Supplemental information for:

A novel peptide restricts ethanol modulation of the BK channel *in vitro* and *in vivo*

Luisa L. Scott, Sangeetha Iyer, Ashley E. Philpo, Melva N. Avalos, Natalie S. Wu, Ted Shi, Brooke A. Prakash, Thanh-Tu Nguyen, S. John Mihic, Richard W. Aldrich, Jonathan T. Pierce

Waggoner Center for Alcohol and Addiction Research, The University of Texas at Austin, Austin, TX, 78712 (LLS, SI, AEP, MNA, NSW, TS, BAP, T-TN, SJM, RWA, JTP)

Department of Neuroscience, The University of Texas at Austin, Austin, TX 78712 (SJM, RWA, JTP)

Center for Learning and Memory, The University of Texas at Austin, Austin, TX 78712 (RWA, JTP)

Running title: *Peptide restricts BK channel modulation by ethanol*

		wild type			*slo-1* null			*hslo(+)*		
	treatment	speed (cm/min)	N	*p*	speed (cm/min)	N	*p*	speed (cm/min)	N	*p*
LS3	vehicle	1.12 ± 0.0292	126		0.505 ± .0186	210		0.507 ± 0.0272	111	
	peptide	0.746 ± 0.0255	162	3.5E-21	0.469 ± 0.0169	205	0.16	0.340 ± 0.0208	110	1.9E-06
	veh+etoh	0.489 ± 0.0194	127		0.545 ± 0.0165	212		0.323 ± 0.0170	68	
	pep+etoh	0.538 ± 0.0210	163	0.16	0.553 ± 0.0167	212	0.70	0.446 ± 0.0264	65	0.00011
LS10	vehicle	1.29 ± 0.0331	149		0.498 ± 0.0315	77		0.674 ± 0.0403	128	
	peptide	1.09 ± 0.0365	162	6.03E-05	0.527 ± 0.0399	80	0.56	0.617 ± 0.0426	116	0.34
	veh+etoh	0.676 ± 0.0486	103		0.562 ± 0.0235	76		0.385 ± 0.0242	74	
	pep+etoh	0.905 ± 0.0510	113	0.0014	0.539 ± 0.0230	74	0.30	0.540 ± 0.0527	60	0.0052
LS11	vehicle	1.31 ± 0.0300	89		0.500 ± 0.0186	103		0.669 ± 0.0387	127	
	peptide	1.11 ± 0.0345	70	1.9E-05	0.528 ± 0.0282	94	0.40	0.418 ± 0.0302	122	7.4E-07
	veh+etoh	0.272 ± 0.0172	67		0.450 ± 0.0180	81		0.320 ± 0.0224	67	
	pep+etoh	0.295 ± 0.0210	59	0.39	0.452 ± 0.0242	77	0.96	0.215 ± 0.0182	58	0.00052
LS13	vehicle	0.828 ± 0.0509	73		0.592 ± 0.0356	93		.579 ± 0.0427	73	
	peptide	0.675 ± 0.0489	67	0.033	0.578 ± 0.0353	121	0.79	0.580 ± 0.0490	56	0.99
	veh+etoh	0.552 ± 0.0439	75		0.344 ± 0.0159	110		0.366 ± 0.0306	67	
	pep+etoh	0.317 ± 0.0269	82	7.0E-06	0.0365 ± 0.0204	115	0.44	0.372 ± 0.0275	58	0.88
LS19	vehicle	0.855 ± 0.0250	164		0.500 ± 0.0219	171		0.583 ± 0.0347	128	
	peptide	0.7419 ± 0.0208	159	0.0012	0.487 ± 0.0241	160	0.72	0.482 ± 0.0325	133	0.034
	veh+etoh	0.392 ± 0.0203	167		0.365 ± 0.0189	159		0.458 ± 0.0327	76	
	pep+etoh	0.291 ± 0.0173	140	0.00024	0.395 ± 0.0209	168	0.22	0.451 ± 0.0353	67	0.89

Table S1. A comparison of crawl speeds between worms treated with vehicle or select peptides

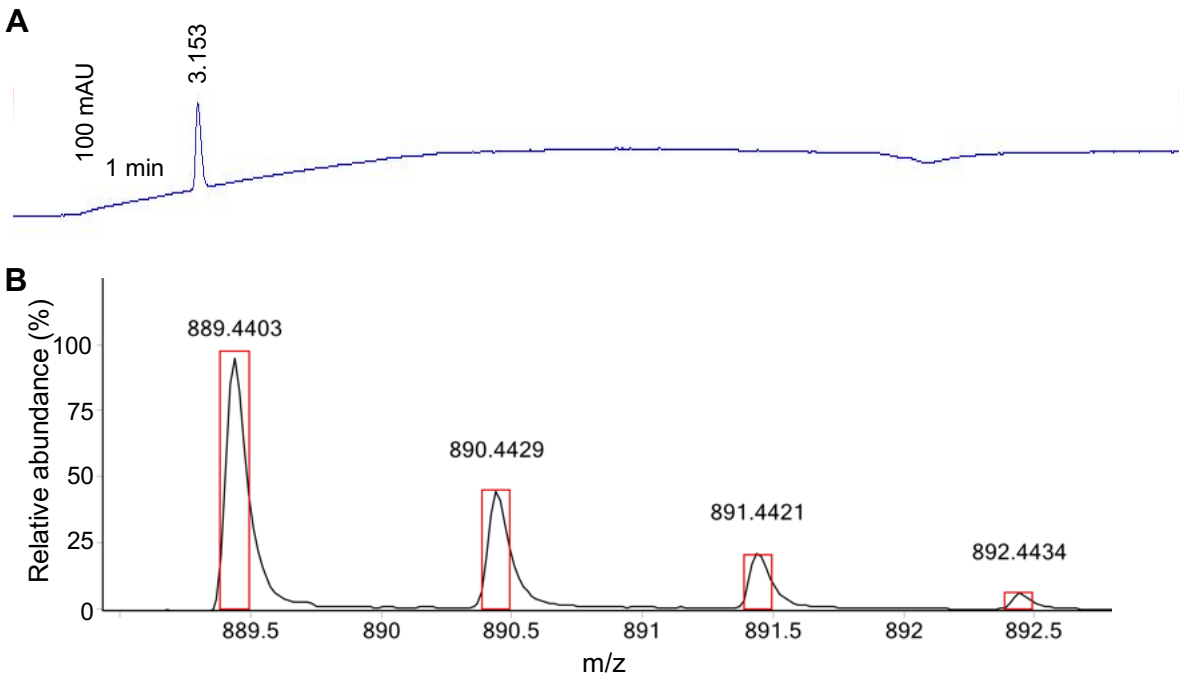

Figure S1. **LC/MS confirmed purity and identity of LS10.** ***A***, HPLC traces showed a single peak absorbance at 3.153 min. 1-17 min of 214 nM trace shown. ***B***, The mass spectrum indicates a molecular weight of 889 g/mol, as predicted for the peptide.

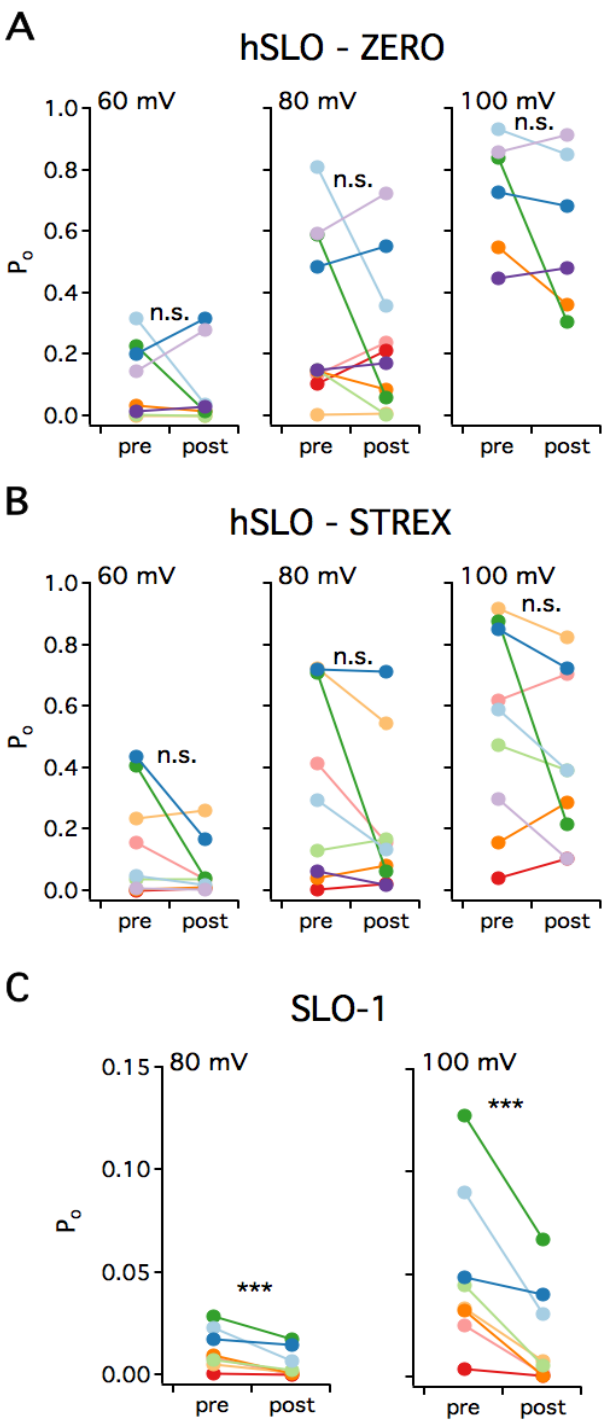

Figure S2. **Peptide LS10 consistently reduces the probability of opening for the worm but not the human BK channel**. Human (hSLO) or worm (SLO-1) BKα channels were recorded in inside-out patches. 500-nM LS10 was applied by diffusion to the extracellular face. The P_o at the start of the recording (pre) was measured using at least five 3-second traces recorded over a duration of ~5 minutes. The P_o was again assessed 15-30 minutes later (post). Each patch represented by a unique color for datasets plotted in ***A***-***C***. ***A***, The ZERO isoform of hSLO did not show a consistent change in P_o in response to LS10 application. ***B***, The STREX isoform of hSLO also did not show a consistent change in P_o. hSLO expressed in HEK293 cells. Intracellular solution: ~750 nM Ca^{2+}, 0 Mg^{2+}. Pre vs. post, paired Student's *t*-tests, n.s. ***C***, SLO-1 showed a significant decrease in P_o in response to LS10 application. SLO-1 expressed in oocytes. Intracellular solution: ~5 μM Ca^{2+}, 0 Mg^{2+}. Pre vs. post, paired Student's *t*-tests, ***$p<0.005$.

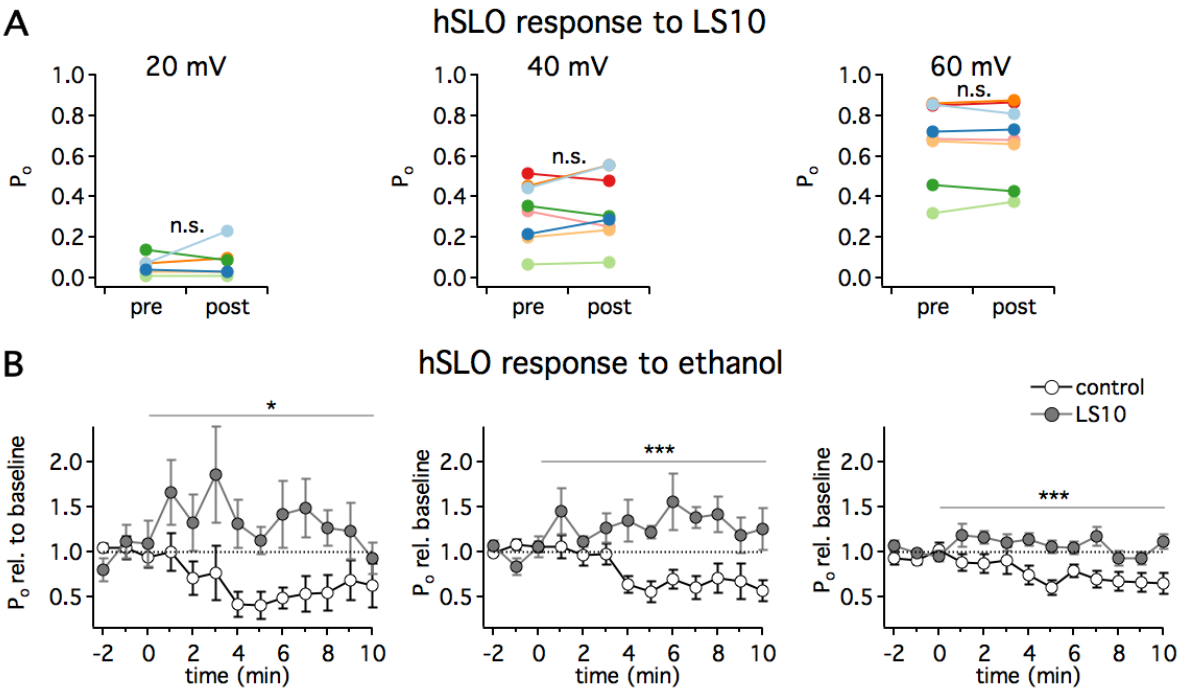

Figure S3. **Peptide LS10 restricts ethanol modulation without altering basal probability of opening for human BK channels**. Human (hSLO-ZERO) BKα channels were recorded in inside-out patches. 500-nM LS10 was applied by diffusion to the extracellular face. The P_o at the start of the recording (pre in ***A***) was measured using at least ten 3-second traces recorded over a duration of several minutes. The P_o was again assessed 15 minutes later (post in ***A***, baseline in ***B***). ***A***, hSLO showed no change in P_o in response to LS10 application. Data for each patch represented by a unique color. Pre vs. post, paired Student's *t*-tests, n.s. ***B***, After bath application of 50-mM ethanol (time = 0), control (open) recordings showed a decrease in P_o. Preincubation with LS10 (shaded) restricted the ethanol-induced decrease in P_o. P_o measured each minute using three 3-second traces and plotted relative to baseline as mean ± SEM. Two-way repeated measures ANOVA showed a main effect of peptide treatment on the response to ethanol: 20 mV, $F(1, 11)=5.70$, $^*p<0.05$; 40 mV, $F(1, 15)=16.63$, $^{***}p<0.001$; 60 mV, $F(1, 16)=21.26$, $^{***}p<0.001$. hSLO expressed in HEK293 cells. Intracellular solution: ~638 nM Ca^{2+}, 2 mM Mg^{2+}.