

Supplementary Table 1

A.)

ids	Status	Segment	Treatment	Format	Substrate	Project #
C103 D0	Differentiated	Colon	Control	3D	Matrigel	1
C103 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C104 D0	Differentiated	Colon	Control	3D	Matrigel	1
C104 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C109 D0	Differentiated	Colon	Control	3D	Matrigel	1
C109 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C111 D0	Differentiated	Colon	Control	3D	Matrigel	1
C111 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C112 D0	Differentiated	Colon	Control	3D	Matrigel	1
C112 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C131 D0	Differentiated	Colon	Control	3D	Matrigel	1
C131 D100	Differentiated	Colon	1,25D3	3D	Matrigel	1
C103 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C103 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
C104 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C104 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
C109 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C109 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
C111 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C111 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
C112 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C112 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
C131 H0	Undifferentiated	Colon	Control	3D	Matrigel	1
C131 H100	Undifferentiated	Colon	1,25D3	3D	Matrigel	1
D102 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D102 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D103 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D103 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D104 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D104 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D109 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D109 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D144 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D144 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D2 D0	Differentiated	Duodenum	Control	3D	Matrigel	1
D2 D100	Differentiated	Duodenum	1,25D3	3D	Matrigel	1
D102 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D102 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
D103 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D103 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
D104 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D104 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
D109 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D109 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
D144 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D144 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
D2 H0	Undifferentiated	Duodenum	Control	3D	Matrigel	1
D2 H100	Undifferentiated	Duodenum	1,25D3	3D	Matrigel	1
C143	Undifferentiated	Colon	Control	3D	Matrigel	2
C144	Undifferentiated	Colon	Control	3D	Matrigel	2
C146	Undifferentiated	Colon	Control	3D	Matrigel	2
C147	Undifferentiated	Colon	Control	3D	Matrigel	2
C152	Undifferentiated	Colon	Control	3D	Matrigel	2
C153	Undifferentiated	Colon	Control	3D	Matrigel	2
C173 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C177 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C183 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C189 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C203 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C209 CD	Undifferentiated	Colon	Control	3D	Matrigel	2
C3 aafA	Differentiated	Colon	aafA	Monolayer	Matrigel	3
C3 eaec	Differentiated	Colon	eaec	Monolayer	Matrigel	3
C3 ehec	Differentiated	Colon	ehec	Monolayer	Matrigel	3
C3 expec	Differentiated	Colon	expec	Monolayer	Matrigel	3
C3 HS	Differentiated	Colon	HS	Monolayer	Matrigel	3
C3 Mock	Differentiated	Colon	Mock	Monolayer	Matrigel	3
C4 aafA	Differentiated	Colon	aafA	Monolayer	Matrigel	3
C4 eaec	Differentiated	Colon	eaec	Monolayer	Matrigel	3
C4 ehec	Differentiated	Colon	ehec	Monolayer	Matrigel	3
C4 expec	Differentiated	Colon	expec	Monolayer	Matrigel	3
C4 HS	Differentiated	Colon	HS	Monolayer	Matrigel	3
C4 Mock	Differentiated	Colon	Mock	Monolayer	Matrigel	3
C9 aafA	Differentiated	Colon	aafA	Monolayer	Matrigel	3
C9 eaec	Differentiated	Colon	eaec	Monolayer	Matrigel	3
C9 ehec	Differentiated	Colon	ehec	Monolayer	Matrigel	3
C9 expec	Differentiated	Colon	expec	Monolayer	Matrigel	3
C9 HS	Differentiated	Colon	HS	Monolayer	Matrigel	3
C9 Mock	Differentiated	Colon	Mock	Monolayer	Matrigel	3
D103 aafA	Differentiated	Duodenum	aafA	Monolayer	Matrigel	3
D103 HS	Differentiated	Duodenum	HS	Monolayer	Matrigel	3
D103 Mock	Differentiated	Duodenum	Mock	Monolayer	Matrigel	3
D3 eaec	Differentiated	Duodenum	eaec	Monolayer	Matrigel	3
D3 ehec	Differentiated	Duodenum	ehec	Monolayer	Matrigel	3
D3 expec	Differentiated	Duodenum	expec	Monolayer	Matrigel	3

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D4 aafA	Differentiated	Duodenum	aafA	Monolayer	Matrigel	3
D4 eaec	Differentiated	Duodenum	eaec	Monolayer	Matrigel	3
D4 ehec	Differentiated	Duodenum	ehec	Monolayer	Matrigel	3
D4 expec	Differentiated	Duodenum	expec	Monolayer	Matrigel	3
D4 HS	Differentiated	Duodenum	HS	Monolayer	Matrigel	3
D4 Mock	Differentiated	Duodenum	Mock	Monolayer	Matrigel	3
D9 aafA	Differentiated	Duodenum	aafA	Monolayer	Matrigel	3
D9 eaec	Differentiated	Duodenum	eaec	Monolayer	Matrigel	3
D9 ehec	Differentiated	Duodenum	ehec	Monolayer	Matrigel	3
D9 expec	Differentiated	Duodenum	expec	Monolayer	Matrigel	3
D9 HS	Differentiated	Duodenum	HS	Monolayer	Matrigel	3
D9 Mock	Differentiated	Duodenum	Mock	Monolayer	Matrigel	3
I103 aafA	Differentiated	Ileum	aafA	Monolayer	Matrigel	3
I103 ehec	Differentiated	Ileum	ehec	Monolayer	Matrigel	3
I103 HS	Differentiated	Ileum	HS	Monolayer	Matrigel	3
I103 Mock	Differentiated	Ileum	Mock	Monolayer	Matrigel	3
I3 eaec	Differentiated	Ileum	eaec	Monolayer	Matrigel	3
I3 expec	Differentiated	Ileum	expec	Monolayer	Matrigel	3
I4 aafA	Differentiated	Ileum	aafA	Monolayer	Matrigel	3
I4 eaec	Differentiated	Ileum	eaec	Monolayer	Matrigel	3
I4 ehec	Differentiated	Ileum	ehec	Monolayer	Matrigel	3
I4 expec	Differentiated	Ileum	expec	Monolayer	Matrigel	3
I4 HS	Differentiated	Ileum	HS	Monolayer	Matrigel	3
I4 Mock	Differentiated	Ileum	Mock	Monolayer	Matrigel	3
I9 aafA	Differentiated	Ileum	aafA	Monolayer	Matrigel	3
I9 eaec	Differentiated	Ileum	eaec	Monolayer	Matrigel	3
I9 ehec	Differentiated	Ileum	ehec	Monolayer	Matrigel	3
I9 expec	Differentiated	Ileum	expec	Monolayer	Matrigel	3
I9 HS	Differentiated	Ileum	HS	Monolayer	Matrigel	3
J11 aafA	Differentiated	Jejunum	aafA	Monolayer	Matrigel	3
J11 eaec	Differentiated	Jejunum	eaec	Monolayer	Matrigel	3
J11 ehec	Differentiated	Jejunum	ehec	Monolayer	Matrigel	3
J11 expec	Differentiated	Jejunum	expec	Monolayer	Matrigel	3
J11 HS	Differentiated	Jejunum	HS	Monolayer	Matrigel	3
J11 Mock	Differentiated	Jejunum	Mock	Monolayer	Matrigel	3
J2 aafA	Differentiated	Jejunum	aafA	Monolayer	Matrigel	3
J2 eaec	Differentiated	Jejunum	eaec	Monolayer	Matrigel	3
J2 ehec	Differentiated	Jejunum	ehec	Monolayer	Matrigel	3
J2 expec	Differentiated	Jejunum	expec	Monolayer	Matrigel	3
J2 HS	Differentiated	Jejunum	HS	Monolayer	Matrigel	3
J2 Mock	Differentiated	Jejunum	Mock	Monolayer	Matrigel	3
J3 aafA	Differentiated	Jejunum	aafA	Monolayer	Matrigel	3
J3 eaec	Differentiated	Jejunum	eaec	Monolayer	Matrigel	3
J3 ehec	Differentiated	Jejunum	ehec	Monolayer	Matrigel	3
J3 expec	Differentiated	Jejunum	expec	Monolayer	Matrigel	3
J3 HS	Differentiated	Jejunum	HS	Monolayer	Matrigel	3
J3 Mock	Differentiated	Jejunum	Mock	Monolayer	Matrigel	3
C135 D0	Differentiated	Colon	Control	3D	Matrigel	4
C136 D0	Differentiated	Colon	Control	3D	Matrigel	4
C136 H0	Undifferentiated	Colon	Control	3D	Matrigel	4
C103 bac 6h	Differentiated	Colon	bac	Transwell	Collagen	5
C103 lto 24h	Differentiated	Colon	lto	Transwell	Collagen	5
C103 lto 6h	Differentiated	Colon	lto	Transwell	Collagen	5
C103 mock 24h	Differentiated	Colon	Mock	Transwell	Collagen	5
C103 mock 6h	Differentiated	Colon	Mock	Transwell	Collagen	5
C103 mock bac 6h	Differentiated	Colon	Mock-bac	Transwell	Collagen	5
C109 bac 6h	Differentiated	Colon	bac	Transwell	Collagen	5
C109 lto 24h	Differentiated	Colon	lto	Transwell	Collagen	5
C109 lto 6h	Differentiated	Colon	lto	Transwell	Collagen	5
C109 mock 24h	Differentiated	Colon	Mock	Transwell	Collagen	5
C109 mock 6h	Differentiated	Colon	Mock	Transwell	Collagen	5
C109 mock bac 6h	Differentiated	Colon	Mock-bac	Transwell	Collagen	5
D103 bac 6h	Differentiated	Duodenum	bac	Transwell	Collagen	5
D103 lto 24h	Differentiated	Duodenum	lto	Transwell	Collagen	5
D103 mock 24h	Differentiated	Duodenum	Mock	Transwell	Collagen	5
D103 mock 6h	Differentiated	Duodenum	Mock	Transwell	Collagen	5
D103 mock bac 6h	Differentiated	Duodenum	Mock-bac	Transwell	Collagen	5
D109 bac 6h	Differentiated	Duodenum	bac	Transwell	Collagen	5
D109 lto 24h	Differentiated	Duodenum	lto	Transwell	Collagen	5
D109 lto 6h	Differentiated	Duodenum	lto	Transwell	Collagen	5
D109 mock 24h	Differentiated	Duodenum	Mock	Transwell	Collagen	5
D109 mock 6h	Differentiated	Duodenum	Mock	Transwell	Collagen	5
D109 mock bac 6h	Differentiated	Duodenum	Mock-bac	Transwell	Collagen	5
I103 bac 6h	Differentiated	Ileum	bac	Transwell	Collagen	5
I103 lto 24h	Differentiated	Ileum	lto	Transwell	Collagen	5
I103 lto 6h	Differentiated	Ileum	lto	Transwell	Collagen	5
I103 mock 24h	Differentiated	Ileum	Mock	Transwell	Collagen	5
I103 mock 6h	Differentiated	Ileum	Mock	Transwell	Collagen	5
I103 mock bac 6h	Differentiated	Ileum	Mock-bac	Transwell	Collagen	5
I109 bac 6h	Differentiated	Ileum	bac	Transwell	Collagen	5
I109 lto 24h	Differentiated	Ileum	lto	Transwell	Collagen	5
I109 lto 6h	Differentiated	Ileum	lto	Transwell	Collagen	5
I109 mock 24h	Differentiated	Ileum	Mock	Transwell	Collagen	5
I109 mock 6h	Differentiated	Ileum	Mock	Transwell	Collagen	5
I109 mock bac 6h	Differentiated	Ileum	Mock-bac	Transwell	Collagen	5

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J11 bac 6h	Differentiated	Jejunum	bac	Transwell	Collagen	5
J11 Ito 6h	Differentiated	Jejunum	Ito	Transwell	Collagen	5
J11 mock 24h	Differentiated	Jejunum	Mock	Transwell	Collagen	5
J11 mock 6h	Differentiated	Jejunum	Mock	Transwell	Collagen	5
J11 mock bac 6h	Differentiated	Jejunum	Mock-bac	Transwell	Collagen	5
J2 bac 6h	Differentiated	Jejunum	bac	Transwell	Collagen	5
J2 Ito 24h	Differentiated	Jejunum	Ito	Transwell	Collagen	5
J2 Ito 6h	Differentiated	Jejunum	Ito	Transwell	Collagen	5
J2 mock 24h	Differentiated	Jejunum	Mock	Transwell	Collagen	5
J2 mock 6h	Differentiated	Jejunum	Mock	Transwell	Collagen	5
J2 mock bac 6h	Differentiated	Jejunum	Mock-bac	Transwell	Collagen	5
D104 MO	Differentiated	Duodenum	Mock	Monolayer	Collagen	6
D104 VO	Differentiated	Duodenum	Virus	Monolayer	Collagen	6
D109 MO	Differentiated	Duodenum	Mock	Monolayer	Collagen	6
D109 VO	Differentiated	Duodenum	Virus	Monolayer	Collagen	6
D4 MO	Differentiated	Duodenum	Mock	Monolayer	Collagen	6
D4 VO	Differentiated	Duodenum	Virus	Monolayer	Collagen	6
D8 MO	Differentiated	Duodenum	Mock	Monolayer	Collagen	6
D8 VO	Differentiated	Duodenum	Virus	Monolayer	Collagen	6
j2 Ito	Differentiated	Jejunum	Ito	3D	Matrigel	7
j2 mock	Differentiated	Jejunum	Mock	3D	Matrigel	7
j7 Ito	Differentiated	Jejunum	Ito	3D	Matrigel	7
j7 mock	Differentiated	Jejunum	Mock	3D	Matrigel	7
J11	Undifferentiated	Jejunum	Control	3D	Matrigel	7
J2	Undifferentiated	Jejunum	Control	3D	Matrigel	7
J11 GCDCA 3h	Differentiated	Jejunum	GCDCA	Monolayer	Collagen	8
J11 gGII.4 10h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J11 gGII.4 24h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J11 gGII.4 6h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J11 GII.4 10h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J11 GII.4 24h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J11 GII.4 6h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J11 media 3h	Differentiated	Jejunum	Media	Monolayer	Collagen	8
J2 GCDCA 3h	Differentiated	Jejunum	GCDCA	Monolayer	Collagen	8
J2 gGII.4 10h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J2 gGII.4 24h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J2 gGII.4 6h	Differentiated	Jejunum	gGII.4	Monolayer	Collagen	8
J2 GII.4 10h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J2 GII.4 24h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J2 GII.4 6h	Differentiated	Jejunum	GII.4	Monolayer	Collagen	8
J2 media 3h	Differentiated	Jejunum	Media	Monolayer	Collagen	8
SOJ11 2	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
SOJ11 3	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
J2soft5	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
J2soft6	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
J2soft7	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
SOJ3 1	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
SOJ3 2	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
SOJ3 3	Differentiated	Jejunum	Soft	Monolayer	Hydrogel	9
MJ11 1	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
MJ11 2	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
MJ11 3	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
J2md5	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
J2med6	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
J2med7	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
MJ3 1	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
MJ3 2	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
MJ3 3	Differentiated	Jejunum	Medium	Monolayer	Hydrogel	9
STJ11 1	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
STJ11 2	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
J11stiff5	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
J2st5	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
J2st6	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
J2st7	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
STJ3 1	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
STJ3 2	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
STJ3 3	Differentiated	Jejunum	Stiff	Monolayer	Hydrogel	9
A96WJ111	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
A96WJ112	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
A96WJ113	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
J2961	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
J2962	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
J2967	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
A96WJ3 1	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
A96WJ3 2	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
J3967	Differentiated	Jejunum	Plastic	Monolayer	Matrigel	9
ULDM1 1	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10
ULDM1 2	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10
ULDM1 3	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10
ULDM2 1	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10
ULDM2 2	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10
ULDM2 3	Differentiated	Jejunum	LDM4 Uninduced NGN3	Transwell	Matrigel	10

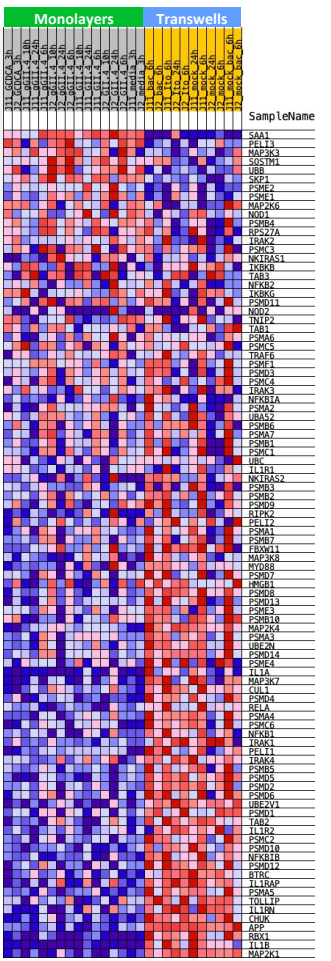
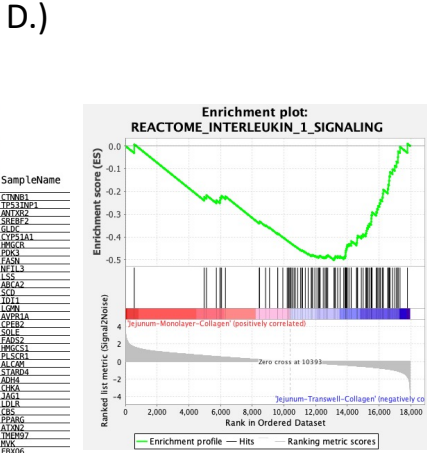
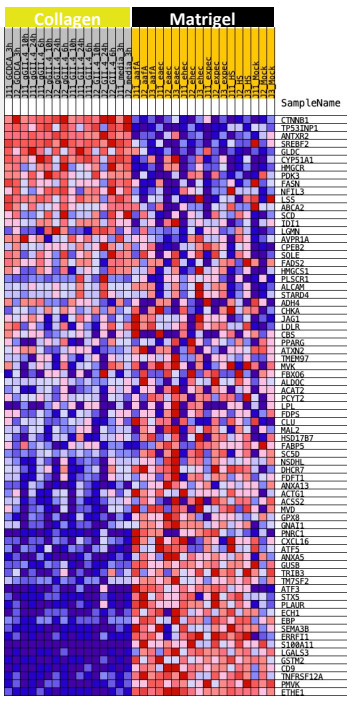
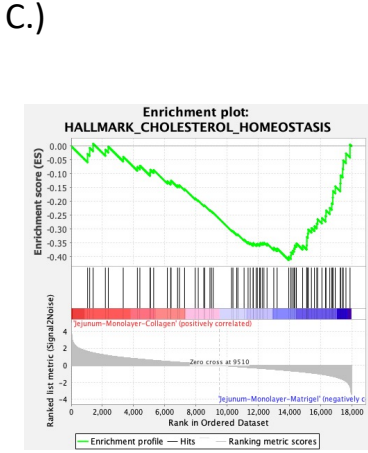
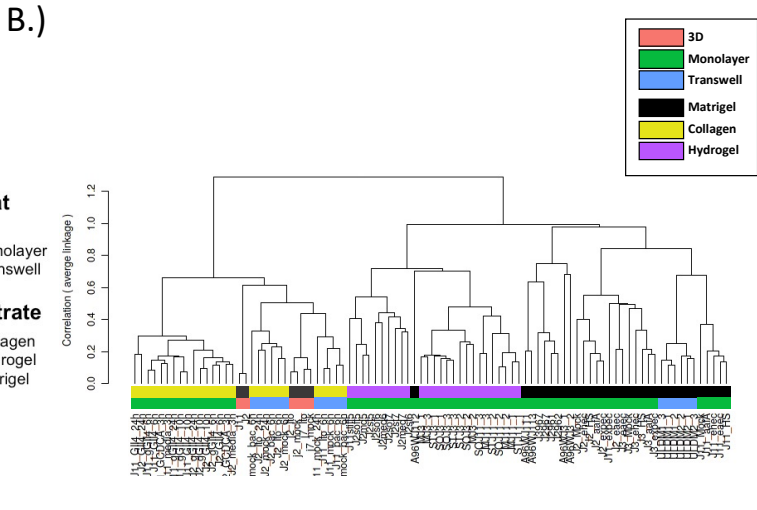
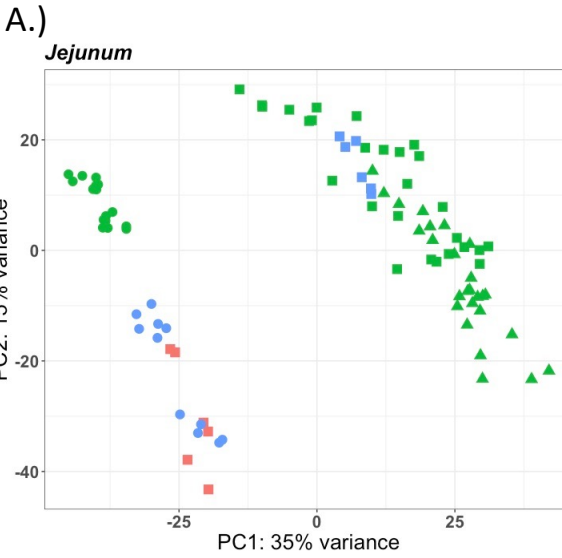
Supplementary Table 1.) Demographics of data used in analysis - Table of Samples A.) Shown is the metadata table with the Status, Segment, Treatment, Format, Substrate and Project for every sample (ids) used in this analysis (n=251).

A.)

DESeq2 Differential Gene Expression Results (FDR \leq 0.01 and a fold change \geq 2 or \leq 0.5)		
	Comparison	DE Genes
Differentiated Duodenum	3D-MATRIGEL_vs_MONOLAYER-MATRIGEL	5139
	MONOLAYER-COLLAGEN_vs_MONOLAYER-MATRIGEL	6004
	MONOLAYER-COLLAGEN_vs_TRANSWELL-COLLAGEN	4695
Differentiated Jejunum	3D-MATRIGEL_vs_MONOLAYER-MATRIGEL	5195
	3D-MATRIGEL_vs_TRANSWELL-COLLAGEN	1305
	MONOLAYER-COLLAGEN_vs_MONOLAYER-HYDROGEL	7113
	MONOLAYER-COLLAGEN_vs_TRANSWELL-COLLAGEN	3740
	MONOLAYER-MATRIGEL_vs_MONOLAYER-COLLAGEN	5148
	MONOLAYER-MATRIGEL_vs_MONOLAYER-HYDROGEL	4341
Differentiated Ileum	MONOLAYER_MATRIGEL_vs_TRANSWELL_COLLAGEN	5702
Differentiated Colon	MONOLAYER-MATRIGEL_vs_3D-MATRIGEL	6049
	MONOLAYER-MATRIGEL_vs_TRANSWELL-COLLAGEN	5253
	TRANSWELL-COLLAGEN_vs_3D-MATRIGEL	7271
Transwells	DUODENUM_VS_JEJUNUM	420
	DUODENUM_VS_ILEUM	3115
	DUODENUM_VS_COLON	3720
	JEJUNUM_VS_ILEUM	2857
	JEJUNUM_VS_COLON	3801
	ILEUM_VS_COLON	2592
Monolayers	DUODENUM_VS_JEJUNUM	1012
	DUODENUM_VS_ILEUM	2184
	DUODENUM_VS_COLON	2750
	JEJUNUM_VS_ILEUM	3206
	JEJUNUM_VS_COLON	4115
	ILEUM_VS_COLON	1266
3D	DUODENUM_vs_COLON_DIFFERENTIATED	2296
	JEJUNUM_vs_COLON_DIFFERENTIATED	5977
	DUODENUM_vs_JEJUNUM_DIFFERENTIATED	5332
	DUODENUM_vs_COLON_UNDIFFERENTIATED	2224
	JEJUNUM_vs_COLON_UNDIFFERENTIATED	3705
	DUODENUM_vs_JEJUNUM_UNDIFFERENTIATED	3873
	DUODENUM: DIFFERENTIATED_vs_UNDIFFERENTIATED	2902
	COLON: DIFFERENTIATED_vs_UNDIFFERENTIATED	2756

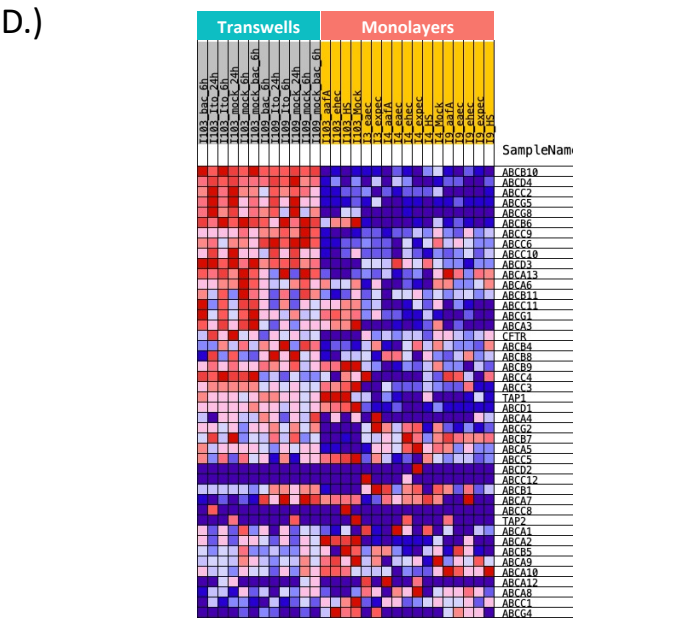
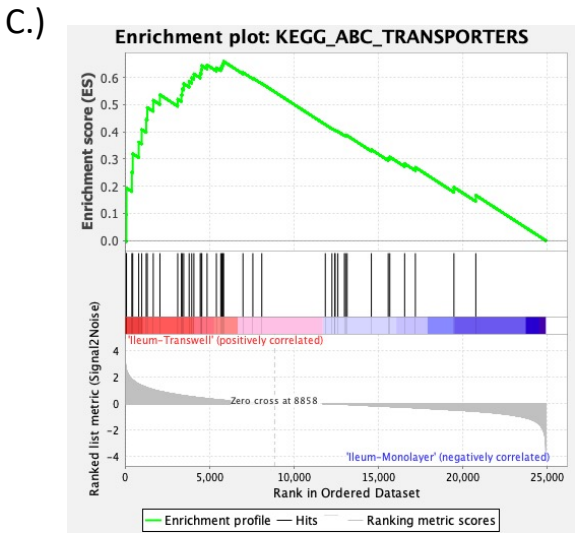
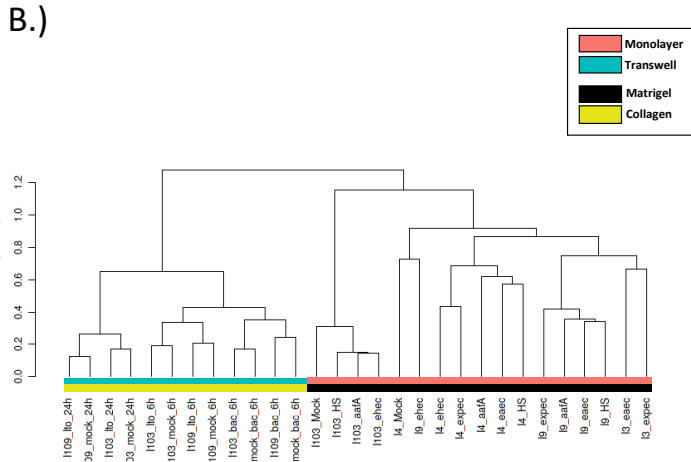
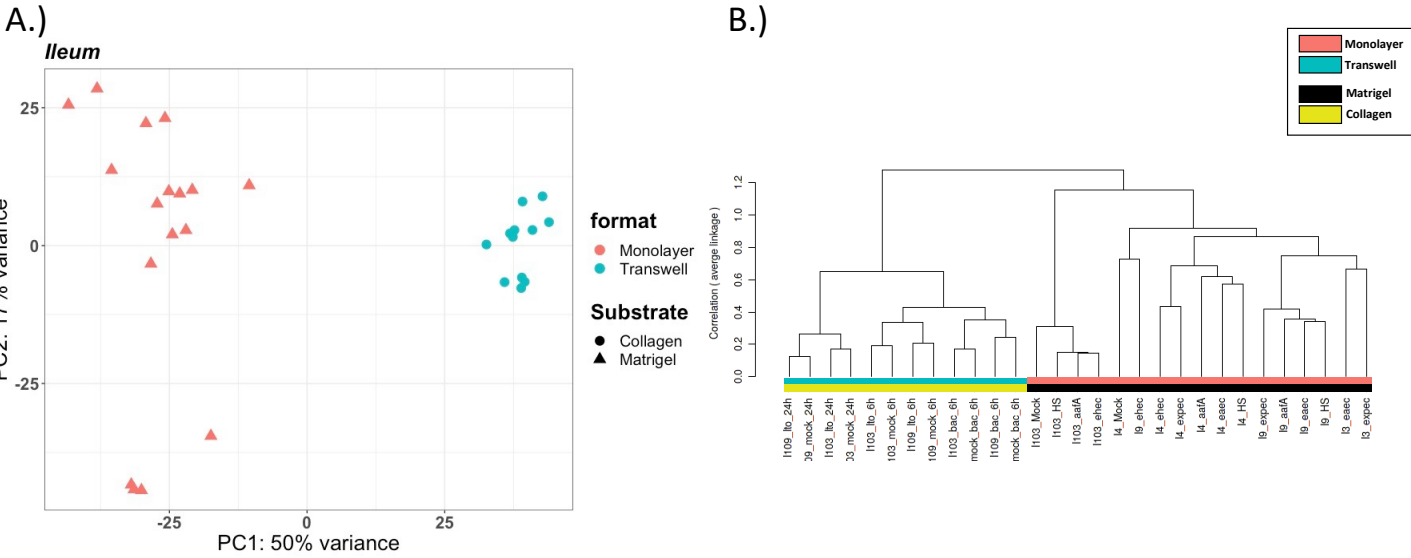
Supplementary Table 2. Differentially expressed genes via DESeq2. Summary table of the number of differentially expressed genes for 33 comparisons performed in DESeq2 (FDR \leq 0.01 & Fold Change \geq 2 and \leq 0.5). DE Gene: Differentially Expressed Genes.

Supplementary Figure 1



Supplementary Figure 1. Cholesterol biosynthesis and Interleukin-1 signaling pathway genes are upregulated in jejunal monolayers grown on Matrigel and jejunal transwells grown on collagen, respectively. A.) PCA of the RNA-sequencing datasets for jejunal enteroids: 3D-Matrigel (n=6), Monolayer-Collagen (n=16), Monolayer-Hydrogel (n=26), Monolayer-Matrigel (n=27), Transwell-Collagen (n=11), and Transwell-Matrigel(n=6); B.) A dendrogram with agglomerative hierarchical clustering of the jejunal gene set from RNA-sequencing. Branch length indicates degree of difference between samples; C.) GSEA showing an enrichment of the hallmark cholesterol homeostasis gene set signature in jejunal monolayers grown on Matrigel when compared to jejunal monolayers grown on collagen; D.) GSEA showing an enrichment of the reactome interleukin-1 signaling gene set signature in jejunal transwells grown on collagen when compared to jejunal monolayers grown on collagen;

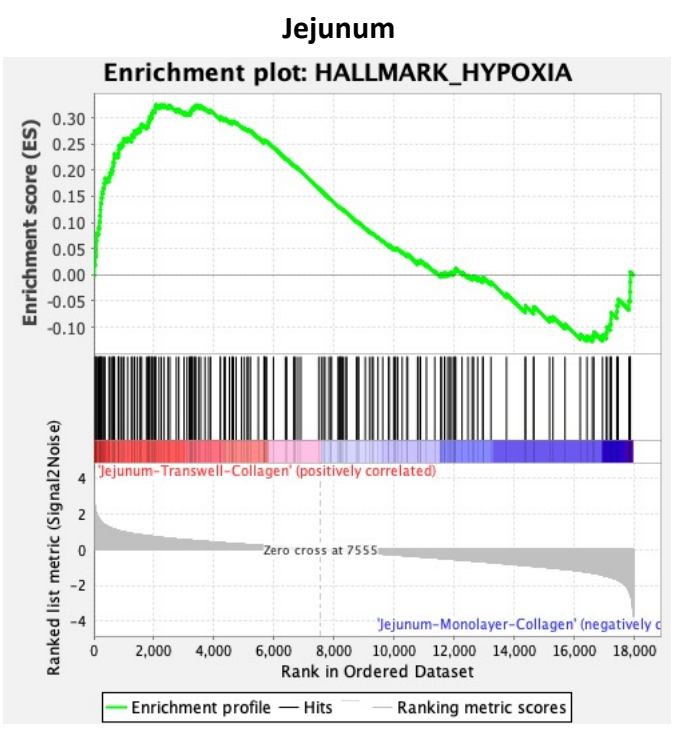
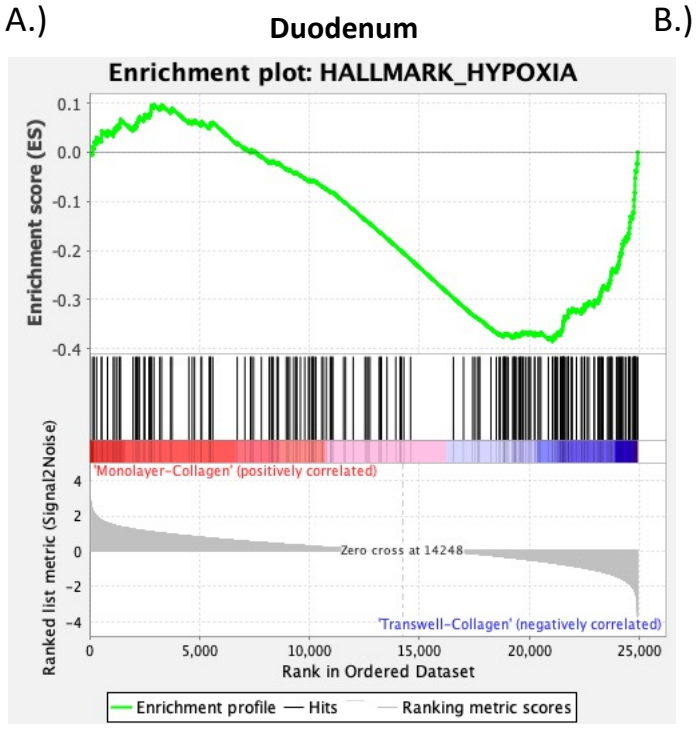
Supplementary Figure 2



Supplementary Figure 2. ABC transporter genes are upregulated in ileal transwells grown on collagen in comparison to ileal monolayers grown on Matrigel. A.) PCA of the RNA-sequencing datasets for ileal enteroids: Monolayer-Matrigel (n=17), and Transwell-Collagen (n=12); B.) A dendrogram with agglomerative hierarchical clustering of the ileal gene set from RNA-sequencing. Branch length indicates degree of difference between samples; C.) GSEA showing an enrichment of the KEGG ABC transporters gene set signature in ileal transwells grown on collagen IV when compared to ileal monolayers grown on Matrigel; D.) Heatmap of the KEGG ABC transporters gene set showing an enrichment in ileal transwells grown on collagen IV (gray) compared to ileal monolayers grown on Matrigel (yellow);

Supplementary Figure 3. The antigen processing cross presentation gene set is upregulated in colonoid monolayers grown on Matrigel compared to differentiated 3D colonoids grown in Matrigel. A.) PCA of the RNA-sequencing datasets for colonic enteroids: 3D-Matrigel (n=39), Monolayer-Matrigel (n=18) and Transwell-Collagen (n=12); B.) A dendrogram with agglomerative hierarchical clustering of the colonic gene set from RNA-sequencing. Branch length indicates degree of difference between samples; C.) GSEA showing an enrichment of the reactome antigen processing cross presentation gene set signature in colonoid monolayers grown on Matrigel when compared to 3D colonoids grown in Matrigel; D.) Heatmap of the reactome antigen processing cross presentation gene set showing an enrichment in colonoid monolayers grown on Matrigel (yellow) compared to 3D colonoids grown in Matrigel (gray);

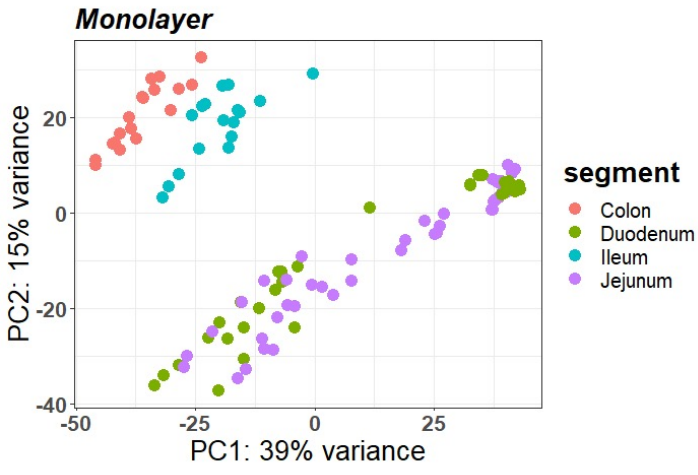
Supplementary Figure 4. Wnt/ β -catenin signaling and transmembrane receptor protein kinase activity genes are enriched in 3D intestinal organoids grown in Matrigel compared to monolayers grown on Matrigel. A.) GSEA showing an enrichment of the hallmark wnt/ β -catenin signaling gene set signature in duodenal (left), jejunal (middle) and colonic (right) 3D organoids grown in Matrigel when compared to monolayers grown on Matrigel; B.) GSEA showing an enrichment of the GO transmembrane receptor protein kinase activity gene set signature in duodenal (left), jejunal (middle) and colonic (right) 3D organoids grown in Matrigel when compared to monolayers grown on Matrigel; C.) Heatmap of the transmembrane receptor protein kinase activity gene set showing an enrichment in 3D duodenal enteroids grown in Matrigel (gray) compared to Duodenal monolayers grown on Matrigel (yellow);



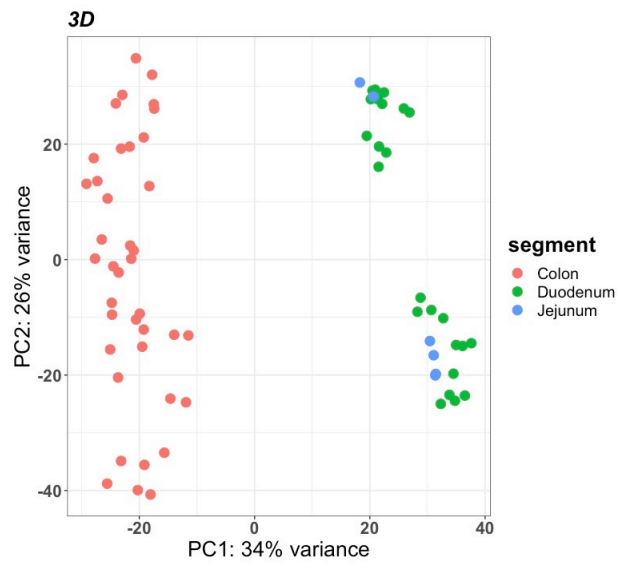
Supplementary Figure 5. Hypoxia gene set is enriched in duodenal and jejunal transwells on Collagen compared to monolayers grown on collagen. A & B.) GSEA showing an enrichment of the hallmark hypoxia gene set signature in (A) duodenal (NES = -1.72 and FDR = 0.001) and (B) jejunal (NES = 1.78 and FDR = 0.003) transwells grown on collagen when compared to duodenal and jejunal monolayers grown on collagen;

Supplementary Figure 6

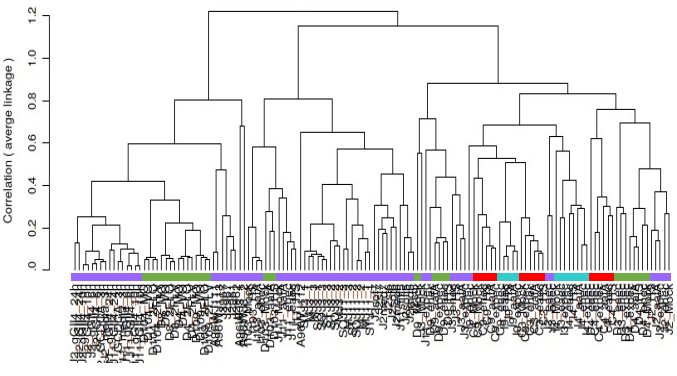
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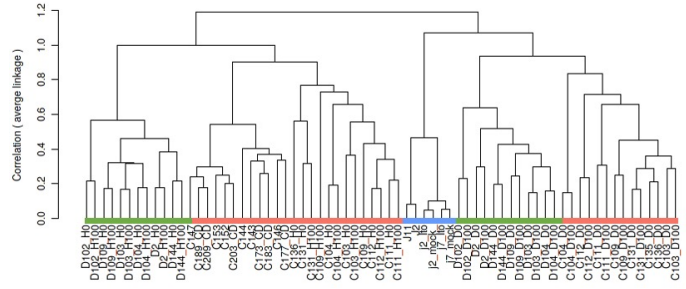
B.)



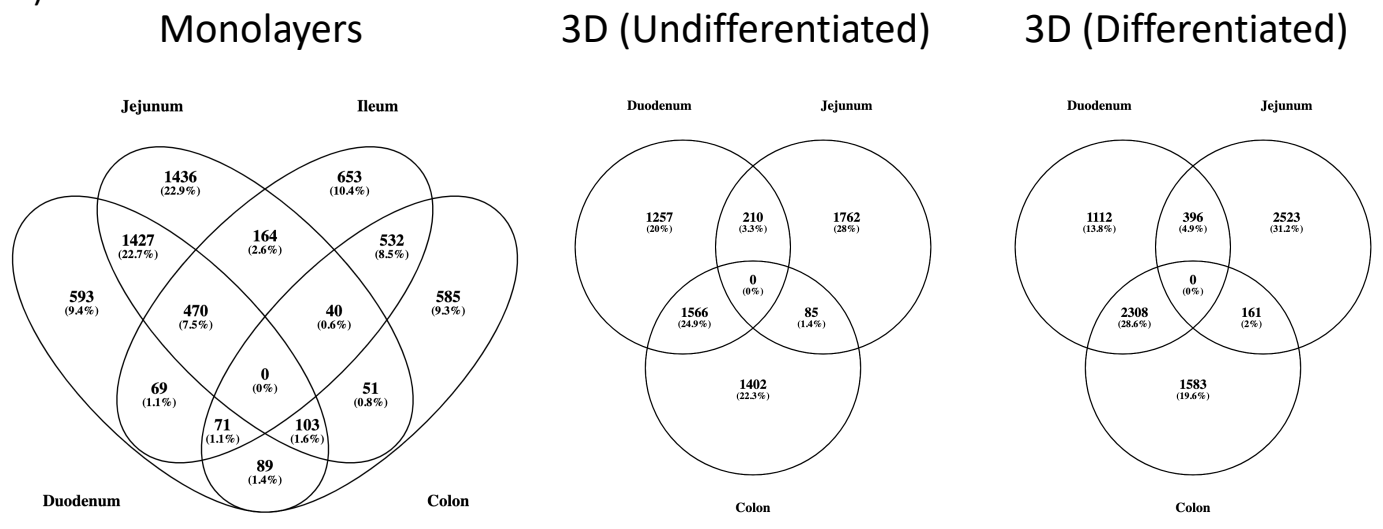
C.)



D.)



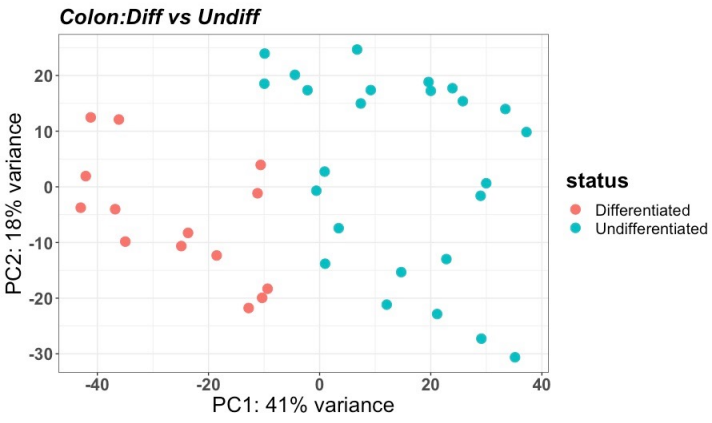
E.)



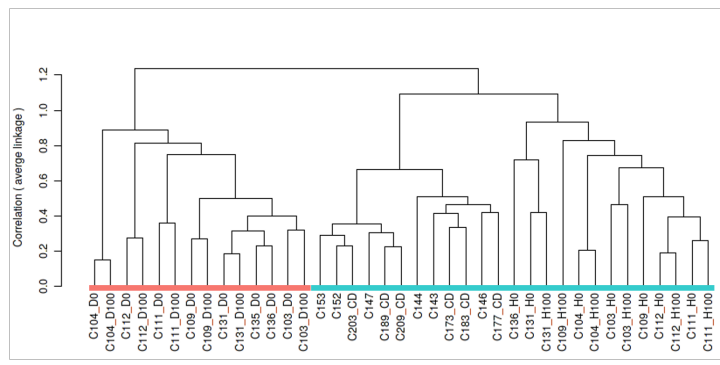
Supplementary Figure 6. Transcriptionally segments vary in intestinal organoids grown in 3D and on monolayers. A.) PCA of the RNA-sequencing datasets for enteroids on monolayers: Duodenum (n=26), Jejunum (n=69), Ileum (n=17), and Colon (n=18); B.) PCA of the RNA-sequencing datasets for enteroids on 3D: Duodenum (n=24), Jejunum (n=6), and Colon (n=39); C.) A dendrogram with agglomerative hierarchical clustering of the monolayer gene set from RNA-sequencing. Branch length indicates degree of difference between samples; D.) A dendrogram with agglomerative hierarchical clustering of the 3D gene set from RNA-sequencing. Branch length indicates degree of difference between samples; E.) Venn diagram displaying the overlap of differentially expressed genes (DEGs) between duodenal, jejunal, ileal and colonic intestinal organoids grown on Monolayers (Left) and in 3D (Undifferentiated (middle) and Differentiated (right)) . DEGs were defined as any gene that was differentially enriched in the segment of interest compared to any other segment with DESeq2 (FDR \leq 0.01 & Fold Change \geq 2 and \leq 0.5);

Supplementary Figure 7

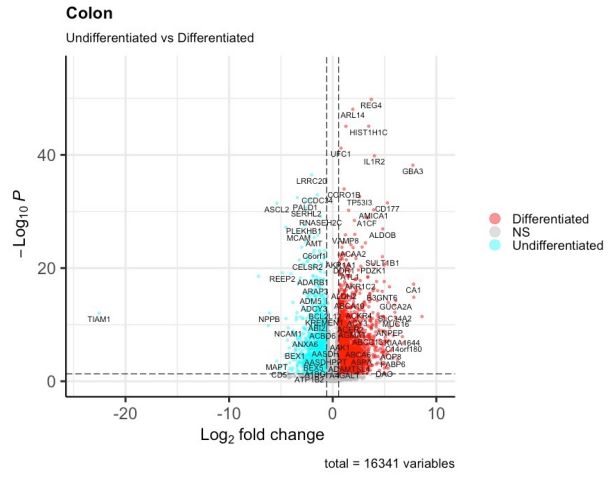
A.)



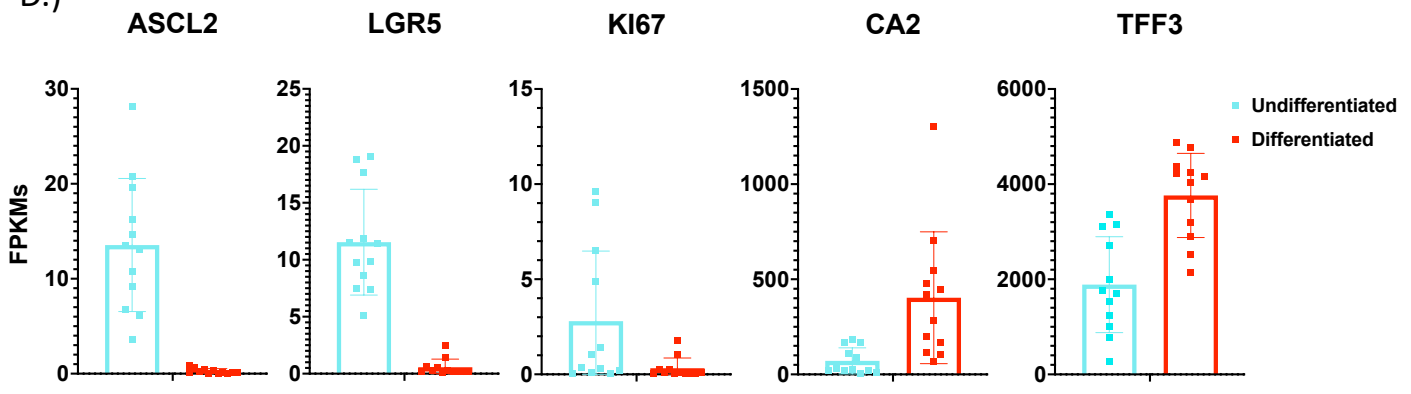
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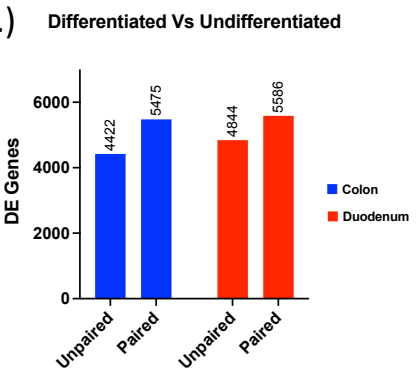
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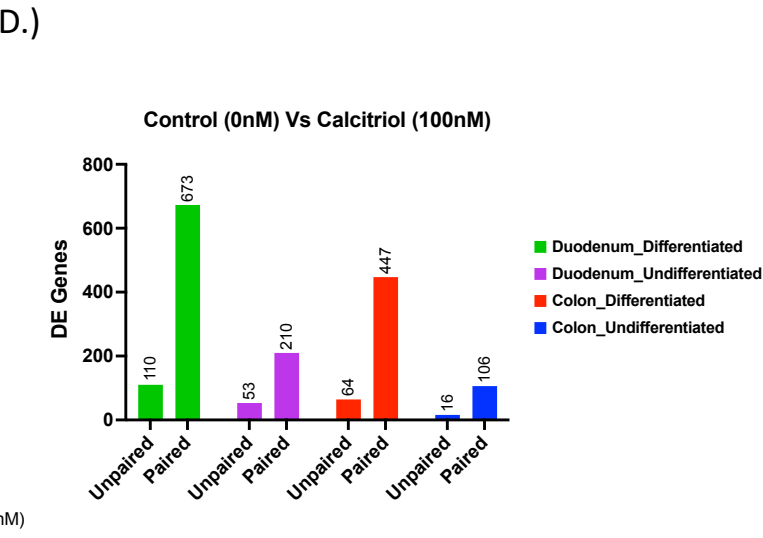
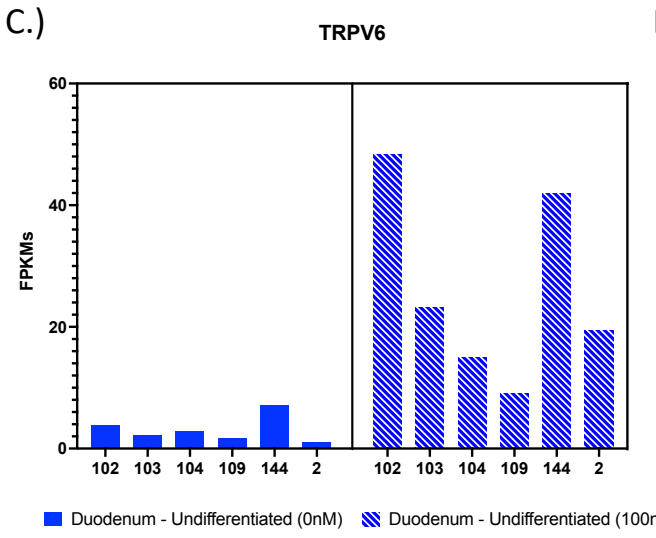
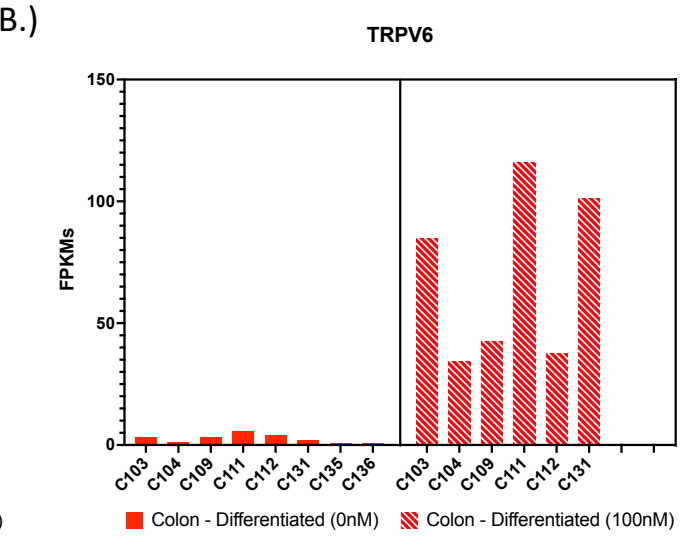
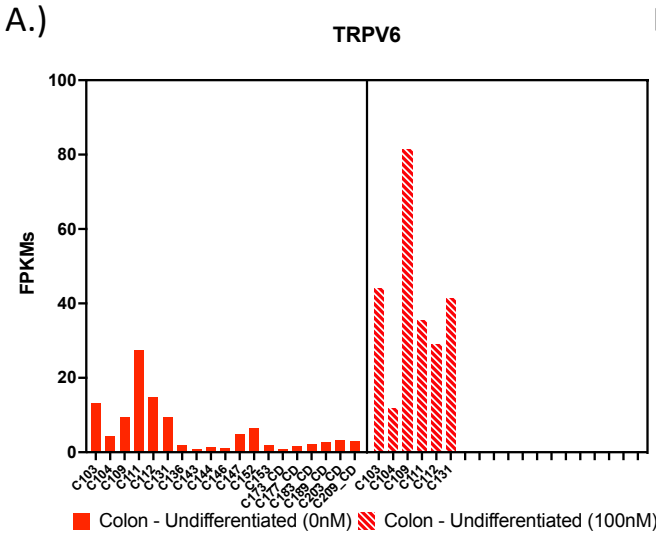
D.)



E.)



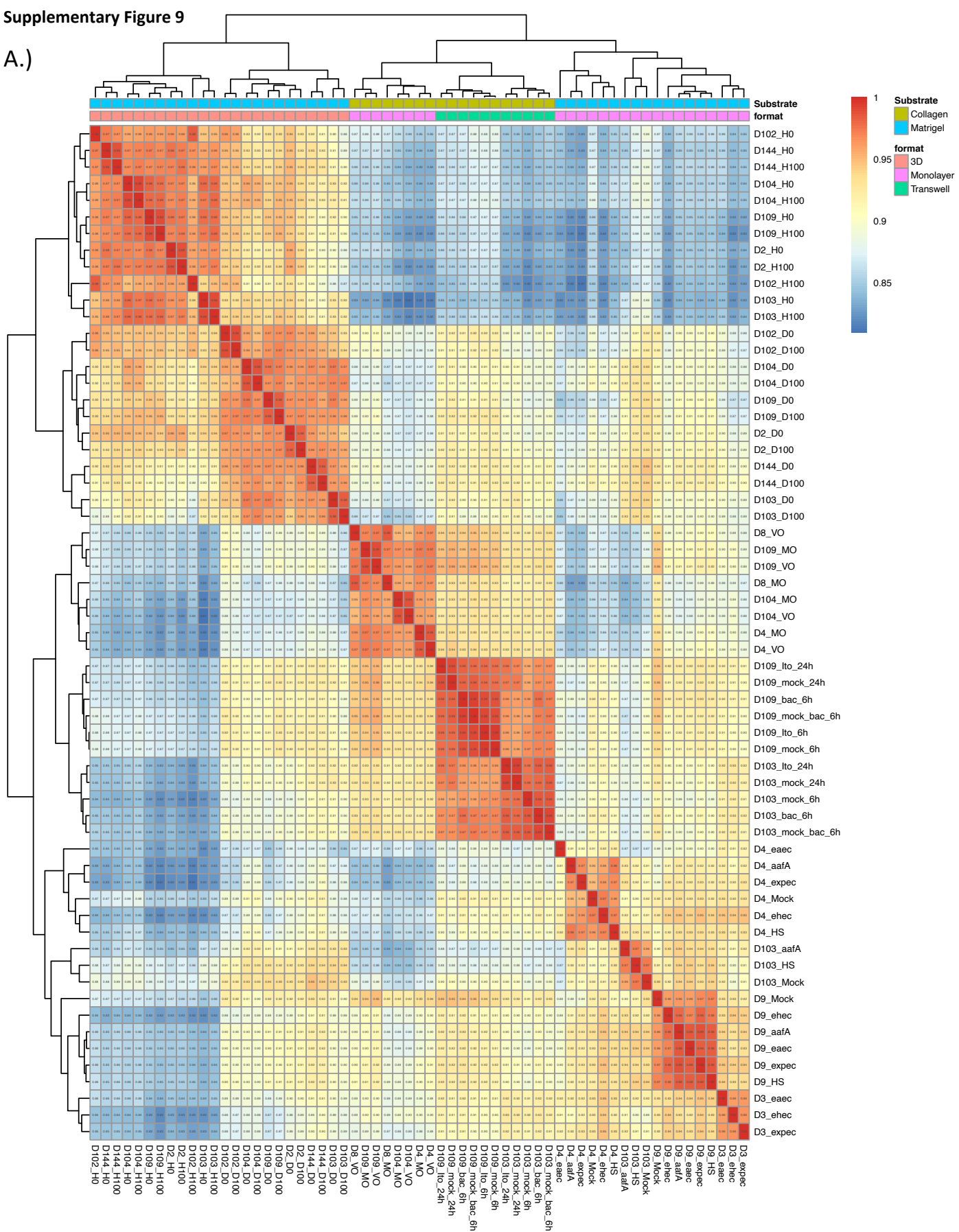
Supplementary Figure 7. Differentiation media conditions drive changes in proliferation and differentiation markers in 3D colonoids. A.) PCA of the RNA-sequencing datasets for 3D Colonic enteroids: Differentiated (n=14) and Undifferentiated (n=25); B.) A dendrogram with agglomerative hierarchical clustering of the 3D colonic gene set from RNA-sequencing. Branch length indicates degree of difference between samples; C.) Volcano plot of differentially expressed genes when comparing differentiated (red) and undifferentiated (cyan) 3D colonoids. Red/cyan dots indicate differentially expressed genes ($FDR \leq 0.01$ and a foldchange ≥ 2 or ≤ 0.5) that are upregulated or downregulated (respectively) in differentiated (1338 genes) vs. undifferentiated (1418 genes) 3D colonoids; D.) Normalized FPKMs of undifferentiated (cyan) and differentiated (red) 3D colonoids for stem cell (*ASCL2*, *KI67* and *LGR5*) and differentiation (*CA2* and *TFF3*) markers; E.) The number of differentially expressed (DE) genes that were output from DESeq2 when comparing differentiated vs undifferentiated 3D duodenal enteroids (red) and colonoids (blue) as a result of using an unpaired (left) or paired (right) experimental design;



Supplementary Figure 8. Patient-to-patient variability results in variable basal gene expression and response to stimuli of TRPV6 in differentiated and undifferentiated duodenal enteroids and colonoids. A.) Normalized FPKMs of TRPV6 expression in undifferentiated patient derived colonoid lines comparing Control (solid) to 100nM Calcitriol treatment (dashed); B.) Normalized FPKMs of TRPV6 expression in differentiated patient derived colonoid lines comparing Control (solid) to 100nM Calcitriol treatment (dashed); C.) Normalized FPKMs of TRPV6 expression in undifferentiated patient derived duodenal enteroids lines comparing Control (solid) to 100nM Calcitriol treatment (dashed); D.) The number of differentially expressed (DE) genes that were output from DESeq2 as a result of using an unpaired (left) or paired (right) experimental design, for differentiated duodenal enteroids (green), undifferentiated duodenal enteroids (purple), differentiated colonoids (red) and undifferentiated colonoids (blue) treated with calcitriol (100nM) calcitriol compared to control (0nM);

Supplementary Figure 9

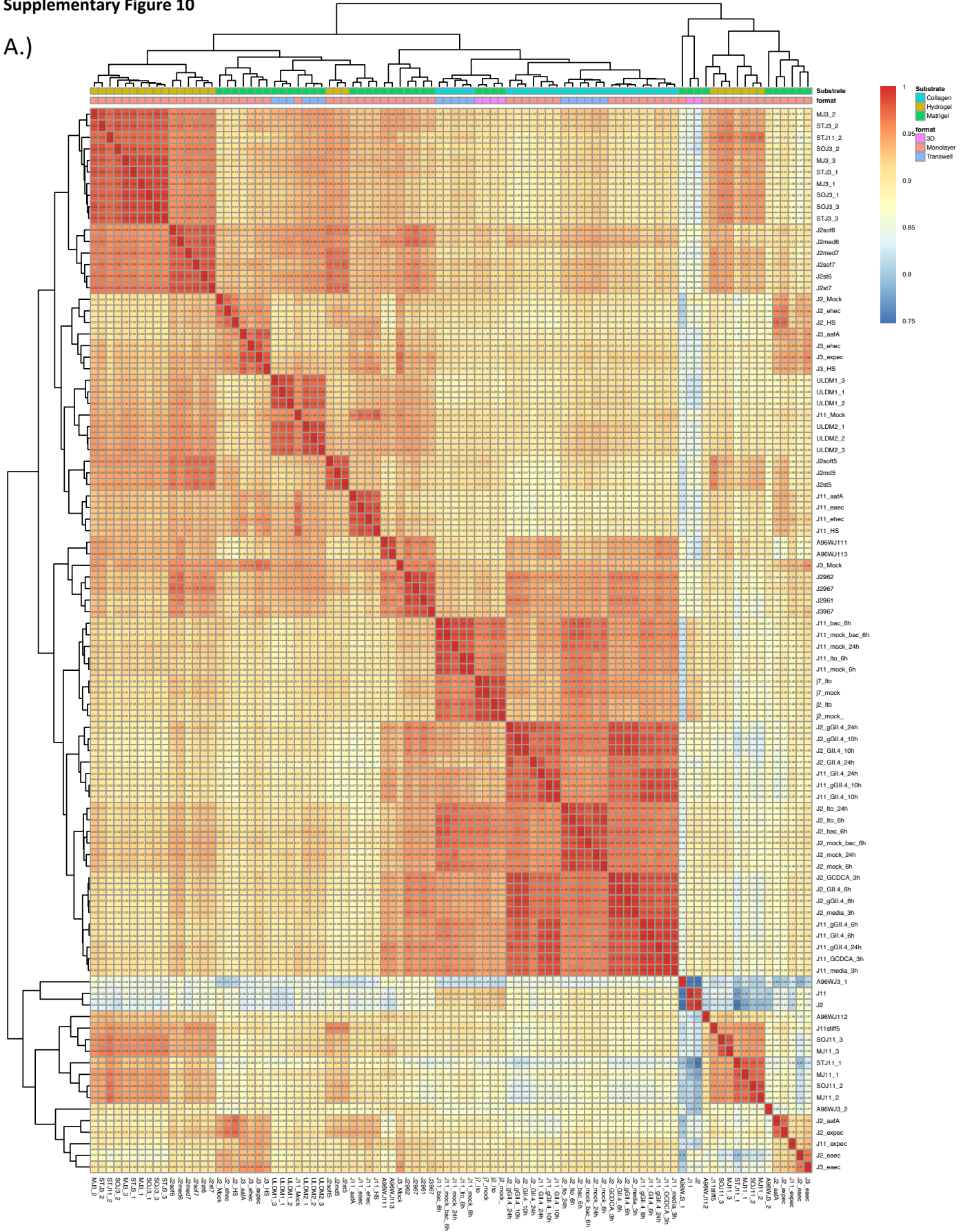
A.)



Supplementary Figure 9. Correlation Matrix of Duodenal Enteroids. A.) A Pearson correlation matrix of Duodenal enteroids

Supplementary Figure 10

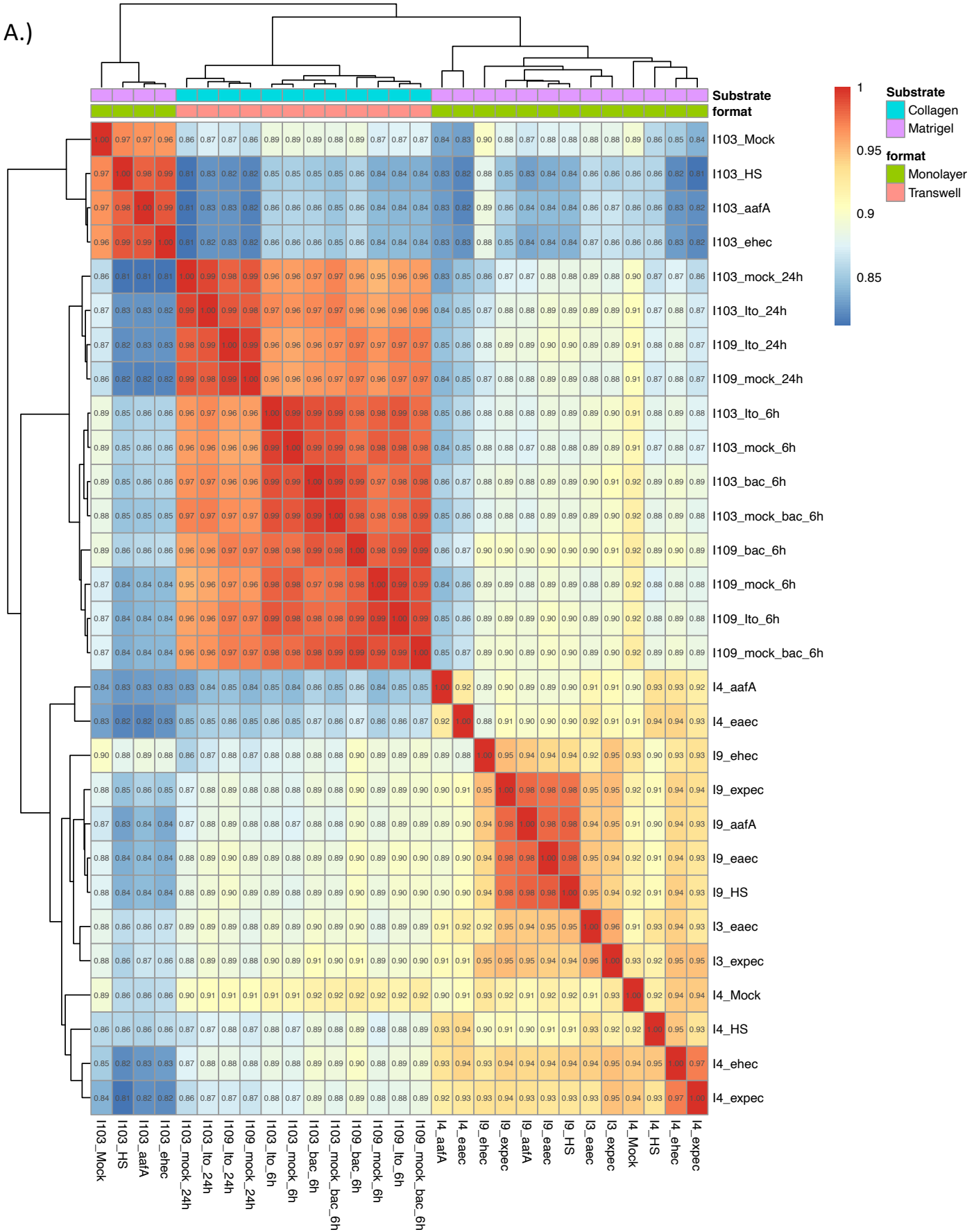
A.)



Supplementary Figure 10. Correlation Matrix of Jejunal Enteroids. A.) A Pearson correlation matrix of Jejunal enteroids

Supplementary Figure 11

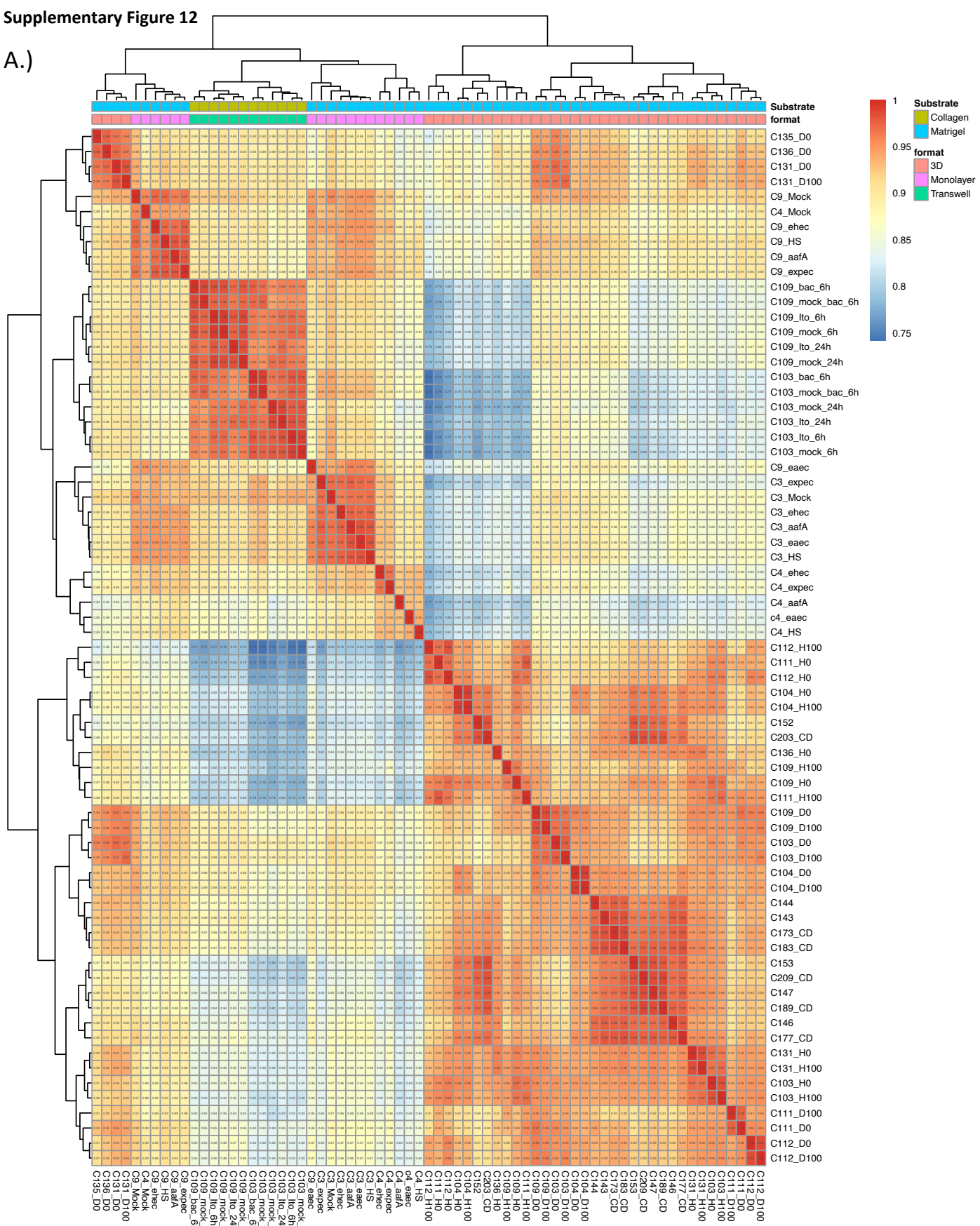
A.)



Supplementary Figure 11. Correlation Matrix of Ileal Enteroids. A.) A Pearson correlation matrix of Ileal enteroids.

Supplementary Figure 12

A.)



Supplementary Figure 12. Correlation Matrix of Colonoids. A.) A Pearson correlation matrix of Colonoids