Supplemental Data

TBL1 and TBLR1 Phosphorylation on Regulated Gene

Promoters Overcomes Dual CtBP and NCoR/SMRT

Transcriptional Repression Checkpoints

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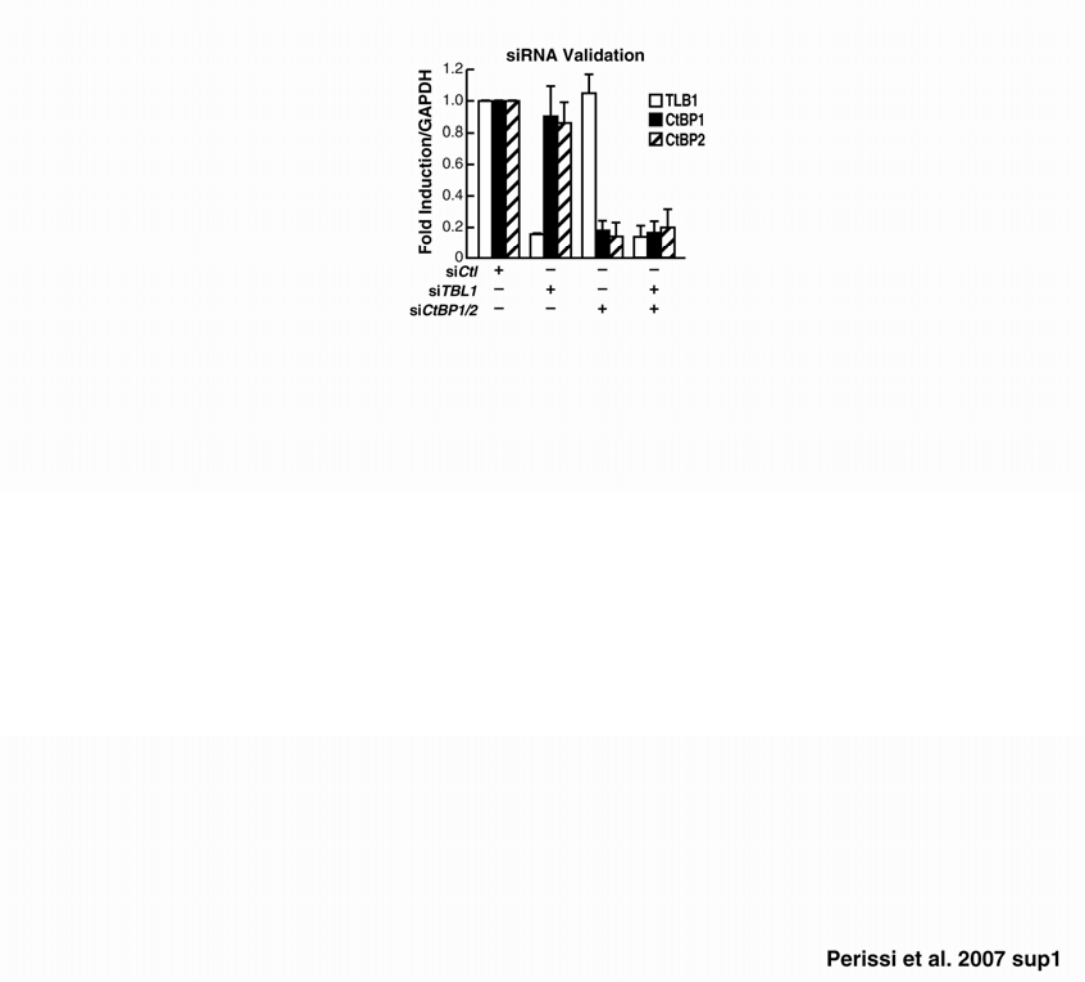
Figure S1. siRNA Validation from Figure 1D

mRNA expression levels of TBL1, CtBP1 and CtBP2 are measured by RT-PCR to validate siRNA efficiency. Normalization is done using the house-keeping gene GAPDH.
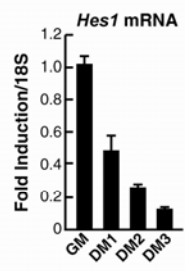
Hes1 mRNA

Fold Induction/18S

GM DM1 DM2 DM3

Figure S2. *Hes1* Gene Expression in C2C12 Cells

RT-PCR analysis of *Hes1* expression in C2C12 cells at different days after induction of skeletal muscle differentiation.
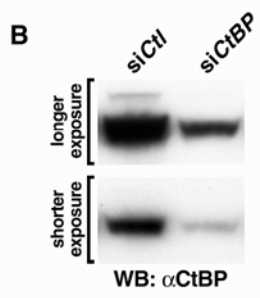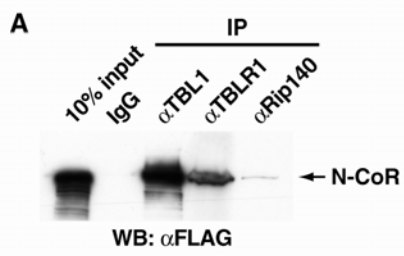
A

10% input

IgG

IP

αTBL1

αTBLR1

αRip140

← N-CoR

WB: αFLAG

B

siCtl

siCtBP

longer exposure

shorter exposure

WB: αCtBP

Figure S3. TBL1 Interacts with CtBP and Mediates Its Degradation

(A) Immunoprecipitation of transiently transfected Flag-NCoR by α-TBLR1 and α-TBLR1 confirming that both antibodies are equally efficient in immunoprecipitating a known interacting protein.

(B) Western blot α–CtBP in transiently transfected U2OS cells confirming the specificity of the bands identified by the antibody.

M-TBLR1: — + — — —
M-TBLR1mut (Δ2): — — + — —
M-TBLR1mut (Δ5): — — — + —
M-TBLR1mut (Δ2,Δ5): — — — — +

10% input — —MycTBLR1
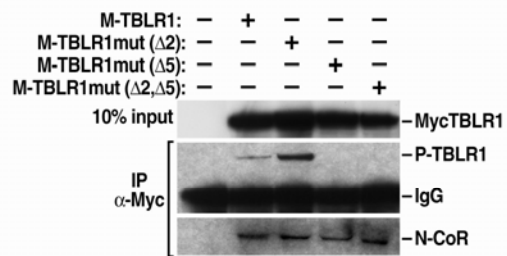
IP
α-Myc

—P-TBLR1

—IgG

—N-CoR

Figure S4. Validation of Mono-P-TBLR1 Antibody

Immunoprecipitation of transiently transfected Flag-TBLR1 (F-TBLR1) or Myc-TBLR1 (M-TBLR1) wildtype and mutated at the two phosphorylation sites ("5" corresponds to Ser123 and "2" corresponds to Ser199/Thr203/Ser204) confirmed site specificity of the α-MonoP-TBLR1.
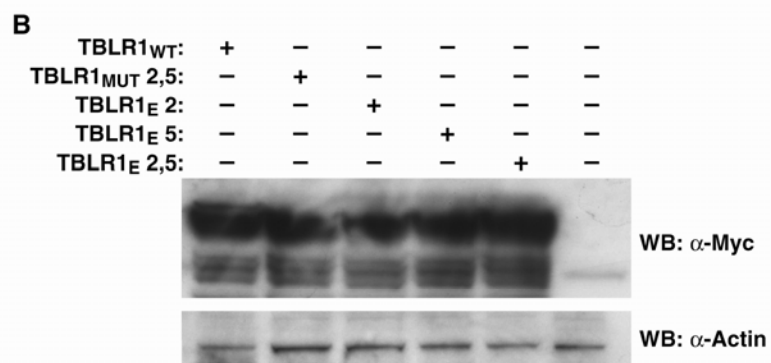
A

TBL1$_{WT}$:	−	+	−	−
TBL1$_{MUT}$ 1,3,4:	+	−	−	−
TBL1→TBLR1:	−	−	+	−

WB: α-FLAG

WB: α-Actin

B

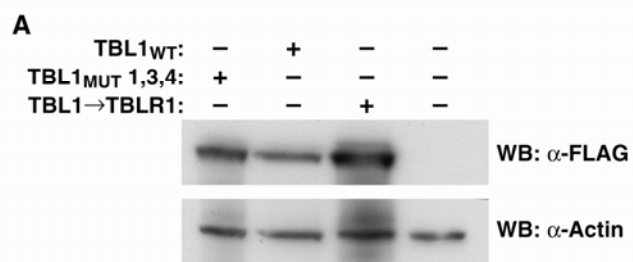|						
TBLR1$_{WT}$:	+	−	−	−	−	−
TBLR1$_{MUT}$ 2,5:	−	+	−	−	−	−
TBLR1$_E$ 2:	−	−	+	−	−	−
TBLR1$_E$ 5:	−	−	−	+	−	−
TBLR1$_E$ 2,5:	−	−	−	−	+	−

WB: α-Myc

WB: α-Actin

Figure S5. Validation of the Expression Plasmids for TBL1 and TBLR1 Wild-Type and Mutants

(**A**) Western blot α-FLAG for TBL1 wild type and mutant expression plasmids.

(**B**) Western blot α-Myc for TBLR1 wild type and mutant expression plasmids.

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CLUSTAL W (1.83) multiple sequence alignment


hTBL1R    MSISSDEVNFLVYRYLQESGFSHSAFTFGIESHISQSNINGALVPPAALISIIQKGLQYV 60
mTBL1R    MSISSDEVNFLVYRYLQESGFSHSAFTFGIESHISQSNINGALVPPAALISIIQKGLQYV 60
hTBL1X    MSITSDEVNFLVYRYLQESGFSHSAFTFGIESHISQSNINGTLVPPAALISILQKGLQYV 60
mTBL1X    MSITSDEVNFLVYRYLQESGFSHSAFTFGIESHISQSNINGTLVPPAALISILQKGLQYV 60
          ***:*************************************:**********:*******


hTBL1R    EAEVSINEDGTLFDGRPIESLSLIDAVMPDVVQTRQQAYRDKLAQQQAAAAAAAAAAA-- 118
mTBL1R    EAEVSINEDGTLFDGRPIESLSLIDAVMPDVVQTRQQAYRDKLAQQHAAAAAAAAAAT-- 118
hTBL1X    EAEISINEDGTVFDGRPIESLSLIDAVMPDVVQTRQQAFREKLAQQQASAAAAAAAATAA 120
mTBL1X    EAEISINEDGTVFDGRPIESLSLIDAVMPDVVQTRQQAFREKLAQQQANAAAAAAAAAAT 120
          ***:*******:**********************:*:****:* ********:

                              5-PKC                          1-CK1
hTBL1R    ------------SQQGSAKNGENTANGEENGAHTIANNHTDMMEVDGDVEIPPNKAVVLR 166
mTBL1R    ------------NQQGSAKNGENTANGEENGAHTIANNHTDMMEVDGDVEIPSNKAVVLR 166
hTBL1X    AT-AATTTSAGVSHQNPSKNREATVNGEENRAHSVNN-HAKPMEIDGEVEIPSSKATVLR 178
mTBL1X    ATSTAATTPAAAQQNPPKNGEATVNGEENGAHAINN-HSKPMEIDGEVEIPESKATVLR 179
          :*...** * *.***** **:: * *:. **:**:****.:**.***

                                       2-CK1/GSK3
hTBL1R    GHESEVFICAWNPVSDLLASGSGDSTARIWNLSENSTSGSTQLVLRHCIREGGQDVPSNK 226
mTBL1R    GHESEVFICAWNPVSDLLASGSGDSTARIWNLSENSTSGPTQLVLRHCIREGGQDVPSNK 226
hTBL1X    GHESEVFICAWNPVSDLLASGSGDSTARIWNLNENSNGGSTQLVLRHCIREGGHDVPSNK 238
mTBL1X    GHESEVFICAWNPVSDLLASGSGDSTARIWNLNENSNGGSTQLVLRHCIREGGHDVPSNK 239
          *******************************.***..*.*************:******


hTBL1R    DVTSLDWNSEGTLLATGSYDGFARIWTKDGNLASTLGQHKGPIFALKWNKKGNFILSAGV 286
mTBL1R    DVTSLDWNSEGTLLATGSYDGFARIWTKDGNLASTLGQHKGPIFALKWNKKGNFILSAGV 286
hTBL1X    DVTSLDWNTNGTLLATGSYDGFARIWTEDGNLASTLGQHKGPIFALKWNRKGNYILSAGV 298
mTBL1X    DVTSLDWNSDGTLLATGSYDGFARIWTEDGNLASTLGQHKGPIFALKWNKKGNYILSAGV 299
          ********:.:*****************:*********************:***:******

                       3-CK1
hTBL1R    DKTTIIWDAHTGEAKQQFPFHSAPALDVDWQSNNTFASCSTDMCIHVCKLGQDRPIKTFQ 346
mTBL1R    DKTTIIWDAHTGEAKQQFPFHSAPALDVDWQSNNTFASCSTDMCIHVCKLGQDRPIKTFQ 346
hTBL1X    DKTTIIWDAHTGEAKQQFPFHSAPALDVDWQNNTTFASCSTDMCIHVCRLGCDRPVKTFQ 358
mTBL1X    DKTTIIWDAHTGEAKQQFPFHSAPALDVDWQNNTTFASCSTDMCIHVCRLGCDRPVKTFQ 359
          ******************************.*.***************:** ***:****


hTBL1R    GHTNEVNAIKWDPTGNLLASCSDDMTLKIWSMKQDNCVHDLQAHNKEIYTIKWSPTGPGT 406
mTBL1R    GHTNEVNAIKWDPTGNLLASCSDDMTLKIWSMKQDNCVHDLQAHNKEIYTIKWSPTGPGT 406
hTBL1X    GHTNEVNAIKWDPSGMLLASCSDDMTLKIWSMKQEVCIHDLQAHNKEIYTIKWSPTGPAT 418
mTBL1X    GHTNEVNAIKWDPSGMLLASCSDDMTLKIWSMKQDACVHDLQAHSKEIYTIKWSPTGPAT 419
          *************:* *******************: *:****** *************.*

          4-CK1/GSK3
hTBL1R    NNPNANLMLASASFDSTVRLWDVDRGICIHTLTKHQEPVYSVAFSPDGRYLASGSFDKCV 466
mTBL1R    NNPNANLMLASASFDSTVRLWDVDRGICIHTLTKHQEPVYSVAFSPDGRYLASGSFDKCV 466
hTBL1X    SNPNSNIMLASASFDSTVRLWDIERGVCTHTLTKHQEPVYSVAFSPDGKYLASGSFDKCV 478
mTBL1X    SNPNSNIMLASASFDSTVRLWDVERGVCIHTLTKHQEPVYSVAFSPDGKYLASGSFDKCV 479
          .***:*:*****************.:**:* *********************:********


hTBL1R    HIWNTQTGALVHSYRGTGGIFEVCWNAAGDKVGASASDGSVCVLDLRK 514
mTBL1R    HIWNTQTGALVHSYRGTGGIFEVCWNAAGDKVGASASDGSVCVLDLRK 514
hTBL1X    HIWNTQSGNLVHSYRGTGGIFEVCWNARGDKVGASASDGSVCVLDLRK 526
mTBL1X    HIWNTQSGSLVHSYRGTGGIFEVCWNARGDKVGASASDGSVCVLDLRK 527
          ******:* ******************* *******************
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Figure S6. TBL1 and TBLR1 Sequence Alignment

CustalW alignment of TBL1 and TBLR1 murine and human aminoacid sequences. Highlighted in yellow are the F-box and the six WD40 domains, in blue the TBL1-specific phosphorylation sites, in red the TBLR1-specific phosphorylation sites with predicted kinases.