

Supplementary data for

**Upregulation of miR-34a-5p, miR-20a-3p and miR-29a-3p by Onconase in A375 melanoma cells
correlates with the downregulation of specific onco-proteins**

by

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The data included are

Supplementary Table S1, Figure S1 and Figure S2

Table S1. miRs expression values recovered in the first experiment performed with ONC-treated or non-treated (control) A375 cells.

miRs	samples	Cq Mean	ΔCq	RQ
miR-30b-5p	+onconase	17.53	-0.30	1.23
miR-30b-5p	control	17.83		
miR-516b-5p	+onconase	30.33	-1.13	2.19
miR-516b-5p	control	31.46		
miR-146a-5p	+onconase	22.49	-0.83	1.77
miR-146a-5p	control	23.32		
miR-128-3p	+onconase	22.95	-1.40	2.64
miR-128-3p	control	24.35		
miR-34a-5p	+onconase	20.18	-1.54	2.91
miR-34a-5p	control	21.72		
miR-20a-3p	+onconase	21.23	-3.32	9.98
miR-20a-3p	control	24.54		
miR-20a-5p	+onconase	16.48	-1.64	3.12
miR-20a-5p	control	18.12		
miR-15b-5p	+onconase	17.60	-1.57	2.98
miR-15b-5p	control	19.18		
miR-24-3p	+onconase	17.09	-1.12	2.17
miR-24-3p	control	18.21		
miR-23a-5p	+onconase	N.A.	-	
miR-23a-5p	control	28.71		
miR-9	+onconase	22.93	-0.63	1.55
miR-9	control	23.56		
mir-29a-3p	+onconase	18.05	-1.80	3.48
mir-29a-3p	control	19.85		
miR-103a-3p	+onconase	23.28	-0.76	1.69
miR-103a-3p	control	24.04		
miR-27a-3p	+onconase	20.41	0.48	0.72
miR-27a-3p	control	19.93		
miR-106a-5p	+onconase	24.22	-1.17	2.25
miR-106a-5p	control	25.39		
miR-22-5p	+onconase	23.29	-2.33	5.03
miR-22-5p	control	25.62		
miR-941	+onconase	25.44	1.13	0.80
miR-941	control	24.31		
miR-21-5p	+onconase	15.42	-2.08	4.24
miR-21-5p	control	17.50		
miR-532-5p	+onconase	22.88	1.45	0.37
miR-532-5p	control	21.43		
miR-140-5p	+onconase	22.36	-0.40	1.32
miR-140-5p	control	22.76		
miR-326	+onconase	28.17	-0.14	1.10
miR-326	control	28.31		
miR-425-5p	+onconase	21.37	0.06	0.96
miR-425-5p	control	21.31		
miR-495-3p	+onconase	30.12	-0.25	1.19
miR-495-3p	control	30.36		
miR-191-5p	+onconase	19.25	0.23	0.85
miR-191-5p	control	19.02		

N.A. = not amplified; RQ = relative quantity.

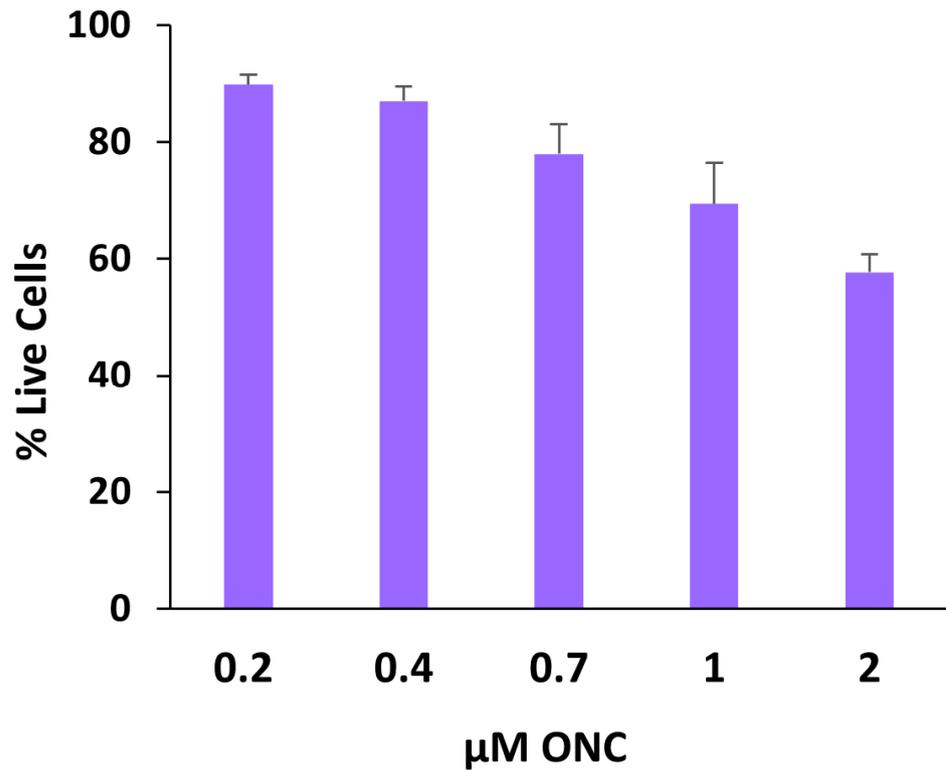


Figure S1. Cell viability assay of FO1 cells treated with different ONC concentrations. Cells were seeded in 96-well plates (2.9×10^3 cells/well). After 24 h, cells were incubated for 72 h with different concentrations of ONC. At the end of the treatment, cells were fixed by adding 25 μL /well of 50% (W/v) TCA directly into the culture medium. Plates were incubated at 4 $^{\circ}\text{C}$ for 1 h, washed four times with ddH₂O and dried at RT. Staining was performed by adding 50 μL /well of 0,04% (W/v) sulforhodamine B (SRB) sodium salt solution (Sigma-Aldrich, Milan, Italy). After 1 h RT incubation, plates were rinsed with 1% HAc and air-dried. SRB was solubilized in 10 mM Tris-base solution pH 10.5 and Abs₅₁₀ measured in the plate reader TECAN NanoQuant Infinite M200 Pro (Tecan Group Ltd.). Four replicates for each condition/data point were performed.

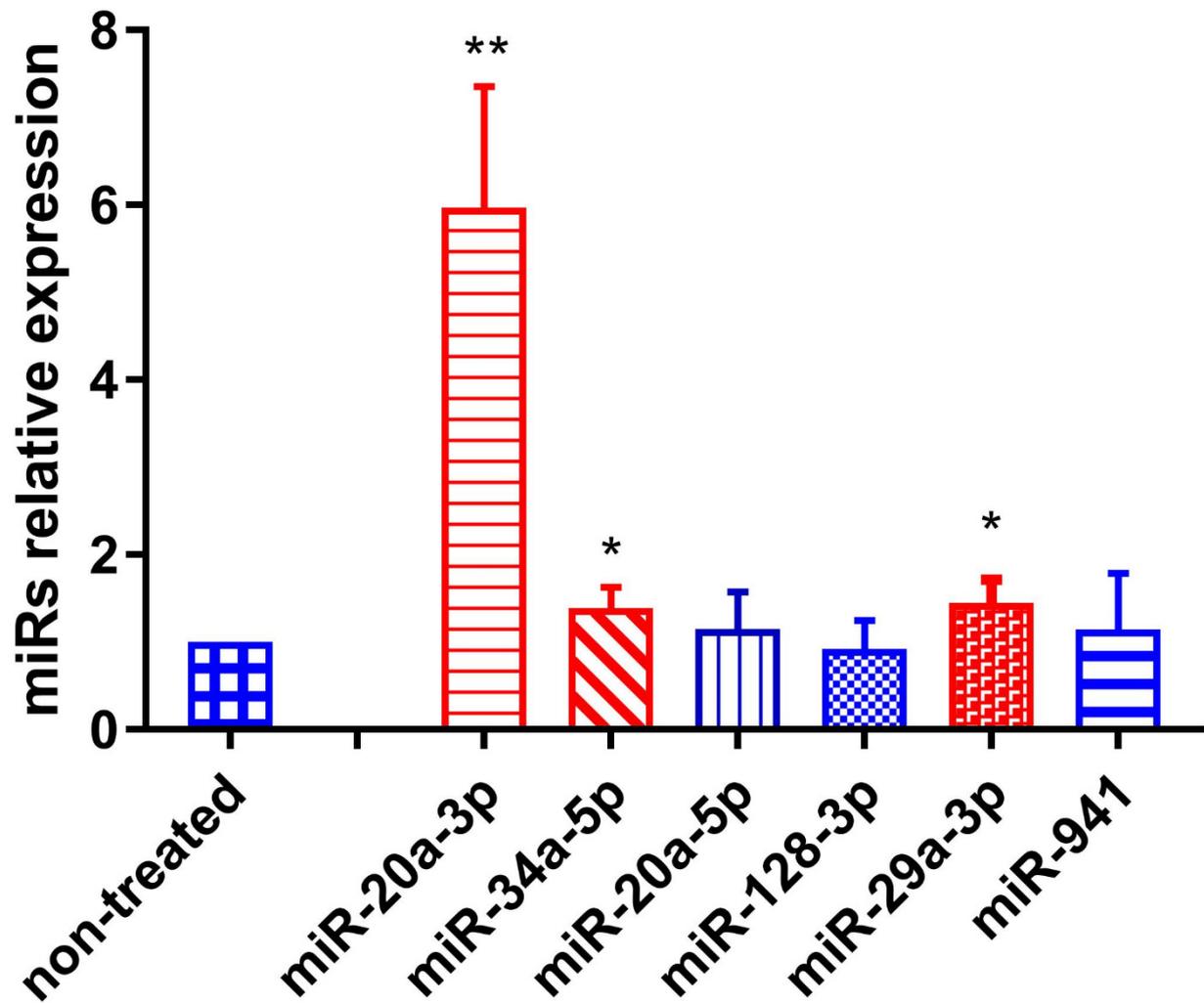


Figure S2. Relative expression of miRNAs after a 48 h ONC incubation of FO1 cells. FO1 cells were cultured for 48 hours after 1 μ M ONC administration in the culture medium. Red color bars refer to the onco-suppressor miRNAs that are upregulated by ONC at a statistically significant level; the blue ones refer instead to miRNAs whose expression level after ONC is not significantly different from untreated control. The mean values \pm S.D. of miRs expression level measured by RT-PCR and deriving from three independent experiments are shown. All comparisons were performed *vs* each control sample after normalization to miR-191 expression; * $p < 0.05$, ** $p < 0.01$.