Supplemental figures

Supplementary Figure 1 ***A***, Comparison of all known synuclein sequences. ***B***, Comparison of human, mouse and α-, β- and γ-synuclein sequences. Program used: ClustalW2 (http://www.ebi.ac.uk/Tools/clustalw2/index.html).

Supplemental Figure 2: Mapping the CK1 phosphorylation sites: WT, S129A and S129E were phosphorylated for 24 h with CK1 and the different phosphorylated species (I,II,III) were separated by semi-preparative RP-HPLC and analyzed by MALDI-MS. ***A,*** The Coomassie blue stained SDS gels of the mono-, di-, and unphosphorylated species of each protein. The S129A α-syn was subjected to further trypsin digestion and tandem mass spectrometry. ***B,*** 4280-4380 m/z inset covering the pseudomolecular region of C-terminal peptide [103-140] of S129A analyzed by MALDI-MS in positive ion mode. The peak corresponding to the monophosphorylated peptide at m/z 4363.4 could not be detected. ***C,*** MALDI-MS spectra in negative ion mode showing the m/z region 1450–1850 of the tryptic digest of unphosphorylated (blue), monophosphorylated (red), and diphosphorylated (green) peaks obtained from an in vitro phosphorylation of S129A with CK1. The peak at m/z 1478.0 corresponds to the sequence TVEGAGSIAAATGFVKK ([81-96]), which contains three potential phosphorylation sites. The difference in mass of 80 or 160 Da is consistent with the addition of one and two phosphate groups, respectively. ***D,*** CID spectrum of the doubly modified peptide [81-96]. Fragmentation pattern of the [M + 2H]2+ 81-96 peptide bearing two phosphates on S87 and T92 residues. (Top right) Sequence coverage based on detected y and b fragment ions. Ions carrying the phosphate group are shown with one or two stars (* and **) on the sequence.

Supplemental Figure 3. CK1-mediated phosphorylation inhibits the fibrillization of S129A and S129E α-syn. ***A,*** ThT fluorescence measurements of CK1-phosphorylated S129A and ***B,*** S129E α-syn (100 μM) (white) and their unphosphorylated control (black). S129A and S129E α-syn were phosphorylated for 24 h with CK1 and then aggregated for the indicated length of time. Negatively stained TEM images of phosphorylated S129A and S129E and their unphosphorylated control after 12 h of incubation at 37°C under agitating conditions (scales bar 0.2 μm). ***C,*** ThT fluorescence measurements of CK1-phosphorylated S129E α-syn (20 μM). S129E was phosphorylated for 24 h with CK1 and then the different phosphorylated species were separated by RP-HPLC. S129E and S129E/S87-P samples were aggregated for the indicated length of time. Negatively stained TEM images of S129E and S129E/S87-P after 24 h of incubation at 37°C under agitating conditions (scales bar 0.2 μm).

Supplemental Figure 4. ***A***, Comparison of two-dimensional 1H -15N HSQC spectra of unphosphorylated WT (blue) and phosphorylated WT (red) α-syn. A dashed rectangle marks glutamine and asparagine side chain resonances. ***B,*** Comparison of 3J(HN,Hα) scalar couplings observed in nonphosphorylated WT (black), nonphosphorylated S129D (blue) and phosphorylated S129D (grey) α-syn at 15 °C. ***C,*** Comparison of 15N R1ρ spin relaxation rates

in nonphosphorylated WT (black) and phosphorylated S129D (grey). The domain organization of α-syn is shown on the top: basic N-terminal domain (red), hydrophobic NAC region (yellow), acidic C-terminal domain (blue) and the six repeats (green).

Supplemental Figure 5. *In vitro* phosphorylation of α-syn monomers and fibrils as a function of time by CK1. Samples were separated on a 12% SDS gel and probed with anti-α-syn (211, 1:500), anti-S129-P (1:5000) or anti-S87-P (1:100) α-syn antibodies.

Suppl. Figure 1

A

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Zebra-finch                    MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVSNVGGAVVTGVTAVAQKTVEGAGNIAAATGLVKKDQL 100
Canary                         MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVSNVGGAVVTGVTAVAQKTVEGAGNIAAATGLVKKDQL 100
Chicken                        MDVFMKGLNKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVSNVGGAVVTGVTAVAQKTVEGAGNIAAATGLVKKDQL 100
Mouse                          MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDQM 100
Rat                            MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDQM 100
Bovine                         MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGRTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGEAVVTGVTAVAQKTVEGAGSIAAATGFGKKDHM 100
Pig                            MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGEAVVTGVTAVAQKTVEGAGSIAAATGFGKKDQL 100
Bonobo                         MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Chimpanzee                     MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Lowland-gorilla                MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Human                          MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Rhesus-macaque                 MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFIKKDQL 100
Cynomolgus-monkey              MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFIKKDQL 100
Red-guenon                     MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Sumatran-orangutan             MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Common-woolly-monkey           MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTSVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDHS 100
Black-handed-spider-monkey     MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTSVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDHS 100
Red-chested-mustached-tamarin  MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVRKDHL 100
Fugu-rubripes                  MDAFMKGFSKAKDGVVAAAEKTKQGVTGAAEMTKDGVMFVGTKTKD----GVTVVAGKTVSGVSQVGGAMVTGVTAVAQKTVESAGSIAAATGLVKKE-P 95

Zebra-finch                    AKQNEEGFLQEGMVNNTGVAVDPENEAYEMPPEEEYQDYEPEA 143
Canary                         AKQNEEGFLQEGMVNNTGAAVDPDNEAYEMPPEEEYQDYEPEA 143
Chicken                        AKQNEEGFLQEGMVNNTDIPVDPENEAYEMPPEEEYQDYEPEA 143
Mouse                          GK-GEEGYPQEGILED--MPVDPGSEAYEMPSEEGYQDYEPEA 140
Rat                            GK-GEEGYPQEGILED--MPVDPSSEAYEMPSEEGYQDYEPEA 140
Bovine                         GK-GEEGASQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Pig                            GK-NEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Bonobo                         GK-NEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Chimpanzee                     GK-NEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Lowland-gorilla                GK-NEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Human                          GK-NEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Rhesus-macaque                 GK-NEEGAPQEGILQD--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Cynomolgus-monkey              GK-NEEGAPQEGILQD--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Red-guenon                     GK-NEEGAPQEGILQD--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Sumatran-orangutan             GK-NEEGATQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Common-woolly-monkey           GK-SEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Black-handed-spider-monkey     GK-SEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Red-chested-mustached-tamarin  GK-SEEGAPQEGILED--MPVDPDNEAYEMPSEEGYQDYEPEA 140
Fugu-rubripes                  GKQGDDAAAPENMAES-----PDVTDPAEATEEDADD----- 127
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B

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Human-α     MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVATVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGSIAAATGFVKKDQL 100
Mouse-α     MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDQM 100
Rat-α       MDVFMKGLSKAKEGVVAAAEKTKQGVAEAAGKTKEGVLYVGSKTKEGVVHGVTTVAEKTKEQVTNVGGAVVTGVTAVAQKTVEGAGNIAAATGFVKKDQM 100
Human-β     MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKTKEGVLYVGSKTREGVVQGVASVAEKTKEQASHLGGAVFS----------GAGNIAAATGLVKREEF 89
Mouse-β     MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKTKEGVLYVGSKT-SGVVQGVASVAEKTKEQASHLGGAVFS----------GAGNIAAATGLVKKEEF 88
Rat-β       MDVFMKGLSMAKEGVVAAAEKTKQGVTEAAEKTKEGVLYVGSKTKEGVVQGVASVAEKTKEQASHLGGAVFS----------GAGNIAAATGLVKKEEF 89
Human-γ     MDVFKKGFSIAKEGVVGAVEKTKQGVTEAAEKTKEGVMYVGAKTKENVVQSVTSVAEKTKEQANAVSEAVVSSVNTVATKTVEEAENIAVTSGVVRKEDL 100
Mouse-γ     MDVFKKGFSIAKEGVVGAVEKTKQGVTEAAEKTKEGVMYVGTKTKENVVQSVTSVAEKTKEQANAVSEAVVSSVNTVANKTVEEAENIVVTTGVVRKEDL 100
Rat-γ       MDVFKKGFSIAREGVVGAVEKTKQGVTEAAEKTKEGVMYVGTKTKGERGTSVTSVAEKTKEQANAVSEAVVSSVNTVATKTVEEAENIVVTTGVVRKEDL 100

Human-α     GK--NEEGAPQEGILED--MP-VDPDNEAYEMPSEEGYQDYEPEA--- 140
Mouse-α     GK--GEEGYPQEGILED--MP-VDPGSEAYEMPSEEGYQDYEPEA--- 140
Rat-α       GK--GEEGYPQEGILED--MP-VDPSSEAYEMPSEEGYQDYEPEA--- 140
Human-β     PTDLKPEEVAQEAAEEPLIEPLMEPEGESYEDPPQEEYQEYEPEA--- 134
Mouse-β     PTDLKPEEVAQEAAEEPLIEPLMEPEGESYEDSPQEEYQEYEPEA--- 133
Rat-β       PTDLKPEEVAQEAAEEPLIEPLMEPEGESYEDSPQEEYQEYEPEAKGP 137
Human-γ     RP----SAPQQE------------GEASKEKEEVAEEAQSGGD----- 127
Mouse-γ     EP----PAQDQ-------------EAKEQEE--NEEAKSGED----- 123
Rat-γ       EP----PAQDQ-------------EAKEQEE--GEEAKSGGD----- 123
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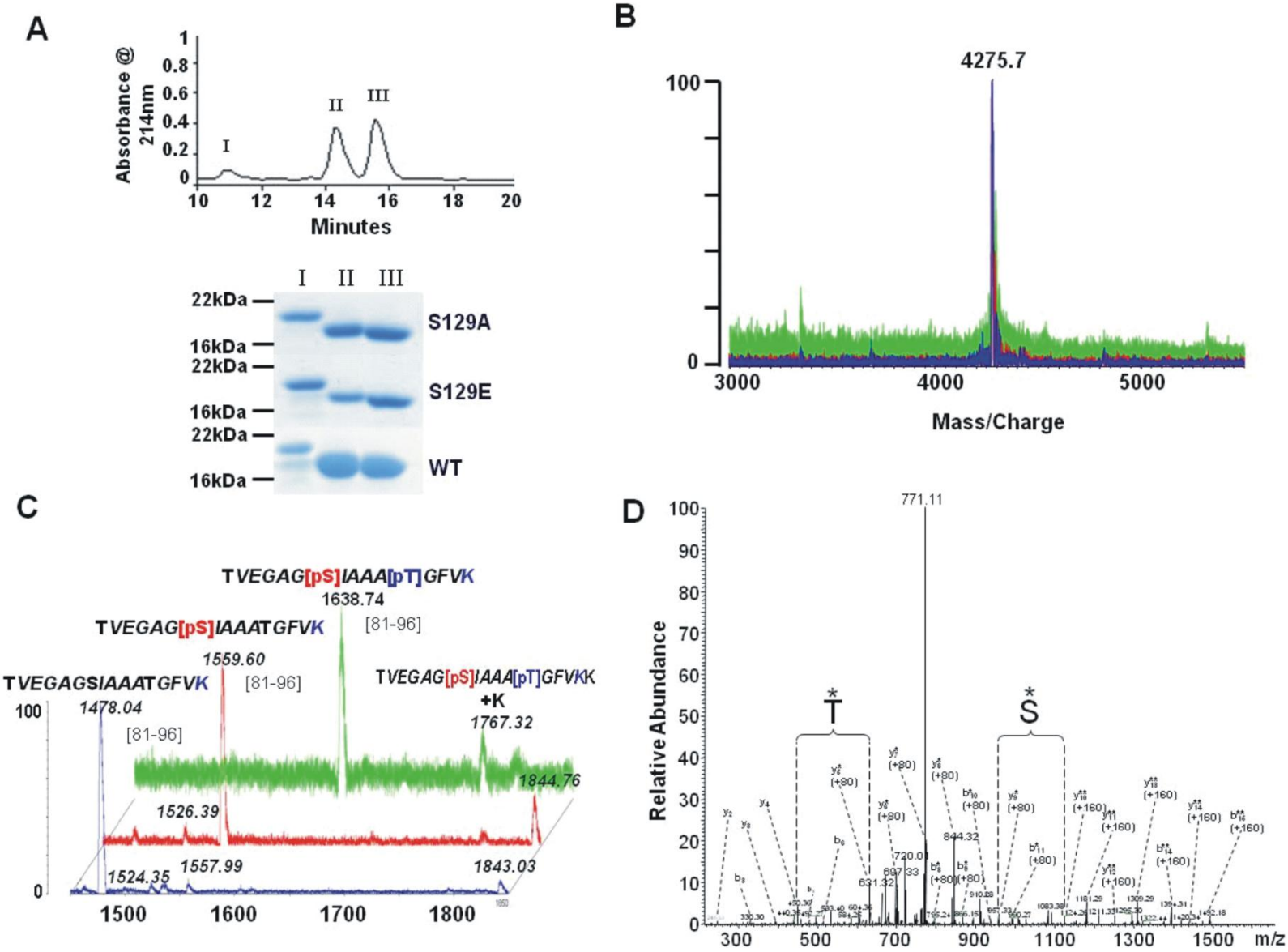
A
Absorbance @ 214nm
Minutes
I
II
III
22kDa
16kDa
S129A
S129E
WT
B
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Mass/Charge
C
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1638.74
[81-96]
TVEGAG[pS]IAAATGFVK
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1478.04
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D
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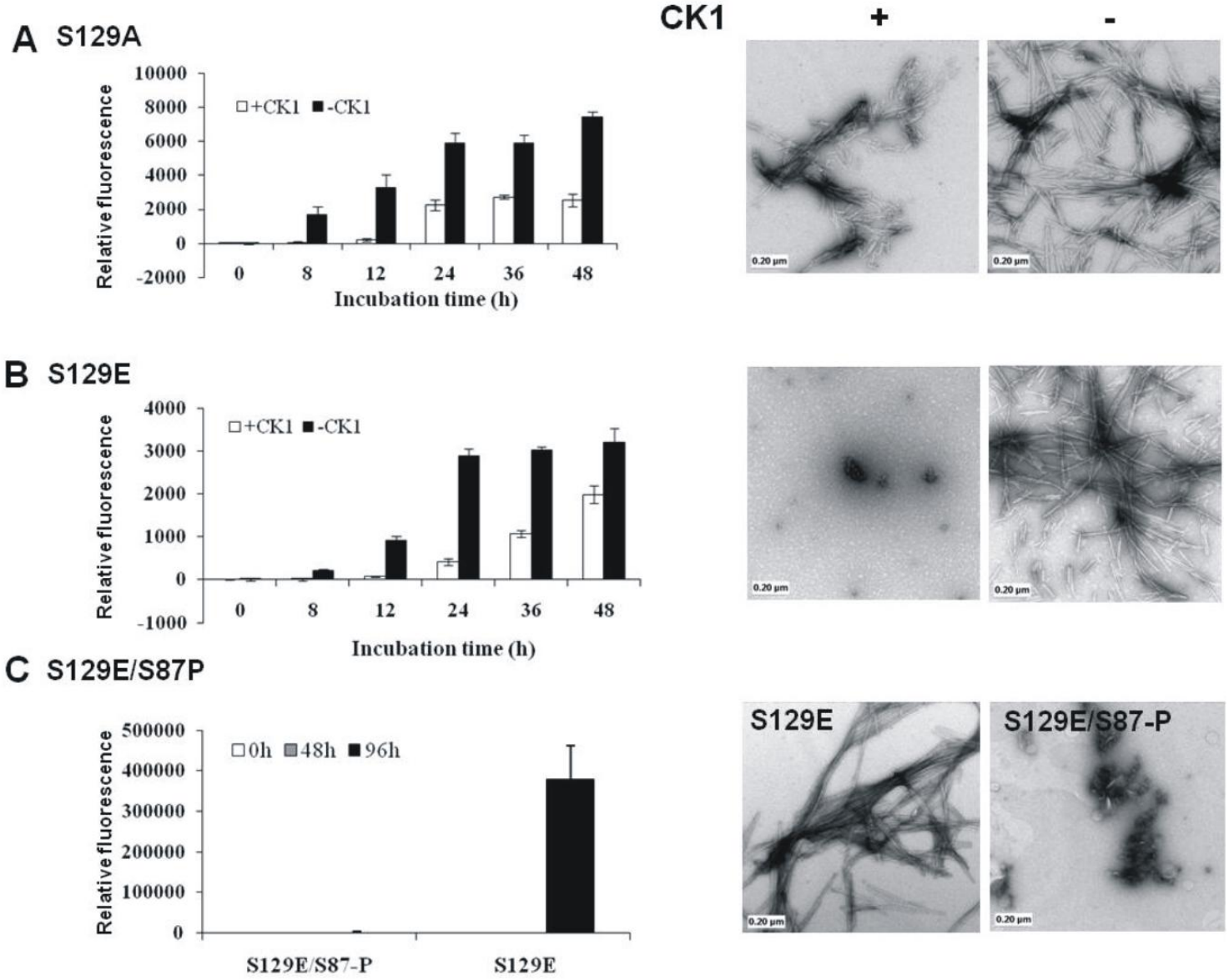
Relative Abundance
m/z


A S129A
Relative fluorescence
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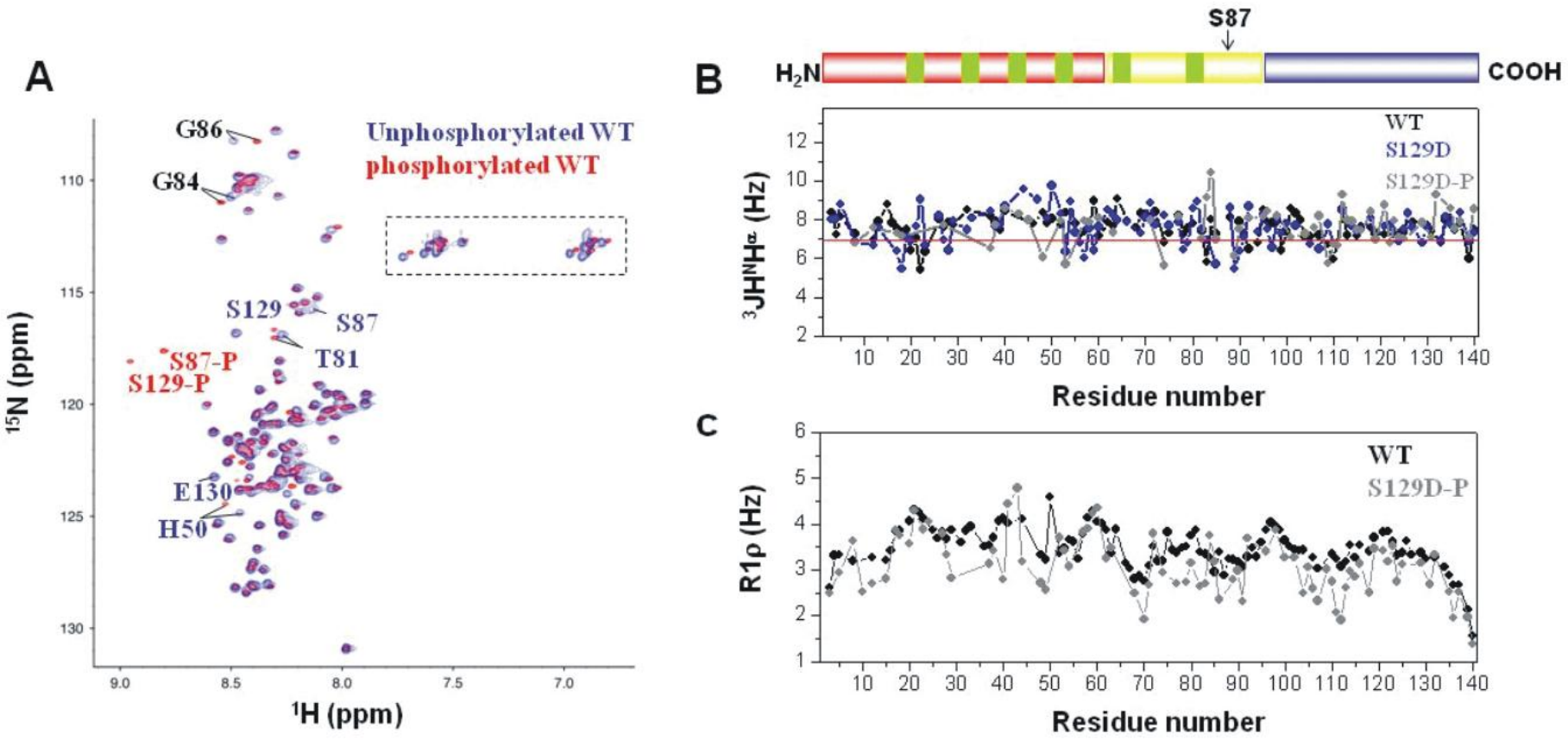
8000
6000
4000
2000
0
-2000
+CK1 -CK1
0
8
12
24
36
48
Incubation time (h)
B S129E
Relative fluorescence
4000
3000
2000
1000
0
-1000
+CK1 -CK1
0
8
12
24
36
48
Incubation time (h)
C S129E/S87P
Relative fluorescence
500000
400000
300000
200000
100000
0
0h 48h 96h
S129E/S87-P
S129E
CK1
+
-
0.20 μm
S129E
S129E/S87-P

Suppl. Figure 4



Suppl. Figure 5