# Supporting Information

## Plikus et al. 10.1073/pnas.1215935110

### SI Text

Movies S1, S2, S3, and S4 are time-lapse recordings from individually cultured *Period2*$^{Luc}$ vibrissa follicles show that circadian cycles of luminescence localize to several follicular areas, most prominently to follicular bulge and bulb. To aid visualization, the highest luminescence levels in Movies S2 and S4 were converted to red color. Each movie starts with the bright-field image of microdissected vibrissa follicles at time point 0 of the experiment; 24-h cycles of actual experimental time are labeled on the right. At the beginning of the experiment, follicles A and D have telogen morphology, whereas follicles B, C, E, and F are in anagen.

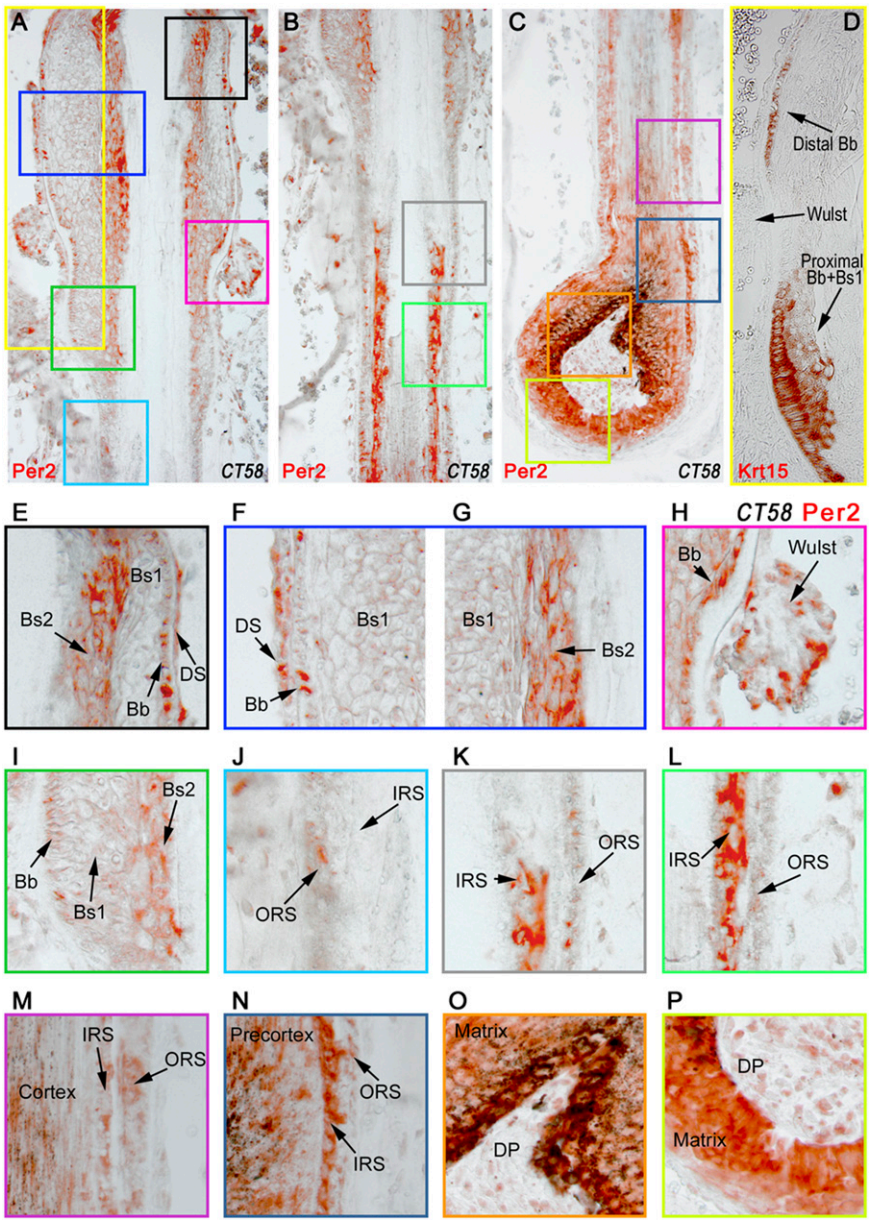



**Fig. S1.** Complex expression of Per2 identifies multiple hair follicle compartments as targets of circadian pathway activity. (*A–C*) Expression of Per2 in distal (*A*), middle (*B*), and proximal (*C*) segments of wild-type (WT) anagen vibrissae at circadian time point (CT)58. (*D*) Two keratin (Krt)15-expressing sites can be identified in mouse vibrissae: the larger proximal site, where both basal (Bb) and suprabasal (Bs1) progenitor cell populations are Krt15-positive; and the smaller distal site, where only Bb cells express Krt15. These distal Krt15-positive Bb basal cells also express nuclear Per2 at CT58 (*F*). (*E–P*) Details of Per2 expression in different compartments of WT anagen vibrissae at CT58. Within the bulge epithelium, expression changes along the proximodistal axis. There are three distinct cell types within vibrissa bulge: Bb and suprabasal (Bs1) progenitor cells and suprabasal nonprogenitor (Bs2) cells. In the most proximal segment, Bb and Bs1 cells are Per2-negative (*I*). At the level of ringwulst (*H*) and in the distal segment (*F* and *G*), several basal Bb cells express nuclear Per2, whereas all suprabasal Bb1 cells do not. The majority of suprabasal Bb2 cells express Per2 along the entire length of the bulge area (*E*, *G*, and *I*). Below the vibrissae bulge, Per2 is mostly absent in the midsegment (*L*) and is weak cytoplasmic in the proximal (*M* and *N*) segment of the outer root sheath. Per2 is very strong, nuclear in the lower epithelial matrix (*P*) and strong, but mostly cytoplasmic, in the upper matrix (*O*). Precortex (*N*) and proximal cortex (*M*) express weak Per2, whereas the distal, differentiated cortex is completely Per2-negative. Within the inner root sheath, proximal segment has notably strong cytoplasmic Per2 expression (*N*), the midsegment has patchy cytoplasmic expression (*M*), and the distal segment has very strong, nuclear/cytoplasmic expression (*L*) that completely and abruptly disappears upon terminal differentiation of the inner root sheath (*K*). Many dermal papilla cells express nuclear Per2 (*P*), whereas the dermal sheath shows nuclear Per2 expression only in (*H*) and above the ringwulst (*F*). Corresponding areas throughout this figure are identified by identically color-coded boxes.

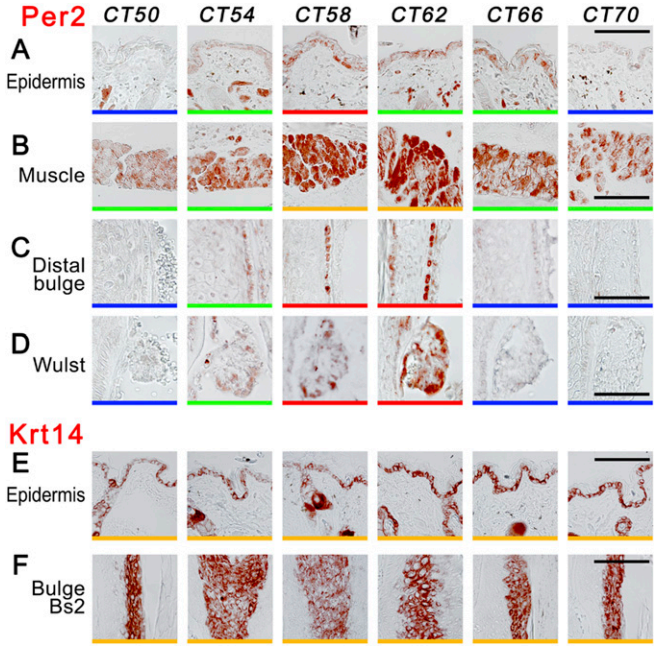



**Fig. S2.** Circadian cycles of Per2 expression exist in multiple compartments of anagen vibrissae follicles. (*A–H*) Circadian profiles of Per2 expression in anagen vibrissae from WT mice entrained to 12:12 12-h light:12-h dark (LD) schedule. In the distal (*Upper*) segment of the follicle, both Per2-positive cluster of epithelial Bb cells and mesenchymal ringwulst show clear circadian cycles of Per2 expression that peaks at CT58/CT62 (*C* and *D*). Within extrafollicular compartments, the epidermis has clear expression cycle of Per2 (*A*). At the same time, skeletal fibers of vibrissae muscles show strong expression of Per2 (*B*) that lacks apparent circadian rhythmicity. (*E* and *F*) The typical noncircadian protein Krt14 has constant expression both in interfollicular epidermis (*E*) and in epithelial Bb2 cells of the vibrissae bulge (*F*) at all time points of the day, from CT50 through CT70. Color bars at the bottom of each image define expression levels: blue, no expression; green, weak, mostly cytoplasmic expression; yellow, strong cytoplasmic and/or weak nuclear expression; red, strong nuclear or mixed nuclear/cytoplasmic expression.

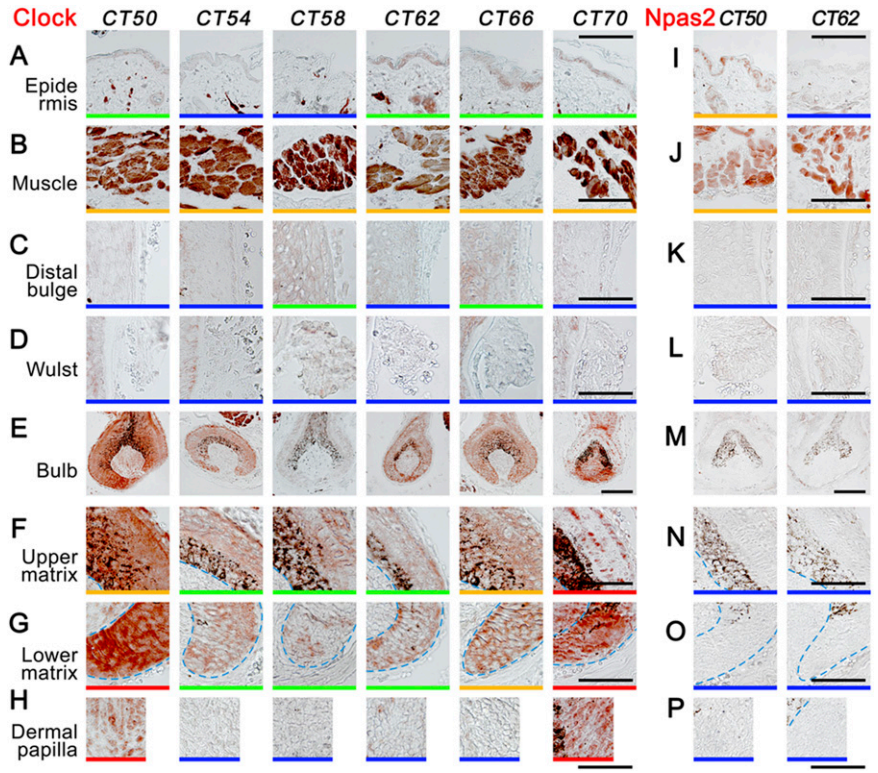



**Fig. S3.** Circadian cycles of Clock and NPAS2 expression exist in multiple compartments of anagen vibrissae follicles. Circadian profiles of Clock (*A–H*) and neuronal PAS domain-containing protein 2 (*I–P*) expression in anagen vibrissae from WT mice entrained to an LD schedule. In the upper (distal) segment of the follicle, the cluster of epithelial Bb cells and mesenchymal ringwulst do not express Clock (*C* and *D*) and Npas2 (*K* and *L*). In the follicular bulb, circadian expression cycles of Clock are prominent in the epithelial matrix and in the mesenchymal dermal papilla. In both compartments, Clock expression peaks at CT50 and CT70 and is at its low at CT58/CT62 (*E–H*). Npas2 is not expressed in either epithelial matrix or dermal papilla of the follicular bulb (*M–P*). Within extrafollicular compartments, epidermis has clear differential expression of Npas2 (*I*), and yet it does not distinctly express Clock (*A*). At the same time, skeletal fibers of vibrissae muscles show strong expression of Clock (*B*) and Npas2 (*J*) that lacks apparent circadian rhythmicity. The definition of color bars is the same as in Fig. S2.

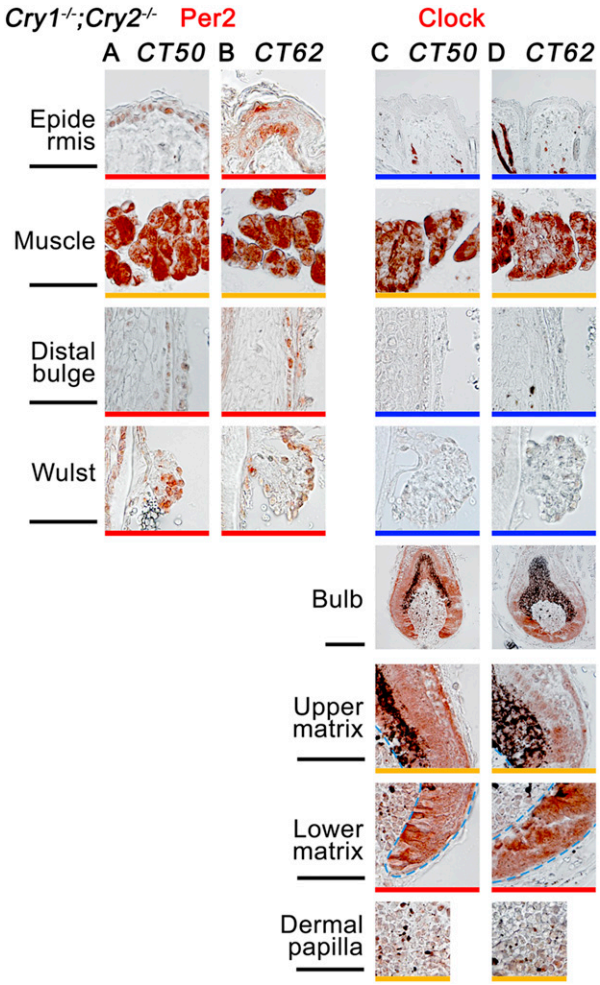



**Fig. S4.** Per2 and Clock expression loses circadian rhythmicity in *Cry1*$^{-/-}$;*Cry2*$^{-/-}$ skin. In *Cry1*$^{-/-}$;*Cry2*$^{-/-}$ vibrissae, both Per2 and Clock expression becomes arrhythmic. Per2 remains high in the distal epithelial bulge Bb cells, mesenchymal ringwulst, and interfollicular epidermis at both CT62 and CT50 (*A* vs. *B*). Clock remains high in epithelial matrix and dermal papilla at CT50 and CT62, whereas it is absent from the distal epithelial bulge Bb cells, mesenchymal ringwulst, and epidermis, where it is not expressed normally (*C* vs. *D*). The definition of color bars is the same as in Fig. S2.

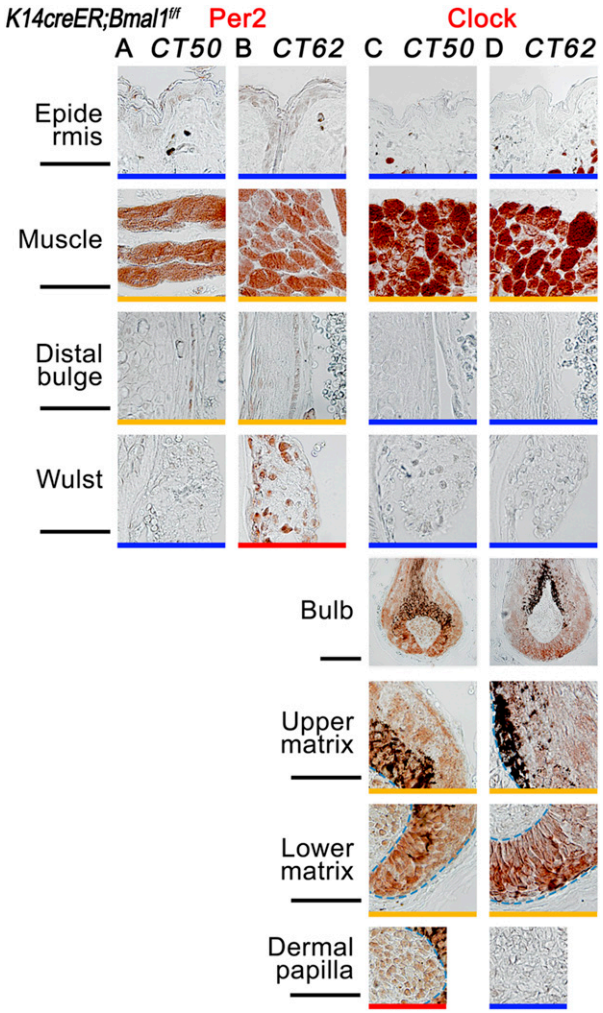



**Fig. S5.** Per2 and Clock expression loses circadian rhythmicity in epithelial compartment of induced *K14creER;Bmal1*$^{f/f}$ mice. In *K14creER;Bmal1*$^{f/f}$ hair follicles, both Per2 and Clock expressions become arrhythmic in epithelial but not in mesenchymal compartments. In *K14creER;Bmal1*$^{f/f}$ anagen vibrissae, Per2 is nearly absent in the interfollicular epidermis and distal bulge but remains high in epithelial matrix at CT50 and CT62 (Fig. 2). Mesenchymal structures (dermal papilla and ringwulst) maintain normal cyclic Per2 expression, which peaks at CT62 (also see Fig. 2). Similarly, cyclic Clock expression is lost in all epithelial structures of the vibrissae follicle but not in dermal papilla, where it peaks at CT50 (*C* and *D*). The definition of color bars is the same as in Fig. S2.

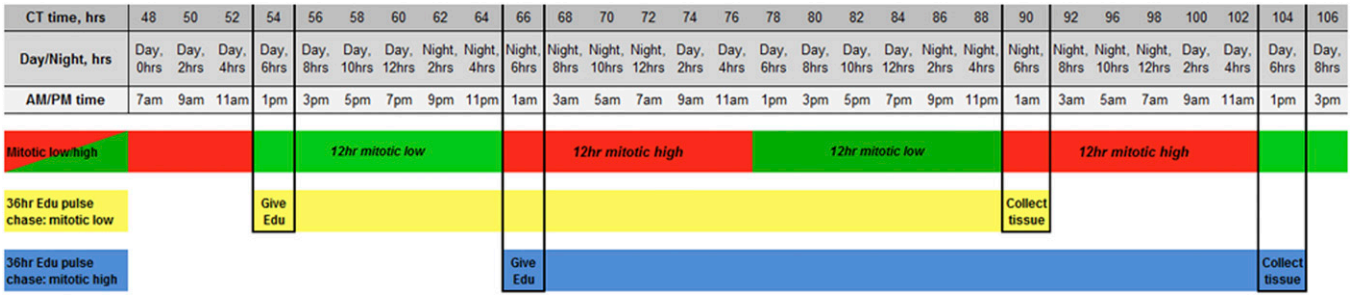



**Fig. S6.** Design of the 36-h 5-ethynyl-2′-deoxyuridine (EdU) pulse-chase experiment. Experimental time points are identified in terms of the CT (*Top*), subjective day and night time of the day (*Middle*), and corresponding objective AM/PM time (*Bottom*). Twelve-hour-long periods of high and low mitotic activity in anagen hair-follicle matrix are marked as interchanging red and green timeline. Yellow and blue lines mark boundaries of the 36-h long EdU pulse-chase experiments that encompass either two 12-h-long mitotic depressions and one 12-h-long mitotic peak (yellow; CT54 groups) or for two mitotic peaks and one mitotic depression (green; CT66 group).

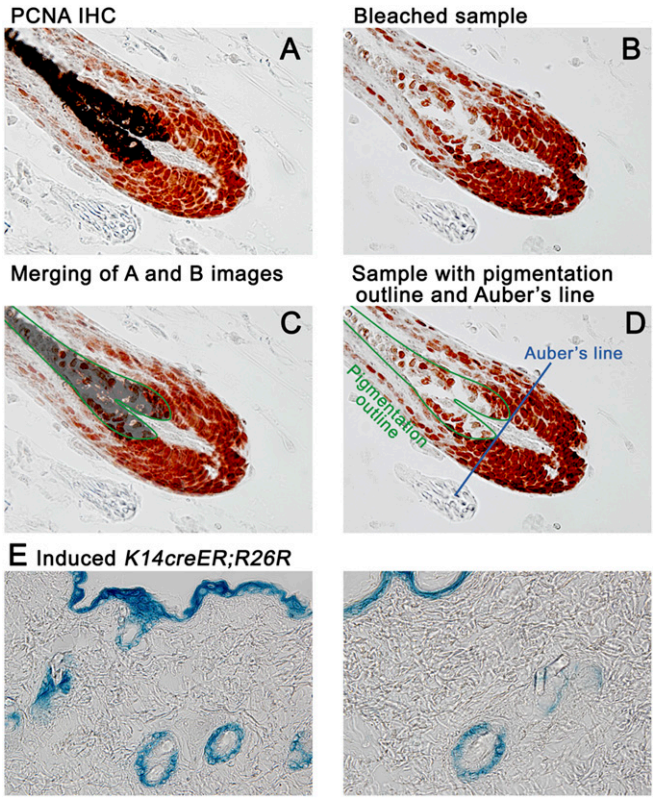



**Fig. S7.** Method of establishing Auber line level in immunohistologic (IHC) samples. Stained anagen hair follicle samples from pigmented mice were treated with $H_2O_2$ to bleach follicular melanin. This procedure reveals stained cells, otherwise concealed by melanin. Samples were photographed before (*A*) and after (*B*) bleaching, and precise outlines of pigmented areas were reconstructed by digitally overlapping before and after images (*C* and *D*). Intensity of AEC substrate color is not affected by $H_2O_2$ treatment. (*E*) Induction efficiency of *K14creER* promoter was verified in *K14creER;R26R* mice.

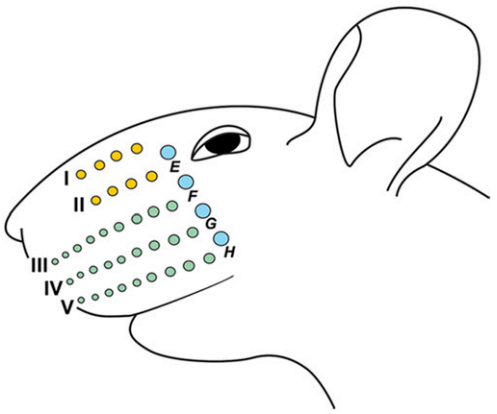



**Fig. S8.** Map of the mystacial vibrissae pad.

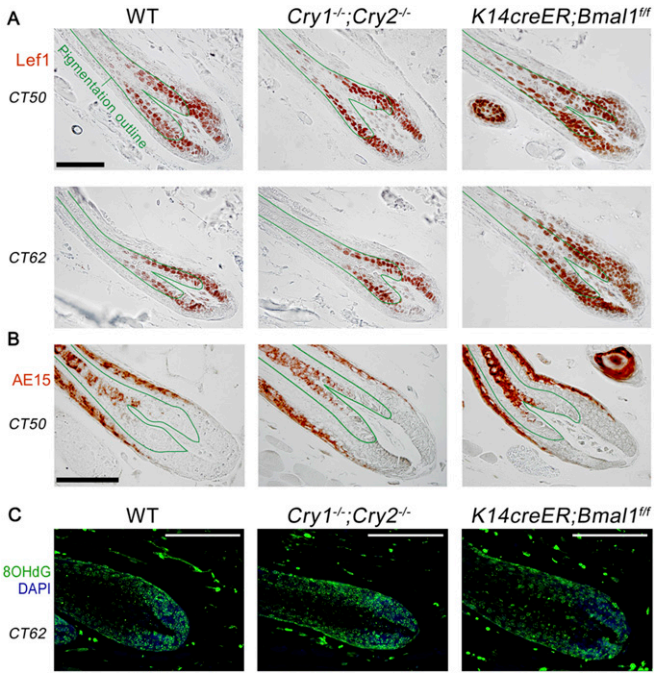



**Fig. S9.** Profiling circadian gene expression patterns and 8-hydroxy-2′-deoxyguanosine (8OHdG) in hair-follicle matrix. (*A*) Generally normal nuclear Lef1 expression is seen in WT, *Cry1*$^{-/-}$;*Cry2*$^{-/-}$, and *K14creER;Bmal1*$^{f/f}$ anagen hair follicles, indicating lack of differentiation defect of the hair shaft's cortex and cuticle. (*B*) There is no significant vertical shift in expression of AE15$^{+}$ trichohyalin in inner root sheath and medulla of hair shaft of circadian mutants. (Scale bars: 50 μm.) (*C*) Hair-follicle matrix cells experience oxidative damage, as measured by immunostaining for 8OHdG, a marker of oxidized DNA nucleosides.

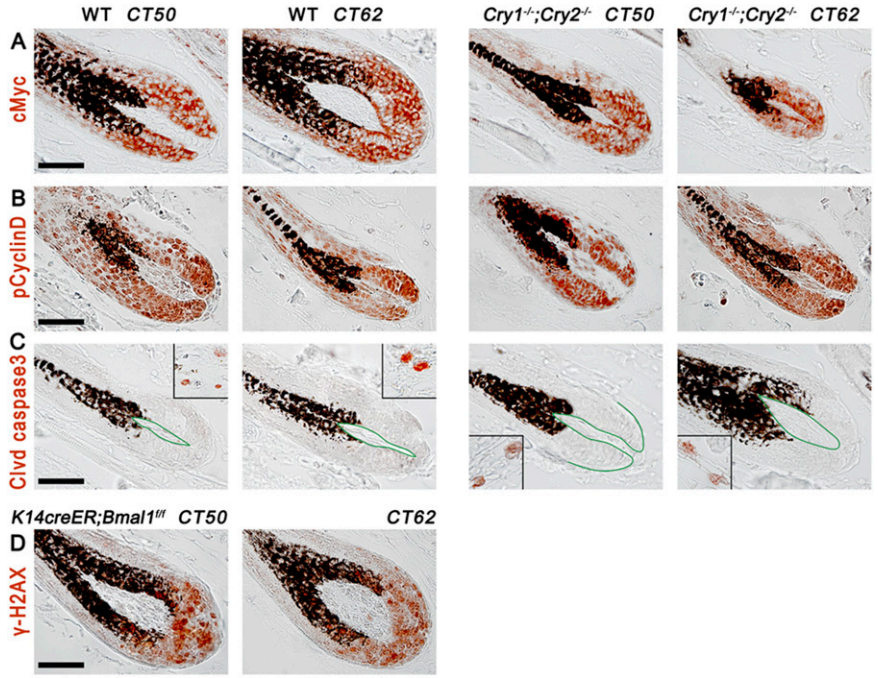
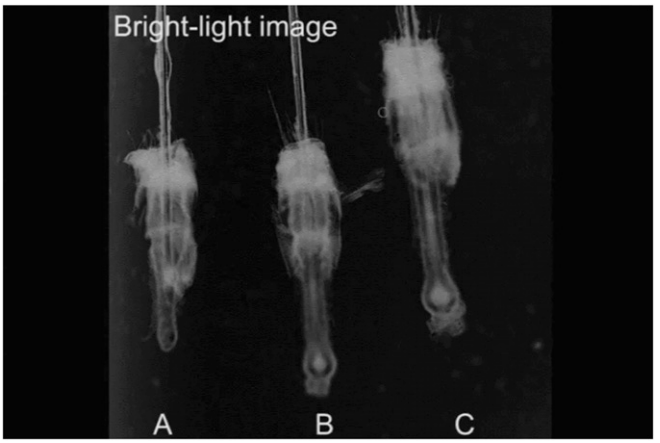
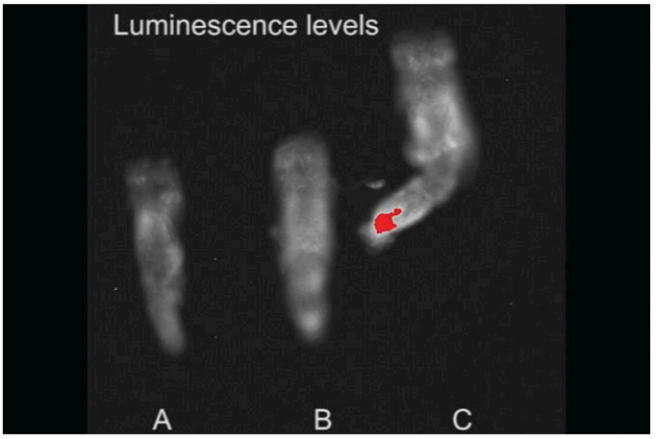



**Fig. S10.** Molecular profiling of cell cycle gating in hair-follicle matrix. (*A* and *B*) cMyc and pCyclinD1 are strongly expressed in the matrix of anagen follicles, and their expression levels do not appear to change at different circadian time points, as well as between WT and *Cry1*$^{-/-}$;*Cry2*$^{-/-}$ mice. (*C*) Virtual absence of cleaved caspase3-positive apoptotic cells in the matrix of both WT and *Cry1*$^{-/-}$;*Cry2*$^{-/-}$ anagen follicles at both CT50 and CT62 time points. (*Insets*) Cleaved caspase3-positive cells in the dermis of the corresponding sample. (*D*) Circadian differences in γH2AX expression disappear in the matrix of *K14creER;Bmal1*$^{f/f}$ anagen hair follicles, where it becomes continuously high.



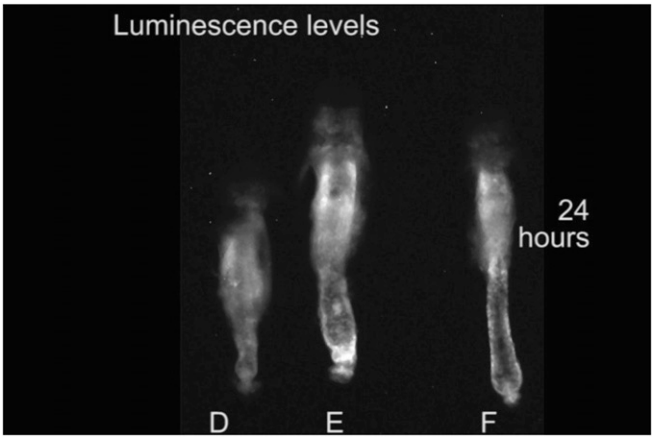

**Movie S1.** Per2Luc luminescence images of a telogen and two anagen vibrissa follicles.

Movie S1



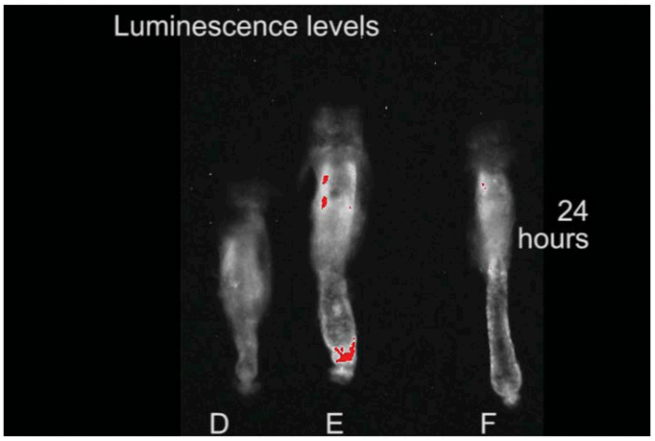

**Movie S2.** False-color rendering of Per2Luc luminescence images of vibrissa follicles shown in Movie S1.

Movie S2




**Movie S3.** Per2Luc luminescence images of a telogen and two anagen vibrissa follicles. This is an independent experiment.

Movie S3




**Movie S4.** False-color rendering of Per2Luc luminescence images of vibrissa follicles shown in Movie S3.

Movie S4