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Spermine-starch nanoparticles with antisense vicR suppress *Streptococcus mutans* cariogenicity

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Appendix

Stability of SNN-ASvicR complex

The released DNAs were carried out with 28.8 μg SNN-AS*vicR* complex which were immersed into 1 ml of PBS or 10 % FBS and incubated in room temperature or 4°C for 1, 2, 3, and 4 days. At each time point, 2μl of leaching liquor was measured with NanoDrop One^{c} respectively to determine the concentration of DNA and quantified at 260 nm (García-Alegría et al, 2020). In addition, the released DNAs were analyzed by 1 % agarose gel electrophoresis in tris-acetate (TAE) buffer at 100V for 30 min. The resulting DNA products were observed under UV irradiation using a Bio-Rad electrophoresis system (Bio-Rad Laboratories, CA, USA).

The inhibitory effects of SNN-ASvicR complex on the Enterococcus faecalis or Streptococcus sanguis

The *Enterococcus faecalis* and *Streptococcus sanguis* strains were provided by the State Key Laboratory of Oral Diseases (Sichuan University, Chengdu, China). *E. faecalis* and *S. sanguis* strains were routinely grown in Brain Heart Infusion (BHI) media overnight at 37°C anaerobically (90% N_2, 5% CO_2, 5% H_2). Then, *E. faecalis and S. sanguis* suspensions were diluted into fresh BHI media at 1:20 ratio and cultured into optical density at 600 nm [OD_{600}] 0.2. For SSN-AS*vicR* treatment, 28.8 μg of SSN-AS*vicR* were added to the 1 mL of bacterial culture and incubated for 3 h. For SSN treatment, 25.6 μg of SSN were added to 1 mL of bacterial and incubated for 3 h. Untreated bacterial suspensions were set as blank control. After SSN-AS*vicR* or SSN treatment, the plating cell counting assay was conducted. Serially diluted ten microliters of bacterial suspensions were coated on BHI plates and incubated for 48h at 37°C anaerobically (90% N_2, 5% CO_2, 5% H_2).

The sequences of AS*vicR-gfp* were synthesized by Sangon Biotech (Shanghai, China). The start sequence of AS*vicR* (676 nucleotides) is underlined. Sequence bold is the starting site of *gfp*. The nucleotides highlighted in yellow indicate the BamHI restriction site and nucleotides highlighted in green indicate EcoRI restriction site.

ggatccAGTCTTCTCCTTATTTGACATAAATTTATTGTTAGCTTTTCTAACATTATATTGTTATACTATCATACCTT
TTCTAAGATGTCAAATATTATCAGAAAATTCAGTAAGATAACTTTCTCTAATAGTTTGAAATAAACTTGTTGGTTTT
CATTTGCCATTGATTTTGATATAATCGTAGAGGGTTACTTTAAGCAGTAAAGCTTTAAGTAAATAAAAATGGTTCTA
ACATAAAGTTTACTCTAAGTGGGCTTGATATAAAAATCAGATAAAAATTAATAAAATAGCATGATTTTTAATCTTTC

TTACTTATTTCATAAACTTCTATTTTAAGACCATAAGCGAGGTATTCATAGAGTAAAATGATTAAAACTGCTGGGGA

AATTGTAAAAATAAGGGACGGATTCAAACACATTGTTGTTTATGACTTCATGGAAAACCAACACCGCGTCGCGTCAA

AATGTACTCTGGACGACTAGGGTATCTTCAATTTTTTCACGCAGACGACGAACAGTAACATCAACAGTACGGACATC

TCCAAAATAATCATAGCCCCAAACCGTTTCAAGTAAATGTTCACGCGTCATCACCTGACCTGTGTGTGTCGCTAAGT

GATGCAACAACTCAAACTCACGGTGAGTTAACTCTACCTCTGTTCCACGTTTTTTAGCAACAAAAGCATCTGGTAAG

ATTTGCAAGTCCCCAATAATAATTTCAGGTATACCTGAAGCATTTTCCTCAGCCACTGCGGATTCAATATTTTCAGT

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ACTTCCTTAGCCACTTCAAGACCGTCTAGTTCTGGTAACATTAAGTCCAATATAATCAAGTCAGGATTTTCTTCTTC

ATATTTACTTAATGCTTCACGCCCATCAAAGGC**ATG**GTGAGCAAGGGCGAGGAGCTGTTCACCGGGGTGGTGCCCAT

CCTGGTCGAGCTGGACGGCGACGTAAACGGCCACAAGTTCAGCGTGTCCGGCGAGGGCGAGGGCGATGCCACCTACG

GCAAGCTGACCCTGAAGTTCATCTGCACCACCGGCAAGCTGCCCGTGCCCTGGCCCACCCTCGTGACCACCCTGACC

TACGGCGTGCAGTGCTTCAGCCGCTACCCCGACCACATGAAGCAGCACGACTTCTTCAAGTCCGCCATGCCCGAAGG

CTACGTCCAGGAGCGCACCATCTTCTTCAAGGACGACGGCAACTACAAGACCCGCGCCGAGGTGAAGTTCGAGGGCG

ACACCCTGGTGAACCGCATCGAGCTGAAGGGCATCGACTTCAAGGAGGACGGCAACATCCTGGGGCACAAGCTGGAG

TACAACTACAACAGCCACAACGTCTATATCATGGCCGACAAGCAGAAGAACGGCATCAAGGTGAACTTCAAGATCCG

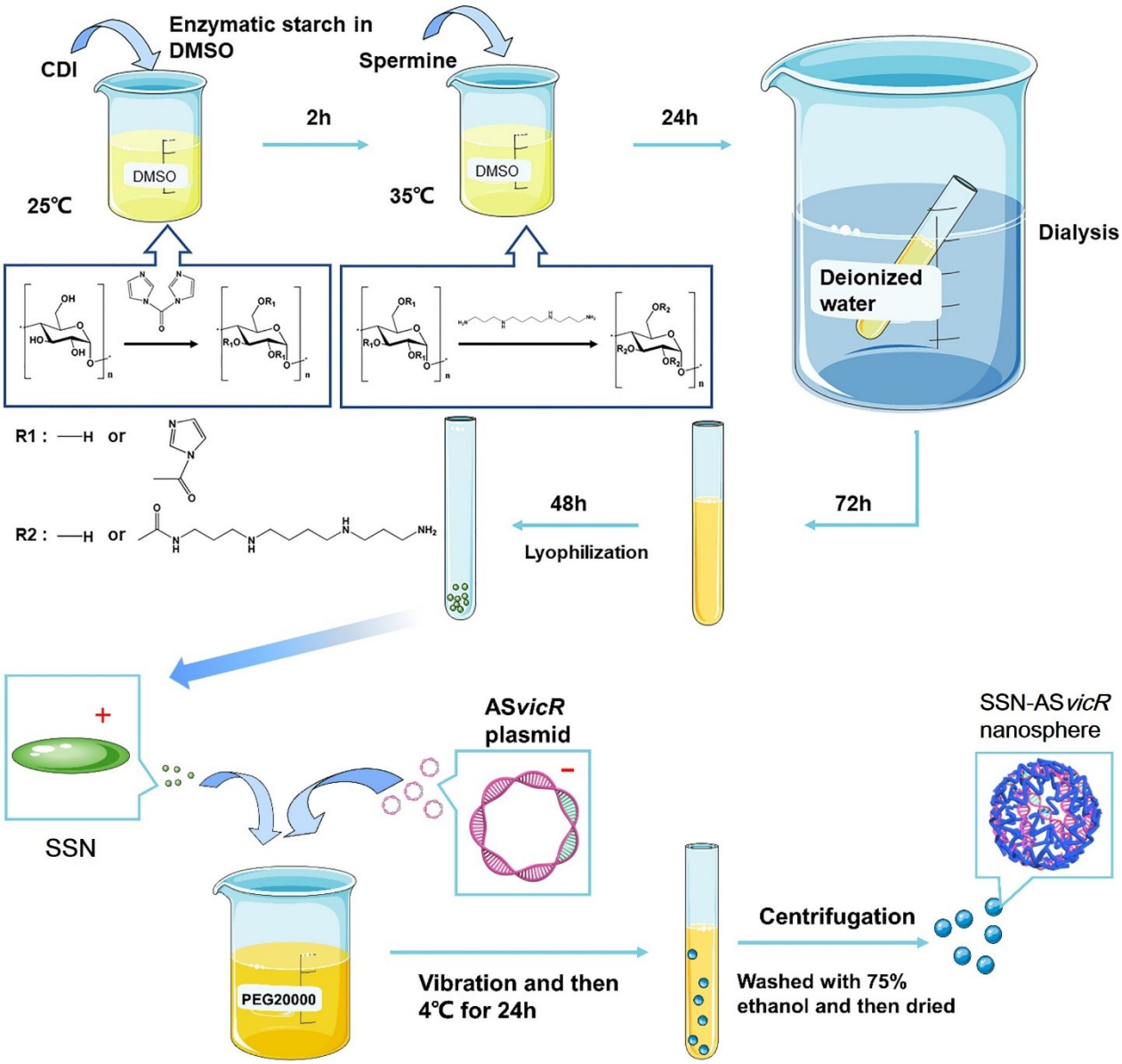
CCACAACATCGAGGACGGCAGCGTGCAGCTCGCCGACCACTACCAGCAGAACACCCCCATCGGCGACGGCCCCGTGC

TGCTGCCCGACAACCACTACCTGAGCACCCAGTCCGCCCTGAGCAAAGACCCCAACGAGAAGCGCGATCACATGGTC

CTGCTGGAGTTCGTGACCGCCGCCGGGATCACTCTCGGCATGGACGAGCTGTACAAGTAAgaattc

Reference

García-Alegría AM, Anduro-Corona I, Pérez-Martínez CJ, Guadalupe Corella-Madueño MA, Rascón-Durán ML, Astiazaran-Garcia H. Quantification of DNA through the NanoDrop Spectrophotometer: Methodological Validation Using Standard Reference Material and Sprague Dawley Rat and Human DNA. Int J Anal Chem. 2020;2020:8896738.



75

76 **Appendix Figure 1.** The composition flowchart of spermine modified starch-AS*vicR* nanoparticles

77 (SSN-AS*vicR*).

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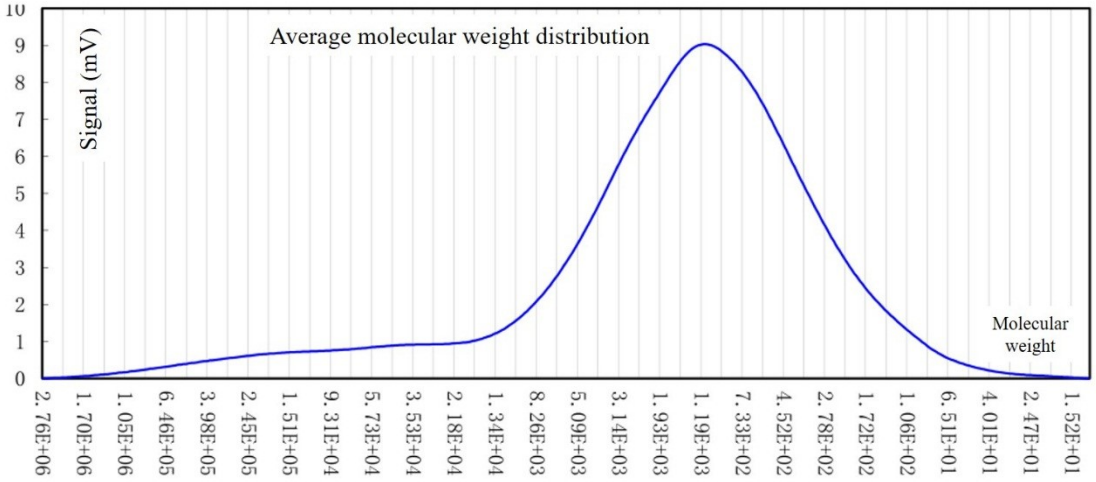
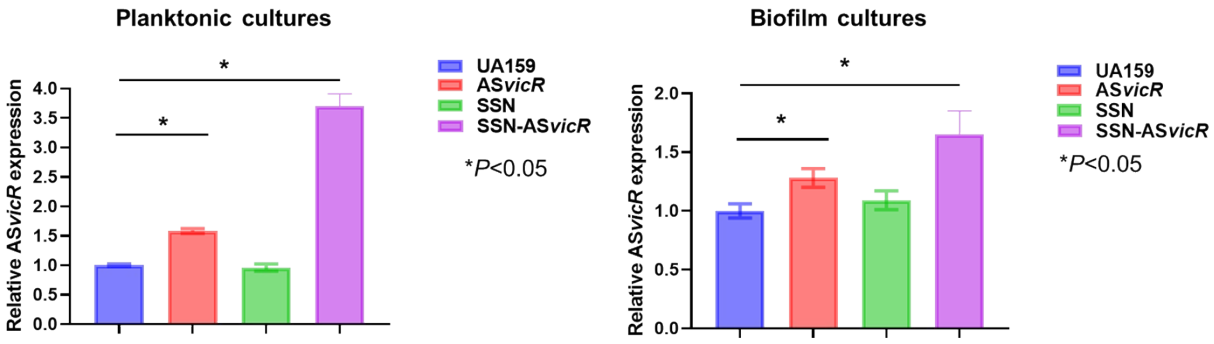
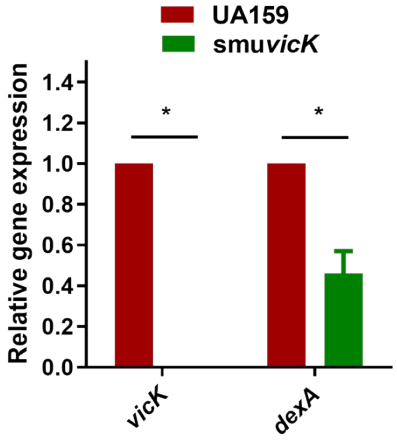


Appendix Figure 2. Molecular weight distribution of cornstarch after enzymolysis.



Appendix Figure 3. The expression of AS*vicR* RNA in planktonic and biofilm cultures of *S. mutans*

Relative gene transcription levels analyzed by quantitative RT-PCR. The *S. mutans* were treated by SSN, AS*vicR* and SSN-AS*vicR* respectively. *S. mutans* UA159 parent strain was used as control group. *gyrA* was set as the internal control with an expression level of 1.0 (mean ± standard deviation, **P* < 0.05). Although the *gyrA* was invariable among the conditions/groups tested, the threshold cycle values (CT) were quantified and the expression of AS*vicR* was normalized relative to the expression of the *gyrA* gene, which was used as an internal reference. Data were calculated according to the $2^{-\Delta\Delta CT}$ method.



94

95 **Appendix Figure 4.** The *dexA* transcription in a *vicK* knock-out mutant was analyzed by quantitative
96 RT-PCR ($P > 0.05$).

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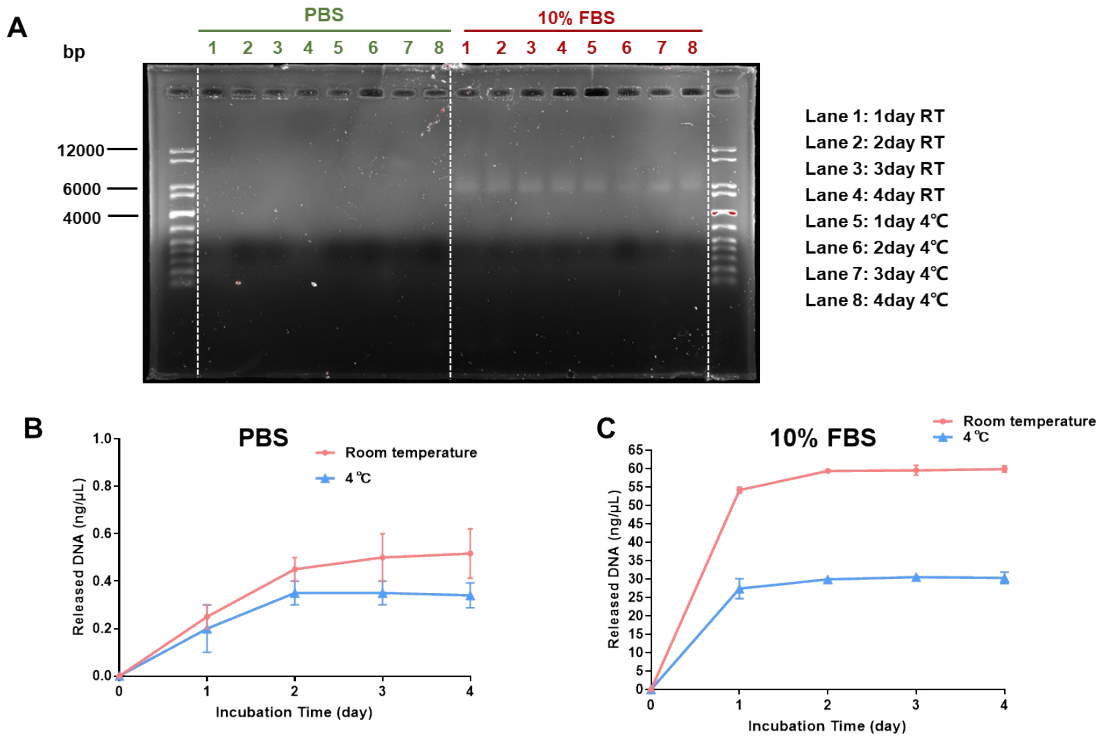
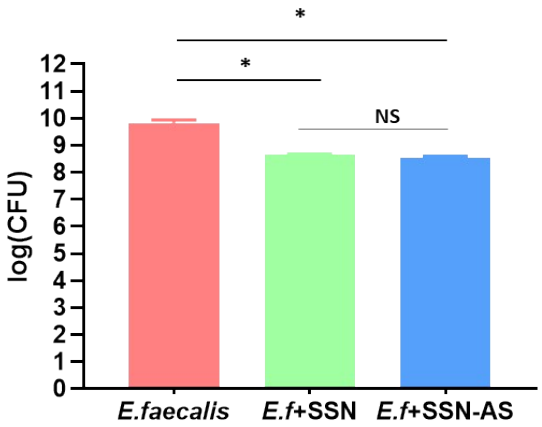
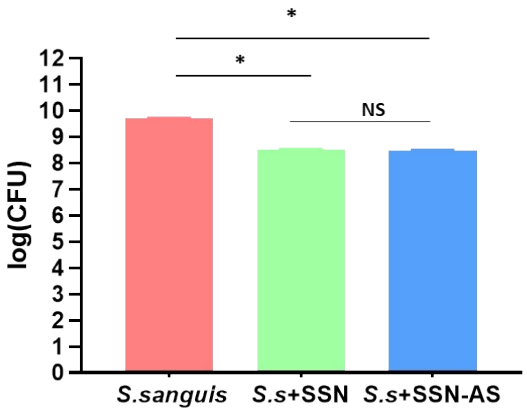


98

99 **Appendix Figure 5**. The released DNAs from the SNN-AS*vicR* complex were measured at 1 day, 2 days,
100 3days and 4 days respectively. **(A)** The released DNAs were analyzed by 1 % agarose gel
101 electrophoresis; **(B)** The released DNAs in PBS were measured at 1 day, 2 days, 3days and 4 days
102 respectively; **(C)** The released DNAs in 10% FBS were measured at 1 day, 2 days, 3days and 4 days
103 respectively.



Appendix Figure 6. The inhibitory effects of SSN or SNN-AS*vicR* complex on the *Enterococcus faecalis*.



Appendix Figure 7. The inhibitory effects of SSN or SNN-ASvicR complex on the *Streptococcus sanguis*.

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119 **Appendix Table 1. Oligonucleotide primers used in this study.**

Primers	Sequence 5’-3’ (Forward/Reverse)	Source or reference
QRT-PCR		
gyrA	5’-ATTGTTGCTCGGGCTCTTCCAG-3’/ 5’-ATGCGGCTTGTCAGGAGTAACC-3’	Mao et al., 2016
vicR	5’-CGCAGTGGCTGAGGAAAATG-3’/ 5’-ACCTGTGTGTGTCGCTAAGTGATG-3’	Mao et al., 2016
gtfB	5’-ACACTTTCGGGTGGCTTG-3’/ 5’-GCTTAGATGTCACTTCGGTTG-3’	Mao et al., 2016
gtfC	5’-CCAAAATGGTATTATGGCTGTCG-3’/ 5’-TGAGTCTCTATCAAAGTAACGCAG-3’	Mao et al., 2016
gbpB	5’-AGCAACAGAAGCACAACCATCAG-3’/ 5’-CCACCATTACCCCAGTAGTTTCC-3’	Mao et al, 2016
dexA	5’-AGGGCTGACTGCTTCTGGAGT-3’/ 5’-AGTGCCAAGACTGACGCTTTG-3’	Yang et al., 2019
gfp	5’-CACATGAAGCAGCACGACTT-3’/ 5’-TCCTTGAAGTCGATGCCCTT-3’	This study
16sR (*S. gordonii* and *S. sanguinis*)	5’- AAGCAACGCGAAGAACCTTA -3’/ 5’- GTCTCGCTAGAGTGCCCAAC -3’	Jiang et al, 2018
S. gordonii vicR	5’- TTGGATTTGATGTTGCCAGA -3’/ 5’- CAAATTCTGAACGTCGCAAA -3’	This study
S. sanguinis vicR	5’- GTCCGAACGAGTTGACCATT -3’/ 5’- CCATACCGTTTCCAGCAAGT -3’	This study
Random 6 mers	5'-(P)NNNNNN-3'	RevertAid First Strand cDNA Synthesis Kit (Thermo Scientific)

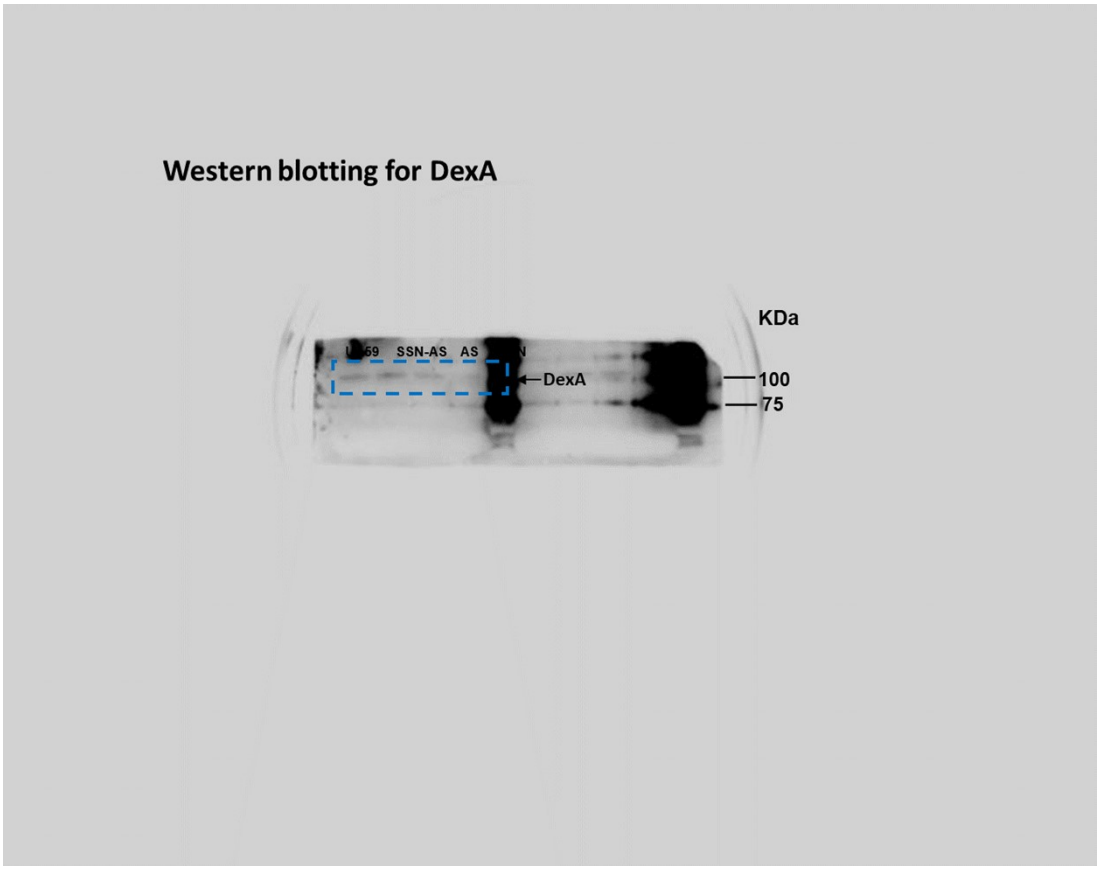
Reverse transcription for noncoding RNA		
First strand cDNA synthesis for AS*vicR*	5'-CCGCAGTGGCTGAGG-3'	Lei et al., 2020
qRT-PCR analysis for AS*vicR*	5'- CACGCAGACGACGAACAG -3'	Lei et al., 2020
EMSA		
dexA promoter	5'-ACTTGTTCGGAAATAGCCACTGCGT-3'/ 5'-AACTCCAAGTATTCATTTTGTCATAT-3'	This study

Appendix Table 2 Caries unit scores

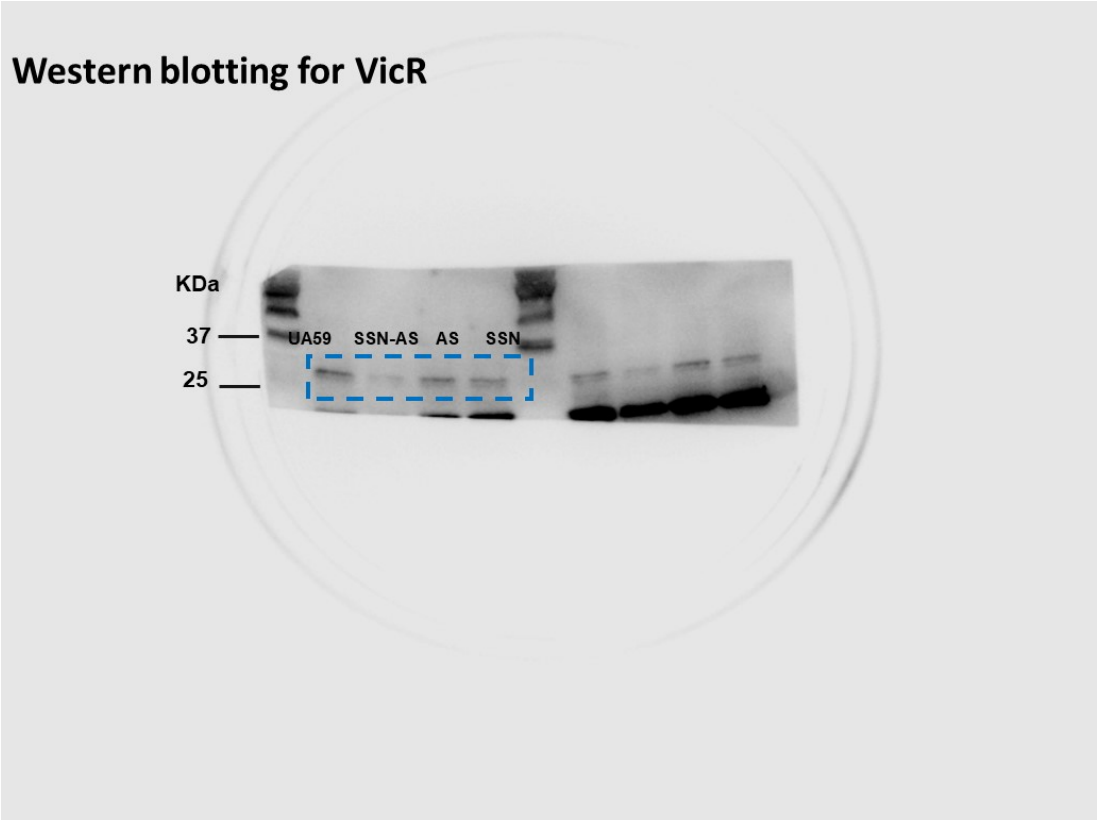
	D_E	D_m	Total Keyes' score
UA159	0.55±0.51	2.27±1.4	2.81±1.3[a]
SSN	0.58±0.53	1.75±1.51	2.33±1.82[b]
AS*vicR*	0.92±0.78	1.16±1	2.1±1.34[b]
SSN-AS*vicR*	0.75±0.45	0.75±0.57	1.5±1.32[c]

The depths of penetration are under 2 headings: enamel only (D_E), and dentinal (D_m). The data represent the carious lesions score according to modified Keyes scores. By using UA159 as positive control, significant differences were determined. Dissimilar letters indicate significantly different values (n=6, P<0.05).

Raw data for the upper lane of Figure 5C-Western blotting for DexA



149 **Raw data for the middle lane of Figure 5C-Western blotting for VicR**



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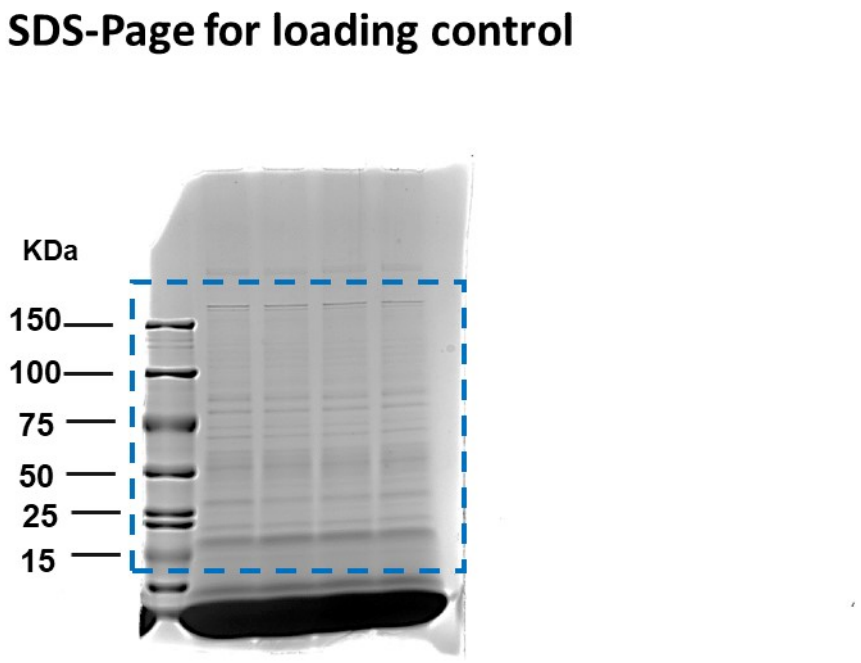
151 **Raw data for the lower lane of Figure 5C- SDS-Page for loading control**



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