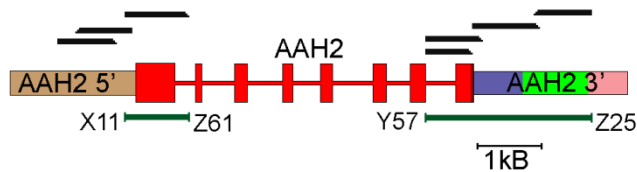
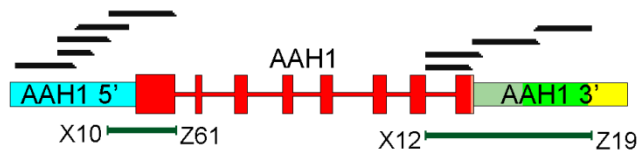
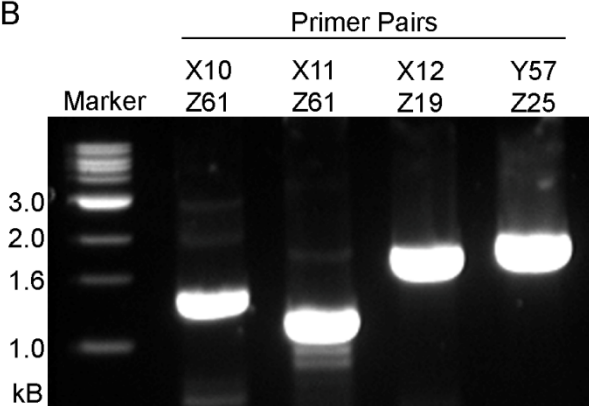


**FIG S1:** Calibration curves of standards for HPLC detection of Dopachrome (Dopac), Dopamine (DA) and Homovanillic acid (HVA), showing area of peak vs amount of standard analysed. Each point represents the average of 5 replicates (3 replicates for Dopac at 1.0ng/ 100uL). Standard errors were less than 2% for DA, HVA, and the two higher concentrations of Dopac, and was 6.92% for Dopachrome at the lowest concentration (0.1ng/ 100uL). The linear regression of these points was used to quantitatively measure catecholamine levels in experimental samples.  $R^2 = 0.9992$  (Dopac), 0.9992 (DA), 0.9997 (HVA).

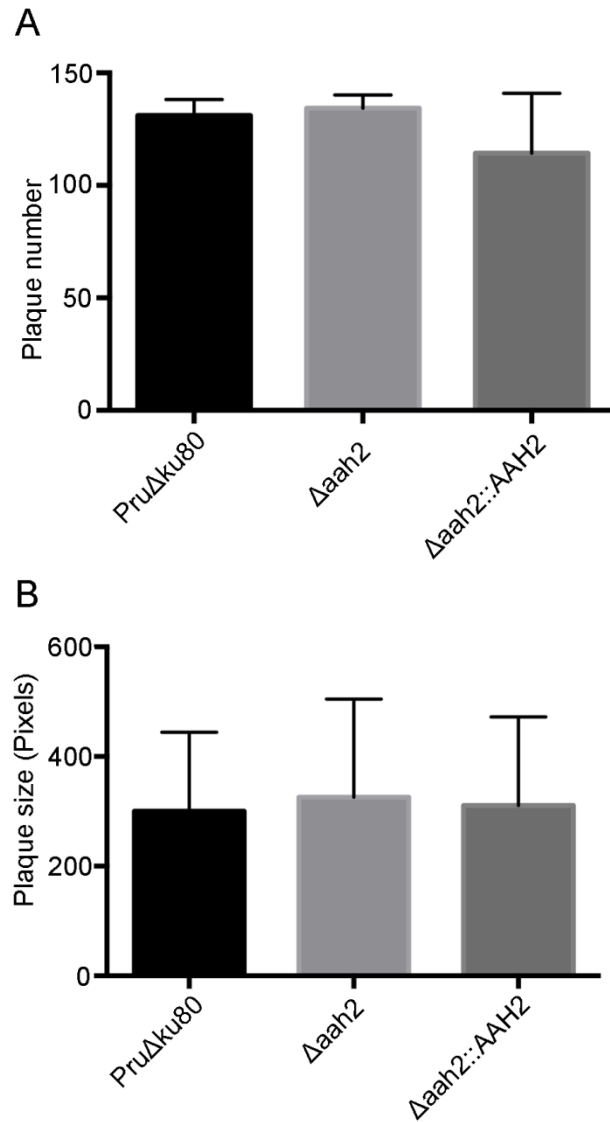
A



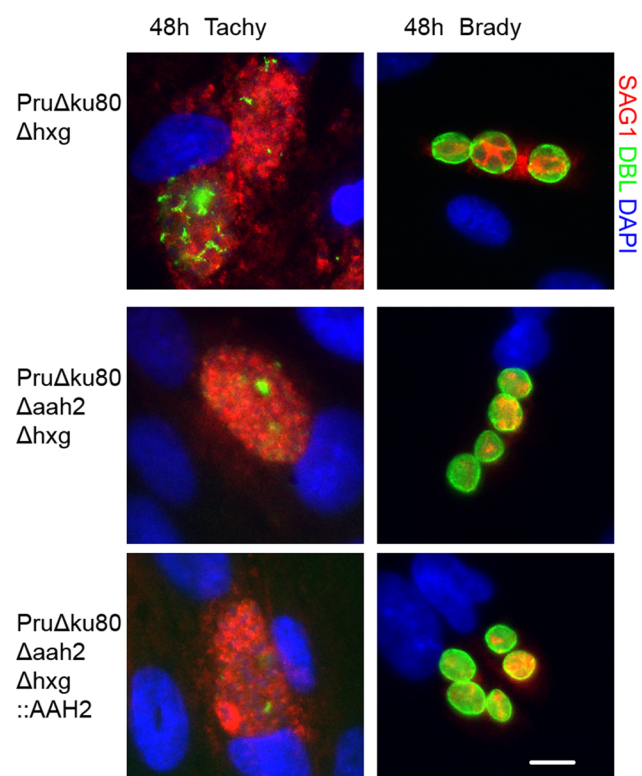
B



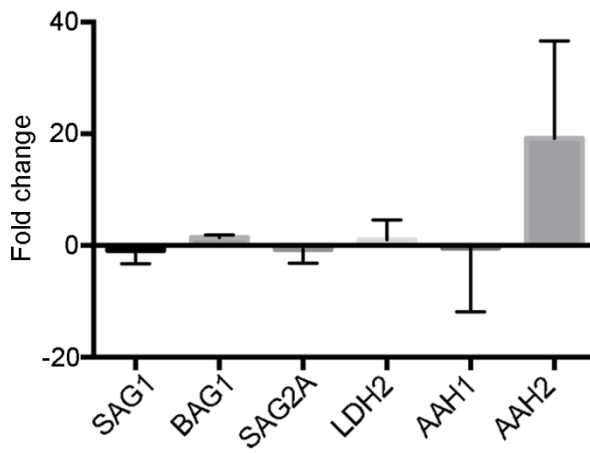
**FIG S2:** Architecture of the genomic loci containing *AAH1* and *AAH2*. (A) PCR reactions and sequenced regions are consistent with the previous assembly of the ME49 genome (ToxoDB Assembly 8). Identical colors indicate regions of >99% homology. Black bars indicate individual Sanger sequencing reads. Green bars indicate PCR products. Map is to scale. (B) PCRs against the Pru $\Delta ku80$  genome extending from the gene into the 5' or 3' UTR show products whose sizes are consistent with the Assembly 8 gene models. Expected sizes for primer pairs: X10/ Z61 (1.318 kB), X11/ Z61 (1.140 kB), Z28/ Y63 (2.418 kB), Z28/ Z25 (2.541 kB).



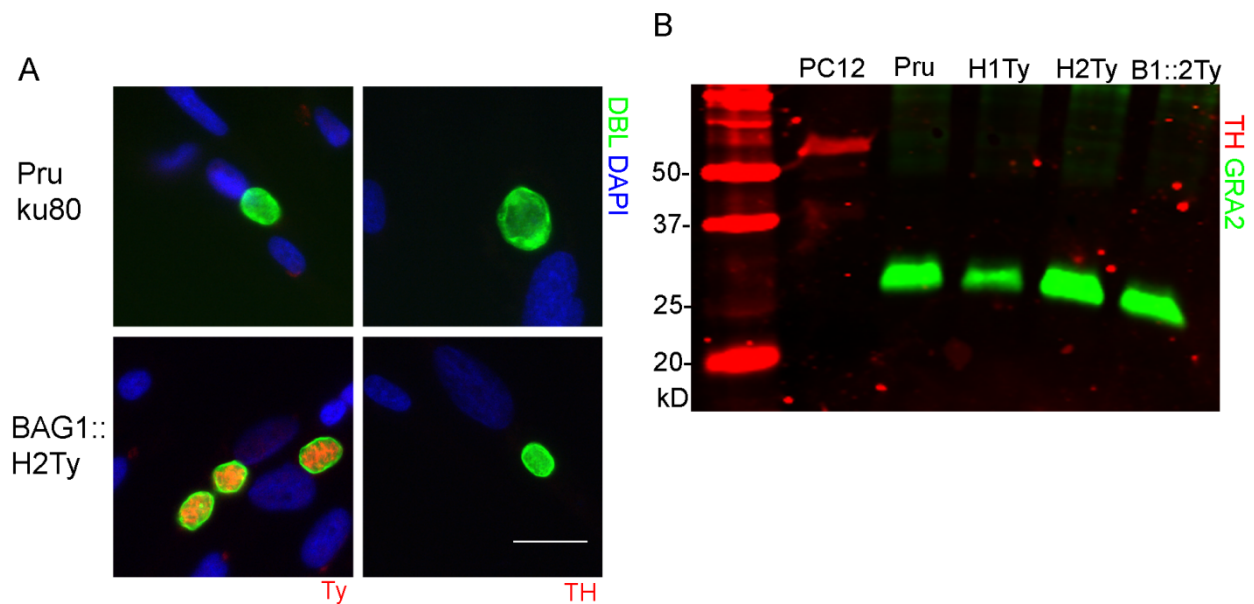
**FIG S3** (A) Plaque numbers after *in vitro* growth of strains on HFF monolayers stained with Crystal violet. No significant difference in plaque number was observed between strains ( $P = 0.34$ , One-way ANOVA). (B) Size of plaques on HFF monolayers, arbitrary units (pixels). No significant difference in plaque size was observed between strains ( $P = 0.86$ , One-way ANOVA).



**FIG S4** Immunofluorescence analysis of WT,  $\Delta aah2$  and  $\Delta aah2::AAH2$  parasites in HFF cells *in vitro*, 48 h post-infection with and without pH stress for bradyzoite induction. Blue = DAPI, Red = SAG1, Green = DBL. Scale bar = 10  $\mu$ m.

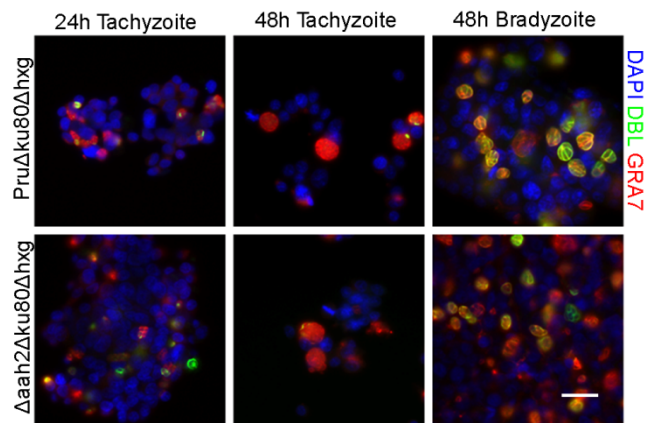


**FIG S5** qPCR comparing the difference in expression of the stage-specific markers *SAG1*, *BAG1*, *SAG2A*, and *LDH2*, and the hydroxylases *AAH1* and *AAH2* of 48hr bradyzoite-induced cultures of WT parasites compared to the *BAG1* promoter-driven *AAH2* overexpressor. N= 3.

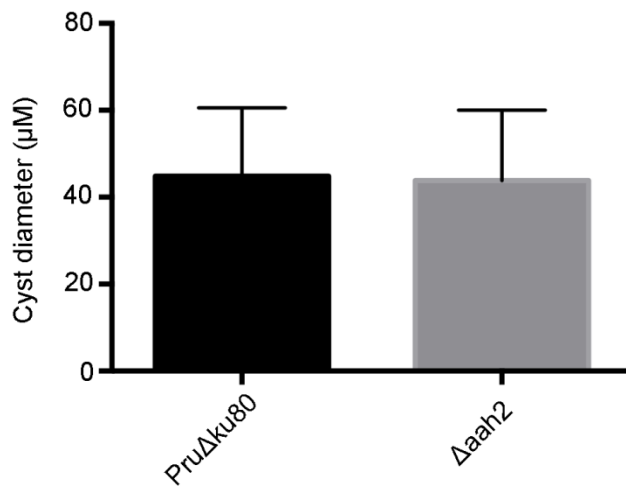


**FIG S6:** Detection and localization of tyrosine hydroxylase. Tyrosine hydroxylase (TH) was detected with a commercial antibody (Abcam ab#112) used to stain *in vitro* bradyzoites of PruΔku80 and BAG1::AAH2Ty overexpressing parasites. (A) Immunofluorescence assay shows presence of AAH2 in the overexpressor detected by Ty tag antibody (BB2). Commercial TH antibody did not cross-react to either the PruΔku80 or BAG1::AAH2Ty overexpressor lines. Scale bar = 10 μM. (B) Western blot analysis of TH in PC12 cells and in bradyzoite-infected HFF cells. The endogenous TH antibody was detected in PC12 cells with the commercial tyrosine hydroxylase antibody. However, there was no cross-reaction in PruΔku80, the Ty-tagged AAH1 strain, the Ty-tagged AAH2 strain, or the BAG1 promoter-driven AAH2::Ty overexpressor strain. GRA2 was detected with Tg17-179 antibody (Charif et al., 1990) and was used as a loading control. Expected sizes: TH 55kD, GRA2 28 kD (GRA2 is predicted to be 19.8 kD, however it typically migrates at ~28kD).

Charif, H., Darcy, F., Torpier, G., Cesbron-Delauw, M.F. & Capron, A. *Toxoplasma gondii*: characterization and localization of antigens secreted from tachyzoites. *Exp. Parasitol.* **71**, 114-124 (1990).

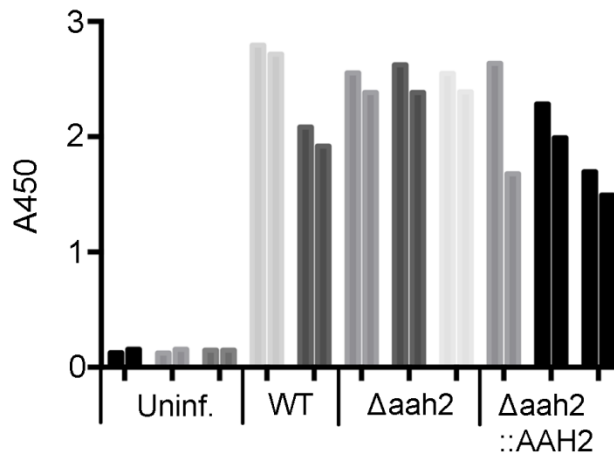


**FIG S7** Immunofluorescence analysis of WT and *Δaah2* parasites in PC12 cells *in vitro*, 24 h and 48 h post-infection at physiological pH, and 48 h post-infection with pH stress for bradyzoite induction. Blue = DAPI, Red = GRA7, Green = DBL. Scale bar = 10  $\mu$ m.

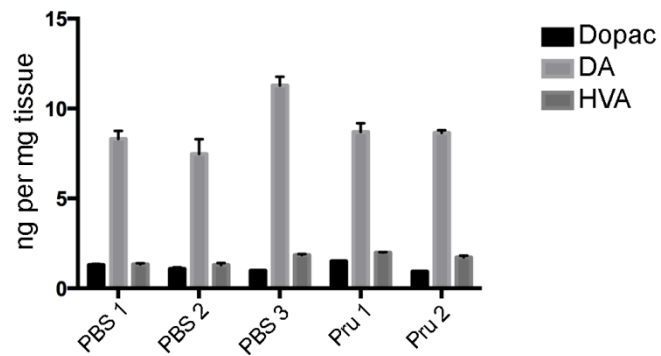


**FIG S8** Size of parasite brain cysts in CD1 mice as measured by fluorescence microscopy in WT and  $\Delta aah2$  parasite infection. No significant difference in cyst size was observed ( $P = 0.86$ , unpaired T-test).  $N = 5$  mice (WT), 4 mice ( $\Delta aah2$ ), 1-7 cysts measured per mouse,  $N = 17$  cysts total (WT), 15 cysts total ( $\Delta aah2$ ).





**FIG S9** ELISA of anti-*T. gondii* antibody titers in naïve CD1 mice fed 5 brain cysts of WT,  $\Delta aah2$ , or  $\Delta aah2::AAH2$  parasites each or PBS-control by oral gavage, 1 mo. post-infection. 2 technical replicates shown per mouse. Antibody titers were significantly increased in mice fed infected cysts ( $P = 0.0001$ , one-way ANOVA). Increased antibody titers were indicative of successful vertical transmission of infection in  $\Delta aah2$  and  $\Delta aah2::AAH2$  parasites.



**FIG S10:** Analysis of Dopamine, DOPAC and HVA in the striatum of mice injected i.p. with PBS control or 1k *PruΔku80* tachyzoites at 1 mo. post-infection. No significant changes in catecholamine concentrations were seen ( $P = 0.1626$ , Friedman test).  $N = 3$  mice (PBS), 2 mice (*PruΔku80*), with 3 technical replicates each.