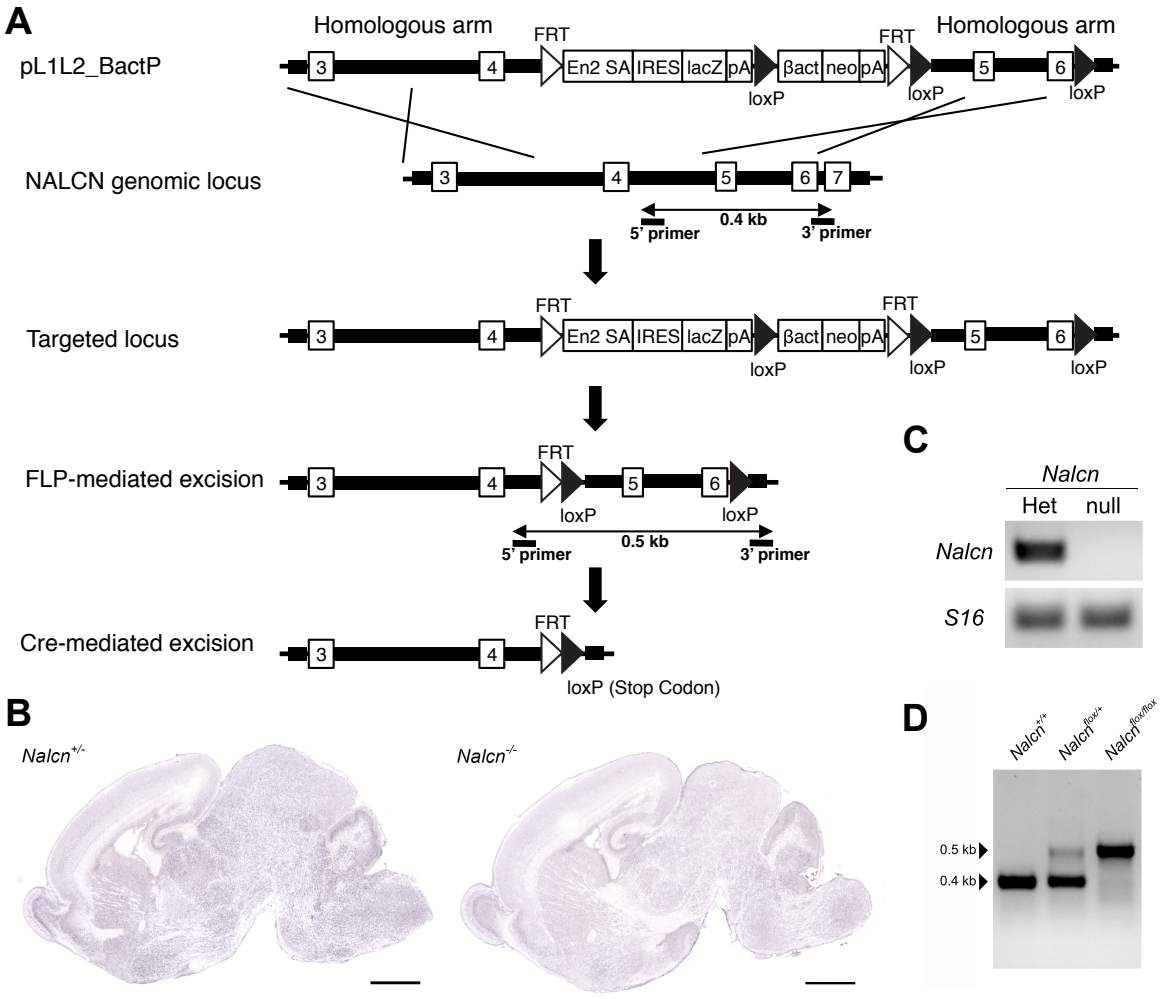


Figure S1. Generation of a targeted *Nalcn* floxed allele (related to STAR Methods).
(A) The L1L2_Bact_P cassette was placed between intact *Nalcn* 5' and 3' homology arms (thick lines) (pL1L2_BactP). Exon 5 and 6 at 3' homology arm are flanked by *loxP* sites. This construct was targeted to the *Nalcn* genomic locus, followed by *in vivo* removal of the L1L2_Bact_P cassette, as depicted. *Nalcn* null mice were generated using $Hprt^{Cre}$ line and showed no *Nalcn* expression, according to both **(B)** *in situ* hybridization (scale bars represent 500 μm) and **(C)** qPCR. **(D)** Genotyping for *Nalcn* floxed allele.

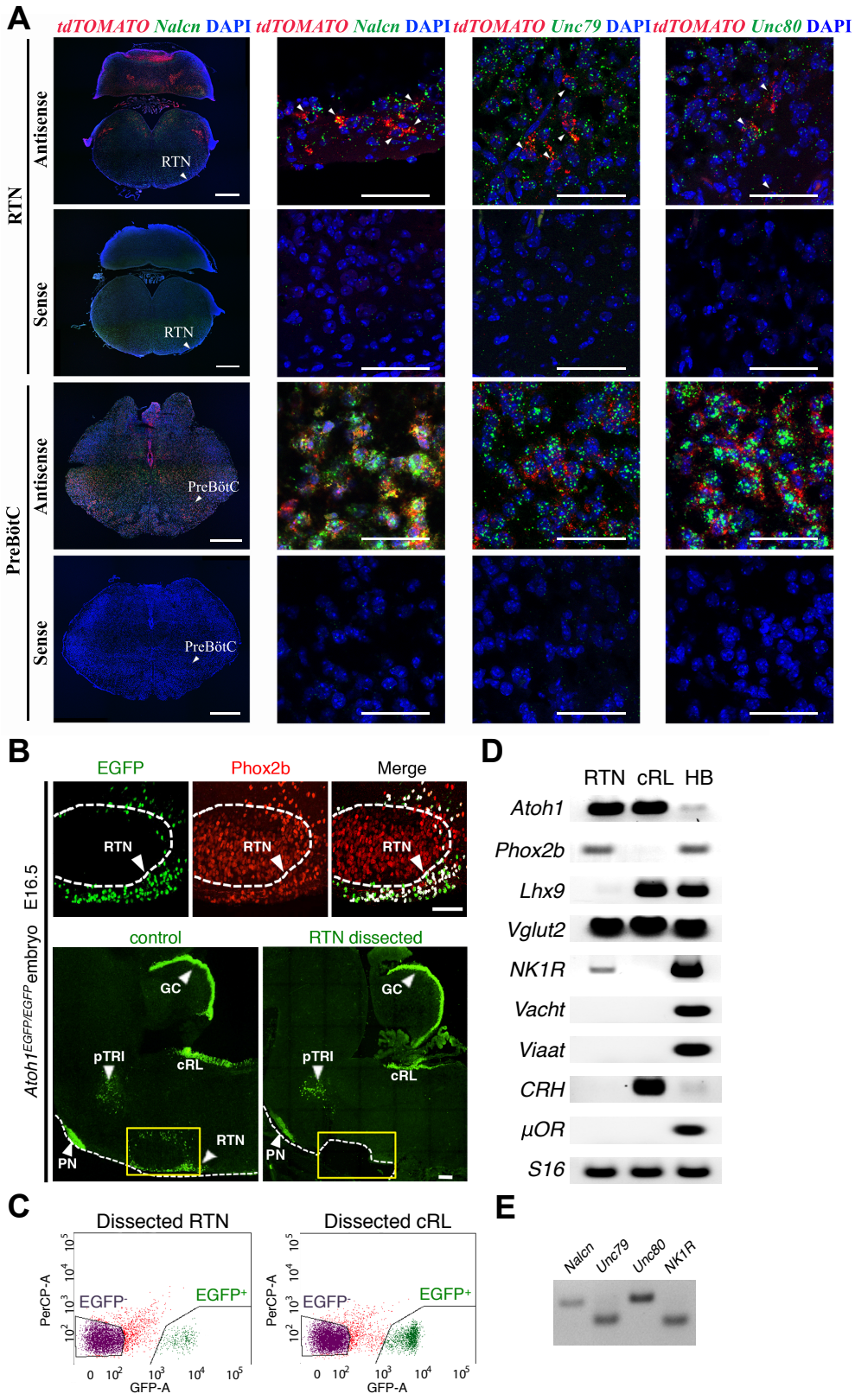

A
tdTOMATO Nalcn DAPI
tdTOMATO Nalcn DAPI
tdTOMATO Unc79 DAPI
tdTOMATO Unc80 DAPI
RTN
PreBötC
Antisense
Sense
RTN
PreBötC
B
EGFP
Phox2b
Merge
E16.5
Atoh1EGFP/EGFP embryo
control
RTN dissected
GC
pTRI
cRL
RTN
PN
C
Dissected RTN
Dissected cRL
PerCP-A
EGFP-
EGFP+
GFP-A
D
RTN
cRL
HB
Atoh1
Phox2b
Lhx9
Vglut2
NK1R
Vacht
Viaat
CRH
μOR
S16
E
Nalcn
Unc79
Unc80
NK1R

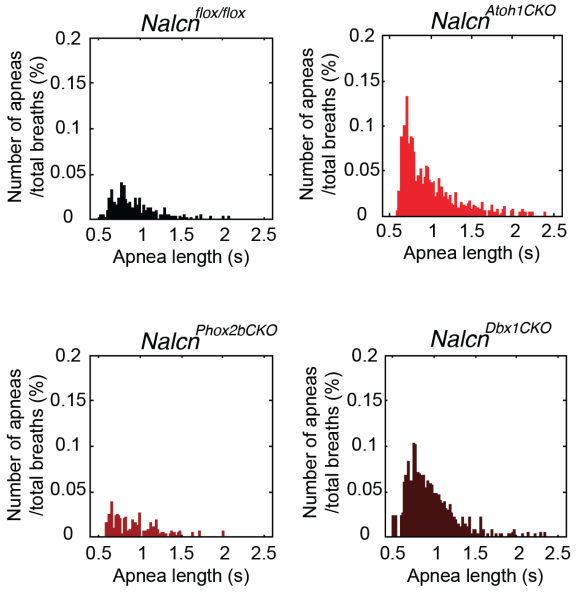
Figure S2. *Nalcn* mRNA is expressed in the RTN and preBötC neurons (related to STAR Methods).
(A) Double *in situ* hybridization using *tdTOMATO*, *Nalcn*, *Unc79*, and *Unc80* antisense probes showes that P0 *tdTOMATO*$^{+}$ RTN (*Atoh1*^{Cre}*; Rosa*$^{LSL\text{-}tdTOMATO}$) (white arrowheads) and preBötC neurons (*Dbx1*^{Cre}*; Rosa*$^{LSL\text{-}tdTOMATO}$) (red) co-express *Nalcn*, *Unc79*, or *Unc80* (green). Scale bar represents 500 and 50 μm.
(B) The Atoh1-EGFP allele marks the Phox2b^{+} RTN neurons at E16.5 (top, white arrowheads), which were selectively dissected (bottom, yellow rectangular box) for cell sorting. Scale bars represent 100 μm.
(C) Representative images for FACS-sorted EGFP^{+} RTN neurons (left) and cRL (right) neurons.
(D) RT-PCR verified the identity of the FACS-isolated RTN (left lane) and cRL (middle lane) neurons and demonstrated that they express corresponding cell type-specific markers. The E16.5 whole hindbrains (HB) were harvested for comparison.
(E) RT-PCR verified the expression of *Nalcn, Unc79,* and *Unc80* in the FACS-isolated RTN.

A

	P0		
	Nalcn$^{flox/flox}$	*Dbx1*^{Cre}; *Nalcn*$^{flox/+}$	*Nalcn*^{Dbx1CKO}
Baseline breathing rate (Br/min)	94.00±17.75	86.80±18.31	82.19±19.08
Tidal volume (mL/breath/g)	0.0047±0.0019	0.0054±0.0015	0.0052±0.0018
Minute ventilation (V_E) (mL/min/g)	0.428±0.001	0.456±0.001	0.418±0.001****
O_2 consumption (VO2) (mL/min/g)	0.063±0.097	0.064±0.065	0.065±0.128
Ventilatory equivalent for oxygen (V_E / VO_2)	6.833±0.011	7.187±0.007	6.652±0.019****

	7-week-old			
	Nalcn$^{flox/flox}$	*Nalcn*^{Atoh1CKO}	*Nalcn*^{Dbx1CKO}	*Nalcn*^{En1CKO}
Baseline breathing rate (Br/min)	239.31±28.73	227.19±13.59	219.63±39.81	211.45±19.34
Hypoxia peak (Br/min)	318.61±33.79	316.75±28.71	284.97±50.56	N/A
Hypercapnia (Br/min) (5% CO_2, 20% O_2 balanced with N_2)	330.39±66.50	335.13±23.51	327.47±19.46	N/A
Hypercapnia (Br/min) (5% CO_2 balanced with O_2)	359.48±29.30	323.54±40.21	313.59±35.60	N/A
Apnea frequency (Apnea/10^4 breaths)	65.9±48.0	145.9±104.0****	212.2±7.0****	112.38±55.46**
Inspiration time (sec)	0.086±0.010	0.085±0.009	0.113±0.015****	0.094±0.005
Expiration time (sec)	0.212±0.024	0.203±0.035	0.204±0.028	0.224±0.033
Tidal volume (mL/breath/g)	0.390±0.081	0.417±0.090	0.470±0.049	0.459±0.053

B



C

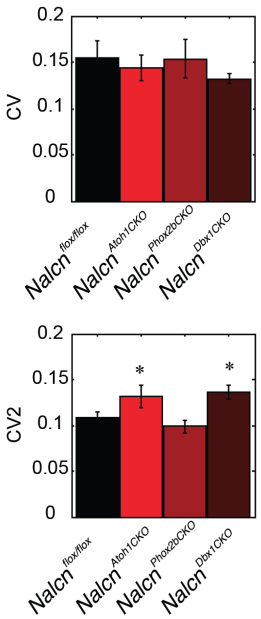


Figure S3. Loss of *Nalcn* in Atoh1^{+} lineage or preBötC neurons remains normal chemoreflex but leads to apneas (related to Figure 1).

(A) Breathing parameters from (1) P0 *Nalcn*$^{flox/flox}$ (n=12), *Dbx1*^{Cre}*; Nalcn*$^{flox/+}$ (n=14), and *Nalcn*^{Dbx1CKO} (n=14) newborns, and (2) 7-week-old *Nalcn*$^{flox/flox}$ (n=22), *Nalcn*^{Atoh1CKO} (n=28), *Nalcn*^{Dbx1CKO} (n=17), and *Nalcn*^{En1CKO} (n=12) mice. (One-way ANOVA; two-way ANOVA for hypoxic and hypercapnic exposure)

(B) Distribution of breaths that meet the criteria of apnea (see Methods) as a percentage of total observed breaths. For each animal the duration of apnea were binned in 0.02 sec segments and number of apneas in each bin was normalized to total number of breaths recorded for that animal. Data is represented as average percentage of apneas with a certain apnea length within each group (wild-type n=16, *Nalcn*^{Atoh1CKO}, n=14, *Nalcn*^{Phox2bCKO}, n=10, *Nalcn*^{Dbx1CKO}, n=8). Numbers of apneas in *Nalcn*^{Atoh1CKO} and *Nalcn*^{Dbx1CKO} mice was significantly higher than in wild-type mice, but duration of apneas was not significantly different between any of the CKO mice and wild-type mice (t-test, p>0. 3735 for all).

(C) The variance (CV) in breath length is not statistically different among different groups (t-test, p>0.0642 for all). *Nalcn*^{Atoh1CKO} mice and *Nalcn*^{Dbx1CKO} mice had significantly higher variability (CV2) in breath lengths between two following breaths (t-test, p=0.0168 and p=0.0197), whereas no increase in variability was observed in *Nalcn*^{Phox2bCKO} mice was observed (t-test, p=0.0890).

Mean ± SD, **: $P < 0.01$; ****: $P < 0.001$

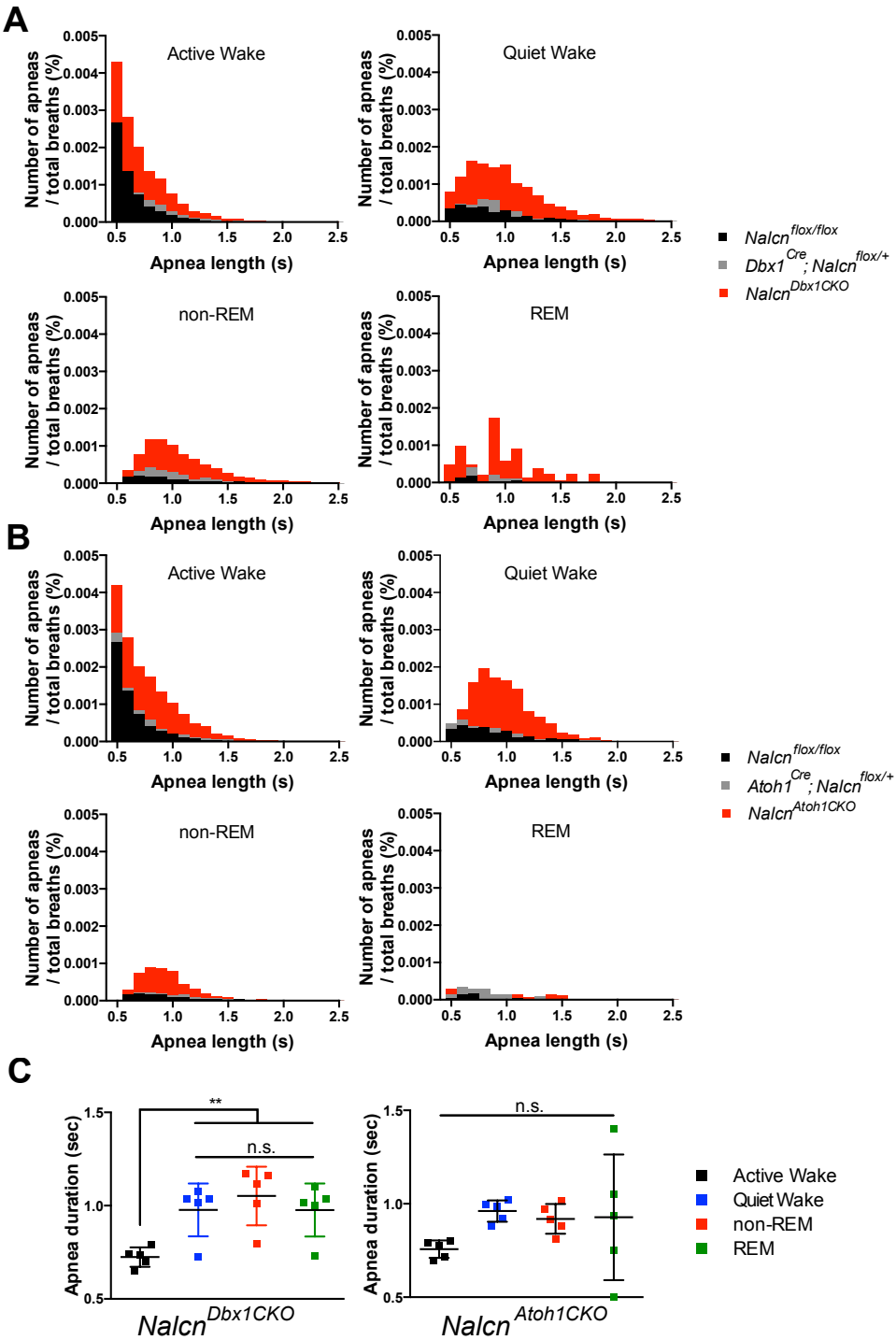


Figure S4. Loss of *Nalcn* in Atoh1^{+} lineage or preBötC neurons leads to apneas during both sleep and wake states (related to Figure 3).

(A) Sleep apnea recording of 12-15-week-old *Nalcn*$^{flox/flox}$ (n=5), *Dbx1*^{Cre}*; Nalcn*$^{flox/+}$ (n=5), and *Nalcn*^{Dbx1CKO} (n=5) mice in active wake, wake, non-REM, REM stages.

(B) Sleep apnea recording of 12-15-week-old *Nalcn*$^{flox/flox}$ (n=5), *Atoh1*^{Cre}*; Nalcn*$^{flox/+}$ (n=5), *Nalcn*^{Atoh1CKO} (n=5) mice in active wake, wake, non-REM, and REM stages.

(C) Apnea duration in different brain functioning stages from (left) *Nalcn*^{Dbx1CKO} (n=5) and (right) *Nalcn*^{Atoh1CKO} (n=5) mice. (One-way ANOVA)

Mean ± SD, **: $P < 0.01$

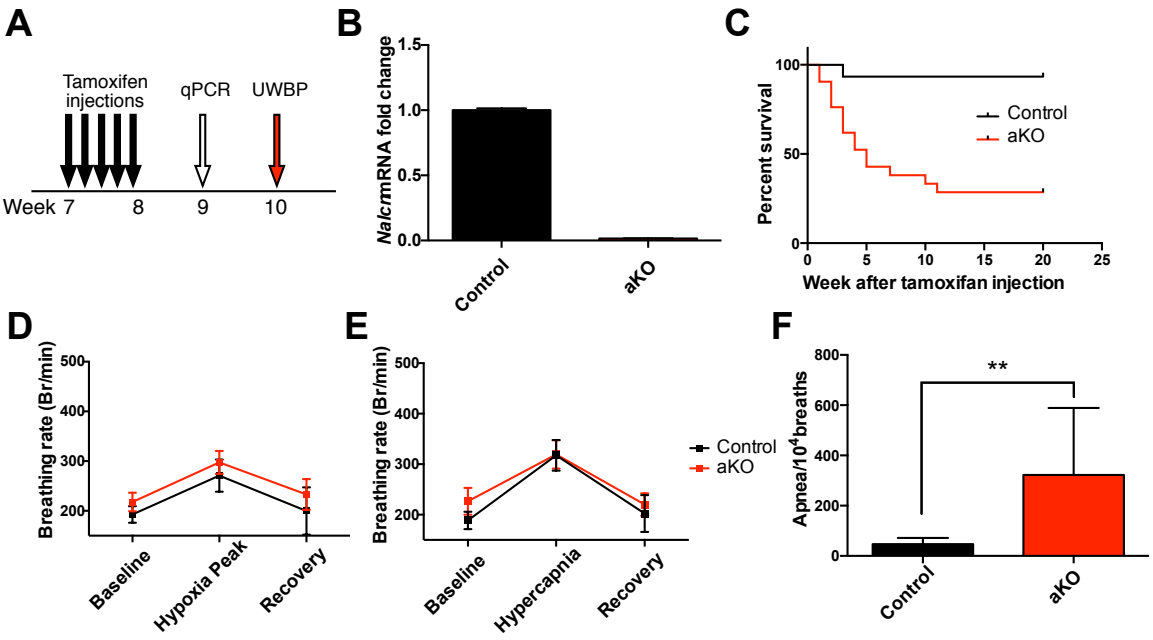


Figure S5. Loss of *Nalcn* in adult mice leads to apneas and premature lethality (related to STAR Methods and discussion).

(A) Schematic representation of the time points for tamoxifen intraperitoneal injections (black arrows), qPCR (white arrow), and UWBP (red arrow).

(B) qPCR showing that *Nalcn* is efficiently knocked-out.

(C) The survival curve for aKO (n=22) and control (n=15) mice. ($p<0.01$, Log-rank test)

(D-F) UWBP of 10-week-old aKO (n=6-12) and control (n=11-13) mice. (Two-way ANOVA and independent sample t-test)

Mean ± SD, **: $P < 0.01$

	Nalcn$^{flox/flox}$	*Nalcn*^{Atoh1CKO}	*Nalcn*^{Dbx1CKO}
pH	7.32 ± 0.05	7.34 ± 0.10	7.31 ± 0.08
Partial Pressure of CO_2 (PCO_2) (mmHg)	37.06 ± 7.36	40.00 ± 8.74	45.56 ± 4.56
Partial Pressure of O_2 (PO_2) (mmHg)	59.60 ± 19.91	62.50 ± 10.79	61 ± 6.56
Sodium (Na^+) (mmol/L)	142.60 ± 2.19	141.20 ± 1.48	145.80 ± 1.64
Potassium (K^+) (mmol/L)	5.78 ± 0.94	5.54 ± 1.21	4.46 ± 0.21
Ionized calcium (mmol/L)	1.09 ± 0.12	1.05 ± 0.10	1.20 ± 0.03
Glucose (mg/dL)	291.20 ± 35.95	301 ± 35.90	239 ± 41.63
Hematocrit (% PCV)	36.20 ± 2.05	39.00 ± 4.06	37.80 ± 1.79
HCO_3^+ (mmol/L)	18.96 ± 2.13	21.34 ± 2.00	23.10 ± 3.12
Total CO_2 (TCO_2) (mmol/L)	20.00 ± 2.65	22.06 ± 2.07	24.40 ± 3.05
Oxygen Saturation (sO_2) (%)	82.80 ± 17.14	91.40 ± 5.81	87.80 ± 4.15
Base Excess (mmol/L)	-7.02 ± 1.92	-4.60 ± 3.05	-3.2 ± 4.55
Hemoglobin (Hb) (g/dL)	12.32 ± 0.67	13.26 ± 1.37	12.84 ± 0.63

Table S1. Loss of *Nalcn* in Atoh1$^+$ lineage or preBötC neurons remains normal systematic CO_2 homeostasis (related to STAR Methods).
Blood gas measurements from *Nalcn*$^{flox/flox}$ (n=5), *Nalcn*^{Atoh1CKO} (n=5), and *Nalcn*^{Dbx1CKO} (n=5). (One-way ANOVA)

A RTN

	Atoh1^{Cre}; *Nalcn*$^{flox/+}$		*Nalcn*^{Atoh1CKO}	
	Baseline	Substance P	Baseline	Substance P
Neuron burst frequency (Hz)	3.22 ± 1.30	4.52 ± 2.14*	1.64 ± 1.09*	1.52 ± 1.03
Resting membrane potential (mV)	-57.39 ± 6.42	N/A	-66.51 ± 7.90**	N/A
Input resistance (MΩ)	1229.52 ± 541.98	N/A	1711.02 ± 639.45*	N/A
Capacitance (pF)	24.62 ± 10.40	N/A	20.21 ± 8.35	N/A
Holding current (pA)	-41.52 ± 11.11	N/A	-31.79 ± 13.72*	N/A
Rheobase (pA)	13.00 ± 3.08	N/A	11.43 ± 2.23	N/A
F_{adap} index	0.492 ± 0.108	N/A	0.495 ± 0.167	N/A

B PreBötC

	Dbx1^{Cre}; *Nalcn*$^{flox/+}$		*Nalcn*^{Dbx1CKO}	
	Baseline	Substance P	Baseline	Substance P
Network burst frequency (Hz)	0.13 ± 0.06	0.16 ± 0.05*	0.07 ± 0.06*	0.07 ± 0.06
Network burst duration (msec)	3583 ± 1350	3815 ± 1698	4511 ± 1480	4935 ± 1430
Neuron burst frequency (Hz)	0.16 ± 0.07	0.22 ± 0.10*	0.10 ± 0.04*	0.12 ± 0.04
Neuron burst duration (msec)	535.6 ± 219.7	703.1 ± 427.1	770.5 ± 374.0	814.4 ± 674.5
Neuron firing per burst	3.64 ± 1.70	5.25 ± 2.34	3.41 ± 0.73	3.04 ± 0.81
Neuron firing frequency (mHz)	6.45 ± 1.54	8.60 ± 4.24	6.40 ± 2.38	6.29 ± 2.68
Resting membrane potential (mV)	-62.29 ± 3.38	N/A	-67.63 ± 3.84**	N/A
Input resistance (MΩ)	889.41 ± 281.26	N/A	1099.13 ± 325.15*	N/A
Capacitance (pF)	30.37 ± 9.41	N/A	23.52 ± 6.24**	N/A
Holding current (pA)	-43.66 ± 10.92	N/A	-34.45 ± 9.68**	N/A
Rheobase (pA)	11.14 ± 5.47	N/A	10.70 ± 3.06	N/A
F_{adap} index	0.242 ± 0.118	N/A	0.339 ± 0.083	N/A

Table S2. Biophysical properties of the RTN and preBötC with *Nalcn* deletions (related to Figure 2).
(A) Full values and SDs of different biophysical properties of $Nalcn^{Atoh1CKO}$ and $Atoh1^{Cre}; Nalcn^{flox/+}$ RTN neurons. Input resistance, holding current, and capacitance: $Nalcn^{Atoh1CKO}$, n=19, and $Atoh1^{Cre}; Nalcn^{flox/+}$, n=12; spiking frequency adaptation index: $Nalcn^{Atoh1CKO}$, n=9 and $Atoh1^{Cre}; Nalcn^{flox/+}$, n=7; rheobase current: $Nalcn^{Atoh1CKO}$, n=7 and $Atoh1^{Cre}; Nalcn^{flox/+}$, n=5.
(B) Full values and SDs of different biophysical properties of $Nalcn^{Dbx1CKO}$ and $Dbx1^{Cre}; Nalcn^{flox/+}$ preBötC neurons. Input resistance, holding current, and capacitance: $Nalcn^{Dbx1CKO}$, n=25; $Dbx1^{Cre}; Nalcn^{flox/+}$, n=23; spiking frequency adaptation index: $Nalcn^{Dbx1CKO}$, n=8 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=4; rheobase current: $Nalcn^{Dbx1CKO}$, n=7 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=10; burst duration: $Nalcn^{Dbx1CKO}$, n=6 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=9; firing per burst: $Nalcn^{Dbx1CKO}$, n=6 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=8; firing frequency within burst: $Nalcn^{Dbx1CKO}$, n=8 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=7; network burst duration: $Nalcn^{Dbx1CKO}$, n=11 and $Dbx1^{Cre}; Nalcn^{flox/+}$, n=10.
Independent sample t-test, mean ± SD, *: $P < 0.05$; **: $P < 0.01$