

*High-Throughput FRET Assays For cAMP
Enable GPCR Agonist Drug Discovery*

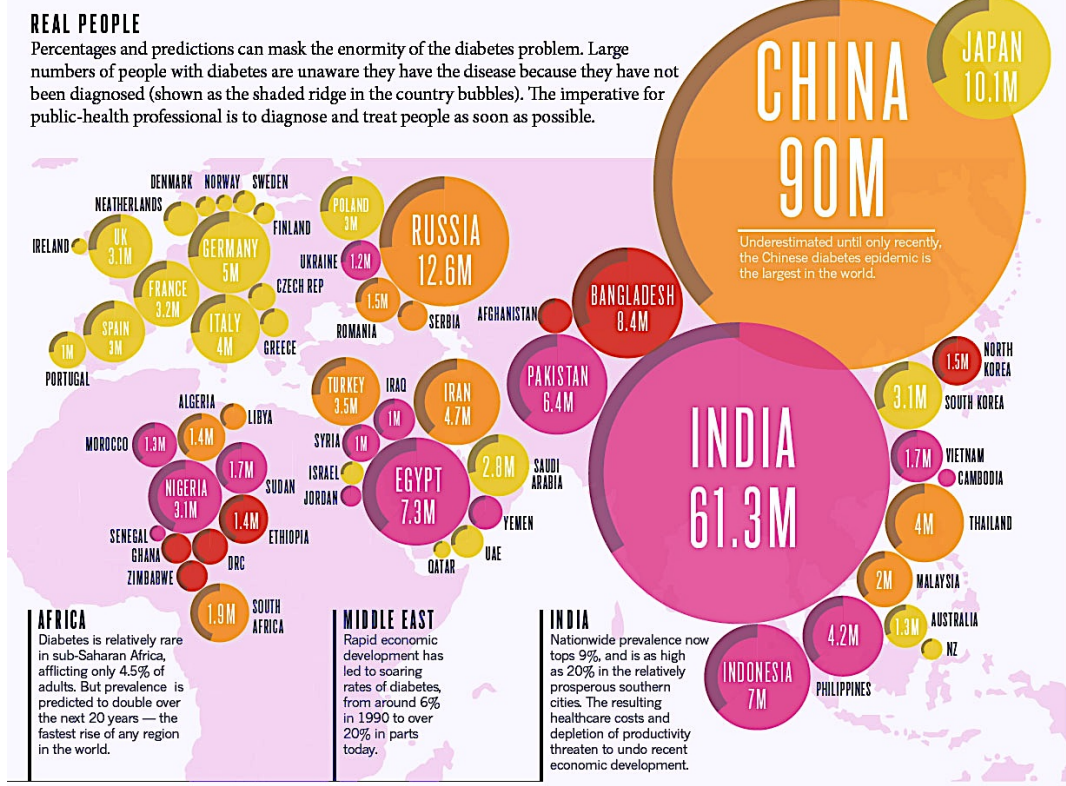
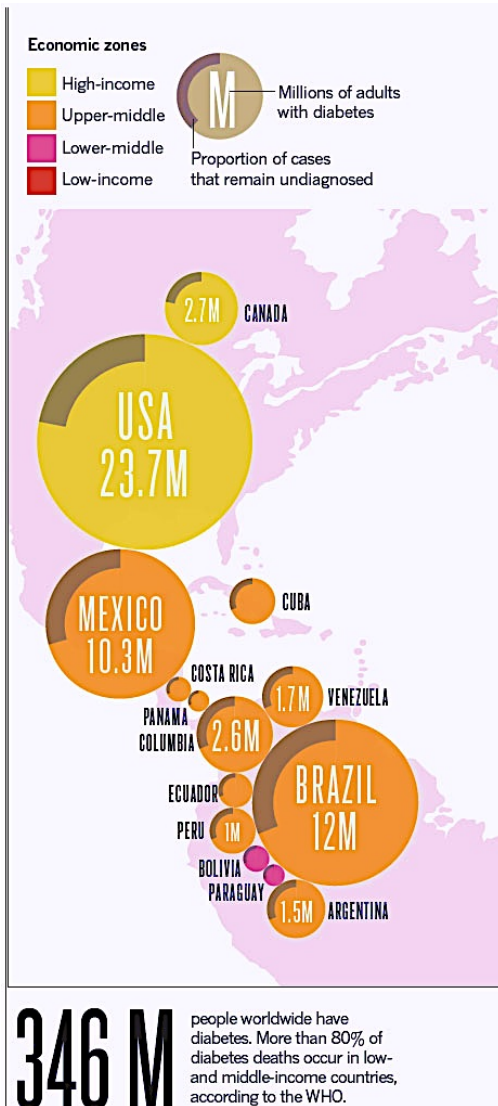
George G. Holz

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*State University of New York
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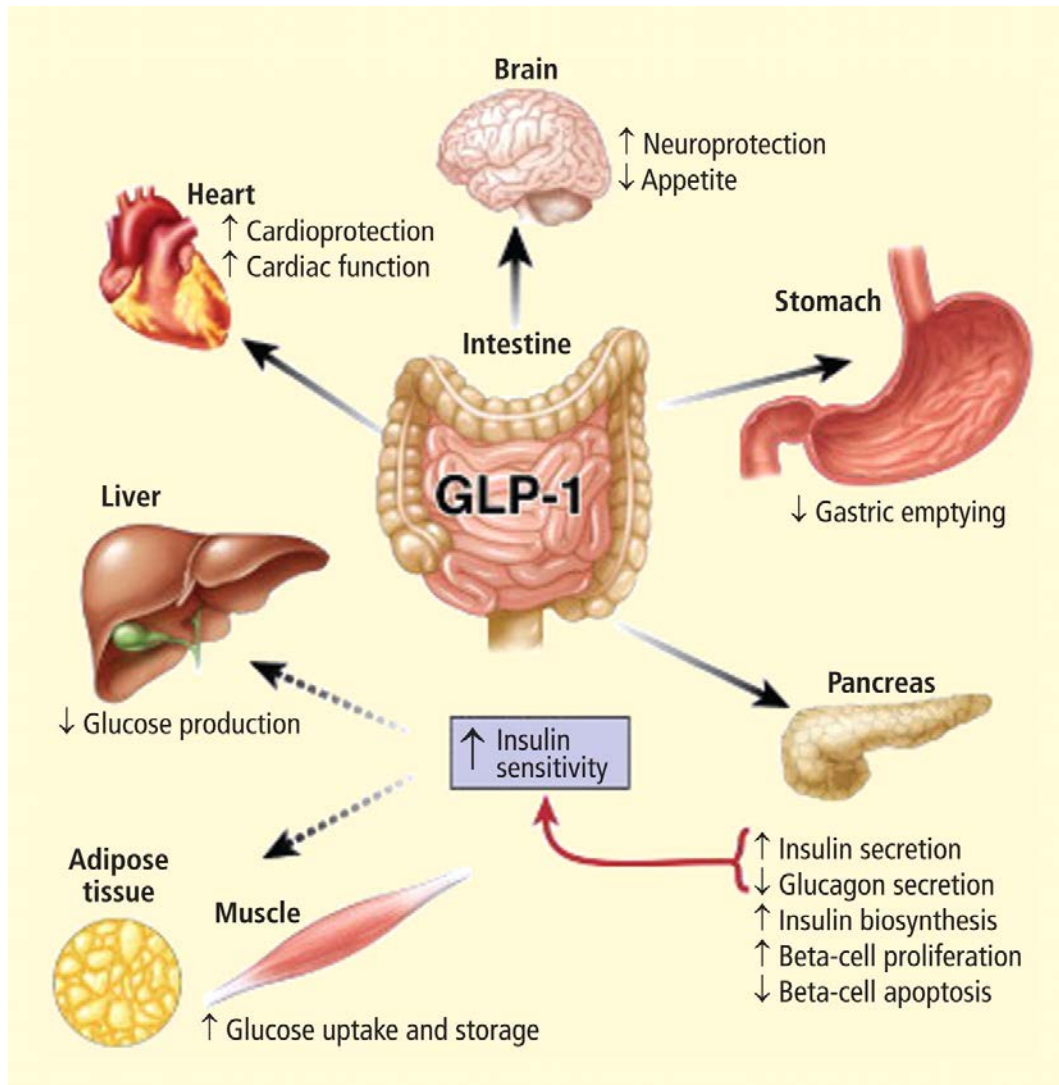


Worldwide Prevalence of Type 2 Diabetes Mellitus (T2DM)

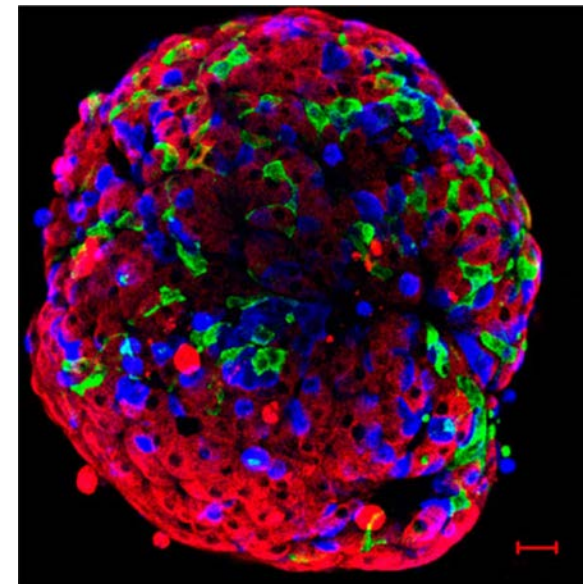


- 346 M people worldwide have diabetes (WHO)
- 7th leading cause of death in 2030 (WHO)
- Global cost in 2014 = \$612 B (\$245 B in US)

A New GLP-1 Based Therapy For Treatment Of T2DM And The Metabolic Syndrome



Mouse Islet of Langerhans



Red: Insulin / β -Cell
 Blue: Glucagon / α -Cell
 Green: Somatostatin / δ -Cell
 — 100 microns

GLP-1

Glucagon-Like Peptide-1-(7-36)-amide

HAEGTFTSDVSSYLEGQAAKEFIAWLVKGR-NH₂

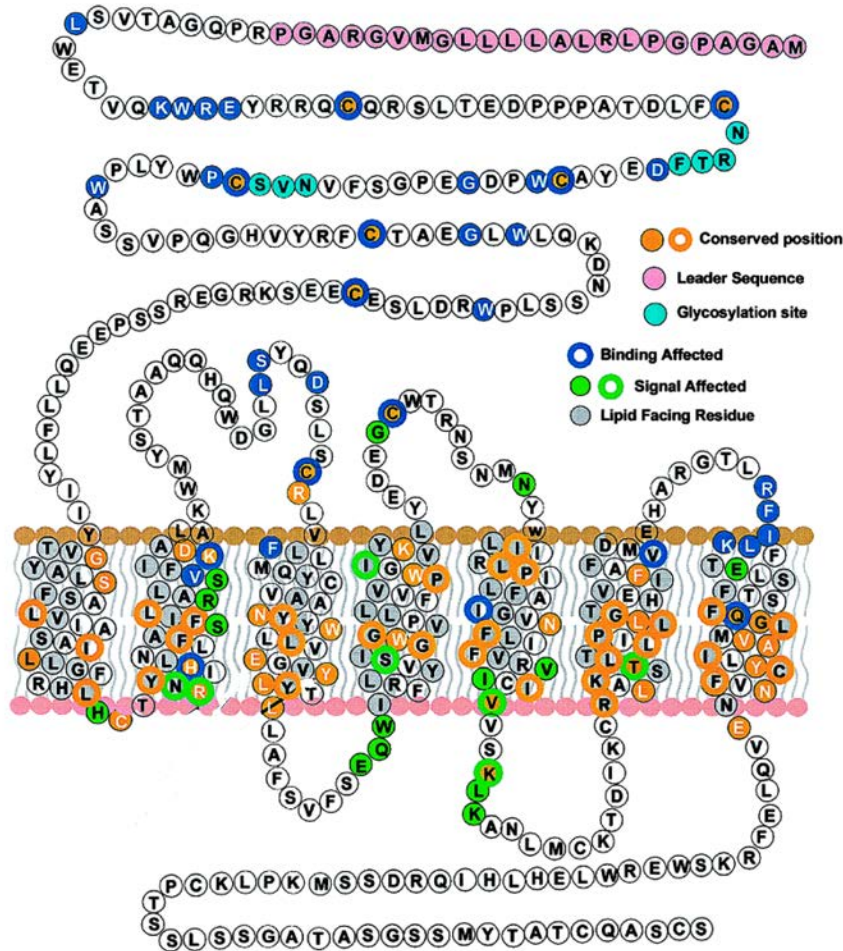
↑ _____ DPP-IV Cleavage Site $t_{1/2}$ 2-5 min (i.v. admin.)

Yellow Indicates Amino Acids Identical To Those In Glucagon

Green Indicates Conserved Amino Acids Found In Glucagon

Red Indicates Amino Acids Not Found Or Conserved In Glucagon

The GLP-1 Receptor Is An Important Target For T2DM Drug Discovery Efforts

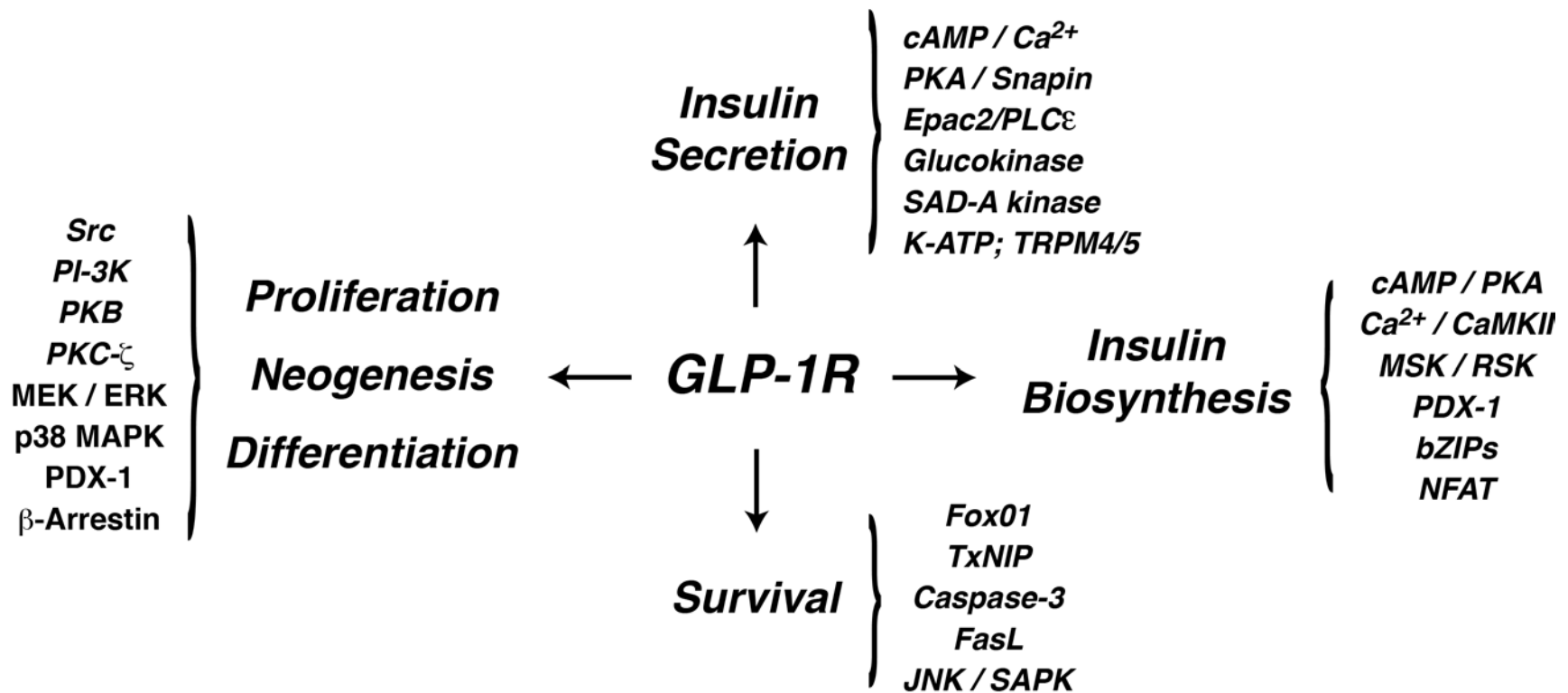


The Actions Of GLP-1 Are Mediated By A “Family B” GPCR Related In Structure To The Glucagon, PTH, Secretin, Calcitonin, PACAP, And VIP Receptors

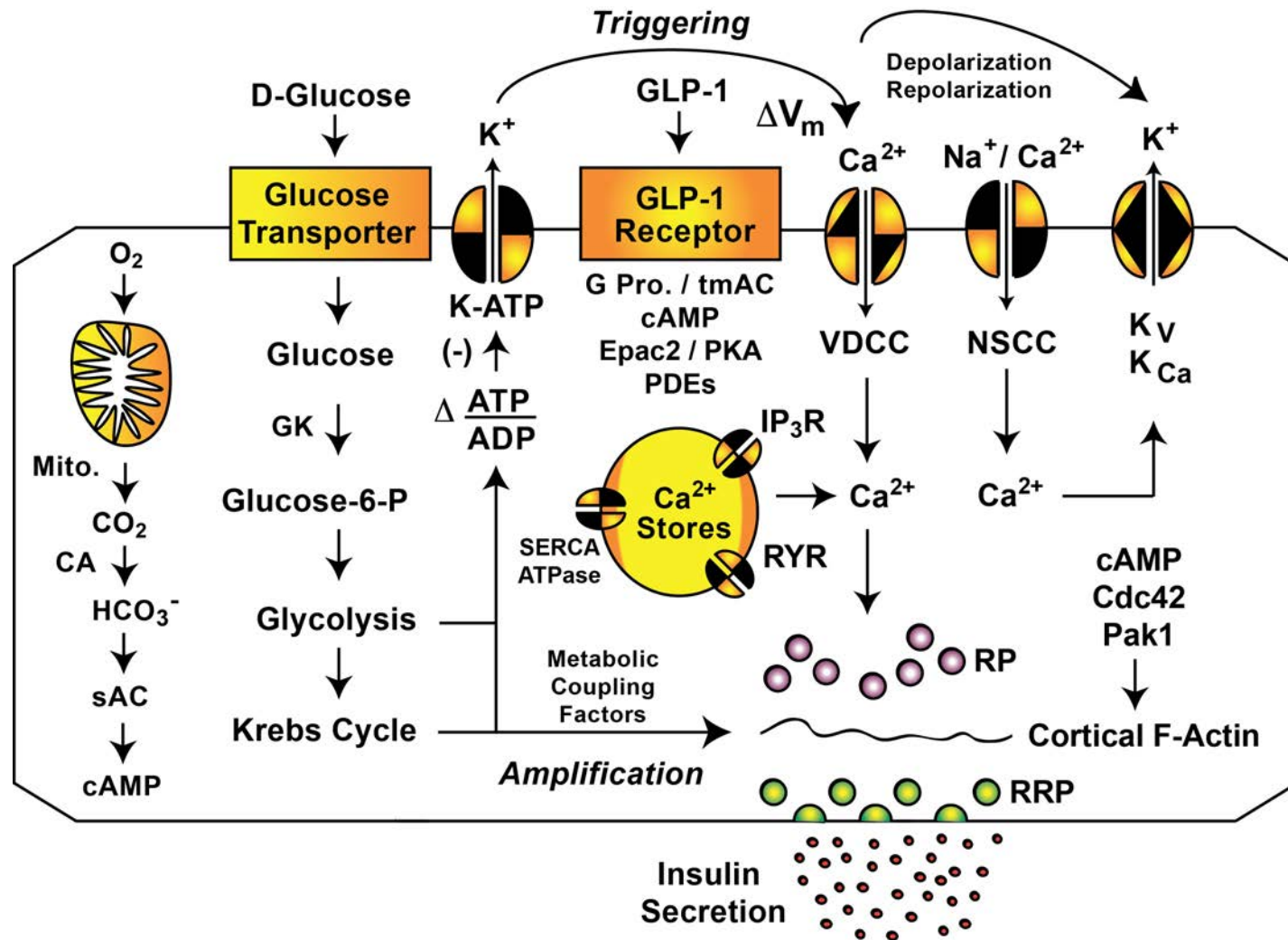
GLP-1R Agonists Used In T2DM Therapeutics Include Bydureon, Victoza, And Trulicity

New “Dual Agonists” Combine GLP-1 With Glucagon Or Other Peptides Such As PYY(3-36)

GLP-1 Receptors Are Coupled To Diverse Signaling Pathways In Pancreatic Beta Cells – Opportunity Exists For GLP-1R “Biased Agonists”

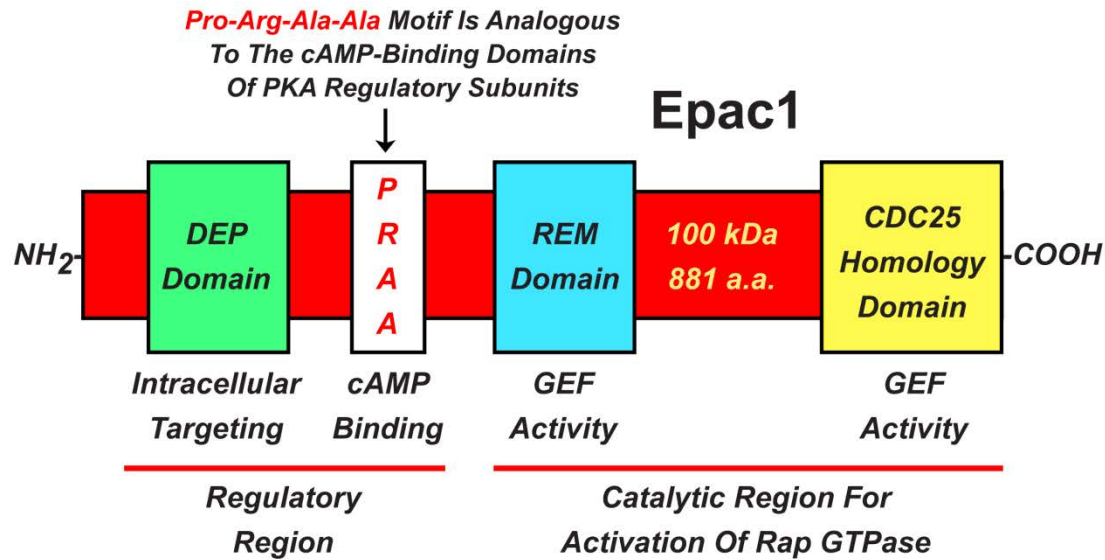


GLP-1R Agonists Potentiate Glucose-Stimulated Insulin Secretion From Pancreatic Beta-Cells

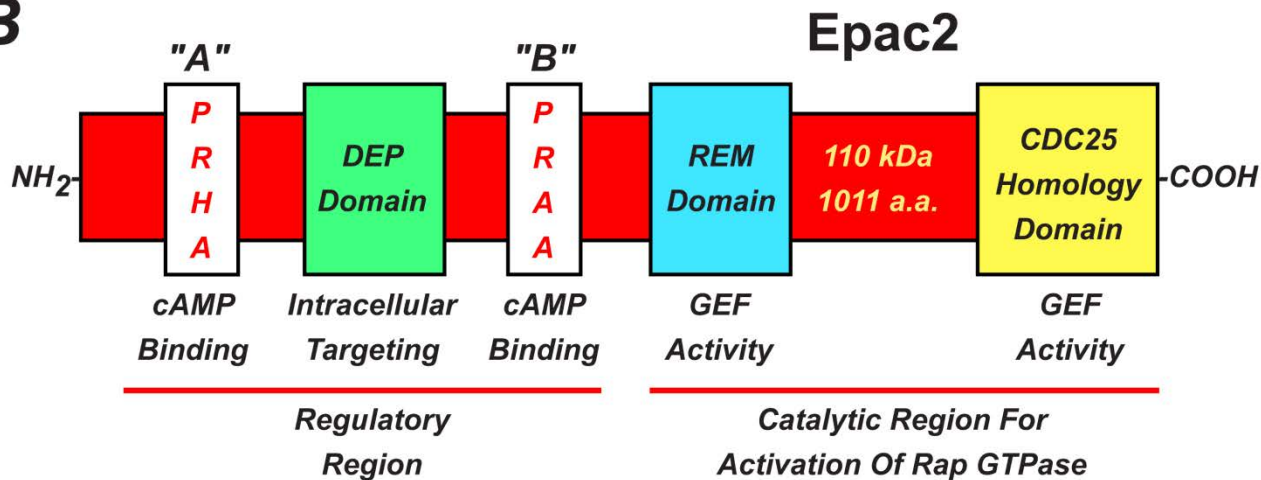


Domain Structures For Epac1 And Epac2 Encoded By RAPGEF3 AND RAPGEF4

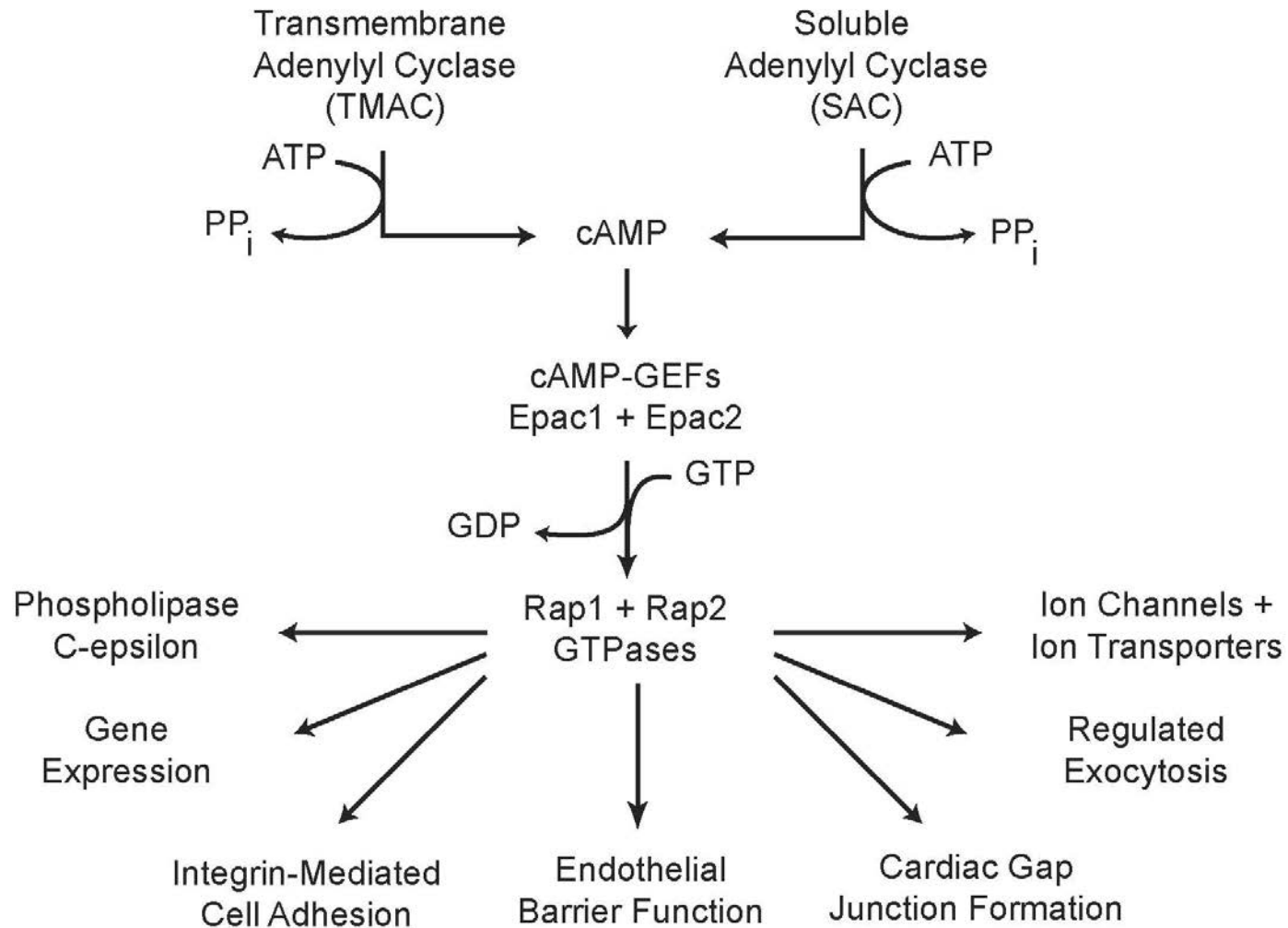
A



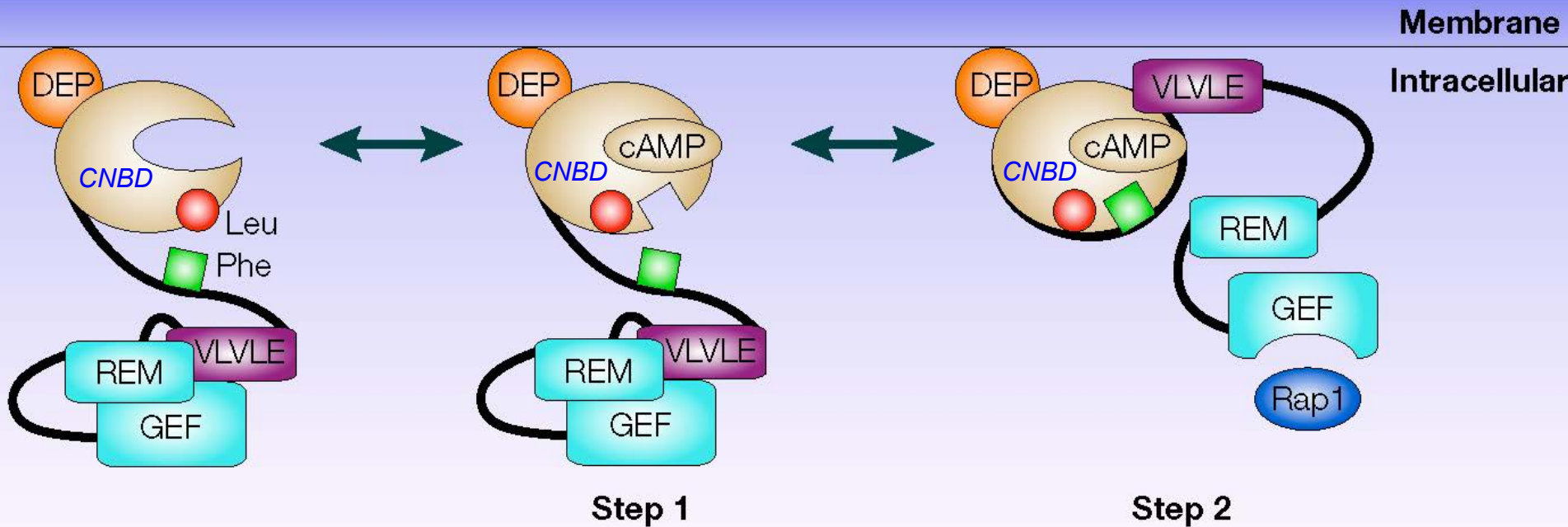
B



Epac1 and Epac2 Are Implicated In The Regulation Of Multiple Cellular Functions



Activation Of Epac GEF Activity Is A Process Of Disinhibition Initiated By cAMP Binding



*Autoinhibited,
No GEF Activity,
No Interaction
With Rap1*

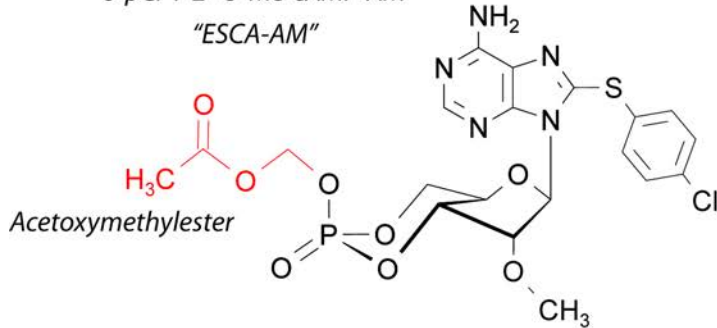
Binding Of cAMP

*Disinhibition Of GEF
Activity And Association
of Epac With Rap1*

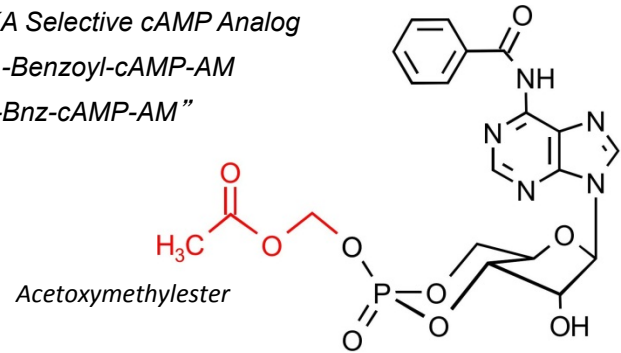
CNBD : Cyclic Nucleotide-Binding Domain

AM-Esters Of cAMP Analogs Are Selective Activators Of Epac And PKA

Epac Selective cAMP Analog
8-pCPT-2'-O-Me-cAMP-AM
"ESCA-AM"

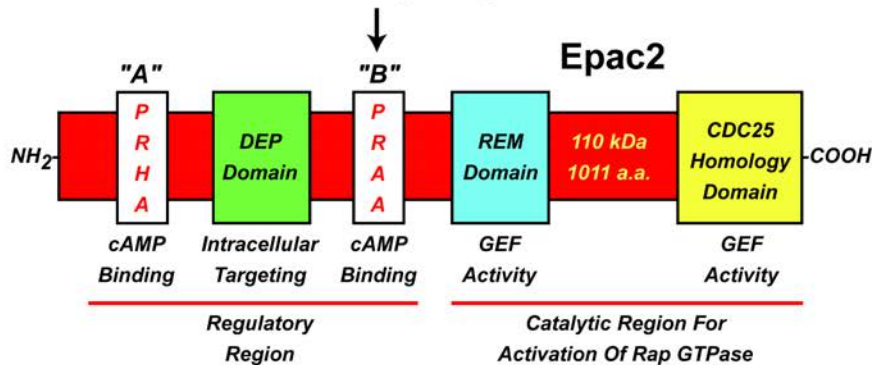


PKA Selective cAMP Analog
N⁶-Benzoyl-cAMP-AM
"6-Bnz-cAMP-AM"

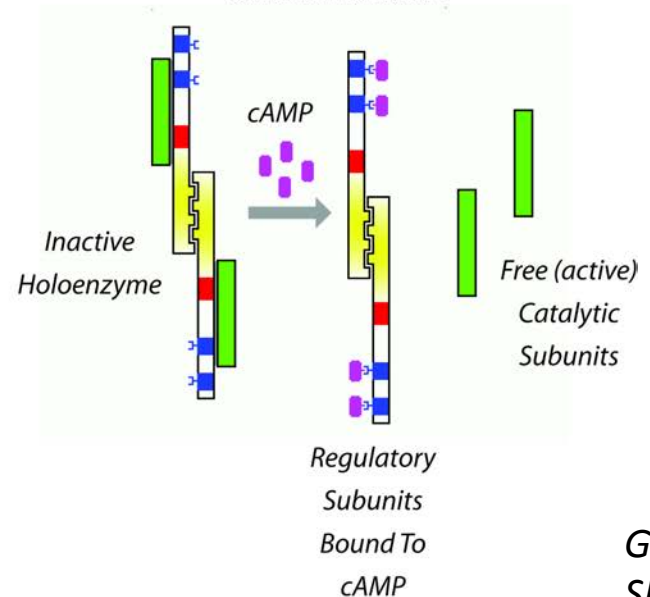


cAMP-Dependent Activation
of Epac1 + Epac2

PRAA Motif Is Analogous To The cAMP-Binding
Domains Of PKA Regulatory Subunits

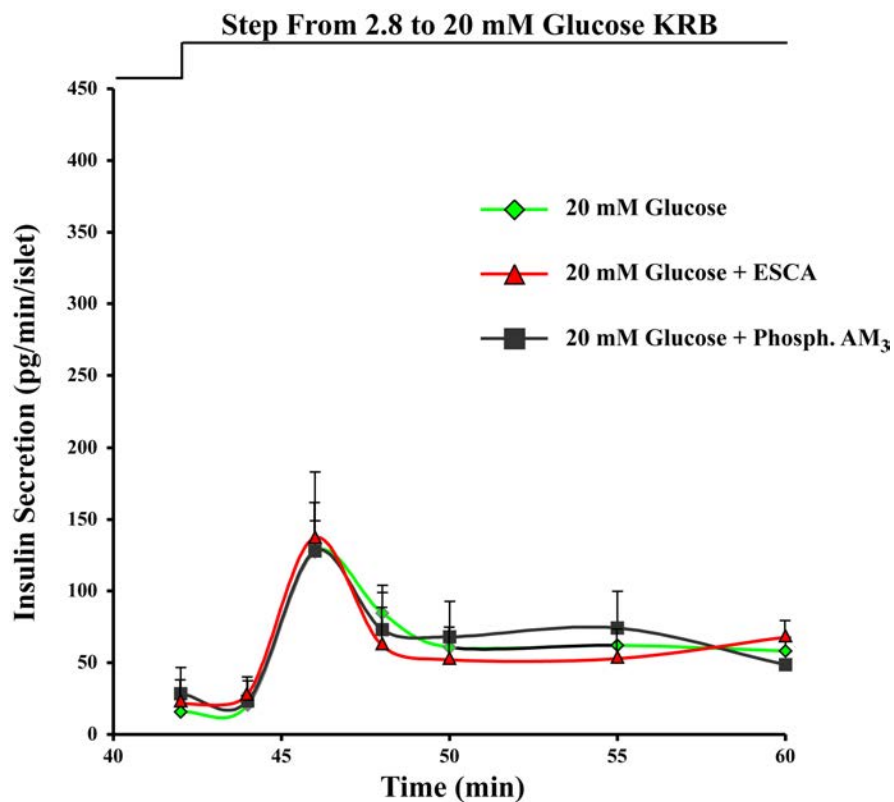


cAMP-Dependent Activation
of Protein Kinase A

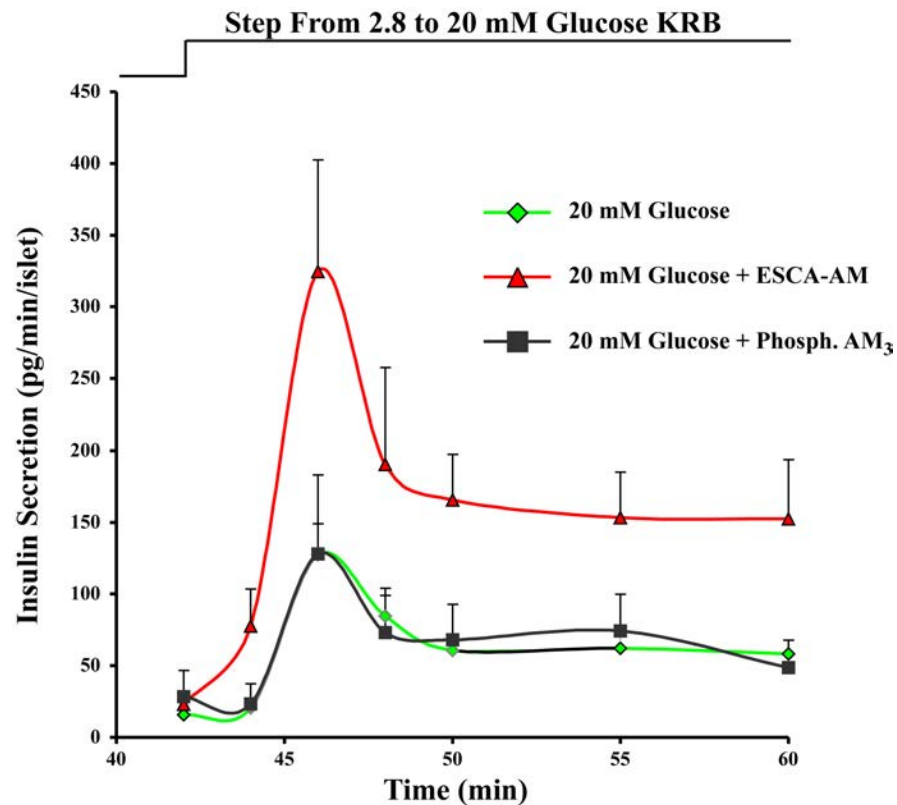


Differential Insulin Secretagogue Properties Of A Non-AM-Ester And An AM-Ester Of 8-pCPT-2'-O-Me-cAMP In Mouse Islets

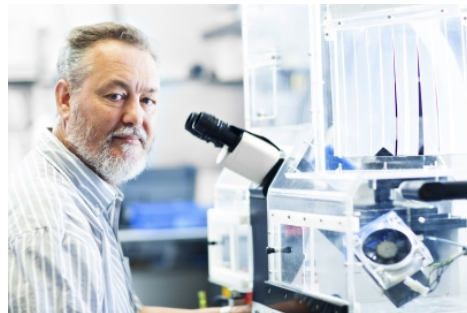
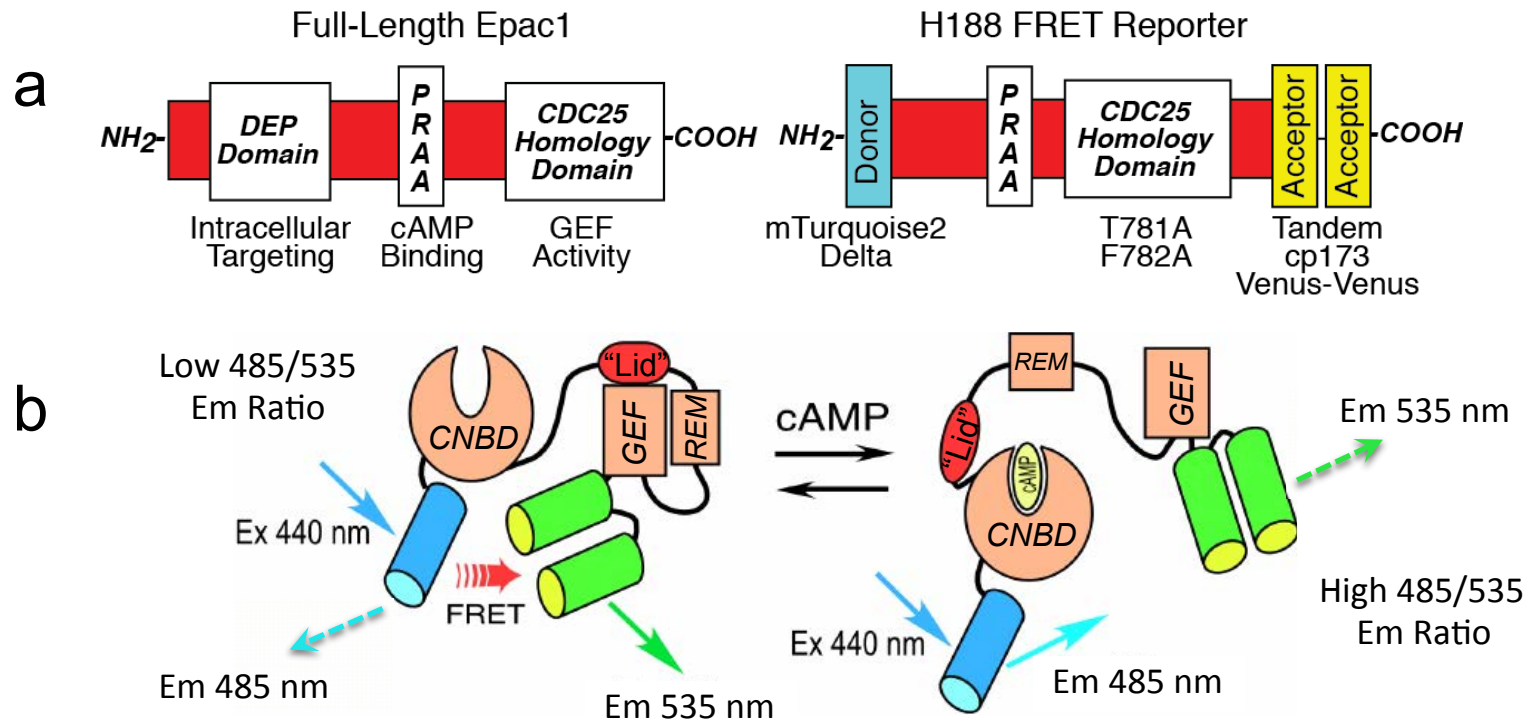
Non-AM-Ester



AM-Ester



Epac1-Based FRET Reporter H188 For Detection Of cAMP In Clonal HEK293-H188-C24 Cells

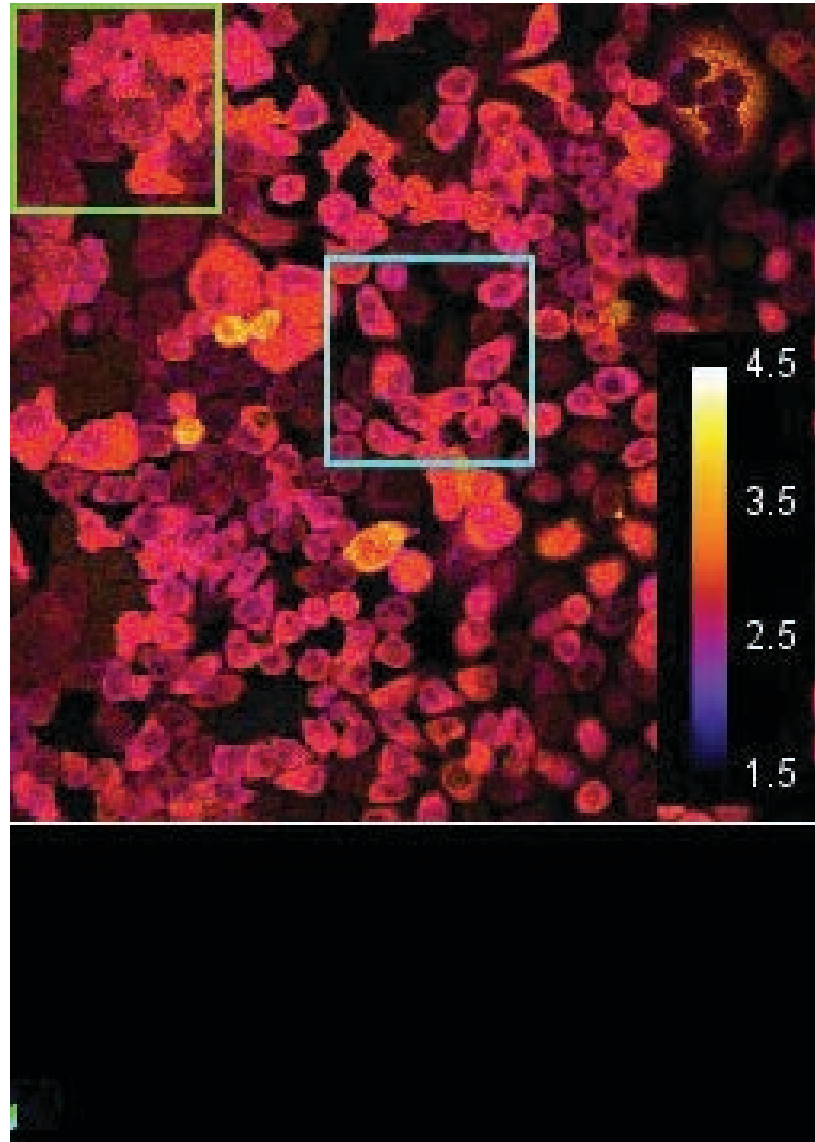
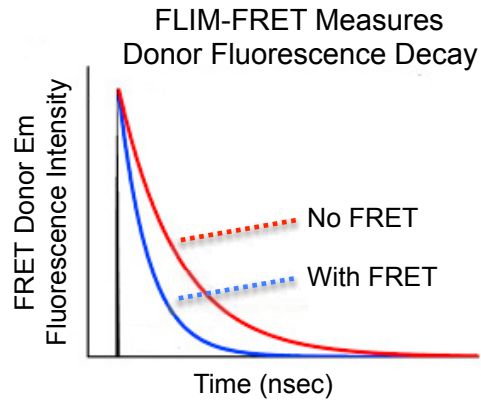


Prof. Kees Jalink
(Netherlands Cancer Inst.)

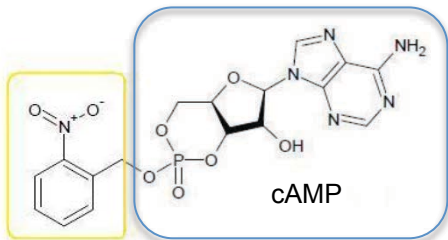


Jeffrey Klarenbeek
(Jalink Lab)

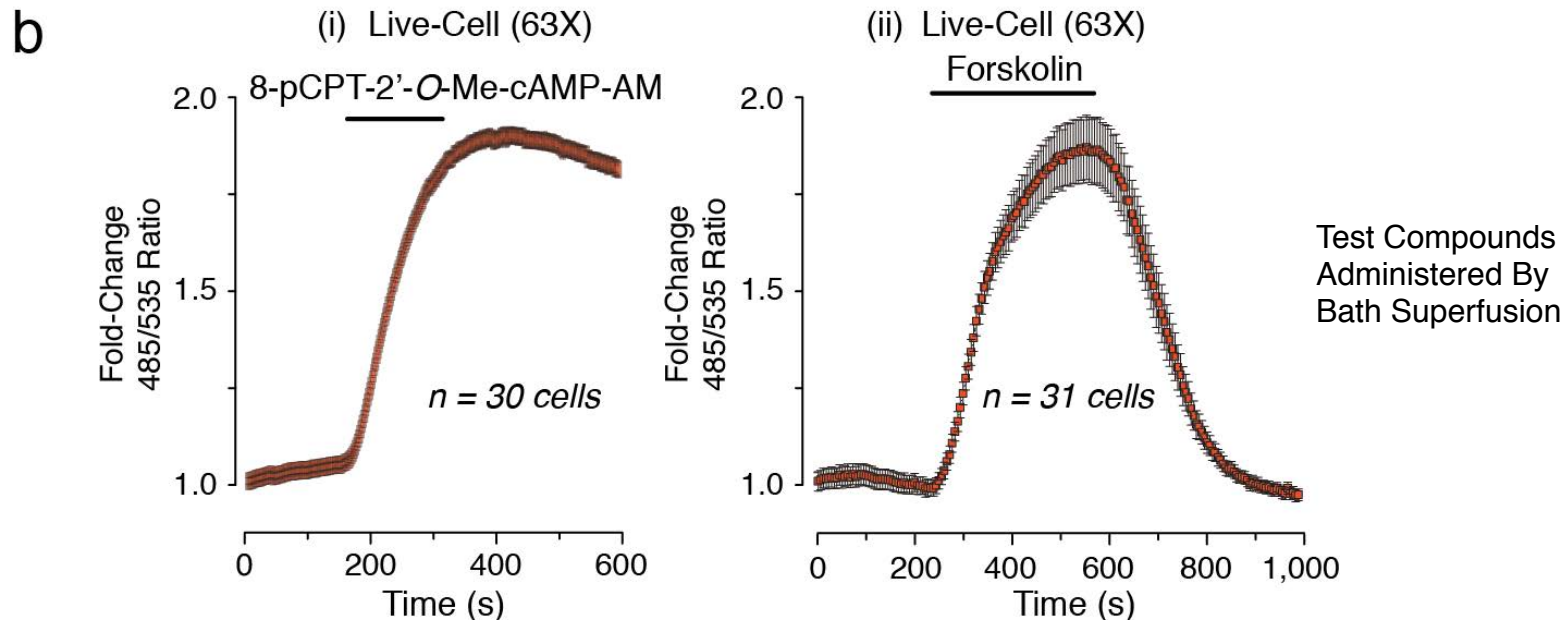
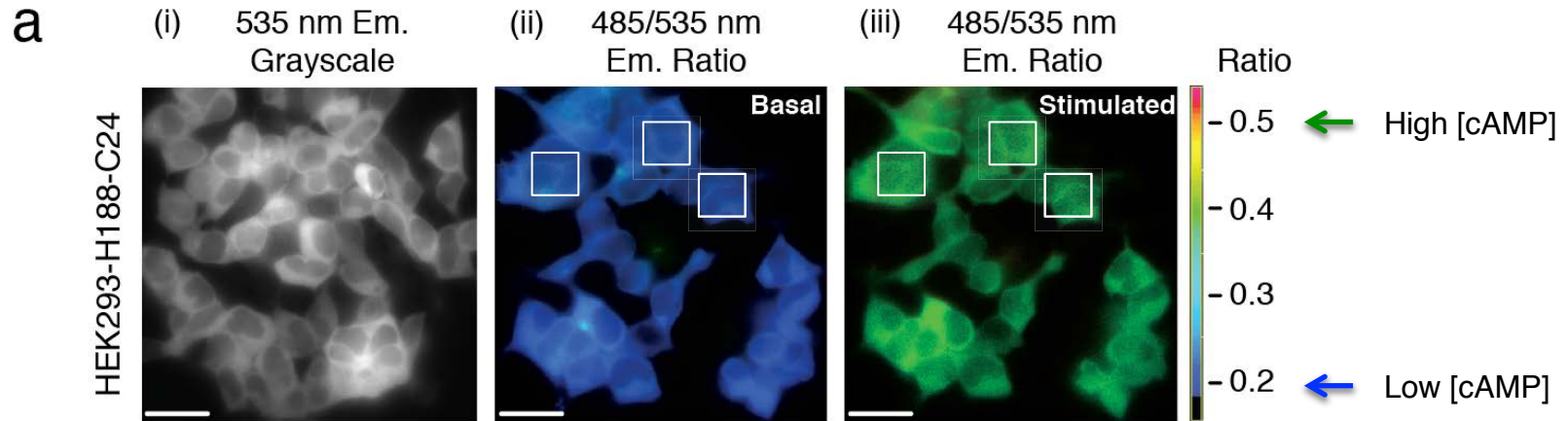
FLIM-FRET Live-Cell Imaging During UV Flash Photolysis Of Caged cAMP In HeLa Cells



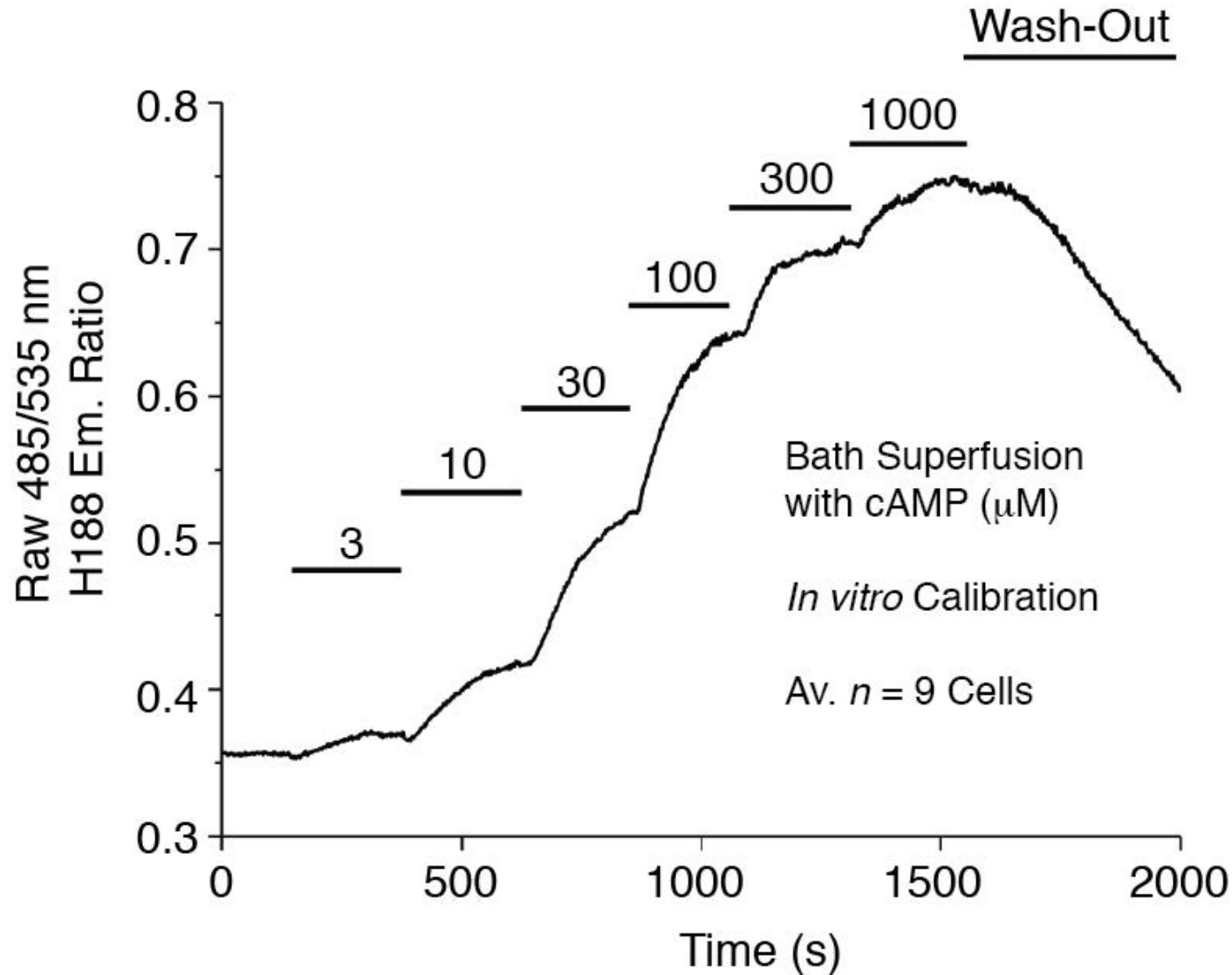
NPE-cAMP
"Caged cAMP"



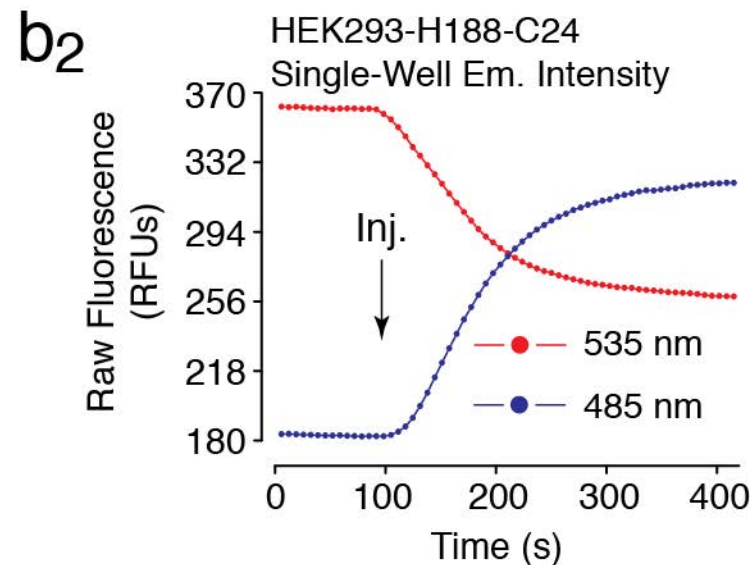
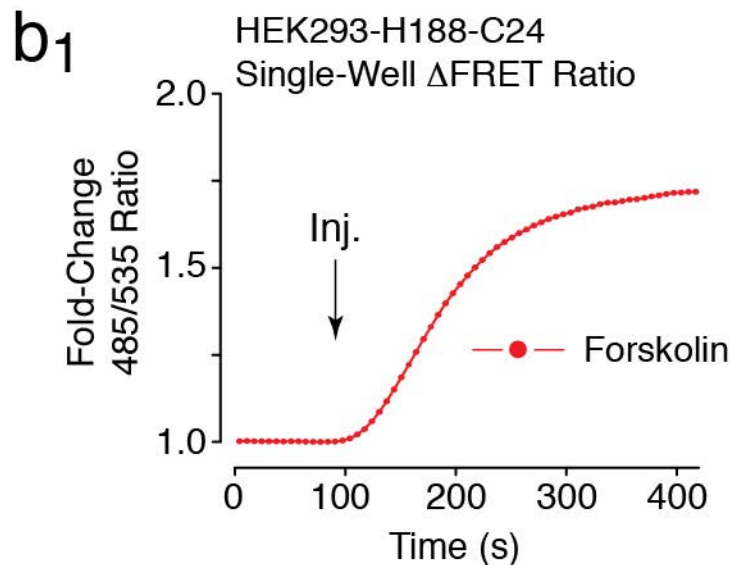
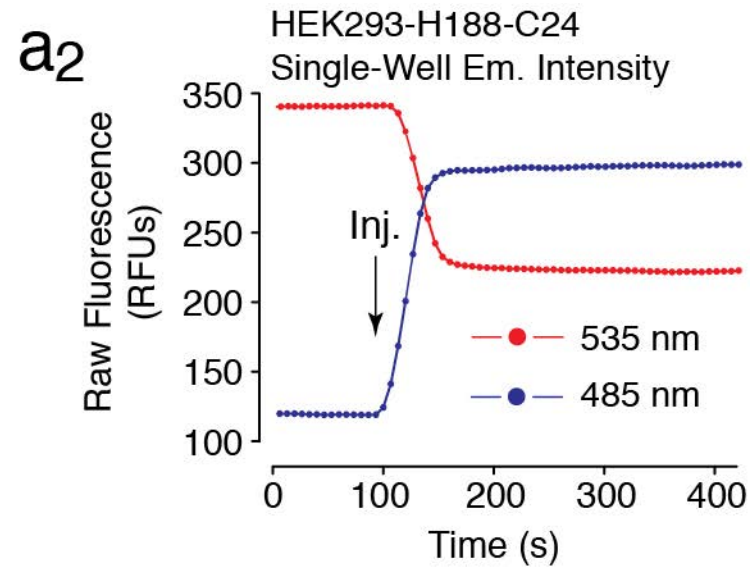
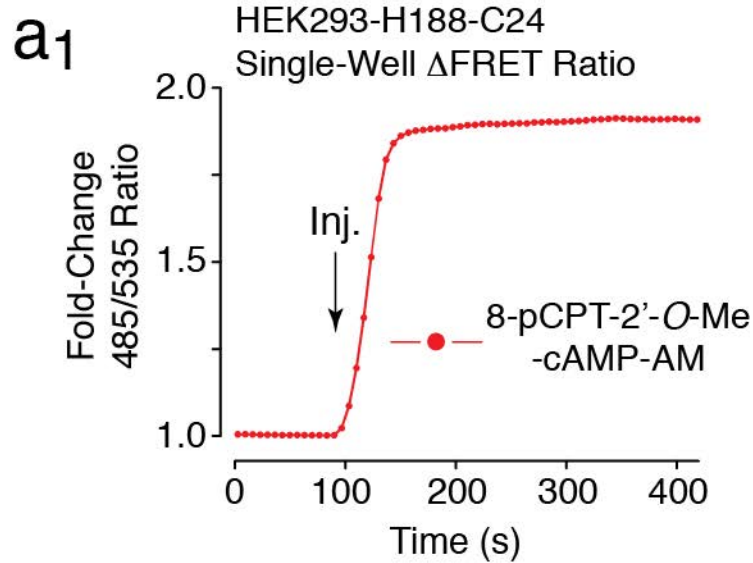
Ratiometric Live-Cell Imaging Of FRET In Clusters Of HEK293-H188-C24 Cells



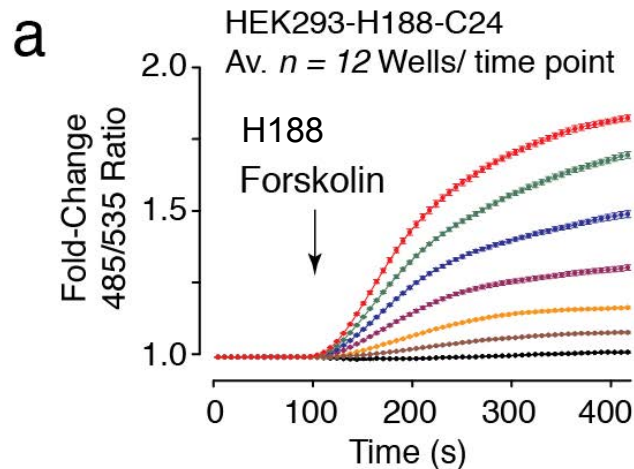
Live-Cell Calibration Of H188 cAMP Sensitivity In Digitonin-Permeabilized HEK293-H188-C24 Cells



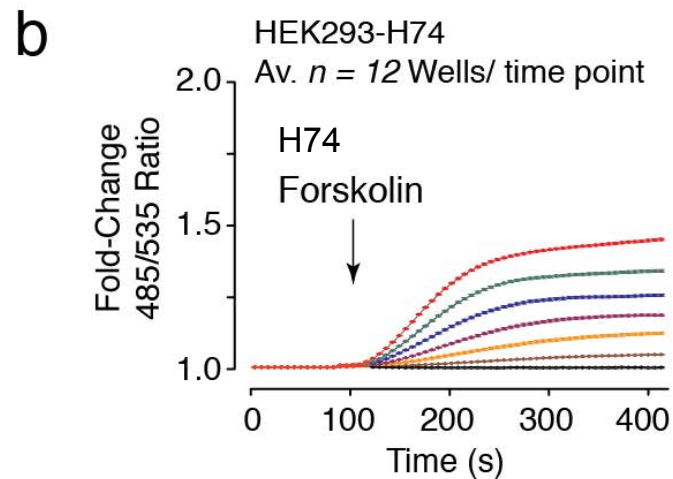
Microplate Reader Single-Well Detection Of FRET In HEK293-H188-C24 Cells



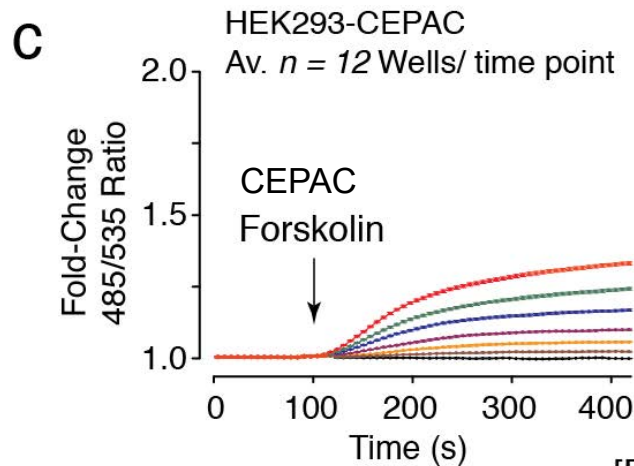
Improved Performance Of H188 Compared To Earlier Epac1-Based FRET Reporters



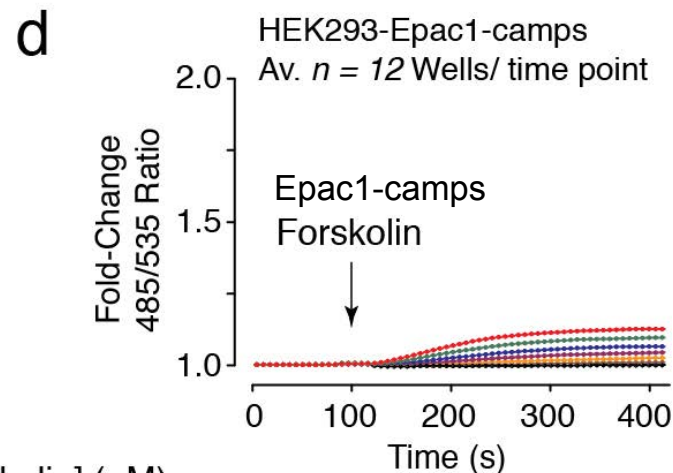
H188
Klarenbeek et al.
PLoS 2015



H74
Klarenbeek et al.
PLoS 2011



CEPAC
Salonikidis et al.
JBC 2011

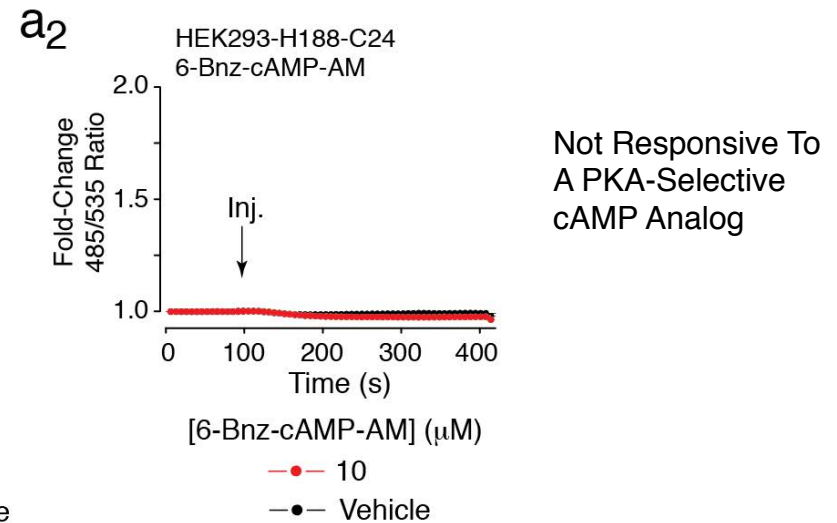
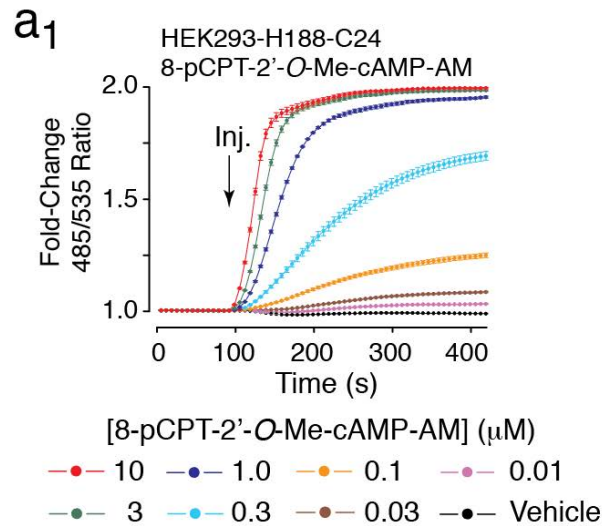


Epac1-camps
Nikolaev et al.
JBC 2004



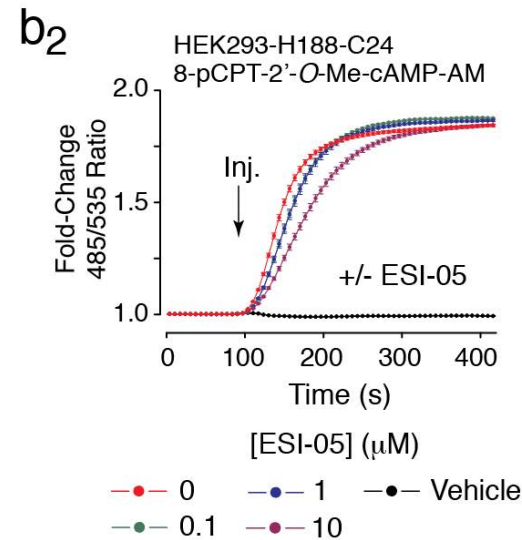
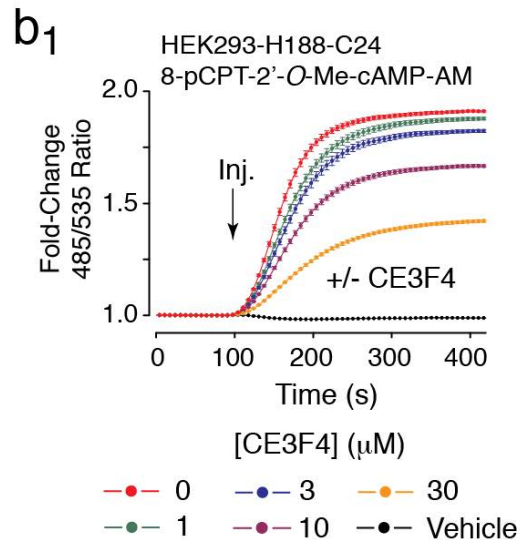
H188 Provides A Platform For Rapid Screening Of Small Molecule Epac1 Activators And Inhibitors

Responsive To An Epac-Selective cAMP Analog



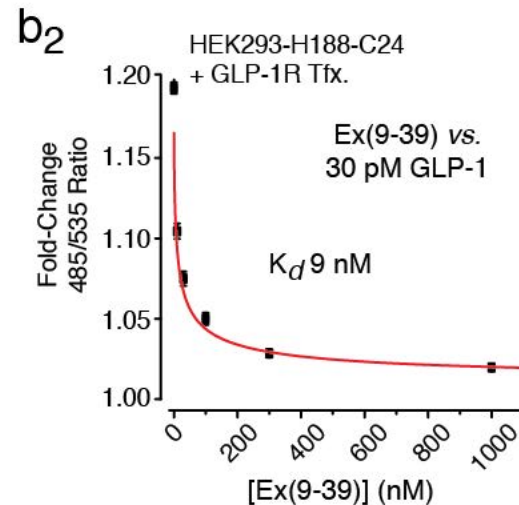
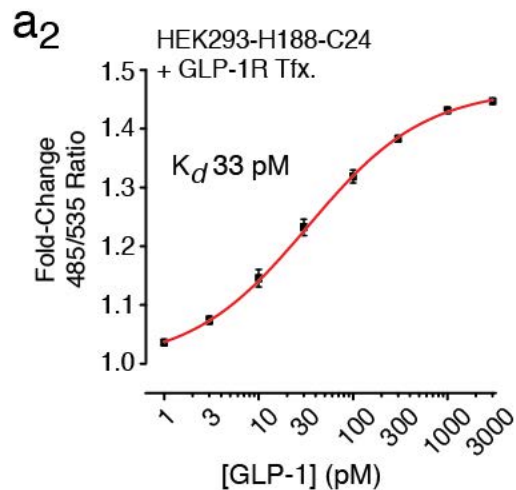
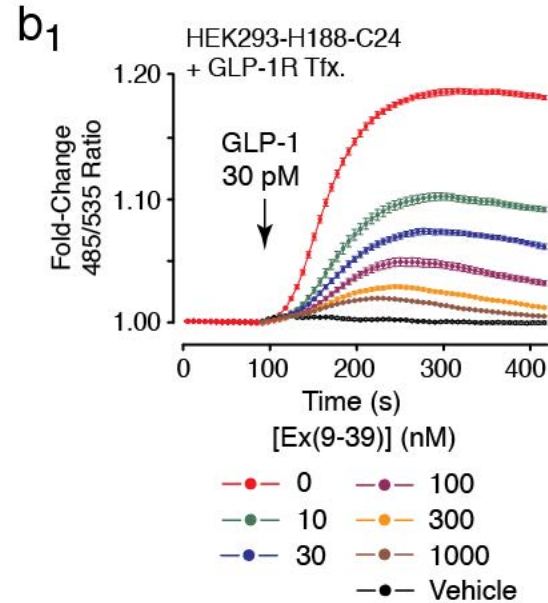
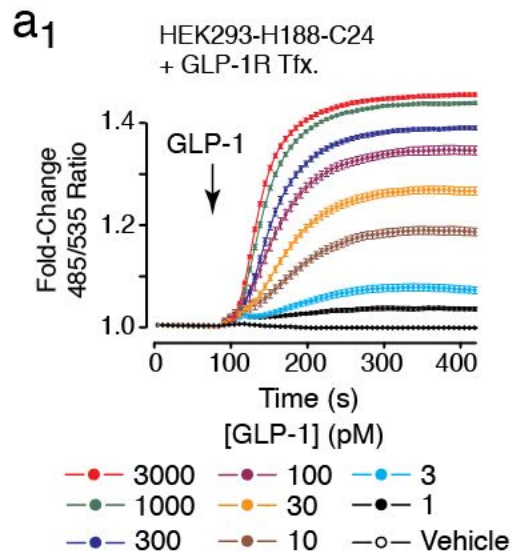
Not Responsive To A PKA-Selective cAMP Analog

Responsive To A Specific Epac1 Inhibitor

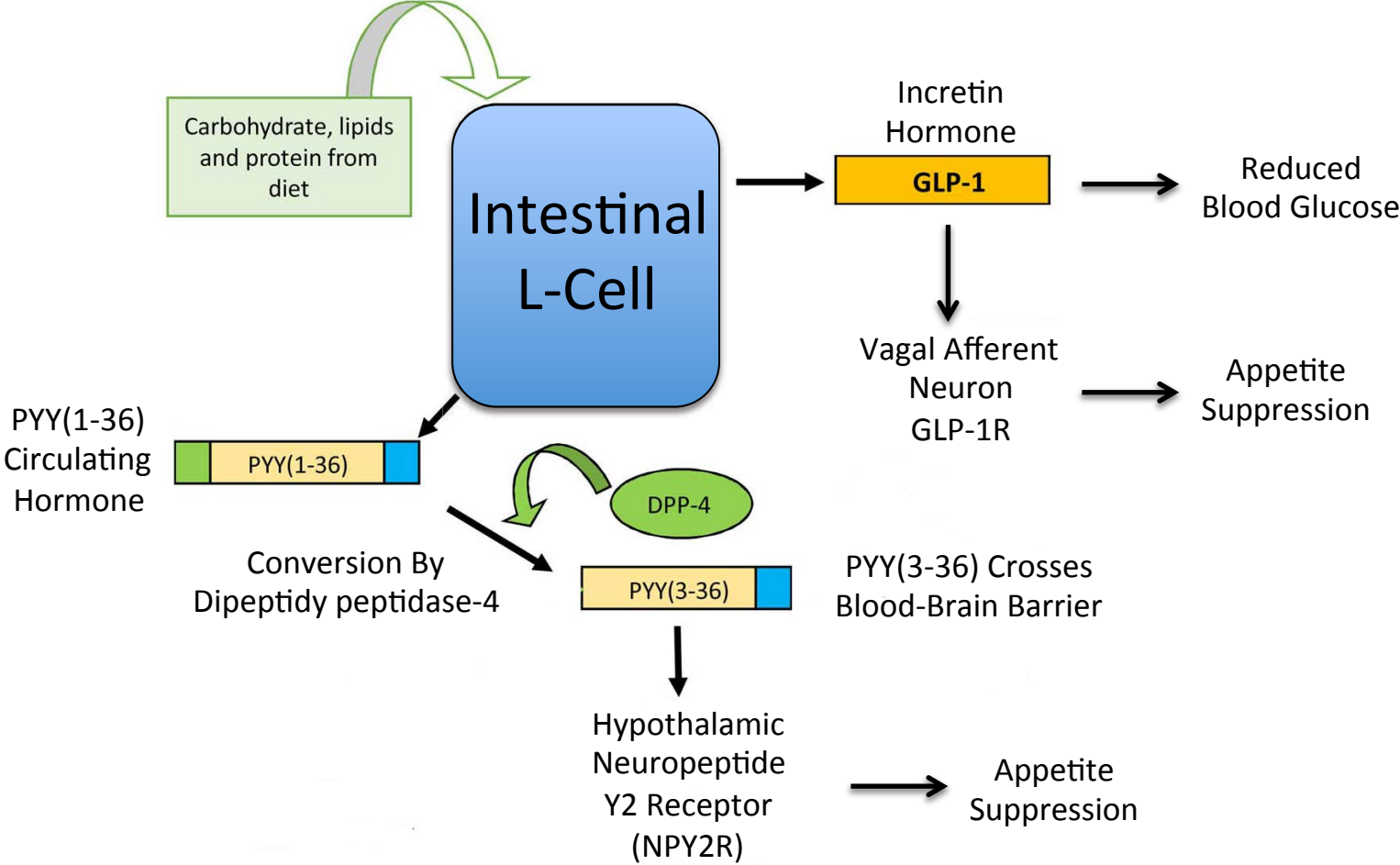


Not Responsive To A Specific Epac2 Inhibitor

Real-Time Analysis Of GLP-1 Receptor Agonist And Antagonist Action In HEK293-H188-C24 Cells



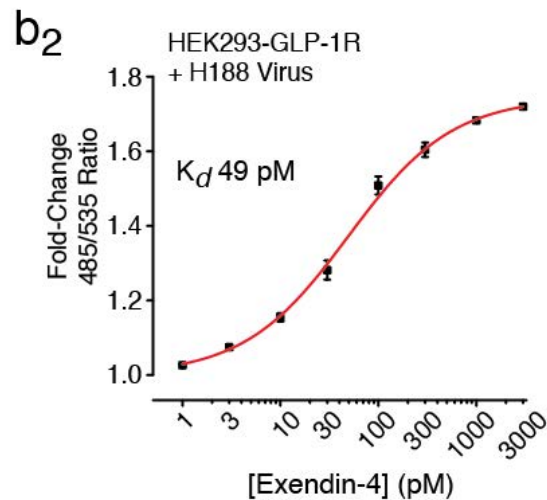
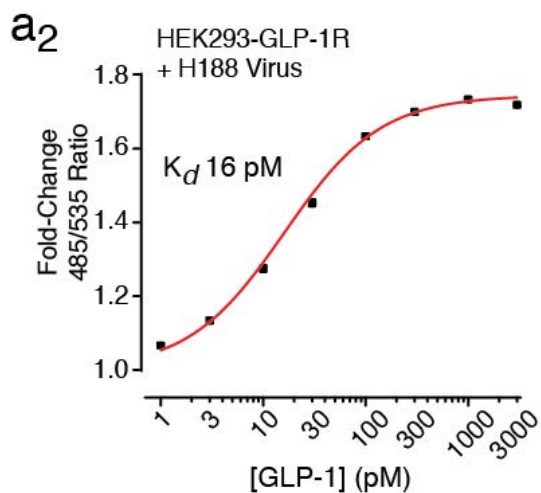
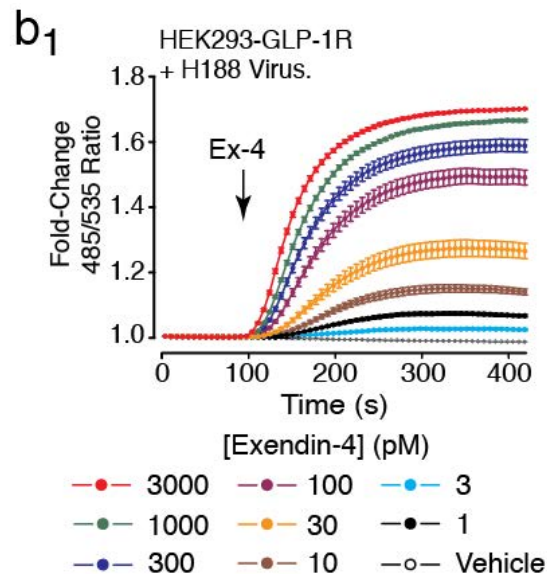
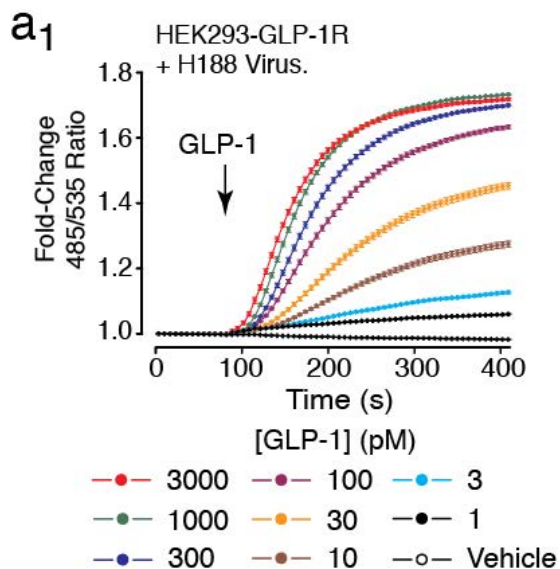
Rationale For A “Dual Agonist” Strategy Using GLP-1 And PYY(3-36) To Treat T2DM And Obesity



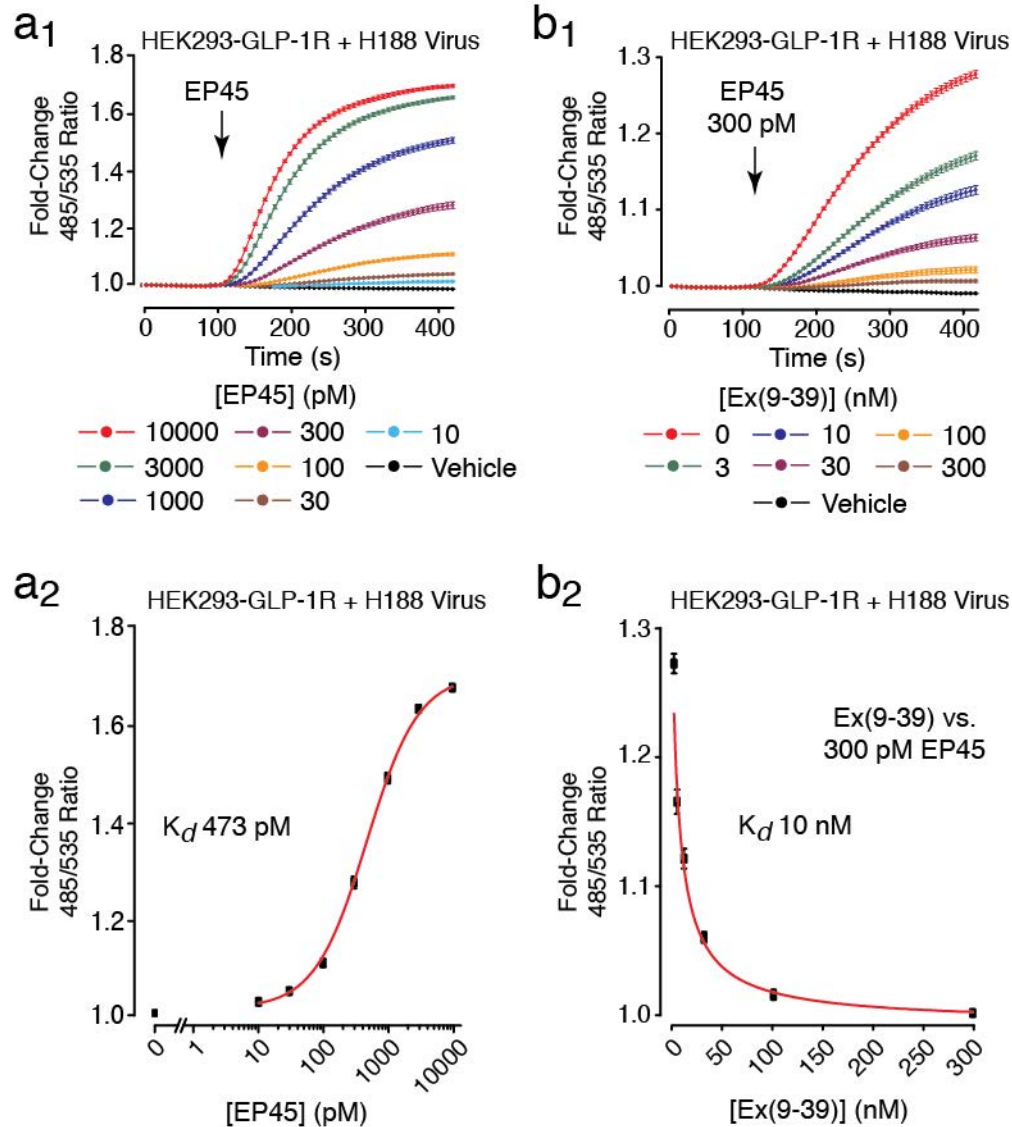
Chimeric Peptide EP45 Incorporates Amino Acid Motifs Present Within GLP-1R Agonist Exendin-4 And NPY2R Agonist PYY(3-36)

Peptide	Sequence	AA
PYY(1-36)	YPIKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH2	36
PYY(3-36)	IKPEAPGEDASPEELNRYYASLRHYLNLVTRQRY-NH2	34
EP45	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSRHYLNLVTRQRY-NH2	45
Ex-4	HGEGTFTSDLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH2	39
Ex(9-39)	DLSKQMEEEAVRLFIEWLKNGGPSSGAPPPS-NH2	31
GLP-1	HAEGTFTSDVSSYLEGQAAKEFIAWLKVG-NH2	30
Glucagon	HSQGTFTSDYSKYLDLRRAQDFVQWLMNT	29
GIP	YAEGTFISDYSIAMDKIHQQDFVNWLLAQKGGKNDWKHNITQ	42

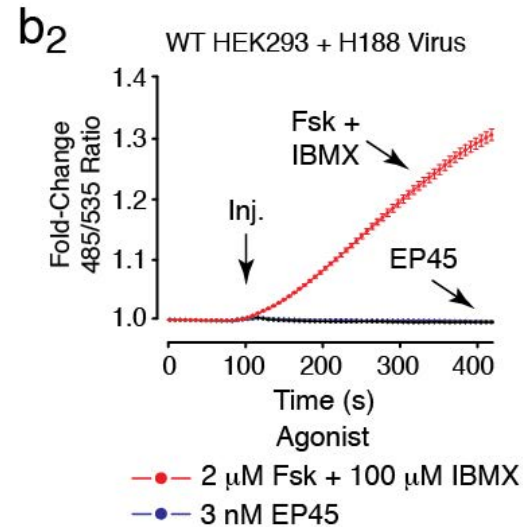
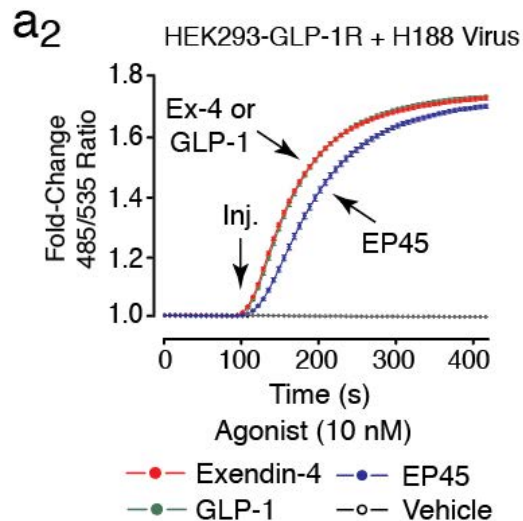
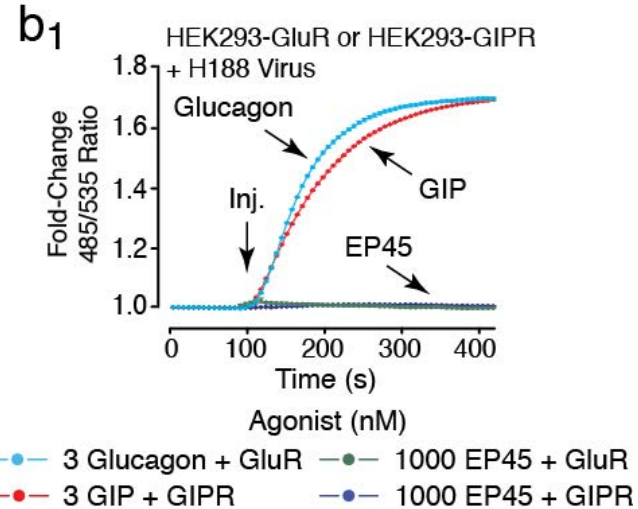
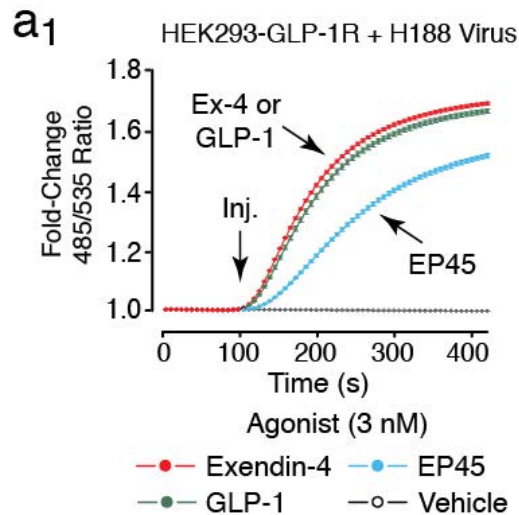
H188 Adenoviral Transduction For Evaluation Of GLP-1 Receptor Agonist Action In HEK293-GLP-1R Cells



GLP-1 Receptor Agonist Properties Of Chimeric Peptide EP45 In HEK293-GLP-1R Cells

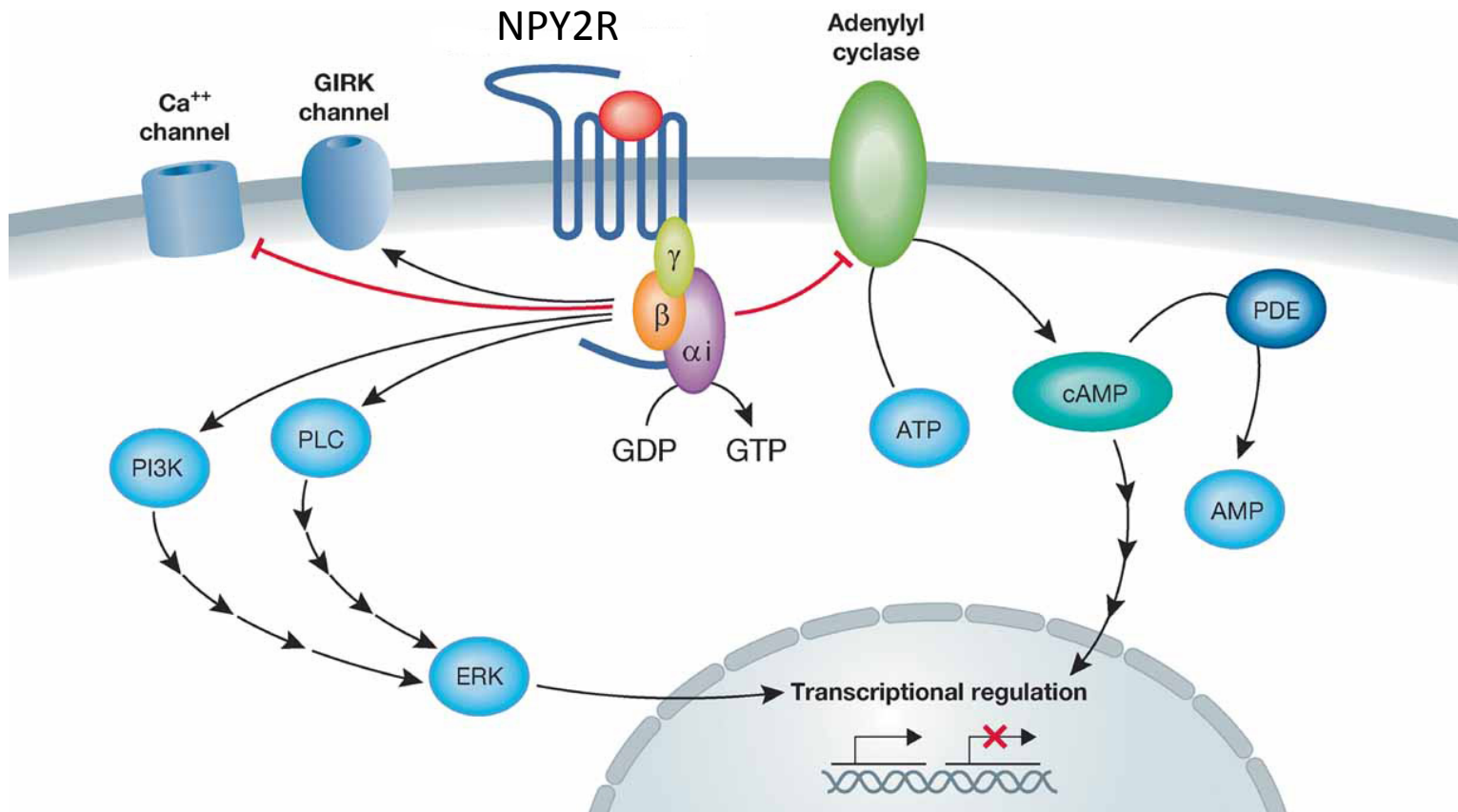


Chimeric Peptide EP45 Is A Full Agonist At The GLP-1R But Fails To Activate Glucagon Or GIP Receptors

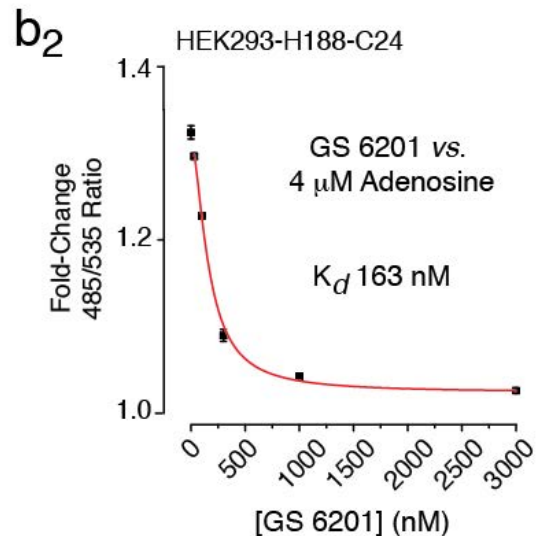
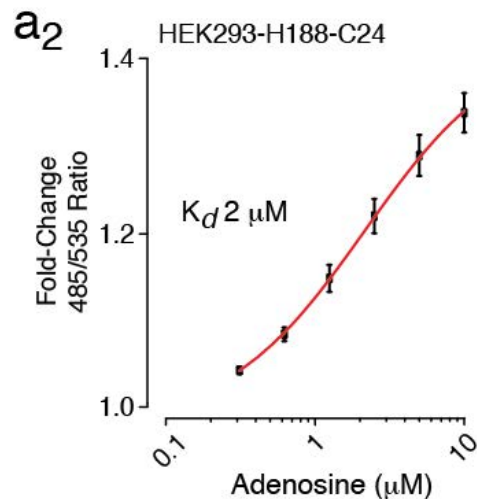
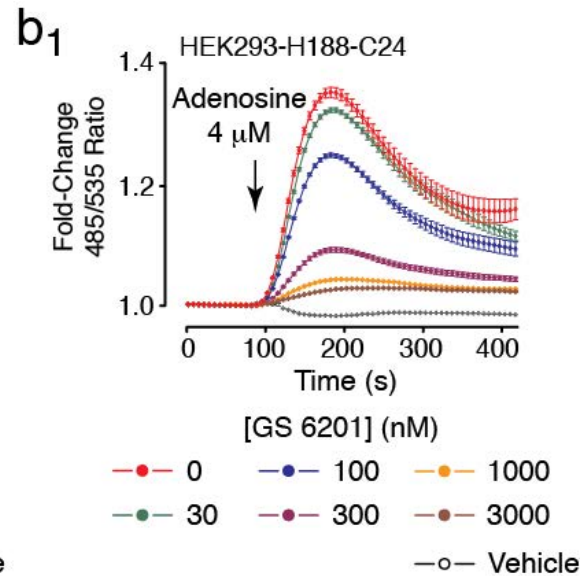
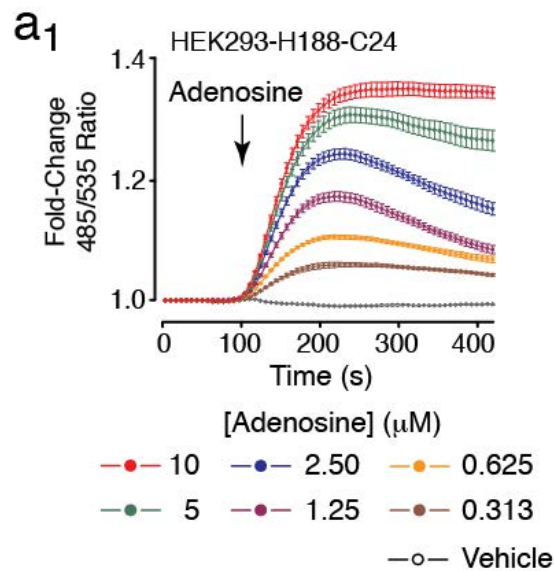


NPY2R Is A "Family A" GPCR That Negatively Regulates cAMP Production

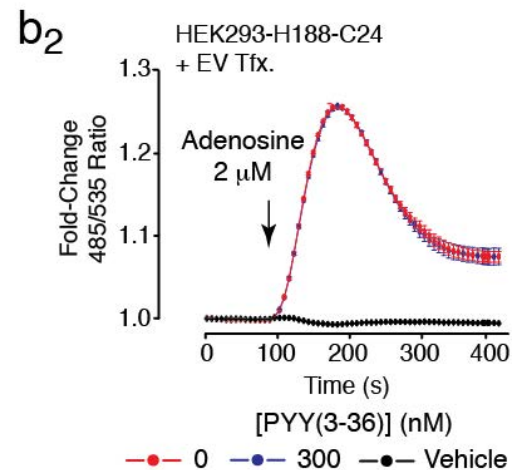
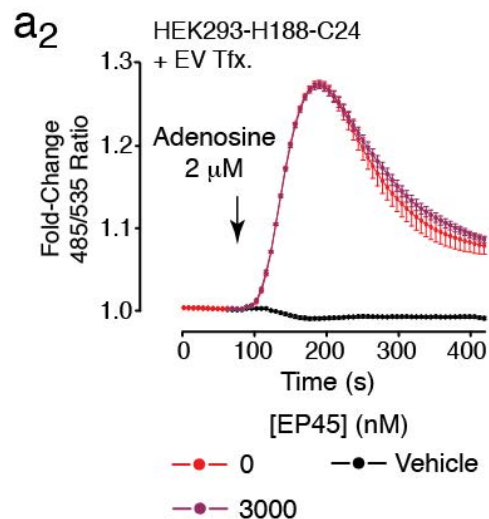
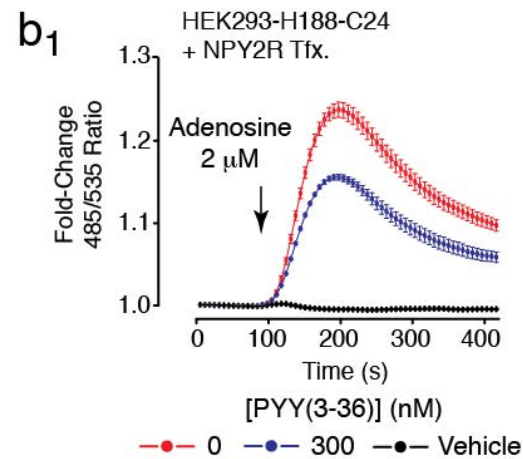
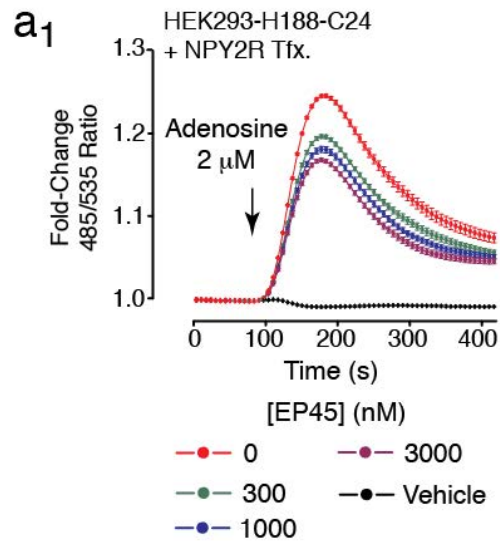
Does EP45 Act As An NPY2R Agonist To Lower Levels Of cAMP In The FRET Assay?



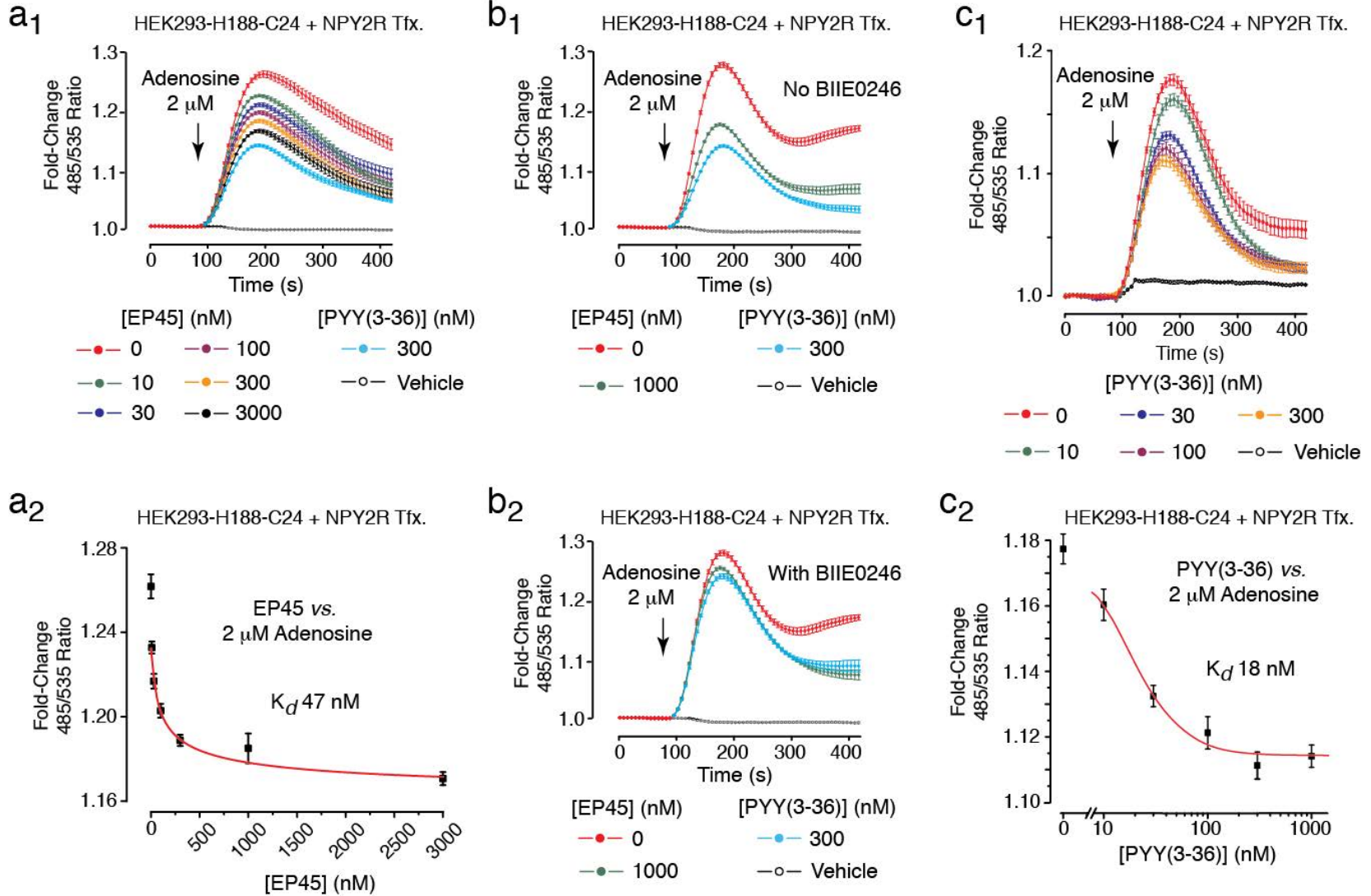
A_{2B} Receptors Mediate The Action Of Adenosine To Stimulate cAMP Production In HEK293-H188-C24 Cells



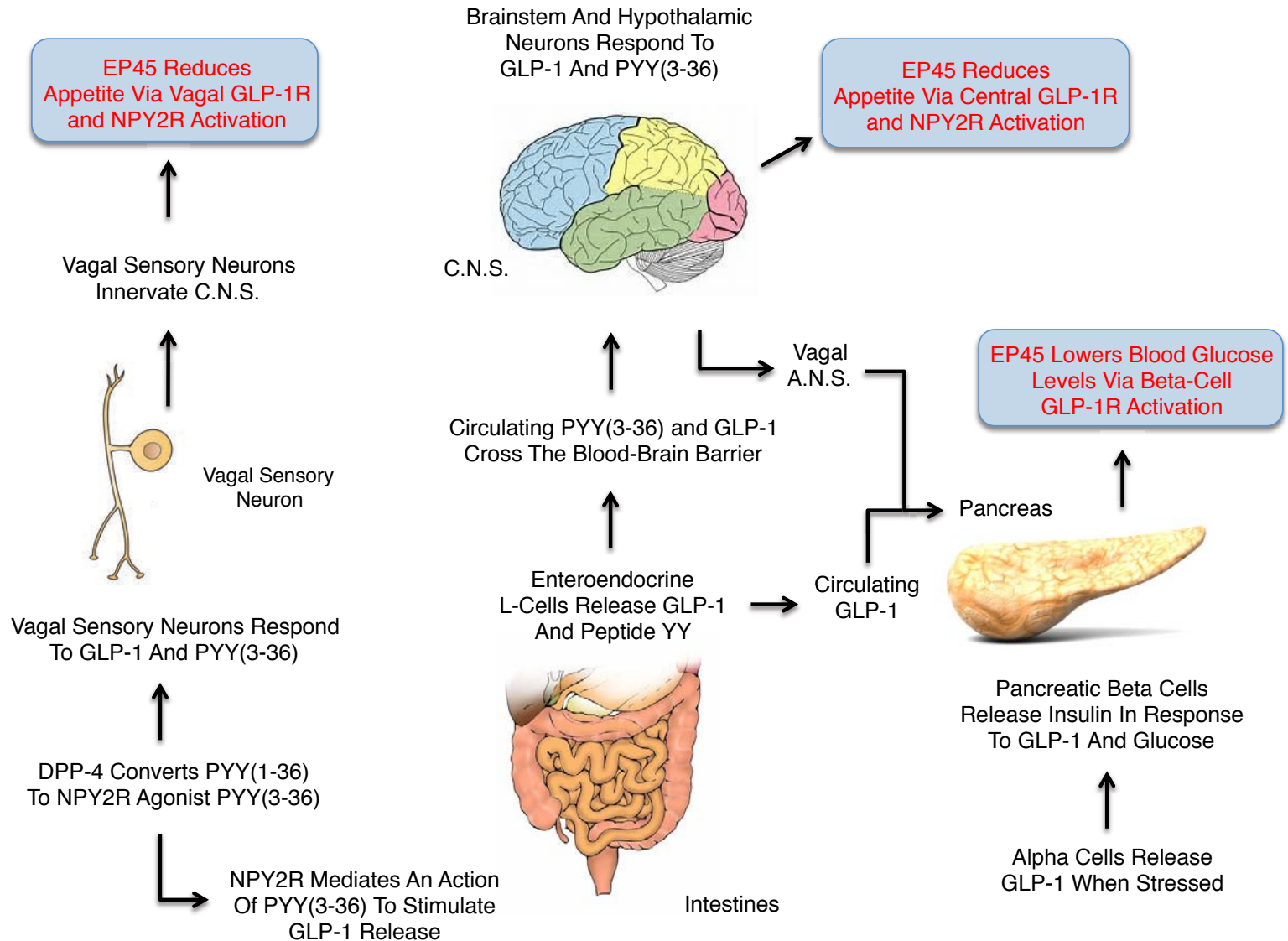
EP45 + PYY(3-36) Counteract A_{2B} Receptor-Stimulated cAMP Production In HEK293-H188-C24 Expressing NPY2R



Relative Potencies Of EP45 And PYY(3-36) At NYPR2 In Assays Of A_{2B} Receptor-Mediated cAMP Production



Summary Conclusion – Dual Agonist Properties Of Chimeric Peptide EP45



Participants In This Project

- **SUNY Upstate**

George G. Holz / Project Head

Oleg G. Chepurny / Holz Lab Staff

Colin A. Leech / Holz Lab Staff

- **Syracuse University**

Robert P. Doyle / Chemist

Ronald L. Bonaccorso / Chemist

George M. Langford / Microscopist

Torsten Wollert / Microscopist

- **BIOLOG Life Sci. Inst.**

Frank Schwede / Chemist

- **University of Washington**

Christian L. Roth / Endocrinologist

- **Funding Provided By -**

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NIH R15 DK097675 (Doyle / Roth)

NIH R01 DK098466 (Roth)

NIH R01 DK104936 (Roth)

