G protein-coupled receptors: Nutritional and therapeutic targets

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UNIVERSITY of GLASGOW

EAAP Annual Meeting 2012 Bratislava

The Danish Council for Strategic Research

FFARMED

Bringing together researchers in Denmark, Germany and the U.K.

- The project aims to identify food constituents acting on free fatty acid receptors and study how these may exert beneficial or detrimental effects on the development of type 2 diabetes (T2D) and other metabolic diseases

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FFA Family of GPCRs

Receptor name	FFA1	FFA2	FFA3
Previous names	GPR40, FFAR1	GPR43, FFAR2	GPR41, FFAR3
Gene location	19q13.1	19q13.1	19q13.1
Amino acid length	300	330	346
% amino acid identity	33% to FFA2 34% to FFA3	33% to FFA1 43% to FFA3	34% to FFA1 43% to FFA2
Activating fatty acid (carbon chain length)	C10-C22, saturated and unsaturated	C1-C6	C1-C6
Synthetic agonists	rosiglitazone; GW9508, TUG-424, TAK-875	ESN-282 4-CMTB	-
Synthetic antagonists	GW1100, DC260126	TUG-942	-
G protein coupling	$G\alpha_{q/11}$	$G\alpha_{i/o}$ and $G\alpha_{q/11}$	$G\alpha_{i/o}$

GPR42 is also located at 19q13.1 only certain polymorphisms are functional

Distribution and function of FFA1-3

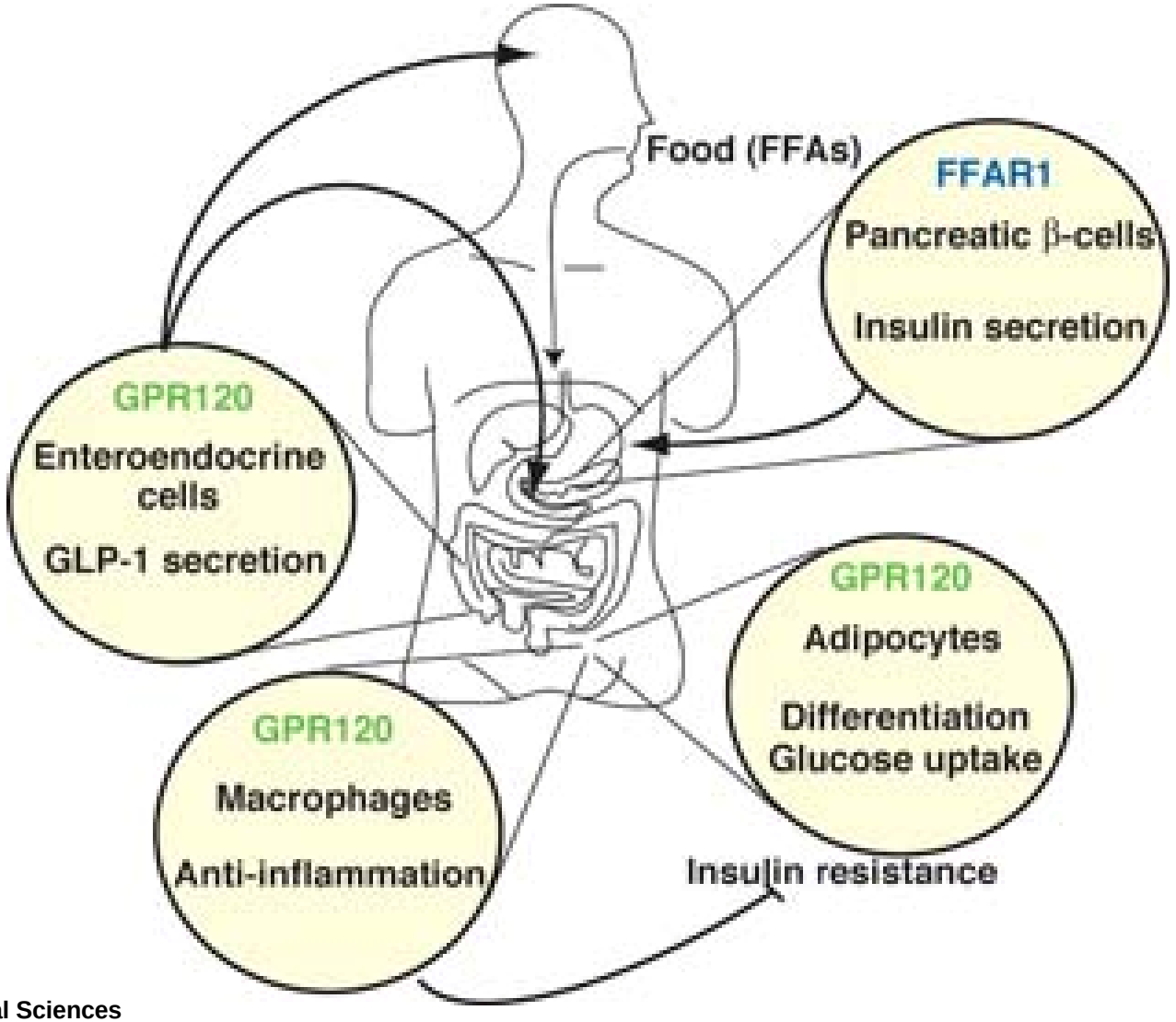
Receptor name	FFA1	FFA2	FFA3
Tissue distribution	Highest expression in pancreatic islets, also in GI tract, brain and monocytes	Immune cells including peripheral blood leukocytes, neutrophils, eosinophils; adipocytes; distal ileum; colon.	Adipose tissue; spleen; lymph node; bone marrow; peripheral blood mononuclear cells
Tissue function	Potentiation of GSIS in pancreatic islets; increase in glucagon secretion	Stimulation of adipogenesis; inhibition of lipolysis; activation of polymorphonuclear cells	Increase in leptin production
Disease	type 2 diabetes	Adiposity, inflammation	Adiposity

Stoddart et al., (2008) Pharmacol. Rev. **60**, 405-417

There are also other GPCRs that respond to fatty acids

Receptor name	FFA1	GPR120	GPR84
Previous names	GPR40	GPR129	Inflammation-related GPCR EX33
Gene location	19q13.1	10q23.33	12q13.13
Amino acid length	300	361 (short) 377 (long)	396
% amino acid identity	33% to FFA2 34% to FFA3	limited	limited
Activating fatty acid (carbon chain length)	C12-C22, saturated and unsaturated	C12-C22, saturated and unsaturated	C7-C11
Synthetic agonists	rosiglitazone; GW9508X, TUG-424, TAK-875	Grifolic acid TUG-891	3,3'-diindoylmethane
Synthetic antagonists	GW1100, DC260126	none	none
G protein coupling	$G\alpha_{q/11}$	β-arrestin, $G\alpha_{q/11}$	$G\alpha_{i/o}$

Free fatty acid receptors FFAR1 and GPR120 are both potential novel therapeutic targets for metabolic disorders



Journal of Pharmaceutical Sciences
Volume 100, Issue 9, pages 3594-3601, 25 MAY 2011 DOI: 10.1002/jps.22639
http://onlinelibrary.wiley.com/doi/10.1002/jps.22639/full#fig2

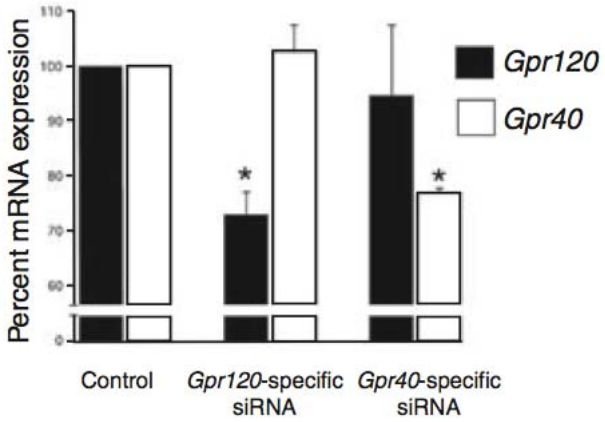
Agonists at FFA1 are currently undergoing clinical trials

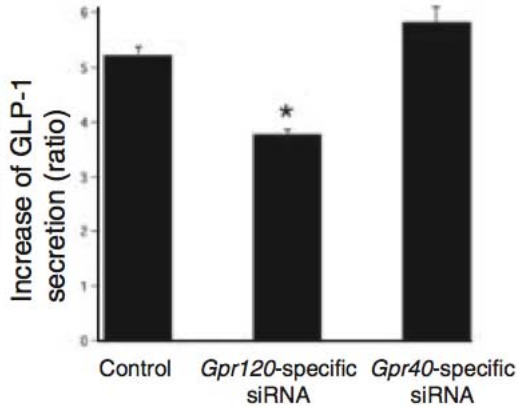
- FFA1 (GPR40) is highly expressed in pancreatic beta-cells and mediates enhancement of glucose-stimulated insulin secretion

- the FFA1 receptor agonist TAK-875 has recently completed phase II clinical trials, meeting key milestones to regulate glycaemia

What about GPR120?

- Identified as a Gq-coupled GPCR for long chain polyunsaturated fatty acids in 2005
- High levels of expression were demonstrated in the intestine
- Received great interest in type 2 diabetes and obesity because it accounts for FFA mediated GLP-1 secretion in enteroendocrine STC-1 cells





GPR120 Background

- GPR120 expression both on adipocytes and macrophages
- GPR120 produces anti-inflammatory effects on macrophages via β-arrestin signaling
- GPR120 enhances glucose uptake in adipocytes via Gq signaling
- Together the GLP-1, anti-inflammatory and insulin-sensitizing effects of GPR120 make it a very attractive target for T2D/obesity

It also helps in validation if genetic studies implicate or link a GPCR in a disease phenotype

- **Research Highlight**
- ***Nature Reviews Gastroenterology and Hepatology* 9, 187 (April 2012) | doi:10.1038/nrgastro.2012.47**
- **OBESITY: GPR120 dysfunction can cause obesity in mice and humans**

- This is a comment on data published recently
- **Ichimura et al., (2012) Nature 483, 350–354**
- that indicated that a polymorphic variant in this GPCR limited signalling and was linked to obesity.

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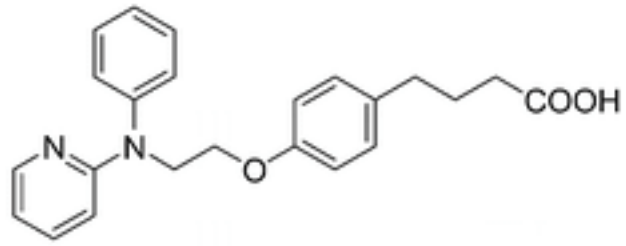
Current limitations in GPR120 research

- Limited current validation
- GPR120 endogenous ligands are all also active at FFA1 (GPR40)
- To date, published synthetic GPR120 ligands are all low potency (pEC_{50}<6) and have limited selectivity over FFA1

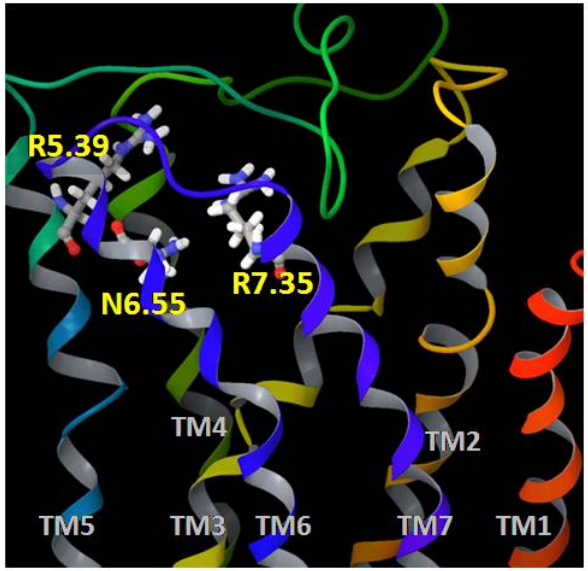


Suzuki et al.
pEC_{50}=5.9
16 Fold Selective over FFA1

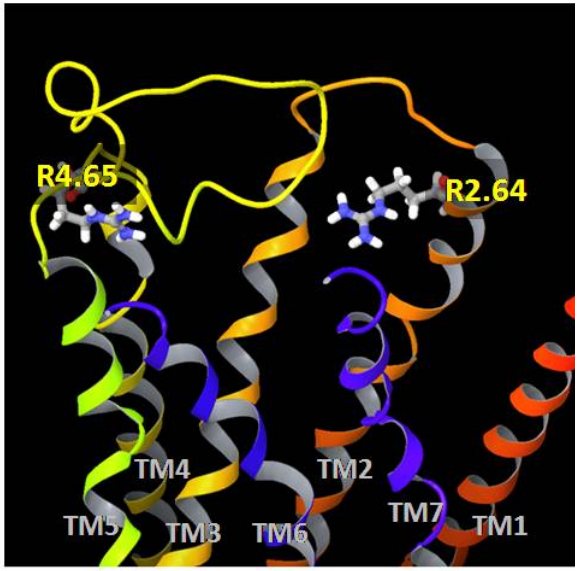
The long isoform of GPR120 is apparently found only in man
And is reported to have a limited range of signals

Although both respond to the same group of endogenous ligands these two GPCRs are markedly different

- GPR120 lacks the cluster of conserved positively charged residues of the FFA1-3 family
- GPR120 does have two Arg residues close to the top of TM helices (and it is known that Arg2.64 but not Arg 4.65 is important)
- Therefore it should be possible to generate highly selective small molecule ligands



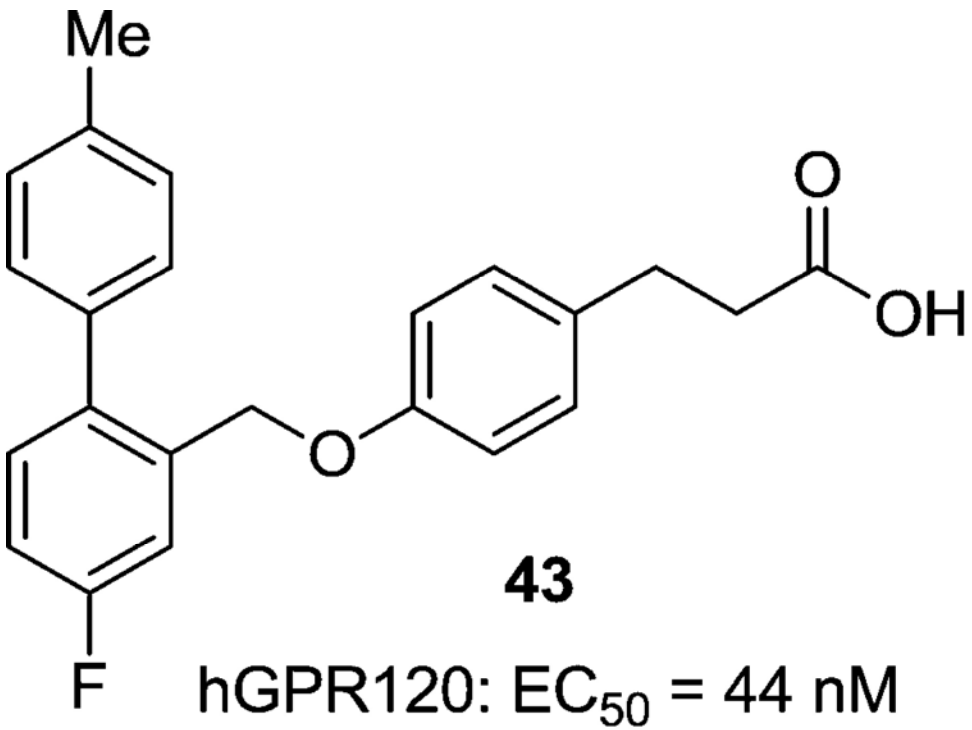
GPR40



GPR120

University of Glasgow

TUG-891: the first high potency and highly selective agonist at GPR120



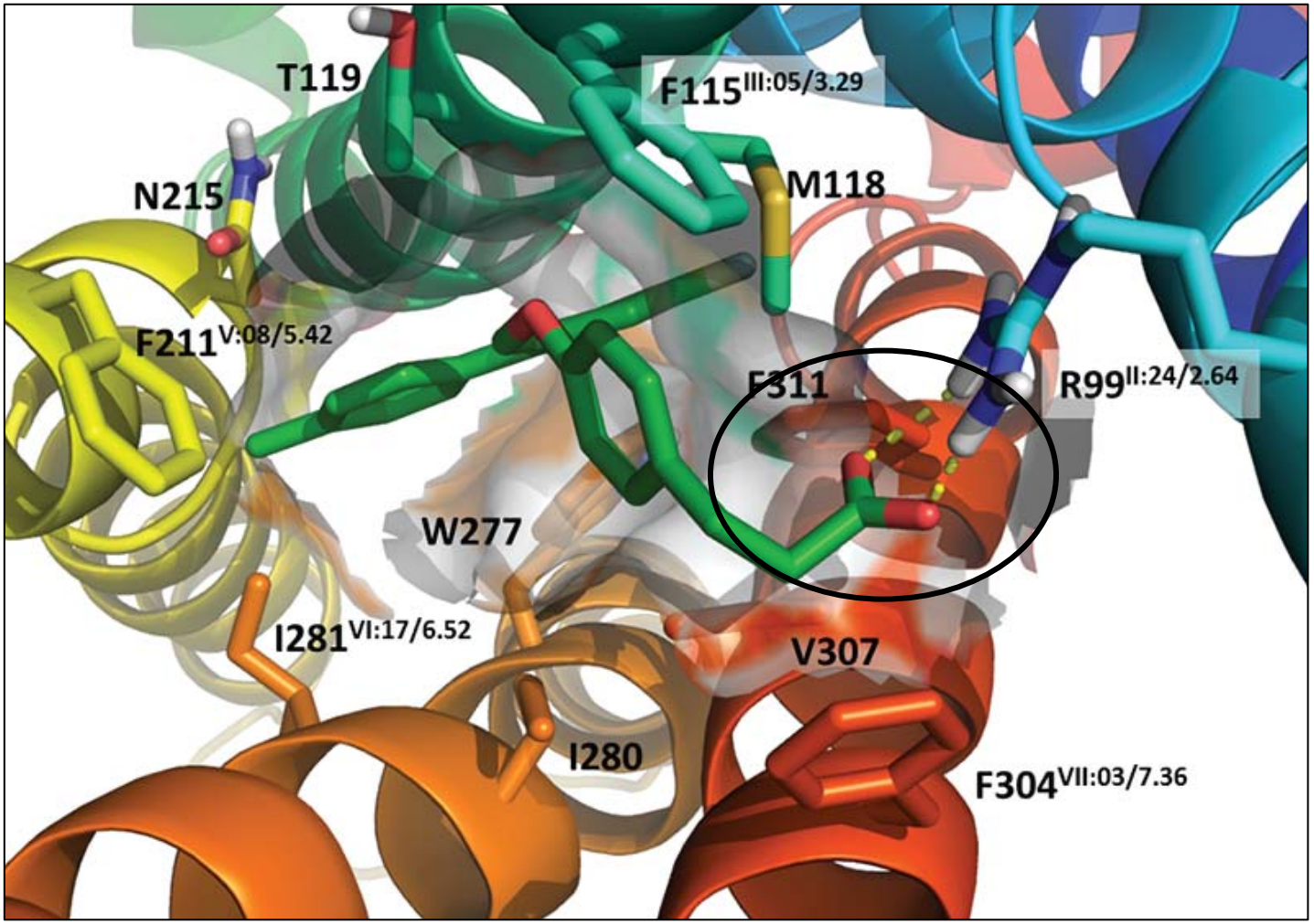
43

hGPR120: EC_{50} = 44 nM

mGPR120: EC_{50} = 17 nM

Published in: Bharat Shimpukade; Brian D. Hudson; Christine Kiel Hovgaard; Graeme Milligan; Trond Ulven; *J. Med. Chem.* **2012,** 55, 4511-4515.
DOI: 10.1021/jm300215x

Potential mode of binding of 891 to human GPR120



Shimpukade et al., 2012, J. Med. Chem. 55, 4511-4515

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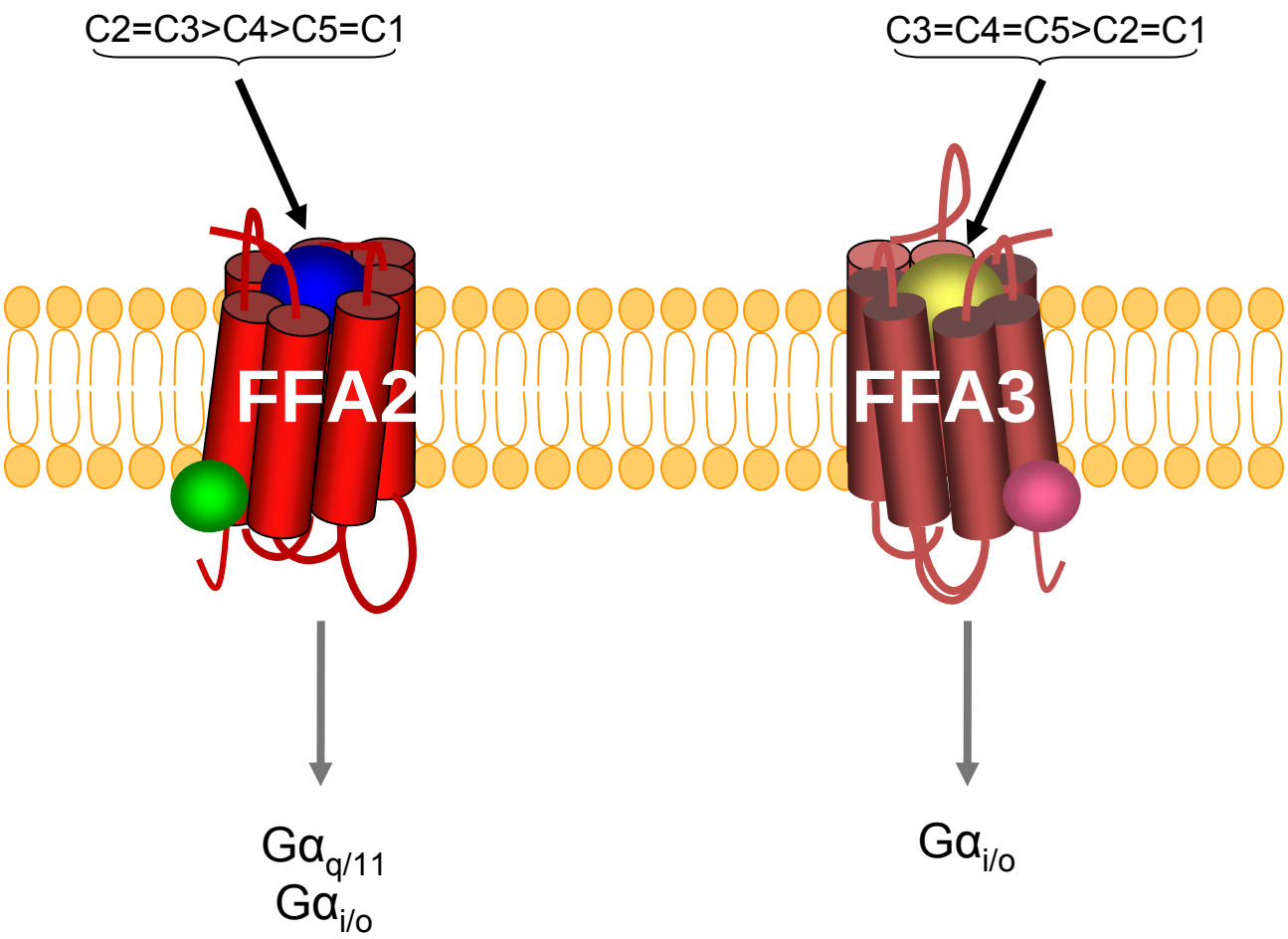
FFA2 and FFA3 respond to short chain fatty acids
produced predominantly by microbial fermentation of non-digestible carbohydrates



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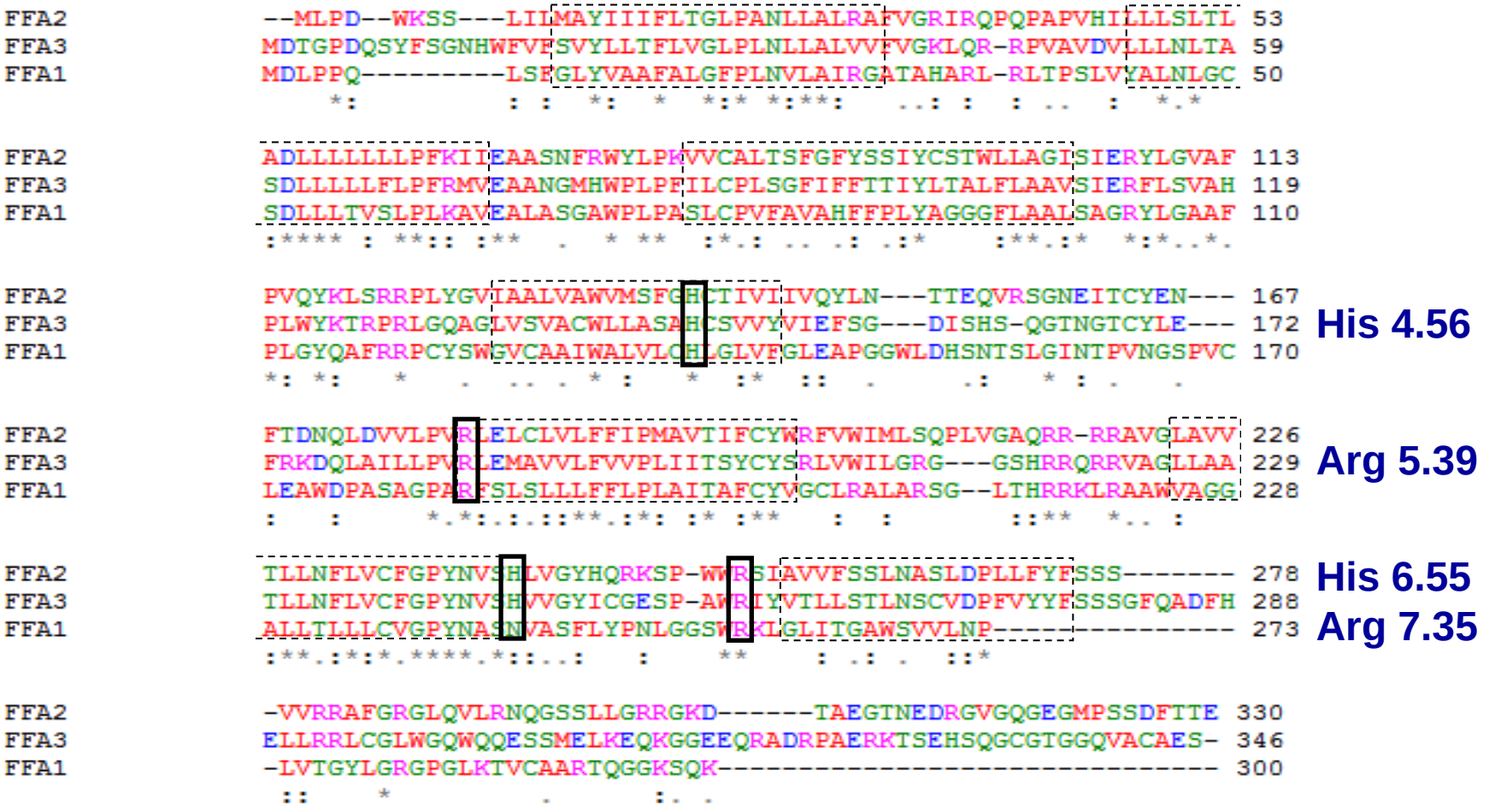
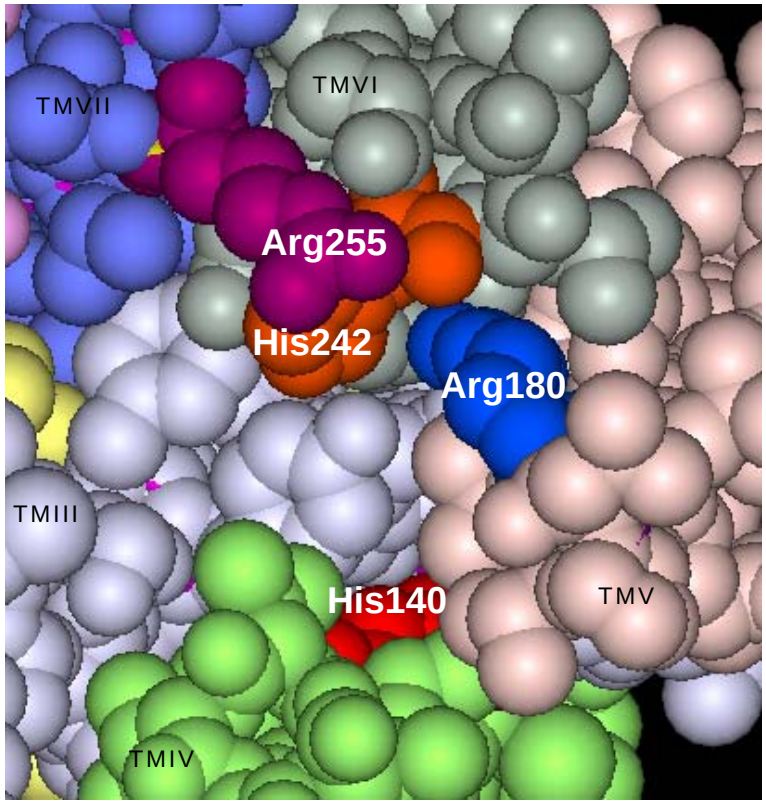
Identification of TM residues that could be involved in fatty acid binding and receptor activation



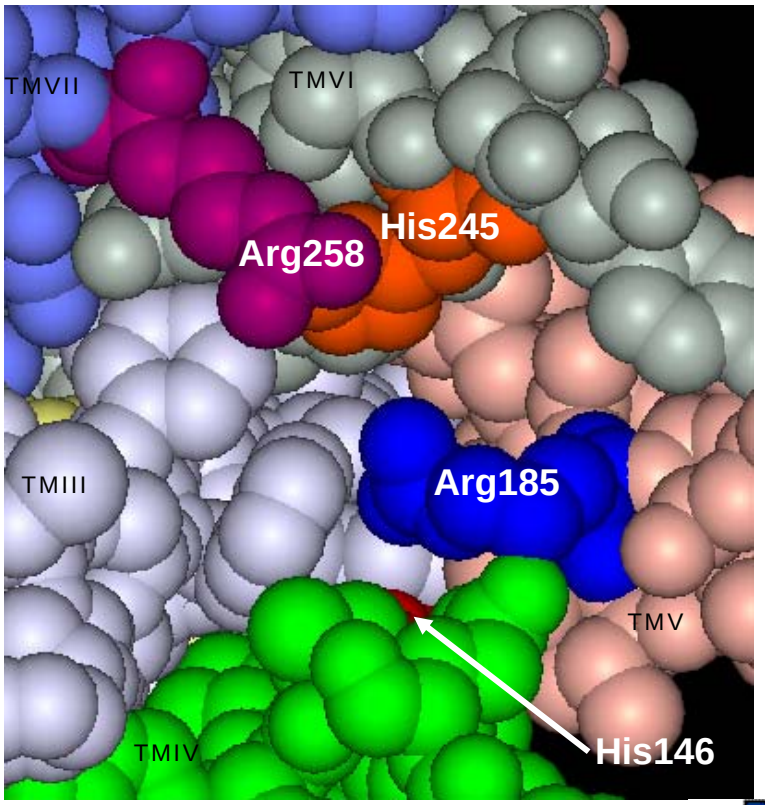
FFA1, FFA2 and FFA3 respond to FFAs but not the corresponding amides

Looking from the extracellular milieu

Human FFA2



Human FFA3



Stoddart et al., (2008) J. Biol. Chem. **283**, 32913-32924

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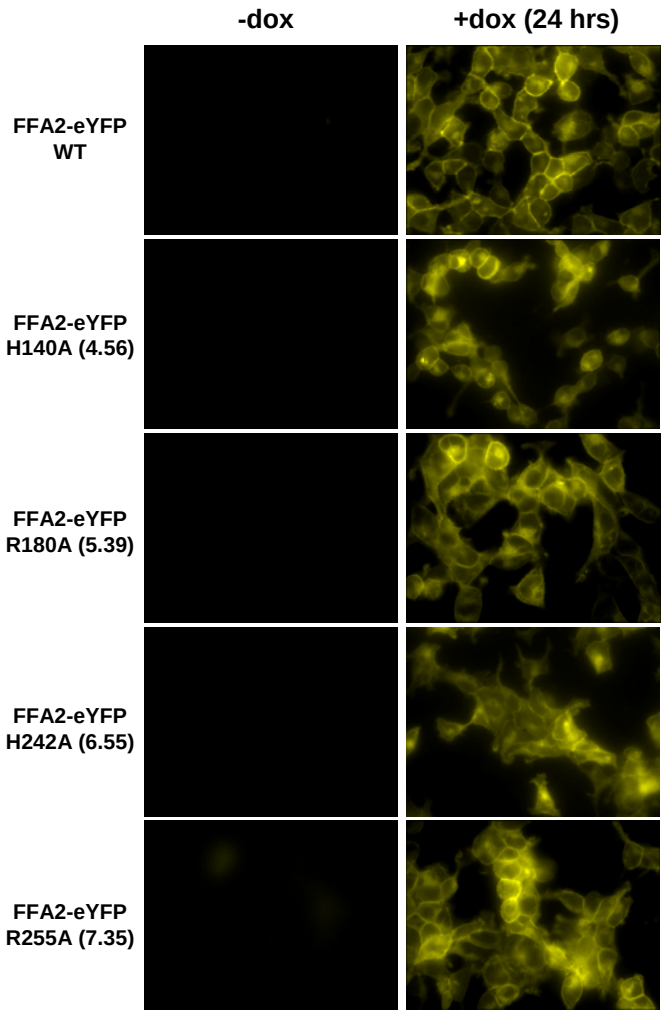
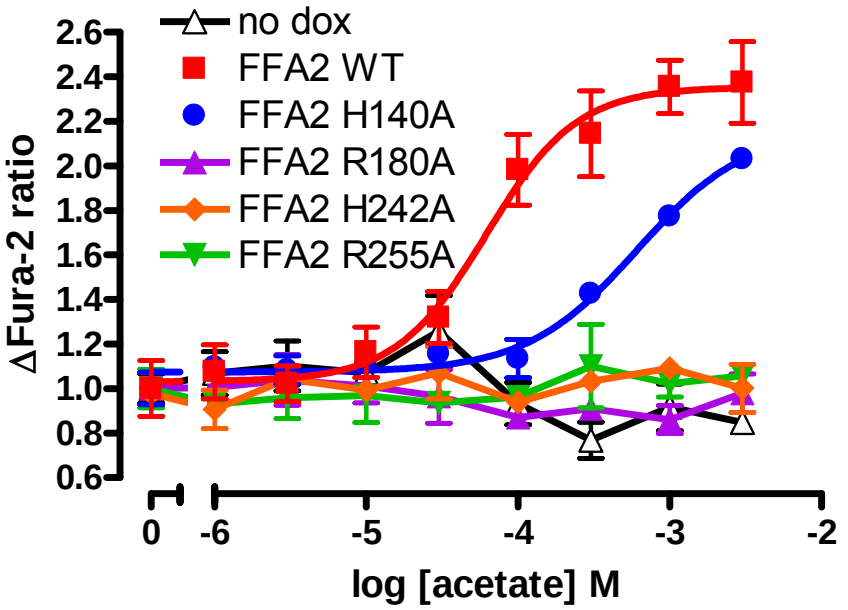
Mutation of Arg180 (5.39), His242 (6.55), or Arg255 (7.35) renders hFFA2 unable to respond to short chain fatty acids





But for His140 (4.56) only potency is reduced

Stoddart et al., (2008) J. Biol. Chem. **283**, 32913-32924

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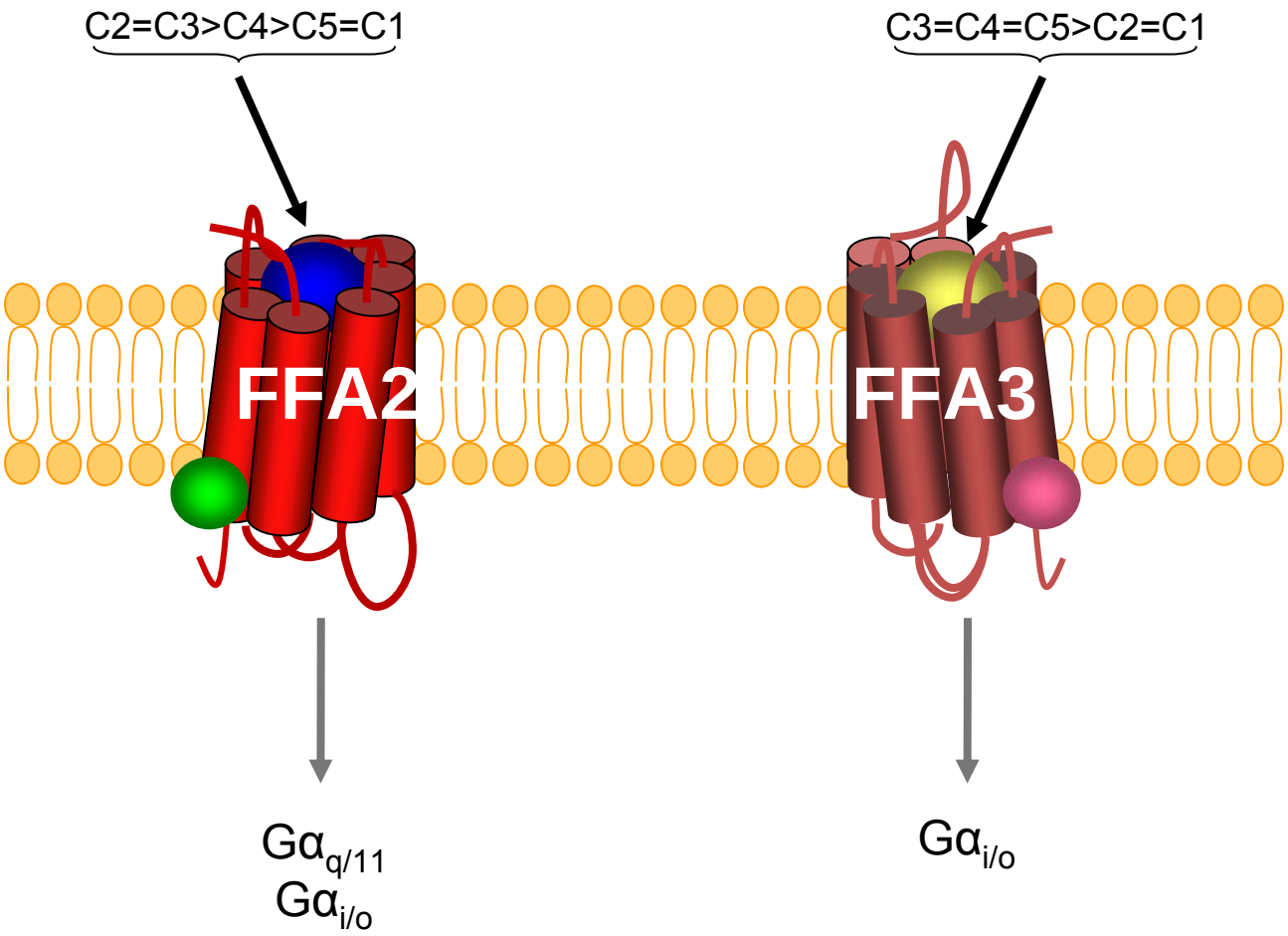
Equivalent mutations in FFA3 also abolish agonist function

However there are differences in SCFA ligand SAR in man



This has resulted in acetate being used as an FFA2 receptor selective agonist

Milligan et al., (2009) Br. J. Pharmacol. **158**, 146-153

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But is this valid if we consider non-human studies?

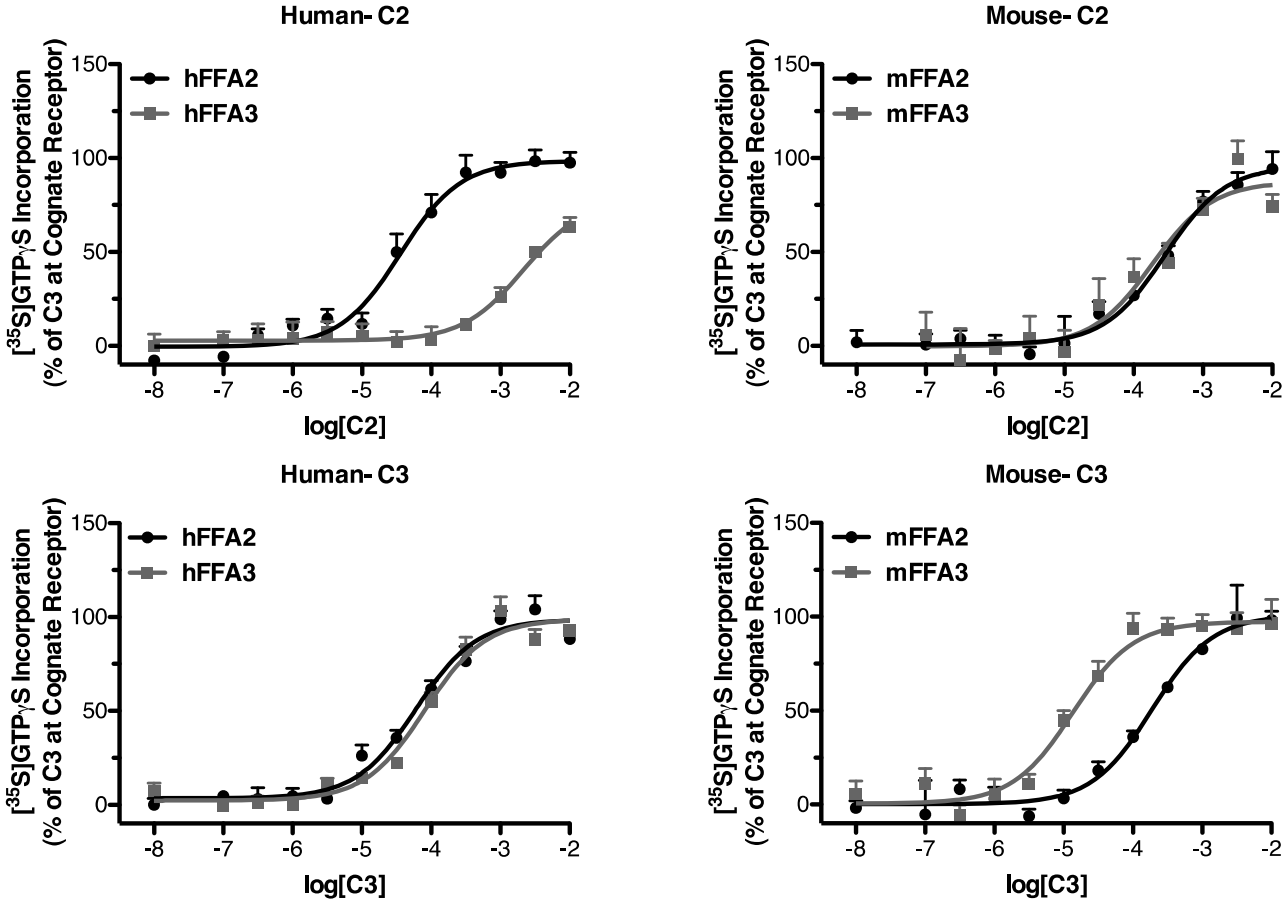
- For example:
- Is this selectivity also seen at the rodent receptor orthologues?

But is this valid if we consider non-human studies?

- For example:
- Is this selectivity also seen at the rodent receptor orthologues?

The answer (of course) is No

C2 and C3 selectivity is not maintained in the mouse orthologs of FFA2 and FFA3



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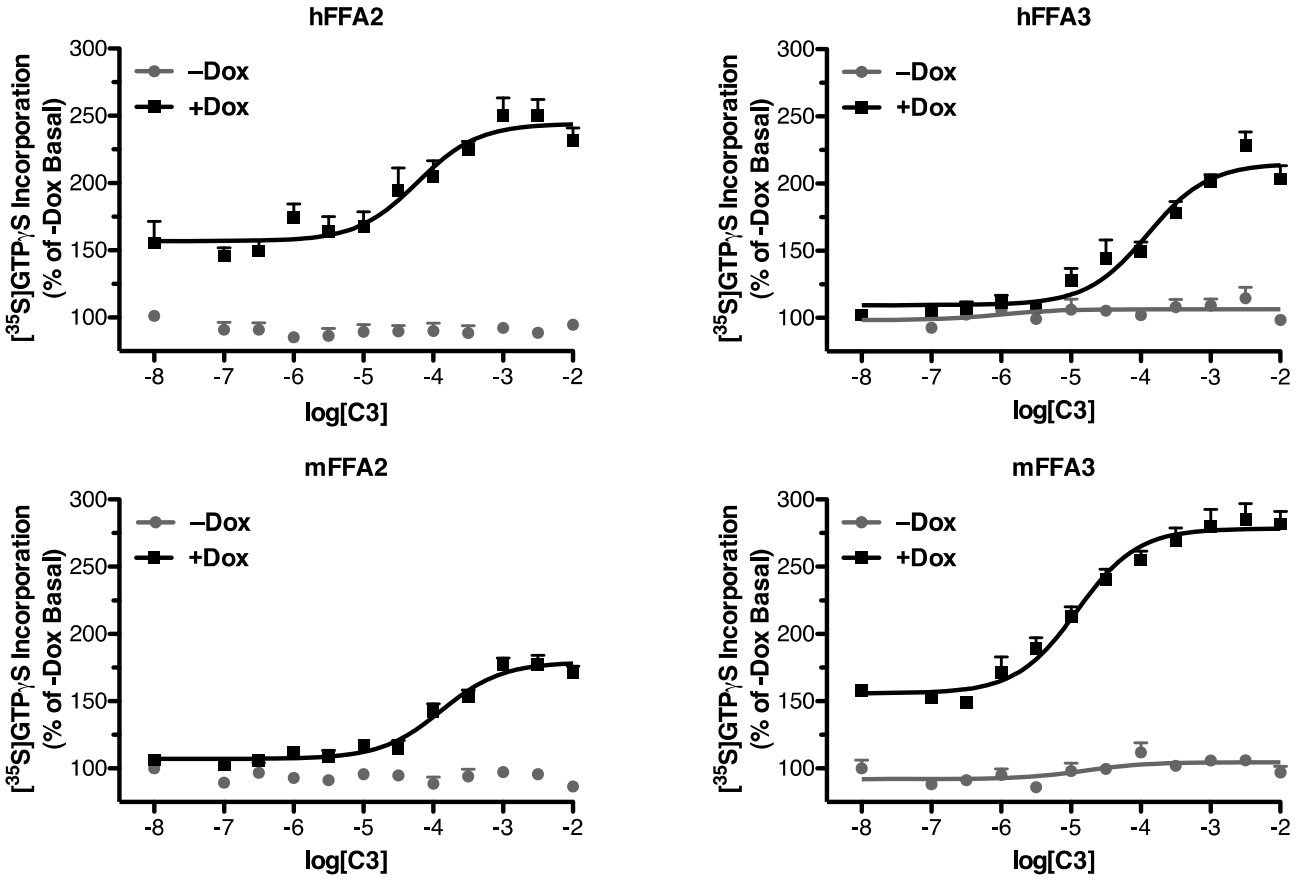
Acetate, therefore, cannot be used to selectivity probe FFA2 function in rodents

Hudson et al., (2012) J. Biol. Chem. (in press)

This is due to markedly different levels of ligand-independent (constitutive activity) in the murine and human orthologs of FFA2 and FFA3



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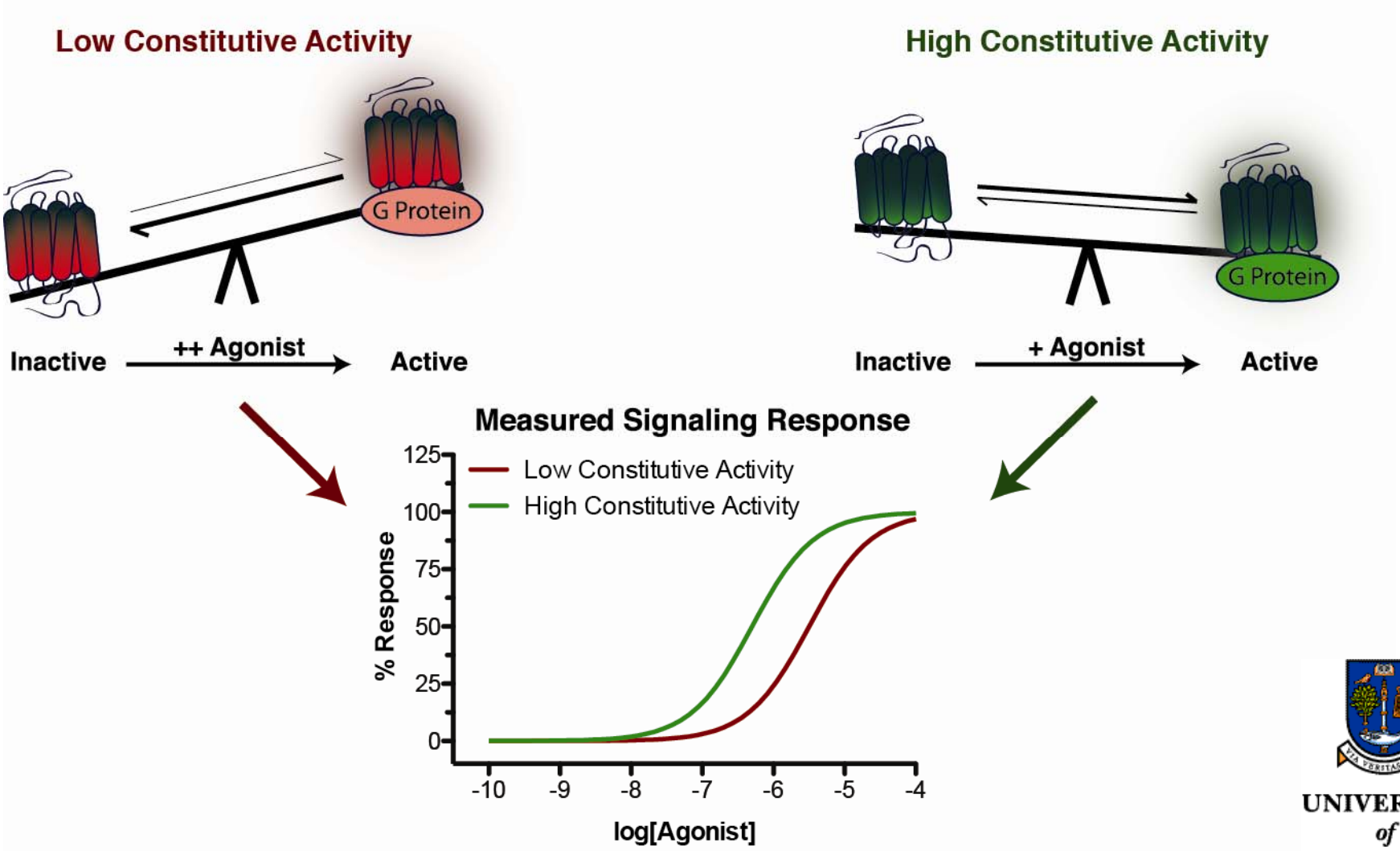
Human FFA2 and murine FFA3 have high agonist-independent activity

Constitutively active forms of receptors are known to display higher agonist potency

Hudson et al., (2012) J. Biol. Chem. (in press)

There is a smaller energy barrier between the Inactive and Active States of Constitutively Active GPCRs: Therefore less agonist is needed for activation



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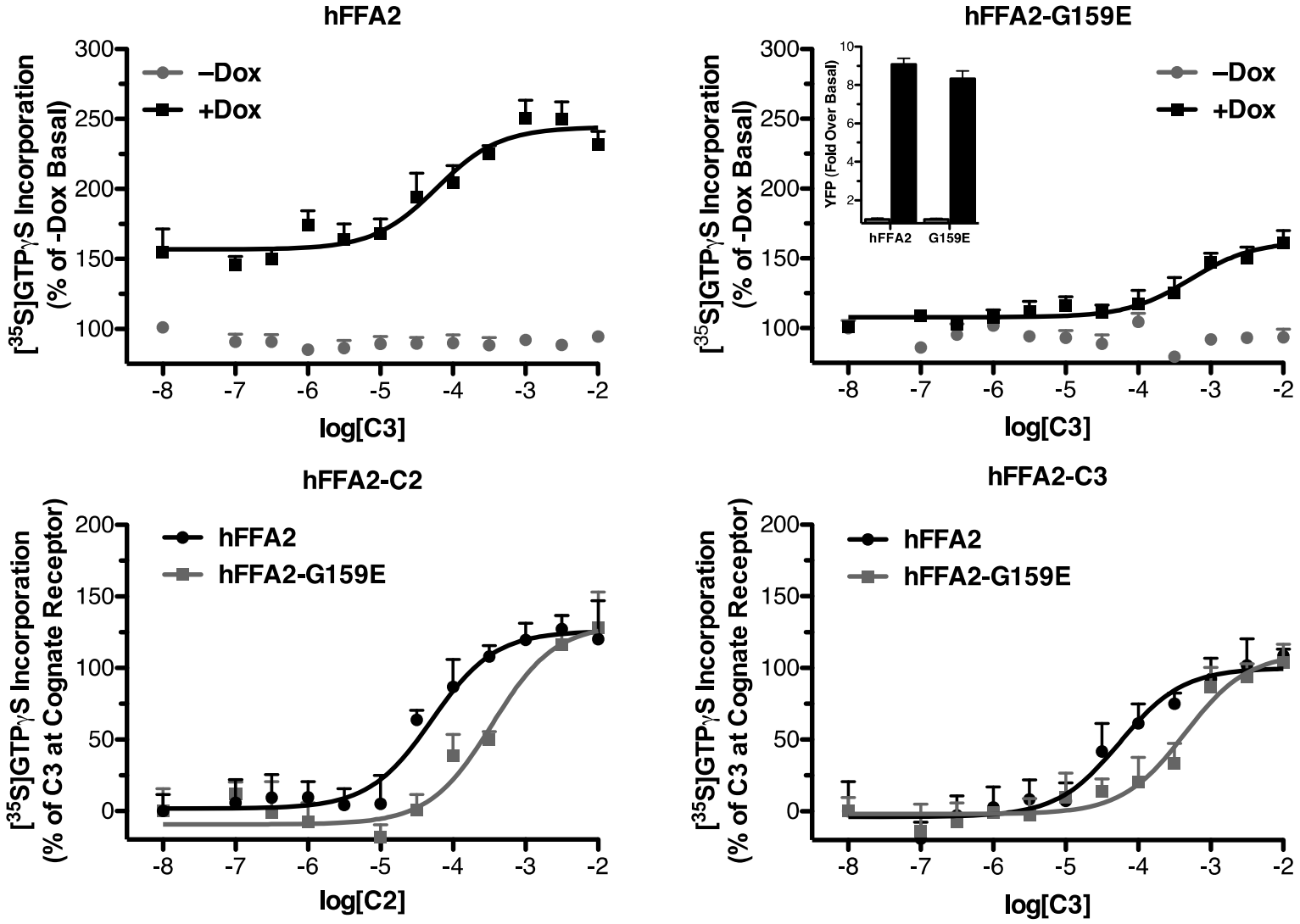
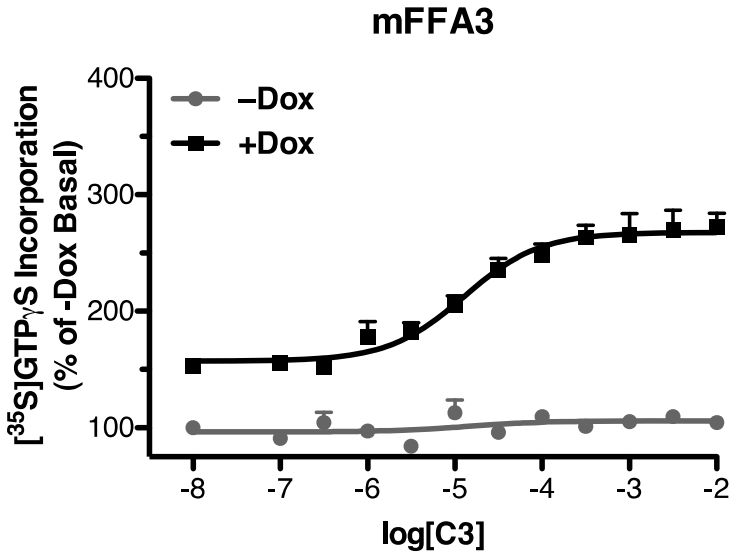
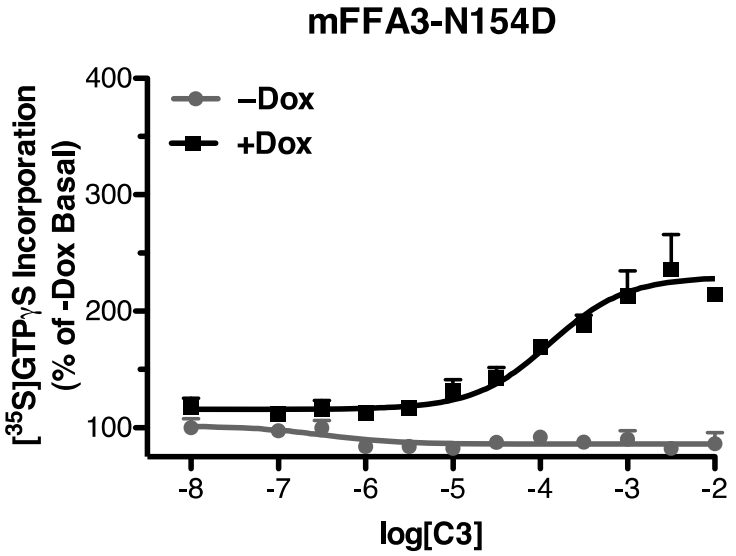
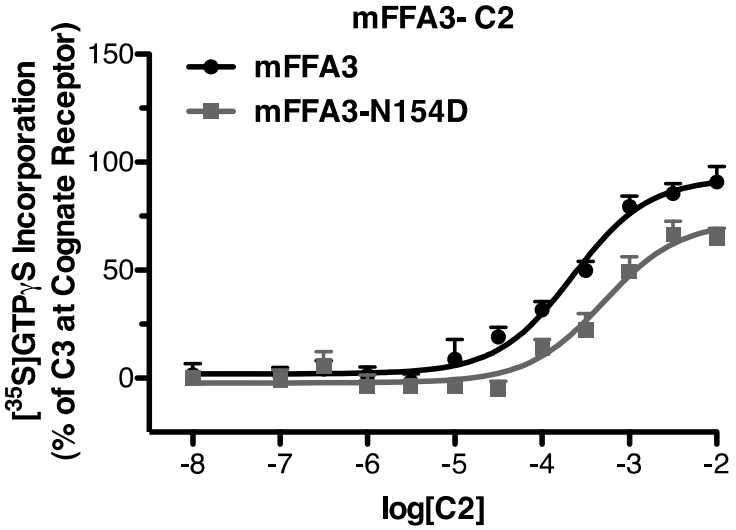
Constitutive activity and SCFA potency at hFFA2 is reduced by introducing a negatively charged Glu residue present in ECL2 of mFFA2 This introduces an extracellular 'ionic lock'

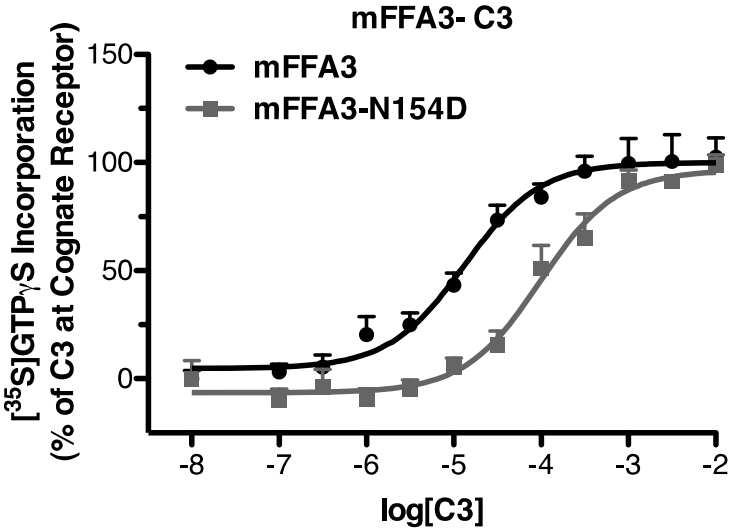


In the same way both constitutive activity and SCFA potency are reduced for mFFA3 by introduction an Asp residue present in ECL2 of hFFA3

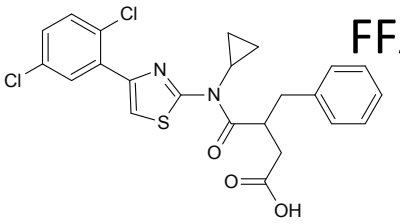
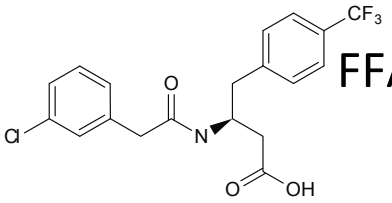
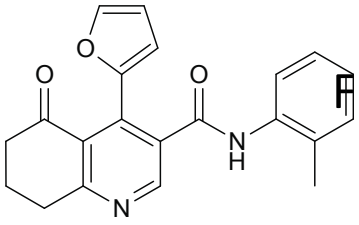








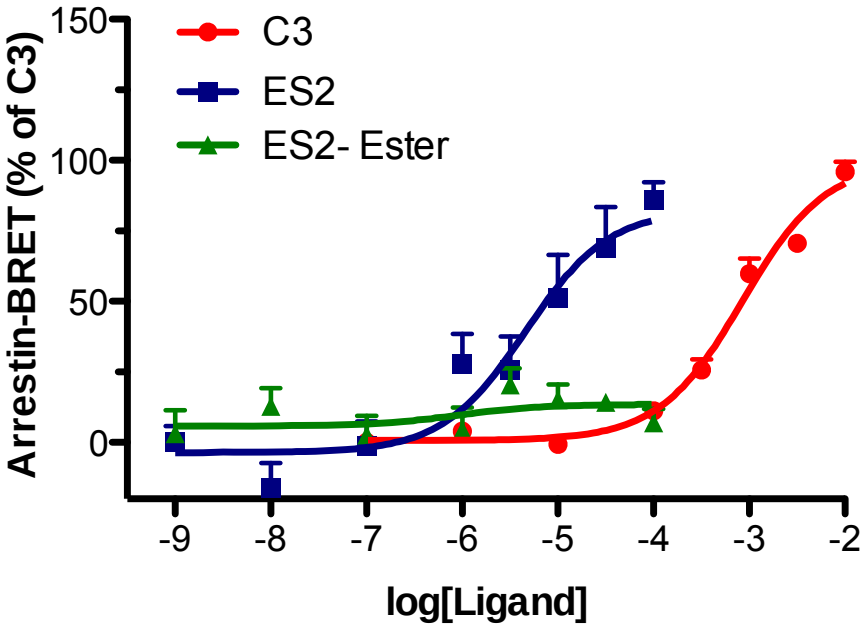
Can synthetic ligands provide information on the respective roles of FFA2 and FFA3?
Ligands from the patent literature can be valuable tools

Compound	Structure	Pharmacology
TUG-800		FFA2 selective agonist
TUG-942		FFA2 selective antagonist
TUG-816		FFA3 selective agonist

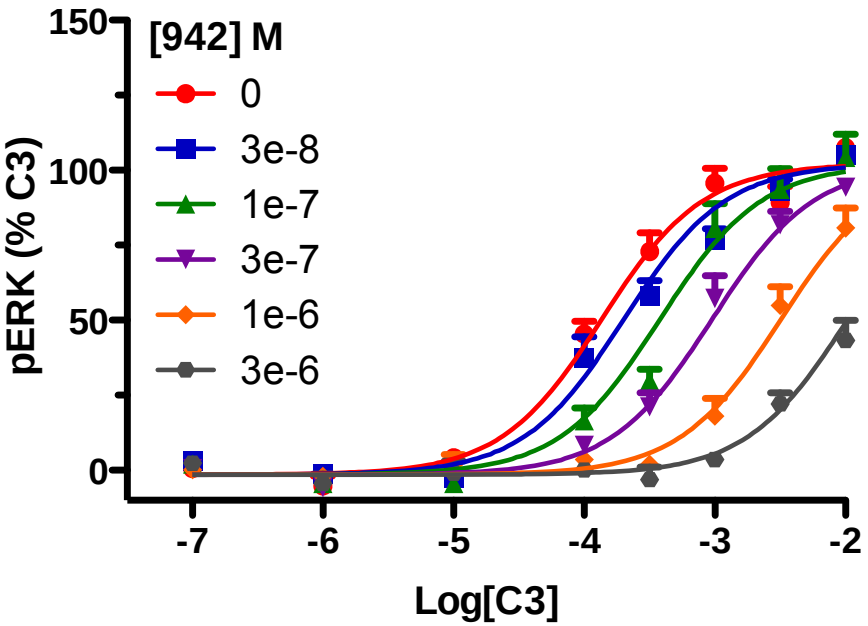
FFA2 ligands originally described by Euroscreen, FFA3 by Arena Pharmaceuticals

FFA2 agonists and an antagonist from Euroscreen can indeed provide useful tools to explore the function of this receptor

Euroscreen FFA2 Agonist



Euroscreen FFA2 Antagonist



At least 100 fold more potent than C3
Orthosteric as the carboxylate moiety is required

Competitive antagonist

Hudson et al., 2012

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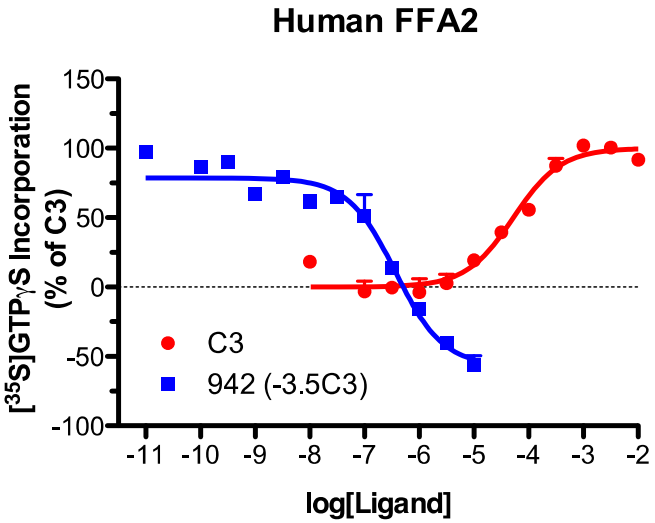
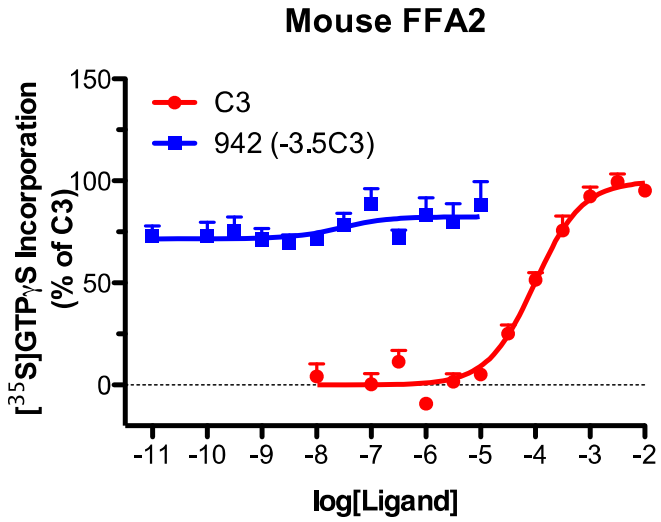
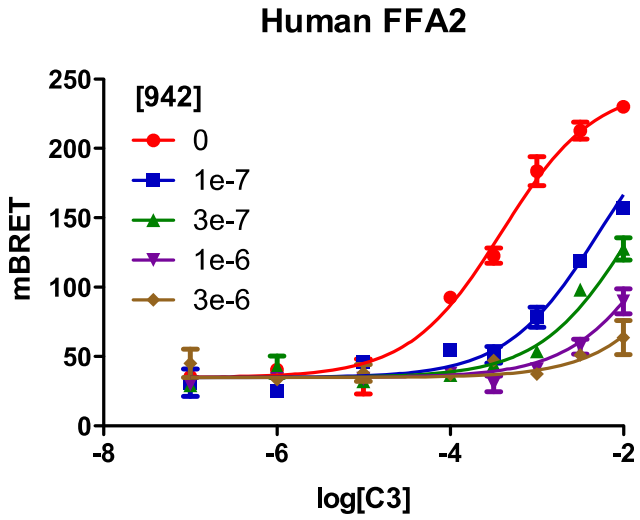
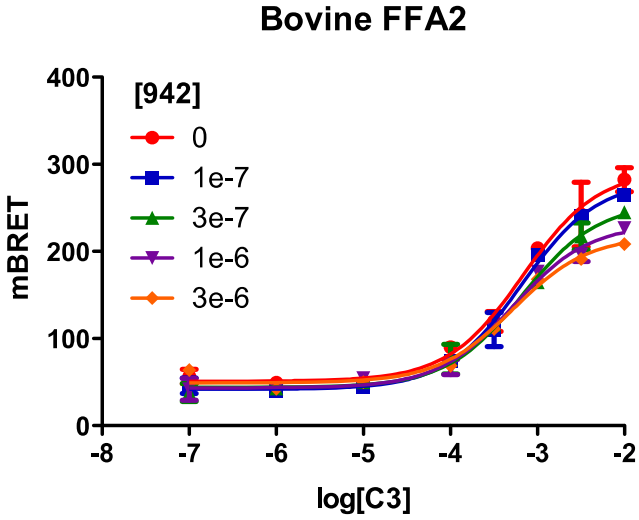
But only at the human receptor, FFA2 antagonism by compound 942 is limited to the human orthologue









Because of this we decided to make a

RASSL version of FFA2

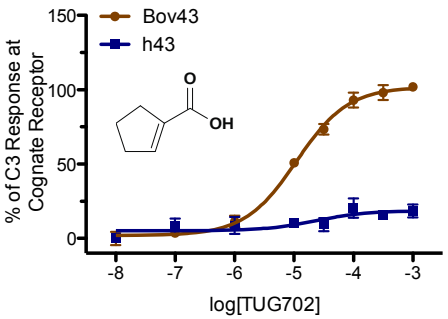
- Receptor Activated Solely by Synthetic Ligand

- Previously achieved for muscarinic receptors using random mutagenesis and molecular evolution

- Here we employed **pharmacological variation between species orthologues** as a design principle

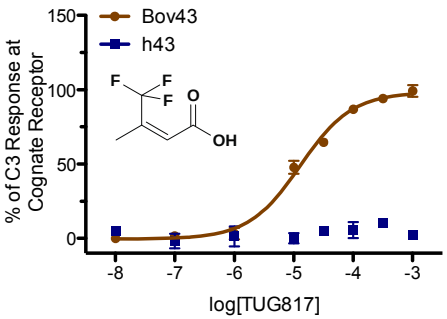
Based on the acceptance of longer chain length fatty acids several extremely selective ligands for BovFFA2 were predicted, and confirmed

702



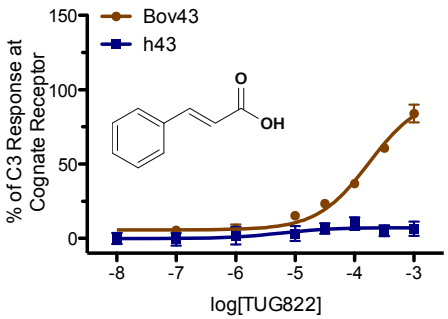
	Bov43
LogEC50	-4.958

817



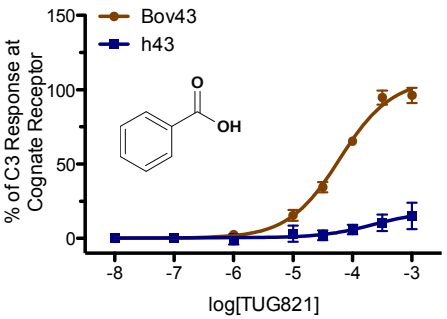
	Bov43
LogEC50	-4.889

822



	Bov43
LogEC50	-3.758

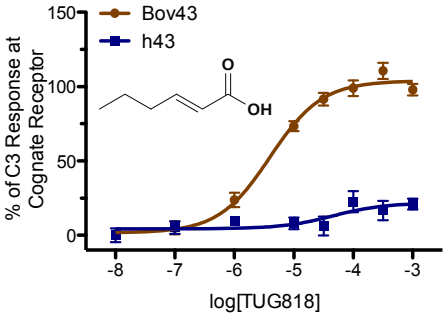
821



	Bov43
LogEC50	-4.215

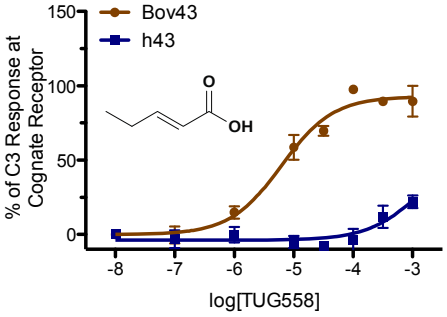
This is SORBIC ACID

818



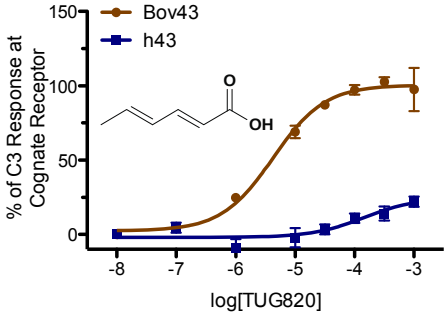
	Bov43
LogEC50	-5.397

558



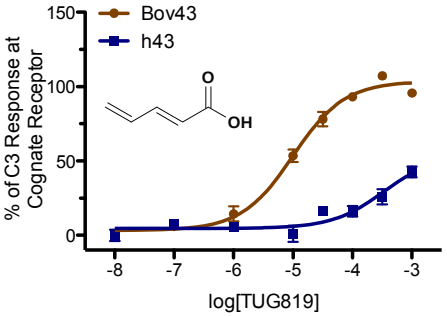
	Bov43
LogEC50	-5.205

820



	Bov43
LogEC50	-5.370

819

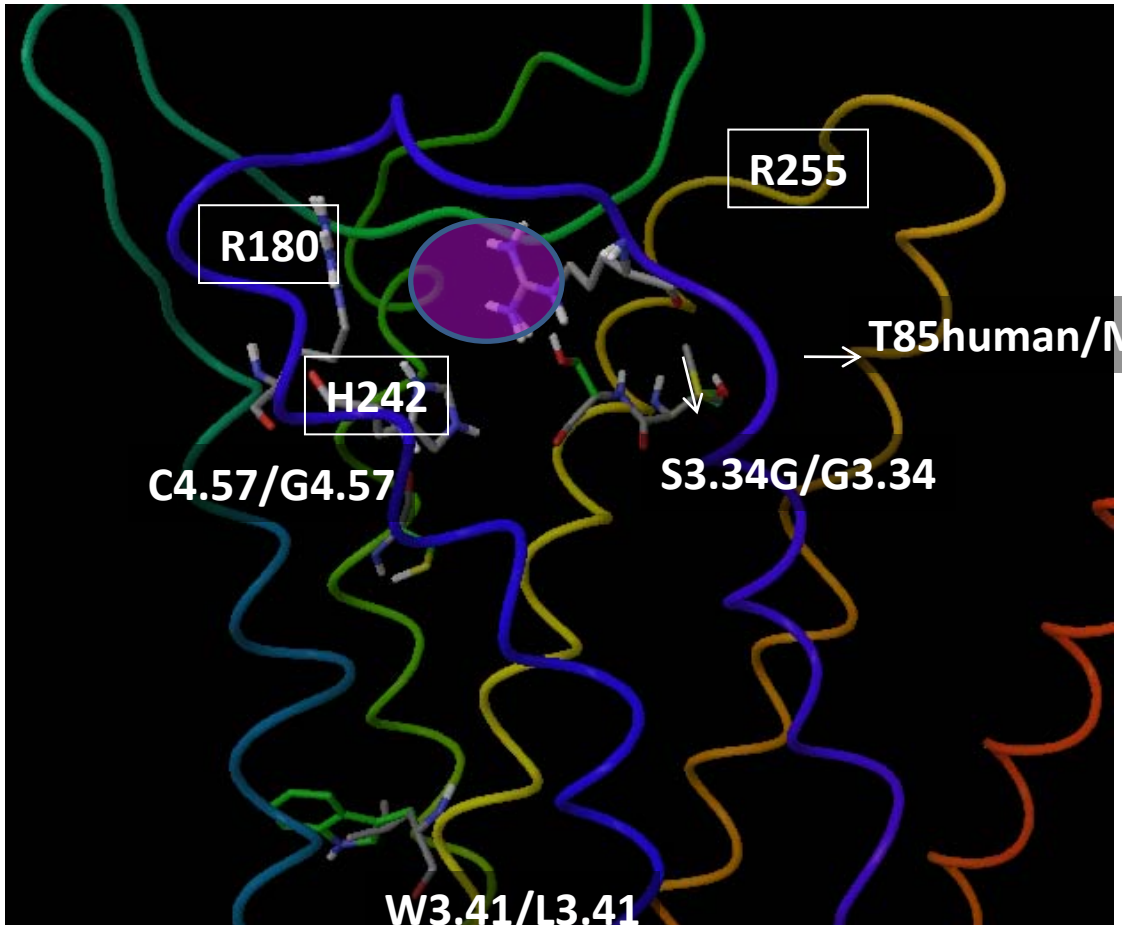


	Bov43
LogEC50	-5.001

Sorbic Acid

- Is a non-toxic preservative used for its anti-fungal properties
- LD50 = 8-10 g/Kg
- Widely used as a preservative in foods including cheese, cured meats and bread
- **Is approved for use as an additive to cattle feed**.
  - Only one study has examined effects of including sorbic acid in cattle feed, finding it did not improve cattle weight gain.
  - **Can anyone tell me at what concentrations?**

Homology modelling of ligand binding to BovFFA2 vs hFFA2
Understanding ligand selectivity



Suggestions for mutagenesis

Within 8Å of the binding site:

hFFA2-S3.34G/BovFFA2-G3.34S
HFFA2-C4.57G/BovFFA2-G4.57C

Additional site predicted to affect binding pocket size:

h43-W3.41L / Bov43-L3.41W

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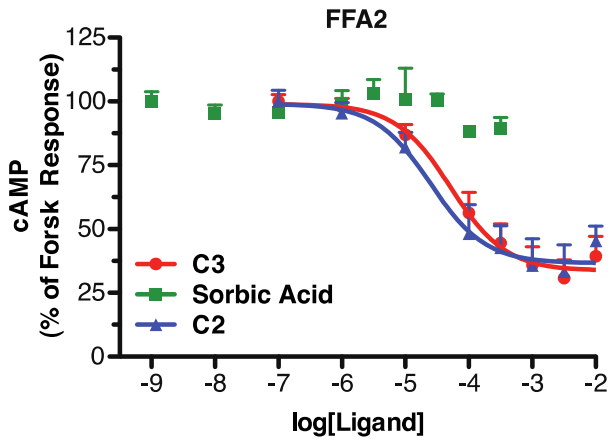
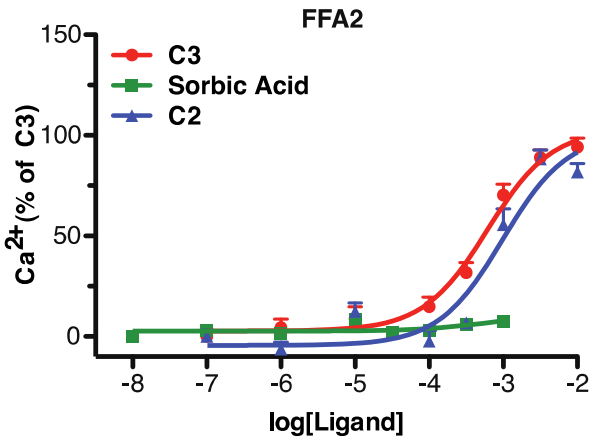
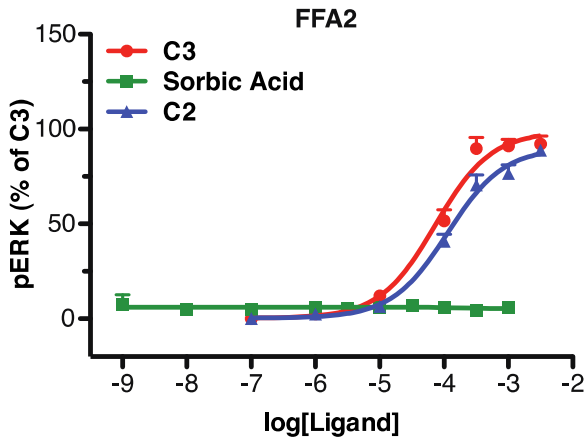
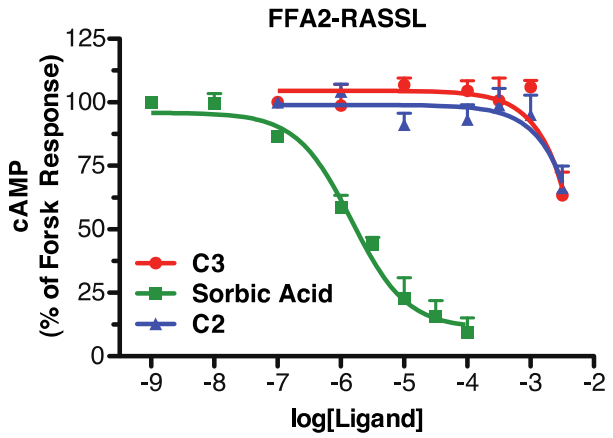
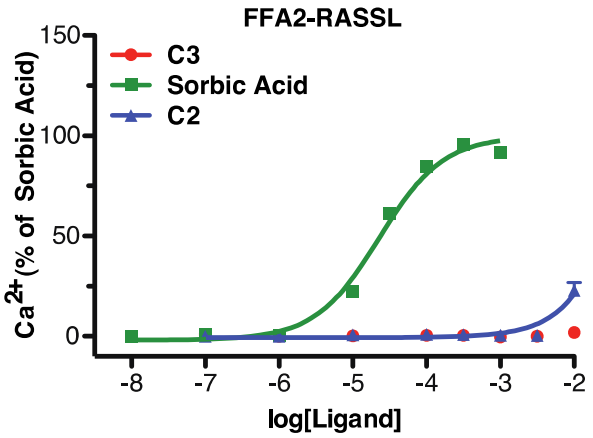
Signaling by Endogenous Ligands at Wild Type and Sorbic Acid at FFA2-RASSL is Similar Across Multiple Assay Readouts

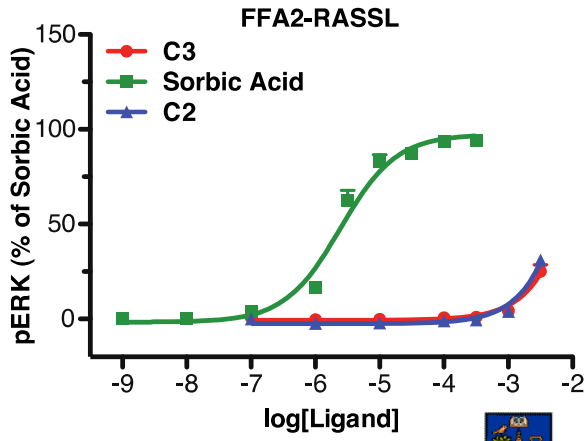












Hudson et al., (2012) FASEB J (in press)

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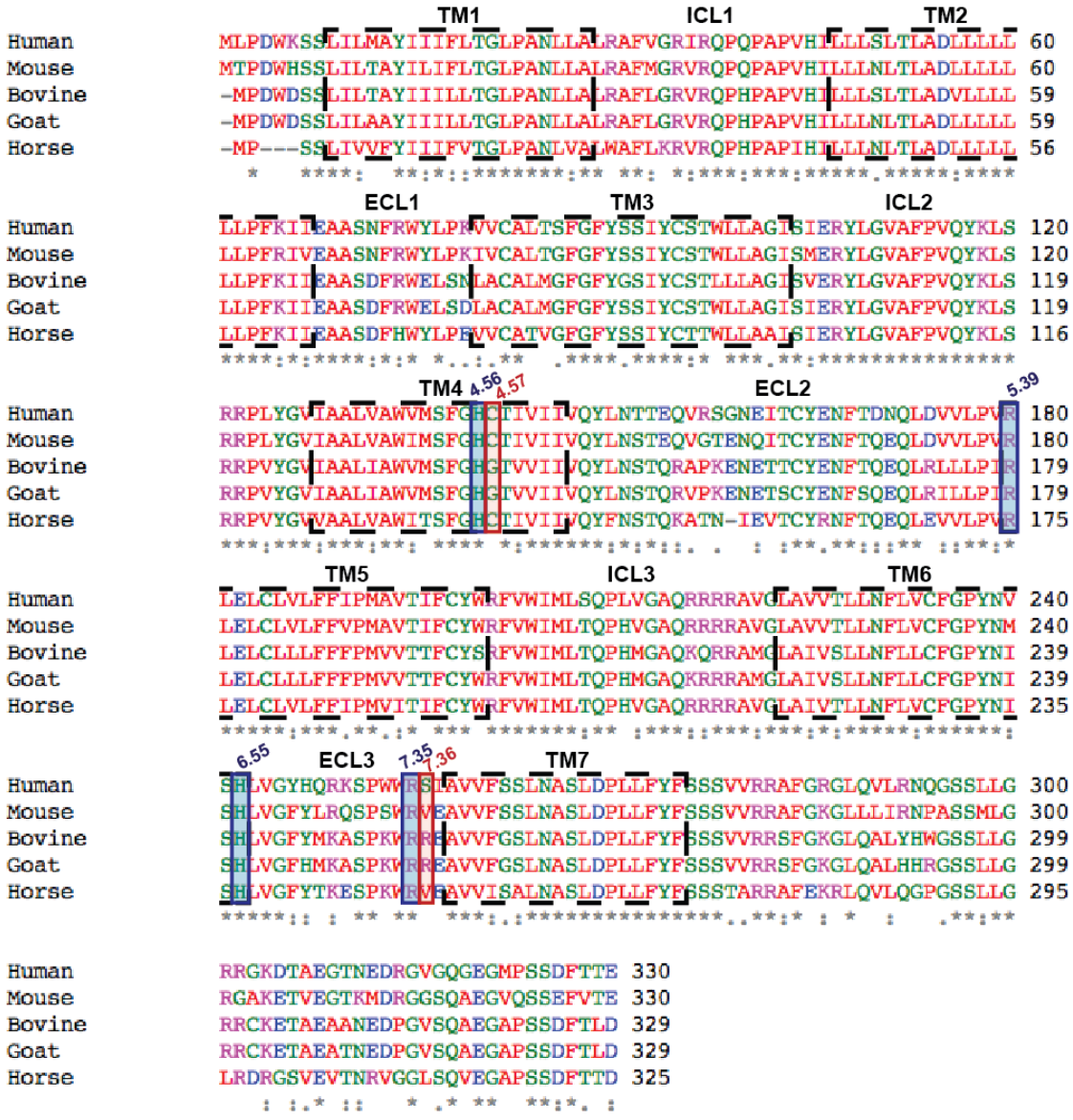
Multi-Species Sequence Alignment of FFA2



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Hudson et al. (2012) FASEB J (in press)

Key observations from the sequence alignments

- The 4 key positively charged residues of the binding pocket for SCFAs (H4.56, R5,39, H6.55, R7.35) are conserved across species
- In ruminants (cow, goat (sheep sequence not available)) position 4.57 is Gly rather than Cys and 7.36 is Arg
- Neither of these is true however for horse
- Predict that horse will have pharmacology akin to human but goat (and possibly sheep) will show pharmacology akin to cow

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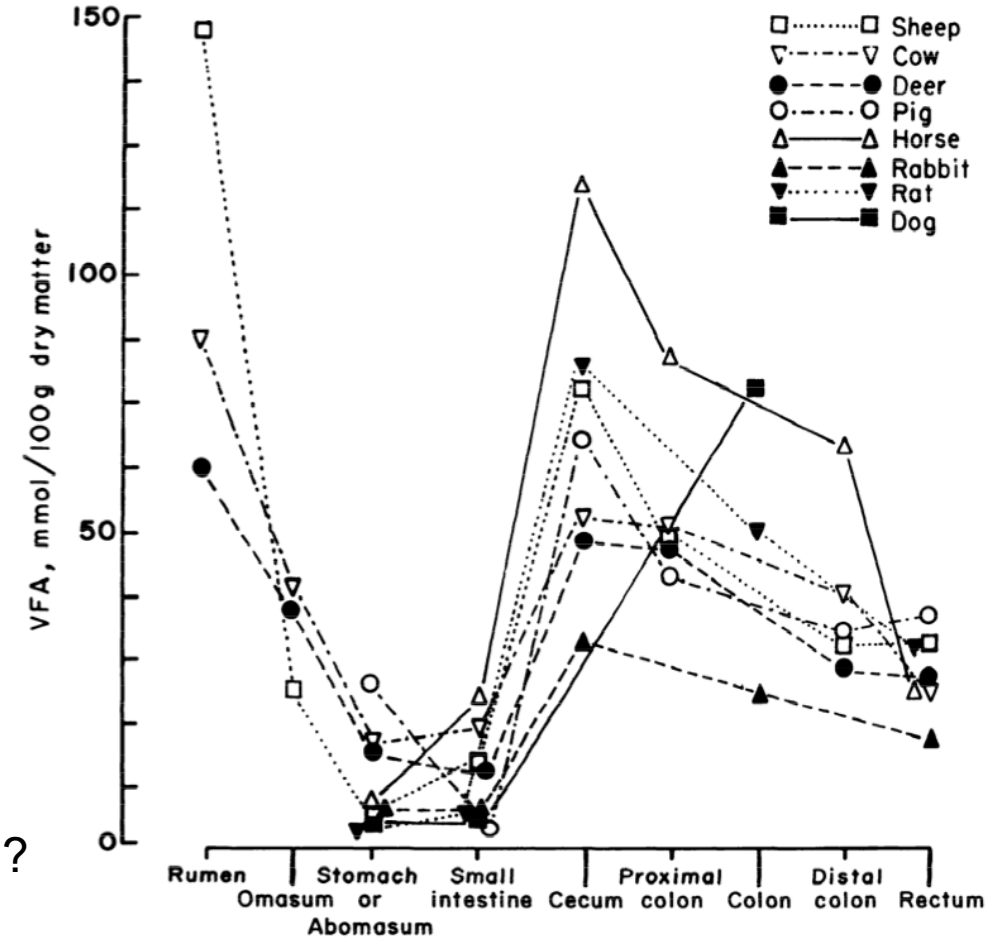
SCFA Concentrations in the Digestive Tract of Various Species

- Highest SCFA concentrations are observed in Rumen
- Although not a ruminant, the horse also shows very high concentrations in lower digestive tract
- Suggests unique pharmacology of ruminant FFA2 may be related directly to rumen function

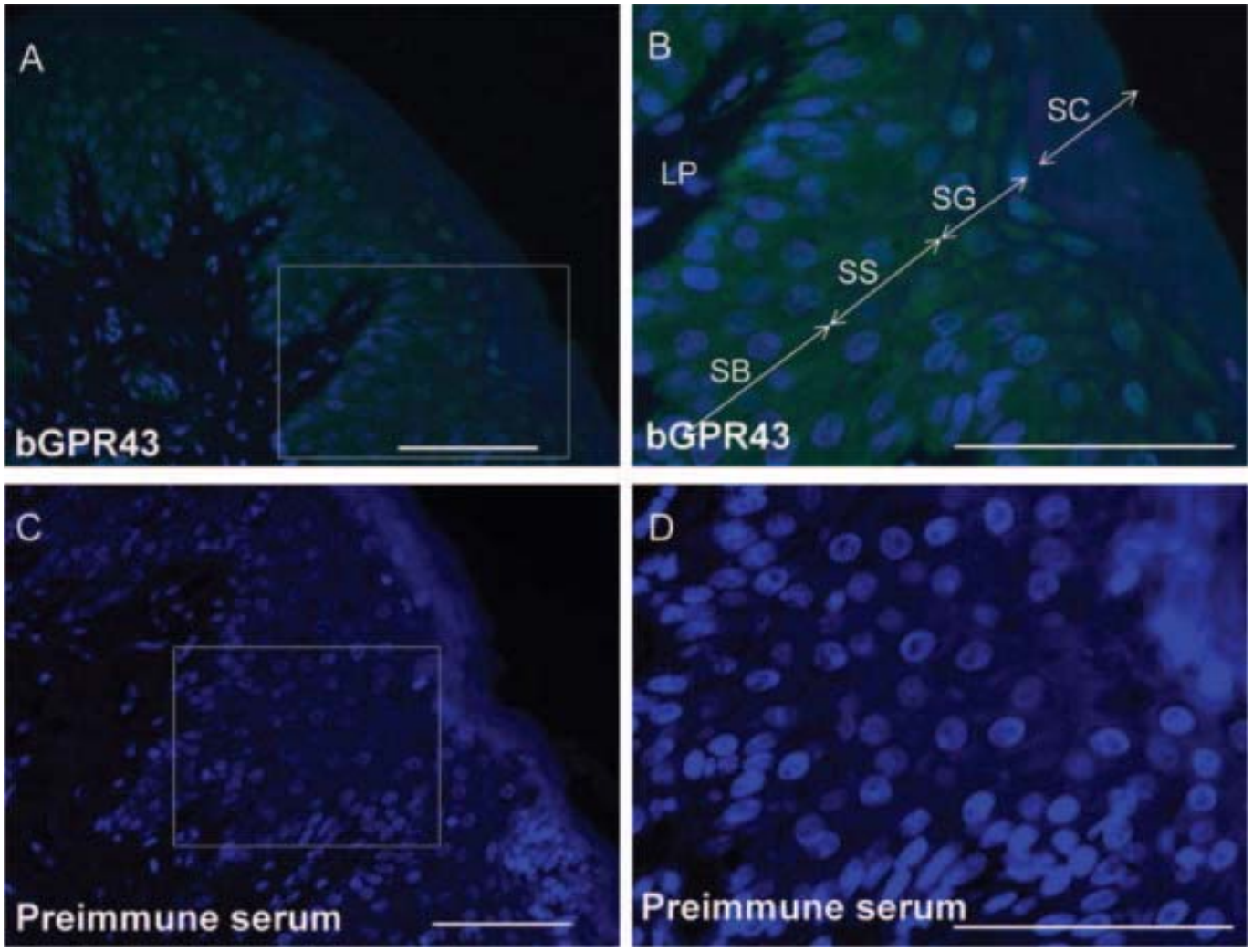
Bovine FFA2 has very low potency for acetate. Is this an adaptation to very high levels of acetate in the rumen?

We predict this will also be true for other ruminants



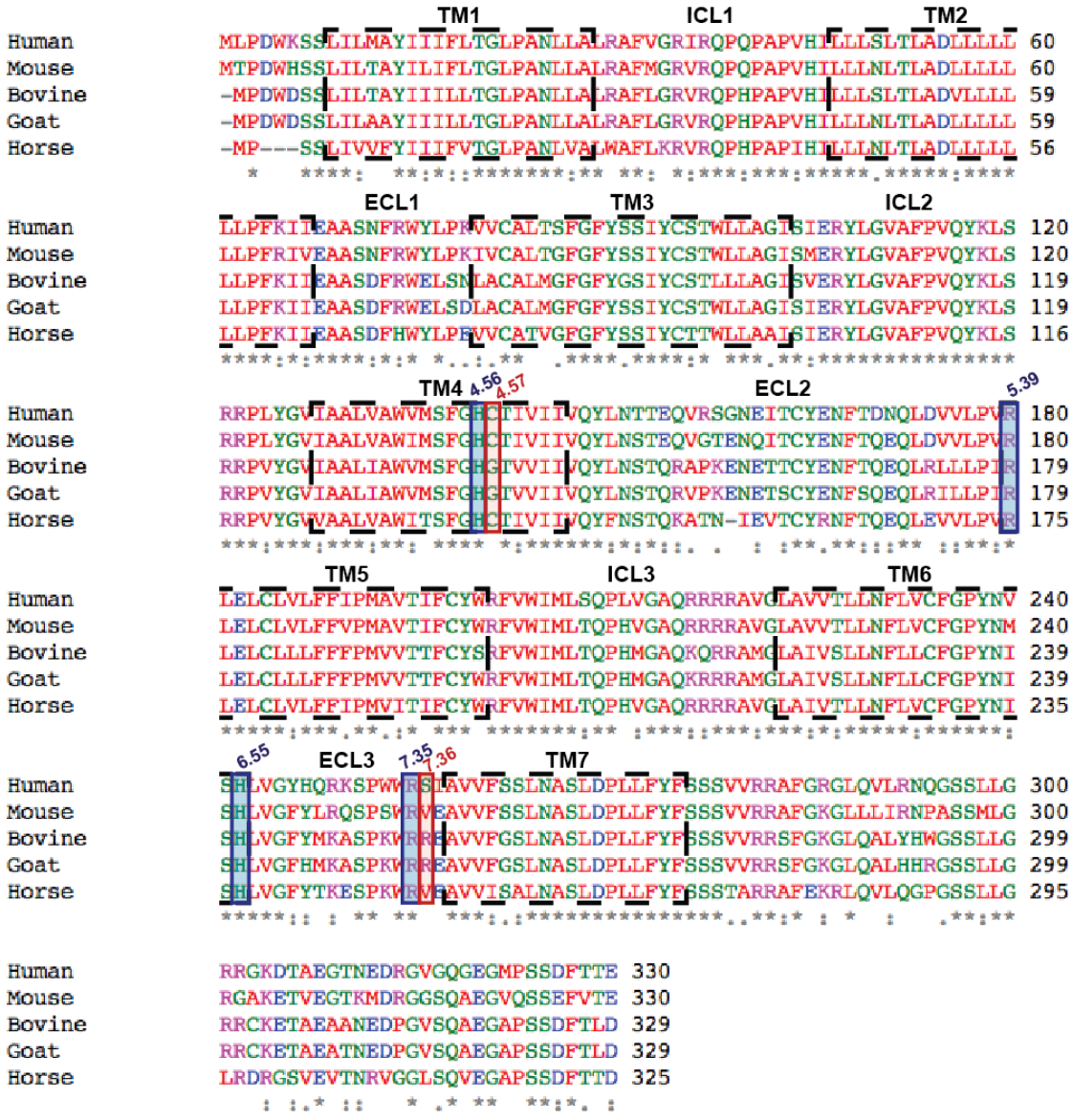
Bergman, 1990. Physiol. Rev. 70, 567-590

FFA2 is Expression in Bovine Rumen



Wang, et al., 2012. J. Dairy Sci. 95, 1371-1375

Multi-Species Sequence Alignment of FFA2



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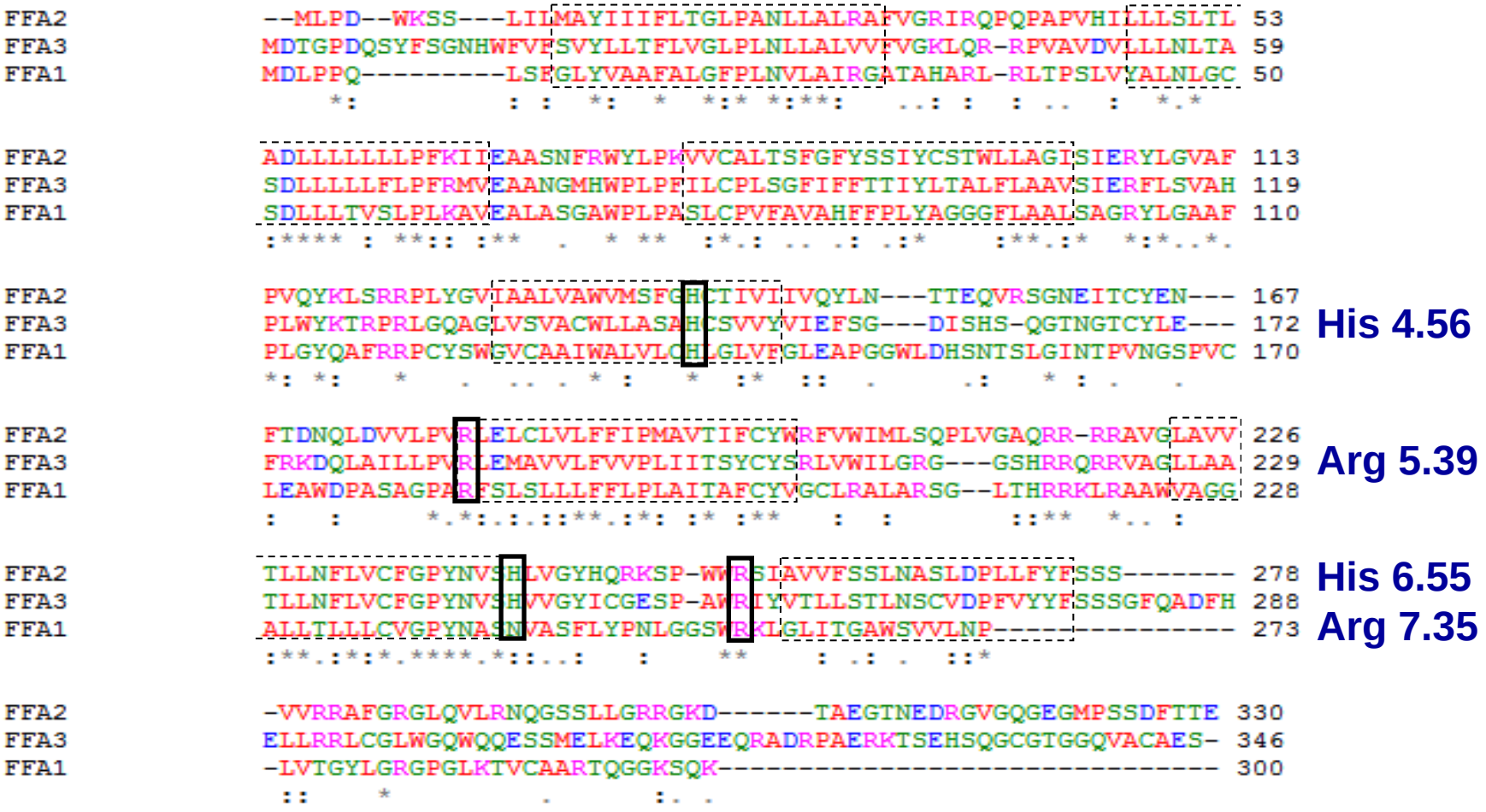
Identification of TM residues that could be involved in fatty acid binding and receptor activation



FFA1, FFA2 and FFA3 respond to FFAs but not the corresponding amides