



**HAL**  
open science

## The hormone Fibroblast Growth Factor 19 stimulates water intake

José Ursic-Bedoya, Carine Chavey, Lucy Meunier, Guillaume Desandré, Anne-Marie Dupuy, Iria Gonzalez-Dopeso Reyes, Thierry Tordjmann, Eric Assénat, Urszula Hibner, Damien Gregoire

► **To cite this version:**

José Ursic-Bedoya, Carine Chavey, Lucy Meunier, Guillaume Desandré, Anne-Marie Dupuy, et al..  
The hormone Fibroblast Growth Factor 19 stimulates water intake. 2021. hal-03441478

**HAL Id: hal-03441478**

**<https://hal.science/hal-03441478>**

Preprint submitted on 22 Nov 2021

**HAL** is a multi-disciplinary open access archive for the deposit and dissemination of scientific research documents, whether they are published or not. The documents may come from teaching and research institutions in France or abroad, or from public or private research centers.

L'archive ouverte pluridisciplinaire **HAL**, est destinée au dépôt et à la diffusion de documents scientifiques de niveau recherche, publiés ou non, émanant des établissements d'enseignement et de recherche français ou étrangers, des laboratoires publics ou privés.

# The hormone Fibroblast Growth Factor 19 stimulates water intake

José Ursic-Bedoya<sup>\*1,2</sup>, Carine Chavey<sup>\*1</sup>, Lucy Meunier<sup>1,2</sup>, Guillaume Desandré<sup>1</sup>, Anne-Marie Dupuy<sup>3</sup>, Iria Gonzalez-Dopeso Reyes<sup>1</sup>, Thierry Tordjmann<sup>4</sup>, Eric Assénat<sup>1,2</sup>, Urszula Hibner<sup>1</sup>, Damien Gregoire<sup>1,✉</sup>

1. Institut de Génétique Moléculaire de Montpellier, University of Montpellier, CNRS, Montpellier, France

2. Department of hepatogastroenterology, Hepatology and Liver Transplantation Unit, Saint Eloi Hospital, University of Montpellier, France

3. Biochemistry and Hormonology Department, Lapeyronie Hospital, University of Montpellier, Montpellier, France

4. Université Paris Saclay, Faculté des Sciences d'Orsay, INSERM U.1193, bât. 443, 91405, Orsay, France

✉ Corresponding author

\* Co-first authorship

## Contact information of the corresponding author

Damien Gregoire, Ph.D.

Institut de Génétique Moléculaire de Montpellier

1919, route de Mende

34293 Montpellier Cedex, France

Email : [damien.gregoire@igmm.cnrs.fr](mailto:damien.gregoire@igmm.cnrs.fr)

Phone : +33 (0)4 34 35 96 59

## 1 Abstract

2 Fibroblast growth factor 19 (FGF19) is a hormone with pleiotropic metabolic functions,  
3 leading to ongoing development of analogues for the treatment of metabolic disorders.  
4 On the other hand, FGF19 is overexpressed in a sub-group of hepatocellular  
5 carcinoma (HCC) patients and has oncogenic properties. It is therefore crucial to  
6 precisely define FGF19 effects, notably chronic exposure to elevated concentrations  
7 of the hormone. We used hydrodynamic gene transfer approach to generate a  
8 transgenic mouse model with long-term FGF19 hepatic overexpression. Here we  
9 describe a novel effect of FGF19, namely stimulation of water intake. This phenotype,  
10 lasting at least over a 6-month period, depends on signaling in the central nervous  
11 system and is independent of FGF21, although it mimics some of its features. We  
12 further show that HCC patients with high levels of circulating FGF19 have a reduced  
13 natremia, indicating diuretic features. The present study provides evidence of a  
14 new activity for FGF19, which could be clinically relevant in the context of FGF19  
15 overexpressing cancers and treatment of metabolic disorders by FGF19 analogues.

16

17

## 1 Introduction

2 Fibroblast growth factor 19, FGF19, (and its mouse ortholog, FGF15), functions  
3 as a hormone, notably controlling bile acids synthesis and nutrient metabolism (Angelin  
4 *et al*, 2012). FGF15/19 has been reported to exert multiple effects in the regulation of  
5 glucose and lipid metabolism, as well as in energy expenditure and body adiposity,  
6 through its actions on liver, muscle, white adipose tissue and brain (Gadaleta &  
7 Moschetta, 2019).

8 Under physiological conditions, FGF19 is expressed during the postprandial  
9 phase by enterocytes of the terminal ileum(Inagaki *et al*, 2005). The expression by  
10 hepatocytes has also been described in cholestasis (Schaap *et al*, 2009; Wunsch *et*  
11 *al*, 2015; Hasegawa *et al*, 2019). FGF19 belongs to the endocrine FGF subfamily,  
12 along with FGF21 and FGF23. Endocrine-FGF lack the heparin binding domain found  
13 in canonical FGFs and act through the binding to a FGF receptor (FGFR 1 to 4) in  
14 complex with a co-receptor,  $\beta$ -klotho (KLB) for FGF19 and FGF21 (Suzuki *et al*, 2008;  
15 Wu *et al*, 2010). While many of FGF19 activities are thought to be mediated by  
16 FGFR4/KLB signaling, FGF19 and FGF21 also display overlapping metabolic  
17 regulations through activation of FGFR1c/KLB signaling (Wu *et al*, 2011), notably in  
18 the hypothalamus (Lan *et al*, 2017; Perry *et al*, 2015). Because of their metabolic  
19 effects, FGF19 and FGF21 pathways are considered as promising therapeutic targets  
20 for several diseases (Degirolamo *et al*, 2016). In particular, FGF19 has generated a  
21 great interest in pharmacological research for treatment of chronic liver diseases such  
22 as nonalcoholic steatohepatitis (Harrison *et al*, 2018, 2020), primary biliary cholangitis  
23 (Mayo *et al*, 2018) and primary sclerosing cholangitis (Hirschfield *et al*, 2019).

24  
25 On top of its metabolic effects, FGF19/FGFR4 pathway shows oncogenic  
26 functions in several cancers, notably hepatocellular carcinoma, the most frequent  
27 primary liver cancer. The 11q13.3 genomic region containing FGF19 is frequently (4-  
28 15%) amplified in HCC tumors (Sawey *et al*, 2011; Wang *et al*, 2013; Schulze *et al*,  
29 2015), and tumor cells overexpression of FGF19 independently of amplification has  
30 also been reported (Caruso *et al*, 2019; Hatlen *et al*, 2019). Moreover, HCC patients  
31 display higher circulating levels of FGF19 than control population (Maeda *et al*, 2019).  
32 These findings led to the development of potent and selective FGFR4 inhibitors, as  
33 well as monoclonal antibodies (Kim *et al*, 2019; Bartz *et al*, 2019), which are currently



1 tested in clinical trials for FGF19-driven hepatocarcinoma, underscoring the  
2 therapeutic promise of targeting the FGF19 pathway in HCC (Gadaleta & Moschetta,  
3 2021).

4 Because of these paradoxical effects on metabolism and oncogenesis, it is  
5 therefore crucial to better delineate the complex interplay of FGF19 roles and actions.  
6 In this study, we generated a mouse model of FGF19 overexpression by hepatocytes,  
7 and identified a new metabolic effect of FGF19, namely stimulation of water intake. We  
8 further report that HCC patients with increased FGF19 circulating concentrations show  
9 features of disturbance of the hydrosodic balance.

10

## 11 Results and Discussion

### 12 Mouse model of FGF19 expression

13 We used hydrodynamic gene transfer (HGT) technique (Liu *et al*, 1999; Zhang  
14 *et al*, 1999) to generate a mouse model in which FGF19 is overexpressed by a fraction  
15 of hepatocytes. The rapid injection of a large volume of solution (10% w/v) in the tail  
16 vein leads to *in vivo* transfection of hepatocytes. Combination of plasmids encoding  
17 the Sleeping Beauty transposase SB100X and FGF19 was used to establish stable  
18 expression of the human hormone (Figure 1A). We routinely detect by epifluorescence  
19 microscopy one to two percent of transfected hepatocytes, which persisted up to 6  
20 months after the injection (Figure 1B). The long-term expression of the transgene was  
21 confirmed by RT-qPCR analysis (Figure 1C). The level of FGF19 in plasma, detected  
22 by ELISA, was around 10 ng/ml (CI<sub>95%</sub>= [3.5-15.0]) two weeks post-transfection and  
23 remained high, despite its gradual decrease, to reach 1 ng/ml at 6 months post-  
24 injection (CI<sub>95%</sub>= [0.76 -1.11]) (Figure 1D). These concentrations are supra-  
25 physiological, as healthy human subjects display circulating concentrations of 50-  
26 590 pg/mL (Angelin *et al*, 2012); however, they are close to those observed in a subset  
27 of HCC patients (Maeda *et al*, 2019). Of note, these pathologically relevant  
28 concentrations are 100 to 1000-fold inferior to those reported in a model of AAV  
29 infection (1 µg/mL (Zhou *et al*, 2017)). FGF19-expressing mice showed the previously  
30 characterized transcriptional repression of *Cyp7a1* and *Cyp8b1*, key enzymes of bile  
31 acids synthesis from cholesterol (Figure 1E). Serum 7α-hydroxy-4-cholesten-3-one  
32 (C4) level, a biomarker of bile acid synthesis, was also significantly reduced on FGF19-  
33 expressing mice (Figure 1E, 10.6 ng/mL CI<sub>95%</sub>= [4.9; 16.2] for controls vs 1.3 ng/mL

1  $CI_{95\%} = [0.8; 1.8]$  for FGF19,  $p=0.0027$ ). As previously reported, FGF19 exerted a  
2 significant effect on the body weight ([Figure 1F](#)) (Lan *et al*, 2017), while histological  
3 analysis of livers revealed no visible effect of FGF19 overexpression ([Figure 1G](#)).  
4 Altogether, these results indicate that the secreted FGF19 is active, and that the  
5 circulating levels are compatible with its physiological functions.

## 7 **FGF19 overexpression increases water intake**

8 While studying metabolic effects in FGF19 transgenic mice, we serendipitously  
9 observed that their cages were very wet compared to those of control mice, suggesting  
10 increased urine production. This prompted us to measure the animals' water intake.  
11 We observed that FGF19 expressing mice drank 6.6 to 7.8 mL/day ( $CI_{95\%}$  of the mean)  
12 compared to 2.9 to 3.1 mL/day for control animals hydrodynamically injected with an  
13 empty vector ( $p$ -value  $<0.0001$ ) ([Figure 2A](#)). Strikingly, this phenotype was stable in  
14 time for at least 5 months. The increase of water intake was associated with a  
15 significant decrease of urine osmolality ([Figure 2B](#), 1744 mOsm/kg/H<sub>2</sub>O  $CI_{95\%} = [1512;$   
16  $1976]$  for controls vs 723 mOsm/kg/H<sub>2</sub>O  $CI_{95\%} = [568; 879]$  for FGF19,  $p<0.0001$ ). We  
17 then measured urine production in individual metabolic cages and found that FGF19<sup>+</sup>  
18 mice have an increased urine output compared to controls ([Figure 2C](#), 490  $\mu$ L/day  
19  $CI_{95\%} = [204; 775]$  for controls vs 2871  $\mu$ L/day  $CI_{95\%} = [1589; 4153]$  for FGF19,  
20  $p=0.0004$ ) and confirmed the phenotype of decreased urine osmolality ([Figure 2D](#)).  
21 Altogether, these results indicate that FGF19 overexpression mimics a phenotype of  
22 diabetes insipidus.

23 Next, we assessed the effect of FGF19 overexpression on water drinking in  
24 another experimental system, in which FGF19 is produced by hepatic tumor cells. To  
25 model FGF19-negative and FGF19-positive hepatic tumors, we realized intrahepatic  
26 allografts of oncogenic cell lines derived from Myc-sgTrp53 or Myc-sgTrp53-FGF19  
27 tumors, both driven c-Myc oncogene in conjunction with inactivation of the p53 tumor  
28 suppressor, associated for the latter with FGF19 expression. Whereas Myc-sgTrp53  
29 tumor development had no impact on water intake, mice injected with FGF19-  
30 expressing cells showed progressive rise of water intake from 3 to 7 mL/day as tumors  
31 grew ([Figure 2E](#)). Mean circulating FGF19 levels at 3 weeks in mice injected with Myc-  
32 sgTp53-FGF19 cell were 153 ng/mL ( $CI_{95\%} = [81; 225]$ ), close to those obtained with  
33 AAV-driven FGF19 liver overexpression (Zhou *et al*, 2017). Thus, FGF19-driven  
34 increase of water intake by FGF19 was confirmed in a second experimental model.

1 Importantly, this phenotype might be relevant for the subset of HCC patients  
2 overexpressing FGF19. It is unclear why the drinking phenotype has not been  
3 previously described in FGF19 overexpressing mice (Zhou *et al*, 2017; Nicholes *et al*,  
4 2002). We note however that it would have probably escaped our notice if we were not  
5 directly handling the cages.

6 To determine if the diabetes insipidus phenotype was sex dependent, we next  
7 overexpressed FGF19 by HGT in C57Bl/6J males. Again, the FGF19 expression  
8 increased water intake, leading to a mean water consumption of 6.5 mL/ day ( $CI_{95\%} =$   
9 [5.1; 7.9]) during the first month after HGT (Figure 2F). We next investigated whether  
10 the overexpression of FGF15, the FGF19 mouse ortholog, also gave rise to the water  
11 drinking phenotype. Hydrodynamic gene transfer of FGF15 encoding plasmid did not  
12 significantly increase water intake nor reduced urine osmolality (Figure 2G). FGF15  
13 shares only 53% of the amino acid sequence with FGF19 (Nishimura *et al*, 1999);  
14 notably, FGF15 has an unpaired cysteine residue (Cys-135) that forms an  
15 intermolecular disulfide bridge to structure a homodimer. It has been suggested that  
16 this particularity leads to a diminished receptor activation and metabolic effects  
17 compared to FGF19 (Zhou *et al*, 2017).

18

### 19 **FGF19 effect on the central nervous system**

20 To test if the role of FGF19 in water homeostasis is central or peripheral, we  
21 performed an intraperitoneal injection to subject mice to an acute increase in water  
22 load. Cumulative urine excretion showed that the FGF19<sup>+</sup> mice displayed a similar  
23 response to water loading compared to control mice. This is in contrast with the  
24 increased urine output observed in FGF19<sup>+</sup> mice when given free access to water  
25 (Figure 3A, 206  $\mu$ L  $CI_{95\%} = [123; 290]$  for controls vs 433  $\mu$ L  $CI_{95\%} = [330; 535]$  for  
26 FGF19,  $p=0.0013$ , and Figure 2C). These data suggest that FGF19 stimulates  
27 voluntary water intake, via the implication of the central nervous system.

28 The phenotype we report is reminiscent of what has been described for FGF21,  
29 where pharmacologic administration of the hormone also increased water  
30 consumption in mice (Camporez *et al*, 2013; Talukdar *et al*, 2016; Song *et al*, 2018;  
31 Turner *et al*, 2018). In a physiological context, FGF21 has been shown to stimulate  
32 water drinking in response to dehydration caused by metabolic stresses such as  
33 ketogenic diet or alcohol consumption (Song *et al*, 2018). Because FGF21 has also  
34 been involved in sugar appetite (Talukdar *et al*, 2016), we submitted control and

1 FGF19 overexpressing mice to a two-bottle preference study between water and 3%  
2 sucrose. Strikingly, we observed the same phenotype as with FGF21, i.e. FGF19  
3 overexpressing mice showed reduced appetite for 3% sucrose water (Figure 3B, at  
4 week 1: 90 %  $CI_{95\%} = [79; 102]$  for controls vs 59%  $CI_{95\%} = [33; 85]$  for FGF19,  $p=0.015$ ).  
5 A possible mechanism for FGF19 to phenocopy the FGF21 effect is that the former  
6 increases the latter's secretion. However, measurements of FGF21 plasmatic levels  
7 indicated that FGF19 expression had no effect on the FGF21 levels, ruling out an  
8 indirect effect (Figure 3C). Overall, we conclude that FGF19 effect on water  
9 homeostasis is central and independent of FGF21, however the exact mechanisms  
10 involved in this regulation remain to be determined. FGF21 impact on water  
11 homeostasis is mediated in part by activation of the neurons of the paraventricular  
12 nucleus in the hypothalamus (Song *et al*, 2018). FGF19 has been shown to exert  
13 effects on the central nervous system through FGFR1c/ $\beta$ -klotho signaling (Marcelin *et*  
14 *al*, 2014; Liu *et al*, 2018; Perry *et al*, 2015), therefore one possibility is that FGF19  
15 activates FGFR1c in the hypothalamus. Another, and non-mutually exclusive,  
16 mechanistic explanation of FGF19 action is via modulation of the bile acid pool  
17 composition, since bile acids function as signaling molecules. Indeed, FGF19 is a  
18 potent inhibitor of bile acids synthesis by the liver and mice overexpressing FGF19  
19 have modified bile acid pool composition, with increased FXR antagonists and  
20 decreased FXR agonists which in turn represses FXR activity (Gadaleta *et al*, 2018).  
21 It has been shown that FXR inactivation leads to a decrease in urine osmolality through  
22 the regulation of aquaporin water channel (aquaporin 2) in the renal collecting ducts  
23 (Zhang *et al*, 2014). Of note, one remarkable observation is the long-term phenotype  
24 after hepatic FGF19 expression: in contrast to effects of FGF21 on water intake after  
25 a single injection, we report a long-lasting diabetes insipidus phenotype.

26

### 27 **HCC patients with high circulating FGF19 have reduced natremia**

28 To explore if the effects of FGF19 on water homeostasis have a clinical relevance, we  
29 considered pathological conditions associated with supra-physiological concentrations  
30 of FGF19. A subset of patients with hepatocellular carcinoma (HCC) have increased  
31 FGF19 serum levels (Maeda *et al*, 2019), and around 10% of tumors are characterized  
32 by the amplification of FGF19 genomic locus (Sawey *et al*, 2011; Schulze *et al*, 2015).  
33 In healthy subjects, FGF19 plasma levels range from 50 to 590 pg/mL (Lundåsen *et*  
34 *al*, 2006). To explore a potential effect on water homeostasis in patients with increased

1 FGF19 circulating levels, we assayed FGF19 concentration in plasma in 173 HCC  
2 patients from the “Liver-pool Cohort”, held in the Montpellier University Hospital. Most  
3 patients were male (89%), had underlying cirrhosis (81%) and multifocal or advanced  
4 HCC stage (BCLC B, C or D stage, 73%). We defined three groups based on the result  
5 of the FGF19 ELISA assay (Figure 4A): “high FGF19” for the upper quartile ([FGF19]  
6 > 603 pg/mL, 43 patients), “intermediate FGF19” for the middle quartiles ([FGF19]  
7 between 218 pg/mL and 603 pg/mL, 87 patients) and “low FGF19” for the lower quartile  
8 ([FGF19] < 218 pg/mL, 43 patients). “High FGF19” group corresponded to supra-  
9 physiological concentrations based on the literature. Interestingly, natremia of the “high  
10 FGF19” group was significantly lower than the two other groups (Figure 4B, 140.1  
11 mmol/L CI<sub>95%</sub>= [139.4; 140.8] for “low FGF19” vs 139.2 mmol/L CI<sub>95%</sub>= [138.5; 139.9]  
12 for “intermediate FGF19” vs 137.1 mmol/L CI<sub>95%</sub>= [136.2; 138] for “high FGF19”,  
13 p<0.0001). This result is suggestive of an imbalance in the regulation of the sodium  
14 and water homeostasis, potentially via an increased water consumption in a context of  
15 effective hypovolemia in this cohort of predominantly cirrhotic patients. Of interest, this  
16 effect remains significant when subjects under diuretic treatment or clinical ascites are  
17 excluded from the analysis (data not shown). Moreover, the natremia difference did  
18 not seem related to an impaired renal function in “FGF19 high” group (Figure 4C).  
19 Thus, our data from HCC patients with increased circulating FGF19 concentrations is  
20 consistent with an effect on water sodium balance in patients. Further work is required  
21 to establish if a reduction in natremia might have a prognostic value for FGF19-driven  
22 hepatocellular carcinoma. Finally, since FGF19 analogs are currently tested in different  
23 clinical trials, we suggest that it would be of great interest to analyze the newly  
24 discovered FGF19 activity, namely natremia and water intake, in response to these  
25 compounds.

## 27 Material and Methods

### 29 Vectors: cloning of FGF19/15

30 Plasmids constructs pSBbi-RN-FGF19 and pSBbi-BB-FGF15 were generated by cloning the human FGF19,  
31 amplified from Huh7 cDNA, and the murine FGF15, amplified from C57Bl/6J mouse ileum cDNA, into psBbi-RN  
32 and psBbi-BB plasmids (gifts from Eric Kowarz, Addgene plasmid #60519 and #60521, respectively), previously  
33 digested by the restriction enzyme SfiI. Primer sets for amplification of the inserts (with SfiI recognition site):  
34 FGF19-SfiI-for-5'-ATCGGGCCTCTGAGGCCAGGGAGGTGCCATGCGGA-3'

1 FGF19-Sfil-rev-5'-CGATGGCCTGACAGGCCGCCCTGGCAGCAGTGAAGA-3'

2 FGF15-Sfil-for-5'-ATCGGGCCTCTGAGGCCCCGAGGTGTCATGGCGAG-3'

3 FGF15-Sfil-rev-5'-CGATGGCCTGACAGGCCCGGAATCCTGTCAATTTCTG-3'

4

## 5 **Animal experiments**

6 All reported animal procedures were carried out in accordance with the rules of the French Institutional Animal Care  
7 and Use Committee and European Community Council (2010/63/EU). Animal studies were approved by institutional  
8 ethical committee (Comité d'éthique en expérimentation animale Languedoc-Roussillon (#36)) and by the Ministère  
9 de l'Enseignement Supérieur, de la Recherche et de l'Innovation (Apafis#10278-2018082809241782v1).

10 Hydrodynamic Gene Delivery: Hydrodynamic injections were performed in 6 to 8 week-old C57Bl/6J female (unless  
11 stated otherwise) mice, as described previously (Zhang *et al*, 1997; Liu *et al*, 1999). Briefly, 0.1 mL/g body weight  
12 of a solution of sterile saline containing plasmids of interest were injected into lateral tail vein over 8-10 s. psBBI-  
13 EF1a-FGF19-dTomato (12.5 µg), psBBI-EF1a-dTomato (12.5 µg), psBBI-EF1a-FGF15-TagBFP (12.5 µg) or  
14 psBBI-EF1a-TagBFP (12.5 µg) were injected together with sleeping beauty transposase SB100X (2.5 µg, ratio of  
15 5:1). pCMV(CAT)T7-SB100 was a gift from Zsuzsanna Izsvak (Addgene plasmid #34879).

16 Allografts: C57Bl/6J female mice (Charles River) mice were anesthetized with intra-peritoneal injection of Xylazine-  
17 Ketamine mixture. After incision of abdominal wall and peritoneum, the left lateral lobe of the liver was pulled out of  
18 the mouse body. 5000 cells, resuspended in 5 µL of 25% Matrigel (BD) - PBS, were injected using a 10µL Hamilton  
19 syringe in the left lobe of the liver. After injection, liver was put back in normal position and the abdomen was  
20 sutured. Mice were monitored daily during 3 weeks, then euthanized before the tissues were collected and fixed  
21 following classical procedures.

22 Water consumption was measured by cages (n=2-5 mice) three times a week. For the double bottle assay, mice  
23 were acclimated to cages with two bottles containing water for one week. They were then given access to bottles  
24 with water containing 3% sucrose (Sigma-Aldrich #16104) or pure water. Water and water-3% sucrose intake was  
25 measured every two days. The position of bottles was changed every two days. Acute water loading was performed  
26 by an intraperitoneal injection of 2 ml of water. Mice were immediately placed in individual cages without access to  
27 food and water. Urine was collected hourly over 6 h.

28

## 29 **Blood samples for ELISA experiments**

30 Mice were fasted for 4-6 hours then killed by anesthetic overdose with isoflurane. Plasma was collected after  
31 centrifugation. Plasma FGF19 and FGF21 were measured by ELISA, according to the manufacturers' instructions:  
32 human FGF19 ELISA kit (Biovendor, RD191107200R), mouse FGF21 ELISA kit (Sigma-Aldrich, EZRMFGF21-  
33 26K).

34

## 35 **RNA isolation, qPCR**

36 RNA was extracted from liver tissue and purified using RNeasy mini kit (Qiagen) according to manufacturer's  
37 protocol. Reverse transcription of total RNA (1 µg) was done with QuantiTect Reverse Transcription kit (Qiagen),  
38 and cDNA quantified using LC Fast start DNA Master SYBR Green I Mix (Roche) with primers detailed below on  
39 LightCycler480 apparatus (Roche). Gene expression levels were normalized with hypoxanthine phospho-  
40 ribosyltransferase (HPRT). Primer pairs used for qPCR: Hprt 5'- GCAGTACAGCCCCAAAATGG-3' and 5'-  
41 GGTCCCTTTTACCAGCAAGCT-3', FGF19 5'-CCAGATGGCTACAATGTGTACC-3' and 5'-  
42 CAGCATGGGCAGGAAATGA-3', Cyp7a1 5'-CTGCAACCTTCTGGAGCTTA-3' and 5'-



1 ATCTAGTACTGGCAGGTTGTTT-3', Cyp8b1 5'- CCTGTTTCTGGGTCCTCTTATTC-3' and 5'-  
2 TCTCCTCCATCACGCTGTC-3'.  
3

#### 4 **Human studies**

5 A total of 173 patients from the prospective HCC “Liverpool” cohort conducted in the Montpellier University Hospital  
6 were included and analyzed retrospectively. All patients provided written consent for research at the time of their  
7 blood collection, in line with international regulations and ICH GCP (International Conference on Harmonization-  
8 Good Clinical Practice, Biobank Registration Number DC 2014-2328 AC 2014-2335). Montpellier University  
9 Hospital Institutional Review Board committee approved this study ((N° 2019\_IRB-MTP\_01-11). All the patients had  
10 FGF19 plasma concentration measured at the inclusion using human FGF19 ELISA kit (Biovendor,  
11 RD191107200R), according to the manufacturers’ instructions.  
12

#### 13 **Statistical Analyses**

14 Data sets were tested with 2-tailed unpaired Student *t* tests or Mann-Whitney U tests, correlations were analyzed  
15 with Pearson's  $\chi^2$  test using Prism Software version 8 (GraphPad). Significant *P* values are shown as: \**P* <0.05,  
16 \*\**P* <0.01, \*\*\**P* <0.001, and \*\*\*\**P* <0.0001.

17 Comparisons of the mRNA expression levels between groups were assessed using Mann-Whitney U test.  
18 Spearman's rank-order correlation was used to test the association between continuous variables. Univariate  
19 survival analysis was performed using Kaplan-Meier curve with log-rank test.  
20

#### 21 **Acknowledgments**

22 We acknowledge Montpellier Biocampus facilities: the imaging facility (MRI), and the  
23 “Réseau d’Histologie Expérimentale de Montpellier” - RHEM facility, supported by  
24 SIRIC Montpellier Cancer (Grant INCa\_Inserm\_DGOS\_12553), the European regional  
25 development foundation and the Occitanie region (FEDER-FSE 2014-2020  
26 Languedoc Roussillon), for the histological analyses. We are grateful to  
27 zootechnicians of IGMM animal housing facility, Cédric Orféo, Eve Lasserre, Zoé  
28 Lebrere, for their work. We thank Emmanuel Vignal for the osmometer and Anne-  
29 Dominique Lajoix for allowing access to metabolic cages. We thank members of our  
30 lab for helpful discussions and comments. This work was funded by EVA-Plan cancer  
31 INSERM THE, and supported by SIRIC Montpellier Cancer Grant  
32 INCa\_Inserm\_DGOS\_12553. The funders had no role in study design, data collection  
33 and analysis or publication process.

1

## 2 Author contributions

3 JUB: Conceptualization, Methodology, Investigation, Writing-original draft, Writing-  
4 review & editing; CC: Conceptualization, Investigation, Writing-review & editing; LM:  
5 Investigation; GD: Investigation; IG: Investigation; AD: Resources, Investigation; TT:  
6 Resources; EA: Supervision, Funding acquisition, Writing-review & editing; UH:  
7 Supervision, Funding acquisition, Writing-review & editing; DG: Conceptualization,  
8 Investigation, Visualization, Supervision, Funding acquisition, Writing-original draft,  
9 Writing-review & editing.

10

## 11 Conflict of interest

12 The authors disclose no conflict of interest.

13

## 14 References

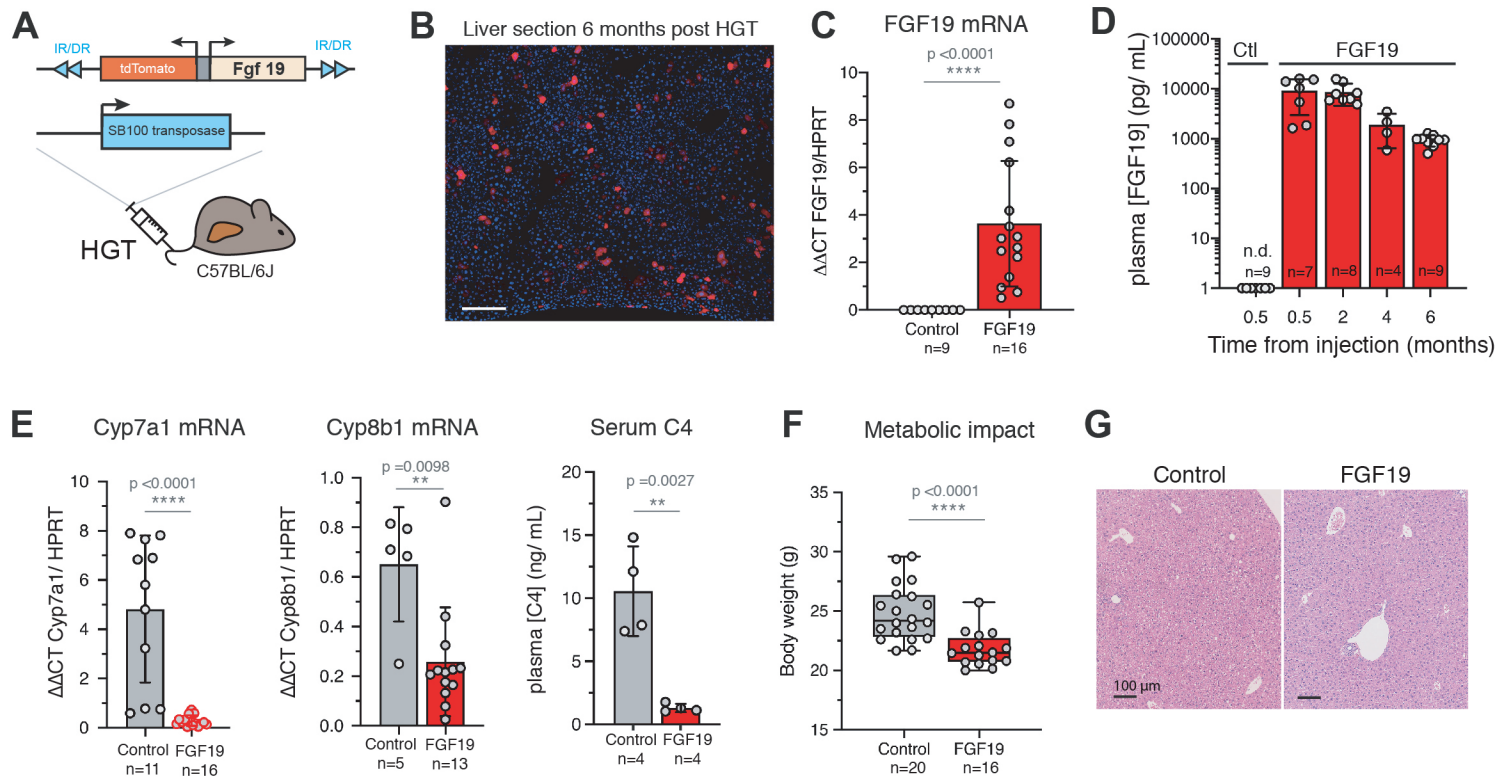
- 15 Angelin B, Larsson TE & Rudling M (2012) Circulating fibroblast growth factors as metabolic regulators - A critical  
16 appraisal. *Cell Metab* 16: 693–705
- 17 Bartz R, Fukuchi K, Ohtsuka T, Lange T, Gruner K, Watanabe I, Hayashi S, Oda Y, Kawaida R, Komori H, *et al*  
18 (2019) Preclinical Development of U3-1784, a Novel FGFR4 Antibody against Cancer, and Avoidance of Its  
19 On-target Toxicity. *Mol Cancer Ther* 18: 1832–1843
- 20 Camporez JPG, Jornayvaz FR, Petersen MC, Pesta D, Guigni BA, Serr J, Zhang D, Kahn M, Samuel VT, Jurczak  
21 MJ, *et al* (2013) Cellular mechanisms by which FGF21 improves insulin sensitivity in male mice.  
22 *Endocrinology* 154: 3099–3109
- 23 Caruso S, Calatayud AL, Pilet J, La Bella T, Rekik S, Imbeaud S, Letouzé E, Meunier L, Bayard Q, Rohr-Udilova  
24 N, *et al* (2019) Analysis of Liver Cancer Cell Lines Identifies Agents With Likely Efficacy Against  
25 Hepatocellular Carcinoma and Markers of Response. *Gastroenterology* 157: 760–776
- 26 Degirolamo C, Sabbà C & Moschetta A (2016) Therapeutic potential of the endocrine fibroblast growth factors  
27 FGF19, FGF21 and FGF23. *Nat Rev Drug Discov* 15: 51–69
- 28 Gadaleta RM & Moschetta A (2019) Metabolic Messengers: fibroblast growth factor 15/19. *Nat Metab* 1: 588–594
- 29 Gadaleta RM & Moschetta A (2021) Dark and Bright Side of Targeting the Fibroblast Growth Factor Receptor 4 in  
30 the Liver. *J Hepatol*
- 31 Gadaleta RM, Scialpi N, Peres C, Cariello M, Ko B, Luo J, Porru E, Roda A, Sabbà C & Moschetta A (2018)  
32 Suppression of Hepatic Bile Acid Synthesis by a non-tumorigenic FGF19 analogue Protects Mice from  
33 Fibrosis and Hepatocarcinogenesis. *Sci Rep* 8: 1–10
- 34 Harrison SA, Rinella ME, Abdelmalek MF, Trotter JF, Paredes AH, Arnold HL, Kugelmas M, Bashir MR, Jaros MJ,  
35 Ling L, *et al* (2018) NGM282 for treatment of non-alcoholic steatohepatitis: a multicentre, randomised, double-  
36 blind, placebo-controlled, phase 2 trial. *Lancet* 391: 1174–1185
- 37 Harrison SA, Rossi SJ, Paredes AH, Trotter JF, Bashir MR, Guy CD, Banerjee R, Jaros MJ, Owers S, Baxter BA,  
38 *et al* (2020) NGM282 Improves Liver Fibrosis and Histology in 12 Weeks in Patients With Nonalcoholic



- 1 Steatohepatitis. *Hepatology* 71: 1198–1212
- 2 Hasegawa Y, Kawai M, Bessho K, Yasuda K, Ueno T, Satomura Y, Konishi A, Kimura T, Ikeda K, Tachibana M, *et*
- 3 *al* (2019) CYP7A1 expression in hepatocytes is retained with upregulated fibroblast growth factor 19 in
- 4 pediatric biliary atresia. *Hepatol Res* 49: 314–323
- 5 Hatlen MA, Schmidt-Kittler O, Sherwin CA, Rozsahegyi E, Rubin N, Sheets MP, Kim JL, Miduturu C, Bifulco N,
- 6 Brooijmans N, *et al* (2019) Acquired on-target clinical resistance validates fgfr4 as a driver of hepatocellular
- 7 carcinoma. *Cancer Discov* 9: 1686–1695
- 8 Hirschfield GM, Chazouillères O, Drenth JP, Thorburn D, Harrison SA, Landis CS, Mayo MJ, Muir AJ, Trotter JF,
- 9 Leeming DJ, *et al* (2019) Effect of NGM282, an FGF19 analogue, in primary sclerosing cholangitis: A
- 10 multicenter, randomized, double-blind, placebo-controlled phase II trial. *J Hepatol* 70: 483–493
- 11 Inagaki T, Choi M, Moschetta A, Peng L, Cummins CL, McDonald JG, Luo G, Jones SA, Goodwin B, Richardson
- 12 JA, *et al* (2005) Fibroblast growth factor 15 functions as an enterohepatic signal to regulate bile acid
- 13 homeostasis. *Cell Metab* 2: 217–225
- 14 Kim RD, Sarker D, Meyer T, Yau T, Macarulla T, Park JW, Choo SP, Hollebecque A, Sung MW, Lim HY, *et al*
- 15 (2019) First-in-human phase I study of fisogatinib (BLU-554) validates aberrant FGF19 signaling as a driver
- 16 event in hepatocellular carcinoma. *Cancer Discov* 9: 1696–1707
- 17 Lan T, Morgan DA, Rahmouni K, Sonoda J, Fu X, Burgess SC, Holland WL, Kliewer SA & Mangelsdorf DJ (2017)
- 18 FGF19, FGF21, and an FGFR1/β-Klotho-Activating Antibody Act on the Nervous System to Regulate Body
- 19 Weight and Glycemia. *Cell Metab* 26: 709–718.e3
- 20 Liu F, Song Y & Liu D (1999) Hydrodynamics-based transfection in animals by systemic administration of plasmid
- 21 DNA. *Gene Ther* 6: 1258–66
- 22 Liu S, Marcelin G, Blouet C, Jeong JH, Jo YH, Schwartz GJ & Chua S (2018) A gut–brain axis regulating glucose
- 23 metabolism mediated by bile acids and competitive fibroblast growth factor actions at the hypothalamus. *Mol*
- 24 *Metab* 8: 37–50
- 25 Lundåsen T, Gälman C, Angelin B & Rudling M (2006) Circulating intestinal fibroblast growth factor 19 has a
- 26 pronounced diurnal variation and modulates hepatic bile acid synthesis in man. *J Intern Med* 260: 530–536
- 27 Maeda T, Kanzaki H, Chiba T, Ao J, Kanayama K, Maruta S, Kusakabe Y, Saito T, Kobayashi K, Kiyono S, *et al*
- 28 (2019) Serum fibroblast growth factor 19 serves as a potential novel biomarker for hepatocellular carcinoma.
- 29 *BMC Cancer* 19: 1088
- 30 Marcelin G, Jo YH, Li X, Schwartz GJ, Zhang Y, Dun NJ, Lyu RM, Blouet C, Chang JK & Chua S (2014) Central
- 31 action of FGF19 reduces hypothalamic AGRP/NPY neuron activity and improves glucose metabolism. *Mol*
- 32 *Metab* 3: 19–28
- 33 Mayo MJ, Wigg AJ, Leggett BA, Arnold H, Thompson AJ, Weltman M, Carey EJ, Muir AJ, Ling L, Rossi SJ, *et al*
- 34 (2018) NGM 282 for Treatment of Patients With Primary Biliary Cholangitis: A Multicenter, Randomized,
- 35 Double-Blind, Placebo-Controlled Trial. *Hepatol Commun* 2: 1037–1050
- 36 Nicholes K, Guillet S, Tomlinson E, Hillan K, Wright B, Frantz GD, Pham TA, Dillard-Telm L, Tsai SP, Stephan JP,
- 37 *et al* (2002) A mouse model of hepatocellular carcinoma: Ectopic expression of fibroblast growth factor 19 in
- 38 skeletal muscle of transgenic mice. *Am J Pathol* 160: 2295–2307
- 39 Nishimura T, Utsunomiya Y, Hoshikawa M, Ohuchi H & Itoh N (1999) Structure and expression of a novel human
- 40 FGF, FGF-19, expressed in the fetal brain. *Biochim Biophys Acta - Gene Struct Expr* 1444: 148–151
- 41 Perry RJ, Lee S, Ma L, Zhang D, Schlessinger J & Shulman GI (2015) FGF1 and FGF19 reverse diabetes by
- 42 suppression of the hypothalamic-pituitary-adrenal axis. *Nat Commun* 6: 1–10
- 43 Sawey ET, Chanrion M, Cai C, Wu G, Zhang J, Zender L, Zhao A, Busuttill RW, Yee H, Stein L, *et al* (2011)
- 44 Identification of a Therapeutic Strategy Targeting Amplified FGF19 in Liver Cancer by Oncogenomic
- 45 Screening. *Cancer Cell* 19: 347–358

- 1 Schaap FG, van der Gaag NA, Gouma DJ & Jansen PLM (2009) High expression of the bile salt-homeostatic  
2 hormone fibroblast growth factor 19 in the liver of patients with extrahepatic cholestasis. *Hepatology* 49:  
3 1228–1235
- 4 Schulze K, Imbeaud S, Letouzé E, Alexandrov LB, Calderaro J, Rebouissou S, Couchy G, Meiller C, Shinde J,  
5 Soysouvanh F, *et al* (2015) Exome sequencing of hepatocellular carcinomas identifies new mutational  
6 signatures and potential therapeutic targets. *Nat Genet* 47: 505–511
- 7 Song P, Zechner C, Hernandez G, Cánovas J, Xie Y, Sondhi V, Wagner M, Stadlbauer V, Horvath A, Leber B, *et*  
8 *al* (2018) The Hormone FGF21 Stimulates Water Drinking in Response to Ketogenic Diet and Alcohol. *Cell*  
9 *Metab* 27: 1338-1347.e4
- 10 Suzuki M, Uehara Y, Motomura-Matsuzaka K, Oki J, Koyama Y, Kimura M, Asada M, Komi-Kuramochi A, Oka S &  
11 Imamura T (2008)  $\beta$ klotho is required for fibroblast growth factor (FGF) 21 signaling through FGF receptor  
12 (FGFR) 1c and FGFR3c. *Mol Endocrinol* 22: 1006–1014
- 13 Talukdar S, Owen BM, Song P, Hernandez G, Zhang Y, Zhou Y, Scott WT, Paratala B, Turner T, Smith A, *et al*  
14 (2016) FGF21 regulates sweet and alcohol preference. *Cell Metab* 23: 344–349
- 15 Turner T, Chen X, Zahner M, Opsahl A, DeMarco G, Boucher M, Goodwin B & Perreault M (2018) FGF21 increases  
16 water intake, urine output and blood pressure in rats. *PLoS One* 13
- 17 Wang K, Lim HY, Shi S, Lee J, Deng S, Xie T, Zhu Z, Wang Y, Pocalyko D, Yang WJ, *et al* (2013) Genomic  
18 landscape of copy number aberrations enables the identification of oncogenic drivers in hepatocellular  
19 carcinoma. *Hepatology* 58: 706–717
- 20 Wu AL, Coulter S, Liddle C, Wong A, Eastham-Anderson J, French DM, Peterson AS & Sonoda J (2011) FGF19  
21 regulates cell proliferation, glucose and bile acid metabolism via FGFR4-dependent and independent  
22 pathways. *PLoS One* 6
- 23 Wu X, Ge H, Lemon B, Vonderfecht S, Weiszmann J, Hecht R, Gupte J, Hager T, Wang Z, Lindberg R, *et al* (2010)  
24 FGF19-induced hepatocyte proliferation is mediated through FGFR4 activation. *J Biol Chem* 285: 5165–5170
- 25 Wunsch E, Milkiewicz M, Wasik U, Trottier J, Kempialska-Podchorodecka A, Elias E, Barbier O & Milkiewicz P (2015)  
26 Expression of hepatic Fibroblast Growth Factor 19 is enhanced in Primary Biliary Cirrhosis and correlates  
27 with severity of the disease. *Sci Rep* 5: 1–13
- 28 Zhang G, Budker V & Wolff JA (1999) High levels of foreign gene expression in hepatocytes after tail vein injections  
29 of naked plasmid DNA. *Hum Gene Ther* 10: 1735–7
- 30 Zhang G, Vargo D, Budker V, Armstrong N, Knechtle S & Wolff JA (1997) Expression of naked plasmid DNA injected  
31 into the afferent and efferent vessels of rodent and dog livers. *Hum Gene Ther* 8: 1763–72
- 32 Zhang X, Huang S, Gao M, Liu J, Jia X, Han Q, Zheng S, Miao Y, Li S, Weng H, *et al* (2014) Farnesoid X receptor  
33 (FXR) gene deficiency impairs urine concentration in mice. *Proc Natl Acad Sci* 111: 2277–2282
- 34 Zhou M, Luo J, Chen M, Yang H, Learned RM, DePaoli AM, Tian H & Ling L (2017) Mouse species-specific control  
35 of hepatocarcinogenesis and metabolism by FGF19/FGF15. *J Hepatol* 66: 1182–1192
- 36

## Figure 1



**Figure 1: *in vivo* stable transfection of hepatocytes with FGF19 leads to long term secretion of biologically active FGF19.**

**A.** Mouse model strategy based on hydrodynamic gene transfer (HGT).

**B.** Cryosection of liver 6 months after HGT, showing stably transfected hepatocytes that express TdTomato. Scale bar: 100µm.

**C.** qPCR quantification of FGF19 mRNA in control and FGF19 transfected livers 6 months post injection.

**D.** Plasma levels of FGF19 detected by ELISA. Plasmids combined with transposase are indicated, control-RFP («Ctl») and FGF19-RFP («FGF19») respectively. The number of mice analyzed for each time point is indicated.

**E.** qPCR quantification of mRNA expression of Cyp7a1 and Cyp8b1, target genes of FGF19/FGFR4 pathway and serum 7α-hydroxy-4-cholesten-3-one (C4) levels, serum biomarker of bile acid synthesis.

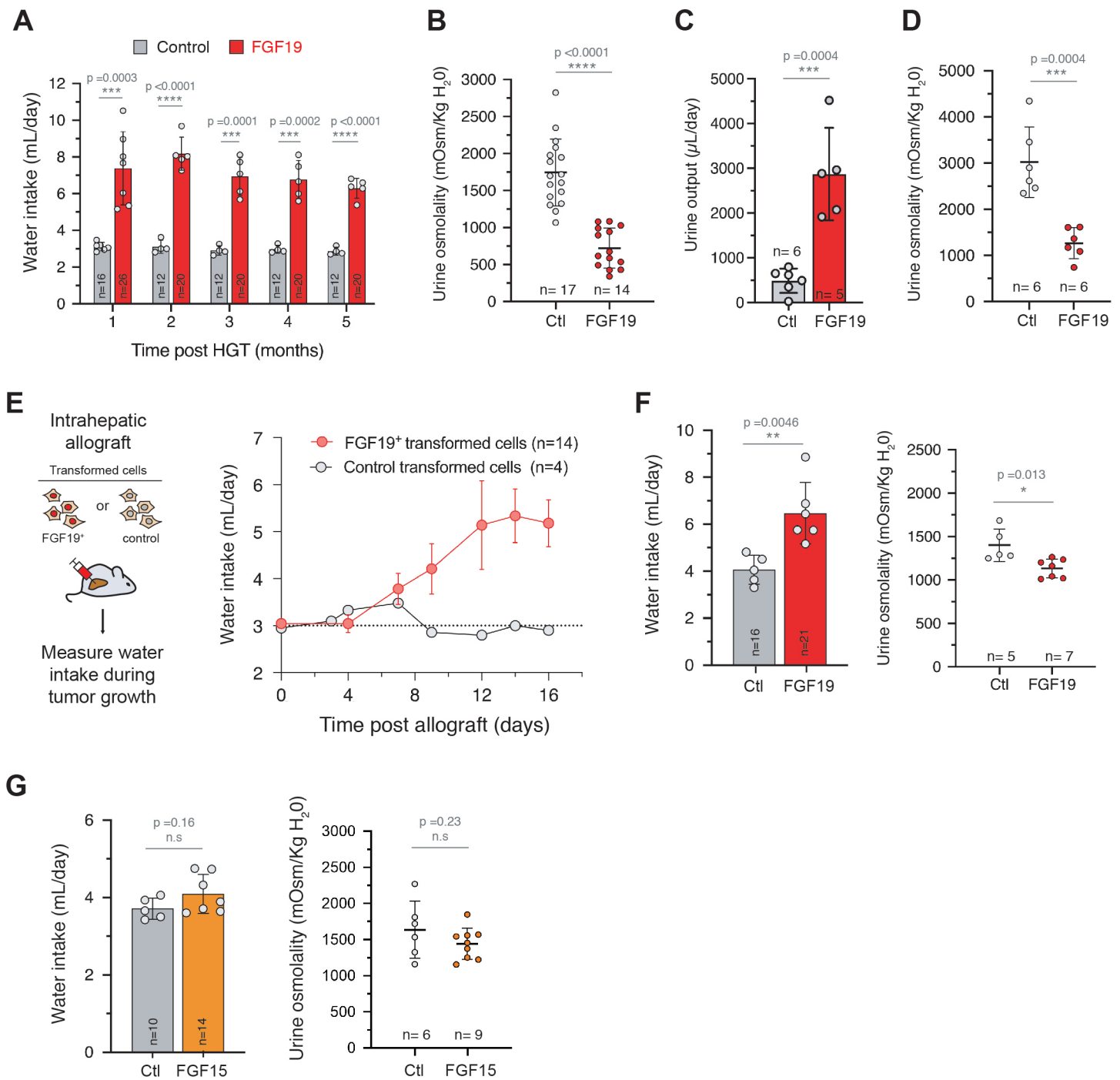
**F.** Body weight of control and FGF19 HGT mice at 6 months.

**G.** HES staining of liver sections of control and FGF19 HGT mice after 6 months, showing no visible alteration on liver morphology.

Data information: In (C-F): data are presented as mean ± SD. \* p≤0.05, \*\*p <0.01, \*\*\* p<0.001, \*\*\*\* p<0.0001.

C-E: Mann-Whitney test, F: unpaired Student's t-test. N.s: not significant. n.d: not detectable.

## Figure 2



### Figure 2: FGF19 increases water intake and urine output.

**A.** Water consumption of control or FGF19+ female mice. The number of mice analyzed for each time point is indicated and each dot represents the mean value of an independent cage over one month.

**B.** Urine osmolality of control and FGF19+ female mice at 3 months post HGT.

**C.** Daily urine output in female mice housed in metabolic cages.

**D.** Urine osmolality of control and FGF19+ female mice housed in metabolic cages.

**E.** Water consumption of female mice following intrahepatic allograft of FGF19+ (MYC-sgTp53-FGF19) and control (MYC-sgTp53) cell lines. Mean water consumption is shown, corresponding to 14 mice (3 cages) or 4 mice (1 cage). Dotted line represents the mean consumption of C57Bl/6J mice on the same period in the animal facility (n=15).

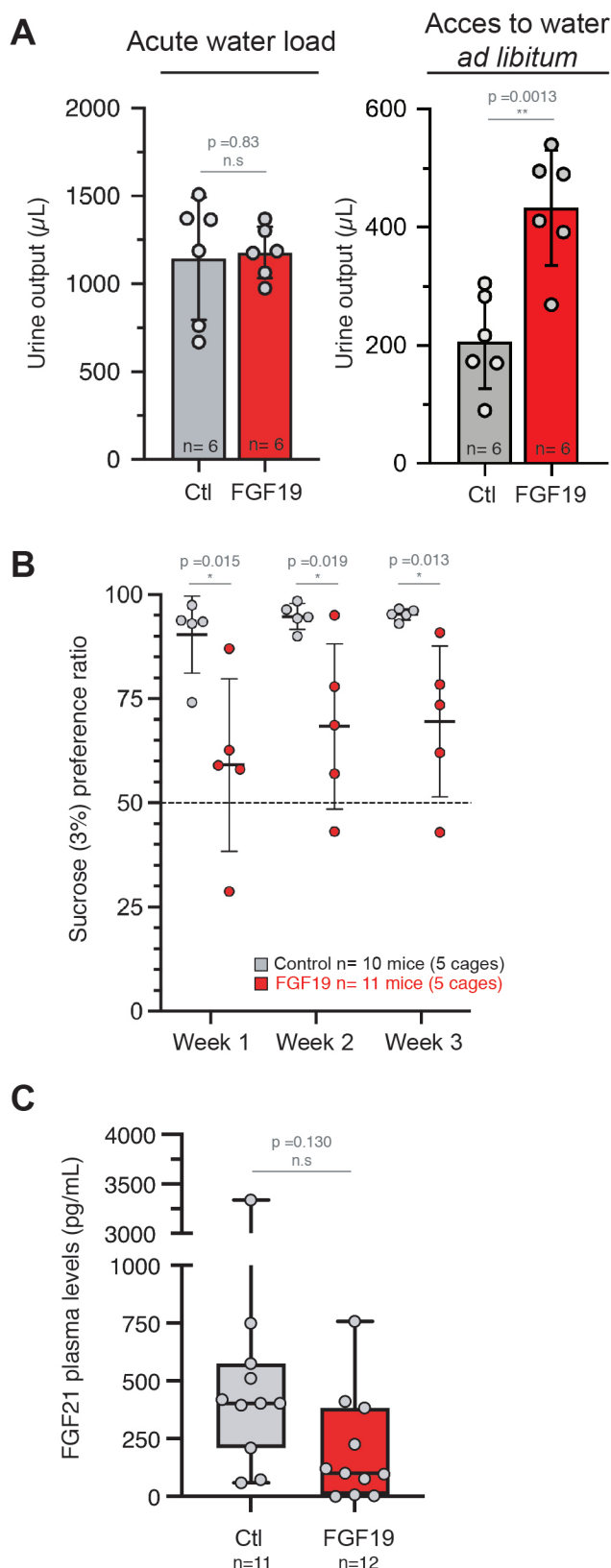
**F.** Effect of FGF19 overexpression on water intake and urine osmolality on male mice 1 month post HGT.

**G.** Water consumption and urine osmolality of female mice injected with FGF15-BFP (FGF15) or BFP plasmid (control, Ctl) 1 month post HGT.

Data information: In (A-G): data are presented as mean  $\pm$  SD. \*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ . n.s: not significant. For water intake measures, each dot represents the mean value of an independent cage with two to five mice. For all panels, unpaired t-test statistical significance is indicated.



## Figure 3



### Figure 3: FGF19 effects on water homeostasis suggest central regulation.

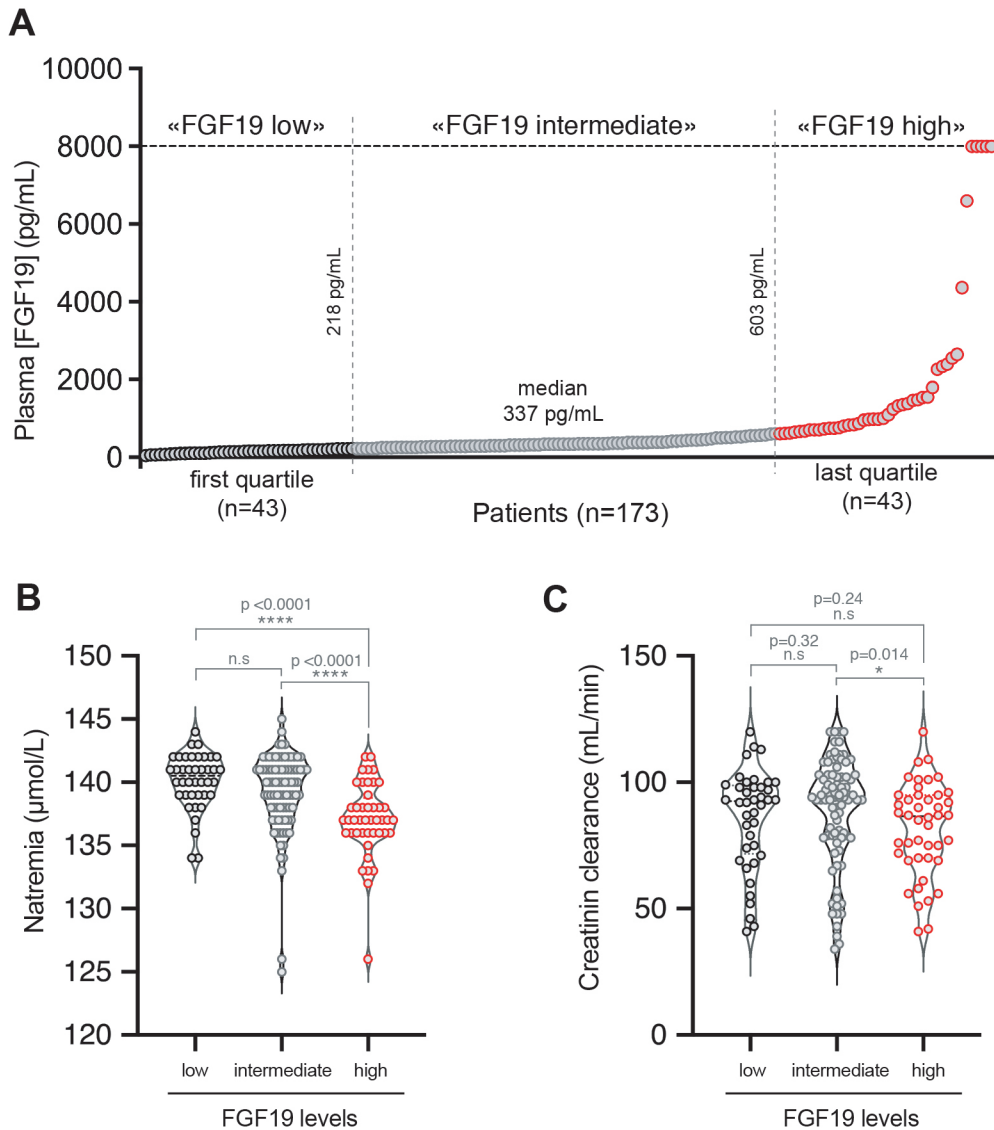
**A.** Urine output and urine osmolality 6h after acute water load or free acces to water. n=6 mice per group.

**B.** Sucrose (3%) preference ratio in a double-bottle experiment. Each dot represents a cage containing two to three animals. The total number of animals is indicated.

**C.** FGF21 plasma levels determined by ELISA in control and FGF19 HGT mice. n= 11-12 mice per group.

Data information: In (A-C): data are presented as mean  $\pm$  SD. \* $P \leq 0.05$  (unpaired Student's t-test). N.s: not significant.

## Figure 4



### Figure 4: Increased FGF19 in hepatocellular carcinoma patients is associated with disturbances of the hydrosodic balance .

**A.** Serum FGF19 distribution in a cohort of 173 patients with advanced HCC (FGF19 measured by ELISA).

**B-C.** Violin-plots of natremia (B) and creatinin clearance (C) in patients of the cohort according to the plasma FGF19 concentration.

Data information: \*  $p \leq 0.05$ , \*\*  $p < 0.01$ , \*\*\*  $p < 0.001$ , \*\*\*\*  $p < 0.0001$ . N.s: not significant. Mann Whitney test significance is indicated.