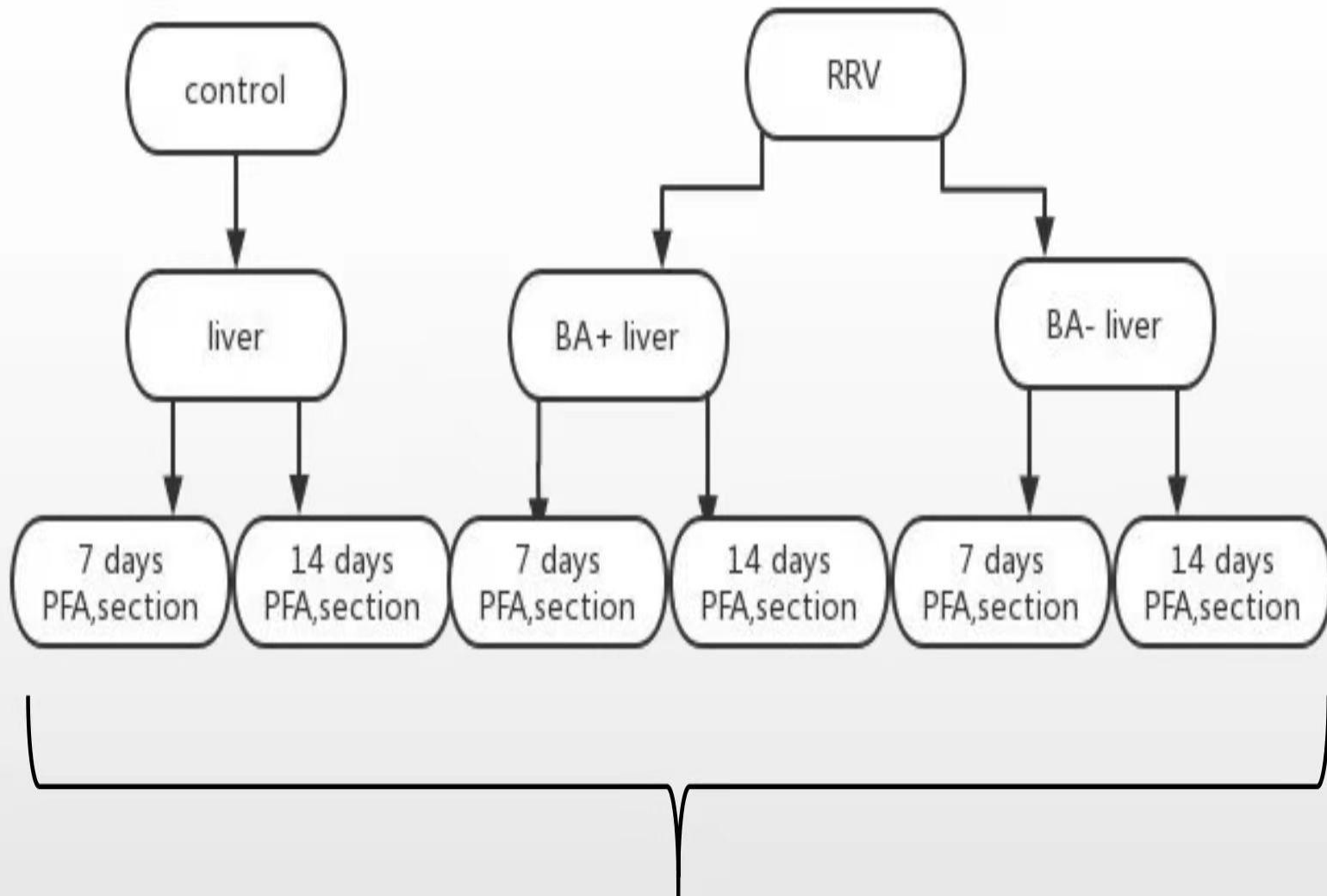


BA Background

- Most of the previous and ongoing research focuses on the operative approach and adjuvant treatment, while there is little knowledge on **pre-operative** treatment.
- Almost all research on **steroids focuses on post-operative usage**, and their therapeutic value as a neo-adjuvant treatment to improve the surgical outcome remains undetermined.

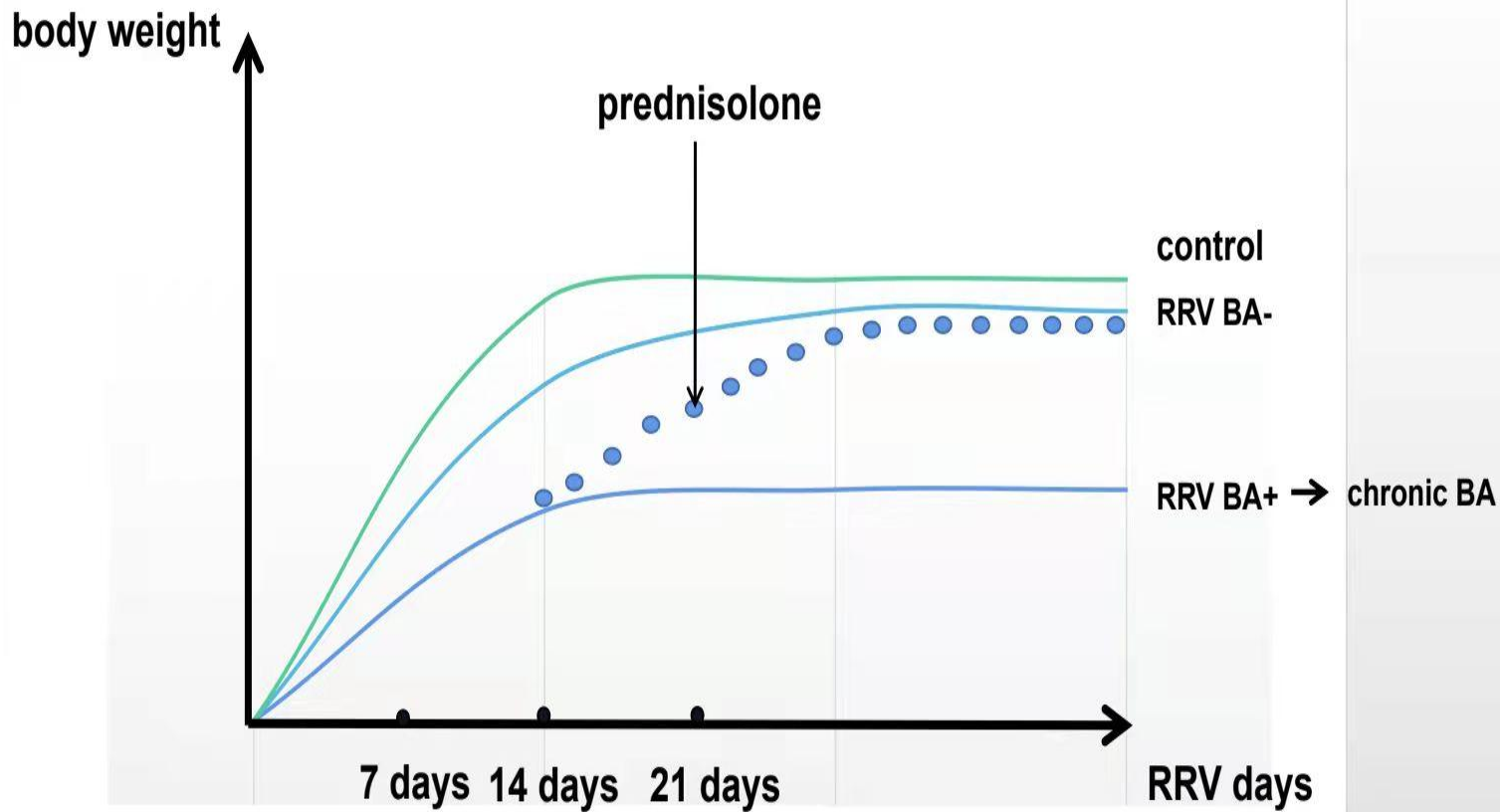
Research Tools

- Animal BA model (RRV infected mice)
- Liver organoids from BA patients and mice
- BA liver organoids co-cultured with prednisolone
- Non-BA liver organoids co-cultured with (Poly I:C; 40µg/ml) induces BA aberrant morphology

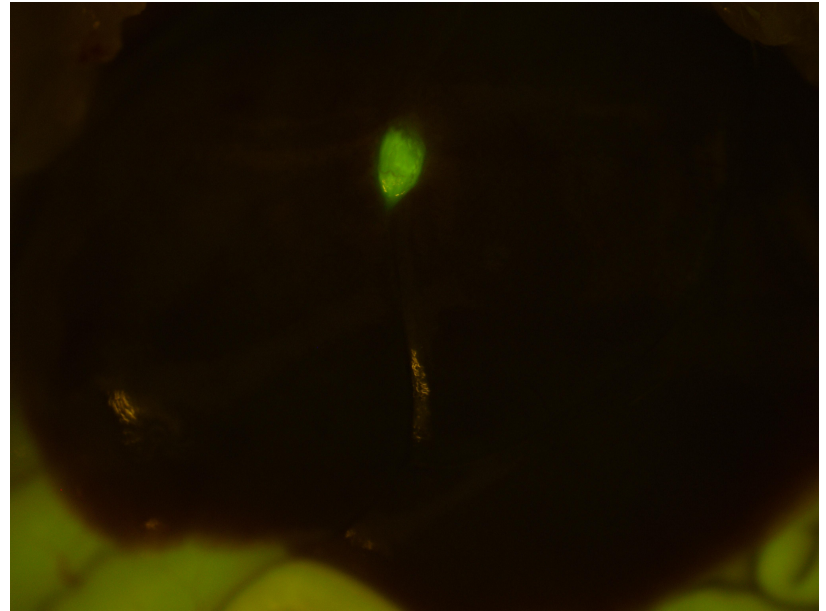
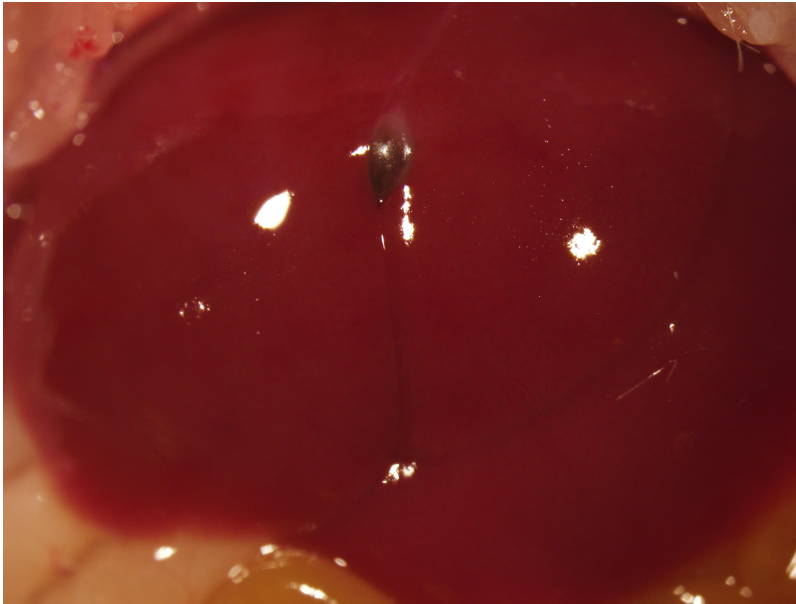


Immne cell test : IHC

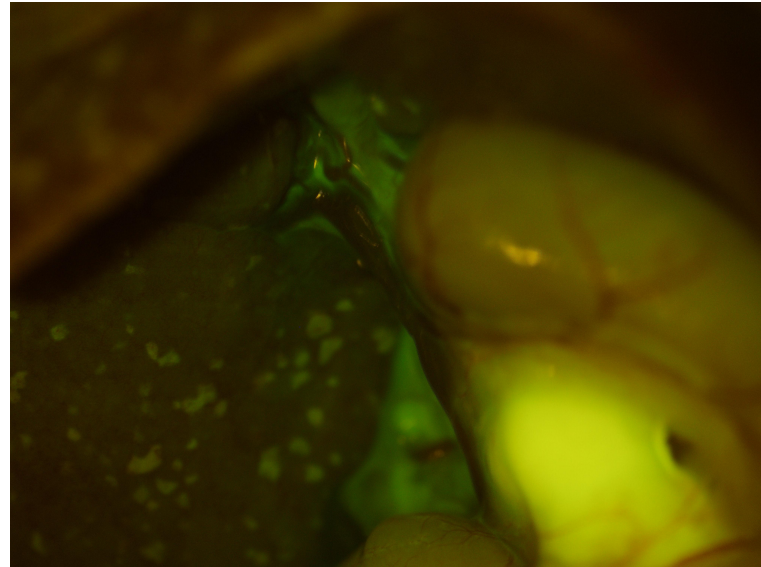
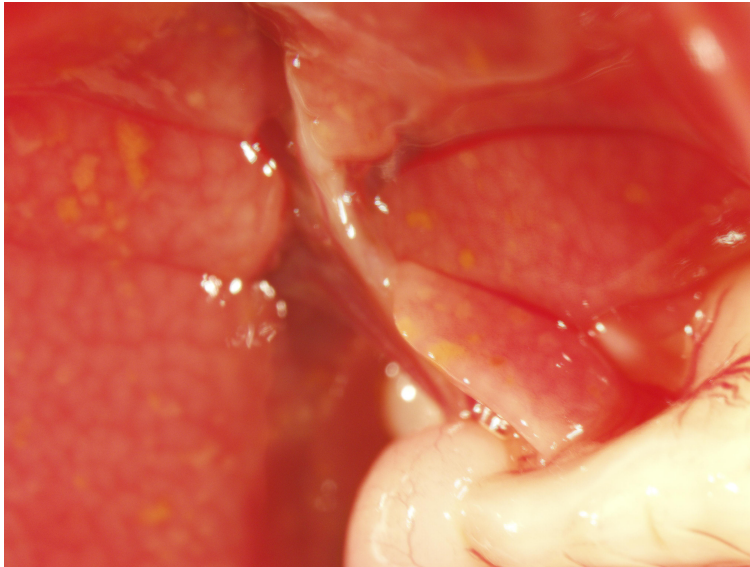
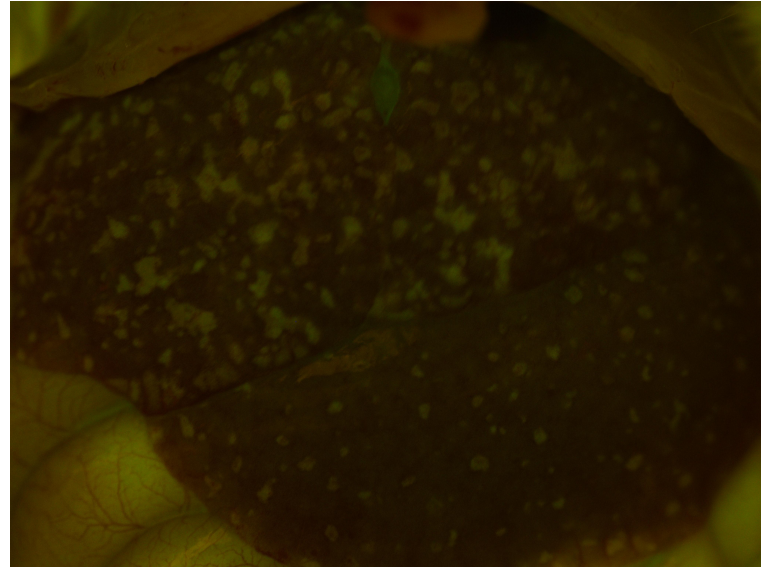
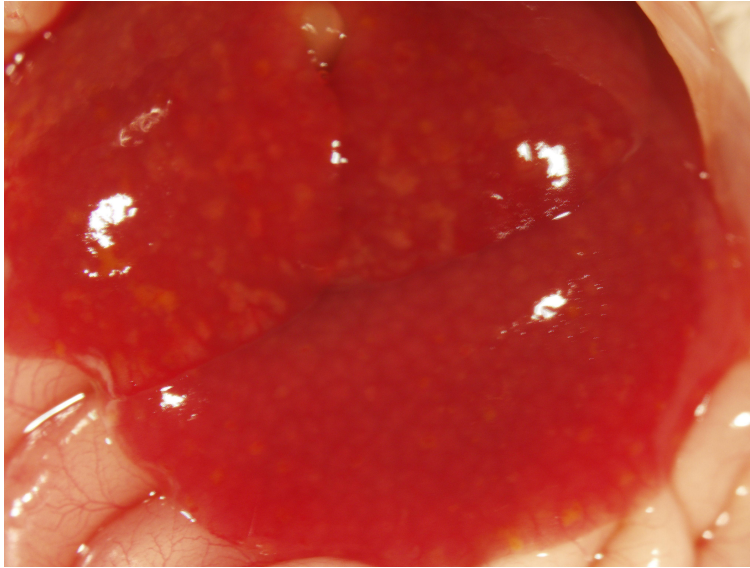
monitor: ①body weight ②jaundice



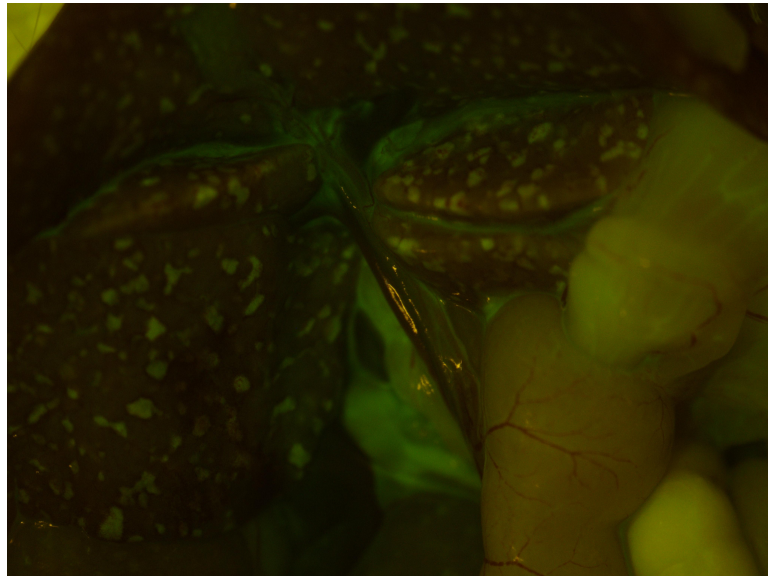
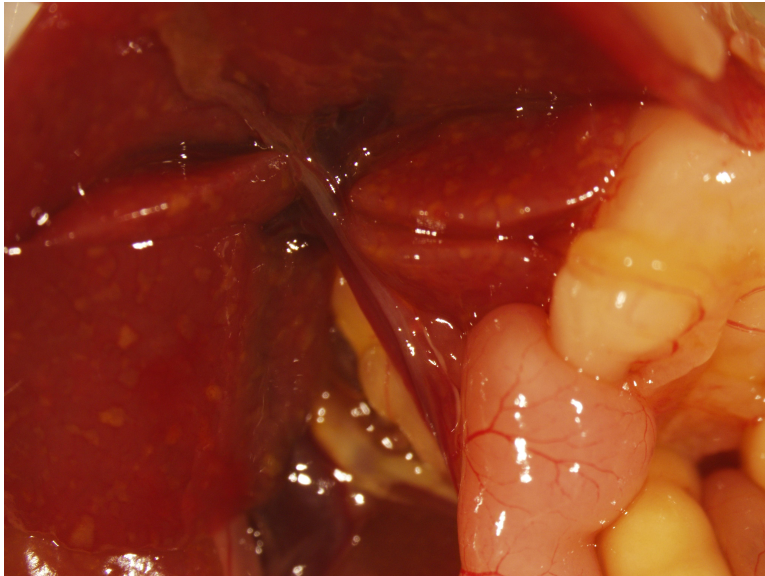
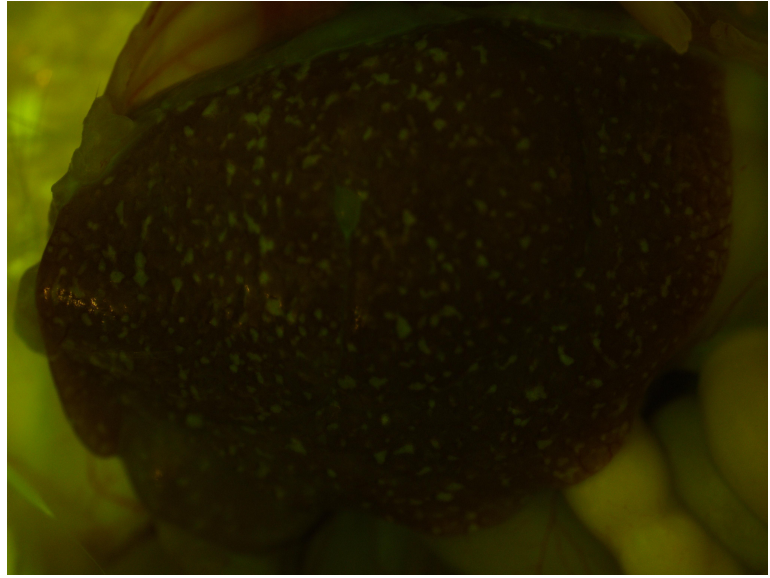
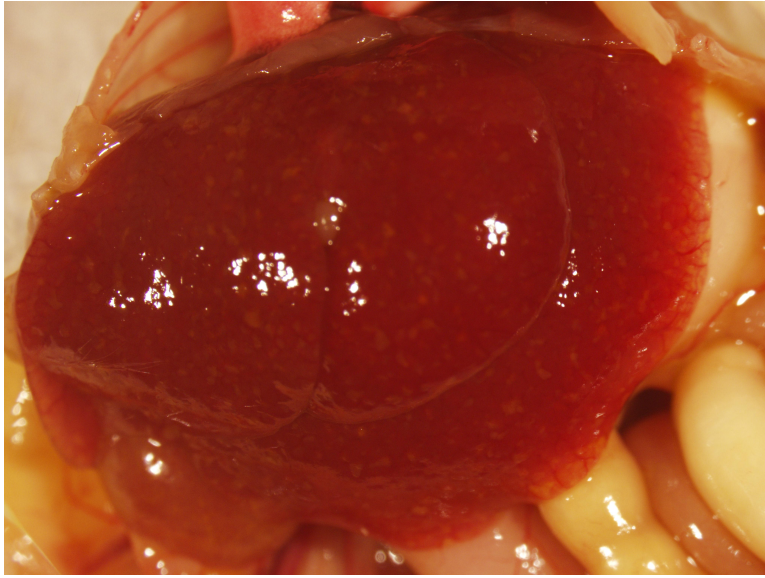
normal mice or RRV no reaction mice



RRV 7days mice



RRV 14days mice



RRV 21days mice

- in raise

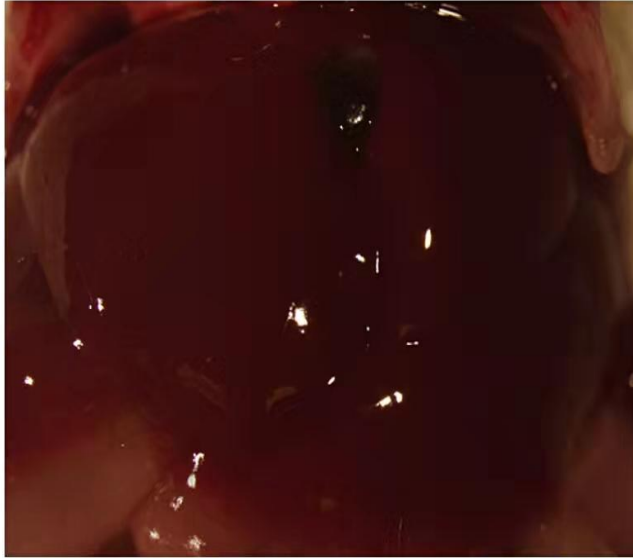
Steroid Inject Concentration

(D) Treatment and analysis of RRA-infected BA mouse with steroid

BA mice will be intraperitoneally injected three times per week with 4mg/kg body weight Prednisolone from post-RRA day 20 until the day of sacrifice (post-RRA day 34, 55, 83 and 111; 10 mice at each time points). The dosage follows the clinical practice currently used in Queen Mary Hospital for post-Kasai usage (19). Mice receiving an equal amount of ^{PBS} ethanol:H₂O (1:3) served as the un-treated group. BA mice (n=10) will also be

RRV21days+prednisilone14days

(reverse BA symptom, but not cured)



1

first cage

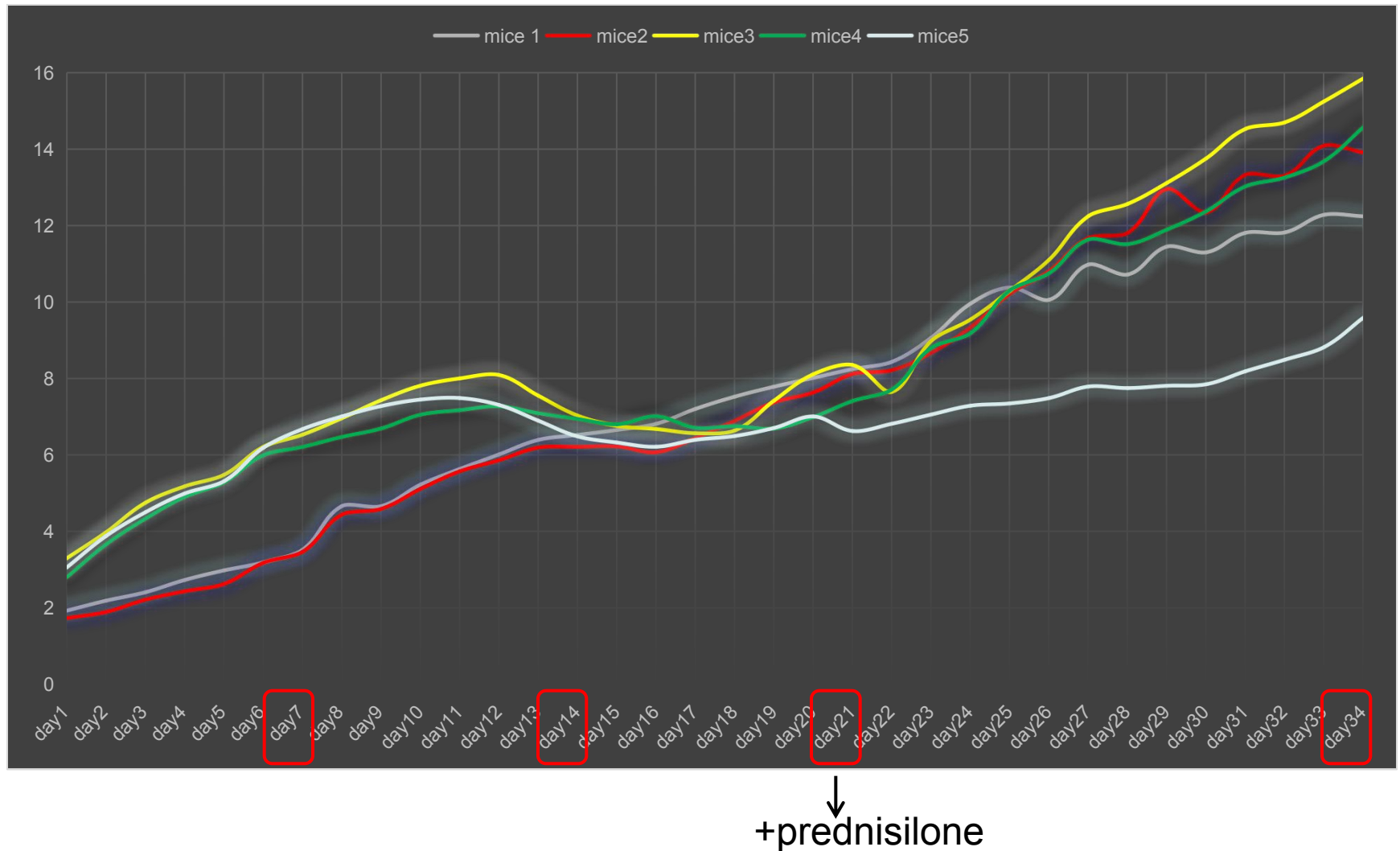
2



Second cage



RRV21days+prednisilone14days mice (body weight)

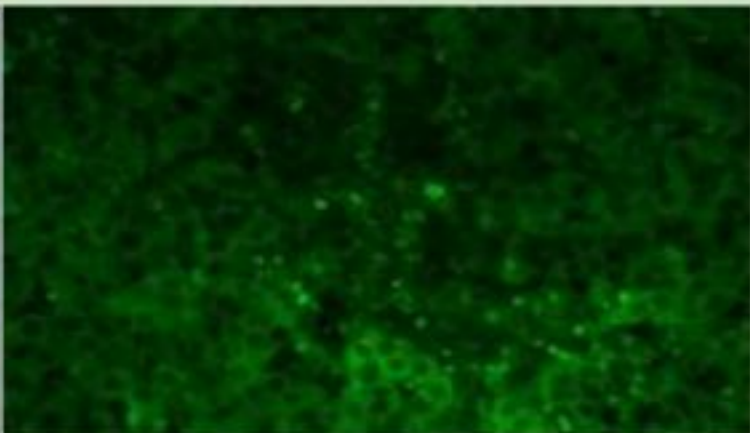
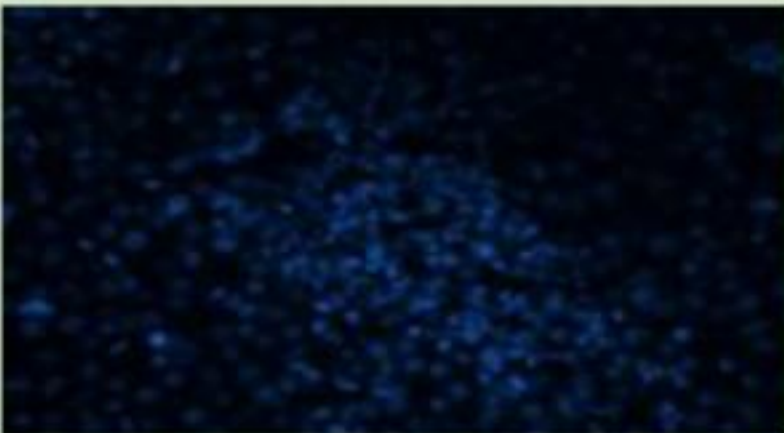


immune cells antibodies for mice paraffin section:

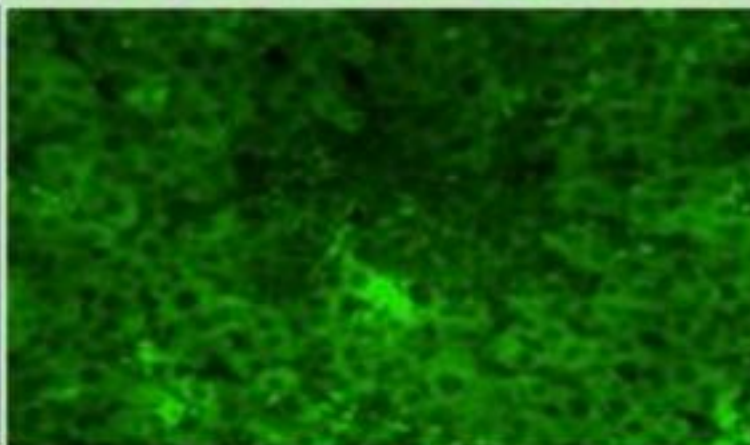
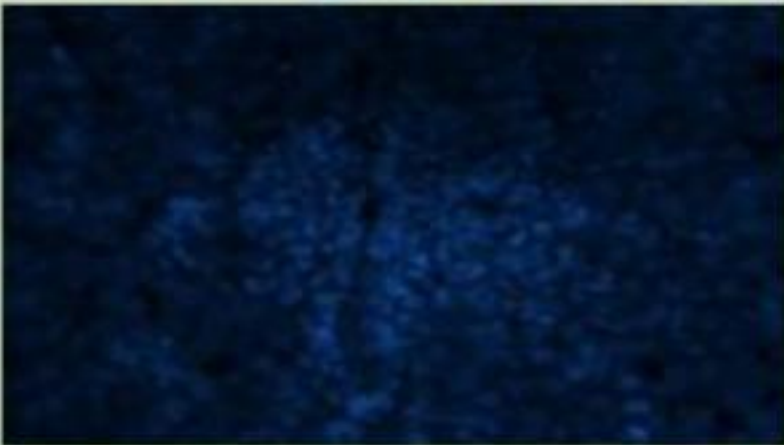
CD3+ CD4+ CD8+ CD11b NK1.1 iNOS FOXP3 Perforin

some mice results:

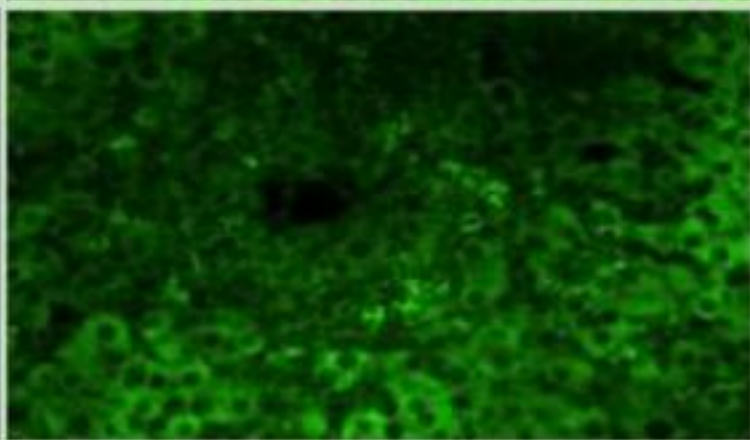
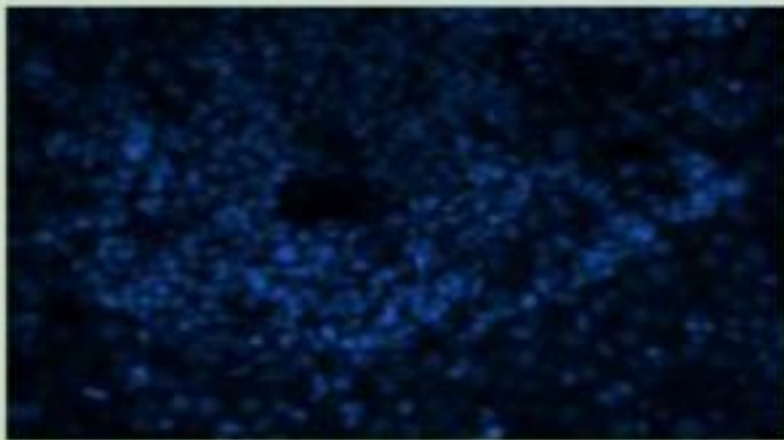
necrosis area	day7	day14
CD3+	+	-
CD4+	+	-
CD11b	+	+
NK1.1	-	-
iNOS	-	-
FOXP3	-	-
Perforin	-	-



day7 CD3

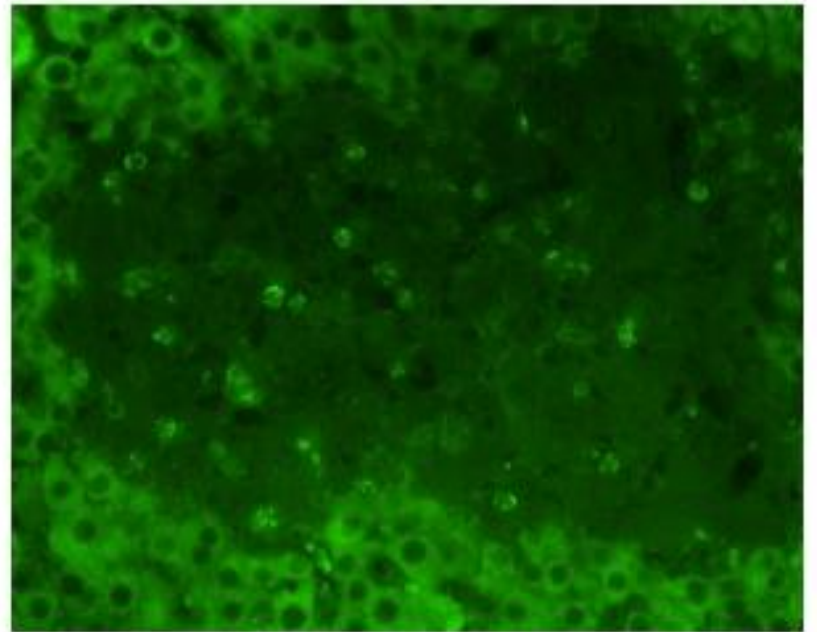
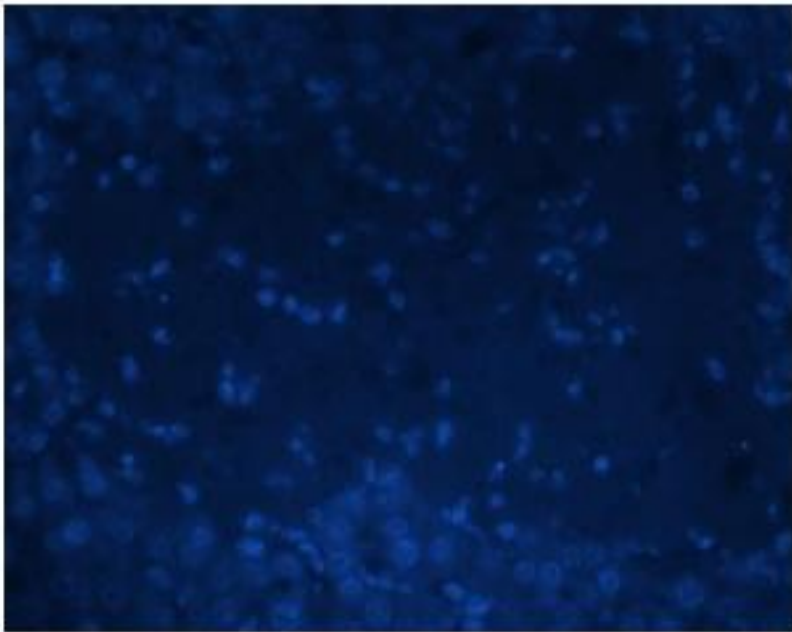


day7 CD4



day7 CD11b

day14 CD11b



Next: stain other mice section

immune cells antibodies for mice **paraffin section:**

CD3+ CD4+ CD8+ CD11b NK1.1 iNOS FOXP3 Perforin

some mice results:

in necrosis area

RRV7days: CD3 +
CD4 ++
CD11b ++

RRV14days: NK1.1 +
CD8 +
CD4 +++
CD11b +++++++

RRV34days: 6只PBSmice CD3++++++
1只PBSmice CD8+

Fluorescent staining of frozen section

RRV34days:

CD8+

CD3+

CD4+ (无)

CD11b

NK1.1

iNOS

FOXP3

Perforin

Fluorescent staining of **frozen section**

- 34days steroid : 11 只
- 34days PBS : 12 只

CD8 T+

CD3 T+

CD11 B+

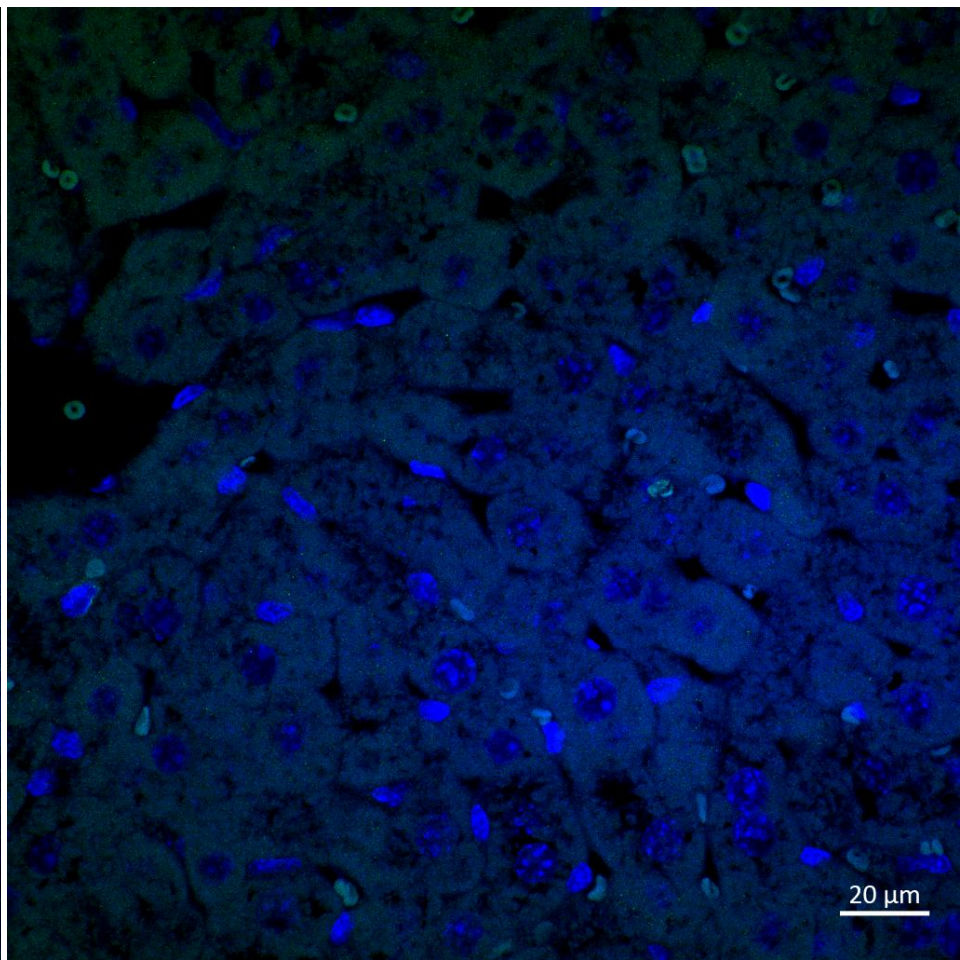
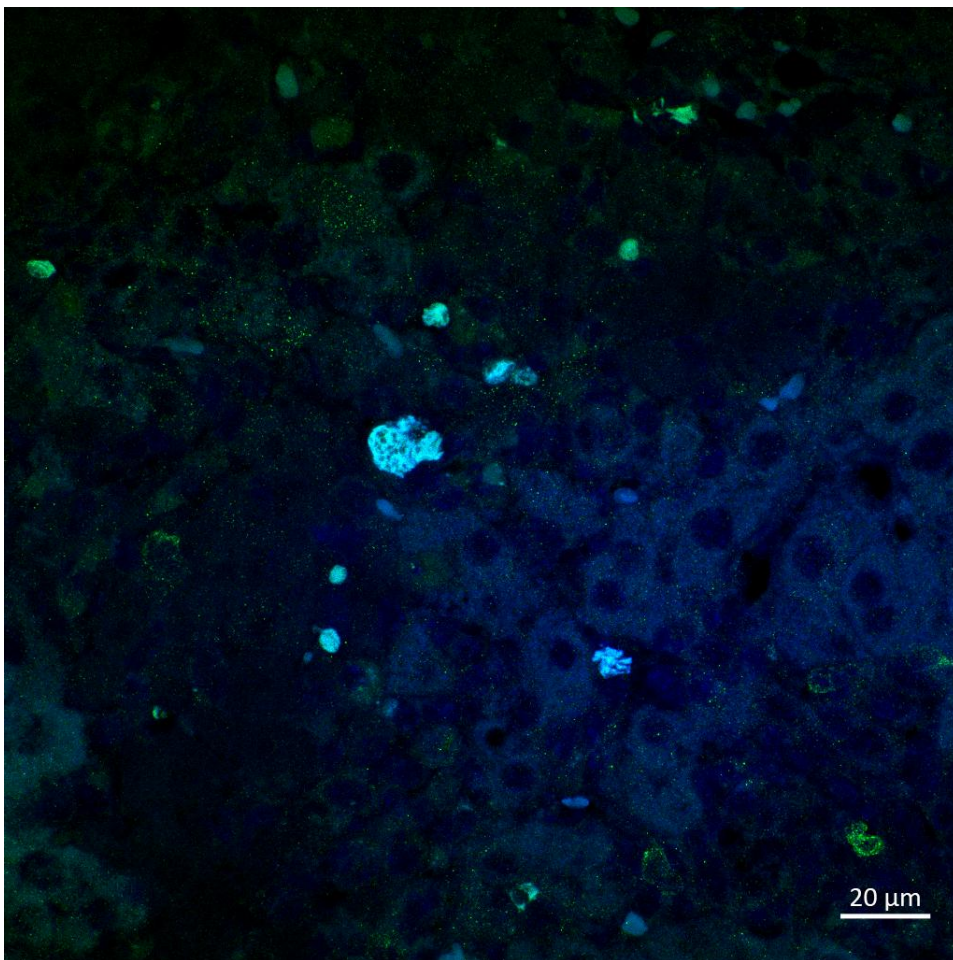
NK 1.1

PBS group have more positive than steroid group

CD3

PBS

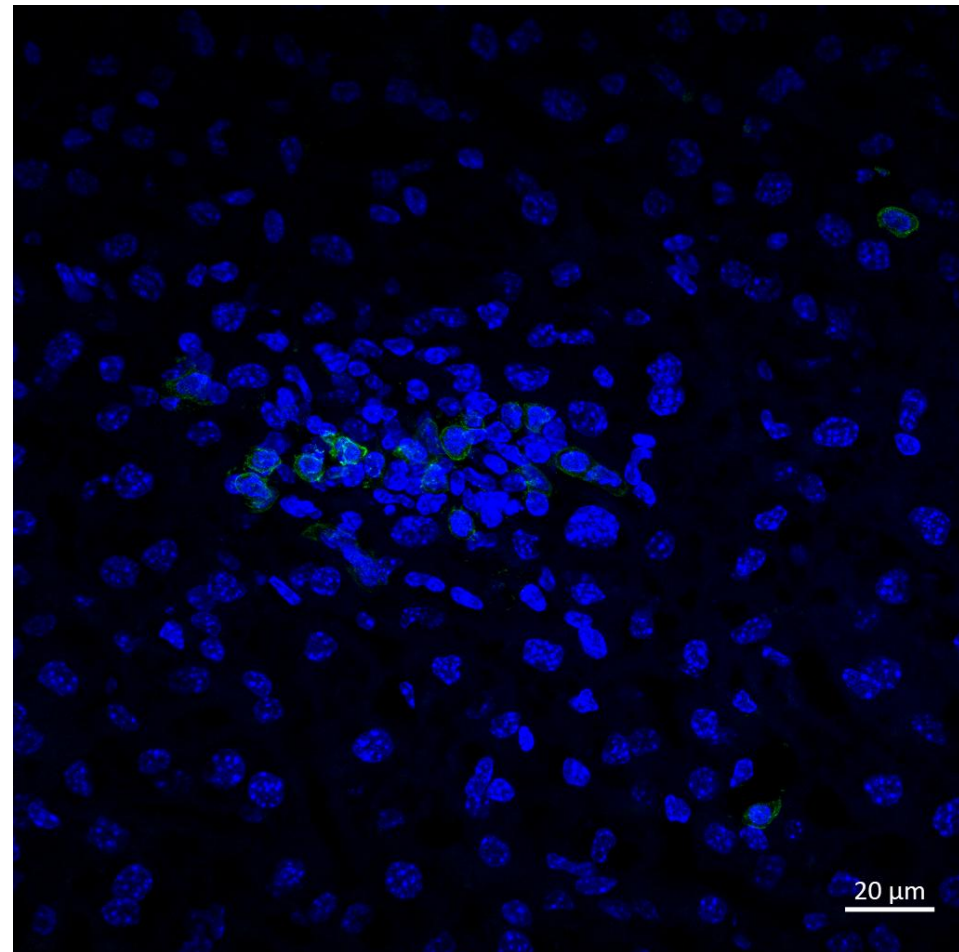
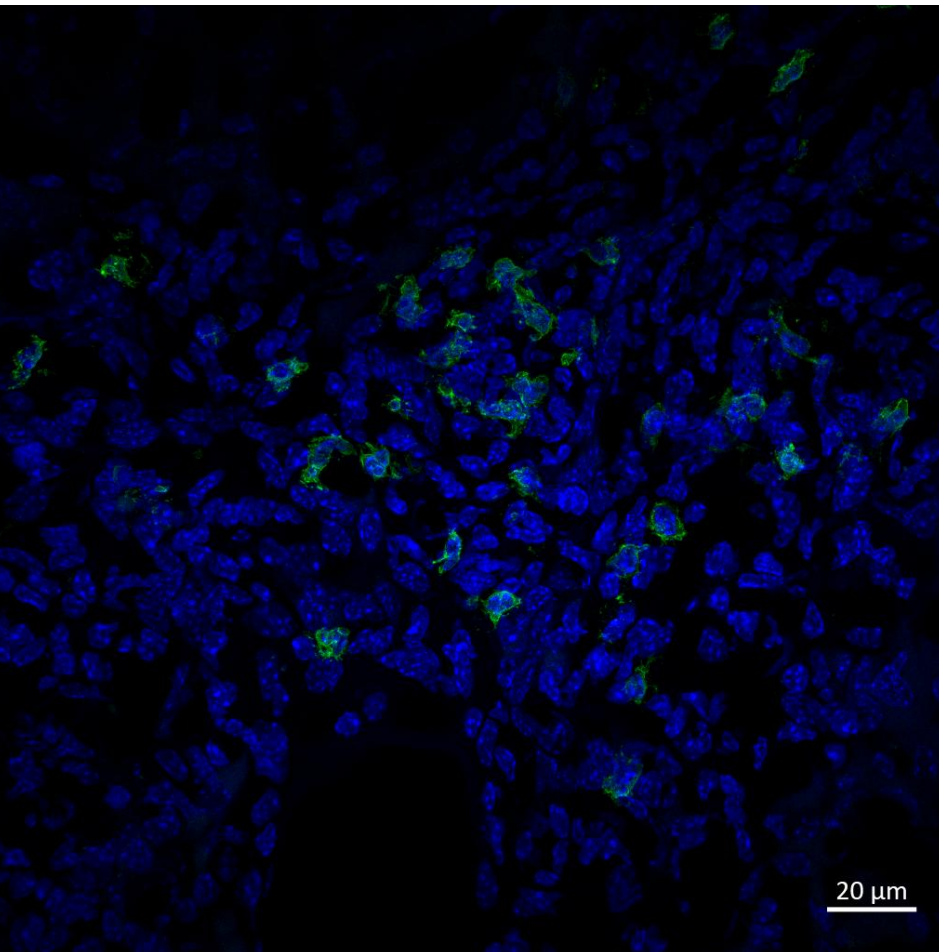
steroid



CD8

PBS

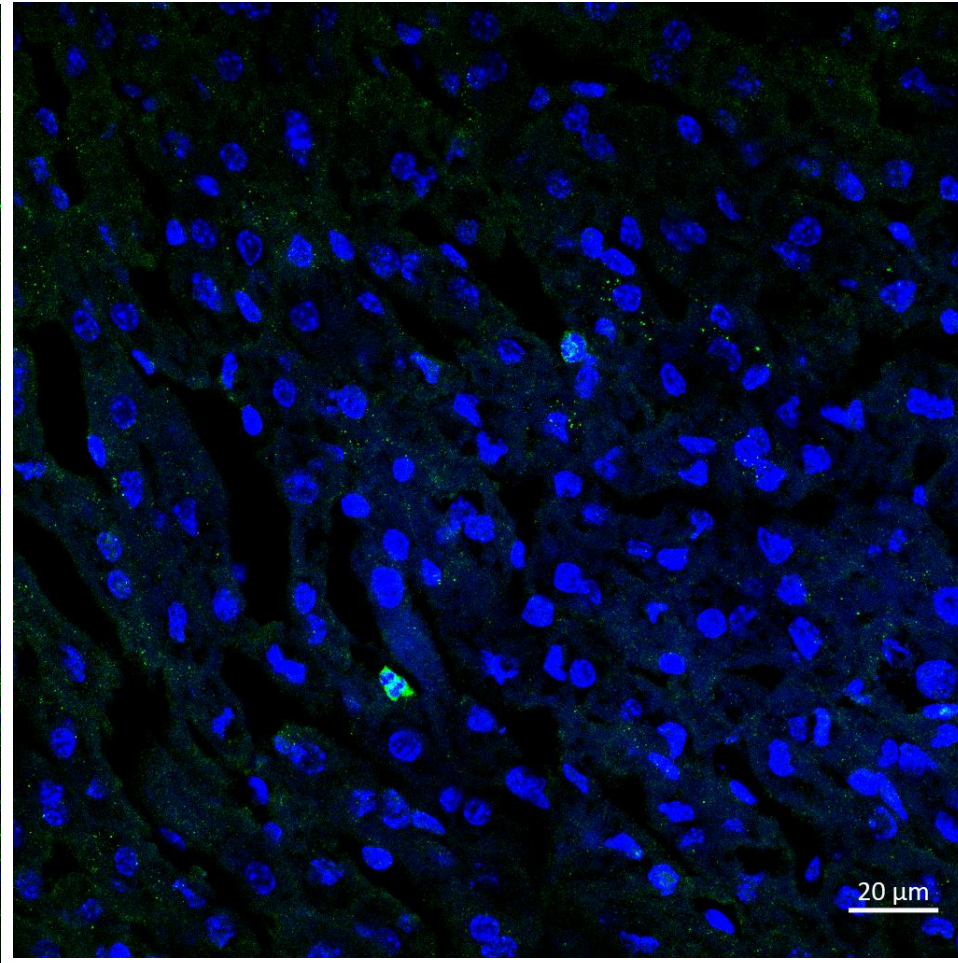
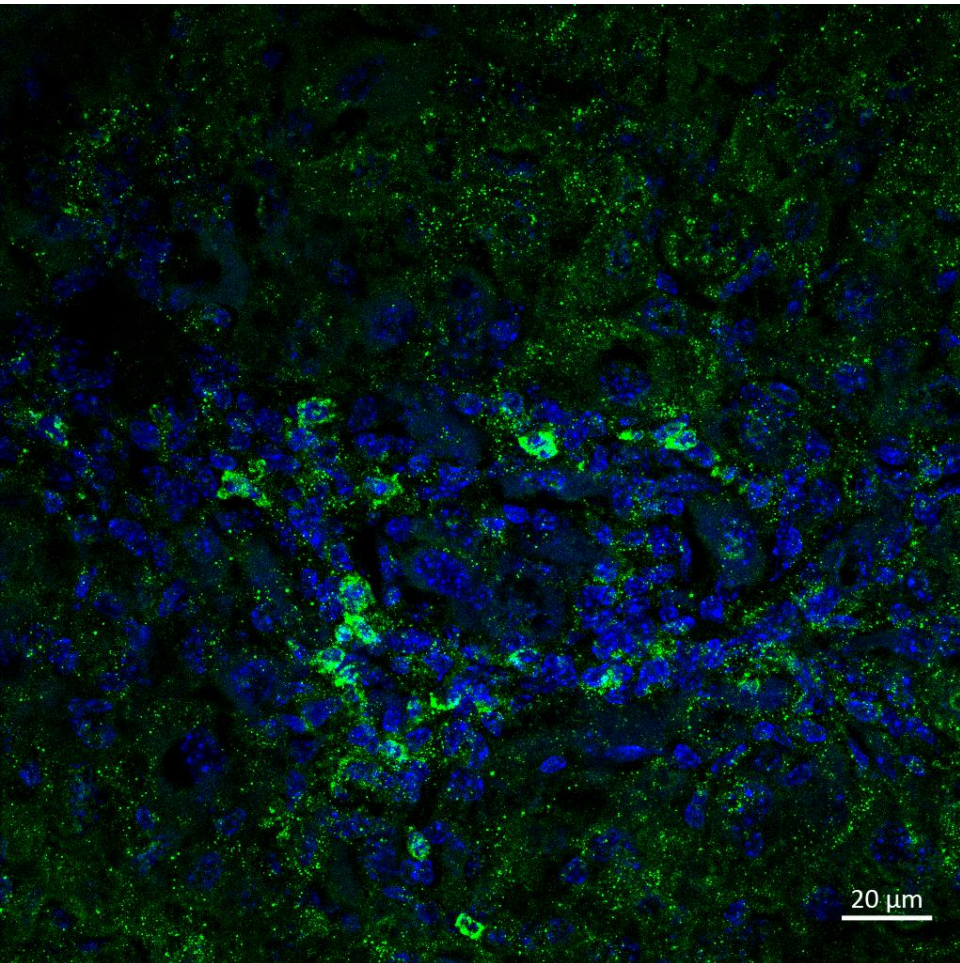
steroid



CD11b

PBS

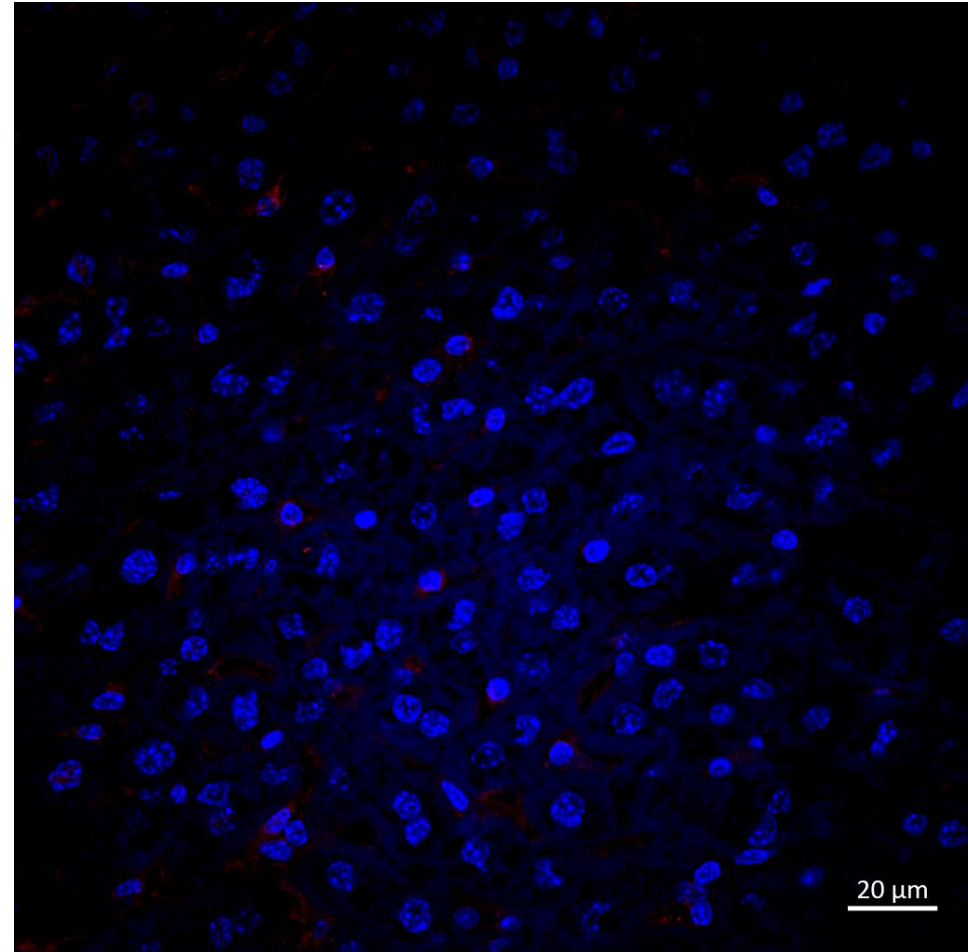
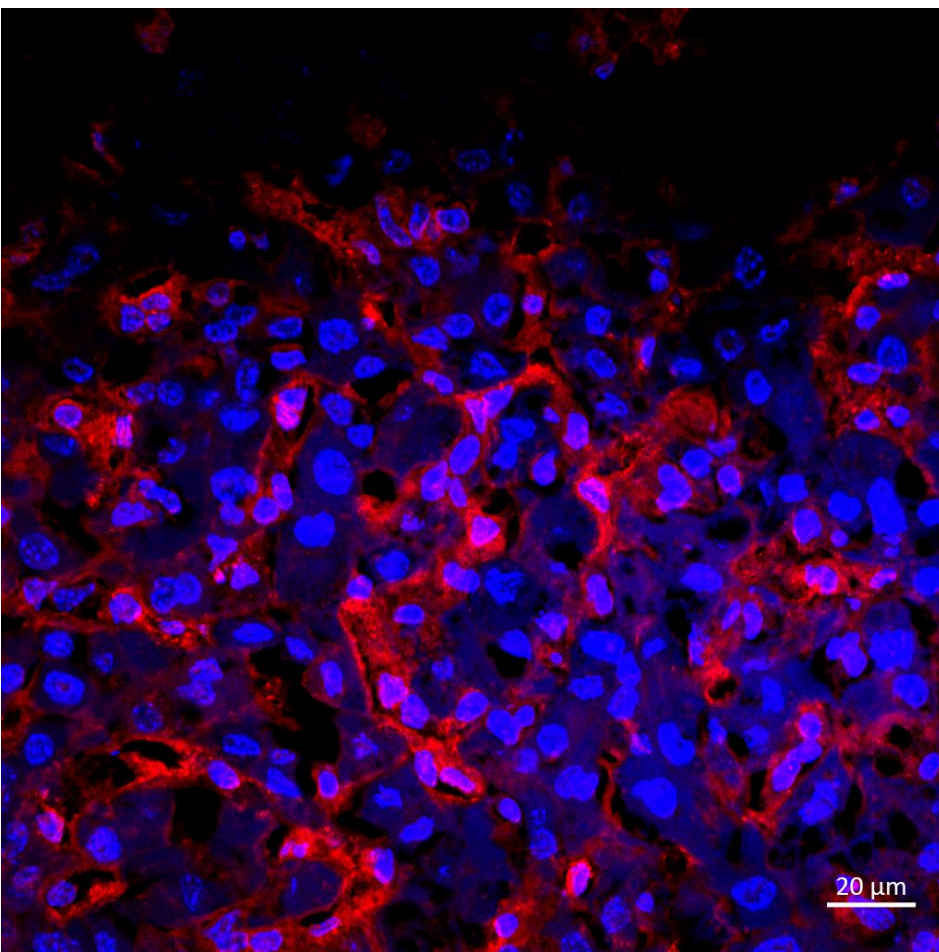
steroid



NK1.1

PBS

steroid



Birth21days+PBS14daysMice (control group 1)

- 3只(section; blood; protein; RNA)

Birth21days+steroid14daysMice (control group 2)

- 3只(section; blood; protein; RNA)

encapsulated steroid

- concentration: 0.2mg/ml
- volume: 1.7ml
- injection during time: 1 week

(RRV 21days+ encapsulated steroid 7days)

mice	post-dilution concentration(mg/kg)	volume(ml)
3只	2	2xweight(g)/0.2
3只	2	2xweight(g)/5
3只	inject PBS	inject PBS

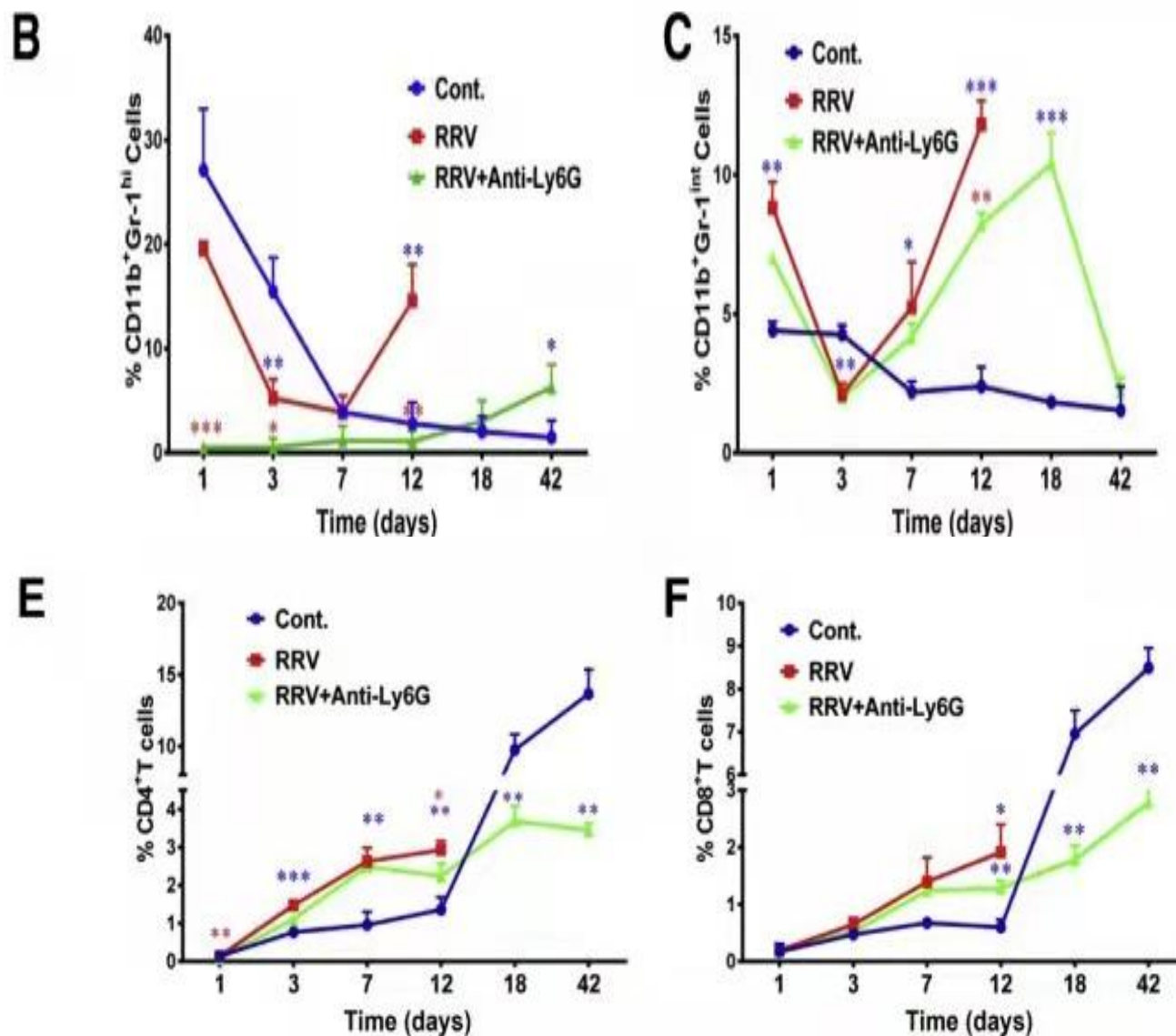


Figure 4 Flow cytometric analysis of immune cells in the spleen of the different treatment groups. The spleens were collected after the experiment, and the mononuclear cells were obtained and stained with the appropriate antibodies. **A** and **D**: Analysis of GR-1 and CD11 (**A**) and CD4 and CD8 (**D**) expression at different days after rhesus rotavirus (RRV) and RRV + anti-Gr-1 antibody treatment compared with a normal control (Cont.) group. **B**, **C**, **E**, and **F**: Graphs showing the expression of CD11b⁺Gr-1^{hi} (**B**), CD11b⁺Gr-1^{int} (**C**), CD4⁺ (**E**), and CD8⁺ (**F**) at different time points for the treatment groups. $n = 3$ to 5 spleens in each group. * $P < 0.05$, ** $P < 0.01$, and *** $P < 0.001$ versus control.

Organoid Procedure

pick organoid→RNA→cDNA

→purification→DNA sample(15ul)

→DNA concentration(2ul)

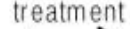
→bioanalyser(3ul)

→high sensitivity DNA sequencing(5ul)

Poly I:C co-culturing

- the majority of the non-BA organoids developed into **multi-vacuole** or **un-expanded** organoids, which resembled the aberrant morphology of **BA organoids**

A

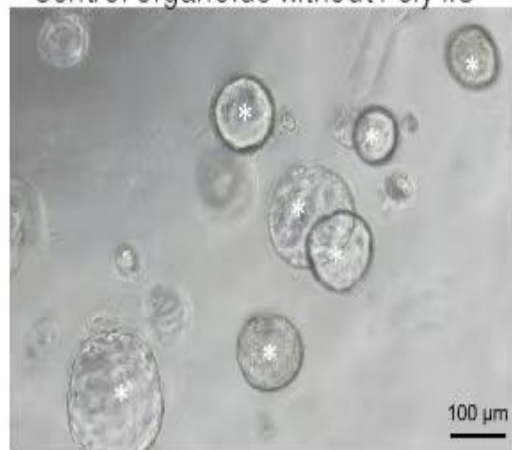
TrypLE
treatmentOrganoid cells
in Matrigel

± PolyI:C (Day 3)

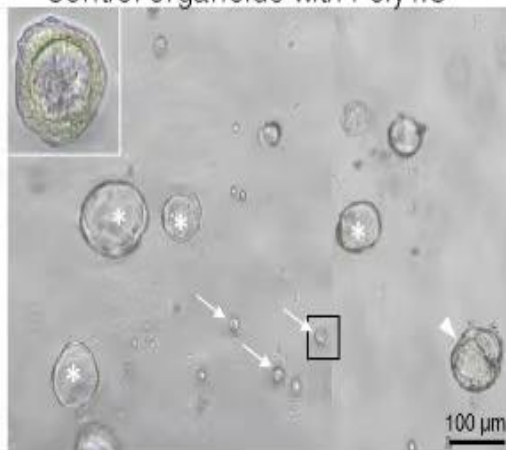
Morphology
examination & RNA-se
q analysis (Day 17)

B

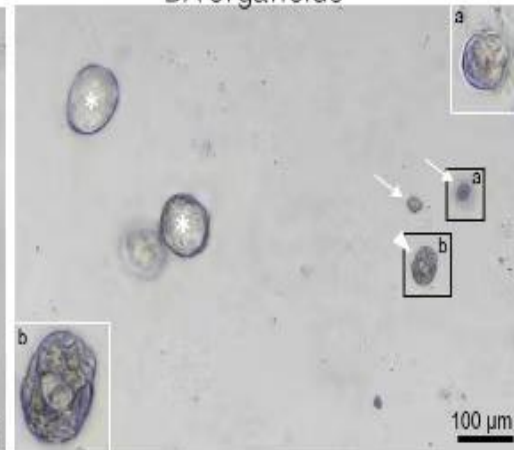
Control organoids without Poly I:C



Control organoids with Poly I:C



BA organoids



Organoid morphology

Well-expanded (% total)

Multi-vacuole (% total)

Un-expanded (% total)

No treatment

100

0

0

Poly I:C (40μg/ml)

18.6±2

15±0.7

66.4±3.5

Biliary Atresia

24.1±3

27.6±1.2

48.3±2

Steroid co-culturing

- In day 3 of organoid culture, co-culture organoid with prednisolone (1 μ g/ml)
- non-BA organoid: Poly I:C+ prednisolone
- BA organoid: prednisolone

Patient

- **BA**: organoid sequencing, single cell
- totally 8 **HB/CC** non-tumor:

in day 3



+poly ic, +prednisilone



transform non-BA organoids to BA organoids

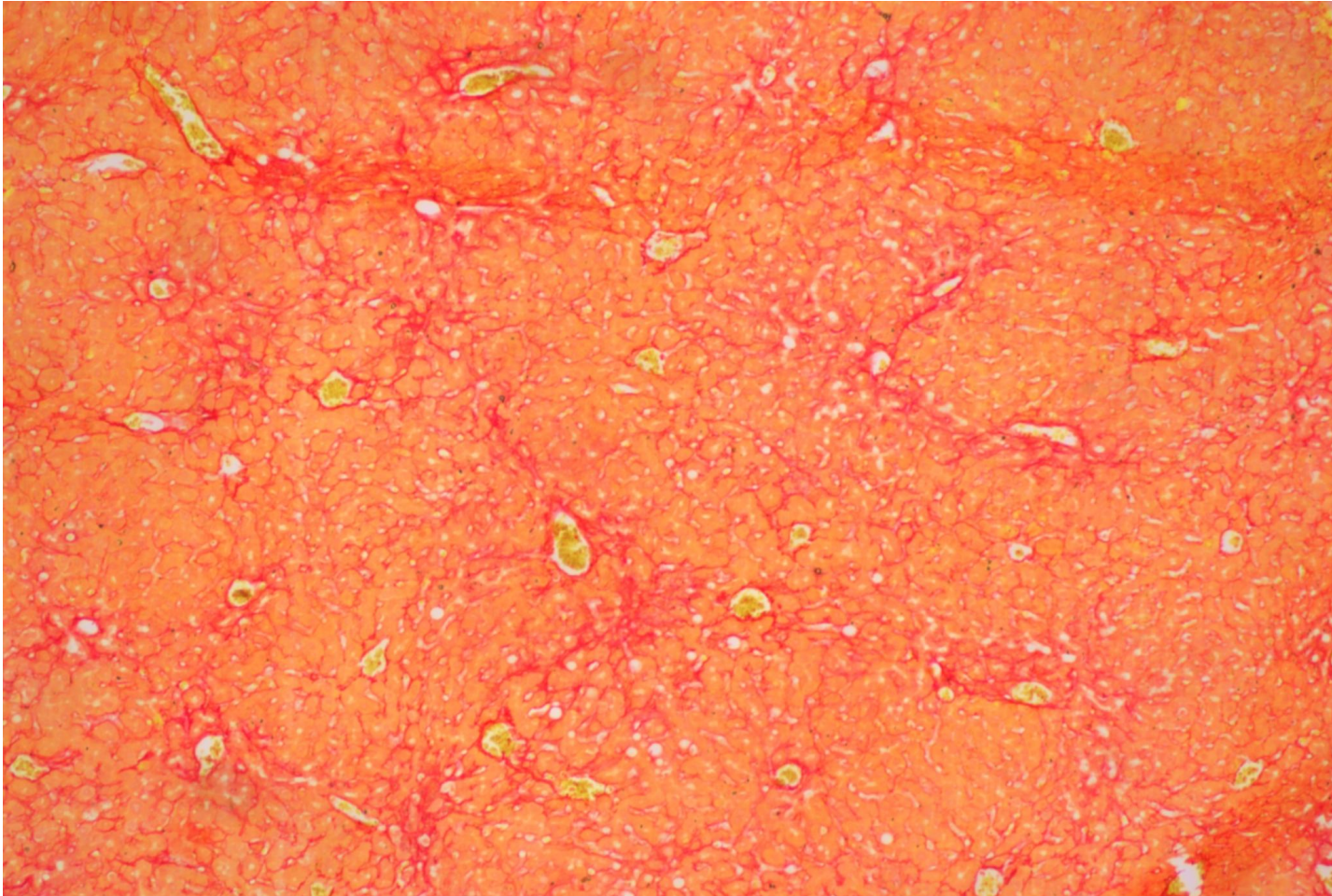


sequencing

Mice

- 34days steroid : 8只
- 34days PBS : 9只
- Fibrosis+ : 9只 PBS, 1只 steroid
- Fibrosis- : 7只 steroid
- new 34days mice : 6只 (3只 PBS, 3只 steroid)
(section; blood; protein; RNA; organoid; single cell)

Fibrosis Positive



Fibrosis Negative

