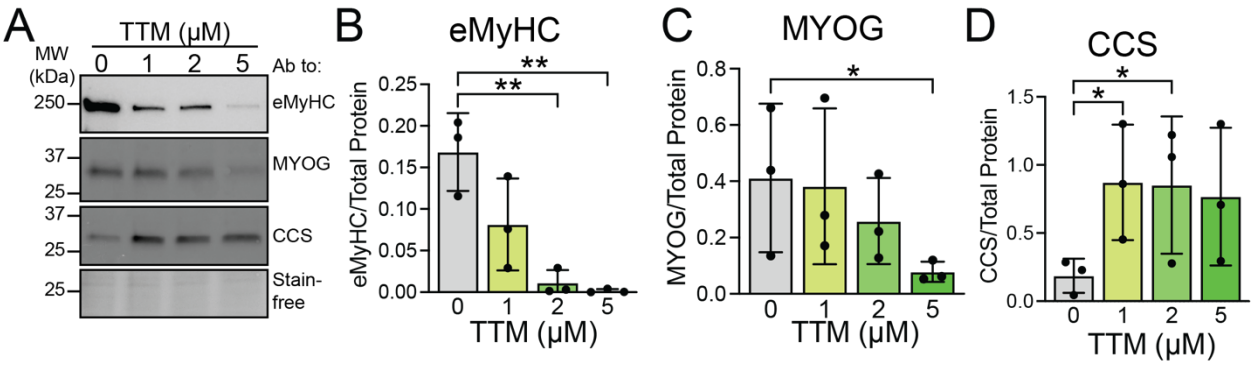
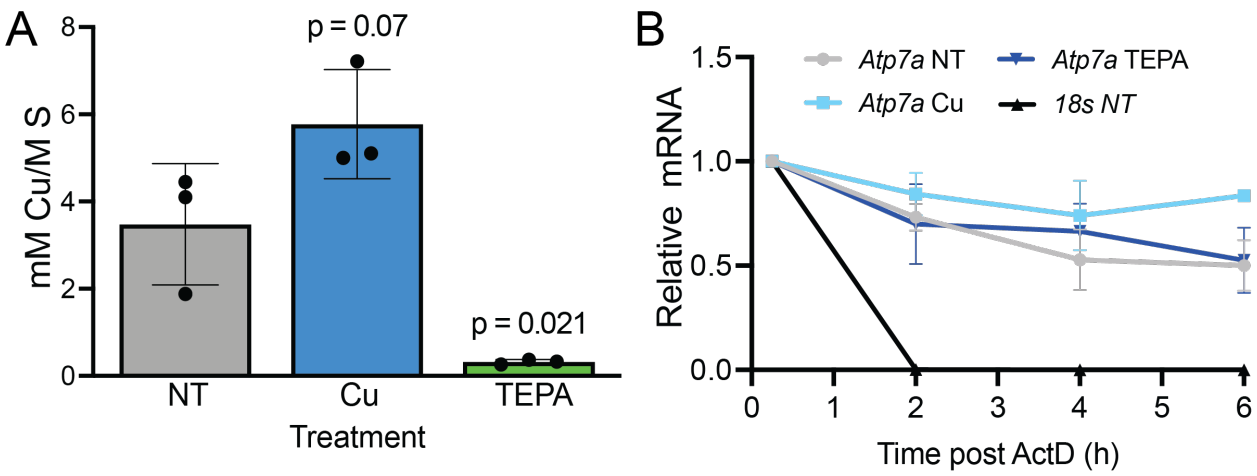
Figure S1



Supplementary Figure 1: TTM impairs C2C12 myoblast differentiation. ***A)*** Immunoblot of lysates from TTM-treated C2C12 myotubes probed with antibodies to eMyHC, myogenin (MYOG), and the Cu chaperone for SOD1 (CCS). Total protein is shown using Stain-free gel imaging technology. Quantification for eMyHC ***(B)***, MYOG ***(C)***, and CCS ***(D)*** as measured by densitometry and normalized to total protein showing significant decreases in MYOG and eMyHC and a significant increase in CCS with TTM treatment. Shown is the mean ± standard deviation for n = 3-4 experiments. For all experiments, statistical significance was determined using one-way ANOVA (* $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$).

Figure S2



Supplementary Figure 2: Extended growth in TEPA decreases intracellular Cu and does not affect *Atp7a* RNA stability. ***A)*** Levels of Cu (Cu) normalized to sulfur (S) from C2C12 in myoblasts (MB) in normal growth medium (NT) or grown in medium containing 20 µM $CuSO_4$ (Cu) or 20 µM TEPA (TEPA) for four days. Values were measured using ICP-OES. ***B)*** Actinomycin D assay showing that *Atp7a* mRNA is stable in C2C12 myoblasts are grown in the presence of Cu but unstable in normal growth medium or TEPA-treated medium. Shown is mean ± standard deviation for n = 3 experiments. Statistical significance was determined by one-way ANOVA.

Figure S3

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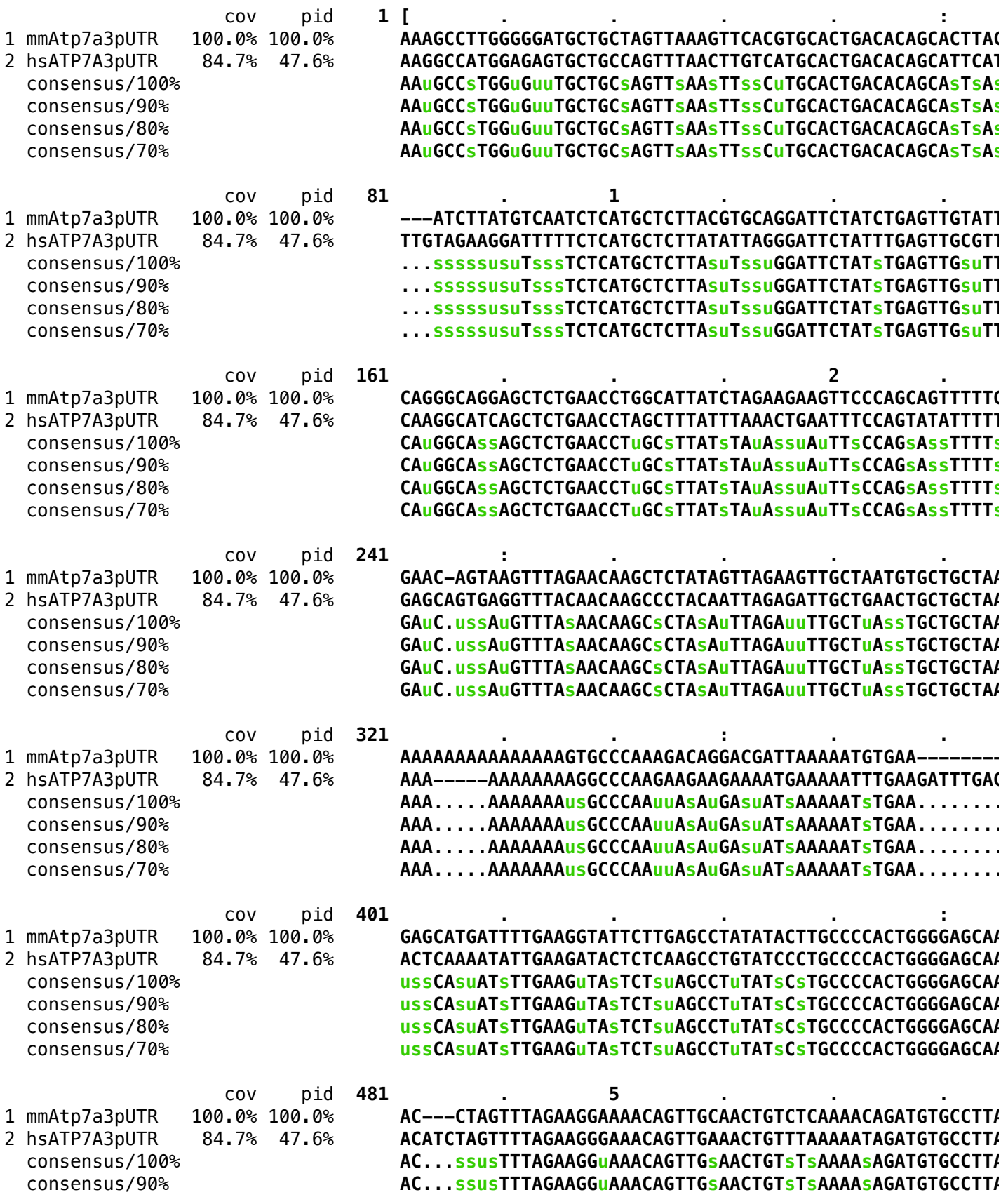

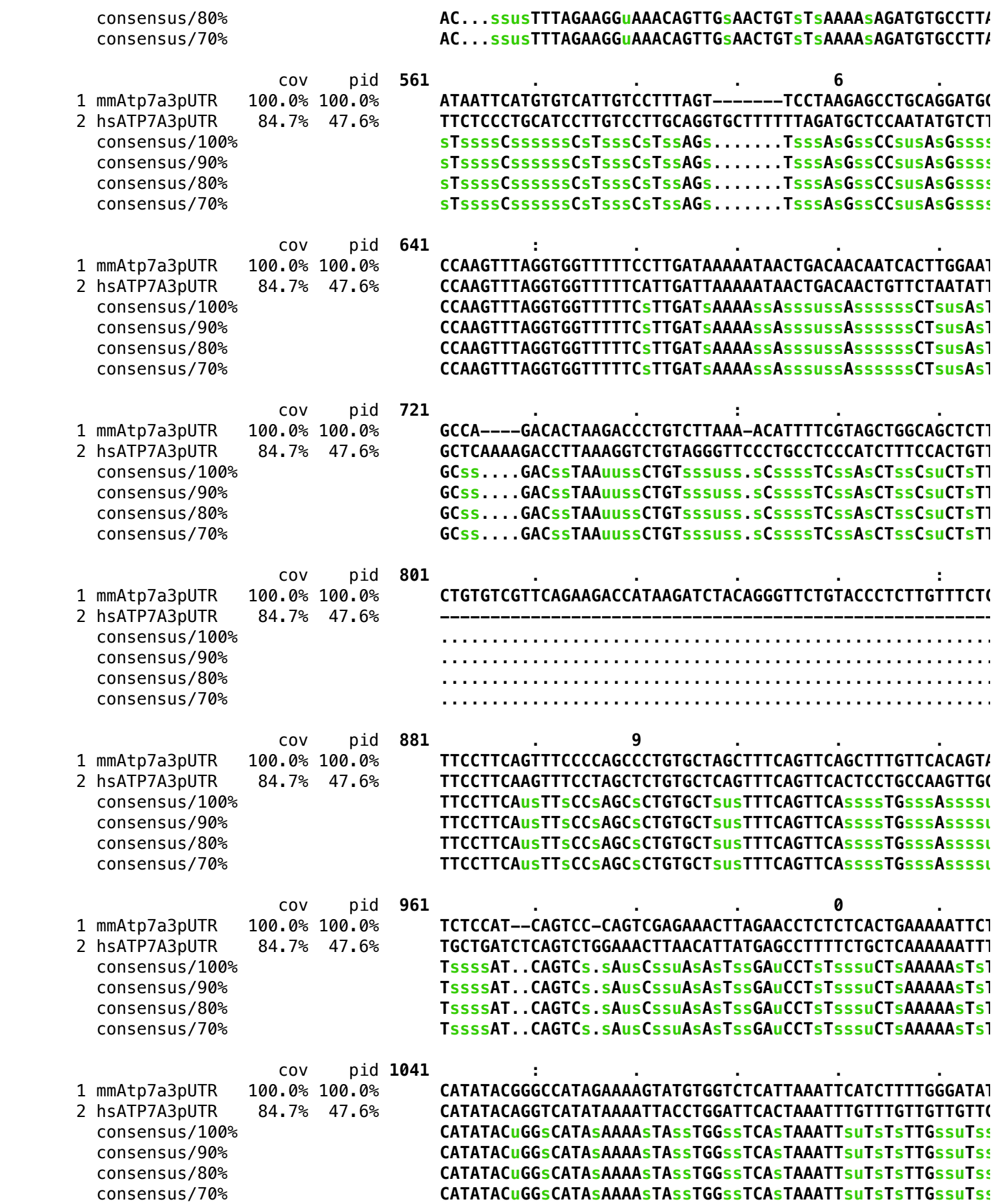
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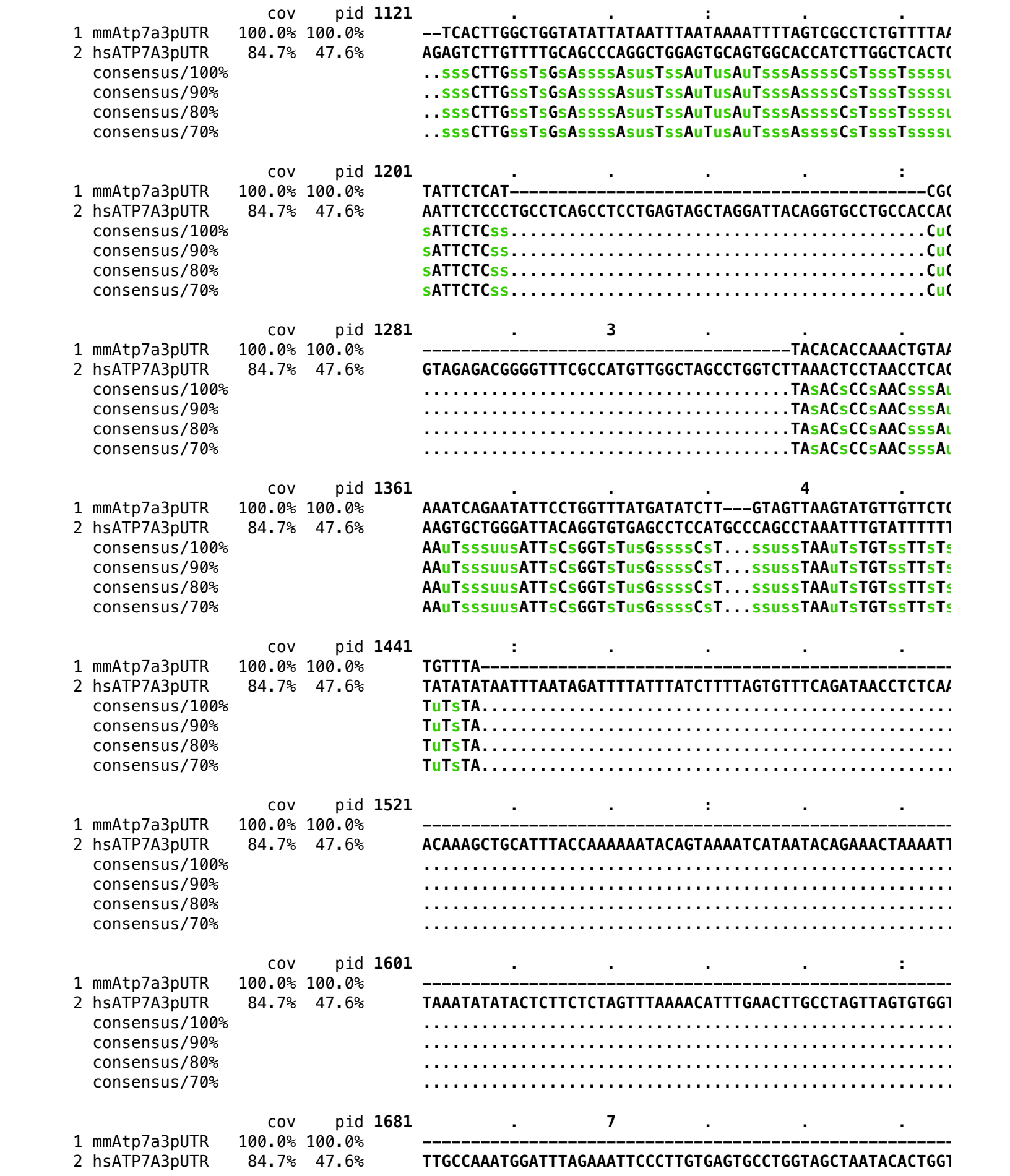
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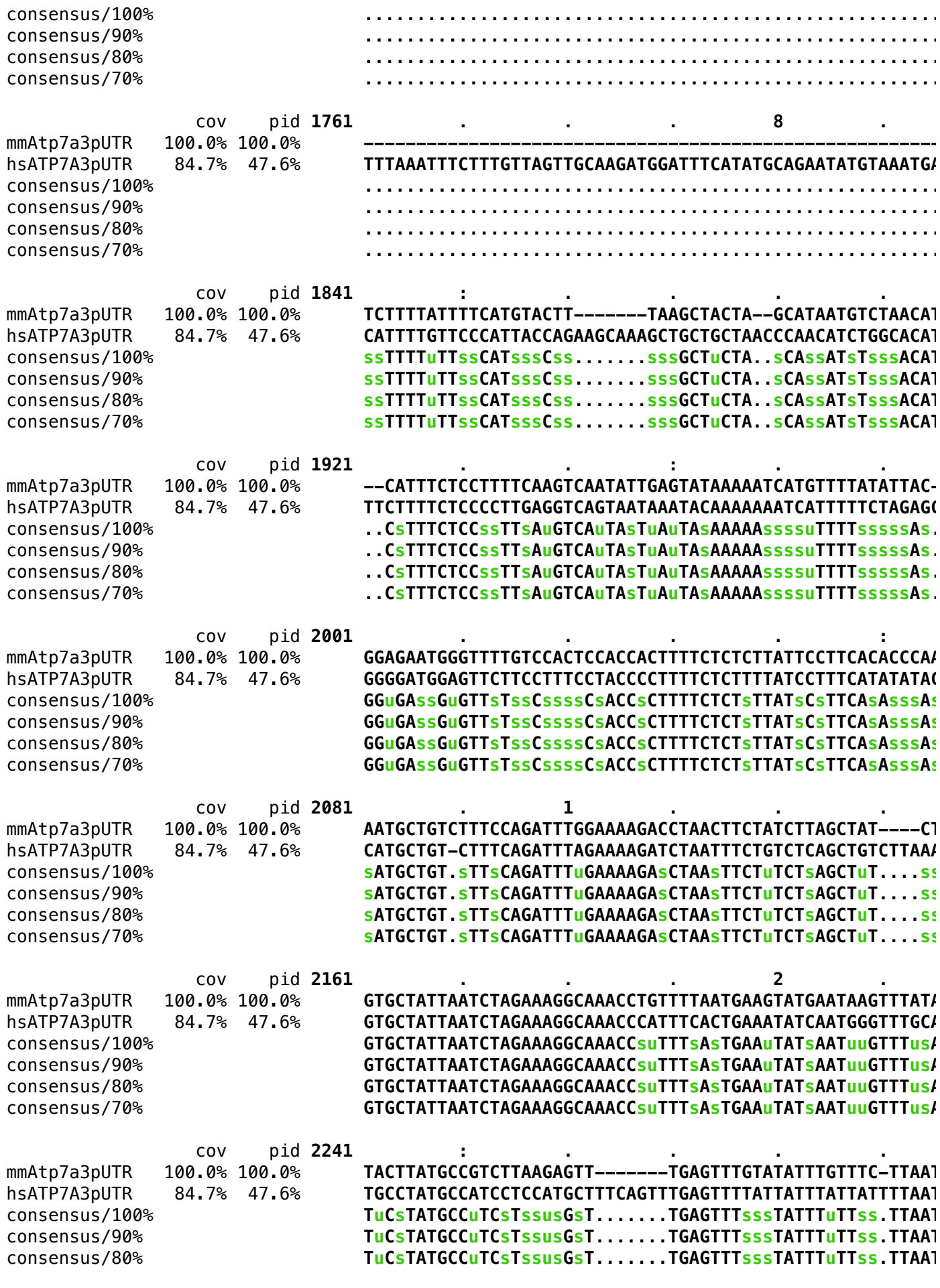
cov pid 1681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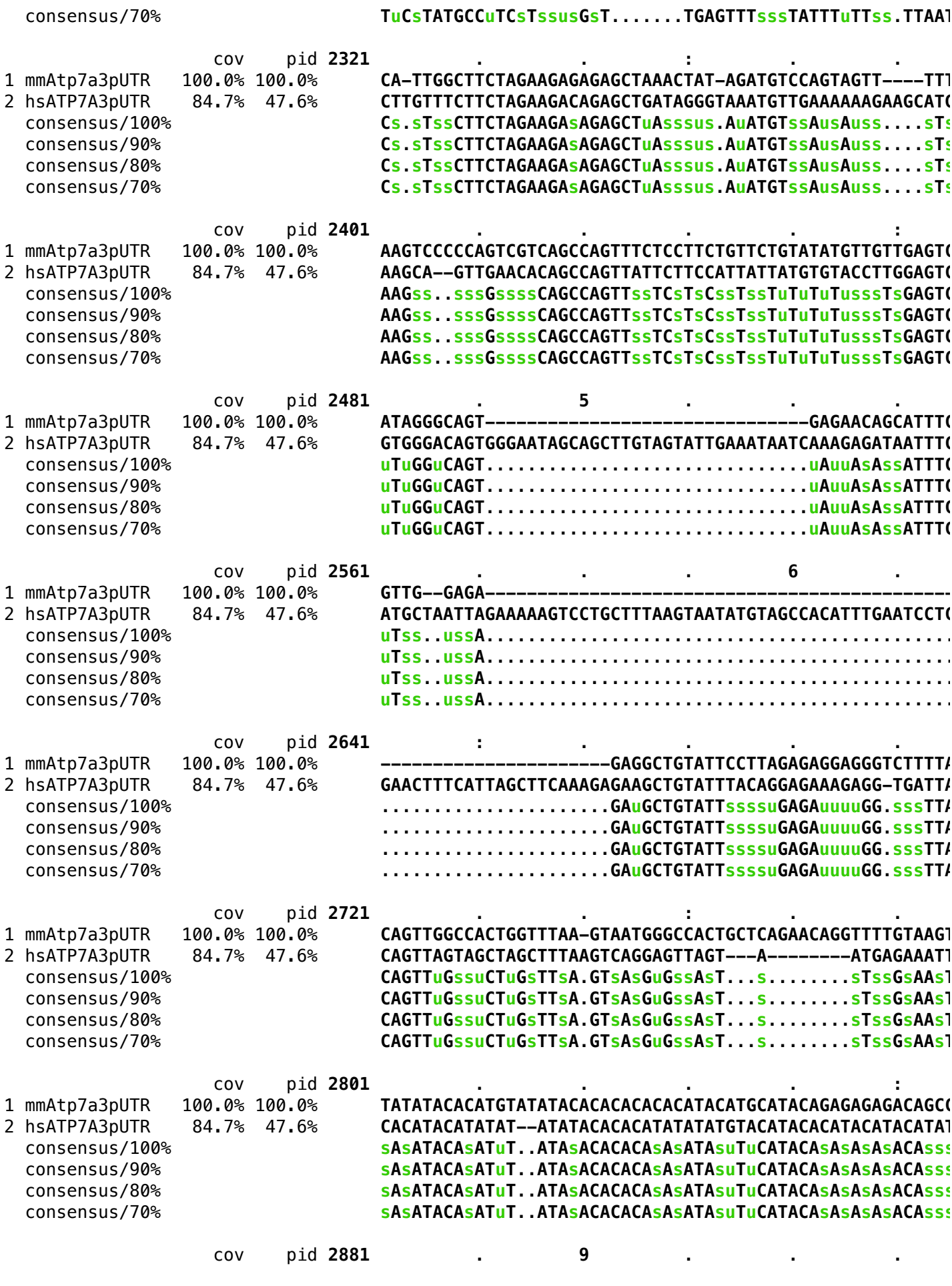
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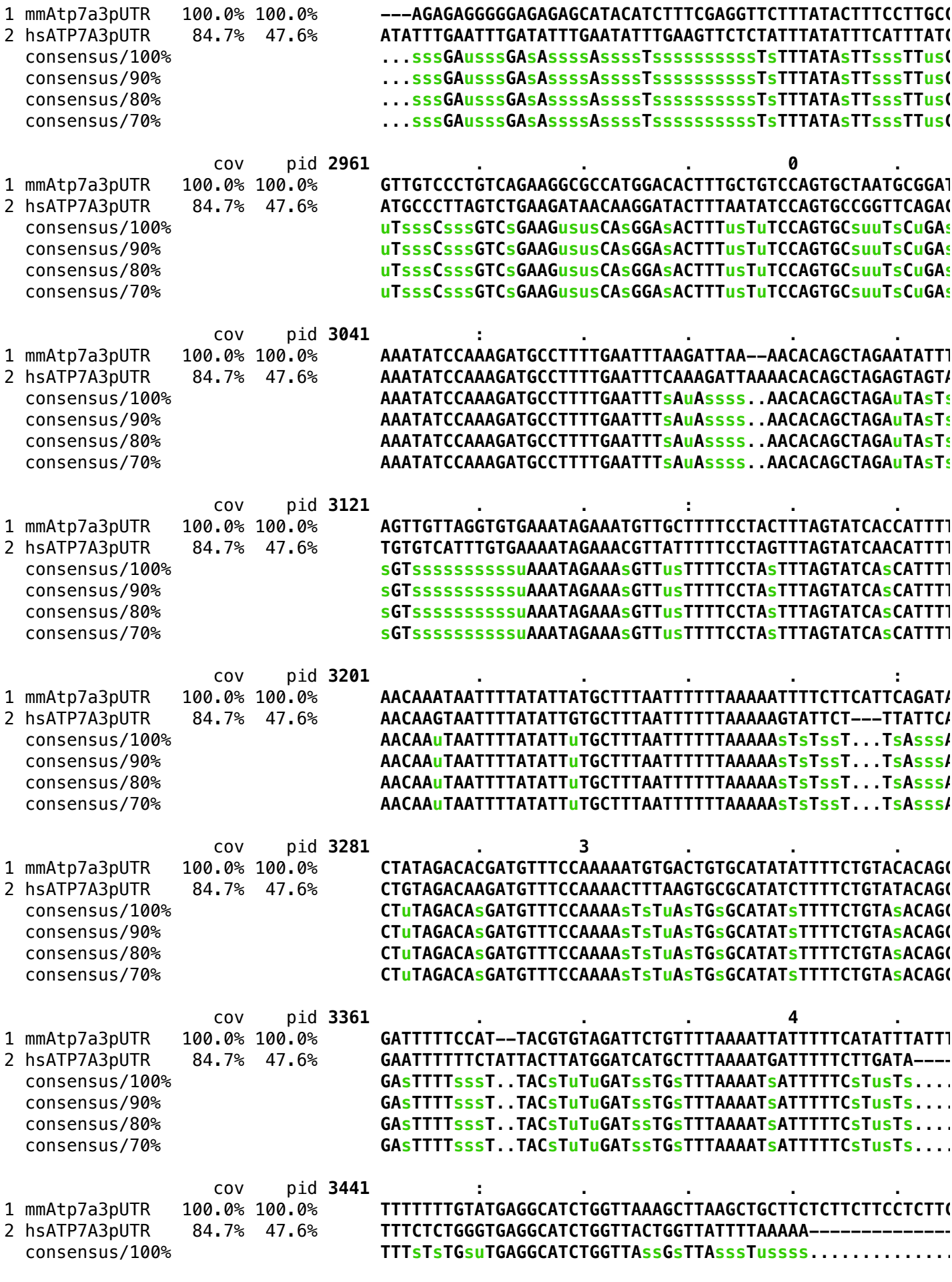
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cov pid 3041
cov pid 3121
cov pid 3201
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cov pid 3441

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  consensus/80%                      ....................................................
  consensus/70%                      ....................................................

                     cov    pid 4321          .         .         :         .      ] 4367
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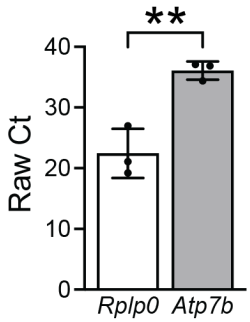
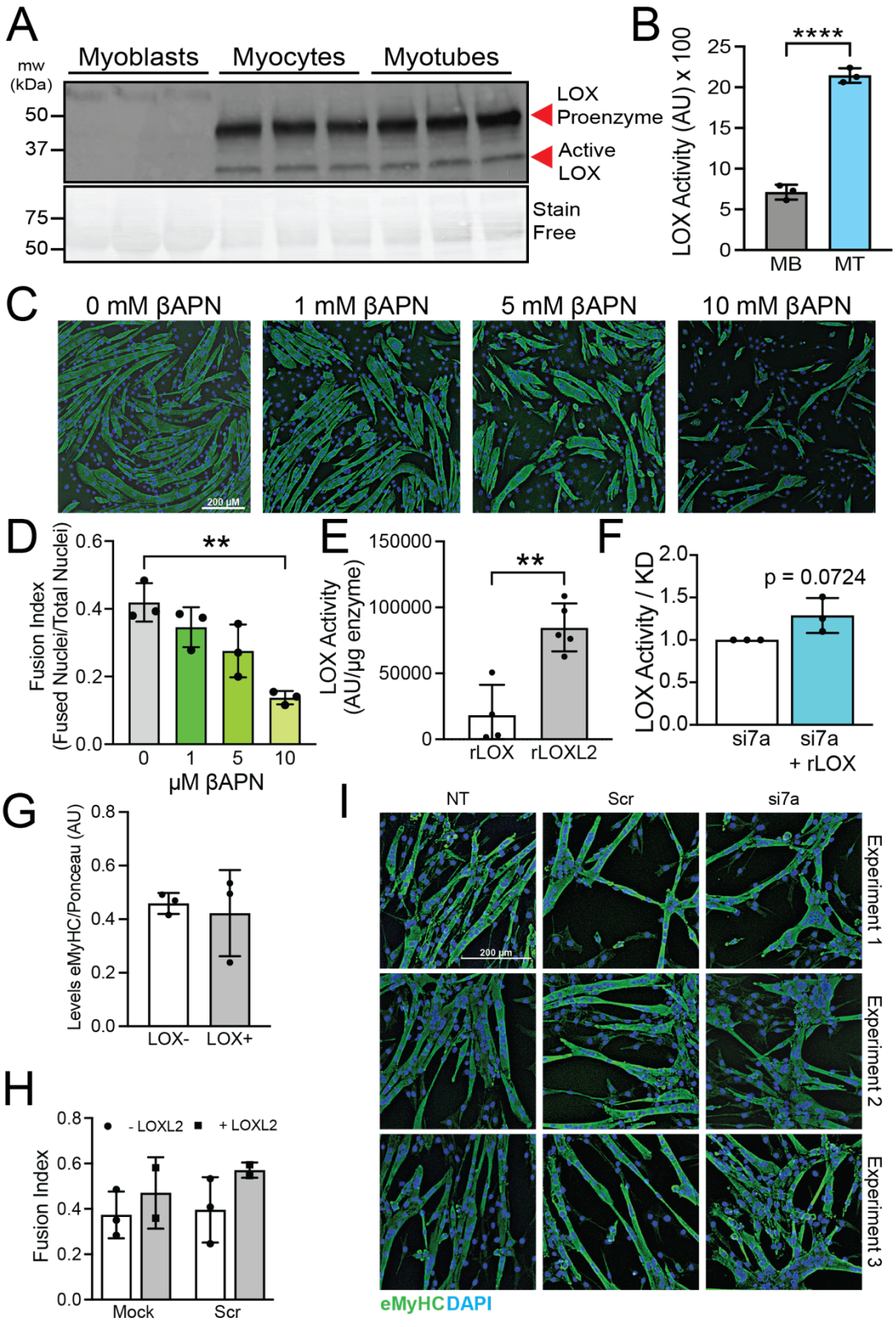
Supplementary Figure 3: Substantial conservation is observed in murine and human *ATP7A* 3' UTRs. Multiple sequence alignment and consensus sequence of murine *Atp7a* 3' UTR (NM_001109757) and human *ATP7A* 3' UTR (NM_001282224) as visualized in MView.

Figure S4



Supplementary Figure 4: *Atp7b* is not detected in myoblasts. Raw Ct values for qRT-PCR using primers to *Rplp0* and *Atp7b* in myoblasts showing Ct values for *Atp7b* at nearly undetectable levels above 30. Shown is mean ± standard deviation for n = 3-4 experiments. Statistical significance was determined by t-test (** $p < 0.01$).

Figure S5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Myocytes
Myotubes
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75
50
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Active LOX
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****
MB
MT
C
0 mM βAPN
1 mM βAPN
5 mM βAPN
10 mM βAPN
200 µM
D
Fusion Index (Fused Nuclei/Total Nuclei)
**
µM βAPN
E
LOX Activity (AU/µg enzyme)
**
rLOX
rLOXL2
F
LOX Activity / KD
p = 0.0724
si7a
si7a + rLOX
G
Levels eMyHC/Ponceau (AU)
LOX-
LOX+
H
- LOXL2
+ LOXL2
Fusion Index
Mock
Scr
I
NT
Scr
si7a
Experiment 1
Experiment 2
Experiment 3
200 µm
eMyHC DAPI

Supplementary Figure 5: LOX activity is required for C2C12 myoblast differentiation. ***A)*** Immunoblot of conditioned medium from C2C12 myoblasts (MB), myocytes (MC), and myotubes (MT) lysates probed with an antibody to LOX and with total protein shown using Stain-free gel technology. The LOX proenzyme is detected at ~45 kDa while process and active LOX is detected at ~30 kDa. ***B)*** LOX activity shown as arbitrary units (AU) x 100 showing increased activity in MT relative to MB. ***C)*** C2C12 myoblasts were induced to differentiate in the presence of 0, 1, 5, or 10 mM of the LOX inhibitor βAPN and stained with an antibody to eMyHC and DAPI to visualize nuclei. ***D)*** Fusion index quantification revealing a significant decrease in myotube formation caused by LOX inhibition with βAPN. ***E)*** In vitro activity of recombinant human LOX (rLOX) and recombinant human LOXL2 (rLOXL2) as AU x 100. ***F)*** Total LOX activity in *Atp7a* knockdown myotubes (si7a) versus knockdown myotubes with added rLOX (si7a + LOX) reported as fold change of knockdown. ***G)*** Immunoblot quantification showing no change in levels of eMyHC in Scr myotubes with or without added LOX. ***H)*** Fusion index showing no change in non-treated or Scr myotubes with or without added LOXL2. ***I)*** Additional representative images of NT, Scr, and si7a myoblasts differentiated on Matrigel showing three independent experiments stained with an antibody to eMyHC with nuclei stained with DAPI. Bar = 200 μm. Shown is the mean ± standard deviation for n = 3 experiments. Statistical significance was determined using t-test or one-way ANOVA (** $p < 0.01$, **** $p < 0.0001$).

Supplementary Table 1: Half-life of *Atp7a* RNA under various conditions

Condition	T(1/2)	p*
MB	5.37	-
MC	7.74	0.0075
MT	8.86	0.16
MB Cu	18.7	0.0005
MB TEPA	6.56	0.66
* Comparison to untreated MB		

Supplementary Table 2: Predicted protein binding sites in the proximal *ATP7A* 3' UTR

RBP	Prediction	RBS location	Score	PhastCons score	Transcript ID	Context
HuR	Deep bind	chrX:77302350-77302400;+	0.364	0.593	ENST00000343533	UTR3
HuR	Deep bind	chrX:77302650-77302700;+	0.21	0.419	ENST00000343533	UTR3
HNRNPA1	TESS	chrX:77302300-77302350;0	0.198	0.703	ENST00000343533	UTR3
HNRNPH1	TESS	chrX:77302550-77302600;0	0.751	1.638	ENST00000343533	UTR3
HNRNPH1	FIMO	chrX:77304800-77304850;0	0.421	0.563	ENST00000341514	UTR3
HNRNPH1	FIMO	chrX:77302400-77302450;0	0.26	0.788	ENST00000350425	UTR3
HNRNPCL1	TESS, FIMO	chrX:77302350-77302400;0	0.364	0.593	ENST00000343533	UTR3
HNRNPCL1	FIMO	chrX:77302250-77302300;0	0.425	0.801	ENST00000343533	UTR3
RBFOX1	Deep bind	chrX:77302400-77302450;+	0.26	0.788	ENST00000343533	UTR3
PABPC5	TESS, FIMO	chrX:77302400-77302450;0	0.26	0.788	ENST00000343533	UTR3
PTBP1	TESS, FIMO	chrX:77302950-77303000;0	0.119	0.482	ENST00000343533	UTR3
HNRNPC	TESS, FIMO	chrX:77302350-77302400;0	0.364	0.593	ENST00000343533	UTR3
HNRNPC	FIMO	chrX:77302250-77302300;0	0.425	0.801	ENST00000343533	UTR3
HNRNPC	FIMO	chrX:77302150-77302200;0	0.113	0.488	ENST00000343533	UTR3
PABPC4	TESS, FIMO	chrX:77302350-77302400;0	0.364	0.593	ENST00000343533	UTR3
QKI	TESS, FIMO	chrX:77302250-77302300;0	0.425	0.801	ENST00000343533	UTR3
YBX2	TESS	chrX:77302250-77302300;0	0.425	0.801	ENST00000343533	UTR3
HNRNPA1L2	TESS, FIMO	chrX:77302150-77302200;0	0.113	0.488	ENST00000343533	UTR3
ESRP2	FIMO	chrX:77302500-77302550;0	0.056	0.456	ENST00000343533	UTR3

Supplementary Table 3: Primary antibodies used in this study

Antibody	Manufacturer information	Experiment	Dilution
eMyHC	DSHB F1.652 hybridoma supernatant	Stain	1:10
eMyHC	DSHB F1.652 bioreator supernatant	Blot	1:10,000
MYOG	DSHB FD5 hybridoma supernatant	Blot	1:100
CCS	Santa Cruz (H-7) sc-55561	Blot	1:1000
ATP7A	Wang, et. al *Plos One*, 2012	Blot	1:3000
ATOX1	Proteintech 226411-1-AP	Blot	1:1000
LOX	Novus Biologicals #NB1002527SS	Blot	1:1000

Supplementary Table 4: Primers used in this study

Primer name	Sequence
Atp7a F	*CCAAGGGTGTGACTGGTGTT*
Atp7a R	*GGCACTCACCACAGATGGAA*
Myc F	*CGCGATCAGCTCTCCTGAAA*
Myc R	*AAGTTCACGTTGAGGGGCAT*
Atp7b F	*CCCAGGAAGAACTTGGCGTC*
Atp7b R	*TTTGGCTGTGACCTGTCTCTC*
Myh3 F	*TCTCTGTCACAGTCAGAGGTGT*
Myh3 R	*TTTTCCGACTTGCGGAGGAA*
Myog F	*CACTGGAGTTCGGTCCCAA*
Myog R	*TGTGGGCGTCTGTAGGGTC*
Acta1 F	*GCGACCTCACTGACTACCTG*
Acta1 R	*GATGTCGCGCACAATCTCAC*