Supplemental material

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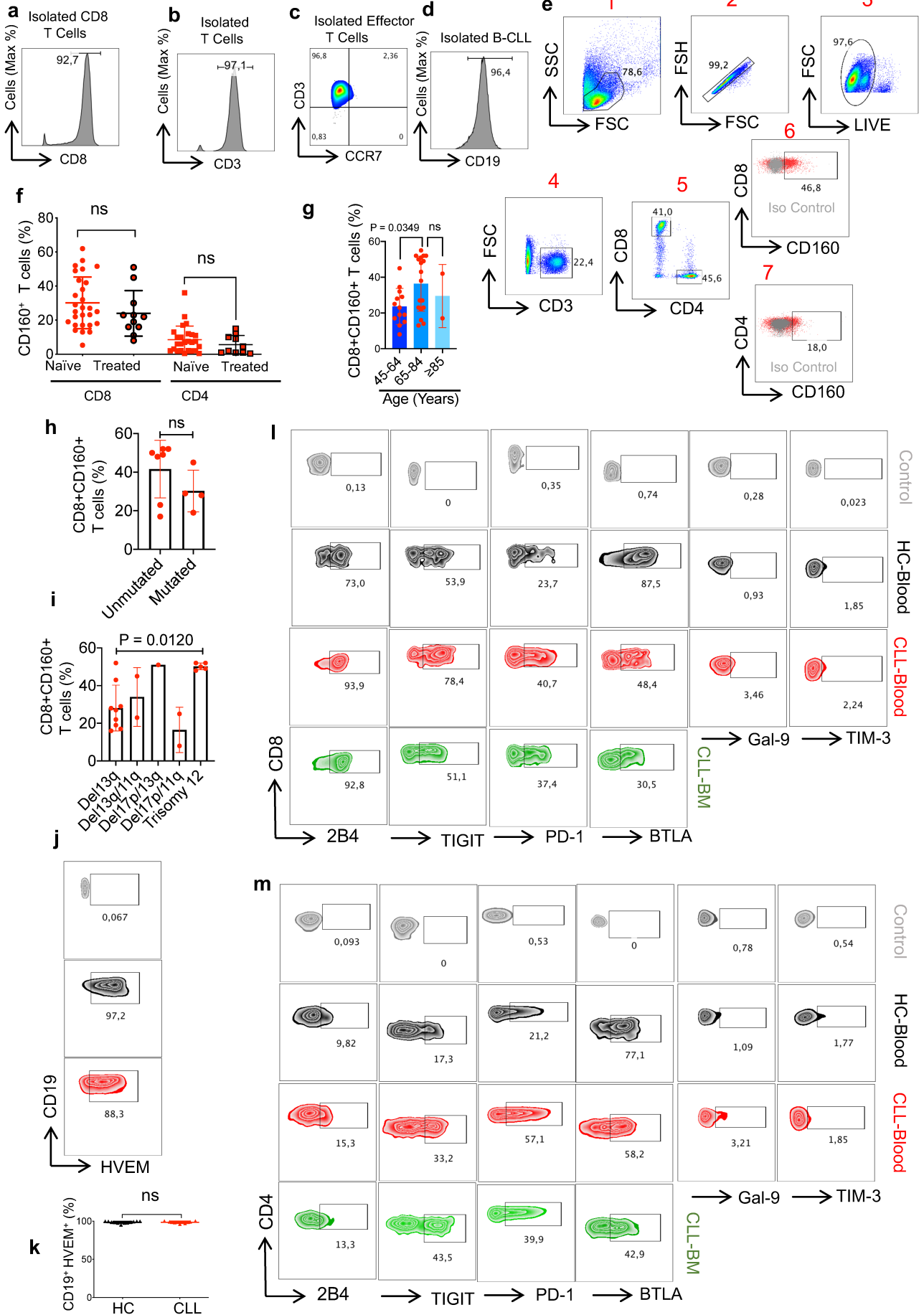
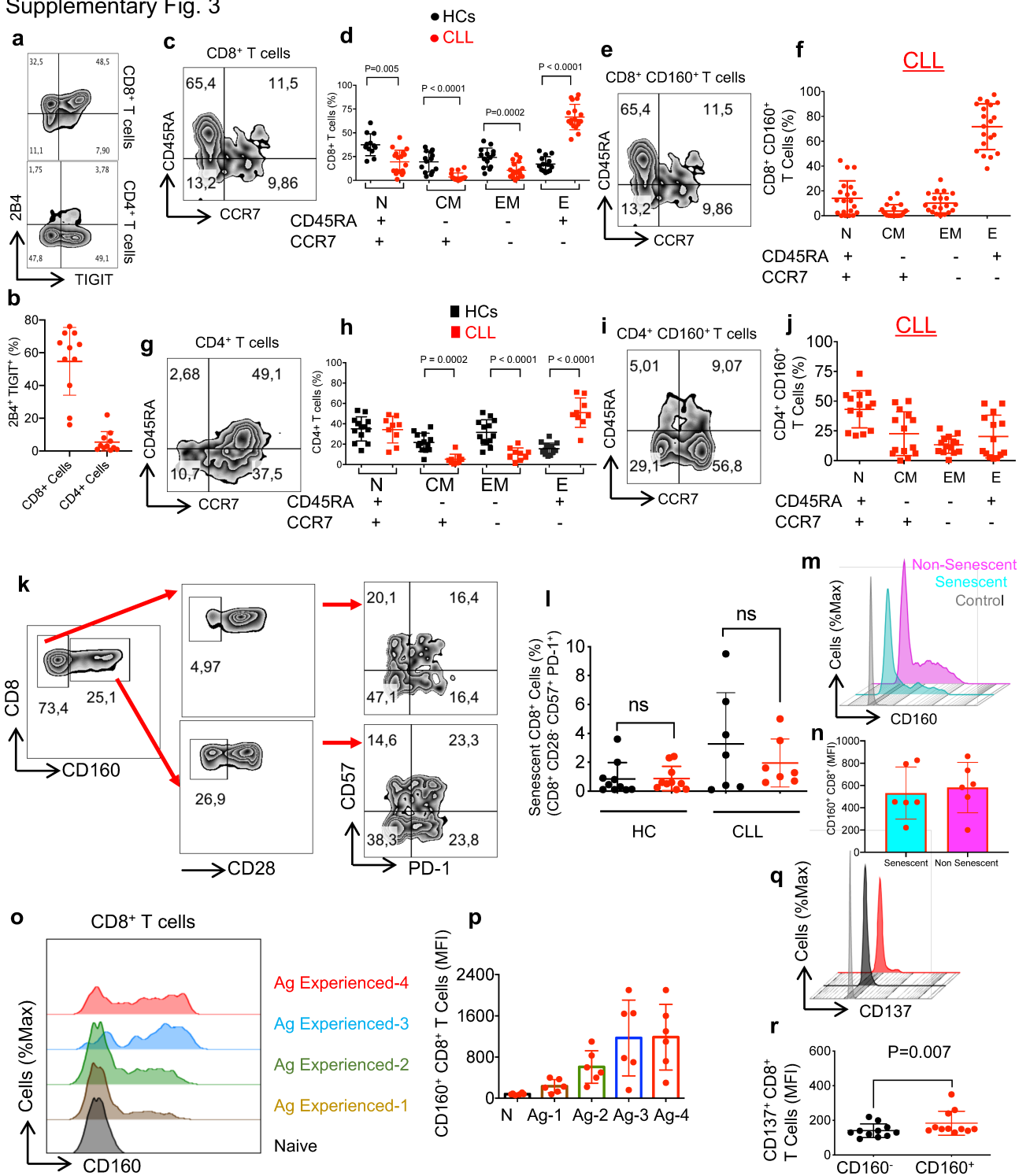*J Immunother Cancer*

Supplementary Fig. 1

Supplementary Fig. 1 (a) Representative flow cytometry purity plots of isolated CD8+ T cells, **(b)** CD3+ T cells, **(c)** CD8+ effector T cells (CD3+CCR7-CD8+), and **(d)** B-CLLs. **(e)** Flow cytometry gating strategy for CD160+CD8+ and CD4+ T cells in CLL patients. **(f)** Cumulative data showing CD160 expression on CD8+ and CD4+ T cells in treatment naïve versus treated CLL patients. **(g)** Cumulative data of percentages of CD160+CD8+ in different age groups of HCs. **(h)** Cumulative data of percentages of CD8+CD160+ in CCL patients with or without IgHV mutation. **(i)** Cumulative data of the FISH analysis in CLL patients. **(j)** Representative flow cytometry plots, and **(k)** cumulative data of HVEM expression in B-CLL versus HCs B cells. **(l)** Representative flow cytometry plots of 2B4, TIGIT, PD-1, BTLA, Gal-9 and TIM-3 expression in CD8+, and **(m)** CD4+ T cells in blood of HCs versus blood and bone marrow of CLL patients. Each dot represents data from a human subject ± SD.
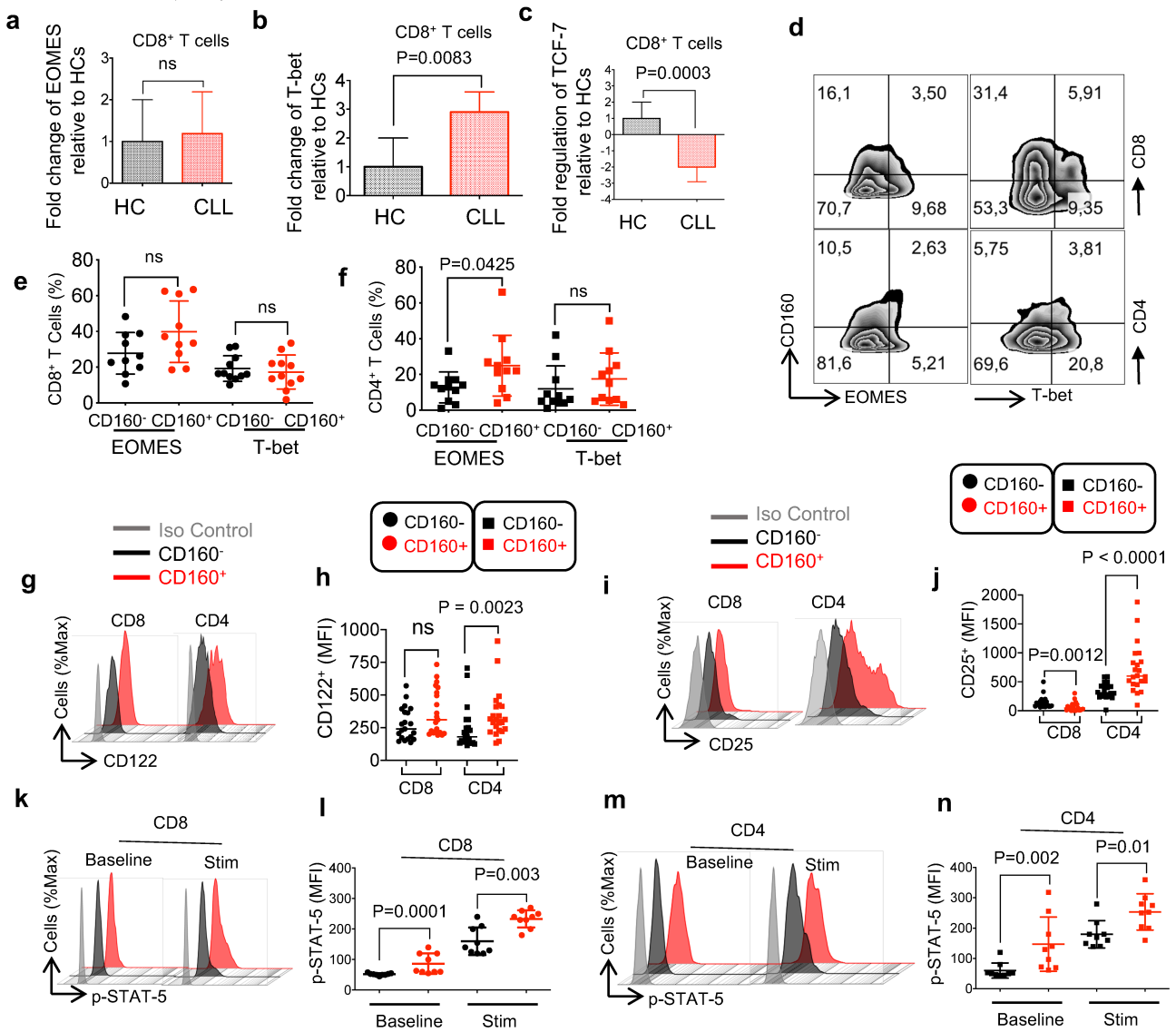
Supplementary Fig. 2
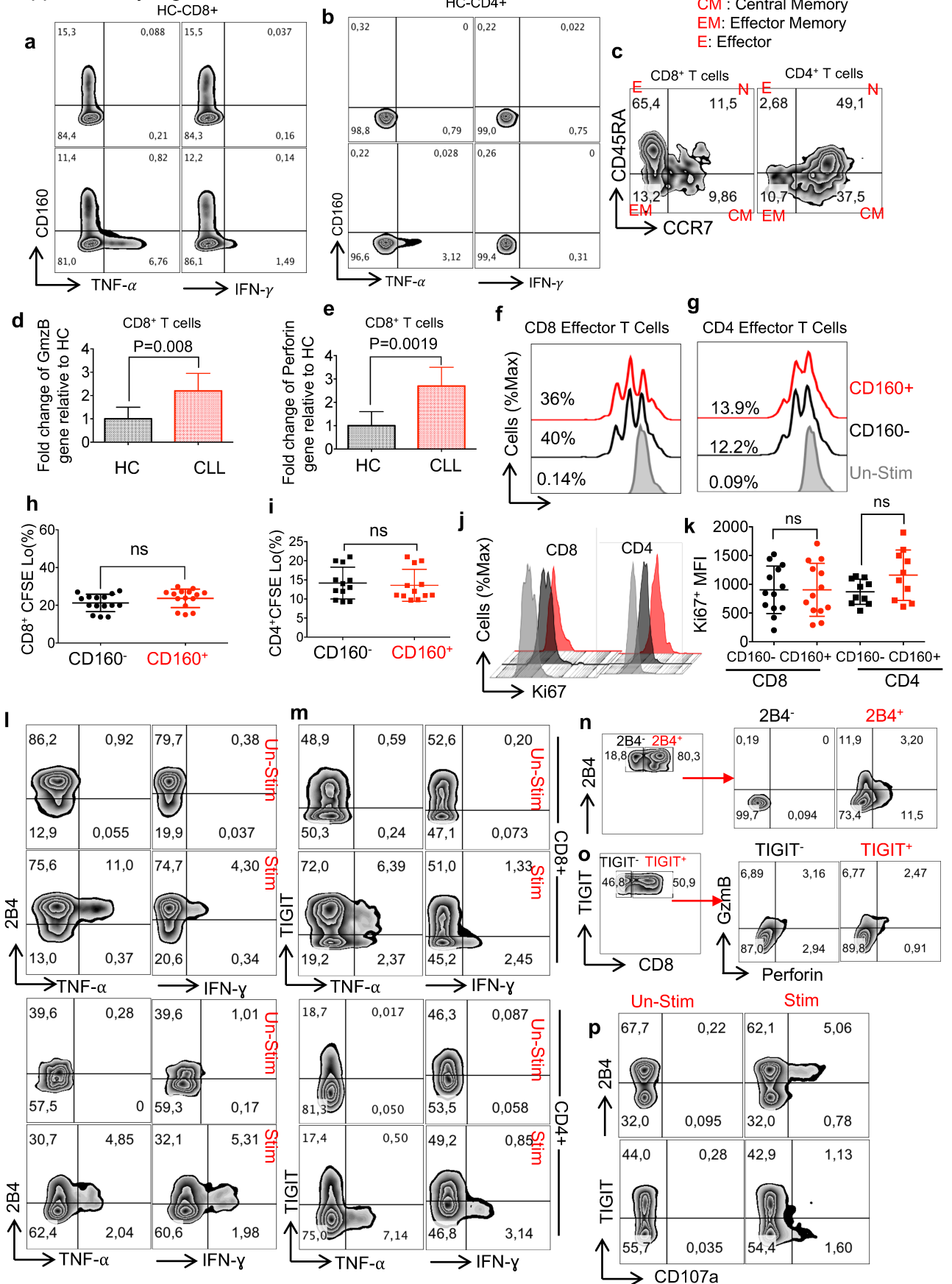
Supplementary Fig. 2 (a) Representative plots of TNF-α and IFN-γ expression in CD8+CD160+ versus CD8+CD160- T cells in HCs. (**b**) Representative plots of TNF-α and IFN-γ expression in CD4+CD160+ versus CD4+CD160- T cells in a HC. (**c**) Representative flow plots of the gating strategy for T cell differential subsets in CD8+ and CD4+ cells. (**d**) Fold regulation of GzmB, and (**e**) perforin mRNA in CD8+ T cells of CLLs relative to HCs blood, data are rom 6 individuals/group. (**f**) Representative plots of proliferation (CFSE lo) in CD160- and CD160+ either CD8+, or (**g**) CD4+ T cells. (**h**) Cumulative data of proliferation (CFSE lo) in CD160- and CD160+ either CD8+, or (**i**) CD4+ T cells. (**j**) Representative histograms, and (**k**) cummulaive data of Ki67 expression in CD8+ and CD4+ T cells in CLL patients. (**l**) Representative flow cytometry plots of TNF-α and IFN-γ expression in 2B4-/2B4+ either CD8+ or CD4+ T cells. (**m**) Representative flow cytometry plots of TNF-α and IFN-γ expression in TIGIT-/TIGIT+ either CD8+ or CD4+ T cells. (**n**) Representative flow cytometry plots of GzmB and Perforin expression in 2B4-/2B4+, and (**o**) TIGIT-/TIGIT+ CD8+ T cells. (**p**) Representative flow cytometry plots of CD107a expression in 2B4-/2B4+ and TIGIT-/TIGIT+ CD8+ T cells. Each dot represents data from a human subject ± SD.

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Supplementary Fig. 3

Supplementary Fig. 3 (a) Representative plots, and (**b**) cumulative data of 2B4 and TIGIT co-expression in CD8^{+} and CD4^{+} T cells of CLL patients. **(c)** Representative flow cytometry plot of CD45RA and CCR7 expression in CD8^{+} T cells in the blood of CLLs**. (d)** Cumulative data showing expression of different subsets of CD8^{+} T cells in CLLs (N: Naive, CM: Central memory, EM: Effector memory, E: Effector) vs HCs. **(e)** Representative flow cytometry plot of the expression of CD45RA and CCR7 in CD160^{+}CD8^{+} T cells. **(f)** Cumulative data showing differential subset distribution of CD160^{+} CD8^{+} T cells in CLL patients. **(g)** Representative flow cytometry plot of CD45RA and CCR7 expression in CD4^{+} T cells in CLLs**. (h)** Cumulative data showing the expression of different subsets of CD4^{+} T cells in CLLs (N: Naive, CM: Central memory, EM: Effector memory, E: Effector) vs. HCs. **(i)** Representative flow cytometry plot of expression of CD45RA and CCR7 in CD160^{+} CD4^{+} T cells. **(j)** Cumulative data showing differential subset distribution of CD160^{+} CD4^{+} T cells. **(k)** Representative flow plots, and (**l**) cumulative data of CD28, CD57, PD-1 expression in CD160^{+} CD8^{+} T cells (CD28^{-} CD57^{+} PD-1^{+} considered as senescent cells). **(m)** Histogram plots, and (**n**) cumulative data showing the mean fluorescence intensity (MFI) of senescent cells (CD28^{-}CD57^{+}PD-1^{+}) in CD160^{+}CD8^{+} T cells. **(o)** Representative flow cytometry plot of percent expression of CD160^{+} in Naive (CD27^{+}CD28^{+}CD45RA^{+}CCR7^{+}CD57^{-}), Ag experienced level-1 (CD27^{+} CD28^{+}CD45RA^{-} CCR7^{+} CD57^{-}), Ag experienced level-2 (CD27^{+}CD28^{+}CD45RA^{-}CCR7^{-}CD57^{+/-}), Ag experienced level-3 (CD27^{+}CD28^{-}CD45RA[+/-]CCR7^{-}CD57^{+/-}) and Ag experienced level-4 (CD27^{-}CD28^{-}CD45RA^{-}CCR7^{-}CD57^{+}) subsets of CD8^{+} T cells. **(p)** Quantification of MFI of CD160^{+} in Naive (CD27^{+}CD28^{+}CD45RA^{+}CCR7^{+}CD57^{-}), Ag experienced level-1 (CD27^{+} CD28^{+}CD45RA^{-} CCR7^{+} CD57^{-}), Ag experienced level-2 (CD27^{+}CD28^{+}CD45RA^{-}CCR7^{-}CD57^{+/-}), Ag experienced level-3 (CD27^{+}CD28^{-}CD45RA[+/-]CCR7^{-}CD57^{+/-}) and Ag experienced level-4 (CD27^{-}CD28^{-}CD45RA^{-}CCR7^{-}CD57^{+}) subsets of CD8^{+} T cells. **(q)** Representative flow cytometry plot, and (**r**) quantification of MFI of CD137+CD160^{-}/CD160^{+} CD8^{+} T cells. Each dot represents data from a human subject ± SD.

Supplementary Fig. 4

Supplementary Fig. 4 (a) Expression of EOMES mRNA in CD8^{+} T cells of CLLs relative to HCs. **(b)** Expression of T-bet mRNA in CD8^{+} T cells of CLLs relative to HCs. **(c)** Expression of TCF-7 mRNA in CD8^{+} T cells of CLLs relative to HCs. **(d)** Representative flow cytometry plots of EOMEs and T-bet expression in CD160^{-} /CD160^{+} CD8^{+} and CD4^{+} T cells. **(e)** Cumulative data showing percent expression of EOMEs and T-bet in CD160^{-}/CD160^{+} CD8^{+} T cells. **(f)** Cumulative data showing percent expression of EOMEs and T-bet in CD160^{-}/CD160^{+} CD4^{+} T cells. **(g))** Representative flow cytometry plots of CD122 (IL-2Rβ) expression in CD160^{-}/CD160^{+} CD8^{+} and CD4^{+} T cells. **(h)** Quantification of MFI for CD122 in CD160^{-}/CD160^{+} CD8^{+} and CD4^{+} T cells. **(i)** Representative flow cytometry plots of CD25 (IL-2Rα) expression in CD160^{-}/CD160^{+} CD8^{+} and CD4^{+} T cells. **(j)** Quantification of MFI for CD25 in CD160^{-}/CD160^{+} CD8^{+} and CD4^{+} T cells. **(k)** Representative flow cytometry plots of pSTAT-5 expression in CD160^{-}/CD160^{+} CD8^{+} T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(l)** Quantification of MFI of pSTAT-5 in CD160^{-}/CD160^{+} CD8^{+} T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(m)** Representative flow cytometry plots of pSTAT-5 expression in CD160^{-}/CD160^{+} CD4^{+} T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). **(n)** Quantification of MFI for pSTAT-5 in CD160^{-}/CD160^{+} CD4^{+} T cells at the baseline and after *in vitro* stimulation with recombinant human IL-2 (100 IU/ml). Each dot represents data from a human subject ± SD, cumulative data in a-c are from 6 human subjects/group.

Supplementary Fig. 5
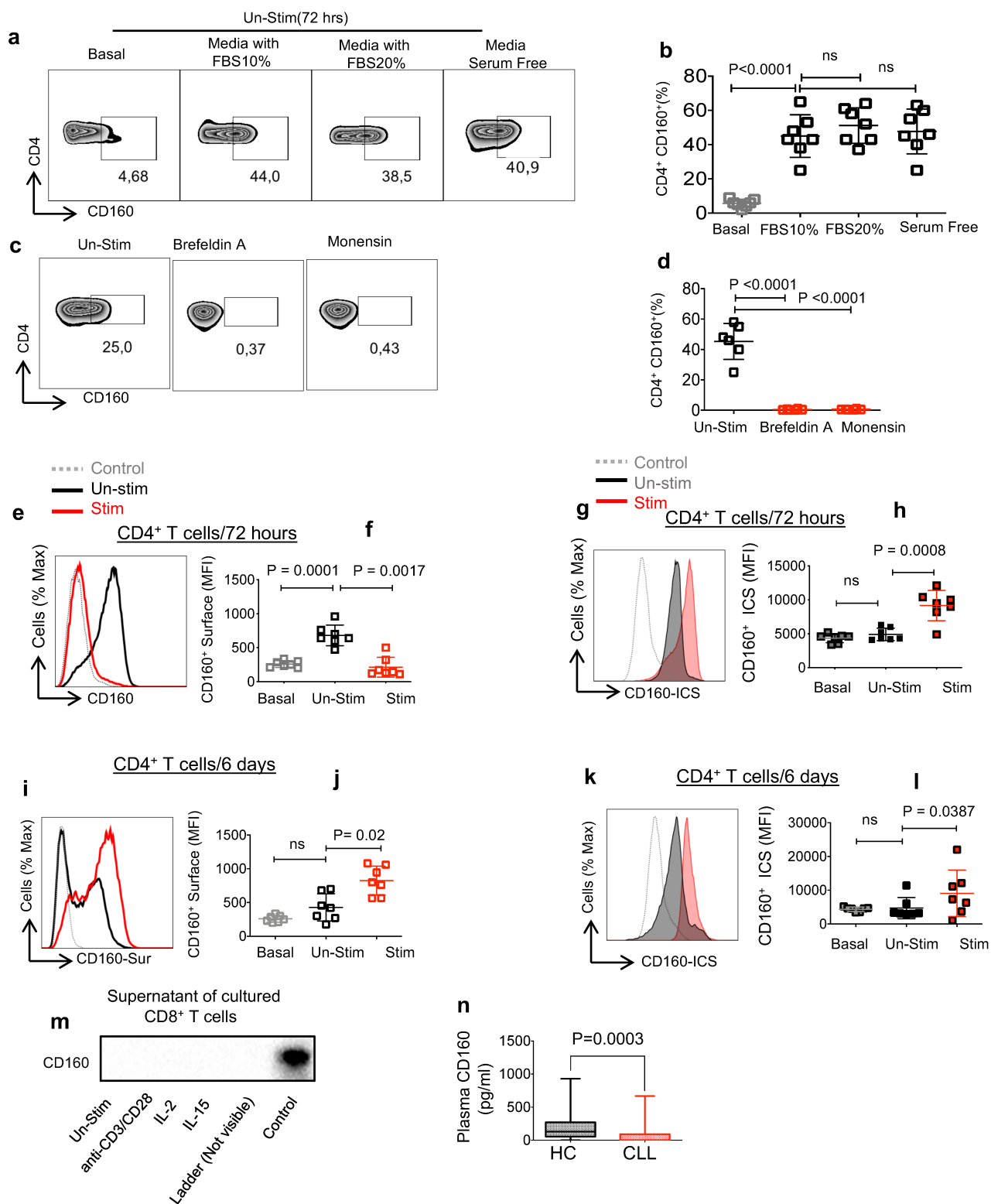
Supplementary Fig. 5 (a) Representative flow plots, and **(b)** cumulative data of CD160 expression at the baseline and following culture in RPMI media supplemented with FBS (10% and 20%) or serum free for 72 hours. **(c)** Representative flow plots, and **(d)** cumulative data of CD160 expression on CD4 T cells after 16 hours culture with supplementation of brefeldin A or Monensin. **(e)** Representative histogram, and **(f)** cumulative data of surface CD160 expression on CD4$^+$ T cells after 72 hours stimulation (Stim) with the anti-CD3/CD28 compared to unstimulated cells (un-stim). **(g)** Representative histogram, and **(h)** and cumulative data of intra-cytoplasmic CD160 expression in CD4$^+$ T cells after 72 hours stimulation with the anti-CD3/CD28 compared to Un-Stim. **(i)** Representative histogram, and **(j)** cumulative data of surface CD160 expression on CD4$^+$ T cells after 6 days of stimulation with the anti-CD3/CD28. **(k)** Representative histogram, and **(l)** cumulative data of intracytoplasmic CD160 expression in CD4$^+$ T cells after 6 days of stimulation stimulated with the anti-CD3/CD28 compared to Un-Stim. Each dot represents data from a human subject ± SD. **(m)** The blot is showing CD160 protein expression in the culture supernatant from PBMCs of a CLL patient either un-stim or stimulated with the anti-CD3/CD28, IL-2, and IL-5 compared to the positive control (cell pellet) measured by the Western blotting. **(n)** Data of quantification of the plasma CD160 in HCs versus CLL patients measured by ELISA. Each dot represents data from a human subject/single experiment ± SD, cumulative data in **(n)** are from 23 HCs and 41 CLL human subjects.

Supplementary Fig. 6

Supplementary Fig. 6. **(a)** ImmageStream plots of Evs uptake by live T cells following overnight culture of isolated and CFSE-labeled of EVs from a CLL patient with PBMCs of a HC compared with PBMCs cultured without (w/o) EVs but co-cultured with CFSE-labeled autologous B cells as control. Bright field (BF). **(b, C)** The heatmap showing differentially detected cytokines and chemokines in the plasma of CLLs and HCs. Each row represents one of the cytokines and each column represents a sample. The color scale calculated according to Z-score and means cytokine concentration standard deviation from the mean with red for the high expression and blue for the low expression levels. **(d)** Representative plots showing viability of non-B-CLL and B-CLL cells before and after 12 hr culture. **(e)** Pie chart showing percentages of patients in different RAI stages of CLL in our cohort.