# Supplementary data

Casein kinase 1 underlies temperature compensation of circadian rhythms in human red blood cells

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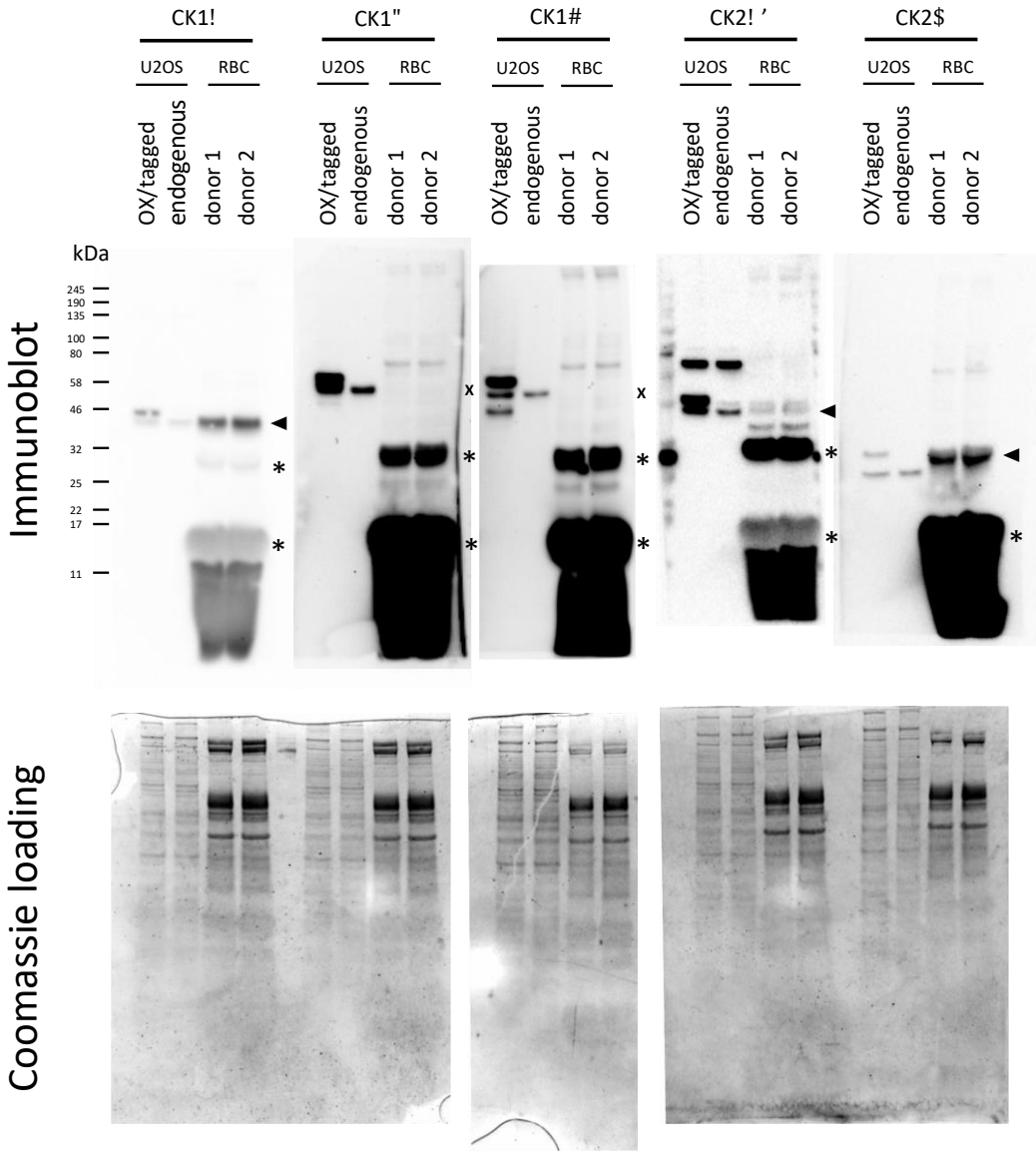
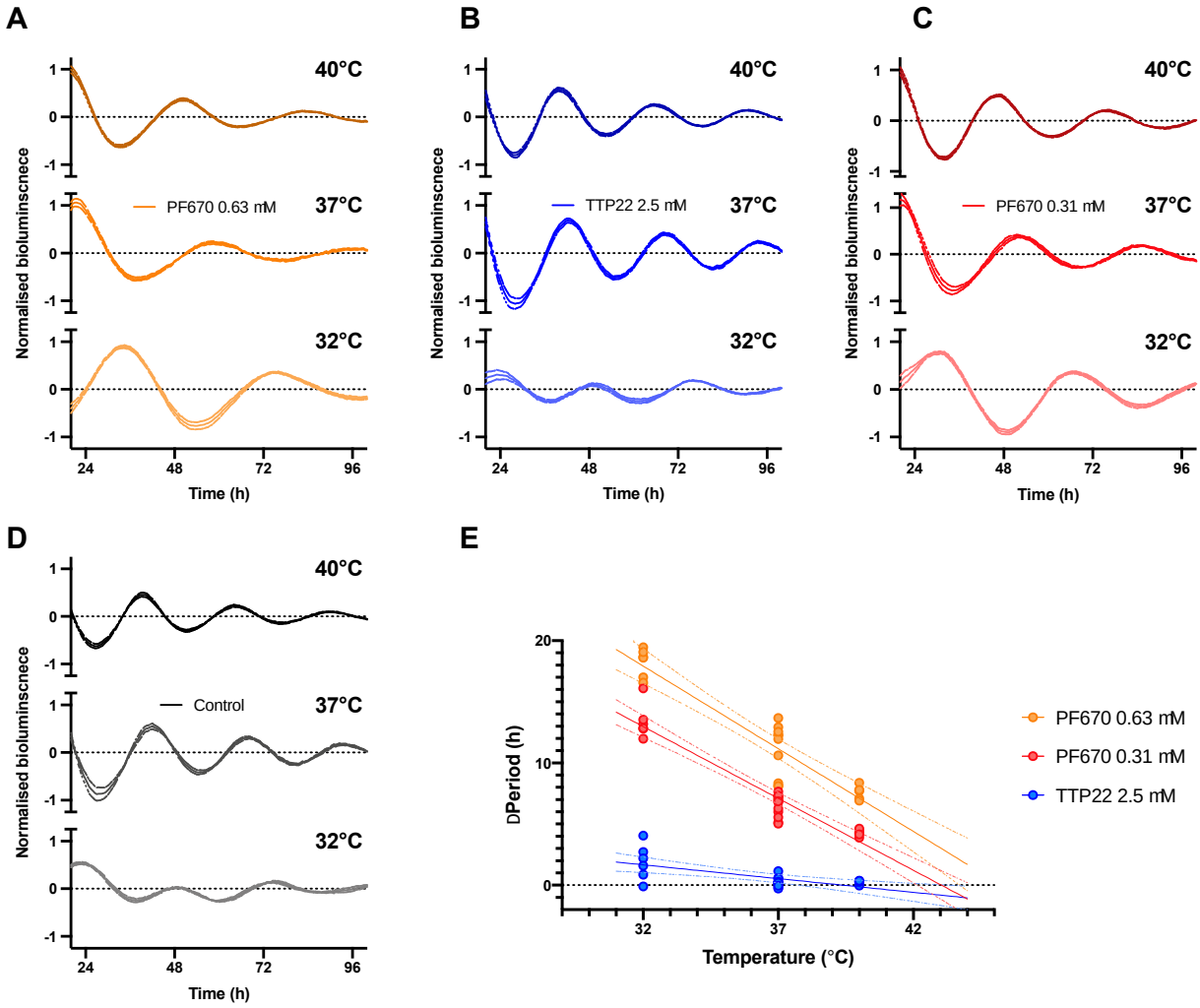



**Supplementary Figure 1. Uncropped immunoblot scans from Figure 2.**

Cut membranes from three different gels were probed with antibodies to the indicated kinase isoform. Lysates of RBCs from two different donors were loaded. Lysates from U2OS expressing a tagged indicated kinase isoform (V5-CK1α, V5-CK1δ, HA-CK1ε, HA-CK2α' and Myc-CK2β) and untransfected U2OS cells served as controls. Membranes were exposed for different lengths of time for detection of signal. Over-exposed blots are shown for CK1δ and CK1ε. ◄ = specific band of interest. **X** = absence of band of interest. * = non-specific band due to peroxidase activity of haemoglobin dimer (32kDa) and monomer (16kDa). A very faint band at 64kDa, corresponding to the haemoglobin tetramer, can be seen on some membranes.




**Supplementary Figure 2. Effect of casein kinase inhibition is temperature dependent.**
Human U2OS cells (*BMAL1:Luc*), synchronised by the change of medium at t=0, were cultured at constant 32 °C, 37 °C or 40°C for 4 days in the presence of 0.63 µM PF-670462 (orange, A), 0.31 µM PF-670462 (red, B) and 2.5 µM TTP22 (blue, C) or vehicle (black, D). Raw data was detrended using a 24- or 30-h moving average for TTP22 or PF-670462 respectively, and period values derived from damped cosine curves fitting using non-linear regression as described previously. (E) Change in period relative to control is shown (mean ± SEM, n = 6 (for 32°C and 40°C) or 12 (for 37°C)). Straight lines were fitted to the data using linear regression in GraphPad Prism and extrapolated to 31°C and 44°C. 95% confidence bands for the straight line fit are shown as dotted lines.

| Kinase | Inhibitor function (% remaining vs control) | | | Presence in | |
|---|---|---|---|---|---|
| | TTP22 | D4476 | PF670 | RBCs | U2OS[1] |
| **CK1$\alpha$** | n.d. | n.d. | 4.4 | + | + |
| **CK1$\alpha$-like** | n.d. | n.d. | 0.9 | n.d. | n.d |
| **CK1$\delta$** | n.d. | 4 | 3.8 | - | + |
| **CK1$\varepsilon$** | n.d. | n.d. | 0.1 | - | + |
| **CK2$\alpha$** | 0.72 (CK2) | 83 (CK2) | 53 | n.d. | + |
| **CK2$\alpha$'** | n.d. | n.d. | 33 | + | + |
| **CK2$\beta$** | n/a | n/a | n/a | + | + |
| **JNK3** | 104 | 82 | 0 | - | - |
| **ROCK1** | 126 | 105 | 100 | + | + |
| **ROCK2** | n.d. | n.d. | 100 | + | + |
| **ASK1/MAP3K5** | 92 | n.d. | 100 | - | - |
| **AURKA/STK6** | 23 | n.d. | 61 | - | + |
| **Reference** | Golub *et al.* (compound 6a, 10 µM) | Bain *et al.* (10µM) | Bibian *et al.* (10µM KINOME) | Figure 2 this manuscript; Beck *et al.*; Bryk and Wiśniewski | |

**Supplementary Table 1.** Data from literature on specificity of the inhibitors used in this study. n/a = not applicable (CK2$\beta$ is a regulatory subunit and has no kinase activity). Where assays do not specify inhibition of a specific isoform of CK1 or CK2, data is given for the function of either CK2 or CK1 after treatment and listed in brackets. n.d. = no data.

**Supplementary References**

1. Beck, M. *et al.* The quantitative proteome of a human cell line. *Mol. Syst. Biol.* **7,** 1–8 (2011).