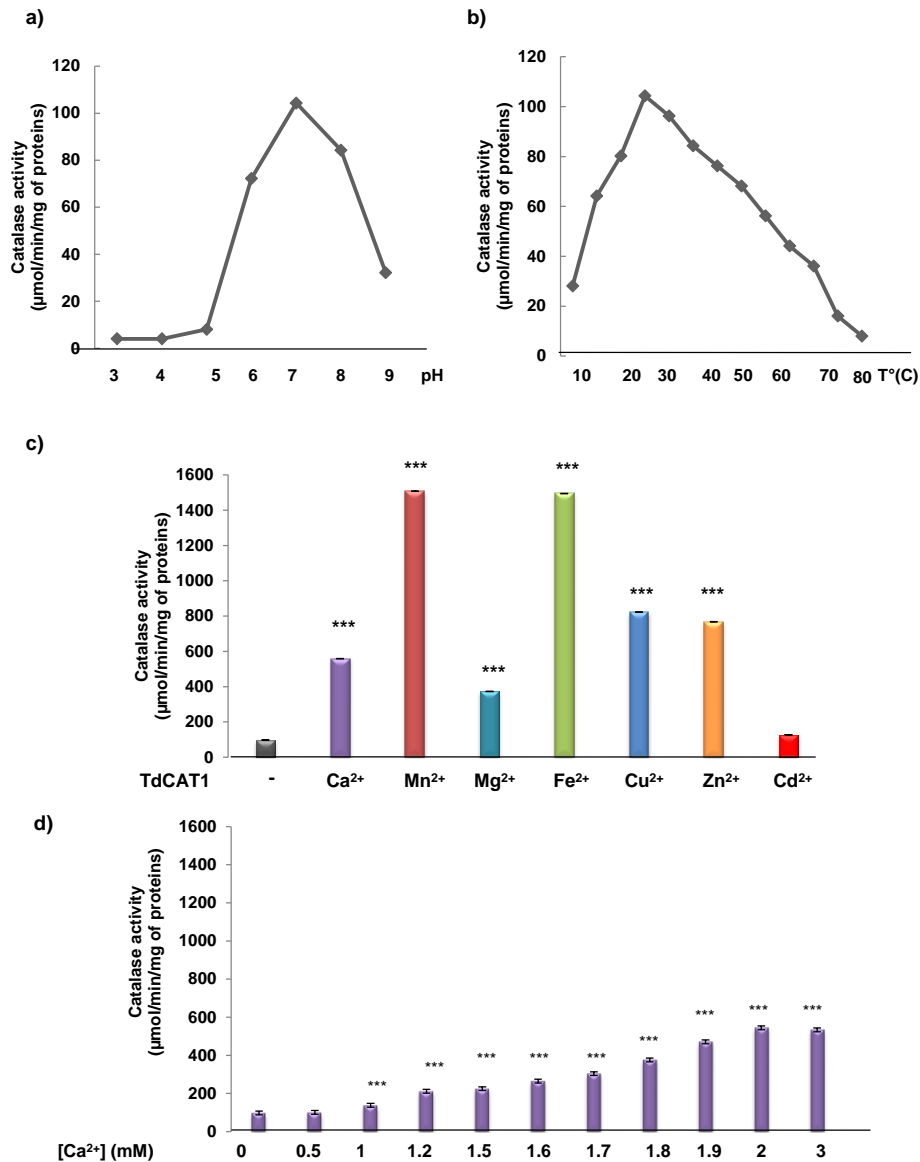
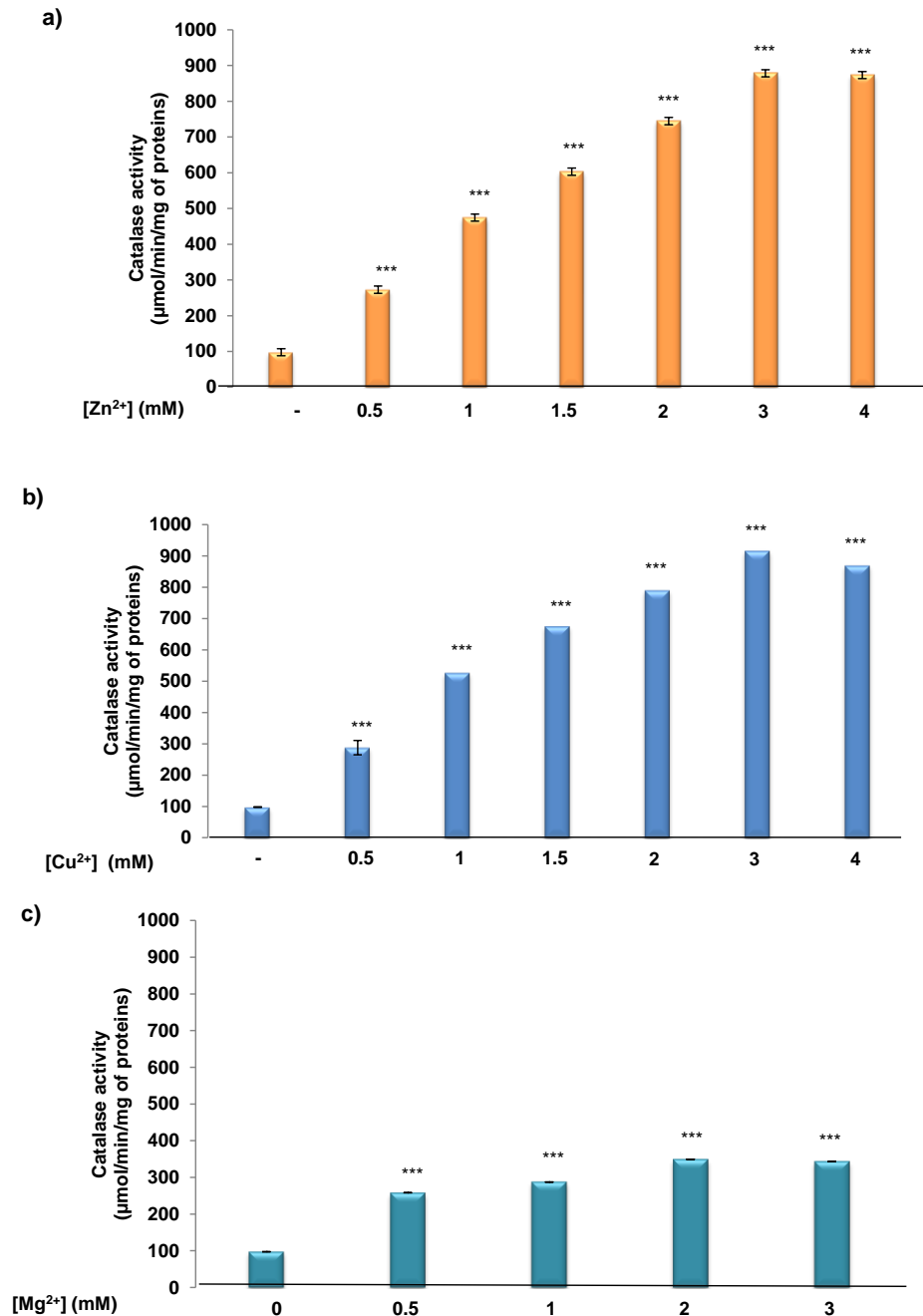


Supplemental Table S1. List of primers used in PCR amplification of TdCAT1 and its truncated forms. The forward primer CF was used for the amplification of all forms with the use of reverse primers corresponding of each one.

Amplified fragments	Nucleotide sequences	Primers names
TdCAT1	TCGAATTCATGGACCCCTACAAGTA ATCTCGAGTTACATGCTCGGCTTGG	CF CR1
TdCAT ₂₀₀	ATCTCGAGGGCGGTAGTCGGCGGG	CR2
TdCAT ₂₉₅	ATCTCGAGCCTCGGGCCACGTCTTG	CR3
TdCAT ₄₆₀	ATCTCGAGGGTGAGGCGGGGGTCC	CR4



Supplemental Figure S1. Functional characterization of TdCAT1. (a) Effect of pH on the catalytic activity of TdCAT1. pH stability of the catalytic activity of TdCAT1 was studied in different pH medium ranging from 3-9 with a maximum activity at pH 7. (b) Effect of temperature on the catalytic activity of TdCAT1. The catalytic activity was studied in presence of different temperature with 5°C difference graduation and maximum activity at 25°C. (c) Stimulatory effects of divalent cations on the *in vitro* phosphatase activity of the recombinant His_TdCAT1. The catalytic activity is stimulated in the presence of 2 mM Mn²⁺, Fe²⁺, Zn²⁺, Cu²⁺, Mg²⁺ and Ca²⁺ but not in presence of Cd²⁺. (d) stimulatory effect of Ca²⁺ cations on the catalase activity of TdCAT1. TdCAT1 activity was assayed with 160 μg of recombinant Protein after incubation for 1 min in presence of 50mM H₂O₂ as a substrate in a 75 mM phosphate buffer at pH 7.0 and 25°C and the absorbance was measured at 240 nm. H₂O₂ decomposition was measured by measuring the decrease of H₂O₂ absorbance and was calculated as μmol H₂O₂ decomposed/mg protein/min. Values are means of initial rates (μmol/min/mg of proteins) ± S.E from at least three independent experiments with identical results.



Supplemental Figure S2. Stimulatory effects of Zn²⁺, Cu²⁺ and Mg²⁺ on the *in vitro* catalase activity of the recombinant His-TdCAT1. TdCAT1 activity was assayed with 160 μg of recombinant His_TdCAT1 and 50 mM H₂O₂ as a substrate, in the presence of increasing concentrations of Zn²⁺ (**a**), Cu²⁺ (**b**), and Mg²⁺ (**c**). Values are means of initial rates (μmol/min/mg of proteins) ± S.E from three independent experiments. (***) indicates values significantly different from the control (basal catalase activity of TdCAT1). Statistical significance was assessed by applying the ANOVA test with <0.005.

a)

Mn²⁺ binding Domain

SlCat3	DYHLL L EKIANFD	12
OsCat	DYH L VEKLANFD	12
ZmCat2	DYH L VEKLANFD	12
AtCat1	DYH L VEKLANFD	12
NtCat1	DYH L VEKLANFD	12
NbCat	DYH L VEKLANFD	12
GhCat1	DYH L VEKLANFD	12
StCat2	DYH L VEKLANFD	12
AtCat2	DYH L VEKLANFD	12
TdCat1	DYH L VEK I ADFD	12
AetCaT1	DYH L VEK I ADFD	12
BdCat1	DYH L VEKIANFD	12
AtCat3	DYH L IEK V ANFT	12
SpCat1	DYH L IEK I QNFT	12
*****:***: :*		

b)

Mg²⁺ binding Domain

AtCat1	T P ER Q ERFIQR	11
AtCat2	T P ER Q ERFIQR	11
NtCat1	T P DR Q ERFIRR	11
NbCat	T P DR Q ERFIRR	11
StCat2	T P DR Q ERFIRR	11
TdCat1	D P AR Q ERFINR	11
BdCat1	D P AR Q ERFINR	11
AetCaT1	D P AR Q ERFINR	11
ZmCat2	D P AR Q ERFITR	11
OsCat	D P AR Q DRFIKR	11
SlCat3	A P DR Q ERFLCR	11
GhCat1	A A DR Q ERFICR	11
AtCat3	A P DR Q DRFVKR	11
SpCat1	A P DR Q DRFINR	11
**:*:*: *		

Supplemental Figure S3. Sequence alignment of Mn²⁺ and Mg²⁺ binding domains from different plant catalase. *Arabidopsis thaliana* [CAT1 (accession no. X94447), CAT2 (accession no. X64271) and CAT3 (accession no. U43147)], rice *Oryza sativa* (OsCAT A; accession no. XP_015629749.1), *Zea mays* (ZmCAT1; accession no. PWZ57146.1), *Nicotiana tabacum* (NtCAT; accession no. U93244), *Nicotiana bentamiana* (NbCAT; accession no. EU998969), *Gossypium hirsutum* (GhCAT1; accession no. XP_016687939.1), *Solanum tuberosum* (StCAT2, accession no. KAH0685113.1), *Triticum turgidum ssp durum* (TdCAT1; Accession no. KP696753), *Aegilops tauchii* (AetCAT1; accession no. XP_020164896.1), *Brachypodium distachyon* (BdCAT1, accession no. XP_003558892.1) and *Sorghum bicolor* (SbCAT1, accession no. XP_021304262.1).

[illegible]

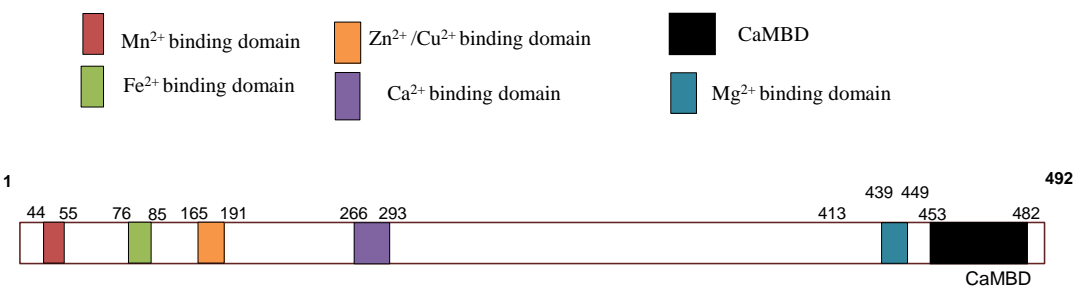
Supplemental Figure S4. Sequence alignment of Zn/Cu binding domain from different plant catalase.

Arabidopsis thaliana [CAT1 (accession no. X94447), CAT2 (accession no. X64271) and CAT3 (accession no. U43147)], rice *Oryza sativa* (OsCAT A; accession no. XP_015629749.1), *Zea mays* (ZmCAT1; accession no. PWZ57146.1), *Nicotiana tabacum* (NtCAT; accession no. U93244), *Nicotiana bentamiana* (NbCAT; accession no. EU998969), *Gossypium hirsutum* (GhCAT1; accession no. XP_016687939.1), *Solanum tuberosum* (StCAT2, accession no. KAH0685113.1), *Triticum turgidum ssp durum* (TdCAT1; Accession no. KP696753), *Aegilops tauchii* (AetCAT1; accession no. XP_020164896.1), *Brachypodium distachyon* (BdCAT1, accession no. XP_003558892.1) and *Sorghum bicolor* (SbCAT1, accession no. XP_021304262.1).

			E	n	-	-	n	n	-	-	n	x	-	y	-	S	G	-	-y	-	-	-	-z
			E									D	X	D NS	IL VF Y W	DE NS TG	D N Q G H R K	G P	LI V M C	DE N Q S T A G C	X	X	DE
TdCAT1			E	W	T	F	Y	I	Q	T	I	D	P	D	H	E	E	R	F	D	-	F	D
BdCAT1			E	W	K	F	Y	I	Q	T	I	D	P	D	H	E	G	R	F	D	-	F	D
OsCAT			E	W	K	L	F	I	Q	T	I	D	P	D	H	E	D	R	F	D	-	F	D
ZmCAT2			E	W	T	L	Y	I	Q	T	M	D	P	E	M	E	D	R	L	D	D	L	D
AtCAT1			E	W	K	L	F	I	Q	I	I	D	P	A	D	E	D	K	F	D	-	F	D
AtCAT2			E	W	K	L	F	I	Q	I	I	D	P	A	D	E	D	K	F	D	-	F	D
AtCAT3			E	W	K	L	F	I	Q	T	M	D	P	A	D	E	D	K	F	D	-	F	D
NtCAT1			E	W	K	L	F	I	Q	T	M	D	P	D	H	E	D	R	F	D	-	F	D
NbCAT1			E	W	K	L	F	I	Q	T	M	D	P	D	H	E	D	R	F	D	-	F	D
SICAT3			E	W	K	L	F	I	Q	T	I	D	P	D	H	E	D	R	F	D	-	F	D
StCAT2			E	W	K	L	F	I	Q	I	M	D	P	D	H	E	D	K	F	D	-	F	D
GhCAT1			E	W	K	L	F	I	Q	T	I	D	P	D	H	E	D	K	F	D	-	F	D
SpCAT1			E	W	K	L	Y	I	Q	V	M	D	P	D	H	E	D	R	F	D	-	F	D

Supplemental Figure S5. Sequence alignment of Ca²⁺ binding domain from different plant catalase. *Arabidopsis thaliana* [CAT1 (accession no. X94447), CAT2 (accession no. X64271) and CAT3 (accession no. U43147)], rice *Oryza sativa* (OsCAT A; accession no. XP_015629749.1), *Zea mays* (ZmCAT1; accession no. PWZ57146.1), *Nicotiana tabacum* (NtCAT, accession no. U93244), *Nicotiana bentamiana* (NbCAT; accession no. EU998969), *Gossypium hirsutum* (GhCAT1; accession no. XP_016687939.1), *Solanum tuberosum* (StCAT2, accession no. KAH0685113.1), *Triticum turgidum ssp durum* (TdCAT1; Accession no. KP696753), *Aeogilops tauchii* (AetCAT1; accession no. XP_020164896.1), *Brachypodium distachyon* (BdCAT1, accession no. XP_003558892.1) and *Sorghum bicolor* (SbCAT1, accession no. XP_021304262.1).

a)



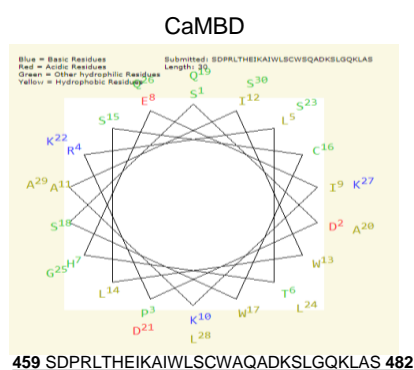
b)

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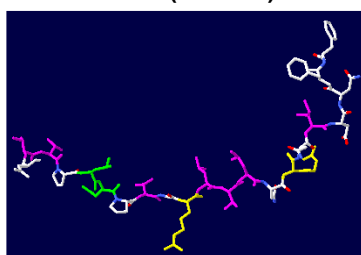
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..... 0000000000 0024444444 4444444444 4420000000 00
  
```

c)



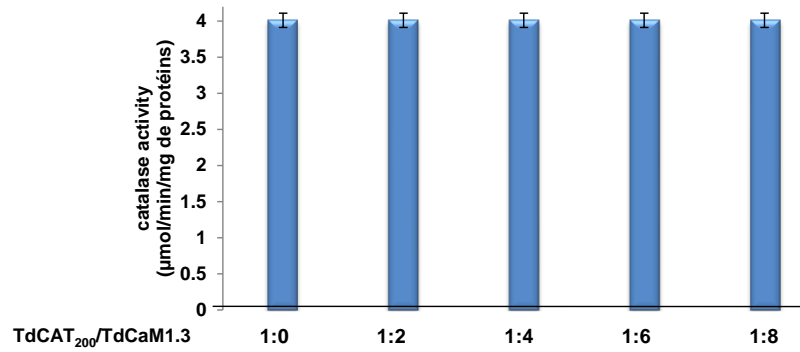
d)

CaMBD (459-482)

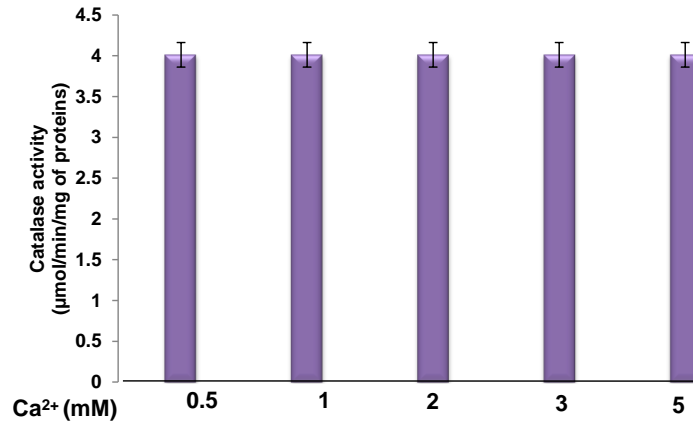


Supplemental Figure S6. TdCAT1 harbors a conserved CaMBD located at the C-terminal part of the protein. (a) Schematic presentation of TdCAT1 with identification of the conserved CaMBD represented in black. (b) Localization and Identification of the calmodulin binding domain as revealed by Calmodulin target Database. (c) Helical wheel projection of the motif for the Calcium dependent calmodulin binding domain (underlined sequences). Dashed lines separate proposed hydrophobic (h) and basic (+) faces of the NT and CT wheels. (d) 3D structure of CaMBD as revealed by spd v 1.4 program. The basic amino acids are represented bin yellow, the hydrophobic amino acids are represented in pink.

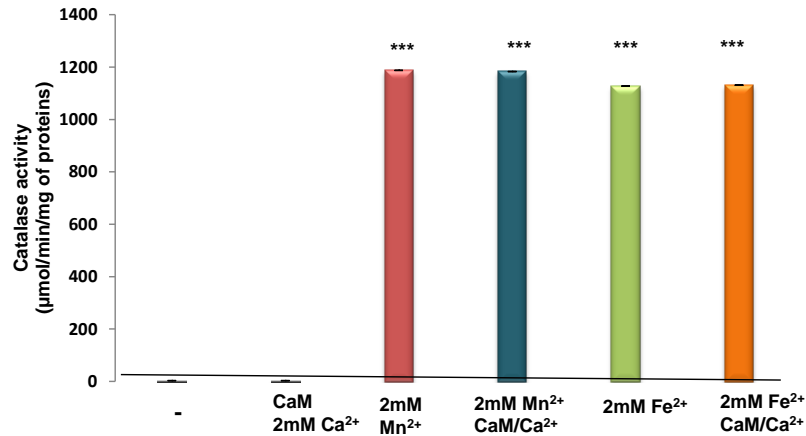
a)



b)



c)



Supplemental Figure S7. The activity of His_TdCAT1₂₀₀ is not affected by TdCaM1.3/Ca²⁺ complex. (a) Catalase activities were measured according to the same conditions indicated before with a His_TdCAT1₂₀₀/TdCaM1.3 molar ratio ranging from 1:0 to 1:8 1:4. **(b)** TdCAT1₂₀₀ activity was not modified in presence of increasing concentrations of Calcium ranging from 0.5 to 5 mM. **(c)** Catalase activity of deleted form His-TdCAT₂₀₀ was stimulated in presence of Mn²⁺ and Fe²⁺ but not with CaM/Ca²⁺ complex. All data are mean values ± S.E of initial rate from three independent assays. (***) indicates value significantly different from the control. Statistical significance was assessed by applying the ANOVA test with <0.005.