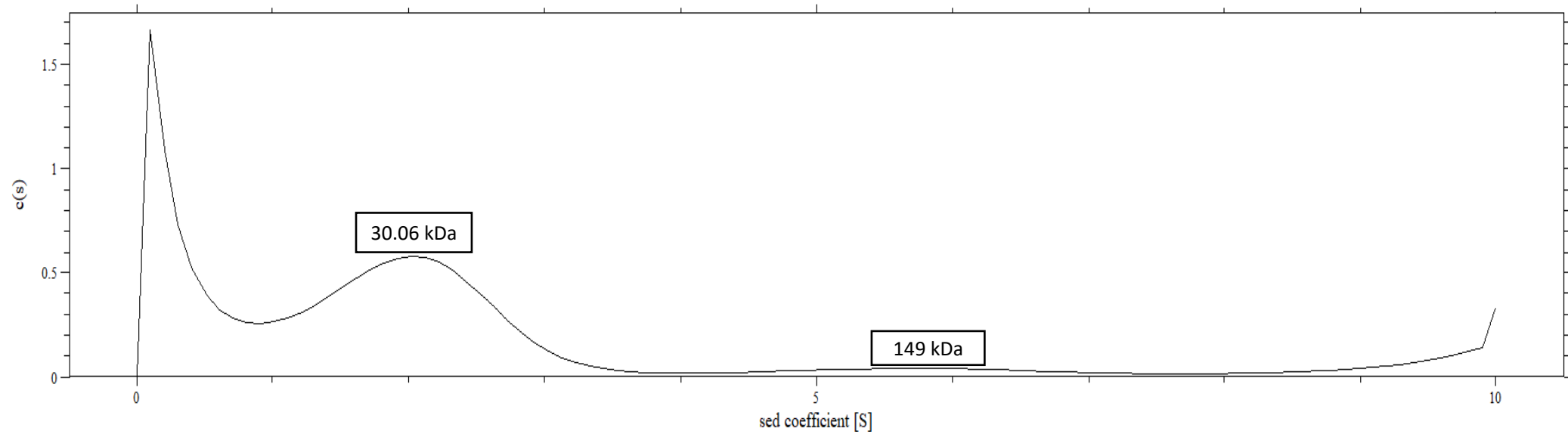
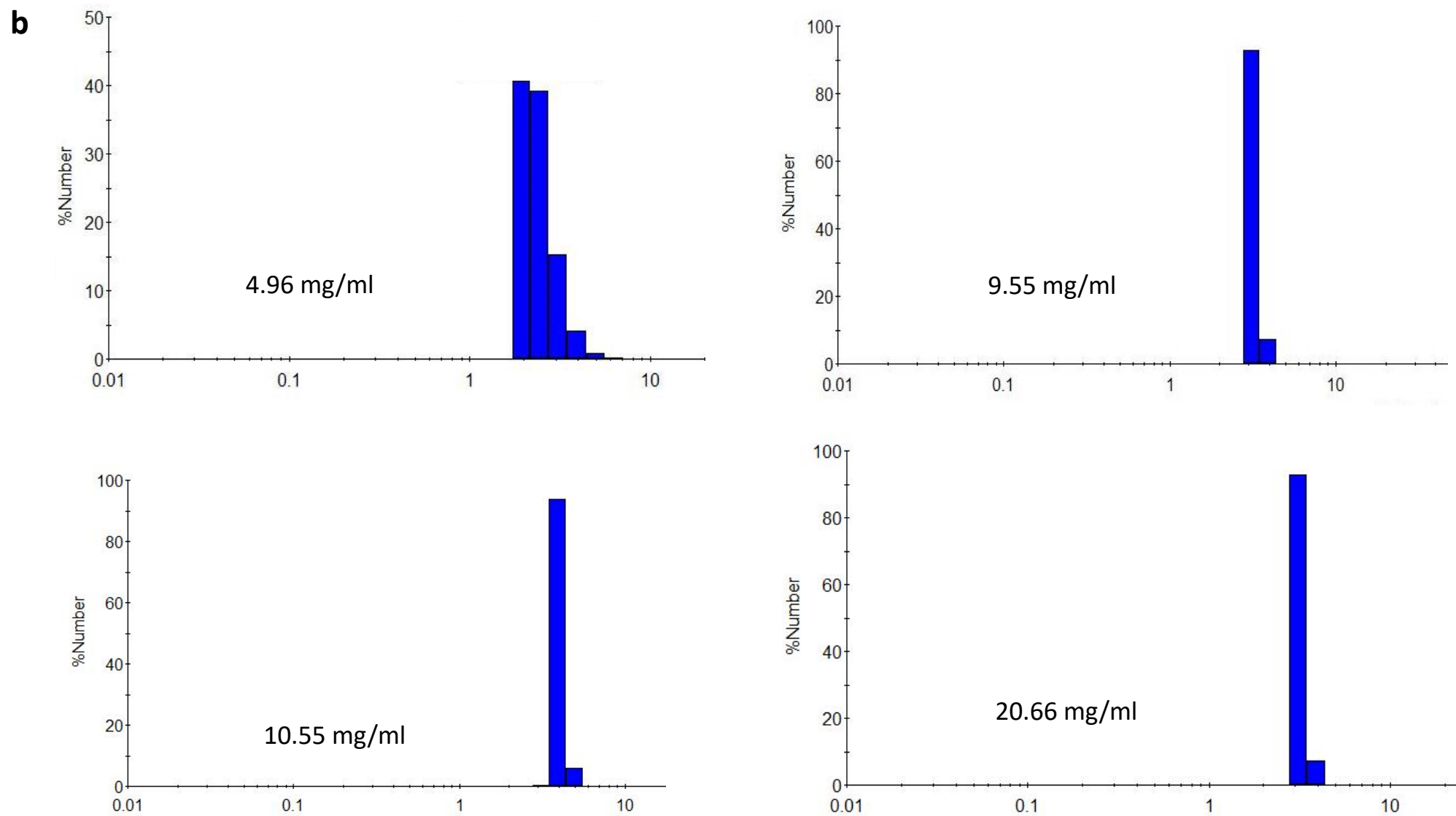


# Supplementary Figure

**a**

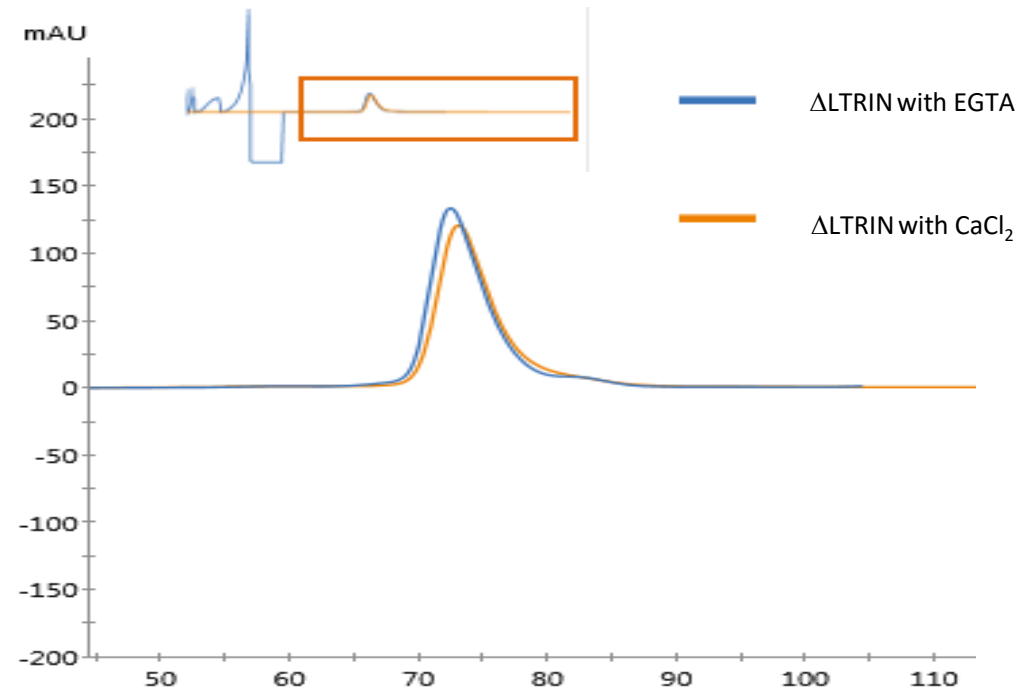


**Supplementary Figure S1:** a) Sedimentation equilibrium analysis of  $\Delta$ LTRIN indicates that it exist in dimer formation. b) Dynamic light scattering data (DLS) of  $\Delta$ LTRIN across different concentration. c) Size exclusion chromatography elution profile and SDS-PAGE of  $\Delta$ LTRIN in the presence of  $\text{Ca}^{2+}$  or EGTA. d) Comparing the DLS data of  $\Delta$ LTRIN in the presence of  $\text{Ca}^{2+}$  or EGTA. e) Size exclusion chromatography elution profile and SDS-PAGE C133Q  $\Delta$ LTRIN. f) DLS data of C133Q  $\Delta$ LTRIN showing that it is in dimer form. g) The CD spectra of  $\Delta$ LTRIN in the presence of 10mM EGTA continue to exhibit a predominant alpha helix secondary structure. h) Secondary structure prediction of full length LTRIN by PSIPRED. The signal peptide is highlighted in red box. The  $\Delta$ LTRIN used is underlined with green.

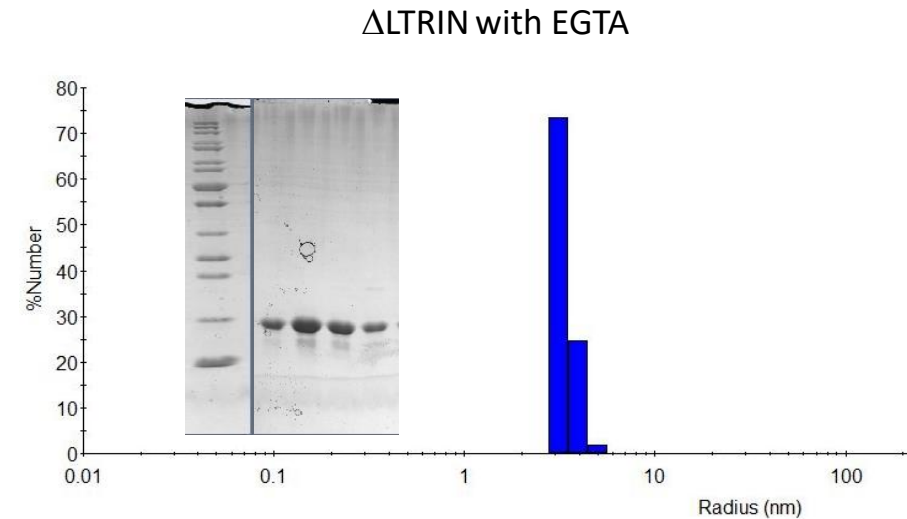
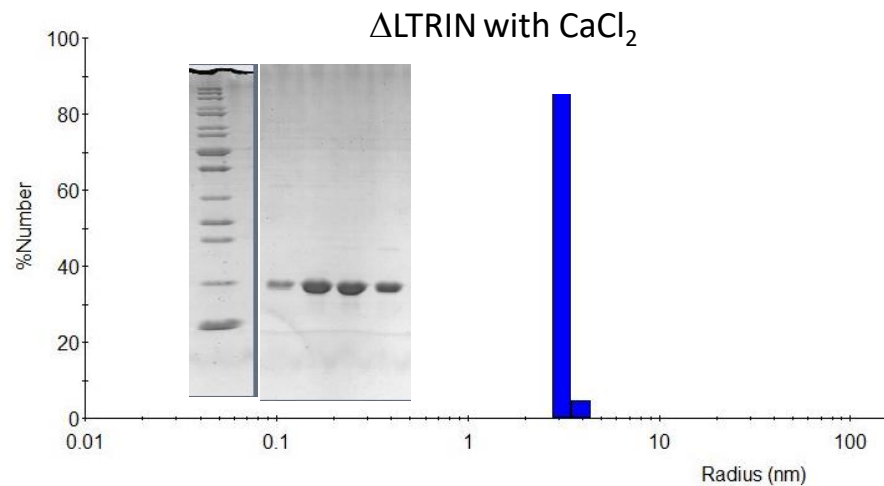


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**c**

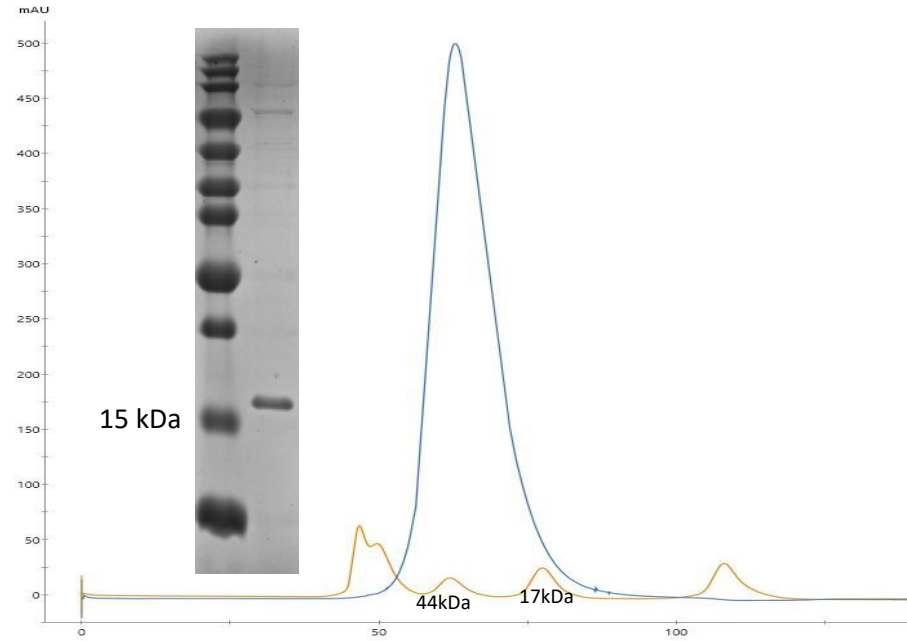


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**d**

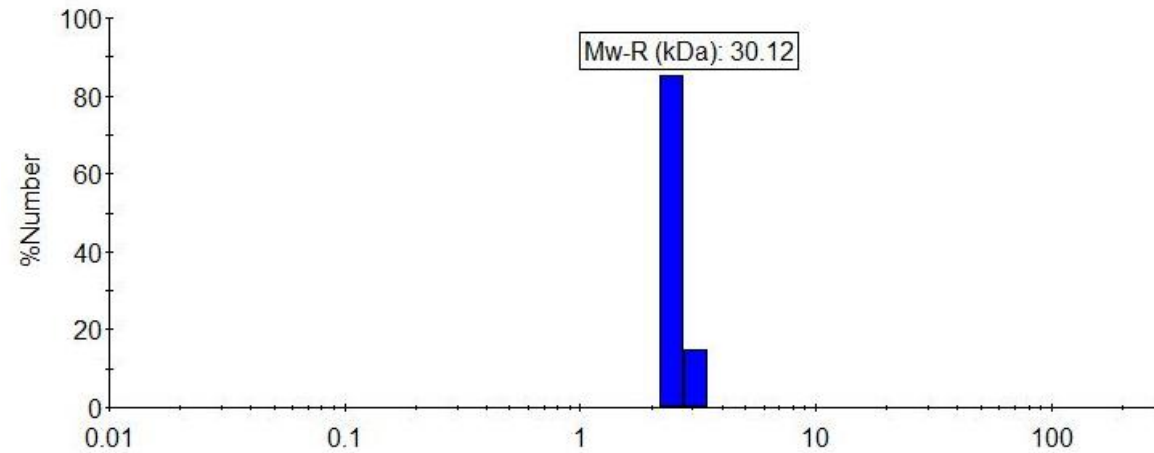
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**e**



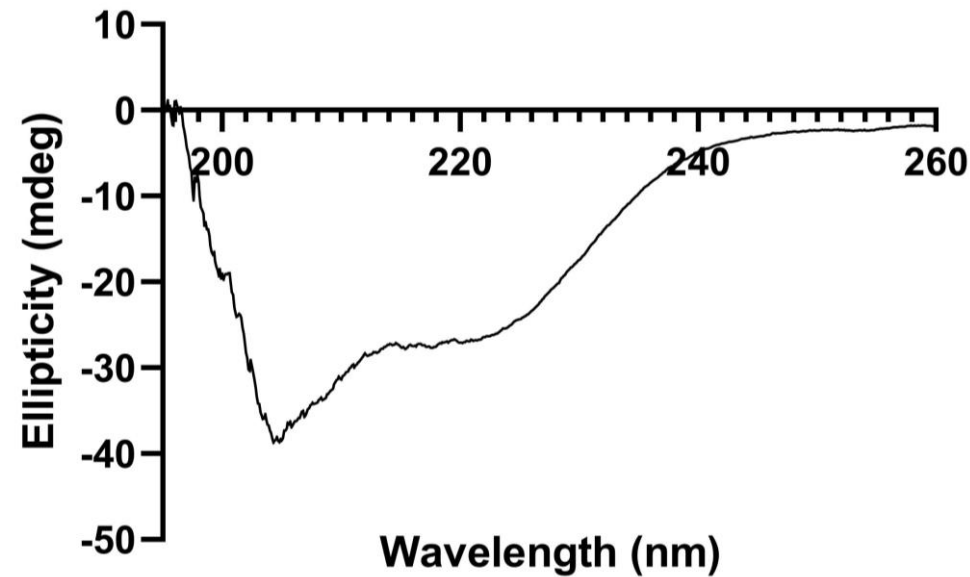
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**f**



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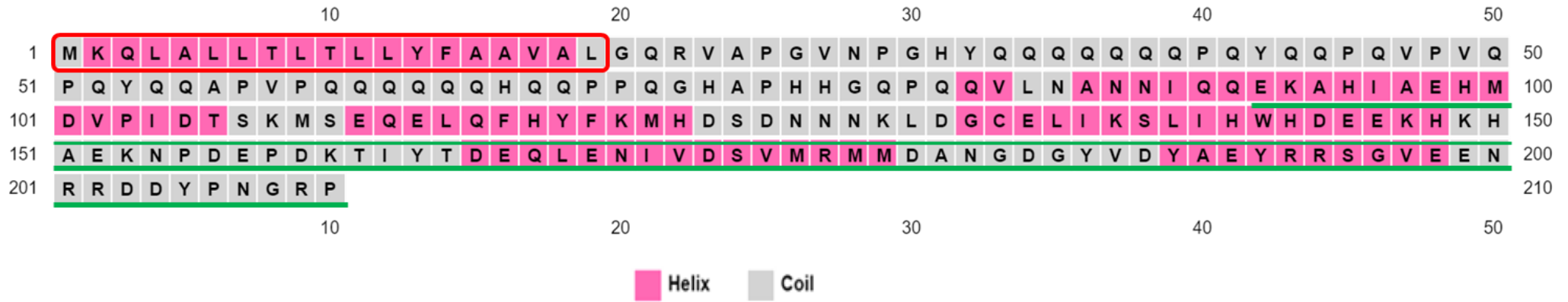
g



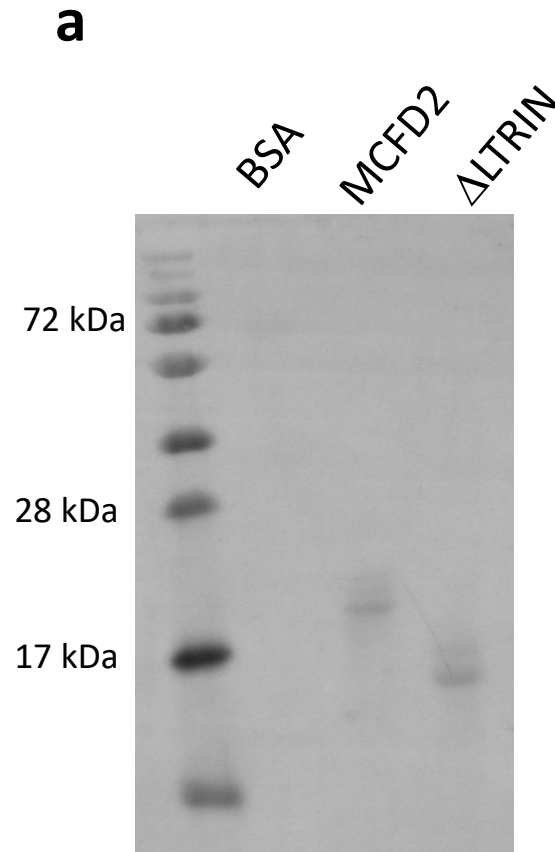
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# h

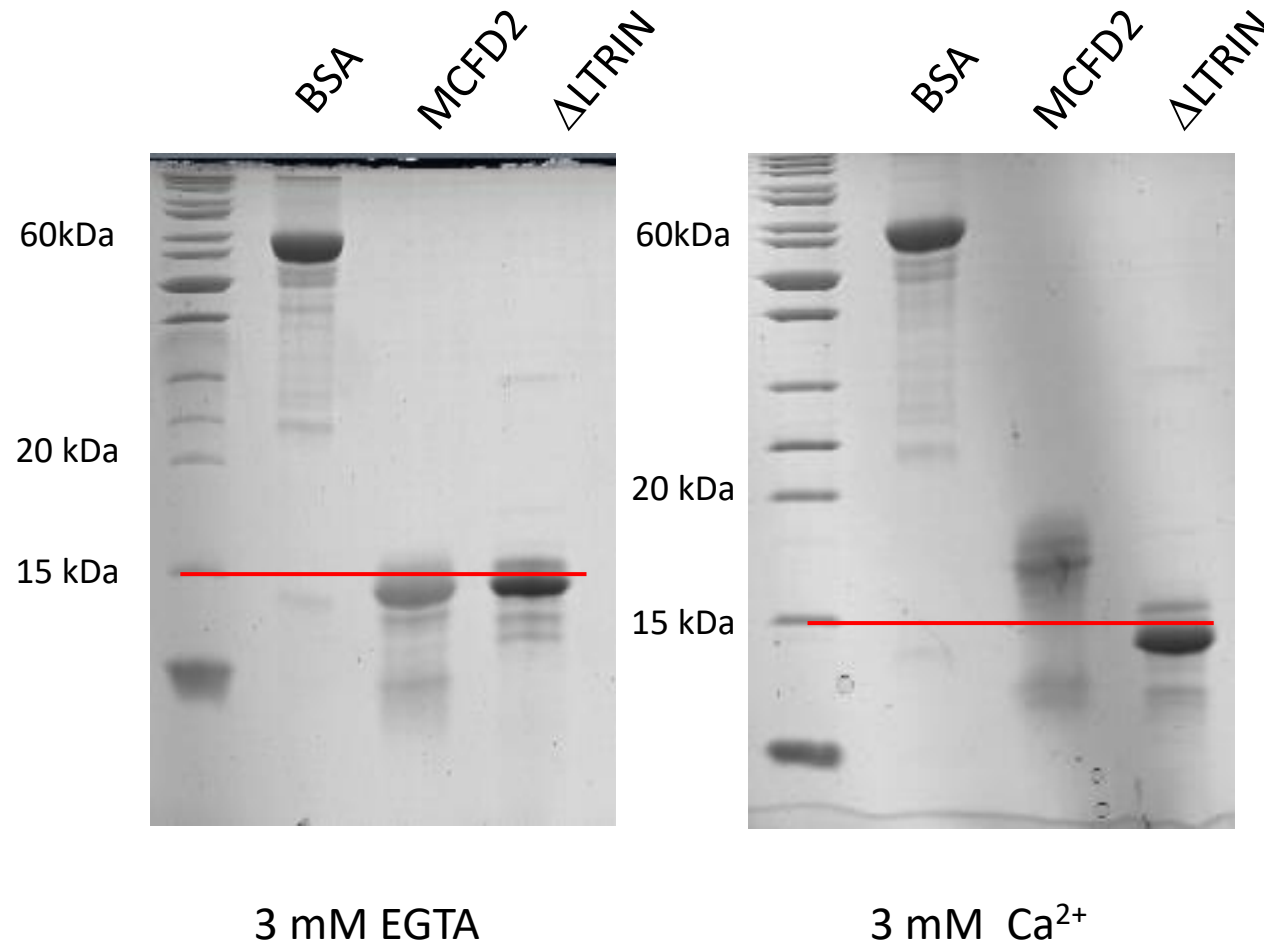


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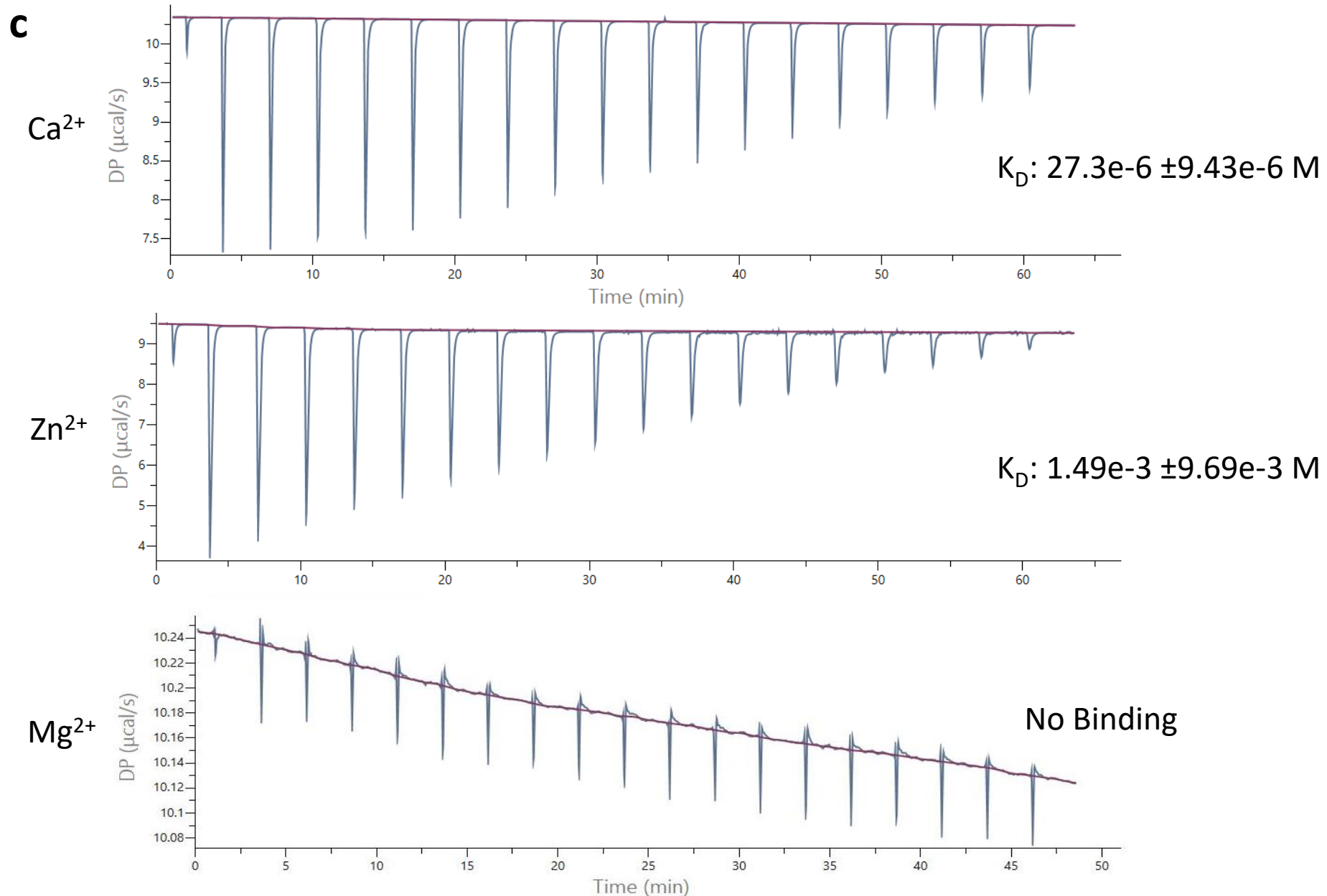


**Supplementary Figure S2:** The EF-hand motif of  $\Delta$ LTRIN a)  $\text{Ca}^{2+}$  binding ability of  $\Delta$ LTRIN is visualised using ruthenium red staining. b) Gel mobility shift assay of  $\Delta$ LTRIN shows that limited structural changes is induced upon bind with  $\text{Ca}^{2+}$ . c)  $\Delta$ LTRIN shows different binding affinity towards different divalent ions. d) The presence of  $\text{Zn}^{2+}$  causes protein precipitation, resulting in a negative outcome.

**b**

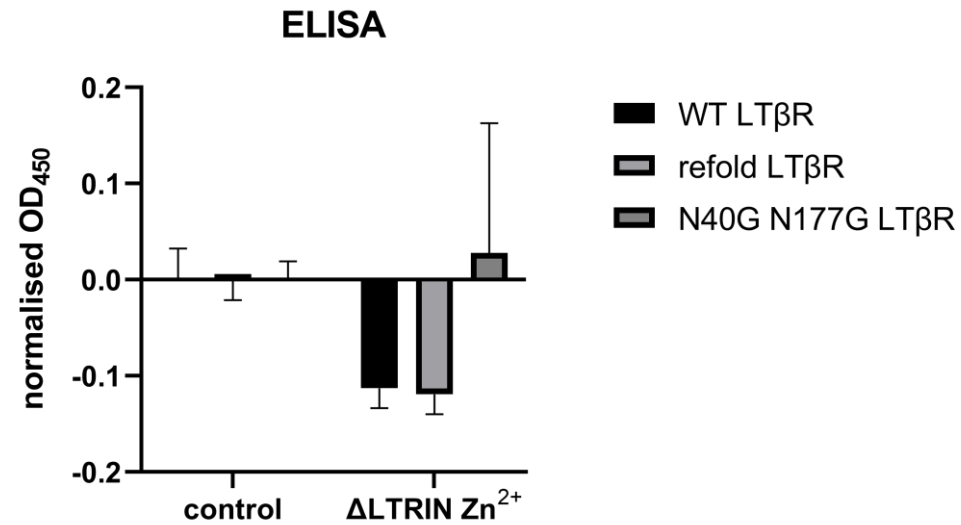


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**Supplementary Figure S2:** The EF-hand motif of  $\Delta\text{LTRIN}$  a)  $\text{Ca}^{2+}$  binding ability of  $\Delta\text{LTRIN}$  is visualised using ruthenium red staining. b) Gel mobility shift assay of  $\Delta\text{LTRIN}$  shows that limited structural changes is induced upon bind with  $\text{Ca}^{2+}$ . c)  $\Delta\text{LTRIN}$  shows different binding affinity towards different divalent ions. d) The presence of  $\text{Zn}^{2+}$  causes protein precipitation, resulting in a negative outcome.

d



**Supplementary Figure S2:** The EF-hand motif of  $\Delta$ LTRIN a)  $\text{Ca}^{2+}$  binding ability of  $\Delta$ LTRIN is visualised using ruthenium red staining. b) Gel mobility shift assay of  $\Delta$ LTRIN shows that limited structural changes is induced upon bind with  $\text{Ca}^{2+}$ . c)  $\Delta$ LTRIN shows different binding affinity towards different divalent ions. d) The presence of  $\text{Zn}^{2+}$  causes protein precipitation, resulting in a negative outcome.

	H(r)	H(d)	S(r)	S(d)	Turn	Unrd
SELCON3	0.214	0.143	0.044	0.070	0.245	0.323
CONTINLL	0.264	0.462	0.00	0.053	0.221	0.00
CDSSTR	0.300	0.257	0.059	0.094	0.151	0.145
AlphaFold	0.586		0.00		0.414	

**Supplementary Table S1:** The CD spectra of  $\Delta$ LTRIN in the presence of 2mM CaCl<sub>2</sub> is employed to assess its secondary structure content through three different methods (SELCON3, CONTINLL, and CDSSTR). The proportion of alpha-helix and loop structures in  $\Delta$ LTRIN as anticipated by AlphaFold was also quantified. H(r): regular alpha helices; H(d): distorted alpha helices; S(r): regular beta-sheets; S(d): distorted beta-sheets; Turn: beta-turn; Unrd: unordered structure.