

# CD138 Expression Promotes Accumulation and Activation of T Cells in Autoimmune MRL/lpr Mice

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## Research Article

**Keywords:** CD138+ T cells, Cellular activation, Cellular apoptosis, Autoimmunity, Systemic lupus erythematosus

**Posted Date:** December 16th, 2021

**DOI:** <https://doi.org/10.21203/rs.3.rs-1136102/v1>

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# Abstract

CD138<sup>+</sup> T cells that accumulated in Fas-deficiency lupus mice, had been identified as autoreactive T cells in SLE which significantly promoted autoantibody secretion. In present study, we found CD138 expression in T cells played a key role in the progression of SLE in MRL/lpr mice. Our results indicated CD138<sup>+</sup> T cells apoptosis was in Fas dependent way. However, CD138 expression of T cells in MRL/lpr mice could significantly prevent T cells apoptosis, contribute to accumulation of T cells and DN T cells and simultaneously promote T cells activation. Importantly, CD138 expression in DN T cells significantly increased FasL expression of DN T cells enhancing the cytotoxicity of DN T cells. Phorbol 12-myristate 13-acetate and Ionomycin (PI) stimulation could significantly prevent CD138<sup>+</sup> T cells accumulation by strikingly inducing their specific apoptosis. Moreover, PI stimulation significantly activated CD138<sup>+</sup> T cells with increased CD69 expression in them. Importantly, our results showed CD69 expression in CD138<sup>+</sup> T cells could significantly increase the apoptosis level of them. That indicated PI stimulation could induce specific apoptosis of CD138<sup>+</sup> T cells via increasing CD69 expression in CD138<sup>+</sup> T cells. In addition, our results showed CD138<sup>-</sup> T cells in MRL/lpr mice had significant defects in activation. However, to activate T cells could significantly prevent CD138 expression in CD3<sup>+</sup> T cells of MRL/lpr mice. Our results suggested CD138 expression in CD3<sup>+</sup> T cells of MRL/lpr mice was probably caused by the failure of activation in autoreactive T cells before self-antigens exposure to immune system.

# Introduction

Systemic lupus erythematosus (SLE) is the autoimmune disease characterized by the production of multiple autoantibodies including anti-nuclear antibody (ANA) and anti-double-stranded DNA (dsDNA) antibody<sup>1,2</sup>. Productions of these autoantibodies make a detrimental effect on multiple tissues and organs<sup>3-5</sup>. However, SLE is the autoimmune disease with a so complex mechanism that both T cells and B cells participate in the progression<sup>6-8</sup>.

CD138 is a marker of plasma cells which have been believed to be originated from activated B cells<sup>9,10</sup>. While recent years, CD138<sup>+</sup> T cells, which express both CD3 and CD138 on their surface, were reported to be plasmablastic B-cell neoplasms in clinical cases<sup>11</sup>, and also have been identified in SLE murine model<sup>12-14</sup>. CD138<sup>+</sup> T cells have been shown to significantly accumulate in Fas-deficiency lupus mice<sup>12-14</sup>. Previous research has demonstrated double negative (DN) T cells play an important role in the progression of lupus and significantly contribute to the tissue injury of SLE<sup>6,15,16</sup>. Plasma cells accumulation were also the cardinal feature of SLE<sup>17,18</sup>. While interestingly, our present studies showed that the majority of CD138<sup>+</sup> cells in SLE murine model were CD138<sup>+</sup> T cells. Moreover, most of CD138<sup>+</sup> T cells were also DN T cells that were CD4 and CD8 double negative. Importantly, recent research has demonstrated that CD138<sup>+</sup> T cells significantly could significantly promote autoantibody production both in vivo and in vitro with a CD4 receptor dependent way indicating that CD138<sup>+</sup> T cells are those autoreactive T cells that accumulated in MRL/lpr mice<sup>12</sup>. So, CD138<sup>+</sup> T cells may be key to uncover the underlying mechanism in the progression of SLE. However, the mechanism of CD138<sup>+</sup> T cells

accumulation in splenocytes of MRL/lpr mice was still undeciphered. In present study, we tried to investigate the mechanism of CD138+ T cells accumulation in MRL/lpr mice.

Phorbol 12-myristate 13-acetate (PMA) and Ionomycin are commonly used to promote cellular activation in vitro culture<sup>19,20</sup>. Surprisingly, we found PMA and Ionomycin (PI) stimulation could significantly prevent CD138+ T cells accumulation in splenocytes of MRL/lpr mice. PI stimulation progressively decrease the CD138+ T cells frequency as the time of PI stimulation increased. Based on these evidences, we further sought the underlying mechanisms of CD138 expression in CD3+ T cells and of PI stimulation preventing CD138-expressing T cells accumulation.

## **Materials And Methods**

### **Animals**

Female MRL/lpr mice and MRL/MPJ mice were purchased from the Slac Laboratory (Shanghai, China). Mice were housed at 22 ± 1°C with a relative humidity of 50–60% with a 12-hour light/dark cycle. All animal experiments were approved by Institutional Animal Care and Use Committee (IACUC) of Beijing Institute of Traditional Chinese Medicine (IACUC Issue No. 2021040202), and were carried out in accordance with with Animal Research: Reporting of In Vivo Experiments (ARRIVE) guidelines and institutional regulations.

### **Cell Culture in vitro**

Mice were euthanized at the 17-18th week age and then we obtained the spleen in mice. After harvesting the spleen, single-cell suspensions of splenocytes were obtained by filtering through a 70um cell strainer (BD Bioscience, USA). Splenocytes were cultured in medium RPMI-1640 (Hyclone, USA) with 10% FSC (Gibco, USA) in vitro at 37°C with 5% CO<sub>2</sub>, or with treatment of PMA (Thermo Fisher Scientific, USA) and Ionomycin (Thermo Fisher Scientific, USA). 50ng/mL PMA and 1ug/mL Ionomycin were performed to stimulate splenocytes for 2 hours or 4 hours as PI stimulation.

### **Measurement of cytokine levels in serum using the Luminex platform**

Serum levels of multiple cytokines were measured using the Luminex assay kits (Thermo Fisher, USA). Measurements were performed according to the manufacturer's instructions. Diluted serum samples were added onto 96-well plates coated with magnetic beads and incubated for 120min after vortexing. The beads were then washed, and the detection antibody mixture was added and incubated for 30min at room temperature. After incubation and plate washing, the samples were analyzed on the Luminex™ platform.

### **Flow cytometry**

Splenocytes were incubated on ice with CD16/CD32 monoclonal antibody (Thermo Fisher Scientific, eBioscience, USA) for 15 minutes, and then red blood cells were lysed using lysis buffer (BD Bioscience, USA). Cells were stained with the following antibodies for flow cytometry analysis: anti-CD3 PE-cy7, anti-CD3 APC-cy7, anti-CD4 FITC, anti-CD8 PerCP, anti-CD8 APC, anti-CD19 APC-cy7, anti-CD138 PE, anti-CD69 PE, anti-CD69 APC, anti-CD25 APC, anti-FasL APC, anti-B220 PerCP, anti-B220 PE-cy7, Annexin V FITC and 7-AAD PerCP (Thermo Fisher Scientific, eBioscience, USA). FACs data were analyzed using the Flowjo software (Tree Star, USA) version 10.6 for PC.

## Immunofluorescence

Frozen tissue sections were used for immunofluorescence assays. Frozen sections were fixed, blocked, and then stained with primary antibodies, i.e., anti-CD3 antibody (ab33429, Abcam) and anti-CD138 antibody (AF3190, R&D Systems). Sections were visualized using Alexa Fluor 488 and Alexa Fluor 594 secondary antibodies (Abcam) both at a dilution of 1:200. Immunofluorescence images were obtained using ZEN Blue lite software (ZEISS, Germany). Representative imaging data were obtained at identical settings of the ZEN Blue lite software and all assays included negative controls, where the primary antibody was omitted (Supplementary Figure A).

## Statistical analysis

Data from all experiments were expressed as mean  $\pm$  SD and were analyzed using the SPSS software (SPSS, Inc., Chicago, IL, USA). Comparisons between the groups were performed for statistical significance using one-way analysis of variance. Differences with *P* values less than 0.05 were considered statistically significant.

## Results

### CD138 expression in T cells leads to defective apoptosis of T cells of MRL/lpr mice.

We observed CD138<sup>+</sup> cells frequencies in CD3<sup>+</sup> T cells were negligible in splenocytes of MRL/MPJ mice (Figure 1A). But these abnormal T cells accumulated in splenocytes of Fas-deficiency MRL/lpr mice (Figure 1A). In addition, CD138<sup>+</sup> T cells also evidently infiltrated in kidney of MRL/lpr mice, but not in kidney of MRL/MPJ mice (Figure 1B). We also observed CD138<sup>+</sup> T cells in MRL/MPJ mice had a very high level of apoptosis, but a low level in MRL/lpr mice (Figure 1C). That indicated CD138<sup>+</sup> T cells apoptosis in mice was in a Fas-dependent way and Fas deficiency in MRL/lpr mice led to the accumulation of CD138<sup>+</sup> T cells. However, we observed CD138<sup>+</sup> T cell in splenocytes of MRL/lpr mice had a significant decrease in number of apoptotic cells and an increase in number of live cells, compared with CD3<sup>+</sup>CD138<sup>-</sup> T cells (Figure 1D). According to these results, we conclude CD138 expression in CD3<sup>+</sup> T cells leads to the defective apoptosis of CD3<sup>+</sup> T cells in Fas-deficiency MRL/lpr mice. Then, we observed CD138<sup>+</sup> T cells in fresh splenocytes of MRL/lpr mice had a significantly increased CD69<sup>+</sup> cells frequency and simultaneously had a decreased CD25<sup>+</sup> cells frequency compared with CD138<sup>-</sup> T cells in

fresh splenocytes of MRL/lpr mice (Figure 1E). CD138 expression significantly promoted FasL expression in CD3<sup>+</sup> T cells of MRL/lpr mice (Figure 1E). Importantly, CD138 expression in DN T cells also strikingly increased FasL expression in DN T cells of MRL/lpr mice (Figure 1F).

## **CD4<sup>+</sup> T cells and Double negative T cells expressed CD138 in MRL/lpr mice**

We observed the majority of CD138<sup>+</sup> T cells in MRL/lpr mice were CD4 and CD8 double negative, only a proportion of CD138<sup>+</sup> T cells expressed CD4 (Supplementary Figure B). CD8<sup>+</sup> T cells had a limited frequency of CD138<sup>+</sup> cells (Figure 1G). Frequency of CD138<sup>+</sup> cells in DN T cells were more significant, compared with CD4<sup>+</sup> T cells (Figure 1G). Previous research also has demonstrated DN T cells frequency among CD3<sup>+</sup> T cells are elevated significantly and progressively as the increase of ages of MRL/lpr mice<sup>6</sup>. According to these results, it indicated that the majority of CD138<sup>+</sup> T cells in MRL/lpr mice would derive from CD138<sup>-</sup> DN T cells in MRL/lpr mice as increased DN T cells accumulation of MRL/lpr mice. Additionally, we observed CD4<sup>+</sup> T cells in MRL/lpr mice comprised two cells subsets including CD4<sup>hi</sup> T cells and CD4<sup>int</sup> T cells (Figure 1H). CD4<sup>int</sup> T cells which have a down regulated expression of CD4 also expressed B220 (Figure 1H). We found CD4<sup>+</sup>CD138<sup>+</sup> T cells which expressed B220 too (Figure 1H) had a significant down regulated CD4 expression and belonged to the B220<sup>+</sup>CD4<sup>int</sup> T cells subsets (Figure 1H).

## **CD138 expression could improve the defective activation of CD138<sup>-</sup> T cell in MRL/lpr mice.**

Further, we observed multiple cytokines levels in serum of MRL/lpr mice including IFN- $\gamma$ , TNF, IL-6, IL-10, IL-17, and IL-2 were significantly increased compared those in MRL/MPJ mice. That indicates MRL/lpr mice was in inflammation in vivo (Figure 2A). CD138<sup>-</sup> T cells in fresh splenocytes of MRL/lpr mice without in vitro stimulation had an increased frequency of CD69<sup>+</sup> cells compared with those in fresh splenocytes of MRL/MPJ mice (Figure 2B). But frequency of CD69<sup>+</sup> cells in CD138<sup>-</sup> T cells of MRL/MPJ mice strikingly elevated compared with that in CD138<sup>-</sup> T cells of MRL/lpr mice after 5 h in vitro stimulation of fresh splenocytes with PMA and Ionomycin (Figure 2C). In addition, both CD69 expressions and FasL expressions in CD138<sup>-</sup> T cells were not significantly increased after in vitro stimulation of splenocytes with 4 h PI stimulation (Figure 2D). These results indicates CD138<sup>-</sup> T cell in MRL/lpr mice has a significantly defective activation.

Importantly, we found CD138<sup>+</sup> T cells in fresh splenocytes of MRL/lpr mice had significantly increased CD69<sup>+</sup> cells frequencies (Figure 1D and 2B) and FasL expressions (Figure 1D) compared with CD138<sup>-</sup> T cells in those of MRL/lpr mice. In addition, CD138<sup>+</sup> T cells in fresh splenocytes of MRL/lpr mice also had a significantly increased CD69<sup>+</sup> cells frequency and FasL expression compared with CD138<sup>-</sup> T cells in those of MRL/lpr mice even after in vitro 5 h stimulation of splenocytes (Figure 2C and 2E). Our results also indicate CD138 expression could improve the defective activation of CD138<sup>-</sup> T cells in MRL/lpr mice.

## **PI stimulation could significantly prevent CD138+ T cells accumulation**

We tried to use PI to stimulate and activate splenocytes of MRL/lpr mice, and then observed the change of CD138+ T cells frequency in splenocytes after PI stimulation. 4 h in vitro culture of splenocytes in MRL/lpr mice without any stimulation caused significant increases in CD138+ T cell frequency in splenocytes of MRL/lpr mice with increased CD138 expression (Figure 3A and 3D). However CD138+ T cells frequencies were progressively reduced as the time of PI stimulation increased (Figure 3A). Besides, PI stimulation also significantly reduced CD138+ cells frequencies both in DN T cells and CD4+ T cells after PI stimulation (Figure 3B and 3C). CD138 expression in CD138+ T cells was also significantly and progressively down regulated as the time of PI stimulation increased (Figure 3D). These results indicated PI stimulation could prevent CD138+ T cells accumulation in splenocytes of MRL/lpr mice.

## **CD138 expression in T cells could contribute to DN T cells accumulation in MRL/lpr mice**

T cells and DN T cells accumulations were evidently observed in splenocytes of MRL/lpr mice (Figure 4A). Both T cells and DN T cells frequencies in splenocytes of Fas-deficiency MRL/lpr mice were significantly increased compared with those in splenocytes of MRL/MPJ mice (Figure 4A and 4B), which contributed to splenomegaly of MRL/lpr mice. However, CD3+ T cells in MRL/MPJ mice proliferated more significantly and rapidly after 24 h in vitro CpGC stimulation of splenocytes compared with those in MRL/lpr mice (Figure 4A). CD3+ T cells frequency in stimulated splenocytes of MRL/MPJ mice was close to that in stimulated splenocytes of MRL/lpr mice (Figure 4A). But DN T cells frequency in CD3+ T cells of MRL/MPJ was still negligible after 24 h PI stimulation of splenocytes (Figure 4B). That indicated CD3+ T cells in MRL/lpr mice have a defective proliferation. That also indicated Fas deficiency in MRL/lpr mice but not in vivo inflammation induced by increased pro-inflammation cytokines level resulted in DN T cells accumulation.

In addition to reducing CD138+ T cells frequency in splenocytes, 4 h PI stimulation also significantly reduced both CD3+ T cells and DN T cells frequency in splenocytes of MRL/lpr mice (Figure 4C and 4D). Moreover, we cultured splenocytes of MRL/lpr mice without any stimulation for 24 h. CD138+ T cells frequency in splenocytes evidently and rapidly increased after 24 h culture of splenocytes in vitro (Figure 4E). The 24 h in vitro culture simultaneously also enhanced CD3+ T cells frequency and DN T cells accumulation in splenocytes (Figure 4E). These results demonstrated CD138 expression could significantly contribute to the increased CD3+ T cells frequency and DN T cells accumulation in splenocytes of MRL/lpr mice.

## **PI stimulation could induce specific apoptosis of CD138+ T cells**

Next, we sought the underlying mechanisms of PI stimulation preventing the accumulation of CD138+ T cells in MRL/lpr mice. Splenocytes activated with 4 h PI stimulation had a significantly increased apoptotic cells number and a decreased live cells number in CD138+ T cells (Figure 5A). But PI stimulation did not evidently affect the numbers of apoptotic and live cells in CD3+CD138- T cells (Figure 5A). Interestingly, PI stimulation even decreased apoptotic cells number and simultaneously increased live cells number in CD3-CD138+ plasma cells (Figure 5B). That indicated PI stimulation specifically induced cellular apoptosis in CD3+CD138+ T cells, but not in CD3+CD138- T cells and CD3-CD138+ plasma cells. So, our results demonstrated PI stimulation significantly prevented CD138+ T cells accumulation by inducing the specific apoptosis of CD138+ T cells. Moreover, the increased levels of apoptosis in CD138+ T cells induced by PI stimulation were not caused by its cytotoxic effect<sup>21,22</sup>.

## **PI stimulation significantly promotes the activation of CD138+ T cells**

Further we observed PI stimulation had significantly elevated the activation level in CD3+ T cells of MRL/lpr mice with increased CD69+ cells frequency and FasL expression in them (Figure 5C). Both CD4+ T cells and DN T cells simultaneously showed significant increases in the frequencies of CD69+ cells after 4 h PI stimulation (Figure 5D). CD69+ cells frequency and FasL expressions in CD138+ T cells of MRL/lpr mice were also significantly increased after 4 h PI stimulation (Figure 5E and 5F). Both CD138+ DN T cells and CD4+CD138+ T cells showed significant increases in FasL expression after 4 h PI stimulation (Figure 5F). However, PI stimulation failed to significantly promote the activation of CD138- T cells in MRL/lpr mice (Figure 2D). That indicates PI stimulation results in the increased apoptosis level of CD138+ T cells, and simultaneously and significantly activates CD138+ T cells but not CD138- T cells in splenocytes of MRL/lpr mice. These results further indicate CD138 expression could promote the activation of CD138- T cells in MRL/lpr mice.

## **The frequency of CD138+ T cells was inversely correlated with the activation level of CD138+ T cells**

We isolated the fresh splenocytes from MRL/lpr mice, and cultured splenocytes in vitro without any stimulation. CD138+ T cells frequency rapidly increased in the 48 h (Figure 6A) in vitro culture accompanied with a down regulated activation level in CD3+ T cells and CD138+ T cells (Figure 6B, 6C and 6D). Then, CD138+ T cells frequency began to decrease (Figure 6A) accompanied with an elevated level of activation in CD3+ T cells and CD138+ T cells after 48 h in vitro culture (Figure 6B, 6C and 6D). That indicated CD138+ T cells frequency was inversely correlated with the activation level of CD3+ T cells and CD138+ T cells in MRL/lpr mice. That suggested that PI stimulation may promote the activation of CD138+ T cells to induce specific apoptosis of CD138+ T cells and to prevent CD138+ T cells accumulation.

# CD69 expression in CD138+ T cells results in significantly increased level of apoptosis in CD138+ T cells

At last, we tried to demonstrate our hypothesis that the activation of CD138+ T cells induced by PI stimulation caused their apoptosis. Consistent with our speculation, we observed CD69+ cells in CD138+ T cells of MRL/lpr mice had a significantly increased apoptosis level compared with CD69- cells in CD138+ T cells (Figure 6E). But CD69 expression failed to increase the apoptosis level of CD138- T cells in MRL/lpr mice (Figure 6F). Our results indicate CD69 expression in CD138+ T cell could promote its specific apoptosis and demonstrate PI stimulation results in increase of specific apoptosis in CD138+ T cells by activating them via increasing their expressions of CD69.

## Discussion

Our study has showed that CD138+ T cells apoptosis in MRL/MPJ mice was in Fas dependent way. But CD138 expression in CD3+ T cells of MRL/lpr mice significantly prevented the apoptosis of CD3+ T cells, contributed to CD3+ T cells and DN T cells subsets accumulation and simultaneously promoted CD3+ T cells activation. Importantly CD138 expression could also increase FasL expression in DN T cells promoting the cytotoxicity of DN T cells. Moreover, decreased apoptotic and increased live numbers of CD138+ T cells led to the accumulation of CD138+ T cells in spleen of MRL/lpr mice. Our results demonstrated PI stimulation prevented CD138+ T cells accumulation and decreased CD138+ cells frequencies in DN and CD4+ T cells by inducing the specific apoptosis of CD138+ T cells. Our results demonstrated PI stimulation caused specific apoptosis in CD138+ T cells via increasing CD69 expression in them. Our results also suggested CD138 expression in CD3+ T cells was probably caused by the failure of activation in autoreactive T cells before self-antigens exposure to immune system.

Fas (CD95) is the member of the tumor necrosis factor receptor family and interacts with Fas ligand (FasL) after T cell receptor (TCR) activation to induce apoptosis<sup>16</sup>. Fas deficiency leads to DN T cells accumulation in MRL/lpr lupus mice resulting in lymphadenectasis and splenomegaly<sup>23,24</sup>. In addition to DN T cells, our results also showed apoptosis of CD138+ T cells was in a Fas-dependent way and indicated Fas deficiency also led to CD138+ T cells accumulation in MRL/lpr mice as the age increased and the lupus developed<sup>12</sup>. Our results showed CD138+ T cells had a high level of CD69 expression but a low level of CD25 expression. Importantly, the majority of CD138+ T cells were CD4 and CD8 double negative. CD138 expression significantly increased FasL expression in CD3+ T cells and their subsets including DN T cells. However, DN T cells in MRL/lpr mice are strongly cytotoxic, overexpressing FasL, which results in autoimmune injuries of multiple tissues that express small amounts of Fas receptor<sup>16,25</sup>. That indicated CD138 expression increased DN T cells cytotoxicity which could promote the lupus development and tissue injuries in MRL/lpr mice.

Our results showed CD3+ T cells accumulated but had a defective proliferation in Fas-deficiency MRL/lpr mice. Moreover the accumulated CD138+ T cells had a significant decrease in apoptotic number of cells

and simultaneously had a higher number of live cells compared with CD138- T cells demonstrating CD138+ T cells have a defective apoptosis in MRL/lpr mice. Importantly, previous study has shown that CD138+ T cells had a lower level of proliferation compared with CD138- T cells subsets<sup>12,14</sup>. According to these results, we conclude that CD138+ T cells accumulation was caused by Fas deficiency leading to their defective apoptosis but not hyper proliferation. In addition, our results also demonstrated CD138 expression greatly contributes to the accumulation of T cells and DN T cells in MRL/lpr mice by significantly decreasing the apoptotic number of T cells and DN T cells.

Production of autoantibodies has a detrimental effect on multiple organs and plays a key role in the progress of SLE<sup>26</sup>. We know immature T cells will experience positive selection and negative selection to be mature single positive T cells that cannot recognize self-antigens<sup>27,28</sup>. Autoreactive T cells will be deleted by Fas-mediated apoptosis during negative selection in the thymus<sup>29</sup>. Fas deficiency give the chance of autoreactive T cells to pass through the negative selection<sup>30,31</sup>. Autoreactive B cells may also avoid the apoptosis in negative selection induced by Fas-dependent apoptosis<sup>32</sup>. Our results has shown Fas deficiency results in the accumulation of CD138+ T cells in MRL/lpr mice. In addition, CD138+ T cells including CD4+CD138+ T cells commonly express B220 which has been demonstrated to express on those autoreactive T cells such as nonselected CD8+ T cells and DN T cells<sup>29,33</sup>. Importantly, CD138+ T cells have been demonstrated to be key in the progression of anti-dsDNA antibody secretion with a CD4 receptor dependent way<sup>12</sup>. CD138+ T cells have been also reported to promote the tissue injuries in condition that self-antigens are exposed to the immune system<sup>7,12</sup>. These results demonstrate accumulated CD138+ T cells had large numbers of the auto-reactive T cells that had avoided Fas-dependent apoptosis in negative selection<sup>12,14</sup>. CD4+CD138+ T cells in MRL/lpr mice had a significant decrease in CD4 expression compared with CD4+CD138- T cells. It had been reported that CD4+ T cells could convert into DN T cells<sup>34,35</sup>. CD4+CD138+ T cells in MRL/lpr mice may be the precursor of DN T cells which were originated from CD4+ T cells that have the potential of conversion into DN T cells.

CD138 expression in T cells plays a key role in the progression of lupus in MRL/lpr mice. Our results showed CD138 expression could contribute to the accumulation of CD3+ T cells by significantly preventing their apoptosis. Moreover, CD138+ T cells showed to be activated more easily compared with CD138- T cells indicating CD138 expression could promote the activation of T cells in MRL/lpr mice. That suggested CD138 expression could contribute to autoreactive T cells accumulation and simultaneously promote autoreactive T cells to be activated by autoreactive B cells, by which abnormal plasma cells formation was increased<sup>12</sup>. However, the mechanisms of CD138 expression in these abnormal T cells were still undeciphered. Our results showed CD138- T cells in MRL/MPJ mice were more easily activated compared with CD138- T cells in MRL/lpr mice. PI stimulation also failed to significantly activate CD3+CD138- T cells in splenocytes of MRL/lpr mice. These results indicated CD138- T cell had an evidently defective activation in MRL/lpr mice. Our results simultaneously showed PI stimulation significantly decreased CD138 expression in CD138+ T cells. PI stimulation was able to significantly reduce frequency of CD138+ cell in CD3+ T cell and its cell subsets with increased activation level of CD3+ T cell and CD138+ T cells. Previous research also has showed TCR activation negatively regulated

CD138 expression frequency in DN T cells<sup>14</sup>. These results demonstrated CD138 expression in CD3+ T cells could be prevented by the stimulation to activate CD3+ T cells.

Isolated splenocytes from MRL/lpr mice were cultured in vitro in the medium without cellular stimulation. We observed CD138+ T cells frequency increased in the 48 h accompanied with decreased activation level of CD3+ T cells and CD138+ T cells, and then decreased gradually after 48 hours accompanied with increased activation level of CD3+ T cells and CD138+ T cells. That indicated CD138+ T cells frequency was inversely correlated with the activation level of CD3+ T cells and CD138+ T cells. Previous research has demonstrated CD138+ T cells had large numbers of autoreactive T cells that promote autoantibody production in the presence of dsDNA, and enhanced disease progression in SLE by rapidly activating autoreactive B cells when self-antigens are exposed to the immune system<sup>12</sup>. These evidences suggest that CD138 expression in CD3+ T cells was probably due to failure of activation in these abnormal T cells in absence of self-antigens exposure.

In our present study, PI stimulation reduced CD138+ T cells frequency in splenocytes of MRL/lpr mice. Moreover, our results showed PI stimulation led to specific apoptosis of CD138+ T cells. PI stimulation simultaneously promoted the activation of CD138+ T cells with increased CD69 cells frequency and FasL expression in them. What is important, CD69+ cells in CD138+ T cells had a significant increase in apoptosis level compared with CD69- cells in CD138+ T cells. According to our results, we confirmed that the increased apoptosis of CD138+ T cells and subsequent decreased accumulation of CD138+ T cells were induced by PI stimulation via increasing CD69 expression in CD138+ T cells. Our research uncovered the role of CD138 expression in CD3+ T cells, and why to activate CD3+ T cells significantly increased the apoptosis of CD138+ T cells and prevented the accumulation of CD138+ T cells in MRL/lpr mice. We also provided the novel insight into the mechanisms preventing the autoreactive T cells accumulation in SLE.

## Conclusion

CD138 expression in T cells played a key role in the progression of lupus in MRL/lpr mice. CD138 expression in CD3+ T cells of MRL/lpr mice prevented apoptosis of T cells, simultaneously contributed to CD3+ T cells and DN T cells accumulation and promoted CD3+ T cells activation. CD138 expression in DN T cells significantly increased their FasL expression enhancing the cytotoxicity of DN T cells. Defective apoptosis levels caused by CD138 expression in CD138+ T cells resulted in CD138+ T cells accumulation in spleen of MRL/lpr mice. Our results demonstrated PI stimulation prevents CD138+ T cells accumulation by increasing the specific apoptosis of them. Our results further demonstrated that PI stimulation could induce specific apoptosis of CD138+ T cells via increasing CD69 expression in CD138+ T cells. Our results also indicated CD138- T cells in MRL/lpr mice had a defective activation and increased activation level of CD3+ T cells prevented CD138 expression in CD3+ T cells of MRL/lpr mice. That suggested CD138 expression in CD3+ T cells of Fas-deficiency MRL/lpr mice may be caused by the failure of activation in autoreactive T cells before self-antigens exposure to immune system.

# Declarations

## Authors' contributions

Tianhong Xie conceived the study and wrote the manuscript. Tianhong Xie and Ping Li designed the experiments. Tianhong Xie and Xin Liu performed the laboratory work. Tianhong Xie and Xin Liu performed data analysis. Ping Li revised and edited the manuscript. All authors read and approved the final version of the manuscript for publication.

## Conflicts of interest statement

The authors declare no conflict of interests.

## Acknowledgements

This work is funded by Postdoctoral Research Activity foundation of Beijing (ZZ2019-23) and the MiaoPu research foundation of the Beijing Institute of Traditional Chinese Medicine (MP-2020-45).

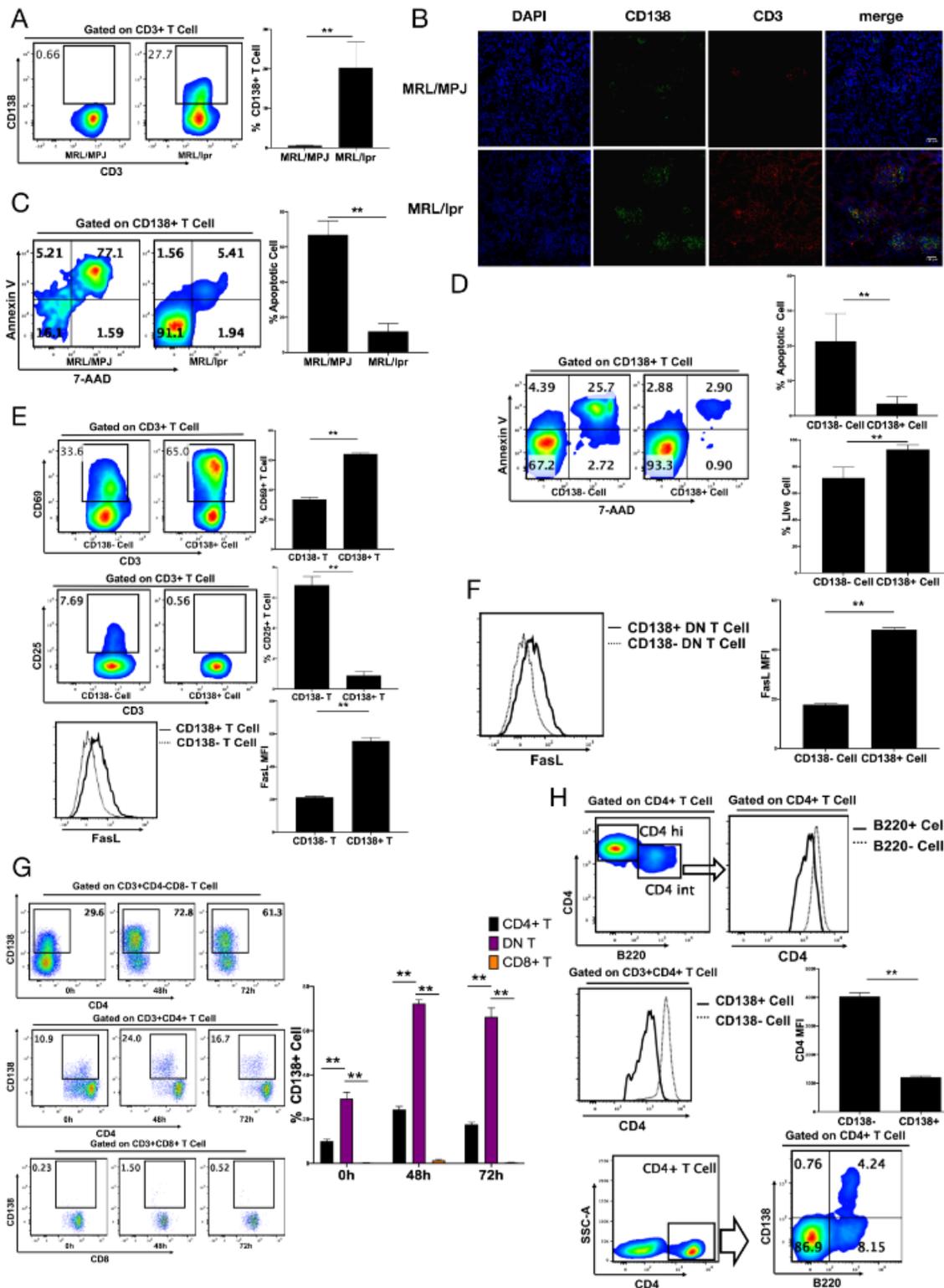
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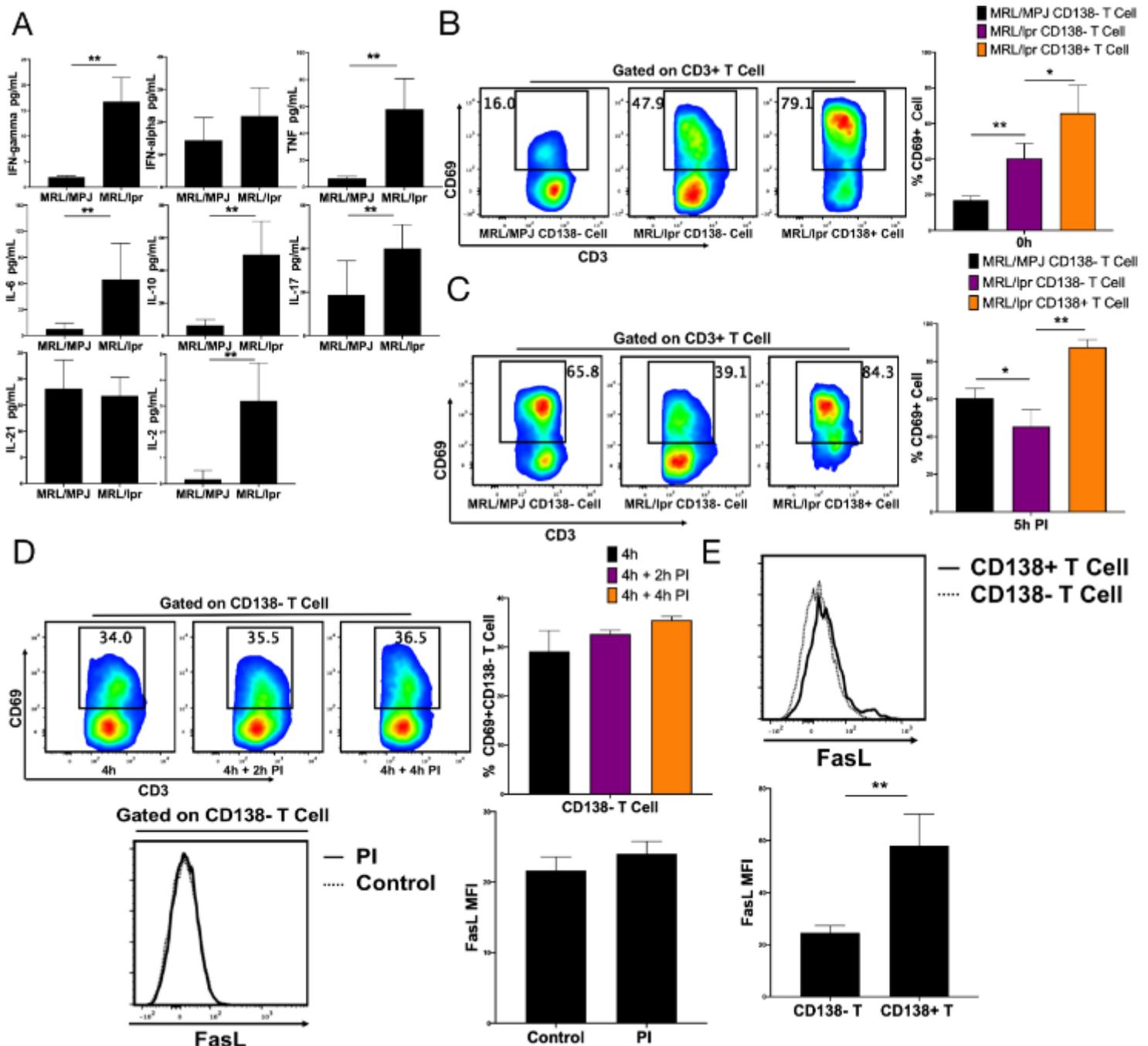
## Figures



**Figure 1**

(A) Flow cytometry analyses and bar charts denote frequencies of CD138+ cells in CD3+ T cells in fresh splenocytes of 17-18 week-age MRL/MPJ and of 17-18 week-age MRL/lpr mice. (B) Representative frozen kidney sections stained with CD3 (red), CD138 (green) and DAPI (blue) are shown to observe CD138+ T cell infiltration in renal tissue in MRL/lpr mice, original magnification: 200X, scale bars: 150  $\mu$ m. (C) Flow cytometry analyses and bar charts denote frequencies of apoptotic cells in CD3+CD138+T cells of fresh

splenocytes in MRL/MPJ and MRL/lpr mice. (D) Flow cytometry analyses and bar charts denote frequencies of apoptotic and live cells in CD3+CD138- and CD3+CD138+ T cells in fresh splenocytes of MRL/lpr mice. (E) Flow cytometry analyses and bar charts denote CD69+ and CD25+ cells frequency and FasL expression in CD3+CD138- and CD3+CD138+ T cells of fresh splenocytes in MRL/lpr mice. (F) Flow cytometry analyses and bar charts denote FasL expression in CD138- and CD138+ DN T cells of fresh splenocytes in MRL/lpr mice. (G) Flow cytometry analyses and bar chart denote CD138+ cells frequencies of DN, CD4+ and CD8+ T cells in fresh splenocytes of MRL/lpr mice, in fresh splenocytes of MRL/lpr mice in vitro cultured for 48 h and 72 h. (H) Flow cytometry analyses of CD4 expression in CD4+ T cells subsets. CD4+ T cells of MRL/lpr mice have two cells subsets including CD4hi and CD4int T cells. CD4 int T cells have significantly down-regulated CD4 expressions and simultaneously express B220. Bar chart denote CD4 expression in CD4+CD138+ and CD4+CD138- T cells in MRL/lpr mice. CD4+CD138+ T cells commonly expressed B220 and belonged to B220+CD4int T cells subsets. n=4-6 per group/experiment. Data are representative of 2-3 three independent experiments with reproducible results; Data are presented as mean±SD; \*\*P < 0.01 by one-way analysis of variance.



**Figure 2**

(A) Bar charts showing serum levels of IFN- $\gamma$ , IFN- $\alpha$ , TNF, IL-6, IL-10, IL-17, IL-21 and IL-2; (B) Flow cytometry analyses and bar chart denote frequencies of CD69+ cells in CD138- T cells of fresh splenocytes in 17-18 week-age MRL/MPJ mice, and in CD138- and CD138+ T cells of fresh splenocytes in 17-18 week-age MRL/lpr mice. (C) Flow cytometry analyses and bar chart denote frequencies of CD69+ cells in CD138- T cells of fresh splenocytes in 17-18 week-age MRL/MPJ mice, and in CD138- and CD138+ T cells of fresh splenocytes in 17-18 week-age MRL/lpr mice after 5 h in vitro PI stimulation. (D) Flow cytometry analyses and bar charts denote CD69+ cells frequencies in CD138- T cells of splenocytes in MRL/lpr mice after 0 h, 2 h and 4 h PI stimulation, respectively and FasL expression in CD138- T cells of splenocytes in MRL/lpr mice with and without 4 h PI stimulation. (E) Flow cytometry analyses and bar

chart denote FasL expression in CD3+CD138+ and CD3+CD138- T cells of 4 h PI-stimulated splenocytes in MRL/lpr mice. n=4-8 per group/experiment. Data are representative of 2-3 independent experiments with reproducible results. Data are presented as mean±SD; \*P < 0.05, \*\*P < 0.01 by one-way analysis of variance.

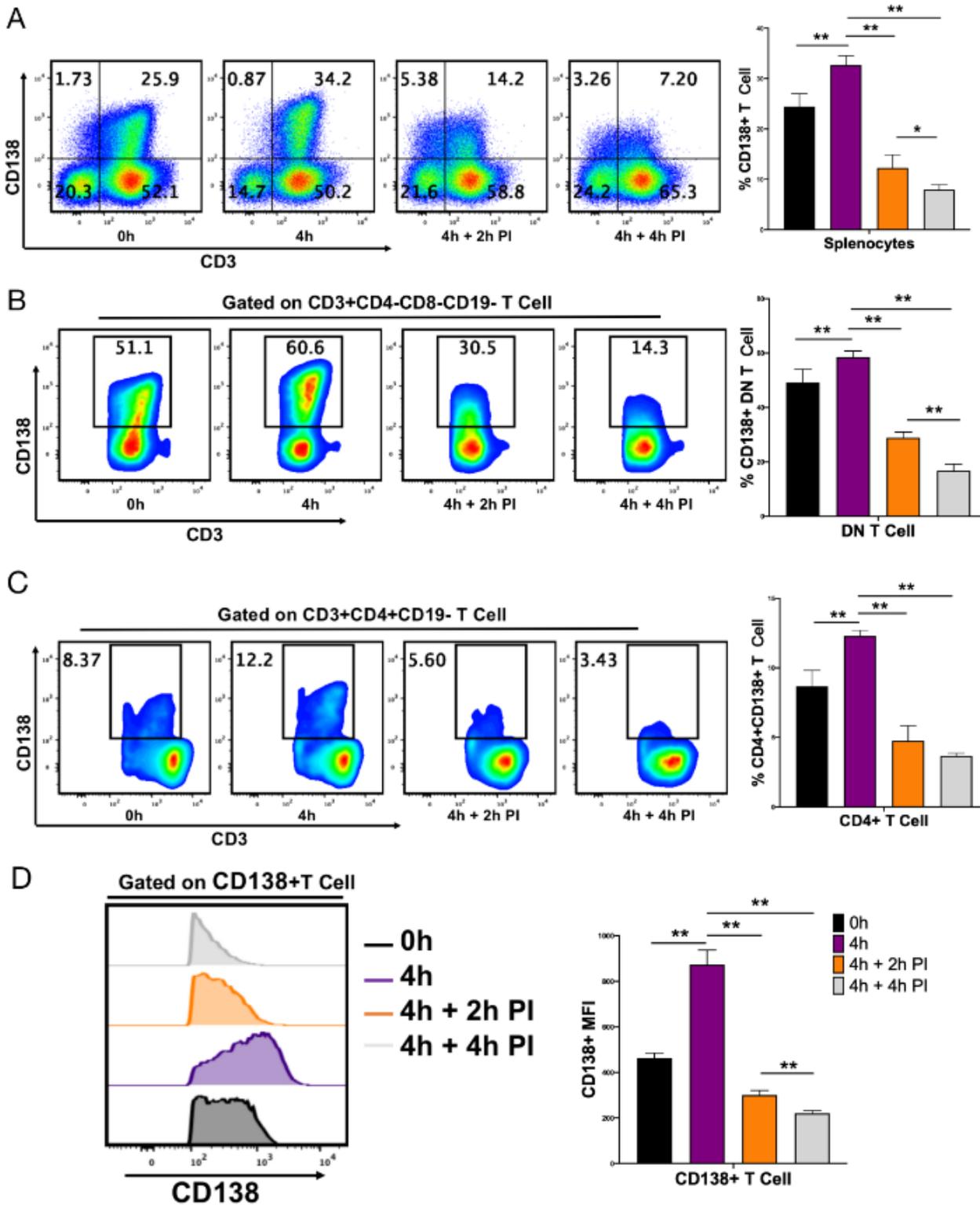
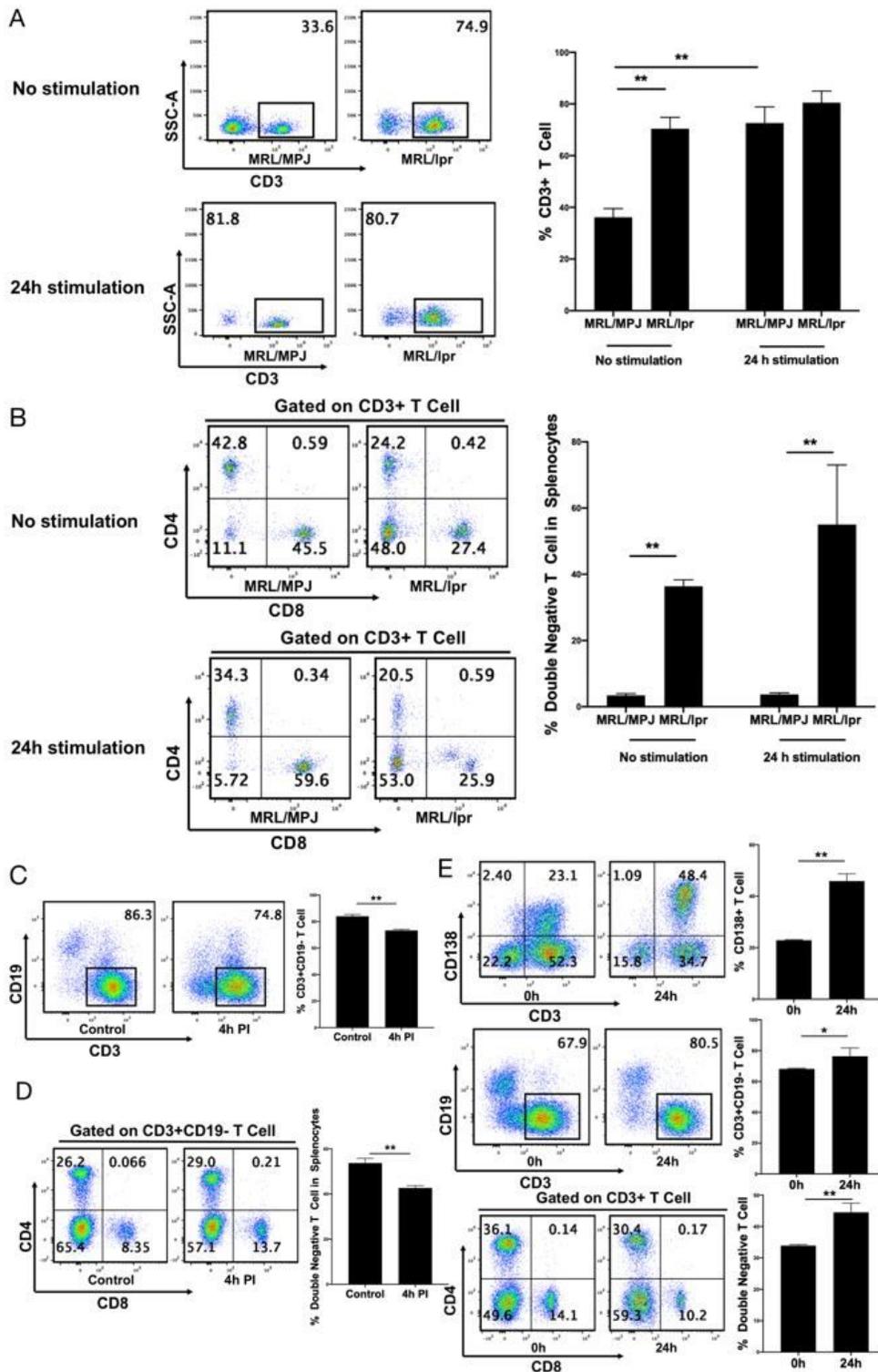


Figure 3

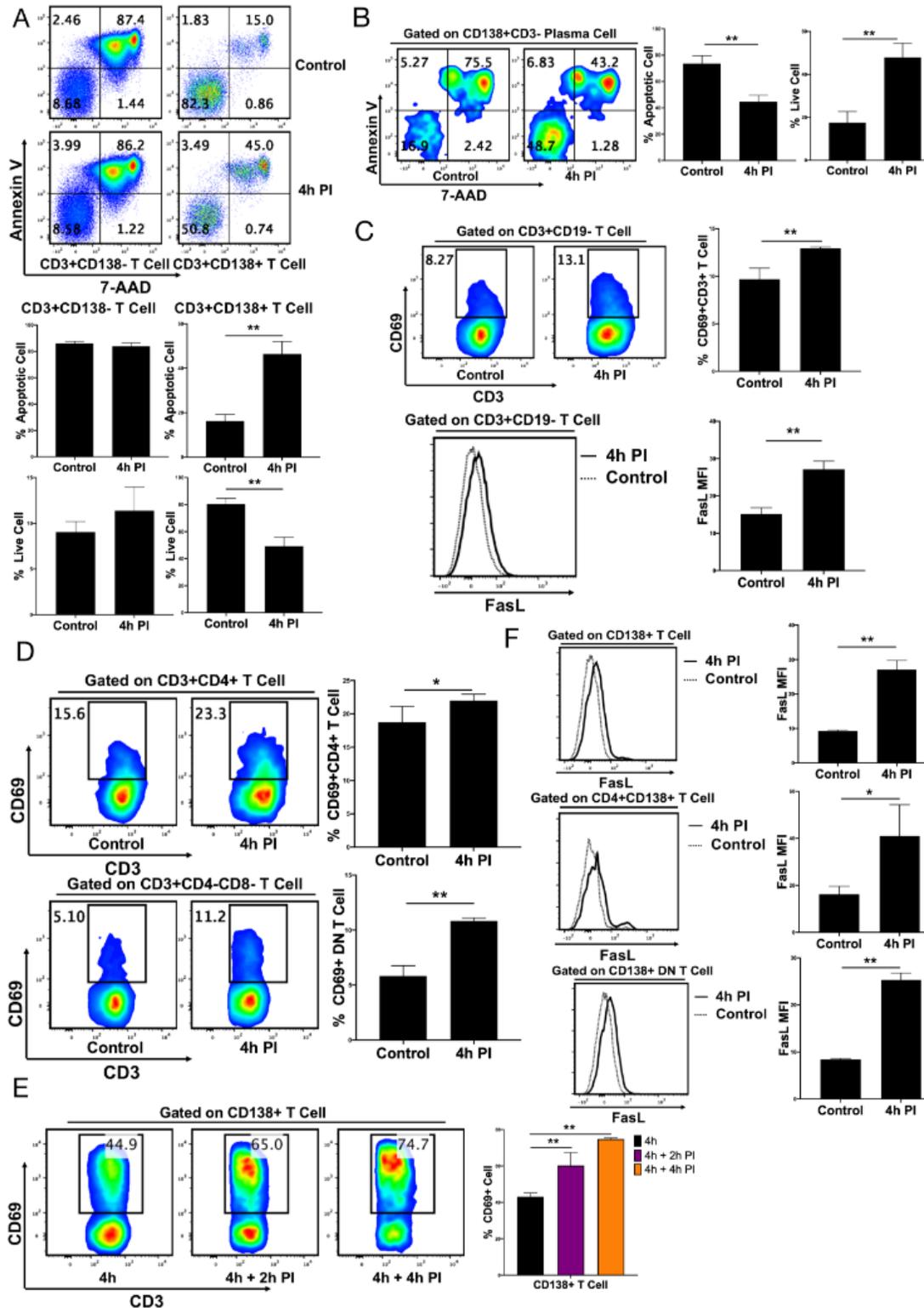
(A) Flow cytometry analyses and bar charts denote CD138<sup>+</sup> T cells frequencies in splenocytes of MRL/lpr mice in vitro cultured for 0 h, 4 h, 4 h + 2 h PI stimulation and 4 h + 4 h PI stimulation. (B) Flow cytometry analyses and bar charts denote CD138<sup>+</sup> cells frequencies in DN T cells of splenocytes in MRL/lpr mice in vitro cultured for 0 h, 4 h, 4 h + 2 h PI stimulation and 4 h + 4 h PI stimulation. (C) Flow cytometry analyses and bar charts denote CD138<sup>+</sup> cells frequencies in CD4<sup>+</sup> T cells of splenocytes in MRL/lpr mice in vitro cultured for 0 h, 4 h, 4 h + 2 h PI stimulation and 4 h + 4 h PI stimulation. (D) Flow cytometry analyses and bar charts denote CD138 MFI for CD138 expression in CD138<sup>+</sup> T cells of fresh splenocytes in MRL/lpr mice in vitro cultured for 0 h, 4 h, 4 h + 2 h PI stimulation and 4 h + 4 h PI stimulation. n=4-5 per group/experiment. Data are representative of 2-3 independent experiments with reproducible results; Data are presented as mean±SD; \*P < 0.05, \*\*P < 0.01 by one-way analysis of variance.



**Figure 4**

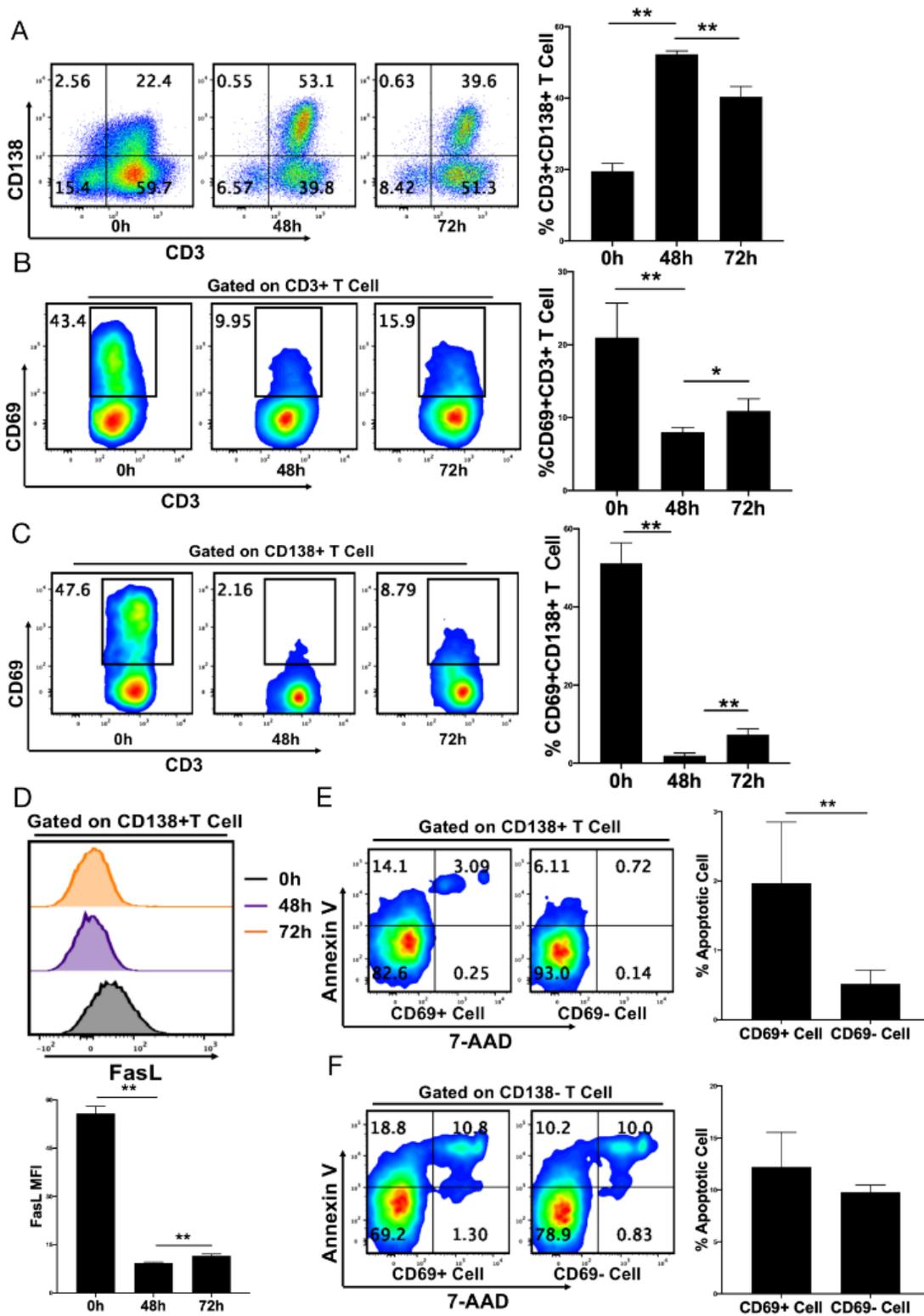
(A) Flow cytometry analyses and bar charts denote frequencies of CD3+ T cells in splenocytes of 17-18 week-age MRL/MPJ and 17-18 week-age MRL/lpr mice with and without 24 h in vitro CpGC stimulation. (B) Flow cytometry analyses and bar charts denote frequencies of DN T cells in CD3+ T cells of 17-18 week-age MRL/MPJ and 17-18 week-age MRL/lpr mice with and without 24 h in vitro CpGC stimulation. (C) Flow cytometry analyses and bar charts denote frequencies of CD3+ T cells in splenocytes of MRL/lpr

mice with and without 4 h in vitro PI stimulation. (D) Flow cytometry analyses and bar charts denote frequencies of DN T cells in splenocytes of MRL/lpr mice with and without 4 h in vitro PI stimulation. (E) Flow cytometry analyses and bar charts denote frequencies of CD138+, CD3+ and DN T cells in splenocytes of MRL/lpr mice with and without 4 h in vitro PI stimulation. n=4-5 per group/experiment. Data are representative of 2-3 independent experiments with reproducible results. Data are presented as mean±SD; \*P < 0.05, \*\*P < 0.01 by one-way analysis of variance.



## Figure 5

(A) Flow cytometry analyses and bar charts denote frequencies of apoptotic and live cells in CD3<sup>+</sup>CD138<sup>-</sup> and CD3<sup>+</sup>CD138<sup>+</sup> T cells in splenocytes of MRL/lpr mice with and without 4 h PI stimulation. (B) Flow cytometry analyses and bar chart denote frequencies of apoptotic and live cells in CD138<sup>+</sup>CD3<sup>-</sup> plasma cells in splenocytes of MRL/lpr mice with and without 4 h PI stimulation. (C) Flow cytometry analyses and bar charts denote CD69<sup>+</sup> cells frequencies and FasL expression in CD3<sup>+</sup> T cells in splenocytes of MRL/lpr mice with and without 4 h PI stimulation. (D) Flow cytometry analyses and bar charts denote CD69<sup>+</sup> cells frequencies in CD4<sup>+</sup> T cells of and in DN T cells of splenocytes in MRL/lpr mice with and without 4 h PI stimulation. (E) Flow cytometry analyses and bar charts denote CD69<sup>+</sup> cells frequencies in CD138<sup>+</sup> T cells of splenocytes in MRL/lpr mice after 0 h, 2 h and 4 h PI stimulation. (F) Flow cytometry analyses and bar charts denote FasL expressions in CD138<sup>+</sup>, CD4<sup>+</sup>CD138<sup>+</sup> and CD138<sup>+</sup> DN T cells of splenocytes in MRL/lpr mice with and without 4 h PI stimulation. n=4-5 per group/experiment. Data are representative of 2-3 independent experiments with reproducible results; Data are presented as mean±SD; \*P < 0.05, \*\*P < 0.01 by one-way analysis of variance.



**Figure 6**

(A) Flow cytometry analyses and bar chart denote frequencies of CD138<sup>+</sup> T cells in fresh splenocytes of MRL/lpr mice, in fresh splenocytes of MRL/lpr mice in vitro cultured for 48 and 72 h. (B) Flow cytometry analyses and bar chart denote CD69<sup>+</sup> cells frequencies in CD3<sup>+</sup> T cells frequency in fresh splenocytes of MRL/lpr mice, in fresh splenocytes of MRL/lpr mice in vitro cultured for 48 and 72 h. (C) Flow cytometry analyses and bar chart denote CD69<sup>+</sup> cells frequencies in CD138<sup>+</sup> T cells in fresh splenocytes of MRL/lpr

mice, in fresh splenocytes of MRL/lpr mice in vitro cultured for 48 h and 72 h. (D) Flow cytometry analyses and bar chart denote FasL MFI for FasL expression in CD138+ T cells in fresh splenocytes of MRL/lpr mice, in fresh splenocytes of MRL/lpr mice in vitro cultured for 48 and 72 h. (E) Flow cytometry analyses and bar charts denote apoptotic cells frequencies of CD69+ and of CD69- cells in CD138+ T cells of fresh splenocytes in MRL/lpr mice. (F) Flow cytometry analyses and bar charts denote apoptotic cells frequencies of CD69+ and of CD69- cells in CD138- T cells of fresh splenocytes in MRL/lpr mice. n=4-6 per group/experiment. Data are representative of 2-3 independent experiments with reproducible results; Data are presented as mean±SD; \*P < 0.05, \*\*P < 0.01 by one-way analysis of variance.

## Supplementary Files

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