Supplementary Table 5: HDX Data Summary and Experimetal Parameters

Data Set	$PPM1D_{(1-420)}$	Hinge and flap deletions	
Related Figures	Figures 4A, 4B, 5A, 5B	Figures 4C, 4D, 4E, 4F, 6B, 6D, Supp. 5F	
States analyzed	$PPM1D_{(1-420)}$ + DMSO $PPM1D_{(1-420)}$ + GSK	+DMSO $PPM1D_{(1-420)}$ $PPM1D_{\Delta flap}$ $PPM1D_{\Delta hinge}$	+GSK $PPM1D_{(1-420)}$ $PPM1D_{\Delta flap}$ $PPM1D_{\Delta hinge}$
HDX reaction details (a)	Final D_2O concentration = 93.6%,		
HDX time course	0.167, 1, 10, 60, 240 minut		
Back-exchange (b)	~35%		
HDX controls	3 undeuterated + DMSO	8 undeuterated; 2-3 per state + DMSO	
Number of peptides	79 followed, 102 identified	96 followed, 148 identified	
Filtering parameters	0.3 products per a.a. 3 consecutive products 8 ppm error File threshold of 2	0.3 products per a.a. 3 consecutive products 10 ppm error File threshold of 4	
Sequence coverage	91.2%	79%	
Average peptide length	13.6	11.3	
Redundancy	2.8	3.26	
Replicates	1-3 technical replicates for eac		
Repeatability (c)	+/- 0.20 relative Da		
Significant differences (d)	> 0.5 Da		

(a) 15-fold dilution with labeling buffer [20 mM HEPES, pD 7.5, 25 mM NaCl, 5 mM MgCl2, 0.1 mM TCEP, 99.9% D2O].
1:1 (v/v) dilution with quench buffer [0.8M guanidine hydrochloride, 0.8% formic acid, H2O].

(b) Back exchange estimated using peptides from the loop region of PPM1D, specifically peptides covering residues 45-9

(c) Average standard deviation across all replicate measurements for all peptides and timepoints for each state analyzed

(d) Global |ΔHX| significance threshold was calculated from experimental standard deviations to be 0.405 Da (Hageman

C-terminal truncations	Loop deletion
Figure 7D	Supplementary Figure 6B
$PPM1D_{(1-420)}$ $PPM1D_{(1-400)}$ $PPM1D_{(1-377)}$	$PPM1D_{(1-420)}$ $PPM1D_{\Delta loop}$
pH_{read} = 7.1	
tes	
3 undeuterated; $PPM1D_{(1-420)}$	3 undeuterated; $PPM1D_{(1-420)}$
128 followed, 193 identified	69 followed, 82 identified
0.3 products per a.a. 3 consecutive products 9 ppm error File threshold of 2	0.3 products per a.a. 3 consecutive products 10 ppm error File threshold of 3
92.9%	86.4%
12.8	10.6
4.21	2
:h state	

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TS and Weis DD. *Anal Chem* 91, 8008-8016, 2019).