Supplementary Materials and Methods

Materials

DMEM Glutamax was from GIBCO (Paisley, Scotland). PKCζ pseudo-substrate (#P1614) and cycloheximide (#C7698) were from Sigma Chemical Corporation (St.Louis, MO, US), as well as all other chemicals used.

Epithelial barrier function measurements.

In vitro, trans-epithelial electric resistance (TER) was measured in the NRC (Normal Rat Cholangiocyte), MDCK II, and MDCK II TGR5-GFP cell lines, either with the Millicell ERS-2 Volt-ohmmeter (Millipore, France) with a chopstick electrode, or with the Cellzscope 2 (Nanoanalytics, Germany). TER was measured in confluent cell monolayers and expressed as ohm.cm^2 after background (empty insert) subtraction, or in percentage compared to WT conditions. 10 kDa FITC-dextran diffusion was measured by adding the dextran solution (2 mg/mL in serum-free culture medium) to the upper chamber, and sampling the lower compartment at 1 hour intervals for 5 hours. Data shown in this study are those obtained after 5 hours. Fluorescence intensity was measured using a Wallac fluorometer (Victor3; PerkinElmer, MA). Data were expressed after correction for background, using a standard curve. For TER and dextran transfer measurements, cells were seeded at 3-4 x 10^5/cm^2 on poly-ethylene transparent inserts (0.4 µm pore size, BD Falcon; BD Biosciences, France) and cultured until a confluent monolayer was obtained.

In vivo, under isoflurane anaesthesia, after laparotomy and ligation of the cystic duct, a 30G needle at the end of a perfusion line (see below) was inserted inside the GB lumen, through the neck wall, then a string was tightly attached around both the GB neck and the needle body. The perfusion line was composed of: an electric syringe pump (PHD 2000, Harvard Apparatus); a 1 ml syringe filled with either a 4 kDa fluorescein isothiocyanate (FITC)-dextran (10 mg/ml) or GCA C13 N15 (10 µM) solution; a 1 mm internal diameter catheter (Plastimed, France); a 3 ways valve; a pressure transducer (Harvard Apparatus, PHD 2000) (**Supplementary Figure 1**). A bolus of 30 µl (WT mice) or 10 µl (TGR5-KO mice) was injected at a 150 µl/min rate, and a tight knot was immediately tied while the

needle was pulled out of the GB. Injection volume has been determined after having performed a volume-pressure curve in WT and TGR5-KO mice, as shown in **Supplementary Figure 1**. As TGR5-KO mice harboured smaller GB than WT animals ([1] and personal data), these control experiments allowed us to perform isobaric injections. For FITC-dextran quantification, blood samples were performed 6 min after injection, and mice were sacrificed. Blood was centrifuged (3000 rpm, 4 °C, 20 min), the plasma was collected and analysed using a Perkin Elmer fluorometer Victor Microplate Reader, at an excitation wavelength of 492 nm and an emission wavelength of 521 nm (data were expressed as arbitrary units (AU)). For GCA C13 N15 quantification, livers were sampled 20 min after GB injection, and stored at -80°C until mass spectrometric assessment of GCA concentration (see below for BA mass spectrometry). Normalization of dextran passage on GB surface was performed by measuring GB length and width from WT and TGR5-KO treated mice, with a callipers square. GB surface was calculated assuming GB shape as an ellipsoid volume. The equation used was the following: $2\pi c^2+2\pi ac.\arcsin(e)/e$, where *a* is GB length, *c* is GB width and *e* is $\sqrt{(a^2-c^2)}/a$.

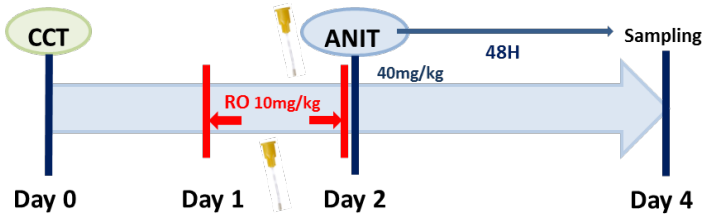
Surgery and other procedures in animals

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals" prepared by the National Academy of Sciences and published by the National Institutes of Health. C57BL/6J Gpbar1$^{-/-}$ (TGR5-KO) and C57BL/6J wild-type (WT) mice were kindly provided by Galya Vassileva and the Merck Research Laboratories (Kenilworth, USA). Pr Elisabetta Dejana (IFOM-IEO Campus, Milan, Italy) provided us with the C57BL/6J JAM-A$^{-/-}$ mice. A backcross with C57BL/6J from Jackson laboratory was performed every 2 years. The study was performed on 12-16 weeks old male mice.

Bile duct ligation with cholecystectomy (CCT): Laparotomy was performed under general anesthesia induced by isoflurane inhalation (4,5%) on fed mice. CCT was performed by ligating the cystic duct and then carefully dissecting the GB bed before cystic duct section.

After CCT, the bile duct was isolated, doubly ligated, and sectioned between the ligatures as previously described [2]. Sham operation consisted in laparotomy, GB and bile duct manipulation without ligation or resection. In series of experiments, mice were treated with vehicle (Veh: Gelatin/NaCl (7.5% / 0.62%)) or the specific TGR5 agonist RO5527239 [3] (10 mg/kg/day, oral gavage) for 2 days before BDL and CCT. Liver fragments were either frozen in nitrogen cooled isopentane or in RNAlater (Qiagen, France) and stored at -80°C until use, or fixed in 4% formaldehyde and embedded in paraffin.

ANIT intoxication. The protocol used was derived from the one previously described [4]. α-Naphtyl-isothiocyanate (ANIT) (Alfa Aesar, Kandel, Germany) was administered to mice by oral gavage (40 mg/kg body weight in olive oil). ANIT was administered after CCT and TGR5 agonist (RO at day 1 and day 2) or vehicle treatment, as described in the following experimental scheme.



Bile flow and biliary dextran excretion. Functional integrity of hepatocyte tight junctions (hepatocyte paracellular permeability) was measured as described [5] [6] [7], by measuring blood to bile passage of 40 kDa FITC-conjugated dextran. Under isoflurane, after laparotomy, bile duct ligation and GB cannulation, bile was collected in pre-tared 1,5 ml tubes during 20 min to determine bile flow rate, and then a 400 µl injection of 40 kDa FITC-dextran (25 mg/ml in saline), was performed in the inferior vena cava. Bile was then collected in 3 min fractions for 30 minutes. Bile was diluted 1/100 in water, and FITC fluorescence was measured using a Perkin Elmer fluorometer Victor Microplate Reader, at an excitation

wavelength of 492 nm and an emission wavelength of 521 nm. Data are reported as fluorescence arbitrary units (AU) per microliter of bile, per min, per g liver.

Patients samples

Liver biopsies came from the Biological Resource Center of Kremlin-Bicêtre Hospital (CRB PARIS SUD - UG 1203, HOPITAL BICETRE – APHP, France). All patients signed an informed consent form. Liver biopsies from 16 patients were analyzed: 4 hepatectomized patients (non-tumoral livers, 3 women and 1 man, mean age 74 ± 3 years); 7 end-stage primary biliary cholangitis (PBC, 6 women and 1 man, mean age 50 ± 3 years); 5 end-stage primary sclerosing cholangitis (PSC, 1 woman and 4 men, mean age 40 ± 6 years). Liver biopsies were analyzed by a pathologist and classified according to the METAVIR score.

Cell culture – cell treatments, transfections, cell lysis.

NRC (Normal Rat Cholangiocytes) were cultured as previously described [8, 9]. NRC were grown in DMEM/F12 supplemented with 5% FCS, non-essential amino acids, vitamins, glutamine, penicillin/streptomycin, pyruvate, lipid concentrate, soybean trypsin inhibitor, bovine pituitary extract, selenium, insulin and transferrin (all from Gibco), dexamethasone, and trio-iodo thyronine (from Sigma). Tissue culture flasks and plates were coated with rat tail collagen I (Sigma). Cells were seeded at 10^5 cells/cm^2 on PET inserts (from BD Biosciences, ref 353180) for TER and dextran transfer measurements (100% confluence approximately reached at day 5). For western blots, cells were seeded on 6 wells plates at 5.10^4 cells/cm^2, grown until 60-70% confluence (day 5), starved (FCS free medium) for 8 hours (overnight), and incubated with the different drugs and inhibitors for different time periods.

MDCK II cells were grown in DMEM Glutamax. MDCK II cells expressing TGR5-GFP were generated by transfecting with pcDNA3.1 expression vector (generous gift from Pr Kristina Schoonjans, EPFL, Switzerland). Stable transfections of plasmids using either Turbofect (Invitrogen, CA, USA) transfection reagents were performed according to the manufacturer's instructions. Transfected cells were grown in DMEM Glutamax supplemented with 100 µg/ml G418 (Euromedex).

MDCK II Tet-Off cells expressing Flag-tagged JAM-A / WT and JAM-A / S285A were used as described in *Iden et al* [10]. Transfected cells were grown in DMEM Glutamax supplemented with 100 µg/ml G418 (Euromedex), 1 µg/ml puromycine (Invitrogen, CA, USA), 150 µg/ml hygromycin B gold (Invivogen, CA, USA), and 100 ng/ml doxycycline (Sigma). Flag-JAM-A/WT and -JAM-A/S285A expression was induced by growing cells in medium lacking doxycycline.

F258 cells were cultured as described [11] (Rat Liver biliary Epithelial Cell line, provided by Dr Lagadic-Gossmann, Rennes, France): this cell line is derived from a primary culture of rat ductules (small bile ducts) [11] and were grown in Williams medium with 10% FCS, 1% glutamine (Gibco), and 1% penicillin/streptomycin/fungizon.

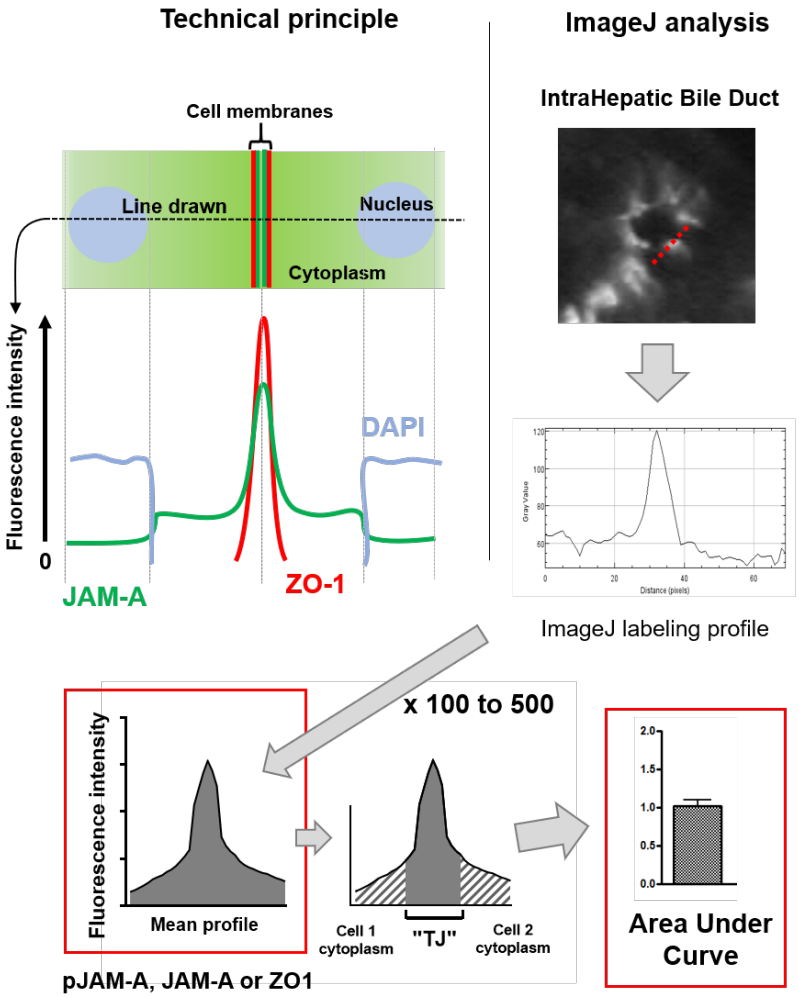
The following lysis buffer (Buffer "L") was used for protein extraction: 50 mM Hepes, pH7.4, 150 mM NaCl, 100 mMNaF, 10% Glycerol (v/v), 10 mM Na4P2O7, 200 µM Na3VO4, 10 mM EDTA, 1% Triton X-100 (v/v), 10 µg/ml Aprotinin, 10 µg/ml Leupeptin, 1 mM PMSF, and one protein inhibitor cocktail tablet (Roche, cOmplete Mini, EDTA free) for 10 ml.

Cycloheximide was used to block protein synthesis in MDCK II cells. Cultured cells were incubated with either cycloheximide alone or together with the TGR5 agonist RO5527239, for 24 hours, at 66 nmol/l. Cells were then processed for western blot analysis of JAM-A and GAPDH.

Immunohistochemistry and histochemistry

H&E staining was performed following standard procedures in the Department of Pathology at the Kremlin Bicêtre Hospital, on 4% formaldehyde-fixed 5µm liver sections. Antibodies against: JAM-A (Invitrogen #361700, 1/100), phospho (S285)-JAM-A (Santa Cruz # Sc-17430-R, 1/50), claudin 1- 5 (Zymed,1/100), Occludin (Zymed, 1/100), cingulin (1/5000), ZO-1 (kind gift from Dr. Stevenson (Salk Institute, La Jolla, CA), 1/100) and ZO-2 (Thermo-Fisher, #71-1400, 1/100), the cholangiocyte marker Cytokeratin-19 (CK-19, TROMA-III, Developmental Studies Hybridoma Bank, University of Iowa, 1/500), were used on ethanol acetone-fixed 10 µm liver cryosections, for 1 hour at 37°C. Liver sections and cells were then incubated with secondary antibodies (Alexa fluor 1/500, Molecular Probes) 30 min at 37°C. Images were acquired by epifluorescence (Axioskope; Zeiss) and confocal (EZ-C2; Nikon) microscopy and analyzed with ImageJ software.

For quantitative analysis of JAM-A, pJAM-A and ZO-1 levels in intrahepatic bile ducts, images were processed using ImageJ (NIH). The selection of a Region of Interest (ROI) consisting in drawing by hand a line going through the tight junction (on the basis of the protein ZO-1labeling). The analysis of each pixel of this line using ImageJ gives a labelling profile for each protein (see scheme). This process is repeated on every bile duct observed on the whole liver section, allowing the acquisition of 100 to 500 profiles for each TJ protein in each condition. These measurements allowed to obtain a "mean" representative profile and to calculate an area under curve (AUC) restricted to the tight junction region only, on the basis of ZO-1 immunostaining (see the scheme below). The same approach was used for quantitative analysis of JAM-A, pJAM-A and ZO-1 levels at hepatocyte TJ (see **Supplementary Figure 6A**).



Confocal microscopy

Double immuno-histofluorescence experiments were performed on livers and GB tissues to detect ZO-1 (λ_{ex}=561 nm, λ_{em}=590 nm) and JAM-A, pJAM-A or PKCζ (λ_{ex}=488 nm, λ_{em}=525 nm) on a Nikon EZ-C2 confocal microscope (TE-2000E, Nikon, France). Image acquisition and analysis were performed with the NIS-Elements AR (Advanced research) software (Nikon Instrument, France). Optical slices (0.3 µm) were performed. Either individual slices or projection of 2-3 slices were shown in corresponding figures.

Immunoblot

Whole cell and liver lysates were prepared using buffer "L" (see above). Proteins were quantified using the DCProtein Assay (Biorad), resolved by 7.5 or 10% SDS–PAGE gel electrophoresis and transferred to nitrocellulose membranes. Membranes were blocked in 5% skim milk, then incubated over night at 4◦C with specific primary antibodies: pan-pPKC (βII Ser660, Cell Signaling Technology, #9371, 1/1000), pPKC ζ-λ (Thr403/410, Cell Signaling Technology #9378, 1/1000), GAPDH (Thermofisher #MA5-15738,1/2000), PKC ζ (Santa-Cruz, USA #sc-216), α-Tubulin (Sigma #9026), alpha-actin (Sigma-Aldrich, #A1978, 1/3000), JAM-A (Invitrogen #361700, 1/500), phospho (S285)-JAM-A (Santa Cruz # Sc-17430-R, 1/250), claudin-1, claudin-3 (Zymed, 1/100), claudin-4 (Novex, #36-4800, 1/100), occludin (Zymed, 1/100), ZO-1 (kind gift from Dr. Stevenson (Salk Institute, La Jolla, CA)), ZO-2 (Thermo-Fisher, #71-1400, 1/1000) and the cholangiocyte marker cytokeratin-19 (CK-19, TROMA-III, Developmental Studies Hybridoma Bank, University of Iowa, 1/500).

The bound antibodies were detected (Fusion, Vilbert-Lourmat, France) using corresponding horseradish peroxidase-conjugated secondary antibodies and chemiluminescence detection kit (Biorad). Quantification of the signals was performed by densitometry using ImageJ software.

RT-qPCR

Total RNA was extracted from frozen mouse liver or GB, using TRI Reagent (Sigma), according to the manufacturer's protocol.

Complementary DNA (cDNA) was prepared by reverse transcription of 0.2 or 1 μg of total RNA using superscript II enzyme and random primers (Invitrogen). cDNA was amplified by PCR in the presence of independent forward and reverse primers detailed in the

Supplementary Table 2. For quantitative PCR, cDNA were amplified using SYBR green PCR kit (Bio-Rad) and Chromo 4 Real-Time detector (Bio-Rad) and normalized to HPRT using Opticon Monitor 3 software (Bio-Rad). PCR conditions were as follows: 95°C 2 min, then 40 cycles at 95° for 15 sec and at 60°C for 1 min.

Biochemical assays

Plasma alanine aminotransferase (ALT), total bilirubin (T.Bili) and alkaline phosphatase (ALP) were measured using a Synchron LX20 Clinical System (Beckman Coulter, France) analyzer.

Bile acids measurements. All chemicals and solvents were of the highest purity available. CA, DCA, CDCA, UDCA, LCA, HCA , glyco and tauro derivatives were obtained from Sigma-Aldrich (Saint Quentin Fallavier, 38297, France) 3-sulfate derivatives were a generous gift of J Goto (Niigita University of Pharmacy and Applied Life Science, 5-13-2 Kamishinei-cho, Niigata 950-8574, Japan). 23- NOR-5β-cholanoic acid-3α,12α diol , all muricholic acids, glyco and tauro derivatives were purchased from Steraloids Inc (Newport, USA). Acetic acid, ammonium carbonate, ammonium acetate and methanol were of HPLC grade and purchased from Sigma-Aldrich (Saint Quentin Fallavier, 38297, France). Bile acids measurements were performed on mouse liver by high-performance liquid chromatography-tandem mass spectrometry as described in [12].

Supplementary Figures legends

Supplementary Fig. 1. Gallbladder injections procedure.

As explained in details in *Supplementary Materials and Methods*, GB was injected with an electric precision syringe pump at a 150 µl/min rate, with 4 kDa FITC-Dextran or modified BA (GCA C13 N15). Isobaric injections were performed, after a volume-pressure curve has been established in WT and TGR5-KO mice.

Supplementary Fig. 2. TGR5 agonist specificity.

WT and TGR5-KO mice were gavaged for 2 days with TGR5 specific agonist RO5527239 (RO) or with the vehicle (Veh). Then GB bile volume was measured by bile puncture. Only WT mice exhibited enhanced GB filling upon RO treatment, as compared with vehicle-treated mice. Data are means±sem from n=5 mice in each group. Students' t test.

Supplementary Fig. 3.

A. TGR5 expression in the different cell lines used. NRC, F258 and MDCK II cells were sampled for RNA extraction and processed as explained in Materials and Methods for RT-qPCR. TGR5 mRNA expression was quantified against GAPDH gene expression as control, and expressed over WT liver. WT hepatocytes are shown for reference. Data are means±sem from n=2-7 samples from each condition. Mann Whitney U test. **B. TER and FITC-dextran diffusion measurements in F258 cells.** F258 cells were grown until confluence. TER and FITC-dextran transfer were measured upon vehicle or RO5527239 (RO) treatment, as explained in Materials and methods. Data are means±sem from n=4 experiments in each group, Students' t test. **C. Compared TGR5 mRNA expression in mouse liver and liver**

cells. TGR5 mRNA was quantified (qPCR) in mouse GB (WT), IBDU (WT), liver (WT and TGR5-KO) and isolated WT hepatocytes (Hep). GB: gallbladder; IBDU: isolated bile duct units. Data are means±sem from at least 5 samples per group.

Supplementary Fig. 4. Tight junction protein expression in WT and TGR5-KO liver and gallbladder.

A. mRNA expression for the main TJ proteins, in WT and TGR5-KO GB. JAM-A, JAM-C, claudins 1-5, claudin 7, occludin, ZO-1. **B-F**. Immunostaining for TJ proteins (claudins 1,2,3,5, occludin, cingulin, ZO-1, ZO-2), CK-19 and E-Cadherin, in WT and TGR5-KO livers and GB.

Supplementary Fig. 5. JAM-A and pJAM-A expression in WT and TGR5-KO gallbladder. Confocal microscopy analysis.

GB from WT and TGR5-KO mice were sampled, frozen in OCT and isopentane, and stored at -80°C until processed for immunohistochemistry and confocal microscopy, as explained in *Materials and methods*. **A.** JAM-A was localized at the TJ only, as revealed by ZO-1 co-localization. Weaker JAM-A staining was reproducibly observed in TGR5-KO mice GB (see ***Figure 2***). **B.** pJAM-A was localized at the TJ, as revealed by ZO-1 co-localization. Weaker pJAM-A staining was reproducibly observed in TGR5-KO mice GB (see ***Figure 2***). Each image corresponds to the projection of 3 confocal optical slices (0.3 μm thick each). Images are representative of n= 4 samples per group.

Supplementary Fig. 6. JAM-A and pJAM-A expression in WT and TGR5-KO gallbladder and liver.

A. Basal state JAM-A expression and phosphorylation (p-JAM-A) in hepatocyte TJ, in WT and TGR5-KO mice. Semi-quantitative analysis of immunostainings (see **Supplementary Materials and Methods**). Data are means of n=4 mice per group, Mann-Whitney U test. **B. Basal state JAM-A expression in WT and TGR5-KO GB.** One representative western blot image, and semi-quantitative analysis: data are means±sem from at least 3-4 pools of 3 GB each per genotype. Two representative lanes are shown for each condition. Analysis was performed after normalization on CK-19 (cholangiocyte marker) expression. While JAM-A was less expressed in TGR5-KO as compared to WT mice, ZO-1 expression was similar in the two genotypes. Mann Whitney U test. **C.** JAM-A and pJAM-A expression in WT and TGR5-KO livers, at basal state and 48 hours after BDL, upon vehicle or TGR5 agonist treatment. Representative western blot images, and semi-quantitative analysis: data are means±sem from at least 5 liver samples per group. Analysis was performed after normalization on GAPDH expression.

Supplementary Fig. 7. JAM-A and pJAM-A expression in WT and TGR5-KO ileum. Confocal microscopy analysis.

A. Basal state JAM-A, pJAM-A and ZO-1 expression in WT and TGR5-KO ileum. JAM-A and pJAM-A were localized at the TJ, as revealed by ZO-1 co-localization. **B**. **JAM-A, pJAM-A and ZO-1 expression in WT and TGR5-KO ileum 48 hours after BDL.** No difference in JAM-A and pJAM-A expression at the TJ has been observed between basal state and BDL conditions. Each image corresponds to the projection of 3 confocal optical slices (0.3 µm thick each). Images are representative of n= 4 samples per group. Scale bar: 30 µm.

Supplementary Fig. 8.

A. Semi-quantitative analysis of JAM-A and pJAM-A immunofluorescent staining in NRC cells. Cells were stimulated with either the vehicle or the TGR5 agonist RO5527239 (30 μM, 30 min), fixed and further processed for JAM-A and pJAM-A immunostaining, as explained in Materials and Methods. Values in green are percentages of cells with fluorescence (JAM-A, pJAM-A or ZO-1) higher than mean ± 2 SD of the control (vehicle) group. One representative image of these experiments is shown in **Figure 3A**. **B. TGR5 agonist-induced JAM-A phosphorylation in biliary epithelial F258 cells.** Western blot and immunofluorescent staining of pJAMA were performed on F258 cells treated or not (vehicle: DMSO) with the TGR5 agonist RO5527239 (RO, 30 μM, 5 up to 120 min). One representative western blot image of these experiments is shown, along with a semi-quantitative analysis: data are means±sem from at least 4 samples per condition. Analysis was performed after normalization on α-tubulin expression. F258 cells were stimulated with either the vehicle or the TGR5 agonist RO5527239 (30 μM, 10 and 30 min), fixed and further processed for pJAM-A immunostaining, as explained in Materials and Methods. One representative image is shown. **C. JAM-A mRNA expression in GB from sham and BDL WT mice.** GB was removed 48 hours after BDL or sham operation in WT mice, stored in RNA later and processed for RNA extraction and RT-qPCR as explained in Material and Methods. Data are means±sem from at least 5 samples per group, Students' t test.

Supplementary Fig. 9.

A. Other TJ proteins than JAM-A are not impacted by TGR5 specific stimulation. Western blot analysis from NRC cells treated with either the vehicle or RO5527239 (RO, 30 μM, 30 min), and processed for immunoblotting against ZO-1, occludin, claudin-3 (Cldn-3)

or claudin-4 (Cldn-4). No significant variation was observed in the expression of these proteins. Representative images are shown and semi-quantitative analysis: data are means±sem from 3 samples per condition. Mann Whitney U test. **B. MDCK II non-phosphorylatable JAM-A mutant (S285) cells.** pJAM-A immunostaining after vehicle (DMSO) or RO5527239 (RO, 30 µM, 30 min) treatment, in the presence or not of Doxycyclin (Dox^+/Dox^-). Representative image of 3 experiments.

Supplementary Fig. 10. TGR5 agonism provides hepatoprotection in ANIT mice.

A. **JAM-A expression and phosphorylation in the biliary epithelium are increased after ANIT treatment.** RO5527239 treatment in ANIT mice further increases JAM-A and pJAM-A. Semi-quantitative analysis of IHBD immunostainings (see *Supplementary Materials and Methods*). Left graphs: Representative profiles of tight junctions fluorescence for each condition. Middle graphs: Areas under curve (AUC) determined from representative profiles for each condition (see Supplementary Materials and Methods). Right images: JAM-A, pJAM-A, ZO-1 and CK-19 immunofluorescence on liver sections from control (Ctrl) and ANIT mice (Veh and RO). Scale bar=50 µm. Data are means of n=3-7 mice per group, Mann-Whitney U test. **B**. **More severe phenotype in TGR5-KO as compared with WT mice.** TGR5 agonist treatment significantly protects WT mice from ANIT-induced liver injury (see the experimental design), as revealed by H&E-stained liver sections (necrosis area, surrounded by doted lines, n=3-7 mice/group (Mann-Whitney U test). Scale bar=100µm.

Supplementary Fig. 11. JAM-A and pJAM-A expression in PBC and PSC patients.

A. **JAM-A expression in the biliary epithelium is increased in PBC and PSC as compared with control (CTRL) patients.** Semi-quantitative analysis of IHBD immunostainings (see *Supplementary*

Materials and Methods). Left graphs: Representative profiles of tight junctions fluorescence for each condition. Middle graphs: Areas under curve (AUC) determined from representative profiles for each condition (see Supplementary Materials and Methods). Right graph: JAM-A mRNA expression in CTRL, PBC (liver) and PSC patients (GB and liver). N=4-5 patients per group. **B. JAM-A, pJAM-A and CK-19 immunofluorescence on liver sections from control (CTRL) PBC and PSC patients.** Scale bar=50 μm. Images are representative from 4-6 patients per group. Data are means of n=3-7 mice per group, Mann-Whitney U test. **C. JAM-A and pJAM-A expression in hepatocytes** (immunofluorescence on liver sections, left images) and whole liver (western blot and semiquantitative analysis, right panels), in CTRL PBC and PSC patients (n=6 patients per group).

Supplementary Fig. 12. TGR5 agonist treatment does not impact cytokine gene induction after BDL.

IL-6, TNFα, IL-1β and TGF-β mRNAs in sham and WT BDL mice livers, upon vehicle or TGR5 agonist treatment (n=6 mice per group). Mann-Whitney U test.

Supplementary Fig. 13. Tight junction protein expression in WT and JAM-A-KO liver.

A, B. Immunostaining for TJ proteins (JAM-A, pJAM-A, claudins 1,2,3,5, ZO-1) in WT and JAM-A-KO livers.

Supplementary Fig. 14. Schematic diagram depicting the TGR5-mediated BA pathway for regulation of biliary epithelial permeability and hepato-protection. BA through TGR5 stimulation induce the TJ protein JAM-A to phosphorylate via a PKCζ-dependent mechanism, leading to its stabilization at the TJ. This results in a reduced biliary epithelial permeability, and an enhanced hepato-protection against BA leakage from bile towards the hepatic parenchyma, especially in the setting of biliary obstruction.

Supplementary Table 1: antibodies for immunohistochemistry and western blot.

Name	Source / Reference	Dilution	
		IHC	WB
α-actin	Sigma, #A-2668	N/A	1/1000
α-tubulin	Sigma, #T-9026	N/A	1/2000
E-cadherin	Novex, #13-1900	1/200	N/A
cingulin	kind gift from Pr Sandra Citi (University of Geneva, Switzerland)	1/5000	N/A
claudin-1	Invitrogen, #51900	1/100	N/A
claudin-2	Zymed, #51-6100	1/100	1/1000
claudin-3	Zymed, #34-1700	1/100	1/1000
claudin-4	Novex, #36-4800	N/A	1/100
claudin-5	Zymed, #34-1600	1/100	N/A
cytokeratin-19	CK-19, TROMA-III, Developmental Studies Hybridoma Bank, University of Iowa	1/500	1/5000
GAPDH	Thermofisher, #MA5-15738	N/A	1/2000
GFP	Santa-Cruz, #sc-9996	N/A	1/500
JAM-A	Invitrogen, #361700	1/100	1/1000
phospho (S285)-JAM-A	Santa Cruz, # Sc-17430-R	1/50	1/500
occludin	Zymed, #33-1500	1/100	1/500
PKC ζ	Santa-Cruz, USA #sc-216	1/200	1/1000
phospho-PKC ζ-λ (Thr403/410)	Cell Signaling Technology, #9378	N/A	1/1000
ZO-1	kind gift from Dr. Stevenson, Salk Institute, La Jolla, CA	Cell culture medium	
ZO-2	Thermo-Fisher, #71-1400	1/100	1/1000

N/A: not applicable.

Supplementary Table 2: primers for qPCR.

Gene	Primer sequences
mHPRT	**Fw :** TCCTCCTCAGACCGCTTTT **Rv :** CCTGGTTCATCATCGCTAATC
mCK19	**Fw :** AGGAGGAAATTACTGCCCTG **Rv :** CTCAATCCGAGCAAGGTAGG
mTGR5	**Fw :** GTCAGCTCCCTGTTCTTTGC **Rv :** CAGGAGGCCATAAACTTCCA
mJAM-A	**Fw :** GCCAGATCACAGCTCCCTAT **Rv :** ACTGATCGTCGGCTTGGATG
hJAM-A	**Fw :** GCG CAA GTC GAG AGG AAA CT **Rw :** AAA AGC CCG AGT AGG CAC AG
mJAM-C	**Fw :** AAGGACGACTCTGGGCAGTA **Rv :** TCCCAGCAATGTTCAAATCA
mClaudin 1	**Fw :** TGGGGCTGATCGCAATCTTT **Rv :** CACTAATGTCGCCAGACCTGA
mClaudin 2	**Fw :** TGTGAATGAACTGAAGGAAAGC **Rv :** ATCCTGCACCCAGCTGTATT
mClaudin 3	**Fw :** ACAGACGTCCGTCAGTTTTC **Rv :** GTGATCTGCGCCGTGATGAT
mClaudin 4	**Fw :** GCTTGGTAGCTGGTGCATCG **Rv :** CCCAGGACCTGTAGTCCCATA
mClaudin 5	**Fw :** GAGCAGAGGCACCAGAATCA **Rv :** CTGCAGACCCCATGGCTAAA
mClaudin 7	**Fw :** GACGCCCATGAACGTTAAGTA **Rv :** TTGCTTTCACTGCCTGGAC
mZO1	**Fw :** GAGCCCCCTAGTGATGTGTG **Rv :** GGTTTTAGGGTCACCCGACG
mOccludin	**Fw :** GGAGGACTGGGTCAGGGAATA **Rv :** CGTCGTCTAGTTCTGCCTGT
mE-Cadherin	**Fw :** CCCAGAGACTGGTGCCATTT **Rv :** TGGCAATGGGTGAACCATCA
rHPRT	**Fw :** AGGACCTCTCGAAGTGT **Rv :** ATCCCTGAAGTGCTCATTATA
h β-actin	**Fw** : GAG CTA CGA GCT GCC TGA CG **Rw** : GTA GTT TCG TGG ATG CCA CAG GAC T
rTGR5	**Fw :** AAAGGTGGCTACAAGTGCTTC **Rv :** TTCAAGTCCAAGTCAGTGCTG
cGAPDH	**Fw :** AGATCCCGCCAACATCAA **Rv :** GGTGAAGACCCAGTGGAC
cTGR5	**Fw :** AGATCCGCAAGCTCCAAAC **Rv :** TGCACTGCAGAGTCGTGTC

1 Li T, Holmstrom SR, Kir S, Umetani M, Schmidt DR, Kliewer SA*, et al.* The G protein-coupled bile acid receptor, TGR5, stimulates gallbladder filling. Mol Endocrinol 2011;**25**:1066-71.
2 Besnard A, Gautherot J, Julien B, Tebbi A, Garcin I, Doignon I*, et al.* The P2X4 purinergic receptor impacts liver regeneration after partial hepatectomy in mice through the regulation of biliary homeostasis. Hepatology 2016;**64**:941-53.
3 Ullmer C, Alvarez Sanchez R, Sprecher U, Raab S, Mattei P, Dehmlow H*, et al.* Systemic bile acid sensing by G protein-coupled bile acid receptor 1 (GPBAR1) promotes PYY and GLP-1 release. Br J Pharmacol 2013;**169**:671-84.
4 Desmet VJ, Krstulović B, Van Damme B. Histochemical study of rat liver in alpha-naphthyl isothiocyanate (ANIT) induced cholestasis. Am J Pathol 1968;**52**:401-21.
5 Han X, Fink MP, Uchiyama T, Yang R, Delude RL. Increased iNOS activity is essential for hepatic epithelial tight junction dysfunction in endotoxemic mice. Am J Physiol Gastrointest Liver Physiol 2004;**286**:G126-36.
6 Yeh TH, Krauland L, Singh V, Zou B, Devaraj P, Stolz DB*, et al.* Liver-specific β-catenin knockout mice have bile canalicular abnormalities, bile secretory defect, and intrahepatic cholestasis. Hepatology 2010;**52**:1410-9.
7 Rahner C, Stieger B, Landmann L. Structure-function correlation of tight junctional impairment after intrahepatic and extrahepatic cholestasis in rat liver. Gastroenterology 1996;**110**:1564-78.
8 Grosse B, Cassio D, Yousef N, Bernardo C, Jacquemin E, Gonzales E. Claudin-1 involved in neonatal ichthyosis sclerosing cholangitis syndrome regulates hepatic paracellular permeability. Hepatology 2012;**55**:1249-59.
9 Vroman B, LaRusso NF. Development and characterization of polarized primary cultures of rat intrahepatic bile duct epithelial cells. Lab Invest 1996;**74**:303-13.
10 Iden S, Misselwitz S, Peddibhotla SS, Tuncay H, Rehder D, Gerke V*, et al.* aPKC phosphorylates JAM-A at Ser285 to promote cell contact maturation and tight junction formation. J Cell Biol 2012;**196**:623-39.
11 Hardonnière K, Huc L, Podechard N, Fernier M, Tekpli X, Gallais I*, et al.* Benzo[a]pyrene-induced nitric oxide production acts as a survival signal targeting mitochondrial membrane potential. Toxicol In Vitro 2015;**29**:1597-608.
12 Humbert L, Maubert MA, Wolf C, Duboc H, Mahe M, Farabos D*, et al.* Bile acid profiling in human biological samples: comparison of extraction procedures and application to normal and cholestatic patients. J Chromatogr B Analyt Technol Biomed Life Sci 2012;**899**:135-45.