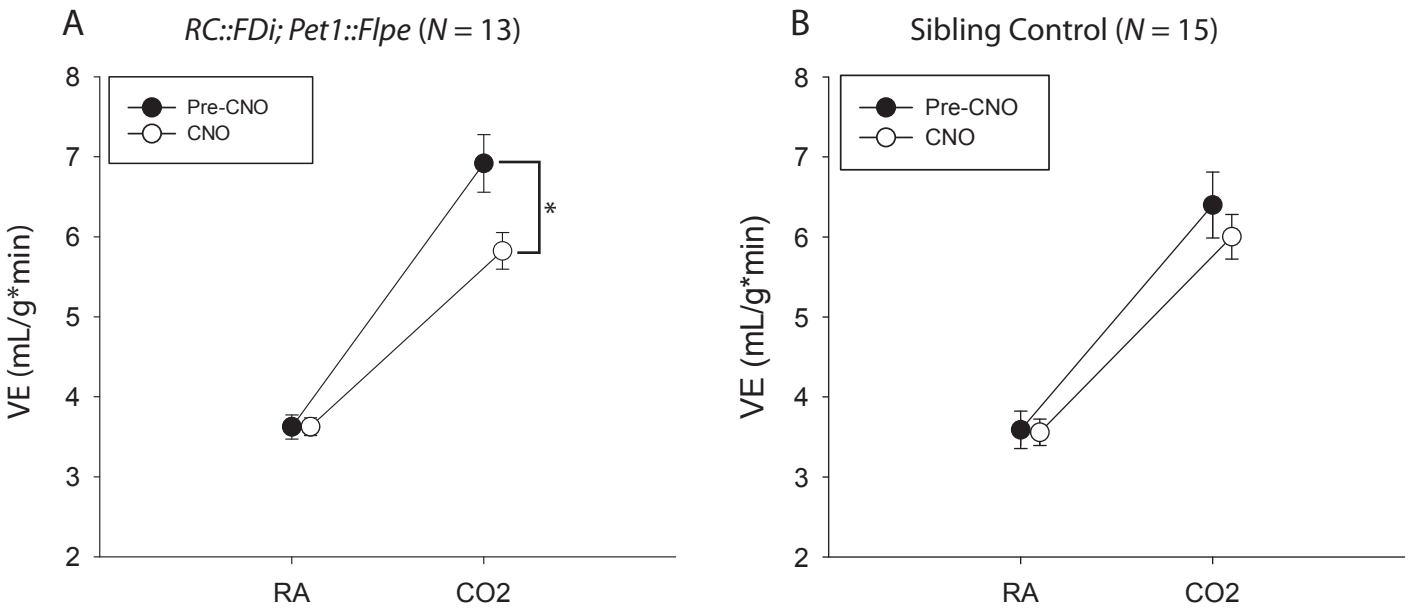
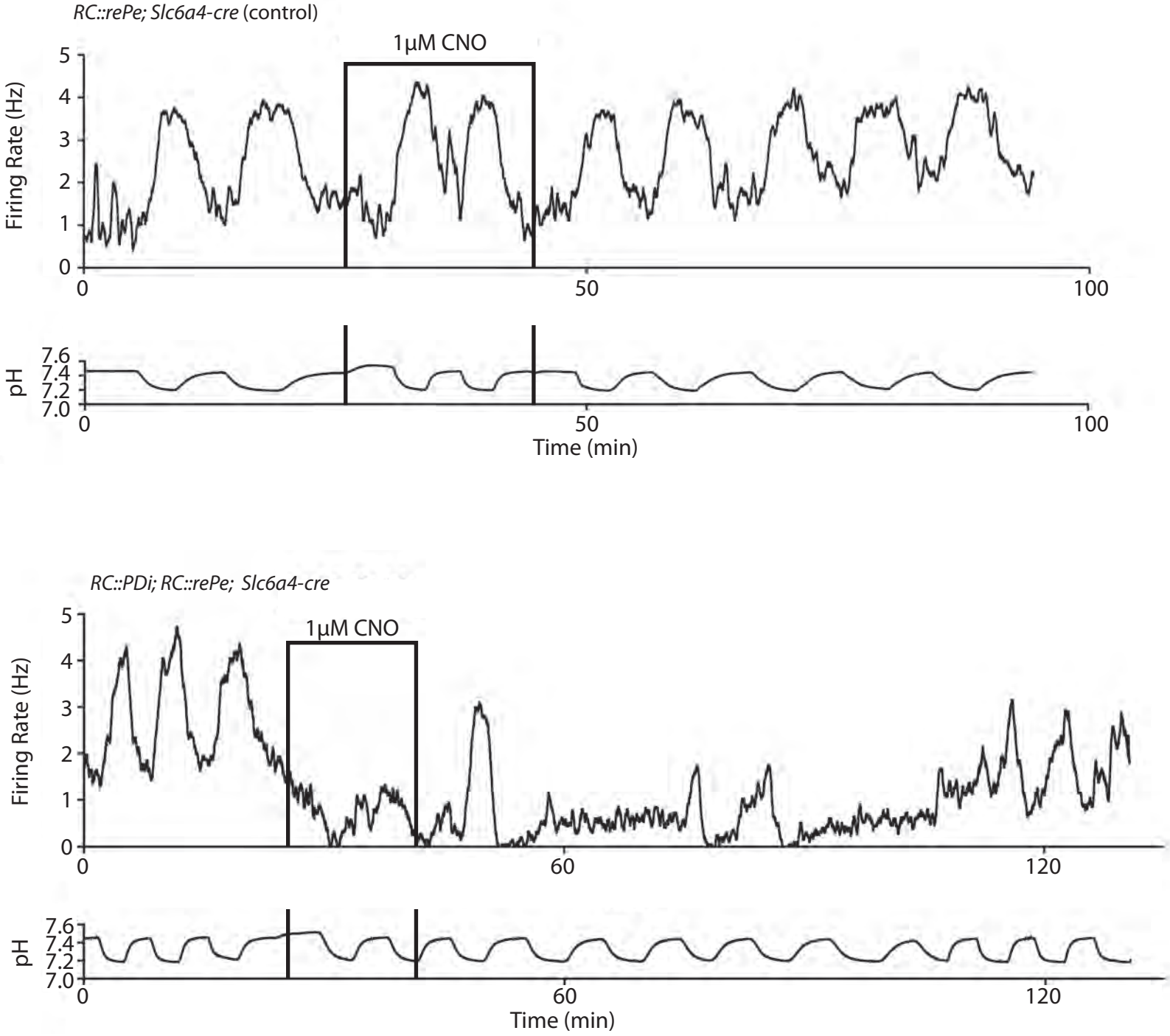
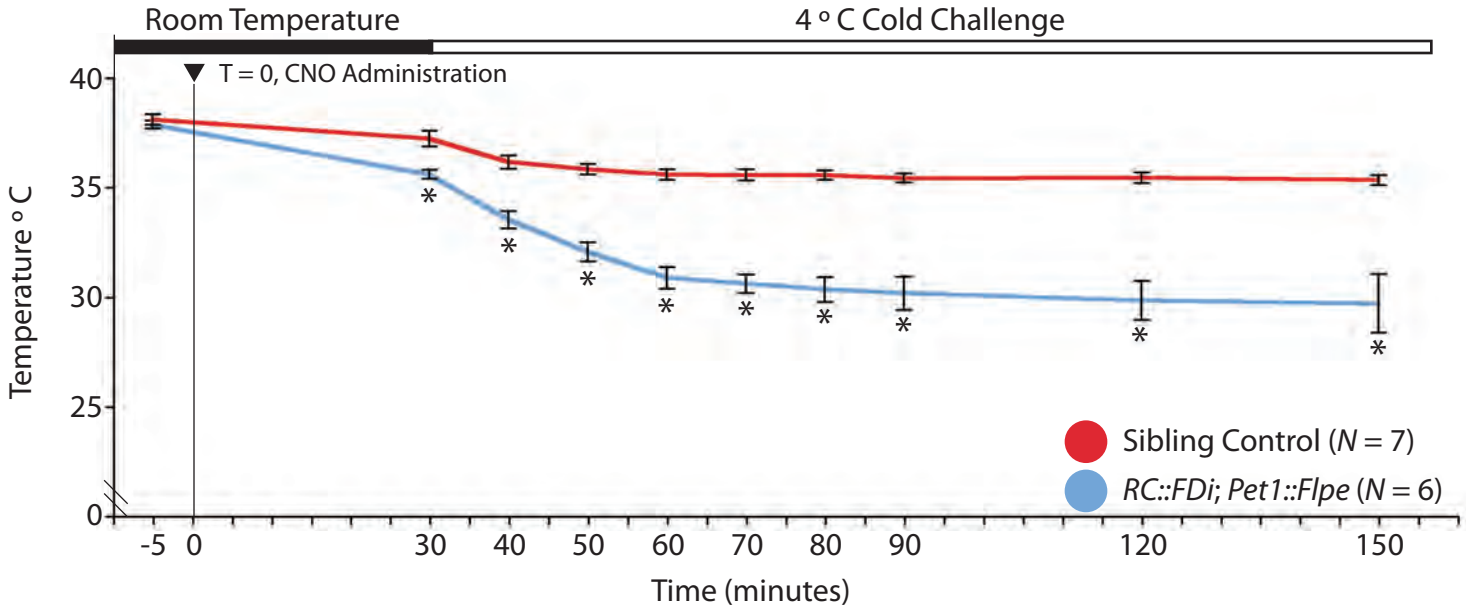


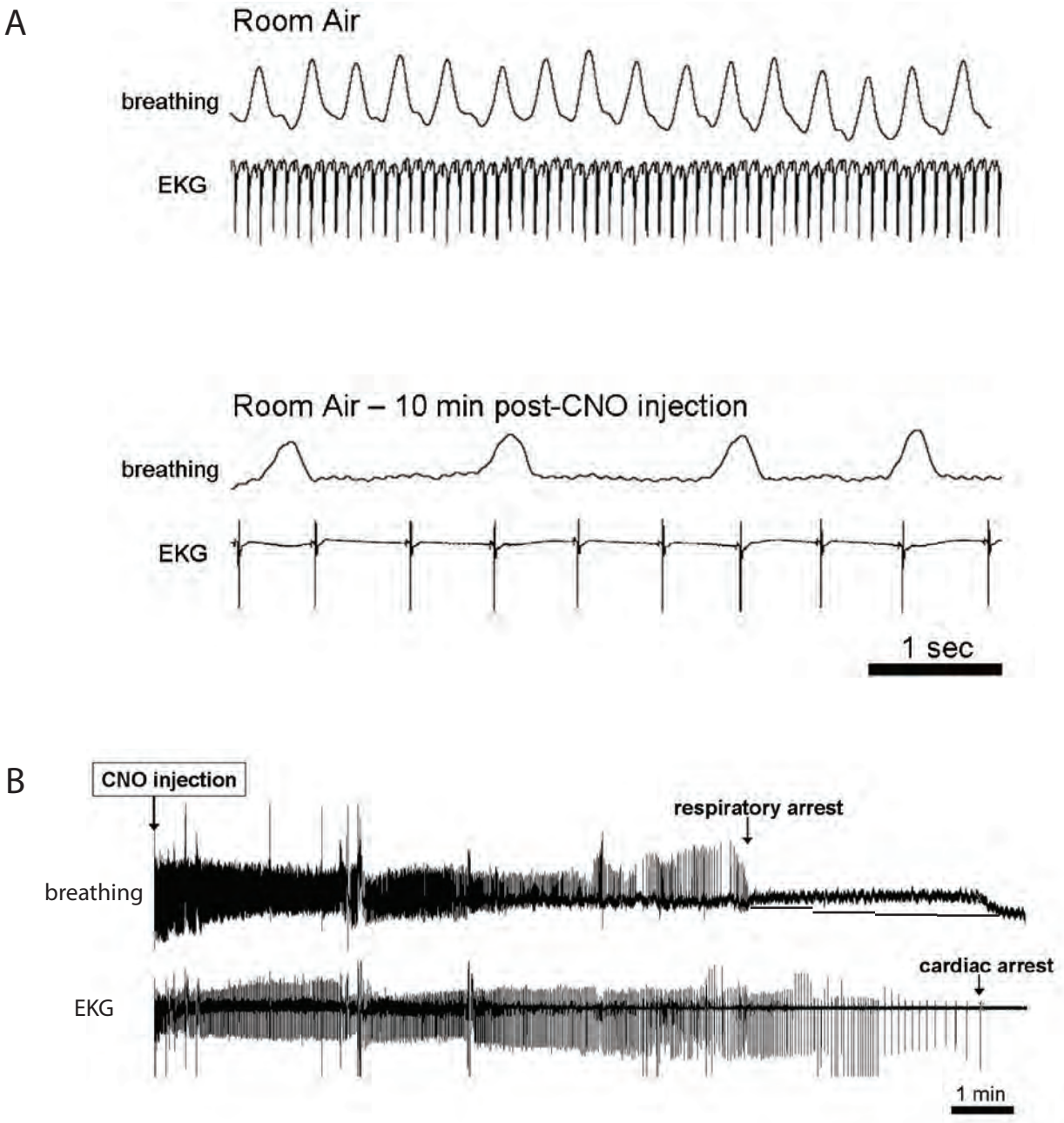
Sup. Fig. 1. CNO/Di-inhibition of *Pet1::Flpe*-defined serotonergic neurons disrupts the central respiratory CO_2 chemoreflex in conscious adult mice. Double transgenic *RC::FDi; Pet1::Flpe* mice were assessed as in Fig. 3. with plethysmograph chamber temperature held at 34ºC. (A) *RC::FDi; Pet1::Flpe* showed reduced hypercapnic ventilatory responses after administration of CNO (10 mg/kg), *p = 0.001 (RM ANOVA followed by Tukey post-hoc). (B) Sibling controls. CNO administered trials shifted slightly to the right for clarity.



Sup. Fig. 2. Reduced firing rate response to 9% CO_2 in *RC::PDi; RC::rePe; Slc6a4*-cre neurons upon 1μM CNO application. Traces of firing rate (Hz) and simultaneous bath pH from cultured medullary serotonergic neurons from control *RC::rePe; Slc6a4*-cre mice (top) and *RC::PDi; RC::rePe; Slc6a4*-cre mice (bottom), similar to Fig. 3 (E).



Sup. Fig. 3. CNO/Di-inhibition of *Pet1::Flpe*-defined serotonergic neurons results in reduced ability to thermo-regulate. Body temperature at room temperature (~23ºC) was assessed 5 min before a single CNO intraperitoneal injection (10 mg/kg) and then again after 30 min. Mice were then subject to ambient conditions of 4ºC with body temperature measurements taken every 10 min for the next hour and then every 30 min. *RC::FDi; Pet1::Flpe* male mice showed an inability to regulate body temperature as compared to control siblings, *p < 0.01 (unpaired t-test).



Sup. Fig. 4. Broad constitutive Di expression is compatible with development and triggers respiratory and cardiac arrest upon CNO injection. (A) Example trace showing that pre-CNO, *RC::Di* adult mice have normal respiratory and heart rates (53) (154 ± 15 (SEM) breaths/min and 478 ± 57 (SEM) beats/min; animals analyzed = 4) that depress severely (55 ± 11 breaths/min, 70 ± 15 beats/min) within 10 min of CNO injection (10mg/kg i.p.). (B) An example full respiratory and heart rate trace showing heart rate reduction within 30 s of CNO injection, with cardic arrest occuring within 20 minutes (4 minutes after respiratory arrest).