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Supplemental Information

Intestinal Stem Cells Exhibit Conditional Circadian Clock Function

Kathyani Parasram, Nathaniel Bernardon, Maha Hammoud, Hanna Chang, Li He, Norbert Perrimon, and Phillip Karpowicz

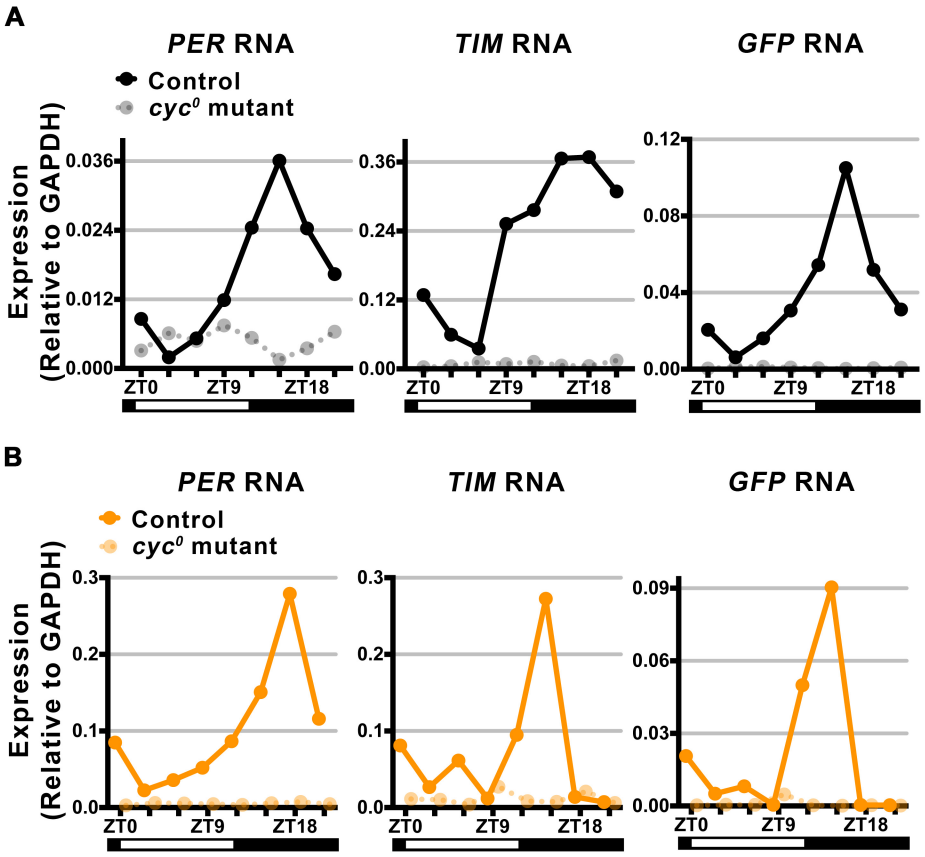

Figure S1: Analysis of *Clock*^{PER} and *Clock*^{TIM} reporter transcription. Related to Figure 1. **(A)** A second representative experiment showing RT-qPCR expression for *Clock*^{PER} (*GFP* RNA), *PER* and *TIM* RNA. Each data point represents a signal obtained from n=10 intestines. Data confirms the expression rhythms shown in Fig 1E. **(B)** RT-qPCR expression for *Clock*^{TIM} (*GFP* RNA), *PER* and *TIM* RNA. Each data point represents a signal obtained from n=10 intestines. As in Fig 1E and above, all three genes in show similar expression phases indicating similar timing of CLK/CYC activity on the *TIM* promoter as on the *PER* promoter.

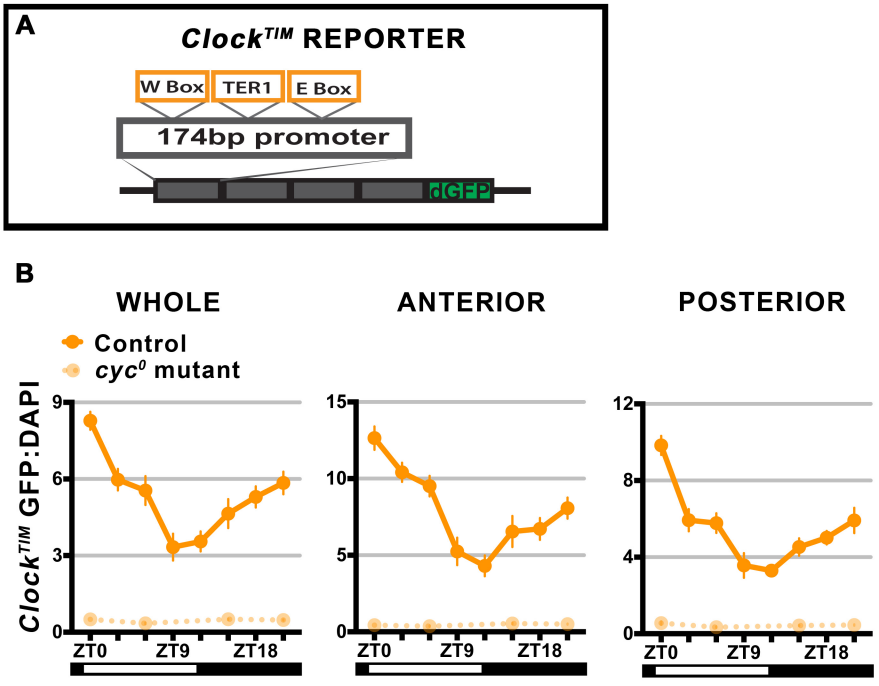

Figure S2: The *Clock*^{TIM} reporter reports circadian clock activity. Related to Figure 1 and Figure 2A. (A) Schematic of the *Clock*^{TIM} reporter shows four copies of the 174bp minimal promoter upstream of destabilized GFP (dGFP) to report temporal changes in circadian clock activity through *TIM* expression. **(B)** Analysis of *Clock*^{TIM} GFP:DAPI signal under LD photoperiod for the whole intestine, anterior (R2), and posterior (R5) regions. All regions show similar phase of clock reporter activity, and the *cyc*^{0} mutant has no circadian transactivation and is GFP low. Data presented as mean of n≥10 intestines, error bars show ± SEM. Control *vs*. *cyc*^{0}: whole gut (2-way ANOVA F=6.52, p=0.0006), anterior (2-way ANOVA F=7.172, p=0.0003), posterior (2-way ANOVA F=11.2, p<0.0001).

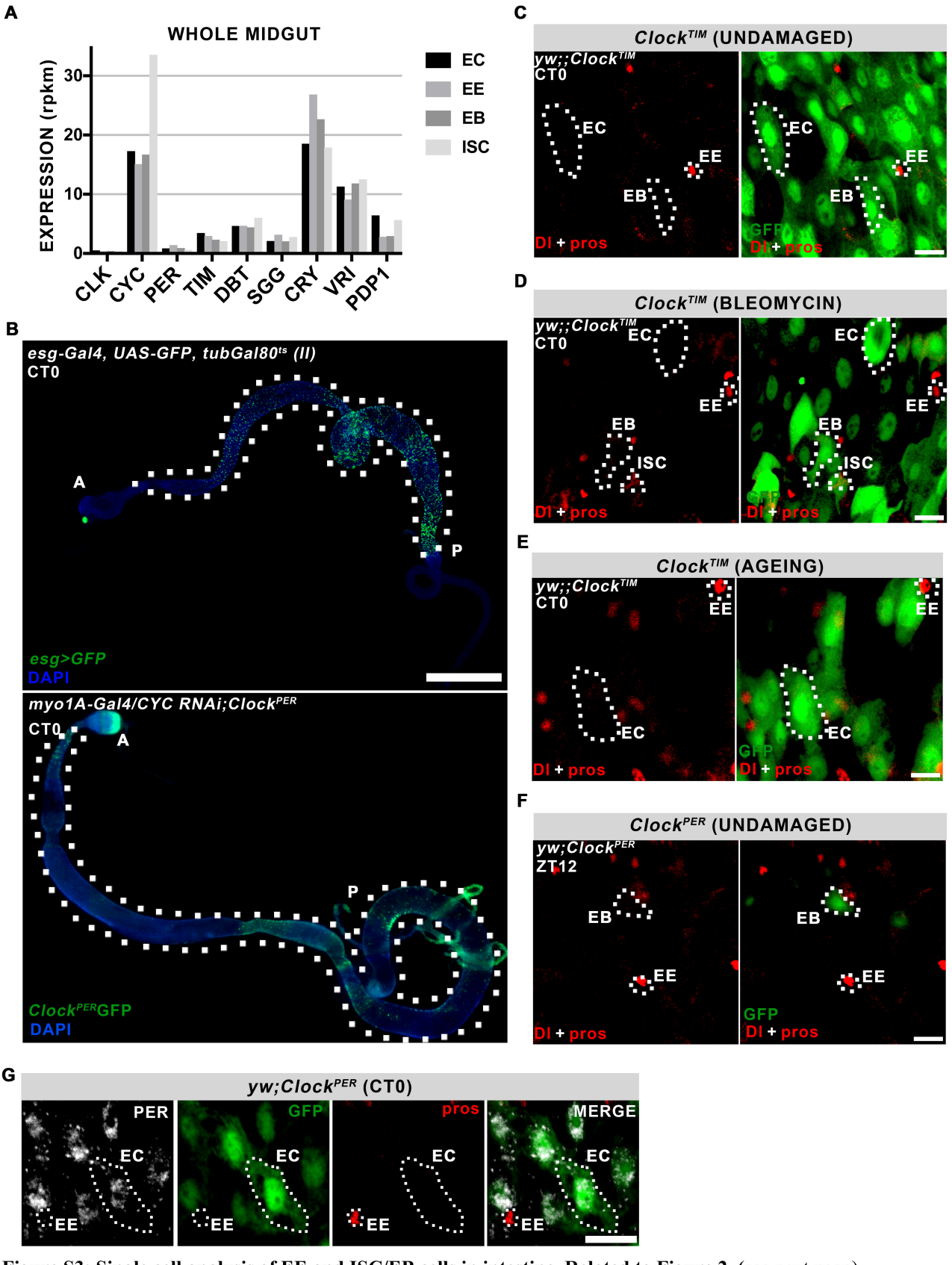

Figure S3: Single cell analysis of EE and ISC/EB cells in intestine. Related to Figure 2. (see next page)

Figure S3: Single cell analysis of EE and ISC/EB cells in intestine. Related to Figure 2. **(A)** Graph depicting the RNA-sequencing expression of clock genes modified from *flygutseq.buchonlab.com*[26]. Data shows similar gene expression levels for all clock genes in all four intestinal epithelial cell types, although we note that the expression of CLK is particularly low in this dataset. The phase of circadian clock activity and the dissection time is not available from this dataset which could greatly affect clock gene levels. **(B)** Representative images of *Drosophila* intestines at CT0 comparing the number of undifferentiated precursors marked by GFP in the *esg-Gal4, UAS-GFP, tubGal80^{TS}* genotype which marks all ISC/EBs when flies are shifted to 29^{o}C temperature, *vs*. the number of undifferentiated precursors marked by *Clock^{PER}* GFP+ when *CYC* is knocked down in ECs (*Myo1A>CYC*) at its highest time of expression. Note that many small GFP+ cells that are present in the former, are absent in the latter. DAPI counterstains nuclei, A: indicates the anterior region, P: posterior. Scale bar is 500µm. **(C)** Representative confocal Z-stack of *Clock^{TIM}* at CT0 shows that EEs do not express GFP+ reporter signal from the *TIM* reporter either. **(D and E)** Confocal section of *Clock^{TIM}* at CT0 shows that EE cells do not express GFP from the *TIM* reporter under bleomycin-treated or ageing conditions. Dl+ and pros+ marking the ISCs and EEs, respectively, are outlined, as is a large polyploid EC and differentiating EB, as indicated, scale bar is 10µm. **(F)** Confocal section of *Clock^{PER}* at ZT12, the trough of GFP+ expression, shows that EE cells do not express *PER* reporter activity in an anti-phasic fashion either. An EE is outlined, as is an EB showing low levels of GFP+ signal at this time, scale bar is 10µm. **(G)** Confocal section showing nuclear PER antibody staining in ECs, but not EEs in the *Clock^{PER}* reporter that co-expresses PER and GFP simultaneously, indicating no circadian clock function. EEs do not show either expression of PER or GFP. Cells of interest are outlined: pros+ marks EEs, ECs are the large polyploid cells. Scale bar is 10µm.

Supplemental Experimental Procedures

Fly Strains

Flies were housed at 25°C under a 12-h light:12-h dark cycle (LD) on media containing 1.2% w/v dry yeast, 0.7% w/v soyflour, 5% w/v cornmeal, 0.4% w/v malt, 0.4% v/v agar, 5.3% v/v with propionic acid, and tegosept (Diamed). Experiments with temperature-induced expression (Fig 5) were carried out in the exact same conditions except at 29°C for 7 days. In all experiments, flies were tested following >5 days synchronization to LD schedules. In the free-running experiments, flies were moved to 24h dark conditions (DD) and tested the following day after moving, or 5 or 10 days after free-running DD conditions (as indicated in the Figures). Female flies <14 days old (at time of dissection) were used for experiments, except for ageing experiments which used female flies aged 35-40 days. For bleomycin-treatment, 2 days prior to the experiment flies were put on food as above but containing 25µg/mL bleomycin (Millipore). Restricted feeding was performed by moving flies from hydration-supplemented vials that contained 2.4g of agar in 500mL water to vials containing food (at the times indicated in the text).

CYC RNAi constructs, which have been previously validated for efficient cell-specific circadian disruption[S1], were used with the *esg-Gal4* driver to disrupt *CYC* in undifferentiated precursors (ISCs + EBs) or with the *Myo1A-Gal4* driver to disrupt *CYC* in differentiated ECs. Our previous work has also shown these *Gal4* drivers are not expressed in clock neurons, and hence do not affect circadian behavior [S1].

The following strains were used:
ClockPER on II (attP40)
ClockPER on III (attP2)
ClockTIM on II (attP40)
ClockTIM on III (attP2)
cyc^{0}, ry^{506}
w^{1118};;cry^{01}
esg-Gal4 (II)
myo1A-Gal4 (II)
yw; PER-HGM
w;TIM-HGM
w;;TIM-HGM
Luc RNAi (Bloomington stock center #31603)
cyc RNAi (Bloomington stock center #42563)
Mer RNAi (Bloomington stock center #28007)
APC RNAi (Bloomington stock center #34869)
N RNAi (Bloomington stock center #33611)
UAS-Yki

Experimental fly strains, organized by figure:

Figure		Fly Strain
1	C	*yw;*$Clock^{PER}$*;*
	D	*yw;*$Clock^{PER}$*;*
	E	*yw;*$Clock^{PER}$*;* *yw;*$Clock^{PER}$*/CyO;*cyc^{0}*,*ry^{506}*/TM6B*
	F	*yw;*$Clock^{PER}$*;* *yw;*$Clock^{PER}$*/CyO;*cyc^{0}*,*ry^{506}*/TM6B*
2	A	*yw;*$Clock^{PER}$*;* *yw;*$Clock^{PER}$*/CyO;*cyc^{0}*,*ry^{506}*/TM6B*
	C	*yw;*$Clock^{PER}$*;*
	D	*yw;*$Clock^{PER}$*;*
	E	w^{1118}
	A	*yw;*$Clock^{PER}$*;*

B	*yw;Clock^{PER};* *;Clock^{PER};cry^{01}*
C	*yw;Clock^{PER};*
D	*yw;Clock^{PER};*
E	*yw;Clock^{PER};*
A	*esg-Gal4,UAS-CD8RFP/Valium20-Cyc;Clock^{PER}/MKRS*
B	*Myo1A-Gal4/Valium20-Cyc;Clock^{PER}/MKRS*
C	*yw;Clock^{PER};*
D	*yw;Clock^{PER};*
E	*Myo1A-Gal4/IF;Clock^{PER}/Valium1-Luc* *Myo1A-Gal4/Valium20-Cyc;Clock^{PER}/MKRS* *esg-Gal4,UAS-CD8RFP/IF;Clock^{PER}/Valium1-Luc* *esg-Gal4,UAS-CD8RFP/Valium20-Cyc;Clock^{PER}/MKRS*
F	same as 4E
G	same as 4E
A	*esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-Cyc* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-APC* *esg-Gal4/Clock^{PER};tubGal80^{TS}/UAS-Yki* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-N*
B	*esg-Gal4/Clock^{PER};tubGal80^{TS}/+* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-Cyc* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-APC* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium10-Mer* *esg-Gal4/Clock^{PER};tubGal80^{TS}/UAS-Yki* *esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-N* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/+* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/Valium20-Cyc* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/Valium20-APC* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/Valium10-Mer* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/UAS-Yki* *esg-Gal4/Clock^{TIM};tubGal80^{TS}/Valium20-N*
C	*esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-APC*
D	*esg-Gal4/Clock^{PER};tubGal80^{TS}/Valium20-N*
A	*yw;Clock^{PER};* *yw;Clock^{PER}/CyO;cyc^{0},ry^{506}/TM6B*
B	*w;Clock^{TIM}* *yw;Clock^{TIM}/CyO;cyc^{0},ry^{506}/TM6B*
B	*w;Clock^{TIM}* *yw;Clock^{TIM}/CyO;cyc^{0},ry^{506}/TM6B*
B	*esg-Gal4,UAS-GFP,tubGal80^{TS};* *Myo1A-Gal4/Valium20-Cyc;Clock^{PER}/MKRS*
C	*w;;Clock^{TIM}*
D	*w;;Clock^{TIM}*
E	*w;;Clock^{TIM}*
F	*yw;Clock^{PER}*
G	*yw;Clock^{PER}*

Generation of Clock reporters

The following primers were used:
gBlock1 forward 5' AACCAACAACTCTAGACTACTGC 3'
gBlock1 reverse 5' ACTGCTGAGTCAGACTATCTGGATCCTCTAGACGTGC 3'
gBlock1 forward 5' AGTCTGACTCAGCAGTAACCAACAACTCTAGACTACTGC 3'
gBlock1 reverse 5'ATCTGGATCCTCTAGACGTGC 3'

Sequence of Per enhancer:
CGAGTTCGCACAACTCATGGTGGGGCAGGGACGGGTCCGAGTGCAAAAAGCGTCCGAGAAACCGTAG
GCAGTGAAAAGCCGCCGCTCACGTGGCGAACTGCGTGACTTGGCCAGCAAATCCGC

Sequence of Tim enhancer:
TTCCGGCGCTGGTTATTCATGTTATCGAACATCGTAGTGGCGGTTGGCAAATAAACGTGCGGCACGTT
GTGATTACACGTGAGCCGATTTCCCCGGCCGTCCGGCATTGAGTGCGAGCGGGACGGAGCAGCGCGGC
GAGAGCTTTCGGCTAGCGTCTTTCGTTCGCACAAACGC

The full-length sequences for the plasmids can be found in the supplementary data file.

RT-qPCR

The following primers were used:
GFP Forward: TTTCATCACCCTCCTCCAAG
GFP Reverse: TTATGCCCGAGTTCTTTTCG
PER Forward: TCATCCAGAACGGTTGCTACG
PER Reverse: CCTGAAAGACGCGATGGTGT
TIM Forward: CCAGCATTCATTCCAAGCAG
TIM Reverse: GCGTGGCAAACTGTGTTATG
GAPDH Forward: CCAATGTCTCCGTTGTGGA
GAPDH Reverse: TCGGTGTAGCCCAGGATT

Tissue Fluorescence Imaging

Flies were synchronized to LD cycles as described above. At each time point ~10 intestines were dissected in PBS (Fisher), and fixed in 4% PFA (Electron microscopy sciences) in PBS for 40 minutes at room temperature. Intestines were rinsed 3x using PBS, and then counterstained with DAPI (ThermoFisher Scientific, 1:5000) in PBS-T (PBS + 0.2% Triton X-100, Fisher) for 5 minutes. Intestines were then washed with PBS-T and mounted on slides with ProLong Gold antifade reagent (ThermoFisher Scentific). Samples were imaged using a slide scanner (Zeiss Axio Scan.Z1) that assembled single images consisting of merged and tiled Z-stacks of the entire tissue sample in a single plane of focus. Images were analyzed using Zen Blue Edition software (Zeiss). Images were processed using Photoshop CS5 (Adobe).

Supplemental Reference

S1. Karpowicz, P., Zhang, Y., Hogenesch, J.B., Emery, P., and Perrimon, N. (2013). The circadian clock gates the intestinal stem cell regenerative state. Cell reports *3*, 996-1004.

Additional Items

A) 4xPER Sequence

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VERSION     .
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REFERENCE   1  (bases 1 to 11609)
  AUTHORS   Li
  TITLE     Direct Submission
  JOURNAL   Exported Sep 17, 2018 from SnapGene Viewer 4.1.5
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B) 4xTIM Sequence

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  TITLE     Direct Submission
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            http://www.snapgene.com
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