SUPPORTING INFORMATION

A phosphorylated intermediate in the activation of WNK kinases

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Table S1. Statistics of crystallographic data and refinement

	5W7T (pWNK1)	**pWNK1+Sucrose**
Space group	P1 21 2	C 1 2 1
Unit cell dimensions (Å)	*a,b,c*=45.18, 62.24,120.90	*a,b,c* =129.83,64.6,45.87
Angles (°)	α,β,γ=90, 92.3,90	α,β,γ = 90.0,108.4,90.0
Wavelength (Å)	0.97112	0.92025
Resolution (Å)	30-2.0 (2.0-2.05)	30.0-2.18 (2.23-2.17)
Unique reflections (last shell)	39436(1844)	16989 (996)
Completeness (%) (last shell)	91 (58)	94 (76)
I/σ (last shell)	12.7 (1.8)	10.8 (1.5)
Rsym, Rpim (last shell)[a]	0.11, 0.06 (0.72, 0.45)	0.08, 0.04, (0.25, 0.12)
Redundancy (last shell)	3.9 (3.3)	5.5 (5.0)
CC1/2 (last shell)	0.8(0.53)	1.0 (0.96)
Wilson B factor	18.5	35.0
Structure		
Rwork/Rfree[b] (last shell)	0.151/0.226 (0.20/0.21)	0.19/0.24(0.21/0.27)
Non-H protein atoms	5355	2405
Waters	825	220
RMSD in bond length (Å)[c]	0.013	0.015
RMSD in bond angles(°)[c]	1.7	2.0
Average B-values (Å^2)	22	40
Ramachandran plot stats. (%)		
Most favored region	98.0	96
Disallowed region	0.18	0.8
Molprobity Score	1.3	2.3
Residues missing from the model	None	A280-I285, 291-293

[a] $R_{sym} = \sum | I_{avg} - I_j | / \sum I_j$.

[b] $R_{factor} = \sum | F_o - F_c | / \sum F_o$, where F_o and F_c are observed and calculated structure factors, respectively, R_{free} was calculated from a randomly chosen 5% of reflections excluded from the refinement, and R_{factor} was calculated from the remaining 95% of reflections.

[c] r.m.s.d is the root-mean-square deviation from ideal geometry.

Table S2 Comparison of Cavities and water content in uWNK1, pWNK1 and sucrose soaked pWNK1 structures

Property	6CN9 (uWNK1) (dimeric)	5W7T (pWNK) (per monomer)	Suc-pWNK1 (monomer)
Cavities (Top 10 $Å^3$)	1900	771	977
Cavities (Top $Å^3$)	650	440	346

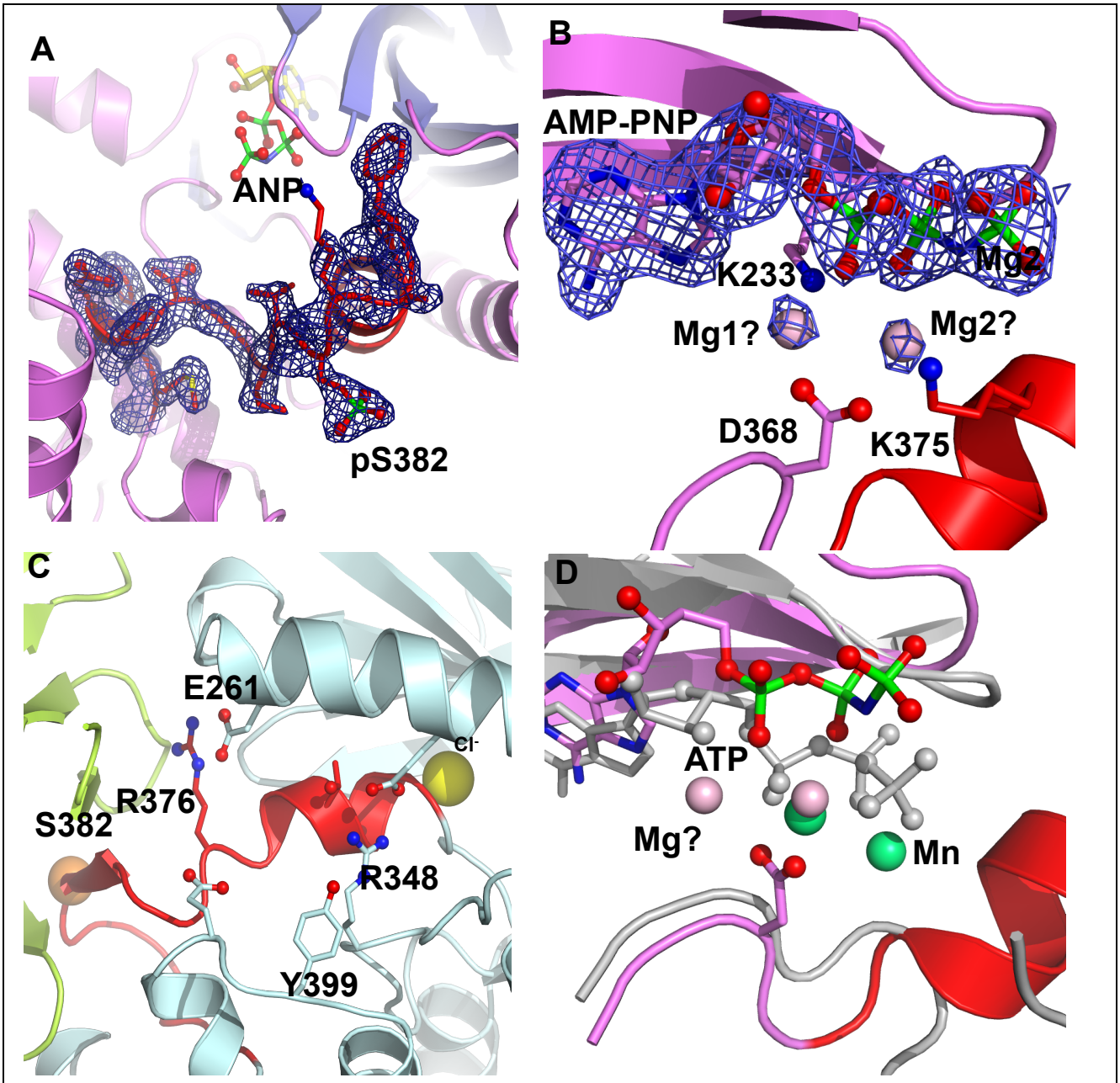

Figure S1. Electron density for pS382 in pWNK1 and comparison to uWNK1 and PKA. (A) Electron density for pS382 and flanking activation loop contoured at 1σ in Coot. pWNK1 is magenta, AMP-PNP is shown in sticks. Putative Mg^{++} ions positions in pWNK1. Electron density contoured at 1σ on AMP-PNP and Mg^{++} ions is blue. (C) The hAL in inactive dimeric uWNK1 (PDB file 6cn9, subunit A cyan, subunit B green). Activation loop red, chloride yellow, cα for S382 is shown as an orange sphere, showing its location in the subunit interface. (D) Superposition of the active site of pWNK1 compared with active PKA (PDB 1ATP) showing putative Mg^{++} ions in pWNK1(pink) and Mn^{++} ions in PKA (cyan), pWNK1 is magenta. PKA is shown in gray.

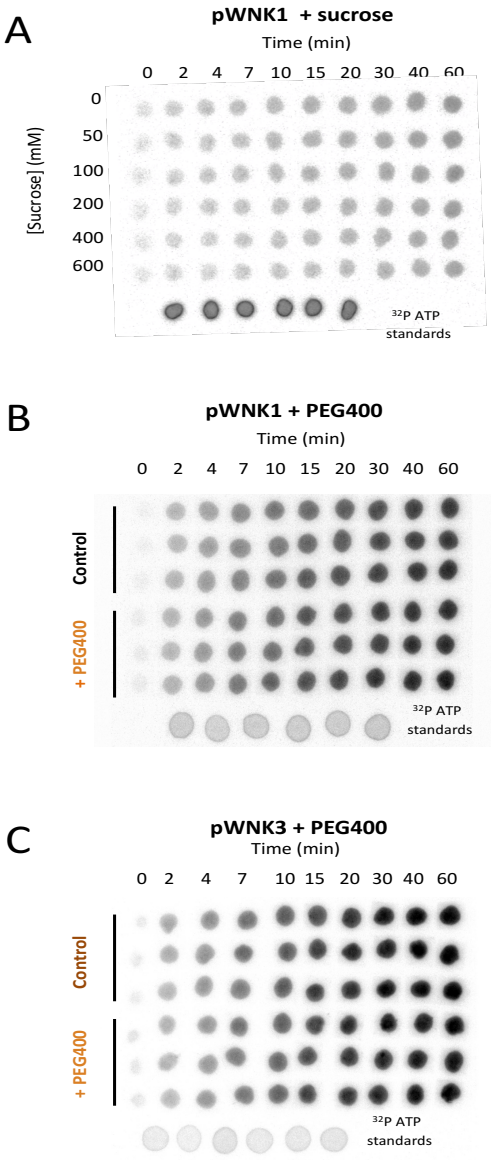

Figure S2. WNK assay autoradiography. Autoradiography for progress curves following total protein incorporation of ^{32}P over time for (A) pWNK1 with increasing sucrose (B) pWNK1 with and without 15%PEG400 in triplicate, and (C) pWNK3 with and without 15%PEG400 in triplicate.

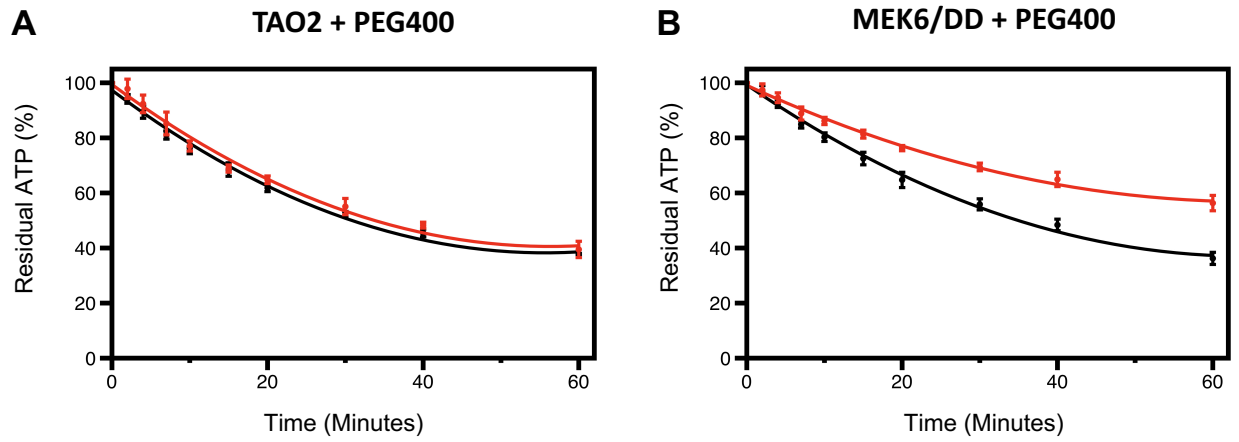

Figure S3. PEG400 effects on control kinases. (A) Progress curves for the activity of TAO2 on Myelin Basic Protein with (red) and without (black) 15%PEG400, tracking the disappearance of ATP using Kinase-Glo®. (B) Progress curves for the activity of MEK6/DD on kinase-dead p38a K53M with (red) and without (black) 15%PEG400, also using Kinase-Glo® as in (B).

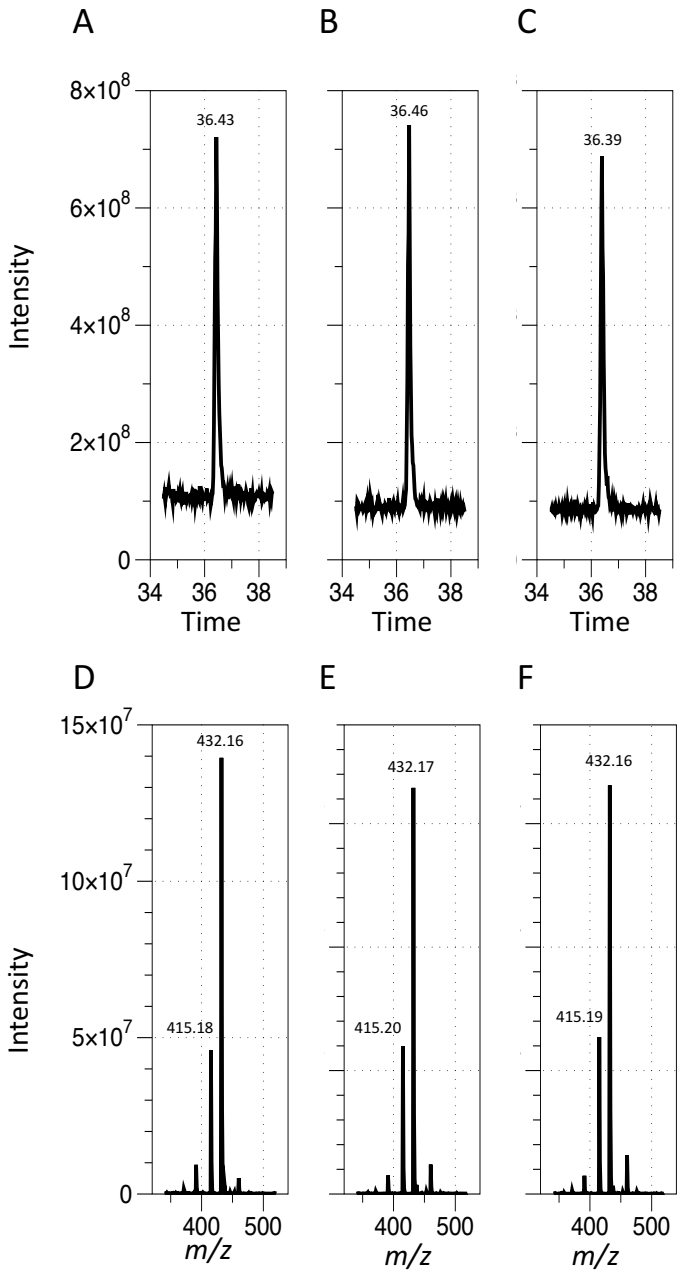

Figure S4 Mass Spectrometry of PEG400. (A – C) TIC (Total Ion Current) traces showing the reverse phase HPLC elution time of the most prevalent ethylene glycol oligomer (PEG) (n=9). Negative control assays lacking ATP (A) or pWNK1 (B) are essentially identical to a full pWNK1 assay (C). Six other ethylene glycol oligomers were observed (n=7,8,10-13) which also showed no elution time change (data not shown). (D-E) Observed *m/z* of HPLC-separated PEG400 n=9 from traces observed in (A-C), respectively. Parental mass [M + H] of oligomer is 415.19, and an accompanying ammonium adduct has an *m/z* of 432.16.