

**BK α subunit
slo1 protein (cbv1 isoform)**

**BK β_1 subunit
KCNMB1 protein**

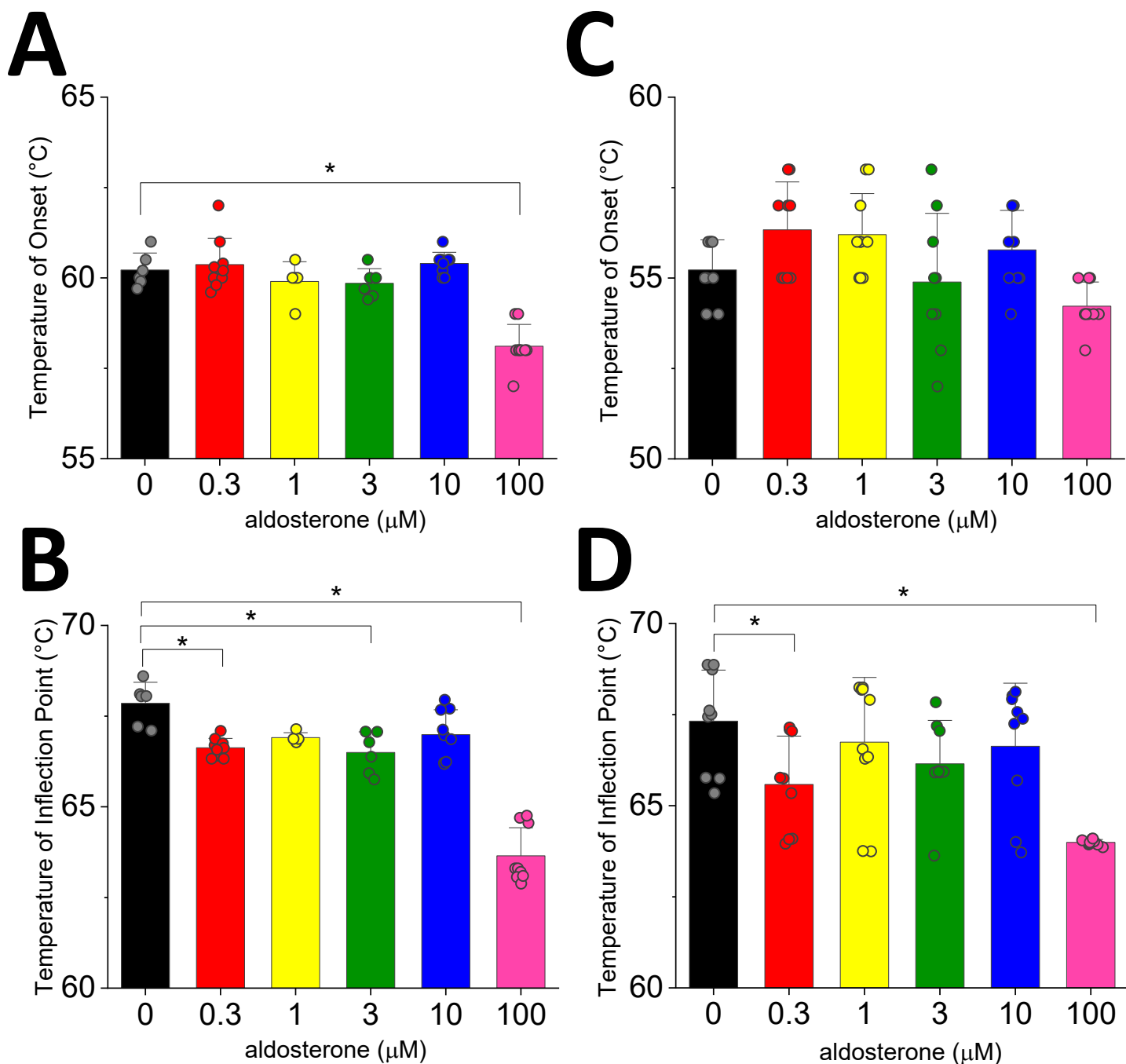


Fig. S1: Onset and Inflection point temperatures of slo1 and KCNMB1 protein thermal unfolding in presence of various aldosterone concentrations. **A.** Bar graph depicting that the onset of protein unfolding for the CBV1 isoform differed between vehicle control (0 μM aldosterone) and 100 μM aldosterone. **B.** Bar graph depicting that the temperature of inflection point for the CBV1 isoform differed from vehicle control at 0.3 μM, 3 μM, and 100 μM aldosterone. **C.** Bar graph showing lack of changes in temperatures of onset for KCNMB1 protein unfolding in presence of different aldosterone concentrations. **D.** Bar graph depicting that the temperature of inflection point for KCNMB1 protein differed from vehicle control at 0.3 μM and 100 μM aldosterone.

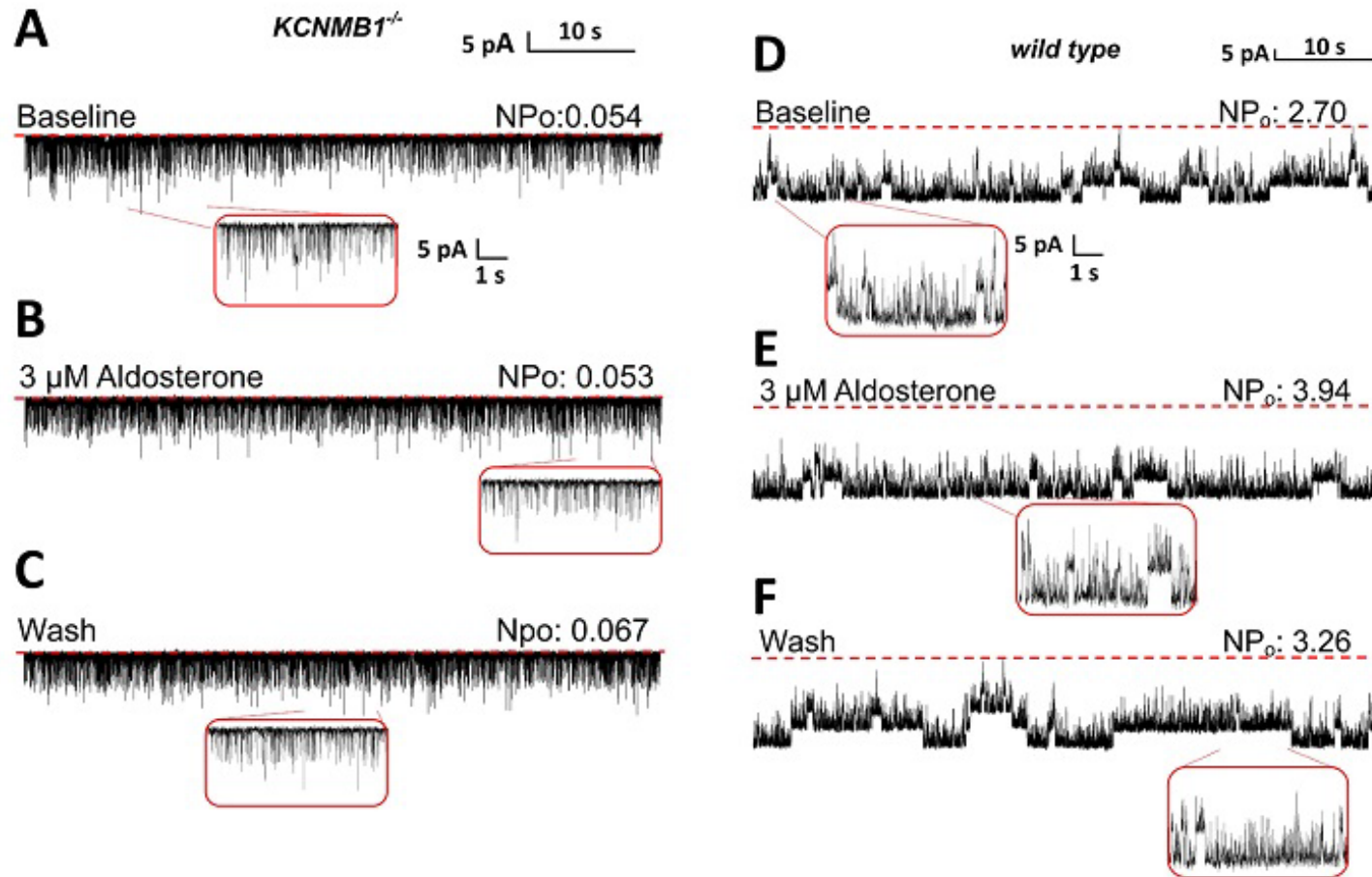


Fig. S2. Aldosterone activates smooth muscle BK channels from *wt* mouse middle cerebral artery (MCA) in a concentration-dependent manner; this action is blunted in myocyte BK channels from *KCNMB1*^{-/-} mice. On the left: channel activity traces before (A) during (B) and after (C) application of 3 μ M aldosterone to the cytosolic side of inside-out (I/O) patches from *KCNMB1*^{-/-} myocytes. Channel steady-state activity (NPo) as function of aldosterone concentration shows mild increase only at 100 μ M. On the right: channel activity traces before (D) during (E) and after (F) application of 3 μ M aldosterone to the cytosolic side of I/O patches from *wt* myocytes. In *wt* cells, steroid action is concentration-dependent: EC₅₀=3 μ M; EC_{MAX}=10 μ M. Red dotted lines indicate the baseline (all channels closed). Time-expanded traces are shown within red boxes.

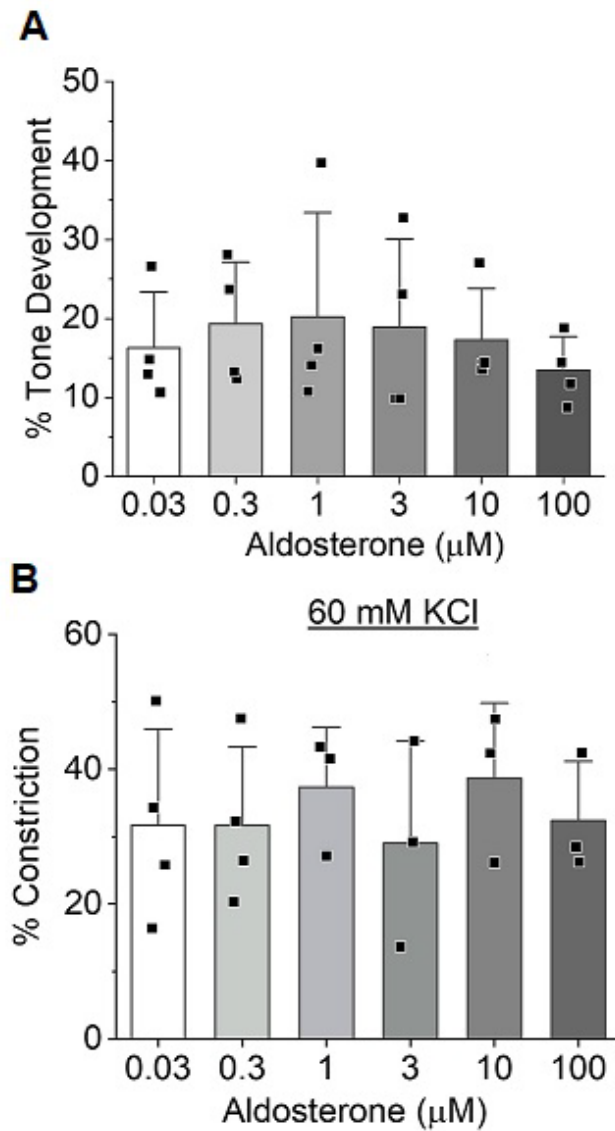


Fig. S3. A. All mouse middle cerebral artery (MCA) segments probed with different concentrations of aldosterone were able to develop myogenic tone after pressurization to 60 mmHg, matching that of previous publications (*Bukiya et al., 2008; 2009; North et al., 2018; 2020*). **B.** After exposure to aldosterone, all MCA segments constricted in response to depolarizing, 60 mM KCl, as previously documented in this preparation by *North et al. (2020)*.