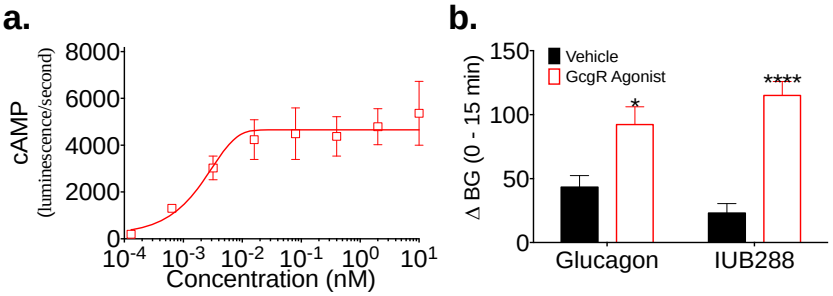
Key Biological and/or Chemical Resources

The studies detailed in this application utilize cell lines, antibodies, oligonucleotides, genetically modified mouse strains, and novel chemical reagents (receptor agonist, antagonist, hormone analogs, and proteolytic inhibitors) obtained from commercial vendors as well as other investigators. All reagents, regardless of source, are validated by our group as detailed below:

1. Cell lines: Our group employs several hepatic cell lines including HepG2 (human), and FAO (rat). Both lines were obtained from a commercial vendor (ATCC). Thawed stocks are validated with respect to isolated primary hepatocytes (which is our preferred experimental platform), and in stocks of similar cell lines from other laboratories in the UAB Comprehensive Diabetes Center. We are especially concerned with the expression of *GcgR* in these lines. In this regard, the FAO line (which lacks GcgR expression) provides a negative control to the HepG2 and primary hepatocytes.
2. Antibodies: The majority of antibodies used in the Habegger lab are commercially produced. Importantly, for this proposal PPARα (H-98) (Santa Cruz) and cFos (Cell Signaling) are routinely validated by western blotting. Any changes in antibody specificity will be noted, and another source will be utilized.
3. Mouse lines: Integral to this proposal are reliable Cre recombinase and floxed (LoxP) mouse lines. As noted in the Research Strategy, the multiple Cre lines proposed are readily available from Jackson labs. Any changes in Cre expression or efficiency noted in our colony will prompt a replacement order with Jackson Labs. The specialized floxed or null mouse lines employed in the proposal (e.g. KLB^{flox}, $GcgR^{flox}$) have been published in the literature extensively (see references in Research Proposal) and will be regularly genotyped and maintained on a pure C57BL/6 background. All mouse knockout models will be validated by immunofluorescence, western blotting, and/or qPCR for genetic recombination.
4. Novel chemical reagents: Our group routinely utilizes advanced pharmacology (receptor-agonists, -antagonist, hormone analogs, and proteolytic inhibitors) in genetically modified mouse lines to elucidate novel aspects of physiology. This strategy relies upon high-quality, well-characterized reagents and we have been fortunate to collaborate with the DiMarchi group at Indiana University for these tools. A primary example of these reagents is the GcgR agonist IUB288. As shown below (and reference[1]), this is a peptide, which replicates native GcgR action, yet is modified to enhance ligand-receptor specificity and extend bio-availability. Likewise, we will utilize the GcgR/GLP1R co-agonist characterized by Day *et al.*[2] to elucidate GcgR action in the absence of its hyperglycemic effects. The DiMarchi group will also provide rhFGF21 and a novel inhibitor of its endopeptidase, FAP. In all cases, peptides are pharmaceutical grade and have been validated for receptor/substrate specificity.



IUB288 stimulates cAMP production in HEK293 cells expressing GcgR (a, n = 2). 15 min GcgR agonism via native glucagon or IUB288 (3 nmol/kg) in C57/B6J mice stimulates elevated blood glucose (b, n=8). All data represent average ± SEM. * p< 0.05, ****

1. Habegger KM, Stemmer K, Cheng C, et al. Fibroblast growth factor 21 mediates specific glucagon actions. Diabetes 2013;62:1453-63.
2. Day JW, Ottaway N, Patterson JT, et al. A new glucagon and GLP-1 co-agonist eliminates obesity in rodents. Nat Chem Biol 2009;5:749-57.