

Supplementary Material

General and specific cytotoxicity of chimeric antisense oligonucleotides in bacterial cells and human cell lines

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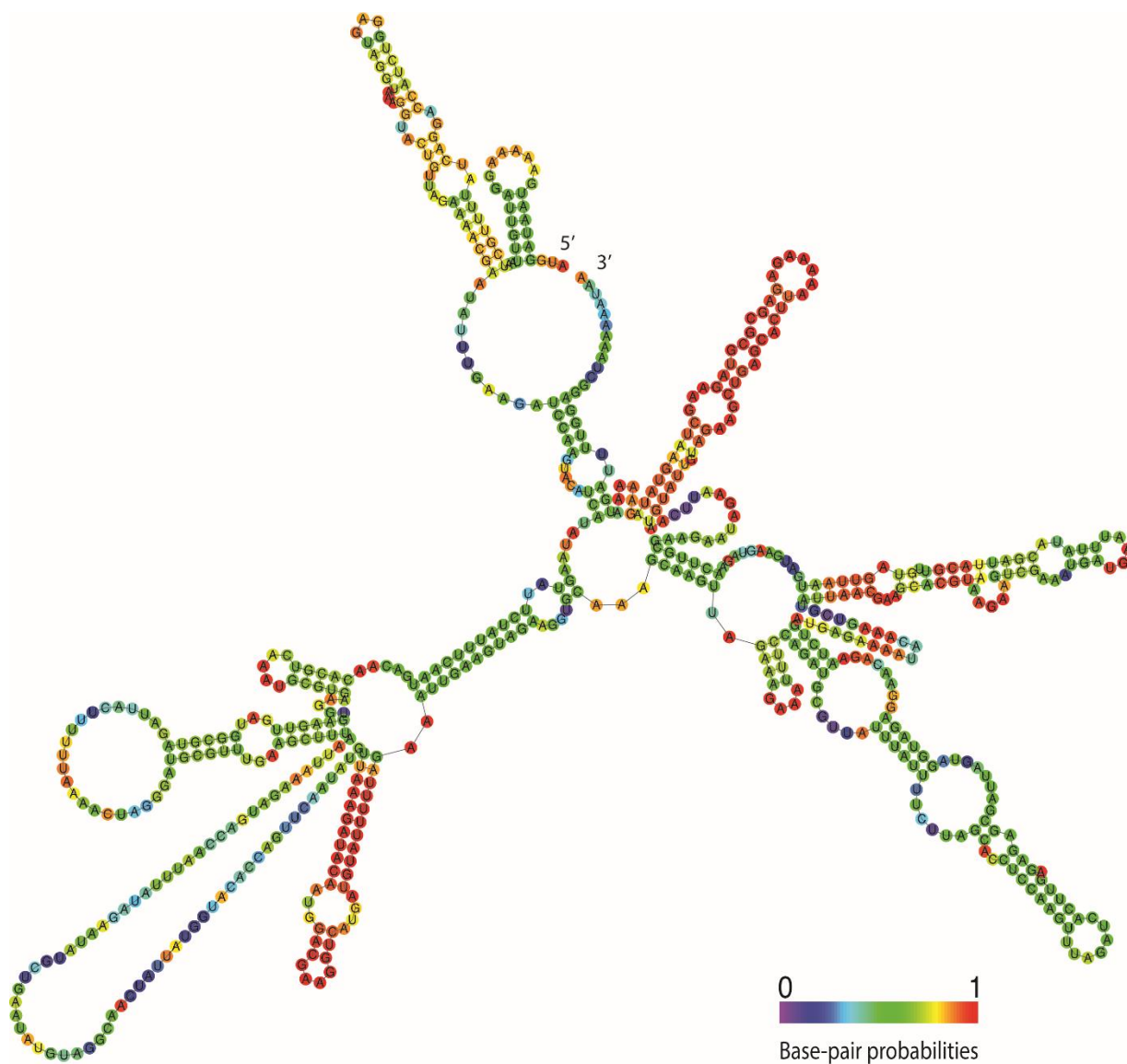


Figure S1. A partition structure of the folding of the mRNA, encoding guanylate kinase in *S. aureus* (strain ATCC 25 923). The model structure is obtained by the RNAfold web server version 2.4.8.

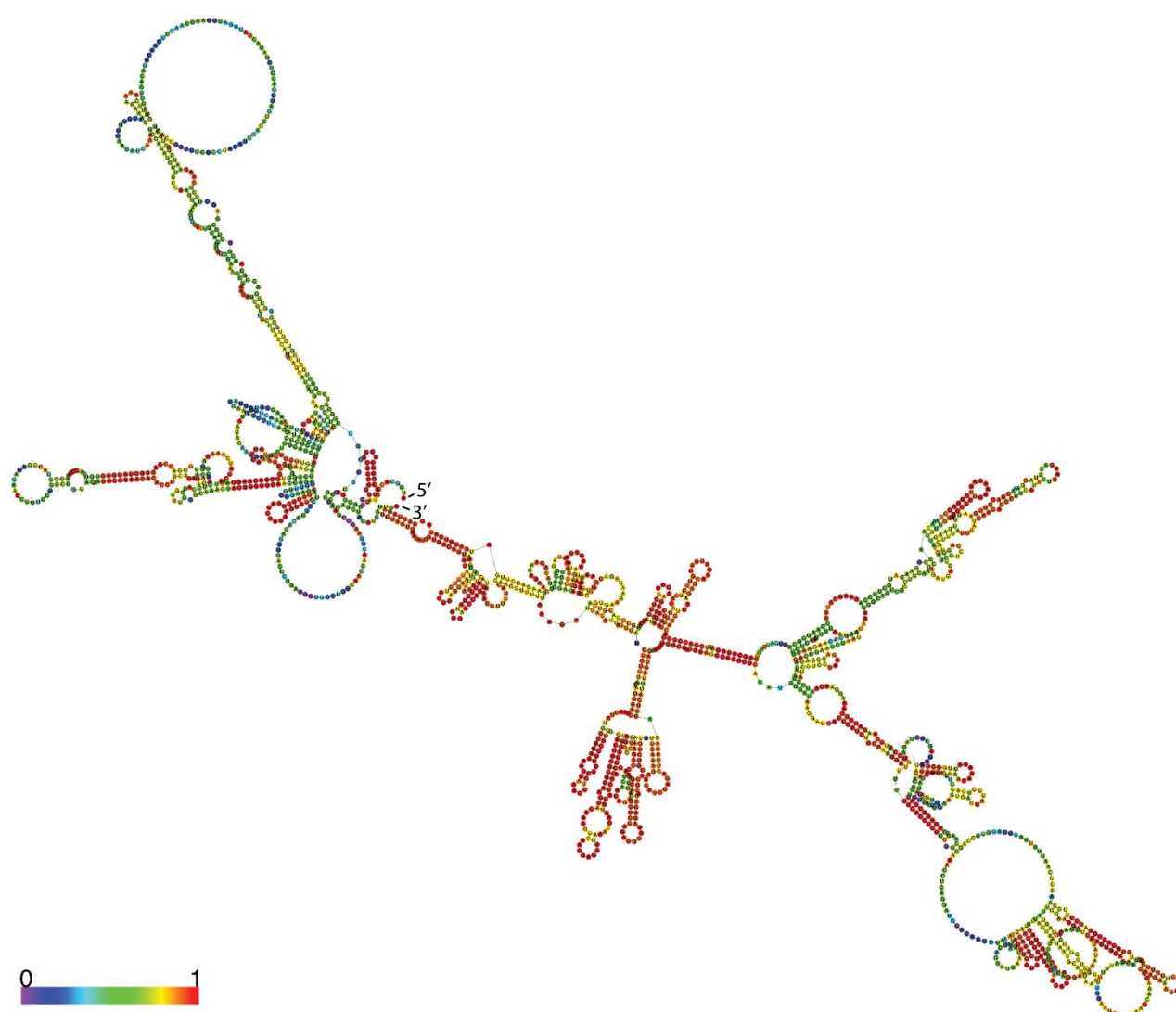


Figure S2. Partition structure of the folding of the mRNA, encoding Homo sapiens UBA2 gene. The model structure is obtained by the RNAfold web server version 2.4.8.

RNA Reference Sequence (RefSeq) was performed for ASO1's target (5'-AAUUAGGUAAAGAAGCAAAAUC) in the GeneBank database to compare it with the curated data in both human and bacterial organisms. The blast results demonstrate the absence of full 100% query coverage in other bacterial organisms with the targeted sequence in the guanylate kinase region in *S. aureus*. Due to varying mismatches, the highest % of similarity is 68%, and it is observed as demonstrated in **Figure S5A**:

- *Prolinoborus fasciculus* (syn. *Aquaspirillum fasciculus*)
- *Legionella lansingensis*

The same analysis was performed for similarities in the human organism. The results demonstrated a maximum % query coverage of 90% with the targeted sequence in the predicted ncRNA 2778 (LINC02778), followed by 81% in transcript variant X3 in LOC105372520 ncRNA (LOC105372520).

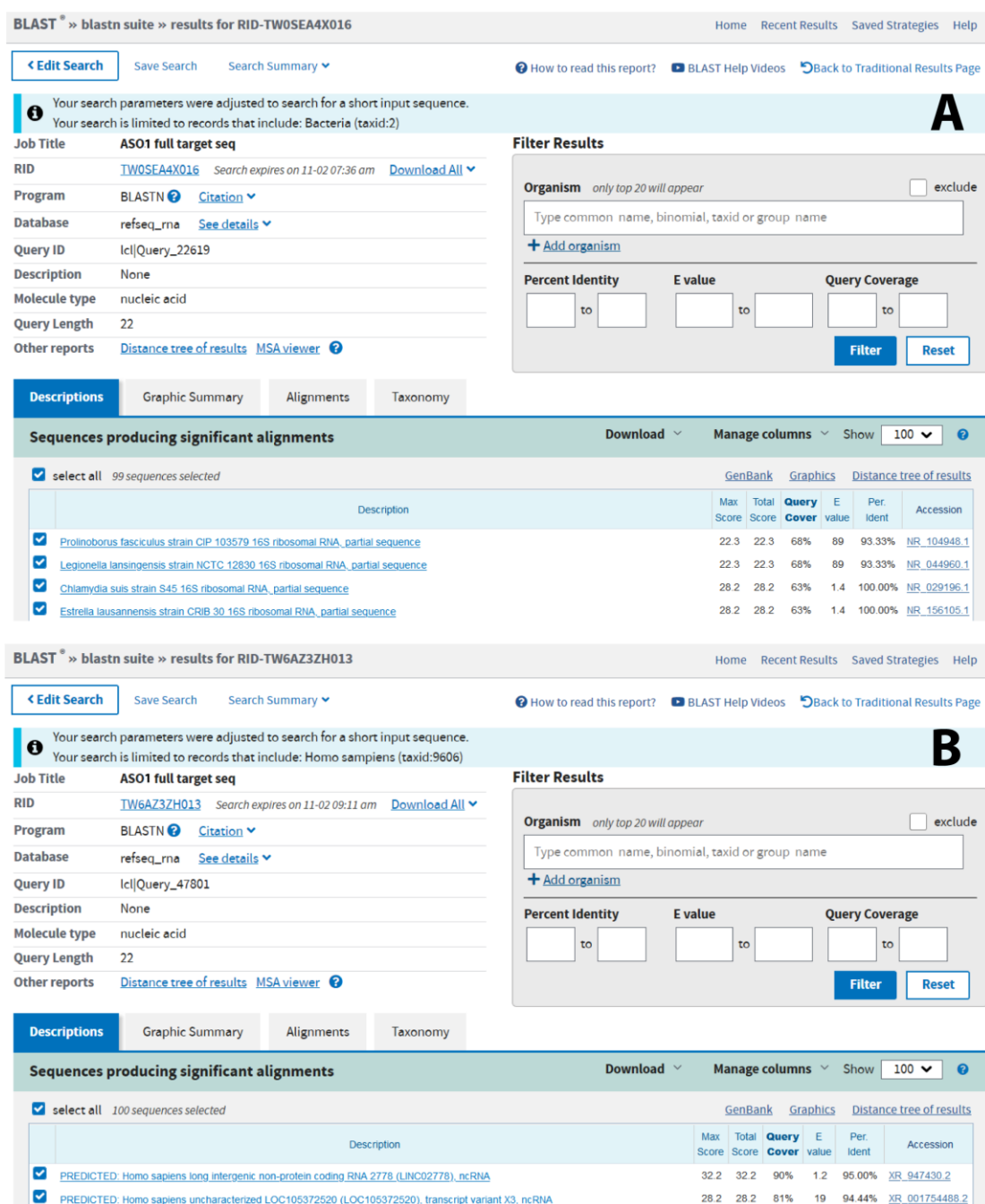


Figure S3. RNA Reference Sequence (RefSeq) analysis for similarities of ASO1's target with bacterial and human organisms in the GeneBank database. (A) RefSeq results for similarities of ASO1's target with bacterial microorganisms. (B) RefSeq for similarities of ASO1's target within the human organism.

RNA Reference Sequence (RefSeq) was performed for ASO2's target (5'-AATGAAGCAAGAAAAGAAGTTG) in the GeneBank database to compare it with the

curated data in both human and bacterial organisms. The BLAST results demonstrate the absence of full 100% query coverage in other bacterial organisms with the targeted sequence in the guanylate kinase region in *S. aureus*. Due to varying mismatches, the highest % of similarity is 86%, which is observed in the Gram-negative *Candidatus blochmannia*, as demonstrated in Figure S5A.

The same analysis was performed for similarities in the human organism. The results demonstrated a maximum % query coverage of 72% between the targeted sequence when there are no gaps (100% identity) and the following:

- 3 transcript variants of the Rho GTPase activating protein 30 mRNA (1, 3, and 4);
- 4 predicted transcript variants of the Rho GTPase activating protein 30 mRNA (X1, X2, X3, and X4);
- 2 predicted transcript variants of uncharacterized LOC105377508 ncPHK (1 and 2).

However, if we allow aligning the results with some gaps or mismatches instead of a 100% identity score, we have several hits that might explain our experimental data. They are in transcription variant of the proto-oncogene BRAF (95% query cover, 95.24% identity), purine-rich element binding protein B (PURB) mRNA (90% query cover, 95% identity), and transcription variants of the center (CNTLN) mRNA (90% query cover, 95% identity). There were also several more predicted variants of those targets among the results.

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Query ID lcl|Query_2689

Description None

Molecule type nucleic acid

Query Length 22

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Descriptions Graphic Summary Alignments Taxonomy

Sequences producing significant alignments

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	Description	GenBank	Graphics	Distance tree of results
		Max Score	Total Score	Query Cover E value Per ident Accession
<input checked="" type="checkbox"/>	Candidatus Blochmannia vafer strain RVAF 23S ribosomal RNA gene, complete sequence	22.3	42.6	86% 89 100.00% NR_076781.1
<input checked="" type="checkbox"/>	Cyanobacterium sp. PCC 10605 strain PCC 10605 23S ribosomal RNA, complete sequence	24.3	64.9	72% 23 100.00% NR_102522.1

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B

Job Title Nucleotide Sequence

RID 3JUF0F2J01R Search expires on 02-28 04:36 am [Download All](#)

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Database refseq_ma [See details](#)

Query ID lcl|Query_304595

Description None

Molecule type nucleic acid

Query Length 22

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	Description	Scientific Name	Max Score	Total Score	Query Cover	E value	Per ident	Acc. Len	Accession
<input checked="" type="checkbox"/>	Homo sapiens R-Raf proto-oncogene, serine/threonine kinase (BRAF), transcript variant 13, mRNA	Homo sapiens	34.2	34.2	95%	0.18	95.24%	5062	NM_001378474.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens uncharacterized LOC105372310 (LOC105372310), transcript variant X4, ncRNA	Homo sapiens	34.2	34.2	95%	0.18	95.24%	1732	XR_001754065.1
<input checked="" type="checkbox"/>	Homo sapiens centelin (CNTLN), transcript variant 2, mRNA	Homo sapiens	32.2	64.7	90%	0.72	95.00%	4666	NM_001114395.3
<input checked="" type="checkbox"/>	Homo sapiens uric acid element binding protein 2 (PUB2), mRNA	Homo sapiens	32.2	54.5	90%	0.72	95.00%	9232	NM_033224.5
<input checked="" type="checkbox"/>	Homo sapiens centelin (CNTLN), transcript variant 1, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	5518	NM_017738.4
<input checked="" type="checkbox"/>	Homo sapiens centelin (CNTLN), transcript variant 5, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	5515	NM_001365029.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X6, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	4554	XM_024447583.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X4, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	5366	XM_006716793.4
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X3, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	4394	XM_017014840.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X10, misc. RNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	3443	XR_929282.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X9, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	3341	XM_017014843.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X8, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	4345	XM_017014842.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X7, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	3344	XM_011517941.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X5, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	5372	XM_017014841.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens centelin (CNTLN), transcript variant X2, mRNA	Homo sapiens	32.2	32.2	90%	0.72	95.00%	5682	XM_017014839.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens uncharacterized LOC105373942 (LOC105373942), transcript variant X2, ncRNA	Homo sapiens	30.2	54.5	86%	2.8	100.00%	7232	XR_001739942.2
<input checked="" type="checkbox"/>	Homo sapiens microtubule associated monooxygenase, calponin and LIM domain containing 2 (MICAL2), tr--	Homo sapiens	30.2	30.2	86%	2.8	94.74%	6658	NM_001282666.1
<input checked="" type="checkbox"/>	Homo sapiens microtubule associated monooxygenase, calponin and LIM domain containing 2 (MICAL2), tr--	Homo sapiens	30.2	30.2	86%	2.8	94.74%	4259	NM_001282665.1
<input checked="" type="checkbox"/>	Homo sapiens lamin subunit alpha 3 (LAMA3), transcript variant 2, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	5618	NM_000227.8
<input checked="" type="checkbox"/>	Homo sapiens lamin subunit alpha 3 (LAMA3), transcript variant 4, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	5450	NM_001127718.4
<input checked="" type="checkbox"/>	Homo sapiens lamin subunit alpha 3 (LAMA3), transcript variant 3, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	10483	NM_001127717.4
<input checked="" type="checkbox"/>	Homo sapiens lamin subunit alpha 3 (LAMA3), transcript variant 1, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	10651	NM_198129.4
<input checked="" type="checkbox"/>	Homo sapiens ral guanine nucleotide dissociation stimulator like 1 (RGL1), transcript variant 4, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	4725	NM_001297871.3
<input checked="" type="checkbox"/>	Homo sapiens FKBP prolyl isomerase 14 (FKBP14), transcript variant 1, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	4978	NM_017946.1
<input checked="" type="checkbox"/>	Homo sapiens ral guanine nucleotide dissociation stimulator like 1 (RGL1), transcript variant 2, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	5076	NM_001297869.3
<input checked="" type="checkbox"/>	Homo sapiens ral guanine nucleotide dissociation stimulator like 1 (RGL1), transcript variant 5, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	4638	NM_001297872.3
<input checked="" type="checkbox"/>	Homo sapiens ral guanine nucleotide dissociation stimulator like 1 (RGL1), transcript variant 1, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	5077	NM_015149.6
<input checked="" type="checkbox"/>	Homo sapiens ral guanine nucleotide dissociation stimulator like 1 (RGL1), transcript variant 3, mRNA	Homo sapiens	28.2	28.2	81%	11	94.44%	4966	NM_001297870.3
<input checked="" type="checkbox"/>	Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant 3, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4197	NM_001287600.2
<input checked="" type="checkbox"/>	Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant 4, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	3811	NM_001287602.2
<input checked="" type="checkbox"/>	Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant 1, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4342	NM_001025588.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens uncharacterized LOC105377508 (LOC105377508), transcript variant X2, ncRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	3572	XR_939398.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens uncharacterized LOC105377508 (LOC105377508), transcript variant X1, ncRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	3589	XR_939397.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant X4, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4178	XM_005245073.3
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant X3, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4256	XM_011509391.2
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant X2, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4023	XM_017009860.1
<input checked="" type="checkbox"/>	PREDICTED: Homo sapiens Rho GTPase activating protein 30 (ARHGAP30), transcript variant X1, mRNA	Homo sapiens	32.2	32.2	72%	0.72	100.00%	4254	XM_005245070.2

Figure S4. RNA Reference Sequence (RefSeq) analysis for similarities of ASO2's target with bacterial and human organisms in the GeneBank database.

(A) RefSeq results for similarities of ASO2's target with bacterial microorganisms. **(B)** RefSeq for similarities of ASO2's target within the human organism. With blue lines and arrows marked, the two types of maximum % hits are either query cover but lack of whole identity or the complete 100% identity with lower query cover than the targeted sequence.

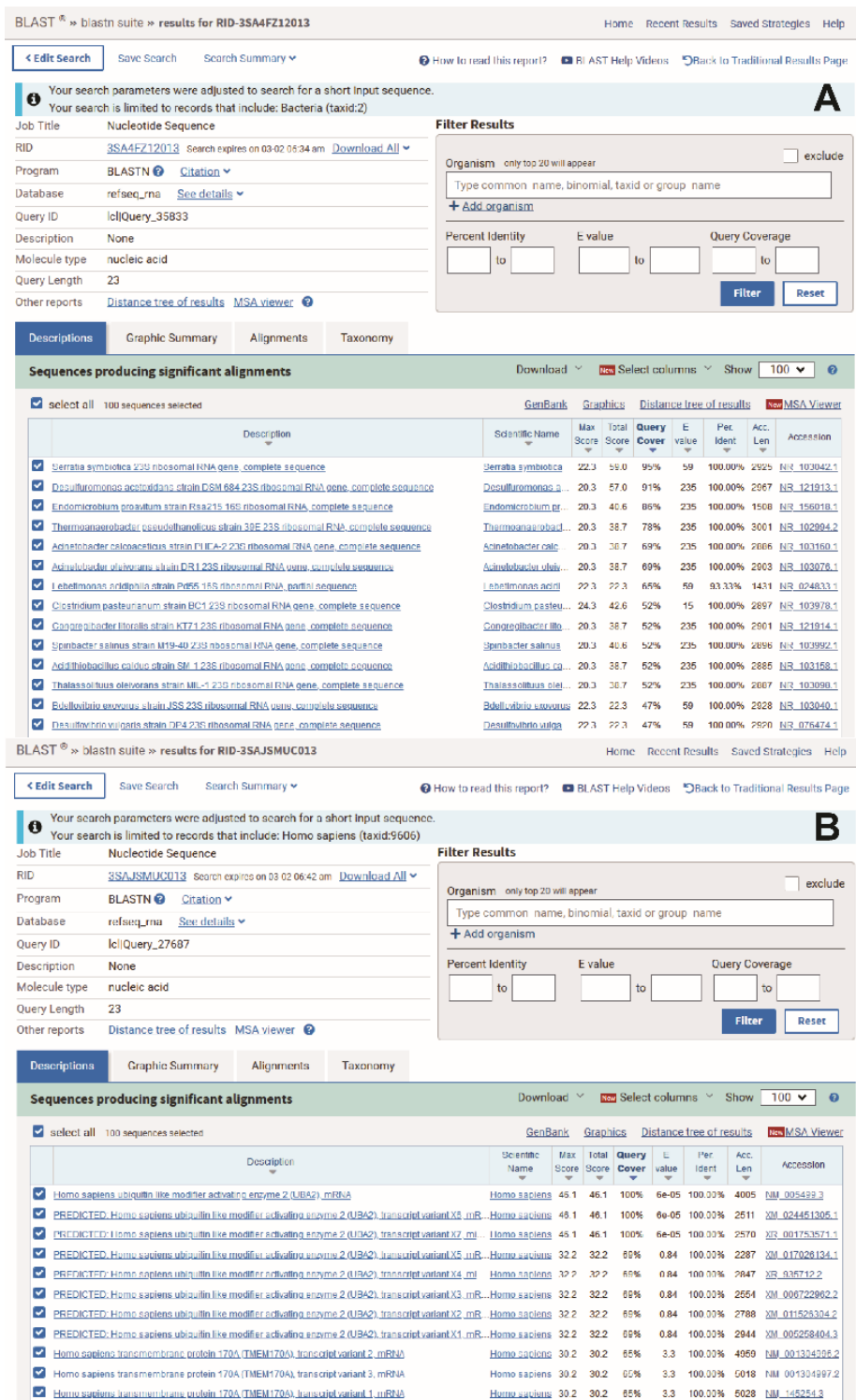


Figure S5. RNA Reference Sequence (RefSeq) analysis for similarities of ASO3's target with bacterial and human organisms in the GeneBank database.

(A) RefSeq results for similarities of ASO3's target with bacterial microorganisms. **(B)** RefSeq for similarities of ASO3's target within the human organism.

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Program	? Citation ▼
Database	refseq_rna See details ▼
Query ID	lcl Query_54379
Description	None
Molecule type	nucleic acid
Query Length	11
Other reports	?

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A No significant similarity found. For reasons why [click here](#)

Figure S6. RNA Reference Sequence (RefSeq) analyzes similarities of ASO4's hypothetical target with all organisms in the GeneBank database.

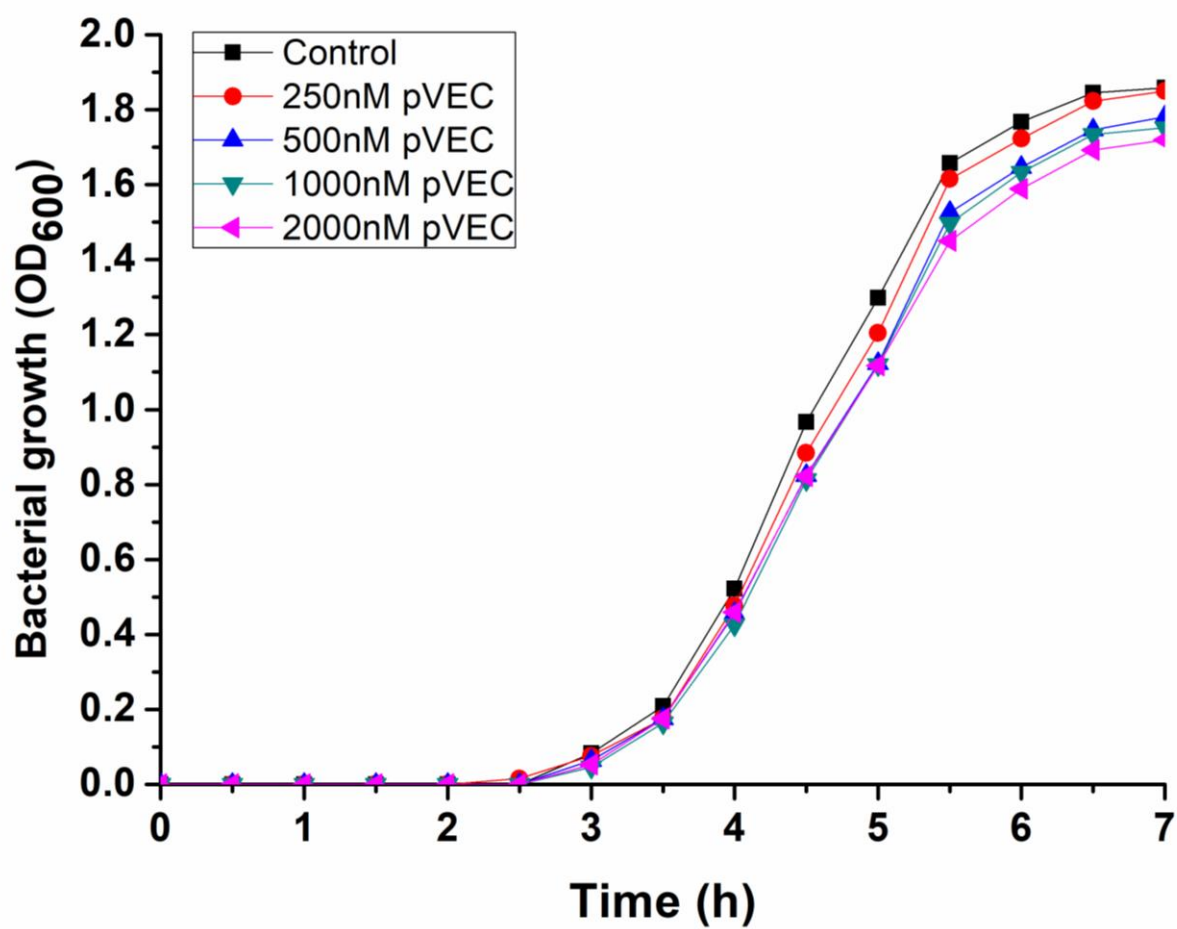


Figure S7. Growth curve of *S. aureus* (strain ATCC 25 923) cultured in LB medium. Bacteria were treated with different concentrations of pVEC: 250nM (red line), 500nM (blue line), 1000nM (green line), and 2000nM (purple line). Treated bacteria were compared to the control with untreated bacteria (black line). The measurements were assessed in cuvettes by spectrophotometer at 600nm wavelength.

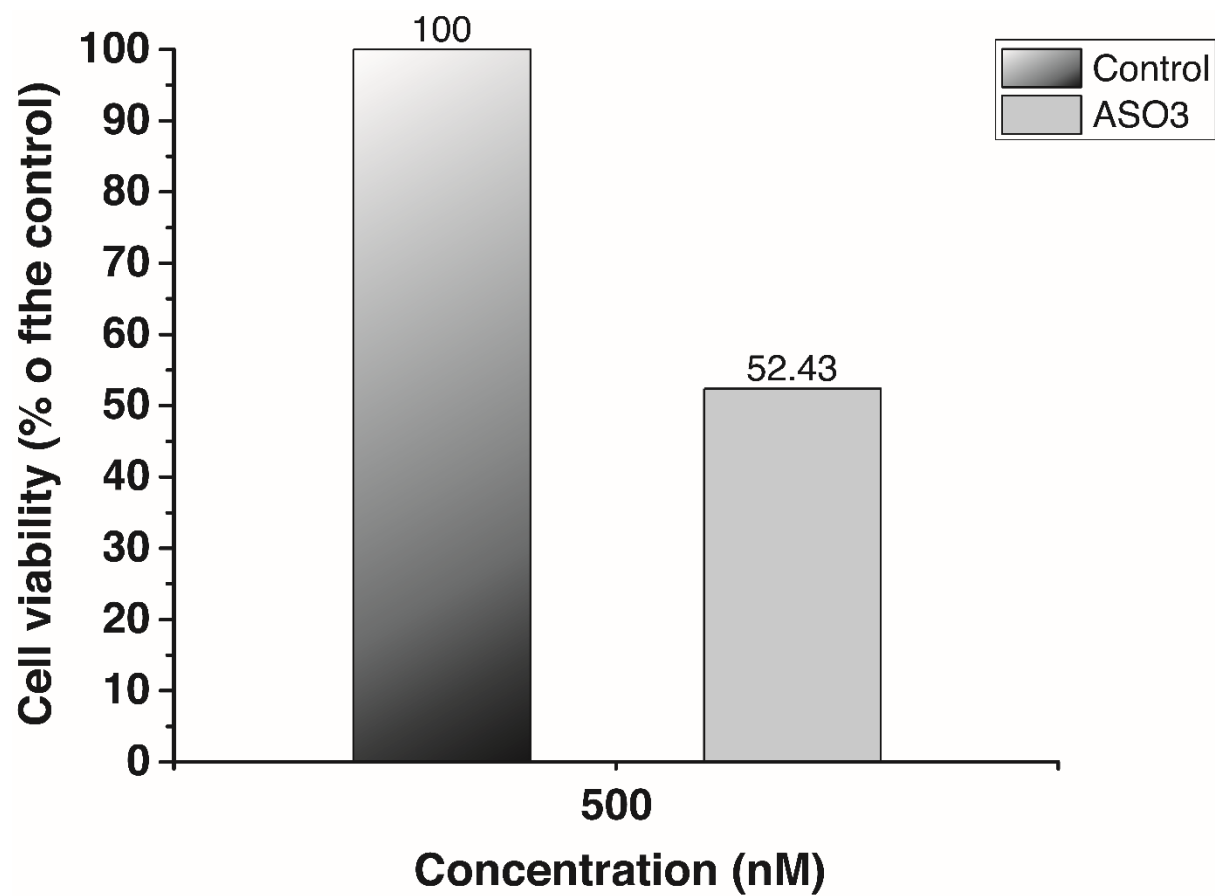


Figure S8. MTT viability assay of the colorectal adenocarcinoma HT29 cell line was measured at 540/620nm. Results are expressed as the percentage of the cell viability relative to the control. The 500nM pVEC-ASO3 is tested after exposure for 72h.