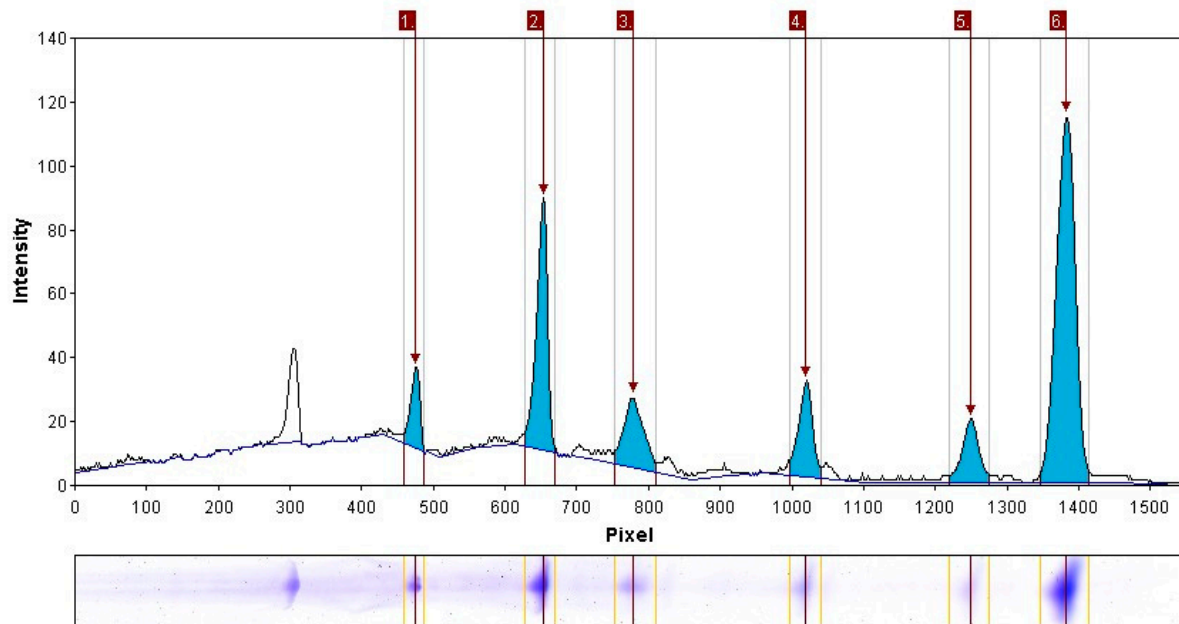


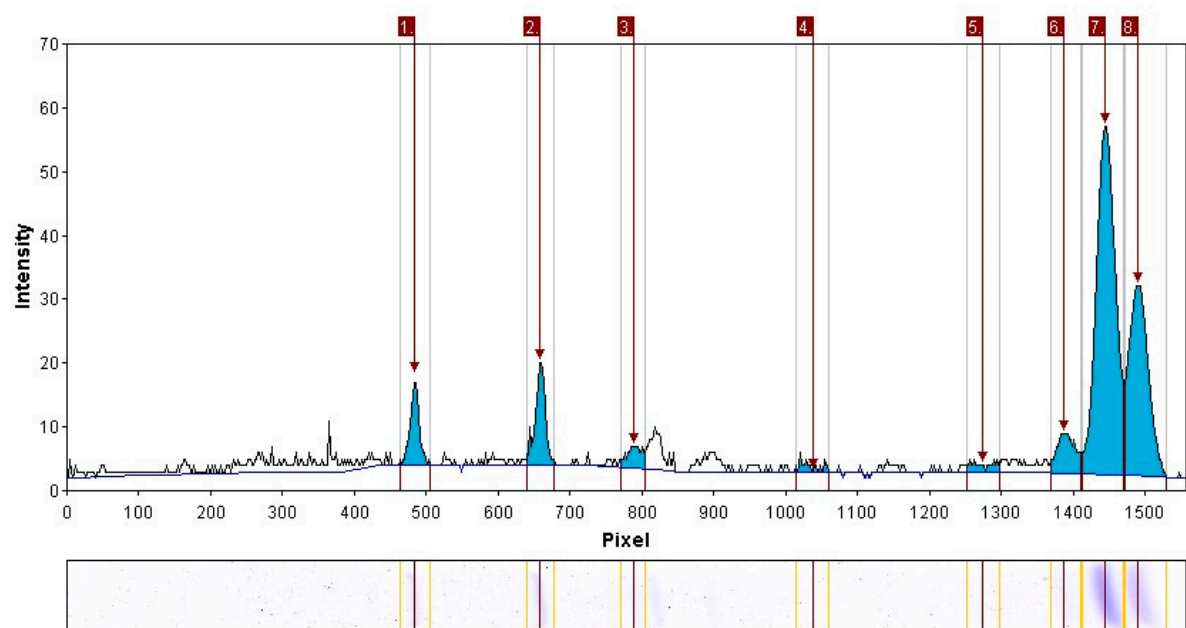
## Supplementary Materials

**Supplementary Figure 1.** Histograms showing band intensity of Nops. Band volume of Nops were measured by densitometer scan of stained SDS-PAGE gel. Band raw volume was obtained using GelAnalyzer software v. 19.1. For comparison the band volume of molecular weight markers are also shown.

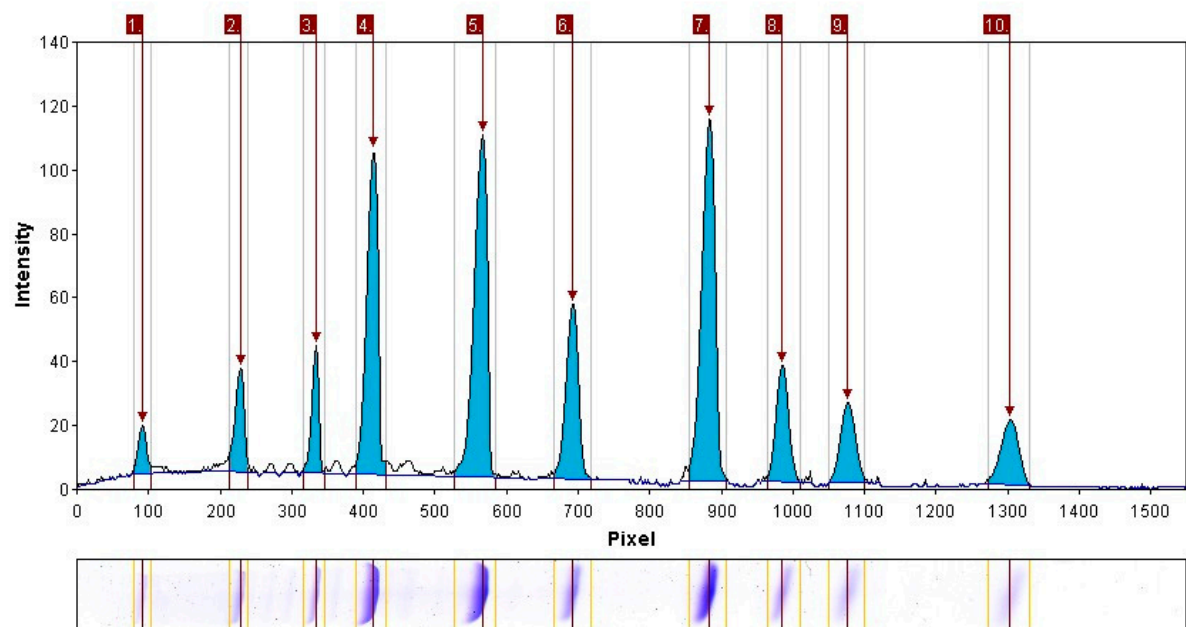
Histogram of Lane 1 from Figure 1, showing band intensity.



Histogram of Lane 6 from Figure 1, showing band intensity.



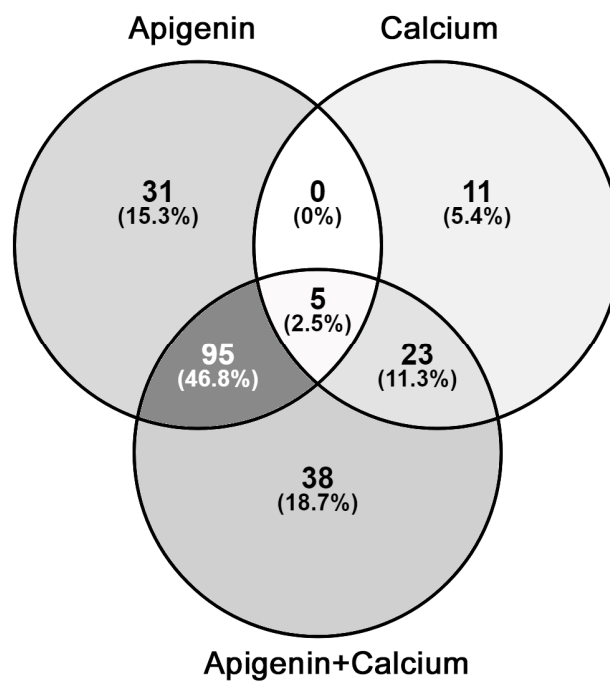
Histogram of Lane M from Figure 1 (molecular weight marker), showing band intensity.



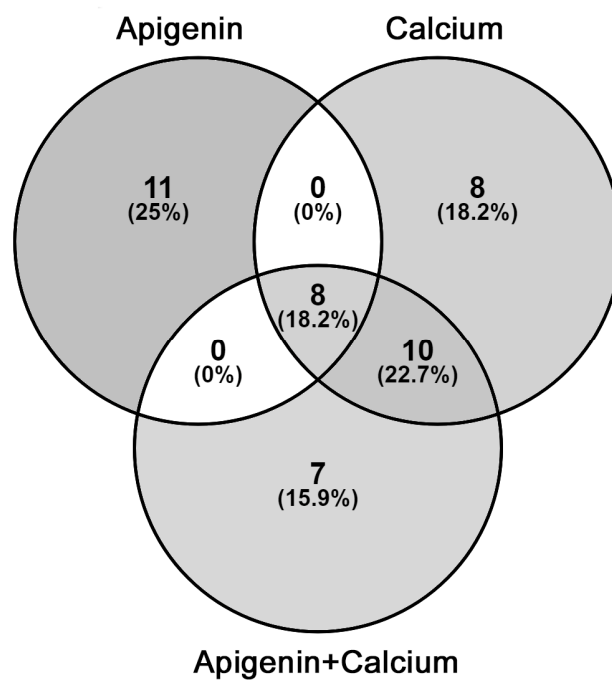
Band volume of Nops measured by densitometer scan of stained SDS-PAGE gel. Band raw volume was obtained using GelAnalyzer software v. 19.1. For comparison the band volume of molecular weight markers are also shown.

<b>Band #</b>	<b>Nop Protein</b>	<b>Lane 1 Volume</b>	<b>Lane 6 Volume</b>		<i>MW</i>	<i>Marker Volume</i>
<b>1</b>	<b>NopX</b>	<b>381</b>	<b>193</b>		250	202
<b>2</b>	<b>NopL</b>	<b>1425</b>	<b>265</b>		150	474
<b>3</b>	<b>NopP</b>	<b>704</b>	<b>88</b>		100	476
<b>4</b>	<b>NopB</b>	<b>691</b>	<b>39</b>		75	1893
<b>5</b>	<b>NopC</b>	<b>538</b>	<b>53</b>		50	2316
<b>6</b>	<b>Nop A</b>	<b>3572</b>	<b>183</b>		37	1087
<b>7</b>	<b>Nop A product</b>	<b>---</b>	<b>1687</b>		25	2468
<b>8</b>	<b>Nop A product</b>	<b>---</b>	<b>959</b>		20	777
					15	625
					10	570

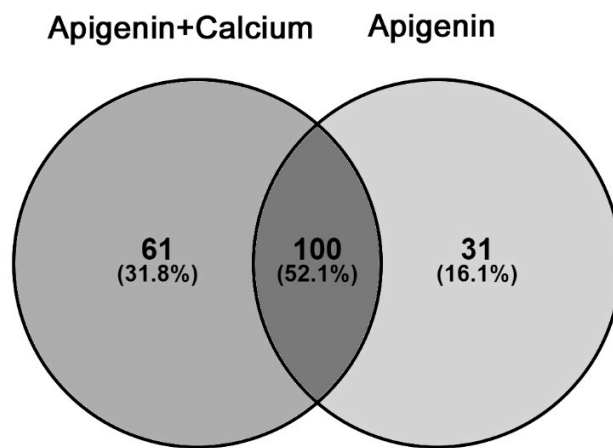
**Supplementary Figure 2.** Venn diagram of the up-regulated sets of the DEGs found in the *S. fredii* NGR234 upon treatments with apigenin, calcium, and calcium+apigenin. Apigenin, calcium, and apigenin+calcium treatment showed 31, 11, and 38 exclusive DEGs, respectively. The shared DEGs were 95 to apigenin and apigenin+calcium treatments, 23 to calcium and apigenin+calcium, and only 5 to the three conditions.



**Supplementary Figure 3.** Venn diagram of the down-regulated sets of the DEGs found in the *S. fredii* NGR234 upon treatments with apigenin, calcium, and calcium+apigenin. Apigenin, calcium, and apigenin+calcium treatment showed 11, 8, and 7 exclusive DEGs, respectively. The shared DEGs were 10 to calcium and apigenin+calcium treatments, and 5 to the three conditions. The apigenin and apigenin+calcium treatments did not share any DEG.



**Supplementary Figure 4.** Venn diagram of the up-regulated sets of the DEGs found in the *S. fredii* NGR234 upon treatments with apigenin and calcium+apigenin. Apigenin+calcium treatment showed 61 exclusive DEGs, 31 to apigenin treatment, and the two conditions share 100 DEGs.



**Supplementary Table 1.** Primers used in this work.

Oligonucleotide	Sequence 5'-3'	Target
NGR_a03410_fw NGR_a03410_rev	GTTCAACGCAAGACCATTTGA CATCGCCACCCTGATAAA	Amplification of part of <i>nodA</i>
NGR_a00550_fw NGR_a00550_rev	AGAACGTAGCGGCCAAAG GTAACAGTGCAGTAGCATA	Amplification of part of <i>nopA</i>
NGR_a00670_fw NGR_a00670_rev	AGCTCTGCTGCCTAAGATAAAG CCAAGTCAGTCGCCAGTAAA	Amplification of part of <i>rhcJ</i>
NGR_a00470_fw NGR_a00470_rev	CTCGAGGCCCTATTGCAGTATC ATGTCACGCAGCCTTGATAG	Amplification of part of <i>syrM2</i>
NGR_a00800_fw NGR_a00800_rev	TAAGGAGCGACGGGTATTCA TAGAGACGGGTTTACGGAGAAA	Amplification of part of <i>ttsI</i>
NGR_b17530_fw NGR_b17530_rev	ATGAAGGCCAACCAGGAAG GGTCGTCGCTGTCGAAAT	Amplification of part of transcriptional regulator
NGR_b20640_fw NGR_b20640_rev	TTTGA CTGTGGCGCTTCT TGTTGAGCAGGATCGATGTC	Amplification of part of <i>TRAP-T</i> family transporter
NGR_c19850_fw NGR_c19850_rev	CTGATCTGCACCAACACCAT CCTCTTTCCTTGAGACGAAGTG	Amplification of part of <i>asr</i>
NGR_b03240_fw NGR_b03240_rev	CGTGCTGGACCTCAATCTG Reverse GACGACGACGATCGGAATTT	Amplification of part of <i>LuxR</i> family transcriptional regulator
NGR_c31220_fw NGR_c31220_rev	CATGCTTCCGAGATGTTCCA CTTGTGGGCGAGCATCTT	Amplification of part of <i>lpdA1</i>
NGR_c17830_fw NGR_c17830_rev	GGATGTATTGAGAGCGTCTTT CTTGAACATTGCTGCCCTTG	Amplification of part of <i>sugar ABC</i> transporter substrate-binding protein
NGR_c30940_fw NGR_c30940_rev	TCATGTTCCGATCCCTTAC CTCGCCGATTTGCAGAGATA	Amplification of part of <i>glpD2</i>
NGR_c07820_fw NGR_c07820_rev	CCAAGGATCTCCGTTTGCT GCAGCACGAACTCACCATA	Amplification of part of <i>cycL</i>
NGR_a00400_fw NGR_a00400_rev	CCTGCGATTGAATGTGTTTACC GTTGGCCTCGACATCATCTT	Amplification of part of <i>nodZ</i>
NGR_b18360_fw NGR_b18360_rev	AAGCTACCCGGACGACTATT TGCTGAACCCTTCGGTTTG	Amplification of part of <i>exoA</i>
NGR_c09220_fw NGR_c09220_rev	AGATGGTTTACGGTGGTGAAG TATACGAGGACCGGGTTCAT	Amplification of part of outer membrane protein
NGR234_16Sr_fw NGR234_16Sr_rev	GCACGTAGGCGGACATTTAAGTCA G GCCACTGGTGTTCCTCCGAATATC	Amplification of part of 16S ribosomal RNA

Supplementary Table 2. Number of total and mapped reads for replicates in each condition.							
	replicon	Seq length	Total Reads	Mapped Reads	Unmapped Reads	%mapped Reads	%unmapped Reads
Wild-type_1	pNGR234a (U00090.2)	536165	804828	801701	3127	99.61	0.39
	pNGR234b (CP000874.1)	2430033	18596888	18523899	72989	99.61	0.39
	Chromosome (CP001389.1)	3925702	30234000	30112211	121789	99.60	0.40
	<b>Total/Mean</b>		<b>49635716</b>	<b>49437811</b>	<b>197905</b>	<b>99.61</b>	<b>0.39</b>
Wild-type_2	pNGR234a (U00090.2)	536165	855624	852407	3217	99.62	0.38
	pNGR234b (CP000874.1)	2430033	20411653	20348312	63341	99.69	0.31
	Chromosome (CP001389.1)	3925702	32784939	32674113	110826	99.66	0.34
	<b>Total</b>		<b>54052216</b>	<b>53874832</b>	<b>177384</b>	<b>99.66</b>	<b>0.34</b>
Wild-type_3	pNGR234a (U00090.2)	536165	907197	903790	3407	99.62	0.38
	pNGR234b (CP000874.1)	2430033	20940958	20874973	65985	99.68	0.32
	Chromosome (CP001389.1)	3925702	35870197	35746948	123249	99.66	0.34
	<b>Total</b>		<b>57718352</b>	<b>57525711</b>	<b>192641</b>	<b>99.66</b>	<b>0.34</b>
Wild-type_Ap_1	pNGR234a (U00090.2)	536165	2209875	2200835	9040	99.59	0.41
	pNGR234b (CP000874.1)	2430033	14178113	14100560	77553	99.45	0.55
	Chromosome (CP001389.1)	3925702	23898500	23760701	137799	99.42	0.58
	<b>Total</b>		<b>40286488</b>	<b>40062096</b>	<b>224392</b>	<b>99.49</b>	<b>0.51</b>
Wild-type_Ap_2	pNGR234a (U00090.2)	536165	2140510	2132140	8370	99.61	0.39
	pNGR234b (CP000874.1)	2430033	13991992	13918012	73980	99.47	0.53
	Chromosome (CP001389.1)	3925702	22984762	22860546	124216	99.46	0.54
	<b>Total</b>		<b>39117264</b>	<b>38910698</b>	<b>206566</b>	<b>99.51</b>	<b>0.49</b>
Wild-type_Ap_3	pNGR234a (U00090.2)	536165	4725217	4710307	14910	99.68	0.32
	pNGR234b (CP000874.1)	2430033	30538295	30414924	123371	99.60	0.40
	Chromosome (CP001389.1)	3925702	50863134	50648487	214647	99.58	0.42
	<b>Total</b>		<b>86126646</b>	<b>85773718</b>	<b>352928</b>	<b>99.62</b>	<b>0.38</b>
Wild-type_Ca_1	pNGR234a (U00090.2)	536165	634856	631940	2916	99.54	0.46
	pNGR234b (CP000874.1)	2430033	15821383	15740104	81279	99.49	0.51
	Chromosome (CP001389.1)	3925702	26281153	26135572	145581	99.45	0.55



	<b>Total</b>		<b>42737392</b>	<b>42507616</b>	<b>229776</b>	<b>99.49</b>	<b>0.51</b>
Wild-type_Ca_2	pNGR234a (U00090.2)	536165	619699	616867	2832	99.54	0.46
	pNGR234b (CP000874.1)	2430033	15490505	15408323	82182	99.47	0.53
	Chromosome (CP001389.1)	3925702	25539792	25393213	146579	99.43	0.57
	<b>Total</b>		<b>41649996</b>	<b>41418403</b>	<b>231593</b>	<b>99.48</b>	<b>0.52</b>
Wild-type_Ca_3	pNGR234a (U00090.2)	536165	650355	647457	2898	99.55	0.45
	pNGR234b (CP000874.1)	2430033	16553146	16469024	84122	99.49	0.51
	Chromosome (CP001389.1)	3925702	27142679	26994394	148285	99.45	0.55
	<b>Total</b>		<b>44346180</b>	<b>44110875</b>	<b>235305</b>	<b>99.50</b>	<b>0.50</b>
Wild-type_ApCa_1	pNGR234a (U00090.2)	536165	3535006	3524321	10685	99.70	0.30
	pNGR234b (CP000874.1)	2430033	16238057	16186563	51494	99.68	0.32
	Chromosome (CP001389.1)	3925702	32575707	32461574	114133	99.65	0.35
	<b>Total</b>		<b>52348770</b>	<b>52172458</b>	<b>176312</b>	<b>99.68</b>	<b>0.32</b>
Wild-type_ApCa_2	pNGR234a (U00090.2)	536165	3399174	3389120	10054	99.70	0.30
	pNGR234b (CP000874.1)	2430033	16152618	16103556	49062	99.70	0.30
	Chromosome (CP001389.1)	3925702	28245060	28150024	95036	99.66	0.34
	<b>Total</b>		<b>47796852</b>	<b>47642700</b>	<b>154152</b>	<b>99.69</b>	<b>0.31</b>
Wild-type_ApCa_3	pNGR234a (U00090.2)	536165	3583779	3572001	11778	99.67	0.33
	pNGR234b (CP000874.1)	2430033	17304645	17246990	57655	99.67	0.33
	Chromosome (CP001389.1)	3925702	30264642	30153312	111330	99.63	0.37
	<b>Total</b>		<b>51153066</b>	<b>50972303</b>	<b>180763</b>	<b>99.66</b>	<b>0.34</b>

**Supplementary Table 3.** Mass spectrometric identification of amino acid sequences of NGR234 low molecular weight proteins isolated from acrylamide gels.

Protein ID ---- Accession	Mascot Score	Peptides (Unique Spectra) ---- % Coverage	MW Thr. / Exp.	(Position)      Peptide Matched
Band A Nodulation protein NopA ( <i>Sinorhizobium fredii</i> ) ---- AY775562.2 (gil499177556)	1536	8 (21) ---- 100%	7,004 / 7,000	(1-25)      (-)MSKIGTLTSAVGAGAAAGQNVAAK(G) (3-25)      (K)IGTLTSAVGAGAAAGQNVAAK(G) (5-25)      (G)TLTSAVGAGAAAGQNVAAK(G) (24-49)      (K)GAGAAAFQAQIAELAAVSAEATAR(S) (31-49)      (F)QAQIAELAAVSAEATAR(S) (48-64)      (R)SMLLRVTTELQTTK(K) (53-64)      (R)TVTTELQTTK(K) (64-71)      (K)AADERVQ(-)
Band B Nodulation protein NopA ( <i>Sinorhizobium fredii</i> ) ---- AY775562.2 (gil499177556)	1061	4 (12) ---- 85%	7,004 / 4,000	(3-25)      (K)IGTLTSAVGAGAAAGQNVAAK(G) (29-49)      (A)AFQAQIAELAAVSAEATAR(S) (53-64)      (R)TVTTELQTTK(K) (64-71)      (K)AADERVQ(-)
Band C Nodulation protein NopA ( <i>Sinorhizobium fredii</i> ) ---- AY775562.2 (gil499177556)	465	4 (7) ---- 87%	7,004 / 3,000	(3-25)      (K)IGTLTSAVGAGAAAGQNVAAK(G) (29-49)      (A)AFQAQIAELAAVSAEATAR(S) (48-64)      (S)MLLRVTTELQTTK(K) (64-71)      (K)AADERVQ(-)