Supplementary Table S1. Summary of single-cell RNA data of thirty ALS patients and ten HC

Sample ID	Sample	Platform	Disease	No. of cells (before QC)	No. of cells (after QC)
ALS3	PBMC	GPL28038	ALS	11972	4122
ALS6	PBMC	GPL28038	ALS	15934	10614
ALS7	PBMC	GPL28038	ALS	10164	1844
ALS8	PBMC	GPL28038	ALS	12395	2680
ALS9	PBMC	GPL28038	ALS	10758	7223
ALS10	PBMC	GPL28038	ALS	11432	8370
ALS11	PBMC	GPL28038	ALS	11962	7752
ALS13	PBMC	GPL28038	ALS	8514	3303
ALS14	PBMC	GPL28038	ALS	8648	4100
ALS16	PBMC	GPL28038	ALS	4746	1132
ALS17	PBMC	GPL28038	ALS	10360	1855
ALS18	PBMC	GPL28038	ALS	12039	2762
ALS19	PBMC	GPL28038	ALS	11668	1679
ALS20	PBMC	GPL28038	ALS	9097	2422
ALS21	PBMC	GPL28038	ALS	12993	8136
ALS22	PBMC	GPL28038	ALS	10360	1763
ALS23	PBMC	GPL28038	ALS	13720	5081
ALS24	PBMC	GPL28038	ALS	11205	1444
ALS25	PBMC	GPL28038	ALS	15220	6581
ALS26	PBMC	GPL28038	ALS	11010	2399
ALS27	PBMC	GPL28038	ALS	13435	2252
ALS28	PBMC	GPL28038	ALS	12960	1651
ALS29	PBMC	GPL28038	ALS	12914	1140
ALS30	PBMC	GPL28038	ALS	8701	2725

ALS31	PBMC	GPL28038	ALS	13677	5763
ALS32	PBMC	GPL28038	ALS	11556	5738
ALS33	PBMC	GPL28038	ALS	11151	3593
ALS34	PBMC	GPL28038	ALS	13377	6278
ALS35	PBMC	GPL28038	ALS	10639	5260
ALS36	PBMC	GPL28038	ALS	10988	3081
C1	PBMC	GPL28038	HC	11434	4497
C2	PBMC	GPL28038	HC	12127	4545
C3	PBMC	GPL28038	HC	11511	2937
C4	PBMC	GPL28038	HC	11150	1884
C5	PBMC	GPL28038	HC	9222	1122
C6	PBMC	GPL28038	HC	6145	1507
C7	PBMC	GPL28038	HC	9132	1136
C8	PBMC	GPL28038	HC	11381	991
C9	PBMC	GPL28038	HC	7772	1349
C10	PBMC	GPL28038	HC	9115	3033

Note. ALS: Amyotrophic lateral sclerosis; HC: healthy controls; QC: quality control.

Supplementary Table S2. Information of cis-QTL and GWAS datasets

Data	Resource	Samples	Population	Wed Site
cis-eQTL	eQTLGen Consortium	31684	European	https://www.eqtlgen.org/cis-eqtls.html
ALS	IEU OpenGWAS project	ncase: 27,205 ncontrol: 110,881	European	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST90027164/
ALS	IEU OpenGWAS project	ncase: 20,806 ncontrol: 59,804	European	https://gwas.mrcieu.ac.uk/datasets/ebi-a-GCST005647/

Note. ALS: Amyotrophic lateral sclerosis; GWAS: Genome-wide association studies; eQTL: expression quantitative trait loci.

Supplementary Table S3. Detailed information about bulk transcriptomes sequencing data

GEO ID	Platform	Total Samples	Number of Cases	Number of Controls	Tissue	Country	Last Update Date
GSE112676	GPL6947	741	233 ALS patients	508 controls	Whole blood	Netherlands	2019

Note. ALS: Amyotrophic lateral sclerosis.

Supplementary Table S4. Sequences of the primers used in this study

Species	Genes	Forward primers	Reverse primers
Homo	S100A6	ACAAGGACCAGGAGGTGAAC	ACCCACCACTGGATTTGACT
Homo	SERPINB6	GTCTCTGTCCAAGGTCGTGC	AGAATCCCGTTGGTCTTGCT
Homo	SMAD7	AGTATTGCTCACCCAGTGCC	AACACACAGGATGGGAGCAG
Homo	TPST2	AGTGCCCAAGGGAGATGTGAG	GCCCGGCACTCTAGCAC
Homo	DIP2A	TCCAAAATCAAGTGGGGCGA	AAATGAGCCCCAGCCTTCTC
Homo	GAPDH	TCCAAAATCAAGTGGGGCGA	AAATGAGCCCCAGCCTTCTC

Supplementary Table S5. Deferentially expressed genes between the cytotoxic CD4+ T cells and the other subtypes

Gene	*p*_val	avg_log2FC	pct.1	pct.2	*p*_val_adj
GNLY	0	2.507389298	0.848	0.235	0
GZMH	0	2.445265201	0.981	0.184	0
FGFBP2	0	2.133775451	0.914	0.161	0
NKG7	0	2.088153392	1	0.316	0
GZMA	0	1.856902404	0.976	0.278	0
CCL5	0	1.817352141	0.998	0.324	0
GZMB	0	1.661908115	0.752	0.156	0
EFHD2	0	1.561954095	0.866	0.289	0
CST7	0	1.516628432	0.954	0.299	0
PLEK	0	1.490022593	0.782	0.19	0
HOPX	0	1.454320713	0.722	0.197	0
CCL4	0	1.442778589	0.67	0.146	0
ZEB2	0	1.383394556	0.613	0.128	0
PRF1	0	1.325297917	0.745	0.213	0
CX3CR1	0	1.286718792	0.532	0.09	0
LGALS1	0	-1.254197624	0.826	0.414	0
KLRB1	0	1.227488312	0.496	0.22	0
TRGC2	0	1.204716203	0.55	0.153	0
S100A4	0	1.182960283	0.999	0.891	0
CTSC	0	1.179379545	0.757	0.382	0
C12orf75	0	1.174689339	0.768	0.332	0
LINC01871	0	1.126759643	0.623	0.202	0
SH3BGRL3	0	1.099074809	0.999	0.952	0
DIP2A	7.38E-55	-0.359622084	0.265	0.168	1.98E-50

ID2	0	1.095939085	0.753	0.396	0
KLRG1	0	1.072701372	0.596	0.184	0
ITGB1	0	1.064794155	0.906	0.539	0
LYAR	0	1.01894114	0.677	0.285	0
HLA-DPB1	0	1.017924043	0.79	0.411	0
SAMD3	0	1.011377382	0.673	0.305	0
RAP1B	0	0.992231277	0.904	0.711	0
C1orf21	0	0.98707455	0.434	0.085	0
MAF	0	0.985516691	0.607	0.253	0
MYBL1	0	0.968261986	0.435	0.137	0
HLA-DRB1	0	0.959942504	0.63	0.261	0
APOBEC3G	0	0.954779622	0.589	0.252	0
SYNE1	0	0.940349817	0.588	0.275	0
CD99	0	0.925017635	0.938	0.717	0
YWHAQ	0	0.899431541	0.708	0.421	0
CD2	0	-0.897686477	0.924	0.785	0
LAIR2	0	0.894183629	0.355	0.102	0
CD81	0	0.888441428	0.797	0.509	0
GZMM	0	0.881931953	0.812	0.506	0
HLA-DPA1	0	0.870753464	0.651	0.33	0
HCST	0	0.868310948	0.96	0.84	0
ABHD17A	0	0.858048751	0.755	0.492	0
CYBA	0	0.846243397	0.975	0.871	0
SRGN	0	0.839871206	0.903	0.688	0
LCP1	0	0.834392711	0.861	0.678	0
CD52	0	0.825309013	0.994	0.979	0

HLA-C	0	0.824135302	1	0.995	0
CLIC1	0	0.816283778	0.89	0.662	0
SPON2	0	0.808274311	0.359	0.092	0
FLNA	1.01E-277	-0.7970482	0.652	0.403	2.72E-273
AHNAK	7.38E-298	0.793320445	0.756	0.496	1.98E-293
ADGRG1	0	0.791251548	0.345	0.074	0
TBX21	0	0.785057064	0.352	0.078	0
CYTOR	0	0.782945055	0.546	0.235	0
HSPA1A	4.81E-57	0.774859371	0.274	0.176	1.29E-52
PRSS23	0	0.769838272	0.323	0.063	0
MSN	7.54E-272	0.757260909	0.731	0.527	2.02E-267
TGFBR3	0	0.753320177	0.339	0.088	0
DSTN	2.71E-241	0.744798787	0.664	0.444	7.27E-237
S100A6	0	0.744622756	0.992	0.952	0
ITGB2	3.35E-282	0.744020104	0.795	0.593	9.01E-278
ANXA1	0	0.738555561	0.957	0.752	0
MATK	0	0.729527913	0.404	0.132	0
MYO1F	2.78E-301	-0.727089995	0.423	0.174	7.46E-297
B2M	0	0.72395869	1	1	0
IFITM2	3.31E-253	0.723753462	0.9	0.81	8.87E-249
PPP2R5C	1.47E-261	0.716623363	0.831	0.644	3.95E-257
PYHIN1	2.33E-243	0.716141552	0.518	0.273	6.25E-239
SYNE2	2.38E-221	0.714237527	0.696	0.478	6.38E-217
S100A10	0	0.713121153	0.972	0.884	0
CTSW	2.21E-292	0.705391359	0.591	0.29	5.92E-288
CD320	3.49E-236	0.704608046	0.387	0.176	9.36E-232

CDC42EP3	1.73E-230	0.701844764	0.456	0.23	4.64E-226
TLN1	6.65E-208	0.698312759	0.645	0.452	1.78E-203
LITAF	7.85E-226	0.692103647	0.737	0.554	2.11E-221
RORA	2.22E-174	0.691235666	0.538	0.348	5.95E-170
CALM1	0	0.689695337	0.996	0.98	0
PFN1	0	0.685604855	0.995	0.978	0
ARPC2	0	0.684577771	0.95	0.856	0
NEAT1	3.64E-127	-0.670757935	0.676	0.54	9.77E-123
RUNX3	3.91E-211	0.669434538	0.479	0.258	1.05E-206
PTPRC	0	0.667454665	0.992	0.962	0
ZBTB38	3.96E-184	0.664582959	0.453	0.25	1.06E-179
ITGA4	5.88E-219	0.659827307	0.653	0.413	1.58E-214
CLEC2B	3.56E-173	0.65643358	0.647	0.467	9.56E-169
CAST	5.80E-185	0.650722377	0.734	0.574	1.56E-180
ADRB2	0	0.649233652	0.275	0.063	0
DBI	5.85E-246	0.645757338	0.772	0.585	1.57E-241
MYL12B	0	0.641263908	0.973	0.914	0
ITGAL	4.09E-212	-0.635827818	0.42	0.207	1.10E-207
RNF213	1.21E-190	0.633844067	0.729	0.562	3.25E-186
SMAD7	1.04E-254	0.633095749	0.325	0.121	2.80E-250
PLAC8	1.31E-139	0.630915294	0.614	0.461	3.51E-135
ARL4C	5.18E-213	0.629101258	0.866	0.747	1.39E-208
FCRL6	0	0.627755666	0.294	0.076	0
MYL6	0	0.617818105	0.977	0.926	0
TRG-AS1	4.84E-141	0.615655341	0.406	0.233	1.30E-136
SLC9A3R1	7.02E-158	0.61038363	0.55	0.369	1.88E-153

TPM3	2.65E-226	0.608221024	0.891	0.792	7.11E-222
PAXX	1.05E-166	0.60728834	0.572	0.387	2.81E-162
GNG2	6.75E-157	0.604765054	0.444	0.261	1.81E-152
VCL	1.84E-228	0.603049506	0.307	0.118	4.93E-224
CD63	3.33E-189	0.599400783	0.628	0.418	8.93E-185
S1PR5	0	0.598726105	0.269	0.058	0
SEPTIN7	1.61E-255	0.593662158	0.916	0.834	4.33E-251
GPR65	4.77E-164	0.592496004	0.381	0.197	1.28E-159
PSMB9	3.44E-207	0.588898997	0.829	0.692	9.24E-203
STOM	2.40E-222	0.588608918	0.336	0.137	6.45E-218
IL32	0	0.5877845	0.998	0.964	0
SYTL3	5.71E-194	0.582043814	0.31	0.13	1.53E-189
CHST12	2.70E-212	0.580211115	0.333	0.139	7.25E-208
HLA-B	0	0.578779383	1	0.998	0
GSTP1	1.03E-167	0.577487376	0.657	0.484	2.76E-163
EMP3	4.45E-249	0.572235604	0.941	0.863	1.20E-244
TNFRSF1B	6.50E-165	0.571453966	0.403	0.213	1.74E-160
YWHAZ	8.33E-191	0.565653509	0.867	0.775	2.23E-186
DHRS7	5.31E-146	-0.563319159	0.6	0.441	1.43E-141
GBP5	9.06E-157	0.562234923	0.46	0.262	2.43E-152
CD300A	6.81E-254	0.562000388	0.254	0.08	1.83E-249
APMAP	1.15E-169	0.561566064	0.368	0.182	3.09E-165
CD226	3.10E-151	0.559834275	0.327	0.162	8.33E-147
ANXA2	9.14E-167	0.558598949	0.544	0.328	2.45E-162
CFL1	1.75E-299	0.55688493	0.987	0.957	4.70E-295
ACTN4	1.32E-157	0.554628359	0.373	0.193	3.55E-153

PLAAT4	1.65E-184	0.550983083	0.851	0.734	4.43E-180
TPST2	4.08E-133	0.583622461	0.299	0.144	1.10E-128
RASSF1	5.97E-166	0.550277685	0.356	0.177	1.60E-161
DNAJC1	6.83E-158	0.548893405	0.342	0.167	1.83E-153
S100A11	9.99E-167	0.544964201	0.804	0.616	2.68E-162
APOBEC3C	6.27E-155	-0.544365518	0.346	0.172	1.68E-150
SSBP3	7.17E-149	0.542886853	0.348	0.179	1.93E-144
HLA-DRA	8.79E-83	0.54079027	0.316	0.184	2.36E-78
HLA-DRB5	2.71E-185	0.533368577	0.325	0.136	7.27E-181
ARPC5L	2.83E-127	0.532743674	0.496	0.331	7.60E-123
MYL12A	1.55E-303	0.532217723	0.993	0.98	4.17E-299
PTP4A2	5.05E-150	0.530640485	0.775	0.665	1.36E-145
CLEC2D	1.61E-115	0.530092978	0.682	0.565	4.33E-111
PPP1CA	1.83E-137	0.526472006	0.636	0.484	4.92E-133
RAP2B	9.46E-109	0.526288647	0.456	0.31	2.54E-104
IL2RG	2.89E-164	0.526285597	0.846	0.748	7.77E-160
MBP	7.23E-116	0.522700625	0.66	0.542	1.94E-111
IQGAP1	5.88E-112	0.520164551	0.517	0.366	1.58E-107
JUN	4.57E-40	0.518265057	0.72	0.68	1.23E-35
IQGAP2	1.96E-102	0.518084725	0.475	0.331	5.27E-98
DYNLL1	7.24E-97	0.515666667	0.63	0.513	1.94E-92
TGFB1	2.91E-110	0.513679977	0.518	0.377	7.81E-106
ATP2B4	2.16E-129	0.513040913	0.329	0.173	5.80E-125
HLA-A	0	0.511592092	0.999	0.993	0
C5orf56	2.31E-118	0.509715964	0.387	0.228	6.19E-114
CD3G	1.07E-188	0.503944035	0.944	0.899	2.88E-184

SSBP4	1.68E-103	0.501712759	0.455	0.312	4.52E-99
GUK1	1.19E-147	0.500324394	0.733	0.607	3.19E-143
SERPINB6	4.45E-22	0.566872367	0.256	0.199	1.20E-17
SPN	8.32E-91	0.50015821	0.433	0.302	2.23E-86

Supplementary Table S6. Results of heterogeneity analysis

outcome	exposure	method	Q	Q_df	Q_pval
Amyotrophic lateral sclerosis || GCST90027164	SERPINB6	MR Egger	0.746266845807175	2	0.688573355258413
Amyotrophic lateral sclerosis || GCST90027164	SERPINB6	Inverse variance weighted	1.09784650298761	3	0.777593971663138
Amyotrophic lateral sclerosis || GCST90027164	SMAD7	Inverse variance weighted	0.825815342174835	1	0.363485364266931
Amyotrophic lateral sclerosis || GCST90027164	TPST2	Inverse variance weighted	0.00544506220760166	1	0.941176955207092
Amyotrophic lateral sclerosis || GCST90027164	DIP2A	MR Egger	0.840993143422345	2	0.656720629946454
Amyotrophic lateral sclerosis || GCST90027164	DIP2A	Inverse variance weighted	0.982303489362248	3	0.805533881655725

Supplementary Table S7. Results of horizontal pleiotropy test

outcome	exposure	egger_intercept	se	pval
Amyotrophic lateral sclerosis || GCST90027164	SERPINB6	0.00634000955114166	0.0106924700628068	0.613337529122061
Amyotrophic lateral sclerosis || GCST90027164	SMAD7	NA	NA	NA
Amyotrophic lateral sclerosis || GCST90027164	TPST2	NA	NA	NA
Amyotrophic lateral sclerosis || GCST90027164	DIP2A	0.00576396729835104	0.0153332610064715	0.743110015744927

Supplementary Table S8. Results of Steiger filtering

Genes	PPH0	PPH1	PPH2	PPH3	PPH4
S100A6	1.88E-21	7.12E-01	4.06E-22	1.54E-01	1.34E-01
SERPINB6	1.37E-302	8.20E-01	5.86E-304	3.49E-02	1.45E-01
SMAD7	5.76E-15	7.01E-01	1.74E-16	2.09E-01	2.78E-01
TPST2	4.91E-78	6.76E-01	2.46E-79	3.36E-02	2.91E-01
DIP2A	6.62E-303	5.28E-01	9.25E-304	7.35E-02	3.99E-01

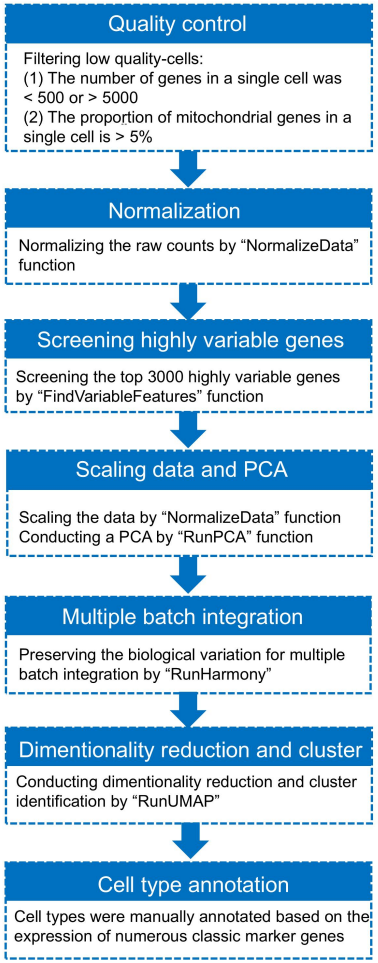
Supplementary Table S9. Results of Bayesian co-localization analysis

exposure	outcome	snp_r2. exposure	snp_r2. outcome	correct_causal_direction	steiger_pval
S100A6	Amyotrophic lateral sclerosis || GCST90027164	3.68E-03	3.87E-05	TRUE	3.50E-10
SERPINB6	Amyotrophic lateral sclerosis || GCST90027164	9.93E-02	1.38E-04	TRUE	3.93E-287
SMAD7	Amyotrophic lateral sclerosis || GCST90027164	3.54E-03	6.02E-05	TRUE	2.49E-09
TPST2	Amyotrophic lateral sclerosis || GCST90027164	1.34E-02	5.90E-05	TRUE	7.58E-36
DIP2A	Amyotrophic lateral sclerosis || GCST90027164	1.06E-01	7.22E-05	TRUE	0.00E+00

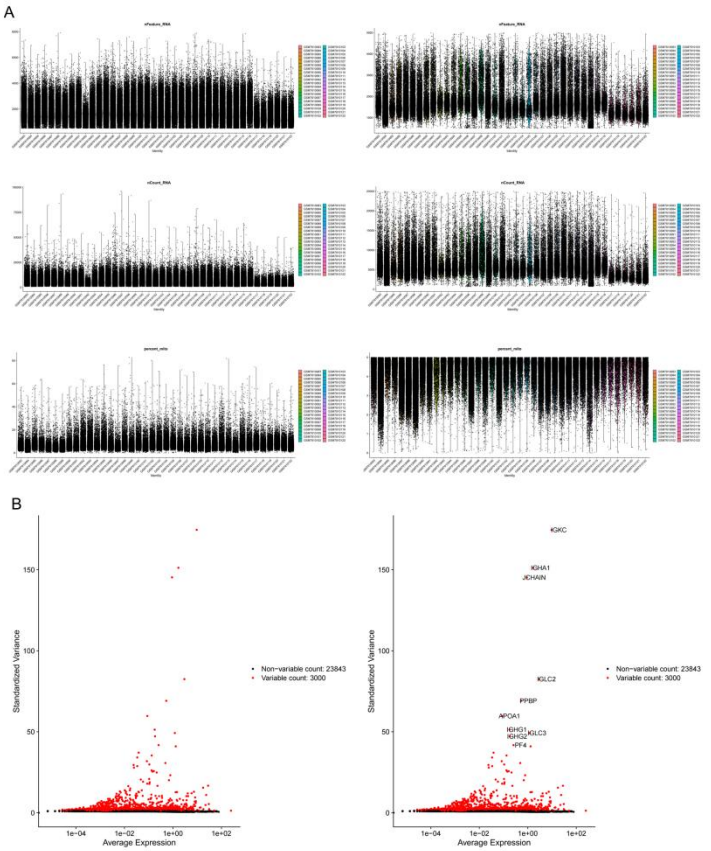
Note. The posterior probability of hypothesis 4 (PPH4) > 0.8 was considered as that the two association signals are consistent with a shared causal variant. H0: no association with either trait; H1: association with ALS GWAS, not with gene; H2: association with gene, not with ALS GWAS; H3: association with gene and ALS GWAS, two independent SNPs; H4: association with gene and ALS GWAS), one shared SNP.

Supplementary Table S10. Result of the phenotype scanning

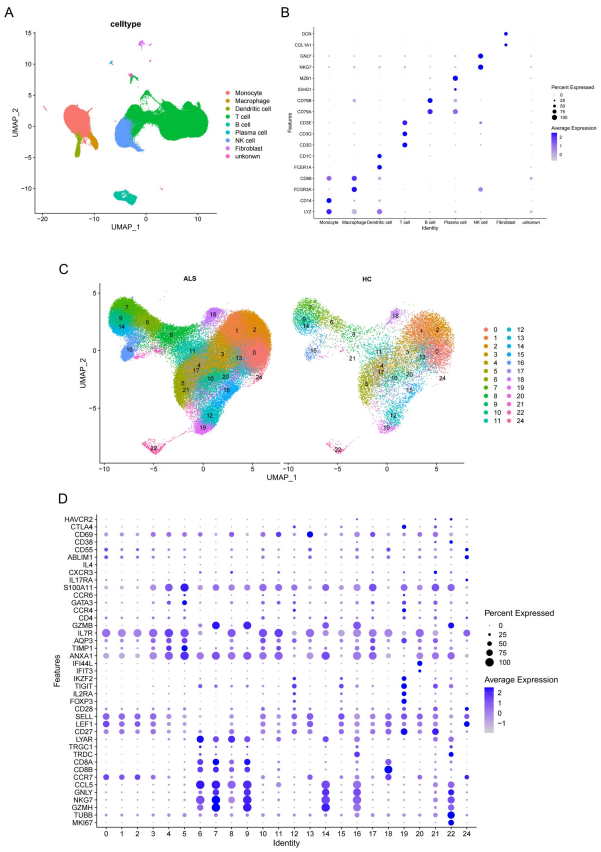
gene	snp	trait	chr	pos	*P*	OR or BETA	95% CI (TEXT)	MAPPED_ GENE	Sample	STUDY ACCESSION
SERPINB6	rs318493	Platelet count	6	2904557	6E-11	0.0142	SERPINB6	34594039	European	GCST90018969
SERPINB6	rs318493	Lymphocyte count	6	2904557	0.00000002	0.0135	SERPINB6	34594039	European	GCST90018962
DIP2A	rs2096507	Neutrophil count	21	46522003	6E-13	0.013913	DIP2A	32888493	European	GCST90002351
DIP2A	rs2096507	White blood cell count	21	46522003	2E-12	0.02647097	DIP2A	30595370	European	GCST007070



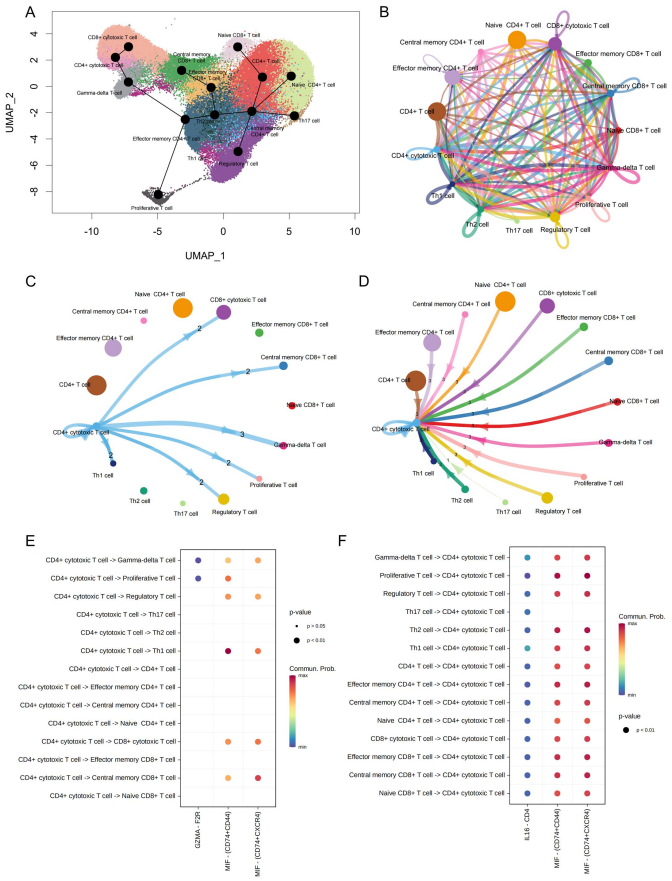
Supplementary Figure S1. Flow chart of processing scRNA-seq data.



Supplementary Figure S2. Quality control of scRNA-seq data of ALS patients and HC. (A) Bar chart shows the number of genes per cell (nFeature_RNA), the number of unique molecular identifers per cell (nCount_RNA), and the percentage of mitochondrial genes per cell (percent_mito) in each ALS patients and HC. (B) Dot diagram shows the average expression of top 3,000 highly variable genes in red (Top 10 highly variable genes are labeled independently).



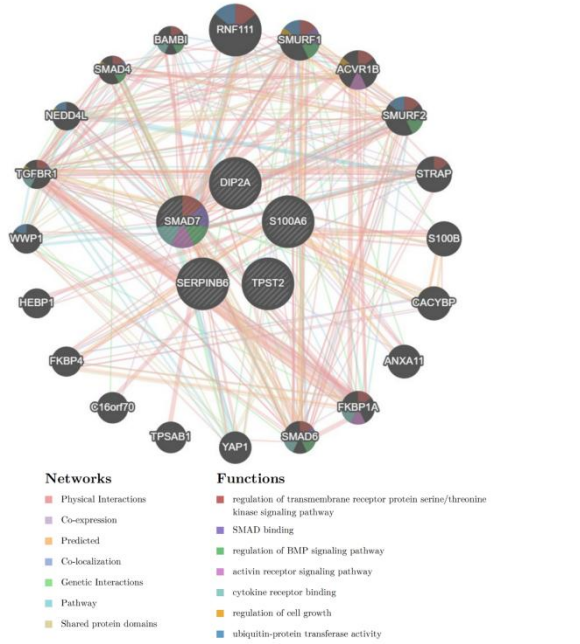
Supplementary Figure S3. Dimensionality reduction clustering analysis and cell annotation of marker genes in each cell cluster. (A) UMAP plot shows annotation of 9 different cell types. (B) Bubble plot shows the expression level of marker genes in each cell types. (C) UMAP plot shows identification of 24 T cell clusters. (D) Bubble plot shows the expression level of marker genes in each T cell cluster.



Supplementary Figure S4. Pseudotime analysis and cell-cell communication analysis. (A) Pseudotime analysis of T cells subtypes using Slingshot algorithms. (B) Circle graph of cell-cell communication analysis of T cell subtypes (Number of interactions). (C–D) Line graph of all interactions between CD4+ cytotoxic T cells and other T cell subtypes. (E–F) Dot plot of ligand-receptor interactions between CD4+ cytotoxic T cells and other T cell subtypes.

Supplementary Figure S5. Functional analysis of metabolic pathways of all T cell subtypes in patients with ALS.

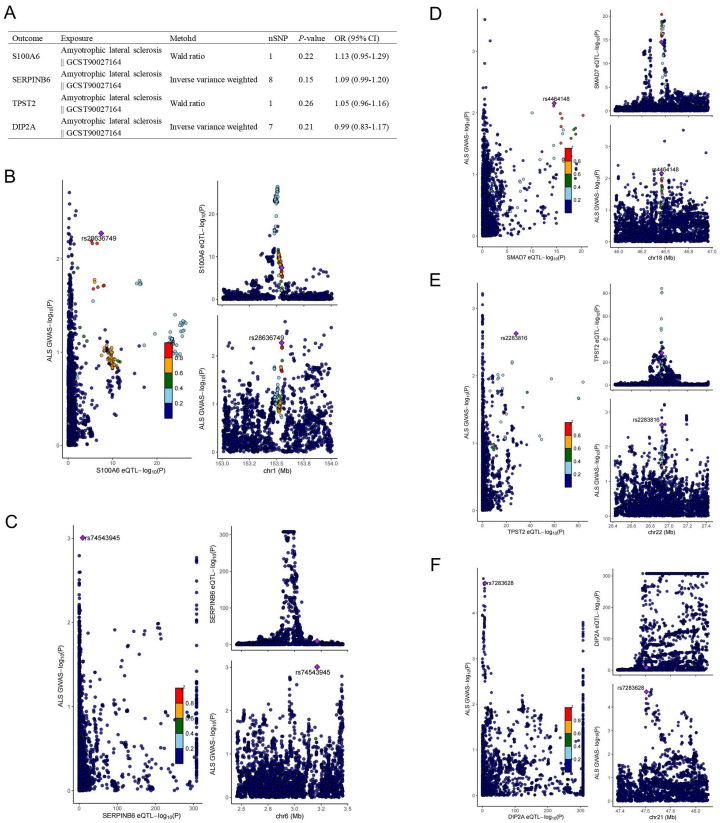
A



B

Term	*P*-value	Adjusted *P*-value	Genes
glibenclamide	9.30E-5	1.15E-2	TPST2;S100A6;SERPINB6;SMAD7
ampyrone	5.59E-4	2.50E-2	TPST2;S100A6;SMAD7
tamoxifen	6.05E-4	2.50E-2	TPST2;SERPINB6;SMAD7
chlorzoxazone	2.40E-3	6.00E-2	TPST2;S100A6;SMAD7
quercetin	2.71E-3	6.00E-2	TPST2;S100A6;DIP2A;SMAD7
MIGLITOL	2.99E-3	6.00E-2	S100A6
lobeline	3.82E-3	6.00E-2	TPST2;S100A6;SMAD7
Methaneseleninic acid	3.87E-3	6.00E-2	SERPINB6;SMAD7
ajmaline	5.84E-3	8.047E-2	S100A6;SMAD7
deltamethrin	6.98E-3	8.66E-2	S100A6

Supplementary Figure S6. Construction of PPI network and prediction of candidate drugs. (A) The PPI network of different interactions between proteins using GeneMANIA. (B) Top 10 drug candidates obtained from DSigDB.



Supplementary Figure S7. Bidirectional MR analysis and Bayesian colocalization analysis. (A) Bidirectional MR analysis of the expression of five causal genes. (B–F) Bayesian colocalization analysis of five causal genes containing S100A6 (B), SERPINB6 (C), SMAD7 (D), TPST2 (E), and DIP2A (F). Purple diamonds highlighted the SNP that had the minimal cumulative *P*-value across eQTL and ALS GWAS studies.