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

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ABSTRACT

Hypertension (HTN) is a pandemic associated with inflammation and excessive production of catecholamines. Previous work has shown that hypertensive patients have reduced plasma levels of Catestatin (CST), a bioactive cleavage product of the prohormone Chromogranin A (CgA). Similarly, 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 the present 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. Additionally, intraperitoneal injection with CST reverses these phenotypes, and ischemic pre-conditioning-induced cardioprotection was also abolished in CST-KO mice. To further explore the relationship between HTN and CST/macrophages, 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.

ABBREVIATIONS

BMDM: bone marrow-derived macrophage;
BP: blood pressure;
BMT: bone marrow transfer;
CCL: C-C motif chemokine ligand
CXC: C-X-C motif chemokine ligand
CDN: chlodronate;
CgA: chromogranin A;
CST: catestatin;
DA: dopamine;
EPI: epinephrine;
HTN: hypertension;
IFN: interferon;
IL: interleukin;
IPC: ischemic preconditioning;
IR: ischemia/reperfusion;
KO: knockout;
LV: left ventricular;
LVDP: LV developed pressure;
LVEDP: LV end diastolic pressure;
LVPWd: LV posterior wall thickness;
MAP: mean arterial pressure;
IVSd: interventricular septum wall thickness;
NE: norepinephrine;
SBP: systolic blood pressure;
TEM: transmission electron microscopy;
TNF: tumor necrosis factor;
WT: wild-type;

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] . Elevated levels of pro- and reduced levels of anti-inflammatory cytokines have been reported in hypertensive or pre-hypertensive patients compared to healthy individuals [4-6] . These 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 dopamine (DA), 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 an unexpected finding of how catecholamine production is attenuated by another secretion product of neuroendocrine cells: the peptide catestatin (CST). Previous work has shown that 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 in HTN [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 decreases the BP and the levels of plasma catecholamines to that seen in control littermates [19]. It is increasingly clear in mouse models of diabetes [20], colitis [21] and atherosclerosis [22] that CST exerts anti-inflammatory effects by inhibiting the activation of

114 macrophages and shifting their differentiation to more anti-inflammatory phenotypes [23].
115 Therefore, we hypothesized that CST exerts its cardioprotective role by skewing
116 macrophages to more anti-inflammatory phenotypes, thereby resulting in lower
117 catecholamine production.

118 To directly discern the role of CST in the regulation of the cardiovascular system,
119 we generated a precise tool: CST-KO mice, which lack only the CST-coding region of the
120 *Chga* gene. As predicted, CST-KO mice display a hypertensive, hyperadrenergic, and
121 inflammatory phenotype which is rescued by exogenous addition of CST. Thus, by
122 exploring our CST-KO mice in conjunction with macrophage depletion via two methods,
123 chlodronate (CDN) liposomes and from bone-marrow transfer (BMT) between CST-KO
124 and wild-type (WT), this study sought to elucidate the neuroendocrine relationship
125 between CST, catecholamine production, and ultimately the anti-inflammatory/anti-HTN
126 functions of macrophages. With an increased understanding of the CST to HTN pathway,
127 this could be an important advance to eventually utilizing CST as a novel target for the
128 treatment and prevention of HTN.
129

METHODS

An expanded Materials and Methods section is available in the Data Supplement. Further 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. Further studies will look into female mice. 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 and sex-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 with exogenous CST, mice were injected intraperitoneally with CST (2 μg/g body weight) at 9:00 AM for 2-4 weeks before collecting feces or 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 either unpaired two-tailed Student's *t*-test for comparison of two groups or one-way or two-way analysis of variance (ANOVA) for comparison of more than two groups followed by Tukey's *post hoc* test if appropriate. All data are presented as mean ± SEM and significance was assumed when $p<0.05$.

RESULTS

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

CST-KO mice are hypertensive. Consistent with the anti-HTN functions of CST [19, 25, 26], we found that the CST-KO mice are hypertensive and display diurnal increases in both systolic and mean arterial BP (Figure 2A & S1). The high BP in CST-KO mice is rescued by intraperitoneal injection of exogenous CST (2 µg/g body weight for 15 days), whereas CST had no impact on normotensive BP in WT mice (Figure 2B). In WT mice, the plasma CST level was 0.86 nM, which increased to 1.72 nM 24 hrs after administration of CST (Figure 2C). In CST-KO mice, plasma CST was 1.17 nM after 24 hr of CST supplementation, indicating that CST supplementation provided a near physiological concentration of CST.

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

respective IR controls. In WT mice, but not in CST-KO mice, IPC also improved recoveries of both the maximum and minimum rates of pressure development in the LV (dP/dt_{max} and dP/dt_{min}) compared to the respective IR controls (Figure S2B). These data show that whereas IPC conferred protection against IR damage in WT mice, with observed improvements in all functional measures in the reperfusion period, CST-KO mice could not be preconditioned.

CST-KO mice have increased inflammation in heart and circulation. CST is an anti-inflammatory peptide [23 28], raising the possibility that CST might regulate cardiovascular function via the immune system. Indeed, in plasma of CST-KO mice, we found increased levels of proinflammatory cytokines TNF-α, IFN-γ, C-C motif chemokine ligand (CCL)-2 and -3, and C-X-C motif chemokine ligand (CXCL)-1 (Figure 3A). By contrast, the anti-inflammatory cytokine IL-10 was decreased in CST-KO mice. Intraperitoneal injection with exogenous CST in CST-KO mice reversed this phenotype: it decreased the levels of most proinflammatory cytokines and increased anti-inflammatory cytokines in plasma of both WT and CST-KO mice (Figure 3A). RT-PCR also revealed inflammation in the heart of CST-KO mice: the expression of anti-inflammatory genes *IL10*, *IL4*, *Mrc1*, *Arg1*, *Clec7a* and *Clec10a* was reduced, whereas the pro-inflammatory genes *Tnfa*, *Ifng*, *Emr1*, *Itgam*, *Itgax*, *Nos2a*, *IL12b CcL2*, and *CxcL1* were upregulated (Figure 3B&C). LV protein levels of the proinflammatory cytokines TNF-α, IFN-γ, CCL-2, CCL-3, CXCL-1, and IL-6 were also elevated in CST-KO mice (Figure 3D). These phenotypes were also reversable by intraperitoneal injection of CST. We also observed increased phosphorylation (Ser177/181) of IKK-β, a component of the cytokine-activated intracellular signaling pathway involved in triggering immune responses via NF-κB (Figure 4A). These findings show that the immune system of CST-KO mice is skewed towards inflammation.

CST reduces pro-inflammatory macrophages *in vitro*. In the next set of experiments, we addressed whether CST would directly shift macrophages to more anti-inflammatory responses *in vitro*. Macrophages were derived from bone-marrow of WT mice (BMDM) and differentiated to an either pro-inflammatory M1-like phenotype or to an anti-inflammatory M2-like phenotype [29]. Culturing these macrophages for 24 hr with 100 nM

CST resulted in a small, but significant, reduction of the production of pro-inflammatory cytokines TNF-α, CCL-2, CCL-3, CXCL-1 and IL-1β (Figure 4B&C). In contrast, the levels of anti-inflammatory IL-10 were increased, especially for the M2-like macrophages.

Since macrophages are a secretory cell type, we also addressed whether macrophages produce CgA and CST. Indeed, Western blotting analysis revealed the presence of both CgA and CST in peritoneal macrophages (Figure 4D), which were isolated after thioglycolate (3% solution in water) and cultured in DMEM with 10% FBS for 48 hr with daily medium changes [29]. To assess the physiological relevance of CST-production by macrophages, we performed BMT experiments in which we irradiated CST-KO mice and then cross-transplanted the marrow from WT mice. We analyzed plasma CST of these mice and found that WT bone-marrow recipient CST-KO mice, but not CST-KO bone-marrow recipients, had near physiologic levels of plasma CST (0.52 nM) (Figure 4E). Thus, macrophages (and possibly other bone-marrow derived cell types) are major producers of CST in circulation.

Macrophages are key effector cells responsible for the anti-inflammatory actions of CST. TEM studies revealed abundant infiltration of macrophages and fibrosis in the intercellular spaces between chromaffin cells in the adrenal medulla of CST-KO mice (Figure S3 and S4**)**. Also, marked cardiac fibrosis and an increased presence of macrophages were observed in the heart of saline-treated CST-KO mice, as shown by TEM and flow cytometry (Figure 5A, S3, S5 and S6A). In both the adrenal gland and heart of CST-KO mice, CST supplementation reduced the abundance of macrophage infiltrates (Figure S3). This was supported by flow cytometry analysis showing a ~38% decrease of $CD11b^{+}F4/80^{+}$ macrophages in CST-supplemented CST-KO heart (Figure 5A and S6B).

We assessed the functional role of the infiltrated macrophages in the heart and adrenal gland of CST-KO mice using two independent approaches. First, we depleted macrophages by CDN liposomes (Figure S6B), which not only depleted macrophages in heart and adrenal gland (Figure S3), but also reversed the hypertensive phenotype of CST-KO mice (Figure 5B). Second, we carried out BMT assays in which we irradiated both WT and CST-KO mice and then cross-transplanted their marrows: bone-marrow from CST-KO mice was transplanted into WT mice and *vice versa*. Both the inflammatory

and hypertensive phenotypes were transferred by BMT: while CST-KO bone-marrow recipient WT mice showed increased BP; elevated levels in plasma and heart of TNF-α, IFN-γ, CCL-2, CCL-3, and CXCL-1; and reduced levels of IL-10, WT bone-marrow recipient CST-KO mice showed the opposite phenotypes (Figure 5B-D). Since WT bone-marrow recipient CST-KO mice had near physiologic levels of plasma CST (Figure 4E), we conclude that macrophages and other immune cells are not only key effectors of the anti-hypertensive actions of CST but are also main producers of CST themselves.

Heightened sympathetic stimulation and hypersecretion of catecholamines in adrenal gland of CST-KO mice. Prior studies in humans, mice, and rats have shown that pro-inflammatory cytokines increase catecholamine production and secretion [3, 8, 9]. In line with this, we found that compared to WT littermates, CST-KO mice has elevated levels of both adrenal and plasma catecholamine levels (Figure 6A). This phenotype was also transferable by BMT: CST-KO bone-marrow recipient WT mice showed increased levels of NE and EPI in the adrenal medulla and plasma, whereas WT bone-marrow recipient CST-KO mice showed reduced levels of NE and EPI (Figure 6A).

Since heightened sympathetic nerve traffic has been documented in young, middle-aged, and elderly hypertensives; in pregnancy-induced hypertension; and in systo-diastolic hypertension or an isolated elevation of BP [30], we measured NE in the LV and kidney of WT and CST-KO mice. In contrast to the adrenal medulla and plasma, we observed reduced levels of NE in the heart and kidney of CST-KO mice (Figure 6A). Decreased NE in CST-KO mice indicates increased cardiac and renal spillover of NE, which is common in hypertensive and heart failure patients [31-34] . The CST-KO adrenal medulla exhibited abundant docked chromaffin granules and decreased acetylcholine-containing vesicles at the sympatho-adreno-medullary synapse (Figure 6B-C and S7), implicating heightened sympathetic nerve activity leading to hypersecretion of catecholamines. Supplementation of CST-KO mice with CST reversed this phenotype (Figure 6B-C and S7) and led to a concomitant decrease in both plasma and adrenal catecholamines (Figure 6A). The elevated BP in CST-KO mice was reversed by the nicotinic acetylcholine receptor antagonist chlorisondamine (Figure S8).

To test whether lack of CST affected heart structure and function, we undertook gravimetry (Figure S9A) and echocardiographic ultrasound imaging (Figure S9B). CST-KO mice showed increased heart weights and sizes compared to WT mice (Figure S9A). Although CST-KO mice maintained a similar level of left ventricular (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 [35, 36] , and a trend towards an increase in interventricular septum wall thickness (IVSd; p=0.07) (Figure S9B). Heart rate, left ventricular internal diameter during systole, and left ventricular 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 [37-39] . For instance, a recent study in the Japanese population found that prolonged low-grade inflammation as evaluated by increased C-reactive protein (CRP) increases arterial stiffness and the consequent development of HTN [40] . CRP is also considered as an independent risk factor for the development of HTN [41] . Besides the importance of peripheral vascular inflammation in hypertension, it has been shown that inducing inflammation in the brainstem triggers hypertension in a normotensive rat [42] .

From this study's data, it is becoming increasingly clear that this inflammation and the development of HTN are counteracted by the anti-inflammatory peptide hormone CST through its feedback inhibition/regulation of macrophages. 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 Ang II or DOCA-salt, while dysregulation of it causes sensitization to these hypertensive challenges [43] . 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 white coat syndrome and borderline hypertensive subjects [44] as well as in established hypertensive subjects of all ages, and the magnitude of this elevation is related to the magnitude of HTN [45] . In addition, hypertensive patients with metabolic risk factors, such as obesity, metabolic syndrome, or diabetes mellitus, also exhibit sympathetic overdrive [46-48] . Like humans, spontaneously hypertensive rats show reduced cardiac parasympathetic nerve activity, elevated sympathetic nerve activity and increased NE release [49] .

Immunoendocrine regulation of BP. The augmented sympathetic nerve activity in HTN is known to activate both myeloid cells and T cells [2], and circulating concentrations of pro-inflammatory cytokines are increased in primary HTN [50] . T-lymphocytes are critical for Angiotensin II and deoxycorticosterone acetate-salt-induced hypertension [51, 52] . Intracerebroventricular administration of IL-6 increases splenic sympathetic nerve activity [53] , while central administration of IL-1β increases adrenal, splenic and renal sympathetic nerve activity [54] . Injection of TNF-α into central sympathetic nuclei, such as the paraventricular nucleus increases sympathetic nerve activity, BP and heart rate in rats [55] .

To our knowledge, the present study is the first to demonstrate increased infiltration of macrophages in the adrenal medulla concomitant with increased secretion of catecholamines and the consequent development of HTN in CST-KO mice, which were normalized after CST supplementation. These findings imply that CST regulates the BP through a novel immunoendocrine regulation of catecholamine secretion via macrophages.

What causes the elevated BP in CST-KO mice? In these mice, the heart rate was not increased, and fractional shortening was unaltered compared to WT, indicating that the increased BP is not driven by elevated cardiac output. Consequently, it might be that the LV hypertrophy in the CST-KO mice, and possibly also the increased posterior wall thickness associated with high BP [35, 36] , is a secondary effect of the BP elevation. It seems therefore likely that the elevated BP is caused by increased vascular resistance,

due to vasoconstriction and/or increased arterial stiffness [40] . 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 [56, 57] . While a subset of macrophages orchestrate monocyte recruitment and contribute to heart failure pathogenesis [58] , others are increased during diastolic dysfunction [59] , myocardial infarction, and acute hemodynamic stress [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 in an 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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FIGURE LEGENDS

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

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

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

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

cytokines (n=8) in supernatant of bone-marrow derived macrophages (M0-BMDMs) differentiated to proinflammatory M1-type **(B)** and anti-inflammatory M2-type phenotypes **(C)**. Cells were treated with 100 nM CST. **(D)** Western blots showing the presence of CgA and CST in peritoneal macrophages (n=4). **(E)** Plasma CST levels in CST-KO mice which received BMT from CST-KO or WT mice (n=6). Panel A-C: one-way ANOVA; panel E: *t*-test; ns, not significant; *p<0.05; **p<0.01; ***p<0.001.

Fig. 5. Macrophages mediate cardioprotective effect of CST. (A) Flow cytometry data showing $CD45^{+}F4/80^{+}CD11b^{+}$ macrophages in CST-KO heart (n=3). Sal: saline control. **(B)** Systolic blood pressure (SBP) after depletion of macrophages by CDN (n=8) and bone-marrow transfer (BMT) into irradiated mice (n=8). Levels of cytokines in plasma **(C)** and heart **(D)** of mice with BMT (n=8). One-way ANOVA: ns, not significant; *p<0.05; **p<0.01; ***p<0.001.

Fig. 6. Increased catecholamine secretion in CST-KO mice. (A) Plasma, adrenal, heart and kidney norepinephrine (NE) and epinephrine (EPI) levels in WT and CST-KO mice. Mice were treated with saline (Sal) or CST or underwent bone-marrow transfer (BMT) into irradiated mice (n=8-12). **(B)** TEM micrographs of chromaffin granules (CG) in the adrenal medulla of CST-KO mice. PM, plasma membrane. **(C)** TEM micrographs of splanchnic-adrenomedullary synapse with acetylcholine vesicles (AChV) and dense core peptidergic vesicles (PdV). CC, chromaffin cell; CG, chromaffin granule. Two-way ANOVA: ns, not significant; *p<0.05; **p<0.01; ***p<0.001.

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. 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
36. Eliakim-Raz N, Prokupetz A, Gordon B, Shochat T, Grossman A. Interventricular septum and posterior wall thickness are associated with higher systolic blood pressure. *J Clin Hypertens (Greenwich)*. 2016;18:703-706
37. Rodriguez-Iturbe B, Pons H, Quiroz Y, Lanaspa MA, Johnson RJ. Autoimmunity in the pathogenesis of hypertension. *Nat Rev Nephrol*. 2014;10:56-62
38. Caillon A, Schiffrin EL. Role of inflammation and immunity in hypertension: Recent epidemiological, laboratory, and clinical evidence. *Curr Hypertens Rep*. 2016;18:21
39. McMaster WG, Kirabo A, Madhur MS, Harrison DG. Inflammation, immunity, and hypertensive end-organ damage. *Circ Res*. 2015;116:1022-1033
40. Tomiyama H, Shiina K, Matsumoto-Nakano C, Ninomiya T, Komatsu S, Kimura K, Chikamori T, Yamashina A. The contribution of inflammation to the development of hypertension mediated by increased arterial stiffness. *J Am Heart Assoc*. 2017;6
41. Blake GJ, Rifai N, Buring JE, Ridker PM. Blood pressure, c-reactive protein, and risk of future cardiovascular events. *Circulation*. 2003;108:2993-2999

42. Waki H, Liu B, Miyake M, Katahira K, Murphy D, Kasparov S, Paton JF. Junctional adhesion molecule-1 is upregulated in spontaneously hypertensive rats: Evidence for a prohypertensive role within the brain stem. *Hypertension*. 2007;49:1321-1327
43. Rudemiller NP, Crowley SD. Interactions between the immune and the renin-angiotensin systems in hypertension. *Hypertension*. 2016;68:289-296
44. Anderson EA, Sinkey CA, Lawton WJ, Mark AL. Elevated sympathetic nerve activity in borderline hypertensive humans. Evidence from direct intraneural recordings. *Hypertension*. 1989;14:177-183
45. Grassi G. Role of the sympathetic nervous system in human hypertension. *J Hypertens*. 1998;16:1979-1987
46. Grassi G, Seravalle G, Dell'Oro R, Turri C, Bolla GB, Mancia G. Adrenergic and reflex abnormalities in obesity-related hypertension. *Hypertension*. 2000;36:538-542
47. Grassi G, Dell'Oro R, Quarti-Trevano F, Scopelliti F, Seravalle G, Paleari F, Gamba PL, Mancia G. Neuroadrenergic and reflex abnormalities in patients with metabolic syndrome. *Diabetologia*. 2005;48:1359-1365
48. Huggett RJ, Scott EM, Gilbey SG, Stoker JB, Mackintosh AF, Mary DA. Impact of type 2 diabetes mellitus on sympathetic neural mechanisms in hypertension. *Circulation*. 2003;108:3097-3101
49. Judy WV, Farrell SK. Arterial baroreceptor reflex control of sympathetic nerve activity in the spontaneously hypertensive rat. *Hypertension*. 1979;1:605-614
50. Libby P, Ridker PM, Maseri A. Inflammation and atherosclerosis. *Circulation*. 2002;105:1135-1143
51. Guzik TJ, Hoch NE, Brown KA, McCann LA, Rahman A, Dikalov S, Goronzy J, Weyand C, Harrison DG. Role of the t cell in the genesis of angiotensin ii induced hypertension and vascular dysfunction. *J Exp Med*. 2007;204:2449-2460
52. Marvar PJ, Thabet SR, Guzik TJ, Lob HE, McCann LA, Weyand C, Gordon FJ, Harrison DG. Central and peripheral mechanisms of t-lymphocyte activation and vascular inflammation produced by angiotensin ii-induced hypertension. *Circ Res*. 2010;107:263-270
53. Helwig BG, Craig RA, Fels RJ, Blecha F, Kenney MJ. Central nervous system administration of interleukin-6 produces splenic sympathoexcitation. *Auton Neurosci*. 2008;141:104-111
54. Niijima A, Hori T, Aou S, Oomura Y. The effects of interleukin-1 beta on the activity of adrenal, splenic and renal sympathetic nerves in the rat. *J Auton Nerv Syst*. 1991;36:183-192
55. Zhang ZH, Wei SG, Francis J, Felder RB. Cardiovascular and renal sympathetic activation by blood-borne tnf-alpha in rat: The role of central prostaglandins. *Am J Physiol Regul Integr Comp Physiol*. 2003;284:R916-927
56. Lavine KJ, Epelman S, Uchida K, Weber KJ, Nichols CG, Schilling JD, Ornitz DM, Randolph GJ, Mann DL. Distinct macrophage lineages contribute to disparate patterns of cardiac recovery and remodeling in the neonatal and adult heart. *Proc Natl Acad Sci U S A*. 2014;111:16029-16034
57. Heo GS, Kopecky B, Sultan D, Ou M, Feng G, Bajpai G, Zhang X, Luehmann H, Detering L, Su Y, Leuschner F, Combadiere C, Kreisel D, Gropler RJ, Brody SL,

Liu Y, Lavine KJ. Molecular imaging visualizes recruitment of inflammatory monocytes and macrophages to the injured heart. *Circ Res*. 2019;124:881-890

58. Bajpai G, Bredemeyer A, Li W, Zaitsev K, Koenig AL, Lokshina I, Mohan J, Ivey B, Hsiao HM, Weinheimer C, Kovacs A, Epelman S, Artyomov M, Kreisel D, Lavine KJ. Tissue resident ccr2- and ccr2+ cardiac macrophages differentially orchestrate monocyte recruitment and fate specification following myocardial injury. *Circ Res*. 2019;124:263-278

59. Hulsmans M, Sager HB, Roh JD, Valero-Munoz M, Houstis NE, Iwamoto Y, Sun Y, Wilson RM, Wojtkiewicz G, Tricot B, Osborne MT, Hung J, Vinegoni C, Naxerova K, Sosnovik DE, Zile MR, Bradshaw AD, Liao R, Tawakol A, Weissleder R, Rosenzweig A, Swirski FK, Sam F, Nahrendorf M. Cardiac macrophages promote diastolic dysfunction. *J Exp Med*. 2018;215:423-440

60. Heidt T, Courties G, Dutta P, Sager HB, Sebas M, Iwamoto Y, Sun Y, Da Silva N, Panizzi P, van der Laan AM, Swirski FK, Weissleder R, Nahrendorf M. Differential contribution of monocytes to heart macrophages in steady-state and after myocardial infarction. *Circ Res*. 2014;115:284-295

NOVELTY AND SIGNIFICANCE

What is new?

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

What is relevant?

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

Summary

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

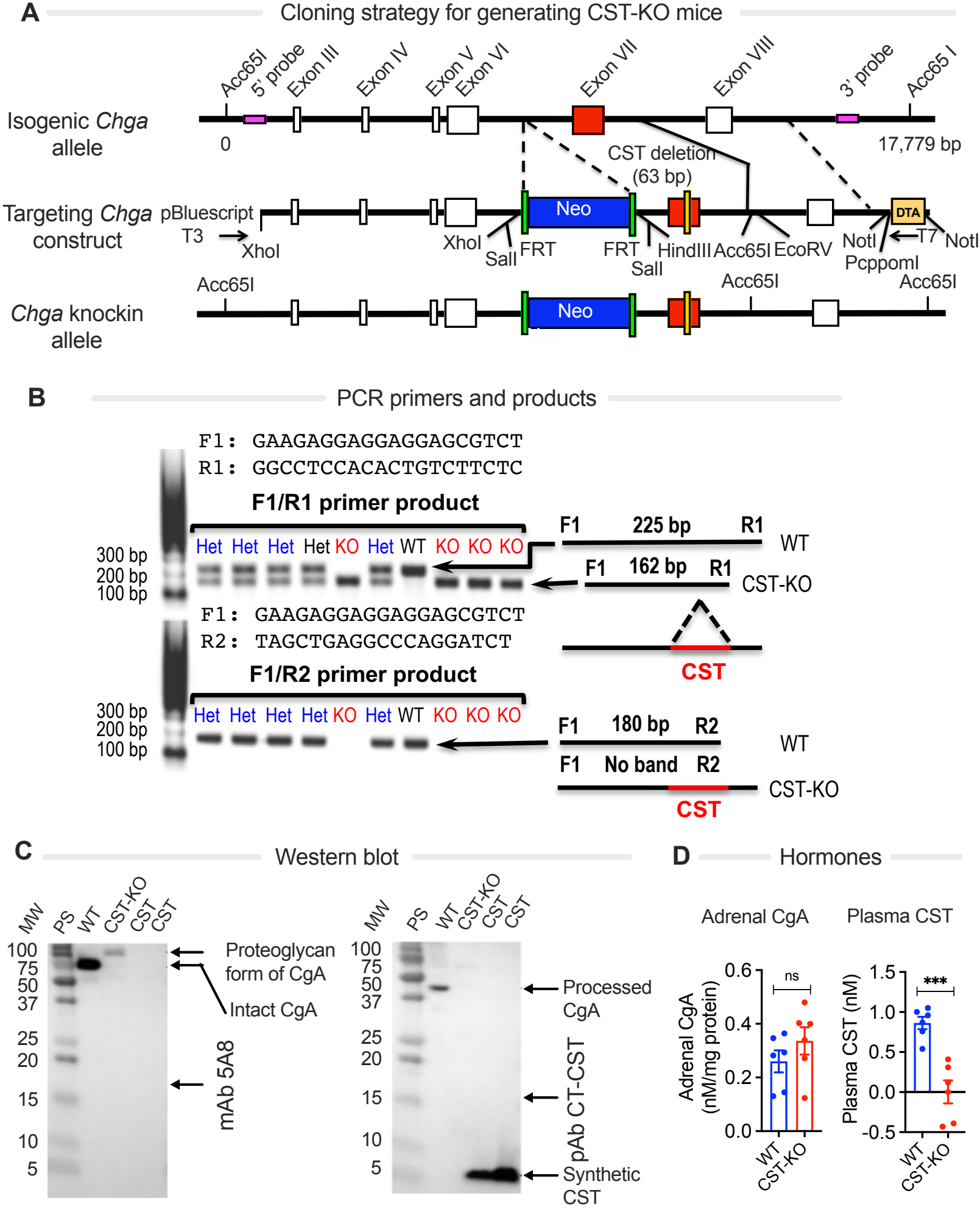
A
Cloning strategy for generating CST-KO mice
Isogenic Chga allele
Acc65I
5' probe
Exon III
Exon IV
Exon V
Exon VI
Exon VII
Exon VIII
3' probe
Acc65 I
0
17,779 bp
CST deletion (63 bp)
Targeting Chga construct
pBluescript T3
XhoI
Neo
DTA
SalI
FRT
HindIII
Acc65I
EcoRV
NotI
PcppomI
T7
Chga knockin allele
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
CST
F1: GAAGAGGAGGAGGAGCGTCT
R2: TAGCTGAGGCCCAGGATCT
F1/R2 primer product
F1 180 bp R2
F1 No band R2
C
Western blot
MW
PS
WT
CST-KO
CST
CST
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)
Plasma CST (nM)
ns
***

Figure 1

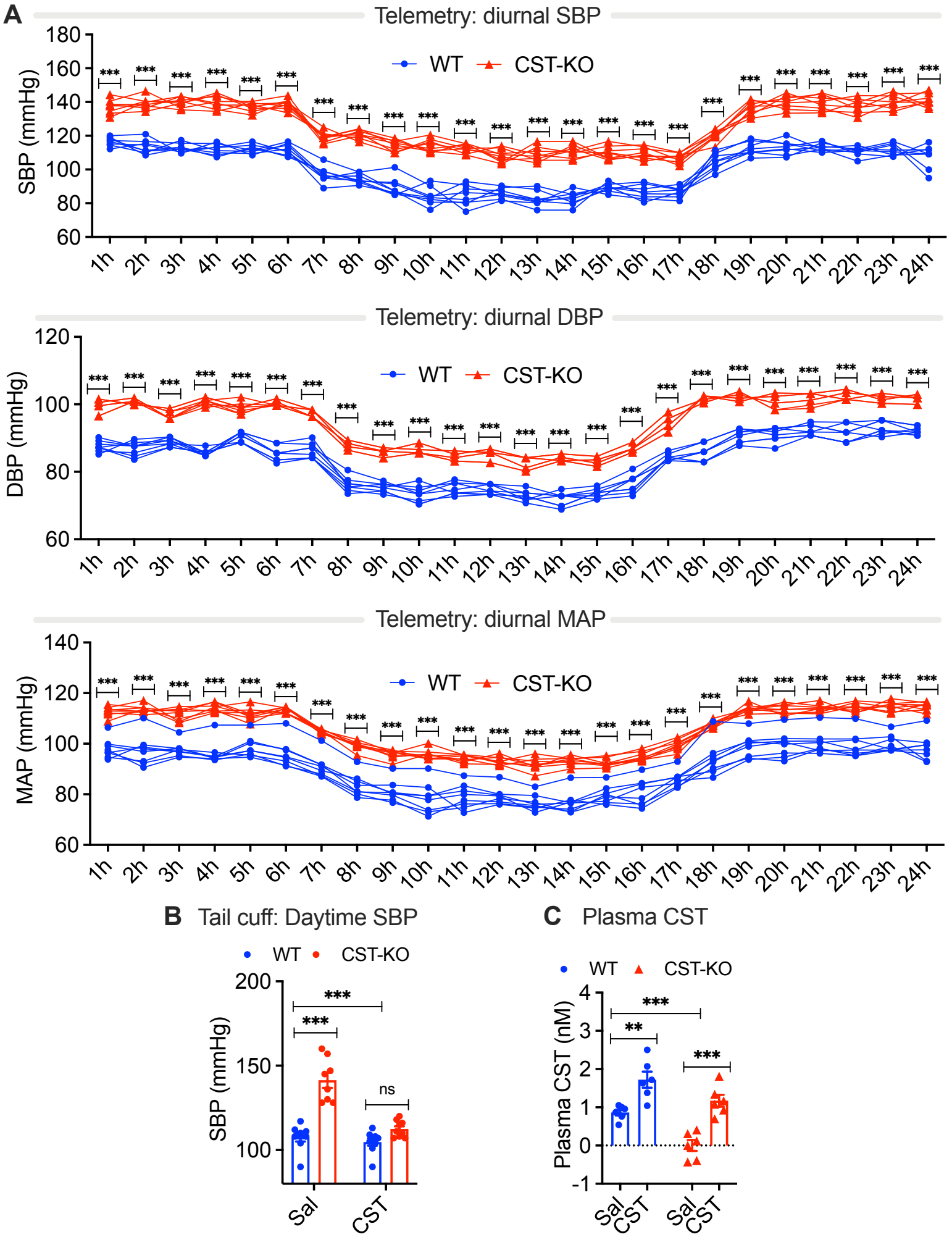
A
Telemetry: diurnal SBP
WT
CST-KO
SBP (mmHg)
180
160
140
120
100
80
60
1h 2h 3h 4h 5h 6h 7h 8h 9h 10h 11h 12h 13h 14h 15h 16h 17h 18h 19h 20h 21h 22h 23h 24h
Telemetry: diurnal DBP
DBP (mmHg)
120
100
80
60
Telemetry: diurnal MAP
MAP (mmHg)
140
120
100
80
60
B Tail cuff: Daytime SBP
SBP (mmHg)
200
150
100
ns
Sal
CST
C Plasma CST
Plasma CST (nM)
4
3
2
1
0
-1
Sal
CST
Sal
CST

Figure 2

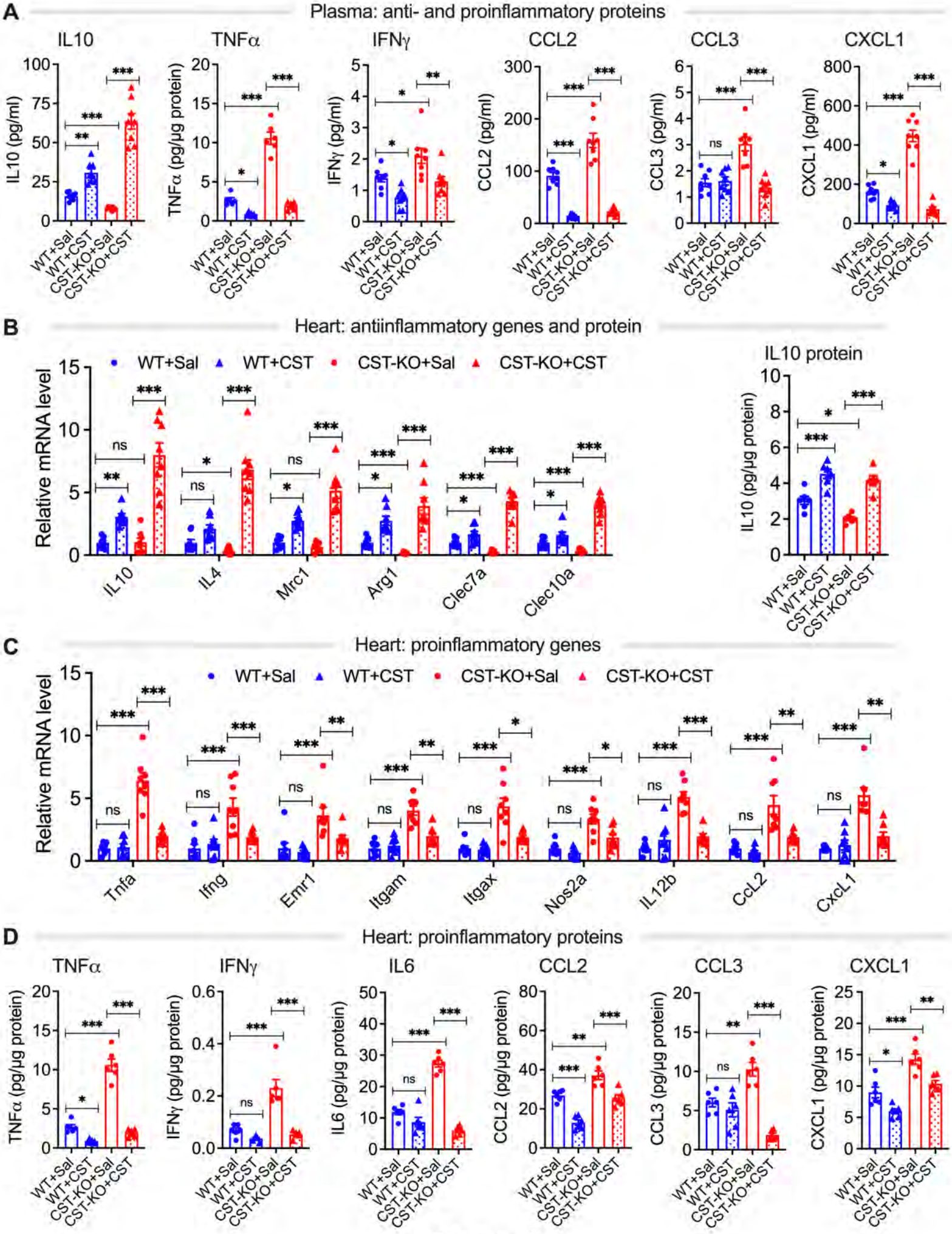
A
Plasma: anti- and proinflammatory proteins
IL10
TNFα
IFNγ
CCL2
CCL3
CXCL1
IL10 (pg/ml)
TNFα (pg/μg protein)
IFNγ (pg/ml)
CCL2 (pg/ml)
CCL3 (pg/ml)
CXCL1 (pg/ml)
WT+Sal
WT+CST
CST-KO+Sal
CST-KO+CST
B
Heart: antiinflammatory genes and protein
Relative mRNA level
IL10
IL4
Mrc1
Arg1
Clec7a
Clec10a
IL10 protein
IL10 (pg/μg protein)
C
Heart: proinflammatory genes
Relative mRNA level
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

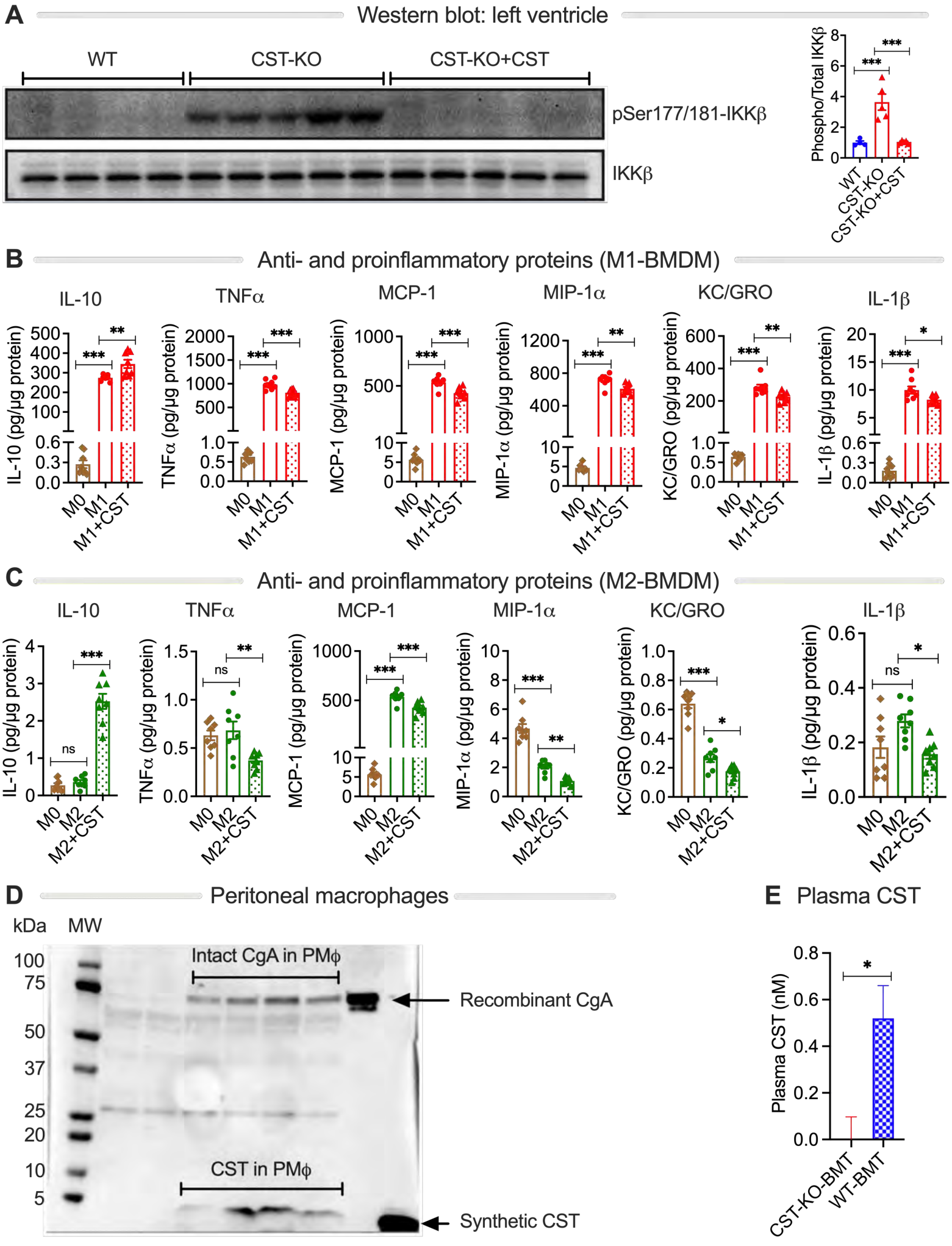
A
Western blot: left ventricle
WT
CST-KO
CST-KO+CST
pSer177/181-IKKβ
IKKβ
Phospho/Total IKKβ
B
Anti- and proinflammatory proteins (M1-BMDM)
IL-10
TNFα
MCP-1
MIP-1α
KC/GRO
IL-1β
IL-10 (pg/μg protein)
TNFα (pg/μg protein)
MCP-1 (pg/μg protein)
MIP-1α (pg/μg protein)
KC/GRO (pg/μg protein)
IL-1β (pg/μg protein)
M0
M1
M1+CST
C
Anti- and proinflammatory proteins (M2-BMDM)
M2
M2+CST
ns
D
Peritoneal macrophages
kDa
MW
Intact CgA in PMϕ
CST in PMϕ
Recombinant CgA
Synthetic CST
E
Plasma CST
Plasma CST (nM)
CST-KO-BMT
WT-BMT

Figure 4

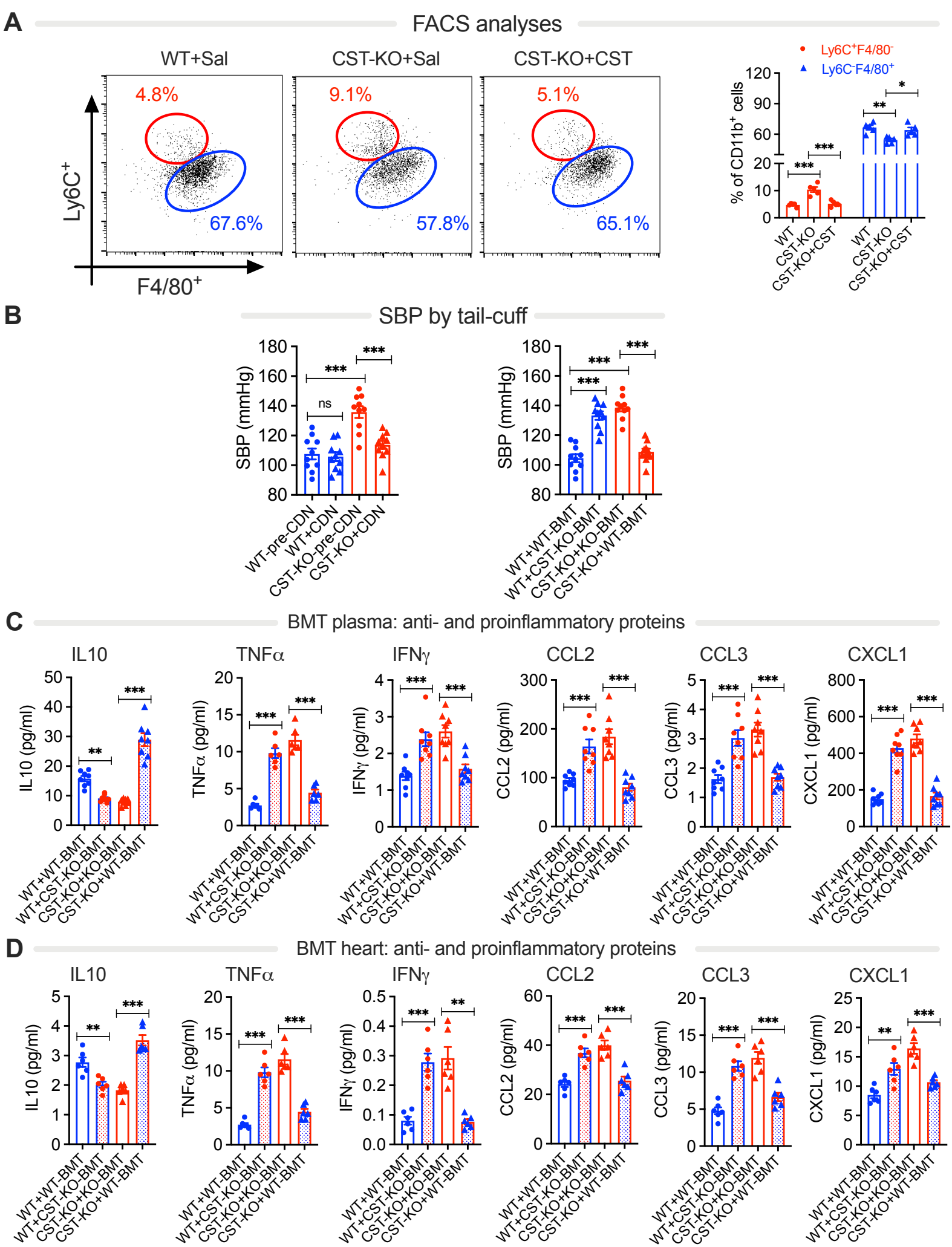
A
FACS analyses
WT+Sal
CST-KO+Sal
CST-KO+CST
4.8%
67.6%
9.1%
57.8%
5.1%
65.1%
Ly6C+
F4/80+
Ly6C+F4/80-
Ly6C-F4/80+
% of CD11b+ cells
WT
CST-KO
CST-KO+CST
B
SBP by tail-cuff
SBP (mmHg)
ns
WT-pre-CDN
WT+CDN
CST-KO-pre-CDN
CST-KO+CDN
WT+WT-BMT
WT+CST-KO-BMT
CST-KO+KO-BMT
CST-KO+WT-BMT
C
BMT plasma: anti-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

Figure 5

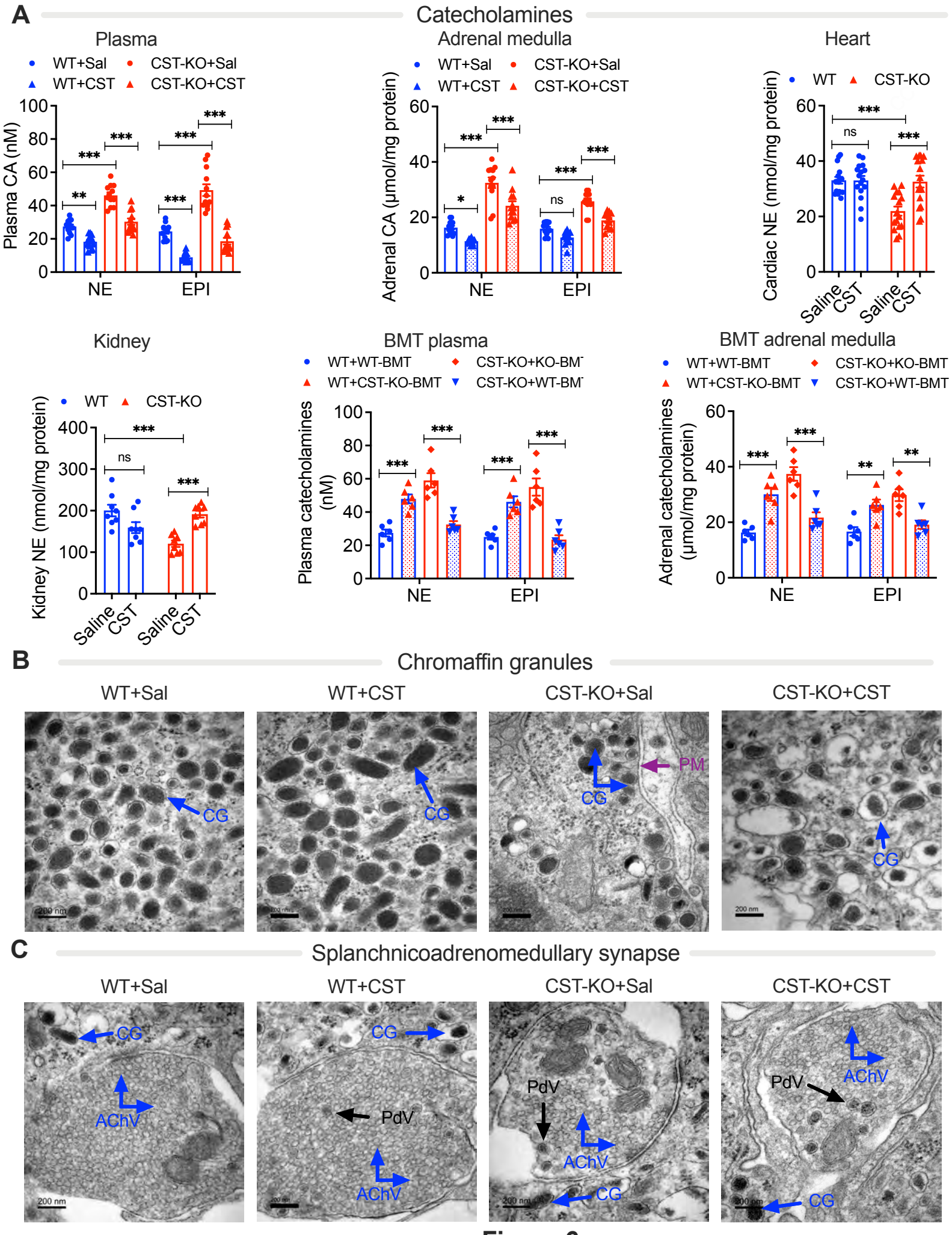
A
Catecholamines
Plasma
WT+Sal
CST-KO+Sal
WT+CST
CST-KO+CST
Plasma CA (nM)
NE
EPI
Adrenal medulla
Adrenal CA (μmol/mg protein)
Heart
WT
CST-KO
Cardiac NE (nmol/mg protein)
Saline
CST
Kidney
Kidney NE (nmol/mg protein)
BMT plasma
WT+WT-BMT
CST-KO+KO-BMT
WT+CST-KO-BMT
CST-KO+WT-BMT
Plasma catecholamines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

Figure 6