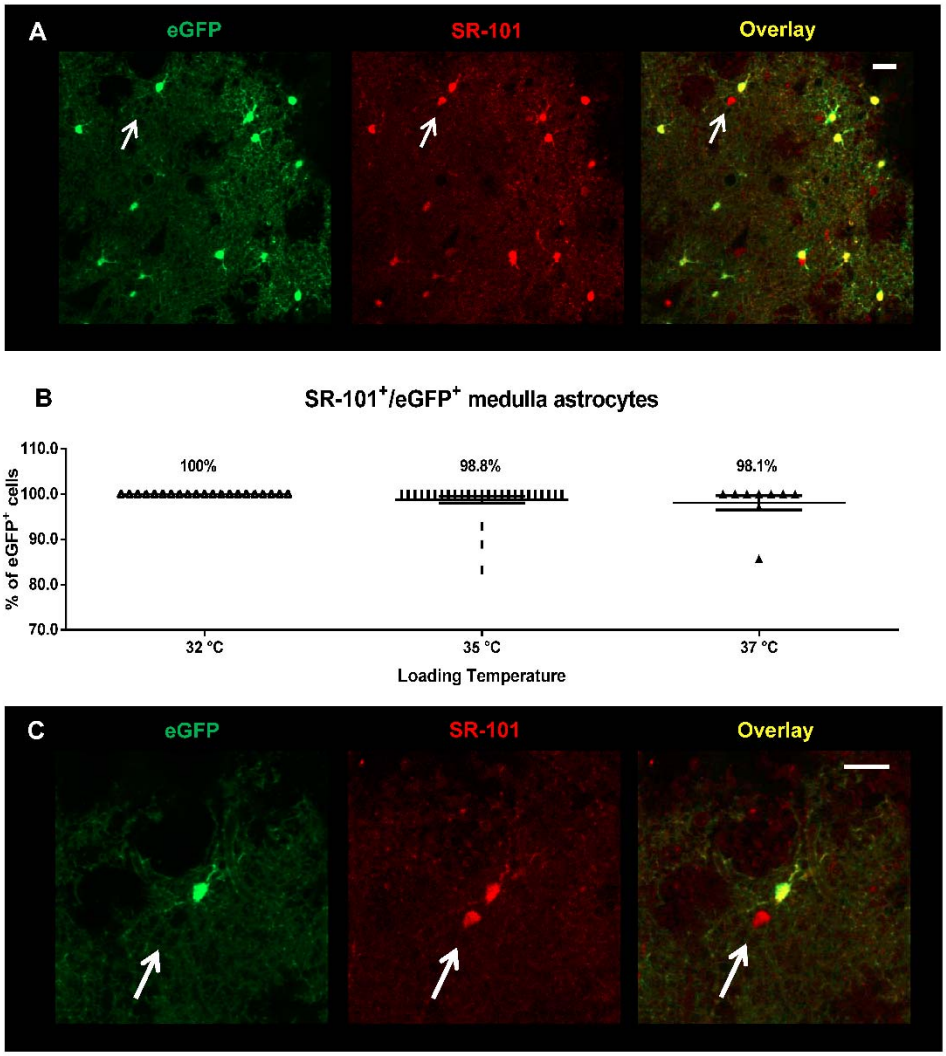

Figure S1. SR-101, an astrocyte marker commonly used to label cortical and hippocampal astrocytes, selectively labels medulla astrocytes *in situ.* Acute brainstem slices containing medulla region were made from *Glt1*-eGFP mice, in which eGFP is expressed in over 85% of the grey matter astrocytes in the brain. (A) The majority of $eGFP^+$ astrocytes were labeled with SR-101 (red) in acute medulla slices from *Glt1*-eGFP mice (magnification: 60X; scale bar: 20 μm). (B) Quantification of percentage of $eGFP^+$ cells that were labeled by SR-101 in acute brainstem slices under three commonly used loading temperatures. Under all three conditions over 98% of the $eGFP^+$ astrocytes were labeled by SR-101 (32˚C: 21 images/126 $eGFP^+$ cells; 35˚C: 28 images/184 $eGFP^+$ cells; 37˚C: 9 images/34 $eGFP^+$ cells). (C) SR-101 labeled a few $eGFP^-$ cells (white arrows) that were morphologically similar to astrocyte (magnification: 60X, zoom: 2X; scale bar: 20 μm). These cells were accounted for 4.5% ~ 7.5% cells labeled with SR-101 across three loading conditions.

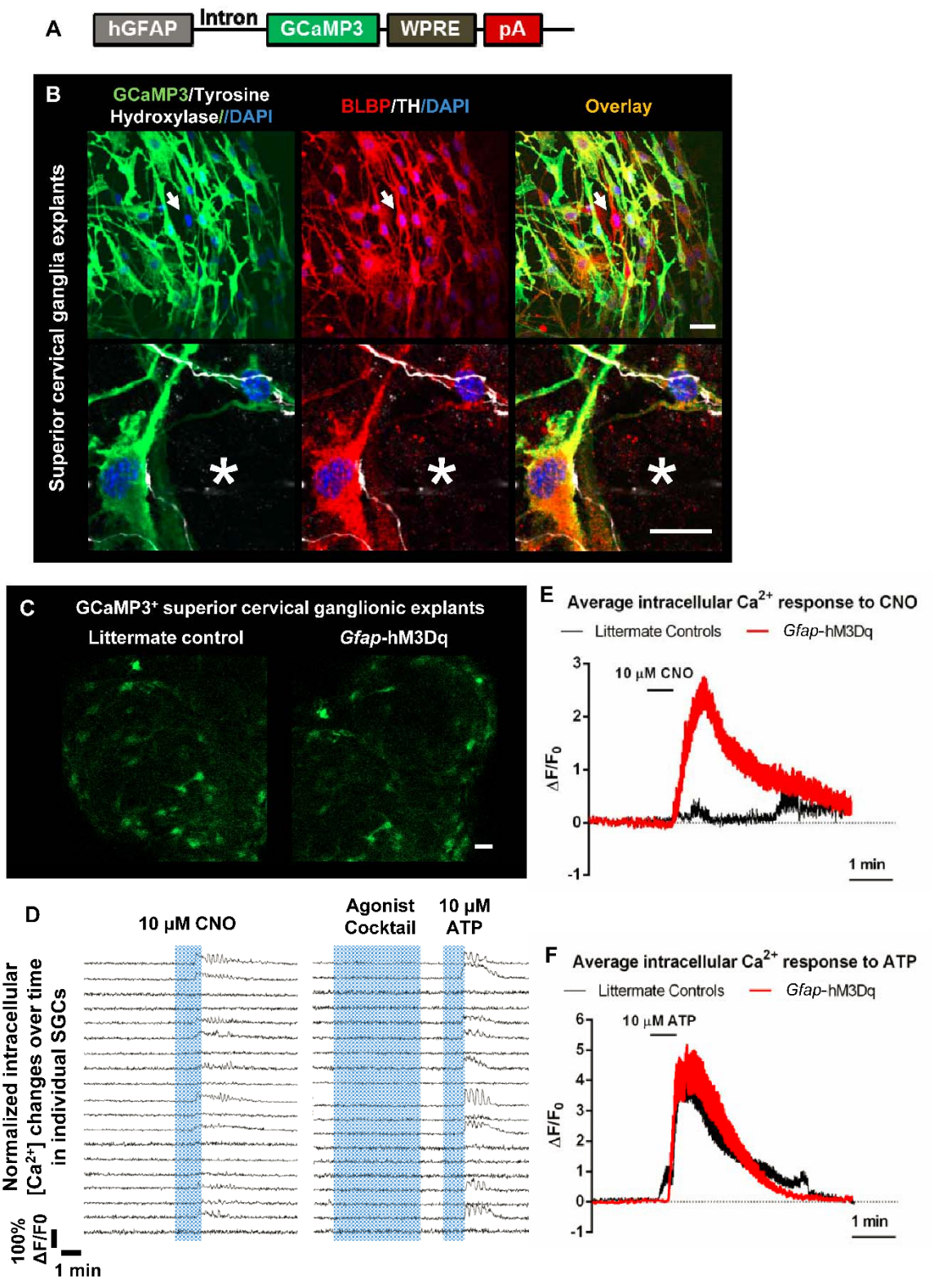

Figure S2. CNO induced intracellular Ca^{2+} elevations in SGCs in superior cervical ganglia explants isolated from *Gfap-GCaMP3*$^{+/-}$*::Gfap -hM3Dq*$^{+/-}$ mice. (A) Schematic of

Gfap-GCaMP3 construct used to prepare transgenic mice. GCaMP3 expression is driven by the fragment of hGFAP promoter. (B) Cytoplasmic GCaMP3 expression in $BLBP^{+}$ satellite glial cells but not in post-ganglionic neuronal soma (asterisk) or neuronal processes (TH^{+}) in the sympathetic superior cervical ganglia explants (magnification: 40X; bottom panels: 2X zoom; scale bar: 20 μm). (C) GCaMP3 expression visualized using confocal microscope from SGCs in cultured superior cervical ganglionic explants isolated from a *Gfap-GCaMP3*$^{+/-}$*::Gfap-hM3Dq*$^{+/-}$ mouse and its littermate control *Gfap-GCaMP3*$^{+/-}$ mice (magnification: 60X; scale bar: 20 μm). (D) Representative intracellular Ca^{2+} elevations in $GCaMP3^{+}$ SGCs in response to CNO and ATP (10 μM each) in ganglionic explants isolated from *Gfap-GCaMP3*$^{+/-}$*::Gfap-hM3Dq*$^{+/-}$ mice. SGCs did not respond to a cocktail of common Gq-GPCR agonists including DHPG, Histamine, and Carbachol (10 μM each) (repeated 4 times). (E-F) Averaged traces from CNO (E) and ATP-induced (F) Ca^{2+} elevations in ganglionic SGCs isolated from *Gfap-GCaMP3*$^{+/-}$*::Gfap-hM3Dq*$^{+/-}$ mouse and its littermate control *Gfap-GCaMP3*$^{+/-}$ mice (*Gfap*-hM3Dq: 48 cells/4 explants/ 2 mice; Littermate controls: 57 cells/5 explants/2 mice). Littermate control mice did not respond to CNO but exhibited similar Ca^{2+} responses to ATP.

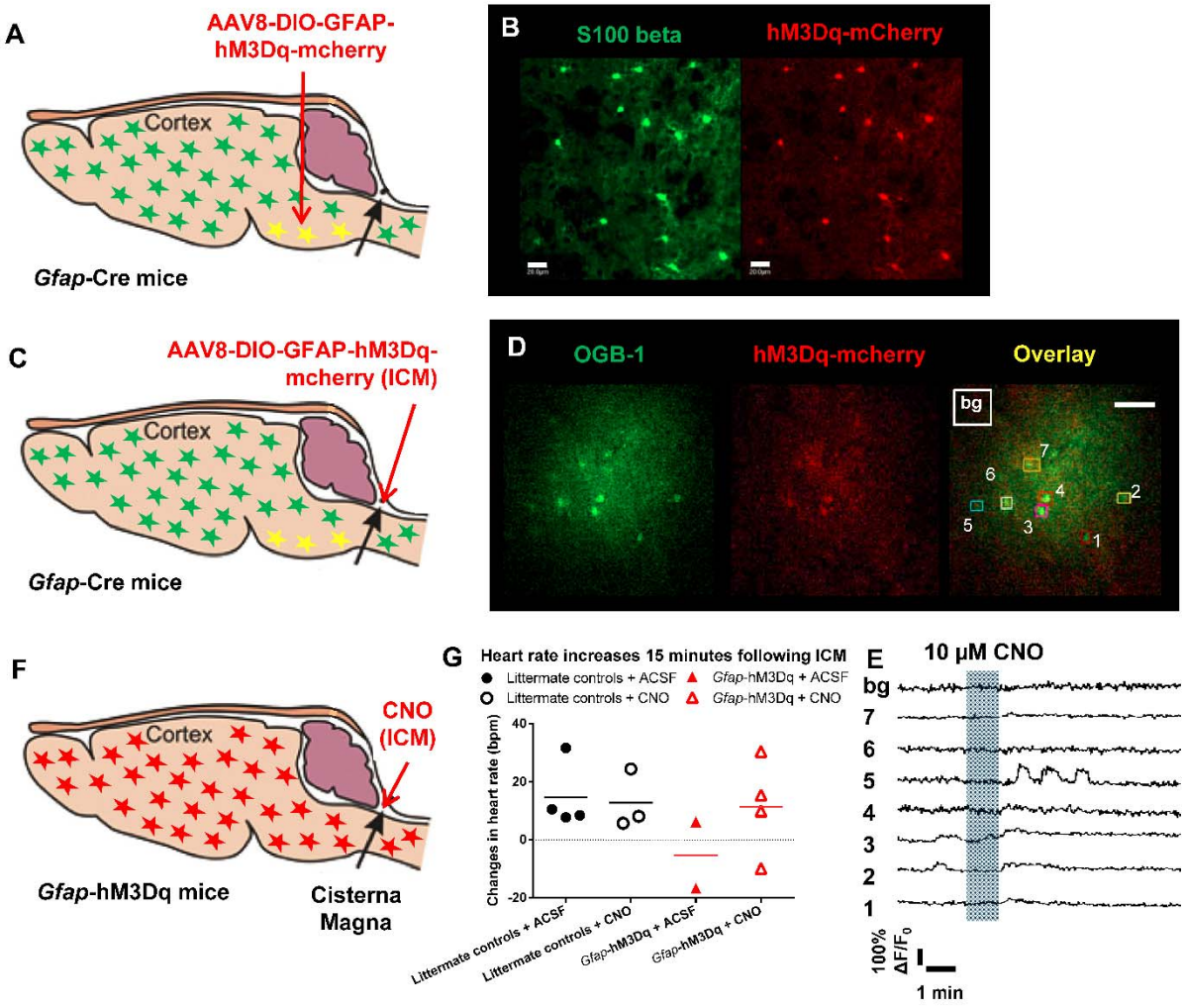

Figure S3. hM3Dq-mediated Gq-GPCR activation in brainstem astrocytes is not responsible for CNO-induced cardiovascular changes. (A) Direct injection of AAV8-DIO-GFAP-hM3Dq-mCherry viral vectors into medulla in order to express hM3Dq in medulla astrocytes (modified from Bazzigaluppi at al., 2015). (B) AAV8-DIO-GFAP-hM3Dq-mCherry injection into brainstem of *Gfap*-Cre mice led to mCherry expression in medulla astrocytes three weeks later (magnification: 60X; scale bar: 20 µm). (C) Alternative method of expressing hM3Dq in medulla astrocytes via viral injections into cisterna magna (ICM). (D) Representative confocal images showing hM3Dq-mCherry expression in OGB-1 loaded medulla astrocytes 3 weeks after ICM viral injections (magnification: 60X; scale bar: 20 µm). (E) Bath application of CNO (10 µM) induces intracellular Ca^{2+} elevations in ~65% astrocytes in medulla (19 out of 29 cells/5 slices/3 mice). (F) Bolus CNO administration into cisterna magna as an alternative method to activate medulla astrocytes in *Gfap*-hM3Dq mice. (G) No CNO-induced tachycardia was observed 15 minutes after CNO ICM injections into *Gfap*-hM3Dq mice (*n*=2-4 mice for each group).