**Supplemental figures:**

**Figure S1: Proteoglycan expression of mouse and human cell lines and CNFY activity.**

**(A)** Normalized RNA expression (NX) of different proteoglycans in HeLa, THP-1 and Molt-4 cells. Data were obtained from https://www.proteinatlas.org.

**(B)** Cells were incubated with 4 nM GST-CNFY and GST-CNFYC866S for 4 h. RhoA was detected by Western blotting using an anti-human RhoA antibody. GAPDH was used as a loading control. The experiments were repeated three times independently with similar results.

**Figure S2: Regression analysis of FACS derived CNFY binding data:** Modelling of a two binding sites Hill kinetic to FACS derived CNFY binding data using the regression function f(x)=Bmax1\*xh1/(KD1h1+xh1)+Bmax2\*xh2/(KD2h2+xh2) | h1=1 for **(A)** HeLa cells (Bmax1=0.4, KD1=11.5, h1=1, Bmax2=0.7, KD2=57.1, h2=2.6) and **(B)** THP-1 cells (Bmax1=0.3, KD1=19.4, h1=1, Bmax2=0.7, KD2=78.6, h2=3.0). The additive regression function describes binding of the ligand to two receptors and consists of f(x)1=Bmax1\*xh1/(KD1h1+xh1) | h1=1 depicted in blue and f(x)2= Bmax2\*xh2/(KD2h2+xh2) depicted in green.

**Figure S3: Detection of RhoA deamidation by the shift assay:** HeLa cells were incubated with 4 nM GST-CNFY in the presence of several GAG (50 µg/ml), respectively as indicated: (dextran sulfate (DxS), heparin (H), fondaparinux (F) and chondroitin sulfate A (CS). RhoA was detected by Western-blotting using an anti-human RhoA antibody. Equal loading was verified by detection of GAPDH. The experiment was repeated three times independently.

**Figure S4: Characterization of the C-terminal heparin-binding motive.**

**(A)** Structure of the CNFY C-terminal domain (709-1014) modeled to the known crystal structure of CNF1 C-terminal domain (709-1014) (35). Amino acids of the heparin-binding motive K774, K775, K777 as well as K786 are highlighted as balls.

**(B)** Flow cytometry analyses of the wildtype and mutant DL488-GST-CNF709-1014 (35 nM) binding to HeLa cells in the presence or absence of 50 µg/ml heparin. Values are average of bound fluorescence ± SD normalized to control cells only incubated with DL488-GST-CNFY (n *=* 3). Statistical analysis was performed using student’s t-test. \*\*\*\* p < 0.0001.