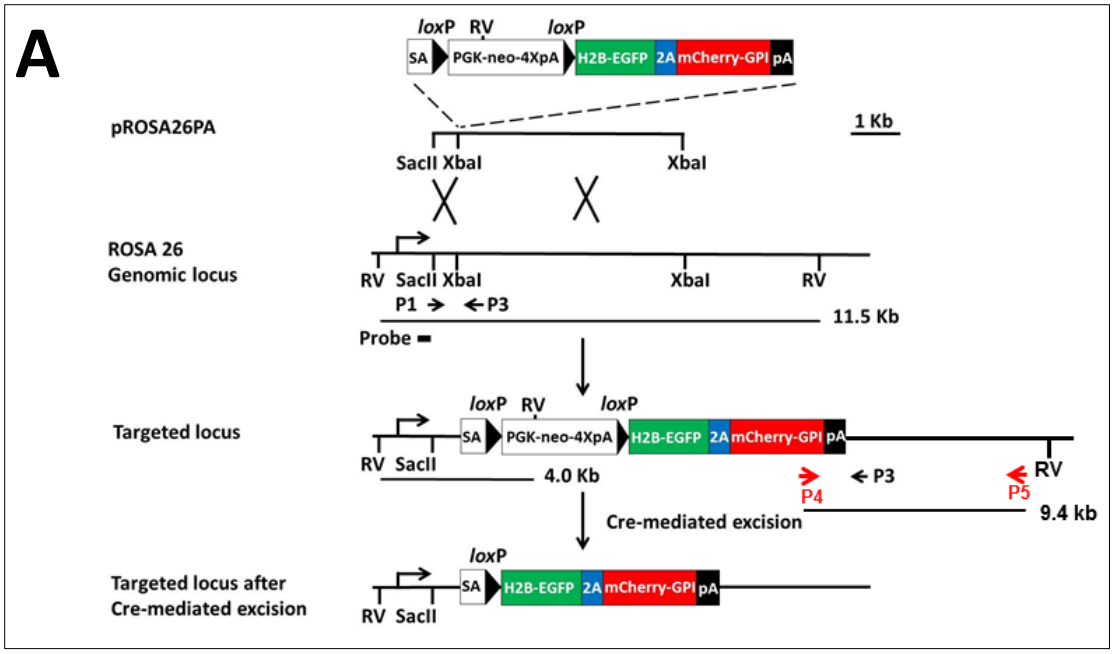
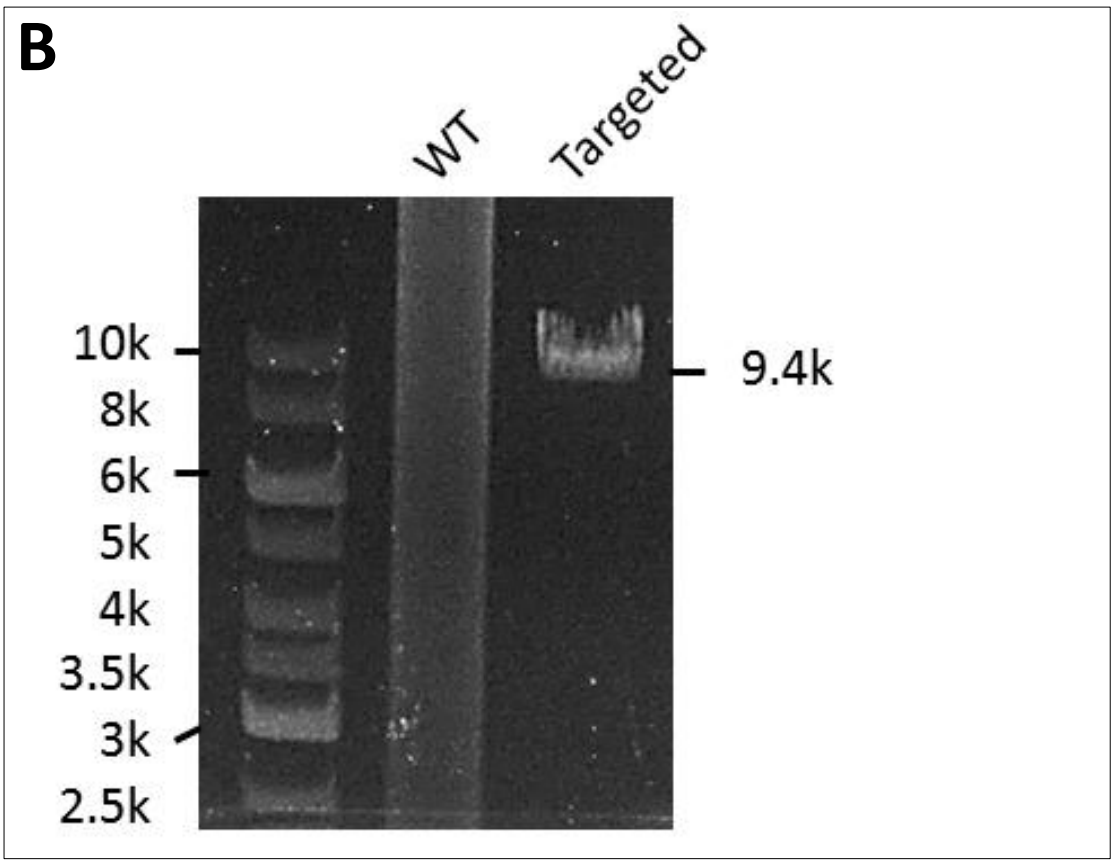
**Figure S1**

The R26 targeting event was reconfirmed by a long PCR experiment using primer sequences from the report construct (P4) and a 3' external genomic region (P5) **(A)**. Only the successfully targeted R26R-GR allele will result in a 9.4 Kb PCR product **(B)**. Genomic DNA from the germline transmitted ES cell clone was used in this study.



The primer sequences used in this study were the followings:

P4: 5'- CAA CGA GGA CTA CAC CAT CGT GGA ACA GTA CGA -3‘
P5: 5'- GGT TGT TTT AGA ATC AGA CTG GTA GCC TAA TGA CT -3‘

The reaction mix was the following:

| | |
|---|---|
| Genomic DNA (dil. 10X) | 5 |
| 10X Expand long template buffer | 2 |
| dNTP (10 mM) | 10 |
| $MgCl_2$(25 mM) | 2 |
| BSA (10 mg/ml) | 0.5 |
| DMSO | 0.5 |
| P4 (10 μM) | 2 |
| P5 (10 μM) | 2 |
| Expand long template enzyme mix (Roche) | 1 |
| $ddH_2O$ | 22 |
| Total | 50 |

The PCR reaction was performed using the following program:

| | | |
|---|---|---|
| 92 °C | 2’ | |
| 92 °C | 10” | 10 cycles |
| 65 °C | 30” | |
| 68 °C | 8’ | |
| 92 °C | 10” | |
| 65 °C | 30” | 25 cycles |
| 68 °C | 8’+20” | |
| 68 °C | 7’ | |
| 14 °C | ∞ | |