

Supporting Information

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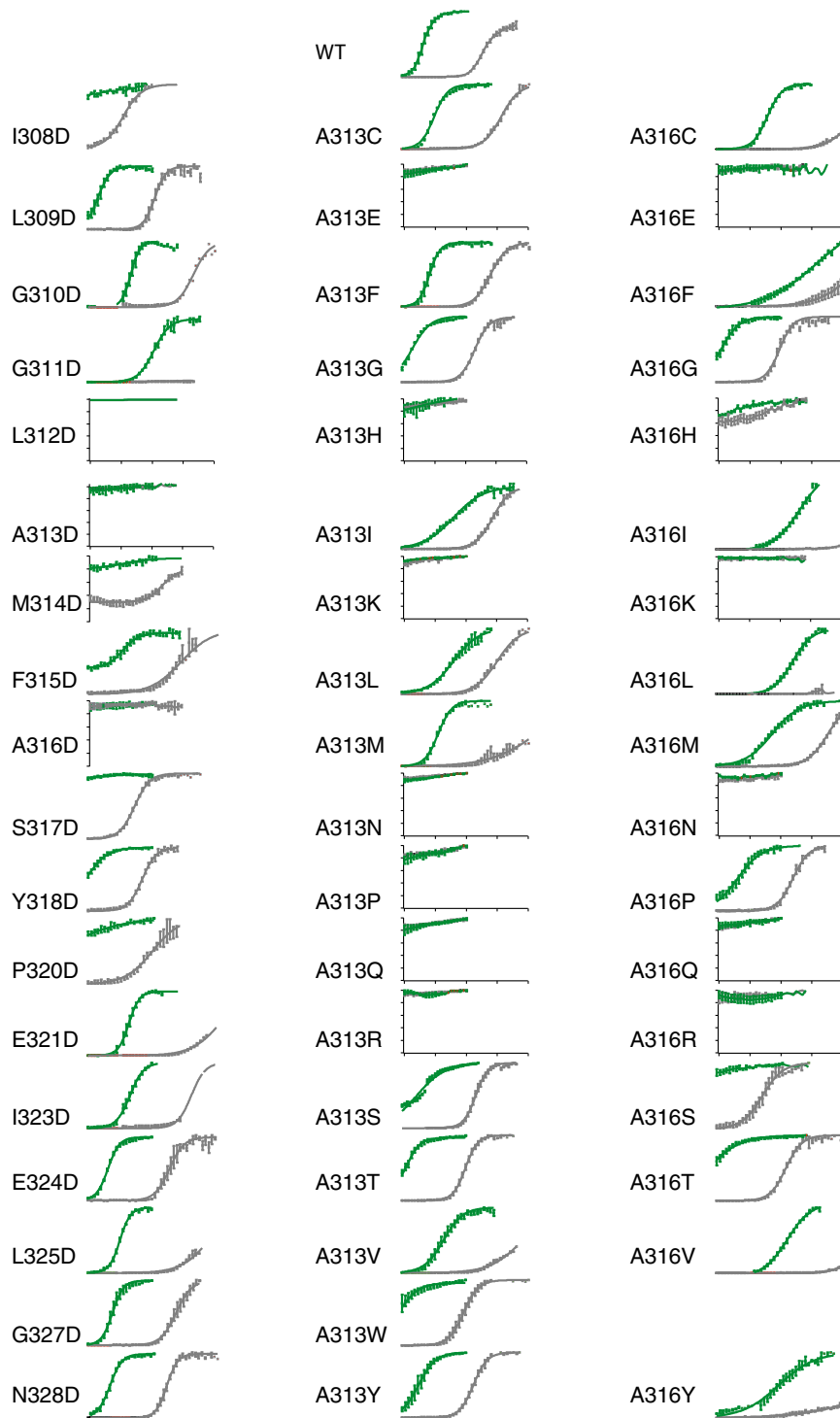


Fig. S1. Steady-state G - V curves of wild-type and mutant BK channels in 0 (gray) and 85 μM Ca^{2+} (green). For each panel, G/G_{max} (ranging from 0 to 1) was plotted against voltage (ranging from -100 to $+300$ mV). Error bars represent SEM.

Table S1. Characterization of steady-state gating for single aspartate substitutions along BK S6

Channel	0 Ca ²⁺			85 μM Ca ²⁺			n
	V _{1/2} , mV	z	G/G _{max} *	V _{1/2} , mV	z	G/G _{max} *	
WT	163 ± 0.8	1.0 ± 0.03	0.5	-34 ± 0.5	1.4 ± 0.04	0.5	3
I308D	11 ± 0.5	0.8 ± 0.03	0.99	NA	NA	0.91	4
L309D	232 ± 1.5	1.0 ± 0.05	0.06	35 ± 0.6	1.7 ± 0.06	0.01	4
G310D	108 ± 1.5	1.4 ± 0.09	0.96	-69 ± 1.1	1.3 ± 0.07	0.86	3
G311D	NA	NA	0	107 ± 1.2	1.0 ± 0.04	0.004	3
L312D	NA	NA	1	NA	NA	1	3
A313D	NA	NA	1	NA	NA	1	3
M314D	130 ± 5.2	1.4 ± 0.11	0.89	NA	NA	1	6
F315D	186 ± 3.0	0.5 ± 0.03	0.38	7 ± 2.3	1.0 ± 0.08	0.49	5
A316D	NA	NA	1	NA	NA	1	6
S317D	45 ± 0.8	1.0 ± 0.03	0.99	NA	NA	0.96	5
Y318D	70 ± 0.6	1.1 ± 0.03	0.98	-107 ± 0.8	0.7 ± 0.02	0.9	6
V319D	NC	NC	NC	NC	NC	NC	NC
P320D	97 ± 2.1	0.6 ± 0.03	0.83	NA	NA	0.86	4
E321D	311 ± 2.0	0.6 ± 0.02	0.03	27 ± 1.9	1.5 ± 0.14	0.02	5
I322D	NC	NC	NC	NC	NC	NC	NC
I323D	225 ± 0.8	1.1 ± 0.04	0.06	32 ± 0.7	1.1 ± 0.03	0.04	4
E324D	156 ± 1.5	1.1 ± 0.06	0.57	-37 ± 0.8	1.4 ± 0.05	0.54	5
L325D	281 ± 3.5	0.6 ± 0.03	0.06	1 ± 0.5	1.5 ± 0.04	0.11	7
I326D	NC	NC	NC	NC	NC	NC	NC
G327D	176 ± 0.7	1.0 ± 0.03	0.37	-23 ± 0.9	1.4 ± 0.06	0.35	3
N328D	140 ± 0.7	1.5 ± 0.05	0.80	-39 ± 0.7	1.3 ± 0.04	0.56	5

NA, not applicable; NC, no current.

*Measured at V_{1/2} of wild type.

Table S2. Characterization of steady-state gating for L312X BK channels

Channel	0 Ca ²⁺			85 μM Ca ²⁺			n
	V _{1/2} , mV	z	G/G _{max} *	V _{1/2} , mV	z	G/G _{max} *	
WT	163 ± 0.8	1.0 ± 0.03	0.5	-34 ± 0.5	1.4 ± 0.04	0.5	3
L312A	31 ± 2.6	1.2 ± 0.05	1	NA	NA	0.93	15, 3
L312C	NA	NA	1	NA	NA	0.89	4
L312D	NA	NA	1	NA	NA	0.99	3
L312E	NC	NC	NC	NC	NC	NC	NC
L312F	186 ± 6.0	1.01 ± 0.12	0.29	1 ± 8.2	1.49 ± 0.01	0.12	3
L312G	NA	NA	1	NA	NA	0.93	5
L312H	NA	NA	NA	NA	NA	NA	NA
L312I	86 ± 3.4	0.93 ± 0.02	0.94	NA	NA	0.5	10, 3
L312K	NA	NA	NA	NA	NA	NA	NA
L312M	170 ± 13.1	1.06 ± 0.11	0.43	-30 ± 5.0	1.39 ± 0.10	0.45	3
L312N	NA	NA	1	NA	NA	1	3
L312P	NC	NC	NC	NC	NC	NC	NC
L312Q	NA	NA	1	NA	NA	1	5
L312R	NC	NC	NC	NC	NC	NC	NC
L312S	NA	NA	0.99	NA	NA	0.92	3
L312T	NA	NA	0.98	NA	NA	0.85	3
L312V	NA	NA	0.98	NA	NA	0.81	4
L312W	NC	NC	NC	NC	NC	NC	NC
L312Y	NC	NC	NC	NC	NC	NC	NC

NA, not applicable; NC, no current.

*Measured at V_{1/2} of wild type.

Table S3. Characterization of steady-state gating for A313X BK channels

Channel	0 Ca ²⁺			85 μ M Ca ²⁺			n
	V _{1/2} , mV	z	G/G _{max} *	V _{1/2} , mV	z	G/G _{max} *	
WT	163 ± 0.8	1.0 ± 0.03	0.5	-34 ± 0.5	1.4 ± 0.04	0.5	3
A313C	212 ± 0.7	0.9 ± 0.02	0.15	1 ± 1.1	1.2 ± 0.05	0.16	3
A313D	NA	NA	1	NA	NA	0.92	3
A313E	NA	NA	1	NA	NA	0.89	4
A313F	178 ± 0.8	0.9 ± 0.02	0.36	-14 ± 0.6	1.5 ± 0.05	0.23	3
A313G	129 ± 1.0	1.1 ± 0.04	0.82	-68 ± 2.4	0.8 ± 0.04	0.76	5
A313H	NA	NA	1	NA	NA	0.87	4
A313I	185 ± 0.7	0.9 ± 0.02	0.31	62 ± 1.5	0.5 ± 0.14	0.12	4
A313K	NA	NA	1	NA	NA	0.97	4
A313L	200 ± 1.3	0.7 ± 0.02	0.26	62 ± 1.2	0.6 ± 0.02	0.08	3
A313M	326 ± 5.7	0.3 ± 0.02	0.1	15 ± 1.4	1.2 ± 0.06	0.09	3
A313N	NA	NA	1	NA	NA	1	4
A313P	NA	NA	1	NA	NA	1	3
A313Q	NA	NA	1	NA	NA	1	3
A313R	NA	NA	1	NA	NA	1	3
A313S	91 ± 0.8	1.0 ± 0.03	0.95	-141 ± 2.6	0.4 ± 0.02	0.87	5
A313T	99 ± 0.4	1.3 ± 0.02	0.96	-86 ± 1.0	1.1 ± 0.05	0.91	6
A313V	269 ± 2.5	0.5 ± 0.02	0.1	27 ± 1.3	0.8 ± 0.03	0.11	9
A313W	94 ± 0.4	1.0 ± 0.02	0.95	-105 ± 3.0	0.8 ± 0.06	0.92	4
A313Y	124 ± 0.4	1.1 ± 0.02	0.85	-48 ± 0.7	0.9 ± 0.02	0.63	7

NA, not applicable; NC, no current.

*Measured at V_{1/2} of wild type.

Table S4. Characterization of steady-state gating for A316X BK channels

Channel	0 Ca ²⁺			85 μ M Ca ²⁺			n
	V _{1/2} , mV	z	G/G _{max} *	V _{1/2} , mV	z	G/G _{max} *	
WT	163 ± 0.8	1.0 ± 0.03	0.5	-34 ± 0.5	1.4 ± 0.04	0.5	3
A316C	343 ± 1.8	0.6 ± 0.01	0.01	64 ± 0.7	1.1 ± 0.03	0.01	5
A316D	NA	NA	0.99	NA	NA	0.99	6
A316E	NA	NA	0.99	NA	NA	0.99	3
A316F	327 ± 1.6	0.4 ± 0.01	0.03	211 ± 6.5	0.3 ± 0.01	0.02	4
A316G	97 ± 2.6	1.1 ± 0.11	0.95	-86 ± 1.1	0.9 ± 0.03	0.86	3
A316H	NA	NA	0.99	NA	NA	0.76	3
A316I	353 ± 39.2	0.7 ± 0.06	0.003	150 ± 6.4	0.6 ± 0.06	0.01	3
A316K	NA	NA	0.98	NA	NA	0.98	3
A316L	NA	NA	0	140 ± 1.1	0.8 ± 0.02	0.003	3
A316M	244 ± 0.6	0.8 ± 0.02	0.07	72 ± 2.0	0.6 ± 0.02	0.07	4
A316N	NA	NA	1	NA	NA	0.92	3
A316P	140 ± 0.7	1.0 ± 0.02	0.72	-34 ± 1.7	0.7 ± 0.03	0.50	6
A316Q	NA	NA	1	NA	NA	0.90	4
A316R	NA	NA	0.99	NA	NA	0.87	3
A316S	40 ± 1.6	0.8 ± 0.03	0.98	NA	NA	0.93	4
A316T	117 ± 1.0	1.0 ± 0.03	0.86	-126 ± 3.2	0.5 ± 0.02	0.86	5
A316V	358 ± 2.4	0.7 ± 0.02	0.003	121 ± 1.3	0.7 ± 0.03	0.01	3
A316W	NC	NC	NC	NC	NC	NC	NC
A316Y	439 ± 10.1	0.2 ± 0.01	0.07	96 ± 2.3	0.4 ± 0.02	0.09	3

NA, not applicable; NC, no current.

*Measured at V_{1/2} of wild type.