

## Additional file 1

**Table S1.** Identified subsets of peripheral blood mononuclear cells defined using cell-surface markers

Identified subsets	Definition by cell-surface markers
CD4 <sup>+</sup> T cells	CD3 <sup>+</sup> CD4 <sup>+</sup>
Naïve CD4 <sup>+</sup> T cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO <sup>-</sup>
Memory CD4 <sup>+</sup> T cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CD45RO <sup>+</sup>
T <sub>reg</sub> cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CD25 <sup>+</sup> CD127 <sup>low</sup>
T <sub>h</sub> 1 cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR3 <sup>+</sup> CCR6 <sup>-</sup>
T <sub>h</sub> 2 cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR3 <sup>-</sup> CCR6 <sup>-</sup>
T <sub>h</sub> 17 cells	CD3 <sup>+</sup> CD4 <sup>+</sup> CXCR3 <sup>-</sup> CCR6 <sup>+</sup> CD161 <sup>+</sup>
CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup>
Naïve CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup> CD45RO <sup>-</sup>
Memory CD8 <sup>+</sup> T cells	CD3 <sup>+</sup> CD8 <sup>+</sup> CD45RO <sup>+</sup>
B cells	CD3 <sup>-</sup> CD20 <sup>+</sup>
Naïve B cells	CD3 <sup>-</sup> CD20 <sup>+</sup> CD27 <sup>-</sup>
Memory B cells	CD3 <sup>-</sup> CD20 <sup>+</sup> CD27 <sup>+</sup>
Natural Killer cells	CD3 <sup>-</sup> CD56 <sup>+</sup>
Non-classical monocytes	CD3 <sup>-</sup> CD14 <sup>+</sup> CD16 <sup>+</sup>
Classical monocytes	CD3 <sup>-</sup> CD14 <sup>+</sup> CD16 <sup>-</sup>

**Table S2.** Correlations between peripheral T cell subsets and serum markers, composite measures of disease activity, and Health Assessment Questionnaire-Disability Index score at baseline. Spearman rank correlation coefficient was used for analysis.

	RF	ACPA	CRP	TJC	SJC	D-VAS	Pt-VAS	HAQ-DI	CDAI	SDAI
<b>CD4<sup>+</sup> T cells / lymphocytes</b>	-0.14	-0.22	0.07	-0.08	0.08	0.00	-0.10	-0.08	0.00	0.01
<b>Naïve CD4<sup>+</sup> T cells / CD4<sup>+</sup> T cells</b>	-0.07	0.11	-0.23	-0.05	0.01	-0.07	-0.27	0.03	-0.11	-0.15
<b>Memory CD4<sup>+</sup> T cells / CD4<sup>+</sup> T cells</b>	0.07	-0.11	0.23	0.05	-0.01	0.07	0.27	-0.03	0.11	0.15
<b>HLA-DR<sup>+</sup>CD4<sup>+</sup> T cells / CD4<sup>+</sup> T cells</b>	-0.25	-0.08	0.17	0.04	0.15	0.15	0.17	-0.05	0.23	0.24
<b>CD38<sup>+</sup>CD4<sup>+</sup> T cells / CD4<sup>+</sup> T cells</b>	-0.10	-0.07	-0.27	-0.09	-0.08	-0.13	-0.17	-0.15	-0.17	-0.20
<b>CD69<sup>+</sup>CD4<sup>+</sup> T cells / CD4<sup>+</sup> T cells</b>	-0.03	0.28	-0.09	-0.07	-0.02	-0.11	-0.29	-0.23	-0.13	-0.14
<b>CD8<sup>+</sup> T cells / lymphocytes</b>	0.15	-0.01	-0.04	0.08	0.21	0.15	0.05	0.21	0.16	0.15
<b>Naïve CD8<sup>+</sup> T cells / CD8<sup>+</sup> T cells</b>	0.35*	0.31*	0.14	0.26	0.34*	0.22	0.18	0.25	0.37*	0.33*
<b>Memory CD8<sup>+</sup> T cells / CD8<sup>+</sup> T cells</b>	-0.35*	-0.31*	-0.14	-0.26	-0.34*	-0.22	-0.18	-0.25	-0.37*	-0.33*
<b>HLA-DR<sup>+</sup>CD8<sup>+</sup> T cells / CD8<sup>+</sup> T cells</b>	0.00	-0.07	0.41*	0.12	0.19	0.18	0.34*	0.30*	0.29	0.34*
<b>CD38<sup>+</sup>CD8<sup>+</sup> T cells / CD8<sup>+</sup> T cells</b>	-0.07	-0.15	0.23	0.10	0.07	-0.08	0.20	-0.11	0.08	0.12
<b>CD69<sup>+</sup>CD8<sup>+</sup> T cells / CD8<sup>+</sup> T cells</b>	0.07	0.00	-0.22	0.00	-0.24	-0.11	-0.11	-0.07	-0.19	-0.20
<b>T<sub>reg</sub> / CD4<sup>+</sup> T cells</b>	0.09	-0.07	0.07	0.21	0.30*	-0.09	0.05	0.00	0.15	0.11
<b>Naïve T<sub>reg</sub> / T<sub>reg</sub></b>	0.06	-0.01	0.42*	-0.11	0.12	0.09	0.14	-0.07	0.10	0.16
<b>Memory T<sub>reg</sub> / T<sub>reg</sub></b>	-0.06	0.01	-0.42*	0.11	-0.12	-0.09	-0.14	0.07	-0.10	-0.16
<b>HLA-DR<sup>+</sup> T<sub>reg</sub> / T<sub>reg</sub></b>	0.21	-0.09	0.08	0.08	-0.15	0.16	0.37*	0.17	0.10	0.10
<b>T<sub>h</sub>1 / CD4<sup>+</sup> T cells</b>	0.21	0.04	0.06	0.09	0.09	0.17	-0.04	0.09	0.09	0.05
<b>HLA-DR<sup>+</sup> T<sub>h</sub>1 / T<sub>h</sub>1</b>	-0.11	0.26	0.11	-0.25	0.03	-0.05	-0.28	-0.17	-0.15	-0.14
<b>T<sub>h</sub>2 / CD4<sup>+</sup> T cells</b>	0.31	0.12	0.11	0.37*	0.24	0.32*	0.35*	0.29	0.37*	0.36*
<b>HLA-DR<sup>+</sup> T<sub>h</sub>2 / T<sub>h</sub>2</b>	-0.18	0.00	-0.28	-0.19	-0.22	-0.10	-0.05	-0.12	-0.22	-0.23
<b>T<sub>h</sub>17 / CD4<sup>+</sup> T cells</b>	0.08	0.00	-0.22	0.00	-0.23	-0.10	-0.11	-0.07	-0.19	-0.19
<b>HLA-DR<sup>+</sup> T<sub>h</sub>17 / T<sub>h</sub>17</b>	0.05	-0.21	-0.14	0.07	0.24	0.10	-0.04	-0.31	0.11	0.07

\* $P < 0.05$

ACPA, anti-citrullinated protein antibody; CRP, C-reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

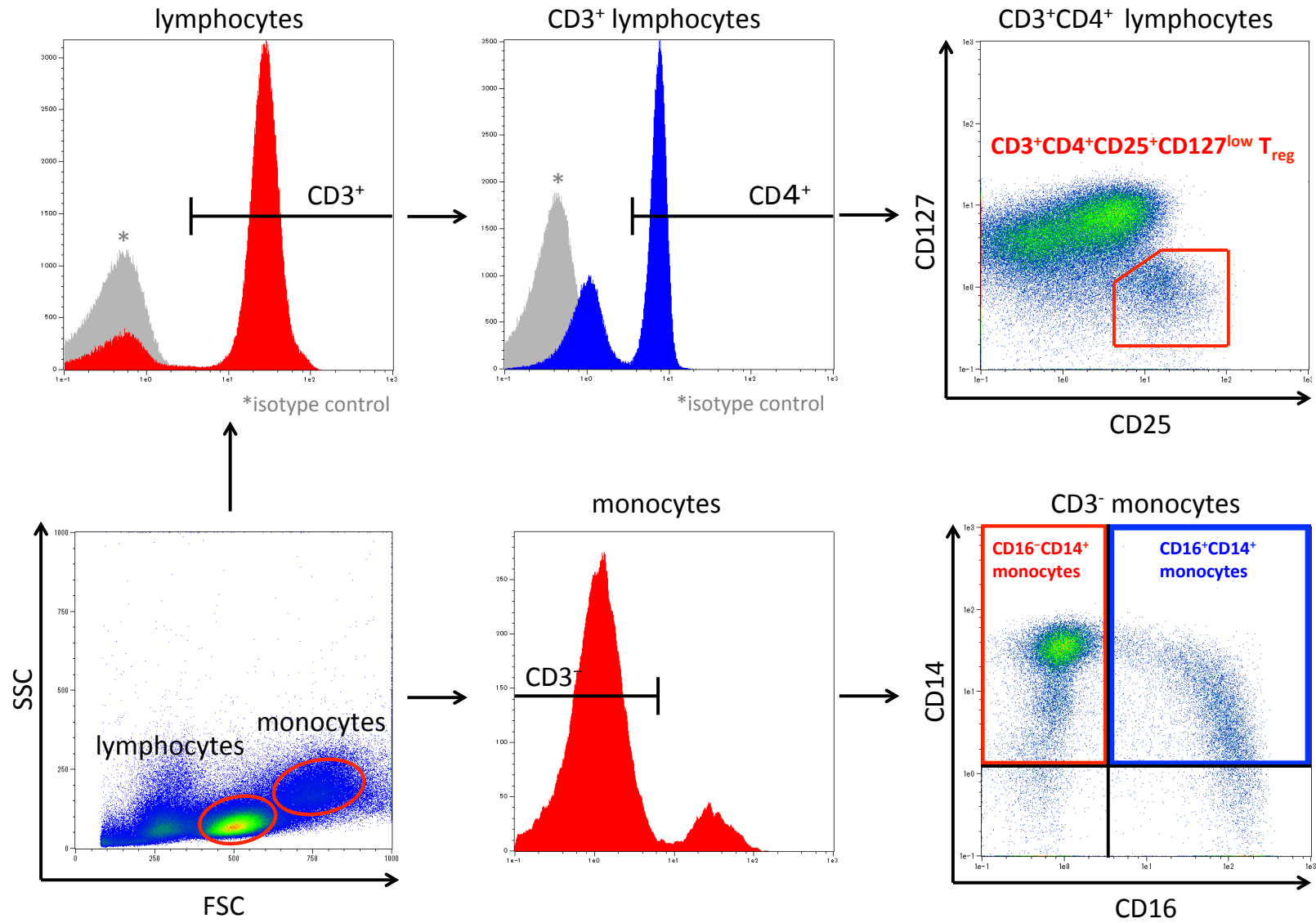
**Table S3.** Correlations between peripheral B cell subsets, NK cells and monocyte subsets and serum markers, composite measures of disease activity, and Health Assessment Questionnaire-Disability Index score at baseline. Spearman rank correlation coefficient was used for analysis.

	RF	ACPA	CRP	TJC	SJC	D-VAS	Pt-VAS	HAQ-DI	CDAI	SDAI
<b>B cells / lymphocytes</b>	-0.01	0.02	0.16	-0.14	0.09	0.03	-0.02	0.11	-0.04	-0.04
<b>CD80<sup>+</sup> B cells / B cells</b>	-0.01	0.04	0.01	0.17	0.14	0.06	0.09	0.00	0.15	0.12
<b>CD86<sup>+</sup> B cells / B cells</b>	0.08	0.22	0.24	-0.09	-0.06	-0.02	-0.02	0.11	-0.07	-0.02
<b>HLA-DR<sup>+</sup> B cells / B cells</b>	-0.25	0.05	-0.16	-0.09	0.01	-0.07	-0.16	-0.14	-0.11	-0.13
<b>Naïve B cells / B cells</b>	0.22	0.26	0.43*	0.22	0.11	-0.01	0.23	0.28	0.20	0.22
<b>Memory B cells / B cells</b>	-0.22	-0.26	-0.43*	-0.22	-0.11	0.01	-0.23	-0.28	-0.20	-0.22
<b>NK cells / lymphocytes</b>	-0.21	-0.01	-0.10	-0.23	-0.30	-0.21	-0.06	-0.12	-0.24	-0.25
<b>CD80<sup>+</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	-0.15	0.06	-0.06	0.04	-0.20	-0.22	-0.12	-0.24	-0.18	-0.18
<b>CD86<sup>+</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	-0.01	0.10	-0.09	0.37	0.23	0.01	0.14	-0.01	0.27	0.21
<b>HLA-DR<sup>+</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	0.09	0.05	-0.11	0.13	-0.06	0.11	0.22	0.06	0.10	0.08
<b>CD69<sup>+</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	-0.16	-0.06	0.01	-0.14	-0.28	-0.23	-0.30*	-0.17	-0.27	-0.26
<b>CD16<sup>+</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	0.24	0.20	0.17	-0.01	0.03	0.06	0.17	0.37*	0.06	0.11
<b>CD16<sup>-</sup> CD14<sup>+</sup> monocytes / CD14<sup>+</sup> monocytes</b>	-0.25	-0.20	-0.17	0.01	-0.03	-0.06	-0.17	-0.37*	-0.06	-0.11

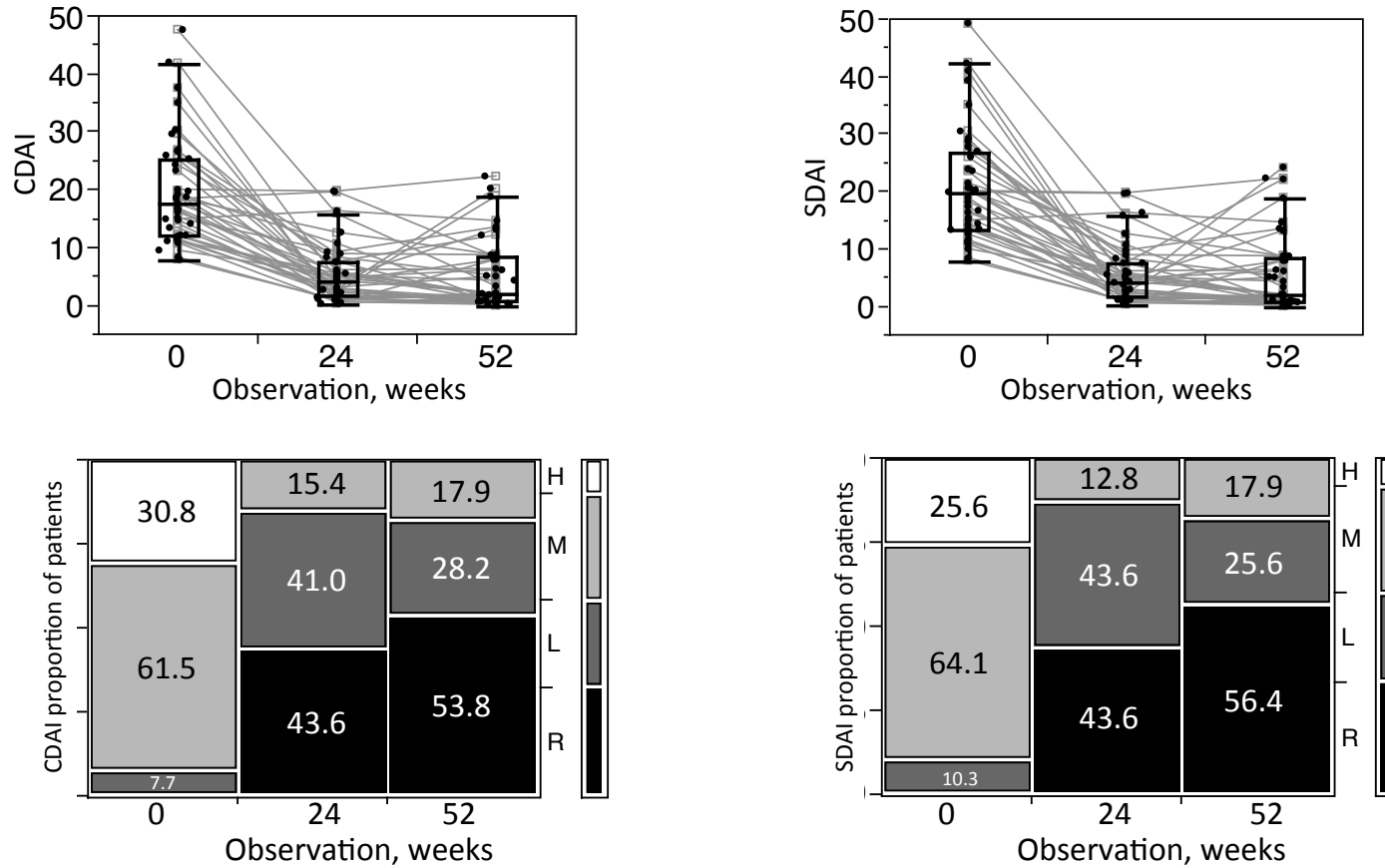
\* $P < 0.05$

ACPA, anti-citrullinated protein antibody; CRP, C-reactive protein; HAQ-DI, Health Assessment Questionnaire Disability Index; RF, rheumatoid factor; SJC, swollen joint count; TJC, tender joint count.

**Figure S1.** Gating strategy for flowcytometry analysis of T<sub>reg</sub> and subsets of monocytes



**Figure S2.** Chronological changes in and proportion of clinical activity from baseline until Week 52 of TCZ treatment. R, remission; L, low disease activity; M, moderate disease activity; H, high disease activity; CDAI, Clinical Disease Activity Index; SDAI, Simplified Disease Activity Index.

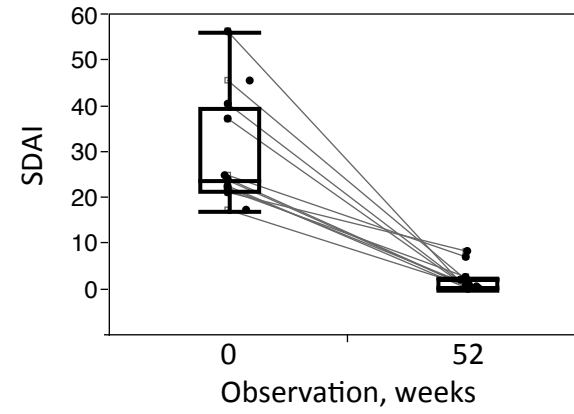
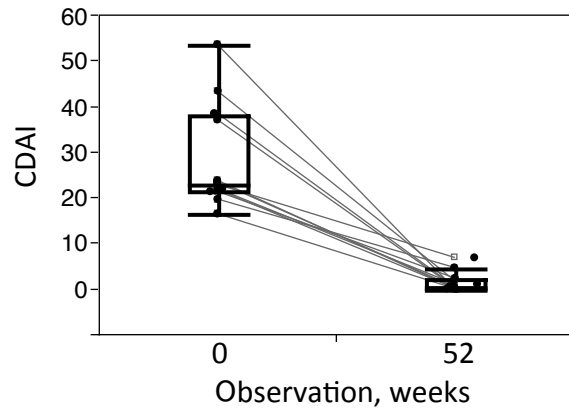


**Table S4.** Baseline demographics and clinical characteristics of the patients treated with MTX

<b>Patient Characteristics, n=12</b>	<b>Mean <math>\pm</math> SD or Number (%)</b>	<b>Median (IQR)</b>
Age, years	57.6 $\pm$ 12.5	58.0 (45.0–71.0)
Female, n (%)	9 (75.0)	–
Disease duration, years	25.7 $\pm$ 45.4	11 (2.3–29.3)
Dose of methotrexate, mg/week	7.3 $\pm$ 1.0	8.0 (6.0–8.0)
Concomitant glucocorticoid, n (%)	0 (0)	–
SDAI	29.6 $\pm$ 12.1	23.7 (21.6–39.5)
CDAI	28.6 $\pm$ 11.5	23.1 (21.4–38.1)
RF positive, n (%)	8 (66.7)	–
ACPA positive, n (%)	6 (50.0)	–
CRP, mg/dl	0.98 $\pm$ 0.92	0.7 (0.2–1.9)
ESR, mm/h	52.0 $\pm$ 32.7	45.5 (25.8–76.8)
MMP-3, ng/ml	112.7 $\pm$ 112.6	69.5 (43.3–162.8)
HAQ-DI	0.7 $\pm$ 0.8	0.63 (0.1–1.2)

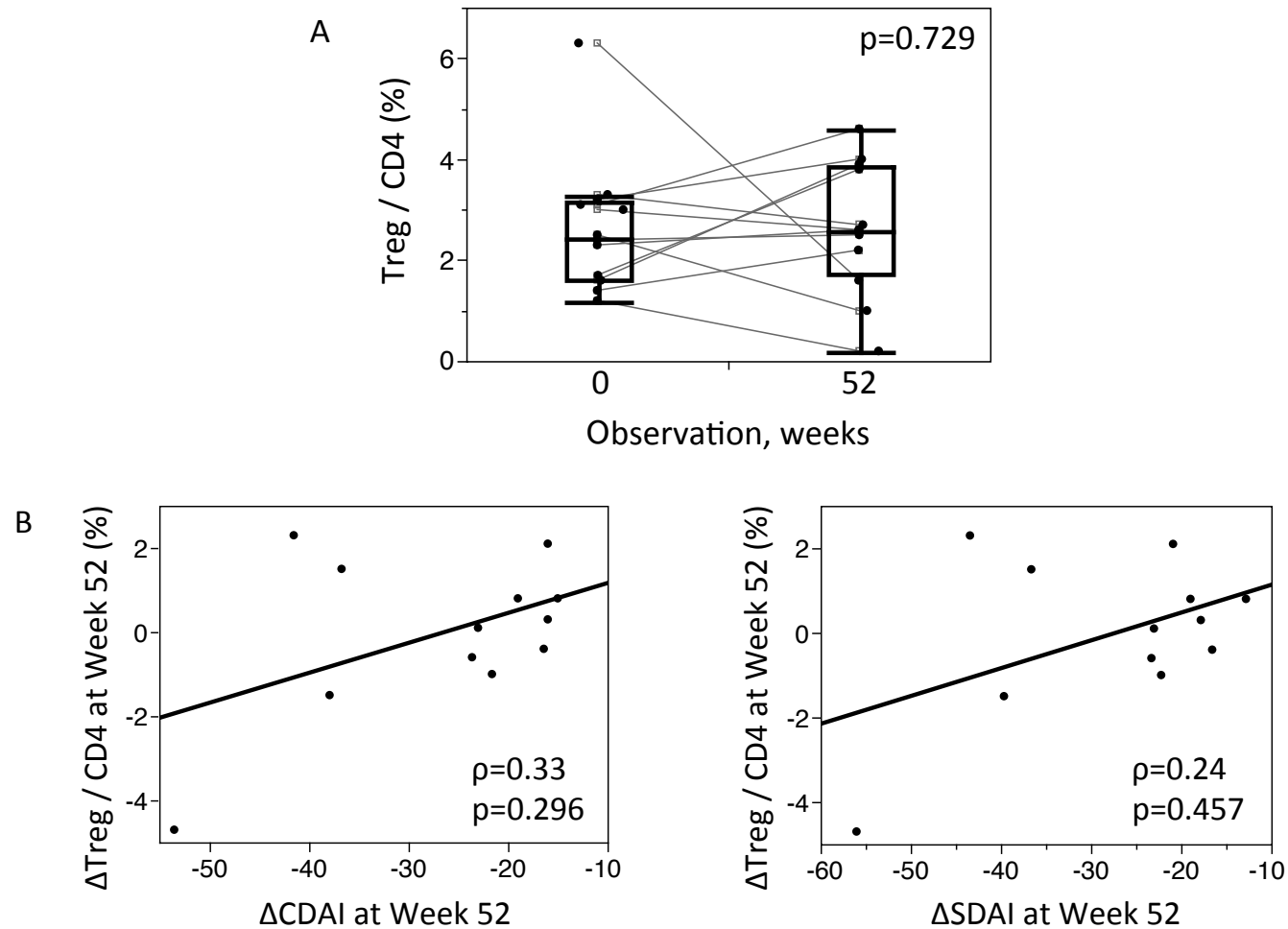
ACPA, anti-citrullinated protein antibody; CDAI, Clinical Disease Activity Index; CRP, C-reactive protein; ESR, erythrocyte sedimentation rate; HAQ-DI, Health Assessment Questionnaire Disability Index score; MMP-3, matrix metalloproteinase 3; MTX, methotrexate; RF, rheumatoid factor; SDAI, simplified disease activity index

**Figure S3.** Chronological changes in clinical activity from baseline until Week 52 of MTX treatment. CDAI, Clinical Disease Activity Index; SDAI, Simplified Disease Activity Index.



**Figure S4.** Relations between effects of MTX on the proportion of  $T_{reg}$  and clinical responses. **(A)** Chronological change in the proportion of  $T_{reg}$  among  $CD4^+$  T cells from baseline to Week 52 of MTX treatment. **(B)** The relations between the change in clinical activities ( $\Delta CDAI$  and  $\Delta SDAI$ ) and the change in proportion of  $T_{reg}$  among  $CD4^+$  T cells ( $\Delta T_{reg} / CD4$ ) at Week 52. The squares signify medians and interquartile ranges.

Data were analyzed by Kruskal-Wallis's test in **A**; Spearman's rank correlation coefficient in **B**.





**Table S5.** Changes in proportions (%) of subsets and surface markers of B cells and monocytes during MTX treatment. Subsets and surface markers were selected as the changed ones in TCZ therapy.

Subsets and surface markers	Median (IQR)		Baseline vs. Week 52
	Baseline	Week 52	<i>P</i> -value
CD80 <sup>+</sup> B cells / B cells	10.6 (6.3–13.6)	9.4 (5.3–14.3)	0.817
CD86 <sup>+</sup> B cells / B cells	74.3 (63.7–84.7)	75.8 (60.5–78.6)	0.729
Naïve B cells / B cells	65.6 (63.5–74.0)	74.8 (72.1–84.0)	0.073
Memory B cells / B cells	34.5 (27.1–36.5)	25.2 (15.8–28.3)	0.065
HLA-DR <sup>+</sup> CD14 <sup>+</sup> monocytes / CD14 <sup>+</sup> monocytes	67.1 (57.6–75.2)	66.2 (61.5–72.8)	0.921
CD69 <sup>+</sup> CD14 <sup>+</sup> monocytes / CD14 <sup>+</sup> monocytes	92.4 (87.5–94.5)	94.8 (93.0–96.0)	0.106
CD16 <sup>+</sup> CD14 <sup>+</sup> monocytes / CD14 <sup>+</sup> monocytes	26.4 (17.6–33.2)	16.9 (8.7–27.7)	0.057
CD16 <sup>-</sup> CD14 <sup>+</sup> monocytes / CD14 <sup>+</sup> monocytes	73.6 (66.8–82.4)	83.1 (72.3–91.3)	0.057