

## Supplementary Materials

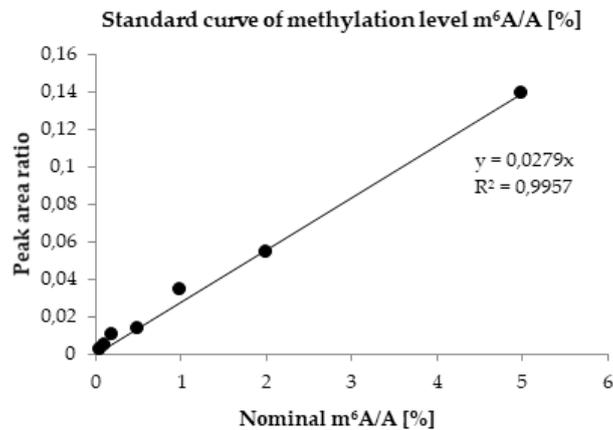


Figure S1. Standard curve of methylation level m<sup>6</sup>A/A [%].

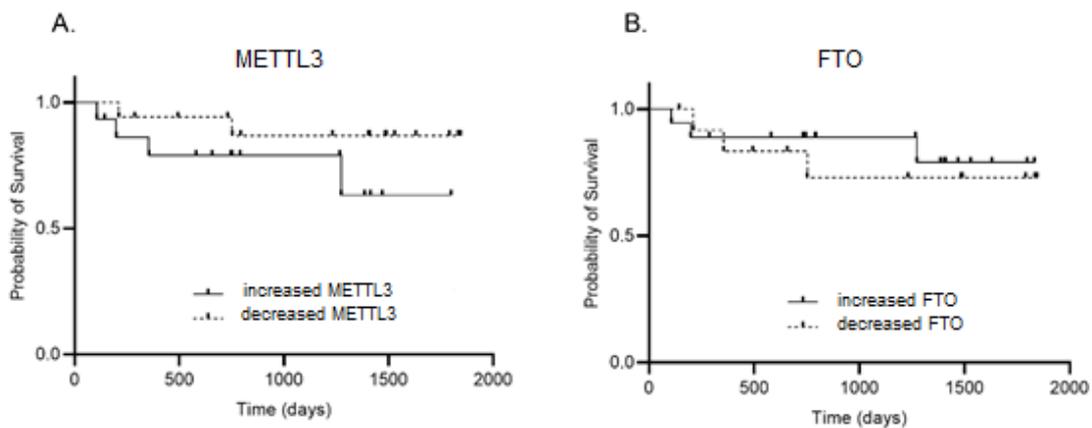


Figure S2. The Kaplan-Meier survival analysis of HNSCC patients according to increased/ decreased mRNA level of METTL3 (A) and FTO (B) genes in cancerous tissues compared to histopathologically unchanged tissues.

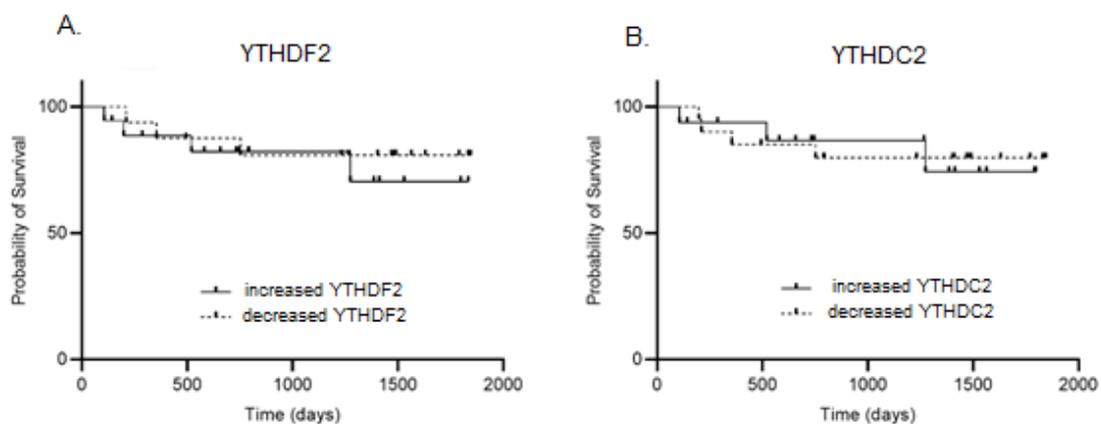


Figure S3. The Kaplan-Meier survival analysis of HNSCC patients according to increased/ decreased mRNA level of YTHDF2 (A) and YTHDC2 (B) genes in cancerous tissues compared to histopathologically unchanged tissues.

Table S1. Primers sequence used in qPCR analysis.

Gene	Sequence (5'-3')
<i>GAPDH</i>	F: GTCTCCTCTGACTTCAACAGCG R: ACCACCCTGTTGCTGTAGCCAA
<i>METTL3</i>	F: TTTTCCGGTTAGCCTTCGGG R: GATAGAGCTCCACGTGTCCG
<i>FTO</i>	F: ACTTGGCTCCCTTATCTGACC R: TGTGCAGTGTGAGAAAGGCTT
<i>YTHDF2</i>	F: AACTGCGACACATTGCGCTA R: TCTCTGGTTCCTCCTTCCCTAA
<i>YTHDC2</i>	F: TTGGATGAGGGCCTTTGGTTT R: CCATTGGTCCCTGTGTGCTCAC

F- forward primer

R- reverse primer

Table S2. m<sup>6</sup>A/A ratio in cancerous and histopathologically unchanged tissue samples from patients with HNSCC

Characteristic	Cancerous tissue	Histopathologically unchanged tissue	p-value
	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	
<b>Age at the time of surgery (years)</b>			
?60	0.11 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.04 <sup>b</sup>	0.74
>60	0.12 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.04 <sup>b</sup>	0.56
<b>Gender</b>			
Male	0.11 $\pm$ 0.03 <sup>b</sup>	0.11 $\pm$ 0.03 <sup>b</sup>	0.86
Female	0.13 $\pm$ 0.04 <sup>b</sup>	0.11 $\pm$ 0.04 <sup>b</sup>	0.23
<b>Tumor stage (TNM classification)</b>			
T1	0.12 $\pm$ 0.05 <sup>b</sup>	0.14 $\pm$ 0.05 <sup>b</sup>	0.64
T2	0.13 $\pm$ 0.05 <sup>b</sup>	0.11 $\pm$ 0.04 <sup>b</sup>	0.20
T3	0.10 $\pm$ 0.03 <sup>b</sup>	0.10 $\pm$ 0.02 <sup>b</sup>	0.82
T4	0.11 [0.08-0.21] <sup>a</sup>	0.11 [0.06-0.18] <sup>a</sup>	0.90
N0	0.11 [0.08-0.22] <sup>a</sup>	0.11 [0.06-0.17] <sup>a</sup>	0.71
N1	0.12 $\pm$ 0.04 <sup>b</sup>	0.12 $\pm$ 0.03 <sup>b</sup>	0.88
N2	0.12 $\pm$ 0.05 <sup>b</sup>	0.13 $\pm$ 0.04 <sup>b</sup>	0.76
N3	0.078 $\pm$ 0.03 <sup>b</sup>	0.084 $\pm$ 0.03 <sup>b</sup>	0.83
<b>Histologic grade</b>			
G1	0.14 [0.08-0.20] <sup>a</sup>	0.11 [0.09-0.18] <sup>a</sup>	0.48
G2	0.11 [0.04-0.22] <sup>a</sup>	0.11 [0.05-0.18] <sup>a</sup>	0.87
G3	0.11 [0.08-0.17] <sup>a</sup>	0.09 [0.06-0.18] <sup>a</sup>	0.39
<b>Anatomical site</b>			
Larynx	0.11 [0.08-0.17] <sup>a</sup>	0.10 [0.07-0.18] <sup>a</sup>	0.71
Oral cavity	0.11 [0.04-0.22] <sup>a</sup>	0.11 [0.05-0.18] <sup>a</sup>	0.95
Oropharynx	0.13 $\pm$ 0.02 <sup>b</sup>	0.09 $\pm$ 0.03 <sup>b</sup>	0.12

Depending on the data distribution we performed the U Mann-Whitney test<sup>a</sup> or unpaired t-test with Welch's correction

<sup>b</sup>.

**Table S3.** *METTL3* transcript levels in cancerous and histopathologically unchanged tissue samples from patients with HNSCC.

Characteristic	Cancerous tissue	Histopathologically unchanged tissue	p-value
	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	
<b>Age at the time of surgery (years)</b>			
?60	0.84 [0.24-2.90] <sup>a</sup>	0.92 [0.17-3.63] <sup>a</sup>	0.89
>60	0.89 [0.26-3.48] <sup>a</sup>	0.70 [0.31-3.38] <sup>a</sup>	0.40
<b>Gender</b>			
Male	0.83 [0.26-2.97] <sup>a</sup>	0.70 [1.7-3.38] <sup>a</sup>	0.73
Female	1.65 $\pm$ 1.17 <sup>b</sup>	1.38 $\pm$ 1.14 <sup>b</sup>	0.56
<b>Tumor stage (TNM classification)</b>			
T1	1.67 $\pm$ 1.62 <sup>b</sup>	2.44 $\pm$ 1.38 <sup>b</sup>	0.57
T2	1.36 [0.41-3.12] <sup>a</sup>	1.31 [0.17-2.33] <sup>a</sup>	0.68
T3	0.65 [0.25-1.51] <sup>a</sup>	0.57 [0.31-3.38] <sup>a</sup>	0.71
T4	0.83 [0.24-2.97] <sup>a</sup>	0.67 [0.25-3.11] <sup>a</sup>	>0.99
N0	0.76 [0.33-3.48] <sup>a</sup>	0.92 [0.31-2.76] <sup>a</sup>	0.93
N1	1.02 [0.24-2.94] <sup>a</sup>	0.84 [0.27-3.36] <sup>a</sup>	0.75
N2	1.43 $\pm$ 0.96 <sup>b</sup>	1.25 $\pm$ 0.97 <sup>b</sup>	0.66
N3	0.54 $\pm$ 0.12 <sup>b</sup>	0.67 $\pm$ 0.60 <sup>b</sup>	0.76
<b>Histologic grade</b>			
G1	0.76 [0.57-3.12] <sup>a</sup>	0.73 [0.17-1.80] <sup>a</sup>	0.93
G2	0.90 [0.24-3.48] <sup>a</sup>	1.28 [0.25-3.38] <sup>a</sup>	0.90
G3	0.71 $\pm$ 0.39 <sup>b</sup>	0.49 $\pm$ 0.10 <sup>b</sup>	0.34
<b>Anatomical site</b>			
Larynx	0.84 [0.25-2.97] <sup>a</sup>	0.63 [0.25-3.38] <sup>a</sup>	0.98
Oral cavity	0.95 [0.24-3.48] <sup>a</sup>	0.90 [0.27-3.63] <sup>a</sup>	0.76
Oropharynx	1.48 $\pm$ 1.42 <sup>b</sup>	0.98 $\pm$ 1.15 <sup>b</sup>	0.80

The *METTL3* transcript levels were measured in triplicates and standardized by *GAPDH* reference gene, relative gene expression were calculate using Pfaffl method. Depending on the data distribution we performed the U Mann-Whitney test<sup>a</sup> or unpaired t-test with Welch's correction<sup>b</sup>.

**Table S4.** *FTO* transcript levels in cancerous and histopathologically unchanged tissue sample from patient with HNSCC.

Characteristic	Cancerous tissue	Histopathologically unchanged tissue	p-value
	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	
<b>Age at the time of surgery (years)</b>			
?60	1.06 [0.31-3.64] <sup>a</sup>	1.65 [0.14-4.07] <sup>a</sup>	0.66
>60	1.20 [0.41-3.10] <sup>a</sup>	0.87 [0.33-3.10] <sup>a</sup>	0.48
<b>Gender</b>			
Male	1.15 [0.31-3.64] <sup>a</sup>	0.95 [0.14-3.10] <sup>a</sup>	0.13
Female	0.96 [0.41-2.58] <sup>a</sup>	1.17 [0.46-4.07] <sup>a</sup>	0.55
<b>Tumor stage (TNM classification)</b>			
T1	0.84 $\pm$ 0.83 <sup>b</sup>	1.94 $\pm$ 0.69 <sup>b</sup>	0.16
T2	1.47 $\pm$ 0.76 <sup>b</sup>	1.61 $\pm$ 1.10 <sup>b</sup>	0.72
T3	0.53 [0.42-1.52] <sup>a</sup>	0.51 [0.33-1.45] <sup>a</sup>	0.71
T4	1.48 $\pm$ 0.97 <sup>b</sup>	0.99 $\pm$ 0.60 <sup>b</sup>	0.10
N0	0.59 [0.42-2.35] <sup>a</sup>	1.03 [0.34-2.06] <sup>a</sup>	0.29
N1	1.34 [0.41-3.64] <sup>a</sup>	1.16 [0.33-4.07] <sup>a</sup>	0.92
N2	1.41 $\pm$ 0.89 <sup>b</sup>	1.12 $\pm$ 0.59 <sup>b</sup>	0.46
N3	0.49 $\pm$ 0.07 <sup>b</sup>	0.57 $\pm$ 0.47 <sup>b</sup>	0.80
<b>Histologic grade</b>			
G1	1.07 $\pm$ 0.52 <sup>b</sup>	1.12 $\pm$ 0.62 <sup>b</sup>	0.88
G2	1.47 [0.31-3.64] <sup>a</sup>	1.11 [0.28-4.07] <sup>a</sup>	0.15
G3	0.54 [0.43-0.71] <sup>a</sup>	0.57 [0.33-3.10] <sup>a</sup>	>0.99
<b>Anatomical site</b>			
Larynx	1.47 [0.42-3.64] <sup>a</sup>	0.78 [0.28-2.38] <sup>a</sup>	0.17
Oral cavity	1.10 [0.31-2.89] <sup>a</sup>	1.16 [0.34-4.07] <sup>a</sup>	0.85
Oropharynx	0.95 $\pm$ 0.54 <sup>b</sup>	1.37 $\pm$ 1.54 <sup>b</sup>	0.69

The *FTO* transcript levels were measured in triplicates and standardized by *GAPDH* reference gene, relative gene expression were calculate using Pfaffl method. Depending on the data distribution we performed the U Mann-Whitney test<sup>a</sup> or unpaired t-test with Welch's correction<sup>b</sup>.

**Table S5.** *YTHDF2* transcript levels in cancerous and histopathologically unchanged tissue sample from patient with HNSCC.

Characteristic	Cancerous tissue	Histopathologically unchanged tissue	p-value
	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	
<b>Age at the time of surgery (years)</b>			
≤60	0.87 [0.07-4.95] <sup>a</sup>	0.92 [0.14-4.50] <sup>a</sup>	0.79
>60	1.39 [0.32-5.33] <sup>a</sup>	1.10 [0.10-4.22] <sup>a</sup>	0.89
<b>Gender</b>			
Male	1.00 [0.07-5.33] <sup>a</sup>	0.99 [0.10-4.22] <sup>a</sup>	0.84
Female	1.35 [0.38-4.95] <sup>a</sup>	1.03 [0.14-4.50] <sup>a</sup>	0.76
<b>Tumor stage (TNM classification)</b>			
T1	0.71 $\pm$ 0.90 <sup>b</sup>	2.76 $\pm$ 2.46 <sup>b</sup>	0.38
T2	1.88 $\pm$ 1.24 <sup>b</sup>	1.60 $\pm$ 1.24 <sup>b</sup>	0.64
T3	0.60 [0.32-2.74] <sup>a</sup>	0.52 [0.10-1.98] <sup>a</sup>	0.80
T4	1.01 [0.32-5.33] <sup>a</sup>	1.00 [0.14-4.22] <sup>a</sup>	0.99
N0	1.10 [0.07-5.33] <sup>a</sup>	1.27 [0.17-4.22] <sup>a</sup>	0.56
N1	1.00 [0.32-2.24] <sup>a</sup>	1.04 [0.13-4.50] <sup>a</sup>	0.95
N2	1.22 [0.38-4.95] <sup>a</sup>	0.98 [0.20-3.32] <sup>a</sup>	0.82
N3	0.67 $\pm$ 0.54 <sup>b</sup>	0.39 $\pm$ 0.37 <sup>b</sup>	0.50
<b>Histologic grade</b>			
G1	1.69 $\pm$ 0.89 <sup>b</sup>	1.29 $\pm$ 0.74 <sup>b</sup>	0.43
G2	0.80 [0.07-5.33] <sup>a</sup>	0.94 [0.13-4.22] <sup>a</sup>	0.74
G3	1.58 $\pm$ 1.15 <sup>b</sup>	0.85 $\pm$ 0.67 <sup>b</sup>	0.34
<b>Anatomical site</b>			
Larynx	1.31 [0.32-5.33] <sup>a</sup>	0.98 [0.10-4.22] <sup>a</sup>	0.75
Oral cavity	0.92 [0.07-4.95] <sup>a</sup>	1.01 [0.13-4.50] <sup>a</sup>	0.75
Oropharynx	2.19 $\pm$ 0.93 <sup>b</sup>	0.76 $\pm$ 0.71 <sup>b</sup>	0.15

The *YTHDF2* transcript levels were measured in triplicates and standardized by *GAPDH* reference gene, relative gene expression were calculate using Pfaffl method. Depending on the data distribution we performed the U Mann-Whitney test<sup>a</sup> or unpaired t-test with Welch's correction<sup>b</sup>.

**Table S6.** *YTHDC2* transcript levels in cancerous and histopathologically unchanged tissue sample from patient with HNSCC.

Characteristic	Cancerous tissue	Histopathologically unchanged tissue	p-value
	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	Median (range) <sup>a</sup> or Mean ( $\pm$ SD) <sup>b</sup>	
<b>Age at the time of surgery (years)</b>			
≤60	0.93 [0.01-3.89] <sup>a</sup>	1.36 [0.18-4.38] <sup>a</sup>	0.29
>60	1.18 [0.12-4.38] <sup>a</sup>	1.40 [0.19-4.52] <sup>a</sup>	0.82
<b>Gender</b>			
Male	0.98 [0.13-4.38] <sup>a</sup>	1.46 [0.19-4.52] <sup>a</sup>	0.25
Female	1.37 [0.01-3.04] <sup>a</sup>	1.03 [0.18-4.38] <sup>a</sup>	0.62
<b>Tumor stage (TNM classification)</b>			
T1	1.47 $\pm$ 1.32 <sup>b</sup>	2.12 $\pm$ 0.87 <sup>b</sup>	0.52
T2	1.42 $\pm$ 0.88 <sup>b</sup>	1.46 $\pm$ 1.23 <sup>b</sup>	0.92
T3	1.11 [0.50-3.14] <sup>a</sup>	2.04 [0.19-2.84] <sup>a</sup>	0.51
T4	0.76 [0.12-4.38] <sup>a</sup>	1.32 [0.26-4.52] <sup>a</sup>	0.75
N0	1.26 [0.38-4.32] <sup>a</sup>	1.93 [0.38-4.52] <sup>a</sup>	0.61
N1	0.70 [0.50-1.77] <sup>a</sup>	1.04 [0.26-2.10] <sup>a</sup>	0.75
N2	1.72 $\pm$ 1.43 <sup>b</sup>	1.66 $\pm$ 1.21 <sup>b</sup>	0.92
N3	1.03 $\pm$ 0.32 <sup>b</sup>	0.63 $\pm$ 0.41 <sup>b</sup>	0.26
<b>Histologic grade</b>			
G1	1.58 $\pm$ 0.55 <sup>b</sup>	1.46 $\pm$ 0.84 <sup>b</sup>	0.80
G2	0.98 [0.01-4.98] <sup>a</sup>	1.57 [0.18-4.52] <sup>a</sup>	0.47
G3	1.54 $\pm$ 1.31 <sup>b</sup>	1.71 $\pm$ 1.37 <sup>b</sup>	0.88
<b>Anatomical site</b>			
Larynx	1.26 [0.55-4.38] <sup>a</sup>	2.10 [0.19-4.52] <sup>a</sup>	0.84
Oral cavity	0.95 [0.01-2.94] <sup>a</sup>	1.31 [0.18-4.38] <sup>a</sup>	0.70
Oropharynx	0.97 $\pm$ 0.73 <sup>b</sup>	0.87 $\pm$ 0.22 <sup>b</sup>	0.85

The *YTHDC2* transcript levels were measured in triplicates and standardized by *GAPDH* reference gene, relative gene expression were calculate using Pfaffl method. Depending on the data distribution we performed the U Mann-Whitney test<sup>a</sup> or unpaired t-test with Welch's correction<sup>b</sup>.