

## **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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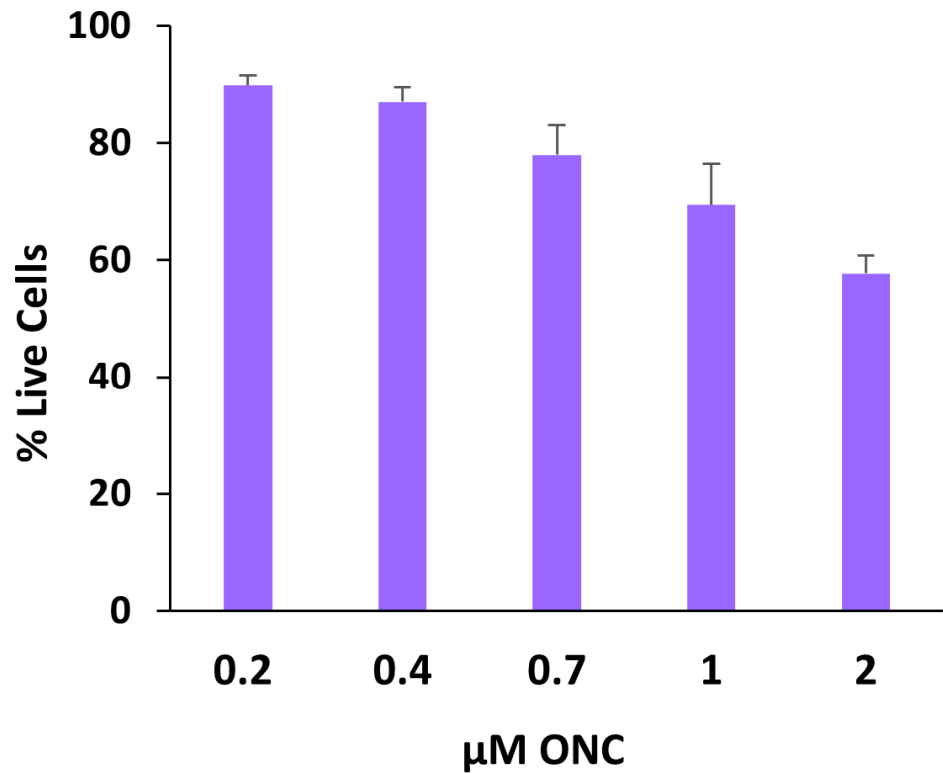
**The data included are**

**Supplementary Table S1, Figure S1 and Figure S2**

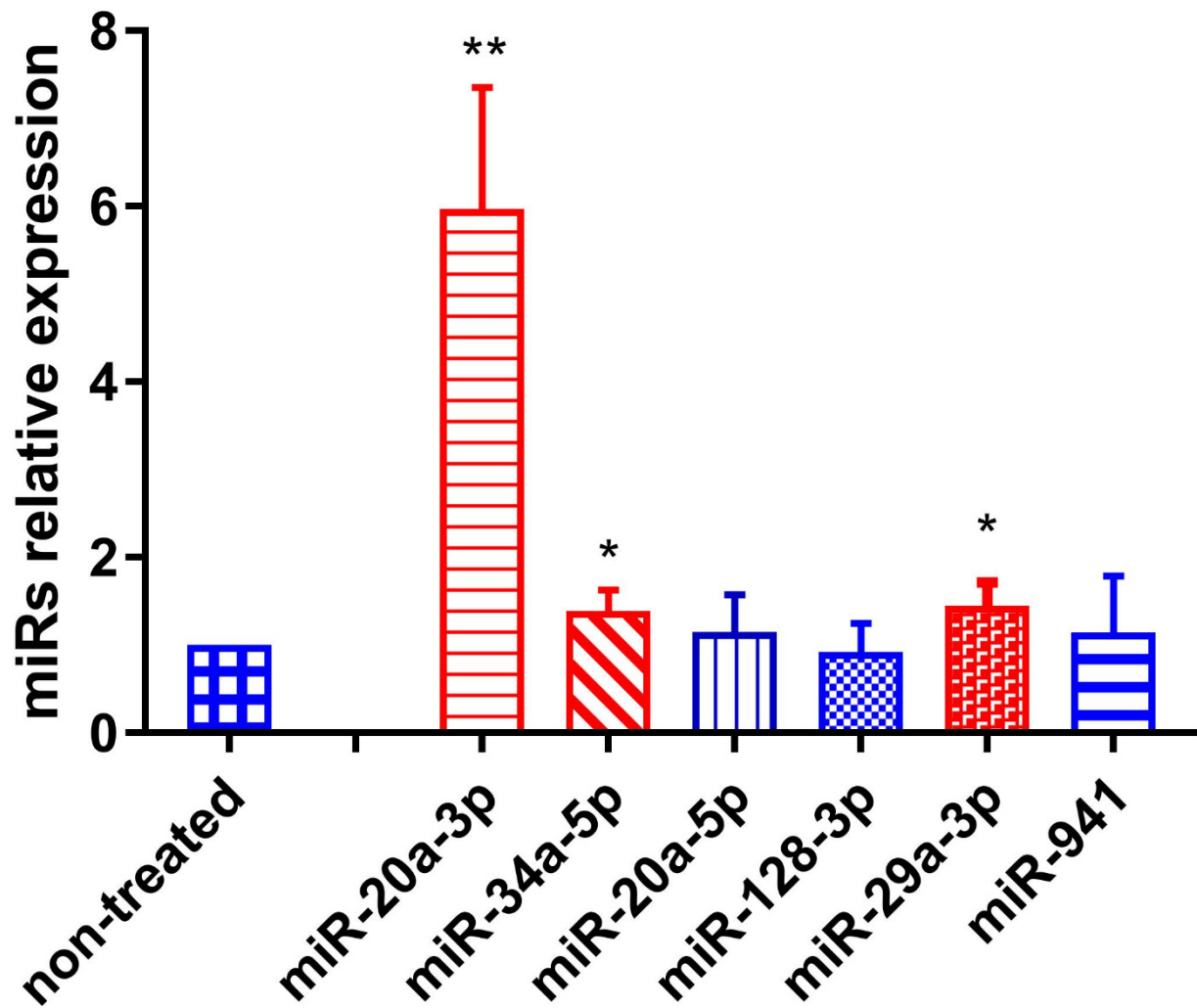
**Table S1.** miRs expression values recovered in the first experiment performed with ONC-treated or non-treated (control) A375 cells.

miRs	samples	Cq Mean	$\Delta Cq$	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 ( $2.9 \times 10^3$  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  $\mu$ L/well of 50% (W/v) TCA directly into the culture medium. Plates were incubated at 4 °C for 1 h, washed four times with ddH<sub>2</sub>O and dried at RT. Staining was performed by adding 50  $\mu$ L/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 Abs<sub>510</sub> 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  $\mu$ 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; \*  $p < 0.05$ , \*\*  $p < 0.01$ .