The oncogenic ΔNp73 isoform contributes to aggressiveness in acute promyelocytic leukemia

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Supplementary Table and Figures

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Supplementary Table 1

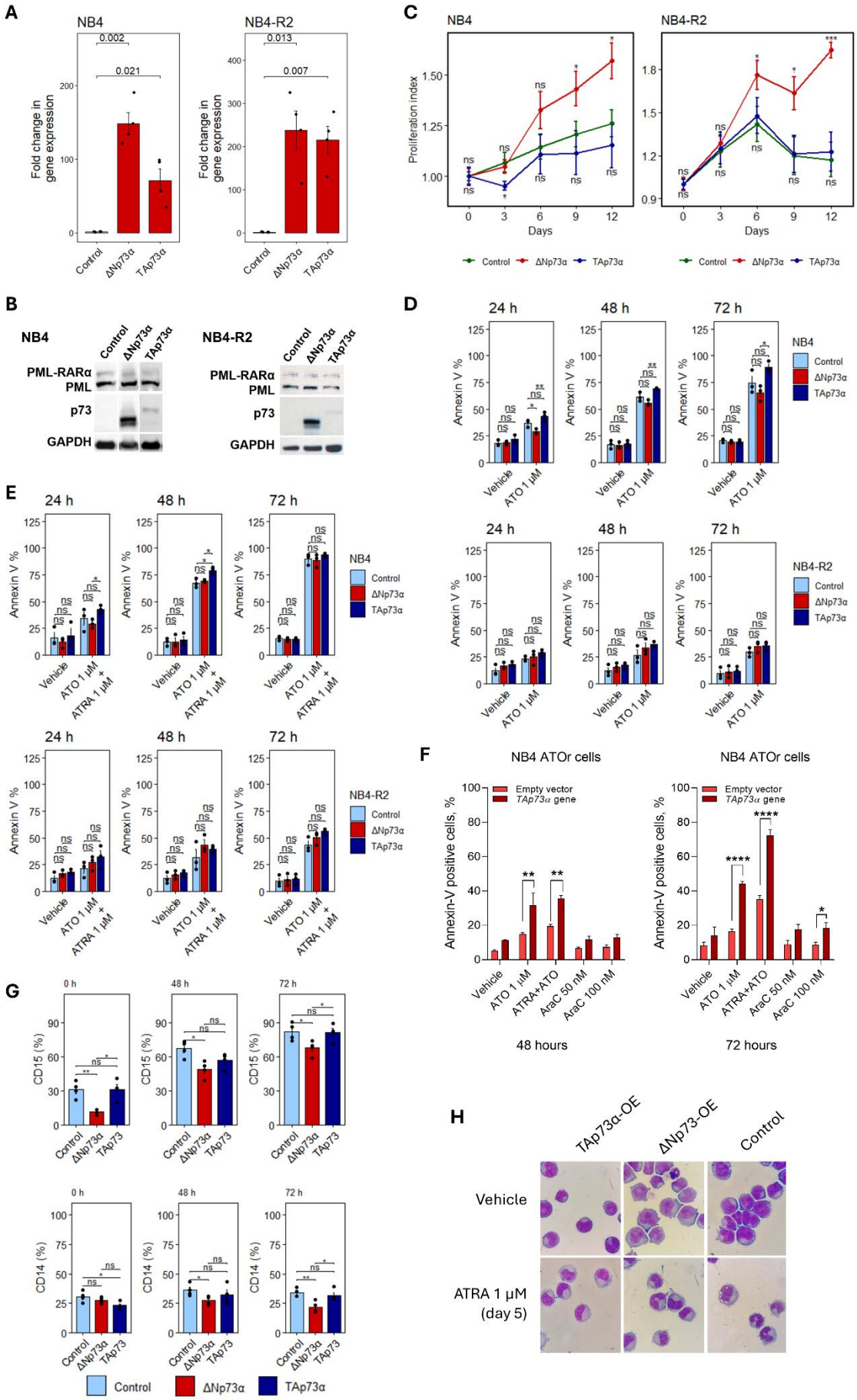
Characteristics		ΔNp73 expression		p
		Low	High	
Number of patients		30	68	
Gender (%)	female	14 (46.7)	34 (50.0)	0.828
	male	16 (53.3)	34 (50.0)	
Age (median [IQR])		36.6 [27.9, 43.4]	34.88 [26.42, 48.34]	0.829
Classification risk (%)	Favorable	6 (20.0)	9 (13.2)	0.641
	Intermediate	12 (40.0)	32 (47.1)	
	Adverse	12 (40.0)	27 (39.7)	
Break cluster region (%)	bcr1	17 (58.6)	37 (62.7)	0.458
	bcr2	2 (6.9)	1 (1.7)	
	bcr3	10 (34.5)	21 (35.6)	
ECOG (%)	0	22 (75.9)	38 (55.9)	0.355
	1	3 (10.3)	13 (19.1)	
	2	3 (10.3)	10 (14.7)	
	3	1 (3.4)	7 (10.3)	
Fever at diagnosis (%)	No	13 (44.8)	35 (52.2)	0.657
	Yes	16 (55.2)	32 (47.8)	
FLT3-ITD (%)	Detected	2 (9.5)	15 (23.4)	0.219
	Non detected	19 (90.5)	49 (76.6)	
WBC, 10^9/L (median [IQR])		5930 [1737.5, 24450]	6125 [1850, 30187]	0.905
WBC, no. (%), x 10^9/L	≤ 10	18 (60.0)	40 (58.8)	1
	> 10	12 (40.0)	28 (41.2)	
Platelet, 10^9/L (median [IQR])		27200 [15750, 49500]	25500 [14000, 39000]	0.459
Platelet count, no. (%), x 10^9/L	> 50	8 (26.7)	11 (16.2)	0.271
	≤ 50	22 (73.3)	57 (83.8)	
Hb, g/dL (median [IQR])		8.55 [7.65, 9.07]	8.85 [7.35, 10.10]	0.464
Hb, no. (%), g/dL	> 10	4 (13.3)	19 (27.9)	0.131
	≤ 10	26 (86.7)	49 (72.1)	
Creatinine, mg/dL (median [IQR])		0.79 [0.66, 0.92]	0.80 [0.70, 1.00]	0.228
Creatinine, no. (%), mg/dL	≤ 1.4	29 (96.7)	65 (97.0)	1
	> 1.4	1 (3.3)	2 (3.0)	
Albumin, g/dL (median [IQR])		4.40 [3.80, 4.60]	4.00 [3.60, 4.30]	0.026
Albumin, no. (%), g/dL	> 3.5	21 (100.0)	42 (79.2)	0.028
	≤ 3.5	0 (0.0)	11 (20.8)	
Uric acid, mg/dL (median [IQR])		3.70 [2.58, 5.67]	3.80 [3.20, 5.00]	0.544
Uric acid, no. (%), mg/dL	≤ 7	26 (92.9)	64 (98.5)	0.215
	> 7	2 (7.1)	1 (1.5)	
Fibrinogen, mg/dL (median [IQR])		149.5 [88.3, 196.5]	159.5 [100, 233.8]	0.374
Fibrinogen, no. (%), mg/dL	> 170	13 (46.4)	28 (42.4)	0.821
	≤ 170	15 (53.6)	38 (57.6)	
Complete remission (%)	No	1 (3.3)	12 (17.6)	0.06
	Yes	29 (96.7)	56 (82.4)	
FAB subtype (%)	M3	28 (93.3)	65 (95.6)	0.64
	M3v	2 (6.7)	3 (4.4)	
Early mortality (%)	No	30 (100.0)	57 (83.8)	0.017
	Yes	0 (0.0)	11 (16.2)	

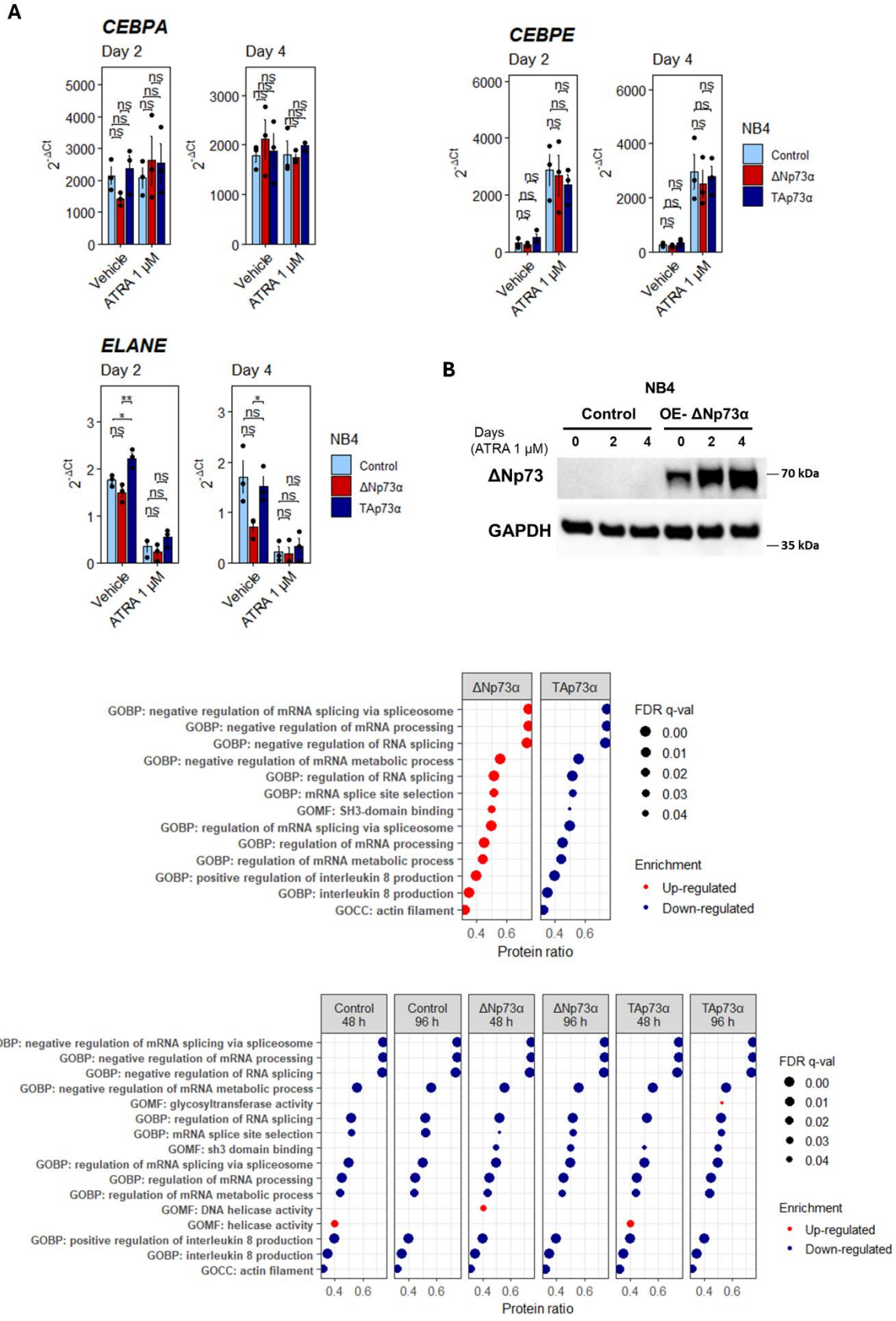
*IQR: Interquartile range. Categorical variables were compared with Fisher's exact test while continuous variables with Mann-Whitney test.

Supplementary Figure legends

Supplementary Figure 1. Effect of ΔNp73α and TAp73α on the proliferation and ATO response of NB4 and NB4-R2 cells. (A) Normalized copy numbers (NCN, N copies of target gene/copies of *ACTB*) of OE cells versus controls (transduced with empty vector). (B) Protein abundance of ΔNp73α and TAp73α in OE NB4, NB4-R2, and control cells. The antibodies used to detect ΔNp73α was the pan anti-p73 antibody (Novus biologicals: NB100-56674, 1:1000) and for TAp73 we used an anti-TAp73 (abcam: ab14430; 1:1000). (C) Proliferation assay of transduced APL cells showing higher proliferation index in ΔNp73α OE-NB4 cells. (D-E) Annexin V apoptosis assay after treatment with ATO (D) or ATO+ATRA (E) in OE-NB4 and -NB4-R2, and control cells. (F) Apoptosis assay in NB4 ATOr cells overexpressing TAp73 indicating resensitization to ATO mediated by TAp73. (G) Percentage levels of CD14 and CD15 markers in ATRA-treated NB4 cells overexpressing p73 isoforms. (H) Wright's stain of transduced NB4 cells after 120 h of ATRA-treatment and controls.

Supplementary Figure 2. Evaluation of ATRA-modulated genes and proteome analysis of ATRA-treated NB4 overexpressing ΔNp73α or TAp73α. (A) CEBPA, CEBPE, and ELANE expressions were evaluated during the ATRA-treatment, finding no impact of ΔNp73α or TAp73α. (B) Increased stability of ΔNp73α protein observed during ATRA treatment. (C) Following a proteomic approach, pathway enrichment analysis revealed RNA splicing processes as top differentially modulated between ΔNp73α and TAp73α in NB4 cells. (D). However, these same pathways were uniformly downregulated during ATRA treatment, regardless of p73 isoform overexpression.


A
NB4
NB4-R2
Fold change in gene expression
0.002
0.021
0.013
0.007
Control
ΔNp73α
TAp73α
B
NB4
NB4-R2
PML-RARα
PML
p73
GAPDH
C
NB4
NB4-R2
Proliferation index
Days
D
24 h
48 h
72 h
Annexin V %
Vehicle
ATO 1 μM
E
ATO 1 μM + ATRA 1 μM
F
NB4 ATOr cells
Empty vector
TAp73α gene
Annexin-V positive cells, %
ATRA+ATO
AraC 50 nM
AraC 100 nM
48 hours
72 hours
G
CD15 (%)
CD14 (%)
TAp73
H
TAp73α-OE
ΔNp73-OE
Control
Vehicle
ATRA 1 μM (day 5)


A
CEBPA
Day 2
Day 4
CEBPE
Day 2
Day 4
ELANE
Day 2
Day 4
NB4
Control
ΔNp73α
TAp73α
Vehicle
ATRA 1 μM
B
NB4
Control
OE- ΔNp73α
Days (ATRA 1 μM)
0 2 4 0 2 4
ΔNp73
GAPDH
70 kDa
35 kDa
C
ΔNp73α
TAp73α
GOBP: negative regulation of mRNA splicing via spliceosome
GOBP: negative regulation of mRNA processing
GOBP: negative regulation of RNA splicing
GOBP: negative regulation of mRNA metabolic process
GOBP: regulation of RNA splicing
GOBP: mRNA splice site selection
GOMF: SH3-domain binding
GOBP: regulation of mRNA splicing via spliceosome
GOBP: regulation of mRNA processing
GOBP: regulation of mRNA metabolic process
GOBP: positive regulation of interleukin 8 production
GOBP: interleukin 8 production
GOCC: actin filament
Protein ratio
FDR q-val
0.00
0.01
0.02
0.03
0.04
Enrichment
Up-regulated
Down-regulated
D
Control 48 h
Control 96 h
ΔNp73α 48 h
ΔNp73α 96 h
TAp73α 48 h
TAp73α 96 h
GOBP: negative regulation of mRNA splicing via spliceosome
GOBP: negative regulation of mRNA processing
GOBP: negative regulation of RNA splicing
GOBP: negative regulation of mRNA metabolic process
GOMF: glycosyltransferase activity
GOBP: regulation of RNA splicing
GOBP: mRNA splice site selection
GOMF: sh3 domain binding
GOBP: regulation of mRNA splicing via spliceosome
GOBP: regulation of mRNA processing
GOBP: regulation of mRNA metabolic process
GOMF: DNA helicase activity
GOMF: helicase activity
GOBP: positive regulation of interleukin 8 production
GOBP: interleukin 8 production
GOCC: actin filament
Protein ratio
FDR q-val
0.00
0.01
0.02
0.03
0.04
Enrichment
Up-regulated
Down-regulated