Supplementary Materials: X-Linked miRNAs Associated with Gender Differences in Rheumatoid Arthritis

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Figure S1. Absence of correlation between the expression levels of miR-146a and miR-223 and biologic parameters of patients with non-active RA disease. Expression levels of X-linked miRNAs in PBMCs from RA patients and healthy subjects were quantified using RT-PCR, and endogenous RNU48 was used for normalization. Correlation between miR-146a (a) and miR-223 (b) expression levels and 5 biological parameters (DAS28, ESR, CRP, RF Anti-CCP and Disease duration) are shown. Abbreviations: RF: Rheumatoid factor, DAS28: Disease Activity Score-28, CRP: C-reactive protein, anti-CCP: Antibodies to cyclic citrullinated peptides, and ESR: Erythrocyte sedimentation rate.
**Figure S2.** Treatment does not influence the expression levels of X-linked miRNAs in RA patients. Expression levels of X-linked miRNAs were detected on PBMCs of RA patients \((n = 21)\) using RT-PCR, and endogenous RNU48 was used for normalization. Data are plotted according to the treatment: Tocilizumab (blue circles), Rituximab (green circles), Infliximab (red circles), Adalimumab (grey circles), Prednisolone (orange circles) and Methotrexate (black circles).

**Figure S3.** Comparison of X-linked miRNAs expression levels in RA and healthy subjects PBMC samples without gender stratification. Expression levels of X-linked miRNAs were detected on PBMCs of RA patients and healthy subjects using RT-PCR, and endogenous RNU48 was used for normalization. Expression levels of miR-221 and miR-222 (a); miR-532 and miR-188 (b); miR-98 and Let7-f (c); miR-652 (e) miR-106a, miR-20d, miR-92a and miR363 (f) are plotted without gender stratification. Results are expressed as mean ± SD of individual samples of 21 RA patients and 21 healthy subjects.
Figure S4. Expression levels of 4 miRNAs located near 2 FOXP3 polymorphisms associated with RA susceptibility without gender stratification. To assess the relationship between genetic variation and the expression level of X-linked miR-221/222 and miR532/188 clusters miR-221, miR-222, miR-532 and miR-188 expression levels were quantified in RA patients using RT-PCR and DNA from RA patients was genotyped using direct PCR sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit. Data are presented according to the FOXP3 genotype, rs3761548 (a) and rs2232365 (b) and to the mutated versus wild type genotype. Box plots are the representation of individual sample of 21 RA patients.

Figure S5. Cell type-specific expression of X-linked miRNAs in healthy donors. The expression levels of 11 X-linked miRNAs in different immune cell types were extracted from the GSE28487 data sets. Eight X-linked miRNAs (miR-221, miR-222, miR-92a, miR-106a, miR-20b, miR-363, miR-98 and miR-652) are similarly expressed between all human immune cells types investigated and 3 miRNAs (miR-188, miR-532, and miR-223) appear to be preferentially expressed in monocytes. Relative miRNA expressions are represented as log2 signal value.
Figure S6. Expression levels of X-linked miRNA precursors in RA patients according to gender stratification and FOXP3 genotypes. The expression level of X-linked miR-221/222 and miR532/188 cluster precursors were quantified using RT-PCR in RA patients (n = 21) and healthy controls (n = 22) and stratified according to the gender (a); The relationship between genetic variation in the FOXP3 promoter and miRNA precursors are presented (b,c). DNA from RA patients was genotyped using direct PCR sequencing with the BigDye Terminator v3.1 Cycle Sequencing Kit. Data are presented according to the FOXP3 genotype: rs3761548 (b) and rs2232365 (c) and to the mutated versus wild type genotypes.