

## Supplemental Material

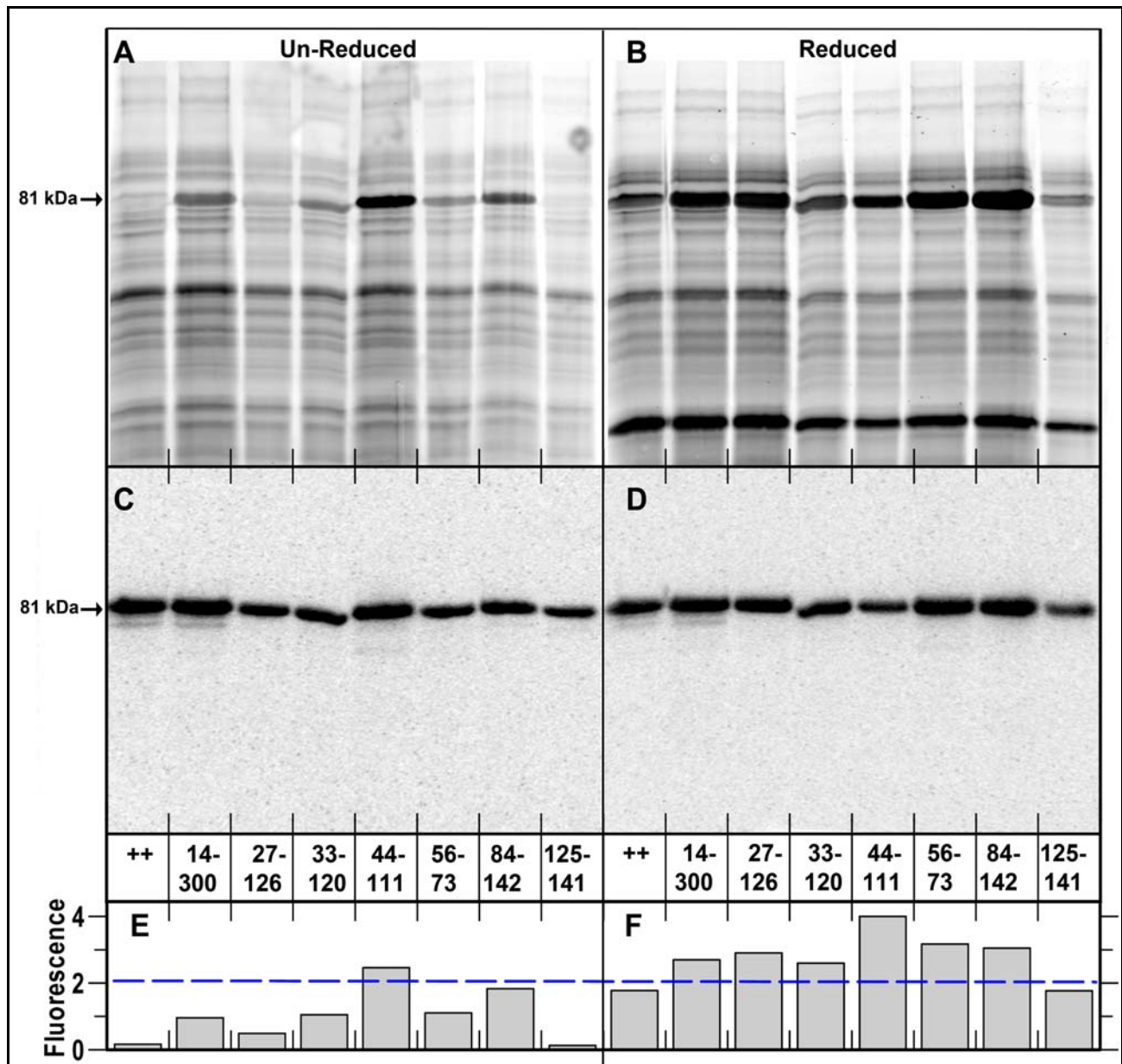
### **N-Domain Rearrangements within FepA during Ferric Enterobactin Transport**

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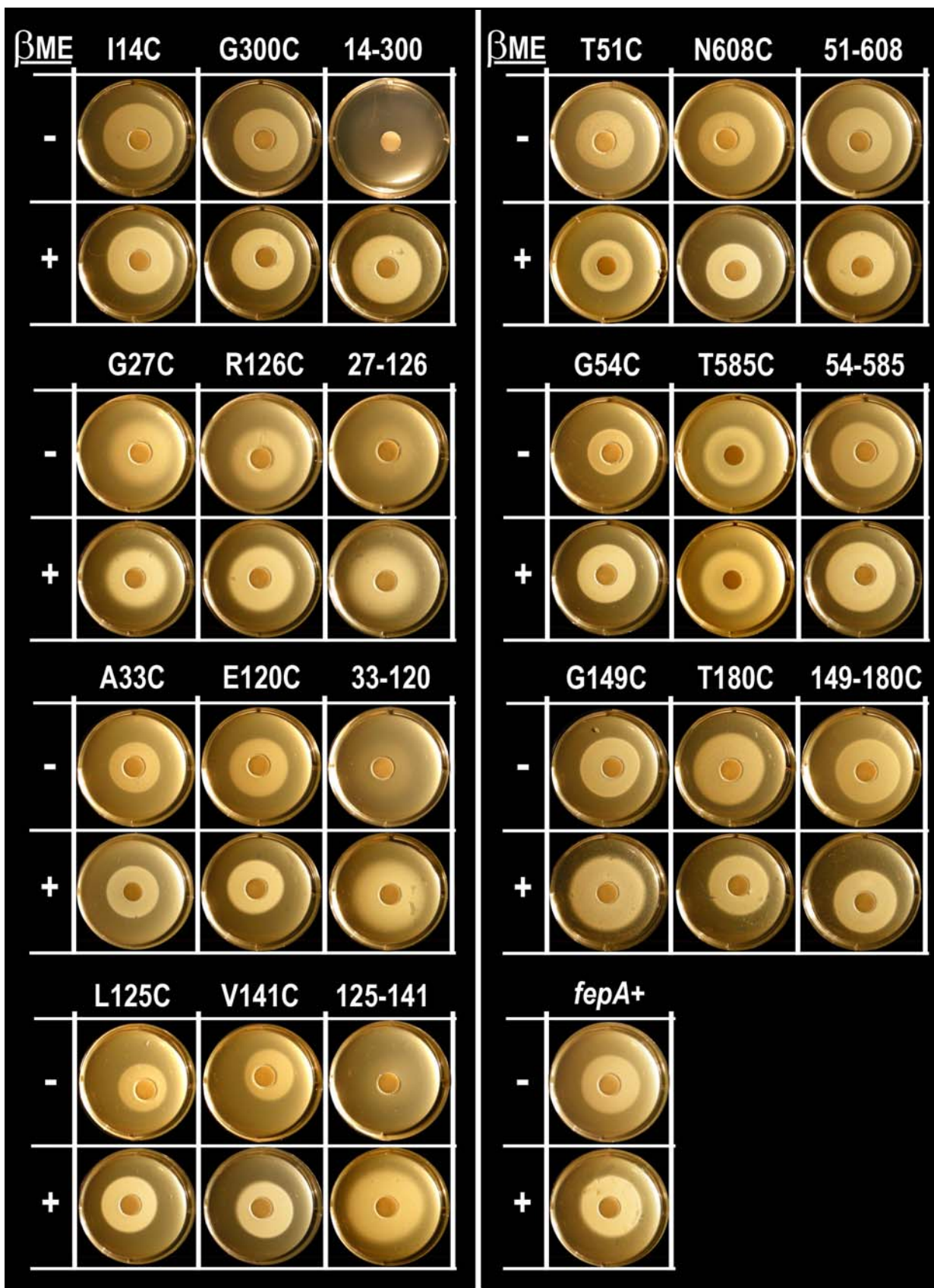
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*Running title: Site-directed Cys-pairs in FepA*

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**Figure S1. FM labeling of FepA Cys-pair mutants.** We prepared cell envelopes from iron-deficient *E. coli* strains expressing FepA Cys-pair mutants and modified them with 5  $\mu$ M FM either before (non-reduced; **panel A**) or after (reduced; **panel B**) exposure to 10 mM  $\beta$ ME. After fluoresceination we resolved the proteins on SDS-PAGE gels and visualized fluorescence on a Typhoon imager at 520 nm, then transferred the proteins to NC and performed immunoblots with anti-FepA MABs 41 and 45 (72), visualized with [ $^{125}$ I]-protein A (**panels C and D**, respectively). This immunoblot, as well as the gel in panel A, contains wild-type FepA (++) that provides an internal molecular weight marker at 81.5 kDa (arrows). **Quantification of FM-labeling (panels E and F).** Using ImageJ, we determined the intensities of the FepA bands in panels A and C (un-reduced) and B and D (reduced), and calculated the relative amounts of free Cys (i.e., accessible to FM-labeling) for each mutant in both conditions. We arbitrarily set the fluorescence of the most heavily labeled protein (44-111) to a level of 4; the blue dashed line represents the expected fluorescence labeling of fully reduced wild-type FepA (++; 2 free Cys). See the text for additional explanations.



**Figure S2. Siderophore nutrition tests of single and double Cys mutants in FepA.** We assayed FeEnt utilization by OKN3 ( $\Delta fepA$ )/pITS23 (*fepA*<sup>+</sup>) and its derivatives carrying engineered Cys substitutions in *fepA*, in the absence and presence of  $\beta$ ME (1 mM). None of the single mutants showed defects in FeEnt uptake, but four intra - N double mutants (left column) were unable to efficiently acquire FeEnt, unless tested in the presence of  $\beta$ ME. Inter - N-C Cys-pairs (right column), on the other hand, did not require  $\beta$ ME for FeEnt transport.