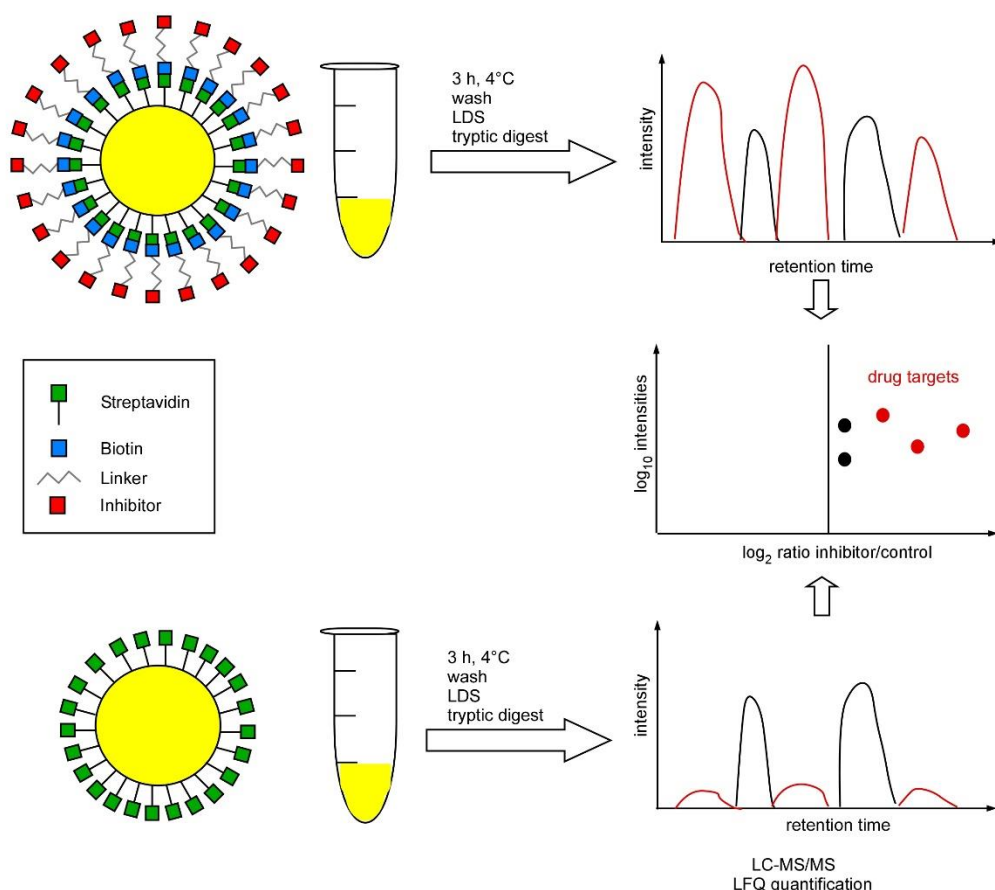
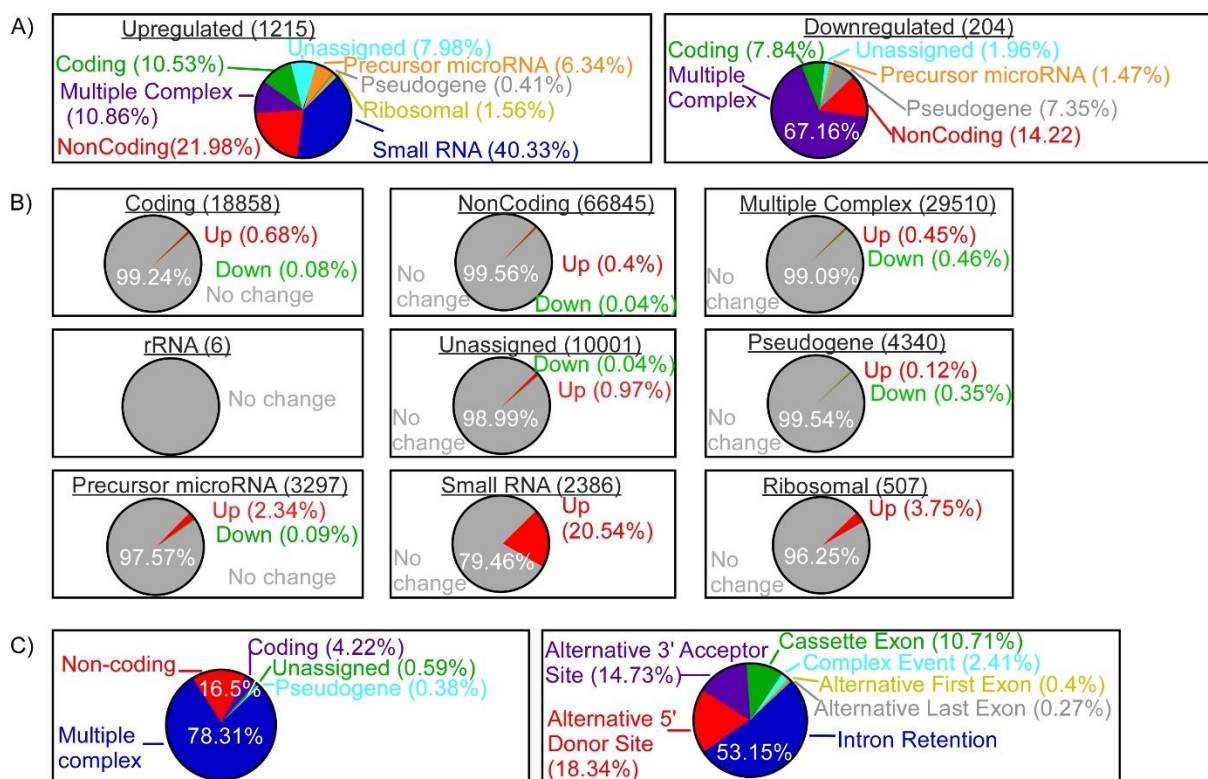


**Figure S1.** Overview of the synthesis of the biotin-labeled ancistrocladinium A derivative 2b.

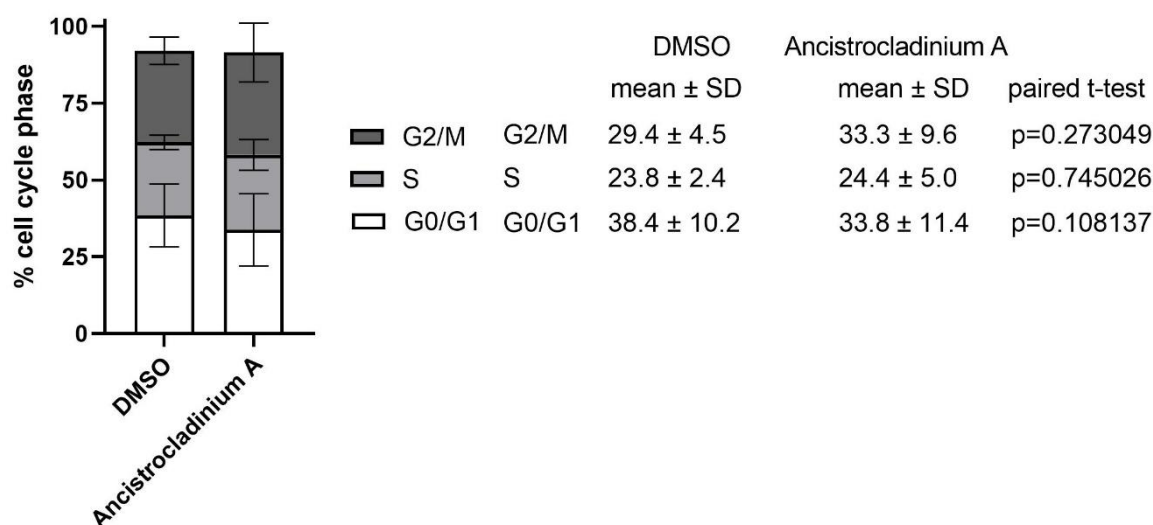


**Figure S2.** Affinity capture experiment for identification of potential target proteins and their interaction partners of ancistrocladinium A. A biotinylated inhibitor was loaded on streptavidin beads; empty beads were used as control. Both beads were incubated with INA-6 cell lysate for 3 h at 4 °C and washed, and captured proteins were eluted with LDS sample buffer. Proteins were digested with trypsin and analyzed by nanoLC-MS/MS. Label-free quantification (LFQ) of both samples was performed for all identified proteins. In the intensity vs. LC-MS/MS LFQ quantification, the different protein sequences with different abundancies are schematically drawn in 2

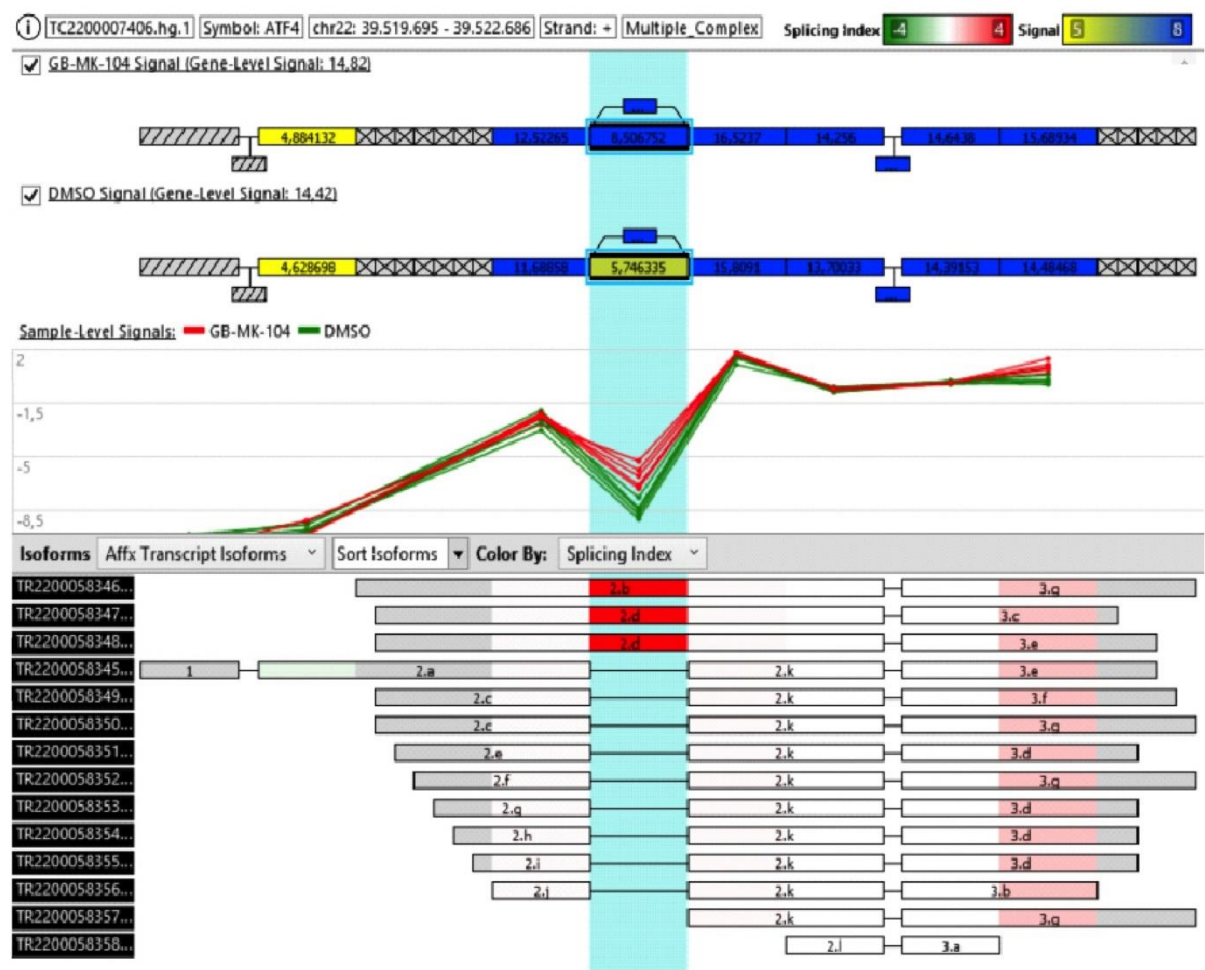
different colors (black = not specifically enriched proteins, red = specifically enriched proteins that bind to ancistrocladinium A). Specifically enriched drug target proteins are in drawn in red in the log<sub>10</sub> intensities vs. log<sub>2</sub> ratio inhibitor/control blot. Not significantly enriched proteins are drawn in black.



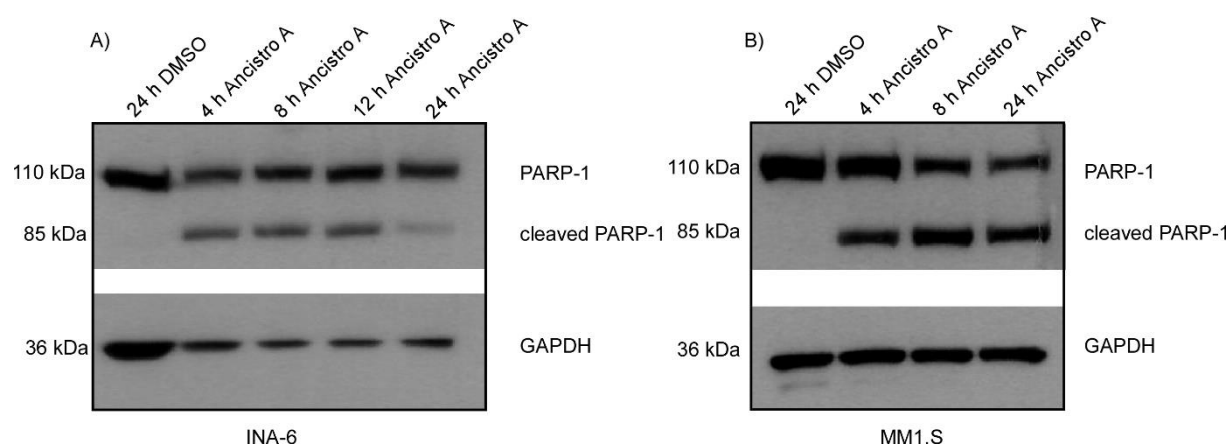
**Figure S3.** Ancistrocladinium A induces gene expression alterations and alternative splicing. INA-6 cells were treated for 4 h with 3  $\mu$ M Ancistrocladinium A or DMSO as control followed by RNA extraction, cRNA synthesis, ss cDNA transcription and hybridization to Affymetrix Clarion D human array. Afterwards, (A & B) gene expression changes and (C) splicing expression alterations were determined.



**Figure S4.** Ancistrocladinium A decreases the cells in G0/G1 phase. INA-6 cells were treated for 24 h with 3  $\mu$ M Ancistrocladinium A and followed by propidium iodide staining and flow cytometry-based cell cycle analysis. Four independent experiments were performed and a paired t-test with multiple comparisons was performed.



**Figure S5.** Ancistrocladinium A induces ATF4 protein expression in INA-6 cells. Splicing effect on ATF4-RNA 4 h after treatment of INA-6 cells with ancistrocladinium A (here labelled as GB-MK-104). Shown is the original low-resolution graph that was generated by the TAC4.0 software. Of note, in Figure 4, this graph was redrawn for better quality.



**Figure S6.** Ancistrocladinium A induces PARP-1 cleavage. **(A)** INA-6 and **(B)** MM1.S cells were treated for 4 h, 8 h, 24 h and INA-6 cells also for 12 h with 3  $\mu$ M Ancistrocladinium A followed by cell lysis and Western blot analysis of PARP-1 cleavage. GAPDH served as loading control.