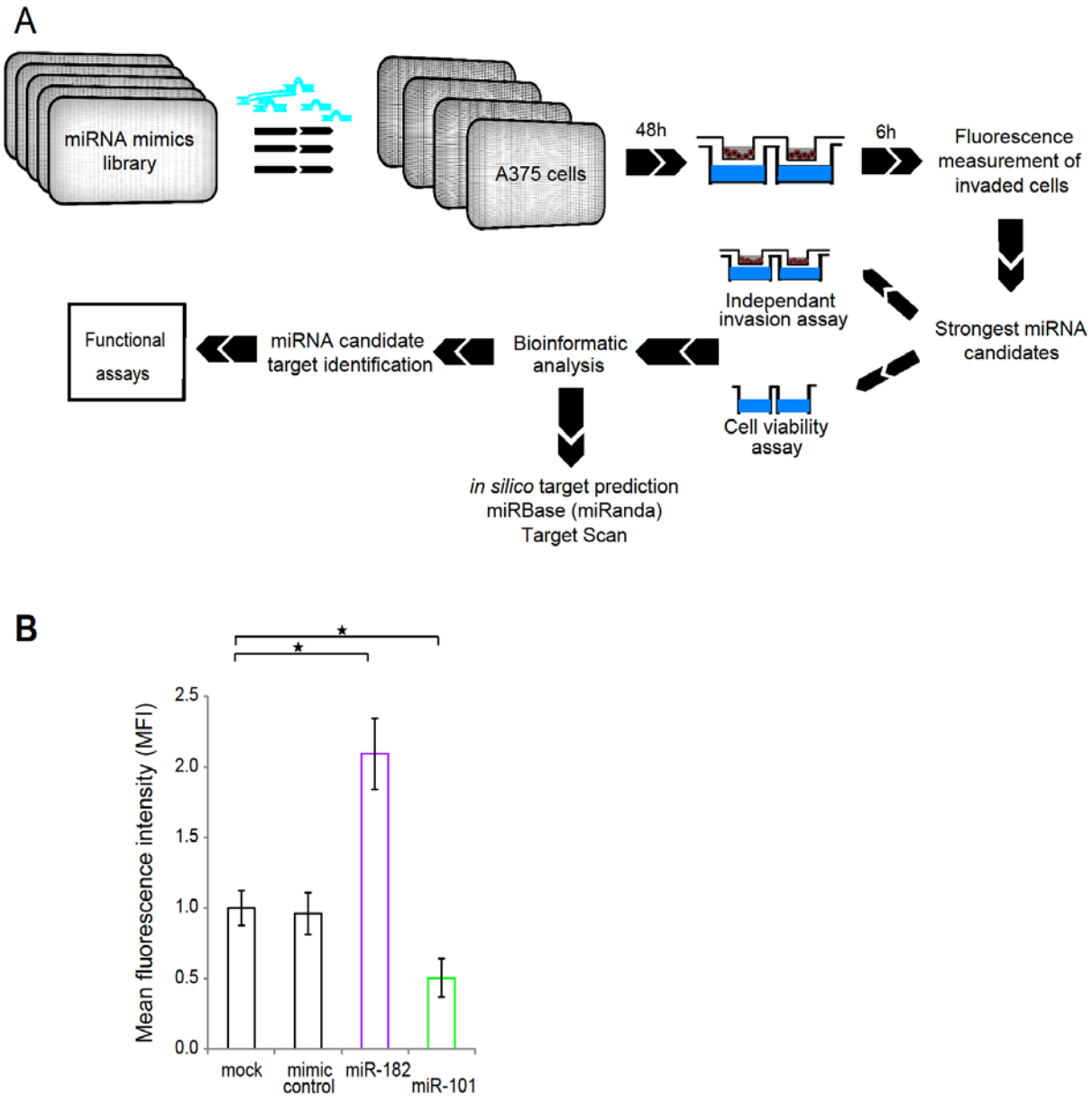
Supplemental Figures



Supplemental Fig. S1. Workflow for a functional screening approach to investigate miRNAs impacting on melanoma cell invasion. (A) A375 melanoma cells were transfected with miRNA mimics from a human library and seeded 48 hours later into a Matrigel-coated Boyden chamber with a FCS gradient. Invasion was analyzed 6 hours later. Subsequent validation of selected miRNA candidates was performed as outlined. **(B)** A375 cells were seeded into the 96-well Boyden chamber wells and the amount of invaded cells was determined by fluorescence staining of living cells. As positive controls, miR-182 known to accelerate melanoma cell invasion (purple) and miR-101 inhibiting melanoma cell invasion (green), were selected. Mean fluorescence intensity (MFI) was measured and normalized to mock treated control cells. Significance at the 0.05 level is indicated by asterisk (*).

Fig. S2. Cell viability assay on 97 most effective miRNA candidates accelerating (A) or inhibiting (B) A375 invasion. A375 cells were seeded into 96-well plates and transfection with the respective miRNA mimics was performed 24h later. After 48h, the number of viable cells was determined by CTG assay. The green and purple lines indicate a deviation in A375 cell viability by more than 20% (green, decreased; purple, increased viability). Data are shown as mean +/- STD of biological triplicates. miRNAs were sorted according to their increasing (A) or decreasing (B) invasive capacity, respectively.

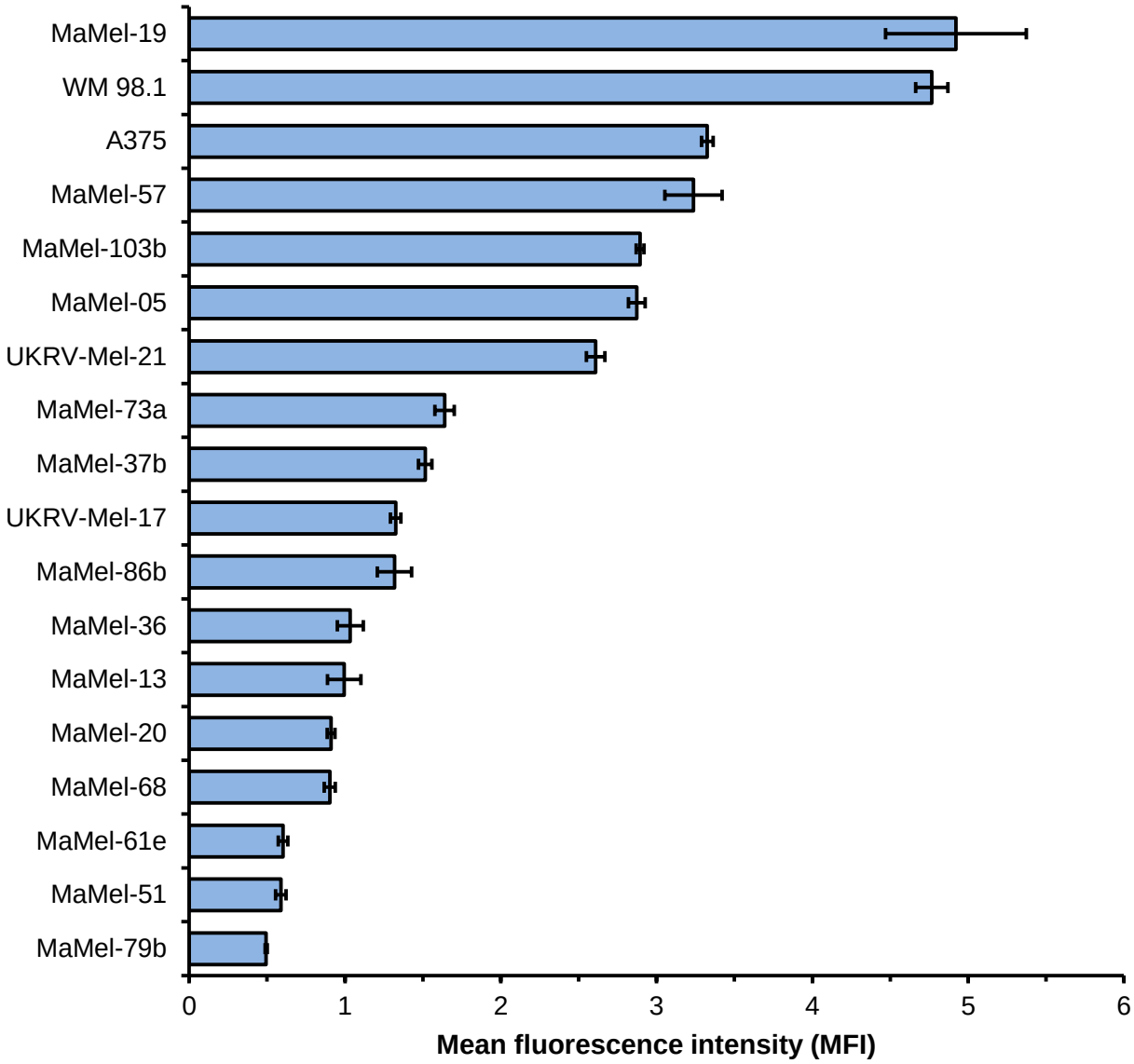


Fig. S3. Invasive capacity of melanoma cell lines. Eighteen melanoma cell lines were tested in the 96-well Boyden chamber assay for their invasive capacity in biological triplicates after an invasion time of 24h.

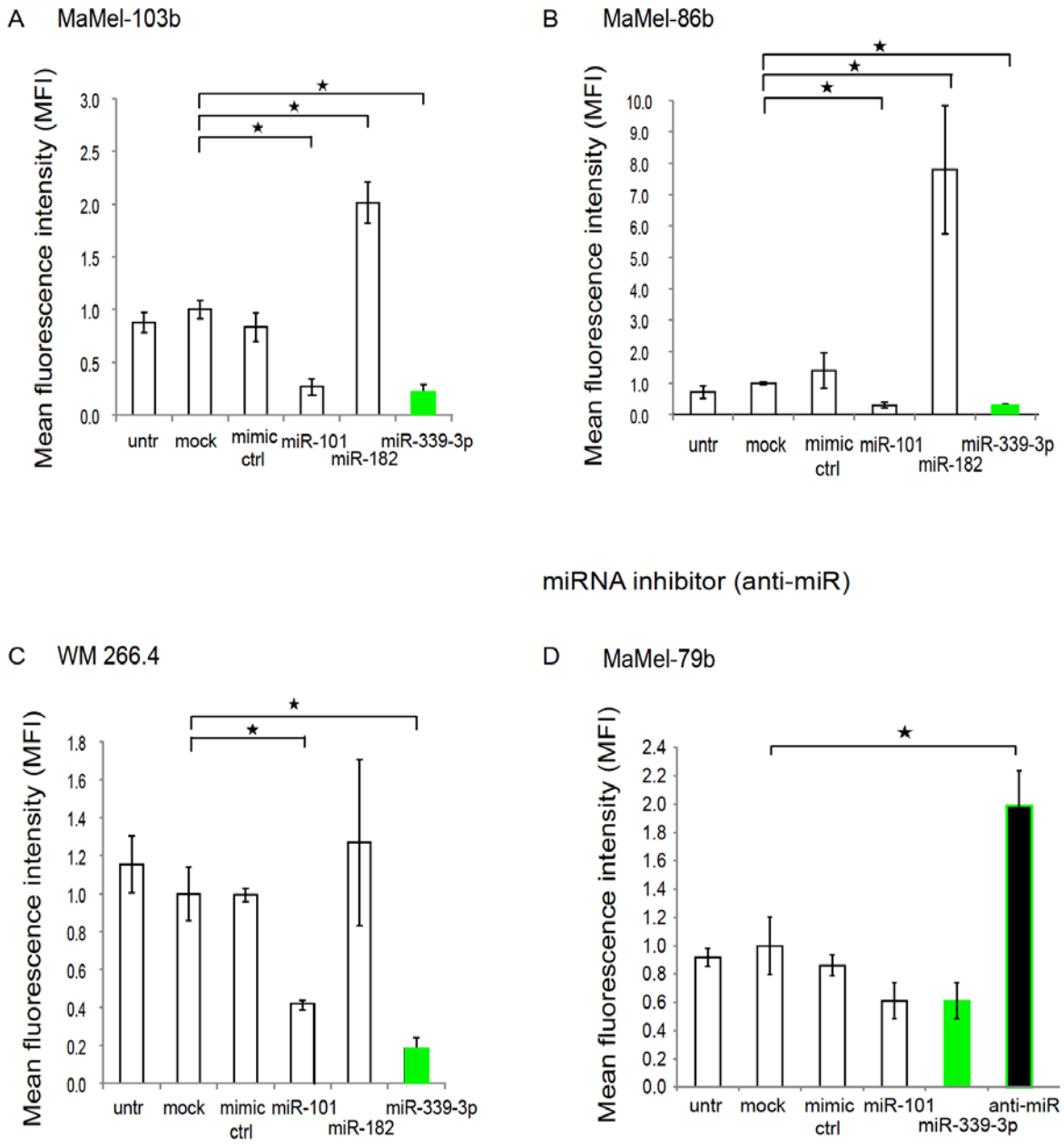


Fig. S4. miR-339-3p inhibits invasion in various melanoma cell lines. miR-339-3p could be shown to inhibit melanoma cell invasion in three different melanoma cell lines, MaMel-103b, MaMel-86b and WM 266.4 in a Boyden chamber assay format **(A-C)**. Anti-miR-339-3p treatment restored the invasive capacity of MaMel-79b **(D)**. Mean fluorescence intensity was measured (MFI) and normalized to mock control treated cells. Significance at the 0.05 level is indicated by asterisk (*).

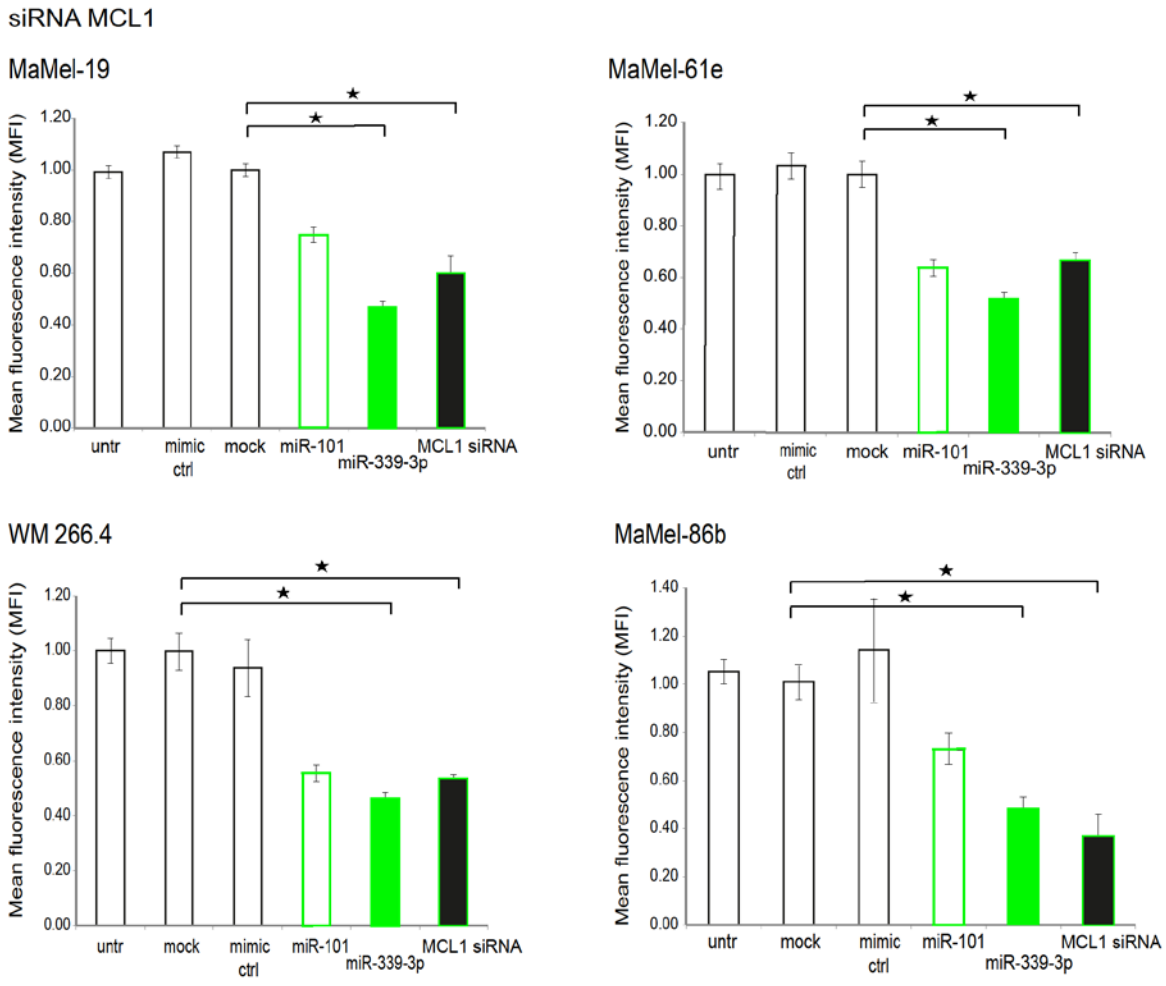


Fig. S5. MCL1 specific siRNA inhibits melanoma cell invasion. siRNA against MCL1 could be shown to mimic the effect of miR-339-3p on melanoma cell invasion in melanoma cell lines MaMel-19, MaMel-61e, WM 266.4 and MaMel-86b in a Boyden chamber assay format. Mean fluorescence intensity (MFI) was measured and normalized to mock control treated cells. Significance at the 0.05 level is indicated by asterisk (*).

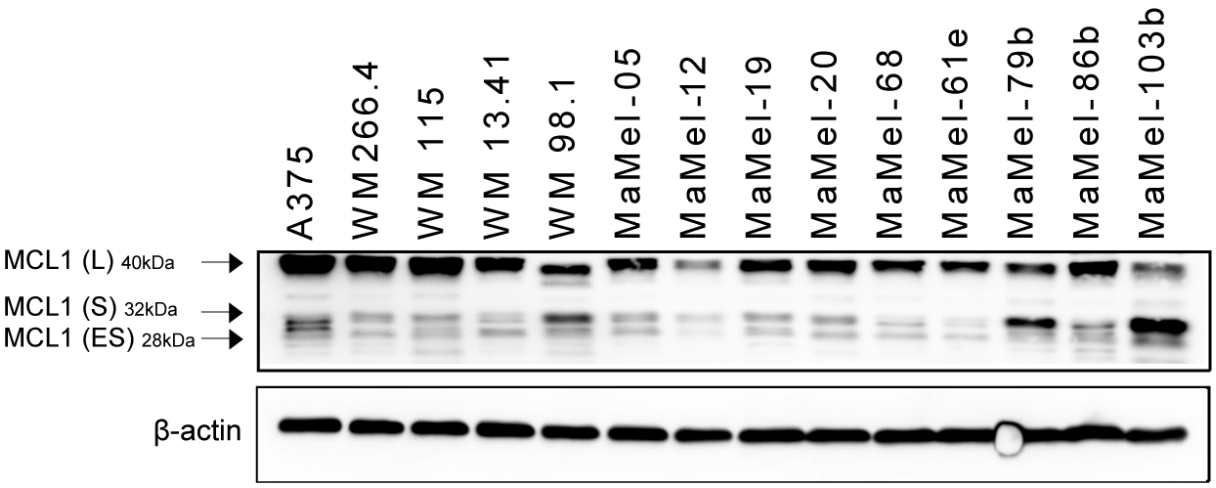


Fig. S6. MCL1 protein expression levels determined in 14 melanoma cell lines.
MCL1splicing variants: long isoform MCL1 (L), short isoforms MCL1 (S) and MCL1 (ES).

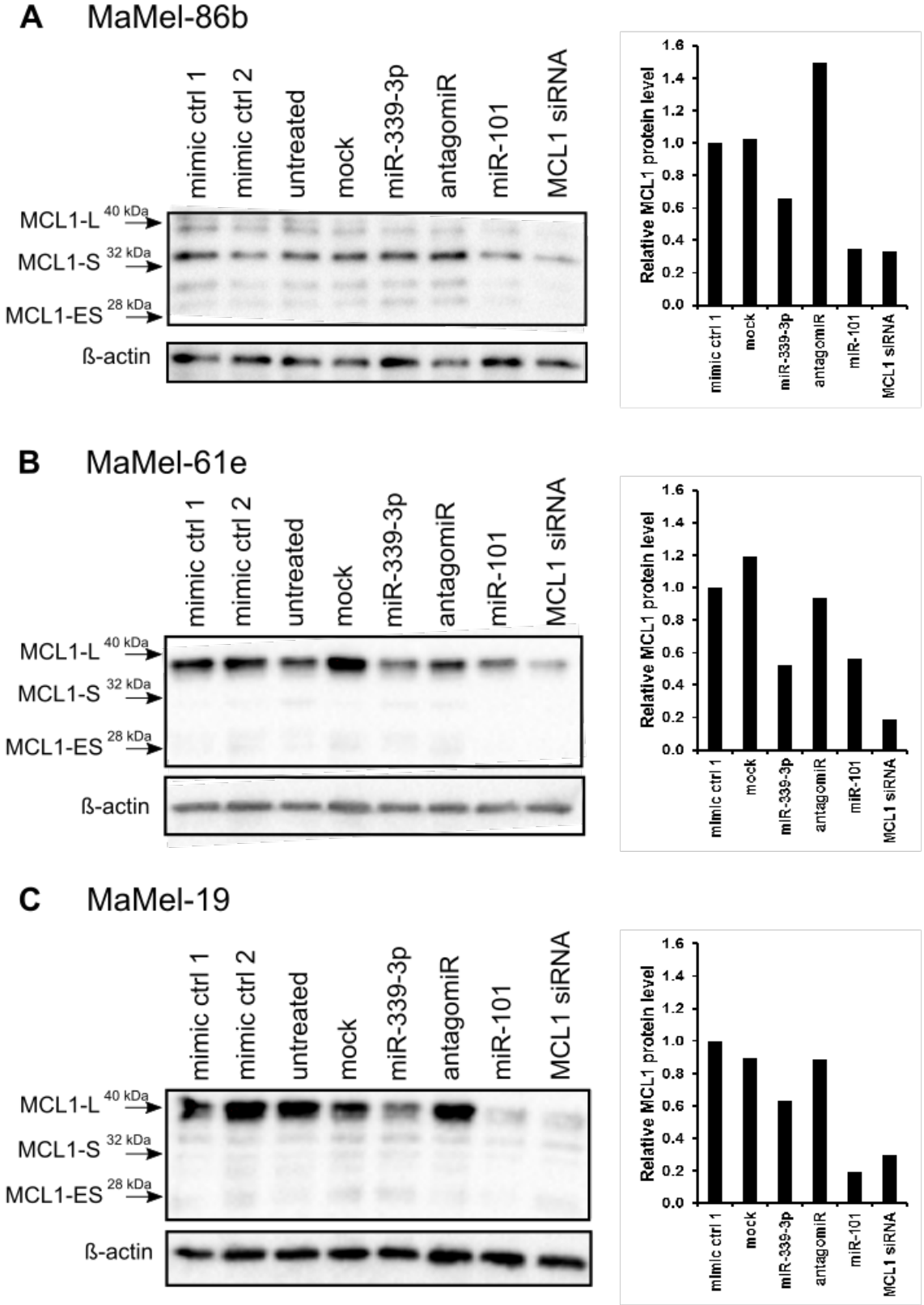


Fig. S7. MCL1 protein expression can be downregulated by miR-339-3p. Cell lines MaMel-86b, MaMel-61e and MaMel-19 were transfected with negative control mimics (ath-miR-416: mimic ctrl 1; cel-miR-243: mimic ctrl 2), miR-339-3p, antagomiR, miR-101, MCL1 siRNA or mock treated. Protein was measured after 48h. Graphs to the right give quantification by densitometry, normalized to β-actin and then to mimic control.