Supplementary data:

Jolanta Grembecka, Amalia M. Belcher, Thomas Hartley, and Tomasz Cierpicki

Molecular basis of the Mixed Lineage Leukemia (MLL) - menin interaction: Implications for targeting MLL leukemias.

Figure S1. MLL46 is natively unstructured protein. **A**. Heteronuclear $^{15}N\{^{1}H\}$ NOEs for MLL^{46} indicate high backbone flexibility. Majority of residues exhibit negative $^{15}N\{^{1}H\}$ NOEs consistent with lack of well ordered structure. The experiment was carried out for 0.7mM MLL46 in 50mM phosphate buffer, pH 6.8 and 50mM NaCl at 20ºC. **B**. Backbone order parameters (S^2) calculated for MLL^{46} based on chemical shifts indicate lack of well ordered structure. The order parameters (S^2) were calculated for Cα, Cβ, Hα, N, H^N chemical shifts employing RCI program (1).

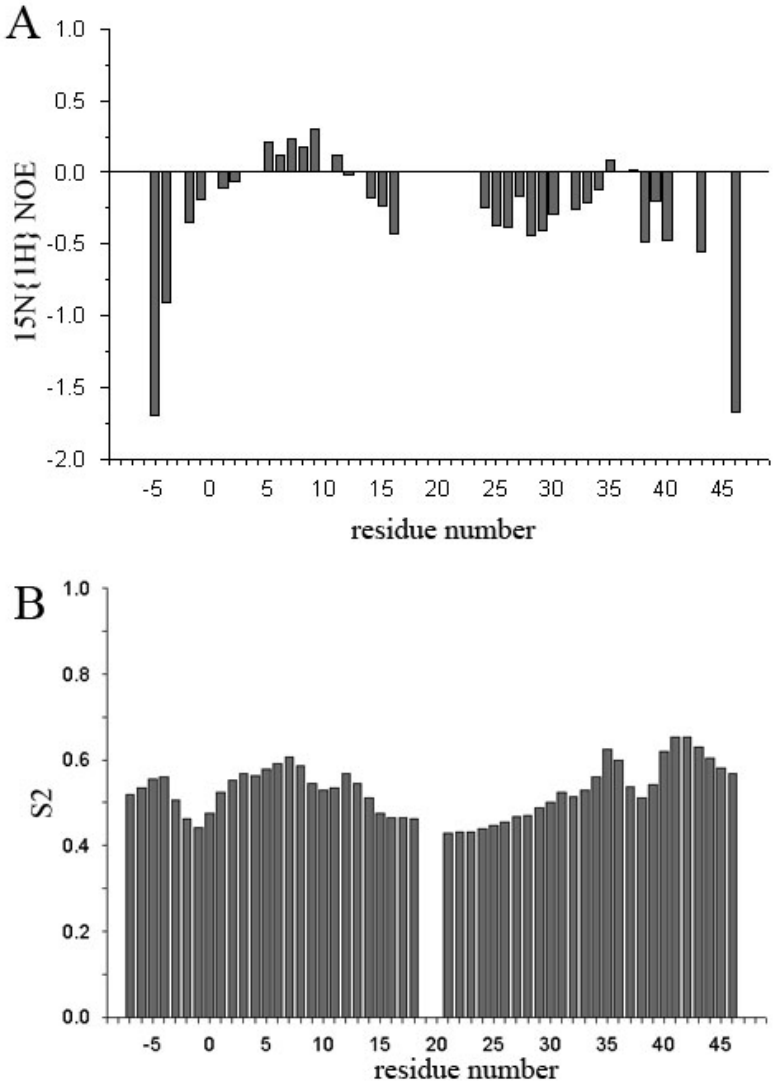


Figure S2. Comparison of ^{1}H-^{15}N HSQC and CACO spectra for 50μM MLL^{46} in 50mM TRIS, pH 7.5, 50mM NaCl measured at 25ºC. Complete set of resonances is observed on CACO spectrum while only about 10 backbone amides can be observed on HSQC spectrum due to fast exchange of amide protons with water. These experiments demonstrate advantage of using ^{13}C-detected experiments for binding studies of natively unstructured proteins.

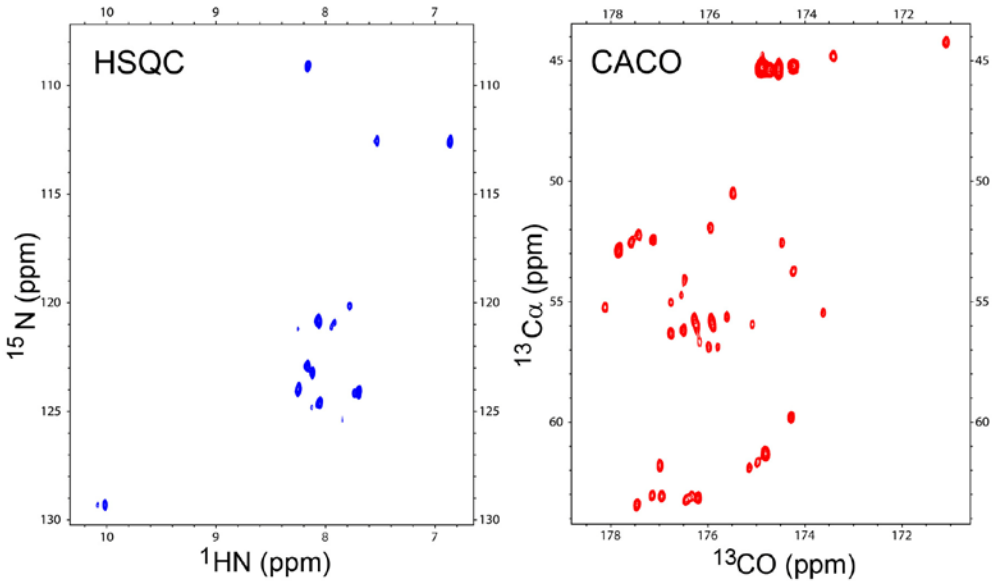


Figure S3. Mapping of MLL^{46} interaction with menin based on the NCO experiment. **A**. Assigned NCO spectrum for 50μM MLL^{46}. **B**. Spectrum for 50μM MLL^{46} in the presence of 40μM menin. Very strong broadening is observed for MBM1 and MBM2 residues and only MLL residues which remain highly flexible in the complex are observed.

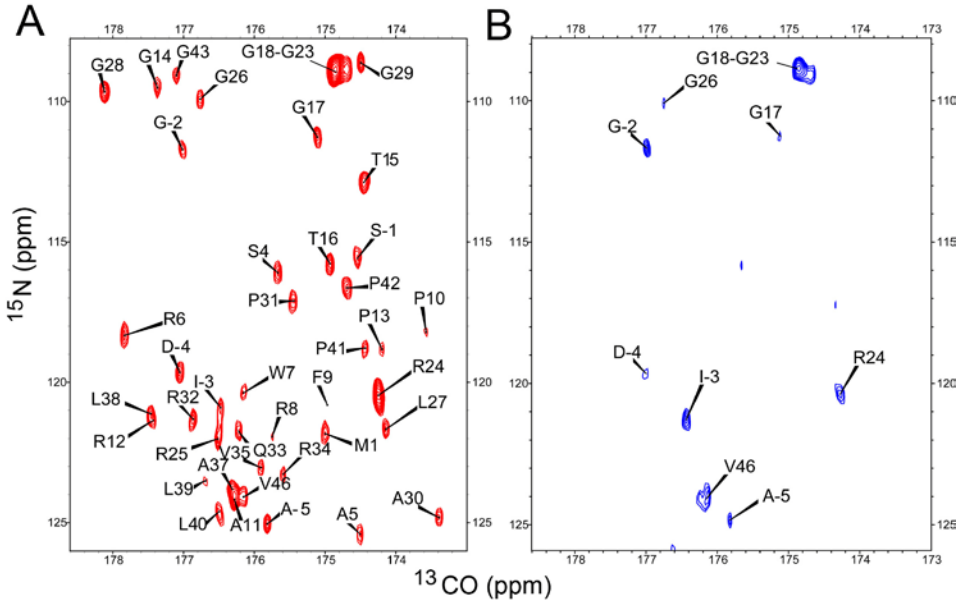
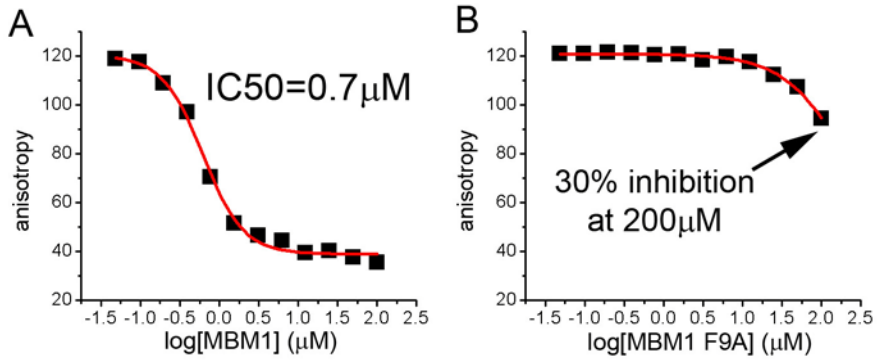


Figure S4. Competition experiment demonstrating the specificity of competition between MBM1 and MBM2 for binding to menin. The fluorescein labeled MBM2 (0.2μM) in complex with menin (2μM) was titrated with MBM1 and MBM1 F9A mutant. While strong competition is observed for MBM1 (**A**), the F9A mutant, which binds to menin very weakly is also a very weak competitor (**B**).



Supplementary Tables

Table S1. IC_{50} values for series of MLL^{4-15} peptides with alanine substitutions. The IC_{50} values were determined in FP competition experiments using fluorescein labeled MLL^{4-15} peptide.

MLL peptide	**IC_{50} μM**
Wt	0.23 ± 0.016
R6A	0.85 ± 0.13
W7A	1.0 ± 0.24
R8A	1.7 ± 0.35
F9A	~ 500
P10A	6.7 ± 1.6
R12A	1.1 ± 0.07
P13A	11.4 ± 1.2

Reference:

1. Berjanskii, M. V., and Wishart, D. S. (2005) *J Am Chem Soc* **127**, 14970-14971