Supplementary Information

for

A common mechanism links activities of butyrate in the colon

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Supplementary Tables

Table S1: List of molecules used for testing hypotheses regarding the effects of butyrate on stem cell proliferation, cytokine assay on BMDMs, and histone deacetylase (HDAC) inhibition. pKa for carboxylic acids refers to the strongest conjugate acid (RCO_2H).

Number	Structure	IUPAC name	pK_a	CAS#	Supplier
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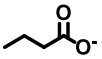
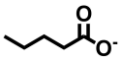
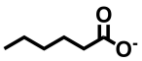
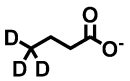
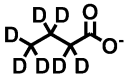
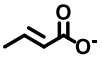
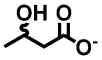
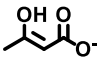
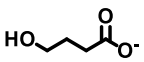
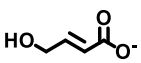
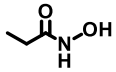
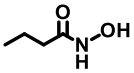
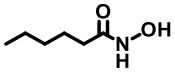
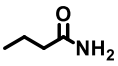
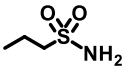
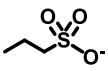
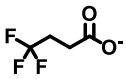
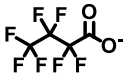
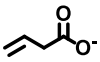
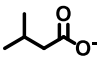
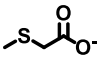
2		Propanoate	4.9	79-09-4	Merck
3		Butanoate	4.8	107-92-6	Sigma-Aldrich
4		Pentanoate	4.8	109-52-4	Merck
5		Hexanoate	4.8	142-62-1	Sigma-Aldrich
6		(4,4,4-$^2H_3$)Butanoate	n/a	36789-14-7	Sigma-Aldrich
7		($^2H_7$)Butanoate	n/a	202468-80-2	Sigma-Aldrich
8		2-Butenoate	4.7	107-93-7	Sigma-Aldrich
9		3-Hydroxybutanoate[a]	4.7	300-85-6	Sigma-Aldrich
10		3-Oxobutanoate	3.6	541-50-4	Sigma-Aldrich
11		4-Hydroxybutanoate[b]	4.7	591-81-1	Sigma-Aldrich
12		(2E)-4-Hydroxy-2-buteonate	n/a	24587-49-3	Thermo Fischer
13		N-hydroxy-acetamide	9.3	546-88-3	Sigma-Aldrich

Number	Structure	IUPAC name	pK_a	CAS#	Supplier
14		N-hydroxy-propanamide	9.3	2580-63-4	Synthesized in-house
15		N-hydroxy-butanamide	9.5	4312-91-8	Sigma-Aldrich
16		N-hydroxy-hexanamide	9.6	4312-93-0	Synthesized in-house
17		Butyramide	n/a	541-35-5	Sigma-Aldrich
18		1-Propanesulfonamide	n/a	24243-71-8	Enamine
19		1-Propanesulfonate	n/a	5284-66-2	Alfa Aesar
20		4,4,4-Trifluorobutanoate	4.2	406-93-9	Sigma-Aldrich
21		Heptafluorobutanoate	1.8	375-22-4	Sigma-Aldrich
22		3-Butenoate	4.3	625-38-7	Sigma-Aldrich
23		3-Methylbutanoate	4.8	503-74-2	Sigma-Aldrich
24		(Methylsulfanyl)acetate	3.7	2444-37-3	Alfa Aesar
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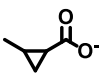
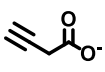
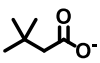
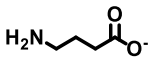
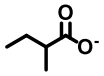
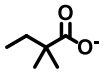
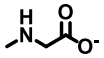
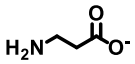
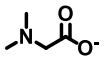
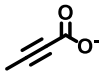
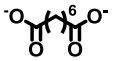
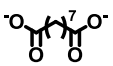
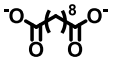
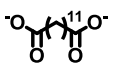
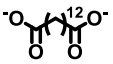
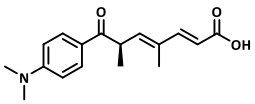
27		2-Methylcyclopropanecarboxylate	5.0	29555-02-0	Alfa Aesar
28		3-Butynoate	3.3	2345-51-9	Sigma-Aldrich
29		3,3-Dimethylbutanoate	5.0	1070-83-3	Sigma-Aldrich

Number	Structure	IUPAC name	pK_a	CAS#	Supplier
30		4-Aminobutanoate	4.2	56-12-2	Sigma-Aldrich
31		2-Methylbutanoate[a]	4.8	116-53-0	Sigma-Aldrich
32		2,2-Dimethylbutanoate	5.0	595-37-9	Sigma-Aldrich
33		N-Methyl-glycine	2.4	107-97-1	Sigma-Aldrich
34		β-Alanine	3.6	107-95-9	Merck
35		N,N-Dimethylglycine	2.1	1118-68-9	Sigma-Aldrich
36		2-Butynoate	2.6	590-93-2	Sigma-Aldrich
37		Octanedioate[c]	4.5	505-48-6	Sigma-Aldrich
38		Nonanedioate[c]	4.6	123-99-9	Sigma-Aldrich
39		Decanedioate[c]	4.7	111-20-6	Sigma-Aldrich
40		Tridecanedioate[c]	4.6	505-52-2	Sigma-Aldrich
41		Tetradecanedioate[c]	4.6	821-38-5	Sigma-Aldrich
TSA		(2E,4E,6R)-7-[4-(Dimethylamino)phenyl]-N-hydroxy-4,6-dimethyl-7-oxo-2,4-heptadienamide or Trichostatin A	8.9	58880-19-6	Abcam

n/a: not available; [a] used in racemic form; [b] approximately 40% is present in the lactone form in the assay buffer, as determined by H-NMR; [c] diacids were tested to ensure that there was no secondary binding site[1] in proximity to the primary one.

Table S2: The EC_{50} values (μM) of GPR43 and GPR41 activation by short-chain fatty acids as determined using a cAMP accumulation assay (in the presence of forskolin) and a fluorescent-based calcium mobilization assay. Data are presented as mean ± S.E. (n=3). Data calculated from Le Poul et al[2].

Short-chain fatty acid	**GPCR43**		**GPCR41**	
	cAMP	**Ca^{2+}**	**cAMP**	**Ca^{2+}**
Acetate	35 ± 4	102 ± 5	1023 ± 94	1072 ± 49
Propionate	14 ± 2	79 ± 4	6 ± 1	20 ± 5
Butyrate	28 ± 7	339 ± 23	42 ± 10	58 ± 8
Pentanoate	1660 ± 585	1905 ± 353	42 ± 14	78 ± 11
Hexanoate	1380 ± 191	>5000	102 ± 17	135 ± 19

Supplementary Figures

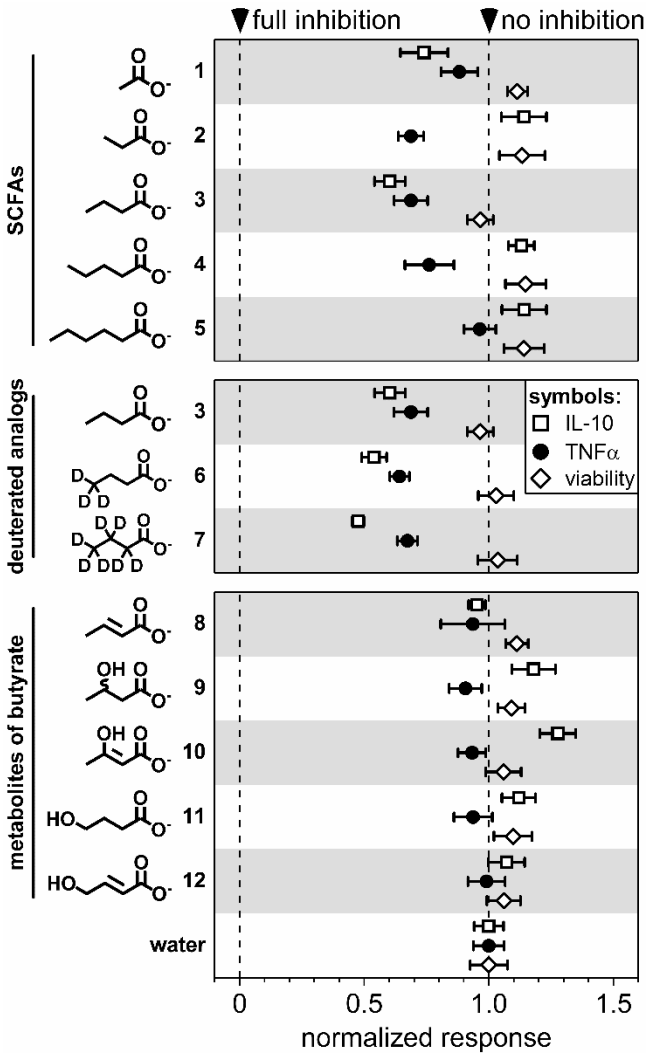


Figure S 1: Expression of interleukin-10 (IL-10) and tumor necrosis factor-α (TNFα) by bone-marrow-derived macrophages (BMDMs). IL-10 and TNFα were measured using enzyme-linked-immunosorbent assay (ELISA) of the supernatant. All responses (in the form of absorbance measurements) were first corrected to a blank sample (baseline-correction) and then normalized to set the activity of the control solvent (i.e. water) sample as 1. The top section of the plot follows the change in chain length for short-chain fatty acids (SCFAs). The middle section of the plot examines the kinetic isotope effect. The bottom section comprises possible

oxidative metabolic products of butyrate. The data are reported as empirical means. Error bars are 95% confidence intervals ($n \geq 7$).

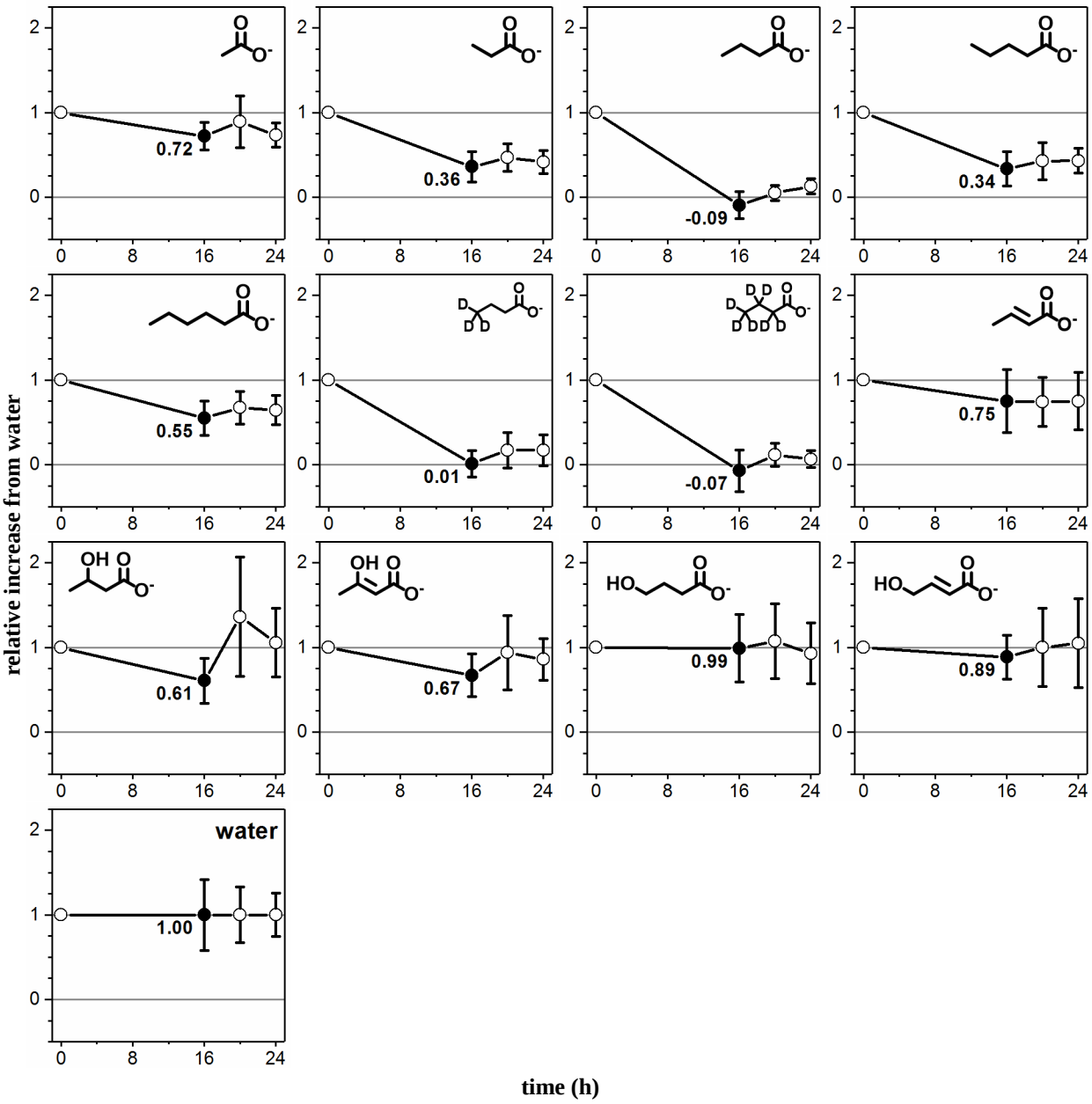


Figure S 2: Time-dependent proliferation of colonic epithelial stem cells (CESCs). Data are measured as luminescence values and then normalized to the initial time-point (0 h) as well as the control solvent (i.e. water) sample (such that the value for the control samples is set to 1). The filled circles highlight the data that were used in Figures 1 and 2. Data are reported as means. Error bars are 95% confidence intervals ($n \geq 7$).

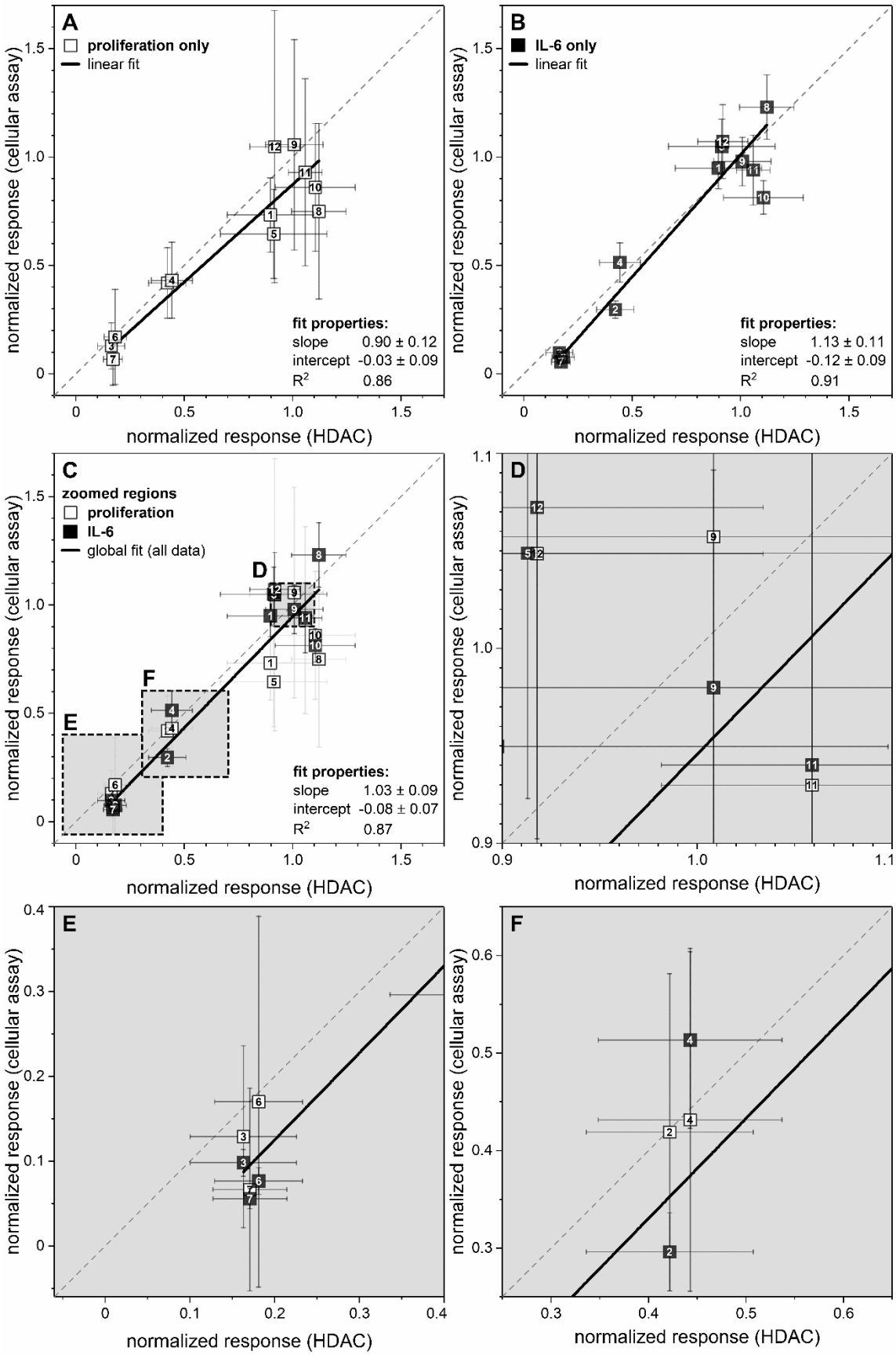

A
proliferation only
linear fit
fit properties:
slope 0.90 ± 0.12
intercept -0.03 ± 0.09
R² 0.86
B
IL-6 only
linear fit
fit properties:
slope 1.13 ± 0.11
intercept -0.12 ± 0.09
R² 0.91
C
zoomed regions
proliferation
IL-6
global fit (all data)
fit properties:
slope 1.03 ± 0.09
intercept -0.08 ± 0.07
R² 0.87
D
E
F
normalized response (cellular assay)
normalized response (HDAC)

Figure S 3: Correlation plots of either A) proliferation of colonic epithelial stem cells (CESCs) or B) expression of IL-6 in bone-marrow-derived macrophages (BMDMs) against the response of molecules in cell-free assay for activity of HDACs. C) Plot shows regions used for magnifying overlaying plots in D, E, and F.

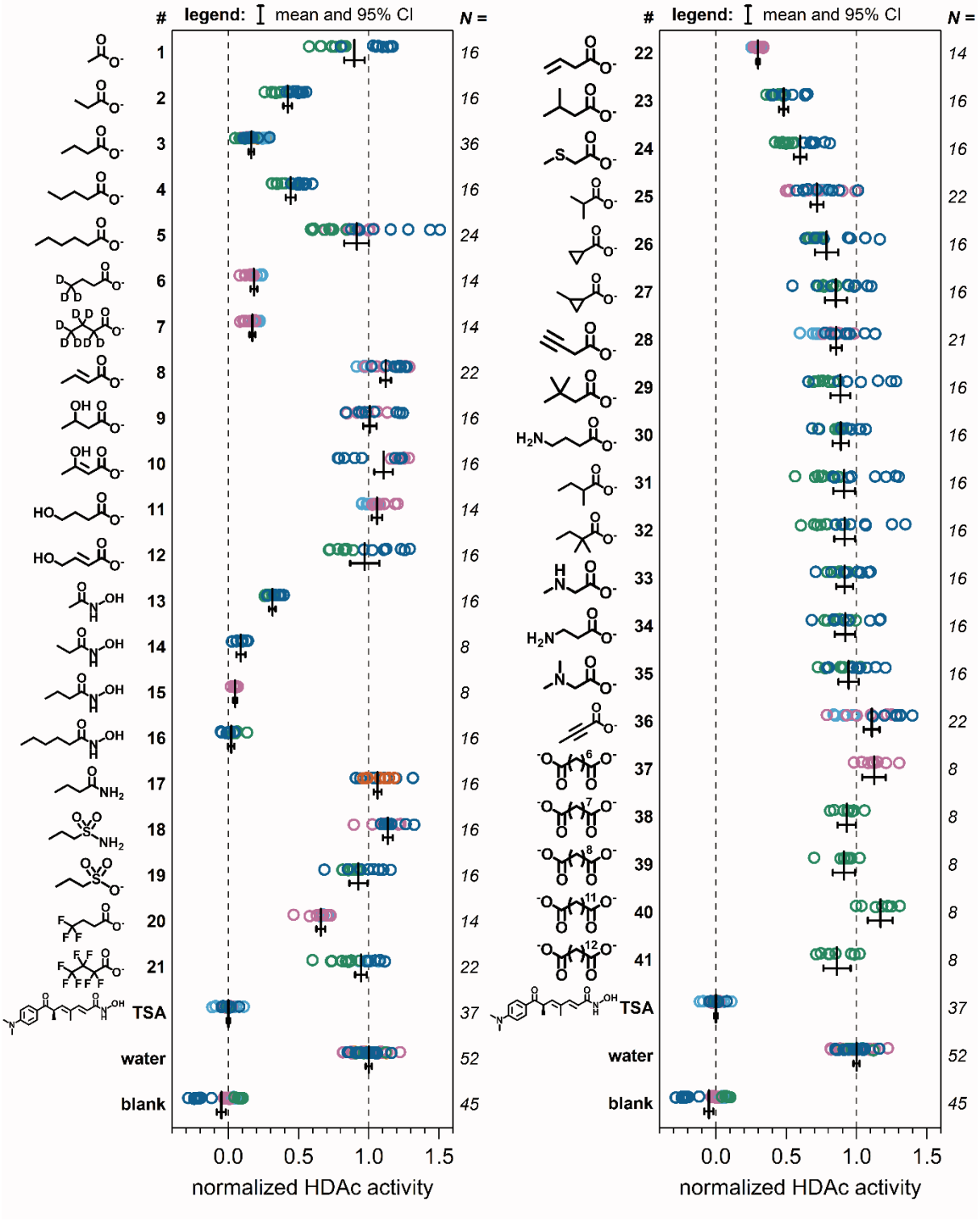


Figure S 4: Response of analogs of butyrate in an assay that measures the activity of HDACs. Data are mean values. Error bars are 95% confidence intervals. Individual datum from multiple

experiments are presented as circles with different colors (batch of crude nuclear extracts from HeLa cell line). Numbers on the left axis are labels for compounds (see Supplementary Table S1 for details). Numbers on the right axis represent sample size.

References

1. Davie, J.R. Inhibition of histone deacetylase activity by butyrate. *J Nutr* **133**, 2485S-2493S (2003).
2. Le Poul, E. et al. Functional characterization of human receptors for short chain fatty acids and their role in polymorphonuclear cell activation. *Journal of Biological Chemistry* **278**, 25481-25489 (2003).