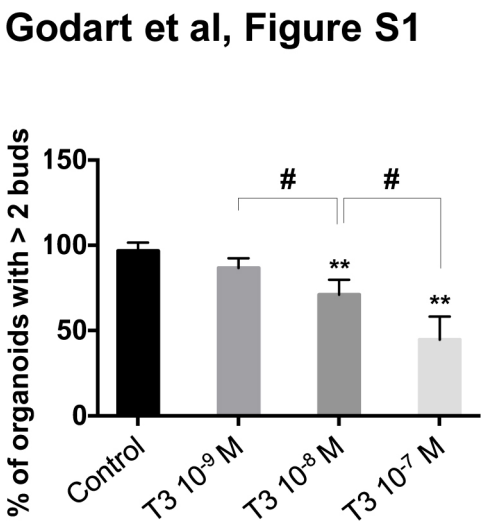
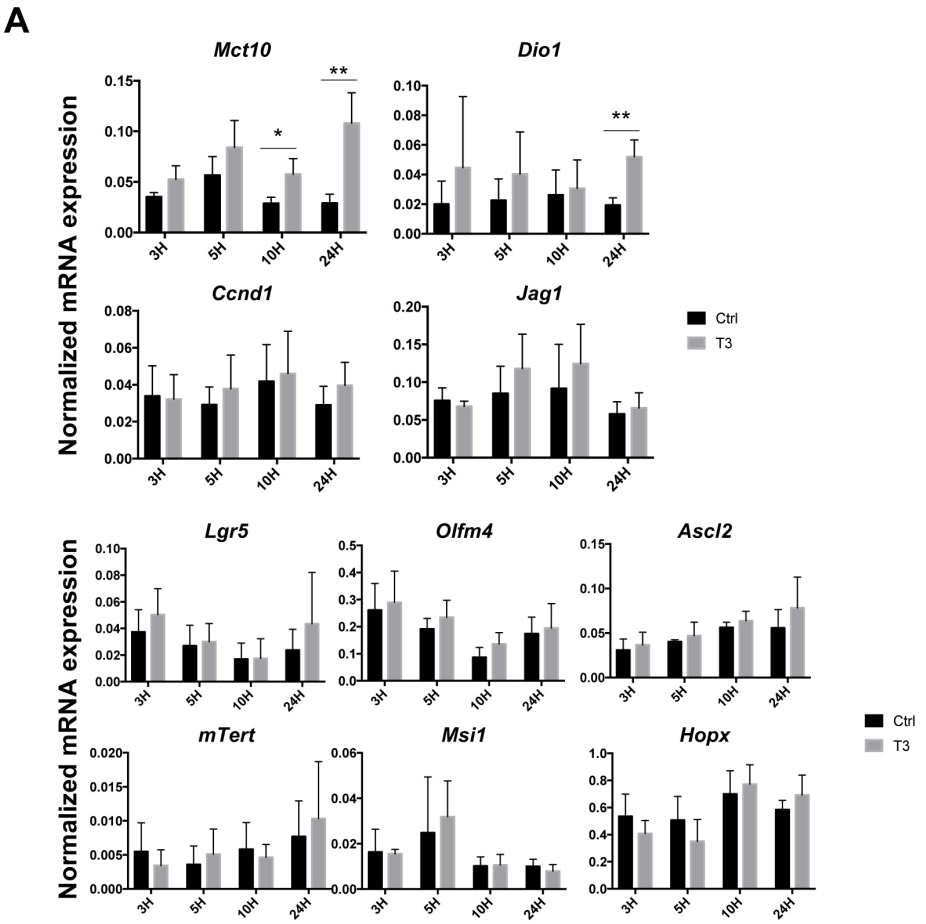


Figure S1. Test of T3 concentrations on intestinal organoids. Replicated organoids were maintained in control condition or treated with T3 at different concentrations (10^{-7} M, 10^{-8} M and 10^{-9} M), as indicated. The percentage of complex organoids (> 2 buds) was analyzed at D4. Histograms represent mean ± SD, n = 6. **, $P < 0.01$ compared to control; #, $P < 0.05$ compared to the T3 10^{-9} M condition. This experiment convinced us to choose the 10^{-7} M concentration of T3 for all following experiments.

Godart et al, Figure S2



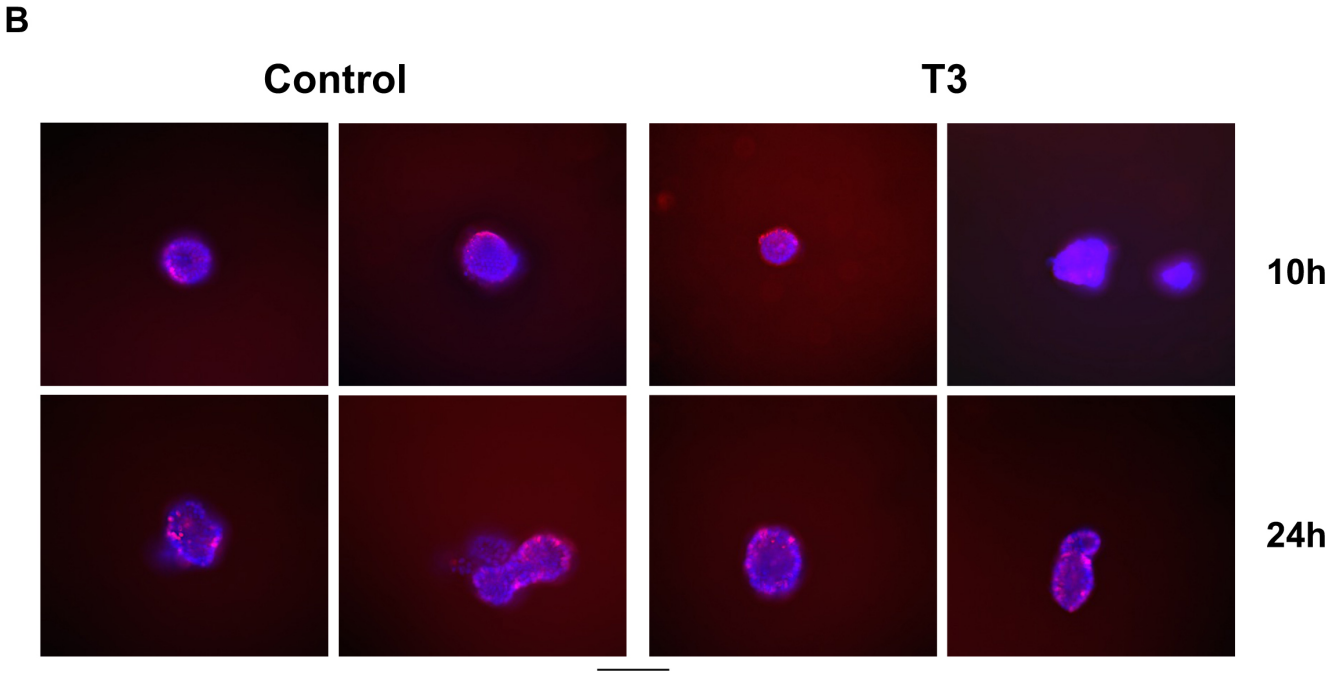


Figure S2. Short-term treatment of TRα $^{0/0}$ organoids with T3. A) Replicated TRα $^{0/0}$ organoids were cultured over 24 h in the absence (Control) or presence of 10^{-7} M T3, as indicated. RT-qPCR experiments were performed at different time points, as indicated, to analyze the mRNA expression of the TH metabolizing enzyme *Dio1* and transporter *Mct10*; *Ccnd1* (Cyclin D1) was used as a proliferative marker and *Jag1* as a direct T3-target gene. In addition, stem cell markers *Lgr5*, *Olfm4*, *Ascl2*, *mTert*, *Msi1* and *Hopx* were also analyzed. Histograms represent mean ± SD, n = 4, after normalization against *Ppib*. *, $P < 0.05$, **, $P < 0.01$ compared to the respective control conditions. B) Proliferation analysis by EdU incorporation was performed on organoids in control condition or treated with T3 for 10 h or 24 h; in both cases EdU was added in the culture medium 2 h before ending cultures. Images show merged EdU labeling (red) and nuclear staining (blue). Bar = 7 µm.

Godart et al, Figure S3

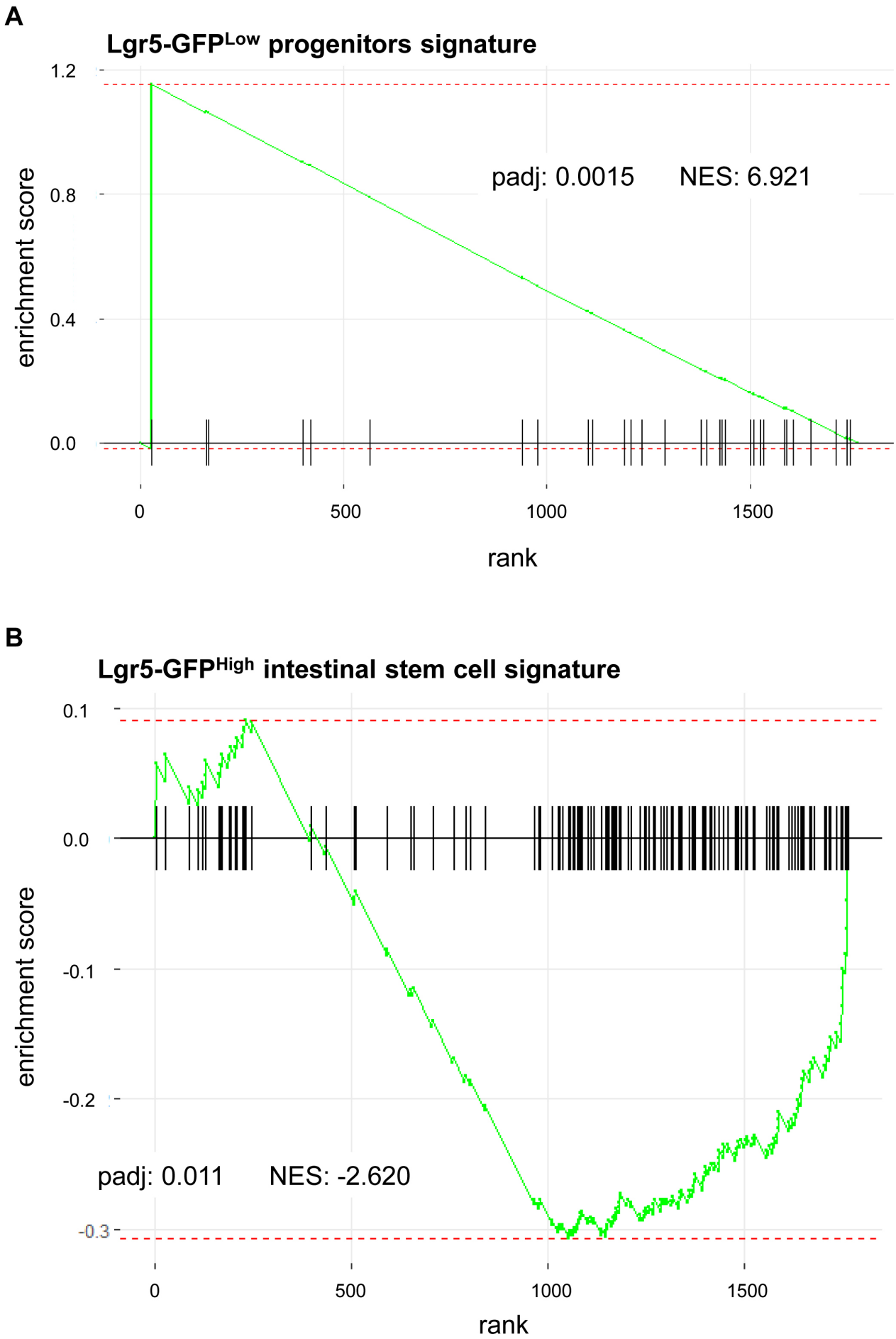
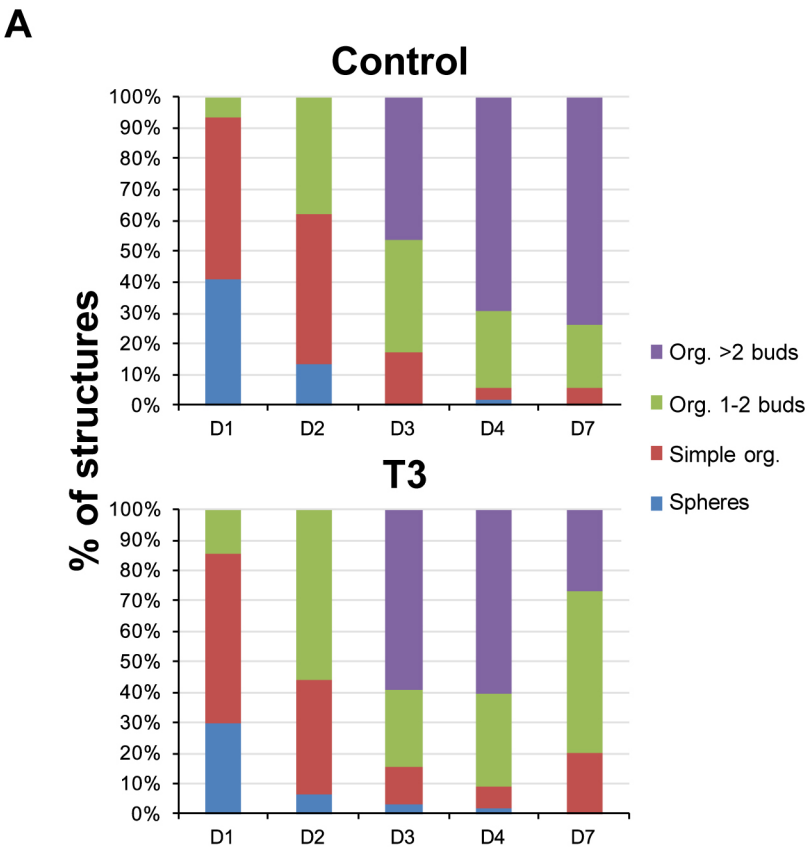


Figure S3. Gene set enrichment analysis (GSEA). Genes are ranked according to their differential expression between T3-treated and control organoids. Image showing the comparison of our DE genes and the genes characterizing progenitors (A) or the genes characterizing intestinal stem cell signature described in Munoz et al., 2012 (B). Black bars below each graph depict the position of common genes. NES: normalized enrichment score.

Godart et al, Figure S4



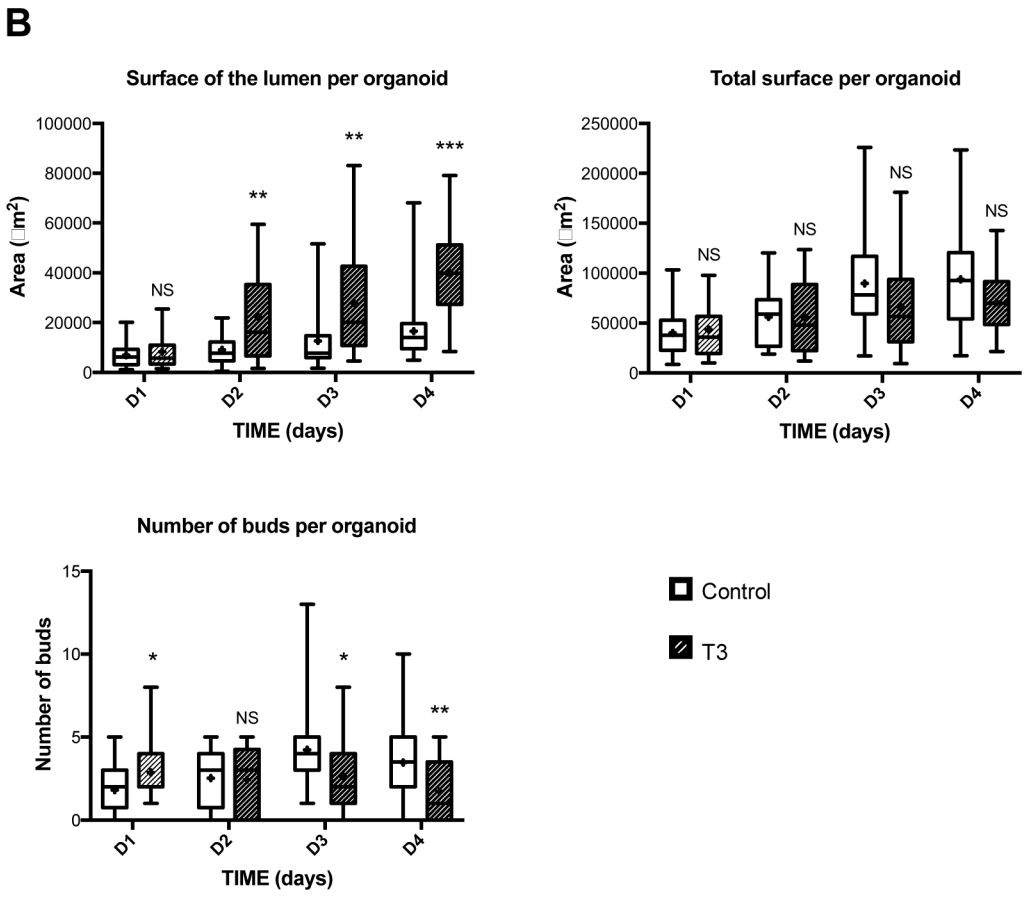


Figure S4. Complementary growth properties and features of cultured crypts upon T3 treatment. Crypts were prepared from *Lgr5*-EGFP intestine and maintained in culture for several days in the absence (Control) or presence of 10^{-7} M T3, as indicated. A) Multilayered histograms represent the mean ± SD, n = 6, of each counted structure in the cultured crypts in control or T3 condition. The percentage of spheres, simple organoids, 1-2 bud organoids and more complex organoids (> 2 buds) was evaluated every day for 1 week. D, days in culture. B) Analysis of the lumen size, the total size and the number of buds per organoid was performed on representative images taken under the inverted microscope by using the ImageJ software. 30-50 organoid per day and per condition were analyzed. D, days in culture. NS, not significant; *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ compared to the respective control conditions.

Godart et al, Figure S5

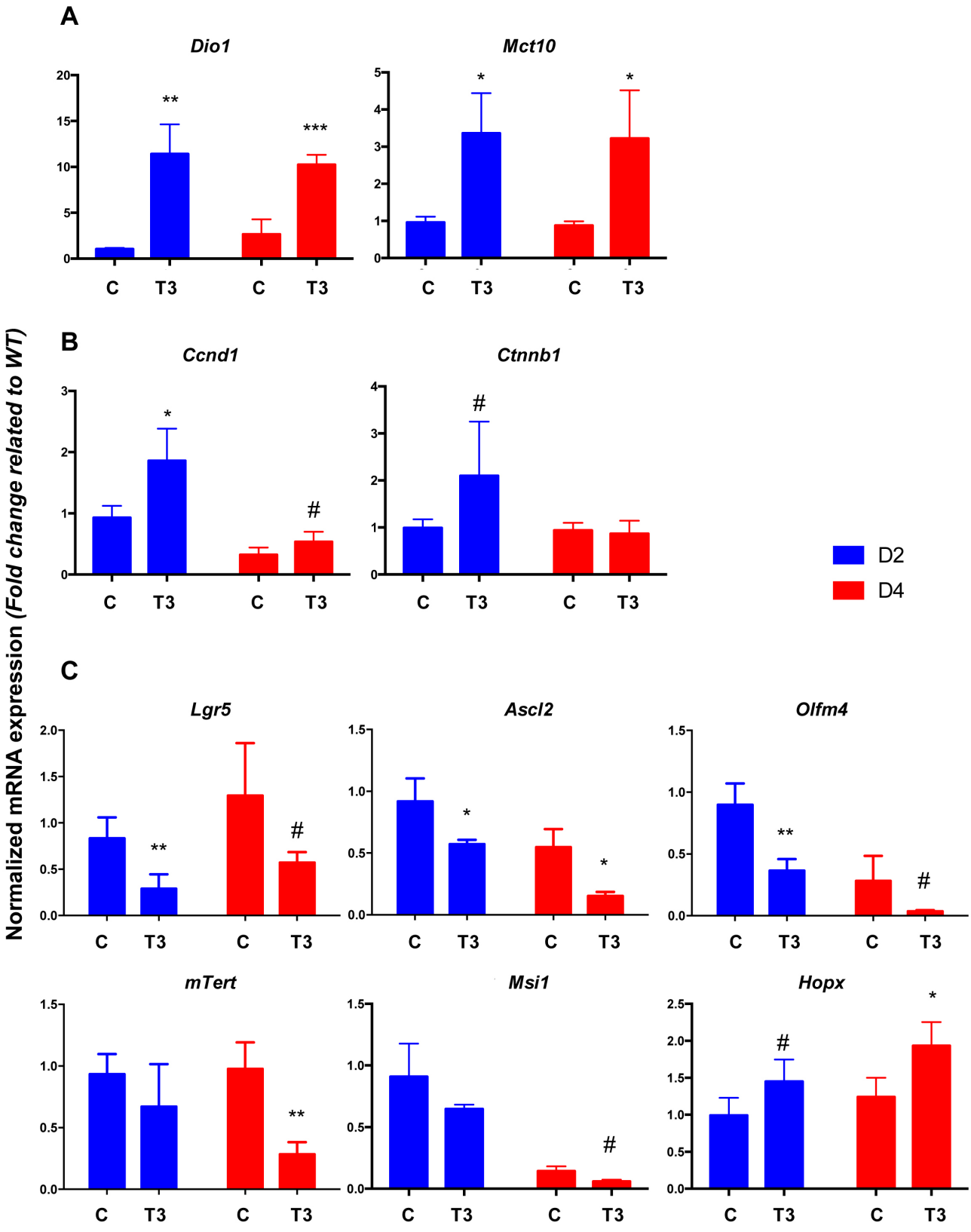


Figure S5. Complementary molecular analyses of T3-treated organoids. Replicated *Lgr5*-EGFP organoids were cultured in the absence (C, control) or presence of 10^{-7} M T3 (T3), as indicated, for 2 (D2) and 4 days (D4). RT-qPCR experiments were performed to analyze the expression of TH metabolizing enzyme mRNA *Dio1* and transporter *Mct10* (A); *Ccnd1* (Cyclin D1) was used as proliferative marker and *Ctnnb1* (β -catenin) as a direct T3-target gene (B). C) Analysis of stem cell markers *Lgr5*, *Olfm4*, *Ascl2*, *mTert*, *Msi1* and *Hopx*. Histograms represent mean ± SD, n = 4, after normalization against *Ppib*. The expression value of each gene is represented as fold change related to the control condition at D2. *, $P < 0.05$, **, $P < 0.01$ and ***, $P < 0.001$ compared to the respective control conditions. #: marginally significant compared to the respective control conditions.

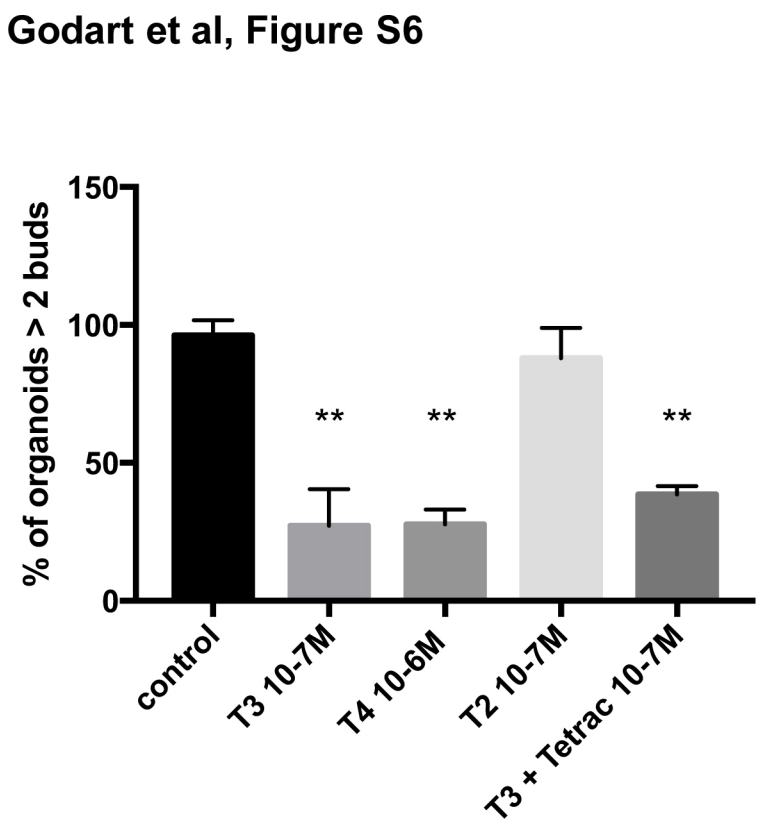
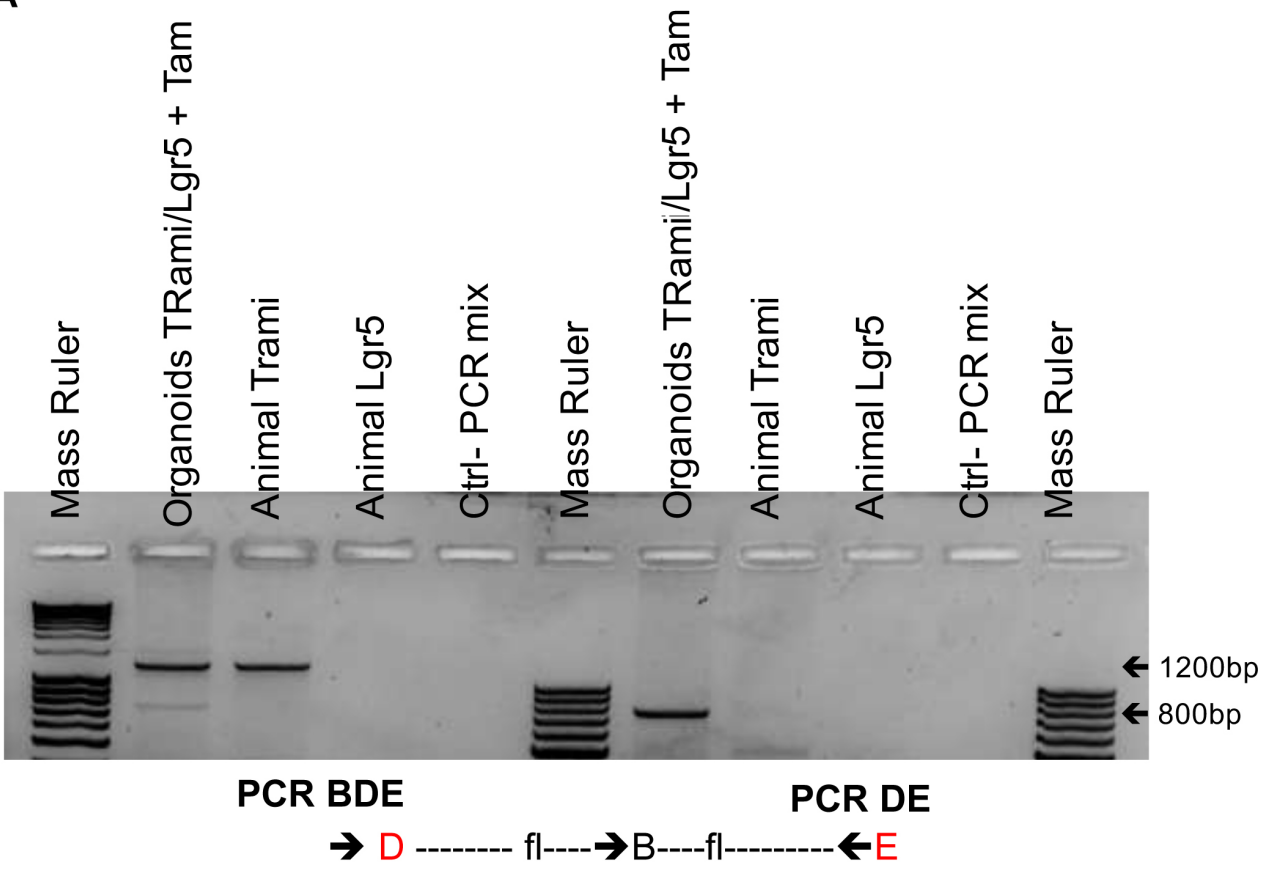


Figure S6. Analysis of different TH-related molecules in organoids. Replicated *Lgr5*-EGFP organoids were maintained in control, T3 (10^{-7} M), T4 (10^{-6} M), 3,3'-T2 (10^{-7} M) or T3 and Tetrac (10-7 M) (T3+Tetrac). The percentage of complex organoids (> 2 buds) was analyzed at D4. Histograms represent mean ± SD, n = 6. **, $P < 0.01$ compared to the control or to the T2 condition.

Godart et al, Figure S7

A



1200 bp : Non-recombined TRami allele
800 bp : Recombined TRami allele and expression of the TRami

B

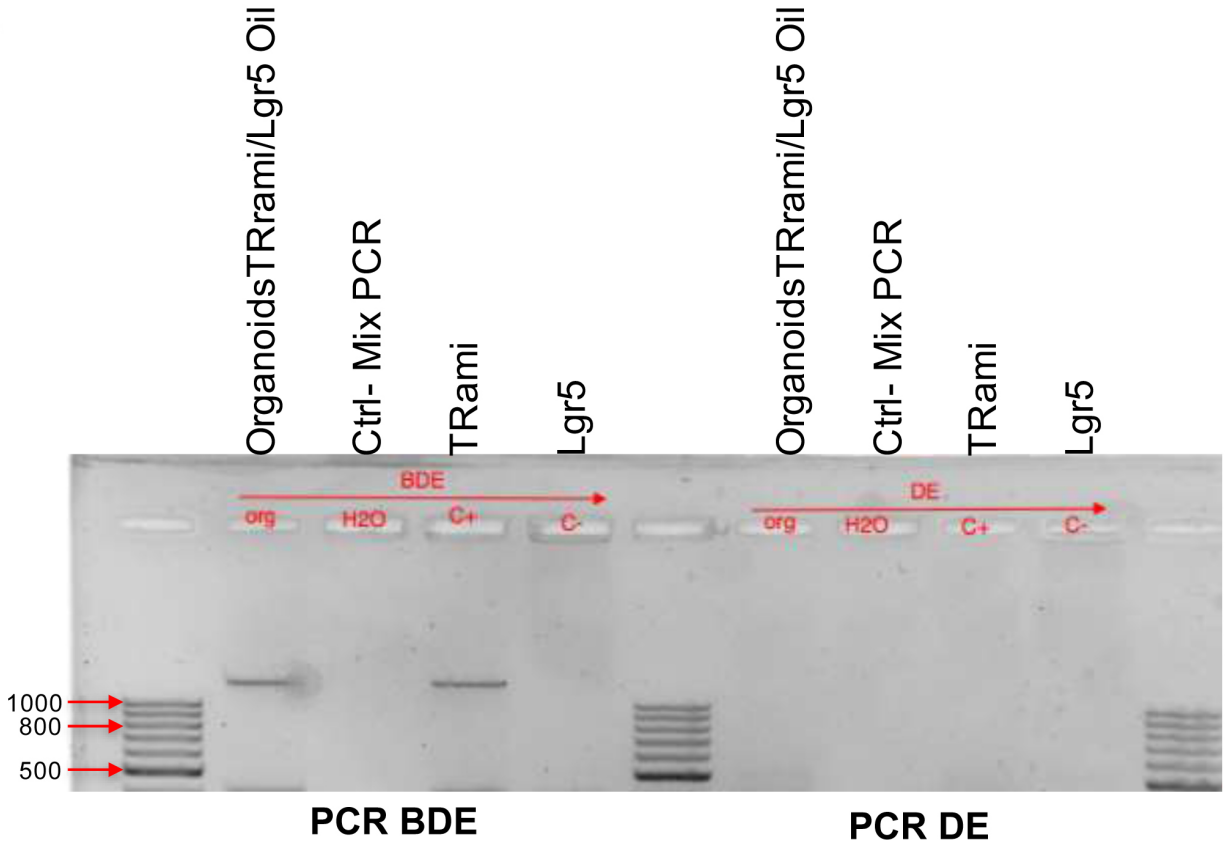


Figure S7. Generation and validation of the TRami/*Lgr5*-EGFP/*Rosa*-Tomato animals and organoids. PCR analysis on gDNA from triple transgenic TRami/*Lgr5*-EGFP/*Rosa*-Tomato organoids from previously tamoxifen- (A) or oil- (B) injected animals. Recombination of gDNA was evaluated after 4 days of culture and compared to controls (gDNA extracted from intestine of TRami or *Lgr5*-EGFP mice, PCR Mix). Combination of two (DE) or three (BDE) primers was used to amplify gDNA by PCR. In the presence of tamoxifen, 800 bp amplicons were detected, revealing the presence of the recombined TRami allele (A) whereas they were not detected when mice were injected with oil (B).

Godart et al, Figure S8

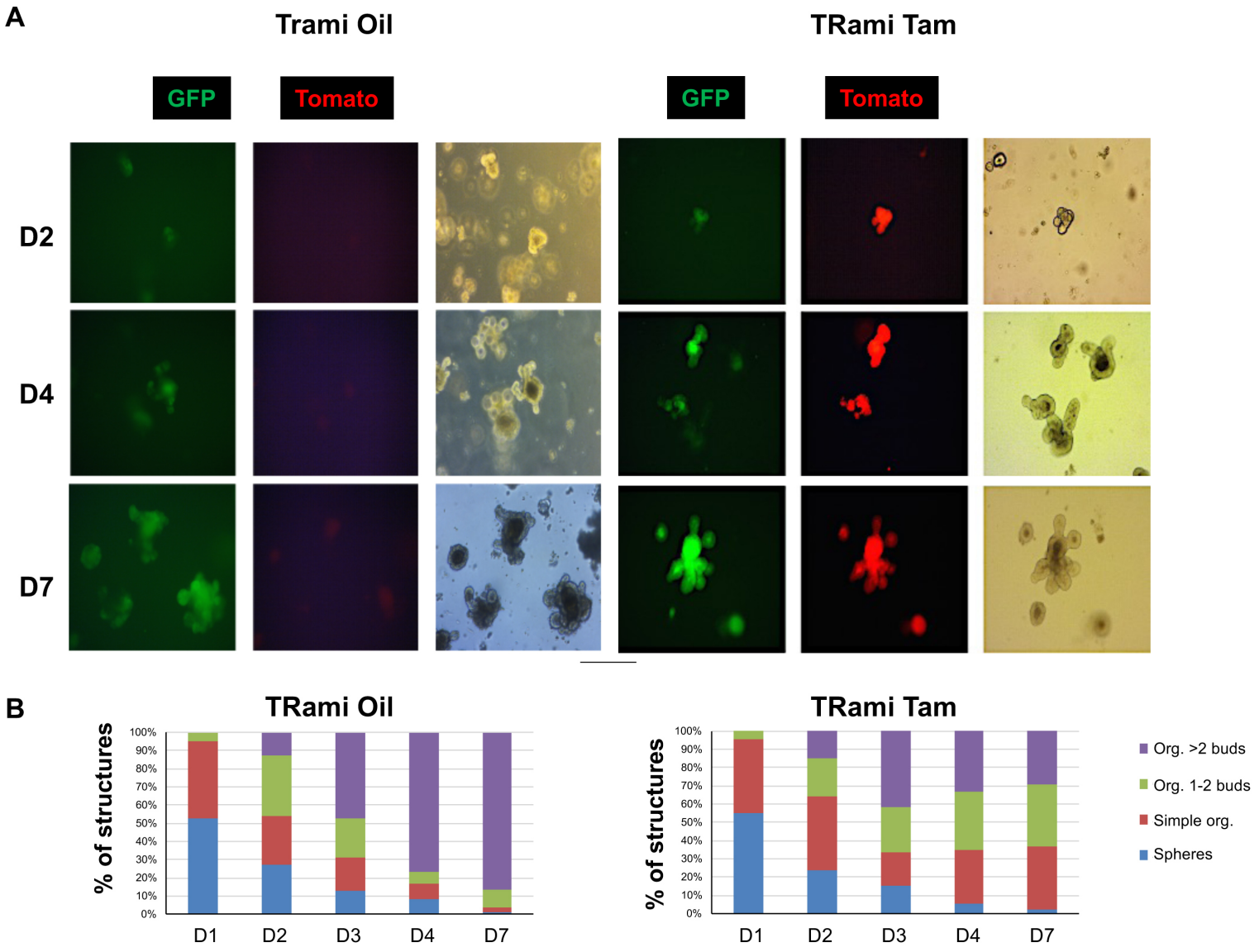


Figure S8. Complementary phenotypic analysis of the TRami/*Lgr5*-EGFP/*Rosa*-Tomato organoids. A) Live GFP and RFP fluorescence analysis of fresh triple transgenic organoids established from tamoxifen- or oil-injected mice, as indicated, and observed at D2, D4 and D7. D, days in culture. In TRami/*Lgr5*-EGFP/*Rosa*-Tomato animals, tamoxifen injections induced deletion of floxed sites enabling the expression of the Tomato protein (red fluorescence) as well as of the TRami allele. No recombination could be observed in organoids established from oil-treated animals, confirmed by the absence of Tomato signal. Left: GFP, Middle: Tomato, Right: Brightfield. Pictures have been taken under an inverted microscope at the indicated days after the start of the culture, and are representative of two independent experiments, each conducted on six replicates (Bar = 50 μm). B) Multilayered histograms represent the mean ± SD, n = 6, of each counted structure in the cultured crypts in oil or Tam condition. The percentage of spheres, simple organoids, 1-2 bud organoids and more complex organoids (> 2 buds) was evaluated every day for 1 week. D, days in culture.

Godart et al, Figure S9

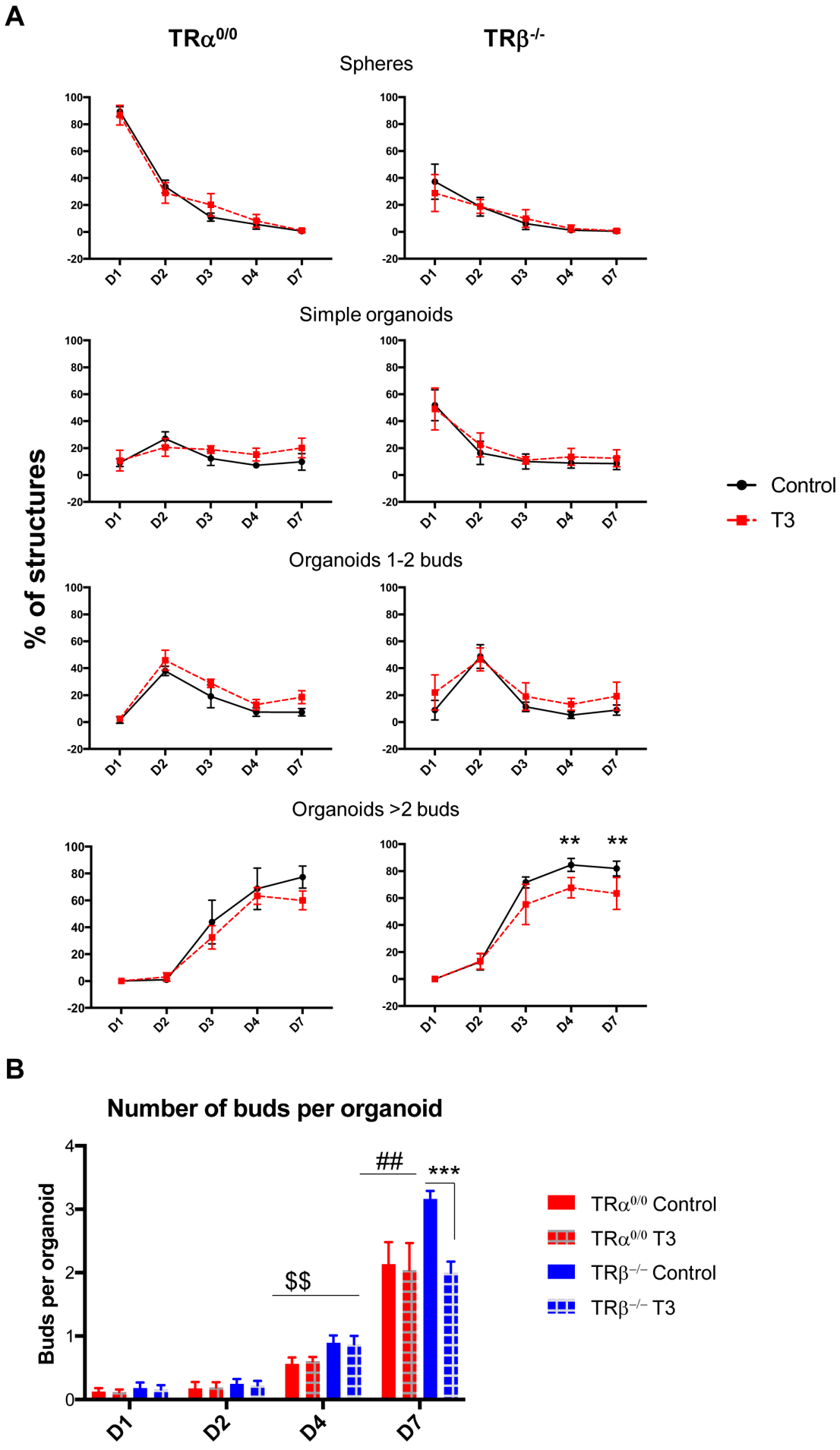


Figure S9. Complementary analyses on organoids from TRα $^{0/0}$ and TRβ $^{-/-}$ mice. A) Crypts were prepared from TRα $^{0/0}$ and TRβ $^{-/-}$ intestine and maintained in culture for several days in the absence (Control) or presence of 10^{-7} M T3, as indicated. The number of simple structures (spheres) or organoids of increasing complexity (1 or 2 buds, more than 2 buds) in control and T3 condition, as indicated, were scored under the inverted microscope during seven days of culture. Graph lines represent the mean ± SD, n = 6, of each structure counted in the cultures from different genotypes and conditions. **, $P < 0.01$ compared to the respective control condition. B) The number of buddings per organoid was scored at different time points in cultured organoids of different genotypes in control and T3 conditions, as indicated. Histograms represent mean ± SD, n = 20. $$, $P < 0.01$ compared to TRβ$^{-/-}$ control or T3 conditions; ##, $P < 0.01$ compared to the TRβ$^{-/-}$ control condition; **, $P < 0.01$ compared to the control condition of the same genotype.

Godart et al, Figure S10

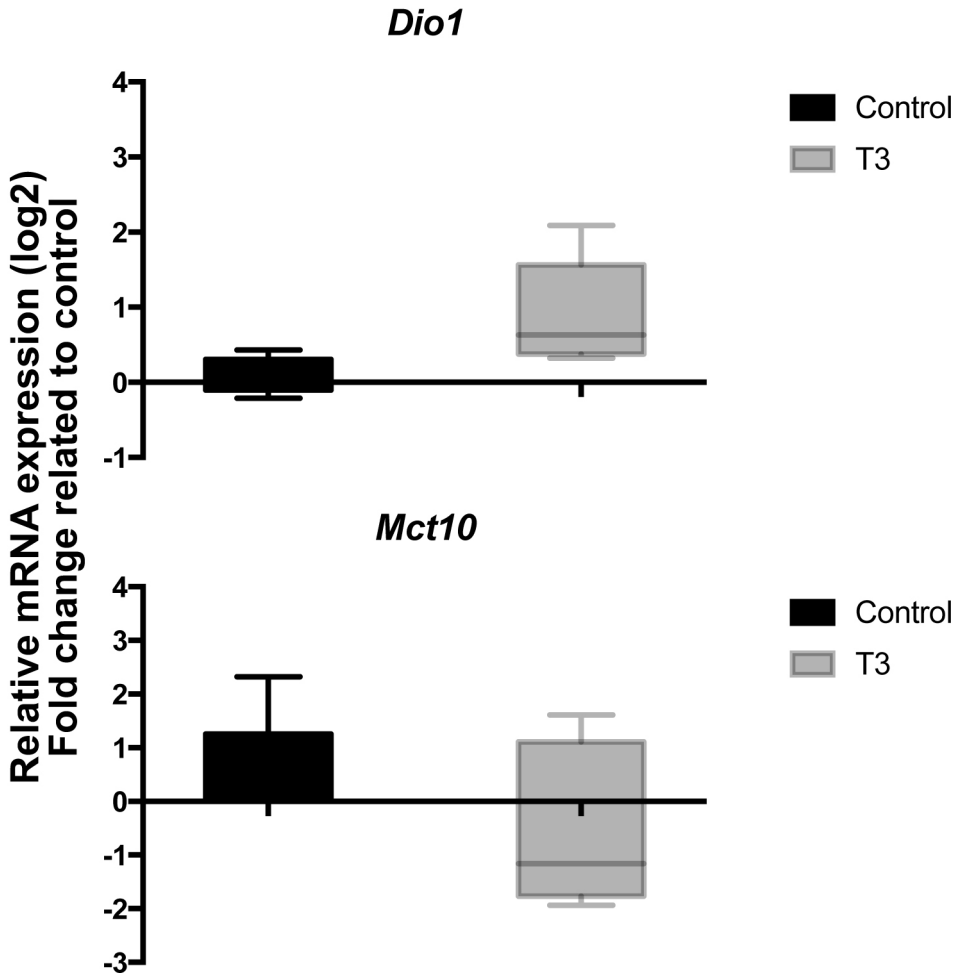


Figure S10. Effect of T3 treatment *in vivo* on *Dio1* and *Mct10* mRNA expression. A) RT-qPCR experiments to analyze the expression of TH metabolizing enzyme *Dio1* and transporter *Mct10* mRNAs. The study was performed on RNA extracted from the distal small intestinal mucosa. Boxplots show the distribution of data and the mean ± SD, n = 6, after normalization against *Ppib*. Data are represented as fold change relative to the control condition.

Godart et al, Figure S11

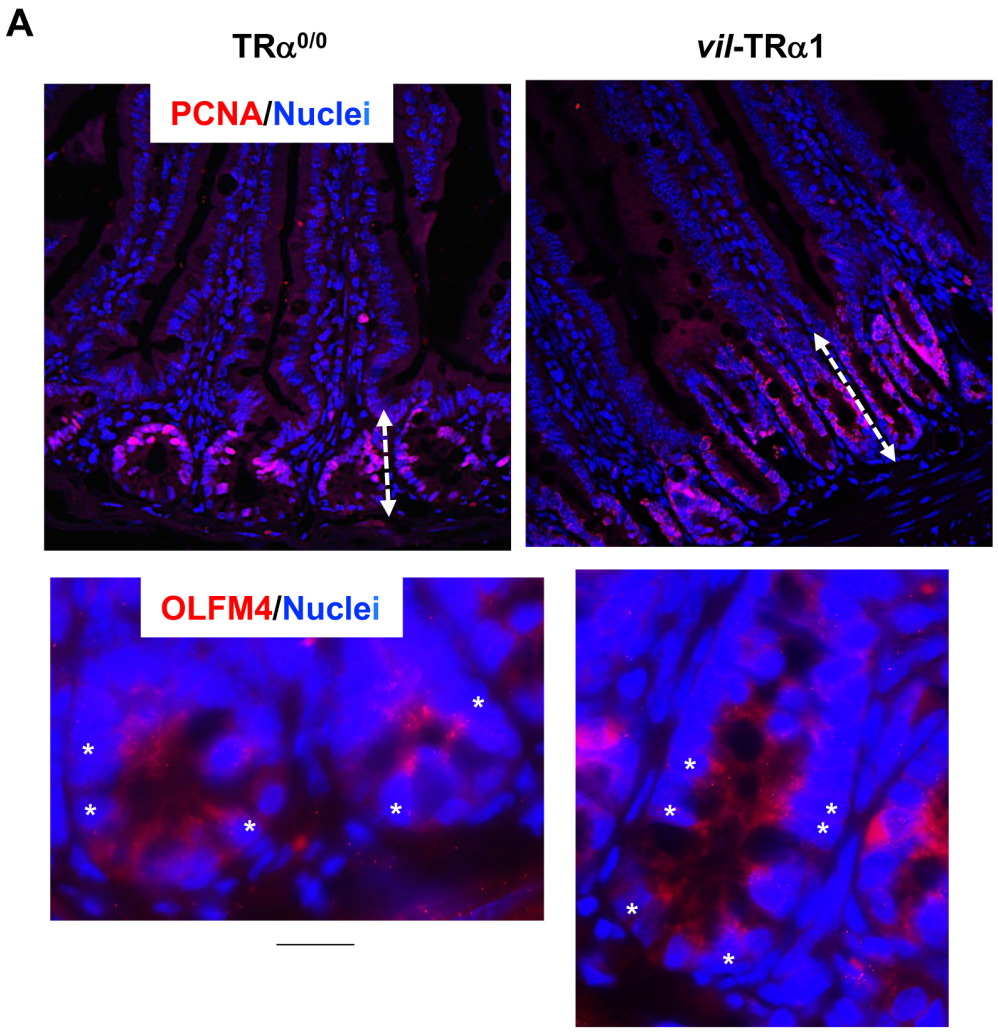


Figure S11. TRα1 modulation *in vivo* affects intestinal stem cells. A) Analysis of PCNA-positive proliferating cells (upper panels) or OLFM4-positive stem cells (lower panels) in distal small intestinal sections from TRα$^{0/0}$ and *vil*-TR 1 animals, representing models of constitutive TRα-knockout and TRα1-targeted overexpression, respectively (Gauthier et al, 2001; Kress et al, 2010). The images show merged PCNA or OLFM4 (red) and nuclear staining (blue). Pictures are representative of three different animals per condition. Bar PCNA = 10 μm; bar OLFM4 = 5 μm. The white dotted double-arrow indicates the PCNA-positive crypt length; white asterisks point to OLFM4-positive cells. B) RT-qPCR analysis on stem cell markers *Lgr5*, *Olfm4*, *Hopx* and *mTert*. The study was performed on RNA extracted from the distal small intestinal mucosa. Boxplots show the distribution of data and the mean ± SD, n = 6, after normalization against *Ppib*. Data are represented as fold change relative to the control condition.

Table S1. Differentially-expressed genes between T3-treated and control organoids.

Click here to download Table S1

Table S2. Comparative analyses between T3-treated and control organoids *vs* SC or progenitor gene signatures.

Click here to download Table S2

Table S3: Genes specifically expressed in Progenitor cells.

Click here to download Table S3

Table S4. Comparative analyses between T3-treated and control organoids *vs* genes described in Kress et al., 2009.

Gene Symbol	Kress 2009	RNA-seq 2019	Function
Acot1	down	down	Palmitoyl-CoA hydrolase activity; hydrolase activity
Acss1	down	down	Acetyl-CoA biosynthetic process; metabolic process
Adh1	down	down	Retinoid metabolic process; alcohol dehydrogenase (NAD) activity; retinol dehydrogenase activity
Akr1c13	down	down	Xenobiotic metabolic process; oxidation-reduction process
Aldh1a1	down	down	Retinol metabolic process; retinoic acid metabolic process; 9-cis-retinoic acid biosynthetic process
Aldh1a7	down	down	Retinoic acid metabolic process
Amn	down	down	Cell adhesion/extracellular matrix
Apobec1	up	up	Nucleai acid metabolism; mRNA processing
Apobec3	up	up	Nucleai acid metabolism
Armcx1	down	down	Membrane proteins/transporters
Atp13a3	down	down	Cation transport; cellular calcium ion homeostasis
Bmp7	down	down	Transforming growth factor beta receptor binding; growth factor activity; BMP receptor binding
Cat	down	down	Stress and apoptosis
Cldn8	down	down	Cell adhesion/extracellular matrix
Cxxc5	down	down	Transcriptional regulation
Cycs	up	up	Metabolism; cytochrome c oxidase complex activity
Dab1	down	down	Cell adhesion/extracellular matrix
Dhrs7	up	up	Metabolic process; oxidation-reduction process
Dock5	up	up	Cell cycle control/proliferation
Elovl6	up	up	Fatty acid elongase activity; transferase activity
Fgf1	down	up	Intestinal crypt formation; response to irradiation
Faah	down	down	Fatty acid catabolic process
Fmo5	down	down	Oxidoreductase activity and NADP binding
Fos	up	up	Proto-Oncogene, Transcription Factor
Gcnt2	down	up	Remodeling glicans; barrier function; regulating Muc expression
Gng10	up	up	G protein, involved as a modulator or transducer in various transmembrane signaling systems
Gp1bb	up	up	Transmembrane signaling receptor activity
Gpx2	up	up	Detoxification of Reactive Oxygen Species
Gsta1	up	up	Glutathione transferase activity
Gstm4	down	down	Glutathione transferase activity
Higd1a	up	up	Mitochondrial respiratory chain that catalyzes the reduction of oxygen
Hmgcs2	down	down	Mitochondrial enzyme that catalyzes the first reaction of ketogenesis
Idh3a	up	up	Pyruvate metabolism and Citric Acid (TCA) cycle
Kcne3	up	up	Voltage-gated potassium channel activity
Klf9	up	up	Regulation of transcription; cellular response to thyroid hormone stimulus
Lbp	up	up	Liver development; lipid transport; inflammatory response
Letm1	up	up	Protein binding; metal ion binding
Marcksl1	down	down	Positive regulation of cell proliferation
Nnt	dwn	down	NADPH regeneration; proton transport; cell redox homeostasis
Notch1	up	down	Stem cell biology; activation in enterocyte progenitor
Psmb8	up	up	Threonine-type endopeptidase activity
Pxmp4	down	down	Peroxisomal membrane; protein binding
Sesn1	up	up	Regulation of protein kinase B signaling; regulation of response to reactive oxygen species
Sh3bgr	up	up	SH3 domain binding
Slc13a1	up	up	Sodium-sulfate symporter activity
Stard5	down	down	Lipid transport; lipid binding; cholesterol binding; bile acid binding
Steap1	up	up	Ion transport; oxidoreductase activity
Tcf23	down	down	Protein dimerization activity
Tcf4	down	down	Transcription factor activity
Tgfbi	up	up	Collagen binding; cell adhesion molecule
Tlr3	up	up	Toll-like receptor signaling pathway; involved in immune response
Tnfrsf14	up	up	Tumor necrosis factor-activated receptor activity; protein binding
Ttr	down	down	Hormone binding; protein binding; thyroid hormone binding
Upp1	up	up	Catalytic activity; uridine phosphorylase activity; transferase activity

Table S5: Expression of *Thra* gene in different crypt populations.

GSE25109 - Stem Cells - Present Genes*	GSE25109 - Paneth Cells - Present Genes*	* Present Gene = flagged as "P" in 4/4 samples
GeneName	GeneName	
Thra	Thra	

GSE23672 -GFPHigh - Present Genes*	GSE23672 - Low - Present Genes*	* Present Gene = flagged as "P" in 4/4 samples
GeneName	GeneName	
Thra	Thra	

GSE25109 - Differential analysis - Stem Cells *vs.* Paneth Cells (Fold-Change > 1.5 & Pvalue < 0.05)

GeneName	SystematicName	Fold-Change (StemCells vs PanethCells)	P-value (StemCells vs PanethCells)	Description
Thra	NM_178060	2.20145311274614	0.00459508961922441	ref|Mus musculus thyroid hormone receptor alpha (Thra), mRNA [NM_178060]

GSE23672 - Differential analysis - High *vs.* Low (Fold-Change > 1.5 & Pvalue < 0.05)

GeneName	SystematicName	Fold-Change (High vs Low)	P-value (High vs Low)	Description
Thra	NM_178060	1.60923971813349	0.0129884032909439	ref|Mus musculus thyroid hormone receptor alpha (Thra), mRNA [NM_178060]

Table S6: oligonucleotides used for RTqPCR studies

Gene symbol	Category	Sequence (5'-3') forward / reverse
Ppib	Housekeeping gene	CAC CAA TGG CTC ACA GTT CTT
		ATG ACA TCC TTC AGT GGC TTG
Dio1	Thyroid hormone deiodinase selenoenzyme	AGA GAG CCA GAT TCC TGT GC
		GCT TGT AGG AAC CAT AGG CAT TGG
Mct10	Thyroid hormone transporter	CAA GGA CGA TGA CAA CAT GG
		GTC CGT GAA GAC ACT CAC GA
Lgr5	Active stem cell marker	GAC AAT GCT CTC ACA GAC
		GGA GTG GAT TCT ATT ATT ATG G
Ascl2	Active stem cell marker	CCT ATG CCT TAC CCA TGC T
		TTT CCA AGT CCT GAT GCT G
mTert	Facultative stem cell marker	GCA GGT GAA CAG CCT CCA GAC AG
		TCC TAA CAC GCT GGT CAA AGG GAA GC
Olfm4	Active stem cell marker	CTG TGG GCA ATT TAT GCA ACT
		CAG ATG GCT TGT ACT GCT TGG
Msi1	Active and reserve stem cell marker	ATG CTG GGT ATT GGG ATG CT
		CGG GGA ACT GGT TGT AA
Hopx	Facultative stem cell marker	CAT CCT TAG TCA GAC GCG CA
		AGG CAA GCC TTC TGA CCG C
Jag1	Voie Notch, TRα1 direct target gene	ACCAAGCTCAAGATCAAAAA
		TTTATTGCCAGGAACAACAC
Ctnnb1	Voie Wnt, TRα1 direct target gene	AGCCGAGATGGCCCAGAAT
		AAGGGCAAGGTTTCGAATCAA
Ccnd1	Cell cycle, cell proliferation	CAGAGGCGGATGAGAACAAGT
		GCGGTAGCAGGAGAGGAAG

Table S7: antibodies used

Western blot			
Antigen	**Brand, Ref**	**Species**	**Dilution**
CASPASE 3	Cell signaling, 9661	Rabbit	1/1000
PHOSPHO H3	Santa cruz, sc-10809	Rabbit	1/500
β-ACTIN	Sigma, A5316	Mouse	1/10000
Secondary antibody HRP-conjugated	Promega, W4011	Anti Rabbit	1/10000
	Promega, W4021	Anti Mouse	1/10000
Immunolabeling			
GFP	Millipore, AB16901	Chiken	1/500
CASPASE 3	Cell signaling, 9661	Rabbit	1/100
CHGA	Zymed, 18-0094	Rabbit	1/500
LYZ	Abcam, ab108508	Mouse	1/500
MUC2	Santa Cruz, sc-15334	Rabbit	1/100
KI67	Abcam, ab16667	Rabbit	1/200
OLFM4	Abcam, ab85046	Rabbit	1/200
PCNA	Dako, M0879	Mouse	1/1000
Secondary antibody Fluorescence-conjugated	Molecular Probes, A10042	Anti Rabbit	1/1000
	Molecular Probes, A11039	Anti Chicken	1/500
	Molecular Probes, A11004	Anti Mouse	1/1000