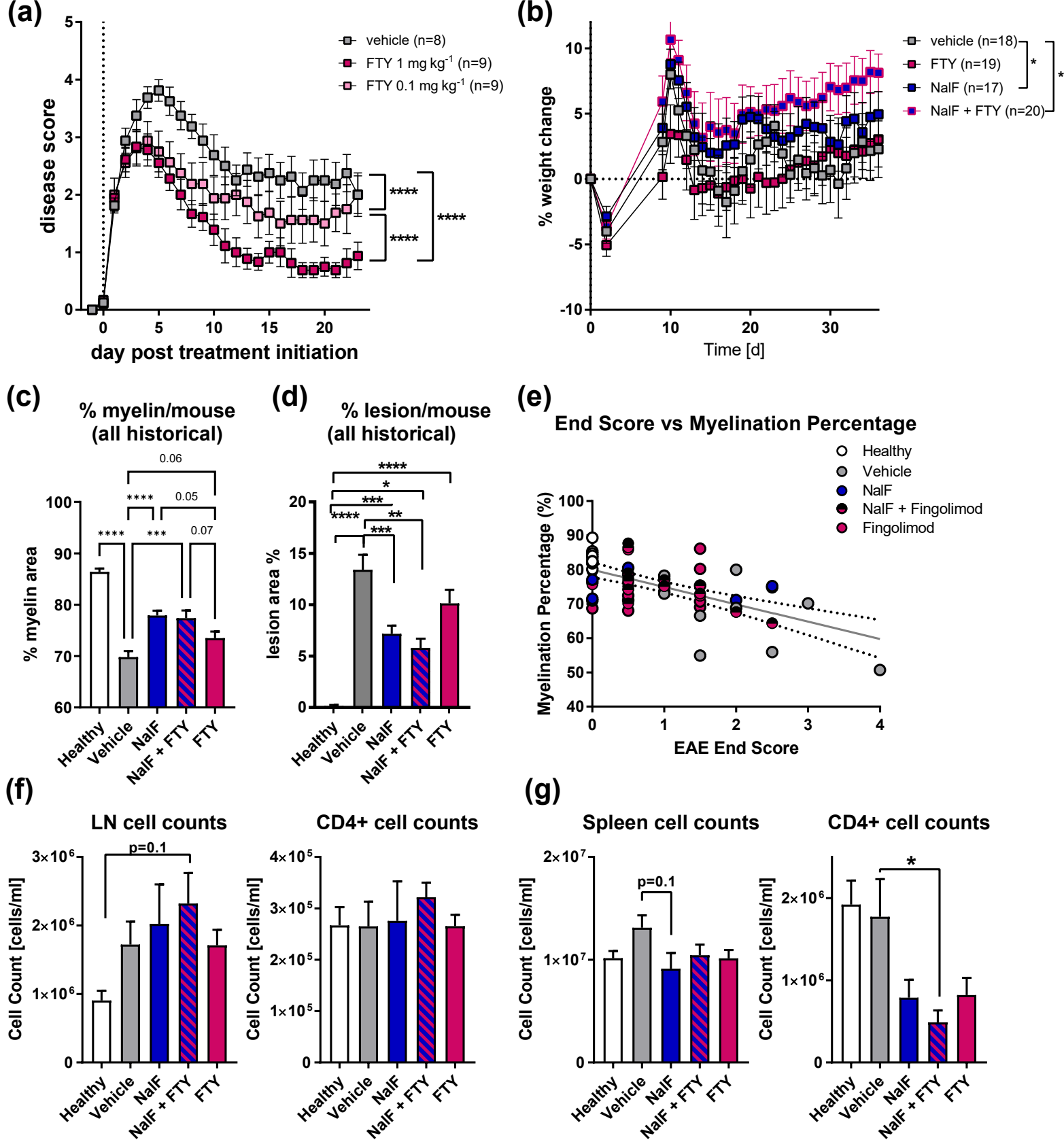
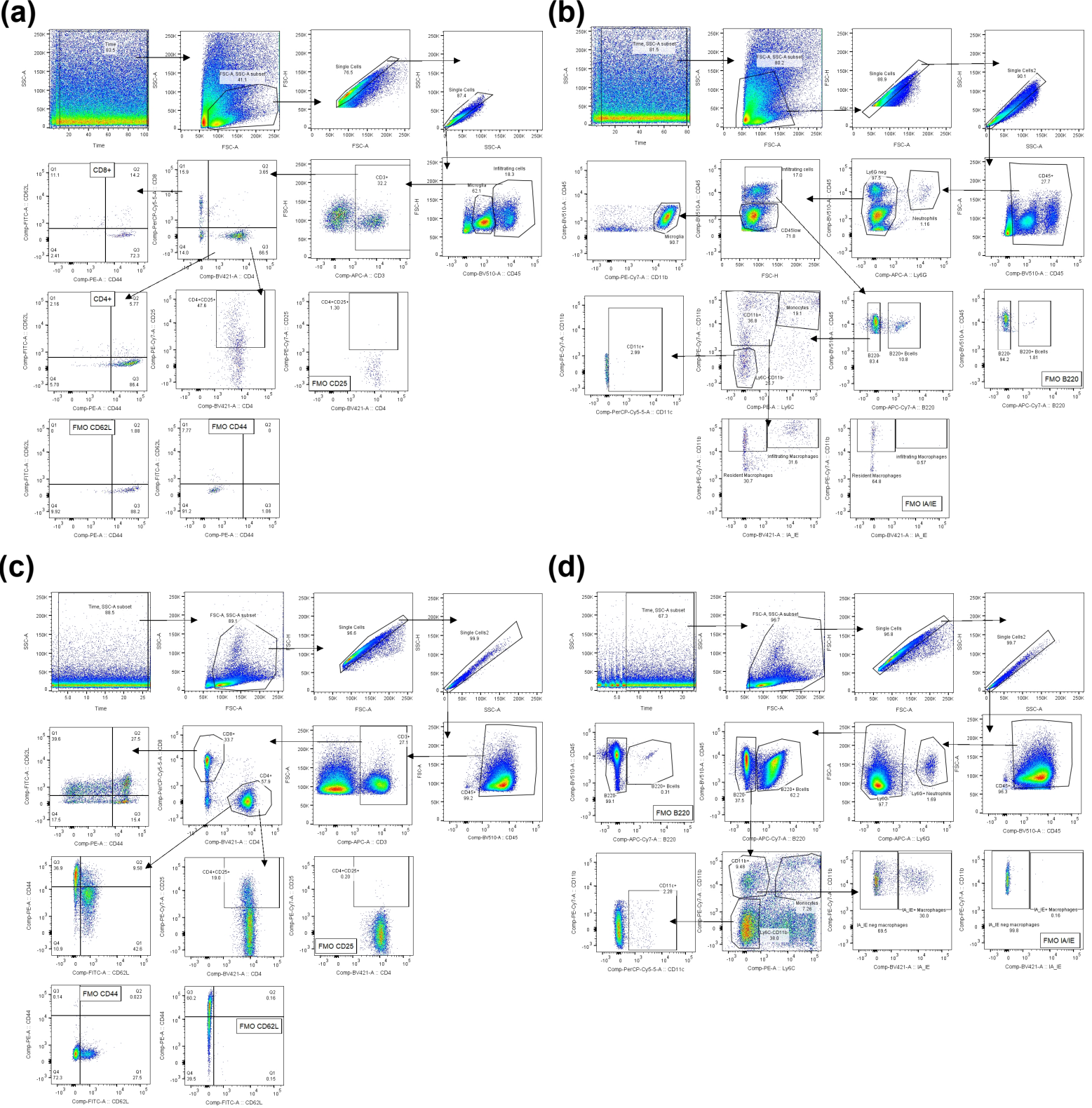
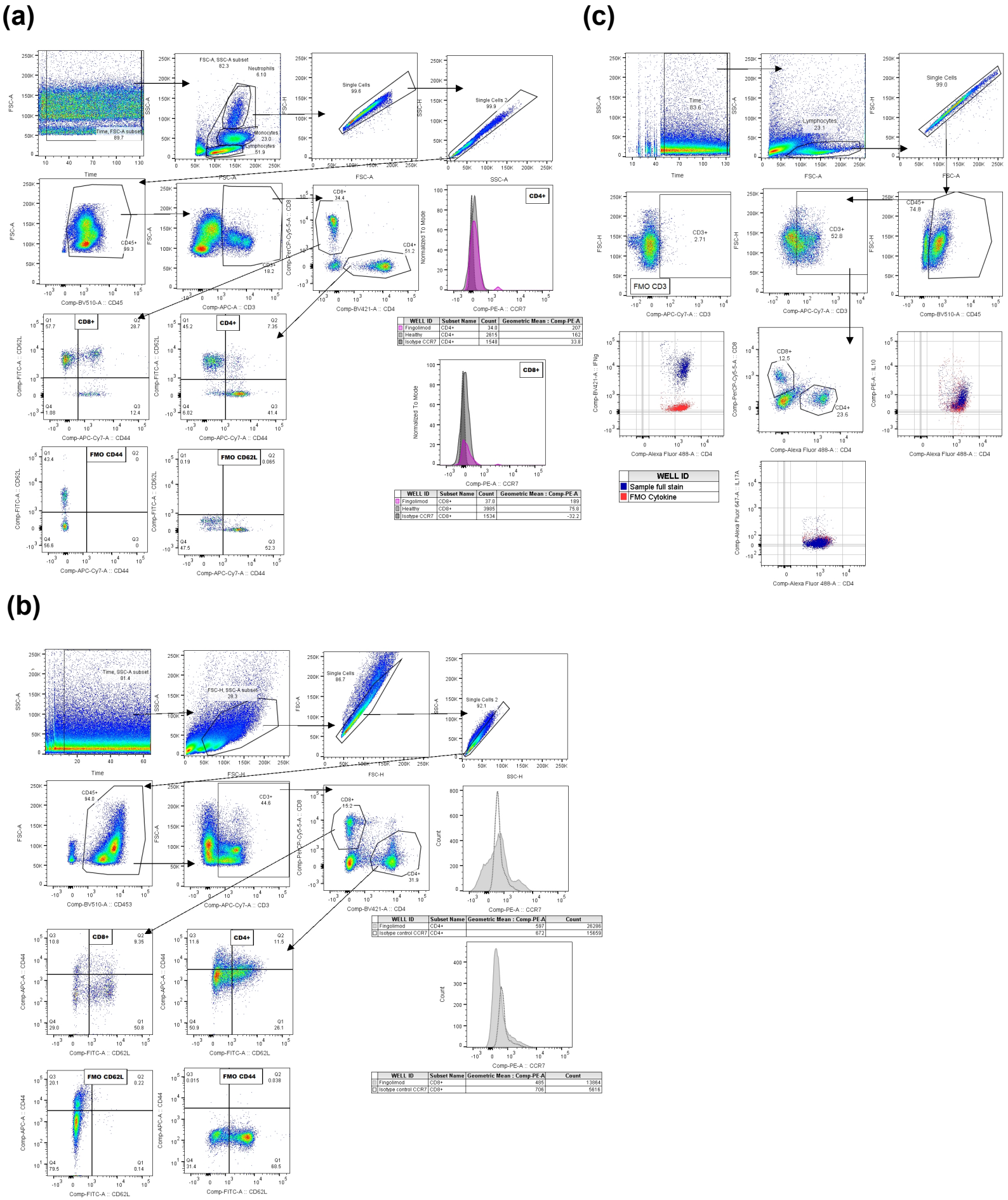


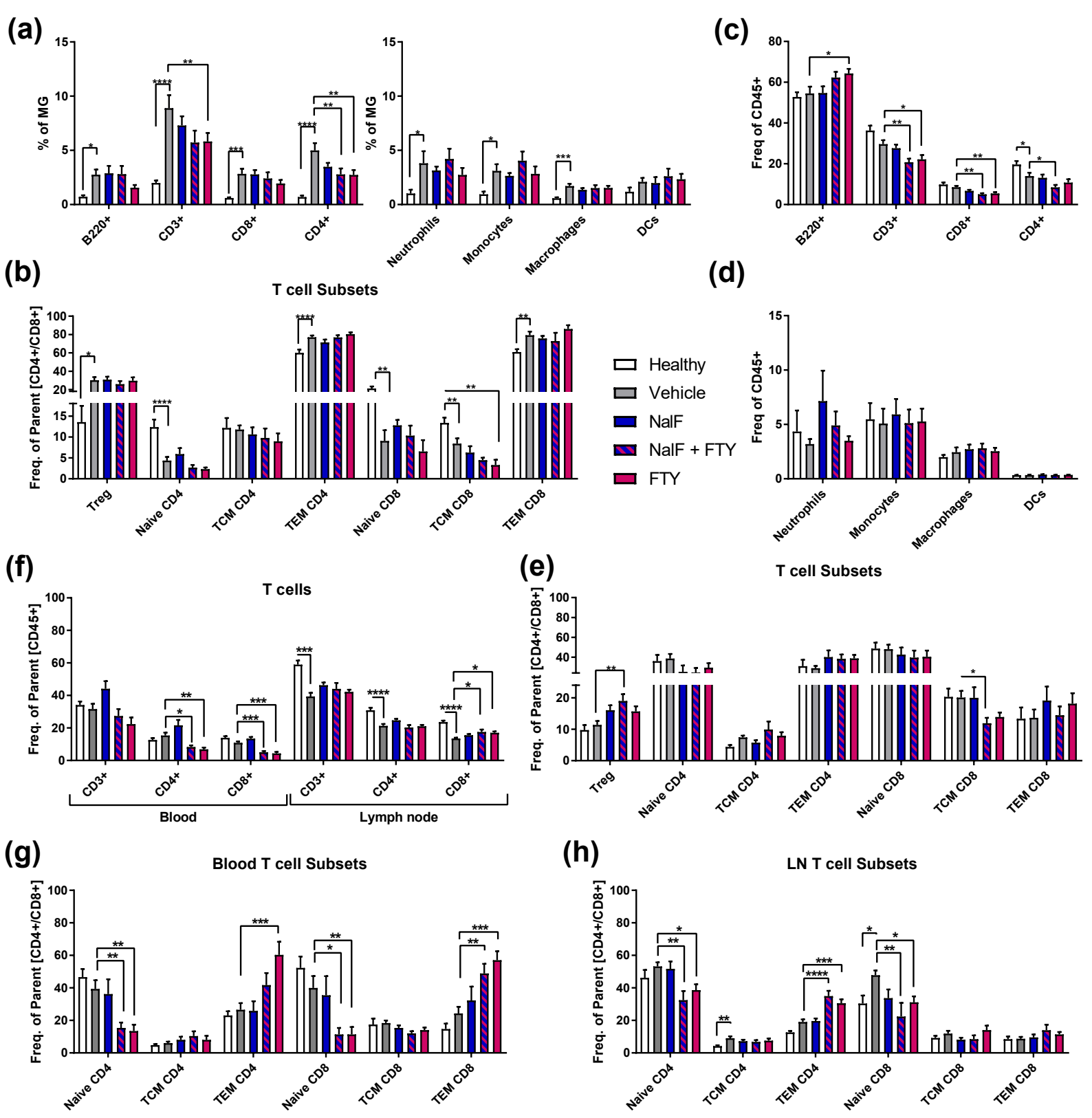
Supplementary Figure 1. (a) Disease scores of vehicle and fingolimod (1 mg kg^{-1} p.o. or 0.1 mg kg^{-1}) treated animals from disease onset (score≥1) to 23 dpt. (b) Weight change of vehicle, nalfurafine (0.01 mg kg^{-1} i.p.), fingolimod (1 mg kg^{-1} p.o.) or the combination treated animals from disease onset (score≥1) to 23 dpt. Weight change as difference to baseline. (c) Myelination and (d) lesion percentage in the spinal cord quantified by Black Gold II staining of all data recorded (published previously in Denny at al. 2021, Paton et al. 2022) and unpublished data. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001 (e) Correlation between myelination percentage in the spinal cord of vehicle, nalfurafine and Fingolimod treated EAE animals and disease end score. (f) Lymph node and (g) Spleen cell counts of lymphocyte populations from healthy, vehicle, nalfurafine (NalF, 0.01 mg kg^{-1}), Fingolimod (FTY, 1mg kg^{-1}) or the combination in EAE animals.



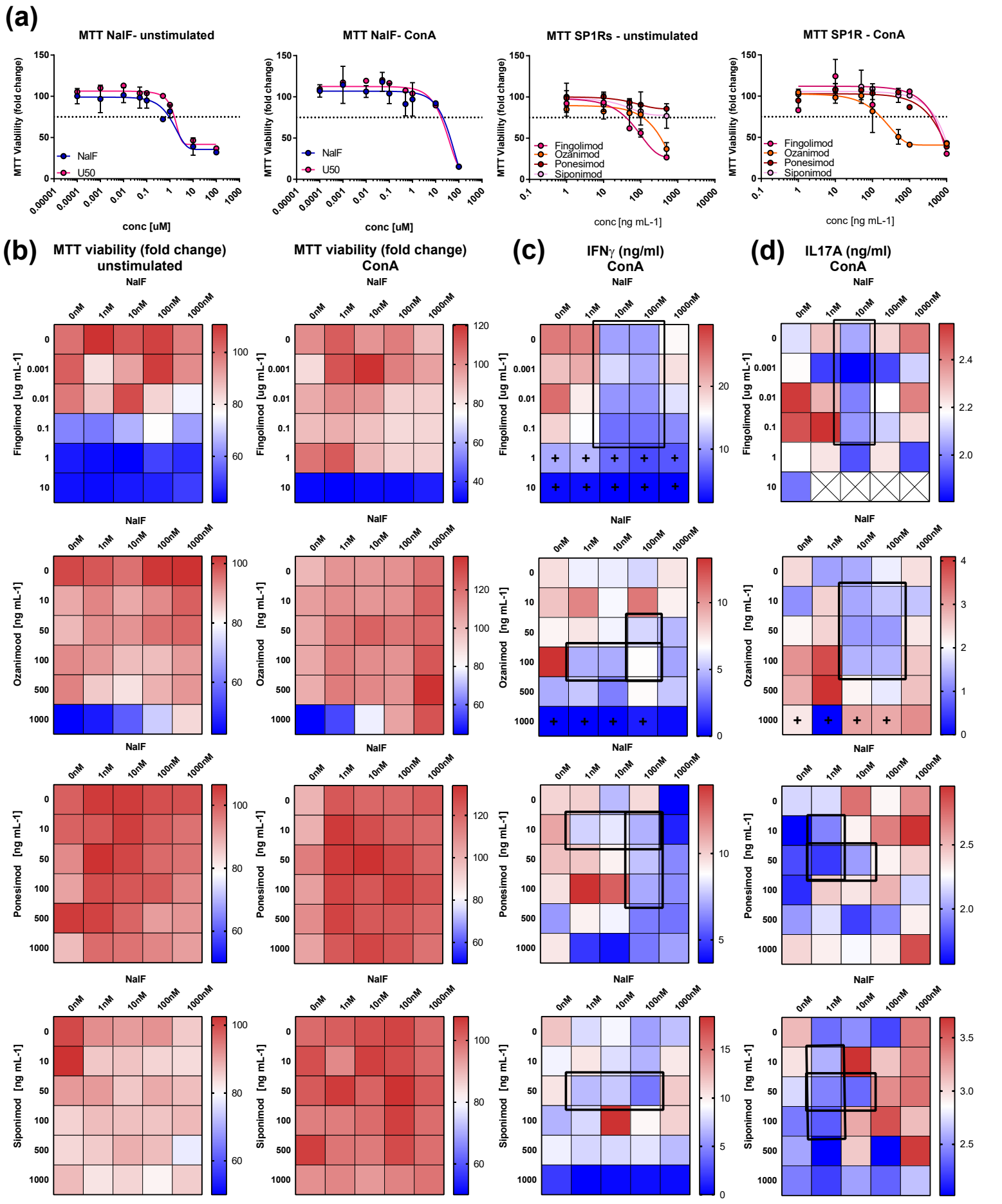
Supplementary Figure 2. Flow cytometry gating strategy for (a,b) brain (T cell Panel (a) and Myeloid Panel (b)) and (c,d) spleen (T cell Panel (c)and Myeloid Panel (d)).



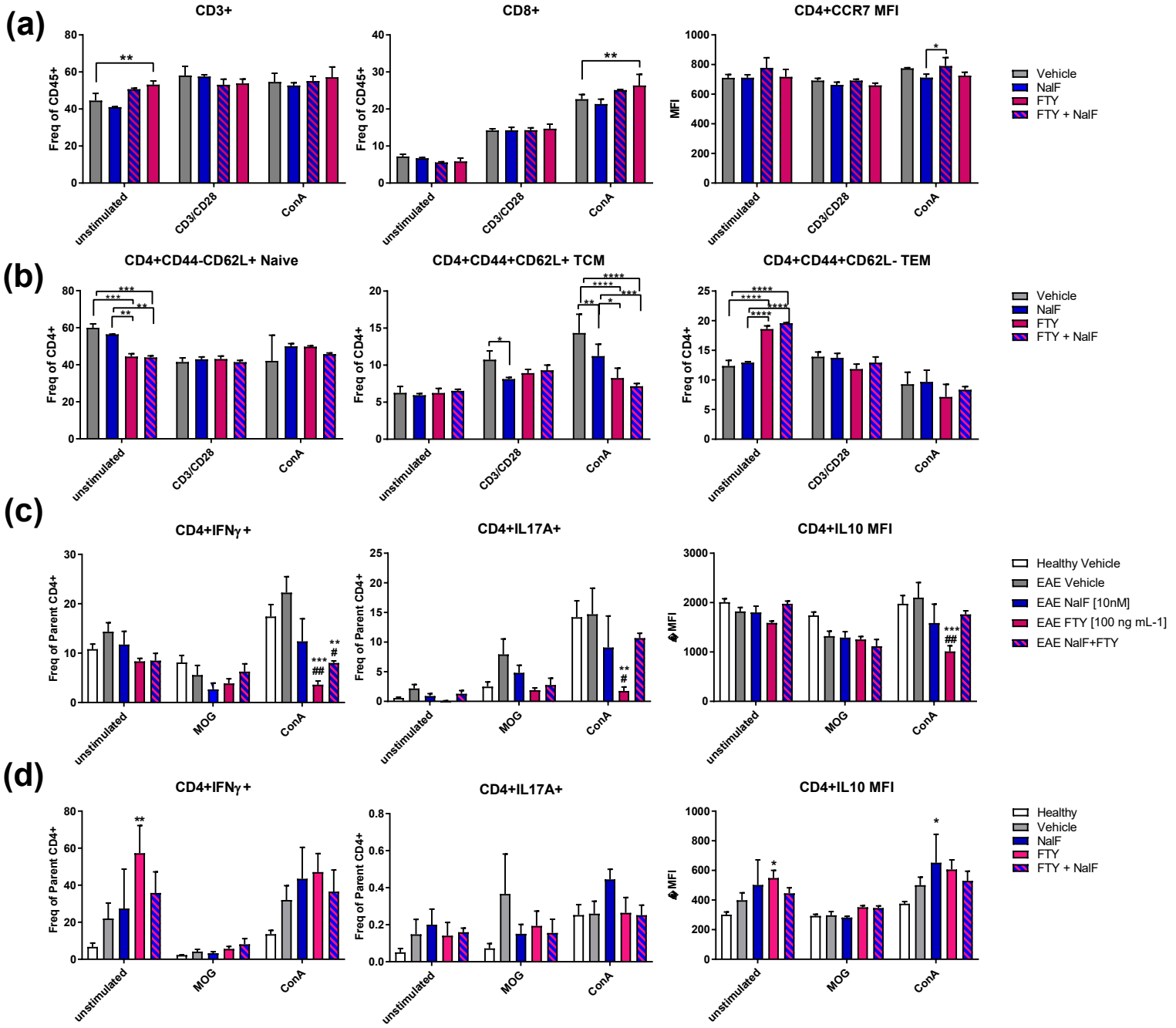
Supplementary Figure 3. (a) Flow cytometry gating strategy for blood and lymph nodes (b) Flow cytometry gating strategy for in vitro analysis of splenocytes left untreated or treated with ConA or CD3/C28 dynabeads. (c) Flow cytometry gating strategy for intracellular cytokine detection of IFNγ, IL17A and IL10 in splenocytes.



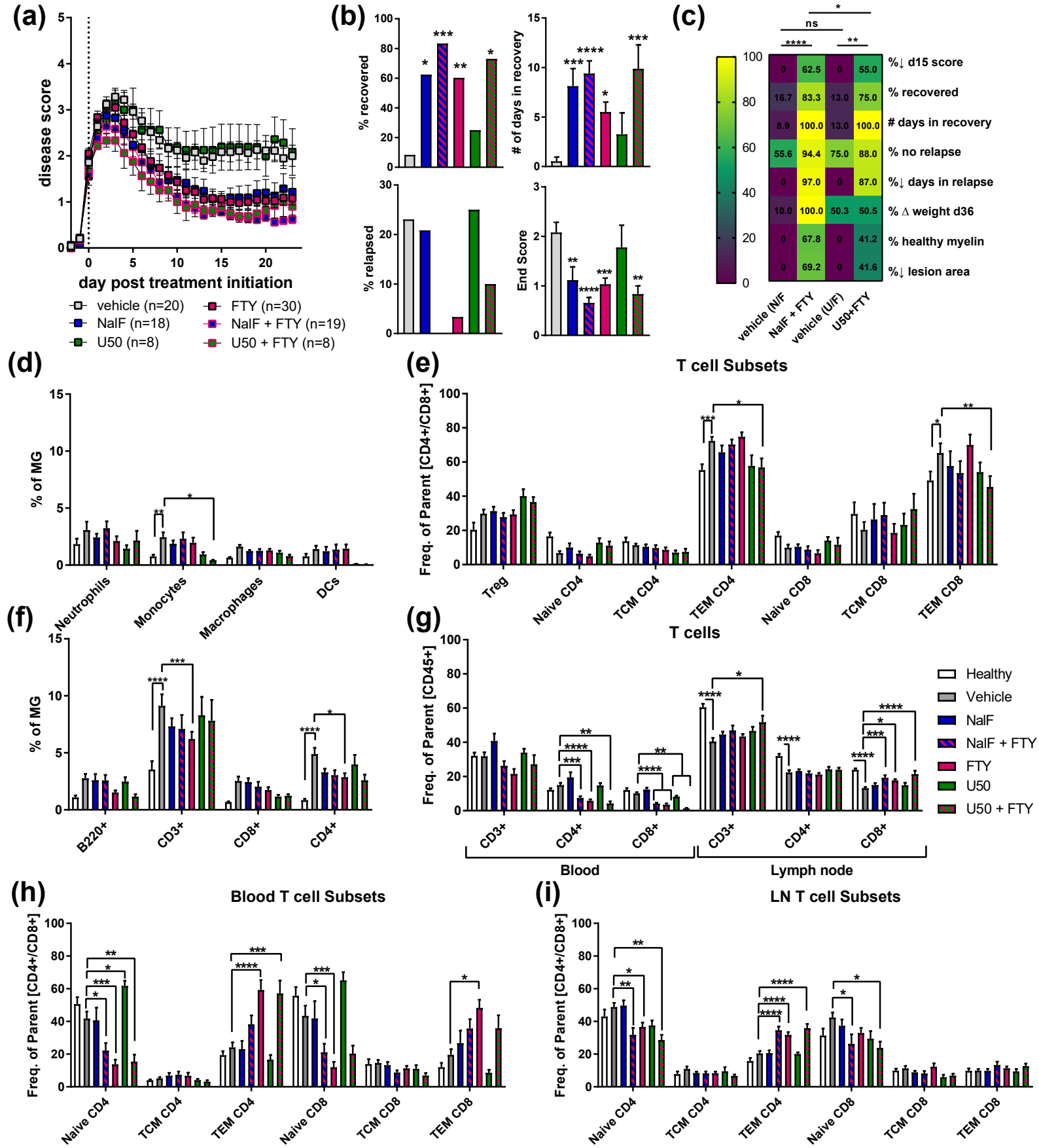
Supplementary Figure 4. Analysis of lymphocyte populations from healthy, vehicle, nalfurafine (NalF, 0.01 mg kg^{-1}), Fingolimod (FTY, 1mg kg^{-1}) or the combination in (a,b) brain tissue, (c-e) spleen tissue or (f-h) blood and lymph nodes. (a,b) All infiltrating immune cells were identified by $CD45^{high}$ expression. The relative number of cells is expressed as a ratio to microglia ($CD45^{int}CD11b^{+}$). Identification of CNS-infiltrating lymphocyte immune cell types by a sequential gating strategy as described in Supplement figure 1. (c-e) lymphocytes in spleen and their specific subsets (e). (f) T cell lymphocytes in blood and lymph node and their specific subsets (g,h), gating strategy in Supplementary figure 2. Shown are the results from 6 independent experiments with 10-18 mice/group for brain data and 3 independent experiments with 8-14 mice/group for blood and lymph node data. Data are mean±SEM. Non-parametric One-way ANOVA with Dunnett multiple comparisons testing, compare the mean of each treatment against vehicle within each cell type. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



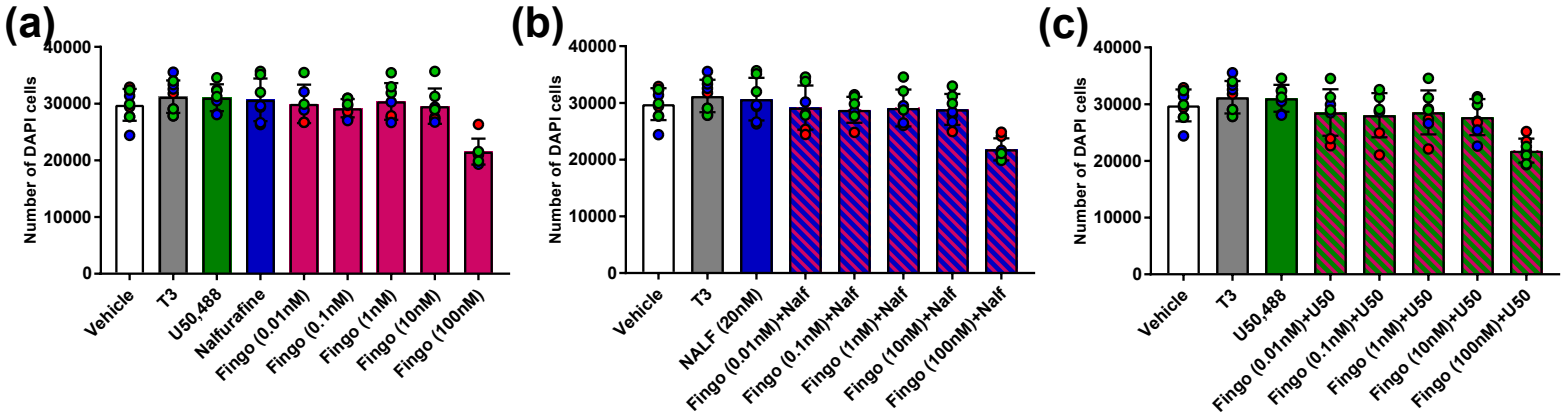
Supplementary Figure 5. MTT and cytokine results after nalfurafine and S1PRs treatments. Splenocytes were left untreated or treated with ConA for 72h in combination with increasing concentration of nalfurafine (NalF), U50,488, Fingolimod, Ozanimod, Ponesimod or Siponimod or a combination of thereof. Viability of single (a) or co-treatment (b) was assessed by MTT assay. (a) 3-5 independent experiments (b) 2 independent experiments. Supernatant was collected and IFNγ (c) and IL17A (d) was measured by ELISA to assess co-treatment effects. (c,d) 2 independent experiments, only ConA stimulated splenocytes are shown. Squared wells – co-treatment effects on downregulation of cytokines. The + symbol indicates the absence of cytokines due to non-viable cells.



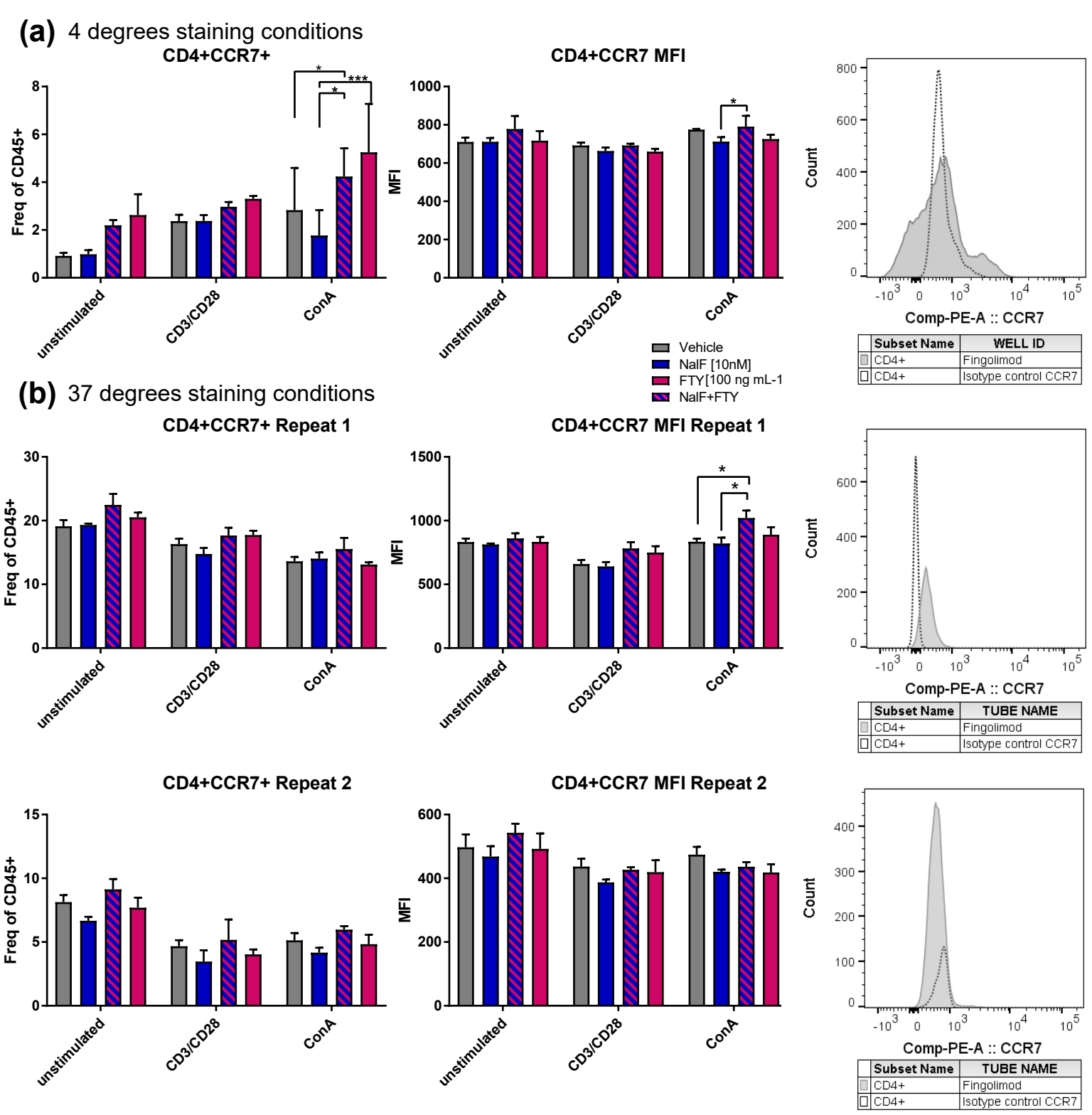
Supplementary Figure 6. In-vitro, ex-vivo and in-vivo data of nalfurafine and fingolimod effect of $CD4^+$ T cell subsets and cytokine production. (a,b) Healthy splenocytes were either treated with vehicle (grey), nalfurafine (blue, 10nM), fingolimod (FTY, pink, 100ng mL^{-1}) or the combination (blue-pink striped) and were left unstimulated or stimulated with ConA or CD3/CD28 for 72h and three different $CD4^+$ T cell subsets (Naïve, TCM – T cell central memory, TEM – T cell effector memory) were quantified. Data are mean±SEM from two individual experiments with n=13/treatment group. 2-way ANOVA with Tukey multiple comparisons testing, compare the mean of each stimulation group. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001. (c) splenocytes from non-sick EAE mice were treated ex-vivo with vehicle (grey), nalfurafine (blue, 10nM), fingolimod (FTY, pink, 100ng mL^{-1}) or the combination (blue-pink striped) and were left unstimulated or stimulated with ConA or MOG for 72h and intracellular cytokine production in $CD4^+$ T cells were quantified. Data are mean±SEM from two individual experiments with n=12/treatment group. 2-way ANOVA with Tukey multiple comparisons testing, compare the mean of each stimulation group. *,#P<0.05, **,##P<0.01, ***P<0.001, ***P<0.0001. * significance tested against vehicle, # significance tested against nalfurafine (d) EAE mice were treated in-vivo with either vehicle, nalfurafine (NalF, 0.01 mg kg^{-1}), Fingolimod (FTY, 1mg kg^{-1}) or the combination. After splenocytes isolation, cells were left untreated or re-stimulated with with ConA or MOG for 72h and intracellular cytokine production in $CD4^+$ T cells were quantified. Data are mean±SEM from two individual experiments with n=5-8/treatment group. 2-way ANOVA with Tukey multiple comparisons testing, compare the mean of each stimulation group. *P<0.05, **P<0.01, ***P<0.001, ***P<0.0001.



Supplementary Figure 7. (a) Disease scores of healthy, vehicle, nalfurafine (NalF, 0.01 mg kg^{-1}), Fingolimod (FTY, 1mg kg^{-1}), U50,488 (U50, 1.6 mg kg^{-1}) or the combination treated animals from disease onset (score≥1) to 23 dpt. Scores aligned to the day of disease onset (day 0 post treatment). Mice were treated daily. Results combined from 1-4 independent experiments (n=5-18, as indicated). (b) Percentage recovery to 23 dpt (recovery = score ≤0.5), Number of days in recovery to 23 dpt. Percentage relapse to 23 dpt (relapse= increase by 1 full point from the lowest score of remission). End Score. Non-parametric One-way ANOVA with Tukey multiple comparisons testing. Analysis of lymphocyte populations in (c-e) brain tissue or (g-i) blood and lymph nodes. (c,d) All infiltrating immune cells were identified by $CD45^{high}$ expression. The relative number of cells is expressed as a ratio to microglia ($CD45^{int}CD11b^{+}$). (g) T cell lymphocytes in blood and lymph node and their specific subsets. (Shown are the results from 2-6 independent experiments with 8-18 mice/group for brain data and 2-3 independent experiments with 8-14 mice/group for blood and lymph node data. Data are mean±SEM. Non-parametric One-way ANOVA with Dunnett multiple comparisons testing, compare the mean of each treatment against vehicle within each cell type. *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001.



Supplementary Figure 8. In-vitro treatment of NalF in combination with Fingolimod (FTY) in primary mouse mixed neuronal/glial culture. Cell counting of DAPI positive cells (a) mouse OPC cultures treated with vehicle (0.1% DMSO), T3 (30 ng mL^{-1}), Nalf (20 nM), U50,488 (10 µM), fingolimod (10 nM, 1 nM, 0.1 nM, 0.01 nM) and (b,c) a combination of fingolimod with NalF or U50,488.



Supplementary Figure 9. In-vitro experiment to understand staining temperature for chemokine receptor expression. Healthy splenocytes were either treated with vehicle (grey), nalfurafine (blue, 10nM), fingolimod (FTY, pink, 100ng mL^{-1}) or the combination (blue-pink striped) and were left unstimulated or stimulated with ConA or CD3/CD28 for 72h. Staining for the chemokine receptor CCR7 on $CD4^+$ T cells was either performed at 4 degrees (a)(figures duplicated from main Figure 4 a, and Supplement Figure 6 a), or at 37 degrees (b). Representative histograms for CCR7 expression after fingolimod treatment in unstimulated splenocytes compared to isotype control are shown. (b) Data are mean±SEM from two individual experiments with n=3 mice/treatment group. 2-way ANOVA with Tukey multiple comparisons testing, compare the mean of each stimulation group. *P<0.05, **P<0.01, ***P<0.001, ***P<0.0001.