Supplementary Information for

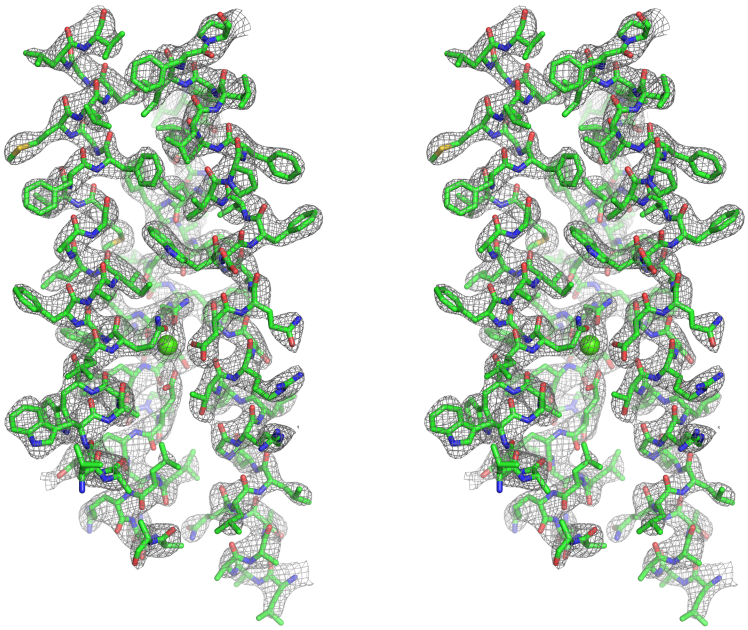
Calcium is an essential cofactor for metal efflux by the ferroportin transporter family

Deshpande et al.

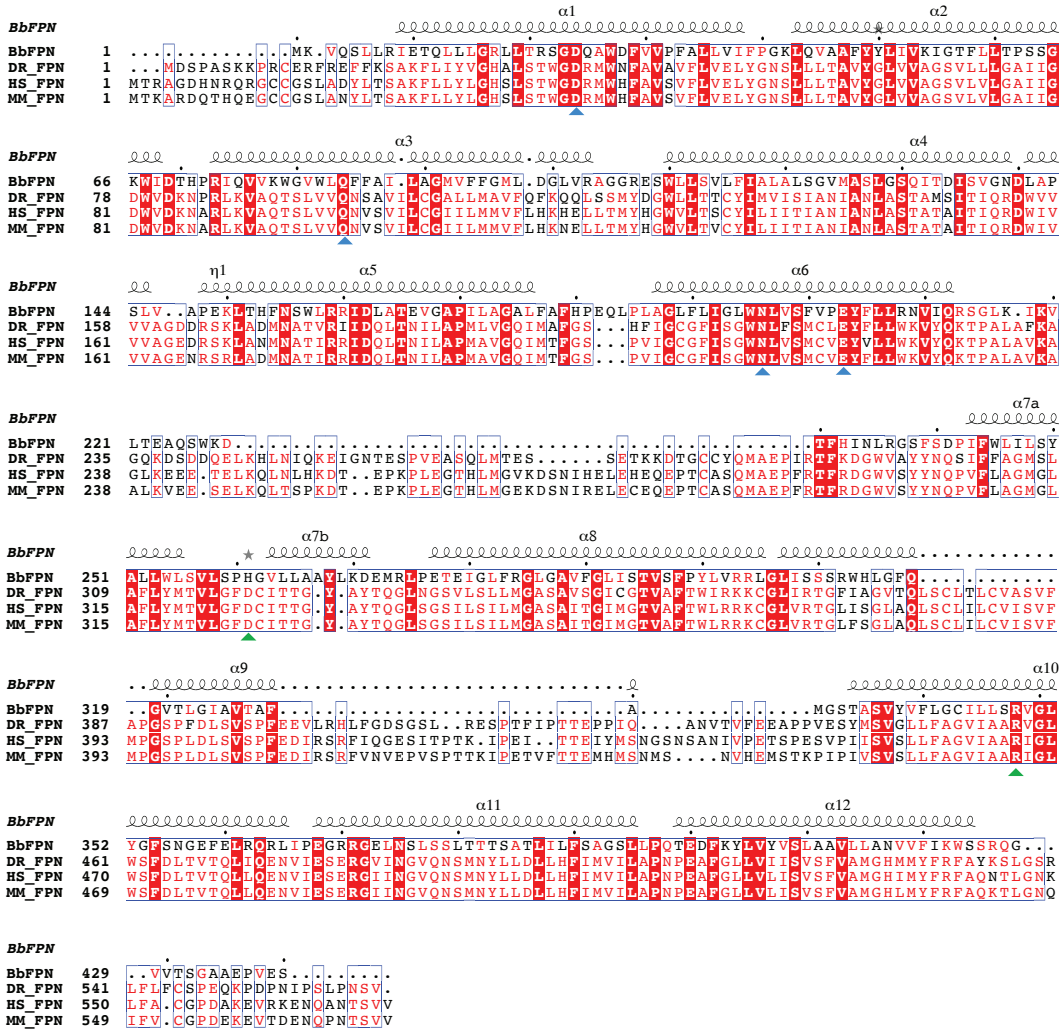
This PDF file includes:

Supplementary Figures 1-11

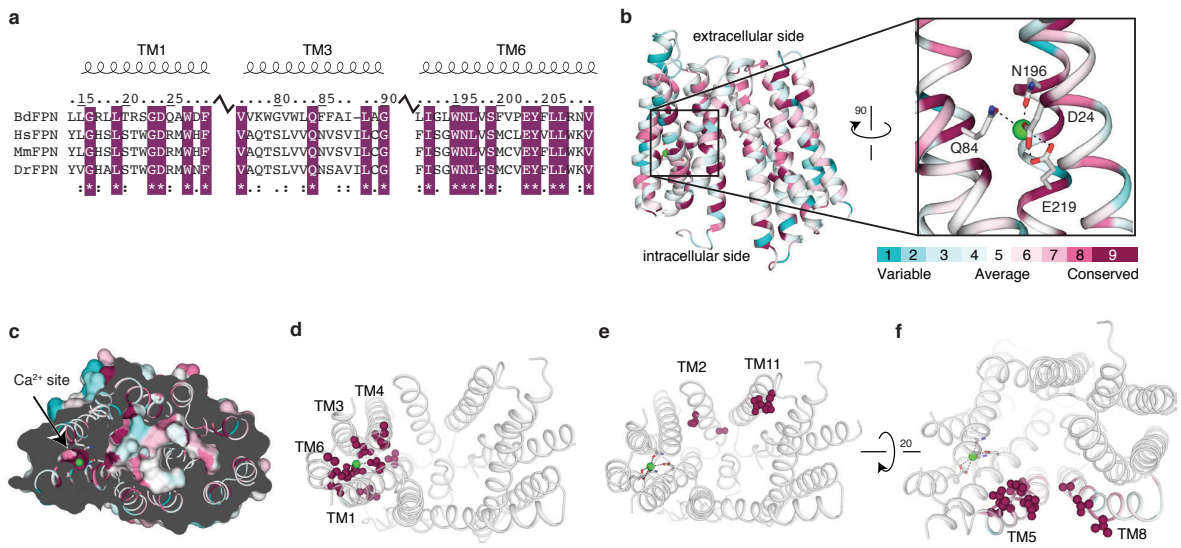
Supplementary Table 1



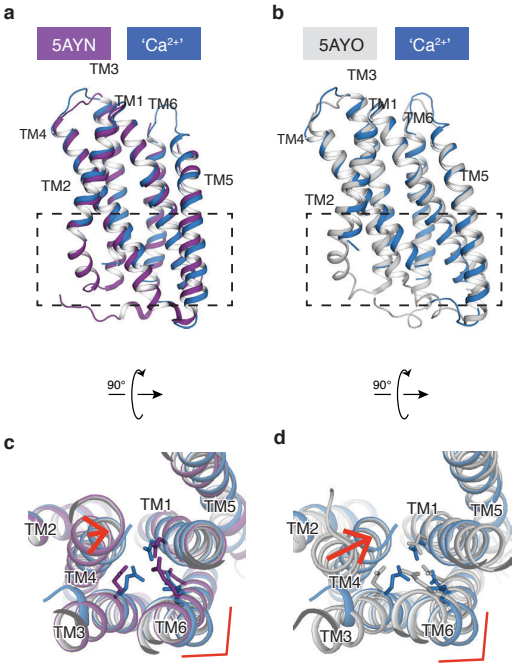
Supplementary Figure 1. Stereo image of representative electron density for BbFpn. Stereo image of the N-terminal domain of BbFpn (green), with $2mF_o$-DF_c electron density (grey) contoured at 1.5σ.



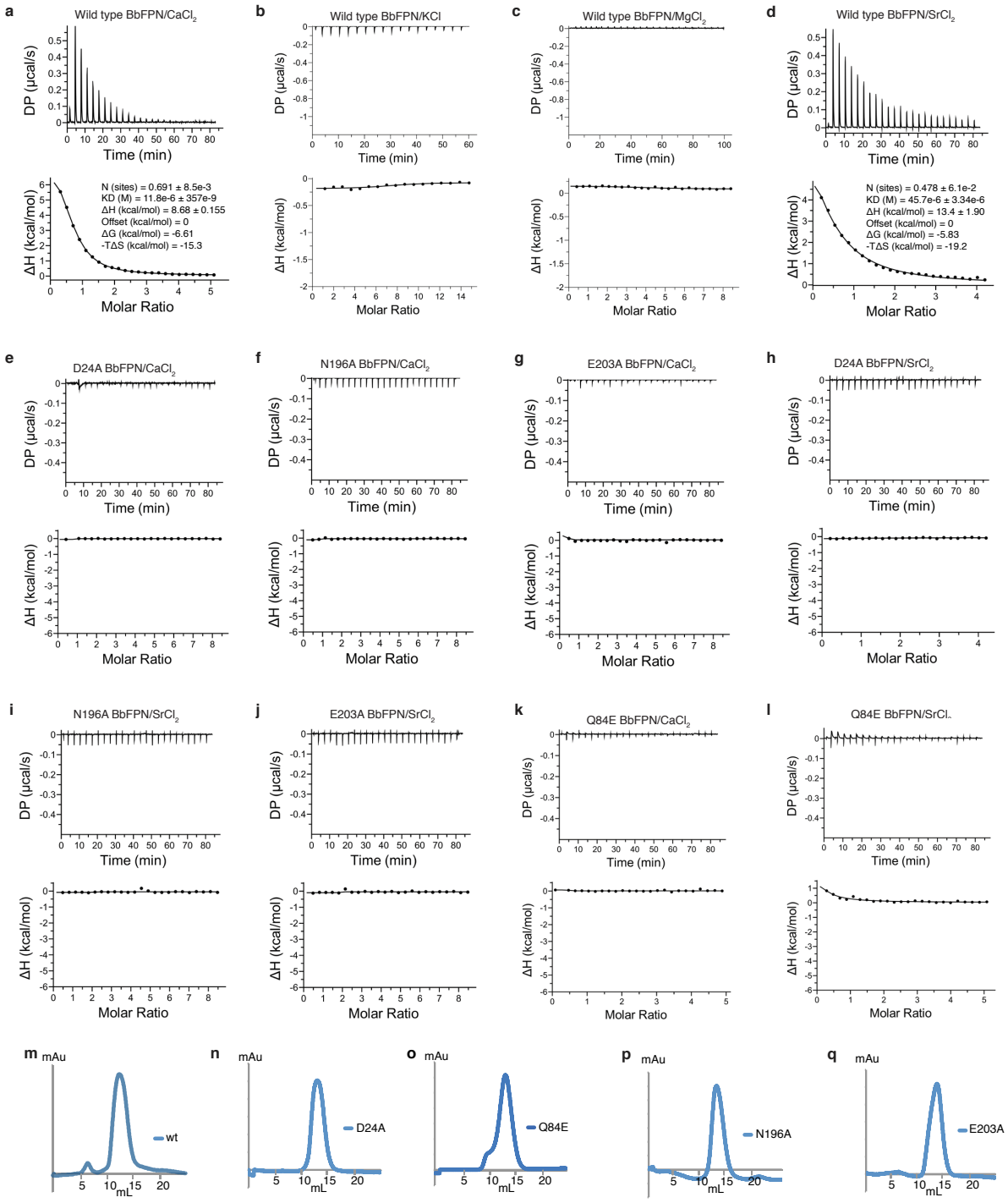
Supplementary Figure 2. Sequence conservation between Fpn proteins. Amino acid sequences for bacterial Fpn (BbFPN; *Bdellovibrio bacteriovorous*), zebrafish Fpn (DR_FPN; *Danio rerio*), human Fpn (HS_FPN), and mouse Fpn (MM_FPN). Secondary structure elements for α-helices are shown based on the structure of BbFpn (PDB entry 5AYN). Fully conserved residues are shown with red panels, and red letters for partially conserved. Residues implicated in Ca^{2+}-ion coordination are indicated with blue triangles, whilst putative substrate ligands are indicated by green triangles.



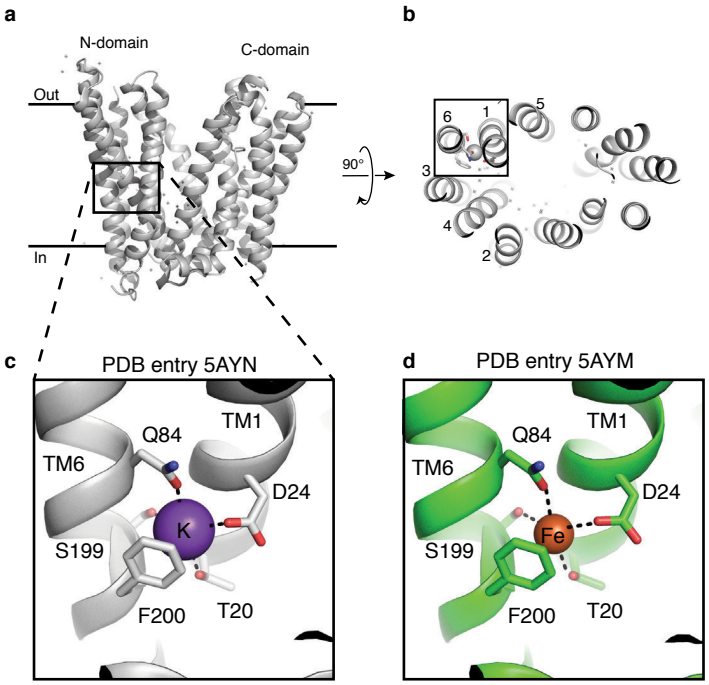
Supplementary Figure 3. Sequence conservation in and around the Ca^{2+} site in Fpn proteins. a Sequence alignment of the Ca^{2+} site, species names as in Supplementary Data Figure 2. **b** Overall structure with the Ca^{2+} site enlarged (right). Figure is coloured from teal (most variable) to magenta (most conserved) on the basis of ConSurf analysis (http://consurfdb.tau.ac.il/). Ca^{2+} ion is shown as a green sphere, and ligands are illustrated in ball-and-stick. **c** Surface rendering and colouring according to ConSurf with a view from the intracellular side of the membrane. The Ca^{2+} ion is located in a conserved occluded pocket. **d** View as in **c**, with the conserved residues in the 4 helixes (TMs 1, 3, 4, and 6) surrounding the Ca^{2+} site illustrated as purple spheres. The conserved residues in these helices are predominantly situated in and around the Ca^{2+}-site. **e-f** View from the intracellular side. Other conserved residues in the N-terminal domain are predominantly located on TM2 and TM5, which forms the main 'hinge' region between the two 6TM domains.



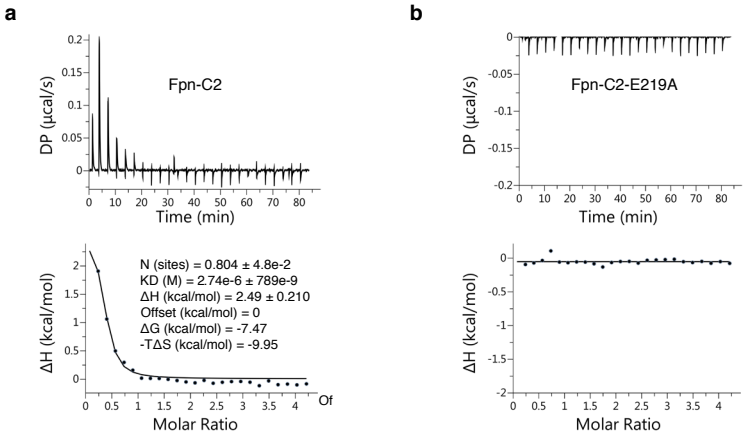
Supplementary Figure 4. Conformational changes in the N-terminal domain. a Superposition of Ca^{2+} bound N-terminal domain with previously determined outward facing conformation (PDB entry 5AYN; purple) and **b** inward facing conformation (PDB entry 5AYO; grey). **c-d** View from the intracellular side, with the structural changes highlighted with red arrows and lines.



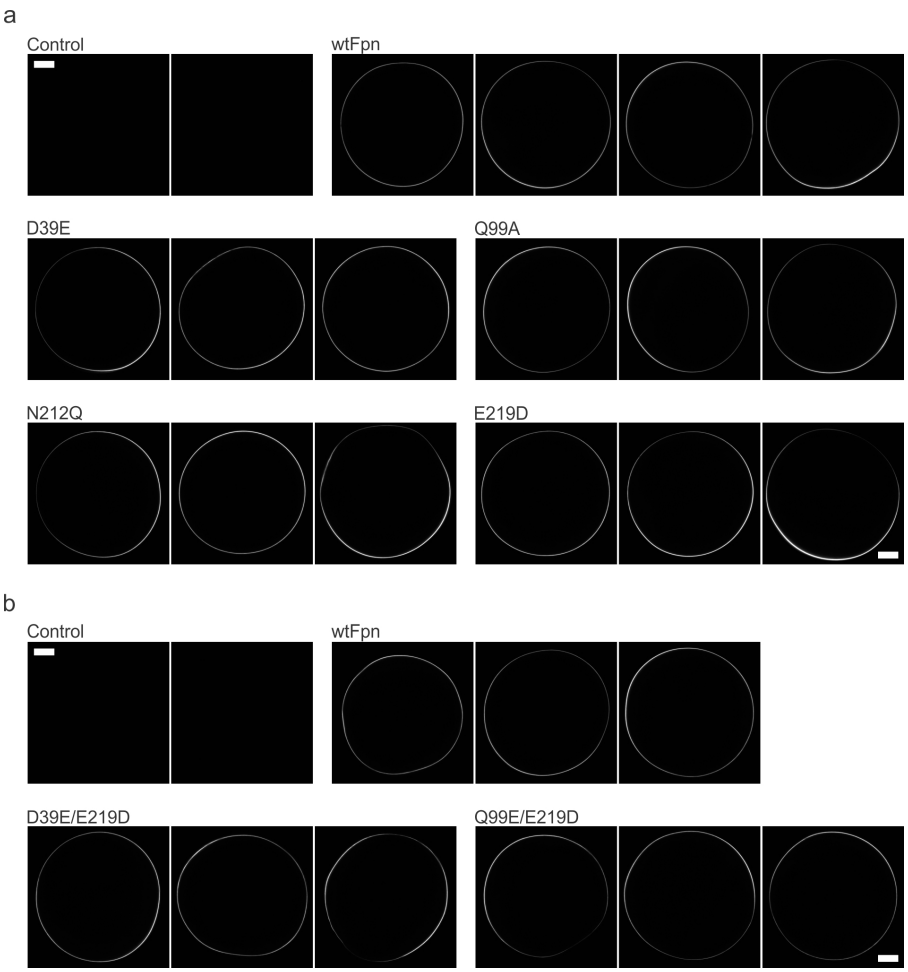
Supplementary Figure 5. Isothermal titration calorimetry data. a-d ITC isotherms of Ca^{2+}, K^{+}, Mg^{2+}, and Sr^{2+} to wild-type BbFpn, respectively. **e-g** ITC measurements of Ca^{2+} binding to D24A, N196A, E203A mutants of BbFpn. **h-j** ITC measurements of Sr^{2+} binding to D24A, N196A, E203A mutants of BbFpn. **k-l** ITC measurements of Ca^{2+} and Sr^{2+} binding to Q84E. **m-q** Size exclusion chromatograms of wild type and D24A, Q84E, N196A, E203A mutants of BbFpn. Samples were run on a Superdex 200 10/300GL (GE Lifesciences), and A280 monitored.



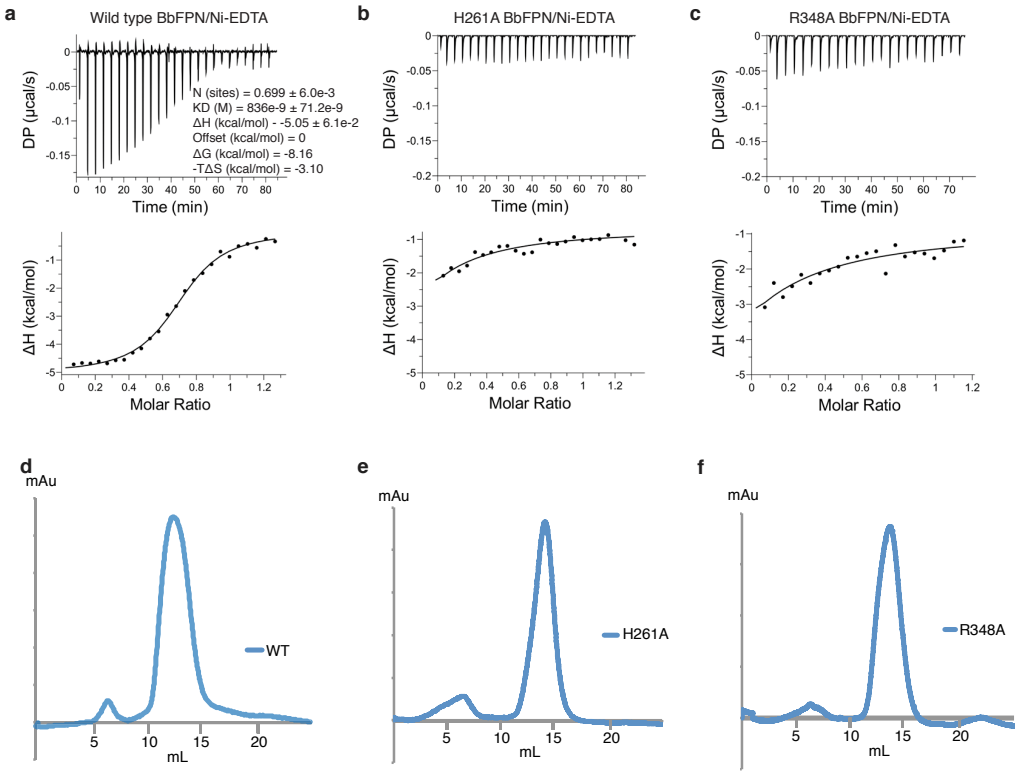
Supplementary Figure 6. Cation binding site in previously determined BbFpn structures. a Cartoon representation of previously determined outward facing conformation of BbFpn (PDB entry 5AYN). The location of the putative substrate binding site is indicated by rectangle box. **b** 5AYN structure viewed from the periplasmic side. Transmembrane (TM) helices 1-6 are numbered and the square box indicates the position of the cation-binding site (between TM1 and TM6), with the K^+ from 5AYN shown as a grey sphere. **c** Close up view of the cation binding site in 5AYN. The K^+ ion (from the crystallization condition) is coordinated by the residues shown in ball-and-stick. **d** Soaking with Fe (5 mM) gave a structure (PDB entry 5AYM) with anomalous difference density at the same site, suggesting Fe binding.



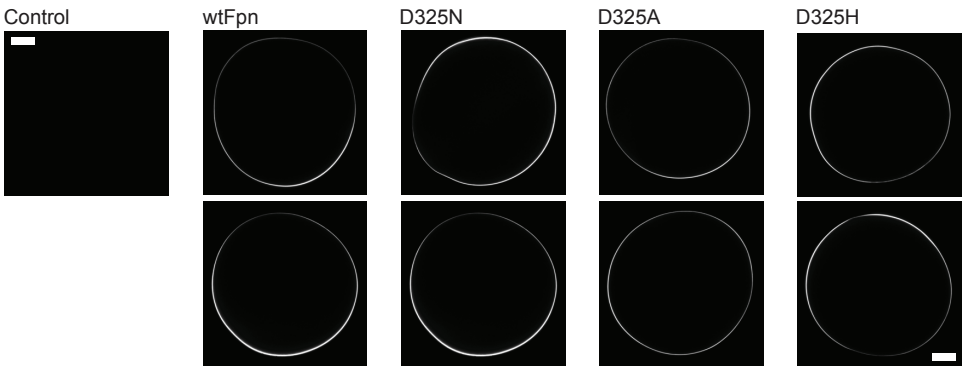
Supplementary Figure 7. Isothermal titration calorimetry data. a ITC isotherms of Ca^{2+} to the thermostabilized mouse Fpn-C2 construct. **b** ITC measurements of Ca^{2+} binding to Fpn-C2-E219A.



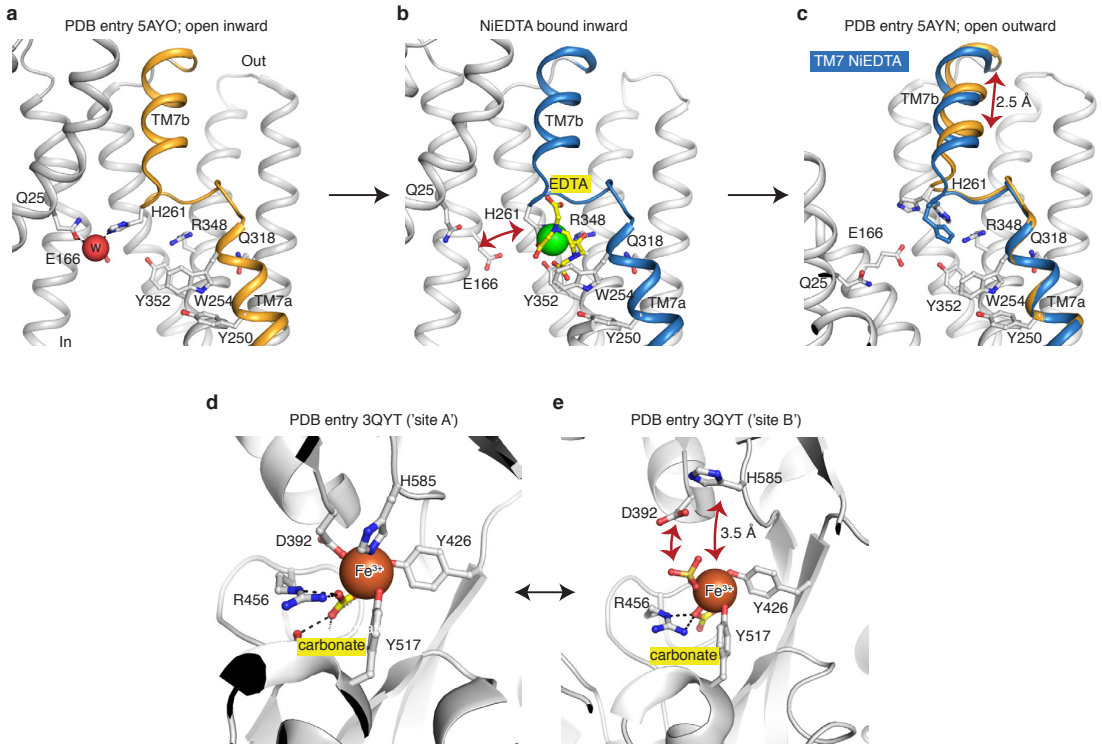
Supplementary Figure 8. Mutagenesis of putative Ca^{2+}-coordinating residues in human Fpn. Live-cell imaging of EGFP-tagged wildtype and mutant Fpn. Each image is of a separate oocyte mounted on the confocal LSM. All images were collected using identical settings. Scale bars, 0.2 mm.



Supplementary Figure 9. Isothermal titration calorimetry data. a-c ITC isotherms of Ni-EDTA binding to wild-type BbFpn, H261A, and R348A, respectively. **d-f** Size exclusion chromatograms of wild type BbFpn and the mutants H261A and R348A. Samples were run on a Superdex 200 10/300GL (GE Lifesciences).



Supplementary Figure 10. Live-cell imaging of oocytes expressing mutants of the putative Fe-binding site of human Fpn. Each image is of a separate oocyte mounted on the confocal LSM. All images were collected using identical settings. Scale bars, 0.2 mm. a, Mutants in which we substituted D325 with N, A, or H, compared with wtFpn in the same oocyte preparation as used in Figure 6E.



Supplementary Figure 11. Proposed substrate binding and release mechanism by Fpn proteins. a Open 'apo' inward conformation (PDB entry 5AYO) of BbFpn viewed parallel to the membrane normal. TM7 is shown in orange and discussed residues are shown in ball-and-stick representation. In this conformation, H261 is oriented away from the substrate-binding pocket, coordinating a water molecule (red sphere) together with Q25 and E166. In this state, the pocket is fully open and accessible for substrate binding. **b** Ni-EDTA bound structure, with TM7 shown in blue. Substrate binding leads to a conformational change in H261, which form a direct ligand to Ni^{2+} (green sphere). **c** Open outward-facing structure (PDB entry 5AYN) with TM7 highlighted in orange. The C-terminal domain from the (Ni-EDTA bound) inward-facing conformation was superimposed and TM7 visualised in blue colour. The superimposition illustrates a shift in TM7b along the membrane normal. We propose that this shift disrupts the metal coordination by H261, which promotes release of both metal and metabolite. **d** Analogous substrate release can be seen in transferrin, in which carbonate and Fe^{3+} binds synergistically. In transferrin, a conserved arginine residue (R456) coordinates the carbonate molecule, which in turn coordinate Fe^{3+} together with surrounding residues. **e** In the substrate release mechanism, the Fe^{3+} ligands D392 and H585 move away from the metal, promoting substrate release.

Supplementary Table 1. Primers used in study.

Construct Name	Primer name	Primer sequence (5' to 3')
BbFPN	BbFPN-F	AAA ACT CGA GAT GAA AGT CCA GAG CTT
BbFPN	BbFPN-R	TTT TGG ATC CGG ATT CAA CGG GTT CAG CA
BbFpn-D24A	D24A-F	CCAGATCCGGGGCTCAGGCCTGGG
BbFpn-D24A	D24A-R	TCCCAGGCCTGAGCCCCGGATCTGG
BbFpn -Q84A	Q84A-F	TGGGGAGTCTGGCTGGCGTTCTTTGCCATTC
BbFpn -Q84A	Q84A-R	CAAGAATGGCAAAGAACGCCAGCCAGACTCCCC
BbFpn -Q84N	Q84N-F	AAGTGGGGAGTCTGGCTGAATTTCTTTGCCATTCTTGCC
BbFpn -Q84N	Q84N-R	GGCAAGAATGGCAAAGAAATTCAGCCAGACTCCCCACTT
BbFpn -Q84E	Q84E-F	TGGGGAGTCTGGCTGGAGTTCTTTGCCATTCTTG
BbFpn -Q84E	Q84E-R	GAATGGCAAAGAACTCCAGCCAGACTCCCC
BbFpn -N196A	N196A-F	GTTCCTGATTGGTCTTTGGGCTCTGGTTTCTTTTG
BbFpn -N196A	N196A-R	ATTCAGGAACAAAAGAAACCAGAGCCCAAAGACCAATC
BbFpn -E203A	E203A-F	AATCTGGTTTCTTTTGTTCCTGCATACTTTCTTTTGCGGAATGTG
BbFpn -E203A	E203A-R	CACATTCCGCAAAAGAAAGTATGCAGGAACAAAAGAAACCAG
hFpn-D39E	D39E-F	GCCACATCCGCTCTCCCCAAGTAGAGAGAG
hFpn-D39E	D39E-R	CTCTCTCTACTTGGGGAGAGCGGATGTGGC
hFpn-Q99E	Q99E-F	GACTGAAACATTCTCTACCACCAGCGAGGTCTG
hFpn-Q99E	Q99E-R	CAGACCTCGCTGGTGGTAGAGAATGTTTCAGTC
hFpn-Q99A	Q99A-F	AGGATGACTGAAACATTCGCTACCACCAGCGAGGTCTG
hFpn-Q99A	Q99A-R	CAGACCTCGCTGGTGGTAGCGAATGTTTCAGTCATCCT
hFpn-N212Q	N212Q-F	GGCTTTATTTCGGGATGGCAGTTGGTATCCATGTGCGTG
hFpn-N212Q	N212Q-R	CACGCACATGGATACCAACTGCCATCCCGAAATAAAGCC
hFpn-E219A	E219A-F	GTGTCCATGTGTGTGGCGTACTTCTTGCTCTGG
hFpn-E219A	E219A-R	CCAGAGCAAGAAGTACGCCACACACATGGACAC
hFpn-E219D	E219D-F	GTATCCATGTGCGTGGATTACGTTCTGCTCTGGAA
hFpn-E219D	E219D-R	TTCCAGAGCAGAACGTAATCCACGCACATGGATAC
hFpn-D325A	D325A-F	CTGTCCTGGGCTTTGCCTGCATCACCACAGG
hFpn-D325A	D325A-R	CCTGTGGTGATGCAGGCAAAGCCCAGGACAG
hFpn-D325H	D325H-F	ATATGACTGTCCTGGGCTTTCACTGCATCACCAC
hFpn-D325H	D325H-R	GTGGTGATGCAGTGAAAGCCCAGGACAGTCATAT
hFpn-D325N	D325N-F	ATATGACTGTCCTGGGCTTTAACTGCATCACCAC