Supplemental Materials

Supplemental Figures

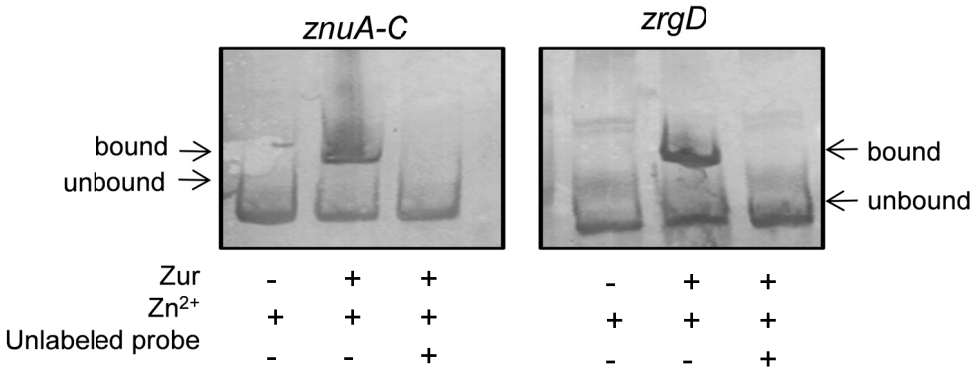

Fig. S1. Zur binds *znu* and *zrg* promoters specifically. 0.2 pmol biotin-labeled *znuA-C* and *zrgD* promoter fragments, with and without 40 pmol unlabeled DNA, were incubated with 20 ng recombinant Zur protein in the reaction binding buffer at room temperature for 20 min. 0.4 μM Zn^{2+} was included in the reaction mix.

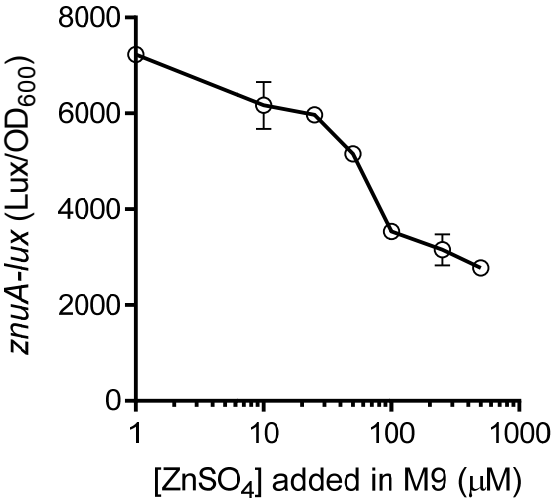

Fig. S2. *znuA* expression in M9 medium. Wild type containing P_{znuA}-*luxCDABE* transcriptional plasmid reporters were grown in M9 minimal medium plus $ZnSO_4$ indicated to mid-log phase at 37ºC. Luminescence was measured and normalized against OD_{600}. Data are mean and s.d. of three independent experiments.

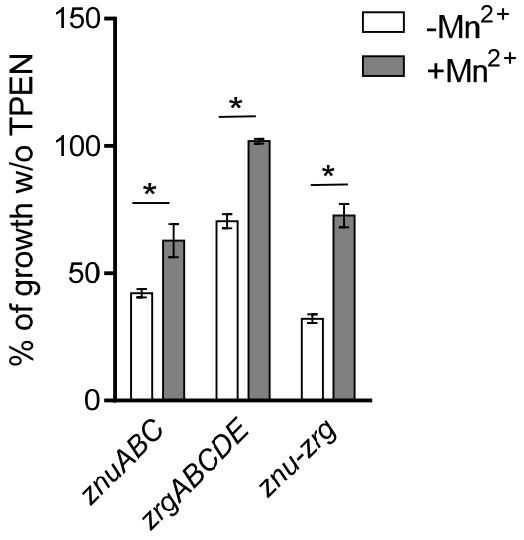

Fig. S3. Mn^{2+} effects on zinc transporter mutants. The overnight cultures of wild type and zinc transporter mutants were inoculated 1:100 into LB medium, LB medium pretreated with 30 μM TPEN, and TPEN-treated LB medium with 10 μM of $MnSO_4$. The cultures were grown statically at 37℃ and at the time points indicated, OD_{600} was measured. Data are mean and s.d. of four independent experiments. *: $P<0.05$.

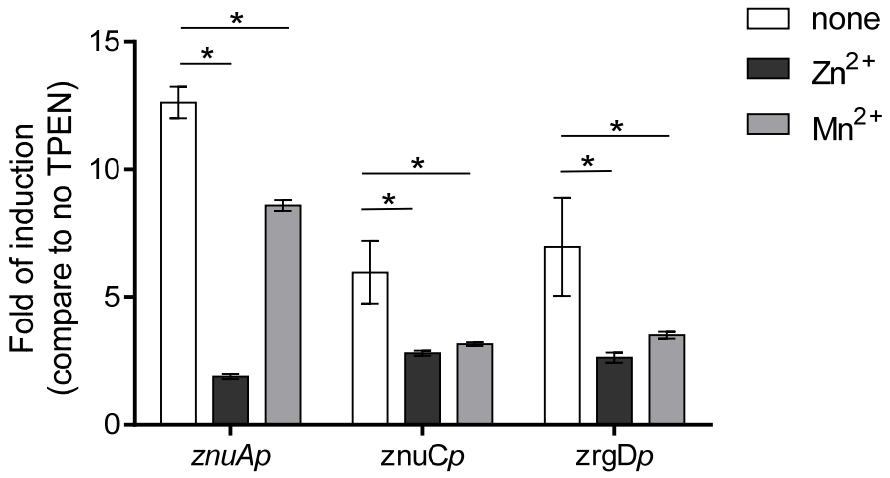

Fig. S4. Mn2+ effects on the expression of zinc transporters. The overnight cultures of wild type containing P_{znuA}-, P_{znuC}, and P_{zrgD}-*luxCDABE* reporters were inoculated 1:100 into LB medium, LB medium pretreated with 30 μM TPEN, and TPEN-treated LB medium with 10 μM of $ZnSO_4$ or $MnSO_4$. The cultures were grown statically at 37℃ to mid-log phase and luminescence and OD_{600} were measured. Fold of induction was calculated by normalizing against Lux/OD_{600} expressed in LB (no TPEN treatment). Data are mean and s.d. of four independent experiments. *: $P<0.05$.

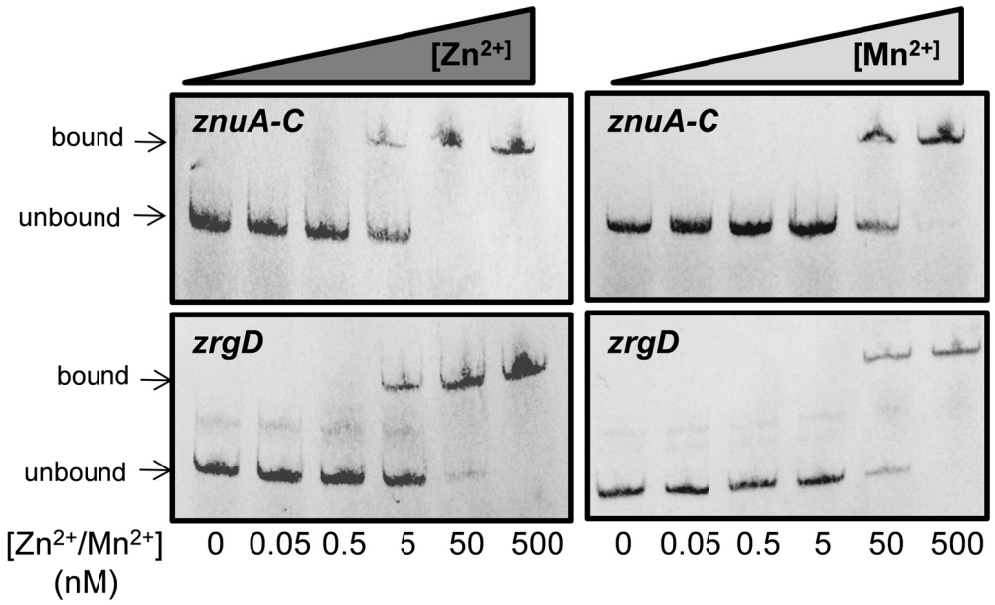

Fig. S5. Mn2^{+} effects on Zur activity. Gel shift assays. 0.2 pmol biotin-labeled *znuA-C* and *zrgD* promoter fragments were incubated with 20 ng recombinant Zur protein in the reaction binding buffer at room temperature for 20 min. Zn^{2+} and Mn^{2+} concentrations included in the reaction mix were indicated.

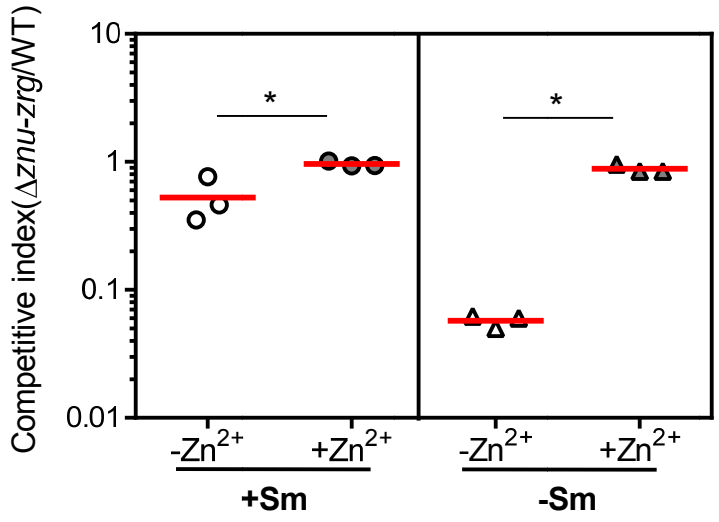

Fig. S6. The effects of supplemental Zn^{2+} on *V. cholerae* colonization. Six-week-old CD-1 mice were fed water containing streptomycin before infected with 10^{8} cells 1:1 mixed wild type and Δ*znu-zrg* mutants. For one set of mice (+Sm), streptomycin was continuously included in drinking water and for the other set (-Sm), streptomycin was removed 12 hrs post *V. cholerae* infection. When indicated, 10 mg/Kg mouse/day of $ZnSO_4$ was intragastically inoculated into

mice. At 5-day post-inoculation, small intestines were collected and the output ratio of the mutant to wild type was determined by plating on LB agar plates containing X-gal.
The competitive index was calculated by output ratio of mutants to wild type normalized against input ratio of mutants to wild type. Red horizontal lines: mean of the competitive index. *: $P<0.05$.