Supplemental_Fig_S2

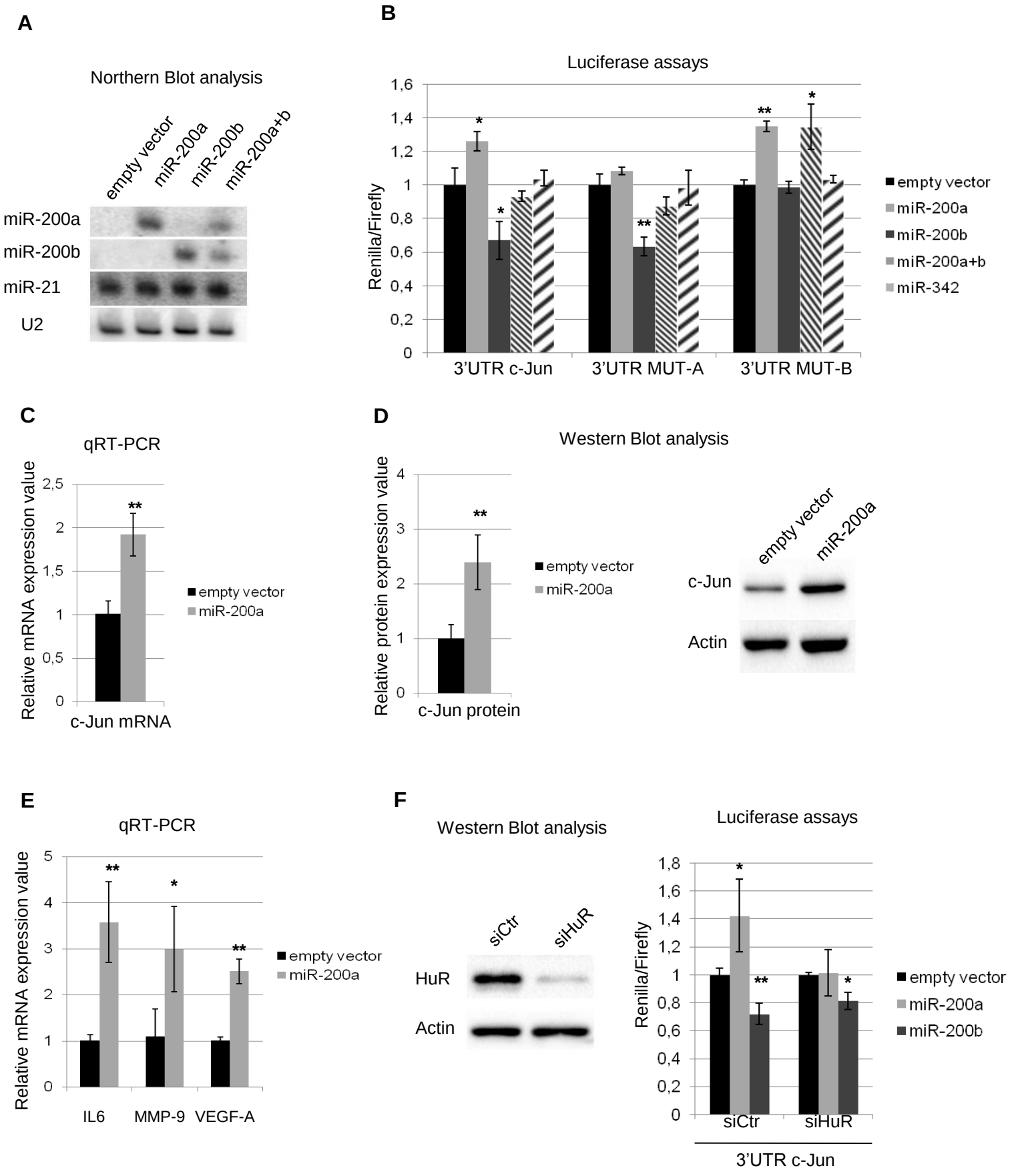

Fig S2. miR-200a as non-canonical regulator of c-Jun in HeLa cells.

Northern Blot analysis showing the expression levels of miR-200a and miR-200b upon transfection of empty vector or miR-200a or miR-200b coding plasmids in HeLa cells. miR-21 expression was verified as positive control. U2 was used to normalize. A representative picture is reported. (B) Histograms of luciferase assay results show the non-canonical and canonical regulation of c-Jun in HeLa cells, mediated respectively by miR-200a and miR-200b. Dual-luciferase constructs containing downstream the Renilla gene the full-length c-Jun 3'UTR (3'UTR c-Jun) or the 3'UTR bearing mutated binding site for miR-200a (3'UTR MUT-A) or for miR-200b (3'UTR MUT-B) were co-transfected with miR-200 coding plasmids (with either miR-200a and miR-200b coding plasmids or a mixture of them) in HeLa cells. The Renilla luciferase activity was measured 24h after the transfection. The empty vector and miR-342 coding plasmid were used as negative controls. Each Renilla luciferase reading was normalized to the related Firefly luciferase value. Data are presented as means±SD from three replicates in one of at least two independent experiments. (C-D-E) miR-200a affects mRNA and protein levels of c-Jun as well as c-Jun activity. mRNA expression levels of both c-Jun (C) and c-Jun-regulated genes (E) were observed 24h after miR-200a coding plasmid transfection by qRT-PCR experiments. The empty vector was used as negative control. GAPDH was used as endogenous control. Relative quantification was performed using the comparative cycle threshold (ΔΔCt) method. Error bars are SD of three biological replicates. (D) Western blot with anti-c-Jun and anti-actin antibodies was performed on protein extracts from HeLa transfected with miR-200a over-expression plasmid or empty vector. Relative quantification to actin is shown in the histogram on the right. Error bars are SD of four biological replicates. (F) Luciferase assay measurements upon HuR depletion indicating that miR-200a-mediated regulation of c-Jun is HuR-dependent. (F left) Western blot analysis of protein extracts from HeLa cells transfected with 50nM siRNA against HuR (siHuR) or negative control (siCtr) two times at 24h interval, confirming HuR depletion.

(F right) HeLa cells were first transfected with siHuR or siCtr and after 24h were again transfected with siRNAs, c-Jun 3'UTR reporter construct and miRNA coding plasmids. After 24h from the last transfection, Renilla luciferase activity was measured and analyzed, as previously described.

For all experiments, asterisks (*) indicate statistically significant modulations with respect to the empty vector according to unpaired Student's t test, *$p < 0.05$, **$p < 0.01$.