Supplementary Material for:

TonB-Dependent Iron Transport by *Caulobacter crescentus*

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Figure S1. Isopycnic sucrose gradients of C. crescentus cell envelope fractions and analysis by SDS-PAGE. After growth in nutrient broth containing either 50 uM FeSO4 (iron-replete conditions) or 200 um BP (iron-deficient conditions; see Results), we pelleted the cells by centrifugation, resuspended them in ice cold 50 mM Tris-Cl, pH 7.4 with trace amounts of DNase and RNase, and lysed them in a French press at 14,000 psi (49). After a low-speed centrifugation to remove unbroken cells, we spun the lysate for 1 hr at 100,000 x g to pellet the cell envelopes, resuspended the pellets and fractionated them on isopycnic sucrose gradients (49). **A. Fractionation of cell envelopes.** Images of the resulting gradients show the apparent separation of high and low density fractions of cell envelope, that typically correspond to IM components at the upper 0.77 - 1.44 M interface and OM components at the lower 1.44 - 2.02 M interface. Putative IM from the iron-replete fraction showed the presence of iron-containing proteins, typically electron transport chain components. **B. SDS-PAGE of component proteins.** Lanes 1-3 derived from iron-replete cultures; lanes 4-6 from iron-deficient cultures. We collected the upper interface (lanes 1 and 4), the intermediate region between the interfaces (lanes 2 and 5), and the lower interface (lanes 3 and 6) of each gradient. After collecting the

membrane components by centrifugation at 100,000 x g for 1 h, we resuspended the pellets and subjected 30 ug of each sample to SDS-PAGE. The coomassie blue stain of the resulting gel revealed that the sucrose gradients failed to separate the IM and OM fractions: we observed the same proteins in different locations of the gradients. Note the presence of protein R2 (identified in Fig. 5 as 00196; 69.2 kDa;) in all three fractions of the iron-replete cell envelopes. Other proteins were not readily distinguished by this fractionation method.

Figure S2. Phylogenetic relationships of negatively iron-regulated E. coli and C. crescentus TBDT. From the protein sequences of putative TonB-dependent proteins annotated in (14), we separately analyzed the TonB-box regions (TBB), N-termini (N), C-termini (C) and full length sequences with CLUSTALW2 (39). *C. crescentus* (Ccr)TBDT that are induced by iron deprivation or repletion (24) are enclosed in black or grey boxes, respectively; Ccr02370 (MalA (26)) is enclosed in purple. The *E. coli* (Eco)TBDT are enclosed in different colored boxes. The iron-regulated Eco and Ccr TBDT grouped in two branches (magenta and blue) of the phylogenetic tree of the full-length proteins; magenta and blue lines connect the proteins through the cladograms of C-domains, N-domains, and TonB-boxes.

Figure S3. Phylogenetic relationships of *C. crescentus* TBDT that are overexpressed during iron repletion. CLUSTALW2 comparisons of the TonB-boxes, N-domains, C-domains and full length protein sequences of 8 Eco and 62 Ccr TBDT, analyzed as described above, highlight the relationships of Ccr TBDT that are overexpressed by iron repletion (23, 24; enclosed in grey boxes). With two exceptions (Ccr01155, Ccr02895), these proteins reside in the green branch of the full length proteins, and are similar to each other in terms of their C- and N-domains.

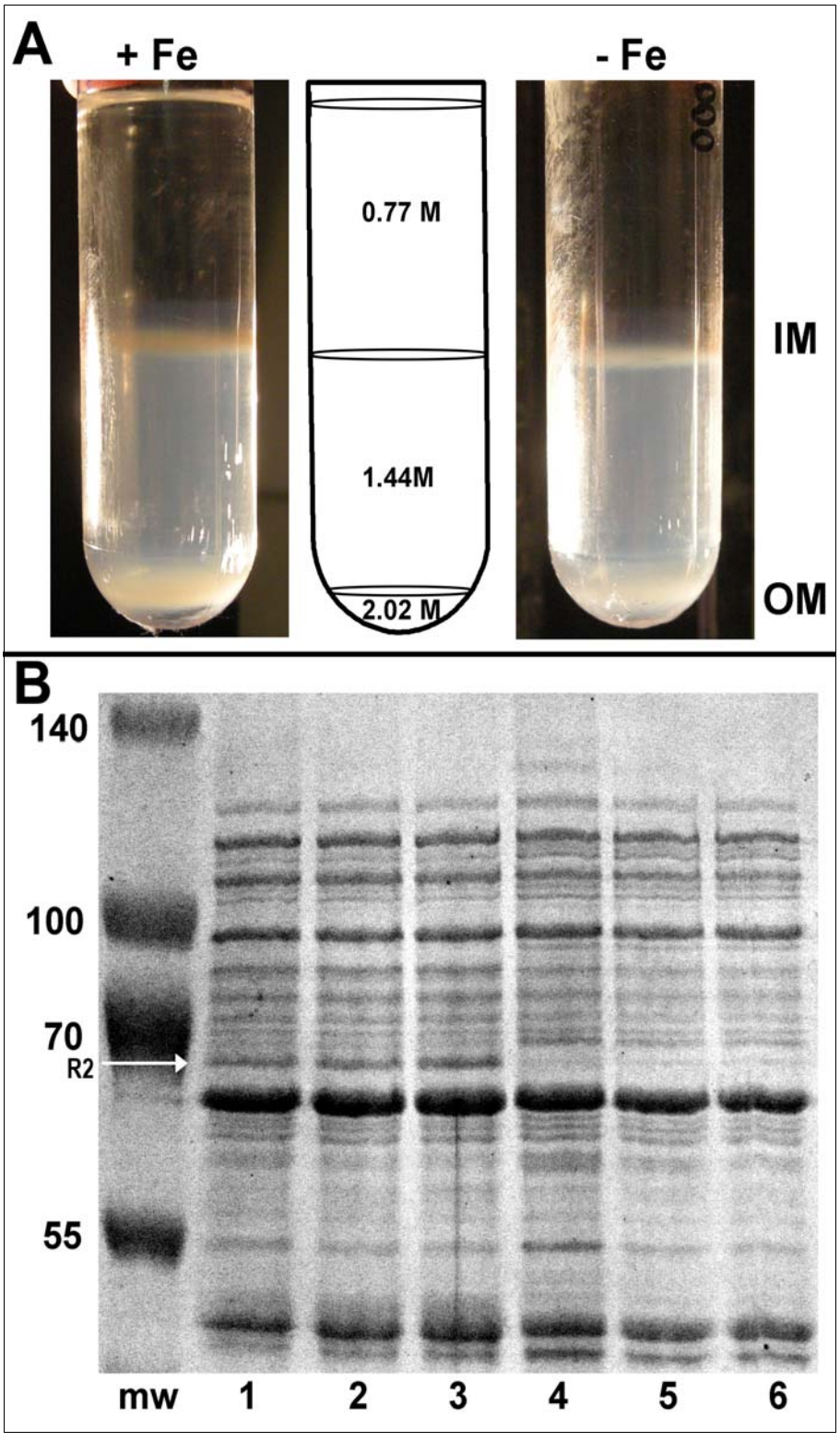

Figure S1

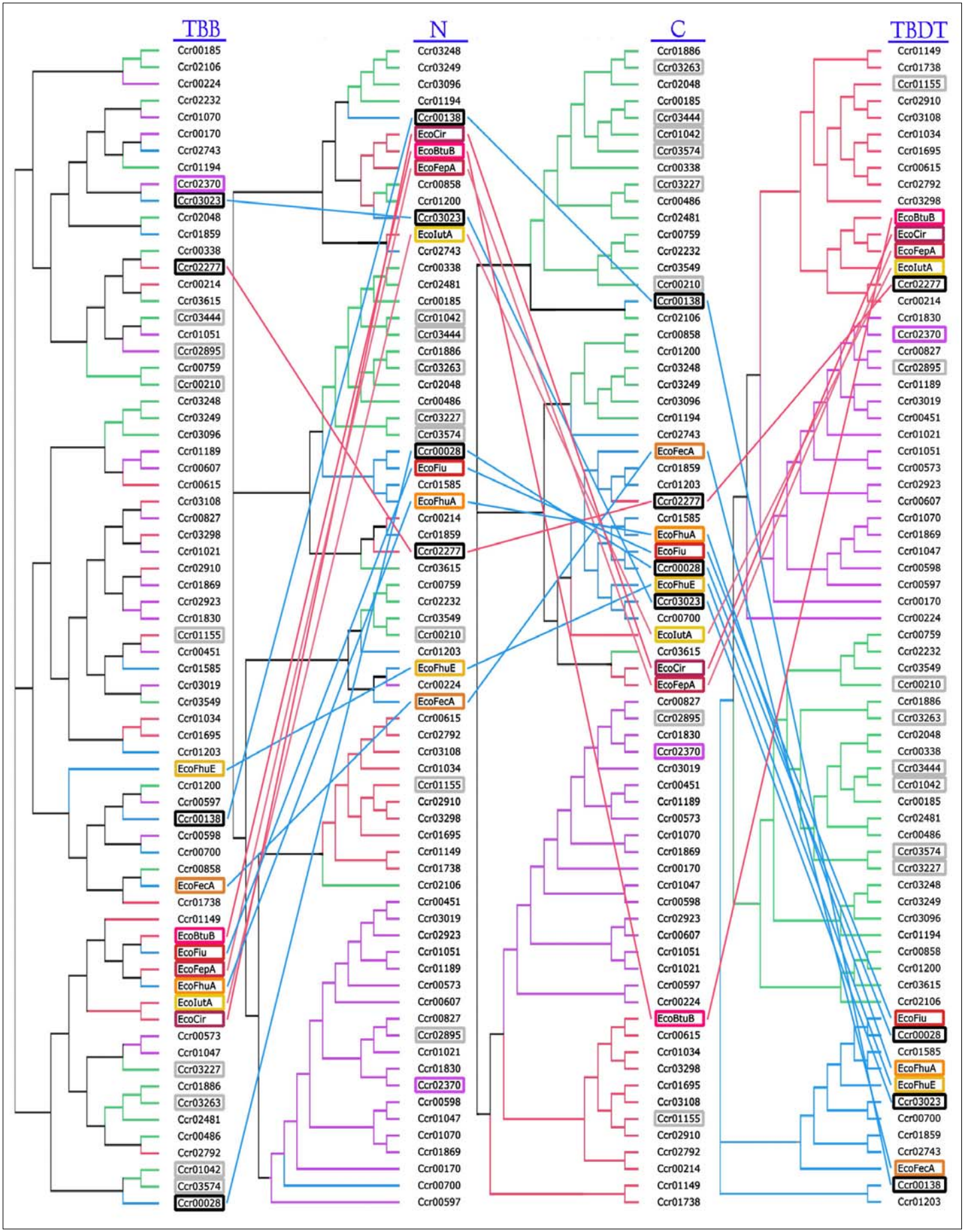
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Figure S2

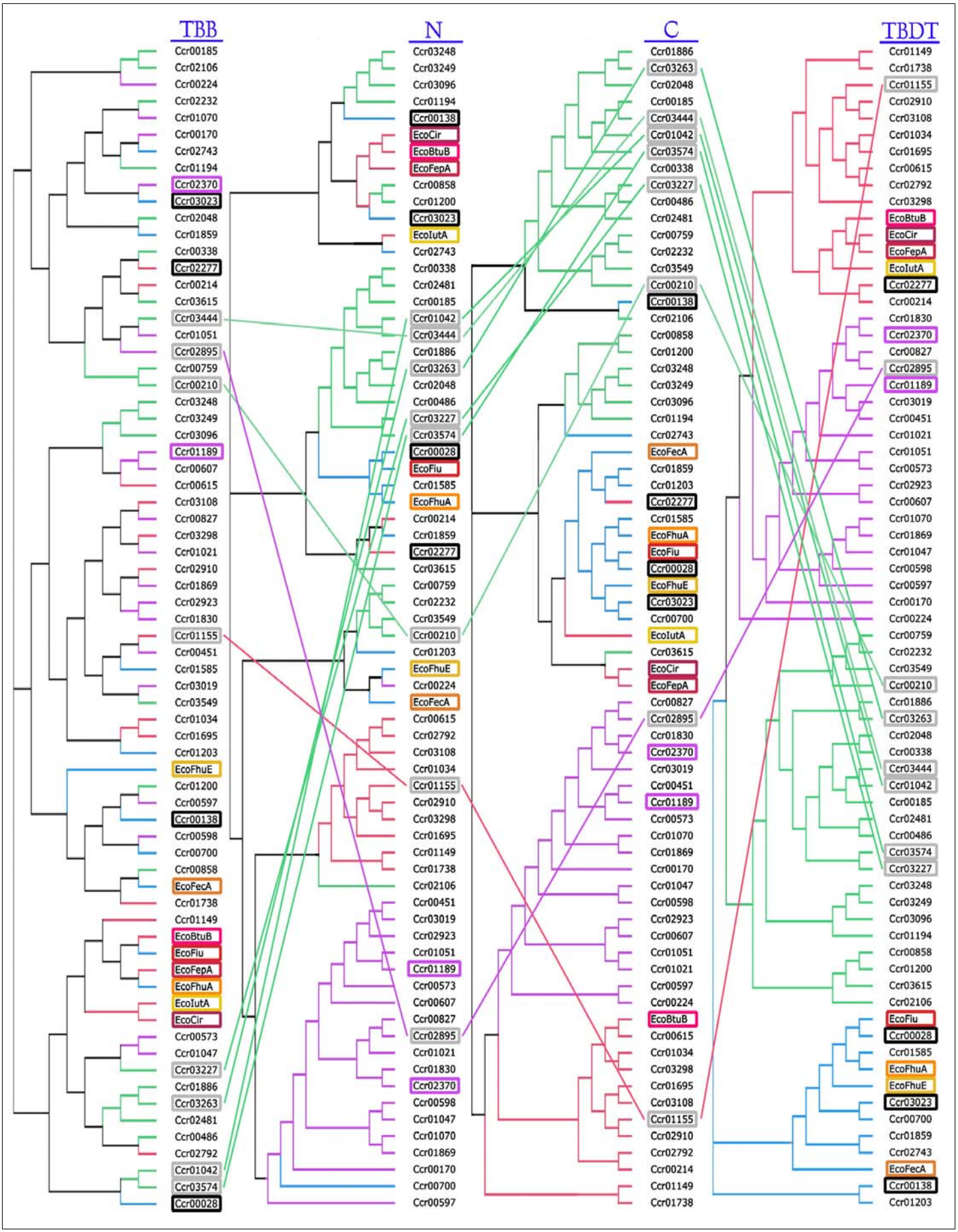
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Figure S3