Substrate specificity and biochemical characteristics of an engineered mammalian chondroitinase ABC

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SUPPORTING INFORMATION:

Supplementary Figures: 2

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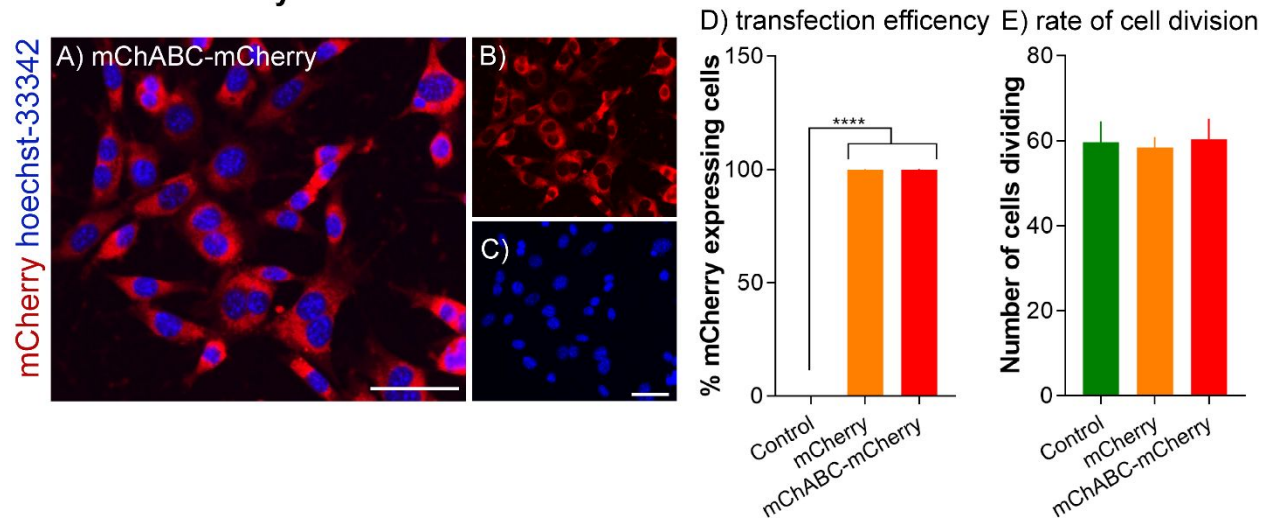**mChABC-mCherry transfection of HEK293 cells**

Figure S1: Expression and secretion of active mChABC-mCherry from HEK293 cells. A-

C) mChABC-mCherry is readily transfected into HEK293 cells. A) HEK293 cells were

either naïve (control), or transfected with mCherry or mChABC-mCherry. Image shows

B) mCherry fluorescence (red) and C) Hoechst-33342 (blue) staining. Images show

individual channels and a merge. Scale bar = 40 µm. D) Transfection efficiency for

mCherry and mChABC-mCherry are ~100% (N=6 independent cell batches assessed per

group) and not divergent from each other (P=0.825) although both are divergent from

control ($F_{(2,15)}$=0.618, P<0.0001; ANOVA with post-hoc Turkey). E) Using anti-Ki67

staining, percentages of HEK cell division was not altered by transfection (N=8 independent cell batches assessed per group; $F_{(2,21)}=0.434$, $P=0.654$; ANOVA with post hoc Turkey). For panels D and E, data shows means ± SD.
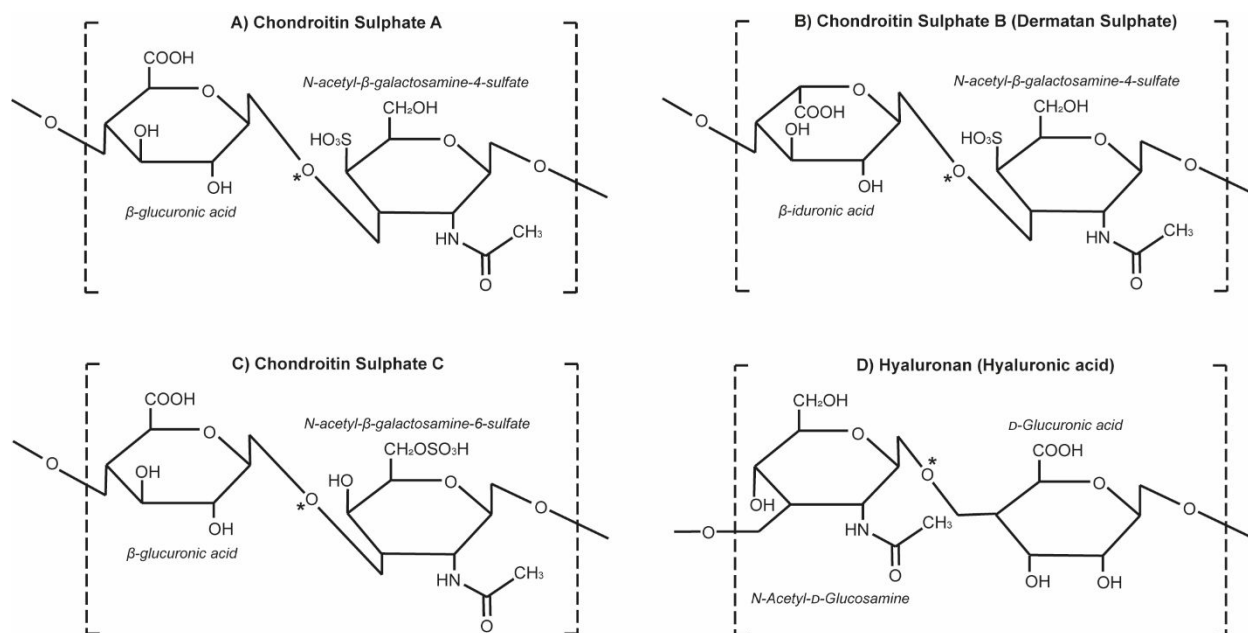

Figure S2: Chemical structure of chondroitin sulphate A (CS-A), chondroitin sulphate B (dermantan sulphate, DS), chondroitin sulphate C (CS-C), and hyaluronan (hyaluronic acid, HA). A-C) Chondroitin sulphate and dermatan sulfate are composed of disaccharides of N-acetylgalactosamine and an uronic acid joined by β(1→4) or β(1→3) linkages, respectively. A) CS-A contains glucuronic acid and is 4 O-sulfated (the

alternating co-polymer β-glucuronic acid-(1-3)-N-acetyl-β-galactosamine-4-sulfate). B)

DS contains N-acetylgalactosamine, but the uronic acid present is L-iduronic acid (the

alternating co-polymer β-iduronic acid-(1-3)-N-acetyl-β-galactosamine-4-sulfate). C) CS-

A contains glucuronic acid and is 6 O-sulfated (the alternating co-polymer β-glucuronic

acid-(1-3)-N-acetyl-β-galactosamine-6-sulfate). D) HA is comprised of alternating β-D-

(1→3) glucuronic acid and β-D-(1→4)-N-acetylglucosamine residues. For all panels: *

represents the point at which chondroitinase ABC would cut break the

disaccharides/residuals apart.