

# Regulation of IL-8 gene expression in gliomas by microRNA miR-93

## Additional File

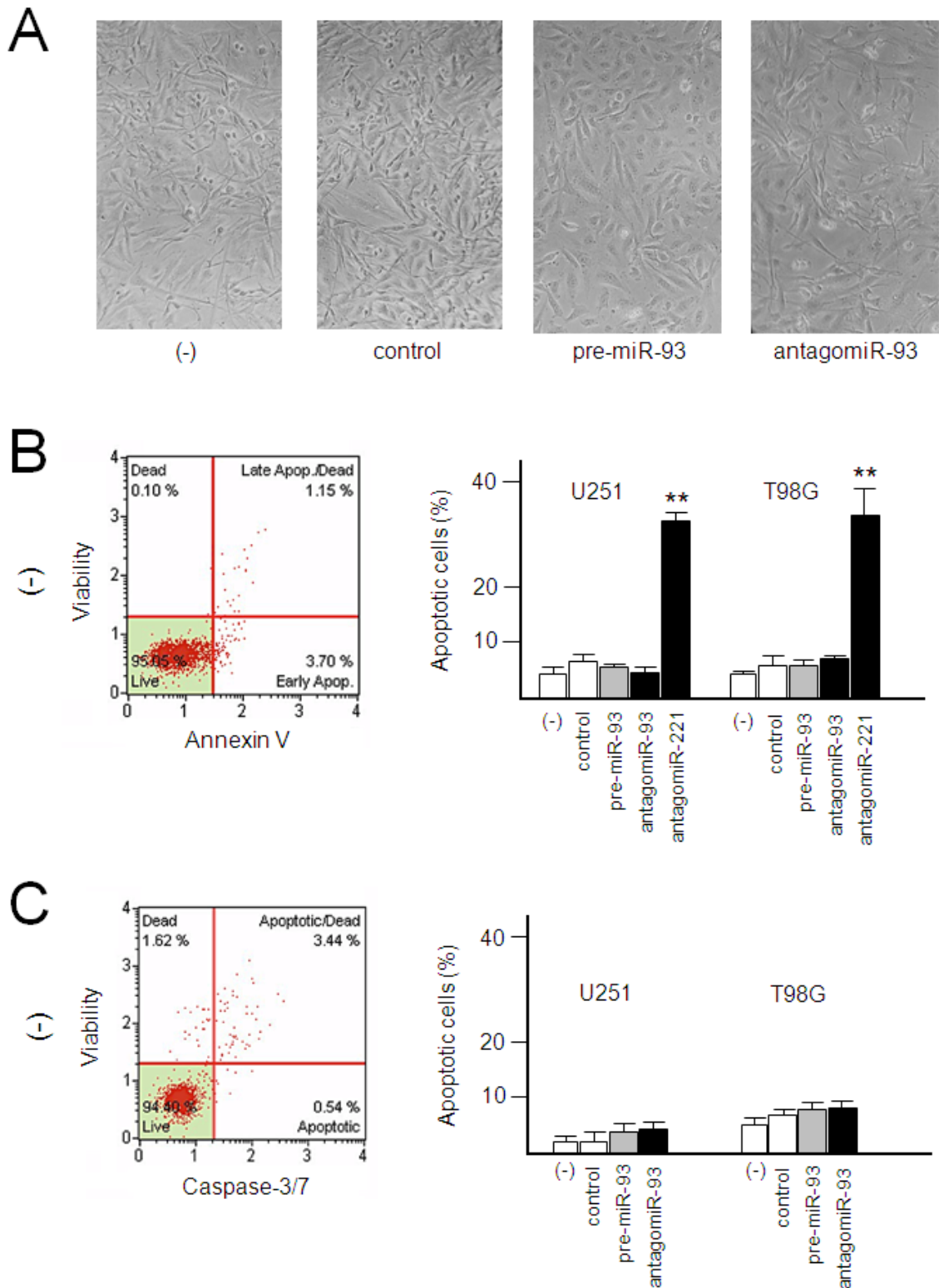
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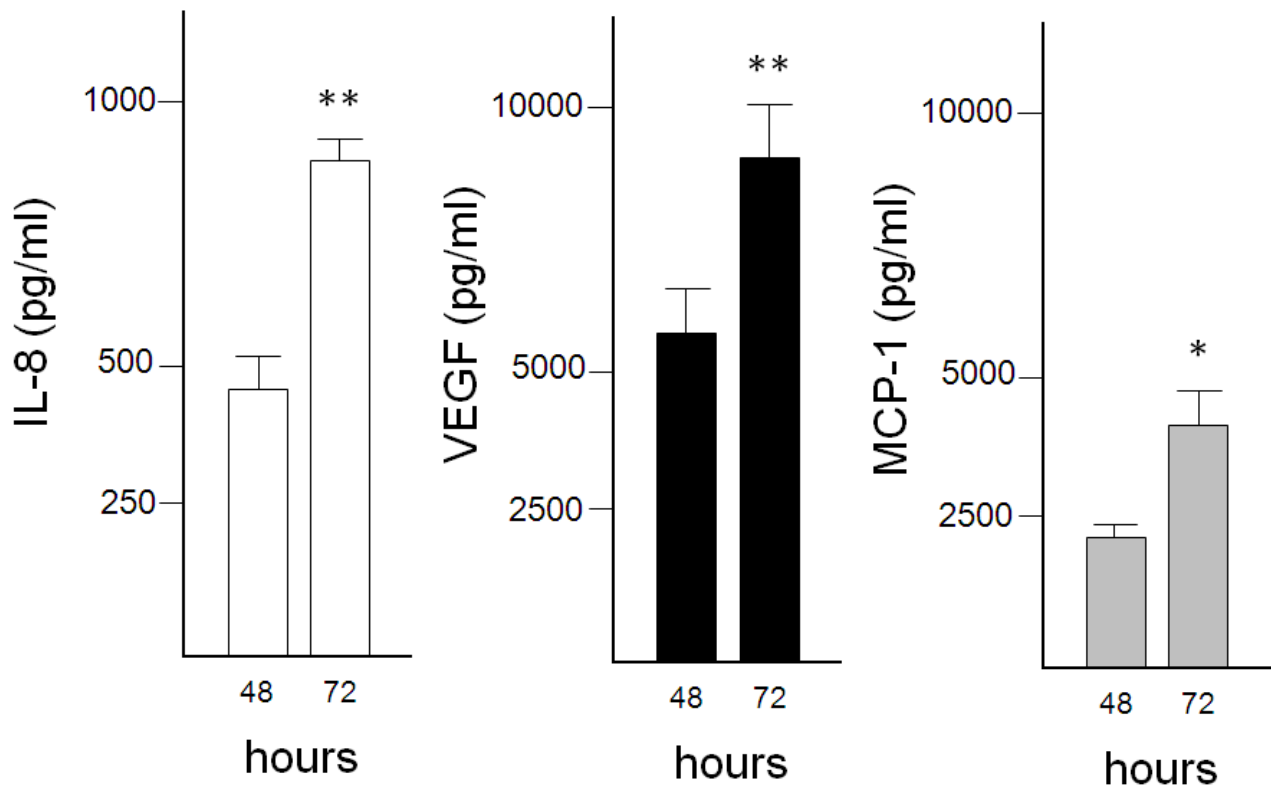
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**Additional File Figure 1.** A. Morphology of U251 glioma cells treated for 24 hours with control, pre-miR-93 and antagomiR-93 molecules (200 nM). B,C. Effects of the different treatments on apoptosis on U251 and T98G glioma cell lines, as indicated. Apoptosis was analyzed by the annexin-V release test [1] (B) or by caspase-3/7 production [2] (C). In panel B, the effects of a positive antagomiR-221 is also shown (see Brognara et al., 2014)[3]. Data represent the average S.D. of three independent experiments. \*\* =  $p < 0.01$ . (-): untreated cellular samples.



**Additional File Figure 2.** Release of IL-8, VEGF and MCP-1 by U251 glioma cells cultured for 48 and 72 hours. Protein release was quantified by Bio-plex analysis. Data represent the average S.D. of three independent experiments. \* =  $p < 0.05$ ; \*\* =  $p < 0.01$ .

## ADDITIONAL METHODS

Apoptosis was analyzed on U251 and T98G glioma cell lines after 48h of treatment with pre-miR-93 or antagomiR-93 (200nM). Cells were washed with sterile PBS (Phosphate-buffered Saline) and then tested with the Muse Annexin V Dead Cell kit (Millipore Corporation, Billerica, MA, USA) or Muse Caspase 3/7 kit (Millipore) [4]. The assays were performed with Muse Instrument (Millipore) [4], according to the instructions provided by the manufacturer.

## **ADDITIONAL REFERENCES**

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[2] McIlwain DR, Berger T, Mak TW. **Caspase Functions in Cell Death and Disease.** *Cold Spring Harb Perspect Biol* 2013;5:a008656

[3] Brognara E, Fabbri E, Bazzoli E, Montagner G, Ghimenton C, Eccher A, Cantù C, Manicardi A, Bianchi N, Finotti A, Breveglieri G, Borgatti M, Corradini R, Bezzetti V, Cabrini G, Gambari R. **Uptake by human glioma cell lines and biological effects of a peptide-nucleic acids targeting miR-221.** *J Neurooncol.* 2014; 118:19-28.

[4] Aboud OA, Wettersten HI, Weiss RH. **Inhibition of PPAR $\alpha$  Induces Cell Cycle Arrest and Apoptosis, and Synergizes with Glycolysis Inhibition in Kidney Cancer Cells.** *Plosone* 2013