Supplemental Information:

Article title: The circadian clock modulates enamel development.

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Figure S1

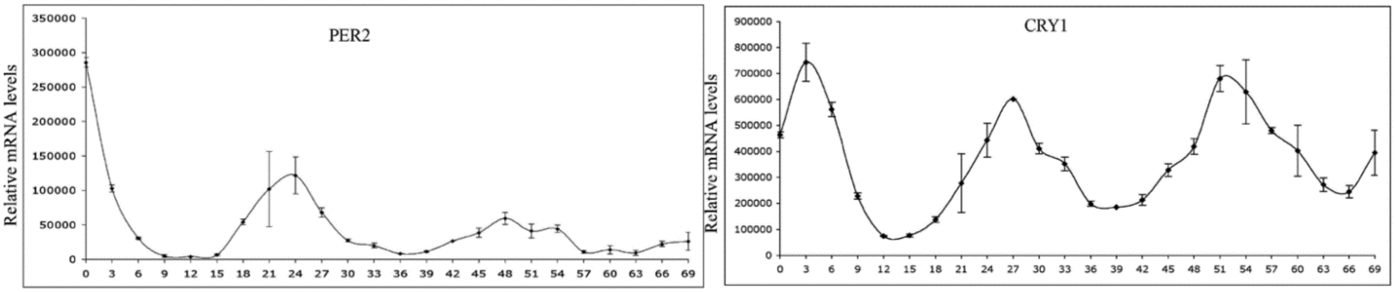

Figure S1. Real time PCR of ameloblsat cells following serum synchronization. To characterize gene expression profiles in enamel producing cells, the ameloblast-like cell line LS8 (Chen et al., 1992) was cultured in 10mm cell culture plates grown in DMEM supplemented with 10% FBS, 5% CO_2 at 37^oC. The circadian cycles of the cells were synchronized using a modified "serum shock" protocol based on that described by Balsalobre et al. (1998). Specifically, cells were treated for 2 hours in DMEM with a high serum concentration (50% FBS). This serum-rich media was removed and replaced with serum-free media. The synchronized cells were cultured for 72 hours and harvested at 3 hour intervals. Harvested cells were stored at -80^oC until the completion of the experiment and then processed for nucleic acid extraction as follows. Homogenized synchronized cells were processed for RNA using a Qiagen RNeasy Mini kit. Reverse-transcribed PCR was performed using an iScript cDNA Synthesis kit (Bio-Rad). Real time PCR reactions were performed using iQ SYBR Green Supermix (Bio-Rad). Table S1 provides all primer sequences. Triplicate samples for each time point and for each animal were processed. All values for the mRNA species were normalized to *β-actin* using methods described in Livak and Schmittgen (2001). The expression of *Per2* and *Cry1* oscillated in phase over 72 hours, with the amplitude of the *Cry1* rhythm maintained throughout the period, whereas the *Per2* rhythm amplitude dampened. The expression of *Per2* and *Cry1* oscillated in phase over 72 hours. The expression of *Cry1* was significantly different between acrophase and bathyphase over the 72 hour period (one-way ANOVA, $P<0.05$) and although the expression of *Per2* continued to oscillate, acrophase and bathyphase were not significant after 48 hours.

Table S1. Primer sequences used for real time PCR.

Gene Symbol	NCBI Gene ID	Forward Primer (5’-3’)	Reverse Primer (5’-3’)	Product Size
Amelx	11704	GGGGACCTGGATTTTGTTTG	AACCATAGGAAGGATACGGCTG	161 bp
NFYa	18044	GGCAGGGAATGTGGTCAAC	TGCGGTGATACTGTTTGGC	146 bp
Per2	18627	AAAGCTGACGCACACAAAGAA	ACTCCTCATTAGCCTTCACCT	151 bp
Cry1	12952	CACTGGTTCCGAAAGGGACTC	CTGAAGCAAAAATCGCCACCT	153 bp
Bmal1	11865	ACAGTCAGATTGAAAAGAGGCG	GCCATCCTTAGCACGGTGAG	124 bp
Car2	12349	GATAAAGCTGCGTCCAAGAGC	GCATTGTCCTGAGAGTCATCAAA	77 bp
Slc4a4	54403	TCCGAGAACTACTCCGACAAA	CTGCTGGACTCATCCGCATT	53 bp
Lamp1	16783	CAGCACTCTTTGAGGTGAAAAAC	CCATTCGCAGTCTCGTAGGTG	91 bp
B-actin	11461	GGGAAATCGTGCGTGACATC	GCGGCAGTGGCCATCTC	76 bp

Table S2. E-box elements located within 10-kb upstream of transcription start site

ene	**Human E-Box (5’-3’)**	**Mouse E-Box (5’-3’)**	**Rat E-Box (5’-3’)**
Amelx	-5349 to -5344 cctattgaaacgtgcaactcag	-9306 to -9301 tattcaatcacgttatgccttc	None
Ambn	-4497 to -4492 tttgtgacaacgtggatgaacc	None	-5145 to -5139 ttctcttacacgtttcaataat
	-5654 to -5649 tcccatgacacgtggggaatat		-7222 to -7217 tagtggagcacgtgtctctttt
	-7739 to -7734 gaagggtccacgtgtgaaaagc		--------
Enam	-3258 to -3253 attacaggcacgtgccaccaag	None	None
Mmp20	-44 to -39 gaagtgcaaacgtgcactgtct	-577 to -572 aatccaaccacgttcaaatcat	-1157 to -1152 gccttgatcacgtgtgcgtctt
	-7211 to -7206 ttgatggacacgttggttgatt	-3636 to -3631 ctttttaacacgtgatcatgta	-1402 to -1397 cgagtttgcacgtgaggaggag
	-7312 to -7307 gttccatccacgttgtggcaaa	-8760 to -8755 atgtaattcacgtgattcagta	-2246 to -2241 ctgggactcacgtgatggaagg
	--------	-9447 to -9442 ttagaggtcacgtggggtgggg	-5691 to -5686 gggaggagcacgttctaattac
	--------	--------	-6669 to -6664 gctatgtgcacgtttcctaccc
Nfya	-1839 to -1834 ccaaaactcacgttgatttaaa	-1439 to -1434 tgatctaaaacgtgcaaaggaa	-1714 to -1709 tcagaactcacgttgacttaac
	-9889 to -9884 ttcatagacacgtttaggaaat	-1591 to -1586 tggaaactcacgttgacttaac	-3661 to -3656 tgataccccacgtggatgcaat
	--------	--------	-4663 to -4658 taagaccccacgtggcaggtta
	--------	--------	-7544 to -7539 gctgtgttaacgtgcagtacca
	--------	--------	-9209 to -9204 acaagaaaaacgtgtgatgtgg
	--------	--------	-9427 to -9422 ctctcagtaacgtgctgttttc

Note: Transcription start sites are based on RefSeq sequences listed in the Methods section. E-box elements are highlighted in gray along with 8 base pairs of flanking sequence on either side.

Table S3. Percentage of filtered RefSeq records containing specific numbers of E-box elements 10-kb upstream of transcription start sites.

Number of elements	Human RefSeq Records[a]		
	5'-CACGTG-3' considered alone[b]	5'-CACGTT-3' considered alone[c]	5'-CACGTG-3' and 5'-CACGTT-3'[d]
0	33.62%	26.0%	9.46%
1	33.18%	33.6%	19.99%
2	19.18%	23.2%	23.83%
3	8.32%	11.0%	19.51%
4	3.49%	4.3%	12.75%
5	1.32%	1.4%	7.09%
6	0.4%	0.4%	3.9%
7	0.2%	0.1%	1.7%
8	0.1%	0.1%	0.8%
9	0.1%	0.0%	0.4%
10	0.0%	0.0%	0.2%
>10	0.0%	0.0%	0.3%

Number of elements	Mouse RefSeq Records[a]		
	5'-CACGTG-3' considered alone[b]	5'-CACGTT-3' considered alone[c]	5'-CACGTG-3' and 5'-CACGTT-3'[d]
0	32.4%	30.1%	10.7%
1	33.4%	34.5%	21.6%
2	19.6%	21.0%	23.1%
3	8.9%	9.6%	18.8%
4	3.7%	3.3%	12.4%
5	1.4%	1.1%	7.2%
6	0.4%	0.3%	3.4%
7	0.1%	0.0%	1.6%
8	0.1%	0.0%	0.7%
9	0.0%	0.0%	0.3%
10	0.0%	0.0%	0.1%
>10	0.0%	0.0%	0.1%

Number of elements	Rat RefSeq Records[a]		
	5'-CACGTG-3' considered alone[b]	5'-CACGTT-3' considered alone[c]	5'-CACGTG-3' and 5'-CACGTT-3'[d]
0	31.1%	28.1%	10.2%
1	32.8%	32.7%	19.3%
2	20.2%	22.2%	22.6%
3	9.7%	10.8%	18.8%
4	3.8%	4.2%	13.0%
5	1.6%	1.4%	8.3%
6	0.4%	0.4%	4.1%
7	0.2%	0.1%	2.0%
8	0.1%	0.0%	0.9%
9	0.0%	0.0%	0.4%
10	0.0%	0.0%	0.2%
>10	0.0%	0.0%	0.1%

[a] The percentage of filtered RefSeq records containing the specified number of E-box elements 10-kb upstream of the transcription start site are provided. Both strands are considered in all cases. [b] Only considers 5'-CACGTG-3' elements. [c] Only considers 5'-CACGTT-3' elements. [d] Considers the sum of the number of 5'-CACGTG-3' and 5'-CACGTT-3' elements.