

Supplementary Information

for

4 Coordination and oxidation properties of ZBTB20: CX₂CX₁₂HX₃H-type zinc fingers

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35 **References**

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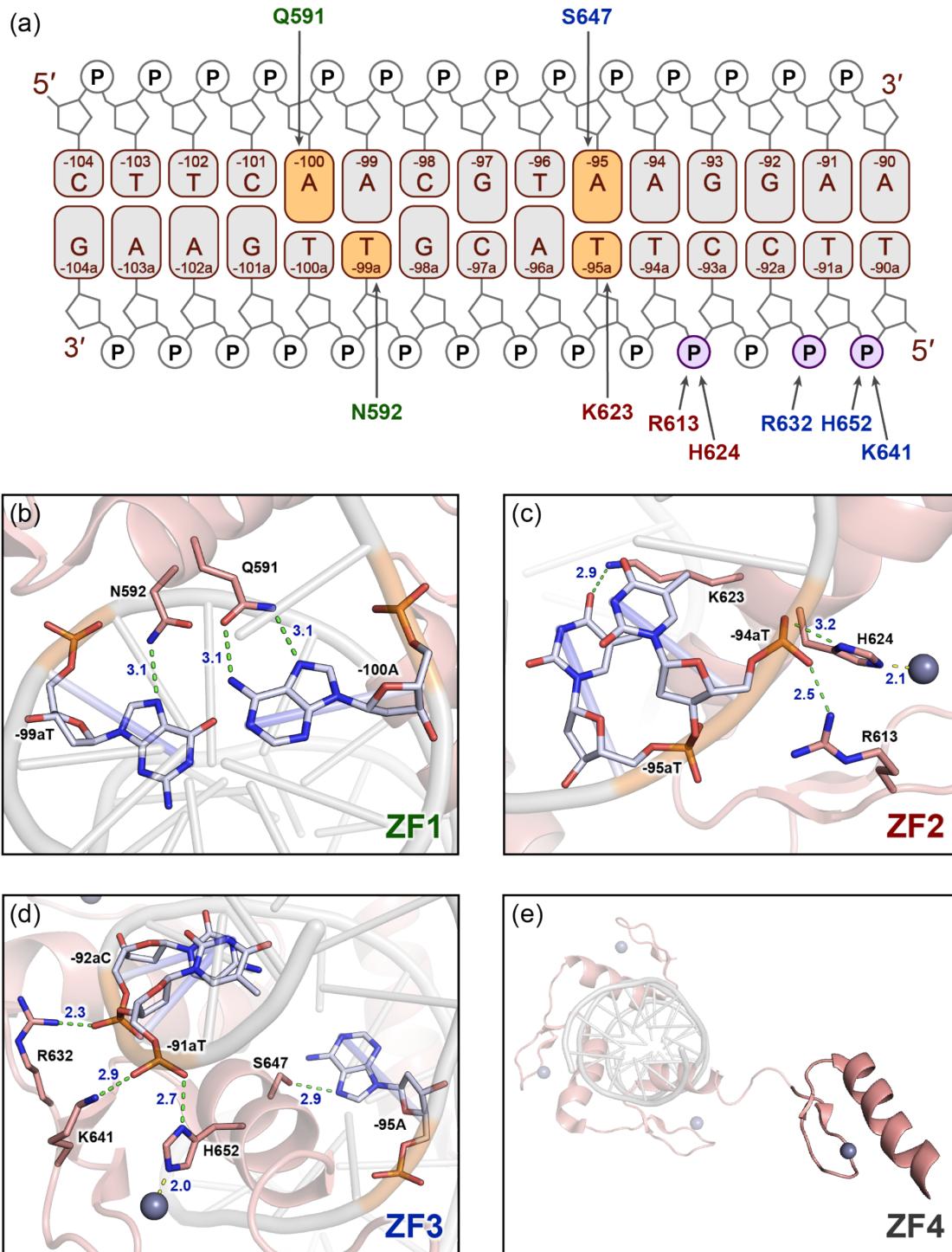
BTB domain

1 MTERIHSINLHNFSNSVLETLNEQRNRGHFCDVTVRIGHGMLRAHRCVLAAGSPFFQDKL 60
 61 LLGYSDIEIPSVVSQSVQKLIDFMYSGVLRVSQSEALQILTAASILQIKTVIDECTRIV 120
 121 SQNVGDVFPGIQDSDQDT PRGTPESGTSGQSSDTESGYLQSHPQHSVDRIYSALYACSMQ 180
 181 NGSGERSFYSGAVVSHHETALGLPRDHMEDPSWITRIHERSQQMERYLSTTPETTHCRK 240
 241 QPRPVRIQTLVGNIHIKQEMEDDYDYYGQQRVQILERNEEECTEDTDQAEGETEPKGE 300
 301 SFDSGVSSSIGTEPD SVEQQFGPGAARD SQAEP TQPEQAAEAPAEGGPQTNQLETGASSP 360
 361 ERSNEVEMDSTVITVSNSDKSVLQQPSVNTS ^{ZF1} SIGQPLPSTQLYLRQTELTSNLRMPLTL 420
 421 TSNTQVIGTAGNTYLPALFTTQ ^{ZF2} PAGSGPKPFLFSLPQPLAGQQTQFVTFQPGLSTFTAQ 480
 481 LPAPQPLASSAGHSTASGQGEKKPYE ^{ZF3} CTLICNKTF TAKQNYVK ^{ZF4} HMFVHTGEKPHQCSICWR 540
 541 SFSLKDYL ^{ZF5} IKHMVTHTGV RAYQCSICCNKRFTQKSSLNV ^{ZF4} HMR LHRGEKSYE ^{ZF5} CYICKKKFSH 600
 601 KTLLERHVALHSASNGTPPAGTPPGARAGPPGVVACTEG ^{ZF5} TTYVC ^{ZF5} SVC PAKFDQIEQFNDH 660
 661 Linker MRMHVSDG 668

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38 **Figure S1.** Amino acid sequence of ZBTB20 from *Homo sapiens* (NCBI: AAH29041.1).¹ The BTB domain
 39 is highlighted in dark blue. Each zinc finger domain (ZF1–5) is highlighted in a distinct color: ZF1 in
 40 orange, ZF2 in light blue, ZF3 in light green, ZF4 in yellow, and ZF5 in salmon pink. Zn²⁺–coordinating
 41 cysteine residues are shown in orange, and histidine residues are indicated in blue. The linker sequence
 42 connecting ZF4 and ZF5 is underlined in blue.

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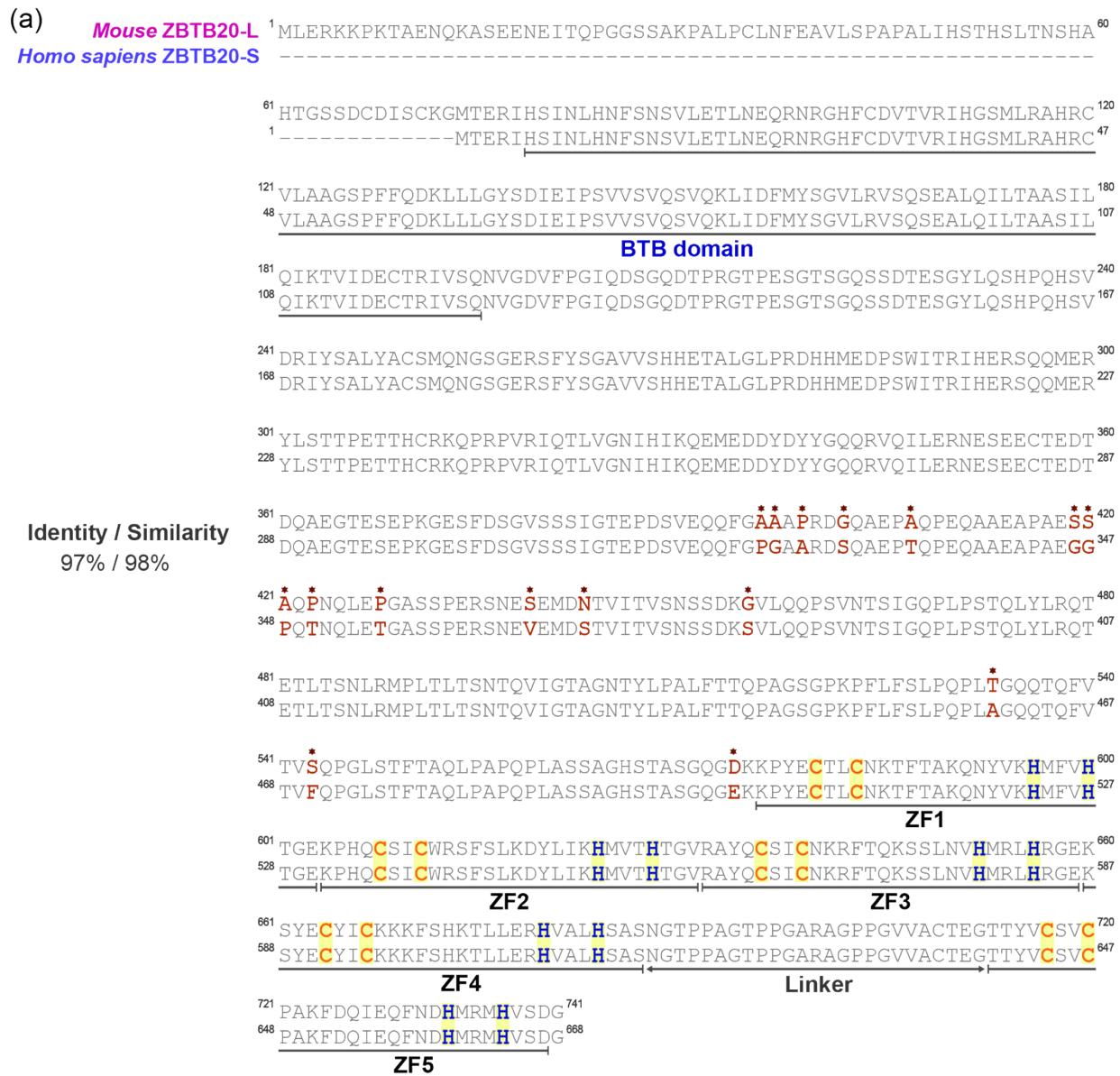


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45 **Figure S2.** Structural basis of the ZBTB20(ZF1–4)-mediated recognition of the *afp* promoter.² (a) Ladder
 46 diagram illustrating the DNA sequence within the mouse *afp* promoter and the specific nucleotides

47 contacted by each ZF domain of mouse ZBTB20. The upper and lower strands represent the sense and
48 antisense strands, respectively, and residues engaging in base-specific or phosphate-backbone interactions
49 are indicated above or below the corresponding nucleotide positions. (b–e) Structural depiction of the
50 contacts formed between the mouse *afp* promoter and (b) ZF1, (c) ZF2, (d) ZF3, (e) ZF4. The gray sphere
51 represents the Zn²⁺ ion. Hydrogen bonds are shown as green dashed lines, and coordination bonds to Zn²⁺
52 are indicated by yellow dashed lines.

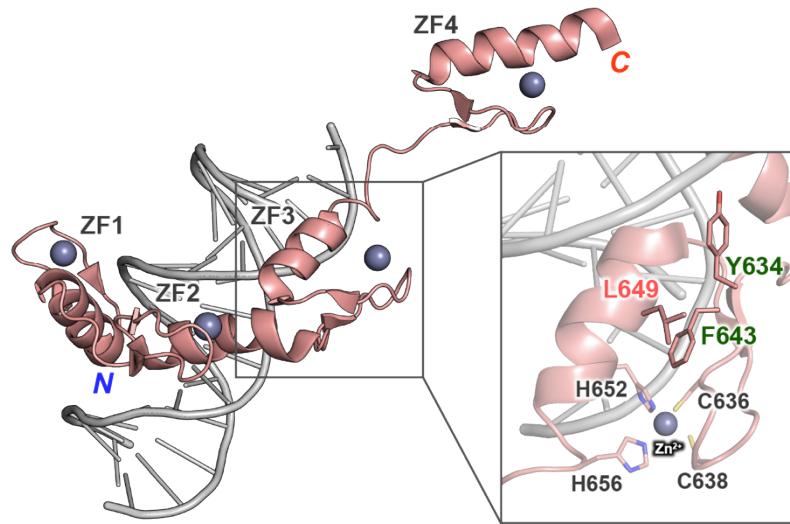
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55 **Figure S3.** Sequence alignment of mouse ZBTB20 and *Homo sapiens* ZBTB20.² Mismatched residues are shown in red bold with a star, and cysteine and histidine in each ZF domain are highlighted with colored boxes.

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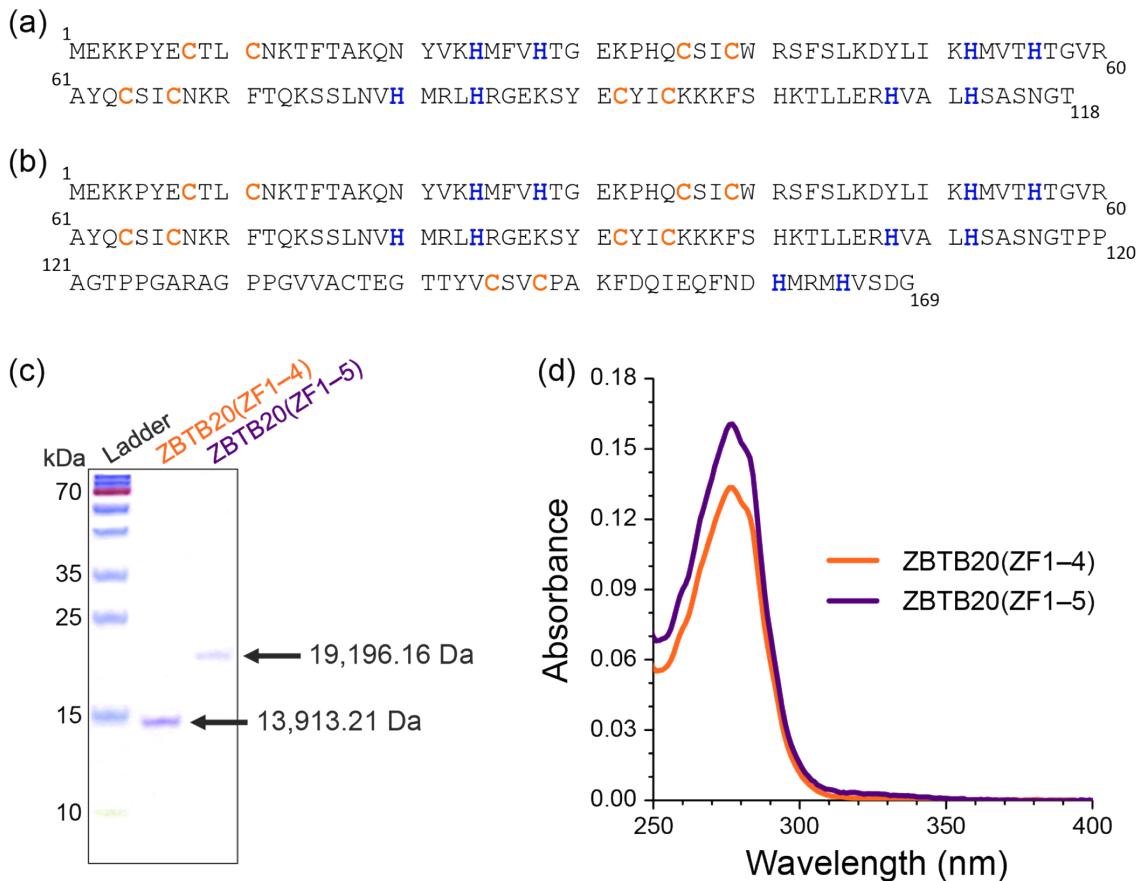
60 **Figure S4.** Crystal structure of mouse ZBTB20(ZF1–4) bound to the mouse *afp* promoter (PDB: 9JZT).²
 61 Zinc ions are depicted as gray spheres. The inset highlights residues in ZF3—Tyr634, Phe643, and
 62 Leu649—that form a hydrophobic core positioned adjacent to the Zn^{2+} -coordinating cysteine and histidine
 63 residues, contributing to local structural stability and maintaining the DNA-binding geometry.³

Homo sapiens zbtb20 → ATG GAA AAA AAG CCT TAT GAG TGC ACT CTC TGC AAC AAG ACT TTC ACC GCC
Codon optimized-zbtb20 → ATG GAG AAA AAA CCG TAT GAA TGC ACC CTG TGC AAC AAA ACG TTT ACC GCG
Homo sapiens ZBTB20 → **M** E **K** **K** P **Y** E **C** **T** **L** C **N** **K** **T** **F** **T** **A**
 AAA CAG AAC TAC GTC AAG CAC ATG TTC GTA CAC ACA GGT GAG AAG CCC CAC CAA TGC AGC ATC TGT
 AAA CAG AAC TAT GTG AAA CAC ATG TTT GTT CAC ACC GGT GAA AAA CCG CAC CAG TGC AGC ATC TGC
 K Q N Y V K H M F V H T G E K P H Q C S I C
 TGG CGC TCC TTC TCC TTA AAG GAT TAC CTT ATC AAG CAC ATG GTG ACA CAC ACA GGA GTG AGG GCA
 TGG CGT AGC TTC TCT CTG AAA GAC TAC CTG ATC AAA CAC ATG GTG ACC CAC ACC GGT GTT CGC GCT
 W R S F S L K D Y L I K H M V T H T G V R A
 TAC CAG TGT AGT ATC TGC AAC AAG CGC TTC ACC CAG AAG AGC TCC CTC AAC GTG CAC ATG CGC CTC
 TAC CAG TGC TCT ATC TGC AAC AAG CGC TTC ACC CAA AAA TCC TCT CTG AAC GTT CAC ATG CGC CTG
 Y Q C S I C N K R F T Q K S S L N V H M R L
 CAC CGG GGA GAG AAG TCC TAC GAG TGC TAC ATC TGC AAA AAG AAG TTC TCT CAC AAG ACC CTC CTG
 CAC CGC GGT GAA AAA TCT TAT GAA TGC TAC ATT TGC AAA AAG AAA TTC TCT CAC AAA ACC CTG CTG
 H R G E K S Y E C Y I C K K K F S H K T L L
 GAG CGA CAC GTG GCC CTG CAC AGT GCC AGC AAT GGG ACC CCC CCT GCA GGC ACA CCC CCA GGT GCC
 GAA CGT CAT GTG GCG CTG CAC AGC GCG AGC AAC GGT ACC CCG CCG GCG ACC CCG CCG GGT GCG
 E R H V A L H S A S N G T P P A G T P P G A
 CGC GCT GGC CCC CCA GGC GTG GTG GCC TGC ACG GAG GGG ACC ACT TAC GTC TGC TCC GTC TGC CCA
 CGT GCG GGT CCG CCG GGT GTT GTG GCA TGT ACT GAA GGT ACC ACC TAC GTT TGC TCC GTG TGC CCG
 R A G P P G V V A C T E G T T Y V C S V C P
 GCA AAG TTT GAC CAA ATC GAG CAG TTC AAC GAC CAC ATG AGG ATG CAT GTG TCT GAC GGA TAA
 GCA AAA TTT GAT CAG ATT GAA CAG TTC AAC GAT CAC ATG CGT ATG CAC GTT AGC GAT GGT TAA
 A K F D Q I E Q F N D H M R M H V S D G

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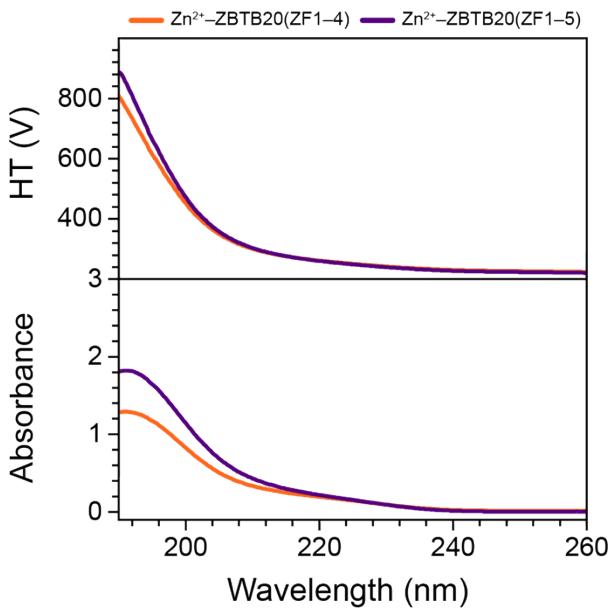
65 **Figure S5.** Codon-optimized DNA sequence of ZBTB20(ZF1–5) from *Homo sapiens*. Comparison of the
 66 *zbtb20* DNA sequence (NCBI: BC029041.1) with its codon-optimized version for *Escherichia coli*
 67 expression. The original sequence is shown in black, and codon-optimized nucleotides are indicated in
 68 orange. The corresponding amino acid sequence of ZBTB20(ZF1–5) is shown in bold purple below each
 69 codon.

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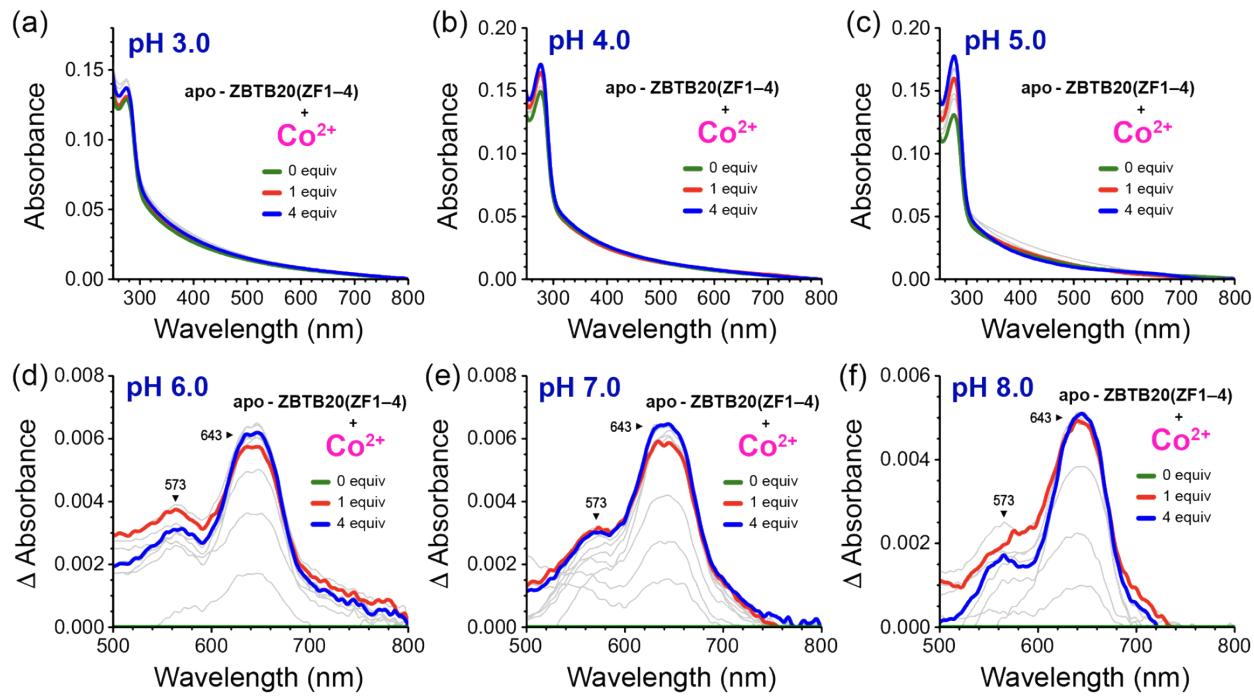
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72 **Figure S6.** Sequence and characterization of purified ZBTB20(ZFs). (a-b) Amino acid sequences of (a)
73 ZBTB20(ZF1-4) and (b) ZBTB20(ZF1-5). Zn²⁺-coordinating cysteine residues are shown in orange and
74 histidine residues in blue. (c-d) SDS-PAGE analysis and UV-Visible spectra of purified (c) ZBTB20(ZF1-
75 4) and (d) ZBTB20(ZF1-5). For SDS-PAGE, ZBTB20(ZF1-4) and ZBTB20(ZF1-5) were diluted to 15
76 μ M and 10 μ M, respectively. UV-Vis spectroscopy was performed using 8 μ M ZBTB20(ZF1-4) and 9 μ M
77 ZBTB20(ZF1-5).



78

79 **Figure S7.** HT voltage and absorbance recorded during CD measurements of ZBTB20(ZFs). Changes in
 80 HT voltage, and absorbance of Zn^{2+} -ZBTB20(ZF1-4) and Zn^{2+} -ZBTB20(ZF1-5). The HT and absorbance
 81 were monitored in parallel during CD measurements. All measurements were performed at a protein
 82 concentration of 20 μM in 20 mM HEPES (pH 7.4) containing 150 mM NaF. CD spectra were collected
 83 using a 0.5-mm quartz cuvette with a 2-nm bandwidth and a scanning speed of 50 $\text{nm}\cdot\text{min}^{-1}$.

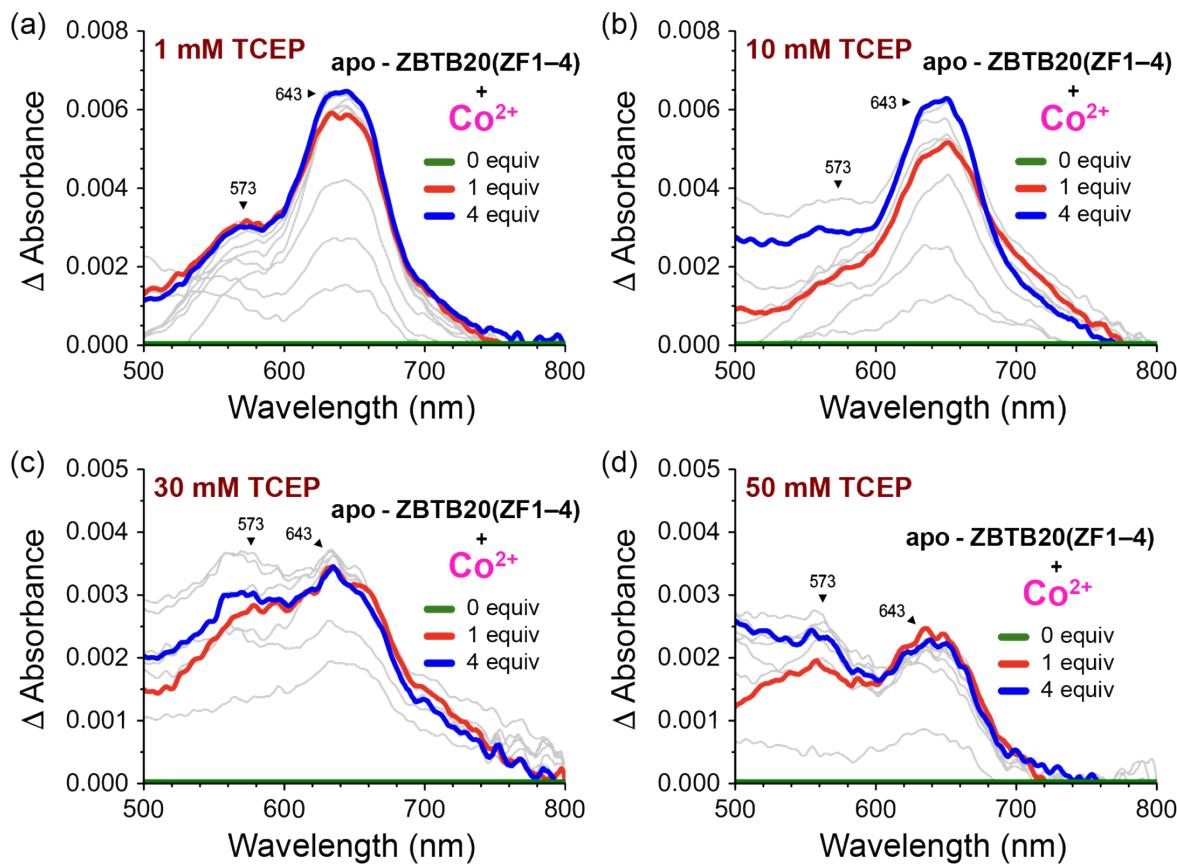


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86 **Figure S8.** pH-dependent oxidation of apo-ZBTB20(ZF1–4). d–d transition bands were measured using a
 87 UV–Visible spectrophotometer, and the % metal bound under each condition was calculated from the
 88 absorbance maxima at 643 nm.⁴ Δ Absorbance represents the changes in absorbance relative to apo–
 89 ZBTB20(ZF1–4) upon Co²⁺ titration. Experiments were conducted at (a–c) pH 3.0, 4.0 and 5.0 (100 mM
 90 sodium acetate), (d) pH 6.0 (100 mM MES monohydrate), (e) pH 7.0 (100 mM MOPS), and (f) pH 8.0 (100
 91 mM Tris), with all buffers containing 150 mM NaCl and 1 mM TCEP. Each experiment was performed
 92 inside an N₂(g)-filled glovebox.

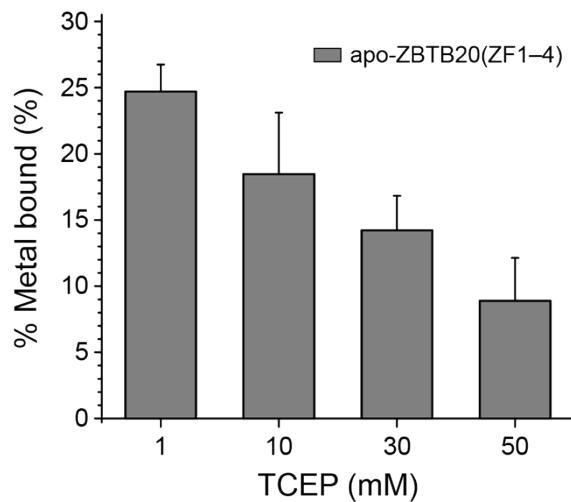
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95 **Figure S9.** TCEP concentration-dependent oxidation of apo-ZBTB20(ZF1–4). Co^{2+} -induced d–d transition
 96 bands between 500 and 800 nm were monitored by UV–Visible spectroscopy, and were observed at 573
 97 nm and 643 nm. Δ Absorbance represents the changes in absorbance relative to apo-ZBTB20(ZF1–4) upon
 98 Co^{2+} titration. Experiments were conducted in a buffer composed of 100 mM MOPS (pH 7.0) and 150 mM
 99 NaCl, with (a) 1 mM TCEP, (b) 10 mM TCEP, (c) 30 mM TCEP, or (d) 50 mM TCEP. Each experiment
 100 was performed inside an $\text{N}_2(\text{g})$ -filled glovebox.

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102

103 **Figure S10.** Comparison of % Metal bound under different TCEP concentrations. The metal binding
104 percentage of apo-ZBTB20(ZF1-4) was determined using the absorbance maximum at 643 nm,
105 corresponding to the Co^{2+} -dependent d-d transition band observed upon metal coordination, and assessed
106 under varying TCEP concentrations. Error bars represent the standard deviation from five measurements.

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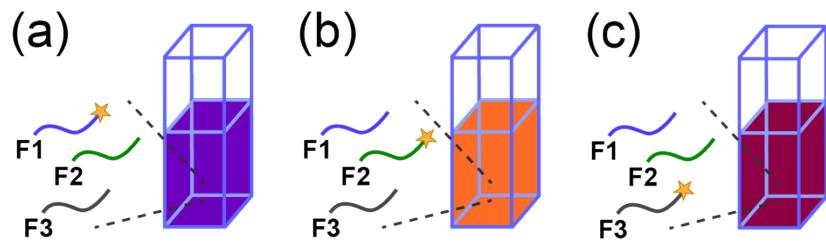
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tcag **cccatttgcttcacacactcttccttcctccaa** gggctaacggaaaagtgcac
 -1,253 **brn2-F1 →** **brn2-F2 →** **brn2-F3 →** -1,204
 ctaccagcgtaggagcacgggaatcgcgcacgcaggcctccgcggcttccggag
 cggccttggcgactgcgcgcgcctacaaggccgcacaccctcgcca~cagcag **+1**
 -1,089 **TSS** -6
 agcaggagcagcaacagaaggcgtcgagcggcgtcgagctgcccgtgtggagaga
 gaggagacagaaagagcgcgaggagaggagcccgaggcgaaaaagtaactgtcaaat
 gcgcgctccttaaccggagcgctcagtcggctccgagagtc **ATG CGC ACC GCA**
BRN2 →

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110 **Figure S11.** Upstream sequence of the *brn2* promoter region from *Homo sapiens* (NCBI: NC_060930.1).⁵

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113 **Figure S12.** Schematic overview of the competition assay. (a–c) Competition assay using (a) 6-FAM
 114 labeled F1, (b) 6-FAM labeled F2, and (c) 6-FAM labeled F3. For each experiment, one fluorescently
 115 labeled *brn2* fraction and two unlabeled double-stranded *brn2* fractions were mixed in a buffer, with each
 116 DNA fraction diluted to 5 nM to maintain a 1:1:1 ratio. ZBTB20(ZF1–4) was titrated into the mixture at
 117 the indicated concentrations, followed by a 2-min incubation before measurement.

Protein	pH	TCEP (mM)	% Metal bound
ZBTB20(ZF1–4)	3.0	1.0	1.6 (± 1.1)
ZBTB20(ZF1–4)	4.0	1.0	1.4 (± 1.3)
ZBTB20(ZF1–4)	5.0	1.0	2.9 (± 2.0)
ZBTB20(ZF1–4)	6.0	1.0	24.8 (± 2.4)
ZBTB20(ZF1–4)	7.0	1.0	24.7 (± 2.0)
ZBTB20(ZF1–4)	7.4	1.0	19.6 (± 3.1)
ZBTB20(ZF1–4)	8.0	1.0	19.0 (± 5.2)
ZBTB20(ZF1–4)	7.0	10.0	18.5 (± 4.6)
ZBTB20(ZF1–4)	7.0	30.0	14.2 (± 2.6)
ZBTB20(ZF1–4)	7.0	50.0	8.9 (± 3.2)
ZBTB20(ZF1–5)	7.4	1.0	49.3 (± 5.4)

119 **Table S1.** % Metal bound of apo-ZBTB20(ZFs) under different pH and TCEP conditions.

120

121 **Reference**

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