Supplementary Information

miRNA-mediated alteration of Sulfatase Modifying Factor 1 expression using self-assembled branched DNA nanostructures

Kanchan Kumari^{1,2}, Avishek Kar¹, Ashok K. Nayak¹, Sandip K. Mishra³, Umakanta Subudhi^{1,4,*}

¹DNA Nanotechnology & Application Laboratory, CSIR-Institute of Minerals & Materials Technology, Bhubaneswar, 751013, India ²Department of Molecular Biology, Umea University, Sweden. ³Cancer Biology Laboratory, Institute of Life Sciences, Bhubaneswar, 751023, India.

⁴Academy of Scientific & Innovative Research (AcSIR), Ghaziabad-201002, Uttar Pradesh, India.

* Corresponding author

Dr Umakanta Subudhi, Senior Scientist,

DNA Nanotechnology & Application Laboratory, CSIR-Institute of Minerals & Materials Technology, Bhubaneswar, 751013. Email id: <u>usubudhi@immt.res.in</u>, <u>subudhisai@gmail.com</u>



Figure S1. Schematic presentation of self-assembled branched DNA (bDNA) nanostructures containing (a) scramble sequences, (b) antimiR sequences of has-miR-106b-5p, (c) antimiR sequences of has-miR-128-3p, (d) antimiR sequences of has-miR-148b-3p, (e) antimiRNAs sequences of 106, 128 and duplicate sequence of antimiR-148 which named as antimiR-bDNA-mix and (f) bDNA-Alex488 having fluorescein labeled at the 5' end of the oligo B.

3'UTR Sequence of SUMF1: Length-998bp

>hg38_knownGene_ENST00000272902.10 range=chr3:4361146-4362143 5'pad=0
3'pad=0 strand=- repeatMasking=none

CAACCAAGGAAAGTCTTCCCCAGTCCAAGGAGCAGTCGTGTCTGACCTAC ATTGGGCTTTTCTCAGAACTTTGAACGATCCCATGCAAAGAATTCCCACC CTGAGGTGGGTTACATACCTGCCCAATGGCCAAAGGAACCGCCTTGTGAG ACCAAATTGCTGACCTGGGTCAGTGCATGTGCTTTATGGTGTGGTGCATC TTTGGAGATCATCACCATATTTTACTTTTGAGAGTCTTTAAAGAGGAAGG GGAGTGGAGGGAACCCTGAGCTAGGCTTCAGGAGGCCCGCGTCCTACGCA GGCTCTGCCACAGGGGTTAGACCCCAGGTCCGACGCTTGACCTTCCTGGG CCTCAAGTGCCCTCCCCTATCAAATGAAGGGATGGACAGCATGACCTCTG GGTGTCTCTCCAACTCACCAGTTCTAAAAAGGGTATCAGATTCTATTGTG ACTTCATAGTGAGAATTTATTATAGATTATTTTTTAGCTATTTTTTCCAT **GTGTGAACCTTGAGTGATACTAATCATGTAAAGTAAGAGTTCTCTTATGT** ATTATTTTCGGAAGAGGGGTGTGGTGACTCCTTTATATTCGTACTGCACT TTGTTTTTCCAAGGAAATCAGTGTCTTTTACGTTGTTATGATGAATCCCA CATGGGGCCGGTGATGGTATGCTGAAGTTCAGCCGTTGAACACATAGGAA TGTCTGTGGGGTGACTCTACTGTGCTTTATCTTTTAACATTAAGTGCCTT TGGTTCAGAGGGGCAGTCATAAGCTCTGTTTCCCCCCTCTCCCCAAAGCCT TCAGCGAACGTGAAATGTGCGCTAAACGGGGGAAACCTGTTTAATTCTAGA TATAGGGAAAAAGGAACGAGGACCTTGAATGAGCTATATTCAGGGTATCC GGTATTTTGTAATAGGGAATAGGAAACCTTGTTGGCTGTGGAATATCCGA TGCTTTGAATCATGCACTGTGTTGAATAAACGTATCTGCTAAATCAGG

Figure S2. The nucleotide sequence of 3'UTR of SUMF1 used to study the miRNA binding sites.



Figure S3. Expression of SUMF1 in BT474 upon transfection with (a,b) miRNA-106, 128 and 148 sequences and (b) bDNA structures containing antimiR sequences for miRNA-106, 128 and 148 as observed in qRT-PCR.

Sl. No.	Oligo name	Sequences						
1	scramble-A	5' TGCAGCTACCTCGCCTTG TTT CTGAGGAGAGCAGCGCCTTGGCCAGCGCCTC TTT TGCAGCTACCTCGCCTTG 3'	72					
2	oligo B	5' CTGAGTGACGTTGTG TTT GAGGCGCTGGCCAAGCGCTGCTCTCCTCAG TTT CGAATGGAGAGGCAG 3'	66					
3	Oligo B/Alex488N	5' Alex488N CTGAGTGACGTTGTG TTT GAGGCGCTGGCCAAGCGCTGCTCTCCTCAG TTT CGAATGGAGAGGCAG 3'	66					
4	oligo C	5' CTGCCTCTCCATTCG TTT TGAGCAGAAGGCAAGTAGCAGGACAGCAGA TTT CACAACGTCACTCAG 3'	66					
5	scramble-D	5' TGCAGCTACCTCGCCTTG TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT TGCAGCTACCTCGCCTTG 3'	72					
6	antimiR-106 & 128A	5' ATCTGCACTGTCAGCACTTTA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT AAAGAGACCGGTTCACTGTGA 3'	78					
7	antimiR-106 A	5' ATCTGCACTGTCAGCACTTTA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT ATCTGCACTGTCAGCACTTTA 3'	78					
8	antimiR-106D	5' ATCTGCACTGTCAGCACTTTA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT ATCTGCACTGTCAGCACTTTA 3'	78					
9	antimiR-128A	5' AAAGAGACCGGTTCACTGTGA TTT CTGAGGAGAGCAGCGCTTGGCCAGCGCCTC TTT AAAGAGACCGGTTCACTGTGA 3'	78					
10	antimiR-128D	5' AAAGAGACCGGTTCACTGTGA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT AAAGAGACCGGTTCACTGTGA 3'	78					
11	antimiR-148A	5' ACAAAGTTCTGTGATGCACTGA TTT CTGAGGAGAGCAGCGCCTTGGCCAGCGCCTC TTT ACAAAGTTCTGTGATGCACTGA 3'	80					
12	antimiR-148D	5' ACAAAGTTCTGTGATGCACTGA TTT TCTGCTGTCCTGCTACTTGCCTTCTGCTCA TTT ACAAAGTTCTGTGATGCACTGA 3'	80					
13	hsa-miR-106b-5p(RvTs)	5' GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGAC ATCTGC 3'	50					
14	hsa-miR-128-3p (RvTs)	5' GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACAAAGAG 3'	50					
15	hsa-miR-148b-3p (RvTs)	5' GTCGTATCCAGTGCAGGGTCCGAGGTATTCGCACTGGATACGACACAAAG 3'	50					
16	miR SL_Universal (RT-R)	5' GTCGTATCCAGTGCAGGGT 3'	21					
17	hsa-miR-148b-3p (RT-F)	5' AACAGTGTCAGTGCATCACAG 3'	18					
18	hsa-miR-106b-5p(RT-F)	5' AACACGTGTAAAGTGCTGACA 3'	20					
19	hsa-miR-128-3p (RT-F)	5' AACAGTGTCACAGTGAACCG 3'	18					
20	antimiR-106b-5p	5' ATCTGCACTGTCAGCACTTTA 3'	21					
21	antimiR-128-3p	5' AAAGAGACCGGTTCACTGTGA 3'	21					
22	antimiR-148b-3p	5' ACAAAGTTCTGTGATGCACTGA 3'	22					
23	hsa-miR-148b-3p	5' TCAGTGCATCACAGAACTTTGT 3'	22					
24	hsa-miR-106b-5p	5' TAAAGTGCTGACAGTGCAGAT 3'	21					
25	hsa-miR-128-3p	5' TCACAGTGAACCGGTCTCTTT 3'	21					
26	Scramble	5' TGCAGCTACCTCGCCTTG 3'	18					
27	SUMF1 F	5' CCATCCCTGCTGGAGTA 3'	17					
28	SUMF1 R	5' GGTCTTCACTTGCTCACTC 3'	19					
29	18S RNA F	5' GTAACCCGTTGAACCCCATT 3'	20					
30	18S RNA R	5' CCATCCAATCGGTAGTAGCG 3'	20					

Table S1. Sequence of oligonucleotides used in the study including the self-assembly of bDNA structures. Primer sequence for reverse transcription of miRNAs, real time primers for genes and miRNA, miRNA oligonucleotides for miRNAs and antimiRs.

Table S2. AllgenPromo-based analysis for identifying binding sites of important transcription factors at 3kb upstream promoter of SUMF1. Factors predicted within a dissimilarity margin less than or equal to 15%.

0	CUTL1 [T00100]	1	HNF-1B [T01950]	2	HNF-1C [T01951]	3	USF2b [T02377]	4	OC-2 [T03259]	5	oJun [T00133]	6	HNF-3beta [T02513]	7	SRY [T00997]
8	LCR-F1 [T01599]	9	1300 [T01427]	10	647 [T00207]	11	Elk-1 [T00250]	12	TCF-1A [T00999]	13	LEF-1 [T02905]	14	TCF-4E [T02878]	15	HMG I(Y) [T02368]
1	6 STAT4 [T01577]	17	o-Es-1 [T00112]	18	R2 [T00712]	19	Pu box binding factor [T00704]	20	HOXD9 [T01424]	21	HOXD10 [T01425]	22	GATA-1 [T00306]	23	GATA3 [T00311]
2	4 PR B [T00696]	25	PR A [T01661]	26	GR-alpha [T00337]	27	POU3F2 [T00630]	28	TGIF [T04076]	29	o-Fos [T00123]	30	Pax-2 [T01823]	31	AP-2alphaA [T00035]
3	2 TBP [T00794]	33	TMF [T00835]	34	GR-beta [T01920]	35	POU2F2 (Oct-2.1) [T00646]	36	DBP [T04875]	37	CDX2 [T03246]	38	FOXP3 [T04280]	39	TFIID [T00820]
4	0 STATSA [T04683]	41	o-Ets-2 [T00113]	42	Cart-1 [T03978]	43	NF-AT4 [T01946]	44	NF-AT3 [T02462]	45	RelA.[T00594]	46	HSF1 (long) [T01042]	47	HSF1 (short) [T02104]
4	8 HNF-3alpha [T02512]	49	Nex2-1 [T00857]	50	TFIIB [T00818]	51	NF-X3 [T01514]	52	Oct-B1 [T00545]	53	POU2F2B [T00662]	54	C/EBPalpha [T00105]	55	C/EBPbeta [T00581]
5	6 LF-A1 [T00467]	57	RORalphal [T01527]	58	COUP-TF1 [T00149]	59	ER-heta [T(4651]	60	RAR-beta [T00721]	61	SF-1 [T02769]	62	ER-alpha [T00261]	63	POU6F1 [T04470]
6	4 POU2F2C [T00665]	65	ENKTF-1 [T00255]	66	HNF-1A [T00368]	67	NFdehaE3A [T00976]	68	AML1 [T01067]	69	AML1a [T02256]	70	AP-4 [T00036]	71	WT11[T01840]
7	2 AP-3 (2) [T00039]	73	NHP-1 [T00621]	74	EllaE-A [T00246]	75	FOXO3a [T02938]	76	Hf[T01071]	77	Pbx1b [T02087]	78	NF-1 [T00539]	79	T3R-beta1 [T00851]
8	0 YY1 [T00915]	81	TCF-4 [T02918]	82	PXR-1:RXR-alpha [T05671]	83	T3R-alpha1 [T01152]	84	My6-3 [T00519]	85	MyoD (T00525)	86	USF2 [T00878]	87	AR.[T00040]
8	8 RAR-alpha1 [T00719]	89	ARP-1 [T00045]	90	RXR-alpha [T01345]	91	MRF-2 [T04675]	92	AIRE [T05990]	93	Nex3-1 [T04255]	94	NFI/CTF [T00094]	95	Pax-6 [T01122]
9	6 oMyb[T00137]	97	AP-1 [T00029]	98	RAR-gamma [T00720]	99	IRF-2 [T01491]	100	p63 [T00671]	101	TGT3 [T00833]	102	FOXJ2 (long isoform) [T04169]	103	NF-Y [T00150]
1	04 Nrf2 [T01443]	105	POU2F1 [T00641]	106	POU2F2 [T00647]	107	octamer-binding factor [T01225]	108	MZF-1 [T00529]	109	NF-AT2 [T01945]	1 10	NF-AT1 [T01948]	111	STATIbeta [T01573]
1	12 MBF1 [T00492]	113	SXR:RXR-alpha [T05670]	114	Myf-5 [T00521]	115	Tal-1 [T00790]	116	RAR-beta:RXR-alpha [T05420]	117	WT11-KTS[T00900]	118	WT1 -KTS [T01839]	1 19	E2F-1:DP-1 [T05204]
1	20 BTEB3 [T05051]	121	Amt [T01346]	122	USF1 [T00874]	123	AhR [T01795]	124	Pax-5 [T00070]	125	AhR: Armt [T05394]	126	Chx10 [T04139]	127	VDR [T00885]
Ľ	28 PEA3 [T00685]	129	DP-1 [T01548]	130	E2F-1 [T01542]	131	HNF-4alpha [T03828]	132	RP58 [T05040]	133	Sp1 [T00759]	134	CREMalpha [T01803]	135	ATF [T00051]
1	36 ATE-1 [T00968]	137	E2F [T00221]	138	AREB6 [T00625]	139	RFX1 [T01673]	140	Fra-1 [T01462]	141	JunD [T01978]	142	Sp3 [T02338]	143	c-Myc [T00140]
ŀ	44 DEC1 [T05838]	145	ETF [T00270]	146	E2F-5 [T01607]	147	HOX11 [T02054]	148	CREB [T00163]	149	ATF-2 [T00167]	150	aMEF-2 [T01006]	151	PPAR-alpha:RXR-alpha [T05221]
1	52 <u>lk-1 (T02702)</u>	153	XBP-1 [T00902]	154	ANF [T00025]	155	FOX11 [T02474]	156	TFII-I [T00824]	157	WT1 [T00899]	158	GATA-2 [T00308]	159	IRF-1 [T00423]
1	60 RF-3 (T04673)	161	NF-AT1 [T00550]	162	Egt-3 [T00243]	163	CRE [T00170]	164	Phx1.[T06000]	165	CTF [T00174]	166	PKNOX1 [T04122]	167	IPF1 [T04362]
1	68 E4F1 [T00223]	169	RORalpha2 [T01528]	170	E12 [T00204]	171	NF-E2 [T00558]	172	54F (T00222)	173	T3R-alpha [T00838]	174	ERRalphal [T05682]	175	RAR-beta2 [T01326]