Specific phosphorylation of microtubule-associated protein 2c by extracellular signal-regulated kinase reduces interactions at its Pro-rich regions

Jitka Plucarová[1,2], Séverine Jansen[2], Subhash Narasimhan[1,2], Alice Laníková[1,2], Marc Lewitzky[3], Stephan M. Feller[3] and Lukáš Žídek[1,2]

From the [1]National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, CZ-625 00, Brno, Czech Republic, [2]Central European Institute of Technology, Masaryk University, Kamenice 5, CZ-625 00, Brno, Czech Republic, [3]Institute of Molecular Medicine, Martin-Luther-University Halle-Wittenberg, Halle, Germany

Running title: *MAP2c phosphorylation by ERK2*

To whom correspondence should be addressed: Lukáš Žídek, phone: +420 54949 8393, fax: +420 54949 2556, lzidek@chemi.muni.cz

Keywords: microtubule-associated protein (MAP), extracellular signal-regulated kinase (ERK), cyclin-dependent kinase (CDK), Src homology 3 domain (SH3 domain), protein kinase A (PKA), nuclear magnetic resonance (NMR), growth factor receptor-bound protein 2 (GRB2)

CONTENTS

TABLES

Table S1: $^{15}N/^{1}H$ Chemical shifts of phosphorylated serines and threonines in samples of ^{15}N-MAP2c phosphorylated by ERK2 and CDK2 for 20 hours. For phosphorylated residues clustered in the sequence, phosphorylated neighbor residues that influence the position of the peak are indicated in parenthesis. *np* indicate that the residue is not phosphorylated by the respective kinase. Unassigned peaks are labled as *nd*.

Residue	**Unphosphorylated**		**ERK2**		**CDK2**	
	^{15}**N**	^{1}**H**	^{15}**N**	^{1}**H**	^{15}**N**	^{1}**H**
Ser028	118.653	8.211	120.111	8.725	119.992	8.710
Ser136	117.533	8.328	118.810	8.712	118.719	8.700
Ser140	117.401	8.370	118.715	8.751	118.645	8.743
Ser178	118.841	8.358	120.580	8.848	120.414	8.835
Thr235	118.495	8.212	122.909	9.142	122.912	9.147
Thr238	118.645	8.168	120.244	8.664	120.295	8.658
Thr238(Thr235)	-	-	nd	nd	119.864	8.618
Thr238(Ser241)	-	-	120.975	8.724	np	np
Ser241	115.314	8.138	117.193	8.904	np	np
Thr245	121.410	8.271	123.783	8.879	123.792	8.885
Thr248	116.811	7.989	119.565	8.893	121.157	8.995
Thr248(Thr245)	-	-	120.736	8.906	120.467	8.867
Thr256	117.782	8.187	121.816	9.087	122.012	9.143
Thr256(Thr259)	-	-	122.221	9.167	nd	nd
Thr259	115.836	7.981	119.373	8.709	119.158	8.662
Thr259(Thr256)	-	-	118.781	8.651	nd	nd
Thr259(Thr262)	-	-	nd	nd	118.679	8.617
Thr262	115.836	7.981	119.384	8.696	119.186	8.694
Thr262(Ser264)	-	-	np	np	119.086	8.642
Ser264	115.321	8.194	np	np	117.712	8.854
Thr268	117.686	8.207	121.177	9.066	121.855	9.102
Thr268(Thr271)	-	-	nd	nd	121.186	9.074
Thr271	116.372	8.031	119.795	8.932	nd	nd
Thr271(Thr268)	-	-	nd	nd	119.268	8.802
Thr271(Ser274)	-	-	120.365	8.907	-	-
Ser274	116.219	8.223	117.829	8.790	np	np
Thr289	118.651	8.205	121.489	9.043	nd	nd
Ser293	118.674	8.342	120.827	8.867	-	-
Ser293(Thr289)	-	-	120.262	8.785	120.175	8.779
Thr296	116.028	8.122	119.152	8.905	nd	nd
Thr296(Ser293)	-	-	119.656	8.967	118.586	8.826
Ser422	118.671	8.393	119.702	8.710	119.615	8.697
Ser430	116.592	8.210	117.582	8.533	117.470	8.508
Ser448	117.567	8.213	119.421	8.726	np	np

Table S2: Primers used to produce the MAP2c phosphorylation mutants, and for cloning of Fyn-SH3

Mutated site	**primers**
S28D	acccccacgacccagagatgaaggaccagggaggctcagggg
	atctctgggtcgtgggggtgtgcagctgcctctgtgagtgagg
S136D	ctgccacctgacccaccaccatcgccagcatcagaacaaac
	tggtggtgggtcaggtggcagattaactgtttcttcagctgc
S140D	caccaccagacccagcatcagaacaaacagctgcactggaagaagcaacaag
	tgatgctgggtctggtggtggggaaggtggcagattaactgtttcttcagc
S178D	ataaccaaggacccagaaaaacgttcttccctcccaagaccttcc
	tttttctgggtccttggttattccatcagtgactttgtccttcgcc
T238E	acacctactgagcctggatctactgcaatcacccctggcactcc
	agatccaggctcagtaggtgttgaggtgccgctttttcctgctctg
T245E	ctgcaatcgagcctggcactcctccaagctactcttcacgtaccccag
	gtgccaggctcgattgcagtagatccaggggtagtaggtgttgaggtgc
T259E	cccaggcgagcctggaaccccgagctatcccaggacaccaggaac
	gttccaggctcgcctggggtacgtgaagagtagcttggaggagtgcc
T262E	acccctggagaaccgagctatcccaggacaccagg
	atagctcggttctccaggggtgcctggggtacgtga ag
S264D	gaaccccggactatcccaggacaccaggaaccccc aaa tct gg
	ctgggatagtccggggttccaggggtgcctgggg
T268E	tatcccagggaaccaggaaccccaaatctggcatcttggtgcccag
	ggttcctggttccctgggatagctcggggttccaggggtgc
T271E	acaccaggagaacccaaatctggcatcttggtgcccagtgagaag
	agatttgggttctcctggtgtcctgggatagctcggggttccag
S274D	cccccaaagatggcatcttggtgcccagtgagaagaaagttgccatcattcgc
	aagatgccatctttgggggttcctggtgtcctgggatagctcggg
T289E	atcattcgcgaacctccaaagtccccagctactcccaagcag
	ctttggaggttcgcgaatgatggcaactttcttctcactgggcac
S293A	ctccaaaggccccagctactcccaagcagcttcggctcattaaccaac
	gtagctggggcctttggaggagtgcgaatgctggcaactttcttctc
S422D	atcacacaggacccaagcaggtcaagcgtggcgtctccccgg
	cctgcttgggtcctgtgtgatgatctcagccccgtggtcgacgcg
Fyn-SH3	actcccatggaaacactctttgtggccctttatg
	gcgggatcctcaaactggagccacataattgctg

FIGURES

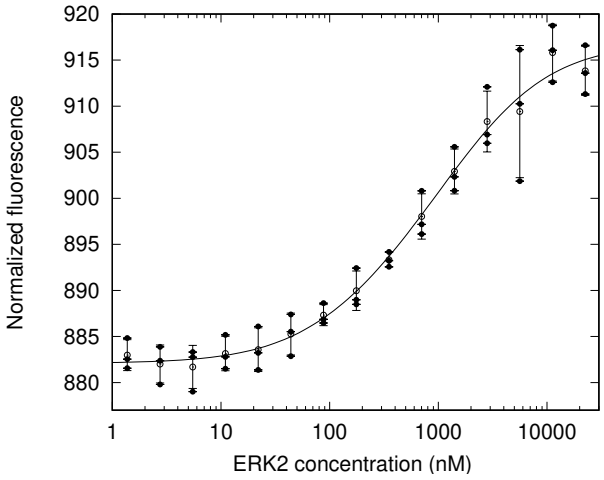

Figure S1: Microscale thermophoretic analysis of the interaction between MAP2c and ERK2. The concentration of MAP2c was 5 nM and ERK2 concentration ranged from 1.3 nM ERK2 to 45 μM. The dissociation constant is $(0.8 \pm 0.4)\ \mu$M

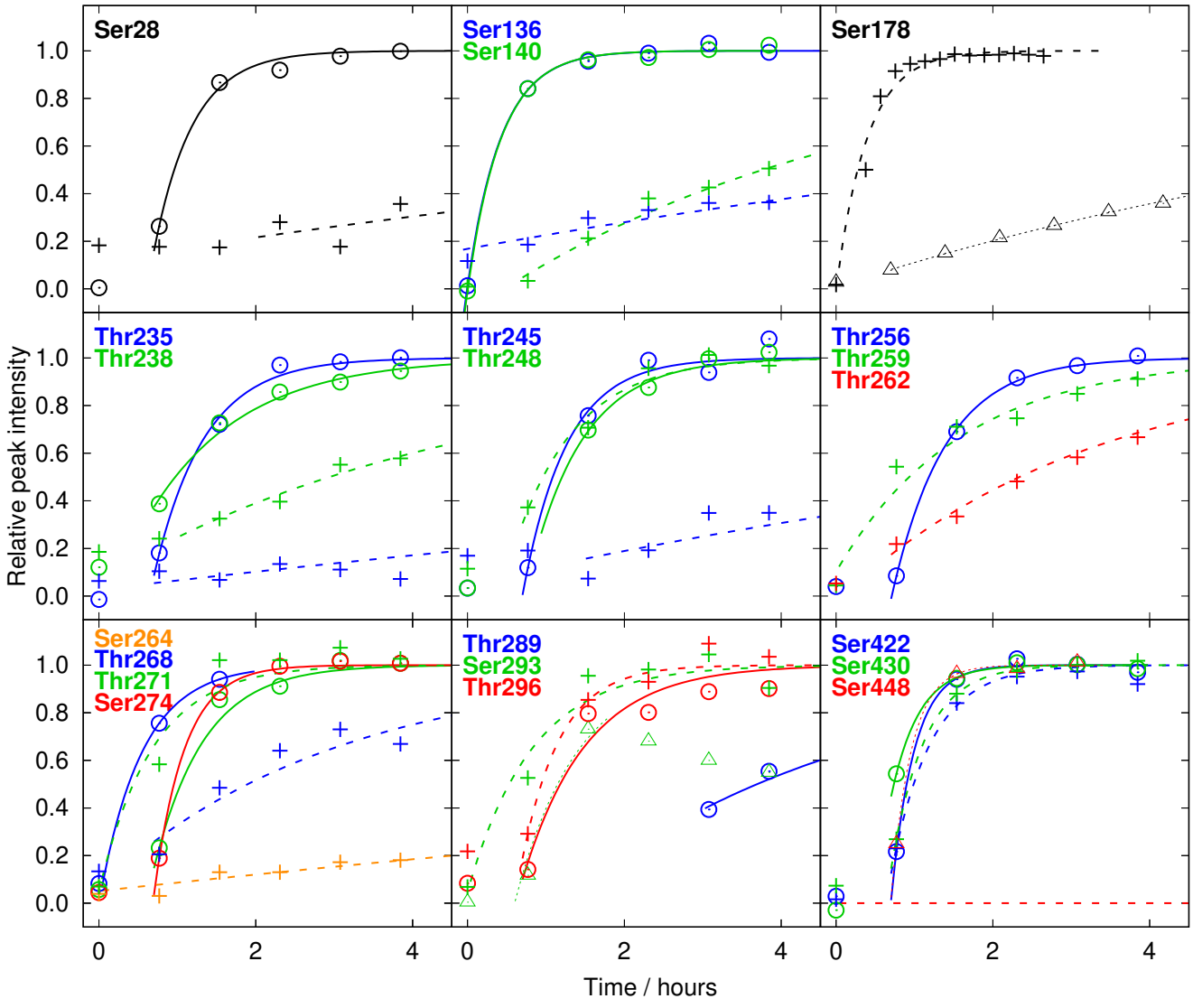

Figure S2: Initial kinetics of phosphorylation of ^{15}N-MAP2c by ERK2 (circles and solid curves) and CDK2 (crosses and dashed lines). Kinetics of phosphorylation of Ser178, Ser293 and Ser448 by ERK2 was determined using MAP2c fragments 1–159, 250–347 and 300–467, respectively (triangles and dashed lines). Note that only two peak heights were used to determine $\tau_{1/2}$ of Ser293 in MAP2c-250–347 because the slowly growing peak height of phosphorylated Ser293 in the vicinity of phosphorylated Thr296 was not measured due to the complexity of the spectra.

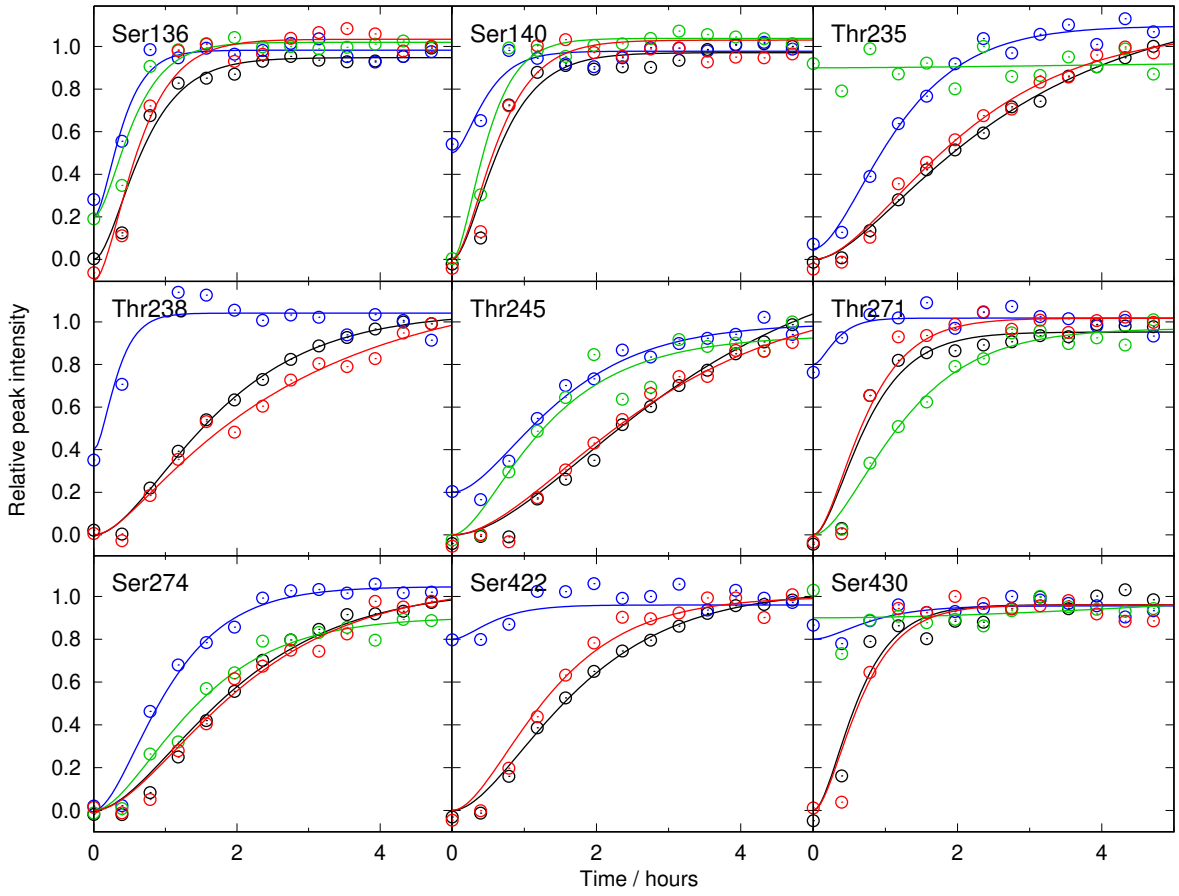

Figure S3: Kinetics of phosphorylation of well-resolved peaks of MAP2c residues by ERK2 with prior priming by CDK2 (blue), GSK3β (green) and PKA (red). Phosphorylation kinetics of unprimed MAP2c are shown in black. Ser136, Thr235, and Ser430 are phosphorylated by GSK3β and CDK2, while Ser140, Thr238, Thr245 and Thr271 are phosphorylated by CDK2. Therefore, peaks of phosphorylated forms of these residues are present already at the beginning of phosphorylation by ERK2. Upon phosphorylation by GSK3β, Thr238 and Ser422 are not resolved in ^{1}H,^{15}N-SOFAST-HMQC. Prior phsophorylation by PKA does not have any effect on the kinetics of phosphorylation by ERK2, while prior phosphorylation by CDK2 moderately increase the rate of ERK2 phosphorylation of Thr235, Thr238, Thr245, and Ser274, and prior phosphorylation by GSK3β moderately increases the rate of phosphorylation of Thr245.

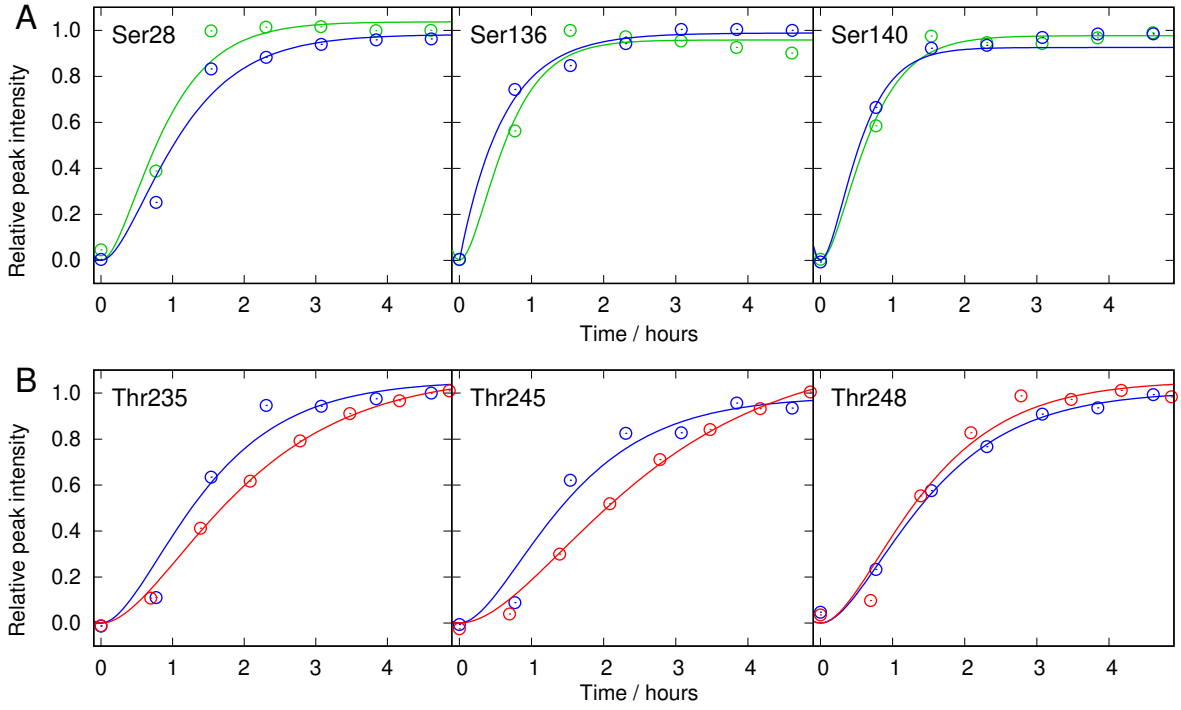

Figure S4: Comparison of the kinetics of phosphorylation by ERK2 of ^{15}N-MAP2c-1–159 (A, green) and ^{15}N-159–254 (B, red), lacking the D-box docking sites, with that of full-length ^{15}MAP2c (blue). Phosphorylation rates of the different residues in absence of the D-boxes are similar to the wild-type MAP2c, indicating that the D-boxes do not influence phosphorylation kinetics significantly.

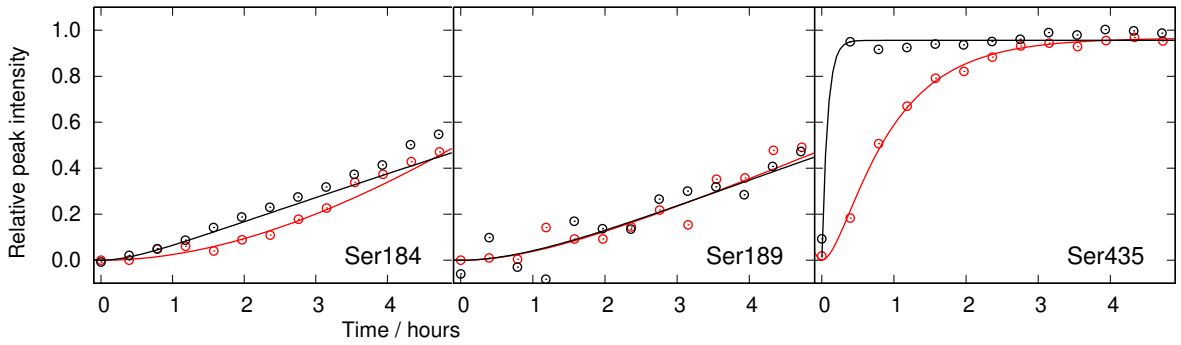

Figure S5: Kinetics of phosphorylation of the main PKA phosphorylation sites (Ser184, Ser189 and Ser435) in ^{15}N-MAP2c by PKA with prior priming by ERK2 (red), compared to unprimed ^{15}N-MAP2c (black). Thr220 was not resolved in the ^{1}H,^{15}N-HSQC spectrum of MAP2c phopshorylated by ERK2. Upon phosphorylation by ERK2, phosphorylation of Ser435 is slower ($\tau_{1/2}$ = (0.85 ± 0.08) hrs, when MAP2c phosphorylated by ERK2, $\tau_{1/2}$ = 0.07 hours in unprimed MAP2c). Phosphorylation of Ser184 is less affected ($\tau_{1/2}$ = 4.1 ± 0.8) hrs and $\tau_{1/2}$ = (2.6 ± 0.1) hrs for primed and unprimed MAP2c, respectively), while Ser189 phosphorylation is not affected.

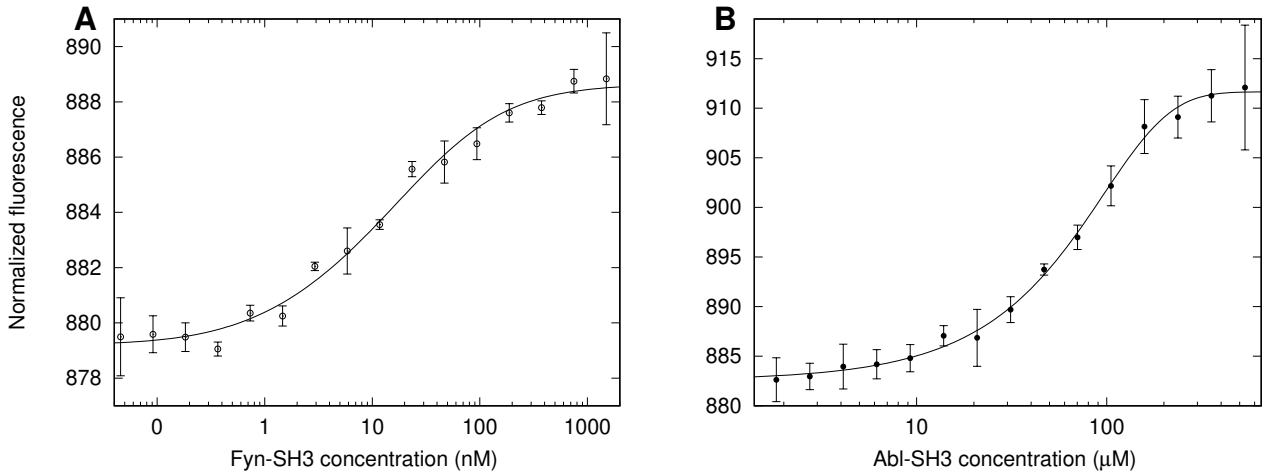

Figure S6: Microscale thermophoresis analysis of the interactions of 5 nM MAP2c with (A) 0.046 nM to 1.5 μM Fyn-SH3 and (B) 1.8 μM to 533 μM Abl-SH3. The dissociation constant are (22 ± 5) nM and (68 ± 10) μM for Fyn-SH3 and Abl-SH3, respectively.

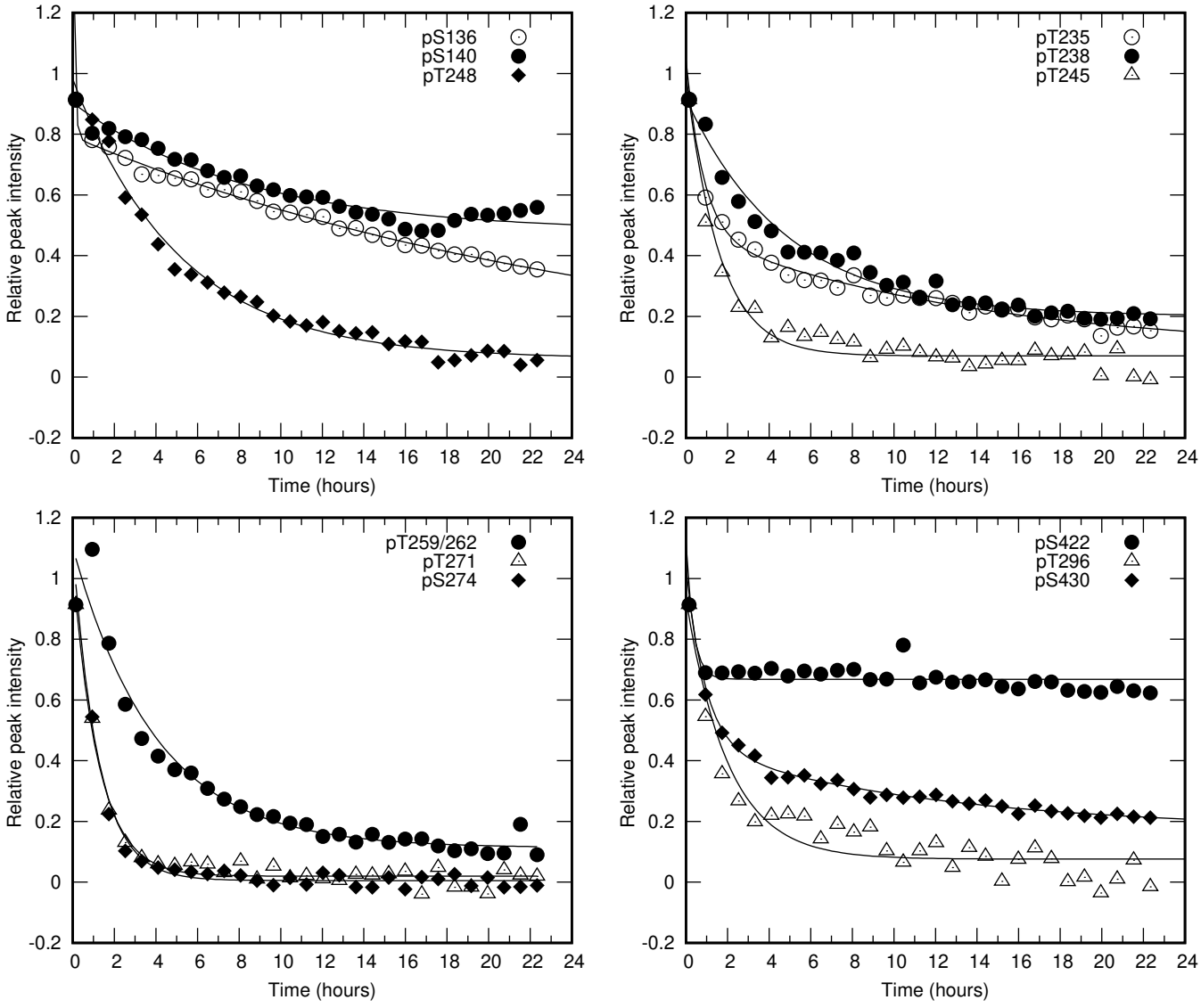

Figure S7: Decrease of the intensities of well resolved serines and threonines of ^{15}N-MAP2c phosphorylated by ERK2 in SH-SY5Y neuroblastoma cell extract.

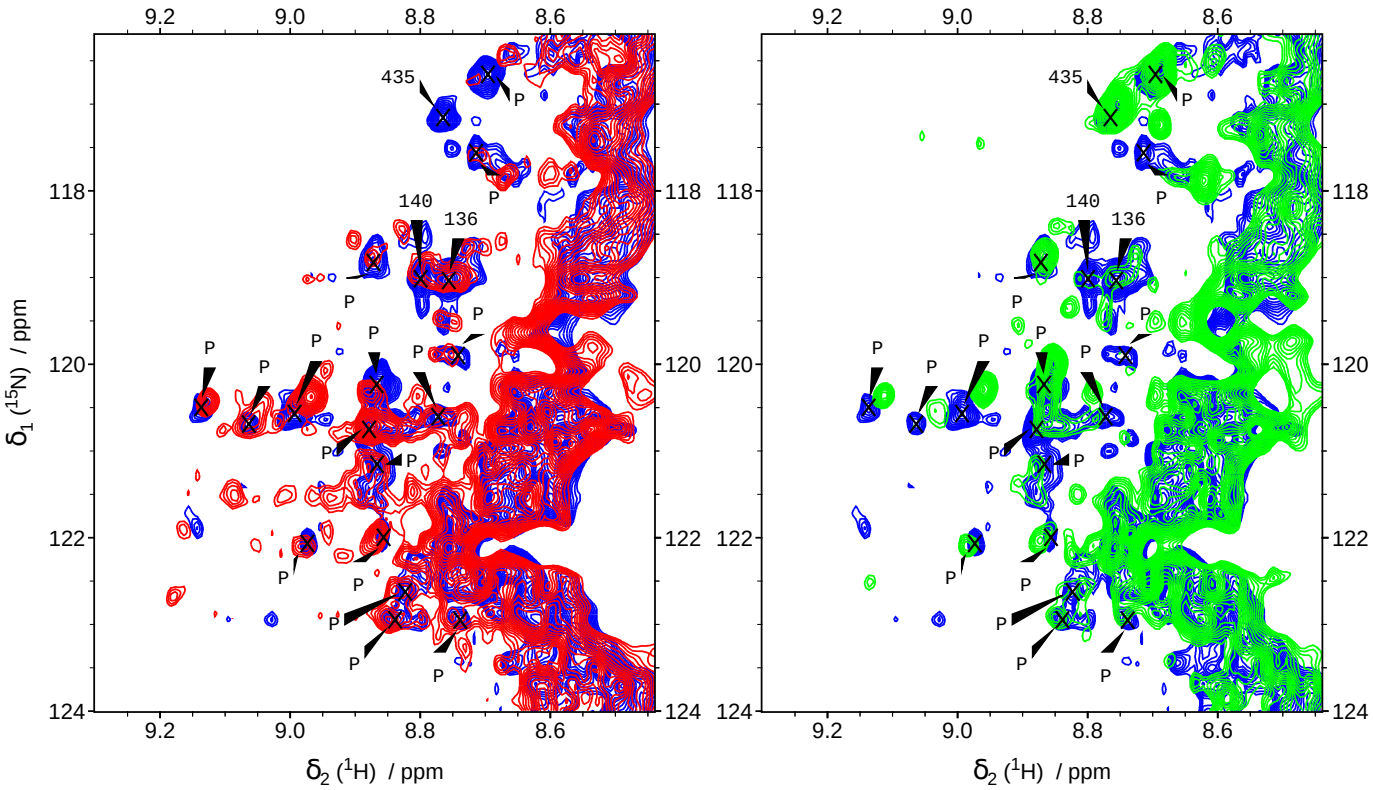

Figure S8: Overlaid ^{1}H,^{15}N-SOFAST-HMQC spectra of ^{15}N-MAP2c-S435D (red), ^{15}N-MAP2c-S140D (green), and ^{15}N-MAP2c wild type (blue, presented separately in Figure 6) phosphorylated in SH-SY5Y neuroblastoma cell extract. Peaks of phosphorylated Ser435 and Ser140 are present in the spectra of wild-type MAP2c (blue, in both panels) but missing in spectra of the corresponding mutants (the peak of Ser435 is missing in the spectrum of the S435D, shown in red in the left panel and the peak of Ser140 is missing in the spectrum of the S140D, shown in green in the right panel).