**SUPPLEMENTAL MATERIAL**

Supplemental Figures and Figure Legends

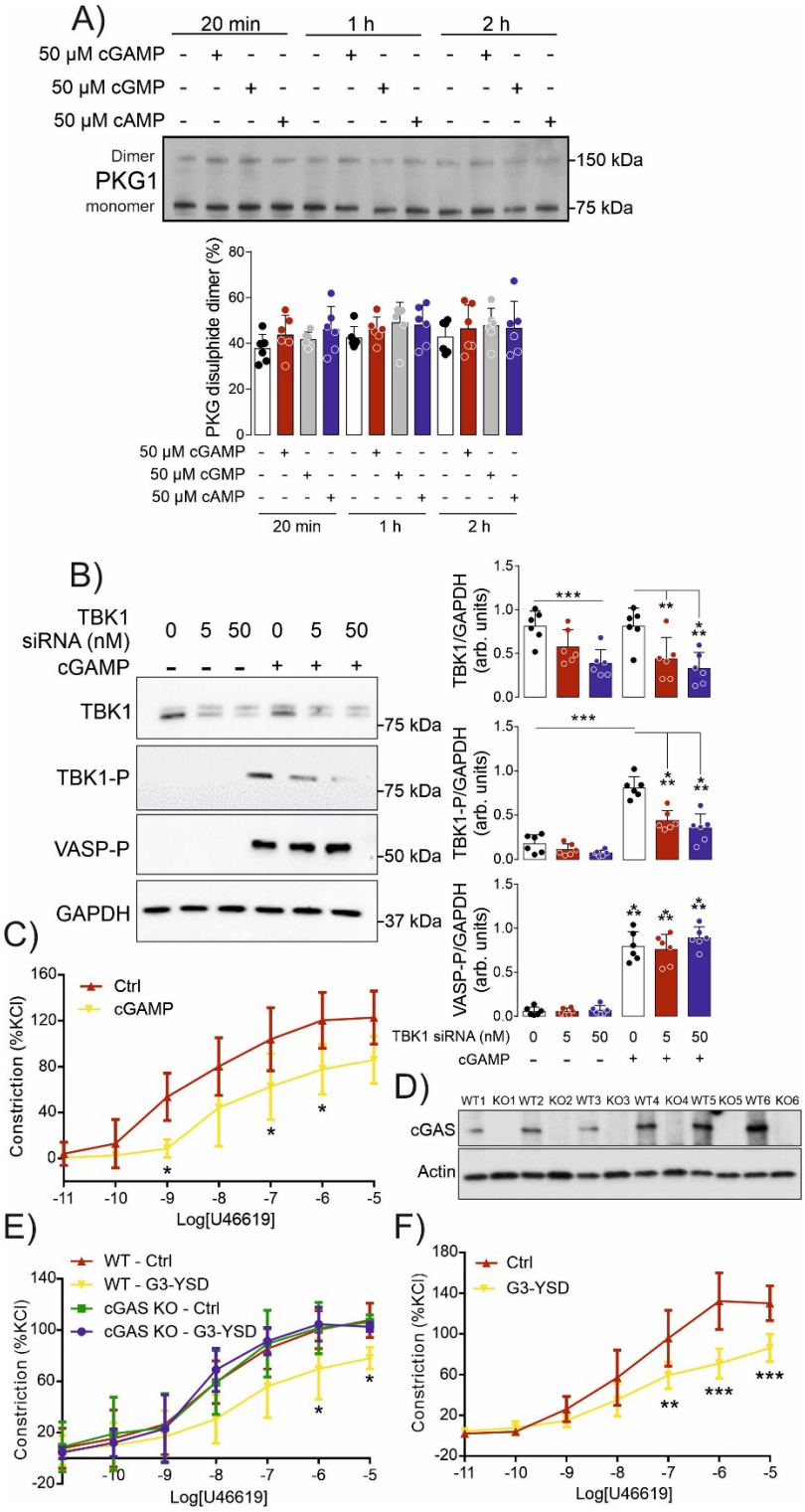


**Fig. S1. cGAS/cGAMP mediates the relaxation of mesenteric vessels. a,** Treatment with cGAMP does not impact on PKGI disulphide dimerization (n=6). **b,** VASP Ser239 phosphorylation in cells treated with cGAMP is independent of TBK1 (n=6). **c,** The vasoconstriction of isolated mesenteric vessels to U46619 in the presence or absence of cGAMP (n=5). **d,** Validation of cGAS knock out in thoracic aorta from cGAS$^{-/-}$ mice. **e**, The vasoconstriction of isolated mesenteric vessels from WT or cGAS$^{-/-}$ littermate mice to U46619 in the presence or absence of G3-YSD (n=5-6). **f,** The vasoconstriction of isolated mesenteric vessels from female mice to U46619 in the presence or absence of G3-YSD (n=5). *P<0.05; **P<0.01; ***P<0.005. Comparisons were made using 1-way ANOVA (**a**) or 2-way ANOVA (**b, c, e, f**) followed by the Tukey post hoc test.

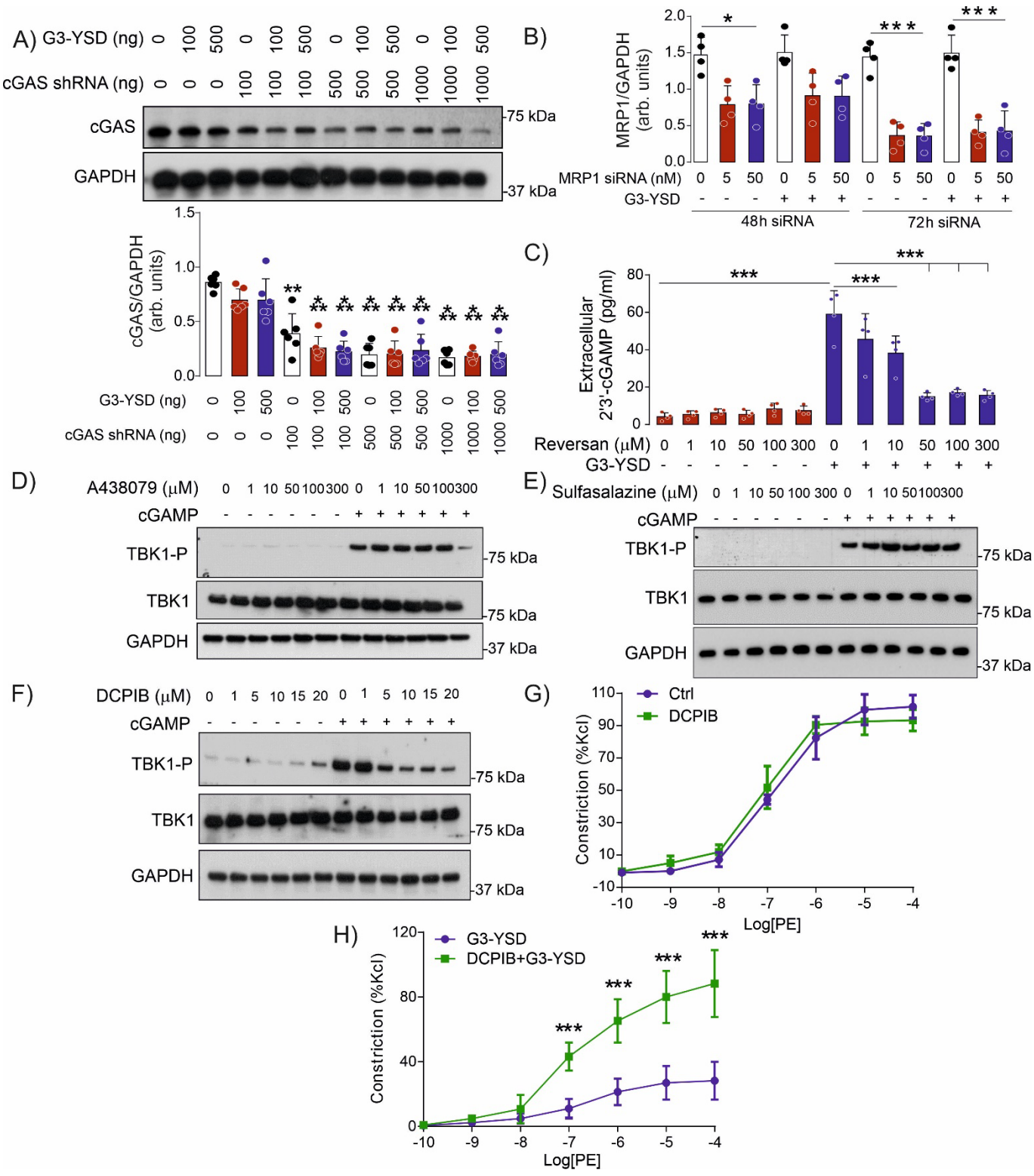


**Fig. S2. cGAS mediates vessel relaxation through VRAC-dependent import of cGAMP.** **a,** Successful knockdown of cGAS expression in endothelial cells (n=6). **b,** Quantification of MRP1 protein abundance (n=4). **c,** The MRP1 inhibitor reversan prevented release of cGAMP from endothelial cells treated with G3-YSD (n=4). Inhibition of P2X7R by A438079, **d,** or SLC19A1 by sulfasalazine, **e,** does not prevent cGAMP-dependent TBK1 phosphorylation. **f**, The VRAC antagonist DCPIB attenuates cGAMP-dependent TBK1 phosphorylation. **g,** The VRAC inhibitor DCPIB does not impact on phenylephrine dependent constriction of isolated aorta (n=3). **h,** The ability of G3-YSD to limit vasoconstriction to phenylephrine is attenuated in the presence of the VRAC inhibitor DCPIB (n=5). *P<0.05; **P<0.01; ***P<0.005. Comparisons were made using 1-way ANOVA (**a, b**) or 2-way ANOVA (**c, g, h**) followed by the Tukey post hoc test.

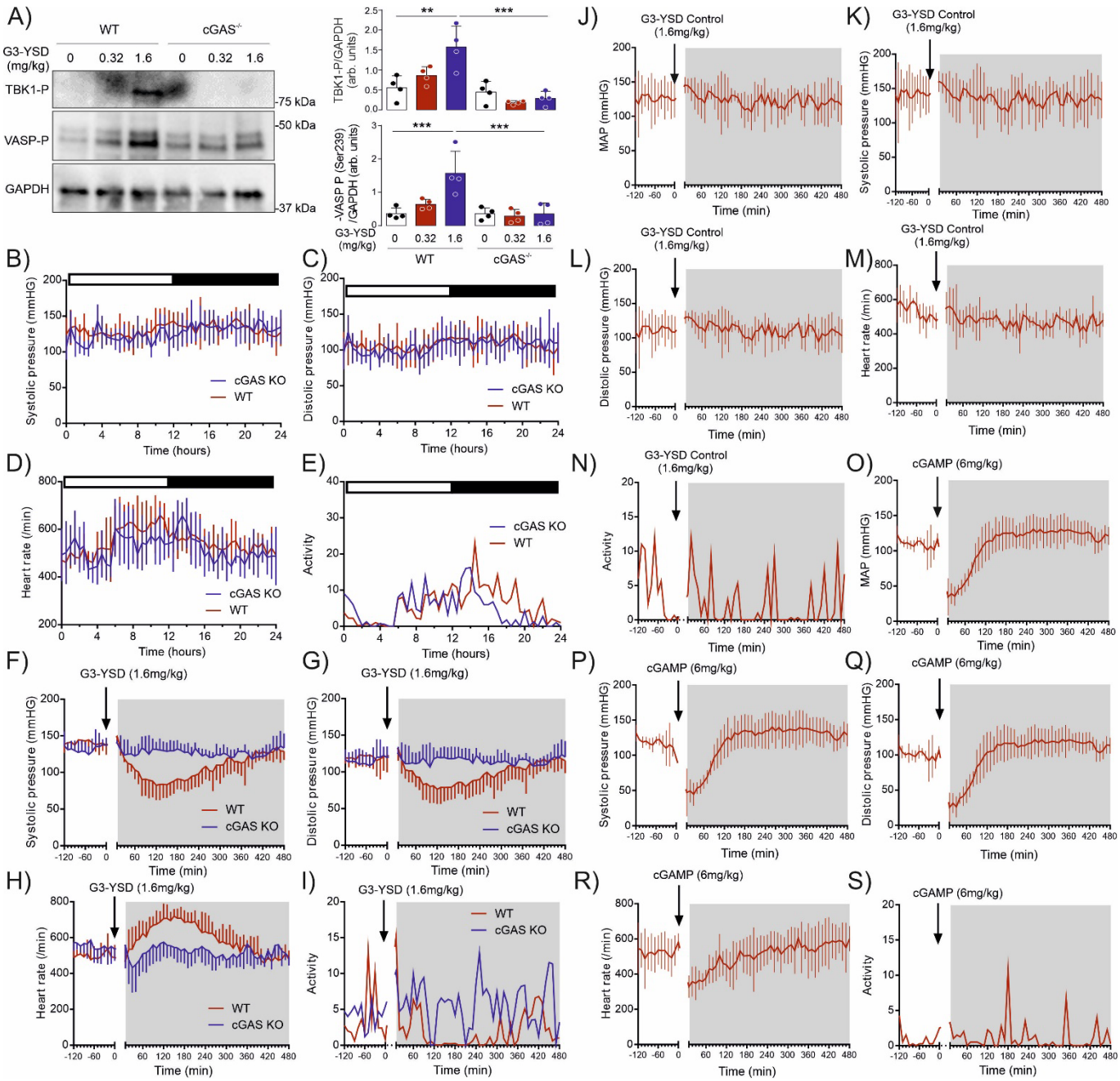


**Fig. S3. cGAS activation or administration with cGAMP induces lowering of blood pressure.** **a,** In vivo administration of G3-YSD led to activation of cGAS and downstream phosphorylation of both TBK1 and VASP in mouse aorta from WT but not cGAS$^{-/-}$ mice (n=4). Baseline telemetry measurements in WT and cGAS$^{-/-}$ mice for **b,** systolic pressure, **c,** diastolic pressure, **d,** heart rate and **e,** activity. Telemetry measurements after administration of G3-YSD for **f,** systolic pressure, **g,** diastolic pressure, **h,** heart rate and **i,** activity (n=5). Telemetry measurements in mice administered G3-YSD-control for **j,** mean arterial pressure **k,** systolic pressure, **l,** diastolic pressure, **m,** heart rate and **n,** activity (n=3). Telemetry measurements in mice administered cGAMP for **o,** mean arterial pressure **p,** systolic pressure, **q,** diastolic pressure, **r,** heart rate and **s,** activity (n=4). **P<0.01; ***P<0.005. Comparisons were made using 2-way ANOVA (**a**) followed by the Tukey post hoc test.

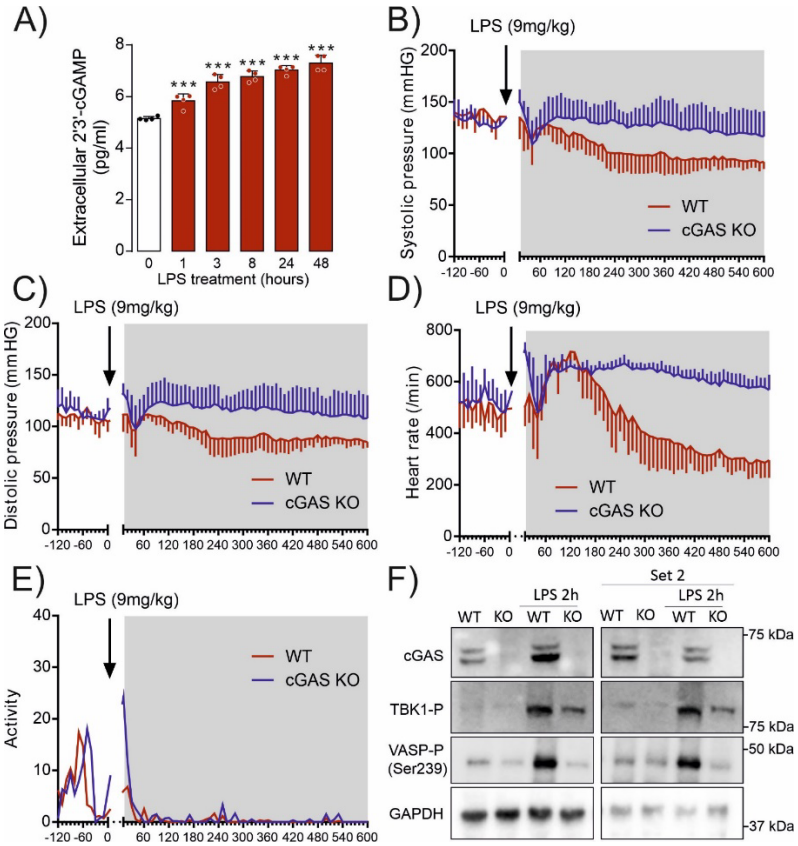


**Fig. S4. Treatment with LPS mediates EC release of cGAMP and lowering of blood pressure.** **a,** Treatment of endothelial cells with LPS leads to a time-dependent accumulation in extracellular cGAMP (n=4). Telemetry measurements in WT and cGAS$^{-/-}$ mice after administration of LPS for **b,** systolic pressure, **c,** diastolic pressure, **d,** heart rate and **e,** activity (n=6). **f,** Enhanced phosphorylation of both VASP and TBK1 in the aorta of mice administered LPS is attenuated in cGAS$^{-/-}$ mice. ***P<0.005. Comparisons were made using 1-way ANOVA (**a**) followed by the Tukey post hoc test.