SUPPLEMENTARY TABLES

Table S1. Hill Coefficients for Symmetrical KCl Spermine Inhibition Dose – Response Curves.

Hill Coefficient Values				
V_j (mV)	**100% sym [KCl]**	**75% sym [KCl]**	**50% sym [KCl]**	**25% sym [KCl]**
9.0 ± 0.1	0.96 ± 0.74	0.40 ± 0.03	0.53 ± 0.05	0.54 ± 0.04
13.8 ± 0.1	0.73 ± 0.28	0.42 ± 0.04	0.49 ± 0.03	0.48 ± 0.04
18.6 ± 0.1	0.57 ± 0.15	0.45 ± 0.02	0.49 ± 0.03	0.52 ± 0.04
23.5 ± 0.1	0.67 ± 0.10	0.45 ± 0.03	0.52 ± 0.05	0.55 ± 0.04
28.4 ± 0.2	0.59 ± 0.09	0.59 ± 0.05	0.64 ± 0.06	0.61 ± 0.04
33.4 ± 0.2	0.56 ± 0.15	0.68 ± 0.06	0.64 ± 0.05	0.72 ± 0.04
38.4 ± 0.2	0.61 ± 0.11	0.71 ± 0.06	0.65 ± 0.06	0.73 ± 0.05
43.5 ± 0.2	0.59 ± 0.10	0.81 ± 0.06	0.69 ± 0.05	0.78 ± 0.05
48.4 ± 0.2	0.45 ± 0.14	0.78 ± 0.06	0.68 ± 0.07	0.72 ± 0.06
Mean ± S.D.*	0.58 ± 0.03	0.64 ± 0.14	0.62 ± 0.08	0.66± 0.10

* Average of Hill Coefficients for given KCl condition excluding the three lowest V_j values.

Table S2. Hill Coefficients for Cx40 Gap Junction-Spermine Inhibition Dose –Response Curves.

Hill Coefficients for Effective K_d Values							
V_j (mV)	**25% *trans* [KCl]**	**50% *trans* [KCl]**	**75% *trans* [KCl]**	**100%-100% [KCl]**	**75% *cis* [KCl]**	**50% *cis* [KCl]**	**25% *cis* [KCl]**
4.3 ± 1.1	---	---	---	---	---	0.7 (fixed)	2.35 ± 0.75
8.9 ± 1.0	1.29 ± 0.48	1.19 ± 1.04	0.6 (fixed)	0.96 ± 0.74	1.17 ± 0.49	0.7 (fixed)	1.78 ± 0.16
13.6 ± 0.9	0.91 ± 0.08	0.90 ± 0.29	0.6 (fixed)	0.73 ± 0.28	0.76 ± 0.12	0.7 (fixed)	1.24 ± 0.13
18.3 ± 0.8	0.68 ± 0.10	0.72 ± 0.14	0.49 ± 0.14	0.57 ± 0.15	0.59 ± 0.08	0.7 (fixed)	0.96 ± 0.20
23.1 ± 0.7	0.54 ± 0.12	0.69 ± 0.10	0.50 ± 0.10	0.67 ± 0.10	0.68 ± 0.07	0.52 ± 0.11	1.03 ± 0.19
28.1 ± 0.7	0.47 ± 0.12	0.58 ± 0.11	0.52 ± 0.08	0.59 ± 0.09	0.68 ± 0.14	0.64 ± 0.18	1.03 ± 0.21
33.1 ± 0.6	0.55 ± 0.10	0.41 ± 0.14	0.65 ± 0.08	0.56 ± 0.15	0.57 ± 0.12	0.76 ± 0.18	1.10 ± 0.23
38.1 ± 0.5	0.71± 0.09	0.43 ± 0.07	0.65 ± 0.09	0.61 ± 0.11	0.60 ± 0.13	0.64 ± 0.15	1.09 ± 0.34
43.2 ± 0.5	0.92± 0.19	0.35 ± 0.07	0.71 ± 0.13	0.59 ± 0.10	0.50 ± 0.10	0.81 ± 0.17	1.25 ± 0.38
48.2 ± 0.5	0.60± 0.10	0.33 ± 0.03	0.61 ± 0.04	0.45 ± 0.14	0.38 ± 0.10	0.85 ± 0.16	1.19 ± 0.58
Mean* ± S.D.	0.64 ± 0.03	0.50 ± 0.04	0.58 ± 0.03	0.58 ± 0.03	0.57 ± 0.03	0.70 ± 0.03	1.09 ± 0.14

*Average of Hill Coefficients for given KCl condition excluding the three lowest and all fixed V_j values.

Table S3. Symmetrical [KCl] On-rate and Off-rate Parameters for Cx40 Spermine Block.

	On-Rates[*]				**Off-Rates**[†]			
% KCl	**Amplitude (ms^{-1} mM^{-1})**	**$V_{k,\ on}$ (mV)**	**Constant (ms^{-1} mM^{-1})**	**r**	**Amplitude (ms^{-1})**	**$V_{k,\ off}$ (mV)**	**Constant (ms^{-1})**	**r**
25%	0.00033 ± 0.000149	11.53 ± 1.63	0.000316 ± 0.000317	0.78	0.1077 ± 0. 0022	-17.17 ± 0.73	0.000841 ± 0.000993	0.83
50%	0.000199 ± 0.000078	12.36 ± 1.42	0.000113 ± 0.000198	0.82	0.1599 ± 0. 0031	-15.96 ± 0.64	0.001331 ± 0.001346	0.84
75%	0.00032 ± 0.000202	13.27 ± 2.96	0.0000252 ± 0.000359	0.71	0.1837 ± 0.0036	-14.38 ± 0.58	0.001905 ± 0.001516	0.84
100%	0.000169 ± 0.000081	13.51 ± 2.08	-0.000099 ± 0.000175	0.77	0.1928 ± 0.0042	-13.55 ± 0.59	0.000104 ± 0.001686	0.81

[*] V_{init} value was the minimum V_j value from Table 2.
[†] V_{init} value was the maximum V_j value from Table 2.

Table S4. On-rate and Off-rate Parameters for Spermine Block Cx40 Gap Junctions.

	On-Rates*				**Off-Rates†**			
% KCl (*cis or trans*)	**Amplitude (ms^{-1} mM^{-1})**	**$V_{k,\ on}$ (mV)**	**Constant (ms^{-1} mM^{-1})**	**r**	**Amplitude (ms^{-1})**	**$V_{k,\ off}$ (mV)**	**Constant (ms^{-1})**	**r**
25% *trans*	0.000126 ± 0.000138	9.87 ±2.55	0.000203 ± 0.000626	0.59	0.0901 ± 0.0027	-17.62 ± 1.16	0.000053 ± 0.00133	0.77
50% *trans*	0.000129 ± 0.000141	10.13 ± 2.72	0.000232 ± 0.000562	0.61	0.1431 ± 0.0034	-17.01 ± 0.89	0.000054 ± 0.00158	0.82
75% *trans*	0.000211 ± 0.000184	12.22 ± 3.07	-0.000024 ± 0.000487	0.63	0.1559 ± 0.0021	-15.55 ± 1.43	0.000123 ±0.000924	0.89
100%-100%	0.000169 ± 0.000081	13.51 ± 2.08	-0.000099 ± 0.000175	0.77	0.1928 ± 0.0042	-13.55 ± 0.59	0.000104 ± 0.001686	0.81
75% *cis*	0.000115 ± 0.000101	16.60 ± 5.33	-0.000106 ± 0.000185	0.70	0.1923 ± 0.0045	-11.22 ± 0.53	0.00455 ± 0.00175	0.82
50% *cis*	0.000087 ± 0.000074	20.46 ± 8.20	-0.000084 ± 0.000097	0.74	0.2250 ± 0.0041	-10.07 ± 0.37	0.00426 ± 0.00154	0.88
25% *cis*	0.000051 ± 0.000062	21.09 ± 14.89	-0.000034 ± 0.000070	0.78	0.2404 ± 0.0080	-9.43 ± 0.67	0.00721 ± 0.00344	0.82

* V_{init} value was the minimum V_j value from Table 3.
† V_{init} value was the maximum V_j value from Table 3.

Table S5. Effective Electrical Distance Values for Spermine Inhibition.*

% KCl (*cis or trans*)	b_{-1}/b_1	S.E.	$z\delta$	S.E.	$\delta^{\dagger}$	C	S.E.	r
100%-100%	115.5	4.8	4.23	0.18	1.06	0.09	0.06	0.97
75% symmetrical	91.1	14.5	4.38	0.22	1.09	0.14	0.05	0.97
50% symmetrical	53.1	1.42	4.49	0.04	1.12	0.13	0.004	0.99
25% symmetrical	59.9	5.4	4.83	0.12	1.21	0.12	0.01	0.99
25% *trans*	73.1	9.8	5.61	0.20	1.40	0.06	0.01	0.98
50% *trans*	86.3	6.5	5.30	0.11	1.33	-0.01	0.01	0.99
75% *trans*	72.9	3.8	4.56	0.07	1.14	0.12	0.01	0.99
75% *cis*	145.8	10.3	4.00	0.10	1.00	-0.03	0.06	0.98
50% *cis*	74.6	11.8	3.07	0.24	0.77	-0.02	0.22	0.96
25% *cis*	42.8	4.4	2.42	0.15	0.61	0.49	0.16	0.98

*Woodhull model curve fits were performed on K_d^{eff} values from V_j = 18 to 43 mV in Tables 2 and 3.

†Spermine has a valence (z) of +4.0 at pH 7.4.

Figure S6. Unitary Cx40 gap junction channel current – voltage relationships. The filled squares (■) are the measured unitary current amplitudes (i_j) obtained during the 30 sec V_j pulses to the indicated command potentials. All data were acquired using the standard polyamine block protocol under the various *cis-trans* [KCl] conditions. (**A**) The $i_j - V_j$ relationship for the Cx40 gap junction channel under control [KCl] conditions. The slope of the linear regression fit of the data obtained from the experiment shown in Figure 4A was 148 pS. (**B**) The Cx40 gap junction channel $i_j - V_j$ relationship after a 25% reduction in the *trans* [KCl] as shown in Figure 4B. Estimation of the apparent γ_j from the i_j fluctuations did not account for the -6.2 mV shift in the equilibrium electrodiffusion potential (E_{rev}). The slope γ_j was 128 pS under these conditions. These results are consistent with previous findings (19). (**C**) The Cx40 gap junction channel $i_j - V_j$ relationship after a 75% reduction in the *trans* [KCl] as shown in Figure 4F. The gap junctional E_{rev} is now -26.5 mV, resulting in significantly under- and over-estimated apparent γ_j values for the observed *trans* and *cis* i_j fluctuations. The linear slope γ_j was reduced to 108 pS under these conditions. Under symmetrical salt conditions, the Cx40 linear slope γ_j was reduced to 120 pS in 75% [KCl] and 70 pS in 25% [KCl] internal pipette solutions (data not shown).

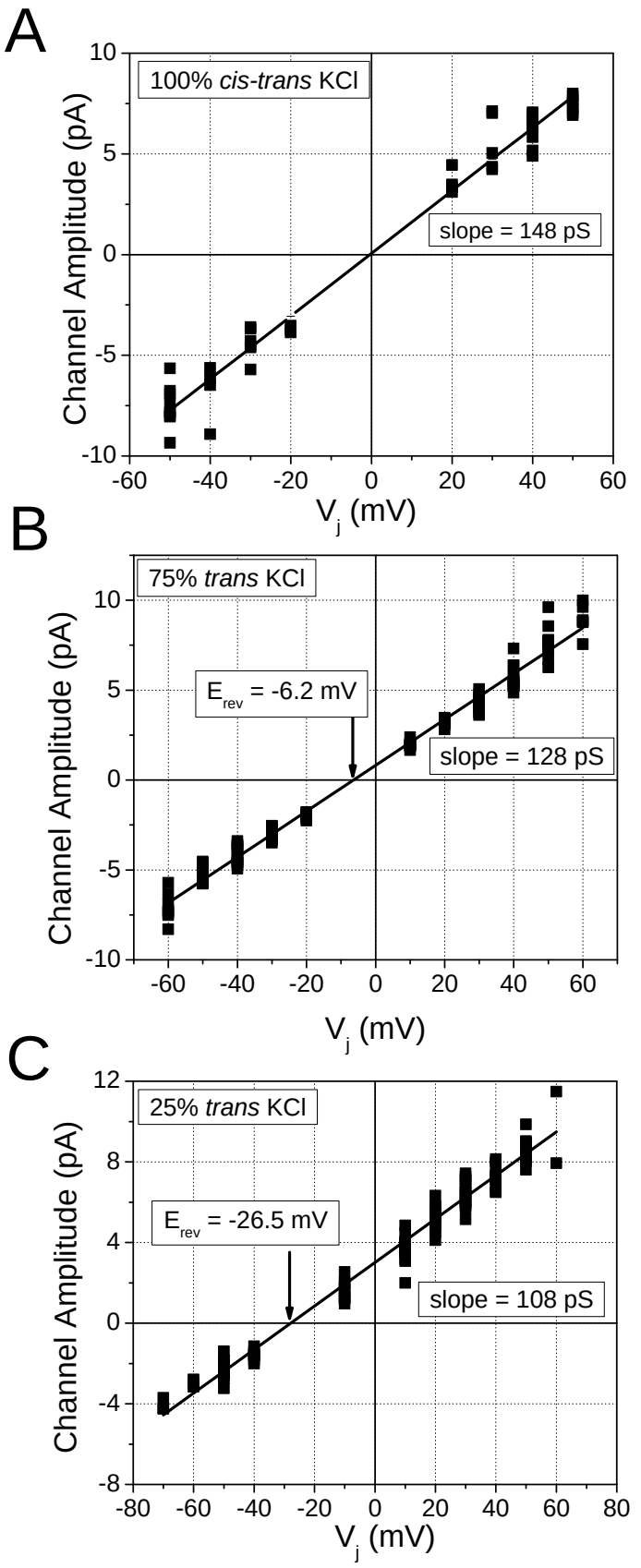

Figure S6
Lin and Veenstra
KCl Alteration of Cx40 Spermine Block