SUPPORTING INFORMATION

SI Figure legends:

Fig. S1. Creation of *Crh*flox and *Crh* KO mice. a) The 5'-incorporation of the targeting vector was validated with two sets of long-range PCR using genomic DNA extracted from ES cells of wild-type (WT) or of targeted ES clones. A common forward primer (5' external primer F) recognized the 5'-flanking sequence of the targeted region, and two reverse primers (internal primer R1 or R2) recognized artificial sequences within the two inserted regions. Amplicons were electrophoresed in agar gel containing ethidium bromide. The five left lanes are from primer set F/R1 and right six lanes (excluding the DNA size marker lane M) are from primer set F/R2. Single amplicons were specifically seen only in the lanes of targeted ES cells. Amplicon identities were confirmed with end-sequencing. b) 3'-incorporation of the targeting vector was validated with Southern blot analysis using genomic DNA that underwent BamHI restriction endonuclease digestion. Digested DNA was loaded on a gel, size-separated, transferred to a nylon membrane, and hybridized with ^{32}P labeled probe which was generated from a PCR product amplified within 3' flanking part of targeted region. Genomic DNA from an independent knock-in ES clone was utilized for band size comparison, from which an appropriate 7.0 kb fragment from the engineered allele and a 8.9kb fragment from the WT allele were generated. All clones of from targeted ES cells exhibited bands of the correct sizes (7.0 kb from the targeted allele without Neo, 7.7kb from the targeted allele with Neo, and 8.9kb from WT allele). Clone 125 in panels (a) and (b) was eventually used for blastocyst injection.

Fig. S2. a) Food intake and b) Body weight are not different between *Crh*WT control (n=8) and *Crh*flox (n=10) mice.

Fig. S3. a) *Crh*WT (control, n=8) and *Crh*flox mice (n=5) revealed comparable basal plasma corticosterone, but *Crh*KO mice (n=10) had markedly decreased levels in both morning (8-9 am) and evening (5-6 pm) levels; b) Following 30 minutes of acute restraint exposure, plasma corticosterone rapidly increased in *Crh*WT (control, n=8) and *Crh*flox mice (n=5), but not in *Crh*KO mice (n=10); *P<0.05 versus 0 minute value.

Fig. S4. Q RT-PCR analysis of the expression of hypothalamic Crh mRNA in whole hypothalamus of *Sim1Crh*KO animals. *Sim1Crh*KO mice (n = 5) showed 30-40% loss of Crh mRNA expression compared to the Crhflox control (n =4), (Control, filled bars; *Sim1Crh*KO, open bars). *P<0.05 versus control.

Fig. S5. a) Adrenal hypoplasia with decreased total adrenal weight, adrenal weight per body weight, and adrenal cross sectional area are present in *Sim1Crh*KO (n=10) compared to control *Crh*flox mice (n=10); b) There are no differences in diurnal plasma corticosterone (morning, 8-9 am; evening, 5-6 pm) among *Crh*flox (n=5), *Crh*WT(n=5) and *Sim1Crh*WT (n=4) animals; c) Restraint-induced corticosterone responses were lower in *Sim1Crh*KO (n = 7) versus *Crh*flox (n = 10) mice. *P<0.05 versus control.

Fig. S6. Shirpa test analysis of body weight and sensory function was not different in *Sim1Crh*KO (n = 7) versus *Crh*flox (n = 10) mice.

Fig. S7. a) Compared to *Crh*flox control mice, *Sim1Crh*KO mice spent more time in the center area; b) *Sim1Crh*KO mice entered into center more frequently compared to

control animals, especially at the beginning of the test period; c) Total distance traveled and velocity are not different between *Sim1Crh*KO (n=7) and control *Crh*flox mice (n=10); *P<0.05, *Sim1Crh*KO versus *Crh*flox control mice.

Fig. S8. Anxiety-like behavior assessment by the holeboard test. (a, b) In the holeboard test, time spent in both the center (a) and periphery (b) were not different between *Sim1Crh*KO mice and *Crh*flox control; c) *Sim1Crh*KO compared to *Crh*flox mice showed a tendency for traveling more in the center area but this did not reach to statistical significance. For all tests, *Sim1Crh*KO (n=7), *Crh*flox control (n=10).

Fig. S9. There were no significant differences for duration in center and closed arms between control (n = 10) and *Sim1Crh*KO mice (n = 7).

Fig. S10. Novel object recognition test in *Sim1Crh*KO mice. a) *Sim1Crh*KO animals spent more time near novel vs. familiar compared to *Crh*flox mice; b) In the same object test, there were no differences for frequency to left object and right object between *Sim1Crh*KO mice and *Crh*flox control animals. The velocities were similar between control and *Sim1Crh*KO animals in same object trials; c) There were no differences between the two genotypes in velocity, or distance travelled around either novel or familiar objects in novel object trials. *P<0.05, *Sim1Crh*KO (n = 7) versus *Crh*flox control mice (n = 10).

Fig. S11. HPA responsiveness after anxiety behavior tests. *Sim1Crh*KO showed attenuated plasma corticosterone level compared to the control animals after the 15 minute open field test (a), 15 minute holeboard test (b), 5 minute EPM test (c), 5 minute

light-dark box test (d), and 5 minutes NORT assessment (e). *P<0.05, *Sim1Crh*KO (n = 7) versus *Crh*flox control mice (n = 10).

Fig. S12. The effect of *Sim1*Cre on anxiety behaviors was assessed. There were no differences among *Crh*flox (n = 5), *Crh*WT (n = 5) and *Sim1Crh*WT (n = 4) animals in the open field test (a), EPM test (b), light-dark box test (c), holeboard test (d), and NORT test (e).

Fig. S13. Representative injection site of Cre-dependent tracer (AAV8-hsyn-DIO-ChR2-mCherry) immunostaining for mCherry (anti-desRed) and visualization with DAB reaction (brown) in a Crh-ires-cre mouse (a). Illustration from Franklin & Paxinos atlas at the approximate level of the injection site (b). LV, lateral ventricle; D3V, dorsal 3rd ventricle; 3V, third ventricle; PVH, paraventricular hypothalamus; opt, optic tract; cc, corpus callosum.

Fig. S14. Tracing of PVHCrh neurons. a-f) Darkfield images of fibers in representative brain regions originating from PVHCrh neurons, from a to f, representative from accumbens shell (a), bed nucleus of the stria terminalis (medial division, anterior) and lateral septal nucleus (c), bed nucleus of the stria terminalis (ventral) (e), medial amygdala nucleus (posterodorsal) (g), ventromedial hypothalamus and arcuate nucleus (i), nucleus of the solitary tract (k). b, d, f, h, j, l) Illustrations from Franklin and Paxinos represent the bregma level corresponding the images in a, c, e, g, i. Red circles indicate the region represented in the dark field images. Abbreviations: LV, lateral ventricle; aca, anterior commissure, anterior; AcbC, Accumbens nucleus, core; LSV, Lateral septal nucleus, ventral part; STMA, bed nucleus of the stria terminalis, medial division, anterior; acp, anterior commissure, posterior; 3V, 3rd ventricle; STV, bed

nucleus of the stria terminalis, ventral; MPA, medial preoptic area; opt, optic tract; BMA, basomedial amygdala nucleus; MePD, medial amygdala nucleus, posterodorsal; MePV, medial amygdala nucleus; posteroventral; mt, mammillothalamic tract; VMH, ventromedial hypothalamus; ARC, arcuate nucleus; 12N, hypoglossal nucleus; AP, area postrema; NTS, nucleus of the solitary tract; sol, solitary tract. Scale bar = 200 um.

Table S1. Efferent projections of PVHCrh-responsive neurons. We traced the efferent projections of PVHCrh-expressing neurons in Crh-ires-Cre mice (Fig. S12a) with the cre-dependent expression of CHR2-mCherry delivered to the PVH by a unilateral injection of AAV8-hsyn-DIO-ChR2-mCherry. Subsequent immunocytochemistry against mCherry (anti-dsRed) allowed visualization of the cell bodies and projections with DAB reaction of CRH expressing cells. We observed moderate to dense projecting fibers in the accumbens nucleus (Acb), bed nucleus of the stria terminalis (Bnst), median preoptic nucleus, medial amygdala (MeA), arcuate nucleus (Arc) and nucleus of the solitary tract (NTS) (Fig. S13a-e). The anatomical distribution of the efferent projecting fibers was analyzed in 2 cases and summarized in the Table. Density of fiber projections was rated as ++++, greatest fiber density; +, lowest fiber density.