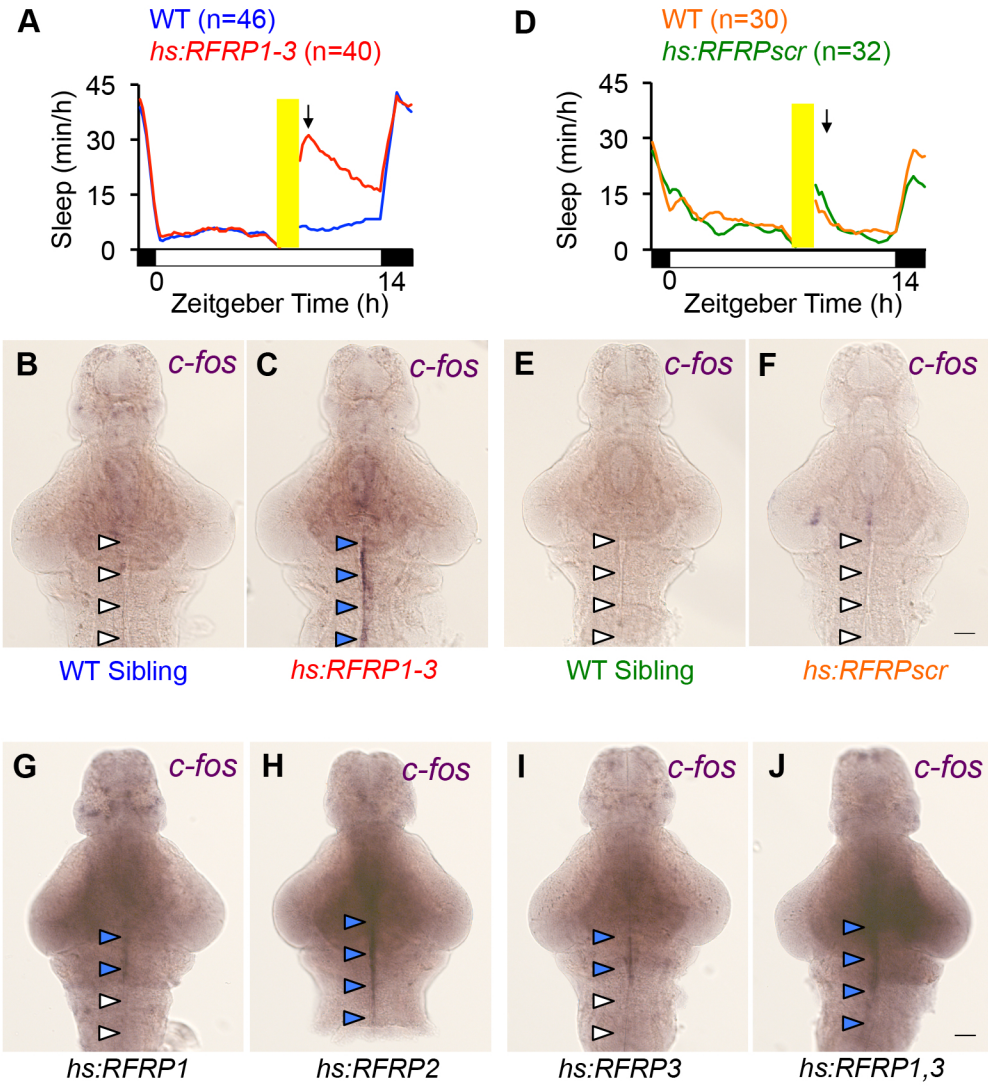


**Figure 2 - Figure Supplement 2.**

***c-fos* expression correlates with overexpression of RFRP peptides that induce sleep.**

One hour after heat-shock, sleep is significantly increased in *Tg(hs:RFRP1-3)* animals compared to their WT siblings (**A**, arrow) but there is no effect on sleep in *Tg(hs:RFRPscr)* animals compared to their WT siblings (**D**, arrow). We assayed neuronal activity at this time point in animals overexpressing different RFRP peptides by performing *c-fos* ISH. In animals overexpressing RFRP1-3, we observed prominent *c-fos* expression along the brain ventricular lining from the hypothalamus to the hindbrain (**C**, 4 blue arrowheads) that was absent in WT siblings (**B**, 4 white arrowheads). Overexpression of specific RFRP peptides affected *c-fos* expression in a manner that correlated with their effects on behavior (**Figure 2E**). No *c-fos* expression was observed in animals overexpressing RFRPscr, in which all 3 RFRP peptides are scrambled (**F**, 4 white arrowheads), weak *c-fos* expression was observed in animals overexpressing either RFRP1 or RFRP3 (**G** and **I**, 2 blue and 2 white arrowheads), and strong *c-fos* expression was observed in animals overexpressing RFRP2, or RFRP1 and RFRP3 (**H** and **J**, 4 blue arrowheads), similar to overexpression of RFRP1-3 (**C**). Yellow bars indicate heat shock. White and black bars under behavioral traces indicate day (14 h) and night, respectively. Scale: 50 μm.