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Supplemental Information

A Transposon-Mediated System for Flexible Control of Transgene Expression in Stem and Progenitor-Derived Lineages

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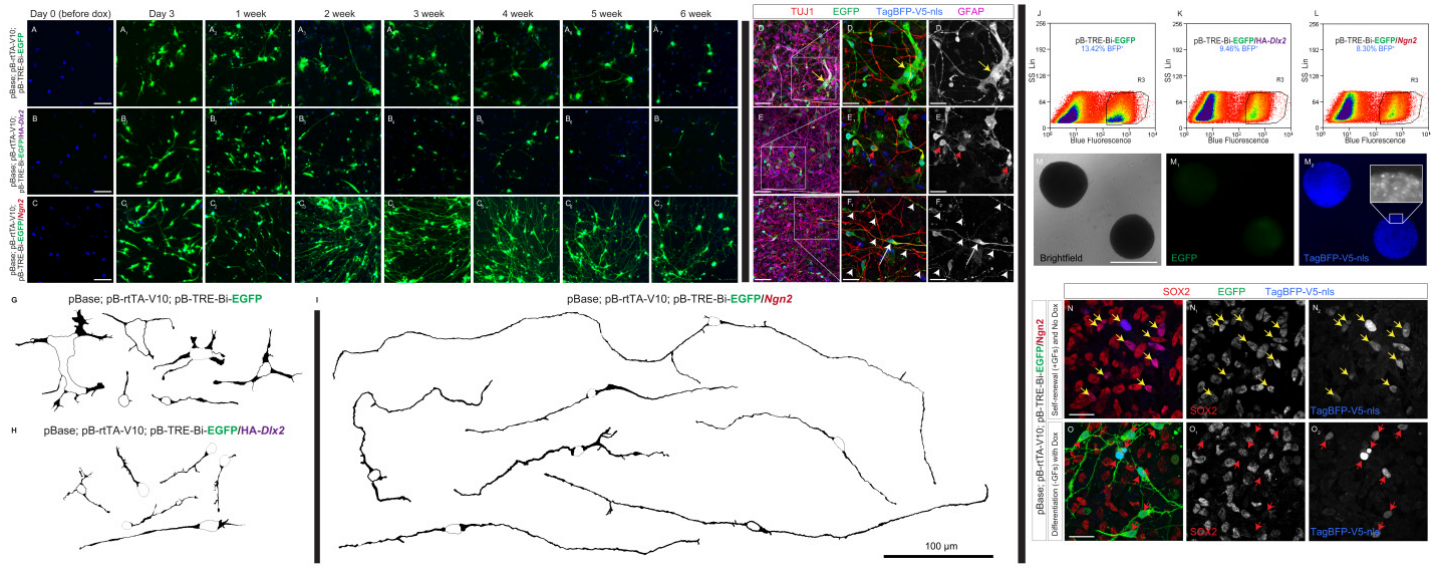

Figure S1. pB-Tet-mediated directed differentiation and utility for sorting of undifferentiated populations.

(A-C_7) Vital imaging of HuNPCs reveals morphological changes in pB-Tet differentiating cells. HuNPCs were nucleofected with pB-TRE-Bi-EGFP (ctrl), pB-TRE-Bi-EGFP/HA-*Dlx2*, or pB-TRE-Bi-EGFP/*Ngn2* and allowed to differentiate without growth factors for 6 weeks. EGFP autofluorescence was captured at denoted time points in live cells.

Scale Bar = 50µm

(D-F_2) Immunocytochemical staining reveals altered differentiation patterns across pB-TRE-Bi-EGFP (ctrl), pB-TRE-Bi-EGFP/HA-*Dlx2*, or pB-TRE-Bi-EGFP/*Ngn2*. Yellow arrow indicates $GFAP^+$ cell with polygonal astrocyte morphology (D-D_2). Red arrows (E-E_2) indicate $TUJ1^+$ cells with small, interneuron-like or migratory neuron morphology. White arrows indicate $TUJ1^+$ cells with long processes containing numerous varicosities (white arrowheads) (F-F_2).

Scale Bar (D, E, F) = 50µm

Scale Bar ($D_{1,2}$, $E_{1,2}$, $F_{1,2}$) = 25µm

(G, H, I) Camera-lucida tracing of GFP^+ cells at two weeks indicating the presence of mainly morphological astrocyte-like cells in the pB-TRE-Bi-EGFP (ctrl) group with few morphological neuronal-like cells. Traced GFP^+ cells revealed morphologically small, potentially migratory interneuron-like cells in the pB-TRE-Bi-EGFP/HA-*Dlx2* group and large projection neuron-like cells in the pB-TRE-Bi-EGFP/*Ngn2* group.

Scale Bar = 100µm

(J, K, L) FACS graphs indicating BFP^+ populations in cells nucleofected with pB-TRE-Bi-EGFP (ctrl), pB-TRE-Bi-EGFP/HA-*Dlx2*, or pB-TRE-Bi-EGFP/*Ngn2*

(M-M_2) Sorted cells expanding as relatively pure populations of nucleospheres that are GFP^-, BFP^+. (pB-TRE-Bi-EGFP/*Ngn2* shown)

Scale Bar = 500µm

(N-O_2) Nucleofected cells remain $SOX2^+$ in the absence of dox, and lose SOX2 expression after dox-induced differentiation. (pB-TRE-Bi-EGFP/*Ngn2* shown)

Scale Bar = 50µm

Figure S1 is linked to main Figure 1

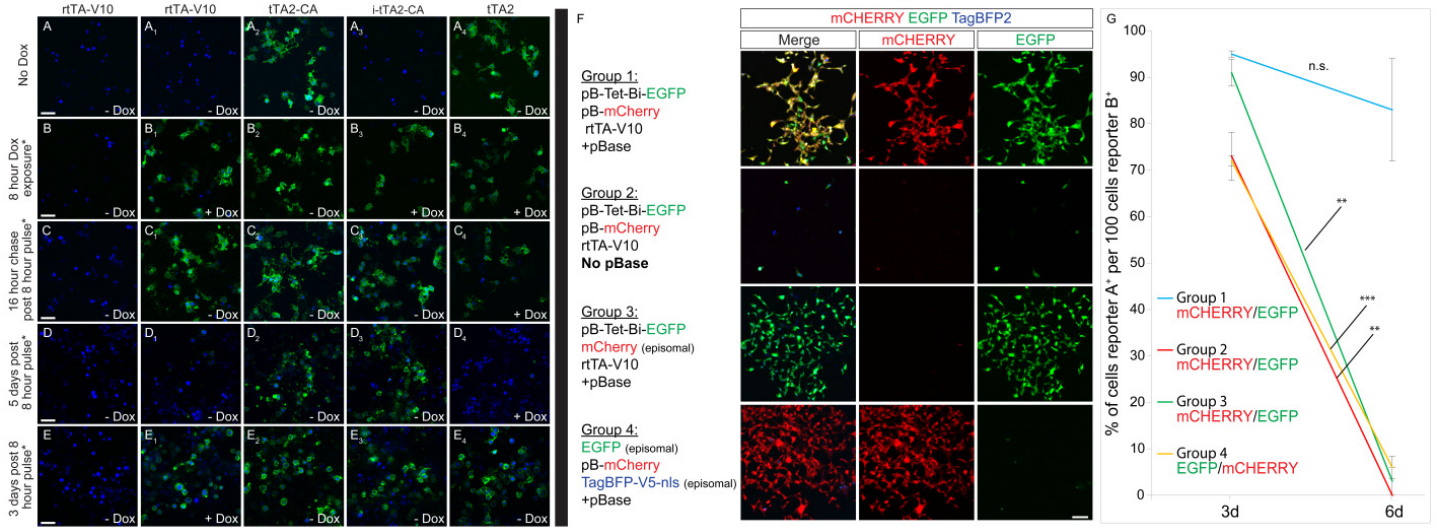

Figure S2. Transactivator variants allow for flexible transgene control

(A-E) Live images of N2a cells transfected with transactivator variants before luciferase cell lysis. Note: no dox was added to cells in columns A-E, and A_2-E_2. Cells in A_3-E_3 received a single pulse of dox immediately after imaging A_3 and before B_3 to activate rtTA-V10 and induce subsequent i-tTA2-CA constitutive activity.

Scale bar = 50µm

(F) Vital confocal imaging of nucleofected neural stem cells at day 6.

(G) Graph of fluorescent protein co-expression at 3 and 6 days post-nucleofection. Percentages are presented as the ratio of cells expressing each fluorescent protein as delineated in the graph legend (e.g. Group 1 is the percentage of mCHERRY cells that are EGFP positive).

Error bars represent mean ± SEM. **p<0.01, ***p<0.001. n = 3 biological replicates per condition per time point.

Scale bar = 100µm

Figure S2 is linked to main Figure 2

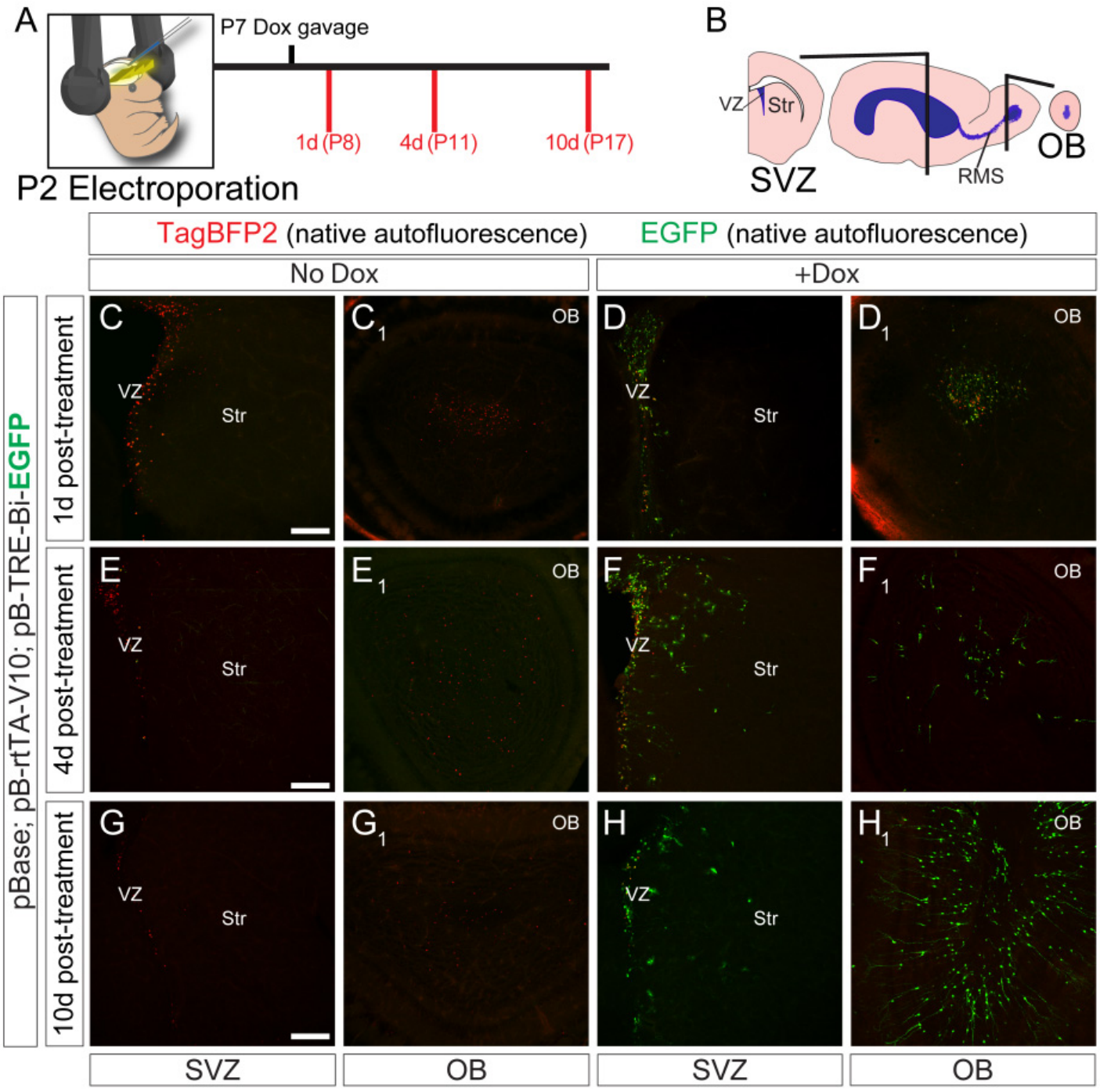

Figure S3. *pB-Tet-GOI* is non-leaky, inducible and reversible in the mouse brain (full panel set related to Figure 3)

(**A**) Experimental timeline for postnatal electroporation and dox activation. (**B**) Brain regions analyzed in **C-H$_1$**. (**C-H$_1$**) Full set of fluorescence images for main **Figure 3**. Minimal EGFP expression is observed in littermates without dox. Examination of the brains of mice electroporated with pBase, pB-rtTA-V10, and pB-TRE-EGFP reveals detectable EGFP expression at 1 day. Littermates used to decrease experimental variation across litters. Results are consistent with >3 independent experiments investigating dox activation kinetics in separate litters.

Scale bar = 200µm

Figure S3 is linked to main Figure 3

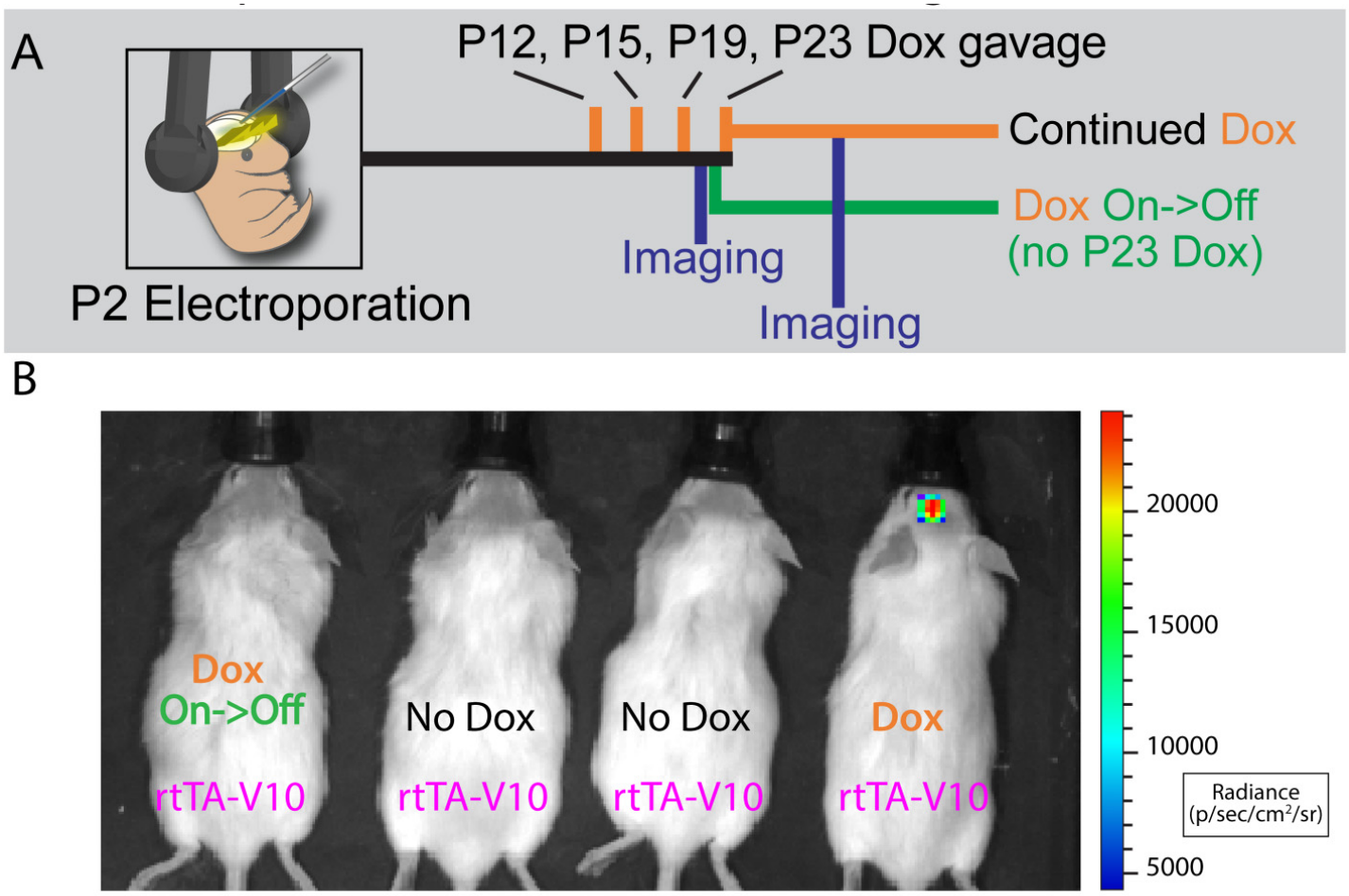

Figure S4. Reversibility of non-invasive bioluminescence imaging of pB-Tet-GOI variants

(**A**) Timeline of experiments for littermates depicted in main **Figure 4A** and **Supp. Figure 4C.**

(**B**) Dox administration was continued (far right) or discontinued (far left) in littermates from Figure 4a.

Figure S4 is linked to main Figure 4

Supplemental Table 1 - Antibodies used.

Immunostaining				
Manufacturer	**Catalog Number**	**Host Species**	**Antigen**	**Concentration Used**
Cell Signaling	C29F4	Rabbit	HA	1:1000
Covance	MMS-101R	Mouse	HA	1:1000
Santa Cruz	SC-19233	Goat	NGN-2	1:100
Sigma	T8660	Mouse	β-tubulin III	1:2000
Dako	Z0334	Rabbit	GFAP	1:500
Abcam	13970	Chicken	EGFP	1:5000
Abcam	95038	Goat	V5	1:1000
Invitrogen	46-0705	Mouse	V5	1:1000
Western Blot				
Manufacturer	**Catalog Number**	**Host Species**	**Antigen**	**Concentration Used**
Covance	MMS-101R	Mouse	HA	1:1000
Santa Cruz	SC-19233	Goat	NGN-2	1:1000
Abcam	13970	Chicken	EGFP	1:10000
Abcam	95038	Goat	V5	1:5000
Invitrogen	46-0705	Mouse	V5	1:5000

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Cloning

Plasmids were generated using In-fusion cloning (Clontech) along with standard molecular biology techniques to incorporate relevant cDNAs into pCAGGS (episomal) or pZGs (piggyBac)-based vectors. Details are available upon request.

Tissue Preparation

After anesthesia, mouse brains were isolated and immersion fixed in 4% ice-cold paraformaldehyde (PFA) overnight. Brains were then embedded in low melting point 4% agarose and sectioned at 70 µm on a vibratome.

Subventricular Zone and Olfactory Bulb Imaging of Native TagBFP2 and EGFP Autofluorescence

Unstained 70 µm SVZ and OB sections were mounted on glass slides and coverslipped. Confocal images were collected using a Nikon A1R inverted laser scanning confocal microscope. Immunohistochemical amplification of BFP and EGFP signals were omitted to preclude the artifactual, non-linear amplification and subsequent normalization of signal intensity observed following antibody staining. The number of cells in the olfactory bulb core was counted using a fixed-size grid centered on the terminus of the RMS. ImageJ was employed to count fluorescent cells and signaling intensities in an automated, unbiased fashion by employing the "Analyze Particles" function after calibration to ensure each cell was counted as a single particle.

Imaging

All confocal images were collected on a Nikon A1R inverted laser scanning confocal microscope with appropriate settings to sequentially image colors and avoid signal crosstalk. The exposure and saturation measures were utilized to capture the maximum dynamic range. Typically, after the exposure was set, the identical setting was reused for the subsequent samples in the group. Live images of HuNPCs were obtained on an EVOS digital fluorescence inverted microscope.

Image Processing

ND2 image files were initially imported into ImageJ for manipulation of confocal Z-stacks or for isolation of individual channels from single z-slices for subsequent editing in Adobe Photoshop CS6. Image curves were adjusted for

consistency of dynamic range and exposure in Photoshop CS6, cropped, and then imported into Adobe Illustrator CS6 for the preparation of final images.

In vitro Phenotypic Quantification

Cell phenotype percentages ($TUJ1^+$ and $GFAP^+$ of all GFP^+ and BFP^+ cells) were quantified in an unbiased manner by an observer blind to the treatment groups. $TUJ1^+$ and $GFAP^+$ quantification was done in triplicates (n= 3 coverslips per condition per timepoint); 100 GFP^+ cells per coverslip, performed in triplicate.

Nucleofection

Human Neural progenitor cell nucleofection was performed using the Amaxa Nucleofector 2b device according to manufacturer's recommendations (Lonza AG). Unless otherwise noted, plasmids were added to the Lonza Nucleofection Solution in the following amounts (per reaction): Response plasmid(s): 7µg, pBase: 1µg, rtTA-V10: 3µg. For transposition experiment (Figure S2, F-G), plasmids were added to their respective groups in the following amounts (per reaction): pBase: 1µg, rtTA-V10: 3µg, all other plasmids: 7µg.

Immunostaining of HuNPCs

Immunocytochemistry was performed as previously described (Breunig et al., 2007b). Briefly, a primary antibody mixture was made in PBS-Triton (PBS, 0.3 % triton) with 3% normal donkey serum (NDS) and the desired primary antibodies at the ratios indicated in Supp. Table 1. Coverslips were incubated with the primary antibody mixture for at least 12 hours at 4°C, followed by three 5 min washes with PBS at room temperature. Secondary antibody mixtures were made with PBS-T and the appropriate secondary antibodies at a 1:1000 dilution (Jackson Immunoresearch; conjugated with Alexa 405, Fitc, Alexa488, Dylight488, Alexa555, Dylight549, Alexa647, or Dylight649).). The secondary antibody mixture was added to the coverslips and incubated at room temperature for 1 hour on a shaker. Coverslips were then washed in PBS and mounted on slides with anti-fade mounting gel medium (Invitrogen ProLong).

Western Blot

Mouse N2a cells were transfected with Lipofectamine 2000 (Invitrogen 11668019), following the manufacturer's protocol. After transfection, the cells were grown for three days at 37°C. Cells were harvested by accutase incubation for 3 min at 37°C, followed by re-suspension in equal amount media, and centrifugation for 3 min at 3000 rpm. The resulting pellet was re-suspended in laemmli buffer and boiled for 15 min at 95°C. Protein concentrations were measured using a ThermoScientific Nano Drop. Protein separation was performed using SDS-PAGE separation and transfered onto nitrocellulose membranes, which were incubated overnight at 4°C using primary antibodies listed in Supp. Table 1 diluted in 5% milk in 0.1% PBS-Tween. All secondary antibodies (Li-cor IRDye®) were used at a 1:15000 dilution. Infrared detection was accomplished by the Li-Cor Odyssey® CLX Imaging System.

FACS

Once harvested cells reached approximately 80% confluency, media was removed and T75 cm^2 flasks were passaged with 2 mL of accutase at 37°C in 5% CO2 for 3 minutes. Accutase was neutralized with 2mL of media and cells were centrifuged at 1350 rpm for 3 minutes. Supernatant was removed and cells were resuspended in 2 mL of fresh media, using a P1000 to disrupt the cells in a careful yet vigorous manner. In increments of approximately 200 µL, cells were filtered through a 70 µm filter (BD Falcon). This filtrate was then placed on ice. Cells were then sorted at the Cedars-Sinai Flow Cytometry Core using a Beckman Coulter MoFlo sorter for blue fluorescence with tight gates making sure only the cells highly expressing BFP were collected, indicative of TagBFP-V5 expression from the response plasmid. Cells were collected into fresh media and kept cold on ice. Once all cells were sorted, the collected cells were pelleted, washed and placed into new T25 cm^2 flasks.

IVIS Live Imaging

Bioluminescence imaging of animals was performed at the Cedars-Sinai Medical Center Imaging Core Facility using the Xenogen Spectrum In Vivo Imaging System (IVIS). Animals were subcutaneously injected with 150mg/kg luciferin (VivoGlo™, Promega 1043). Following a 10 minute waiting period to
allow for circulation of substrate, animals were imaged in the Xenogen IVIS while under isoflurane anesthesia. Luciferase expression was analyzed and quantified by substracting the background noise (e.g. signal hindlimb regions distal from CNS) from each respective animal.

Dual-Luciferase Reporter Assay

Mouse N2a cells were transfected with Lipofectamine 2000 (Invitrogen 11668019), following the manufacturers protocol. Cells were grown in a 24-well plate as a monolayer in quadruplets (n=4 per condition) and imaged at respective time points followed by lysis and dual-luciferase activity assessment using the Promega Dual-Luciferase® Assay E910 according to the manufacturer's protocol. Briefly, cells were passively lysed by adding 100ul of Promega® PLB buffer and shaking at room temperature for 15 minutes. 20ul of lysate and 50ul of Promega® LARII substrate was added to a 96-well assay plate. Firefly luciferase activity was measured using the Wallac Envision Manager Software with a 10 second delay per well. After firefly luciferase activity was measured, 50ul of Promega Stop & Glo® was added and *Renilla* activity was measured.

Statistical Analysis

Statistical analyses were carried out using Excel (Microsoft). We compared groups using two sample t tests. For cell counts, at least 100 cells per biological replicate were counted in all cases with the exception of Group 2 in Supplemental Figure S2, where 99 cells were counted.