

***Glycyrrhiza uralensis* water extract enhances dendritic cell maturation and antitumor efficacy of HPV dendritic cell-based vaccine**

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Fuchun Zhang<sup>a</sup>, Jianhua Yang<sup>a,d</sup>, Jinyao Li<sup>a,\*</sup>

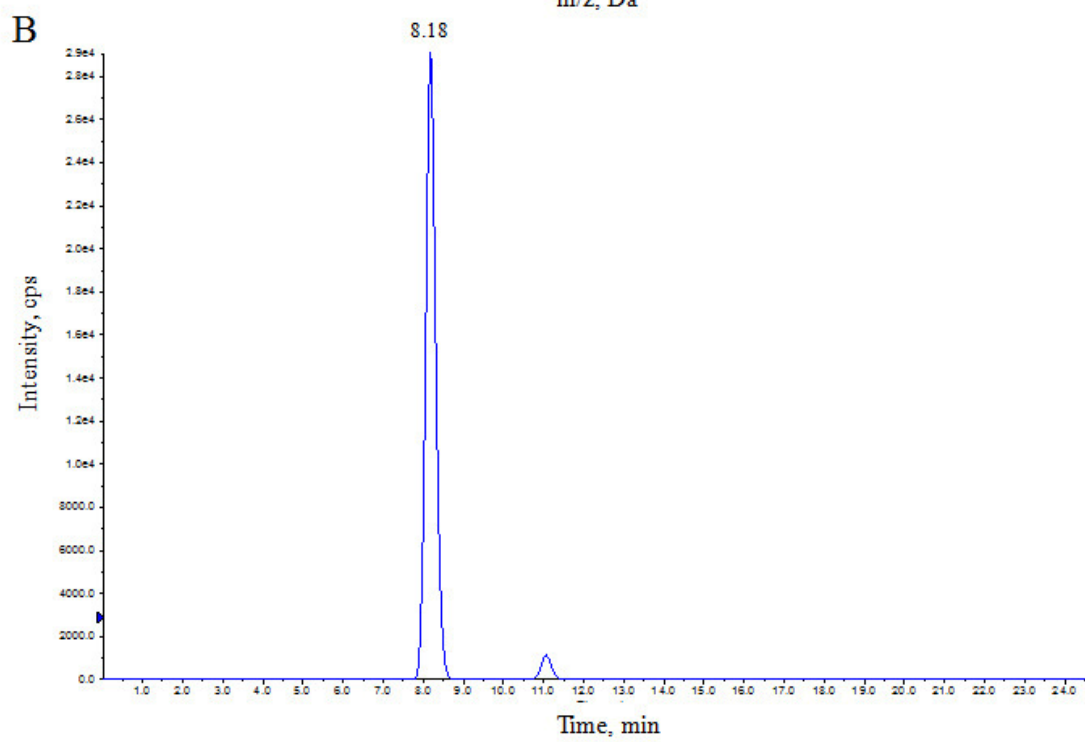
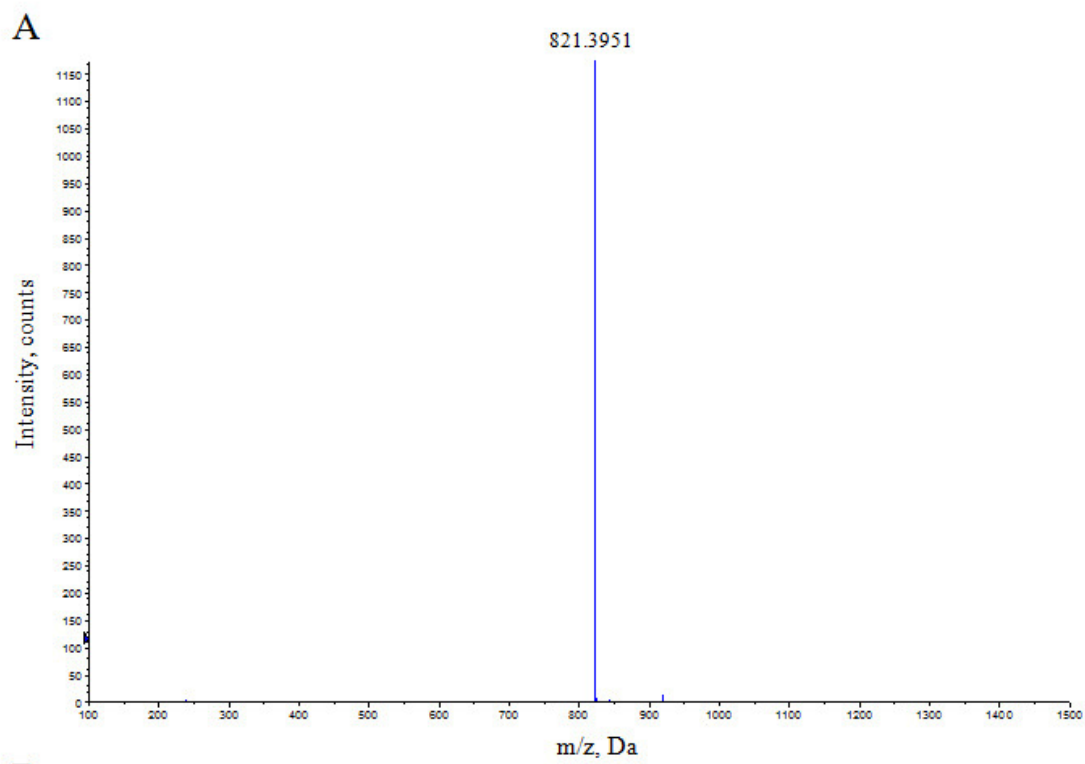
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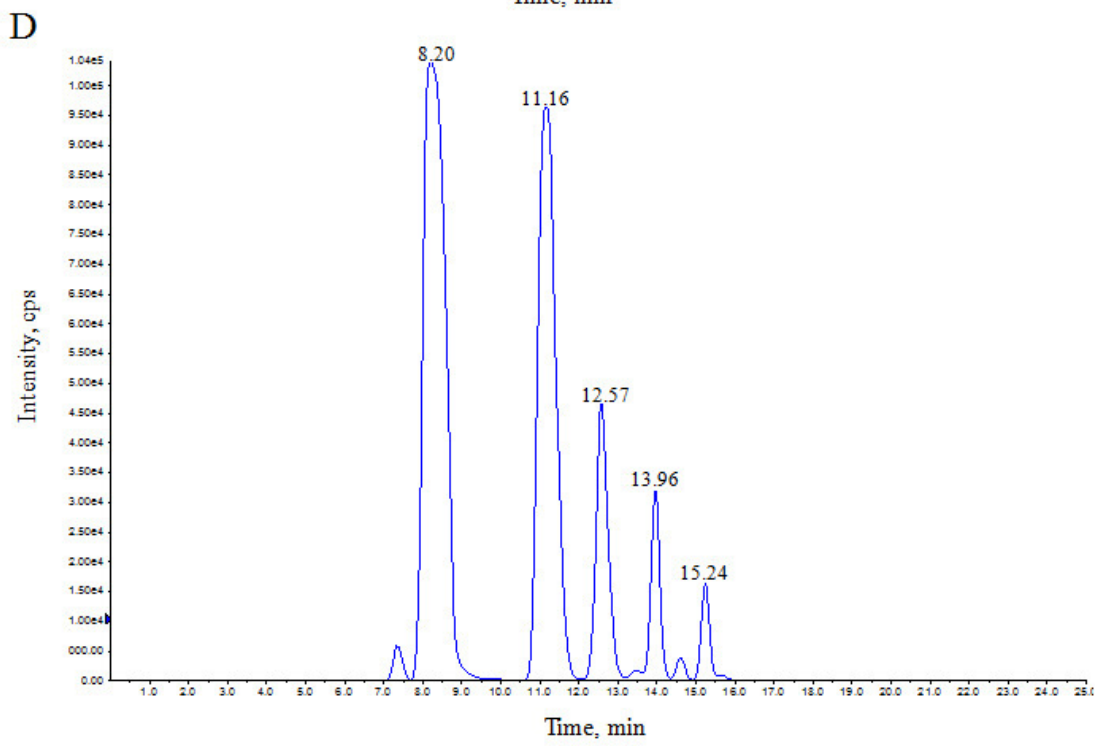
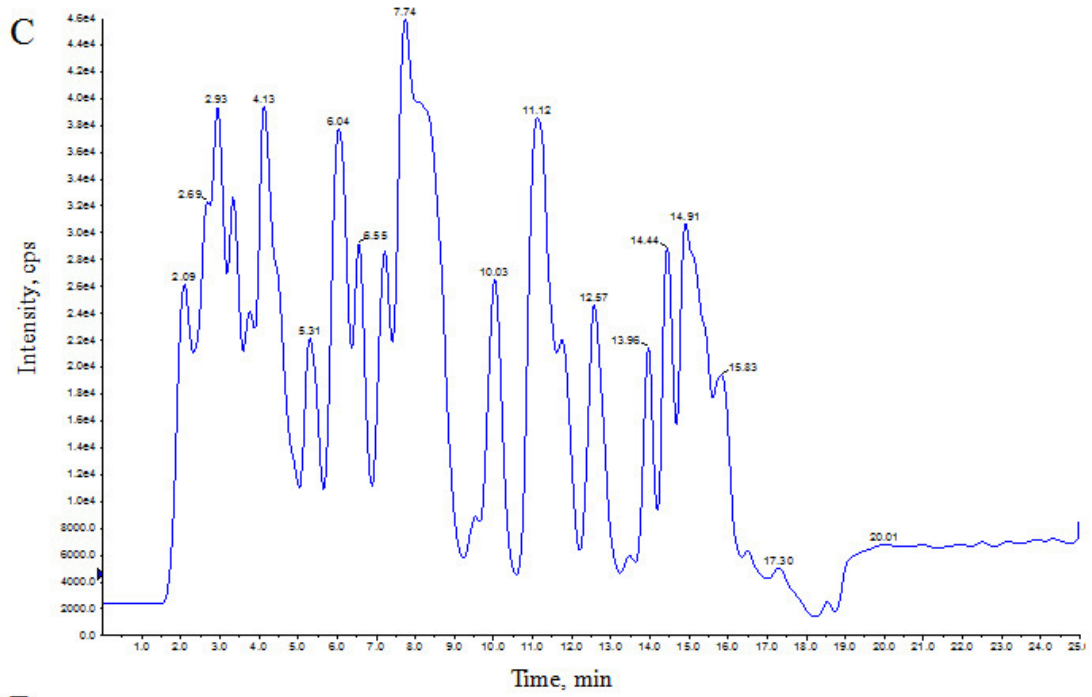
<sup>b</sup> Key laboratory of Xinjiang Uighur Medicine, Xinjiang Institute of Materia Medica, 140 Xinhua South Road, Urumqi 830004, China

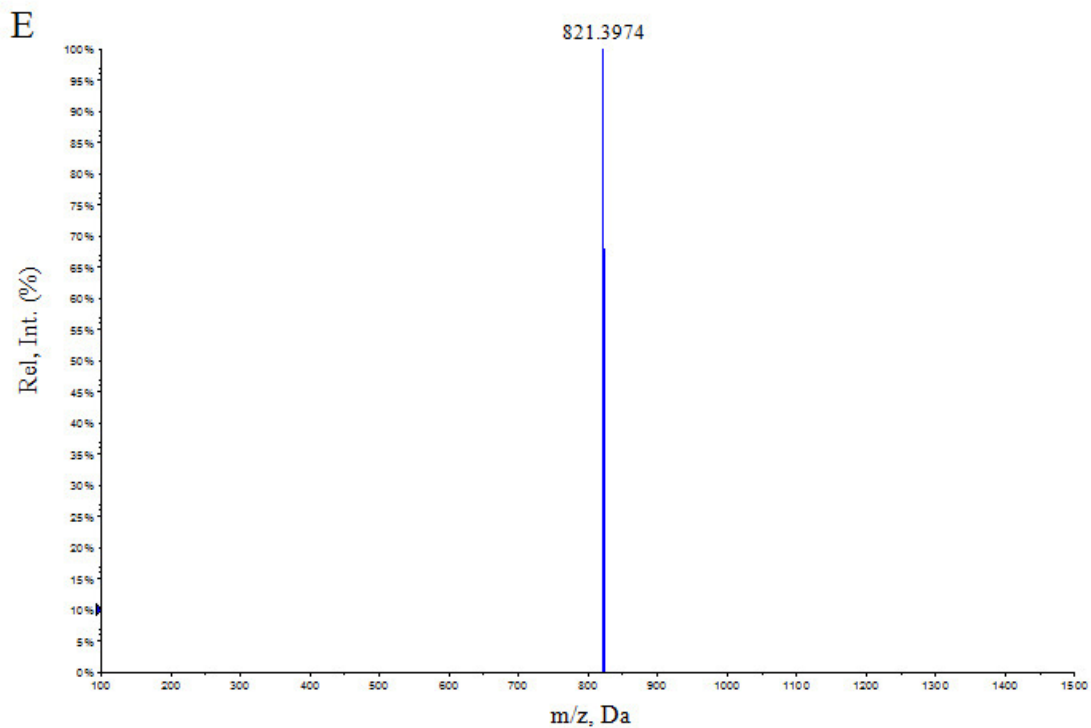
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<sup>d</sup> Texas Children's Cancer Center, Department of Pediatrics, Dan L. Duncan Cancer Center, Baylor College of Medicine, TX 77030, USA

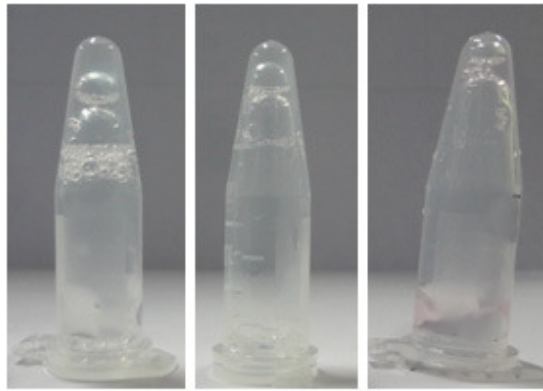
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**Supplementary Figure S1. The quality control of GUWE by LC-MS/MS.** The standard glycyrrhizin and GUWE were analyzed by LC-MS/MS. (A) The m/z of glycyrrhizin is shown. (B) The retention time ( $t_R$ ) of glycyrrhizin is shown. (C) The total ion chromatogram of GUWE is shown. (D) The retention times of components with m/z 821.4 in GUWE are shown. (E) The m/z of one component ( $t_R=8.2$  min) is shown.

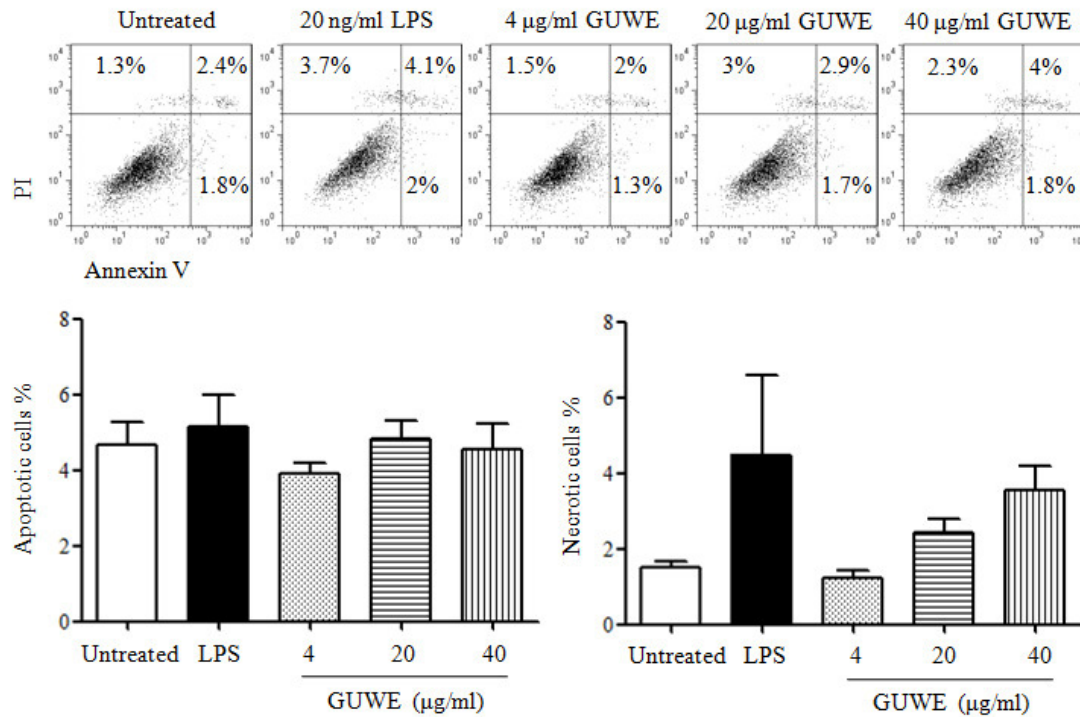


Positive  
control

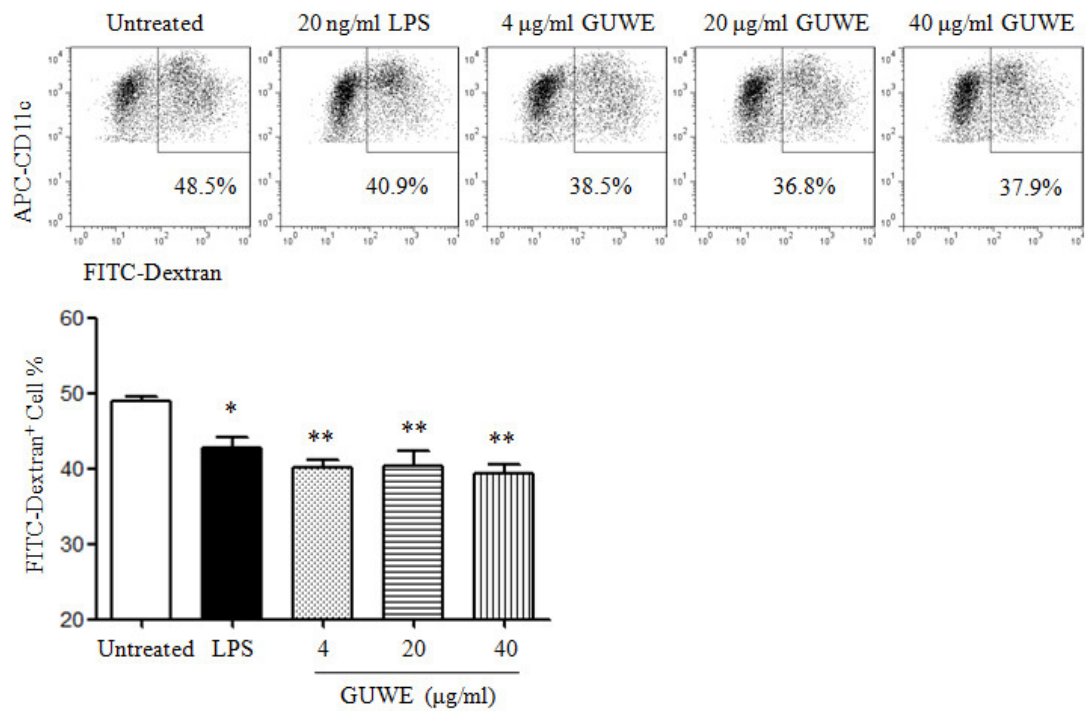
Negative  
control

GUWE

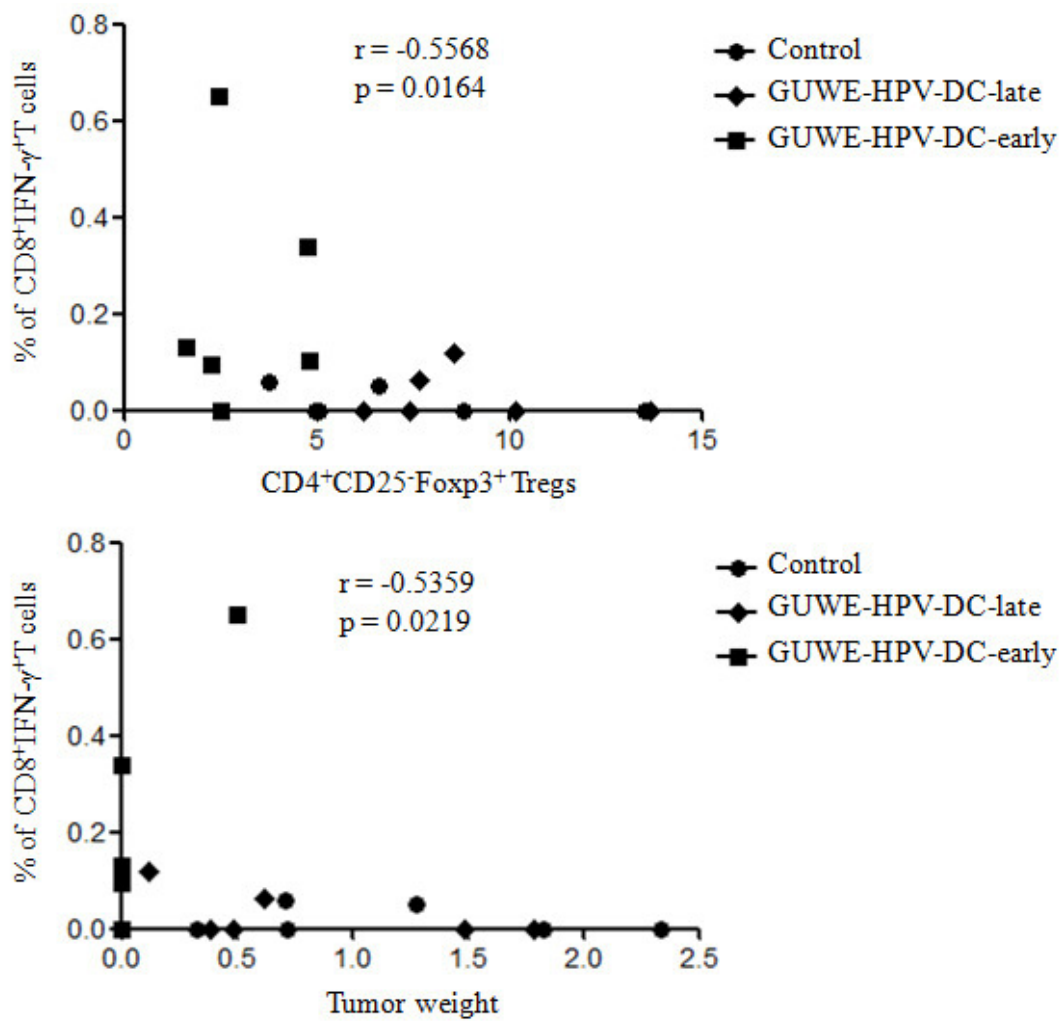
**Supplementary Figure S2. Endotoxin detection of GUWE.**



**Supplementary Figure S3. The effect of GUWE on the viability of DCs.** DCs were induced from bone marrow in the presence of GM-CSF. On day 7, DCs were treated with different concentrations of GUWE or LPS. After 12 h, DCs were collected and stained with Annexin V and PI. Samples were analyzed with flow cytometry. Representative dot plots are shown in upper panels. The frequencies of apoptosis and necrosis are shown in lower panels.



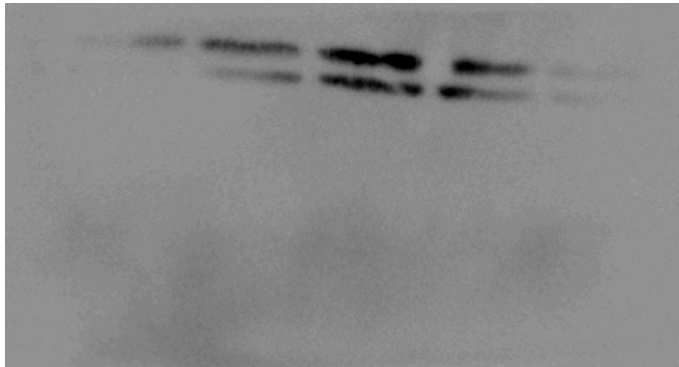
**Supplementary Figure S4. The capacity of antigen up-take of DCs upon GUWE treatment.** DCs were treated with different concentrations (4, 20 and 40 µg/ml) of GUWE or LPS for 12 h, and then inoculated with FITC-Dextran for 1 h. After staining with APC-CD11c, samples were analyzed with flow cytometry. The representative dot plots are shown in upper panels and the summary data (mean ± SEM) are shown in lower panel. Data are from 3 independent experiments and analyzed by ANOVA. \*  $p < 0.05$ ; \*\*  $p < 0.01$  compared to untreated DCs.



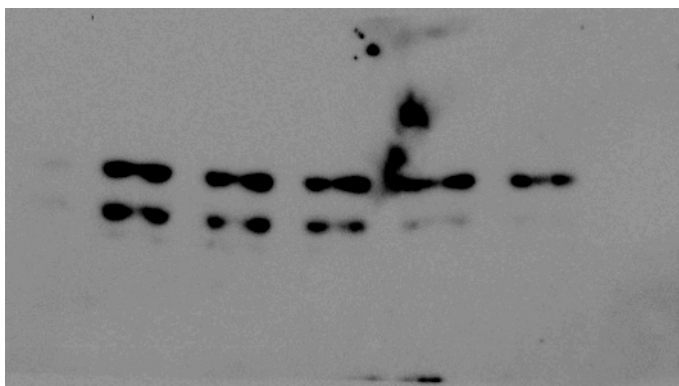
**Supplementary Figure S5. The correlation of CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells with iTregs and tumor weight.** The upper panel shows the correlation between CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells and iTregs. The lower panel shows the correlation between CD8<sup>+</sup>IFN- $\gamma$ <sup>+</sup> T cells and tumor weight.



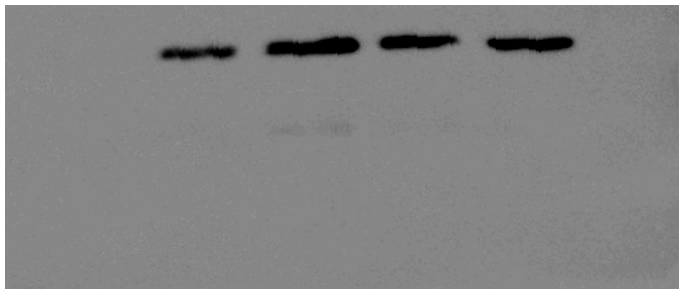
Full-length blots for Figure 4.



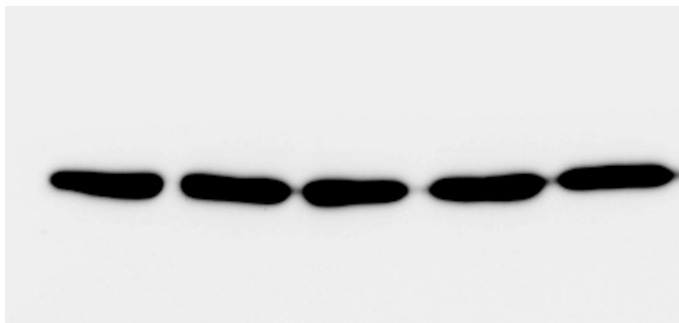
P-JNK



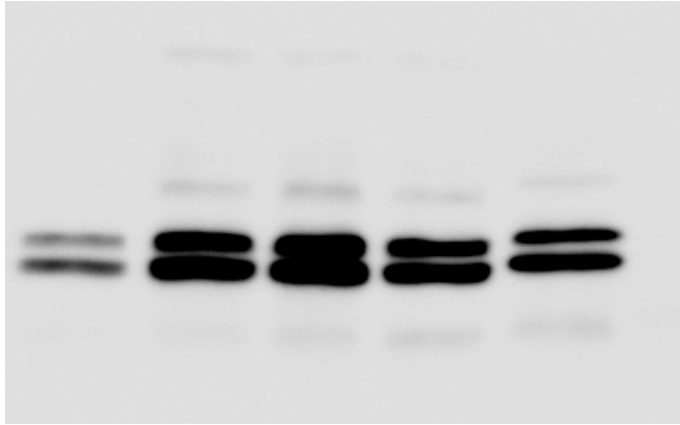
JNK



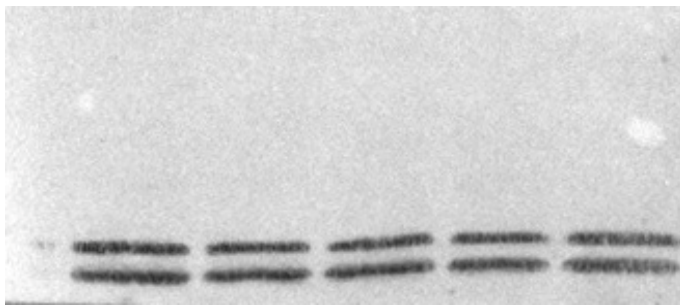
P-p38



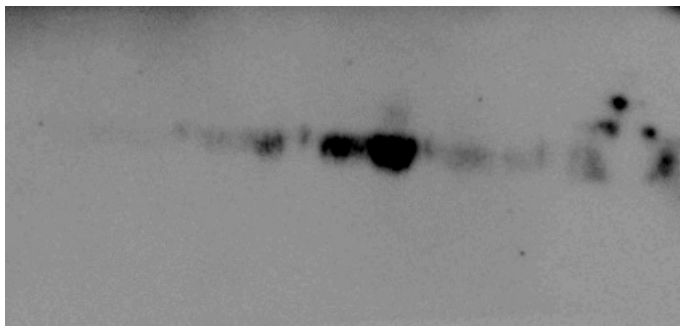
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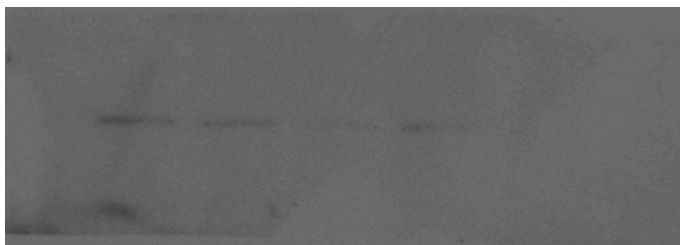
P-ERK



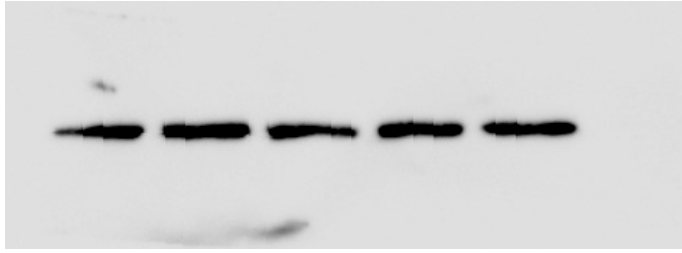
ERK



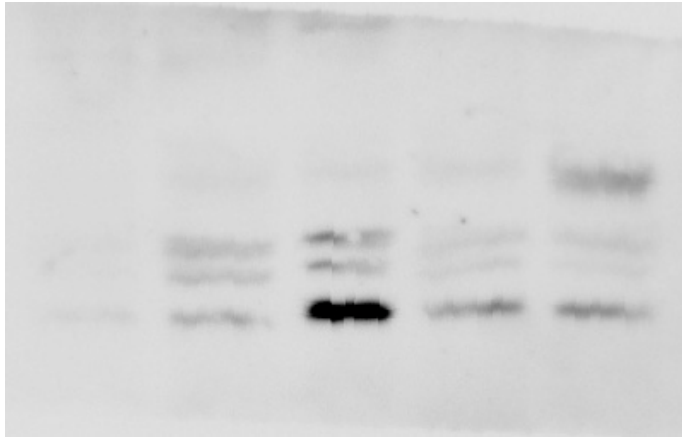
P-IKK $\alpha/\beta$



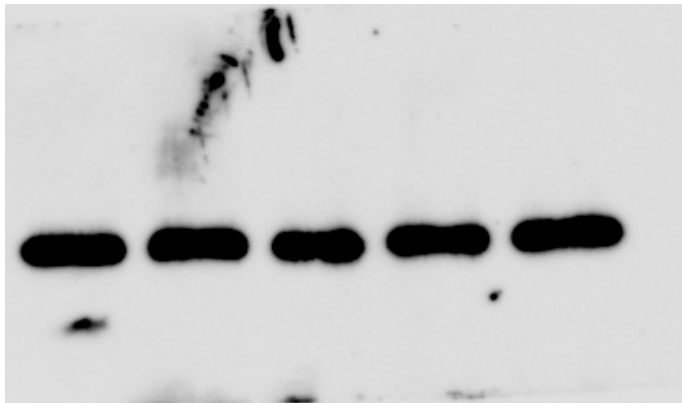
IKK $\alpha$



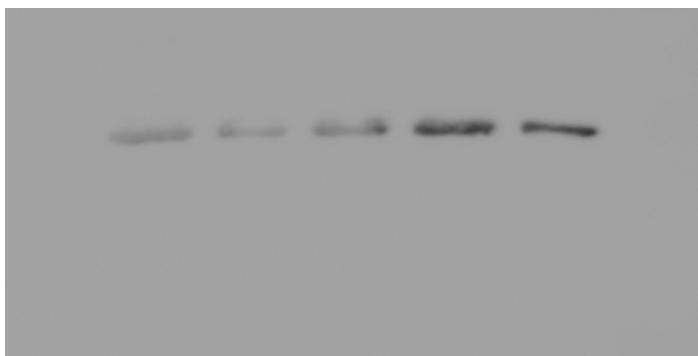
IKKβ



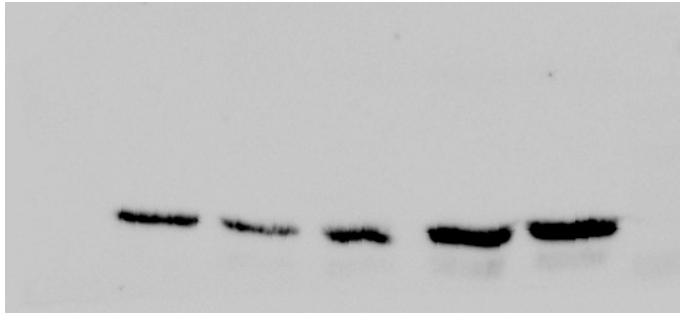
P-IκB



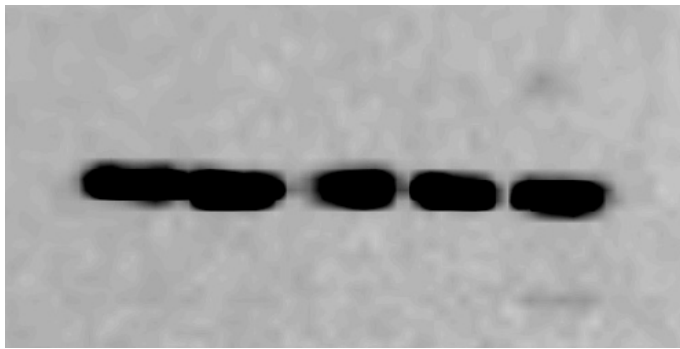
IκB



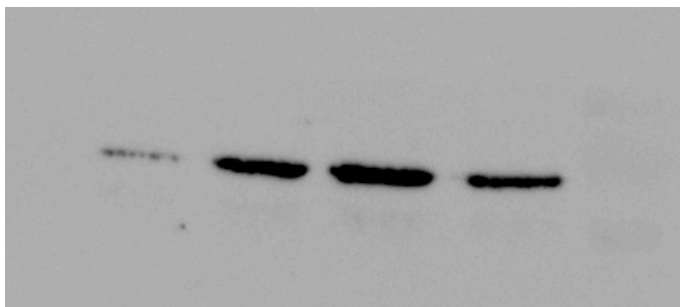
P-NF-κBp65 in cytoplasm



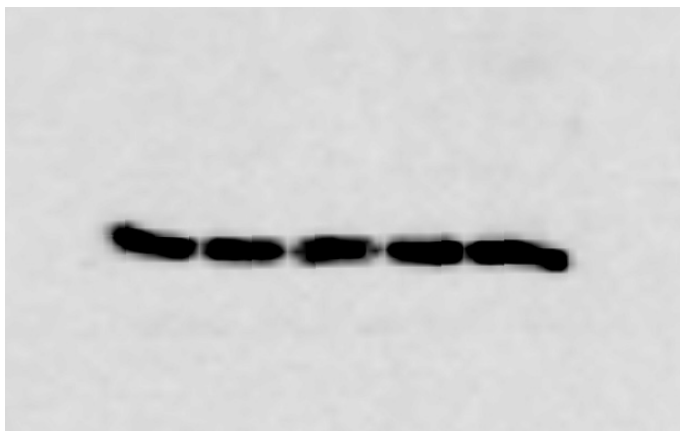
NF-κBp65 in cytoplasm



β-actin



NF-κBp65 in nuclear



Histone