Supporting Information

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SI Text

Because of the complex history of nomenclature regarding Mediator subunits and differences in protein names between mammals and *Drosophila*, it is sometimes thought that mammalian TRAP150 (thyroid hormone receptor-associated protein-150, also known as THRAP3) is the same protein as Mediator subunit 23 (MED23). It should be noted that TRAP150 and MED23 are unrelated proteins encoded by different genes (mouse *Trap150*/*Thrap3* gene ID: 230753 on chr4; *Med23* gene ID: 70208 on chr10) and that TRAP150 is not considered a structural component of mediator (1).

1. Taatjes DJ (2010) The human Mediator complex: A versatile, genome-wide regulator of transcription. *Trends Biochem Sci* 35(6):315–322.

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Range = chr4: 125863816 -125864974

Thrap3 (*Trap150*) gene, promoter and 5' UTR

Fig. S1. Promoter region of *Trap150* gene (*Thrap3*) includes circadian locomotor output cycles kaput (CLOCK)-brain, muscle Arnt-like 1 (BMAL1) circadian clock regulatory sites. Shown are 1,000 nucleotides immediately upstream of the putative transcriptional start site (transcribed sequences shown in capital letters) and 158 nucleotides of the 5′ UTR. Canonical (shaded) and noncanonical (bold) E-box elements are highlighted.

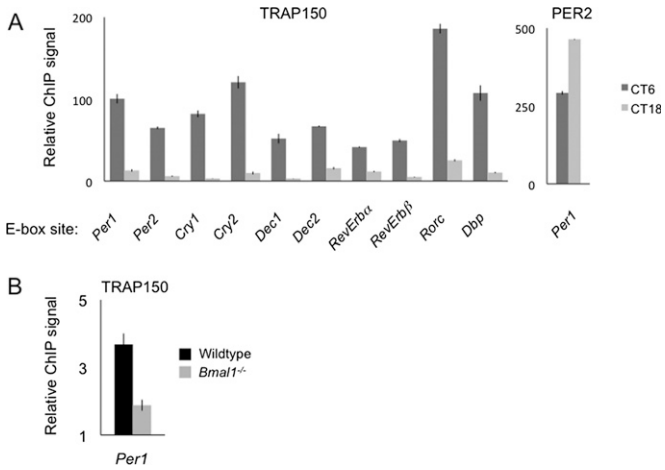

Fig. S2. Association of TRAP150 with E-box sites. (*A*) Circadian rhythm of TRAP150 at E-box sites of multiple CLOCK-BMAL1 circadian target genes. (*Left*) ChIP assays for TRAP150 from mouse liver at circadian time (CT)6 (phase of CLOCK-BMAL1 transcriptional activation) or CT18 (phase of negative-feedback repression) at E-box sites of the genes indicated at bottom. (*Right*) A control ChIP experiment from the same chromatin samples assaying the occupancy of the PERIOD (PER)2 protein at the proximal *Per1* E-box site at CT6 and CT18. The expected profile (roughly opposite to that of TRAP150) was observed. Shown are mean ± SEM of triplicate experiments, representative of three independent experiments. The signal was normalized to a parallel IgG control ChIP. (*B*) Shown are ChIP assays for TRAP150 at the *Per1* E-box site from mouse liver (CT4) from wild-type and *Bmal1*$^{-/-}$ littermates. Shown are mean ± SEM of triplicate experiments, representative of two independent experiments. Signal was normalized to a parallel IgG control ChIP.

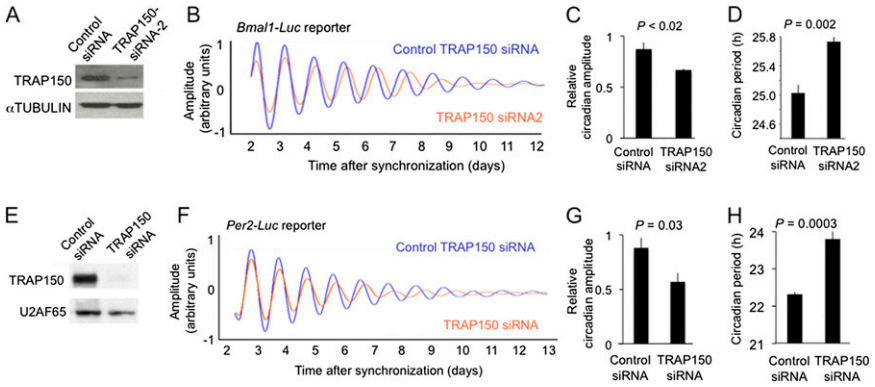

Fig. S3. Depletion of TRAP150 from mammalian cells decreases circadian amplitude and increases circadian period length. (*A–D*) Human *Bmal1-Luc* U2OS circadian reporter cells. (*A*) Western blot showing the effect of control siRNA or TRAP150 siRNA2 (different siRNA than that for the experiments shown in Fig. 3) on a steady-state level of endogenous TRAP150. α-TUBULIN, loading control. (*B*) Real-time circadian oscillations of bioluminescence in synchronized cells after electroporation of TRAP150 siRNA2 (red) or control siRNA (blue). Representative of three experiments. (*C* and *D*) Group data for circadian amplitudes and period lengths, respectively, after depletion of TRAP150 (mean ± SEM; $n = 3$ for each; *t* test, two-tailed). (*E–H*) Human *Per2-Luc* U2OS circadian reporter, a different cell line than that used for the experiments shown in Fig. 3 or *A–D* above. Data displayed as for *A–D*, above. (*F*) Representative of nine experiments. (*G* and *H*) $n = 9$.

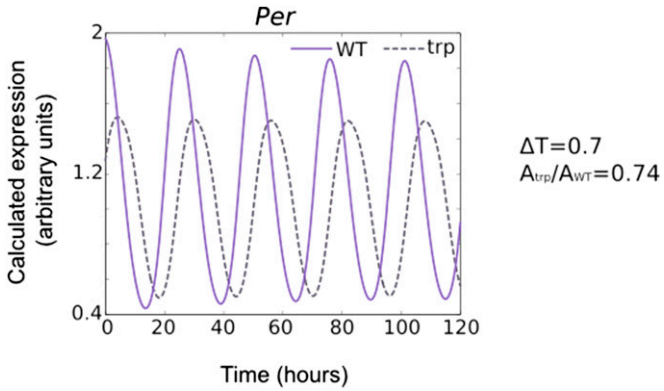

Fig. S4. Modeling of clock properties after simulated decrease in *Per* and Cryptochrome (*Cry*) transcription comparable to that observed following depletion of TRAP150 (Fig. 3*B*). A model of the core mammalian circadian clock (1) was implemented using Matlab R2010a (Mathworks), with a solver for nonstiff systems (ODE45) that implements a Runge–Kutta method. We used a relative and absolute tolerance of $10^{-}(-9)$. The plots represent the last 120 h of a 2,000-h simulation, changing parameters that affect transcription rate of *Per* (40% reduction) and *Cry* (25% reduction). WT, computed wild-type trace; trp, computed trace after TRAP150 depletion; ΔT, increase in calculated period length in hours in trp trace; A, amplitude.

1. Relógio A, et al. (2011) Tuning the mammalian circadian clock: Robust synergy of two loops. *PLOS Comput Biol* 7(12):e1002309.

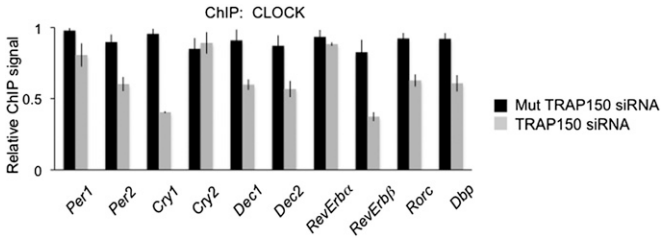

Fig. S5. TRAP150 promotes binding of CLOCK-BMAL1 to E-boxes of CLOCK-BMAL1 circadian target genes. ChIP assays showing the effect of TRAP150 depletion on the occupancy of CLOCK on E-box sites of the indicated genes. Data show the mean ± SEM of triplicate experiment. For each gene, signals were normalized to the highest value among the six measurements (triplicate control and triplicate TRAP150 depletion). Data are representative of three independent experiments. ChIP assays for CLOCK typically had lower signals and higher variability than those for BMAL1 shown in Fig. 4.

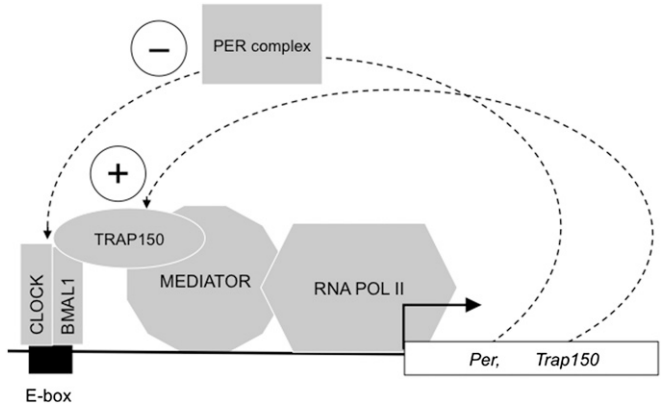

Fig. S6. Model of the role of TRAP150 in the core circadian clock feedback loop. Diagram depicts a CLOCK-BMAL1-TRAP150 complex (at the peak of the TRAP150 circadian oscillation) bound to an E-box site upstream of the *Per* genes and to an E-box site upstream of the *Trap150* gene. Dashed curves: +, positive feedback loop; —, negative feedback loop. Negative feedback action of the PER complex is delayed by ∼4–6 h with respect to the peak action of TRAP150. RNA Pol II, RNA polymerase II preinitiation complex. Arrow denotes start site of transcription. It is possible that the interaction between TRAP150 and CLOCK-BMAL1 is indirect. The protein–protein associations likely involve looping of the underlying DNA.

Table S1. Primer sequences for mature mRNA analysis

Gene	Assay ID (IDT)	Exon location
Gapdh	Mm.PT.39.1	Exon 2–3 junction
Thrap3	Mm.PT.49.11647924	Exon 1–2 junction
Thrap3	Mm.PT.49a.16458483	Exon 2–3 junction
Dbp	Mm.PT.49a.5314479	Exon 3–4 junction

Table S2. siRNA sequences

siRNA	Sequence
Thrap3_A	5′-rGrUrA rCrArA rGrArU rCrCrA rArGrC rArGrU rUrUrG rGrUrC rCrCrA-3′
	5′-rGrGrA rCrCrA rArArC rUrGrC rUrUrG rGrArU rCrUrU rGrUA C-3′
Thrap3_Mut_A	5′-rGrUrA rCrArA rGrArU rCrCrA rArUrC rArGrU rUrUrG rGrUrC rCrCrA-3′
	5′-rGrGrA rCrCrA rArArC rUrGrA rUrUrG rGrArU rCrUrU rGrUA C 3′
Thrap3_B	5′-rGrGrA rGrArG rArGrA rUrCrU rUrArA rGrCrG rArGrG rUrAA A-3′
	5′-rUrUrU rArCrC rUrCrG rCrUrU rArArG rArUrC rUrCrU rCrUrC rCrUrU-3′
Thrap3_Mut_B′	5′-rCrCrU rCrArG rArCrA rUrCrU rUrArA rGrCrG rArGrG rUrAA A-3′
	5′-rUrUrU rArCrC rUrCrG rCrUrU rArArG rArUrG rUrCrU rGrArG rG-3′
Thrap3_C	5′-rGrGrA rUrGrC rUrGrA rUrGrC rUrUrG rGrArC rUrUrU rGrUT T-3′
	5′-rArArA rCrArA rArGrU rCrCrA rArGrC rArUrC rArGrC rArUrC rCrUrU-3′
Thrap3_Mut_C	5′-rCrCrA rUrArA rUrCrA rUrGrC rUrUrG rGrArC rUrUrU rGrUT T-3′
	5′-rArArA rCrArA rArGrU rCrCrA rArGrC rArUrG rArUrU rArUrG rGrUrU-3′

Table S3. Primer sequences for pre-mRNA analysis

Gene	Sequence
Per1	
Forward primer	GAGCAGCCATCCTGAACCTAA
Reverse primer	GACTCCCGAGTGTCGAGCAA
Probe	AGACCTTTAGCGAACACGACCCCTTTACAC
Per2	
Forward primer	TCAGGCACTCCAGGCAAGTC
Reverse primer	GACATCACACCAGAGATTTTAAAGAAAA
Probe	TTGTCCCAAAGCCACCACCCAGC
Cry1	
Forward primer	TGTGTCTAGGTAACTCATAGTGTGGAGAA
Reverse primer	TGCTTTTCCTCCTCTAACCTCAA
Probe	CGGGCTCCGGATCCAGCTTTCT
Cry2	
Forward primer	CTCTCCCCCACGCCTCTT
Reverse primer	CTGATCACCACCACGTTTGTCT
Probe	TCCCATCCCGCGTAACCTCTCTTCA
Bhlhe40 (Dec1)	
Forward primer	TGTGTGCTGTGATCCCTTCAAG
Reverse primer	CAGAGATGCCCCAAGTTGAGA
Probe	CTCCACTCCTAACCTTGTGTTCCGCGA
Bhlhe41(Dec2)	
Forward primer	CTGGATAGTTAATTAGGGCCATTGA
Reverse primer	GGAAAGCTCAGGGCTGGAAT
Probe	TCGCAAGGAAACTCTGCCCGCA
Rps27	
Forward primer	TGGTCCAGGTTTCGACAGAGA
Reverse primer	CCCGCAATTAGCGCAAGA
Probe	CTCGCTCTTGGGTCCGCACGG
Rps20	
Forward primer	CGCCAAGGAAAAGAATCTGAA
Reverse primer	CTCGAGTCTACCTGTCCCTTACCT
Probe	AGGACCGGTGCGCATGCCTACC
Rps13	
Forward primer	TCTGGCAAGCTCACAGTGATG
Reverse primer	AACCCAACATGGAAAGTCCTTCT
Probe	TGGTGTTCCAACATTCGCAGTTTCCA
Rpl30	
Forward primer	GAATCCGAGAGGCTCTTTGTATG
Reverse primer	GTCATCTAAAACCAAACTCAGTTCCA
Probe	TCTCAGACATCATGAAAGGGTTCAGTTCCA
Oaz1	
Forward primer	GGGCCACAGTGCTGAGATG
Reverse primer	CACTGCCCCTCACCTGTGT
Probe	CACTGCCTGTGAGGCCTGTCTTGC

Table S4. Primers for promoter analysis

Gene	Sequence
Per1	
Forward primer	ATCCTGATCGCATTGGCTGACTGA
Reverse primer	TCTCTTCCTGGCATCTGATTGGCT
Per2	
Forward primer	ATGTGACAGCGGAGGGCGAC
Reverse primer	CTCGGCCCGTCACTTGGTGC
Cry1	
Forward primer	CTCAGGAGACCCGCAGAG
Reverse primer	TCACGTTTCTGAAGTGTGTTTACT
Cry2	
Forward primer	GCAGCACAGGGAGAACAAA
Reverse primer	CACTGGAGAGGAGGGTTCAC
Dec1 (Bhlhe40)	
Forward primer	CAGATCCCTTGCAGCCCGGTG
Reverse primer	GCCGGAGCTCGGCTCACTTG
Dec2 (Bhlhe41)	
Forward primer	GTGATTAGTGTCACCGCAATTC
Reverse primer	CAAGTGGTTCTGGCAACAGT
Reverbα (Nr1d1)	
Forward primer	AGGCACACTCCACCTACATTGTCA
Reverse primer	TGGAGCAGGTACCATGTGATTCCA
Reverbβ (Nr1d2)	
Forward primer	ACGTGCACGGTGTGTTTAC
Reverse primer	CACCCAGCTCGCATTATGTA
Rorc	
Forward primer	GCTGGGAGCGAAGGGACAGC
Reverse primer	TCGCCCACTCCCTCCACCTG
Dbp	
Forward primer	AAAACCAGTGCTGCACATTC
Reverse primer	GCCTACAGCTTCCCCTTTC
Hprt	
Forward primer	TGTGAAAGAACTGGGCCTAAA
Reverse primer	TCCCAGAGGATTCCCAGATA
Gapdh	
Forward primer	TTGAGCTAGGACTGGATAAGCAGGG
Reverse primer	ATTTATAGGAACCCGGATGGTGGG
Rpl30	
Forward primer	CAGAACAAACGCCCAGAAC
Reverse primer	TGCGGTAGTTGGTTGCTATG
Rps27	
Forward primer	CGCCACAGCAGCTAAATTC
Reverse primer	TTCGAATGCAACGGAATG
Oaz1	
Forward primer	TGTCTTCCCTCCCACAGAGT
Reverse primer	CCTACTCCGGCTTCTGATTG