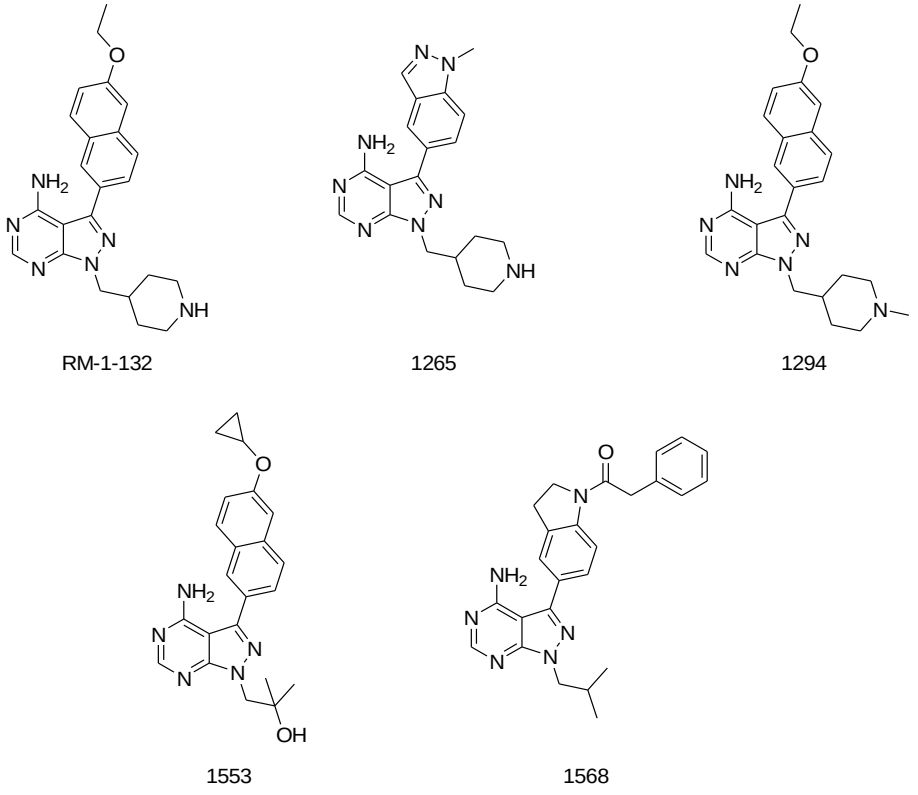

Fig S1 . Structures of bumped kinase Inhibitors

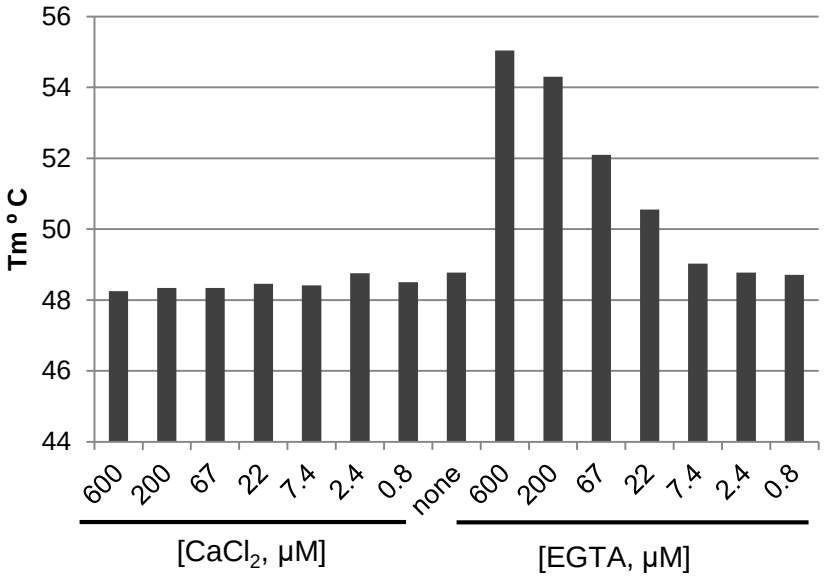

Figure S2. Calcium chelation stabilizes purified CDPK1 in TSAs. The addition of calcium to the TSA had no effect, while the addition of EGTA increased the T_m. The standard assay buffer ("none") contained no added calcium nor did it contain EGTA. However, it is possible that CDPK1 bound calcium in *E. coli* and/or that buffers contained trace amounts of calcium.

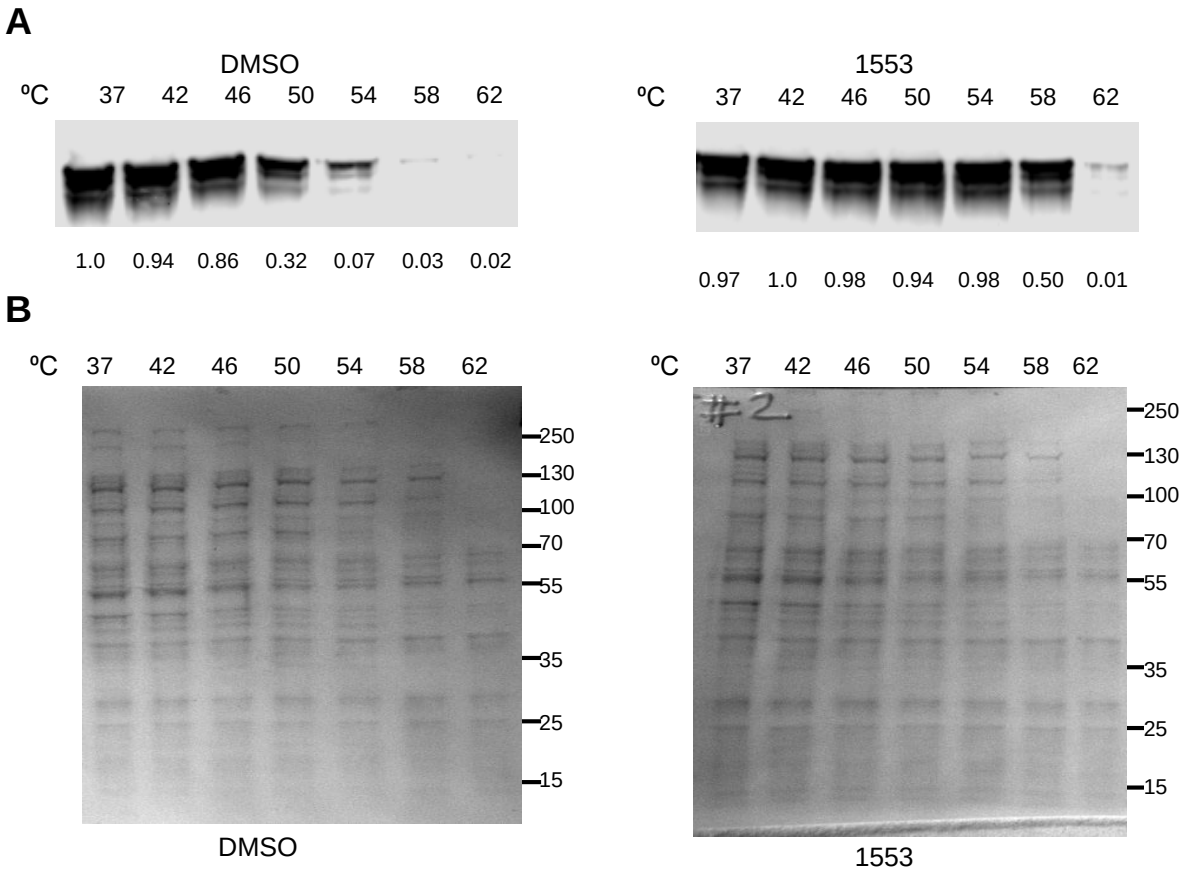

Figure S3. Example of an L-CETSA assay of CDPK1 using BKI 1553 compared to vehicle alone (DMSO). Following incubation at the indicated temperature, samples were centrifuged and the soluble material was loaded onto an SDS-PAGE gel and then blotted.

A) Western blot using anti-CDPK1 followed by goat anti-rabbit IgG coupled to IRDye 800. Signal was revealed by scanning on the LICOR Odyssey. Below each lane , signal is quantified as compared to the highest signal in the series.

B) Ponceau stained blot demonstrating that BKI 1553 does not cause non-specific protein aggregation.

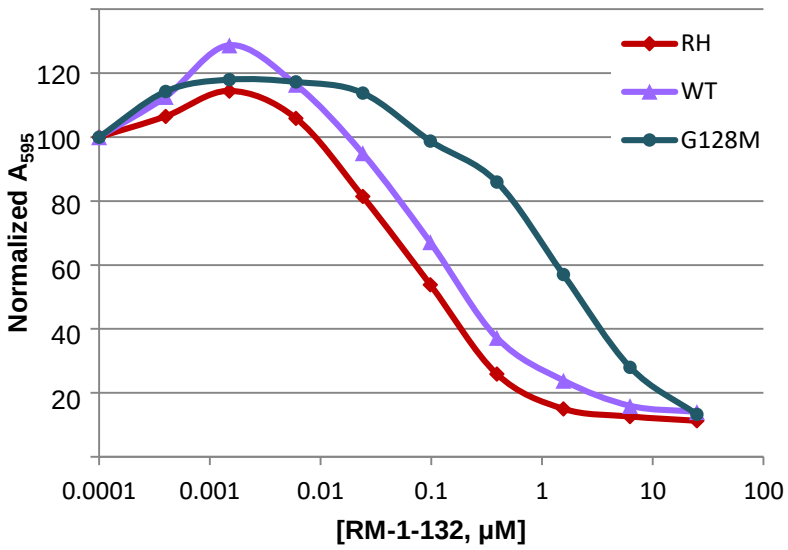

Figure S4. **Overexpression of CDPK1 with G128M gatekeeper mutation renders *T. gondii* resistant to BKI RM-1-132.** RH, the parental d11 clone used in RM1-132 selection. Experiments; WT and G128M, overexpression of epitope-tagged wild-type and gatekeeper mutant CDPK1 respectively. Compound was added prior to invasion of host cells and a β-galactosidase reporter was used to quantify the parasites at 44 hours using the colorometric substrate chlorophenol red β-galactopyranose. The average of two independent experiments with triplicate data points is shown.

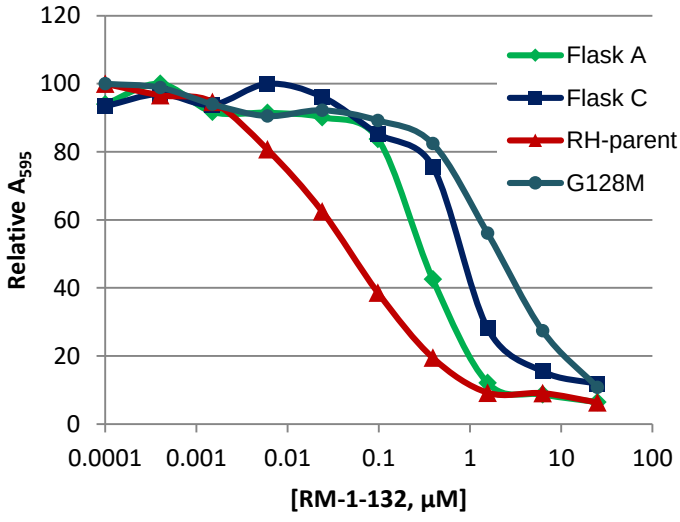

Fig. S5. Invasion/growth assays for *T. gondii* selected with RM-1-132. Uncloned lines were tested after 16 passages of selection. EC_{50}s calculated in this experiment are (µM): Parent 0.04, G128M 2.25, flask A 0.32, flask C 0.72. The assay was conducted as described in Fig. S4.