

G protein-coupled receptors: Nutritional and therapeutic targets

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The Danish Council for Strategic Research



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	$\text{G}\alpha_{q/11}$	$\text{G}\alpha_{i/o}$ and $\text{G}\alpha_{q/11}$	$\text{G}\alpha_{i/o}$

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

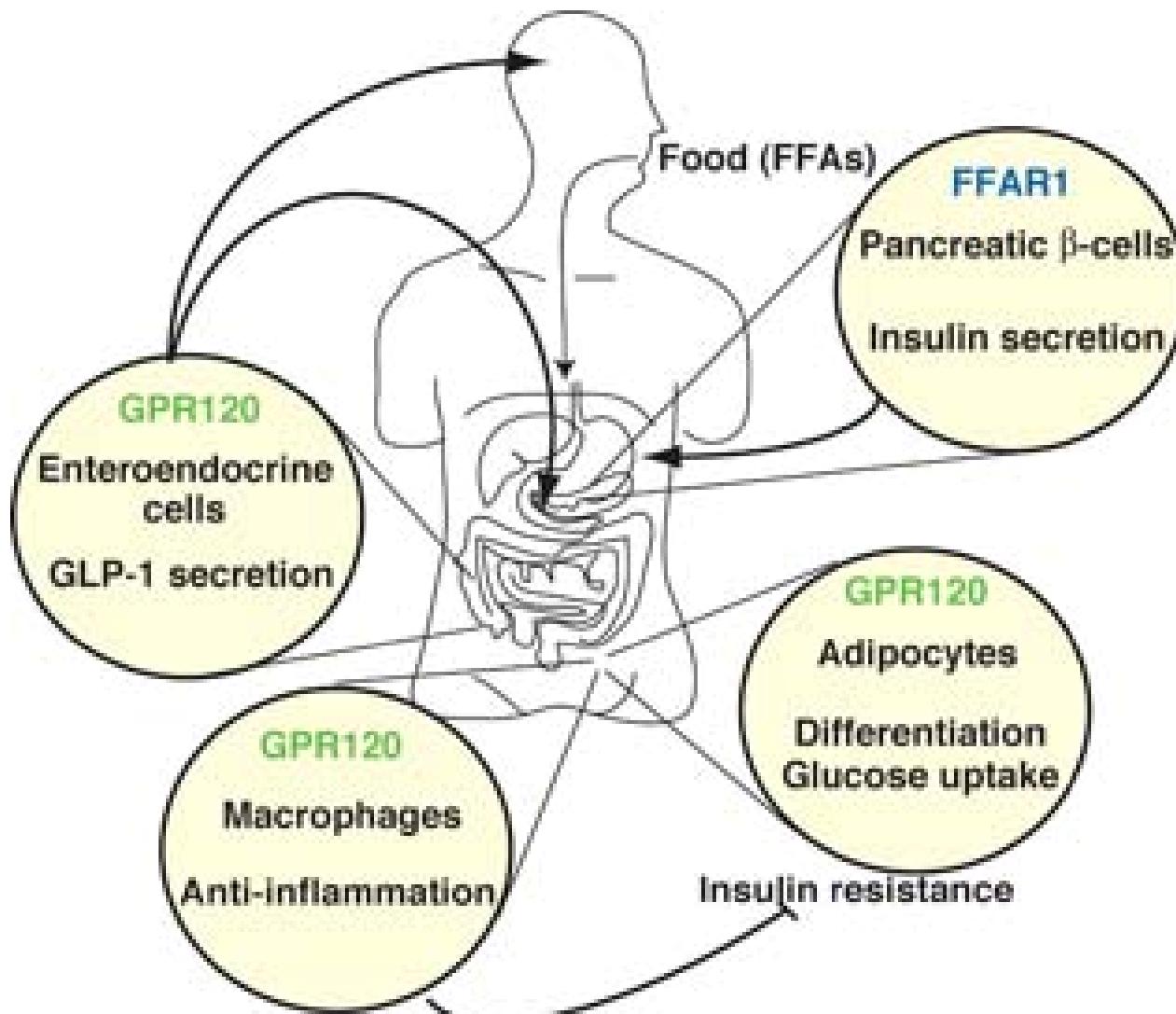
Distribution and function of FFA1-3

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

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'-diindolylmethane
Synthetic antagonists	GW1100, DC260126	none	none
G protein coupling	$\text{G}\alpha_{q/11}$	β -arrestin, $\text{G}\alpha_{q/11}$	$\text{G}\alpha_{i/o}$

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

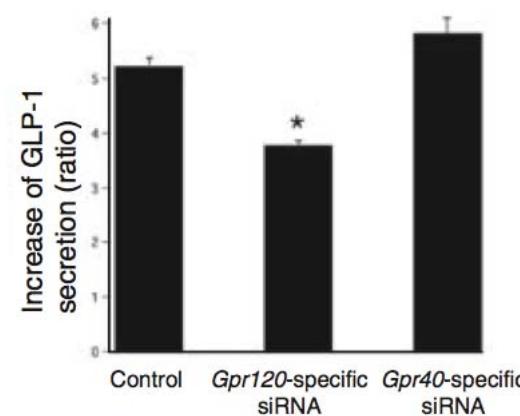
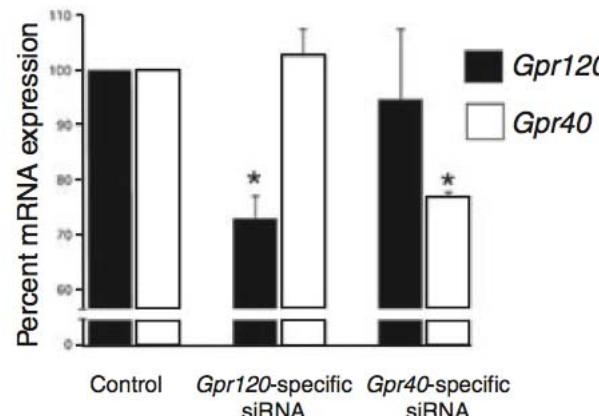


Agonists at FFA1 are currently undergoing clinical trials

- 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

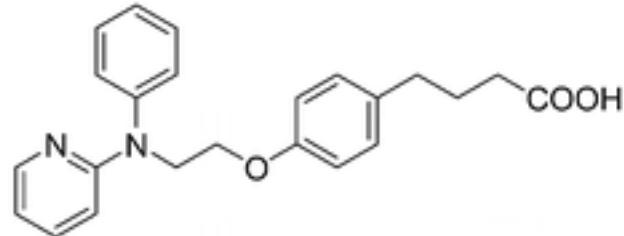
It also helps in validation if genetic studies implicate or link a GPCR in a disease phenotype

- 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.



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

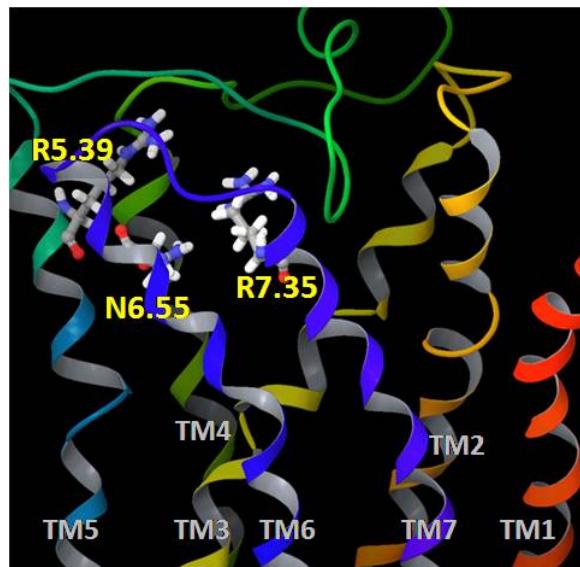


Suzuki et al.
 $pEC_{50}=5.9$
16 Fold Selective over FFA1

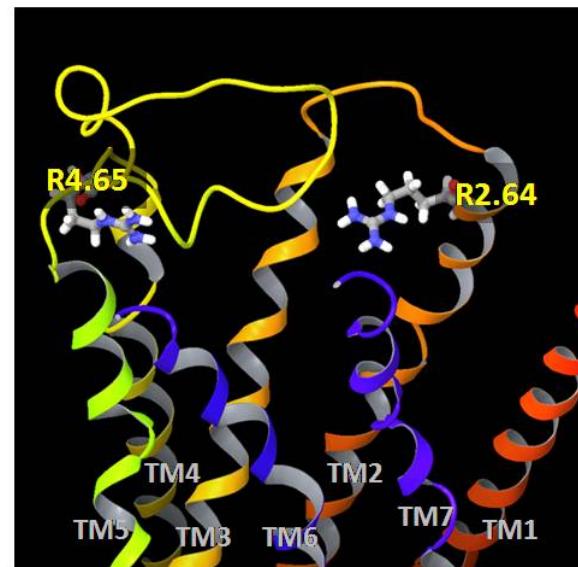
The long isoform of GPR120 is apparently found only in man
And is reported to have a limited range of signals

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

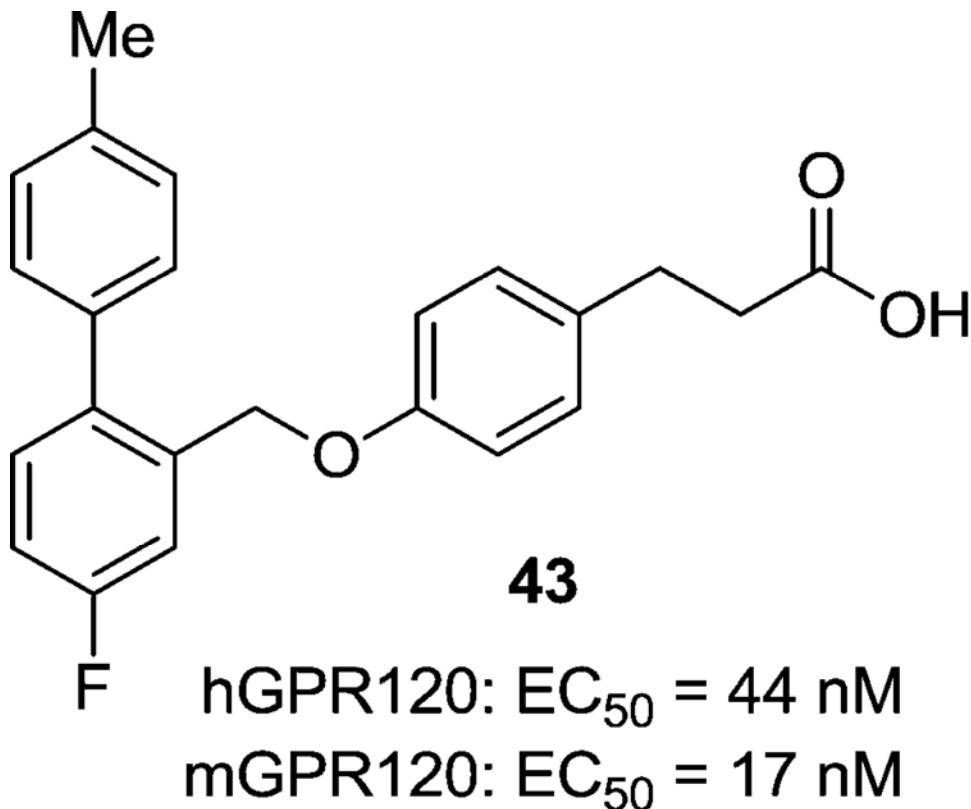


GPR40



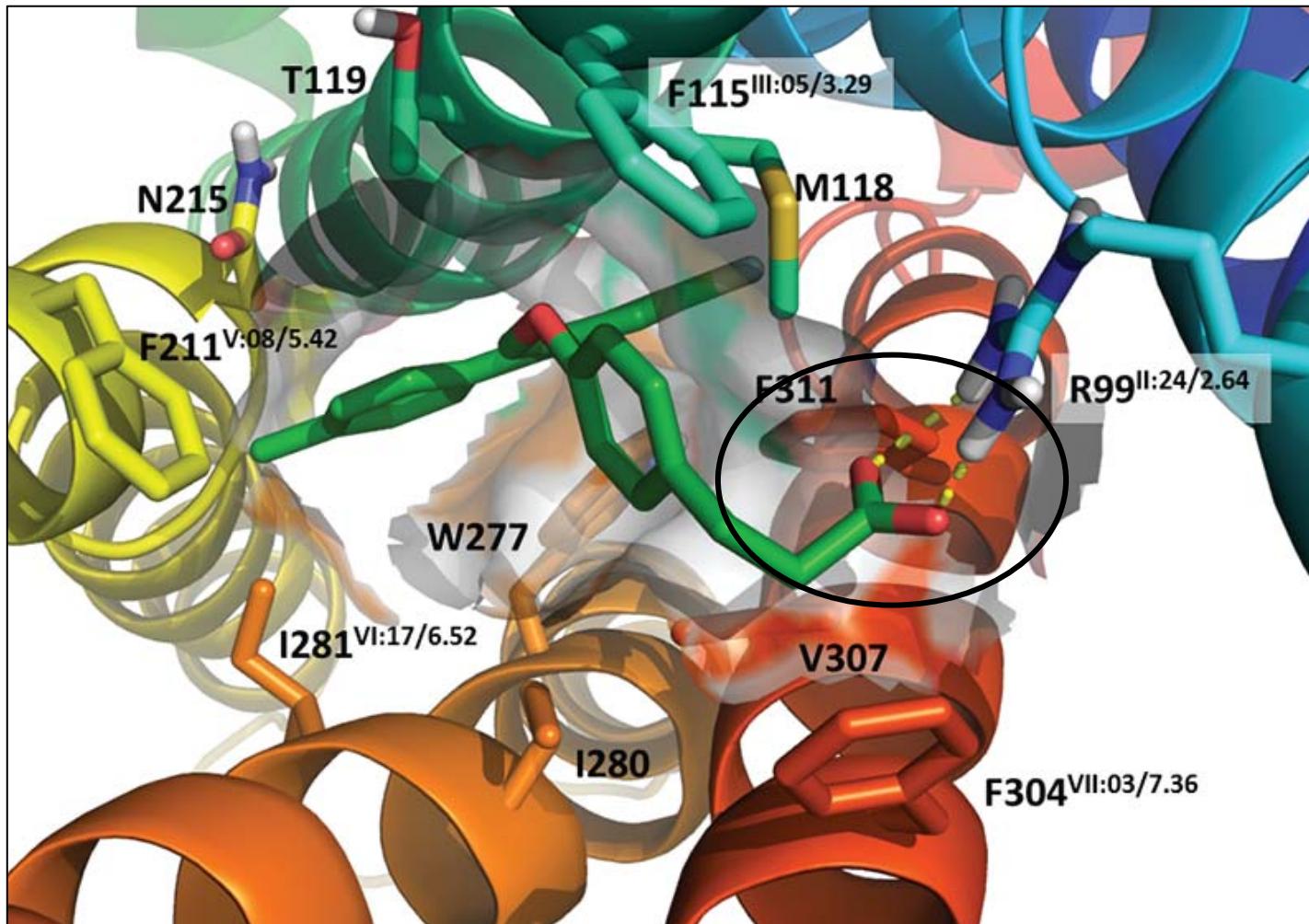
GPR120

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



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
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Potential mode of binding of 891 to human GPR120

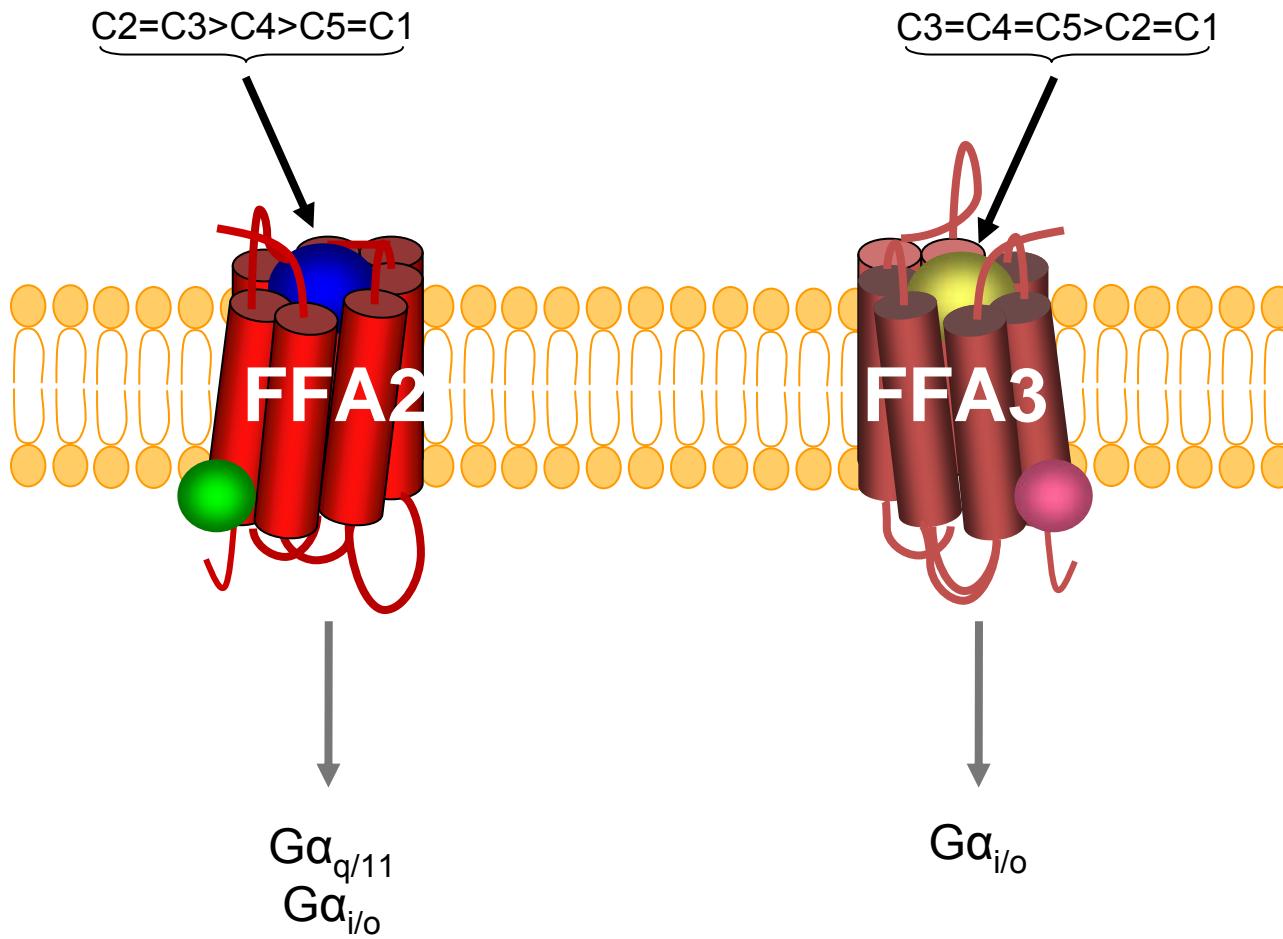


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FFA2 and FFA3 respond to short chain fatty acids produced predominantly by microbial fermentation of non-digestible carbohydrates



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

FFA2	--MLPD--WKSS--LILMAYIIIIFLTGLPANLLALRAFVGRIRQPQPAPVHILLLSITL--	53
FFA3	MDTGPDPQSYFSGNHWFVFSVYLLTFLVGLPLNLLALVVVFVGKLR-RPVAVDVILLNLTA	59
FFA1	MDLPPQ--LSEGLYVAAFALGFPLNVLAIRGATAHARL-RLTPSLVYALNLGC	50
	*: : * : * * : * : * : * : .	
FFA2	ADLLLLLPLPKIIEAASNFRWYLPKVVCALTSGFYSSIFYCSTWLLAGISIERYLGVAF	113
FFA3	SDLLLLLFLPFRMVEAANGMHWPPLFILCPLSGFIFFFFTIYLTLAFLAAVSIERFLSAH	119
FFA1	SDLLLTVSPLPKAVEALASGAWPLPASLCPVFAVAHFFPLYAGGGFLAALSAGRYLGAAF	110
	:**** : * * : * : * . . * * : * :	
FFA2	PVQYKLSRRPLYGVIAALVAWVMSFG[RE]CTIVLIVQYLN--TTEQVRSGNEITCYEN--	167
FFA3	PLWYKTRPRLGQAGLVSVACWLLASAHC[VYYVIEFSG--DISHS-QGTNGTCYLE--	172
FFA1	PLGYQAFRRPCYSWGVCAAIIWALVLCHLGLVFGLEAPGGWLDHSNTSLGINTPVNGSPVC	170
	*: *: * .	
FFA2	FTDNQLDVVLPPV[RE]ELCLVLFFIPMAVTIFCYWRFVWIMLSQPLVGAQRR-RRAVGLAVV	226
FFA3	FRKDQLAILLPPV[RE]EMAVVLFVVPLIITSYCYSRLVWILGRG--GSHRRQRRAVGLAA	229
FFA1	LEAWDPASAGPARFSLSLLLFFLPLAITAFCYVGCLRALARSG-LTHRRKLRAAWVAGG	228
	: .	
FFA2	TLLNFLVCFGPYNVSH[RE]LVGYHQRKSP-W[RE]SIAVVFSSLNASLDPLLFYFSSS--	278
FFA3	TLLNFLVCFGPYNVSH[RE]VGYICGESP-AWRIYVTLLSTLNCSVDPFVYYFSSSGFQADFH	288
FFA1	ALLTILLCVGPyNASN[RE]ASFLYPNLGGSWRKGLGITGAWSVVLNP	273
	:***:***:***:***: .	
FFA2	-VVRRAFGRGLQVLRNQGSSLLGRRGKD-----TAEGTNEDRGVGQGEQMPSSDFTTE	330
FFA3	ELLRRLCGLWGQWQQESSMELKEQKGEEQRADRPAERKTSEHSQCGTGQQVACAES-	346
FFA1	-LVTGYLGRGPGLRTVCAARTQGGKSQK-----	300
	:: .	

His 4.56

Arg 5.39

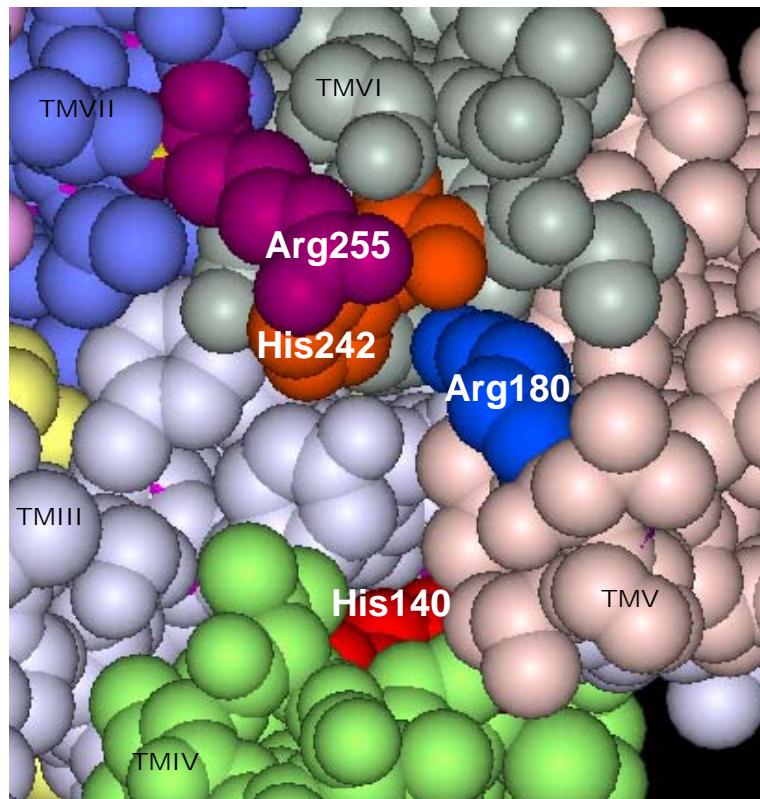
His 6.55

Arg 7.35

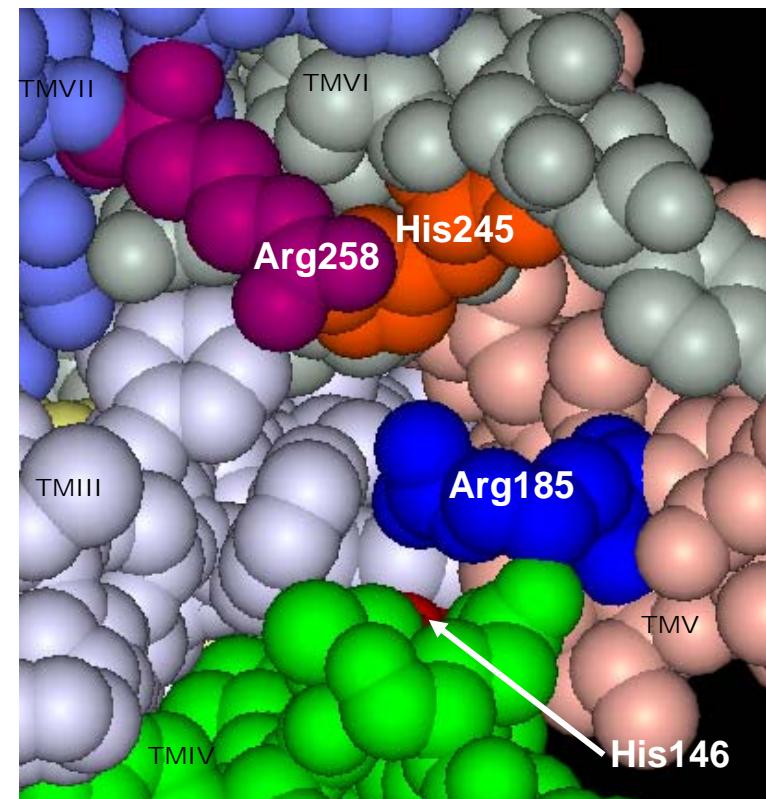
FFA1, FFA2 and FFA3 respond to FFAs but not the corresponding amides

Looking from the extracellular milieu

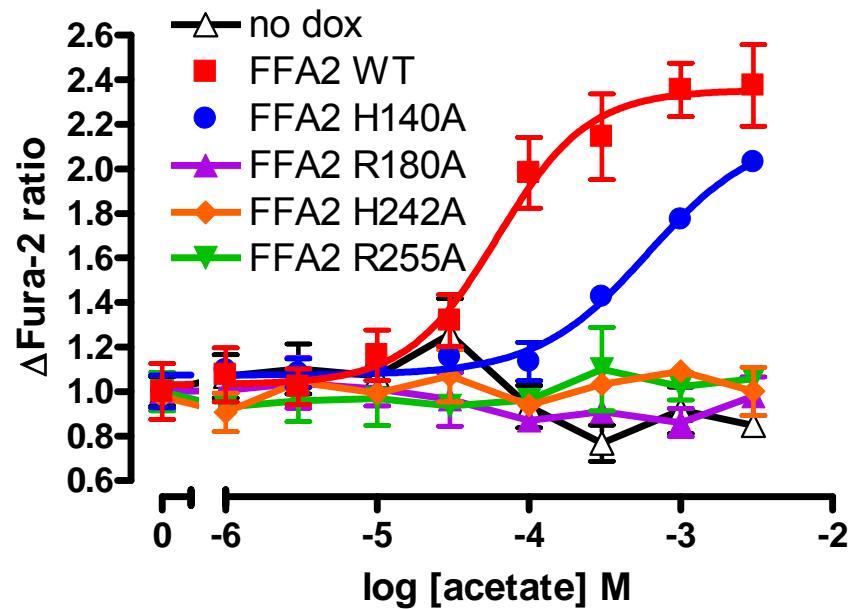
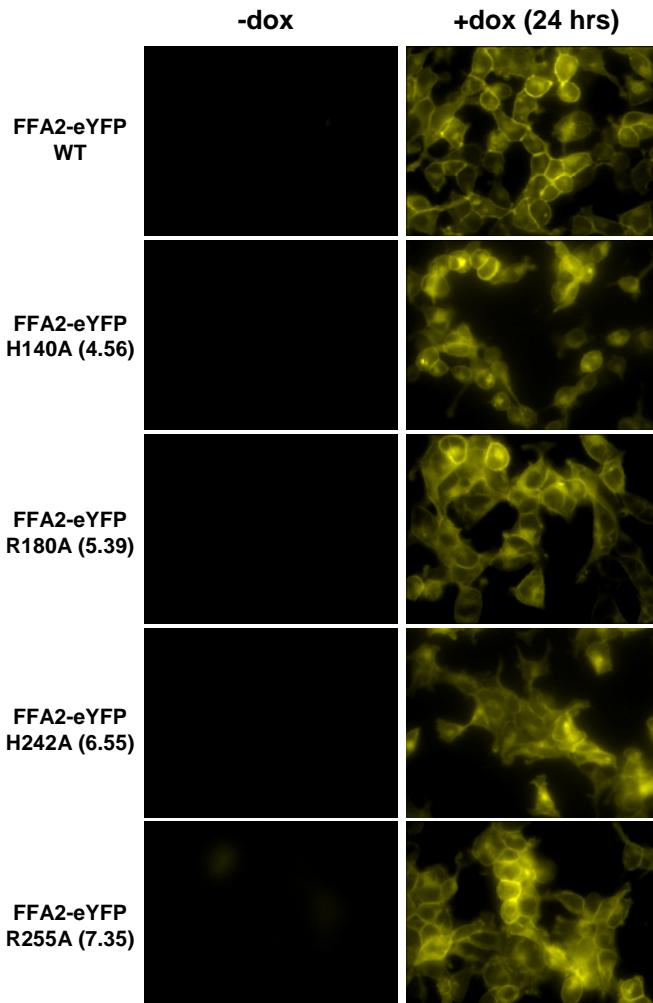
Human FFA2



Human FFA3



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



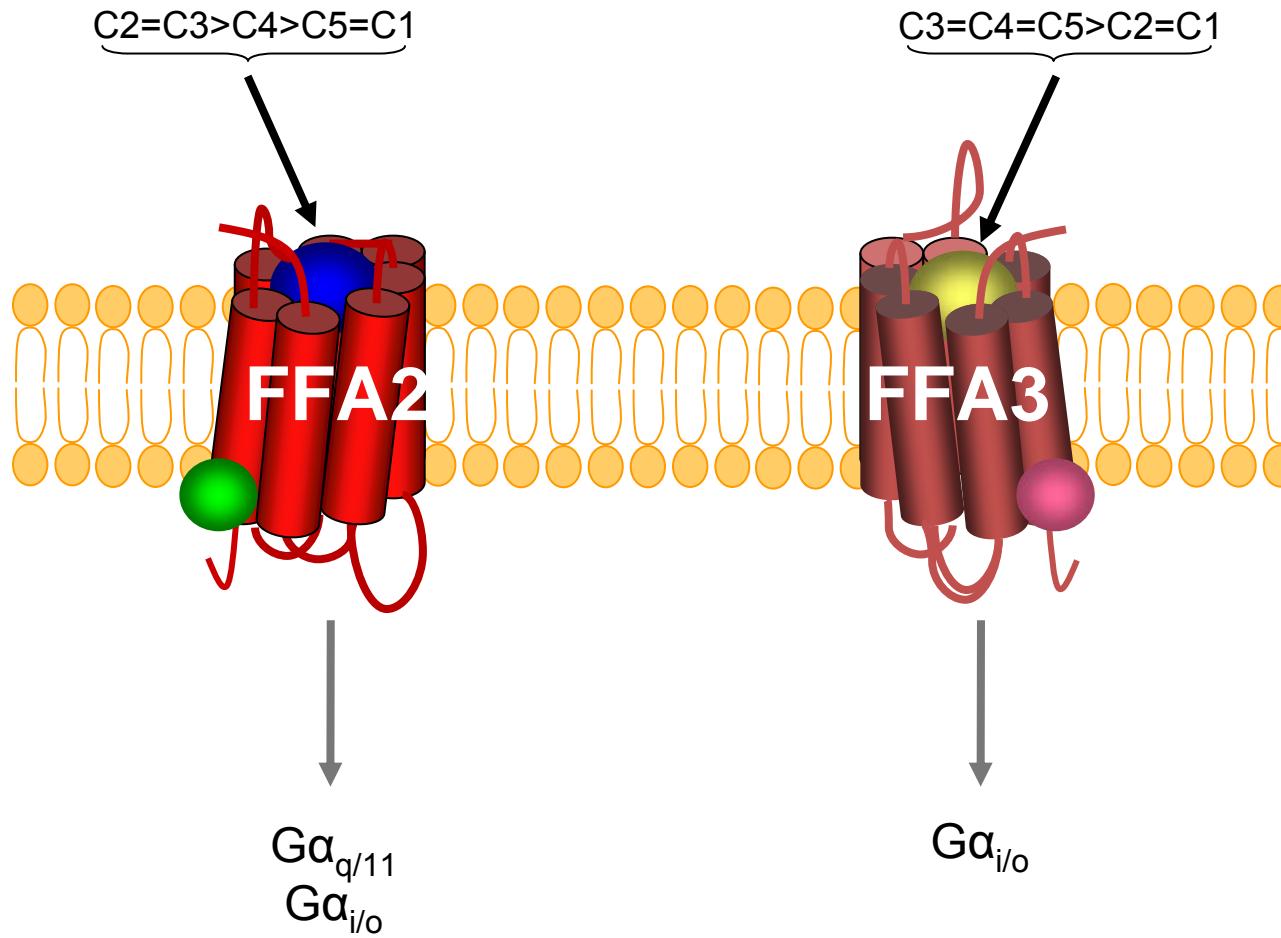
But for His140 (4.56) only potency is reduced



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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?

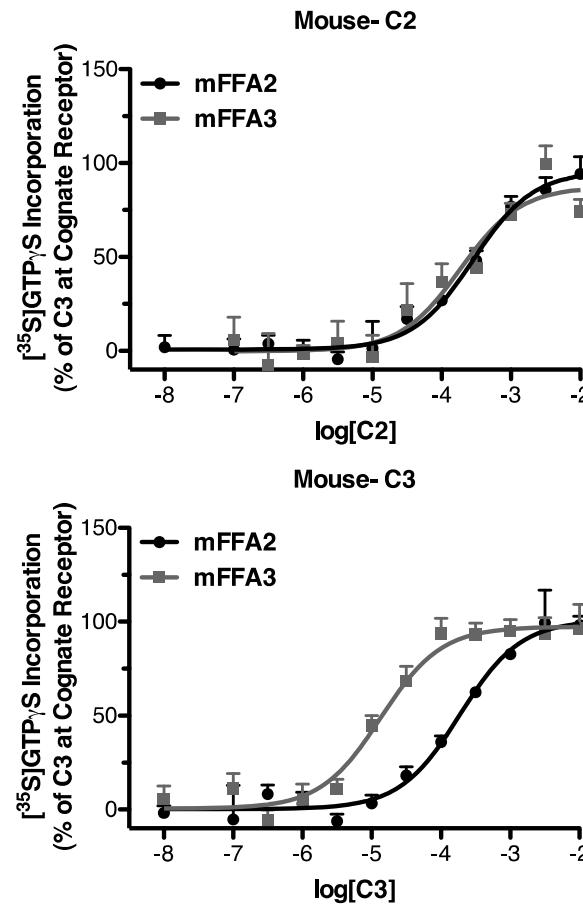
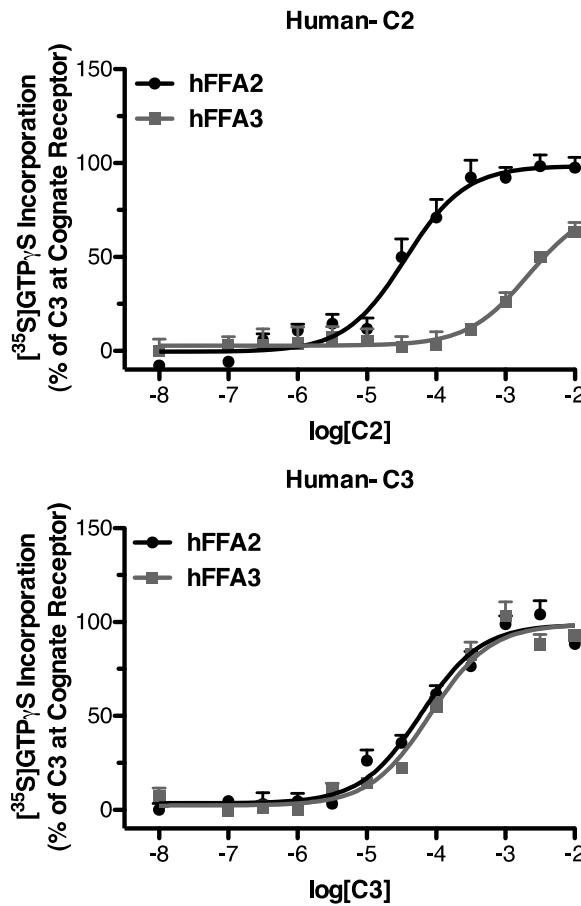
But is this valid if we consider non-human studies?

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

The answer (of course) is No



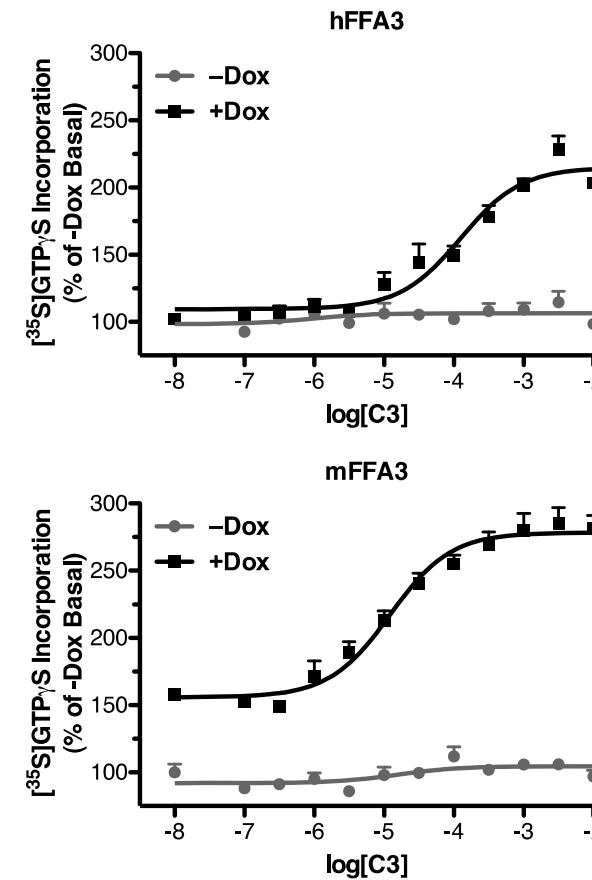
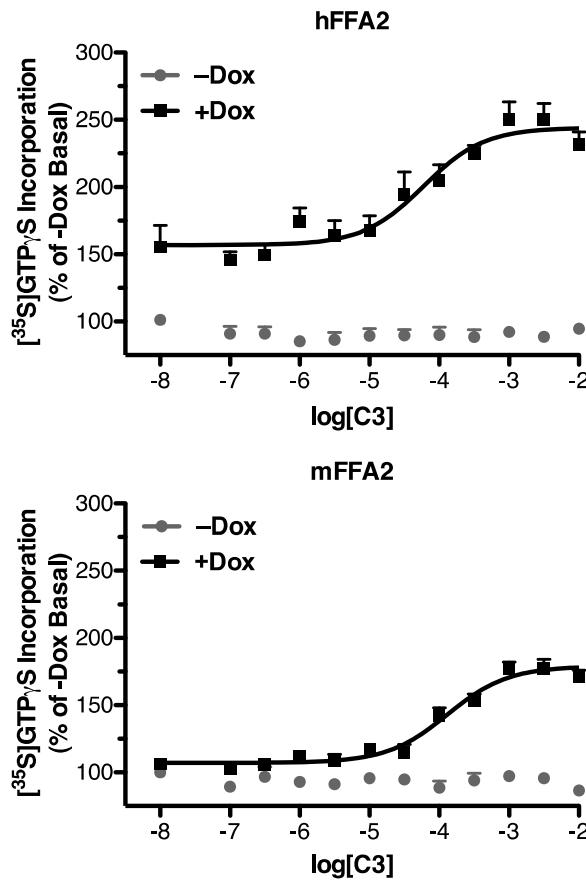
C2 and C3 selectivity is not maintained in the mouse orthologs of FFA2 and FFA3



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

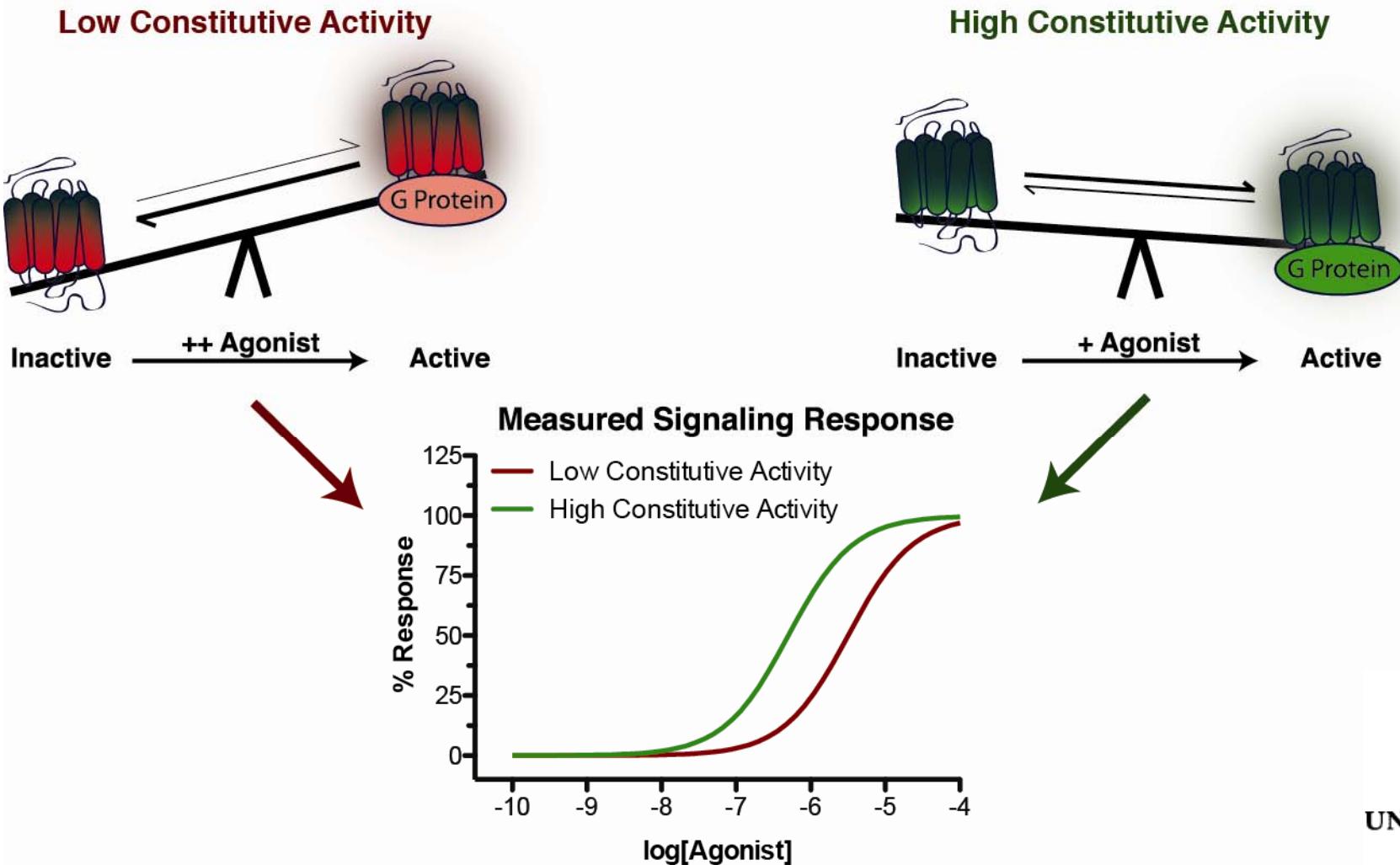
This is due to markedly different levels of ligand-independent (constitutive activity) in the murine and human orthologs of FFA2 and FFA3



Human FFA2 and murine FFA3 have high agonist-independent activity

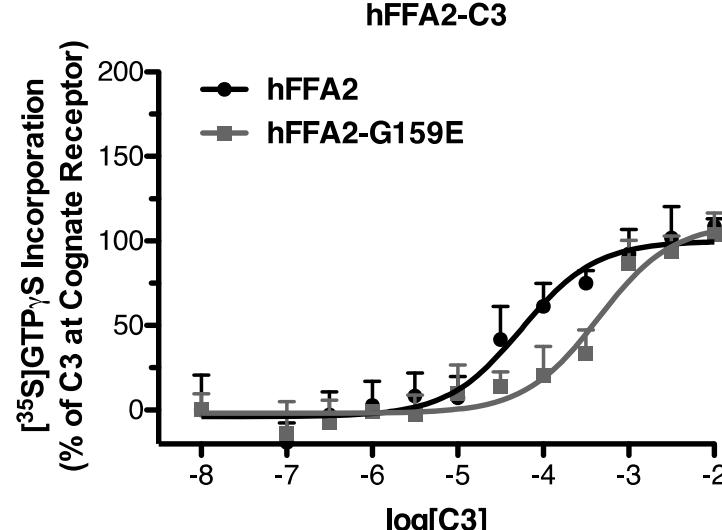
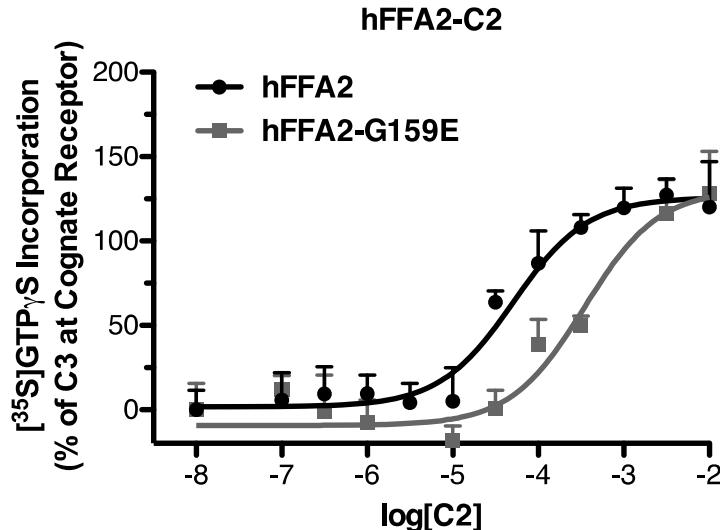
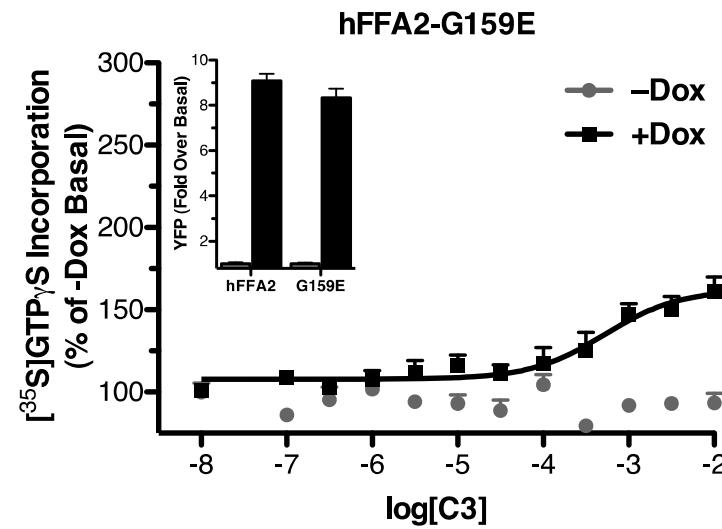
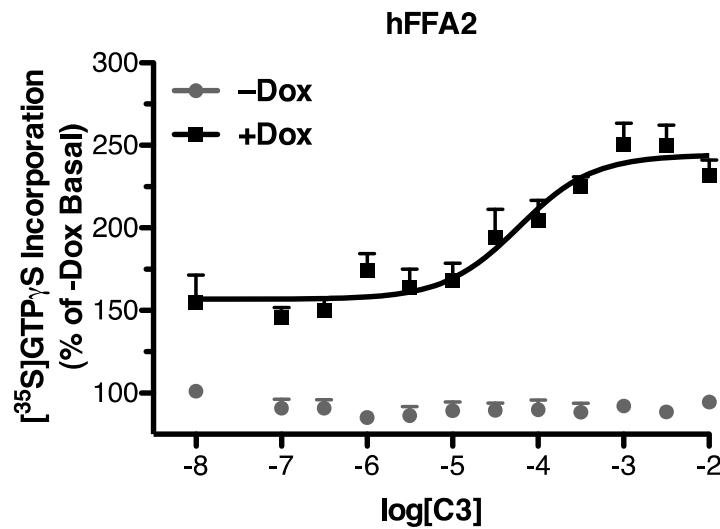
Constitutively active forms of receptors are known to display higher agonist potency

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

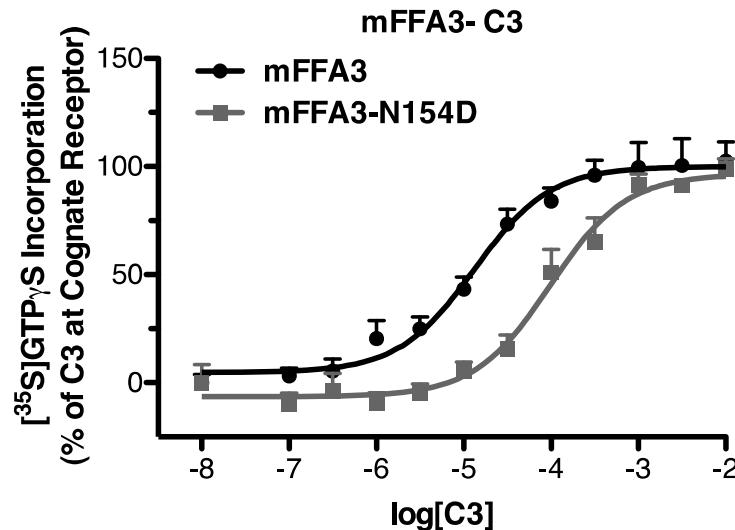
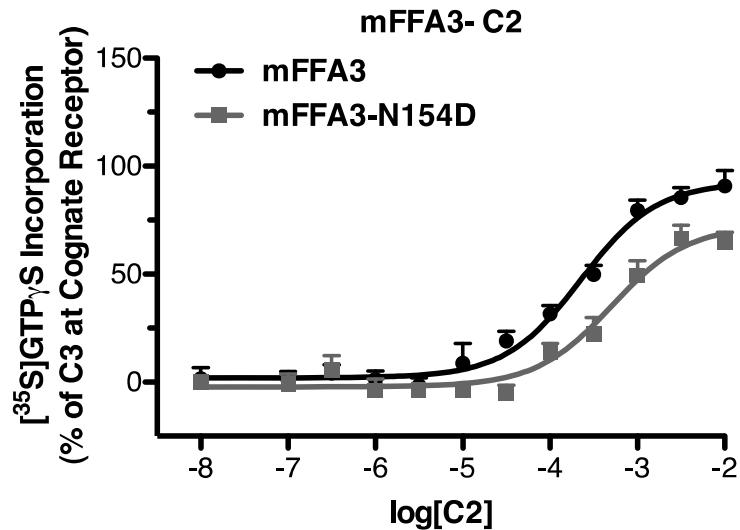
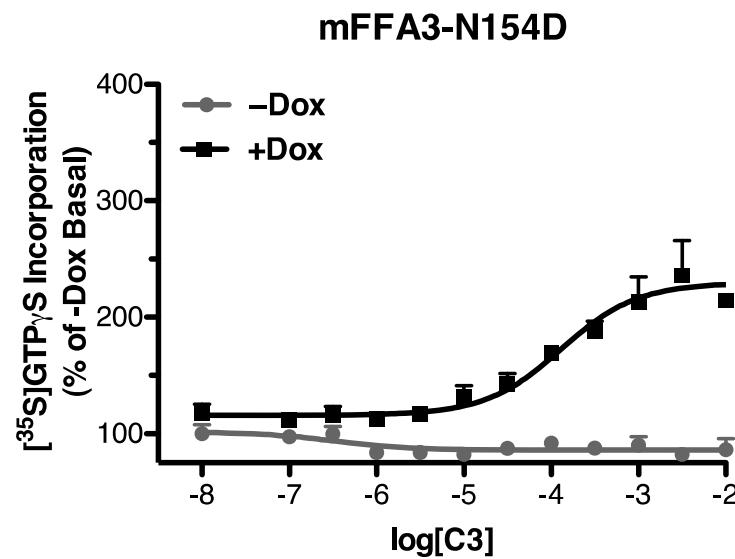
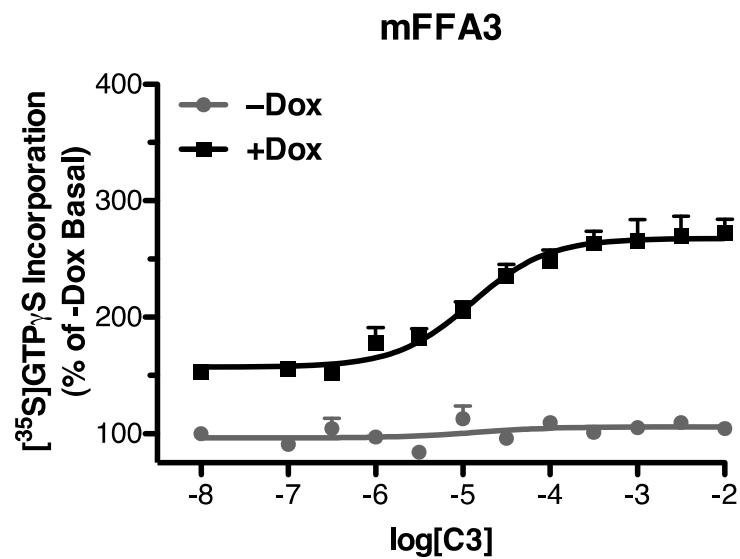


Constitutive activity and SCFA potency at hFFA2 is reduced by introducing a negatively charged Glu residue present in ECL2 of mFFA2

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

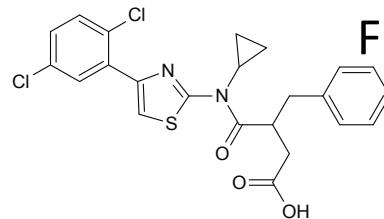


Can synthetic ligands provide information on the respective roles of FFA2 and FFA3?

Ligands from the patent literature can be valuable tools

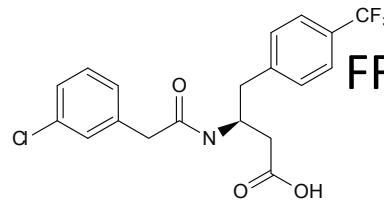
Compound Structure Pharmacology

TUG-800



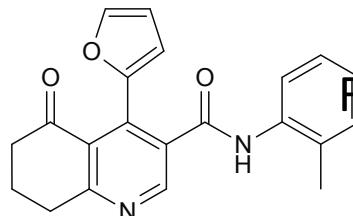
FFA2 selective agonist

TUG-942



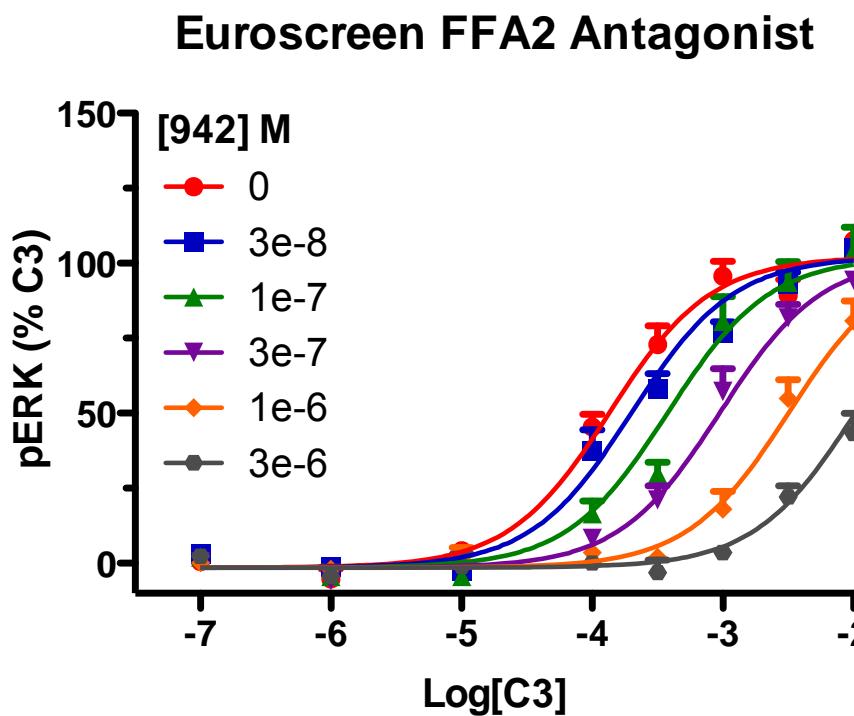
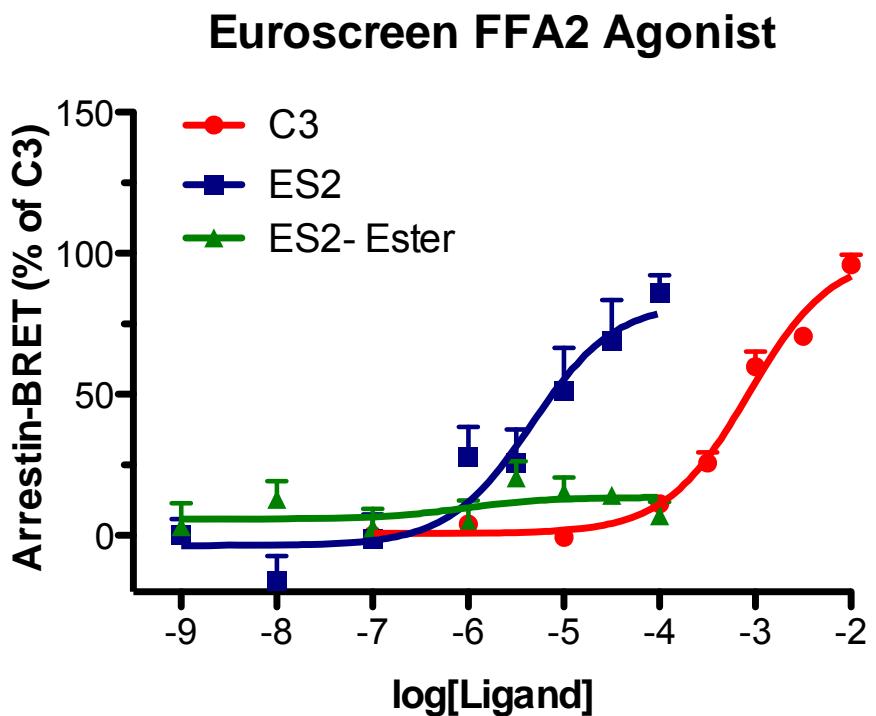
FFA2 selective antagonist

TUG-816



FFA3 selective agonist

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



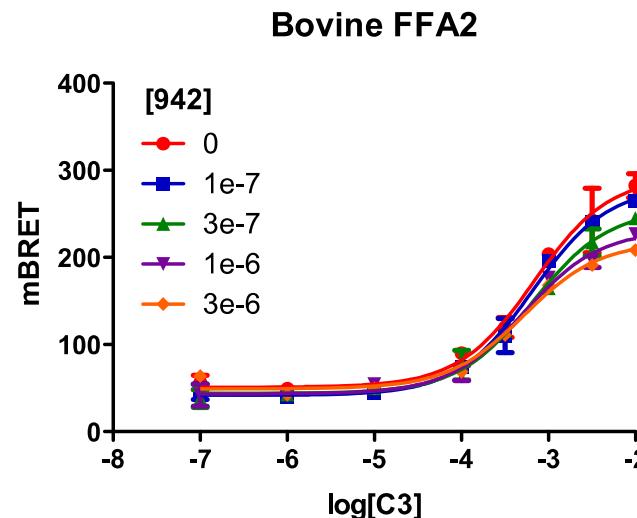
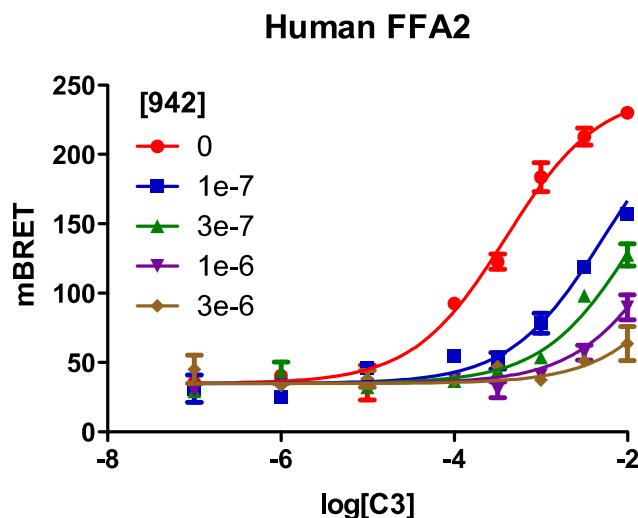
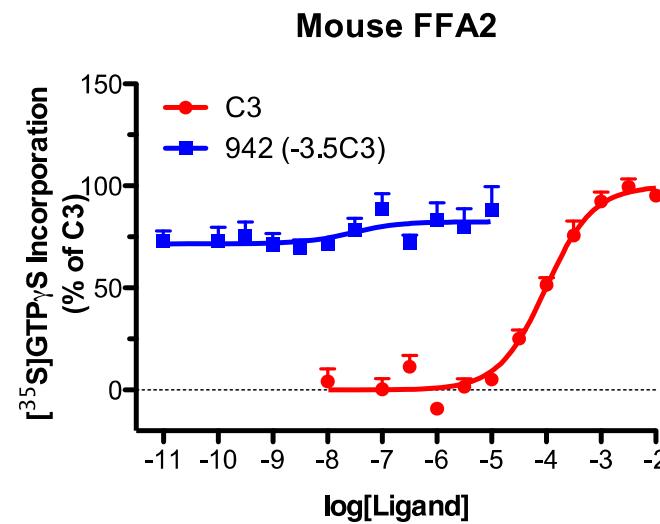
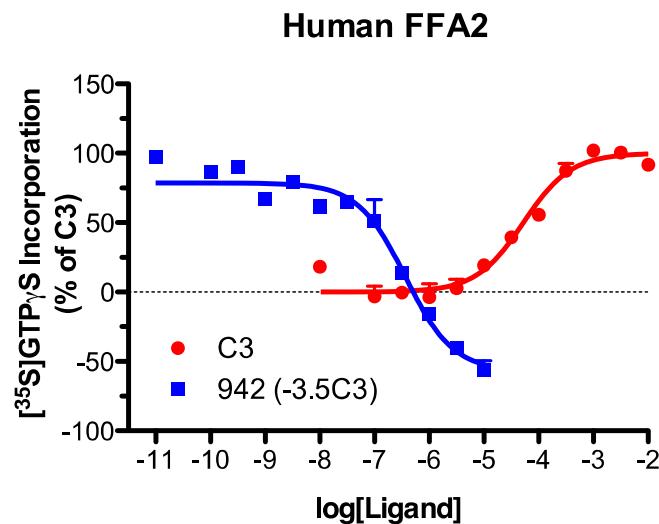
At least 100 fold more potent than C3

Orthosteric as the carboxylate moiety is required

Competitive antagonist



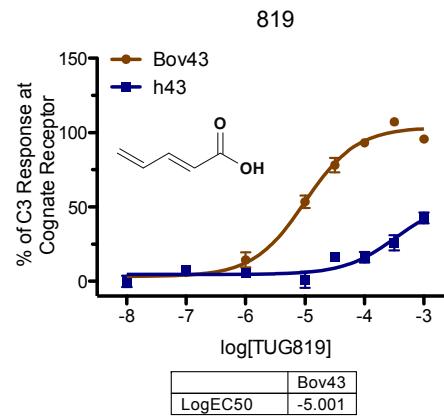
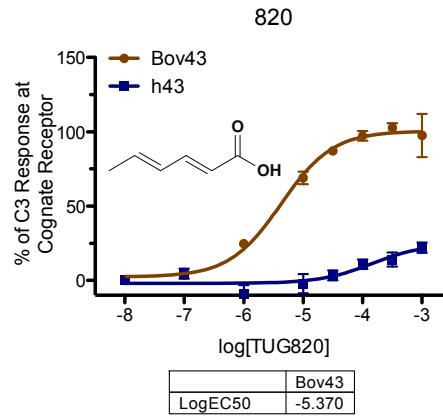
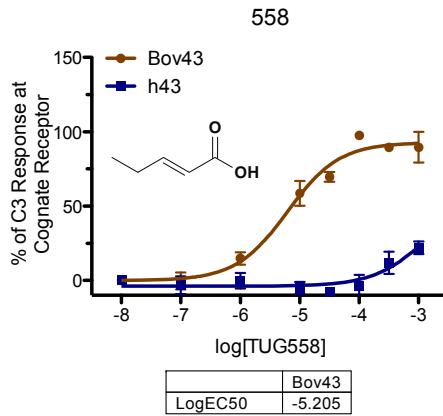
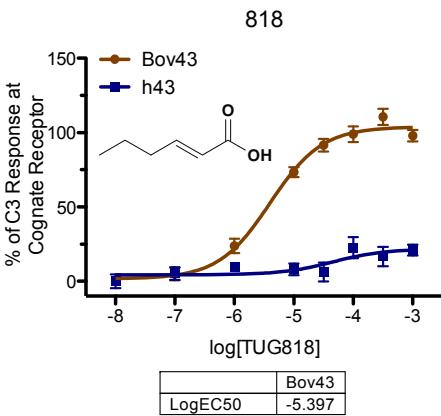
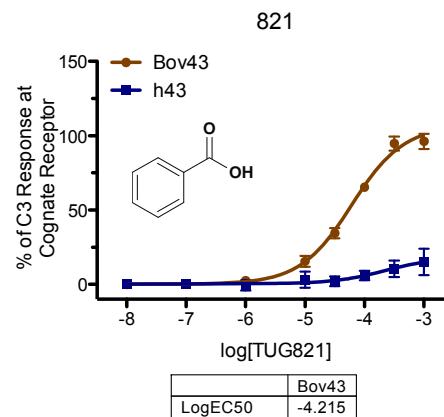
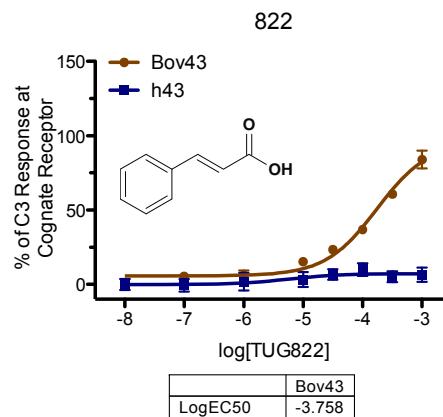
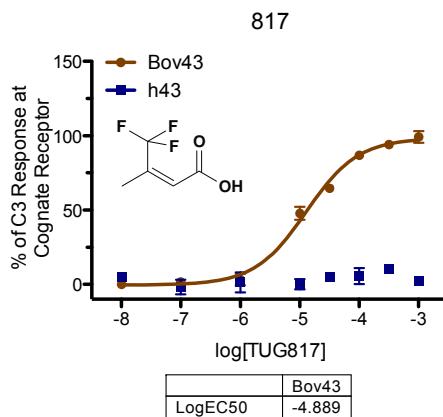
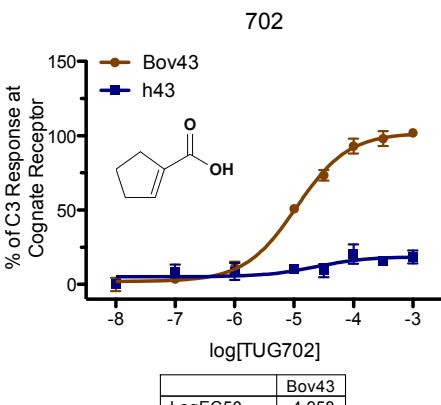
But only at the human receptor, FFA2 antagonism by compound 942 is limited to the human orthologue



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



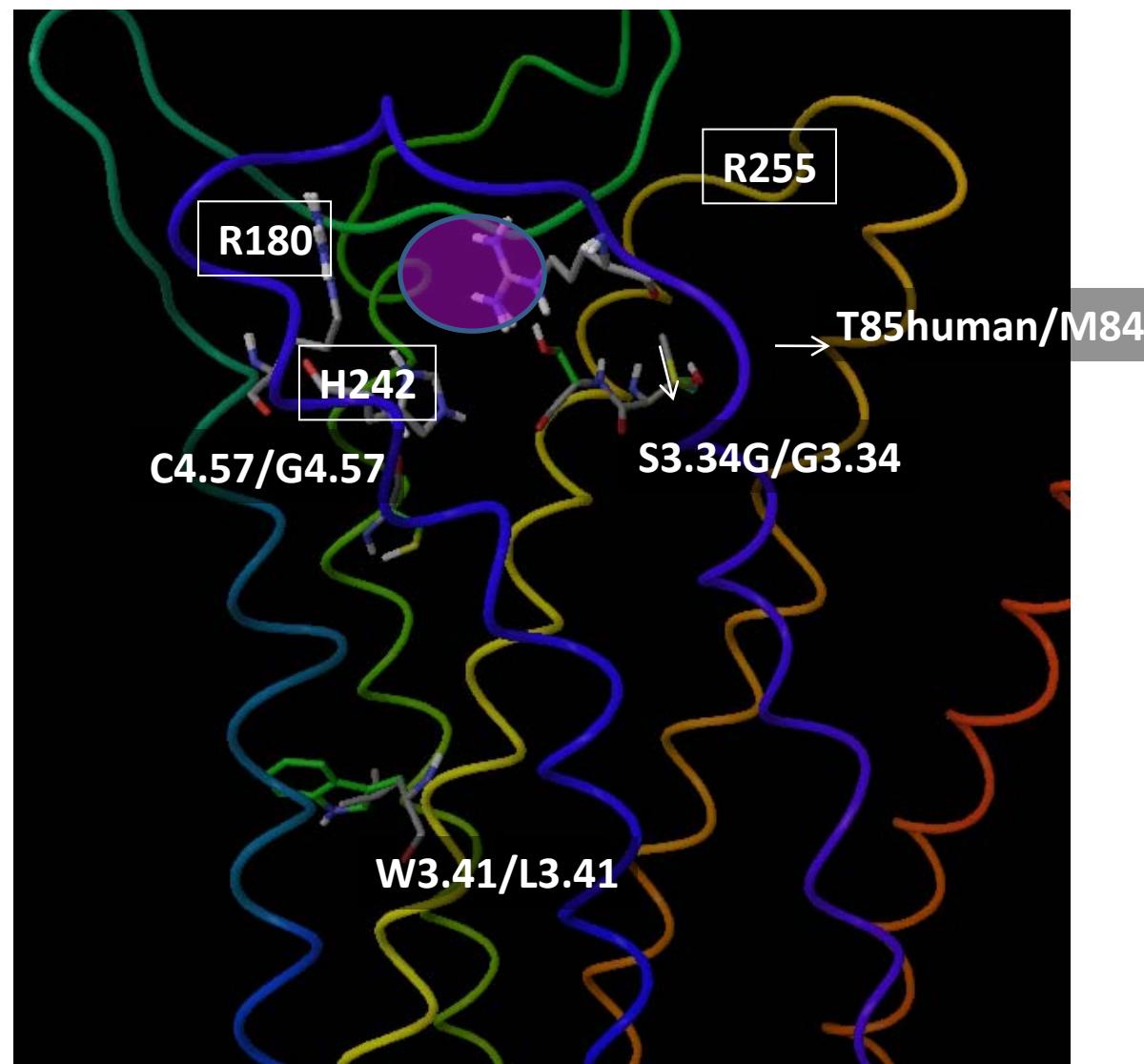
This is SORBIC ACID

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:

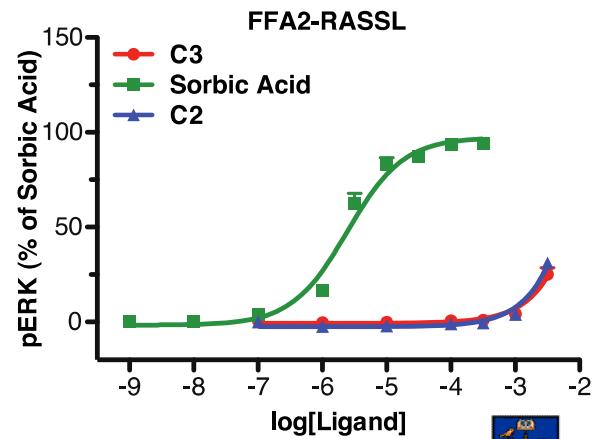
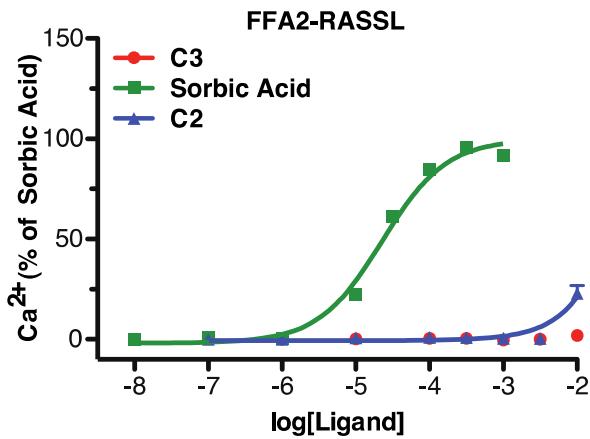
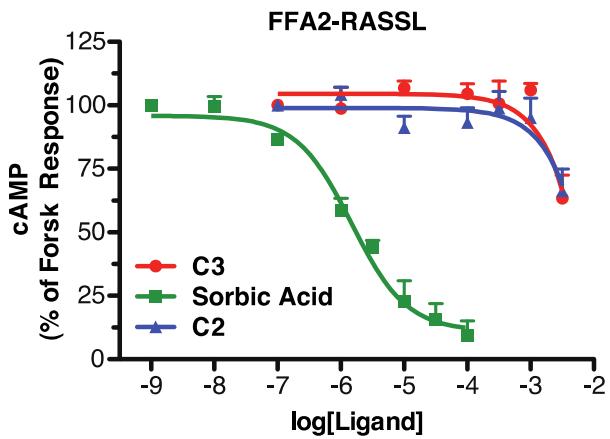
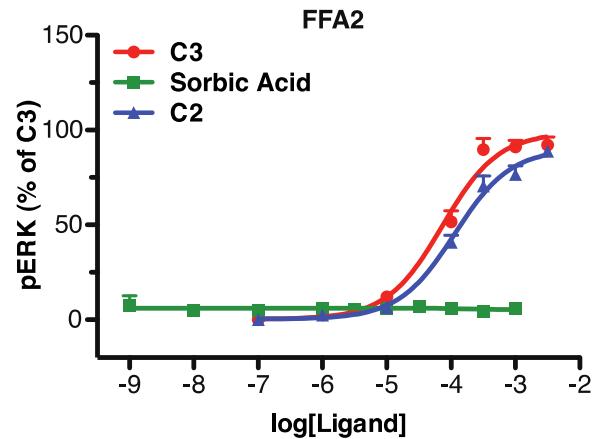
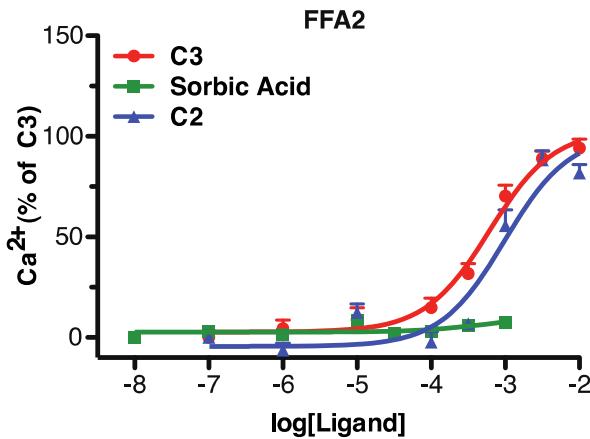
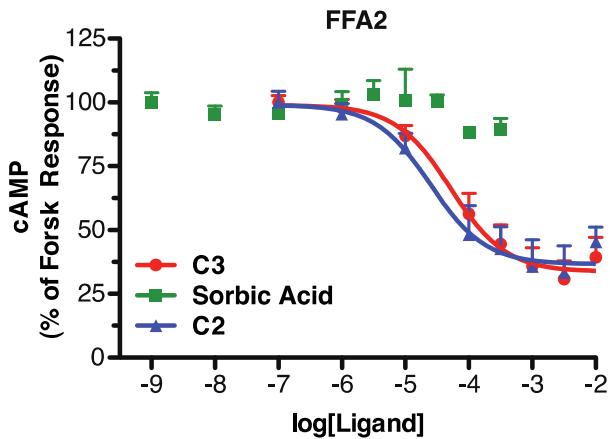
hFFA2-S3.34G/BovFFA2-G3.34S
HFFA2-C4.57G/BovFFA2-G4.57C

Additional site predicted to affect binding pocket size:

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



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



Multi-Species Sequence Alignment of FFA2

	TM1	ICL1	TM2	
Human	MLPDWKSS LILMAYIIIFLTGLPANLLA R ^A FVGRIRQPQPA ^{PVH} I LLS L T A D LLL LL			60
Mouse	MTPDWHSSL LILTAYIIIFLTGLPANLLA R ^A F ^M GRVRQPQPA ^{PVH} I LLS L T A D LLL LL			60
Bovine	-MPDWDSS LILTAYIIIFLTGLPANLLA R ^A F ^L GRVRQPHPA ^{PVH} I LLS L T A D V LLL			59
Goat	-MPDWDSS LILAAIYIIIFLTGLPANLLA R ^A F ^L GRVRQPHPA ^{PVH} I LLS L T A D V LLL			59
Horse	-MP---SS LIVVFYIIIFVTGLPANLVALW AFLKRV ^R QPHPA ^{PVH} I LLS L T A D V LLL			56
	*****	*****	*****	*****
	ECL1	TM3	ICL2	
Human	LLPFK IIEAASNFRWYLPNNVCALTSFGFYSSIIYCSTWLLAGISI E ^R YLGVAFPVQYKLS			120
Mouse	LLPFRI VEAASNFRWYLPKIVCALTGFGFYSSIIYCSTWLLAGISI MERYLGVAFPVQYKLS			120
Bovine	LLPFK IIIEAASDFRWELSNLACALMGFGFYGSIIYCSTWLLAGISI SVERYLGVAFPVQYKLS			119
Goat	LLPFK IIIEAASDFRWELSDLACALMGFGFYSSIIYCSTWLLAGISI E ^R YLGVAFPVQYKLS			119
Horse	LLPFK IIIEAASDFHWYLPFVVCATVGFGFYSSIIYCCTWLLAAISI E ^R YLGVAFPVQYKLS			116
	*****	*****	*****	*****
	TM4 4.56 A.51	ECL2	5.39	
Human	RRPLYGV IAALVAWVMSFGHC TIVIIQYLNNTTEQVRSGNEITCYENFTDNQL D VVLPVR			180
Mouse	RRPLYGV IAALVAWIMSF GHC TIVIIQYLNSTEQVG TENQITCYENFTQEQ Q LDVVLPVR			180
Bovine	RRPVYG VIAALIAWVMSFGHG TIVIIQYLNSTQRAPKENETTCYENFTQEQ Q LRLLLPIR			179
Goat	RRPVYG VIAALIAWVMSFGHG TIVIIQYLNSTQRVPKENETSCYENFSQEQLRILLPIR			179
Horse	RRPVYG WAALVAWITSFGHC TIVIIQYFNSTQKATN-IEVTCYRNFTQEQ Q LEVVLPIR			175
	*****	*****	*****	*****
	TM5	ICL3	TM6	
Human	LELC LVLFIPMAVTIFCYWRFVWIMLSQLPLVGAQRRRAVGLAVV TLLN F LG C PG P YN			240
Mouse	LELC LVLFIPMAVTIFCYWRFVWIMLSQLPHVGAQRRRAVGLAVV TLLN F LG C PG P YN			240
Bovine	LELC LLLLFFFPMVVTTFCYS RFVWIMLTQPHMGAQ K QRRAM L AIV S LLN F LLC F PG P YN			239
Goat	LELC LLLLFFFPMVVTTFCYS RFVWIMLTQPHMGAQ K QRRAM L AIV S LLN F LLC F PG P YN			239
Horse	LELC LVLFIPMVITIFCYWRFVWIMLSQLPHVGAQRRRAVGLAIV TLLN F LG C PG P YN			235
	*****	*****	*****	*****
	6.56 ECL3 1.35 3.6 TM7			
Human	SHLVGYH QRKSPW IAAVFSSLN A SLD P LLF Y FSSSV R RAF G RGL Q VL R N Q GSS I LG			300
Mouse	SHLVGFY LRQSPSW VEAVFSSLN A SLD P LLF Y FSSSV R RAF G K L LI R N P ASS M LG			300
Bovine	SHLVGFYM KASP PK R REAVVFGSLN A SLD P LLF Y FSSSV R RSFG K GL Q ALY H WGSS I LG			299
Goat	SHLVGFHM KASP PK R REAVVFGSLN A SLD P LLF Y FSSSV R RSFG K GL Q ALHHRGSS I LG			299
Horse	SHLVGFYT KESPKW RAV V ISAL N ASLD P LLF Y FSSSTARRAF E KRL Q VL Q GPSS I LG			295
	*****	*****	*****	*****
Human	RRGKD TAEGTNEDRGV GQQGE GMPS SDFTTE			330
Mouse	RGA KETVEGT KMDRG GSQAEGVQSSEFV TE			330
Bovine	RRCK ETAEEANEDPGV SQAE GAPSSDFTL D			329
Goat	RRCK ETAATNEDPGV SQAE GAPSSDFTL D			329
Horse	LRDRGS VEVTNRVGG LSQVE GAPSSDFTTD			325
	*****	*****	*****	*****



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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

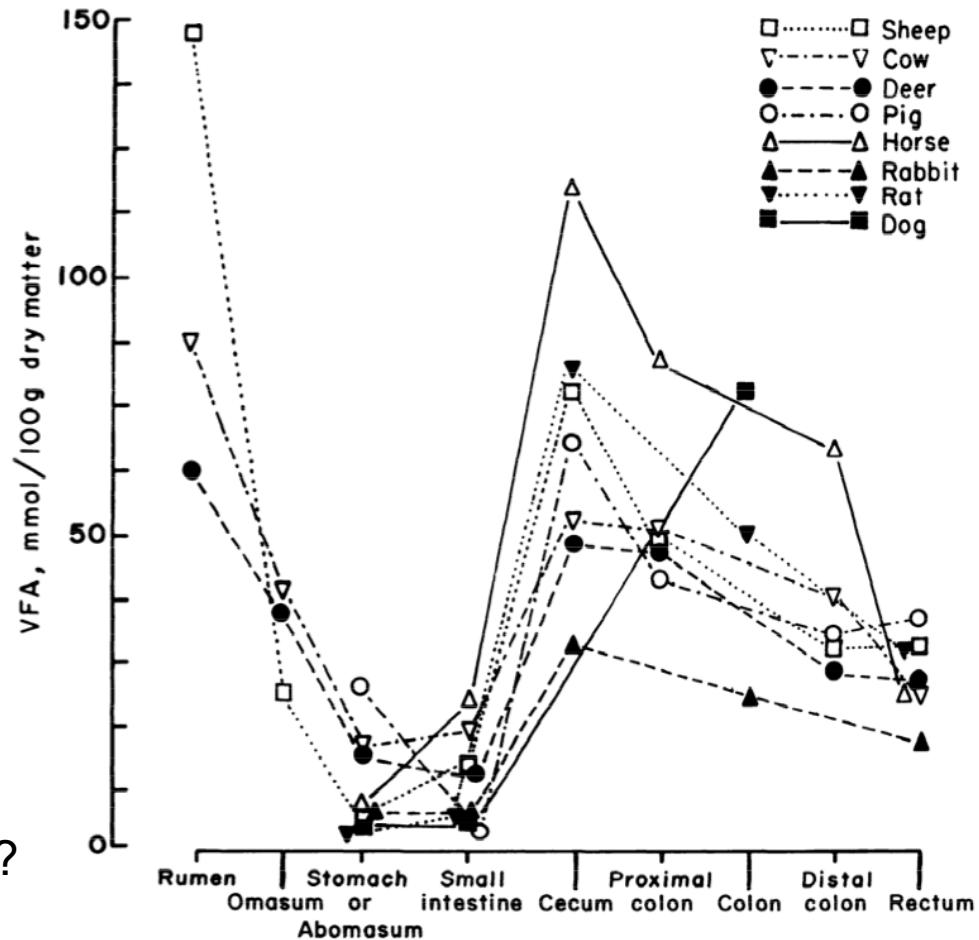


SCFA Concentrations in the Digestive Tract of Various Species

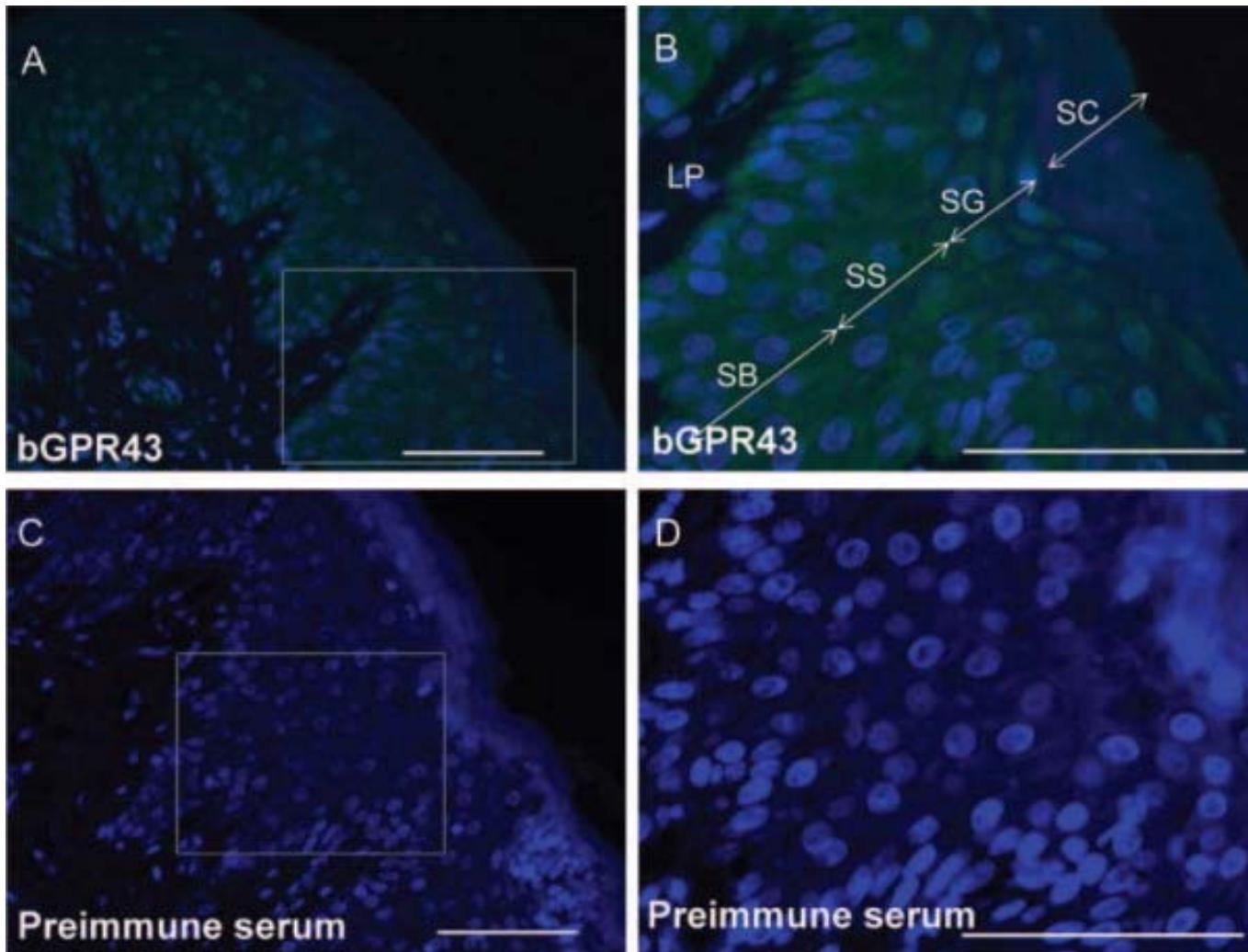
- Highest SCFA concentrations are observed in Rumen
- Although not a ruminant, the horse also shows very high concentrations in lower digestive tract
- Suggests unique pharmacology of ruminant FFA2 may be related directly to rumen function

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



FFA2 is Expression in Bovine Rumen



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

Multi-Species Sequence Alignment of FFA2

	TM1	ICL1	TM2	
Human	MLPDWKSS LILMAYIIIFLTGLPANLLA R ^A FVGRIRQPQPA ^{PVH} ILLSLTLADLLL L			60
Mouse	MTPDWHSSL LILTAYIIIFLTGLPANLLA R ^A F ^M G ^V RQPQPA ^{PVH} ILLSLTLADLLL L			60
Bovine	-MPWD DSS LILAAYIIILTGLPANLLA R ^A F ^L G ^R VQPHPA ^{PVH} ILLSLTLADLLL L			59
Goat	-MPWD DSS LILAAYIIILTGLPANLLA R ^A F ^L G ^R VQPHPA ^{PVH} ILLSLTLADLLL L			59
Horse	-MP---SS LIVVFYIIIFV TGLPANLVALWAFLKRV ^R QPHPA ^I I ^H ILLNLTLADLLL L			56
	*****	*****	*****	*****
	ECL1	TM3	ICL2	
Human	LLPFK TEAASNFRWYLP P ^N V ^C ALTSFGFYSS IYCSTWLLAGISIERYLGVAFPVQYKLS			120
Mouse	LLPF ^R IVE EAASNFRWYLP KIVCALTG ^F GYSS IYCSTWLLAGISMERYLGVAFPVQYKLS			120
Bovine	LLPFK III E AASDFRWELS N LACALMGFGFYGS IYCSTWLLAGISVERYLGVAFPVQYKLS			119
Goat	LLPFK III E AASDFRWELS D LACALMGFGFYSS IYCSTWLLAGISIERYLGVAFPVQYKLS			119
Horse	LLPFK III E AASDFHWYLP P ^V V CATVGFGFYSS IYCCTWLLAAISIERYLGVAFPVQYKLS			116
	*****	*****	*****	*****
	TM4 4.56 A.51	ECL2	5.39	
Human	RRPLYGV IAALVAWVMSFGHC TIVII QYLNTTEQVRSGNEITCYENFTDNQLD VVLPVR			180
Mouse	RRPLYGV IAALVAWIMSFGHC TIVII QYLNSTQRAPKENETTCYENFTQEQQLR LLLPIR			180
Bovine	RRPVYG VIAALIAWVMSFGHG TIVII QYLNSTQRAPKENETTCYENFTQEQQLR LLLPIR			179
Goat	RRPVYG VIAALIAWVMSFGHG TIVII QYLNSTQRPVKENETSCYENFSQEQLR LLLPIR			179
Horse	RRPVYG VWAALVAWITSFGHC TIVII QYFNSTQKATN-IEVTCYRNFTQEQGLEVVLPVR			175
	*****	*****	*****	*****
	TM5	ICL3	TM6	
Human	LELC LVLFFIPMAVTIFCYWRFVWIMLSQLPLVGAQR RRRAV G LAVV TLLNPLVCFGPY N			240
Mouse	LELC LVLFFVPMAVTIFCYWRFVWIMLTQPHVGAQR RRRAV G LAVV TLLNPLVCFGPY N			240
Bovine	LELC LLLFFFPMVVTTFCYS R FWIWIMLTQPHMGAQ K Q R RAM L AIV S LLN F LL C FG P Y N I			239
Goat	LELC LLLFFFPMVVTTFCYS R FWIWIMLTQPHMGAQ K Q R RAM G LAIV S LLN F LL C FG P Y N I			239
Horse	LELC LVLFFIPMVITIFCYWRFVWIMLTQPHVGAQR RRRAV G LAI V T L LN F IV C FG P Y N I			235
	*****	*****	*****	*****
	6.56 ECL3 1.35 3.6 TM7			
Human	SHLVGYH QRKSPW I AVV FSSLN ASLDPLLFYFSSSV V RRAFGRGLQVLRNQGSSLLG			300
Mouse	SHLVGFY LRQSPS W V AVV FSSLN ASLDPLLFYFSSSV V RRAFGKGLLIRNPASSMLG			300
Bovine	SHLVGFYM KASP PK V REAVV F GSLN A SLDPLLFYFSSSV V RRSF G KGLQALYHWGSSLLG			299
Goat	SHLVGFHM KASP PK V REAVV F GSLN A SLDPLLFYFSSSV V RRSF G KGLQALHHRGSSLLG			299
Horse	SHLVGFYT KESPKW V AVV I SLN A SLDPLLFYFSSSTARRAF E KRLQV L QGPSSLLG			295
	*****	*****	*****	*****
Human	RRGKD TAEGTNEDRGV GQQGE GMPS SDFTTE			330
Mouse	RGA KETVEGT KMDRG GQS AE GV QSSE FV TE			330
Bovine	RRCK ETAEEANEDPGV SQAE GA EPSSDFT TL D			329
Goat	RRCK ETAEEATNEDPGV SQAE GA EPSSDFT TL D			329
Horse	LRDRGS VEVTNRV GG L SQ V EG A PS S DDFT TD			325
	*****	*****	*****	*****

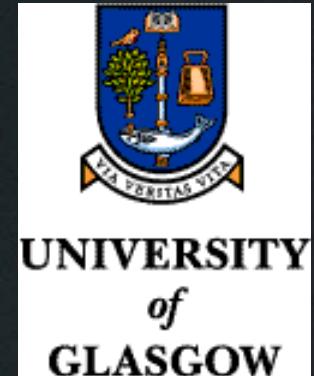


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Identification of TM residues that could be involved in fatty acid binding and receptor activation

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