

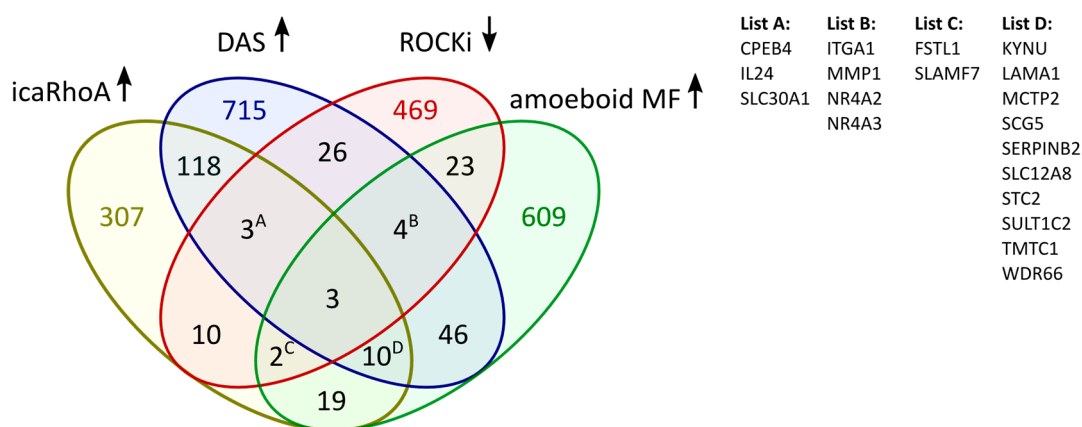
Increased Level of Long Non-Coding RNA MALAT1 is a Common Feature of Amoeboid Invasion

Ladislav Merta, Aneta Gandalovičová, Vladimír Čermák, Michal Dibus, Tony Gutschner, Sven Diederichs, Daniel Rösel and Jan Brábek

Supplementary Materials

A

Genes UPREGULATED in amoeboid form



B

Genes DOWNREGULATED in amoeboid form

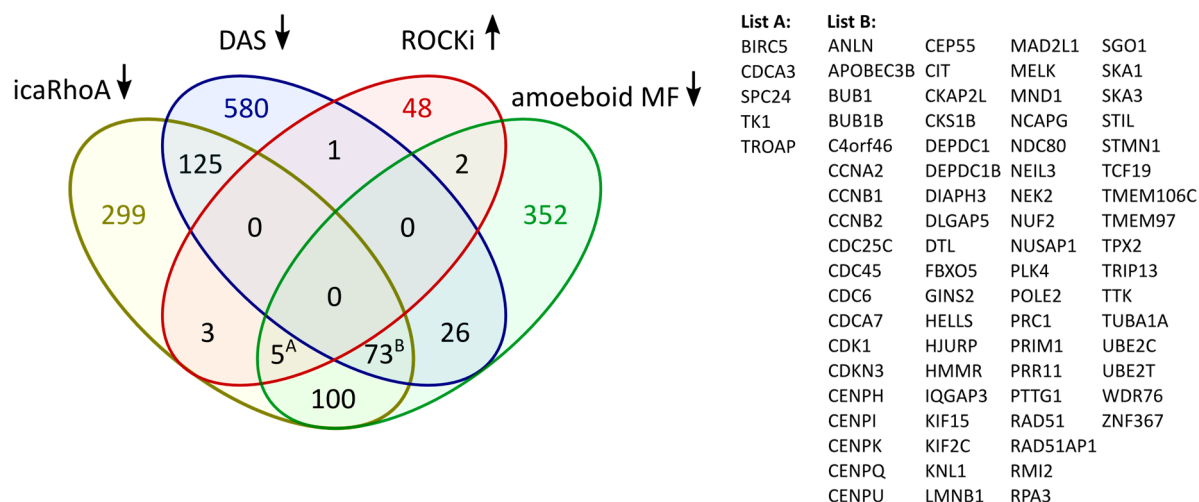


Figure 1. Transcriptomic overlaps of only 3 datasets used for meta-analysis. (A) Venn diagram of gene sets upregulated in the amoeboid HT1080 cells and macrophages, and suppressed in A375m2 cells by ROCK inhibitors (ROCKi). (B) Venn diagram of gene sets downregulated in the amoeboid HT1080 cells and macrophages, and upregulated in A375m2 cells by ROCK inhibitors. Lists of the genes are provided for overlaps of 3 gene sets.

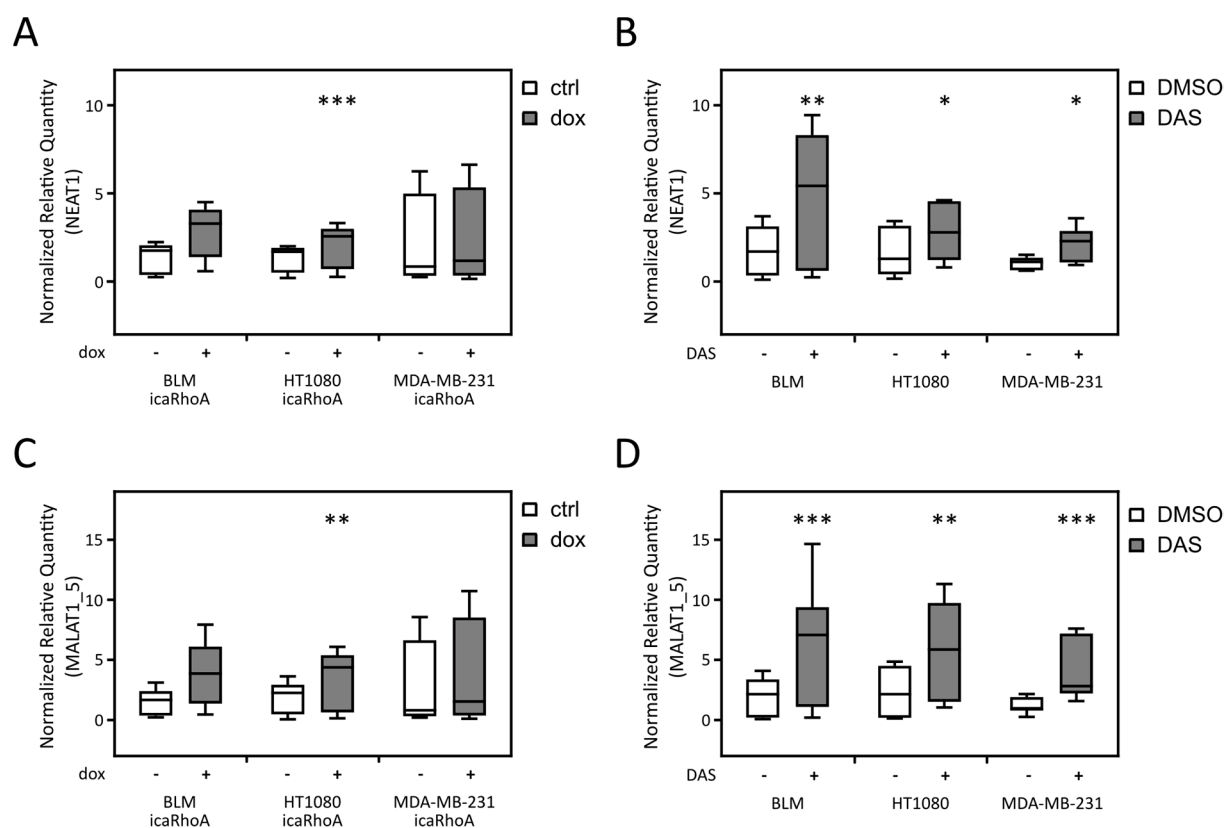


Figure 2. RT-qPCR analysis of NEAT1 and second pair of primers for MALAT1 in cells undergoing MAT. (A-B) Analysis of the NEAT1 gene expression in cells undergoing MAT by induction of constitutively active RhoA (A) or by dasatinib treatment (B). (C-D) Analysis of the MALAT1 gene expression (using primer pair pairing with 5' end of the transcript) in cells undergoing MAT by induction of constitutively active RhoA (C) or by dasatinib treatment (D). Median values are marked in the box plots, whiskers represent min to max value. P-values: *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$. All data are a representation of at least 3 independent experiments.

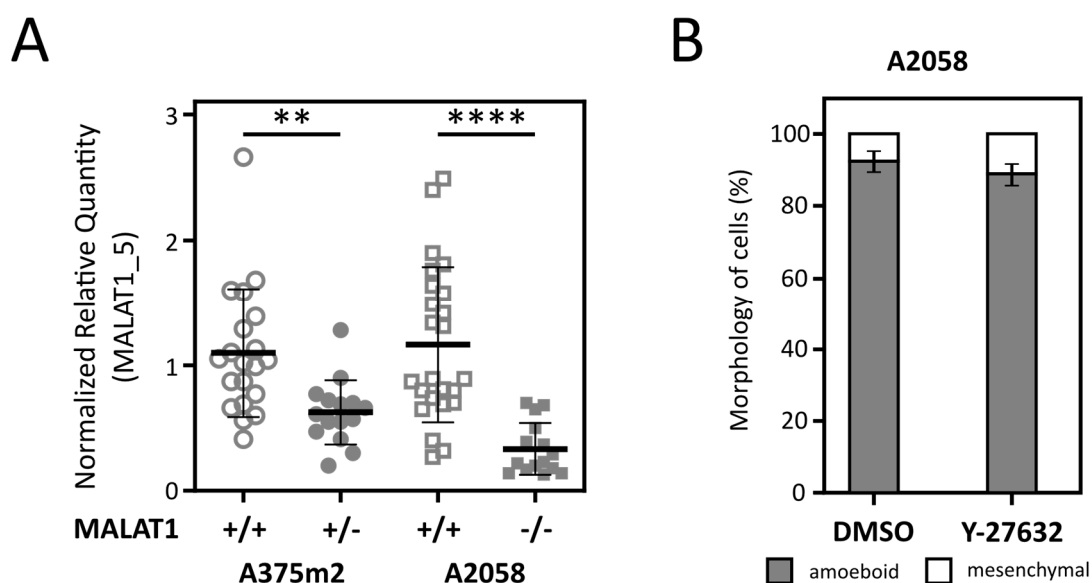


Figure 3. Complementary RT-qPCR quantification of MALAT1 level in clones derived from A375m2 and A2058 cell lines and quantification of cell morphology of A2058 cells after treatment with Y-27632. (A) RT-qPCR analysis of MALAT1 level in clones derived from the A375m2 and A2058 cell lines using second pair of primers pairing with the 5' end of MALAT1 transcript. N(A375m2 MALAT1 +/+) = 20 clones; N(A375m2 MALAT1 +/-) = 15 clones; N(A2058 MALAT1 +/+) = 24 clones; N(A2058 MALAT1 -/-) = 15 clones. Data represent mean \pm SD. (B) morphology of A2058 cells after treatment with ROCK inhibitor Y-27632. Data represent mean \pm SD. P-values: **** $p < 0.0001$, ** $p < 0.01$. All data are a representation of at least 3 independent experiments.

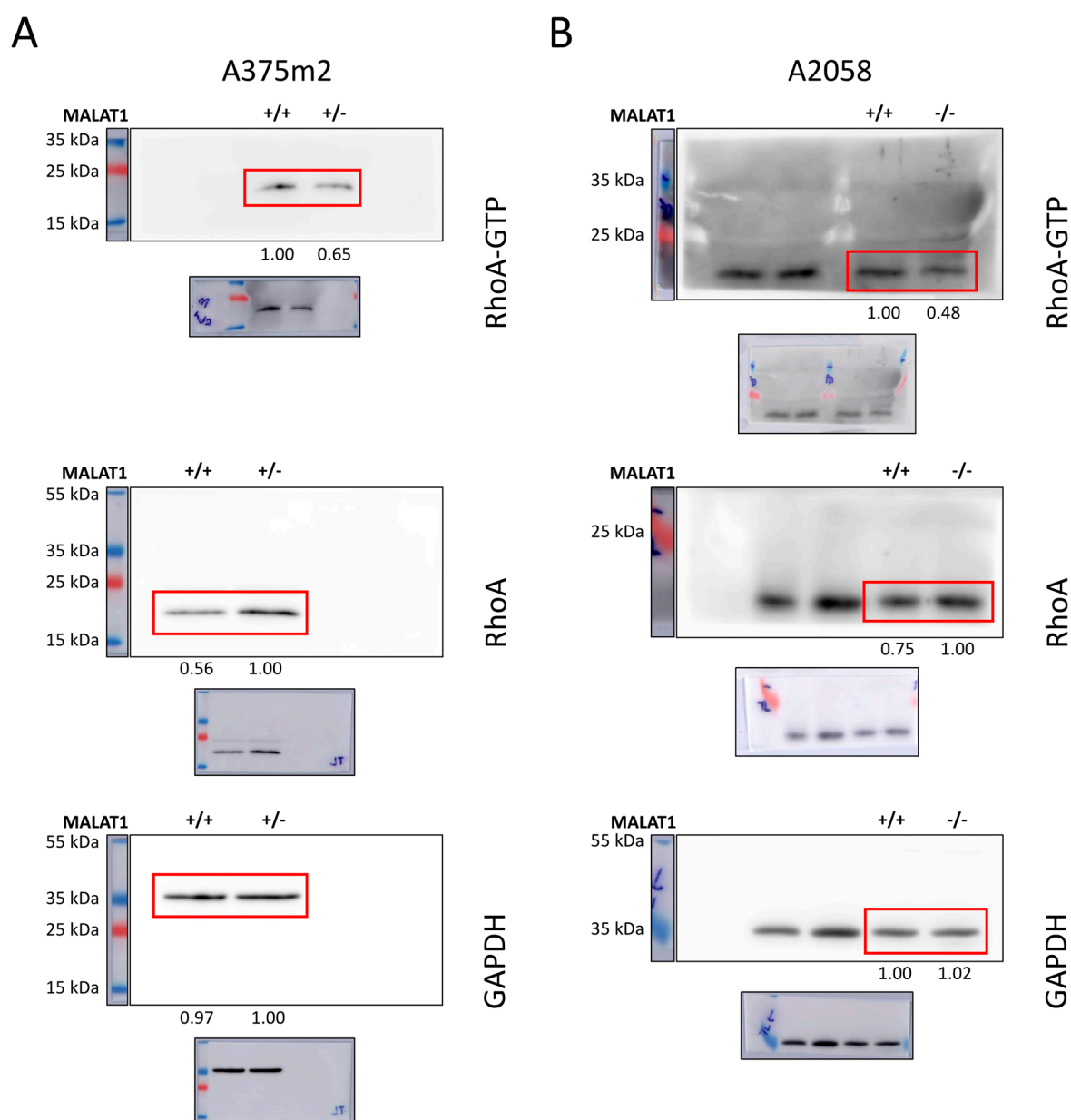


Figure 4. Uncropped Western blots used in the study. (A) Uncropped blots used in Figure 3D. (B) Uncropped blots used in Figure 4C. Red rectangle marks the area presented in the main text. The numbers below the bands represent the intensity ratio. Because the machine used to visualize the blots uses different channels of photo acquisition, each part consists of the HRP signal (the channel from which the cropped images were made) + added marker (upper part) and the whole membrane HRP signal and transmitted light composite channel (lower part).

Table 1. RT-qPCR primers used in the work.

TRANSCRIPT	ACC. NUMBER	PRIMER	SEQUENCE	nt	%GC	T _m (°C)	PRODUCT (bp)	Eff
EIF4H	NM_022170	EIF4Ha	CCTTCTGGCTACGGGAACCAT	21	57.1	63.3	95	1.977
		EIF4Hs	CTTCGACACCTACGACGATCG	21	57.1	63.3		
GAPDH	NM_002046	GAPDH _a	GCATGGACTGTGGTCATGAG	20	55.0	60.5	87	2.012
		GAPDH _s	CTGCACCACCAACTGCTTAG	20	55.0	60.5		
HNRNPL	NM_001533	HNRNPL _a	GCGCTCACTTTTGCCTGAGAA	21	52.4	61.3	96	1.993
		HNRNPL _s	CTGGAGGTGACCGAGGAGAA	20	60.0	62.5		
MALAT1	NR_002819	MALAT1_3 _a	GGTCTGTGCTAGATCAAAAGG	21	47.6	59.4	71	2.010
		MALAT1_3 _s	AAAGCAAGGTCTCCCCACAAG	21	52.4	61.3		
MALAT1_5	NR_002819	MALAT1_5 _a	GTTTCATCCTACCACTCCCAAT	22	45.5	60.3	85	1.992
		MALAT1_5 _s	GAATTGCGTCATTTAAAGCCTA	22	36.4	56.5		
NEAT1	NR_131012	5NEAT1 _f	GTGGTAGGAAATGCAGGTTGA	21	47.6	59.4	122	1.904
		5NEAT1 _r	CCAAGCAACAACCTTAACCAACA	22	40.9	58.4		
PPIA	NM_021130	PPIA _f	GCCGAGGAAAACCGTGTAATA	21	52.4	61.3	106	1.941
		PPIA _r	CTGCAAAACAGCTCAAAGGAGAC	22	50.0	62.1		
PSMA1	NM_148976	PSMA1 _a	AGACCAACTGTGGCTGAACCT	21	52.4	61.3	95	1.945
		PSMA1 _s	GACAATGATGTCACCTGTTTGAG	23	43.5	61.1		

Nt; Number of nucleotides, T_m; Melting temperature, Eff; Amplification efficiency determined by standard curve analysis.

Table 2. Reference genes used for respective cell lines in RT-qPCR analysis.

Cell line/clones derived from cell line	Reference genes
BLM (DMSO/DAS)	EIF4H, GAPDH
BLM icaRhoA (-dox/+dox)	EIF4H, HNRNPL
HT1080 (DMSO/DAS)	EIF4H, GAPDH
HT1080 icaRhoA (-dox/+dox)	EIF4H, GAPDH
MDA-MB-231 (DMSO/DAS)	HNRNPL, PSMA1
MDA-MB-231 (-dox/+dox)	EIF4H, HNRNPL
A375m2 clones	EIF4H, PPIA
A2058 clones	PPIA, PSMA