Supplemental material:

Supplementary Figure 1: D7L1 *and* D7L2 *gene organization and sgRNA sequences.* (A) D7 genes are arranged as an inverted pair on chromosome 2. Approximate distance from each respective transcriptional termination region is indicated in kilobase pairs (kbp). **(B** and **C)** *D7L1* and *D7L2* gene models showing the approximate position of the ORF across each of five exons (E1-E5). Approximate scale is shown in base pairs (bp). Blue arrows indicate sgRNAs designed in the plus DNA strand and green boxes show sgRNAs designed on the minus DNA strand. (**D** and **E**) sgRNA sequences used to target *D7L1* gene **(C)** and *D7L2* **(E).** Cas9 cleavage sites are indicated by a lightning bolt.

Supplementary Figure 2: *Generation of* D7L1$^{(-/-)}$ *and* D7L2$^{(-/-)}$ *homozygous lines by CRISPR/Cas9.* **(A)** High-resolution melt curve analysis (HRMA) of heterozygous lines (*D7L1*$^{(+/-)}$ and *D7L2*$^{(+/-)}$, red) and WT (grey) at generation 5 (G5) **(B)** Genomic DNA sequence of WT and CRISPR-induced mutations (*D7L1*$^{(-/-)}$ and *D7L2*$^{(-/-)}$). Underlined texts indicate the sgRNA target sequences and lightning bolt symbols the genomic cleavage points. PAM sequence 5′-NGG-3′ are indicated in blue. Red nucleotides indicate insertions and hyphens show deletions. **(C)** Electropherograms of *D7L1* and *D7L2* genes from the heterozygous and homozygous *D7L1*-KO and *D7L2*-KO mosquitoes. Red arrows indicate the genomic cleavage points. **(D)** Translation of the conceptual RNA from CRISPR-induced mutant alleles. Mutated nucleotides are indicated in blue.

Supplementary Figure 3: *Immunolocalization of AeD7 proteins in the salivary glands of female mosquitoes.* First row corresponds to bright field (BF) images. Second and third rows correspond to immunofluorescent images of glands incubated with anti-rabbit IgG conjugated with Alexa Fluor 594 (red) or Phalloidin conjugated with Alexa Fluor 488 (green), respectively. To ensure specificity, protein A-purified IgG antibodies were pre-adsorbed.

Supplementary Figure 4: *Multidimensional scaling plot (MDS) of similarity between WT, D7L1 and D7L2 replicate samples.* Differential expression analyses were done with the number of unique mapping peptides detected by mass spectrometry of salivary samples. The salivary samples indicated in the plot are as follows: WT mosquitoes (WT.S3 and WT.S4), *D7L1*-KO mosquitoes (AeD7L1KO.S9 and AeD7L1.S10) and *D7L2*-KO mosquitoes (AeD7L2KO.S11 and AeD7L2.S12).

Supplementary Figure 5: *Lack of D7 reingested proteins in* D7*-KO mosquito midguts.* (A) Schematic representation of the immunochemistry studies. Immediately after blood feeding on chicken, mosquitoes were fixed in paraformaldehyde, imbibed in paraffin, and used to make 5 µm-thick sections. Sections of WT **(B)**, *D7L1*$^{(-/-)}$ **(C)**, and *D7L2* $^{(-/-)}$ **(D)** mosquitoes incubated with specific antibodies (IgG α-D7L1 and IgG α-D7L1). Positive signal was detected by DAB chromogen and visualized as dark brown spots (indicated by black arrows). A certified pathologist checked the immunohistochemistry slides, took the pictures and blindly determined positivity or negativity of the signal for each sample. Scale bars are indicated.

Supplementary Figure 6: *Detailed images of blood-fed mosquito sections.* Immediately after blood-feeding on chicken, mosquitoes were fixed and sections prepared for immunohistochemistry analysis. **(A)** Longitudinal mosquito section stained with hematoxylin and eosin (H&E). Images of salivary glands and midgut containing nucleated avian blood are amplified below. (**B)** Transversal section of thorax (left) and midgut (right) from a WT mosquito after staining with IgG α-D7L1 antibody. Red arrows indicate positive signal spots **(C)**. Longitudinal section of a *D7L1*-KO mosquito stained with H&E (left) and specific IgG α-D7L2 (right). Positive signal was detected by DAB chromogen and visualized as dark brown spots. A certified pathologist checked the immunohistochemistry slides, took the pictures and blindly determined positivity or negativity of the signal for each sample. Scale bars are shown.

Supplementary Figure 7: Plasmodium gallinaceum *oocyst levels in midguts from* D7*-KO and WT mosquitoes exposed to infected chicken with high and low parasitemia.* (A) Oocyst levels from mosquito groups fed on a chicken with high parasitemia (50.0%). **(B)** Oocyst levels from mosquito groups fed on a chicken with low parasitemia (2.4%). Bars indicate medians of at least 25 mosquitoes analyzed

per group. For multiple comparisons, WT was used as the control group and samples were compared using the non-parametric Kruskal-Wallis test. P values are indicated as follows: ns: $p > 0.05$; *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$; ****$p < 0.0001$. **(C)** Mercurochrome-stained midguts from WT, D7L1$^{(-/-)}$ and D7L2$^{(-/-)}$ mosquitoes at day 6 after exposure to a *P. gallinaceum* infected chicken with high parasitemia (50%). Number of mosquitoes dissected (N); infection prevalence (prev).

Supplementary Figure 8: Plasmodium gallinaceum *transmission experiment.* **(A)** Experimental plan of mosquito infections and parasite transmissions by mosquito bites. **(B)** Sporozoite counts from mosquito groups fed on infected chicken. Bars indicate means ± SEM of 4 pools of salivary glands from 5 mosquitoes per group. **(C-F)** Clinical signs and parasitemia levels were measured daily during 12 days after mosquito challenge. Two chicken per mosquito group were used. Each chicken was exposed to 5 infected mosquitoes. For multiple comparisons, WT was used as the control group and samples were compared using the one-way ANOVA for the sporozoite levels, and two-way ANOVA for the clinical signs and parasitemia determinations. P values are indicated as follows: *$p < 0.05$; **$p < 0.01$.

Supplementary Figure 9: Plasmodium gallinaceum *oocyst levels in midguts from* D7*-KO and WT mosquitoes exposed to infected chicken blood through artificial membrane system.* Oocyst levels per midgut from mosquito groups fed on infectious chicken blood adjusted to 2.4% parasitemia with fresh naïve chicken blood through an artificial membrane system. A representation of two biological replicates is shown. Bars indicate medians of at least 56 mosquitoes analyzed per group. For multiple comparisons, WT was used as the control group and samples were compared using the non-parametric Kruskal-Wallis test.

Supplementary Table 1: *Mass spectrometry results.*
Whole proteomes from saliva from either WT or *D7*-KO mosquitoes, duplicates designated as WT-S3 and WT-S4; *D7L1*-KO-S9 and *D7L1*-KO-S10; and *D7L2*-KO-S11 and *D7L2*-KO-S12. A FASTA description map is shown and peptides detected by mass spectrometry of samples number of unique mapping peptides determined by EdgeR.

Supplementary Table 2: *Probing time parameters.*
Range and median of probing time across mosquito groups fed on C57BL/6 mice, leukotriene deficient mice (B6.129S2-Alox5), chicken, and membrane feeder.

Supplementary Table 3: *Primers used in this study.*
Primers used for genotyping (sequencing and High-Resolution Melting Analysis), gene expression analysis and *Plasmodium gallinaceum* infection study.