

Table S1 Primers used in our experiments

Primers	Primer sequences(5'→3')	Introduction	
301waaIUP1	<u>cgggatccgg</u> tatgggaagaatcaag	Construction of ligase-defective strain	
301waaIUP2	gc <u>gtc</u> gactcagaaatgctacgggtg		
301waaIDP1	cca <u>agctt</u> ggaccttagacaatcaa		
301waaIDP2	ccctc <u>gag</u> atgtaggaagcataaccg		
50973waaIUP1	<u>cgggatccagg</u> cttgactatgtgga	Construction of ligase-defective strain	
50973waaIUP2	gc <u>gtc</u> gacatctggcgatagtagtatg		
50973waaIDP1	cca <u>agctt</u> tagtgcaggcatattggg		
50973waaIDP2	ccctc <u>gag</u> tatcacctcgcagaacct		
PilE-U	c <u>ggaatt</u> catgaacaccctcaaaaaggttt	Amplifying PilE	
PilE-D	ccca <u>agctt</u> tagtgggtggtggtggtg		
pglL-C-A-F	gccgcaaactgccgatttaaatcctgcctgcttc	Construction of PglL _{mut}	
pglL-C-A-R	ttaaatcggcagtttggcggcggggtgcgcgcccga		
SacI-U	c <u>gagct</u> ctgcataattcgtgctcaaggcgcaactc		
PstI-D	aact <u>gcagg</u> gcagtttatggcggg		
Primers below were applied to create mutation of amino acids by using nested-PCR SacI-U was the upstream primer for the following amplifications.			
5573-D1(5569-D1)	agaagtgttggccggccattcgcctcggccgcatgcgctaaa cgct	Shorten the sequence of glycosylation site	
5573-D2	gg <u>actag</u> ttttgatttcagaggaggtgccacgccgagagaagtgtgt tgccggg		
5569-D2	gg <u>actag</u> tgagggtgccacgccgagagaagtgtgttgccggg		
5566-D	gg <u>actag</u> tcacgccgagagaagtgtgttgccggccattcgcctc gccgcatgcgctaaacgct		
5866-D	gg <u>actag</u> tcacgccgagagaagtgtgttgccggctcggccgcca tgcgctaaac		
5566AA(5666A A,5766AA and 5866AA)-D1	gtcgaggtcgcgcccacgttgcgcggtcctcggcccgatgcg ctaaacgct		
5566AA-D2	cacgccgagagaagtgtgttgccggccattcgcctcagagtcgc cgcca		
5666AA-D2	cacgccgagagaagtgtgttgccggccattcgtcagagtcgcgc cgacttg		
5766AA-D2	cacgccgagagaagtgtgttgccggccagtcagagtcgcgcgc acgttg		
5866AA-D2	cacgccgagagaagtgtgttgccgggtcagagtcgcgcgcgac gtt		
5566AA(5666A A,5766AA and 5866AA)-D3	gg <u>actag</u> tcgcgcccgttgcgggctgcacgccgagagaagtgt		
5566AA-D1	As shown above		Alanine scanning

5766AA-57A-D2	cgggctgcacgccggcagaagtgttggccggcgctcaggtc gccgccgacgt	of shortened glycosylation site
5766AA-58A-D2	cgggctgcacgccggcagaagtgttggccggccagtcaggtc gccgccgacgt	
5766AA-59A-D2	cgggctgcacgccggcagaagtgttggccggccagtcaggtc gccgccgacgt	
5766AA-60A-D2	cgggctgcacgccggcagaagtgttggccggccagtcaggtc gccgccgacgt	
5766AA-61A-D2	cgggctgcacgccggcagaagtggcgttggccggccagtcaggtc gccgccgacgt	
5766AA-62A-D2	cgggctgcacgccggcagaagcgttggccggccagtcaggtc gccgccgacgt	
5766AA-57A(58^62A)-D3	<u>ggactagtgcgcggcggttggccggctgcacgccggcaga</u>	
5766AA-65A,66A-D2	agaagtgttggccggccagtcaggtcggccgacgttg	
5766AA-65A-D3	<u>ggactagtgcgcggcggttggccggctgcacggcggcagaagtgtt</u> ggtgccgggcca	
5766AA-66A-D3	<u>ggactagtgcgcggcggttggccggctgcgcggcggcagaagtgtt</u> ggtgccgggcca	
5566AA-D1	As shown above	Optimization of the recognition motif between WP and S
NNT-D2	ccgggctgcacgccggcagaagtgttggccagtcaggtcgc cgccgacgt	
NT-D2	ccgggctgcacgccggcagaagtgttggccagtcaggtcgc cgacgt	
GNT-D2	ccgggctgcacgccggcagaagtgttggccagtcaggtcgc cgccgacgt	
GT-D2	ccgggctgcacgccggcagaagtggccggccagtcaggtcgc cgacgt	
GAT-D2	ccgggctgcacgccggcagaagtggcggccagtcaggtcgc cgccgacgt	
GAA-D2	ccgggctgcacgccggcagaagcggcgccggccagtcaggtc gccgccgacgt	
WPAAAS(AA A)-D2	ccgggctgcacgccggcagaagcggcgccggccagtcaggtc gccgccgacgt	
WPAAS-D2	ccgggctgcacgccggcagaagcggcgccggccagtcaggtcgc cgccgacgt	
WPAS-D2	ccgggctgcacgccggcagaagcggcgccggccagtcaggtcgc cgccgacgt	
WPS-D2	ccgggctgcacgccggcagagggccagtcaggtcggccgacg t	
NNT(NT, GNT, GT, GAT, GAA,	<u>ggactagtgcgcggcggttggccggctgcacgccggcaga</u>	

AAA, WPAAS, WPAS and WPS)-D3		
5566AA-D1	As shown above	Optimization of the recognition motif after S
SAG(SA, S, SAP and SP)-D2	agaagcggcggcggccagtcgaggtcgcgcgcgcacgt	
SAG-D3	ggactagtgcgcggcggtttgccgggctggccggcagaagcggcg gcgggcca	
SA-D3	ggactagtgcgcggcggtttgccgggctgggcagaagcggcg ggcca	
S-D3	ggactagtgcgcggcggtttgccgggctgagaagcggcg ca	
SAP-D3	ggactagtgcgcggcggtttgccgggctggggggcagaagcggcg gcgggcca	
SP-D3	ggactagtgcgcggcggtttgccgggctggggagaagcggcg ggcca	
5566AA-D1	As shown above	
SAG-D2	As shown above	
3A-AP-D3	ggactagtgcgcggcggtttgccgggctggggggcagaagcggcg gcgggccaagtcg	
3A-QP-D3	ggactagtgcgcggcggtttgccgggctgggggtgagaagcggcg cgggccagtcg	
3A-FP-D3	ggactagtgcgcggcggtttgccgggctggggaaagaagcggcg gcgggccaagtcg	
3A-DP-D3	ggactagtgcgcggcggtttgccgggctggggatcagaagcggcg cgggccagtcg	
3A-HP-D3	ggactagtgcgcggcggtttgccgggctggggatgagaagcggcg gcgggccaagtcg	
3A-KP-D3	ggactagtgcgcggcggtttgccgggctggggctagaagcggcg cgggccagtcg	
3A-SP-D3	ggactagtgcgcggcggtttgccgggctggggactagaagcggcg cgggccagtcg	
3A-PP-D3	ggactagtgcgcggcggtttgccgggctgggggtgagaagcggcg gcgggccaagtcg	
3A-WP-D3	ggactagtgcgcggcggtttgccgggctggggccaagaagcggcg gcgggccaagtcg	
3A-YP-D3	ggactagtgcgcggcggtttgccgggctggggataagaagcggcg cgggccagtcg	
3A-EP-D3	ggactagtgcgcggcggtttgccgggctggggttcagaagcggcg cgggccagtcg	
3A-RP-D3	ggactagtgcgcggcggtttgccgggctggggacgagaagcggcg gcgggccaagtcg	

3A-GP-D3	ggactagtgcgcggcggtttgccgggctgggggtccagaagcggcgg cggggccagtcg	
3A-VP-D3	ggactagtgcgcggcggtttgccgggctgggggacagaagcggcg gcggggccagtcg	
3A-LP-D3	ggactagtgcgcggcggtttgccgggctgggggagagaagcggcg gcggggccagtcg	
3A-IP-D3	ggactagtgcgcggcggtttgccgggctgggggatagaagcggcg gcggggccagtcg	
3A-CP-D3	ggactagtgcgcggcggtttgccgggctggggacaagaagcggcg gcggggccagtcg	
3A-MP-D3	ggactagtgcgcggcggtttgccgggctggggcatagaagcggcgg cggggccagtcg	
3A-NP-D3	ggactagtgcgcggcggtttgccgggctggggattagaagcggcgg cggggccagtcg	
3A-TP-D3	ggactagtgcgcggcggtttgccgggctggggagtagaagcggcg gcggggccagtcg	
P30-P2-D1	accctcaacaaaagctaacggtaaaattattaaactggcccgcat gcgctaaacgc	Replaced hydrophilic fragments with P30 and P2
P30-P2-D2	gcggcgggccattctaaatgactagcagatacttaggaaccctcaac caaaagctaac	
P30-P2-D3	atttagaatttgctttatatactggggggcagaagcggcggcggcca ttctaaatg	
P30-P2-D4	ggactagtctcagttatactataaatttagaatttgctttatat	
5566AA-D1	As shown above	Replaced hydrophilic fragment at C-terminal with P30, gp100 ⁴⁴⁻⁵⁹ or HA ³⁰⁷⁻³¹⁹
P30-D2	aaccaaagctaacggtaaaattattaaagggggcagaagcggcgg cggggccagtcgaggtcggccgacg	
P30-D3	ggactagttctaaatgactagcagatacttaggaaccctcaacaaa agctaacggtaaaat	
gp-D2	ccattcgggatacagttggcggtccagggggcagaagcggcggcg ggccagtcgaggtcggccgacgt	
gp-D3	ggactagtgtcgagcgctgggcctcagtcattcgggatacagttgg cgg	
HA-D2	gtgttctgttgactactggggccagaagcggcggcggggccagtc gaggtcggccgacgt	
HA-D3	ggactagtggcgcgagcttcagagtggtctgttgactacttggg	