Bandyopadhyay, Douglass, et al.
Figure S1.

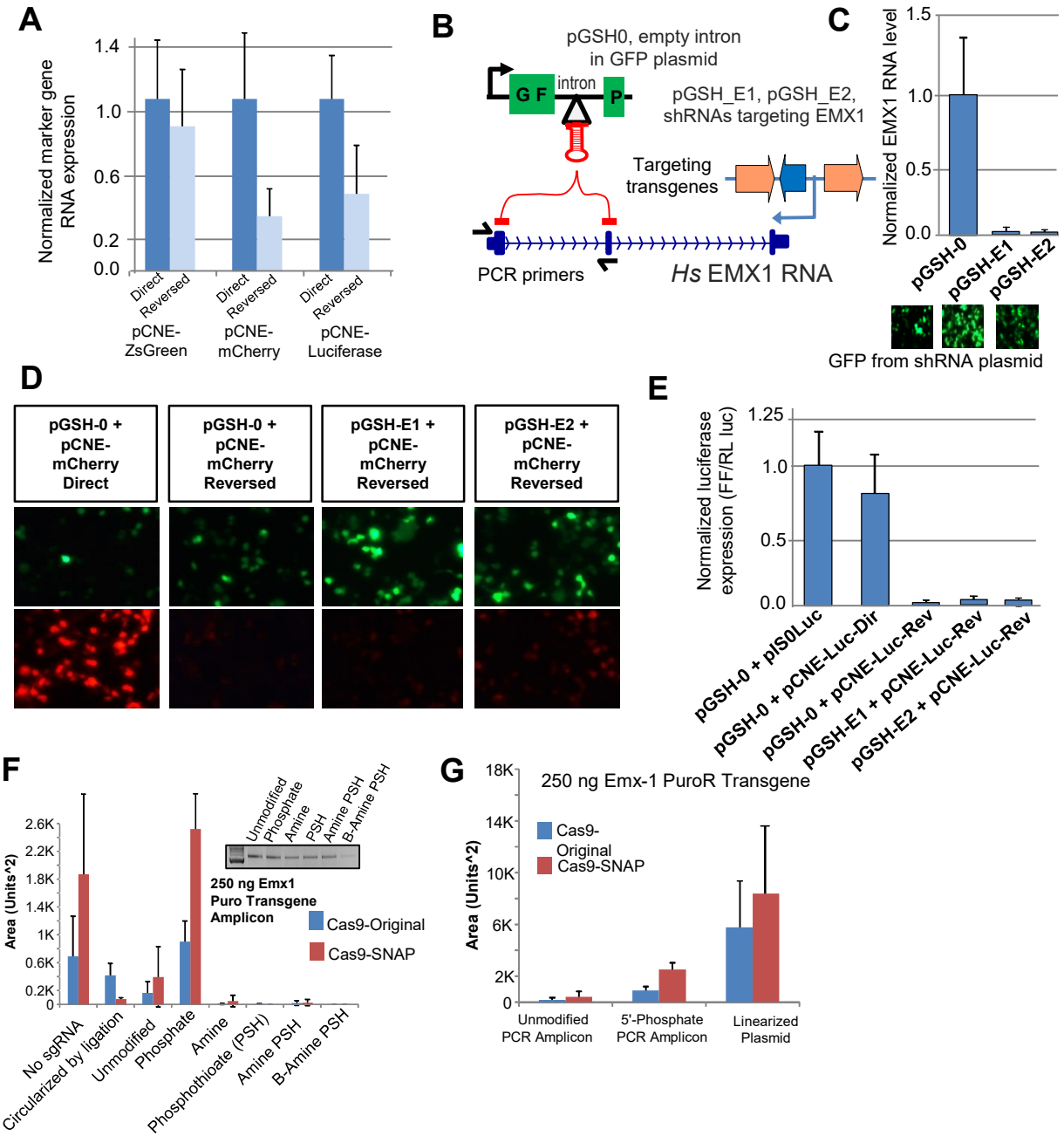

Figure S1. Directionality of homology arms can impact selection marker expression, while blunt end dsDNA PCR amplicons are less suitable templates for Cas9-stimulated HDR.
A) Quantitative RT-PCR analysis of reporter gene expression from "Direct" (Dir) and "Reversed" (Rev) pCNE-based plasmids. B) Design of shRNAs; C) and effective knockdown of endogenous EMX1 transcripts in HEK293FT cells. D) Top panels with GFP expression confirm shRNA transfection, but this does not improve the poor expression of mCherry cloned into pCNE-plasmid. E) Similar lack of luciferase expression that is not improved by knocking down EMX1 with shRNAs. F) Comparisons of unmodified dsDNA PCR amplicons versus amplicons with primers that have a 5' end modifications. Error bars represent standard deviation of three biological replicates measuring the amount of puromycin-selected cells. Agarose gel showing similar levels of each dsDNA PCR amplicon with modified 5' ends are comparable to the unmodified dsDNA amplicon. G) Comparison of a PCR amplicon versus a plasmid template, where transfections contained the same molar amount of puromycin marker as well as balanced same mass of DNA with carrier plasmid added to the PCR amplicon.