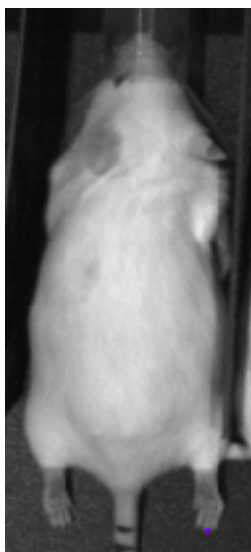


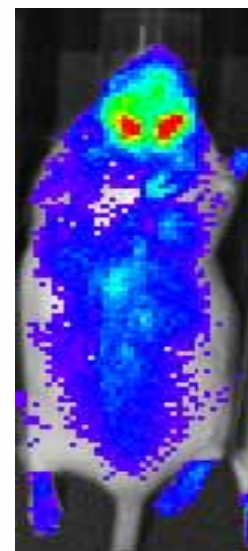


# The CRE Luc Reporter Mouse Model

## A transgenic bioimaging mouse model to assay ligand activation of GPCRs



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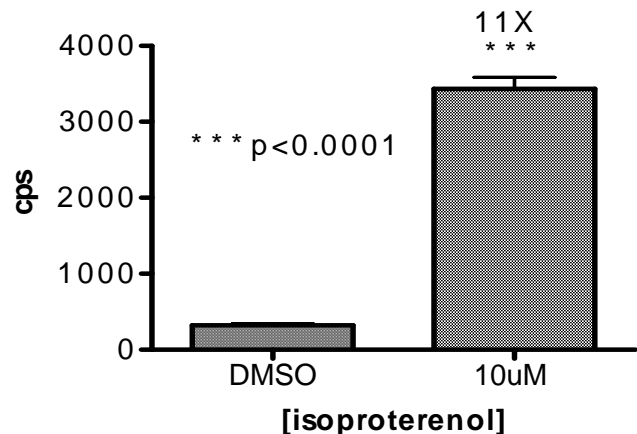




# Isoproterenol response of CRE Luc primary neurons (and Gs or Gi agonist profiles)

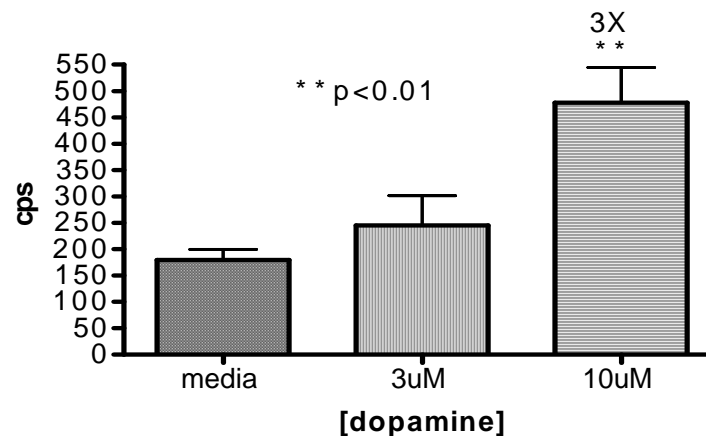
**Gs: ADR $\beta$ 1/2,  
isoproterenol**

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



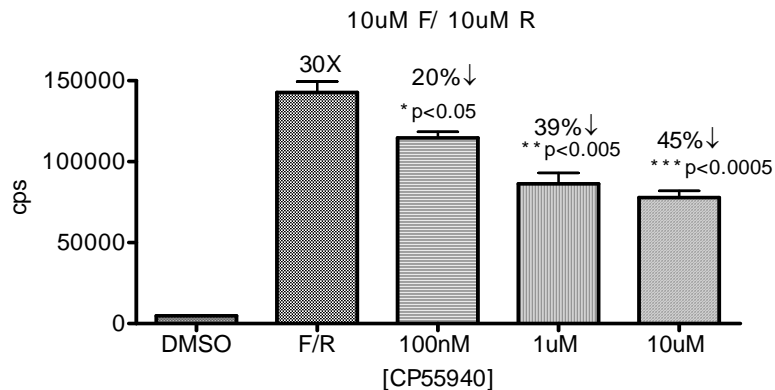
**Gs: DRD,  
dopamine**

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



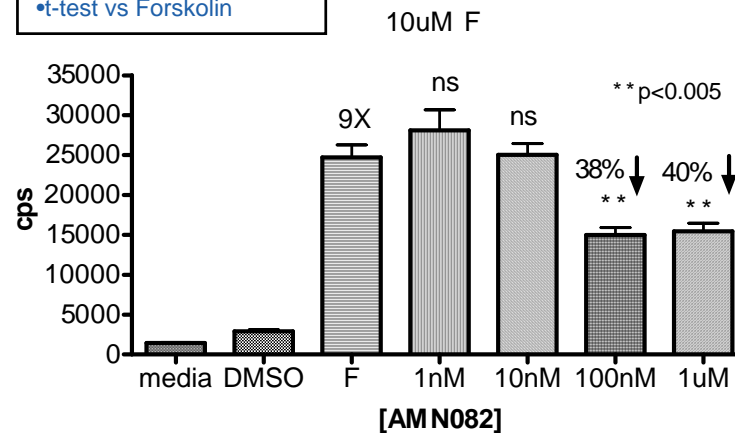
**Gi: CB1,  
CP55,940**

- E18, d3 cortical neurons
- 10 $\mu$ M forskolin/ 10 $\mu$ M rolipram
- 8 hour treatment
- t-test vs Forskolin Rolipram



**Gi: mGluR7, AMN082**

- E18, d3 cortical neurons
- 10 $\mu$ M forskolin
- 4 hour treatment
- t-test vs Forskolin





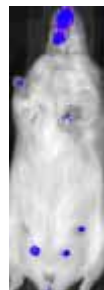
# Pancreatic specific induction of luciferase by a GLP1 agonist

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

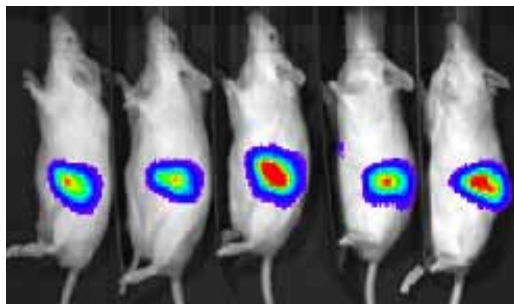
Basal



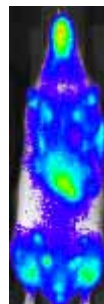
Basal



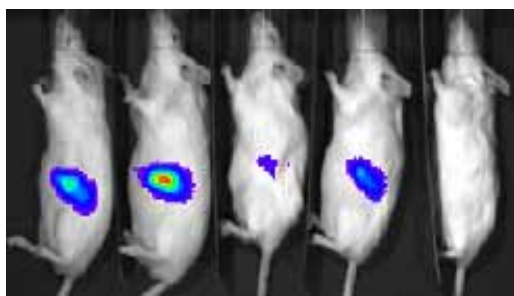
Induction by GLP1 agonist



Induction by Forskolin/rolipram

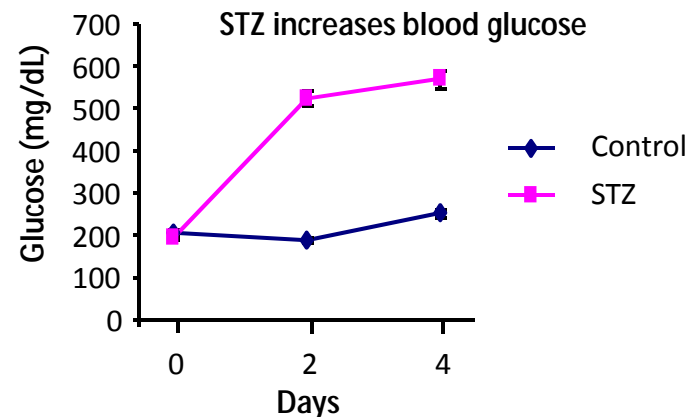


Induction by GLP1 after STZ treatment

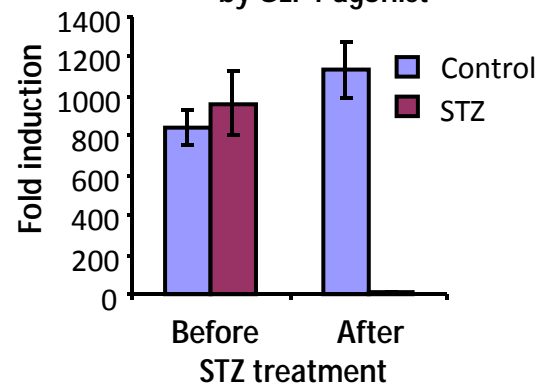


Control

STZ

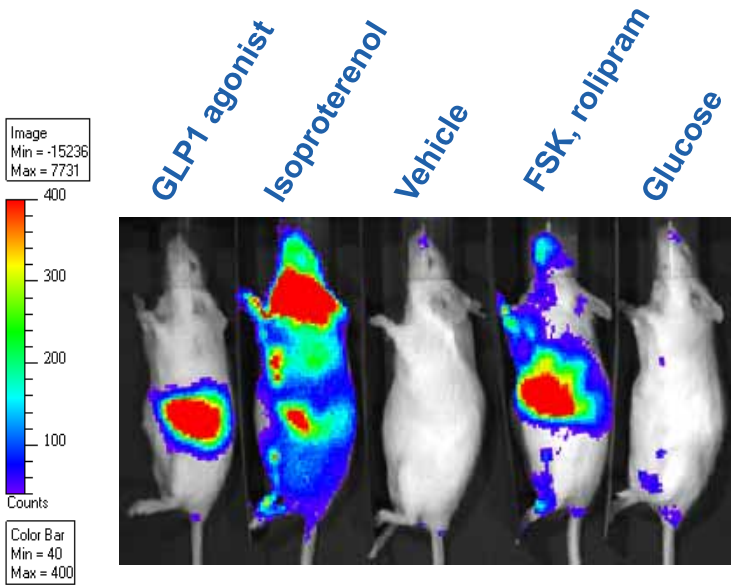


STZ (day 4) blocks the induction of luci by GLP1 agonist



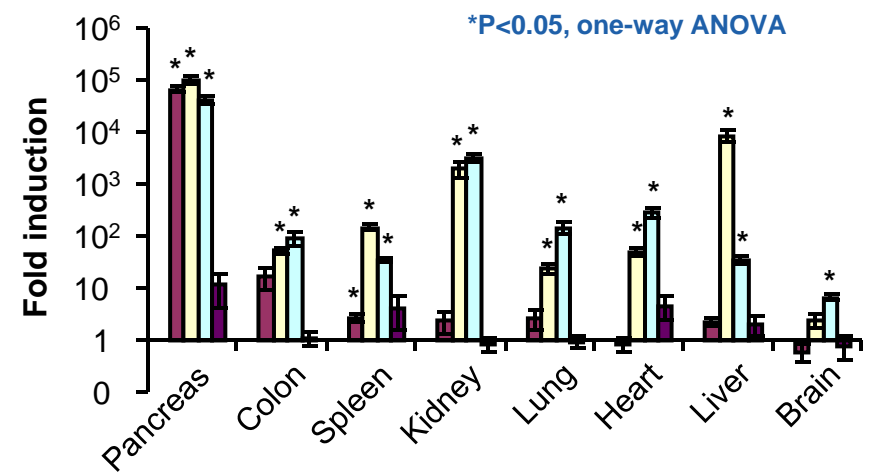
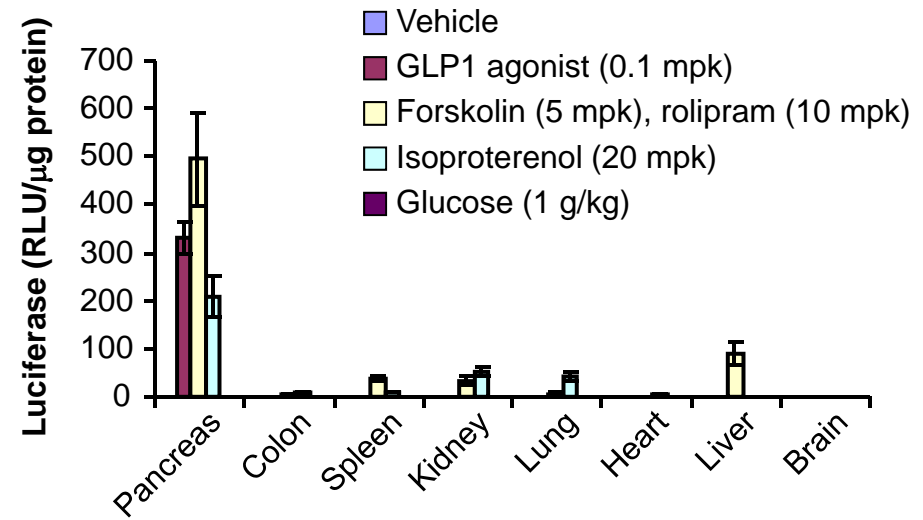
# GLP1 agonist induces luciferase expression mainly in the pancreas

- 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.

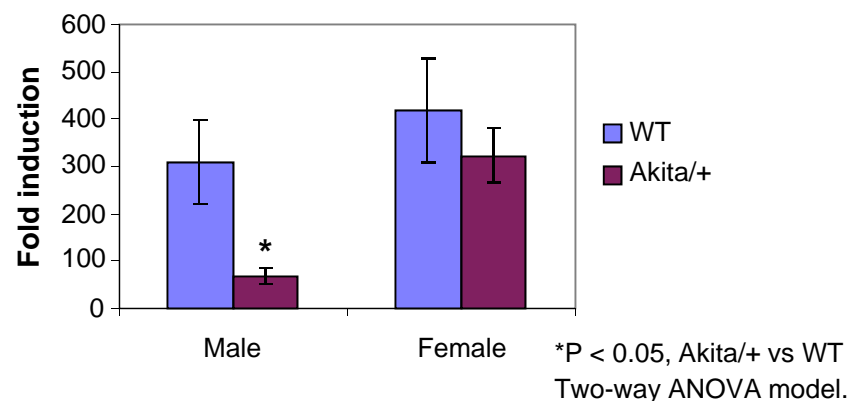
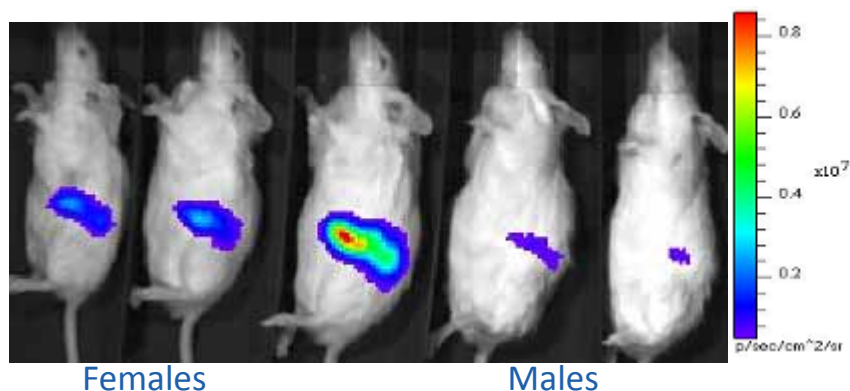
## Ex vivo assay on tissue homogenates



# Pancreatic luciferase response in CRE Luc-*Ins2*<sup>Akita</sup> mice

- *Ins2*<sup>Akita</sup> 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  $\beta$  cells.
- Crossed CRE Luc with *Ins2*<sup>Akita</sup> (FVB/N background) to see if CRE-Luc induction is correlated with  $\beta$  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/+



- 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*.

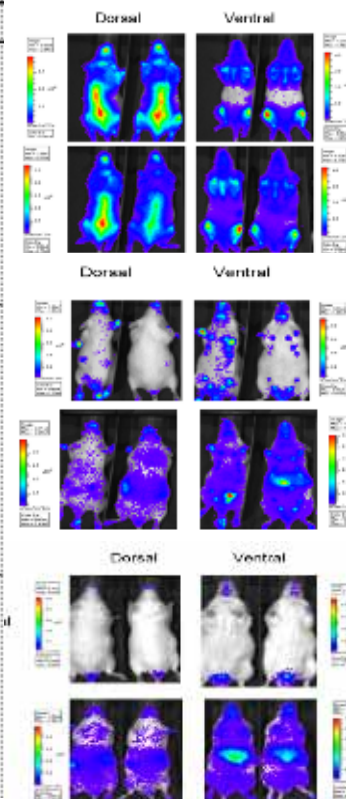
*Profiling responses for various GPCRs have been tested in the following combinations*

- **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**

	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	
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, $\beta$ AR-isoproterenol • BM engraftment into NSG mice (potential use for Gi agonists)	•adipose, int.panc, lung, spl, br Gs: Adrb3-CL316,243		
64	Jax sperm Het (QC to live born) CFL sperm Ho	Ho (BRWCRL)	spleen, kidney	•BM engraftment into NSG mice (potential use for Gi agonists) Gs: $\beta$ AR-isoproterenol	•T cells Gs: DP- BW245C, $\beta$ AR-isoproterenol, EP2-EX00000173A •B cells Gs: DP- BW245C •microglie Gs: DP- BW245C, PGD2, in house antagonists	•adipose, int.panc, lung, spl, br Gs: Adrb3-CL316,243		
69	Jax sperm Het (QC to live born) CFL sperm Ho	Ho (BRWCRL)	spleen, kidney, liver, brain		•neurons Gi agonists: CBI- CP55,940 Gq agonist: PROKR2-PROK2 peptide •whole splenocytes Gs: DP-BW245C, EP2, EX00000173A, $\beta$ AR-isoproterenol	•adipose, int.panc, lung, spl, br Gs: Adrb3-CL316,243		



## ■ 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

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## ■ CRE-Luc model availability

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