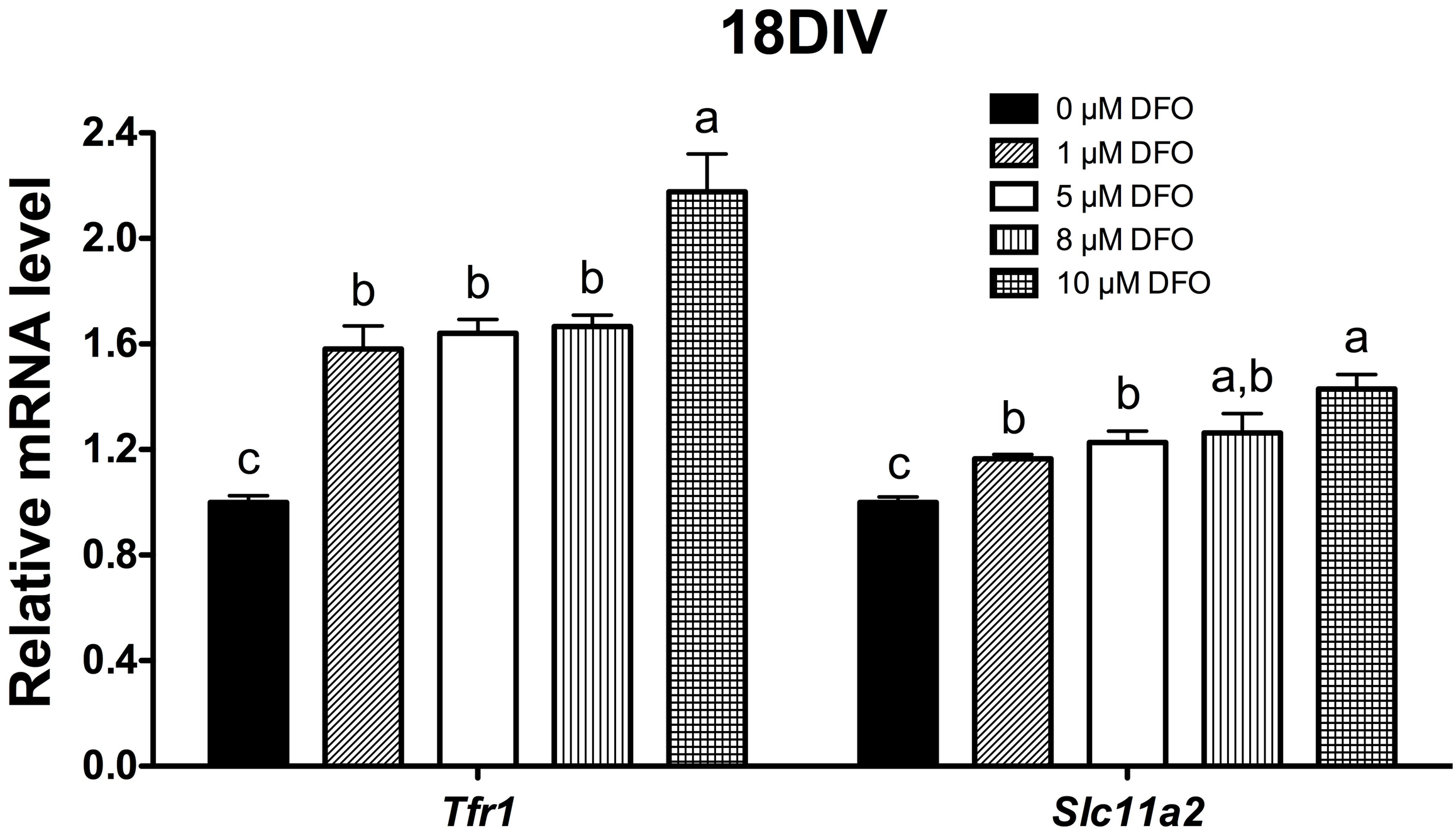
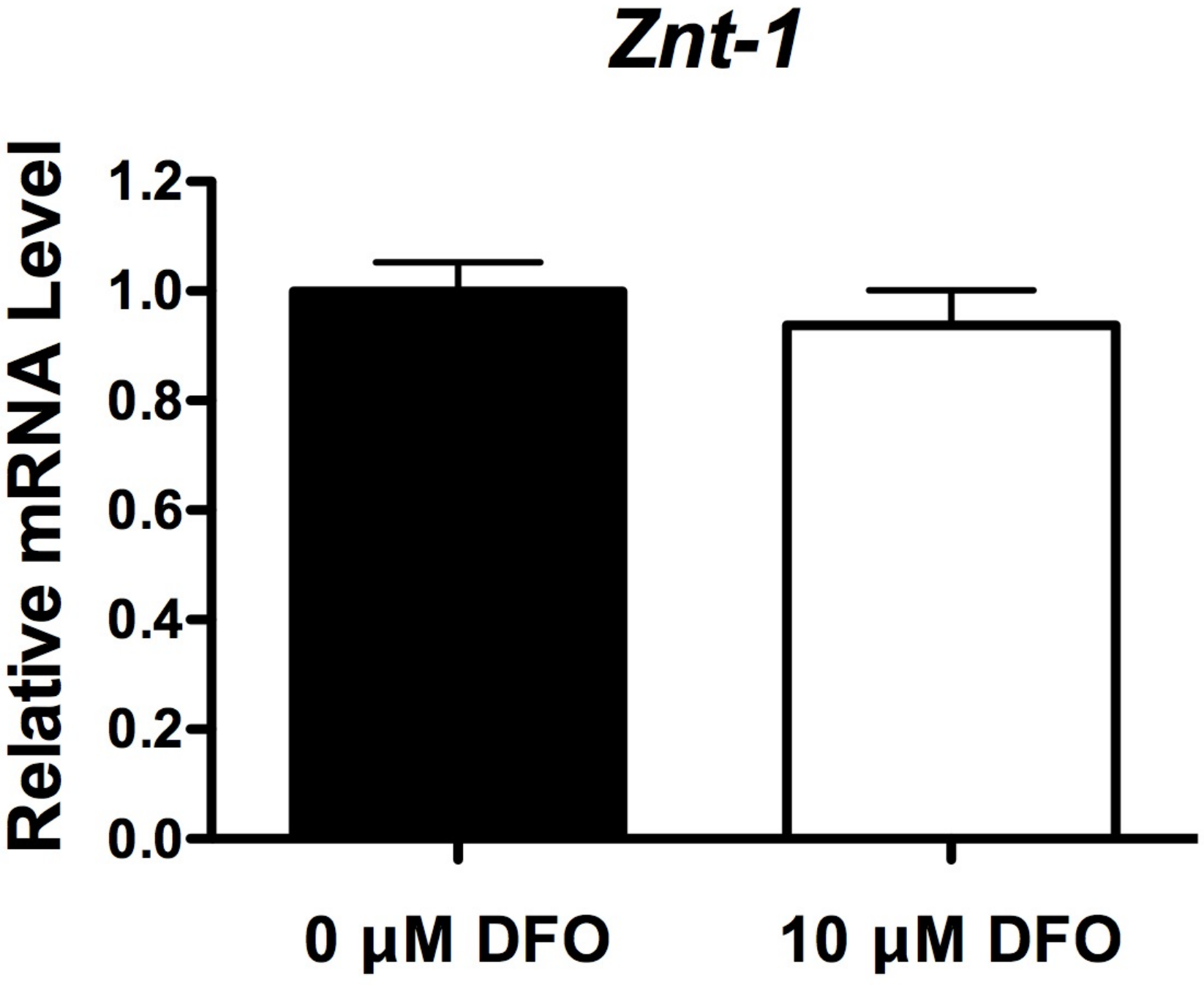

18DIV
Relative mRNA level
2.4
2.0
1.6
1.2
0.8
0.4
0.0
0 μM DFO
1 μM DFO
5 μM DFO
8 μM DFO
10 μM DFO
c
b
b
b
a
c
b
b
a,b
a
Tfr1
Slc11a2


Znt-1
Relative mRNA Level
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0 µM DFO
10 µM DFO

Supplemental Table 1: Seahorse Assay Protocol

| Command | Time (min) | Drug Injection | Cycles |
|---|---|---|---|
| Calibrate | | | |
| Mix | 3:00 | | |
| Wait | 2:00 | None | 3 |
| Measure | 3:00 | | |
| Mix | 3:00 | | |
| Wait | 2:00 | Oligomycin | 3 |
| Measure | 3:00 | | |
| Mix | 3:00 | | |
| Wait | 2:00 | FCCP | 3 |
| Measure | 3:00 | | |
| Mix | 3:00 | | |
| Wait | 2:00 | Antimycin A/Rotenone | 3 |
| Measure | 3:00 | | |

**Supplemental Figure Legends:**

**Supplemental Figure 1: The effect of various DFO doses on *Tfr1* and *Slc11a2* gene expression at 18DIV.** Hippocampal neurons cultured from E16 mice were treated with various DFO doses (i.e., 0, 1, 5, 8, or 10 μM) and 5-FU beginning at 3DIV. At 18DIV, cells were collected, total RNA was extracted, and cDNA was synthesized. Quantitative real-time PCR (qPCR) was performed for *Tfr1* and *Slc11a2*, two IRE/IRP-regulated genes involved in neuronal iron uptake that index neuronal iron status. Relative mRNA levels are calculated relative to an internal control cDNA sample and a reference gene (i.e., *Tbp*). The data from 1-3 independent cultures were pooled and are presented as mean ± SEM. Groups not sharing a common letter (a > b > c) are significantly different by one-way ANOVA and Tukey's post-hoc multiple comparison test ($p < 0.05$). n=4-10.

**Supplemental Figure 2: The effect of DFO treatment on neuronal zinc status.** Hippocampal neurons cultured from E16 mice were treated with DFO and 5-FU beginning at 3DIV. At 11DIV, cells were collected, total RNA was extracted, and cDNA was synthesized. Quantitative real-time PCR (qPCR) was performed for *Znt-1*, a gene coding for a zinc transporter, to index neuronal zinc status. Relative mRNA levels are calculated relative to an internal control cDNA sample and

a reference gene (i.e., *Tbp*). The data are presented as mean ± SEM. There was no statistical difference between groups by Student's t-test ($p < 0.05$). n=5.