Evolution shapes the responsiveness of the D-box enhancer element to light and reactive oxygen species in vertebrates.

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Supplementary material

Figure S1

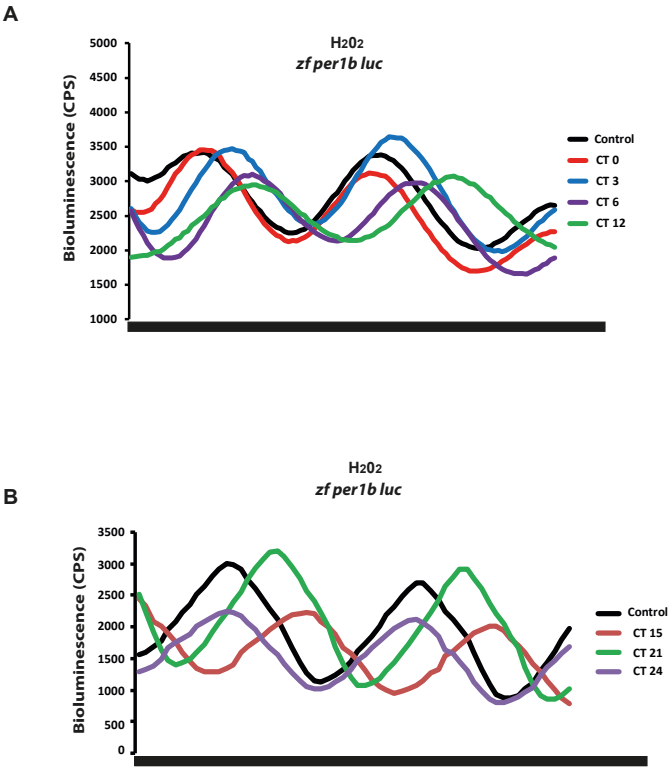

Figure S1: H_2O_2 entrains the zebrafish cell circadian clock. (A, B) Luciferase expression profile of the *zf per1b-luc* luciferase construct stably expressed in PAC-2 cells upon H_2O_2 treatment delivered at different time points during the subjective **(A)** day and **(B)** night (CT). Relative bioluminescence is plotted on the y-axis and time on the x-axis. Each trace is plotted as the mean from a minimum of n=4 independent wells.

Figure S2

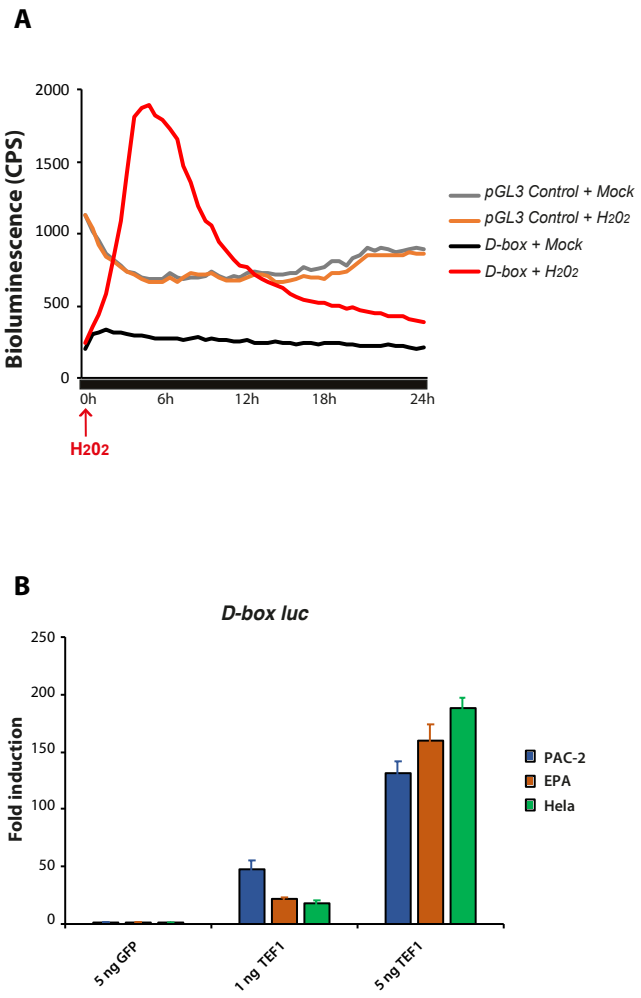

Figure S2: D-box activation by H_2O_2 . (A) Real time luciferase expression profile of D-box and SV40-driven (*pGL3 Control*) luciferase reporters in PAC-2 cells upon treatment with 300µM H_2O_2, during a 24 hours period. As a negative control for the H_2O_2 treatment, PAC-2 cells were mock treated with L15 medium. The black bar below the panel indicates the constant darkness during the experimental procedure. Relative bioluminescence is plotted on the y-axis and time on the x-axis. Each trace is plotted as a mean of n=4 independent wells. **(B)** *in vitro* luciferase assay of PAC-2, EPA and HeLa cells co-transfected with the *D-box Luc* reporter and two concentrations (1ng and 5ng) of the expression vectors for the TEF1 transcription factor or EGFP (negative control). Mean (n=3) ± SD of the relative fold induction of bioluminescence relative to the control is plotted on the y-axis and the expression vector with its concentration is indicated on the x-axis.

Figure S3

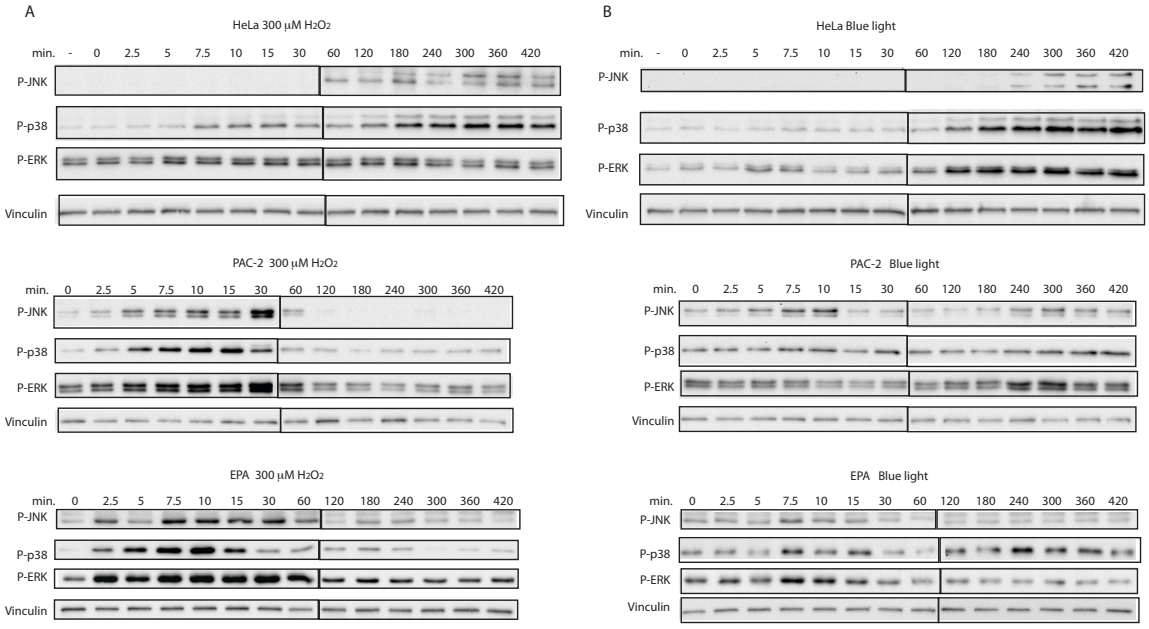

Figure S3: Regulation by light and ROS of stress-induced MAP kinases. (A, B) Representative western blots for P-JNK, P-p38, P-ERK and vinculin as a loading control in HeLa (upper panels), PAC-2 (central panels), and EPA (lower panels), treated for 420 minutes (7 hours) with **(A)** 300 μM H_2O_2 or **(B)** blue light. Each panel is assembled from cropped western blotting images (see Supplementary material file for the original images). Specifically, for all PAC-2 and HeLa data, each time course is assembled from two identically and simultaneously processed western blots, where the first blot contained samples from time 0 to 30 minutes of treatment and the second contained samples from 60 to 420 minutes. In the case of the EPA cell data, the first blot contained samples from time 0 to 60 minutes of treatment and the second from time 120 to 420 minutes.

Table S1:

Reagent	Stock Concentration	Final Concentration	Solvent:
Diphenyleneiodonium (DPI)	Sigma Aldrich D2926 - 10mg	1- 15 μM	DMSO
N-acetylcysteine (NAC)	Sigma Aldrich A9165-5 mg	2 - 6 mM	H_2O
VAS2870	Enzo Life Sciences BML–EI395-0010 -10 mg	5 - 20 μM	DMSO
U0126	Sigma Aldrich	1-40 μM	DMSO

Table S2: qRT-PCR zebrafish (*zf*) and *P.andruzzii* cavefish (*cf*) primers sequences

Gene:	**Forward primer**	**Reverse primer**
zfβ-actin	F: GCCTGACGGACAGGTCAT	R: ACCGCAAGATTCCATACCC
zfper2	F: CTTCACCACACCATACAGG	R: GTCTGACGGGGACGAGTCT
zfcry1a	F: TCCGCTGTGTGTACATCCTC	R: CAAACACTGCAGCAAAAACC
cfcry1a	F: GGCTCCACGACAATCCTTCA	R: TGGGGAAGACATCGGTAGGT
cfper2	F: CCGCAAAGTTTCCTTCGTCA	R: CATTACTGCCCAGACTCCCA
cfβ-actin	F: GATGAGGAAATCGCTGCCCT	F: GTCCTTCTGTCCCATGCCAA

Table S3: Antibodies

Sigma-Aldrich V9131 **α- vinculin**	α –vinculin monoclonal clone hVIN-1
Cell Signaling 9211S **α- Phospho-p38 MAPK**	Thr180/Tyr182
Cell Signaling 9101S **α- Phospho-p44/p42 MAPK**	Thr202/Tyr204
Cell Signaling 9251S **α- Phospho SAPK/JNK**	T183/Y185

Table S4: Luciferase reporters and expression constructs.

zf per1b-Luc	Vallone, Gondi et al. 2004 [25]
D-box$_{cry1a}$-Luc	Mracek et al. PLOS One, 2012 [26]
pcDNA3.1/myc-His/lacZ	Invitrogen
pGL3 Control	Promega
EGFP	*pCS2 EGFP* was a gift from Prof. Steffen Scholpp (University of Exeter, UK)
DN-p38	*pcDNA-3 Flag p38 alpha (agf)* a gift from Roger Davis (Addgene plasmid #20352) Enslen, Raingeaud et al. 1998 [66]

DN-JNK1	*pcDNA-3 Flag Jnk1a1(apf)* a gift from Roger Davis (Addgene plasmid # 13846) Derijard, Hibi et al. 1994 [67]
TEF1	*pcDNA3.1-TEF1* Vatine et al. PLOS Biology, 2009 [37]