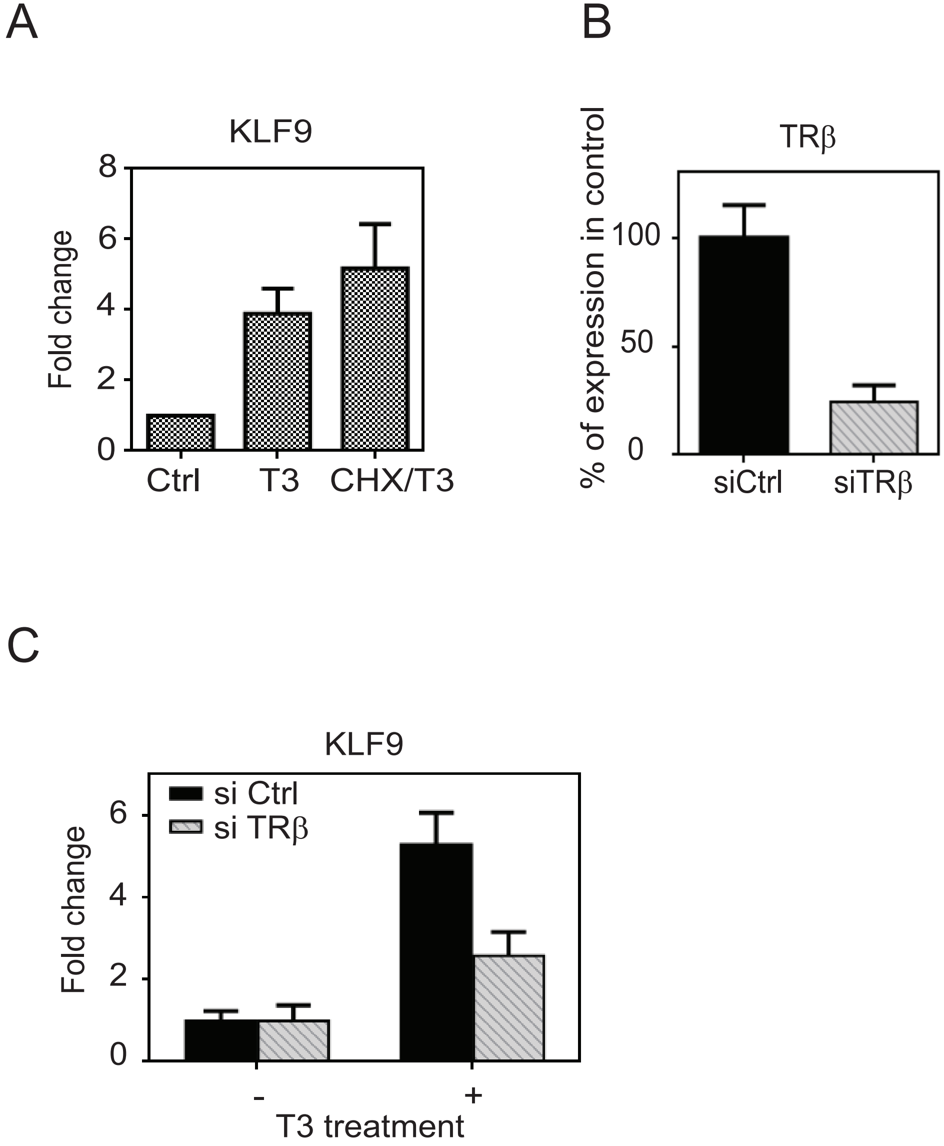
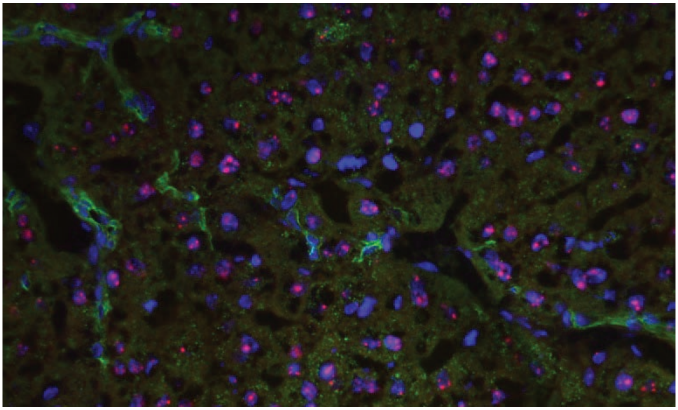
Filename	Format	Size	Description
stem1875-sup-0001-suppfig1.eps		1287K	T3 induction of KLF9 in HepG2 cells is a direct effect. (A): CHX treatment does not abrogate KLF9 responsiveness to T3. HepG2 cells were pretreated with CHX followed by treatment with T3. (B): Transfection of HepG2-TRβ cells with KLF9 siRNA resulted in 75% knockdown of TRβ relative to siRNA control and more than 50% reduction in T3 response (C): KLF9 mRNA levels were assessed as in Figure 1 of the main text.
stem1875-sup-0002-suppfig2.eps		3968K	KLF9 is not expressed in cholangiocytes, as judged by absence of colocalization of KLF9 with cholangiocytes marker CK19.
stem1875-sup-0003-suppfig3.eps		783K	(A): HepG2-TRβ cells were transfected with KLF9 siRNA at 50 nM final concentration. RNA and protein were prepared for qPCR, and Western blots, respectively. (B): Three days after KLF9 knockdown, cells were treated with 100 nM T3 for 18 h, and KLF9 mRNA was measured by qPCR. All data are represented as mean μ SD.
stem1875-sup-0004-suppfig4.eps		842K	Venn diagram of genes regulated by KLF9 or T3 (TR). Diagrams represent the number of genes regulated after KLF9 knockdown or treatment with T3 in HepG2-TRβ cells. The number in the middle (overlap) represents the genes commonly regulated by KLF9 and T3. Microarray data are deposited in the Gene Expression Omnibus (GEO); accession number GSE54699;
stem1875-sup-0005-suppfig5.eps		1107K	Effect of KLF9 knockdown on T3 response appears false-negative on ALPI in microarray analysis. Basal expression level of ALPI mRNA is significantly decreased after KLF9 knockdown but magnitude of gene response to T3 is the same. mRNA levels were assessed as in Figure 1.
stem1875-sup-0006-suppfig6.eps		1070K	TRα and TRβ mRNA expression levels in different cell types and during different stages of differentiation were measured by qPCR. All data are represented as mean μ SD.
stem1875-sup-0007-suppfig7.eps		4185K	iKCL004, iKCL011 and KCL034 were differentiated to endoderm. During definitive endoderm differentiation qRT-PCR analysis revealed decrease of pluripotent markers Nanog and Oct4 and emergence of endoderm markers MIXL1 and SOX17 (A). GATA4+ immunostaining confirmed differentiation of the majority of cells into endoderm (B).
stem1875-sup-0008-suppfig8.eps		921K	qPCR verification of Notch pathway genes LFNG and DLK1, identified as T3 targets in KCL034 and iKCL004 cells. All data are represented as mean μ SD.
stem1875-sup-0009-suppfig9.eps		1452K	Model of TR/KLF9 regulation of Notch pathway. TR and KLF9 cooperate to regulate multiple branches of the Notch pathway. A schematic of the Notch signaling pathway is presented with TR or/and KLF9 targets marked in orange.



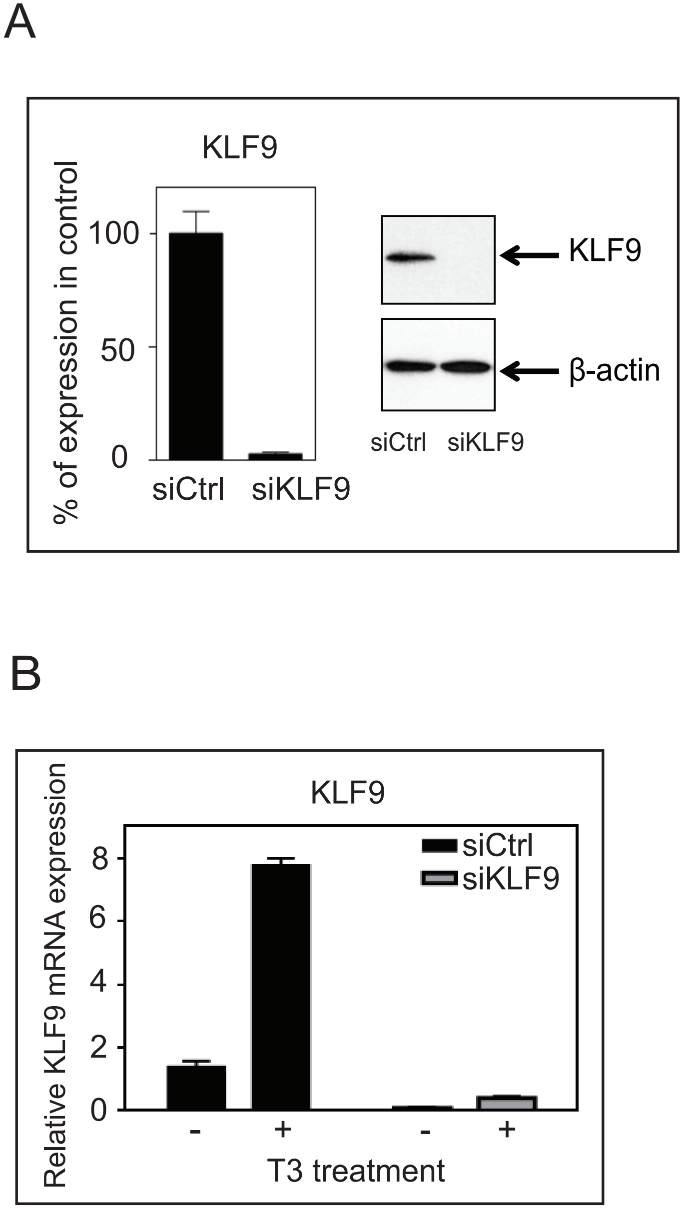
Supplemental Figure 1, Webb P.

KLF9/CK19 expression in mouse liver



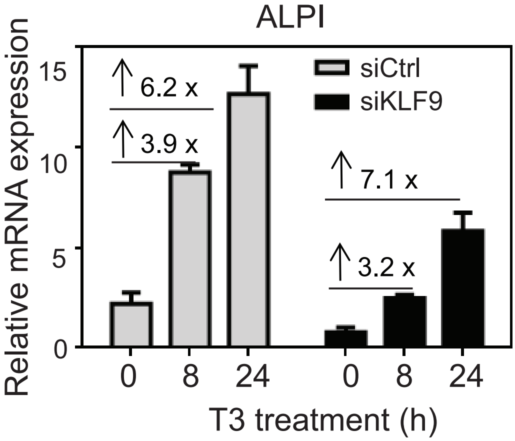
KLF9 (red)
CK19 (green)

Supplemental Figure 2, Webb P.

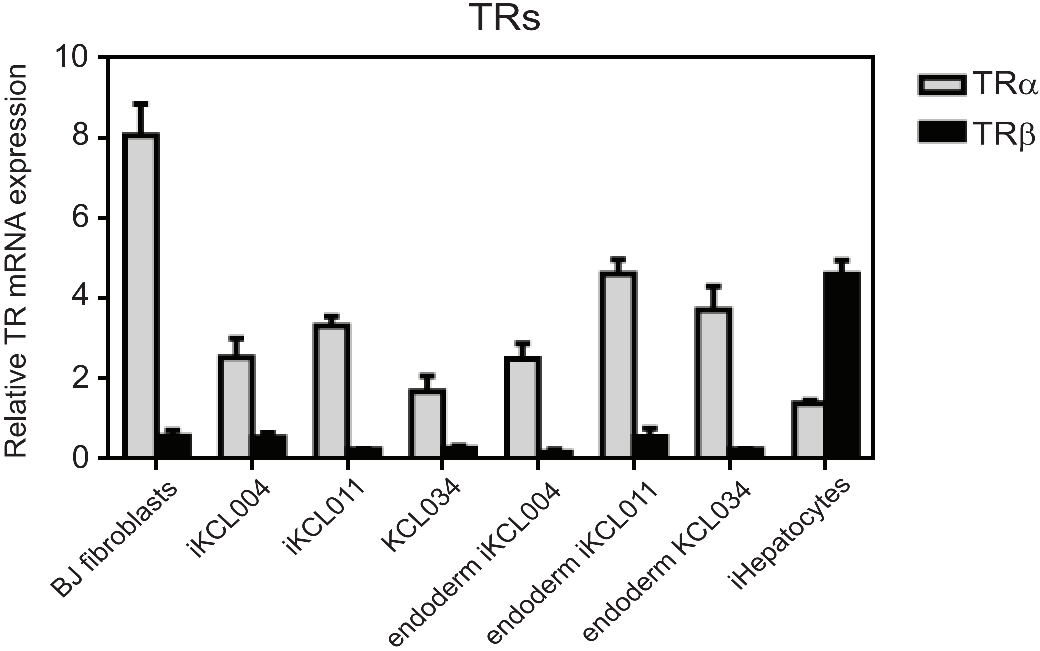


Supplemental Figure 3, Webb P.

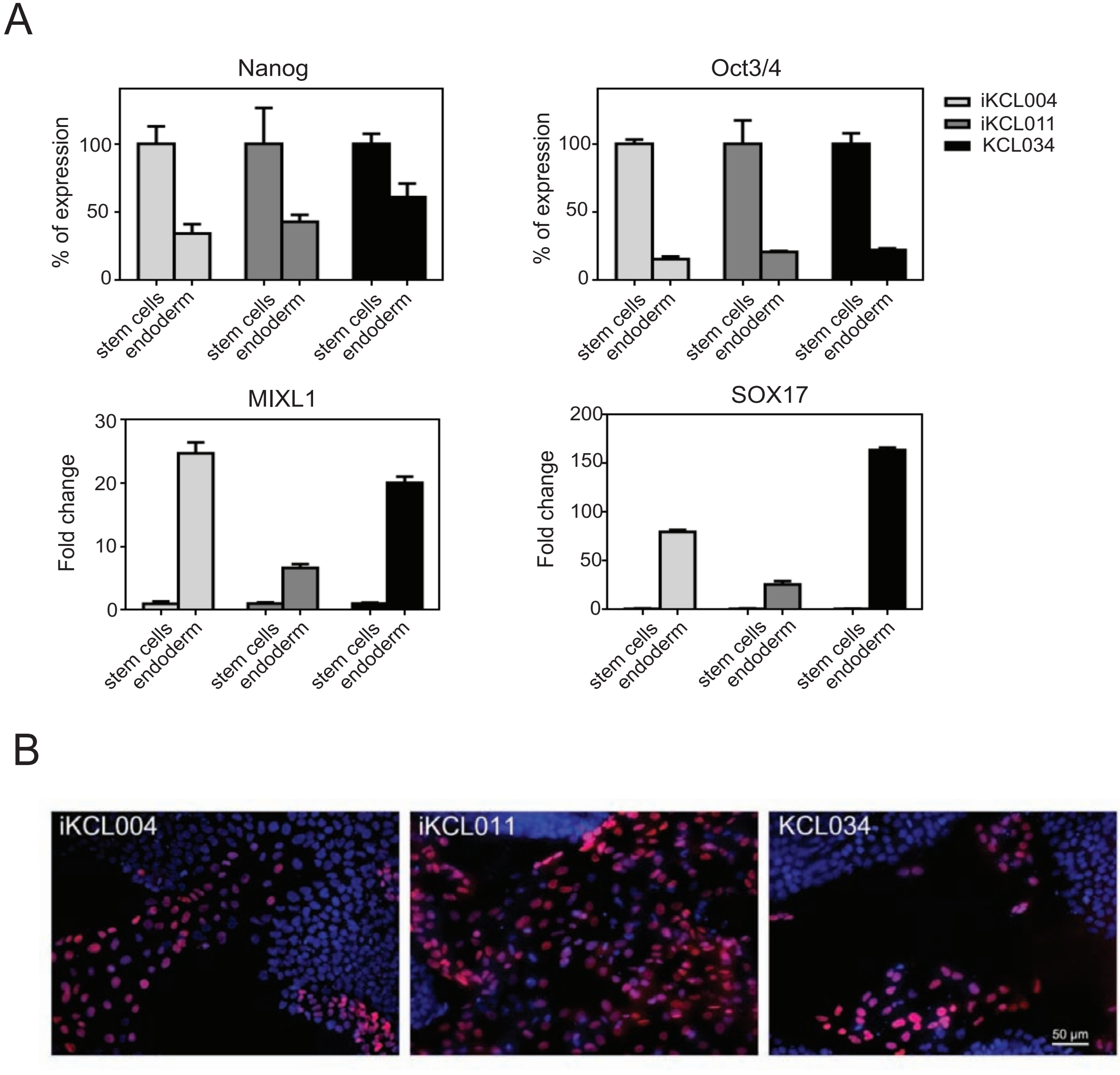
Supplemental Figure 4, Webb P.



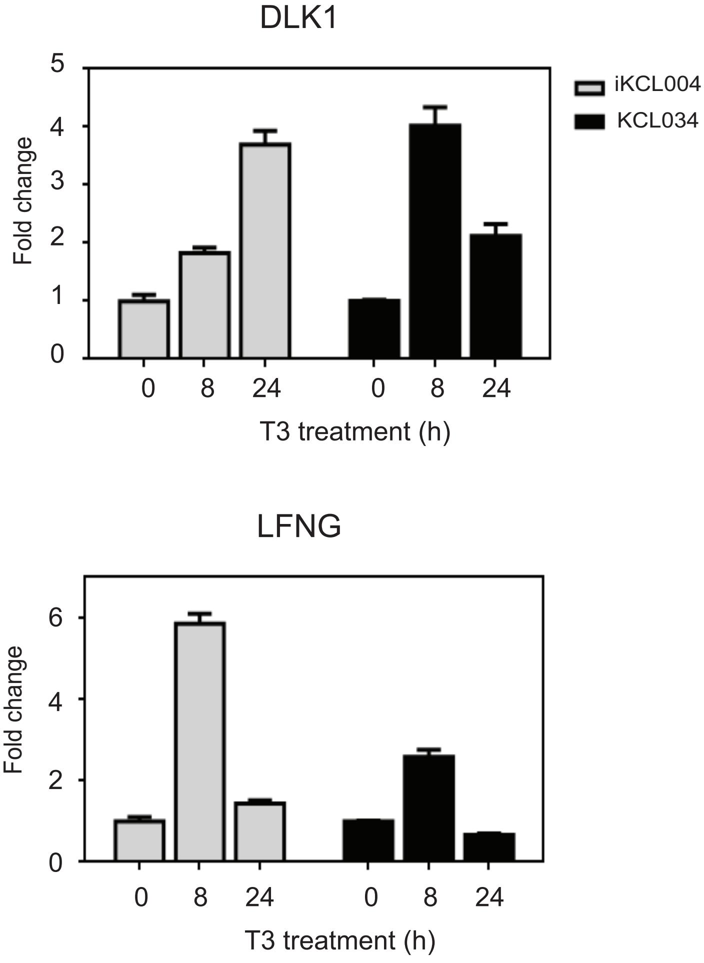
Supplemental Figure 5, Webb P.



Supplemental Figure 6, Webb P.



Supplemental Figure 7, Webb P.



Supplemental Figure 8, Webb P.

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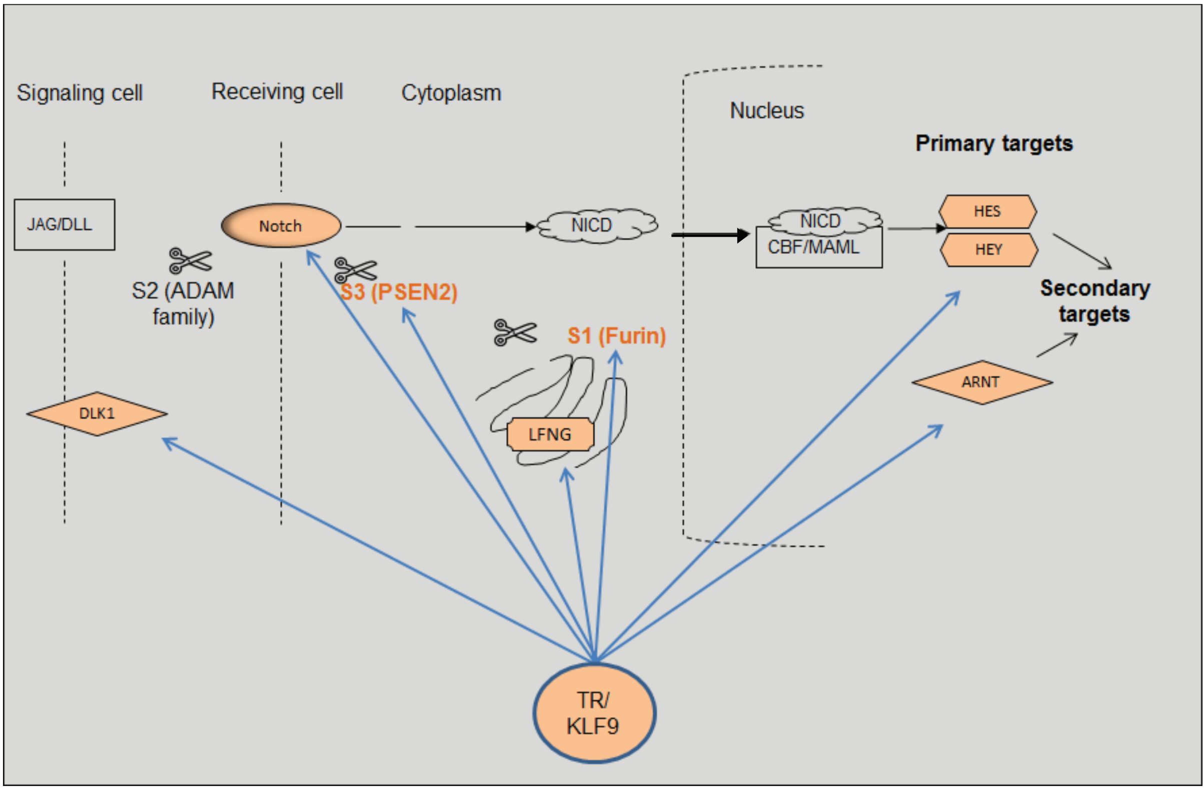

Supplemental Figure 9, Webb P.

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Supplemental Table 1. Summary of genes regulated by T3 in control and KLF9-silenced HepG2-TRβ cells. Microarray data are deposited in the Gene Expression Omnibus (GEO); http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?token=sxkvckgwhxmfdgx&acc=GSE54699; accession number GSE54699).

HepG2-TRβ	Treatment	Regulated genes
siControl	T3 8 h	295
	T3 24 h	468
	T3 total	**530**
siKLF9	T3 8 h	355
	T3 24 h	448
	T3 total	**576**

Supplemental Table 2. KLF9 mediated processes. Canonical processes obtained from GeneCodis using SlimProcess database. Gene co-occurence annotation found by Genecodis for the genes differentially expressed (FC > 2, *P* < 0.05 corrected for multiple testing) between siCtrl versus siKLF9 HepG2-TRβ samples. *P*-values have been obtained through hypergeometric analysis (Hyp) corrected by FDR method (Hyp*).

NGR	**NG**	**Hyp**	**Hyp***	**Annotations**
1176	28	3.18E-06	4.45E-05	GO:0007165: signal transduction (BP)
556	17	1.36E-05	9.51E-05	GO:0007155: cell adhesion (BP)
114	7	7.94E-05	0.0003704	GO:0007568: aging (BP)
519	13	0.0009204	0.0025772	GO:0030154: cell differentiation (BP)
156	6	0.0029387	0.0068569	GO:0008219: cell death (BP)
630	13	0.0048978	0.0097956	GO:0055085: transmembrane transport (BP)
242	7	0.0064812	0.0113423	GO:0007267: cell-cell signaling (BP)
182	5	0.0245919	0.038254	GO:0006457: protein folding (BP)
200	5	0.0348663	0.0488129	GO:0034641: cellular nitrogen compound metabolic process (BP)

NGR=Number of annotated genes in the reference list
NG= Number of annotated genes in the input list
Hyp=Hypergeometric pValue
Hyp*= Corrected hypergeometric pValue

Supplemental Table 3. Differentially expressed genes in the KLF9 pathways obtained from GeneCodis using Panther pathways database (Table 1, upper section)

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Annotations (Panther Pathways)	Gene Symbol
P00052: TGF-beta signaling pathway	FOSL1,GDF11,SMAD7,BMP6,FKBP1A,INHBE,JUN
P00003: Alzheimer disease-amyloid secretase pathway	CACNB2,PRKCE,PRKCZ,BACE2,PSEN2
P00021: FGF signaling pathway	SPRY4,PRKCE,PRKCZ,FGF6,PPP2R1B
P00057: Wnt signaling pathway	DKK1,WNT11,PRKCE,PRKCZ,EDN1,KREMEN2,CDH15,ADSSL1
P00006: Apoptosis signaling pathway	PRKCE,DIABLO,BIRC3,JUN,RELB
P00031: Inflammation mediated by chemokine and cytokine signaling pathway	CCL2,PRKCE,PRKCZ,CCL5,IL8,JUN

Supplemental Table 4. Differentially expressed genes in the KLF9-mediated processes obtained from GeneCodis using SlimProcess database (Supplemental Table 2).

Annotations	**Gene Symbol**
GO:0007165: signal transduction (BP)	IL7R,WNT11,RARB,CLIC3,SLC44A2,OCRL,COL15A1,IGF1,TNFRSF21,NR2E3,ABL2,BAIAP2L2,TNFAIP6,IL8,FKBP1A,PRKCE,CHL1,FGF6,CCL2,HMOX1,PRKCZ,MDK,DKK1,ULK1,ARHGAP28,NEDD9,PLAU,RERGL
GO:0007155: cell adhesion (BP)	CLDN1,TNFRSF12A,DSC2,F11R,F8,COL15A1,CDH15,THBS1,TNFAIP6,MYBPC2,CHL1,CCL2,HES1,EPHA2,COL9A1,NEDD9,OLR1
GO:0007568: aging (BP)	ALDOC,IL6,DCN,CCL2,JUN,CCL5,SERPINF1
GO:0030154: cell differentiation (BP)	ANG,TNFRSF12A,ARNT,SEMA4B,COL15A1,SLIT2,DUSP6,CHL1,FGF6,MDK,ZSCAN2,SEMA4F,FOXO4
GO:0008219: cell death (BP)	ANG,TNFRSF12A,HYAL1,HMOX1,PSEN2,OLR1
GO:0055085: transmembrane transport (BP)	SLC2A12,ATP2B4,SLC44A2,CACNA1H,SLC22A3,SLC25A42,ATP6V1G2,SLC16A14,HMOX1,KCNU1,CLCNKA,SLC39A7,SLC22A18
GO:0007267: cell-cell signaling (BP)	EDN1,IL11,NFE2,TNFAIP6,FGF6,CCL5,SEMA4F
GO:0006457: protein folding (BP)	TUBB2B,FKBP1A,TUBB4,DNAJC4,DNAJB6
GO:0034641: cellular nitrogen compound metabolic process (BP)	ARG2,TMLHE,QDPR,GPT,HPD

Supplemental Table 5. T3 mediated pathways and processes. Canonical pathways and processes obtained from GeneCodis using Panther pathways and SlimProsses database, respectively. Gene co-occurence annotation found by GeneCodis for the genes differentially expressed (FC > 2, *P* < 0.05 corrected for multiple testing) between Ctrl versus T3-treated HepG2-TRβ samples. *P*-values have been obtained through hypergeometric analysis (Hyp) corrected by FDR method (Hyp*).

T3 targeted pathways

NGR	NG	Hyp	Hyp*	Annotations (Panther Pathways)
96	9	6.92E-06	9.00E-05	P00036: Interleukin signaling pathway
66	5	0.00209362	0.00544341	P00003: Alzheimer disease-amyloid secretase pathway
72	5	0.00306684	0.00569556	P00019: Endothelin signaling pathway
126	7	0.00177089	0.0057554	P00029: Huntington disease
70	5	0.00271231	0.00587668	P00016: Cytoskeletal regulation by Rho GTPase
108	6	0.00372057	0.00604593	P00027: Heterotrimeric G-protein signaling pathway-Gq alpha and Go
280	11	0.00175866	0.00762087	P00057: Wnt signaling pathway
153	8	0.00125551	0.0081608	P00005: Angiogenesis
198	8	0.00609162	0.008799	P00031: Inflammation mediated by chemokine and cytokine signaling
150	6	0.0173269	0.0204772	P00026: Heterotrimeric G-protein signaling pathway-Gi alpha and Gs
107	5	0.0158559	0.0206127	P00006: Apoptosis signaling pathway
114	5	0.0202859	0.0219764	P00018: EGF receptor signaling pathway
118	5	0.0231471	0.0231471	P00004: Alzheimer disease-presenilin pathway

T3 targeted processes

NGR	NG	Hyp	Hyp*	Annotations
1176	53	3.29E-14	5.93E-13	GO:0007165: signal transduction (BP)
242	21	2.54E-11	2.28E-10	GO:0007267: cell-cell signaling (BP
519	23	9.90E-07	5.94E-06	GO:0030154: cell differentiation (BP)
200	10	0.000459	0.001376	GO:0034641: cellular nitrogen compound metabolic process (BP)
128	8	0.00039	0.001403	GO:0009790: embryo development (BP)
556	18	0.000731	0.00188	GO:0007155: cell adhesion (BP)
114	7	0.000992	0.002231	GO:0007568: aging (BP)
604	18	0.001835	0.003669	GO:0006810: transport (BP)
73	5	0.003256	0.005328	GO:0030198: extracellular matrix organization (BP)
312	11	0.004021	0.006032	GO:0008283: cell proliferation (BP)
106	5	0.015281	0.019647	GO:0007010:cytoskeleton organization (BP)

NGR=Number of annotated genes in the reference list
NG= Number of annotated genes in the input list
Hyp=Hypergeometric pValue;
Hyp*= Corrected hypergeometric pValue

Supplemental Table 6. Processes regulated by the putative TR/KLF9 axis target genes: integration of microarray data obtained with KLF9 knockdown (Fig. 2A) and TR/KLF9 dependent targets (Fig. 2C). Analysis was done by GeneCodis using SlimProcess database.

NGR	NG	Hyp	Hyp*	Annotations
1176	63	4.05E-14	1.58E-12	GO:0007165: signal transduction (BP)
556	31	5.90E-08	1.15E-06	GO:0007155: cell adhesion (BP)
242	18	6.48E-07	8.43E-06	GO:0007267: cell-cell signaling (BP)
114	12	1.33E-06	1.30E-05	GO:0007568: aging (BP)
519	26	4.87E-06	3.80E-05	GO:0030154: cell differentiation (BP)
49	7	2.99E-05	0.00016665	GO:0006520: cellular amino acid metabolic process (BP)
34	5	0.000368	0.00119456	GO:0040007: growth (BP)
128	9	0.000616	0.00184698	GO:0009790: embryo development (BP)
630	24	0.000711	0.00198062	GO:0055085: transmembrane transport (BP)
106	7	0.003463	0.00710914	GO:0007010: cytoskeleton organization (BP)
604	21	0.004336	0.00845465	GO:0006810: transport (BP)
312	13	0.005448	0.0101169	GO:0008283: cell proliferation (BP)
200	9	0.012029	0.0203965	GO:0034641: cellular nitrogen compound metabolic process (BP)
241	10	0.014178	0.0221182	GO:0006629: lipid metabolic process (BP)
291	11	0.019492	0.0281563	GO:0005975: carbohydrate metabolic process (BP)
156	7	0.025542	0.0355767	GO:0008219: cell death (BP)

Supplemental Table 7. Differentially expressed genes in the TR/KLF9-mediated pathways (Panther Pathways database) presented in Table 1, lower section.

Annotations (Panther Pathways)	**Gene Symbol**
P00052: TGF-beta signaling pathway	FOSL1,CITED2,LEFTY1,GDF11,SMAD7,BMP6,FKBP1A,INHBE,JUN
P00057: Wnt signaling pathway	DKK1,ACTA2,TLE1,WNT11,TLE6,PRKCE,AXIN2,PRKCZ,PCDHA1, FZD4,CDH6,EDN1,LEF1,KREMEN2,CDH15,ADSSL1
P00006: Apoptosis signaling pathway	PRKCE,DIABLO,TNFRSF10B,BCL2L1,CREM,TNFSF10,BIRC3,JUN, RELB
P00003: Alzheimer disease-amyloid secretase pathway	CACNB2,PRKCE,PRKCZ,MAPK4,BACE2,PCSK1,PSEN2
P00016: Cytoskeletal regulation by Rho GTPase	MYH10,SSH1,ACTA2,TUBB4,RHOU,TUBB2B,CFL2,RAC3
P00018: EGF receptor signaling pathway	SPRY4,PRKCE,SFN,PRKCZ,PHLDB2,STAT4,HBEGF,SHC1,RAC3
P00004: Alzheimer disease-presenilin pathway	ACTA2,WNT11,NOTCH3,JUP,BACE2,FZD4,PCSK1,PSEN2,LEF1
P00045: Notch signaling pathway	HEY1,NOTCH3,HES1,PSEN2,HEY2
P00021: FGF signaling pathway	SPRY4,PRKCE,SFN,PRKCZ,FGF6,PPP2R1B,SHC1,RAC3
P00019: Endothelin signaling pathway	PRKCE,PRKCZ,ECEL1,PCSK1,EDN1,ADCY7
P00036: Interleukin signaling pathway	IL11,MKNK2,STAT4,IL8,IL6,RPS6KA2,SHC1
P00031: Inflammation mediated by chemokine and cytokine signaling pathway	CCL2,MYH10,ACTA2,PRKCE,PRKCZ,CCL5,IL8,SHC1,JUN,RAC3
P04393: Ras Pathway	RPS6KA2,RGL1,SHC1,JUN,RAC3
P00029:Huntington disease	ACTA2,CYFIP2,TUBB4,TUBB2B,JUN,RAC3
P00047: PDGF signaling pathway	PDGFRL,MKNK2,STAT4,RPS6KA2,SHC1,JUN

Supplemental Table 8. Differentially expressed genes in the TR/KLF9-mediated processes (Slim Process) presented in Supplemental Table 6.

Annotations	**Gene Symbol**
GO:0007165: signal transduction (BP)	TLE1,CHN1,CDS1,IL7R,WNT11,MFAP4,UNC5A,RARB,TLR3,CLIC3,TNFRSF25,SLC44A2,TNFRSF10B,OCRL,LRRN2,COL15A1,IGF1,CREM,IGFBP1,TNFRSF21,TNFRSF1A,NR2E3,RASD1,INSL4,ABL2,RASL11B,BAIAP2L2,RTKN2,VIPR2,RASSF4,TNFAIP6,ICOSLG,HBEGF,GAS6,IL8,RGL1,FKBP1A,INHA,SFN,MPP2,PRKCE,NR3C1,CHL1,RPS6KA2,PTGES,FGF6,CCL2,ADM,HMOX1,PRKCZ,TNC,MDK,DKK1,INPP5D,ULK1,ARHGAP28,NEDD9,TNFSF10,PDE1C,TNFRSF18,PLAU,RHBDL1,RERGL
GO:0007155: cell adhesion (BP)	CLDN1,TNFRSF12A,DSC2,PCDHA1,ADAM12,LEF1,MFAP4,F11R,F8,LRRN2,COL15A1,L1CAM,SIRPA,PVRL2,PDZD2,CDH15,THBS1,CDH6,TNFAIP6,MYBPC2,LAMB3,CHL1,PTPRU,CCL2,TNC,HES1,EPHA2,EPHA4,COL9A1,NEDD9,OLR1
GO:0007267: cell-cell signaling (BP)	GJB2,EDN1,HCN2,IL11,EFNA1,INSL4,NFE2,VIPR2,TNFAIP6,INHA,FGF6,ADM,CRB1,CCL5,PCSK1,SEMA4F,TNFSF10,TSHR
GO:0007568: aging (BP)	ALDOC,IL6,DCN,HCN2,NR3C1,CCL2,ADM,JUN,SHC1,CCL5,PTGS1,SERPINF1
GO:0030154: cell differentiation (BP)	PEG10,ANG,TNFRSF12A,ECSCR,SEMA6B,PAQR8,ARNT,SEMA4B,COL15A1,CREM,SEMA6D,L1CAM,GPM6B,SLIT2,MDGA1,DUSP6,INHA,ZC3H12A,CHL1,FGF6,PTPRU,MDK,TXNDC3,ZSCAN2,SEMA4F,FOXO4
GO:0006520: cellular amino acid metabolic process (BP)	SLC7A2,CTH,ACY1,QDPR,AGXT2L1,SDSL,SDS
GO:0040007: growth (BP)	BCL2L1,BMP6,GDF11,INHBE,VEGFA
GO:0009790: embryo development (BP)	FOXF1,FOXO1,FOXF2,STOX2,FZD4,FOXO6,PCSK1,FOXO4,PSEN2
GO:0055085: transmembrane transport (BP)	SLC39A3,SLC2A12,HCN2,ATP2B4,SLC44A2,SLC7A2,CACNA1H,SLC22A3,SLC27A4,SLC25A42,ADCY7,ATP6V1G2,SLC26A6,ABCB1,SLC16A14,ABCD1,HMOX1,KCNU1,ABCB9,SCN4A,CLCNKA,SLC39A7,SLC16A9,SLC22A18
GO:0007010: cytoskeleton organization (BP)	BRWD1,RHOU,PVRL2,ABLIM2,CCL2,NEDD9,DES
GO:0006810: transport (BP)	GJB2,GABRE,PTGDS,ATP2B4,SLC7A2,CACNA1H,SLC27A4,CETP,STRA6,ABCB1,HBE1,ABCD1,HBA2,STEAP1,SRPR,ABCA13,SLC25A35,FDXR,CLCNKA,MB,CACNB2
GO:0008283: cell proliferation (BP)	FES,MYH10,STAT4,UCN2,INSL4,CITED2,INSIG1,GAS6,SFN,FGF6,RAB4B,SERPINF1,CYR61
GO:0000902: cell morphogenesis (BP)	IL7R,BCL6,JUP,SSH1
GO:0034641: cellular nitrogen compound metabolic process (BP)	ARG2,SLC7A2,TMLHE,KYNU,QDPR,GPT,HPD,PCSK1,ALDH4A1
GO:0006629: lipid metabolic process (BP)	ACSF2,LCN15,PTGDS,OCRL,RBM12,SLC27A4,CETP,PCK1,PTGS1,CEL
GO:0005975: carbohydrate metabolic process (BP)	ALDOC,PGM2L1,HYAL1,TFF1,PYGM,PFKFB2,PCK1,PPP1R3C,PCK2,PC,PFKFB4
GO:0008219: cell death (BP)	ANG,TNFRSF12A,L1CAM,HYAL1,HMOX1,PSEN2,OLR1

Supplemental Table 9. Summary of genes regulated by T3 in KCL034, iKCL004 and BJ cells. Microarray data have been deposited in NCBI's Gene Expression Omnibus (GEO); http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?tacc=GSE58273; accession number GSE58273.

Cell line	T3-regulated genes
KCL034	820
iKCL004	826
BJ	0

Supplemental Table 10. Pathway enrichment analysis of T3 dependent transcriptome in KCL034 and iKCL004 cells: Canonical pathways obtained from GeneCodis using Panther Pathways database as described in Table 1.

	KCL034		**iKCL004**	
Annotations (Panther Pathways)	**NG**	**Hyp***	**NG**	**Hyp***
P00057 Wnt signaling pathway	28	1.75E-09	29	1.92E-10
P00005: Angiogenesis	16	4.50E-06	18	1.22E-07
P04374 5HT2 type receptor mediated sign. p.	12	4.23E-07	9	0.000122
P00019 Endothelin signaling pathway	11	4.40E-06	6	0.02040
P00012 Cadherin signaling pathway	13	9.87E-05	19	5.81E-09
P00042 Muscarinic acetylcholine receptor 1 and 3 signaling pathway	9	3.30E-05	8	0.00022
P04385 Histamine H1 receptor mediated sign.	9	5.82E-06	7	0.000202
P00045 Notch signaling pathway	6	0.000767	5	0.017203
P00021 FGF signaling pathway	9	0.003079	7	0.033244
P00004 Alzheimer disease-presenilin pathway	9	0.004535	12	0.000104
P00027 Heterotrimeric G-protein signaling pathway-Gq alpha and Go alpha mediated pat.	9	0.003085	9	0.00317
P00029 Huntington disease	8	0.021204	8	0.024924
P00031 Inflammation mediated by chemokine and cytokine signaling pathway	10	0.032948	13	0.002324
P00043 Muscarinic acetylcholine receptor 2 and 4 signaling pathway	5	0.021579	5	0.028328
P04391 Oxytocin receptor mediated signaling pathway	10	3.85E-06	8	0.000216
P04394 Thyrotropin-releasing hormone receptor signaling pathway	10	4.56E-06	8	0.00022

NG=Number of annotated genes in the input list
Hyp*= Corrected hypergeometric pValue

Supplemental Table 11. List of the differentially expressed genes after T3 treatment in the enriched Notch pathway (Panther Pathways database) in both KCL034 and iKCL004 cells.

Annotations (Panther Pathways)	**Gene Symbol**
P00045: Notch signaling pathway	HES4,HEY2,LFNG,DLK1