Supplementary Figure 1. Kv7 channel pharmacological activation with ML213 leads to an inhibition of spontaneous phasic contractions in DSM isolated strips. A-D) Cumulative concentration-response curves for ML213 on DSM phasic contraction: A) amplitude, B) muscle force, C) duration and D) frequency in the absence and presence of the Kv7 channel inhibitor XE991 (n=6, N=6; *P<0.05, **P<0.01, and ***P<0.001). #P<0.05, ##P<0.01, and ###P<0.001 indicate statistical significance in the effects of ML213 on spontaneous phasic contractions in the absence (control) versus presence of XE991 (n=6, N=6; two-way ANOVA test followed by Bonferroni post-test). Data are represented as means ± SD. See Table 1 for potency and maximum efficacy values.
Supplementary Figure 2. Kv7 channel pharmacological activation with ML213 leads to an inhibition of 20 mM KCl-induced phasic contractions in DSM isolated strips. A-D) Cumulative concentration-response curves for the inhibitory effects of ML213 on DSM phasic contraction: A) amplitude, B) muscle force, C) duration and C') frequency (n=7, N=7; *P<0.05 and ***P<0.001; two-tailed paired Student’s t-test). Data are represented as means ± SD. See Table 1 for potency and maximum efficacy values.
Supplementary Figure 3. Differential inhibitory effects of ML213 and nifedipine on 60 mM KCl-induced tonic contractions in DSM isolated strips. Summarized data, displayed as scatterplots with means, demonstrating the differential inhibitory effects of ML213 (10 µM) and nifedipine (1 µM) on DSM 60 mM KCl-induced tonic contraction (n=8, N=4; ###P<0.001 vs. control, and **P<0.001 ML213 vs. nifedipine, respectively; two-tailed paired Student’s t-test).
Supplementary Figure 4. Kv7 channel pharmacological activation with ML213 leads to an inhibition of CCh-induced DSM phasic contractions. A-D) Cumulative concentration-response curves for ML213 on CCh (0.1 or 1 µM)-induced DSM phasic contraction A) amplitude, B) muscle force, C) duration, and D) frequency (n=6, N=6 for both data sets; #P<0.05, ###P<0.001; two-tailed paired Student’s t-test). Data are represented as means ± SD. See Table 1 for potency and maximum efficacy values.
Supplementary Figure 5. Kv7 channel pharmacological activation with ML213 leads to an inhibition of nerve-evoked contractions induced by 10 Hz and 20 Hz EFS in DSM isolated strips. A-B) Cumulative concentration-response curves for ML213 on DSM EFS-induced: A) contraction amplitude and B) muscle force (n=7, N=6 for 10 Hz; n=6, N=6 for 20 Hz; *P<0.05 for 10 vs. 20 Hz; two-tailed paired Student’s t-tests). Data are represented as means ± SD. See Table 1 for potency and maximum efficacy values.
Supplementary Figure 6. ML213 decreases 0.5-50 Hz EFS-induced contractions in DSM isolated strips. A-B) Cumulative frequency-response curves demonstrating the inhibitory effects of A) 1 µM ML213 and B) 3 µM ML213 on the amplitude of 0.5-50 Hz EFS-induced contractions in DSM isolated strips (n=6, N=6 for both data sets; *P<0.05, **P<0.01, and ***P<0.001; two-tailed paired Student’s t-test). Data are represented as means ± SD.
Supplementary Figure 7. ML213 decreases global intracellular Ca\(^{2+}\) concentrations in DSM isolated strips. Summarized data, shown as scatterplots with medians, representing the reduction in global Ca\(^{2+}\) levels by 10 µM ML213 in DSM isolated strips (n=8, N=6; **P<0.01; two-tailed Wilcoxon matched-pairs signed rank test).
Supplementary Figure 8. ML213 (10 µM) enhanced whole cell Kv7 currents in freshly-isolated DSM cells. The two curves represent the current–voltage relationship expressed as normalized current in the absence or presence of 10 µM ML213 (n=7, N=5, *P<0.05; two-tailed paired Student’s t-test). Data are represented as means ± SD.
Supplementary Figure 9. Time course of ML213-induced activation of Kv7 channel currents in freshly-isolated DSM cells. Summary data, displayed as scatterplots with medians, for the percentage of Kv7 currents in the presence of ML213 compared to control at -10 and +40 mV respectively (n=8, N=5, **P<0.01; two-tailed Wilcoxon matched-pairs signed rank test). Summarized data illustrate the average of 4 to 6 points of each steady-state current.
Supplementary Figure 10. ML213 hyperpolarizes the resting membrane potential in freshly-isolated DSM cells. A) Summarized data for the hyperpolarizing effects of ML213 (10 µM) on the DSM cell membrane potential (n=5, N=5; **P<0.01, two-tailed paired Student’s t-test). B) Summarized data for XE991 (10 µM)-induced depolarization in freshly-isolated DSM cells and the lack of ML213 (10 µM) hyperpolarizing effects in the presence of 10 µM XE991 (n=8, N=4; *P<0.05; two-tailed paired Student’s t-test). Data are represented as scatterplots with means. NS – non-significant.
Supplementary Figure 11. ML213-induced hyperpolarization is reversed by Kv7 channel inhibition with XE991. Summary data for the hyperpolarizing effects of ML213 (10 µM) on the DSM cell membrane potential (n=7, N=5, *P<0.05, effects reversible by 10 µM XE991 (n=7, N=5, #P<0.05; two-tailed paired Student’s t-test).