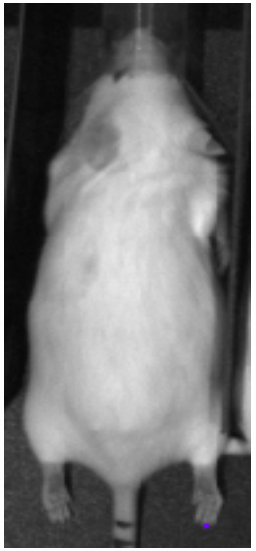
The CRE Luc Reporter Mouse Model
A transgenic bioimaging mouse model to assay ligand activation of GPCRs



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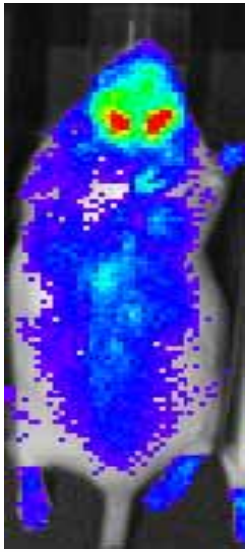
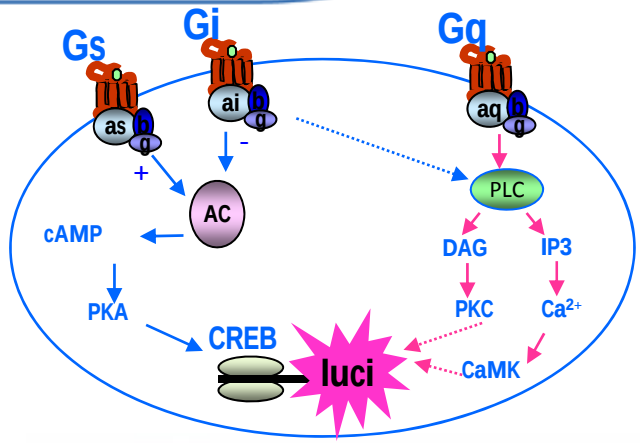
Keystone Symposia: G Protein-Coupled Receptors
February 20, 2012



The CRE Luc mouse model
background and objectives

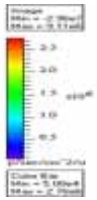
CRE-luciferase reporter system

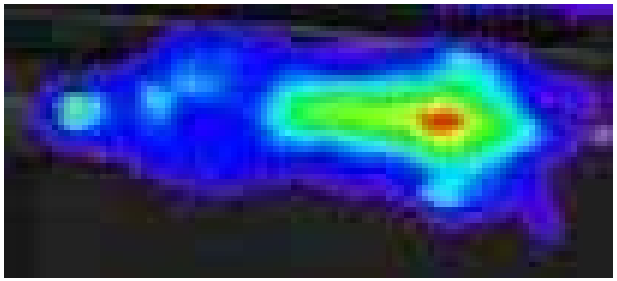
- CRE promoter is responsive to the activation of CREB via the cAMP or PLC pathway
- Luciferase reporter expression is modulated to reflect GPCR activity through a transcriptional readout
- Assay can be used for all 3 GPCR classes: Gs ,Gi and indirectly Gq

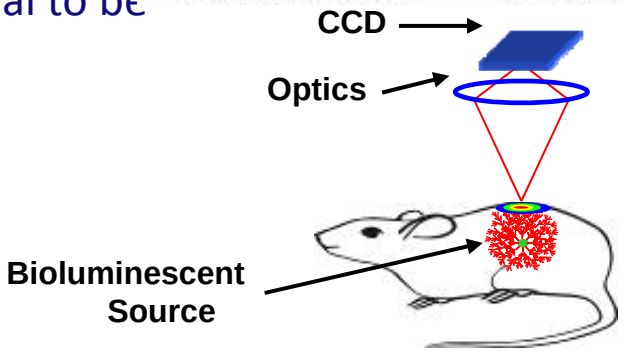


Bioimaging

- Real-time *in vivo* imaging utilizes the light emitted by a bioluminescent reporter gene (luciferase) expressed *in vivo*
- Allows for quantification of the signal non-invasively
- Temporal and spatial data can be collected from the same animal which reduces variation and allows each animal to be its own control



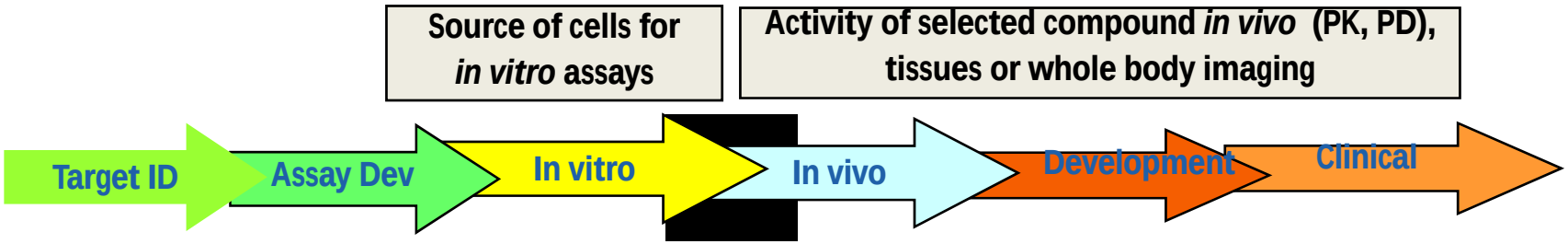




The CRE Luc mouse model
background and objectives

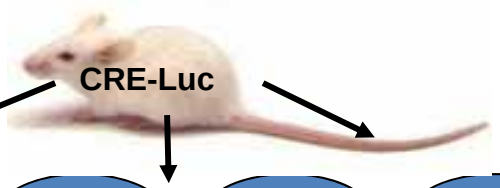
- **Model goal:** Combine a GPCR reporter system with real-time *in vivo* bioimaging to assay GPCR ligand receptor interactions in primary cells, tissues or live animals.
  - Same reporter system utilized for both *in vitro* and *in vivo* assays
  - Profiling of compounds selected from *in vitro* assays for rapid PK/PD
  - CRE Luc mouse models support rapid application to ligand:receptor pharmacological assays *in vitro*
  - GPCR ligand interactions can be assayed in a native system avoiding difficult to transfect primary cells and engineered cell lines

- **Model application:** The CRE Luc model has broad applications to GPCR ligand and receptor interactions.
  - Addresses the transition from cells to animal model profiling of leads in GPCR drug development



CRE Luc Reporter Mouse Model Application Strategy

- Starting with a variety of luciferase expression profiles, pilot studies defined the model's potential impact on drug development projects.
  - Typical pilots started with CRE Luc primary cell responses followed by *in vivo* experiments



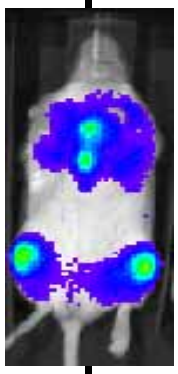
Feasibility profiles

No-Go Go

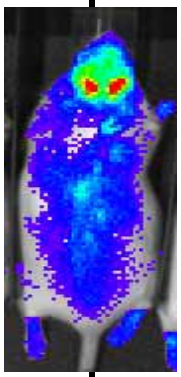
Specific pilots to reach project application decision

No-Go Go

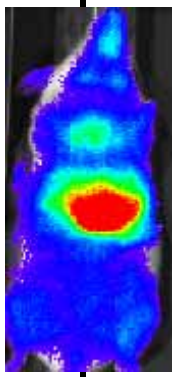
Project application


T,B,macs.

64


CNS

187


Liver

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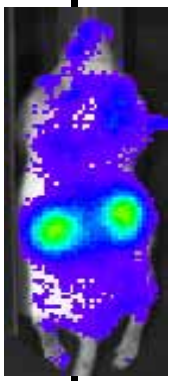
Kidney

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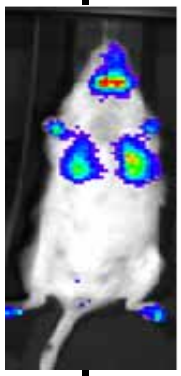
Lung

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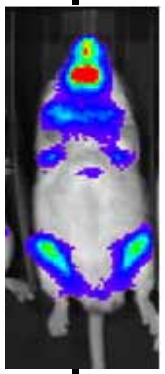
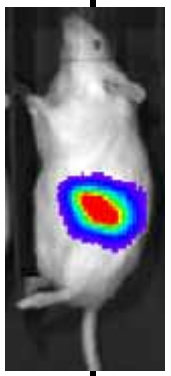

Adipose

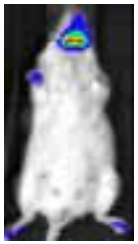

Pancreas

11, 16

Tg lines for model applications

Studying the GPCR cAMP signaling pathway using CRE Luc mouse

In vivo | *In vitro* | *Ex vivo*

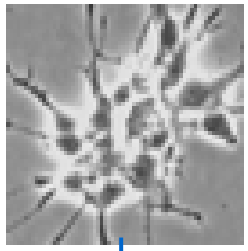


Baseline imaging

Compound dosing

Re-imaging

IVIS bioimaging
Whole live animal imaging
Simple, quick
Limited resolution



Compound dosing

Tissue homogenates

Luciferase assay

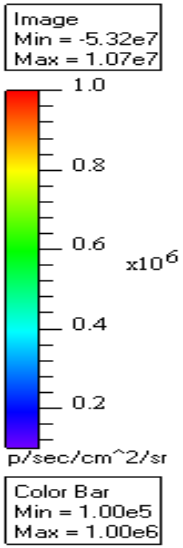
Microplate reader
Sensitive, accurate
Better organ resolution
Time-consuming

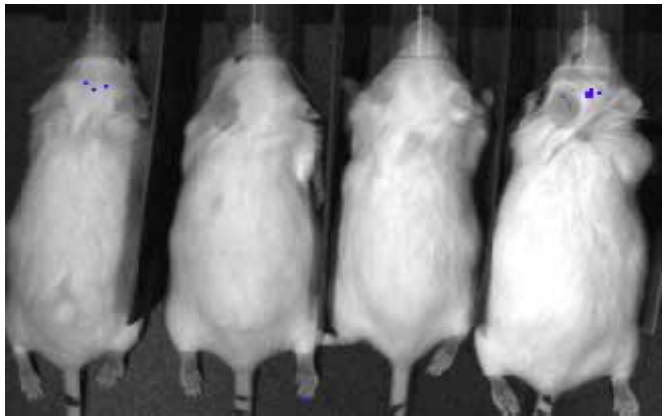
Radiance/RLU
Fold induction

Next set of slides demonstrates this diversity of data with isoproterenol

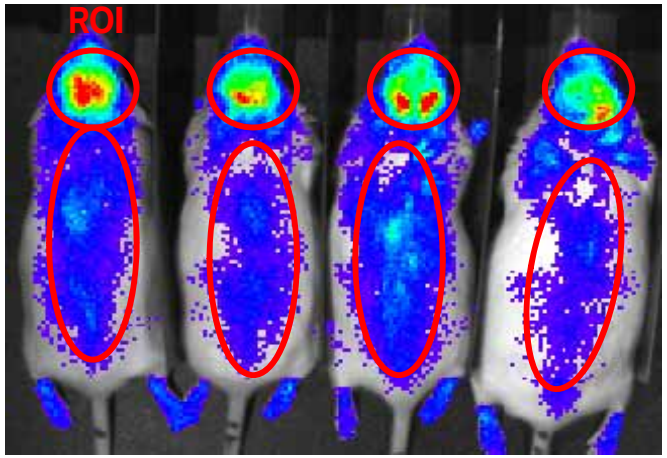
Isoproterenol *in vivo* response in CRE Luc

- **Response to Isoproterenol in line 187 with CNS predominate luci expression**
  - Treatment: isoproterenol, 10MPK, ip
  - Imaging at T=0 and 5 hours
  - Statistically significant increase in quantitative CNS response over baseline

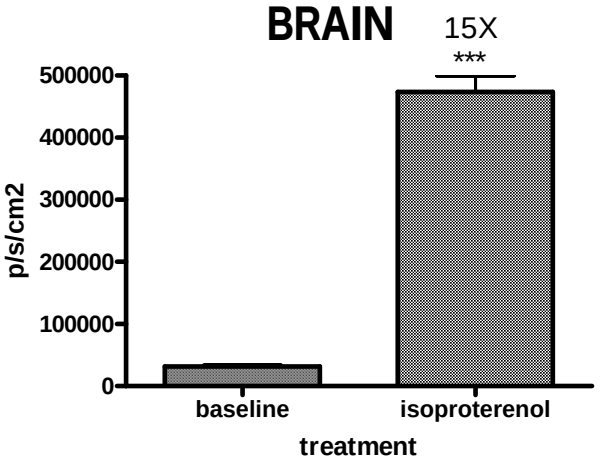


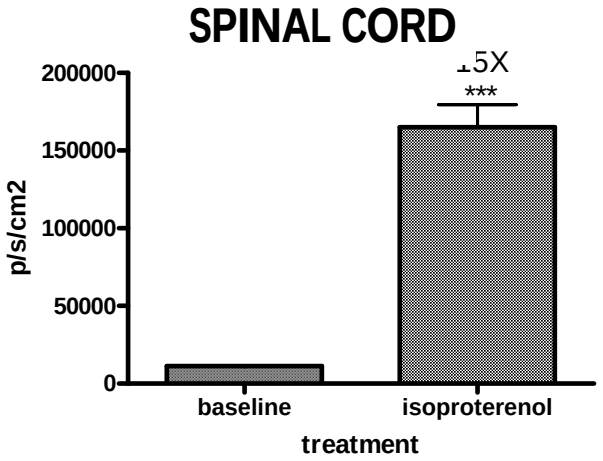


T=0



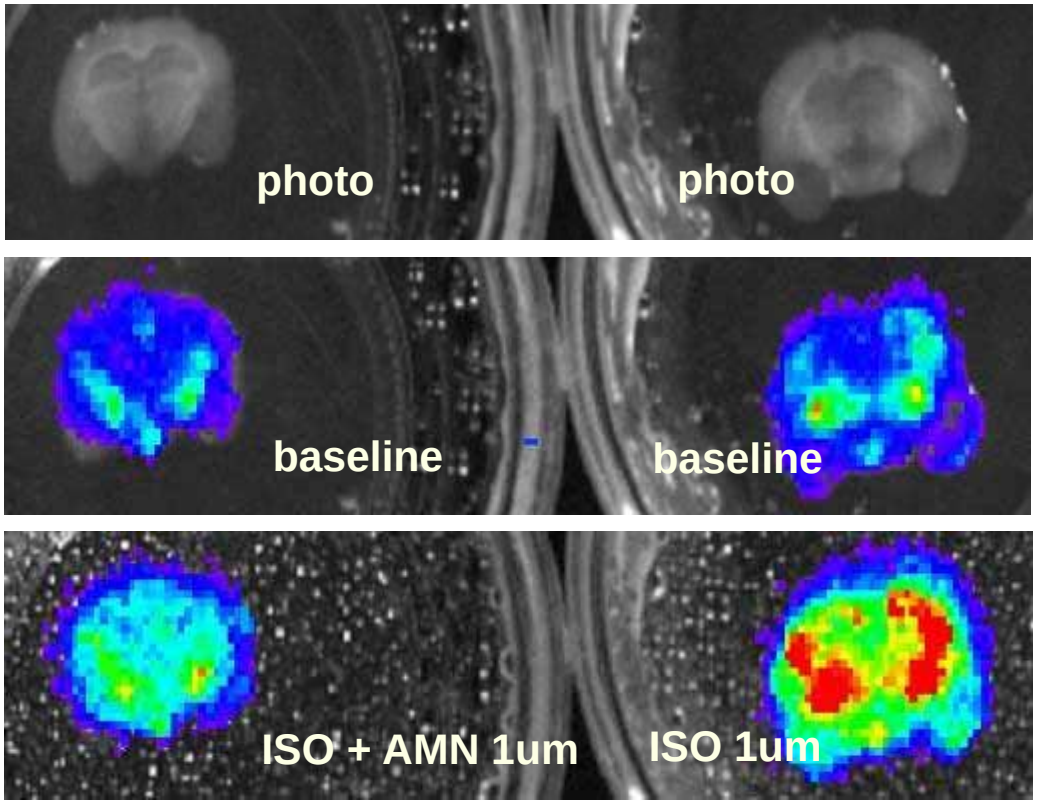
T=5hrs



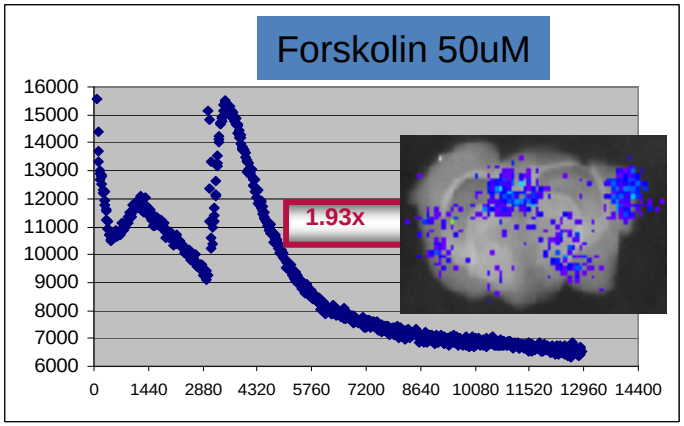


Isoproterenol *ex vivo* (brain slice) response in CRE Luc line 187

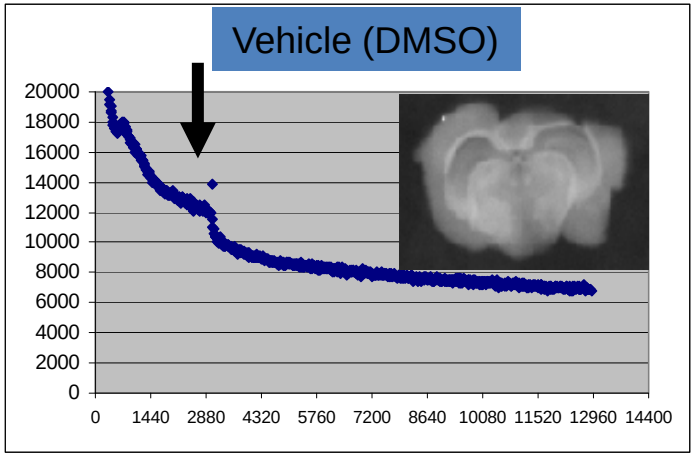
- **Compound induced changes in luciferase levels in brain slices can be detected and quantified by bioimaging**
  - **Gs agonist: isoproterenol signal is diminished by Gi agonist AMN087**
- **Strategy to identify the region specific expression of the transgene and drug interaction**



Slice 1



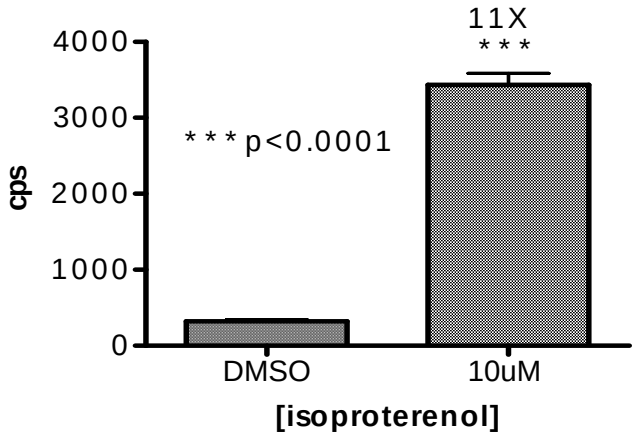
Slice 2



Isoproterenol response of CRE Luc primary neurons (and Gs or Gi agonist profiles)

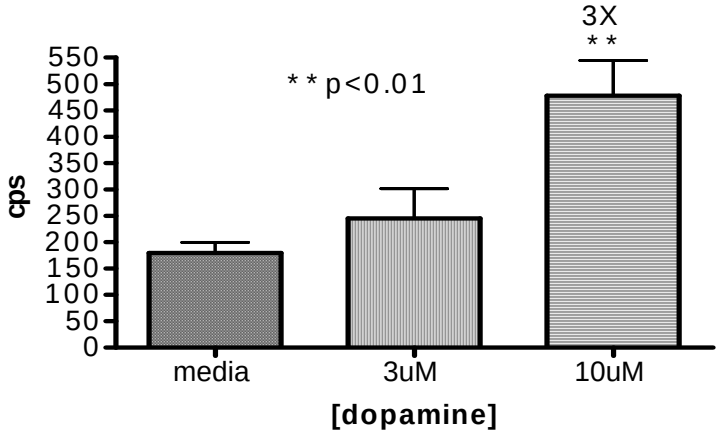
Gs: ADRβ1/2, isoproterenol

- E18, d3 cortical neurons
- t-test vs DMSO
- 4 hour treatment



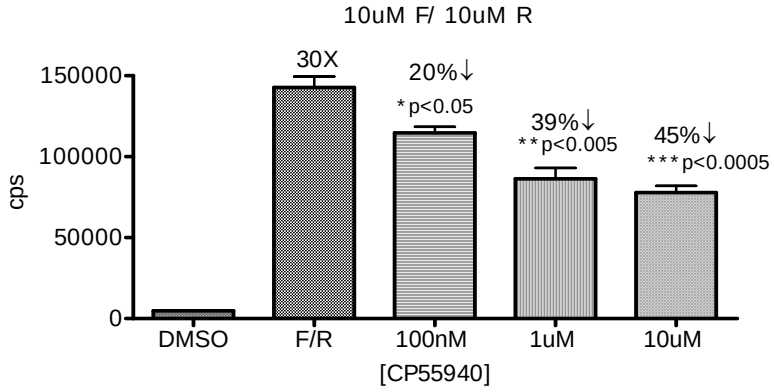
Gs: DRD, dopamine

- E14, d4 striatal neurons
- t-test vs media
- 5 hour treatment



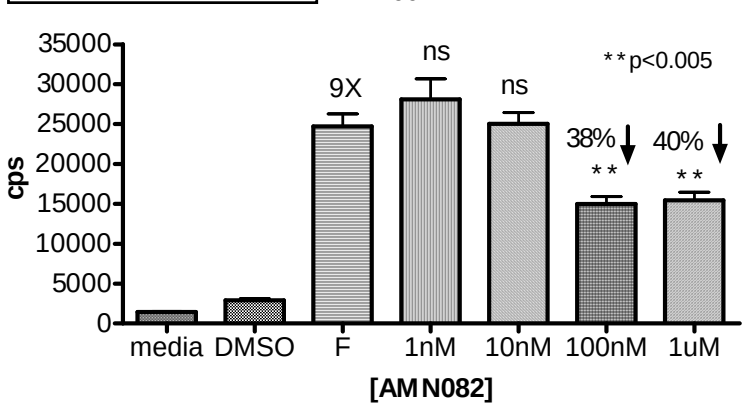
Gi: CB1, CP55,940

- E18, d3 cortical neurons
- 10μM forskolin/ 10μM rolipram
- 8 hour treatment
- t-test vs Forskolin Rolipram



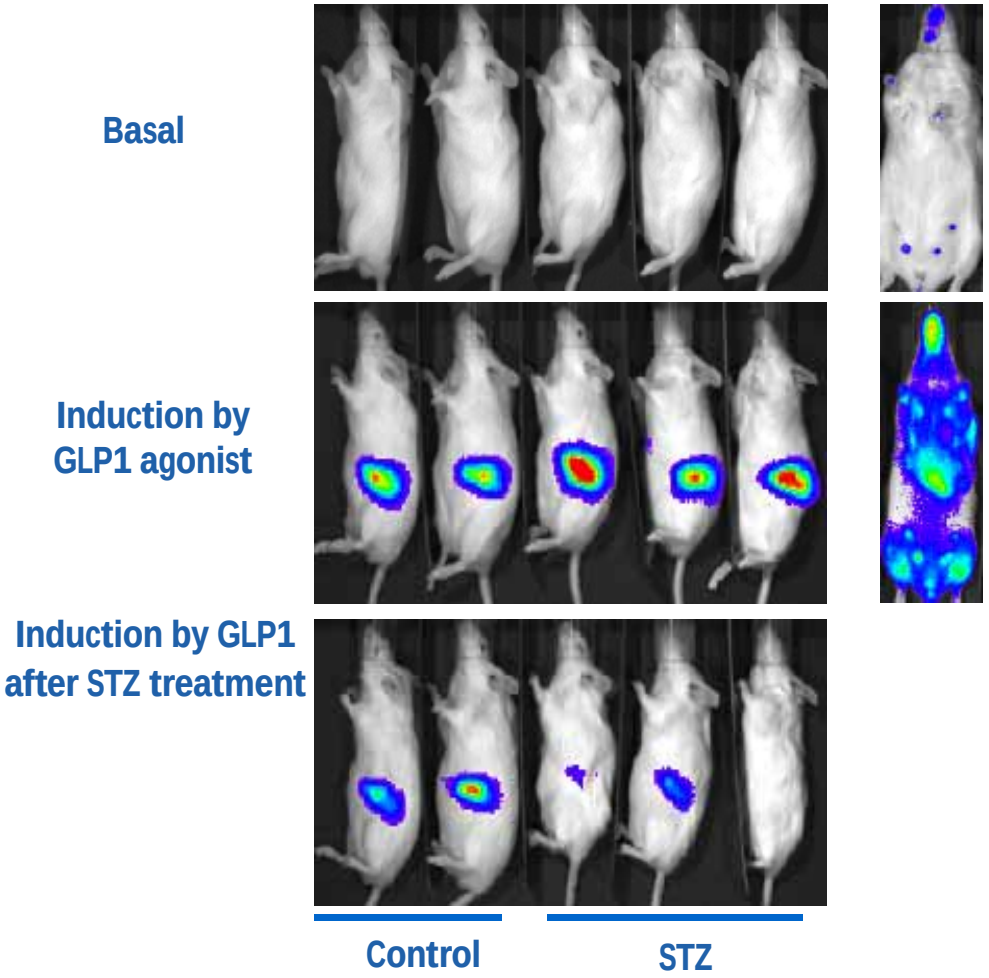
Gi: mGluR7, AMN082

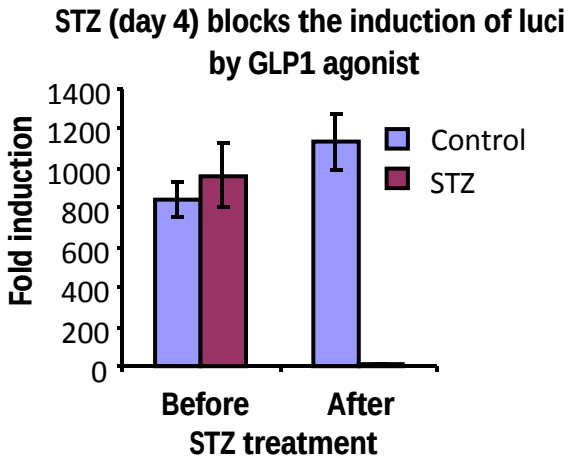
- E18, d3 cortical neurons
- 10μM forskolin
- 4 hour treatment
- t-test vs Forskolin



Pancreatic specific induction of luciferase by a GLP1 agonist

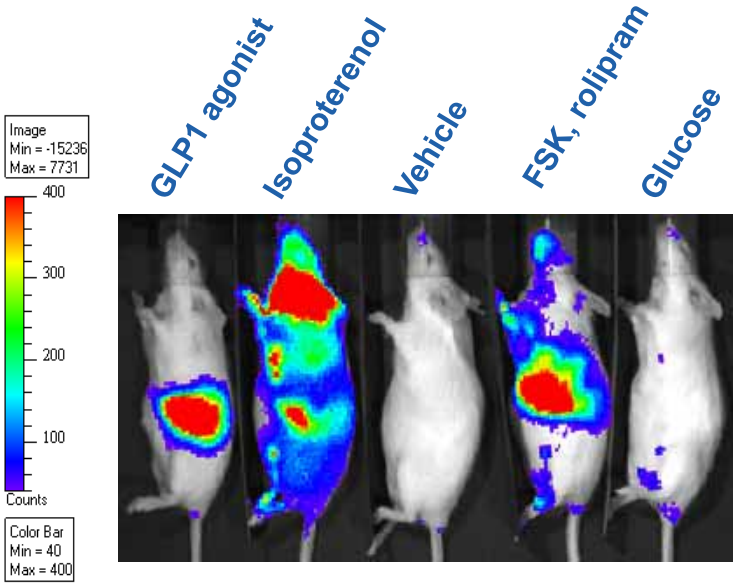
- Pancreatic specific induction of luciferase by the GLP1 agonist is blocked by streptozotocin treatment due to the destruction of β-cells





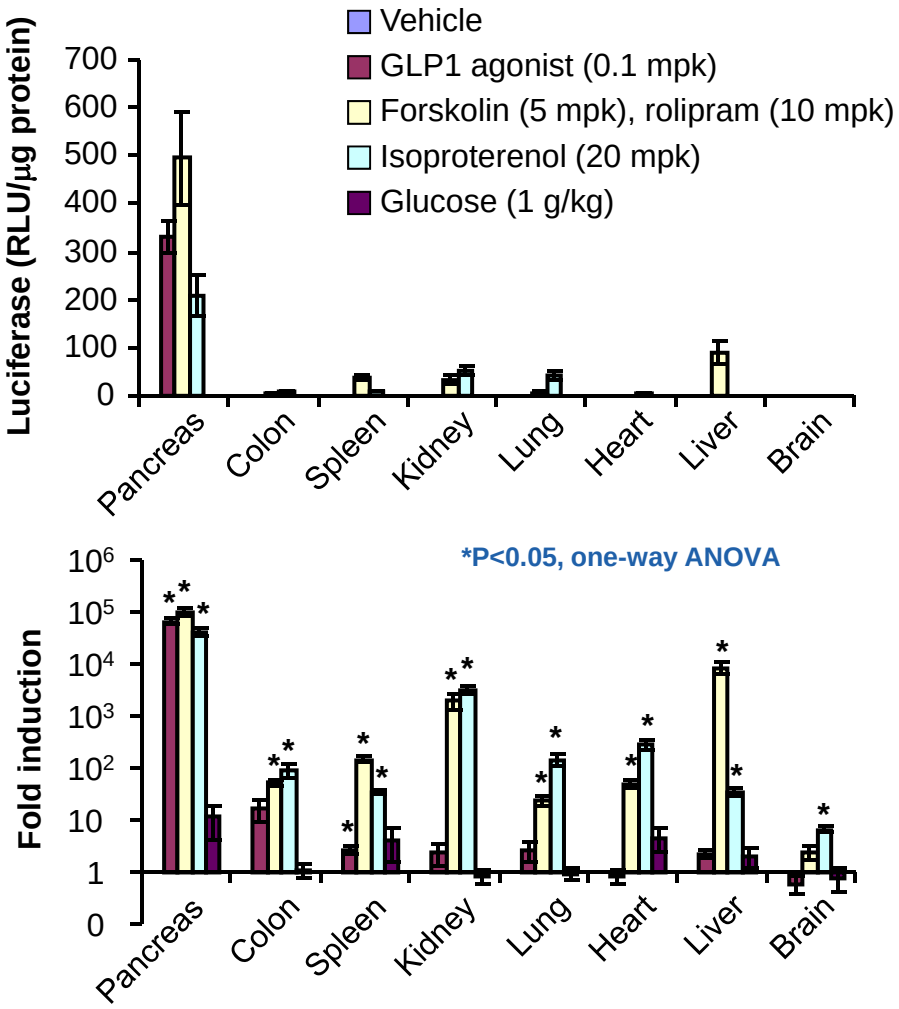
GLP1 agonist induces luciferase expression mainly in the pancreas

- GLP1R found in multiple tissues, however compound activity is only seen in pancreas.
- CRE Luc model defines the site of action for a compound *in vivo* (rapid PK/PD).



- Compound dependent patterns of luciferase expression, suggesting that pancreas-specific activity of the GLP1 agonist is unlikely an transgenic artifact.
- Strong induction in the pancreas by the GLP1 agonist, isoproterenol, and forskolin plus rolipram was observed.

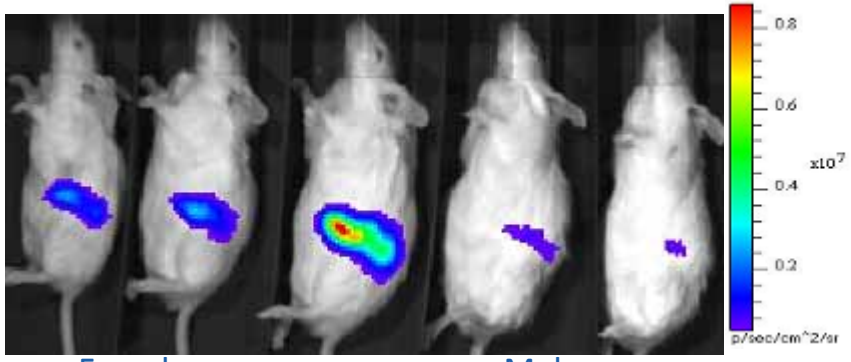
Ex vivo assay on tissue homogenates



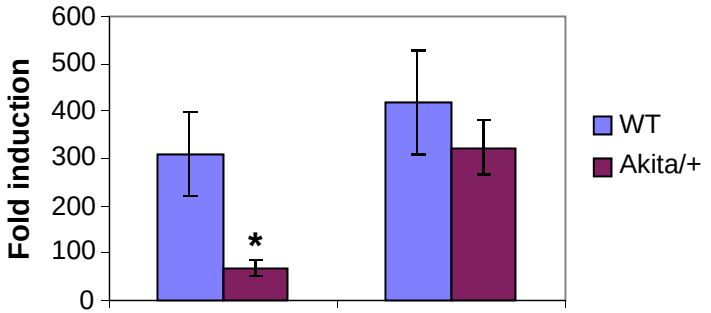
Pancreatic luciferase response in CRE Luc-Ins2^{Akita} mice

- *Ins2*^{Akita} is an autosomal dominant mutation that causes early onset hyperglycemia in the absence of obesity, due to a missense mutation resulting in mis-folding of proinsulin and death of β cells.
- Crossed CRE Luc with *Ins2*^{Akita} (FVB/N background) to see if CRE-Luc induction is correlated with β cell function in this T1DM model.
- 8-week old mice were subject to baseline imaging on day 1 and treatment with GLP1 agonist (0.1mpk, sc) followed by re-imaging at 4 hr on day 2.

Akita/+ Akita/+ WT Akita/+ Akita/+



Females Males



*P < 0.05, Akita/+ vs WT
Two-way ANOVA model.

- Decreased CRE Luc induction by the GLP1 agonist (0.1 mpk, sc, 4 hrs) in the highly diabetic male mice. This effect was not significant in the less diabetic female littermates.
- *In vivo* signals were confirmed by *ex vivo* luciferase assay in a subset of animals.

Summary

- **From initial studies, we have demonstrated the utility of the CRE Luc model to profile compounds in whole animals, tissue extracts, slices, and primary cells *in vitro.***

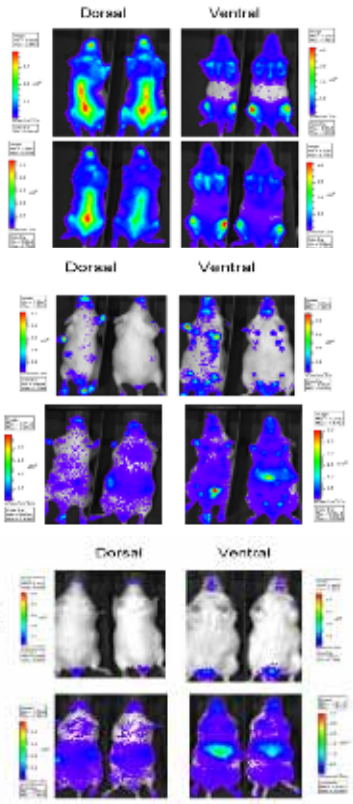
Profiling responses for various GPCRs have been tested in the following combinations

- **Gs agonist:**
  - *In vitro with microglia, neurons, cardiomyocytes, MEFs and brain slices*
  - *In vivo in the pancreas, brain, spinal cord*
- ***Gs antagonists:***
  - *In vitro: microglia, neurons, and T cells*
  - *In vivo: brain, spinal cord*
- ***Gi agonists:***
  - *In vitro: neurons, Tcells, brain slices*
- ***Gi antagonists:***
  - *In vitro: neruons, Tcells, brain slices*

Characterization of the CRE Luc lines

- **Details of the profiling assays with the CRE Luc transgene have been summarized in a single table** (available upon request)
- **Eight CRE Luc lines are available through Taconic Biosciences**

	A	B	C	D	E	F	G	H
1	Line number	Frozen	Breeding status	Primary tissues expressing luci transgene (bioimaging / enzyme assay)	Reference compound validation assays			Bioimaging picture
2					In vivo assays	in vitro assays	ex vivo / in vivo	
9	44	Jax sperm Het (QC to live born)	Het (BRW)	bone marrow (high basal), spl high basal expression in bones (BM), brain		•BM and splenocytes used for RNAi •whole splenocytes Gs: DP-BW245C, EP2, EX00000173A, βAR-isoproterenol • BM engraftment into NSG mice (potential use for Gi agonists)	•adipose, int.panc, lung, spl, br Gs: Adrb3- CL316,243	G:\cre luc\line bioimaging pictures\
10	64	Jax sperm Het (QC to live born) CRL sperm Ho	Ho (BRWCRL)	spleen, kidney	•BM engraftment into NSG mice (potential use for Gi agonists) Gs: βAR-isoproterenol	•T cells Gs: DP- BW245C, βAR-isoproterenol, EP2-EX00000173A •B cells Gs: DP- BW245C •microglia Gs: DP- BW245C, PGD2, in house antagonists	•adipose, int.panc, lung, spl, br Gs: Adrb3- CL316,243	G:\cre luc\line bioimaging pictures\
	69	Jax sperm Het (QC to live born) CRL sperm Ho	Ho (BRWCRL)	spleen, kidney, liver, brain		•neurons Gi agonists: CB1- CP55,940 Gq agonist: PROKR2-PROK2 peptide •whole splenocytes Gs: DP-BW245C, EP2, EX00000173A, βAR-isoproterenol	•adipose, int.panc, lung, spl, br Gs: Adrb3- CL316,243	G:\cre luc\line bioimaging pictures\



Acknowledgements and Model Availability

- **Immunology Experimental Pharmacology**
  - Holly Dressler (PTL, model generation, development, and applications)
  - Fernando Camacho (psoriasis)
  - Kyriakos Economides (psoriasis)
  - Andy Giovanni (brain slices)
  - Sarah Favara (linage profiling, CNS)
  - Zhen Pang (diabetes, Metabolism)
  - Nancy Wu (dibaetes, Metabolism)

- **CRE-Luc model information**
  - Greg Polites: greg.polites@sanofi-aventis.com or hgpolites3@gmail.com
- **CRE-Luc model availability**
  - Taconic Biosciences
    email: info@taconic.com