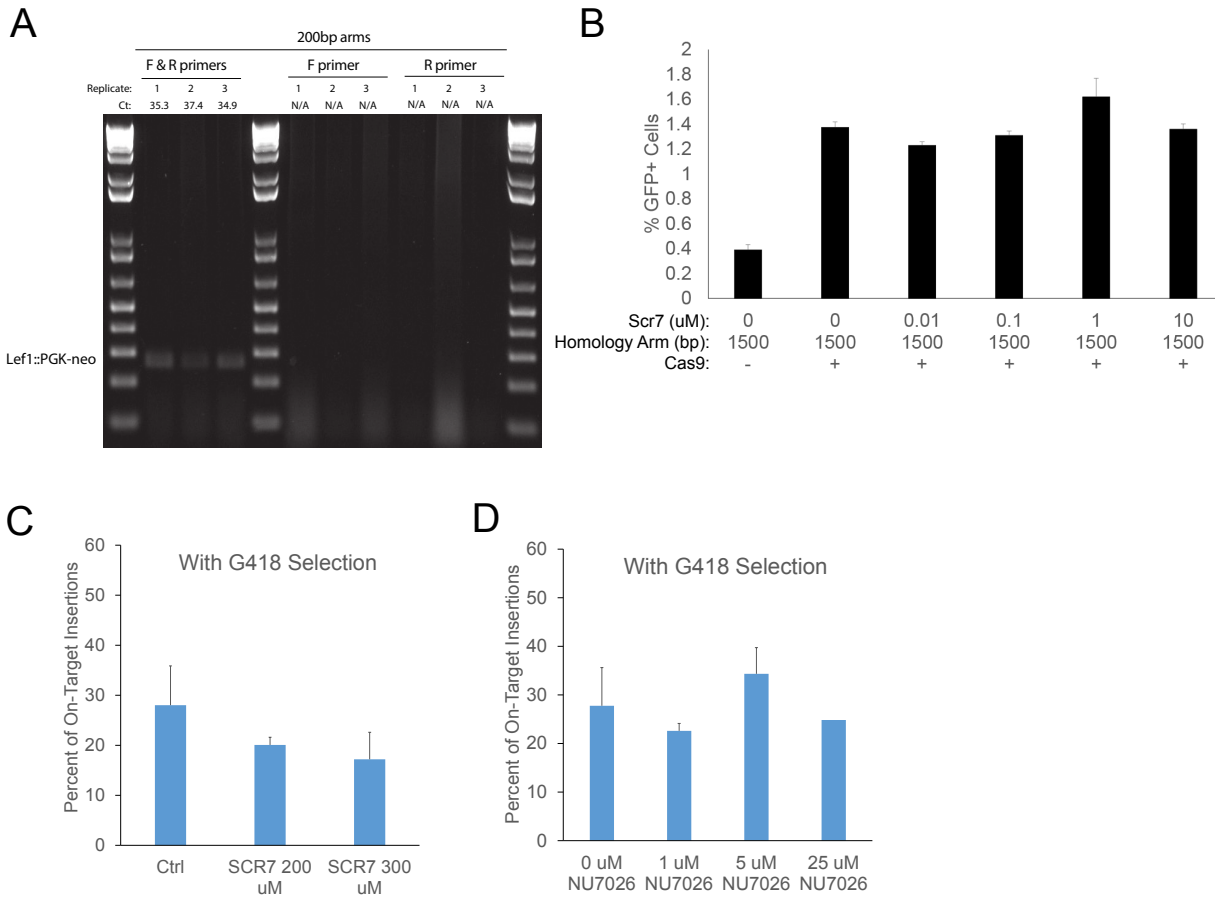
FIGURE S1



Supplemental Figure 1.

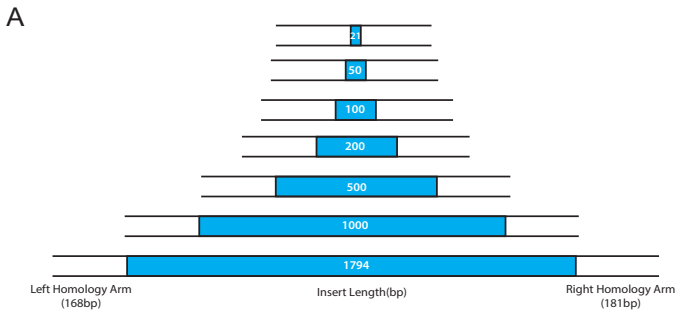
(A) qPCR products for reactions containing forward and reverse primers as well as forward and reverse primers only.

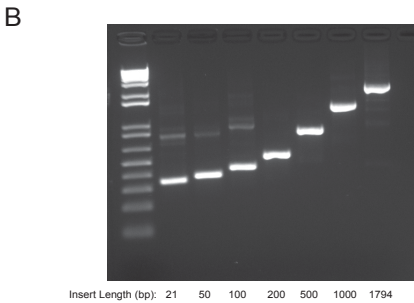
(B) Insertion frequency of GFP expression cassette using 1500bp homology arms to the *Lef1* gene was quantified by flow cytometry for cells grown in 0.01 – 10μM SCR7.

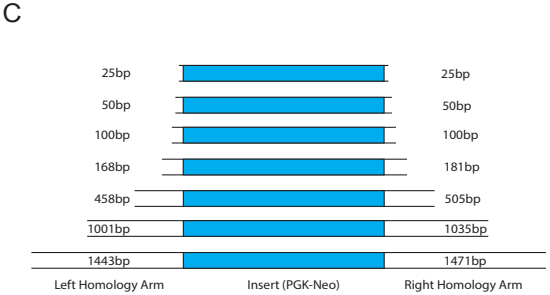
(C) qPCR analysis of on-target insertions for cells grown in 200-300μM SCR7. Values shown for cells surviving G418 selection. Bars represent mean +/- SD for biological and technical duplicates.

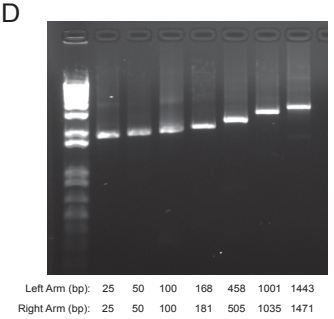
(D) qPCR analysis of on-target insertions for cells grown in 0-25μM NU7026. Values shown for cells surviving G418 selection. Bars represent mean +/- SD for biological and technical duplicates.

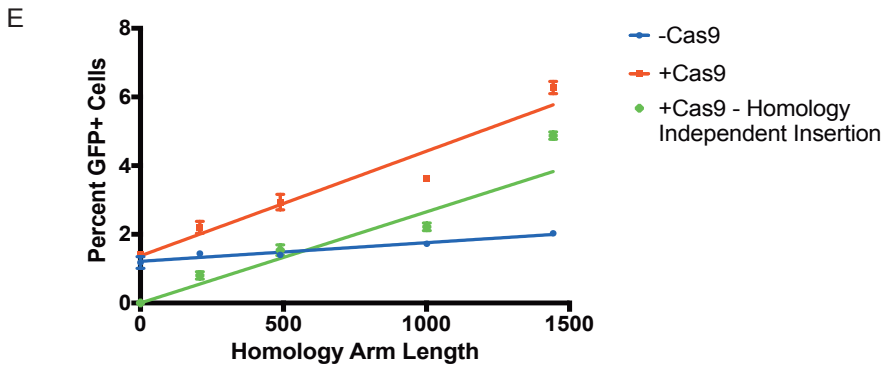
FIGURE S2











	-Cas9	+Cas9	+Cas9 - Homology Independent Insertion
Comparison of Fits			
Null hypothesis	YIntercept = 0	YIntercept = 0	YIntercept = 0
Alternative hypothesis	YIntercept unconstrained	YIntercept unconstrained	YIntercept unconstrained
P value	0.0003	0.0390	0.9498
Conclusion (alpha = 0.05)	Reject null hypothesis	Reject null hypothesis	Do not reject null hypothesis
Preferred model	YIntercept unconstrained	YIntercept unconstrained	YIntercept = 0
F (DFn, DFd)	409.1 (1, 3)	12.38 (1, 3)	0.004677 (1, 3)

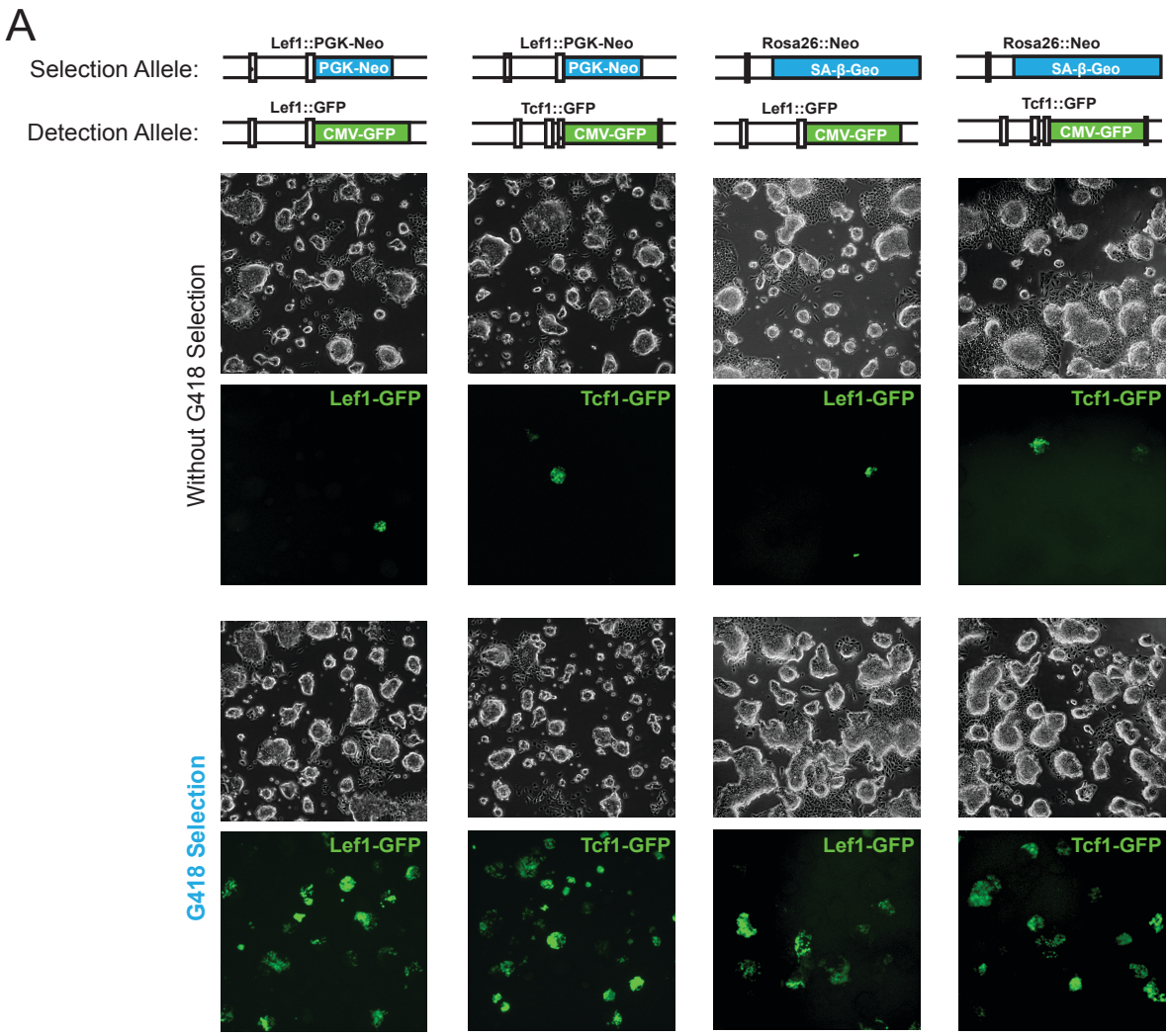
Supplemental Figure 2.

(A and B) DNA donor templates used in figure 2B. Homology arm length was held constant at 168bp on the left and 202bp on the right. Insert length was varied from 21bp up to 1794bp. Schematic (A) and PCR products (B) are shown.

(C and D) DNA donor templates used in figure 2C-E. Homology arm length was varied while holding insert length constant. Arm lengths are illustrated in the schematic (C) and PCR products are shown (D).

(E) Linear regression analysis of figure 2D to determine if subtracting the empirically determined homology-independent repair from the +Cas9 samples would result in a fit where y-intercept = 0 as predicted. The linear fits (top panel) and regression analysis for y-intercept (bottom panel) are shown.

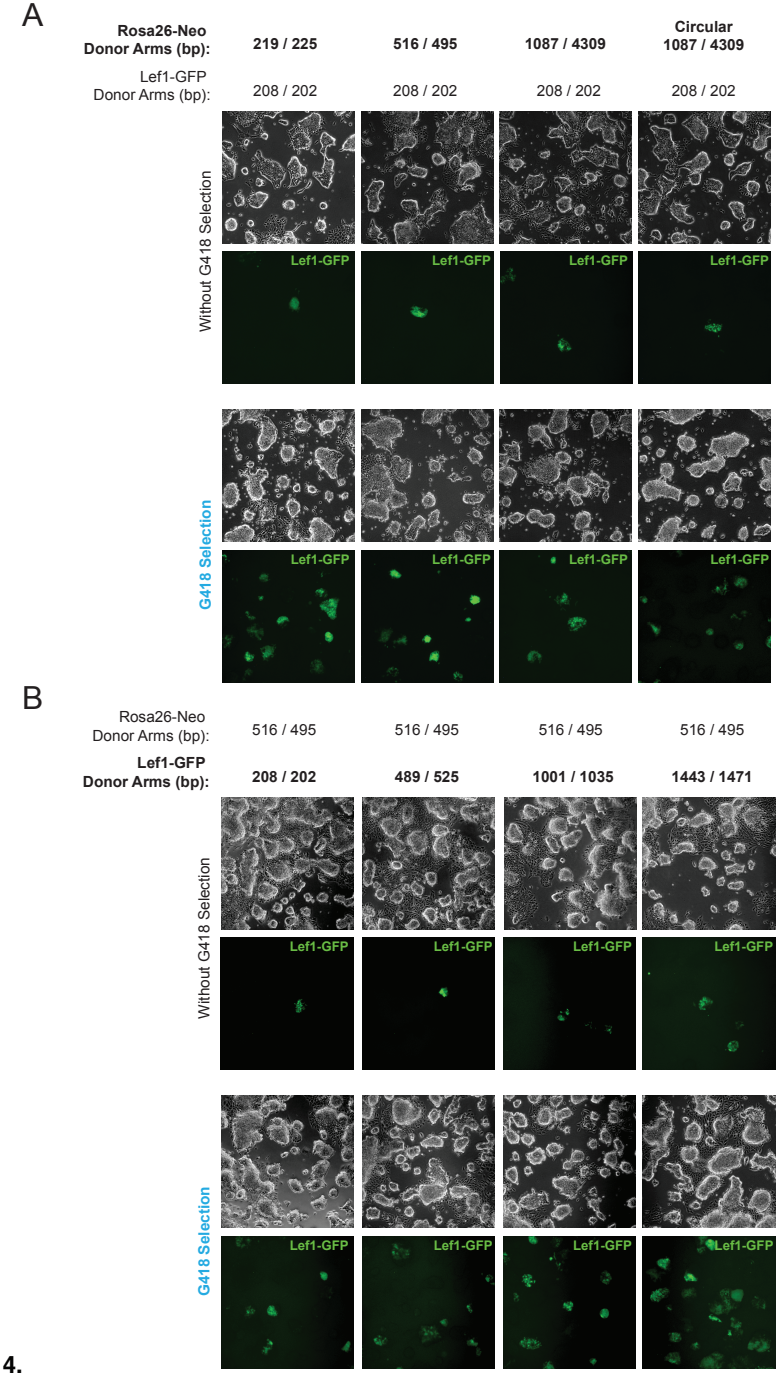
FIGURE S3



Supplemental Figure 3.

(A) Representative DIC and fluorescent microscopy images for the co-incidental insertion experiments shown in Figure 3 using sgRNA and donor DNA target insertion at distinct genes; Lef1, Tcf1, and Rosa26. The combination of selection and detection donor DNA are noted at the top of each column.

FIGURE S4



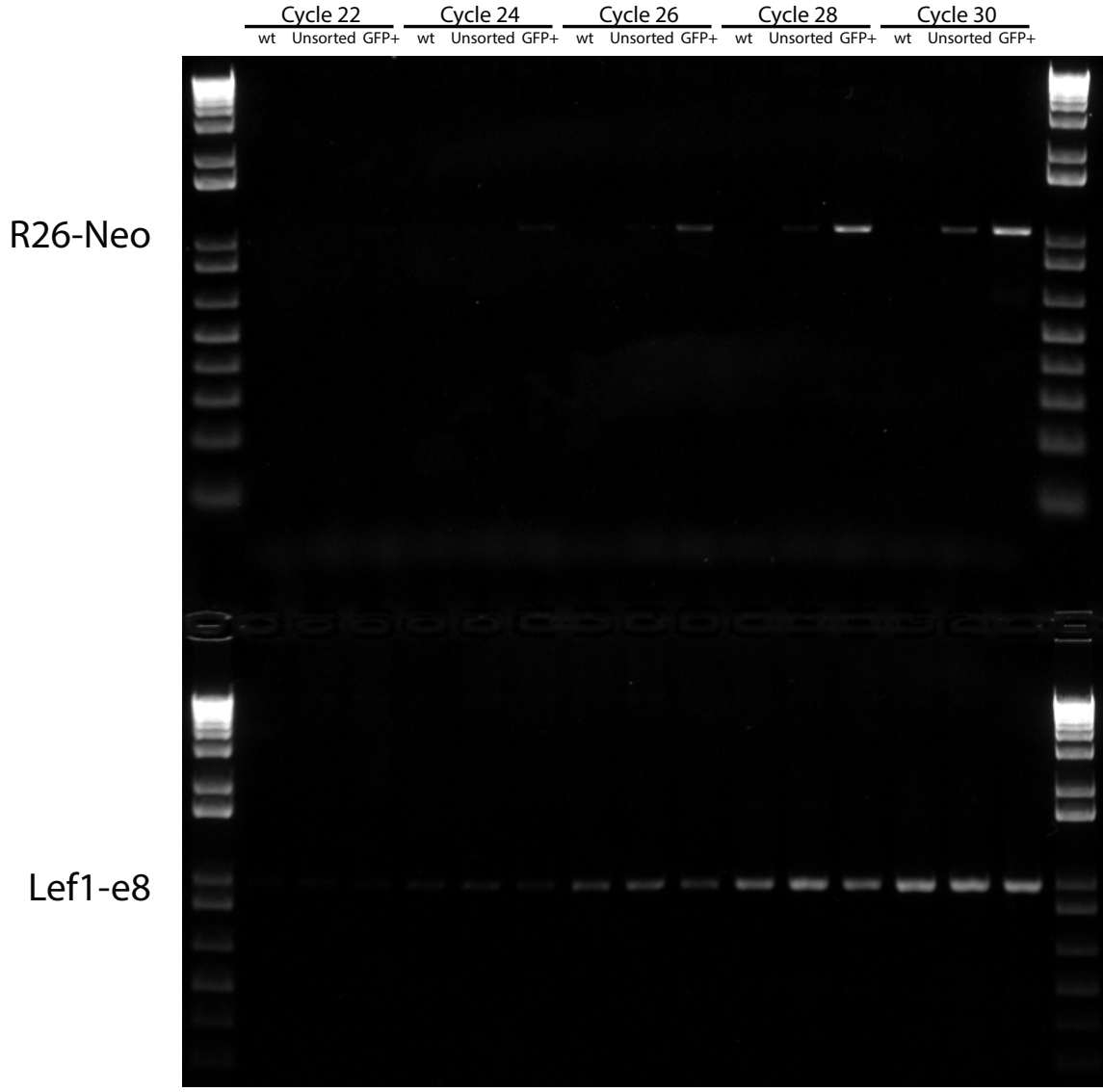
Supplemental Figure 4.

(A) Representative DIC and fluorescent microscopy images of using Lef1-CMV-GFP as the donor DNA for detection and Rosa26-Neo as the donor DNA for selection. Transfected cells (top) and cells following G418 selection (bottom) are shown. As indicated at the top of each column, homology arm length was varied for the Rosa26-Neo donor DNA and kept constant at 208/202bp for Lef1-GFP.

(B) Similar to (A), except homology arm length was varied for Lef1-CMV-GFP and kept constant for Rosa26-Neo at 516/495bp.

FIGURE S5

A



Supplemental Figure 5.

(A) Semi-quantitative PCR cycle gradient for R26-Neo for co-incidental insertion experiments shown in Fig 5 following fluorescence activated cell sorting of Ctnnb1::EGFP cells.