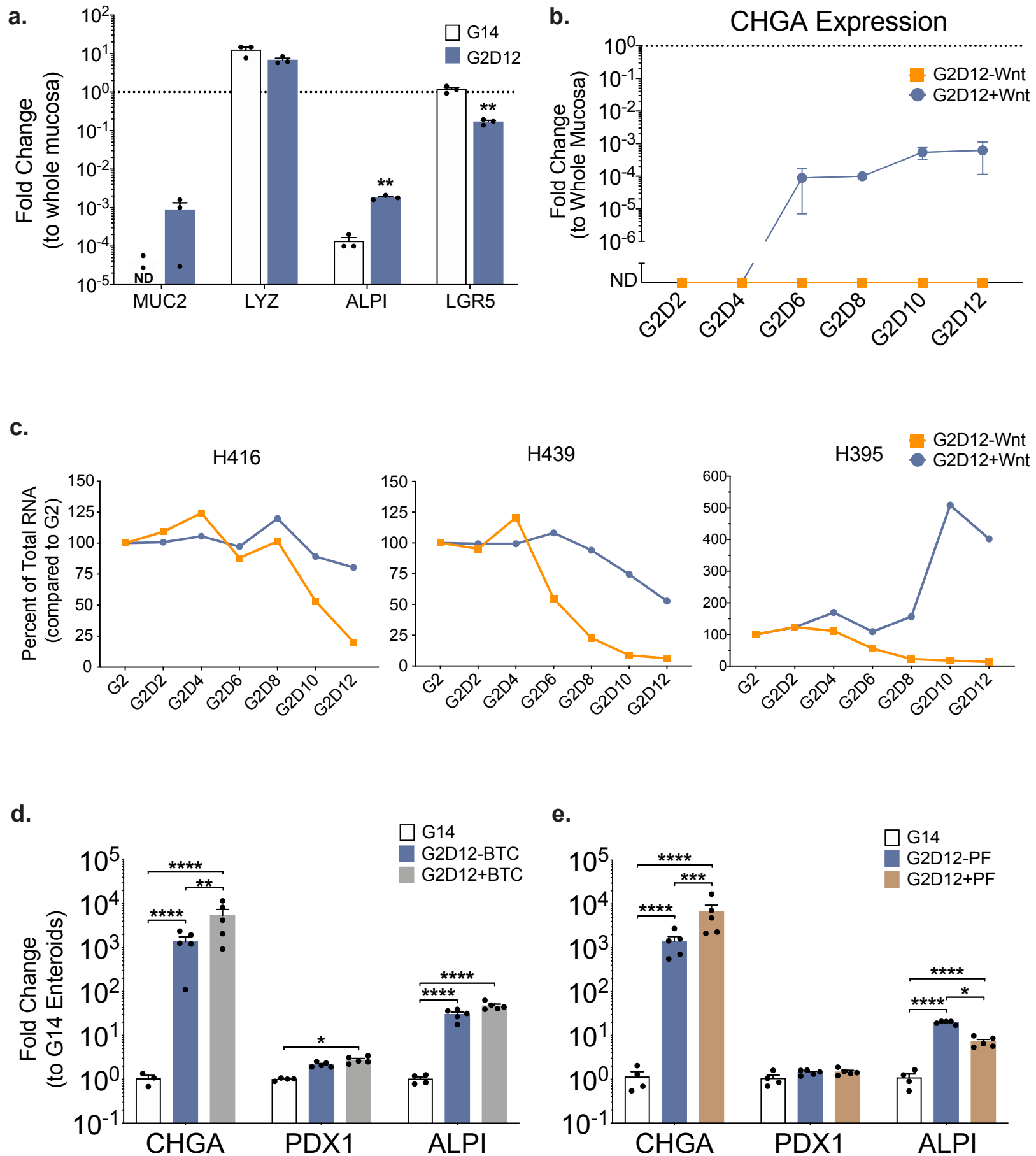


# Supplementary Figure 1



845 **Supplementary Figure 1. Base Differentiation Media Components Important for**  
846 **Enteroendocrine Marker Expression**

847 (a) qPCR analysis of intestinal lineage markers of enteroids grown in either G14 or G2D12  
848 compared to whole mucosa and normalized to *18S*. Dotted line denotes expression level in  
849 whole mucosa. Representative experiment showing n = 3 wells from each condition from a  
850 single enteroid line. MUC2 = mucin 2, LYZ = lysozyme, ALPI = intestinal alkaline phosphatase,  
851 LGR5 = leucine-rich repeat-containing G-protein coupled receptor 5, ND = not detectable in 1 or  
852 more samples.

853 (b) qPCR analysis of chromogranin A (*CHGA*) expression over time of enteroids grown in  
854 G2D12 with Wnt (G2D12+Wnt) or G2D12 without Wnt (G2D12-Wnt) compared to whole  
855 mucosa and normalized to *18S*. RNA was collected every two days after the start of  
856 differentiation. Dotted line denotes expression level in whole mucosa. Representative  
857 experiment showing n = 3 wells from each condition and timepoint from a single enteroid line.  
858 For G2D12+Wnt, only two of three wells expressed *CHGA* at G2D8 with the nondetectable  
859 sample not being included in analysis. ND = not detectable.

860 (c) Time course study of total mRNA levels from three enteroid lines, shown as a percent  
861 compared to RNA levels two days after starting experiment (G2), grown in G2D12+Wnt or  
862 G2D12-Wnt. RNA was collected every two days after start of experiment. Representative  
863 experiment showing n = 3 wells from each condition from a single enteroid line.

864 (d) qPCR analysis of intestinal lineage markers of enteroids grown in G2D12 with betacellulin  
865 (G2D12+BTC) and G2D12 without betacellulin (G2D12-BTC) compared to enteroids grown in  
866 G14 and normalized to *18S*. Representative experiment showing n = 3-5 wells from each  
867 condition from a single enteroid line. *CHGA* = chromogranin A, *PDX1* = pancreatic and  
868 duodenal homeobox 1.

869 (e) qPCR analysis of intestinal lineage markers of enteroids grown in G2D12 with PF06260933  
870 (G2D12+PF) and G2D12 without PF06260933 (G2D12-PF) compared to enteroids grown in

871 G14 and normalized to 18S. Representative experiment showing n = 3-5 wells from each  
872 condition from a single enteroid line.  
873 Bars and line graph (b) show mean  $\pm$  SEM, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ .  
874 Each experiment repeated with at least three different enteroid lines. Unless otherwise stated,  
875 specific conditions were excluded from statistical analysis if the data from 1 or more samples  
876 was labeled as not detectable.

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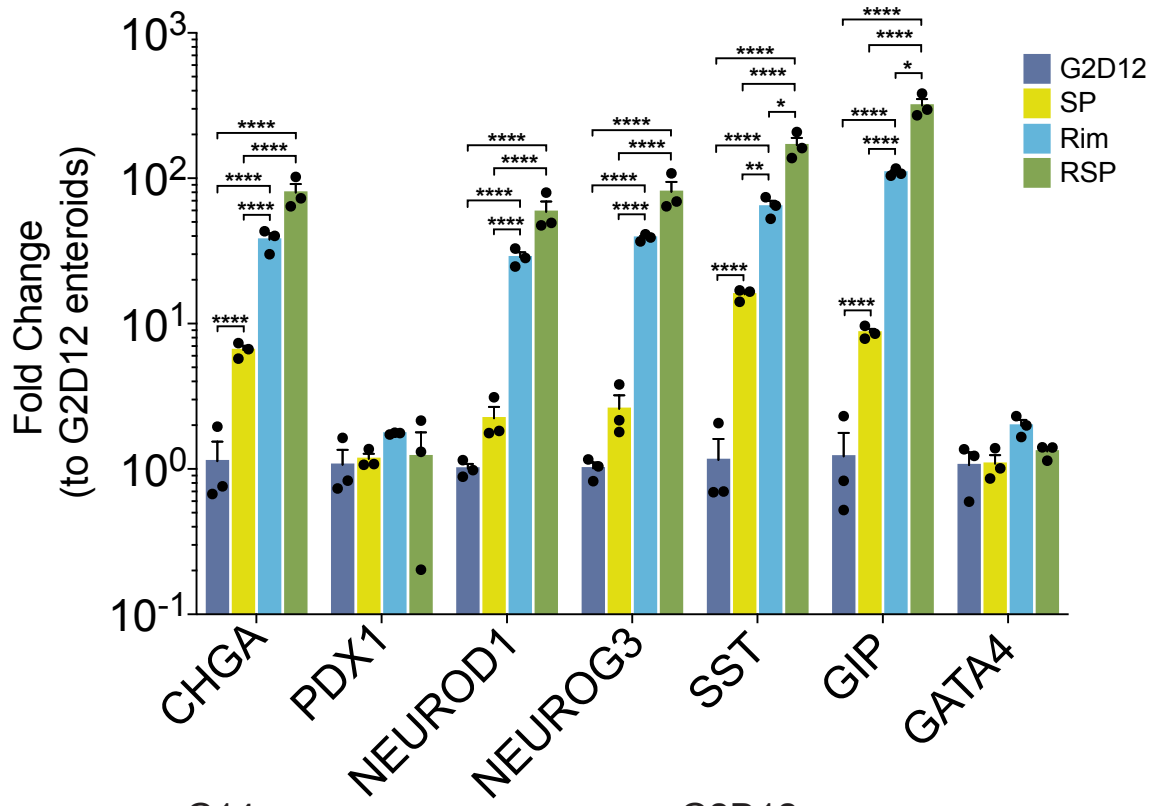
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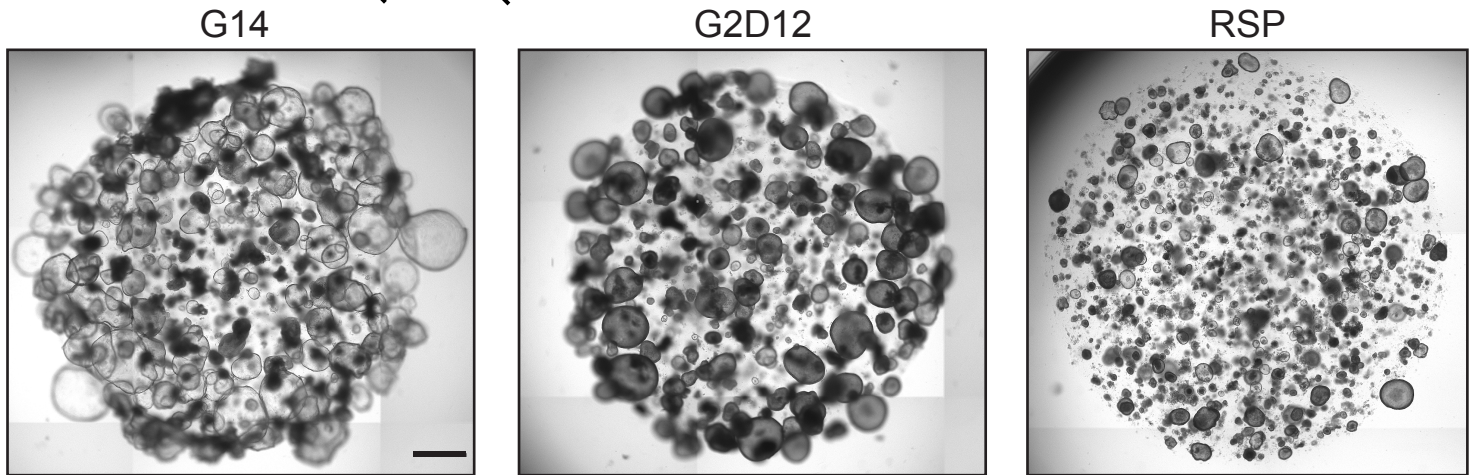
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# Supplementary Figure 2

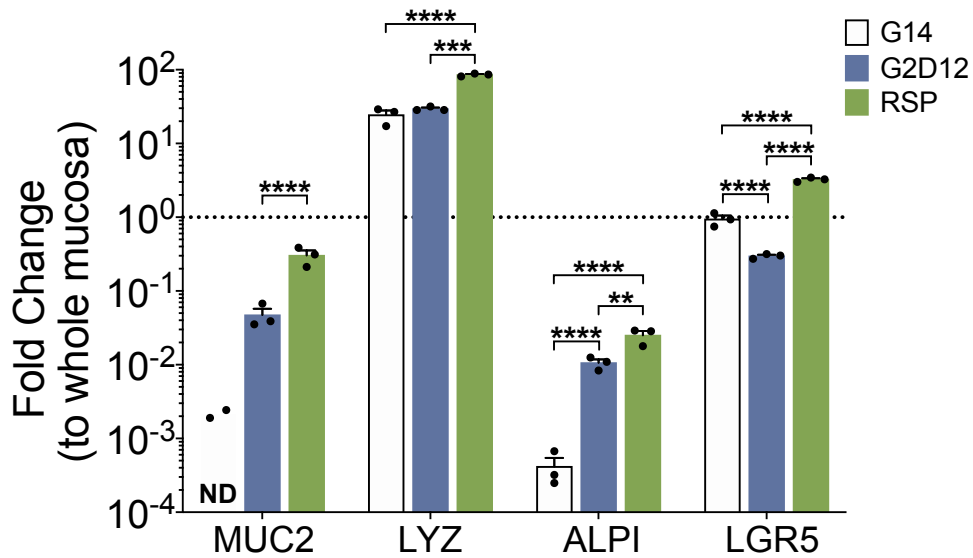
a.



b.



c.



897 **Supplementary Figure 2. Combination of Rimonabant and SP600125 Induces**

898 **Enteroendocrine and other Intestinal Lineage Markers**

899 (a) qPCR analysis of enteroendocrine markers of enteroids grown in G2D12 with rimonabant  
900 (Rim), G2D12 with SP600125 (SP), and G2D12 with rimonabant and SP600125 (RSP)

901 compared to enteroids grown in G2D12 and normalized to *18S*. Representative experiment  
902 showing  $n = 3$  wells from each condition from single enteroid line. CHGA = chromogranin A,

903 PDX1 = pancreatic and duodenal homeobox 1, NEUROD1 = neuronal differentiation 1,

904 NEUROG3 = neurogenin 3, SST = somatostatin, GIP = glucose-dependent insulinotropic

905 peptide, GATA4 = GATA binding protein 4.

906 (b) Representative light microscopy of enteroids (whole well) grown in G14, G2D12, and RSP.

907 Scale bar = 1mm.

908 (c) qPCR analysis of intestinal lineage markers of enteroids grown in G14, G2D12, and RSP

909 compared to whole mucosa and normalized to *18S*. Dotted line denotes expression level in

910 whole mucosa. Representative experiment showing  $n = 3$  wells from each condition from a

911 single enteroid line. MUC2 = mucin 2, LYZ = lysozyme, ALPI = intestinal alkaline phosphatase,

912 LGR5 = leucine-rich repeat-containing G-protein coupled receptor 5, ND = not detectable in 1 or

913 more samples.

914 Bars show mean  $\pm$  SEM, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Each experiment

915 repeated with at least three different enteroid lines. Specific conditions were excluded from

916 statistical analysis if the data from 1 or more samples was labeled as not detectable.

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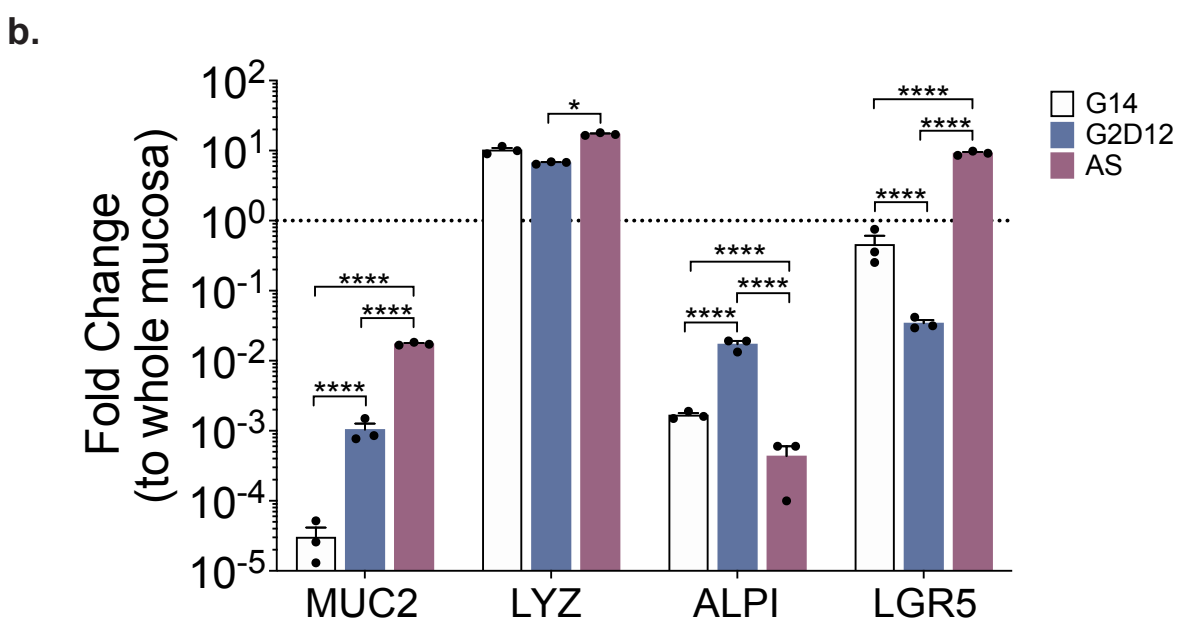
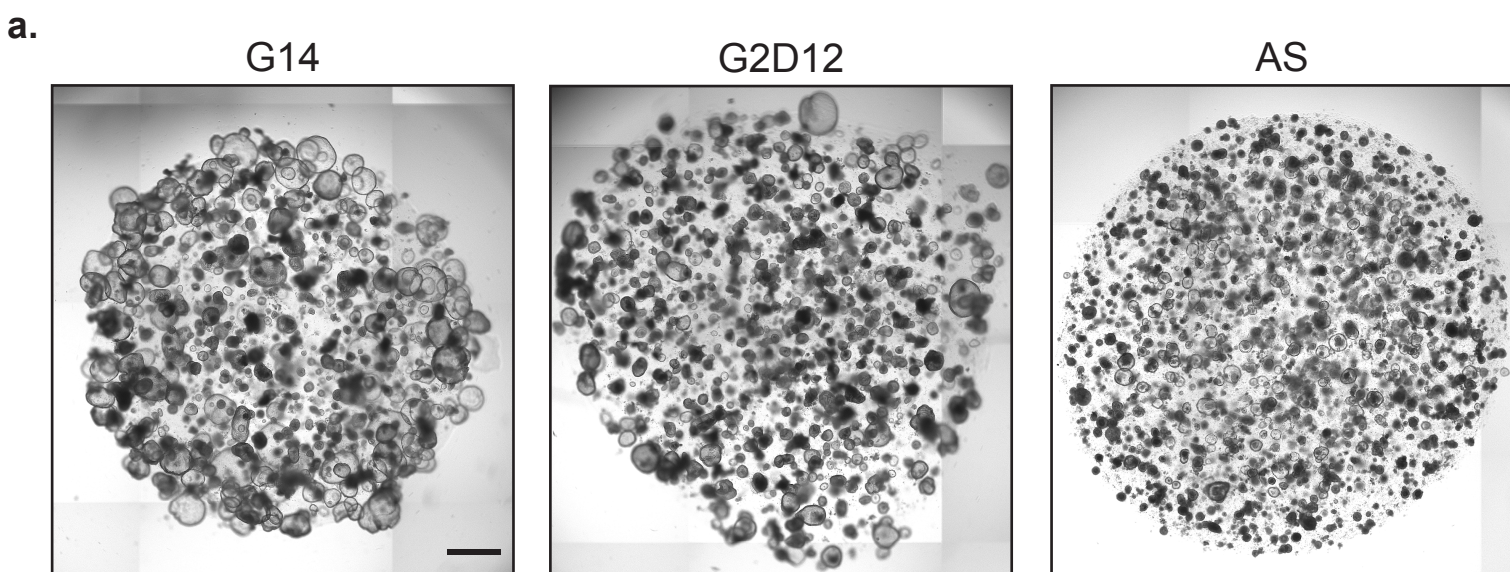
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# Supplementary Figure 3



923 **Supplementary Figure 3. AS1842856 Induces Specific Intestinal Lineage Markers**

924 (a) Representative light microscopy of enteroids (whole well) grown in G14, G2D12, and G2D12  
925 with AS1842856 (AS). Scale bar = 1mm.

926 (b) qPCR analysis of intestinal lineage markers of enteroids grown in G14, G2D12, and AS  
927 compared to whole mucosa and normalized to *18S*. Dotted line denotes expression level in  
928 whole mucosa. Representative experiment showing  $n = 3$  wells from each condition from single  
929 enteroid line. MUC2 = mucin 2, LYZ = lysozyme, ALPI = intestinal alkaline phosphatase, LGR5  
930 = leucine-rich repeat-containing G-protein coupled receptor 5.

931 Bars show mean  $\pm$  SEM, \* $p < 0.05$ , \*\*\*\* $p < 0.0001$ . Each experiment repeated with at least  
932 three different enteroid lines.

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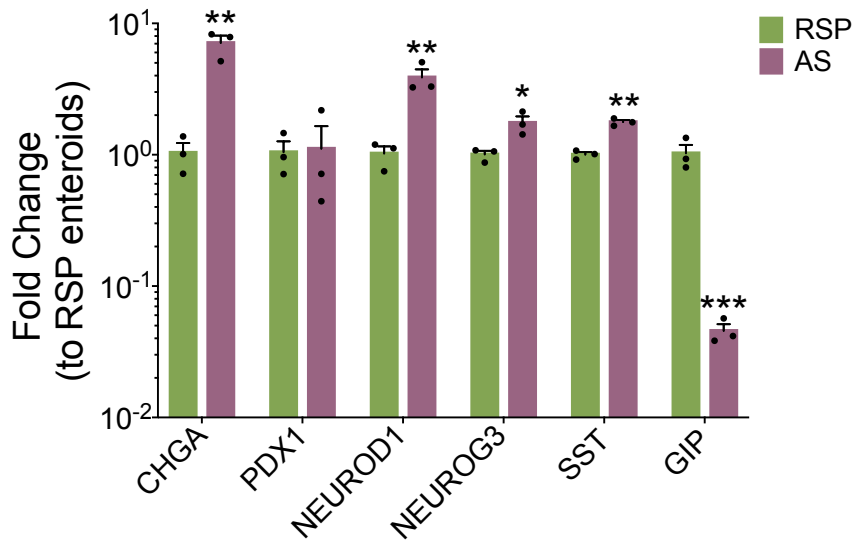
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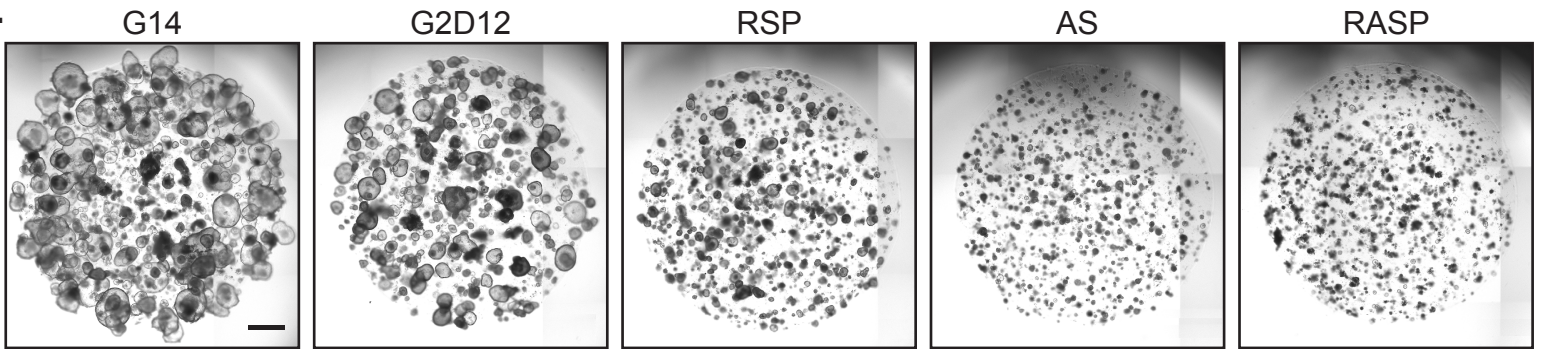
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# Supplementary Figure 4

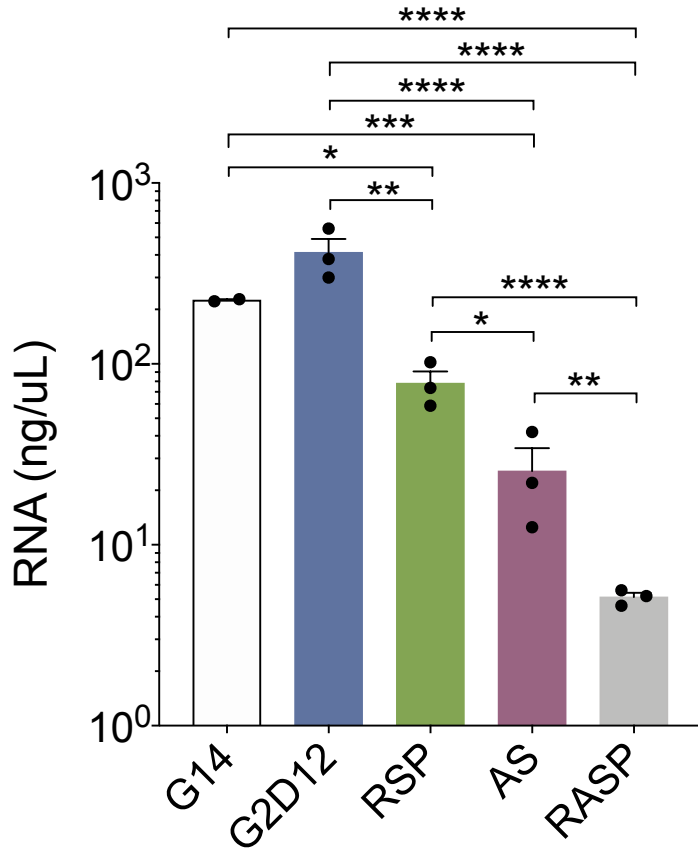
a.



b.



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948 **Supplementary Figure 4. Combination of AS1842856 and Rimonabant/SP600125 For All**  
949 **of Differentiation Leads to Reduction in Isolated RNA**

950 (a) qPCR analysis of enteroendocrine markers of enteroids grown in AS compared to RSP and  
951 normalized to 18S. Representative experiment showing n = 3 wells from each condition from a  
952 single enteroid line. CHGA = chromogranin A, PDX1 = pancreatic and duodenal homeobox 1,  
953 NEUROD1 = neuronal differentiation 1, NEUROG3 = neurogenin 3, SST = somatostatin, GIP =  
954 glucose-dependent insulinotropic peptide.

955 (b) Representative light microscopy of enteroids (whole well) grown in G14, G2D12, AS, RSP,  
956 and G2D12 with AS and RSP (RASP). Scale bar = 1mm.

957 (c) Total mRNA levels from enteroids grown in G14, G2D12, AS, RSP, and RASP.

958 Representative results from n = 2-3 wells from each condition from a single enteroid line.

959 Bars show mean  $\pm$  SEM, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001. Each experiment  
960 repeated with at least three different enteroid lines.

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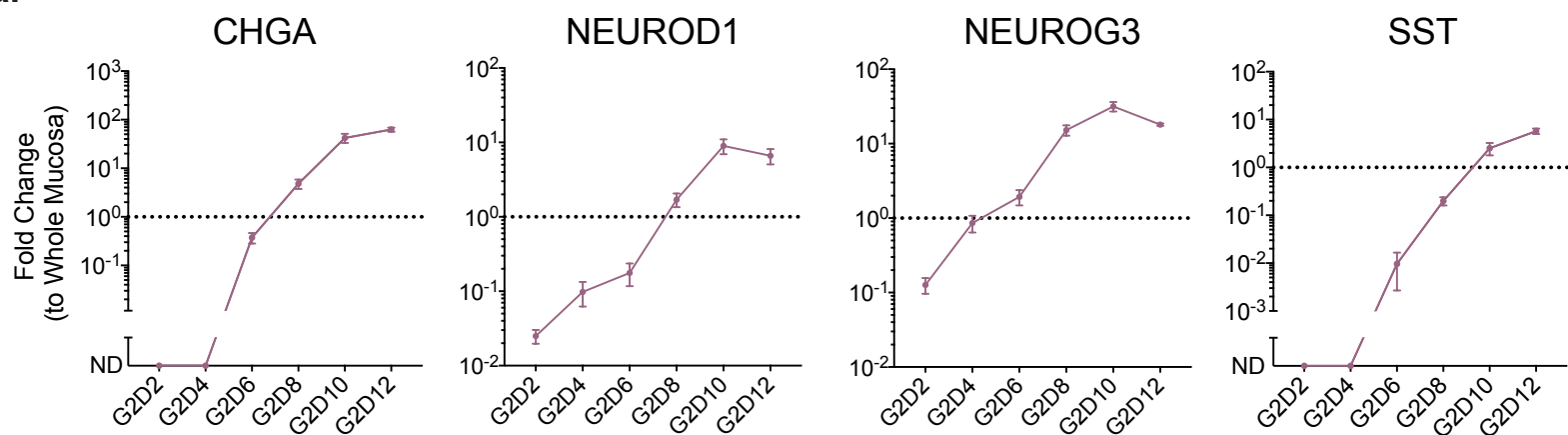
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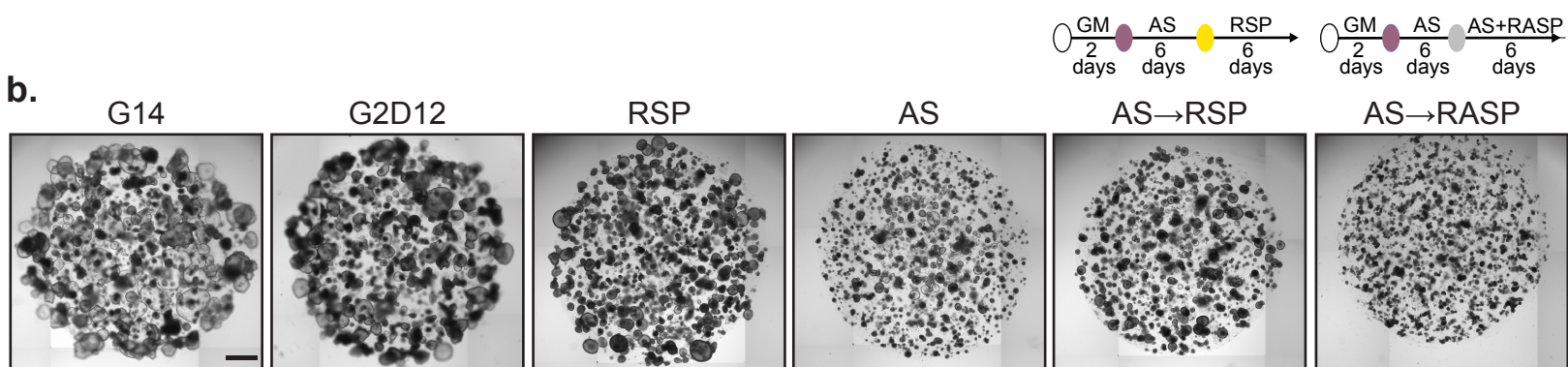
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# Supplementary Figure 5

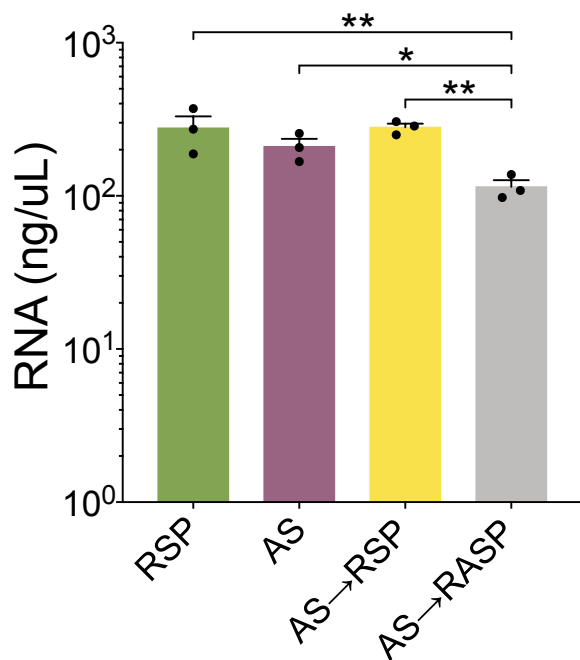
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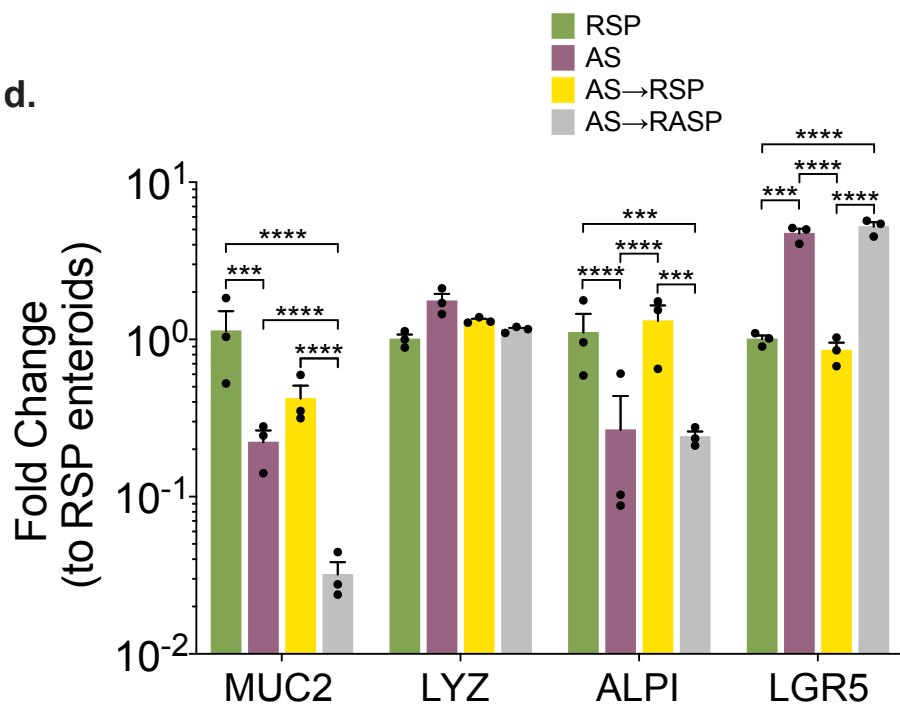
b.



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d.



973 **Supplementary Figure 5. Different Small Molecule Combinations Induce Specific Gene**  
974 **Expression Changes**

975 (a) qPCR analysis of enteroendocrine marker expression over time from enteroids grown in AS  
976 compared to whole mucosa and normalized to *18S*. RNA was collected every two days after  
977 start of differentiation. Dotted line denotes expression level in whole mucosa. Representative  
978 experiment showing  $n = 3$  wells from each timepoint, except  $n = 2$  for G2D12, from a single  
979 enteroid line. At G2D2, only two of three wells expressed NEUROD1 and NEUROG3, with  
980 nondetectable samples excluded from analysis. CHGA = chromogranin A, NEUROD1 =  
981 neuronal differentiation 1, NEUROG3 = neurogenin 3, SST = somatostatin, ND = not detectable.

982 (b) Representative light microscopy of enteroids (whole well) grown in G14, G2D12, AS, RSP,  
983 AS→RSP, and AS→RASP. Specific culture schematics of AS→RSP and AS→RASP located  
984 above each panel, respectively. Scale bar = 1mm.

985 (c) Total mRNA levels from enteroids grown in AS, RSP, AS→RSP, and AS→RASP.  
986 Representative experiment showing  $n = 3$  wells from each condition from a single enteroid line.

987 (d) qPCR analysis of intestinal lineage markers of enteroids grown in AS, AS→RSP, and  
988 AS→RASP compared to RSP and normalized to *18S*. Representative experiment showing  $n = 3$   
989 wells from each condition from single enteroid line. MUC2 = mucin 2, LYZ = lysozyme, ALPI =  
990 intestinal alkaline phosphatase, LGR5 = leucine-rich repeat-containing G-protein coupled  
991 receptor 5.

992 Bars and line graph show mean  $\pm$  SEM, \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$ . Each  
993 experiment repeated with at least three different enteroid lines.

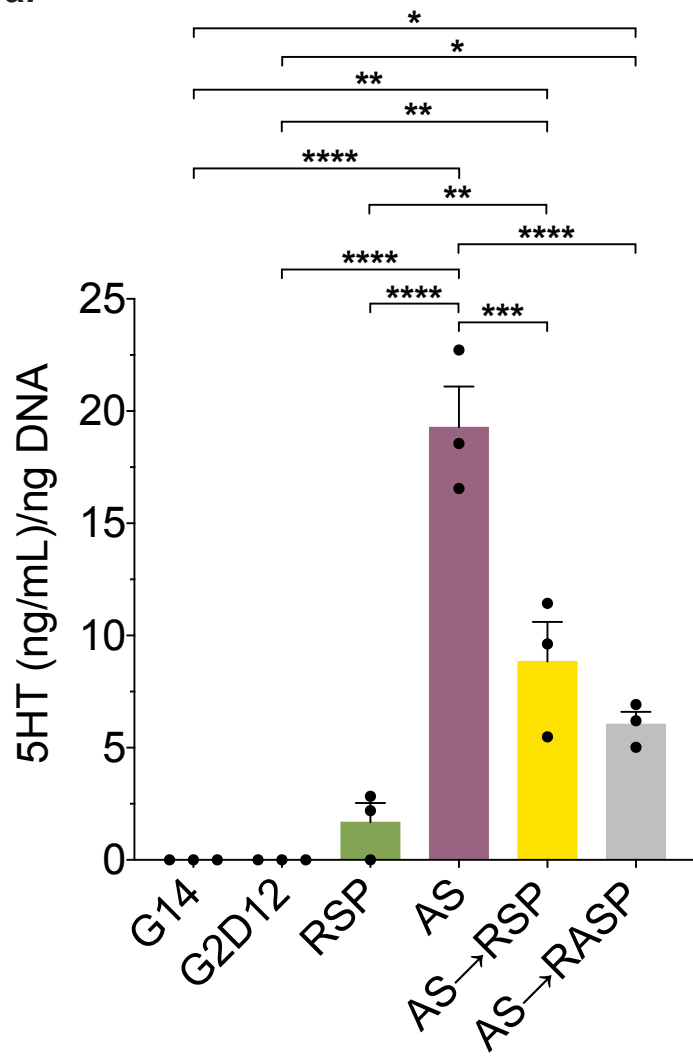
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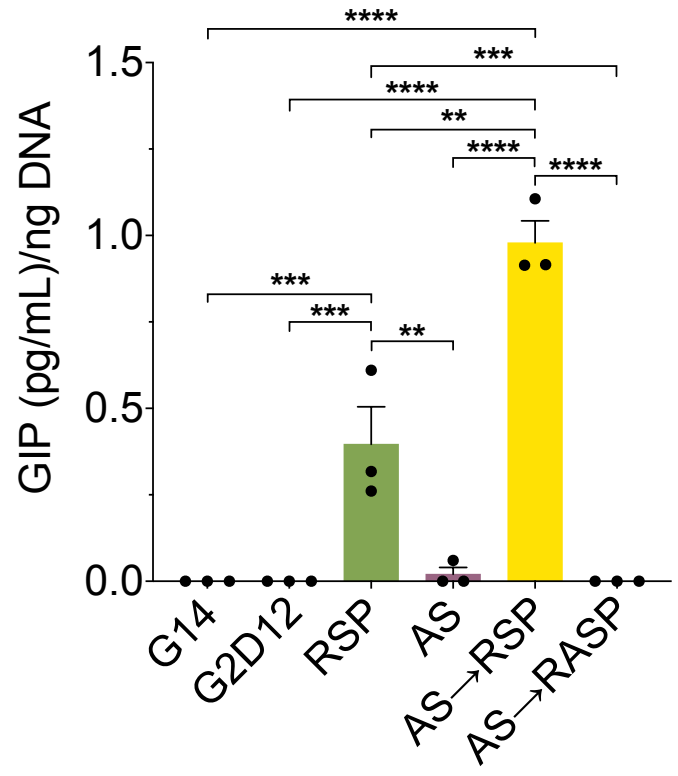
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# Supplementary Figure 6

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997 **Supplementary Figure 6. Hormone Secretion of Different Conditions Controlled for Total**  
998 **DNA**

999 (a) Serotonin (5HT) ELISA of conditioned media from the last two days of differentiation of  
1000 enteroids grown in G14, G2D12, AS, RSP, AS→RSP, and AS→RASP controlled for total DNA  
1001 from each sample. Representative experiment showing n = 3 wells from each condition from a  
1002 single enteroid line.

1003 (b) Glucose-dependent insulintropic peptide (GIP) ELISA of conditioned media from the last  
1004 two days of differentiation of enteroids grown in G14, G2D12, AS, RSP, AS→RSP, and  
1005 AS→RASP controlled for total DNA from each sample. Representative experiment showing n =  
1006 3 wells from each condition from a single enteroid line.

1007 Bars show mean ± SEM, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001, \*\*\*\*p < 0.0001. To control for  
1008 enteroid number, protein concentrations were divided by DNA concentration. Each experiment  
1009 repeated with at least three different enteroid lines.

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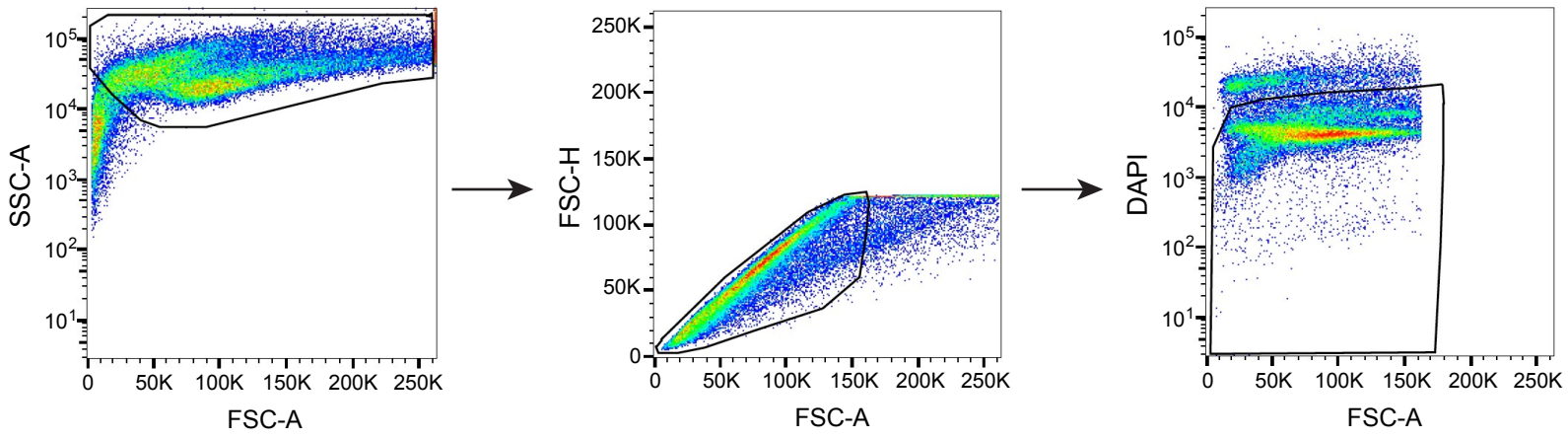
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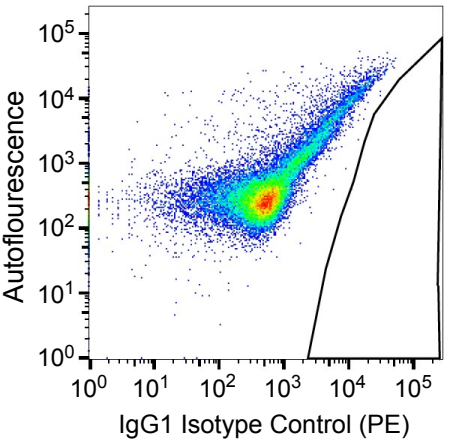
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# Supplementary Figure 7

**a.**



**b.**



1021 **Supplementary Figure 7. Gating Strategy for Flow Cytometry**

1022 (a) Enteroid cells are differentiated from cellular debris based on their forward and side scatter  
1023 area (FSC-A and SSC-A, respectively) parameters. Cells are then examined based on their  
1024 FSC-A and FSC-Height (H) to exclude doublets. 4',6-diamidino-2-phenylindole (DAPI) staining  
1025 is then utilized to identify dead cells, with DAPI high-positive cells being excluded from further  
1026 gating.

1027 (b) The CHGA-positive gate is set by using an IgG1 K isotype control conjugate with  
1028 phycoerythrin (PE).

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1 **Supplementary Table 1. Growth and Differentiation Media Components**

<b>Growth Media</b>	<b>Differentiation Media</b>
L-WRN conditioned media (50% v/v)	
DMEM/F12 (45% v/v)	
Glutamax (1% v/v)	
N-2 Supplement (1% v/v)	
B-27 Supplement (1% v/v)	
HEPES (10mM)	
Primocin (100µg/mL)	
Normocin (100µg/mL)	
A83-01 (500nM)	
N-Acetyl-cysteine (500µM)	
Recombinant Murine EGF (50ng/mL)	
Human [Leu15] Gastrin I (10nM)	
Nicotinamide (10mM)	DAPT (20µM)
SB202190 (10µM)	Betacellulin (20ng/mL)
	Tubastatin-A (10µM)
	PF06260933 (6µM)
	Tranylcypromine (1.5µM)
	Rimonabant (10µM)
	SP600125 (10µM)
	AS1842856 (100nM)

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820 **Supplemental Table 1. Growth and differentiation media components**

821 Growth factors, supplements and small molecules common to both growth and differentiation  
822 medias are listed first. Individual components on the left correspond to growth media; those on  
823 the right correspond to differentiation media. L-WRN cell line used to produce conditioned  
824 media containing Wnt3a, noggin and R-spondin 3. HA-R-Spondin1-Fc 293T cell line used to  
825 produce conditioned media with only R-spondin 1. This conditioned media, with supplemented  
826 noggin (100ng/mL), was used to make Wnt3a-free differentiation media.

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3 **Supplementary Table 2. Description of samples**

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<b>Identification</b>	<b>Age</b>	<b>Sex</b>	<b>Application</b>
H357	13 years	Male	Enteroid line
H368	14 years	Female	Enteroid line
H389	14 years	Male	Enteroid line
H393	15 years	Female	Enteroid line
H395	18 years	Male	Enteroid line
H407	13 years	Male	Enteroid line
H416	21 years	Female	Enteroid line
H439	19 years	Female	Enteroid line
H522	82 years	Female	Whole mucosa RNA
H544	55 years	Female	Whole mucosa RNA
H545	70 years	Male	Whole mucosa RNA

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21 **Supplementary Table 3. Taqman qPCR Primers**

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<b>Name</b>	<b>Abbreviation</b>	<b>Identifier</b>
18S	<i>18S</i>	Hs99999901_s1
Intestinal alkaline phosphatase	<i>ALPI</i>	Hs00357579_g1
Chromogranin A	<i>CHGA</i>	Hs00900370_m1
GATA binding protein 4	<i>GATA4</i>	Hs00171403_m1
Glucose-dependent insulinotropic polypeptide	<i>GIP</i>	Hs00175030_m1
Leucine-rich repeat-containing G-protein coupled receptor 5	<i>LGR5</i>	Hs00969422_m1
Lysozyme	<i>LYZ</i>	Hs00426232_m1
Mucin 2	<i>MUC2</i>	Hs03005103_g1
Neuronal differentiation 1	<i>NEUROD1</i>	Hs01922995_s1
Neurogenin 3	<i>NEUROG3</i>	Hs01875204_s1
Somatostatin	<i>SST</i>	Hs00356144_m1

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