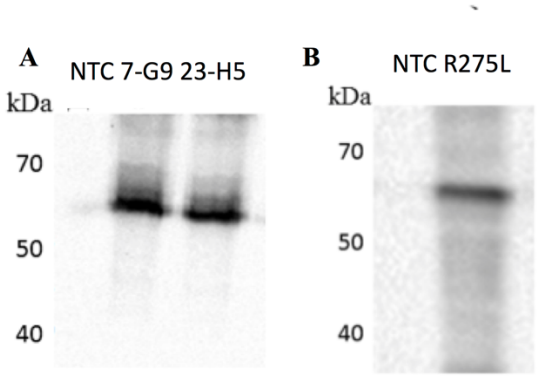

Figure S1. Full length 3xFLAG-NadB was expressed in two independent *nadB* transposon mutants (*nadB*::Tn (7-G9) and *nadB*::Tn (23-H5)) (**A**) and full length 3xFLAG-R275L-NadB was expressed in the *nadB* mutant (**B**). Mouse anti-Flag IgG antibody diluted 1/1000 in 1 % (w/v) skim milk in PBS-T was used as a primary antibody and the secondary antibody (anti-mouse IgG HRP) was diluted to 1/3000 in 1% (w/v) skim milk in PBS-T. The bands detected corresponded to the predicted molecular weight of 61.5 kDa. NTC = negative control (untransformed *C. burnetii*).

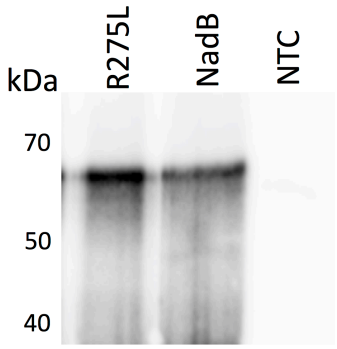

Figure S2. THP-1 cells infected with the *C. burnetii nadB* mutant expressing either 3xFLAG-R275L-NadB (R275L) or 3xFLAG-NadB (NadB) were harvested 3 days after infection and and analysed using SDS-PAGE electrophoresis and immunoblotting. Mouse anti-Flag IgG antibody diluted 1/1000 in 1 % (w/v) skim milk in PBS-T was used as a primary antibody and the secondary antibody (anti-mouse IgG HRP) was diluted to 1/2000 in 1% (w/v) skim milk in PBS-T. The bands detected corresponded to the predicted molecular weight of 61.5 kDa. NTC = negative control (THP-1 cells infected with untransformed *C. burnetii*)

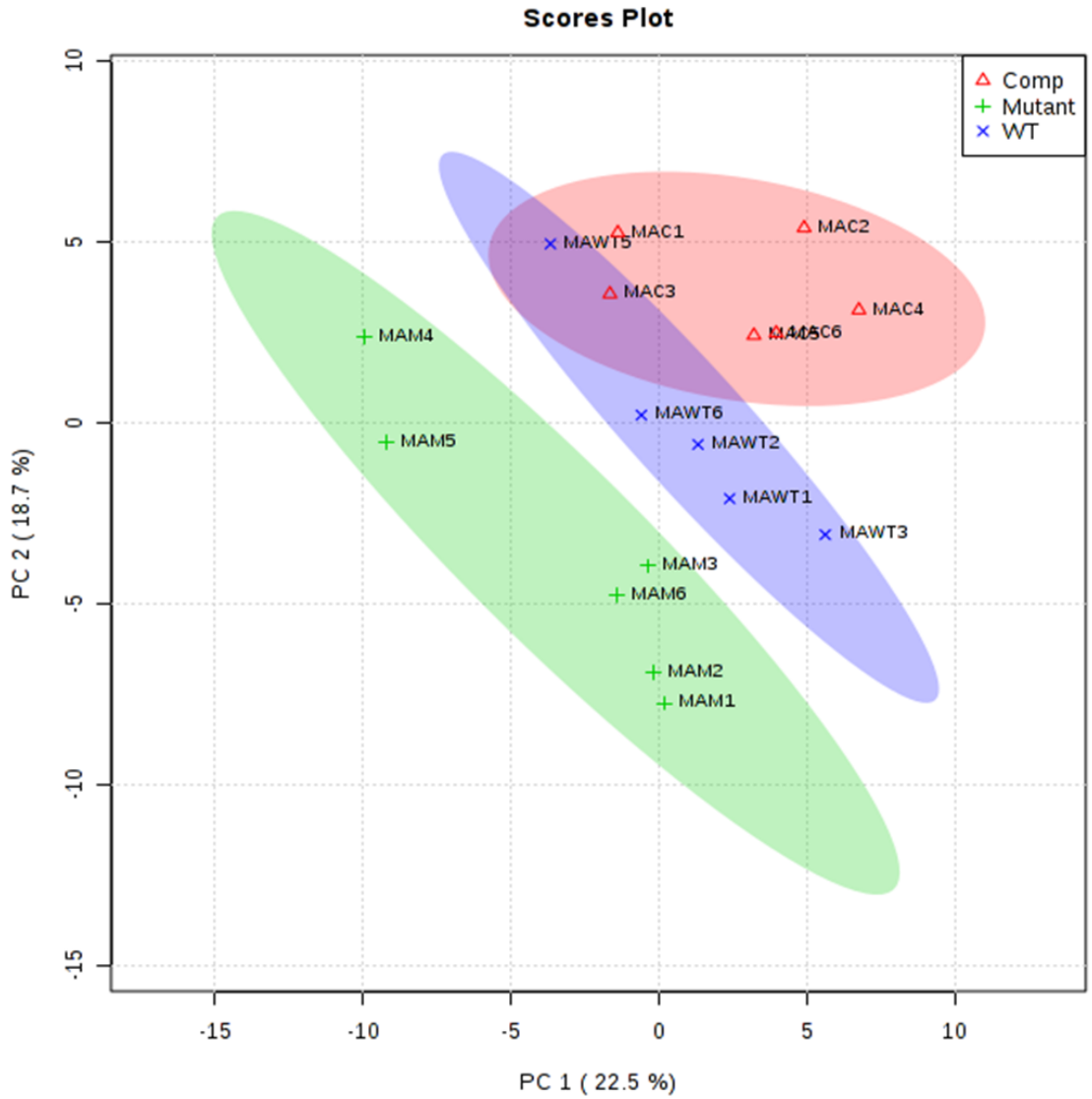

Figure S3. Scores plot. Principal component analysis derived from combined analysis comprising all the 3 groups to highlight their discriminatory potential. WT = wild type *C. burnetii*, Mutant = *nadB* mutant, Comp = complemented *nadB* mutant. Individual points and labels refer to individual biological replicates.

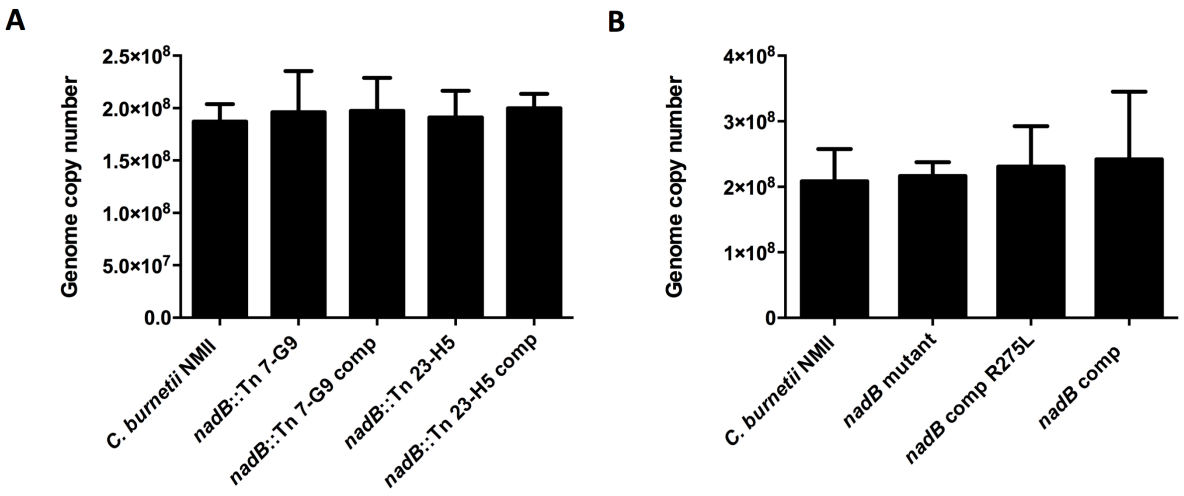

Figure S4. Growth of *C. burnetii* strains in axenic culture (prior to the intracellular replication experiments described in Fig. 1 and Fig. 6). Data represents the average genome copy number at day 6 from three independent biological replicates, as determined by *ompA* specific PCR. Error bars represent the standard error of the mean and no significant differences were observed between strains.