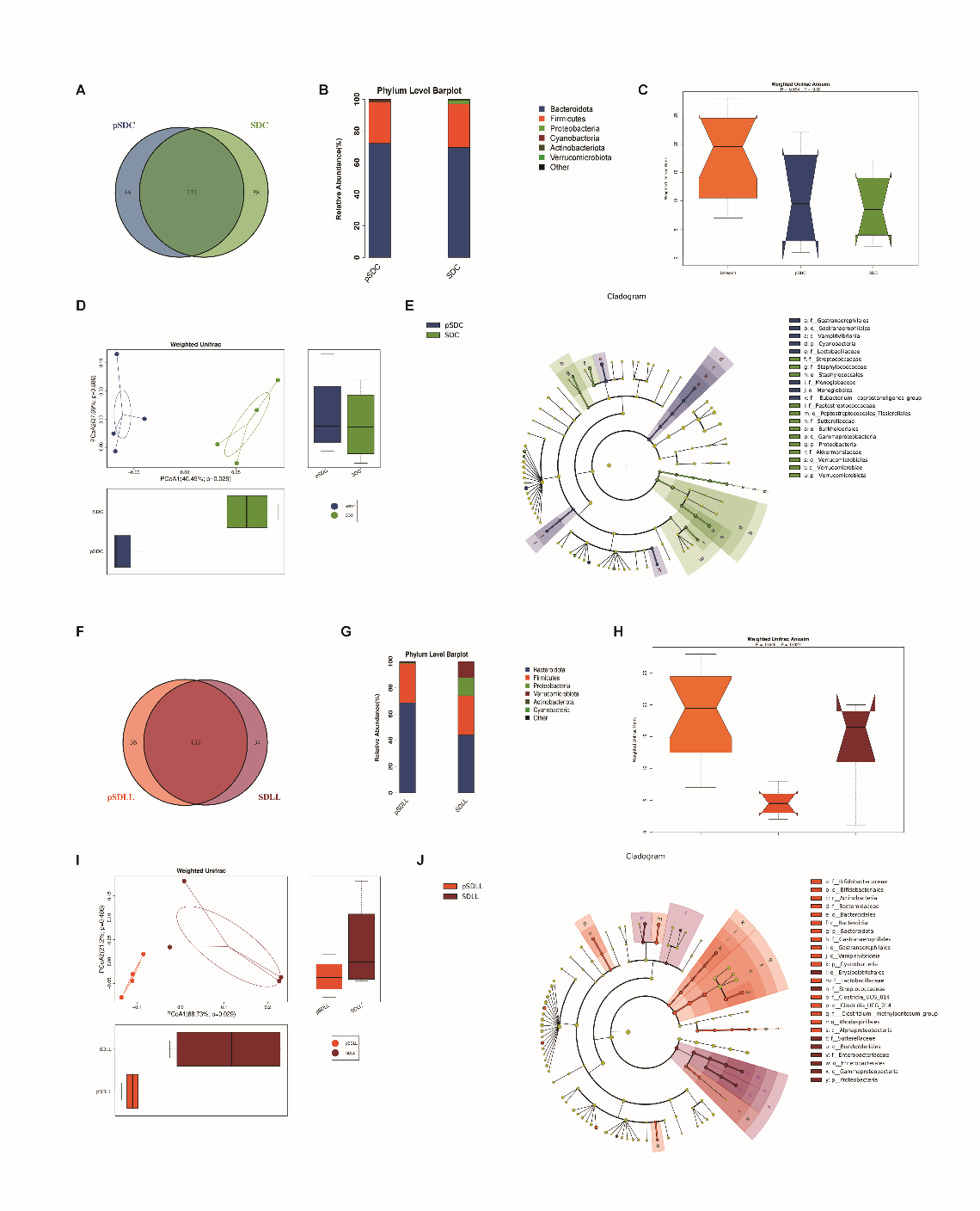


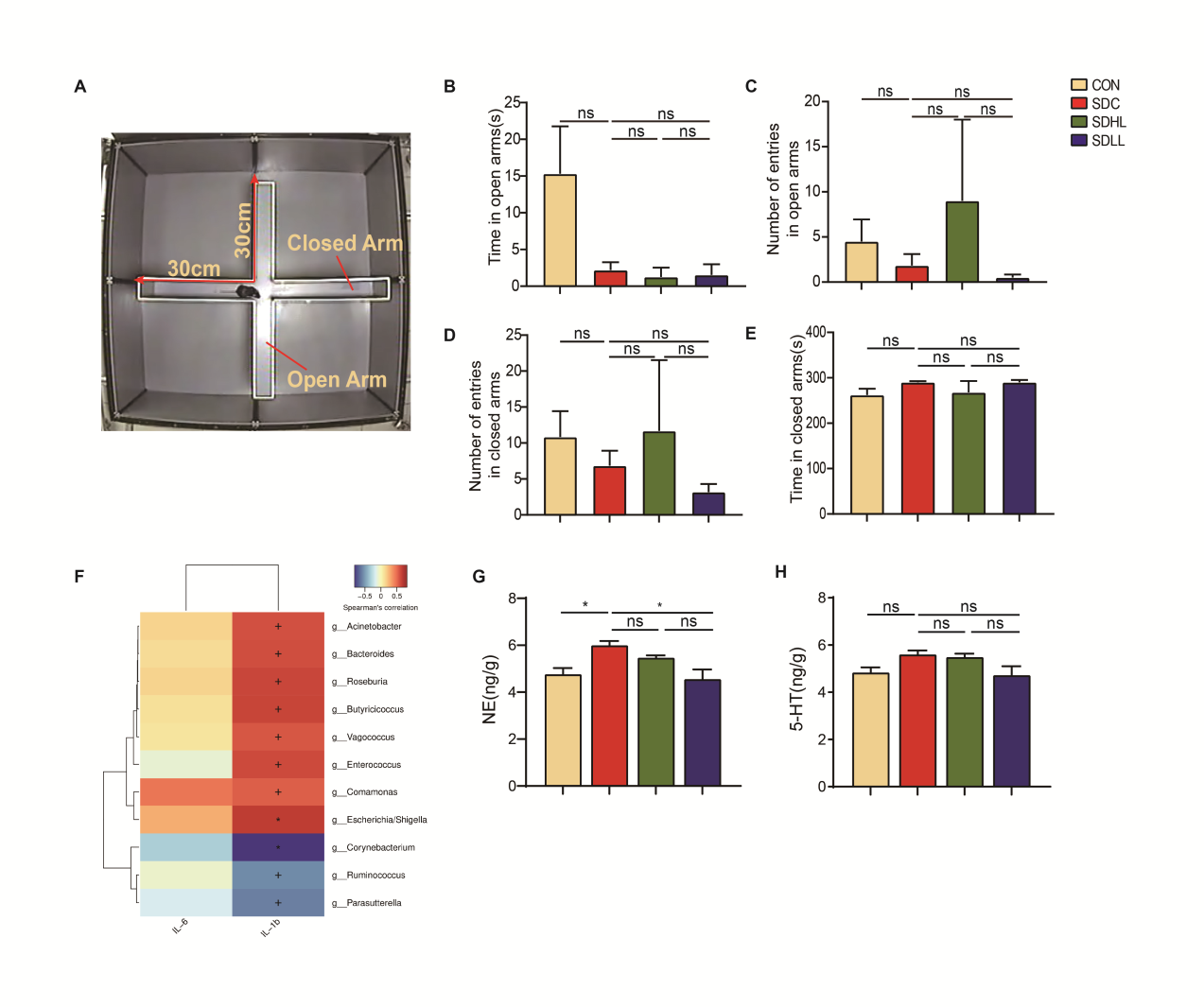
**Supplementary Figure 1. Stress level before molding and SCFAs concentration of feces.**

**(A)** Stress level was detected using sucrose preference test. **(B)** GC-MS was used to detect and quantify SCFA molecules in fecal samples. *LGG*, *Lacticaseibacillus rhamnosus GG*; CON, control group + PBS gavage; SDC, sleep deprivation + PBS gavage; SDHL, sleep deprivation + heat-killed *LGG*; SDLL, sleep deprivation + live-*LGG*; GC-MS, Gas chromatography-mass spectrometry; SCFA, short chain fatty acid. N=4-12 in every group. \**p*<0.05, ns: no significance.



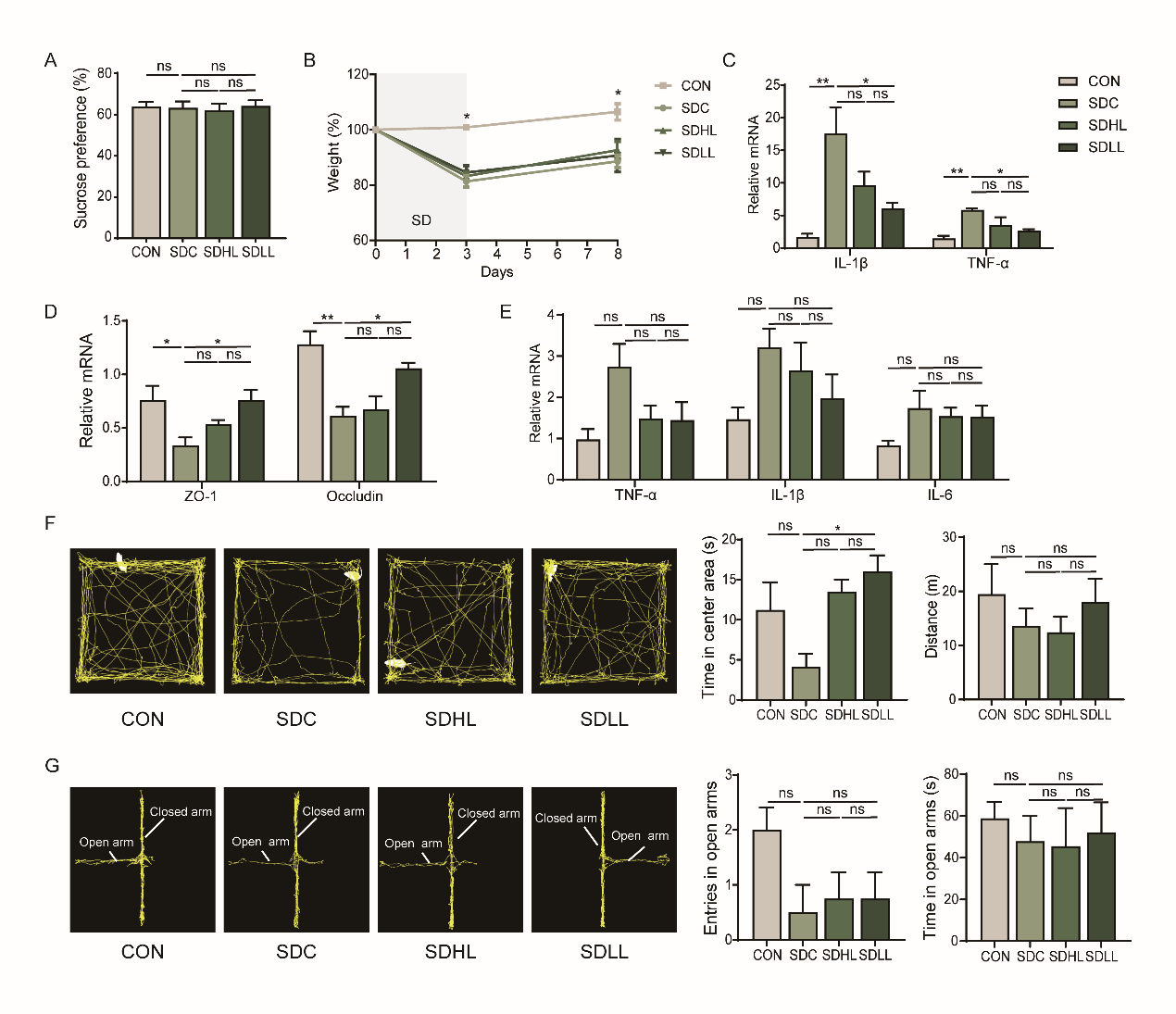
**Supplemental Figure 2. SD destroyed the microecological balance of gut microbiota of mice.**

16S rDNA sequencing was conducted to assess gut microbiota between **(A-E)** pSDC and SDC, **(F-J)** pSDLL and SDLL. **(A, F)** Venn diagram of OTUs, **(B, G)** relative abundance of microbial taxa at the phylum level, **(C, H)** PCoA on Weighted Unifrac, **(D, I)** Weighted Unifrac ANOSIM analysis, and **(E, J)** LEfSe analysis were shown. SDC, sleep deprivation + PBS gavage; pSDC, prior stage of sleep deprivation + PBS gavage; SDLL, sleep deprivation + live-*LGG*; SDLL, prior stage of sleep deprivation + live-*LGG*; PCoA, principal coordinate analysis; ANOSIM, analysis of similarities; LEfSe, LDA EffectSize. N = 4 in every group.



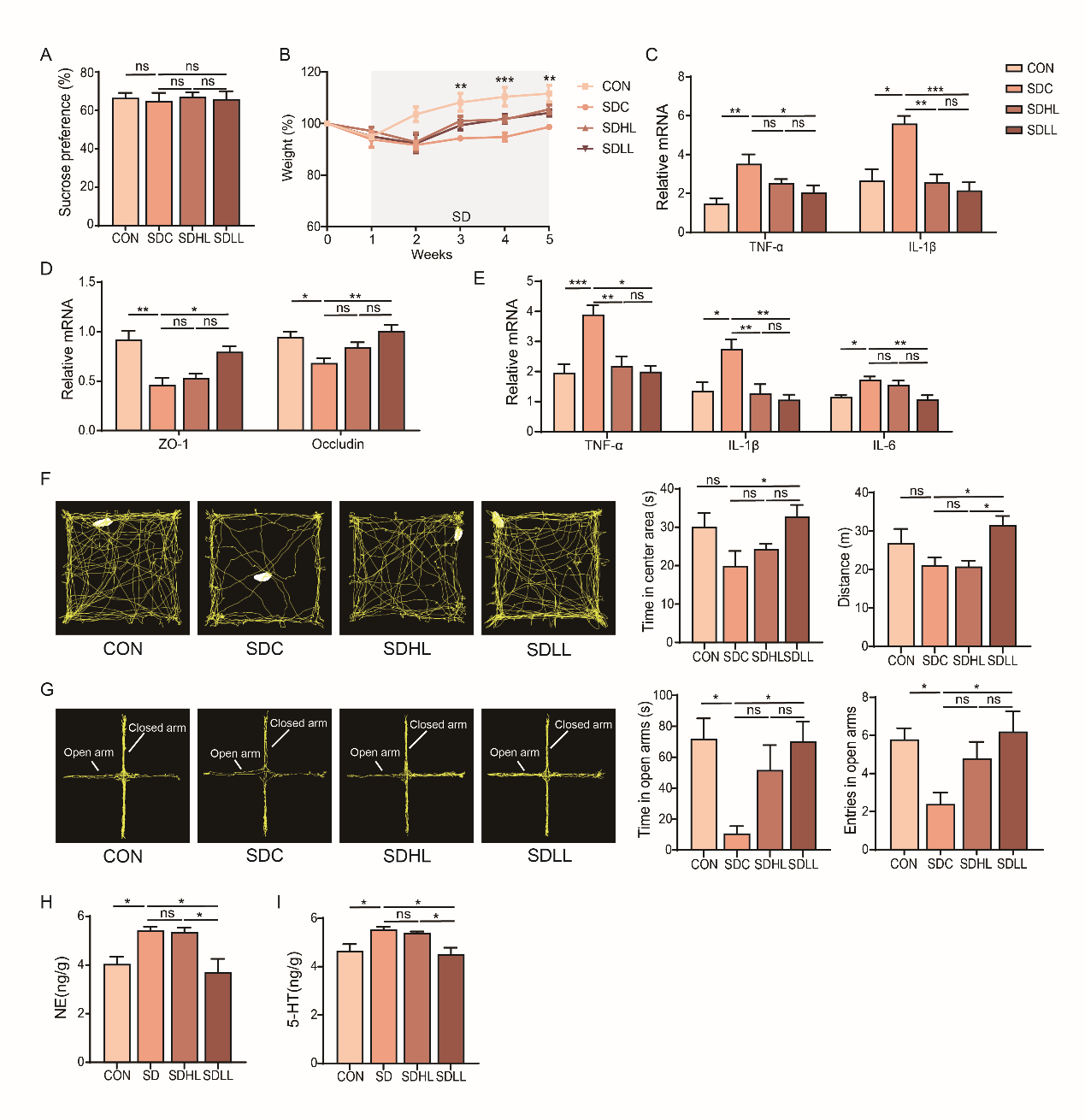
**Supplementary Figure 3. *LGG* supplementation had no significant effect on anxiety-like behavior in mice with sleep deprivation.**

**(A-E)** Anxiety-like behavior was detected using elevated plus maze test. Number of entries into open arms, time in open arms, number of entries into closed arms, and time in closed arms were shown. **(F)** The correlation analysis between gut microbiota and brain tissue measurement indicators was revealed. The concentration of **(G)** NE and **(H)** 5-HT in brain tissue was measured. *LGG*, *Lacticaseibacillus rhamnosus GG*; CON, control group + PBS gavage; SDC, sleep deprivation + PBS gavage; SDHL, sleep deprivation + heat-killed *LGG*; SDLL, sleep deprivation + live-*LGG*; N=6 in every group. \**p*<0.05, ns: no significance.



**Supplementary Figure 4. The intestinal barrier could be improved by supplementing *LGG* at the beginning of SD.**

**(A)** Sucrose preference test was conducted before molding. **(B)** Bodyweight of mice was recorded. **(C-D)** RNA was extracted from colonic tissues in mice from four groups. Realtime-PCR analysis was conducted to evaluate mRNA expression of TNF-α, IL-1β, IL-6, ZO-1, and Occludin. **(E)**Relative mRNA levels of inflammatory cytokines in prefrontal cortex including TNF-α, IL-1β, and IL-6 were demonstrated. **(F)** Open field test was prepared for evaluating mouse exploratory activity. Time in center area, and distance were shown. **(G)** Elevated plus maze test was prepared for evaluating mouse exploratory activity. Entries into open arms, and time in open arms were shown. *LGG*, *Lacticaseibacillus rhamnosus GG*; CON, control group + PBS gavage; SDC, sleep deprivation + PBS gavage; SDHL, sleep deprivation + heat-killed *LGG*; SDLL, sleep deprivation + live-*LGG*; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1β; IL-6, interleukin-6; ZO-1, zonula occludens-1. N=4 in every group. \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, \*\*\*\**p*<0.0001, ns: no significance.



**Supplementary Figure 5. *LGG* alleviated intestinal injury and anxiety-like behavior in chronic sleep-deprived mice.**

**(A)** Sucrose preference test was conducted before molding. **(B)** Bodyweight of mice was recorded. **(C-D)** RNA was extracted from colonic tissues in mice from four groups. Realtime-PCR analysis was conducted to evaluate mRNA expression of TNF-α, IL-1β, ZO-1, and Occludin. **(E)** Relative mRNA levels of inflammatory cytokines in prefrontal cortex including TNF-α, IL-1β, and IL-6 were demonstrated. **(F)** Open field test was prepared for evaluating mouse exploratory activity. Time in center area, and distance were shown. **(G)** Elevated plus maze test was prepared for evaluating mouse exploratory activity. Entries into open arms, and time in open arms were shown.The concentration of **(H)** NE and **(I)** 5-HT in brain tissue was measured. *LGG*, *Lacticaseibacillus rhamnosus GG*; CON, control group + PBS gavage; SDC, sleep deprivation + PBS gavage; SDHL, sleep deprivation + heat-killed *LGG*; SDLL, sleep deprivation + live-*LGG*; TNF-α, tumor necrosis factor alpha; IL-1β, interleukin-1β; IL-6; interleukin-6; ZO-1, zonula occludens-1; NE, Norepinephrine; 5-HT, 5-hydroxytryptamine. N=5 in every group. \**p*<0.05, \*\**p*<0.01, \*\*\**p*<0.001, \*\*\*\**p*<0.0001, ns: no significance.