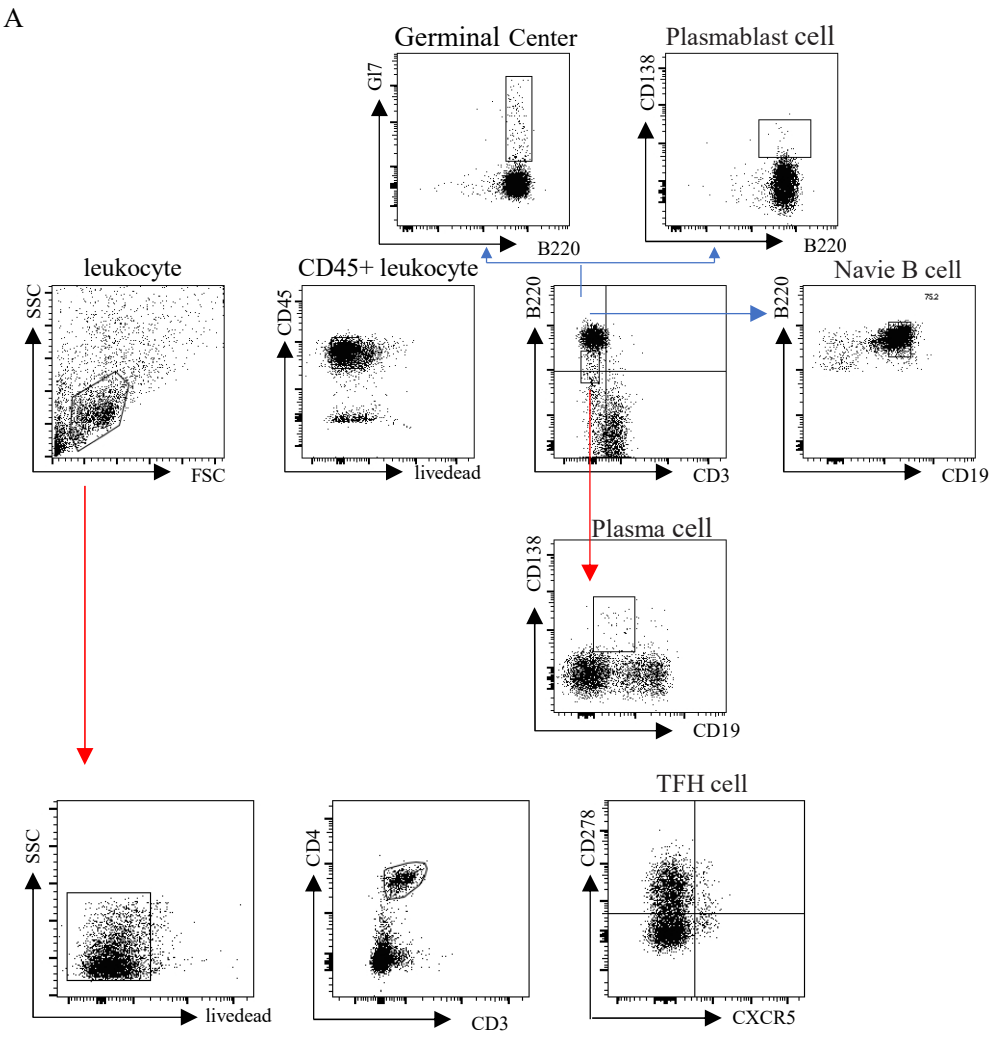
Figure S1 Gating strategies



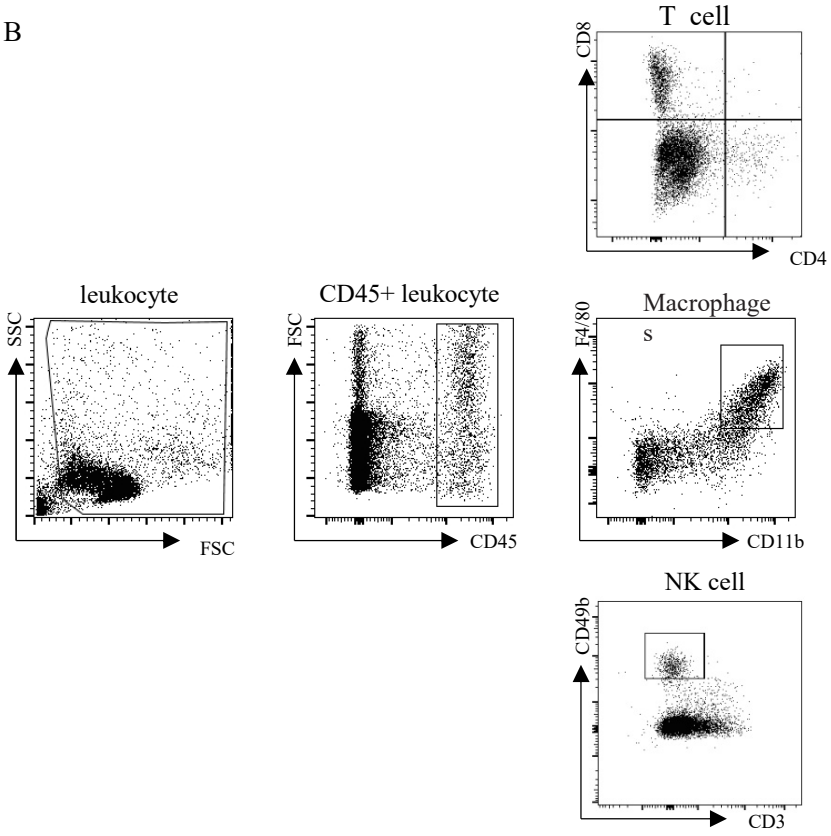


Figure S1. Gating strategy. (A) T and B cells subset from the spleen of AMR mice or Oxidized ATP treated AMR mice recipients were were analyzed. Naïve B cells (CD45+CD3-B220+CD19+); Germinal Center (CD45+CD3-B220+CD19+Gl7+); Plasmablasts (CD45+CD3-B220+CD138+); Plasma cells (CD45+CD3-B220lowCD19lowCD138+); TFH (CD45+CD3+CD4+CD278+CXCR5). (B) Graft infiltration by inflammatory cells of renal allograft recipient mice. T cells (CD45+CD3+); macrophages (CD45+F4/80+CD11b+); NK cells (CD45+CD49b+CD3-).

Figure S2

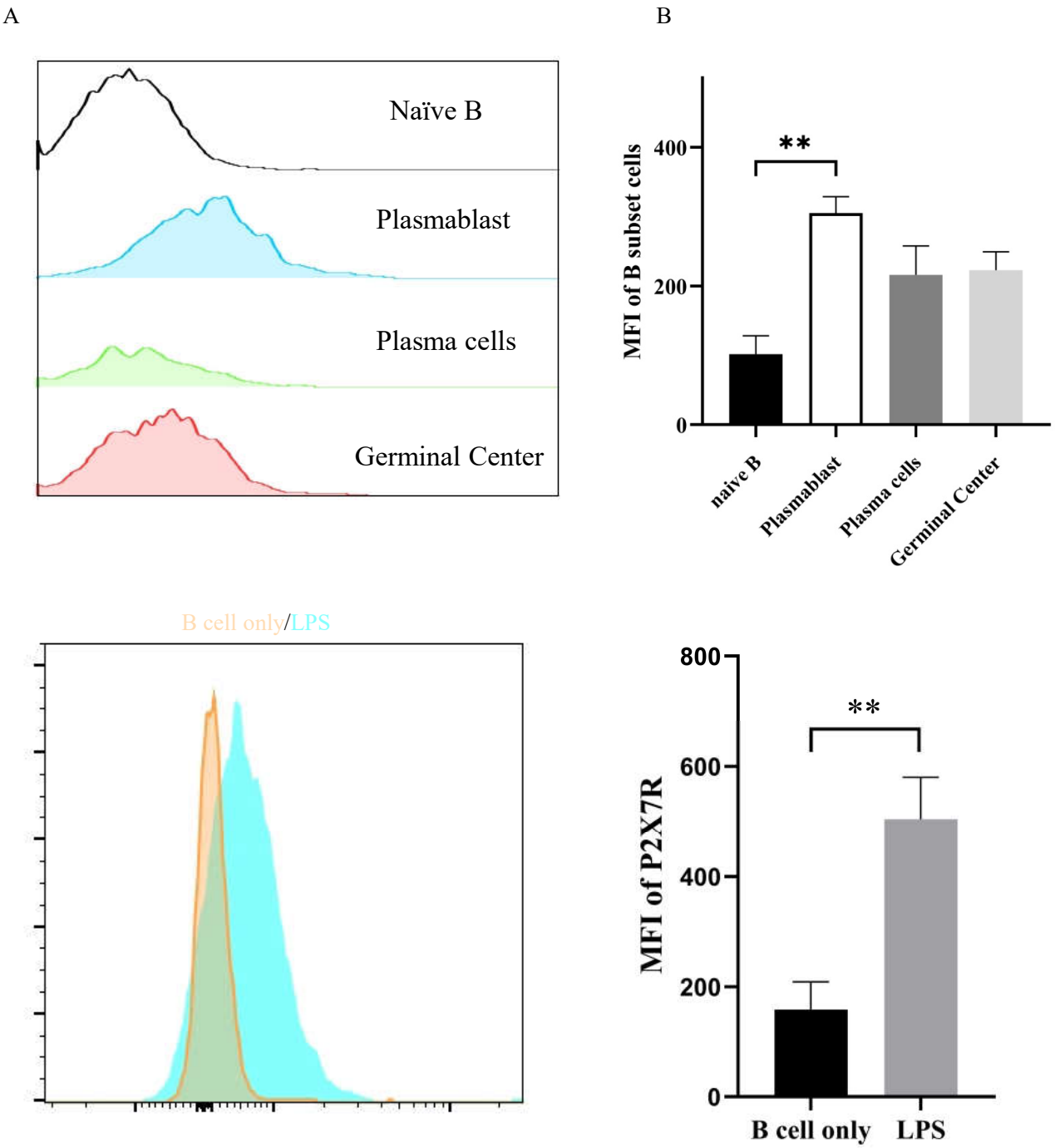
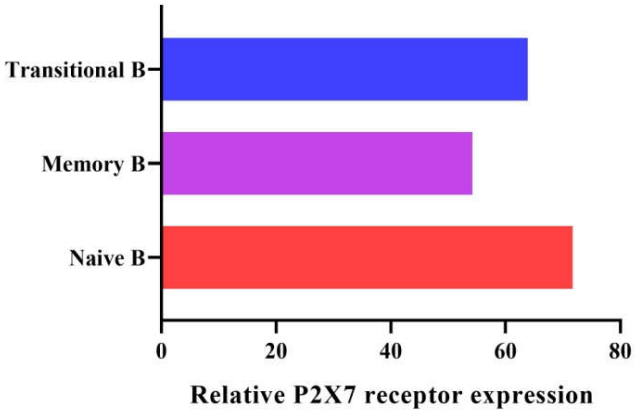


Figure S2. P2X7R expression on mouse B cell subsets. **(A)** B cell subsets from the spleen in AMR mice recipients on day 5. MFI of P2X7R expression on Naïve B cells (CD45+CD3-B220+CD19+); Germinal Center (CD45+CD3-B220+CD19+Gl7+); Plasmablasts (CD45+CD3-B220+CD138+); Plasma cells (CD45+CD3-B220lowCD19lowCD138+) were summarized. (B) Representative flow cytometric histograms and MFI of P2X7R expression on B cells in the presence of LPS or without LPS for 48 h. Data are mean ± SEM (n=5). **P < 0.01.

Figure S3

A



B

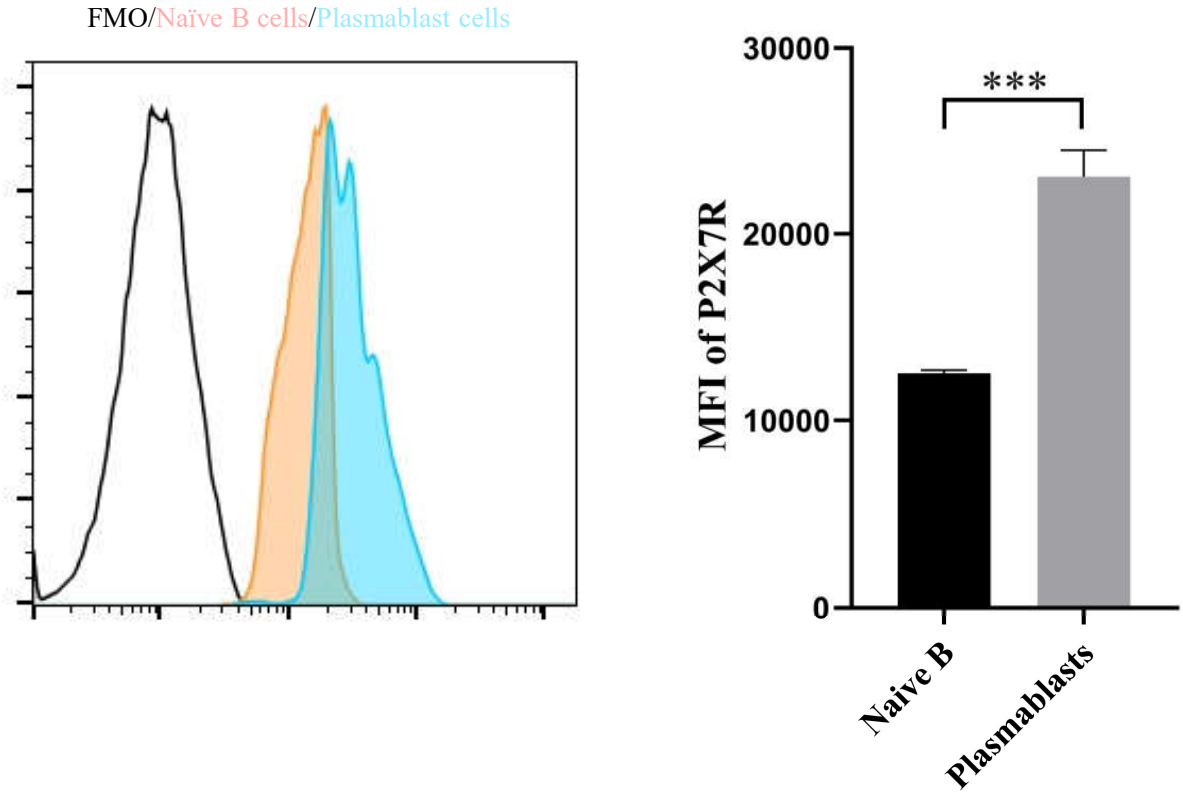


Figure S3. Expression of P2X7 receptor in subsets of human B lymphocytes. **(A)** Transcriptomic data from peripheral blood mononuclear cell of healthy individuals. *Naive B*, sorted as CD19+IgD+CD27-CD5-; *Memory B*, sorted as CD19+IgD-CD27+CD38-; *Transitional B*, sorted as CD19+IgD+CD27-CD5+. All data were taken from www.immgen.org and normalized by DESeq2. **(B)** P2X7B expression on human B cells were unregulated in a vitro model of differentiation of naive B cells (CD19+CD27-) into plasmablasts (CD19+CD27+CD138+) by use of human anti-CD40 and anti-IgM antibody. Data are mean ± SEM of three independent experiments. ***P < 0.001.

Figure S4

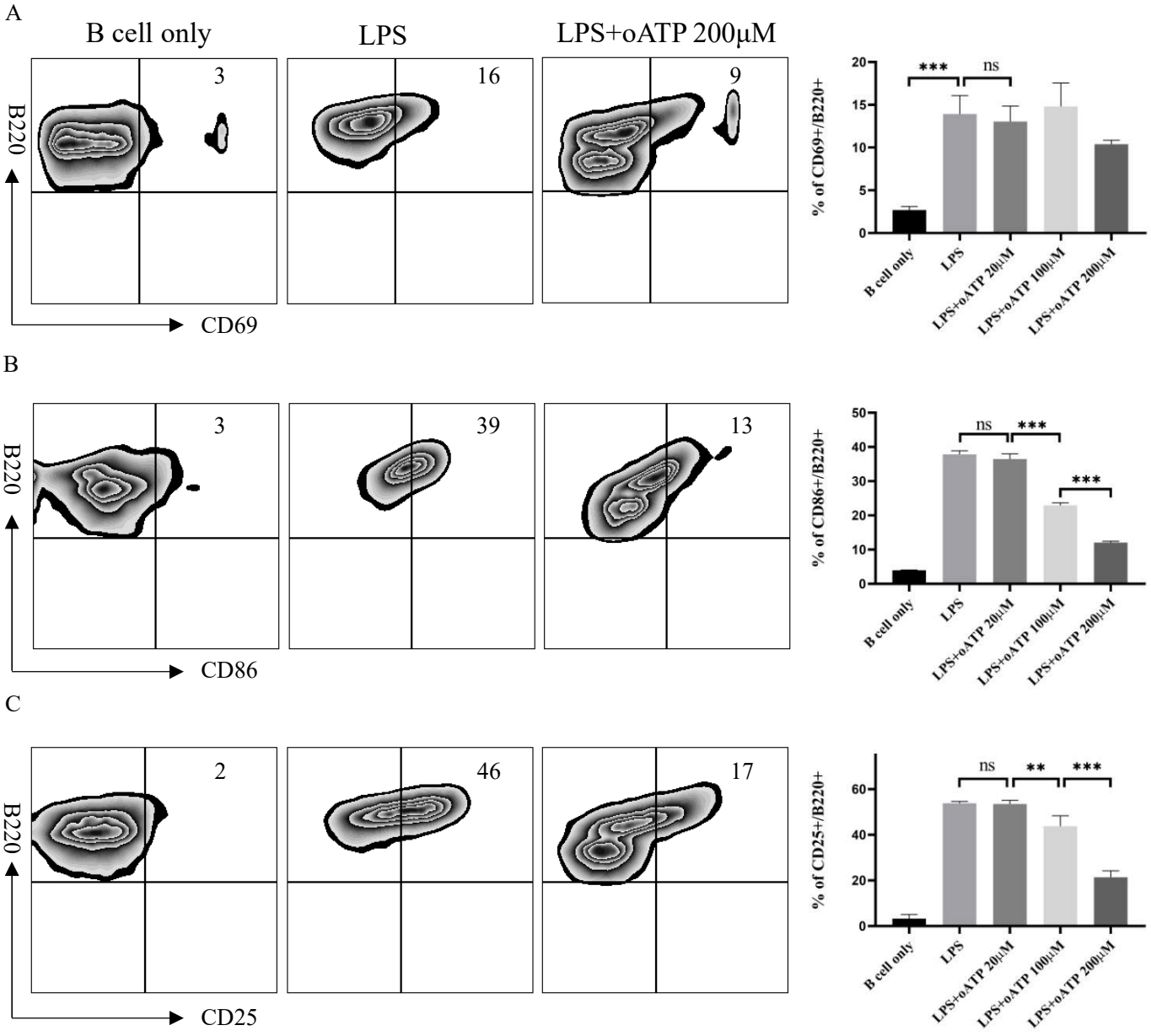


Figure S4. Oxidized ATP suppresses B cell activation *in vitro*. Primary cultures of B cells labeled with CFSE were treated with Oxidized ATP, then analyzed by flow cytometry for the presence of B cells in early stages of activation, based on staining against the markers **(A)** CD69 or **(B)** CD86; or in late stages of activation, based on staining against the marker **(C)** CD25. Representative flow cytograms are shown in the *left,* where the numbers within the plots refer to proliferation percentage of target cells. Quantitation of flow cytometric results are shown on the *right.* Data are mean ± SEM of three independent experiments. **P< 0.01, ***P< 0.001; ns, not significant.

Figure S5

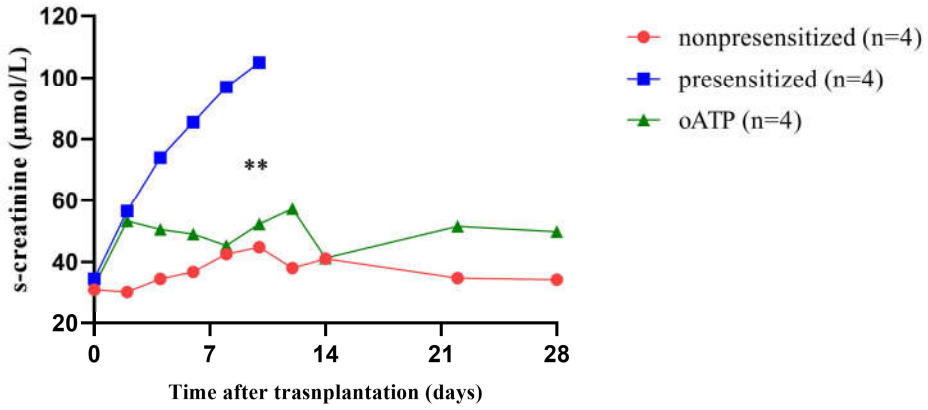
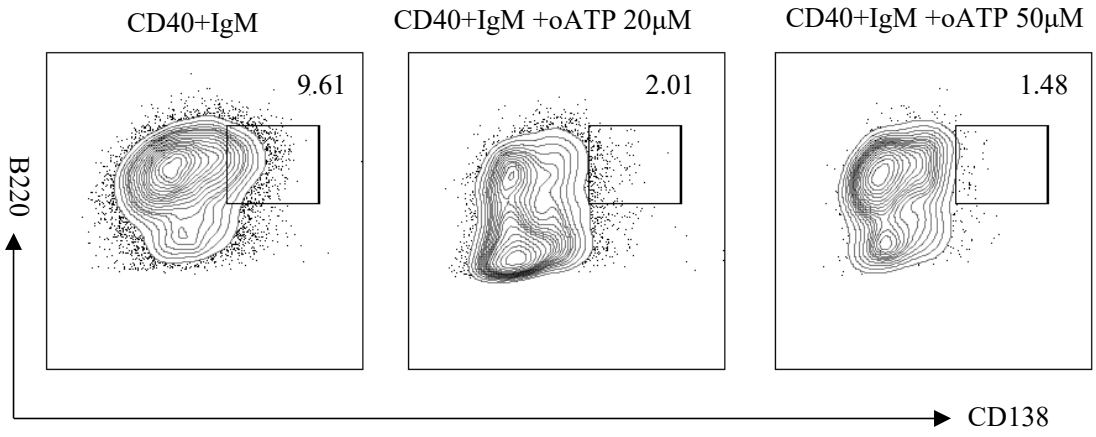


Figure S5. Oxidized ATP protects the renal graft function. Serum creatinine in the peripheral blood was detected at appointed day. Data are mean ± SEM (n=4). **P< 0.01.

Figure S6

A



B

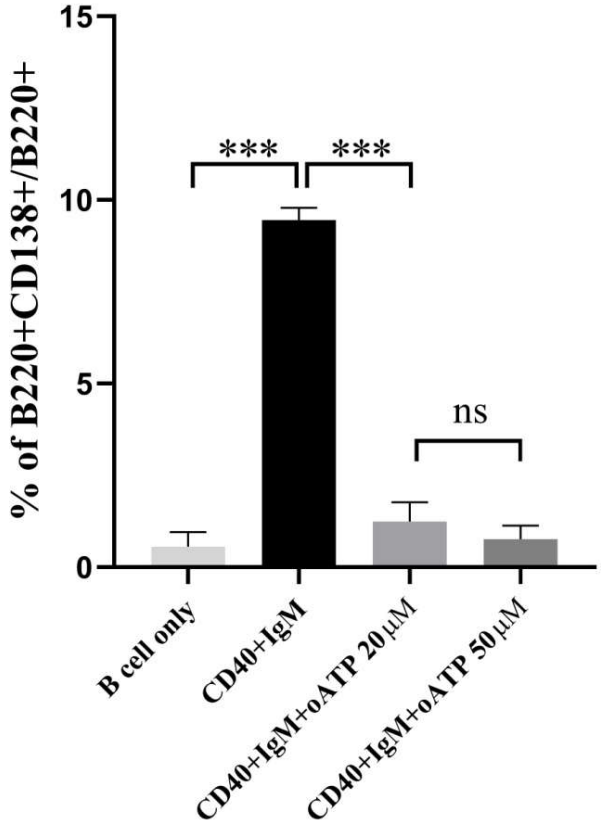


Figure S6. Oxidized ATP suppresses activation of B cells in culture with anti-CD40 antibody and anti-IgM antibody in a dose-dependent manner. **(A)** Representative flow cytometric histograms of B cells exposed for 6 days to the indicated concentrations of oxidized ATP with CD40 and IgM. The numbers within the histograms refer to proportion of cell proliferation. **(B)** Quantification of the flow cytometric experiments. Data are mean ± SEM of three independent experiments. ***P < 0.001; ns, not significant.

Figure S7

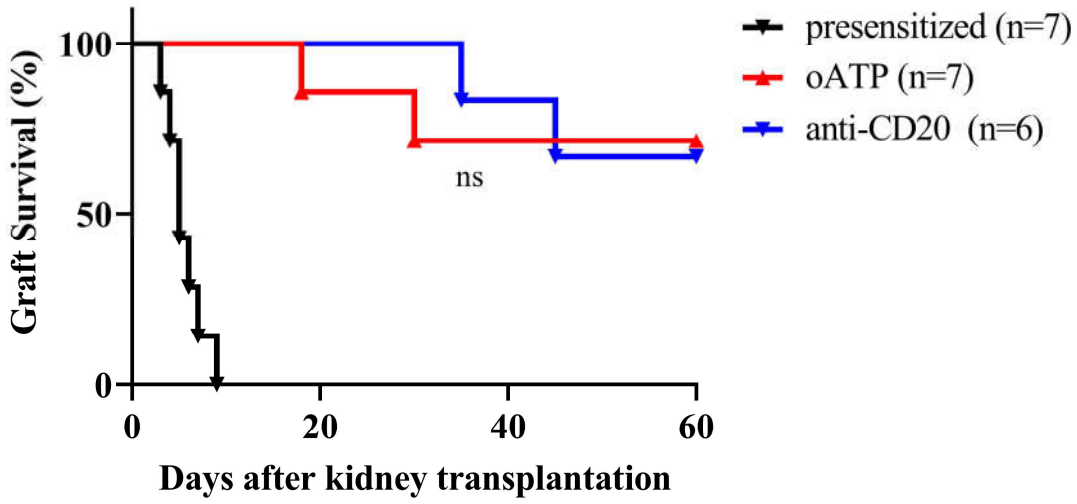


Figure S7. Graft survival curves of control animals that were presensitized (black) or presensitized animals that were treated with oxidized ATP (red) or anti-CD20 (blue) (6-7 animals per condition). The dose of oxidized ATP were given to the recipients as 0.01 mg/g per day from days 1 to 5 after skin transplantation and days 1 to 11 after kidney transplantation. 250 μg mab was given one day before and every one week after kidney transplantation in the anti-CD20 group. Data are mean ± SEM. ns, not significant.

Figure S8

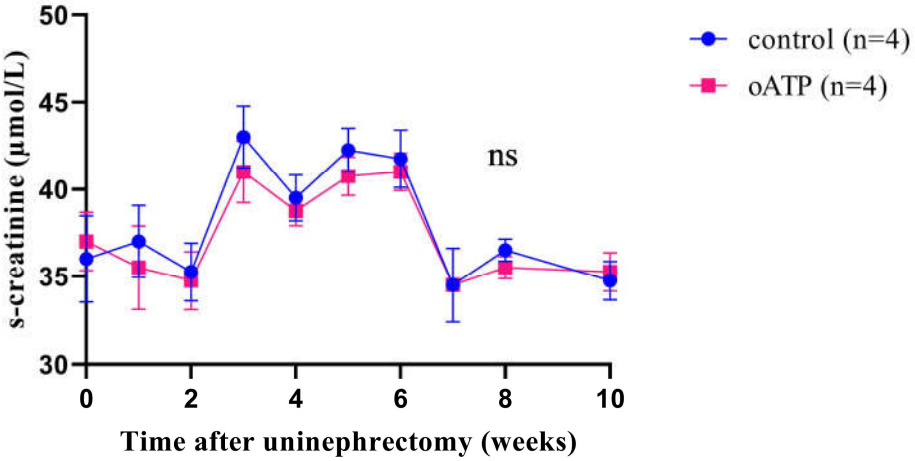
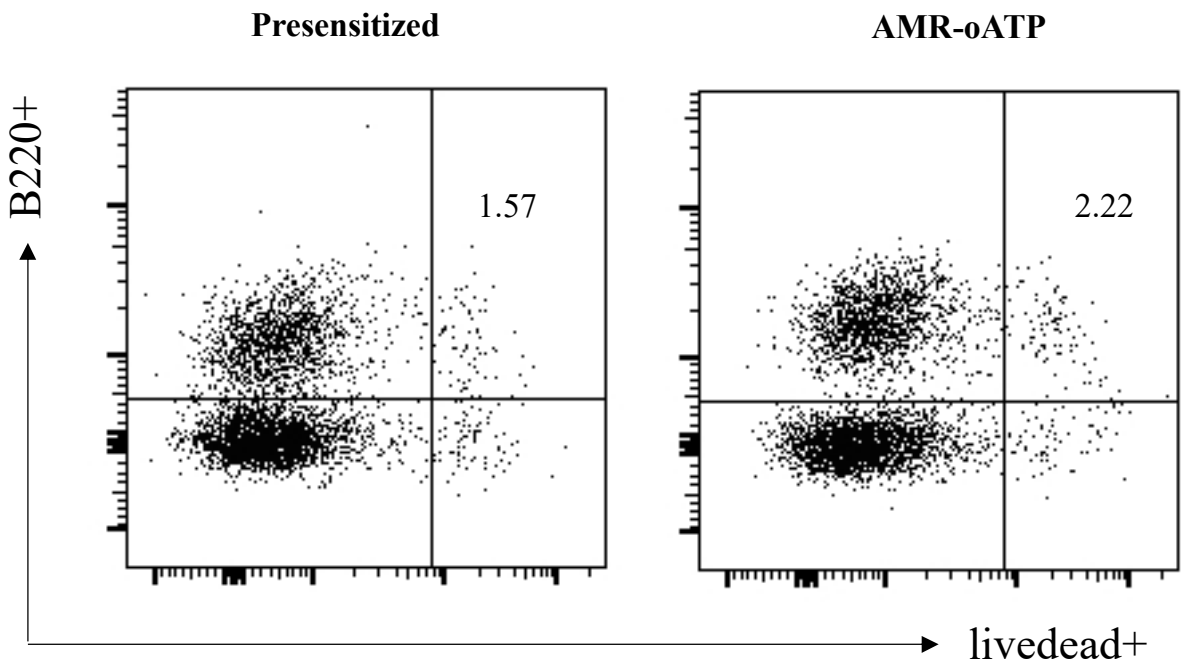


Figure S8. Oxidized ATP drug toxicity experiment by use of normal Balb/C mice with unilateral nephrectomy. No treatment of oxidized ATP as control group. Serum creatinine in the peripheral blood was detected every one week. Data are mean ± SEM (n=4); ns, not significant.

Figure S9

A



B

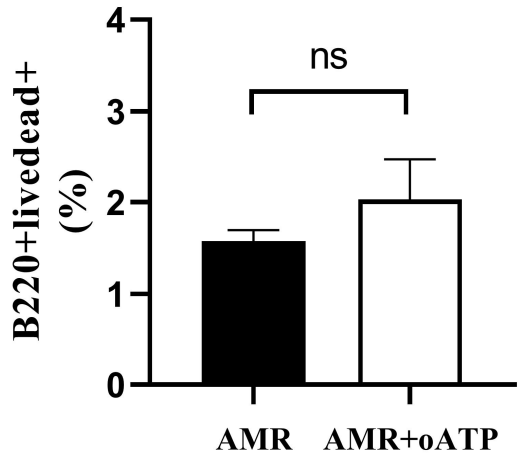


Figure S9. Oxidized did not induce B cells apoptosis of the spleen in vivo. **(A)** Representative flow cytometric histograms of B cells labeled with B220+ and Zombie red^{Tm} Cell active dye. The numbers within the histograms refer to proportion of cell apoptosis. **(B)** Quantification of the flow cytometric experiments. Data are mean ± SEM (n=4). ns, not significant.

Figure S10

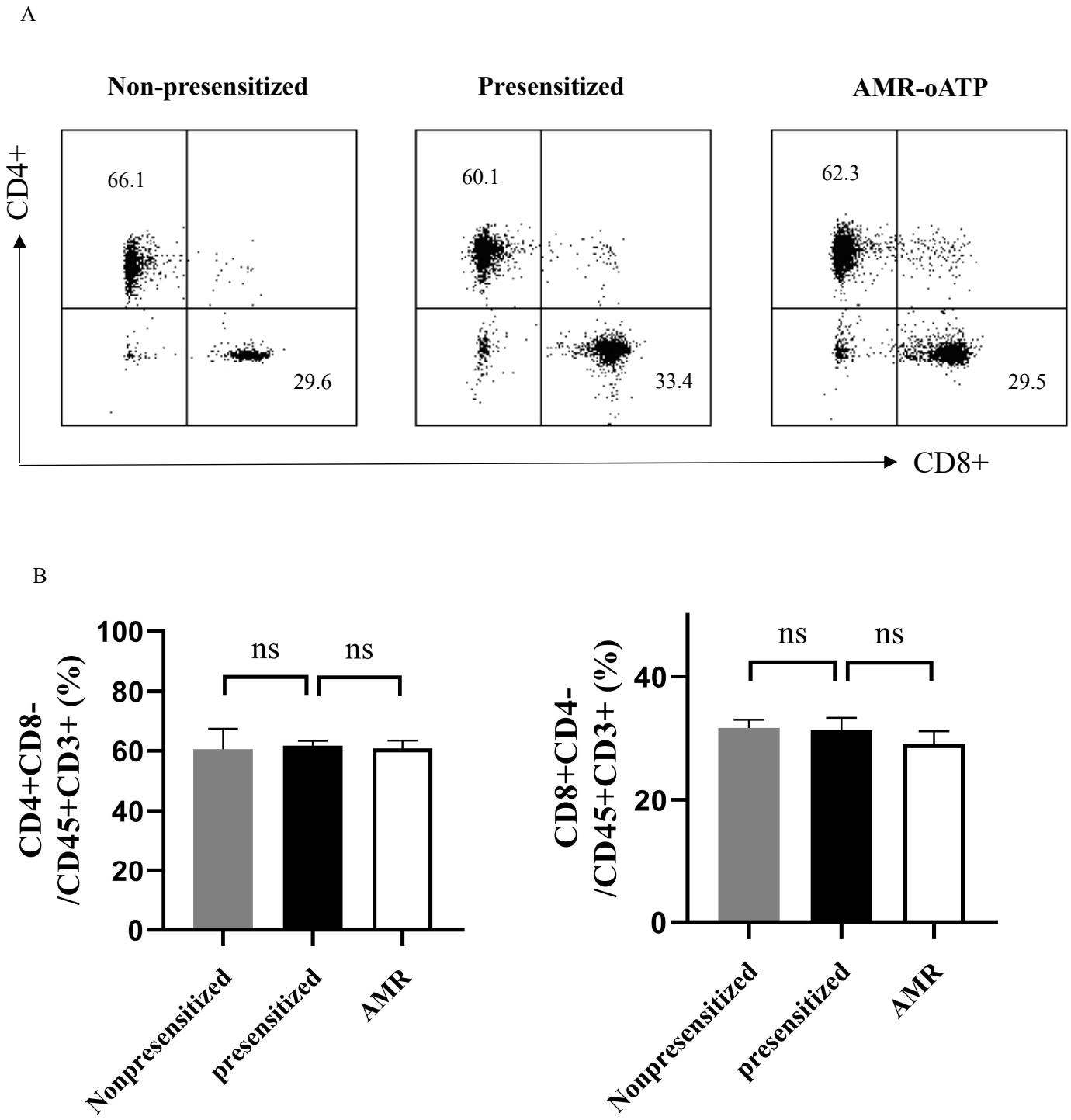


Figure S10. Oxidized ATP did not change the percentage of CD4+ and CD8+ in total CD45+CD3+ T cells of the spleen in presensitized group and oxidized ATP treated group. **(A)** Representative flow cytograms of CD45+CD3+CD4+ T cells and CD45+CD3+CD8+ T cells in the spleen; **(B)** Quantification of the flow cytometric experiments. Data are mean ± SEM (n=4). ns, not significant.