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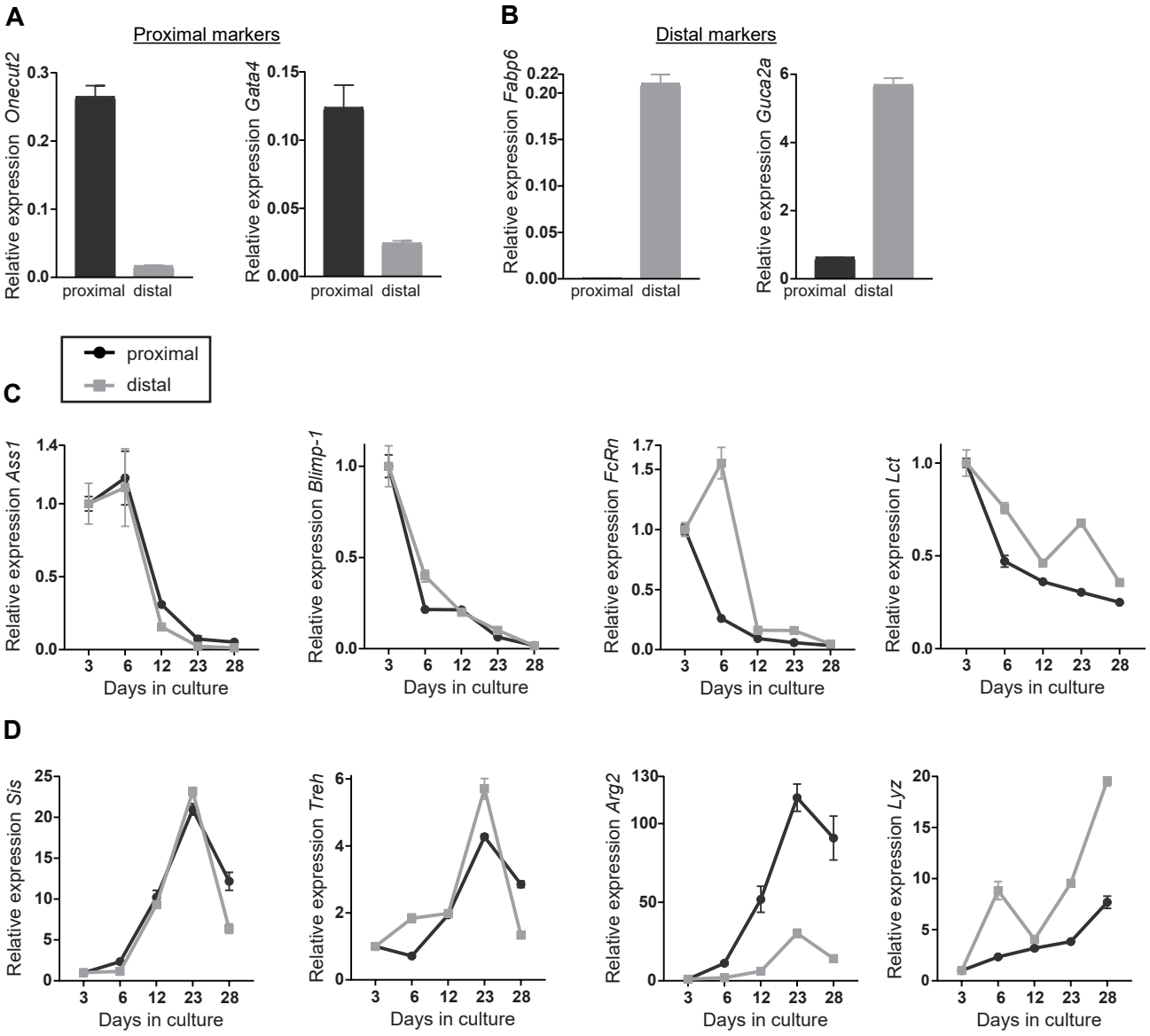
Appendix Figure S1



Appendix Figure S1: *In vitro* maturation follows similar pattern in both proximal and distal organoids.
A Proximal markers *Onecut2* and *Gata4* are highly expressed in organoids isolated from the proximal region of the small intestine.

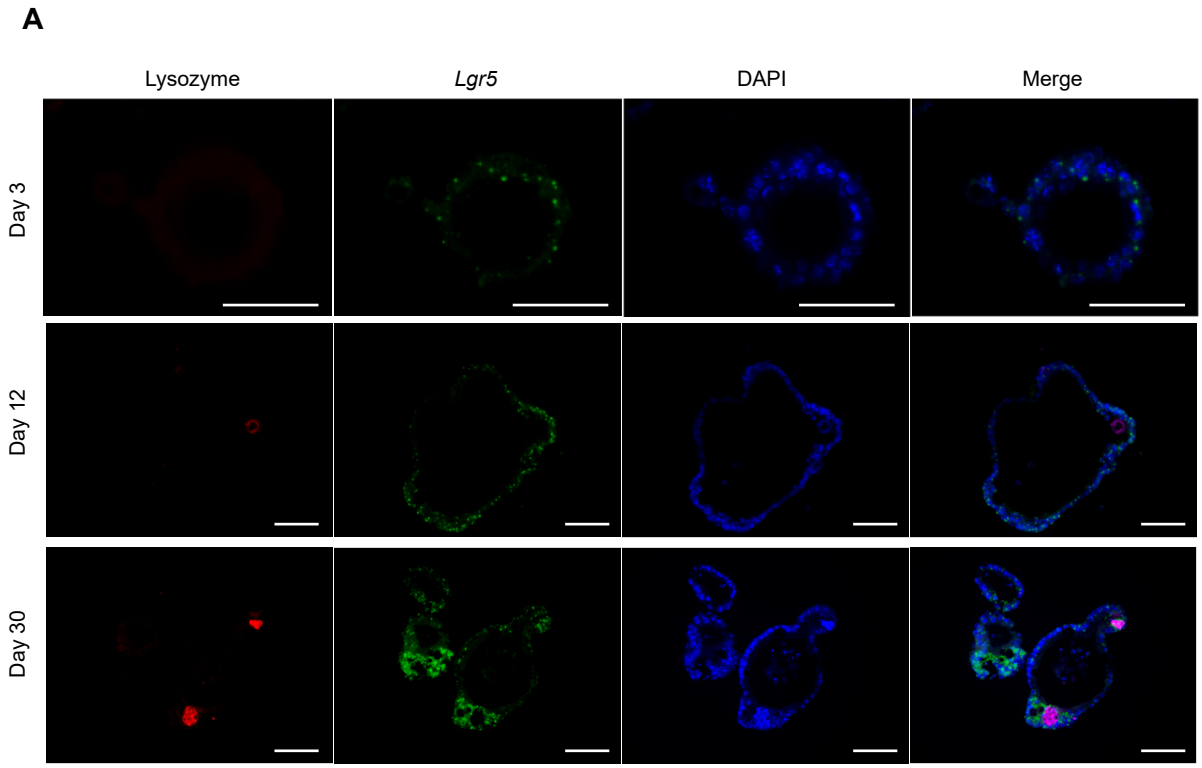
B Distal markers *Fabp6* and *Guca2a* are highly expressed in organoids isolated from the distal region of the small intestine.

C Expression of the neonatal markers *Ass1, Blimp-1, FcRn* and *Lct* decreases in the same way in both proximal and distal organoids.

D Adult markers *Sis, Treh, Arg2* and *Lyz* similarly increase their expression in both proximal and distal organoids.

Data information: Data are presented as mean ± SEM (n=3, individual wells from single organoid culture (see material and methods). Experiment was repeated in two independent organoid cultures with similar results)

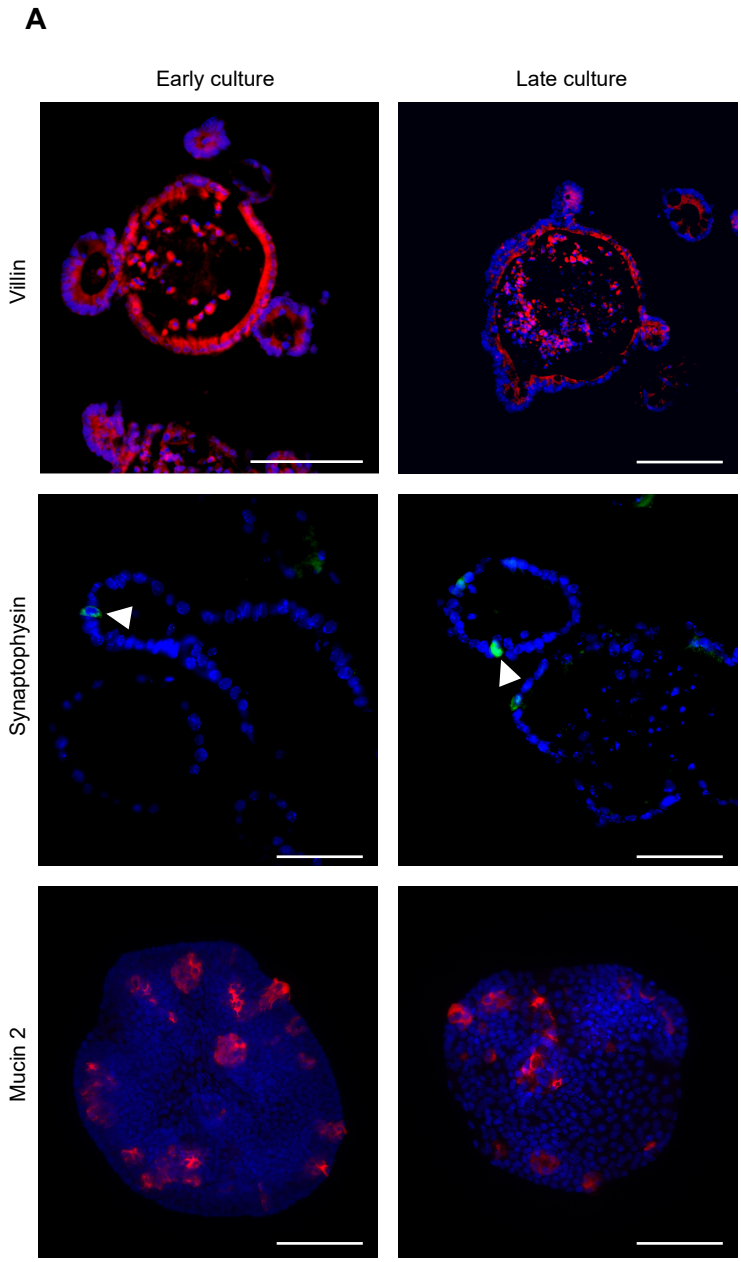
Appendix Figure S2



Appendix Figure S2: Expression of stem cell marker *Lgr5* and Paneth cell marker lysozyme in fetal organoids over time.

A – *Lgr5* is present at day 3 and its expression increases and becomes localized in the crypt region, while lysozyme is absent in the beginning of the culture and starts to appear at the crypt region after day 12.

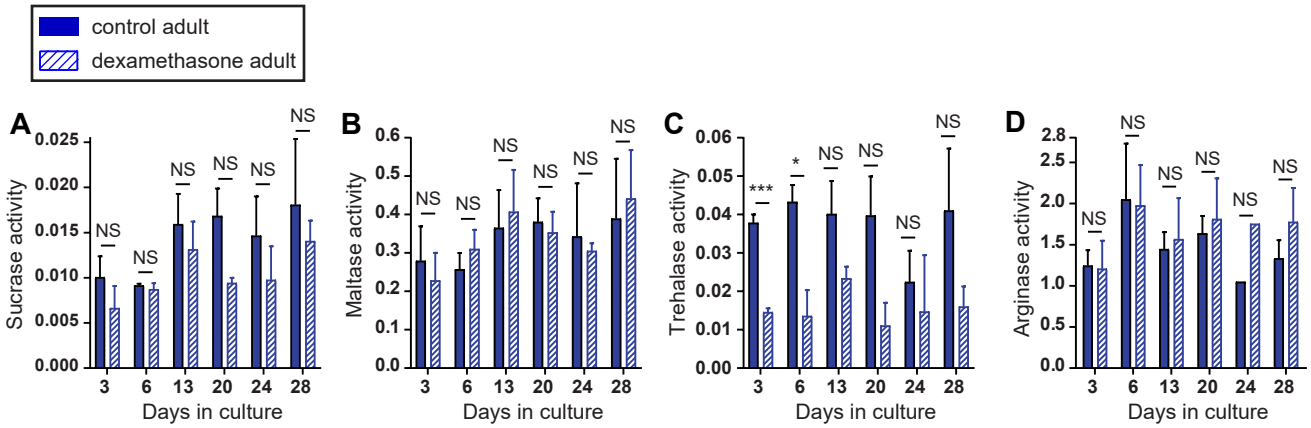
Appendix Figure S3



Appendix Figure S3: Differentiation markers are similarly expressed in fetal organoids during culture.

A – Enterocyte marker Villin, enteroendocrine marker Synaptophysin and goblet cell marker Muc2 are present at early and late culture.

Appendix Figure S4



Appendix Figure S4: Dexamethasone does not affect enzyme activity of adult organoids.

A-D Enzyme activity levels in adult organoids treated with dexamethasone (▨) remain the same compared to control adult organoids (■). The graph in this experiment is reusing treated adult levels from experiment presented in Fig3 I-L including the untreated adult control samples. Activity is given in µM glucose/µg protein·min-1. NS = 2. Data plotted as mean ±SEM. *p<0.05, NS not significant calculated by two-way ANOVA. Activity values of control adult organoids are the same as Figure 3I-L (n=3, independent organoid cultures).

Appendix Table S1. Composition of organoid culture medium (ENR).

Compound	Concentration	Company	Product nr.
Advanced DMEM/F12		Invitrogen	12634-028
Glutamax 100x	1x	Invitrogen	35050-038
Hepes 1M	0.01 M	Invitrogen	15630-056
Pen/Strep	0.2 U/ml	Invitrogen	15140-122
N2 supplement 100x	1x	Invitrogen	17502-048
B27 supplement 100x	1x	Invitrogen	17504-044
n-Acyetylcysteïne	1.25 mM	Sigma-Aldrich	A9165-5G
EGF	0.05 µg/ml	Invitrogen	PMG8045
mNoggin conditioned medium	20%	Home made*	
Rspo1 conditioned medium	10%	Home made*	

* Noggin-Fc and Rspo1-Fc conditioned media were produced by stably transfected HEK cells.

Appendix Table S2. List of primers used in the qRT-PCR.

Gene	Forward primer (5'-3')	Reverse primer (5'-3')	NM number
Ass1	CATTGGAATGAAGTCCCGAG	GATTTTGCGTACTTCCCGAT	NM_007494
Arg2	TAGGGTAATCCCCTCCCTGC	AGCAAGCCAGCTTCTCGAAT	NM_009705
Blimp-1	ACCAAGGAACCTGCTTTTCA	TAGACTTCACCGATGAGGGG	NM_007548
ChgA	GTCTCCAGACACTCAGGGCT	ATGACAAAAGGGGACACCAA	NM_007693
Cnx43	TGGGGGAAAGGCGTGAGGGA	ACCCATGTCTGGCACCTCTCTT	NM_010288
Cramp	AATCTTCTCCCCACCTTTGC	GCCGCTGATTCTTTTGACAT	NM_009921
Cyclo	ATGGTCAACCCCACCGTGT	TTCTGCTGTCTTTGGAACTTTGTC	NM_008907
Defcr1	AAGAGACTAAAACTGAGGAGCAGC	CGACAGCAGAGCGTGTA	NM_010031
Defcr5	AGGCTGATCCTATCCACAAAACAG	TGAAGAGCAGACCCTTCTTGGC-	NM_007851
Fabp6	ACGTGATTGAAAGGGGACGTAACTT	CATTCTTTGCCAATGGTGAACTTGT	NM_008375.2
FcRn	CTTCAGGCGCATAGACGG	CTAAACTCTTGTCCGGAGCG	NM_010189; NM_001357117
Gata4	CTGGAGGCGAGATGGGACGGGACACTAC	CCGCAGGCATTACATACAGGCTCACC	NM_008092.4; NM_001310610.1
Guca2a	GATCCTGCAGAGGCTAGAGG	CCACATGGGCTGAGAGAAAG	NM_008190.1
Lct	TATGCAGGCTACGGCACCGG	GCCAAGTTCTGGCATGCGCC	NM_001081078
Lgr5	TGTGTCAAAGCATTTCCAGC	CAGCGTCTTCACCTCCTACC	NM_010195
Lyz1	GGATGGCTACCGTGGTGTCAAGC	TCCCATAGTCGGTGCTTCGGTC	NM_013590
Muc2	GAAGCCAGATCCCGAAACCA	GAATCGGTAGACATCGCCGT	NM_023566
Onecut2	AGAGGGTTCTATGCCGGTCT	GGGATTTCTTCTGCGAGTTG	NM_194268.2
Sis	AGGTGTCCGCCTGAGCAAGGT	ATGGACGCCAGCAACAGCCA	NM_001081137
Treh	GCACCTGCTGCTTCTGCT	CATCTGAACTTGGTGCAGGA	NM_021481
Vil1	ATCTCCCTGAGGGTGTGGAC	AGAGAAGGCAGCTGGAGTCA	NM_009509