

**Supplemental 1. *m/z* values of nitrous acid generated HS oligosaccharides**

The calculations are based on the major stable isotopes of C-12, H-1, O-16, N-14 and S-32 with the equations:

$$m/z = (340.09 + 379.11(p-n) + 337.10n + 79.96q - z)/z$$

*p*, number of total internal disaccharides; *n*, number of free amine containing disaccharides;

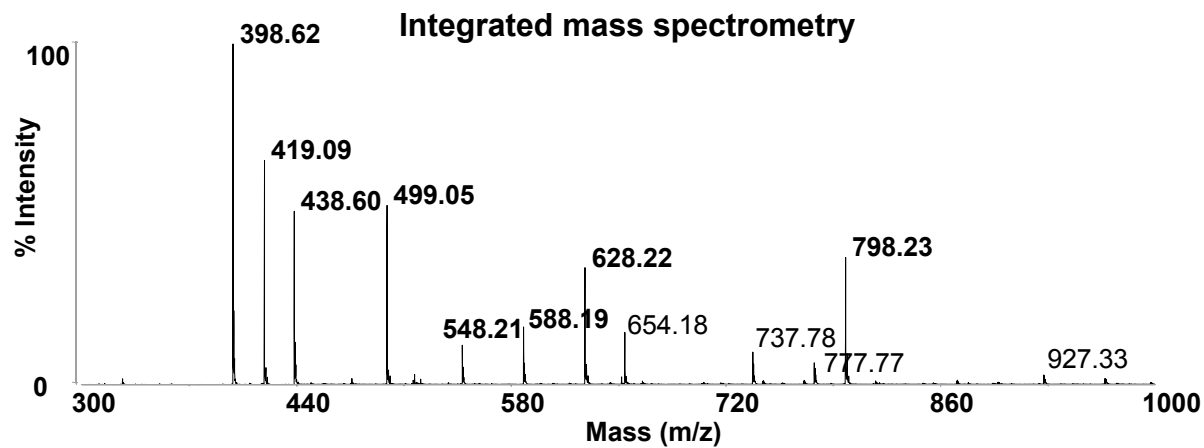
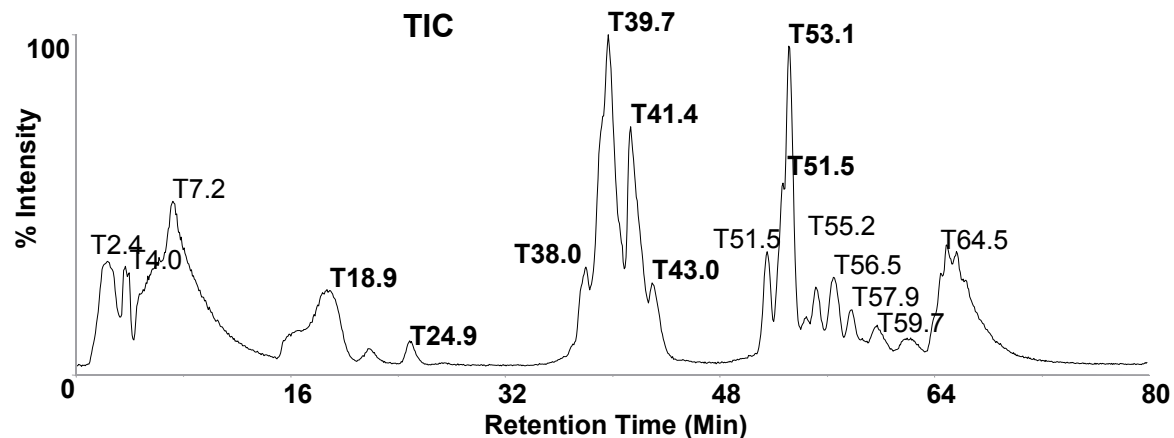
*q*, number of sulfates; *z*, number of negative charges.

Binding of dibutylamine molecule (NH(C<sub>4</sub>H<sub>9</sub>)<sub>2</sub>) to an oligosaccharide will increase *m/z* by 129.15/*z*.

dpX	dp2	dp4			dp6			
<i>p-n</i>	0	1	0	2	1			
<i>n</i>	0	0	1	0	1			
<i>z</i>	1	1	2	2	2	3	2	
<i>q</i>	0	339.09	718.2	358.6	337.60	548.15	365.1	527.15
	1	419.05	798.16	398.58	377.58	588.13	391.75	567.13
	2	499.00	878.12	438.56	417.56	628.11	418.41	607.11
	3	578.96	958.07	478.54	457.54	668.09	445.06	647.09

dpX	dp8			dp10			dp12			
<i>p-n</i>	3	2	4	3	5	4				
<i>n</i>	0	1	0	1	0	1				
<i>z</i>	2	3	2	2	3	3	2	3		
<i>q</i>	0	737.71	491.47	716.71	927.26	617.84	906.26	1116.82	744.21	730.21
	1	777.69	518.12	756.69	967.24	644.49	946.24	1156.8	770.87	756.87
	2	817.66	544.78	796.66	1007.22	671.15	986.22	1196.78	797.52	783.52
	3	857.64	571.43	836.64	1047.20	697.8	1026.20	1236.75	824.17	810.17

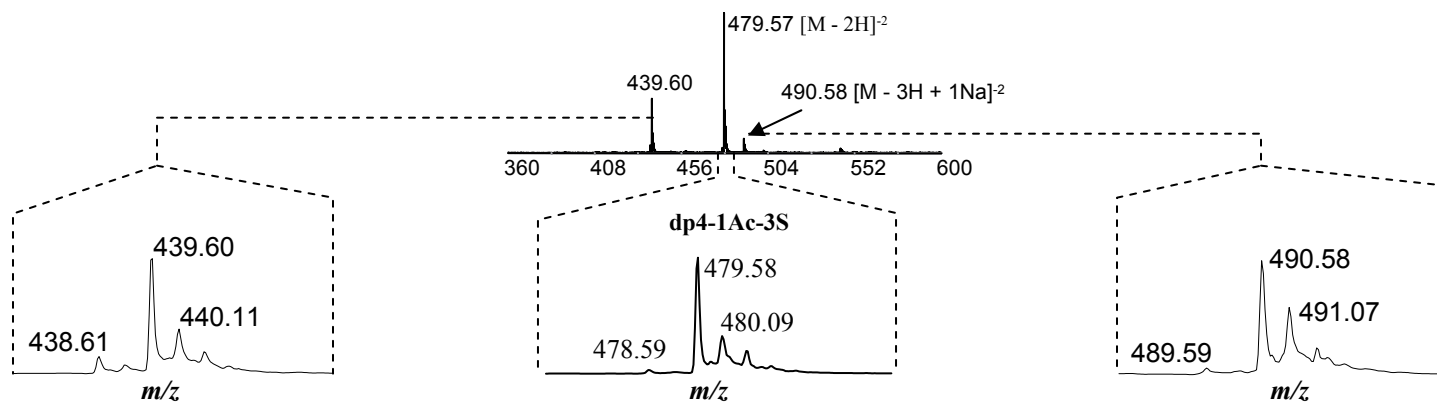
Supplemental 2. Total ion current chromatogram (TIC) and integrated mass spectrum of low pH nitrous acid digested bovine kidney HS



## About supplemental 2

- Upper part: total ion current of low pH nitrous acid digested bovine kidney heparan sulfate.
- Lower part: the integrated mass spectrum from  $m/z$  300 to 1000, where most of the oligosaccharides had signals. The integrated mass spectrum is the average of 1200 individual mass spectra collected from the entire run of HPLC. Almost all  $m/z$  peaks can be ascribed to specific oligosaccharides according to supplemental 1.

### Supplemental 3. 3-OST-1 labeled dp4-1Ac-3S had three m/z clusters

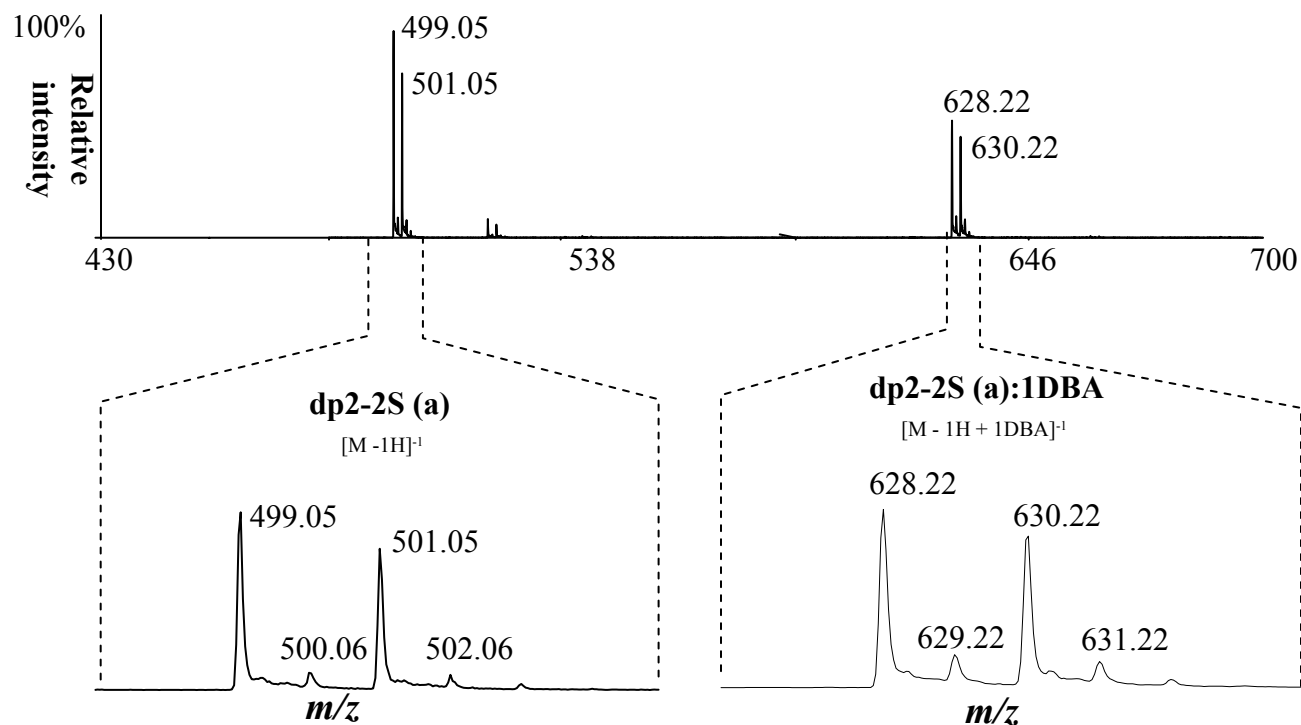


The major cluster was around  $m/z$  479.57 and was chosen for sulfation degree calculation.

The cluster around  $m/z$  439.60 was caused by a sulfate loss.

The cluster around  $m/z$  490.58 was caused by a sodium adduct.

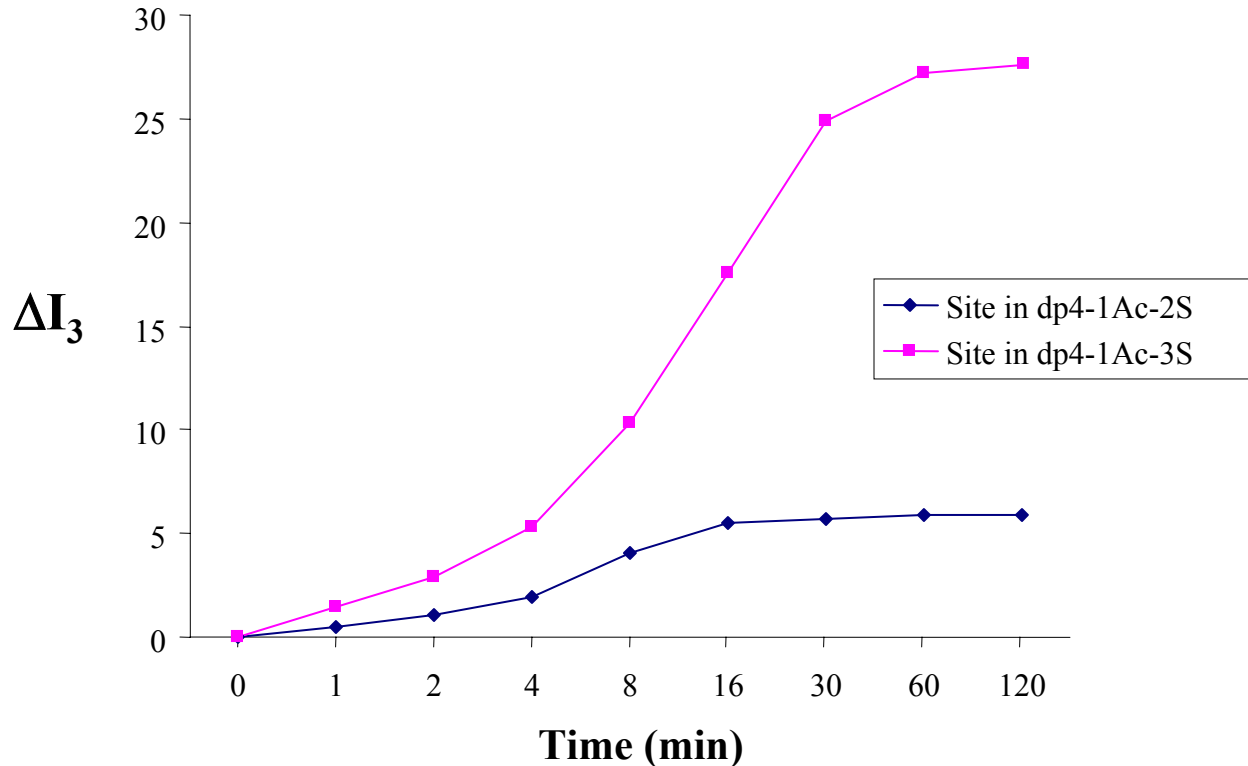
## Supplemental 4. 6-OST-1 labeled dp2-2S(a) had two m/z clusters



The two *m/z* clusters are almost the same. The *m/z* cluster of dp2-2S (a) was used for sulfation degree calculation.

## Supplemental 5

### Time course for the $\Delta I_3$ values of 3-OST-1 sites

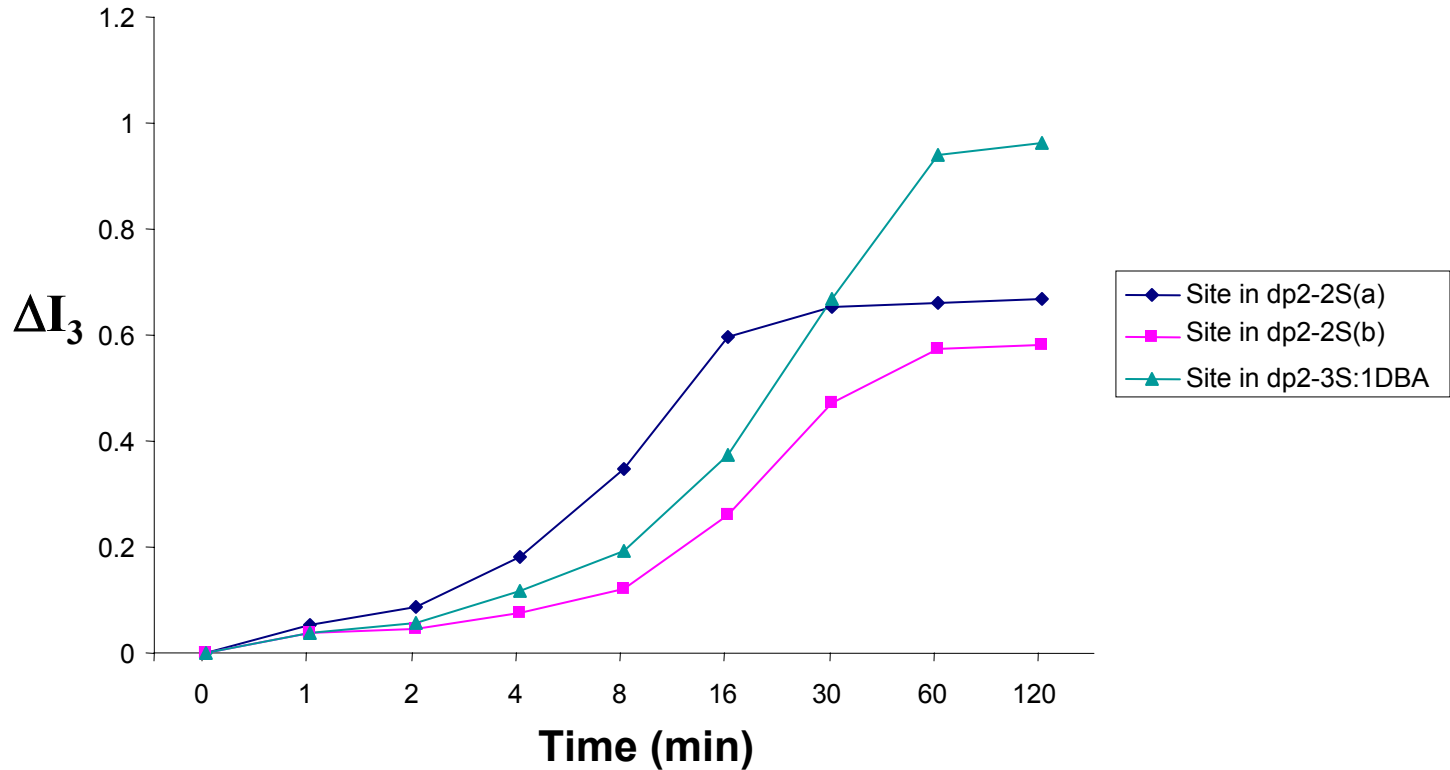


In a 20  $\mu\text{l}$  reaction, 10  $\mu\text{g}$  of HS, 10  $\mu\text{l}$  of labeling buffer (2X), 2  $\mu\text{l}$  of PAP<sup>34</sup>S (3 mM), 70 ng of the expressed 3-OST-1 and sufficient amount of water were assembled. Incubation was kept at 37°C.

The increase of the relative intensity of isotopic peak  $M+2$  ( $\Delta I_3$ ) was plotted against the incubation time.

## Supplemental 6

### Time courses for the $\Delta I_3$ values of 6-OST-1 sites



In a 20  $\mu$ l reaction, 10  $\mu$ g of HS, 10  $\mu$ l of labeling buffer (2X), 2  $\mu$ l of PAP<sup>34</sup>S (3 mM).

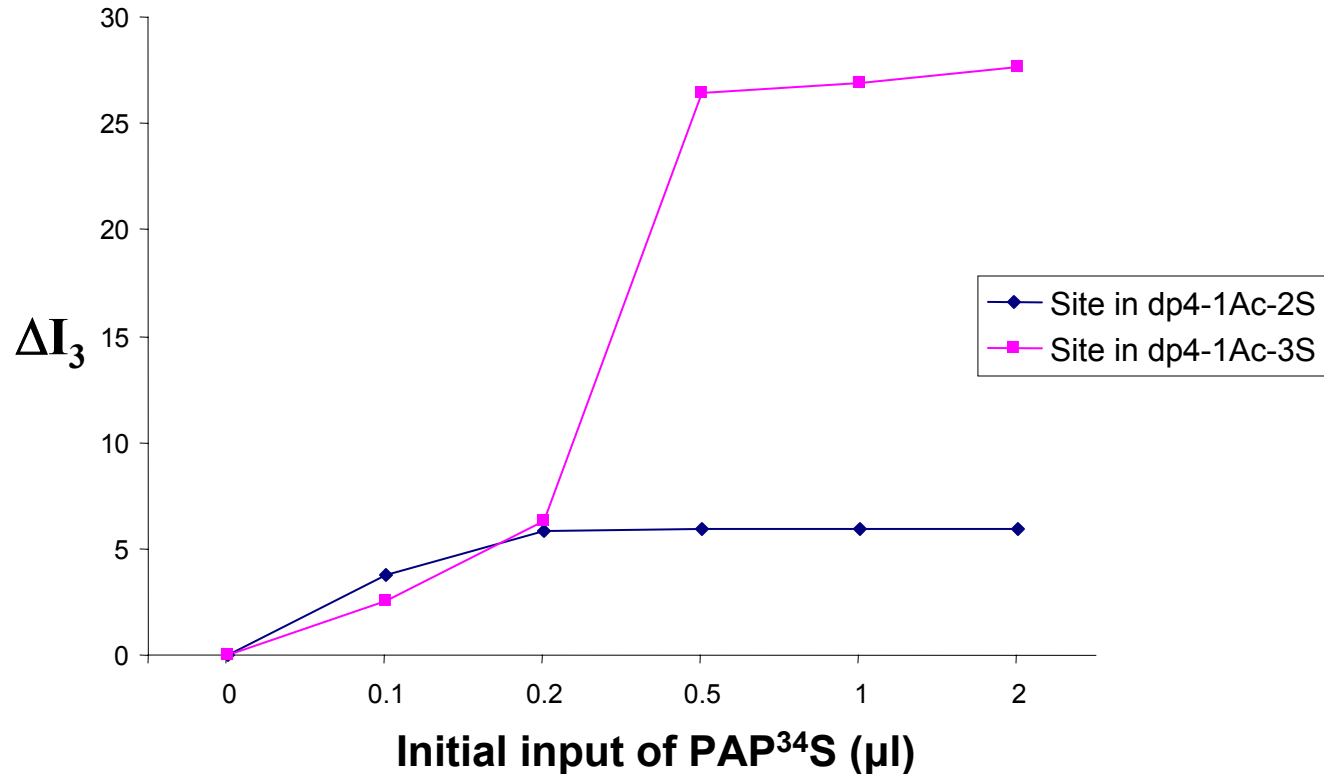
70 ng of the expressed 6-OST-1 and sufficient amount of water was assembled.

Incubation was kept at 37°C.

The increase of the relative intensity of isotopic peak  $M+2$  ( $\Delta I_3$ ) was plotted against the incubation time.

## Supplemental 7

### The $\Delta I_3$ values for 3-OST-1 sites versus the input of PAP<sup>34</sup>S



In a 20  $\mu\text{l}$  reaction, 10  $\mu\text{g}$  of HS, 10  $\mu\text{l}$  of labeling buffer (2X), variable amount of PAP<sup>34</sup>S, 70 ng of the expressed 3-OST-1 and sufficient amount of water was assembled.

The incubation was kept at 37°C for 2 hours.

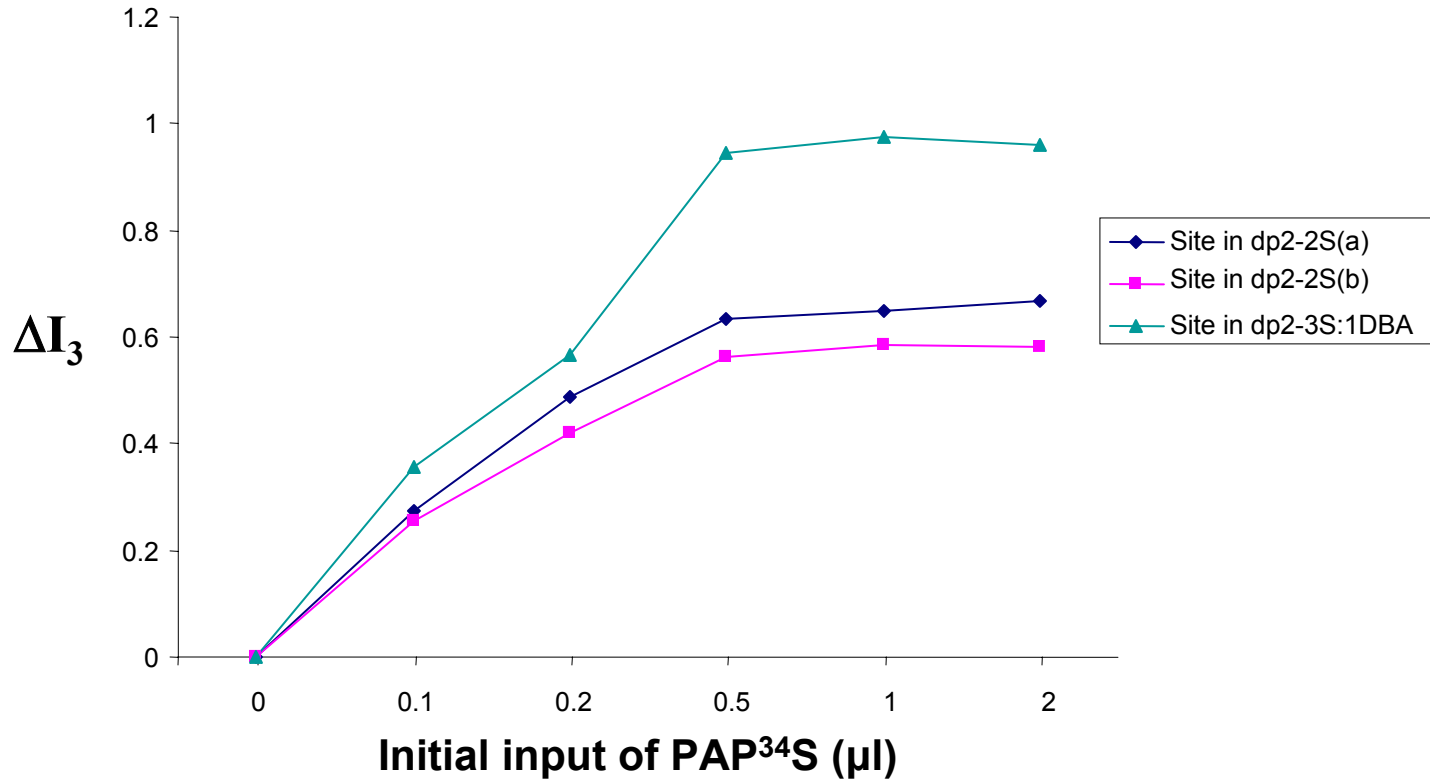
The concentration of PAP<sup>34</sup>S was 3 mM.

$\Delta I_3$  was plotted against the initial input of PAP<sup>34</sup>S.



## Supplemental 8

### The $\Delta I_3$ values for 6-OST-1 sites versus the input of PAP<sup>34</sup>S



In a 20  $\mu\text{l}$  reaction, 10  $\mu\text{g}$  of HS, 10  $\mu\text{l}$  of labeling buffer (2X), variable amount of PAP<sup>34</sup>S, 70 ng of the expressed 6-OST-1 and sufficient water was assembled.

The incubation was kept at 37°C for 2 hours.

The concentration of PAP<sup>34</sup>S was 3 mM.

$\Delta I_3$  was plotted against the initial input of PAP<sup>34</sup>S.