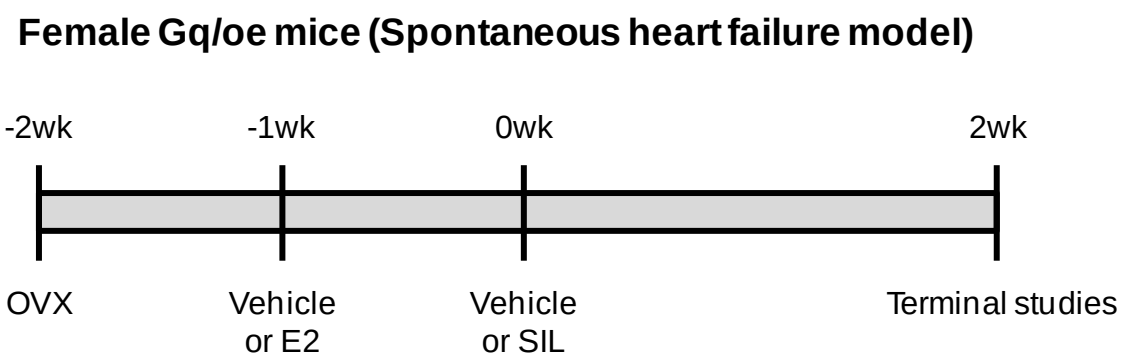
**SUPPLEMENTAL DATA**

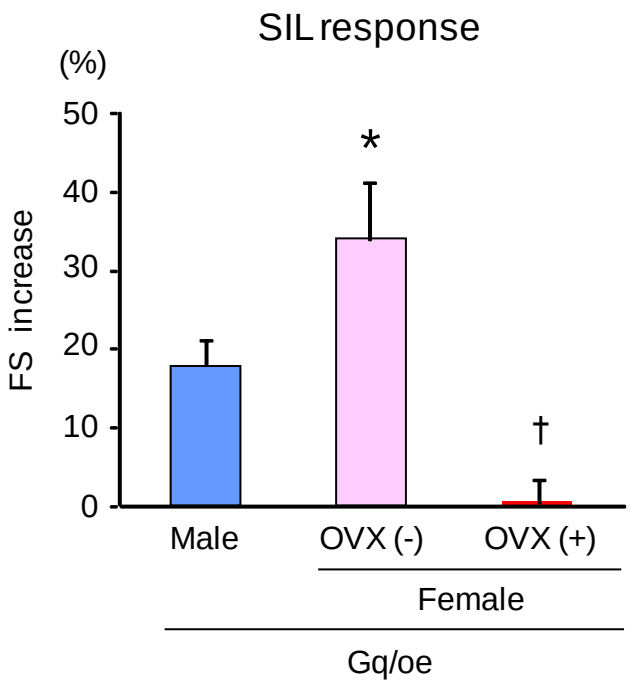
# Estrogen dependence of PDE5 inhibitor efficacy in female heart disease

Hideyuki Sasaki, Takahiro Nagayama, Robert M. Blanton, Kinya Seo, Manling Zhang, Guangshuo Zhu, Dong I. Lee, Djahida Bedja, Steven Hsu, Osamu Tsukamoto, Seiji Takashima, Masafumi Kitakaze, Michael E. Mendelsohn, Richard H. Karas, David A. Kass and Eiki Takimoto

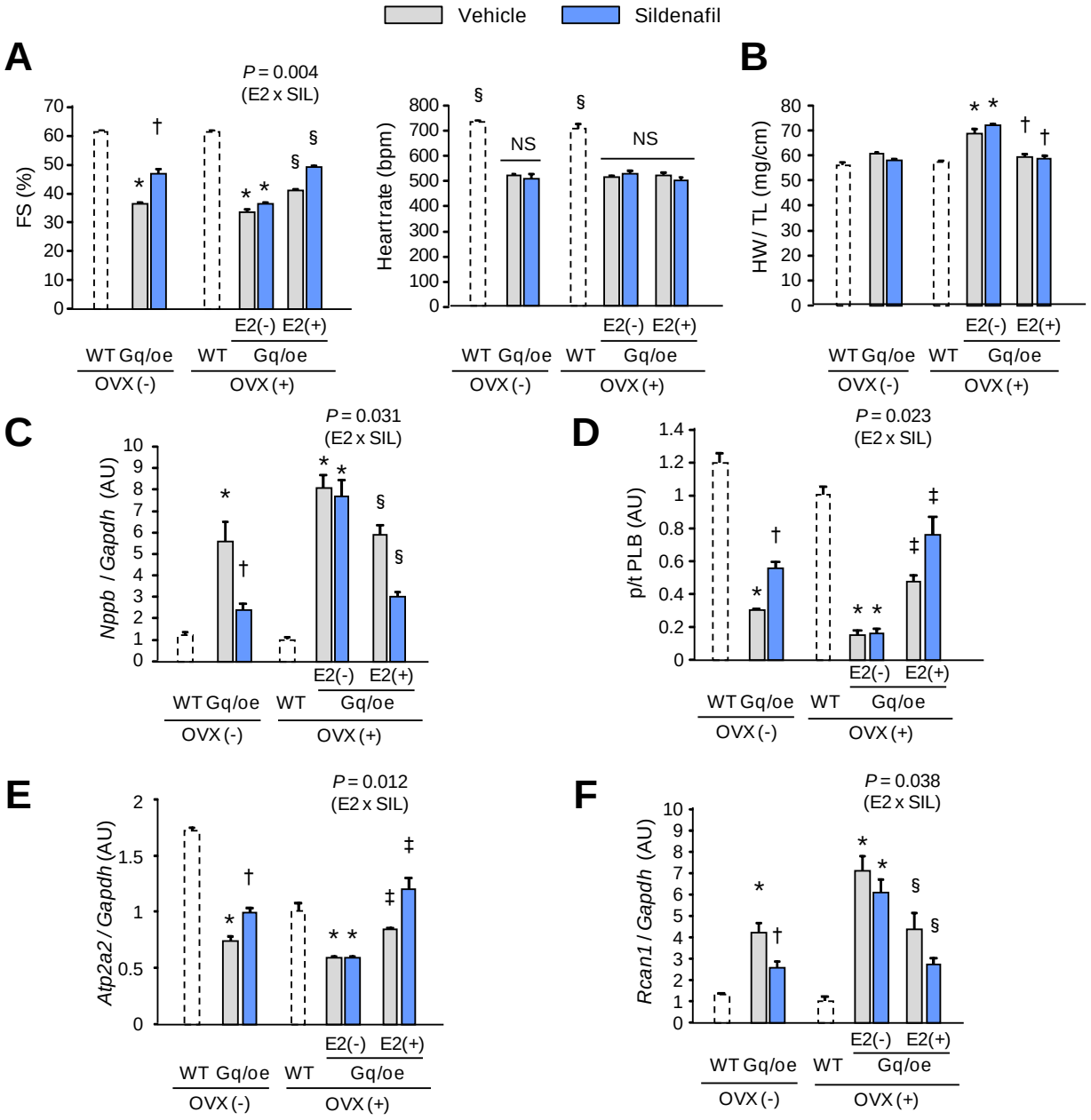
**Contents:** Supplemental Figures 1 to 9 and Legends



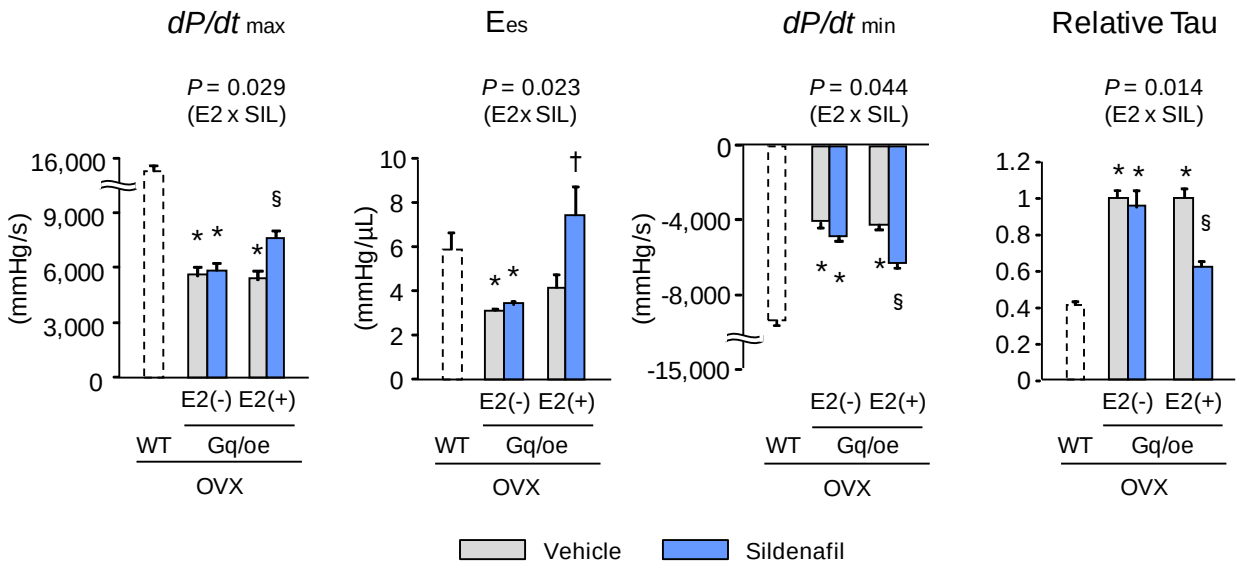

**Supplemental Figure 1. A schematic of the study protocol for female Gq/oe mice.** Gαq over-expressing (Gq/oe) female animals underwent ovariectomy (OVX) at the age of 6-7 weeks. One week after OVX, 17β-estradiol (E2) was replaced by subcutaneous implantation of a 60-day release pellet containing 0.25mg of E2. One week after E2 supplementation, sildenafil (SIL) was orally provided at a dose of 100mg/kg/day mixed in rodent chow. After 2wk-sildenafil treatment, terminal studies were performed.




**Supplemental Figure 2. Gender differences in sildenafil responses in Gq/oe hearts.** Two wk-SIL treatment was more effective in female than male failing Gq/oe hearts but such benefit was abolished by OVX. Summary data for fractional shortening (FS) increase (n=7 per group). **P* < 0.05 versus Gq/oe male; †*P* < 0.05 versus other groups.

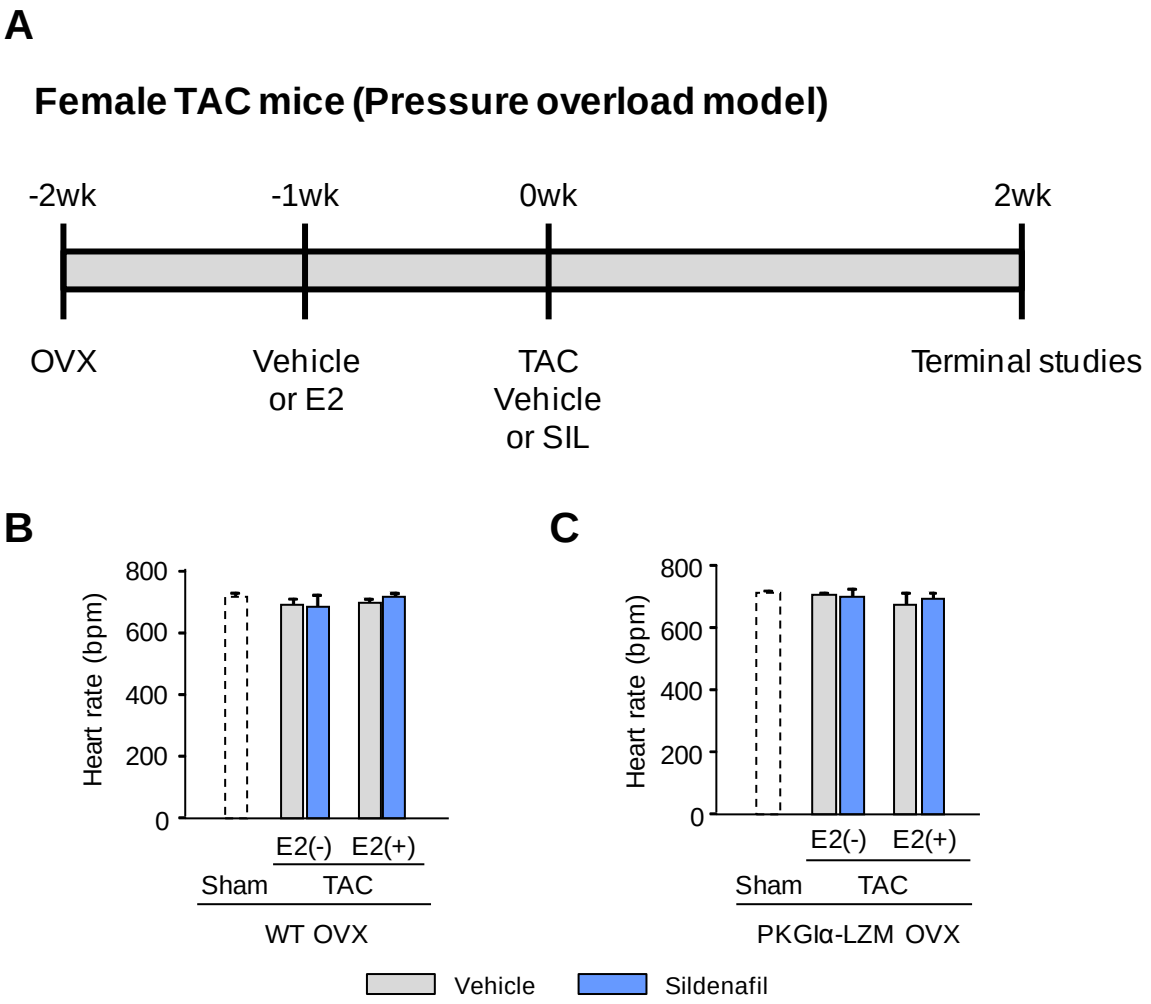



**Supplemental Figure 3. Estrogen dependence of sildenafil efficacy in female Gq/oe hearts.** **(A)** Summary data for FS and heart rate after 2 wk-sildenafil treatment. Sildenafil impact on FS was virtually abrogated without estrogen (after OVX) than that with E2 replacement. Gq/oe itself lowered heart rate. Summary data for **(B)** heart weight (HW) normalized to tibia length (TL). The slight increase in heart weight in Gq/oe (n=5-8 per group) was virtually normalized by E2 supplementation alone. Sildenafil response in **(C)** myocardial BNP (*Nppb*) mRNA expression, **(D)** phosphorylated/total expression ratio for phospholamban (p/t PLB), **(E)** gene expression of sarcoplasmic reticulum $Ca^{2+}$ ATPase 2a (SERCA2a; *Atp2a2*) (n=5-8 per group) and **(F)** *Rcan1* expression (n=6-10 per group) in female Gq/oe hearts with or without E2 replacement. *$P < 0.05$ versus WT groups in non-OVX or OVX mice; †$P < 0.05$ versus vehicle without E2 groups in non-OVX or OVX Gq/oe mice; ‡$P < 0.05$ versus other groups in OVX Gq/oe mice; §$P < 0.05$ versus other groups in non-OVX or OVX mice. *P* values shown are for interactions between E2 and SIL treatments (E2 x SIL; 2-way ANOVA).

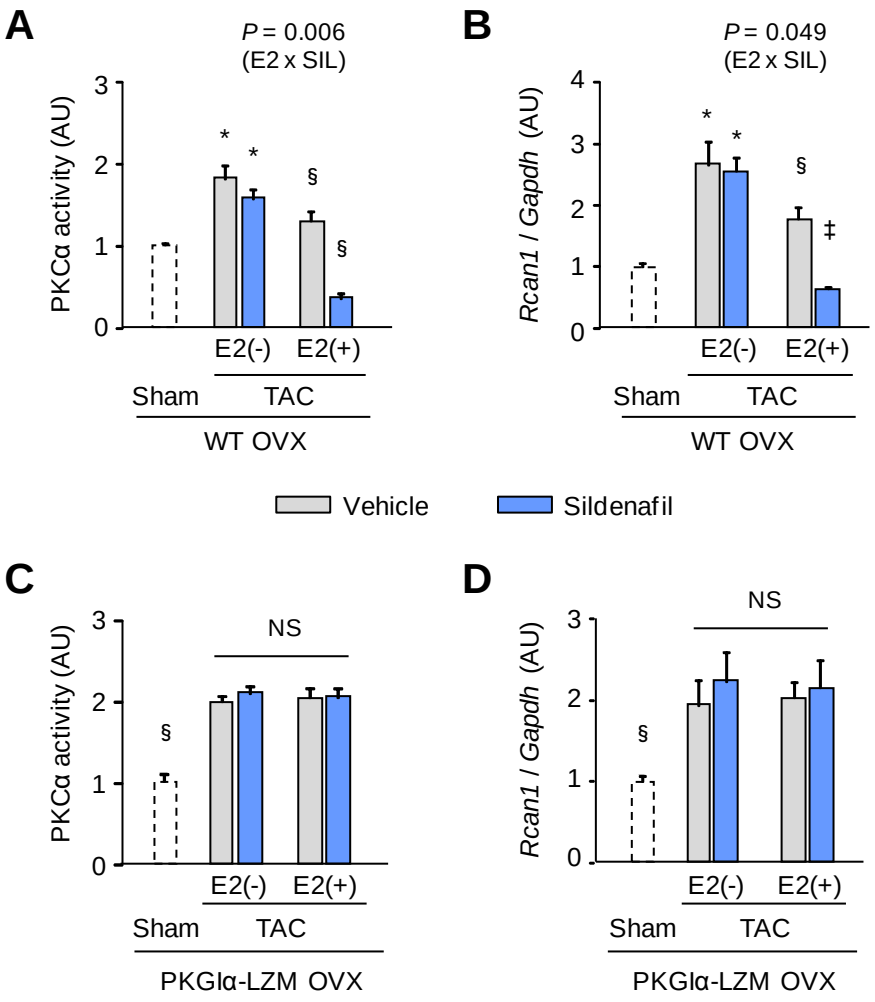



**Supplemental Figure 4. Cardiac functional response to sildenafil in female Gq/oe hearts.**

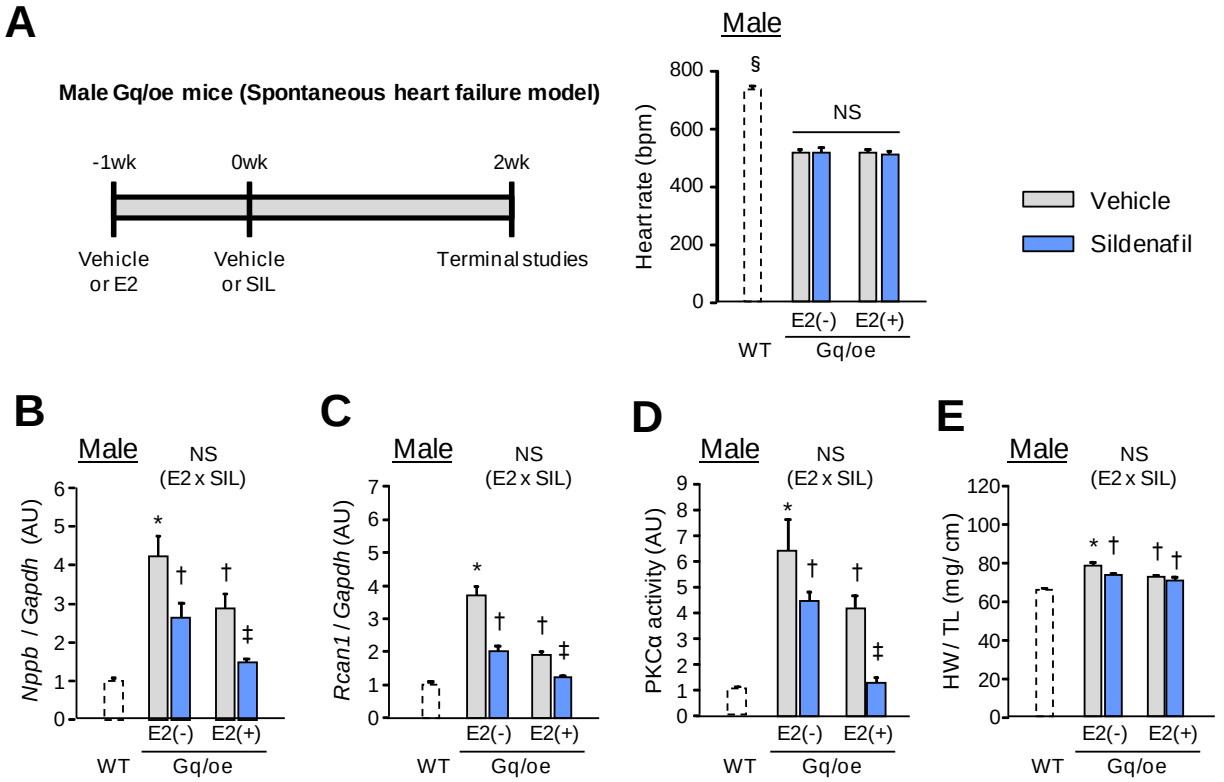
Cardiac function assessed using pressure-volume analysis supports the essential role of estrogen in sildenafil impacts (n=4-7 per group). *dP/dt* $_{max}$, peak rate of left ventricular (LV) pressure rise; Ees, end-systolic elastance (contractility indices). *dP/dt* $_{min}$, peak rate of LV pressure decline; relative (normalized to respective vehicle) Tau, relaxation time constant (diastolic indices). **P* < 0.05 versus WT group; $^{\dagger}$*P* < 0.05 versus other groups in Gq/oe mice; $^{\S}$*P* < 0.05 versus all other groups. *P* values shown are for interactions between E2 and SIL treatments (E2 x SIL; 2-way ANOVA).



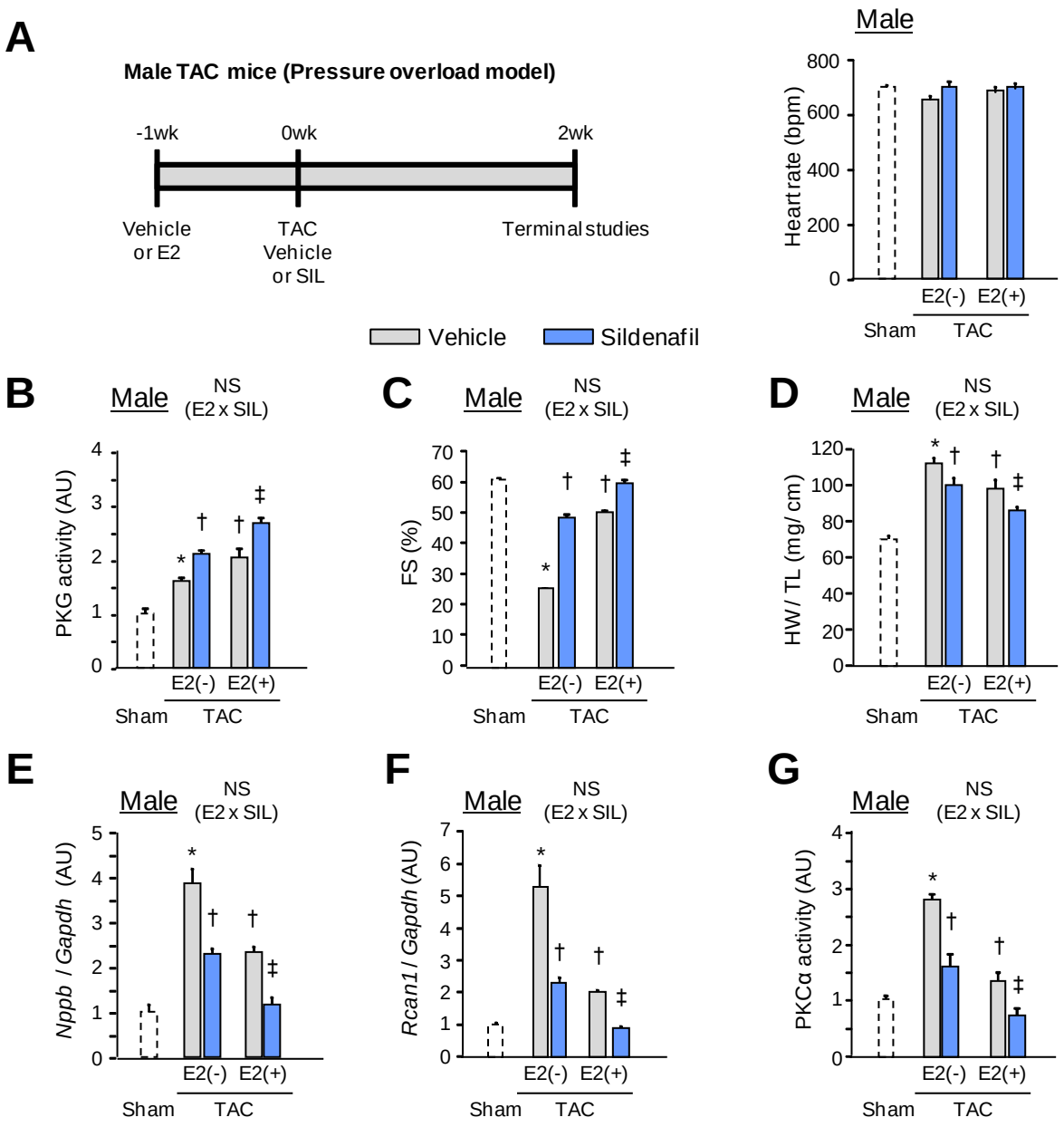

**Supplemental Figure 5. (A) A schematic of the study protocol for the female animals exposed to pressure -overload (TAC).** WT, PKGIα-LZM, eNOS-deficient, or NPRA-deficient animals underwent OVX at the age of 8-10 weeks. Similar to the protocol employed in Gq/oe mice, one week after E2 supplementation, pressure overload was induced by transverse aortic constriction (TAC) and then SIL was orally provided for 2 weeks. Neither **(B)** WT nor **(C)** PKGIα-LZM mice showed significant difference in HR among all groups throughout the TAC protocol.



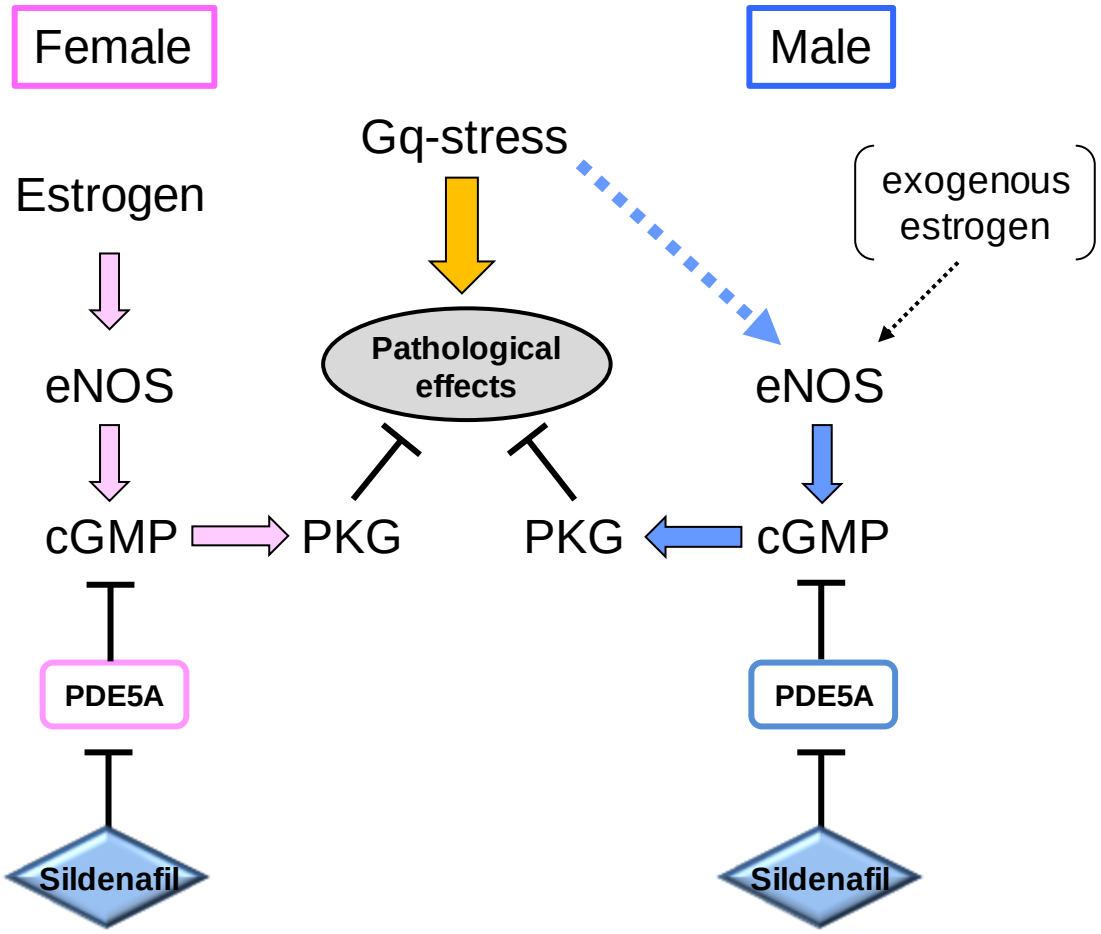

**Supplemental Figure 6. Estrogen dependence of sildenafil efficacy in female pressure-overloaded hearts and the essential role of PKGIα.** **(A)** PKCα activity and **(B)** *Rcan1* gene expression were markedly decreased by sildenafil in OVX mice receiving E2, but little impacted OVX mice alone (n=5-8 per group). **P* < 0.05 versus sham group; ‡*P* < 0.05 versus other groups in TAC mice; §*P* < 0.05 versus all other groups. *P* values shown are for interactions between E2 and SIL treatments (E2 x SIL; 2-way ANOVA). Neither sildenafil, E2, nor their combination ameliorated **(C)** PKCα activity or **(D)** *Rcan1* gene expression in PKGIα-LZM OVX TAC hearts with or without E2 replacement (n=4-6 per group). §*P* < 0.05 versus all other groups.




**Supplemental Figure 7. Sildenafil efficacy in male Gq/oe hearts with or without E2 treatment.** **(A)** A schematic of the study protocol for the Gq/oe male mice. An E2 pellet was implanted at the age of 7-8 weeks and then sildenafil was provided in the same way as in Gq/oe females. Gq/oe itself lowered heart rate. Sildenafil response in **(B)** myocardial BNP (*Nppb*) mRNA expression, **(C)** *Rcan1* expression, and **(D)** PKCα activity in male Gq/oe hearts with or without E2 treatment. **(E)** The slight increase in heart weight (HW/TL) in Gq/oe was normalized by sildenafil or E2 treatment alone. (n=5-9 per group). **P* < 0.05 versus WT group; $^{\dagger}$*P* < 0.05 versus vehicle without E2 group in Gq/oe mice; $^{\ddagger}$*P* < 0.05 versus other groups in Gq/oe mice; $^{\S}$*P* < 0.05 versus all other groups. Two-way ANOVA results are shown for interactions between E2 and SIL treatments (E2 x SIL).




**Supplemental Figure 8. Sildenafil efficacy in male WT hearts exposed to 2wk-TAC with or without E2 treatment. (A)** A schematic of the study protocol for the male animals exposed to pressure-overload (TAC). WT male animals underwent E2 implantation at the age of 9-11 weeks. One week after E2 administration, pressure overload was induced by TAC and then SIL was orally provided for 2 weeks. Heart rate was comparable among all groups. Sildenafil response in **(B)** myocardial PKG activity, **(C)** FS, **(D)** HW/TL, **(E)** myocardial BNP (*Nppb*) mRNA expression, **(F)** *Rcan1* expression, and **(G)** PKCα activity in male WT TAC hearts with or without E2 treatment (n=5-10 per group). **P* < 0.05 versus WT group; †*P* < 0.05 versus vehicle without E2 group in TAC; ‡*P* < 0.05 versus other groups in TAC. Two-way ANOVA results are shown for interactions between E2 and SIL treatments (E2 x SIL).




**Supplemental Figure 9. Schematic diagram of the gender-specific cGMP synthesis in cardiac myocytes.** Female hearts maintain constitutive activation of eNOS by estrogen, providing a tonic synthesis of cGMP that is targeted by PDE5A, whereas male hearts are equipped with Gq stress-responsive eNOS activation mechanism to produce cGMP. Exogenous estrogen enhances eNOS-cGMP synthesis in male hearts.