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Supplemental Information

Germline Transgenic Methods for Tracking Cells and Testing Gene Function during Regeneration in the Axolotl

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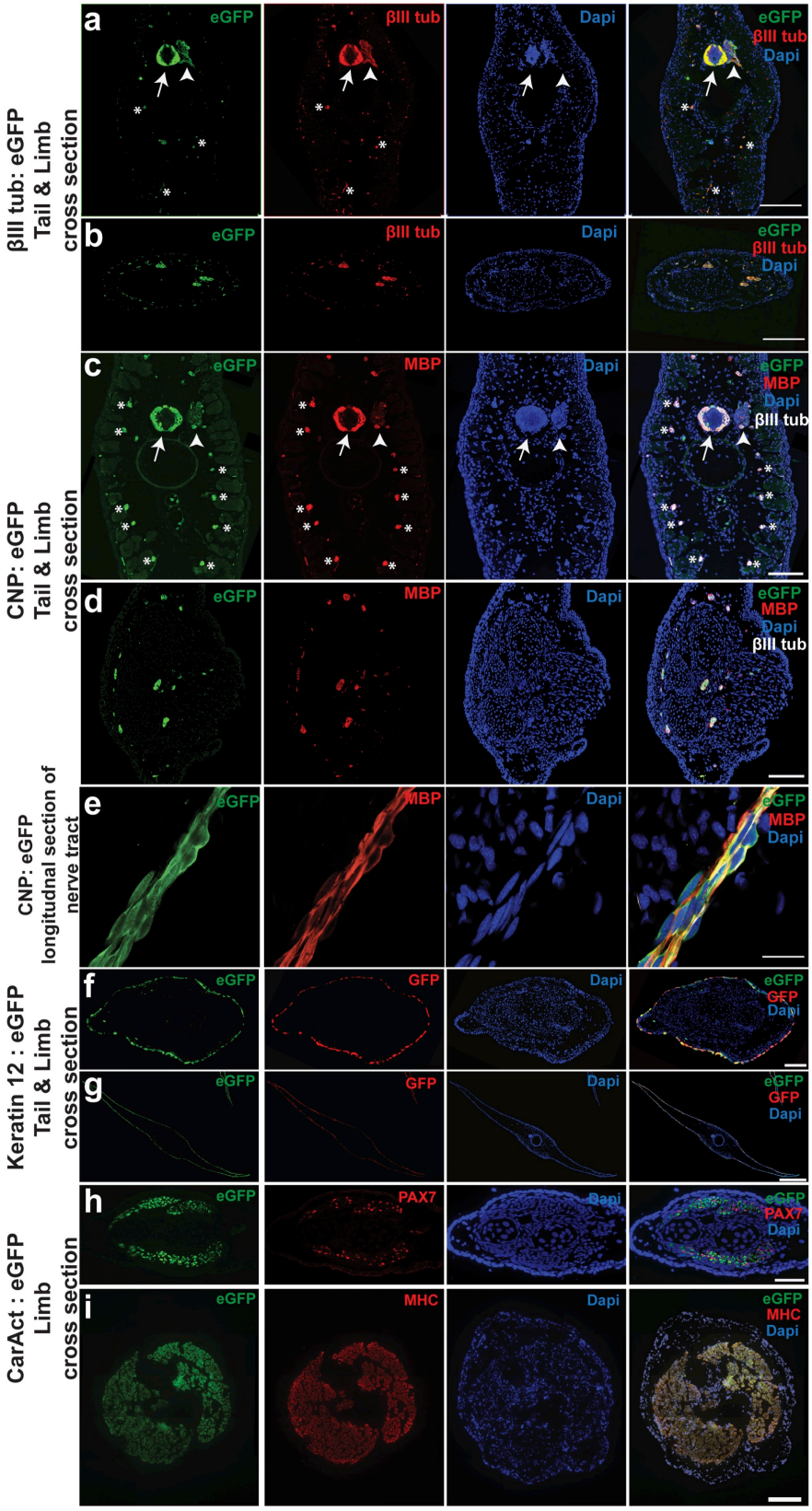

Figure S1-Khattak et al

Figure S1. Immunohistochemical confirmation of tissue-specific eGFP expression in transgenic Axolotls (Related to Figure 1).

a,b. Cross section of tail (a) and limb from F1 of a homozygous *bIIItub:GAP43-eGFP* transgenic animal. Green, eGFP; Red, bIII-tubulin; Blue, Dapi. Arrow points to the spinal cord, arrowhead point a DRG, and asterisks mark peripheral nerve.

c,d. Cross section of tail (c) and limb (d) of *CNP:eGFP* animal. Green is endogenous *eGFP* expressed from the mouse *CNP* promoter. Myelin basic protein (MBP) is in red while nuclei are stained with Dapi (in blue). Merged images are shown at the far right of the panel. Co-localization of eGFP with MBP confirms that eGFP positive cells are MBP positive. βIII tubulin immunofluorescence signal is shown in white (merged image only). Arrow points to the spinal cord, arrowhead point a DRG, and asterisks mark peripheral nerve.

e. Confocal image of longitudinal section from *CNP:eGFP* limb immunostained with MBP antibody. Green, eGFP expressed from CNP promoter; Red, MBP; Blue, Dapi

f,g. Cross section of limb (f) and tail (g) from transgenic *Keratin 12:eGFP* animal showing epidermal specific expression of the promoter. Anti-eGFP antibody was used to enhance the signal (red). Nuclei are stained with Dapi (blue) and merged image is shown at the right of the panel.

h,i. Fluorescence image of limb cross sections from transgenic animal expressing eGFP under the control of *Xenopus Cardiac Actin* promoter. Green, eGFP; Red, PAX7 (h) and MHC (i) Blue, Dapi; and merged image confirming that the *Cardiac Actin* promoter labeling is in muscle and exclusive of muscle satellite cells.

Scale Bars: a, c: 400 μm, d, h, i, f, b: 200 μm, e: 50 μm, g: 1 mm

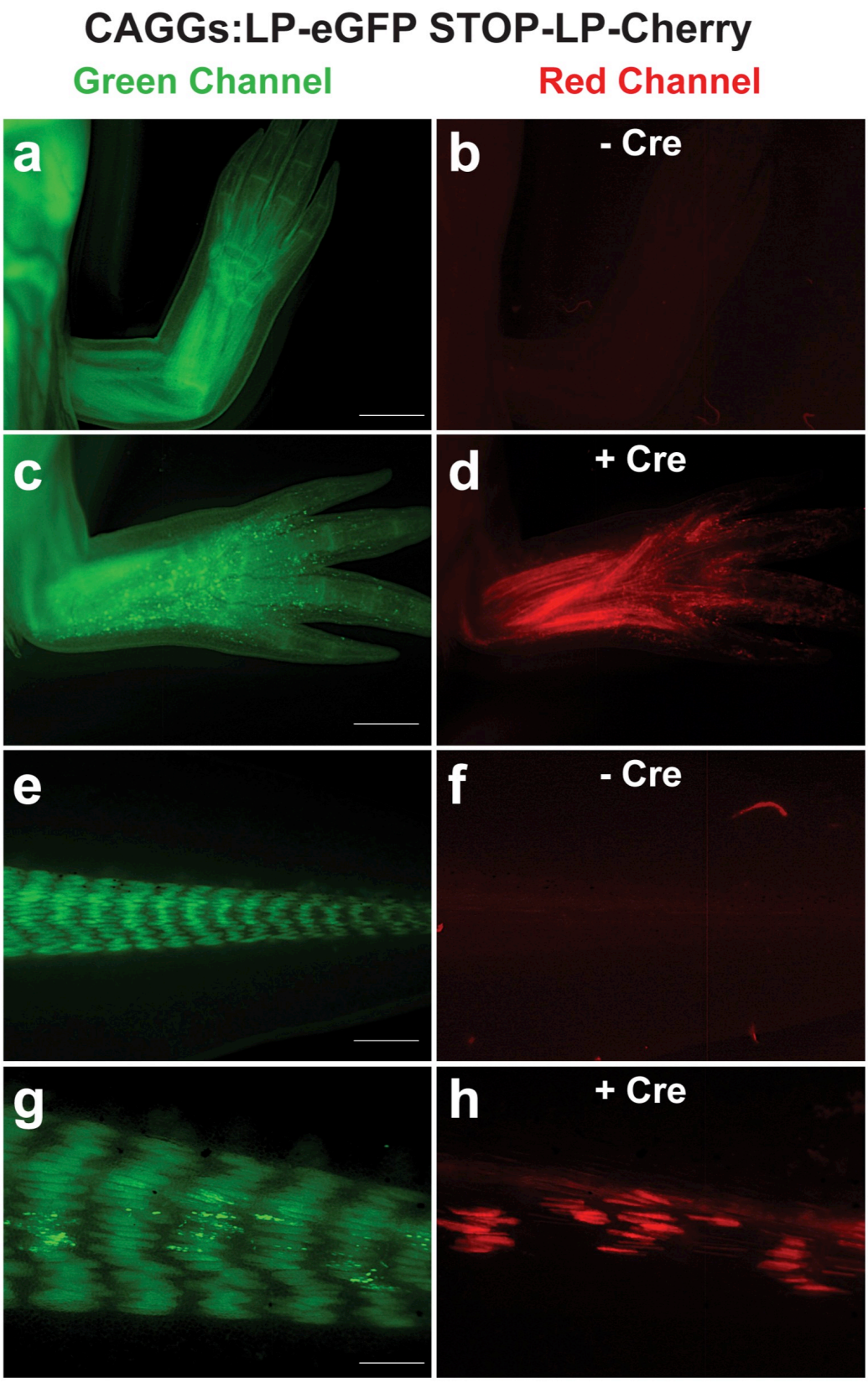

Figure S2- Khattak et al

Figure S2. Characterization of CRE in floxed Cherry reporter transgenic axolotls (Related to Figure 4)

a,b,e,f. Limb (a,b) and Tail (e,f) of floxed Cherry reporter animal (*CAGGs:loxP-eGFP-loxP-Cherry*) is shown prior to electroporation with a mixture of *Cre* and a nuclear eGFP expression constructs in respective green and red channels. c,d,g,h. Limb (c,d) and tail (g,h) after electroporation with a mixture of *Cre* and a *nuclear eGFP* expression constructs (to track electroporated cells). All strong nuclear-GFP positive (CRE-positive) cells expressed Cherry, indicating that CRE can recombine the *LoxP* sites integrated in axolotl genome.

Scale Bars: a,e: 2 mm; c,g: 1 mm

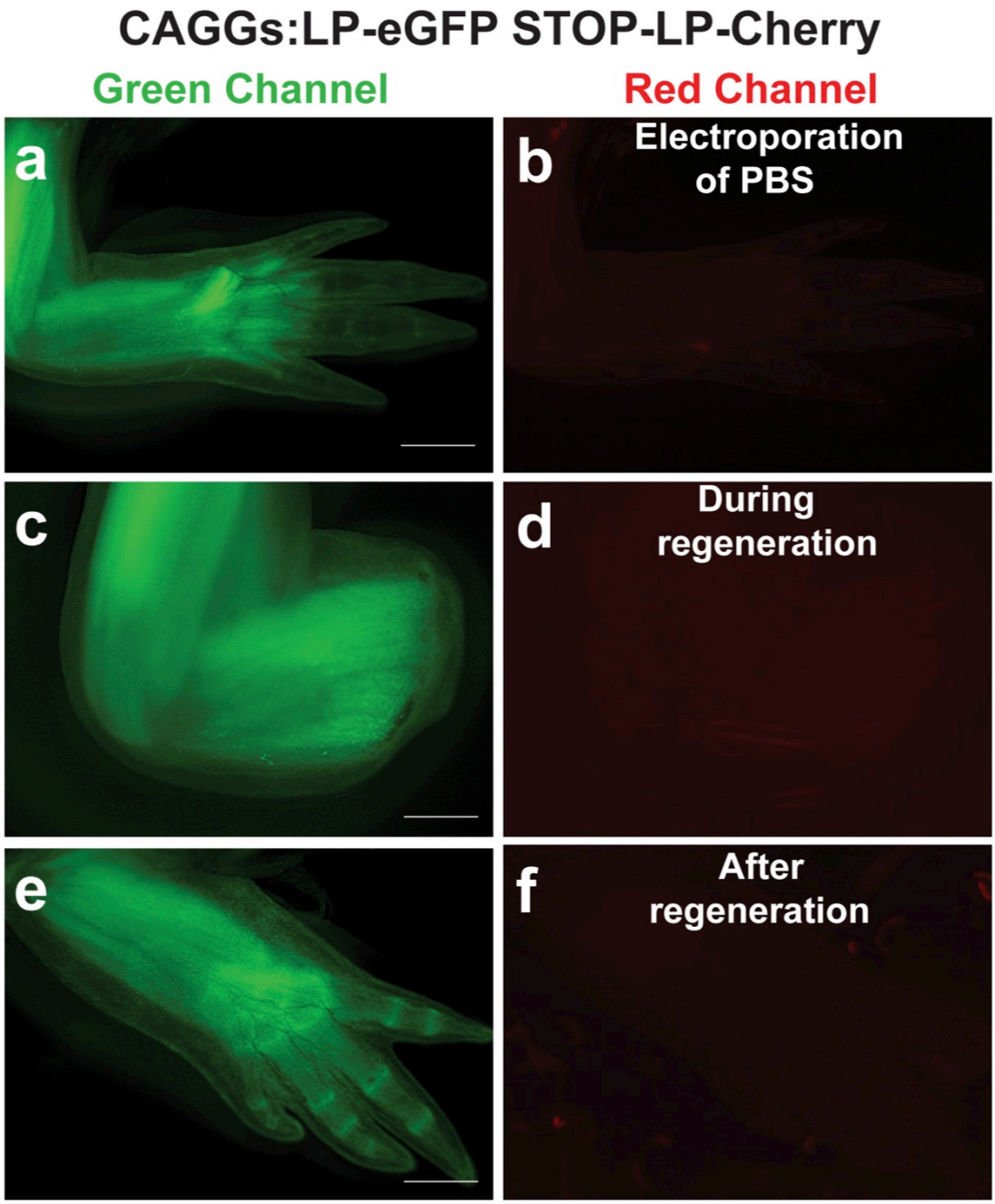

Figure S3-Khattak et al

Figure S3: No spontaneous recombination is observed with the absence of CRE in Lox P reporter line (Related to Figure 4)

a,b. Green and Red channel image of *loxP* reporter limb after electroporation of PBS and/or pUC19 plasmid.

c,d. Limb shown in (a,b) after amputation and regeneration shows no Cherry expression (d).

e,f. Limb after complete regeneration showing comparable level of eGFP expression indicating that there is no transgene silencing and no spontaneous CRE-mediated recombination during or after complete regeneration (f).

Scale Bars: a: 2mm; e: 1mm

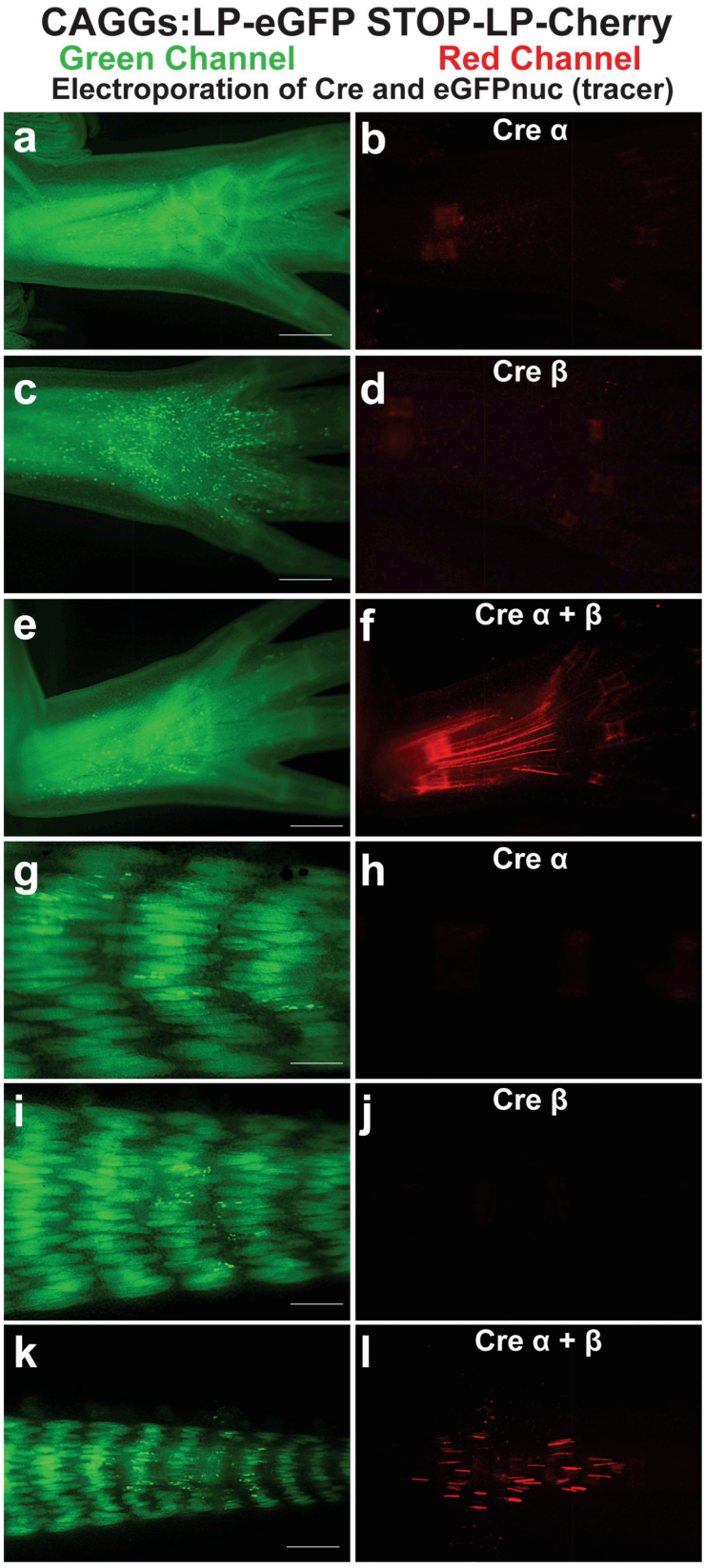

Figure S4-Khattak et al

Figure S4: Characterization of split CRE activity in floxed Cherry reporter transgenic axolotls (Related to Figure 4)

Limbs of floxed Cherry reporter animals for eGFP and Cherry expression are analyzed for split *Cre* complementation. No recombination is seen when only the N-terminus of *Cre* (CRE α, co-expressed with nuclear eGFP) is electroporated into the limb (a,b) or tail (g,h) of the floxed Cherry reporter transgenic animal. Similarly, no Cherry fluorescence is observed when the C-terminus of *Cre* (CRE β) is electroporated into limbs (c,d) or tails (i,j) of floxed Cherry animals. Robust Cherry expression is seen in limbs (e,f) and tails (k,l) after co-electroporation of both N- and C- terminus expressing plasmids of *Cre*.

Scale Bars: a-i: 1mm, k: 2mm

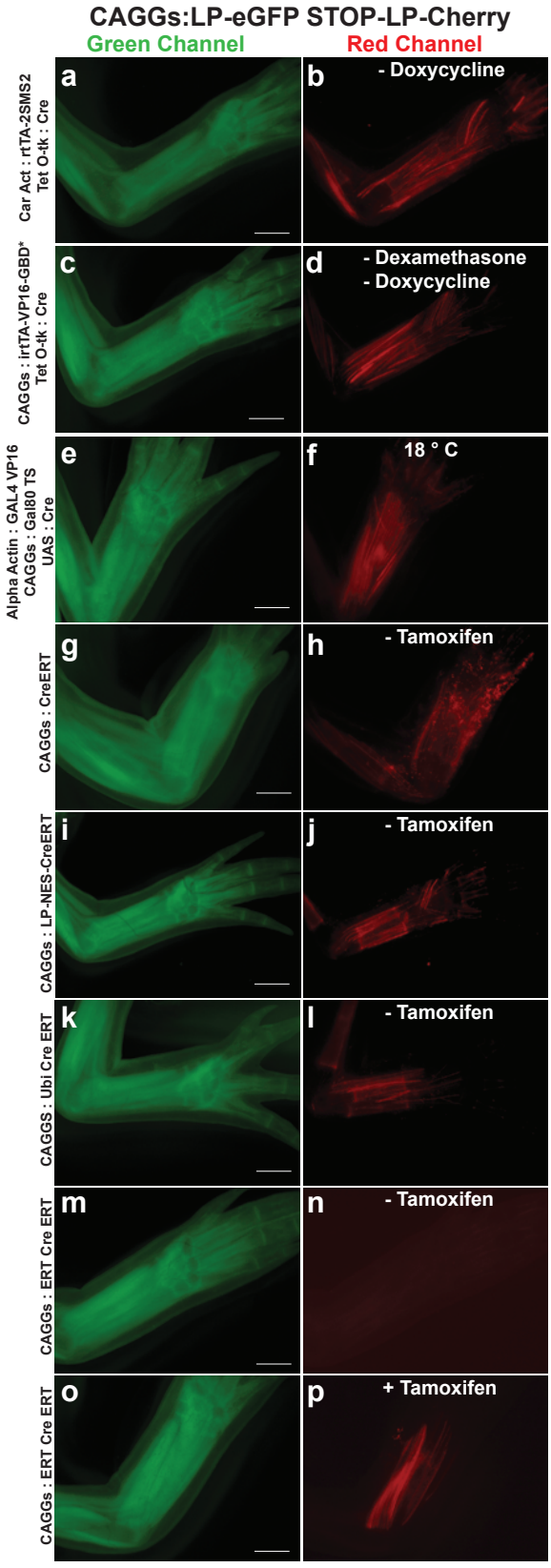

Figure S5-Khattak et al

Figure S5: Testing various inducible systems via electroporation into *loxP* reporter animal line (Related to Figure 4)

a-d. TET-On System: CarAct: rtTA-2^{s}-M2 and TetO-tk: Cre plasmids (a,b) and CAGGs: irtTA-VP16-GBD* and Tet O-tk: Cre plasmids (c,d) and were co-electroporated into the limb of *loxP* reporter animal (*CAGGs:loxP-eGFP-loxP-Cherry*). CRE-mediated recombination resulting in Cherry expression is observed without administration of the doxycycline and Dexamethasone inducer (b,d).

e,f. Temperature sensitive control of Gal4-UAS system: *Alpha Actin: Gal4VP16, CAGGs: Gal80-^{TS}* and *UAS:Cre* were electroporated into *loxP* reporter limbs. Animals were kept at 18°C (Gal4 activity blocked by Gal80^{TS}) resulted in CRE-mediated recombination and subsequent Cherry expression (f).

g-l: Various modifications of CreERT2 to check for tightly induced versus spontaneous recombination. CRE-mediated recombination without tamoxifen administration occurred resulting in Cherry expression when *CAGGs: CreER^{T2}* (g,h), *CAGGs: LoxP-NES-CreER^{T2}* (i,j) or *CAGGs: Ubi-CreER^{T2}* (k,l) were electroporated into the *LoxP* reporter limbs.

m-p. Tight control of CRE-mediated recombination using *CAGGs: ER^{T2}-Cre-ER^{T2}-T2A-eGFP-nuc*. No CRE-mediated recombination was observed prior to tamoxifen administration when *CAGGs: ER^{T2}-Cre-ER^{T2}-T2A-eGFP-nuc* was electroporated (m,n). Cherry expression was seen when the same animal was injected intraperitoneally with tamoxifen (o,p) showing that *ER^{T2}-Cre-ER^{T2}* is not leaky and CRE-mediated recombination can be induced via tamoxifen using this system.

Scale Bars: 1mm