Supplementary Files

Figures S1-S5, Tables S1-S4

Supplementary Figure S1. Expression of miR-885-5p and miR-331-3p in neuroblastoma cell lines and stable clones (a) Conservation of *miR-885-5p*. Comparison of *miR-885-5p* sequences across species. The yellow box indicates the genomic sequence corresponding to mature miR-885-5p. **(b)** The expression of miR-885-5p and miR-331-3p was assessed using northern blotting. The precursor and mature forms of the miRNAs are indicated. **(c)** The genomic regions containing miR-885-5p and miR-331-3p were cloned into pcDNA3.1 vector and transfected into SH-EP and KELLY cells. Transcription of miR-885-5p and miR-331-3p in miR-885-5p-SH-EP, miR-885-5p-KELLY, miR-331-3p-SH-EP and miR-331-3p-KELLY cells was verified by RT-qPCR as described in Materials and Methods.

Supplementary Figure S2. Multinucleation, centrosome amplification and heterozygous *TP53* mutation at codon 15 were identified in the HDN33 cell line. Multinuclear cells were calculated after crystal violet staining. The mean of three independent experiments is presented (±SD). Centrosomes were visualized with immunofluorescent analysis using antibody to γ-tubulin (Dianova) as described by Meraldi et al (Meraldi et al., 2002). The sequencing of p53 exons was performed as described by Idbaih et al (Idbaih et al., 2007).

Supplementary Figure S3. Caspase-3 activity in miR-885-5p and miR-331-3p mimic-transfected SH-EP, IMR32, KELLY, HDN33 and SK-N-BE(2)c. Caspase-3 activity was measured using the Caspase-3 fluorimetric assay kit (BioVision) according to manufacturer's instructions. Fluorescence was detected using the FluorStar Optima microplate fluorescence reader (BMG Labtech). Caspase-3 activity (bar = mean relative caspase activity ±SD) was assessed after miR-885-5p or miR-331-3p transfection using colorimetric assay. The lowest activity value was set to 1, and caspase-3 activities are plotted as fold-increase relative to the lowest value.

Supplementary Figure S4. Stable expression of miR-885-5p leads to inhibition of cell proliferation and activation of p53 pathway. (a) (b) anchorage-dependent growth of miR-885-5p-

SH-EP-miR-885-5p, miR-885-5p-KELLY, miR-331-3p-SH-EP, miR-331-3p-KELLY, vector-transfected and parental SH-EP and KELLY cells was evaluated in 96-well plates by crystal violet staining and Alamar Blue assay (right panels). Numbers indicate cells initially seeded per well, and representative results from one of two independent experiments are shown. Anchorage-independent growth was assessed in soft agar (middle panels). 5000 cells were seeded per well, and cultured for 3 weeks. SA-ß-Gal assays were performed; the positively stained cells were counted (mean percentages ± SD of three experiments are presented). The mean numbers of colonies ± SD are indicated (left panels). **(c) (d)** expression of p53, $p21^{waf1}$, CDK2 and MCM5 are shown in western blots of miR-885-5p-SH-EP, miR-885-5p-KELLY, miR-331-3p-SH-EP, miR-331-3p-KELLY, vector-transfected and parental SH-EP and KELLY cells.

Supplementary Figure S5. Caspase-3 is targeted by miR-885-5p and its depletion can lead to $p21^{waf1}$ accumulation.

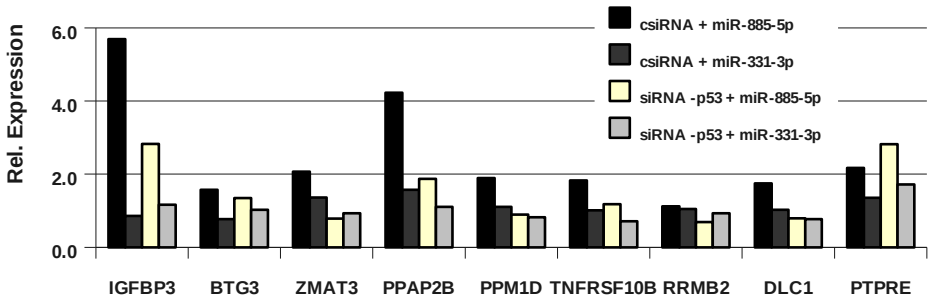
(a) predicted miR-885-5p target sites in the *CASP3* 3'UTRs. Luciferase reporter assays of stably transfected cells and miRNA-mimic-transfected cells (indicated on x-axis) transiently transfected with either empty vector or vector containing wild-type or mutant miR-885-5p binding sites (indicated as bar color) were performed as described in the Materials and Methods. Firefly luciferase values were normalized to renilla activity. The highest normalized luciferase activity of the stable transfected cells or cells transfected with miRNA mimics was set to 1, and values are reported as fold increase (mean relative luciferase activity ± SD). **, $P =< 0.05$; versus vector with mutant miR-885-5p binding sites. **(b)** protein expression of $p21^{waf1}$, p53, CASP3 and CDK2 from siRNA transfected SH-EP and IMR32 cells and antimiR-transfected IMR32 cells is shown in western blots. The relative densitometry values are shown.

Supplementary Table S1. Transcripts with altered expression upon miR-885-5p introduction. Genes differentially expressed after either transfection of miR-885-5p or miR-331-3p are indicated as overlap. Verified p53 targets and transcripts containing a miR-885-5p binding site are marked with

“p53 target” and “miR-885-5p target site”.

Supplementary Table S2. Transcripts with altered expression upon miR-331-3p introduction. Genes upregulated or downregulated after either miR-885-5p or miR-331-3p expression are marked as overlap. Established p53 targets and transcripts containing a miR-331-3p binding site are marked.

Supplementary Table S3. miR-885-5p upregulated p53 target genes are dependent on functional p53. Kelly cells 3 were profiled 3 days after miR-885-5p or miR-331-3p mimic transfections in the presence of p53 targeting siRNA or control siRNA.



Supplementary Table S4. Sequences containing miR-885-5p predicted binding sites used to construct pmiRGLO plasmids.

Plasmid name	Sequence
pmiRGLO-CDK2-site	5'-AAACTAGCGGCCGCCACCTTGGGGTTTTGTAATGACT-3'; 5'-CTAGAGTCATTACAAAACCCCAAGGTGCGGCCGCTAGTTT-3'
pmiRGLO-CDK2-mut-site	5'-AAACTAGCGGCCGCCACCTTGGGGTTTTGATTTGACT-3'; 5'-CTAGAGTCAAATCAAAACCCCAAGGTGGCGGCCGCTAGTTT-3'
pmiRGLO-MCM5-site	5'-AAACTAGCGGCCGCAGAGGAAGGAGCTGTAGTGTT-3'; 5'-CTAGACACTACAGCTCCTTCCTCTGCGGCCGCTAGTTT-3'
pmiRGLO-MCM5-mut-site	5'-AAACTAGCGGCCGCCACCTTGGGGTTTTGATTTGACT-3'; 5'-CTAGAGTCAAATCAAAACCCCAAGGTGGCGGCCGCTAGTTT-3'
pmiRGLO-CASP3-site1	5'-AAACTAGCGGCCGCTAGTAAGTTAAACATTGAAGTAATGAATTTT-3'; 5'-CTAGAAAATTCATTACTTCAATGTTTAACTTGCGGCCGCTAGTTT-3'
pmiRGLO-CASP3-mut-site1	5'-AAACTAGCGGCCGCTAGTAAGTTAAACATTGAACAATTGAATTTT-3'; 5'-CTAGAAAATTCAATTGTTCAATGTTTAACTTGCGGCCGCTAGTTT-3'
pmiRGLO-CASP3-site2	5'-AAACTAGCGGCCGCTAGTGTAAATACACATAGCATGTAATGGTATCTTT-3'; 5'-CTAGAAAGATACCATTACATGCTATGTGTATTTACACTAGCGGCCGCTAGTTT-3'
pmiRGLO-CASP3-mut-site2	5'-AAACTAGCGGCCGCTAGTGTAAATACACATTGCACGCTGTGGTATCTTT-3'; 5'-CTAGAAAGATACCACAGCGTGCAATGTGTATTTACACTAGCGGCCGCTAGTTT-3'

References

Meraldi P, Honda R, Nigg EA. Aurora-A overexpression reveals tetraploidization as a major route to centrosome amplification in p53−/− cells. EMBO J 2002; 21: 483–492.

Idbaih A, Boisselier, B., Sanson, M., Criniere, E., Livad, S., Marie, Y., Carpentier, C., Paris, S., Laigle-Donadey, F., Mokhtari, K., Kujas, M., Hoang-Xuan, K., Delattre, O., Delattrea, J-Y.Tumor genomic profiling and TP53 germline mutation analysis of first-degree relative familial gliomas. Cancer Genetics Cytogenetics 2007; 176: 121-126