

Supplemental Materials

***Lactococcus lactis* subsp. *cremoris* C60 promotes immunoglobulin A production from B cells through functional modification of dendritic cells in intestinal environment**

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Noriko M. Tsuji, Ph.D.

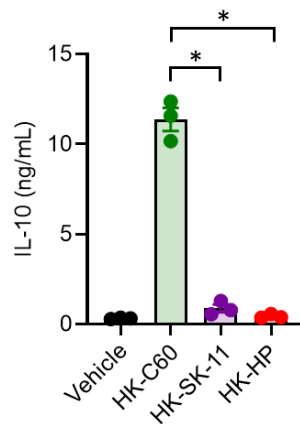
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Supplemental Table 1. Primer sequences in real-time PCR analysis

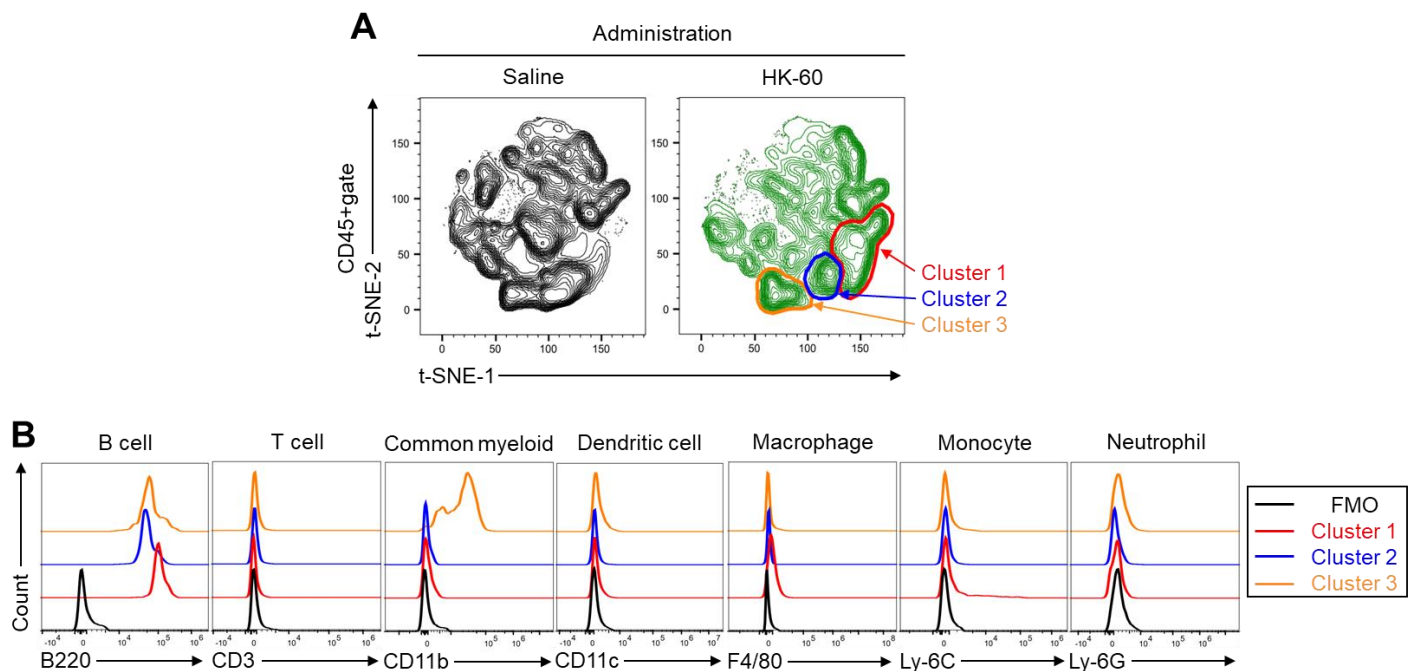
Target	Forward (5'-3')	Reverse (5'-3')
<i>Il1b</i>	GCAACTGTTCTGAACTCAACT	ATCTTTTGGGGTCCGTCAACT
<i>Il4</i>	GGTCTCAACCCCAGCTAGT	GCCGATGATCTCTCTCAAGTGAT
<i>Il6</i>	CCAAGAGGTGAGTGCTTCCC	CTGTTGTTGAGACTCTCTCCCT
<i>Il8</i>	CAAGGCTGGTCCATGCTCC	TGCTATCACTTCCTTTCTGTTGC
<i>Il10</i>	GCTCTTACTGACTGGCATGAG	CGCAGCTCTAGGAGCATGTG
<i>Il12a</i>	CTGTGCCTTGGTAGCATCTATG	GCAGAGTCTCGCCATTATGATTC
<i>Il12b</i>	TGGTTTGCCATCGTTTTGCTG	ACAGGTGAGGTTCACTGTTTCT
<i>Il23a</i>	ATGCTGGATTGCAGAGCAGTA	ACGGGGCACATTATTTTAGTCT
<i>Ifna</i>	GGATGTGACCTTCCTCAGACTC	ACCTTCTCCTGCGGGAATCCAA
<i>Ifnb</i>	CAGCTCCAAGAAAGGACGAAC	GGCAGTGTAACCTTCTGCAT
<i>Ifng</i>	ATGAACGCTACACACTGCATC	CCATCCTTTTGCCAGTTCCTC
<i>Tnfa</i>	GACGTGGAAGTGGCAGAAGAG	TTGGTGGTTTGTGAGTGTGAG
<i>Tgfb</i>	CTCCCGTGGCTTCTAGTGC	GCCTTAGTTTGGACAGGATCTG
<i>Tlr1</i>	TGAGGGTCCTGATAATGTCTAC	AGAGGTCCAAATGCTTGAGGC
<i>Tlr2</i>	GCAAACGCTGTTCTGCTCAG	AGGCGTCTCCCTCTATTGTATT
<i>Tlr3</i>	GTGAGATACAACGTAGCTGACTG	TCCTGCATCCAAGATAGCAAGT
<i>Tlr4</i>	ATGGCATGGCTTACACCACC	GAGGCCAATTTGTCTCCACA
<i>Tlr5</i>	GCCCCGTGTTGGTAATATCTC	ATCTGGGTGAGGTTACAGCCT
<i>Tlr6</i>	TGAGCCAAGACAGAAAACCCA	GGGACATGAGTAAGGTTCTGTT
<i>Tlr7</i>	ATGTGGACACGGAAGAGACAA	GGTAAGGGTAAGATTGGTGGTG
<i>Tlr8</i>	GAAAACATGCCCCCTCAGTCA	CGTCACAAGGATAGCTTCTGGAA
<i>Tlr9</i>	ATGGTTCTCCGTGAAGGACT	GAGGCTTCAGCTCACAGGG
<i>Gapdh</i>	TGTGTCCGTCGTGGATCTGA	TTGCTGTTGAAGTCGCAGGAG

Supplemental Figures



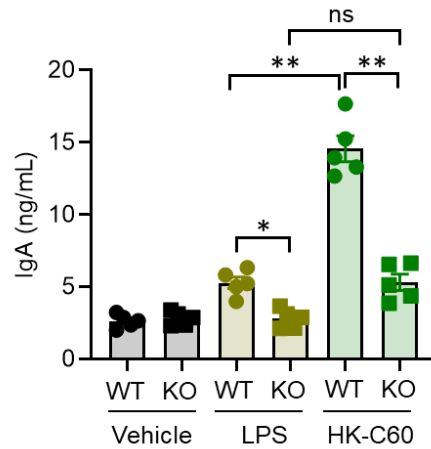
Supplemental Figure 1. *In vitro* IL-10 production assay in BMDCs

BMDCs ($1.0 \times 10^6/\text{mL}$) were cultured with vehicle (PBS), HK-C60 (5.0×10^8 CFU/mL), HK-SK-11 (5.0×10^8 CFU/mL) or HK-HP (5.0×10^8 CFU/mL) at 37°C for 24 h. The IL-10 concentration in cultured medium was measured by ELISA. The data were shown as mean $\pm$ SEM of three samples from independent experiments. One-way ANOVA was used to analyze data for significant differences. Values of $*p < 0.001$ were regarded as significant.



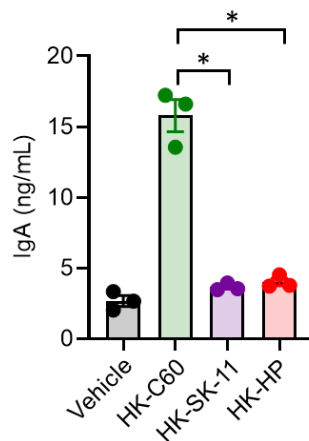
Supplemental Figure 2. Global immunophenotyping in small intestinal PP

The mice received i.g. administration of saline or HK-C60 for 2 weeks ($n=3$ in each group). The small intestinal PP-derived cells were subjected to flow cytometry analysis. A) A t-SNE plot generated by PP-derived cell staining multi markers targeting immune cells. Total three clusters were identified as unique populations in HK-C60 administered group. B) Independent identification of cell type following specific marker expression in three unique clusters.



Supplemental Figure 3. *In vitro* IgA production assay in PP-derived cells originated from MyD88-KO mice

Small intestinal PP-derived cells were prepared from naïve WT mice or MyD88-KO mice (KO) (n=5 in each group), and the cells (1.0×10^7 /mL) were subjected to *in vitro* culture with vehicle (PBS), LPS ($1 \mu\text{g/mL}$) or HK-C60 (5.0×10^8 CFU/mL) at 37°C for 72 h. The IgA concentration in cultured medium was measured by ELISA. The data were shown as mean $\pm$ SEM of five samples from independent experiments. One-way ANOVA was used to analyze data for significant differences. Values of $*p < 0.01$ and $p^{**} < 0.001$ were regarded as significant. ns: not significant.



Supplemental Figure 4. *In vitro* IgA production assay in PP-derived cells

Small intestinal PP-derived cells were prepared from naïve WT mice (n=3), and the cells (1.0×10^7 /mL) were subjected to *in vitro* culture with vehicle (PBS), HK-C60 (5.0×10^8 CFU/mL), HK-SK-11 (5.0×10^8 CFU/mL) or HK-HP (5.0×10^8 CFU/mL) at 37°C for 72 h. The IgA concentration in cultured medium was measured by ELISA. The data were shown as mean $\pm$ SEM of three samples from independent experiments. One-way ANOVA was used to analyze data for significant differences. Values of $*p < 0.001$ were regarded as significant.