Figure 1





**





20µM zinc treatment (min) DMAT















20µM zinc treatment (min) Wild-type ZIP7 **Mutant ZIP7**





Figure 5



B

Proximity ligation assay in TamR cells with ZIP7 and CK2



Figure 6

A

 Zinc
 0
 2
 5

 EGF +
 0
 2
 0
 5

 Ionomycin
 0
 0
 0
 0

20µM zinc treatment (min)



Figure 7

| Time in minutes | 0 | 2 | 5 | 10 | 15 | 20 |
|---------------------------|---|--------------|-----------------------|-------------------------|--------------|--------------|
| CK2 binds to ZIP7 | | √√√ | ✓ | ✓ | | |
| Ser phos of ZIP7 | | √ √ √ | √ √ | ✓ | | |
| ZIP7-dependant Zn release | | | ✓ | √ √ | √ √ √ | √ √ √ |
| Phospho-Tyrosine | | ✓ | √ √ | √ √ | √ √ √ | √ √ √ |
| Phosphorylated AKT | | ✓ | ✓ | √ √ | √ √ | √ √ √ |
| Phosphorylated ERK1/2 | | | ✓ | ✓ ✓ | √ √ | √ √ |



Supplementary Figures



B



Treatment time (min)



zinc treatment time (min)



Proximity Ligation assay images and control results.(A) Representative figures of proximity ligation assay of ZIP7 and CK2 α stimulated with zinc in Fig 1E Scale bar = 10 μ m. (B) Representative figures of no antibody controls for proximity ligation assay in Figs 1E and 4B. Scale bar = 10 μ m. (C) Control results for proximity ligation assay lacking single and both antibodies and representative of n=3 experiments and expressed as mean ±SD



Densitometry analysis of pooled Western Blot results for Fig. 2. (A) Densitometry analysis of pooled Western Blot results from Fig 2D from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$). (B) Densitometry analysis of pooled Western Blot results from Fig 2E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) and ** denotes P ≤ 0.001 . (C) Densitometry analysis of pooled Western Blot results from Fig 2E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) and ** denotes P ≤ 0.001 . (C) Densitometry analysis of pooled Western Blot results from Fig 2E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) .



Characterisation of ZIP7 mutants and ZIP7 antibody.(A) Sequencing demonstration of S275 and S276 mutated to A275 and A276 in ZIP7 sequence. (B) MCF-7 cells transfected with either wild-type or mutant ZIP7 and probed with V5 antibody conjugated to Alexa-Fluor 488 (green). Nuclei stained blue with DAPI. Scale bar = 10 μ m. (C) MCF-7 cells transfected with wild-type ZIP7 and probed with ZIP7 antibody conjugated to Alexa-Fluor 488 (green), V5 antibody conjugated to Alexa-Fluor 594 (red) and nuclei stained blue with DAPI. Scale bar = 10 μ m.

Figure S4



Densitometry analysis of pooled Western Blot results for Fig.3.(A) Densitometry analysis of pooled Western Blot results from Fig 3A from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) and ** denotes ($P \le 0.001$). (B) Densitometry analysis of pooled Western Blot results from Fig 3C from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$). (C) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes from time zero ($P \le 0.05$). (C) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.001$). (D) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.001$). (D) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) and ** denotes ($P \le 0.001$). (D) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) and ** denotes ($P \le 0.001$). (D) Densitometry analysis of pooled Western Blot results from Fig 3E from n=3 experiments and expressed as mean ±SD. * denotes significance from time zero ($P \le 0.05$) .

Figure S5

A



Treatment time (min)

B



Proximity ligation assay images and CK2 α antibody control. (A) Representative figures of proximity ligation assay of ZIP7 and CK2 α stimulated with EGF and ionomycin in Fig 4B Scale bar = 10 μ m. (B) CK2 α antibody recognises a single band on Western Blot of appropriate size in TamR cells