Recovery of central memory and naive peripheral T cells in Follicular Lymphoma patients receiving rituximab-chemotherapy based regimen

by

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Supplementary Table S1. Patient clinical outcomes and time points of blood sample collection.

Patient i.d.[*]	Timepoint T1 (FL-T1)	Response after induction treatment[$]	Timepoint T2 (FL-T2)	Clinical outcomes (Second line therapy)
001	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
002	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
003	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
004	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	Progression (Bendamustine and Idelalisib)
005	After 5 cures of R-CHOP	PR	After 3 cures of R-Benda	Progression (R-DHAX and autograft)
006	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
007	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	Progression (R-DHAX and autograft)
008	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
009[†]	After 5 cures of R-CHOP	CR	-	CR
010	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
011	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
016	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
024	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
026	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
027	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
028	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
029[§]	After 5 cures of R-CHOP	PR	At the end of induction therapy (6 cures of R-CHOP)[§]	CR
030	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
031[†]	After 5 cures of R-CHOP	CR	-	CR

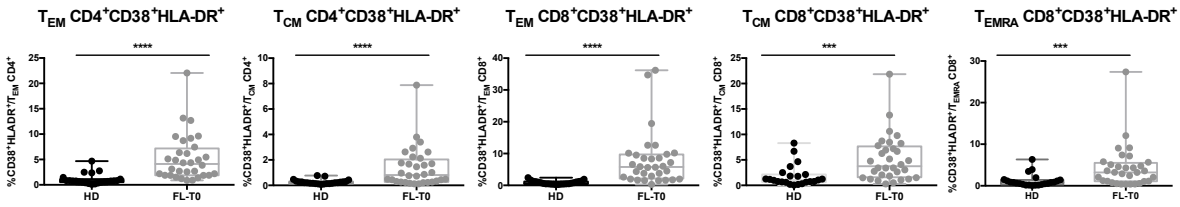
032	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
033	After 5 cures of R-CHOP	CR	After 4 cures of R-maintenance	CR
034	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
035	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
036	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
037	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
038	After 5 cures of R-CHOP	PR	After 2 cures of R-Benda	CR
039	After 5 cures of R-CHOP	CR	After 1 cure of R-maintenance	CR
040	After 5 cures of R-CHOP	PR	After 2 cures of R-Benda and 4 cures R-DHAX	Progression (Autograft)
041	After 5 cures of R-CHOP	PR	After 2 cures of R-Benda and 2 cures of R-maintenance	CR
042	After 5 cures of R-CHOP	CR	After 3 cures of R-maintenance	CR
043	After 5 cures of R-CHOP	CR	After 2 cures of R-maintenance	CR
044	After 5 cures of R-CHOP	CR	After 4 cures of R-maintenance	CR
045	After 5 cures of R-CHOP	PR	After 6 cures of R-Benda	Progression (R-GDP and Selinexor)

CR = Complete Response. PR = Partial Response. R = rituximab. CHOP = cyclophosphamide, doxorubicine, vincristine, prednisolone. Benda = bendamustine. DHAX = dexamethasone, cytarabine, oxaliplatin. GDP = gemcitabine, dexamethasone, cisplatin. * Patients 012-015, 017-023 and 025 were not included in the present study. $ All patients received an induction therapy (R-CHOP) consisting of 6 cycles every 21 days of rituximab combined to CHOP chemotherapy. For patients with CR after 6 cycles of R-CHOP, the induction therapy was followed by a maintenance regimen with cycles of rituximab as a single agent every three month for two years (R-maintenance). Patients with PR to induction therapy received a consolidation rituximab/chemotherapy regimen (R-DHAX or R-Benda) followed by R-maintenance for responder patients. † Severe side effects led to withdrawn after two cycles of R-maintenance. § After induction therapy, this patient received a consolidation therapy based on 4 cures of R-DHAX followed by R-maintenance.
Blood samples were collected from all patients before treatment (T0 also termed FL-T0).

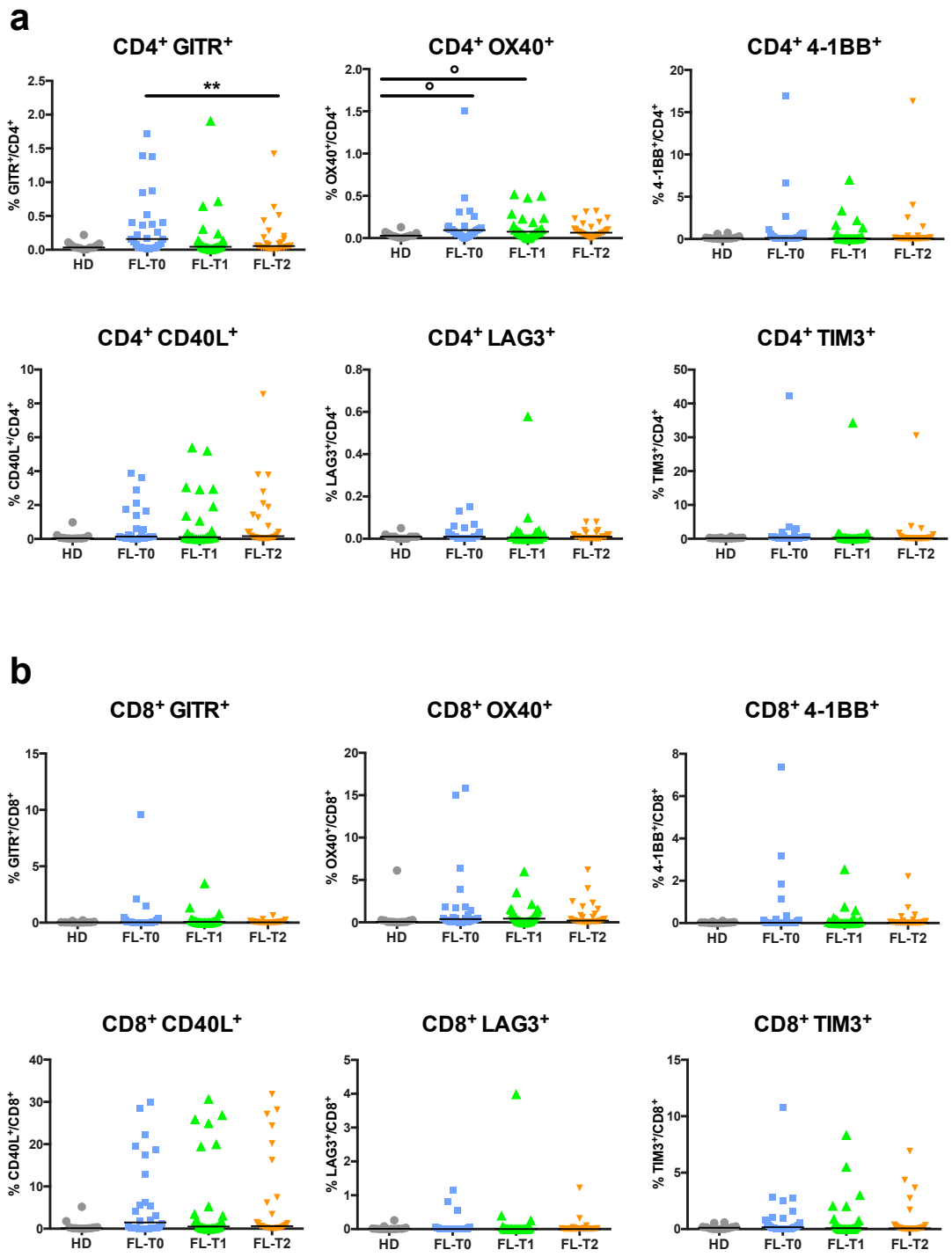
Supplementary Table S2. Antibody panels used in flow cytometry analyses of peripheral T-cell subsets.

Fluorochrome	Panel 1	Panel 2	Panel 3	Panel 4
FITC	CD8 (RPA-T8[*])	CD26 (M-A261[*])	GITR (eBioAITR[$])	CD8 (RPA-T8[*])
PerCP-eF710	CD3 (SK7[*])	CTLA-4 (14D3[$])	TIGIT (MBSA43[$])	TIGIT (MBSA43[$])
PE	-	CD45RA (HI-100[*])	LAG3 (3DS223H[$])	CD3 (OKT3[$])
PE-CF594	CD45RA (2H4LDH11LDB9[*])	CD39 (TU66[*])	-	CD45RA (2H4LDH11LDB9[*])
PE-Cy7	CD28 (28.2[#])	CD25 (M-A251[*])	TIM3 (F38-2E2[$])	CD25 (M-A251[*])
APC	-	CD127 (HIR-7R-M21[*])	OX40 (ACT35[#])	CD127 (HIR-7R-M21[*])
AF700	-	-	CD8 (RPA-T8[*])	-
APC-Cy7	HLA-DR (G46-6[*])	-	CD40L (24-31[#])	HLA-DR (G46-6[*])
BV421	CCR7 (150503[*])	CD4 (OKT-4[$])	4-1BB (4B4-1[#])	CCR7 (150503[*])
Amcyan	Viability (Live/dead cell dye)[£]	Viability (Live/dead cell dye)[£]	Viability (Live/dead cell dye)[£]	Viability (Live/dead cell dye)[£]
BV605	CD38 (HB7[*])	CD3 (OKT3[$])	CD3 (OKT3[$])	CD38 (HB7[*])
BV650	CD4 (SK3[*])	-	CD4 (SK3[*])	CD4 (SK3[*])
BV786	CD27 (L128[*])	PD-1 (EH12.2H7[#])	-	PD-1 (EH12.2H7[#])

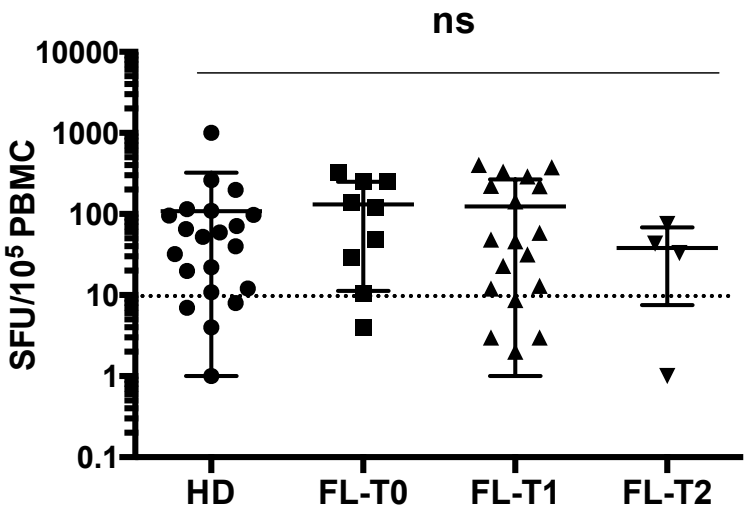
Antibody clones are indicated into brackets. Conjugated antibodies or fluorescent dyes were purchased from [*] BD Bioscience, [$] eBioscience, [#] Biolegend, [£] ThermoFisher Scientific.



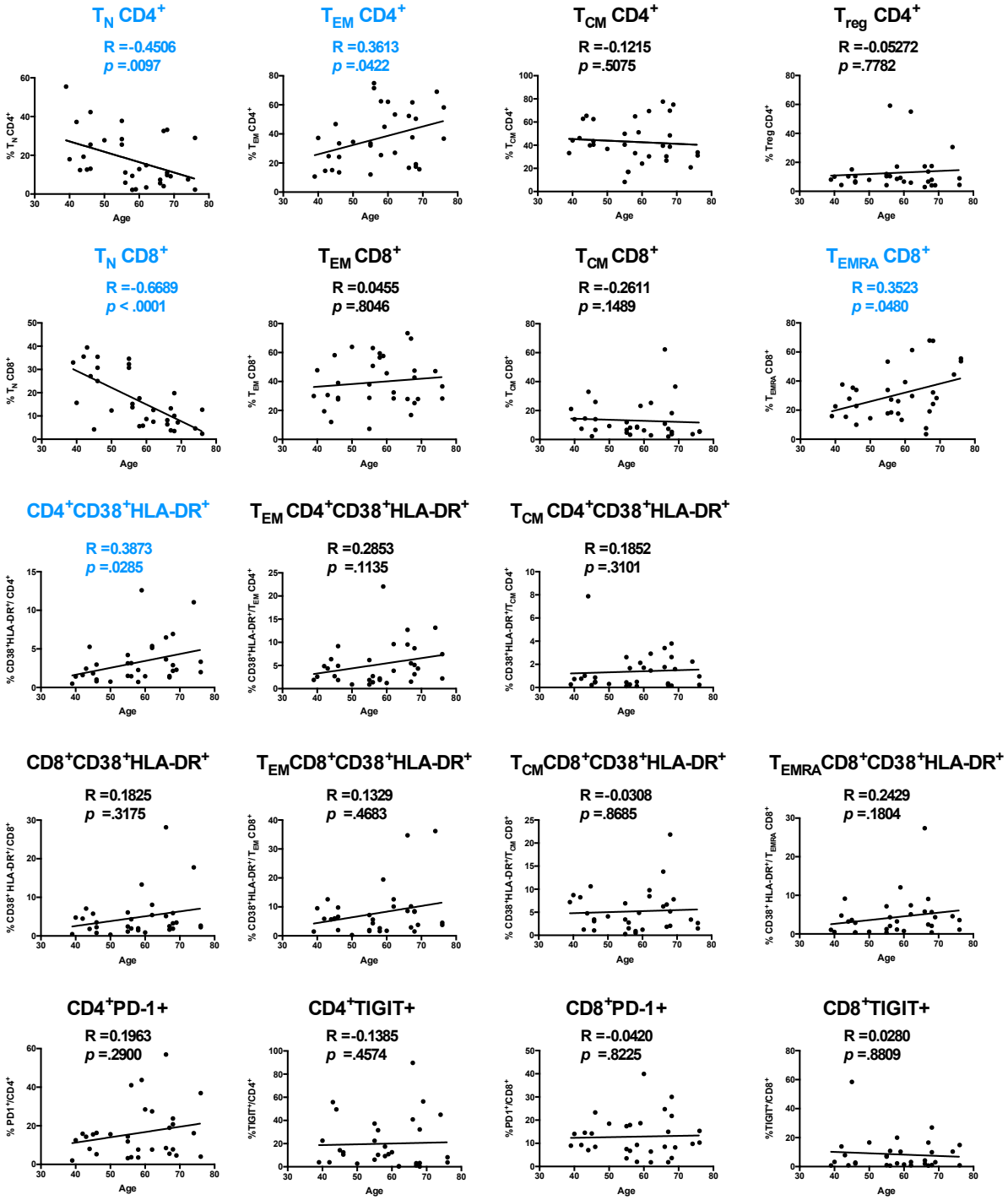
Supplementary Figure S1. Percentages of peripheral T-cell subsets expressing CD38 and HLA-DR activation markers before treatment of FL patients. Box-and-whisker plots of flow cytometry data obtained from blood samples of healthy donors (HD, $n = 23$) and FL patients before treatment (FL-T0, $n = 32$). T_{EM} and T_{CM} ($CD4^+$ and $CD8^+$) and T_{EMRA} ($CD8^+$) were analyzed. A Mann-Whitney test was performed for statistical analyses. *** $p < .001$; **** $p < .0001$.



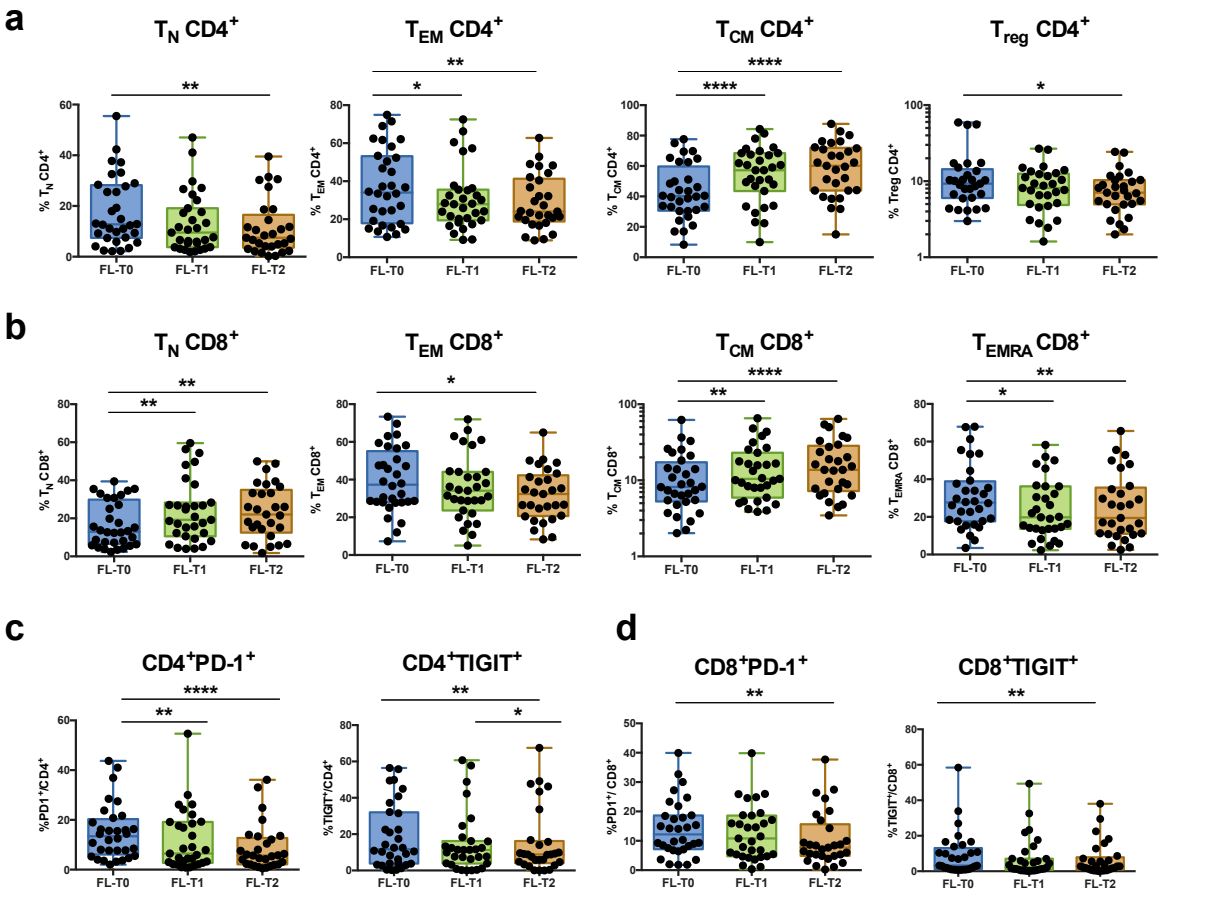
Supplementary Figure S2. Percentages of peripheral T cells expressing immune checkpoints (ICP) in FL patients before and during rituximab-based therapy. PBMC from healthy donors (HD) (n = 12) and from FL patients (n = 28) before treatment (FL-T0) and during therapy (FL-T1; FL-T2) were analyzed by flow cytometry. (**a**) Percentages of GITR$^+$, OX40$^+$, 4-1BB$^+$, CD40L$^+$, LAG3$^+$, TIM3$^+$ among CD4$^+$ T cells. (**b**) Percentages of GITR$^+$, OX40$^+$, 4-1BB$^+$, CD40L$^+$, LAG3$^+$, TIM3$^+$ among CD8$^+$ T cells. Friedman test (comparison between different time points in FL patients) and Kruskal-Wallis test (healthy donors *vs* patients) were performed for statistical analyses. Both tests were followed by Dunn's multiple comparison test. ° $p < .05$ (Kruskal-Wallis test); ** $p < .01$ (Friedman test).



Supplementary Figure S3. *In vitro* IFN-γ responses to virus-derived peptides in FL patients. IFN-γ production in response to CEFT (CMV, EBV, influenza, tetanus toxin)-derived peptides by PBMC from healthy donors (HD; n = 21) and FL patients before (FL-T0, n = 9) and during (FL-T1, n = 18; FL-T2, n = 4) therapy, was assessed by ELISPOT assays. Results were expressed as spot-forming units (SFU) per 10^5 cells. A positive threshold was set at ≥ 10 spot-forming-units (SFU) per 10^5 cells after subtracting the background noise as previously described[50]. Each sample was tested in triplicate and the mean value was reported. A Kruskal-Wallis test followed by Dunn's multiple comparison test was performed for statistical analyses. ns: not significant.



Supplementary Figure S4. Age-dependence of peripheral T-cell phenotype in FL patients before treatment. Spearman correlation tests were performed to analyze the relationship between the percentage of different T-cell subsets (indicated above each diagram) and age of the patients before treatment. R, correlation coefficient. Variables were considered as significantly correlated with $p < .05$ (in blue).



Supplementary Figure S5. Modifications of peripheral T-cell subsets during therapy. Box-and-whisker plots of flow cytometry data obtained from FL patients (n = 29) before (FL-T0) and during treatment (FL-T1; FL-T2). (**a**) Percentages of T_N, T_{EM}, T_{CM} and T_{reg} $CD4^+$ T cells. (**b**) Percentages of T_N, T_{EM}, T_{CM} and T_{EMRA} $CD8^+$ T cells. (**c**) Percentages of $CD4^+$ $PD\text{-}1^+$, $CD4^+$ $TIGIT^+$ T cells. (**d**) Percentages of $CD8^+PD\text{-}1^+$, $CD8^+TIGIT^+$ T cells. Friedman test followed by Dunn's multiple comparison test was performed for statistical analyses. * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$.