Supplementary Information

In vitro selection of Giardia duodenalis for Albendazole resistance identifies a β -tubulin mutation at amino acid E198K

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Supplementary Tables:

Supplementary Table 1. Homology modelling results of *Giardia* beta-tubulin monomer with respect to its template.

Template	Species	Sequence Identity (%)	GMQE	QMEAN
5C8Y_D	Sus barbatus	88.28	0.81	-1.30
1SA0_B	Bos taurus	88.76	0.77	-5.67
3N2G_D	Ovis aries	88.03	0.76	-5.58

Supplementary Figure Legends:

Supplementary Figure S1: Dose response curves of the 13 BZ class compounds, as well as podophyllotoxin and metronidazole, which were tested against AlbS and AlbR isogenic lines.

Supplementary Figure S2: Dose response curves of the 9 Alb structural analogues which were tested against AlbS and AlbR isogenic lines.

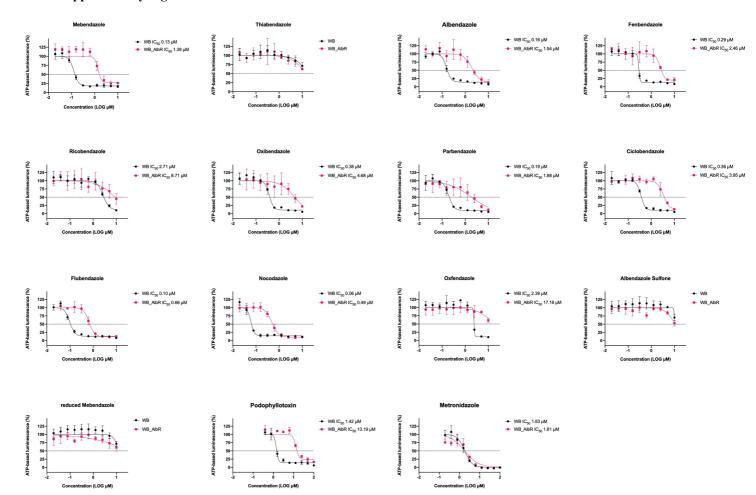
Supplementary Figure S3: Multiple sequence alignment of β -tubulin from *Giardia duodenalis* and other microaerophilic protists with sequences from helminths, fungi and higher eukaryotes showing the conserved residues indicated with an arrow include those implicated in anthelmintic resistance indicated (F167, E198, F200) as well as the *Giardia*-specific C136 modelled to form a hydrogen-bond with Alb in Figure 4A.

Supplementary Figure S4: One binding mode of Alb, where all conserved residues (F167, E198, F200) are forming non-bonded interaction with *Giardia* β -tubulin, but none are involved in hydrogen bonding.

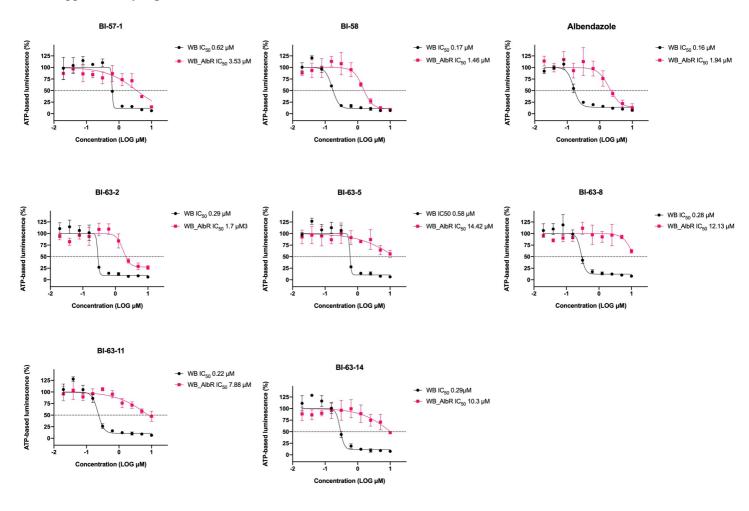
Supplementary Figure S5: Sequencing chromatograms for the three technical replications of AlbS (DMSO1-3) and AlbR (Alb1-3) showing the trace for each of the sequencing regions including nucleotides corresponding to amino acids 134, 165, 167, 198 and 200 of *Giardia* β -tubulin. The E198K heterozygous single-nucleotide polymorphism (SNP) is shown in the three Alb replicates via a red arrow.

Supplementary Figure S2: IC₅₀ dose response curves for albendazole (above) and 3 other BZ compounds (mebendazole, parbendazole, oxibendazole (below) in MtzR isogenic lines WB-MtzR (WB-M3) and WB-MtzS (WB-1B) (Ansell et al., 2017). These demonstrate the MtzR line always has enhanced tolerance to Albendazole varying between 25-75% maximal inhibition compared to its MtzS parent (above), but no change in IC₅₀ or shift in the dose response, indicative of enhanced survival at higher Alb concentration. The same response was also observed for other BZ compounds (below), and was equivalent between BZ-compounds with 5' pharmacophores which undergo redox-based metabolism (Alb, mebendazole) and those which do not (parbendazole, oxibendazole).

Supplementary Figure S1:



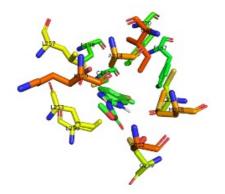
Supplementary Figure S2:



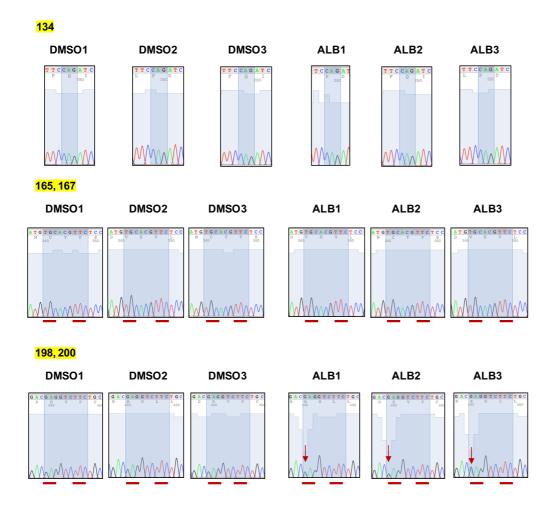
Supplementary Figure S3:

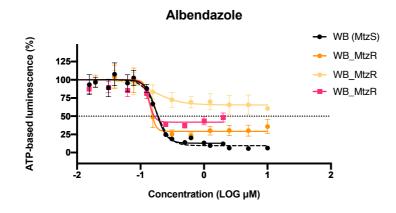
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Supplementary Figure S4:



Supplementary Figure S5:





BZ-Susceptibility in MtzR (Replicate 1)

BZ-Susceptibility in MtzR (Replicate 2)

