SUPPLEMENTAL DATA

Human Metabolome-Derived Cofactors are Required for the Antibacterial Activity of Siderocalin in Urine

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Figure S1. DSF screen identifies fractions with SCN-binding character. HPLC fractions from four solid-phase elutions were individually tested for SCN binding by DSF. Fractions were reconstituted in water and mixed with 3 µM SCN in PBS with or without an additional 20 µM iron as indicated. Plots are divided into subgroups of sequential fractions for visibility. Pooled restrictive (left column of panel) or permissive (right column of panel) urines (equal volumes of urine from four donors for each condition) were fractionated over Chrom P SPE and collected as the flow-through (a,b), water wash (c,d), 50% methanol elution (e,f), and 100% methanol elution (g,h). DSF was performed as described in the methods; plots are shown as the first derivative of the fluorescence signal. In each plot, SCN protein alone is shown as a red line for comparison.
**Figure S2. Urinary aryl alcohols perturb SCN thermal stability by DSF.** Candidate metabolites and known SCN ligands were obtained commercially and tested for SCN binding in the presence and absence of iron. Compounds were mixed in a 3:1 molar ratio with FeCl₃ (in red) or an equivalent volume of PBS (in blue) and added to SCN (3 μM) before measuring thermal stability by DSF. Plots are shown as the first derivative of the fluorescence signal (arbitrary units) with respect to temperature; black lines show SCN with buffer alone. Based on the behavior of a known ligand (catechol), Tₘ shifts or signal ablation were considered positive results to be analyzed further. Axes for all plots are as shown in the catechol plot.