

Supplementary Material to “Glycosylation of $\beta 1$ subunit plays a pivotal role in the toxin sensitivity and activation of BK channels”

A

(N80A)
 Primer S1: 5' TGTGGGTCGCCGTGTCAGC 3'
 Primer A1: 5' GGCATGGGTACTGGGGCAC 3'
 (N142A)
 Primer S2: 5' GCCGAAACCAGCGTCTATTCC 3'
 Primer A2: 5' ACCCCGAGGTGCGGAGAAG 3'
 (N80Q)
 Primer S3: 5' TGTGGGTCCAGGTGTCAGC 3'
 Primer A3: 5' GGCATGGGTACTGGGGCAC 3'
 (N142Q)
 Primer S4: 5' GCAGGAAACCAGCGTCCTATTC 3'
 Primer A4: 5' CCTCTAGGTGCGGAGAAGCAGTAG 3'

B

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1450 1460 1470 1480 1490 1500 1510 1520 1530
Beta1_subunit.seq AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC
Beta1_N80A_N142A.seq AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC
Consensus AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC

1630 1640 1650 1660 1670 1680 1690 1700 1710
Beta1_subunit.seq AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC
Beta1_N80A_N142A.seq AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC
Consensus AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC

1450 1460 1470 1480 1490 1500 1510 1520 1530
Beta1_subunit.seq AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC
Beta1_N80Q_N142Q.seq AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC
Consensus AACTGAAAGGCAAGAAGTCCCCAGTACCCATGCTGTGGGTGAGTGTGAGTCCCGGAGGTGGGCTGTGCTGTACACAC

1630 1640 1650 1660 1670 1680 1690 1700 1710
Beta1_subunit.seq AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC
Beta1_N80Q_N142Q.seq AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC
Consensus AAATTCGAAGAGCAGCAGGTCCTTCTACTGCTTCTCCGACCTGGGGTGCAGAACAGGCTCTATTCCAGGCCCTACGGGGC
    
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C

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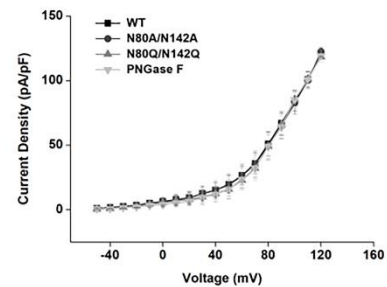
61 70 80 90 100
Beta1 subunit RDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS
Beta1 N80A/N142A RDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS
Consensus RDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS

110 120 130 140 150
Beta1 subunit VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL
Beta1 N80A/N142A VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL
Consensus VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL

60 70 80 90 100
Beta1 subunit IRDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS
Beta1 N80Q/N142Q IRDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS
Consensus IRDQEEELKGGKVPQYPCLAVNVAAGRMAVLYHTEDTRDQNCQCSYIPGS

110 120 130 140 150
Beta1 subunit VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL
Beta1 N80Q/N142Q VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL
Consensus VDNVYQATARADVEKVRKRFQEQVYCFPSAPRGNETSVLFRQLYGPQALL
    
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D



Additional file 1. The construction of N80A/N142A and N80Q/N142Q mutants of $\beta 1$ subunit. **(A)** The complementary primer sequences were designed to construct N80A/N142A mutant and N80Q/N142Q mutant of $\beta 1$ subunit. Primer 1 (S1, A1) and primer 2 (S2, A2) were used to construct N80A/N142A mutant, and primer 3 (S3, A3) and primer 4 (S4, A4) were used to construct N80Q/N142Q mutant. **(B)** Nucleic acid sequence alignment between wild-type, N80A/N142A and N80Q/N142Q mutants of $\beta 1$ subunit. **(C)** Amino acid sequence alignment between wild-type, N80A/N142A and N80Q/N142Q mutants of $\beta 1$ subunit. **(D)** Statistical analysis of current density of wild-type BK channels ($\alpha + \beta 1$) ($n = 8$) and N80A/N142A ($n = 8$, ns, $p > 0.05$) or wild-type BK channels ($\alpha + \beta 1$) ($n = 8$) and N80Q/N142Q ($n = 8$, ns, $p > 0.05$) or wild-type BK channels ($\alpha + \beta 1$) ($n = 8$) and BK ($\alpha + \beta 1$) channels pretreated with PNGase F ($n = 8$, ns, $p > 0.05$).