# Expanded View Figures

Figure EV1. FR prevents 5-HT-induced constriction and $G\alpha_q/G\alpha_{11}$ can be downregulated by lentiviral transduction.

(A) Original traces of 5-HT dose–response curves (10^{-9} M–10^{-5} M) after pre-incubation with DMSO, Ket (10^{-6} M) or FR (10^{-6} M) in PAs. (B) PCR analysis of Gq protein subtypes in native mPASMCs and mPASMCs transduced with lentiviral sh-G11, sh-Gq RNA (negative controls). Murine lung tissue was used as positive control. (C–F) Statistical analysis of relative $G\alpha_{11}$ (C), $G\alpha_q$ (D), $G\alpha_i$ (E), and $G\alpha_s$ (F) mRNA expression in native mPASMCs ($n = 3$) and mPASMCs transduced with lentivirus (sh-control (ctrl), sh-G11, sh-Gq RNA or both, $n = 3$ independent experiments normalized to 18S housekeeping gene. (G, H) Original Western Blot (G) and analysis (H) of $G\alpha_{q/11/14}$ protein expression of native mPASMCs and mPASMCs transduced with lentivirus (sh-control (ctrl), sh-G11, sh-Gq RNA or both, $n = 3$ independent experiments). GAPDH was used as housekeeper. Data information: Values are expressed as mean ± SEM. (C–F, H) One-way ANOVA, Tukey's post hoc test. Source data are available online for this figure.

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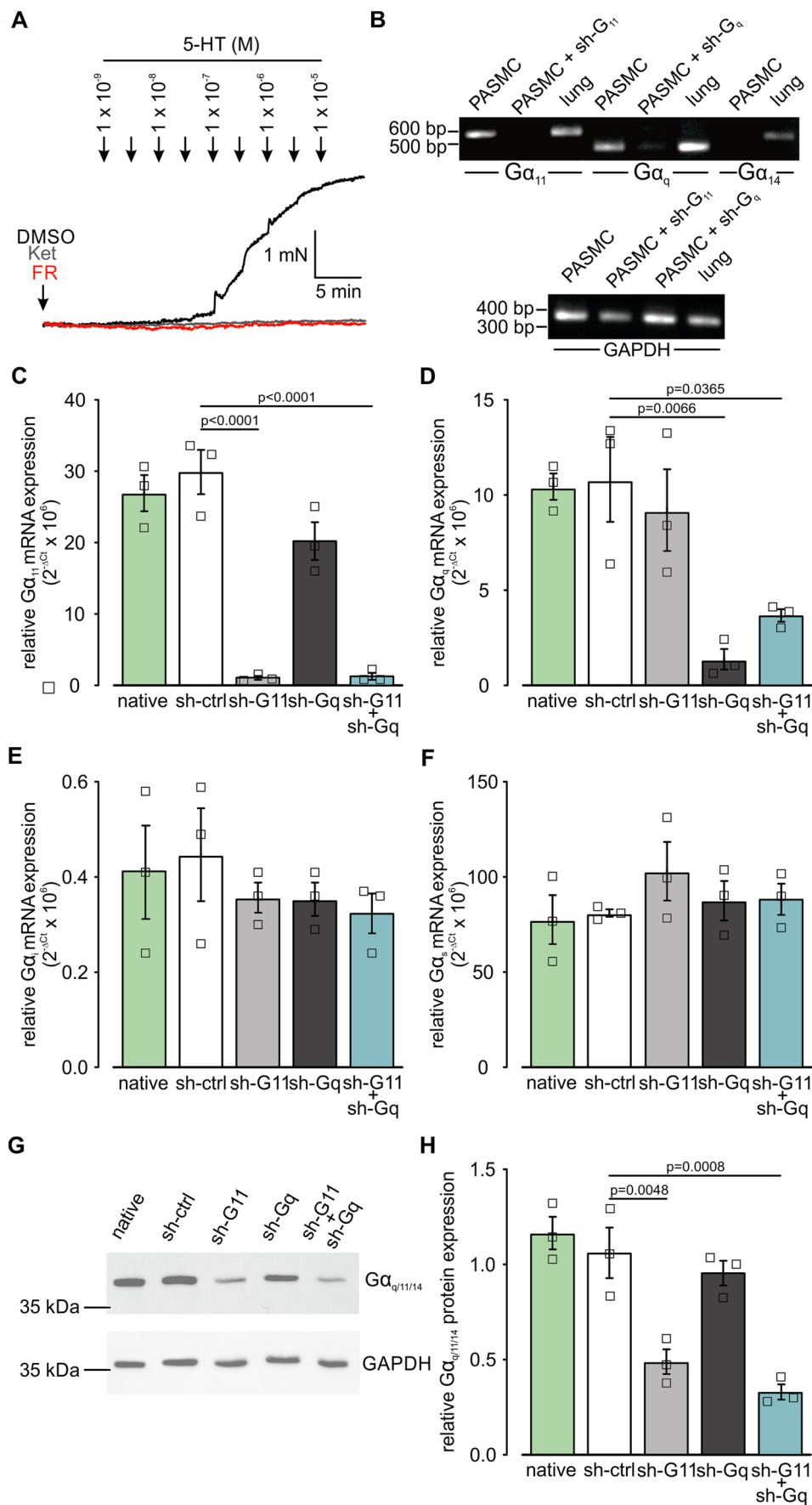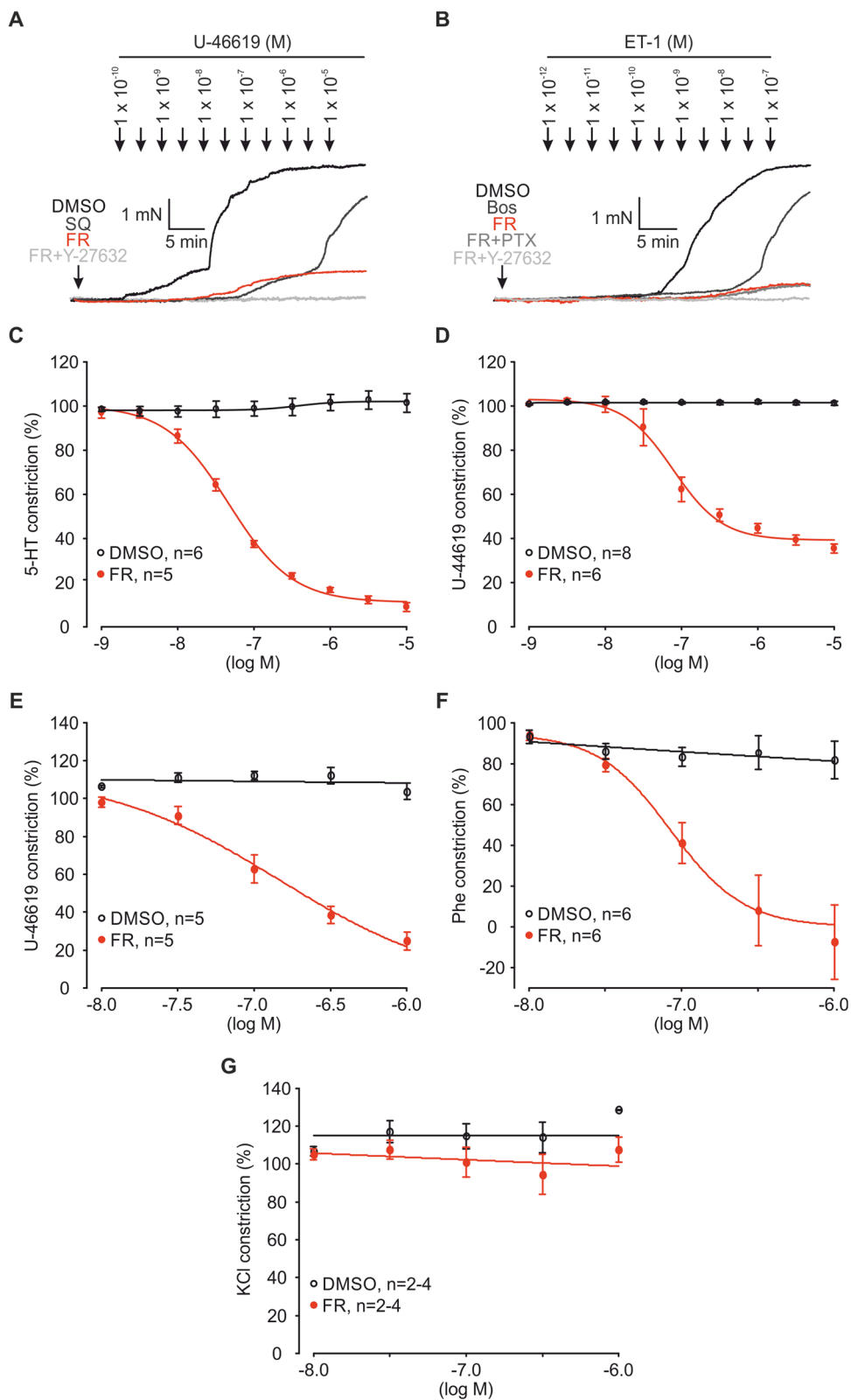
◀ **Figure EV2. FR prevents and reverses Gq-mediated constriction in mouse and pig PAs.**

(A) Original traces of U-46619 dose–response curves (10^{-10} M–10^{-5} M) after pre-incubation with DMSO, SQ (10^{-6} M), FR (10^{-6} M), or FR + Y-27632 (10^{-5} M) in mouse PAs. (B) Original traces of ET-1 dose–response curves (10^{-12} M–10^{-7} M) after pre-incubation with DMSO, Bos (10^{-6} M), FR (10^{-6} M), FR + PTX (1 µg/ml) or FR + Y-27632 (10^{-5} M) in mouse PAs. (C, D) Dose–response curves of DMSO and FR (10^{-9} M–10^{-5} M) after 5-HT (5×10^{-7} M, DMSO: $n = 6$, FR: $n = 5$, C) or U-46619 (10^{-7} M, DMSO: $n = 8$, FR: $n = 6$, D) pre-constriction in murine PAs. (E–G) Dose–response curves of DMSO and FR (10^{-8} M–10^{-6} M) after U-46619 (3×10^{-7} M, DMSO: $n = 5$, FR: $n = 5$, E), Phe (3×10^{-5} M, DMSO: $n = 6$, FR: $n = 6$, F), or KCl (3×10^{-2} M, DMSO: $n = 2$–4, FR: $n = 2$–4, G) pre-constriction in porcine PAs. Data information: Values are expressed as mean ± SEM. Source data are available online for this figure.
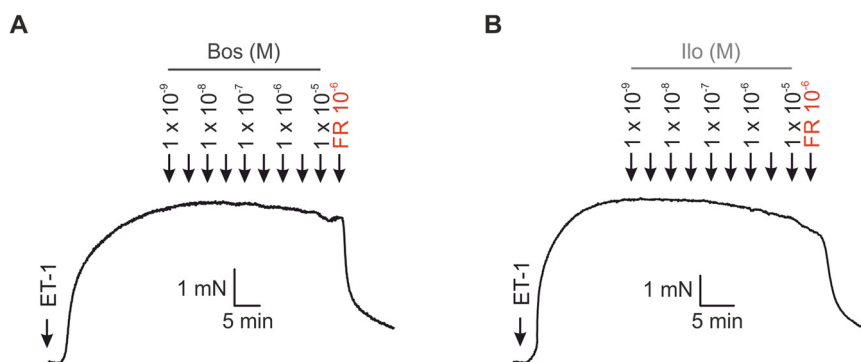
Figure EV3. FR strongly relaxes PAs ex vivo.

(A, B) Original traces of Bos (A) or Ilo (B) dose–response curves (10^{-9} M – 10^{-5} M) followed by single dose FR (10^{-6} M) application after pre-constriction with ET-1 (3×10^{-9} M) in mouse PAs.
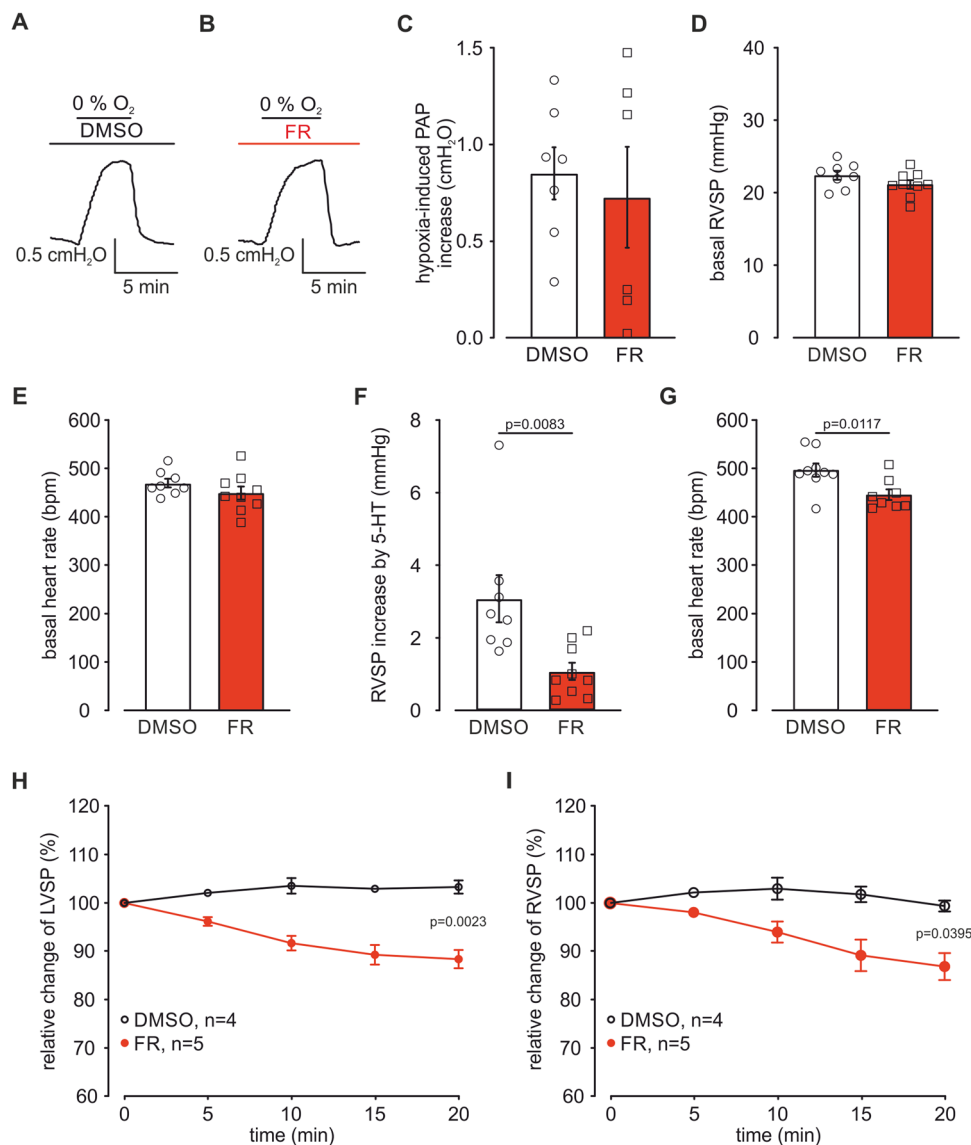
Figure EV4. FR does not affect HPV but reduces RVSP in vivo.

(A, B) Original traces of PAP in the IPL model during perfusion with DMSO (A) or FR (10^{-6} M, B) and exposure to hypoxic air (0% O_2/100% N_2). (C) Statistical analysis of PAP increase evoked by hypoxic air during DMSO ($n = 7$ mice) or FR ($n = 6$ mice) perfusion. (D) Statistical analysis of basal RVSP 1 h after DMSO ($n = 8$) or FR (2.5 μg/mouse, $n = 8$) i.t. application in healthy mice housed under normoxic (21% O_2) conditions. (E) Statistical analysis of basal heart rate in these mice (DMSO: $n = 8$; FR: $n = 8$). (F) Statistical analysis of RVSP increase in response to 5-HT (5×10^{-3} M, 10 μl) i.v. bolus injection in these mice (DMSO: $n = 8$; FR: $n = 8$). (G) Basal heart rate 1 h after DMSO ($n = 9$) or FR (2.5 μg/mouse, 1 h before, $n = 8$) application in mice with pre-existing Hx-induced PH (DMSO: $n = 9$; FR: $n = 8$). (H, I) Relative change of LVSP (H) and RVSP (I) after acute DMSO ($n = 4$) or FR (10 μg/mouse i.p., $n = 5$) application in mice with pre-existing Hx-induced PH. Data information: Values are expressed as mean ± SEM. (C–G) Unpaired student's t-test. (H, I) Two-way ANOVA, Bonferroni post hoc test. Source data are available online for this figure.
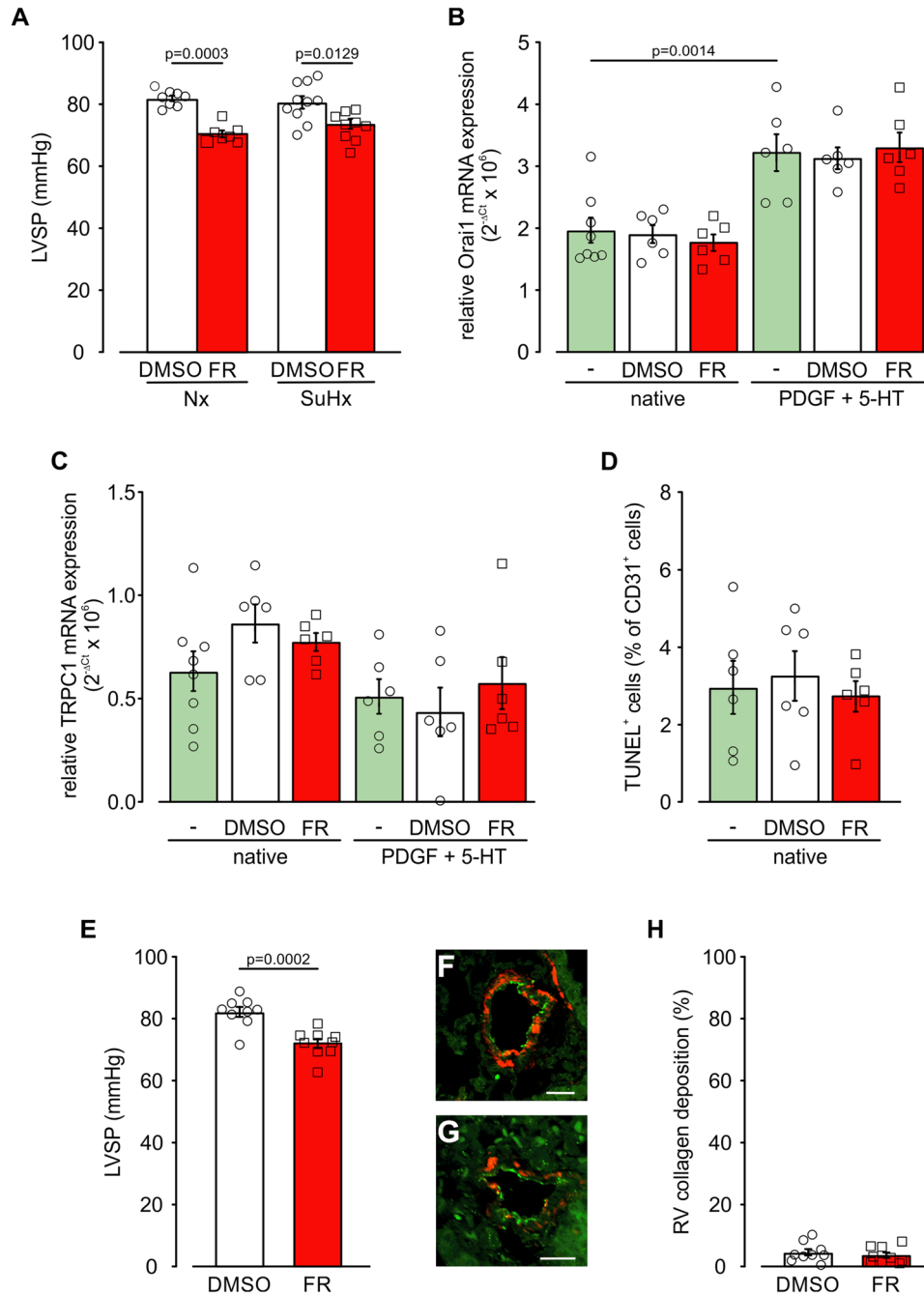
Figure EV5. FR effects on Hx-induced PH in vivo and mPASMCs as well as mLECs in vitro.

(A) Statistical analysis of LVSP in mice treated with the solvent DMSO or FR (10 μg/mouse i.p., Monday to Friday) during exposure to Nx (21% O_2, DMSO: $n = 8$, FR: $n = 7$) or SuHx (10% O_2, DMSO: $n = 10$, FR: $n = 10$) for 3 weeks. (B, C) Statistical analysis of relative Orai1 (B) and TRPC1 (C) mRNA expression in native mPASMCs ($n = 8$) and mPASMCs treated with solvent DMSO or FR (10^{-6} M) with or without additional PDGF (40 ng/ml) + 5-HT (10^{-6} M) stimulation for 12 h, each $n = 6$ normalized to 18 S housekeeping gene, ns indicate different wells derived from at least two different passages. (D) Amount of TUNEL^{+} CD31^{+} mLECs after 2 days without treatment ($n = 6$) or with DMSO ($n = 6$) or FR (10^{-6} M, $n = 6$) treatment. (E) Statistical analysis of LVSP in mice treated with the solvent DMSO ($n = 9$) or FR (10 μg/mouse i.p., Monday to Friday, $n = 9$) in the last 2 weeks of 5 weeks SuHx exposure. (F, G) vWF/α-SMAC staining of PAs in lung sections from SuHx-DMSO (F) and SuHx-FR-treated mice (G), scale bars: 20 μm. (H) Statistical analysis of collagen deposition in the right ventricle in mice treated with the solvent DMSO ($n = 9$) or FR (10 μg/mouse i.p., Monday to Friday, $n = 9$) in the last 2 weeks of 5 weeks SuHx exposure. Data information: Values are expressed as mean ± SEM. (A–D) One-way ANOVA, Tukey's post hoc test, (E, H) Unpaired student's t-test. Source data are available online for this figure.