

## Supporting Information

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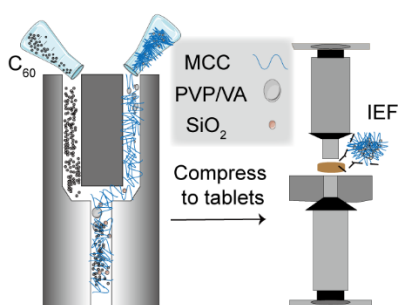
Oral Immunotherapy Reshapes Intestinal Immunosuppression via Metabolic Reprogramming to Enhance Systemic Anti-Tumor Immunity

*Xinran Cao, Yuan Xu, Chen Zhou, Jiawei Huo, Shenge Su, Lei Liu, Ziran Zhu, Lei Li, Wang Jia, Chunru Wang\* and Mingming Zhen\**

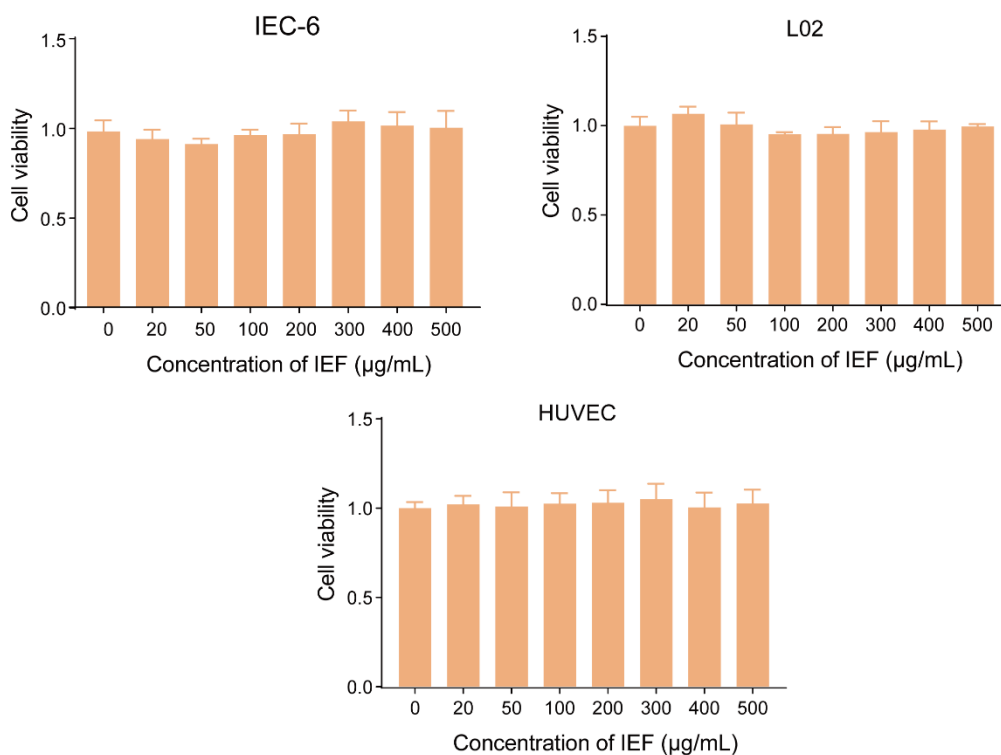
## Supporting Information

### Oral immunotherapy reshapes intestinal immunosuppression via metabolic reprogramming to enhance systemic anti-tumor immunity

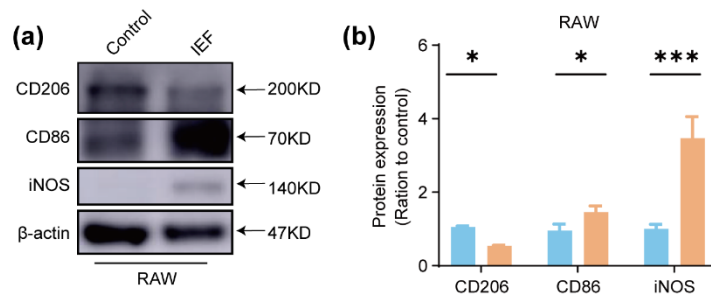
Xinran Cao<sup>†</sup>, Yuan Xu<sup>†</sup>, Chen Zhou, Jiawei Huo, Shenge Su, Lei Liu, Ziran Zhu, Lei Li, Wang Jia, Chunru Wang\* and Mingming Zhen\*



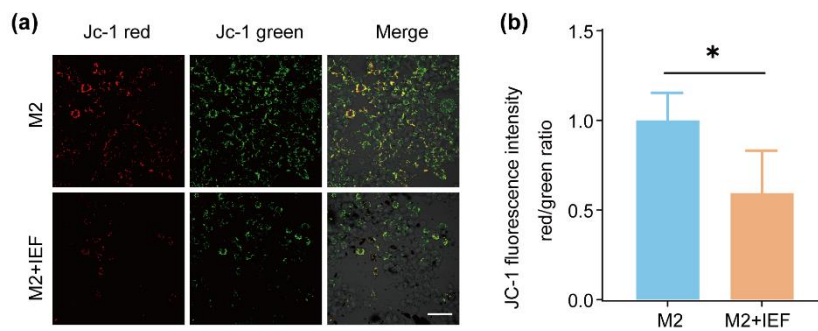
**Fig. S1. Schematic illustration of IEF preparation.**



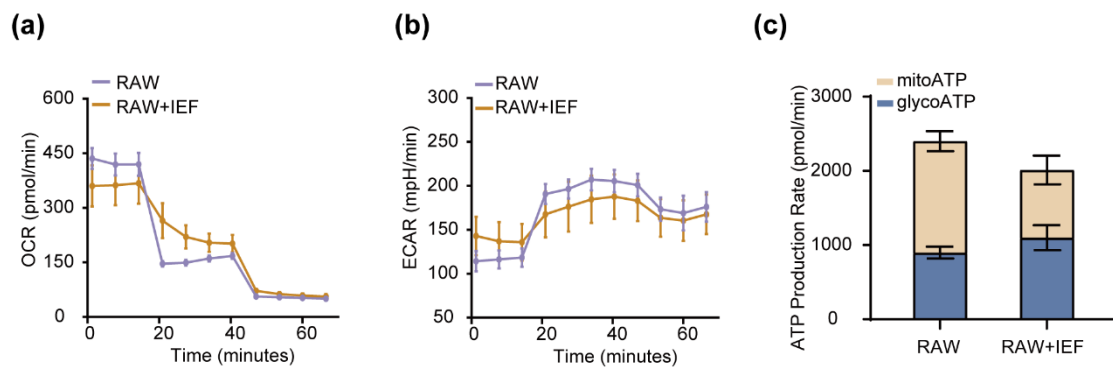
**Fig. S2. Cellular viabilities of IEC-6, L02, and HUVEC after co-incubation with different concentrations of IEF (n=6).**



**Fig. S3. Polarization ability of IEF on RAW macrophages. a-b)** The protein expressions of M2 marker (CD206), and M1 markers (CD86, iNOS) in RAW macrophages before and after co-incubation with IEF (n=3). The data are shown as the mean  $\pm$  s.d. Student's t-test, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.

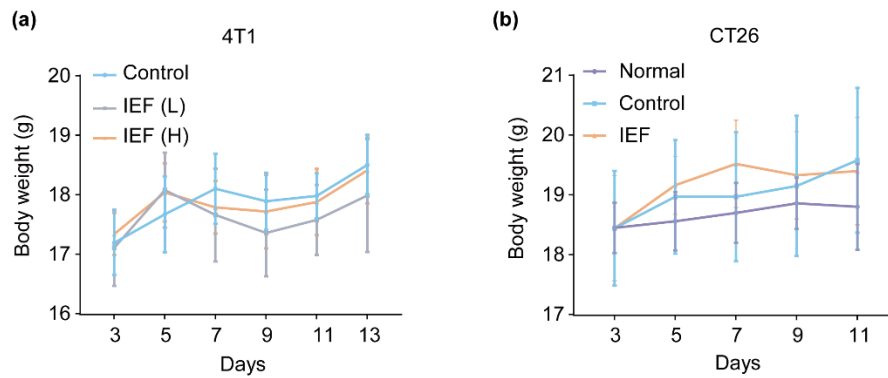


**Fig. S4. Effect of IEF on mitochondrial membrane potential in M2 macrophages. a-b)** Representative fluorescence images in mitochondrial membrane potential (JC-1) before and after co-incubation with IEF of M2 macrophages, (b) and quantification (n=4). Scale bar, 40  $\mu$ m.

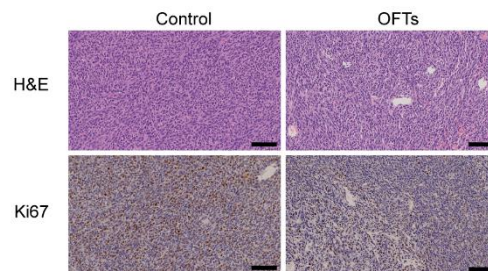


**Fig. S5. Analysis of energy metabolism in RAW macrophages treated with IEF. a-c)** The measurement flow and quantitative analysis of mitochondrial ATP (mitoATP)

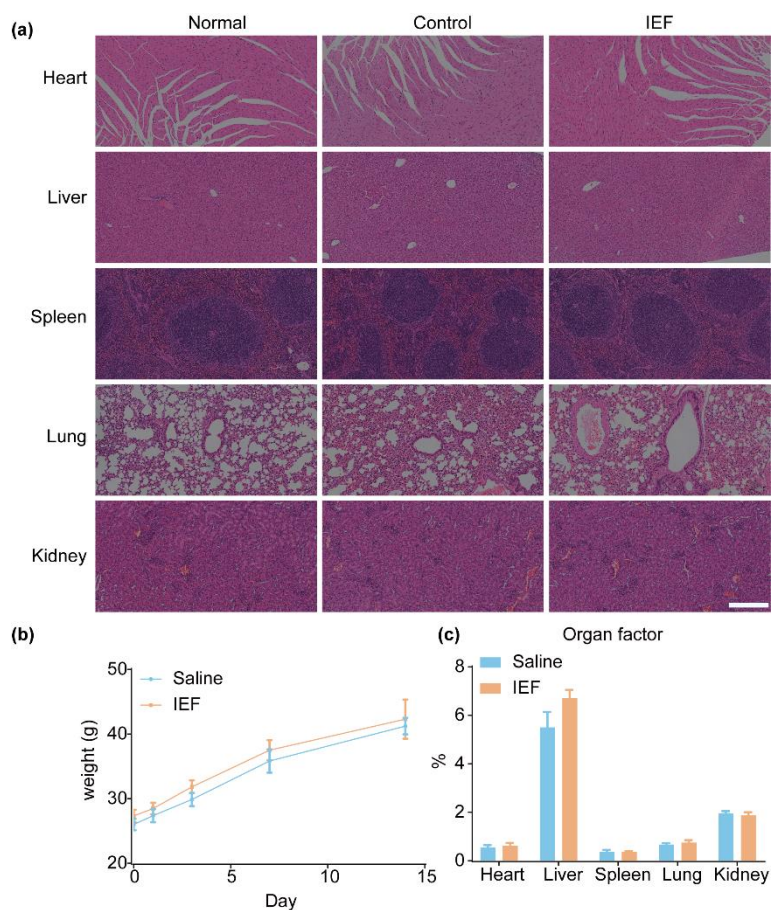
production rate and glycolytic ATP (glyATP) production rate of M0-type macrophages after IEF treatment (n=6). The data are shown as the mean  $\pm$  s.d. Student's t-test, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Fig. S6. Changes in body weight of different mice before and after IEF treatment (n=8).**



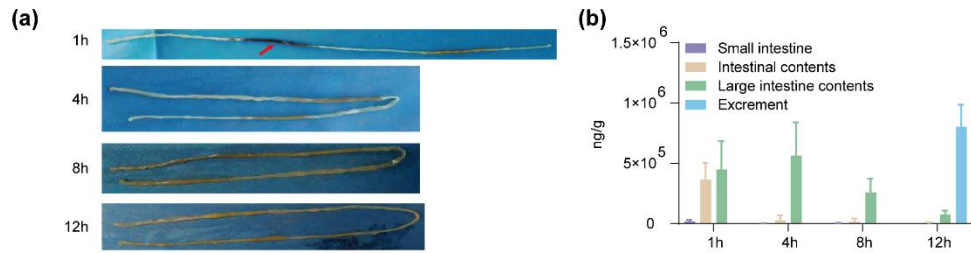
**Fig. S7. Representative images of H&E, and Ki67 staining sections of tumor from different treatment groups.** Images were representative of four biologically independent mice in each group. Scale bar, 100  $\mu$ m.



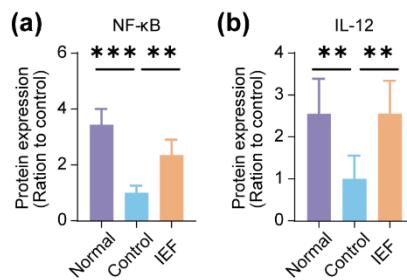
**Fig. S8. The bio-safety of IEF.** **a)** Representative images of H&E staining slices of heart, liver, spleen, lung, and kidney at the end of treatment in mice with CT26 cells. Scale bar, 250  $\mu$ m. **b)** Weight growth curve of KM mouse acute toxicity experiment. **c)** Organ coefficient of KM mouse acute toxicity experiment (n=10).

	Liver	Spleen	Lung	Kidney	Blood	Tumor	Urine
1 h	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	
4 h	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	
8 h	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	
12h	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND	ND $\pm$ ND

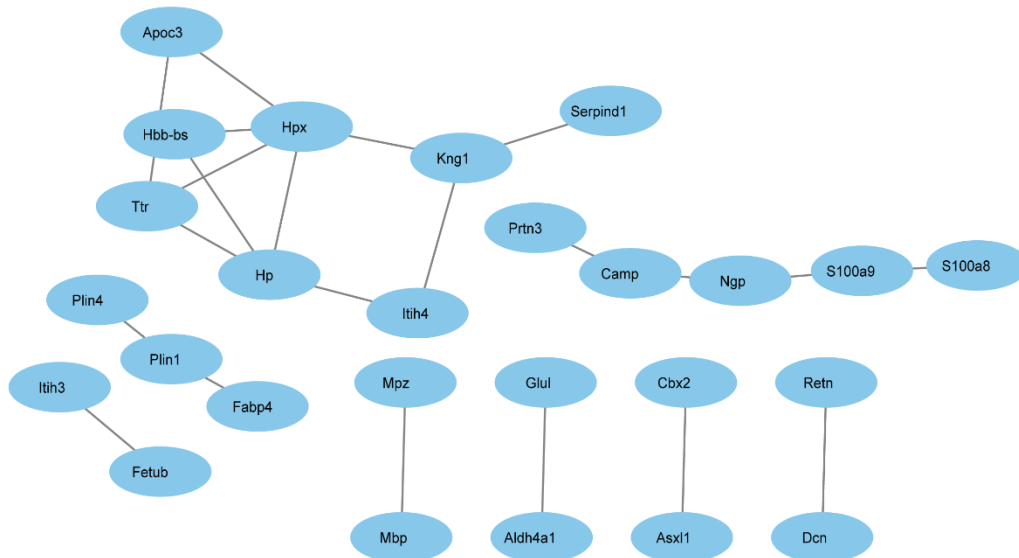
**Table S1. The distribution of IEF in mice at different time points.** (n=6; ND represents no detection; The minimum detection limit is 50 ng/g.)



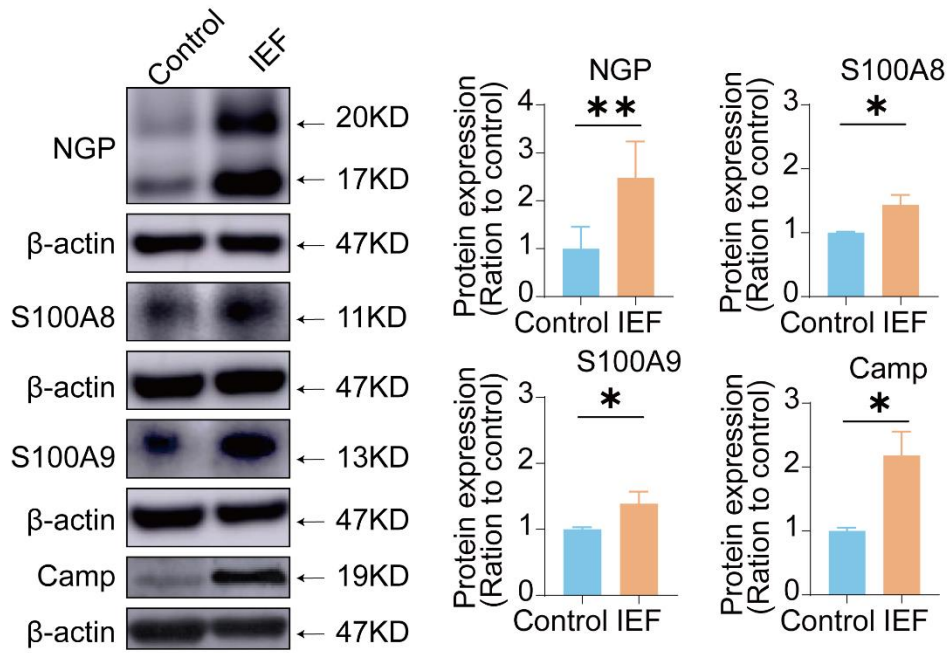
**Fig. S9. The biodistribution of IEF.** a) Distribution of IEF in the small intestine at different time points. Red arrow, IEF. b) Quantitative distribution of IEF in different parts and contents of the intestine at different time points (n=6).



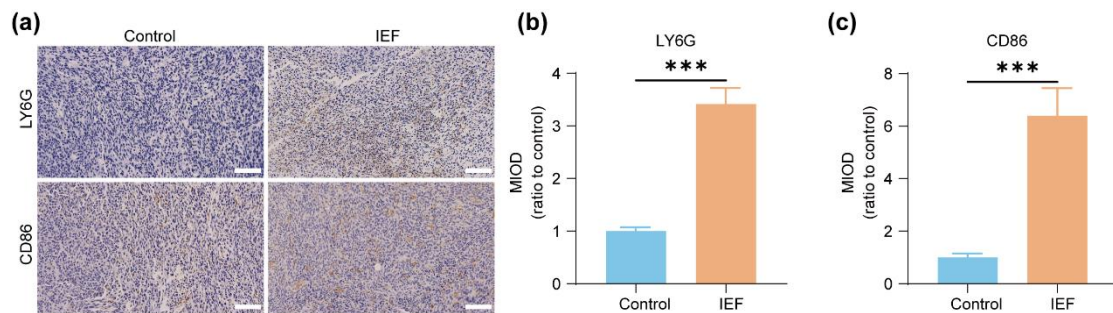
**Fig. S10. The protein expression of NF-κB and IL-12 in the ileum was measured by ELISA (n=5).** The data are shown as the mean ± s.d. One-way ANOVA, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Fig. S11. Tumor site proteomics analysis.** Protein interaction network between differential expression proteins in tumors.



**Fig. S12. Detection of immune-related proteins at tumor sites.** WB assay and quantitative protein expressions of NGP, S100A8, S100A9 and CAMP (n=3). The data are shown as the mean  $\pm$  s.d. Student's t-test, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.



**Fig. S13. Intrinsic immune response at the tumor site.** a) Immunohistochemical staining of neutrophil (labeled by LY6G) and M1-type macrophages (labeled by CD86) in tumor. Scale bar, 100  $\mu$ m. b) Quantification of neutrophils expression (LY6G) in the tumor. c) Quantification of M1 macrophage expression (CD86) in the tumor (n=5). The data are shown as the mean  $\pm$  s.d. Student's t-test, \*p < 0.05, \*\*p < 0.01, \*\*\*p < 0.001.