Figure S1

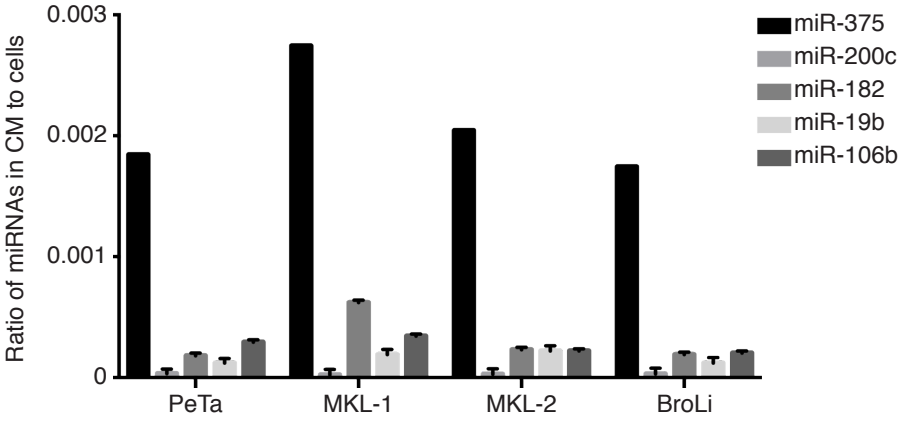

Fig. S1: miR-375 demonstrates by far the highest ratio of extracellular to cellular miRNA
The presence of miR-375, miR-200c, miR-182, miR-19b and miR-106b in 200μl of conditioned medium from the MCC cell lines culture (48 hours culture of 10^6 cells per ml) and in the cells themselves was determined by RT-qPCR in triplicate. The ratio of respective miRNA calculated by the 2-ΔΔCq method in conditioned medium to the cells is depicted.

Figure S2

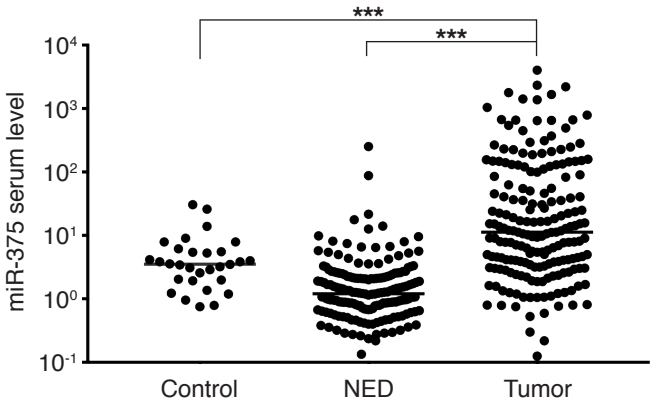

Fig. S2: Circulating cell-free miR-375 in serum discriminates healthy donors from MCC patients with tumor burden

cf miR-375 in sera of healthy donors (n=30) and MCC patients (n=105) was determined by RT-qPCR in duplicate, normalized to spiked-in cel-mir-39 and values were calculated relative to the serum of an MCC patient with no evidence of disease (Graz cohort) by the 2-ΔΔCq method. Results are depicted in Cleveland dot plots categorized by healthy donors and patients with no (NED) or with evidence of disease. The horizontal lines indicate the medians, statistical analyses were performed as described in Material and Methods; p***<0.001

Figure S3

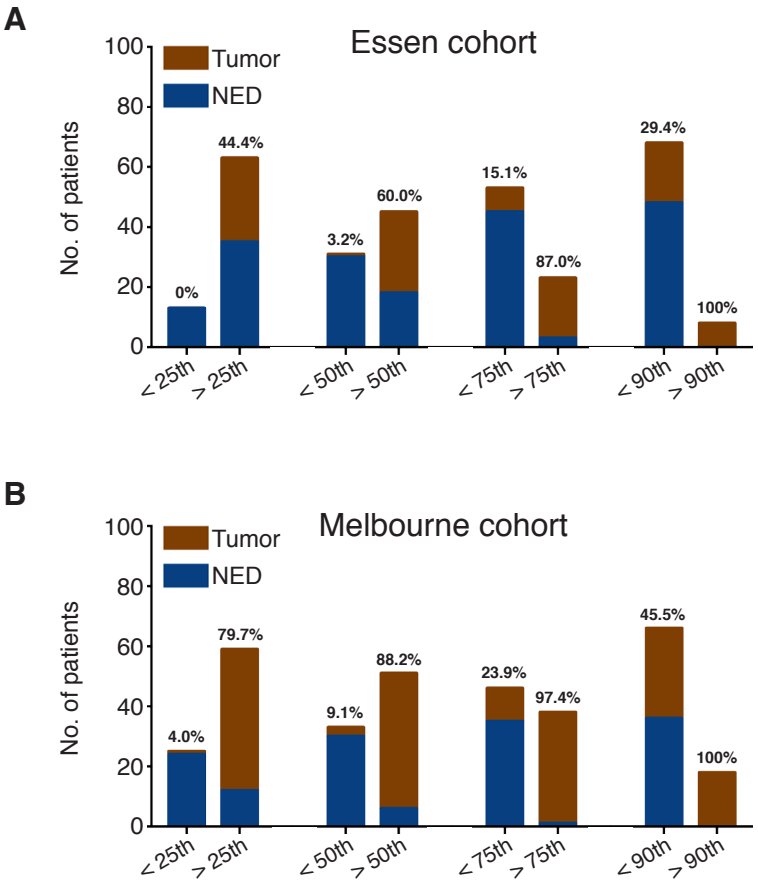

Fig. S3: cf miR-375 serum level percentiles of retrospective discovery and training cohorts were applied to discriminate tumor or NED in prospective validation patient cohorts

Percentiles (25th, 50th, 75th, and 90th) of cf miR-375 serum levels were calculated for the combined discovery and training cohorts. The proportions of MCC patients from the two prospective validation cohorts (**A**: Essen cohort; **B**: Melbourne cohort) with miR-375 serum levels above or below these respective percentiles are depicted. Percentages of MCC patients with detectable tumor burden are given for each group.

Figure S4

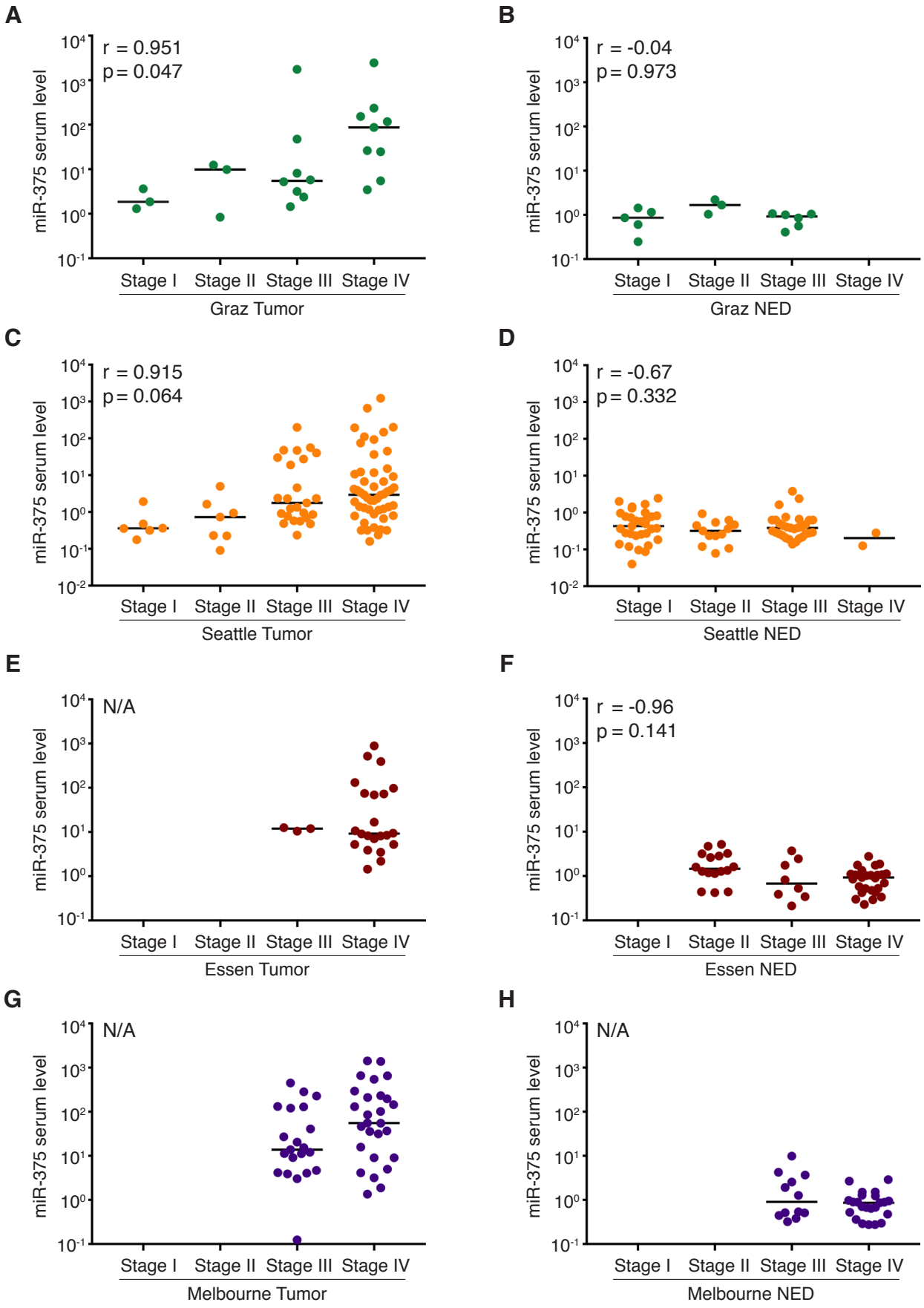

Fig. S4 Correlation analysis was performed between cf miR-375 serum levels and disease stages in four cohorts separately. N/A means too few pairs for correlation analysis. The horizontal lines indicate the medians, statistical analyses were performed as described in Material and Methods.

Figure S5

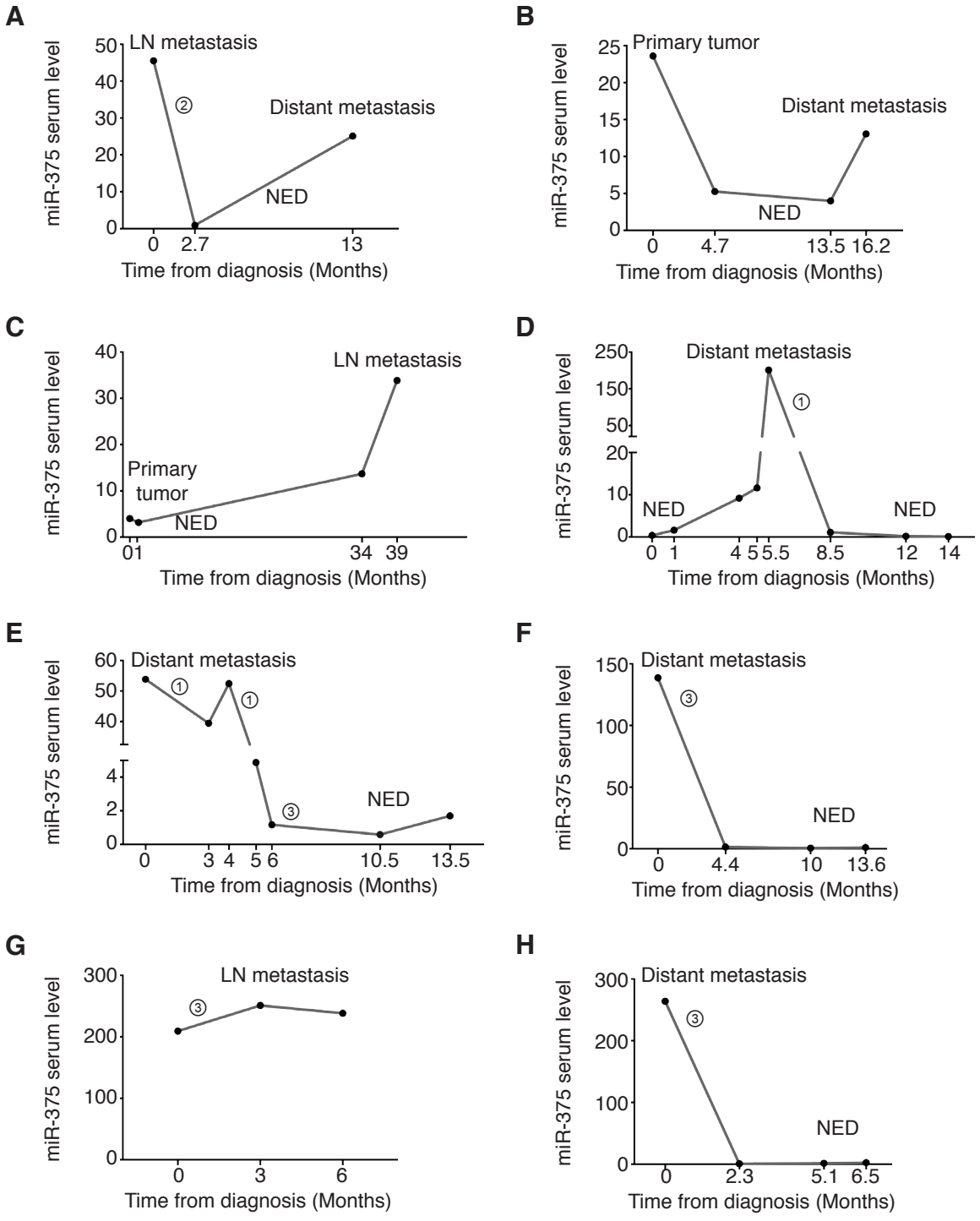

Fig. S5 : Tracking the course of disease in MCC patients using circulating cell-free miR-375
cf miR-375 in sera of MCC patients was determined by RT-qPCR in duplicate, normalized to spiked-in cel-mir-39, and values were calculated relative to the serum of a MCC patient with no evidence of disease (Graz cohort) by the 2-ΔΔCq method. Results are plotted over time together with the narrative description of the course of disease. (**A**: t_#3 and **B**: t_#6 from Graz cohort; **C**: c_#08, **D**: c_#30 and **E**: c_#31 from Seattle Cohort; **F**: e_#4, **G**: e_#2 and **H**: e_#8 from Essen cohort). The numbers circled indicate the different therapies: ① Radiation therapy, ② Chemotherapy and ③ Immunotherapy.

Figure S6

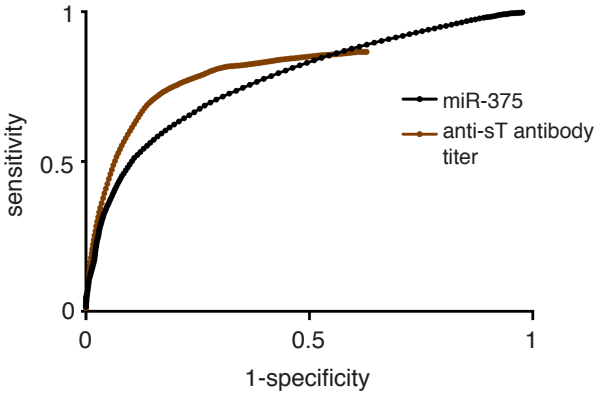

Fig. S6: Comparison of receiver operating characteristic (ROC) curves for anti-MCPyV oncogenic protein antibodies and circulating cell-free miR-375 in serum of MCC patients. Smooth ROC curves of anti-MCPyV small T antigen antibody titer and miR-375 serum level are depicted for the Seattle cohort patients.