
Supplementary information

Development of two mouse strains conditionally expressing bright luciferases with distinct emission spectra as new tools for in vivo imaging

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Supplementary figures and table

Figure S1

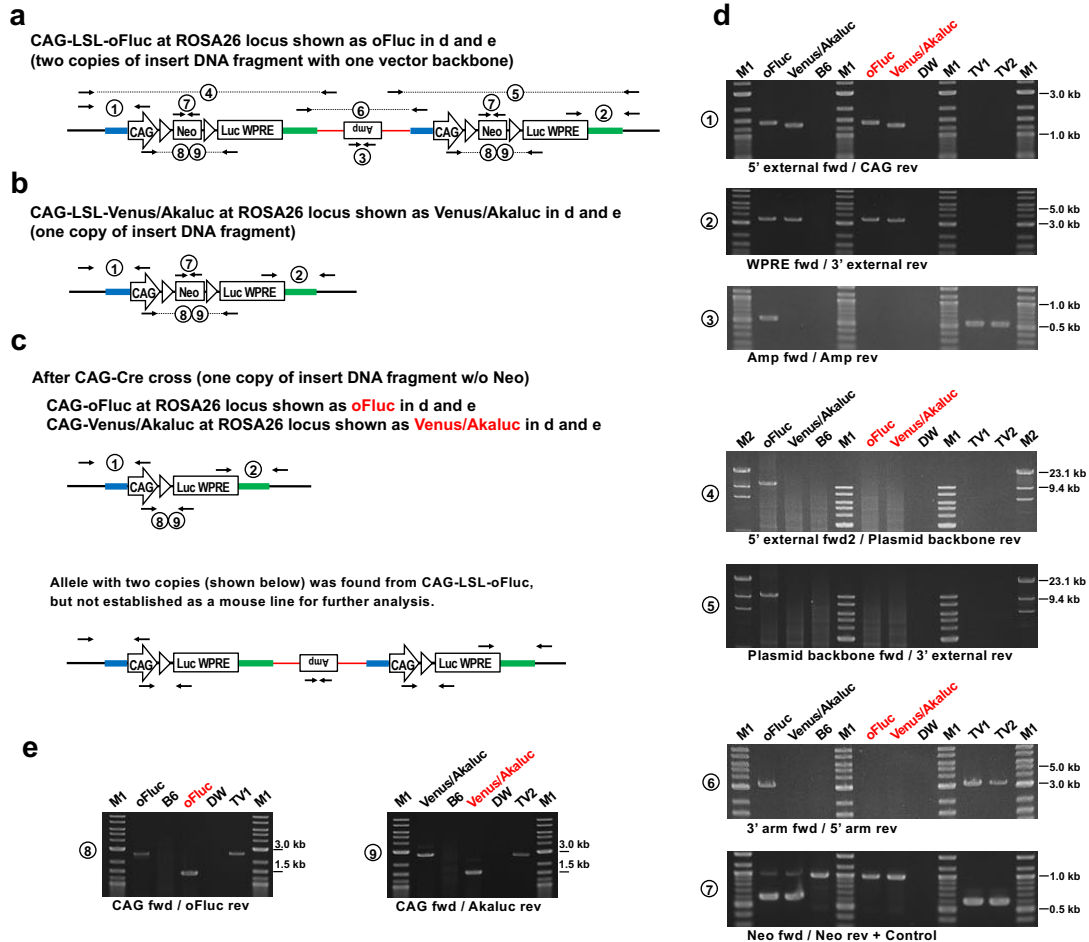


Figure S1. PCR confirmation of targeted knock-in at the *ROSA26* locus in luciferase reporter mice

(a) CAG-LSL-oFluc mice carried two copies of the insert DNA fragment (Figure 1a) with a vector backbone at the *ROSA26* locus. (b) CAG-Venus/Akaluc mice carried one copy of the insert DNA fragment at the *ROSA26* locus. (c) After the CAG-Cre cross, both CAG-oFluc and CAG-Venus/Akaluc mice had one copy of the insert DNA fragment with

deletion of the *Neo* cassette. The locations of the PCR primers are indicated by numbers corresponding to the gel images indicated in (d) and (e). (d) Confirmation of the targeted knock-in at the *ROSA26* locus by PCR. The PCR primer sets used in each panel are indicated by numbers in (a) to (c). oFluc in black (CAG-LSL-oFluc), Venus/Akaluc in black (CAG-LSL-Venus/Akaluc), B6 (C57BL/6), oFluc in red (CAG-oFluc), Venus/Akaluc in red (CAG-Venus/Akaluc), DW (distilled water, negative control), TV1 (targeting vector plasmid for oFluc), and TV2 (targeting vector plasmid for Venus/Akaluc). M1 and M2 are DNA size markers (i.e., Quick-Load 1 kb Plus DNA Ladder and λ DNA-HindIII Digest, respectively (NEB, Japan)). (e) Confirmation of Cre-mediated recombination by PCR. The samples and PCR primers are the same as those described in (d).

Figure S2

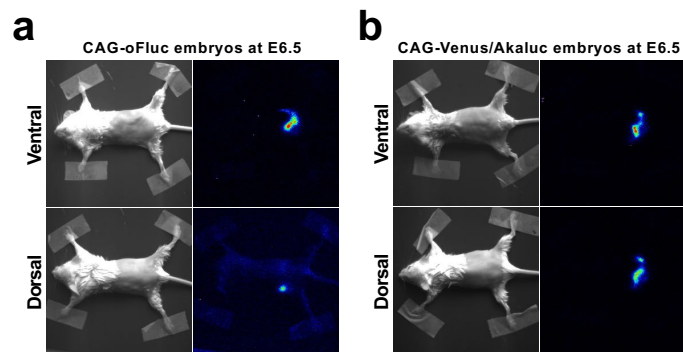


Figure S2. In vivo BLI of pregnant wild-type mothers carrying CAG-oFluc (a) and CAG-Venus/Akaluc (b) embryos at 6.5 days after conception. The hair on the lower abdomen and back of the mice was shaved.

Figure S3

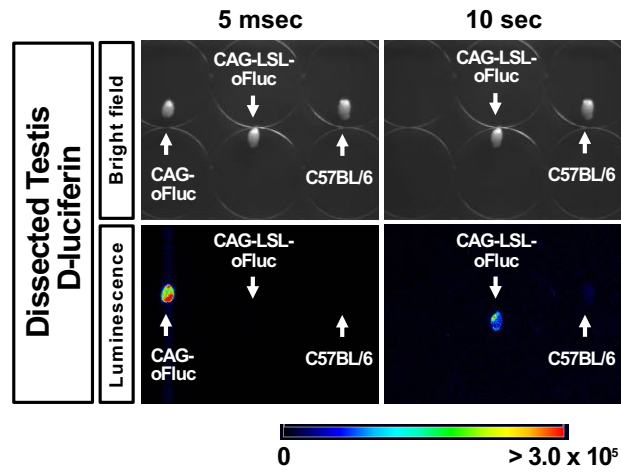


Figure S3. Ex vivo imaging of dissected testes incubated with D-luciferin (1 mM). For longer exposure, the testis dissected from a CAG-oFluc mouse was removed during BLI because its bright luminescence lit up other testes, thus leading to false-positive signals.

Figure S4

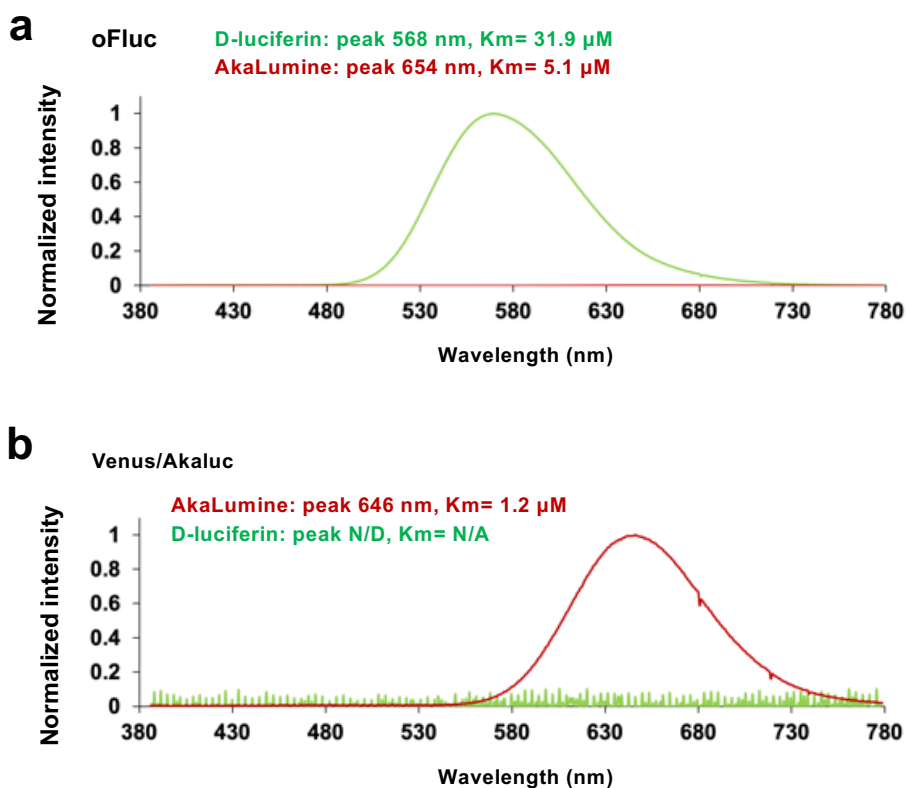


Figure S4. Emission spectra of the recombinant proteins of oFluc (a) and Venus/Akaluc (b), where D-luciferin or AkaLumine was used as their substrate. The intensity was normalized by dividing the measured intensity along each wavelength by the peak intensity of the matched luciferase/luciferin pairs, i.e., the peak intensity of D-luciferin at 568 nm for oFluc (a) and the peak intensity of AkaLumine at 646 nm for Venus/Akaluc (b). Emission spectra of the matched luciferase/luciferin pairs in this figure were reused from Figure 1d.

Figure S5

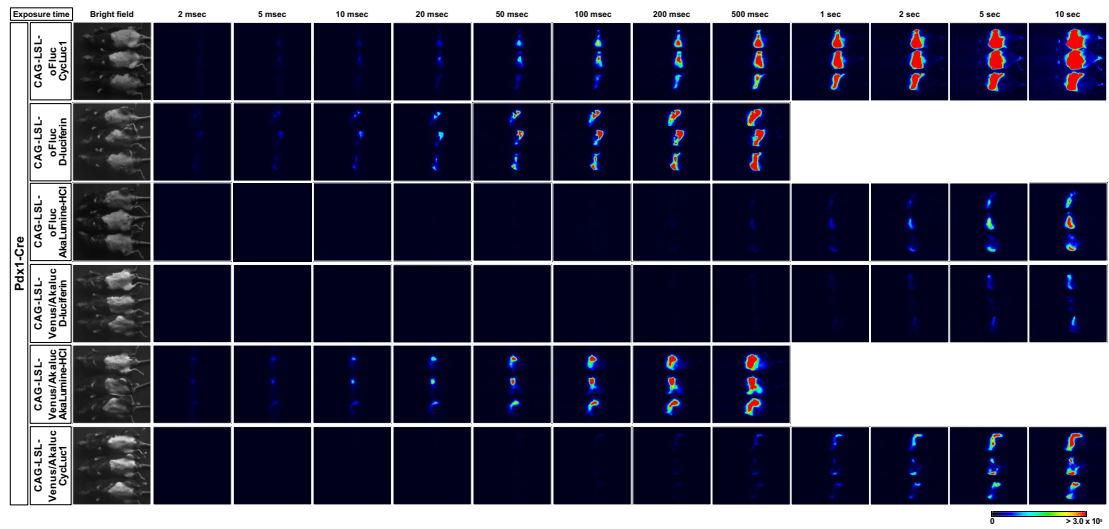


Figure S5. In vivo BLI of oFluc and Akaluc in the pancreas by crossing Pdx1-Cre mice with CAG-LSL-oFluc or CAG-LSL-Venus/Akaluc mice. Images were acquired using multiple exposure times after the administration of one of the three substrates. Data from these images were quantified as reported in Figure 3a. Scale, photon/s/cm².

Figure S6

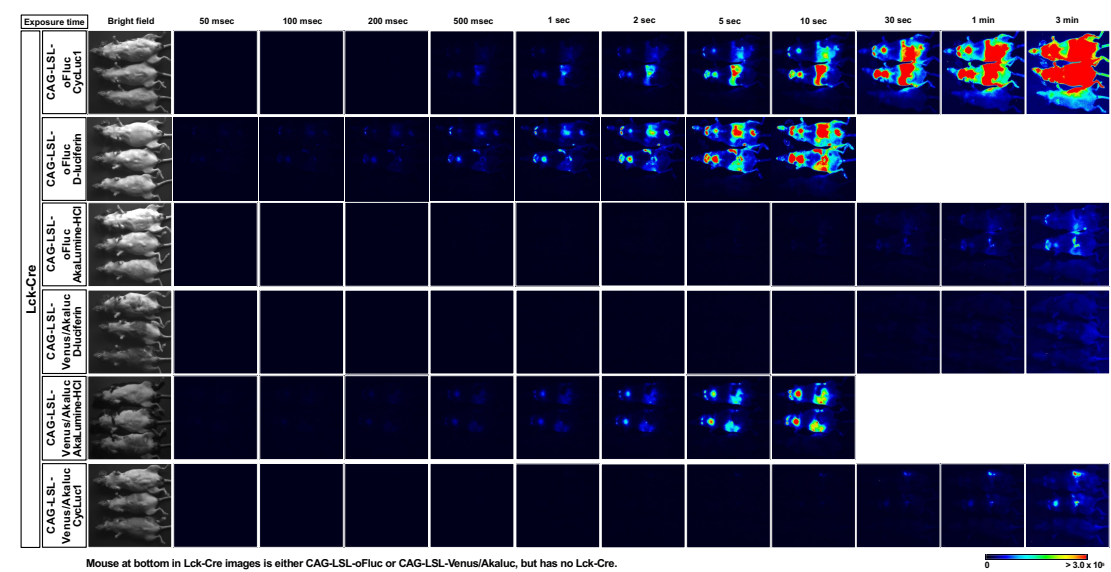


Figure S6. In vivo BLI of oFluc and Akaluc in T cells by crossing Lck-Cre mice with CAG-LSL-oFluc or CAG-LSL-Venus/Akaluc mice. Images were acquired using multiple exposure times after the administration of one of the three substrates. Data from these images were quantified as depicted in Figure 3b. Scale, photon/s/cm².

Figure S7

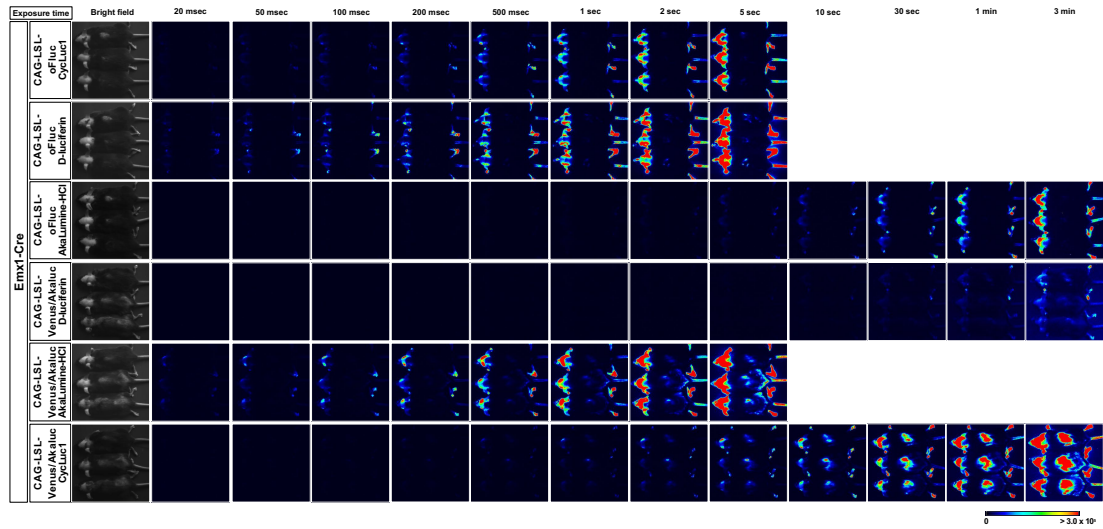


Figure S7. In vivo BLI for oFluc and Akaluc in mice that were double positive for Emx1-Cre and the reporters. Images were acquired using multiple exposure times after the administration of one of the three substrates. Data from these images were quantified as depicted in Figure 4a. Scale, photon/s/cm².

Figure S8

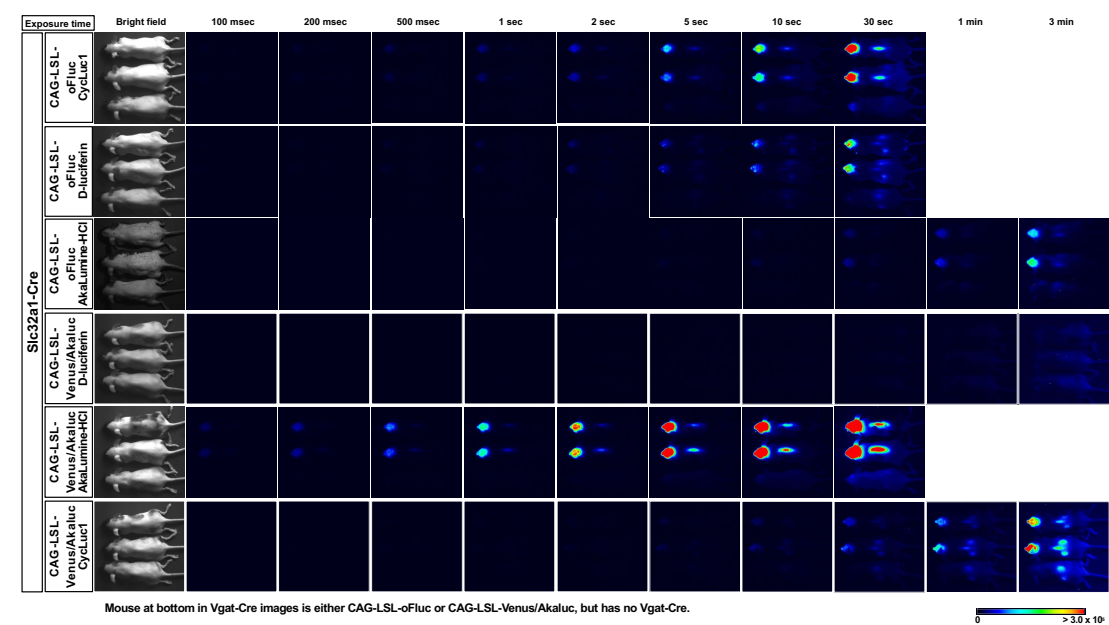


Figure S8. In vivo BLI for oFluc and Akaluc in mice that were double positive for Vgat-Cre and the reporters. Images were acquired using multiple exposure times after the administration of one of the three substrates. Data from these images were quantified as depicted in Figure 4b. Scale, photon/s/cm².

Figure S9

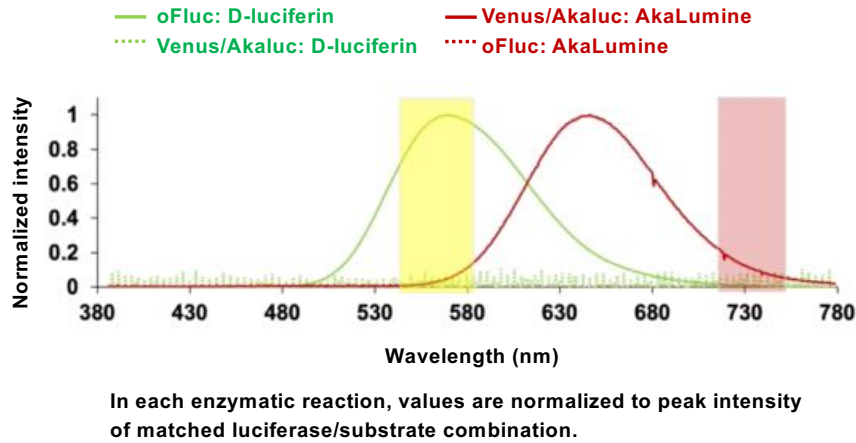


Figure S9. Emission spectra of the recombinant proteins of oFluc and Venus/Akaluc.

Solid lines, matched luciferase/luciferin pairs; dashed lines, mismatched luciferase/luciferin pairs. The yellow and red boxes indicate the two wavelength ranges that can pass through the filters used in the *in vivo* dual-color BLI, as depicted in Figure 6 and Supplementary Figures S10 and S11. Yellow box, 565 ± 40 BP filter; red box, 730 ± 45 BP filter. This figure was generated by combining Figure S4a and S4b.

Figure S10

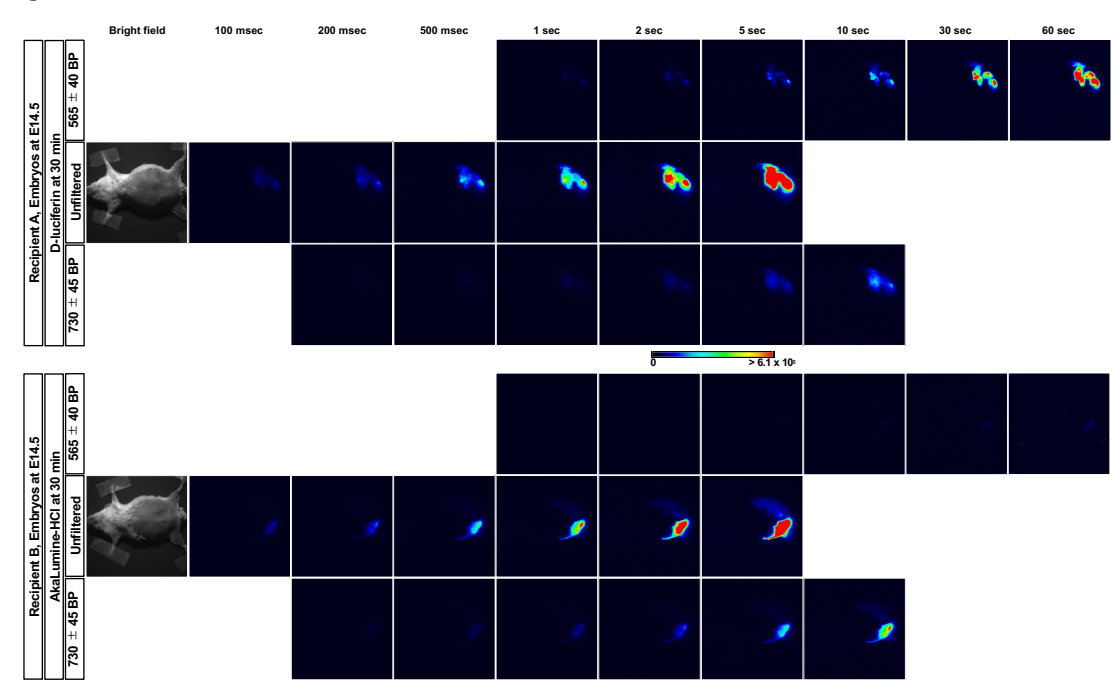


Figure S10. Filter segregation of oFluc and Akaluc after injecting the first substrate, as depicted in Figure 6.

Figure S11

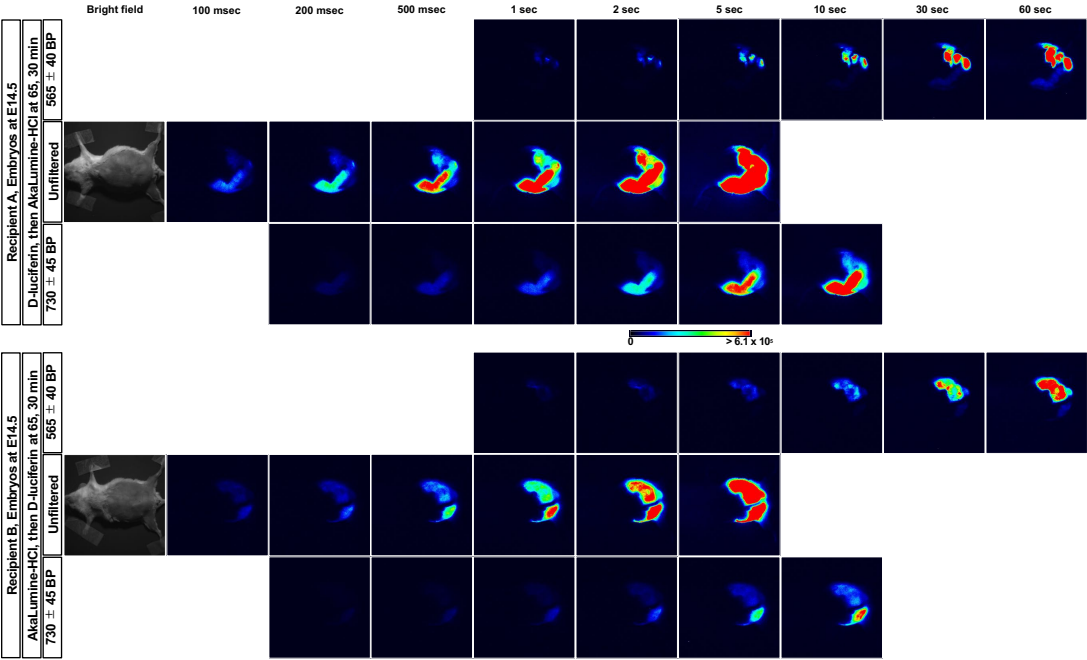


Figure S11. Filter segregation of oFluc and Akaluc after injecting the second substrate, as depicted in Figure 6.

Table S1

PCR test	Primer name	Primer sequence	CAG-LSL- oFluc	CAG-LSL- Venus/Akaluc	B6 (C57BL/6)	CAG-oFluc	CAG- Venus/Akaluc	DW (Distilled water)	TV1	TV2
1	5' external fwd	GCCACCGCCCCACACTTAT	1452 bp	1350 bp	N/A	1452 bp	1350 bp	N/A	N/A	N/A
	CAG rev	ACGTCAATGGAAAGTCCCTATTGGCGTTAC								
2	WPRE fwd	GTCCCTTCGGCCCTCAATCCAGCG	3541 bp	3472 bp	N/A	3541 bp	3472 bp	N/A	N/A	N/A
	3' external rev	GCCTATTACCGGAGAAATCCATGTCCCA								
3	Amp fwd	TTGCCGGAAGCTAGAGTAA	560 bp	N/A	N/A	N/A	N/A	N/A	560 bp	560 bp
	Amp rev	TTTGCTTCTGTGTTTGTCT								
4	5' external fwd2	GCCACCGCCCCACACTTATTGG	9916 bp	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	Plasmid backbone rev	TTGTGAGCGGATAACAATTCACACAGG								
5	Plasmid backbone fwd	CGCTTACAATTTCATTGCGCATTCAGG	10060 bp	N/A	N/A	N/A	N/A	N/A	N/A	N/A
	3' external rev	GCCTATTACCGGAGAAATCCATGTCCCA								
6	3' arm fwd	CTGCAACAAGATATGTAGACTAAAGTTCTGCC	3348 bp	N/A	N/A	N/A	N/A	N/A	3348 bp	3351 bp
	5' arm rev	GTCCGGTGGAGACTTTTCGCTCC								
7	Neo fwd	TGGATTGCACGCAGGTTCTC	618 bp	618 bp	N/A	N/A	N/A	N/A	618 bp	618 bp
	Neo rev	CGGCCACAGTCGATGAATCC								
	Control fwd	TACCCAGTAGAGTCTATAGG	1007 bp	1007 bp	1007 bp	1007 bp	1007 bp	N/A	N/A	N/A
	Control rev	AAATCCAAGTGAGGGTACGC								
8	CAG fwd	GCCTTCTCTTTTCTCAGCTCC	2629 bp	N/T	N/A	1391 bp	N/T	N/A	2629 bp	N/T
	oFluc rev	TGTCGATCAGGGCATTGGTGCC								
9	CAG fwd	GCCTTCTCTTTTCTCAGCTCC	N/T	2755 bp	N/A	N/T	1550 bp	N/A	N/T	2755 bp
	Akaluc rev	GTGCGGTGCGGTAGGGCTACGCC								