Supplemental Figures

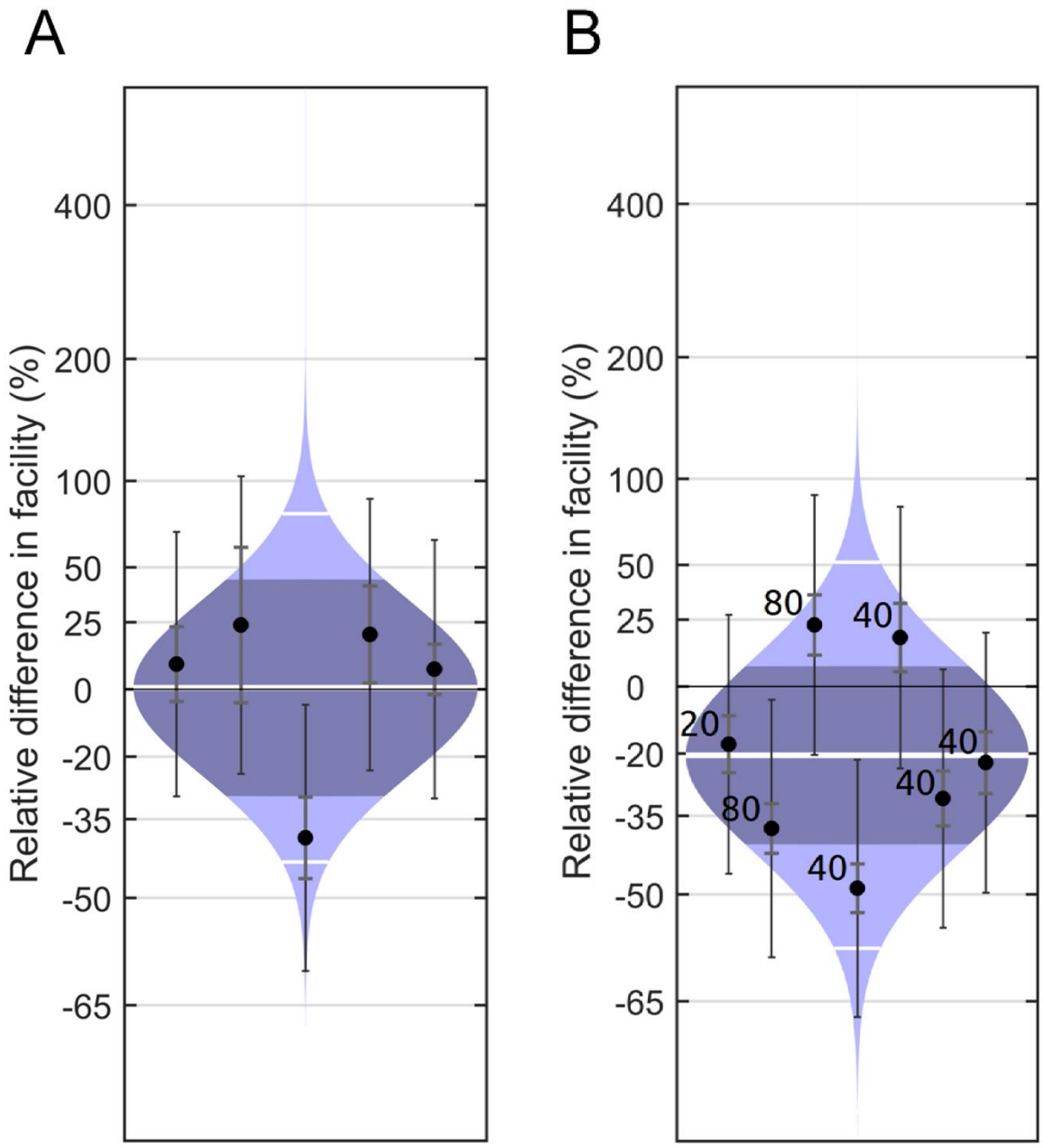

Figure S1. Perfusion with (A) cromakalim at 40 µM or (B) dephosphorylated CKLP1 at 20 – 80 µM did not significantly affect outflow facility when perfused into enucleated eyes from C57BL/6 mice. The relative facility difference between treated and vehicle-treated contralateral eyes was 0% [-30 to 43%] (mean [95% CI]; *P* = 0.9, N = 5) for cromakalim and -21% [-41 to 7%] (*P* = 0.1, n = 7) for dephosphorylated

CKLP1. Each data point represents the relative difference in C_r for an individual mouse between treated and vehicle-treated contralateral eyes. The inner error bars on each data point represent the 95% CI from the fitting to Equation 1, while the outer error bars represent the additional variability between contralateral eyes, estimated from paired untreated eyes in a previous study.[44] Colored regions represent the best estimates of the log-normal sample distributions, with the geometric mean and two-sigma levels of the distributions shown by the central and peripheral horizontal white lines, respectively. Dark central bands represent the 95% CI on the means.

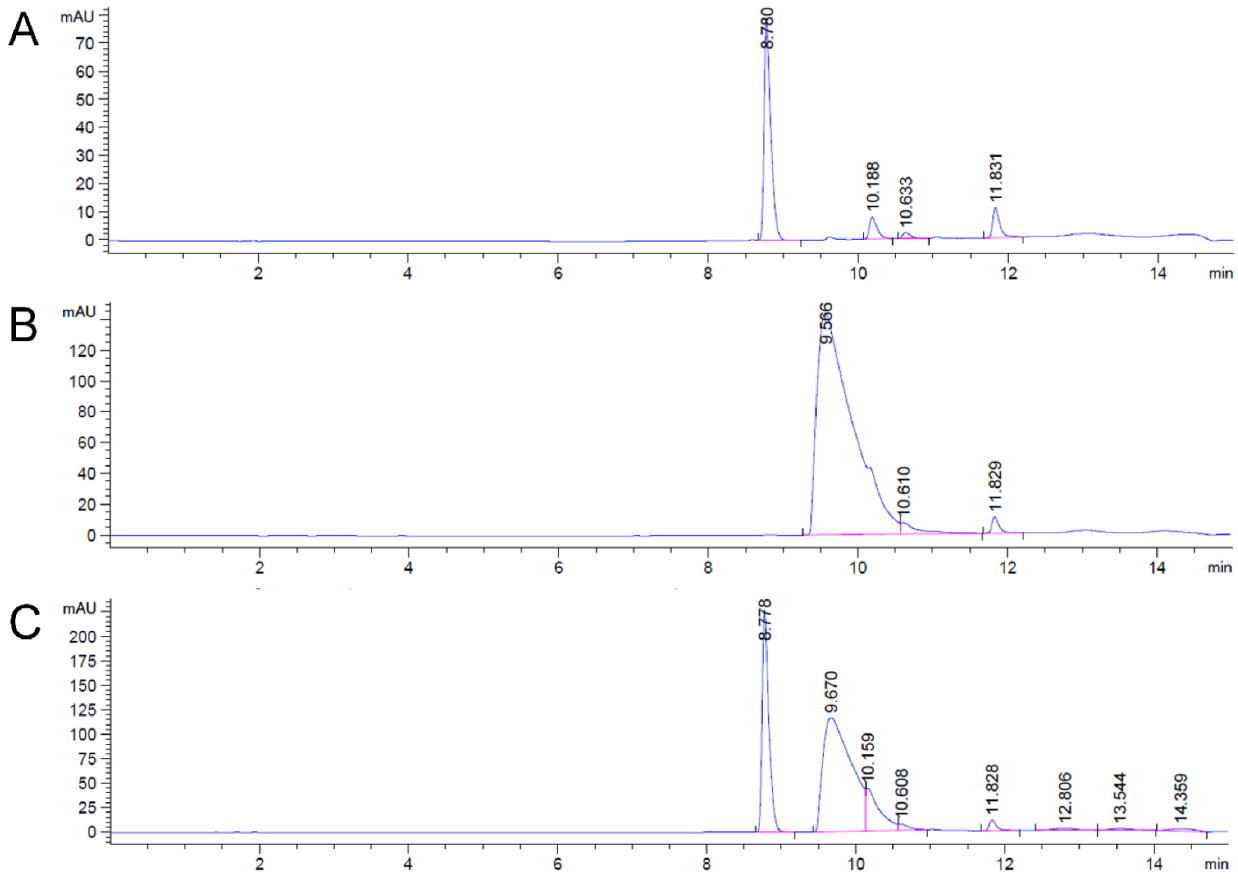

Figure S2. Ultraviolet liquid chromatography spectrum for (A) cromakalim at 20 mM, (B) CKLP1 at 20 mM and **(C)** dephosphorylated CKLP1 at 20 mM. Dephosphorylation of CKLP1 yields the parent

compound cromakalim, identified by a peak at 8.78 minutes observed in panels A and C. CKLP1 can be identified by a peak near 9.6 minutes in panel B and C.

Supplemental Methods

Cromakalim and dephosphorylated CKLP1 were used to determine their effect on outflow facility in enucleated eyes from C57BL/6 mice. Either compound was perfused directly into the conventional outflow pathway while outflow facility was measured using iPerfusion following methods described above.

40 mM of aqueous CKLP1 was dephosphorylated with 10 units of calf intestinal alkaline phosphatase (M0290, New England Biolabs, Ipswich, MA, USA) for 30 minutes in a 37°C water bath. An aliquot was analyzed using Liquid chromatography-mass spectrometry (LC-MS). A vehicle control consisting of equivalent buffer, water and enzyme was treated to the same conditions. De-phosphorylated CKLP1 and vehicle control were diluted in DBG prior to perfusion into ex vivo mouse eyes. LC-MS was performed on an Agilent 6130 Quadrupole LC-MS coupled to an Agilent 1260 Infinity LC using a 150 X 4.6 mm Phenomenex Gemini NX-C18 column with a 110 Å pore size and 5 µm particle size. Ultrapure water and acetonitrile, each containing 0.1% (v/v) formic acid (VWR) by volume, were used for the mobile phase at a flow rate of 1 ml/min. Samples were eluted with a gradient of 95% (v/v) water to 95% (v/v) acetonitrile over 10 min. The electrospray source was operated with a capillary voltage of 3.2 kV and a cone voltage of 25 V with nitrogen used as the nebulizer and desolvation gas at a total flow of 600 l/h. Detection was between 100-1000 Da in both positive and negative ionization mode.