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Supporting information for article:

The structure of vanin 1: a key enzyme linking metabolic disease and inflammation

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**Figure S1** Biosynthesis and secretion of wild-type and mutant vanin 1 isoforms in transiently transfected HEK293T cells. Small-scale cultures of HEK293T cells were transiently transfected with the expression vector pAPEX-3P encoding wild type and mutant vanin isoforms. 72 hours after transfection, supernatants were harvested and reduced samples fractionated by SDS-PAGE and transferred to a PVDF membrane. Western blotting was performed with an anti-FLAG-horse radish peroxidise conjugated monoclonal antibody (Sigma) in combination with chemiluminescent detection.
**Figure S2** Rod shaped crystals of kifunesine treated vanin 1. These crystals were grown in 20.5% PEG 2000, 10% Malate-MES-Tris buffer pH 6.5 - similar to those used for data collection. The longest dimension of the double-arrow headed crystal on the bottom right is 350 microns.
Figure S3  Differential scanning fluorimetry traces of kifunensine treated vanin-1 proteins. Native vanin 1 protein is shown in the top panel, the E249Q variant is in the middle panel and the E439Q variant is shown in the lower panel. Each of the three panels shows (triplicate repeat) melt curves where the protein in the original TBSA formulation is shown in red, the protein in 50 mM Bis-tris pH 6.5, 50 mM NaCl is shown in blue and for comparison, the unfolding profile of the protein in a formulation of 50 mM CHES pH 9, 50 mM NaCl is shown in green. For all three proteins, the Tm of the TBSA unfolding is 333-335 K, the unfolding in the lower pH, Bis-tris buffer is 349, 344 or 342 K (for native, E249Q and E439Q respectively), and the higher pH CHES formulation gives a Tm of 310, 302 and 308 K (for native, E249Q and E439Q respectively). The higher pH melting transition (CHES buffer) appears well behaved in the native and E439Q variant, but the E249Q variant is not well behaved in this formulation. Notice that the E249Q variant appears to have a bimodal unfolding in TBSA (the first unfolding event occurring at 314 K, and the second at 333.5 K); no indication of this two part transition is seen at the lower pH.
**Figure S4** SAXS data for native and mutant vanin-1 proteins. a- Agreement between the measured SAXS data and the reported crystal structure of vanin 1. Data from the wild-type protein is shown with the E249Q and E439Q mutant forms offset 10 and 100 units along the Y axis respectively. The inset shows the straight line fits in the Guinier regime with the wild-type and mutant forms being similarly offset along the Y axis. Experimental data is represented by grey circles and model fits by black lines. b- Distance distribution functions calculated from the vanin 1 SAXS data. The wild-type protein is shown in black while the two mutant forms are shown in grey with almost perfect overlay.