Figure S1

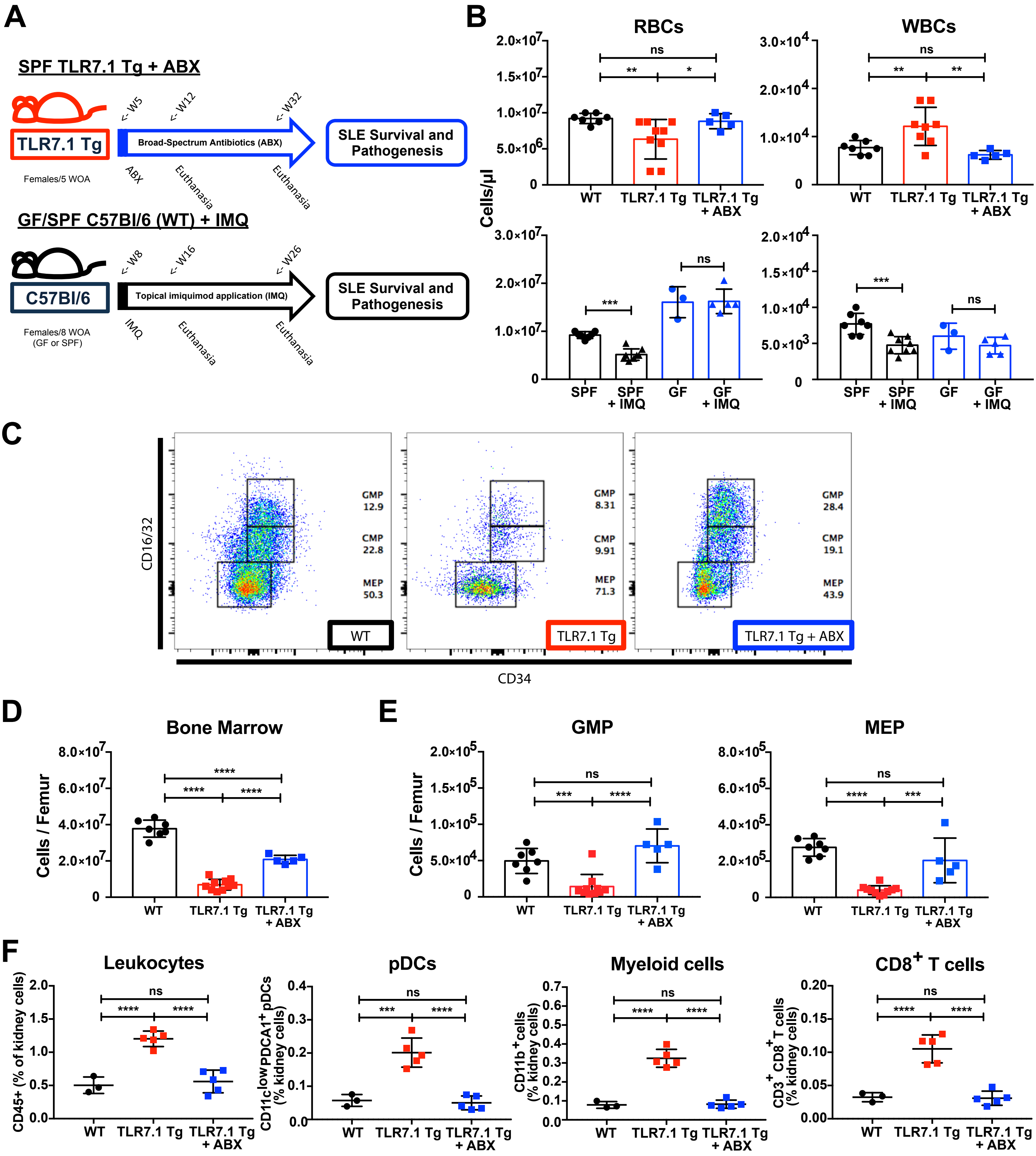

A
SPF TLR7.1 Tg + ABX
TLR7.1 Tg
Females/5 WOA
<- W5
<- W12
<- W32
Broad-Spectrum Antibiotics (ABX)
ABX
Euthanasia
Euthanasia
SLE Survival and Pathogenesis
GF/SPF C57Bl/6 (WT) + IMQ
C57Bl/6
Females/8 WOA (GF or SPF)
<- W8
<- W16
<- W26
Topical imiquimod application (IMQ)
IMQ
Euthanasia
Euthanasia
SLE Survival and Pathogenesis
B
RBCs
WBCs
Cells/µl
2.0×10^{7}
1.5×10^{7}
1.0×10^{7}
5.0×10^{6}
0
3.0×10^{4}
2.0×10^{4}
1.0×10^{4}
ns
**
*
**
WT
TLR7.1 Tg
TLR7.1 Tg + ABX
3.0×10^{7}
2.0×10^{7}
1.0×10^{7}
2.0×10^{4}
1.5×10^{4}
1.0×10^{4}
5.0×10^{3}
***
ns
SPF
SPF + IMQ
GF
GF + IMQ
C
CD16/32
CD34
GMP 12.9
CMP 22.8
MEP 50.3
WT
GMP 8.31
CMP 9.91
MEP 71.3
TLR7.1 Tg
GMP 28.4
CMP 19.1
MEP 43.9
TLR7.1 Tg + ABX
D
Bone Marrow
Cells / Femur
8.0×10^{7}
6.0×10^{7}
4.0×10^{7}
2.0×10^{7}
0
****
WT
TLR7.1 Tg
TLR7.1 Tg + ABX
E
GMP
MEP
Cells / Femur
2.0×10^{5}
1.5×10^{5}
1.0×10^{5}
5.0×10^{4}
8.0×10^{5}
6.0×10^{5}
4.0×10^{5}
2.0×10^{5}
0
ns
***
****
F
Leukocytes
pDCs
Myeloid cells
CD8+ T cells
CD45+ (% of kidney cells)
CD11c^{low}PDCA1^{+} pDCs (% kidney cells)
CD11b^{+}cells (% kidney cells)
CD3^{+}CD8^{+} T cells (% kidney cells)
2.0
1.5
1.0
0.5
0.0
0.4
0.3
0.2
0.1
0.7
0.6
0.20
0.15
0.10
0.05
0.00
ns
***
****
WT
TLR7.1 Tg
TLR7.1 Tg + ABX

Figure S1. Related to Figure 1. The gut microbiota exacerbates lupus-related mortality and pathogenesis. (A) Overview of the experimental design: TLR7.1 Tg lupus-prone mice were treated with ABX and C57Bl/6 mice were treated with topical IMQ to induce a lupus-like syndrome. Age in weeks is listed along the arrows describing the intervention. Starting age is listed below the mouse strains. Experimental cohorts of TLR7.1 Tg and C57Bl/6 mice were sacrificed at 12 and 16 weeks of age, respectively. (B) Red and white blood cell counts. Bone marrow cells were isolated from the left femur and granulocyte-monocyte progenitors as well as megakaryocyte-erythrocyte progenitors were analyzed by flow cytometry. (C) Representative FACS plots of bone marrow progenitors. (D) Bone marrow cell counts. (E) Total granulocyte-monocyte and megakaryocyte-erythrocyte progenitor numbers in the bone marrow. Cells from kidneys were isolated and analyzed by flow cytometry. (F) Frequencies of leukocytes, pDCs, myeloid cells, and CD8+ T cells in the kidneys. The results are expressed as mean ± SEM (n = 3-20 mice per group). The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ***P<0.001; ****P<0.0001; ns = not significant; WOA = weeks of age; RBCs = red blood cells; WBCs = white blood cells; GMP = granulocyte-monocyte progenitors; CMP = common myeloid progenitors; MEP = megakaryocyte-erythrocyte progenitors.

Figure S2

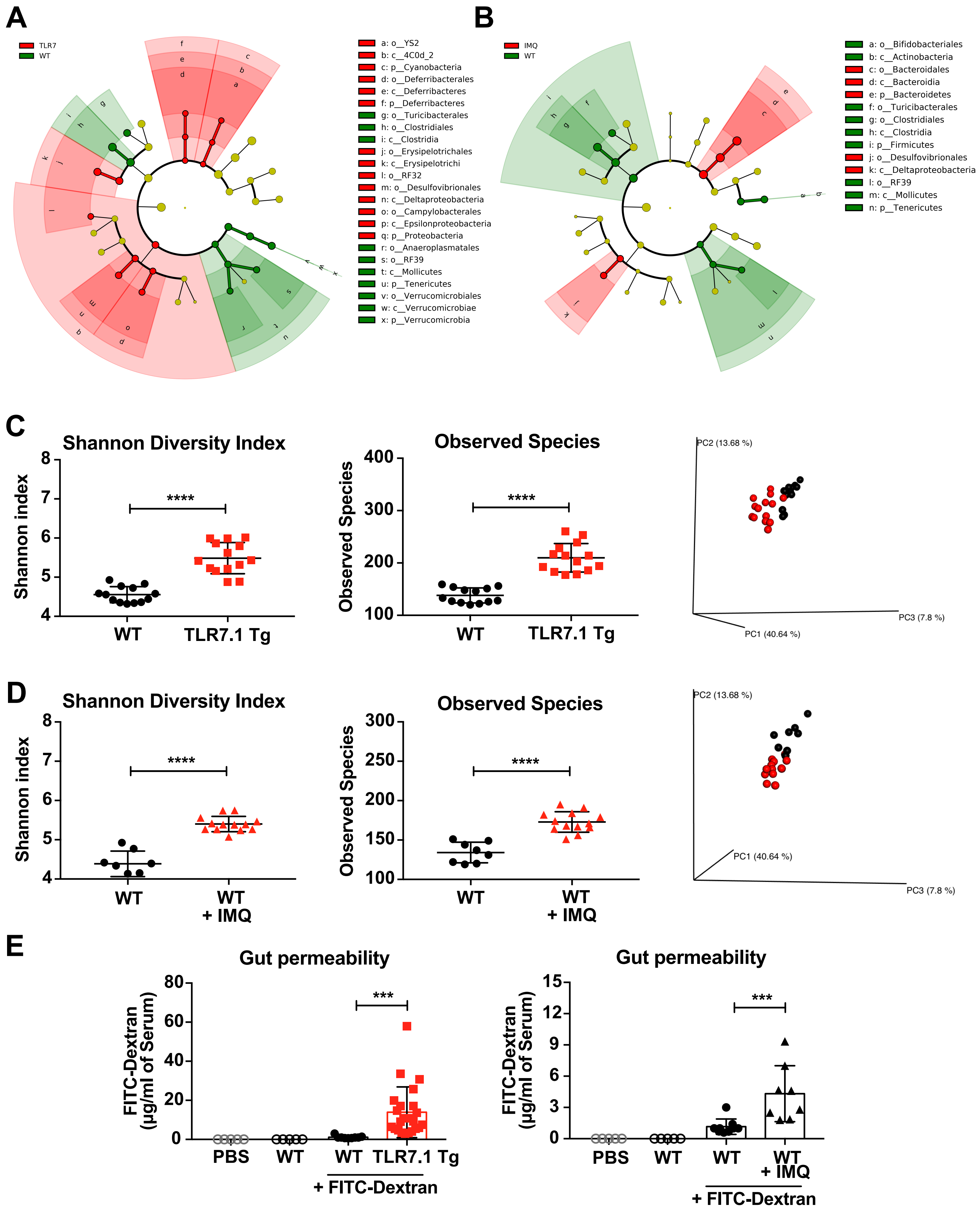
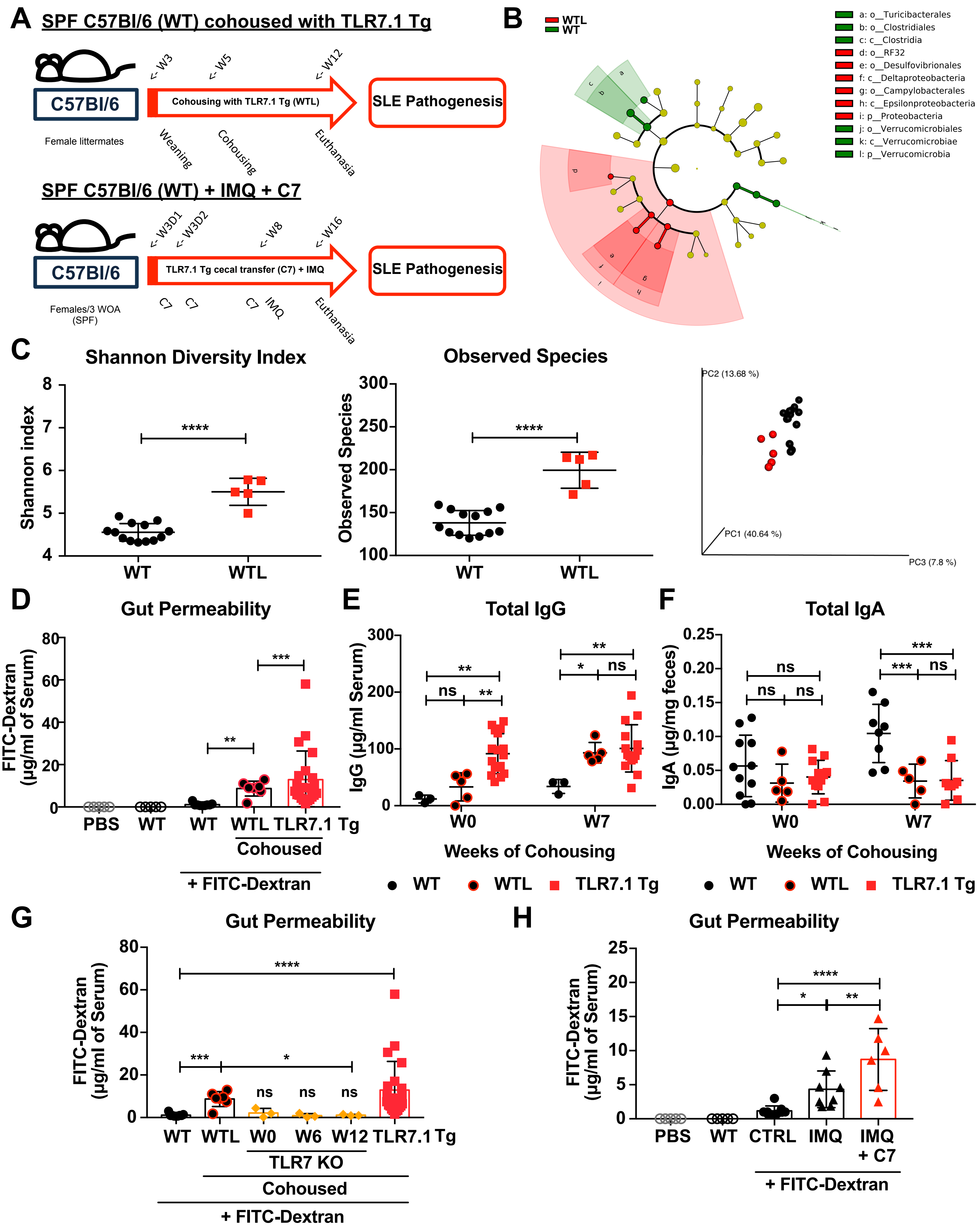

A
TLR7
WT
a: o__YS2
b: c__4C0d_2
c: p__Cyanobacteria
d: o__Deferribacterales
e: c__Deferribacteres
f: p__Deferribacteres
g: o__Turicibacterales
h: o__Clostridiales
i: c__Clostridia
j: o__Erysipelotrichales
k: c__Erysipelotrichi
l: o__RF32
m: o__Desulfovibrionales
n: c__Deltaproteobacteria
o: o__Campylobacterales
p: c__Epsilonproteobacteria
q: p__Proteobacteria
r: o__Anaeroplasmatales
s: o__RF39
t: c__Mollicutes
u: p__Tenericutes
v: o__Verrucomicrobiales
w: c__Verrucomicrobiae
x: p__Verrucomicrobia
B
IMQ
WT
a: o__Bifidobacteriales
b: c__Actinobacteria
c: o__Bacteroidales
d: c__Bacteroidia
e: p__Bacteroidetes
f: o__Turicibacterales
g: o__Clostridiales
h: c__Clostridia
i: p__Firmicutes
j: o__Desulfovibrionales
k: c__Deltaproteobacteria
l: o__RF39
m: c__Mollicutes
n: p__Tenericutes
C
Shannon Diversity Index
Shannon index
8
7
6
5
4
****
WT
TLR7.1 Tg
Observed Species
Observed Species
400
300
200
100
****
WT
TLR7.1 Tg
PC2 (13.68 %)
PC3 (7.8 %)
PC1 (40.64 %)
D
Shannon Diversity Index
Shannon index
8
7
6
5
4
****
WT
WT + IMQ
Observed Species
Observed Species
300
250
200
150
100
****
WT
WT + IMQ
PC2 (13.68 %)
PC1 (40.64 %)
PC3 (7.8 %)
E
Gut permeability
FITC-Dextran (µg/ml of Serum)
80
60
40
20
0
***
PBS
WT
WT
TLR7.1 Tg
+ FITC-Dextran
Gut permeability
FITC-Dextran (µg/ml of Serum)
15
12
9
6
3
0
***
PBS
WT
WT
WT + IMQ
+ FITC-Dextran

Figure S2. Related to Figure 2. Increased abundance and translocation of lactobacilli in lupus. Fecal pellets from WT, TLR7.1 Tg, and C57Bl/6 mice treated with IMQ were collected; bacterial DNA was isolated and sequenced. Cladograms of altered taxa in (A) TLR7.1 Tg and (B) C57Bl/6 mice treated with IMQ compared to WT mice. Shown are significantly altered taxa (increased in red, decreased in green). Alpha and beta diversity plots of (C) TLR7.1 Tg and (D) C57Bl/6 mice treated with IMQ compared to WT mice. (E) Mice were fasted for 4h and gavaged with FITC-Dextran. Serum FITC-dextran levels in TLR7.1 Tg and C57Bl/6 mice treated with IMQ. The results are expressed as mean ± SEM (n = 3-20 mice per group). Data are representative of at least three independent experiments. *P<0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant.

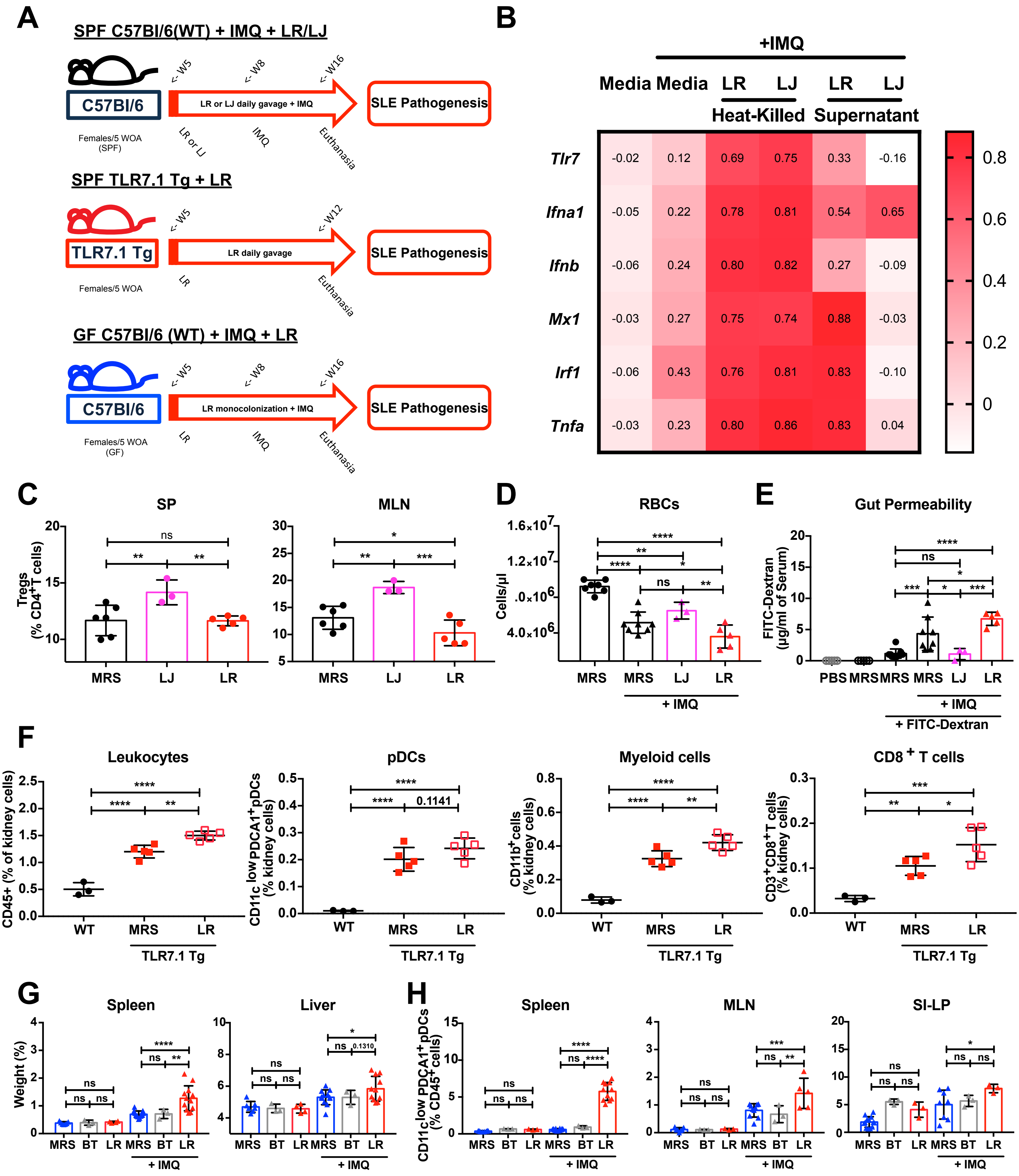
Figure S3



A
SPF C57Bl/6 (WT) cohoused with TLR7.1 Tg
C57Bl/6
Female littermates
<- W3
<- W5
<- W12
Cohousing with TLR7.1 Tg (WTL)
Weaning
Cohousing
Euthanasia
SLE Pathogenesis
SPF C57Bl/6 (WT) + IMQ + C7
C57Bl/6
Females/3 WOA (SPF)
<- W3D1
<- W3D2
<- W8
<- W16
TLR7.1 Tg cecal transfer (C7) + IMQ
C7
C7
C7
IMQ
Euthanasia
SLE Pathogenesis
B
WTL
WT
a: o__Turicibacterales
b: o__Clostridiales
c: c__Clostridia
d: o__RF32
e: o__Desulfovibrionales
f: c__Deltaproteobacteria
g: o__Campylobacterales
h: c__Epsilonproteobacteria
i: p__Proteobacteria
j: o__Verrucomicrobiales
k: c__Verrucomicrobiae
l: p__Verrucomicrobia
a
b
c
d
e
f
g
h
i
j
k
C
Shannon Diversity Index
Shannon index
8
7
6
5
4
****
WT
WTL
Observed Species
Observed Species
300
250
200
150
100
****
WT
WTL
PC2 (13.68 %)
PC1 (40.64 %)
PC3 (7.8 %)
D
Gut Permeability
FITC-Dextran (µg/ml of Serum)
80
60
40
20
0
***
**
PBS
WT
WT
WTL
TLR7.1 Tg
Cohoused
+ FITC-Dextran
E
Total IgG
IgG (µg/ml Serum)
300
200
100
0
**
ns
**
**
*
ns
W0
W7
Weeks of Cohousing
WT
WTL
TLR7.1 Tg
F
Total IgA
IgA (µg/mg feces)
0.25
0.20
0.15
0.10
0.05
0.00
ns
ns
ns
***
***
ns
W0
W7
Weeks of Cohousing
WT
WTL
TLR7.1 Tg
G
Gut Permeability
FITC-Dextran (µg/ml of Serum)
80
60
40
20
0
****
***
*
ns
ns
ns
WT
WTL
W0
W6
W12
TLR7.1 Tg
TLR7 KO
Cohoused
+ FITC-Dextran
H
Gut Permeability
FITC-Dextran (µg/ml of Serum)
25
20
15
10
5
0
****
*
**
PBS
WT
CTRL
IMQ
IMQ + C7
+ FITC-Dextran

Figure S3. Related to Figure 3. Bacterial communities increased in TLR7-dependent lupus models are transferable and exacerbate lupus-related pathogenesis. (A) Overview of the experimental design: C57Bl/6 littermates (WTL) were cohoused with TLR7.1 Tg mice. SPF C57Bl/6 mice were treated with IMQ and gavaged with cecal content of TLR7.1 Tg mice (TLR7-derived cecal microbiota; C7). Age in weeks is listed along the arrows describing the intervention. Starting age is listed below the mouse strains. Fecal pellets from WT, WTL, and C57Bl/6 mice treated with IMQ and C7 were collected; bacterial DNA was isolated and sequenced. (B) Cladogram of altered taxa in WTL compared to WT mice. Shown are significantly altered taxa (increased in red, decreased in green). (C) Alpha and beta diversity plots of WTL compared to WT mice. (D-F) WTL were cohoused with TLR7.1 Tg mice and gavaged with FITC-Dextran after 7 weeks of cohousing, at which point sera an fecal pellets were collected. (D) Serum FITC-dextran was measured 3 hours post-gavage. (E) Total IgG levels in WTL sera were measured. (F) Total IgA levels in fecal pellets supernatants. TLR7 KO mice were cohoused with TLR7.1 Tg mice and gavaged with FITC-Dextran before (W0), 6 weeks (W6), and 12 weeks (W12) after cohousing, at which point sera were collected. (G) FITC-dextran levels in TLR7 KO mice sera. (H) FITC-dextran levels in C57Bl/6 mice treated with IMQ and gavaged with C7. The results are expressed as mean ± SEM (n = 5-20 mice per group). The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant; WOA = week of age.

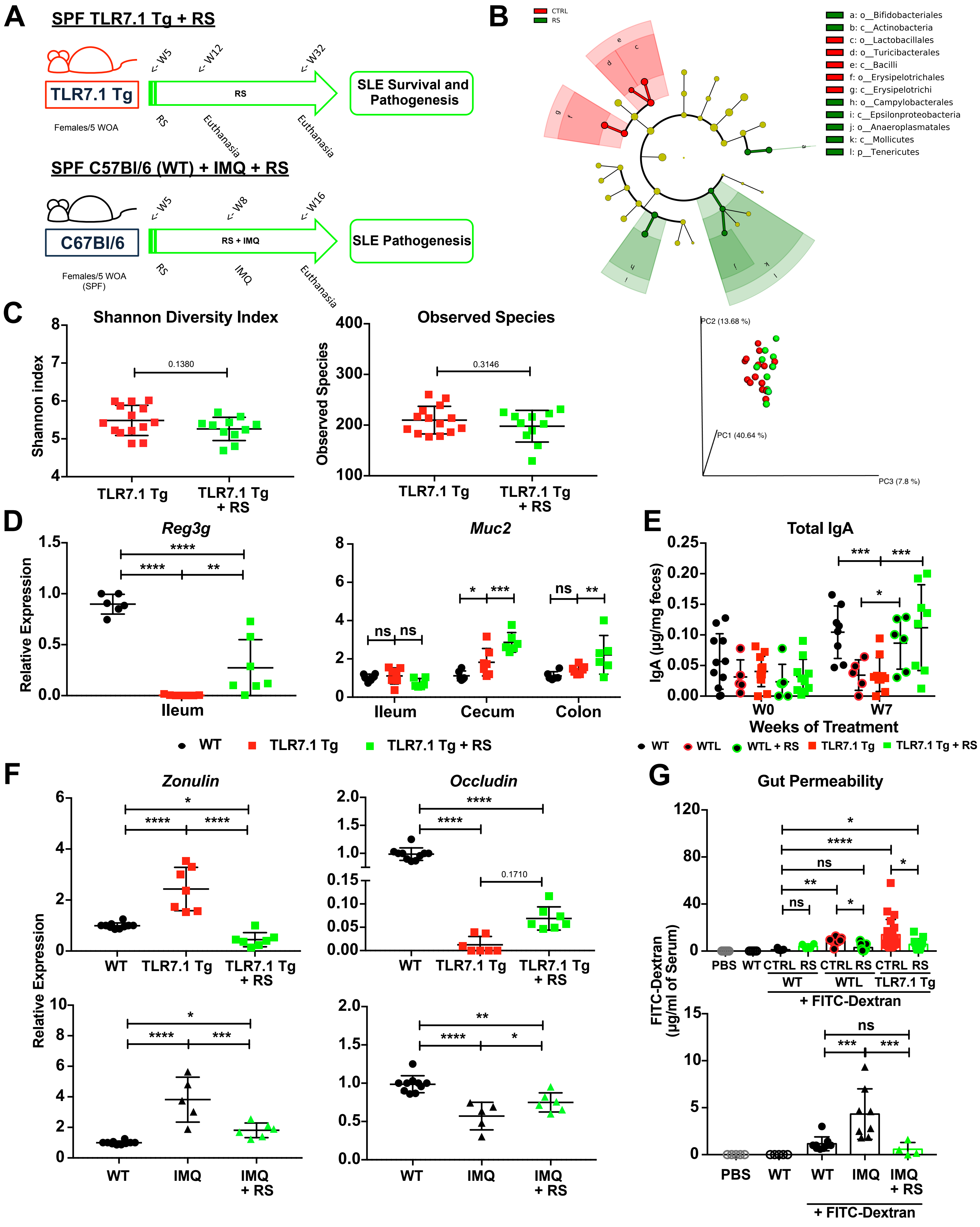
Figure S4



A
SPF C57Bl/6(WT) + IMQ + LR/LJ
C57Bl/6
Females/5 WOA (SPF)
W5
W8
W16
LR or LJ daily gavage + IMQ
LR or LJ
IMQ
Euthanasia
SLE Pathogenesis
SPF TLR7.1 Tg + LR
TLR7.1 Tg
Females/5 WOA
W5
W12
LR daily gavage
LR
Euthanasia
SLE Pathogenesis
GF C57Bl/6 (WT) + IMQ + LR
C57Bl/6
Females/5 WOA (GF)
W5
W8
W16
LR monocolonization + IMQ
LR
IMQ
Euthanasia
SLE Pathogenesis
B
+IMQ
Media Media LR LJ LR LJ
Heat-Killed Supernatant
Tlr7 -0.02 0.12 0.69 0.75 0.33 -0.16
Ifna1 -0.05 0.22 0.78 0.81 0.54 0.65
Ifnb -0.06 0.24 0.80 0.82 0.27 -0.09
Mx1 -0.03 0.27 0.75 0.74 0.88 -0.03
Irf1 -0.06 0.43 0.76 0.81 0.83 -0.10
Tnfa -0.03 0.23 0.80 0.86 0.83 0.04
0.8
0.6
0.4
0.2
0
C
SP
MLN
Tregs (% CD4⁺T cells)
ns
**
**
*
**
***
MRS LJ LR
MRS LJ LR
D
RBCs
Cells/µl
1.6×10⁷
1.2×10⁷
.0×10⁶
4.0×10⁶
****
**
****
*
ns
**
MRS MRS LJ LR
+ IMQ
E
Gut Permeability
FITC-Dextran (µg/ml of Serum)
****
ns
*
***
*
***
PBS MRS MRS MRS LJ LR
+ IMQ
+ FITC-Dextran
F
Leukocytes
CD45+ (% of kidney cells)
****
****
**
pDCs
CD11c low PDCA1⁺pDCs (% kidney cells)
****
****
0.1141
Myeloid cells
CD11b⁺cells (% kidney cells)
****
****
**
CD8⁺ T cells
CD3⁺CD8⁺T cells (% kidney cells)
***
**
*
WT MRS LR
TLR7.1 Tg
G
Spleen
Liver
Weight (%)
ns
ns
ns
****
ns
**
ns
ns
ns
*
ns
0.1310
MRS BT LR MRS BT LR
+ IMQ
H
Spleen
MLN
SI-LP
CD11c low PDCA1⁺pDCs (% CD45⁺cells)
ns
ns
ns
****
ns
****
ns
ns
ns
***
ns
**
ns
ns
ns
*
ns
ns
MRS BT LR MRS BT LR
+ IMQ

Figure S4. Related to Figure 4. *L. reuteri* exacerbates lupus-related pathogenesis. (A) Overview of the experimental design: C57Bl/6 mice were treated with imiquimod (IMQ) and gavaged daily with De Man, Rogosa and Sharpe broth (MRS), *L. reuteri* (LR), or *L. johnsonii* (LJ) primary tissue isolates harvested from TLR7.1 Tg mice. TLR7.1 Tg mice were gavaged with MRS or LR. GF C57Bl/6 mice were treated with IMQ and monocolonized with LR. Age in weeks is listed along the arrows describing the intervention. Starting age is listed below the mouse strains. (B) Splenic pDCs from SPF C57Bl/6 mice were isolated and cultured with heat-killed *L. reuteri*, *L. johnsonii*, or their bacterial supernatants in the presence of IMQ. The expression of type I interferon-related genes was analyzed in pDC-derived RNA. Heat-map is shown including the log-fold changes of the relative expression of type I interferon-related genes compared to untreated pDCs. (C-E) SPF C57Bl/6 mice received MRS, *L. reuteri*, or *L. johnsonii*. (C) Treg frequencies in spleen and MLN, and (D) red blood cell (RBC) counts in whole blood were determined by flow cytometry and cell counting, respectively. (E) Mice were fasted for 4h and gavaged with FITC-dextran. Serum FITC-dextran levels were measured 3 hours post-gavage. (F) Kidneys from *L. reuteri*-gavaged TLR7.1 Tg mice were harvested for immunologic analysis. Shown are frequencies of leukocytes, pDCs, myeloid cells, and CD8+ T cells in the kidneys. (G-H) GF C57Bl/6 mice were treated with IMQ and monocolonized with *L. reuteri* or *B. thetaiotaomicron* (BT). Mice were sacrificed for organ weights and immunologic analysis. (G) Shown are weights of spleen and liver. (H) Cells from spleen, MLN, and SI-LP were isolated and analyzed by flow cytometry. Shown are frequency of pDCs in spleen, MLN, and SI-LP. The results are expressed as mean ± SEM (n = 3-16 mice per group). The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant; WOA = week of age.

Figure S5



A
SPF TLR7.1 Tg + RS
TLR7.1 Tg
Females/5 WOA
<- W5
<- W12
<- W32
RS
RS
Euthanasia
Euthanasia
SLE Survival and Pathogenesis
SPF C57Bl/6 (WT) + IMQ + RS
C67Bl/6
Females/5 WOA (SPF)
<- W5
<- W8
<- W16
RS + IMQ
RS
IMQ
Euthanasia
SLE Pathogenesis
B
CTRL
RS
a: o__Bifidobacteriales
b: c__Actinobacteria
c: o__Lactobacillales
d: o__Turicibacterales
e: c__Bacilli
f: o__Erysipelotrichales
g: c__Erysipelotrichi
h: o__Campylobacterales
i: c__Epsilonproteobacteria
j: o__Anaeroplasmatales
k: c__Mollicutes
l: p__Tenericutes
C
Shannon Diversity Index
Shannon index
0.1380
TLR7.1 Tg
TLR7.1 Tg + RS
Observed Species
Observed Species
0.3146
TLR7.1 Tg
TLR7.1 Tg + RS
PC2 (13.68 %)
PC1 (40.64 %)
PC3 (7.8 %)
D
Reg3g
Relative Expression
Ileum
Muc2
Ileum
Cecum
Colon
E
Total IgA
IgA (μg/mg feces)
W0
W7
Weeks of Treatment
● WT
■ TLR7.1 Tg
■ TLR7.1 Tg + RS
● WT
● WTL
● WTL + RS
■ TLR7.1 Tg
■ TLR7.1 Tg + RS
F
Zonulin
Relative Expression
WT
TLR7.1 Tg
TLR7.1 Tg + RS
Occludin
0.1710
WT
TLR7.1 Tg
TLR7.1 Tg + RS
WT
IMQ
IMQ + RS
WT
IMQ
IMQ + RS
G
Gut Permeability
FITC-Dextran (μg/ml of Serum)
PBS
WT
CTRL
RS
CTRL
RS
CTRL
RS
WT
WTL
TLR7.1 Tg
+ FITC-Dextran
PBS
WT
WT
IMQ
IMQ + RS
+ FITC-Dextran

Figure S5. Related to Figure 5. RS modulates the gut microbiome and decreases *L. reuteri* translocation. (A) Overview of the experimental design: TLR7.1 Tg mice were fed with RS and C57Bl/6 mice were treated with IMQ and fed with RS. Age in weeks is listed along the arrows describing the intervention. Starting age is listed below the mouse strains. Fecal pellets and gut segments from WT, TLR7.1 Tg, and TLR7.1 Tg mice fed with RS were collected; bacterial DNA was isolated and sequenced. (B) Cladogram of altered taxa in TLR7.1 Tg mice fed with RS compared to control (CTRL) diet. Shown are significantly altered taxa (increased in red, decreased in green). (C) Alpha and beta diversity plots of TLR7.1 Tg mice fed with RS compared to CTRL diet. Gut segments and fecal samples were collected from TLR7.1 Tg mice for RNA expression analysis and IgA quantification. (D) *RegIIIg* and *mucin* relative mRNA expression in ileum, cecum and colon. (E) Total IgA levels in fecal supernatants. (F) *Zonulin* and *occludin* relative mRNA expression in ileum. (G) Mice were fasted for 4h and gavaged with FITC-dextran. Serum FITC-Dextran levels were measured 3 hours post-gavage in RS-fed mice. The results are expressed as mean ± SEM (n = 3-20 mice per group). Data are representative of at least three independent experiments. *P<0.05 was considered statistically significant; **P <0.01; ***P<0.001; ****P<0.0001; ns = not significant; WOA = week of age.

Figure S6

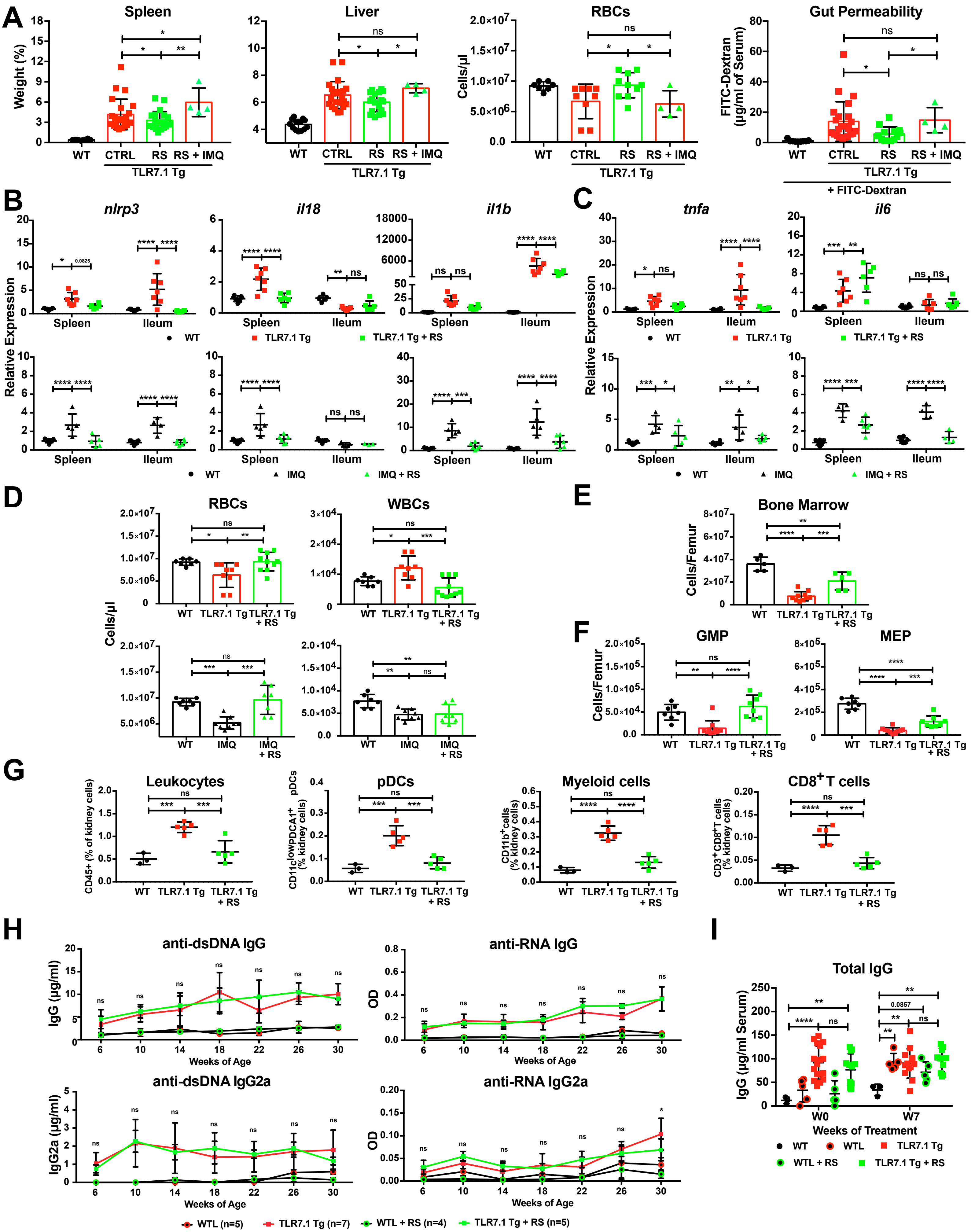
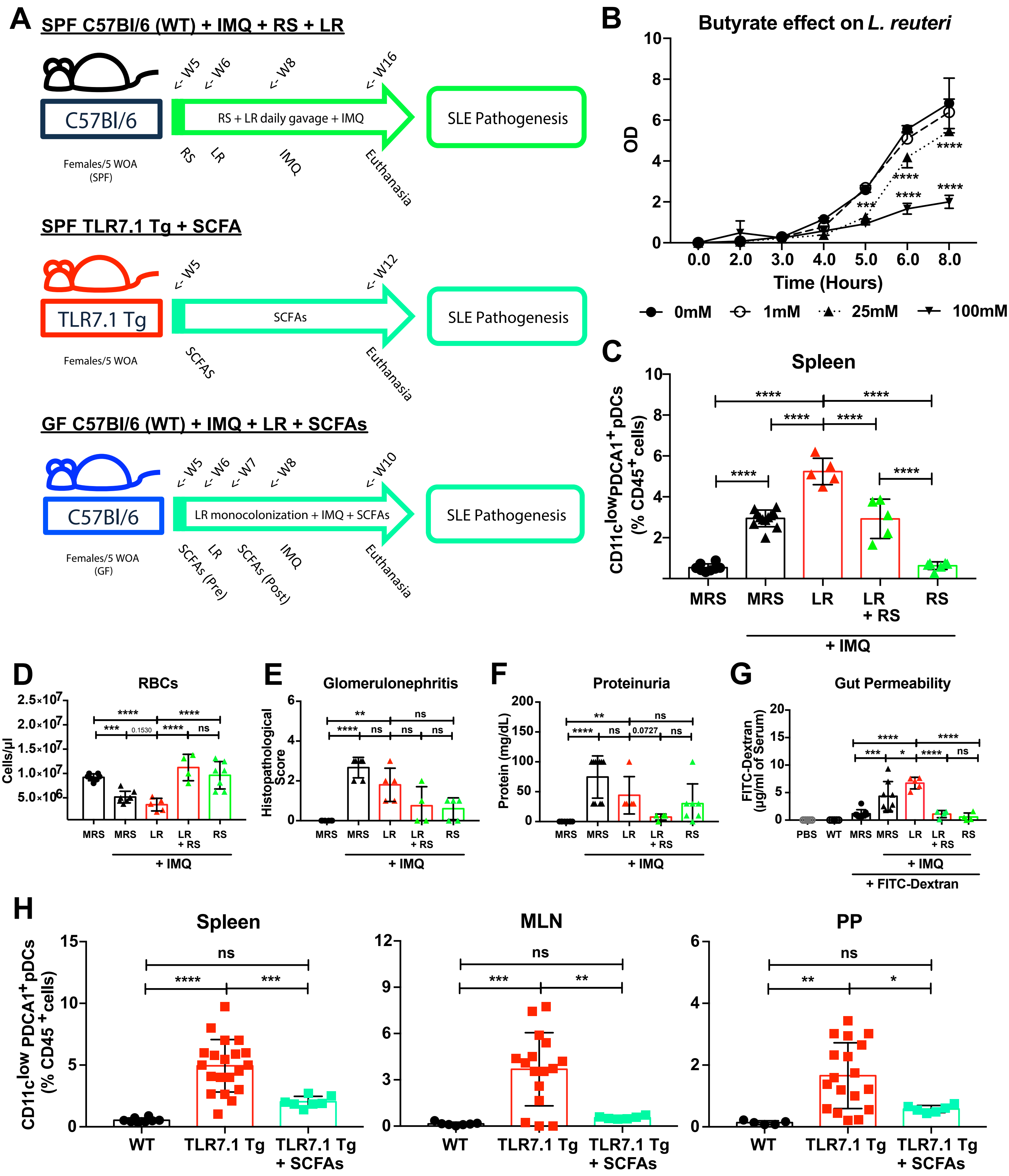

A
Spleen
Weight (%)
WT
CTRL
RS
RS + IMQ
TLR7.1 Tg
Liver
RBCs
Cells/µl
Gut Permeability
FITC-Dextran (µg/ml of Serum)
+ FITC-Dextran
ns
B
nlrp3
il18
il1b
Relative Expression
Spleen
Ileum
0.0825
WT
TLR7.1 Tg
TLR7.1 Tg + RS
IMQ
IMQ + RS
C
tnfa
il6
D
RBCs
WBCs
Cells/µl
TLR7.1 Tg + RS
IMQ + RS
E
Bone Marrow
Cells/Femur
F
GMP
MEP
G
Leukocytes
CD45+ (% of kidney cells)
pDCs
CD11c^low PDCA1+ pDCs (% kidney cells)
Myeloid cells
CD11b+ cells (% kidney cells)
CD8+ T cells
CD3+CD8+ T cells (% kidney cells)
H
anti-dsDNA IgG
IgG (µg/ml)
Weeks of Age
anti-RNA IgG
OD
anti-dsDNA IgG2a
IgG2a (µg/ml)
anti-RNA IgG2a
WTL (n=5)
TLR7.1 Tg (n=7)
WTL + RS (n=4)
TLR7.1 Tg + RS (n=5)
I
Total IgG
IgG (µg/ml Serum)
W0
W7
Weeks of Treatment
0.0857
WT
WTL
TLR7.1 Tg
WTL + RS
TLR7.1 Tg + RS

Figure S6. Related to Figure 6. RS suppresses lupus-related mortality and pathogenesis. RS-fed TLR7.1 Tg mice were treated with IMQ for 4 weeks and were sacrificed at 12 weeks of age. (A) Weights of spleen and liver, red blood cell counts, and serum FITC-dextran levels in RS-fed IMQ-treated mice. Experimental cohorts of TLR7.1 Tg and C57Bl/6 mice treated with IMQ were fed RS and sacrificed at weeks 12 and 16, respectively. (B) *Nlrp3*, *il18*, and *il1b* relative mRNA expression in spleen and ileum. (C) *Tnfa* and *il6* relative mRNA expression in spleen and ileum. (D) Red blood cells and white blood cells counts. Bone marrow cells were isolated from the left femur and granulocyte-monocyte progenitors as well as megakaryocyte-erythrocyte progenitors were analyzed by flow cytometry. (E) Bone marrow cell counts. (F) Total granulocyte-monocyte and megakaryocyte-erythrocyte progenitor numbers in the bone marrow. (G) Cells from kidneys of RS-fed TLR7.1 Tg mice were isolated and analyzed by flow cytometry. Shown are frequencies of leukocytes, pDCs, myeloid cells, and CD8+ T cells in the kidneys. (H) WTL, TLR7.1 Tg, and TLR7.1 Tg mice fed with RS were bled longitudinally and sera were collected for ELISA. Shown are IgG and IgG2a anti-dsDNA and anti-ssRNA autoantibodies. (I) WT, WTL, and TLR7.1 Tg mice were bled before and after RS feeding. Shown are total IgG levels. The results are expressed as mean ± SEM (Survival cohorts n = 8-28 mice per group; experimental cohorts n = 3-20 mice per group). The results are representative of at least two independent experiments. *P <0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant; RBCs = red blood cells; WBCs = white blood cells; GMP = granulocyte-monocyte progenitors; CMP = common myeloid progenitors; MEP = megakaryocyte-erythrocyte progenitors.

Figure S7



A
SPF C57Bl/6 (WT) + IMQ + RS + LR
C57Bl/6
Females/5 WOA
(SPF)
W5
W6
W8
W16
RS + LR daily gavage + IMQ
RS
LR
IMQ
Euthanasia
SLE Pathogenesis
SPF TLR7.1 Tg + SCFA
TLR7.1 Tg
Females/5 WOA
W5
W12
SCFAs
SCFAS
Euthanasia
SLE Pathogenesis
GF C57Bl/6 (WT) + IMQ + LR + SCFAs
C57Bl/6
Females/5 WOA
(GF)
W5
W6
W7
W8
W10
LR monocolonization + IMQ + SCFAs
SCFAs (Pre)
LR
SCFAs (Post)
IMQ
Euthanasia
SLE Pathogenesis
B
Butyrate effect on L. reuteri
OD
Time (Hours)
0.0
2.0
3.0
4.0
5.0
6.0
8.0
0
2
4
6
8
10
***
****
****
****
****
0mM
1mM
25mM
100mM
C
Spleen
CD11c^low PDCA1^+ pDCs (% CD45^+ cells)
0
2
4
6
8
10
****
****
****
****
****
****
MRS
MRS
LR
LR + RS
RS
+ IMQ
D
RBCs
Cells/µl
5.0×10^6
1.0×10^7
1.5×10^7
2.0×10^7
2.5×10^7
****
****
***
0.1530
****
ns
MRS
MRS
LR
LR + RS
RS
+ IMQ
E
Glomerulonephritis
Histopathological Score
0
2
4
6
**
ns
****
ns
ns
ns
MRS
MRS
LR
LR + RS
RS
+ IMQ
F
Proteinuria
Protein (mg/dL)
0
50
100
150
200
**
ns
****
ns
0.0727
ns
MRS
MRS
LR
LR + RS
RS
+ IMQ
G
Gut Permeability
FITC-Dextran (µg/ml of Serum)
0
5
10
15
20
****
****
***
*
****
ns
PBS
WT
MRS
MRS
LR
LR + RS
RS
+ IMQ
+ FITC-Dextran
H
Spleen
CD11c^low PDCA1^+ pDCs (% CD45^+ cells)
0
5
10
15
ns
****
***
WT
TLR7.1 Tg
TLR7.1 Tg + SCFAs
MLN
0
3
6
9
12
ns
***
**
WT
TLR7.1 Tg
TLR7.1 Tg + SCFAs
PP
0
2
4
6
ns
**
*
WT
TLR7.1 Tg
TLR7.1 Tg + SCFAs

Figure S7. Related to Figure 7. RS-induced SCFAs inhibit *L. reuteri* growth in vitro and *L. reuteri*-mediated autoimmunity in vivo. (A) Overview of the experimental design: SPF C57Bl/6 WT mice were treated with imiquimod (IMQ), fed with RS, and gavaged daily with MRS media, *L. reuteri* (LR), or *L. johnsonii* (LJ) primary tissue isolates harvested from TLR7.1 Tg mice. TLR7.1 Tg mice were given SCFAs in their drinking water. GF C57Bl/6 mice received SCFAs one week before (Pre) or after (Post) *L. reuteri* monocolonization and were treated with IMQ. Age in weeks is listed along the arrows describing the intervention. Starting age is listed below the mouse strains. (B) Relative growth of LR cultured anaerobically with increasing doses of butyrate compared to MRS media alone. (C-H) RS-fed *L. reuteri*-gavaged IMQ-treated mice were sacrificed for immunological analysis and organ involvement. (C) Frequencies of pDCs in spleen. (D) Red blood cells counts. (E) Histopathologic scoring for glomerulonephritis. (F) Quantification of proteinuria. (G) Serum FITC-dextran levels 3 hours post-gavage with FITC-dextran. (H) Frequencies of pDCs in spleen, MLN, and PP of SCFAs-treated TLR7.1 Tg mice. The results are expressed as mean ± SEM (n = 3-20 mice per group). The results are representative of at least two independent experiments. *P<0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant; WOA = week of age; RBCs = red blood cells.

Table S1. Related to Figure 2. List of modulated bacterial taxa in TLR7.1 Tg mice.

Table S1. List of modulated taxa in TLR7.1 Tg mice

WT vs. TLR7.1 Tg

Enriched [a]

Rank [c]	Family	Genus	Species	P value
1	*Rikenellaceae*	*AF12*	not identified	2.73E-06
2	*Helicobacteraceae*	not identified	not identified	2.74E-06
3	*Paraprevotellaceae*	*Paraprevotella*	not identified	2.81E-06
4	***Lactobacillaceae***	***Lactobacillus***	***reuteri***	**2.82E-06**
5	*Desulfovibrionaceae*	not identified	not identified	3.74E-06
6	*Rikenellaceae*	not identified	not identified	4.81E-06
7	*Paraprevotellaceae*	not identified	not identified	7.64E-06
8	*Paraprevotellaceae*	*Prevotella*	not identified	7.64E-06
9	*Odoribacteraceae*	*Odoribacter*	not identified	9.82E-06
10	*Odoribacteraceae*	not identified	not identified	9.82E-06
11	*Porphyromonadaceae*	*Parabacteroides*	not identified	1.58E-05
12	*Porphyromonadaceae*	not identified	not identified	1.58E-05
13	*Coriobacteriaceae*	not identified	not identified	2.22E-05
14	*Desulfovibrionaceae*	*Desulfovibrio*	not identified	3.12E-05
15	*Helicobacteraceae*	*Helicobacter*	not identified	7.65E-05
16	*Deferribacteraceae*	*Mucispirillum*	not identified	2.01E-04
17	*Deferribacteraceae*	not identified	not identified	2.01E-04
18	*Deferribacteraceae*	*Mucispirillum*	*schaedleri*	2.01E-04
19	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	6.12E-04
20	*Erysipelotrichaceae*	not identified	not identified	2.23E-03
21	*Erysipelotrichaceae*	*Allobaculum*	not identified	3.08E-03
22	*Desulfovibrionaceae*	*Bilophila*	not identified	9.25E-03
23	*Rikenellaceae*	*Rikenella*	not identified	2.00E-02

Suppressed [b]

Rank [c]	Family	Genus	Species	P value
1	*Lachnospiraceae*	*Anaerostipes*	not identified	7.55E-06
2	*Verrucomicrobiaceae*	not identified	not identified	7.58E-06
3	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	7.58E-06
4	*Verrucomicrobiaceae*	*Akkermansia*	not identified	7.58E-06
5	*Turicibacteraceae*	not identified	not identified	1.34E-04
6	*Turicibacteraceae*	*Turicibacter*	not identified	1.34E-04
7	*Anaeroplasmataceae*	*Anaeroplasma*	not identified	1.08E-03
8	*Anaeroplasmataceae*	not identified	not identified	1.08E-03
9	***Clostridiaceae***	**not identified**	**not identified**	**5.50E-03**
10	*Lachnospiraceae*	not identified	not identified	5.67E-03
11	*Mitochondria*	not identified	not identified	1.69E-02
12	*Bifidobacteriaceae*	*Bifidobacterium*	not identified	1.82E-02
13	*Lachnospiraceae*	*Ruminococcus*	not identified	1.85E-02
14	*Lachnospiraceae*	*Ruminococcus*	*gnavus*	1.85E-02

[a] Bacterial taxa that was significantly enriched in TLR7.1 Tg mice fecal pellets.

[b] Bacterial taxa that was significantly suppressed in TLR7.1 Tg mice fecal pellets.

[c] Bacterial taxa modulated after False Discovery Rate analysis (q<0.05)

Table S2. Related to Figure 2. List of modulated bacterial taxa in IMQ-treated mice.

Table S2. List of modulated taxa in IMQ-treated mice				
WT vs. WT + IMQ				
Enriched [a]				
Rank [c]	Family	Genus	Species	P value
1	*Desulfovibrionaceae*	*Desulfovibrio*	not identified	1.07E-04
2	*Desulfovibrionaceae*	not identified	not identified	1.25E-04
3	*Prevotellaceae*	not identified	not identified	1.39E-04
4	*Prevotellaceae*	*Prevotella*	not identified	1.39E-04
5	*Rikenellaceae*	not identified	not identified	1.94E-04
6	***Lactobacillaceae***	***Lactobacillus***	***reuteri***	**2.92E-04**
7	*Coriobacteriaceae*	not identified	not identified	7.10E-04
8	*Bifidobacteriaceae*	*Bifidobacterium*	not identified	1.36E-03
9	*Bifidobacteriaceae*	not identified	not identified	1.36E-03
Suppressed [b]				
Rank [c]	Family	Genus	Species	P value
1	*Lachnospiraceae*	*Anaerostipes*	not identified	1.60E-05
2	*Turicibacteraceae*	not identified	not identified	2.98E-05
3	*Turicibacteraceae*	*Turicibacter*	not identified	2.98E-05
4	***Clostridiaceae***	**not identified**	**not identified**	**2.75E-04**
5	*Erysipelotrichaceae*	*Coprobacillus*	not identified	1.19E-02

[a] Bacterial taxa that was significantly enriched in WT + IMQ mice fecal pellets.

[b] Bacterial taxa that was significantly suppressed in WT + IMQ mice fecal pellets.

[c] Bacterial taxa modulated after False Discovery Rate analysis (q<0.05)

Table S3. Related to Figure 3. List of modulated bacterial taxa in cohoused mice.

Table S3. List of modulated taxa in cohoused mice				
WT vs. WTL				
Enriched [a]				
Rank [c]	Family	Genus	Species	P value
1	*Rikenellaceae*	*AF12*	not identified	4.74E-05
2	*Desulfovibrionaceae*	*Desulfovibrio*	not identified	4.96E-05
3	*Helicobacteraceae*	not identified	not identified	5.03E-05
4	*Paraprevotellaceae*	*Paraprevotella*	not identified	5.03E-05
5	***Lactobacillaceae***	***Lactobacillus***	***reuteri***	**5.03E-05**
6	*Odoribacteraceae*	*Odoribacter*	not identified	5.03E-05
7	*Odoribacteraceae*	not identified	not identified	5.03E-05
8	*Desulfovibrionaceae*	not identified	not identified	1.36E-04
9	*Helicobacteraceae*	*Helicobacter*	not identified	1.68E-04
10	*Rikenellaceae*	not identified	not identified	2.66E-04
11	*Coriobacteriaceae*	not identified	not identified	2.69E-04
12	*Porphyromonadaceae*	*Parabacteroides*	not identified	5.33E-04
13	*Porphyromonadaceae*	not identified	not identified	5.33E-04
14	*Paraprevotellaceae*	not identified	not identified	7.91E-04
15	*Paraprevotellaceae*	*Prevotella*	not identified	7.91E-04
16	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	8.84E-04
17	*Deferribacteraceae*	*Mucispirillum*	not identified	1.22E-02
18	*Deferribacteraceae*	not identified	not identified	1.22E-02
19	*Deferribacteraceae*	*Mucispirillum*	*schaedleri*	1.22E-02
20	*Mycoplasmataceae*	not identified	not identified	1.90E-02
21	*Rikenellaceae*	*Rikenella*	not identified	1.90E-02
22	*Erysipelotrichaceae*	not identified	not identified	2.05E-02
23	*Erysipelotrichaceae*	*Allobaculum*	not identified	2.05E-02
24	*Alcaligenaceae*	not identified	not identified	2.49E-02
25	*Alcaligenaceae*	*Sutterella*	not identified	2.49E-02
Suppressed [b]				
Rank [c]	Family	Genus	Species	P value
1	*Lachnospiraceae*	not identified	not identified	1.89E-03
2	*Lachnospiraceae*	*Anaerostipes*	not identified	2.58E-03
3	*Verrucomicrobiaceae*	not identified	not identified	2.59E-03
4	*Verrucomicrobiaceae*	*Akkermansia*	*muciniphila*	2.59E-03
5	*Verrucomicrobiaceae*	*Akkermansia*	not identified	2.59E-03
6	*Ruminococcaceae*	*Ruminococcus*	not identified	4.94E-03
7	*Turicibacteraceae*	not identified	not identified	8.30E-03
8	*Turicibacteraceae*	*Turicibacter*	not identified	8.30E-03
9	*Anaeroplasmataceae*	*Anaeroplasma*	not identified	1.11E-02
10	*Anaeroplasmataceae*	not identified	not identified	1.11E-02
11	***Clostridiaceae***	**not identified**	**not identified**	**2.62E-02**
12	*Lachnospiraceae*	*Ruminococcus*	not identified	2.65E-02
13	*Lachnospiraceae*	*Ruminococcus*	*gnavus*	2.65E-02
14	*Ruminococcaceae*	not identified	not identified	2.66E-02

[a] Bacterial taxa that was significantly enriched in WTL (WT littermate cohoused mice) fecal pellets.

[b] Bacterial taxa that was significantly suppressed in WTL mice (WT littermate cohoused mice) fecal pellets.

[c] Bacterial taxa modulated after False Discovery Rate analysis (q<0.05)

Table S4. Related to Figure 5. Composition of diets.

Table S4. Composition of diets				
% By weight	**Standard Diet**	**RS Diet**	**GF Standard Diet**	**GF RS Diet**
Protein	18.6	13.7	18.6	13.7
Carbohydrate	44.2	53.9	44.2	53.7
Fat	6.2	4.1	6.2	4.1
Kcal/g	3.1	3.1	3.1	3.1

Standard diet: Teklad Global 18% Protein Rodent Diet (2018).
RS diet: Unpurified Teklad Custom Diet TD.150492 - 40% HM-260 (RS) (2018).
GF standard diet: Teklad Global 18% Protein Rodent Diet (Sterilizable, 2018S).
GF RS diet: Unpurified Teklad Custom Diet TD.160604 - 40% HM-260 (RS) (2018S).

Table S5. Related to Figure 5. List of modulated bacterial taxa in RS-fed mice.

Table S5. List of modulated bacterial taxa in RS-fed mice				
TLR7.1 Tg vs. TLR7.1 Tg + RS				
Enriched [a]				
Rank [c]	Family	Genus	Species	P value
1	*Bacteroidaceae*	*Bacteroides*	*acidifaciens*	5.00E-05
2	*Anaeroplasmataceae*	*Anaeroplasma*	not identified	3.03E-03
Suppressed [b]				
Rank [c]	Family	Genus	Species	P value
1	*Erysipelotrichaceae*	not identified	not identified	1.91E-04
2	***Lactobacillaceae***	***Lactobacillus***	***reuteri***	**1.23E-03**
3	*Lactobacillaceae*	*Lactobacillus*	not identified	2.60E-03

[a] Bacterial taxa that was significantly enriched in TLR7.1 Tg + RS mice fecal pellets.
[b] Bacterial taxa that was significantly suppressed in TLR7.1 Tg + RS mice fecal pellets.
[c] Bacterial taxa modulated after False Discovery Rate analysis (q<0.05).

Table S6. Related to Figure 6. Frequencies of key immune populations in RS-fed mice.

Table S6. Frequencies of key immune populations in RS-fed mice					
Tregs	**WT**	**TLR7.1 Tg**	**TLR7.1 Tg + RS**	**P value [a]**	**Significance**
Blood	7.500±0.995	18.474±3.204	21.362±4.971	0.5659	ns
Spleen	8.699±0.589	14.712±1.962	12.798±1.909	0.4388	ns
MLN	6.370±0.528	14.361±1.814	8.342±1.779	0.0216	*
PP	6.352±0.645	18.116±1.834	17.883±2.496	0.9339	ns
SI-LP	9.853±1.324	17.866±2.138	13.801±1.706	0.1640	ns
SI-IEL	7.102±1.268	10.472±1.835	9.877±1.443	0.8036	ns
Th17	**WT**	**TLR7.1 Tg**	**TLR7.1 Tg + RS**	**P value [a]**	**Significance**
Spleen	1.377±0.198	3.197±0.330	2.240±0.267	0.0088	**
MLN	0.542±0.053	1.422±0.179	0.930±0.246	0.1460	ns
Neutrophils	**WT**	**TLR7.1 Tg**	**TLR7.1 Tg + RS**	**P value [a]**	**Significance**
Blood	0.371±0.134	1.290±0.230	1.711±0.427	0.3059	ns
Spleen	0.443±0.067	3.659±0.604	2.357±0.448	0.0436	*
MLN	0.033±0.018	0.386±0.110	0.261±0.050	0.2354	ns
PP	0.035±0.021	0.205±0.038	0.045±0.014	0.0001	****
SI-LP	0.244±0.086	1.477±0.546	1.489±0.619	0.9849	ns
SI-IEL	0.165±0.049	0.859±0.535	1.040±0.639	0.7708	ns
$CD44^+CD4^+$ T cells	**WT**	**TLR7.1 Tg**	**TLR7.1 Tg + RS**	**P value [a]**	**Significance**
Spleen	18.267±2.488	85.025±1.221	59.125±3.895	0.0001	****
MLN	9.387±0.598	39.175±4.695	23.750±1.690	0.0017	**
$CD44^+CD8^+$ T cells	**WT**	**TLR7.1 Tg**	**TLR7.1 Tg + RS**	**P value [a]**	**Significance**
Spleen	18.067±1.097	89.875±0.985	59.100±5.387	0.0001	****
MLN	6.970±0.119	40.150±2.911	26.050±1.051	0.0022	**

[a] P values listed represent TLR7.1 Tg vs. TLR7.1 Tg + RS comparisons.

The results are expressed as the mean percentage ± SEM (n = 3-21 mice per group).

*P<0.05 was considered statistically significant; **P<0.01; ***P<0.001; ****P<0.0001; ns = not significant; MLN = mesenteric lymph nodes; PP = Peyer's patches; SI-LP = small intestine lamina propria; SI-IEL = small intestine intraepithelial leukocytes

Table S7. Related to Figure 7. SCFAs levels in RS-fed mice.

Table S7. SCFAs levels in RS-fed mice

	Sample	SCFAs	WT	WT + RS	P value [a]
GF mice	**Fecal Pellets (μmol/g)**	Acetate	na	na	na
		Propionate	na	na	na
		Butyrate	na	na	na
	Colon (μmol/g)	Acetate	1.905±0.99	1.511±0.22	0.9421
		Propionate	0.139±0.12	0.036±0.01	0.8990
		Butyrate	0.166±0.15	0.047±0.02	0.9212
	Cecum (μmol/g)	Acetate	0.825±0.15	0.382±0.04	0.9350
		Propionate	0.021±0.01	0.010±0.01	0.9901
		Butyrate	0.017±0.01	0.007±0.01	0.9927

	Sample	SCFAs	WT	WT + RS	P value [b]	TLR7.1 Tg	TLR7.1 Tg + RS	P value [c]	P value [d]
SPF mice	**Fecal Pellets (μmol/g)**	Acetate	10.625±0.47	14.950±1.27	0.1313	25.000±1.48	21.000±1.61	0.1746	**0.0001**
		Propionate	4.088±0.11	3.783±0.29	0.7308	7.190±0.58	7.367±0.80	0.8344	**0.0002**
		Butyrate	2.534±0.24	4.007±0.64	**0.0327**	3.509±0.56	3.405±0.44	0.8746	0.1123
	Colon (μmol/g)	Acetate	25.780±6.78	23.018±4.07	0.5830	14.449±4.85	7.016±2.08	0.0897	**0.0215**
		Propionate	3.779±0.52	5.551±0.41	**0.0233**	4.102±1.06	2.066±0.61	**0.0030**	0.6523
		Butyrate	5.215±1.27	3.710±1.09	0.1812	1.515±0.63	1.349±0.52	0.8616	**0.0011**
	Cecum (μmol/g)	Acetate	28.725±4.14	29.200±3.30	0. 9246	28.192±2.15	19.464±2.59	**0.0478**	0.9109
		Propionate	4.478±0.35	6.286±0.32	**0.0209**	5.162±0.26	4.629±0.24	0.4127	0.3434
		Butyrate	5.472±0.63	9.202±0.86	**0.0017**	3.628±0.62	4.009±0.93	0.6895	0.0865

[a] P values listed represent GF WT vs. GF WT + RS comparisons.
[b] P values listed represent SPF WT vs. SPF WT + RS comparisons.
[c] P values listed represent TLR7.1 Tg vs. TLR7.1 Tg + RS comparisons.
[d] P values listed represent SPF WT vs. TLR7.1 Tg comparisons.
The results are expressed as the mean ± SEM (n = 3-10 mice per group).
*P values in bold represent statistical significance; na = not analyzed