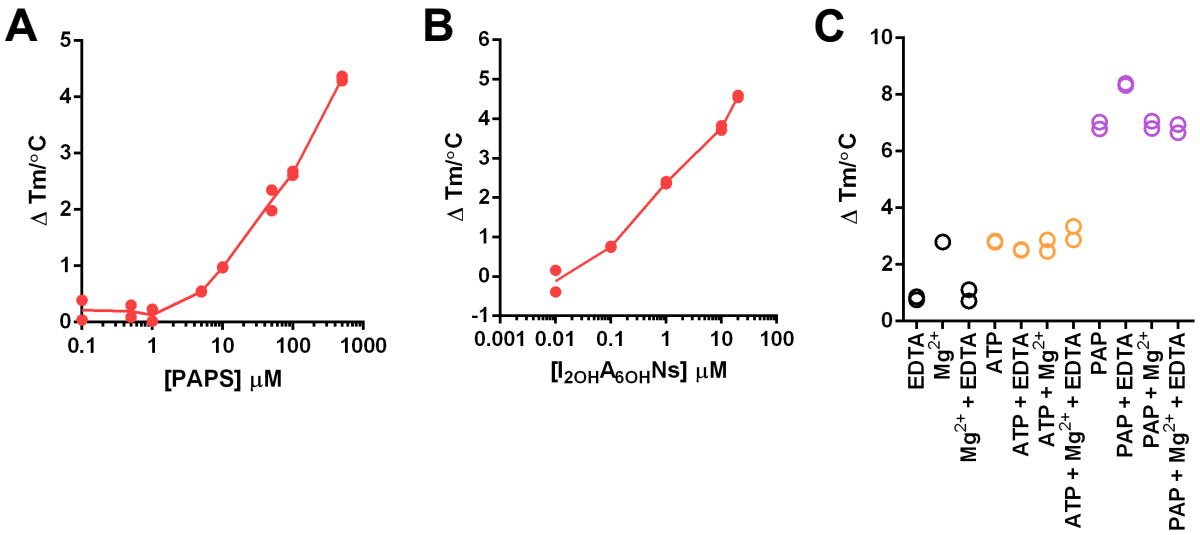
**New tools for carbohydrate sulphation analysis: Heparan Sulphate 2-*O*-sulphotransferase (HS2ST) is a target for small molecule protein kinase inhibitors**

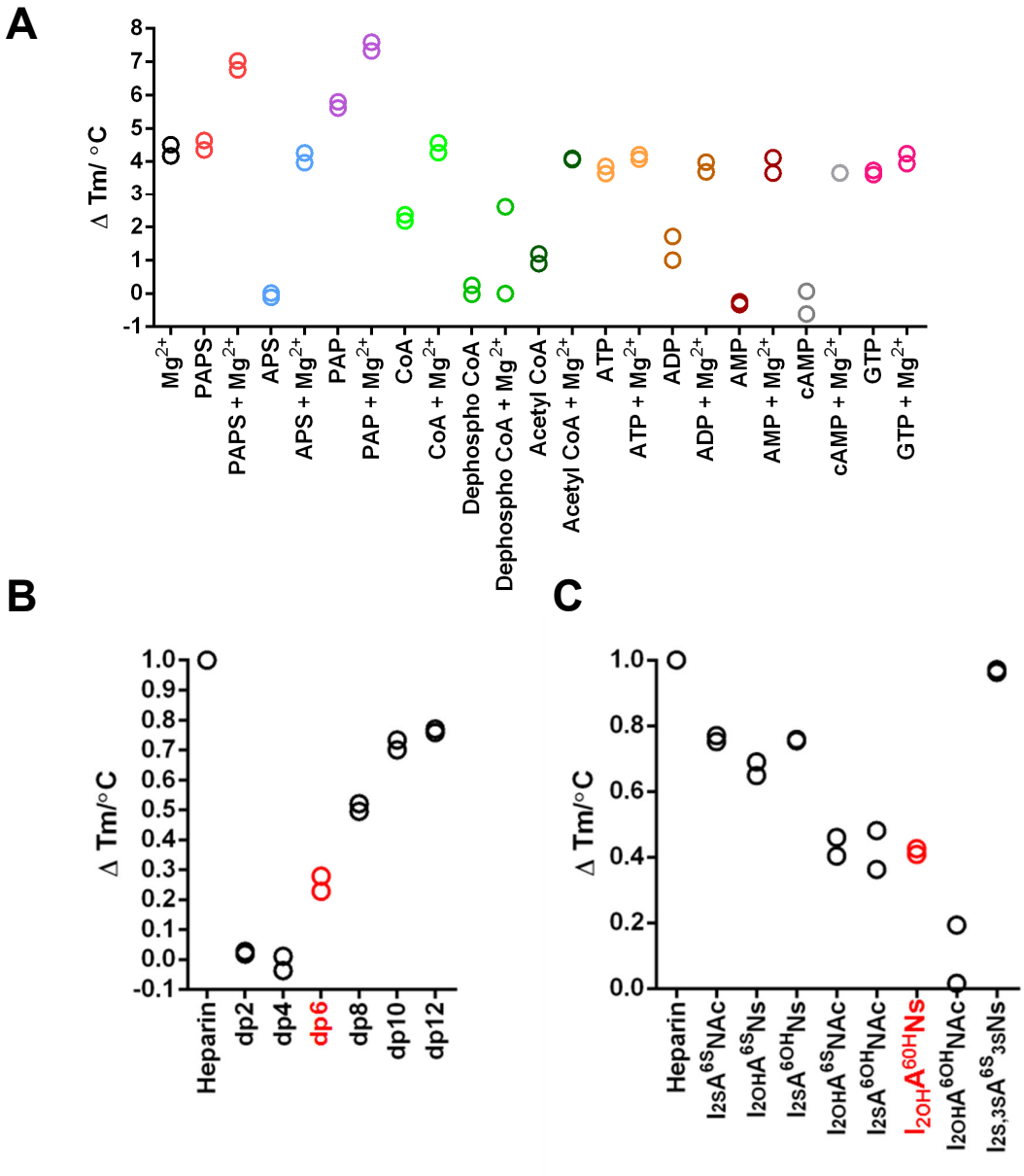
Dominic P. Byrne, Yong Li, Krithika Ramakrishnan, Igor L. Barsukov, Edwin A. Yates, Claire E. Eyers, Dulcé Papy-Garcia, Sandrine Chantepie, Vijayakanth Pagadala, Jian Liu, Carrow Wells, David H. Drewry, William J. Zuercher, Neil G. Berry, David G. Fernig and Patrick A. Eyers.

**Supplementary Material**



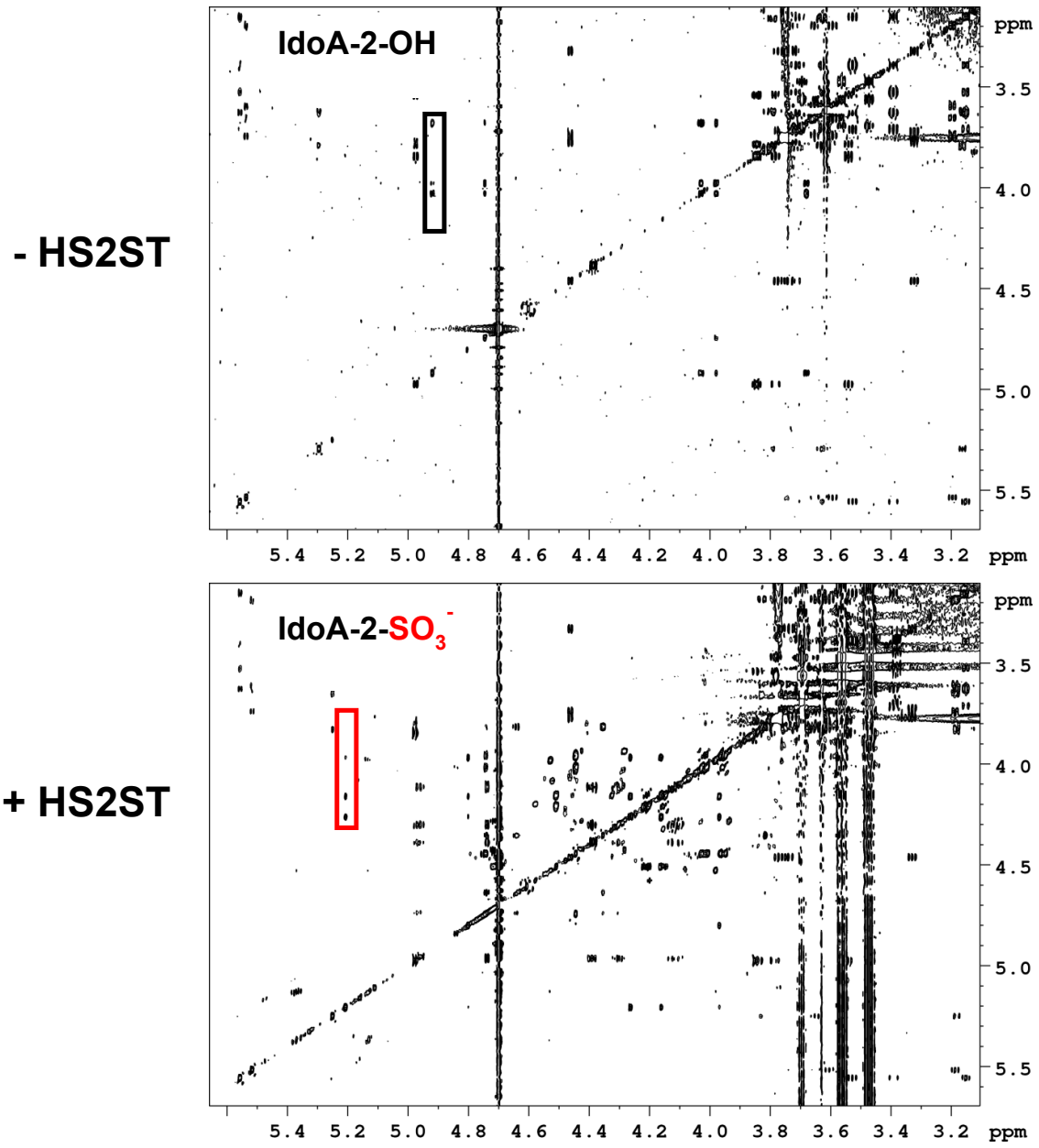

**Supplementary Figure 1. Thermal stability analysis of MBP-HS2ST.**

Concentration-dependent thermal profiling of MBP-HS2ST in the presence of **(A)** PAPS or **(B)** the chemically modified heparin-derivative $I_{2OH}A^{60H}N_S$ (compound 7, see Table 1). $\Delta T_m$ values were calculated by DSF as previously described. **(C)** TSA assay showing changes in MBP-HS2ST thermostability induced by PAP and ATP, and the effects of EDTA and $Mg^{2+}$. Thermal stability of HS2ST was measured as a function of compound binding by DSF. $\Delta T_m$ values of HS2ST protein (5 μM) incubated with 0.5 mM of the indicated nucleotide ± 10 mM $MgCl_2$ ± 10 mM EDTA are shown.



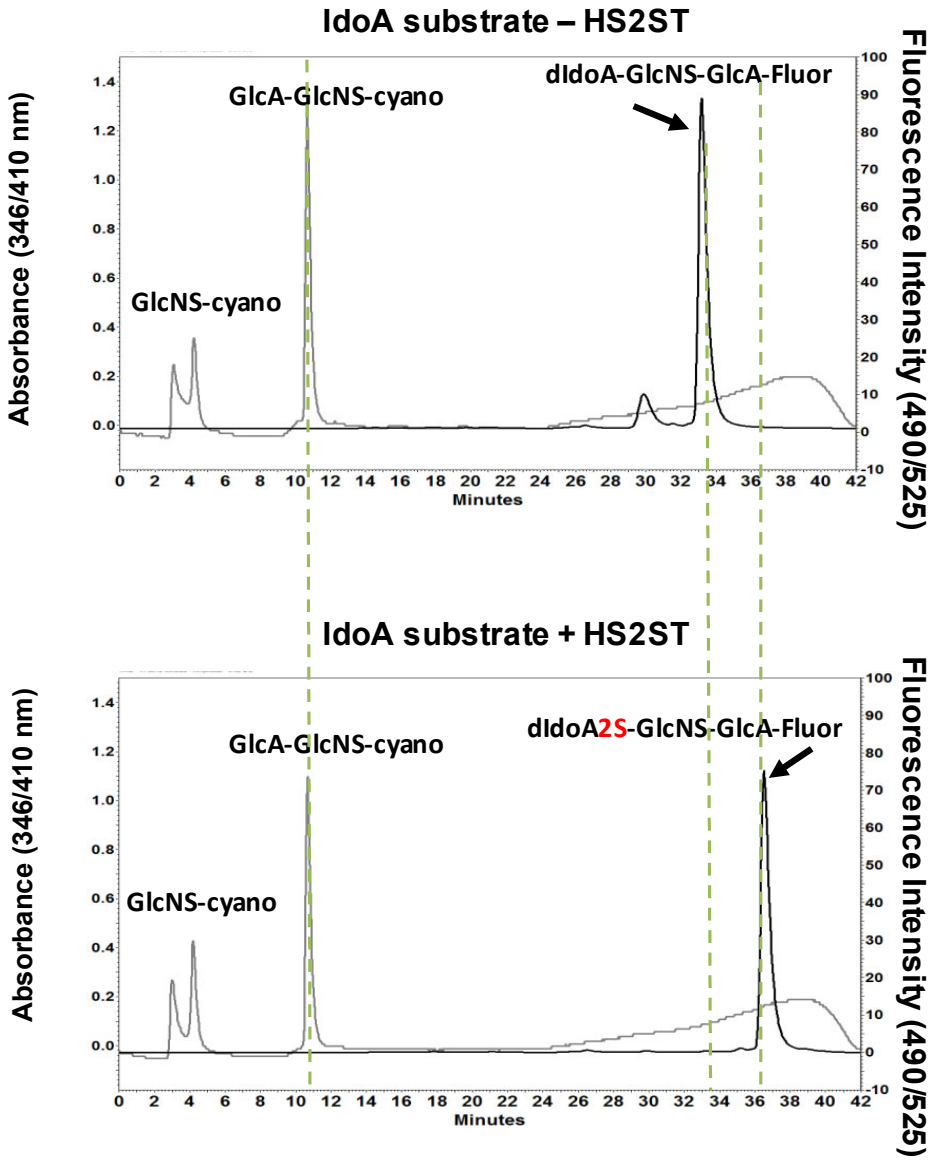

**Supplementary Figure 2. MBP-HS2ST Nucleotide and polysaccharide analysis.**

**(A)** TSA showing MBP-HS2ST binding of nucleotides by DSF. Thermal stability was measured as a function of nucleotide binding by DSF. $\Delta T_m$ values of HS2ST protein (5 µM) incubated with 0.5 mM of the indicated nucleotide ± 10 mM $MgCl_2$ are shown. DSF analysis showing thermal shift (stabilization) of 5 µM HS2ST in the presence of 10 µM size separated oligosaccharide fragments, dp (degree of polymerisation) equivalent to disaccharide (dp2), tetrasaccharide (dp4), hexasaccharide (dp6), octasaccharide (dp8), decasaccharide (dp10) or dodecasaccharide (dp12) **(B)** or chemically modified heparin derivatives **(C)**. The minimal hexasaccharide binding substrate in (B) and the putative HS2ST substrate $I_{2OH}A^{6OH}Ns$ in (C) are both shown in red. $\Delta T_m$ values (calculated as previously described) are normalized relative to heparin. dp=degree of polymerisation.



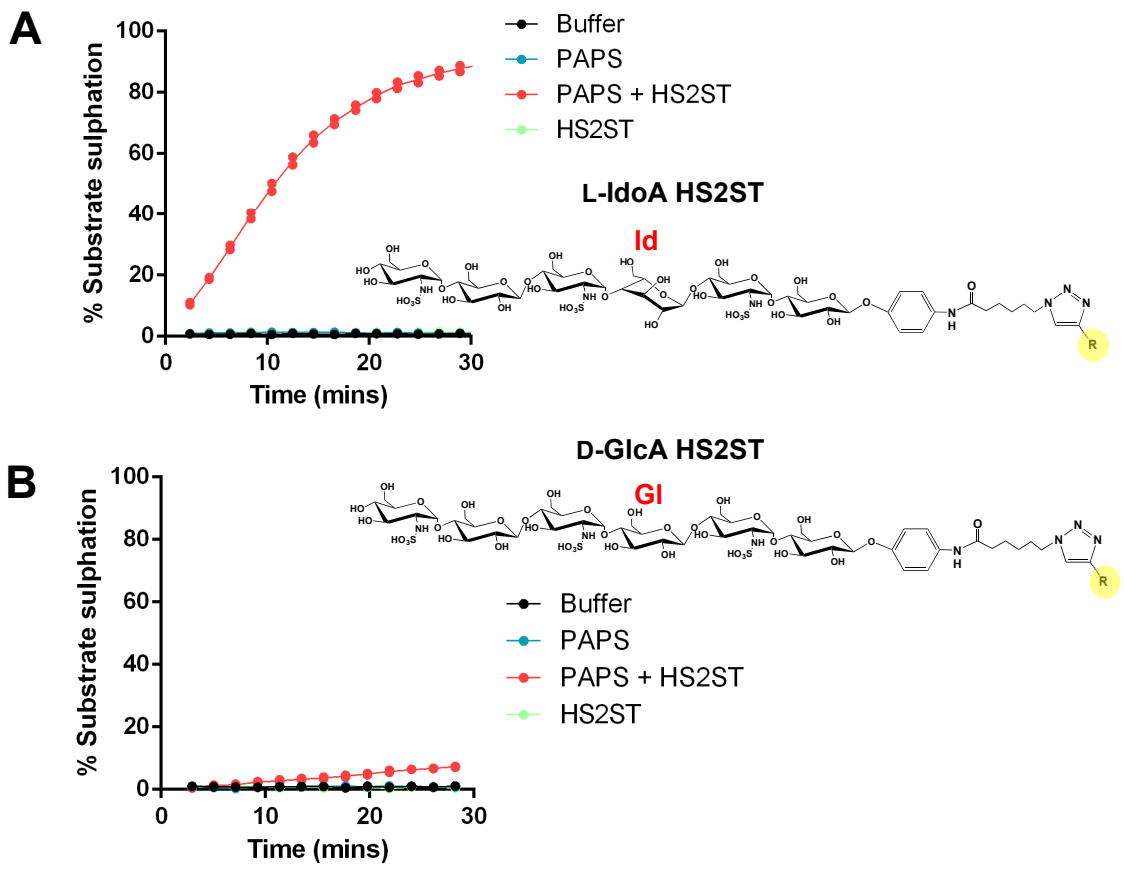

**Supplementary Figure 3. NMR spectra of sulphated and non-sulphated fluorescent polysaccharide substrate.**

TOCSY spectra of the L-IdoA-containing hexameric fluorescein-labelled HS2ST substrate (top) and the 2-O-sulphated product (bottom) generated by incubation with HS2ST, including the full spectrum of all carbohydrate hydrogens detected. Selected spectral regions, including the diagnostic shift caused by 2-O-sulphation, are expanded in Figure 2B in the main text, and are highlighted here by black and red boxes respectively.



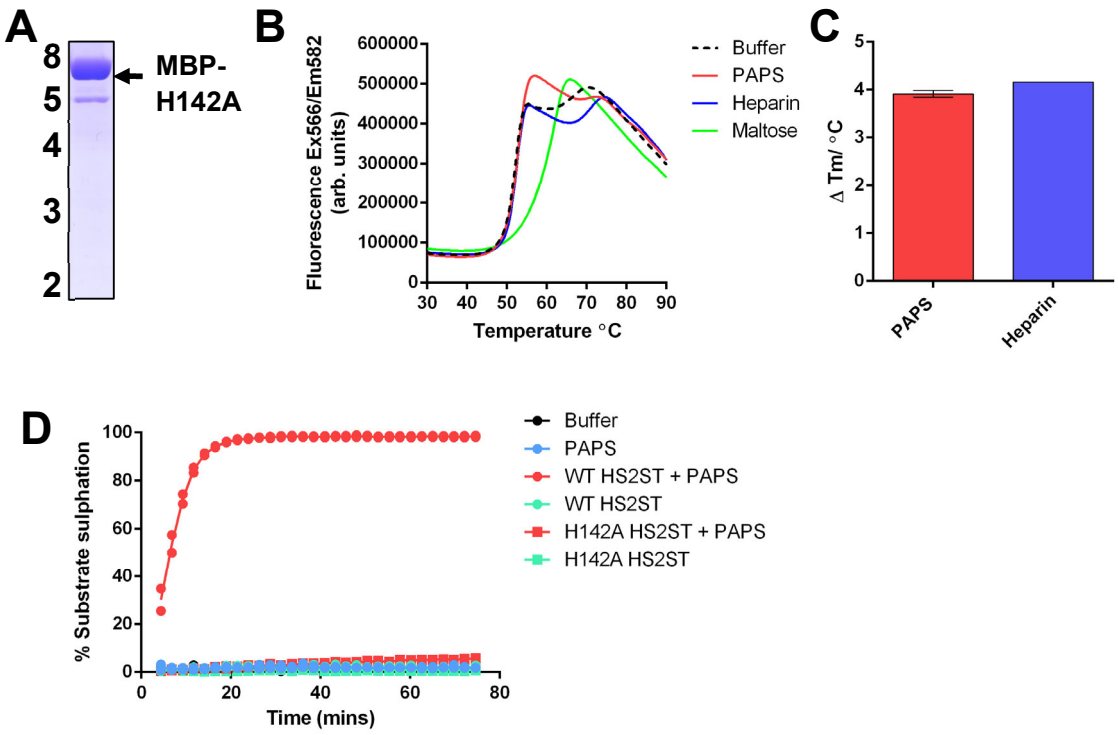

**Supplementary Figure 4. HPLC analysis of sulphated and non-sulphated fluorescent polysaccharide substrate.**

HPLC separation of cyanoacetamide or fluorescein-labelled saccharides obtained from heparitinase digestion of GlcNS-GlcA-GlcNS-IdoA-GlcNS-GlcA-Fluorescein HS2ST substrate. Elution profiles of digested polysaccharide after anion exchange chromatography are shown. The non-sulphated IdoA-containing hexameric substrate (eluting at ~34 min, top) and the 2-*O*-sulphated product (eluting at ~37 min, bottom) were confirmed by comparison of the different peaks in the fluorescence spectra (dashed lines), with the later eluting sulphated product highlighted in red. dIdoA refers to the double bond formed by β-elimination between C4 and C5 in the IdoA and 2-*O*-IdoA oligosaccharides.



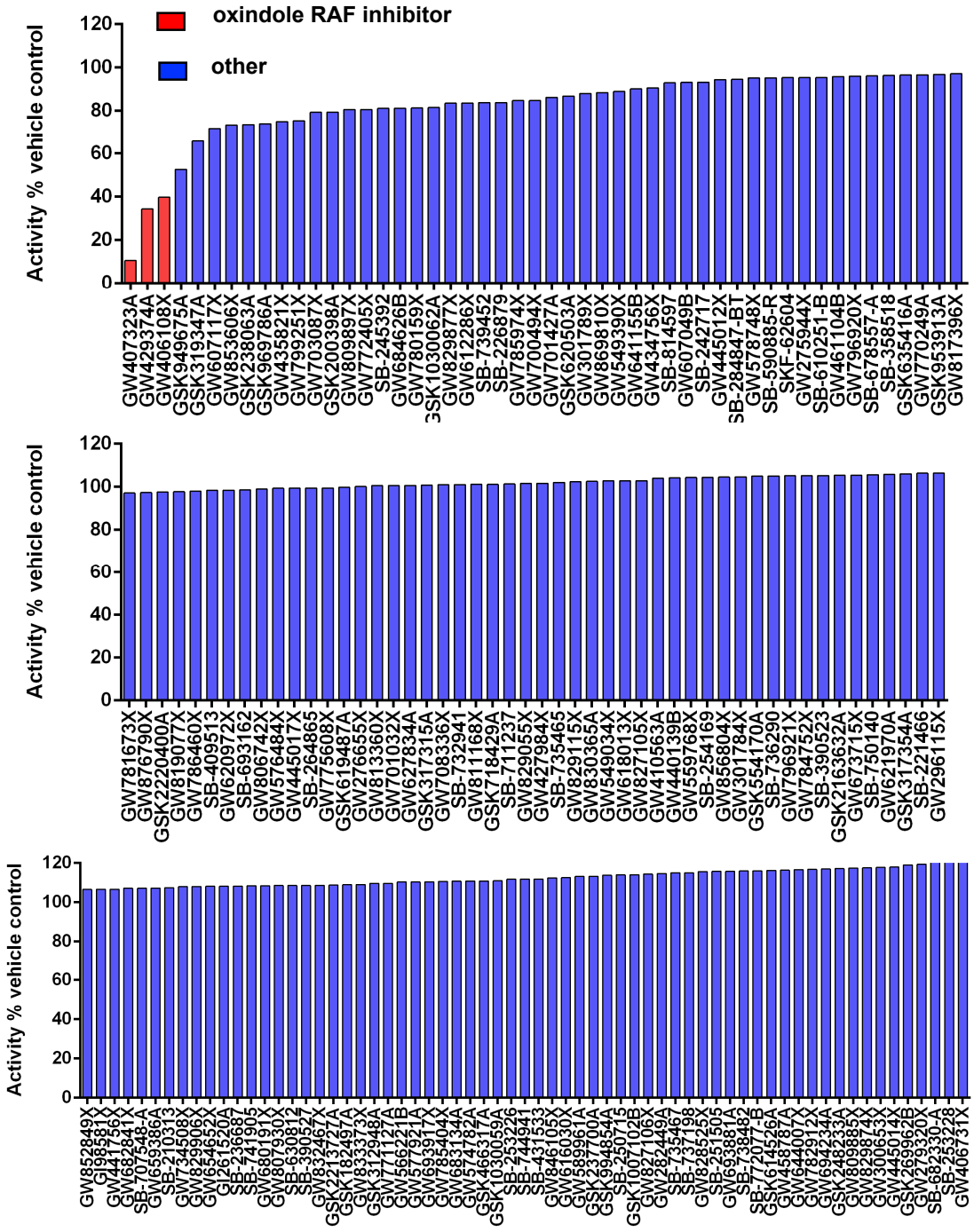

**Supplementary Figure 5. HS2ST glycan residue substrate-specificity analysis.**

Efficient sulphation of a hexasaccharide substrate by HS2ST requires an L-IdoA residue at the appropriate position in the oligosaccharide. Direct microfluidic sulphotransferase assays demonstrating time-dependent sulphation of the fluorescein-tagged hexasaccharide substrate containing either **(A)** L-IdoA or **(B)** D-GlcA residue at the third residue from the fluorescein-conjugated (reducing) end. R=fluorescein. The IdoA or GlcA residues are indicated in red.



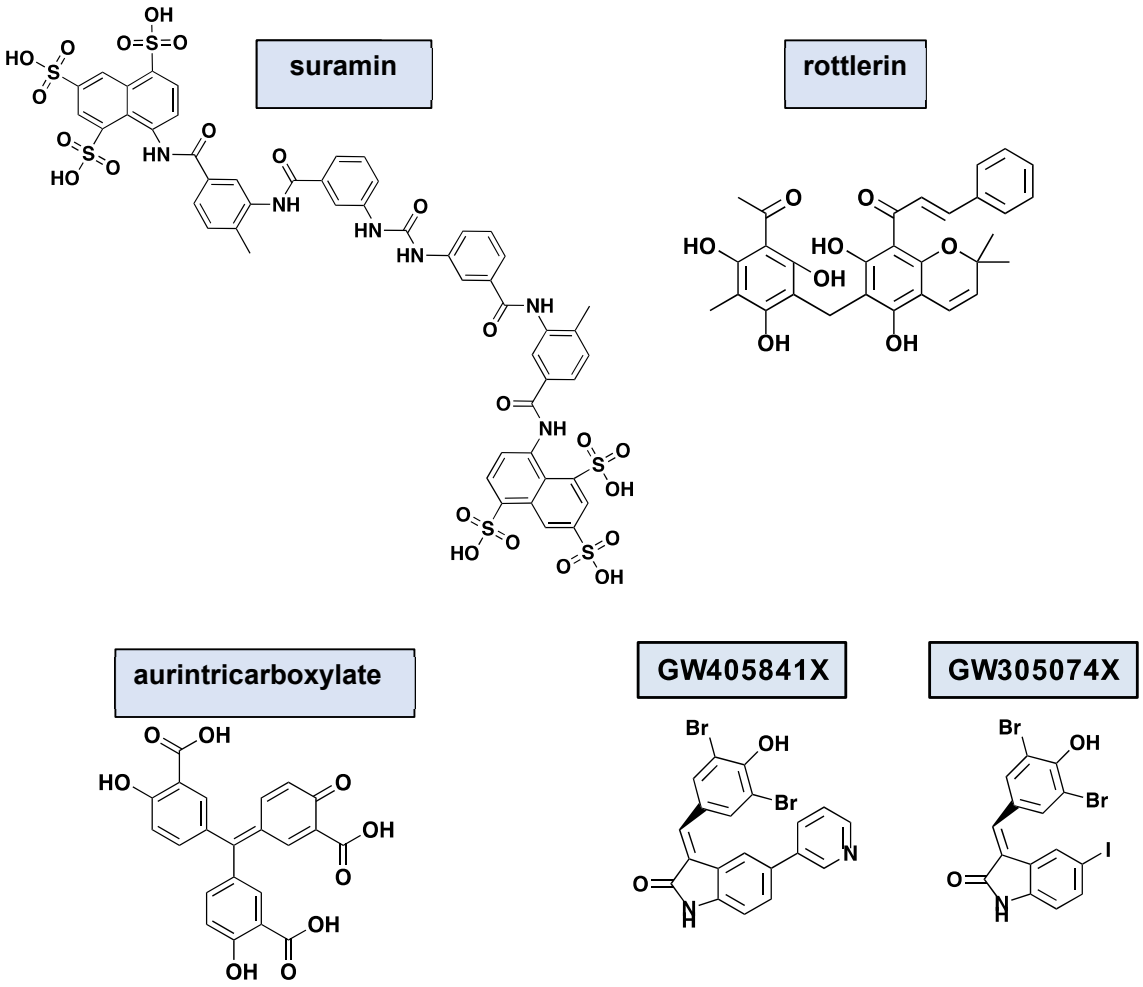

**Supplementary Figure 6. Analysis of purified recombinant H142A MBP-HS2ST1.**

**(A)** Coomassie blue staining of H142A MBP-HS2ST protein. 2 µg of the mutant enzyme was analysed after SDS-PAGE. **(B)** Thermal denaturation profiles for H142A MBP-HS2ST (5 µM) in the presence of 0.5 mM PAPS (red), 10 µM heparin (blue) or 5 mM maltose (green). Buffer control is shown by the black dashed line. **(C)** $\Delta T_m$ values for H142A MBP-HS2ST measured in the presence of 0.5 mM PAPS (red) or 10 µM heparin (blue). Values were obtained by DSF and calculated by subtracting control $T_m$ values (buffer, no ligands) from the measured $T_m$ values. N=2, assayed in triplicate. **(D)** Direct microfluidic kinetic sulphotransferase assay comparing PAPS-dependent sulphation of fluorescein-tagged L-IdoA hexasaccharide by either WT MBP-HS2ST (red circles) or H142A MBP-HS2ST (red squares).



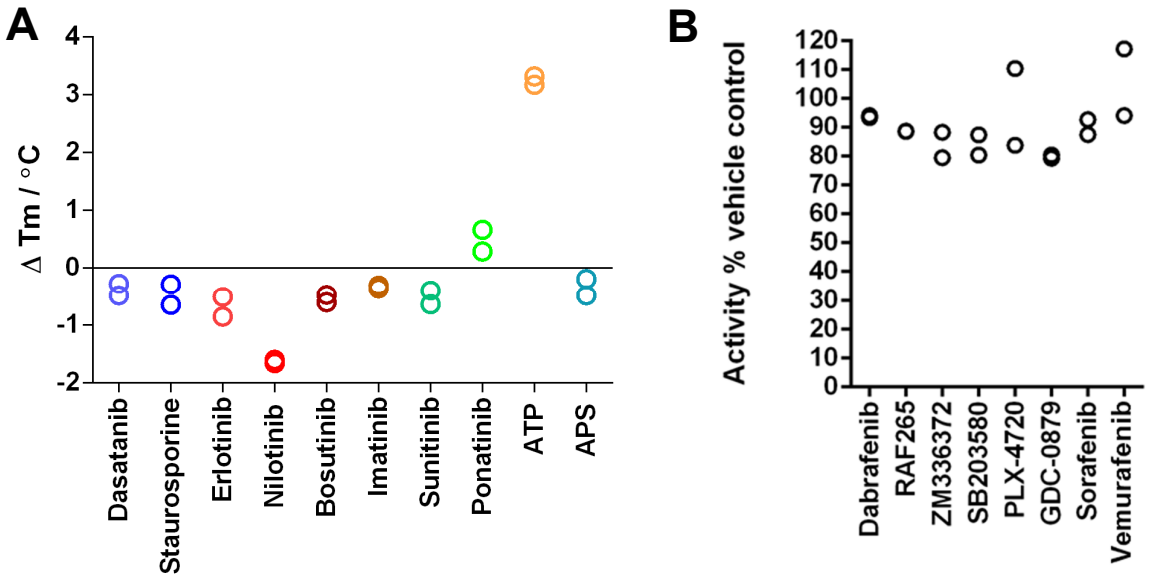

**Supplementary Figure 7. HS2ST enzymatic PKIS compound screen.**

Inhibition of HS2ST catalytic activity by selected PKIS members. Data are presented as HS2ST activity relative to DMSO control, assayed in duplicate. The most notable 'hit' inhibitors from the oxindole chemical class are shaded in red.




**Supplementary Figure 8. Chemical structures of HS2ST inhibitory ligands.**

Chemical structures of suramin, rottlerin, aurintricarboxylic acid and selected PKIS compounds.




**Supplementary Figure 9. Lack of HS2ST inhibition by various kinase inhibitors.**

DSF screening (left panel) or enzyme-based inhibitor assay (right panel) evaluating staurosporine, FDA-approved kinase inhibitors and several chemically distinct RAF kinase inhibitors.