

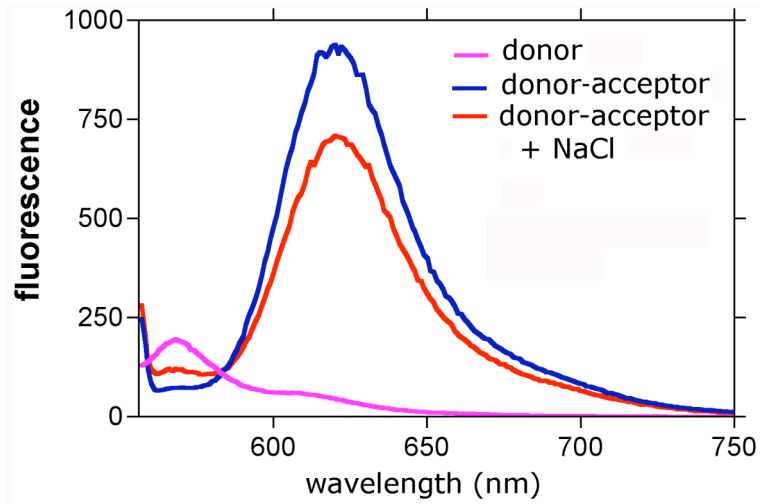
Supplementary Information for:

Mutational and energetic studies of Notch1 transcription complexes

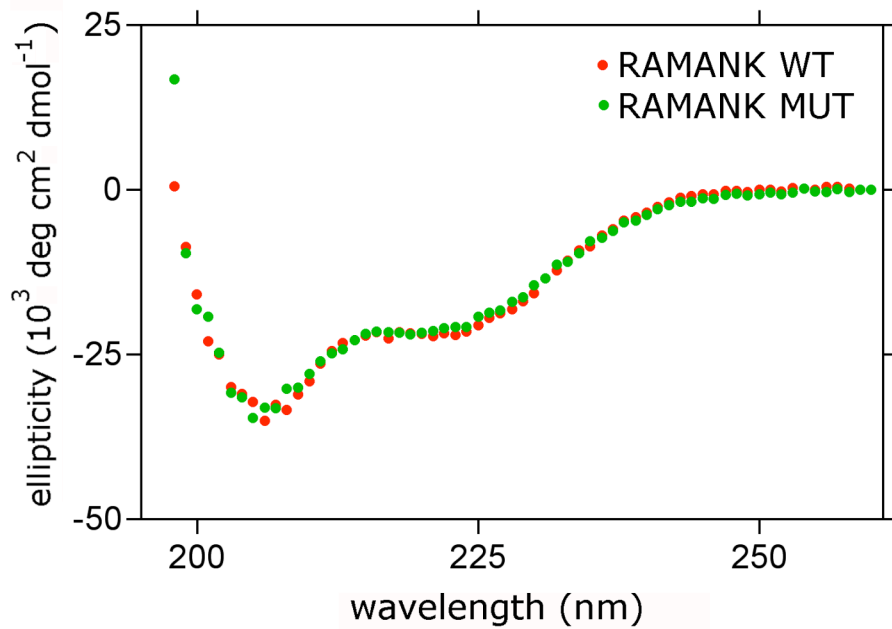
Cristina Del Bianco¹, Jon C. Aster¹ and Stephen C. Blacklow¹

¹Department of Pathology, Brigham and Women's Hospital and Harvard Medical School,

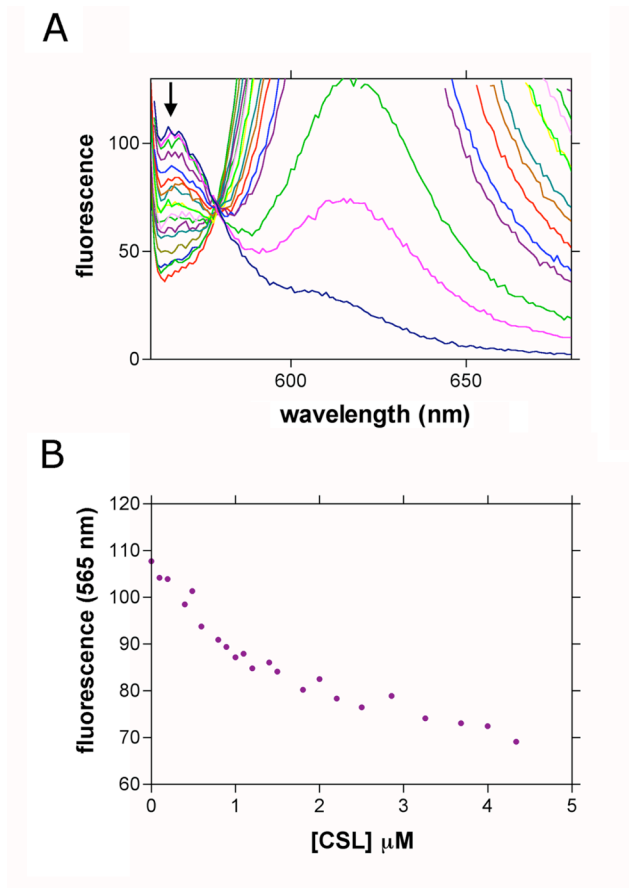
Boston, MA 02115



Supplementary Figure S1. Effect of ionic strength on FRET. Emission spectra of the donor RAMANK F2085C-Alexa Fluor 555 alone (pink) and of complexes exhibiting energy transfer to the acceptor CSL-DNA-Alexa Fluor 594 (blue). Addition of NaCl (0.8 M) causes loss of energy transfer, as judged by increased donor and decreased acceptor emission, respectively (red).



Supplementary Figure S2. Circular dichroism spectroscopy. The spectra were acquired on an Aviv 62DS spectropolarimeter equipped with a thermoelectric temperature controller. Samples of normal RAMANK and RAMANK-mut (10 μ M) were dialyzed against 20 mM sodium phosphate buffer (pH 8.0), containing 150 mM NaCl and 1 mM DTT before measurement. RAMANK-mut contains the mutations C1872A, C1891A, R1963A, R2005E, R2071E, E2072R, and F2085C. All spectra were recorded at 25 °C in a 1 mm path length cuvette as the average of five scans using a 3 sec acquisition time.



Supplementary Figure S3. **A.** Fluorescence emission spectra showing energy transfer from donor (RAMANK F2085C~Alexa Fluor 555) to acceptor upon increasing addition of CSL-DNA~Alexa Fluor 594. **B.** Decrease of fluorescence at 565 nm plotted against increasing concentration of CSL.