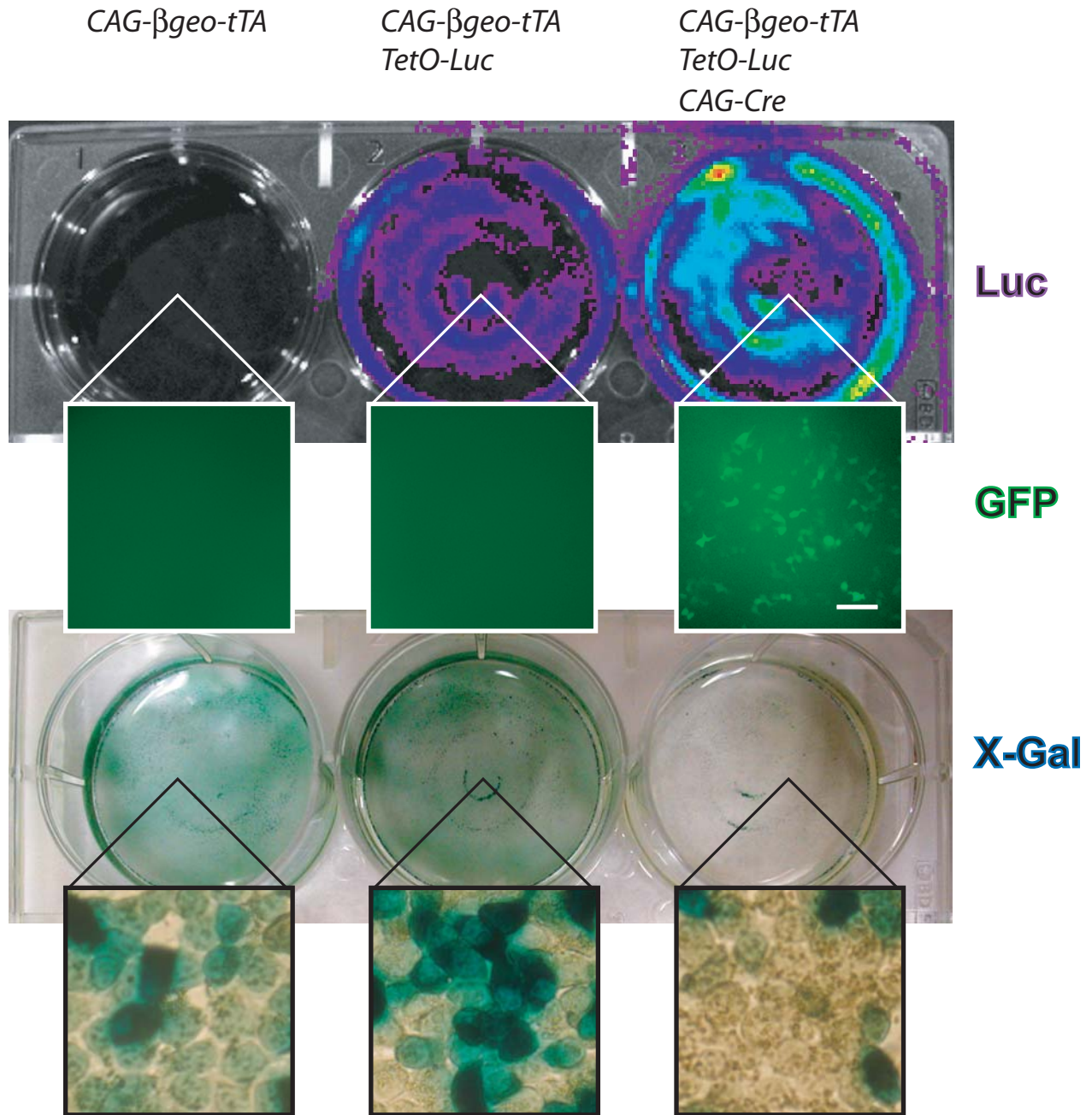


Supplemental Fig. 1

Test of functionality of the CAG- β geo-tTA transgene in 293T cells.

Bioluminescence imaging (Luc) and GFP expression analysis as well as X-gal staining of 293T cells that were transiently transfected with three plasmids (CAG- β geo-tTA, TetO-Luc, CAG-Cre) and their controls. Bar represents 100 μ m.



To test the functionality of all components of the transgene, the CAG-loxP- β geo-loxP-tTA-IRES-GFP (referred to as CAG- β geo-tTA) construct was transfected into 293T cells along with a CAG-Cre transgene as well as reporter construct that expresses the firefly luciferase under the Tet operon (TetO-Luc). GFP and luciferase were highly upregulated in cells that carried the Cre transgene in addition to the CAG- β geo-tTA and TetO-Luc constructs. Luciferase expression was detected, albeit at much lower levels, in the absence of Cre recombinase, which typically occurs in transient transfection assays using the TetO-Luc reporter even in the absence of the tTA (not shown). Moreover, the lack of GFP expression in control cultures without Cre recombinase indicated that the triple poly(A) sites preceding the transactivator prohibited the transcriptional activation of the tTA-IRES-GFP cassette. After examining the expression of GFP and luciferase in viable cells using fluorescence microscopy and bioluminescence imaging (IVIS200), the cells were fixed and processed for X-gal staining. As expected, the expression of the lacZ (β geo) was clearly diminished, which indicates a proper excision of the selectable marker by Cre recombinase.