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Supplemental Information

Thyroid Hormone Transporters MCT8 and OATP1C1 Control Skeletal Muscle Regeneration

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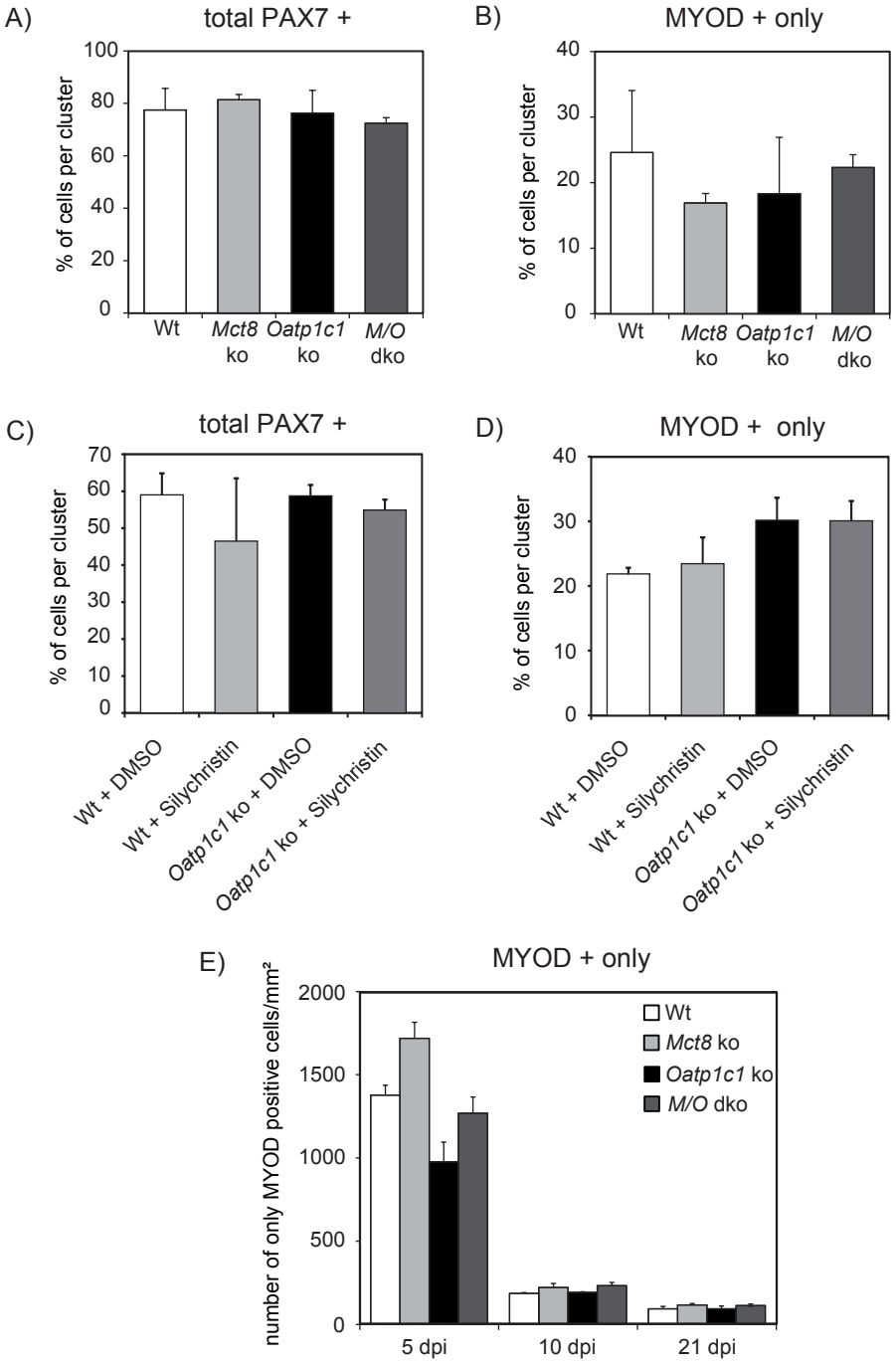
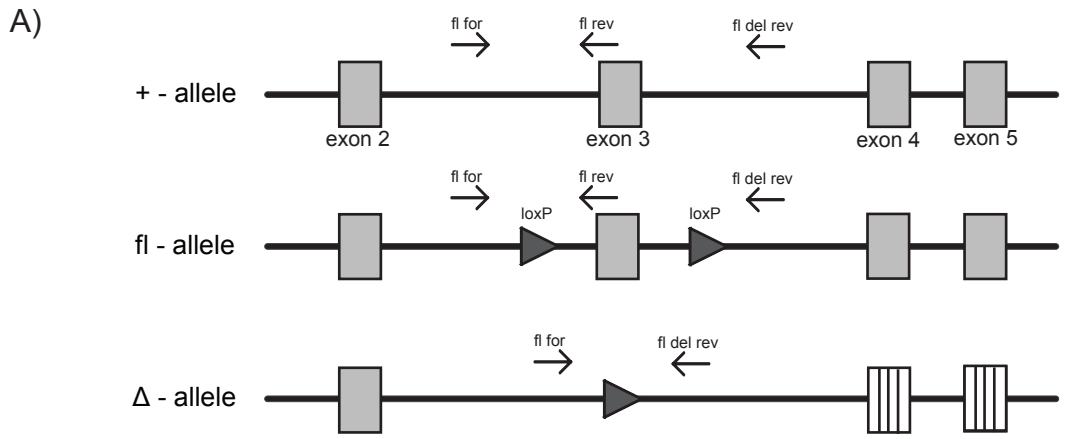
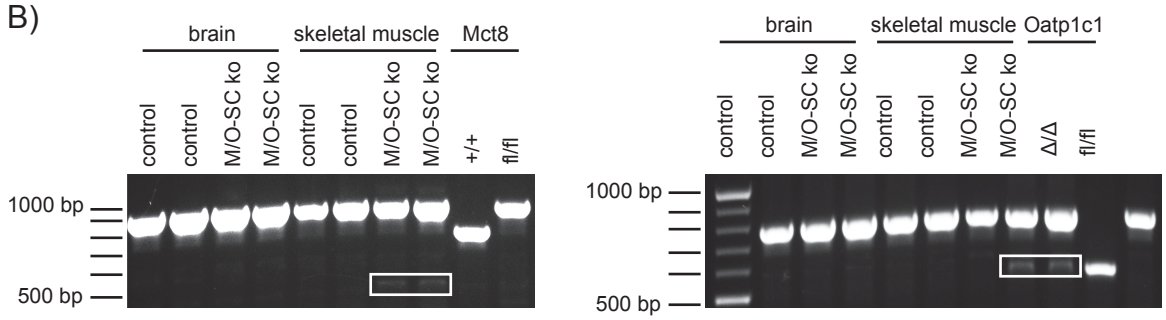
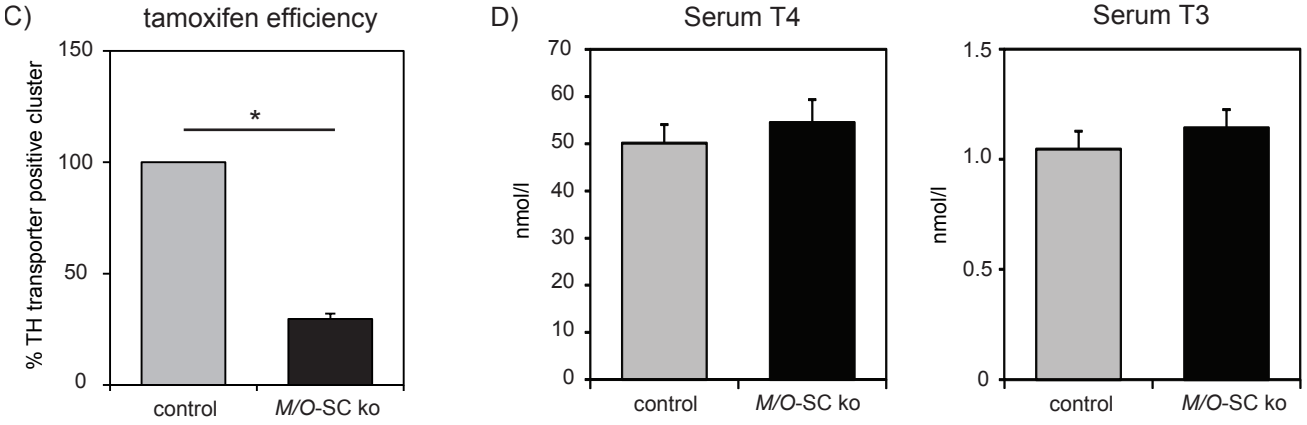
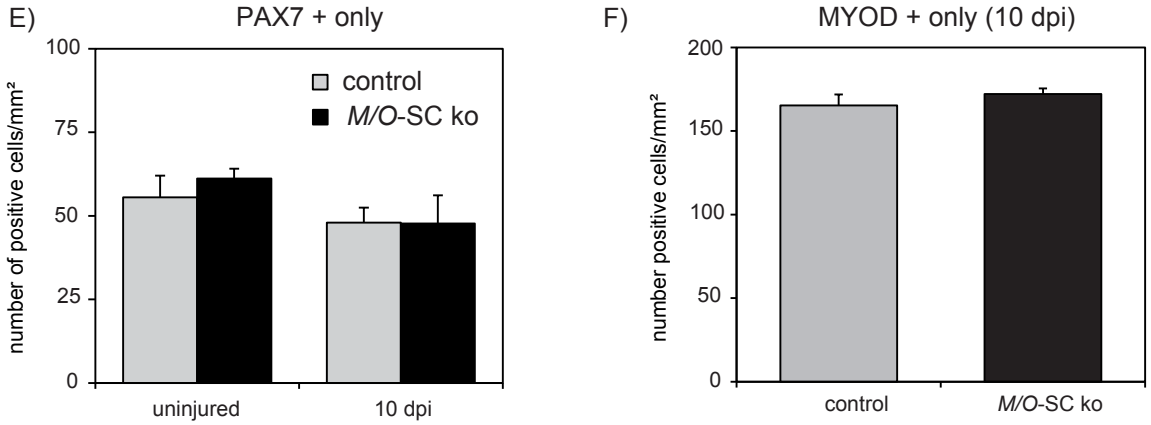
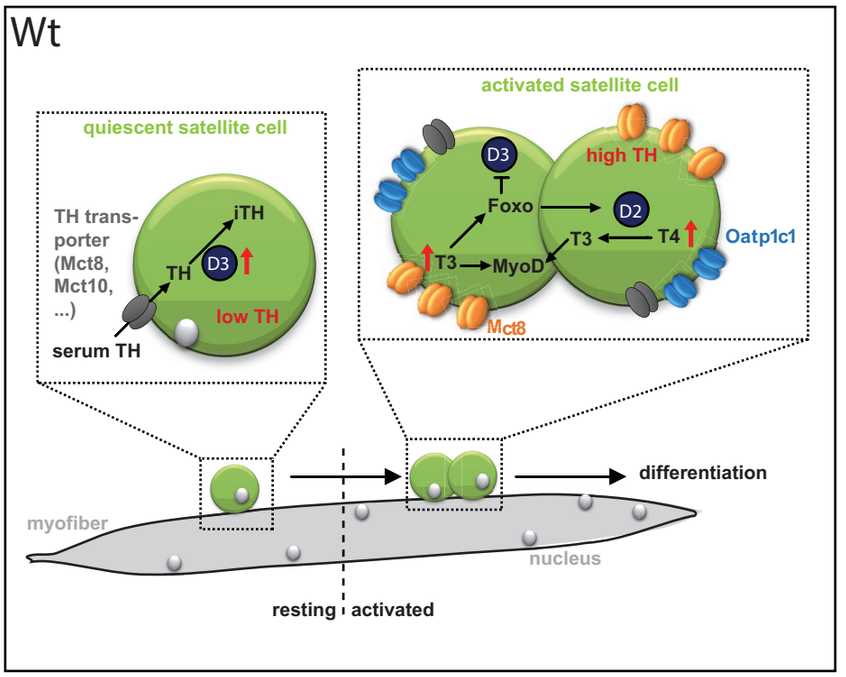

A)
total PAX7 +
% of cells per cluster
100
80
60
40
20
0
Wt
Mct8 ko
Oatp1c1 ko
M/O dko
B)
MYOD + only
% of cells per cluster
40
30
20
10
0
Wt
Mct8 ko
Oatp1c1 ko
M/O dko
C)
total PAX7 +
% of cells per cluster
70
60
50
40
30
20
10
0
Wt + DMSO
Wt + Silychristin
Oatp1c1 ko + DMSO
Oatp1c1 ko + Silychristin
D)
MYOD + only
% of cells per cluster
40
30
20
10
0
Wt + DMSO
Wt + Silychristin
Oatp1c1 ko + DMSO
Oatp1c1 ko + Silychristin
E)
MYOD + only
number of only MYOD positive cells/mm²
2000
1500
1000
500
0
Wt
Mct8 ko
Oatp1c1 ko
M/O dko
5 dpi
10 dpi
21 dpi

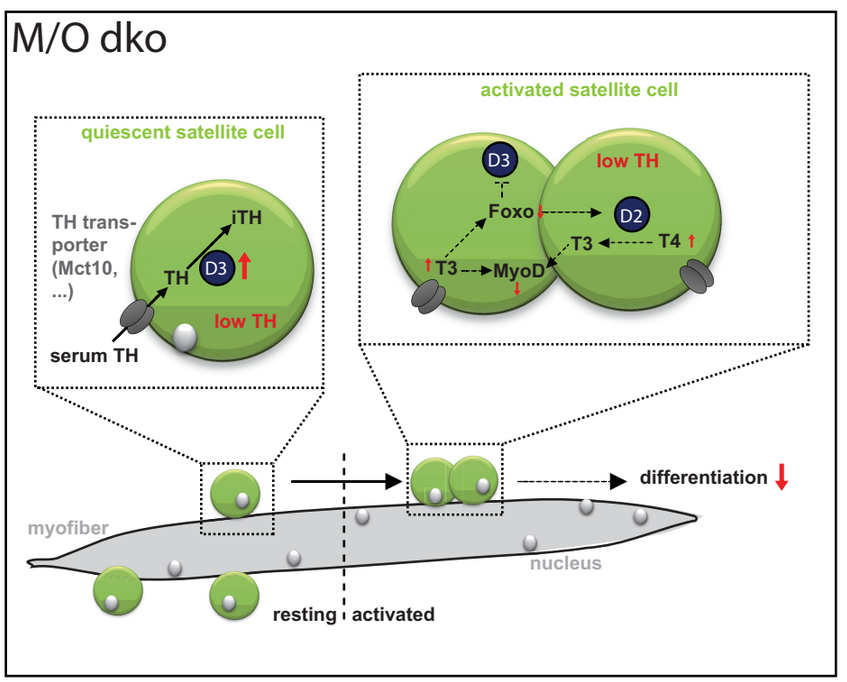

A)
fl for
fl rev
fl del rev
+ - allele
exon 2
exon 3
exon 4
exon 5
fl for
fl rev
fl del rev
loxP
loxP
fl - allele
fl for
fl del rev
Δ - allele


B)
brain
skeletal muscle
Mct8
control
control
M/O-SC ko
M/O-SC ko
control
control
M/O-SC ko
M/O-SC ko
+/+
fl/fl
1000 bp
500 bp
brain
skeletal muscle
Oatp1c1
control
control
M/O-SC ko
M/O-SC ko
control
control
M/O-SC ko
M/O-SC ko
Δ/Δ
fl/fl
1000 bp
500 bp


C)
tamoxifen efficiency
% TH transporter positive cluster
150
100
50
0
*
control
M/O-SC ko
D)
Serum T4
nmol/l
70
60
50
40
30
20
10
0
control
M/O-SC ko
Serum T3
nmol/l
1.5
1.0
0.5
0
control
M/O-SC ko


E)
PAX7 + only
number of positive cells/mm²
100
75
50
25
0
control
M/O-SC ko
uninjured
10 dpi
F)
MYOD + only (10 dpi)
number positive cells/mm²
200
150
100
50
0
control
M/O-SC ko


Wt
activated satellite cell
quiescent satellite cell
D3
high TH
Foxo
D2
T3
T4
Oatp1c1
T3
MyoD
Mct8
TH trans-
porter
(Mct8,
Mct10,
...)
iTH
TH
D3
low TH
serum TH
differentiation
myofiber
nucleus
resting
activated


M/O dko
activated satellite cell
quiescent satellite cell
D3
low TH
Foxo
D2
T3
T4
T3
MyoD
TH trans-
porter
(Mct10,
...)
iTH
TH
D3
low TH
serum TH
differentiation
myofiber
nucleus
resting
activated

Suppl. Fig.1: Muscle stem cell activation *in vitro* and *in vivo.*

Wt, *Mct8* and/or *Oatp1c1* deficient primary EDL myofibers (n=3) were cultured for 72 h, incubated with antibodies directed against PAX7 and the activation and differentiation marker MYOD, and marker positive cells were quantified. No differences in the percentage of PAX7 positive nuclei (A) nor in the percentage of only MYOD positive myoblasts per cluster (B) were observed in TH transporter ko mice. Similarly, incubation of Wt (n=4) and *Oatp1c1* ko (n=6) EDL fibers with DMSO alone or Silychristin (25 µM) for 72 h followed by staining for PAX7 and MYOD did not generate effects on the overall percentage of PAX7 positive nuclei per cluster (C) nor the abundance of only MYOD positive myoblasts (D). (E) 5, 10, and 21 days after CTX injury the number of only MYOD immunopositive myoblasts was quantified in Wt, *Mct8* ko, *Oatp1c1* ko, and *M/O* dko animals, but did not reveal significant alterations between the genotypes. Group mean+SEM are shown. n = 4 Wt 5 dpi, *M/O* dko 5 dpi; n=3 all other groups. 2-way ANOVA and Bonferroni-Holm post hoc testing.

Suppl. Fig.2: Generation and analysis of SC-specific *M/O* deficient (*M/O*-SC ko) mice.

(A) Schematic illustration of the targeting strategy to generate conditional Mct8 mutant mice by flanking exon 3 of the murine *Mct8* gene by loxP sites (fl-allele; dark grey arrowheads). Cre-mediated excision of exon 3 results in a frameshift mutation from exon 4 to 5 (Δ-allele; Δ-allele dashed boxes). Black arrows indicate binding sites for genotyping primers. (B) Genotyping PCRs were conducted on brain and skeletal muscle (gastrocnemius) biopsies for *Mct8* (left panel) and *Oatp1c1* (right panel). In brain samples of tamoxifen-induced control and *M/O*-SC ko mice, only PCR products specific for the respective *Mct8* and *Oatp1c1* fl-allele could be detected. Likewise, PCR products depicting the respective *Mct8* and *Oatp1c1* delta allele were absent

from control skeletal muscle samples but could be detected in skeletal muscle of *M/O*-SC ko mice (white boxes). Tail biopsies of *Mct8* +/+ and *Mct8* fl/fl mice as well as *Oatp1c1* Δ/Δ and fl/fl animals served as controls. (C) EDL cultures prepared from control and *M/O*-SC ko mice (n=3) were immunostained for PAX7, MCT8 and OATP1C1 72 h after isolation. Only 29.7% of all clusters contained PAX7 positive cells that were also immunopositive for either MCT8 or OATP1C1 indicating that tamoxifen treatment was efficient in activating both TH transporters in about 70% of all satellite cells. (D) Circulating T4 and T3 concentrations do not differ between tamoxifen-treated control and *M/O*-SC ko animals (n=9). (E) PAX7 and MYOD immunopositive cells were counted in cryosections of CTX injured TA muscle 10 dpi and the respective contralateral uninjured muscles. Quantification of PAX7 positive only satellite cells (E) and MYOD only positive myoblasts (F) revealed no differences (n=4). Group mean+SEM are shown. * $p < 0.05$. unpaired 2-tailed Student's *t* test.

Suppl. Fig.3: Model of TH transporter-dependent activation of SCs.

SCs (green) located adjacent to mature muscle fibers (grey) express basal amounts of TH transporters such as MCT8 or MCT10 (in dark grey) in order to sense circulating T3 and T4 concentrations in the resting state. In quiescent SCs, T3 and T4 are inactivated (iTH) and TH levels are kept low by D3 activity. Following activation of muscle stem cells due to various stimuli (e.g. injury), MCT8 (orange) levels rise while OATP1C1 (blue) becomes transiently expressed thereby consequently accelerating uptake of T3 and T4 into activated SCs. In a T3-induced feed-forward loop FOXO transcription factors inhibit D3 action while D2 is induced in turn generating supraphysiological T3 concentrations in activated SCs (Dentice et al., 2010). Increased cellular TH content is essential for timely differentiation and progression within the myogenic program by inducing expression of the master regulator of myogenesis, *MyoD*. In *M/O* dko mice, SC might still sense circulating TH via MCT10. However, upon activation, major TH uptake routes are blocked most

likely decreasing the efficiency of the feed-forward loop thereby delaying differentiation programs and hence muscle regeneration.

Supplementary experimental procedures:

qPCR

Total tissue RNA was isolated using the NucleoSpin® RNA II Kit (Macherey-Nagel). Synthesis of cDNA was performed using the Transcriptor High Fidelity cDNA Synthesis Kit (Roche). To exclude the presence of genomic DNA, one sample without reverse transcriptase was included as well. Quantitative Real-Time PCR (qPCR) was performed using the iQ SYBR Green Supermix (Bio-Rad) and the CFX Connect™ Real-Time PCR Detection System (Bio-Rad). Ten nanograms of cDNA were employed in one qPCR reaction. Three to five samples per genotype were subjected to the analysis in triplicate. As a housekeeping gene for normalization *Glyceraldehyde 3-phosphate dehydrogenase* (*Gapdh*) was used. The following primers were chosen to generate the PCR fragments: *Gapdh* 5'-ATGCCAGTGAGCTTCCCGTC-3' and 5'-CATCACCATCTTCCAGGAGC-3'; Hr 5'-AAGCTAAATAGGGGATCCTG-3' and 5'- ATTTGTAGAACGGACCACAC-3'; MHCI 5'-AGTCCCAGGTCAACAAGCTG-3' and 5'-TTCCACCTAAAGGGCTGTTG-3'; *MhcIIa* 5'-AGTCCCAGGTCAACAAGCTG-3' and 5'-GCATGACCAAAGGTTTCACA-3'; *MhcIIb* 5'-AGTCCCAGGTCAACAAGCTG-3' and 5'-TTTCTCCTGTCACCTCTCAACA-3'; *MhcIIx* 5'-AGTCCCAGGTCAACAAGCTG-3' and 5'-CCTCCTGTGCTTTCCTTCAG-3'; *MyoD* 5'-CTACAGTGGCGACTCAGAT-3' and 5'-CACTGTAGTAGGCGGTGTC-3';. The annealing temperature was 55°C for all primer pairs.