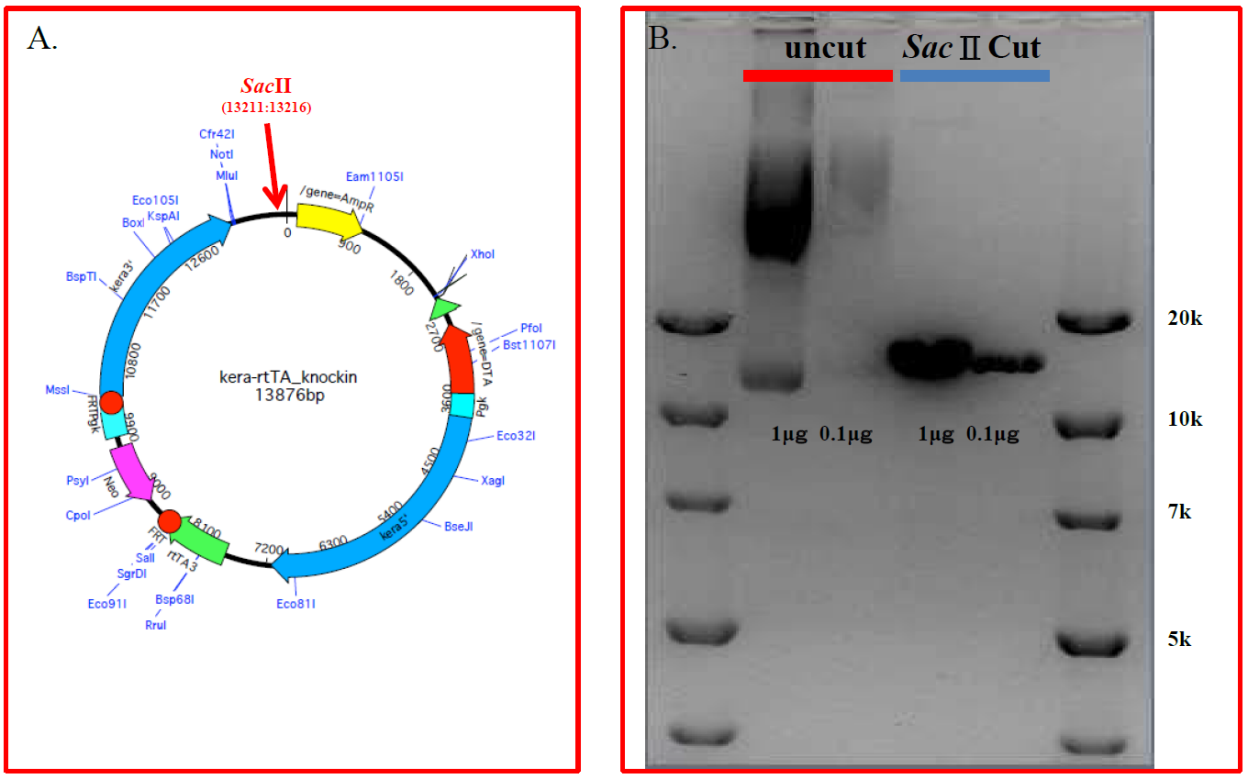
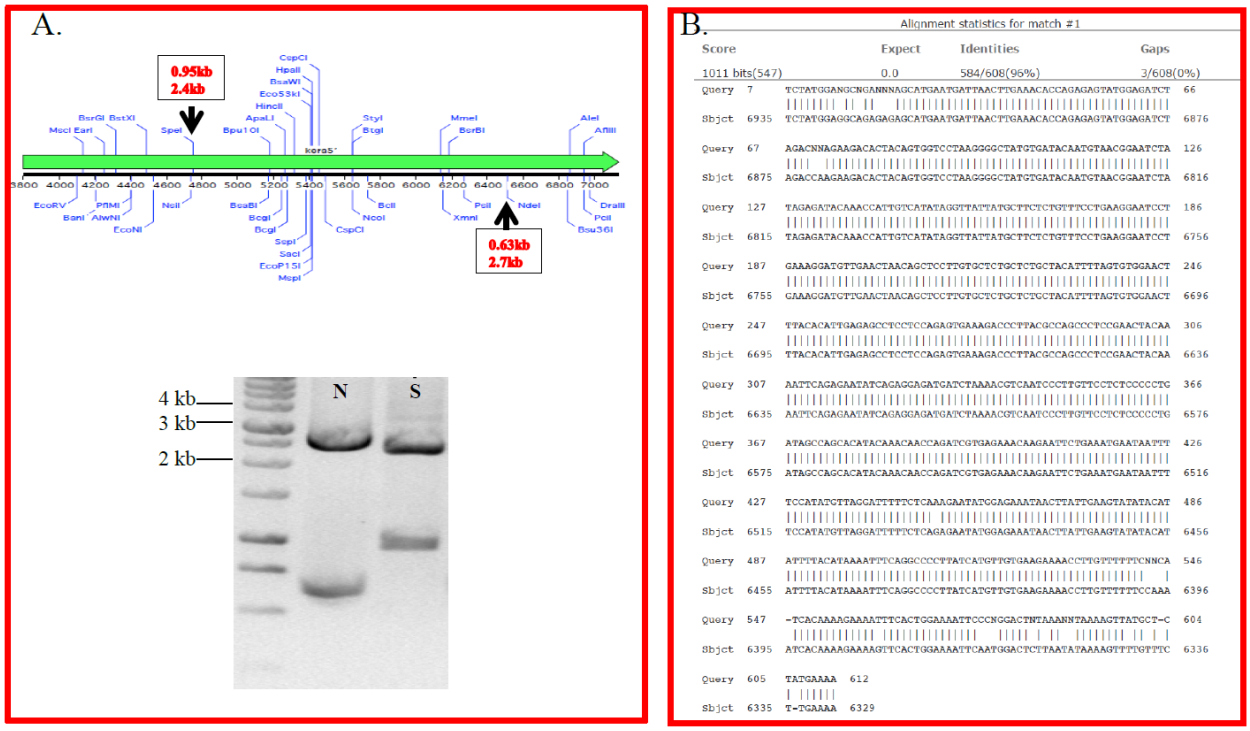
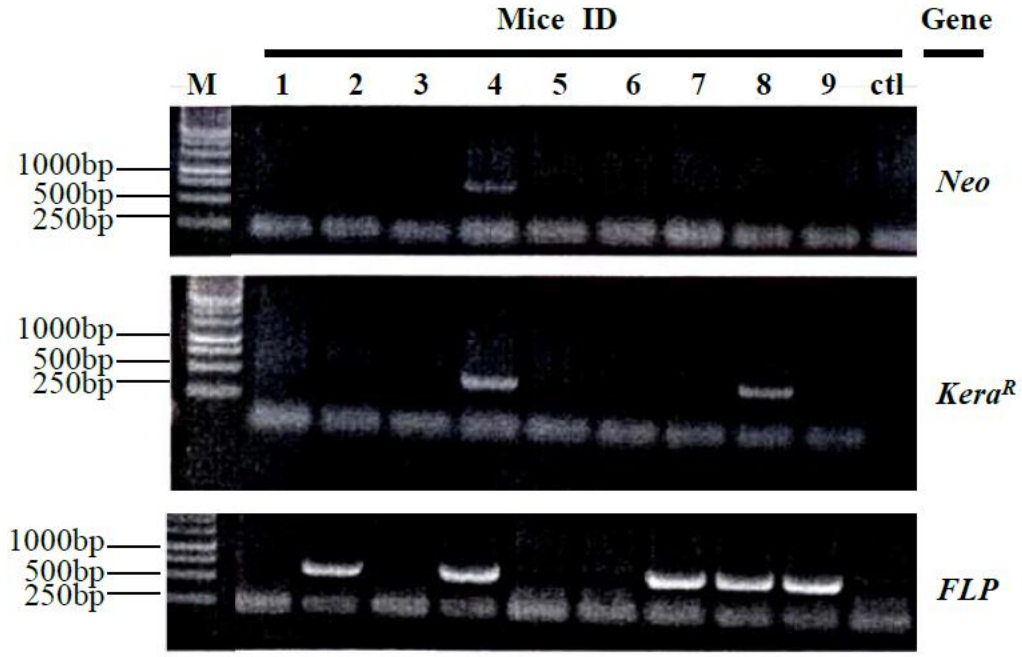
Supplementary materials



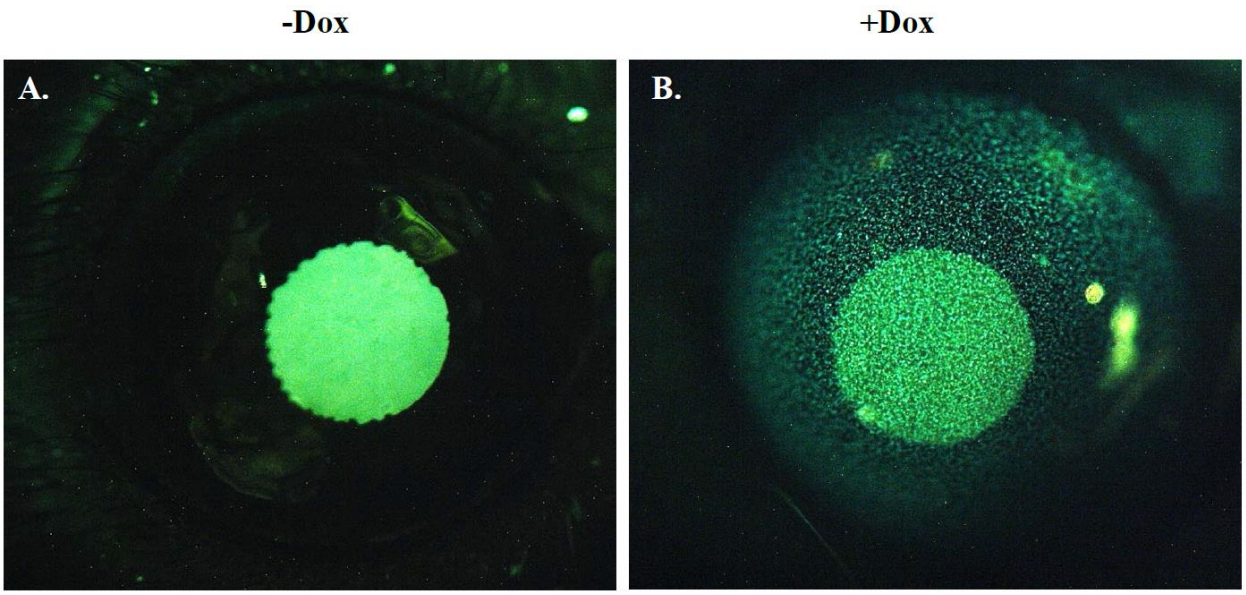
Supplementary Fig.1. Map of the generated targeting construct. (A) Schematic showing the main components of the targeting vector. (B) Shows the targeting construct DNA linearized by *Sac*II.



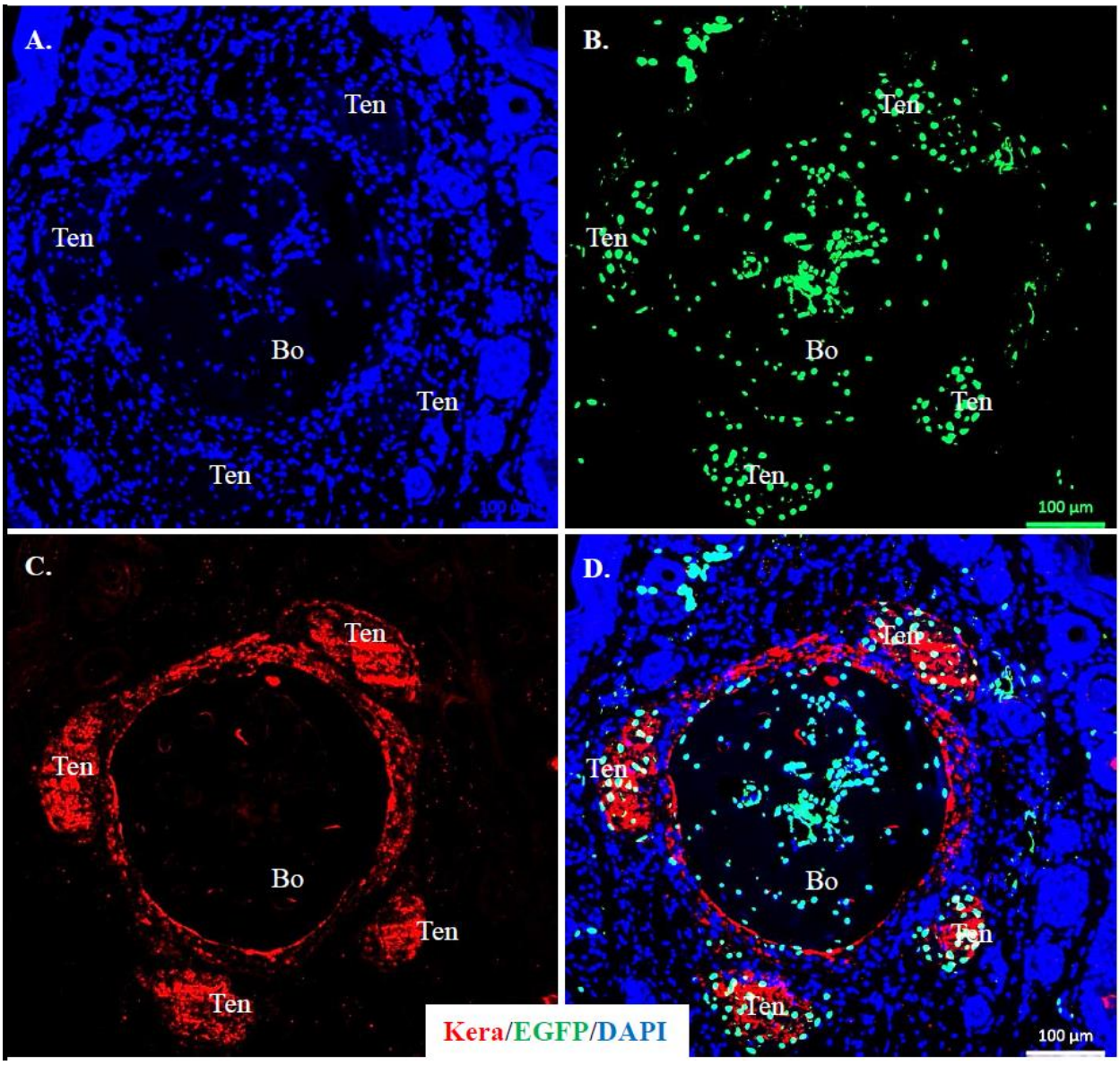
Supplementary Fig. 2. Identification of homologous recombinant ES clones by enzyme digestion and DNA sequencing. (A) Restriction enzymes analysis of the 5′-arm PCR product using primer pair #1 and #2. (B) One example of enzyme digestion of the 5′-arm PCR product using N (*Nde*I) and S (*Spe*I). (C) Alignment of the original targeting construct with the 5′-arm PCR product. Alignment between the original targeting vector with the 5′-arm PCR product from the targeted ES cell DNA which shows the junction region is correct**.**



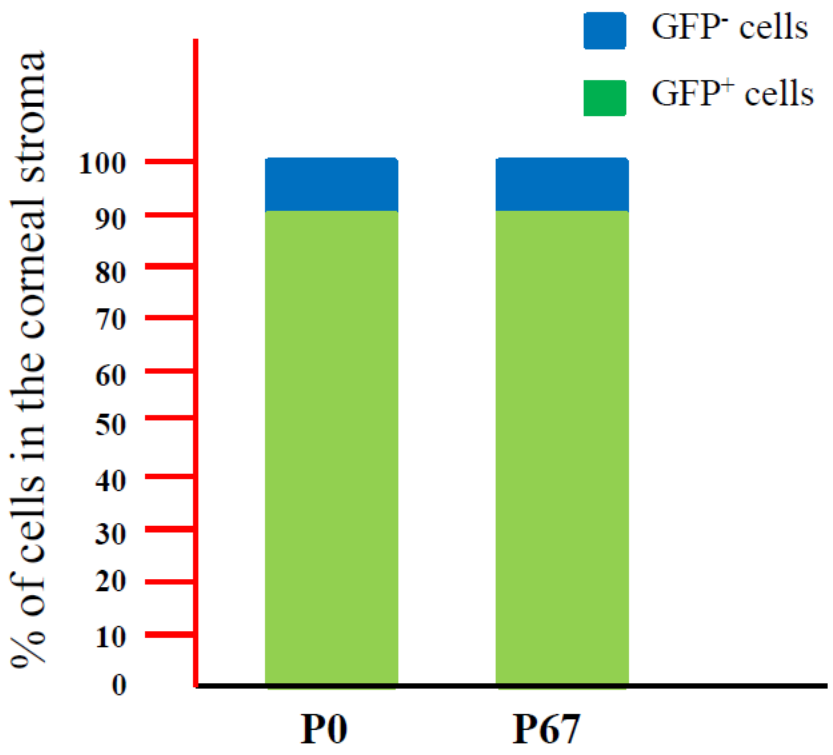
Supplementary Fig. 3. Genotyping of double transgenic mice obtained by crossing *Kera^{RT}* with FLP mice. It is noticed that #4 and #8 both are double transgenic mice. The Neo gene was only removed in #8 by FLP while *Neo* was not removed in #4.



Supplementary Fig.4. GFP expression in double transgenic mice *Kera^{RT}/TH$_{2B}$-EGFP* only after Dox induction. GFP was present in the cornea (B) after Dox induction of 2-month old adult double transgenic mice for 2 days, while there was no GFP detected before induction (A).



Supplementary Fig. 5. GFP is expressed in the tail of double transgenic mice *Kera^{RT}/TH$_{2B}$-EGFP* after Dox induction from P21 to P45. Immunohistochemistry staining showed that keratocan was expressed in tail tendon (C, D). GFP fluorescence was present in the tenocytes (B, D) which was co-localized with keratocan protein. Abbreviations: Ten: tail tendon; Bo, tail bone.



Supplementary Fig. 6. Quantitative analyses of GFP positive cells (GFP^{+}) and negative cells (GFP^{-}) in corneal stroma. Quantitative analyses were done with *Kera^{RT}/TH$_{2B}$-EGFP* double transgenic mice Dox-induced from E12.5 to P0 and P60-P67, respectively. Note that the quite similar percentage of GFP negative cells presented among the whole corneal stromal keratocytes even induced at different time.

Supplementary Table 1. PCR primer used in cloning the target vector Kera-IRES-rtTA3

Primer name	primer sequence	purpose
pgkNeo-F	AGGTCGAGGGACCTA GAAGTTCCTATTCTCTAGAAAGTATAGGAACTTCATTAAGGGTTCCGGATCAGCTTGATTCGAGCCCCAGC	FRT-pgkNeo-FRT cassette
PgkNeo-R	GTGGATCCGGAACCCGAAGTTCCTATACTTTCTAGAGAATAGGAACTTCTAGGTCCCTCGACCTGCAGGAATTCTACCGGGTAGGG	
3'arm-F	GCCTAGTTTAAACCCGGTCCTAGACCACACTTGCAATTGTTCTTACCCACC	2.9-kb 3' homology arm
3'arm-R	CAATTGATGCATCCCCATCTTAGGATGTTTTTAATTCTGGAATATTTCCTTTAGTACTTT	
5'arm-F	CCGGTAGAATTCGATGCCATAAAGATGTGCCTGTGACCAAAATGCCC	3.3-kb 5' homology arm
5'arm-R	CTCATACCACATACATCTACCCTTAAACCAGTTTGGG	
IRES-F	TGTATGTGGTATGAGGCCCCTCTCCCTCCCCCCCCCCTAACGTTA	IRES
IRES-R	TGTGGCCATATTATCATCGTGTTTTTCAAAGGAAAACCACG	
rtTA-F	GATAATATGGCCACAACC ATGAGTAGACTGGACAAGAGCAAAGTC	rtTA3
rtTA-R	ATCGATAAGCTTGATCCGGATTACCCGGGGAGCATGTCAAGGTC	

Supplementary Table 2. Summary of targeted clones and chimera

Clone ID	Original screening	3'-end verification	5'-end verification	Expanding	Injected clones	Chimera	founder
Q3C1-B7	+	+	+	+	+	2	
Q3C1-C7	+	+	+	+	+	1	
Q3C1-A5	+			+			
Q3C1C1	+			+			
Q3C1-A10	+			+			
Q3C2-C2	+	+	+	+	+	0	
Q3C2-G11	+	+	+	+			
Q3C2-C1	+			+			
Q3C2-F1	+			+			
Q3W1-E3	+	+	+	+	+	4 (3 of more than 90%)	
Q3W2-B8	**+**	**+**	**+**	**+**	**+**	**3**	**1**
Q3W2-A12	+			+			
Q3W2-C1	+			+	+	0	