Supplemental Dataset 1. Three-group comparison of differential 2D gel display data using Kruskal-Wallis Statistic.

Supplemental Dataset 2. Proteins identified in the proteome analysis of *Shigella flexneri* grown in vitro, in the intracellular environment of Henle cells, or extracellular in the presence of Henle cells.

Supplemental Figure 1: Network of *S. flexneri* transport and metabolic pathways that are proposed to play a role in the adaptation of *Shigella* to the host intracellular environment. In the schematic, the box symbols denoting major global regulators (Fnr, Fis, ArcA, Fur/RyhB, and Cra) have a distinct color for identification and are either full or open to indicate positive and negative regulation, respectively. Positive regulation by Fur is generally indirect, through repression of the negative regulator RyhB. To focus on regulators with the broadest activities based on our proteomic data and keep the schematic simple, other regulators influencing expression of the depicted genes/proteins are not included in the schematic (see Supplementary Dataset 2 for more details). Except for the data on iron acquisition systems where *S. flexneri* gene expression has been studied, regulatory data are inferred from those of orthologous *E. coli* K-12 genes in the curated EcoCyc database (www.ecocyc.org). Furthermore, the predicted subcellular localization of all processes is shown in the schematic: the green arch depict the outer membrane, the brown arch the inner membrane. Dotted arrows represent transport functions, solid arrows enzymatic activities. Words and abbreviations colored black represent

enzymatically processed and transported metabolites. Abbreviations not defined by IUPAC nomenclature are (b)P for (bi)phosphate, N-AcGln for N-acetylglucosamine, F for fructose, DHAP for dihydroxyacetone-phosphate, R-OH for long chain alcohols, PMF for proton motive force. Protein short names are colored blue, red, green and magenta: Red; increased abundance in *S. flexneri* during growth in Henle cells (*intracellular* vs. *extracellular*). Magenta; decreased abundance (*intracellular* vs. *extracellular*). Blue; abundance changes during *in vivo* (*intracellular* and *extracellular*) vs. *in vitro* growth. Green; no significant abundance changes (*intracellular* vs. *extracellular* and *intracellular* vs. *in vitro*). Binding of Fe-S (iron-sulfur) cofactors is denoted because of special interest in the effects of iron starvation on diverse metabolic pathways dependent on Fe cofactors.