

1 **Supplemental information**

2 **A missense mutation in *Muc2* promotes gut microbiome and**
3 **metabolome-dependent colitis-associated tumorigenesis.**

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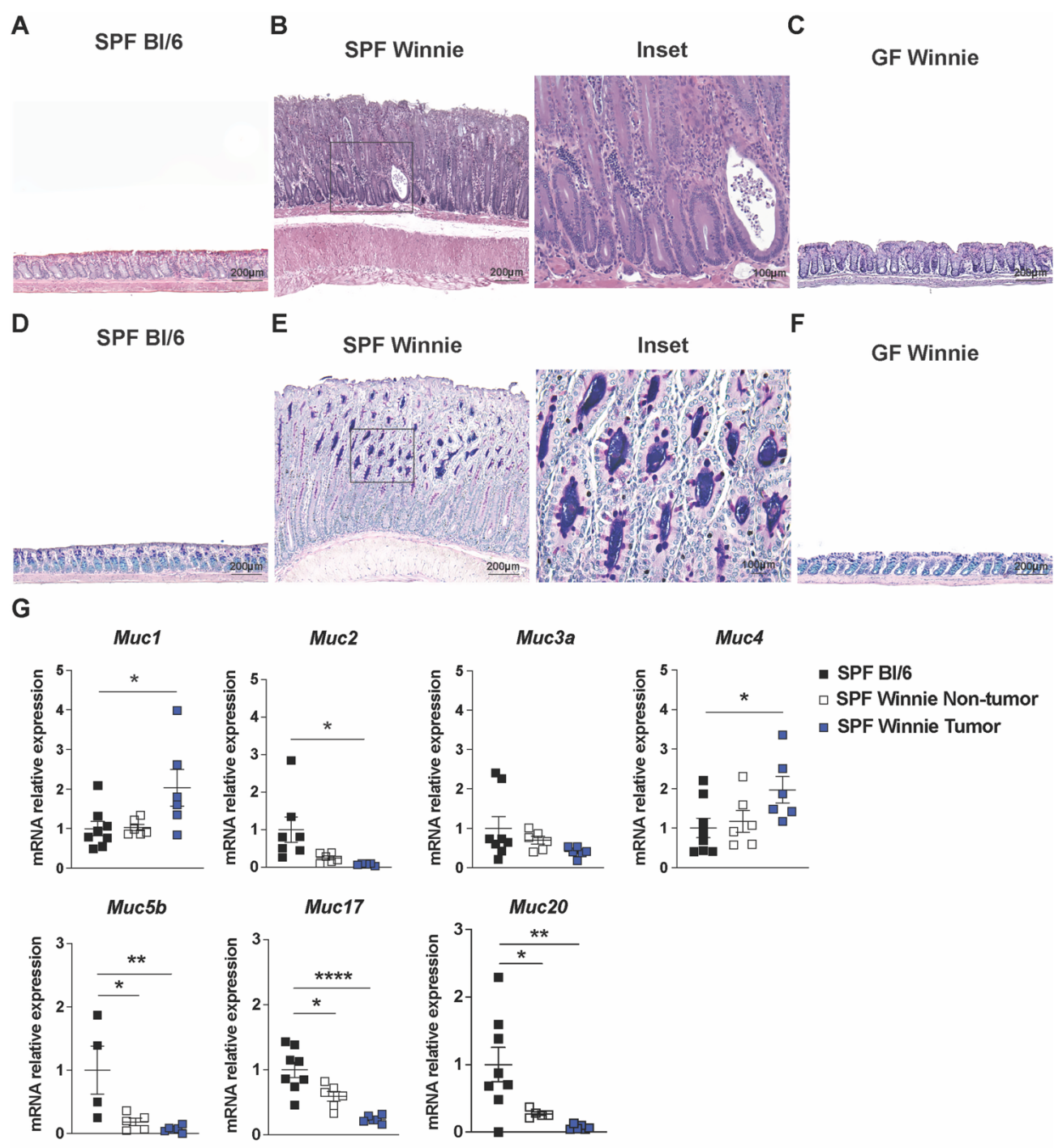
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32 **Conflicts of interest:** The authors declare no potential conflicts of interest.

33 **Supplemental Figure 1.**



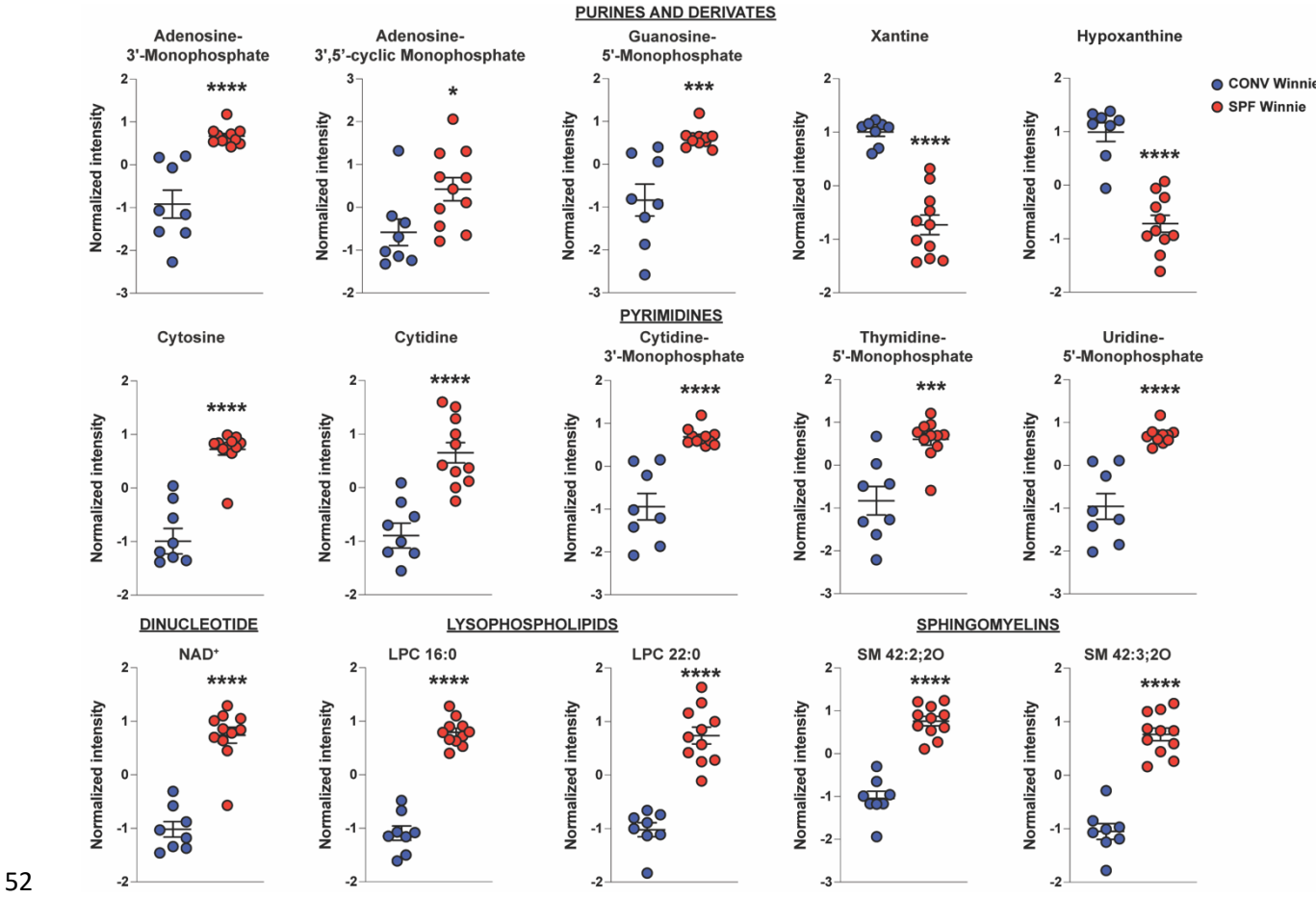
35 **Supplemental Figure 1: Mucus composition is markedly different in SPF Winnie mice**
36 **compared to BI/6 and GF Winnie mice.**

37 (A-C) Representative H&E images of colon tissue from 20-week-old SPF BI/6 (A), SPF Winnie (B),
38 and GF Winnie (C) mice; inset from SPF Winnie mice highlights aberrant crypts, inflammatory
39 infiltrates, and crypt abscesses. Magnification: 5X+2, except for SPF Winnie 5X+1.65 and its inset:
40 20X+1.25. (D-F) Representative images of PAS/Alcian-stained colon from 20-week-old SPF BI/6 (D),
41 SPF Winnie (E), and GF Winnie (F) mice; inset highlights low staining for acid mucins (light blue)
42 and an increase of neutral mucins (magenta) in aberrant crypts of 20-week-old SPF Winnie mice.
43 Magnification: 5X+2, except for SPF Winnie, inset: 20X+2. (G) Quantification of mRNA expression
44 for several intestinal mucins shows increased levels of *Muc1* and *Muc4* and decreased levels of
45 *Muc2*, *Muc5b*, *Muc17*, and *Muc20* in SPF Winnie tumor areas compared to BI/6 mice. In addition,

46 *Muc5b*, *Muc17*, and *Muc20* were also downregulated in SPF Winnie non-tumor areas compared to
47 controls. Data presented in the dot plots are expressed as mean $\pm$ SEM, with *P*-values calculated
48 by ordinary one-way ANOVA followed by Dunnett's test; *n*=5-6 (SPF mice), *n*=4-8 (Bl/6); **P*<0.05;
49 ***P*<0.01; *****P*<0.0001.

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51 **Supplemental Figure 2.**



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53 **Supplemental Figure 2: Metabolipidomic profiles differ in tumor-bearing SPF vs. CONV**
54 **Winnie mice, with alterations in purine and derivatives, pyrimidines, dinucleotide,**
55 **lysophospholipids, and sphingomyelins.**

56 Dot plots for normalized intensity of highly significant metabolites and lipids found in 20-week-old
57 SPF (red) vs. CONV (blue) Winnie stool divided by compound class, i.e., purine and derivatives,
58 pyrimidines, dinucleotide, lysophospholipids, sphingomyelins. Data presented in the dot plots are
59 expressed as mean $\pm$ SEM, with *P*-values calculated by unpaired *t*-test or Mann-Whitney; *n*=12 (SPF
60 mice), *n*=8 (CONV mice); **P*<0.05; ****P*<0.0005; *****P*<0.0001.

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63 **Supplemental Table 1. Mouse Primers used for qPCR**

Mouse Gene name	Forward 5' → 3'	Reverse 5' → 3'
<i>Actb</i>	CACTGTCGAGTCGCGTCC	TCATCCATGGCGAACTGGTG
<i>Muc1</i>	CCACACTCACGGACGCTAC	CATTACCTGCCGAAACCTCCTC
<i>Muc2</i>	CCGGGAAATGCTGTCCAGTT	TGGAGATCCGGGCTGGTATT
<i>Muc3a</i>	GGAAATCCTGTCCTTGAGGAGG	CCTGACAGCTGTTGCCATCT
<i>Muc4</i>	GCCCGCTCATCCACTATCTG	CTCCAGCATACTTAGGTTCAAGC
<i>Muc5b</i>	AGTCAGAGAGTGTATAGTGAATCCA	GGTGTAAGGCGCTCATGCTA
<i>Muc17</i>	CGGAAGTGTGTGGGATGGAG	CTGTTTTCCCCGTCTGTGGTT
<i>Muc20</i>	CTTGTACGGCTGACCGTGG	TTCACGGCGGAGCTGGTTC

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