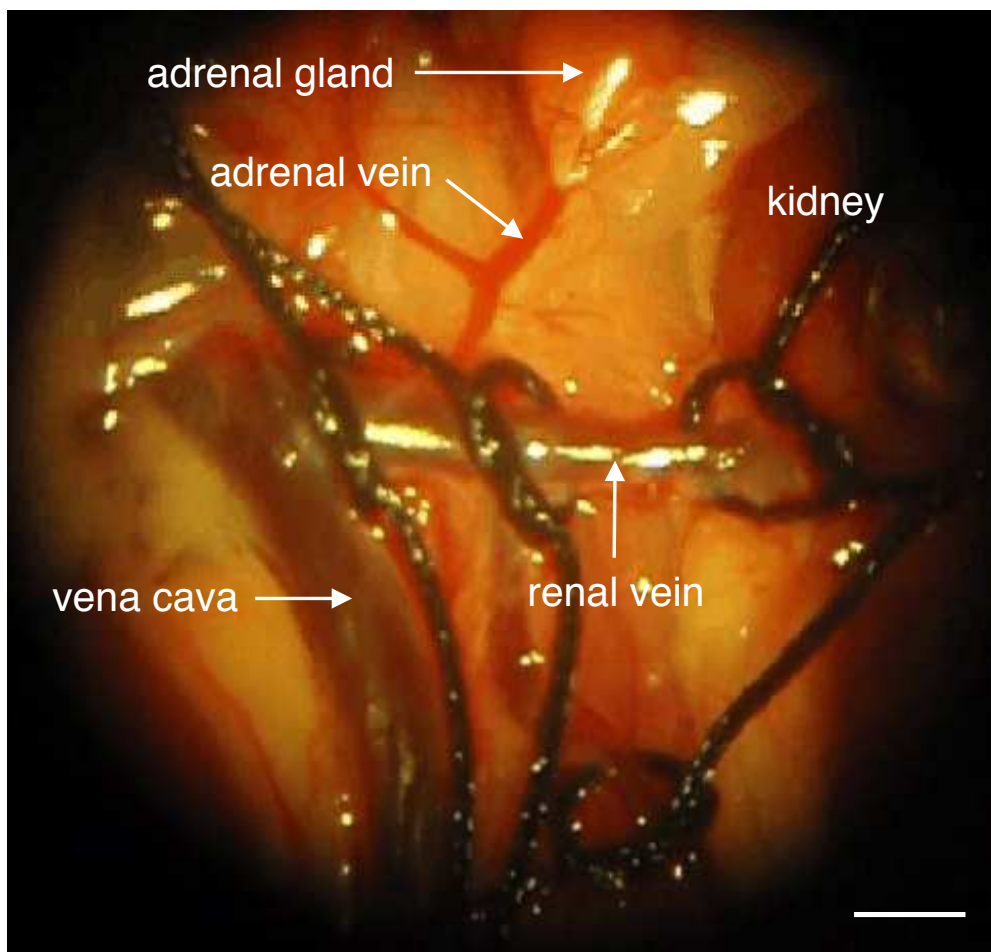


## SUPPLEMENTARY INFORMATION

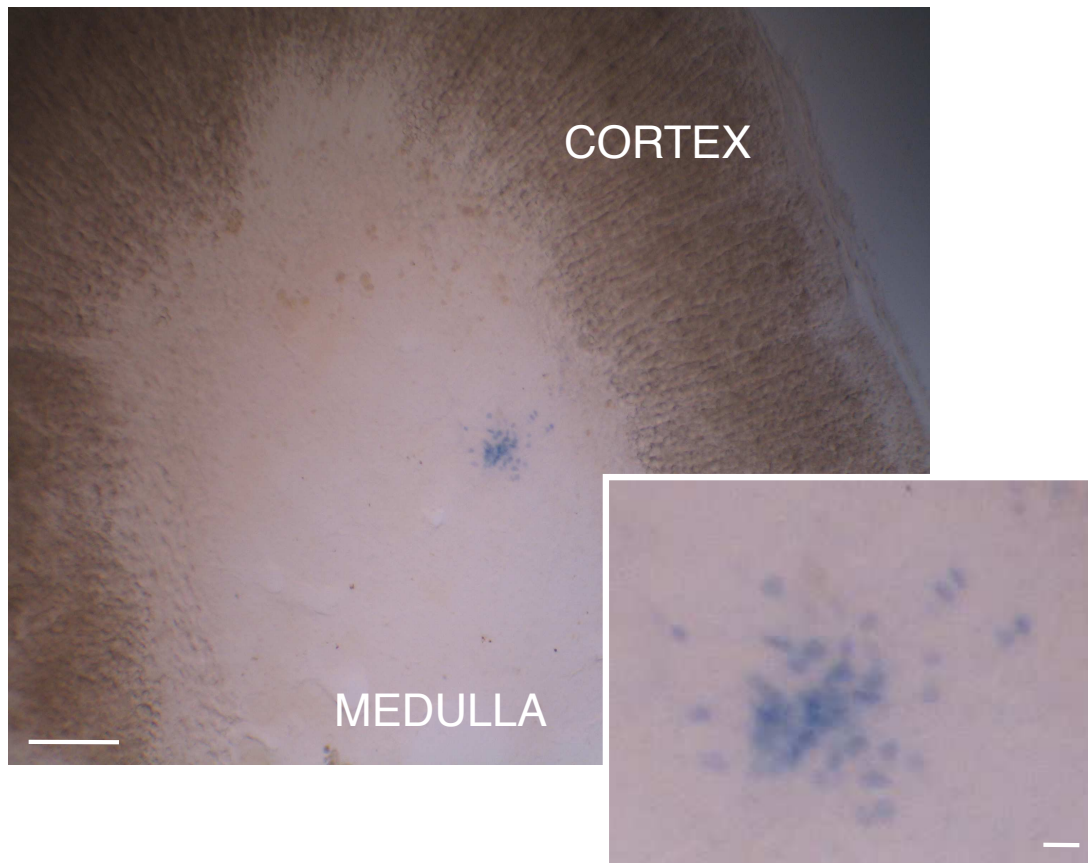
### Gap junction signaling is a stress-regulated component of adrenal neuroendocrine stimulus-secretion coupling *in vivo*

Michel G. Desarménien, Carole Jourdan, Bertrand Toutain, Emilie Vessières, Sheriar G. Hormuzdi and Nathalie C. Guérineau

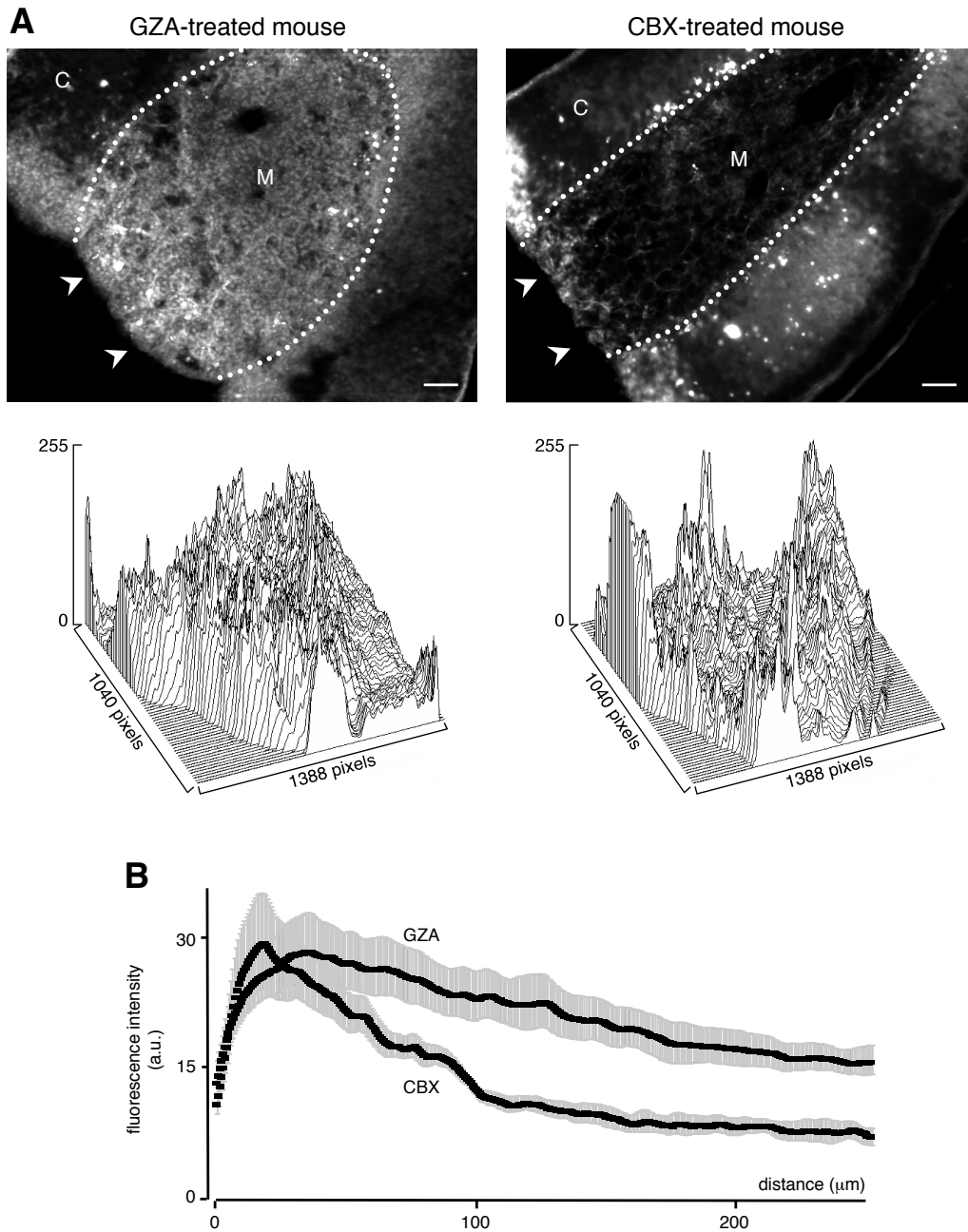


#### Supplementary Figure S1: Macroscopic anatomy of the surgical field after laparotomy.

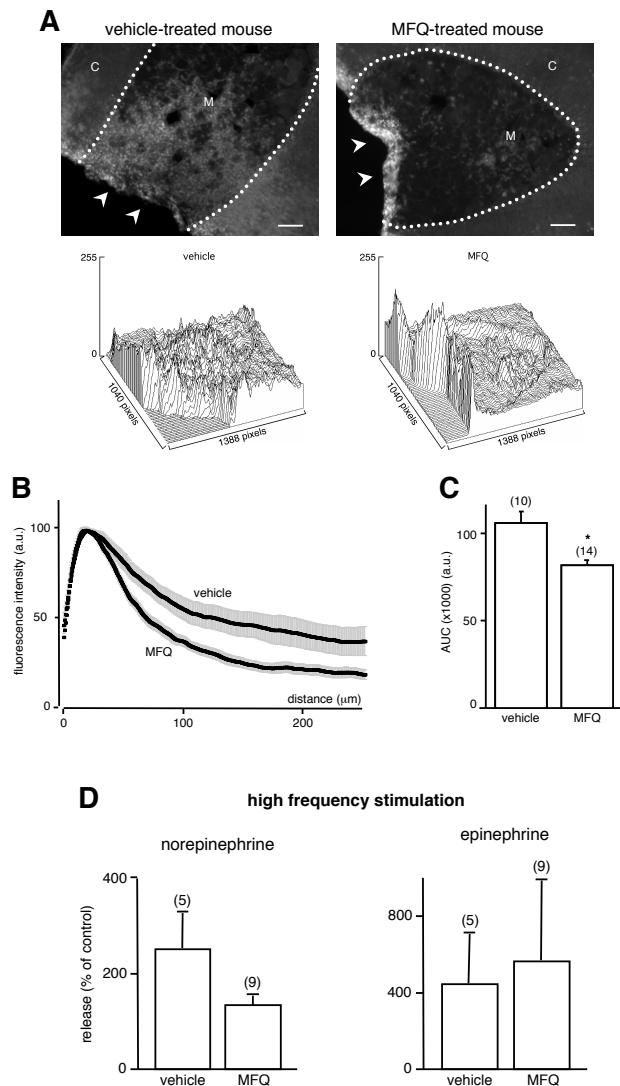
The photograph shows the left adrenal gland and the adrenal vein connecting the gland to the renal vein. Three ligatures are prepared on the renal vein, the two extremes will serve to close the vein and form a reservoir in which the adrenal vein will flow, the middle one will allow tightening a catheter for blood collection. Scale bar, 1 mm.



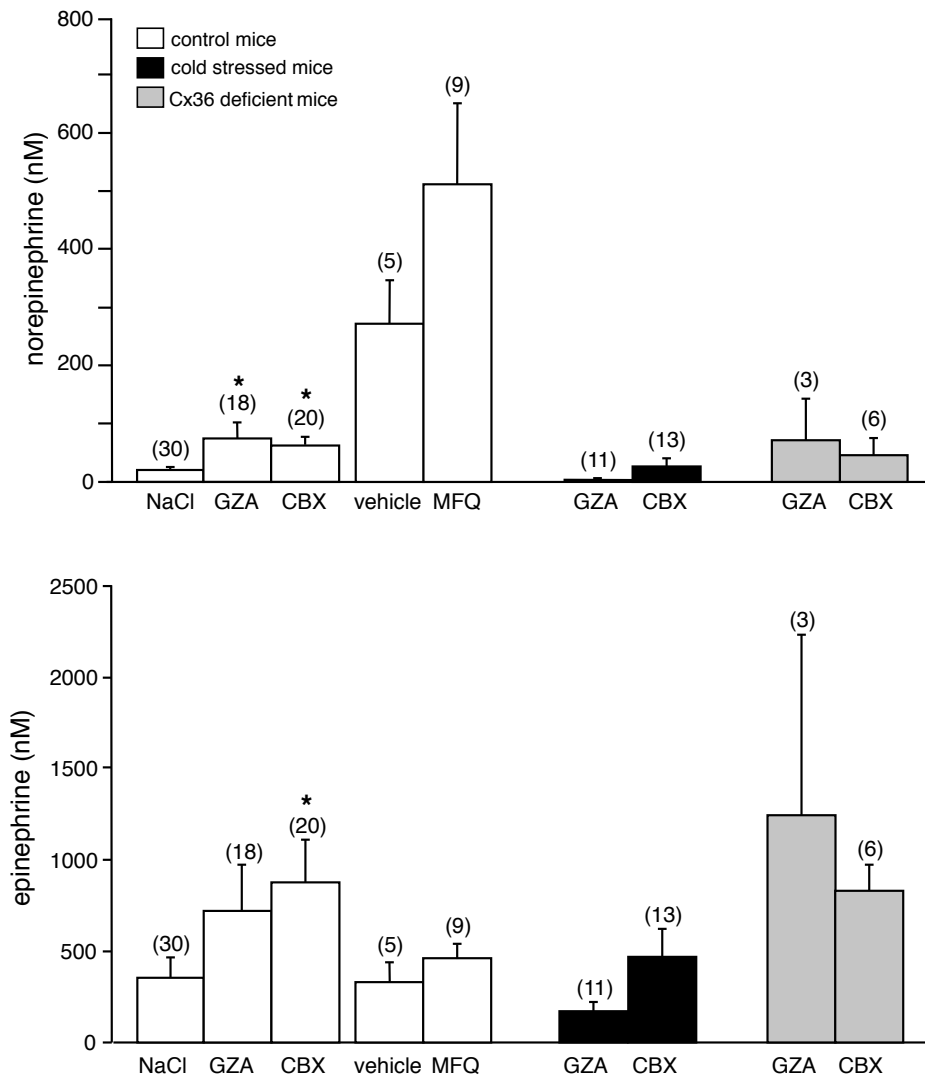
**Supplementary Figure S2: Example of a Chicago Sky Blue 6B-labeled recording area within the medullary tissue.** Microphotograph (x10 objective, scale bar, 150  $\mu\text{m}$ ) of an adrenal gland demonstrating that electrical recording was performed in the medulla. The dye (2% in the recording solution) passively diffused from the microelectrode into the recording area. After *in vivo* recordings, the adrenal was removed, fixed in paraformaldehyde and then sliced (50  $\mu\text{m}$  thickness) using a vibratome. Inset is a higher magnification (x4, scale bar, 15  $\mu\text{m}$ ).



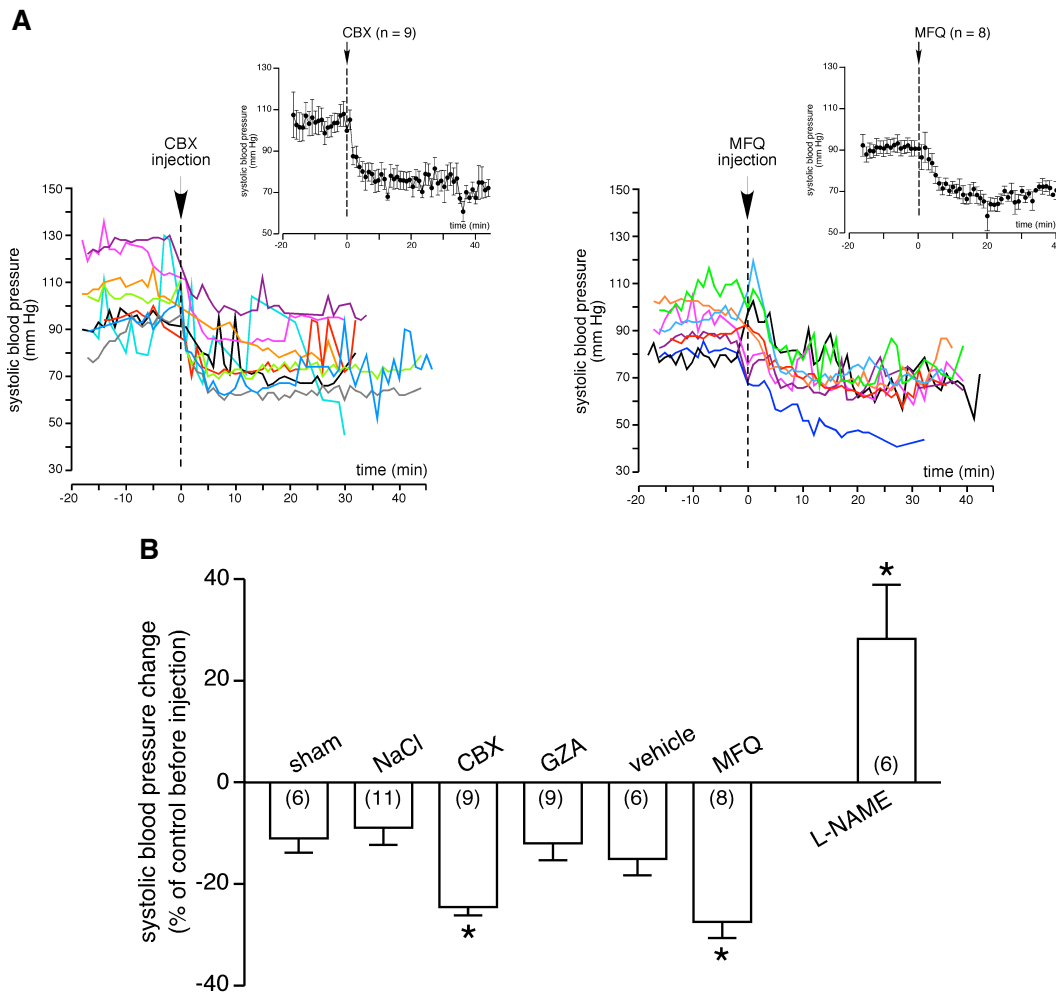
**Supplementary Figure S3: Restricted diffusion of fluorescent cadaverine in the adrenal gland after *i.p.* injection of carbenoxolone.** Intra-peritoneal injection of carbenoxolone (CBX) attenuates Alexa Fluor® 594-Cadaverine diffusion when compared to glycyrrhizic acid (GZA). **A.** Representative snapshots and associated wireframe plots illustrating the cutting edge. Scale bar, 100  $\mu\text{m}$ . **B.** Quantification of LY diffusion measured as the fluorescence intensity *versus* distance from the edge. Data are obtained from 7 GZA- and 5 CBX-treated mice.



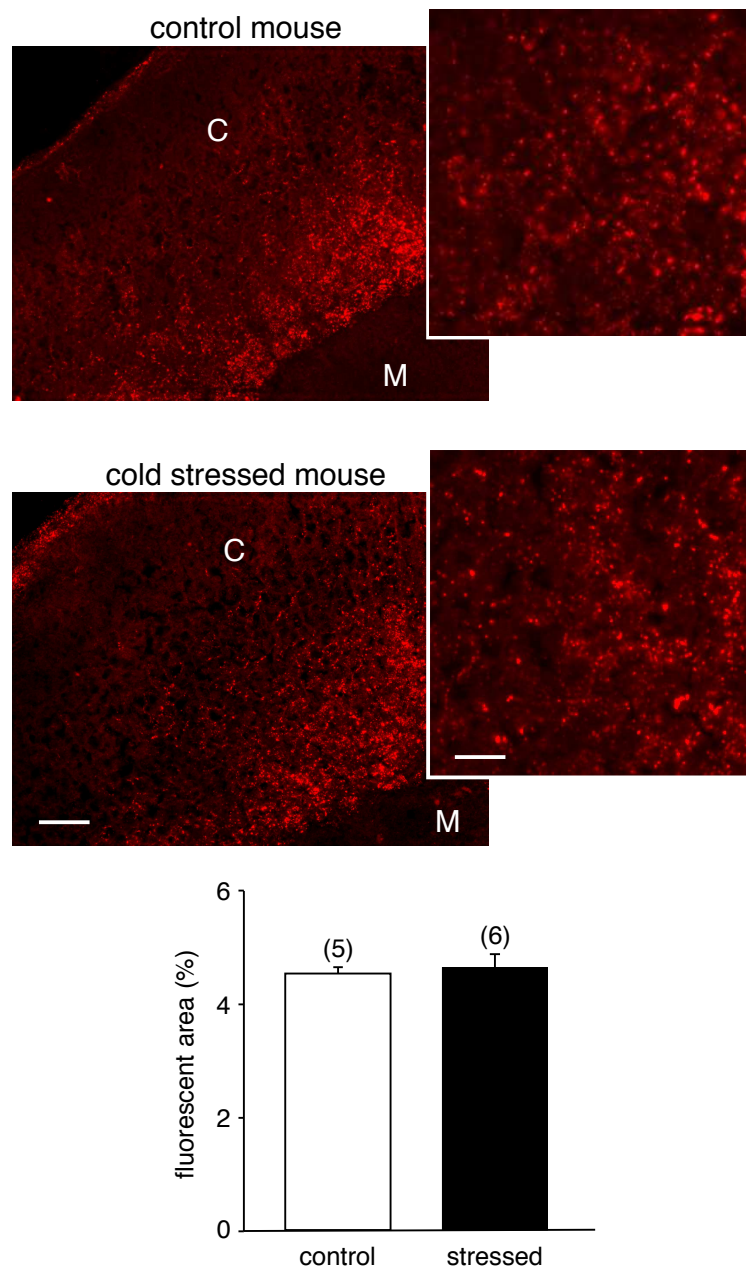
**Supplementary Figure S4: Restricted diffusion of Lucifer Yellow in the adrenal gland after *i.p.* injection of mefloquine.** Mefloquine (MFQ) was injected 45 min before adrenal removal. Intra-peritoneal injection of vehicle (DMSO + H<sub>2</sub>O, 75/25 v/v) was used as control. **A.** Representative snapshots and associated wireframe plots illustrating the cutting edge. Scale bar, 100  $\mu\text{m}$ . **B and C.** Quantification of LY diffusion measured as the fluorescence intensity *versus* distance from the edge (**B**) and as the area under the curve (**C**, \*,  $p < 0.05$  versus vehicle, unpaired Student's *t* test,  $n = 10$ -14 glands from 5-7 mice. Values represent mean  $\pm$  s.e.m.). In MFQ-treated mice, LY diffusion was significantly reduced compared to vehicle-injected mice. **D.** Catecholamine release assay evoked by a high frequency stimulation (4 Hz). MFQ reduced the release of norepinephrine but not epinephrine (Mann Whitney *U* test,  $n = 5$ -9 mice. Values represent mean  $\pm$  s.e.m.).



**Supplementary Figure S5: Basal catecholamine concentrations assayed from adrenal-venous blood in control, cold stressed and Cx36<sup>-/-</sup> mice *i.p.* injected with saline (NaCl), glycyrrhizic acid (GZA), carbenoxolone (CBX), mefloquine (MFQ) or its vehicle (DMSO + H<sub>2</sub>O).** Blood samples were collected alternatively during 2 or 5 min. Since no difference was observed, data were pooled. Note that the injection of NaCl was performed in control mice only, because the accurate controls for CBX-injected mice are the GZA-injected animals. Vehicle injection was used as control for MFQ-treated mice. Compared to NaCl, both GZA and CBX increased basal secretion. In MFQ-treated mice, catecholamine amounts did not significantly differ from those measured in vehicle-injected animals. Nevertheless, basal norepinephrine concentration was dramatically enhanced in mice injected either with the vehicle or with MFQ when compared to NaCl-injected animals, indicative of a vehicle-triggered effect. \*,  $p < 0.05$  versus NaCl, unpaired Student's  $t$  test,  $n = 18-30$  mice. Values represent mean  $\pm$  s.e.m.



**Supplementary Figure S6: Effects of CBX, GZA and MFQ on systolic arterial blood pressure monitored by tail-cuff plethysmography in anesthetized mice.** A systolic blood pressure (SBP) value was collected every minute, at least 15 min before drug injection and 40-45 min after injection. **A.** Chart recordings illustrating the time-lapse changes in SBP in CBX- (left panel), and MFQ-treated mice (right panel), before and after injection. The insets show the mean SBP decrease. **B.** Histogram illustrating the mean change of SBP measured 40 min after injection. For each experimental condition, SBP changes were expressed as the percentage of the mean SBP before injection. Sham animals were manipulated but not injected. L-NAME, which is known to have hypertensive properties, was used as positive control. \*,  $p < 0.05$  versus sham, Mann-Whitney  $U$  test,  $n = 6-9$  mice. Values represent mean  $\pm$  s.e.m.



**Supplementary Figure S7: Cx43 immunostaining in the adrenal cortex of control and cold stressed mice.** Representative examples of Cx43 immunolabeling in the adrenal cortex of a control (top) and a cold stressed (bottom) mouse. Scale bar, 100  $\mu\text{m}$  (inset: scale bar, 50  $\mu\text{m}$ ). Quantification of the immunofluorescent area reveals that adrenocortical Cx43 expression is not modified after an episode of cold stress (Mann Whitney *U* test,  $n = 5\text{-}6$  mice. Values represent mean  $\pm$  s.e.m.).

**Supplementary Table S1: Primer sequences used for quantitative RT-PCR**

gene name	NCBI reference sequence	protein name	forward primer (5'-3')	reverse primer (5'-3')
<i>Gjc3</i>	NM_080450.4	Cx29	aagagccattttgagatgcag	gccacctcactggatgtattg
<i>Gjd2</i>	NM_010290.2	Cx36	ggaatggaccatcttggaga	caccaccacagtcaacagga
<i>Gja1</i>	NM_010288.3	Cx43	tcctttgacttcagcctcca	ccatgtctgggcacctct
<i>Chrna3</i>	NM_145129.2	$\alpha$ 3nAChR	ccagatcatggaaaccaacc	catttcagcttgtagtcattcca
<i>Chrna7</i>	NM_007390.3	$\alpha$ 7nAChR	ccctggctttgctggtatt	gcatgaagacagtcagagaaagtaa
<i>Chrna9</i>	NM_001081104.1	$\alpha$ 9nAChR	ggccatgactgtatttcagcta	ggccatggtagctatgtagtattttc
<i>Chrnb2</i>	NM_009602.4	$\beta$ 2nAChR	actctatggcgctgctgttc	tcctctgtgtcagtacccaaaa
<i>Chrnb4</i>	NM_148944.4	$\beta$ 4nAChR	gttgccctgacatcgtgttgta	ttgcaggcactctttagatg
<i>Gusb</i>	NM_010368.1	Gus	ctctgggtggccttacctgat	cagttgtgtcaccttcacctc
<i>Hprt</i>	NM_013556.2	Hprt	tgatagatccattcctatgactgtaga	aagacattctttccagttaaagttgag
<i>Gapdh</i>	NM_008084.2	Gapdh	ccggggctggcattgctctc	gggggtgggtgggtccagggtt