Supplemental Material

BosR Is A Novel Fur Family Member Responsive to Copper and Regulating Copper Homeostasis in *Borrelia burgdorferi*

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Running Head: Metalloregulation of BosR

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Table S1. Comparison of methods for protein concentration determination

BosR Samples [a]	Bradford (µg/mL)	A280 (µg/mL) [b]	Bradford/A280
1.	568	582	0.98
2.	680	666	1.02
3.	383	409	0.94
4.	67,348	70,530	0.95
5.	74,732	77,920	0.96

[a.] Samples 1-3 were assayed directly, without any dilution. Samples 4-5 were diluted 100-fold for the Bradford assay and diluted 10-fold for measurement of absorbance at 280 nm.

[b.] Extinction coefficient for BosR was calculated as 15,930 M^{-1} cm^{-1} or 0.788 $(g/L)^{-1}$ cm^{-1} using Expasy's ProtParam tool.

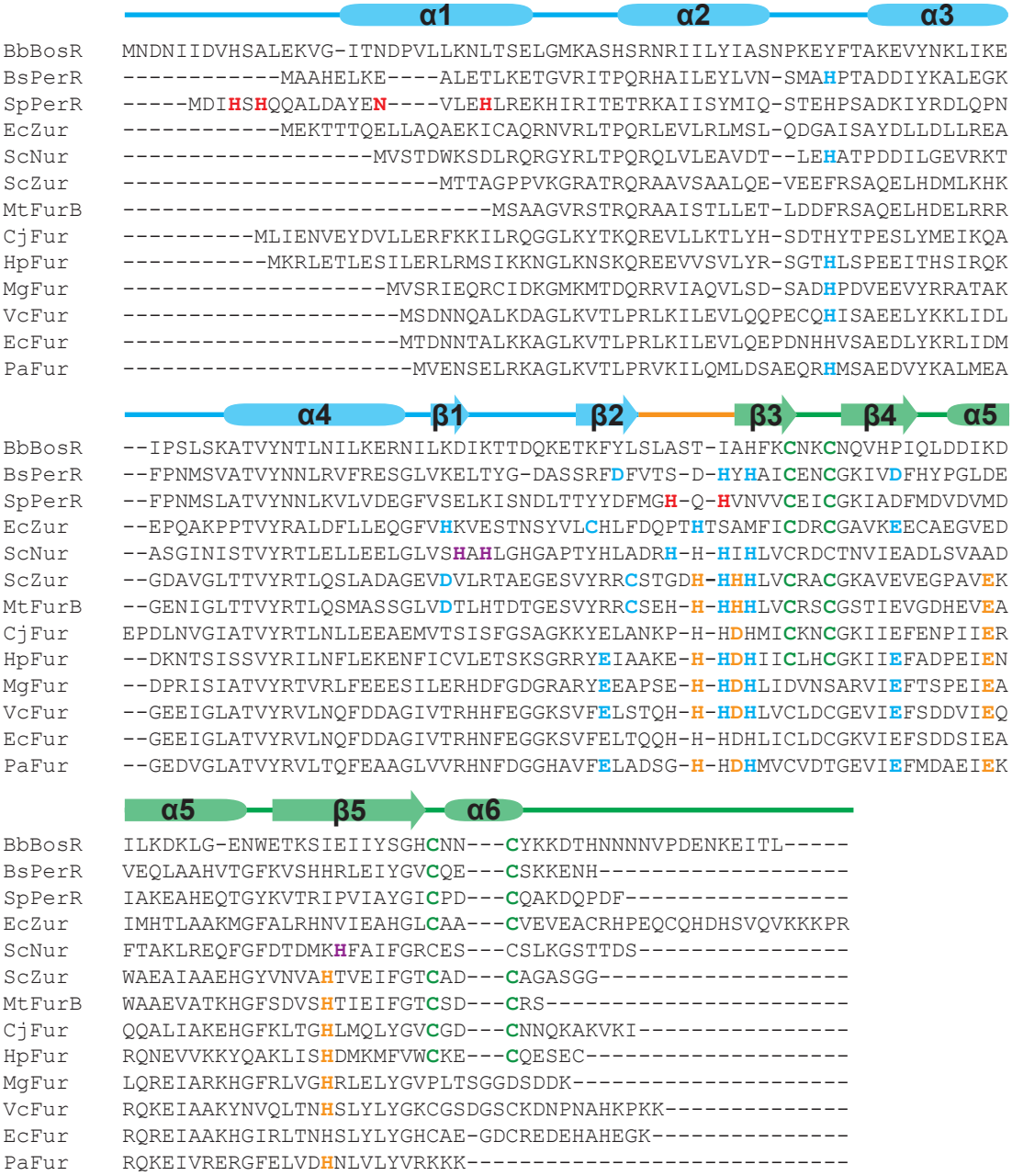

Figure S1. Sequence alignment of BosR with the 12 Fur family members that have crystal structures available. The amino acid sequences of *Borrelia burgdorferi* BosR (BbBosR; NP_212781), *Bacillus subtilis* PerR (BsPerR; NP_388753), *Streptococcus pyogenes* PerR (SpPerR; WP_002986328), *Escherichia coli* Zur (EcZur; NP_418470), *Streptomyces coelicolor* Nur (ScNur; NP_628356) and Zur (ScZur; NP_626748), *Mycobacterium tuberculosis* FurB (MtFurB; NP_216875), *Campylobacter jejuni* Fur (CjFur; WP_002854230), *Helicobacter pylori* Fur (HpFur; NP_207817), *Magnetospirillum gryphiswaldense* Fur (MgFur; WP_024081295), *Vibrio cholerae* Fur (VcFur: NP_231738), *E. coli* Fur (EcFur; NP_415209), *Pseudomonas aeruginosa* Fur (PaFur; NP_253452), were aligned using ClustalW2. Secondary structures were indicated above the sequences, with the DNA-binding domain (DBD) colored in blue, the dimerization domain (DD) in green, and the hinge in orange. The amino acid residues involved in coordination of metal ions are color-coded, with the homologous metal-binding site from different proteins in the same color: S1, green; S2, blue; S3, orange. The unique metal-binding sites found in SpPerR and ScNur are colored in red and purple, respectively. With the exception of the putative S1 residues of BosR, the involvement of all other residues in metal-coordination is supported by structural data.

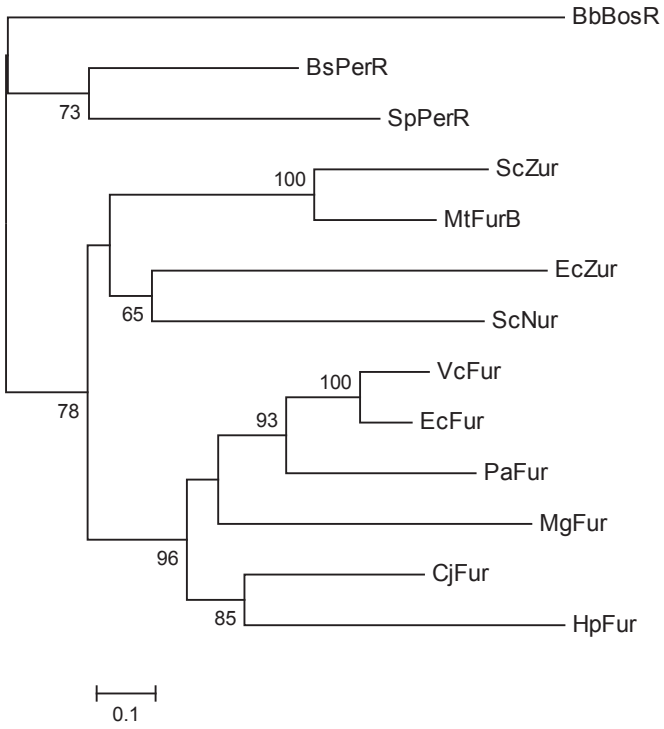

Figure S2. Evolutionary relationships of the Fur family members. The evolutionary history was inferred using the Neighbor-Joining method. The optimal tree with the sum of branch length = 6.74588684 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Poisson correction method and are in the units of the number of amino acid substitutions per site. Evolutionary analyses were conducted in MEGA7. Abbreviations for the Fur family members are the same as described in Figure S1 legend.

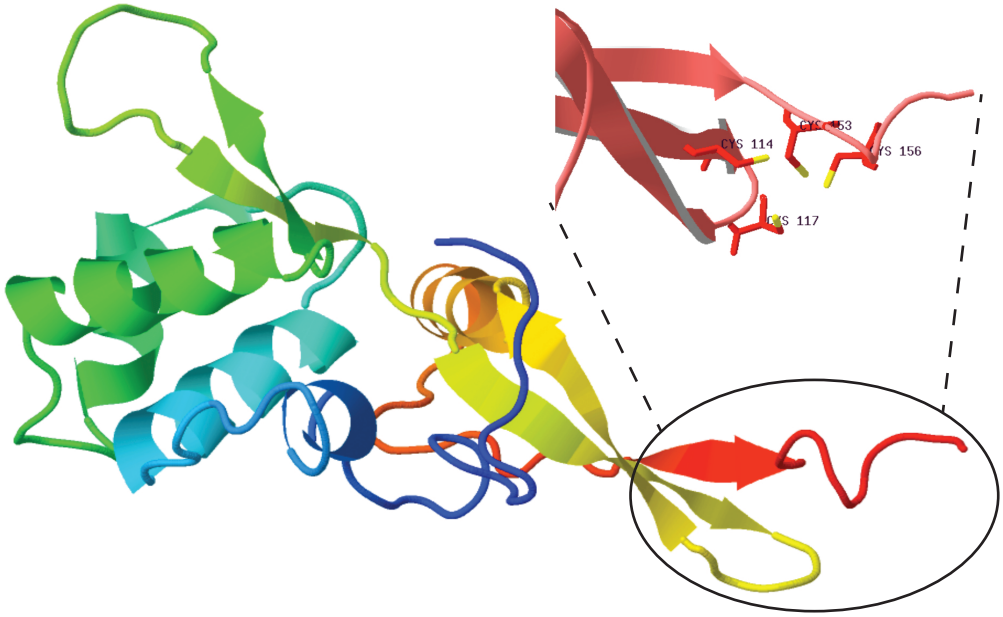

Figure S3. A predicted model of BosR. The model was generated by SWISS-MODEL using a crystal structure of *Streptococcus pyogenes* PerR (PDB, 4I7H) as template. The structural Zn site comprised of four cysteines (shown in the inset) is conserved in BosR.

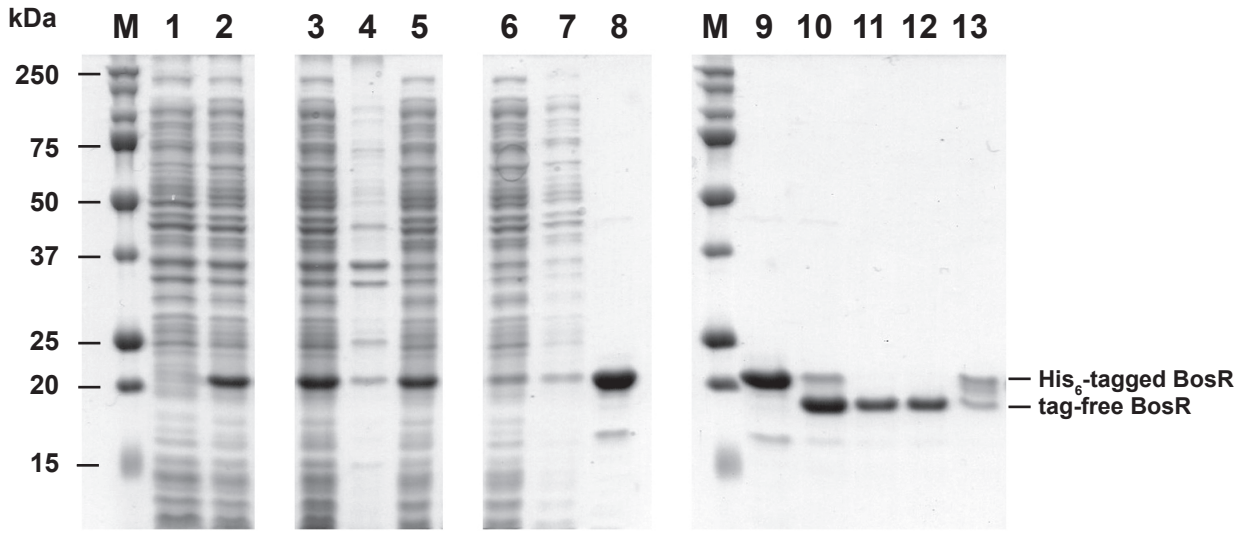

Figure S4. Expression and purification of recombinant BosR. SDS-PAGE analysis of whole cell lysates of *E. coli* before (lane 1) and after (lane 2) IPTG induction indicate that the position of the IPTG-inducible protein is consistent with an estimated size of 21.3 kDa for the His_6-tagged BosR. *E. coli* cells expressing the His_6-tagged BosR were lysed in B-PER (lane 3) and then separated into the insoluble (lane 4) and the soluble fractions (lane 5) by centrifugation. The recombinant BosR is largely present in the soluble fraction. The soluble faction of the cell lysate was filtrated through a 0.22-μm filter, and then loaded onto the HisPur cobalt resin. Analysis of the flowthrough (lane 6), the wash (lane 7), and the elution (lane 8) from the column indicate that the His_6-tagged BosR can be purified to >90% homogeneity. The His_6-tagged BosR was then digested with dipeptidyl aminopeptidase I at 4 ºC overnight, analysis of the protein sample before (lane 9) and after (lane 10) the digestion indicate that the tag was cleaved off from most but not all BosR protein. A second round of purification on the HisPur cobalt resin was carried out to remove any remaining tagged protein. Analysis of the flowthrough (lane 11), the wash (lane 12), and the elution (lane 13) from this column indicated that only tag-free BosR was present in the flowthrough and the wash, which was pooled and used for functional analysis. Molecular weights of protein standards (M) are indicated to the left. Positions of the His_6-tagged BosR and the tag-free BosR are indicated to the right.

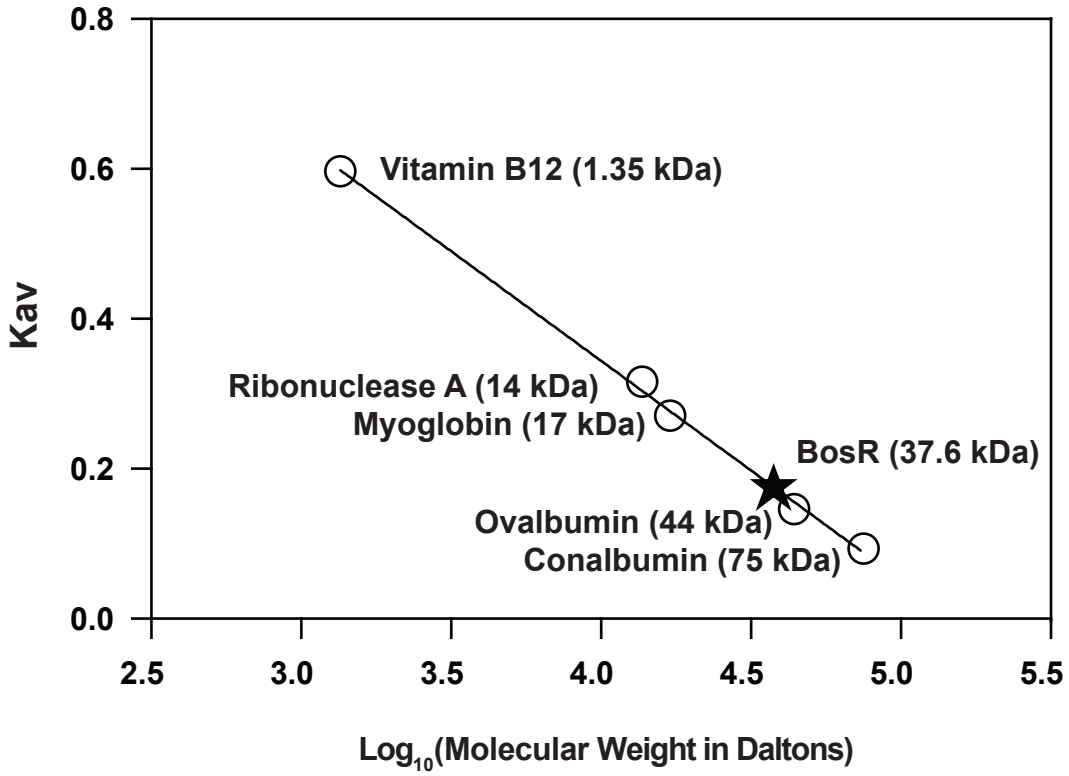

Figure S5. Molecular weight determination of BosR by size-exclusion chromatography. A Superdex 75 (10/300) column was calibrated using known standards, including blue dextran (for determination of the void volume), conalbumin, ovalbumin, myoglobin, ribonuclease A, and Vitamin B12. The calculated molecular weight of BosR is 37.6 kDa, which corresponds to a dimer.

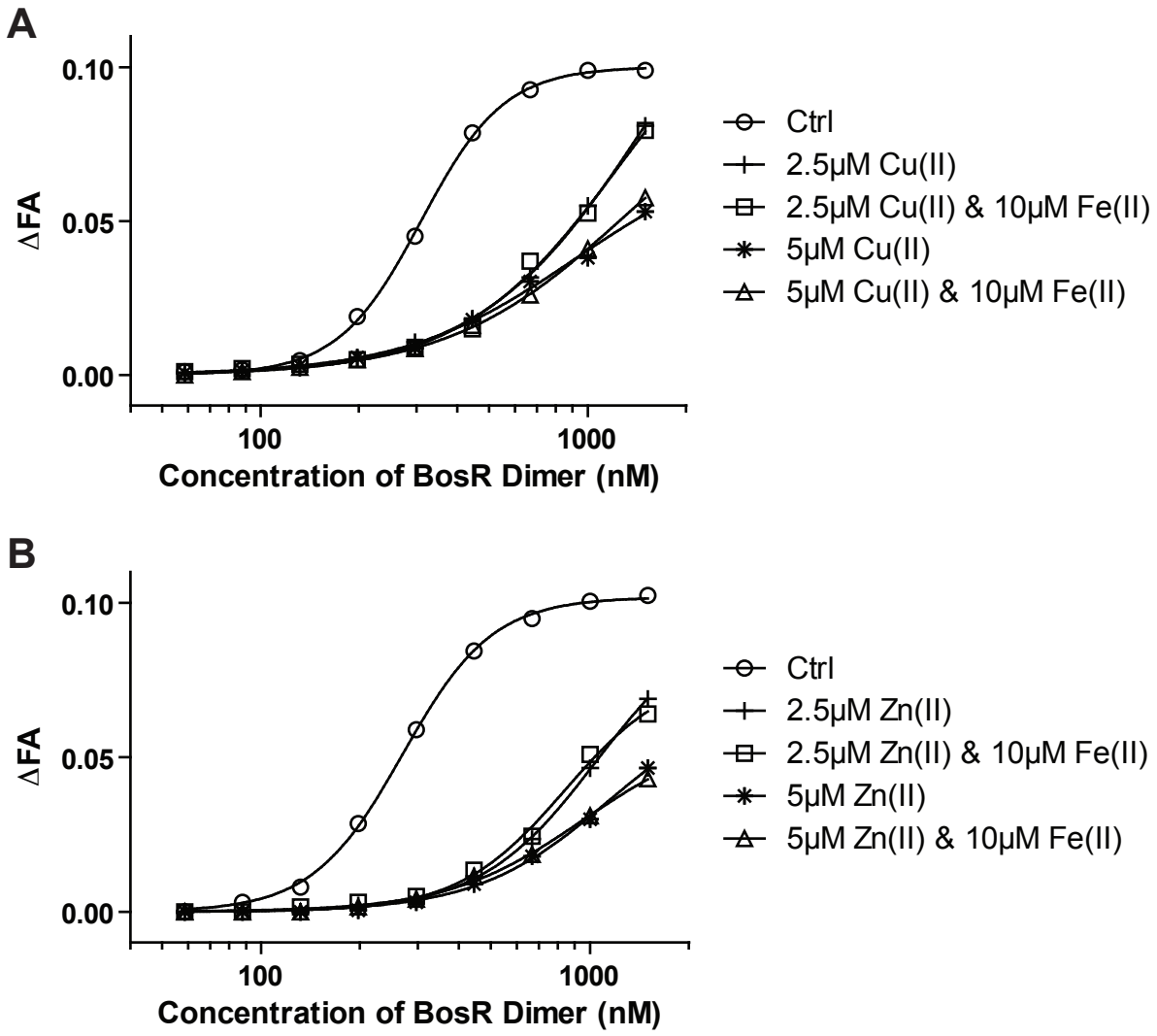

Figure S6. Both Cu(II) and Zn(II) have a higher affinity than Fe(II) for the regulatory sites of BosR. Binding reactions were carried out with or without 2.5 or 5 μM of (A) Cu(II) or (B) Zn(II). When indicated, Fe(II) was added to reactions to a final concentration of 10 μM prior to the addition of BosR. The lack of an effect of Fe(II) on dampening the inhibitory effect of Zn(II) and Cu(II) on the DNA-binding activity of BosR suggests that Zn(II) and Cu(II) have a higher affinity than Fe(II) for the regulatory metal-binding sites of BosR.

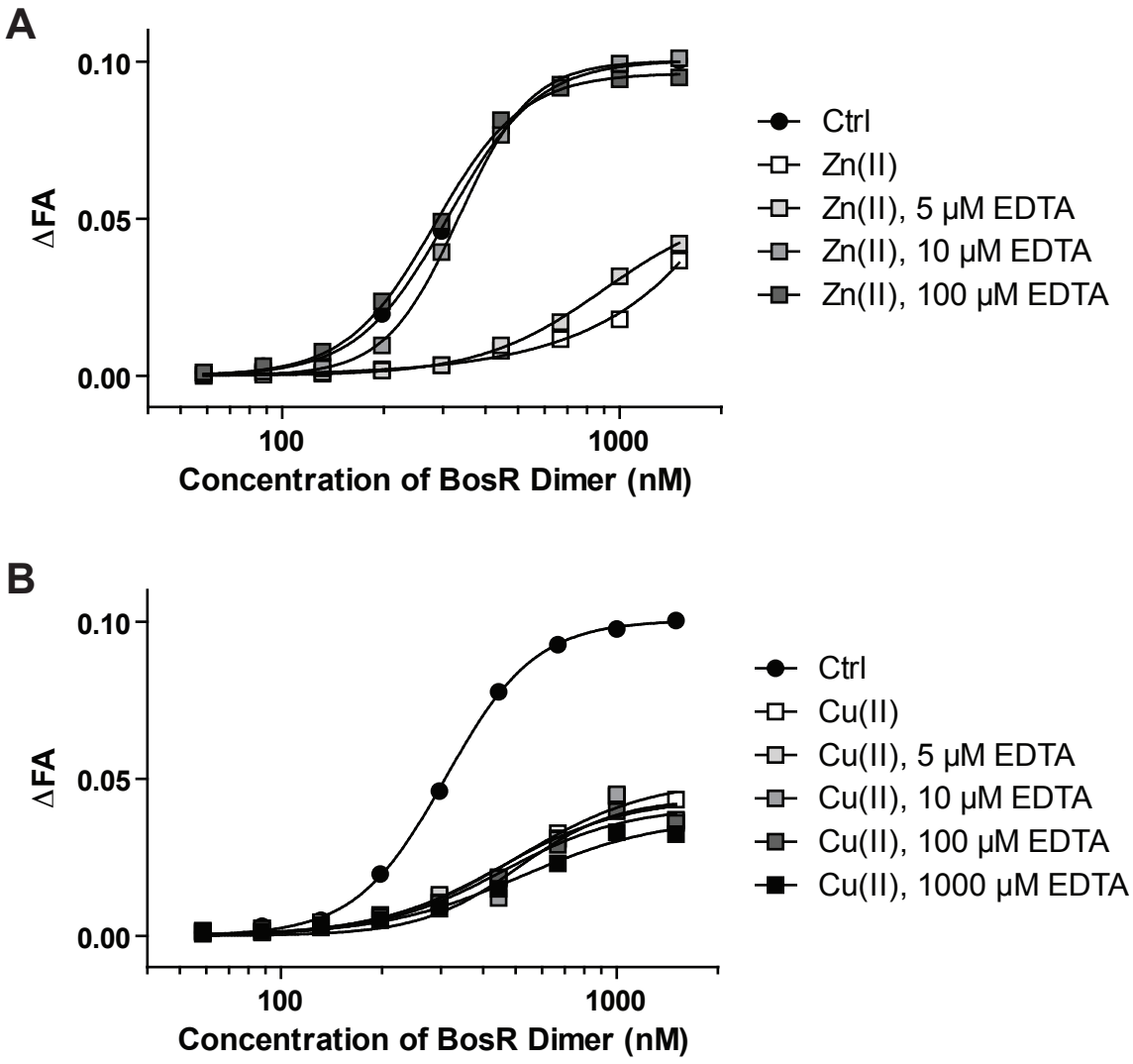

Figure S7. EDTA could reverse the inhibitory effect of Zn(II) on BosR activity but not that of Cu(II). BosR was allowed to bind to the fluorecently labeled probe FP_{ospAB} in the absence (Ctrl) or presence of 10 μM of (A) Zn(II) or (B) Cu(II). When indicated, EDTA was added subsequently to reactions to a final concentration of 5, 10, 100, or 1000 μM.

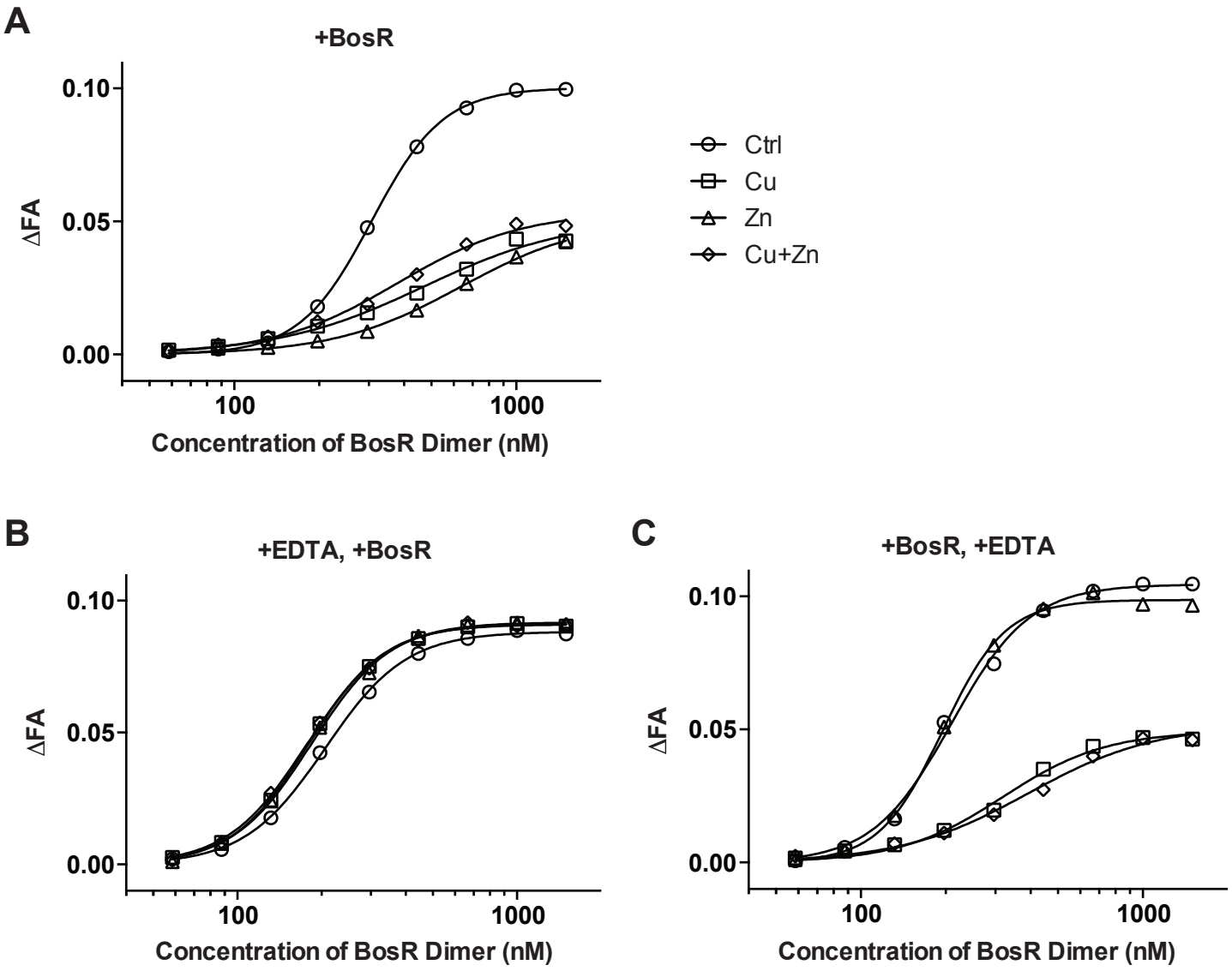

Figure S8. Cu(II) has a higher affinity than Zn(II) for the regulatory sites of BosR. (A) When indicated, Cu(II) and/or Zn(II) were added to binding reactions to a final concentration of 10 μM each. (B) Reactions were set up similarly as (A) except that EDTA was added to all binding reactions to a final concentration of 50 μM prior to the addition of BosR. (C) Reactions were set up similarly as (A) except that EDTA was added to all binding reactions to a final concentration of 50 μM after the addition of BosR. When both Cu(II) and Zn(II) are present, the binding curves behave the same as those with Cu(II) alone, meaning the inhibitory effect on BosR could not be reversed with EDTA, which suggests that Cu(II) has a higher affinity than Zn(II) for the regulatory metal-binding sites of BosR.

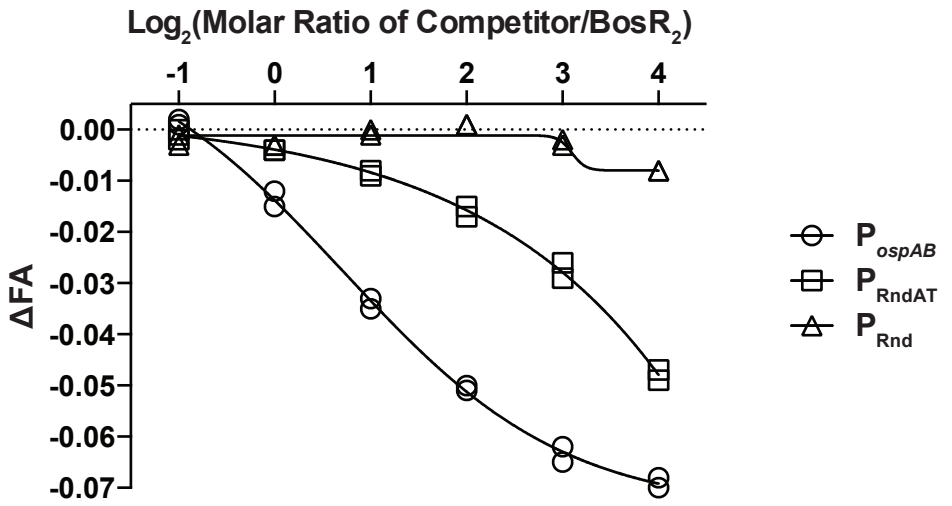

Figure S9. Sequence specificity of BosR binding to the FAM-labeled DNA probe FP_{ospAB}. Pre-incubation of BosR with the unlabeled probe P_{ospAB} (which has the same sequence as FP_{ospAB}) inhibited BosR binding to FP_{ospAB} in a dose-dependent manner. In comparison, the unlabeled probe P_{RndAT} (which has a random sequence of As and Ts) resulted in much weaker inhibition and pre-incubation with P_{Rnd} (which has a random sequence of As, Ts, Gs, and Cs) had no inhibitory effect. The moderate inhibitory effect observed of P_{RndAT} is consistent with BosR preferentially binding to AT-rich sequences.

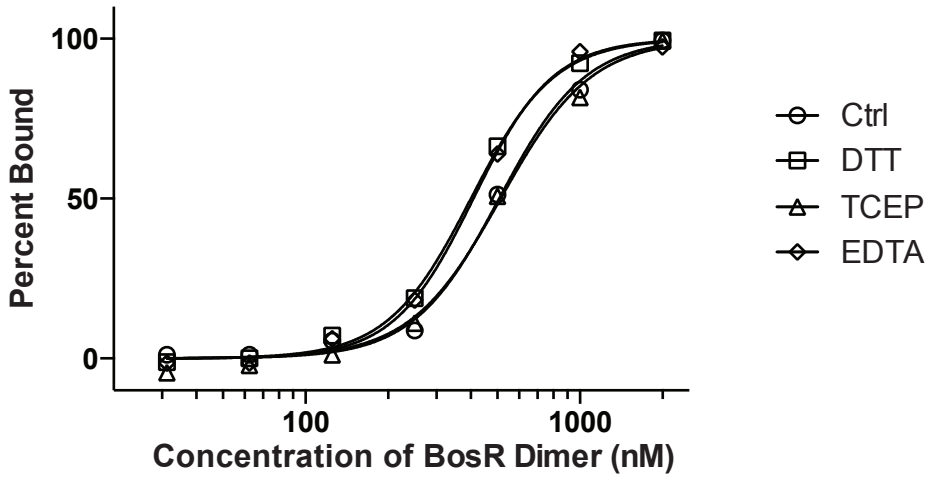

Figure S10. Effects of DTT, TCEP, and EDTA on BosR DNA-binding activity. The fluorescently labeled DNA probe FP_{ospAB} (10 nM) was incubated with BosR (30 - 2000 nM of dimer) in the Tris/KCl buffer (Ctrl) or the same buffer containing 1 mM DTT, 1 mM TCEP, or 10 μM EDTA. Changes in FA were used to calculate the percent of probe bound by BosR.