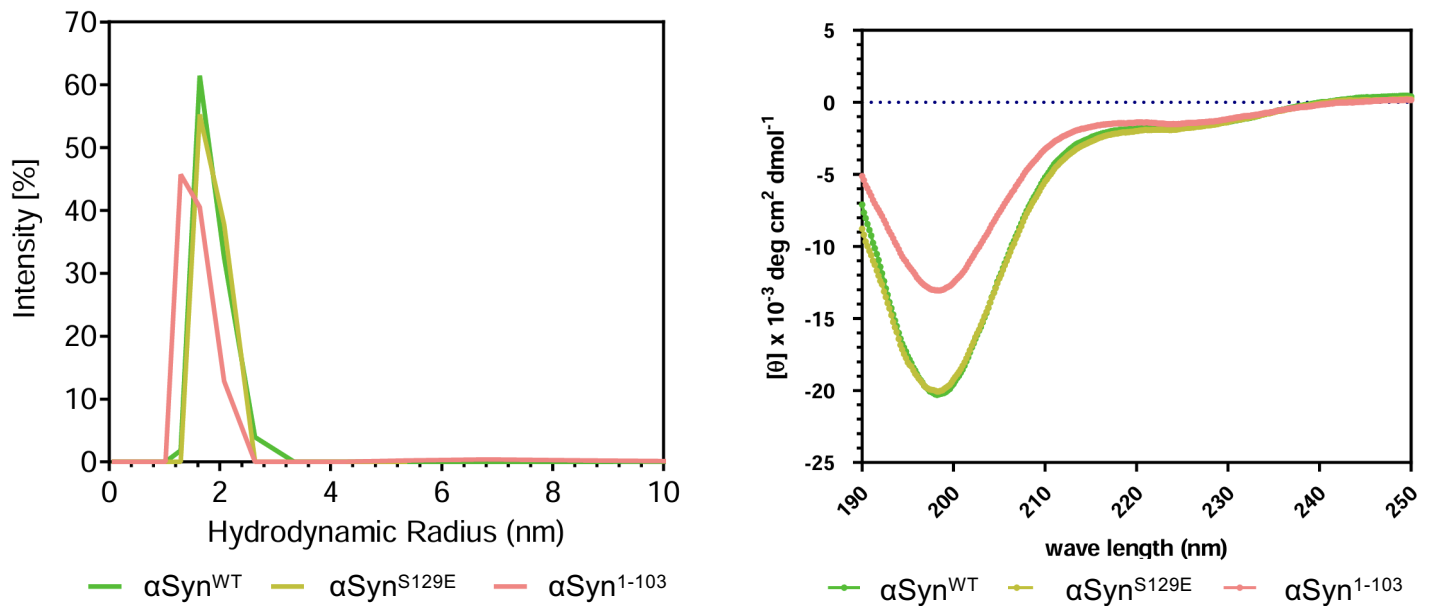


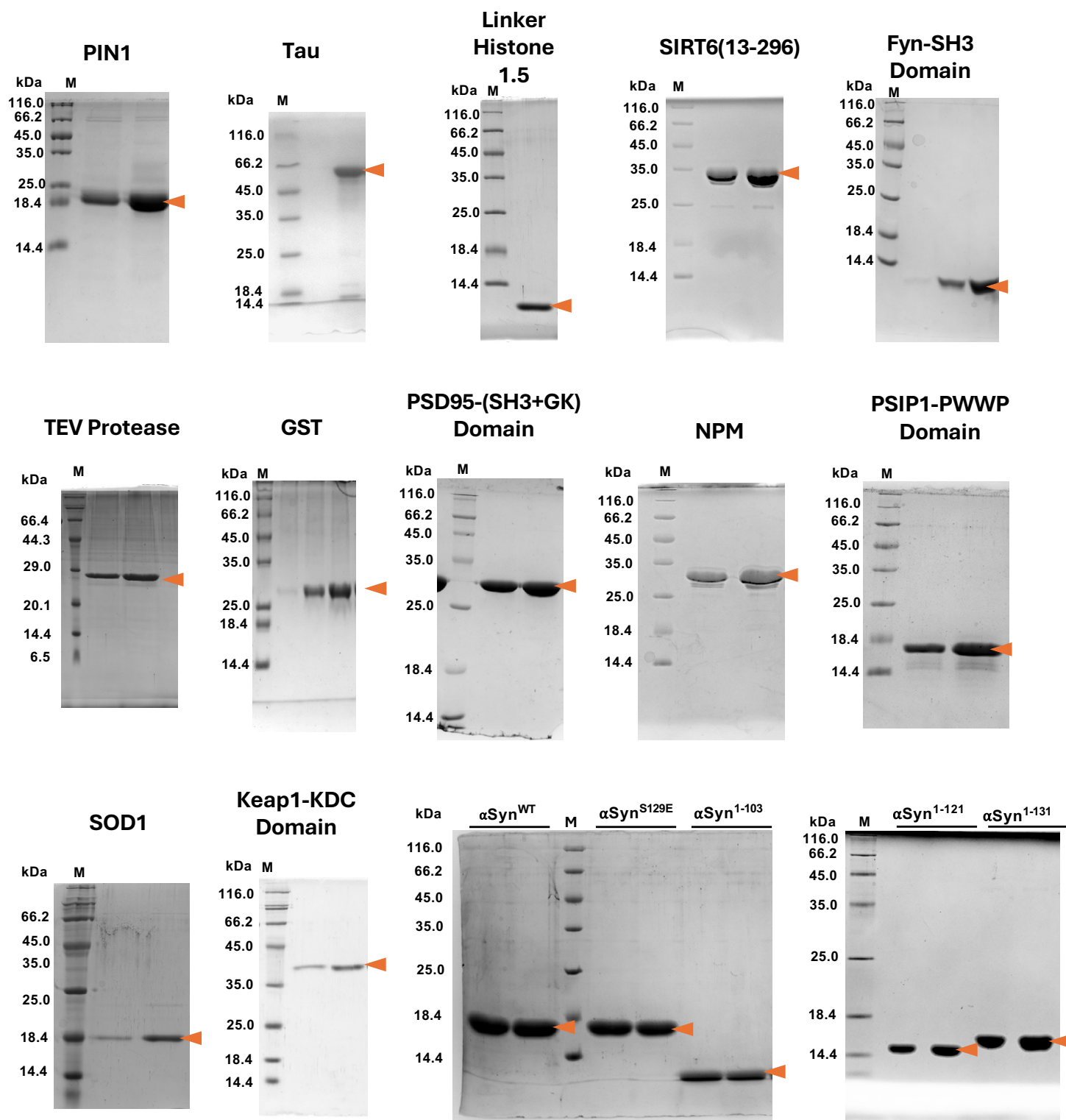
Parkinson's disease-specific α -Synuclein variants potentially drive Lewy body formation by engaging in promiscuous and non- functional interactions

Sneha Jos¹, Niharika Shivanandaswamy¹, Akshya Sharma², Thazhe Kootteri Prasad³,
Rachana Kashyap⁴, Neelagandan Kamariah³, Deepak Sharma², M M Srinivas Bharath⁵,
Sangeeta Nath⁴, Balasundaram Padmanabhan¹, Sivaraman Padavattan^{1#}

Supplementary Figures 1-10



Supplementary Figure 1: Characterization of α Syn proteins by circular dichroism (CD) and dynamic light scattering (DLS). (A) Dynamic light scattering (DLS) analysis performed on the day of purification (day 0) shows that the proteins exist in a monomeric state, with measured hydrodynamic radii (R_h) of 2.1 nm for α Syn^{WT}, 2.2 nm for α Syn^{S129E}, and 1.8 nm for α Syn¹⁻¹⁰³. (B) Far-UV CD spectra of α Syn^{WT}, α Syn^{S129E}, and C-terminally truncated α Syn¹⁻¹⁰³ confirm that all three proteins predominantly adopt a random coil conformation.



Supplementary Figure 2: SDS-PAGE analysis of purified cellular proteins. α Syn and other purified cellular proteins were resolved by SDS-PAGE. Molecular weight markers (M) are shown alongside each sample. Protein of interest are indicated with an arrow (orange).

α -Synuclein¹⁻¹⁰³-104C binding affinity analysis

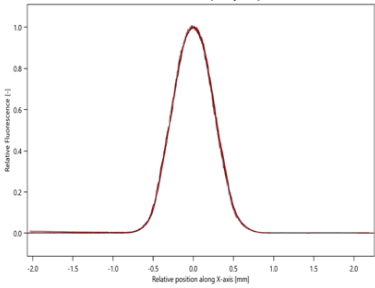
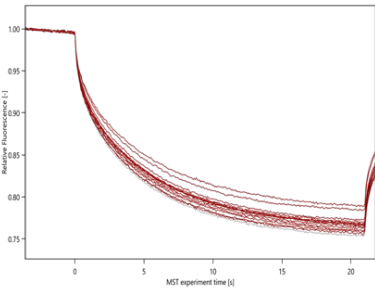
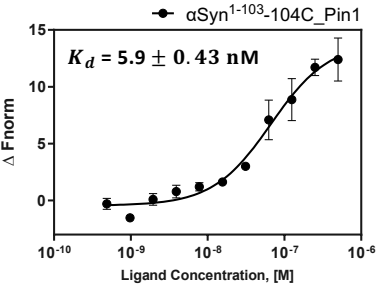
CELLULAR PROTEINS

MST BINDING CURVE

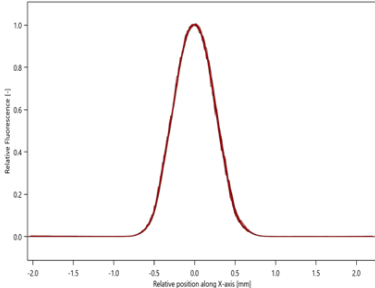
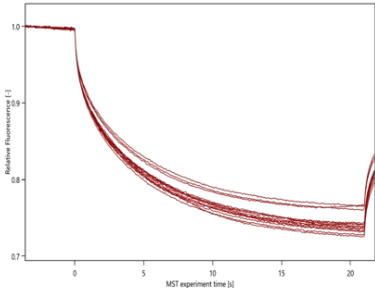
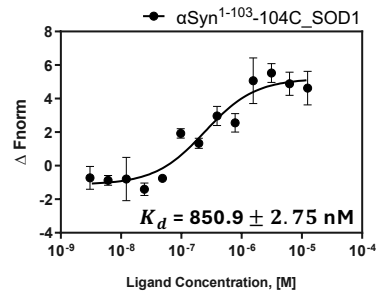
MST TRACES

MST CAPILLARY SCAN

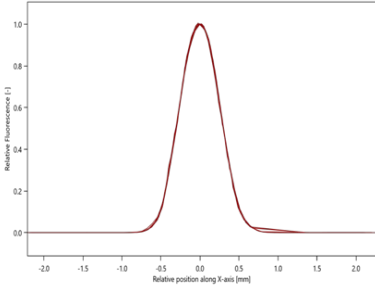
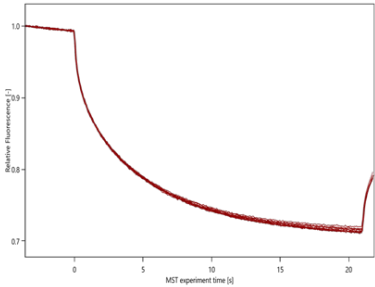
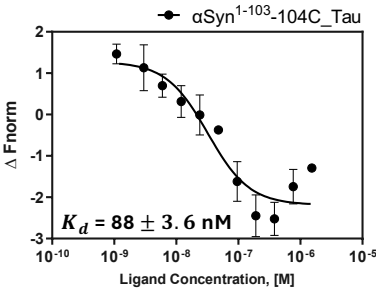
Pin1



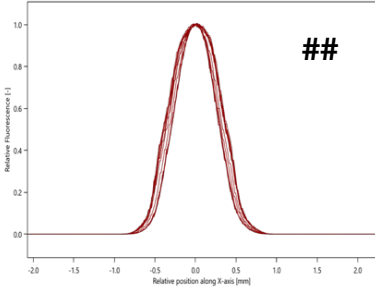
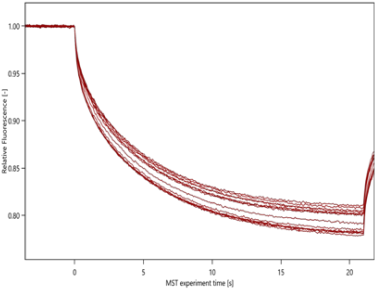
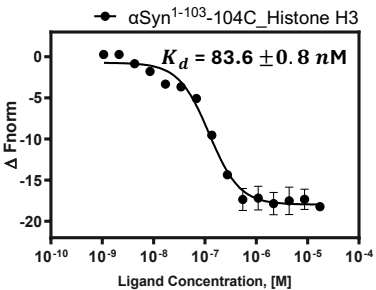
SOD1



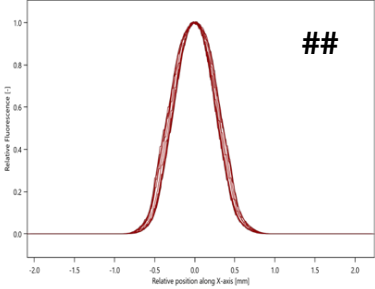
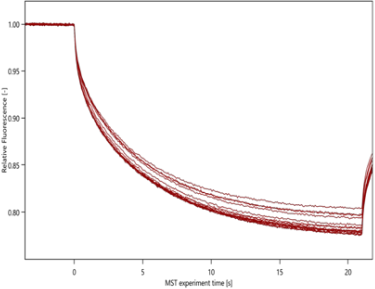
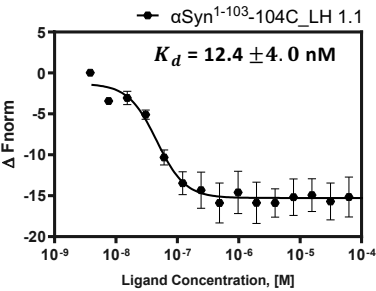
Tau



Histone H3



Linker
Histone H1.1



##- Moderate Adsorption to capillary

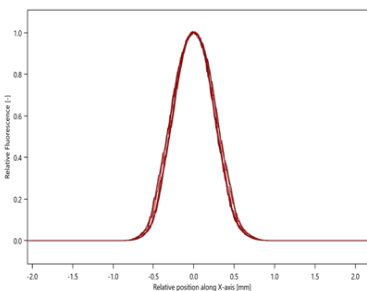
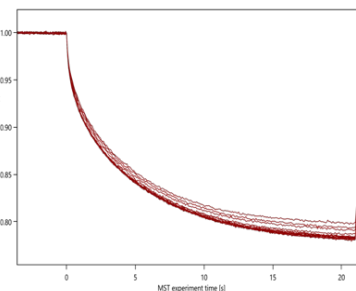
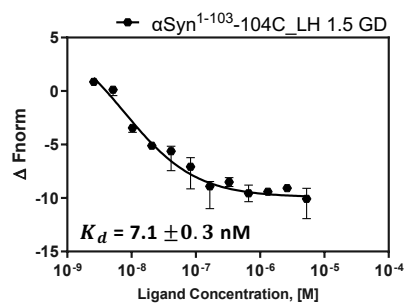
CELLULAR PROTEINS

MST BINDING CURVE

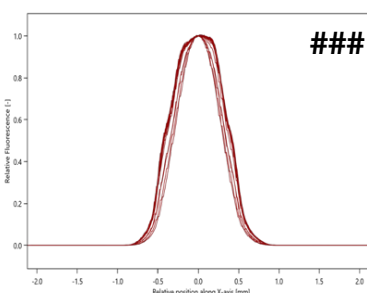
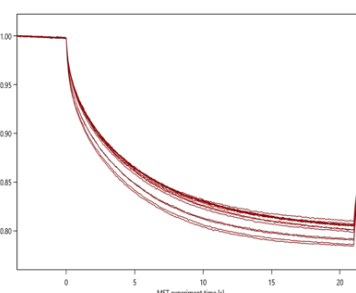
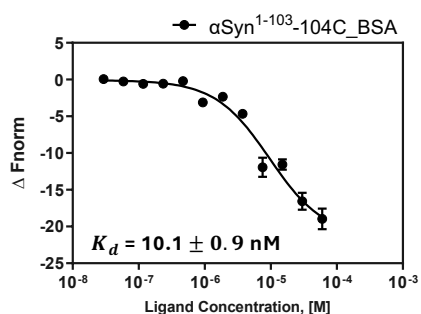
MST TRACES

MST CAPILLARY SCAN

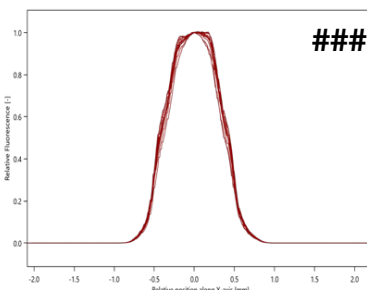
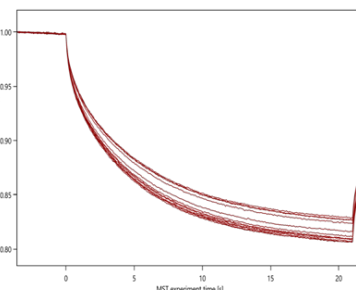
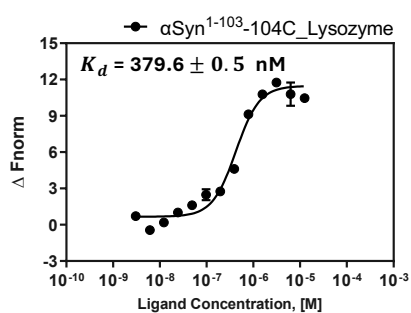
Linker
Histone H1.5



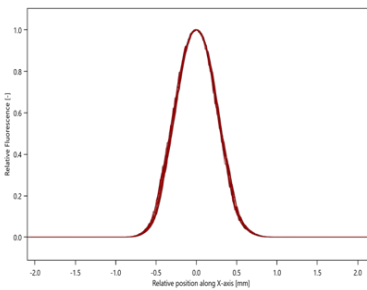
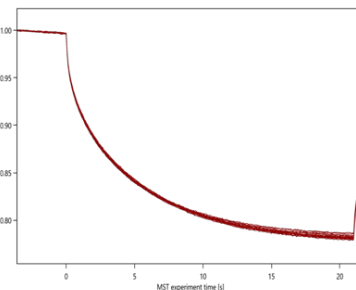
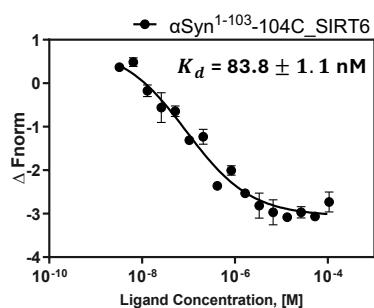
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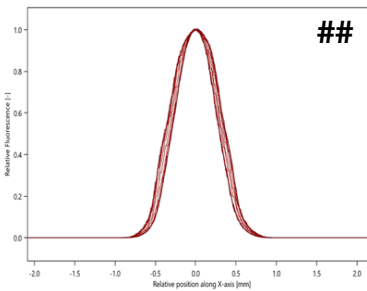
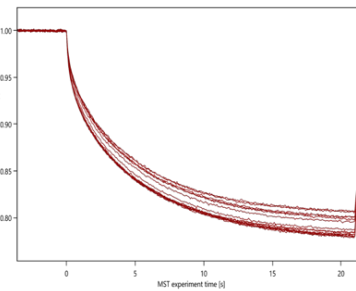
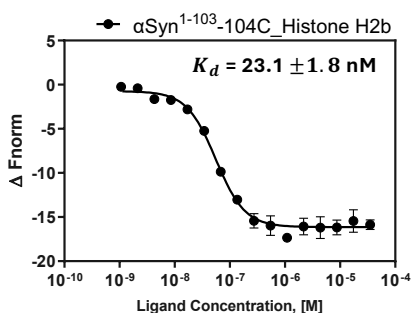
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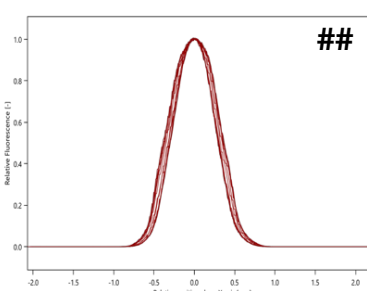
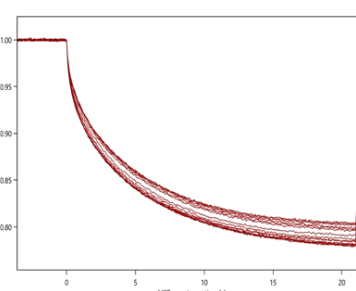
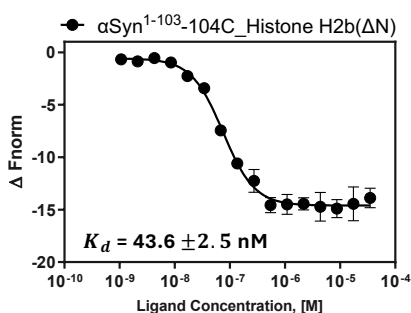
SIRT6



Histone H2b



Histone H2b(ΔN)



##- Moderate Adsorption to capillary

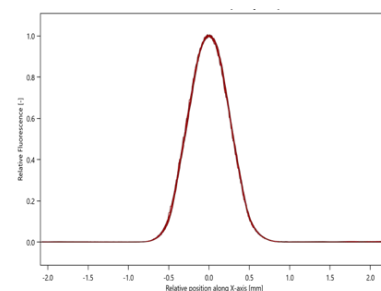
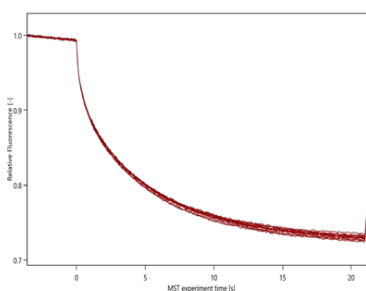
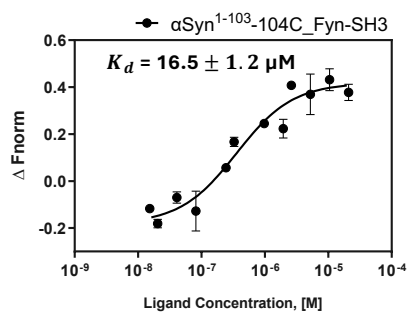
###- High Adsorption to capillary

CELLULAR PROTEINS

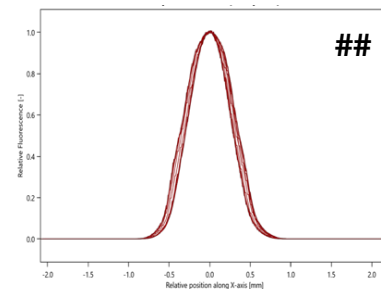
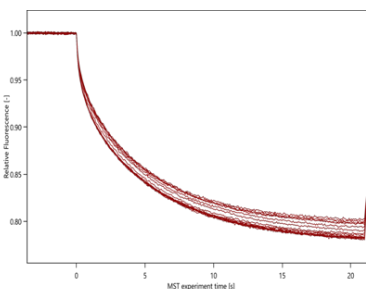
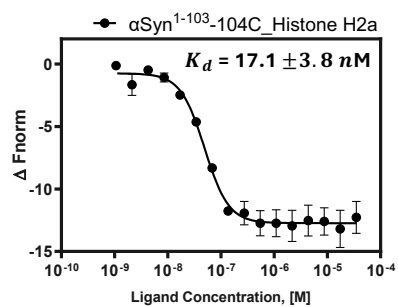
MST BINDING CURVE

MST TRACES

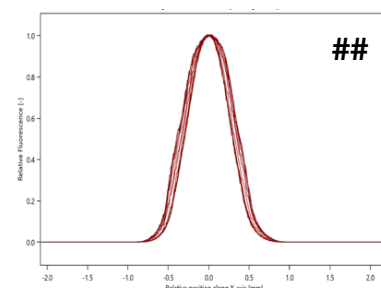
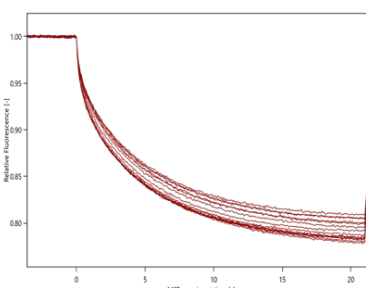
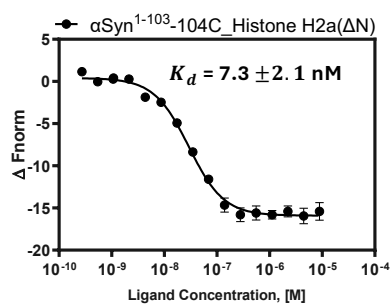
MST CAPILLARY SCAN

Fyn-SH3
Domain

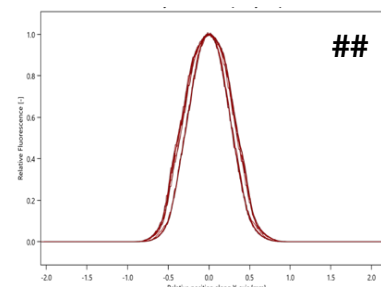
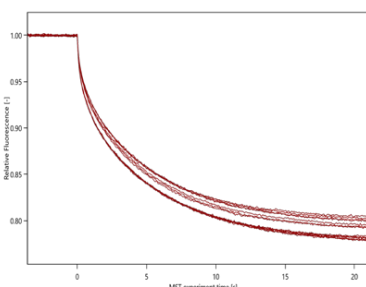
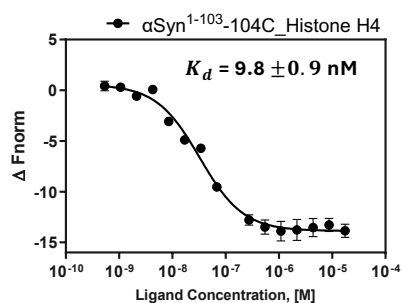
Histone H2a



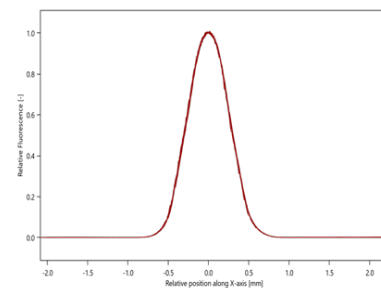
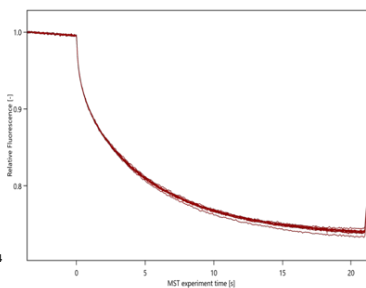
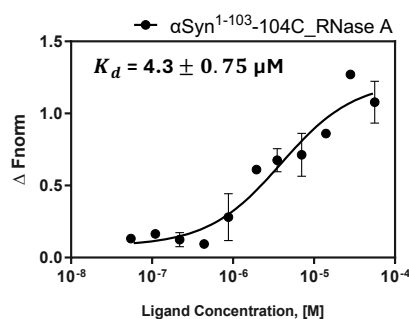
Histone H2a(ΔN)



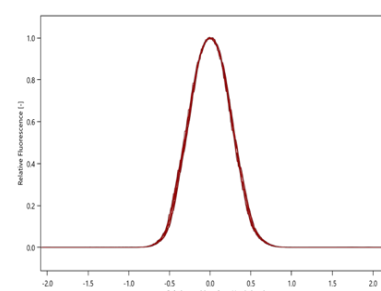
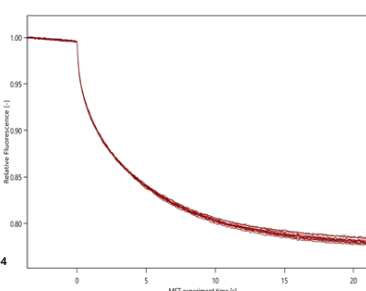
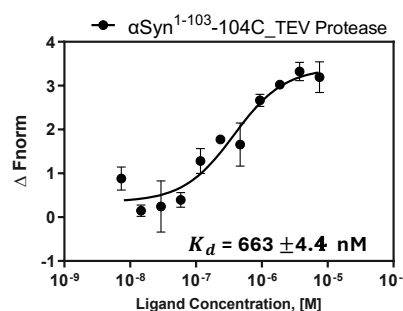
Histone H4



RNase



TEV Protease



##- Moderate Adsorption to capillary

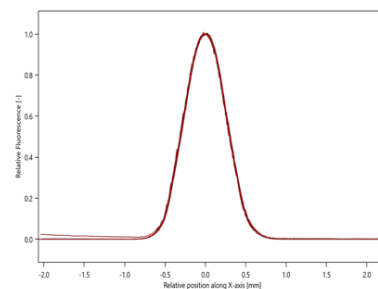
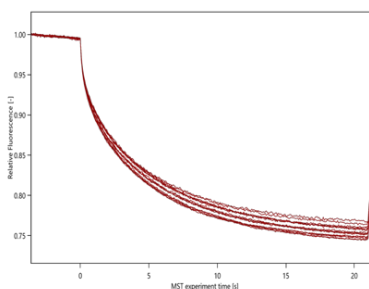
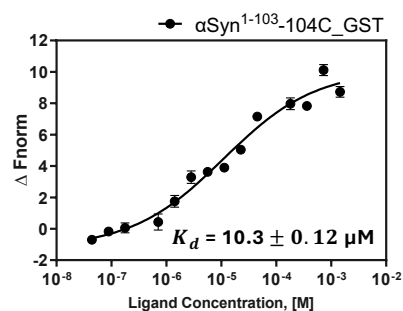
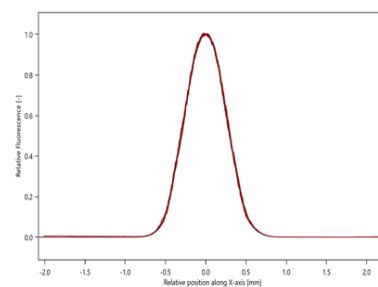
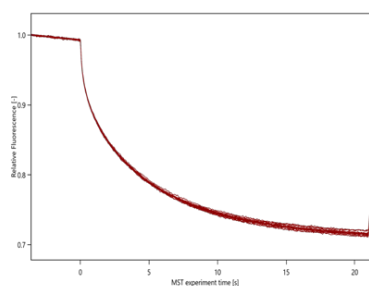
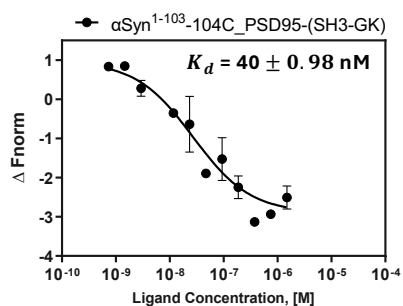
CELLULAR PROTEINS

MST BINDING CURVE

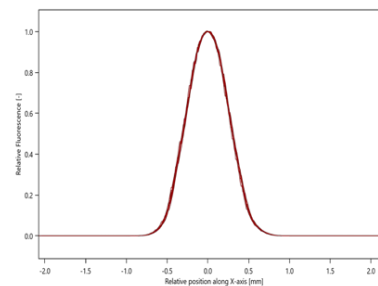
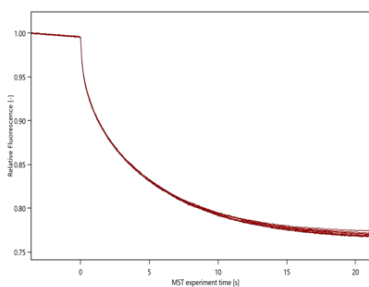
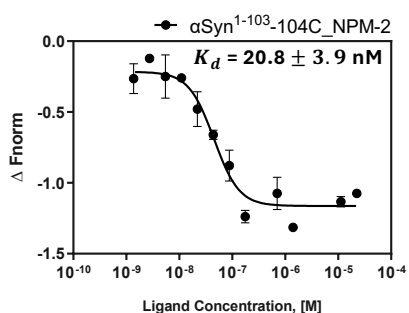
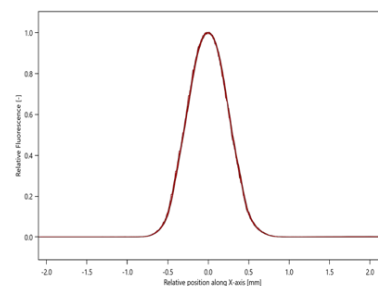
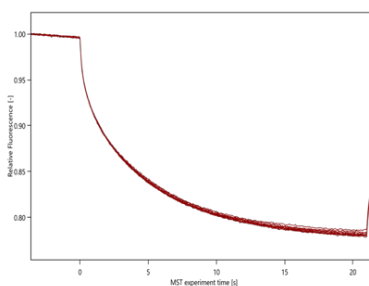
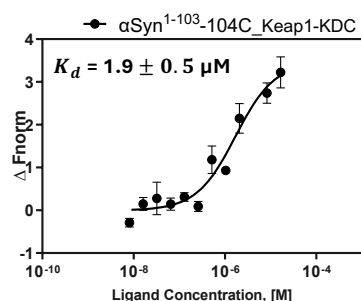
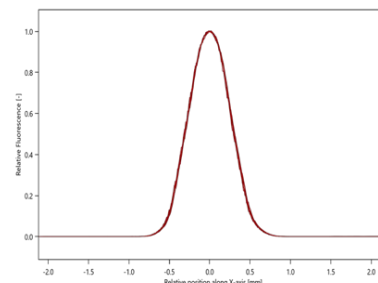
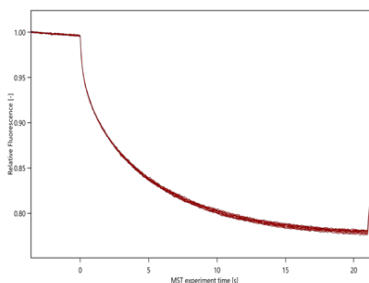
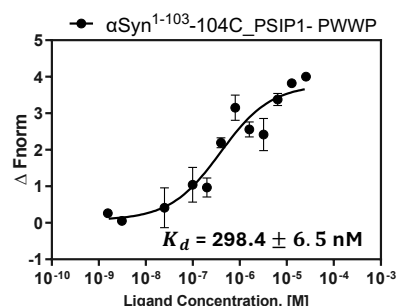
MST TRACES

MST CAPILLARY SCAN

GST

PSD95-
(SH3+GK)
Domain

NPM-2

Keap1_KDC
DomainPSIP1- PWWP
Domain

Supplementary Figure 3: Binding affinity analysis of C-terminal truncated $\alpha\text{Syn}^{1-103}\text{-104C}$ with various cellular proteins. Microscale thermophoresis (MST) binding curves (Right) showing mean K_d values with SD ($n = 3$) for $\alpha\text{Syn}^{1-103}\text{-104C}$ with various cellular proteins. Capillary scans (Left) reveal the presence or absence of protein adsorption to the capillary walls. Adsorption level is indicated using #. Thermophoretic MST traces (Middle), indicate no aggregation in solution. All capillary scan and MST trace plots represent raw output directly generated by the instrument software. Error bar represents SD ($n = 3$).

α -Synuclein^{WT} binding affinity analysis

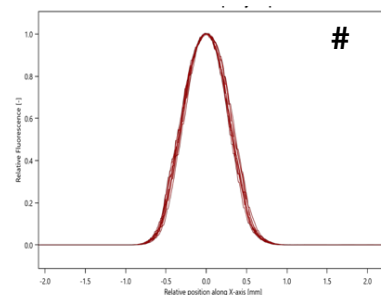
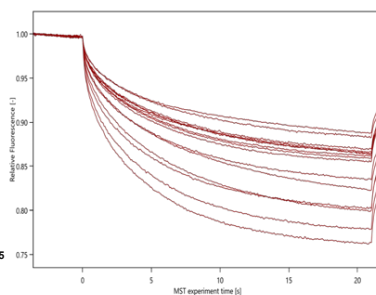
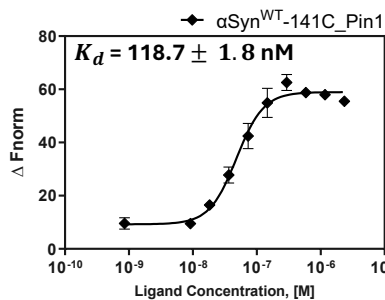
CELLULAR PROTEINS

MST BINDING CURVE

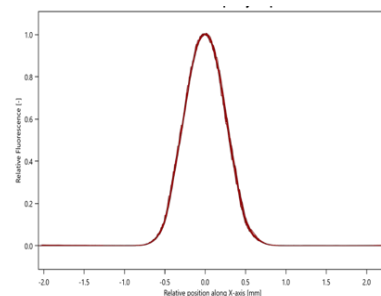
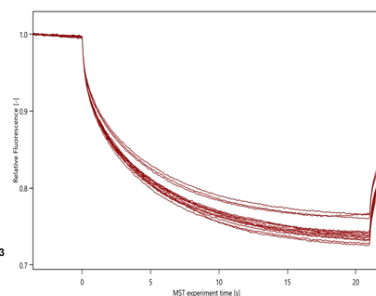
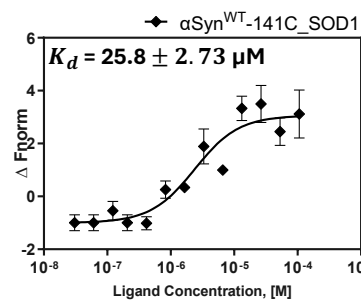
MST TRACES

MST CAPILLARY SCAN

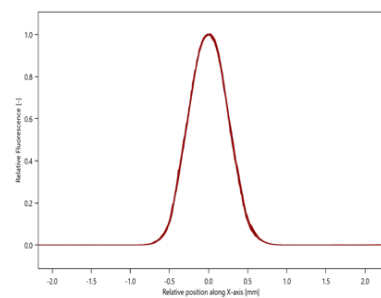
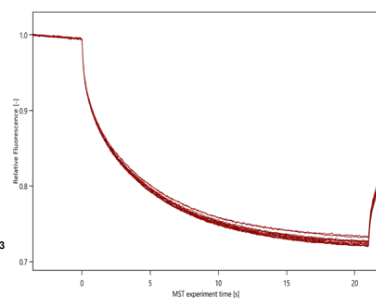
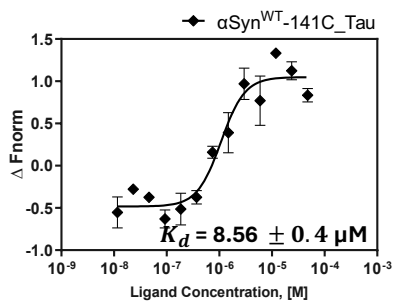
Pin1



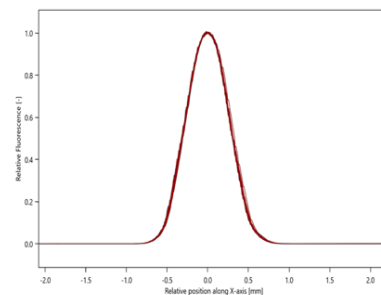
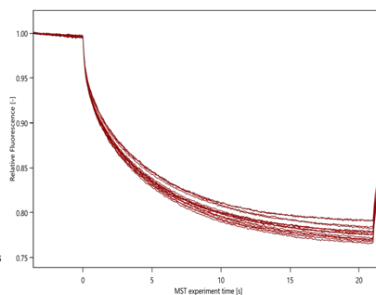
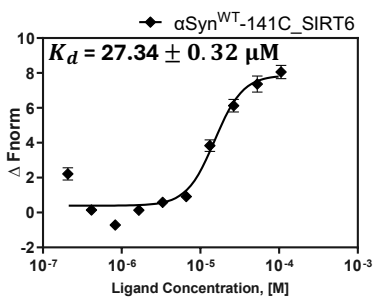
SOD1



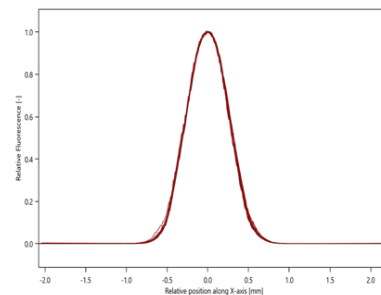
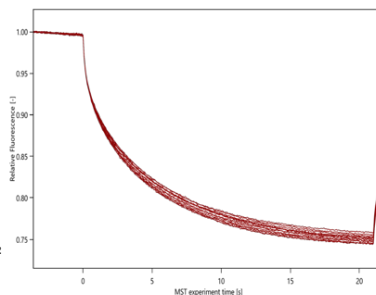
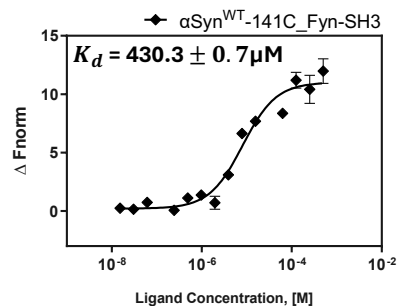
Tau



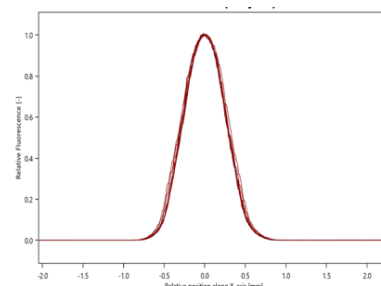
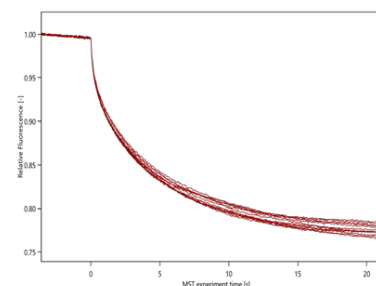
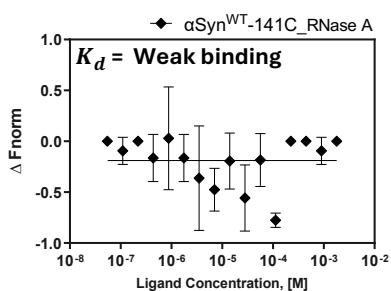
SIRT6



Fyn-SH3
Domain



RNase



#- Slight Adsorption to capillary

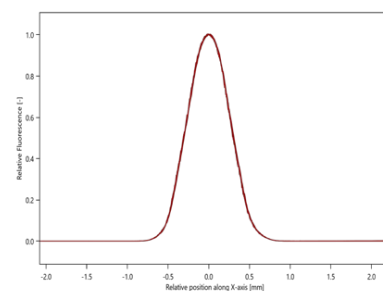
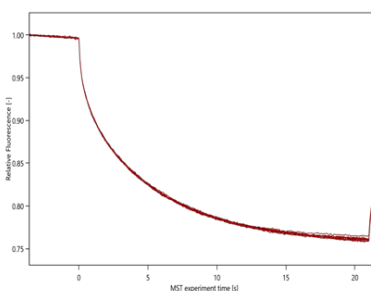
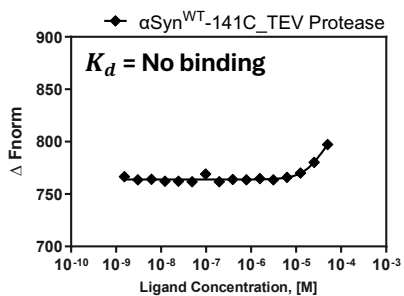
CELLULAR PROTEINS

MST BINDING CURVE

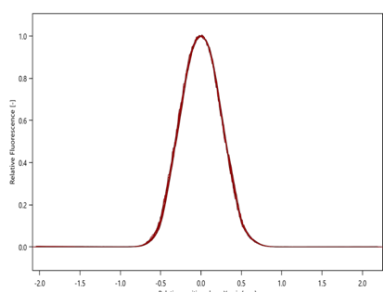
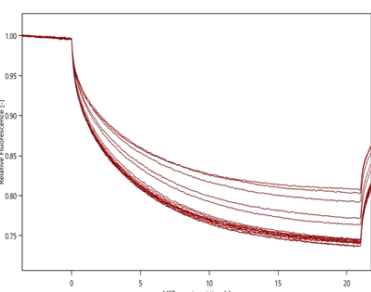
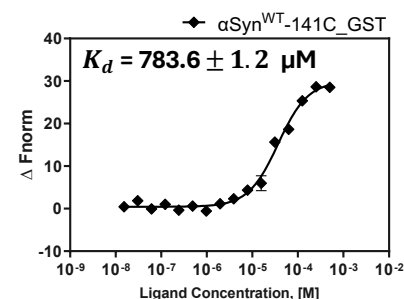
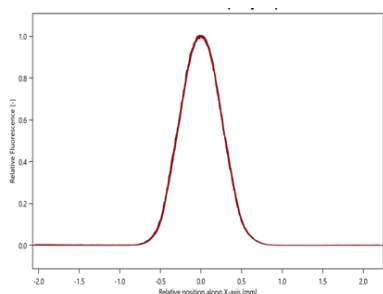
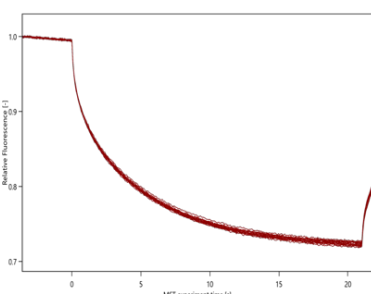
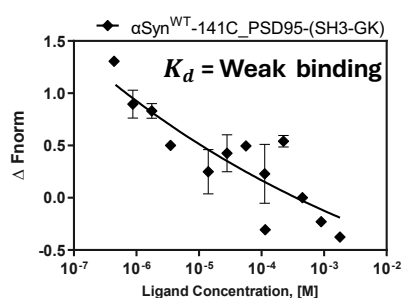
MST TRACES

MST CAPILLARY SCAN

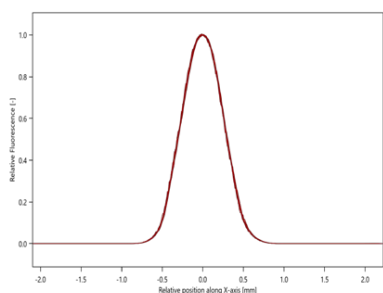
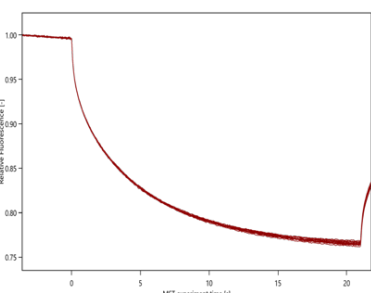
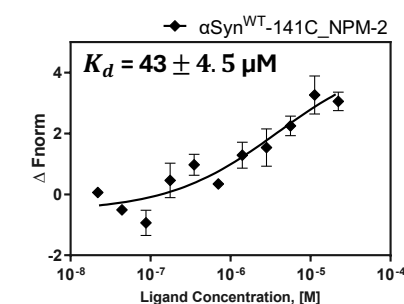
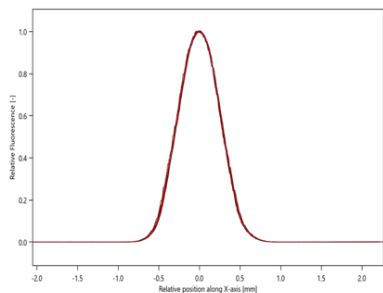
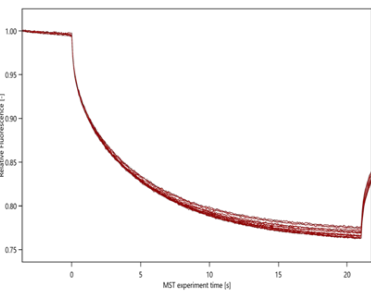
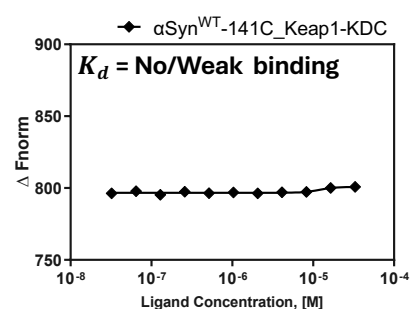
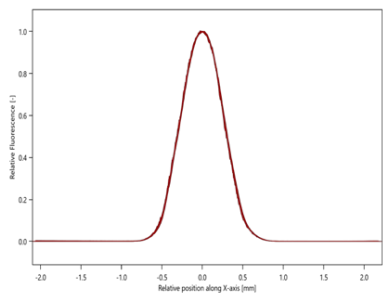
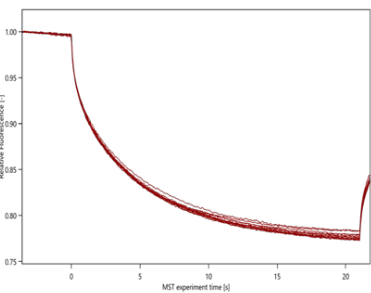
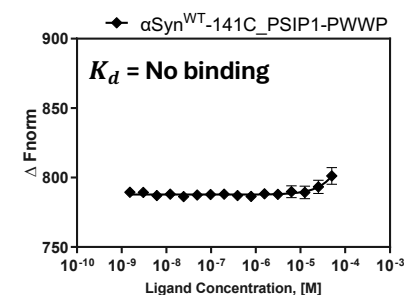
TEV Protease



GST

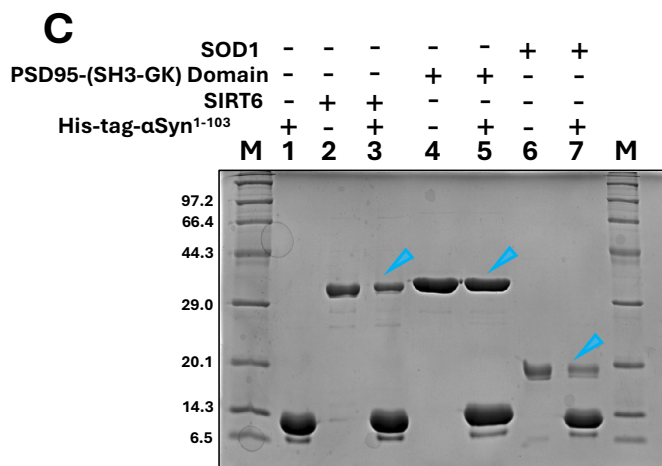
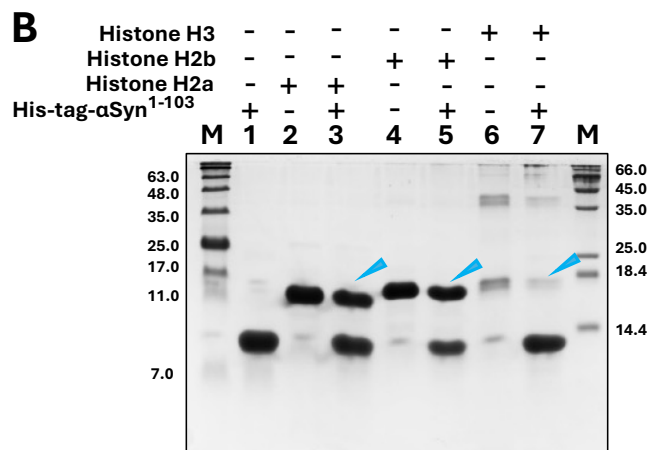
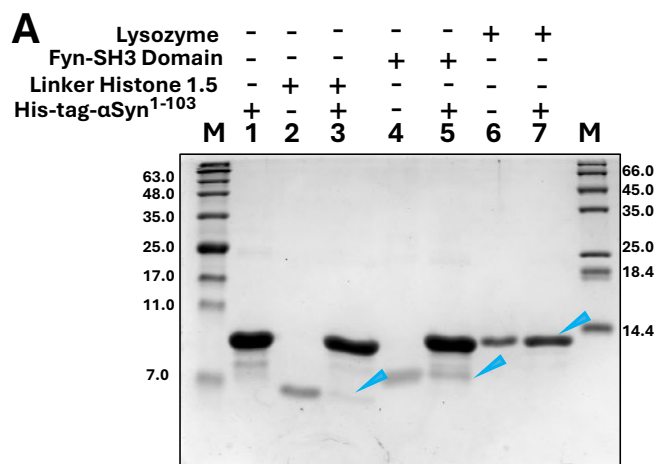
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(SH3+GK)
Domain

NPM-2

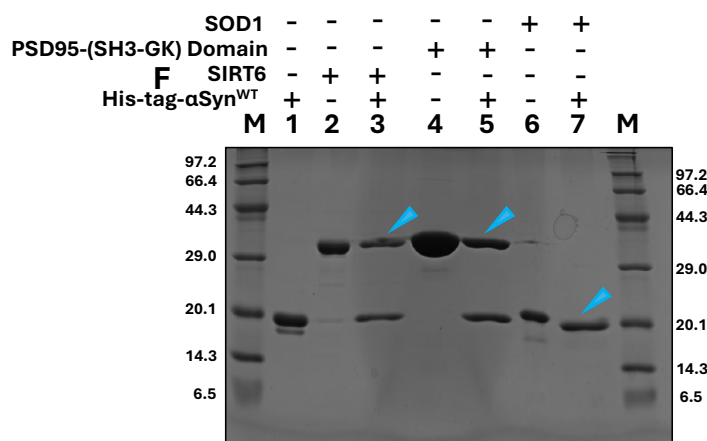
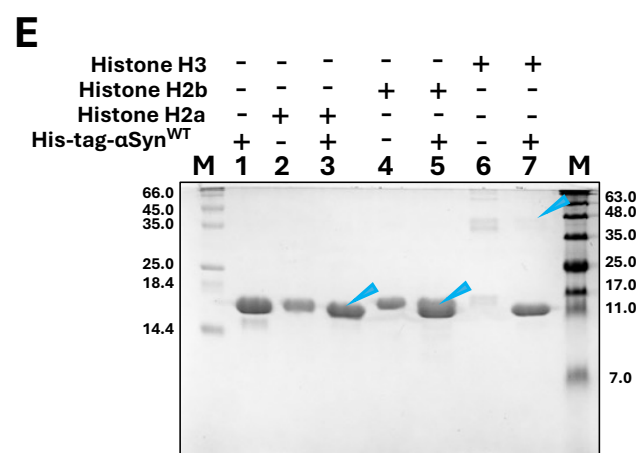
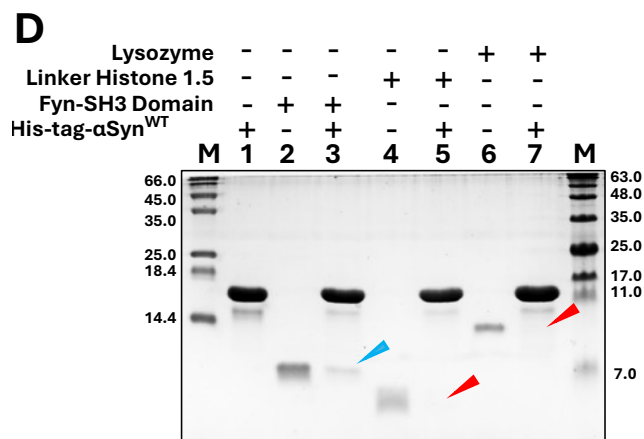
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DomainPSIP1- PWWP
Domain

Supplementary Figure 4: Binding affinity analysis of αSyn^{WT} with various cellular proteins. Microscale thermophoresis (MST) binding curves (Right) showing individual K_d values with SD ($n = 3$) for αSyn^{WT}-141C with various cellular proteins. Capillary scans (Left) reveal the presence or absence of protein adsorption to the capillary walls. Adsorption level is indicated using #. Thermophoretic MST traces (Middle), indicate no aggregation in solution. All capillary scan and MST trace plots represent raw output directly generated by the instrument software. Error bar represents SD ($n = 3$).

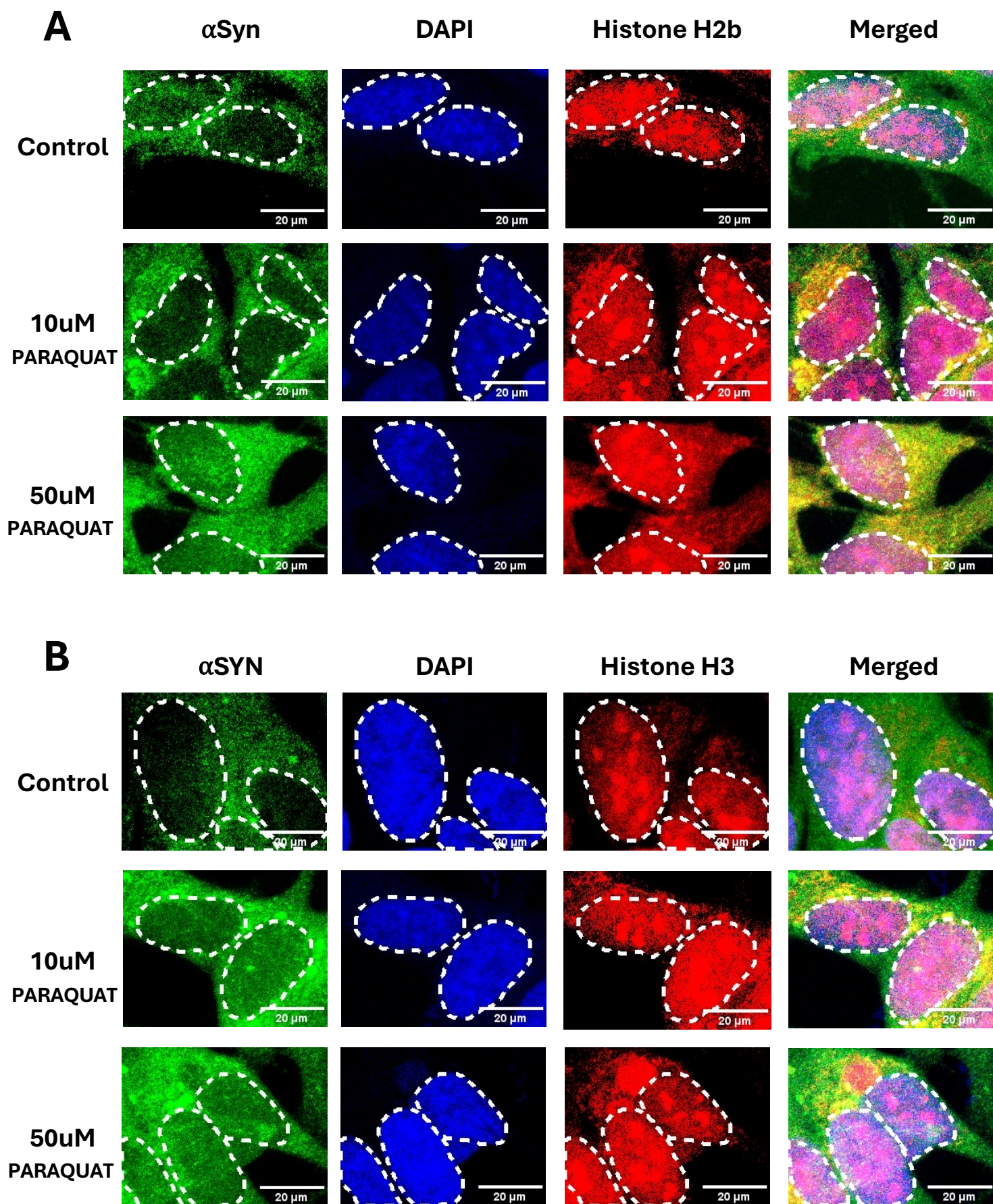
Pull down assay with αSyn^{1-103}



Pull down assay with $\alpha\text{Syn}^{\text{WT}}$



Supplementary Figure 5: Pull-down assay comparing interactions of αSyn^{1-103} and $\alpha\text{Syn}^{\text{WT}}$ with cellular proteins and analysed using SDS-PAGE. Both αSyn variants were incubated with an identical panel of proteins lysozyme, Fyn-SH3, Linker Histone 1.5-globular domain, core histones (H2A, H2B, H3), SIRT6, PSD-95(SH3-GK), and SOD1, and the bound fractions were analysed by SDS-PAGE. **(A-C)** Pull-down gels showing interactions of αSyn^{1-103} with the respective proteins. All tested proteins displayed detectable binding (blue arrows). Lysozyme could not be visualized due to its molecular weight overlapping with αSyn^{1-103} on SDS-PAGE. **(D-F)** Pull-down gels showing interactions of $\alpha\text{Syn}^{\text{WT}}$ with the same set of cellular proteins. Linker Histone 1.5-globular domain and lysozyme did not exhibit detectable binding (red arrows), whereas Fyn-SH3 displayed weak interaction consistent with MST results. Core histone H2A and SOD1 bands overlapped with $\alpha\text{Syn}^{\text{WT}}$ and could not be resolved. SIRT6 and PSD-95(SH3-GK) showed clear binding (blue arrows). Protein marker lanes (M) are included for reference.



Supplementary Figure 6: Subcellular localization of endogenous α Syn in SH-SY5Y cells visualized by confocal microscopy. Representative images show α Syn (green) co-immunostained with antibodies against (A) histone H2B (red) and (B) histone H3 (red), along with DAPI nuclear staining (blue). Images include both untreated cells and those exposed to 10 μ M and 50 μ M Paraquat. Merged channels are shown. Scale bar: 10 μ m.

α -Synuclein^{S129E} binding affinity analysis

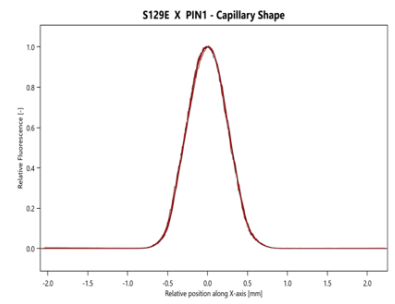
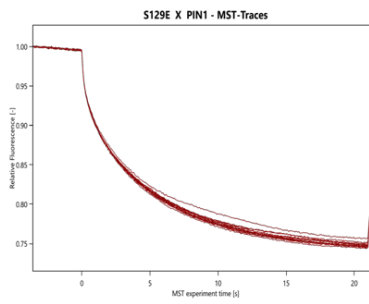
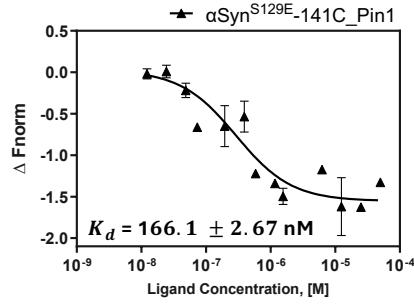
CELLULAR PROTEINS

MST BINDING CURVE

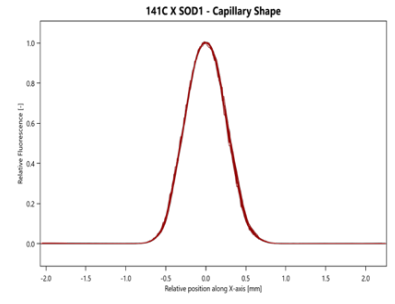
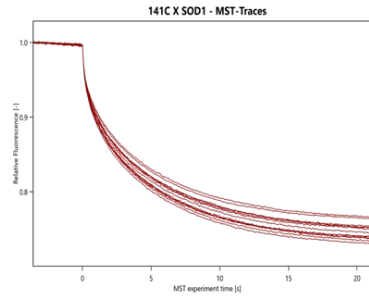
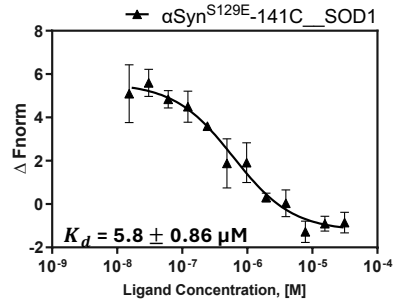
MST TRACES

MST CAPILLARY SCAN

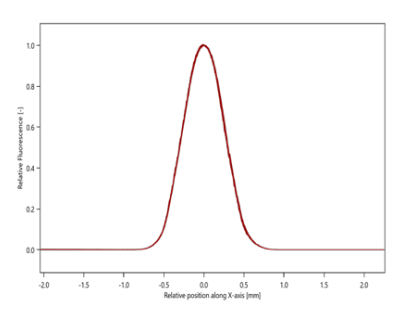
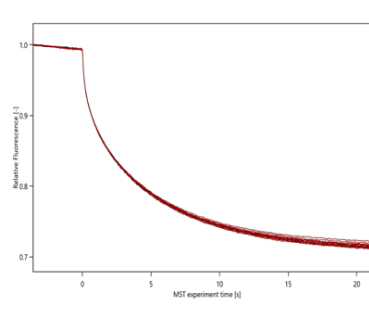
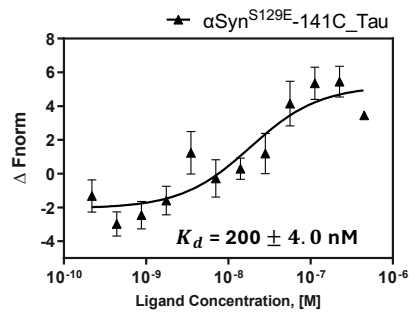
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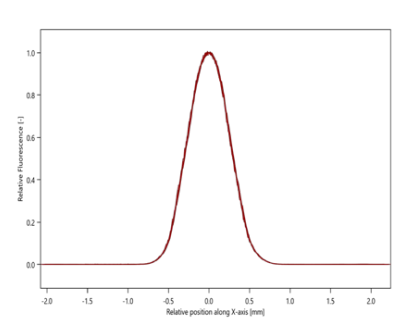
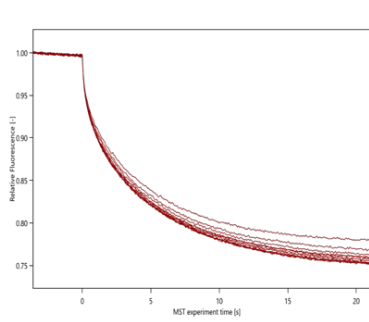
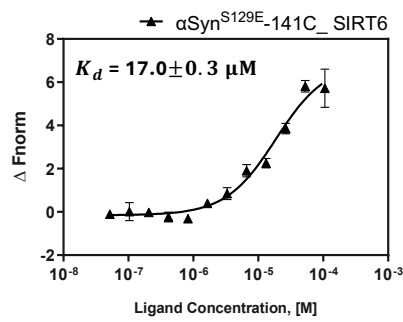
SOD1



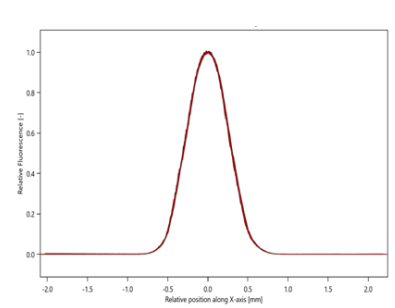
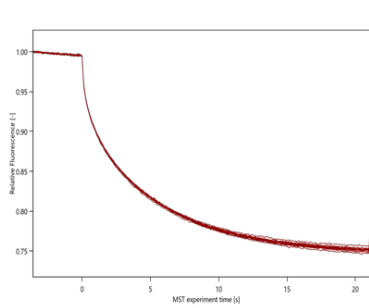
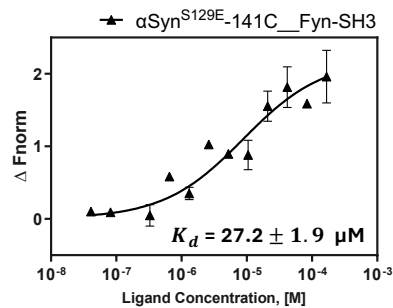
Tau



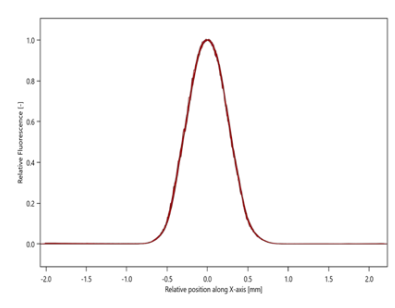
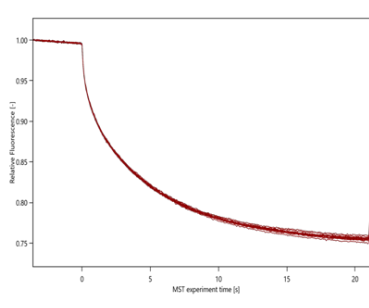
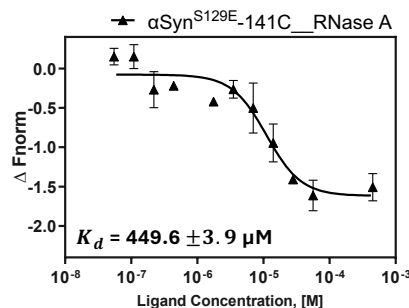
SIRT6



Fyn-SH3
Domain



RNase



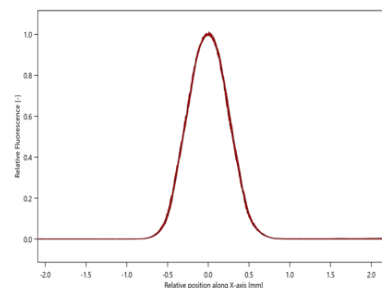
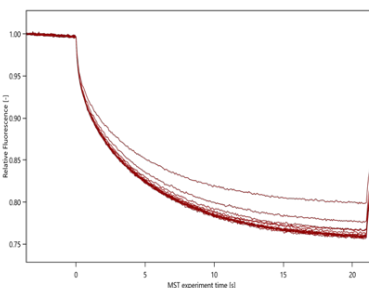
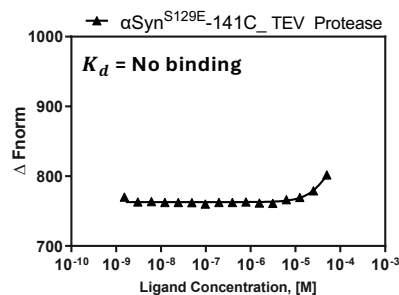
CELLULAR PROTEINS

MST BINDING CURVE

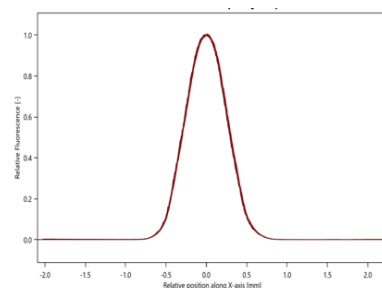
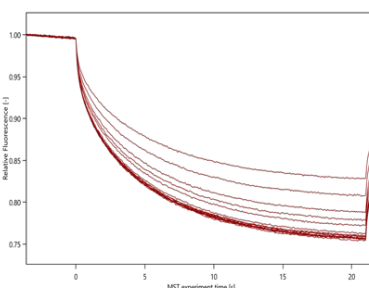
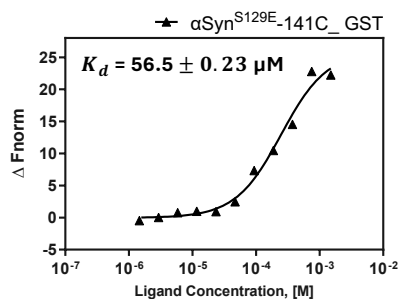
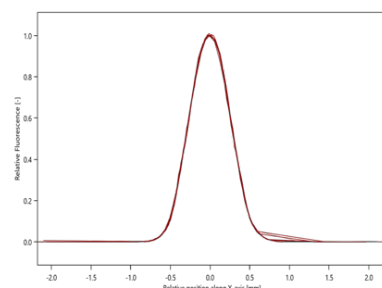
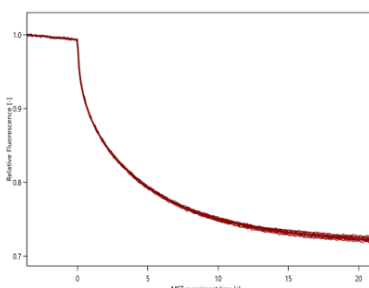
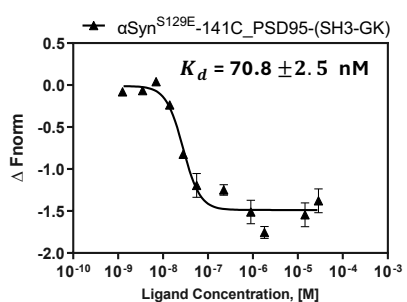
MST TRACES

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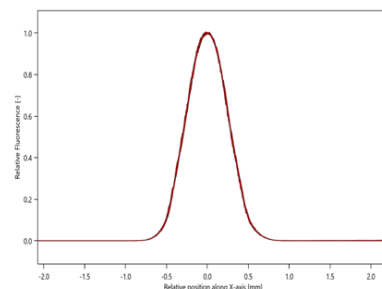
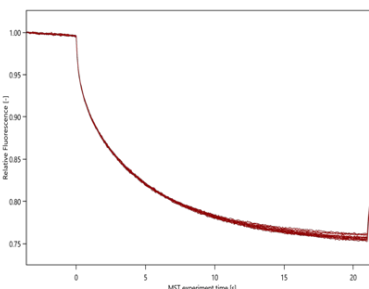
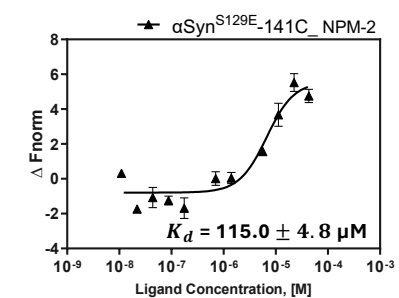
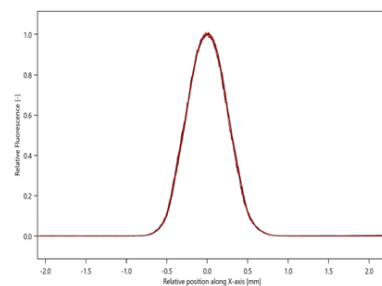
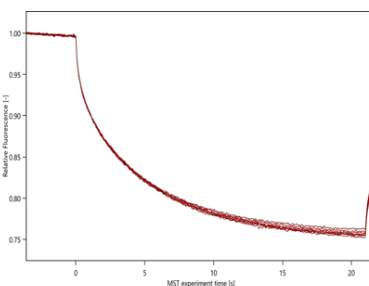
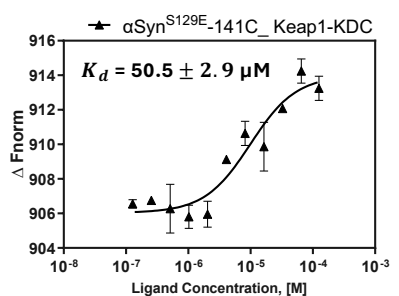
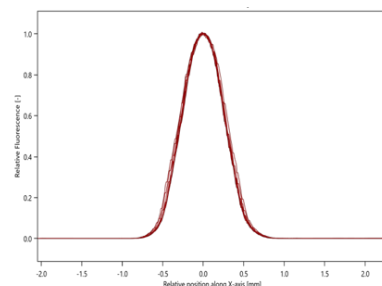
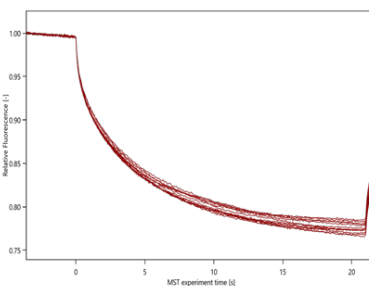
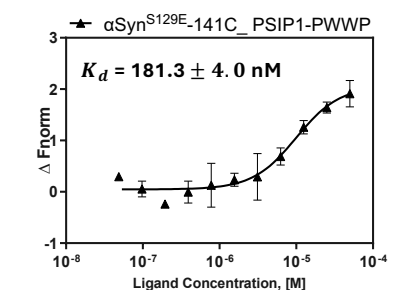
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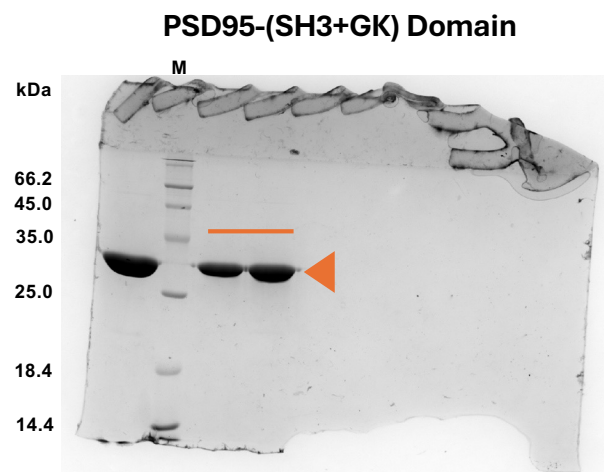
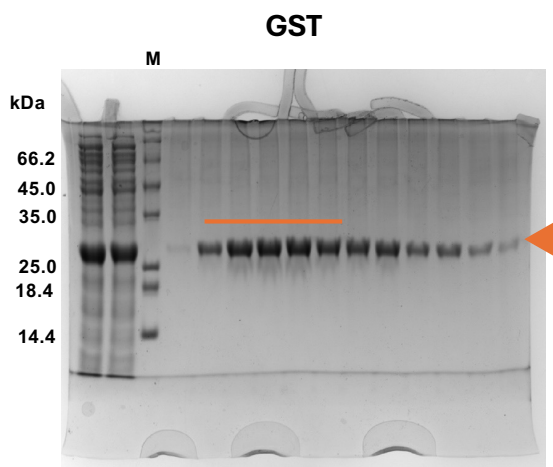
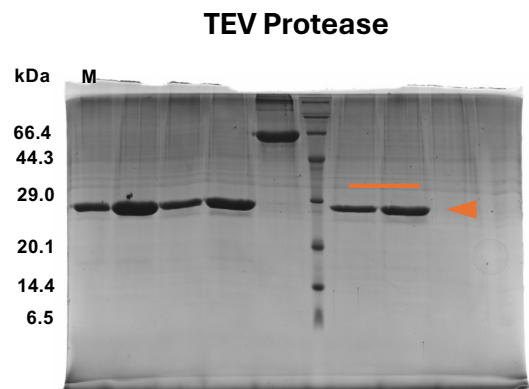
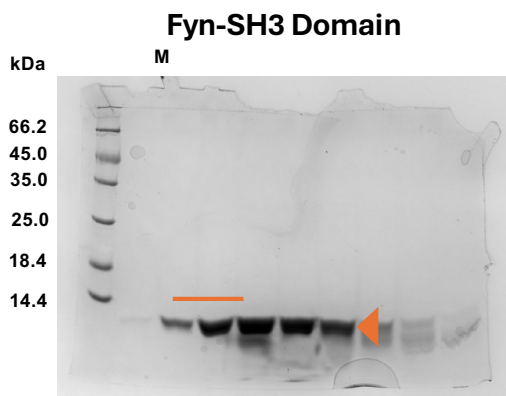
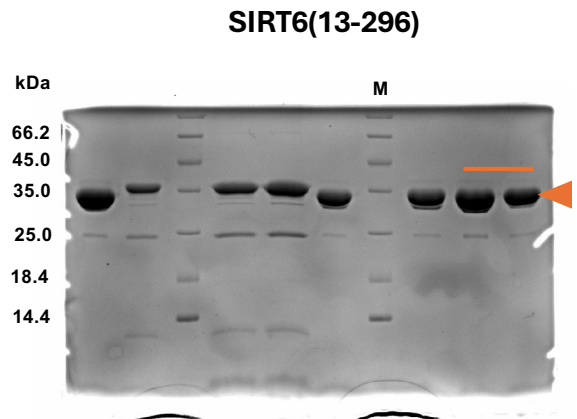
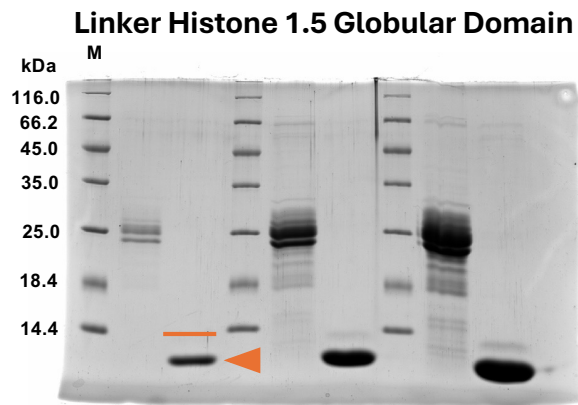
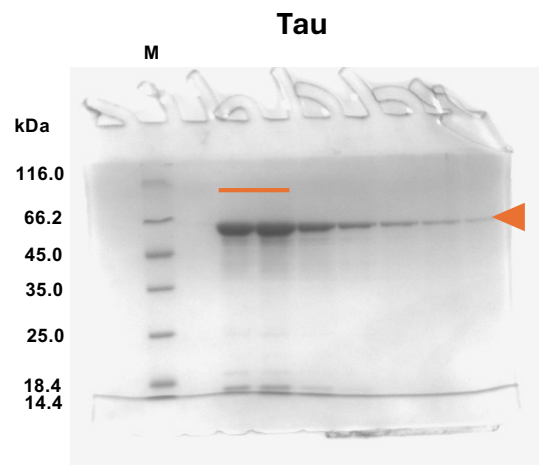
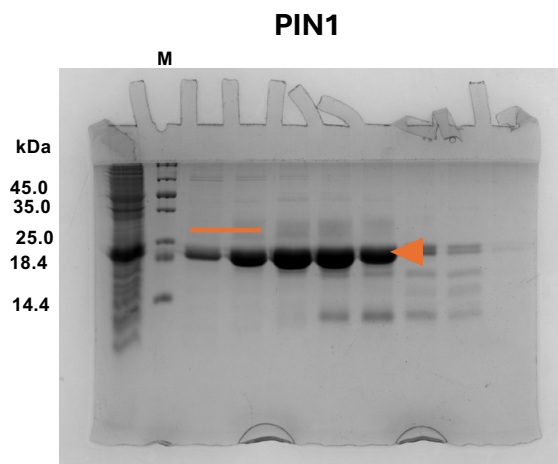
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Domain

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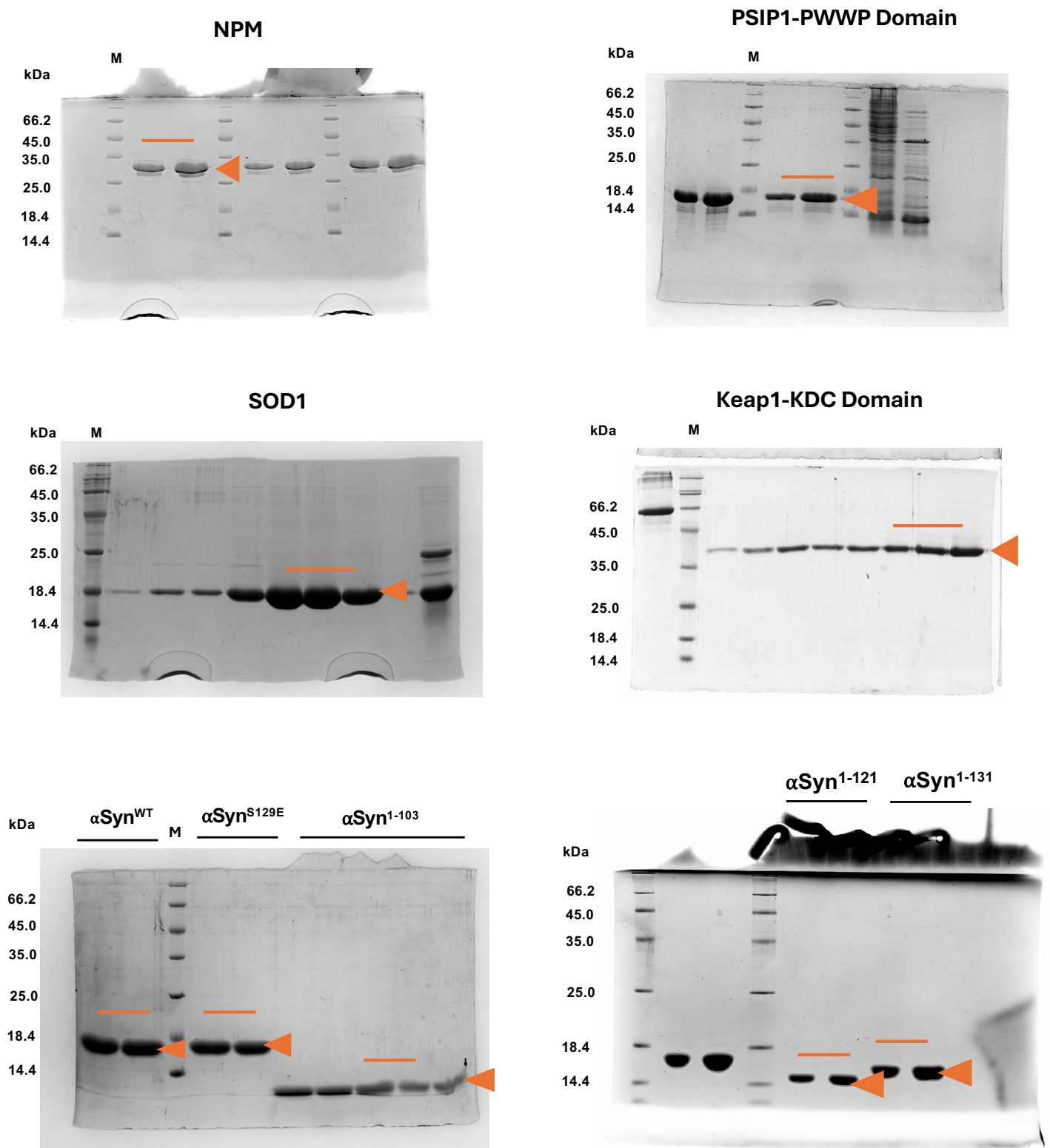
Keap1_KDC
DomainPSIP1- PWWP
Domain

Supplementary Figure 7: Binding affinity analysis of αSyn^{S129E} with various cellular proteins. Microscale thermophoresis (MST) binding curves (right) showing individual K_d values with SD ($n = 3$) for αSyn^{S129E}-141C with various cellular proteins. Capillary scans (left) reveal absence of protein adsorption to the capillary walls. Thermophoretic MST traces (middle), indicate no aggregation in solution. All capillary scan and MST trace plots represent raw output directly generated by the instrument software. Error bar represents $\pm$ SD ($n = 3$).

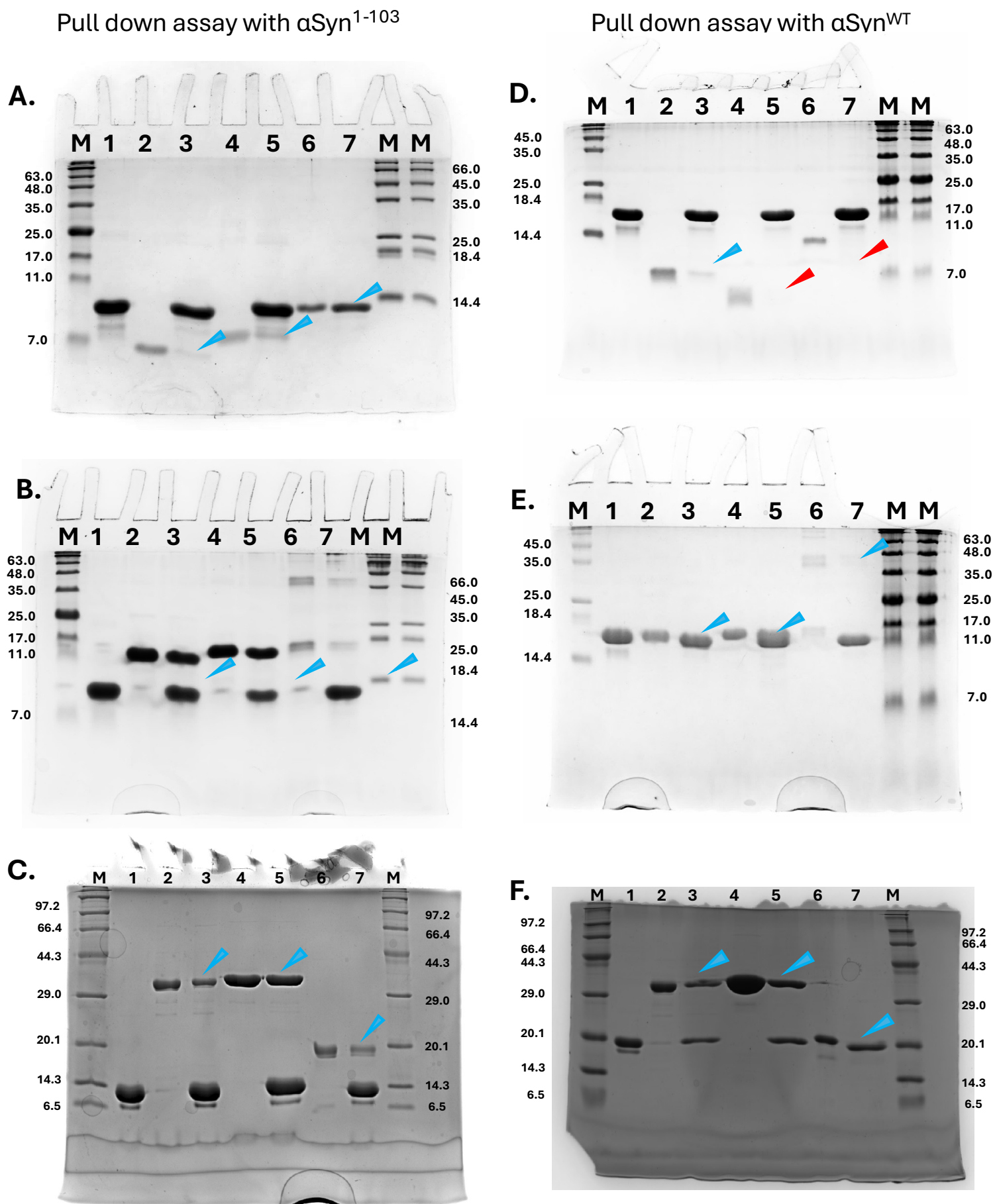
UNCROPPED ORIGINAL GEL IMAGES



Supplementary Figure 8: Original uncropped SDS-PAGE gel images of purified cellular proteins Pin1, Tau, Linker Histone 1.5 globular domain, SIRT6, Fyn-SH3 domain, TEV protease, GST, and PSD95-(SH3-GK) domain. Orange lines indicate the proteins of interest, and orange arrows mark the cropped regions displayed in the main figure. Lane M: Protein marker.



Supplementary Figure 9: Original uncropped SDS-PAGE gel images of purified cellular proteins NPM, PSIP-PWWP domain, SOD1, Keap1-KDC domain, and all purified constructs of αSyn used in this study. Orange lines indicate the proteins of interest, and orange arrows mark the cropped regions displayed in the main figure. Lane M: Protein marker.



Supplementary Figure 10. Original uncropped SDS-PAGE gel images corresponding to Supplementary Figure 5. Gels show αSyn^{1-103} (A–C) and $\alpha\text{Syn}^{\text{WT}}$ (D–F) incubated with equimolar concentrations of lysozyme, Fyn-SH3, Linker Histone 1.5-globular domain, core histones (H2A, H2B, H3), SIRT6, PSD-95(SH3-GK), and SOD1. M: Protein marker.