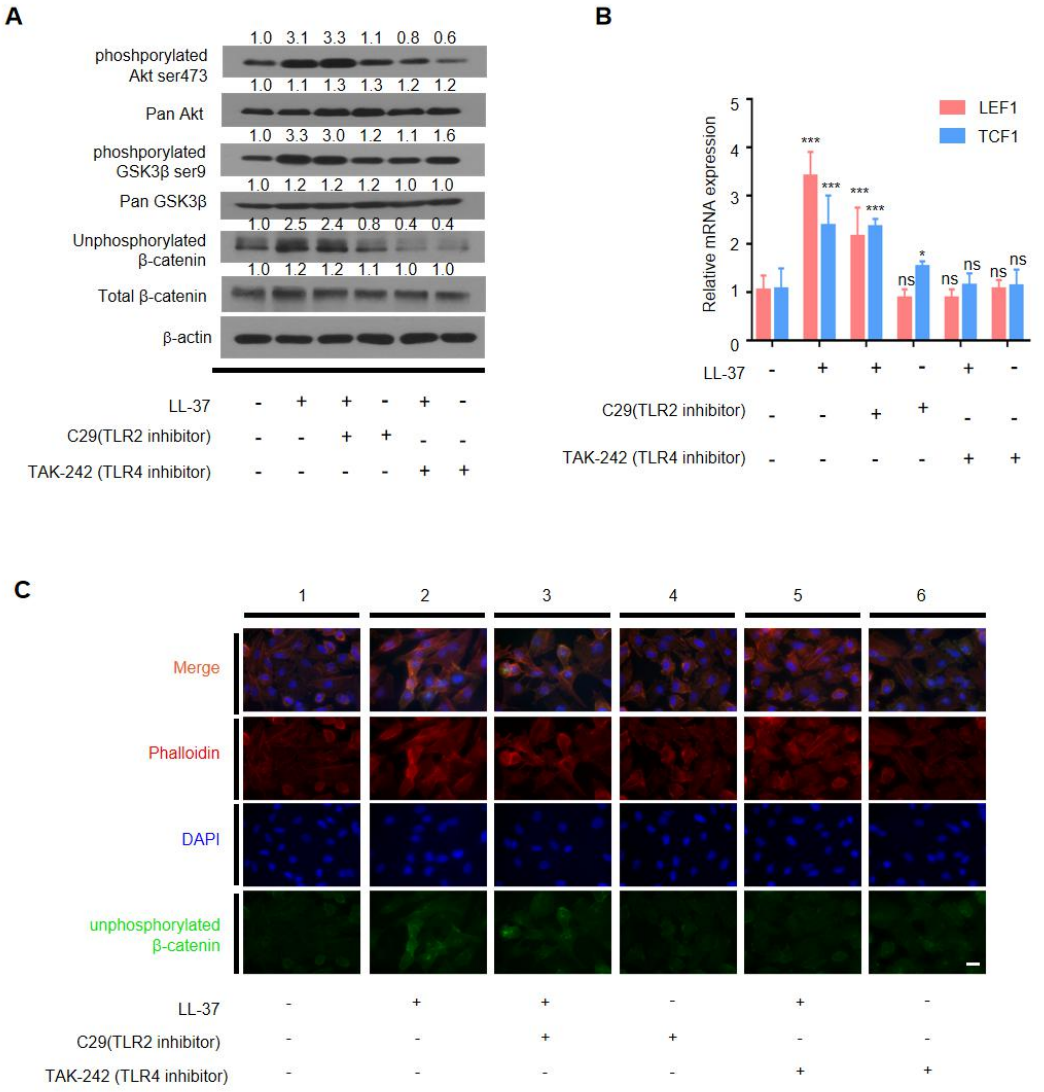

Figure S6. (A) Western blot analysis of unphosphorylated β-catenin, total β-catenin, and β-actin in A549 cells after indicated treatments. (B) qRT-PCR analysis of LEF1 and TCF1 in A549 cells after indicated treatments. (C) Immunofluorescent staining shows subcellular unphosphorylated β--catenin and actin (phalloidin) localization in A549 cells after indicated treatments. Scale bar, 20 μm.