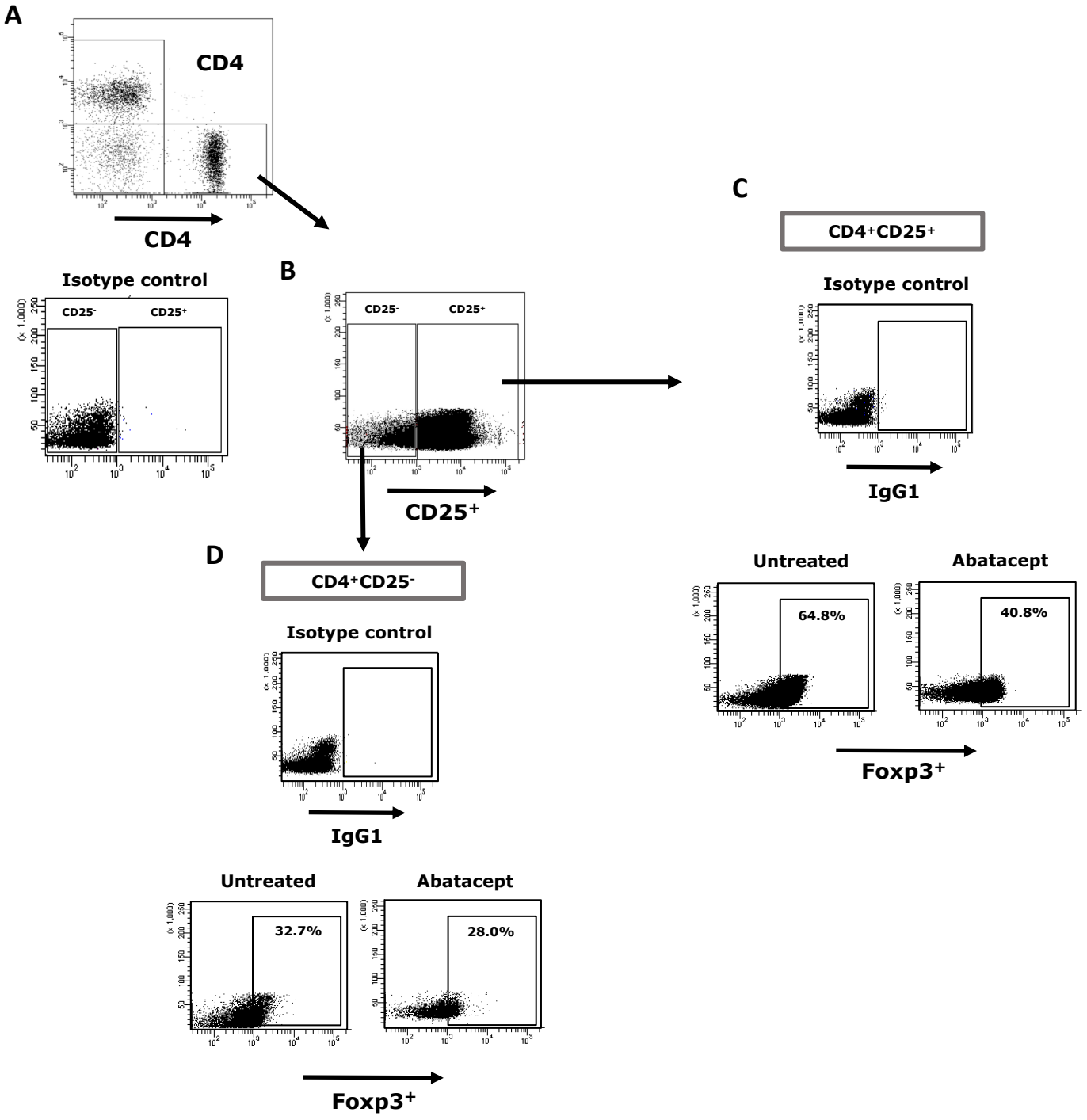
Abatacept Enhances Blood Regulatory B Cells of Rheumatoid Arthritis Patients to a Level that Associates with Disease Remittance

Maha Fahad Alenazy, Fatemeh Saheb Sharif-Askari, Mohammed A. Omair, Mohammed Elwatidy, Maha A. Omair, Hossam Mitwalli, Saleh Al-Muhsen, Abeer Al Masri, Qutayba Hamid, and Rabih Halwani

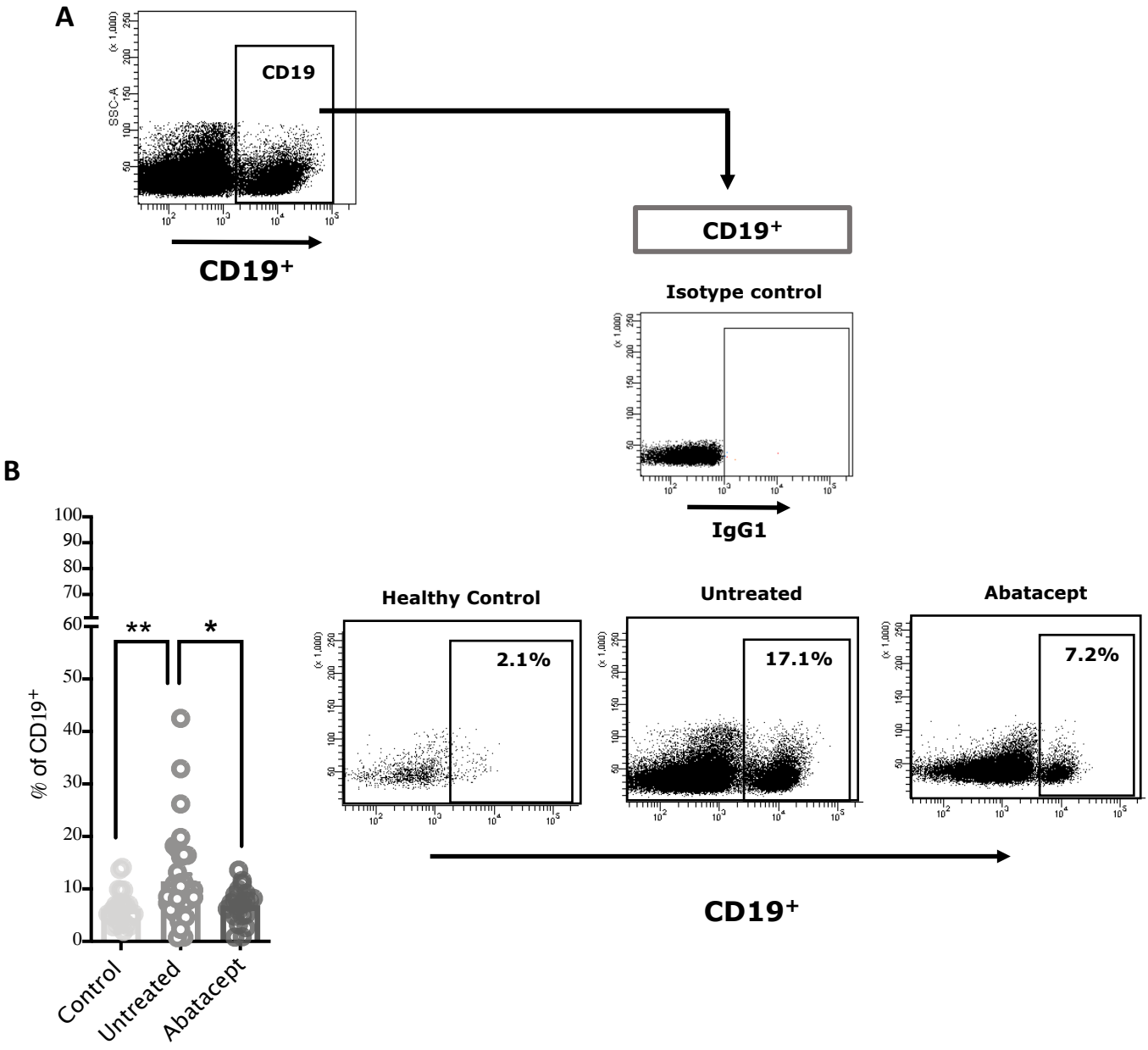
Supplementary Table 1. Association of blood levels $CD138^+CD1d^+EBi3^+IL\text{-}10^+$ Bregs or $CD25^+Foxp3^+LAG3^+$ Tregs or $CD25^-Foxp3^+LAG3^+$ uTregs with serum levels of cytokines.

Cytokines	**Pearson's correlation coefficient (r)**	***P* value**
$CD138^+CD1d^+EBi3^+IL\text{-}10^+$ Bregs		
IL-10	0.301	0.317
IL-35	-0.141	0.646
IFN-β	-0.052	0.866
$CD25^+Foxp3^+LAG3^+$ Tregs		
IL-10	0.145	0.637
IL-35	0.771	0.002
IFN-β	0.672	0.012
$CD25^-Foxp3^+LAG3^+$ Tregs		
IL-10	-0.052	0.866
IL-35	0.801	0.001
IFN-β	0.713	0.006

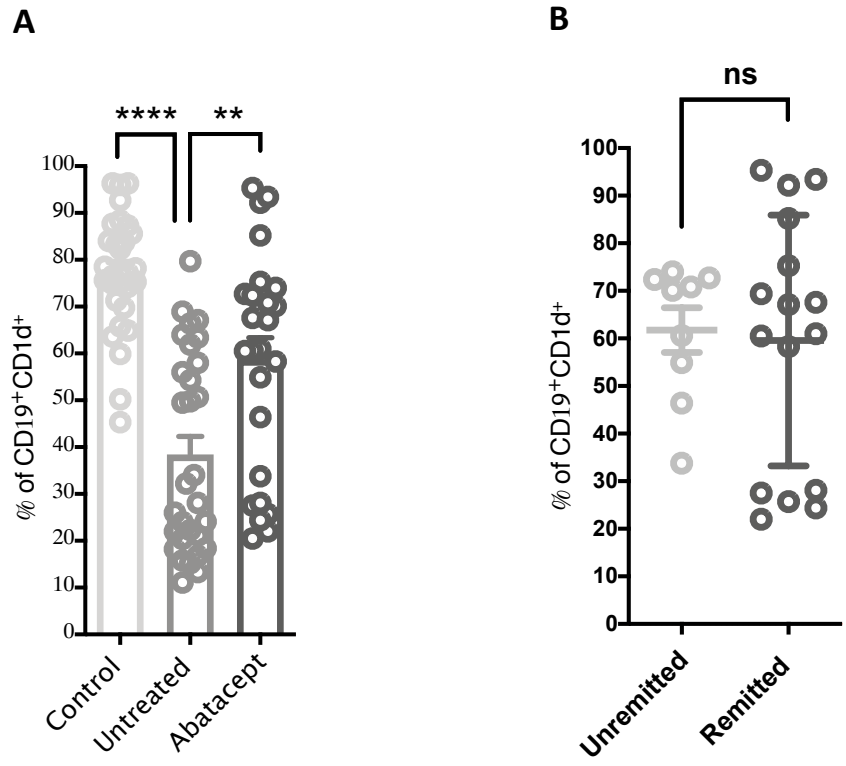
Statistics significant: $P < 0.05$.



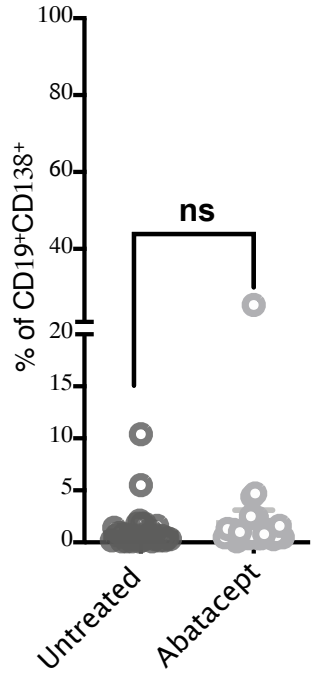
Supplementary Figure 1. Effects of the abatacept on the frequency of conventional Tregs (cTregs) and unconventional Tregs (uTreg) in the PBMCs of RA patients. (A and B) Gating was performed according to CD4 T cells expression and CD25 markers in PBMCs of RA patients. (C) Percentage of cTregs (Foxp3$^+$ cells within CD4$^+$CD25$^+$) in the PBMCs of abatacept-treated and untreated RA patients. Representative FACS data showing no difference in the percentage of these cells in PBMCs following treatment with the abatacept. (D) Percentage of uTregs (Foxp3$^+$ cells within CD4$^+$CD25$^-$) in the PBMCs of abatacept-treated and untreated RA patients. Representative FACS data showing no difference in the percentage of these cells in PBMCs following treatment with the abatacept. Events were recorded and analysed by using BD FACSDiva version 8.0.



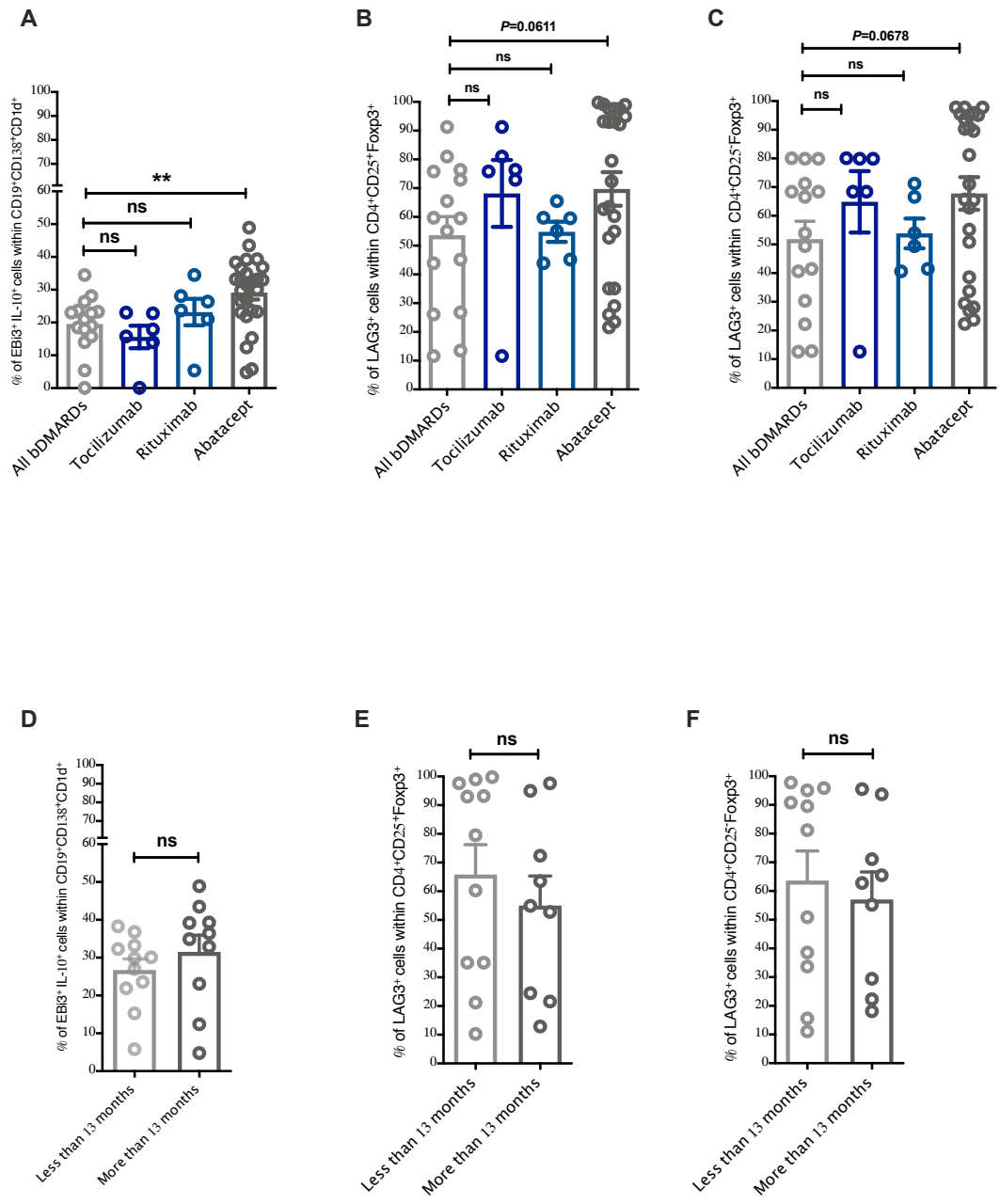
Supplementary Figure 2. Effects of the abatacept on the frequency of CD19$^+$ cells in the PBMCs of RA patients. (A) The gating for CD19$^+$ cells in PBMCs of RA patients. (B) Percentage of CD19$^+$cells in the PBMCs of abatacept-treated and untreated RA patients, and in the healthy controls. Representative FACS data showing a decrease in the percentage of CD19$^+$ in PBMCs following treatment with the abatacept. Events were recorded and analysed by using BD FACSDiva version 8.0. Two-way comparison was done using t-test. * $P<0.05$, ** $P<0.01$.



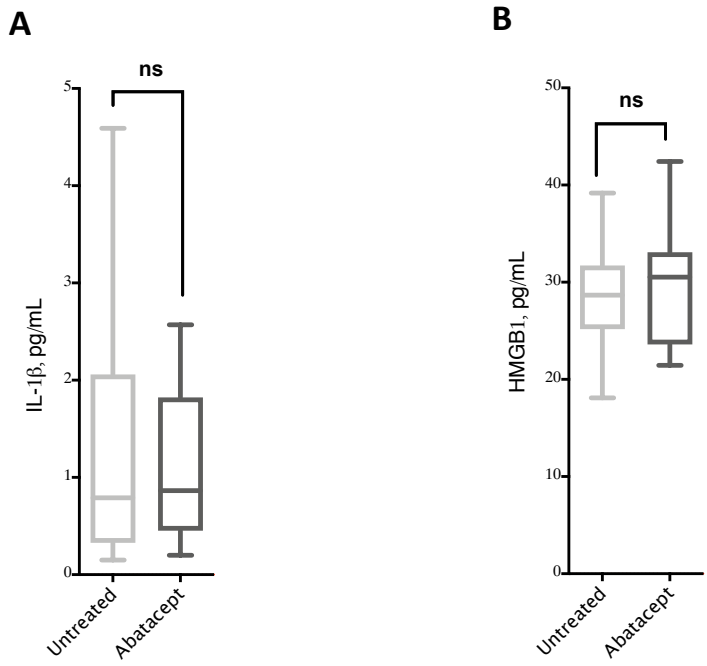
Supplementary Figure 3. Effects of the abatacept on the frequency of $CD19^{+}CD1d^{+}$ Bregs in the PBMCs of RA patients. (A) Percentage of $CD19^{+}CD1d^{+}$ Bregs in the PBMCs of abatacept-treated and untreated RA patients. Representative FACS data showing an increase in the percentage of $CD19^{+}CD1d^{+}$ Bregs in PBMCs following treatment with the abatacept. (B) No different in the blood level of this Breg subtype was observed between remitted and unremitted abatacept-treated groups. DAS28-CRP score lower than 2.6 indicates remission. Two-way comparison was done using t-test. ns: non-significant. ** $P<0.01$, **** $P<0.0001$.



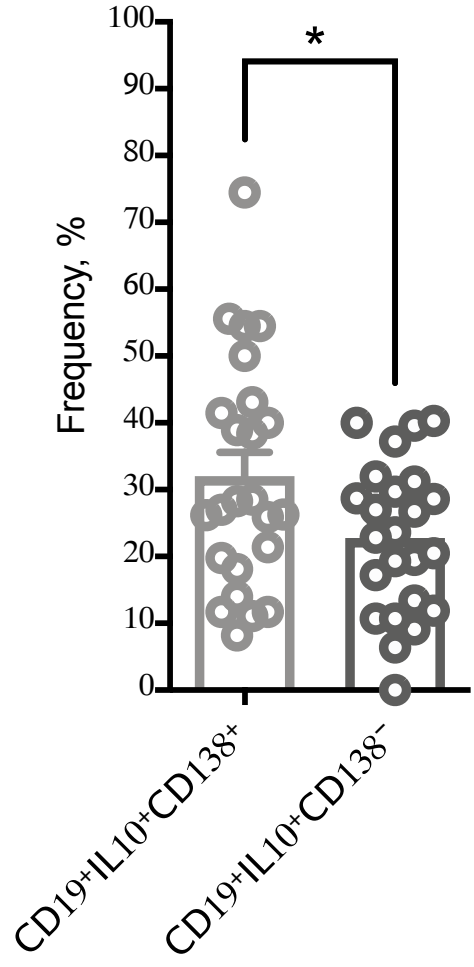
Supplementary Figure 4. Frequency of $CD19^{+}CD138^{+}$cells in the PBMCs of abatacept-treated and untreated RA patients. Representative data showing no difference in the percentage of $CD19^{+}CD138^{+}$cells in PBMCs following treatment with the abatacept. Two-way comparison was done using t-test. ns: non-significant. * $P<0.05$.



Supplementary Figure 5. Frequency of (A) IL-35$^+$IL-10$^+$ Bregs (B) LAG3$^+$ cTregs, and (C) LAG3$^+$ uTregs in the PBMCs of abatacept-treated patients; or RA patients treated with other bDMARDs, including tocilizumab or rituximab for an average of 13 months. Representative data showing an increase in the percentage of these cells in PBMCs following treatment with the abatacept. Frequency of (D) IL-35$^+$IL-10$^+$ Bregs, (E) LAG3$^+$ cTregs, and (F) LAG3$^+$ uTregs in the PBMCs of abatacept-treated patients who were on more or less than 13 months of therapy. Representative data showing no significant difference in the average frequency of IL-35$^+$IL-10$^+$ Bregs, LAG3$^+$ cTregs, and LAG3$^+$ uTregs between these two groups. Two-way comparison was done using t-test. ns: non-significant. * $P<0.05$, ** $P<0.01$.



Supplementary Figure 6. Effects of abatacept on the cytokine levels in the serum of RA patients. Serum levels of (A) IL-1β and (B) HMGB1 in abatacept treated and untreated groups. Data show no significant change in of the serum levels of IL-1 β and HMGB1 of abatacept treated compared to untreated RA groups. The cytokine levels were estimated using a human Luminex assay. Two-way comparison was done using t-test. ns: non-significant. * $P<0.05$.



Supplementary Figure 7. Frequency of $CD19^{+}IL\text{-}10^{+}CD138^{+}$ and $CD19^{+}IL\text{-}10^{+}CD138^{-}$ cells in the PBMCs of abatacept-treated RA patients. Representative data showing an increase in the percentage of $CD19^{+}IL\text{-}10^{+}CD138^{+}$ cells in PBMCs following treatment with abatacept. Two-way comparison was done using t-test. * $P<0.05$.