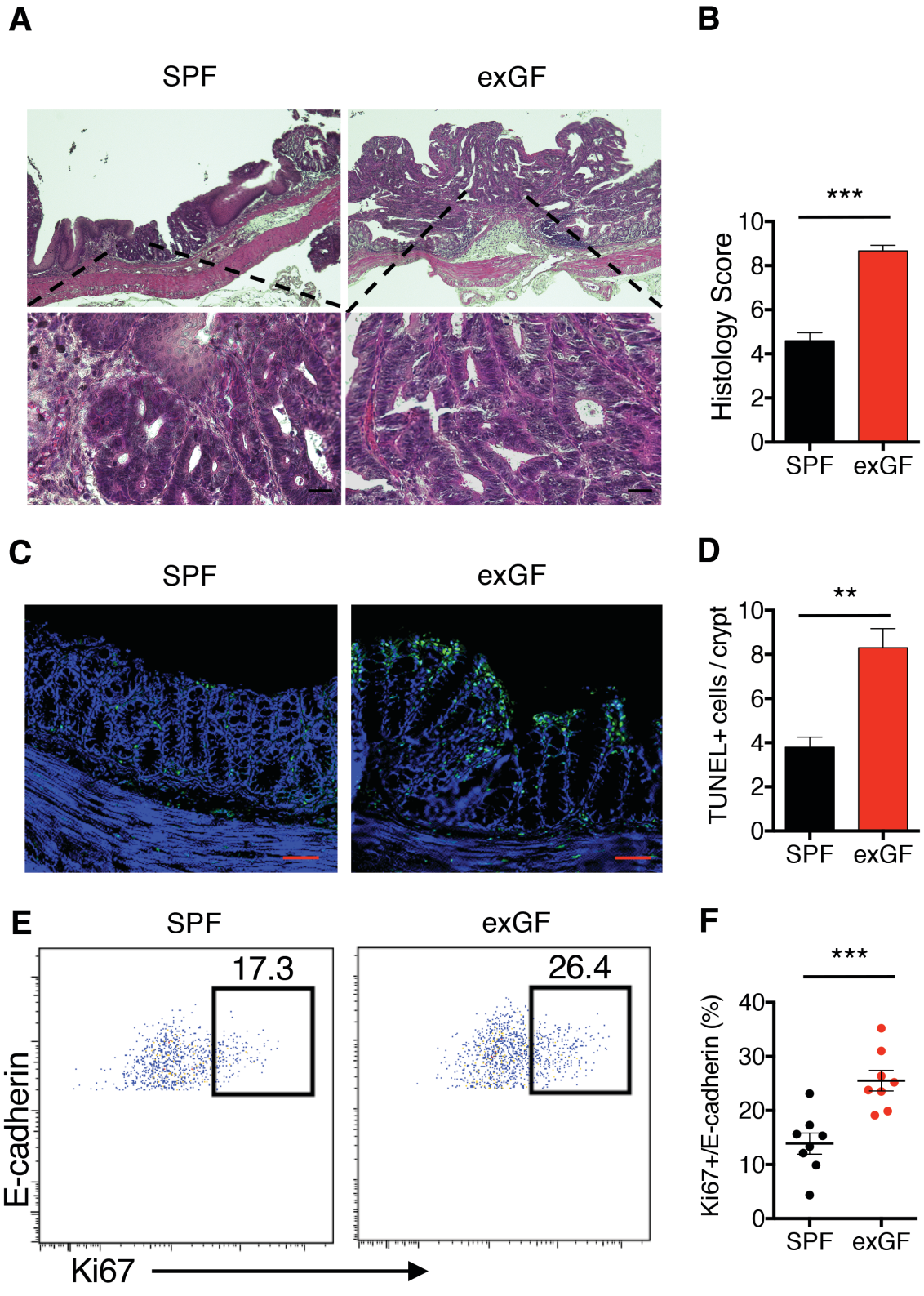
A
SPF
exGF
B
***
Histology Score
10
8
6
4
2
0
SPF
exGF
C
SPF
exGF
D
**
TUNEL+ cells / crypt
10
8
6
4
2
0
SPF
exGF
E
SPF
exGF
17.3
26.4
E-cadherin
Ki67
F
***
Ki67+/E-cadherin (%)
40
30
20
10
0
SPF
exGF

Figure S1.

Enhanced colonic tumor size and epithelial turnover in exGF mice. **A**, Representative of H&E-stained sections of colonic tissue and histology scores from AOM/DSS-treated SPF and exGF mice at day 122 are shown (scale bar, 20 μm). **B**, Epithelial cell apoptosis was analyzed by terminal deoxynucleotidyl transferase deoxyuridine triphosphate nick-end labeling (TUNEL). **C**, Representative confocal images of TUNEL assay: TUNEL, green; DNA, blue. Scale bar, 50μm. **D,** TUNEL+ DAPI+ cells were counted and averaged per crypt. Proliferative activity of intestinal epithelial cells *in situ*. **E**, Intestinal epithelial cells were isolated from colonic tissue and frequencies of Ki67+ among E-Cadherin+CD45- cells in colonic mucosa from SPF (n=8; 4 males and 4 females) and exGF (n=8; 4 males and 4 females) mice were analyzed using flow cytometry. **F,** each dot represents the frequency of the Ki67+ among E-Cadherin+CD45- cells in the colonic mucosa from individual mice. Data are the means ± SEM. Data are the means ± SEM. $^{**}P < 0.01$, $^{***}P < 0.001$.

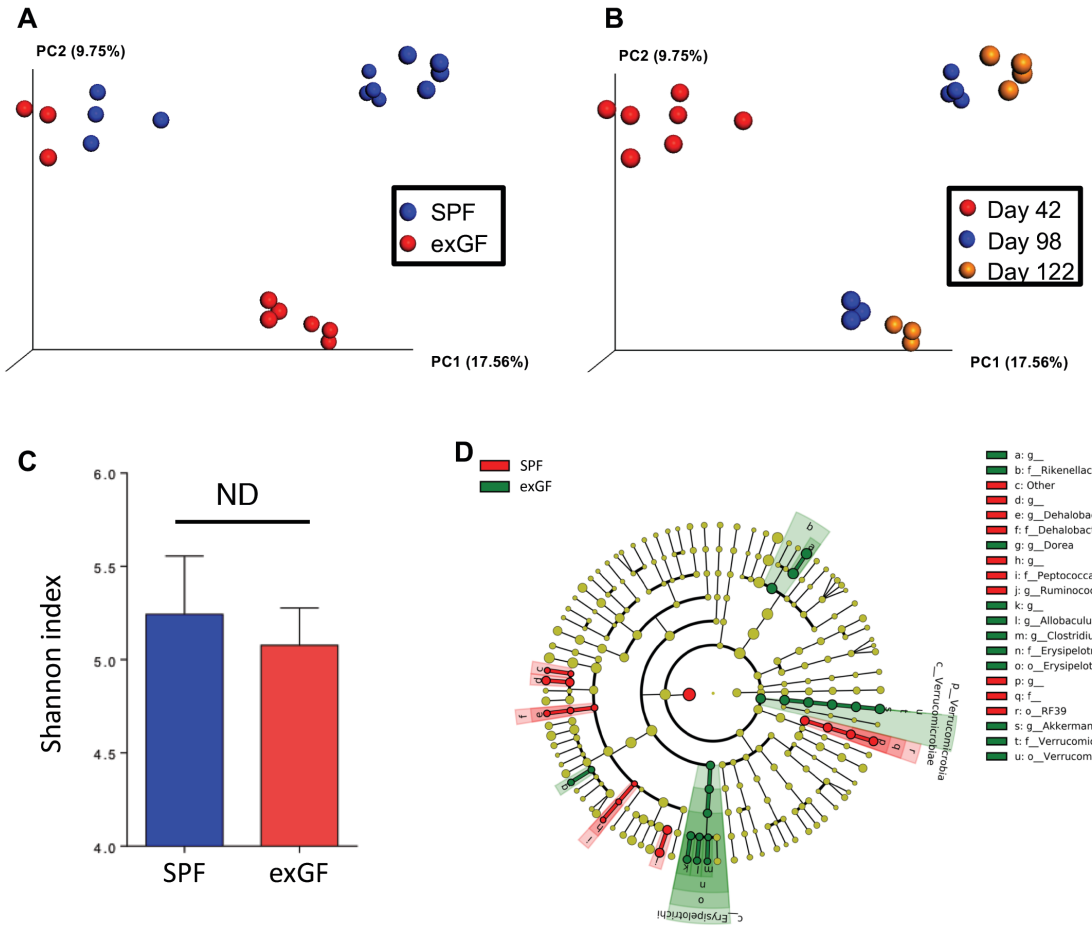
A
PC2 (9.75%)
PC1 (17.56%)
SPF
exGF
B
PC2 (9.75%)
PC1 (17.56%)
Day 42
Day 98
Day 122
C
ND
Shannon index
6.0
5.5
5.0
4.5
4.0
SPF
exGF
D
SPF
exGF
a: g__
b: f__Rikenellaceae
c: Other
d: g__
e: g__Dehalobacterium
f: f__Dehalobacteriaceae
g: g__Dorea
h: g__
i: f__Peptococcaceae
j: g__Ruminococcus
k: g__
l: g__Allobaculum
m: g__Clostridium
n: f__Erysipelotrichaceae
o: o__Erysipelotrichales
p: g__
q: f__
r: o__RF39
s: g__Akkermansia
t: f__Verrucomicrobiaceae
u: o__Verrucomicrobiales
p__Verrucomicrobia
c__Verrucomicrobiae
c__Erysipelotrichi
E
P:Bacteroidetes, C:Bacteroidia, O:Bacteroidales, F:Rikenellaceae
class: SPF
class: ex_GF
Relative abundance
P:Firmicutes, C:Clostridia, O:Clostridiales, F:Lachnospiraceae, G:Dorea
class: SPF
class: ex_GF
Relative abundance
P:Firmicutes, C:Erysipelotrichi, O:Erysipelotrichales, F:Erysipelotrichaceae, G:Clostridium
class: SPF
class: ex_GF
Relative abundance
P:Verrucomicrobia, C:Verrucomicrobiae , O:Verrucomicrobiales, F:Verrucomicrobiaceae, G:Akkermansia, S:muciniphila
class: SPF
class: ex_GF
Relative abundance

Figure S2.

exGF mice are characterized by altered microbiota composition. **A-B**, Microbiota composition was analyzed by 16S rRNA gene Illumina sequencing. Principal coordinate analysis (PCoA) of the unweighted Unifrac distance, with samples colored by experimental group (SPF and exGF; **A**) or by day (42, 98 and 122; **B**). **C**, Analysis of microbiota alpha diversity using the Shannon index between SPF and exGF mice. **D-E**, LEfSe (LDA Effect Size) was used to investigate bacterial community differences between SPF and exGF groups. **D**, Taxonomic cladogram obtained from LEfSe analysis of 16S rRNA genes sequencing by comparing SPF and exGF groups. Red, significantly increased in SPF; Green, significantly increased in exGF. **E**, Relative abundances of bacterial taxa significantly altered in one group compared to the other.

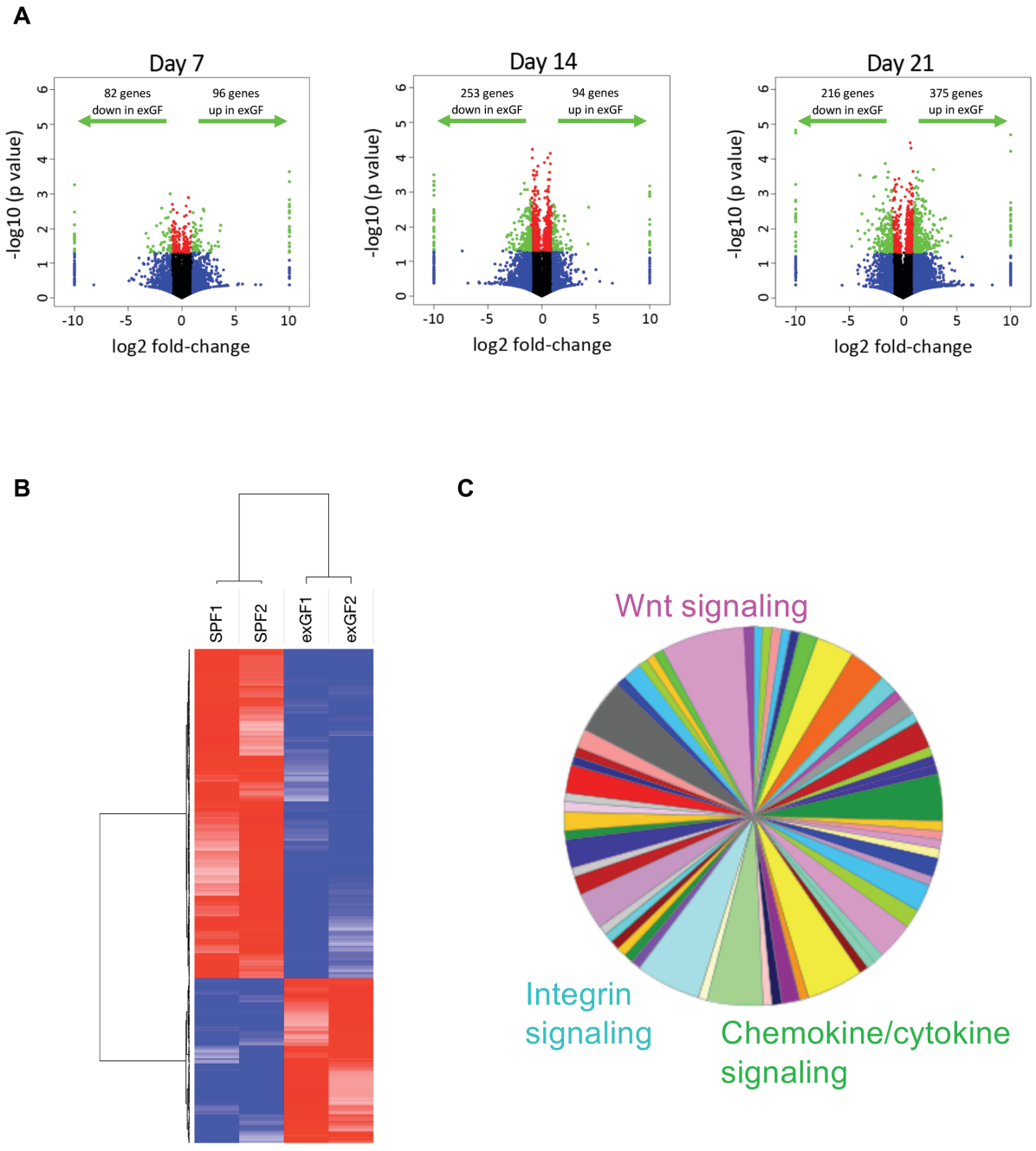

Figure S3.

exGF mice are characterized by altered colonic gene expression. **A**, RNAseq data were visualized on a volcano plot comparing gene expression in SPF and exGF mice at day 7, 14, and 21 of life.

For each gene, the difference in abundance between the two groups is indicated in log2 fold change on the *x*-axis (with positive values corresponding to increased expression in exGF mice compared to SPF mice, and negative values corresponding to decreased expression in exGF mice compared to SPF mice, and significance between the two groups is indicated by -log10 *P*-value on the *y*-axis. Red dots correspond to genes with a *P*-value <0.05 between exGF and SPF mice. Blue dots correspond to genes with at least 2-fold decreased or increased expression in exGF and SPF mice. Green dots correspond to genes with at least 2-fold decreased or increased abundance in exGF and SPF mice and with a *P*-value <0.05. **B**, Intestinal gene expression for replicate SPF and exGF samples were analyzed by RNAseq technology at days 42 of life (prior to AOM/DSS) and the 551 genes significantly altered in one group compared to the other was plotted on the heatmap. **C**, http://pantherdb.org was used to analyze the 551 genes significantly altered in one group compared to the other.

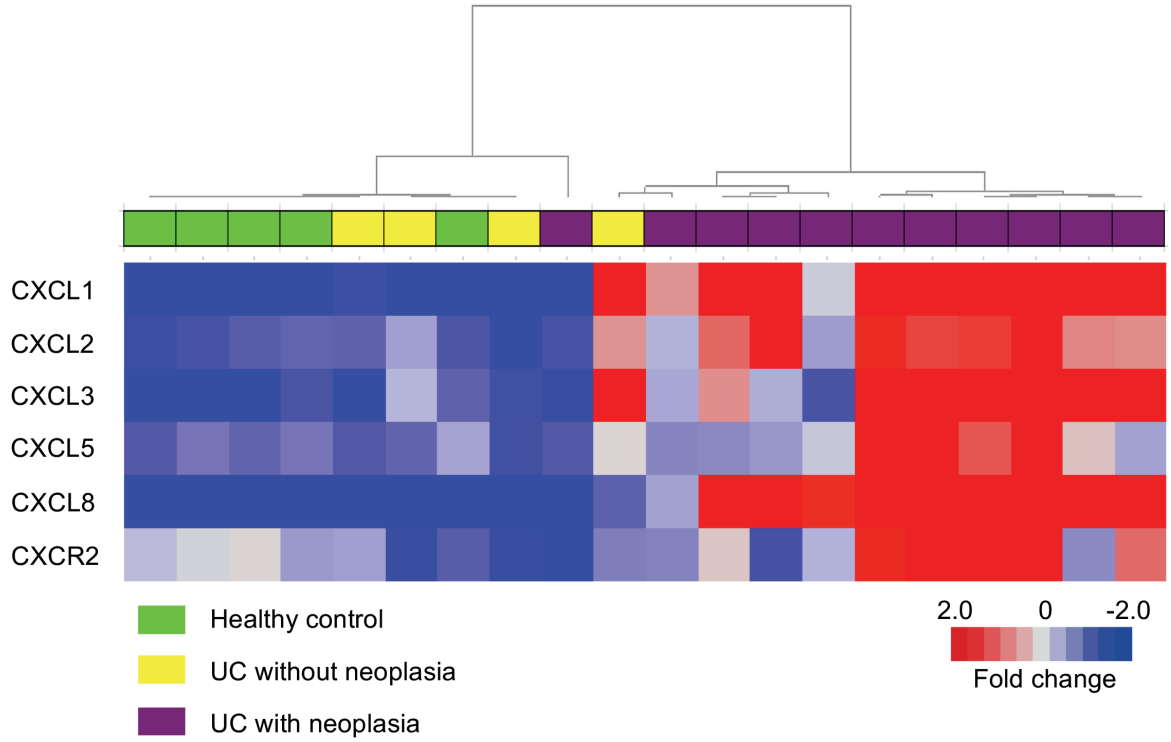

Figure S4.

CXCR2 and CXCR2 ligand mRNA expression is enhanced in colonic tissue UC patients with neoplasia. Colonic biopsy samples were obtained from healthy controls (n=5), ulcerative colitis (UC) patients without colonic dysplasia (n=4) and UC patients harboring remote colonic neoplasia (n=11) during colonoscopy. DNA microarray analysis was performed on 20 RNA samples isolated from the biopsy samples. Gene expressions of CXCL1, CXCL2, CXCL3, CXCL5, CXCL8 and CXCR2 in each group were shown and expressed as a clustering heatmap.