Heterologous expression of nattokinase from *Bacillus subtilis* natto using *Pichia pastoris* GS115 and assessment of its thrombolytic activity

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The optimized ORF encoding NK-Bs

atggctggtaagtcttctactgaaaagaagtacattgttggttttaagcagactatgtctgctatgagttctgctaagaagaagg
atgttatttccgaaaagggtggtaaggttcaaaagcaatttaagtacgttaatgctgccgctgctactttggatgaaaaggctg
ttaaggaattgaaaaaggacccctctgttgcttacgttgaagaagatcatattgctcatgaatacgctcaatctgttccatacgg
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tggtacttacggtgcttacaacggcacttctatggctactccacatgttgctggtgctgctgctttgattttgtctaagcatccaa
cttggactaacgctcaagttagagatagattggaaagtactgctacttacttgggtaactctttttactacggtaagggtttgatt
aacgttcaagcagccgcacaa

Supplementary Table1
Primers for the amplification of ORF encoding NK-Bs

Primer names	sequences
Natt-1	GTCAATGGCTGGTAAGTCTTCTACTGAAAAGAAGTACATTGTTGGTTTTAAG
Natt-2	GGCCATTATTGTGCGGCTGCTTGAACGTTAATCAAACCC

Note: the underlined Natt-1 and Natt-2 sequences matched the sticky ends generated by *Cpo*I and *Not*I, respectively.

Supplementary Fig. 1

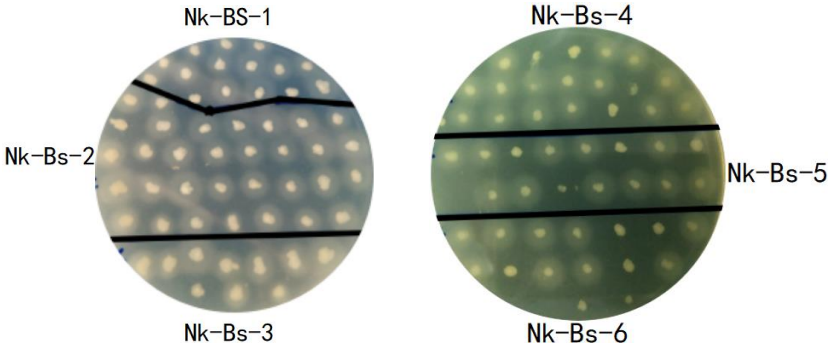

Fig. S1 analyzing the relation between expression levels of NK-Bs and the copy number of *apr*N gene with plate assay.

Supplementary Table 2

The ratio of the opaque halo diameter to the colony diameter

Strains	d_{oh}/d_c

NK-Bs-1	2.31±0.31
NK-Bs-2	2.92±0.28
NK-Bs-3	3.50±0.50
NK-Bs-4	4.36±0.44
NK-Bs-5	5.05±0.35
NK-Bs-6	4.85±0.48

Note: the average diameters of the colonies and halo were measured using plates in Fig. S1.