Supplemental Material

Metabolic dependency of chorismate in *Plasmodium falciparum*

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Running title: *Chorismate dependency in P. falciparum*

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Table S1. RPMI minimal and complete composition used in this study.

Nutrient	Final Concentration (µM or as indicated)	Minimal Medium (MM)	Complete RPMI Medium (CM)
L-Arginine	200	+	+
L-Asparagine	200	+	+
L-Aspartic acid	150	+	+
L-Cysteine	200	+	+
L-Glutamic acid	136	+	+
L-Glutamine	200	+	+
Glycine	133	+	+
L-Histidine	97	+	+
Hydroxy-L-proline	153	+	+
L-Isoleucine	100	+	+
L-Leucine	381	+	+
L-Lysine	200	+	+
L-Methionine	101	+	+
L-Proline	174	+	+
L-Serine	200	+	+
L-Threonine	168	+	+
L-Valine	171	+	+
Choline Chloride	0.0214	+	+
D-Biotin	0.00082	+	+
D-Calcium pantothenate	0.00052	+	+
Myo-Inositol	0.194	+	+
Niacinamide	0.0081	+	+
Pyridoxine hydrochloride	0.0048	+	+
Riboflavin	0.00052	+	+
Thiamine hydrochloride	0.00296	+	+
Vitamin B12	0.00037	+	+
Reduced Glutathione	3,254	+	+
$Ca(NO_3)_2$	609	+	+
HEPES	20,967	+	+
KCl	5,365	+	+
$MgSO_4$	407	+	+
NaCl	102,669	+	+
Na_2HPO_4•7 H_2O	2,797	+	+
D-Glucose	22,203	+	+
$NaHCO_3$	26,784	+	+
Gentamicin	41,876	+	+
Hypoxanthine	367	+	+
Albumax II	5 (g/L)	+	+
Folic Acid	**2.2**		**+**
***p*-Aminobenzoate**	**7.3**		**+**
L-Phenylalanine	**90**		**+**
L-Tryptophan	**24.5**		**+**
L-Tyrosine	**111**		**+**
***p*-Hydroxybenzoate**	**7.3**		**+**

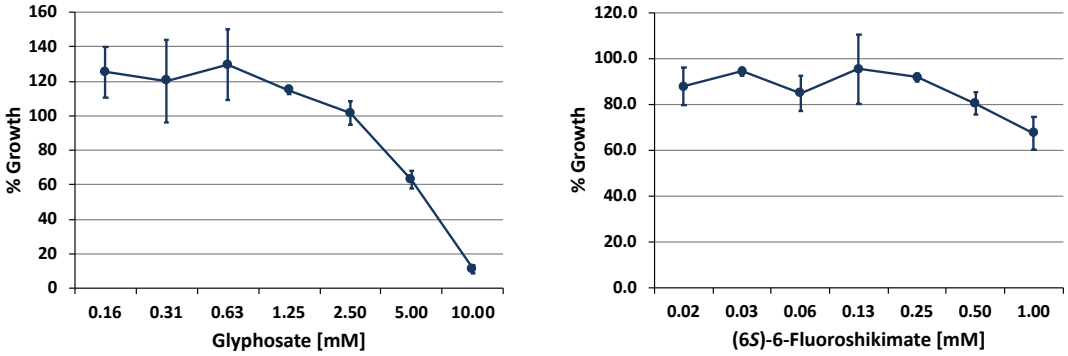

Fig. S1. Effect of glyphosate and (6*S*)-6-fluoroshikimate on *in vitro* growth of *P. falciparum*. Dose-dependent growth inhibition was determined after incubation for 72 h in the presence of increasing concentrations of the inhibitor in MM. Parasite growth was assessed by SYBR green. Results represent means ± S.E.M. of two independent assays, with each assay performed in triplicate.

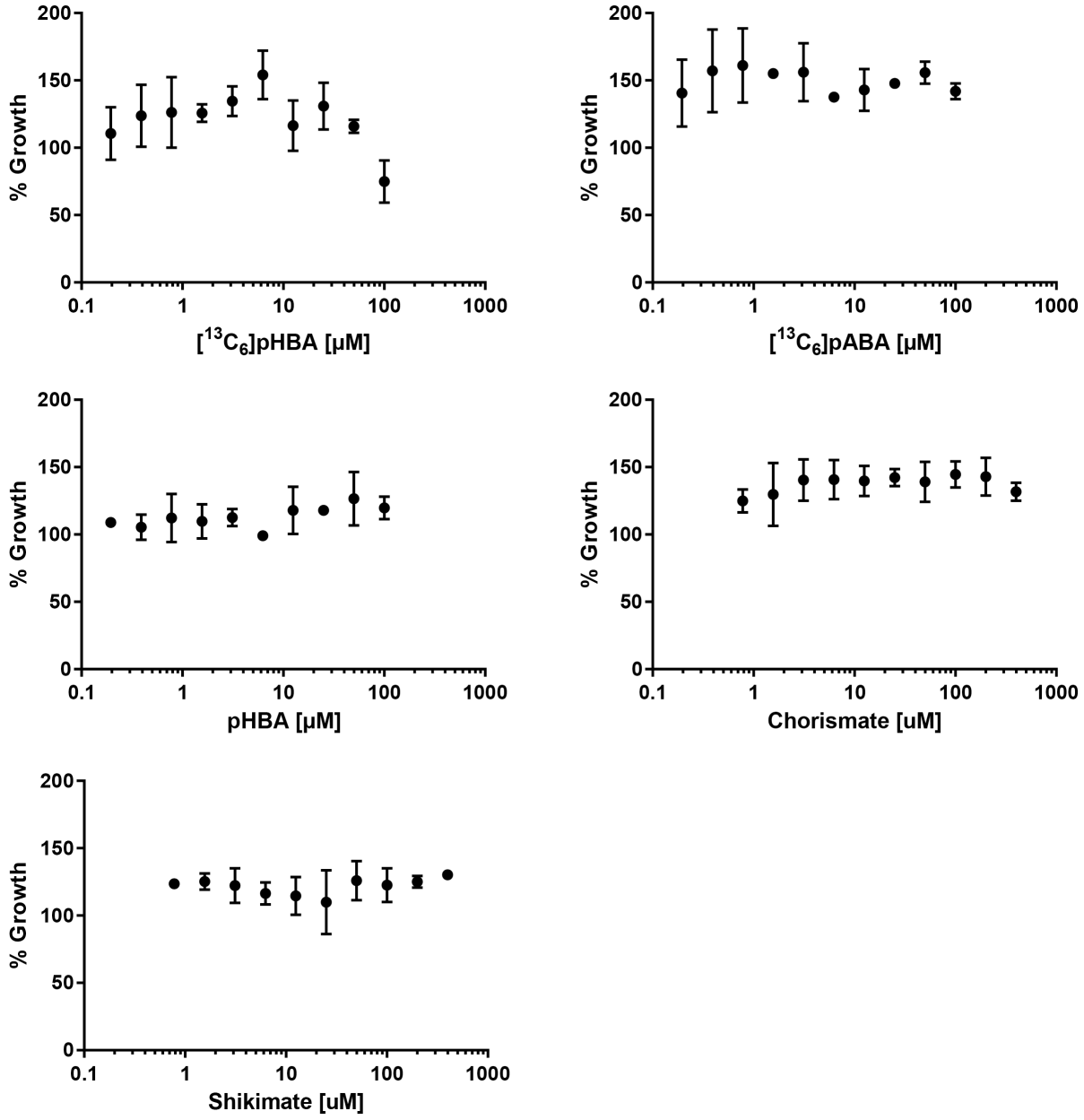

Fig. S2. Effect of metabolites on *P. falciparum in vitro* growth. Concentration-dependent potential metabolite toxicity was assessed in MM by SYBR green assay after 72 h incubation.

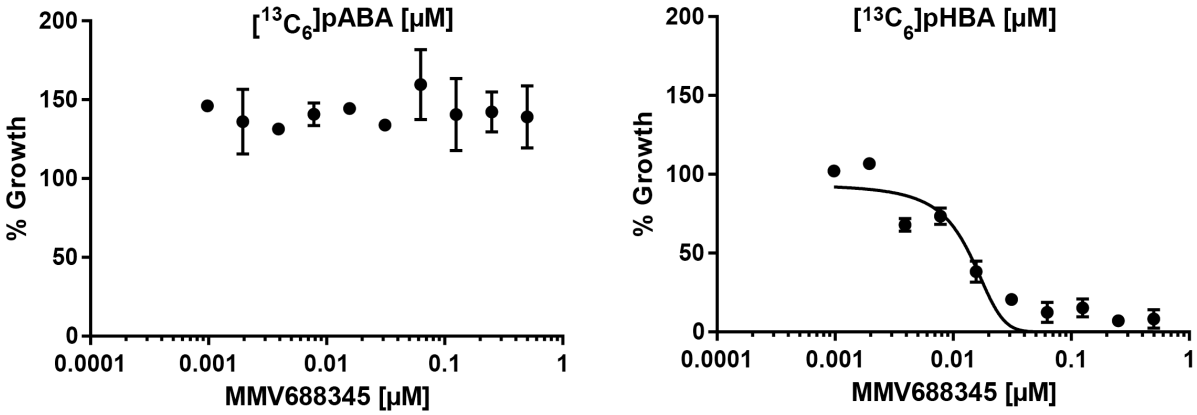

Fig. S2. (continuation) Reversal of growth inhibition by MMV688345. *P. falciparum in vitro* reversal of growth inhibition by MMV688345 in the presence of 7.3 µM [$^{13}C_6$]pHBA or [$^{13}C_6$]pABA was assessed in MM by SYBR green assay after 72 h incubation.

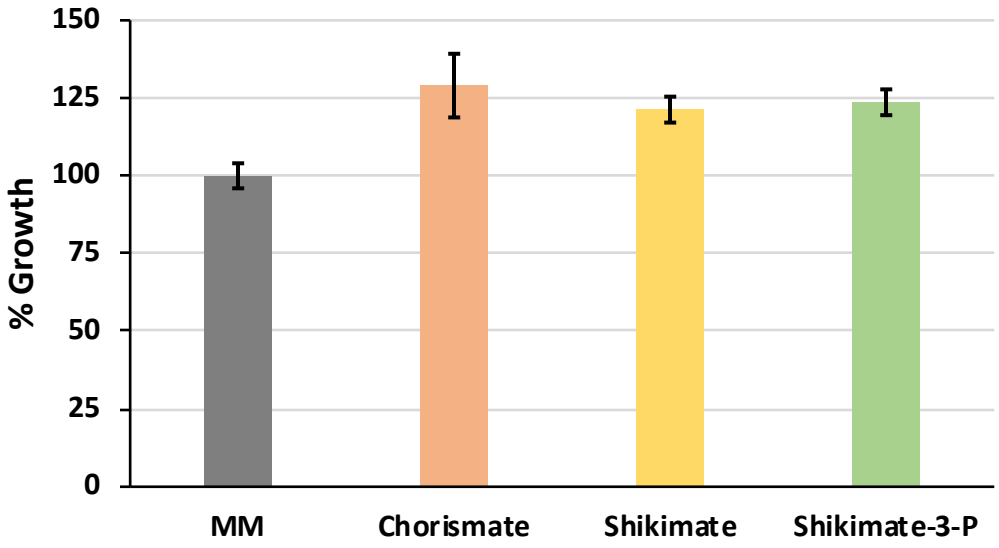

Fig. S3. *P. falciparum* growth in MM in the presence of different metabolites from the shikimate pathway. Cultures were supplemented with chorismate (12.5 μM), shikimate (25 μM) or shikimate-3-phosphate (25 μM) and growth was assessed by SYBR green assay. Values represent the mean ± S.E.M. from at least three independent assays performed in triplicate. *P* values for each supplemented metabolite with respect to control are: (*) < 0.00001, (**) < 0.000004 and (***) < 0.000009.

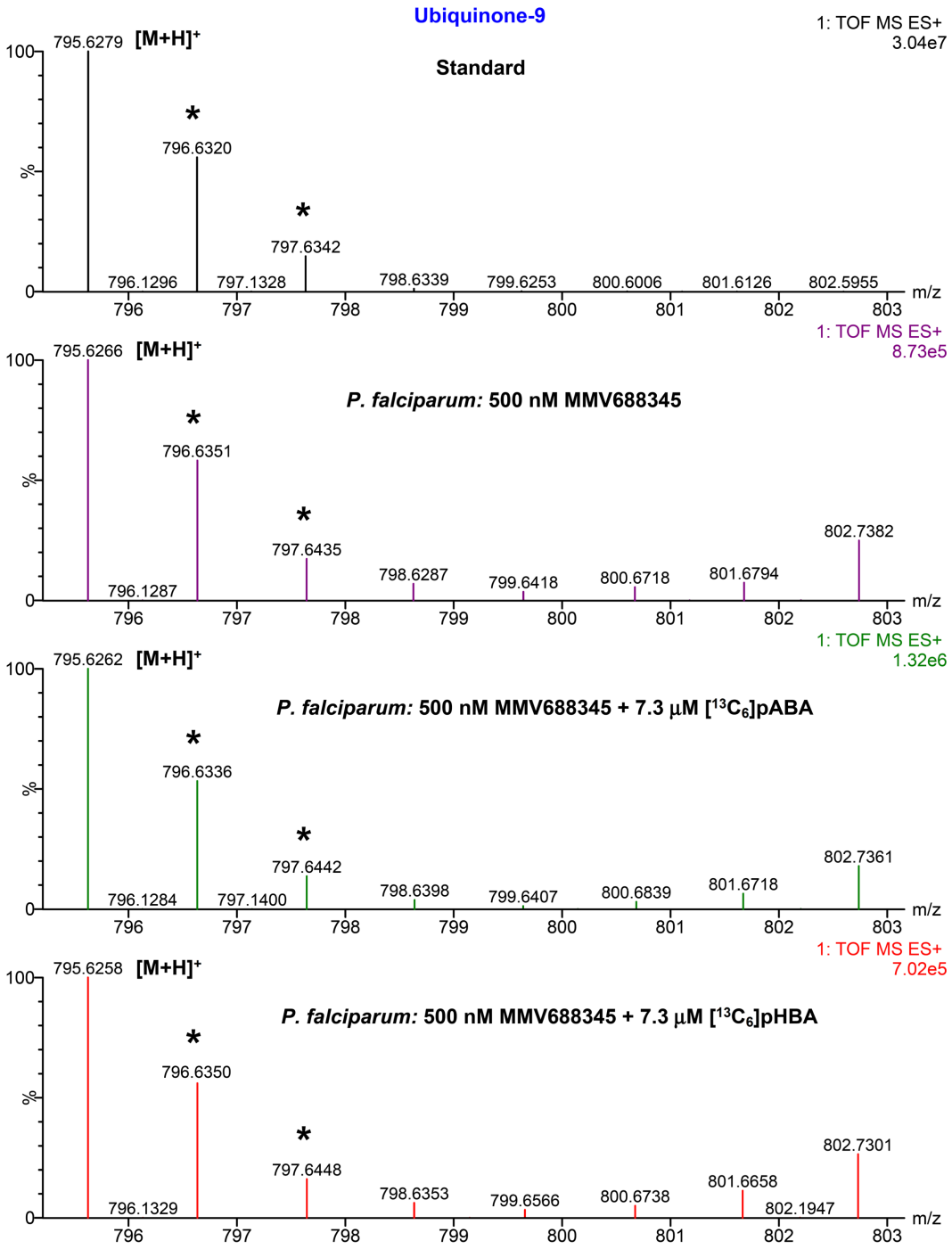

Fig. S4. LC-HRMS positive-ion mode spectra of ubiquinone-9 to assess whether *P. falciparum* is able to use [$^{13}C_6$]pHBA and [$^{13}C_6$]pABA as a metabolic precursor for ubiquinone biosynthesis. $[M+H]^+$ indicates the positive-ion corresponding to the mass of ubiquinone-9 and (*) indicates its natural isotopic distribution. The ion corresponding to the [$^{13}C_6$]ubiquinone-9 ($[M+H]^+$ expected = 801.6487) for [$^{13}C_6$]pHBA or [$^{13}C_6$]pABA incorporation into the head group of ubiquinone-9 was not detected.

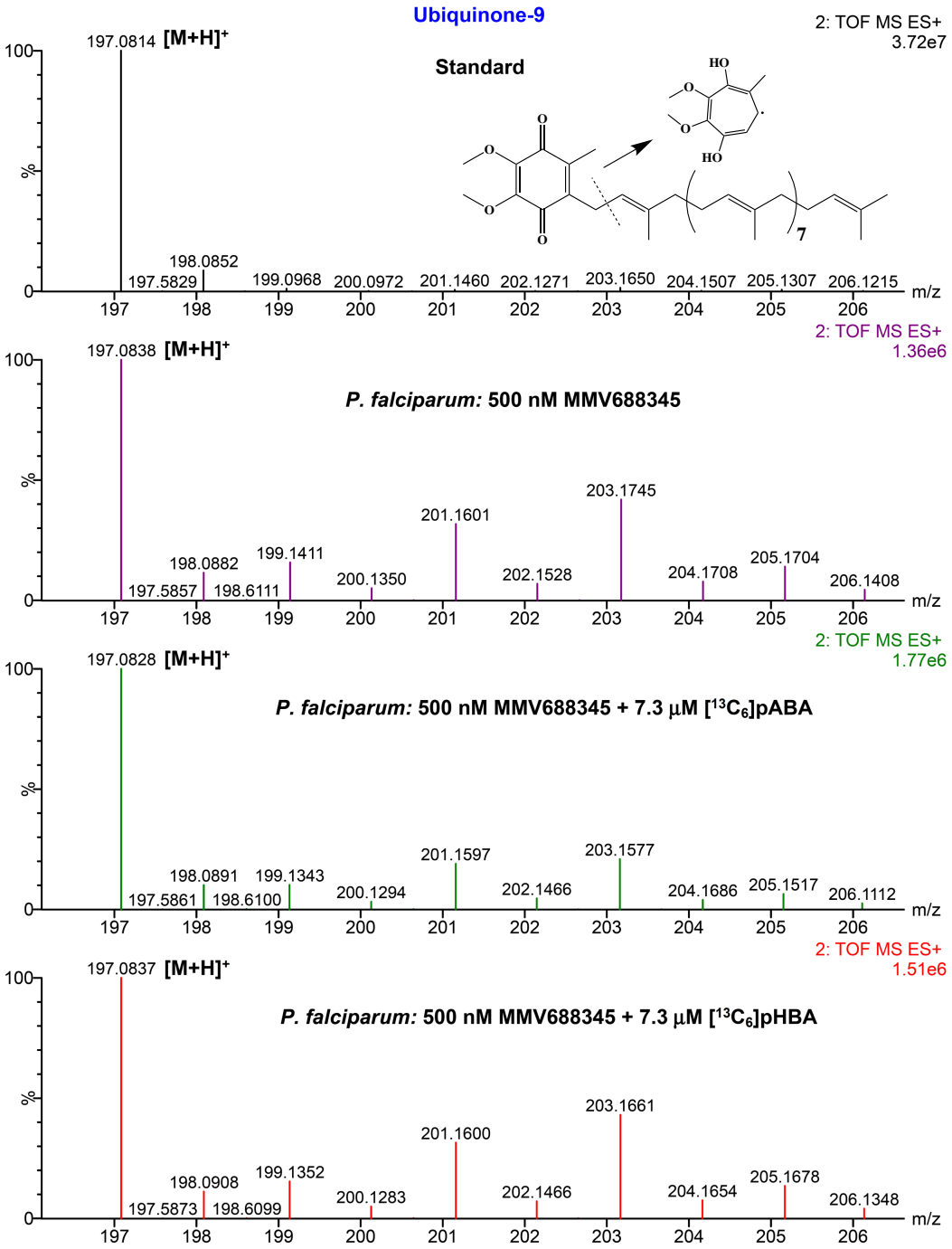

Fig. S4. (continuation) Mass fragmentation profile of ubiquinone-9. $[M+H]^+$ indicates the positive-ion corresponding to the mass of the ubiquinone-9 tropylium ion ($[M]^+$ expected = 197.0808). The predicted fragmentation is shown. The $[^{13}C_6]$tropylium ion ($^{13}C_6$-$[M]^+$ expected = 203.1010) was not detected.

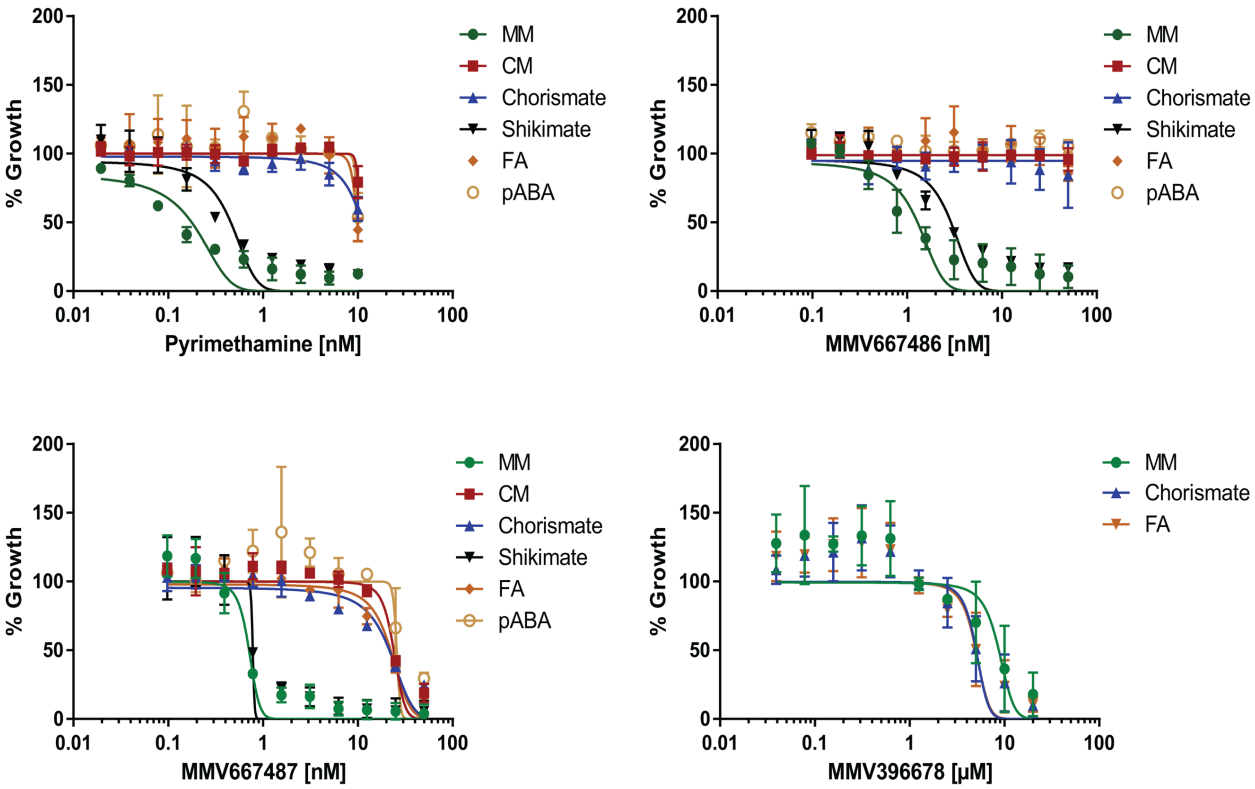

Fig. S5. Growth inhibition observed in MM by pyrimethamine (IC_{50} = 0.18 ± 0.03 nM), MMV667486 (IC_{50} = 1.28 ± 0.17 nM) and MMV667487 (IC_{50} = 0.71 ± 0.05 nM) was reversed in CM while growth inhibition by MMV396678 (IC_{50} = 8,723 ± 1.3 nM) was not reversed by FA. Chorismate, FA and pABA but not shikimate, also reversed growth inhibition by pyrimethamine, MMV667486 and MMV667487 similar to MMV688345. The following metabolite concentrations were used: 25 µM of shikimate, 12.5 µM of chorismate and 7.3 µM of FA.