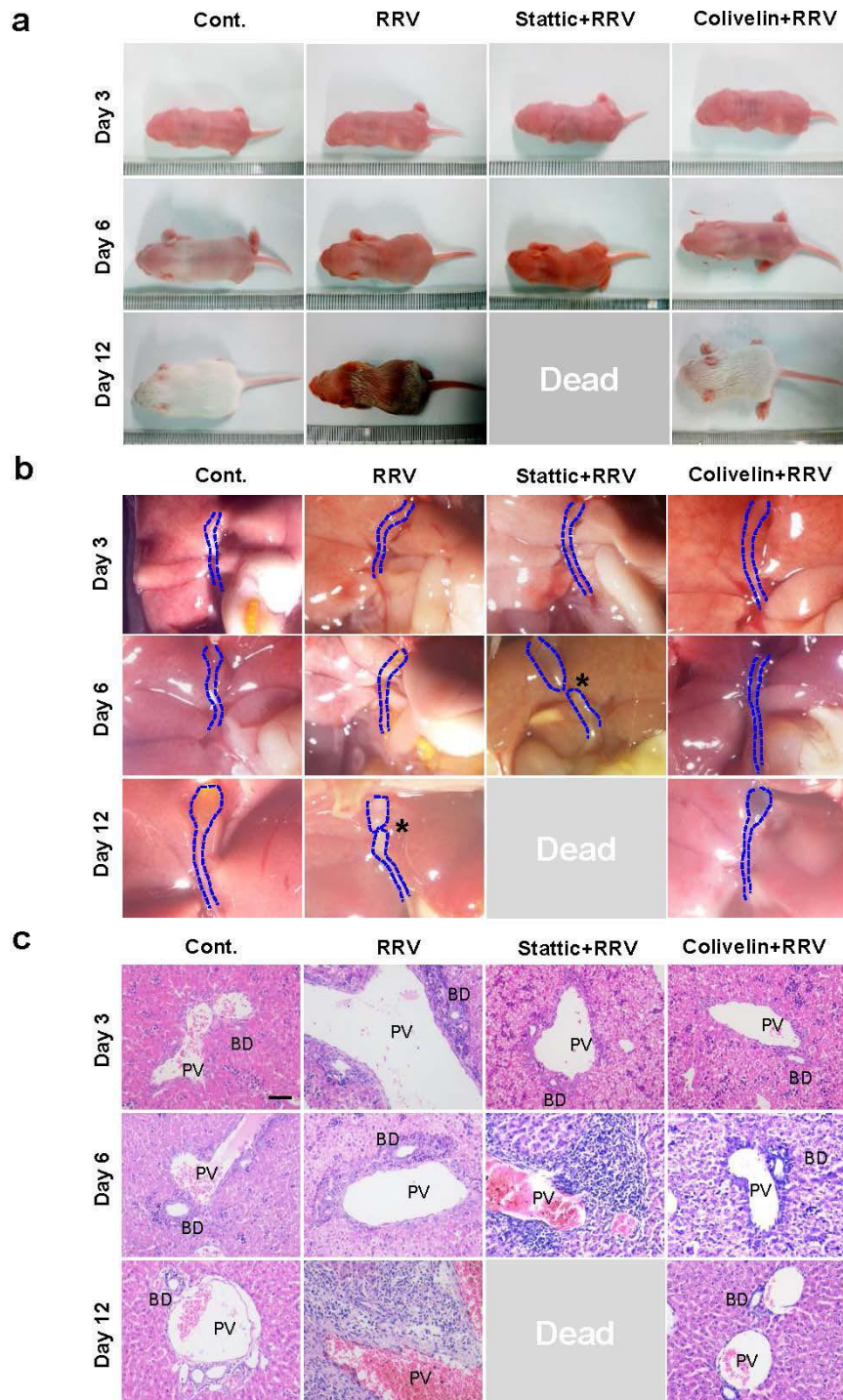


Supplementary figure 1

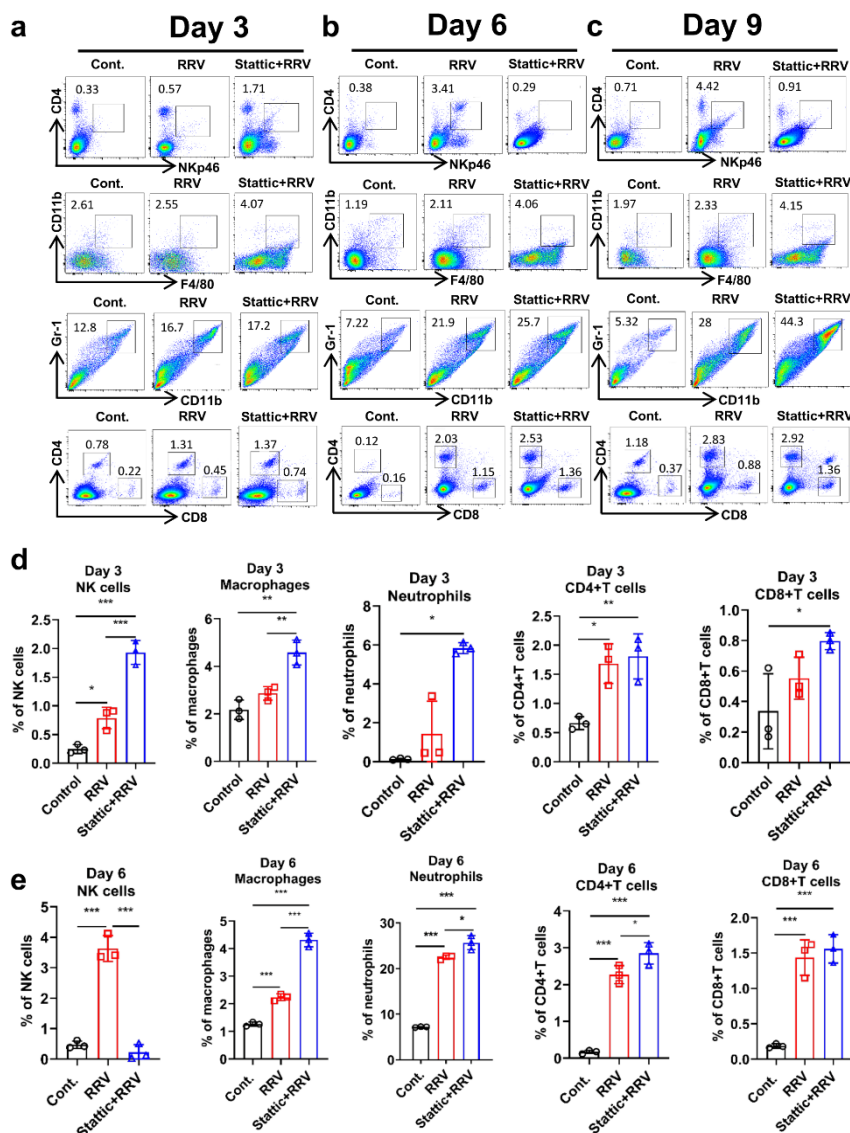


Supplementary figure 1 Effect of STAT3 inhibition or activation on a mouse model of biliary atresia on days 3, 6 and 12. The STAT3 inhibitor Stattic (25 $\mu\text{g/g}$ body weight) or the activator Colivelin (1 $\mu\text{g/g}$ body weight) was given 4 hours before RRV inoculation. **a** Effects of Stattic and Colivelin on BA syndrome. Photographs of the mice were taken on days 3, 6 and 12 after RRV inoculation. **b** Photographs of the portal area after dissection

under a dissecting microscope. Cholangiography was achieved by injection of 0.4% methylene blue. The blue line shows the outline of the gallbladder and bile duct. The asterisks depict BA. **c** HE staining revealing the hepatic bile ducts and cellular infiltration in the Stattic and Colivelin treatment groups. The scale bar represents 50 μm . BD, bile duct; PV, portal vein.

Supplementary figure 2

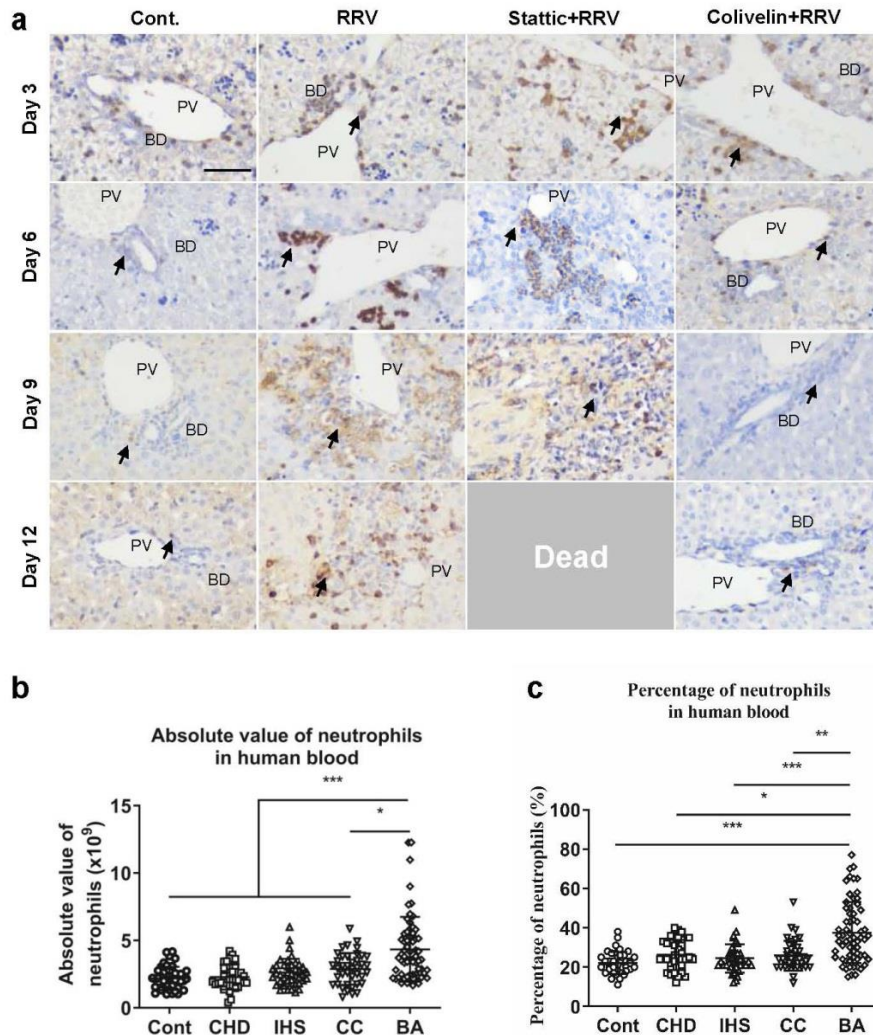
Suppl. Fig. 2



Supplementary figure 2 Effects of STAT3 inhibition on hepatic immune cell regulation on days 3 and 6 after RRV inoculation, and comparison of neutrophils in different neonatal diseases. **a, b and c** STAT3 inhibition induced changes in NK cell, macrophage, neutrophil, CD4+ T cell and CD8+ T cell numbers in the liver as measured by flow cytometry. Cell suspensions were obtained from the liver at the end of experiments and labelled with appropriate antibodies for immune cell analysis. At least three sets of experiments were performed, and one representative set of results is presented here. Data were obtained on days 3 (**a**), 6 (**b**) and 9 (**c**) after

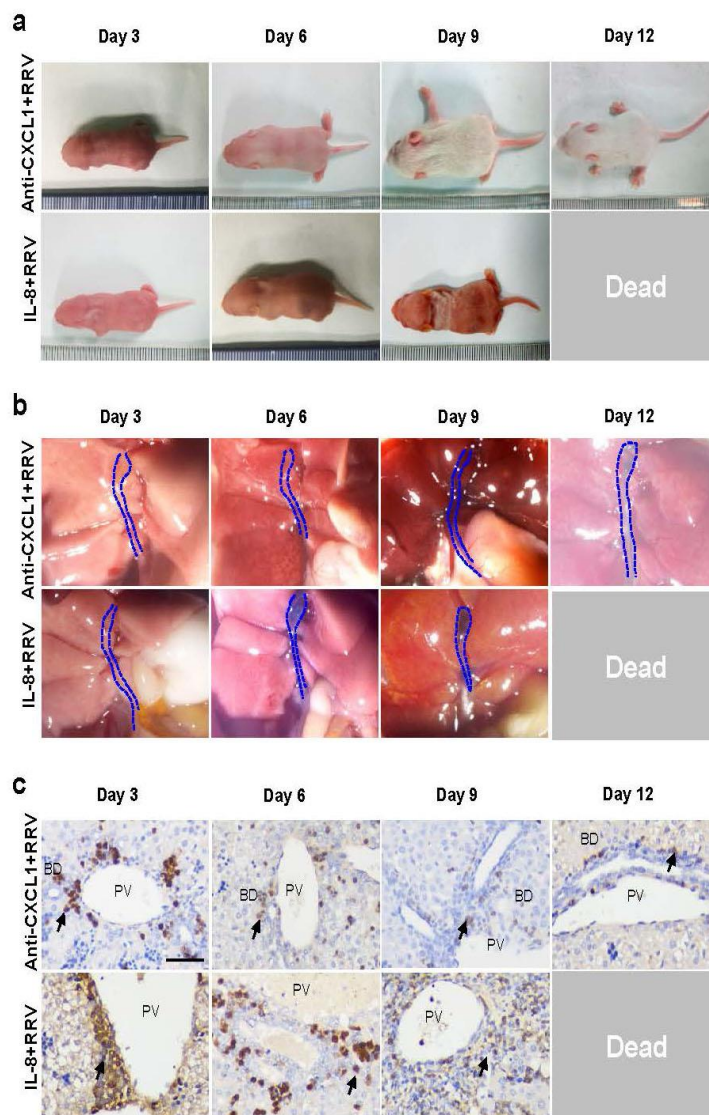
RRV inoculation. **d and e** Data were collected and on days 3 (**d**) and 6 (**e**) after RRV inoculation and evaluated by statistical analysis. *P<0.05, **P<0.01, ***P<0.001.

Supplementary figure3



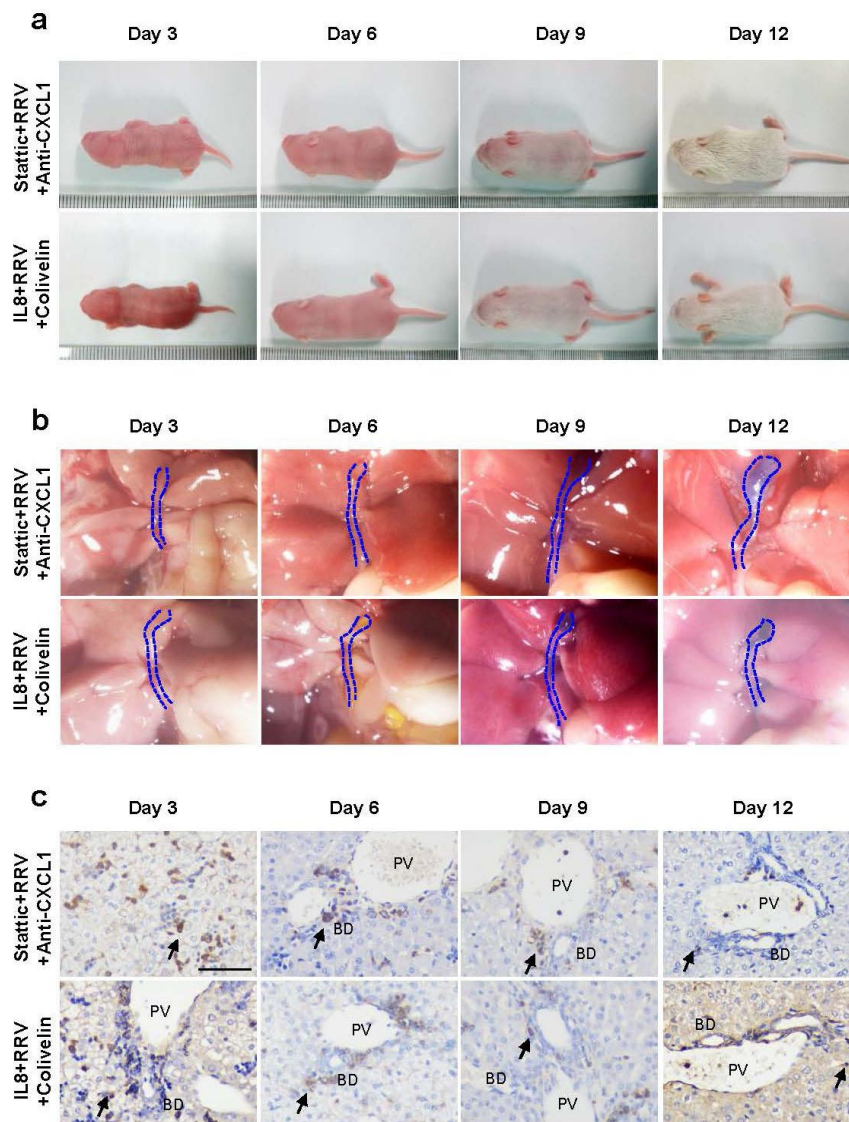
Suppl. Fig. 3. Effects of STAT3 inhibition or activation on neutrophil accumulation and the content of neutrophil in human peripheral blood. **a** Effects of STAT3 inhibition or activation on neutrophil accumulation determined by immunohistochemical staining on days 3, 6, 9 and 12 after RRV inoculation. BD, bile duct; PV, portal vein; the black arrows represent neutrophils; the scale bar represents 50 μ m. **b** Absolute value of neutrophils from clinical analysis of the peripheral blood of BA patients compared with that of patients with other neonatal diseases: Cont., healthy control (n=40); CHD, congenital heart disease (n=35); IHS, infant hepatitis syndrome (n=40); CC (n=40); and BA (n=63). *P<0.05, ***P<0.001. **c** Percentages of neutrophils in the peripheral blood for the same patient populations. *P<0.05, **P<0.01, ***P<0.001.

Supplementary figure4



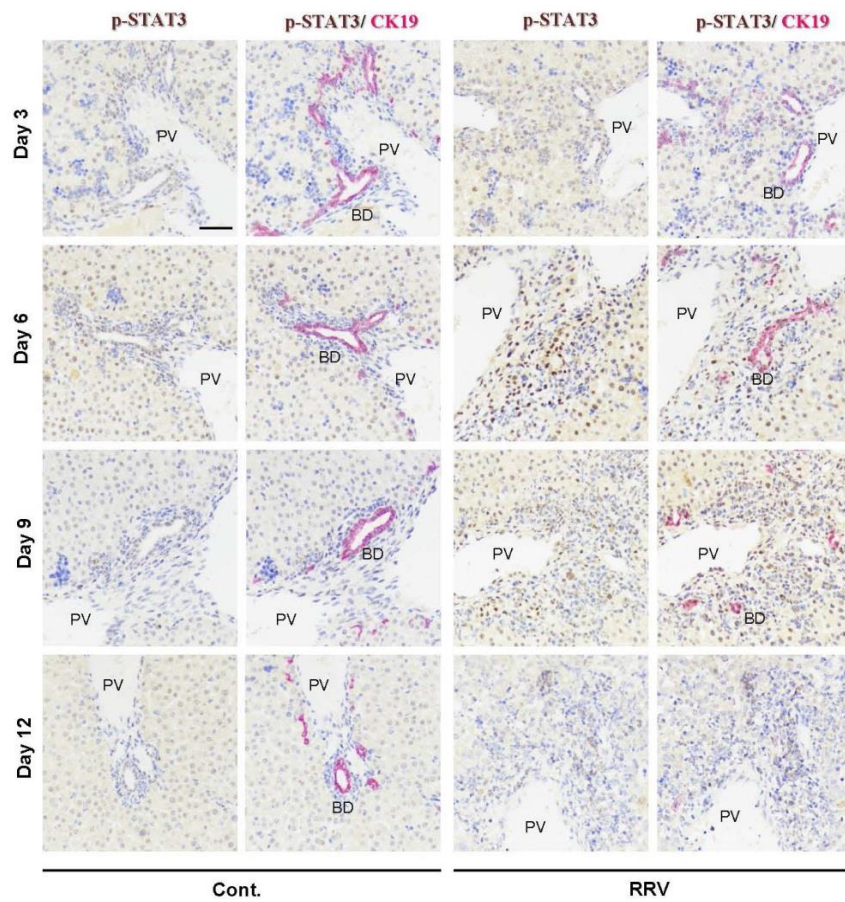
Supplementary figure 4 Administration of an antibody against the chemoattractant CXCL1 or human recombinant IL-8 to BA model mice. **a** Morphological changes on days 3, 6, 9 and 12 after RRV inoculation in BA model mice after administration of anti-CXCL1 and anti-IL-8 antibodies. **b** Photographs of portal area changes in the treatment groups mentioned above. Cholangiography was achieved by injection of 0.4% methylene blue. The blue line shows the outline of the gallbladder and bile duct. **c** Changes in neutrophils in the liver in the test and control groups detected by immunohistochemical staining. The black arrows represented neutrophil. The scale bar represents 50 μ m. BD, bile duct; PV, portal vein.

Supplementary figure 5



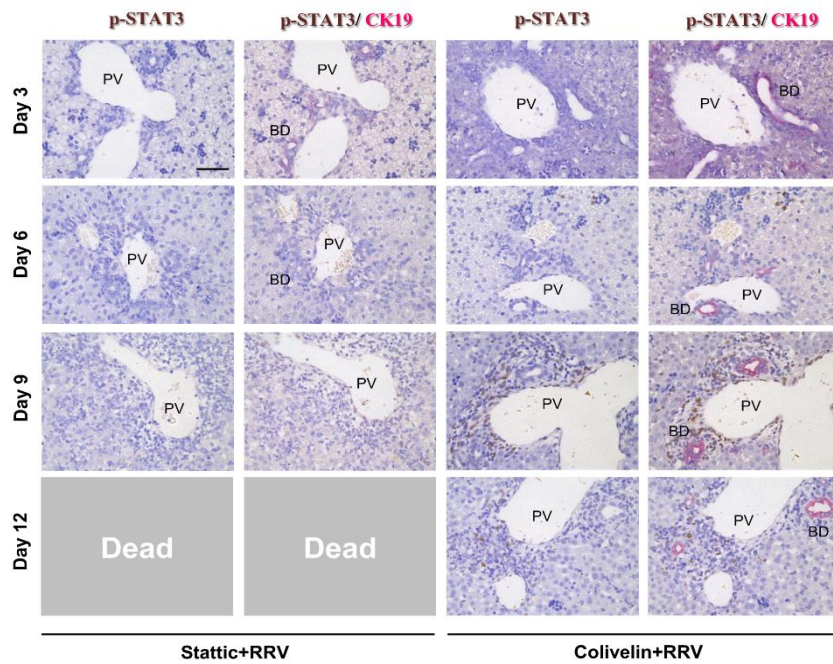
Supplementary figure 5 Combined treatments of the anti-CXCL1 antibody/STAT3 inhibitor Stattic and human recombinant IL-8/the STAT3 activator Colivelin in the BA mouse model. **a** Changes in morphology in BA mice in response to treatment with either the anti-CXCL1 antibody combined with the STAT3 inhibitor or recombinant IL-8 combined with the STAT3 activator Colivelin on days 3, 6, 9 and 12 after RRV inoculation. **b** Photographs of the portal areas in the treatment groups mentioned above. Cholangiography was achieved by injection of 0.4% methylene blue. The blue line shows the outline of the gallbladder and bile duct. **c** Changes in neutrophils in the liver as measured by immunohistochemical staining. The black arrows represented neutrophil. The scale bar represents 50 μ m. BD, bile duct; PV, portal vein.

Supplementary figure 6



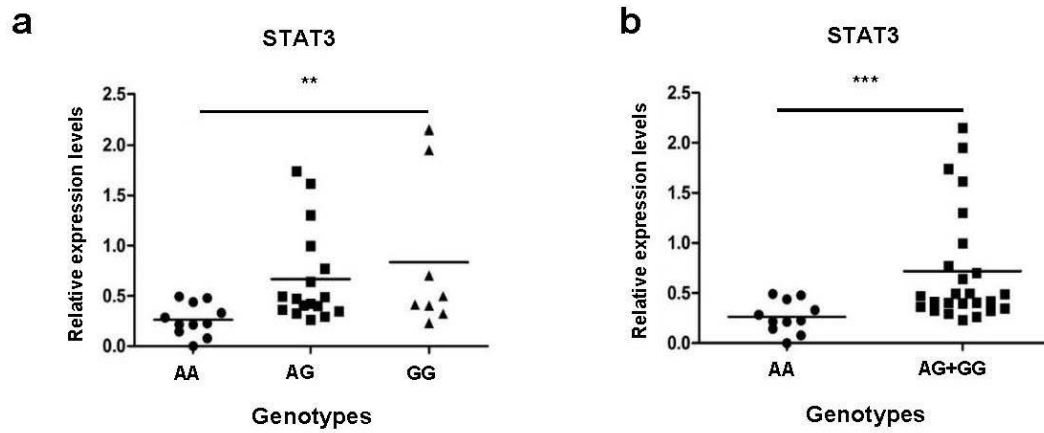
Supplementary figure 6 The effect of STAT3 activation on a BA mouse model. Immunohistochemical double staining for p-STAT3 was performed in BA model mice on days 3, 6, 9 and 12. Brown and pink color indicated p-STAT3 and CK19 for bile duct. The scale bar represents 100 μm .

Supplementary figure 7



Supplementary figure 7 The effect of STAT3 activator and inhibitor on a BA mouse model. Immunohistochemical double staining for p-STAT3 was performed in BA model mice on days 3, 6, 9 and 12. Brown and pink color indicated p-STAT3 and CK19 for bile duct. The scale bar represents 100 μ m.

Supplementary figure8



Supplementary figure 8 Genetic association and expression of STAT3 in biliary atresia. **a and b** Expression of STAT3 in patients with the risk genotype (AA) of SNP rs7211777 was compared to that in patients with the GG or AG genotype, and statistical significance was evaluated (n=8-15 for each genotype).

Supplementary table 1: Reagents/antibodies used in the experiments

Reagents/antibody	Reactivity	Company	Catalogue # (Lot #)	Dilution
CK19	Human	Santa Cruz Biotechnology	sc-376126 (K2613)	1:100
Ck19	Mouse	Abcam	ab133496 (GR3271,57-2)	1:200
STAT3	Human / mouse	Cell Signalling	9139S	1:600
p-STAT3	Human / mouse	Cell Signalling	9145S	1:400
p-STAT3	Human	Abcam	ab30647	1:50
CXCL1	Human / mouse	Abcam	ab86436	1:250
CD11b-FITC	Mouse	Thermo Fisher Scientific	11-0112-82	1:100
Gr-1-PE	Mouse	Thermo Fisher Scientific	12-5931-82	1:100
Gr-1-FITC	Mouse	Thermo Fisher Scientific	11-5931-82	1:100
Ly6G-PE	Mouse	Thermo Fisher Scientific	12-9668-82	1:100
Ly6C-PerCP-Cyanine5.5	Mouse	Thermo Fisher Scientific	45-5932-82	1:100
CD4-PerCP-Cyanine5.5	Mouse	Thermo Fisher Scientific	45-0042-82	1:100
CD3e-Alexa Fluor 488	Mouse	Thermo Fisher Scientific	53-0031-82	1:100
CD8a-APC	Mouse	Thermo Fisher Scientific	17-0081-82	1:100
MHC-II-PE	Mouse	Thermo Fisher Scientific	12-5321-82	1:100
F4/80 -APC	Mouse	Thermo Fisher Scientific	17-4801-82	1:100
CD11c -PE	Mouse	Thermo Fisher Scientific	12-0114-82	1:100
STAT3 inhibitor (Stattic)	Mouse	Santa Cruz Biotechnology	sc-202818	-

STAT3 activator (Colivelin)	Mouse	Santa Cruz Biotechnology	sc-361153	-
Recombinant IL-8	human	BioLegend	574206	-
Anti-CXCL1 antibody	Mouse	Thermo Fisher Scientific	48415	-

Supplementary Table 2: Demographic and Clinical Characteristics of the Study population

Variable	Choledochal cysts (CC, n=20)	Biliary atresia (BA, n=28)
Age (days)	272.5 (92, 1640)	78.5 (70, 89.5)
Males, n (%)	3(15%)	16(57.14%)
Females, n (%)	17(85%)	12(42.86%)
Underlying disease, n (%)		
Thalassemia	0(0)	1(3.57%)
Congenital intestinal atresia	0(0)	1(3.57%)
Acute pancreatitis	1(5%)	0(0)
Hernia	1(5%)	3(10.71%)
DBIL (umol/L)	45.32±55.74	137.3±48.1
TBIL (umol/L)	64.28±67.79	153.9±60.07
GGT (U/L)	612.8±809.3	772.8±598.6
Total bile acid (umol/L)	80.31±126.2	178±82.31

Age: median(25% percentile, 75% percentile)

Supplementary Table 3: Expression of STAT3 in Gr-1+ and it's sub clusters.

	NC		RRV	
	Ly6G ⁺	Ly6C ⁺	Ly6G ⁺	Ly6C ⁺
Total Cell Number	3608	973	2631	1122
STAT3 ⁺ cells	270	146	138	117
%	7.5%	15.0%	5.2%	10.4%

Supplementary Table 4: The association of SNP rs7211777 in STAT3 with BA susceptibility using 503 cases and 495 controls.

CHR	SNP	BP	A1/A2	TEST	Patients	Controls	OR (CI 0.95)	P
				DOM	389/69	375/107	1.61(1.15~2.25)	5.31E-03
17	rs7211777	42382057	A/G	REC	155/303	160/322	1.03(0.79~1.35)	0.833
				ADD	544/372	535/429	1.23(1.02~1.48)	0.033
				GENO_2DF	155/234/69	160/215/107	NA	0.015

CHR: Chromosome; SNP: Single nucleotide polymorphism; BP: Base Pair; A1/A2: Allele1/Allele2; TEST: test of genetic model; Patients: genotype/allele numbers in patients; Control: genotype/allele numbers in controls; OR: Odds Ratio; CI: Confident Interval; P: P value of disease association.