**Supplementary Material**

Table 1 Formula of High-fat diet

|  |  |
| --- | --- |
| Formula | **g/kg** |
| Casein | 195 |
| Maltodextrin | 225 |
| Sucrose | 89 |
| Soybean Oil | 33 |
| Lard | 301 |
| Cellulose | 69 |
| Mineral Mix, M1021 | 68 |
| Vitamin Mix, V1010 | 14 |
| L-Cystine | 3 |
| Choline Bitartrate | 3 |
| TBHQ | 0.067 |
| Total | 1000 |

Table 2 Formula of DFB

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Chinese name | Botanical name | Dosage used | Family | Parts used |
| Dahuang | *Rheum palmatum L* | 9g | Polygonaceae | Root |
| Fuzi | *Aconitum carmichaeli Debx* | 10g | Ranunculaceae | Root |
| Xixing | *Asarum sieboldii Miq* | 3g | Aristolochiaceae | Root |
| Baijiangcao | *Herba Patriniae* | 10g | Painaceae | Whole grass |
| Yiyiren | *Semen Coicis* | 30g | Poaceae | Seed |

Table 3 Gradient elution system used for HPLC analyses of DFB

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| Time(min) | Mobile phase A(%) | Mobile phase B(%) | Flow rate（mL/min） | Voltage(mA) |
| 0 | 80 | 20 | 1.0 | 600 |
| 10 | 70 | 30 | 1.0 | 600 |
| 20 | 75 | 35 | 1.0 | 600 |
| 25 | 60 | 40 | 1.0 | 600 |
| 30 | 58 | 42 | 1.0 | 600 |
| 40 | 55 | 45 | 1.0 | 600 |
| 50 | 50 | 50 | 1.0 | 600 |
| 65 | 45 | 55 | 1.0 | 600 |
| 75 | 42 | 58 | 1.0 | 600 |
| 77 | 37 | 63 | 1.0 | 600 |
| 120 | 20 | 80 | 1.0 | 600 |
| 125 | 10 | 90 | 1.0 | 600 |

Table 4 Flow cytometry antibody

|  |  |  |  |
| --- | --- | --- | --- |
| Index | Fluorescein | Laser channel | Product number |
| CD3 | APC-Cy7 | 640 nm | 561042 |
| CD4 | FITC | 488 nm/561nm | 553046 |
| CD8 | Alexa Fluor® 700 | 640 nm | 557959 |
| PD1 | BV421 | 405 nm | 562584 |
| TIM3 | PE | 488 nm/561nm | 566346 |
| CD39 | BV605 | 405 nm | 63-0391-82 |
| IFN-γ | BV605 | 405 nm | 5058400 |
| TNF-α | AF488 | 488 nm/561nm | 557719 |
| TOX | APC | 640 nm | 130-118-335 |
| TCF-1 | AF700 | 640 nm | B8224N-100 |

Table 5 Auxiliary reagent

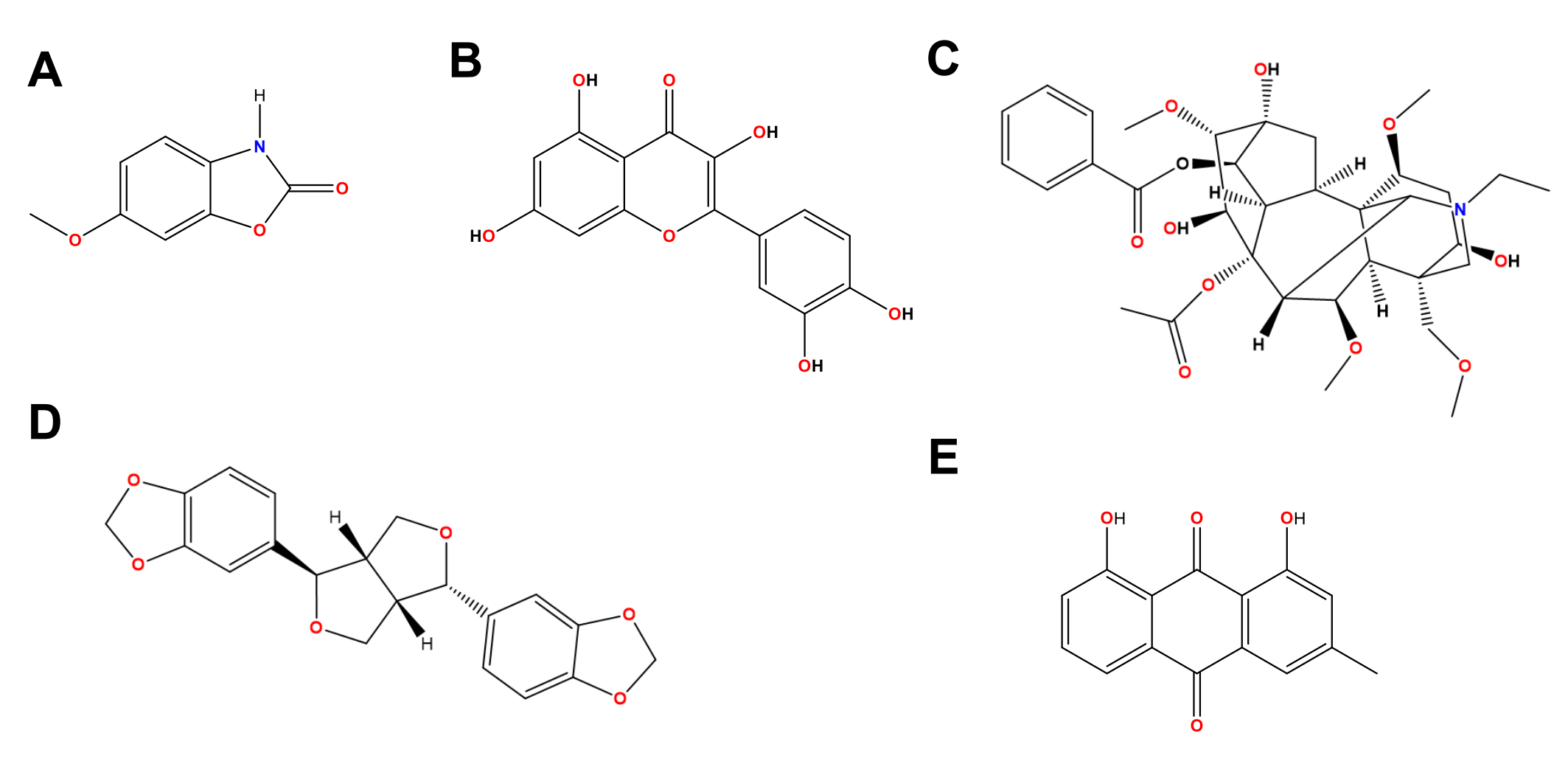
|  |  |  |
| --- | --- | --- |
| Auxiliary reagent | Product number | Product name |
| Fixed nuclear membrane | 00-5523-00 | eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set |
| Fixed ruptured membrane | 554714 | Cytofix/Cytoperm Soln Kit |
| Irritation blocker | 550583 | Leuko Act Cktl With GolgiPlug |
| Compensating microspheres | A10497 | AbC™ Total Antibody Compensation Bead Kit |
| Fc Block**TM** | 553141 | Purified Rat Anti-Mouse CD16/CD32 |

Table 6 Elisa Set

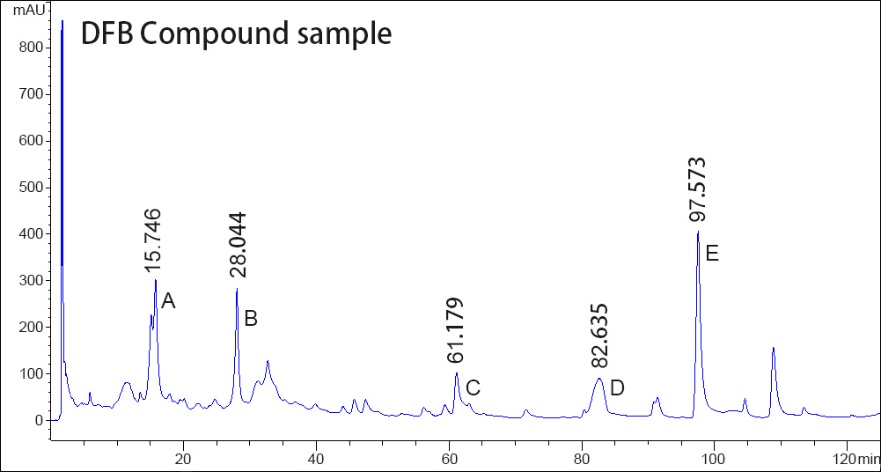
|  |  |  |
| --- | --- | --- |
| Product name | Product number | |
| BD OptEIATM human TGF-β1 ELISA Set | | Cat 559119 |
| BD OptEIATM Mouse MCP-1 ELISA Set | | Cat 555260 |
| BD OptEIATM Mouse IL-6 ELISA Set | | Cat 555240 |

Table 7 Other reagents

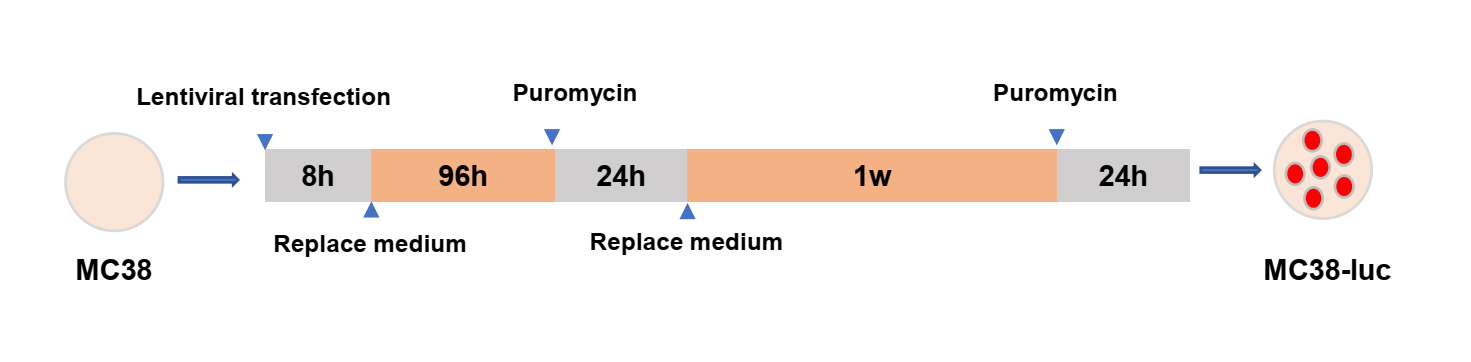
|  |  |
| --- | --- |
| Product name | Category name |
| In vivo Mab anti-mouse PD1 | BioX Cell(BE0146) |
| CCR2-RA-[R] | MedChemExpress(HY-50081) |
| 60% high-fat dietary feed | Trophic Animal Feed High-Tech Co.,Ltd,China |
| D-Luciferin, Potassium Salt | Shanghai Yisheng Biological Technology Co., Ltd. |



S1. (A)Structure of Coixol(Monomer of Semen Coicis);(B) Structure of Quercetin(Monomer of Herba Patriniae);(C) Structure of Aconitine(Monomer of Aconitum carmichaeli Debx) ;(D) Structure of Asarinin(Monomer of Asarum sieboldii Miq) ;(E)Structure of Chrysophanol ((Monomer of Rheum palmatum L)).



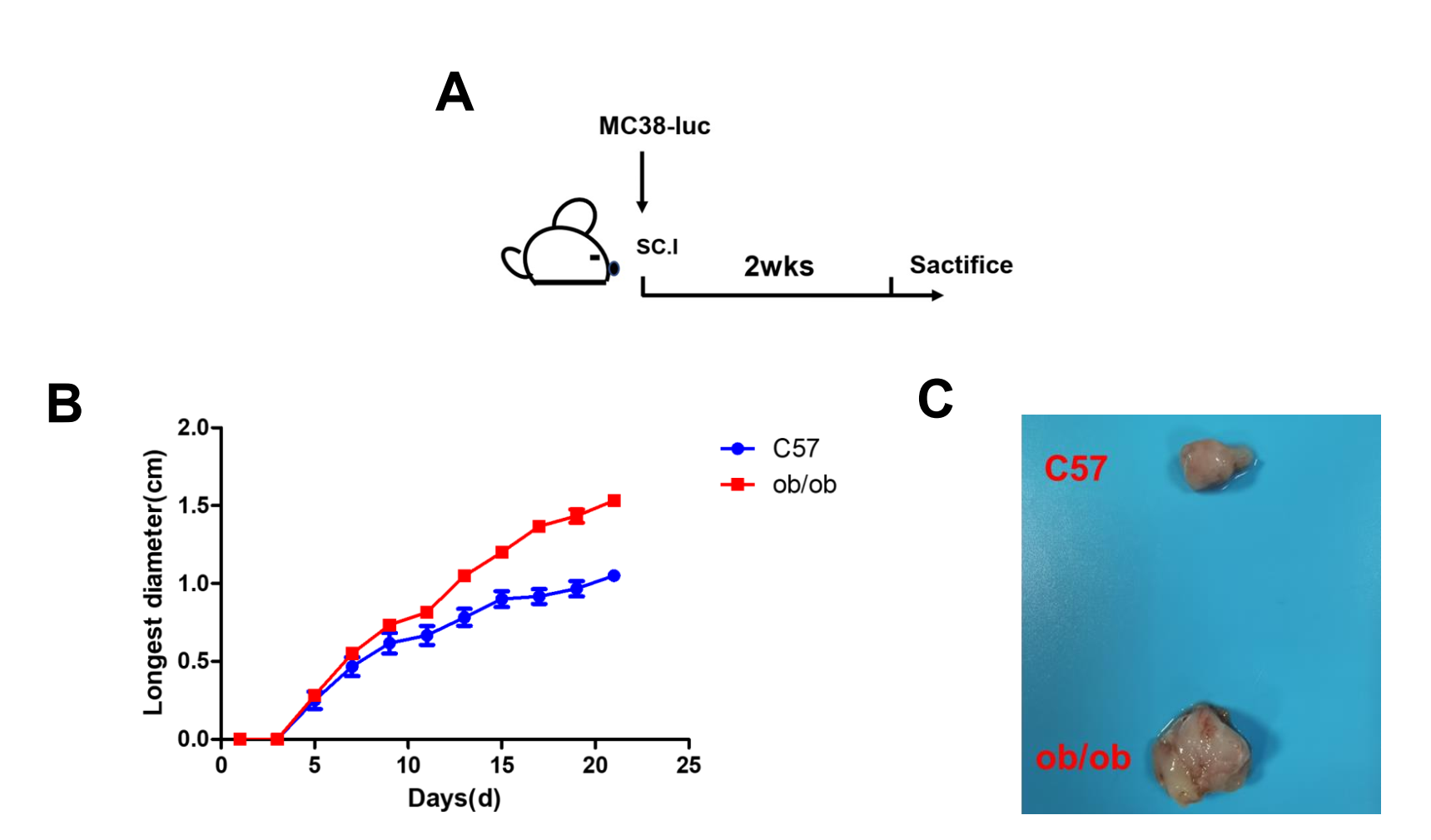
S2. Compositional analysis of DFB by High-Performance Liquid Chromatographic (HPLC) analysis. A: Coixol; B: Quercetin; C: Aconitine; D: Asarinin; E: Chrysophanol.



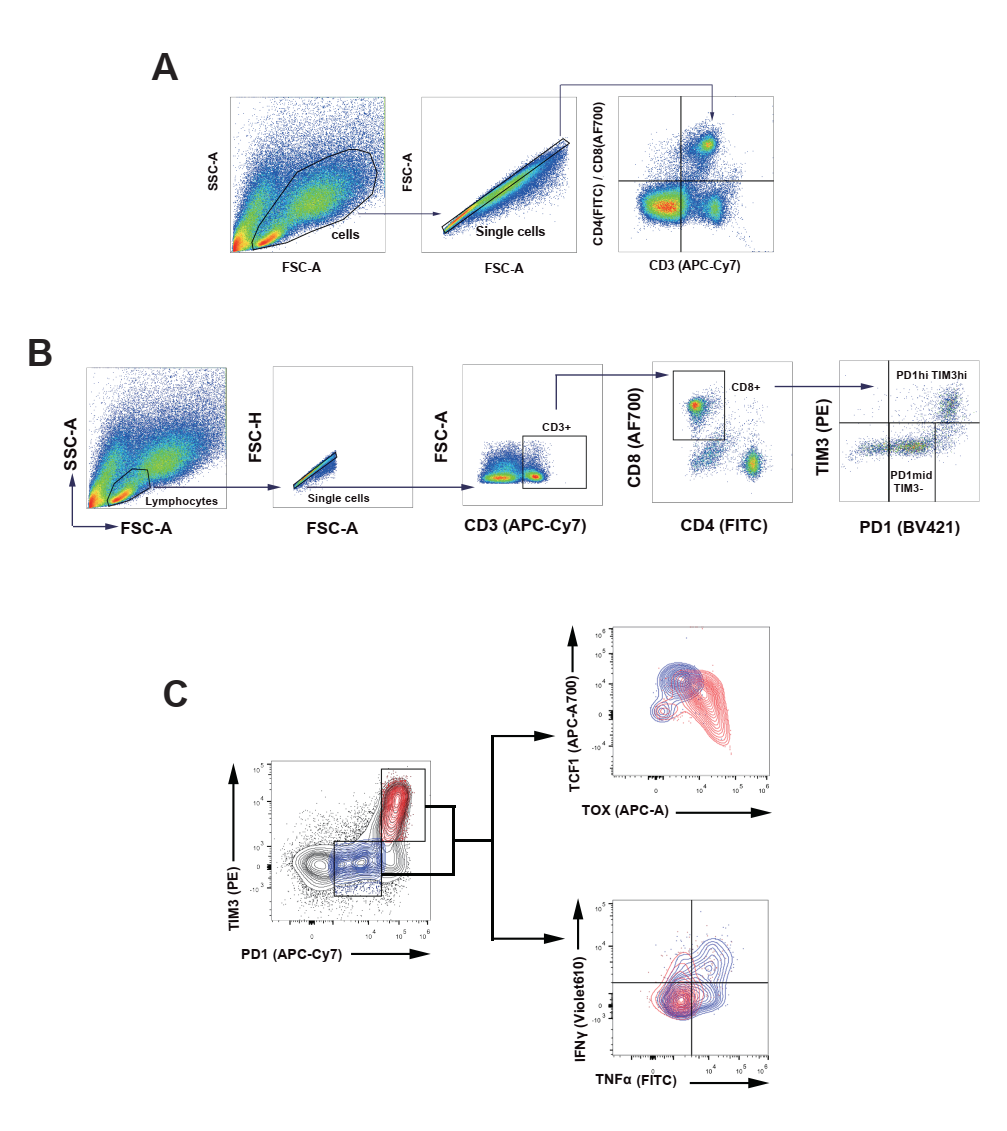
S3. Experimental protocol for MC38-luc cell lines Construct



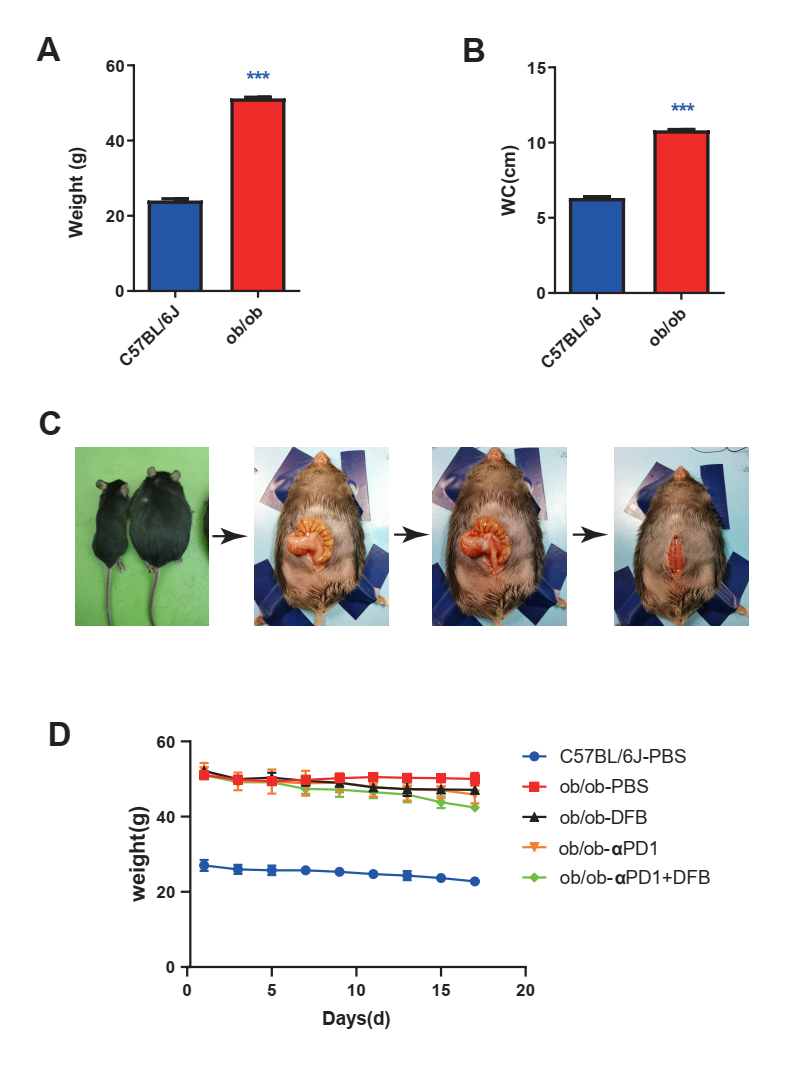
S4. DFB inhibit the growth of colorectal cancer (A) Flow chart of mouse modeling (B) tumor weight（C）and tumor volume (D) Photograph of tumor in mice. (n=5 mice per group). (E) Weight of tumor. Data were presented as mean± SD (n=5 mice per group); \*\*P<0.01; \*\*\*P<0.001.



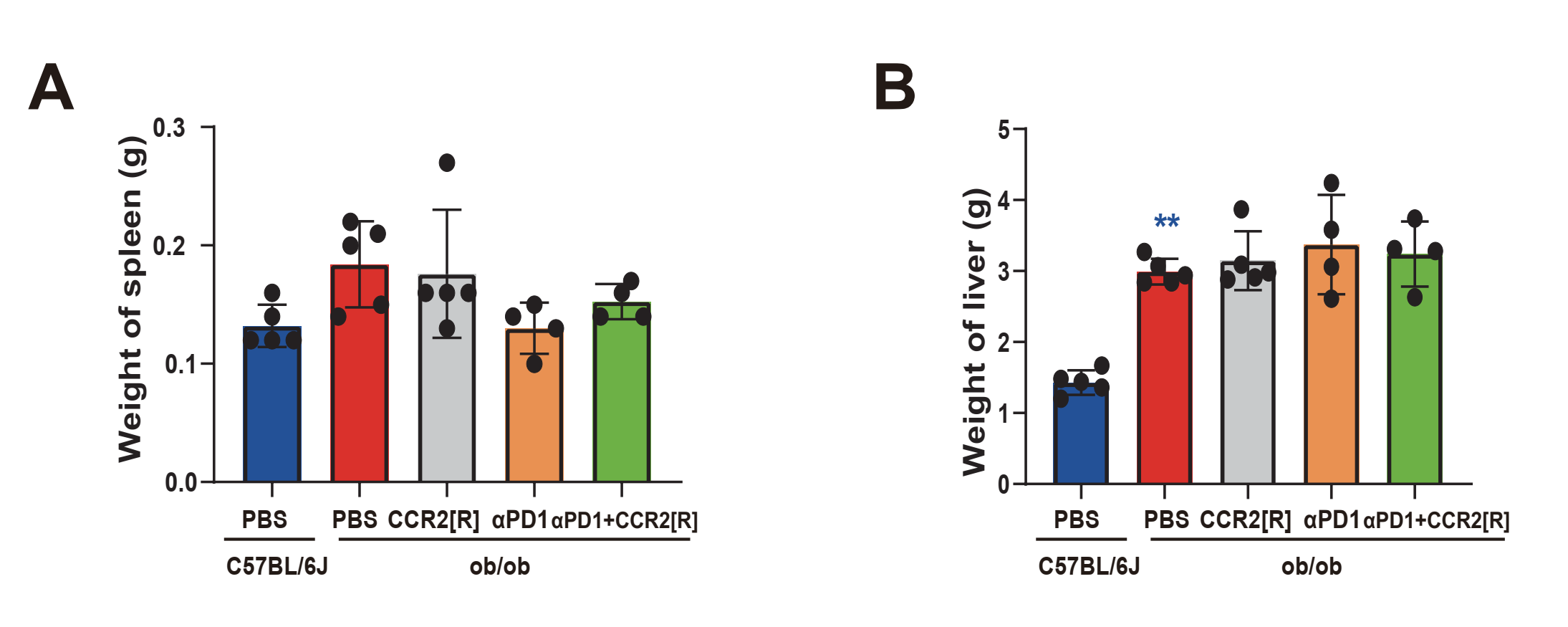
S5. (A) Schematic depicting experimental setup. (B) Changes in the longest diameter of subcutaneous tumors. (C) Picture of tumor after modeling.



S6. Cell population gate logic diagram in flow cytometry



S7. The comparison of weight (A) and abdominal circumference(WC)(B) between C57BL/6J and ob/ob (C) Photograph of Colorectal cancer heterotopic transplantation（D）Changes in mouse body weight during modeling \*\*P<0.01; \*\*\*P<0.001.



S8. The comparison of weight of spleen(B) and liver(C) during modeling \*\*P<0.01; \*\*\*P<0.001.

**Blinding**

Blinding was not performed in mouse experiments. The investigator needed to know the treatment groups in order to perform the study. Tumor weights (an objective measurement) were carried out only at the study endpoints after mice were euthanized and tumors were  
harvested.

**Sample size**

No sample size calculation was done either for in vitro or in vivo studies. For in vivo studies, sample sizes were determined based on our preliminary experiments. In our experience, n=3-5 mice per group is sufficient to detect meaningful biological differences with good  
reproducibility.