Supplementary information

Elucidating genetic backgrounds of myasthenia gravis in Japanese by genome-wide association studies and multi-omics analyses of thymoma

Corresponding to:
Yukinori Okada, MD, PhD
Department of Genome Informatics, Graduate School of Medicine, the University of Tokyo
7-3-1 Hongo, Bunkyo-ku, Tokyo, Japan.
Tel: +81-3-5841-1860
E-mail: yuki-okada@m.u-tokyo.ac.jp

Masanori P. Takahashi, MD, PhD
Department of Clinical Laboratory and Biomedical Sciences, Graduate School of Medicine, the University of Osaka, Suita, Japan.1-7 Yamadaoka Suita, Osaka 565-0871, Japan.
Tel: +81-6-6829-2587
E-mail: mtakahas@sahs.med.osaka-u.ac.jp

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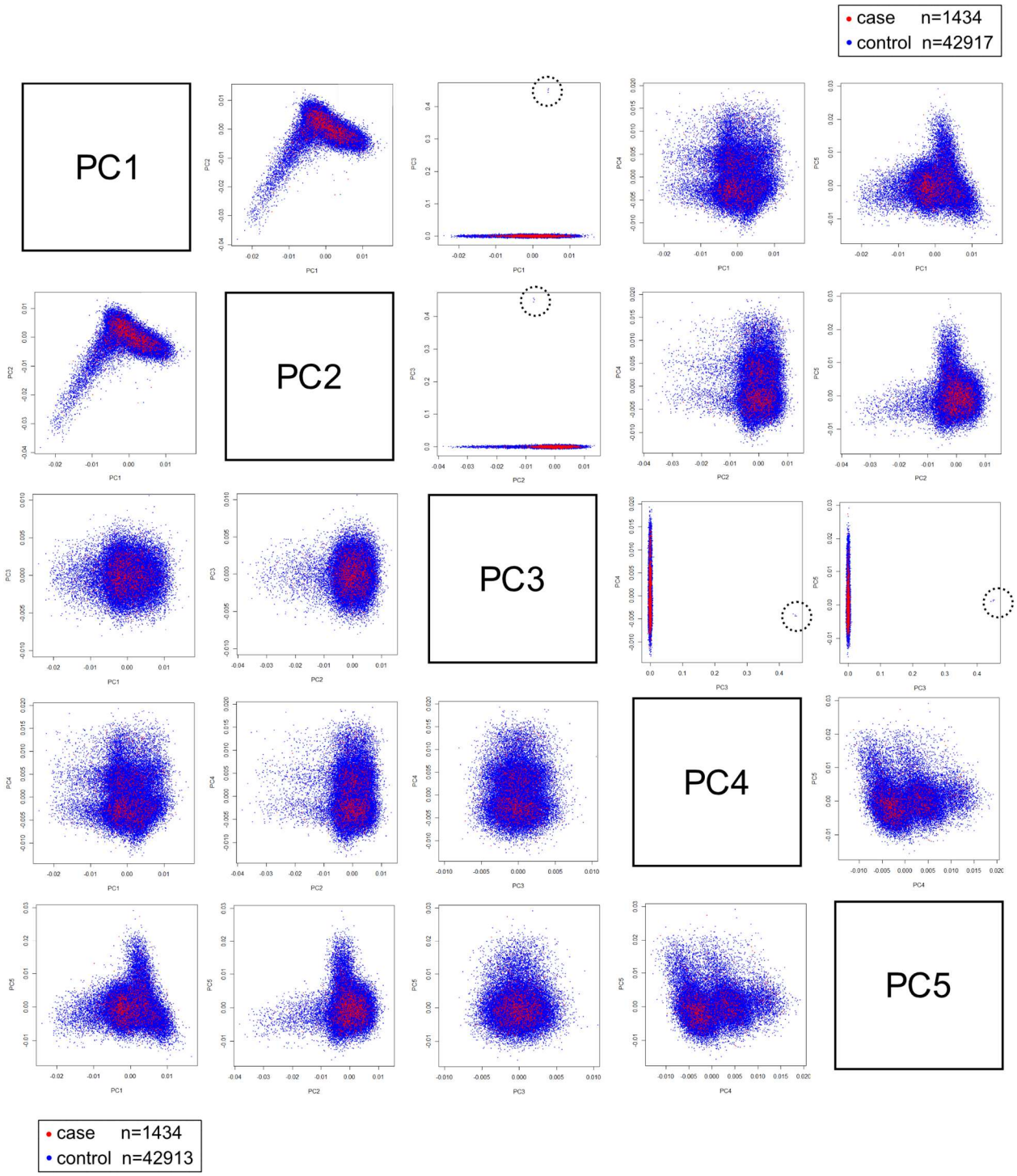
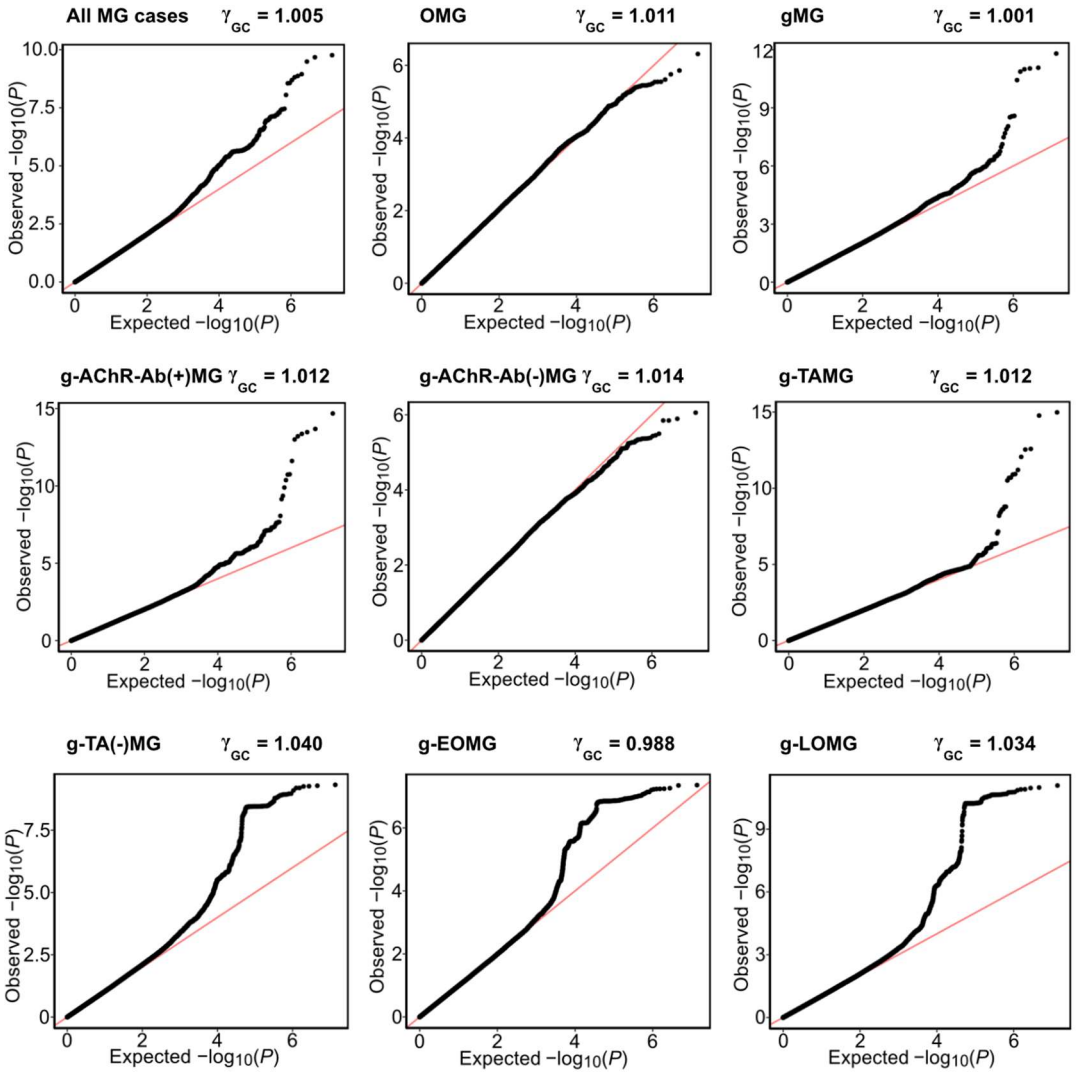
Supplementary Figures and Tables

Supplementary Figure 1. Principal component analysis of GWAS subject datasets



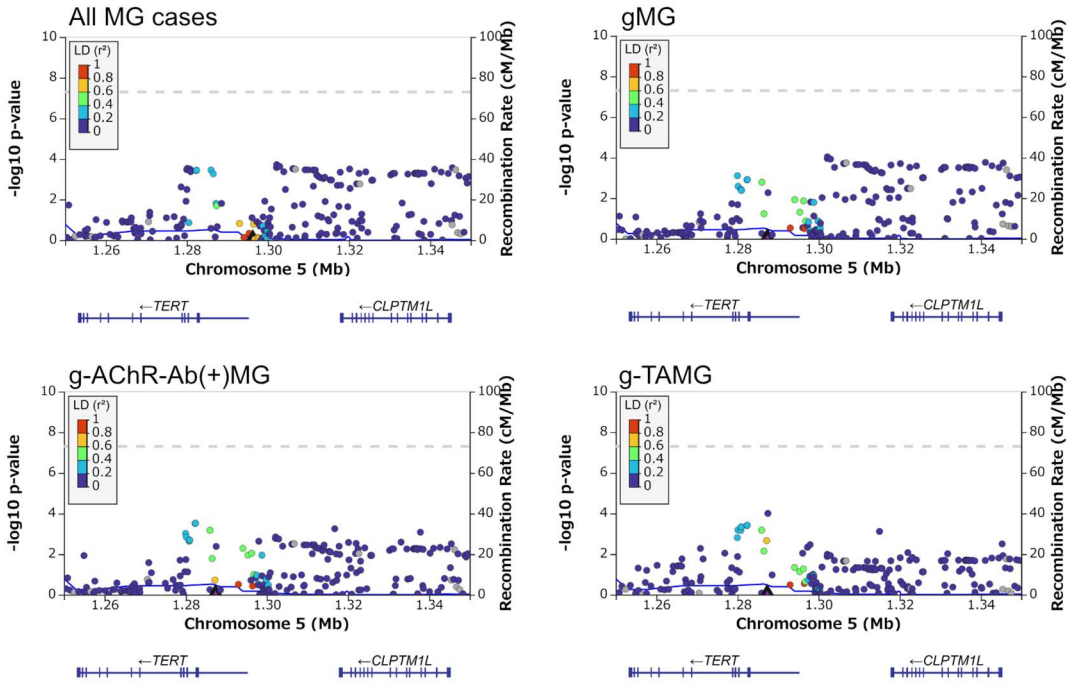
Scatter plots of principal components (PCs) 1 through 5. Each point represents a sample: red for MG cases (n = 1,434), blue for controls (n = 42,917). The upper right panels show the distribution before outlier exclusion. Dotted circles indicate four control samples identified as PC outliers, which were excluded from subsequent GWAS analysis. The lower left panels show the distribution after exclusion of these outliers.

Supplementary Figure 2. The quantile-quantile plots of GWAS of each MG subtype



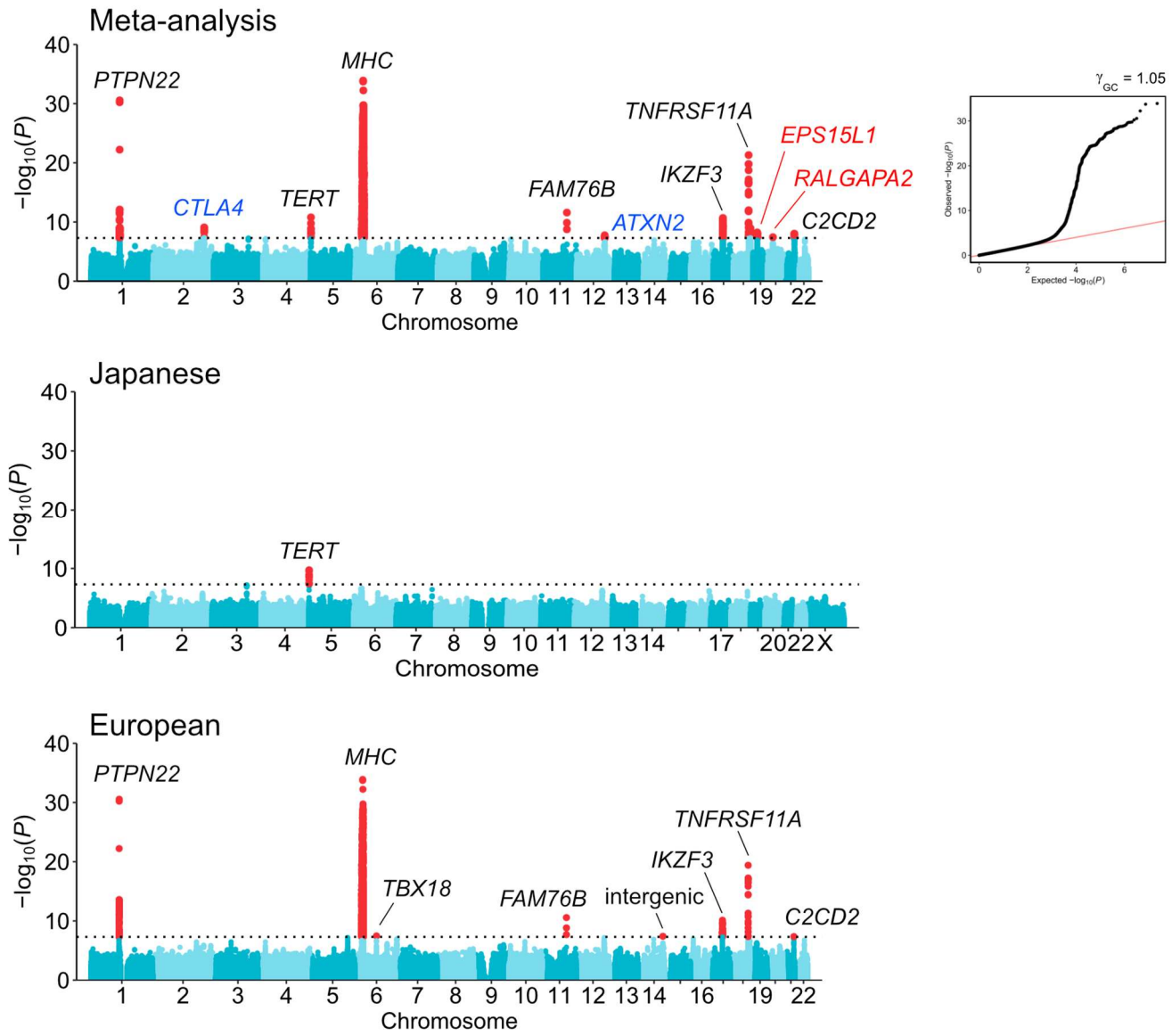
The horizontal and vertical axes indicate expected and observed -log$_{10}$(*P* values) for the association tests. 6,889,986 variants with minor allele frequency (MAF) ≥ 0.02 which passed post imputation QC of Rsq ≥ 0.5 were tested including both autosomal and X chromosomes.

Supplementary Figure 3. Conditional association study with the *TERT* lead variant



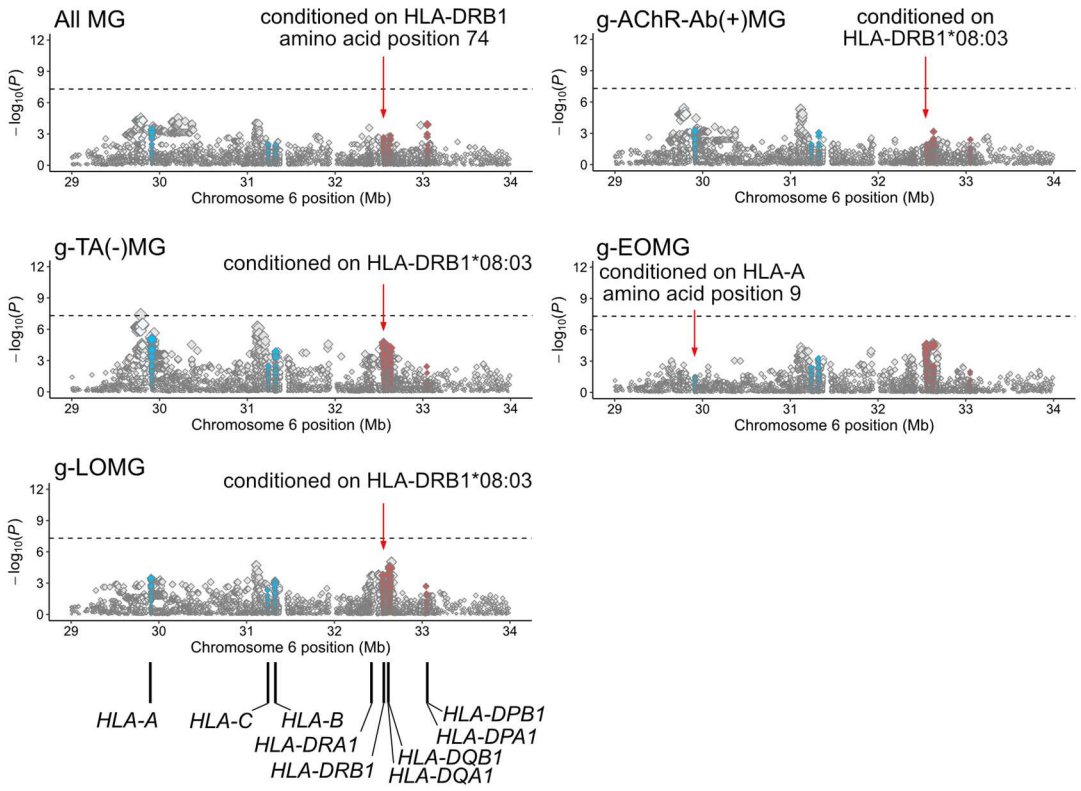
Regional plots show results for the *TERT* region in the conditioned analysis of the lead variant. The lead variants were Rs3215401 for All MG cases and rs2736099 for gMG, g-AChR-Ab(+)MG, and g-TAMG. Color of the dots indicates the r^2 value of the linkage disequilibrium (LD) of each variant with the lead variant.

Supplementary Figure 4. Cross-population GWAS meta-analysis by integrating the Japanese GWAS with those of the Europeans



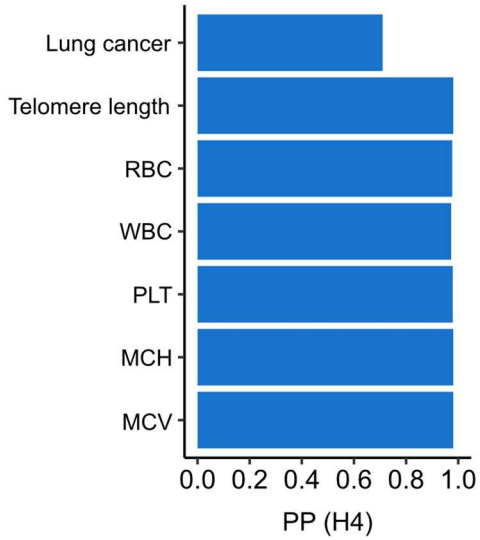
A Manhattan plot and a quantile-quantile plot show the result of cross-population GWAS meta-analysis by integrating the Japanese GWAS with those of the Europeans. Manhattan plots of the Japanese and European GWAS are shown below the meta-analysis plot for comparison. The dotted horizontal black line indicates the genome-wide significance level ($P = 5.0\times10^{-8}$), and red circles indicate SNPs that satisfied genome-wide significance level. Newly identified loci are labeled in red, and previously reported loci that also reached genome-wide significance by meta-analysis are shown in blue.

Supplementary Figure 5. Condition analysis of HLA variants within the MHC region



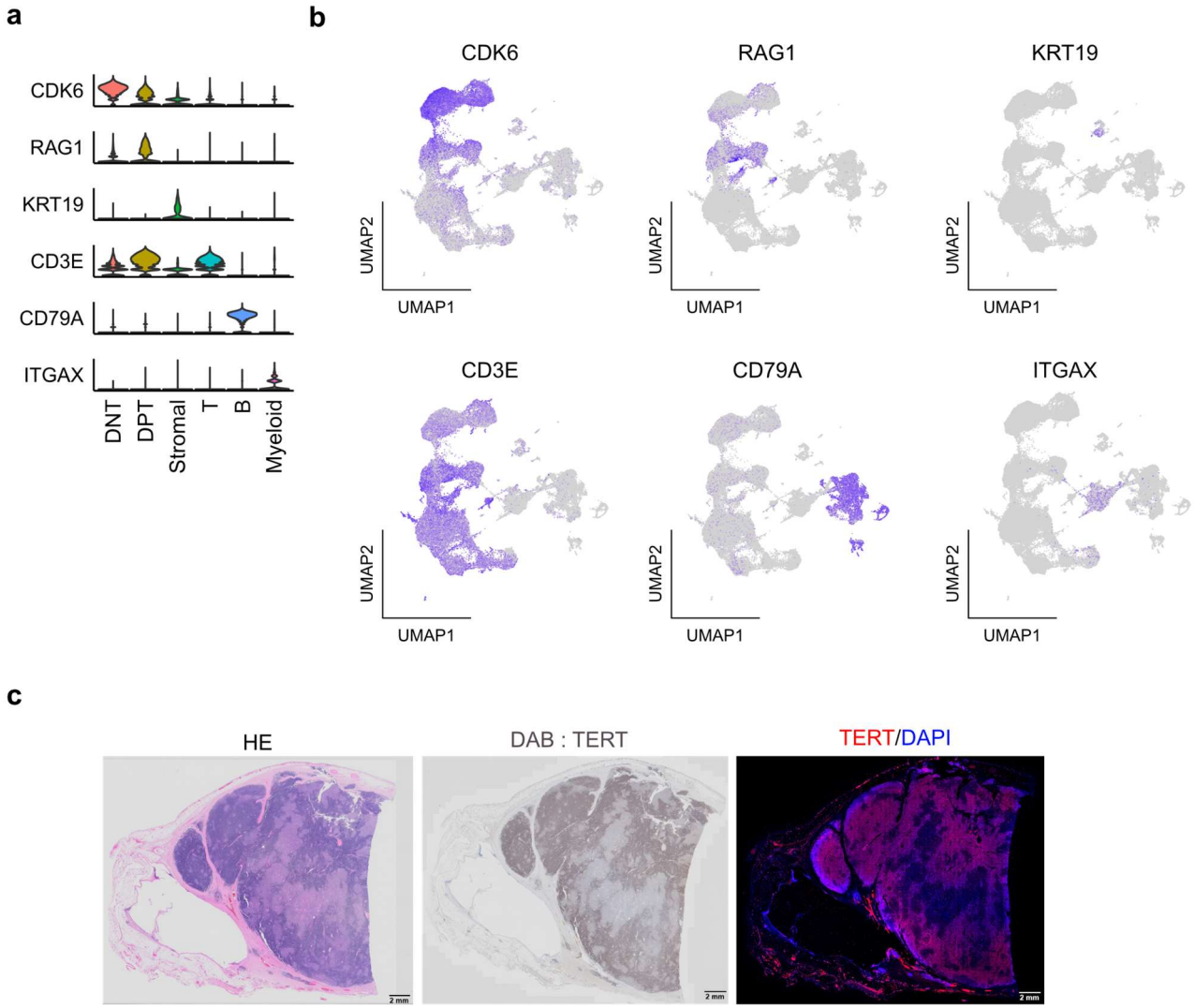
Regional plots show the result of conditional analysis of HLA variants in the MHC region. Diamonds represent -$\log_{10}$(*P* values) for the tested HLA variants, including SNPs, classical HLA alleles, and amino acid polymorphisms. Red arrows indicate conditioned alleles in each subtype. The dotted horizontal black lines indicate genome-wide significance threshold of $P = 5.0\times10^{-8}$. Blue and Red Diamonds indicate variants in HLA class I and class II alleles, respectively.

Supplementary Figure 6. Colocalization of the *TERT* regional associations with MG



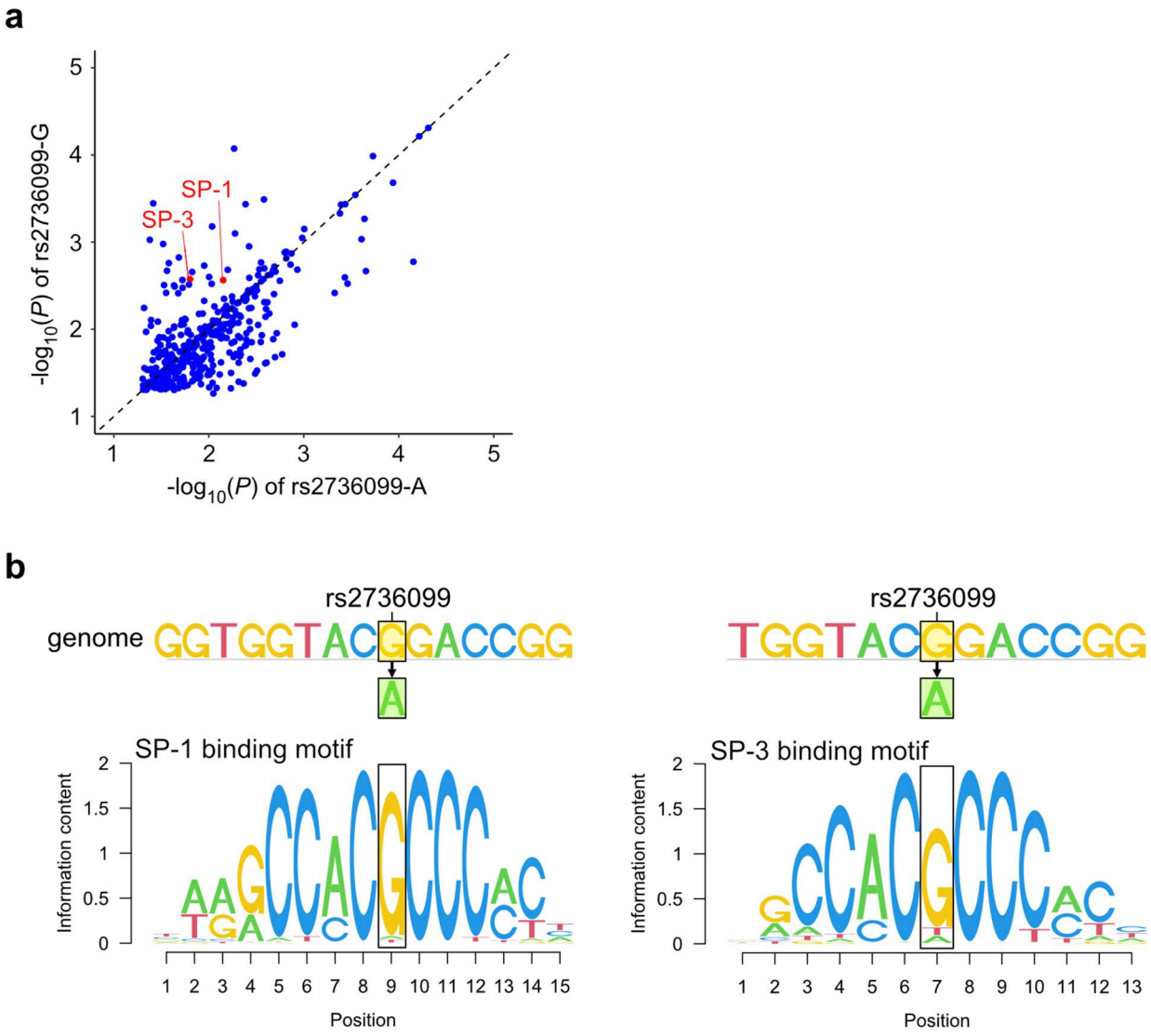
A bar plot shows calculated posterior probability (PP) for sharing the causal variant (= H4) of each trait.

Supplementary Figure 7. Cell type annotation in single cell RNA sequence of thymomas and PBMCs and immunostaining of thymoma specimen



a, b) Violin plots and UMAP show expression of each cell marker gene in single cell RNA sequence of thymomas and PBMCs. **c)** Stained image of a histopathology specimen of a thymoma specimen. HE stands for hematoxylin-eosin staining, DAB for immunostaining with 3,3'-diaminobenzidine, and DAPI for fluorescent staining with 4',6-diamidino-2-phenylindole.

Supplementary Figure 8. TF binding affinity analysis for the rs2736099 alleles



a) A scatter plot shows *P* values for sequence match of rs2736099-A and rs2736099-G with TF binding motifs. **b)** Allele information of rs2736099 in the genome sequence and binding motif sequence of transcription factors SP-1 and SP-3.

Supplementary Table 1. Genome-wide significant variants associated with myasthenia gravis at 5p13.

Supplementary Table 1 Genome wide significant variants associated with myasthenia gravis in Chromosome 5

subtype	SNP (rsID)	Position (GRCh37)	Reference/ Effect allele	Case		Control		OR (95% CI) of Allele2	*P* value
				n	Effect allele frequency	*n*	Effect allele frequency		
All MG	rs7734992	5:1280128	T/C	1,434	0.412	42,913	0.361	1.24 (1.15-1.35)	3.5×10^{-8}
	rs4975538	5:1280830	G/C		0.405		0.354	1.24 (1.15-1.35)	3.7×10^{-8}
	rs7726159	5:1282319	C/A		0.406		0.351	1.27 (1.17-1.37)	2.8×10^{-9}
	rs7725218	5:1282414	G/A		0.407		0.352	1.26 (1.17-1.37)	2.7×10^{-9}
	rs7705526	5:1285974	C/A		0.410		0.354	1.27 (1.17-1.37)	2.0×10^{-9}
	rs2736100	5:1286516	A/C		0.441		0.387	1.25 (1.16-1.35)	8.9×10^{-9}
	rs2736099	5:1287340	G/A		0.350		0.295	1.30 (1.19-1.41)	3.3×10^{-10}
	rs2853672	5:1292983	A/C		0.396		0.341	1.27 (1.18-1.38)	1.3×10^{-9}
	rs2736098	5:1294086	C/T		0.302		0.250	1.31 (1.21-1.43)	2.1×10^{-10}
	rs2853669	5:1295349	A/G		0.305		0.255	1.30 (1.19-1.41)	1.1×10^{-9}
	rs3215401	5:1296255	A/AG		0.305		0.253	1.32 (1.21-1.43)	1.7×10^{-10}
	rs2735940	5:1296486	G/A		0.395		0.340	1.27 (1.18-1.38)	1.5×10^{-9}
	rs2736109	5:1296759	C/T		0.238		0.197	1.29 (1.18-1.42)	4.1×10^{-8}
	rs2736108	5:1297488	C/T		0.233		0.192	1.29 (1.18-1.42)	3.6×10^{-8}
gMG	rs7726159	5:1282319	C/A	1,134	0.413	42,913	0.351	1.30 (1.19-1.42)	2.7×10^{-9}
	rs7725218	5:1282414	G/A		0.414		0.352	1.30 (1.19-1.42)	2.6×10^{-9}
	rs7705526	5:1285974	C/A		0.416		0.354	1.30 (1.19-1.42)	3.0×10^{-9}
	rs2736100	5:1286516	A/C		0.447		0.387	1.28 (1.17-1.40)	1.3×10^{-9}
	rs2853677	5:1287194	A/G		0.361		0.304	1.30 (1.18-1.42)	8.9×10^{-9}
	rs2736099	5:1287340	G/A		0.364		0.295	1.38 (1.26-1.52)	1.6×10^{-12}
	rs2853672	5:1292983	A/C		0.411		0.341	1.35 (1.24-1.48)	1.0×10^{-11}
	rs2736098	5:1294086	C/T		0.314		0.250	1.39 (1.26-1.53)	8.5×10^{-12}
	rs2853669	5:1295349	A/G		0.317		0.255	1.37 (1.25-1.51)	3.7×10^{-11}
	rs3215401	5:1296255	A/AG		0.317		0.253	1.39 (1.26-1.53)	9.3×10^{-12}
	rs2735940	5:1296486	G/A		0.409		0.340	1.35 (1.24-1.48)	1.4×10^{-11}
	rs2736109	5:1296759	C/T		0.245		0.197	1.34 (1.21-1.48)	2.1×10^{-8}
	rs2736108	5:1297488	C/T		0.238		0.192	1.33 (1.20-1.48)	3.3×10^{-8}
g-AChR-Ab(+)MG	rs2075785	5:1278584	C/T	934	0.398	42,913	0.465	1.33 (1.20-1.46)	8.7×10^{-9}
	rs10054203	5:1279964	G/C		0.446		0.384	1.30 (1.18-1.43)	4.8×10^{-8}
	rs7734992	5:1280128	T/C		0.425		0.361	1.31 (1.19-1.44)	2.2×10^{-8}
	rs4975538	5:1280830	G/C		0.417		0.354	1.31 (1.19-1.44)	3.3×10^{-8}
	rs6897196	5:1280938	A/G		0.417		0.353	1.31 (1.19-1.44)	2.5×10^{-8}
	rs1561204283	5:1280940	GAGCCCACC/G		0.417		0.353	1.31 (1.19-1.44)	2.5×10^{-8}
	rs7726159	5:1282319	C/A		0.428		0.351	1.38 (1.26-1.52)	1.9×10^{-11}
	rs7725218	5:1282414	G/A		0.429		0.352	1.38 (1.26-1.52)	1.8×10^{-11}
	rs7705526	5:1285974	C/A		0.430		0.354	1.38 (1.26-1.52)	4.3×10^{-11}
	rs2736100	5:1286516	A/C		0.462		0.387	1.36 (1.24-1.50)	1.3×10^{-10}
	rs2853677	5:1287194	A/G		0.381		0.304	1.41 (1.28-1.56)	2.5×10^{-12}
	rs2736099	5:1287340	G/A		0.382		0.295	1.49 (1.35-1.65)	2.1×10^{-15}
	rs2853672	5:1292983	A/C		0.427		0.341	1.44 (1.31-1.59)	3.4×10^{-14}
	rs2736098	5:1294086	C/T		0.330		0.250	1.50 (1.35-1.66)	2.1×10^{-14}
	rs2853669	5:1295349	A/G		0.333		0.255	1.48 (1.33-1.64)	1.0×10^{-13}
	rs3215401	5:1296255	A/AG		0.332		0.253	1.49 (1.34-1.65)	4.3×10^{-14}
	rs2735940	5:1296486	G/A		0.425		0.340	1.44 (1.31-1.59)	6.2×10^{-14}
	rs2736109	5:1296759	C/T		0.256		0.197	1.42 (1.27-1.59)	4.4×10^{-10}
	rs2736108	5:1297488	C/T		0.250		0.192	1.42 (1.27-1.59)	7.2×10^{-10}
	rs4975612	5:1300310	T/G		0.432		0.369	1.31 (1.19-1.45)	2.2×10^{-8}
g-TAMG	rs2075785	5:1278584	C/T	335	0.354	42,913	0.465	1.61 (1.37-1.90)	6.4×10^{-9}
	rs7734992	5:1280128	T/C		0.472		0.361	1.59 (1.36-1.86)	4.2×10^{-9}
	rs4975538	5:1280830	G/C		0.465		0.354	1.59 (1.36-1.87)	3.3×10^{-9}
	rs6897196	5:1280938	A/G		0.467		0.353	1.61 (1.37-1.88)	1.6×10^{-9}
	rs1561204283	5:1280940	GAGCCCACC/G		0.467		0.353	1.61 (1.37-1.88)	1.6×10^{-9}
	rs7726159	5:1282319	C/A		0.478		0.351	1.70 (1.45-1.99)	2.1×10^{-11}
	rs7725218	5:1282414	G/A		0.479		0.352	1.70 (1.45-1.99)	2.0×10^{-11}
	rs7705526	5:1285974	C/A		0.480		0.354	1.69 (1.44-1.98)	3.1×10^{-11}
	rs2736100	5:1286516	A/C		0.484		0.613	1.70 (1.45-1.99)	1.3×10^{-11}
	rs2853677	5:1287194	A/G		0.548		0.696	1.90 (1.62-2.22)	1.7×10^{-15}
	rs2736099	5:1287340	G/A		0.558		0.705	1.92 (1.64-2.26)	1.1×10^{-15}
	rs2853672	5:1292983	A/C		0.530		0.659	1.72 (1.47-2.01)	6.3×10^{-12}
	rs2736098	5:1294086	C/T		0.378		0.250	1.86 (1.57-2.20)	2.6×10^{-13}
	rs2853669	5:1295349	A/G		0.381		0.255	1.83 (1.55-2.17)	8.7×10^{-13}
	rs3215401	5:1296255	A/AG		0.382		0.253	1.86 (1.57-2.20)	2.9×10^{-13}
	rs2735940	5:1296486	G/A		0.534		0.660	1.72 (1.43-2.01)	1.2×10^{-11}
	rs2736109	5:1296759	C/T		0.292		0.197	1.71 (1.43-2.05)	2.5×10^{-9}
	rs2736108	5:1297488	C/T		0.286		0.192	1.72 (1.44-2.06)	2.3×10^{-9}

Associations were estimated using a generalized mixed model (two-sided).

Supplementary Table 2. The replication study of European meta GWAS in Japanese.

Gene	Chr	Pos (GRCh37)	rsID	Effect/Other allele	European			JAMG-R	All MG cases	
					Effect allele frequency	OR (95%CI)	*P* value	Effect allele frequency	OR (95%CI)	*P* value
PTPN22	1	114377568	rs2476601	A/G	0.130	1.46 (1.37-1.56)	2.8×10^{-31}	NA	NA	NA
MAGI3	1	114217705	rs7522138	A/G	0.470	0.88 (0.83-0.94)	6.8×10^{-9}	0.340	1.02	0.57
CHRNA1	2	175616667	rs6433501	G/A	0.112	1.17 (1.10-1.24)	3.5×10^{-7}	0.942	1.00	0.74
CTLA4	2	204734487	rs231779	T/C	0.451	1.11 (1.06-1.16)	1.8×10^{-6}	0.621	1.17 (1.08-1.27)	1.0×10^{-4}
TNIP1	5	150447128	rs6861227	G/T	0.128	1.17 (1.10-1.24)	7.4×10^{-8}	0.112	0.94	0.28
MHC	6	30063652	rs1264706	C/G	0.072	1.62 (1.53-1.71)	1.3×10^{-34}	NA	NA	NA
TBX18	6	85513783	rs215918	A/G	0.374	0.89 (0.85-0.93)	3.4×10^{-8}	0.403	1.03	0.47
RNASET2	6	167437988	rs2301436	T/C	0.477	1.12 (1.07-1.17)	1.1×10^{-7}	0.418	1.03	0.43
FAM76B	11	95311422	rs4409785	C/T	0.169	1.19 (1.13-1.26)	2.7×10^{-11}	0.076	1.17 (0.99-1.38)	0.030
ATXN2	12	111904371	rs4766578	T/A	0.406	1.12 (1.07-1.18)	6.7×10^{-8}	NA	NA	NA
IKZF3	17	37912377	rs12946510	T/C	0.493	1.15 (1.10-1.20)	7.6×10^{-11}	0.325	1.08	0.068
TNFRSF11A	18	60005046	rs7239261	A/C	0.436	1.21 (1.17-1.27)	4.0×10^{-20}	0.326	1.14 (1.05-1.24)	1.3×10^{-3}

Gene	Chr	Pos	rsID	Effect/Other allele	OMG		gMG		g-AChR-Ab(+)MG		g-AChR-Ab(-)MG	
					OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
PTPN2	1	114377568	rs2476601	A/G	NA	NA	NA	NA	NA	NA	NA	NA
MAGI3	1	114217705	rs7522138	A/G	0.90	0.25	1.06	0.21	1.07	0.16	1.00	0.97
CHRNA1	2	175616667	rs6433501	G/A	0.90	0.58	1.06	0.52	1.03	0.76	1.22	0.37
CTLA4	2	204734487	rs231779	T/C	1.36 (1.13-1.63)	5.4×10^{-4}	1.12 (1.02-1.24)	8.5×10^{-3}	1.15 (1.03-1.28)	5.2×10^{-4}	1.02	0.82
TNIP1	5	150447128	rs6861227	G/T	0.80	0.083	0.98	0.76	1.01	0.88	0.83	0.26
MHC	6	30063652	rs1264706	C/G	NA	NA	NA	NA	NA	NA	NA	NA
TBX18	6	85513783	rs215918	A/G	1.01	0.94	1.04	0.43	1.02	0.62	1.10	0.37
RNASET2	6	167437988	rs2301436	T/C	1.02	0.79	1.03	0.44	1.07	0.18	0.89	0.27
FAM76B	11	95311422	rs4409785	C/T	1.44 (1.03-2.03)	0.017	1.10	0.23	1.12	0.21	1.04	0.85
ATXN2	12	111904371	rs4766578	T/A	NA	NA	NA	NA	NA	NA	NA	NA
IKZF3	17	37912377	rs12946510	T/C	1.11	0.24	1.07	0.14	1.08	0.12	1.02	0.86
TNFRSF11A	18	60005046	rs7239261	A/C	1.14	0.13	1.14 (1.04-1.25)	4.0×10^{-3}	1.16 (1.04 - 1.28)	3.2×10^{-3}	1.04	0.70

Gene	Chr	Pos	rsID	Effect/Other allele	g-TAMG		g-TA(-)MG		g-EOMG		g-LOMG	
					OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value	OR (95%CI)	*P* value
PTPN2	1	114377568	rs2476601	A/G	NA	NA	NA	NA	NA	NA	NA	NA
MAGI3	1	114217705	rs7522138	A/G	1.04	0.62	1.09	0.17	1.04	0.66	1.14	0.13
CHRNA1	2	175616667	rs6433501	G/A	1.00	0.99	1.05	0.72	0.99	0.95	1.14	0.46
CTLA4	2	204734487	rs231779	T/C	1.20 (1.00-1.44)	0.024	1.12	0.068	1.09	0.31	1.12	0.18
TNIP1	5	150447128	rs6861227	G/T	1.02	0.87	1.00	0.96	0.92	0.51	1.09	0.51
MHC	6	30063652	rs1264706	C/G	NA	NA	NA	NA	NA	NA	NA	NA
TBX18	6	85513783	rs215918	A/G	0.96	0.59	1.07	0.29	1.10	0.27	1.03	0.71
RNASET2	6	167437988	rs2301436	T/C	0.98	0.83	1.11	0.065	1.07	0.42	1.17	0.067
FAM76B	11	95311422	rs4409785	C/T	1.02	0.88	1.17	0.14	1.15	0.38	1.14	0.21
ATXN2	12	111904371	rs4766578	T/A	NA	NA	NA	NA	NA	NA	NA	NA
IKZF3	17	37912377	rs12946510	T/C	1.09	0.3	1.08	0.22	1.08	0.39	1.07	0.43
TNFRSF11A	18	60005046	rs7239261	A/C	1.03	0.77	1.23 (1.09-1.40)	5.0×10^{-4}	1.09	0.33	1.35 (1.14-1.60)	3.1×10^{-4}

Associations were estimated using a generalized mixed model (two-sided). 'NA' indicates that the SNPs were neither genotyped nor imputed in the JAMG-R dataset. Chr: Chromosome.

Supplementary Table 3. Associations of the HLA variants with MG disease subtypes

			Case		Control			
Subtype	HLA variant	position	*n*	frequency	*n*	frequency	OR (95% CI)	*P* value
All MG cases	AA_DRB1_74	6:32551948	1,434	-	42,913	-	-	1.1×10^{-8}
g-TA(-)MG	HLA_DRB1_08:03	6:32552086	599	0.131	42,913	0.081	1.71 (1.45-2.03)	5.6×10^{-9}
g-EOMG	AA_A_9	6:29910558	293	-	42,913	-	-	2.4×10^{-8}
	AA_A_246	6:29912088		-		-	-	2.7×10^{-8}
	AA_A_299	6:29912349		-		-	-	2.7×10^{-8}
	AA_A_334	6:29913038		-		-	-	2.7×10^{-8}
g-LOMG	AA_B_76	6:31324509	306	-	42,913	-	-	1.9×10^{-8}
	HLA_B_46:01	6:31323307		0.105		0.047	2.32 (1.78-3.03)	1.9×10^{-8}
	HLA_DRB1_08:03	6:32552086		0.163		0.081	2.18 (1.75-2.71)	1.2×10^{-10}

Associations were estimated by logistic regression model (two-sided). Odds ratios compare the presence versus absence of each HLA variant. HLA variants starting with “AA” represent amino acid polymorphisms. We conducted an omnibus test that considers multiple alleles at each amino acid position; therefore, odds ratios for individual polymorphisms are not available.

Supplementary Table 4. Sample size for the MG disease subtypes and treatment response

response	All MG cases	OMG	gMG	g-AChR-Ab(+)MG	g-AChR-Ab(-)MG	g-TAMG	g-TA(-)MG	g-EOMG	g-LOMG
poor	317 (22.1%)	19 (6.3%)	298 (26.3%)	203 (21.7%)	95 (48.0%)	73 (21.8%)	130 (21.7%)	75 (25.6%)	55 (18.0%)
intermediate	206 (12.6%)	33 (11.0%)	173 (15.2%)	134 (14.3%)	39 (19.5%)	38 (11.3%)	96 (16.0%)	56 (19.1%)	50 (13.1%)
good	911 (65.3%)	248 (82.7%)	663 (58.5%)	597 (63.9%)	66 (33.0%)	224 (66.9%)	373 (62.3%)	162 (55.3%)	211 (69.0%)
total	1,434	300	1,134	934	200	335	599	293	306
Association between rs2736099-A and treatment response									
OR (95% CI) of rs2736099-A	1.23 (0.98-1.55)	1.16 (0.55-2.48)	1.16 (0.94-1.43)	1.41 (1.09-1.81)	0.94 (0.53-1.69)	1.49 (0.98-2.25)	1.42 (1.01-1.99)	1.95 (1.29-2.96)	0.98 (0.62-1.55)
P value	0.040	0.70	0.17	4.2×10^{-3}	0.85	0.060	0.021	8.5×10^{-4}	0.93

Associations were estimated using a generalized mixed model (two-sided). The percentages shown in parentheses indicate the percentages of patients with poor, intermediate, and good treatment responses within each disease subtype. The association between rs2736099-A and treatment response represents the odds ratio of patients with a poor response compared to those with a good response.

Supplementary Table 5. PheWAS analysis of *TERT* rs2736099

Disease	ICD10	n	OR (95%CI)	*P*
Lung Cancer	C34	7,986	1.17 (1.14-1.22)	4.1×10^{-20}
ILD	J84.1/J84.8/J84.9/J93	2,614	0.82 (0.77-0.87)	2.5×10^{-10}
Colorectal cancer	C18	16,134	0.93 (0.91-0.96)	8.1×10^{-8}
Uterine fibroids	D25	17,545	1.06 (1.03-1.08)	3.1×10^{-5}
Graves' disease	E05.0	3,806	0.90 (0.85-0.95)	1.8×10^{-4}
Quantitative traits	**Category**	**n**	**Effect size (95%CI)**	***P***
RBC	Blood cell count	206,158	0.037 (0.031-0.043)	4.8×10^{-31}
Hb	Blood cell count	206,037	0.014 (0.0080-0.020)	5.3×10^{-6}
Ht	Blood cell count	206,036	0.017 (0.011-0.024)	3.9×10^{-8}
MCV	Blood cell count	194,734	-0.047 (-0.054 - -0.040)	2.1×10^{-42}
MCH	Blood cell count	192,950	-0.048 (-0.055 - -0.040)	1.1×10^{-43}
WBC	Blood cell count	202,881	0.031 (0.024-0.037)	6.8×10^{-19}
Neutrophil	Blood cell count	136,819	0.028 (0.020-0.036)	6.1×10^{-12}
Monocyte	Blood cell count	136,819	0.015 (0.0067-0.023)	3.4×10^{-4}
PLT	Blood cell count	197,859	0.040 (0.033-0.047)	1.0×10^{-31}

Binary traits were tested by logistic regression analysis, continuous traits by linear regression analysis. ILD: interstitial lung disease, RBC: red blood cell, Hb: hemoglobin, Ht: hematocrit, MCV: mean corpuscular volume, MCH: mean corpuscular hemoglobin, WBC: white blood cell, PLT: platelet count.

Supplementary Table 6. Colocalization of the *TERT* regional associations with MG

Binary traits	*n*	n_{SNPs}	PP (H4)
Lung Cancer	4,444	3,733	0.710
Quantitative traits	***n***	n_{SNPs}	**PP (H4)**
RBC	153,512	3,733	0.977
MCV	129,832	3,733	0.981
MCH	128,028	3,733	0.981
WBC	154,355	3,733	0.973
PLT	148,623	3,733	0.979
Telomere length	35,570	7,134	0.981

PP: posterior probability

Supplementary Table 7. Diseases excluded from control as autoimmune diseases

systemic lupus erythematosus
Sjogren syndrome
dermatopolymyositis
localized scleroderma
systemic scleroderma
Bechet's disease
sarcoidosis
polymyaglia rheumatica with giant cell artertis
polymyaglia rheumatica
adult-onset Still's disease
microscopic polyangiitis
Wegener's granulomatosis
Churg-Strauss syndrome
Takayasu disease
thrombotic microangiopathy
IgG4-related disease
mixed connective tissue diseases
SAPHO syndrome