**Supplementary data**

# Nuclear Magnetic Resonance affects the Circadian Clock and Hypoxia Inducible Factor isoforms in Zebrafish

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**Running head:** NMR and the circadian clock

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**Supplemental Figure S1: Treatment and sampling schedules of the different experiments: experimental setup A** was used for the experiment on early transcriptional response (S3); **experimental setup B** was used for zebrafish cells and larvae (Figure 1, Figure 2, Figure 3); **experimental setup C** was used for circadian protein expression of Hif-1α and Hif-3α (Figure 4) and **experimental setup D** was used for the experiment on redox markers (Figure 5);

**Supplemental Figure S2:** MBST® control unit with chip card (left) and MBST® NMR device with a metal free fish tank placed in the center (right); in the fish tank two breeding boxes are visible, in which zebrafish larvae were held throughout the experiment.

**Supplemental Figure S3: mRNA copy numbers of selected genes after varying NMR treatment durations of 1h, 2h and 4h, respectively**: No early transcriptional response upon NMR irradiation was detected for the genes (**A**) *per1b* , (**B**) *cry1aa,* (**C**) *hif1-α* and (**D**) *hif-3α* after a single 1h, 2h or 4h treatment when measured once directly afterwards; Black bars represent sham treated cells, turquoise bars those treated with NMR; data are presented as means ± standard error (n=4 to 6).

**Supplemental Figure S4:** *per1b* promoter activity driving luciferase in the Zebrafish cell line DAP49: A single treatment with NMR for four hours in the ascending part of *per1b* oscillation does not lead to any alteration in promoter activity; sham treated cells (black line), NMR treated cells (turquoise line); presented as means ± standard error, (n=6).

**Supplemental Figure S5: A:** protein levels Prx-SO2/3 ($Prx_{ox}$) increasing with increasing concentrations of extracellular $H_2O_2$; **B:** Hif-3α protein levels after a single 1h treatment compared to a single 4h treatment with either sham or NMR; data are presented as means ±

standard error (n=3) for $Prx_{ox}$, (n=8) for Hif-3α; asterisks mark significant differences after applying Two Way ANOVAs (GraphPad Prism version 6.00), significance was accepted for $p \leq 0.05$.

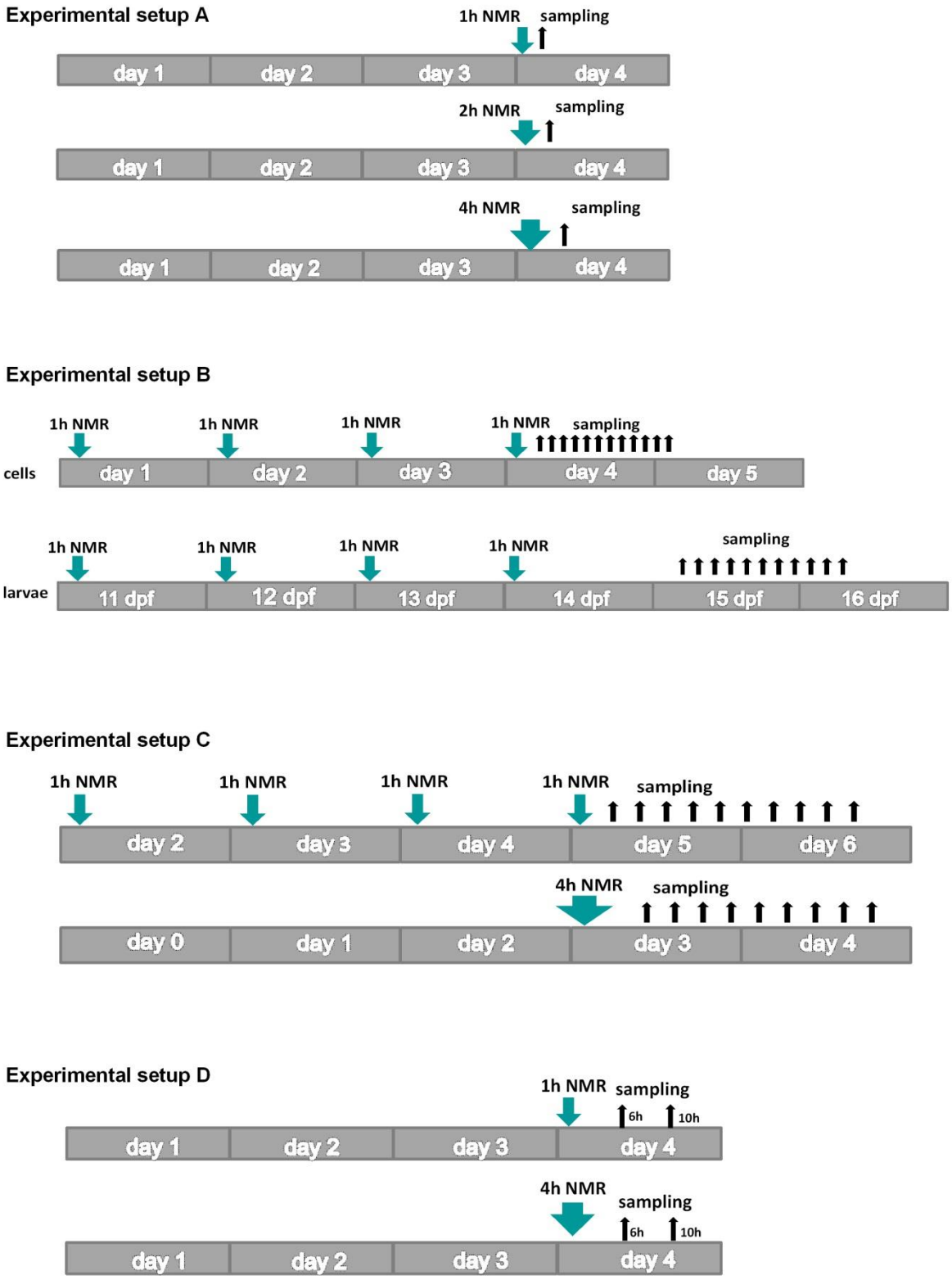

Experimental setup A
1h NMR
sampling
day 1
day 2
day 3
day 4
2h NMR
sampling
day 1
day 2
day 3
day 4
4h NMR
sampling
day 1
day 2
day 3
day 4
Experimental setup B
1h NMR
1h NMR
1h NMR
1h NMR
sampling
cells
day 1
day 2
day 3
day 4
day 5
1h NMR
1h NMR
1h NMR
1h NMR
sampling
larvae
11 dpf
12 dpf
13 dpf
14 dpf
15 dpf
16 dpf
Experimental setup C
1h NMR
1h NMR
1h NMR
1h NMR
sampling
day 2
day 3
day 4
day 5
day 6
4h NMR
sampling
day 0
day 1
day 2
day 3
day 4
Experimental setup D
1h NMR
sampling
6h
10h
day 1
day 2
day 3
day 4
4h NMR
sampling
6h
10h
day 1
day 2
day 3
day 4

**Figure S1**

**Figure S2**

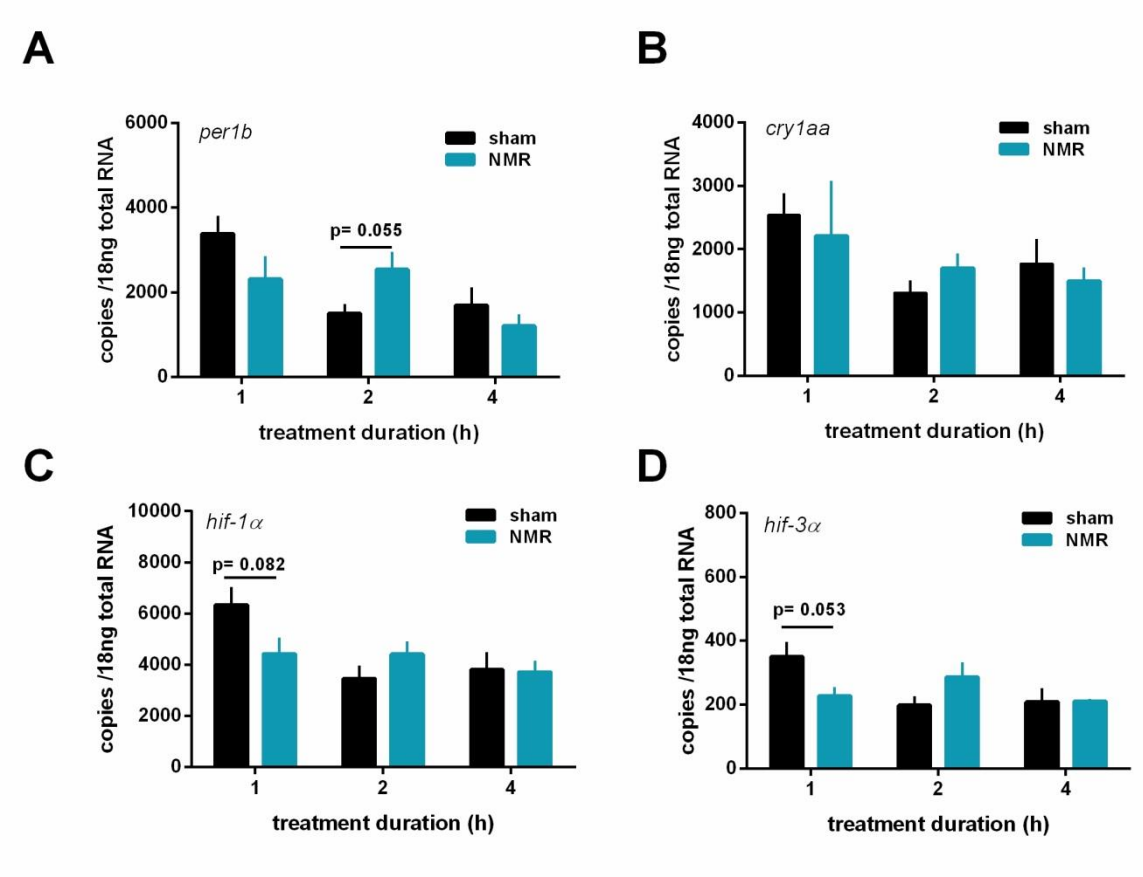
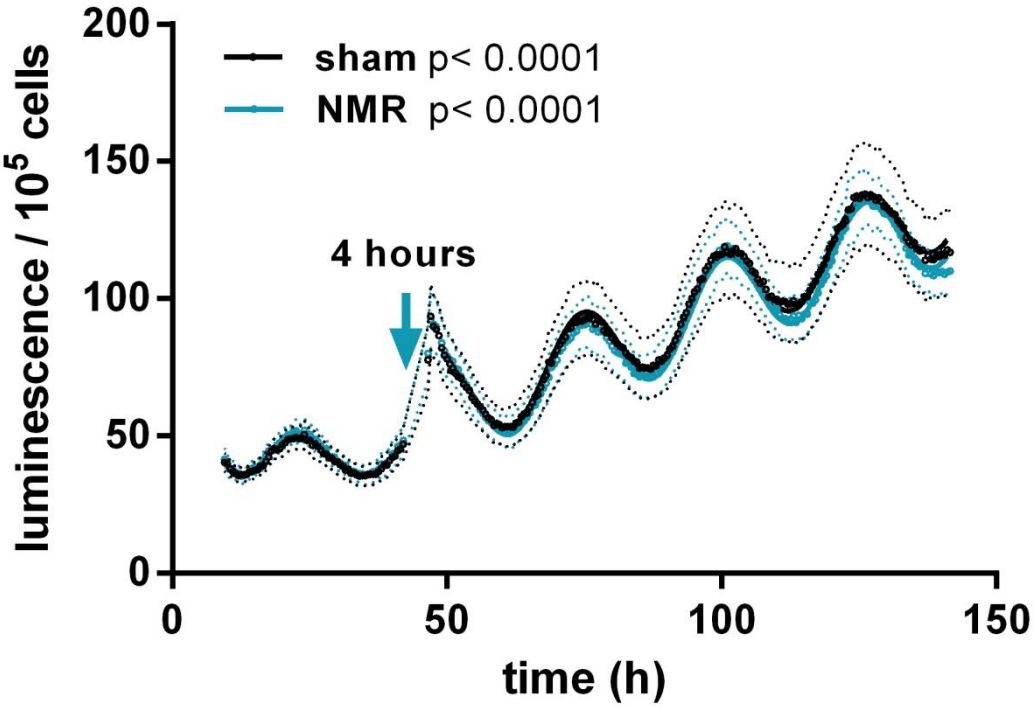

A
per1b
sham
NMR
p= 0.055
copies /18ng total RNA
6000
4000
2000
0
1
2
4
treatment duration (h)
B
cry1aa
sham
NMR
copies /18ng total RNA
4000
3000
2000
1000
0
1
2
4
treatment duration (h)
C
hif-1α
sham
NMR
p= 0.082
copies /18ng total RNA
10000
8000
6000
4000
2000
0
1
2
4
treatment duration (h)
D
hif-3α
sham
NMR
p= 0.053
copies /18ng total RNA
800
600
400
200
0
1
2
4
treatment duration (h)

**Figure S3**

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65 **Figure S4**

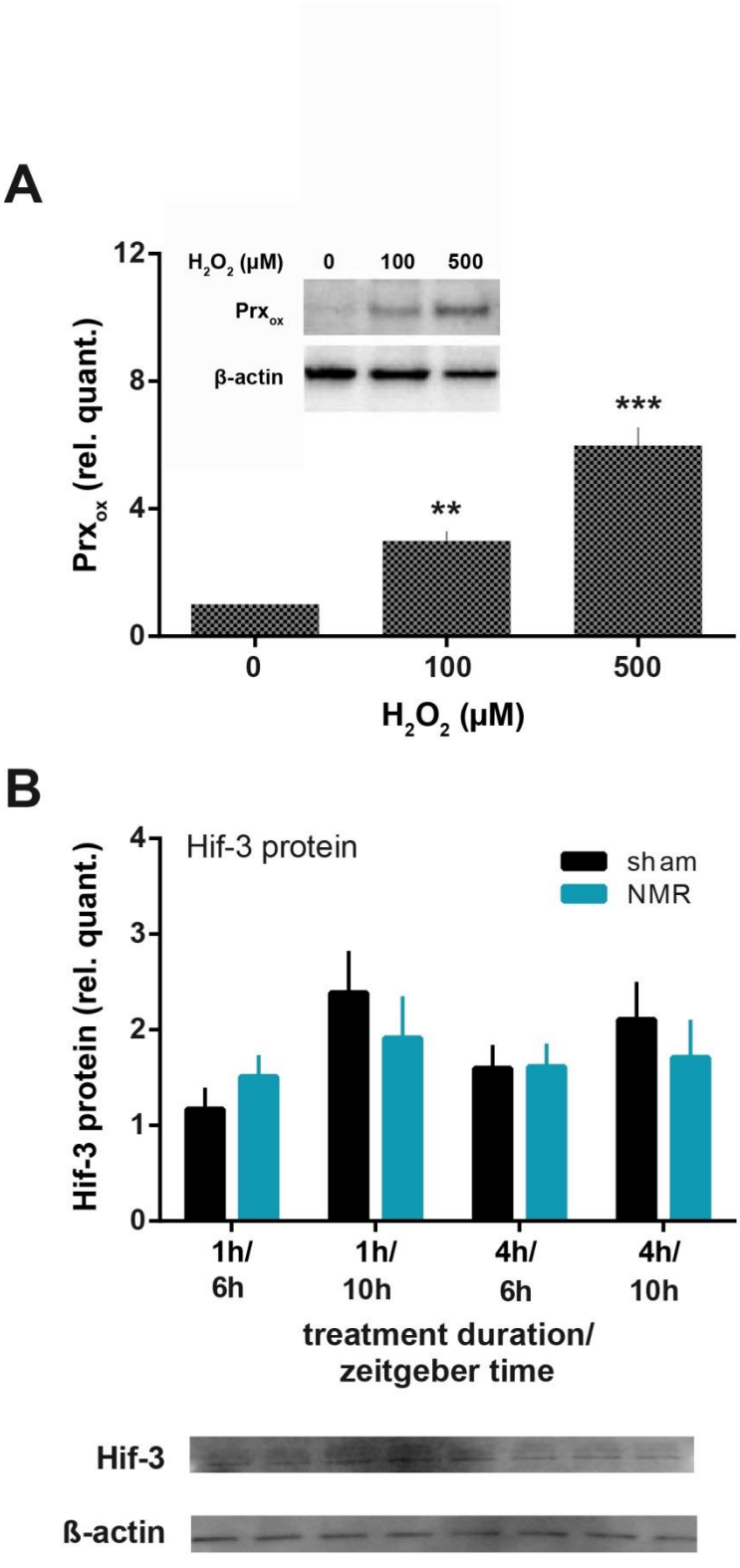
A
H2O2 (µM)
0
100
500
Prxox
β-actin
Prxox (rel. quant.)
12
8
4
0
**
***
0
100
500
H2O2 (µM)
B
Hif-3 protein
sham
NMR
Hif-3 protein (rel. quant.)
4
3
2
1
0
1h/ 6h
1h/ 10h
4h/ 6h
4h/ 10h
treatment duration/ zeitgeber time
Hif-3
ß-actin

**Figure S5**