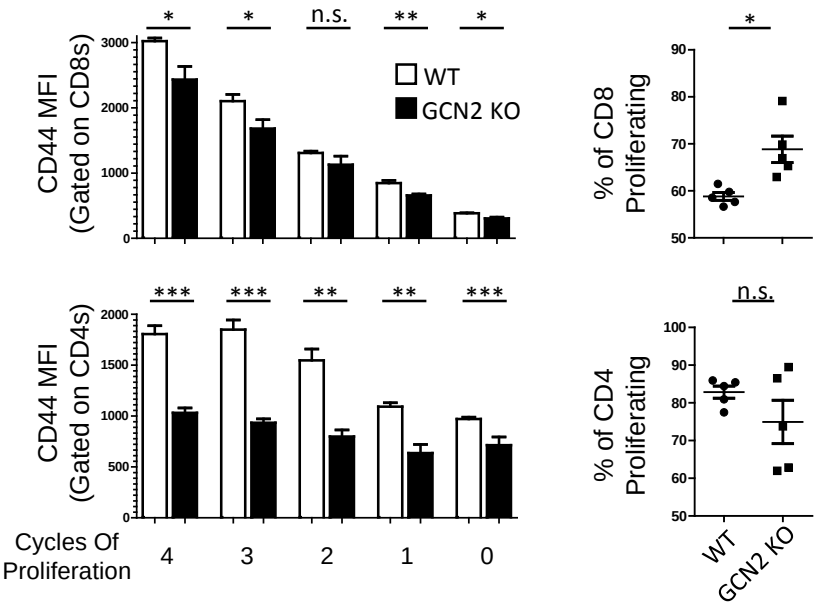
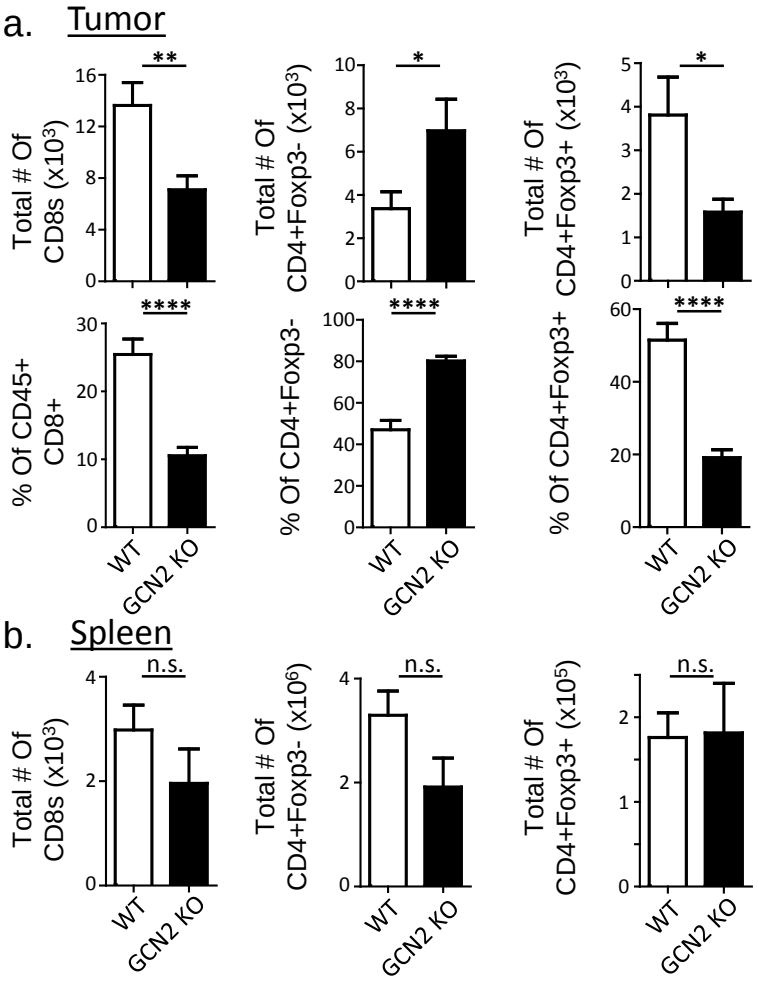
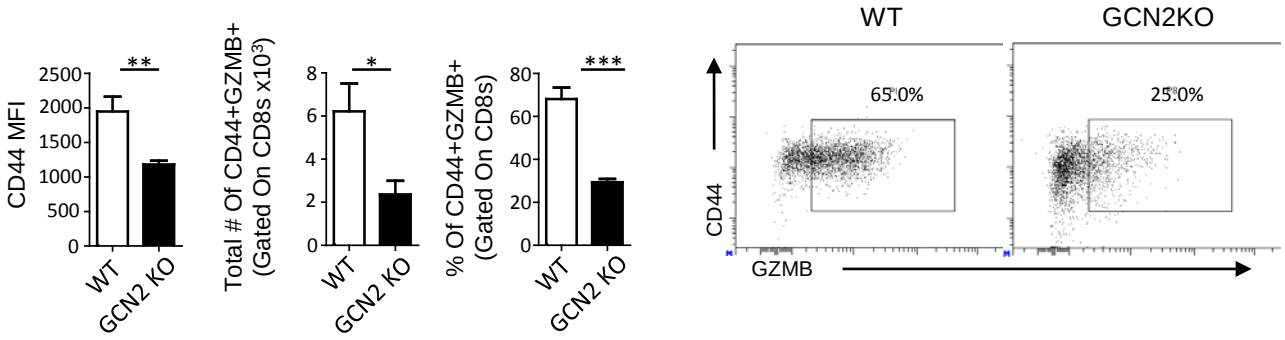


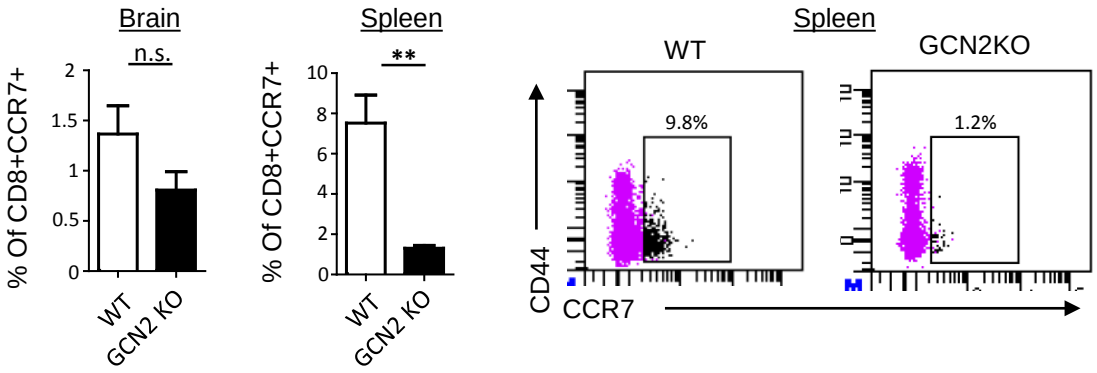
Supplementary Figure 1: Under optimal conditions, absence of GCN2 has minimal effect on T-cell proliferation *in-vitro*. T-cells were isolated from the spleen of WT and GCN2 KO mice, then labeled with CTV dye and stimulated with IL2, CD3, and CD28 for 72 hours. After 72 hours cells were lifted and their MFI for CD44 and proliferation rate were measured using flow cytometry. Flow cytometry statistics calculated and shown as percentage positive population, from n=3. Unpaired T-test analysis was used to calculate significance. $p<0.05$*; $p<0.01$**; $p<0.001$***.



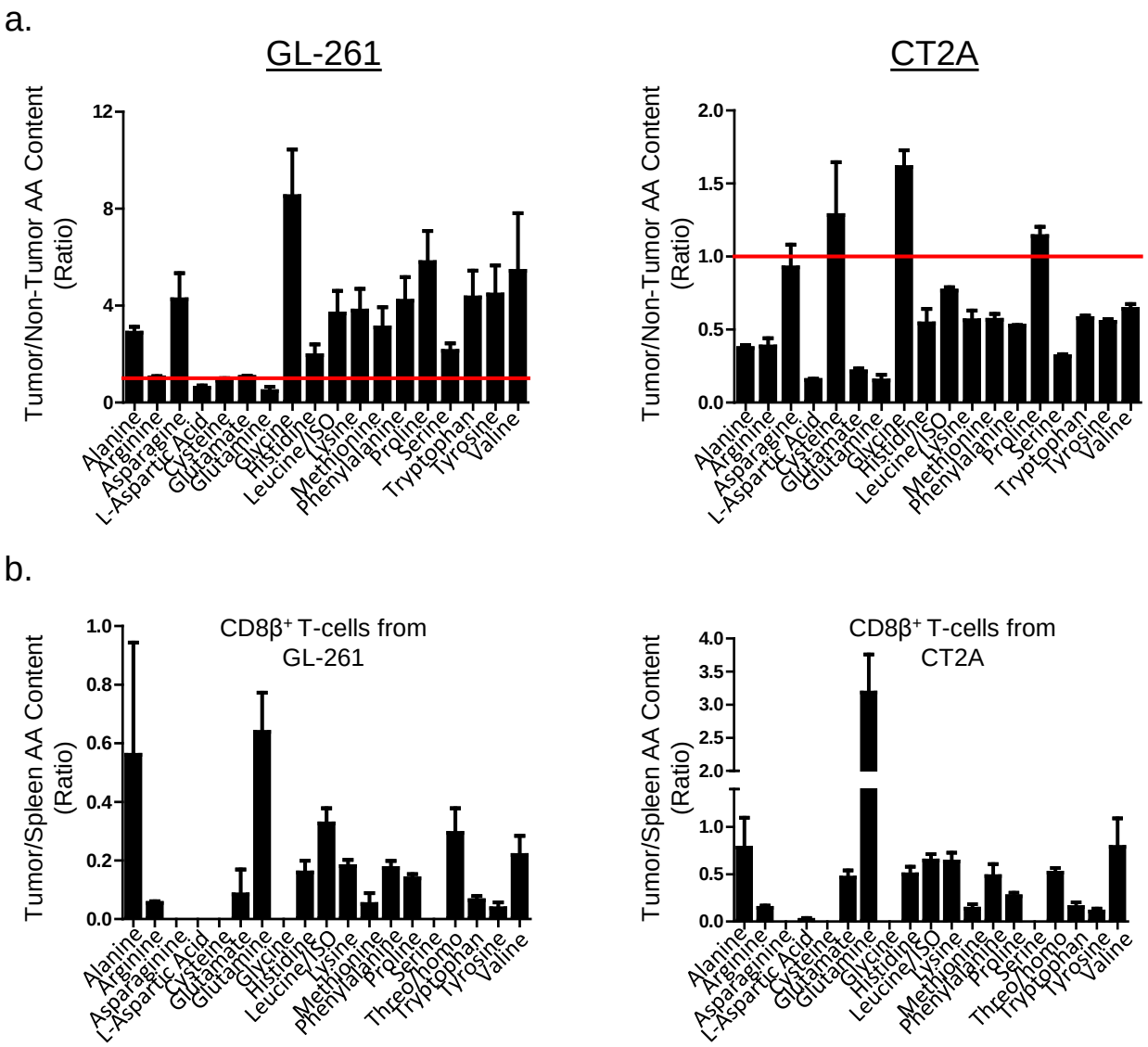
Supplementary Figure 2: Reduction in CD8$^+$ T-cell infiltration was recapitulated using a different glioma murine cell line (CT2A). WT and GCN2 KO mice were injected *i.c.* with 2X10^5 CT2A tumor cells and after 14 days, they were sacrificed for flow cytometric analysis. In **(a)** flow cytometric analysis of the total number and percentage of the different T-cell subsets infiltrating the brain. In **(b)** flow cytometric analysis of the total number of the different T-cell subsets from the spleen. Flow cytometry statistics calculated and shown as total number and percentage positive population, n=7 per group, representative of two experiments. Unpaired T-test analysis was used to calculate significance. $p<0.05$*; $p<0.01$**; $p<0.001$***.



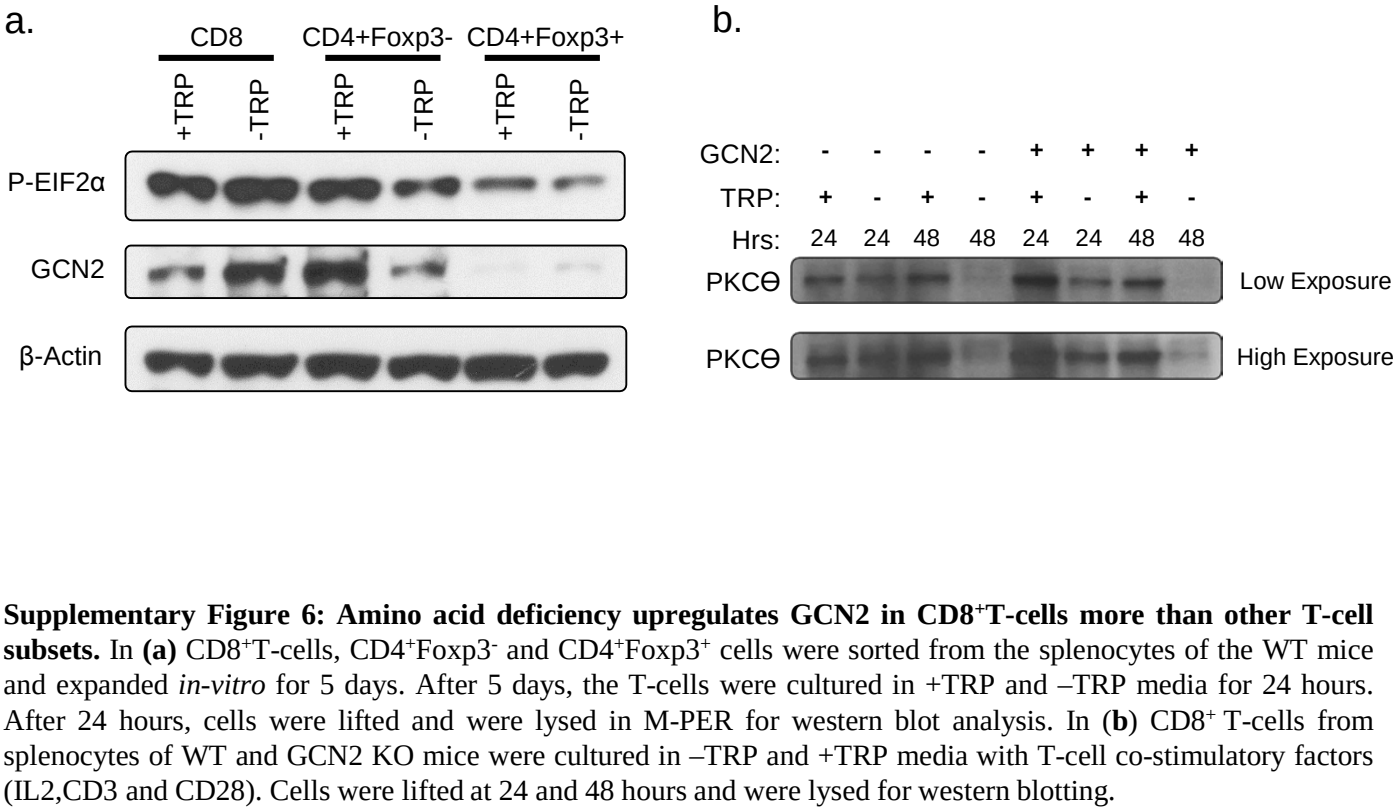
Supplementary Figure 3: Tumor infiltrating CD8$^+$ T-cells in GCN2 KO mice are less activated using a different glioma murine cell line (CT2A). WT and GCN2 KO mice were injected *i.c.* with 2X10^5 CT2A tumor cells and after 14 days, they were sacrificed. Tumor infiltrating lymphocytes were stimulated for 5 hours and after 5 hours they were stained and analyzed for CD44 MFI and cytokine secretion with flow cytometry. Flow cytometry statistics calculated and shown as total number and percentage positive population, n=7 per group, representative of two experiments. Unpaired T-test analysis was used to calculate significance. $p<0.05$*; $p<0.01$**; $p<0.001$***.



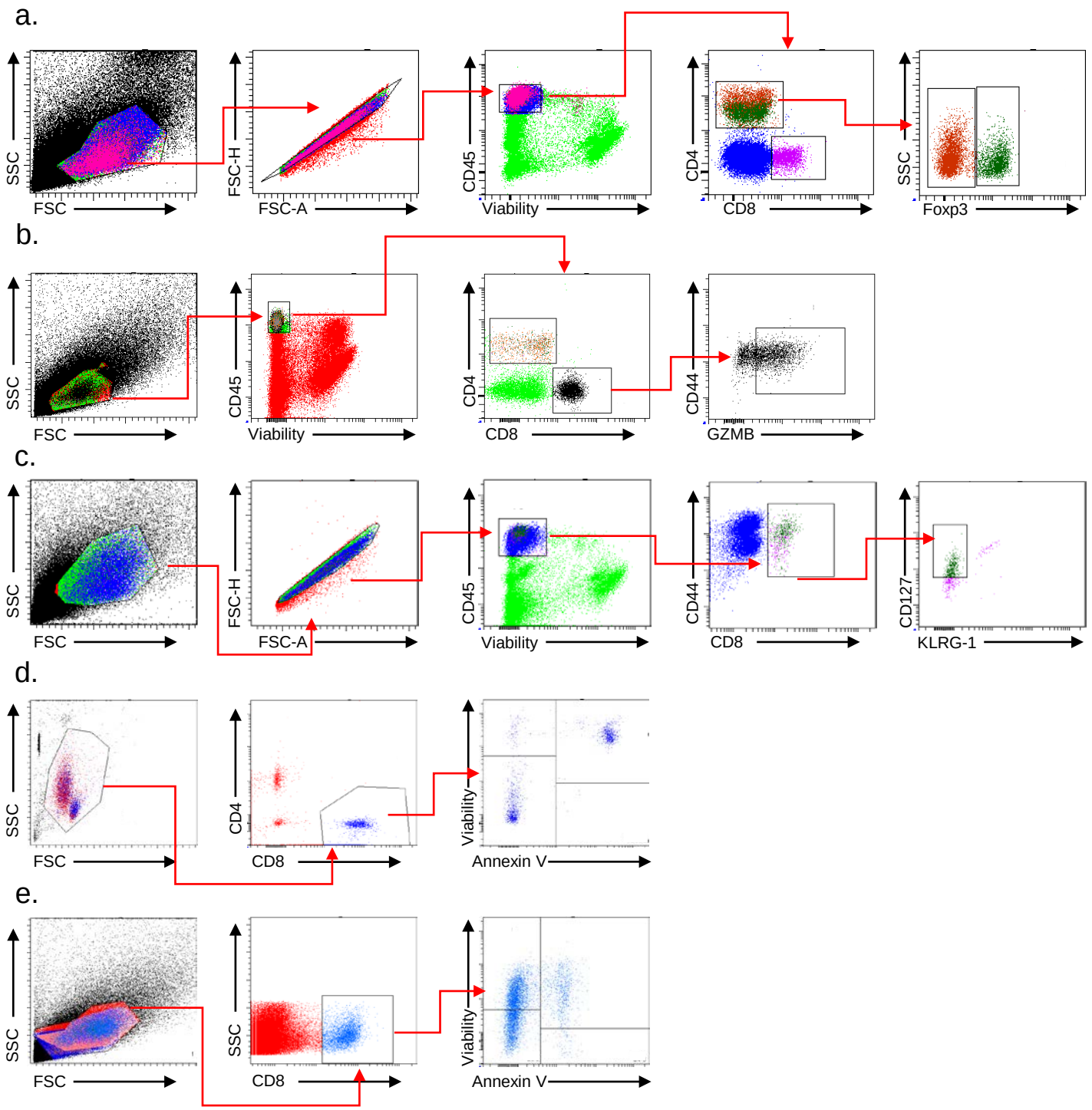
Supplementary Figure 4: Tumor infiltrating CD8$^+$ T-cells in GCN2 KO mice can migrate into the brain. WT and GCN2 KO mice were injected *i.c.* with 2X10^5 GL-261 tumor cells and after 14 days, they were sacrificed. Tumor infiltrating and splenic lymphocytes were stained for CCR7 expression in the state of activation (CD44) for flow cytometric analysis. Flow cytometry statistics calculated and shown as total number and percentage positive population, n=5 per group, representative of two experiments. Unpaired T-test analysis was used to calculate significance. $p<0.05$*; $p<0.01$**; $p<0.001$***.



Supplementary Figure 5: Amino acid deficiency in CD8⁺ T-cells infiltrating the tumor microenvironment. In (**a-b**) WT mice were injected with $2x10^5$ GL-261 (**Left Panel**) and CT2A (**Right Panel**) murine glioma cells (3 mice per group (**a**), 6 mice per group (**b**)). In (**a**) after 14 days mice were sacrificed and their bulk tumor (Right Hemisphere) and non-tumor brain (Left Hemisphere) were isolated and all of their metabolites were measured using LC-MS. In (**b**) after 14 days mice were euthanized, their CD8β⁺ T-cells were isolated from brain (Tumor) and spleen and their metabolites were measured using LC-MS. In (**a-b**) data are representative of ratio of normalized peak area of each AA from tumor to non-tumor (**a**) and from tumor to spleen(**b**).
AA: Amino acid



Supplementary Figure 6: Amino acid deficiency upregulates GCN2 in CD8^{+}T-cells more than other T-cell subsets. In **(a)** CD8^{+}T-cells, CD4^{+}Foxp3^{-} and CD4^{+}Foxp3^{+} cells were sorted from the splenocytes of the WT mice and expanded *in-vitro* for 5 days. After 5 days, the T-cells were cultured in +TRP and –TRP media for 24 hours. After 24 hours, cells were lifted and were lysed in M-PER for western blot analysis. In (**b**) CD8^{+} T-cells from splenocytes of WT and GCN2 KO mice were cultured in –TRP and +TRP media with T-cell co-stimulatory factors (IL2,CD3 and CD28). Cells were lifted at 24 and 48 hours and were lysed for western blotting.



Supplementary Figure 7: Gating strategies for flow cytometry panels in main figures. (**a**) is the gating strategy related to flow cytometry panels in **Fig. 1**. (**b**) is the gating strategy related to flow cytometry panels in **Fig. 2**. (**c**) is the gating strategy related to flow cytometry panel in **Fig.4**. (**d**) is the gating strategy related to flow cytometry panel in **Fig.5c** and **Fig. 6**. (**e**) is the gating strategy related to flow cytometry panel in **Fig. 5d**.