Supplementary Information

Crystal Structure of the N-terminal region of Human Ash2L Reveals a Winged Helix Motif Involved in DNA Binding

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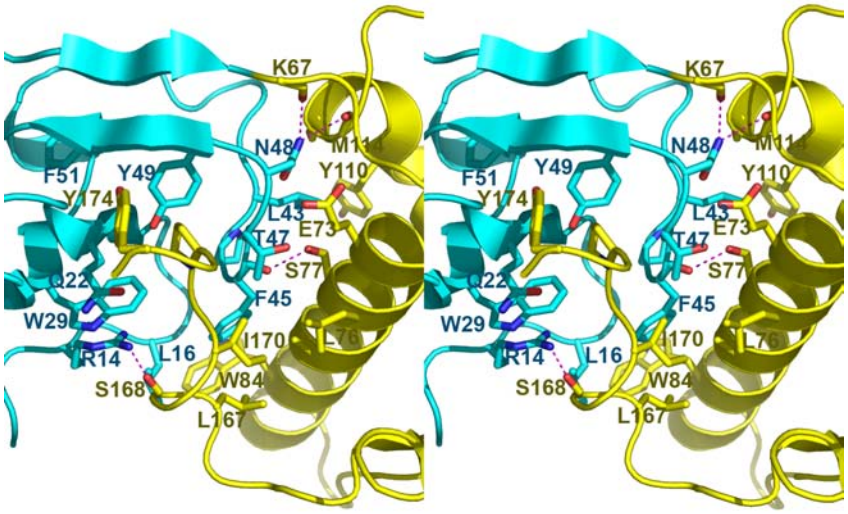
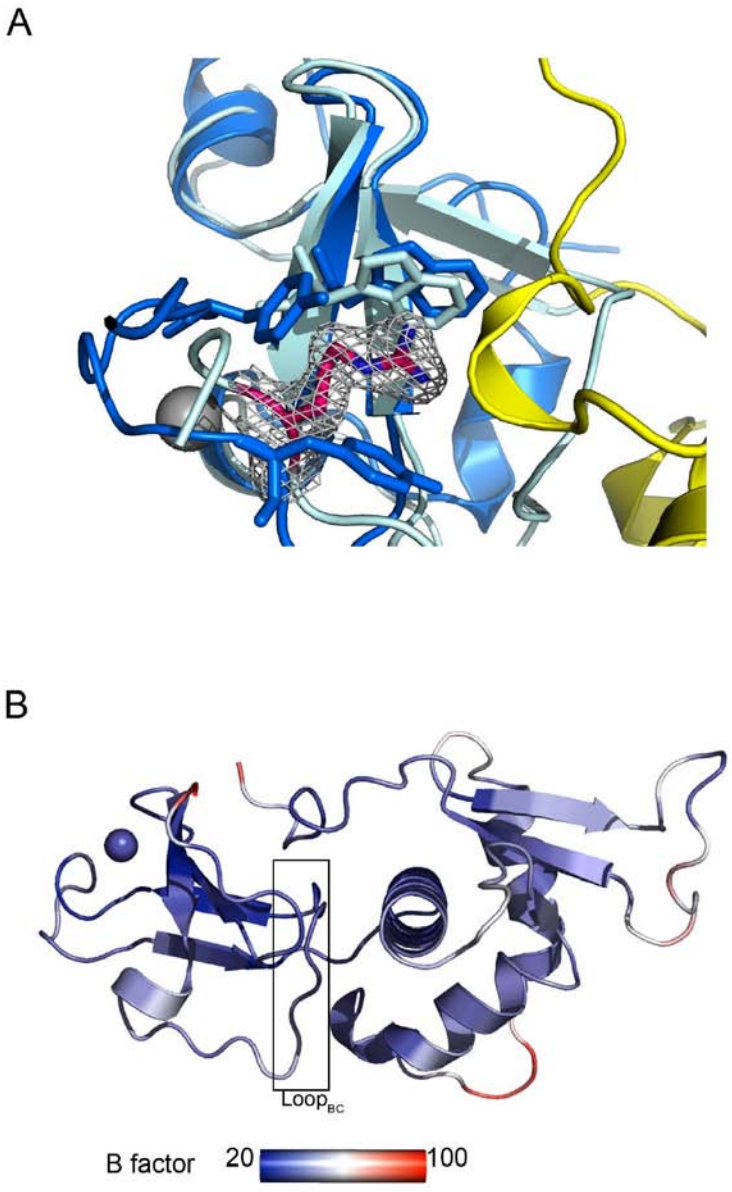
SUPPLEMENTARY FIGURES

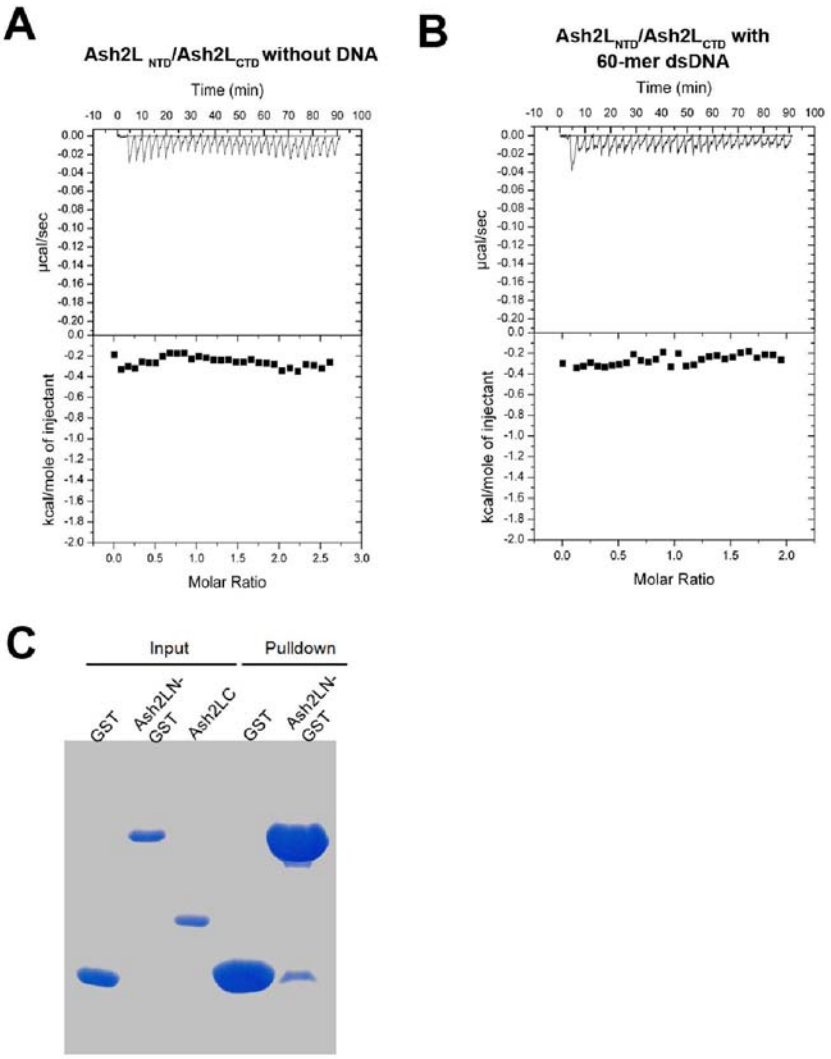
Supplementary Fig S1. Multiple equence alignment of Ash2L proteins from five species, *Homo sapiens, Mus musculus, Drosophila melanogaster, Anopheles gambiae,* and *Caenorhabditis elegans*. Domains of Ash2L are highlighted by colored bars above the sequences (PHD in cyan, WH in yellow, SPRY in green and SDI in red).



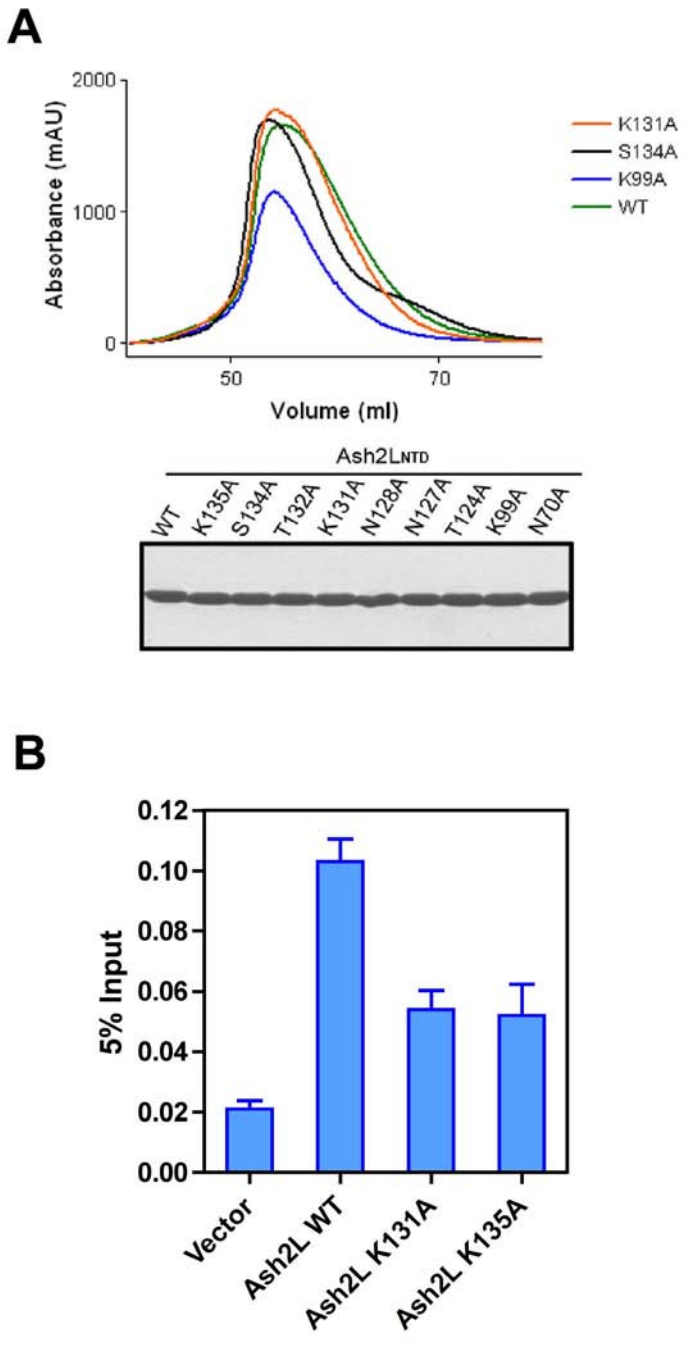
Supplementary Fig S2. Stereo view of the interface between the PHD finger (cyan) and the WH motif (yellow) of Ash2L. $Ash2L_{PHD}$ and $Ash2L_{WH}$ pack across an extensive interface, involving both hydrophobic and electrostatic interactions (shown as magenta dotted lines).



Supplementary Fig S3. Ash2L$_{NTD}$ is not a histone-binding module. (A) Structure superimposition of Ash2L$_{PHD}$ (pale cyan) and PHF8$_{PHD}$ (marine). The electron density (2Fo-Fc) map, contoured at 1.0 σ is shown for Arg14 of Ash2L$_{NTD}$. (B) The structure of Ash2L$_{NTD}$ is colored by B-factor. The lowest B factor is in blue and the highest in red. The loop between strands βB and βC has low B factor and its conformation is well defined.



Supplementary Fig S4. Ash2L$_{NTD}$ does not directly interact Ash2L$_{CTD}$. (A) *In vitro* ITC measurement of the interaction between Ash2L$_{NTD}$ and Ash2L$_{CTD}$ showed that there is no direct interaction between these two domains of Ash2L. (B) *In vitro* ITC measurement of the interaction between Ash2L$_{NTD}$ and Ash2L$_{CTD}$ in the presence of 60mer dsDNA. (C) In vitro pull-down assay showed no interaction between Ash2L$_{NTD}$ and Ash2L$_{CTD}$ in the presence of DNA. GST-Ash2L$_{NTD}$ was incubated with Ash2L$_{CTD}$ in the present of 60-mer dsDNA.



Supplementary Fig S5. Mutational studies of Ash2L. (A) Preparation of mutant $Ash2L_{NTD}$ proteins. Upper panel: gel filtration chromatography profiles (Hiload Superdex 75) of wild-type and three representative mutant $Ash2L_{NTD}$. Lower panel: SDS-PAGE of wild-type and nine mutant $Ash2L_{NTD}$ proteins corresponding to the peak fractions in the gel filtration profiles. (B) Mutants K131A and K135A decreased Ash2L localization to the *HOXC8* locus in cells, as shown by ChIP assay. Error bars in the graph represent standard deviation.

Supplementary Table S1. Data collection, phasing and refinement statistics for human Ash2L$_{NTD}$

	Se-Met Ash2L$_{NTD}$
Data collection	
Space group	$P3_121$
Cell dimensions	
a, b, c (Å)	49.984, 49.984, 165.519
a, b, g (°)	90, 90, 120
Wavelength (Å)	0.9785 (Se peak)
Resolution (Å)	100-2.1
R_{merge}	0.063(0.525)*
I / σI	73.7(3.8)
Completeness (%)	99.4(97.9)
Redundancy	19(10.2)
Refinement	
Resolution (Å)	35-2.1
No. reflections	26822
R_{work} / R_{free} (%)	21.3/25.4
No. atoms	
Protein	1336
Ligand (Zn)	1
Water	75
B-factors (Å^2)	
Protein	44.73
Ligand (Zn)	35.21
Water	44.57
R.m.s deviations	
Bond lengths (Å)	0.011
Bond angles (°)	1.117

*Values in parentheses are for highest-resolution shell.