Methodology Report

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1.Cell Base Experiments

- 1. Cell culture
- 2. MTT assay
- 3. Elisa
- 4. Western blot
- 5. Isolation and purification of primary smooth muscle skin cells and lymphocytes in spleen
- 6. Immunofluorescence
- 7. Transient transfection
- 8. Lentivirus transduction for target gene over expression
- 9. qPCR for detecting the expression of target genes
- 10 Flow cytometer for apoptosis analysis

MTT Assay

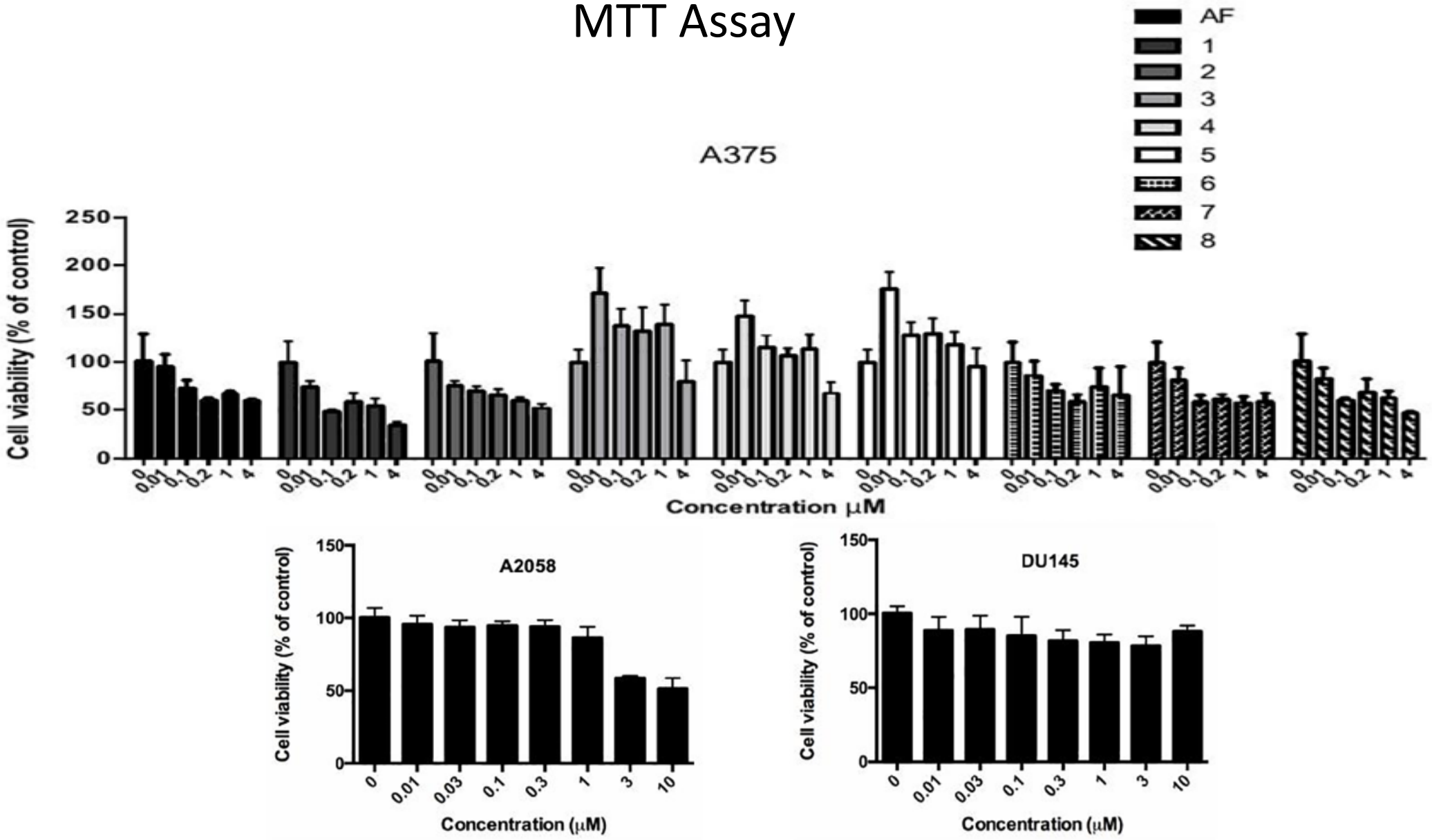
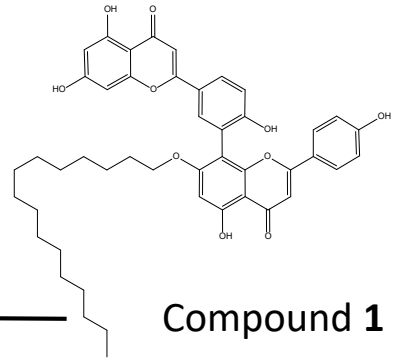
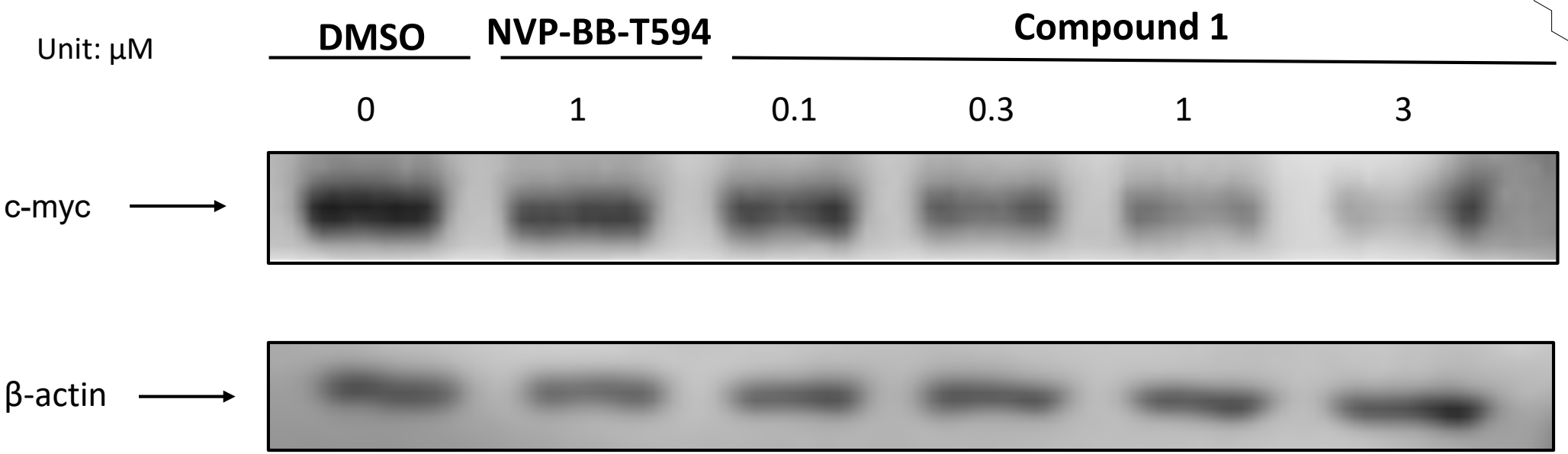


Fig 1. A375 cells were treated with 0.01-04um of compounds or amentoflavone for 48h. A2058 and DU145 cells were treated with the same concentration of compound 1 for 48h.[1]

Western Blot



Compound **1**



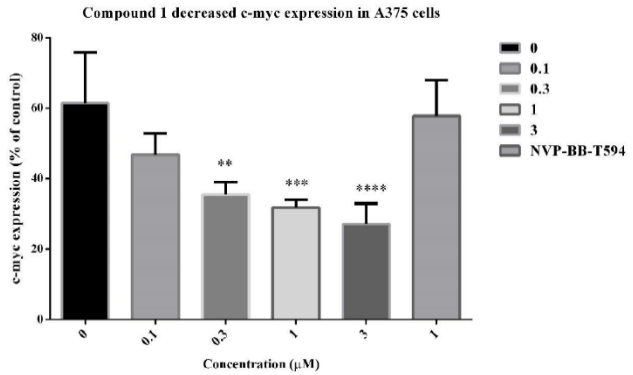
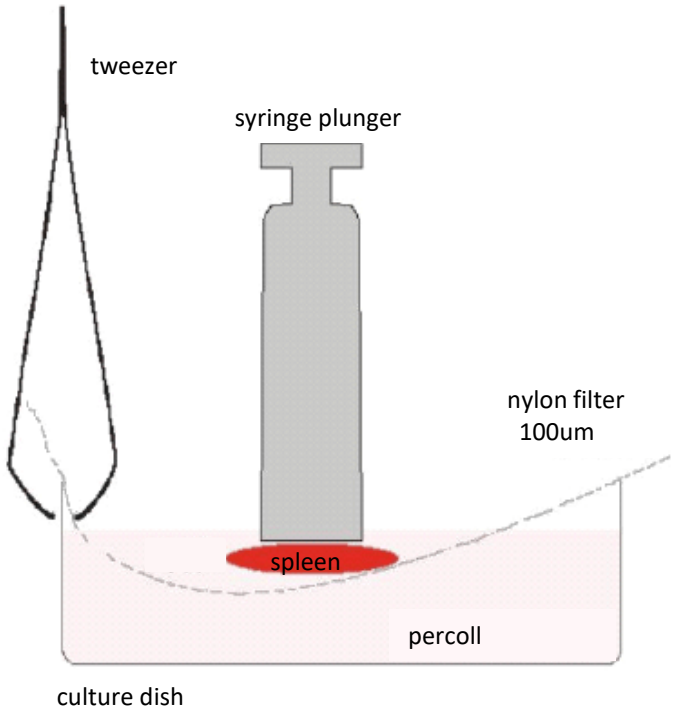
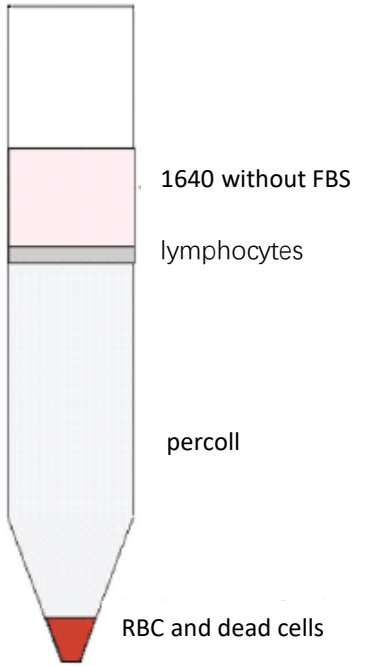


Fig 2. Compound 1 decreased c-myc expression in a dose dependent manner.

Isolation of Lymphocytes in Spleen





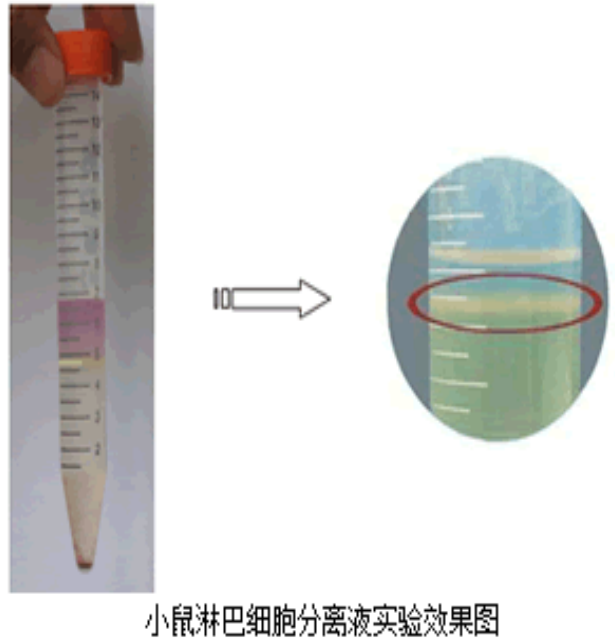


Fig 3. Diagrammatic of lymphocytes isolation.

Apoptosis Analysis

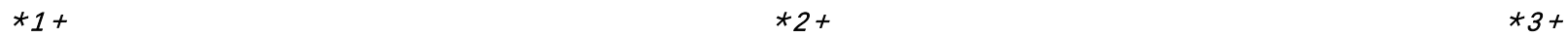
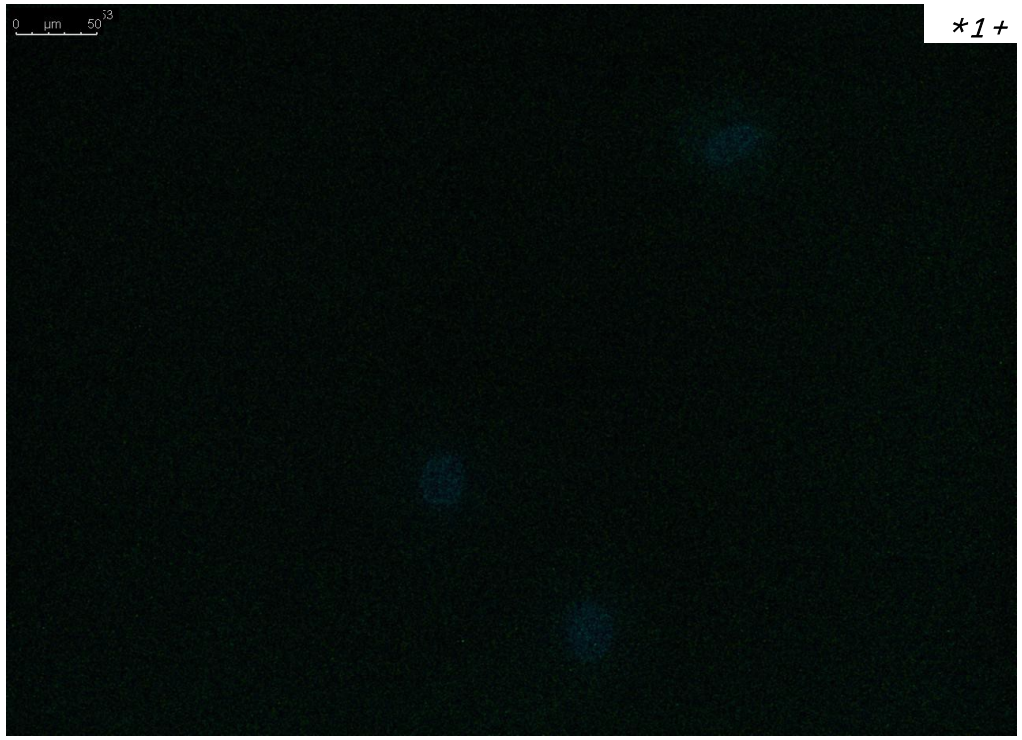


Fig 4. Tumor cells were treated with anti-cancer drug or cytokines for 6h. (1) control group; （2）anti-cancer drug treated group; (3)cytokines treated group

Immunofluorescence



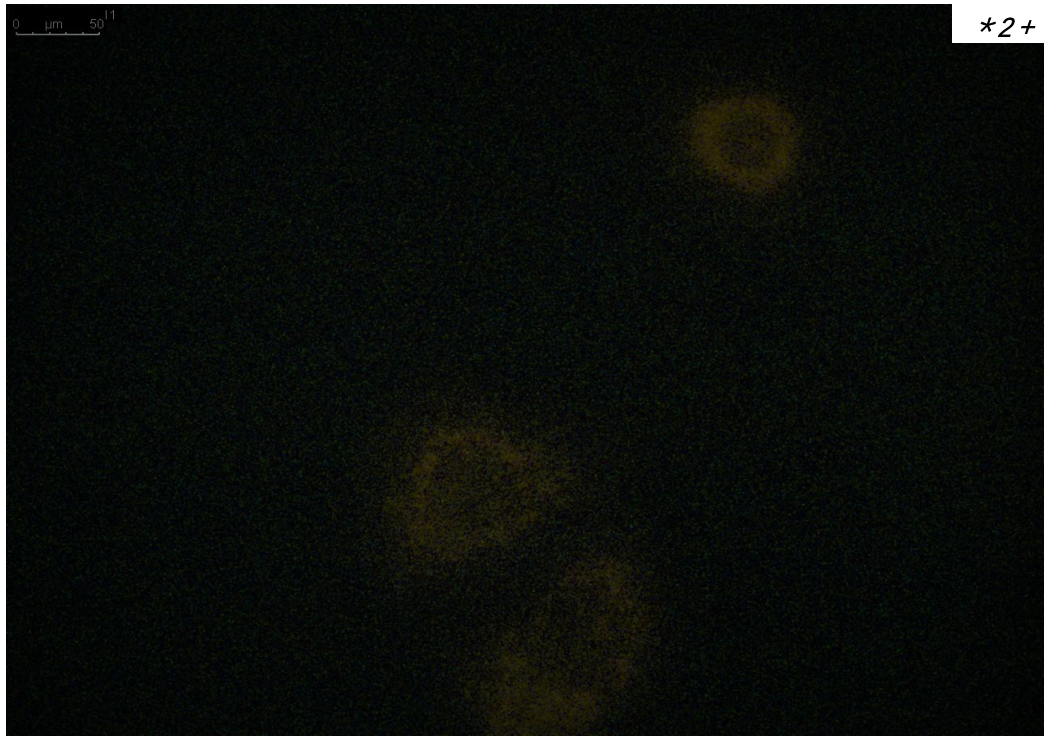
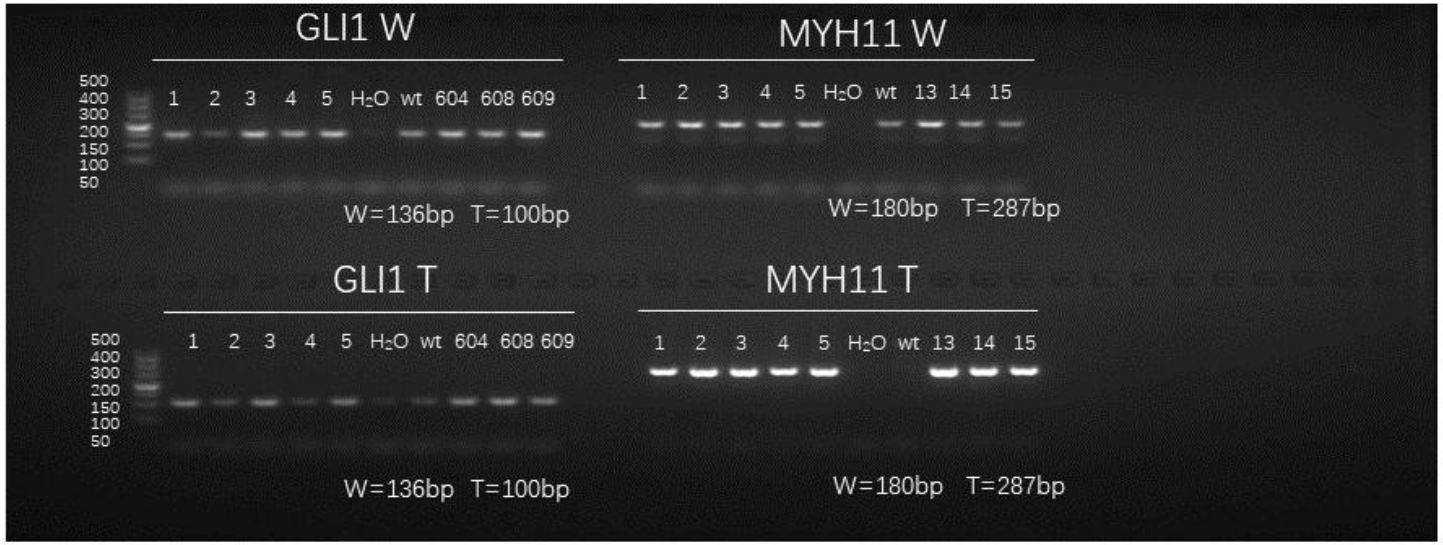


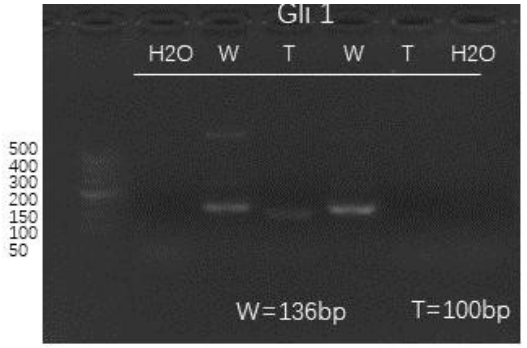
Fig 5. Through time difference of cells attachment, the purity of primary smooth muscle skin cells were verified by the expression of α-SMA.(1) stain of nuclei; (2) stain of α-SMA

2.Animial experiment

- 1. PCR for genotyping
- 2. Animal model of human disease: asthma, acute lung injury
- 3. Preparing for the paraffin section and cryo-section
- 4. HE stain
- 5. Immunofluorescence
- 6. In vivo t Imaging on the IVIS platform
- 7. Bronchoalveolar Lavage
- 8. Lung function test using the Buxco® FinePointe RC
- 9. Preparation of single cells suspension of tissue
- 10. Flow cytometer

PCR Genotyping





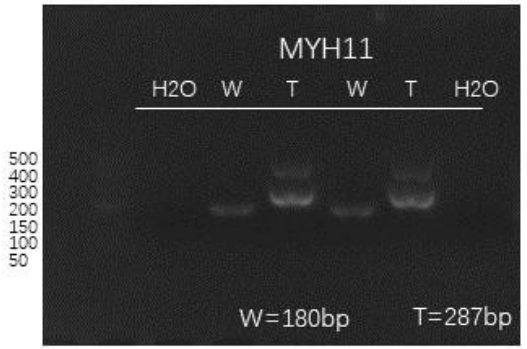
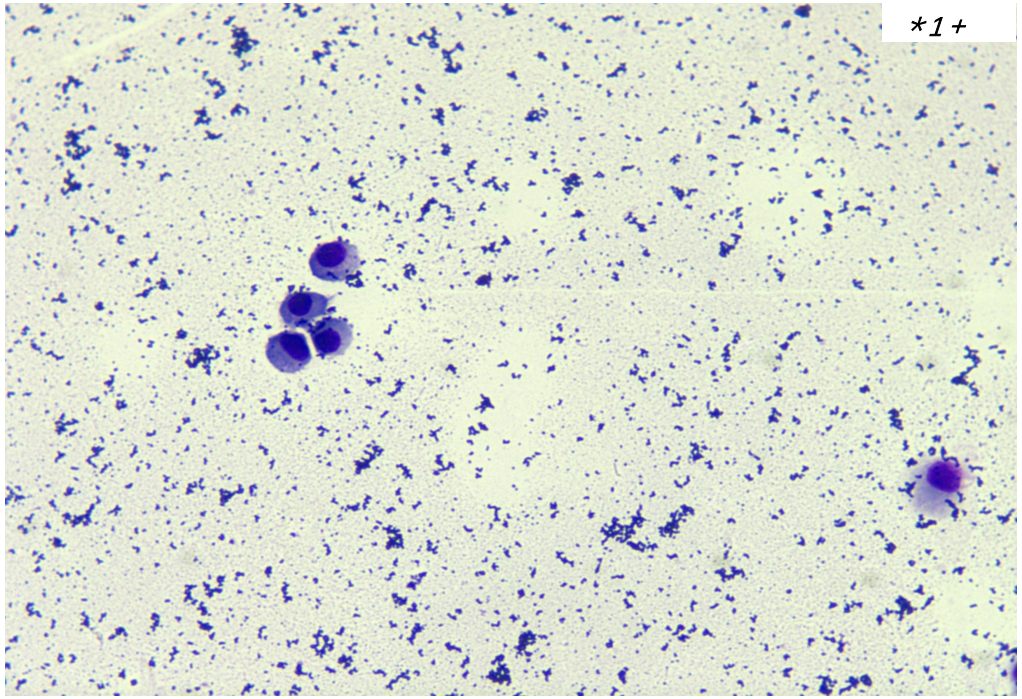


Fig 6. No.1,3,5 moue were both Gli1 positive and MYH11 positive.

EYFP	T	384 bp
	W	142 bp
Gli 1	T	100 bp
	W	136 bp
MYH11	T	287 bp
	W	180 bp

Jamsa Stain



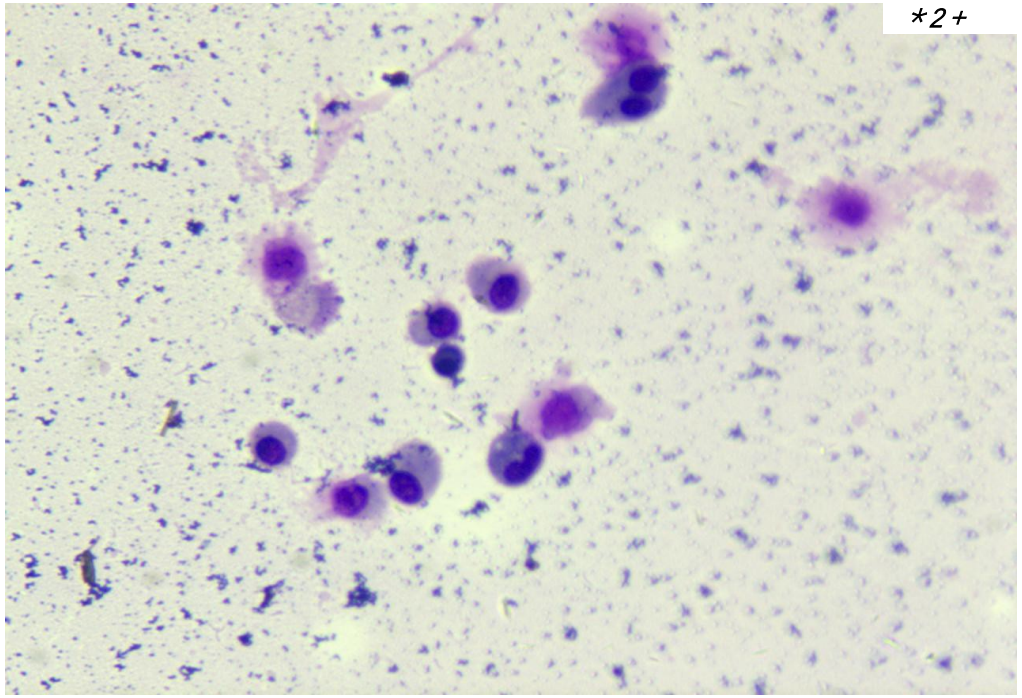
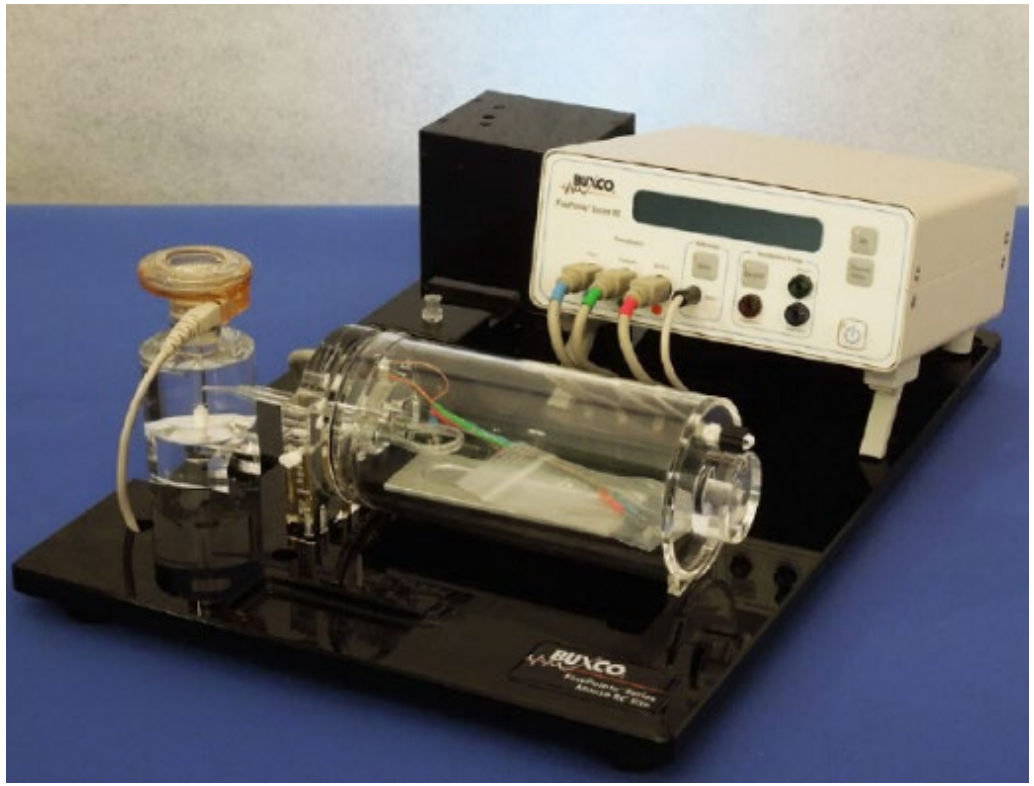


Fig 7. BALF analysis by Jamsa stain. (1) control group; (2) asthma group. increase in asthma model

Lung Function Test



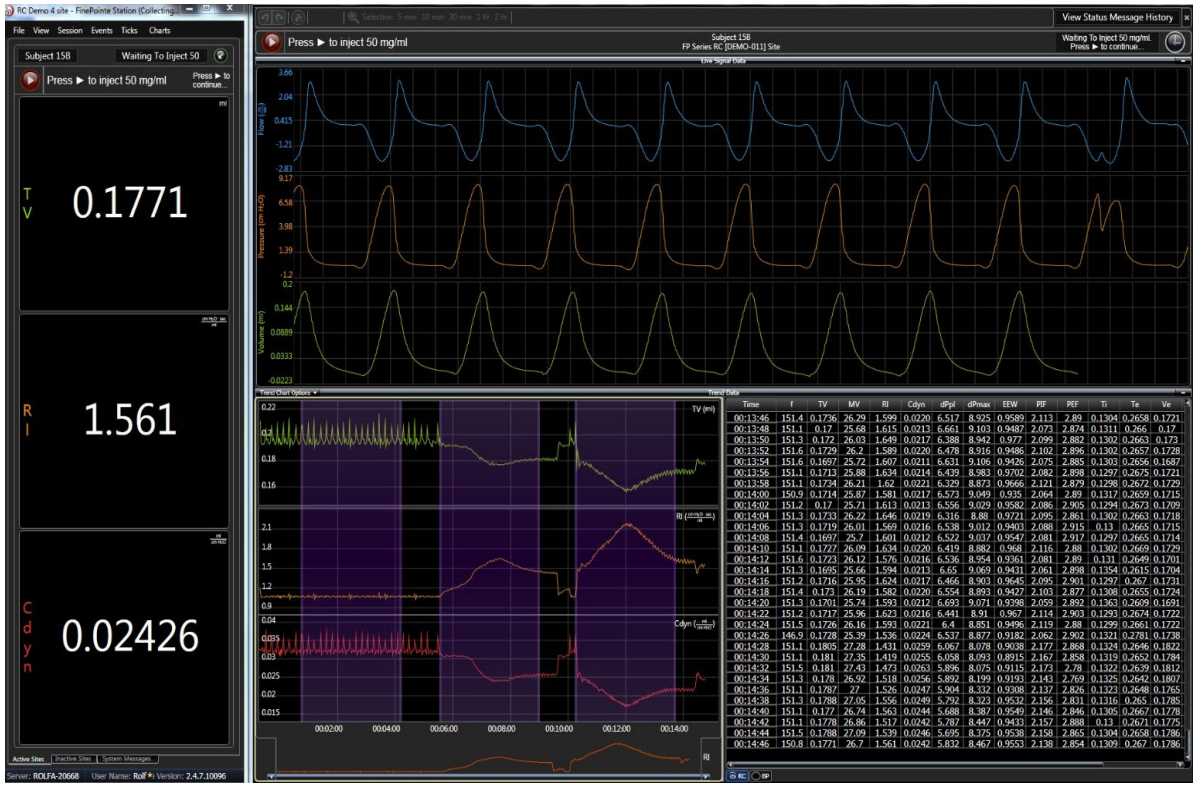


Fig 8. Lung function test using Buxco® FinePointe RC. lung function can be evaluated by measuring lung pressure and airway airflow[2].

In vivo Imaging of Tumors Using IVIS Spectrum

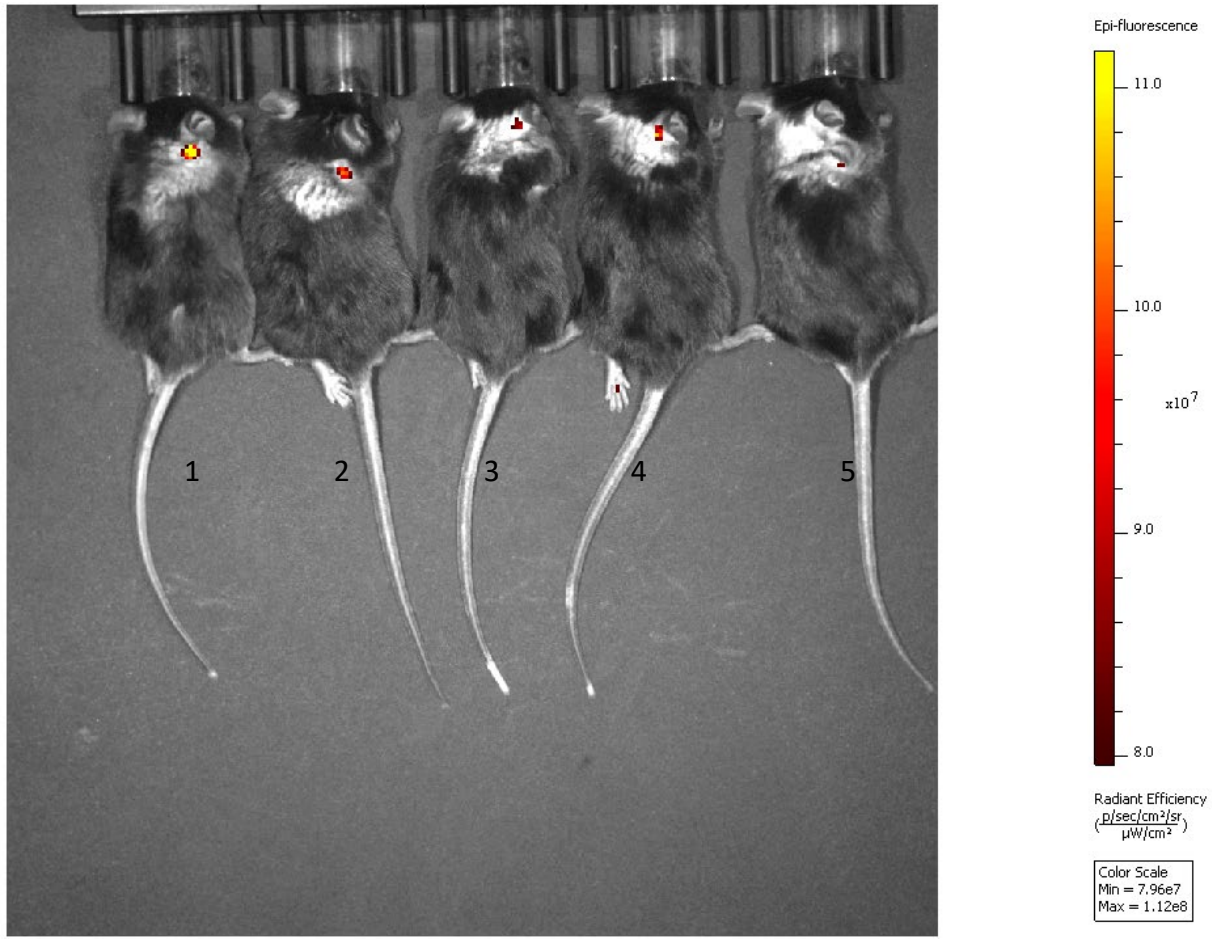
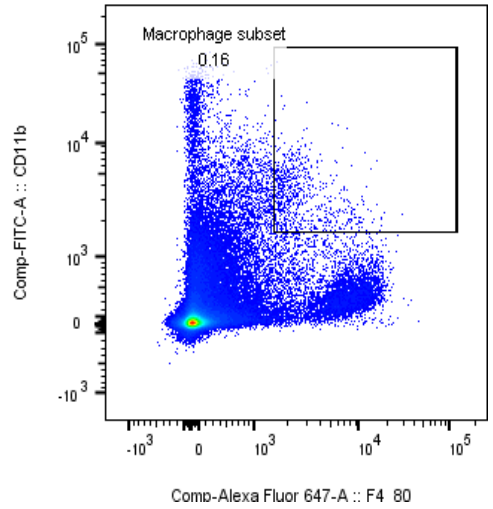
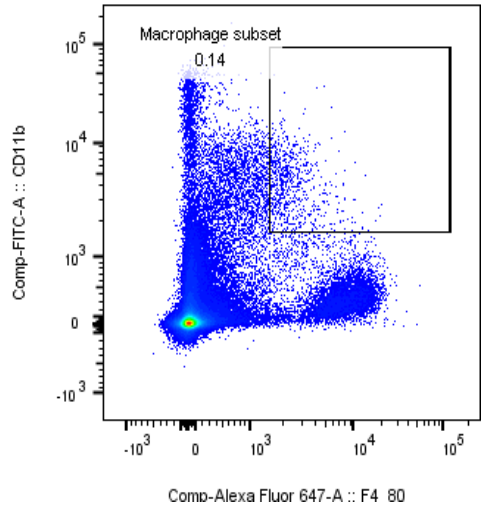


Fig 9. Evaluating the tumor growth. No.1-2 were control group; No.3-5 were drug treatment group.

Proliferation Analysis





*1 *2 *3 *4

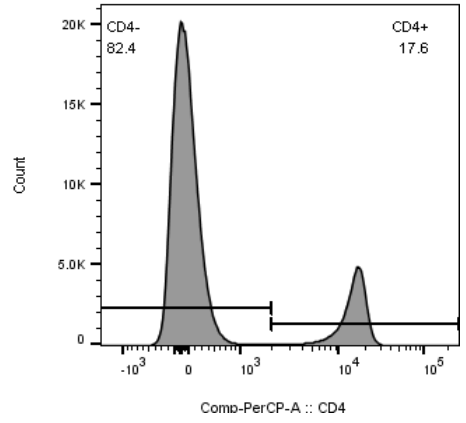
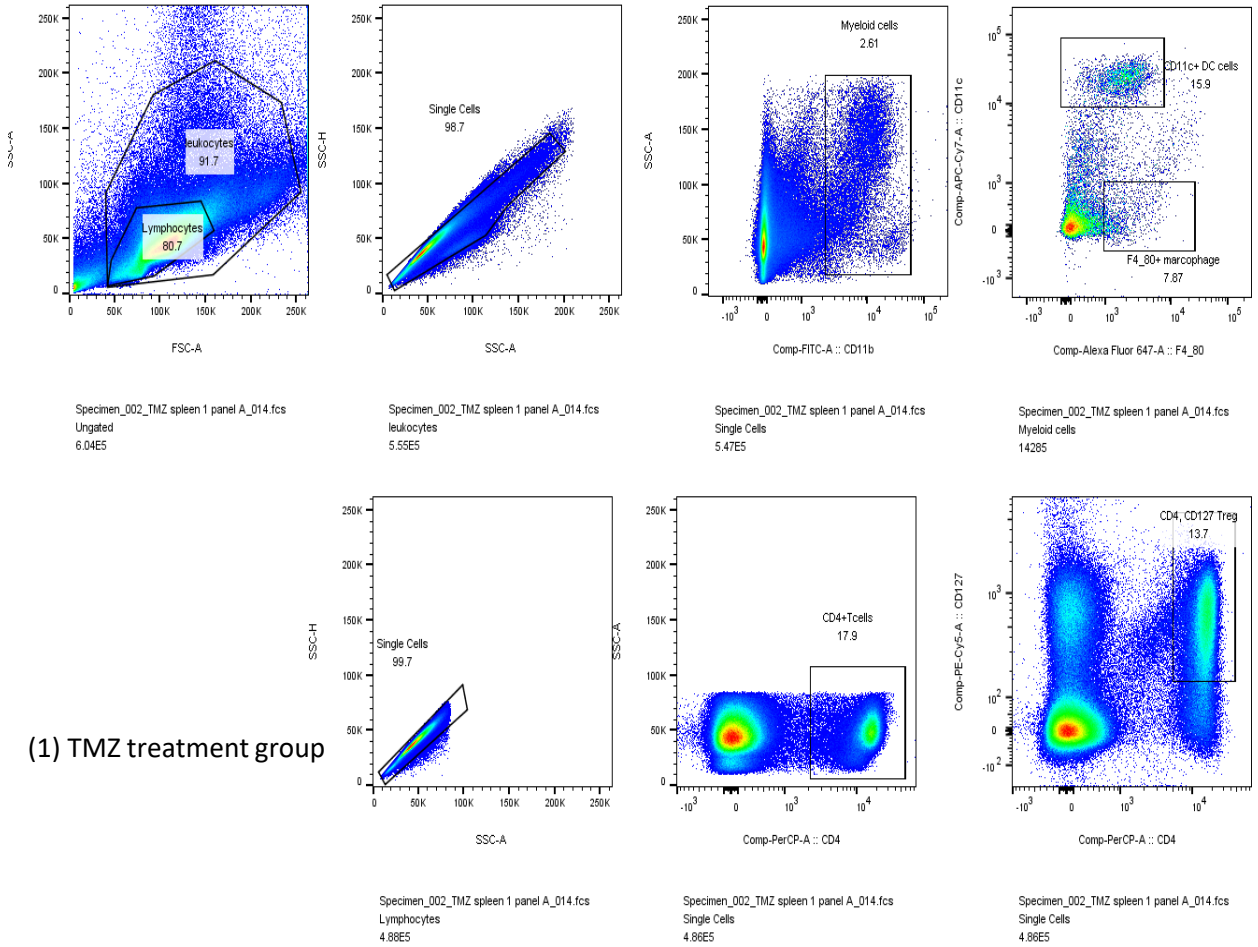
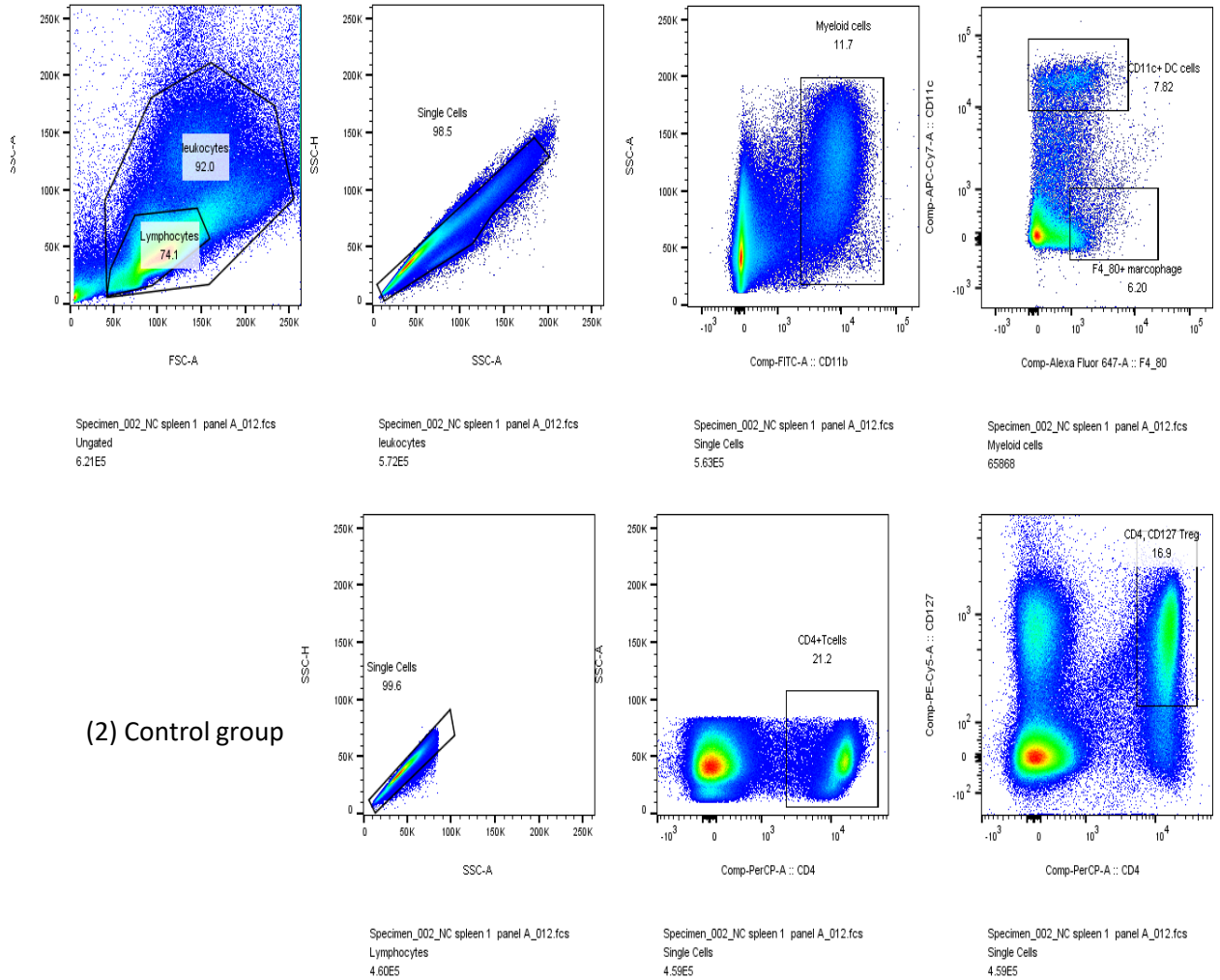


Fig 10. Compared with control group (No. 1,2), The medication group (No. 3,4) had a lower amount of macrophage in spleen while CD4+ cells showed no difference.

FACS Analysis



(1) TMZ treatment group



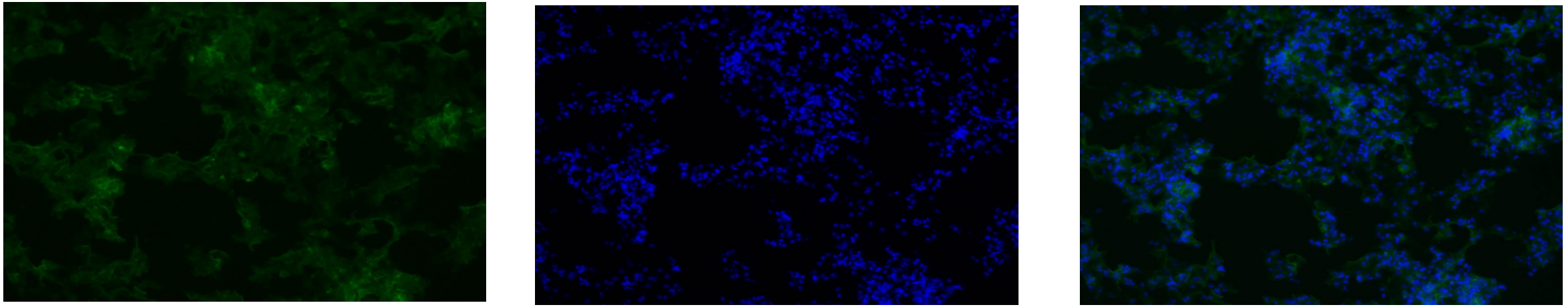
(2) Control group

Fig 5. Spleen were harvested from tumor bearing mouse. (1) control group; （2） anti-cancer drug treated group; (3)cytokines treated group

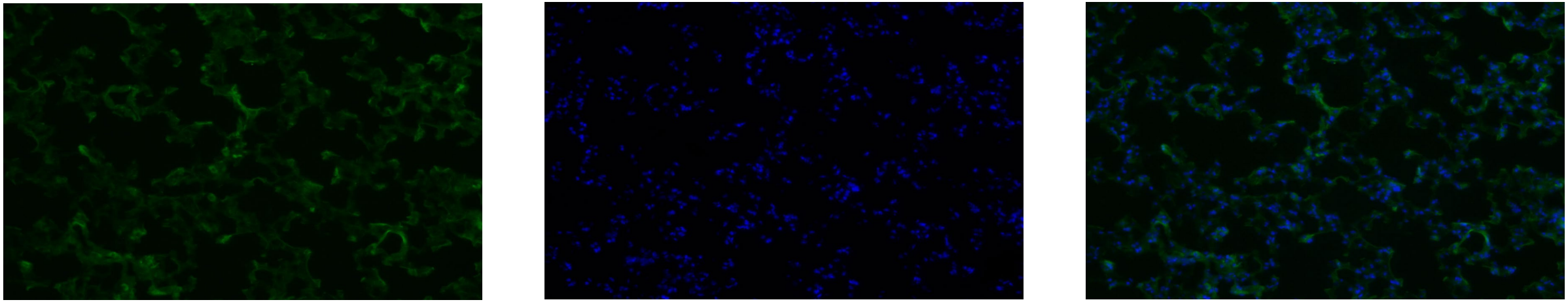
Cecal Ligation Puncture Procedure

Fig 11. Cecal ligation puncture procedure induce acute lung injury.

Immunofluorescence



B6.Gli 1 $^{-/-}$



B6.Gli 1 $^{+/+}$

Fig 12. The expression of Gli 1 in lung tisssue.

3.Others

- 1. Primer design
- 2. Bacterial culture
- 3. Plasmid extration

4. Reference

- 1. Ke-Jia Wu, Jie-min Huang, Hai-Jing Zhong, Zhen-Zhen Dong, Kasipandi Vellaisamy, Jin-Jian Lu, Xiuping Chen, Pauline Chiu, D. W. J. Kwong, Quan-Bin Han, Dik-Lung Ma and Chung-Hang Leung. A natural product-like JAK2/STAT3 inhibitor induces apoptosis of malignant melanoma cells. PloS one (2017).
- 2.https://www.datasci.com/products/buxco-respiratory-products/finepointe-resistance-and-compliance