Supplemental Figure Legends

Fig. S1. Criteria for identifying enriched proteins. To identify proteins enriched in each preparation of interest compared to a control or comparison preparation, we calculated the fold enrichment for each protein (based on analysis of spectral counts, using QuasiTel) and also calculated the statistical significance of differences in numbers of spectral counts assigned to each protein in the two preparations (using Fisher's exact test with a Bonferonni correction). The x axes indicate fold enrichment and the y axes indicate values corresponding to (1 – p value). We then analyzed data for proteins annotated as outer membrane proteins compared to data for proteins predicted to have a non-surface-exposed localization. These analyses allowed us to choose appropriate criteria for identifying proteins that were enriched in one sample compared to another sample. False discovery rates were calculated by comparing a positive control group of proteins (annotated outer membrane proteins for panels A and B, or an expanded list of putative outer membrane proteins selected based on previous studies and protein annotation for panels C and D) to a negative control group of proteins (27 ribosomal proteins for panels A and B, a set of predicted inner membrane proteins (IMP) selected based on previous studies and protein annotation for panel C, or selected proteins predicted to have non-outer membrane localizations for panel D) (see details in Table S2). For each analysis, we defined a p value of < 0.05 with Bonferonni correction as significant, and arbitrarily selected cutoffs for fold-enrichment to allow the identification of a maximum number of positive control proteins while limiting the false discovery rate to < 10%. Proteins were considered enriched if the numbers of assigned spectra were significantly higher in the preparation of interest compared to control preparations, and if there was a minimum fold enrichment compared to the respective counterpart. **(A)** Biotin compared to unlabeled control (Biotin > Control), **(B)** membrane proteins compared to soluble proteins (TM > CP, PP), **(C)** Triton X-100 insoluble proteins compared to Triton X-100 soluble proteins (OM > IM). **(D)** Proteinase K-treated bacteria compared to non-treated bacteria (PK susceptible > PK non-susceptible). Vertical lines indicate the minimum levels of enrichment selected as cutoffs: Biotin > Control = 2, TM > CP, PP = 5.5, OM > IM = 2, PK susceptible > PK non-susceptible = 5.

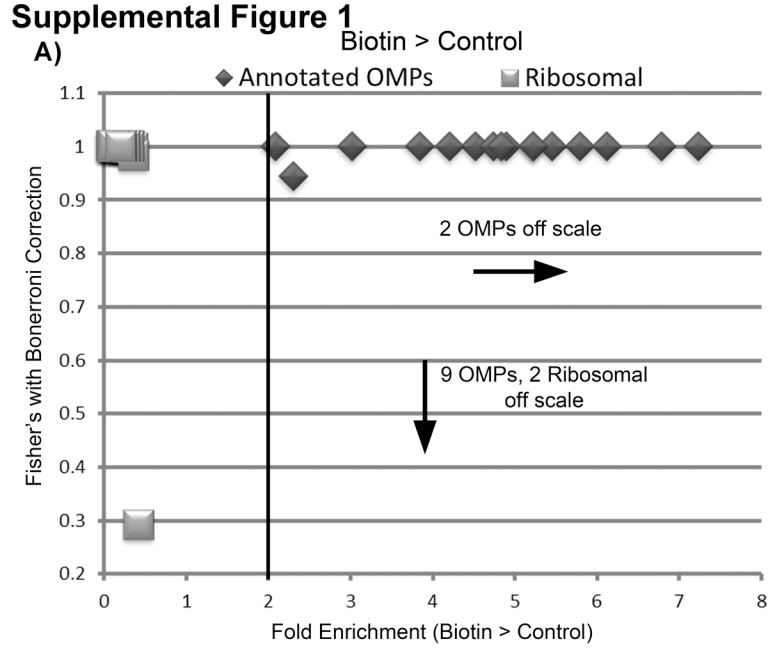
Fig. S2. Enrichment of proteins with an "outer membrane protein" annotation. The proportion of spectra and proportion of proteins annotated as outer membrane proteins were analyzed for each set of preparations. **(A)** Black bars indicate the proportion of spectral counts in each preparation that were attributed to proteins previously annotated as Hop or Hor outer membrane proteins. **(B)** Black bars indicate the proportion of proteins identified in each preparation that were previously annotated as Hop or Hor outer membrane proteins. CP, PP: Soluble proteins, predicted to be localized to

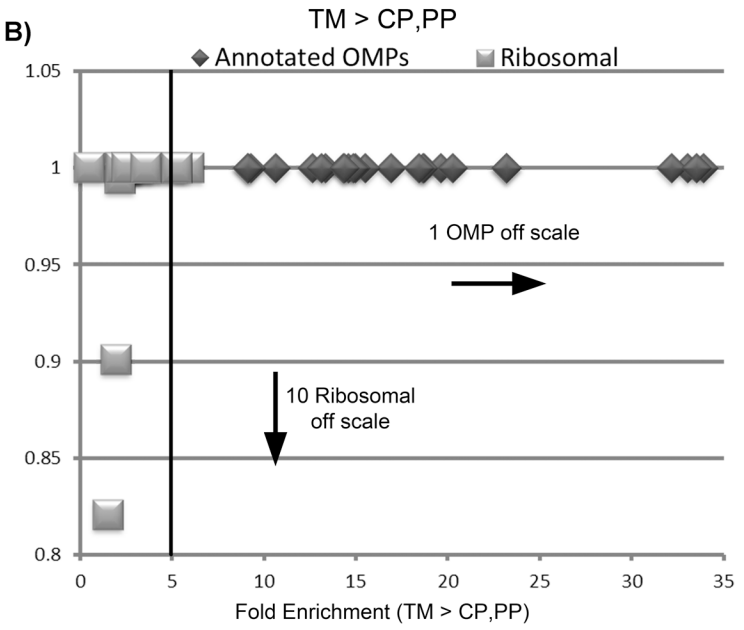
cytoplasmic and periplasmic compartments; TM: insoluble proteins predicted to be localized to membranes; IM: Triton X-100-soluble proteins, predicted to localize to the inner membrane; OM: Triton X-100-insoluble proteins, predicted to localize to the outer membrane; Control: unlabeled control used in biotinyation experiment; Biotin: preparation of purified biotinylated proteins. Biotin > Control: proteins identified as enriched in the biotinylation preparation compared to control. TM > CP, PP: proteins enriched in the TM preparation compared to the CP, PP preparation. OM > IM: proteins enriched in the OM fraction compared to the IM fraction. All Three: proteins meeting three criteria consistent with outer membrane localization (enriched in the biotinylated, TM and OM preparations, compared to respective controls). All data represent the combined results from three independent experiments. ****, $p < 0.001$ for the indicated comparisons (Fisher's Exact test). Values for the set of proteins designated "All Three" in section (A) were significantly higher than values for all other protein sets ($p < 0.001$).

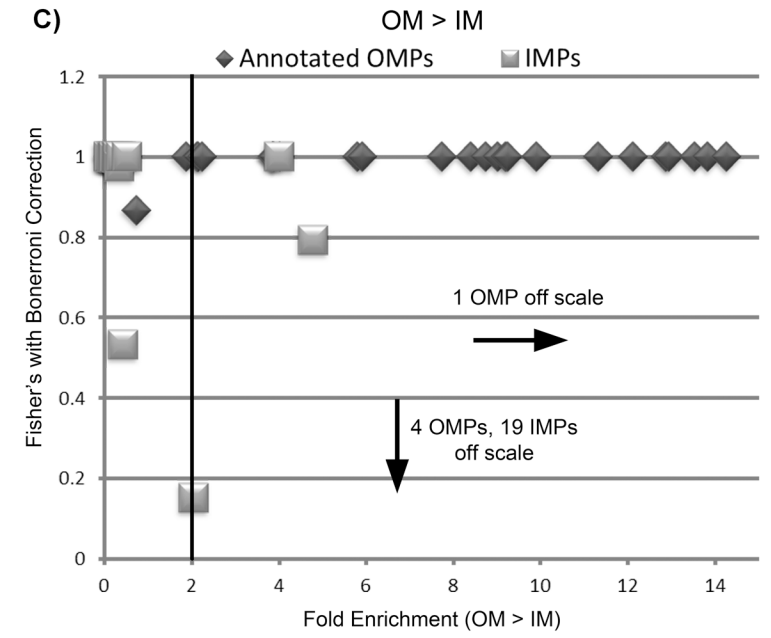
Fig. S3. Comparison of surface-exposed proteins identified by carboxyl-reactive biotinylation compared to amine-reactive biotinylation. In experiments with an amine-reactive biotinylation reagent, we identified 85 proteins that were significantly enriched in the biotinylated preparation compared to a control preparation (Table S3). In experiments with a carboxyl-reactive biotinylation reagent, we identified 32 proteins that were significantly enriched in the biotinylated preparation compared to a control preparation (Table S8). The Venn diagram compares proteins identified using these two biotinylation methods, as well as the set of 39 proteins shown in Table 2. The proteins identified in experiments with the carboxyl-reactive biotinylation reagent were similar to the proteins identified in experiments using an amine-reactive biotinylation reagent.

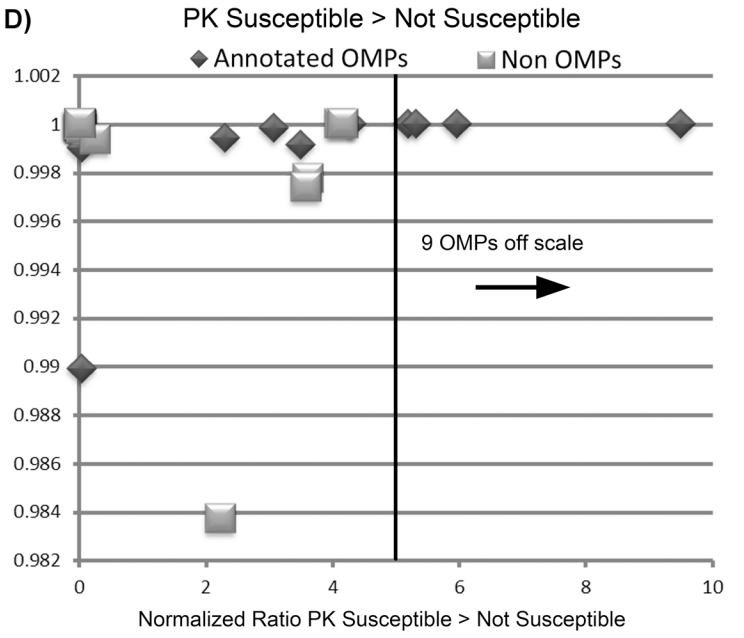
Fig. S4. Subset of surface-exposed outer membrane proteins that are susceptible to digestion by proteinase K. Among the proteins identified using methods based on biotinylation or differential detergent solubility (Figure 2 and Table 2), 13 were susceptible to digestion by extracellular proteinase K. TM/OM corresponds to proteins enriched in the total membrane preparation compared to soluble protein preparation (TM > CP,PP) or enriched in the Triton-insoluble fraction compared to Triton-soluble fraction (OM > IM).

Supplemental Figure 1

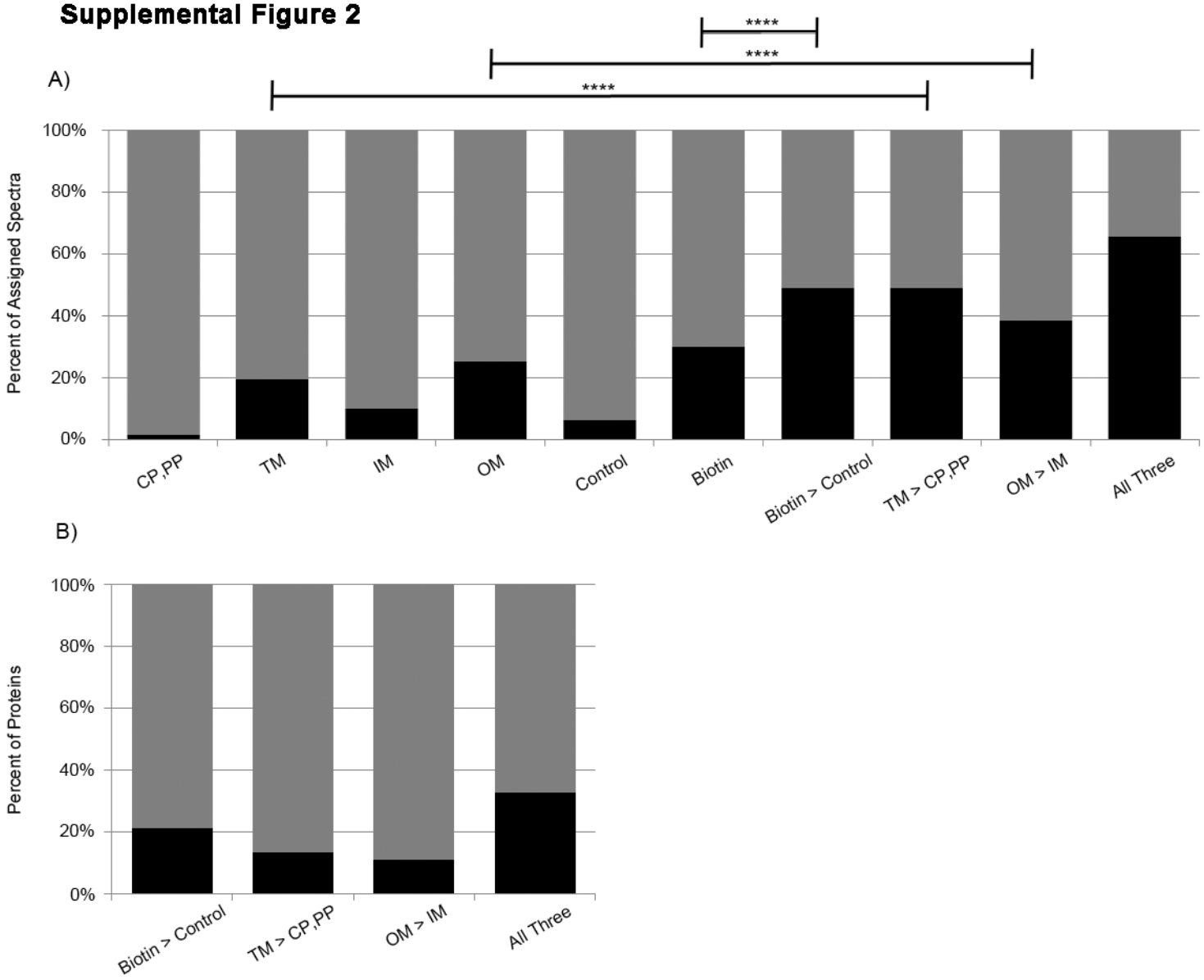




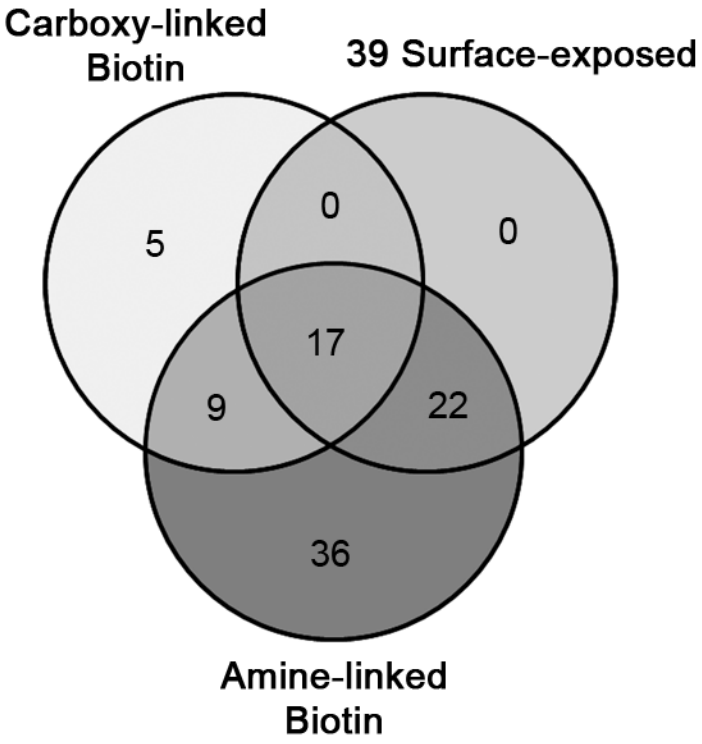




Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4