Fig. S1:

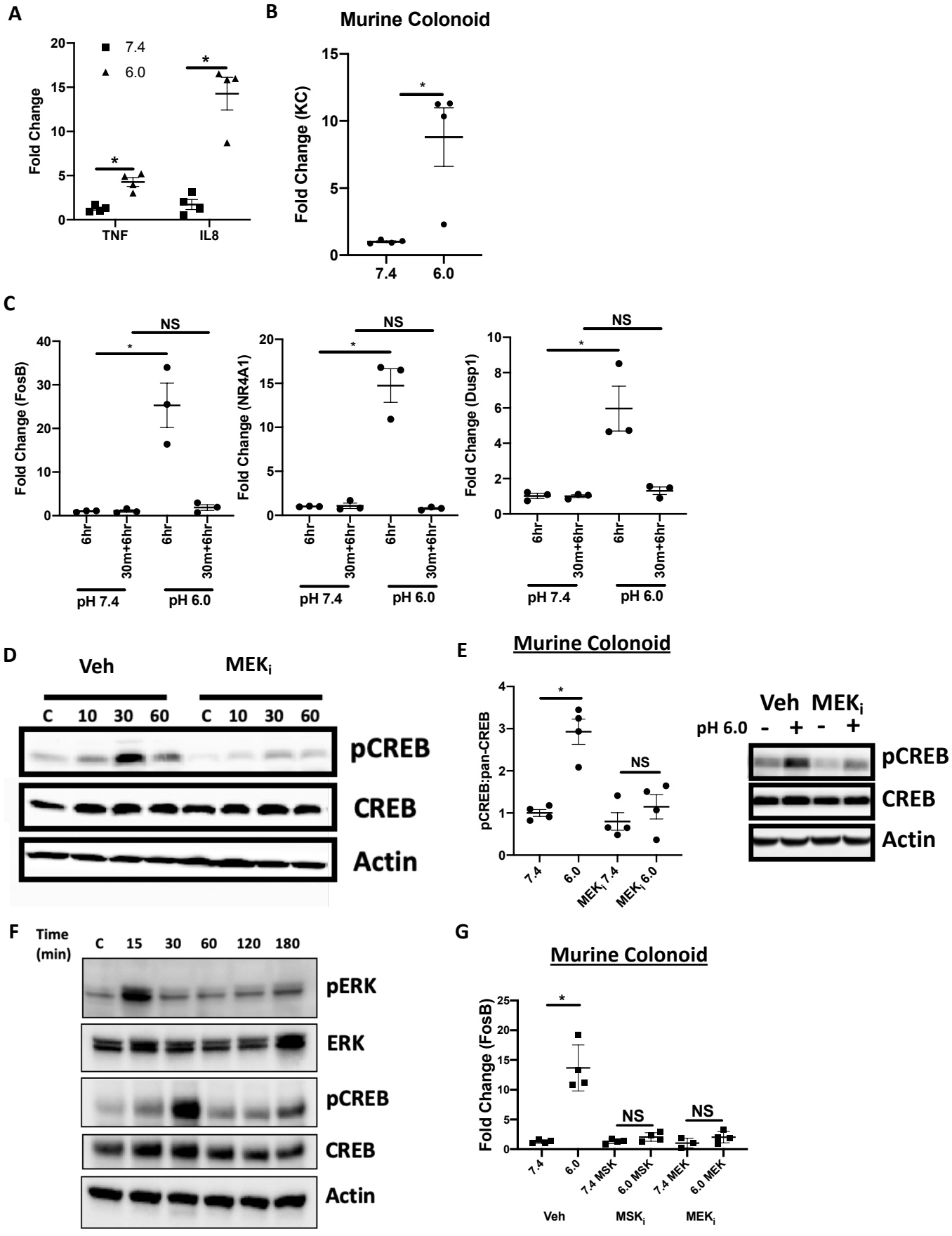

A
7.4
6.0
Fold Change
TNF
IL8
B
Murine Colonoid
Fold Change (KC)
7.4
6.0
C
NS
Fold Change (FosB)
Fold Change (NR4A1)
Fold Change (Dusp1)
6hr
30m+6hr
pH 7.4
pH 6.0
D
Veh
MEKi
C 10 30 60 C 10 30 60
pCREB
CREB
Actin
E
Murine Colonoid
pCREB:pan-CREB
7.4
6.0
MEKi 7.4
MEKi 6.0
Veh MEKi
pH 6.0 - + - +
pCREB
CREB
Actin
F
Time (min)
C 15 30 60 120 180
pERK
ERK
pCREB
CREB
Actin
G
Murine Colonoid
Fold Change (FosB)
7.4
6.0
7.4 MSK
6.0 MSK
7.4 MEK
6.0 MEK
Veh
MSKi
MEKi

Figure S1: Acidosis induced proinflammatory cytokines. a. TNF and IL8 transcript expression in T84 IEC following exposure to pH 7.4 and 6.0 HBSS+ for 6 h (n=4). **b.** KC transcript expression in C57Bl/6 murine colonoids following exposure to pH 7.4 and 6.0 HBSS+ for 6 h (n=4). **c**. FosB, NR4A1, and Dusp1 transcript expression in T84 IEC following a 30 min exposure to pH 6.0 or 7.4 HBSS+ followed by 6 h exposure to 7.4 HBSS+ (n=3). **d**. Representative western blot of phospho-CREB and pan-CREB in T84 IEC pretreated with 10 μm MEK_i at pH 7.4 or exposed to pH 6.0 for 10, 30, and 60 min. **e.** Densitometry analysis and representative western blot of phospho-CREB and pan-CREB in murine colonoids pretreated with 10 μm MEK_i following exposure to pH 6.0 media for 30 min (n=4). **f.** FosB transcription expression in C57Bl/6 murine colonoids pretreated with 10 μm MSK_i or MEK_i following 30 min exposure to pH 6.0 media (n=4). Data from each experiment was pooled and expressed as mean ± SEM and the *p*-value determined by T-test, *p<0.01.

Fig. S2:

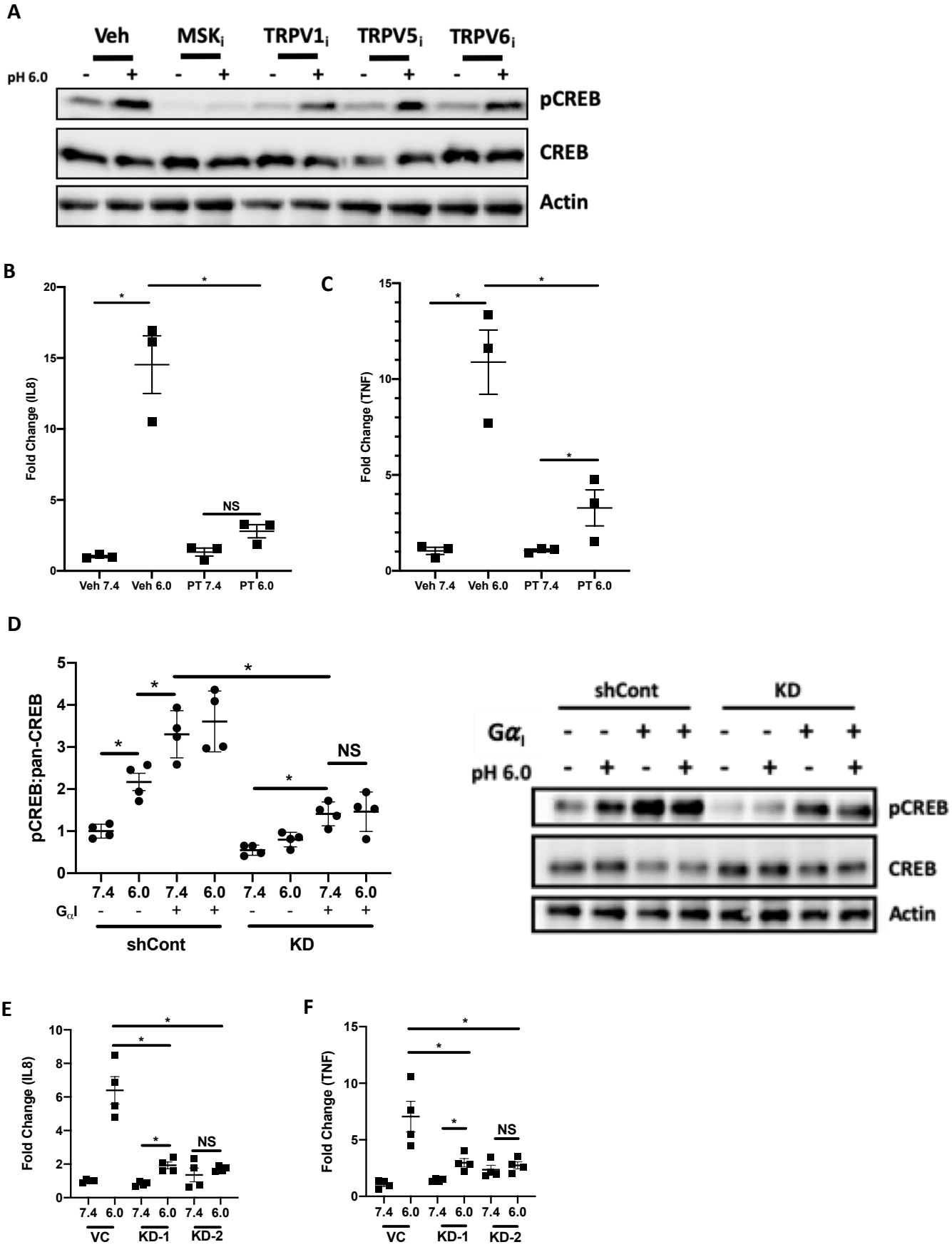

A
Veh
MSK$_i$
TRPV1$_i$
TRPV5$_i$
TRPV6$_i$
pH 6.0
pCREB
CREB
Actin
B
Fold Change (IL8)
Veh 7.4
Veh 6.0
PT 7.4
PT 6.0
NS
C
Fold Change (TNF)
D
pCREB:pan-CREB
shCont
KD
Gα_i
E
F
VC
KD-1
KD-2

Figure S2: Acidosis signaling through $G\alpha_i$ associated surface receptors. a. Western blot of phosphorylated and pan-CREB in T84 IEC treated with 10 μm MSK_i, $TRPV1_i$, $TRPV5_i$, or 100 μm $TRPV6_i$ following 30 min exposure to pH 6.0. **b and c.** IL8 (**b**) and TNF (**c**) transcriptional expression in T84 IEC treated with 1 μg/ml PT following 6 h exposure to pH 6.0 (n=4). **d.** Densitometry analysis and representative western blot of phosphorylated and pan-CREB in shCont and MSK1 KD T84 IEC treated with 10 μm $G\alpha_i$ activator following 30 min exposure to pH 6.0 media (n=4). **e. and f.** IL8 (**e**) and TNF (**f**) transcriptional expression in vector control and GPR31 KD T84 IEC following 30 min exposure to pH 6.0 media (n=4). Data from each experiment was pooled and expressed as mean ± SEM and the *p*-value determined by T-test, *p<0.01.

Fig. S3:

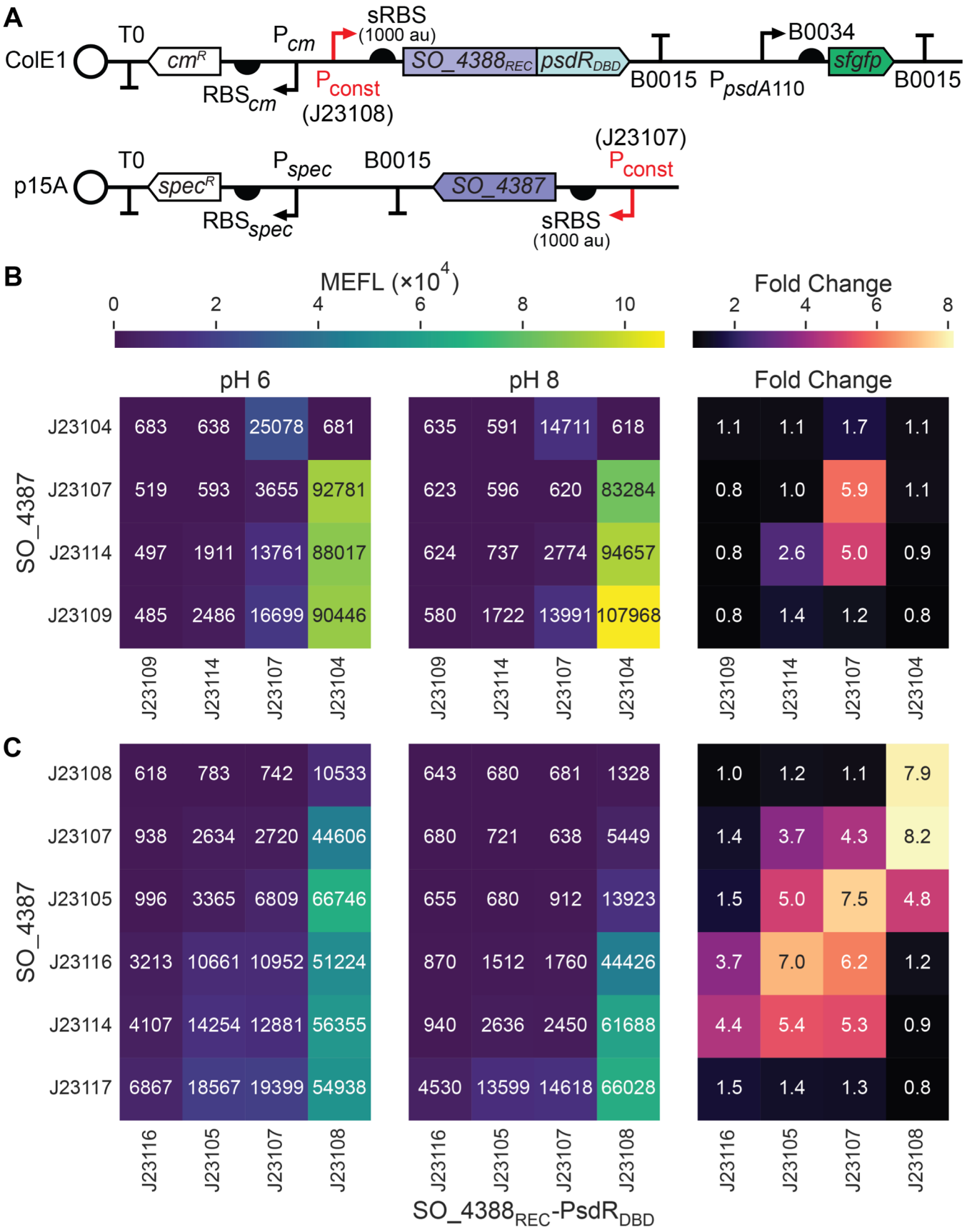
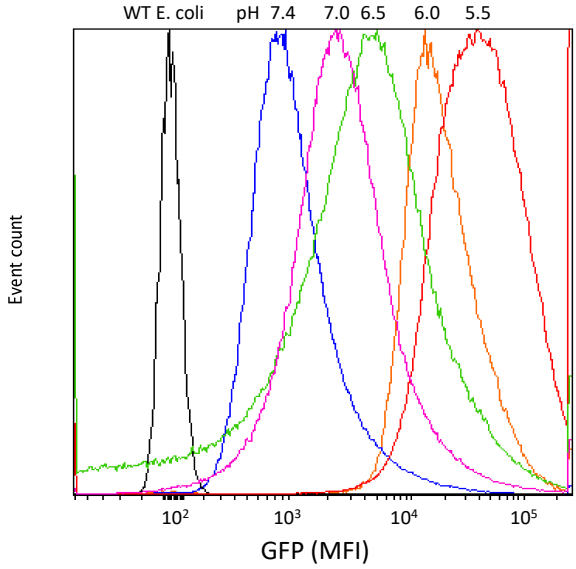

A
ColE1
T0
cmR
RBScm
Pcm
sRBS
(1000 au)
Pconst
(J23108)
SO_4388REC
psdRDBD
B0015
PpsdA110
B0034
sfgfp
B0015
p15A
T0
specR
RBSspec
Pspec
B0015
SO_4387
(J23107)
Pconst
sRBS
(1000 au)
B
MEFL (×10^4)
0 2 4 6 8 10
Fold Change
2 4 6 8
pH 6
pH 8
Fold Change
SO_4387
J23104 683 638 25078 681 635 591 14711 618 1.1 1.1 1.7 1.1
J23107 519 593 3655 92781 623 596 620 83284 0.8 1.0 5.9 1.1
J23114 497 1911 13761 88017 624 737 2774 94657 0.8 2.6 5.0 0.9
J23109 485 2486 16699 90446 580 1722 13991 107968 0.8 1.4 1.2 0.8
J23109 J23114 J23107 J23104
C
SO_4387
J23108 618 783 742 10533 643 680 681 1328 1.0 1.2 1.1 7.9
J23107 938 2634 2720 44606 680 721 638 5449 1.4 3.7 4.3 8.2
J23105 996 3365 6809 66746 655 680 912 13923 1.5 5.0 7.5 4.8
J23116 3213 10661 10952 51224 870 1512 1760 44426 3.7 7.0 6.2 1.2
J23114 4107 14254 12881 56355 940 2636 2450 61688 4.4 5.4 5.3 0.9
J23117 6867 18567 19399 54938 4530 13599 14618 66028 1.5 1.4 1.3 0.8
J23116 J23105 J23107 J23108
SO_4388REC-PsdRDBD

Figure S3: Design and optimization of an acidic pH reporter *E. coli* strain for *in vivo* use. a. Genetic diagram of constitutive pH sensor plasmids (constitutive promoter sites in red, optimal SO_4387 and SO_4388$_{REC}$ -PsdR$_{DBD}$ promoter names in parentheses). **b-c.** Fluorescence signal at pH 6 and pH 8 for constitutive promoter sets evaluated in first (**b**) and second (**c**) rounds of promoter optimization, with corresponding fold changes.

Fig. S4:

A



B

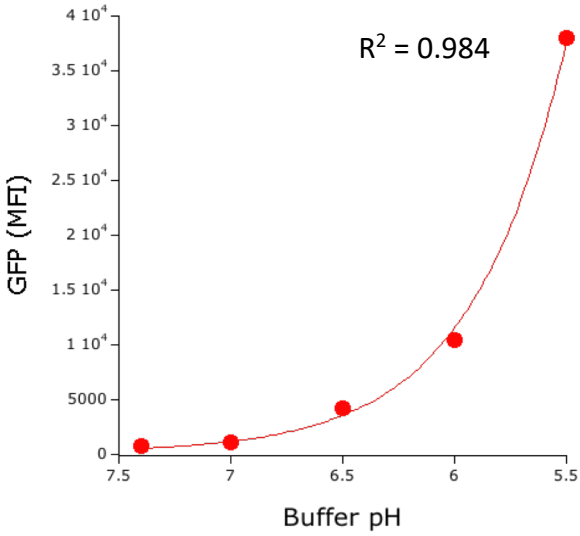


Figure S4: Flow cytometer analysis of sfGFP expression in pH reporter K12 *E. coli* a. Flow analysis of sfGFP intensity in WT and pH reporter K12 *E. coli* exposed to Hanks+ at pH 7.4, 7.0, 6.5, 6.0, and 5.5. **b.** Dose response curve of sfGFP intensity with line of best fit.

Fig. S5:

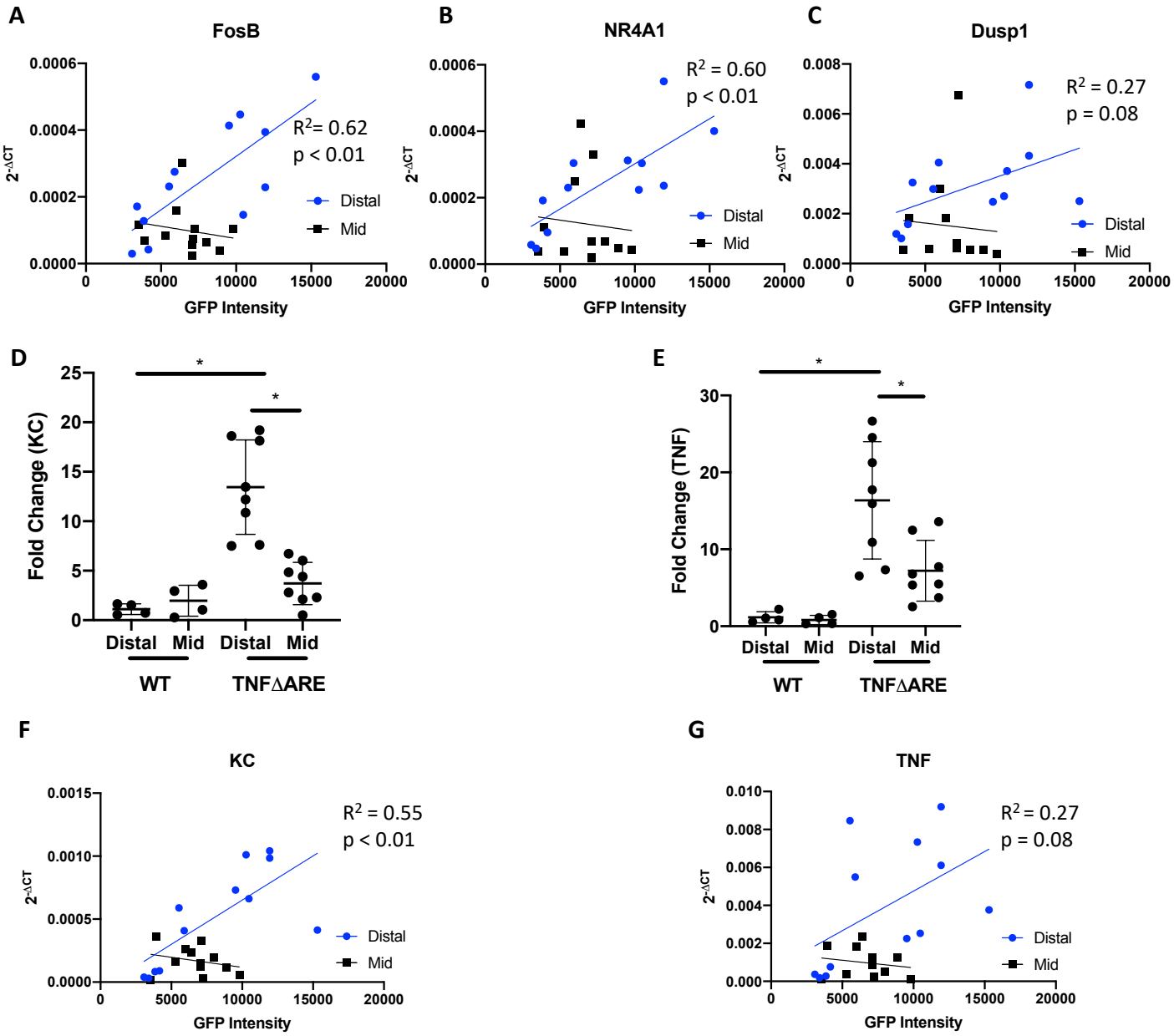


Figure S5: Correlation between acidosis-associated gene expression and mucosal acidity. a-c. Correlation plots comparing sfGFP intensity of pH reporting K12 *E. coli* collected from the mid and distal ileum of WT and TNFΔARE mice and transcriptional expression of FosB (**a**), NR4A1 (**b**), and DUSP1 (**c**) (n=4 and 8 for WT and TNFΔARE mice, respectively). **d-e.** Transcriptional expression of KC (**d**) and TNF (**e**) in tissue collected from the mid and distal ileum of WT and TNFΔARE mice (n=4 and 8 for WT and TNFΔARE mice, respectively). **f-g.** Correlation plots comparing sfGFP intensity of pH reporting *E. coli* collected from the mid and distal ileum of WT and TNFΔARE mice and transcriptional expression of KC (**f**) and TNF (**g**) (n=4 and 8 for WT and TNFΔARE mice, respectively). Data from each experiment was pooled and expressed as mean ± SD and the *p*-value determined by T-test, *p<0.01.

Fig. S6:

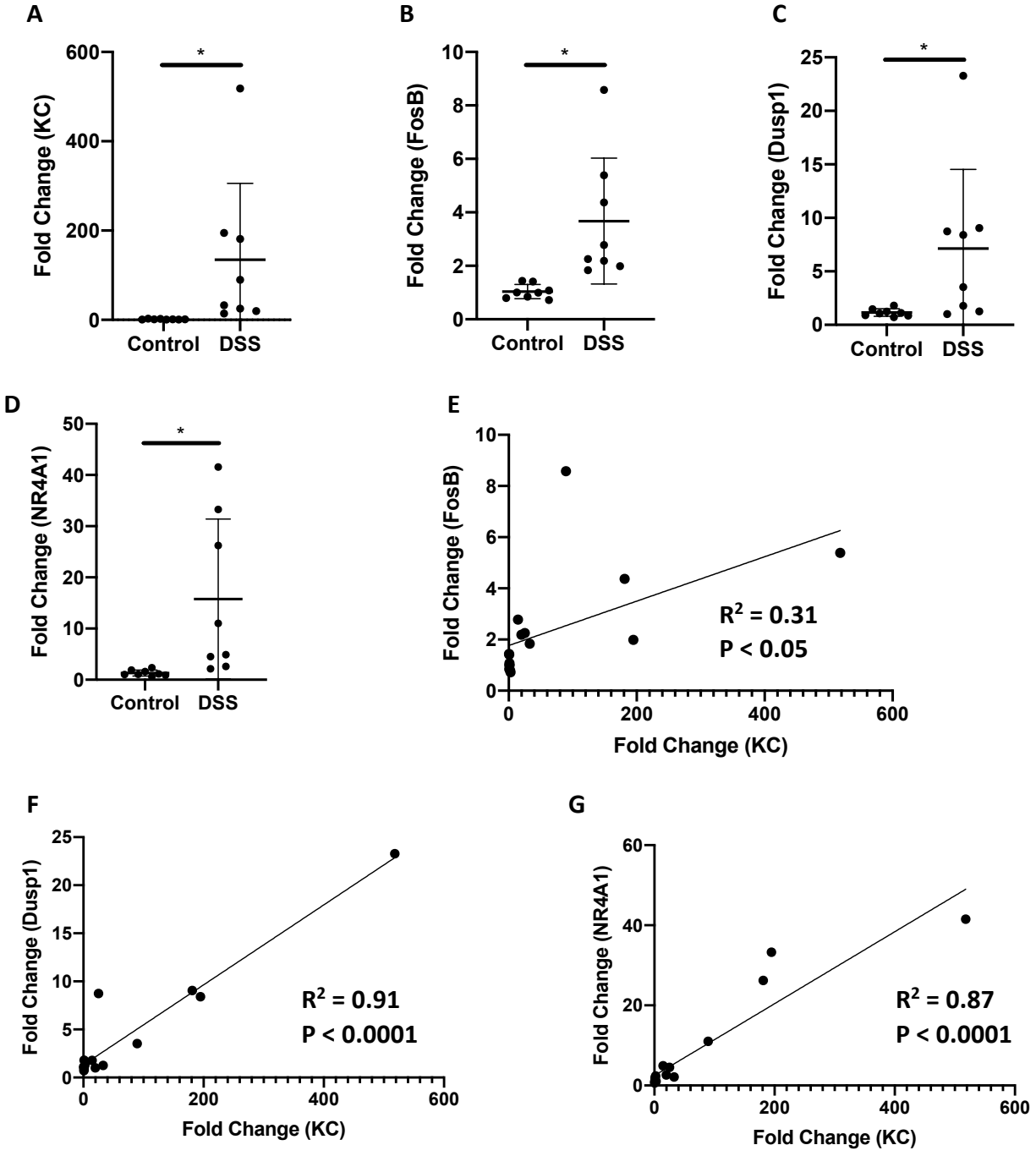


Figure S6: Correlation between acidosis-associated gene expression and KC. a-d. Transcriptional expression of FosB (**a**), Dusp1 (**b**), NR4A1 (**c**), and KC (**d**) in tissue collected from the distal colon of mice treated with 2% DSS and water controls (n=8). **e-g.** Correlation plots comparing transcription al expression of KC and transcriptional expression of FosB (**e**), Dusp1 (**f**), and NR4A1 (**g**) (n=8). Data from each experiment was pooled and expressed as mean ± SD and the *p*-value determined by T-test, *p<0.01.