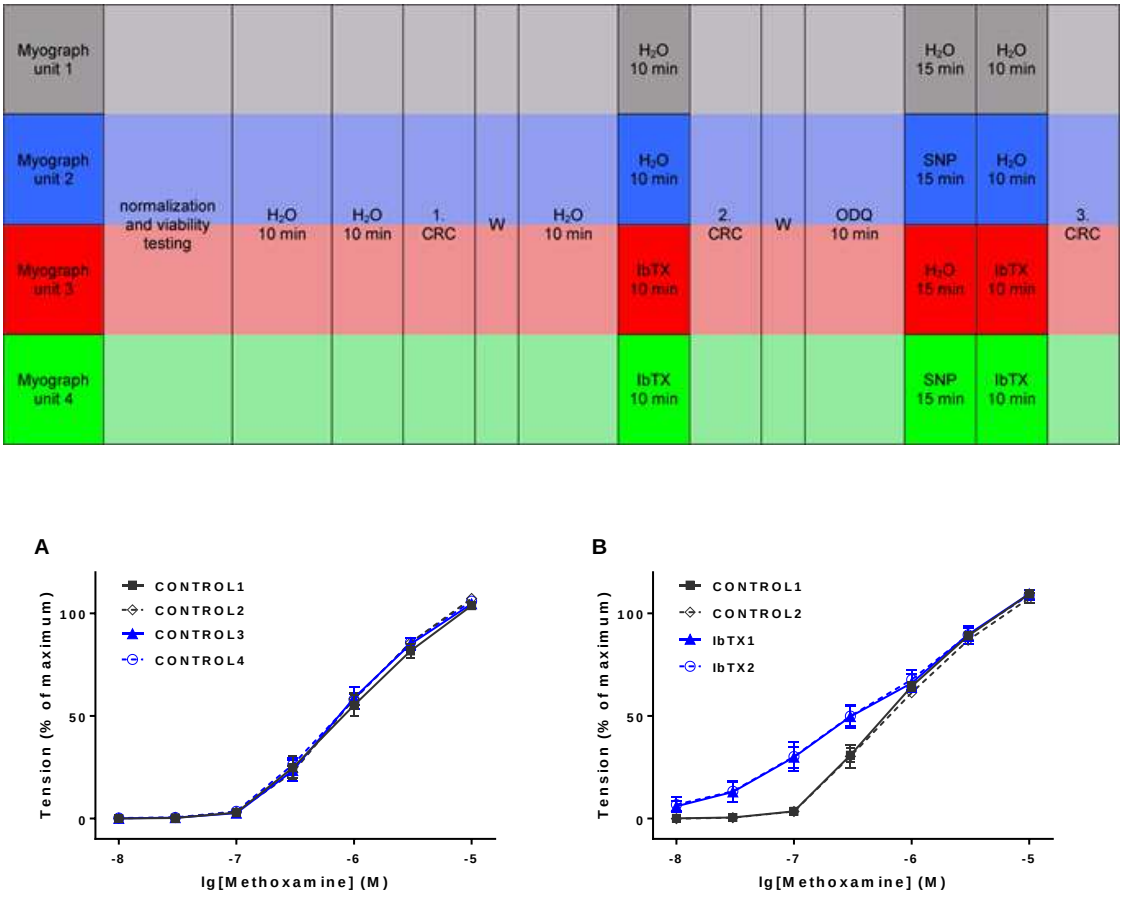
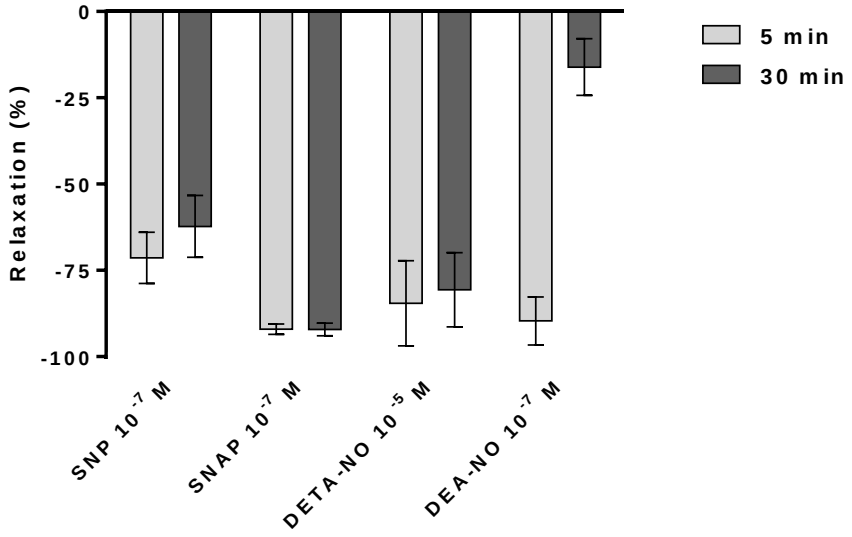
SUPPLEMENTAL MATERIAL

Figure. S1 Standardized protocol of most of the experiments performed in this study.



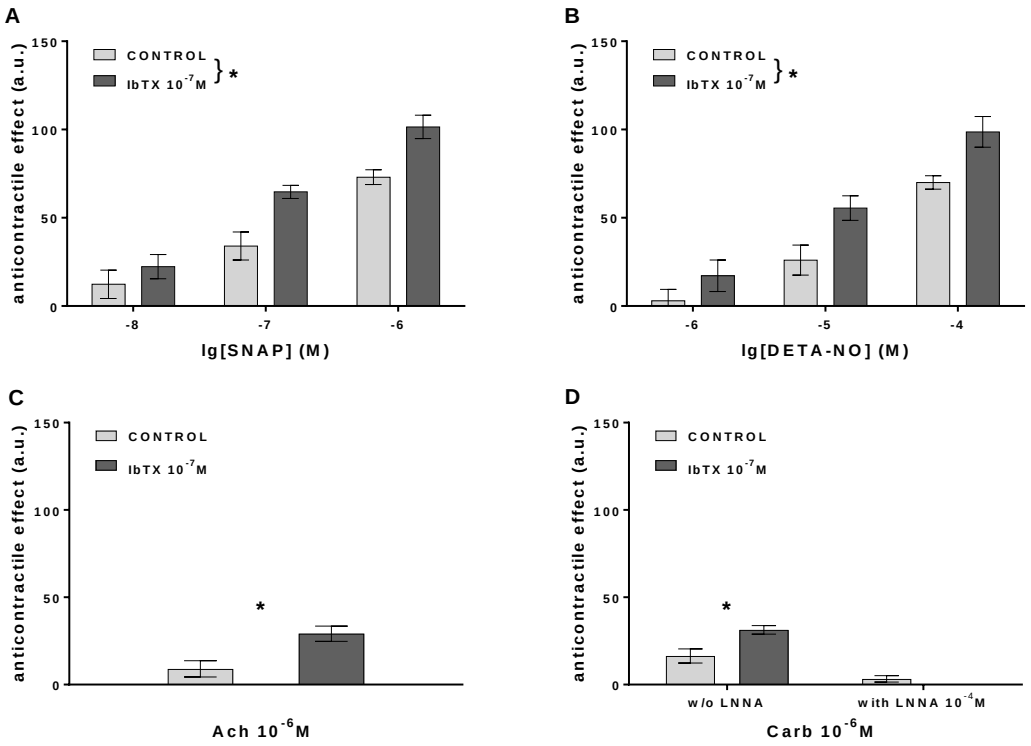
Here, as an example, the protocol of an experiment is shown in which the effect of ODQ (a blocker of the soluble guanylyl cyclase) on the vessel response to the NO-donor SNP was studied in vessels with and without blockage of the BK channel with IbTX. Myograph unit 1-4 reflects different experimental groups; W stands for wash; CRC stands for concentration-response relationship. **A)** MX-induced contractions straight after the viability test (1. CRC). Vessel tension in 4 different experimental groups (CONTROL1 – CONTROL4) (1-way ANOVA: n=7; p=0.94). **B**) Effect of IbTX on MX-induced contractions. Vessel tension in the absence of IbTX (CONTROL1, CONTROL2) and in the presence of IbTX (IbTX1, IbTX2) (2. CRC) (t-test CONTROL1 vs CONTROL2: n=7; p=0.58 and IbTX1 vs IbTX2: n=7; p=0.95). All statistical comparisons are based on the area under the concentration-response relationships.

Figure S2. Stability of NO-donor-induced relaxation.



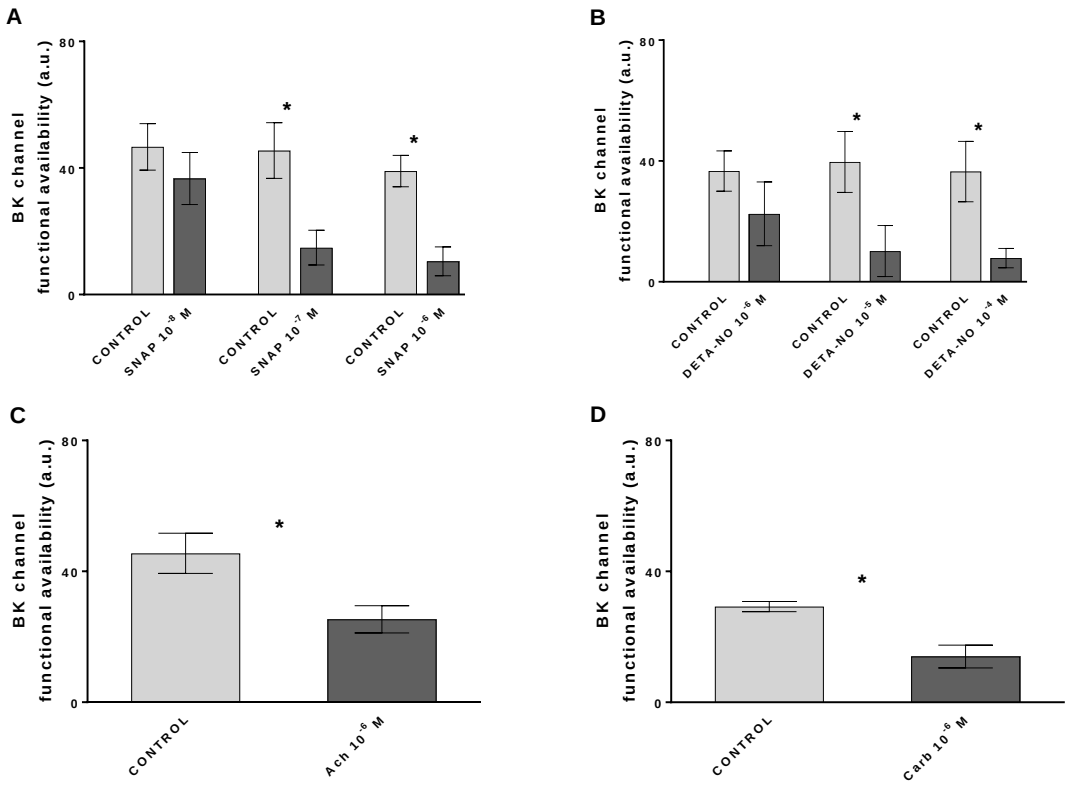
Vessels were pre-constricted with 10^{-6} M methoxamine. The NO-donor-induced relaxation (in %) after 5 minutes and after 30 minutes (paired t-test; SNP: n=10; p=0.11; SNAP: n=7; p=0.92; DETA-NO: n=6; p=0.09; DEA-NO: n=6; p<0.001)

Figure S3. Contribution of the BK channel to the anti-contractile effect of NO.



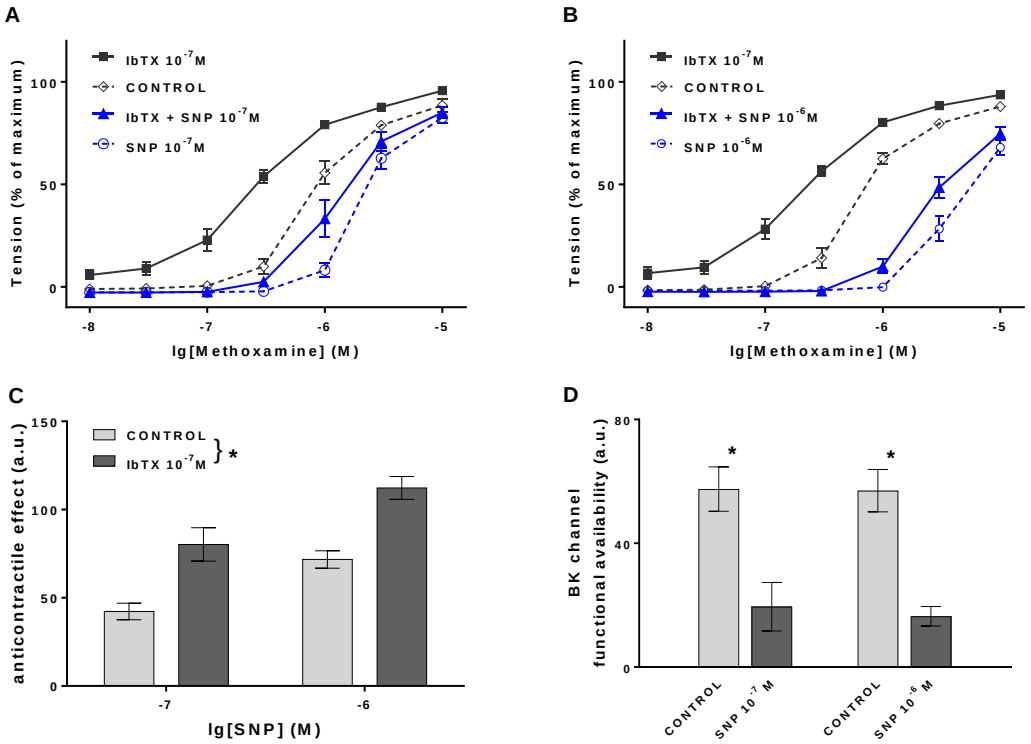
A) The anti-contractile effect of SNAP in the absence (CONTROL) and presence of iberiotoxin (IbTX) (two-way ANOVA for effect of SNAP: n=6-8; $p<0.001$; * - two-way ANOVA control vs IbTX: n=6-8; $p<0.001$). **B)** The anti-contractile effect of DETA-NO in the absence (CONTROL) and presence of iberiotoxin (IbTX) (two-way ANOVA for effect of DETA-NO: n=6; $p<0.001$; * - two-way ANOVA control vs IbTX: n=6; $p<0.001$). **C)** The anti-contractile effect of acetylcholine (Ach) in the absence (CONTROL) and presence of iberiotoxin (IbTX) (t-test for effect of Ach: n=6; $p<0.01$; * - t-test control vs IbTX: n=6; $p<0.05$). **D)** The anti-contractile effect of carbachol (Carb) in the absence (CONTROL) and presence of iberiotoxin (IbTX) (t-test for effect of Carb: n=7; $p<0.001$; * - t-test control vs IbTX: n=8; $p<0.01$); The anti-contractile effect of carbachol (Carb) without and with IbTX in the presence of 10^{-4} M L-NNA (t-test control+LNNA vs Carb+LNNA: n=7; $p=0.69$; t-test IBTX+LNNA vs Carb+IBTX+LNNA: n=7; $p=0.79$)

Figure S4. Contribution of the BK channel to the anti-contractile effect of NO.



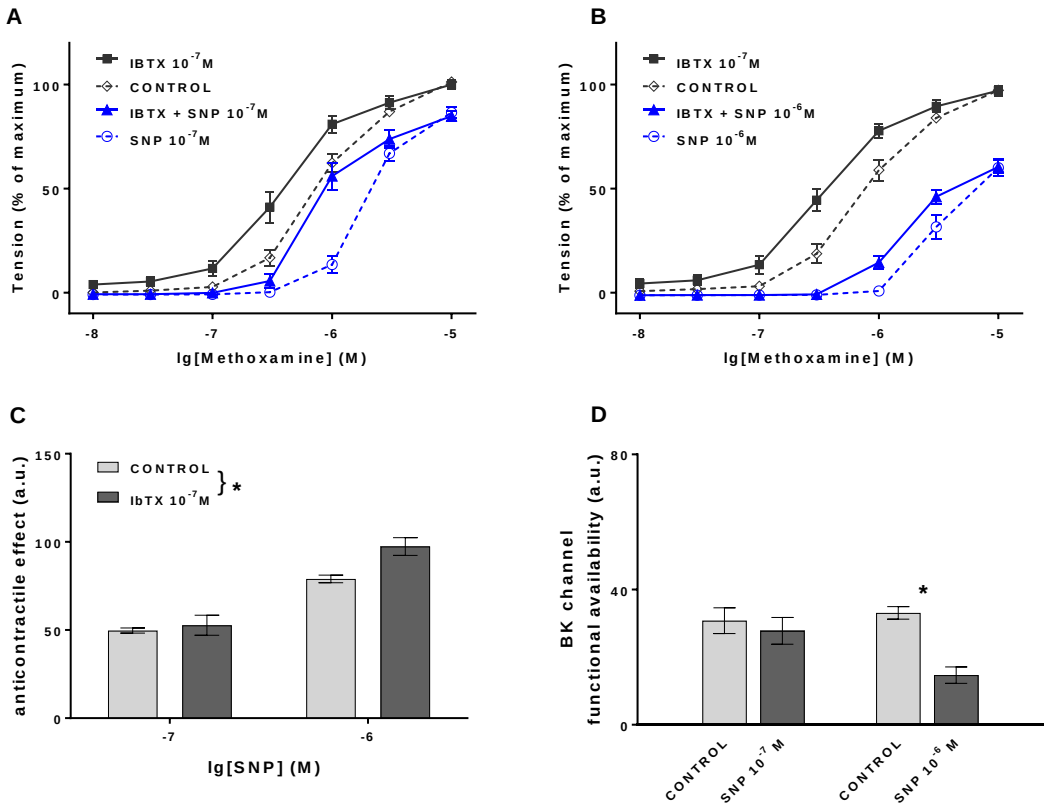
A) Functional availability of the BK channel in the absence (CONTROL) and presence of SNAP (SNAP) (t-test: n=6, p=0.39; * - n=6, $p<0.05$ and n=8, $p<0.001$ for SNAP 10^{-8}, 10^{-7}, and 10^{-6} M, respectively). **B)** Functional availability of the BK channel in the absence (CONTROL) and presence of DETA-NO (DETA-NO) (t-test: n=6, p=0.28; * - n=6, $p<0.05$ and n=6, $p<0.05$ for DETA-NO 10^{-6}, 10^{-5}, and 10^{-4} M, respectively). **C)** Functional availability of the BK channel in the absence (CONTROL) and presence of acetylcholine (Ach) (* - t-test: n=6, $p<0.05$). **D)** Functional availability of the BK channel in the absence (CONTROL) and presence of carbachol (Carb) (* - t-test: n=8, $p<0.01$)

Figure S5. Contribution of the BK channel to the anti-contractile effect of SNP in rat saphenous arteries.



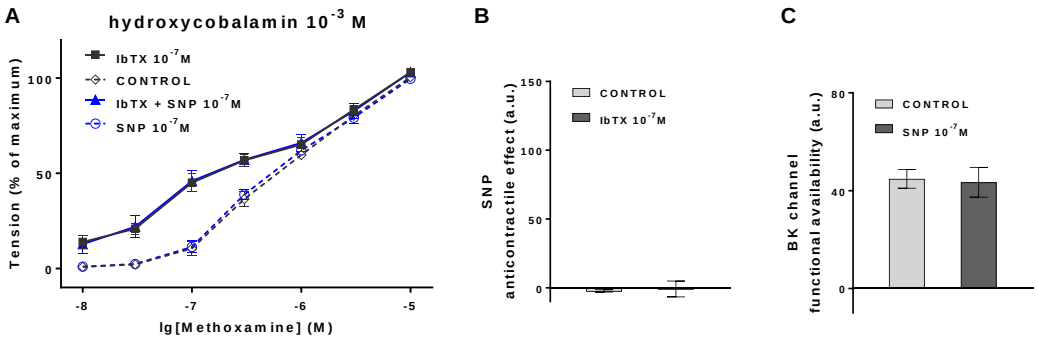
Effect of SNP and IbTX on MX-induced contractions. Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7} M **(A)** and 10^{-6} M **(B)** SNP. **C)** The anti-contractile effect of SNP in the absence (CONTROL) and presence of iberiotoxin (IbTX) (two-way ANOVA for effect of SNP: n=7,8; $p<0.01$; * - two-way ANOVA control vs IbTX: n=7,8; $p<0.001$). **D)** Functional availability of the BK channel in the absence (CONTROL) and presence of SNP (SNP) (* - t-test: n=8, $p<0.01$; n=7, $p<0.001$ for SNP 10^{-7} and 10^{-6} M, respectively)

Figure S6. Contribution of the BK channel to the anti-contractile effect of SNP in mouse tail arteries.



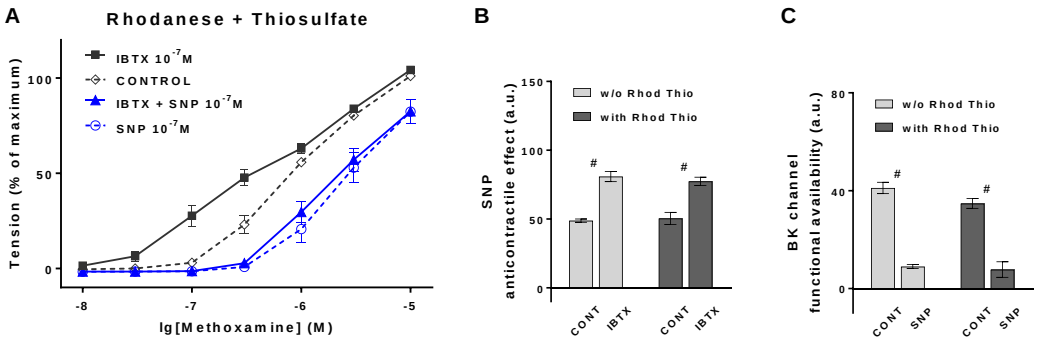
Effect of SNP and IbTX on MX-induced contractions. Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7} M **(A)** and 10^{-6} M **(B)** SNP. **C)** The anti-contractile effect of SNP in the absence (CONTROL) and presence of iberiotoxin (IbTX) (two-way ANOVA for effect of SNP: n=10; $p<0.001$; * - two-way ANOVA control vs IbTX: n=10; $p<0.05$). **D)** Functional availability of the BK channel in the absence (CONTROL) and presence of SNP (SNP) (* - t-test: n=10, $p<0.001$)

Figure S7. Effect of the NO-scavenger hydroxycobalamin on the anti-contractile effect of SNP.



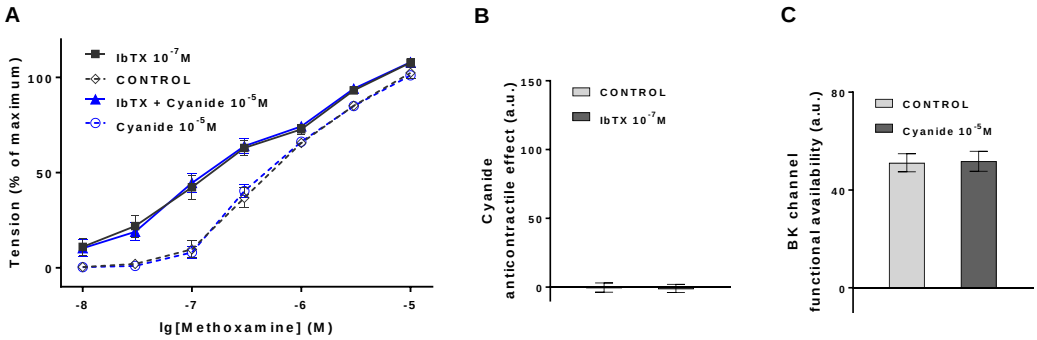
A) Effect of SNP and IbTX on MX-induced contractions in the presence of hydroxycobalamin (10^{-3}M). Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7}M SNP (repeated measures ANOVA: n=7, p=0.78 for CONTROL vs SNP and n=7, p=0.96 for IbTX vs IbTX+SNP). **B)** The anti-contractile effect of SNP in the absence (CONTROL) and presence of iberiotoxin (IbTX) (t-test: n=7; p=0.81). **C)** Functional availability of the BK channel in the absence (CONTROL) and presence of SNP (SNP) (t-test: n=7, p=0.85)

Figure S8. Effect of rhodanese and sodium thiosulfate, which inactivate cyanide, on the anti-contractile effect of SNP.



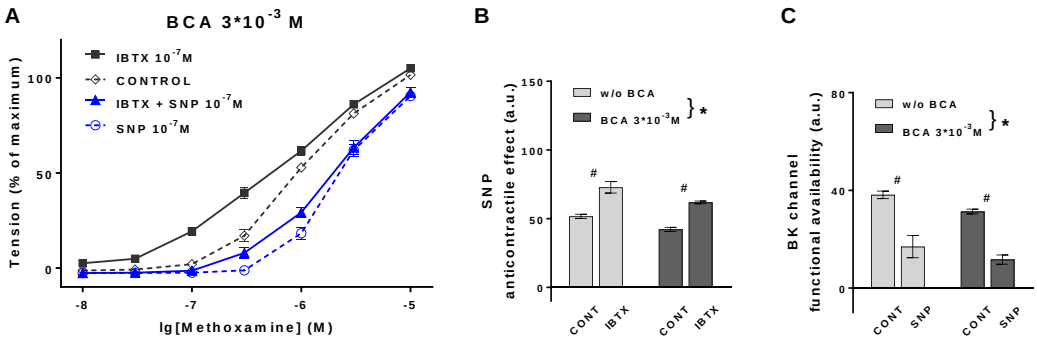
A) Effect of SNP and IbTX on MX-induced contractions in the presence of 30 U/L rhodanese and $1.25*10^{-4}$ M sodium thiosulfate. Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7} M SNP (repeated measures ANOVA: n=8, $p<0.001$ for CONTROL vs SNP and n=8, $p<0.001$ for IbTX vs IbTX+SNP). **B)** The anti-contractile effect of SNP in the absence (CONT) and presence of iberiotoxin (IbTX) (two-way ANOVA without (w/o) vs with rhodanese+sodium thiosulfate: n=8; p=0.78; # - t-test cont vs IbTX: w/o Rhod Thio n=8; $p<0.001$; with Rhod Thio n=8; $p<0.001$). **C)** Functional availability of the BK channel in the absence (CONT) and presence of SNP (SNP) (two-way ANOVA without vs with rhodanese+sodium thiosulfate: n=8; p=0.10; # - t-test cont vs SNP: w/o Rhod Thio n=8; $p<0.001$; with Rhod Thio n=8; $p<0.001$)

Figure S9. Effect of cyanide.



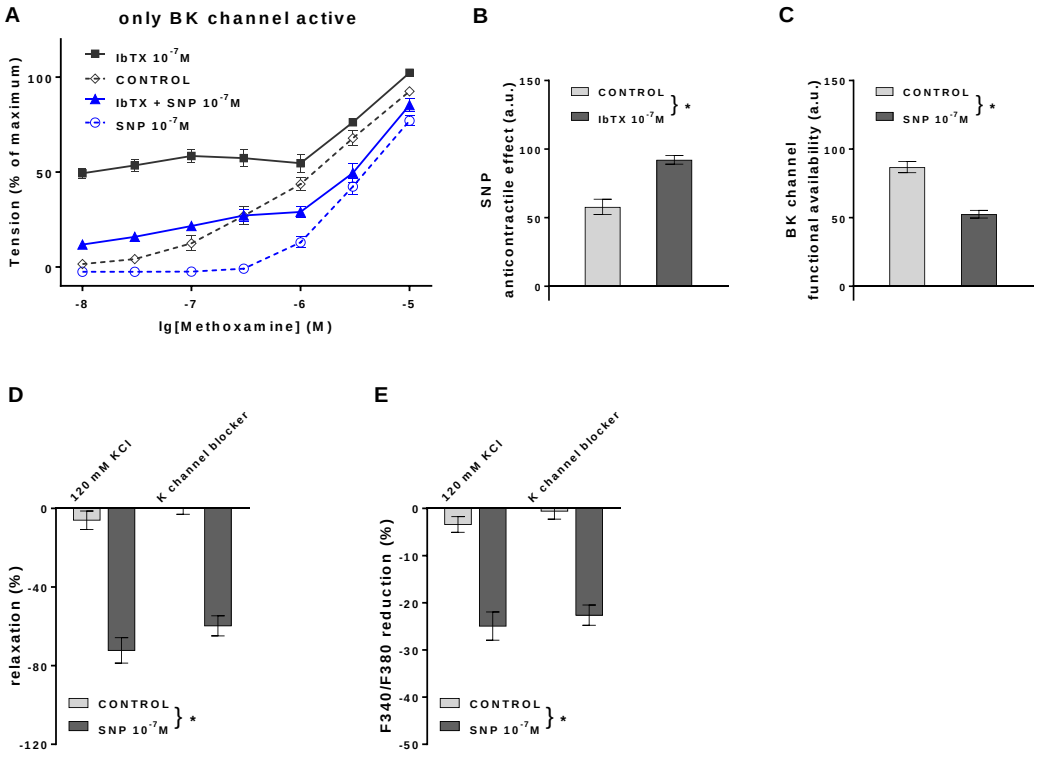
A) Effect of cyanide (CN) at 10^{-5} M and IbTX on MX-induced contractions. Vessel tension in the absence of IbTX and CN (CONTROL), in the presence of IbTX alone (IbTX), in the presence of CN alone (Cyanide), and in the combined presence of IbTX and CN (IbTX + Cyanide) (repeated measures ANOVA: n=8, p=0.99 for CONTROL vs CN and n=8, p=0.94 for IbTX vs IbTX+CN). **B)** The effect of CN in the absence (CONTROL) and presence of iberiotoxin (IbTX) (t-test: n=8; p=0.89). **C)** Functional availability of the BK channel in the absence (CONTROL) and presence of CN (Cyanide) (t-test: n=8, p=0.91)

Figure S10. Effect of β-cyano-L-alanine, an inhibitor of the H_2S-generating enzymes cystathionine γ-lyase and cystathionine β-synthase, on the anti-contractile effect of SNP.



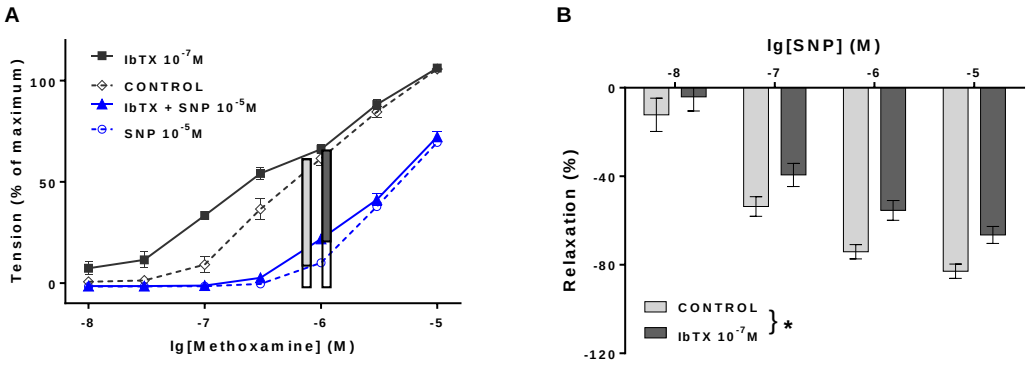
A) Effect of SNP and IbTX on MX-induced contractions in the presence of $3*10^{-3}$ M β-cyano-L-alanine (BCA). Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7} M SNP (repeated measures ANOVA: n=6, $p<0.001$ for CONTROL vs SNP and n=6, $p<0.001$ for IbTX vs IbTX+SNP). **B)** The anti-contractile effect of SNP in the absence (CONT) and presence of iberiotoxin (IbTX) (* - two-way ANOVA without (w/o) vs with BCA: n=6; $p<0.001$; # - t-test cont vs IbTX: w/o BCA n=6; $p<0.01$; with BCA n=6; $p<0.001$). **C)** Functional availability of the BK channel in the absence (CONT) and presence of SNP (SNP) (* - two-way ANOVA without vs with BCA: n=6; $p<0.05$; # - t-test cont vs SNP: w/o BCA n=6; $p<0.01$; with BCA n=6; $p<0.001$)

Figure S11. Effect of blocking non-BK channels on the anti-contractile effect of SNP.



A) Effect of SNP and IbTX on MX-induced contractions in the presence of 10^{-6} M DPO-1, 10^{-7} M stromatoxin, $3*10^{-6}$ M XE991, $3*10^{-5}$ M barium chloride and 10^{-6} M glibenclamide. Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-7}M SNP (repeated measures ANOVA: n=6, $p<0.001$ for CONTROL vs SNP and n=6, $p<0.001$ for IbTX vs IbTX+SNP). **B)** The anti-contractile effect of SNP in the absence (CONTROL) and presence of iberiotoxin (IbTX) (* - t-test cont vs IbTX: n=6; $p<0.001$). **C)** Functional availability of the BK channel in the absence (CONTROL) and presence of SNP (SNP) (* - t-test cont vs SNP: n=6; $p<0.001$). **D)** Effect of SNP on vessel tension induced by 120 mM KCl or 10^{-6} M DPO-1, 10^{-7} M stromatoxin, $3*10^{-6}$ M XE991, $3*10^{-5}$ M barium chloride, 10^{-6} M glibenclamide and 10^{-7} M IbTX (* - t-test cont vs SNP. 120 mM KCl: n=5; $p<0.001$; K channel blocker: n=5; $p<0.001$). E) Effect of SNP on $[Ca]_i$ response induced by 120 mM KCl or 10^{-6} M DPO-1, 10^{-7} M stromatoxin, $3*10^{-6}$ M XE991, $3*10^{-5}$ M barium chloride, 10^{-6} M glibenclamide and 10^{-7} M IbTX (* - t-test cont vs SNP. 120 mM KCl: n=5; $p<0.001$; K channel blocker: n=5; $p<0.001$)

Figure S12. Contribution of the BK channel to the anti-contractile effect of SNP.



A) Effect of SNP and IbTX on MX-induced contractions. Vessel tension in the absence of IbTX and SNP (CONTROL), in the presence of IbTX alone (IbTX), in the presence of SNP alone (SNP), and in the combined presence of IbTX and SNP (IbTX + SNP) at 10^{-5} M SNP. Data were analyzed with an approach used in previously published reports: (i) selection of a submaximal pre-constriction similar in amplitude in the absence and presence of IbTX (black curves, 10^{-6} M MX); (ii) determination of vessel tension in SNP pre-treated vessels in the absence as well as in the presence of IbTX (blue curves, 10^{-6} M MX) and calculation of the differences to the tensions at pre-constriction (light gray and dark gray parts of the vertical bars) and (iii) determination of SNP-induced relaxation in percent by dividing the differences calculated in (ii) by the respective tensions at pre-constriction. **B)** The SNP-induced relaxation (in %) in the absence (CONTROL) and presence of iberiotoxin (IbTX) (* - two-way ANOVA control vs IbTX: $p<0.001$)