

# Internal Mammary Arteries as a Model to Demonstrate Restoration of the Impaired Vasodilation in Hypertension, Using Liposomal Delivery of the CYP1B1 Inhibitor, 2,3',4,5'-Tetramethoxystilbene

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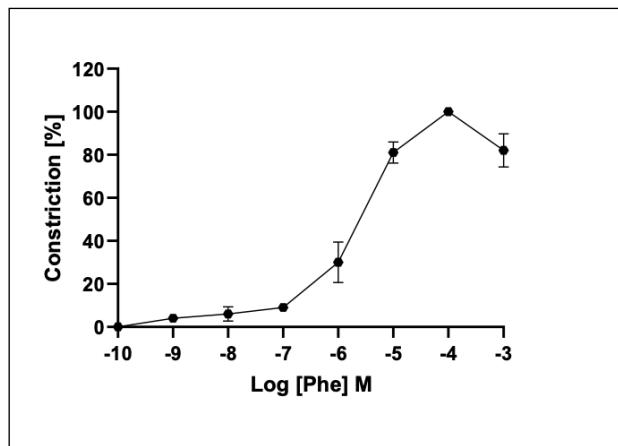
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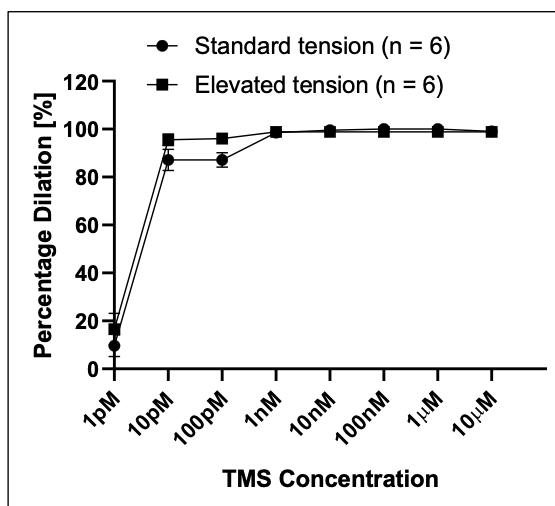
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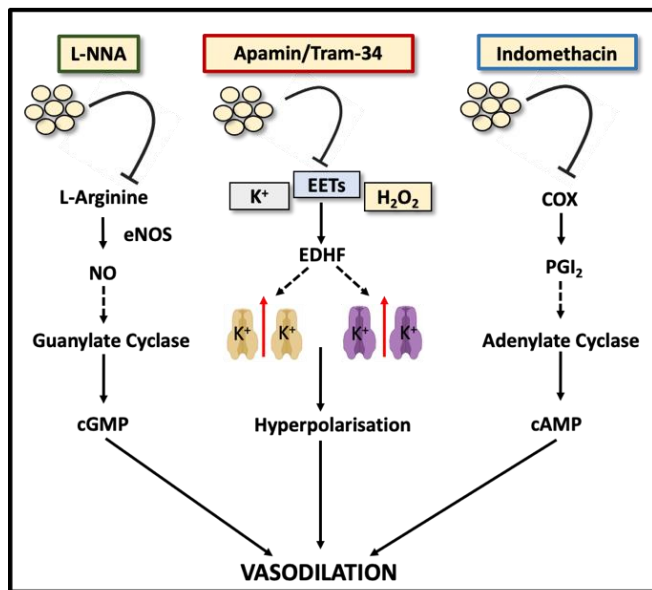
## Supplemental Figures



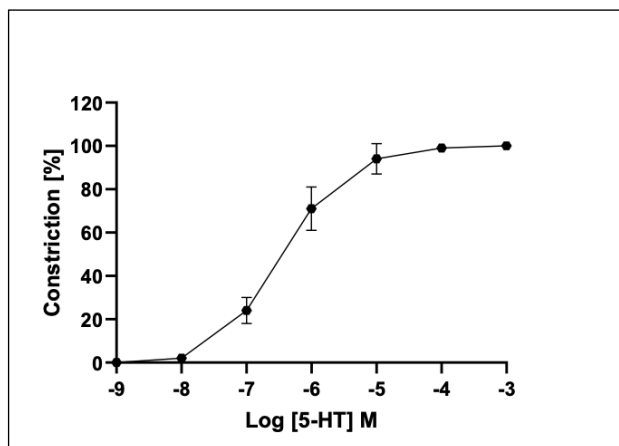
**Figure S1.** Constrictor responses of isolated human internal mammary arteries (IMAs) to phenylephrine (Phe). The dose-response effect of phenylephrine (Phe) ( $10^{-9}$ – $10^{-3}$ M) in isolated IMAs from CABG patients under standard tension (2.5g). All vessels constricted to Phe in a dose-dependent manner, with sub-maximal dose identified as  $10^{-5}$ M (n=3).



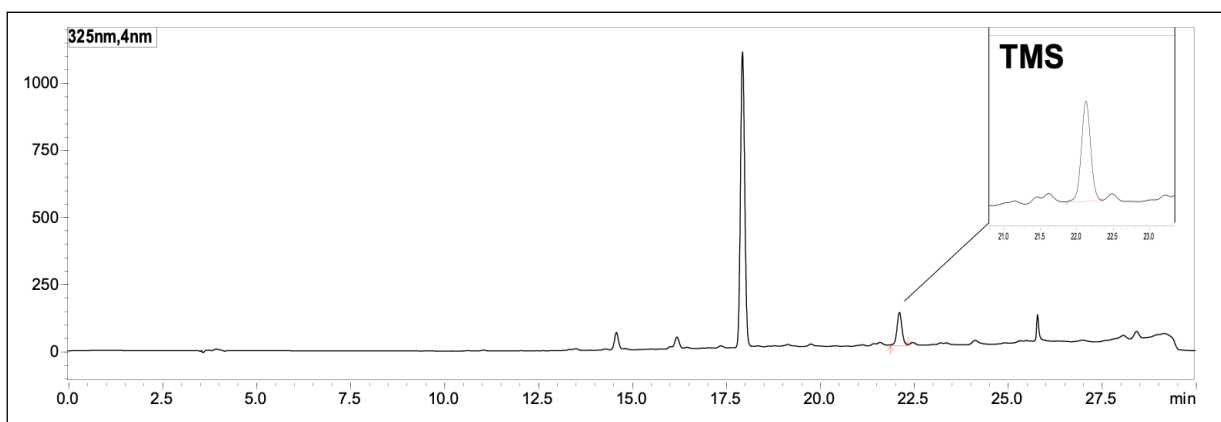
**Figure S2.** The effect of Tetramethoxystilbene (TMS)-loaded liposomes on vascular responses of isolated rat aortic vessels. Dilator responses to cumulative doses of TMS-loaded liposomes (1 pM – 10 µM) after standard (2 g; 1 hour) or elevated tension (4 g; 1 hour) in phenylephrine (Phe;  $10^{-5}$  M) pre-constricted vessels. Maximal dilation was achieved at a minimal dose of 1 nM (n=6). Data were analyzed using a one-way ANOVA followed by a Dunnett's multiple comparison. Data are presented as mean  $\pm$  standard error of mean.



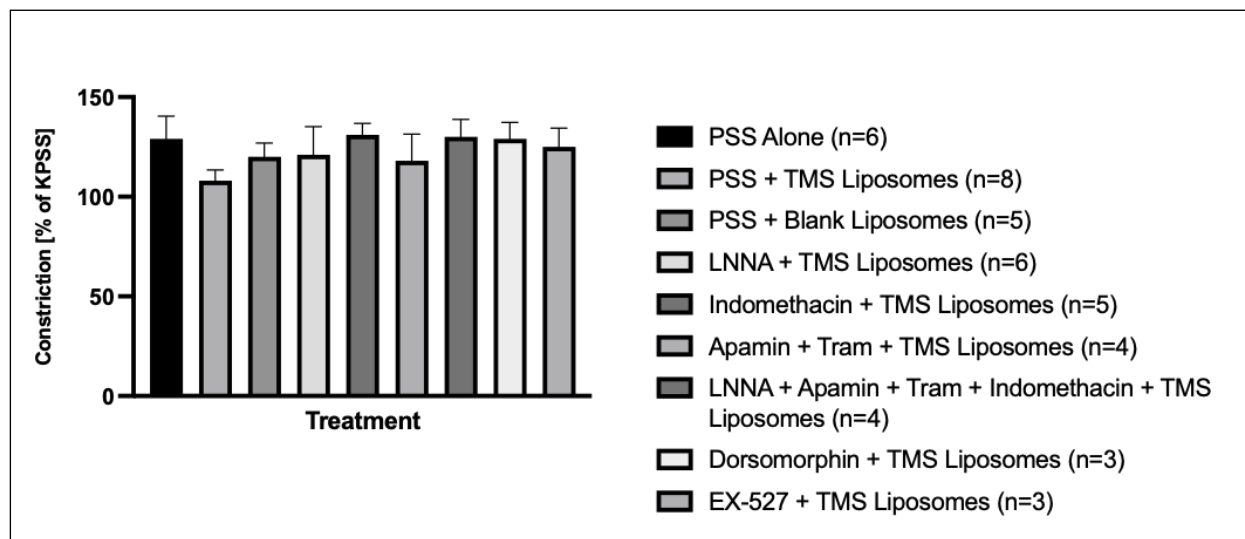
**Figure S3.** Schematic illustration showing pathways and targets implicated following treatment with pharmacological inhibitors used in this study.



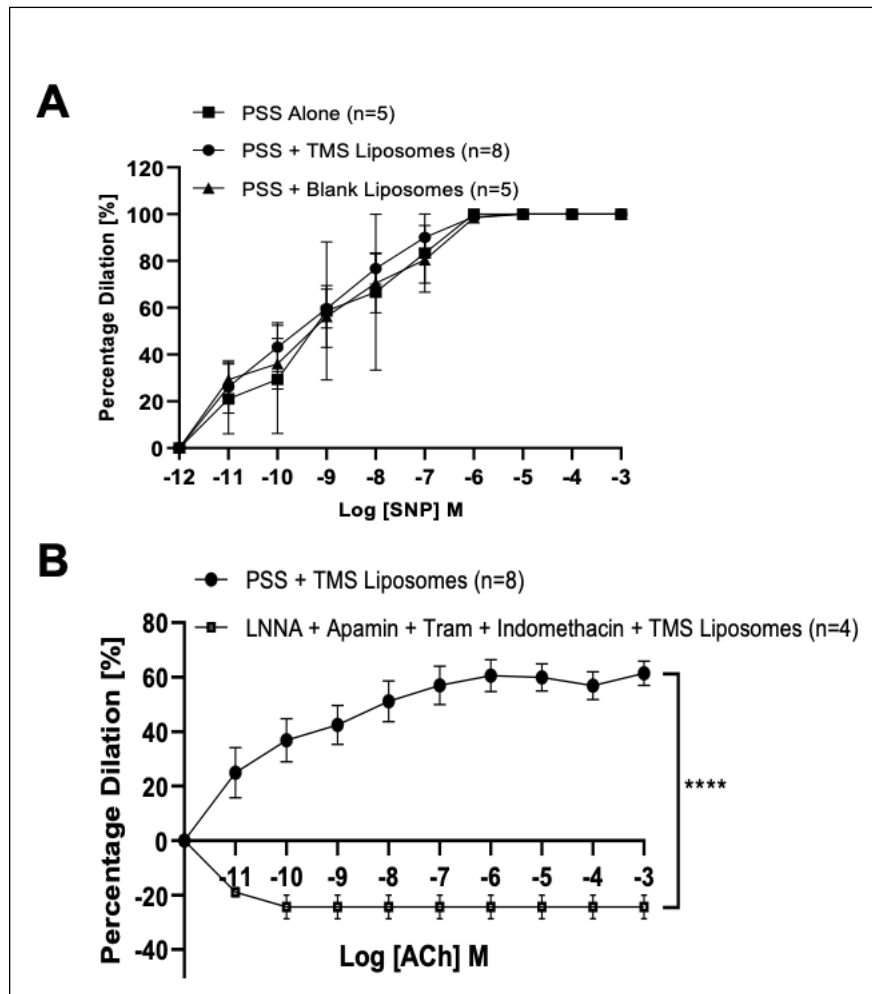
**Figure S4.** Constrictor responses of isolated rat coronary arteries to serotonin (5-HT). The dose-response effect of 5-HT ( $10^{-9}$ – $10^{-3}$ M) in coronary arteries maintained under normotensive pressure (60mmHg; 1hour). All vessels constricted to 5-HT in a dose-dependent manner, with sub-maximal dose identified as  $10^{-6}$ M (n=4).



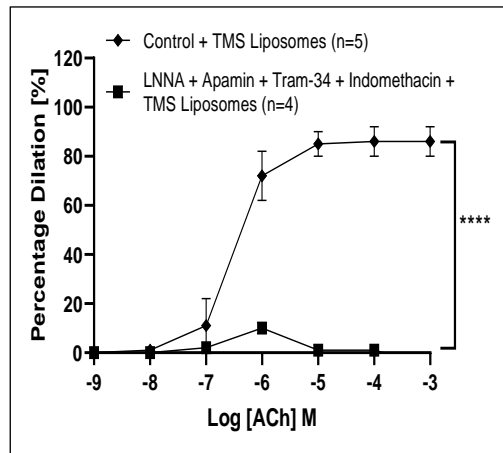
**Figure S5.** HPLC analysis of TMS. TMS content in TMS buffer solution after 4hours incubationwith murine coronary segments. TMS eluted after 22.18min and was identified by coelution with an authentic standard at 325nm.



**Figure S6.** Pre-constrictor responses to phenylephrine (Phe) in isolated left internal mammary arteries (IMAs) from coronary artery bypass graft (CABG) patients. Treatment had no effect on Phe ( $10^{-5}$ M) - induced vasoconstrictor responses of isolated IMAs, *ex vivo*, were similar for all vessels examined in each treatment/experimental group. One-way ANOVA followed by a Dunnett's multiple comparisons test.



**Figure S7.** The influence of Tetramethoxystilbene (TMS)-loaded liposomes on vascular responses of isolated left internal mammary arteries (IMAs) from coronary artery bypass graft (CABG) patients, *ex vivo*. **(A)** Endothelial-independent sodium nitroprusside (SNP) induced responses following co-incubation with TMS-loaded liposomes. **(B)** The influence of N $\omega$ -nitro-L-arginine (LNNA), Indomethacin, Apamin and TRAM-34 in combination on endothelial-dependent ACh-induced dilator responses following co-incubation with TMS- loaded liposomes. The area under the curve for two groups were compared using Two-way ANOVA followed by Tukey's multiple comparisons post-test. Data are presented as mean  $\pm$  SEM. \*\*\*\* $P$ <0.0001.



**Figure S8.** Characterization of dilator responses potentiated by Tetramethoxystilbene (TMS)-loaded liposomes following acute pressure elevation in isolated coronary arteries ex vivo. The influence of N $\Omega$ -nitro-L-arginine (LNNA) + Apamin and TRAM-34 + Indomethacin, in combination, on endothelium-dependent acetylcholine (ACh) responses following acute pressure elevation. The area under the curve for two groups were compared using Two-way ANOVA followed by Tukey's multiple comparisons post-test. Data are presented as mean  $\pm$  SEM. \*\*\*\*P < 0.0001.