

1 **SUPPLEMENTAL FIGURE LEGENDS**

2 **Supplemental Figure 1: The effect of *Klf5* deletion on the intestinal phenotypes in male and**

3 **female mice.** (A) Kaplan-Meier curve of CO and TAM treatment for *Klf5<sup>ΔIND</sup>* mice with male (n=15)

4 and female (n=15) mice. (B) Quantification of clinical scores of CO- or TAM-treated *Klf5<sup>Ctrl</sup>* and

5 *Klf5<sup>ΔIND</sup>* male and female mice (n=10). Max for the clinical score is 12 and quantified with three

6 categories; stool consistency, weight loss, and fecal blood. (C) Hematoxylin-eosin staining of

7 whole colon tissue from *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* male mice treated with CO or TAM. (D) Quantification

8 of histological score of CO- and TAM-treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* male and female mice. Max for

9 the histological score is 11 and quantified with three categories; crypt damage, inflammatory cells

10 in lamina propria, and ulcers (n=10). (E) Hematoxylin-eosin staining of ileal tissue from *Klf5<sup>Ctrl</sup>* and

11 *Klf5<sup>ΔIND</sup>* male and female mice treated with CO or TAM. The red square highlights areas of villus

12 blunting in the small intestine of TAM-treated female *Klf5<sup>ΔIND</sup>* mice. Data from graphs represent

13 mean with SEM. \*\*P<0.01; \*\*\*P<0.001; One-way ANOVA. Scale Bars on images indicate 70μm.

14

15 **Supplemental Figure 2: Different recombination efficiencies of *Klf5* deletion between male**

16 **and female *Klf5<sup>ΔIND</sup>* mice.** (A) Immunohistochemical staining of KLF5 in whole colon tissues from

17 male *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice treated with CO or TAM. (B) RT-qPCR analysis of *Klf5* transcript

18 levels comparing TAM-treated over CO-treated *Klf5<sup>ΔIND</sup>* mice in male and female (n=9). (C) DNA

19 gel electrophoresis of PCR products from primers flanking *loxP* sites upstream of *Klf5* exon 2 and

20 *Actb*. Data from graph B represent mean, \*\*\*P<0.001; Unpaired T-test. Data from graph D

21 represent mean, \*P<0.05; \*\*\*P<0.001; One-way ANOVA. Scale bars on images indicate 70μm.

22

23 **Supplemental Figure 3: Comparison of transcriptional profiles between CO- or TAM-**

24 **treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice.** (A) RT-qPCR analysis comparing CO- to TAM-treated *Klf5<sup>Ctrl</sup>*

25 mice (n=3). (B) RT-qPCR analysis comparing CO-treated *Klf5<sup>Ctrl</sup>* mice, and CO-treated *Klf5<sup>ΔIND</sup>*

1 mice (n=3). (C) RT-qPCR analysis comparing TAM-treated *Klf5<sup>Ctrl</sup>* mice and TAM-treated  
2 *Klf5<sup>ΔIND</sup>* mice (n=3). (D) GSEA pathway analysis positively correlated to CO-treated *Klf5<sup>ΔIND</sup>*  
3 mice. Data from graphs represent mean with SEM. \*\*\*P<0.001; Unpaired T-test.  
4

5 **Supplemental Figure 4: Gating strategy for pan-leukocyte flow analysis.** Gating strategy for  
6 identification of CD45, neutrophil, B cell, T cell, NK cells, monocytes, macrophages, and dendritic  
7 cells.  
8

9 **Supplemental Figure 5: Comparison of additional leukocyte populations between CO- or**  
10 **TAM-treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice.** (A) NK cells, (B) B cells, (C) monocytes, (D) neutrophils,  
11 (E) macrophages, (F) cDC1, (G) Th1, (H) Th2, and (I) ILC3 cells (n=4). (J) 40X brightfield image  
12 of ileum and colon of TAM-treated *Klf5<sup>ΔIND</sup>* mice. Red arrows highlight neutrophil involvement in  
13 cryptitis and crypt abscesses. Data from graphs represent mean with SEM. The small intestine  
14 image of TAM-treated *Klf5<sup>ΔIND</sup>* mice in Supplemental Figure 5J is a zoomed-in image of a section  
15 of the ileal sample from TAM-treated female *Klf5<sup>ΔIND</sup>* mice in Supplemental Figure 1E. One-way  
16 ANOVA. Scale Bar represents 70 μm.  
17

18 **Supplemental Figure 6: Comparison of Th17-inducing factors between CO- or TAM-treated**  
19 ***Klf5<sup>ΔIND</sup>* mice.** *Klf5<sup>ΔIND</sup>* mice were treated with CO or TAM for five days and RT-qPCR analysis  
20 performed on whole colon tissues for (A) IL-1β, (B) IL-23, and (C) SAA. Cytokine array analyses  
21 were performed at days 1 and 5 of CO or TAM treatment for (D) IL-1β, (E) IL-23, and (F) SAA.  
22 (G) ELISA of IL-23 concentrations on days 1, 3, 5, and 7 following CO or TAM treatment. Data  
23 from graphs A-F represent mean, \*P<0.05; \*\*P<0.01; One-way ANOVA test. Data from graph G  
24 represent mean, \*\*\*\*P<0.0001; Two-way ANOVA test.  
25

1

2 **Supplemental Figure 7: Effects of *Klf5* deletion on pSTAT3-related upstream and**

3 **downstream signaling pathways.** (A) Immunohistochemical staining of STAT3 and

4 phosphorylated STAT3 in the colon of CO- or TAM-treated *Klf5*<sup>ΔIND</sup> mice. Scale bars on images

5 indicate 70μm. The pSTAT3 images are lower magnifications of the same images shown in

6 Figure 6A. (B) Quantification of percentage of pSTAT3<sup>+</sup> cells per crypt (n=5). (C) RNA-seq (left

7 graph; n=4) and RT-qPCR (right graph; n=7) analysis of IL-22RA1 transcript levels. (D) RNA-

8 seq (left graph; n=4) and RT-qPCR (right graph; n=6) analysis of IL-22RA2 levels. (E) RNA-seq

9 TPM fold-change for S100A8, S100A9, b-defensin-2, and SOCS3 (n=4). Data from graph B

10 represent mean. \*\*\*P<0.001; One-way ANOVA test. Data from graph C-E represent mean by

11 unpaired T-test. Scale bar represents 70μm.

12

13 **Supplemental Figure 8: Effect of KLF5 deletion on pSTAT3 localization in response to IL-**

14 **6.** IF staining of DAPI and pSTAT3 of C2BBE<sup>Ctrl</sup> and C2BBE<sup>ΔIND</sup> cells treated with DMSO or

15 DOX and PBS or IL-6. Scale bar represents 70μm.

16

17 **Supplemental Figure 9: Cellular localization of pSTAT3 in human colonic specimens.** IHC

18 staining of pSTAT3 in colonic biopsy specimens from normal subject, UC patient, and patient

19 with colon adenocarcinoma. Scale bars represent 70μm.

20

21 **Supplemental Figure 10: The role of KLF5 in the maintenance and survival of colonoids.**

22 (A) Western blots and (B) quantification of KLF5 levels in colonoid cultures from *Klf5*<sup>ΔIND</sup> mice

23 treated with PBS or 4-OHT (n=6). (C) Bright field images of colonoid culture from days 1, 3, and

24 5 of treatment with PBS or 4-OHT. Scale bars on images indicate 70μm. (D) Quantification of

25 percentages of budding colonoids over total number of colonoids on days 0, 1, 3, and 5 (n=8).

1 Data from graph B represent mean. \*\*\*\*P<0.001; Unpaired T-test. Data from graph D represent  
2 mean, \*P<0.05; \*\*P<0.01; Two-way ANOVA. Scale bars on images indicate 70µm.

3

4 **Supplemental Figure 11: Effect of IL-22 on survivability of colonoids derived from *Klf5<sup>ΔIND</sup>***

5 **mice.** (A) Bright field images of *Klf5<sup>ΔIND</sup>* colonoid cultures from days 1 and 5 of treatment with PBS  
6 or 4OHT. Scale bars represent 70µm. (B) Quantification of percentages of budding colonoids over  
7 total number of colonoids on days 1 and 5 (n=10). Data from graph B represent mean, \*P<0.05;  
8 \*\*P<0.01; Two-way ANOVA.

9

10 **Supplemental Figure 12: Comparison of phylogenetic classes in colonic microbiota**

11 **between CO- and TAM-treated *Klf5<sup>ΔIND</sup>* mice.** Linear discriminant analysis of 16s rRNA

12 sequencing in the microbiota from the colon of CO- and TAM-treated *Klf5<sup>ΔIND</sup>* mice (n=9).

13

14 **Supplemental Figure 13: Comparison of colonic microbiota abundance in CO- or TAM-**

15 **treated mice.** (A) Phylogenetic tree of LDA analysis comparing TAM-treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>*

16 mice (n=9). (B) LDA score graph showing significantly different phylogenetic orders between

17 TAM treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice (n=4). (C) Phylogenetic tree of LDA analysis comparing

18 CO- and TAM-treated *Klf5<sup>Ctrl</sup>* mice. (D) LDA score graph showing significantly different

19 phylogenetic orders between CO and TAM treated *Klf5<sup>Ctrl</sup>* mice (n=4). (E) Phylogenetic tree of

20 LDA analysis comparing CO treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice. (F) LDA score graph showing

21 significantly different phylogenetic orders between CO treated *Klf5<sup>Ctrl</sup>* and *Klf5<sup>ΔIND</sup>* mice (n=9).

22

23 **Supplemental Figure 14: Effect of *Klf5* deletion on abundance of two Th17 pathobionts.**

24 qPCR analysis of 16s rRNA of segmented filamentous bacteria (SFB) (A) and Bacteroides (BAT)

1 (B) in the colon of CO- or TAM-treated mice (n=6). Data from graph represent mean. \*P<0.05;  
2 \*\*P<0.01; One-way ANOVA.

3

4 **Supplemental Figure 15: Effect of antibiotic treatment on the weight of CO- and TAM-**  
5 **treated *Klf5*<sup>ΔIND</sup> mice.** (A) Raw quantification of weight for each mouse in the experiment (n=3).  
6 (B) IHC staining of KLF5 in proximal and distal colons from CO- and TAM-treated *Klf5*<sup>ΔIND</sup> mice  
7 with water or antibiotic treatment. Scale Bars on images indicate 70μm.

8

9

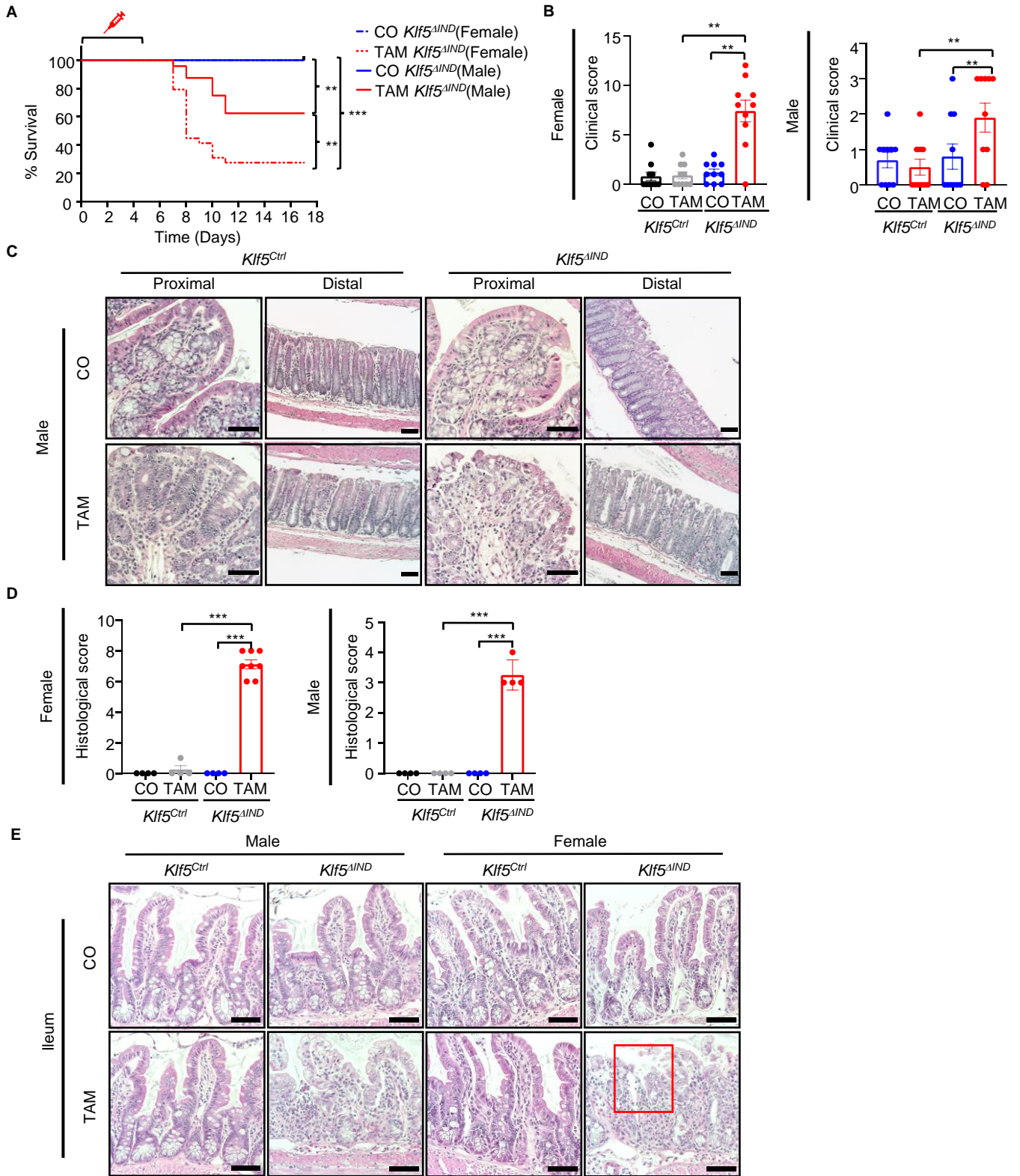
#### 10 **SUPPLEMENTAL TABLE LEGENDS**

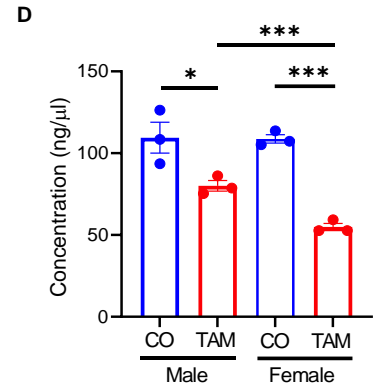
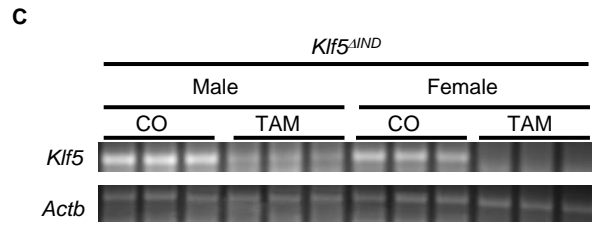
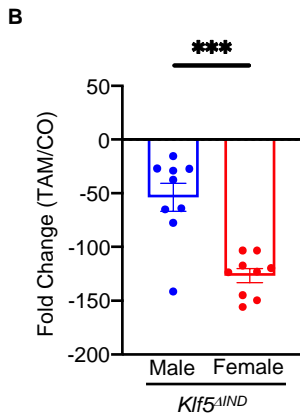
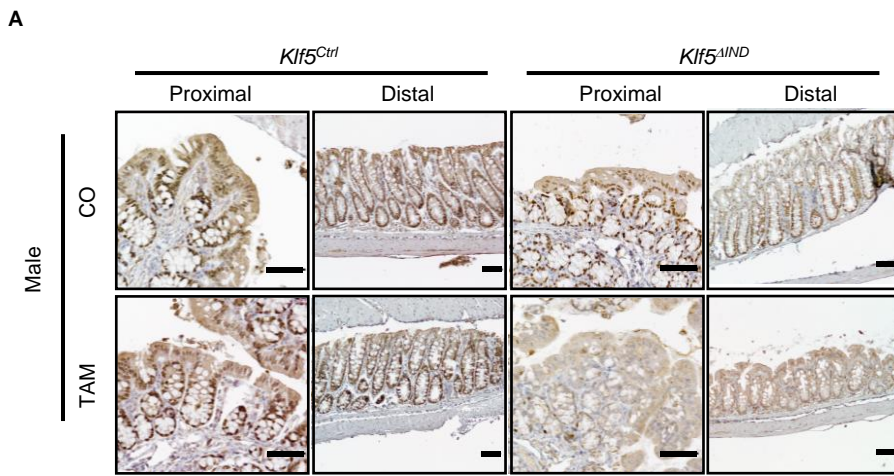
11 **Supplemental Table 1: List of fluorophore-conjugated antibodies for FACS analysis.** and  
12 Catalog number, clone, and fluorophores are listed to the right of each antibody marker.

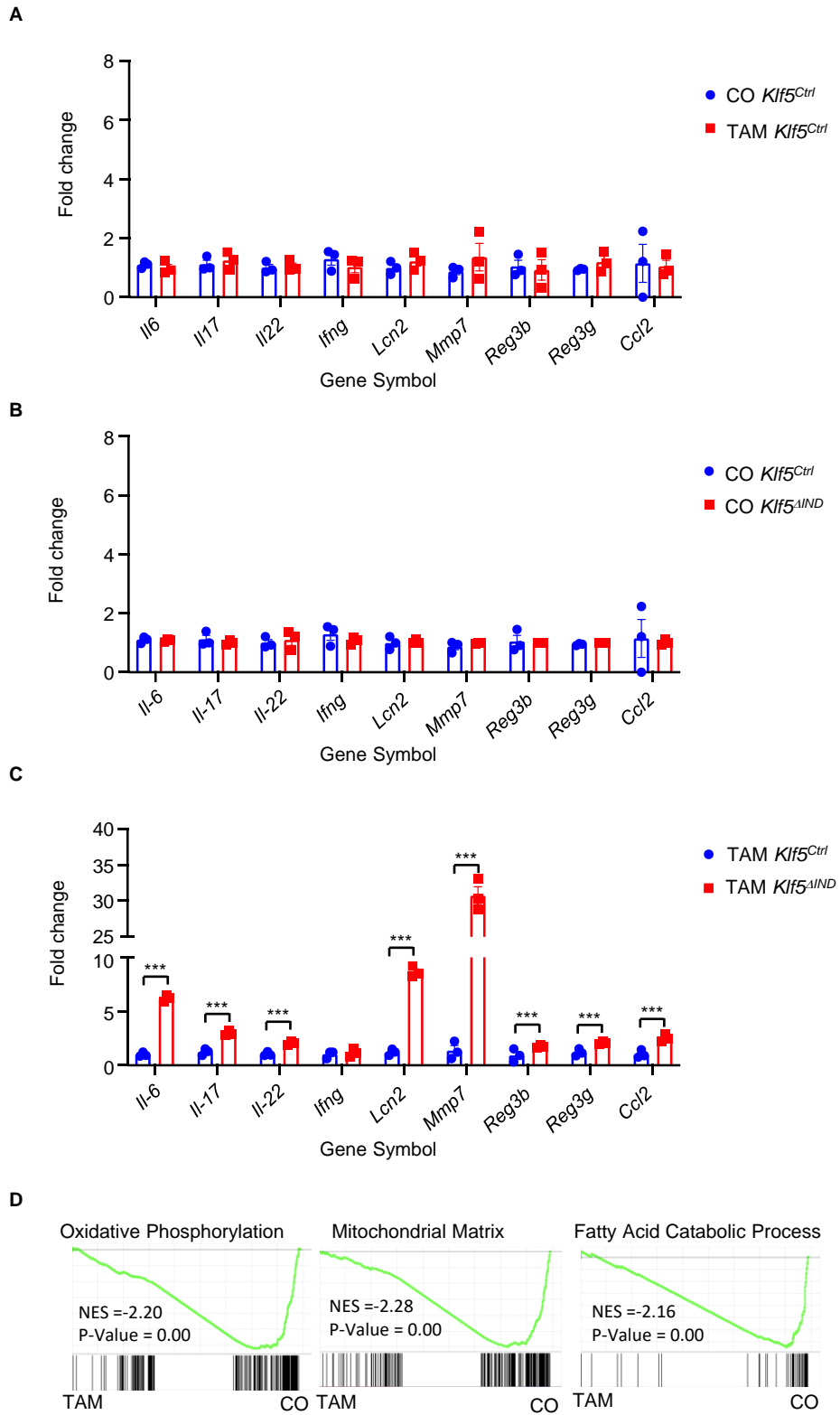
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14 **Supplemental Table 2: List of primary antibodies used for IF staining and western blot.** The  
15 catalog number, clone, and concentrations used for each type of staining protocol.

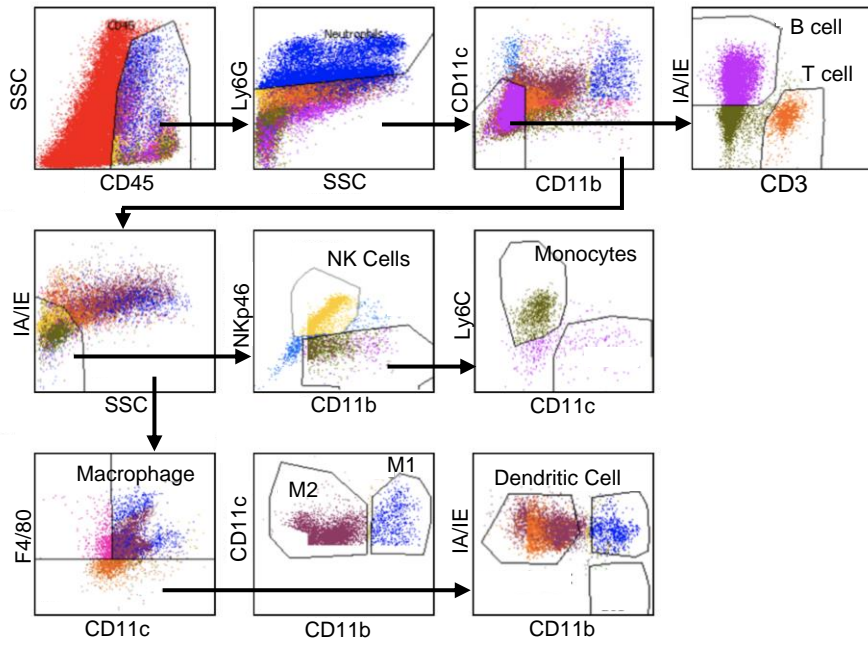
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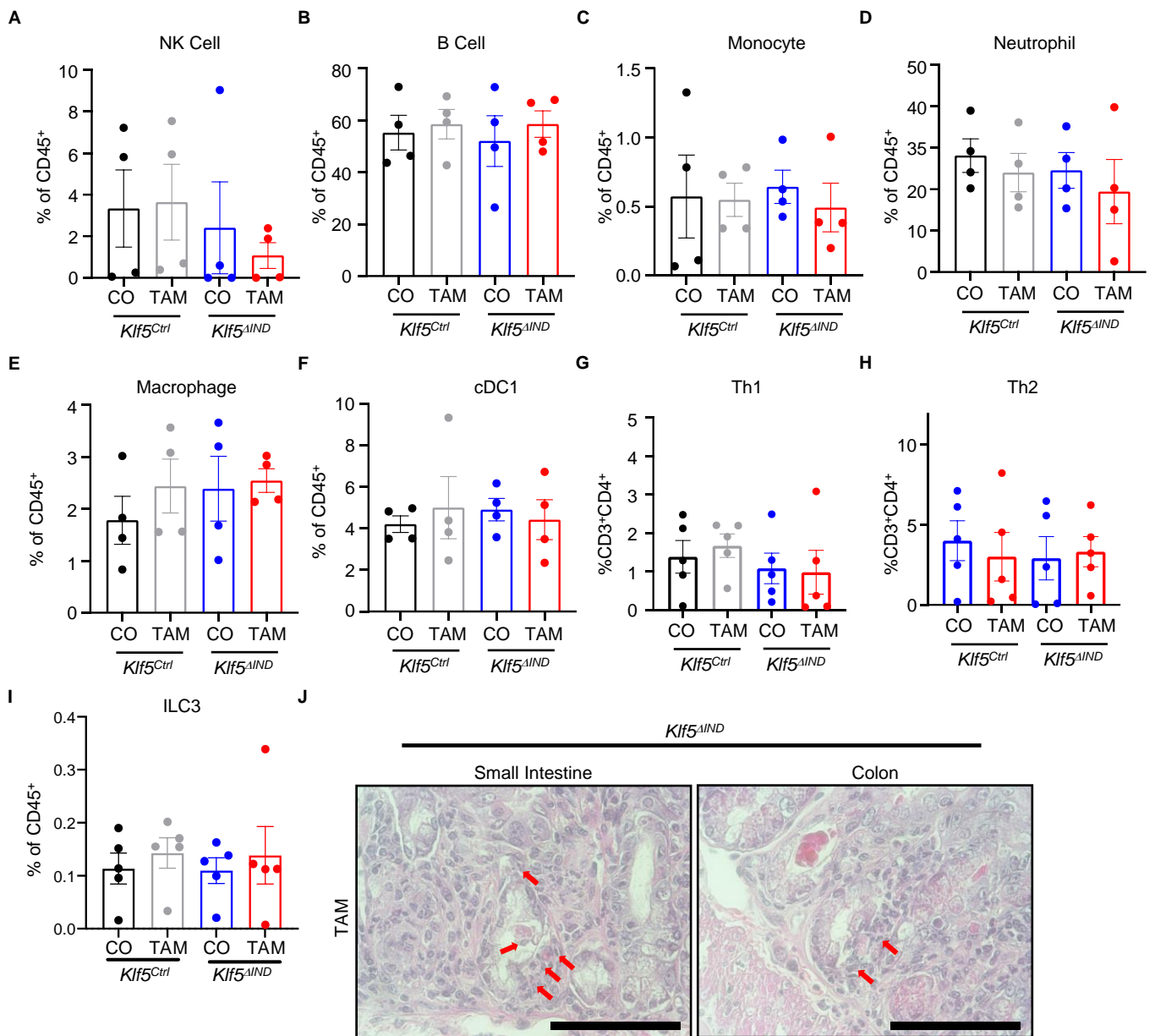


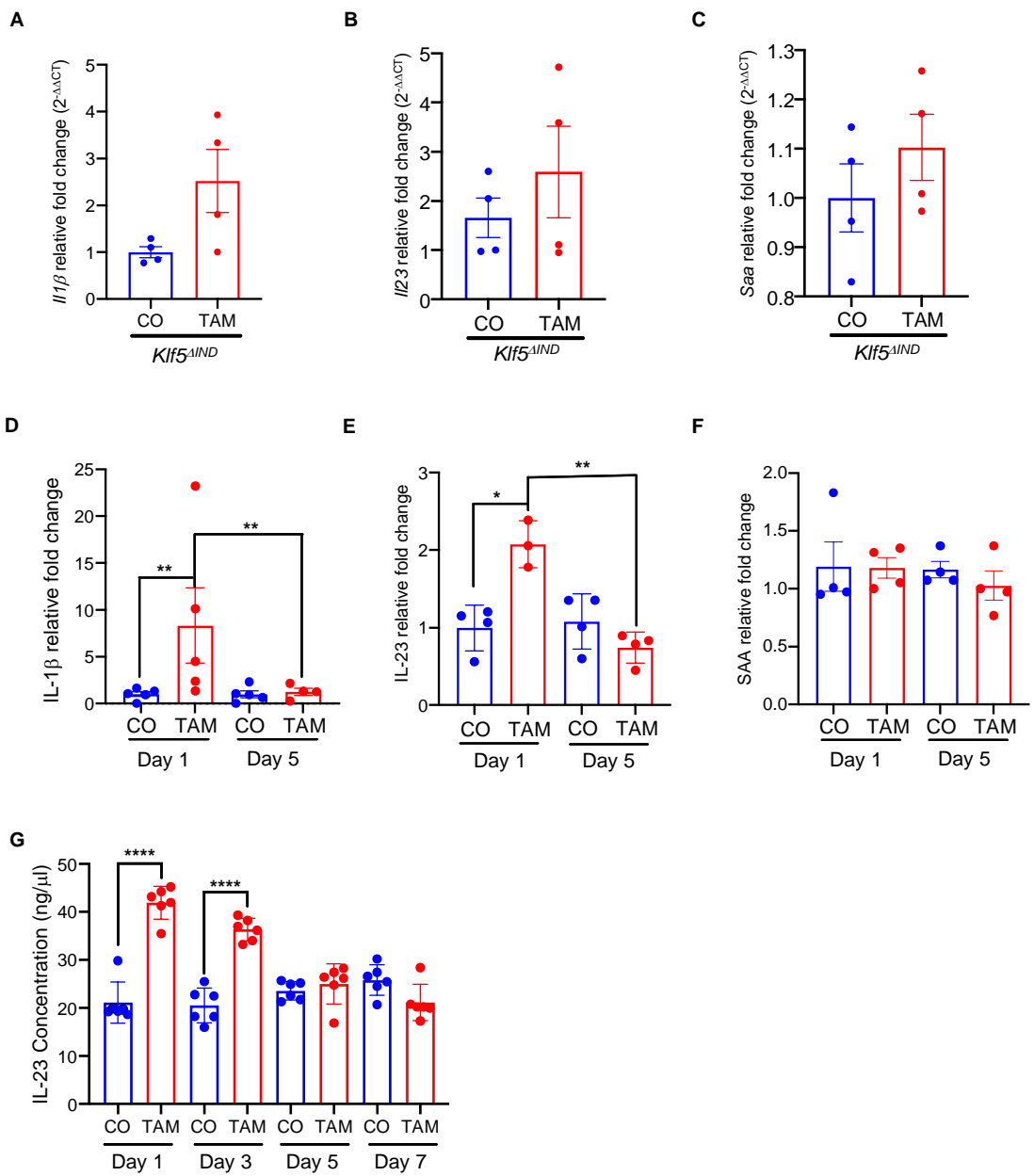




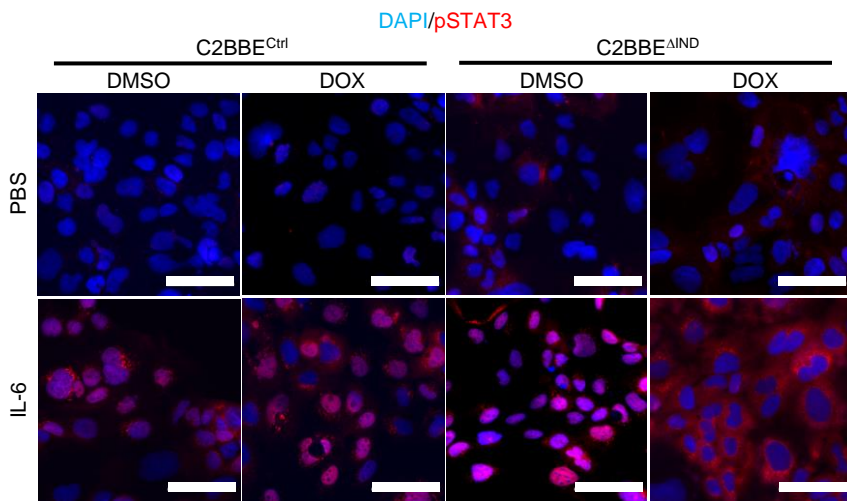




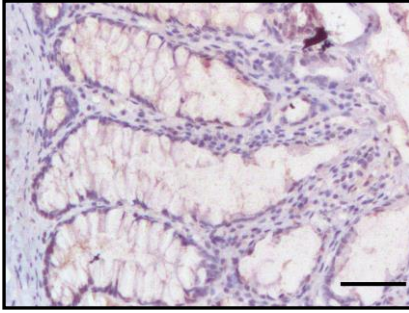




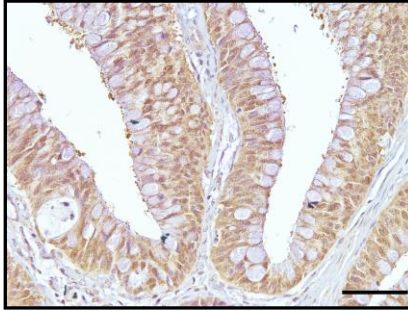




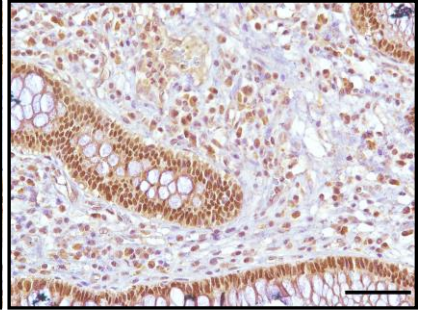
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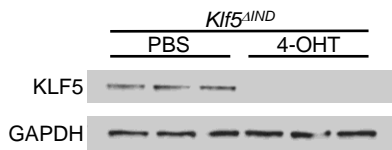
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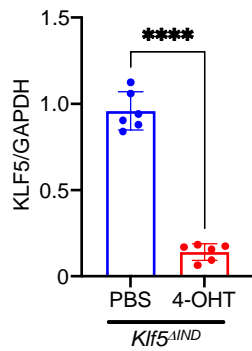
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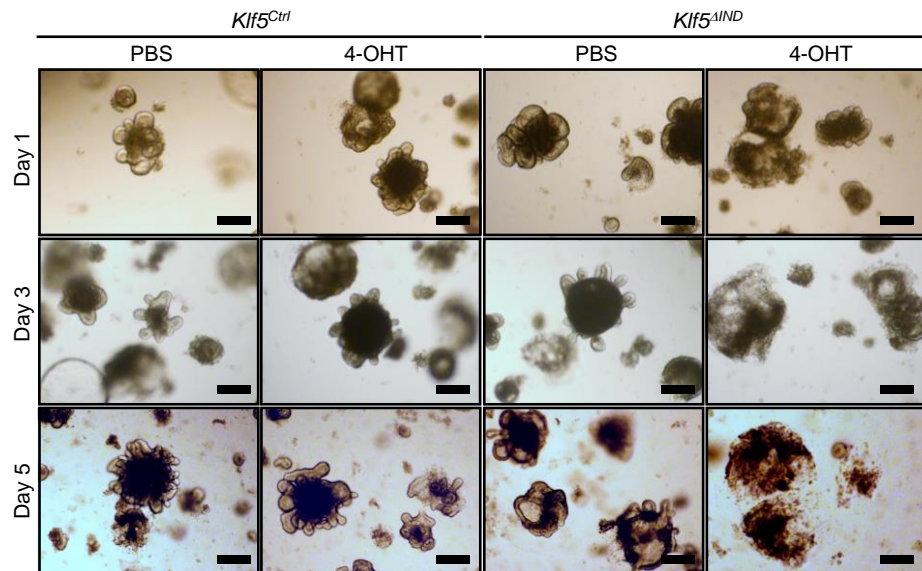
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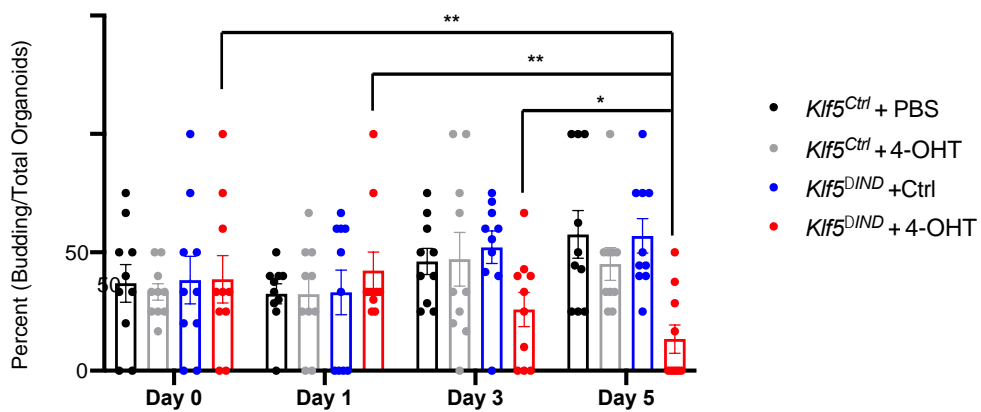
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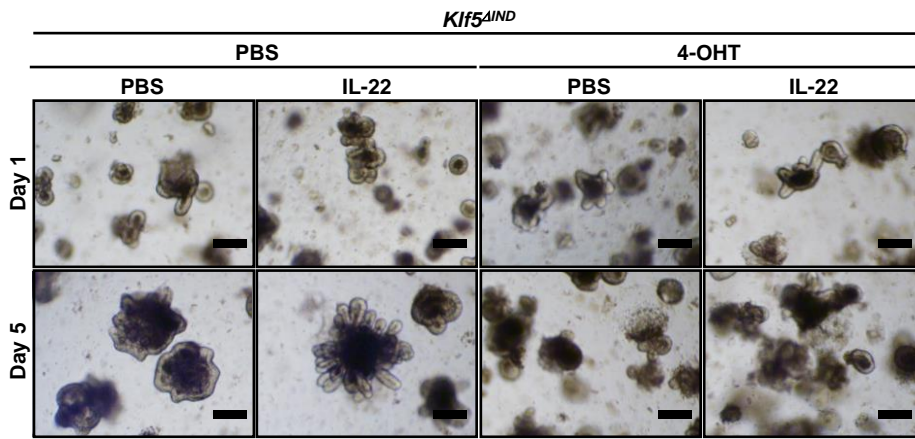
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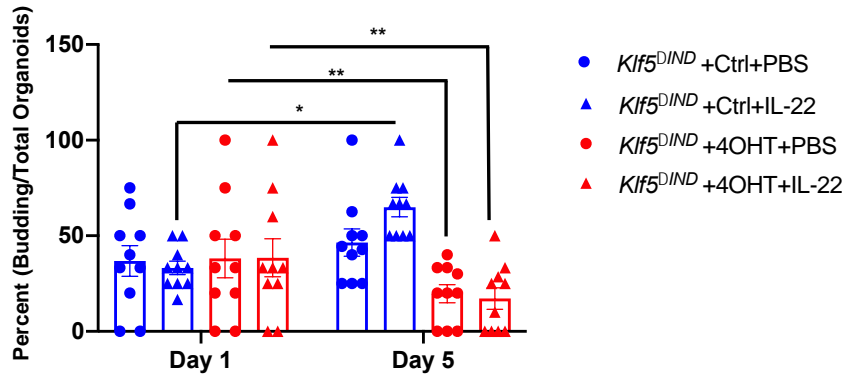
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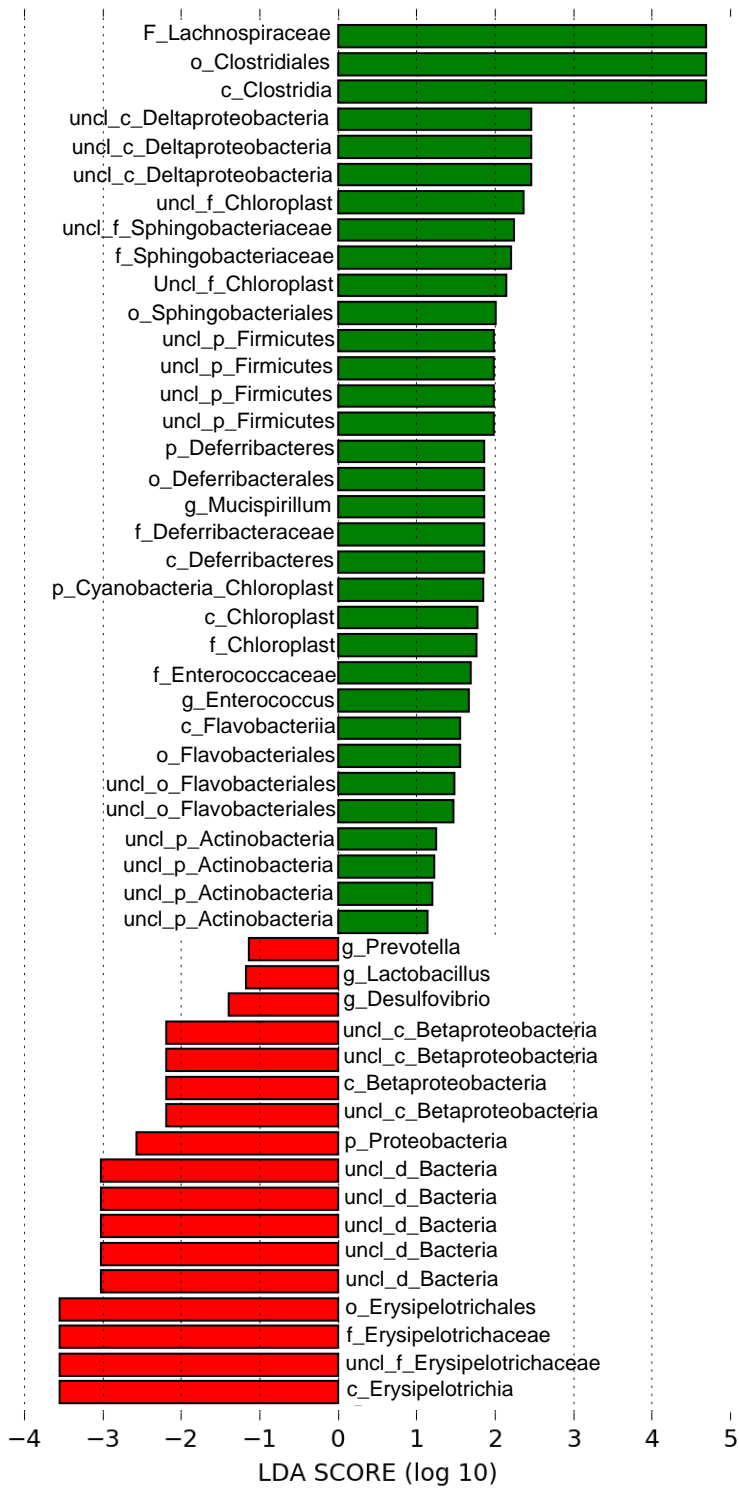
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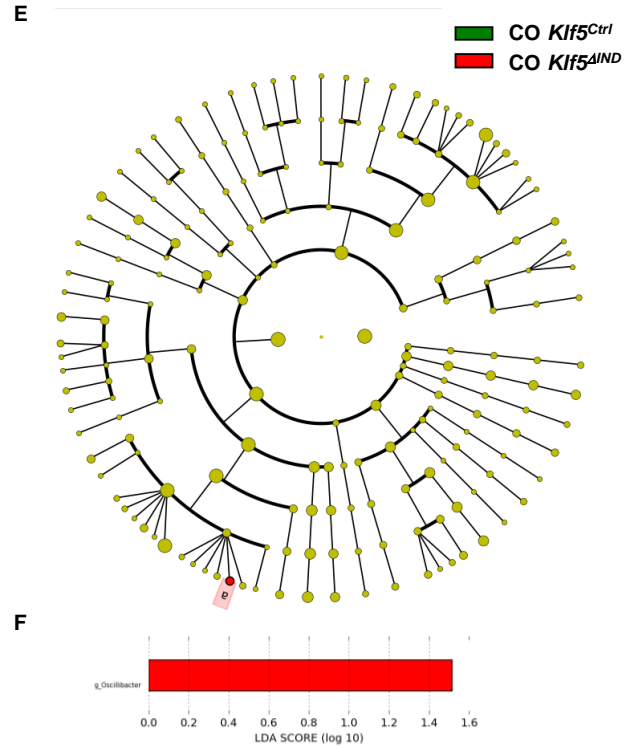
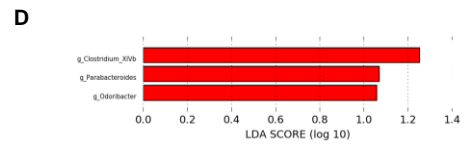
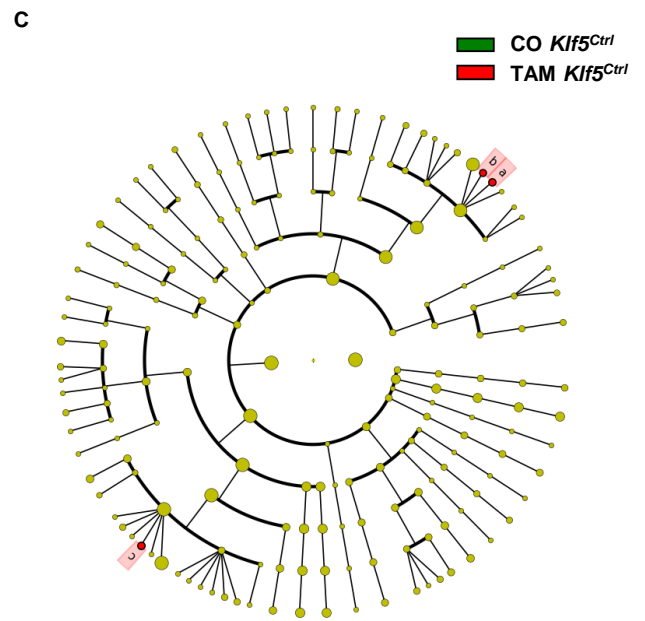
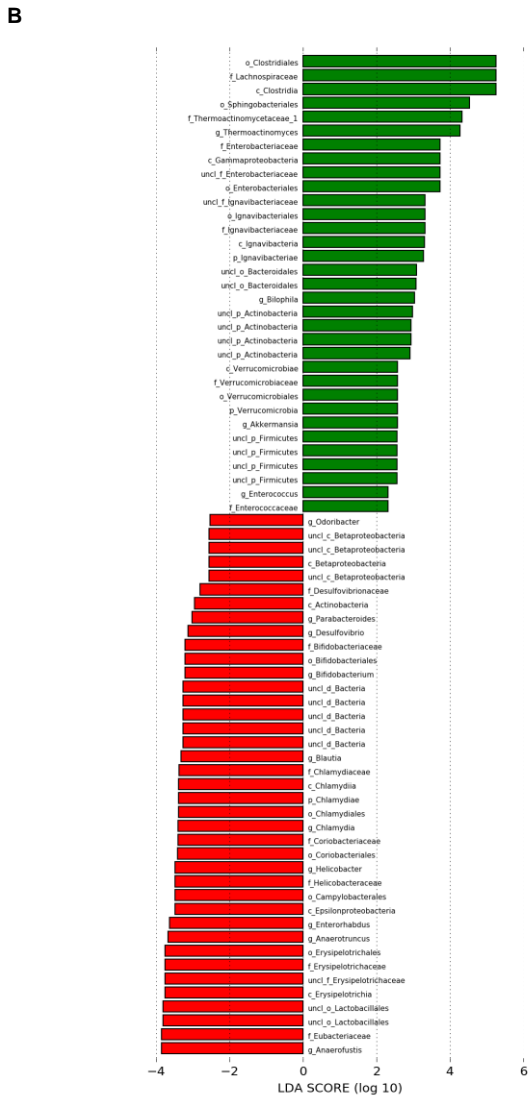
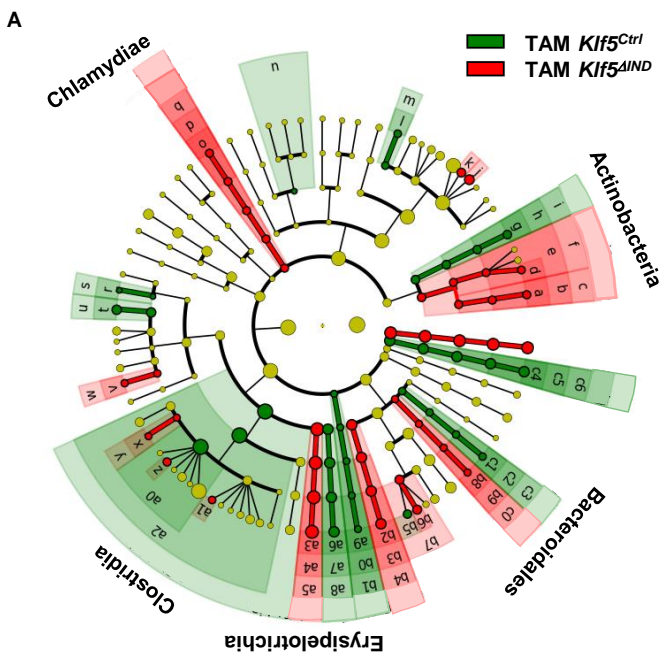


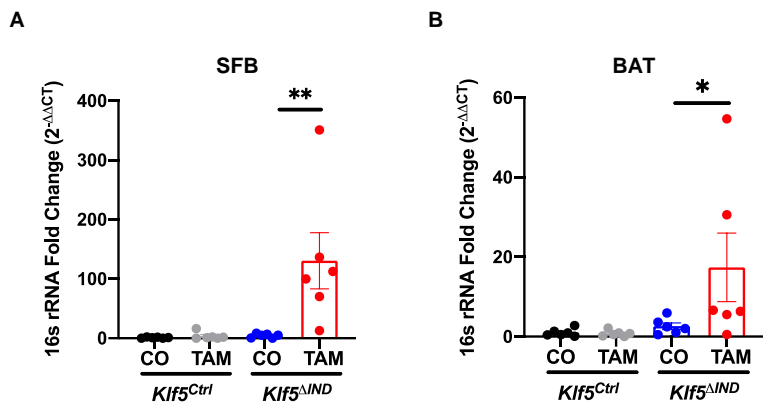
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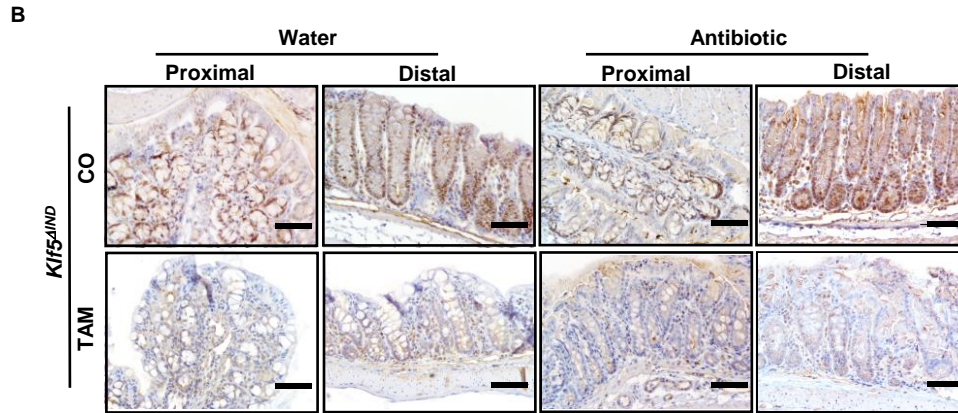
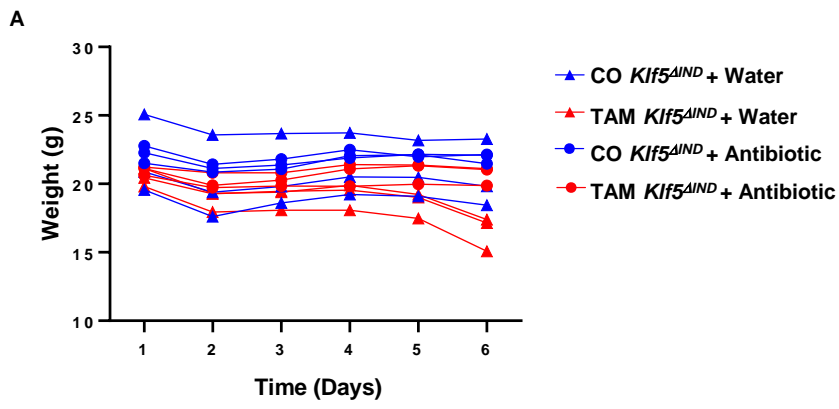












<b>Antibodies</b>	<b>Catalog No. and Company</b>	<b>Clone</b>	<b>Fluorophore</b>
CD45	103122, Biolegend	30-F11	APC
Ly6C	128016, Biolegend	HK1.4	FITC
IA/IE (MHCII)	107645, Biolegend	M5/114.15.2	PerCP 5.5
CD11c	117308, Biolegend	N418	PE
Ly6G	127618, Biolegend	1A8	PE/Cy7
F4/80	123131, Biolegend	BM8	BV421
NKp46	137619, Biolegend	29A1.4	BV605
CD11b	101259, Biolegend	M1/70	BV650
FoxP3	15-5773-80, Thermo Fisher	FJK-16s	PECy5
RORgT	12-6981-82, Thermo Fisher	B2D	PE
GATA3	653813, Biolegend	16E10A23	BV421
CD3	100229, Biolegend	17A2	BV650
CD8b	100229, BD	H35-17.2	BV786
CD4	100425, Biolegend	GK1.5	FITC
CD4	100429, Biolegend	GK1.5	AF700
CD4	100426, Biolegend	GK1.5	APC
CD4	100433	GK1.5	PerCP/Cy5.5
CD4	100407, Biolegend	GK1.5	PE
CD4	100409, Biolegend	GK1.5	PE/Cy5
CD4	100421, Biolegend	GK1.5	PE/Cy7
CD4	100437, Biolegend	GK1.5	BV421
CD4	100451, Biolegend	GK1.5	BV605
CD4	100469, Biolegend	GK1.5	BV650
CD4	100453, Biolegend	GK1.5	BV785
CD326	118211, Biolegend	G8.8	AF647
pSTAT3	sc-8059 AF488, Santa Cruz	B-7	FITC
STAT3	678007, VWR	4G4B45	PE

<b>Antibodies</b>	<b>Catalog No. and Company</b>	<b>Clone</b>	<b>Western Blot</b>	<b>Immunofluorescence/ Immunohistochemistry</b>
Rabbit Anti-STAT3	4904L, Cell Signaling	79D7	1:1000 diluted in 5% Milk	1:100 diluted in 5% BSA w/ TTBS
Rabbit Anti-pSTAT3 (Tyr705)	9145L, Cell Signaling	D3A7	1:1000 diluted in 5% BSA w/ PBS	1:100 diluted in 5% BSA w/ TTBS
Rabbit Anti-KLF5	Ab137676, Abcam	Polyclonal	1:2000 diluted in 5% Milk	1:200 diluted in 5% BSA w/ TTBS
Mouse Anti-GAPDH	CB1001, EMD Millipore	6C5	1:3000 diluted in 5% Milk	N/A
Rabbit Anti-H3	Ab1791, Abcam	Polyclonal	1:3000 diluted in 5% Milk	N/A
Rabbit Anti-IL-17A	Ab79056, Abcam	Polyclonal	N/A	1:100 diluted in 5% BSA w/ TTBS
Rabbit Anti-IL-22	Ab18564, Abcam	Polyclonal	N/A	1:100 diluted in 5% BSA w/ TTBS
Mouse Anti-PanCK	CM011B, Biocare Medical	AE1/AE3	N/A	Prediluted
Rabbit Anti-Ki-67	NB500, Novus Biologicals	Polyclonal	N/A	1:200 diluted in 5% BSA w/ TTBS
Anti-HA	Sc-7392, Santa Cruz	F-7	1:1000 diluted in 5% Milk	N/A
EdU Kit	C10639, Thermo Scientific	N/A	N/A	Kit Manual