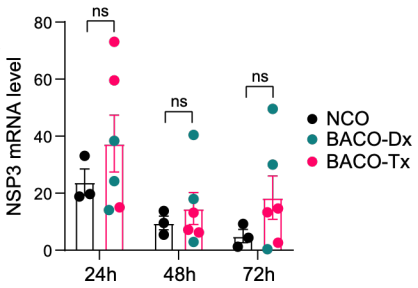
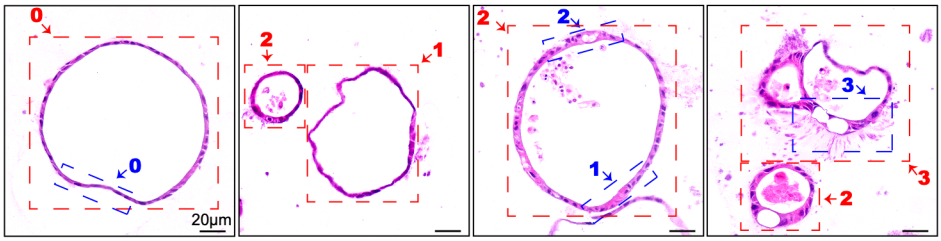
## SUPPLEMENTARY INFORMATION

A

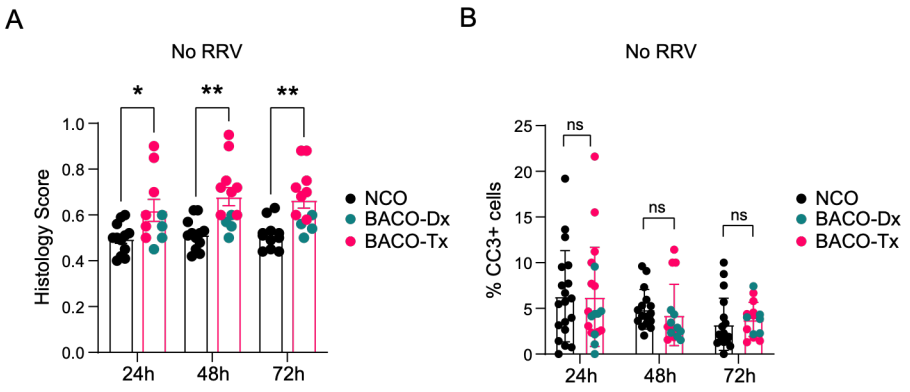



B

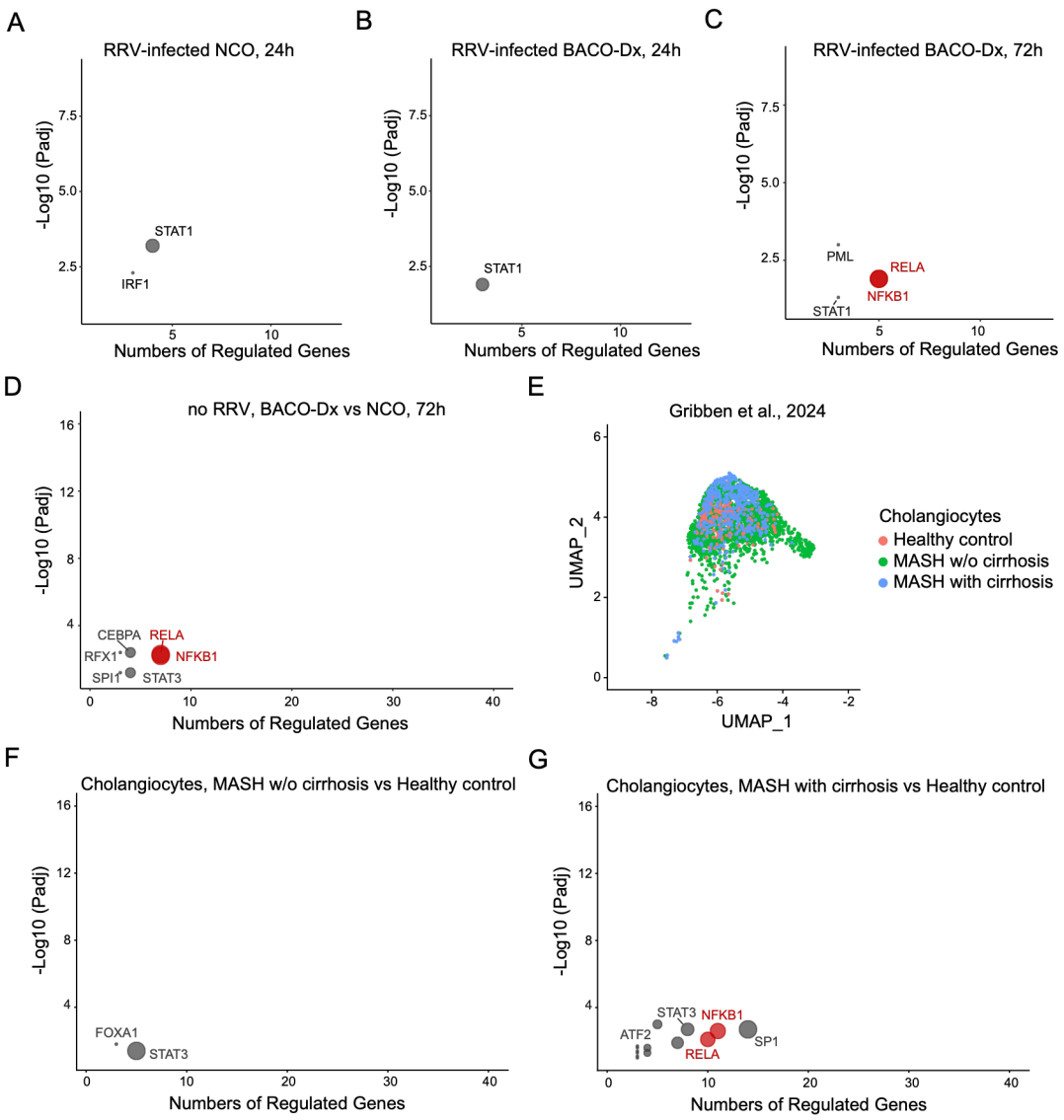



| Structural integrity | |
|---|---|
| 0 | Normal structure, fully intact epithelial architecture. |
| 1 | Mild alteration, subtle architectural irregularity, lumen clear. |
| 2 | Moderate disruption, structural distortion without collapse, lumen with debris. |
| 3 | Severe structural damage, loss of organized architecture with lumen disruption. |
| **Cytopathic activity** | |
| 0 | No cytopathy. |
| 1 | Mild cytopathic changes: sparse vacuoles, mild cellular swelling. |
| 2 | Moderate cytopathic injuries: moderate vacuoles, cellular swelling, membrane instability. |
| 3 | Extensive cellular damages: large vacuoles, cellular bursting, membrane rupture. |

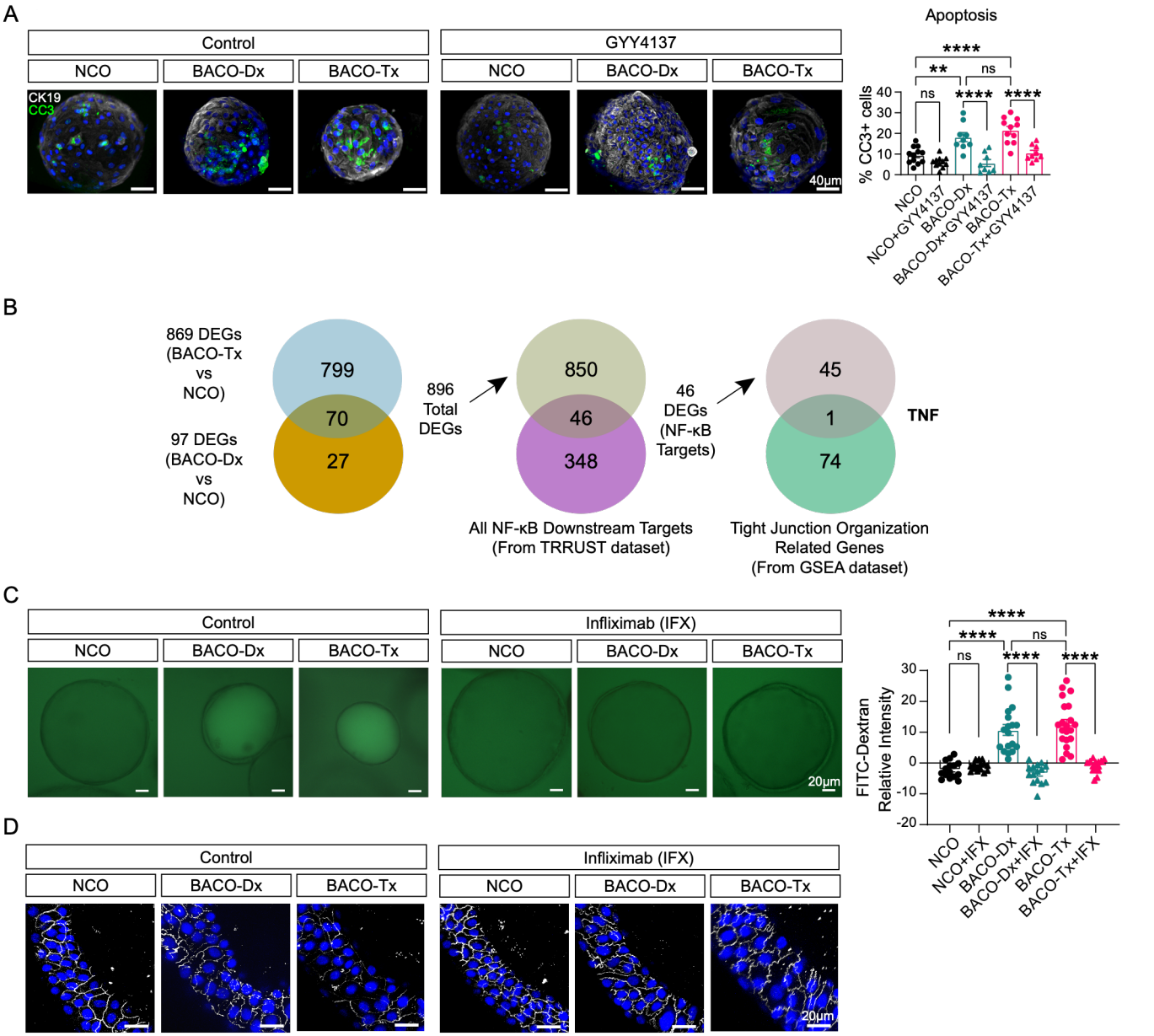
**Supplementary Figure 1. Additional characterization of RRV infection in NCO and BACO. (A)** Quantitative real-time PCR detected mRNA level of viral transcript NSP3 in NCO and BACO with RRV infection. NCO, n = 3 donors; BACO, n = 6 patients with 3 from BA at diagnosis and 3 from BA at transplant; in triplicates. Statistical analysis was performed using unpaired student's t-test; ns, not significant. **(B)** Two-layer histology scoring system including structural integrity (Red) and cytopathic activity (Blue) for Figure 2A. Dashed rectangle marks representative field with each respective score in either of two layers.




**Supplementary Figure 2. Baseline levels of histology score and apoptosis in NCO and BACO without RRV infection. (A)** Baseline levels of histology score. Related to Figure 2A. n=2-4 sections for each donor/patient at each time point. **(B)** Quantification of the ratio of CC3 positive cells in each organoid in uninfected groups (baseline). n=5-8 organoids for each donor/patient at each time point. NCO, n = 3 donors; BACO, n = 3 patients; in triplicates. Statistical analyses were performed using unpaired student's t-test. **P < 0.01; *P < 0.05; ns, not significant.




**Supplementary Figure 3. Transcription factor enrichment in NCO, BACO-Dx and non-BA cirrhosis cholangiocytes.** Plots showing transcription factors enriched from DEGs in RRV-infected NCO compared with uninfected NCO at 24h **(A)**, RRV-infected BACO-Dx compared with uninfected BACO-Dx at 24h **(B)** and 72h **(C)**, uninfected BACO-Dx compared with NCO at 72h **(D)**, cholangiocytes from MASH without cirrhosis compared with healthy control **(F)** and from MASH with cirrhosis compared with healthy control **(G)**. Cholangiocytes populations used for non-BA cirrhosis analysis are shown in **(E)**.




**Supplementary Figure 4. Additional characterization of NF-κB signaling in BA cholangiocytes.** **(A)** Whole mount immunostaining of cleaved caspase 3 (CC3, green) and CK19 (white) in RRV-infected NCO, BACO-Dx and BACO-Tx with and without GYY4137 treatment. Graph describes quantification of CC3 positive cells in each organoid. n=3-5 organoids for each donor/patient. **(B)** Schematic of exploration of downstream NF-κB target, by first identifying all differentially expressed

genes (DEGs) in BACO-Dx and BACO-Tx and overlapping sequentially with all NF-κB downstream targets and genes related with tight junction organization. **(C)** Immunofluorescent images depict diffusion of FITC-dextran into the lumen of NCO, BACO-Dx and BACO-Tx with and without infliximab treatment. Graph indicates quantification of fluorescence intensity of FITC-dextran signal in the lumen of each organoid. n=5-7 organoids for each donor/patient. **(A,C)** Statistical analysis was performed using one-way ANOVA, followed by Tukey post-hoc test. **(D)** Whole mount immunostaining of ZO-1 (white) in NCO, BACO-Dx and BACO-Tx with and without infliximab treatment. ****$P < 0.0001$; ns, not significant. NCO, n = 3 donors; BACO-Dx, n=3 patients; BACO-Tx, n = 3 patients; in triplicates.

**Supplementary Table 1. Differentially expressed genes (DEGs) comparing RRV-infected normal cholangiocyte organoids (NCO) and cholangiocyte organoids from BA patients at diagnosis (BACO-Dx) and at transplant (BACO-Tx) with their respectively uninfected controls.** Lists of differentially expressed genes between RRV-infected NCO, BACO-Dx, BACO-Tx and their respectively uninfected controls at 24, 48, and 72 hours post infection (see accompanying table).

**Supplementary Table 2. Differentially expressed genes (DEGs) comparing uninfected normal cholangiocyte organoids (NCO) and cholangiocyte organoids from BA patients at transplant (BACO-Tx).** Lists of differentially expressed genes between uninfected NCO and BACO-Tx (see accompanying table).

**Supplementary Table 3. List of patient donor samples.**

| No. | NCO | BACO-Dx | BACO-Tx |
|---|---|---|---|
| 1 | 13 yr, M | 3 wk, M | 6 mo, F |
| 2 | 17 yr, M | 9 wk, F | 8 mo, F |
| 3 | N/A, M | 2 mo, M | 17 mo, M |
| 4 | 10 mo, M (urea cycle defect) | 6 wk, M | 2 yr, M |
| 5 | – | – | 1 yr, M |
| 6 | – | – | 9 mo, F |
| **Donors used in each experiment** | | | |
| Figure 1B | 2,3,4 | – | – |
| Figure 1C | 2,3,4 | – | – |
| Figure 2A | 1,2,3 | 3 | 1,4 |
| Figure 2B | 1,2,3 | 3 | 1,4 |
| Figure 2C | 1,2,4 | 3 | 2,4 |
| Figure 2D | 2,3,4 | 3 | 2,4 |
| Figure 3A–C | 1,2,3,4 | 1,2,3 | 1,2,4 |
| Figure 3D | – | – | 1,2,4 |
| Figure 3E | 1,2,3,4 | – | 1,2,4 |
| Figure 4A | 1,2,3 | 2,3,4 | 1,4,5 |
| Figure 4B | 1,2,3 | 2,3,4 | 1,3,4 |
| Figure 4C | 1,2,3 | 2,3,4 | 1,3,4 |
| Figure 4D | 1,2,3 | 2,3,4 | 1,3,4 |
| Supplemental 1A | 2,3,4 | 1,2,3 | 1,2,4 |
| Supplemental 2A–B | 1,2,3 | 3 | 1,4 |
| Supplemental 3A | 1,2,3,4 | – | – |
| Supplemental 3B-C | – | 1,2,3 | – |
| Supplemental 3D | 1,2,3,4 | 1,2,3 | – |
| Supplemental 4A | 1,2,3 | 2,3,4 | 2,4,6 |
| Supplemental 4C-D | 1,2,3 | 2,3,4 | 1,4,5 |

M, Male; F, Female; wk, week; mo, month. yr, year.

**Supplementary Table 3. Source and classification of human cholangiocyte organoids.**

Organoids were established from liver tissue from normal donors (NCO), liver biopsies from patients with biliary atresia obtained at diagnosis (BACO-Dx), and liver explants from patients with biliary atresia at the time of liver transplantation (BACO-Tx). Donors/patients used for each experiment were labeled with their respective numbers classified in the top section of the table.

**Supplementary Table 4. Antibodies used in this study.** Company and catalog information of the antibodies used in this study are listed below.

| Anbitbody | Catalog | Company |
|---|---|---|
| Rotavirus | PA1-85845 | Invitrogen |
| Cytokeratin 19 | MBS439148 | MyBioSource |
| Cleaved Caspase-3 | 9664S | Cell Signaling Technology |
| ZO-1 (for human organoids) | 33-9100 | Invitrogen |
| NF-κB p65 (phospho S536) | ab86299 | Abcam |
| ZO-1 (for mouse IHC) | 40-2200 | Invitrogen |
| anti-human TNFα (Infliximab Biosimilar) | SIM0006 | Bio X Cell |