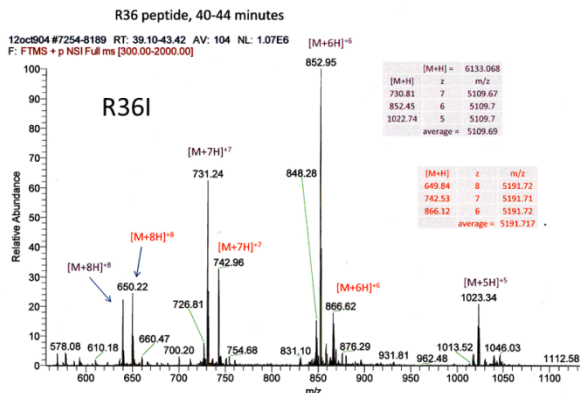
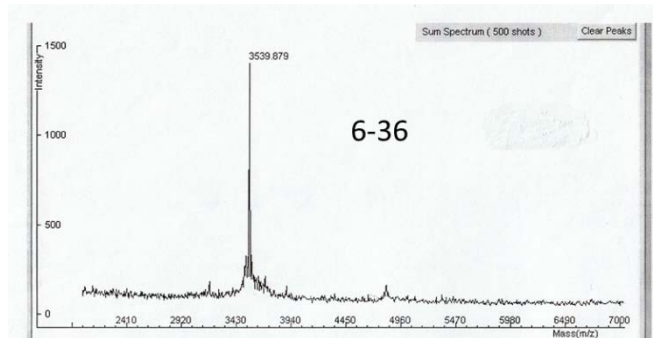
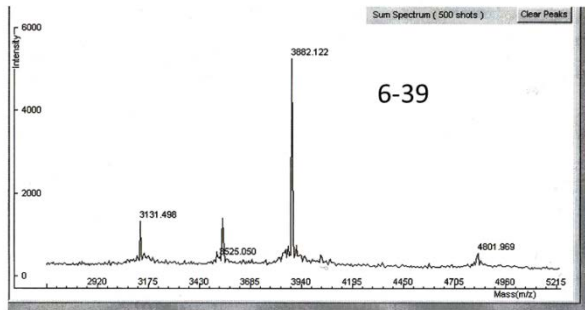
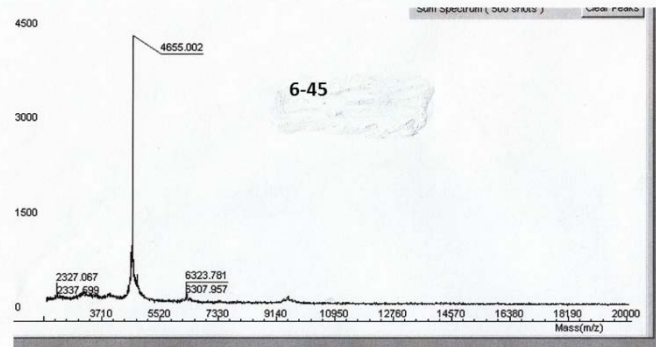
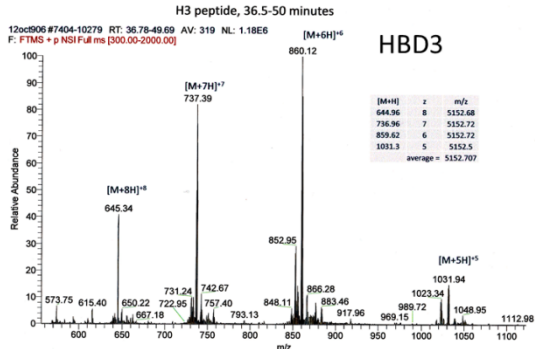
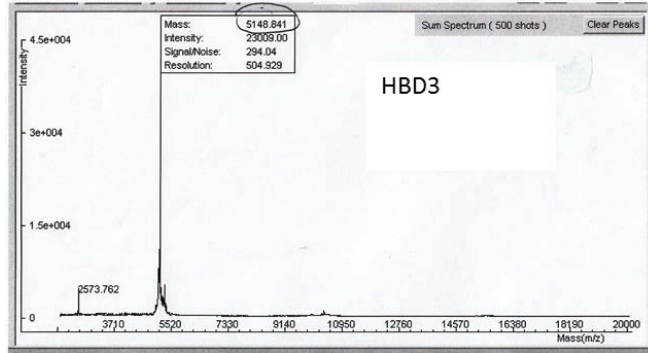
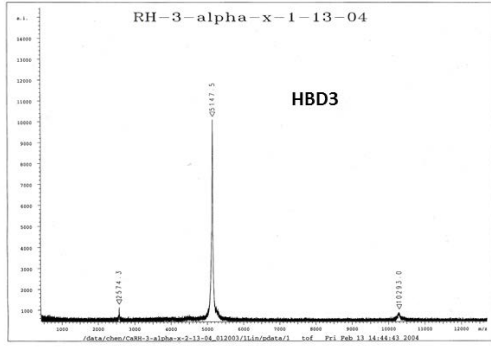
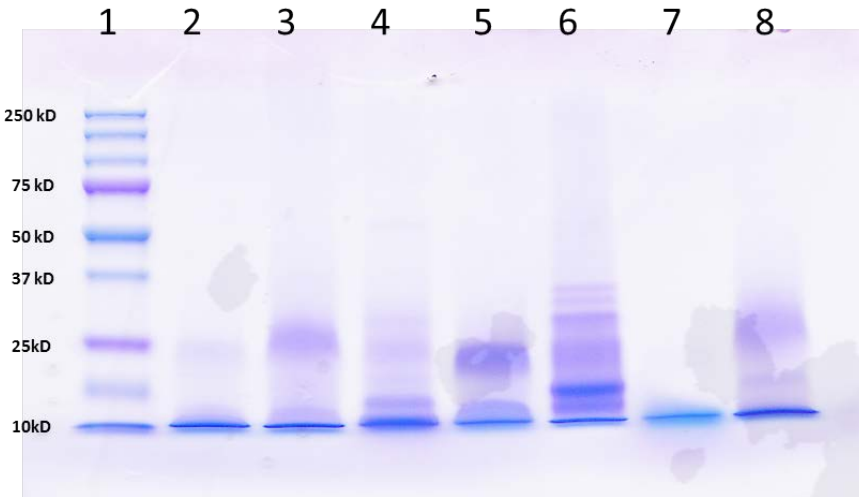


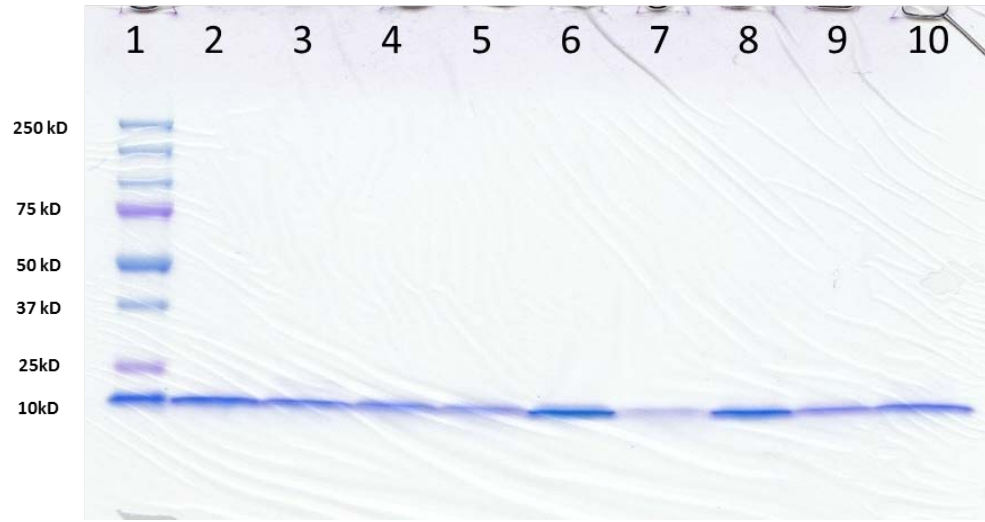
**Figure S1. Example mass results for w/t hBD-3 and some mutants.** Mass spectra of w/t hBD-3 and mutant 6-45 (delete first 5 residues), 6-39 (delete first 5 and last 6 residues), 6-36 (delete first 5 and last 9 residues), and R36I (replace R36 with I36).



**Figure S2. Non-reducing SDS-PAGE of w/t hBD-3 and some mutants.** W/t hBD-3 and respective mutants were boiled in SDS-loading buffer without reducing reagent and applied to 12% Bio-Rad ready-gel. After running at 100V for 1hr, gel was fixed and stained. 1: standard; 2: Helix (insert EYLEKALN at C-terminus of hBD-3); 3: 4AAs (insert EYLE at C-terminus of hBD-3); 4: CA (R42E/R43E/K44S/K45S-hBD3); 5: 8-32-36 (K8A/K32S/R36I); 6: CM2 (C18S-hBD3); 7: CM1-6 (C11S/C18S/C23G/C31I/C40G/C41Y); 8: w/t hBD-3.



**Figure S3. Reducing SDS-PAGE of w/t hBD-3 and some mutants.** W/t hBD-3 and respective mutants were boiled in regular SDS-loading buffer with reducing reagent and applied to 12% Bio-Rad ready-gel. After running at 100V for 1hr, gel was fixed and stained. 1: standard; 2: Helix (insert EYLEKALN at C-terminus of hBD-3); 3: 4AAs (insert EYLE at C-terminus of hBD3); 4: CA (R42E/R43E/K44S/K45S-hBD3); 5: 8-32-36 (K8A/K32S/R36I); 6: CM2 (C18S-hBD3); 7: CM1-6 (C11S/C18S/C23G/C31I/C40G/C41Y); 8: CM1 (C11S-hBD3); 9: NA2(N4E/T5E/K8A-hBD3); 10: w/t hBD-3.



**Table S1. List of hBD-3 mutants.** T: template; F: forward primer; R: reverse primer.

Name	Mutations	Template and Primers
CM1	C11S	T: pET-30c-hBD3 F: C ACA TTA CAG AAA TAT TAT TCC CGA GTC AGA GGC GGC R: CT GAC TCG GGA ATA ATA TTT CTG TAA TGT GTT TAT GAT TCC C
CM2	C18S	T: pET-30c-hBD3 F: GGC CGG TCT GCA GTG CTC AGC GGT CTA CCA AAG GAG R: GCT GAG CAC TGC AGA CCG GCC GCC TCT GAC TCG GG
CM3	C23G	T: pET-30c-hBD3 F: CGG TGT GCT GTG CTC AGC GGT CTA CCA AAG GAG GAA CAG R: C CTT TGG TAG ACC GCT GAG CAC AGC ACA CCG GCC GCC
CM4	C33I	T: pET-30c-hBD3 F: G GAG GAA CAG ATC GGC AAG ATA TCG ACG CGT G R: GCCTTTTCGGCCACGCGTCGATATCTTGCCGATC
CM56	C40G/C41Y	T: pET-30c-hBD3 F: GGC CGA AAA GGC TAC CGA AGA AAG AAA TAA GCG GCC GC R: CTT TCT TCG GTA GCC TTT TCG GCC ACG CGT CGA GCA C
CM 1-6	C11S/ C18S/ C23G/ C40G/ C41Y/ C33I	T: CM15632 Primers: same as CM4
K8A	K8A	T: T: pET-30c-hBD3 F: C GAC AAG GGA ATC ATA AAC ACA TTA CAA GCT TAT TAT TGC R: CC GCC TCT GAC TCT GCA ATA ATA AGC TTG TAA TGT G
K32S	K32S	T: pET-30c-hBD3 F: GAA CAG ATC GGA TCC TGC TCG ACG CGT GGC CGA AAA TGC R: CG CGT CGA GCA GGA TCC GAT CTG TTC CTC CTT TGG AAG
R36I	R36I	T: pET-30c-hBD3 F: C TGC TCG ACG ATC GGC CGA AAA TGC TGC CGA AGA AAG R: CAT TTT CGG CCG ATC GTC GAG CAG GAT CCG ATC TGT TC
8-32	K8A/K32S	T: K8A Primers: same as K32S
8-32-36	K8A/ K32S/ R36I	T: 8-32 Primers: same as R36

Helix	Insert EYLEKALN at C-terminus	T: pET-30c-hBD3 F: GC TGC CGA AGA AAG AAA GAG TAC TTA GAA AAG GCA CTC AAT TAA GCG GCC GC R: CTC GAG TGC GGC CGC TTA ATT GAG TGC CTT TTC TAA GTA CTC TTT CTT TCT TC
8AAs	Insert EKGAYNEL at C-terminus	T: pET-30c-hBD3 F: GC TGC CGA AGA AAG AAA GAG AAG GGA GCA TAC AAT GAG CTC TAA GCG GCC GC R: CTC GAG TGC GGC CGC TTA GAG CTCATTGTATGCTCCCTTCTTTCTTTCTTC
4AAs	Insert EYLE at C-terminus	T: pET-30c-hBD3 F: GC TGC CGA AGA AAG AAA GAG TAC TTA GAA TAA GCG GCC GC R: CTC GAG TGC GGC CGC TTA TTC TAA GTA CTC TTT CTT TCT TC
SSKK	R42S/ R43S	T: T: pET-30c-hBD3 F: CG CGT GGC CGA AAA TGC TGC AGC AGT AAG AAA TAA GC R: GAG TGC GGC CGC TTA TTT CTT ACT GCT GCAGCATTTTCG
RRGG	K44G/ K45G	T: pET-30c-hBD3 F: CG ACG CGT GGC CGA AAA TGC TGC AGA AGA GGG GGA TAA GCG GCC GC R: GTG CTC GAG TGC GGC CGC TTA TCC CCC TCTTCTGCAGCATTTTCG
AAGG	R42A/ R43A/ K44G/ K45G	T: pET-30c-hBD3 F: CG ACG CGT GGC CGA AAA TGC TGC GCT GCA GGG GGA TAA GCG GCC GC R: GTG CTC GAG TGC GGC CGC TTA TCC CCC TGC AGC GCA GCATTTTCG
RRKKRRRK	Insert RRRK at C-terminus	T: pET-30c-hBD3 F: GC TGC CGA AGA AAG AAA CGT AGA CGT AAA TAA GCG GCC GC R: CTC GAG TGC GGC CGC TTA TTT ACG TCT ACG TTT CTT TCTTC
CA	R42E/ R43E/ K44S/ K45S	T: pET-30c-hBD3 F: CG ACG CGT GGC CGA AAA TGC TGC GAA GAG AGC TCA TAA GCG GCC GC R: GTG CTC GAG TGC GGC CGC TTA TGA GCT CTC TTC GCA GCATTTTCG
CA-NC	N4R/ T5R/ R42E/ R43E/ K44S/ K45S	T: CA F: GAC GAC AAG GGA ATC ATA CGT AGA CTA CAG AAA TAT TAT TG R: C TCT GCA ATA ATA TTT CTG TAG TCT ACG TAT GAT TCC CTT G
H2N	Replace G1 through Y10 with GIGDPVT	T: pET-30c-hBD3 F: CC GAC GAC GAC GAC AAG GGA ATC GGG

		GAT CCA GTA ACG TGC AGA GTC AGA GGC GGC R: CCG GCC GCC TCT GAC TCT GCA CGT TAC TGG ATC CCC GAT TCC CTT GTC GTC GTC G
NA2	N4E/ T5E/ K8A	T: pET-30c-hBD3 F: GAC GAC GAC GAC AAG GGA ATC ATA GAA GAG CTC CAG GCA TAT TAT TGC AGA G R: GCC GCC TCT GAC TCT GCA ATA ATA TGC CTG GAG CTC TTC TAT GAT TCC CTT G
NC	N4R/ T5R	T: pET-30c-hBD3 Primers: same as CA-NC
6-45	Delete G1 through T5	T: pET-30c-hBD3 F: CTG GGT ACC GAC GAC GAC GAC AAG TTA CAG AAA TAT TAT TGC R: CT GAC TCT GCA ATA ATA TTT CTG TAA CTT GTC GTC GTC GTC G
11-45	Delete G1 through Y10	T: 6-45 F: GGT ACC GAC GAC GAC GAC AAG TGC AGA GTC AGA GGC GGC R: CA CCG GCC GCC TCT GAC TCT GCA CTT GTC GTC GTC GTC GG
6-41	Delete G1 through T5 and R42 through K45	T: 6-45 F: CG ACG CGT GGC CGA AAA TGC TGC TAA GCG GCC GCA CTC GAG R: GTG CTC GAG TGC GGC CGC TTA GCA GCA TTT TCG GCC ACG