Supplementary Figure 1. Chloroquine sensitivity is unaltered by *gch1* overexpression. Chloroquine IC_{50} values were calculated for each *gch1* line (**A-E**) and compared to parasites transfected with a *Renilla* expressing control plasmid grown under the same concentration of blasticidin. IC50 differences were not significant in all lines confirming the specific role of *gch1* overexpression in pyrimethamine resistance.

Supplementary Figure 2. *gch1* amplification in D6 increases proguanil resistance. Dose response curves from six independent experiments all show a consistent increase in resistance to proguanil for D6 GCH(20) compared to D6 Ren(20). The magnitude of the shift varied between experiments likely due to varied stability of the drug as well as potential off-target effects. However in each experiment the trend was the same; a shift to a higher IC50 in lines with overexpression of *gch1* compared to *Renilla*.

Supplementary Figure 3. *Renilla* overexpression in 5 parasite isolates. Bar graphs show *Renilla* and *bsd* copy number (A) and expression levels (B) in parasites grown under 5 µg ml^{-1} blasticidin and 20 ug ml^{-1} blasticidin.

Supplementary Figure 4. Transfection of Dd2 and V1/S with a *gch1* episome shows no significant increase in *gch1* expression. Bar graphs show *gch1* and *bsd* copy number (A,C) and expression levels (B,D) in parasites grown under 5 µg ml^{-1} blasticidin, GCH (5) and 20 ug ml^{-1} blasticidin, GCH(20).

Supplementary Figure 5. V1/S clones are isogenic. Genomic DNA from each V1/S clone was isolated, digested with the indicated restriction enzymes, separated on a 0.8% agarose gel, blotted and hybridized to a *var* exon 2 probe. Procedures for Southern blotting were performed as described previously (Frank *et al.*, 2006). There

are no apparent differences in the banding pattern between the two clones confirming that they are genetically isogenic.

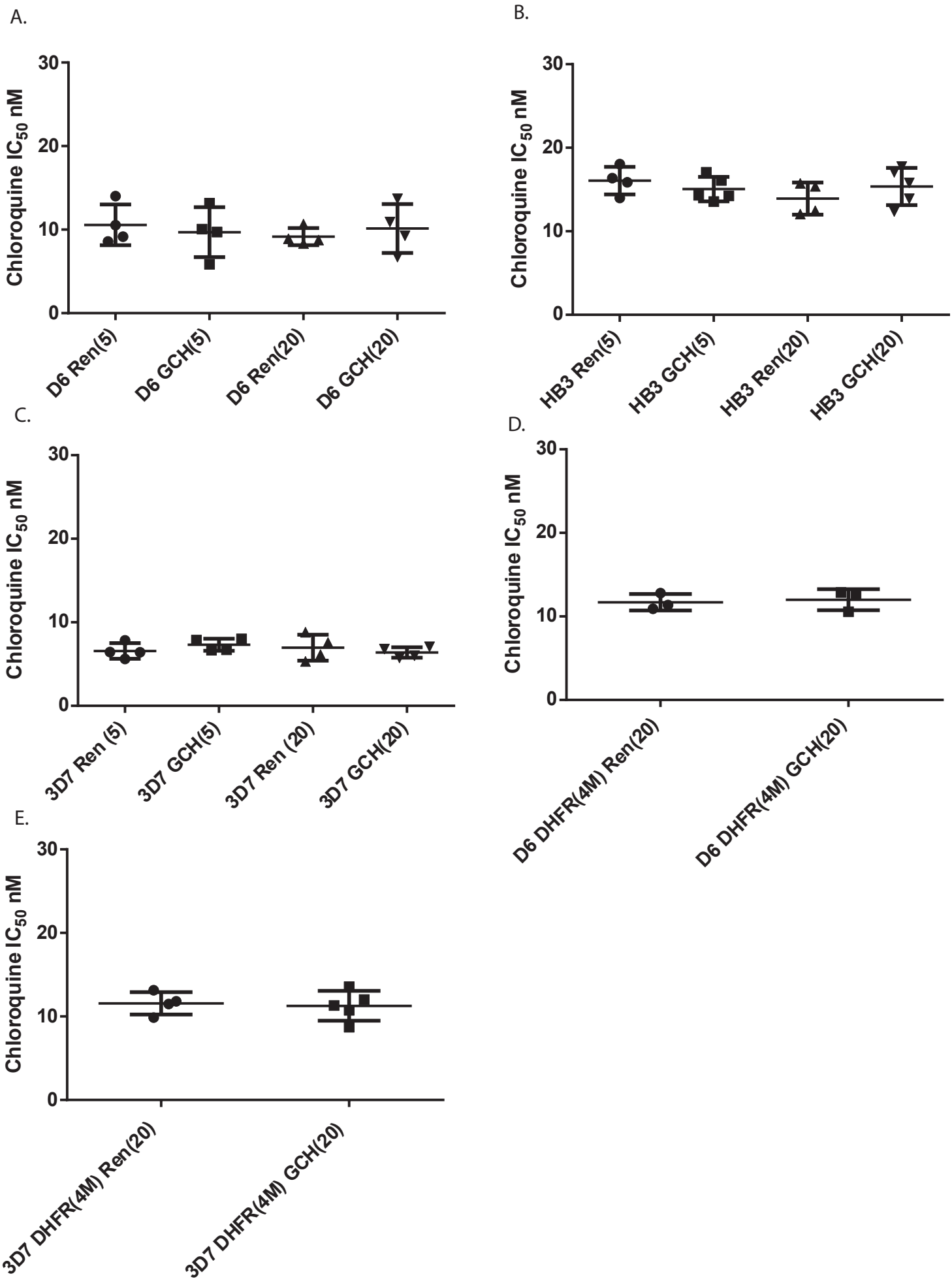
Supplementary Table 1. Primers used in this study. The table lists the primers used in Q-PCR (A) and plasmid construction (B). NotI and SacI restriction sites are underlined.

Supplementary File 1. Raw Ct values and calculated copy number/expression of *gch1* and *bsd.* When multiple synchronizations and transfections were performed additional Ct values are given in adjacent columns.

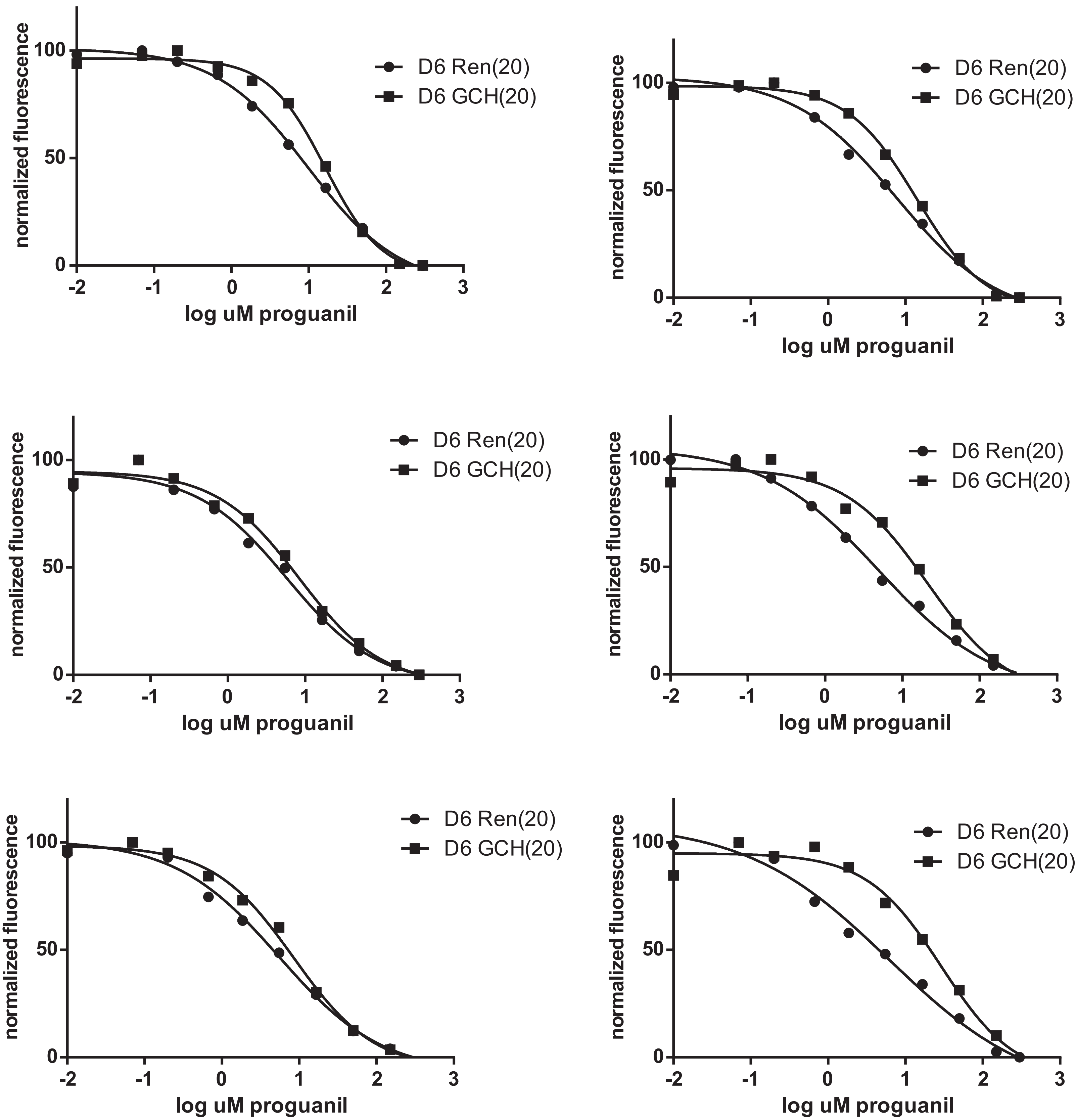
Supplementary Reference

Frank, M., R. Dzikowski, D. Costantini, B. Amulic, E. Berdougo & K. Deitsch, (2006) Strict pairing of var promoters and introns is required for var gene silencing in the malaria parasite Plasmodium falciparum. *J Biol Chem* 281: 9942-9952.

Supplmentary Figure 1

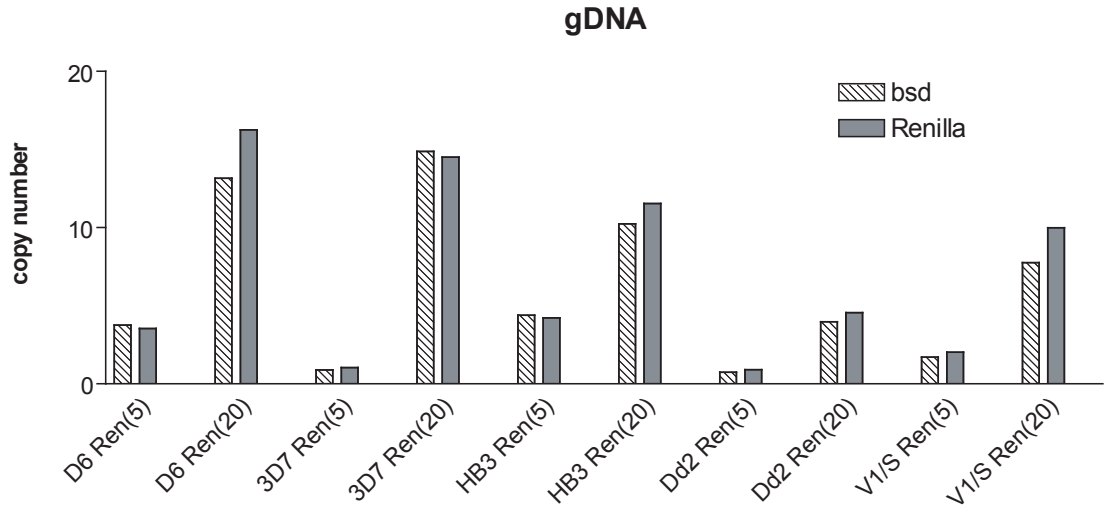


Supplementary Fig 2

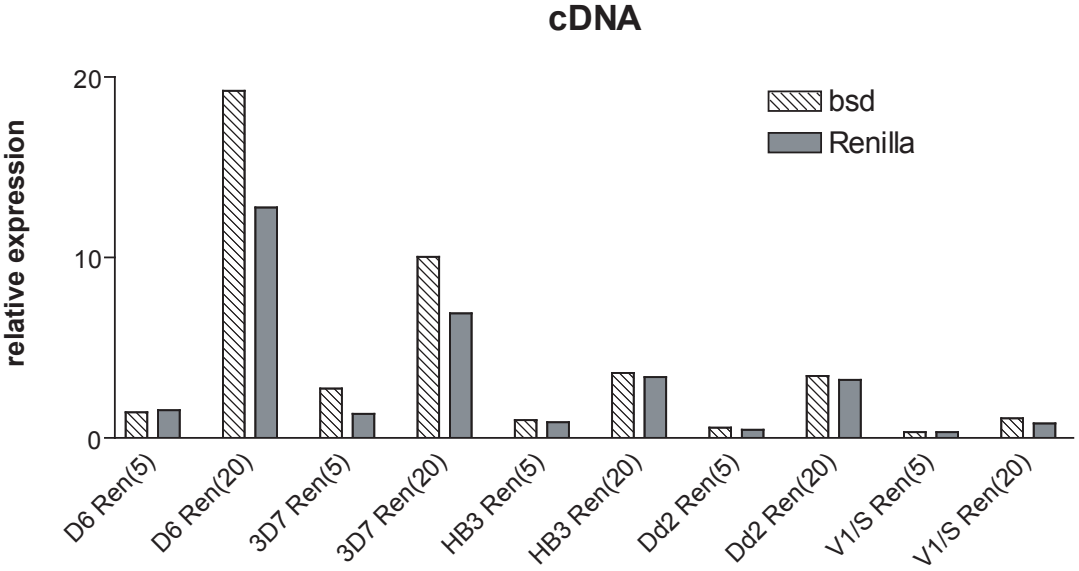


Supplementary Figure 3

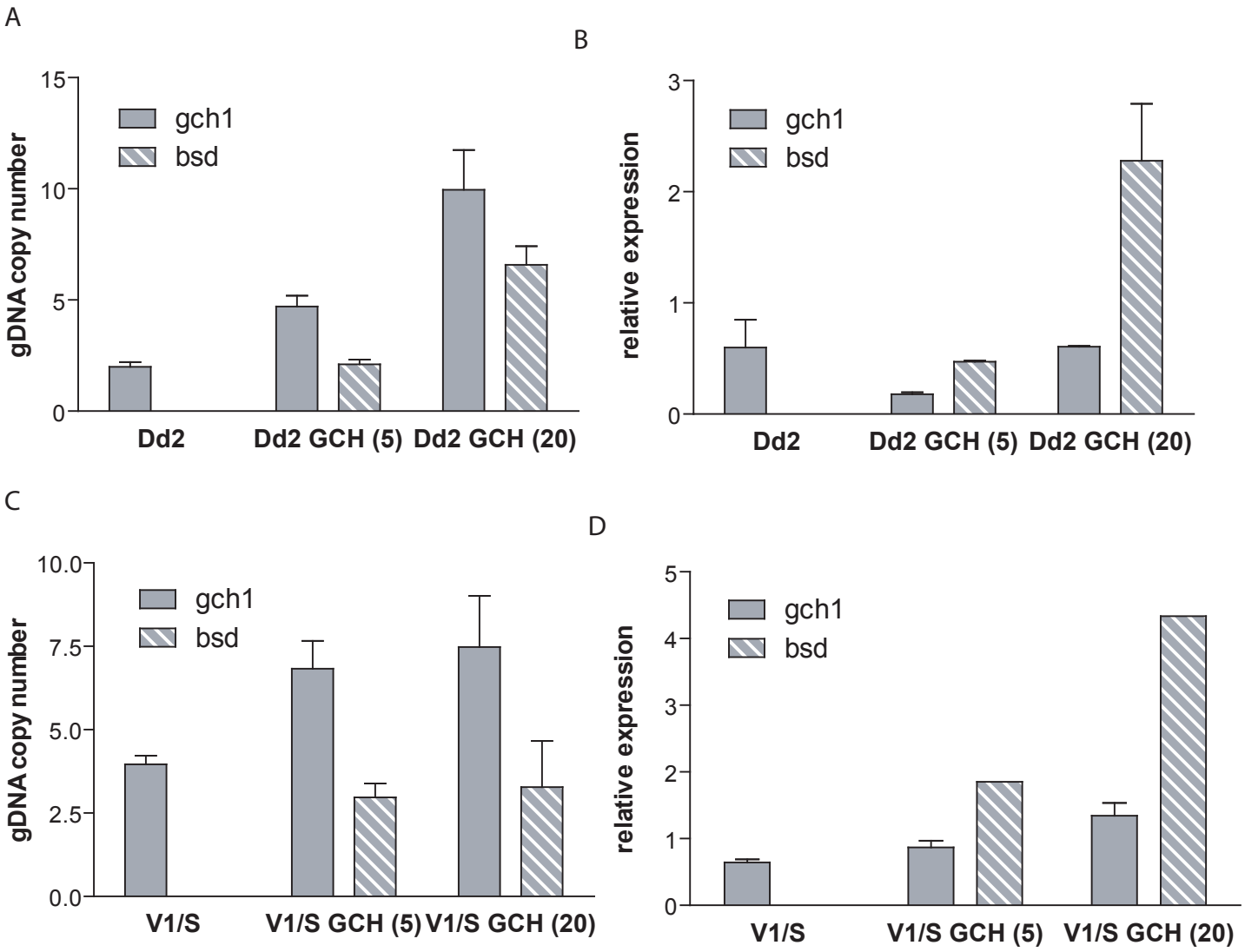
A



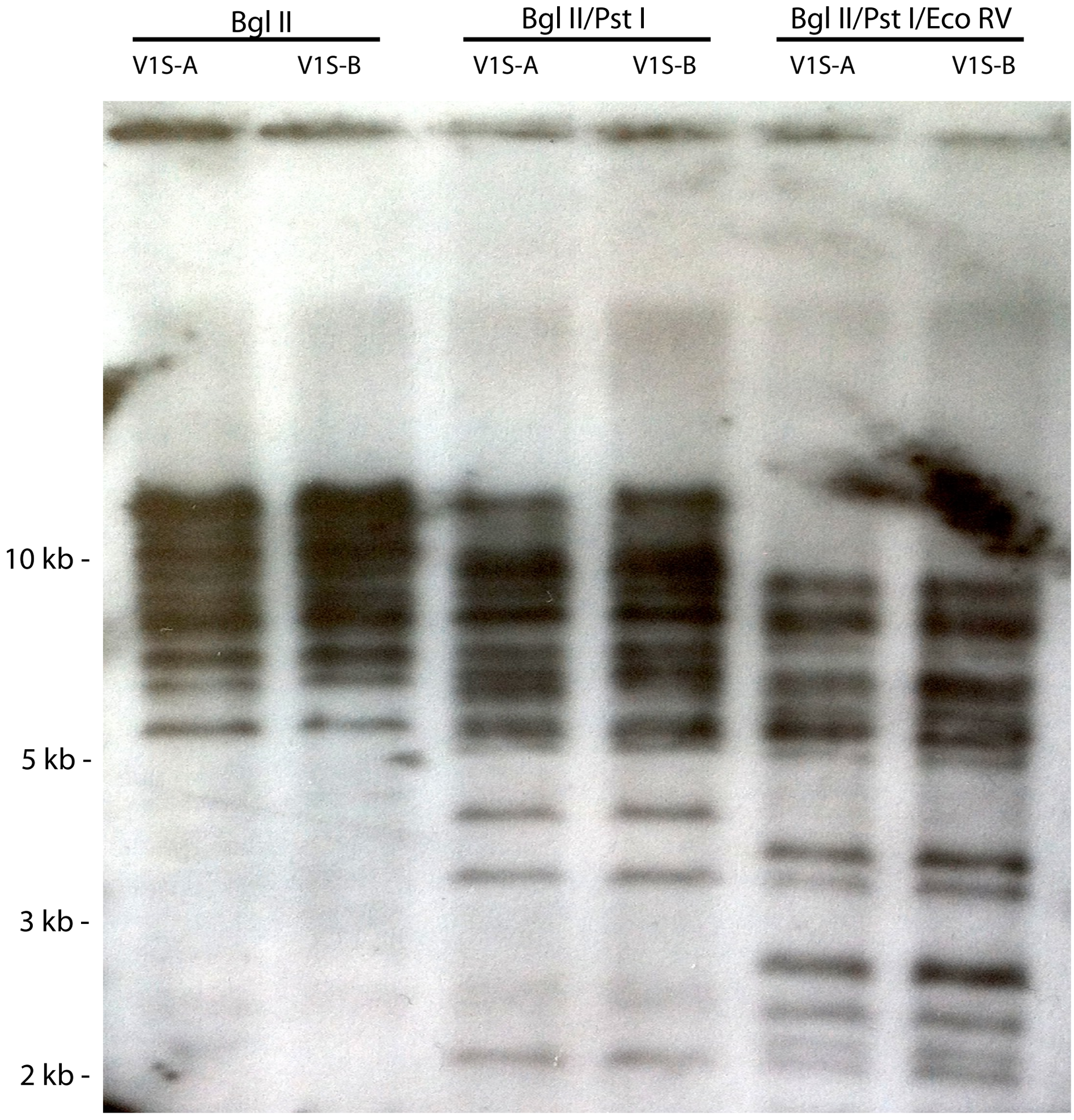
B



Supplemental Figure 4



Supplemental Figure 5



Primers used for Quantitative PCR

A.

	Forward primer	Reverse primer
Seryl-tRNA synthetase	AAGTAGCAGGTCATCGTGGTT	TTCGGCACATTCTTCCATAA
GTP cyclohydrolase	ATGAAACACATAATATGGAAGAAAAA	TCCTTTTCATCTATCACAACAAGG
Blasticidin-s-deaminase	TTTGTCTCAAGAAGAATCCA	TCCCCCAGTAAAATGATATAC
Renilla luciferase	ATGGGATGAATGGCCTGATA	TGTTGGACGACGAACTTCAC

B. Primers used for plasmid construction

	Forward primer	Reverse primer
GCH1	CTGCGGCCGCATTATGTAT AAATATACGTCAATAAAC	CTGAGCTCCTAATTTAAATTTTCCA CAG
Mutant DHFR-TS	CTGCGGCCGCATTATGATG GAACAAGTCTGCGA	CTGAGCTCTTAAGCAGCCATATCC ATTG