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Supplementary Materials:

Modulation of XPC Peptide on Binding Tb^{3+} to *Euplotes octocarinatus* Centrin

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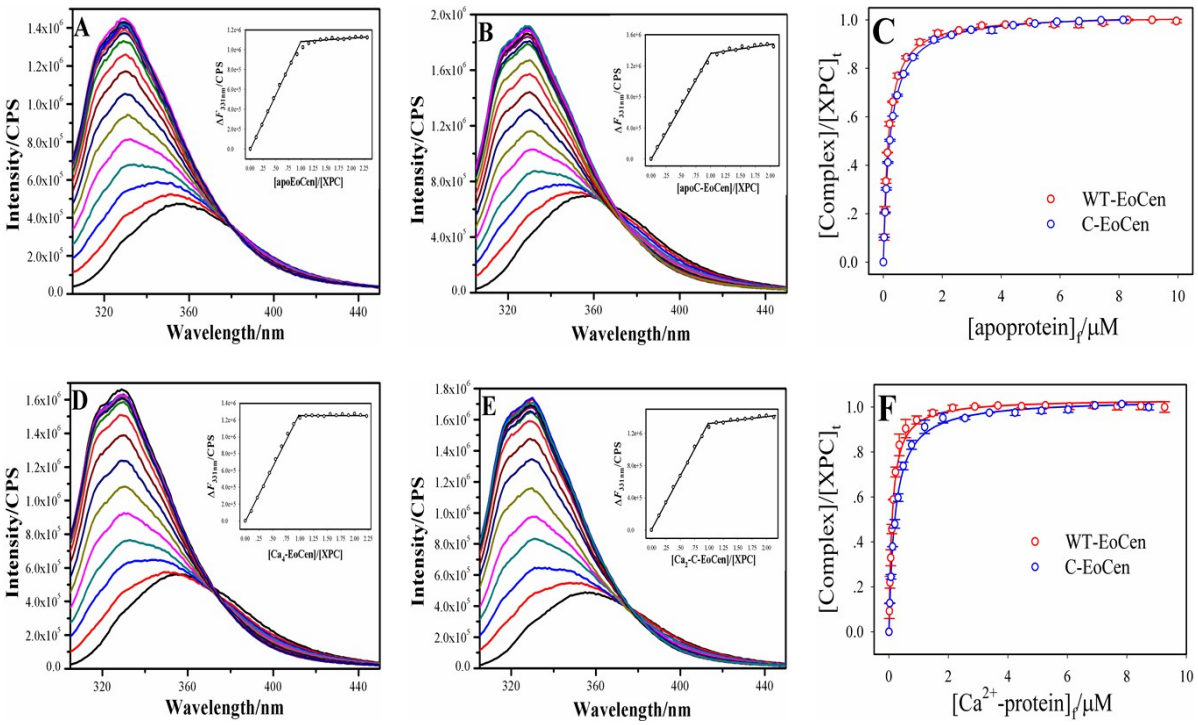

Fig. S1 Interaction of XPC peptide with EoCen monitored by the unique Trp fluorescence of XPC peptide in 10 mM Hepes, 100 mM KCl (pH 7.4) in the presence of EDTA and Ca^{2+}. Fluorescence titration spectra of XPC peptide by EoCen (A) and C-EoCen (B) in the presence of EDTA, and EoCen (D) and C-EoCen (E) in the presence of Ca^{2+}. Inset: $\Delta F_{331\ nm}$ vs [protein]/[XPC]. λ_{ex}=295 nm. Binding isotherms for the interaction of XPC peptide with EoCen (red) and C-EoCen (blue) in the presence of EDTA (C) and Ca^{2+} (F). Data points are average of three experiments. Solid lines represent the best fits, according to described previously.[32]

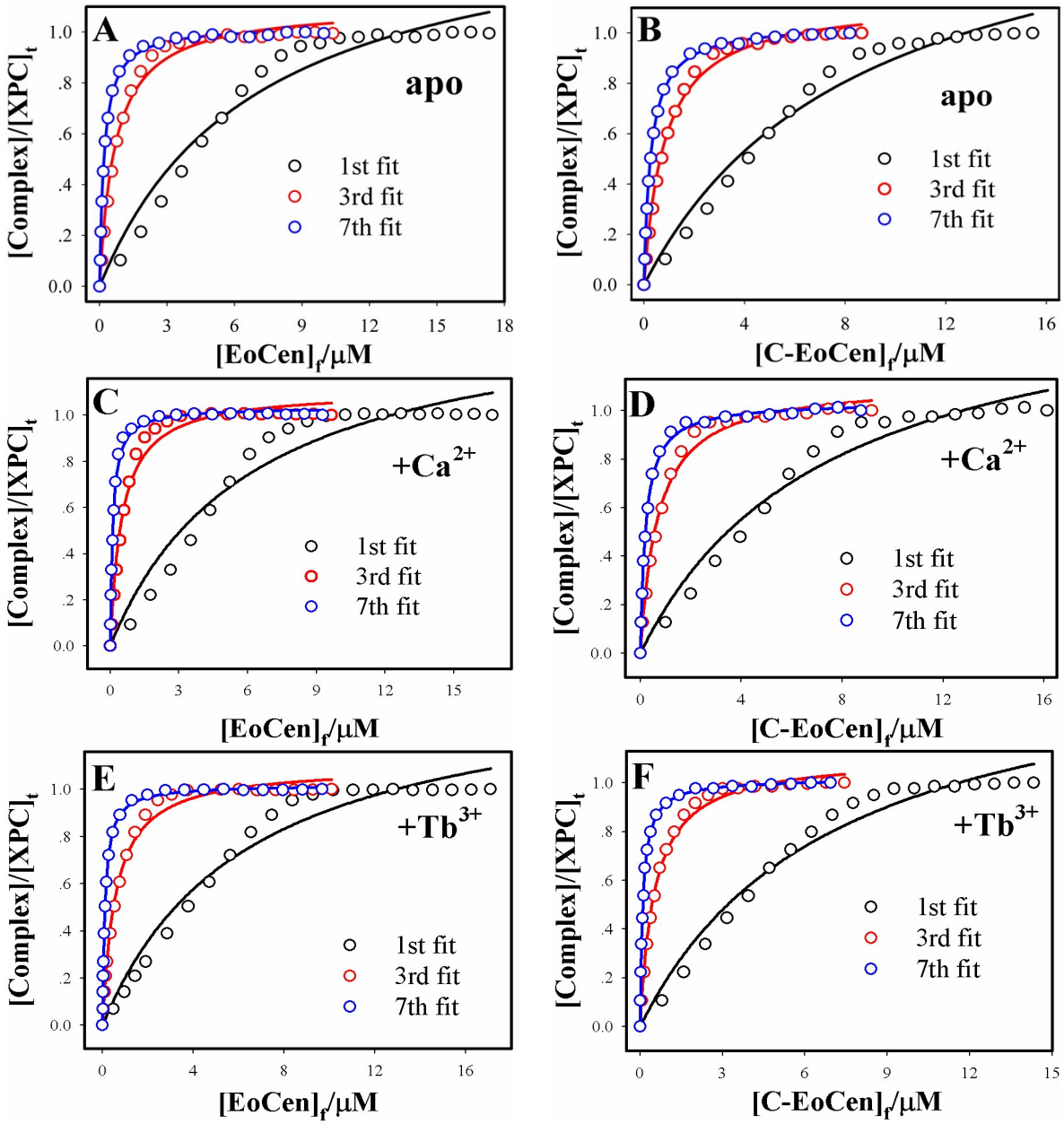

Fig. S2 Fit of [protein-XPC]/[XPC]$_t$ as a function of free concentration of EoCen (A) and C-EoCen (B) in the presence of EDTA, EoCen (C) and C-EoCen (D) in the presence of Ca^{2+}, and EoCen (E) and C-EoCen (F) in the presence of Tb^{3+}, respectively, to a single-site binding model using iteration method by Sigma Plot 10.0.

The calculation of the dissociation constant between Tb^{3+} and protein in the absence and presence of XPC peptide

1. To investigate the effect of XPC peptide on the affinity of Tb^{3+} and protein, titration of EoCen or C-EoCen and N-EoCen were performed with Tb^{3+} stock solution in the absence and presence of XPC peptide. Due to non-radiative energy transfer, Sensitized emission of bound Tb^{3+} was observed at 545nm. So the fluorescence intensity at 545 nm can be attributed to the Tb-protein. If the concentration of protein was *c*, there were 2 Tb^{3+}-binding sites and they were independent and identical in protein. In order to best fitting to a one-site model, assuming the initial concentration of protein was $a=2c$, and there was only one Tb^{3+}-binding site. The concentration of Tb^{3+} ion was *b*. The concentrations of species for proteins and Tb^{3+} ion can be calculated by the following formulas (1)-(6):

$$\text{protein} + Tb^{3+} \leftrightharpoons \text{protein-Tb} \quad (1)$$

t=0	*a*	*b*	0
t=t	*a*-*x*	*b*-*x*	*x*

$$K_d = \frac{[\text{protein}]_f [Tb^{3+}]_f}{[\text{protein-Tb}]} = \frac{(a-x)(b-x)}{x} \quad (2)$$

$$[\text{protein}]_f = [\text{protein}]_t - [\text{protein-Tb}] \quad (3)$$

$$\frac{[\text{protein-Tb}]}{[\text{protein}]_t} = \frac{[Tb^{3+}]_f}{K_d + [Tb^{3+}]_f} \quad (4)$$

$$\frac{F}{F_{\max}} = \frac{[\text{protein-Tb}]}{[\text{protein}]_t} \quad (5)$$

$$[Tb^{3+}]_f = b - x = b - \frac{(a+b+K_d) - \sqrt{(a+b+K_d)^2 - 4ab}}{2} \quad (6)$$

Where $[\text{protein}]_t$ represented the total concentration of protein. $[Tb^{3+}]_f$, $[\text{protein}]_f$ and [protein-Tb] represented the concentration of free Tb^{3+} ion, free protein and the protein-Tb, respectively. *F* and $F_{\max}$ represented the fluorescence intensity in each titration point, and the saturated intensity (at the saturation concentration), at 545 nm, respectively.

Firstly, $[Tb^{3+}]_t$ took place of $[Tb^{3+}]_f$. Fit of $[\text{protein-Tb}]/[\text{protein}]_t$ *vs* $[Tb^{3+}]_t$ was performed using SigmaPlot 10.0 software to a single-site binding model. Secondly, according to K_d and equation (6),

$[Tb^{3+}]_f$ was calculated. Then fit of [protein-Tb]/$[protein]_t$ *vs* $[Tb^{3+}]_f$ was performed again by using the obtained $[Tb^{3+}]_f$. The rest could be done in the same manner until the K_d value approached the approximate value for the *n*th time and the (*n+1*)th time. That is so-called iteration method.

2. The Tb^{3+} binding ability of N-terminal domain of intact EoCen had been influenced after EoCen-XPC complex formed. If the concentration of protein was *c*, there were 2 Tb^{3+}-binding sites and they were independent and identical in protein. In order to best fitting to a one-site model, assuming the initial concentration of protein was *a*=2*c*, and there was only one Tb^{3+}-binding site. The association constant between Tb^{3+} and N-terminal domain of intact EoCen could be estimated by the following formulas (7)-(10):

$$[\text{protein-Tb}] = \frac{F}{F_{\max}}[\text{protein}]_t \qquad (7)$$

$$[\text{protein}]_f = [\text{protein}]_t - [\text{protein-Tb}] \qquad (8)$$

$$[Tb^{3+}]_f = [Tb^{3+}]_t - [\text{protein-Tb}] \qquad (9)$$

$$K_a = \frac{[\text{protein-Tb}]}{[Tb^{3+}]_f[\text{protein}]_f} \qquad (10)$$

Where $[protein]_t$ and $[Tb^{3+}]_t$ represented the total concentration of protein and Tb^{3+}, respectively. $[Tb^{3+}]_f$, $[protein]_f$ and [protein-Tb] represented the concentration of free Tb^{3+} ion, free protein and the protein-Tb, respectively. *F* and F_{max} represented the fluorescence intensity in each titration point in the presence of XPC peptide, and the saturated intensity (at the saturation concentration) in the absence of XPC peptide, at 545 nm, respectively. Finally, K_a could be obtained from the average value of each titration point.

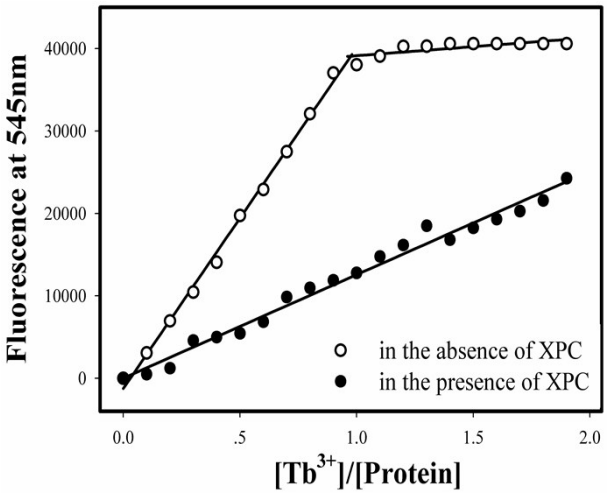

Fig. S3 Tb^{3+} titration curves of the N-terminal domain of intact EoCen in the absence and presence of XPC peptide.

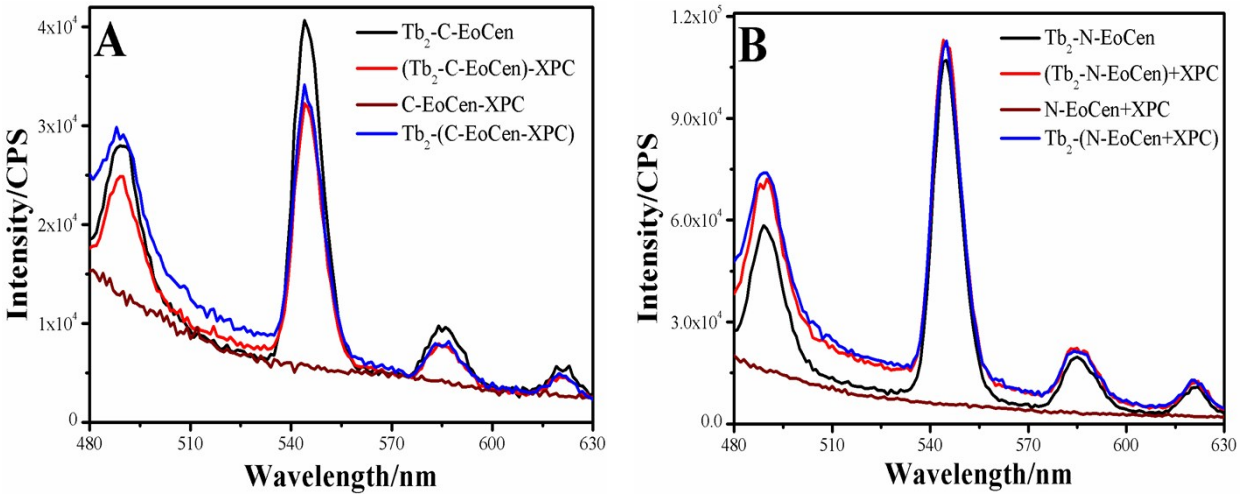

Fig. S4 Effect of the order of species used on Tb^{3+} sensitized emission. After the species in bracket were incubated, then were incubated with corresponding amount specie outside the bracket again. The concentration of protein was 8 μM. λ_{ex}=295 nm, 360 nm light filter was used in the present condition. The number of subscript denoted stoichiometric ratio of species.

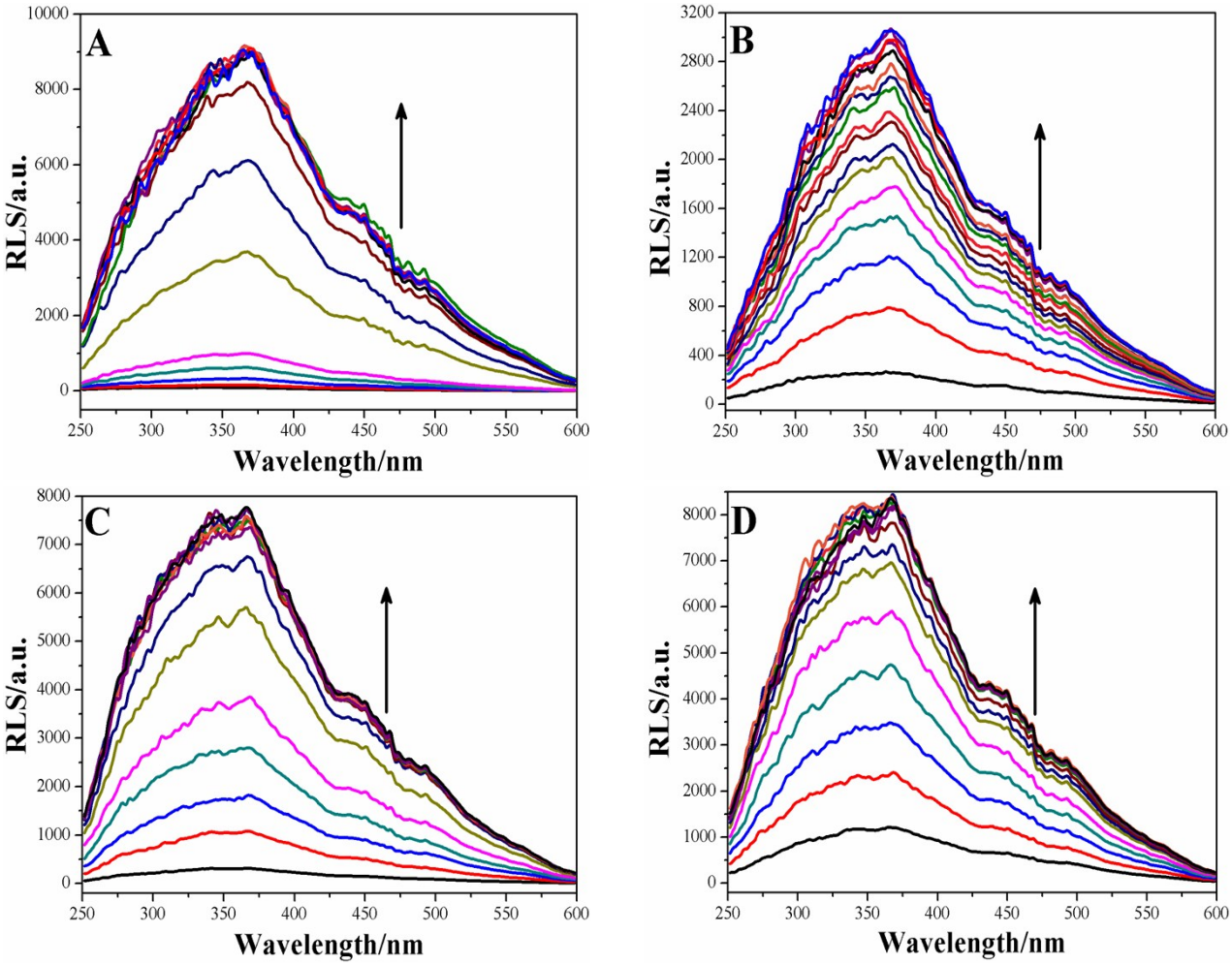

Fig. S5 RLS spectra of Tb^{3+} adding to EoCen in the absence of XPC (A) and in the presence of XPC (B), to N-EoCen in the absence of XPC (C) and in the presence of XPC (D).