Figure Supplementary 1.
Panel A. Luminescence interaction quantification assays were performed with the mammalian CheckMate system (Promega) in HeLa cells, and the firefly luciferase/renilla luciferase (F/R) signals ratio is shown. Compared to full-length NMT2, truncation of the C-terminal domain eliminated interaction with ACBD6. Association with ZNF23 protein, previously identified in a yeast two-hybrid screen (62), could not be confirmed in mammalian cells. Error bars represent the standard deviations of three measurements.
Panel B. Expression of ecNMT2 in E. coli is low (left gel) and a significant amount of the protein was lost in the insoluble fraction, even in presence of 0.1% Triton X-100 (middle panel). The protein was purified by metal-affinity chromatography (right panel).
Panel C. Real-time colorimetric measurements of the activity of purified NMT2 protein. Shown are examples of the trace of absorbance at 412nm obtained in presence of 50μM C14-CoA and 2μM ecNMT2 in the absence or presence of 100μM of the target peptide. The effect of temperature on the reaction is shown in the inset.
Panel D. Examples of traces obtained in the presence of various concentrations of peptide (10-50-100μM), and of C14-CoA (5-50μM) and of ecNMT2 (1x and 2x) as function of time are shown.