

Supplemental Data

Intestinal epithelial cell-derived LKB1 suppresses colitogenic microbiota

Running title: Epithelium LKB1 suppresses colitogenic microbiota

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Supplemental Table 1. Distant matrix for unifracs analysis of 16S RNAseq data related to Fig.4

Distant matrix for unifracs analysis by 16S RNAseq sampling from feces of LKB1^{WT} (WT) and LKB1^{ΔIEC} (KO) mice 0 or 5 days(D) after DSS treatment – related to Fig.4A

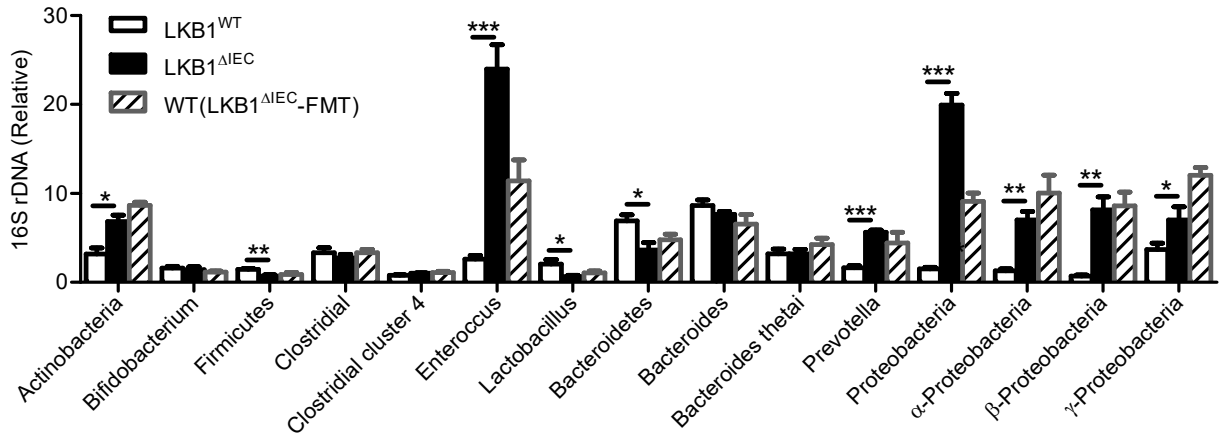
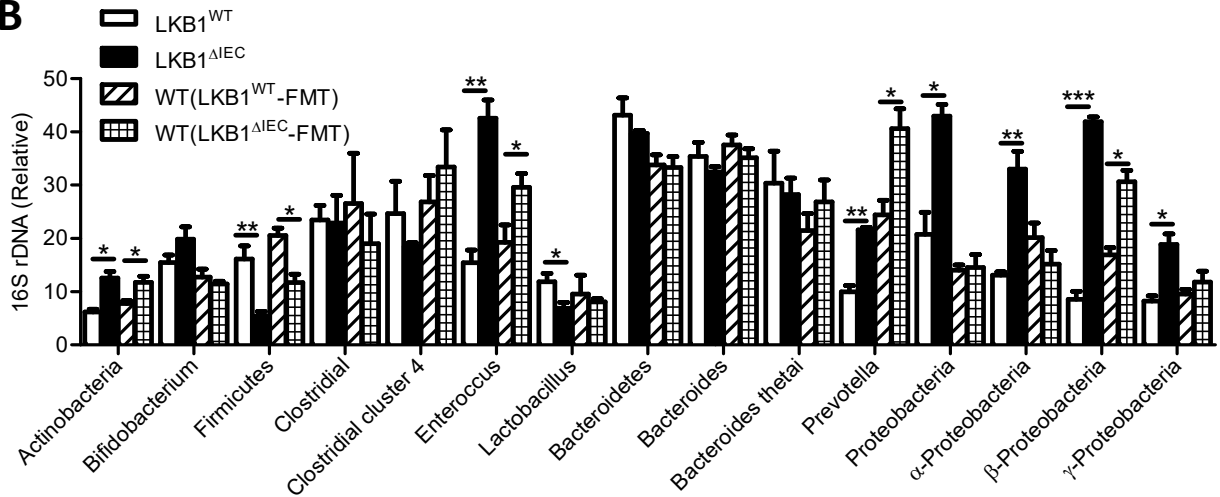
	WT1.D0	WT2.D0	WT3.D0	WT4.D0	WT5.D0	KO1.D0	KO2.D0	KO3.D0	KO4.D0	KO5.D0	WT1.D5	WT2.D5	WT3.D5	WT4.D5	WT5.D5	KO1.D5	KO2.D5	KO3.D5	KO4.D5	KO5.D5
WT1.D0	0.00	0.21	0.24	0.28	0.29	0.38	0.38	0.36	0.40	0.32	0.46	0.44	0.44	0.43	0.43	0.46	0.46	0.46	0.45	0.48
WT2.D0	0.21	0.00	0.20	0.24	0.28	0.34	0.34	0.33	0.35	0.29	0.44	0.44	0.44	0.42	0.41	0.45	0.42	0.44	0.46	0.44
WT3.D0	0.24	0.20	0.00	0.21	0.26	0.30	0.32	0.30	0.31	0.27	0.40	0.41	0.39	0.40	0.40	0.41	0.40	0.42	0.41	0.43
WT4.D0	0.28	0.24	0.21	0.00	0.26	0.33	0.34	0.33	0.34	0.31	0.40	0.39	0.39	0.40	0.40	0.40	0.38	0.40	0.40	0.42
WT5.D0	0.29	0.28	0.26	0.26	0.00	0.35	0.37	0.35	0.38	0.35	0.39	0.40	0.38	0.41	0.39	0.40	0.40	0.39	0.41	0.41
KO1.D0	0.38	0.34	0.30	0.33	0.35	0.00	0.17	0.17	0.16	0.17	0.44	0.45	0.43	0.45	0.43	0.36	0.36	0.35	0.36	0.36
KO2.D0	0.38	0.34	0.32	0.34	0.37	0.17	0.00	0.18	0.12	0.18	0.43	0.44	0.43	0.44	0.42	0.35	0.37	0.34	0.33	0.36
KO3.D0	0.36	0.33	0.30	0.33	0.35	0.17	0.18	0.00	0.17	0.20	0.45	0.45	0.43	0.45	0.42	0.35	0.34	0.35	0.36	0.36
KO4.D0	0.40	0.35	0.31	0.34	0.38	0.16	0.12	0.17	0.00	0.19	0.46	0.46	0.44	0.45	0.42	0.34	0.35	0.34	0.34	0.35
KO5.D0	0.32	0.29	0.27	0.31	0.35	0.17	0.18	0.20	0.19	0.00	0.44	0.46	0.43	0.44	0.41	0.36	0.36	0.35	0.34	0.39
WT1.D5	0.46	0.44	0.40	0.40	0.39	0.44	0.43	0.45	0.46	0.44	0.00	0.13	0.18	0.17	0.26	0.33	0.35	0.36	0.33	0.35
WT2.D5	0.44	0.44	0.41	0.39	0.40	0.45	0.44	0.45	0.46	0.46	0.13	0.00	0.11	0.10	0.24	0.33	0.33	0.36	0.33	0.35
WT3.D5	0.44	0.44	0.39	0.39	0.38	0.43	0.43	0.43	0.44	0.43	0.18	0.11	0.00	0.15	0.26	0.34	0.31	0.36	0.34	0.35
WT4.D5	0.43	0.42	0.40	0.40	0.41	0.45	0.44	0.45	0.45	0.44	0.17	0.10	0.15	0.00	0.23	0.32	0.34	0.34	0.34	0.33
WT5.D5	0.43	0.41	0.40	0.40	0.39	0.43	0.42	0.42	0.42	0.41	0.26	0.24	0.26	0.23	0.00	0.30	0.32	0.29	0.31	0.30
KO1.D5	0.46	0.45	0.41	0.40	0.40	0.36	0.35	0.35	0.34	0.36	0.33	0.33	0.34	0.32	0.30	0.00	0.24	0.17	0.24	0.19
KO2.D5	0.46	0.42	0.40	0.38	0.40	0.36	0.37	0.34	0.35	0.36	0.35	0.33	0.31	0.34	0.32	0.24	0.00	0.24	0.24	0.25
KO3.D5	0.46	0.44	0.42	0.40	0.39	0.35	0.34	0.35	0.34	0.35	0.36	0.36	0.36	0.34	0.29	0.17	0.24	0.00	0.22	0.21
KO4.D5	0.45	0.46	0.41	0.40	0.41	0.36	0.33	0.36	0.34	0.34	0.33	0.33	0.34	0.31	0.24	0.24	0.22	0.00	0.23	0.23
KO5.D5	0.48	0.44	0.43	0.42	0.41	0.36	0.36	0.36	0.35	0.39	0.35	0.35	0.35	0.33	0.30	0.19	0.25	0.21	0.23	0.00

Distant matrix for unifracs analysis by 16S RNAseq sampling from feces of 2 cage of LKB1^{WT} (WT) and two cages of LKB1^{ΔIEC} (KO) – related to Fig.4C

	WT1.1	WT1.2	WT1.3	WT1.4	WT1.5	KO1.1	KO1.2	KO1.3	KO1.4	KO1.5	WT3.1	WT3.2	WT3.3	WT3.4	WT3.5	KO3.1	KO3.2	KO3.3	KO3.4	KO3.5
WT1.1	0.00	0.19	0.25	0.19	0.25	0.42	0.39	0.37	0.35	0.37	0.24	0.25	0.24	0.22	0.25	0.38	0.39	0.37	0.39	0.40
WT1.2	0.19	0.00	0.20	0.18	0.23	0.44	0.42	0.40	0.39	0.39	0.24	0.25	0.28	0.20	0.24	0.41	0.43	0.42	0.43	0.42
WT1.3	0.25	0.20	0.00	0.19	0.14	0.44	0.44	0.40	0.40	0.39	0.28	0.22	0.27	0.17	0.27	0.41	0.44	0.42	0.41	0.41
WT1.4	0.19	0.18	0.19	0.00	0.22	0.43	0.40	0.36	0.37	0.37	0.24	0.25	0.28	0.18	0.18	0.38	0.40	0.38	0.40	0.40
WT1.5	0.25	0.23	0.14	0.22	0.00	0.41	0.40	0.40	0.40	0.39	0.32	0.23	0.26	0.18	0.30	0.41	0.42	0.41	0.41	0.40
KO1.1	0.42	0.44	0.44	0.43	0.41	0.00	0.23	0.28	0.32	0.24	0.42	0.39	0.40	0.41	0.44	0.24	0.25	0.23	0.25	0.24
KO1.2	0.39	0.42	0.44	0.40	0.40	0.23	0.00	0.23	0.30	0.25	0.42	0.41	0.42	0.41	0.40	0.23	0.22	0.21	0.22	0.24
KO1.3	0.37	0.40	0.40	0.36	0.40	0.28	0.23	0.00	0.21	0.20	0.37	0.37	0.41	0.39	0.35	0.23	0.25	0.22	0.23	0.22
KO1.4	0.35	0.39	0.40	0.37	0.40	0.32	0.30	0.21	0.00	0.24	0.38	0.37	0.41	0.40	0.39	0.28	0.29	0.27	0.26	0.26
KO1.5	0.37	0.39	0.39	0.37	0.39	0.24	0.25	0.20	0.24	0.00	0.40	0.34	0.39	0.37	0.39	0.20	0.23	0.22	0.19	0.18
WT3.1	0.24	0.24	0.28	0.24	0.32	0.42	0.42	0.37	0.38	0.40	0.00	0.27	0.29	0.28	0.26	0.39	0.41	0.39	0.41	0.42
WT3.2	0.25	0.25	0.22	0.25	0.23	0.39	0.41	0.37	0.37	0.34	0.27	0.00	0.25	0.22	0.29	0.36	0.39	0.38	0.37	0.35
WT3.3	0.24	0.28	0.27	0.28	0.26	0.40	0.42	0.41	0.41	0.39	0.29	0.25	0.00	0.21	0.30	0.41	0.41	0.42	0.40	0.41
WT3.4	0.22	0.20	0.17	0.18	0.18	0.41	0.41	0.39	0.40	0.37	0.28	0.22	0.21	0.00	0.23	0.39	0.38	0.39	0.39	0.38
WT3.5	0.25	0.24	0.27	0.18	0.30	0.44	0.40	0.35	0.39	0.39	0.26	0.29	0.30	0.23	0.00	0.39	0.38	0.39	0.40	0.40
KO3.1	0.38	0.41	0.41	0.38	0.41	0.24	0.23	0.23	0.28	0.20	0.39	0.36	0.41	0.39	0.39	0.00	0.17	0.19	0.17	0.17
KO3.2	0.39	0.43	0.44	0.40	0.42	0.25	0.22	0.25	0.29	0.23	0.41	0.39	0.41	0.38	0.38	0.17	0.00	0.20	0.16	0.20
KO3.3	0.37	0.42	0.42	0.38	0.41	0.23	0.21	0.22	0.27	0.22	0.39	0.38	0.42	0.39	0.39	0.19	0.20	0.00	0.21	0.21
KO3.4	0.39	0.43	0.41	0.40	0.41	0.25	0.22	0.23	0.26	0.19	0.41	0.37	0.40	0.39	0.40	0.17	0.16	0.21	0.00	0.14
KO3.5	0.40	0.42	0.41	0.40	0.40	0.24	0.24	0.22	0.26	0.18	0.42	0.35	0.41	0.38	0.40	0.17	0.20	0.21	0.14	0.00

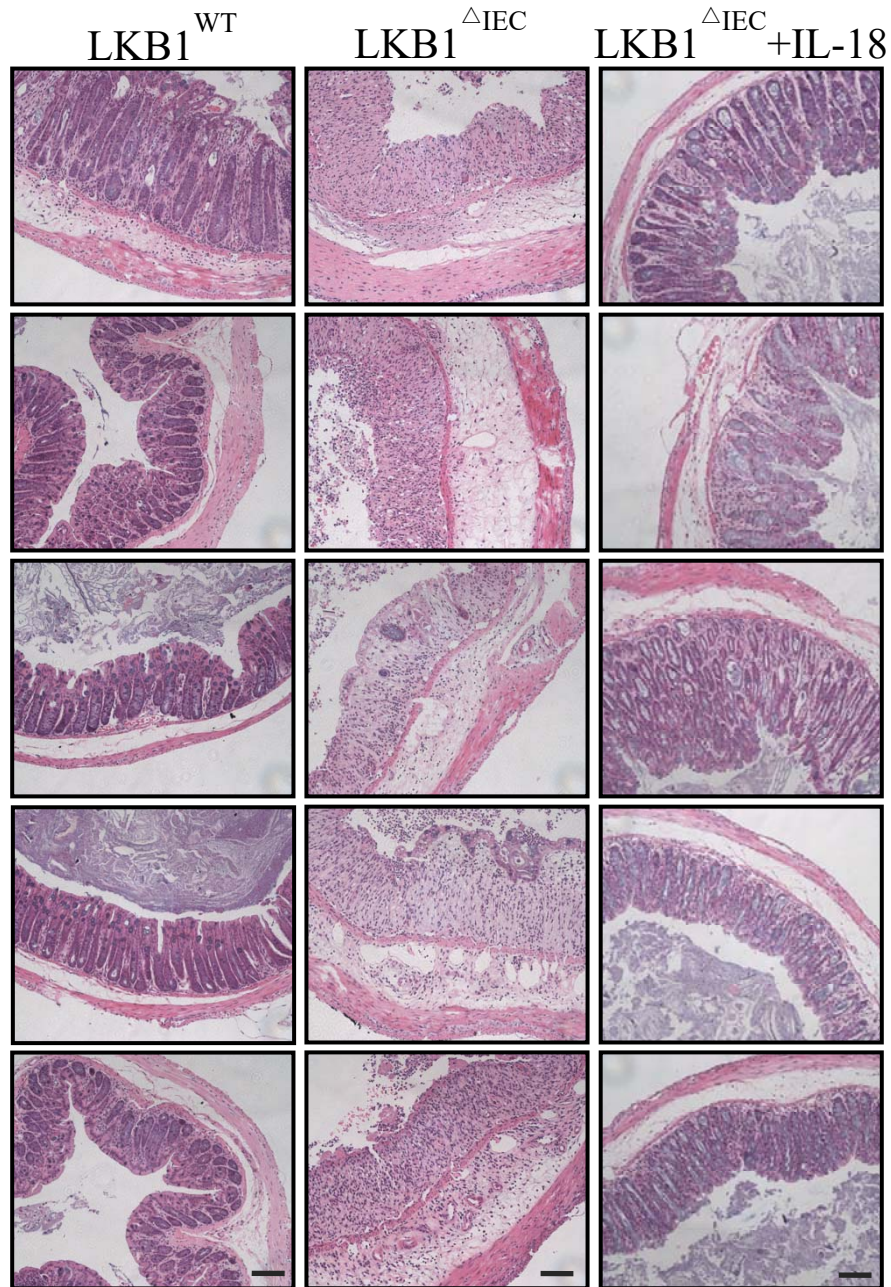
Distant matrix for unifracs analysis by 16S RNAseq sampling from feces of 2 cage of separately-housed WT(WT.D0) and KO mice(KO.D0) and 2 cages of cohoused WT(CO.WT) and KO mice(CO.KO)– related to Fig.4D

	WT1.D0	WT2.D0	WT3.D0	WT4.D0	WT5.D0	KO1.D0	KO2.D0	KO3.D0	KO4.D0	KO5.D0	CO.WT1	CO.WT2	CO.WT3	CO.WT4	CO.WT5	CO.KO1	CO.KO2	CO.KO3	CO.KO4	CO.KO5
WT1.D0	0.00	0.59	0.58	0.66	0.57	0.65	0.66	0.67	0.65	0.74	0.61	0.61	0.54	0.65	0.61	0.67	0.69	0.65	0.68	0.73
WT2.D0	0.59	0.00	0.60	0.55	0.54	0.58	0.56	0.60	0.61	0.58	0.62	0.58	0.62	0.63	0.61	0.65	0.65	0.62	0.62	0.64
WT3.D0	0.58	0.60	0.00	0.54	0.53	0.59	0.59	0.65	0.60	0.66	0.54	0.63	0.55	0.56	0.61	0.59	0.62	0.67	0.59	0.62
WT4.D0	0.66	0.55	0.54	0.00	0.59	0.54	0.56	0.52	0.56	0.60	0.63	0.60	0.68	0.61	0.56	0.63	0.61	0.63	0.57	0.61
WT5.D0	0.57	0.54	0.53	0.59	0.00	0.60	0.58	0.64	0.60	0.67	0.59	0.63	0.57	0.64	0.62	0.58	0.67	0.64	0.64	0.63
KO1.D0	0.65	0.58	0.59	0.54	0.60	0.00	0.52	0.51	0.43	0.48	0.63	0.59	0.68	0.63	0.59	0.60	0.57	0.56	0.61	0.55
KO2.D0	0.66	0.56	0.59	0.56	0.58	0.52	0.00	0.51	0.50	0.54	0.64	0.61	0.63	0.62	0.55	0.56	0.57	0.64	0.63	0.60
KO3.D0	0.67	0.60	0.65	0.52	0.64	0.51	0.51	0.00	0.47	0.45	0.69	0.59	0.70	0.58	0.62	0.61	0.58	0.54	0.61	0.59
KO4.D0	0.65	0.61	0.60	0.56	0.60	0.43	0.50	0.47	0.00	0.46	0.67	0.56	0.67	0.57	0.58	0.58	0.54	0.57	0.60	0.61
KO5.D0	0.74	0.58	0.66	0.60	0.67	0.48	0.54	0.45	0.46	0.00	0.71	0.65	0.71	0.63	0.62	0.60	0.57	0.54	0.58	0.58
CO.WT1	0.61	0.62	0.54	0.63	0.59	0.63	0.64	0.69	0.67	0.71	0.00	0.63	0.60	0.64	0.63	0.69	0.67	0.71	0.63	0.70
CO.WT2	0.61	0.58	0.63	0.60	0.63	0.59	0.61	0.59	0.56	0.65	0.63	0.00	0.65	0.57	0.60	0.62	0.62	0.67	0.62	0.68
CO.WT3	0.54	0.62	0.55	0.68	0.57	0.68	0.63	0.70	0.67	0.71	0.60	0.65	0.00	0.68	0.67	0.69	0.70	0.71	0.69	0.71
CO.WT4	0.65	0.63	0.56	0.61	0.64	0.63	0.62	0.58	0.57	0.63	0.64	0.57	0.68	0.00	0.60	0.65	0.62	0.66	0.58	0.62
CO.WT5	0.61	0.61	0.61	0.56	0.62	0.59	0.55	0.62	0.58	0.62	0.63	0.60	0.67	0.60	0.00	0.59	0.57	0.59	0.62	0.58
CO.KO1	0.67	0.65	0.59	0.63	0.58	0.60	0.56	0.61	0.58	0.60	0.69	0.62	0.69	0.65	0.59	0.00	0.56	0.58	0.64	0.57
CO.KO2	0.69	0.65	0.62	0.61	0.67	0.57	0.57	0.58	0.54	0.57	0.67	0.62	0.70	0.62	0.57	0.56	0.00	0.66	0.59	0.61
CO.KO3	0.65	0.62	0.67	0.63	0.64	0.56	0.64	0.54	0.57	0.54	0.71	0.67	0.71	0.66	0.59	0.58	0.66	0.00		

A**B**

Supplemental Figure 1. Changes in bacterial populations after Fecal Microbiota Transplantation

(A) LKB1^{WT} mice were pretreated with a cocktail of neomycin (1g/L), ampicillin (1g/L), metronidazol (0.5g/l) and vancomycin (0.5 g/L) in normal drinking water for two weeks followed by fecal microbiota transplantation(FMT) from LKB1^{ΔIEC} mice every other day for a total 3 times at 300 μl/mouse each time. 16S rDNA real-time PCR analysis of microbiota of feces before and after FMT were performed. (B) Colon feces from LKB1^{WT} and LKB1^{ΔIEC} mice were collected independently and homogenized in PBS (50 mg/ml). Feces homogenates were transplanted into unrelated WT recipients every other day for a total 3 times at 300 μl/mouse each time. 16S rDNA real-time PCR analysis of microbiota of feces from donor mice and unrelated WT recipient mice after FMT were performed. n=5 mice/group in each experiment, both sex-matched male and female mice were used. Data represents three independent experiments, Error bar, SEM; *p < 0.05, **p<0.01, ***p < 0.001.



Supplemental Figure 2. More representative Hematoxylin and eosin staining are shown, relating to Fig.6E

LKB1^{ΔIEC} mice were i.p injected with IL-18 at a concentration of 1μg/mouse once a day consecutively for 5 days. LKB1^{WT} and LKB1^{ΔIEC} mice injected with PBS were included as controls and rested for 2 days, then mice were subjected to DSS-induced acute colitis with 2.5% DSS. Hematoxylin and eosin staining of colon tissues from indicated groups 9 days after DSS treatment were shown. Data are representative of 3 independent experiments. n=5 mice per group in each experiment. Both sex-matched male and female mice were used.