IUCrJ

Volume 7 (2020)

Supporting information for article:

Structural definition of polyspecific compensatory ligand recognition by P-glycoprotein

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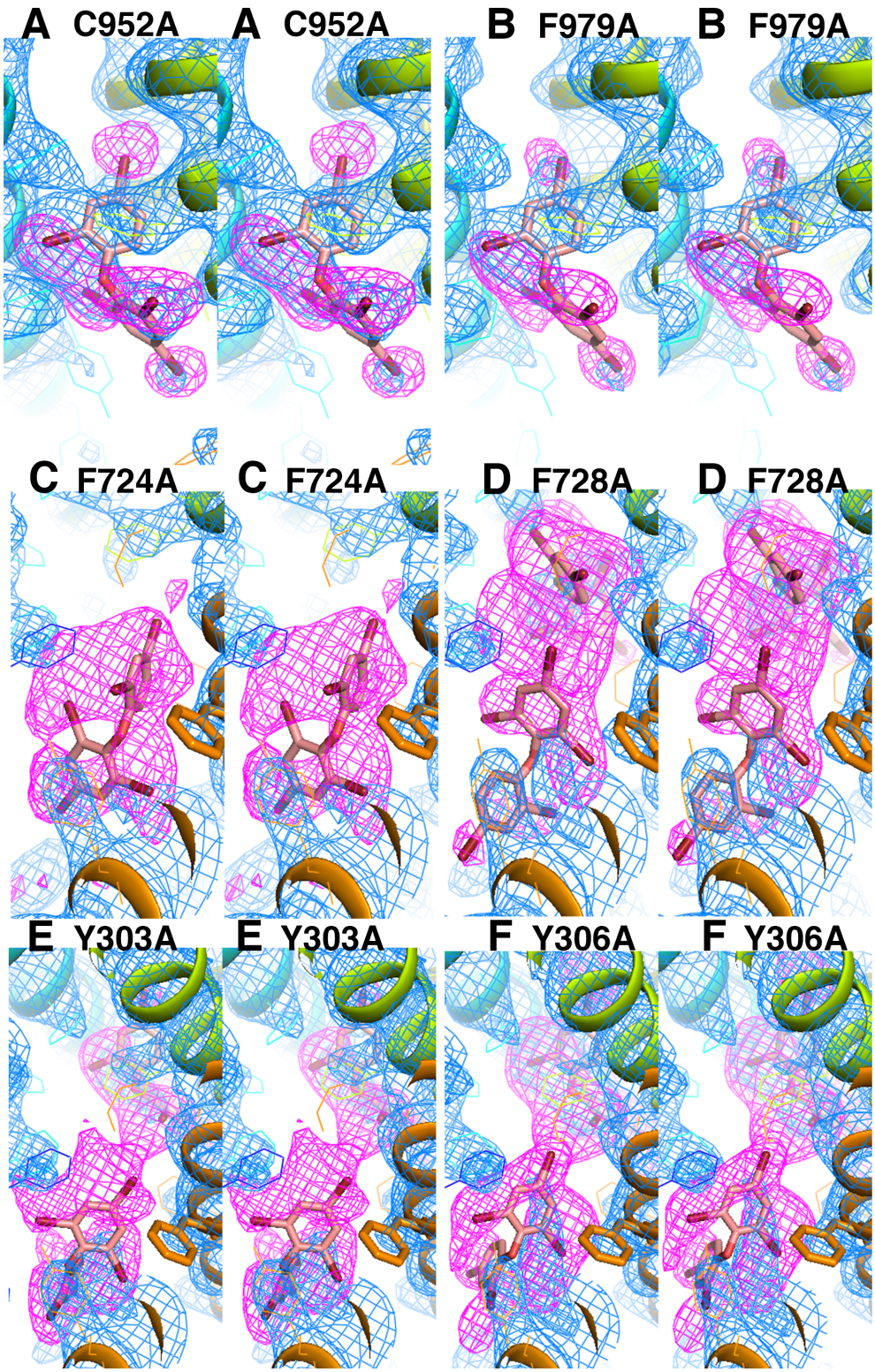

Figure S1 Electron density of six Pgp mutants and localization of BDE-100 by x-ray crystallography. Stereo views are from within the membrane roughly parallel to the membrane plane. Blue mesh represents $2mF_o$-DFc density (B-factor sharpened by 50-75 A^2) contoured to 1.0σ. Magenta mesh represents anomalous difference fourier electron density from the bromine atoms of BDE-100 using x-ray data collected at the bromine anomalous absorption peak (13.48 keV). Contour levels as follows: 5.5σ for C952A, 5.0σ for F979A, 4.0σ for F724A, 3.8σ for F728A, 4.2σ for Y303A and Y306A. BDE-100 in each structure is shown in stick representation with bromine atoms colored red.

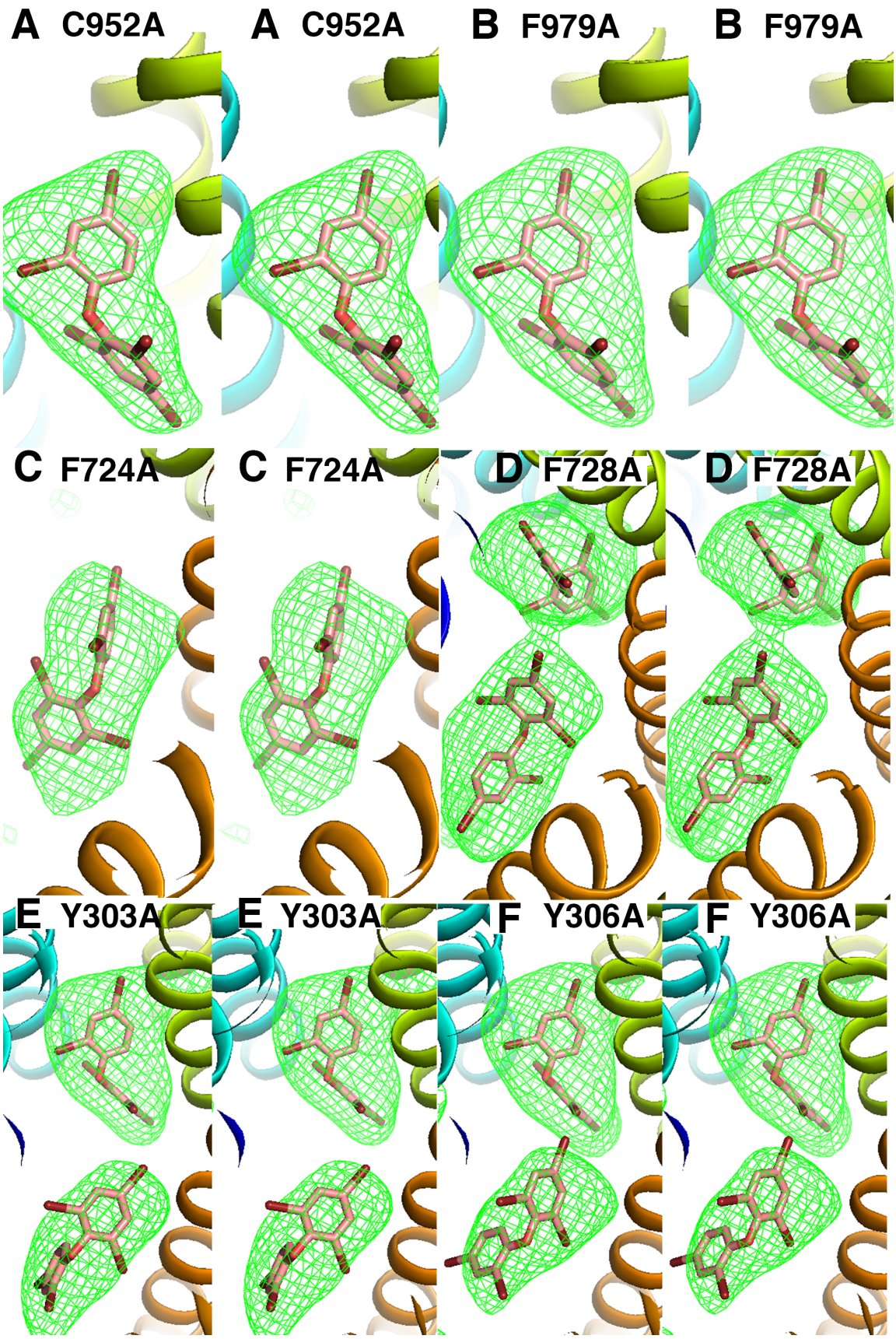

Figure S2 BDE-100 omit electron density maps (Fo-Fc) for the six mutations of Pgp (wall eyed stereo). Omit maps are shown in green (contour levels of 3σ for C952A and 979A, 2.5σ for Y303A and Y306A, and 2.0σ for F724A and F728A).

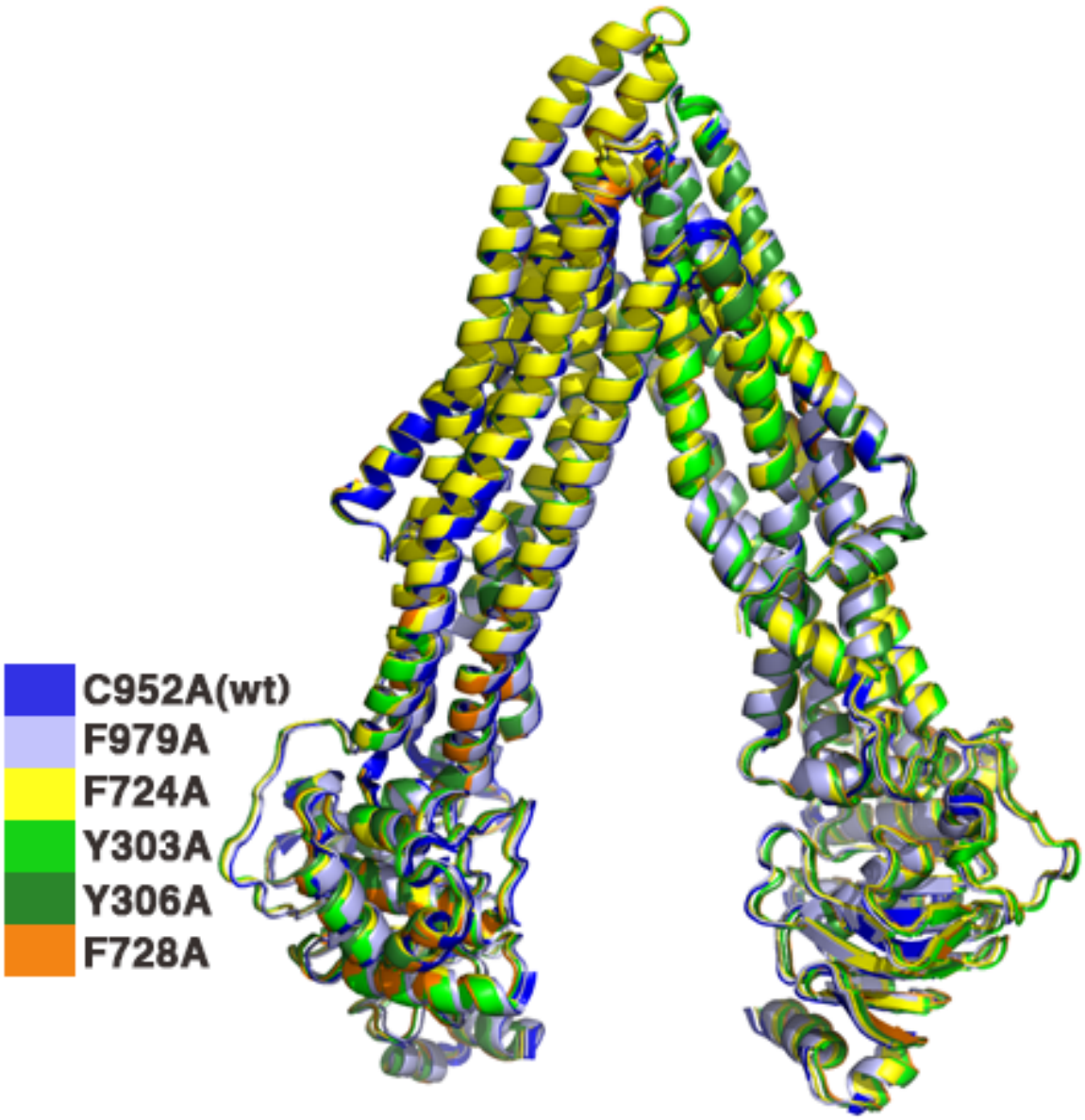

Figure S3 Superposition of all six crystal structures of single site mutations of Pgp determined in this work. C952A is shown in dark blue, F979A in light blue, F724A in yellow, Y303A is shown in green, Y306A dark green, and F728A in orange. Although there were minute changes in some of the TM helices and nucleotide binding domains, the global structures are nearly identical.

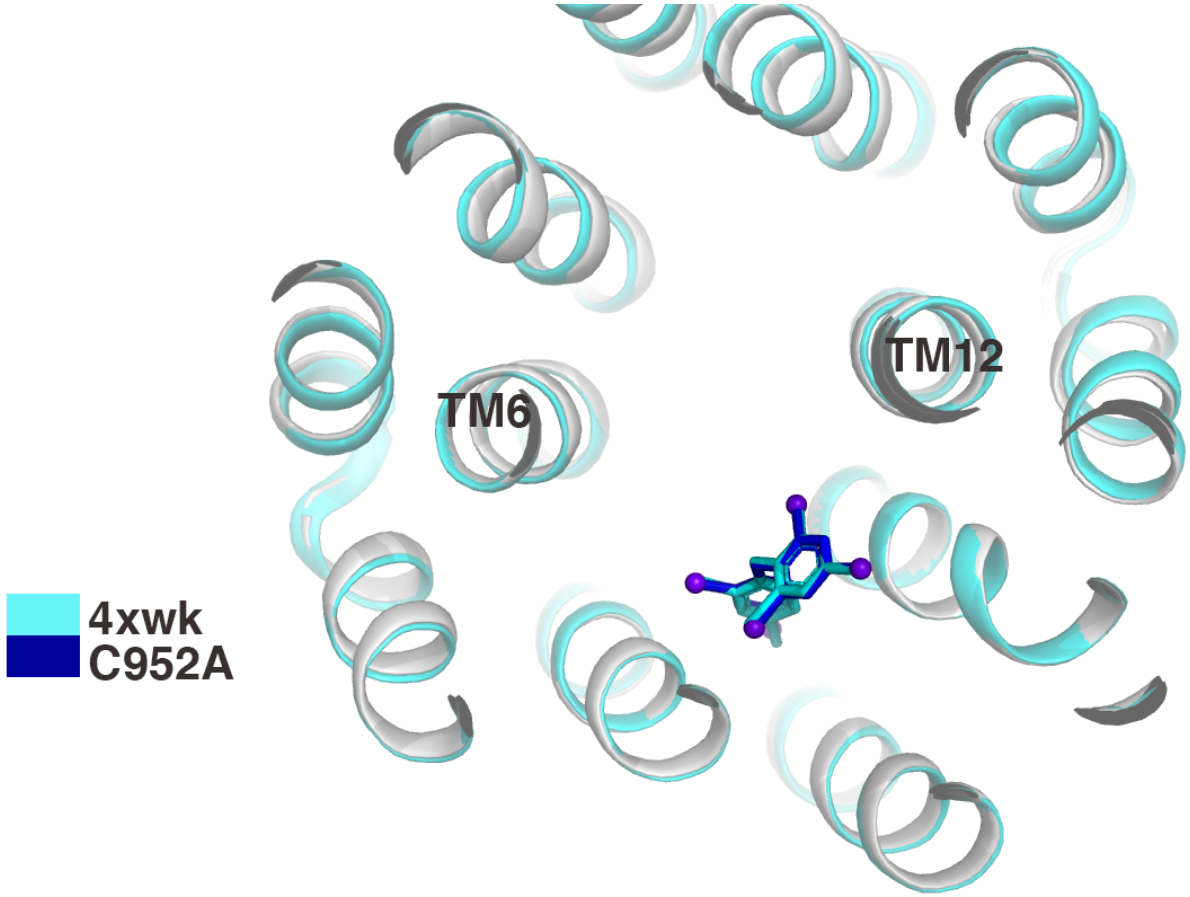

Figure S4 Alignment of C952A and 4xwk. C952A BDE100 is shown in dark blue while the structure is in grey and 4xwk is shown in light blue. Although there were minute changes in some of the TM helices and nucleotide binding domains, the position of BDE100 between both structures are identical.

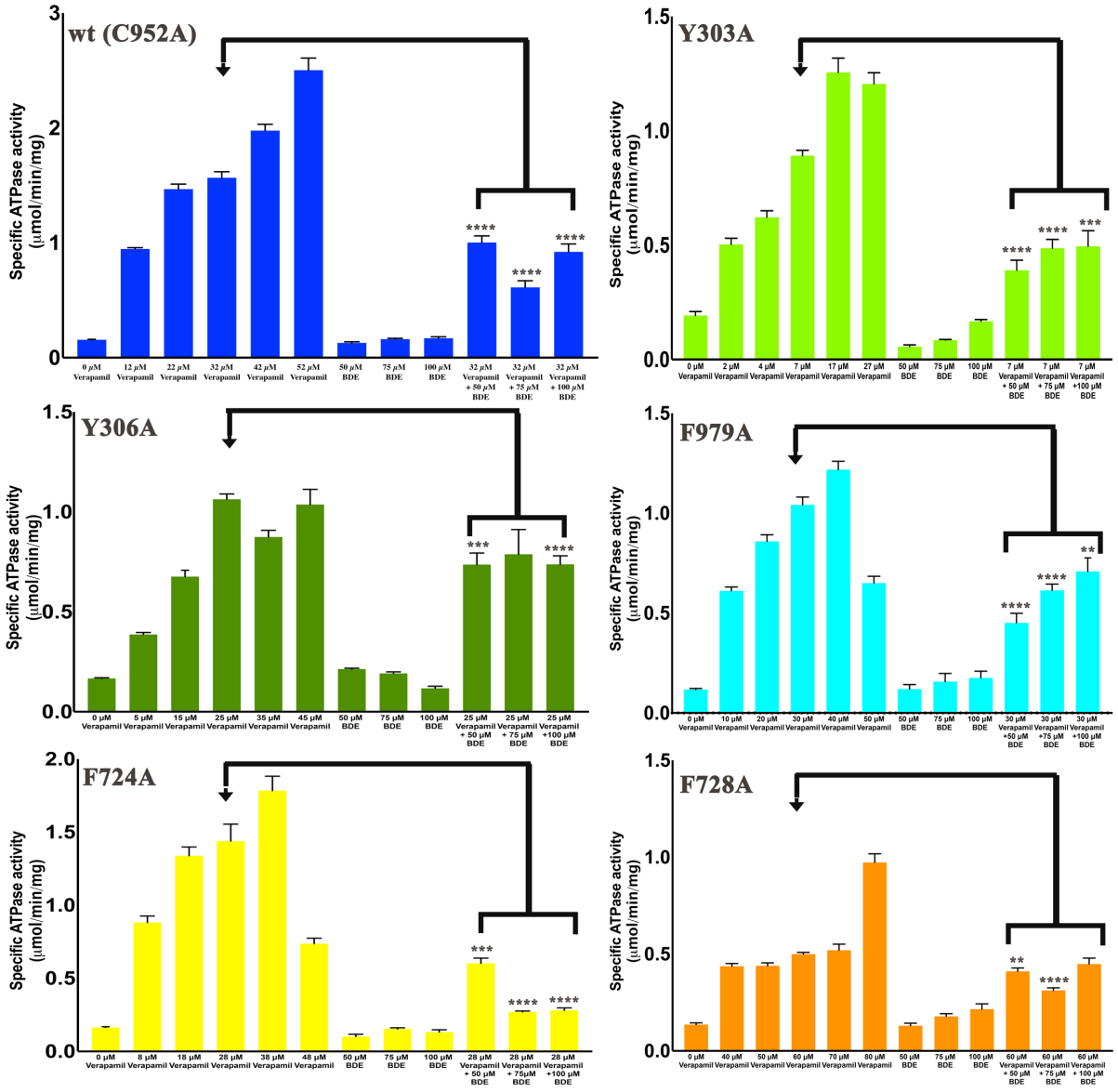

Figure S5 Dose response curve of Verapamil stimulated ATPase activity for the six site-specific mutations of Pgp. Verapamil concentrations clustered around the pre-determined EC_{50} for Verapamil (Figure 4) were chosen to yield a more finely sampled dose response for this figure. BDE-100 alone had little effect on Pgp ATPase activity. Inhibitory effects of BDE-100 at 50 µM, 75 µM and 100 µM on the Verapamil-EC_{50} specific to each mutant were determined. All measurements were determined with seven independent measurements (n=7).

PDB code	phase error (˚)	resolution (Å)
4M1M	27.5	3.80
6UJN	28.2	3.89
6UJP	28.7	3.89
6UJT	29.9	4.17
6UJS	30.2	4.17
5KO2	30.5	3.30
5KOY	31.0	3.85
6UJW	31.5	4.15
6UJR	31.6	4.10
4M2T	32.1	4.20
4M2S	32.7	4.30
5KPD	34.1	3.35
4Q9I	34.1	3.78
4KSD	34.2	4.10
4Q9J	34.3	3.60
4XWK	34.5	3.50
5KPI	35.2	4.00
4Q9L	35.5	3.80
4Q9H	35.7	3.40
4KSC	35.9	4.00
4Q9K	36.2	3.80
4KSB	38.4	3.80
5KPJ	40.4	3.50

Table S1. Phase error statistic and resolution shown for all published x-ray crystal structures of mouse P-glycoprotein.