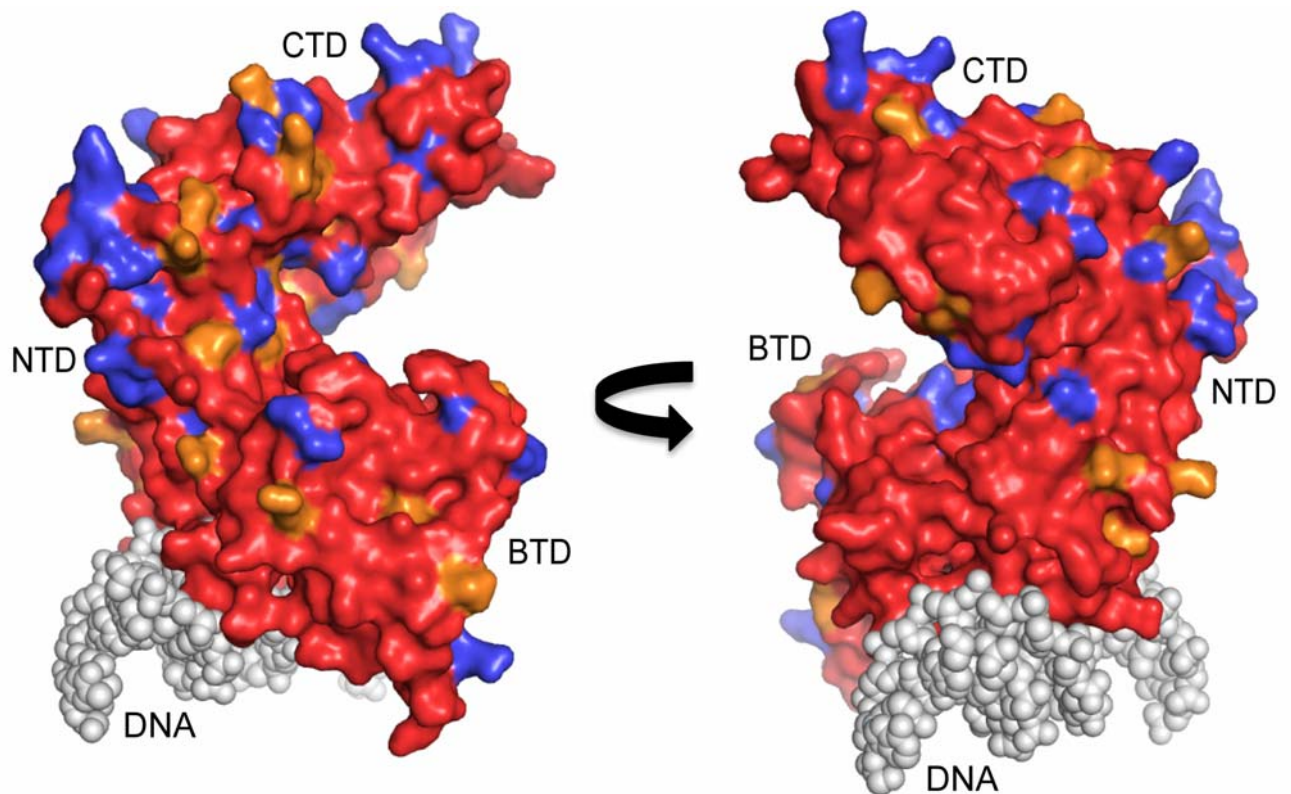


Supplemental Data to

Structural and functional analysis of the repressor complex in the Notch signaling pathway of *D. melanogaster*

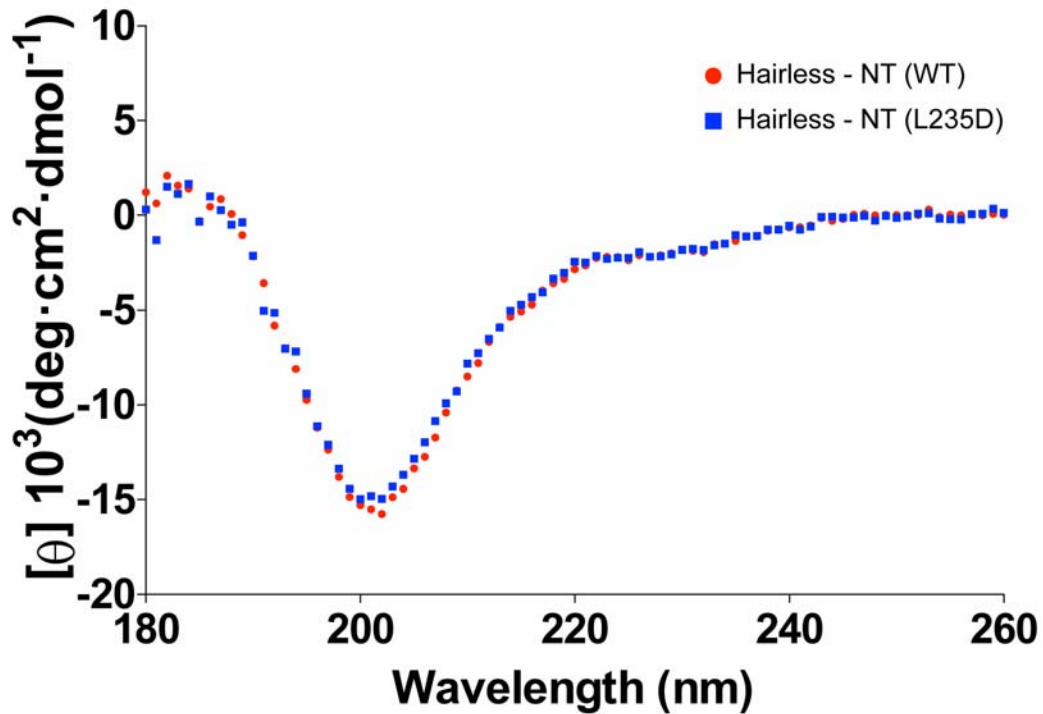
Dieter Maier, Patricia Kurth, Adriana Schulz, Andrew Russell, Zhenyu Yuan, Kim Gruber, Rhett A. Kovall, and Anette Preiss

Figure S1 Conservation of CSL protein structure.



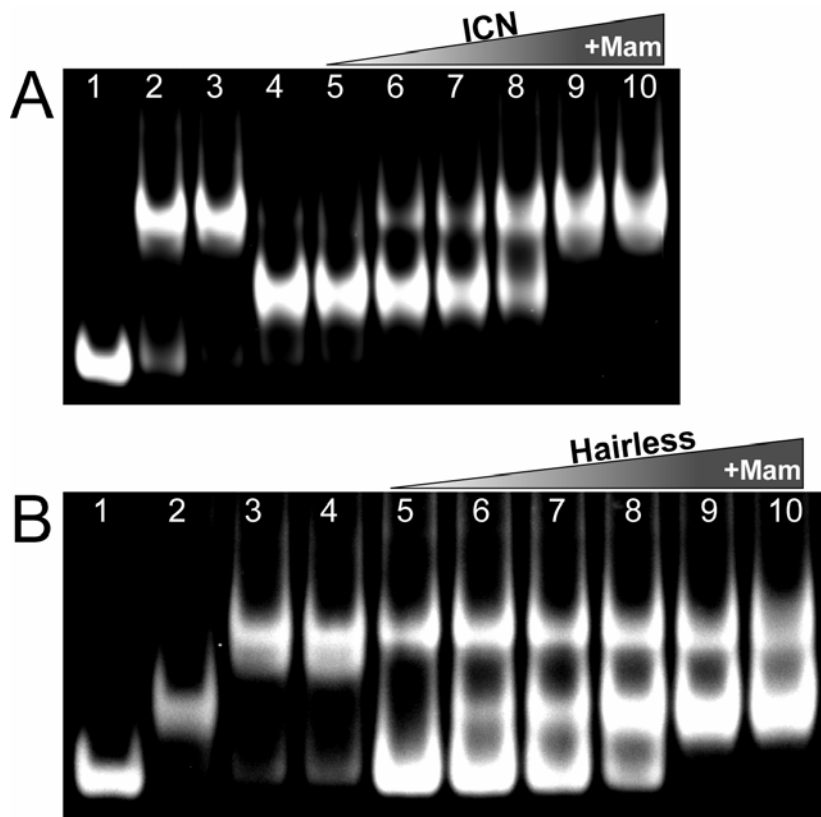
The sequence conservation between fly and mammalian proteins has been mapped onto the surface of the CSL structure. Approximately 180° views are shown. Red, orange, and blue coloring represent residues that are identical, similar, and unconserved, respectively.

Figure S2 The L235D mutation in Hairless has no apparent effect on secondary structure.



Far-UV circular dichroism spectroscopy for wild-type and Hairless mutant L235D (NT-LD). Experiments were performed at 25°C in a buffer containing 50mM sodium phosphate pH 6.5 and 150mM NaF with protein concentrations of 190 μM and 210μM for wild-type and L235D proteins, respectively. The NMRSD parameter values for wild-type and L235D were 0.05 and 0.07, respectively. Similar to wild-type Hairless NT, the CD spectrum of Hairless NT-LD has a minimum at ~200 nm, which is characteristic of random coil.

Figure S3 Mam has no influence on the competition between ICN and Hairless for binding Su(H).



A) Mam has no observable effect on the displacement of Hairless from Su(H) by ICN; control lanes 1-5: Su(H), Su(H)-ICN, Su(H)-ICN-MAM, Su(H)-Hairless, Su(H)-Hairless-Mam complexes, respectively; lanes 6-10: preformed Su(H)-Hairless complexes titrated with increasing concentrations of ICN (0.5 μM, 1 μM, 2 μM, 4 μM, 6 μM) with Mam.

B) The inclusion of Mam has only a modest effect on the displacement of ICN from Su(H) by Hairless; control lanes 1-5: Su(H), Su(H)-Hairless, Su(H)-ICN-MAM, Su(H)-ICN, Su(H)-ICN-Mam (1 μM ICN) complexes, respectively; lanes 6-10: preformed Su(H)-ICN-Mam (1 μM ICN) complexes titrated with increasing concentrations of Hairless (0.5 μM, 1 μM, 2 μM, 5 μM, 20 μM).

For all EMSAs, unless otherwise noted, the concentrations of DNA, Su(H), Hairless (NT), ICN (RAMANK), and Mam are 1 μM, 0.1 μM, 4 μM, 4 μM, and 5 μM, respectively.