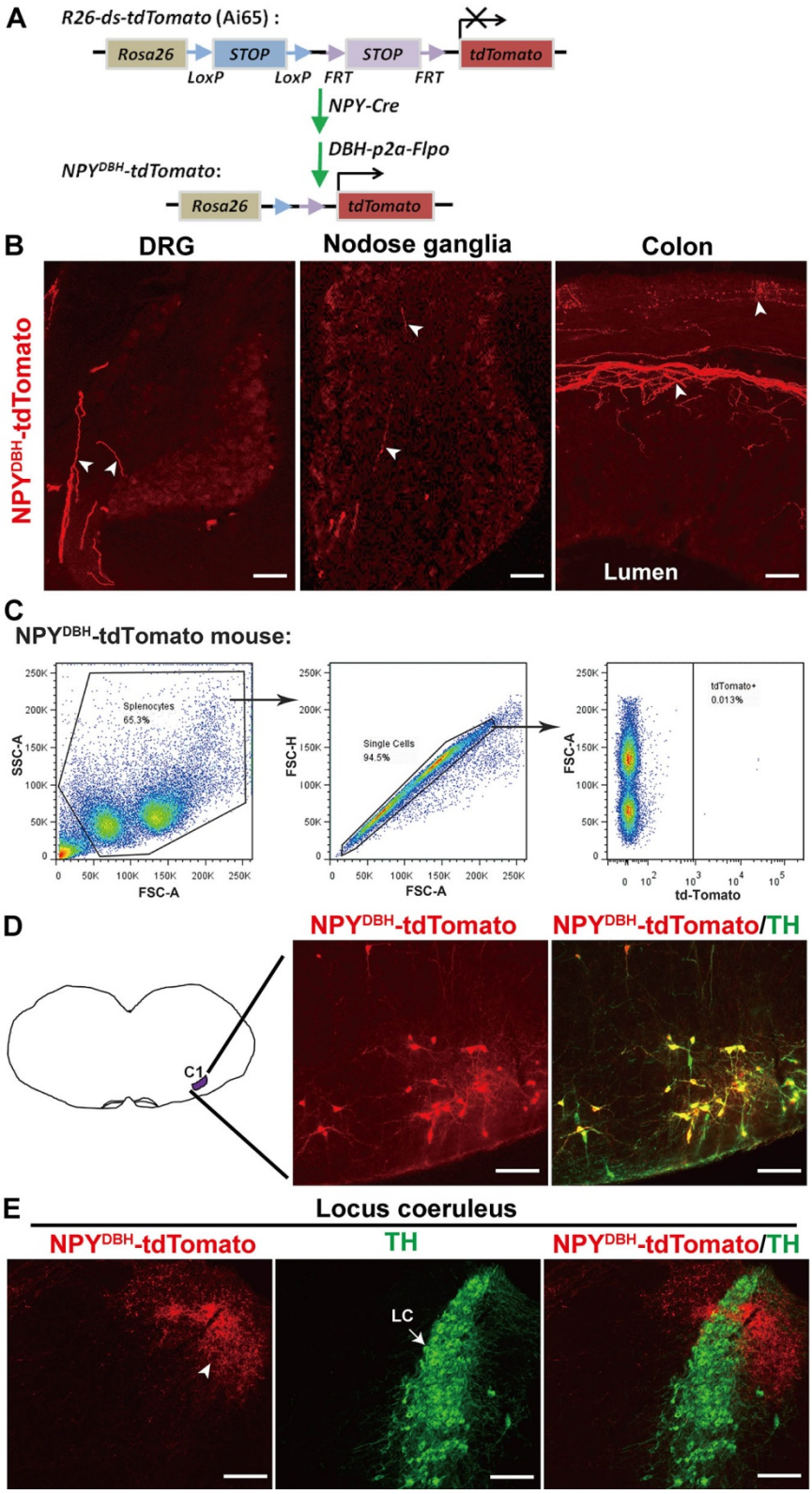

Figure S1. Intersectional marking and characterization of NPY^{DBH} cells. Related to Figure 1.

(A) Schematic showing intersectional genetic strategy to generate *NPY^{DBH}-tdTomato* mice, in which tdTomato expression is confined to NPY^{DBH} sympathetic cells defined by

coexpression of NPY-Cre and DBH-p2a-Flpo, upon removal of two STOP cassettes from the *Rosa26* allele.

(B) Representative sections through dorsal root ganglia ("DRG"), nodose ganglia, and the colon. tdTomato$^+$ fibers, but not somas, were detected in these peripheral tissues, and the fibers are likely from sympathetic ganglia.

(C) No tdTomato signals were detected in splenocytes. Left, forward scatter area (FSC-A) versus side scatter area (SSC-A) gating to isolate suspended cells from debris. Middle, FSC-A versus forward scatter height (FSC-H) gating to isolate single cells from doublets. Right, tdTomato signal versus FSC-A gating to identify tdTomato$^+$ versus tdTomato-negative cells, revealing virtually no tdTomato$^+$ splenocytes (0.013%). n = 5 mice.

(D) A representative section through the caudal hindbrain medulla, showing tdTomato expression in neurons marked by the expression of tyrosine hydroxylase (TH) and located in the ventrolateral region, thereby corresponding to the C1 cluster of catecholaminergic neurons. NPY^{DBH} also mark the more rostral A1 cluster of catecholaminergic neurons (data not shown).

(E) A representative section through the rostral pontine region, showing that tdTomato was not detected in TH$^+$ noradrenergic neurons located in the locus coeruleus ("LC"); the tdTomato$^+$ fibers passing through, or terminating around the dorsal part of, LC (E) might represent innervation from the A1/C1 catecholaminergic cells. Scale bars, 100 μm.

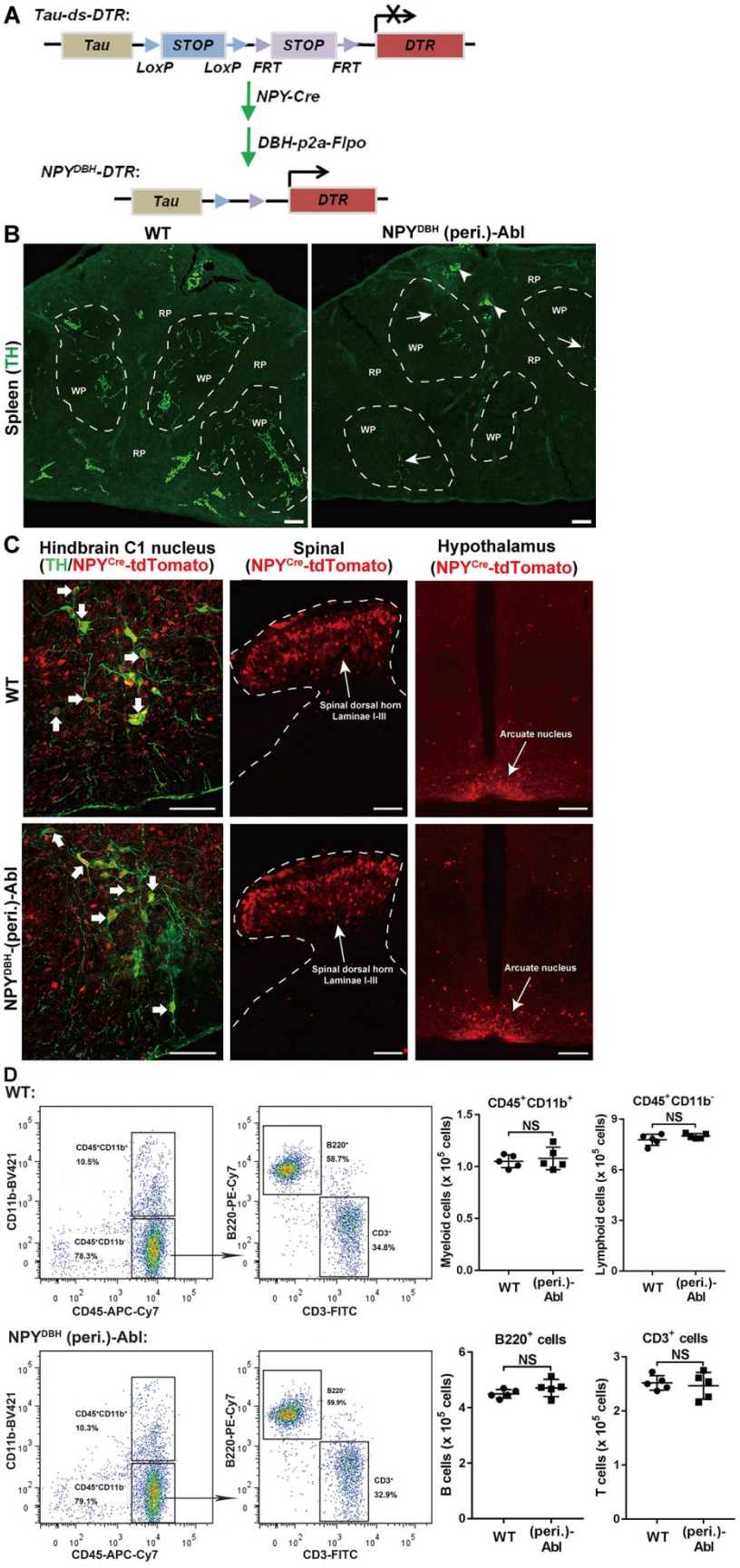

Figure S2. No ablation of NPY^{Cre}-tdTomato^{+} cells in the spinal cord, brain regions or immune organs in NPY^{DBH} (peri.)-Abl mice. Related to Figure 2.

(A) Schematics showing intersectional genetic strategy to drive DTR expression in NPY^{DBH} sympathetic cells, upon removal of two STOP cassettes from the intersectional allele of *Tau*, a pan-neural gene. A Cre-dependent tdTomato allele driven from the *Rosa26* promoter was included (not shown) to label all NPY-Cre^{+} cells with tdTomato.

(B) Representative images through the spleen, showing ablation of most TH^{+} sympathetic fibers in NPY^{DBH} (peri.)-Abl mice compared with wild type (WT) mice, with few remaining in white pulps (WP, arrows) and in trabeculae (arrowheads). RP: red pulps.

(C) i.p. PEGyDT injection in *NPY^{DBH}-DTR* mice did not cause a loss of i) hindbrain C1 and A1 (not shown) catecholaminergic cells marked by coexpression of NPY^{Cre}-tdTomato and TH ("arrows", left column), ii) $tdTomato^{+}$ neurons in superficial laminae (I-III) of the spinal cord, or iii) neurons in the arcuate nuclei of the hypothalamus.

(D) Cell sorting did not reveal changes in splenic immune cells in NPY^{DBH} (peri.)-Abl mice compared with WT mice, one month after PEGyDT injections (two-side student's unpaired *t*-test; NS, not significant: for $CD45^{+}CD11b^{+}$ myeloid cells, $t_8 = 0.504$, $P = 0.628$; for $CD45^{+}CD11b^{-}$ lymphoid cells, $t_8 = 1.36$, $P = 0.211$; for $CD3^{+}$ T cells, $t_8 = 0.414$, $P = 0.689$; for $B220^{+}$ B cells, $t_8 = 1.353$, $P = 0.213$).

n = 5 mice for all experimental groups. Data are shown as mean ± SEM. Scale bars, 100 μm.

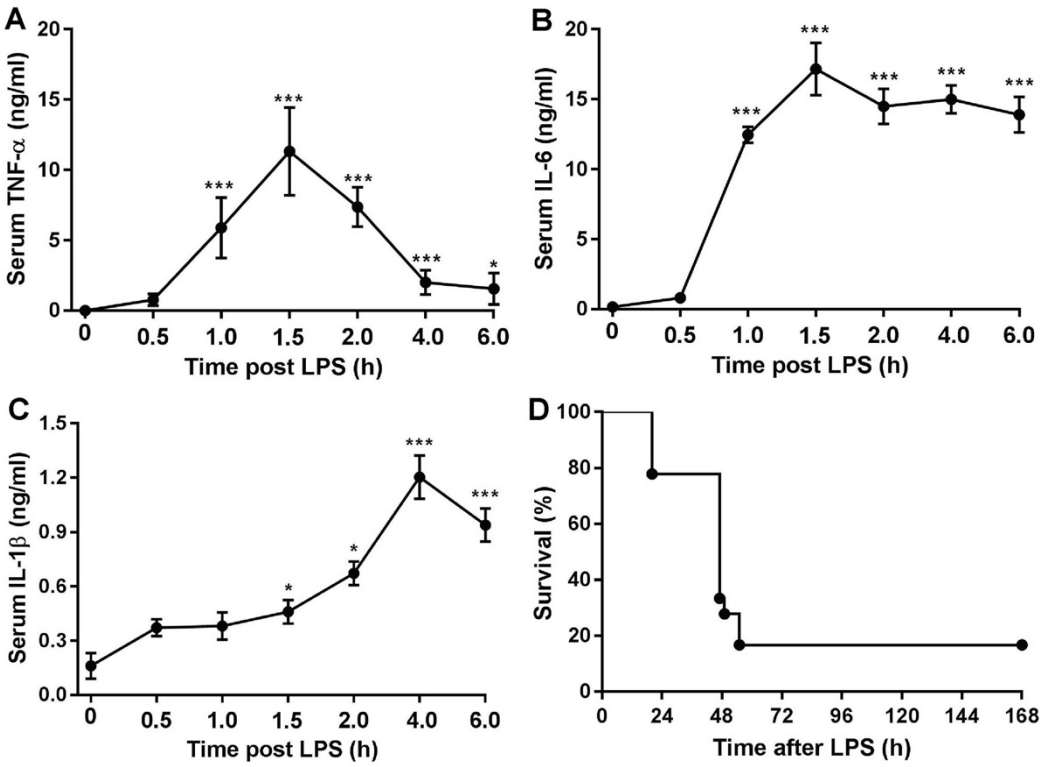

Figure S3. A cytokine storm and lethality induced by LPS. Related to Figure 3.

(A-C) Serum levels of TNF-α, IL-6 and IL-1β at indicated time points after injection of 8 mg/kg LPS in C57B6/J male mice (one-way ANOVA, n = 6 mice per group, $F_{6, 41}$ = 93.125 for TNF-α, 88.267 for IL-6, and 109.301 for IL-1β, $P < 0.001$). *Post hoc* Tukey test revealed increased levels at indicated time points compared with baseline levels (the 0 h time point) (*$P < 0.005$, ***$P < 0.001$).

(D) Kaplan-Merier analysis (n = 21) showing LPS induced nearly 80% lethality within a one-week period. Data are shown as mean ± SEM.

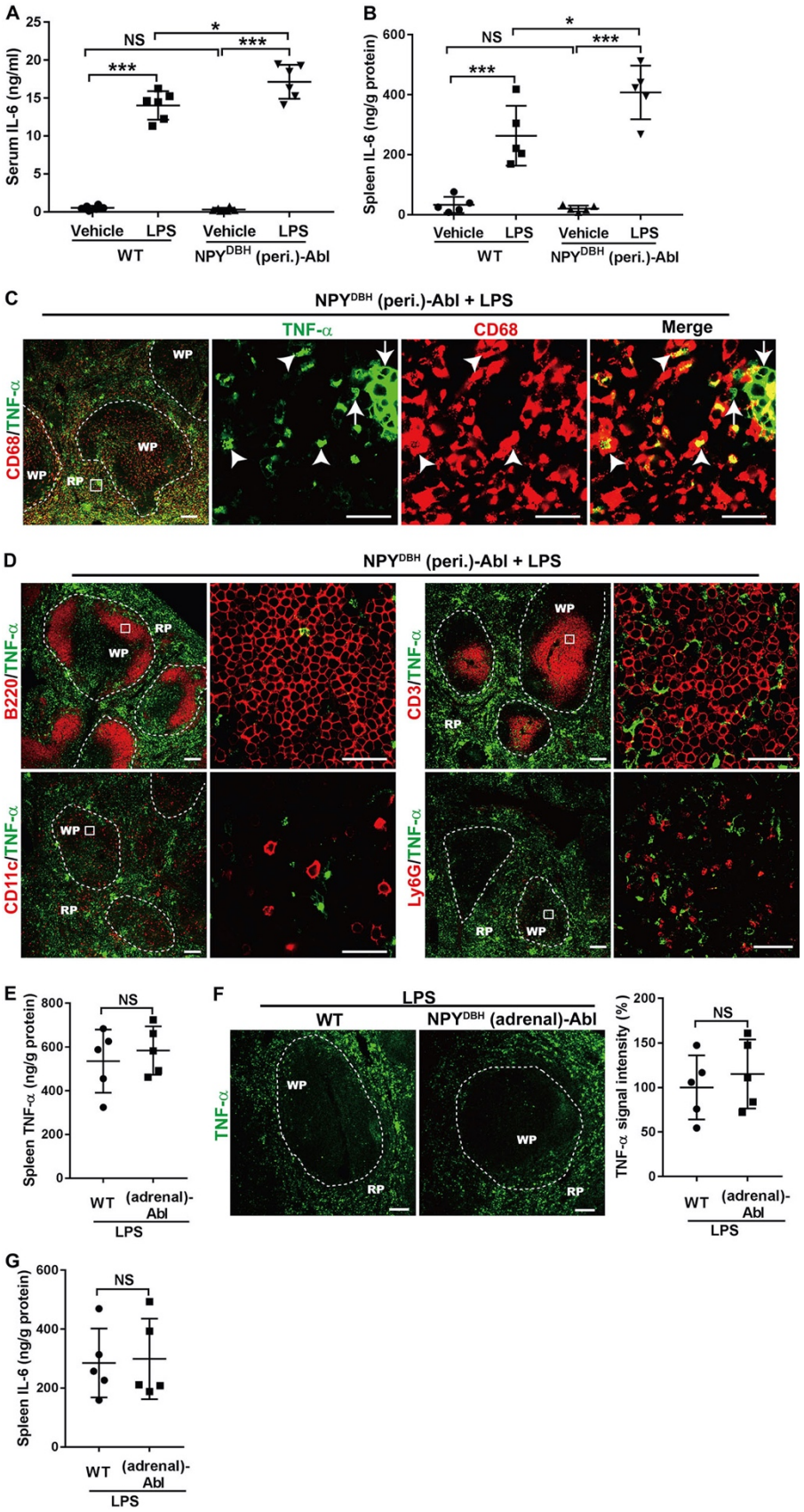

Figure S4. Impact of NPY^{DBH} cell ablation on LPS-induced splenic IL-6 and TNF-α production. Related to Figure 3.

(A and B) LPS induced a larger increase of serum IL-6 in NPY^{DBH} (peri.)-Abl mice compared with wild type ("WT") mice (A: two-way ANOVA, n = 6 mice per group, $F_{1, 20} = 31.275$, $P = 0.013$; *post-hoc* Tukey test: ***$P < 0.001$; *$P = 0.027$; NS = not significant, $P = 0.833$) and in spleen (B: two-way ANOVA, n = 5 mice per group, $F_{1, 16} = 7.122$, $P = 0.010$; *post-hoc* Tukey test: ***$P < 0.001$; *$P = 0.012$; NS, not significant, $P = 0.937$).

(C) Representative images showing that in splenic red pulps (RPs) of LPS-treated NPY^{DBH} (peri.)-Abl mice, TNF-α immunoreactivity was mainly colocalized with $CD68^+$ macrophages ("arrowheads"), but also in some CD68-negative cells ("arrows").

(D) Lack of TNF-α immunoreactivity in $B220^+$ B cells, $CD3^+$ T cells, $CD11c^+$ dendritic cells, and $Ly6G^+$ neutrophils 1 hour after LPS injection.

(E) No difference in splenic TNF-α levels 1 hour after LPS injection in WT versus NPY^{DBH} (adrenal)-Abl mice (two-side student's unpaired *t*-test, n = 5 mice per group, $t_8 = 0.599$; NS, not significant, $P = 0.566$).

(F) Representative images through the spleen, showing that following LPS treatment, WT and NPY^{DBH} (adrenal)-Abl mice displayed similar TNF-α immunostaining patterns, mainly confined to RPs, but not white pulps (WPs). No difference in the overall immunostaining intensity as well (two-side student's unpaired *t*-test, n = 5 mice per group, $t_8 = 0.639$, NS, not significant, $P = 0.541$).

(G) No difference in splenic IL-6 levels 1 hour after LPS injection in WT versus NPY^{DBH} (adrenal)-Abl mice (two-side student's unpaired *t*-test, n = 5 mice per group, $t_8 = 0.164$; NS, not significant, $P = 0.856$). Data are shown as mean $\pm$ SEM. Scale bars, 100 μm.

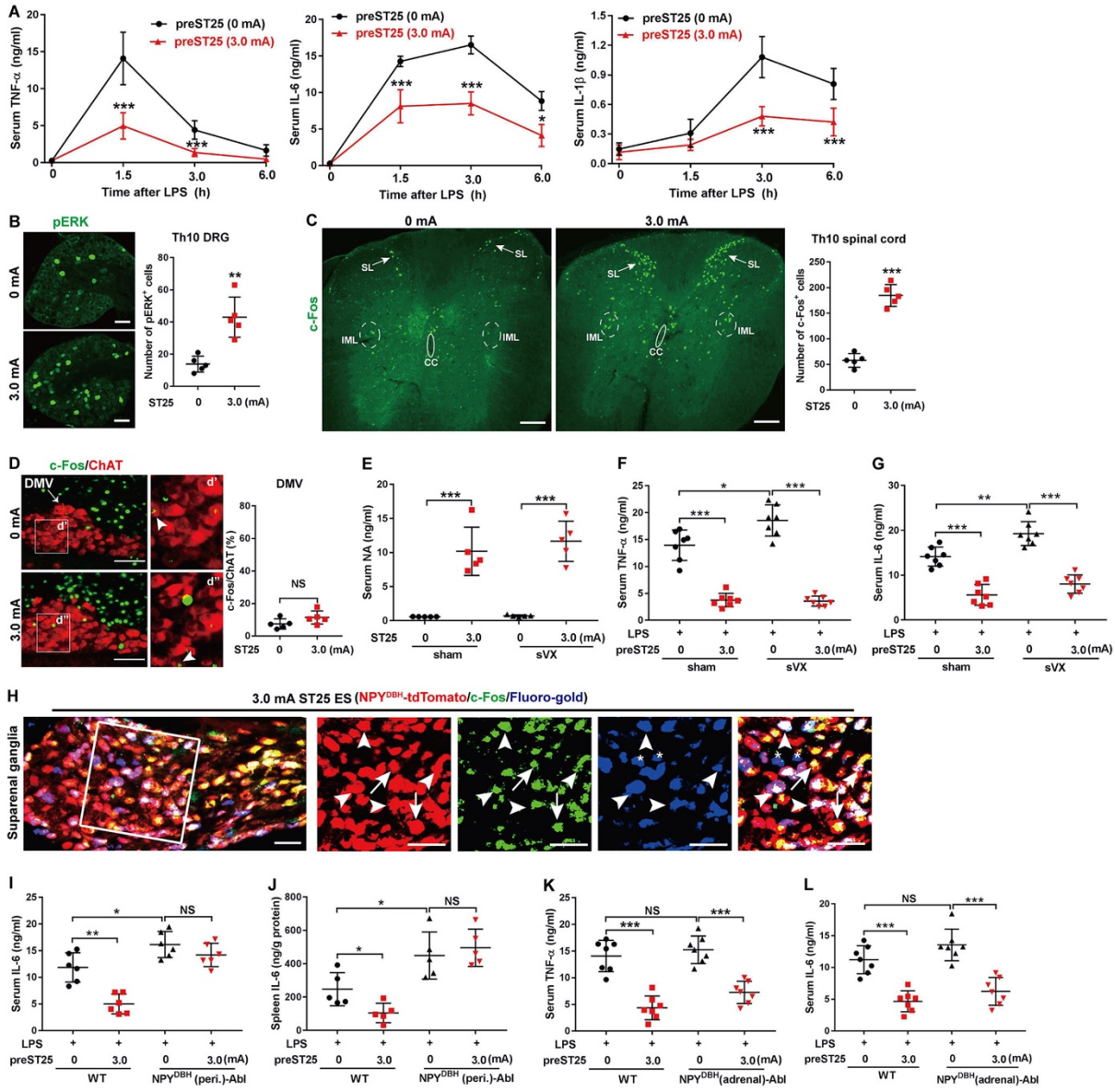

Figure S5. 3.0 mA preST25 ES activated NPY^{DBH} splenic sympathetic neurons and suppressed systemic inflammation in vagal nerve- and NPY^{DBH} chromaffin cell-independent manners. Related to Figure 4.

(A) 3.0 mA preST25 ES suppressed cytokines induced by LPS in adult C57BL/6 mice compared with sham ES ($F_{1, 30}$ = 194.512 for TNF-α, 106.857 for IL-6, and 119.108 for IL-1β, $P < 0.001$; *P = 0.031, ***$P < 0.001$).

(B) pERK and (C) c-Fos induction by 3.0 mA ST25 ES in dorsal root ganglia (DRG) and in the spinal cord at the thoracic Th10 level (pERK: t_8 = 4.851, P = 0.009; c-Fos: t_8 = 11.27, ***P < 0.001). pERK: phosphorylated extracellular signal-regulated kinase, which is one of markers activated by noxious stimuli (Ji et al., 1999). In the spinal cord, 3.0 mA ST25 ES

induced c-Fos in the medial portion, from superficial lamina ("SL") to the central cannel regions ("cc"), as well as the intermediolateral nuclei ("IML") where spinal sympathetic preganglionic neurons (PGNs) are located (see also Figure 4C).

(D) No difference in c-Fos induction in $ChAT^{+}$ neurons in the dorsal motor nuclear of the vagus ("DMV") evoked by 3.0 mA vs 0 mA ES (t_8 = 1.74; NS, P = 0.121).

(E) Serum noradrenaline (NA) release evoked by 3.0 mA ST25 ES compared with 0 mA ES was unaffected by subdiaphragmatic vagotomy ("sVX") compared with sham surgery ($F_{1, 16}$ = 0.611, P = 0.446; ***P < 0.001).

(F and G) sVX did not affect ES-evoked reduction of TNF-α and IL-6 compared with sham surgery ("sham") (for TNF-α: $F_{1, 20}$ = 0.8361, NS, P = 0.425; *P = 0.019, ***P < 0.001; for IL-6: $F_{1, 20}$ = 2.315, NS, P = 0.367;**P = 0.003, ***P < 0.001). For LPS-treated mice receiving sham ES, vagotomy led to an increase in TNF-α production compared with sham surgery, consistent with an anti-inflammatory role of endogenously activated vagal pathways [(Borovikova et al., 2000; Song et al., 2012), but see also (Martelli et al., 2014)].

(H) 3.0 mA preST25 activated splenic sympathetic neurons in suprarenal ganglia. c-Fos induction was confined (94.3%, 3414/3619) to NPY^{DBH}-$tdTomato^{+}$ neurons. Among $tdTomato^{+}$ cells, c-Fos was detected in 95.1% (2105/2213) of $Fluoro\text{-}gold^{+}$ splenic sympathetic cells ("blue"), higher than 38.2% (1309/3424) seen in Fluoro-gold-negative non-splenic cells (P < 0.001, Chi-square test). Arrowheads indicating c-Fos in $tdTomato^{+}$;$Fluoro\text{-}Gold^{+}$ neurons, arrows indicating c-Fos in $tdTomato^{+}$;Fluoro-Gold-negative, and "*" indicating few retrograde labeled cells that did not show c-Fos induction.

(I and J) Loss of 3.0 mA preST25 ES-evoked reduction of IL-6 in NPY^{DBH}(peri.)-Abl mice compared with wild type (WT) mice, both in serum ($F_{1,20}$ = 27.164.113, P < 0.001; **P = 0.009; *P = 0.038; NS, P = 0.637) and in spleen ($F_{1, 16}$ = 9.254, P = 0.031; *P < 0.05; NS, not significant, P = 0.914).

(K and L) 3.0 mA preST25 ES reduced LPS-induced TNF-α and IL-6 compared with 0 mA ES in both WT and (adrenal)-Abl mice (TNF-α: $F_{1, 24}$ = 3.620, P = 0.135; ***P < 0.001, NS, P = 0.079; IL-6: $F_{1, 24}$ = 7.540, P = 0.207; ***P < 0.001, NS, P = 0.103). n = 5-7 mice for all groups. Two-side student's unpaired *t*-test (B, C). Two-way ANOVA plus *post hoc* Tukey test (A, E-G, I-L). NS, not significant. Data are shown as mean ± SEM. Scale bars, 100 μm.

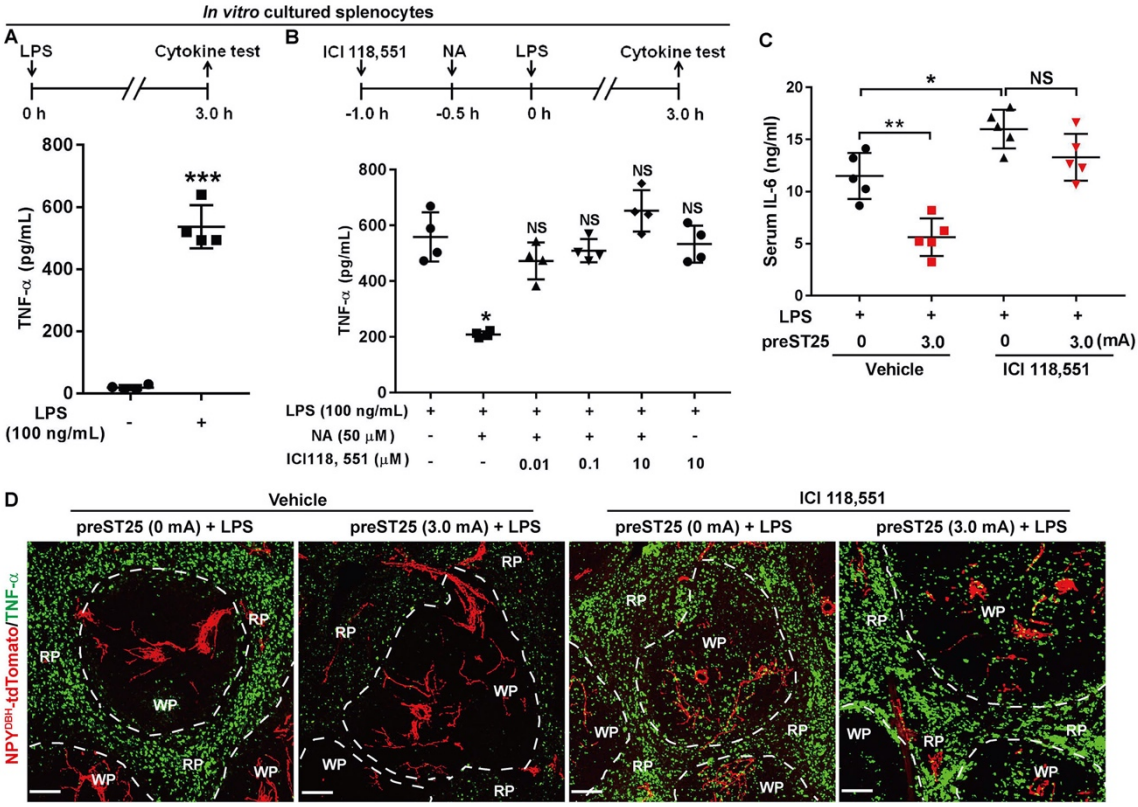

Figure S6. 3.0 mA preST25 ES suppressed systemic inflammation via β2 adrenergic receptors (ARs). Related to Figure 4.

(A and B) Pre-noradrenaline treatment (NA, 50 μM, 30 min before LPS application) suppressed LPS-induced TNF-α release from primary cultured splenocytes, via activation of β2 ARs. (A) LPS (100 ng/ml) induced TNF-α release compared with vehicle (two-side student's unpaired *t*-test, n = 4 mice per group, t_6 = 12.16, ***P < 0.001). (B) Preincubation of noradrenaline (NA) reduced LPS-induced TNF-α levels and this effect can be blocked by ICI118,551 [one-way ANOVA, n = 4 mice per group, $F_{5, 23}$ = 22.472, P < 0.001; *post hoc* Dunnett's test on LPS alone (the first column) versus remaining individual treatment groups: *P = 0.011; NS, not significant, P > 0.05]. The schematics shows the experimental scheme.

(C) 3.0 mA preST25 ES reduced serum IL-6 in LPS-treated C57BL/6 mice that was blocked by ICI 118,551 (2 mg/kg, a β2 AR antagonist, i.p. 30 mins before ES) in comparison with vehicle injection (two-way ANOVA, n = 5 mice per group, $F_{1, 16}$ = 41.059, P < 0.001; *post hoc* Tukey test: **P = 0.004; *P = 0.024; NS, not significant, P = 0.227).

(D) Representative images through the spleen of the NPY^{DBH}-tdTomato mice, showing that reduction of LPS-induced TNF-α immunoreactivity signal evoked by 3.0 mA preST25 ES

compared with sham ES was eliminated by ICI 118,551. Note that for mice with sham ES, TNF-α signals were detected in red pulps (“RPs”), but not in white pulps (“WPs”) in naïve mice, but were expanded to RPs in mice with ICI 118,551 treatment.

Data are shown as mean $\pm$ SEM. Scale bars, 100 μm.

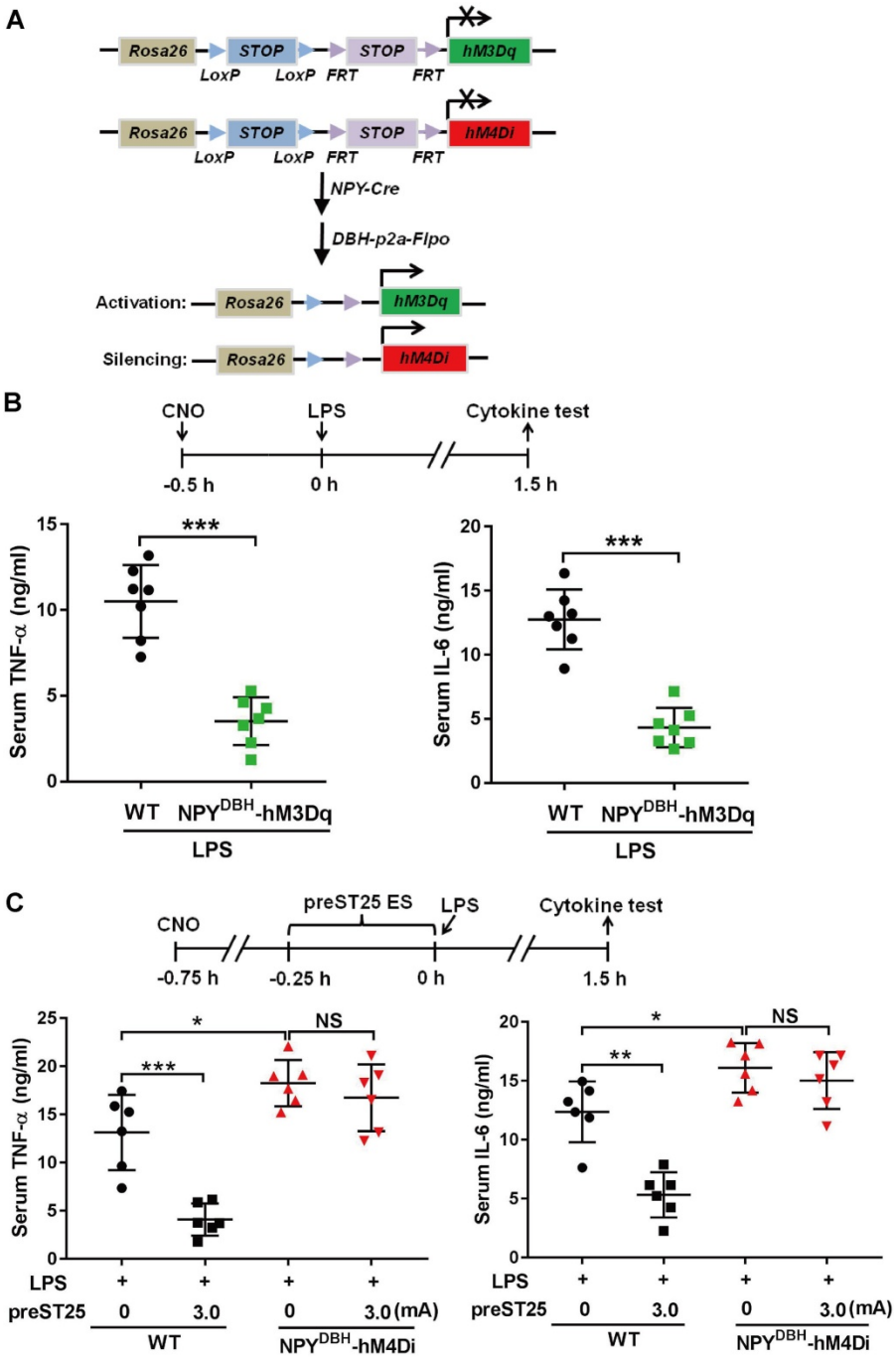

Figure S7. Impact of chemical activation or silencing of NPY^{DBH} cells on LPS-induced TNF-α and IL-6 production and ES-evoked anti-inflammatory effects. Related to Figure 4.

(A) Schematics showing the intersectional strategy of driving hM3Dq or hM4Di expression in NPY^{DBH} cells defined by the coexpression of NPY-Cre and DBH-Flpo. hM3Dq and hM4Di are modified G-protein coupled receptors, whose activation in response to the synthetic ligand CNO can lead to activation or silencing of neuronal cells (Armbruster et al., 2007). (B)

NPY^{DBH} cell activation reduced LPS-induced systemic TNF-α and IL-6 levels. Two-side student's unpaired *t*-test for TNF-α (t_{12} = 7.28, ***P < 0.001) and for IL-6 (t_{12} = 7.99, ***P < 0.001).

(C) Loss of 3.0 mA preST25 ES-evoked reduction of TNF-α and IL-6 after silencing NPY^{DBH} cells via CNO-mediated activation of hM4Di (for TNF-α: two-way ANOVA, n = 6 mice per group; $F_{1,20}$ = 16.355, P < 0.001, *post-hoc* Tukey test: ***P < 0.001; *P = 0.040; NS, not significant, P = 0.722; for IL-6: two-way ANOVA, n = 6 mice per group; $F_{1,20}$ = 22.163, P < 0.001, *post-hoc* Tukey test: **P = 0.008; *P = 0.037; NS, not significant, P = 0.803). It should be noted that NPY^{DBH} marks not only peripheral sympathetic cells, but also the A1/C1 cluster of catecholaminergic neurons in the hindbrain. Thus, the impact of systemic inflammation by activating or silencing NPY^{DBH} cells could be caused by changes of activity in peripheral sympathetic cells, the A1/C1 cluster of catecholaminergic neurons, or both. Data are shown as mean ± SEM.

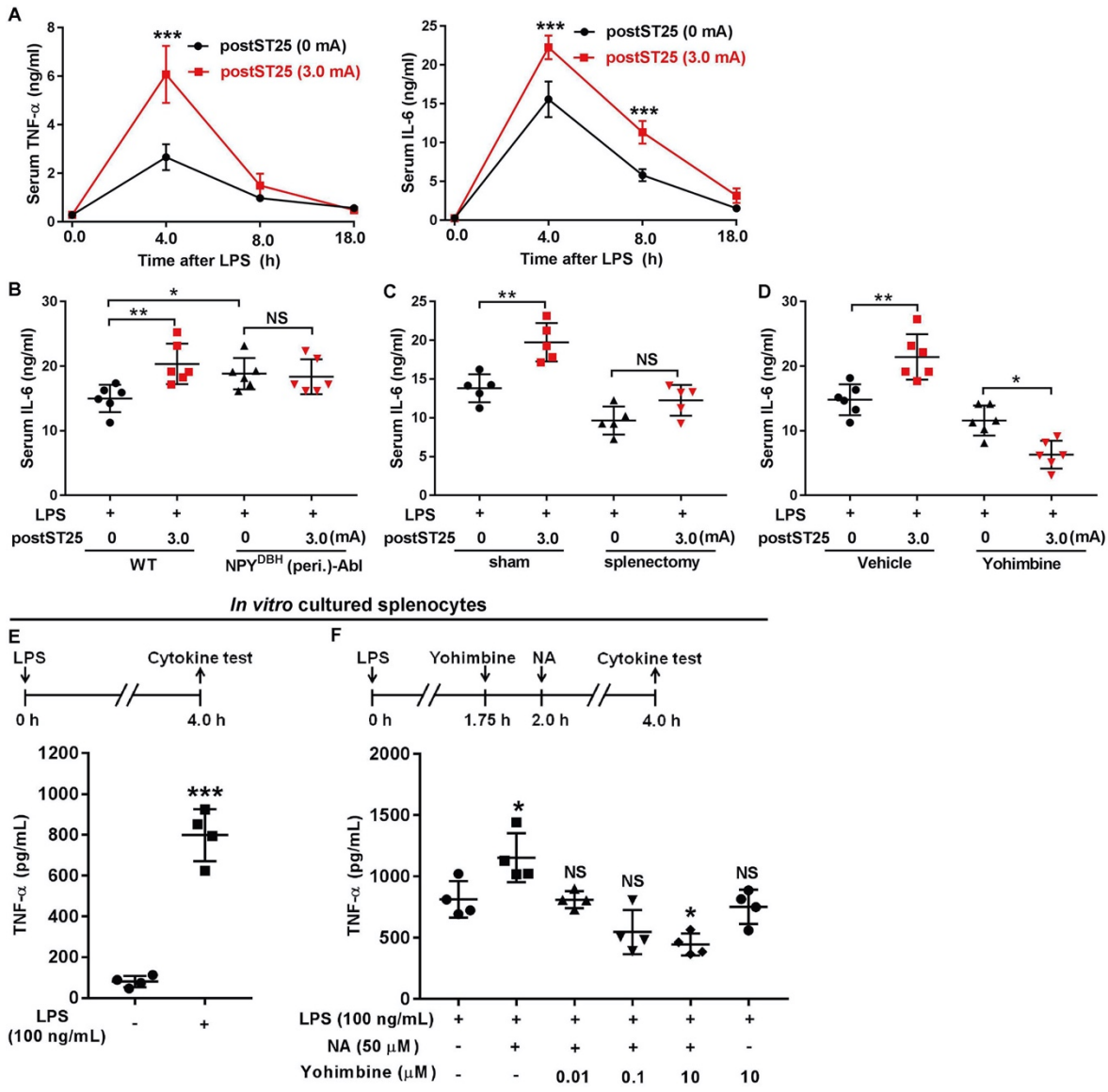

Figure S8. 3.0 mA postST36 ES promoted pro-inflammatory cytokine release via activation of α2 adrenergic receptors (ARs). Related to Figure 5.

(A) 3.0 mA postST25 ES (1.5 hours after LPS injection) increased serum levels of TNF-α and IL-6 at indicated time points in adult C57BL/6 mice, in comparison with sham ES [two-way ANOVA, n = 6 mice per group, $F_{1, 30}$ = 50.595 ("TNF-α") and 110.746 ("IL-6"), P < 0.001 for both cytokines; *post hoc* Tukey test: ***P < 0.001].

(B) Loss of 3.0 mA postST25 ES-evoked increase in serum IL-6 levels in NPY^{DBH} (peri.)-Abl mice compared with wild type (WT) mice (two-way ANOVA, n = 6 per group, $F_{1, 20}$ = 13.255, P < 0.05; *post hoc* Tukey test: **P = 0.007; *P = 0.019; NS, not significant, P = 0.736).

(C) Splenectomy, compared with sham surgery, blocked postST25 ES-evoked increase in serum IL-6 (two-way ANOVA, n = 5 per group, $F_{1, 16}$ = 39.871, P < 0.001; *post hoc* Tukey test:

**P = 0.002; NS, not significant, P = 0.199).

(D) α2 AR antagonist Yohimbine (0.5 mg/kg, 15 mins before ES) blocked postST25 ES-evoked increase of IL-6 (two-way ANOVA, n = 6 per group, $F_{1, 20}$ = 69.464, P < 0.001; *post hoc* Tukey test: **P = 0.003; *P = 0.019).

(E and F) Post noradrenaline treatment promoted LPS-induced TNF-α release from cultured splenocytes, via activation of α2 ARs. (E) LPS (100 ng/ml) induced TNF-α release compared with vehicle, 4 hours after LPS application, in comparison with vehicle treatment (two-side student's unpaired *t*-test, n = 4 mice per group, t_6 = 9.698, P < 0.001). (F) post-LPS incubation of noradrenaline (NA) promoted LPS-induced TNF-α release and this effect can be blocked by Yohimbine (a selective α2 AR antagonist) [one-way ANOVA, n = 4 mice per group, $F_{5, 23}$ = 11.511, P < 0.001; *post hoc* Dunnett's test on LPS alone (the first column) versus remaining individual treatments: *P = 0.025; NS, not significant, P > 0.05]. NA was added 2 hours after giving LPS, and Yohimbine was administered 15 mins prior to NA. TNF-α in the conditioned medium was analyzed at 4 hours after LPS treatment. Note that with the presence of Yohimbine at different doses, NA treatment progressively reduced TNF-α release in comparison with an increase without Yohimbine.

Data are shown as mean $\pm$ SEM.

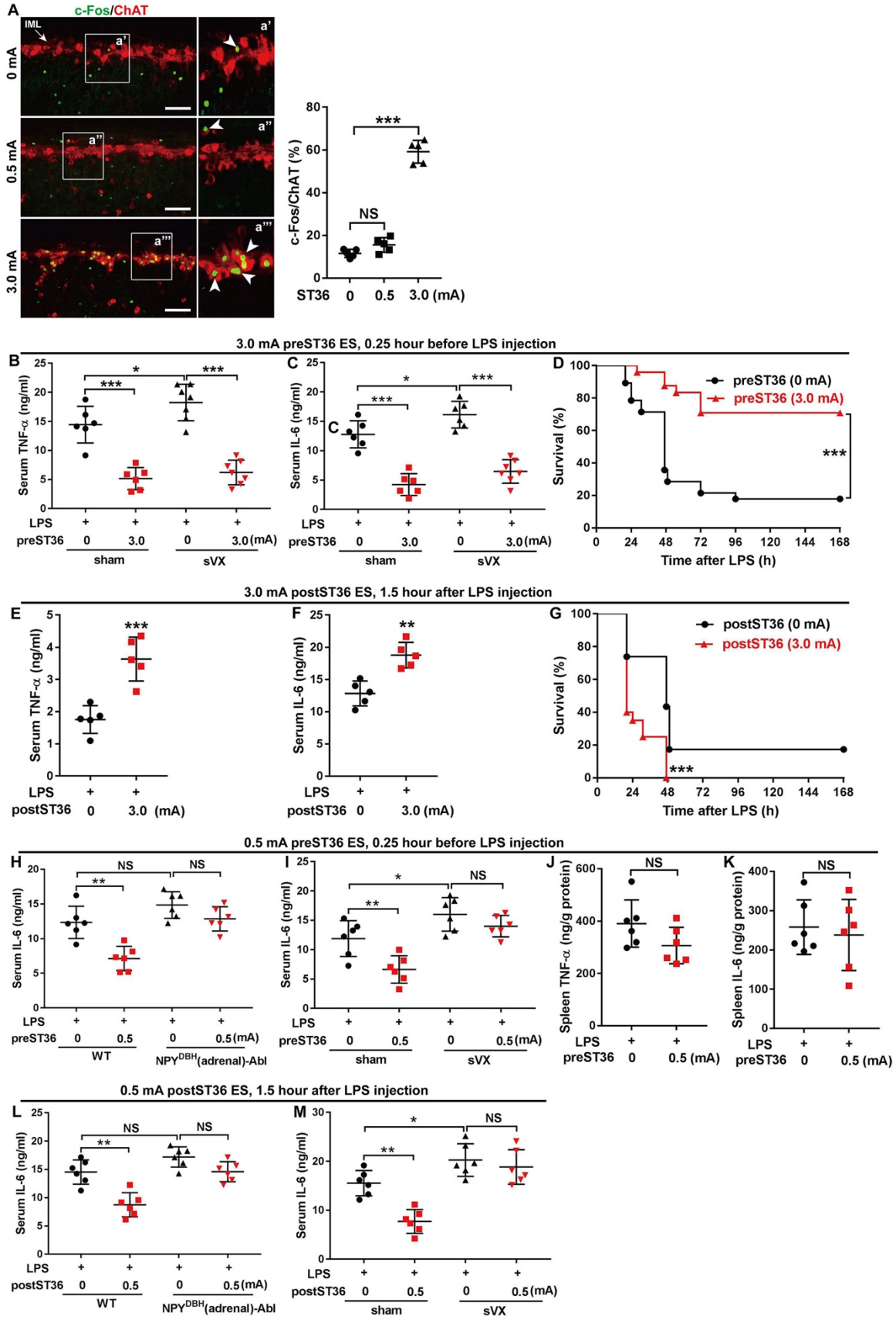

Figure S9. Modulation of systemic inflammation by ES at ST36. Related to Figure 6.

(A) Representative images showing c-Fos immunostaining on sections through the spinal

intermediolateral nuclei ("IML") at T6-T10 levels. a'-a'": higher magnifications of boxed regions. 3.0 mA, but not 0.5 mA ES at ST36 induced c-Fos in $ChAT^+$ preganglionic sympathetic neurons in IML ($F_{2, 12}$ = 73.155, P < 0.001; ***P < 0.001; NS, P = 0.163).

(B and C) 3.0 mA preST36 ES-evoked reduction of LPS-induced TNF-α and IL-6 in serum (ES performed 15 min before LPS injection), compared with sham ES, was unaffected by subdiaphragmatic vagotomy ("sVX") compared with sham surgery ("sham") (for TNF-α: $F_{1, 22}$ = 5.483, P = 0.125; *P = 0.014, ***P < 0.001 and for IL-6: $F_{1, 22}$ = 9.316, P = 0.085; *P = 0.034, ***P < 0.001).

(D) 3.0 mA preST36 ES improved survival compared with 0 mA ES (survival: log-rank test; 0 mA, n = 28; 3.0 mA, n = 22; ***P < 0.001).

(E-F) 3.0 mA postST36 ES (ES performed 1.5 hours after LPS injection) increased serum TNF-α and IL-6 levels compared with 0 mA ES (two-side student's unpaired t-test; for TNF-α: t_8 = 1.695, ***P < 0.001 and for IL-6: t_8 = 4.808, **P = 0.001).

(G) 3.0 mA postST36 ES (ES performed 1.5 hours after LPS injection) reduced survival rate (0 mA, n = 23; 3.0 mA, n = 23, log-rank test, ***P < 0.001).

(H) 0.5 mA preST36 ES reduced LPS-induced serum IL-6 in WT, but not in (adrenal)-Abl mice ($F_{1, 20}$ = 26.157, P < 0.001; **P = 0.002; NS, left, P = 0.181/right, P =0.352).

(I) 0.5 mA preST36 ES reduced LPS-induced serum IL-6 in mice with sham surgery, but not with subdiaphragmatic vagotomy ("sVX") ($F_{1, 20}$ = 45.231, P < 0.001; P = 0.046; **P = 0.009; NS, P = 0.628).

(J-K) 0.5 mA preST36 ES had no effect on LPS-induced TNF-α or IL-6 expression in spleen (TNF-α: t_{10} = -1.28, NS, P = 0.574; IL-6: t_{10} = 0.432, NS, P = 0.674).

(L) 0.5 mA postST36 ES reduced LPS-induced serum IL-6 in WT, but not in (adrenal)-Abl mice ($F_{1, 20}$ = 49.154, P < 0.001; ***P < 0.001; NS, left-P = 0.129/right-P =0.142).

(M) 0.5 mA postST36 ES reduced LPS-induced serum IL-6 in mice with sham surgery, but not with subdiaphragmatic vagotomy ("sVX") ($F_{1, 20}$ = 105.362, P < 0.001; *post hoc* Tukey's test: **P = 0.001; *P = 0.041; NS, not significant, P = 0.837).

n = 5-7 mice for all experimental groups (except D, G). Two-side student's unpaired t-test (E, F, J, K). One-way (A) or two-way (B, C, H, I, L, M) ANOVA plus *post hoc* Tukey test. NS, not significant. Data are shown as mean ± SEM. Scale bars, 100 μm.