## **Supporting information**

## Title:

Bioactivity and bactericidal mechanism of histidine-rich  $\beta$ -hairpin peptide against Gramnegative bacteria

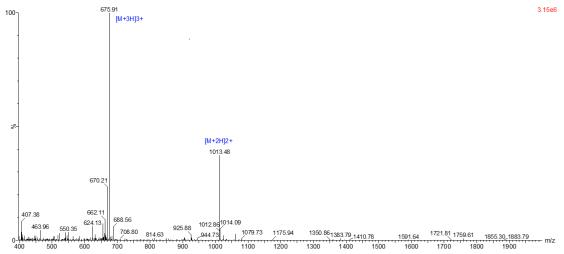
## **Authors:**

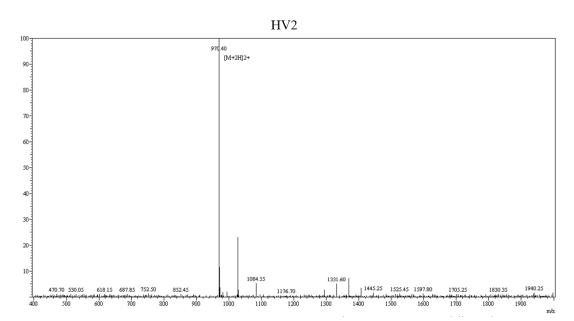
Na Dong, Chensi Wang, Tingting Zhang, Lei Zhang, Chenyu Xue, Xinjun Feng, Chongpeng Bi and Anshan Shan\*

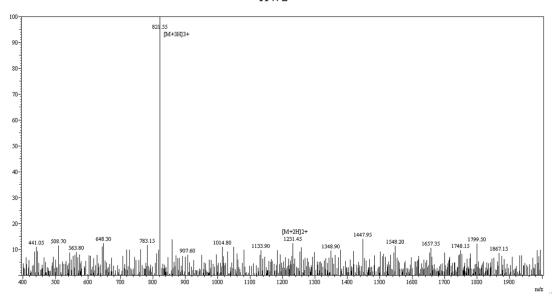
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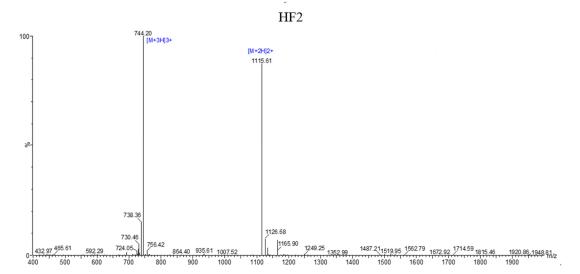
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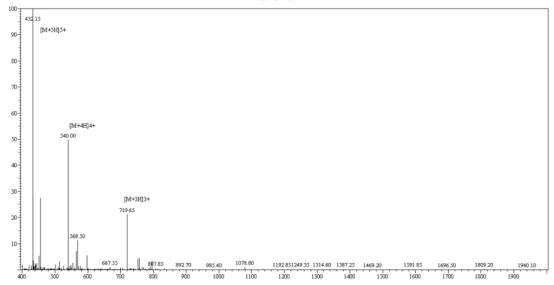




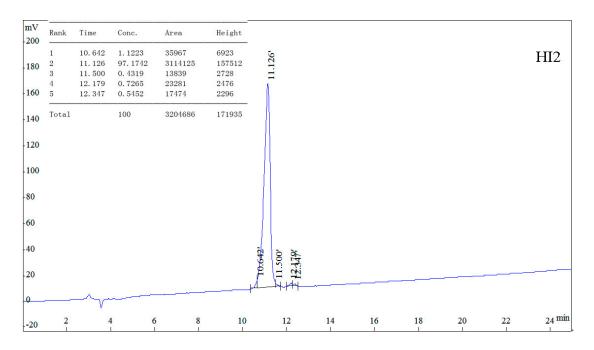


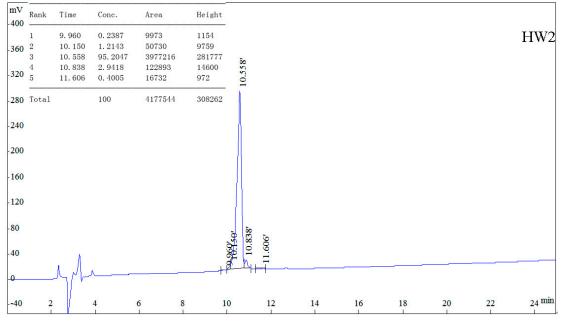


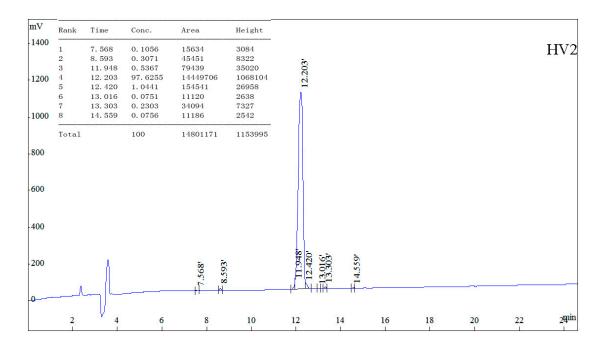


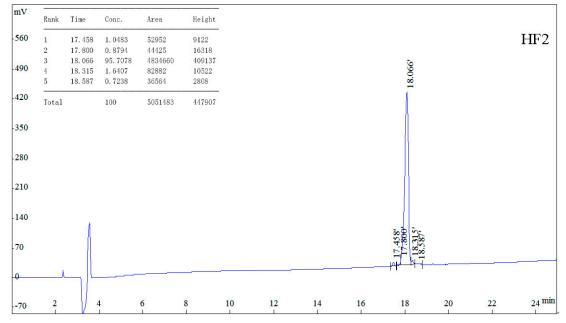


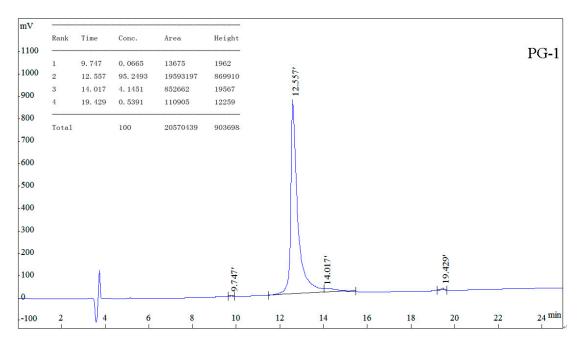
**Supplementary Figure S1:** MALDI-TOF MS of the engineered peptides



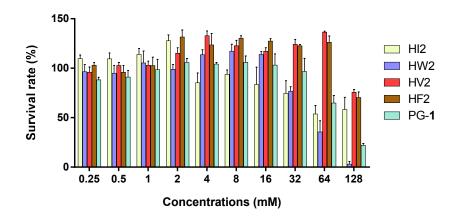








**Supplementary Figure S2:** HPLC spectra of the engineered peptides



Supplementar Figure S3: Cytotoxicity of peptides against HEK 293T cells

Supplementary Table S1: The MICs<sup>a</sup> of HV2, Polymyxin B, Gentamicin, Vancomycin and Ceftazidime against Bacteria

	HV2	Polymyxin B	Gentamicin	Vancomycin	Ceftazidime
MIC(μM)					
Gram-					
E.coli 25922	8	2	1	8	2
E. coli K88	4	0.5	1	2	1
E. coli K99	8	1	0.5	4	0.5
E.coli UB1005	8	1	0.5	16	1
S.pullorum C7913	8	1	1	8	1
P. auruginosa 27853	8	2	1	>128	2
Gram+					
S.aureus 29213	>128	64	1	0.5	16
S.aureus 43300	>128	64	8	0.5	16
S.epidermidis 12228	>128	32	1	0.5	16
MBC <sup>b</sup> (GM)					
Gram (-)	7.13	1.12	0.79	11.31	1.12
Gram (+)	256	50.8	2	0.5	16
Gram (+,-)	23.52	4	1.08	4	2.72

<sup>&</sup>lt;sup>a</sup> Minimum inhibitory concentrations (MIC) were determined as the lowest concentration of peptides that prevented visible turbidity.

 $<sup>^</sup>b$  The geometric mean (GM) of the peptides MICs against all four bacterial strains was calculated. When no detectable antimicrobial activity was observed at 128  $\mu M$ , a value of 256  $\mu M$  was used to calculate the therapeutic index.