Supplementary information

A whole gut virome analysis of 476 Japanese revealed a link between phage and autoimmune disease

Tomofuji Y et al.

Corresponding to Yukinori Okada (yokada@sg.med.osaka-u.ac.jp)

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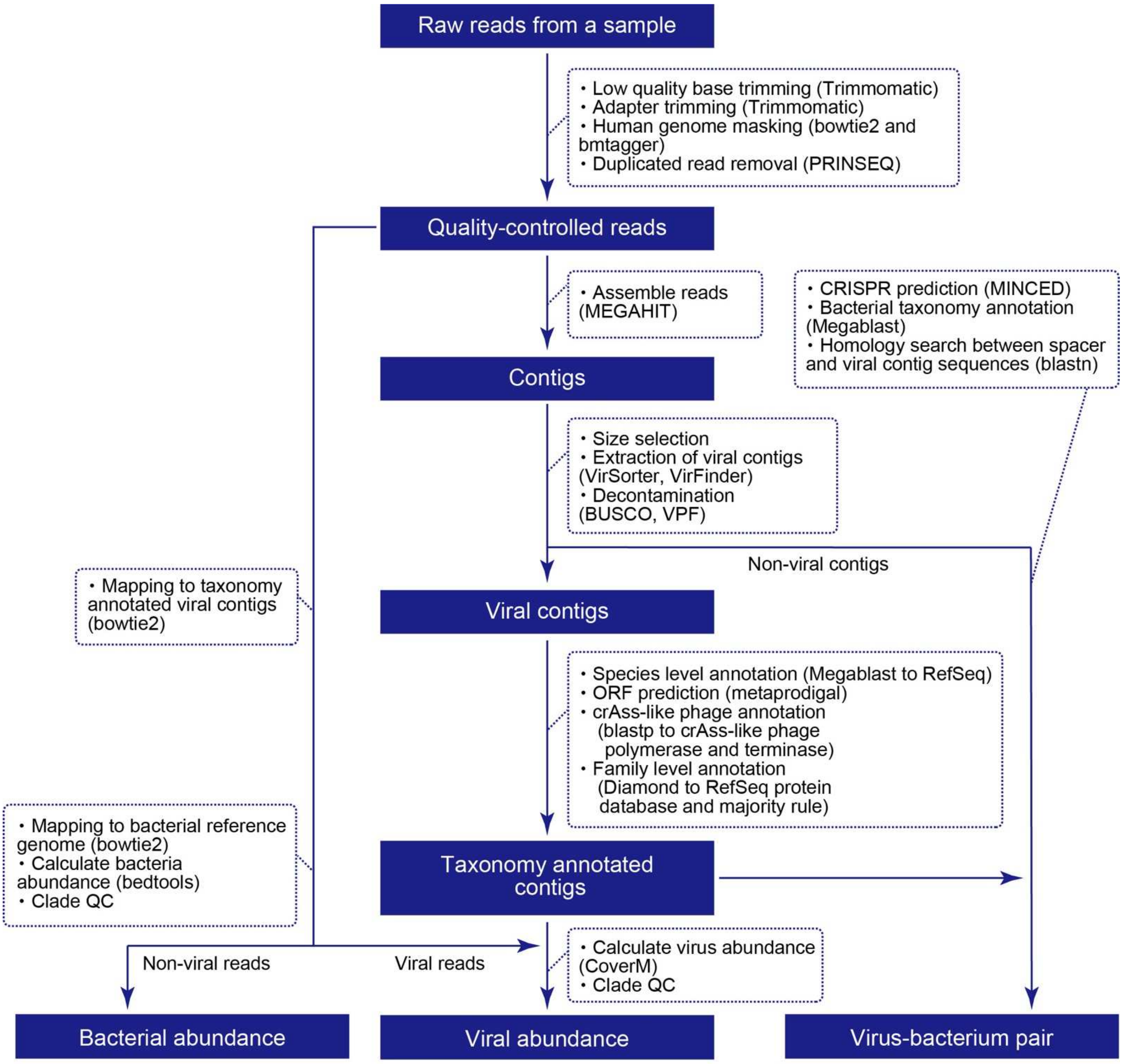
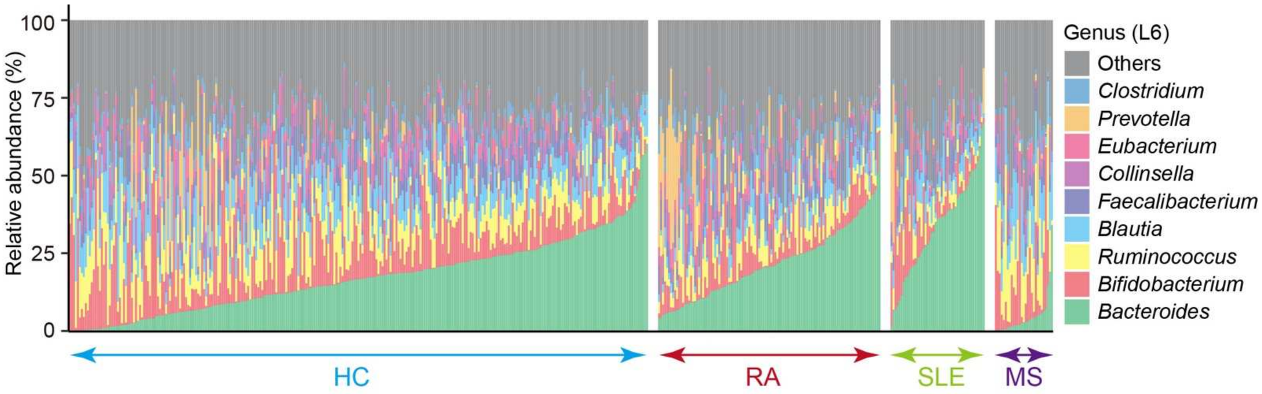
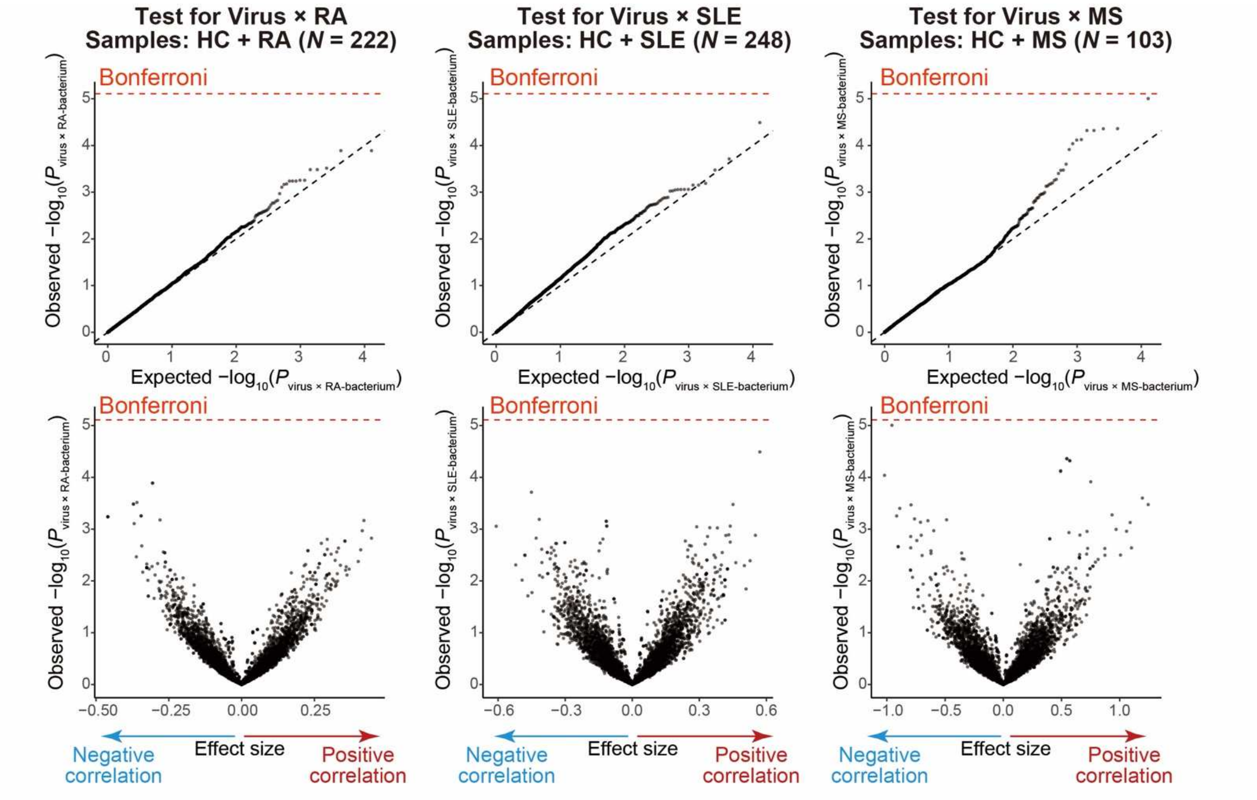


Supplementary Figure 1. Pipeline for virome analysis from shotgun sequencing data. Whole-genome shotgun sequencing reads of the gut microbiome were processed following this pipeline. This pipeline produces three outputs (bacterial abundance, viral abundance, and virus-bacterium pair). CRISPR, clustered regularly interspaced short palindromic repeat; ORF, open reading frame; QC, quality check; RefSeq, NCBI Reference Sequence Database.

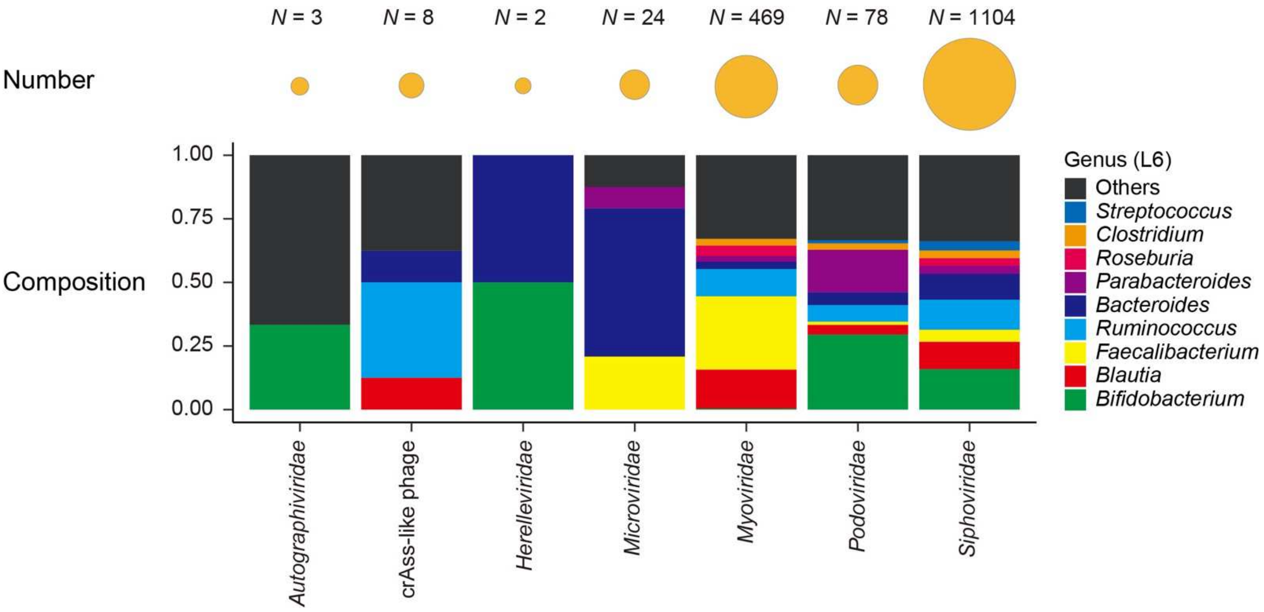


Supplementary Figure 2. Bacterial relative abundance at the genus level (L6).

HC, healthy control; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.



Supplementary Figure 3. Association tests for disease-specific virus-bacterium interaction. (Top), A quantile–quantile plot of the p-values from the disease specific virus-bacterium association analysis (left RA, middle SLE, right MS). The x-axis indicates $-\log_{10}(P_{\text{virus × disease-bacterium}})$ expected from uniform distribution. The y-axis indicates observed $-\log_{10}(P_{\text{virus × disease-bacterium}})$. The diagonal dashed line represents $y = x$, which corresponds to the null hypothesis. The horizontal red dashed line indicates the Bonferroni-corrected threshold ($\alpha = 0.05$). **(Bottom)**, A volcano plot. The x-axis indicates the effect sizes of virus × diseases term in linear regression. The y-axis, horizontal dashed lines are the same as in (top). HC, healthy control; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.



Supplementary Figure 4. Analysis of the bacterial target of viruses based on the CRISPR spacers.

Composition of bacterial genus with CRISPR spacers related to each viral family is represented as bar plot. Numbers of bacterium-virus pairs detected in this analysis are indicated above the bar. CRISPR, clustered regularly interspaced short palindromic repeat; HC, healthy control; MS, multiple sclerosis; RA, rheumatoid arthritis; SLE, systemic lupus erythematosus.

Supplementary Table 1. Characteristics of the study population.

	RA (*N* = 111)	SLE (*N* = 47)	MS (*N* = 29)	Control (*N* = 289)
Mean age (sd)	61.4 (15.0)	42.9 (15.9)	45.6 (9.0)	33.2 (9.0)
Sequencing batch 1	15 (13.5%)	0	0	14 (4.8%)
Sequencing batch 2	67 (60.4%)	5 (10.6%)	0	27 (9.3%)
Sequencing batch 3	29 (26.1%)	10 (21.3%)	0	70 (24.2%)
Sequencing batch 4	0	0	29 (100%)	74 (25.6%)
Sequencing batch 5	0	32 (68.1%)	0	104 (36.0%)
Female	88 (79.3%)	43 (91.5%)	24 (82.8%)	140 (46.2%)
New onset	70 (63.1%)	18 (38.3%)	2 (6.9%)	-
Not new onset	24 (21.6%)	29 (61.7%)	27 (93.1%)	-
Antibiotics use	0	11 (23.4%)	0	0
Proton pump inhibitor use	13 (11.7%)	27 (57.4%)	4 (13.8%)	-
No treatment	63 (56.8%)	14 (29.8%)	2 (6.9%)	-
With treatment	43 (38.7%)	33 (70.2%)	27 (93.1%)	-
Comorbidity				
Diabetes	7 (6.3%)	1 (2.1%)	0	-
Hypertension	21 (18.9%)	4 (8.5%)	3 (10.3%)	-
Dyslipidemia	19 (17.1%)	5 (10.6%)	3 (10.3%)	-
Cardiovascular disorders	4 (3.6%)	1 (2.1%)	0	-
Not evaluated	6 (5.4%)	0	0	-
Treatment for RA				
Steroid	15 (13.5%)	-	-	-
Methotrexate	26 (23.4%)	-	-	-
Salazosulfapyridine	11 (9.9%)	-	-	-
Iguratimod	1 (0.9%)	-	-	-
Tacrolimus	1 (0.9%)	-	-	-
Certolizumab pegol	1 (0.9%)	-	-	-
Abatacept	1 (0.9%)	-	-	-
Atlizumab	1 (0.9%)	-	-	-
DAS-C, mean (sd)	4.1 (1.3)	-	-	-
DAS-C > 4.1	48 (43.2%)	-	-	-
Rheumatoid factor				
Strong positive	60 (54.1%)	-	-	-
Positive	19 (17.1%)	-	-	-
Negative	27 (24.3%)	-	-	-
Not evaluated	5 (4.5%)	-	-	-
ACPA				
Strong positive	62 (55.9%)	-	-	-
Positive	10 (9.0%)	-	-	-
Negative	31 (27.9%)	-	-	-

Not evaluated	8 (7.2%)	-	-	-
Sero-positivity				
Positive	85 (76.6%)	-	-	-
Negative	26 (23.4%)	-	-	-
Steinbrocker stage				
I	58 (52.2%)	-	-	-
II	18 (16.2%)	-	-	-
III	3 (2.7%)	-	-	-
IV	3 (2.7%)	-	-	-
Not evaluated	29 (26.1%)	-	-	-
Interstitial lung disease	6 (5.4%)	-	-	-
Treatment for SLE				
Steroid	-	32 (68.1%)	-	-
Hydroxychloroquine	-	7 (14.9%)	-	-
Tacrolimus	-	5 (10.6%)	-	-
Ciclosporin	-	1 (2.1%)	-	-
Mycophenolate Mofetil	-	8 (17%)	-	-
Cyclophosphamide	-	1 (2.1%)	-	-
SLE-DAI, mean (sd)	-	11.1 (8.7)	-	-
SLE-DAI ≥ 8	-	28 (59.6%)	-	-
Manifestation of SLE				
Psychosis	-	1 (2.1%)	-	-
Vision impairment	-	1 (2.1%)	-	-
Headache	-	4 (8.5%)	-	-
Cerebrovascular disease	-	1 (2.1%)	-	-
Vasculitis	-	1 (2.1%)	-	-
Arthritis	-	14 (29.8%)	-	-
Myositis	-	2 (4.3%)	-	-
Urinary cast	-	9 (19.1%)	-	-
Hematuria	-	5 (10.6%)	-	-
Proteinuria	-	13 (27.7%)	-	-
Pyuria	-	7 (14.9%)	-	-
Rash	-	15 (31.9%)	-	-
Alopecia	-	7 (14.9%)	-	-
Mucosal ulcer	-	5 (10.6%)	-	-
Pleurisy	-	5 (10.6%)	-	-
Pericarditis	-	2 (4.3%)	-	-
Low complement	-	34 (72.3%)	-	-
Increased DNA binding	-	30 (63.8%)	-	-
Fever	-	14 (29.8%)	-	-
Thombocytopenia	-	2 (4.3%)	-	-

Leucopenia	-	12 (25.5%)	-	-
Seizure	-	0	-	-
Organic brain syndrome	-	0	-	-
Cranial nerve disorder	-	0	-	-
Lupus nephritis	-	20 (42.6 %)	-	-
Treatment for MS				
Steroid	-	-	6 (20.7%)	-
Dimethyl fumarate	-	-	9 (31.0%)	-
Fingolimod	-	-	7 (24.1%)	-
Interferon	-	-	4 (13.8%)	-
Glatiramer acetate	-	-	6 (20.7%)	-
EDSS, mean (sd)	-	-	2.6 (2.0)	-
EDSS ≥ 4.5	-	-	8 (27.6%)	-
Spinal cord involvement	-	-	18 (62.1%)	-

ACPA, anti-citrullinated protein/peptide antibody; DAS-C, Disease Activity Score 28 using C-reactive protein; EDSS, Expanded Disability Status Scale; MS, multiple sclerosis; RA, rheumatoid arthritis; RF, rheumatoid factor; sd, standard deviation; SLE, systemic lupus erythematosus; SLE-DAI, SLE Disease Activity Index.

[*1] Information about the date of onset could not be obtained for 17 RA patients.

[*2] Information about treatment and proton pump inhibitor usage could not be obtained for five RA patients.

[*3] Information about the presence of interstitial lung disease for six RA patients.

[*4] DAS-C could not be obtained for 11 RA patients.

[*5] Information about antibiotics usage could not be obtained for a SLE patient.

Supplementary Table 2. Detection ratio of the viral clades.

	Sequencing batch 1	Sequencing batch 2	Sequencing batch 3	Sequencing batch 4	Sequencing batch 5
Number of samples	29	99	109	103	136
Detection ratio (%)					
Autographiviridae	41.4	29.3	30.3	21.4	25.0
crAss-like phage	72.4	62.6	64.2	66.0	63.2
Herelleviridae	65.5	42.4	33.0	21.4	38.2
Microviridae	55.2	51.5	45.9	24.3	62.5
Myoviridae	100.0	100.0	100.0	100.0	100.0
Phycodnaviridae	86.2	53.5	61.5	44.7	63.2
Podoviridae	96.6	93.9	98.2	95.1	97.1
Siphoviridae	100.0	100.0	100.0	100.0	100.0

Supplementary Table 3. Association of age and sex to the viral abundances.

Age (N = 476)

Viruses	Effect size	SE	P
Autographiviridae	3.8×10^{-4}	0.0020	0.85
crAss-like phage	0.0064	0.0036	0.076
Herelleviridae	0.0031	0.0021	0.13
Microviridae	3.4×10^{-5}	0.0025	0.99
Myoviridae	0.0016	8.3×10^{-4}	0.047
Phycodnaviridae	−0.0016	0.0027	0.54
Podoviridae	0.0026	0.0021	0.21
Siphoviridae	7.8×10^{-4}	4.5×10^{-4}	0.087

Male (N = 476)

Viruses	Effect size	SE	P
Autographiviridae	−0.026	0.056	0.64
crAss-like phage	−0.024	0.10	0.81
Herelleviridae	0.029	0.059	0.63
Microviridae	0.047	0.072	0.51
Myoviridae	0.035	0.024	0.14
Phycodnaviridae	0.040	0.077	0.61
Podoviridae	−0.027	0.059	0.64
Siphoviridae	−0.0060	0.013	0.64

SE, standard error.

Supplementary Table 4. Sub-analysis of the crAss-like phage abundance for RA.

Case–control comparison			
Sample set	Effect size	SE	*P*
All	−0.476	0.173	0.0060
Remove male	−0.568	0.197	0.0040
Remove non-new onset patients	−0.385	0.200	0.055
Remove patients with proton pump inhibitors	−0.441	0.183	0.016
Remove patients with steroids	−0.475	0.183	0.010
Remove patients with treatment for RA	−0.520	0.211	0.014
Comparison within case			
Objective	Effect size	SE	*P*
Not treated	−0.008	0.207	0.97
New onset	0.313	0.271	0.25
DAS-C > 4.1	0.018	0.219	0.93
CRP > 1.0 mg / L ($N_{\text{CRP high}}$ = 52 vs $N_{\text{CRP low}}$ = 52)	0.291	0.227	0.20

CRP, C-reactive protein; DAS-C, Disease Activity Score 28 (DAS28) using C-reactive protein; SE, standard error; RA, rheumatoid arthritis.

Supplementary Table 5. Sub-analysis of the viral abundance for SLE.

crAss-like phage

Case–control comparison

Sample set	Effect size	SE	*P*
All	−0.514	0.206	0.012
Remove male	−0.484	0.218	0.027
Remove non-new onset patients	−0.946	0.356	0.0078
Remove patients with antibiotics	−0.372	0.225	0.099
Remove patients with proton pump inhibitors	−0.724	0.319	0.023
Remove patients with steroids	−0.799	0.353	0.023
Remove patients with treatment for SLE	−0.945	0.399	0.018

Comparison within case

Objective	Effect size	SE	*P*
Not treated	−0.806	0.564	0.15
New onset	−0.593	0.580	0.31
SLE-DAI ≥ 8	−1.147	0.634	0.070

Podoviridae

Case–control comparison

Sample set	Effect size	SE	*P*
All	−0.947	0.330	0.0041
Remove male	−0.653	0.357	0.067
Remove non-new onset patients	−1.231	0.469	0.0087
Remove patients with antibiotics	−0.601	0.371	0.11
Remove patients with proton pump inhibitors	−0.591	0.519	0.26
Remove patients with steroids	−1.238	0.492	0.012
Remove patients with treatment for SLE	−1.350	0.519	0.0093

Comparison within case

Objective	Effect size	SE	*P*
Not treated	−0.289	0.593	0.63
New onset	−0.165	0.573	0.77
SLE-DAI ≥ 8	0.313	0.736	0.67

SE, standard error; SLE, systemic lupus erythematosus; SLE-DAI, SLE Disease Activity Index.

Supplementary Table 6. Sub-analysis of the crAss-like phage abundance for combined autoimmune diseases (RA, SLE, and MS).

Case–control comparison

Sample set	Effect size	SE	*P*
All	−0.429	0.126	6.5×10^{-4}
Remove male	−0.464	0.140	9.2×10^{-4}
Remove non-new onset patients	−0.534	0.166	0.0013
Remove patients with antibiotics	−0.387	0.131	0.0031
Remove patients with proton pump inhibitors	−0.417	0.140	0.0029
Remove patients with steroids	−0.406	0.147	0.0057
Remove patients with treatment for combined autoimmune diseases	−0.549	0.180	0.0022
Remove sequencing batch 4	−0.499	0.137	2.6×10^{-4}

Case–control comparison (Per sequencing batch analysis)

Sequencing batch	Effect size	SE	*P*
Batch 1	−0.988	0.551	0.073
Batch 2	−0.270	0.248	0.28
Batch 3	−0.437	0.296	0.14
Batch 4	0.375	0.463	0.42
Batch 5	−0.451	0.269	0.094

Comparison within the case

Objective	Effect size	SE	*P*
Not treated	−0.087	0.179	0.63
New onset	−0.007	0.204	0.97

SE, standard error.

Supplementary Table 7. Meta-analysis for the association between combined autoimmune diseases (RA, SLE, and MS) and the viral abundances.

	Fixed-effect meta-analysis			Random effect meta-analysis				
Viruses	Effect size	SE	*P*	Effect size	SE	*P*	*Q*	*Q-P*
Autographiviridae	0.027	0.241	0.91	0.027	0.241	0.91	0.51	0.77
crAss-like phage	−0.392	0.139	0.0047	−0.392	0.139	0.0047	3.03	0.22
Herelleviridae	−0.474	0.267	0.076	−0.474	0.267	0.076	3.71	0.16
Microviridae	0.302	0.193	0.12	0.302	0.193	0.12	1.32	0.52
Myoviridae	0.169	0.569	0.77	0.030	0.688	0.97	2.08	0.35
Phycodnaviridae	−0.131	0.194	0.50	−0.150	0.225	0.51	2.36	0.31
Podoviridae	−0.215	0.256	0.40	−0.215	0.256	0.40	1.75	0.42
Siphoviridae	−1.746	1.127	0.12	−1.746	1.127	0.12	1.36	0.51

SE, standard error.

Supplementary Table 8. Case–control comparison of the absence/presence state of crAss-like phages for combined autoimmune diseases (RA, SLE, and MS).

Viruses	Effect size	SE	*P*	*N* detection in case	*N* detection in control
crAss-like phage	−0.734	0.259	0.0046	113 (60.4%)	194 (67.1%)

SE, standard error.

Supplementary Table 9. Case–control comparison of *crAssphage* (NC_024711.1) and other crAss-like phages for combined autoimmune diseases (RA, SLE, and MS).

Viruses	Effect size	SE	*P*
crAssphage (NC_024711.1)	−0.378	0.130	0.0036
Other crAss-like phages	−0.374	0.159	0.019

SE, standard error.

Supplementary Table 10. Virus-bacterium pairs with significant association.

Viruses	Bacteria	Level of bacteria	Effect size	SE	$P_{\text{virus-bacterium}}$	q
Podoviridae	*Faecalibacterium sp.*	Species (L7)	0.192	0.035	7.9×10^{-8}	5.1×10^{-4}
Podoviridae	*Faecalibacterium cf. prausnitzii*	Species (L7)	0.186	0.038	1.3×10^{-6}	4.2×10^{-3}

SE, standard error.

Supplementary Table 11. Sub-analysis for the virus-bacterium association analysis.

Podoviridae* and *Faecalibacterium sp.

Sample set	Effect size	SE	$P_{\text{virus-bacterium}}$
All	0.192	0.035	7.9×10^{-8}
Remove male	0.204	0.044	4.7×10^{-6}
Remove non-new onset patients	0.180	0.040	7.3×10^{-6}
Only HC	0.194	0.048	6.2×10^{-5}
Remove patients with antibiotics	0.179	0.035	5.8×10^{-7}
Remove patients with proton pump inhibitors	0.192	0.036	2.1×10^{-7}
Remove patients with steroids	0.188	0.038	8.4×10^{-7}
Remove patients with treatment for combined autoimmune diseases (RA, SLE, and MS)	0.198	0.041	1.6×10^{-6}

Podoviridae* and *Faecalibacterium cf. prausnitzii

Sample set	Effect size	SE	$P_{\text{virus-bacterium}}$
All	0.186	0.038	1.3×10^{-6}
Remove male	0.191	0.047	5.7×10^{-5}
Remove non-new onset patients	0.189	0.042	1.1×10^{-5}
Only HC	0.209	0.051	6.0×10^{-5}
Remove patients with antibiotics	0.181	0.038	2.4×10^{-6}
Remove patients with proton pump inhibitors	0.200	0.040	6.4×10^{-7}
Remove patients with steroids	0.196	0.040	1.8×10^{-6}
Remove patients with treatment for combined autoimmune diseases (RA, SLE, and MS)	0.206	0.044	3.6×10^{-6}

HC, healthy control; MS, multiple sclerosis; RA, rheumatoid arthritis; SE, standard error; SLE, systemic lupus erythematosus.