The immunosuppression of macrophages underlies the cardioprotective effects of catestatin (CST)

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Running Title: Catestatin regulates cardiovascular function

ABSTRACT

Hypertension (HTN) is associated with inflammation and excessive production of catecholamines. Hypertensive patients have reduced plasma levels of Catestatin (CST), a bioactive cleavage product of the prohormone Chromogranin A (CgA). In mouse models, HTN symptoms can be reduced by administration of CST, but the role of CST in the regulation of cardiovascular function is unknown. In this study, we generated mice with knockout (KO) of the region of the CgA gene coding for CST (CST-KO) and found that CST-KO mice are not only hypertensive as predicted, but also display left ventricular hypertrophy, have marked macrophage infiltration of the heart and adrenal gland, and have elevated levels of pro-inflammatory cytokines and catecholamines. Intraperitoneal injection with CST reversed these phenotypes, and ischemic pre-conditioning-induced cardioprotection was also abolished in CST-KO mice. Experiments with chlodronate depletion of macrophages and bone-marrow transfer showed that macrophages produce CST and that the anti-hypertensive effects of CST are mediated in part via CST's immunosuppression of macrophages as a form of feedback inhibition. The data thus implicate CST as a key autocrine attenuator of the cardiac inflammation in HTN by reducing macrophage inflammation.

Keywords: Catestatin, hypertension, Chromogranin A, macrophages, inflammation

54 **ABBREVIATIONS**
55

56 BMDM: bone marrow-derived macrophage;
57 BP: blood pressure;
58 BMT: bone marrow transfer;
59 CCL: C-C motif chemokine ligand
60 CXC: C-X-C motif chemokine ligand
61 CDN: chlodronate;
62 CgA: chromogranin A;
63 CST: catestatin;
64 EPI: epinephrine;
65 HTN: hypertension;
66 IFN: interferon;
67 IL: interleukin;
68 IPC: ischemic preconditioning;
69 IR: ischemia/reperfusion;
70 KO: knockout;
71 LV: left ventricular;
72 LVDP: LV developed pressure;
73 LVEDP: LV end diastolic pressure;
74 LVPWd: LV posterior wall thickness;
75 MAP: mean arterial pressure;
76 IVSd: interventricular septum wall thickness;
77 NE: norepinephrine;
78 SBP: systolic blood pressure;
79 TEM: transmission electron microscopy;
80 TNF: tumor necrosis factor;
81 WT: wild-type;
82

INTRODUCTION

Hypertension (HTN) is an important risk factor for cardiovascular disease and mortality [1]. The burden of HTN and the estimated HTN-associated deaths have increased substantially over the past 25 years. The immune system is well recognized for the genesis and progression of HTN [2, 3]. Compared to healthy individuals, hypertensive or pre-hypertensive patients have elevated levels of pro- and reduced levels of anti-inflammatory cytokines [4-6]. Inflammatory cytokines can lead to vascular and renal dysfunction and progression of HTN [7]. Moreover, inflammatory cytokines can increase blood pressure (BP) by increasing the production of catecholamines in the adrenal gland. Specifically, studies in animal models and cultured neuroendocrine cells show that inflammatory cytokines such as interleukin (IL)-1β, interferon (IFN)-α, IL-6 and tumor necrosis factor (TNF)-α can elevate production of norepinephrine (NE), and epinephrine (EPI) [3, 8, 9]. The dysregulation of the production of catecholamines has been well recognized in HTN [10, 11].

Here, we reveal how catecholamine production is attenuated by another secretion product of neuroendocrine cells: the peptide catestatin (CST). CST is a bioactive proteolytical fragment from the pro-hormone Chromogranin A (CgA; $hCgA_{352-372}$) [12], which is co-stored and co-released with catecholamines in neuroendocrine cells [13]. Likely as a consequence of higher catecholamine production [3], CgA levels are elevated in humans with essential HTN [14] and in rodent genetic models of HTN [14]. However, unlike CgA, plasma CST levels are diminished not only in essential HTN [14, 15], but also in the normotensive offspring of patients with HTN [15], suggesting dysregulation in the processing of CgA to CST [14]. Moreover, HTN-associated single nucleotide polymorphisms within the CST segment of CgA have been identified [16-18].

Animal experiments also indicate a role for CST in HTN: both CgA heterozygote and complete knockout (KO; CgA-KO) mice are hypertensive, and treatment with CST normalizes the BP and plasma catecholamine levels [19]. In mouse models of diabetes [20], colitis [21] and atherosclerosis [22], CST exerts anti-inflammatory effects by inhibiting the activation of macrophages and shifting their differentiation to more anti-inflammatory phenotypes [23]. Therefore, we hypothesized that CST exerts its cardioprotective role by

114 skewing macrophages to more anti-inflammatory phenotypes, thereby resulting in lower
115 catecholamine production.
116 To directly discern the role of CST in the regulation of the cardiovascular system,
117 we generated CST-KO mice, which lack only the CST-coding region of the *Chga* gene.
118 As predicted, CST-KO mice displayed a hypertensive, hyperadrenergic, and inflammatory
119 phenotype which was rescued by exogenous addition of CST. Macrophage depletion by
120 chlodronate (CDN) liposomes and from bone-marrow transfer (BMT) between CST-KO
121 and wild-type (WT) mice revealed that macrophages produce CST and are responsible
122 for the anti-inflammatory/anti-HTN effects of CST. CST might be a novel target for the
123 treatment and prevention of HTN.
124

METHODS

Full Materials and Methods are available in the Data Supplement. Data and protocols are also available upon reasonable request from the corresponding author.

Mice. We used male WT and CST-KO (20-24 weeks old) in C57BL/6 background unless indicated otherwise. Since CgA is especially overexpressed in male patients with hypertension [24], we used only male mice in this study. Mice were kept in a 12 hr dark/light cycle and fed a normal chow diet (NCD: 13.5% calorie from fat; LabDiet 5001, TX). Animals were age-matched, and randomly assigned for each experiment. Control and experimental groups were blinded. Power calculations were conducted to determine the number of mice required for each experiment. For rescue experiments, mice were injected intraperitoneally with CST (2 μg/g body weight) at 9:00 AM for 2-4 weeks before harvesting tissues. All studies with mice were approved by the UCSD and Veteran Affairs San Diego Institutional Animal Care and Use Committees and conform to relevant National Institutes of Health guidelines.

Statistics. Statistics were performed with PRISM 8 (version 8.4.3) software (San Diego, CA). Data were analyzed using unpaired two-tailed Student's *t*-test for comparison of two groups or one-way or two-way analysis of variance (ANOVA) for more than two groups followed by Tukey's *post hoc* test if appropriate. All data are presented as mean ± SEM. Significance was assumed when $p<0.05$.

148 **RESULTS**

149

150 **Generation and validation of CST-KO mice.** The CST coding region ($mCgA_{364-384}$; 63
151 bp) was removed from Exon VII of the *Chga* gene (Figure 1A&B). Using a mouse
152 monoclonal antibody (5A8), we detected full-length CgA (~70 kDa) in WT mice and a
153 proteoglycan form of CgA in CST-KO mice in adrenal gland lysates (Figure 1C), indicating
154 the presence of CgA in CST-KO mice. Blots using a polyclonal antibody directed against
155 the C-terminal domain of CST (CT-CST) showed a proteolytically processed CgA (~46
156 kDa) corresponding to $mCgA_{1-385}$ in WT littermates, but not in CST-KO mice (Figure 1C).
157 Because this antibody detects synthetic CST (positive control for antibody specificity), we
158 conclude that CST-KO mice indeed lack CST. Adrenal CgA content was comparable in
159 WT and CST-KO mice (Figure 1D). CST was not detectable in CST-KO mice (Figure 1D).

160

161 **CST-KO mice are hypertensive.** Consistent with the anti-HTN functions of CST [19, 25, 26],
162 we found that the CST-KO mice were hypertensive and displayed diurnal increases in
163 both systolic and mean arterial BP (Figure 2A & S1). The high BP in CST-KO mice was
164 rescued by intraperitoneal injection of exogenous CST (2 μg/g body weight for 15 days),
165 whereas CST did not affect normotensive BP in WT mice (Figure 2B). In WT mice, the
166 plasma CST level was 0.86 nM, which increased to 1.72 nM 24 hrs after administration
167 of CST (Figure 2C). In CST-KO mice, plasma CST was 1.17 nM after 24 hr of CST
168 supplementation, indicating that CST supplementation provided a near physiological
169 concentration of CST.

170

171 **Ischemic pre-conditioning-induced cardioprotection is impaired in CST-KO mice.**
172 Since CST promotes cardioprotection in rats [27], we tested whether pre-conditioning-
173 induced cardioprotection is affected in CST-KO mice. We subjected WT and CST-KO
174 hearts to ischemia/reperfusion (IR) followed by ischemic preconditioning (IPC). IPC
175 significantly increased the post-ischemic left ventricular developed pressure (LVDP) and
176 lowered the left ventricular end diastolic pressure (LVEDP) in WT hearts compared to
177 CST-KO hearts and their respective IR controls (Figure S2A). Furthermore, neither LVDP
178 nor LVEDP was significantly modified in IPC-treated CST-KO hearts compared to the

179 respective IR controls. In WT mice, but not in CST-KO mice, IPC also improved recoveries
180 of both the maximum and minimum rates of pressure development in the LV (dP/dt_{max}
181 and dP/dt_{min}) compared to the respective IR controls (Figure S2B). These data show that
182 whereas IPC conferred protection against IR damage in WT mice, CST-KO mice could
183 not be preconditioned.
184
185 **CST-KO mice have increased inflammation in heart and circulation.** CST is an anti-
186 inflammatory peptide [23 28], raising the possibility that CST might regulate cardiovascular
187 function via the immune system. Indeed, in plasma of CST-KO mice, we found increased
188 levels of proinflammatory cytokines TNF-α, IFN-γ, C-C motif chemokine ligand (CCL)-2
189 and -3, and C-X-C motif chemokine ligand (CXCL)-1 (Figure 3A). By contrast, the anti-
190 inflammatory cytokine IL-10 was decreased in CST-KO mice. Intraperitoneal injection with
191 exogenous CST in CST-KO mice reversed this phenotype: it decreased the levels of most
192 proinflammatory cytokines and increased anti-inflammatory cytokines in plasma of both
193 WT and CST-KO mice (Figure 3A). RT-PCR also revealed inflammation in the heart of
194 CST-KO mice: the expression of anti-inflammatory genes *IL10*, *IL4*, *Mrc1*, *Arg1*, *Clec7a*
195 and *Clec10a* was reduced, whereas the pro-inflammatory genes *Tnfa*, *Ifng*, *Emr1*, *Itgam*,
196 *Itgax*, *Nos2a*, *IL12b CcL2*, and *CxcL1* were upregulated (Figure 3B&C). LV protein levels
197 of the proinflammatory cytokines TNF-α, IFN-γ, CCL-2, CCL-3, CXCL-1, and IL-6 were
198 also elevated in CST-KO mice (Figure 3D). These phenotypes were also reversable by
199 intraperitoneal injection of CST. We also observed increased phosphorylation
200 (Ser177/181) of IKK-β, a component of the inflammatory NF-κB signaling pathway (Figure
201 4A). These findings show that the immune system of CST-KO mice is skewed towards
202 inflammation.
203
204 **CST reduces pro-inflammatory macrophages *in vitro*.** In the next set of experiments,
205 we addressed whether CST would directly shift macrophages to more anti-inflammatory
206 responses *in vitro*. Macrophages were derived from bone-marrow of WT mice (BMDM)
207 and differentiated to an either a pro-inflammatory M1-like phenotype or an anti-
208 inflammatory M2-like phenotype [29]. Culturing these macrophages for 24 hr with 100 nM
209 CST resulted in a small, but significant, reduction of the production of pro-inflammatory

210 cytokines TNF-α, CCL-2, CCL-3, CXCL-1 and IL-1β (Figure 4B&C). In contrast, the levels
211 of anti-inflammatory IL-10 were increased in the macrophages.
212 Since macrophages are a secretory cell type, we also addressed whether
213 macrophages produce CgA and CST. Indeed, Western blotting analysis revealed the
214 presence of both CgA and CST in cultured peritoneal macrophages (Figure 4D) [29]. To
215 assess the physiological relevance of CST-production by macrophages, we performed
216 BMT experiments in which we irradiated CST-KO mice and then cross-transplanted the
217 marrow from WT mice. We analyzed plasma CST of these mice and found that WT bone-
218 marrow recipient CST-KO mice, but not CST-KO bone-marrow recipients, had near
219 physiologic levels of plasma CST (0.52 nM) (Figure 4E). Thus, macrophages (and
220 possibly other bone-marrow derived cell types) are major producers of CST in circulation.
221
222 **Macrophages are key effector cells responsible for the anti-inflammatory actions**
223 **of CST.** TEM studies revealed abundant infiltration of macrophages and fibrosis in the
224 intercellular spaces between chromaffin cells in the adrenal medulla of CST-KO mice
225 (Figure S3 and S4**)**. Also, marked cardiac fibrosis and an increased presence of recruited
226 monocytes were observed in the heart of saline-treated CST-KO mice, as shown by TEM
227 and flow cytometry (Figure 5A, S3, S5 and S6A). In both the adrenal gland and heart of
228 CST-KO mice, CST supplementation reduced the abundance of macrophage infiltrates
229 (Figure S3). Flow cytometry analysis showed a decrease of the fraction of F4/80$^-$Ly6C$^+$
230 monocytes compared to total CD11b$^+$ cells in CST-supplemented CST-KO heart (Figure
231 5A and S6B).
232 We assessed the functional role of the infiltrated macrophages in the heart and
233 adrenal gland of CST-KO mice using two independent approaches. First, we depleted
234 macrophages by CDN liposomes (Figure S6B), which not only depleted macrophages in
235 heart and adrenal gland (Figure S3), but also reversed the hypertensive phenotype of
236 CST-KO mice (Figure 5B). Second, we carried out BMT assays in which we irradiated
237 both WT and CST-KO mice and then cross-transplanted their marrows: bone-marrow
238 from CST-KO mice was transplanted into WT mice and *vice versa*. Both the inflammatory
239 and hypertensive phenotypes were transferred by BMT: while CST-KO bone-marrow
240 recipient WT mice showed increased BP; elevated levels in plasma and heart of TNF-α,

241 IFN-γ, CCL-2, CCL-3, and CXCL-1; and reduced levels of IL-10, WT bone-marrow
242 recipient CST-KO mice showed the opposite phenotypes (Figure 5B-D). Since WT bone-
243 marrow recipient CST-KO mice had near physiologic levels of plasma CST (Figure 4E),
244 we conclude that macrophages and other immune cells are not only key effectors of the
245 anti-hypertensive actions of CST but are also main producers of CST themselves.
246
247 **Heightened sympathetic stimulation and hypersecretion of catecholamines in**
248 **adrenal gland of CST-KO mice.** Prior studies have shown that pro-inflammatory
249 cytokines increase catecholamine production and secretion [3, 8, 9]. In line with this, we
250 found that compared to WT littermates, CST-KO mice have elevated levels of both
251 adrenal and plasma catecholamine levels (Figure 6A). This phenotype was also
252 transferable by BMT: CST-KO bone-marrow recipient WT mice showed increased levels
253 of NE and EPI in the adrenal medulla and plasma, whereas WT bone-marrow recipient
254 CST-KO mice showed the opposite phenotype (Figure 6A).
255 Since heightened sympathetic nerve traffic has been documented in hypertensive
256 patients [30], we measured NE in the LV and kidney of WT and CST-KO mice. In contrast
257 to the adrenal medulla and plasma, we observed reduced levels of NE in the heart and
258 kidney of CST-KO mice (Figure 6A). Decreased NE in CST-KO mice indicates increased
259 cardiac and renal spillover of NE, which is common in hypertensive and heart failure
260 patients [31-34]. The CST-KO adrenal medulla exhibited abundant docked chromaffin
261 granules and decreased acetylcholine-containing vesicles at the sympatho-adreno-
262 medullary synapse (Figure 6B-C and S7), implicating heightened sympathetic nerve
263 activity leading to hypersecretion of catecholamines [35, 36]. Supplementation of CST-KO
264 mice with CST reversed this phenotype (Figure 6B-C and S7) and led to a concomitant
265 decrease in both plasma and adrenal catecholamines (Figure 6A). Since CST acts as an
266 endogenous inhibitor of nicotine-induced catecholamine secretion [37], we reasoned that
267 ganglionic blockade would reverse elevated BP in CST mice. Indeed, the elevated SBP,
268 DBP, and MAP in CST-KO mice were reversed by the long-acting nicotinic acetylcholine
269 receptor antagonist chlorisondamine (Figure S8A-C). This compound also decreased the
270 heart rate (HR) (Fig. S8D) as reported by others [38].

To test whether lack of CST affected heart structure and function, we undertook gravimetry and echocardiographic ultrasound imaging. CST-KO mice showed increased heart weights and sizes compared to WT mice (Figure S9A). Although CST-KO mice maintained a similar level of LV function (fractional shortening) to age-matched WT mice, there were significant abnormalities in LV remodeling as evidenced by the significant increase in LV posterior wall thickness (LVPWd), which has been associated with high BP [39, 40] , and a trend towards an increase in interventricular septum wall thickness (IVSd; p=0.07) (Figure S9B). HR, LV internal diameter during systole, and LV internal diameter during diastole were comparable between WT and CST-KO mice (Figure S9B).

DISCUSSION

Inflammation and hypertension. Inflammation is well understood to contribute to the development of hypertension by inducing vascular damage, renal damage, and/or abnormal central neural regulation [41-43]. This study showed that inflammation and the development of HTN are counteracted by the anti-inflammatory hormone CST through its feedback inhibition/regulation of macrophages and the anti-inflammatory actions of CST are likely needed to maintain the tolerance of the immune system. Previous studies showing low levels of CST in hypertensive subjects [15] and normalization of BP in CgA-KO mice by CST [19] as well as decreasing BP in spontaneously hypertensive rats [25] indicate that CST is sufficient to reverse HTN. The findings from this study that CST-KO mice are hypertensive with a skewed immune system towards inflammation, and that these phenotypes can be rescued by exogenous administration of recombinant CST, add to this and demonstrate that CST is not only sufficient but also necessary for regulating HTN. Since the inflammation and BP can be reduced by administration of exogenous CST, CST might be a therapeutic target for the treatment of HTN.

Suppression of the immune system attenuates the development of HTN when induced by Angiotensin II or deoxycorticosterone acetate-salt, while dysregulation of it causes sensitization to these hypertensive challenges [44]. Surprisingly, CST-KO mice already show elevated BP in absence of an additional challenge, raising the question of other mechanisms in addition to the immune activation are involved in the HTN phenotype in these mice. However, since bone-marrow transplant from CST-KO to WT mice already suffices to elicit HTN, and these mice only harbor CST lacking immune cells while the autonomic system is normal, it might be that an abnormal activation of the immune system triggers HTN.

Neurohumoral regulation of BP. We found a neuro-adrenergic overdrive-induced HTN in CST-KO mice. Existing literature reveals heightened sympathetic nerve activity in hypertensive subjects [45, 46]. In addition, hypertensive patients with metabolic risk factors, such as obesity, metabolic syndrome, or diabetes mellitus, also exhibit sympathetic overdrive [47-49]. Like humans, spontaneously hypertensive rats show reduced cardiac

313 parasympathetic nerve activity, elevated sympathetic nerve activity and increased NE
314 release [50].
315
316 **Immunoendocrine regulation of BP.** The augmented sympathetic nerve activity in HTN
317 is known to activate both myeloid cells and T cells [2], and circulating concentrations of pro-
318 inflammatory cytokines are increased in primary HTN [51]. T-lymphocytes are critical for
319 Angiotensin II and deoxycorticosterone acetate-salt-induced hypertension [52, 53].
320 Intracerebroventricular administration of IL-6 increases splenic sympathetic nerve activity
321 [54], while central administration of IL-1β increases adrenal, splenic and renal sympathetic
322 nerve activity [55]. Injection of TNF-α into central sympathetic nuclei, such as the
323 paraventricular nucleus increases sympathetic nerve activity, BP and heart rate in rats [56].
324 To our knowledge, the present study is the first to demonstrate increased
325 infiltration of macrophages in the adrenal medulla concomitant with increased secretion
326 of catecholamines and the consequent development of HTN in CST-KO mice, which were
327 normalized after CST supplementation. These findings imply that CST regulates the BP
328 through a novel immunoendocrine regulation of catecholamine secretion via
329 macrophages.
330 What causes the elevated BP in CST-KO mice? Since nicotine-induced increases
331 in BP are associated with an increase in plasma catecholamine secretion [57], it is
332 consistent that lack of CST (an endogenous inhibitor of nicotine-evoked catecholamine
333 secretion [37]) in CST-KO mice resulted in increased plasma catecholamines with
334 consequent increase in BP. We have confirmed this by using chlorisondamine, which
335 decreased BP in CST-KO mice as has been reported in rats [38]. However, in CST-KO
336 mice, the HR was not increased, and fractional shortening was unaltered compared to
337 WT, indicating that the increased BP is not driven by elevated cardiac output. Further
338 studies are required to explain what causes hypertension in CST-KO mice. It might be
339 that the LV hypertrophy in the CST-KO mice, and possibly also the increased posterior
340 wall thickness associated with high BP [39, 40], is a secondary effect of the BP elevation. It
341 seems therefore possible that the elevated BP is caused by increased vascular
342 resistance, due to vasoconstriction and/or increased arterial stiffness [58]. It is possible that

increased cardiac and renal spillover of NE also contribute to the development of BP in CST-KO mice [31-34].

Perspectives. This study provides a key mechanism as to how CST regulates inflammation. Cardiac macrophages are critical for myocardial homeostasis [59, 60]. We found an abundance of infiltrated macrophages in the heart and adrenal gland of CST-KO mice. Using *in vitro* experiments with cultured BMDMs and two parallel approaches (CDN macrophage depletion and BMT) to decrease macrophage activity *in vivo*, we found that macrophages are key effector cells for the anti-hypertensive actions of CST. We also found that bone-marrow originated cells, possibly macrophages, are the main source of circulating CST.

We therefore propose that the macrophages (and chromaffin cells) produce CST, which reduces inflammation by autocrine/feedback inhibition fashion. These anti-inflammatory actions underlie the anti-hypertensive effects of CST, since without CST, macrophages are more reactive, infiltrate the heart, and alter the ultrastructure, physiological makeup, and molecular makeup of the myocardium. Additionally, the data implicate CST as a key mediator of the observed crosstalk between systemic and cardiac inflammation in HTN, which hence plays a central role in cardiovascular homeostasis by regulating the immunoendocrine axis.

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Disclosures

None

REFERENCES

1. Bromfield S, Muntner P. High blood pressure: The leading global burden of disease risk factor and the need for worldwide prevention programs. *Curr Hypertens Rep*. 2013;15:134-136
2. Norlander AE, Madhur MS, Harrison DG. The immunology of hypertension. *J Exp Med*. 2018;215:21-33
3. Byrne CJ, Khurana S, Kumar A, Tai TC. Inflammatory signaling in hypertension: Regulation of adrenal catecholamine biosynthesis. *Front Endocrinol (Lausanne)*. 2018;9:343
4. Chrysohoou C, Pitsavos C, Panagiotakos DB, Skoumas J, Stefanadis C. Association between prehypertension status and inflammatory markers related to atherosclerotic disease: The attica study. *Am J Hypertens*. 2004;17:568-573
5. Peeters AC, Netea MG, Janssen MC, Kullberg BJ, Van der Meer JW, Thien T. Pro-inflammatory cytokines in patients with essential hypertension. *Eur J Clin Invest*. 2001;31:31-36
6. Stumpf C, Auer C, Yilmaz A, Lewczuk P, Klinghammer L, Schneider M, Daniel WG, Schmieder RE, Garlichs CD. Serum levels of the th1 chemoattractant interferon-gamma-inducible protein (ip) 10 are elevated in patients with essential hypertension. *Hypertens Res*. 2011;34:484-488
7. Agita A, Alsagaff MT. Inflammation, immunity, and hypertension. *Acta Med Indones*. 2017;49:158-165
8. Kannan H, Tanaka Y, Kunitake T, Ueta Y, Hayashida Y, Yamashita H. Activation of sympathetic outflow by recombinant human interleukin-1 beta in conscious rats. *Am J Physiol*. 1996;270:R479-485
9. Corssmit EP, Heijligenberg R, Endert E, Ackermans MT, Sauerwein HP, Romijn JA. Endocrine and metabolic effects of interferon-alpha in humans. *J Clin Endocrinol Metab*. 1996;81:3265-3269
10. Floras JS. Epinephrine and the genesis of hypertension. *Hypertension*. 1992;19:1-18
11. Goldstein DS. Plasma catecholamines and essential hypertension. An analytical review. *Hypertension*. 1983;5:86-99
12. Mahata SK, O'Connor DT, Mahata M, Yoo SH, Taupenot L, Wu H, Gill BM, Parmer RJ. Novel autocrine feedback control of catecholamine release. A discrete chromogranin a fragment is a noncompetitive nicotinic cholinergic antagonist. *J Clin Invest*. 1997;100:1623-1633
13. Winkler H, Fischer-Colbrie R. The chromogranins a and b: The first 25 years and future perspectives. *Neuroscience*. 1992;49:497-528
14. O'Connor DT, Zhu G, Rao F, Taupenot L, Fung MM, Das M, Mahata SK, Mahata M, Wang L, Zhang K, Greenwood TA, Shih PA, Cockburn MG, Ziegler MG, Stridsberg M, Martin NG, Whitfield JB. Heritability and genome-wide linkage in us and australian twins identify novel genomic regions controlling chromogranin a: Implications for secretion and blood pressure. *Circulation*. 2008;118:247-257
15. O'Connor DT, Kailasam MT, Kennedy BP, Ziegler MG, Yanaihara N, Parmer RJ. Early decline in the catecholamine release-inhibitory peptide catestatin in humans at genetic risk of hypertension. *J Hypertens*. 2002;20:1335-1345

16. Wen G, Mahata SK, Cadman P, Mahata M, Ghosh S, Mahapatra NR, Rao F, Stridsberg M, Smith DW, Mahboubi P, Schork NJ, O'Connor DT, Hamilton BA. Both rare and common polymorphisms contribute functional variation at chga, a regulator of catecholamine physiology. *Am J Hum Genet*. 2004;74:197-207
17. Rao F, Wen G, Gayen JR, Das M, Vaingankar SM, Rana BK, Mahata M, Kennedy BP, Salem RM, Stridsberg M, Abel K, Smith DW, Eskin E, Schork NJ, Hamilton BA, Ziegler MG, Mahata SK, O'Connor DT. Catecholamine release-inhibitory peptide catestatin (chromogranin a(352-372)): Naturally occurring amino acid variant gly364ser causes profound changes in human autonomic activity and alters risk for hypertension. *Circulation*. 2007;115:2271-2281
18. Angelone T, Quintieri AM, Brar BK, Limchaiyawat PT, Tota B, Mahata SK, Cerra MC. The antihypertensive chromogranin a peptide catestatin acts as a novel endocrine/paracrine modulator of cardiac inotropism and lusitropism. *Endocrinology*. 2008;149:4780-4793
19. Mahapatra NR, O'Connor DT, Vaingankar SM, Hikim AP, Mahata M, Ray S, Staite E, Wu H, Gu Y, Dalton N, Kennedy BP, Ziegler MG, Ross J, Mahata SK. Hypertension from targeted ablation of chromogranin a can be rescued by the human ortholog. *J Clin Invest*. 2005;115:1942-1952
20. Ying W, Mahata S, Bandyopadhyay GK, Zhou Z, Wollam J, Vu J, Mayoral R, Chi NW, Webster NJG, Corti A, Mahata SK. Catestatin inhibits obesity-induced macrophage infiltration and inflammation in the liver and suppresses hepatic glucose production, leading to improved insulin sensitivity. *Diabetes*. 2018;67:841-848
21. Rabbi MF, Labis B, Metz-Boutigue MH, Bernstein CN, Ghia JE. Catestatin decreases macrophage function in two mouse models of experimental colitis. *Biochem Pharmacol*. 2014;89:386-398
22. Kojima M, Ozawa N, Mori Y, Takahashi Y, Watanabe-Kominato K, Shirai R, Watanabe R, Sato K, Matsuyama TA, Ishibashi-Ueda H, Koba S, Kobayashi Y, Hirano T, Watanabe T. Catestatin prevents macrophage-driven atherosclerosis but not arterial injury-induced neointimal hyperplasia. *Thromb Haemost*. 2018;118:182-194
23. Muntjewerff EM, Dunkel G, Nicolasen MJT, Mahata SK, van den Bogaart G. Catestatin as a target for treatment of inflammatory diseases. *Front Immunol*. 2018;9:2199
24. Chen Y, Rao F, Rodriguez-Flores JL, Mahata M, Fung MM, Stridsberg M, Vaingankar SM, Wen G, Salem RM, Das M, Cockburn MG, Schork NJ, Ziegler MG, Hamilton BA, Mahata SK, Taupenot L, O'Connor DT. Naturally occurring human genetic variation in the 3'-untranslated region of the secretory protein chromogranin a is associated with autonomic blood pressure regulation and hypertension in a sex-dependent fashion. *J Am Coll Cardiol*. 2008;52:1468-1481
25. Avolio E, Mahata SK, Mantuano E, Mele M, Alo R, Facciolo RM, Talani G, Canonaco M. Antihypertensive and neuroprotective effects of catestatin in spontaneously hypertensive rats: Interaction with gabaergic transmission in amygdala and brainstem. *Neuroscience*. 2014;270:48-57

26. Biswas N, Gayen J, Mahata M, Su Y, Mahata SK, O'Connor DT. Novel peptide isomer strategy for stable inhibition of catecholamine release: Application to hypertension. *Hypertension*. 2012;60:1552-1559
27. Penna C, Alloatti G, Gallo MP, Cerra MC, Levi R, Tullio F, Bassino E, Dolgetta S, Mahata SK, Tota B, Pagliaro P. Catestatin improves post-ischemic left ventricular function and decreases ischemia/reperfusion injury in heart. *Cell Mol Neurobiol*. 2010;30:1171-1179
28. Mahata SK, Corti A. Chromogranin a and its fragments in cardiovascular, immunometabolic, and cancer regulation. *Ann N Y Acad Sci*. 2019;1455:34-58
29. Ying W, Cheruku PS, Bazer FW, Safe SH, Zhou B. Investigation of macrophage polarization using bone marrow derived macrophages. *J Vis Exp*. 2013
30. Mancia G, Grassi G. The autonomic nervous system and hypertension. *Circ Res*. 2014;114:1804-1814
31. Hasking GJ, Esler MD, Jennings GL, Burton D, Johns JA, Korner PI. Norepinephrine spillover to plasma in patients with congestive heart failure: Evidence of increased overall and cardiorenal sympathetic nervous activity. *Circulation*. 1986;73:615-621
32. Rundqvist B, Elam M, Bergmann-Sverrisdottir Y, Eisenhofer G, Friberg P. Increased cardiac adrenergic drive precedes generalized sympathetic activation in human heart failure. *Circulation*. 1997;95:169-175
33. Grassi G. Assessment of sympathetic cardiovascular drive in human hypertension: Achievements and perspectives. *Hypertension*. 2009;54:690-697
34. Sata Y, Head GA, Denton K, May CN, Schlaich MP. Role of the sympathetic nervous system and its modulation in renal hypertension. *Front Med (Lausanne)*. 2018;5:82
35. Berg T, Jensen J. Simultaneous parasympathetic and sympathetic activation reveals altered autonomic control of heart rate, vascular tension, and epinephrine release in anesthetized hypertensive rats. *Front Neurol*. 2011;2:71
36. Mathar I, Vennekens R, Meissner M, Kees F, Van der Mieren G, Camacho Londono JE, Uhl S, Voets T, Hummel B, van den Bergh A, Herijgers P, Nilius B, Flockerzi V, Schweda F, Freichel M. Increased catecholamine secretion contributes to hypertension in trpm4-deficient mice. *J Clin Invest*. 2010;120:3267-3279
37. Mahata SK, Mahapatra NR, Mahata M, Wang TC, Kennedy BP, Ziegler MG, O'Connor DT. Catecholamine secretory vesicle stimulus-transcription coupling in vivo. Demonstration by a novel transgenic promoter/photoprotein reporter and inhibition of secretion and transcription by the chromogranin a fragment catestatin. *J Biol Chem*. 2003;278:32058-32067
38. Santajuliana D, Hornfeldt BJ, Osborn JW. Use of ganglionic blockers to assess neurogenic pressor activity in conscious rats. *J Pharmacol Toxicol Methods*. 1996;35:45-54
39. Lim PO, Donnan PT, MacDonald TM. Blood pressure determinants of left ventricular wall thickness and mass index in hypertension: Comparing office, ambulatory and exercise blood pressures. *J Hum Hypertens*. 2001;15:627-633

512 40. Eliakim-Raz N, Prokupetz A, Gordon B, Shochat T, Grossman A. Interventricular
513 septum and posterior wall thickness are associated with higher systolic blood
514 pressure. *J Clin Hypertens (Greenwich)*. 2016;18:703-706
515 41. Rodriguez-Iturbe B, Pons H, Quiroz Y, Lanaspa MA, Johnson RJ. Autoimmunity in
516 the pathogenesis of hypertension. *Nat Rev Nephrol*. 2014;10:56-62
517 42. Caillon A, Schiffrin EL. Role of inflammation and immunity in hypertension: Recent
518 epidemiological, laboratory, and clinical evidence. *Curr Hypertens Rep*.
519 2016;18:21
520 43. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, immunity, and
521 hypertensive end-organ damage. *Circ Res*. 2015;116:1022-1033
522 44. Rudemiller NP, Crowley SD. Interactions between the immune and the renin-
523 angiotensin systems in hypertension. *Hypertension*. 2016;68:289-296
524 45. Anderson EA, Sinkey CA, Lawton WJ, Mark AL. Elevated sympathetic nerve
525 activity in borderline hypertensive humans. Evidence from direct intraneural
526 recordings. *Hypertension*. 1989;14:177-183
527 46. Grassi G. Role of the sympathetic nervous system in human hypertension. *J*
528 *Hypertens*. 1998;16:1979-1987
529 47. Grassi G, Seravalle G, Dell'Oro R, Turri C, Bolla GB, Mancia G. Adrenergic and
530 reflex abnormalities in obesity-related hypertension. *Hypertension*. 2000;36:538-
531 542
532 48. Grassi G, Dell'Oro R, Quarti-Trevano F, Scopelliti F, Seravalle G, Paleari F,
533 Gamba PL, Mancia G. Neuroadrenergic and reflex abnormalities in patients with
534 metabolic syndrome. *Diabetologia*. 2005;48:1359-1365
535 49. Huggett RJ, Scott EM, Gilbey SG, Stoker JB, Mackintosh AF, Mary DA. Impact of
536 type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension.
537 *Circulation*. 2003;108:3097-3101
538 50. Judy WV, Farrell SK. Arterial baroreceptor reflex control of sympathetic nerve
539 activity in the spontaneously hypertensive rat. *Hypertension*. 1979;1:605-614
540 51. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*.
541 2002;105:1135-1143
542 52. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J,
543 Weyand C, Harrison DG. Role of the t cell in the genesis of angiotensin ii induced
544 hypertension and vascular dysfunction. *J Exp Med*. 2007;204:2449-2460
545 53. Marvar PJ, Thabet SR, Guzik TJ, Lob HE, McCann LA, Weyand C, Gordon FJ,
546 Harrison DG. Central and peripheral mechanisms of t-lymphocyte activation and
547 vascular inflammation produced by angiotensin ii-induced hypertension. *Circ Res*.
548 2010;107:263-270
549 54. Helwig BG, Craig RA, Fels RJ, Blecha F, Kenney MJ. Central nervous system
550 administration of interleukin-6 produces splenic sympathoexcitation. *Auton*
551 *Neurosci*. 2008;141:104-111
552 55. Niijima A, Hori T, Aou S, Oomura Y. The effects of interleukin-1 beta on the activity
553 of adrenal, splenic and renal sympathetic nerves in the rat. *J Auton Nerv Syst*.
554 1991;36:183-192
555 56. Zhang ZH, Wei SG, Francis J, Felder RB. Cardiovascular and renal sympathetic
556 activation by blood-borne tnf-alpha in rat: The role of central prostaglandins. *Am J*
557 *Physiol Regul Integr Comp Physiol*. 2003;284:R916-927

558 57. Kubler W, Haass M. Cardioprotection: Definition, classification, and fundamental
559 principles. *Heart*. 1996;75:330-333
560 58. Tomiyama H, Shiina K, Matsumoto-Nakano C, Ninomiya T, Komatsu S, Kimura K,
561 Chikamori T, Yamashina A. The contribution of inflammation to the development
562 of hypertension mediated by increased arterial stiffness. *J Am Heart Assoc*. 2017;6
563 59. Lavine KJ, Epelman S, Uchida K, Weber KJ, Nichols CG, Schilling JD, Ornitz DM,
564 Randolph GJ, Mann DL. Distinct macrophage lineages contribute to disparate
565 patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc*
566 *Natl Acad Sci U S A*. 2014;111:16029-16034
567 60. Heo GS, Kopecky B, Sultan D, Ou M, Feng G, Bajpai G, Zhang X, Luehmann H,
568 Detering L, Su Y, Leuschner F, Combadiere C, Kreisel D, Gropler RJ, Brody SL,
569 Liu Y, Lavine KJ. Molecular imaging visualizes recruitment of inflammatory
570 monocytes and macrophages to the injured heart. *Circ Res*. 2019;124:881-890
571

572 **NOVELTY AND SIGNIFICANCE**

573

574 **What is new?**

575 - Mice that lack the peptide hormone catestatin (CST) are hypertensive.
576 - CST skews macrophages to an anti-inflammatory phenotype.
577 - CST reduces inflammation in heart and adrenal gland.

578 **What is relevant?**

579 - Hypertension is associated with inflammation.
580 - Hypertensive patients have reduced plasma levels of CST.
581 - In mouse models, hypertension can be reduced by exogenous CST.

582 **Summary**

583 - The anti-hypertensive effects of CST are mediated via CST's immunosuppression
584 of macrophages.
585 - CST is a key autocrine attenuator of the cardiac inflammation in hypertension.

586

587

588 **FIGURE LEGENDS**
589

590 **Fig. 1. Generation of CST-KO mice. (A)** Schematic diagram showing the cloning
591 strategy for generating CST-KO mice. DTA, diphtheria toxin; FRT, *Flp* recognition target.
592 **(B)** Screening of CST-KO mice by PCR. Primer set 1 flanks the CST domain; expected
593 PCR products: 162 bp, CST-KO; 225 bp, WT mice. Reverse primer 2 binds within CST-
594 coding region; no band, CST-KO, 180 bp WT mice. **(C)** Western blots showing the
595 presence of CgA and CST in WT and CST-KO mice using monoclonal antibody (mAb)
596 5A8 that does not recognize CST, and rabbit polyclonal antibody directed against the C-
597 terminus of CST (pAb CT-CST), which does not recognize CgA beyond CST domain. A
598 truncated CgA (CgA_{1-384}) was present in WT mice but not in CST-KO mice, confirming
599 the deletion of CST domain. **(D)** Adrenal CgA content (n=12) and plasma CST levels
600 (n=6). Unpaired two-tailed *t*-test: ns, not significant; ***p<0.001.
601

602 **Fig. 2. Hypertension in CST-KO mice. (A)** Diurnal systolic (SBP), diastolic (DBP) and
603 mean arterial (MAP) blood pressure by telemetry in wild-type (WT) and CST-KO mice
604 (n=8). **(B)** Daytime SBP by tail-cuff (n=9) and **(C)** plasma CST levels (n=6) of WT and
605 CST-KO mice treated with CST or saline (Sal). Two-way ANOVA: ns, not significant;
606 **p<0.01; ***p<0.001.
607

608 **Fig. 3. CST-KO mice display systemic and cardiac inflammation, which can be**
609 **reversed with exogenous CST. (A)** Plasma cytokines in WT and CST-KO mice (n=8).
610 Sal: saline. **(B&C)** RT-qPCR data showing steady-state mRNA levels of anti- **(B)** and pro-
611 inflammatory **(C)** genes in left ventricle (n=8). **(B&D)** Protein levels of IL-10 **(B)** and pro-
612 inflammatory cytokines **(D).** One-way ANOVA: ns, not significant; *p<0.05; **p<0.01;
613 ***p<0.001.
614

615 **Fig. 4. CST exerts anti-inflammatory effects in isolated macrophages and**
616 **macrophages are major producers of CST. (A)** Western blot analysis of
617 phosphorylated (Ser177/181) and total IKK2 in heart of 4 WT mice, 5 CST-KO mouse
618 and 5 CST-KO mice with intraperitoneal injection of CST. **(B&C)** Protein levels of

619 cytokines (n=8) in supernatant of bone-marrow derived macrophages (M0-BMDMs)
620 differentiated to proinflammatory M1-type **(B)** and anti-inflammatory M2-type phenotypes
621 **(C)**. Cells were treated with 100 nM CST. **(D)** Western blots showing the presence of CgA
622 and CST in peritoneal macrophages (n=4). **(E)** Plasma CST levels in CST-KO mice which
623 received BMT from CST-KO or WT mice (n=6). Panel A-C: one-way ANOVA; panel E: *t*-
624 test; ns, not significant; *p<0.05; **p<0.01; ***p<0.001.
625
626 **Fig. 5. Macrophages mediate cardioprotective effect of CST. (A)** Flow cytometry data
627 showing $CD45^{+}F4/80^{+}CD11b^{+}Ly6c^{-}$ macrophages and $CD45^{+}F4/80^{-}CD11b^{+}Ly6c^{+}$
628 recruited monocytes in CST-KO heart (n=3). Sal: saline control. **(B)** Systolic blood
629 pressure (SBP) after depletion of macrophages by CDN (n=8) and bone-marrow transfer
630 (BMT) into irradiated mice (n=8). Levels of cytokines in plasma **(C)** and heart **(D)** of mice
631 with BMT (n=8). One-way ANOVA: ns, not significant; *p<0.05; **p<0.01; ***p<0.001.
632
633 **Fig. 6. Increased catecholamine secretion in CST-KO mice. (A)** Plasma, adrenal,
634 heart and kidney norepinephrine (NE) and epinephrine (EPI) levels in WT and CST-KO
635 mice. Mice were treated with saline (Sal) or CST or underwent bone-marrow transfer
636 (BMT) into irradiated mice (n=8-12). **(B)** TEM micrographs of chromaffin granules (CG) in
637 the adrenal medulla of CST-KO mice. PM, plasma membrane. **(C)** TEM micrographs of
638 splanchnic-adrenomedullary synapse with acetylcholine vesicles (AChV) and dense core
639 peptidergic vesicles (PdV). CC, chromaffin cell; CG, chromaffin granule. Two-way
640 ANOVA: ns, not significant; *p<0.05; **p<0.01; ***p<0.001.
641

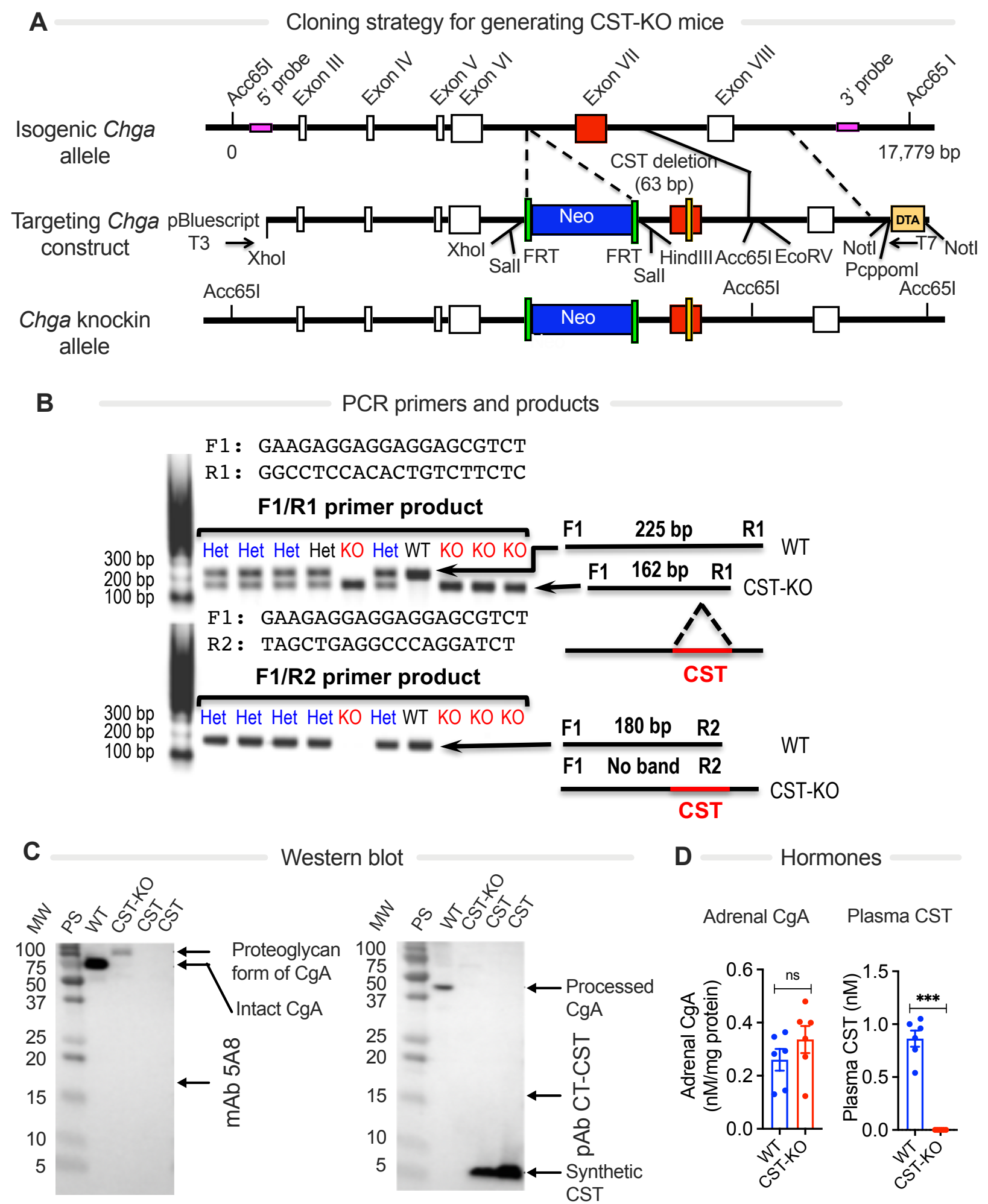
A
Cloning strategy for generating CST-KO mice
Isogenic Chga allele
Acc65I
5' probe
Exon III
Exon IV
Exon V
Exon VI
Exon VII
Exon VIII
3' probe
Acc65 I
0
17,779 bp
CST deletion (63 bp)
Targeting Chga construct
pBluescript
T3
XhoI
XhoI
SalI
FRT
Neo
FRT
SalI
HindIII
Acc65I
EcoRV
DTA
NotI
PcppomI
T7
NotI
Acc65I
Acc65I
Acc65I
Chga knockin allele
Neo
B
PCR primers and products
F1: GAAGAGGAGGAGGAGCGTCT
R1: GGCCTCCACACTGTCTTCTC
F1/R1 primer product
Het Het Het Het KO Het WT KO KO KO
300 bp
200 bp
100 bp
F1 225 bp R1
WT
F1 162 bp R1
CST-KO
F1: GAAGAGGAGGAGGAGCGTCT
R2: TAGCTGAGGCCCAGGATCT
CST
F1/R2 primer product
Het Het Het Het KO Het WT KO KO KO
300 bp
200 bp
100 bp
F1 180 bp R2
WT
F1 No band R2
CST-KO
CST
C
Western blot
MW
PS
WT
CST-KO
CST
CST
100
75
50
37
25
20
15
10
5
Proteoglycan form of CgA
Intact CgA
mAb 5A8
Processed CgA
pAb CT-CST
Synthetic CST
D
Hormones
Adrenal CgA
Plasma CST
Adrenal CgA (nM/mg protein)
0.6
0.4
0.2
0.0
ns
Plasma CST (nM)
1.5
1.0
0.5
0.0
***
WT
CST-KO

Figure 1

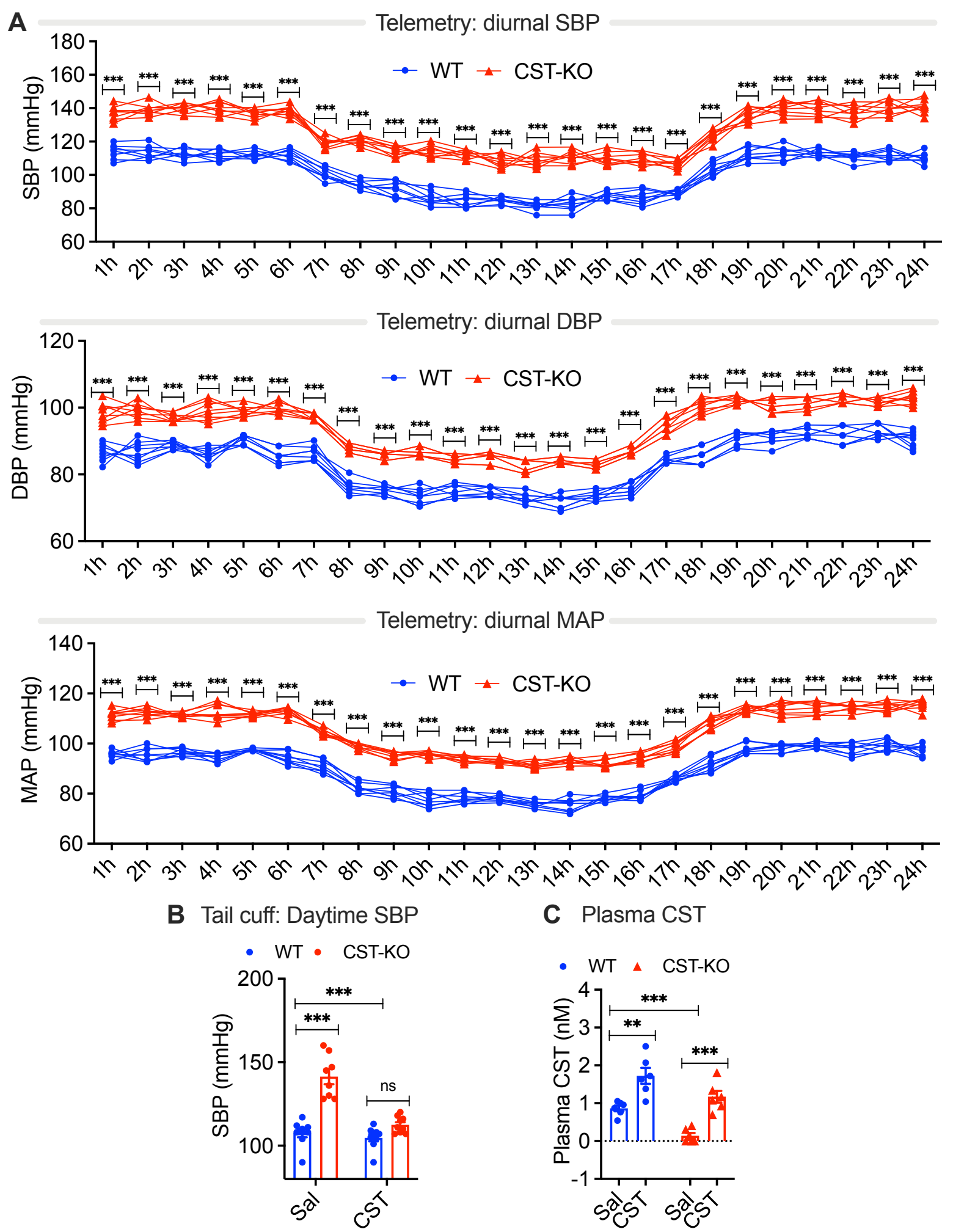
A
Telemetry: diurnal SBP
SBP (mmHg)
WT
CST-KO
Telemetry: diurnal DBP
DBP (mmHg)
Telemetry: diurnal MAP
MAP (mmHg)
B Tail cuff: Daytime SBP
SBP (mmHg)
ns
Sal
CST
C Plasma CST
Plasma CST (nM)

Figure 2

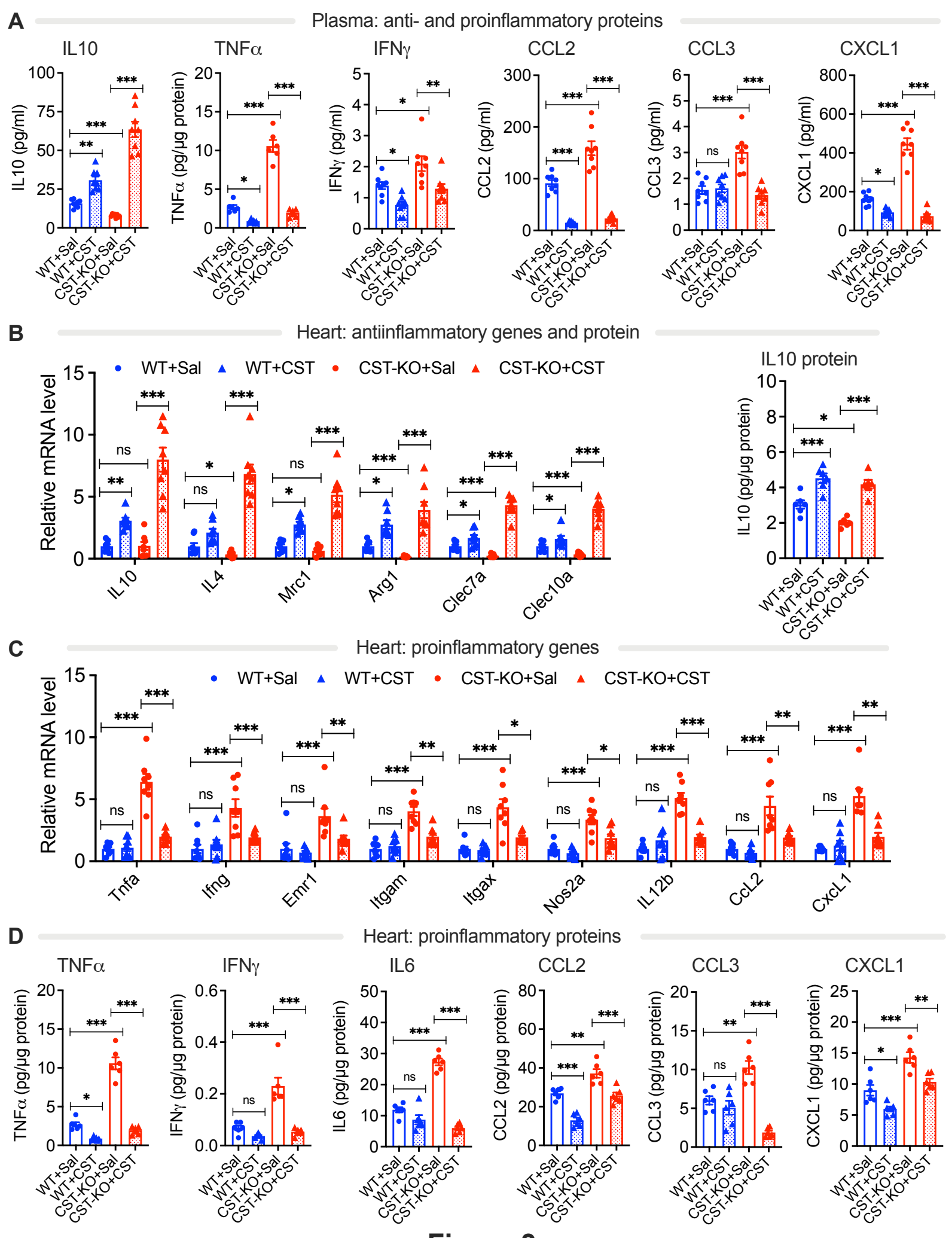
A
Plasma: anti- and proinflammatory proteins
IL10
TNFα
IFNγ
CCL2
CCL3
CXCL1
IL10 (pg/ml)
TNFα (pg/μg protein)
IFNγ (pg/ml)
CCL2 (pg/ml)
CCL3 (pg/ml)
CXCL1 (pg/ml)
WT+Sal
WT+CST
CST-KO+Sal
CST-KO+CST
B
Heart: antiinflammatory genes and protein
Relative mRNA level
IL10
IL4
Mrc1
Arg1
Clec7a
Clec10a
IL10 protein
IL10 (pg/μg protein)
C
Heart: proinflammatory genes
Tnfa
Ifng
Emr1
Itgam
Itgax
Nos2a
IL12b
Ccl2
Cxcl1
D
Heart: proinflammatory proteins
TNFα (pg/μg protein)
IFNγ (pg/μg protein)
IL6 (pg/μg protein)
CCL2 (pg/μg protein)
CCL3 (pg/μg protein)
CXCL1 (pg/μg protein)

Figure 3

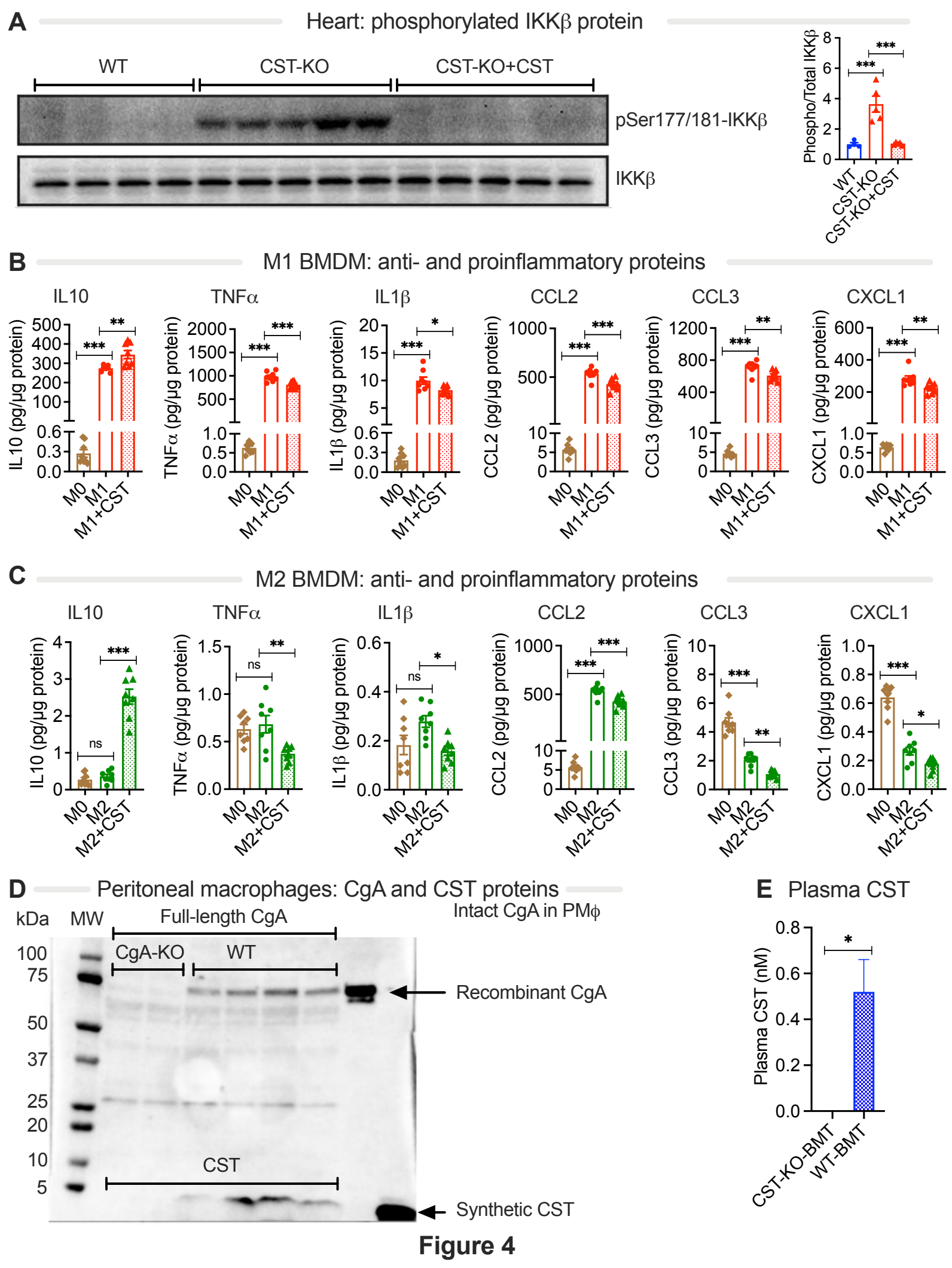
A
Heart: phosphorylated IKKβ protein
WT
CST-KO
CST-KO+CST
pSer177/181-IKKβ
IKKβ
Phospho/Total IKKβ
B
M1 BMDM: anti- and proinflammatory proteins
IL10
TNFα
IL1β
CCL2
CCL3
CXCL1
IL10 (pg/μg protein)
TNFα (pg/μg protein)
IL1β (pg/μg protein)
CCL2 (pg/μg protein)
CCL3 (pg/μg protein)
CXCL1 (pg/μg protein)
M0
M1
M1+CST
C
M2 BMDM: anti- and proinflammatory proteins
M2
M2+CST
ns
D
Peritoneal macrophages: CgA and CST proteins
kDa
MW
Full-length CgA
Intact CgA in PMϕ
CgA-KO
WT
Recombinant CgA
CST
Synthetic CST
E
Plasma CST
Plasma CST (nM)
CST-KO-BMT
WT-BMT

Figure 4

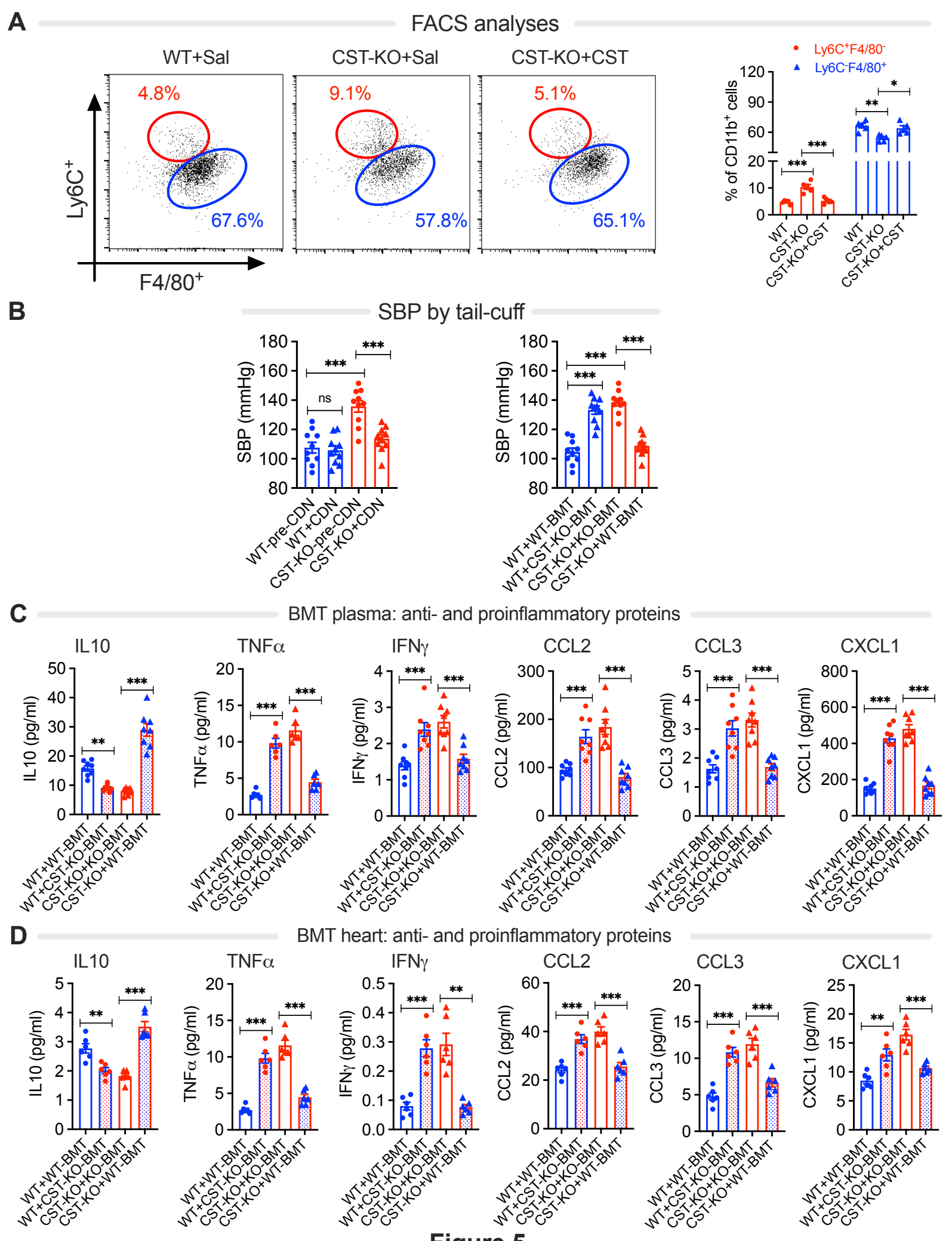
A
FACS analyses
WT+Sal
CST-KO+Sal
CST-KO+CST
4.8%
67.6%
9.1%
57.8%
5.1%
65.1%
Ly6C+
F4/80+
Ly6C+F4/80-
Ly6C-F4/80+
% of CD11b+ cells
WT
CST-KO
CST-KO+CST
B
SBP by tail-cuff
SBP (mmHg)
ns
WT-pre-CDN
WT+CDN
CST-KO-pre-CDN
CST-KO+CDN
WT+WT-BMT
WT+CST-KO-BMT
CST-KO+KO-BMT
CST-KO+WT-BMT
C
BMT plasma: anti- and proinflammatory proteins
IL10
TNFα
IFNγ
CCL2
CCL3
CXCL1
IL10 (pg/ml)
TNFα (pg/ml)
IFNγ (pg/ml)
CCL2 (pg/ml)
CCL3 (pg/ml)
CXCL1 (pg/ml)
D
BMT heart: anti- and proinflammatory proteins

Figure 5

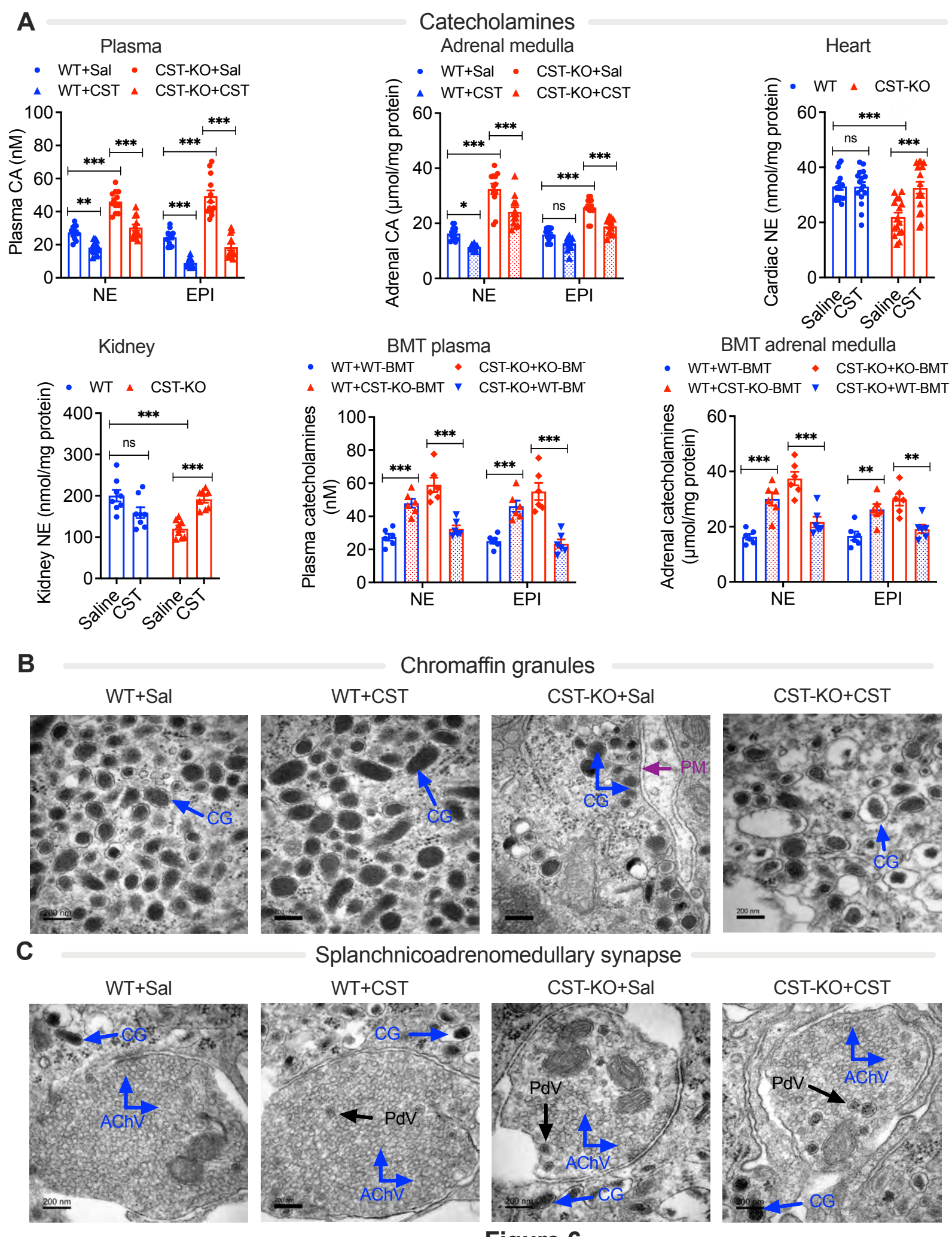
A
Catecholamines
Plasma
WT+Sal
CST-KO+Sal
WT+CST
CST-KO+CST
Plasma CA (nM)
NE
EPI
Adrenal medulla
Adrenal CA (μmol/mg protein)
Heart
WT
CST-KO
Cardiac NE (nmol/mg protein)
Saline
CST
Kidney
Kidney NE (nmol/mg protein)
BMT plasma
WT+WT-BMT
CST-KO+KO-BMT
WT+CST-KO-BMT
CST-KO+WT-BMT
Plasma catecholamines (nM)
BMT adrenal medulla
Adrenal catecholamines (μmol/mg protein)
B
Chromaffin granules
WT+Sal
WT+CST
CST-KO+Sal
CST-KO+CST
CG
PM
200 nm
C
Splanchnicoadrenomedullary synapse
AChV
PdV

Figure 6