

Overexpression of pericentromeric HSAT2 DNA increases expression of EMT markers in human epithelial cancer cell lines

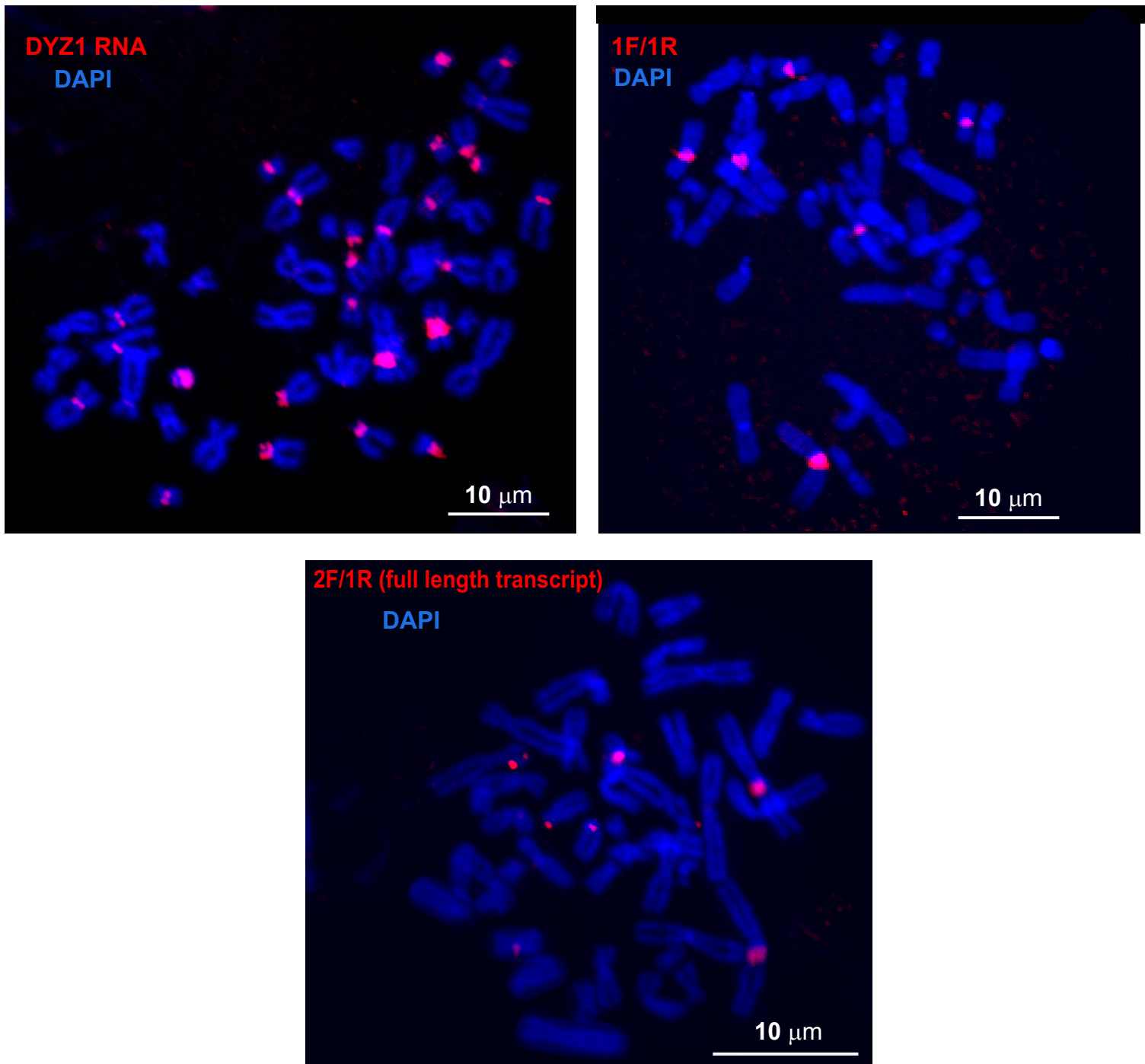
Nikita Ponomartsev^{1*}, Danil Zilov^{1,2*}, Ekaterina Gushcha¹, Alexandra Travina¹, Alexander Sergeev¹, Natella Enukashvily^{1*} •

¹Institute of Cytology, Russian Academy of Sciences, St. Petersburg, 194064, Russia;

²Applied Genomics Laboratory, SCAMT Institute, ITMO University, Saint Petersburg 197101, Russia;

- Two authors have contributed equally

*Correspondence: n.enukashvily@incras.ru (N.E.), ponomartsev@yandex.ru (N.P.)



Supplementary Figure S1. Pericentromeric localization of DYZ1, 1F/1R and 2F/1R sequences probed revealed by FISH.

Pericentromeric location of DYZ1 (used for fishing out 2F/1R RNA from transcriptome in our previous work [17 in the main text]), 1F/1R (cDNA 5') and 2F/1R (a full length transcript registered with TPA Acc No Bk063198) on mitotic chromosomes of human umbilical cord mesenchymal stem cell obtained as described in our previous publications (DOI: <https://doi.org/10.1016/j.jephar.2020.173182> and <https://doi.org/10.17816/mechnikov201810111-19>). All probes are shown in red. Chromosomes are counterstained with DAPI (blue). Scale bars are shown in the images.

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1 Institute of Cytology, Russian Academy of Sciences, St. Petersburg, 194064, Russia;

2 Applied Genomics Laboratory, SCAMT Institute, ITMO University, Saint Petersburg 197101, Russia;

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2F/1R      -----TTTTTTTTTTTTTTTTTTTTTTTTTTTTTCGATGATTGTTCCATTGATTCTG 53
HS1.1      ATATA-AGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGATTCTG 118
HS1.2      ATATATAGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGATTCTG 120
HS1.3      ATATAATGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGATTCTG 120
HS4.1      ATATAATGCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGATTCTG 120
HS4.2      ATATAA-GCAGAGCTGGTTTAGTGAACCGTCAGATCCGCTAGCGCTACCGGTCGATTCTG 119
           *   *   *   *   *   *   *   *   *   *   *   *   *   *   *

2F/1R      TTCGGTGATTCCATTGATTCCATTGATAATGATTCCGTCGAGACCATTGCGATGATTC 113
HS1.1      TTCGGTGATTCCATTGACTTAATTGATAATGATTCCATTGAGACCATTGCGATGATTC 178
HS1.2      TTCGGTGATTCCATTGACTTAATTGATAATGATTCCATTGAGACCATTGCGATGATTC 180
HS1.3      TTCGGTGATTCCATTGACTTAATTGATAATGATTCCATTGAGACCATTGCGATGATTC 180
HS4.1      TTCGGTGATTCCATTGACTTAATTGATAATGATTCCATTGAGACCATTGCGATGATTC 180
HS4.2      TTCGGTGATTCCATTGACTTAATTGATAATGATTCCATTGAGACCATTGCGATGATTC 179
           ***** * ***** * ***** * ***** * *****

2F/1R      CATTCAATCCATTCAATAAAGATTCCATTGAGTCCATTGATTCCCTTCAAGTCC 173
HS1.1      TATTCAATCCATTCAATAATGATTCCATTGAGTCCATTCAACGATTCCATTCAAGTCC 238
HS1.2      TATTCAATCCATTCAATAATGATTCCATTGAGTCCATTCAACGATTCCATTCAAGTCC 240
HS1.3      TATTCAATCCATTCAATAATGATTCCATTGAGTCCATTCAACGATTCCATTCAAGTCC 240
HS4.1      CATTCAATCCATTCAATAATGATTCCATTGAGTCCATTCAATGATTCCATTCCAGTCC 240
HS4.2      CATTCAATCCATTCAATAATGATTCCATTGAGTCCATTCAATGATTCCATTCCAGTCC 239
           ***** * * * * ***** * ***** * * * *

2F/1R      ATTCGATGATTCCATCGAGTCCATTCAATGAATCCATTGATTCATTGATGATGATT 233
HS1.1      ATTCGATGATTCCATCTGATTCCATTCAATGAATCCATGCGATTCCATTCTATGATGATT 298
HS1.2      ATTCGATGATTCCATCTGATTCCATTCAATGAATCCATGCGATTCCATTCTATGATGATT 300
HS1.3      ATTCGATGATTCCATCTGATTCCATTCAATGAATCCATGCGATTCCATTCTATGATGATT 300
HS4.1      ATTCGATGATTCCATCTGATTCCATTCAATGAATCCATGCGATTCCATTCTATGAGGACT 300
HS4.2      ATTCGATGATTCCATCTGATTCCATTCAATGAATCCATGCGATTCCATTCTATGAGGACT 299
           ***** * * * * ***** * * * *

2F/1R      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATTCCATTCAATTC 293
HS1.1      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATTCCATTCCATTCC 358
HS1.2      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATTCCATTCCATTCC 360
HS1.3      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATTCCATTCCATTCC 360
HS4.1      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATACCATTCGATTC 360
HS4.2      CCATTCAATCCATCTGATGATGATTCCATTGATTCATTCAATGATACCATTCGATTC 359
           ***** * * * * ***** * * * *

2F/1R      CATTGATGATGATTCAATCAATTCATTGCGTGATTCCATTGGAATCCACTCGATGAT 353
HS1.1      CATTGATGATGATTCAATCAATTCATTGCGTGATTCCACTGGAATCCACTCGATGAT 418
HS1.2      CATTGATGATGATTCAATCAATTCATTGCGTGATTCCACTGGAATCCACTCGATGAT 420
HS1.3      CATTGATGATGATTCAATCAATTCATTGCGTGATTCCACTGGAATCCACTCGATGAT 420
HS4.1      CATTGATGATGATTCAATCAATTTATTGATGATTCATTGGAATCCACTCGATGAT 420
HS4.2      CATTGATGATGATTCAATCAATTTATTGATGATTCATTGGAATCCACTCGATGAT 419
           ***** * * * * ***** * * * *

2F/1R      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 413
HS1.1      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 478
HS1.2      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 480
HS1.3      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 480
HS4.1      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 480
HS4.2      GAGTCCATCCATTCAATTCATGATAATTCCATTGTTTCCTTCGATGGCGTTCCAT 479
           *****

2F/1R      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 473
HS1.1      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 538
HS1.2      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 540
HS1.3      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 540
HS4.1      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 540
HS4.2      TCGATTCCATTGATGTTGATTCCATTGTTTCATTGGATGATGATTCCGTTTCGTGTC 539
           *****

2F/1R      ATTCGATGATGATCATATTGGATTTCATTCCATAATTCTATTGGAATCCATTGATGAT - 532
HS1.1      ATTCGATGATGATCATATTGGATTTCAGCAGTCGACGGTACCGCGGGCCCGGGATCCACC 598
HS1.2      ATTCGATGATGATCATATTGGATTTCAGCAGTCGACGGTACCGCGGGCCCGGGATCCACC 600
HS1.3      ATTCGATGATGATCATATTGGATTTCAGCAGTCGACGGTACCGCGGGCCCGGGATCCACC 600
HS4.1      ATTCGATGATGATCATATTGGATTTCAGCAGTCGACGGTACCGCGGGCCCGGGATCCACC 600
HS4.2      ATTCGATGATGATCATATTGGATTTCAGCAGTCGACGGTACCGCGGGCCCGGGATCCACC 599
           ***** * * *
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GenBank Acc No for the annotated sequence:
2F/1R - BankIt2670656 HSAT2 Bk063198

GenBank Acc No for cloned sequences:
HS1.1 - OP912403.2 ,
HS1.2 - OP912404.2,
HS1.3 - OP912405.2,
HS4.1 - OP912406.2,
HS4.2 - OP912407.2

Supplementary Figure S2. Alignment of 5 cloned sequences and the HSAT2 transcript's sequence (2F/1R) from assembled transcriptome. The sequence annotated in our previous work (registered with Acc No BankIt2670656 HSAT2 Bk063198) was used for 2F/1R primer set design. Genomic DNA was amplified using 2F/1R primers set (see Table 2 of the main text). The amplified fragments (HS1.1, HS1.2, HS1.3, HS4.1, HS4.2) were cloned and sequenced. All the obtained clones were aligned to original annotated sequence to confirm the results of cloning. Accession numbers for all the sequences are given in the purple frame in the image. BLAST tool was used for the alignment [57 of the main text]

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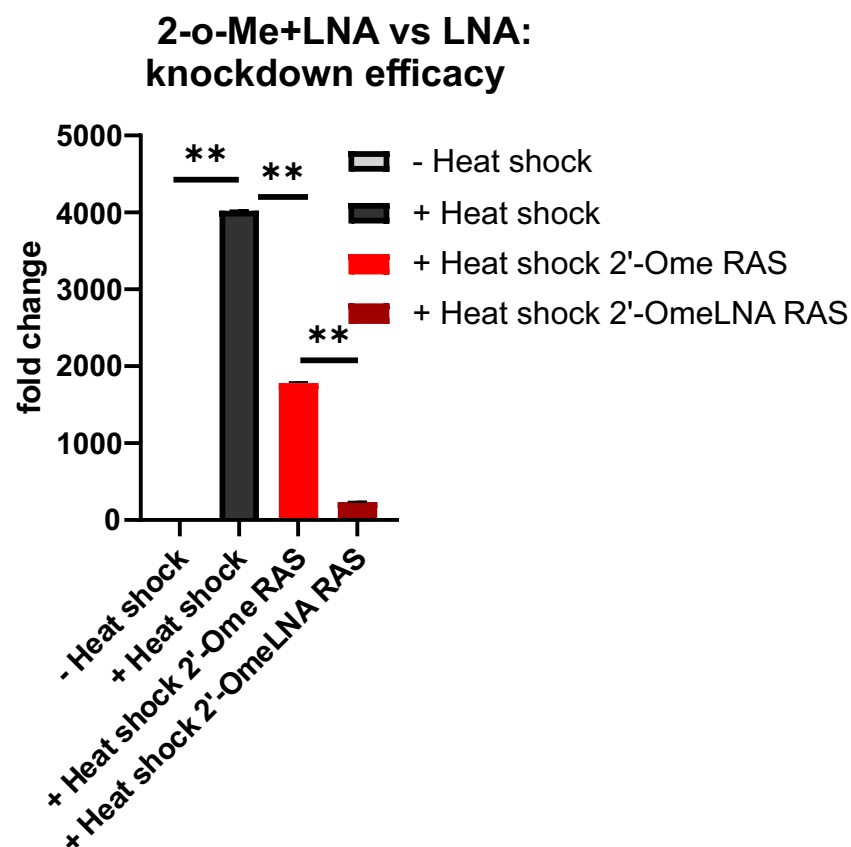
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Supplementary Figure S3. Efficacy of 2-o-Me+LNA oligonucleotides and LNA oligonucleotides in knockdown experiments on heat-shocked A549 cells

Heat shock is known as the most potent stimulator of HSAT2, HSAT3 transcription. Therefore, we used the model to choose a better modification pattern of antisense oligonucleotides. A549 were subjected to heat shock (60 min, 43°C). In an hour of recovery period, cells were collected and used for RNA purification, polydT cDNA synthesis and qPCR with 2F/1R primer set (see Table 2 of the main text) to quantify HSAT2 transcription in cells with (+Heat shock) and without (-Heat shock) heat shock treatment. RAS oligonucleotide (Table 2 of the main text) was modified: in 2'-OmeLNA RAS, four nucleotides at 3' and 5' ends were 2-o-methylated while the remaining core nucleotides were locked nucleic acid-(LNA)-nucleotides; in 2'-Ome RAS, all nucleotides were 2'-o-methylated. HSAT2 transcription level in cells of '-Heat shock' group (not stressed, not transfected with modified RAS) was set to 1. Transcription in other groups was quantified and measured as a fold change ratio to -Heat shock cells. ** - $p < 0.01$.