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Supplemental Information

EBI2 Expression and Function: Robust in Memory Lymphocytes and Increased by Natalizumab in Multiple Sclerosis

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Figure S1. Related to Figure 1.

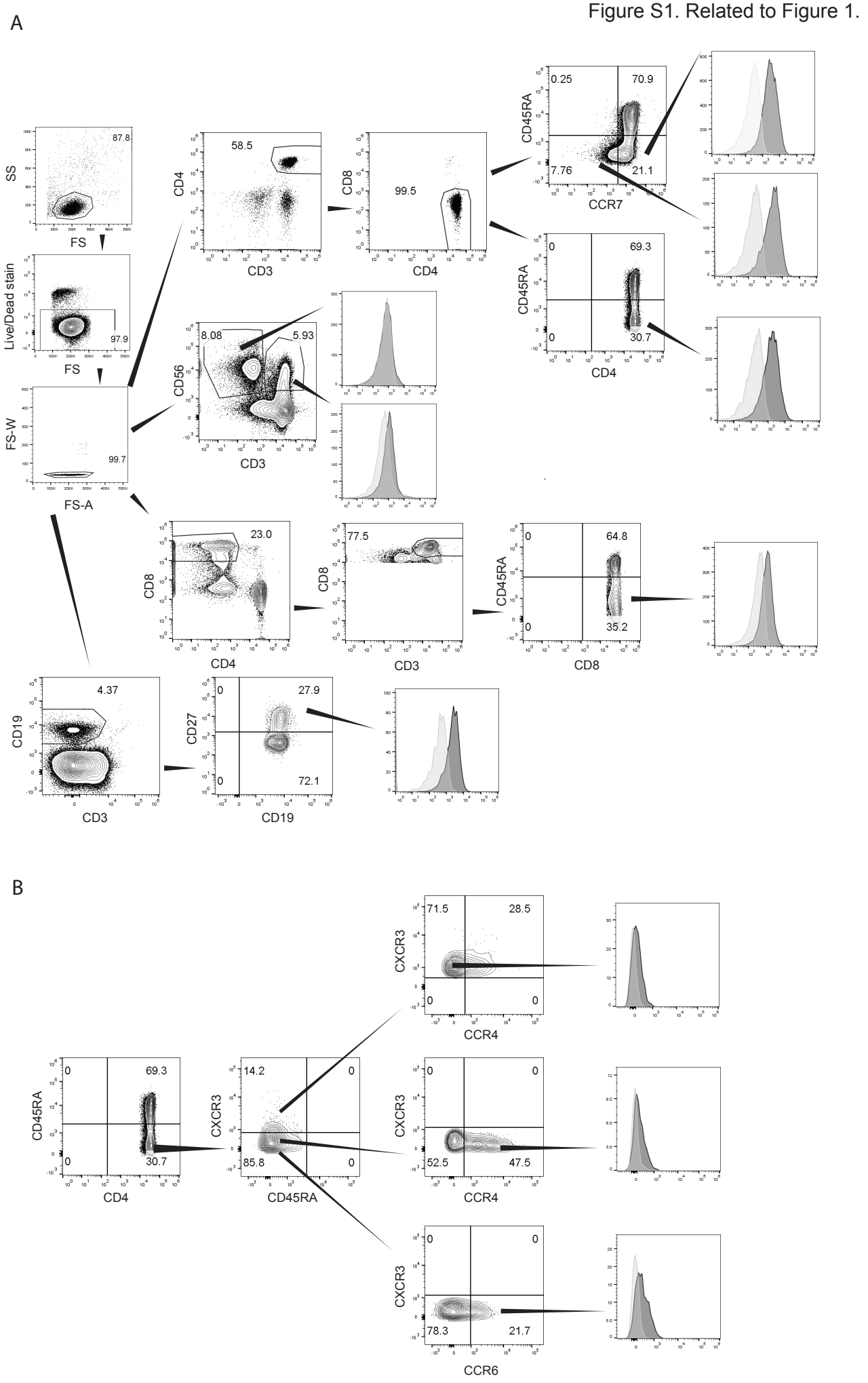
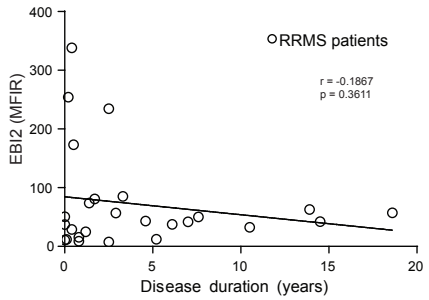
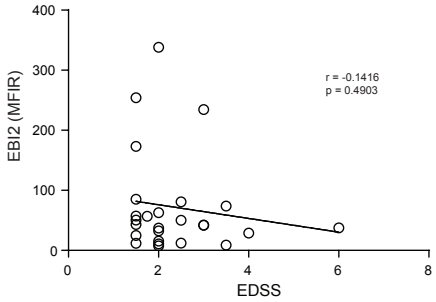


Figure S1. **Related to Figure 1. Flow cytometry gating strategy of lymphocyte subpopulations.** (A) Naive and memory (defined as $CD45RA^+$ and $CD45RA^-$, respectively) $CD4^+$ and $CD8^+$ T cells, central memory ($CCR7^+$) and effector memory ($CCR7^-$) $CD4^+$ T cells, naive and memory (defined as $CD27^-$ and $CD27^+$, respectively) $CD19^+$ B cells, NK ($CD3^-CD56^+$) and NKT ($CD3^+CD56^+$) cells. (B) Th1 ($CXCR3^+CCR4$-), Th2 ($CXCR3^-CCR4^+$) and Th17 ($CXCR3^-CCR6^+$) memory $CD4^+$ T cell subsets. FS means forward scatter, with A for Area or W for Width for doublets exclusion; SS means side scatter. Histograms show EBI2 in dark grey and isotype in light grey.

Figure S2. Related to Figure 4.

A





B

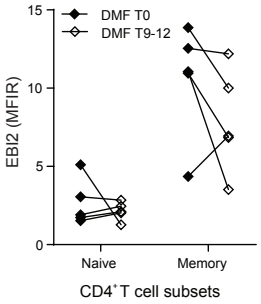


Figure S2. Related to Figure 4. EBI2 expression is not increased with disease duration, elevated EDSS score or dimethyl fumarate treatment. (A) EBI2 expression with corresponding disease duration in years (left panel) or EDSS score (right panel) of RRMS patients (*n=26*) with or without disease modifying treatment (except natalizumab). EDSS means expanded disability status scale. RRMS means relapsing remitting multiple sclerosis patients. (B) Flow cytometric analysis of EBI2 expression on naive ($CD45RA^-$) and memory ($CD45RA^+$) $CD4^+$ T cells before (T0) and after 9-12 months (T9-12) of dimethyl fumarate (DMF) treatment (*n=5*). EBI2 expression shown as mean fluorescence intensity ratios (MFIR).

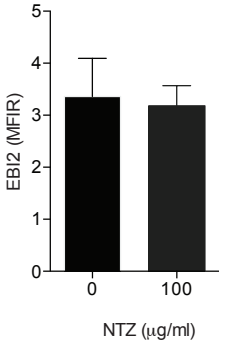
Table S1. Related to Figure 4. Dimethyl fumarate treated patients.

	Gender	Mean Age (± *SEM*)	n	Mean EDSS at T0 (±*SEM*)	Mean EDSS at T9-12 (±*SEM*)	Disease mean duration at blood draw (± *SEM*)	Previous DMT
DMF patients	female	32.33 (*5.78*)	3	1.5 (*0.00*)	2.00 (*0.5*)	4.67 (*1.20*)	1 Glatiramer acetate 1 Fingolimod (stopped since two months) 1 untreated
	male	40.5 (*7.5*)	2	2.25 (*0.75*)	2.00 (*0.5*)	16.5 (*3.5*)	2 Interferon-beta

RRMS : relapsing remitting MS patients
DMF : dimethyl fumarate treated patients
Age in years. Disease duration in years.
EDSS: expanded disability status scale

Figure S3. Related to Figure 4

A



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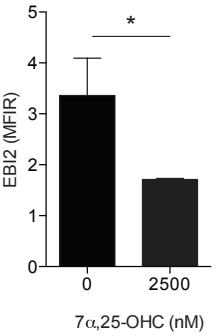

Figure S3. **Related to Figure 4. NTZ is not able to promote EBI2 expression *in vitro*.** Human PBMC were kept after an overnight resting in culture medium for 40 minutes at 37°C with either only culture medium or additionned (A) with NTZ or (B) 7α,25-OHC at the indicated concentrations (n=3). The T cells were analyzed by flow cytometry. EBI2 expression is shown as mean fluorescence intensity ratios (MFIR) in memory $CD3^{+}CD4^{+}$ T cells, gated after dead cell and doublet exclusion. Statistical analysis using unpaired t test with a p value ≤ 0.05 considered significant.