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Imaging of Tissue-specific and Temporal Activation of GPCR Signaling using DREADD Knock-In Mice

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Abstract

Engineered G-protein coupled receptors (DREADDs, designer receptors exclusively activated by designer drugs) are convenient tools for specific activation of GPCR signaling in many cell types. DREADDs have been utilized as research tools to study numerous cellular and physiologic processes, including regulation of neuronal activity, behavior, and metabolism. Mice with random insertion transgenes and adeno-associated viruses have been widely used to express DREADDs in individual cell types. We recently created and characterized *ROSA26-GsDREADD* knock-in mice to allow Cre recombinase-dependent expression of a G_s-coupled DREADD (GsD) fused to GFP in distinct cell populations *in vivo*. These animals also harbor a CREB-activated luciferase reporter gene for analysis of CREB activity by *in vivo* imaging, *ex vivo* imaging or biochemical reporter assays. In this article we provide detailed methods for breeding GsD animals, inducing GsD expression, stimulating GsD activity and measuring basal and stimulated CREB reporter bioluminescence in tissues *in vivo*, *ex vivo* and *in vitro*. These animals are available from our laboratory for non-profit research.

Keywords

Gs-DREADD; GPCR; cAMP; CREB luciferase reporter; *in vivo* bioluminescence imaging; clozapine N-oxide

1. Introduction

G-protein coupled receptors (GPCRs) are the largest class of receptors in mammals and regulate multiple physiologic processes [1,2]. Dysregulated GPCR signaling underlies many diseases including cancer, diabetes and fertility disorders [3–5]. Endogenous GPCRs are commonly expressed in numerous cell types, so results of pharmacologic experiments *in*

vivo using ligands for endogenous GPCRs can be difficult to interpret due to activation of signaling in numerous cell types. In addition, many GPCRs activate more than one intracellular signaling pathway [6–8]. To enable mechanistic and physiological studies of GPCR-activated G-protein signaling, DREADD (designer receptors exclusively activated by designer drugs) technology was developed as a chemical-genetic tool [9–13].

DREADDs (also originally termed RASSLs, receptors activated solely by synthetic ligands [9]) are derived from M3- or M4-muscarinic receptors and have point mutations in the transmembrane domains rendering the receptors unresponsive to the endogenous ligand acetylcholine and yet highly responsive to synthetic drug clozapine *N*-oxide (CNO) [10–13]. DREADD receptors coupled to each class of G-proteins G α s, G α q, G α i have been developed [14]. For *in vivo* applications, viral DREADD expression vectors or transgenic animals expressing DREADDs are commonly used [15–18]. In DREADD-expressing mice, activation of DREADD receptor signaling is achieved by administering CNO by injections or in drinking water.

Two *ROSA26*-based knock-in mice have been generated to accomplish Cre recombinase-dependent expression of G α q or G α i [19,20]. We recently reported generation of a third *ROSA26*-based knock-in mouse line expressing G α s-coupled DREADD fused to GFP in a Cre-dependent manner for tissue-specific activation of cAMP signaling (Fig 1A). Without Cre recombinase expression, the GsD gene is silenced due to an intervening lox-stop-lox (LSL) sequence between the promoter and the GsD cDNA [21]. The GsD receptor can be expressed in any tissue or cell type for which a tissue-specific Cre driver or suitable viral vector is available.

The knock-in allele in the *ROSA26-GsD* mice also encodes luciferase under control of a minimal promoter harboring four full cAMP-response elements (CRE) [21], which are directly bound by CREB [24], to monitor CREB activity by *in vivo* or *ex vivo* bioluminescence assays. The reporter is encoded on the opposite strand, does not require Cre recombinase activity to be expressed, and is sensitive to increased CREB activity due to GsD activation by CNO as well as by endogenous cAMP signaling. The vast diversity of transgenic Cre drivers and viral vectors encoding Cre and widely available instrumentation for bioluminescence reporter imaging should enable investigators to easily utilize these mice to manipulate and monitor cAMP signaling in select cell types *in vivo*.

Our laboratory has utilized this mouse line to drive cAMP pathway activity selectively in mouse hepatocytes and skeletal muscle using AAV-Cre vectors as well as a genetic skeletal muscle-specific Cre driver ([21] and unpublished). In this article, we detail the methods to breed and utilize *ROSA26-GsD* animals for stimulation of G α s signaling *in vivo* and bioluminescence reporter assays to assess resultant CREB activity. We also discuss consideration of control groups and highlight methods to confirm appropriate GsD expression and activity.

2. Materials

2.1. Induction of GsD receptor expression *in vivo*

1. Male or female heterozygous *ROSA26^{GsD/+}* mice (see Note 1), aged 6-14 weeks, housed at 22°C in individually ventilated cages with free access to food and water with a 12h light/dark cycle (7 a.m./7 p.m.) in a facility approved by the local Institutional Animal Care and Use Committee (IACUC). We recommend maintaining the line on the albino B6 (*B6(Cg)-Tyr^{c-2J/J}*) background (Jackson Laboratories, stock #000058) for optimum imaging sensitivity (see Note 2).
2. Mouse chow diet.
3. Adeno-associated viruses (AAV), adenoviruses (AdV) or transgenic mice expressing Cre recombinase.
4. Tamoxifen if using a vector or mouse line encoding Cre-ER. Make a 100 mg/ml solution in pure ethanol. To facilitate TMX solubilization heat the tube at 37°C for 2-3 min. Store this solution at -20°C and use within two weeks.
5. Syringes and needles. Small gauge needles (25-30G) are suitable for luciferin injection. Larger gauge needles (20-22G) are more appropriate for tamoxifen (see Note 3).

2.2. Detection of GsD receptor expression

1. Modified RIPA-T buffer: 20 mM HEPES (pH 7.5), 137 mM NaCl, 1% Triton X-100, 2% SDS, 0.5% Na-DOC, 0.5 mM EDTA, 5 mM Na₄P₂O₇, 20 mM β-glycerophosphate, 50 mM NaF. Store buffer at 4°C. Before use add 1 mM Na₃VO₄, protease inhibitors and 10 μM MG-132.
2. Polyacrylamide gel electrophoresis and Western blotting equipment
3. PVDF membrane, 45 μm pore size
4. 1x Tris-buffered saline (TBS): 10 mM Tris-HCl (pH 7.6), 100 mM NaCl
5. 1x Tris-buffered saline containing 0.1% Tween 20 (TBS-T)
6. Bovine serum albumin (Fraction V): make 3% (w/v) solution in TBS-T
7. Nonfat dry milk: make 5% (w/v) solution in TBS-T
8. Anti-GFP antibody (Rockland Immunochemical)
9. Donkey anti-goat Alexa Fluor 488

¹We recommend using heterozygote *ROSA26^{GsD/+}* mice for all experiments. In *ROSA26^{GsD/GsD}* homozygote mice, the GsD receptor will be expressed at two-fold higher levels than in heterozygotes, with corresponding increases in cAMP signaling. For generating experimental cohorts, we routinely cross homozygous *ROSA26^{GsD/GsD}* with WT or transgenic Cre mice.

²Hair attenuates bioluminescent signals. This is especially problematic in black mice. We recommend shaving mice with hair clippers to expose bare skin before imaging. This is not critical for very strong luminescent signals in albino mice, such as in liver-expressed GsD mice, but can be critical for imaging black mice especially for tissues that produce low intensity signals.

³TMX solutions are not stable, so it is preferable to prepare only small amounts of solution sufficient for injections in the current animal cohort and keeping the remaining TMX powder at -20°C. For injections we recommend using large gauge needles, 20-22G, because of the high viscosity of oil.

10. Donkey anti-goat HRP

2.3. Stimulation and monitoring of GsD activity *in vivo* and *ex vivo*

1. Dimethyl sulfoxide (DMSO)
2. 10 mM clozapine *N*-oxide (CNO) stock solution: approximately 0.2 ml of dimethyl sulfoxide (DMSO) per 25 mg CNO. Bring to the required final volume with 0.9% saline (final concentration of DMSO is 2.5%). Store stock solution at -20°C in 0.5 ml aliquots for up to four months.
3. $1\times$ phosphate-buffered saline (PBS)
4. 120 mg/ml D-luciferin potassium salt stock solution: reconstitute D-luciferin powder in sterile saline or $1\times$ PBS to make a colloid. Mix well and make 0.2 ml aliquots, protect from light and store at -20°C .
5. IVIS Lumina XR instrument (PerkinElmer) or similar (see Note 4) connected to isoflurane vaporizer with anesthesia chamber and equipped with 3 or 5 position nose cone manifold to maintain anesthesia during imaging.
6. Living Image software (PerkinElmer)
7. pSer133 CREB antibody (87G3) (Cell Signaling clone 87G3)
8. Total CREB antibody (48H2) (Cell Signaling clone 48H2)
9. Direct cAMP ELISA kit (Enzo Life Sciences)

2.4. *Ex vivo* bioluminescence CREB reporter imaging of mouse tissues

1. NKH477 adenylyl cyclase activator
2. 3-isobutylmethylxanthine (IBMX)
3. 120 mg/ml D-luciferin stock solution.
4. IVIS Lumina XR instrument
5. Living Image software

3. Methods

3.1. Induction of GsD receptor expression *in vivo*

The GsD receptor can be expressed in any tissue or cell type for which a tissue-specific Cre driver or suitable viral vector is available.

⁴*In vivo* imaging systems are also available from other manufacturers including Li-Cor (Pearl® Trilogy Imager) and Berthold Technologies (NightOWL II In Vivo Imaging System). Some instruments such as IVIS Lumina XR also allows taking X-ray images. For *ex vivo* imaging of small tissue slices or cells, more simple and less expensive systems, such as the Olympus LV200, can be used.

3.1.1. AAV-Cre vectors

1. For liver-specific GsD expression inject intravenously recombinant AAV2/8-TBG-Cre (AAV8.TBG.PI.Cre.rBGH) or AAV2/8-TBG-GFP (AAV8.TBG.PI.eGFP.WPRE.bGH) using 2×10^{11} viral genomes (v.g.)/mouse.
2. Detect receptor expression and activity (see Sections 3.2 and 3.3). Typically, this can be detected as few as seven days after AAV injection.

3.1.2. Activation of Cre-ER recombinase by tamoxifen

1. Dilute tamoxifen stock solution (see Section 2.1, item 4) in corn oil to 15 mg/ml.
2. Inject in mice subcutaneously or intraperitoneally at 75 mg/kg animal weight (see Note 3).

3.2. Detection of GsD receptor expression

We recommend testing GsD receptor expression by native GFP fluorescence, immunofluorescence using anti-GFP antibody (see Section 2.2, items 8,9 and Fig 1B) or Western blotting with an anti-GFP antibody (see Note 5).

1. For Western blotting, lyse tissues in modified RIPA-T buffer containing 2% SDS (see Section 2.2, item 1).
2. Resolve proteins on 7.5% polyacrylamide gels and transfer to PVDF membranes.
3. Block membranes by incubating in 5% milk in TBS-T for 30 min.
4. Incubate membranes with primary GFP antibody (diluted 1:2000 in 3% BSA in TBS-T) overnight at 4°C.
5. Remove primary antibody and wash blots in TBS-T, 3×15 min
6. Incubate with secondary antibody (donkey anti-goat HRP, diluted 1:4000 in 5% milk in TBS-T) for 45 min.
7. Remove secondary antibody and wash blots in TBS-T, 3×15 min
8. Expose membranes to film and develop film

3.3. Stimulation and monitoring of GsD activity *in vivo* and *ex vivo*

The DREADD system is a chemical-genetic approach that theoretically requires both receptor expression and ligand delivery to initiate signaling to downstream effector pathways (see Note 6).

⁵In most cases, native GFP fluorescence is difficult to observe in cells expressing GsD-GFP (unpublished observation). We recommend performing immunofluorescence staining using a GFP antibody. This is especially convenient for detection of GsD expressed in specific cell types, especially small cell populations, for which detection by Western blotting of whole tissue lysate is not appropriate. We successfully used immunolabeling to detect GsD expressed in skeletal muscle fibers (Fig 1B).

⁶The Gs-DREADD construct we employed has detectable basal signaling activity [21], though less than that of previous versions [22]. We observed elevated basal CREB activity in mice expressing GsD in liver, without CNO stimulation, as evidenced by increased CREB reporter bioluminescence and increased expression of CREB target genes (Fig 2A, B in ref [21]). Therefore, we strongly recommend incorporation of a GsD + vehicle (no CNO) control group in the experimental design.

1. Administer CNO 2-6 hr before imaging to allow for luciferase cDNA transcription and translation (see Note 7). Strong bioluminescence signal is typically detected 6 hr after CNO injection in mice expressing GsD in liver [21] and 4 hr after CNO injection in mice expressing GsD in skeletal muscle fibers (Fig 2).
2. Proceed with imaging (see Section 3.3.1) or biochemical analysis of GsD signaling (see Section 3.3., items 3 and 4).
3. Lyse tissues and proceed with Western blot (see Section 3.2) using the following antibody dilutions: phospho-CREB (Ser133) 1:3000 in 3% BSA in TBS-T, total CREB 1:4000 in 3% BSA in TBS-T.
4. Determine cAMP concentration using cAMP kit (see Section 2.3, item 9). Follow the acetylation protocol provided by the manufacturer.

3.3.1. *In vivo* bioluminescence CREB reporter imaging

1. Turn on the IVIS Lumina XR instrument. Check oxygen supply and isoflurane level in the vaporizer.
2. Open Living Image software and click 'Initialize' in the 'IVIS Acquisition Control Panel'. Wait until the temperature indicator turns from red to green, which indicates that the heated stage reached 37°C.
3. Adjust image acquisition settings: select 'Luminescent' and 'Photograph' imaging modes, which will instruct the software to first take a photograph and then a luminescent image, and to overlay pseudocolored luminescence image onto the photograph.

'Binning' has low, medium or high settings. Binning increases signal-to-noise ratio and high binning helps in detection of low intensity signals but does so at the expense of reduced resolution. We use 'medium' binning (binning factor 4).

'F/stop' adjusts the amount of light accessible to camera. Small aperture F/16 is usually used in 'Photograph' mode, while large aperture F/1 is used in 'Luminescent' mode to maximize sensitivity.

'Field of View' adjusts the area captured by the camera and has four positions, A through D. Use position D to image whole mouse or multiple mice. Use other positions to capture smaller areas, such as part of mouse body.

'Exposure time' should be adjusted according to the intensity of the signal. In our experience, liver bioluminescence induced by GsD-CNO requires only a

⁷CNO dose and delivery method require optimization for the tissue targeted and physiology under study. We have successfully used 1 mg/kg CNO injected subcutaneously for activation of GsD expressed in liver and 10 mg/kg for activation of GsD in skeletal muscle satellite cells (unpublished). Other laboratories report DREADD activation by administering CNO in drinking water [18]. CNO is widely used to activate DREADD receptors in animals and has been considered to be pharmacologically inert. A recent report, however, demonstrates that CNO is converted to clozapine in mice when delivered at 10 mg/kg, a concentration often used to activate DREADD signaling *in vivo* [23]. In this study, mice were trained to associate clozapine with a food reward. When CNO, but not other drugs, was administered, the animals responded in the same way as to clozapine. Therefore, the authors concluded that CNO is not inert. To control for possible off-target effects of CNO, it is important to include control animals that do not express DREADD but are treated with CNO in parallel with the experimental animals.

5-10 second exposure. When imaging for the first time, select auto exposure. This mode automatically sets optimal binning and F/stop. For analysis of the signal intensity the image should not be oversaturated (see Note 8).

Once acquisition settings are selected for a group of animals, they should not be changed throughout the experiment. If acquisition settings are altered, signals may not be directly quantitatively compared among different experimental time points.

4. Anesthetize the mice in an isoflurane chamber, weigh the mouse and calculate volume of 24 mg/ml D-luciferin to be injected to achieve dose of 100 mg/kg animal weight (see Note 9).
5. For injections, mix colloid D-luciferin stock solution well and dilute to 24 mg/ml in sterile saline or 1x PBS. Inject D-luciferin intraperitoneally at 100 mg/kg animal weight. For liver imaging, waiting 2 minutes before acquiring the images is sufficient.
6. Place the mice in the imaging chamber with the nose inserted in the nose cone for isoflurane inhalation. A 3-position manifold allows imaging up to 3 mice simultaneously. If several mice are imaged at the same time, separate the animals by black dividers (such as black construction paper folded like a fan) to prevent signal interference between mice.
7. Acquire image. It is helpful to acquire several images using different exposure times.

3.3.2 Image analysis

1. Open images in Living Image software. To compare signal intensity in separate images, make sure that acquisition parameters, especially exposure time, are identical for all images to be compared. Image settings and appearance can be controlled from the 'Tool Palette' menu in Living Image software.
2. Switch units to photons. In this mode, luminescence is displayed as photons per second per centimeter squared per steradian (photons/second/cm²/sr).
3. In 'Tool Palette' > 'Image Adjust' set minimum and maximum for visualization color. Changing color scale only affects signal visualization and does not alter actual photon data.
4. Using 'Tool Palette' > 'ROI Tools' outline the area producing signal, known as region of interest (ROI). To compare similar ROIs in multiple images, such as in images of one mouse after different treatments, use the same ROI area. The best way to select the same ROI is to use 'Copy ROI' and 'Paste ROI' functions.

⁸.For accurate analysis of signal intensity, the luminescent image should contain between 1000 to 60,000 counts. If 'image is oversaturated' message is displayed in Living Image, decrease the exposure time.

⁹.We recommend weighing mice and calculating the volume of 24 mg/ml D-luciferin to be injected in each mouse before the experiment. This saves time during the imaging session, especially if a large cohort of animals is analyzed.

Multiple ROIs can be drawn in one image, which is useful when similar areas need to be quantified.

5. After outlining ROIs, click the ‘Measure ROIs’ icon. This measures all ROIs in all images that are currently open and generates table with ROI data: total flux (photons/sec), minimal, maximal and average radiance (photons/sec/cm²/sr). These results can be copied and analyzed in a different software such as Microsoft Excel. We analyze and report total bioluminescence flux expressed in photons/sec.

3.3.3. Testing the responsiveness of the CRE-luciferase reporter—The responsiveness of the CRE-luciferase reporter gene in liver should be verified in all weanlings following exposure to CREB-activating stimuli such as fasting and/or glucagon (Fig 3 and ref [25]) (see Note 10).

1. Take ventral bioluminescence images under *ad libitum* fed conditions at approximately ZT10 (see Section 3.3.1).
2. After animals recover from anesthesia, fast them by moving the animals to fresh cages with synthetic bedding and no food, but with free access to water for 16 hr (overnight) to activate endogenous CREB. An important consideration is the time of day images are collected, as CREB activity is under circadian control in many tissues (see Note 11).
3. Take ventral bioluminescence images under fasted conditions at approximately ZT0.
4. Analyze imaging data (see Section 3.3.2).

3.4. *Ex vivo* bioluminescence CREB reporter imaging of mouse tissues

Especially convenient for imaging of small organs, such as brown adipose, pituitary gland and other specific regions of brain, this method entails removing organs from mice and imaging them *ex vivo*. It can also be used to image small pieces of larger organs such as liver and heart if it is required to keep the remainder of the organ for other analyses. The *ex vivo* approach has several advantages over whole mouse imaging. First, it allows imaging of organs that are difficult to image in intact mice, such as heart, kidney, lung, and brain, detection of which signals *in vivo* is limited by low intensity, small size, depth, shielding by bone or other factors that attenuate light transmission [26]. Second, it allows the source of luminescence signals *in vivo*, which may arise from multiple overlapping organs, to be

¹⁰ During analysis of CREB reporter signals in fasted animals [21], we observed animal-to-animal variability in bioluminescence. Moreover, although most *GsD*⁺ mice respond to fasting by a several-fold increase in hepatic luminescence, some mice do not have any increase in CREB reporter activity after fasting. We believe that this results from epigenetic silencing of the CRE-containing promoter or luciferase cDNA. We do not observe silencing of the *GsD* portion of the transgene. In experiments that incorporate the CREB reporter portion of the allele, it is therefore crucial to pre-screen all prospective experimental mice using the simple overnight fasting procedure to eliminate mice lacking bioluminescence response prior to assignment to cohorts. When planning mouse breeding for cohorts, we recommend breeding to obtain 20-25% extra mice to obtain a sufficient number of *GsD* animals after excluding non-responders. It is particularly important to use only well-responding mice as breeders, as reporter silencing appears to be heritable.

¹¹ Hepatic cAMP/PKA/CREB signaling is regulated in a circadian manner [27]. We have not observed strong circadian regulation of CREB activity in liver in CREB luciferase reporter mice which express the same CREB reporter as *GsD* mice [25]. However, other tissues may demonstrate pronounced circadian regulation. We therefore recommend testing CREB reporter bioluminescence at different times during the light cycle.

unambiguously identified (Fig 4A). Third, it is possible to cut freshly excised complex organs such as brain, heart, or kidney, into smaller sections in order to obtain more detailed localization of luminescence signal in the organ. Fourth, using a 96-well format allows the effects of *in vitro* pharmacological stimulation to be studied (Fig 4B).

3.4.1. Tissue isolation and analysis of tissue immediately post-mortem

1. Euthanize mouse by CO₂ inhalation. Quickly excise tissues and rinse in 1x PBS.
2. To image tissues immediately post-mortem, lay out freshly isolated tissues of interest on a petri dish or multi-well plates. For smaller tissues this may be the entire tissue, or for larger tissues, a sub-section may be studied. In some cases, it may also be beneficial to sub-divide or dissect tissues so that CREB reporter induction within internal structures that may otherwise be masked by the opacity of the tissue can be visualized. For example, CREB reporter activity in the renal medulla can only be observed if the kidney is bisected.
3. Proceed directly to imaging (see Section 3.4.3) and/or perform *ex vivo* treatments (see Section 3.4.2).

3.4.2. Analysis of tissue responses to *ex vivo* drug treatment

1. Divide tissues of interest into multiple even pieces, ~1-2 mm across using a scalpel blade, being careful to consider that internal structure within organs may result in different signal intensities from different pieces.
2. Transfer tissue pieces into individual wells of a black 96-well microtiter plate containing DMEM media (100-200 µl). Add *ex vivo* treatments to activate GsD or native cAMP formation pathways. The water-soluble forskolin analogue, NKH477 (1 µM) may be used to provide a positive control of maximal CREB reporter induction (Fig 4B). Signal intensity may be improved by addition of the non-specific phosphodiesterase inhibitor IBMX (100 µM), however, this requires careful consideration within the design of each experiment as addition of IBMX can mask the effect of some treatments either mechanistically or by saturating induction of the CREB reporter.
3. Incubate plates in a 37°C tissue culture incubator for the required amount of time. In our experience, 4 hr provides an optimal time for induction of the CREB reporter in response to GsD activation by CNO and agents that acutely induce endogenous cAMP formation. However, the stimulation time may require further optimization depending on experimental design and the nature of the treatments.

3.4.3. Imaging

1. For both approaches, either immediately post-mortem or at the desired time point, add D-luciferin to the tissues to a final concentration of 15 mg/ml – either by addition of 120 mg/ml (8x) concentrated stock to the media, or by applying pre-diluted 15 mg/ml D-luciferin directly to the tissue surface. Transfer plate to IVIS Lumina XR machine.

2. Start acquiring images as described in Section 3.3.1. Typical image settings for this type of experiment are: Binning: 'Medium', Field of View: 'D', Exposure time: '1 min'. These settings may require optimization depending on experimental design. It may also be helpful to initially acquire several images using multiple exposure times.
3. Image data should be analyzed and described as in Section 3.3.2. For individual tissue analysis, quantification as maximal radiance may be particularly appropriate, as this measure is not directly influenced by the size of each tissue or tissue piece studied.

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Figure 1.

GsD expression is induced by Cre recombinase in a tissue-specific manner. A) *ROSA26-LSL-GsDREADD-CRE-Luc* allele. Arrows, transcriptional start sites. B) GsD expression in skeletal muscle fibers of *ROSA26^{GsD/+};HSA-MerCreMer/+* mice following tamoxifen treatment. HSA-MerCreMer is a tamoxifen inducible Cre recombinase under control of the human α -skeletal muscle promoter [28]. Green = GFP immunolabeling, blue = DAPI. npc = no primary antibody control. Scale bar = 20 μ m.

Figure 2.

A) Bioluminescent imaging of CRE-luciferase reporter in *ROSA26^{GsD/+};MCM/+* mice before (0 h) and after CNO injection (2 h, 4 h). B) Quantification of bioluminescence in regions outlined in (A). Significance determined by t-test; *significance vs 0 h within the same genotype, # significance vs 0 h of TMX, vehicle control.

Figure 3. CREB luciferase reporter imaging in *ROSA26^{GSD/+}* mice. Bioluminescence signal in *ROSA26^{GSD/+}* mice *ad libitum* fed (ZT10) and after 16 hr fasting (ZT2).

Figure 4.
Ex vivo imaging of CREB luciferase reporter in *ROSA26^{Gsd/+}* mice. Representative images showing (A) basal CREB-luciferase activity in a panel of tissues freshly excised from *ROSA26^{Gsd/+}* mice and (B) treatment of tissue *ex vivo* to induce CREB reporter activity using NKH477 (1 μ M) and IBMX (100 μ M).