Infections with several flatworm parasites also represent group 1 biological carcinogens, i.e. definite causes of cancer. Infection with the food-borne liver fluke *Opisthorchis viverrini* causes cholangiocarcinoma, bile duct cancer. Whereas the causative agent for most cancers, including CCA in the West, remains obscure, the principal risk factor for CCA in Thailand has long been established – infection with *O. viverrini*. We utilized this established link between infection and cancer to explore the molecular carcinogenesis of *O. viverrini*-induced CCA. Here we report a gene-editing protocol for *O. viverrini* to enable in-depth investigation of pathogenesis and carcinogenesis. We targeted the *Ov-grn-1* gene of *O. viverrini* for knockout by deletion mutation of the coding region of the gene. Both adult and infective larval flukes (newly excysted juveniles, or NEJ) were transfected with a plasmid encoding a guide RNA sequence specific for 20 nucleotides 5'- to a prototypic adjacent motif in exon-1 of the *Ov-grn-1* gene and also encoding the Cas9 nuclease of *Streptococcus pyogenes*. Illumina based deep sequencing of amplicon libraries from genomic DNAs from the parasites demonstrated the presence of Cas9-catalyzed indels within the *Ov-grn-1* locus, and tandem analyses by RT-PCR and western blots revealed rapid depletion of *Ov-grn-1* transcripts and liver fluke granulin. Infection of hamsters with CRISPR/Cas9-edited NEJ enabled studies of liver fluke infection and biliary tract disease in hamsters. Following introduction of CRISPR/Cas9 nuclease plasmid into NEJ, marked reduction of *Ov-grn-1* gene transcripts was evident within days. When hamsters were infected with the gene-edited NEJ, liver fluke infection established within the biliary tract. The findings demonstrated transfection of NEJ with the gene editing plasmid, that gene knockout abolished expression of *Ov-grn-1* by >95%, and infectivity of wild type (WT) and *Ov-grn-1*knockout gene-edited NEJ for hamsters. *Ov-grn-1*$^{-/-}$ knockout flukes induced significantly less pathology in the hamster bile ducts. *Ov-grn-1*$^{-/-}$ parasites were infectious, colonized the biliary tract, grew and developed, were active and motile, and induced a clinically relevant pathophysiological tissue phenotype that significantly differed from WT liver flukes.