SUPPLEMENTARY MATERIAL:

Molecular determinants of KA1 domain-mediated autoinhibition and phospholipid activation of MARK1 kinase

Ryan P. Emptage, Mark A. Lemmon, and Kathryn M. Ferguson

Table S1

Additional SAXS parameters for the data reported in Figure 2C

Ratio	R_g (Å)	Sample (mg/ml)	Norm. I_0/c	D_{max} (Å)	Total estimate (Fit)
1:0	28.1 ± 0.4	5.3	1.00 ± 0.02	75	0.945
1:1	30.3 ± 0.6	7.3	1.02 ± 0.04	80	0.953
1:2	30.0 ± 1.0	9.3	1.15 ± 0.22	95	0.934
1:3	31.9 ± 0.2	11.3	1.37 ± 0.09	100	0.917
0:3	20.8 ± 0.1	6	0.51 ± 0.05	65	0.941
HEWL	14.3 ± 0.1	10.5	0.44 ± 0.01	35	0.962

SAXS analysis was performed for ΔN-KIN^{WT}-UBA MARK1 to which the MARK1 KA1 domain had been added at the noted ratio (ΔN-KIN^{WT}-UBA:KA1), or for hen egg white lysozyme (HEWL) as a control. Relative increases in the normalize I_0/c value are plotted in Figure 2C. These are proportional to the weight-averaged molecular weight (M_W), and have been normalized to 1.0 for ΔN-KIN^{WT}-UBA. With this normalization, the numbers equate to fold increases in M_W. Radii of gyration (R_g) and D_{max} values are listed and are consistent with complex formation.

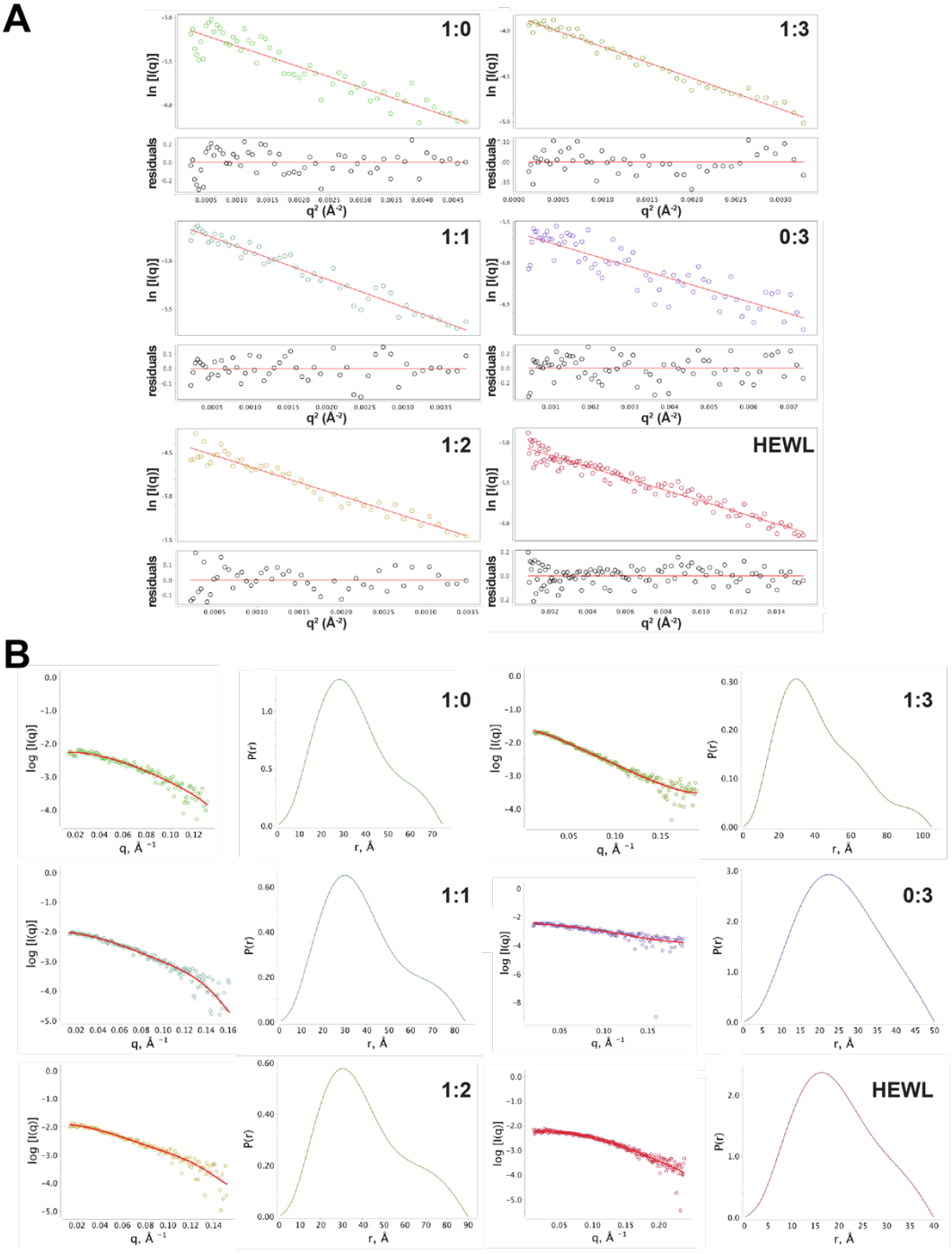

Figure S1 Representative SAXS data analysis

Analysis of the representative SAXS data used to generate Figure 2C. The ratio of kinase-UBA fragment to KA1 domain is indicated for each sample. The hen egg white lysozyme (HEWL) control is also shown. (**A**) The Guinier region is linear for all samples. (**B**) The left hand of each pair of plots shows intensity against q. Open circles represent the data and the red line is the fit from GNOM [1]. The right hand of each pair of graphs shows radial distance distributions (normalized P(r) curves).

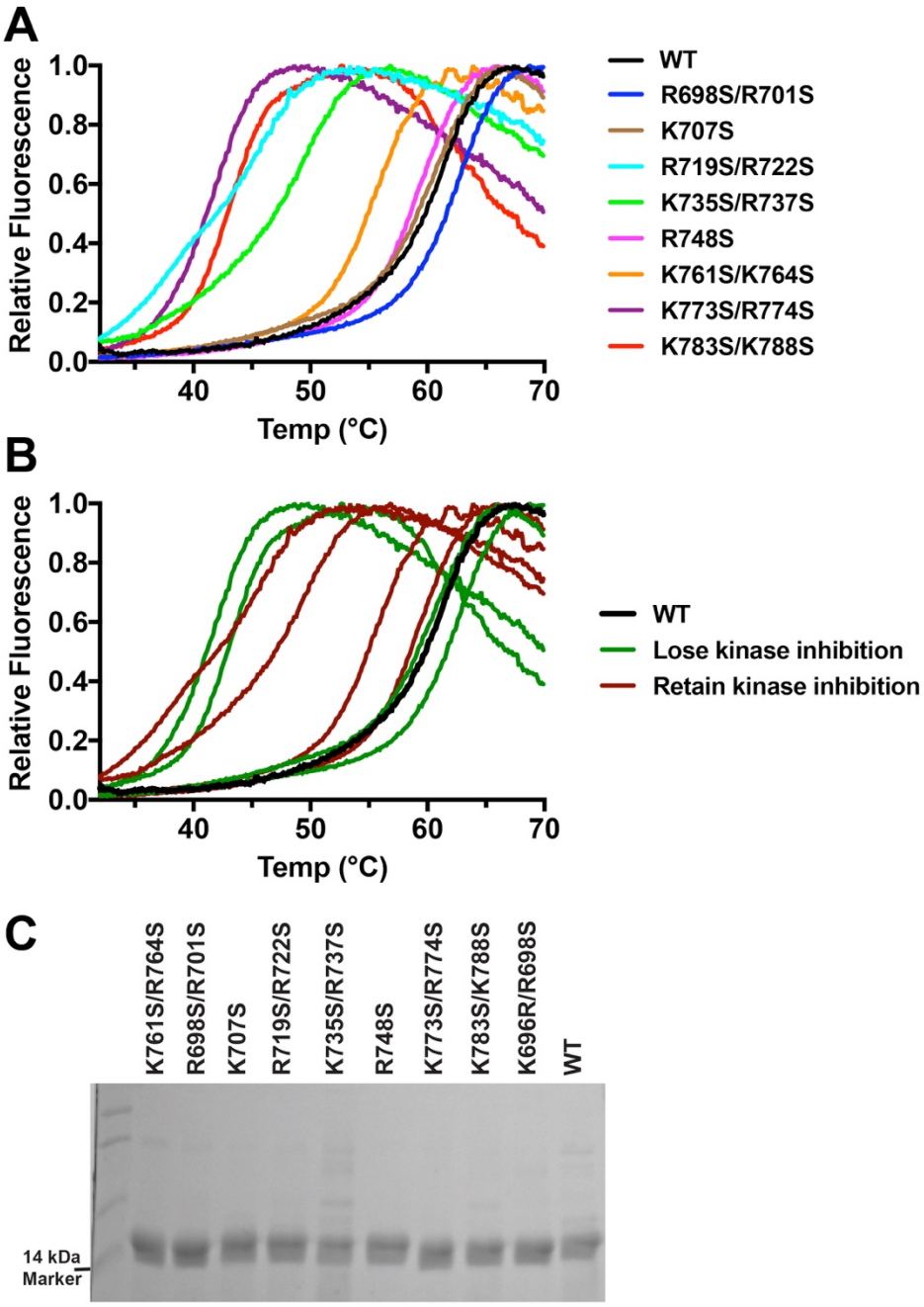

Figure S2 Relative stability of mutated KA1 domain variants.
(**A**) A melting curve for each mutated variant of the MARK1 KA1 domain used in this study was generated by monitoring fluorescence of SYPRO Orange, a dye that binds hydrophobic side-chains and shows an increase in fluorescence as proteins unfold. No variant had a melting temperature less than ~40˚C. The same plot is shown in (**B**), but with the traces colored as wild-type (black), mutations that abolish kinase inhibition by the KA1 domain (green), or those that do not affect kinase inhibition (red). There is no correlation between the effect of a mutation on kinase inhibition and its melting temperature. In (**C**), a purified sample of each KA1 domain was analyzed by SDS-PAGE (5 μg protein per lane), showing similar expression levels and purity.

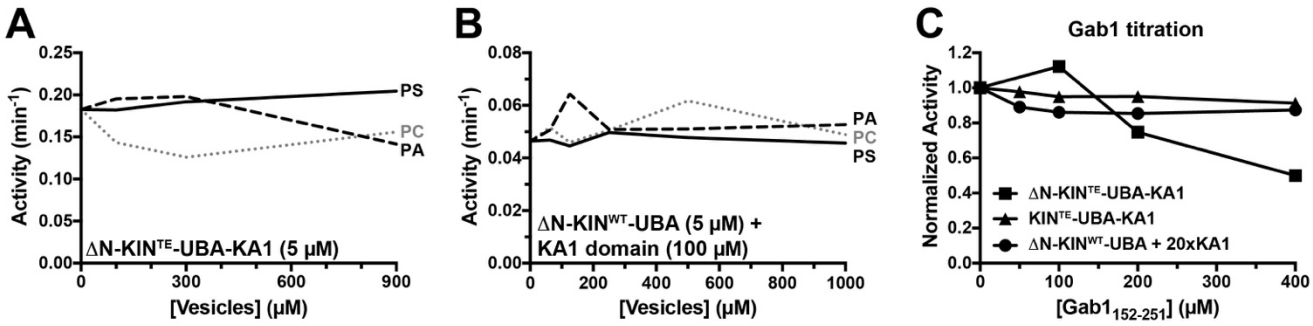

Figure S3 Neither anionic phospholipids alone nor Gab1 activate MARK1.
(**A**) The standard MARK1 kinase assay was performed for 5 μM ΔN-KIN^{TE}-UBA-KA1 'mini' MARK1 in the presence of DOPC vesicles (up to 0.9 mM total lipid) containing no anionic lipids (grey dotted line), 20% (mole/mole) phosphatidylserine (solid black line), or 20% (mole/mole) phosphatidic acid (black dashed line). (**B**) The same result was obtained with 5 μM ΔN-KIN^{WT}-UBA *trans*-inhibited by 100 μM MARK1 KA1 domain. In multiple repeats of these experiments, no activation could be detected unless DOGS-Ni-NTA lipids were included, in which case PA and PS could activate the KA1 domain-containing MARK1 variants (Figure 6). (**C**) 5 μM ΔN-KIN^{TE}-UBA-KA1 (squares), 5 μM KIN^{TE}-UBA-KA1 (triangles), or 5 μM ΔN-KIN^{WT}-UBA inhibited in *trans* with 100 μM MARK1 KA1 domain (circles), were incubated with various concentrations of Gab1$_{152-251}$ [2] in order to assess its ability to activate MARK1. Activities were normalized to those measured with no added Gab1$_{152-251}$ for visual comparison, and no significant activation could be detected across at least three repetitions.

References

1 Svergun, D.I. (1991) Determination of the regularization parameter in indirect-transform methods using perceptual criteria. *J. Appl. Crystallogr.* **25**, 495-503

2 Yang, Z., Xue, B., Umitsu, M., Ikura, M., Muthuswamy, S.K. and Neel, B.G. (2012) The signaling adaptor GAB1 regulates cell polarity by acting as a PAR protein scaffold. *Mol. Cell* **47**, 469-483 doi: 10.1016/j.molcel.2012.06.037