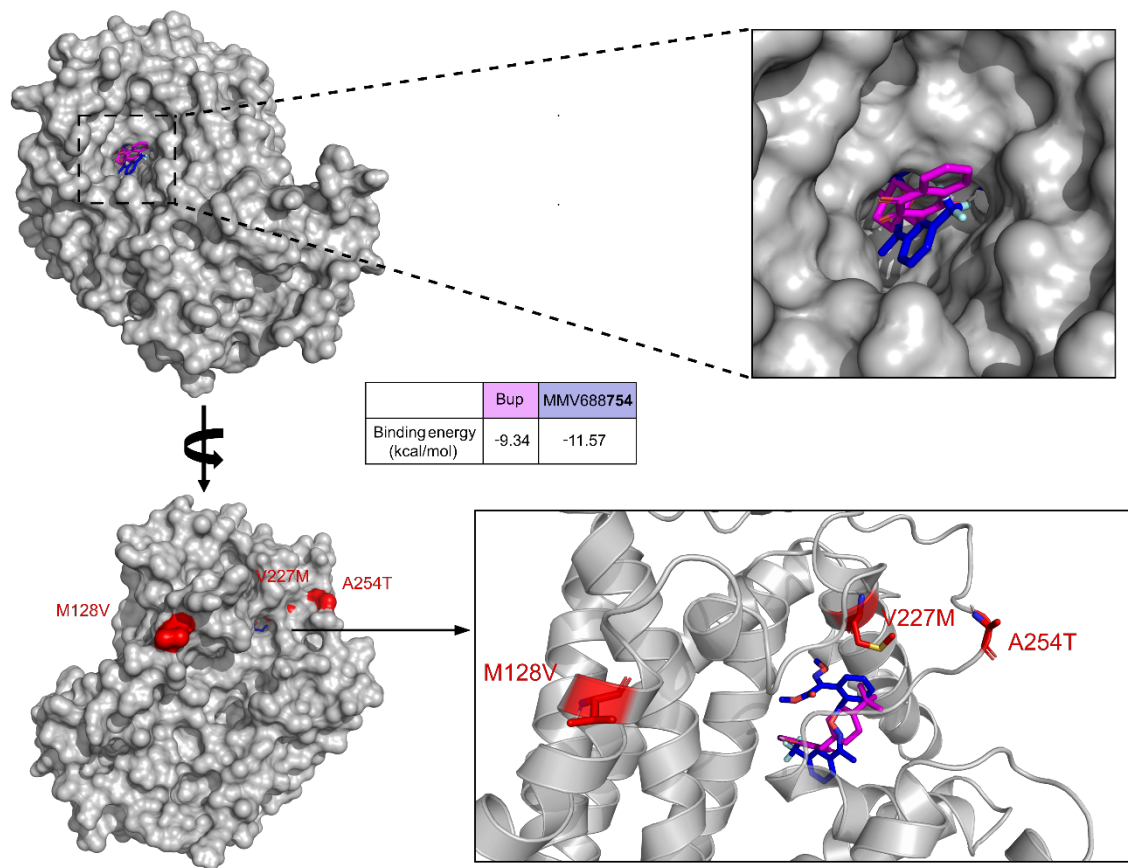


### Supplementary Figure 1: Sequence of the *TaPin1* gene in TBL3 cell lines

Sequence alignment of TaPin1 proteins in TBL3 parental cells and Buparvaquone-resistant TBL3 cell lines. The sequences were identical before and after the generation of drug resistance and both proteins vary slightly from the reference sequence for TaPin1 protein (TA18945 from Piroplasmadb.org).



### Supplementary Figure 2: Model prediction of TaCytB protein structure and drug interactions

Homology prediction model for parasite TaCytB protein (in gray) based on high similarity with chicken cytochrome B (4U3F)<sup>38</sup>. Buparvaquone (in purple) and MMV688754 (in blue) were computationally docked in the q0 site of the enzyme and the predicted binding energy of each compound-enzyme complex was evaluated. TaCytB mutations that are present in the Buparvaquone-resistant TBL3 cell line are displayed in red.

Compound (Plate_Well)	Survival % of DMSO < 25%		MMV	Compound name	Family
	10 $\mu$ M	2 $\mu$ M			
A_A11	3.6				
A_B11	3.2	18.4	MMV676477	CHEMBL2098184	TUBERCULOSIS
A_C04	3.1				
A_F04	2.9				
A_H09	6.3				
A_H11	5.4				
B_A11	9.8				
B_B03	10.8	17.7	MMV676604	AZD5438	KINETOPLASTIDS
B_C03	21.8	6.8	MMV676600	Danusertib	KINETOPLASTIDS
B_C11	4.27				
B_G06	4.9	0.4	MMV003152	Mebendazole	REFERENCE COMPOUNDS
B_G10	23.4				
B_H02	0.02				
B_H03	13.8				
B_H08	0.05				
C_A05	3.2				
C_A10	6.8				
C_C03	0.9				
C_D11	23.5				
C_E11	6.7				
C_F04	2.9				
C_F08	0.8				
C_F10	10				
C_G03	22.5				
C_G04	0.5				
C_G05	0.4				
D_A05	2.3				
D_B07	0.2				
D_B09	9.5				
D_B10	0.2				
D_D11	0.2				
D_E02	19.6				
D_E05	2.4				
D_F03	5.9				
D_F06	3.5				
D_F11	0.05				
D_G08	0.1	11.1	MMV688372	CHEMBL3115821	KINETOPLASTIDS
D_G11	8.9				
D_H09	1.1	0.9	MMV675968	CHEMBL88430	CRYPTOSPORIDIOSIS
E_D05	8.2				
E_E11	0.6	3.7	MMV153413	Thiophene-4	TUBERCULOSIS
E_F08	0.05				
E_G04	4.3	2.8	MMV021013	CHEMBL530275	TUBERCULOSIS
E_G06	9	15.9	MMV688754	Trifloxystrobin	KINETOPLASTIDS

Supplementary Table 1

Target genes	Primers sequence Forward	Primers sequence Reverse
Actin	GGCATCCTGACCCTCAAGTA	CACACGGAGCTCGTTGTAGA
Tasp	AGCAGCCCCTTGTCATGGG	TAATAGCTTTTGCACGGAGGA
MMP9	CCCATTAGCACGCACGACAT	CACGTAGCCCACATAGTCCA
HK2	CGACCAAGTGCAGAAGGTTG	CGTCTGGAGTAGACCTCAC
miR155	TGGCGCAAACCAGGAAGGGGA	ACAGCCCACAGCAAGCCTCC
PDK1	CTAGGCGTCTGTGTGATTG	GATAGAGGTGGGATGGTAC
HSP70	ACGCAAATGGAATCCTCAAC	TATTCGTCGTGCTCTGCTAA
TamR1	CCACTCCTGTAGCGGGTAAA	TTGGGAGGTAAGTACCCAAA
TaCytB	TACCGAGCTCGGATCATGAATTTGTTTAACTCACATTTGC	GCCCTCTAGACTCGATTATGCACGAAGTCTTGC

**Supplementary Table 2**