Supplementary Information

The miR-106b~25 cluster promotes bypass of doxorubicin-induced senescence and increase in motility and invasion by targeting the E-cadherin transcriptional activatior EP300

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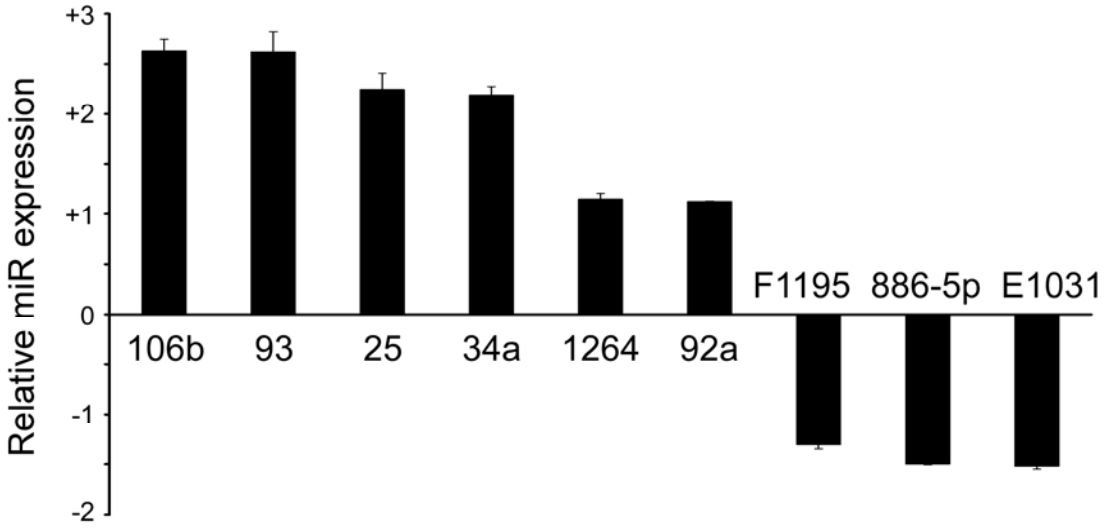
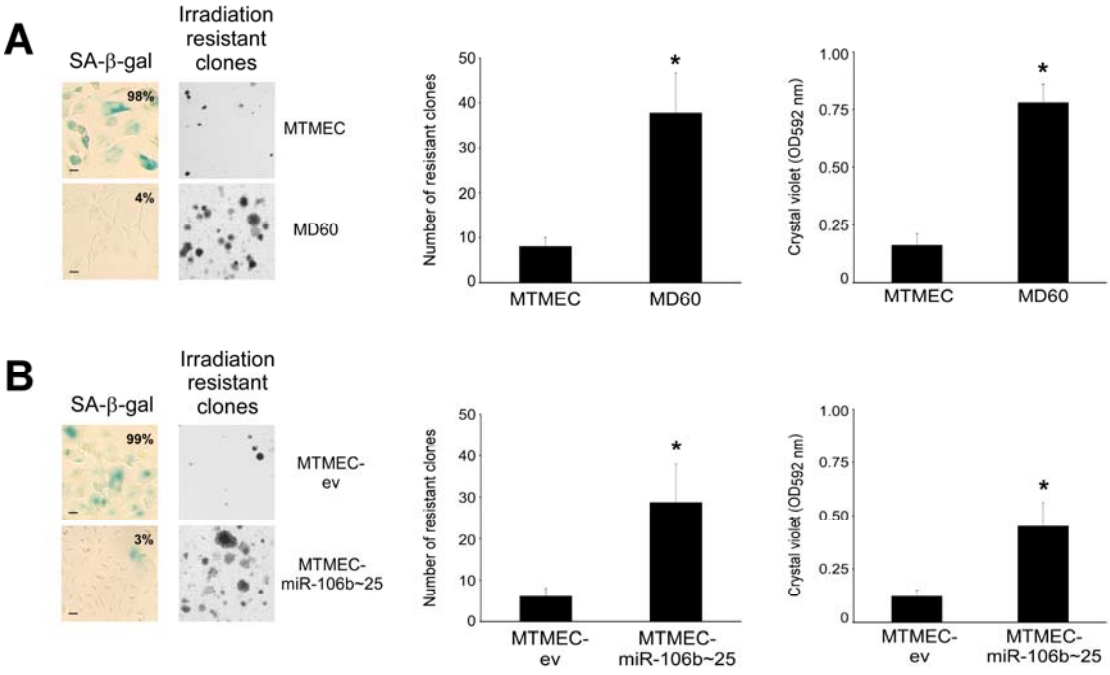


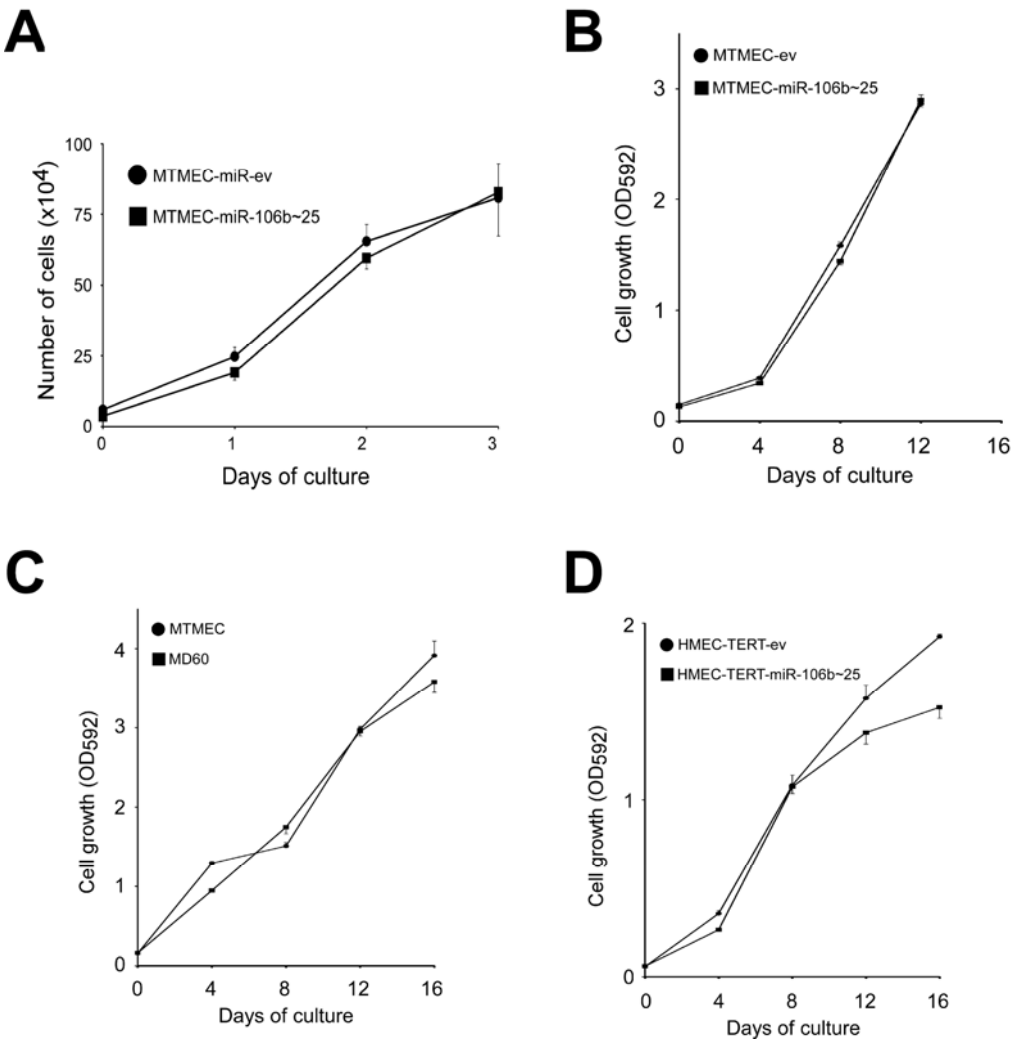
Supplementary Figure S1. Differential miR expression in doxorubicin resistant minimally transformed mammary epithelial cells.

A customary differential miR expression array (miRCURY™ LNA Array version 5th Generation) containing all human miRs in the miRBASE version 15.0 was performed by Exiqon. Only 9 miRs were differentially expressed more than 1-fold, and of these, only 4 more than two-fold, between drug naïve minimally transformed mammary epithelial cells (MTMEC) and their doxorubicin-resistant derivative, MD60. Data shown represents the average ± SD of three independent biological replicates.



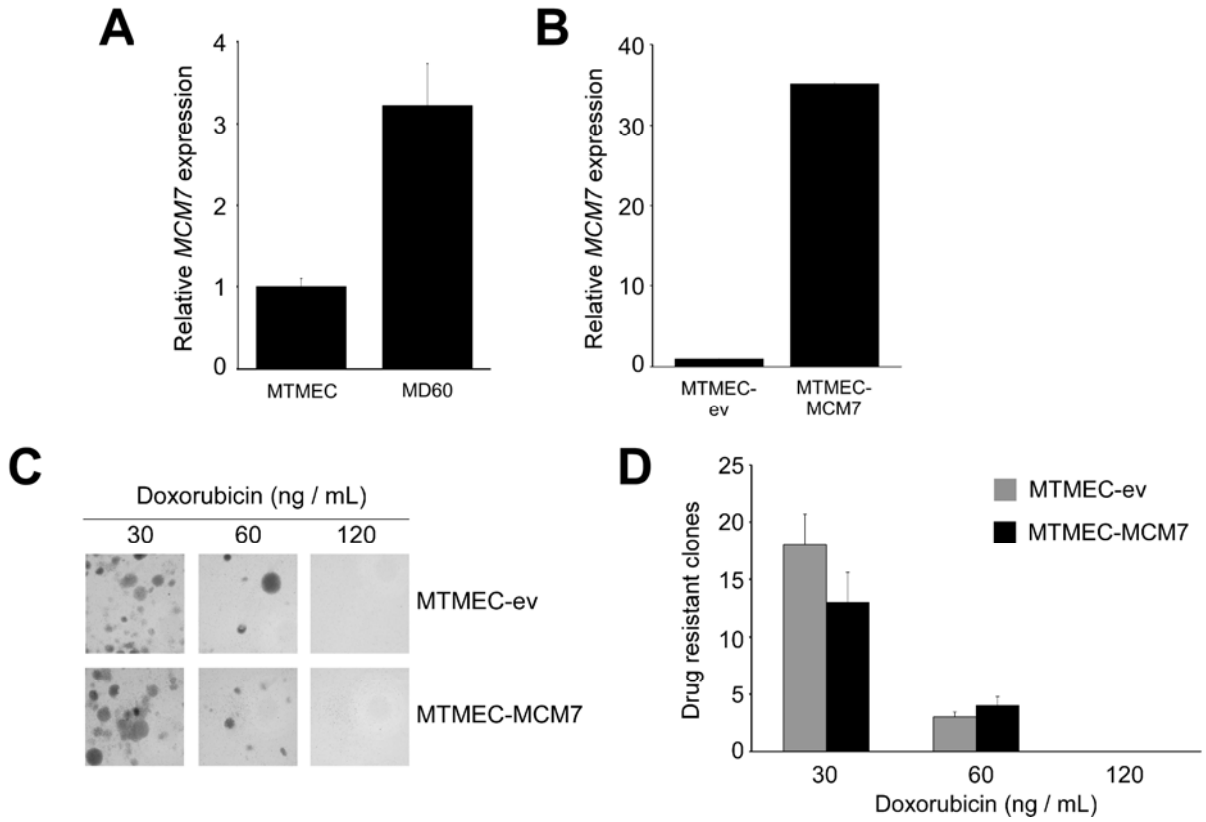
Supplementary Figure S2. The miR-106b~25 cluster enables cells to bypass γ-irradiation induced senescence.

A) MTMEC and MD60 cells received a dose of 9 Gy γ-irradiation and the percentage of cells staining positive for SA-β-galactosidase determined after 7 days (*left picture*). Percentage of senescent cells is indicated after monitoring at least 6 fields of view (typical variation approximately 10%). Bar represents 30 μm. Three weeks after irradiation, resistant clones were visualized by crystal violet staining (*right picture*), counted (*left histogram*) and the amount of cell-bound dye measured at OD_{592} nm after its solubilization (*right histogram*). B) MTMEC cells overexpressing the miR-106b~25 cluster or empty vector control cells were irradiated and assessed as in A. Numerical data represent the average of three independent experiments ± SD (*$p<0.05$). Pictorial data shows a representative of at least three independent replicates.



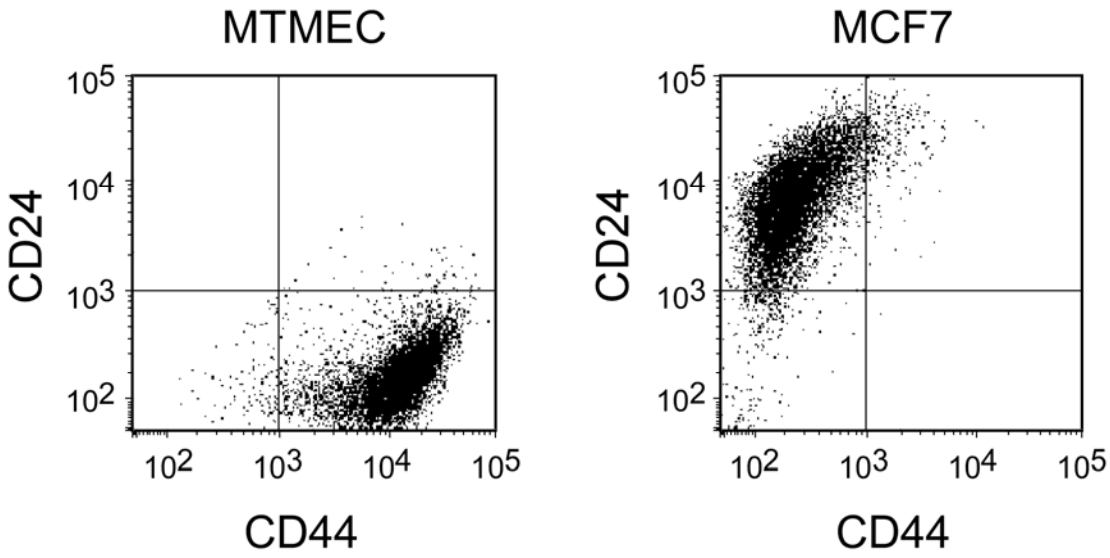
Supplementary Figure S3. Experimental up-regulation of miR-106b~25 cluster expression does not increase cell proliferation in mammary epithelial cells under drug-free conditions.

MTMEC-miR-106b~25 and MTMEC-ev control cells were seeded at approximately 5,000 cells / cm^2 (A) and 125 cells / cm^2 (B). Cell growth was monitored after trypsinization and Typtan blue exclusion (A) or by crystal violet staining, solubilisation and by optical density at 592 nm (B). Drug naïve MTMEC and drug resistant MD60 (C) and immortalized primary HMEC cells by telomerase expression (HMEC-TERT) over- expressing miR-106b~25 (D) were seeded and growth monitored as in B. Data represent the average of three independent experiments ± SD.



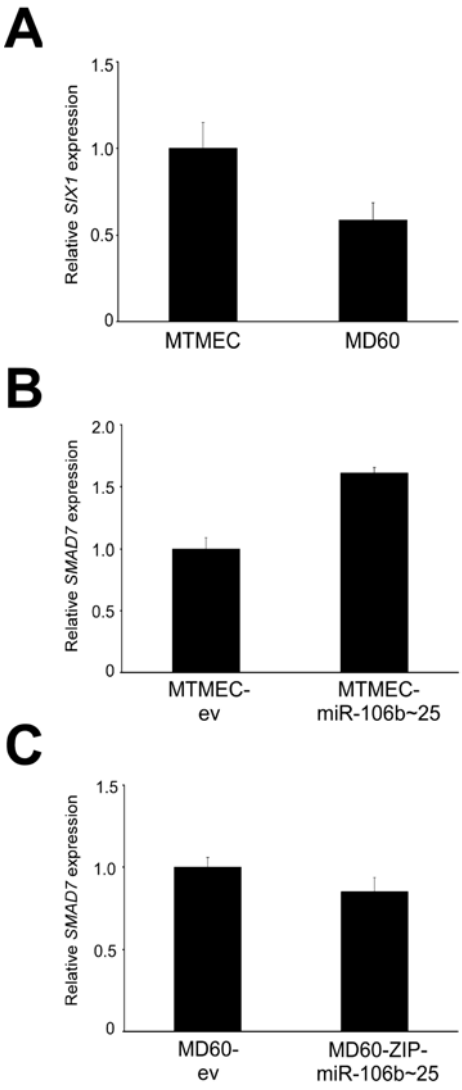
Supplementary Figure S4. Generation of doxorubicin resistance by the miR-106b~25 cluster is independent of the host gene.

A) *MCM7* mRNA is up-regulated in drug resistant MD60 cells. B) Experimental up-regulation of *MCM7* in MTMEC cells after infection with a lentiviral vector harbouring *MCM7* coding region or empty (control cells). *MCM7* expression was determined by reverse transcription and real-time PCR and was normalized to the expression of *RPS14* mRNA. C, D) Clonogenic assay 3 weeks after a single 24-h doxorubicin treatment. C) Crystal violet staining. D) Number of doxorubicin-resistant clones. Numerical data represent the average of three independent experiments ± SD. Pictorial data shows a representative of at least three independent replicates.

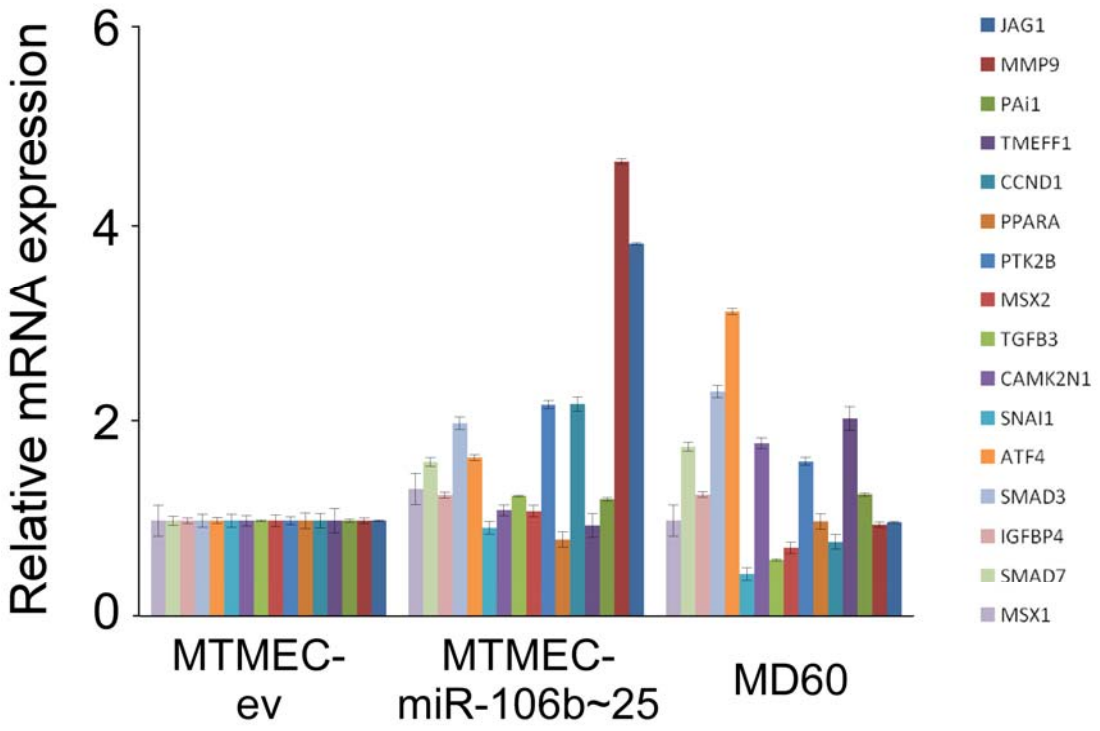


Supplementary Figure S5. MTMEC cells are CD44^{high}.

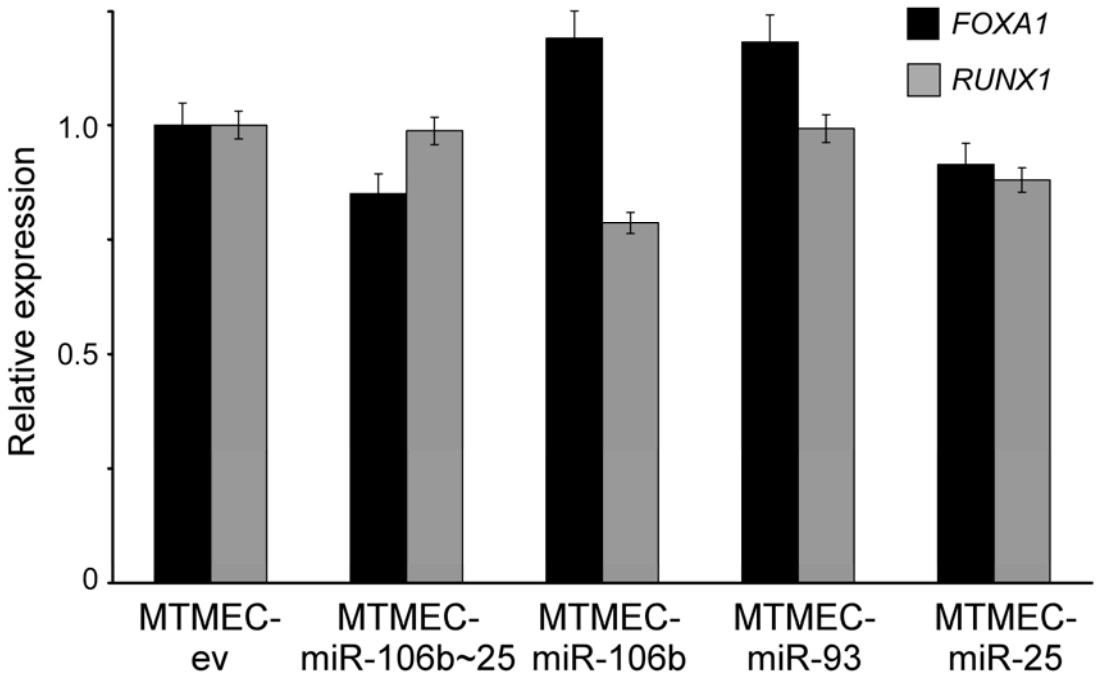
Flow cytometry analysis of CD24 and CD44 in MTMEC and MCF7 cells using phycoerythrin-conjugated anti CD24 IgG and FITC- conjugated anti CD44 IgG. Contrary to MCF7 cells, MTMEC cells are CD44 ^{high}/CD24^{low}. Plot data shows a representative experiment of at least two independent replicates.



Supplementary Figure S6. The miR-106b~25 cluster acts in a *SIX1/SMAD7*-independent fashion in minimally transformed mammary epithelial cells.
A) *SIX1*, a transactivator of the miR-106b~25 cluster, is not up-regulated in drug resistant MD60 cells. B) *SMAD7*, a potential target of the miR-106b~25 cluster, is not down-regulated in MTMEC-106b~25 cells. C) Down-regulation of miR-106b~25 cluster in MD60 cells (MD60-ZIP-miR-106~25) does not up-regulate *SMAD7* expression. Expression data was determined by reverse transcription and real-time PCR and was normalized to the expression of *RPS14* mRNA and represents the average ± SD of at least two experiments.

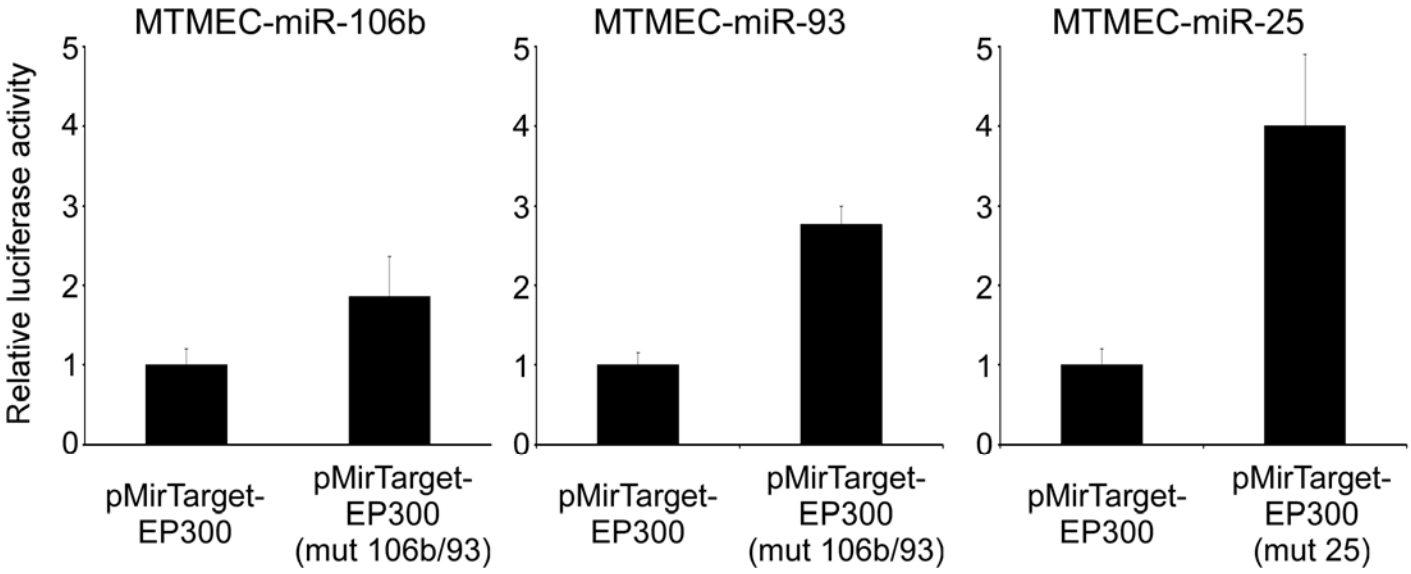


Supplementary Figure S7. Expression of TGF-β down-stream targets.
Expression of a panel of 16 TGF-β targets was determined by reverse transcription and real-time PCR in MTMEC cells overexpressing the miR-106b~25 cluster and drug resistant MTMEC cells, MD60, and normalized to the expression of *RPS14* mRNA. Data is expressed relative to drug naïve MTMEC cells transfected with empty lentiviral vector (MTMEC-ev) and represents the average ± SD of at least two experiments.



Supplementary Figure S8. *FOXA1* and *RUNX1* are not targets of the mir-106b~25 cluster.

Expression of the miR-106b~25 cluster, or its individual miRs, does not lead to down-regulation of either *FOXA1* or *RUNX1*. Expression analysis by reverse transcription and real-time PCR normalized to the expression of *RPS14* mRNA. Data show the average ± SD of at least two experiments



Supplementary Figure S9. The miR-106b~25 cluster targets EP300.

The putative miR target sequence in *EP300* 3'-UTR was mutated to abrogate miR-106b/93 [*pMirTarget-EP300 (mut 106b/93)*] or miR-25 binding [*pMirTarget-EP300 (mut 25)*] and used to transiently transfect MTMEC-miR-106b (*left panel*), MTMEC-miR-93 (*middle panel*) and MTMEC-miR-25 (*right panel*) cells. In all cases cells were co-transfected with a *Renilla* luciferase expression vector to normalize for transfection efficiency. Normalized firefly luciferase activity is shown relative to that from the reporter with the *EP300* 3'-UTR (*pMirTarget-EP300*). Data show the average ± SD of at least two experiments.