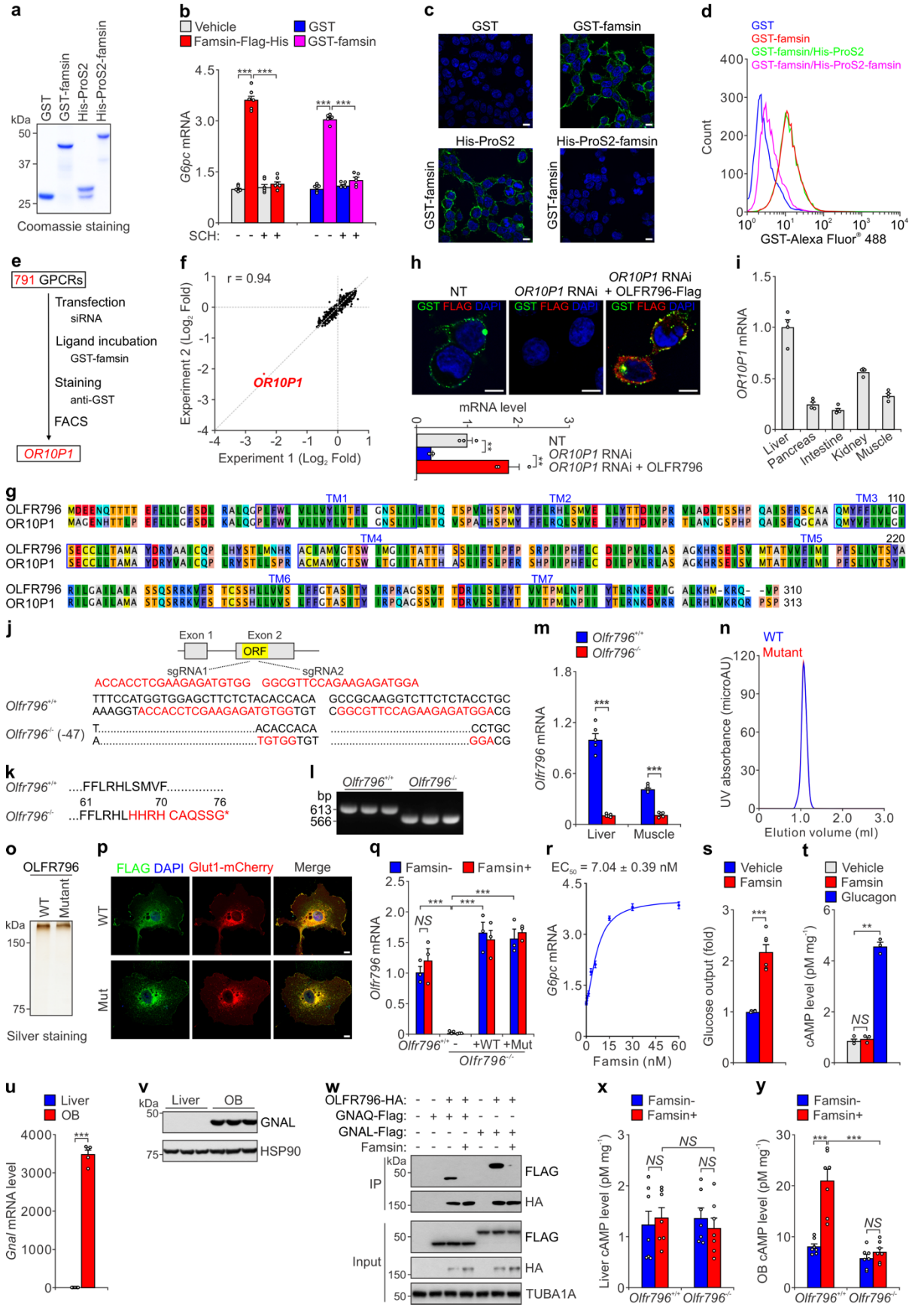
a
GST
GST-famsin
His-ProS2
His-ProS2-famsin
kDa
50
37
25
Coomassie staining
b
Vehicle
Famsin-Flag-His
GST
GST-famsin
G6pc mRNA
SCH:
c
GST
GST-famsin
His-ProS2
His-ProS2-famsin
d
GST
GST-famsin
GST-famsin/His-ProS2
GST-famsin/His-ProS2-famsin
Count
GST-Alexa Fluor® 488
e
791 GPCRs
Transfection
siRNA
Ligand incubation
GST-famsin
Staining
anti-GST
FACS
OR10P1
f
r = 0.94
Experiment 2 (Log2 Fold)
Experiment 1 (Log2 Fold)
OR10P1
h
NT
OR10P1 RNAi
OR10P1 RNAi + OLFR796-Flag
GST FLAG DAPI
mRNA level
NT
OR10P1 RNAi
OR10P1 RNAi + OLFR796
i
OR10P1 mRNA
Liver
Pancreas
Intestine
Kidney
Muscle
g
OLFR796
OR10P1
TM1
TM2
TM3
TM4
TM5
TM6
TM7
j
Exon 1
Exon 2
ORF
sgRNA1
sgRNA2
ACCACCTCGAAGAGATGTGG GGCGTTCCAGAAGAGATGGA
Olfr796+/+
Olfr796-/- (-47)
k
Olfr796+/+ ....FFLRHLSMVF.....
61 70 76
Olfr796-/- ...FFLRHLHHRH CAQSSG*
l
Olfr796+/+
Olfr796-/-
bp
613
566
m
Olfr796+/+
Olfr796-/-
Olfr796 mRNA
Liver
Muscle
n
WT
Mutant
UV absorbance (microAU)
Elution volume (ml)
o
OLFR796
WT
Mutant
kDa
150
75
Silver staining
p
FLAG DAPI Glut1-mCherry
Merge
WT
Mut
q
Famsin-
Famsin+
Olfr796 mRNA
NS
Olfr796+/+ - +WT +Mut
Olfr796-/-
r
EC50 = 7.04 ± 0.39 nM
G6pc mRNA
Famsin (nM)
s
Vehicle
Famsin
Glucose output (fold)
t
Vehicle
Famsin
Glucagon
cAMP level (pM mg-1)
NS
u
Liver
OB
Gnal mRNA level
v
kDa
Liver
OB
GNAL
HSP90
50
75
w
OLFR796-HA:
GNAQ-Flag:
GNAL-Flag:
Famsin:
IP
Input
FLAG
HA
TUBA1A
x
Famsin-
Famsin+
Liver cAMP level (pM mg-1)
NS
Olfr796+/+ Olfr796-/-
y
Famsin-
Famsin+
OB cAMP level (pM mg-1)
NS
Olfr796+/+ Olfr796-/-

**Supplementary information, Fig. S4. Identification of OLFR796 as a receptor of famsin.**

**a** Coomassie staining showing SDS-PAGE separation of GST, GST-famsin, His-ProS2 and His-ProS2-famsin purified from *E. coli*. **b** Effect of famsin-Flag-His purified from Hi-5 cells and GST-famsin purified from *E. coli* on relative *G6pc* mRNA levels in mouse primary hepatocytes in the presence or absence of SCH-202676 (SCH, 10 μM). Data are shown as mean ± s.e.m. Comparison of different groups was carried out using two-way ANOVA followed by Tukey's test. ***p < 0.001. n = 6. **c**-**d** Binding assay on HEK293T cells evaluated by immunostaining (**c**) or flow cytometry (**d**). 100 nM GST or GST-famsin was incubated with HEK293T cells. For competition binding, cells were pretreated with 5 μM His-ProS2 or His-ProS2-famsin for 30 min. Scale bars, 10 μm. **e** siRNA library screening for GPCRs that modulate the binding of GST-famsin in HEK293T cells. **f** Correlation between the two replicates of the primary siRNA screen. Results are shown as fold change (on a $\log_2$ scale) of famsin binding in siRNA knockdown cells relative to cells transfected with non-targeting siRNA. Spearman's rank correlation coefficient (r = 0.94) between the two replicates is shown. **g** Alignment of human OR10P1 and mouse OLFR796. The predicted transmembrane domains (TM) are surrounded by the blue boxes. **h** Effect of *OR10P1* knockdown or addition of OLFR796-Flag on GST-famsin binding (top panel) and mRNA level of *OR10P1* or *Olfr796* (bottom panel) in HEK293T cells. A mixture of siRNAs (5'-CGATCATCCCGCACTTTCT-3', 5'-CCTCTTACATCCGCATCCT-3' and 5'-TGATGACAGCCACCATAGT-3') that target *OR10P1* was used in this assay. NT, non-target RNAi. Scale bars, 10 μm. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using one-way ANOVA followed by Tukey's test. **p < 0.01. n = 3. **i** qPCR results showing relative mRNA levels of *OR10P1* in human tissues. Data are shown as mean ± s.e.m. n = 4. **j** Generation of *Olfr796*$^{-/-}$ mice by CRISPR-Cas9. A deletion of 47 bp was introduced in exon 2 of the *Olfr796* gene. **k** The partial N-terminal sequence of WT OLFR796 and the full sequence of the truncated OLFR796 protein product in *Olfr796*$^{-/-}$ mice are shown. The frame-shifted amino acids of OLFR796 generated by the deletion in *Olfr796*$^{-/-}$ mice are shown in red. **l** PCR analysis showing *Olfr796* fragments generated from *Olfr796*$^{+/+}$ and *Olfr796*$^{-/-}$ mice. **m** Relative mRNA levels of *Olfr796* in the liver or skeletal muscle extracts from 8-10-week-old male *Olfr796*$^{+/+}$ and *Olfr796*$^{-/-}$ mice. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using unpaired two-tailed Student's *t*-test. ***p < 0.001. n = 5 mice. **n** A representative chromatogram from size exclusion chromatography of OLFR796-Flag-His or its mutant (R187D, R195D and E197A) purified from Hi-5 cells. microAU, micro-ultraviolet absorbance at 280 nm. **o** Silver staining showing purified OLFR796-Flag-His and its mutant (R187D, R195D and E197A) separated by SDS-PAGE. **p** Similar cellular localization of WT OLFR796 and its mutant (R187D, R195D and E197A) evaluated by anti-FLAG staining in Cos7 cells. Scale bars, 10 μm. **q** qPCR results showing relative expression of *Olfr796* in *Olfr796*$^{+/+}$ or *Olfr796*$^{-/-}$ primary hepatocytes with or without overexpression of Olfr796 in the presence or absence of famsin (30 nM). Data are shown as mean ± s.e.m. Comparison of different groups was carried out using two-way ANOVA followed by Tukey's test. ***p < 0.001. *NS*, no statistical significance. n = 3. **r** Relative *G6pc* mRNA levels following treatment with different doses of famsin in mouse primary hepatocytes. Data are shown as mean ± s.e.m. n = 6. **s** Effect of famsin (30 nM) on glucose output in mouse primary hepatocytes. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using unpaired two-tailed Student's *t*-test. ***p < 0.001. n = 6. **t** Effect of 10 min treatment with famsin (30 nM) or glucagon (100 nM) on cellular cAMP levels in mouse primary hepatocytes. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using one-way ANOVA followed by Tukey's test. **p < 0.01. *NS*, no statistical significance. n = 3. **u** qPCR results showing relative expression of *Gnal* in the liver or OB extracts from 8-10-week-old male mice. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using unpaired two-tailed Student's *t*-test. ***p < 0.001. n = 5 mice. **v** Immunoblot showing relative expression of GNAL in the liver or OB extracts from 8-10-week-old male mice. **w** Co-immunoprecipitation showing the association of OLFR796-HA with GNAQ-Flag or GNAL-Flag in HEK293T cells treated with or without famsin (30 nM) for 10 min. **x**-**y** Effect of famsin on cAMP levels of liver (**x**) or OB (**y**) extracts from 8-10-week-old *Olfr796*$^{+/+}$ and *Olfr796*$^{-/-}$ male mice. Data are shown as mean ± s.e.m. Comparison of different groups was carried out using two-way ANOVA followed by Tukey's test. ***p < 0.001. *NS*, no statistical significance. n = 5 mice.