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Supporting information for article:

Structure of a superoxide dismutase from a tardigrade: *Ramazzottius varieornatus* strain YOKOZUNA-1

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Table S1 Sequence of *Rv*SOD15 used in this study.

Original sequence of *Rv*SOD15 RvY_13070.1 (Accession number: GAV02514.1)

MTRPLALIIFLVAILTNTDPSRSDAGSDPVNYLFRGPVTAVAAIAGEGEHAGIKGSL TFLQKSLDGRTVINGTISGLPEGKHGLHIVDSGDMTKGCYITTAKGHLNPFNLSHG APSDSARHVGDLGNIYADDTGISVINLTDTVISLFPTPAFVIGRILVIHTTYDDLGRG GSPVSKVNGNAGGRLACGIISYV

Table S2 Primers used in this study.

Protein	**Primers**	**Sequence (5'-3')**
*Rv*SOD15	*Rv*SOD15_OE_PCR_F1	AACTTGTATTTCCAGTCCAATCAT AGCGATCCGGTGAA
	*Rv*SOD15_ OE_PCR_F2	CATCATCATCACGAGAACTTGTA TTTCCAGTCC
	*Rv*SOD15_ OE_PCR_F3	CATCATCATCATCATCACGAGAA CTTGTA
	*Rv*SOD15_ OE_PCR_F4	AGGAGATATACCATGCATCATCA TCATCATCACGA
	*Rv*SOD15_ OE_PCR_R1	GTTAGCAGCCGGATCTTAAACAT AGCTGATGATACC
	*Rv*SOD15_p28_F	AGGAGATATACCATGCATCATCA TCATCATCACGA
	*Rv*SOD15_p28_R	GTTAGCAGCCGGATCTTAAACAT AGCTGATGATACC
*Rv*SOD15-V61H	*Rv*SOD15-V61H_F	CACGACAGCGGTGATATGACCAA GGGC
	*Rv*SOD15-V61H_R	TATCACCGCTGTCGTGAATGTGC AGGCCATGTTTACCC
pET-28a(+) vector	pET28_TAA_F	TAAAGATCCGGCTGCTAACAA
	pET28_ATG_R	CATGGTATATCTCCTTCTTAAAG

Table S3 Amino acids sequence employed in the sequence alignment.

Organism species	Gene IDs
Bombyx mori	*Bm*SOD: NP_001037084.1
Bos taurus	*Bt*SOD: NP_777040.1
Caenorhabditis elegans	*Ce*SOD: NP_001021956.1
Homo sapiens	*Hs*SOD: NP_000445.1
Hypsibius exemplaris	*He*SOD: OQV16439.1
Taenia solium	*Ts*SOD: AAL66230.1
Ramazzottius varieornatus	*Rv*SOD_CuZN1: GAU87854.1 *Rv*SOD_CuZN2: GAU87855.1 *Rv*SOD_CuZN3: GAU87856.1 *Rv*SOD_CuZN4: GAU87857.1 *Rv*SOD_CuZN5: GAU91516.1 *Rv*SOD_CuZN6-1: GAU91519.1 *Rv*SOD_CuZN6-2: GAU60520.1 *Rv*SOD_CuZN6-3: GAU60521.1 *Rv*SOD_CuZN7: GAU92919.1 *Rv*SOD_CuZN8: GAV05942.1 *Rv*SOD_CuZN9: GAV07483.1 *Rv*SOD_CuZN10: GAU95120.1 *Rv*SOD_CuZN11: GAU98317.1 *Rv*SOD_CuZN12: GAU99963.1 *Rv*SOD_CuZN13: GAU99964.1 *Rv*SOD_CuZN14: GAU99965.1 *Rv*SOD_CuZN15: GAV02514.1 *Rv*SOD_CuZN16-1: GAV02928.1 *Rv*SOD_CuZN16-2: GAV02930.1

Table S4 Homology search of *Rv*SOD_CuZNs' and SODs used in this study using HHpred PDB mmcif70_31_Jul.

Gene	Score	Identities (%)	Selected best hit	PDB
*Rv*SOD_CuZN1	146.65	36.0	*Onchocerca volvulus* SOD	5IN2
*Rv*SOD_CuZN2	102.90	45.0	*Bombyx mori* SOD	3L9Y
*Rv*SOD_CuZN3	167.53	50.0	*Homo sapiens* ALS SOD	4A7U
*Rv*SOD_CuZN4	147.11	67.0	*Homo sapiens* ALS SOD	4A7U
*Rv*SOD_CuZN5	151.31	63.0	*Homo sapiens* ALS SOD	4A7U
*Rv*SOD_CuZN6 variant 1	155.13	55.0	*Alvinella pompejana* SOD	3F7L
*Rv*SOD_CuZN6 variant 2	123.94	40.0	*Homo sapiens copper chaperone*	3F7L
*Rv*SOD_CuZN6 variant 3	97.25	35.0	*Cryptococcus liquefaciens* SOD	3CE1
*Rv*SOD_CuZN7	37.23	45.0	*Alvinella pompejana* SOD	3F7L
*Rv*SOD_CuZN8	162.84	29.0	*Candida albicans* SOD	4N3T
*Rv*SOD_CuZN9	184.84	49.0	*Homo sapiens* ALS SOD	4A7U
*Rv*SOD_CuZN10	147.35	50.0	*Cryptococcus liquefaciens* SOD	3CE1
*Rv*SOD_CuZN11	166.58	52.0	*Bombyx mori* SOD	3L9Y
*Rv*SOD_CuZN12	133.32	47.0	*Bombyx mori* SOD	3L9Y
*Rv*SOD_CuZN13	125.93	49.0	*Alvinella pompejana* SOD	3F7L
*Rv*SOD_CuZN14	54.21	45.0	*Alvinella pompejana* SOD	3F7L
*Rv*SOD_CuZN15	137.01	47.0	*Alvinella pompejana* SOD	3F7L
*Rv*SOD_CuZN16 variant 1	198.28	43.0	*Onchocerca volvulus* SOD	5IN2
*Rv*SOD_CuZN16 variant 2 **	326.00	46.5	C6 domain containing protein	-
*He*SOD	133.01	50.0	*Alvinella pompejana* SOD	3F7L
*Ce*SOD	166.10	100.0	*Caenorhabditis elegans* SOD	3KBE
*Ts*SOD	149.97	66.0	*Alvinella pompejana* SOD	3F7L

*Hs*SOD1	155.57	99.0	*Homo sapiens* ALS SOD	4A7U
*Bt*SOD	157.87	82.0	Homo sapiens ALS SOD	4A7U
*Bm*SOD	170.03	99.0	*Bombyx mori* SOD	3L9Y

** *Rv*SOD16 variant 2 hit result was replaced with UniRef50.

Table S5 Anomalous X-ray diffraction datasets of *Rv*SOD15.

	Anomalous CU	**Anomalous Zn**
PDB ID		
Data Collection		
X-ray source	SPring-8 BL44XU	Spring-8 BL44XU
Detector	EIGER X 16 M	EIGER X 16 M
Wavelength (Å)	1.3799	1.2799
Space group	P_1	P_1
Cell Dimensions		
a, b, c(Å)	66.26 68.19 68.29	66.27 68.23 68.32
α, β, γ (°)	94.91 108.36 114.41	94.91 108.39 114.40
Resolution (Å)	45.35-3.5 (3.83-3.50)	45.37-3.5 (3.83-3.5)
Total reflections	43360 (10433)	43134 (9913)
Unique reflections	12238 (2920)	12262 (2920)
Rmerge†(%)	0.057 (0.084)	0.053 (0.074)
Rmeas‡(%)	0.068 (0.099)	0.063 (0.088)
Rpim‡(%)	0.036 (0.052)	0.034 (0.047)
I/σ(I)	12.5 (8.9)	23.5 (16.0)
CC1/2§	0.996 (0.992)	0.996 (0.992)
Completeness (%)	97.0 (97.1)	97.2 (97.3)
Anomalous completeness (%)	91.1 (92.5)	89.8 (91.8)
Redundancy	3.5 (3.6)	3.5 (3.4)
Anomalous redundancy	1.7 (1.8)	1.7 (1.7)

Table S6 *Rv*SODs functional prediction.

Gene	SOD Fold	Ligand	SOD Type	Group
*Rv*SOD_CuZN1	EL and metal-binding loops deletion, open catalytic site	-	Cu-only SOD like	Delta
*Rv*SOD_CuZN2	EL deletion, alike Cu-only SOD, open catalytic site	Cu*	Cu-only SOD like	Gamma
*Rv*SOD_CuZN3	Standard, *β*1, *β*8-sheets elongated	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN4	Standard	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN5	Standard	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN6 variant 1	Standard	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN6 variant 2	EL loop deletion, alike Cu-only SOD, open catalytic site	Cu, Zn	Cu-only SOD like	Gamma
*Rv*SOD_CuZN6 variant 3	Dislike SOD	-	unknown	Epsilon
*Rv*SOD_CuZN7	Dislike SOD	-	unknown	Epsilon
*Rv*SOD_CuZN8 Domain 1	Electrostatic loop deletion, alike Cu-only SOD, open catalytic site	-	Cu-only SOD like	Gamma
*Rv*SOD_CuZN8 Domain 2	Electrostatic loop deletion, alike Cu-only SOD, open catalytic site	Cu*	Cu-only SOD like	Gamma
*Rv*SOD_CuZN9	Standard, *β*6-sheet elongated	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN10	Standard, *β*4, *β*8-sheets elongated	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN11	Standard, *β*4-sheets elongated	Cu, Zn	CuZn-SOD	Alpha
*Rv*SOD_CuZN12	*β*4, *β*6-sheets deletion	Cu, Zn	CuZn-SOD	Beta
*Rv*SOD_CuZN13	Standard	Cu, Zn	CuZn-SOD	Alpha

*Rv*SOD_CuZN14	Dislike SOD	-	unknown	Epsilon
*Rv*SOD_CuZN15	Elongated metal-binding loop	Cu, Zn	CuZn-SOD	Beta
*Rv*SOD_CuZN16 variant 1	*β*4-sheet elongation and *β*4-sheet shorten	Cu, Zn*	CuZn-SOD	Beta

*Required experimental further validation

Figure S1 Sequence alignment of *R. varieornatus* CuZnSODs. *Rv*SOD8 and *Rv*SOD16 variant 2 are excluded from sequence alignment. *Rv*SOD8 has a tundem repreat of SOD-like domains and may be a so-called copper-only SOD-repeat protein. *Rv*SOD_CuZNSOD16 variant 2 does not show sequence similarity to CuZnSODs.

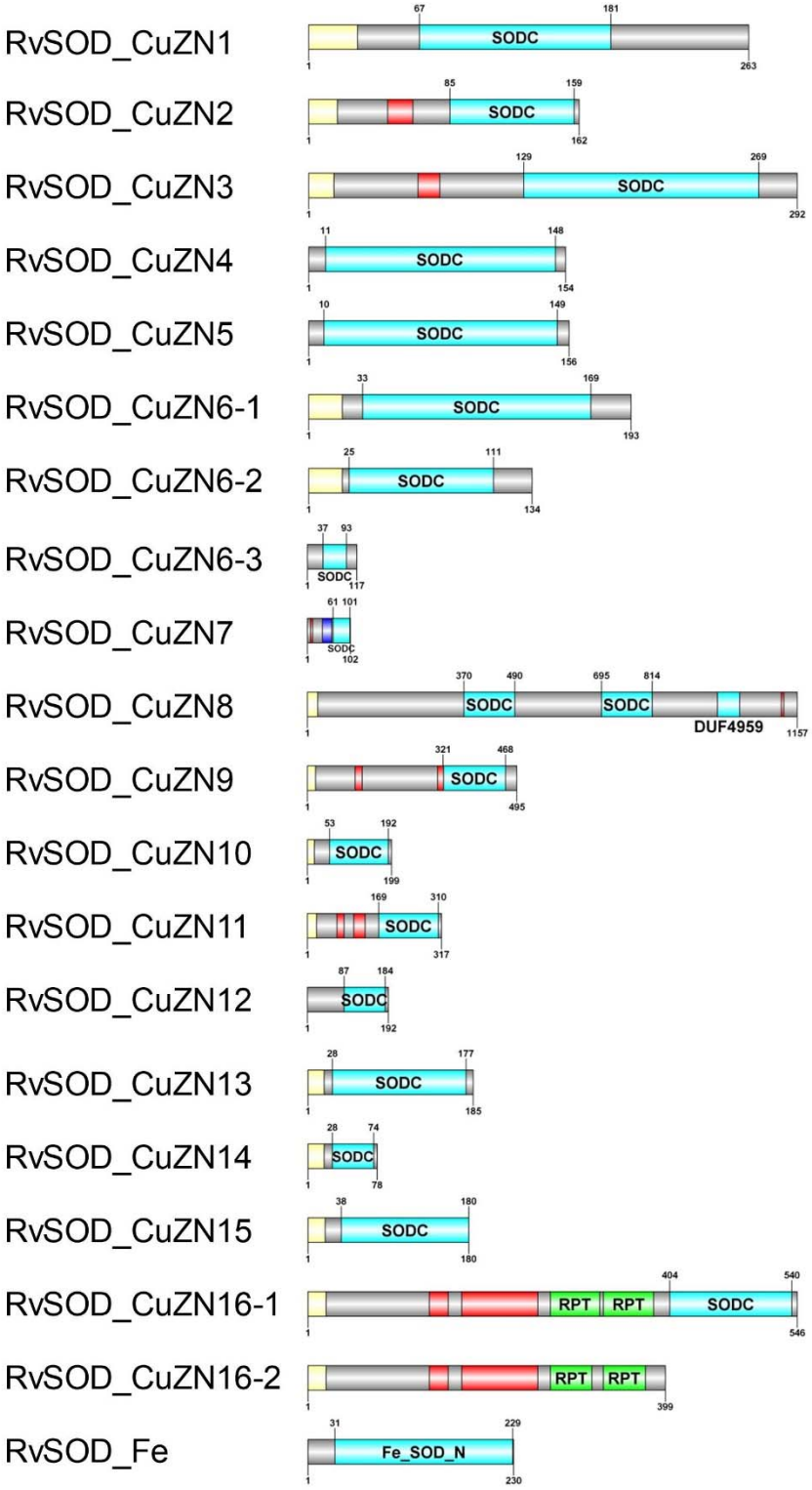

Figure S2 The putative domain structure of *R. varieornatus* CuZnSODs. Digits show the amino acid positions. Yellow box, signal peptide; cyan box, SOD domain; red box, low complexity region; green box, repeat (RPT) domain; blue box, transmembrane domain. *Rv*SOD_CuZNSOD16 variant 2 does not show similarity to the SODC domain.

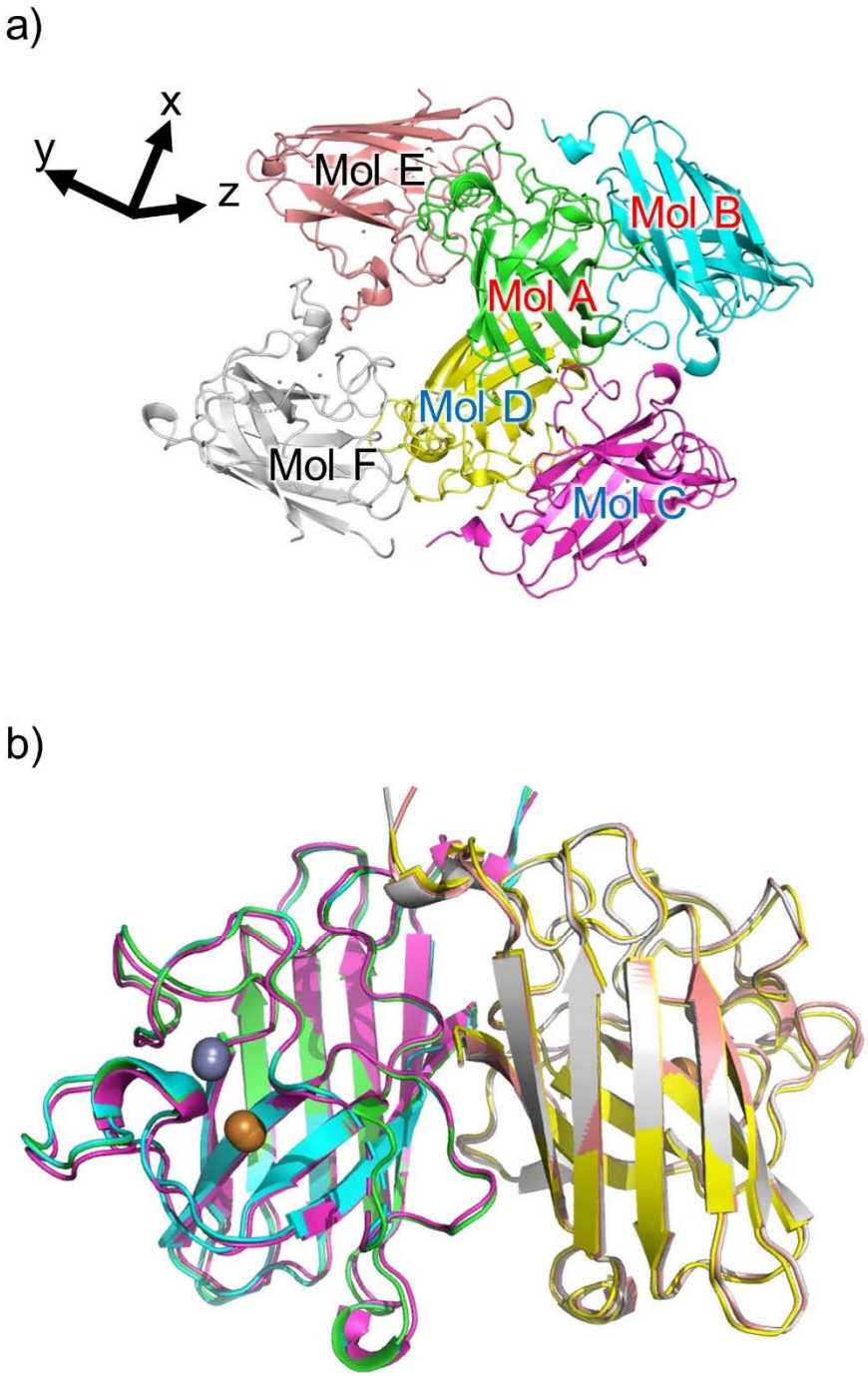

Figure S3 Crystal structure of wild-type *Rv*SOD15. **a)** Components of the unit cell. Molecule A and E form a dimer, Molecule B and F form another dimer. Molecule C and D form a dimer through a symmetry operation. **b)** Structural alignment of three dimers contained in the unit cell.

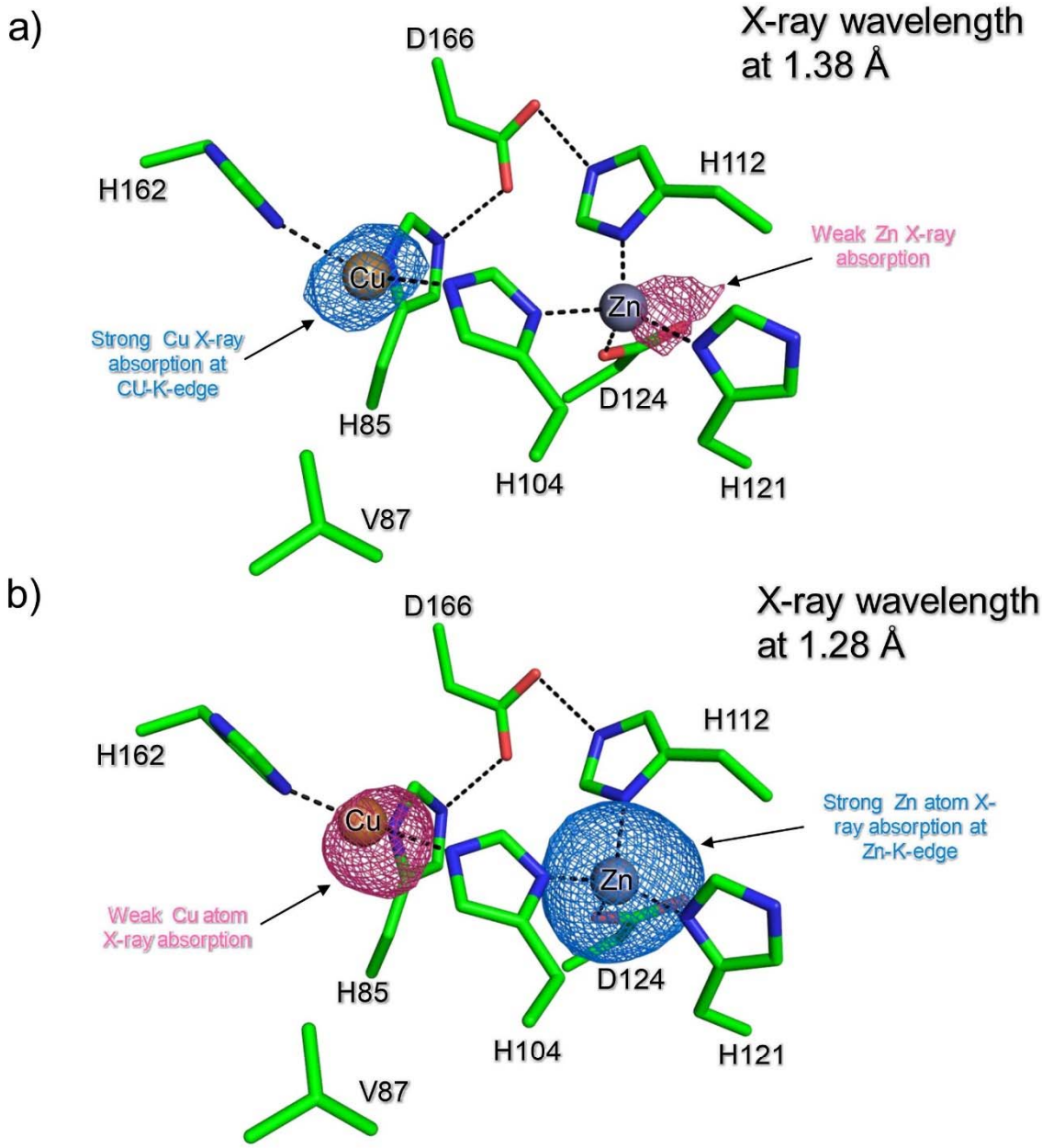
a)
X-ray wavelength at 1.38 Å
D166
H112
H162
Weak Zn X-ray absorption
Cu
Zn
Strong Cu X-ray absorption at CU-K-edge
D124
H85
H104
H121
V87
b)
X-ray wavelength at 1.28 Å
D166
H112
H162
Strong Zn atom X-ray absorption at Zn-K-edge
Cu
Zn
Weak Cu atom X-ray absorption
D124
H85
H104
H121
V87

Figure S4 Anomalous signal obtained from Cu and Zn anomalous diffractions. **a)** Copper anomalous signal at 3.5 sigma observed when 1.38 Å wavelength X-ray was used. Cu atom show a strong anomalous signal at K-edge (cyan) compare to Zn atom show a weaker anomalous signal (warmpink, at 2.5 sigma) at 1.38 Å wavelength X-ray. **b)** Zinc anomalous signal at 3.5 sigma observed when 1.28Å wavelength X-ray was used. Zn atom show a strong anomalous signal at K-edge (cyan) compare to Cu atom show a weaker anomalous signal (warmpink) at 1.28 Å wavelength X-ray.

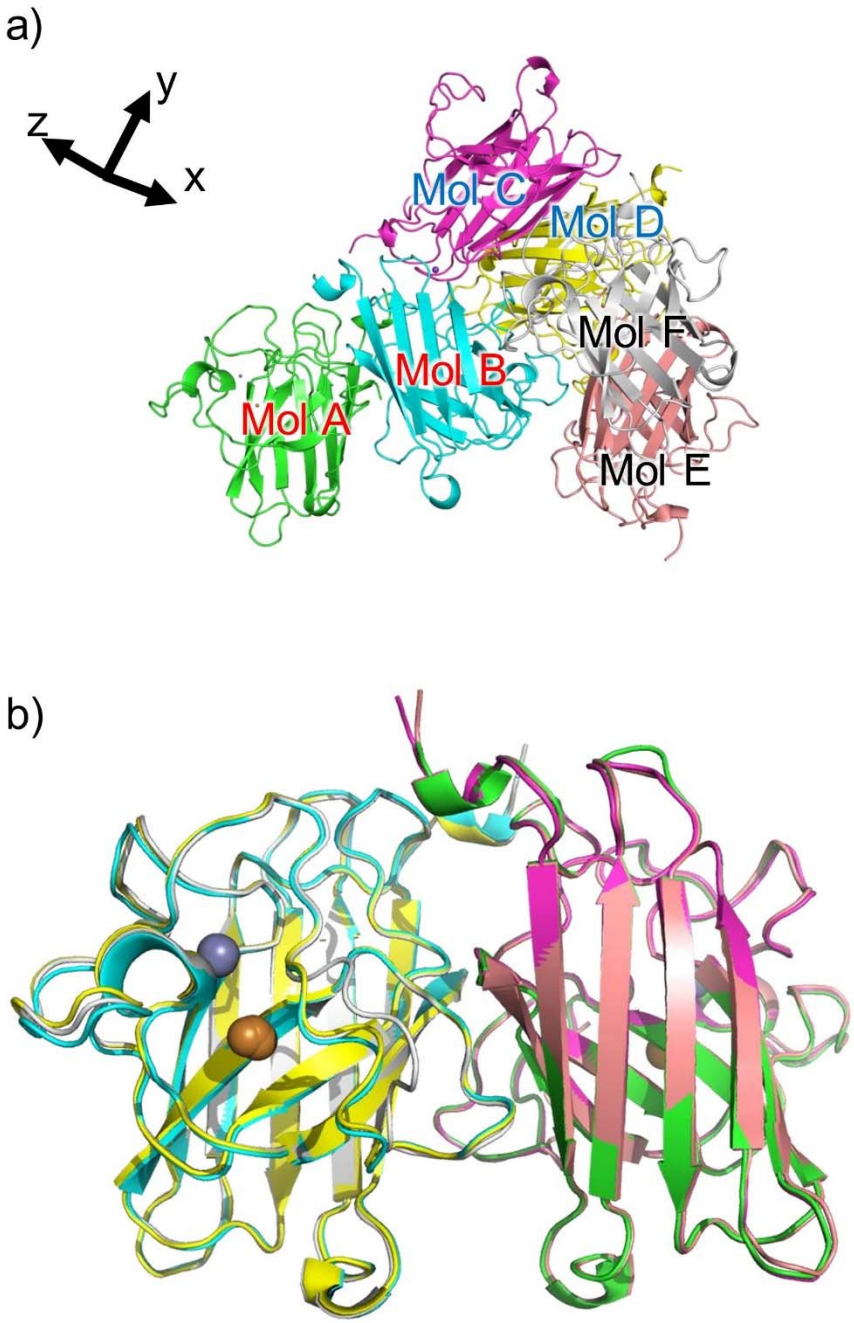
a)
y
z
x
Mol C
Mol D
Mol F
Mol B
Mol A
Mol E
b)

Figure S5 Crystal structure of V87H *Rv*SOD15 mutant. **a)** Components of the asymmetric unit. Molecule A and B form a dimer. Molecule C and D and Molecule E and F respectively form dimers through symmetry operations. **b)** Structural alignment of three dimers contained in the unit cell.

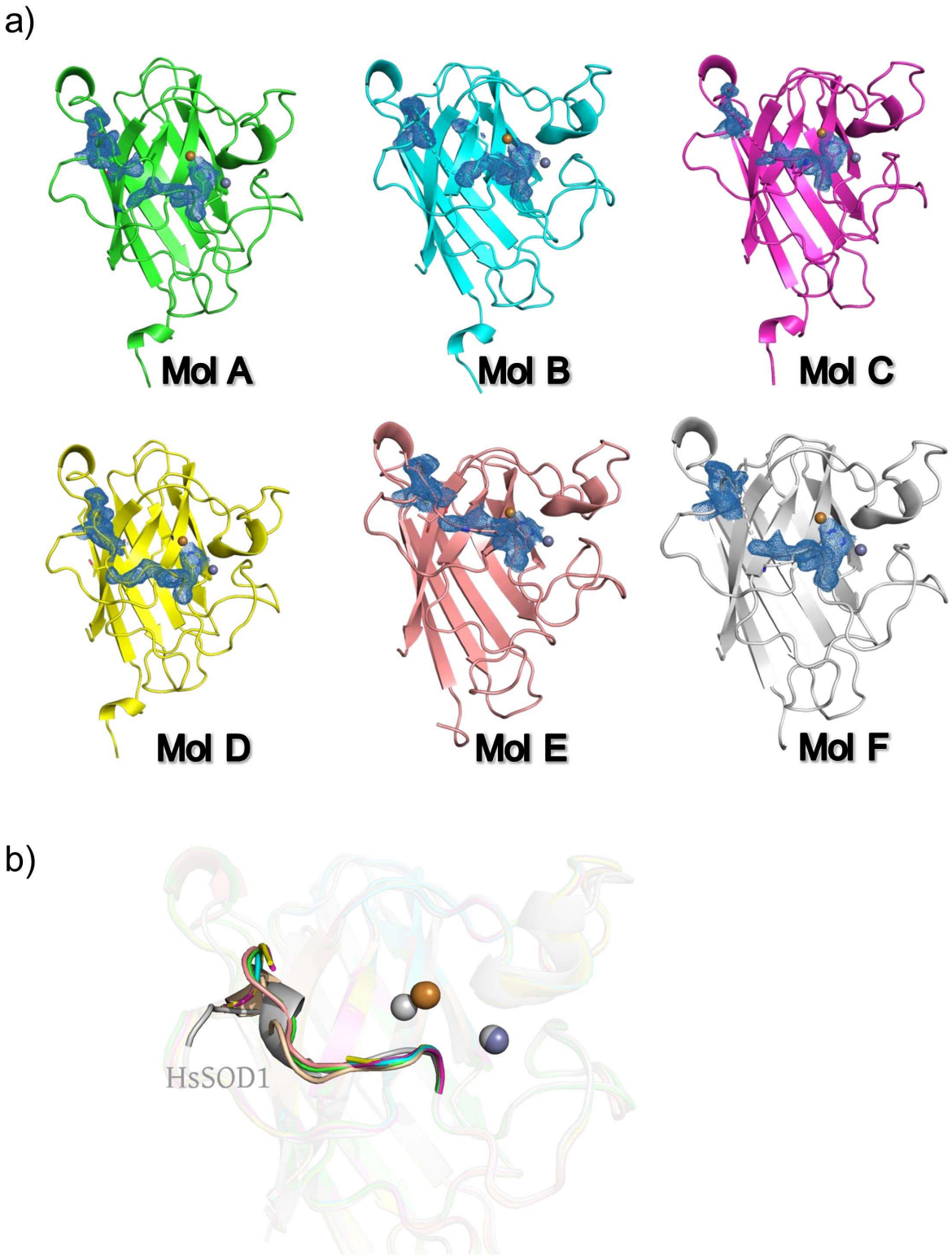

Figure S6 Structural variations of the metal-binding loop in the wild-type structure. **a)** Sigma-A-weighted $2mF_o$-DF_c map for the region from Cys96 to His104 (1 σ) are shown by blue meshes. **b)**

Comparison of the metal-binding loop of *Rv*SOD15 with that of *Hs*SOD1 (PDB code ID: 1PU0, Chain B).

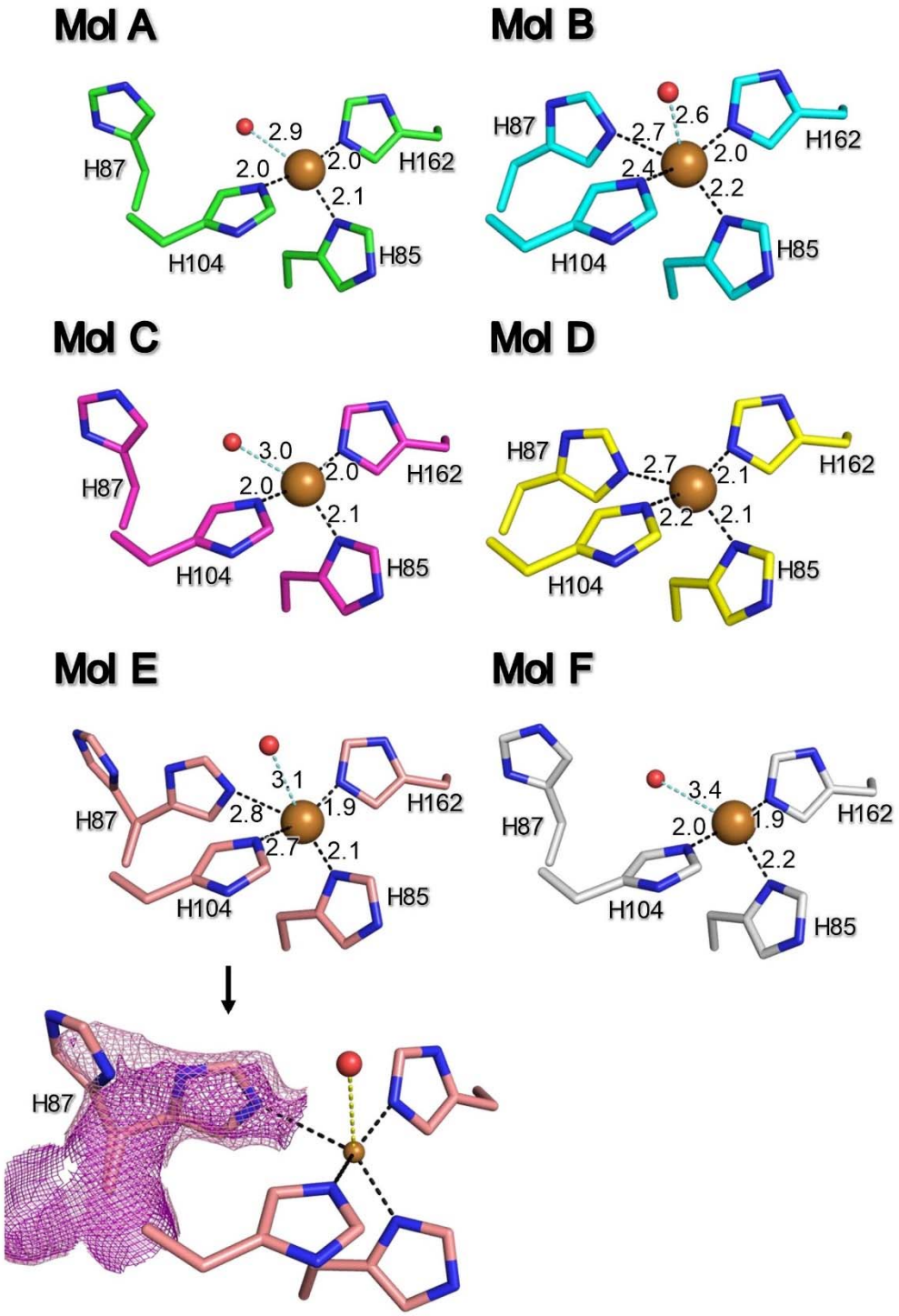

Figure S7 Structures of the reconstructed Cu site in the V87H *Rv*SOD15 mutant structure. Coordination bonds are shown by dotted black lines. Water molecules are shown by red spheres. Weak interactions of the water ligands with the Cu center are shown by dotted cyan lines. Distances are shown by the ångström unit. Sigma-A-weighted $2mF_o$-DF_c map for His87 are shown by purple (1 σ) and pink (0.7 σ) meshes.

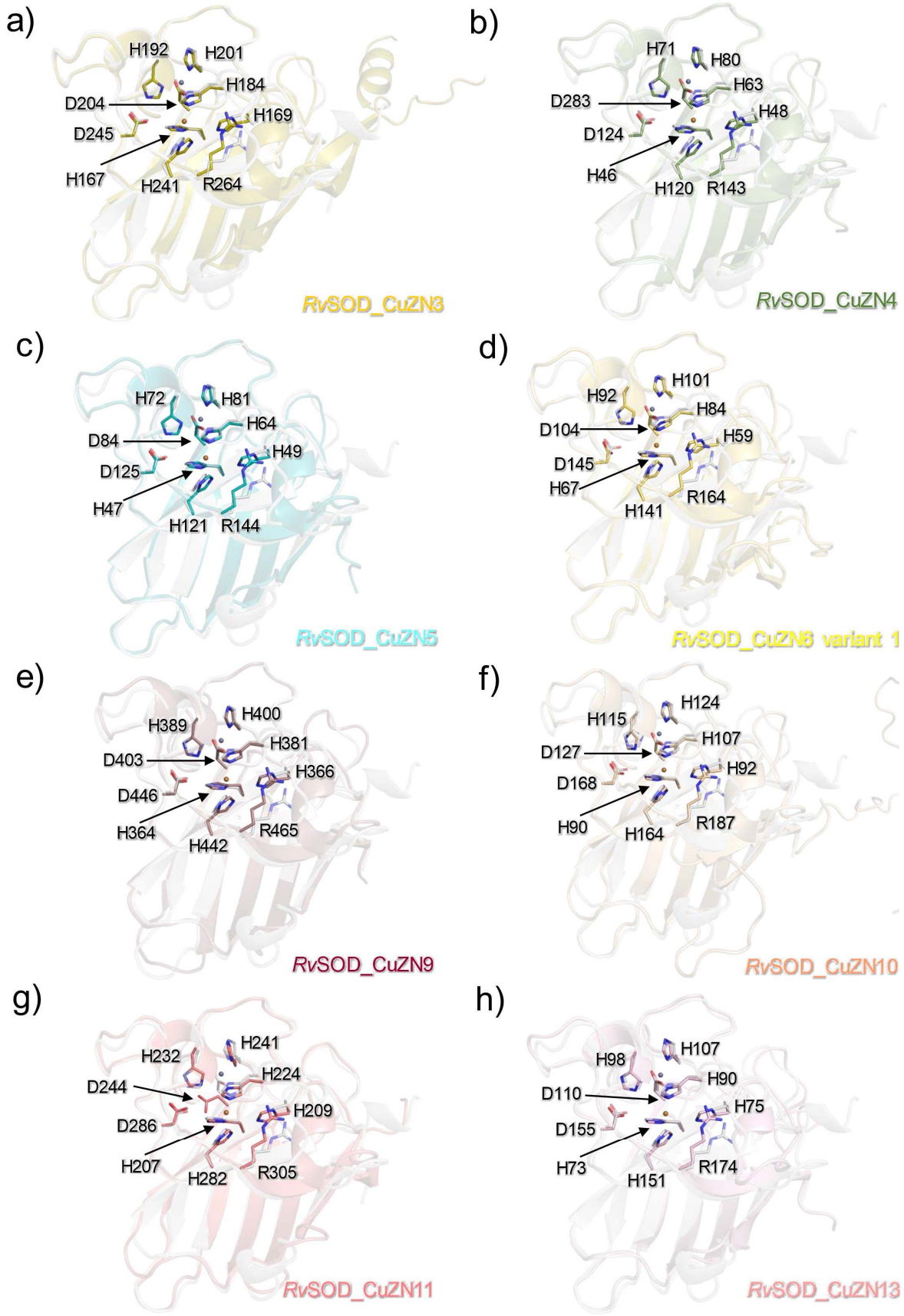

Figure S8 Structural comparison between *Rv*SOD_CuZN15 and alpha-group of typical *Rv*SOD_CuZnSOD model structure. The N-terminal signal peptide is removed from modeling and

metal-binding ligands are shown by sticks. The *Rv*SOD15 structure is shown in grey. a) *Rv*SOD_CuZN3 (Overall pLDDT 89.61; *r.m.s.d of* Cα atoms value of 0.74 Å). b) *Rv*SOD_CuZN4 (Overall pLDDT 97.51; *r.m.s.d of* Cα atoms value of 0.69 Å). c) *Rv*SOD_CuZN5 (Overall pLDDT 97.32; *r.m.s.d of* Cα atoms value of 0.60 Å). d) RvSOD6_CuZN6 variant 1 (Overall pLDDT 87.11; *r.m.s.d of* Cα atoms value of 0.60 Å). e) *Rv*SOD_CuZN9 (Overall pLDDT 94.71; *r.m.s.d of* Cα atoms value of 0.61 Å). f) *Rv*SOD_CuZN10 (Overall pLDDT 85.36; *r.m.s.d of* Cα atoms value of 0.65 Å). g) *Rv*SOD_CuZN11 (Overall pLDDT 70.98; *r.m.s.d of* Cα atoms value of 0.63 Å). h) *Rv*SOD_CuZN13 (Overall pLDDT 94.42; *r.m.s.d of* Cα atoms value of 0.78 Å).

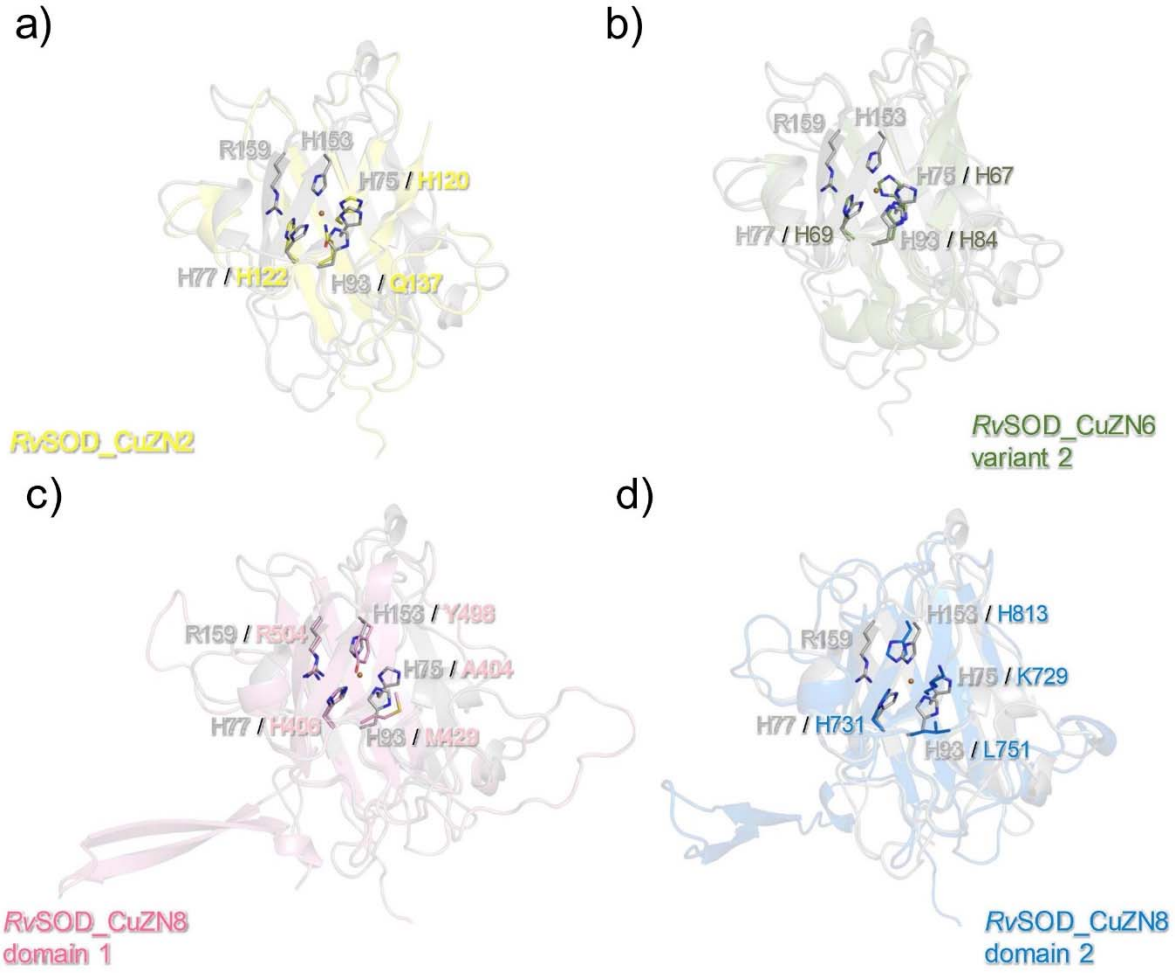

Figure S9 Structural comparison between *Rv*SOD_CuZN15 and gamma group of typical Cu-only SOD-like model structures. Structural superimposition was performed against Cu-only SOD5 from *Candida albicans*, PDB: 4N3T. The similarity to Cu-only SOD cause by the deletion of electrostatic loop. Yet, *Rv*SODs Cu-SOD-like acquired numerous mutations at Cu-binding site. a) *Rv*SOD2 (yellow) and *r.m.s.d of* Cα atoms value of 2.52 Å. b) *Rv*SOD6 variant 2 (smudge) and *r.m.s.d of* Cα atoms value of 1.72 Å. c) *Rv*SOD8 domain 1 (pink) and *r.m.s.d of* Cα atoms value of 0.76 Å. d) *Rv*SOD8 domain 2 (marine) and *r.m.s.d of* Cα atoms value of 1.01 Å.

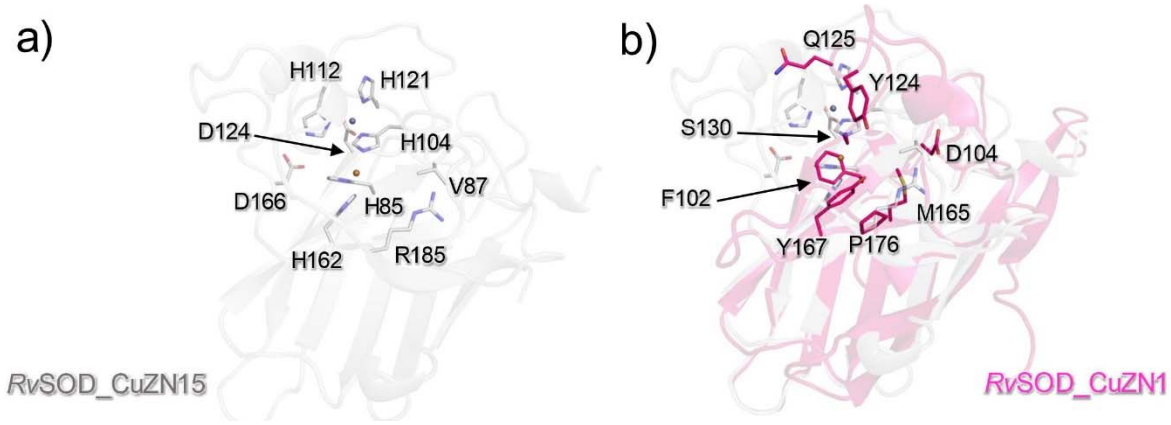

Figure S10 Structural comparison between *Rv*SOD_CuZN15 and delta-group of atypical Cu-only SOD-like *Rv*SODs. The *Rv*SOD15 structural alignment shown in grey. a) *Rv*SOD15. b) *Rv*SOD_CuZN1 (Overall pLDDT value is 85.03 and *r.m.s.d of* Cα value of 1.00 Å).

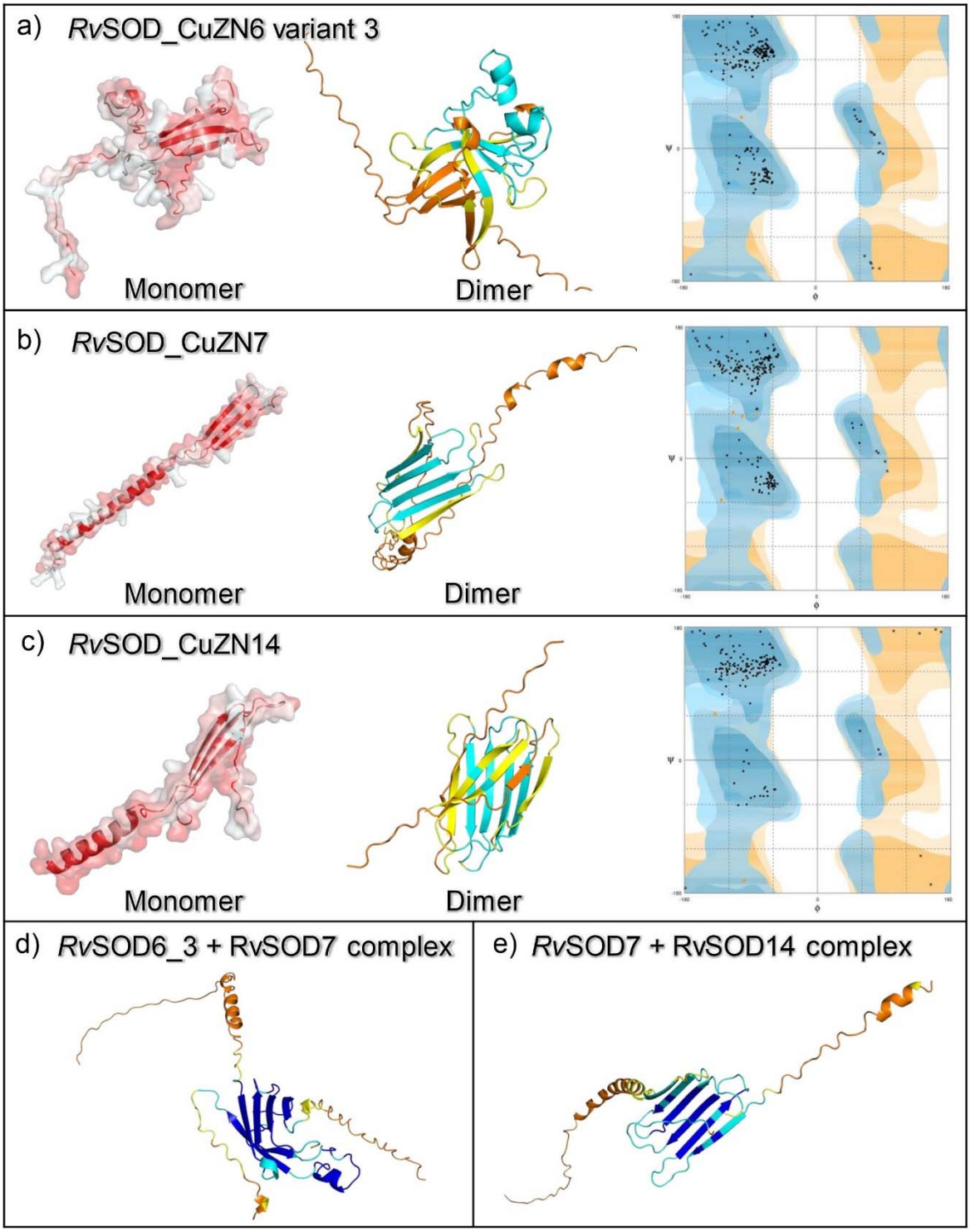

Figure S11 Model structures of epsilon group *Rv*SODs. This group of *Rv*SODs does not resemble to any SOD structures. a) *Rv*SOD_CuZN6 variant 3 monomer and its predicted dimeric conformations. The overall monomer pLDDT score is 76.15, but oligomer overall pLDDT score is 46.93. Left, monomer, red is hydrophobic residue; Middle, local pLDDT of dimer conformation; Right, Ramachandran plot with 99.5% of favoured and allowed regions. b) *Rv*SOD_CuZN7 monomer and dimeric conformations. The overall monomer pLDDT score is 74.82, but the oligomer overall

pLDDT score is 47.93. Left, monomer, red is hydrophobic residue; Middle, local pLDDT of dimer conformation; Right, Ramachandran plot with 100.0% of favoured and allowed regions. c) *Rv*SOD_CuZN14 monomer and its predicted dimeric conformations. The overall monomer pLDDT score is 80.64, but the oligomer overall pLDDT score is 58.37. Left, monomer, red is hydrophobic residue; Middle, local pLDDT of dimer conformation; Right, Ramachandran plot with 100.0% of favoured and allowed regions. d) Predicted *Rv*SOD_CuZN6 variant 3 and *Rv*SOD7 complex structure. The structure colored according to local pLDDT. The overal pLDDT score is 61.56. e) Predicted *Rv*SOD_CuZN7 variant 3 and *Rv*SOD_CuZN14 complex structure. The structure colored according to local pLDDT. The overal pLDDT score is 62.83. The inter-molecular complex shows a lower overall pLDDT score as each protein containing local unique regions. The local pLDDT score is rendered in Blue, pLDDT = (90-100); Cyan, pLDDT = (70-90); Yellow, pLDDT = (50-70); Orange, pLDDT = (0-50).

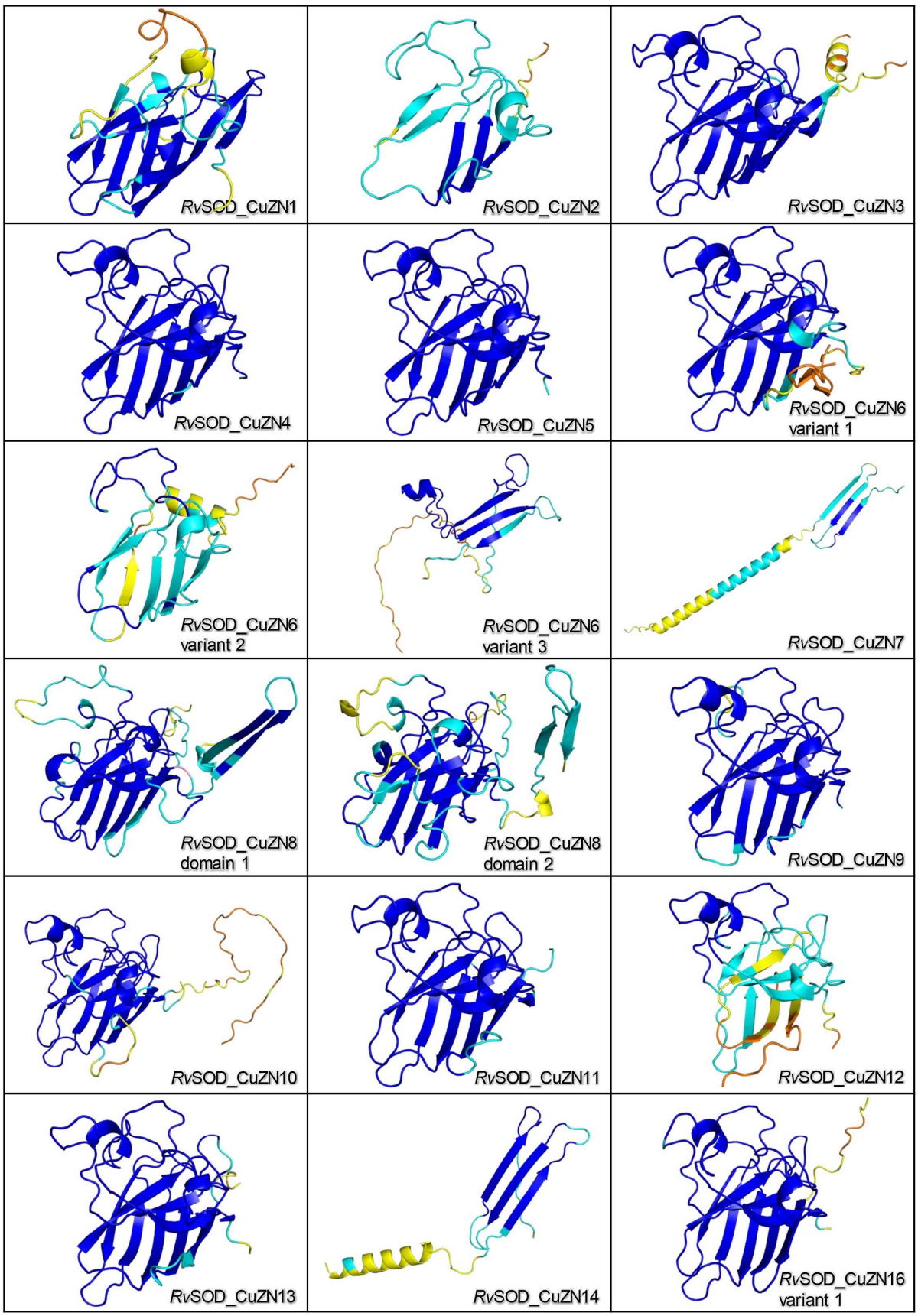

Figure S12 Local pLDDT of *Rv*SODs model structure. pLDDT score is rendered in Blue, pLDDT = (90-100); Cyan, pLDDT = (70-90); Yellow, pLDDT = (50-70); Orange, pLDDT = (0-50).