Supplemental Material

Supplemental Table 1. Background profile of the 10 healthy subjects, 10 pSS patients and 5 disease-control IgG4-RD patients.

ID	Sex	Age (yrs)	HLA-DRB1	Predicted epitope (aa)	M3R peptides used for stimulation (aa)
Healthy subjects					
HS1	F	32	09:01/ 09:01	34-48, 192-207, 219-245	2)16-35, 3) 31-50 18) 188-200, 19) 196-215, 20) 211-230, 21) 226-245
HS2	F	33	04:05/ 04:05	151-167, 216-236	15)151-170, 20) 211-230, 21) 226-245
HS3	F	35	09:01/ 09:01	34-38, 192-207, 219-245	3) 31-50, 18) 188-200, 19) 196-215, 20) 211-230 21) 226-245
HS4	F	54	04:03/ 15:02	96-112, 122-142	9) 91-110, 10) 106-117, 12) 121-140, 13) 136-147
HS5	M	40	14:06/ 16:02	163-180, 202-219	15) 151-170, 16)166-185, 19) 196-215, 20) 211-230
HS6	F	62	09:01/ 08:03	34-48, 163-180, 192-206, 219-244	3) 31-50, 15) 151-170, 16) 166-185, 18) 188-200, 19) 196-215, 20) 211-230, 21) 226-245
HS7	F	42	04:05/ 14:06	151-167, 216-236	15) 151-170, 20) 211-230, 21) 226-245
HS8	F	38	15:01/ 15:02	83-99, 122-141	8) 83-95, 9) 91-110, 12) 121-140, 13) 136-147
HS9	F	49	09:01/ 15:02	96-112, 122-142	9) 91-110, 10) 106-117, 12) 121-140, 13) 136-147
HS10	M	62	04:03/ 15:01	81-101, 158-173, 220-234	7) 76-87, 8) 83-95, 9) 91-110, 15) 151-170, 16) 166-185, 20) 211-230, 21) 226-245
pSS patients					
pSS1	F	80	04:10/ 13:02	84-100, 504-524	8) 83-95, 9) 91-110, 40) 503-515, 41) 511-530
pSS2	F	63	04:03/ 15:02	96-112, 122-142	9) 91-110, 10) 106-117, 12) 121-140, 13) 136-147
pSS3	F	61	08:03/ 15:01	81-101, 158-180	7) 76-87, 8) 83-95, 9) 91-110, 15) 151-170, 16) 166-185
pSS4	F	30	04:05/ 08:02	151-167, 217-236	15) 151-170, 20) 211-230, 21) 226-245

pSS5	M	65	01:01/ 15:01	81-101, 158-180	7) 76-87, 8) 83-95, 9) 91-110, 15) 151-170, 16) 166-185
pSS6	F	45	04:05/ 08:03	164-180, 216-236	15) 151-170, 16) 166-185, 20) 211-230, 21) 226-245
pSS7	F	22	04:05/ 08:02	151-167, 216-236	15) 151-170, 20) 211-230, 21) 226-245
pSS8	F	79	09:01/ 15:01	81-101, 158-172, 219-234	7) 76-87, 8) 83-95, 9) 91-110, 15) 151-170, 16) 166-185, 20) 211-230, 21) 226-245
pSS9	F	73	08:02/ 13:02	36-51, 84-101, 528-546	3) 31-50, 4) 46-65, 8) 83-95, 9) 91-110, 42) 526-545, 43) 541-560
pSS10	M	80	01:01/ 08:03	67-83, 163-181, 461-476	5) 61-72, 6) 68-80, 7) 76-87, 15) 151-170, 16) 166-185, 36) 451-470, 37) 466-485
Disease-control IgG4-RD patients					
IgG4-RD1	F	72	09:01/ 15:01	83-100, 158-172, 219-234	8) 83-95, 9) 91-110, 15) 151-170, 16) 166-185, 20) 211-230, 21) 226-245
IgG4-RD2	M	60	04:05/ 08:02	151-167, 216-236	15) 151-170, 20) 211-230, 21) 226-245
IgG4-RD3	M	51	12:02/ 13:02	82-100, 528-546	7) 76-87, 8) 83-95, 9) 91-110, 42) 526-545, 43) 541-560
IgG4-RD4	M	70	01:01/ 01:01	66-86, 145-159, 193-207	5) 61-72, 6) 68-80, 7) 76-87, 14) 143-155, 15) 151-170, 18) 188-200, 19) 196-215
IgG4-RD5	M	51	04:06/ 15:01	81-101, 158-173, 220-234	15) 151-170, 16) 166-185, 19) 196-215, 20) 211-230

All participants underwent HLA-DRB1 typing, and HLA binding 15-mer peptides from the full sequence M3R (590AA), were predicted by Immune Epitope Database (IEDB) ranked by IC50 value. We selected top 10 ranking M3R peptides for each case, assuming that the T cell epitope would be included in the selected peptides. Table shows the selected M3R peptides for stimulation for each subject. Numbers represent the number of M3R full sequence amino acid described in Supplementary Table 1. The clinical profiles of the subjects were collected from the medical records. aa: amino acid number.

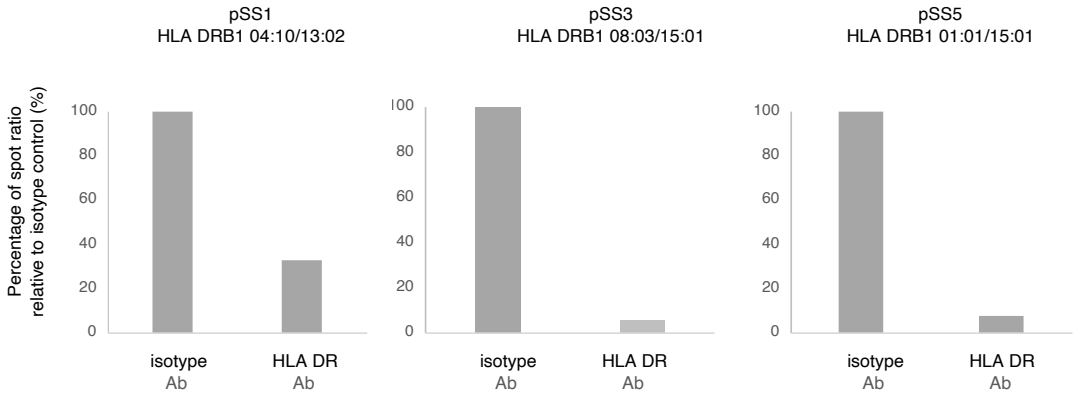
Supplemental Table 2. Synthesized M3R peptides.

n	AA	portion
1	1-20	NT
2	16-35	NT
3	31-50	NT
4	46-65	NT
5	61-72	NT-TM1
6	68-80	TM1
7	76-87	TM1
8	83-95	TM1-CL1
9	91-110	TM1-CL1-TM2
10	106-117	TM2
11	113-125	TM2
12	121-140	TM2-EL1
13	136-147	EL1-TM3
14	143-155	TM3
15	151-170	TM3-CL2
16	166-185	CL2-TM4
17	181-192	CL2-TM4
18	188-200	TM4
19	196-215	TM4-EL2
20	211-230	EL2-TM5
21	226-245	EL2-TM5
22	241-260	TM5-CL3
23	256-275	CL3
24	271-290	CL3
25	286-305	CL3
26	301-320	CL3
27	316-335	CL3
28	331-350	CL3
29	346-365	CL3
30	361-380	CL3
31	376-395	CL3
32	391-410	CL3
33	406-425	CL3
34	421-440	CL3
35	436-455	CL3
36	451-470	CL3
37	466-485	CL3
38	481-500	CL3-TM6
39	496-507	TM6
40	503-515	TM6-EL3
41	511-530	EL3
42	526-545	EL3-TM7

43	541-560	TM7-CT
44	556-575	CT
45	571-590	CT

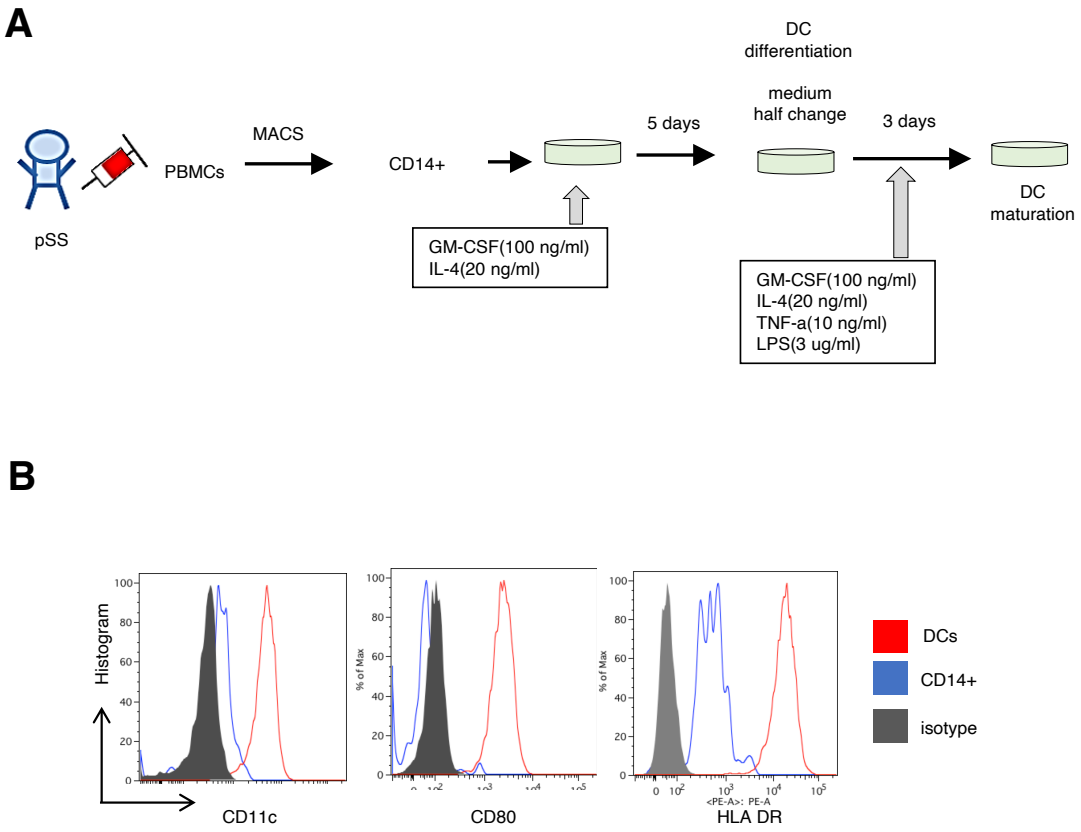
45 types of M3R peptides, each composed of 12-20-mers with overlapping 4-5-mers, were prepared, altogether covering the full M3R sequence. Data are the 45 types of M3R peptides and the portions of the M3R peptides. n: Number of peptides, AA: Amino acid number from the M3R full sequence, NT: N terminal, TM: Transmembrane, CL: Cytoplasmic loop, EL: Extracellular loop, CT: C terminal

Supplemental Figure 1



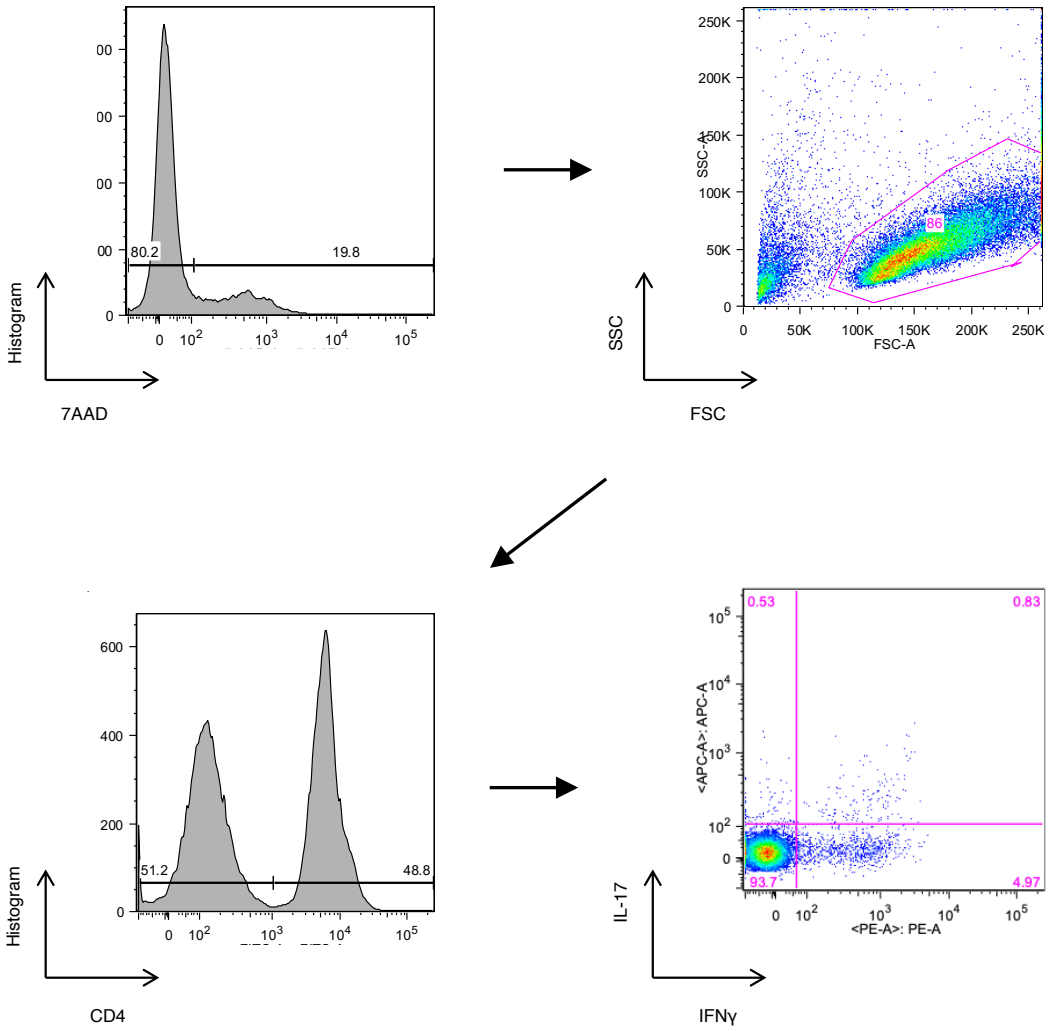
Supplemental Figure 1. HLA-DR blocking of M3R AA83-95 reactivity.
HLA DR restriction of M3R AA83-95 reactivity of Th17 cells was assessed using ELISpot after HLA DR blocking with anti HLA-DR antibody. Three pSS patients positive for M3R-reactive Th17 cells were evaluated, all showing decreased spots after HLA DR blocking procedure compared with isotype antibody. Inhibition ratio of HLA DR antibody, compared with isotype antibody described as 100% are shown. Replicate three wells were examined under each condition.

Supplemental Figure 2



Supplemental Figure 2. Generation of monocyte-derived dendritic cells. Dendritic cells (DCs) were generated from $CD14^+$ monocyte isolated from PBMCs using the method described in detail previously (20).
(A) Procedure used to prepare DCs from $CD14^+$ cells. $CD14^+$ cells isolated by positive fraction of MACS, were cultured with GM-CSF (100 ng/ml) and IL-4 (10 ng/ml). After 5-day culture, the cells were treated with LPS (3 μg/ml), IL-4 (20 ng/ml), and TNF-α (10 ng/ml) for 3 days. The purity of the isolated $CD14^+$ cells was>95%.
(B) Representative FACS data of monocyte-derived DCs. Differentiation of DCs was confirmed by flow cytometry and staining for HLA-DR-PE (clone L243), CD80-PECy7 (clone 2D10), and CD11c-APC (clone 3.9). CD11c, CD80, HLA DR were highly expressed in mature DCs compared to isolated $CD14^+$ cells.

Supplemental Figure 3



Supplemental Figure 3. Gating strategy of M3R induced IL-17, IFNγ production with flow cytometry.
7-AAD was stained to gait out dead cells. Among scatter gate for lymphocyte, CD4 positive cells were assessed for IL-17, and IFNγ production.