SUPPLEMENTAL MATERIAL

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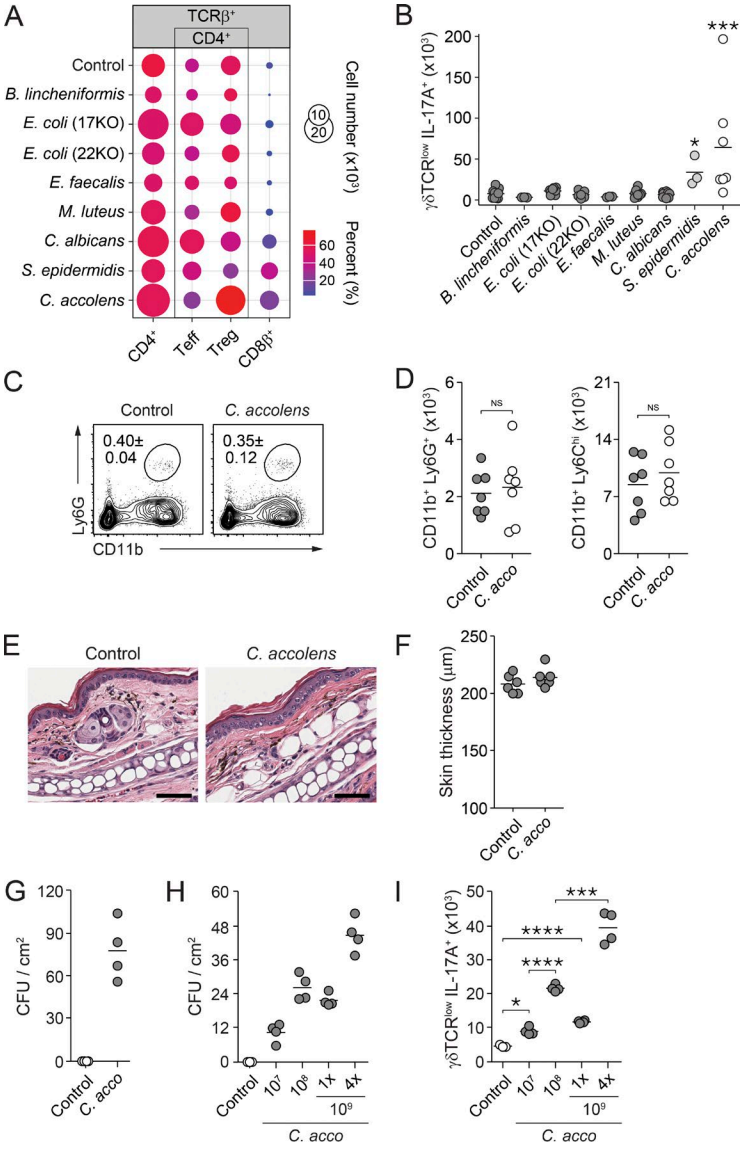

Figure S1. **Effects of topical association with *C. accolens* on the skin immune system. (A)** Mean of absolute numbers (represented by the size of the circles) and frequencies (represented by the colors of the circles) of various populations of immune cells isolated from the skin of mice previously associated or not with diverse skin commensal microbes: TCRβ$^+$ CD4$^+$, TCRβ$^+$ CD4$^+$ Foxp3$^+$ (Treg), TCRβ$^+$ CD4$^+$ Foxp3^{neg} (Teff), and TCRβ$^+$ CD8β$^+$. **(B)** Absolute number of skin CD45$^+$ CD90.2$^+$ γδ TCR^{low} cells producing IL-17A in mice 14 d after the first topical association with diverse skin commensal microbes. Data were collected after in vitro restimulation with PMA and ionomycin in the presence of BFA. *, P < 0.05; ***, P < 0.001 (one-way ANOVA with Holm-Šídák's multiple comparison test). Results shown in A and B are representative of three independent experiments with three to nine animals per group. **(C)** Frequencies (mean ± SEM) and absolute numbers of CD45$^+$ CD11b$^+$ Ly6G$^+$ neutrophils in the skin of mice 14 d after the first topical association with *C. accolens*. Control mice were left unassociated. **(D)** Absolute numbers of neutrophils (CD45$^+$ CD11b$^+$ Ly6G$^+$) and Ly6C^{hi} monocytes (CD45$^+$ CD11b$^+$ Ly6G^{neg} Ly6C^{hi}) in the skin of unassociated and *C. accolens*–associated mice (14 d after the first topical association). **(E)** Representative histopathological comparison of the ear pinnae of unassociated and *C. accolens*–associated mice at day 14 after the first topical association. Bars, 50 µm. **(F)** Measurement of ear thickness in unassociated mice (n = 6) and *C. accolens*–associated mice (n = 6) 14 d after the initial association. Data shown in C–F are representative of three independent experiments. **(G)** Enumeration of CFUs per square centimeter from the ear pinnae of mice 18 h after topical application of *C. accolens* (*C. acco*, 10^9 CFU/ml). Control mice were left unassociated. Each dot represents an individual mouse. **(H and I)** Mice were topically associated with various doses of *C. accolens* (*C. acco*): 10^7 CFU/ml (four topical applications), 10^8 (four topical applications), or 10^9 CFU/ml (1×, one topical application; 4×, four topical applications). Control mice were not associated with *C. accolens*. (H) Enumeration of CFUs per square centimeter from the ear pinnae 2 wk after the first topical application with *C. accolens*. (I) Absolute numbers of CD45$^+$ CD90.2 γδ TCR^{low} cells producing IL-17A (upon PMA/Iono restimulation in presence of BFA) in the skin 2 wk after the first topical application with *C. accolens*. Each dot represents an individual mouse. In G–I, data shown are representative of two independent experiments with four mice per group (*, P < 0.05; ***, P < 0.001; ****, P < 0.0001 as calculated by two-tailed, unpaired Student's *t* test).

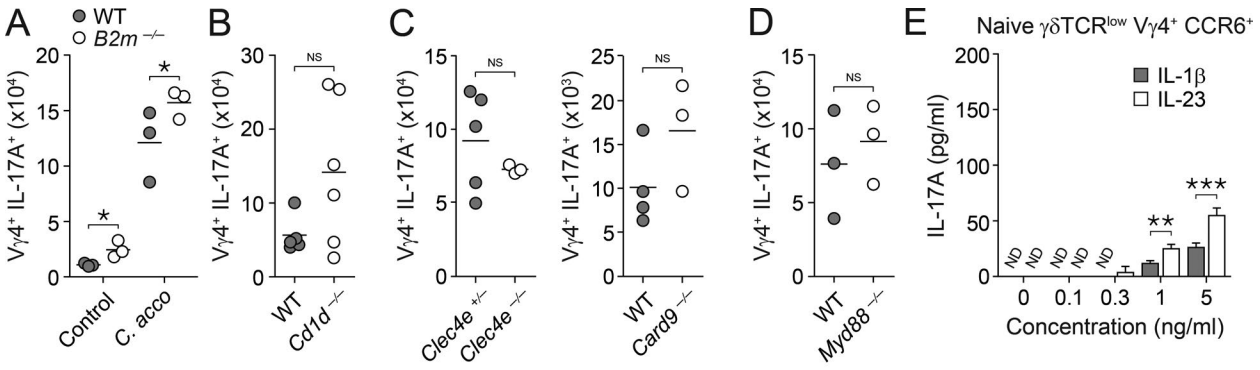

Figure S2. **The effect of *C. accolens* on γδ T17 cells does not require β2m, CD1d, MINCLE, CARD9, or MyD88.** Mice were topically associated with *C. accolens* (*C. acco*) every other day for a week, and the effect on CD45$^+$ CD90.2$^+$ γδ TCR^{low} cells was examined 14 d after the first topical association. Control mice were left unassociated. Absolute numbers of skin IL-17A–producing γδ TCR^{low} Vγ4$^+$ in associated C57BL/6 *B2m*$^{-/-}$ (*B2m*$^{-/-}$; A), C57BL/6 *Cd1d*$^{-/-}$ (*Cd1d*$^{-/-}$; B), *Clec4e*$^{-/-}$ (*Mincle*$^{-/-}$; C), C57BL/6 *Card9*$^{-/-}$ (*Card9*$^{-/-}$; C), or C57BL/6 *Myd88*$^{-/-}$ (*Myd88*$^{-/-}$; D) mice compared with associated littermate control (*Clec4e*$^{+/-}$) or WT C57BL/6 (WT) mice. *B2m*$^{-/-}$, *Card9*$^{-/-}$, and *Myd88*$^{-/-}$ were backcrossed to C57BL/6 for at least 10 generations. Data shown are representative of three independent experiments (three to six mice per group). *, $P < 0.05$; NS, not significant (two-tailed, unpaired Student's *t* test). **(E)** Concentration of IL-17 in the supernatant of overnight cultures of naive γδ TCR$^+$ Vγ4$^+$ CCR6$^+$ cells (purified from the skin and ear draining lymph nodes of unassociated mice) treated with various concentrations of recombinant mouse IL-1β or IL-23. Each bar graph represents the mean concentration (±SD) of triplicate cultures. ND, not detected. Data shown are representative of two independent experiments. **, $P < 0.01$; ***, $P < 0.001$ as calculated by two-tailed, unpaired Student's *t* test.

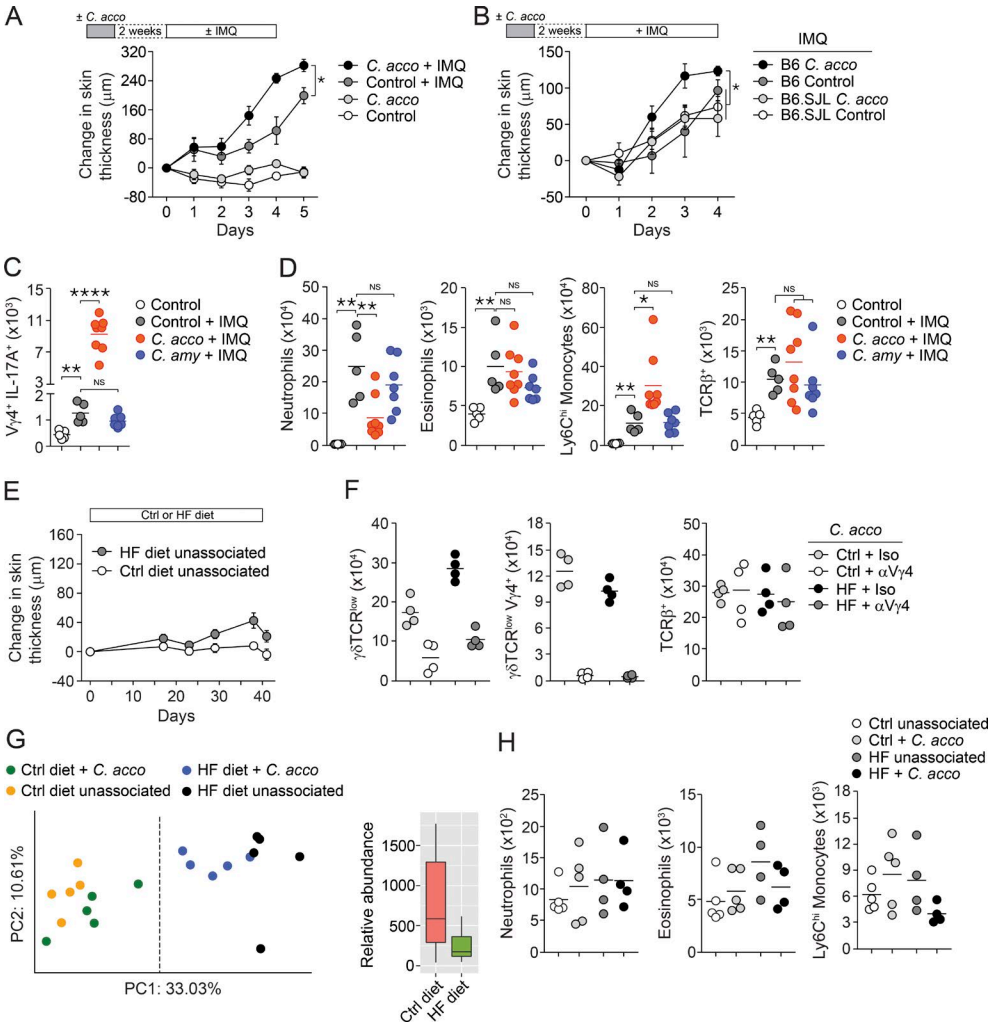

Figure S3. **Impact of *C. accolens* in mice treated with IMQ or high-fat diet. (A)** IMQ was applied daily on the ears of unassociated (Control) or *C. accolens*–associated (*C. acco*) C57BL/6 mice starting 2 wk after the first topical association. The daily ear-skin thickness measurement is reported as the change in ear-skin thickness (mean ± SEM) relative to baseline at day 0 (first day of IMQ application). **(B)** IMQ was applied daily on the ears of unassociated or *C. accolens*–associated C57BL/6 (B6) or B6.SJL mice starting 2 wk after the first topical association. The daily ear-skin thickness measurement is reported as the change in ear-skin thickness (mean ± SEM) relative to baseline at day 0 (first day of IMQ application). In A and B, data shown are representative of two to three independent experiments with three to five mice per group. *, $P < 0.05$ as calculated using two-way ANOVA with Holm-Šídák's correction for multiple hypothesis. **(C and D)** IMQ was applied daily on the ears of unassociated mice (Control), *C. accolens*–associated mice (*C. acco*), or *C. amycolatum*–associated mice (*C. amy*) starting 2 wk after the first topical association. (C) Absolute numbers of skin γδ TCR^{low} Vγ4$^+$ IL-17A$^+$ cells at day 5 after the start of IMQ application. (D) Absolute numbers of neutrophils (CD45$^+$ CD11b$^+$ Ly6G$^+$), eosinophils (CD45$^+$ Ly6G^{neg} MHCII^{neg} CD64^{neg} CD49b$^+$ Siglec F$^+$), Ly6C^{hi} monocytes (CD45$^+$ CD11b$^+$ Ly6G^{neg} Ly6C^{hi}), and TCRβ$^+$ T cells (CD45$^+$ CD90.2$^+$ TCRβ$^+$) in the skin at day 5 after the start of IMQ application. Results shown in C and D are representative of two independent experiments with five to eight mice per group. *, $P < 0.05$; **, $P < 0.01$; ****, $P < 0.0001$; NS, not significant as calculated by two-tailed, unpaired Student's *t* test. **(E)** 3-wk-old mice were placed given a control (Ctrl) or high-fat (HF) diet regiment and left unassociated. The ear-skin thickness measurement is reported as the change in ear-skin thickness (mean ± SEM) relative to baseline at day 0 (first day given the high-fat or control diet). Data are representative of three independent experiments with three to five mice per group. **(F–H)** 3-wk-old mice were fed either a high-fat (HF) or a control (Ctrl) diet for 1 mo before topical association with *C. accolens* (*C. acco*). (F) Absolute numbers of CD45$^+$ CD90.2$^+$ γδ TCR^{low}, CD45$^+$ CD90.2$^+$ γδ TCR^{low} Vγ4$^+$, and CD45$^+$ CD90.2$^+$ TCRβ$^+$ cells after treatment with anti-Vγ4 (αVγ4) or isotype control antibodies (Iso) for 14 d starting on the first day of *C. accolens* topical association. Results shown are representative of two independent experiments with four mice per group. (G) Principal Coordinate (PC) Analysis plot of unweighted UniFrac distance from samples collected from unassociated controls or *C. accolens*–associated animal fed either a control (Ctrl) or high-fat (HF) diet. Graph on the right illustrates the relative abundance (in number of reads) of green genes operational taxonomic unit 470219 assigned to species of *Corynebacterium* in associated animals fed a control of high-fat diet. Samples were rarefied to 5,000 reads per sample. (H) Absolute numbers of neutrophils (CD45$^+$ CD11b$^+$ Ly6G$^+$), eosinophils (CD45$^+$ Ly6G^{neg} MHCII^{neg} CD64^{neg} CD49b$^+$ Siglec F$^+$), Ly6C^{hi} monocytes (CD45$^+$ CD11b$^+$ Ly6G^{neg} Ly6C^{hi}), and TCRβ$^+$ T cells (CD45$^+$ CD90.2$^+$ TCRβ$^+$) in the skin of unassociated mice or 2 wk after association with *C. accolens*. In G and H, data shown are representative of three independent experiments with four to five mice per group.

Table S1. **Gene list of the custom NanoString CodeSet**

Gene	Accession number	Gene	Accession number	Gene	Accession number
5830411N06Rik	NM_001128145.1	*Gusb*	NM_010368.1	*Lgals3*	NM_001145953.1
Actb	NM_007393.1	*Gzma*	NM_010370.2	*Mrc1*	NM_008625.1
Ahr	NM_013464.4	*Gzmb*	NM_013542.2	*Myd88*	NM_010851.2
Alas1	NM_020559.2	*Hprt*	NM_013556.2	*Oaz1*	NM_008753.4
Areg	NM_009704.3	*Icos*	NM_017480.1	*Pdcd1*	NM_008798.1
Bcl2	NM_009741.3	*Ifng*	NM_008337.1	*Prdm1*	NM_007548.3
Bcl2l1	NM_009743.4	*Ikzf1*	NM_009578.2	*Prf1*	NM_011073.2
Bcl2l11	NM_001284410.1	*Ikzf2*	NM_011770.4	*Rora*	NM_013646.1
Bcl6	NM_009744.3	*Ikzf3*	NM_011771.1	*Rorc*	NM_011281.2
Card9	NM_001037747.1	*Ikzf4*	NM_011772.2	*Runx1*	NM_001111021.1
Ccl3	NM_011337.1	*Il10*	NM_010548.1	*Runx3*	NM_019732.2
Ccl4	NM_013652.1	*Il10ra*	NM_008348.2	*S1pr1*	NM_007901.4
Ccl5	NM_013653.1	*Il10rb*	NM_008349.5	*Sell*	NM_001164059.1
Ccr10	NM_007721.4	*Il11ra1*	NM_010549.3	*Sh2d1a*	NM_011364.3
Ccr2	NM_009915.2	*Il12rb1*	NM_008353.2	*Skint1*	NM_001102662.1
Ccr4	NM_009916.2	*Il12rb2*	NM_008354.3	*Slamf1*	NM_013730.4
Ccr5	NM_009917.5	*Il13*	NM_008355.2	*Sox13*	NM_011439.2
Ccr6	NM_001190333.1	*Il15ra*	NM_008358.1	*Stat1*	NM_009283.3
Ccr7	NM_007719.2	*Il17a*	NM_010552.3	*Stat3*	NM_213659.2
Ccr8	NM_007720.2	*Il17f*	NM_145856.2	*Stat4*	NM_011487.4
Ccr9	NM_009913.6	*Il17ra*	NM_008359.1	*Stat5a*	NM_011488.2
Cd27	NM_001042564.1	*Il17rb*	NM_019583.3	*Stat6*	NM_009284.2
Cd3e	NM_007648.4	*Il18r1*	NM_001161842.1	*Tbx21*	NM_019507.1
Cd4	NM_013488.2	*Il1r1*	NM_001123382.1	*Tgfbr1*	NM_009370.2
Cd40lg	NM_011616.2	*Il1r2*	NM_010555.4	*Tgfbr2*	NM_009371.2
Cd44	NM_009851.2	*Il1rap*	NM_134103.2	*Tgfbr3*	NM_011578.3
Cd69	NM_001033122.3	*Il1rl1*	NM_001025602.2	*Tlr1*	NM_030682.1
Cd8a	NM_001081110.2	*Il1rl2*	NM_133193.3	*Tlr11*	NM_205819.2
Cd8b1	NM_009858.2	*Il2*	NM_008366.2	*Tlr12*	NM_205823.2
Clec4d	NM_001163161.1	*Il21*	NM_021782.2	*Tlr13*	NM_205820.1
Clec4e	NM_019948.2	*Il21r*	NM_021887.1	*Tlr2*	NM_011905.2
Clec4n	NM_001190320.1	*Il22*	NM_016971.1	*Tlr3*	NM_126166.2
Clec7a	NM_020008.2	*Il23r*	NM_144548.1	*Tlr4*	NM_021297.2
Ctla4	NM_009843.3	*Il2ra*	NM_008367.2	*Tlr5*	NM_016928.2
Cxcl1	NM_008176.1	*Il2rb*	NM_008368.3	*Tlr6*	NM_011604.3
Cxcl10	NM_021274.1	*Il4*	NM_021283.1	*Tlr7*	NM_133211.3
Cxcr3	NM_009910.2	*Il4ra*	NM_001008700.3	*Tlr8*	NM_133212.2
Cxcr6	NM_030712.4	*Il5*	NM_010558.1	*Tlr9*	NM_031178.2
Ebi3	NM_015766.2	*Il6ra*	NM_010559.2	*Tnf*	NM_013693.1
Eef1g	NM_026007.4	*Il7r*	NM_008372.3	*Tnfrsf4*	NM_011659.2
Eomes	NM_010136.2	*Il9*	NM_008373.1	*Zbtb16*	NM_001033324.2
Foxp3	NM_054039.2	*Itgae*	NM_008399.1		
G6pdx	NM_008062.2	*Klf2*	NM_008452.2		
Gapdh	NM_008084.3	*Klrb1f*	NM_153094.2		
Gata3	NM_008091.3	*Klrg1*	NM_016970.1		