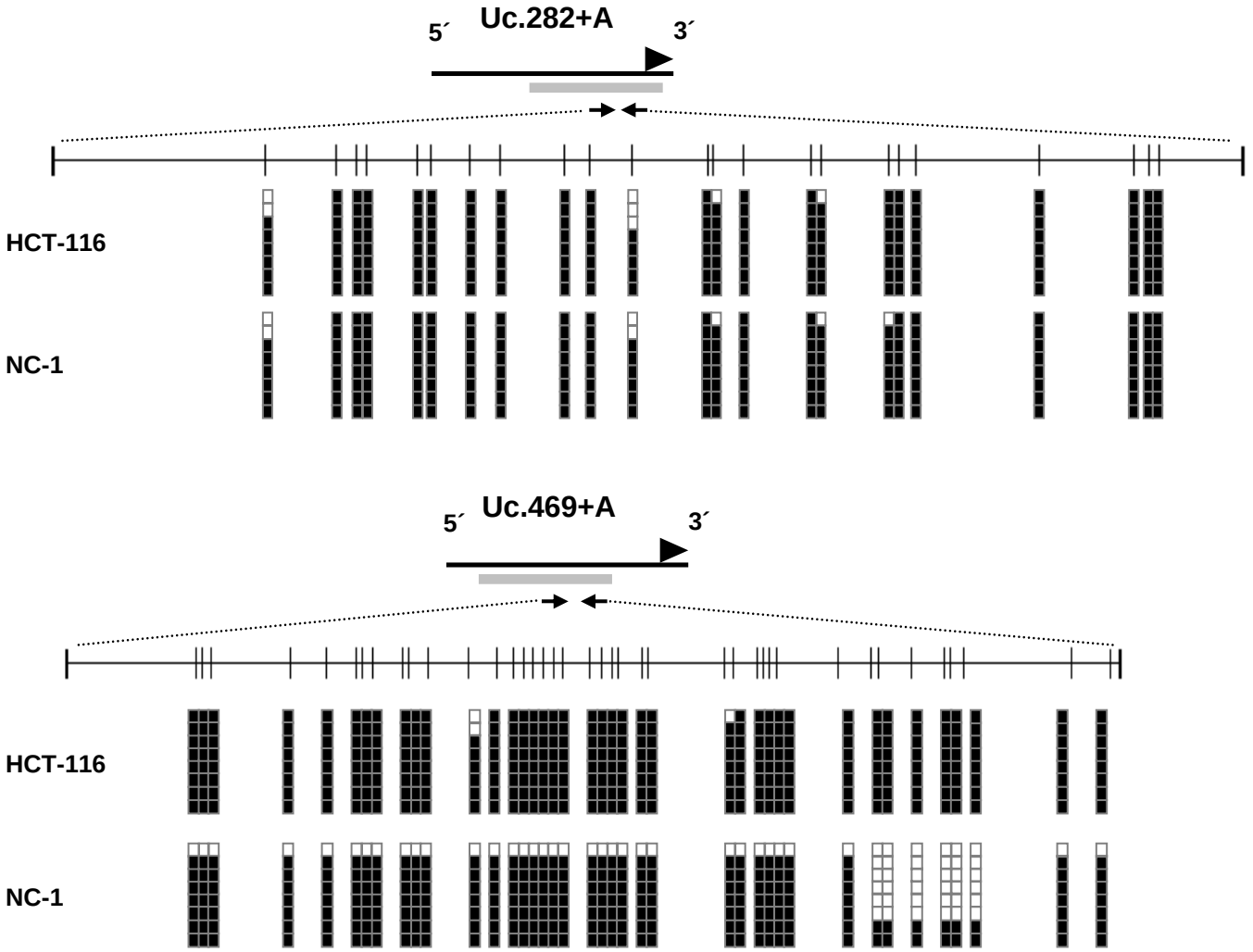
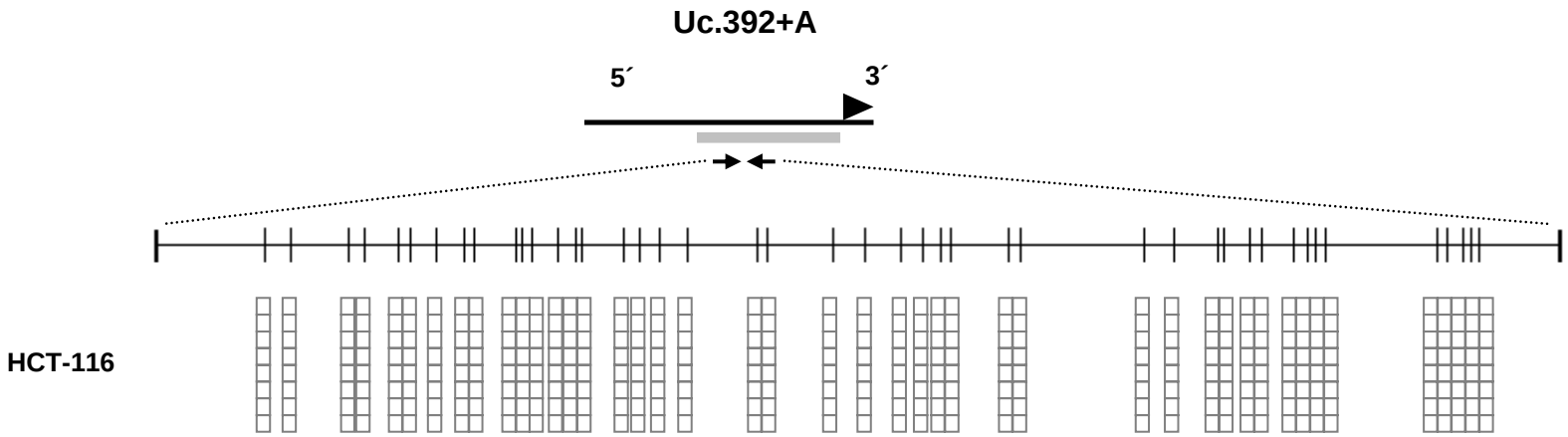
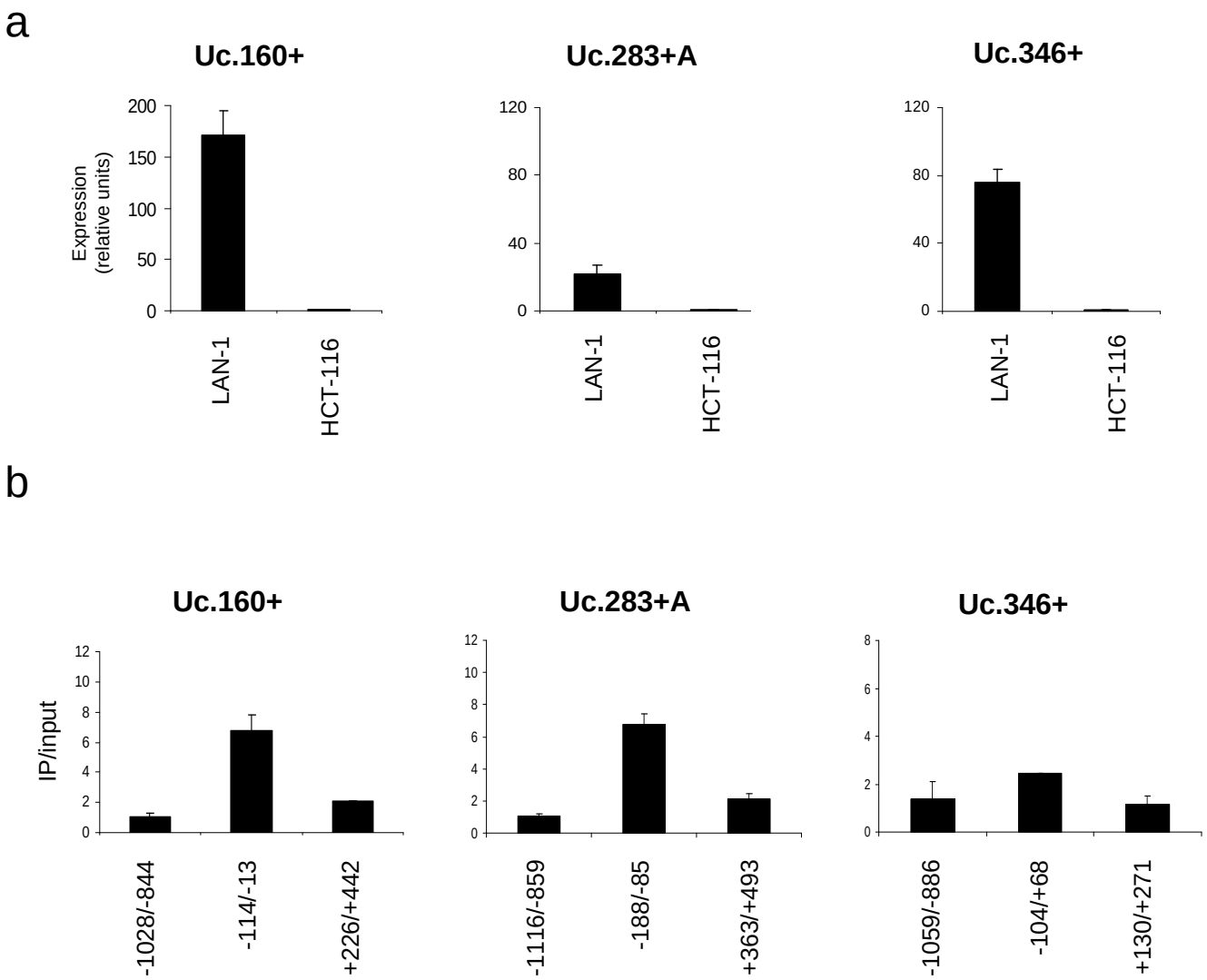


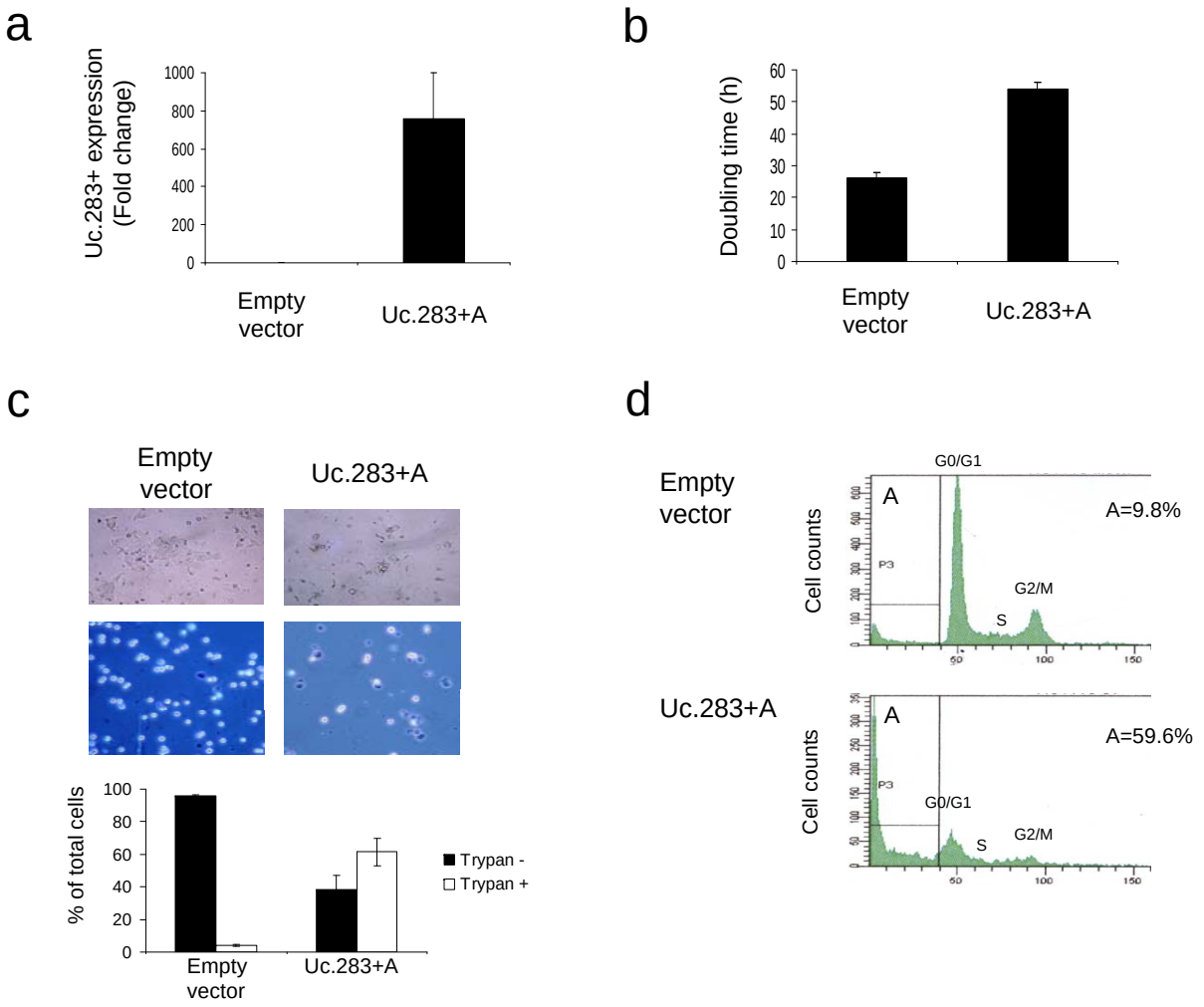
Supplementary Figure 1. Bisulfite genomic sequencing analysis of Uc.282+A and Uc.469+A CpG island methylation status in HCT-116 cells and normal colon mucosa (NC-1). Eight single clones are represented: unmethylated (white squares) and methylated (black squares) CpGs are indicated. Location of the T-UCR probe is shown as an arrow black head. The CpG island is indicated by a grey line. Black arrows indicate genomic bisulfite sequencing primers.



Supplementary Figure 2. Bisulfite genomic sequencing analysis of Uc.392+A CpG island methylation status in HCT-116 cells. Eight single clones are represented: unmethylated (white squares) and methylated (black squares) CpGs are indicated. Location of the T-UCR probe is shown as an arrow black head. The CpG island is indicated by a grey line. Black arrows indicate genomic bisulfite sequencing primers.



Supplementary Figure 3. (a) Expression levels of Uc.160+, Uc.283+A and Uc.346+, evaluated by qRT-PCR, in the CpG island unmethylated neuroblastoma cell line LAN-1. (b) Quantitative chromatin immunoprecipitation (qChIP) analysis of the pattern of RNA polymerase II occupancy around the transcription start site of T-UCRs in CpG island unmethylated LAN-1 cells. Numbers on x–axis correspond to PCR product relative to the transcription start site.



Supplementary Figure 5. Growth inhibitory effects of Uc.283+A transfection. (a) Expression levels of Uc.283+A upon transfection in HCT-116 cells. (b) Uc.283+A transfected cells showed a significant increase in doubling time. The same cell number was platted in 12 well plates by triplcate and left to grow for 12, 24,36, 48 and 60 hours. Cells were tripsinized and counted using a Neubauer chamber at the time points upper mentioned. The doubling time was calculated as a mean of (in hours) = h*ln(2)/ln(c2/c1) at the different time points. (c) Cell death at 60 hours after transfection. On the top panel, an image of the cell culture plates from empty vector and Uc.283+A transfected HCT-116 cells. On the middle panel, representative image of the same culture plates upon counting in the Trypan blue exclusion test of cell viability. On the bottom panel, percentage of dead and live cells in the Trypan blue exclusion test of cell viability. (d) Cell cycle analysis. Effect of Uc.283+A transfection after 60 hours on DNA distribution evaluated by cytofluorymetric analysis. The distribution of cells in the subdiploid peak (A) is indicated within each panel.