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TOXOPLASMA STRAINS

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The inventors have generated multiple gene knockout strains of *T. gondii*, listed below.

RHΔhxΔku80 TIR1 (RHΔku80Δhxgprt/pTub 5'UTR-OsTir1-3xFlag-DHFR 3'UTR-CAT):

The inventors used the CRISPR/Cas9 system for genome editing, comined with the AID degradation system to generate the *Toxoplasma gondii* strain. It was used to study plasma membrane association and PKG function in *Toxoplasma gondii*.

Publication: Plasma Membrane Association by N-Acylation Governs PKG Function in Toxoplasma gondii

DiCre KB1 (RHΔku80Δhxgprt/DiCre):

To generate a DiCre recipient strain expressing both subunits in the same genomic locus, p5RT70DiCre-HX was transfected into RH *hxgprt*$^-$ (RH *hxgprt*$^-$*/diCre*, referred to here as RH DiCre). Expression of Cre recombinase subunits was confirmed by western blot analysis with antibodies to FKBP12 and FRB.

The *ku80Δ::diCre* recipient strain was generated by replacing *HX* with *diCre* in the *ku80Δ::HX* strain by homologous recombination (*ku80Δ::HXΔ::diCre*, referred to here as *ku80Δ::diCre)*. The 5′ UTR–*DiCre*–*ku80* 3′ UTR cassette was transfected into *ku80Δ::HX* strain, and subsequently *ku80Δ::diCre* parasites were selected using 6-thioxanthine to remove *HX*. Integration of *diCre* into the *ku80* locus was confirmed by analytical PCR on genomic DNA using ku80*Δ::HX* fw (1) and ku80 rv (1′) primer pair to check for the presence of *hx* in the *ku80* locus.

Publication: Conditional genome engineering in Toxoplasma gondii uncovers alternative invasion mechanisms

Pru Δku80 SL1 (PruΔku80Δhxgprt clone SL1):

The inventors used electroporation to transfect the parental Pru (Pru**Δ**ku80**Δ**hxgprt) *T. gondii* strain. All transfected plasmids were linearized 5' of the 5' target DNA flank prior to transfection using unique restriction enzyme sites designed into the targeting plasmids. Forward selections to integrate the pmini-HXGPRT selectable marker were performed in mycophenolic acid and xanthine. Negative selections to excise HXGPRT were performed in 6-thioxanthine. Negative selections using the cytosine deaminase (CD) selectable marker were performed in 5-fluorocytosine. Negative selections to delete UPRT were performed in 5-fluorodeoxyuridine (FUDR). After transfection, parasites were allowed to replicate for 24 h without selection to allow replication and ramp up homologous recombination, and then selections were launched and continuously maintained through verification steps of cloned isolates

Publication: Type II Toxoplasma gondii KU80 Knockout Strains Enable Functional Analysis of Genes Required for Cyst Development and Latent Infection

ME49 TIR1 (ME49Δku80::LuciferaseΔhxgprt/pTub 5'UTR-OsTir1-3xFlag-DHFR 3'UTR-CAT)

ME49Δhx Δku80 SL1 (ME49Δku80::LuciferaseΔhxgprt clone SL1)