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Supplementary Materials for

A single-nucleotide polymorphism in a *Plasmodium berghei* ApiAP2 transcription factor alters the development of host immunity

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The PDF file includes:

Other Supplementary Material for this manuscript includes the following:

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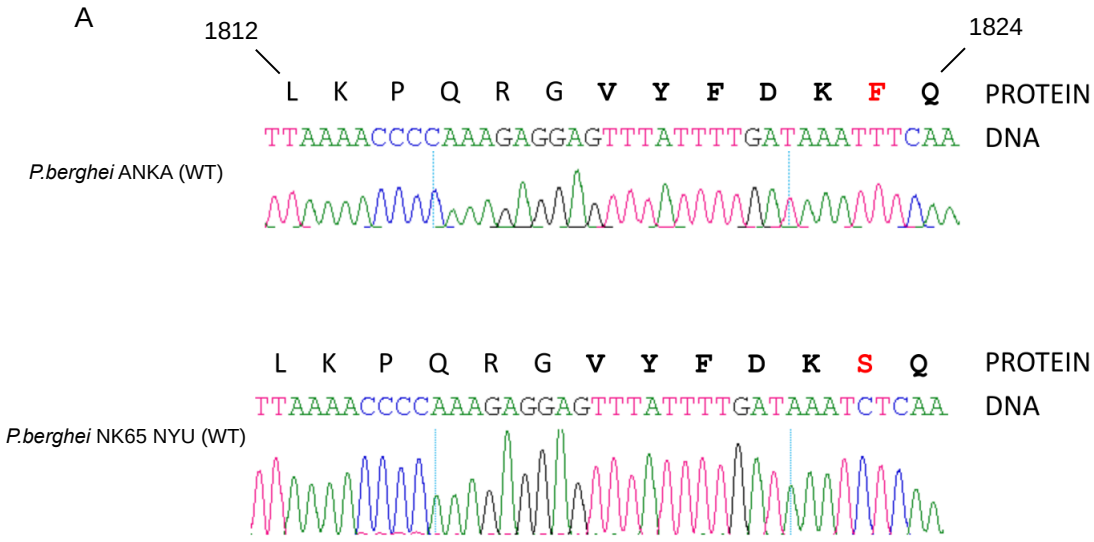
Supplementary Figures

Supplementary Figure 1



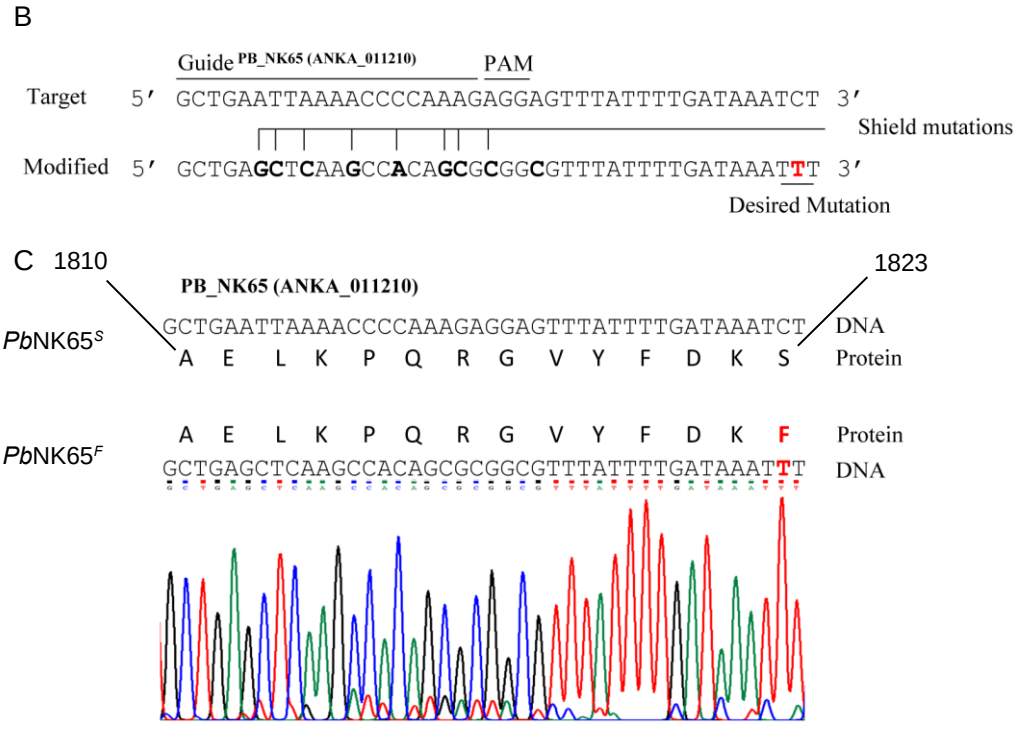


Fig. S1. CRISPR-Cas9 gene modification strategy is outlined. (**A**) Chromatograms showing the DNA sequence of the region of PBANKA_011210 ApiAP2 TF. harboring the SNP in WT *P.berghei* ANKA and WT *P.berghei* NK65 NYU. (**B**) The design of the genetic modification showing the predicted alteration in gene sequence in *P. berghei* NK65 NYU. (**C**) The DNA sequence analysis of WT (*Pb*NK65^{S}) and Crisper Cas-9 modified (*Pb*NK65^{F}) *P. Berghei* NK65 parasite confirming the existence of the planned genetic modifications.

Supplementary Figure 2

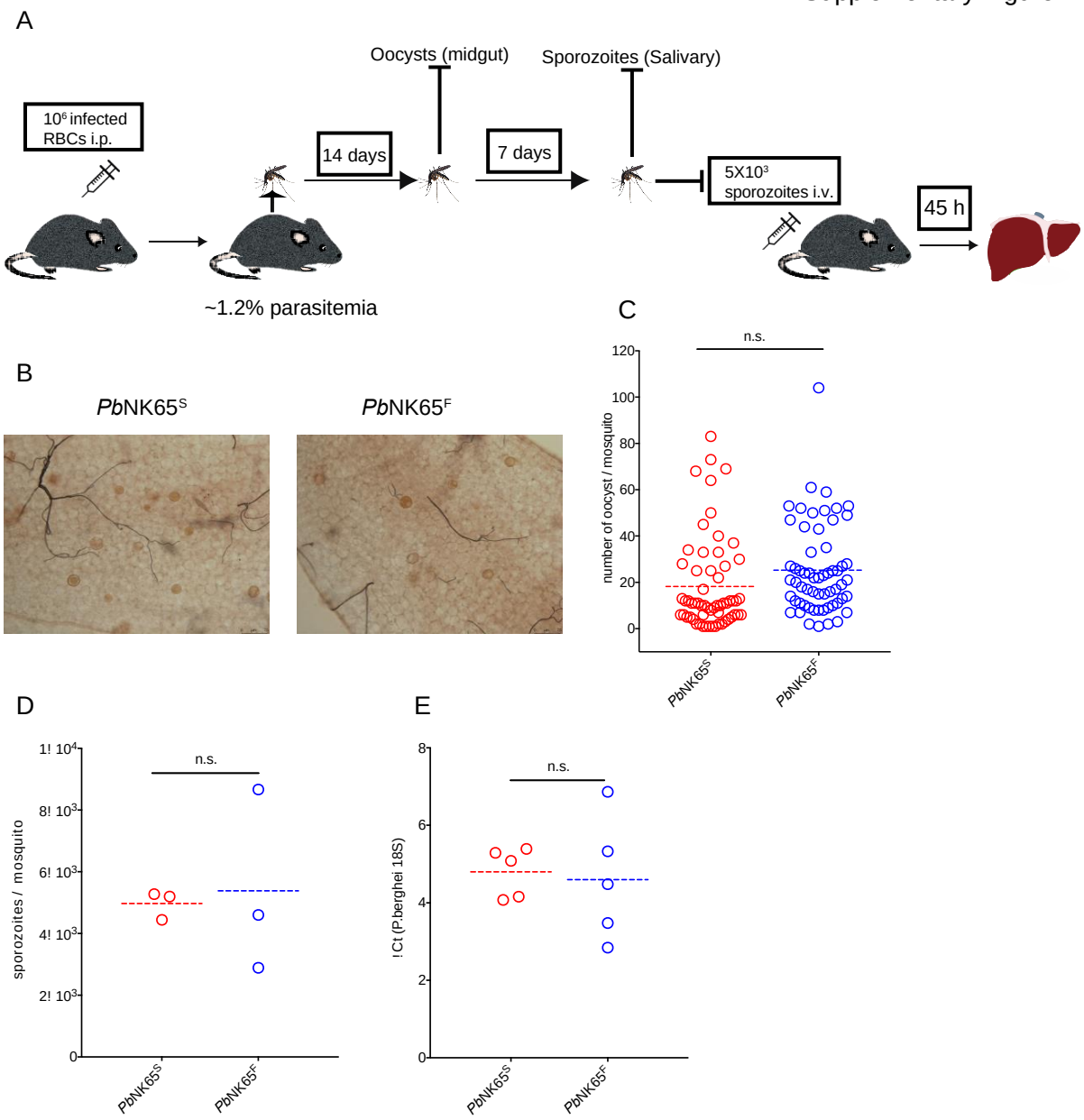


Fig. S2. The SNP in PBANKA_011210 ApiAP2 TF does not interfere with the progression of sexual and pre-erythrocytic stages of the *P. berghei* life cycle. (**A**) Schematic illustration of the experimental strategy used to address the mosquito and liver stages of the *P. berghei* infection: C57BL/6 mice were infected with 10^6 iRBCs (i.p.) taken from either *Pb*NK65^F or *Pb*NK65^S infected donors (3 mice per group). Once parasitemia reached to 1.2%, female *A. Stephensi* mosquitos (approximately 80-100/ cup) were fed on these mice. Mosquitos that did not feed were removed and the remainder were kept in the incubator with frequent sucrose-water feeding. A portion of mosquitos were dissected at day 14 post feeding and midguts were visualized under microscope (**B**). Number of oocysts/ mosquito were counted and graphed (**C**). Each circle represents an individual mosquito. Remaining mosquitos were kept alive and dissected on day 21 post infection. Dissected mosquitos were pooled in groups of 10 and sporozoites were isolated from salivary glands. Graph show the quantification of sporozoites (**D**). Each circle represents 10 mosquitos. $5x10^3$ sporozoites were injected i.v. to C57BL/6 mice (5 per group) and relative amounts of parasites in liver were quantified with qPCR at 46 h post injection (**E**). Data represents two independent experiments. Lines in each graph indicate means. (n.s.= $p>0.05$) Welch's t-test.

Supplementary Figure 3

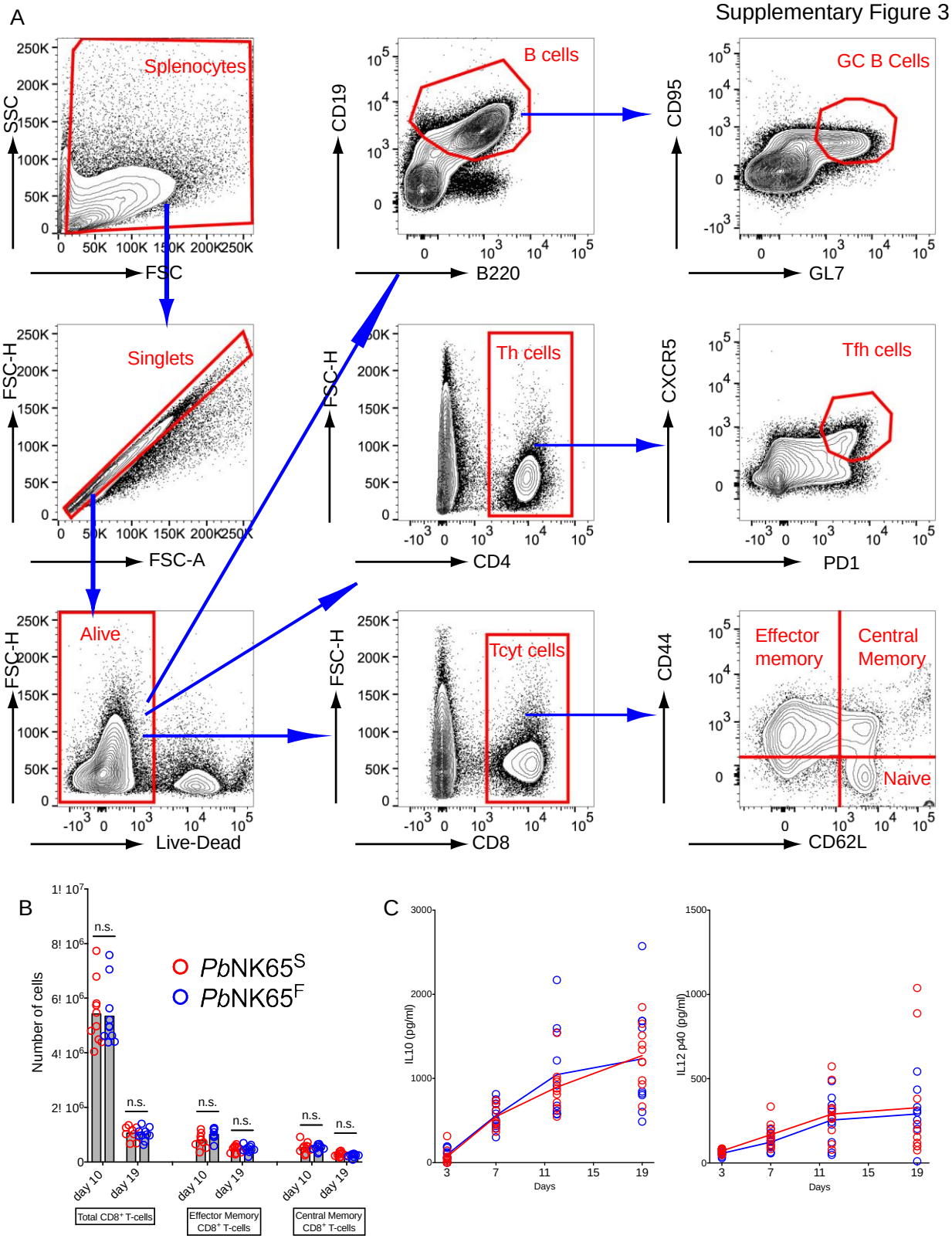
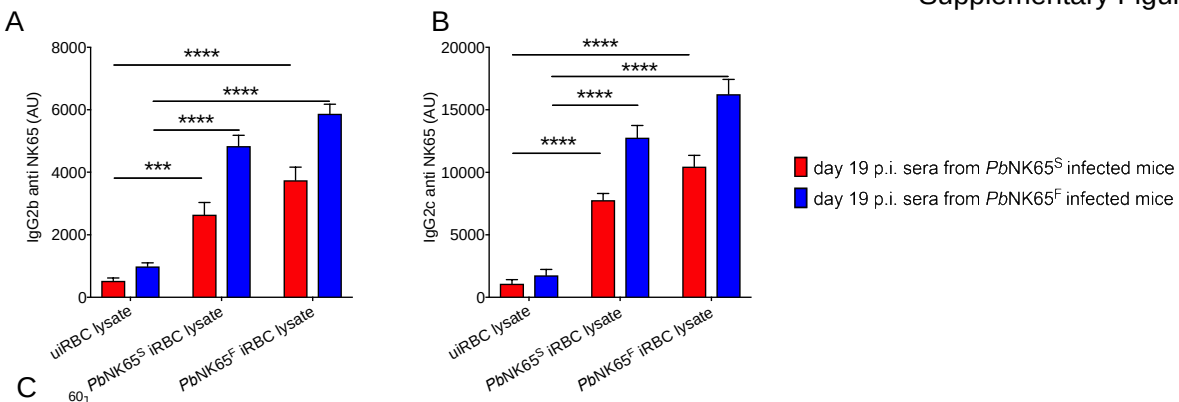
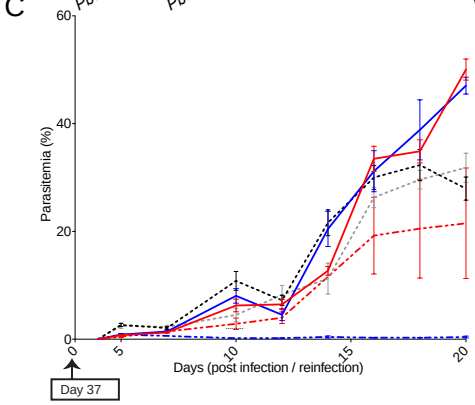


Fig. S3. Flow cytometry gating strategy. (**A**) Stepwise gating strategy used to identify the populations in (Fig. 3). (**B**) Graphs showing the actual numbers of different $CD8^+$ T cell subsets of infected mice from the experiment outlined in (Fig. 3A-C). Each circle represents an individual mouse. Bars show the mean values. (n.s.= $p>0.05$) Welch's t-test.) (**C**) Serum IL-10 and IL12p40 levels of *Pb*$NK65^F$ and *Pb*$NK65^S$ -infected mice from the experiment outlined in (Fig. 2D).

Supplementary Figure 4





	Day 0 Infection	Treatment	Day 37 Infection
	None	None	PbNK65^{S}
	None	None	PbNK65^{F}
	None	CQ	PbNK65^{S}
	None	CQ	PbNK65^{F}
	PbNK65^{F}	CQ	PbNK65^{F}
	PbNK65^{F}	CQ	PbNK65^{S}

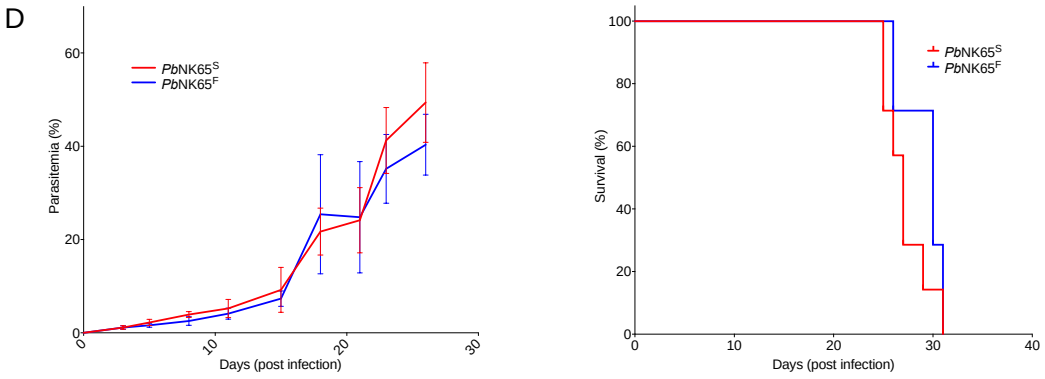


Fig. S4. Graphs comparing mice infected with *Pb*NK65^{F} and *Pb*NK65^{S}. (**A**-**B**) Representative graphs showing relative serum levels of parasite specific and self-reactive IgG2b (A) and IgG2c (B) antibodies at day 19 post infection in mice from the experiment outlined in Fig.5 Bars and error bars indicate mean and standard error of the mean respectively (***=0.0001<p≤0.001; ****= p≤0.0001) (One Way ANOVA with Tukey's multiple comparisions test). (**C**)Parasitemia graph of the experiment outlined in Fig. 6D. (**D**) IFN- γ KO mice were infected with *Pb*NK65^{F} and *Pb*NK65^{S} parasites at 10^2 infected RBC per mouse.

Table S1. Summary of changes observed between the WT and CRISPR-Cas9–mutated parasites based on whole-genome sequencing analysis.

CHROMOSOMAL POSITION	REFERENCE	CHANGE	COMMENT
LT614627.1:436179	A	G	CRISPR MUTATION (SILENT)
LT614627.1:436180	T	C	CRISPR MUTATION (SILENT)
LT614627.1:436182	A	C	CRISPR MUTATION (SILENT)
LT614627.1:436185	A	G	CRISPR MUTATION (SILENT)
LT614627.1:436188	C	A	CRISPR MUTATION (SILENT)
LT614627.1:436191	A	G	CRISPR MUTATION (SILENT)
LT614627.1:436192	A	C	CRISPR MUTATION (SILENT)
LT614627.1:436194	A	C	CRISPR MUTATION (SILENT)
LT614627.1:436197	A	C	CRISPR MUTATION (SILENT)
LT614627.1:436214	C	T	CRISPR MUTATION (S--->F)

Supplementary information 2

The 900-nucleotide synthetic sequence of AP2 (PBANKA_011210) gene

The following 900-nucleotides were used, as homology arm, for editing the AP2 gene in *P. berghei* NK65 to carry the SNP (5467 TCT to TTT) as in the *P. berghei* ANKA AP2.

ccatggATATCTTGTGGAAATAATAATACAAATATTTTAAGATCAAACAAAAATAAATT
T
GGAAAAAAAAACAATTATAATAATTTGAATGAAGGGGAACATAAAGAAAATAATG
AACCTC
ACAGAAATACAGATAATCAAAAAATCGGAATAAAAGTAGATAAAAATGGTAAAAA
TGGAAA
TATATCTATGAACGGTTTATTTGTAAGTTATGGACGTGGACAAAGGACAAAAGGATT
AGAA
AAAAATAAAAACTCAGCGGACAATGCAAGTTCGAATAAGAATAGTGGAAACACAA
AAGGAA
ATGGATCTAAAAGAGGCAATGGAAAAGATAAACAAAATGGTGGATGTTATGTAGAC
ATTGG
TGAAAATTATGAACTTAAATATACAGTTGCTGAGCTCAAGCCACAGCGCGGCGTTTA
TTTT
GATAAATTTCAAAAAGCATGGATAGGAAGTTGGTATGAAGAAGGCAAACAAATAAA
ACGT
CGATTTAAAATTAAATATTATGGTTGGGACGAAGCTAAAGAATTAGCAACAAAAGC
TAGA

TTTTCTTTTGAAAATCGAATCAAAAATTTAGAAGGAAATAACGGAAAAGGTAATTCT
AGT
ACATCTAAAAATAATACAAATTCAGGAGAAAAGAATGGAACAAAACTTACAGTAGT
TAAT
AATAAAAAAAGTGGAATTCAGGATATAGAAAATAATAATAATGATAGAATAAAAAC
AAGA
AACAGTTCAAAAGATGTAGAAGAAAAAAATAATAATGAAAATGCTGGATTTGATGA
AAAT
AATTTTAATGATATAGATAATCATAATAATCCAAATGAAAAAAATAATTCTGATGAT
AAA
AAAAAAAATAATAATATAGATGATGAAATATGGAGTAATCAAGAATCTCAAAATTG
TGATctcgag

The underlined sequences are the restriction sites for NcoI and XhoI, respectively that were used to sub-clone the homology arm in the pYC plasmid. The nucleotides in red corresponds to the 5467-5469 position in the mRNA sequence of the AP2 gene. The nucleotides highlighted in red and underlined are shield mutations introduced to avoid repeated cutting of the edited locus by the Cas9 endonuclease.