

Identification of Tumor Antigens and Immune Subtypes for the Development of mRNA Vaccines and Individualized Immunotherapy in Soft Tissue Sarcoma

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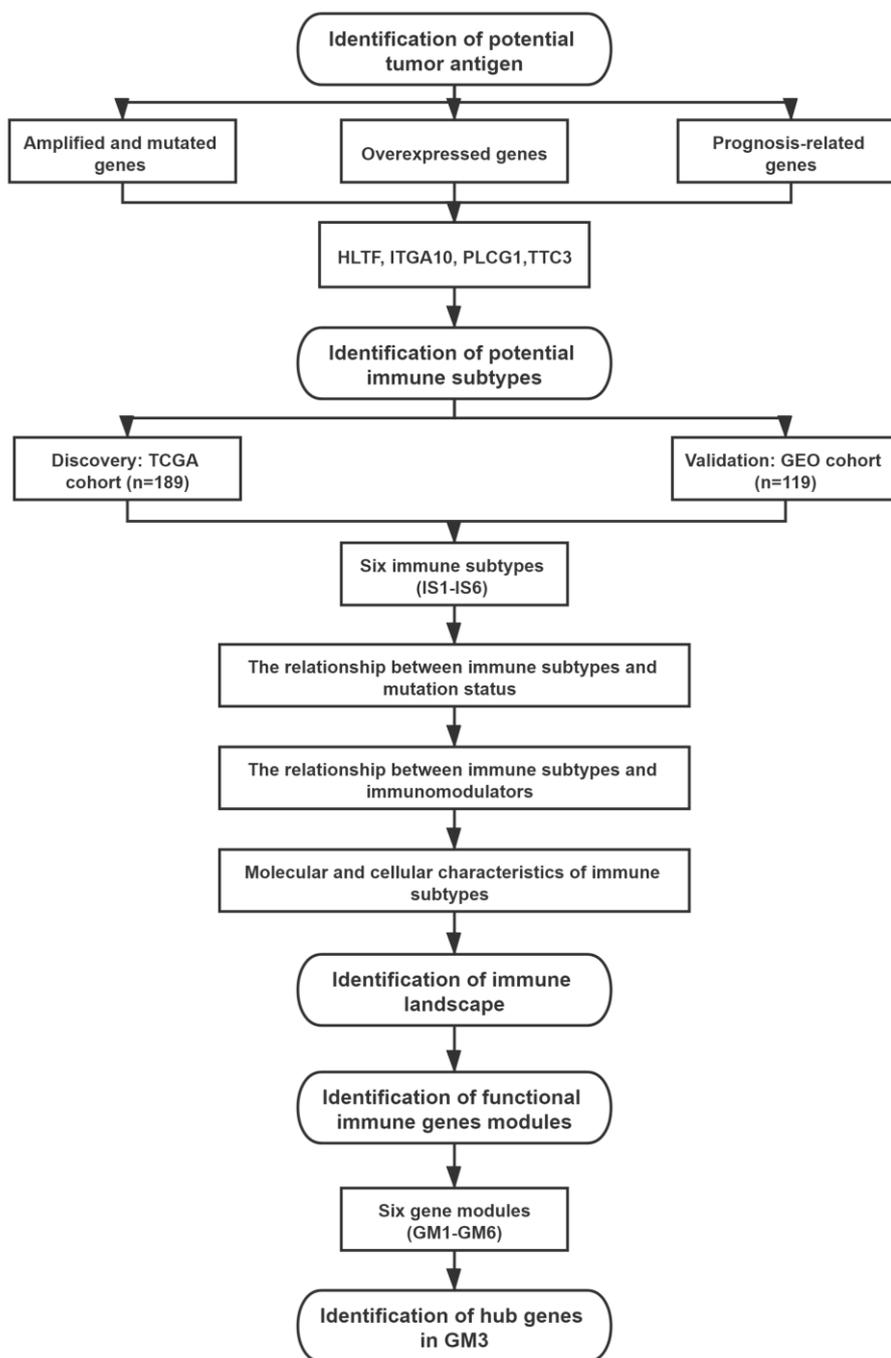
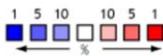


Figure S1. Experimental Setup.

Analysis Type by Cancer	Cancer vs. Normal																	
	ATP2A3	SLC19A1	TTC3	PLCG1	ITGA10	HLTF	MTRFR1	LIMS2	SIX4									
Bladder Cancer		2	2		2	2		4										
Brain and CNS Cancer		3	3			4			3									
Breast Cancer	4	4	6	2	1	7	8	2	4	19	17							
Cervical Cancer			2				4	2			1							
Colorectal Cancer		15	2	4	2		2	13	4	4								
Esophageal Cancer	5		3	1			1				3							
Gastric Cancer		2	3		1			2	1									
Head and Neck Cancer		2		2			5	3		1	3							
Kidney Cancer			2	5			2	1	1	1	1							
Leukemia	2	3	1	5	5	1	1	2	1	2	4							
Liver Cancer					1		4	1	1		1							
Lung Cancer				2			4	11	11		8	4						
Lymphoma	2	5	5	1	1	8	5	1	2	1	1	1						
Melanoma		1	3		2			2	1		2	1						
Myeloma																		
Other Cancer	1	1	2	2	5	2	2	1		5	6	3	3	2	2			
Ovarian Cancer								1				1	1	2				
Pancreatic Cancer					2			1		1								
Prostate Cancer			4	1	3		1		3	3	1		3	2				
Sarcoma	1	4	1	1	7		2		2	8			1	5				
Significant Unique Analyses	15	32	33	12	42	12	24	2	4	16	67	11	48	10	7	50	44	1
Total Unique Analyses	420		435		445		422		407		443		439		343		276	



Cell color is determined by the best gene rank percentile for the analyses within the cell.

Figure S2. Identification of potential overexpressed tumor antigens in STS. Four potential tumor antigens overexpressed in STS compared to normal tissue in the OncoPrint database. The red indicates that the gene expression is up-regulated and the blue indicates that the gene expression is down-regulated. The number indicates the number of analyses.

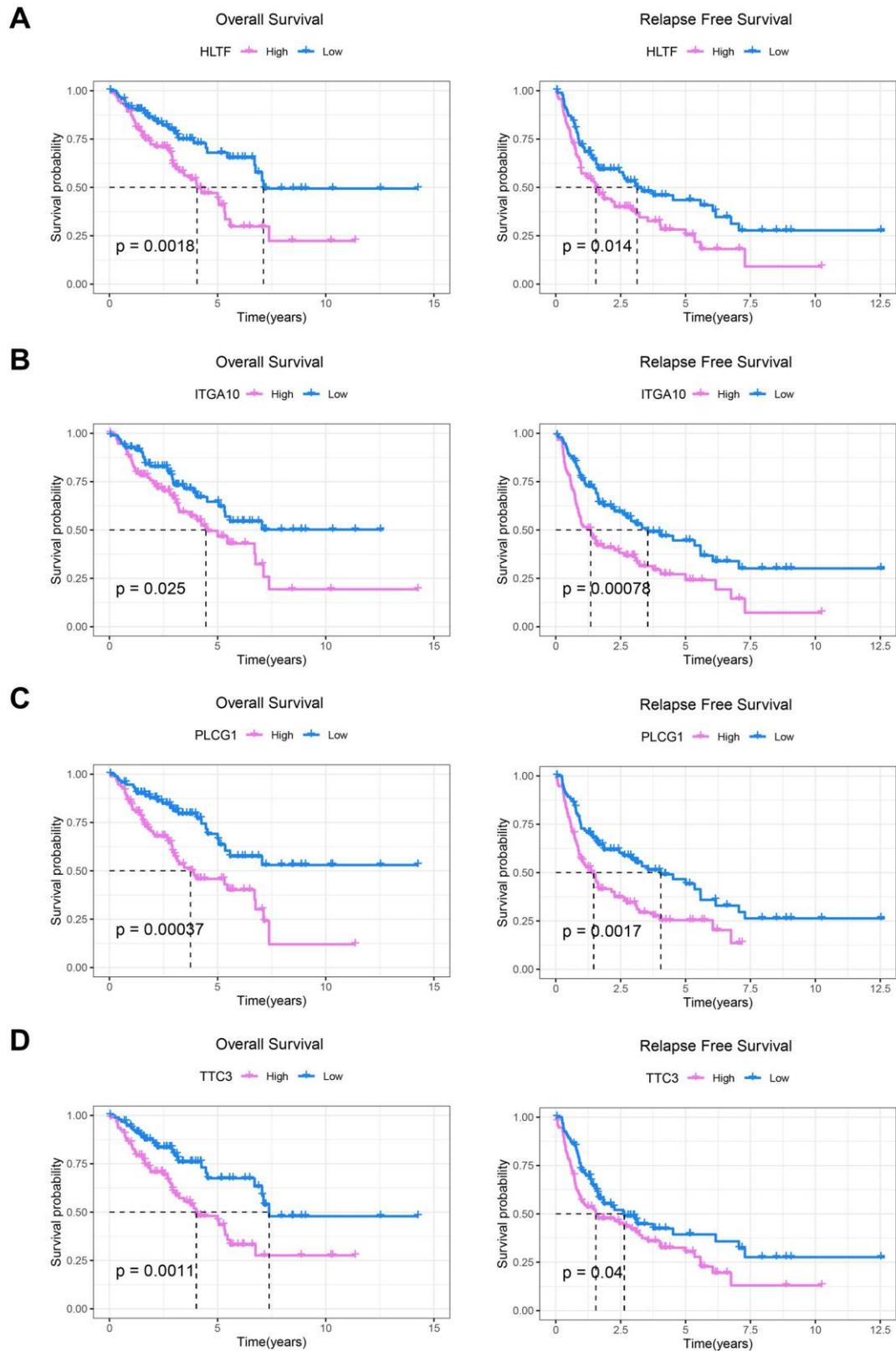


Figure S3. Identification of tumor antigens associated with STS prognosis. A-D Kaplan-Meier curves comparing OS and RFS in STS for groups with different expression of HLTF (A), ITGA10 (B), PLCG1 (C) and TTC3 (D).

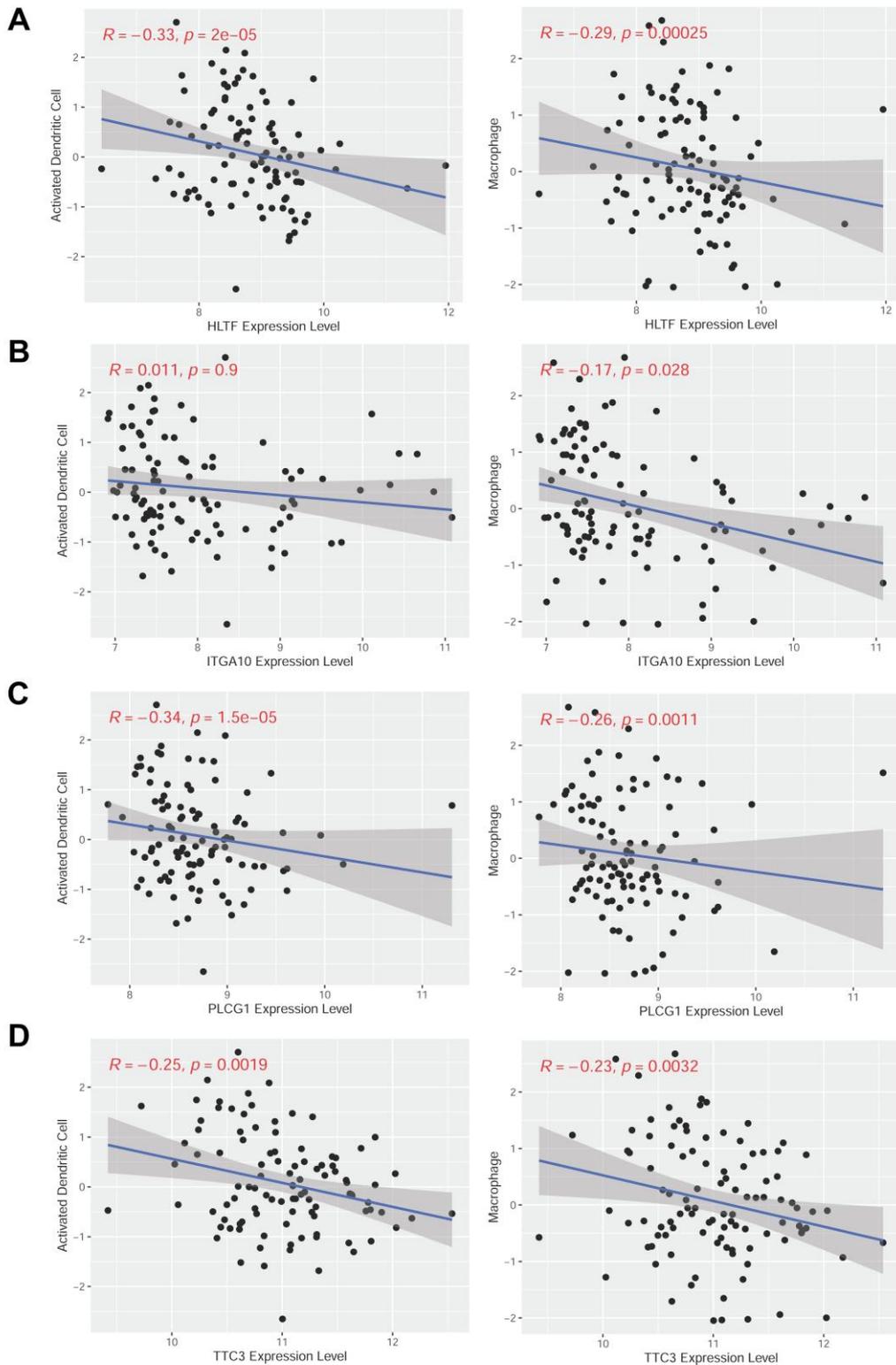


Figure S4. Identification of tumor antigens associated with infiltration of antigen-presenting cells in the GEO cohort. A-D Correlation between expression of HLTF (A), ITGA10 (B), PLCG1 (C) and TTC3 (D) and infiltration levels of dendritic cells and macrophages in STS.

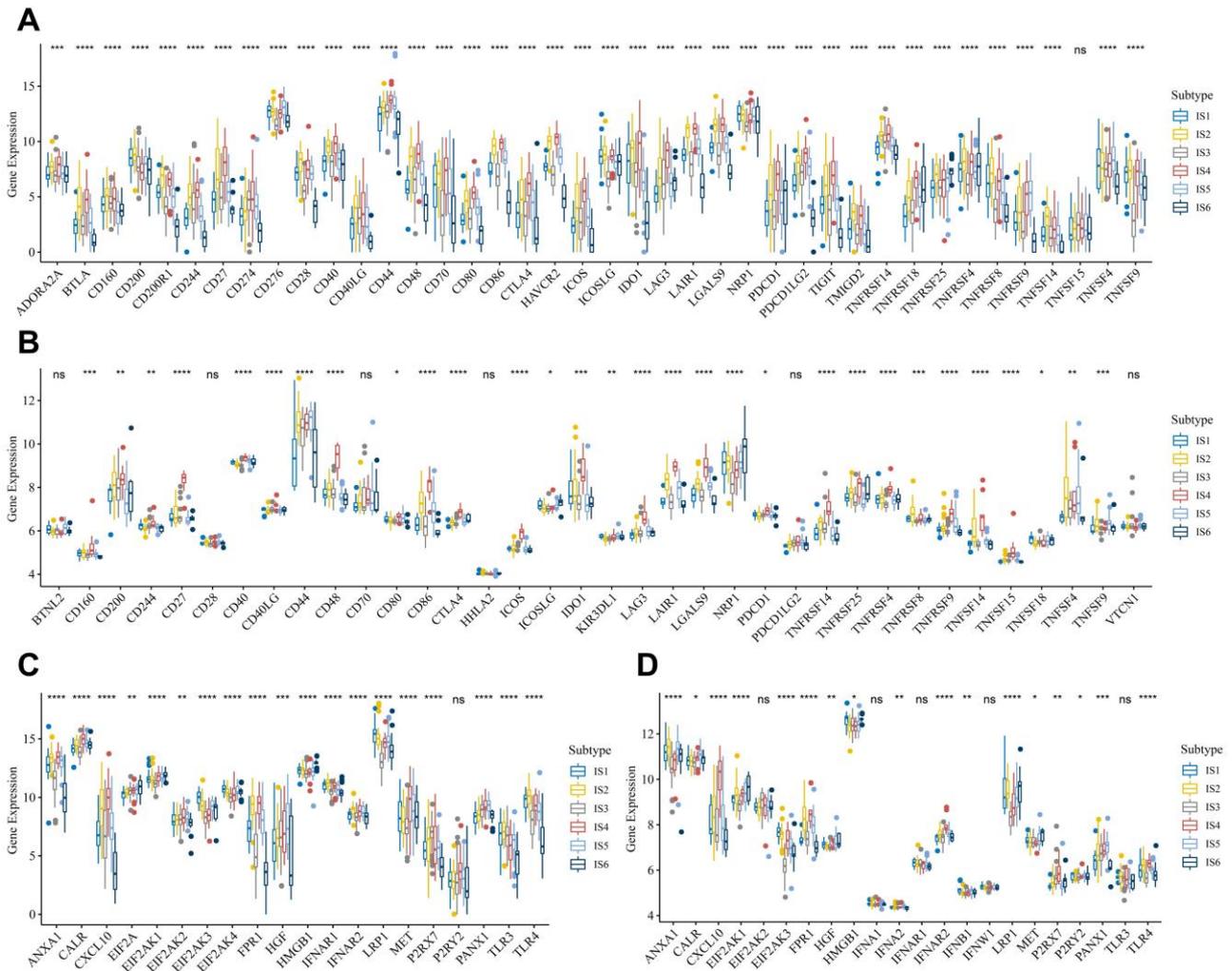


Figure S5. Association between immune subtypes and immunomodulators in STS. **A,B** Differences in expression levels of ICP-related genes among STS immune subtypes in TCGA (**A**) and GEO (**B**) cohorts. **C,D** Differences in expression levels of ICD-related genes among STS immune subtypes in TCGA (**C**) and GEO (**D**) cohorts. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

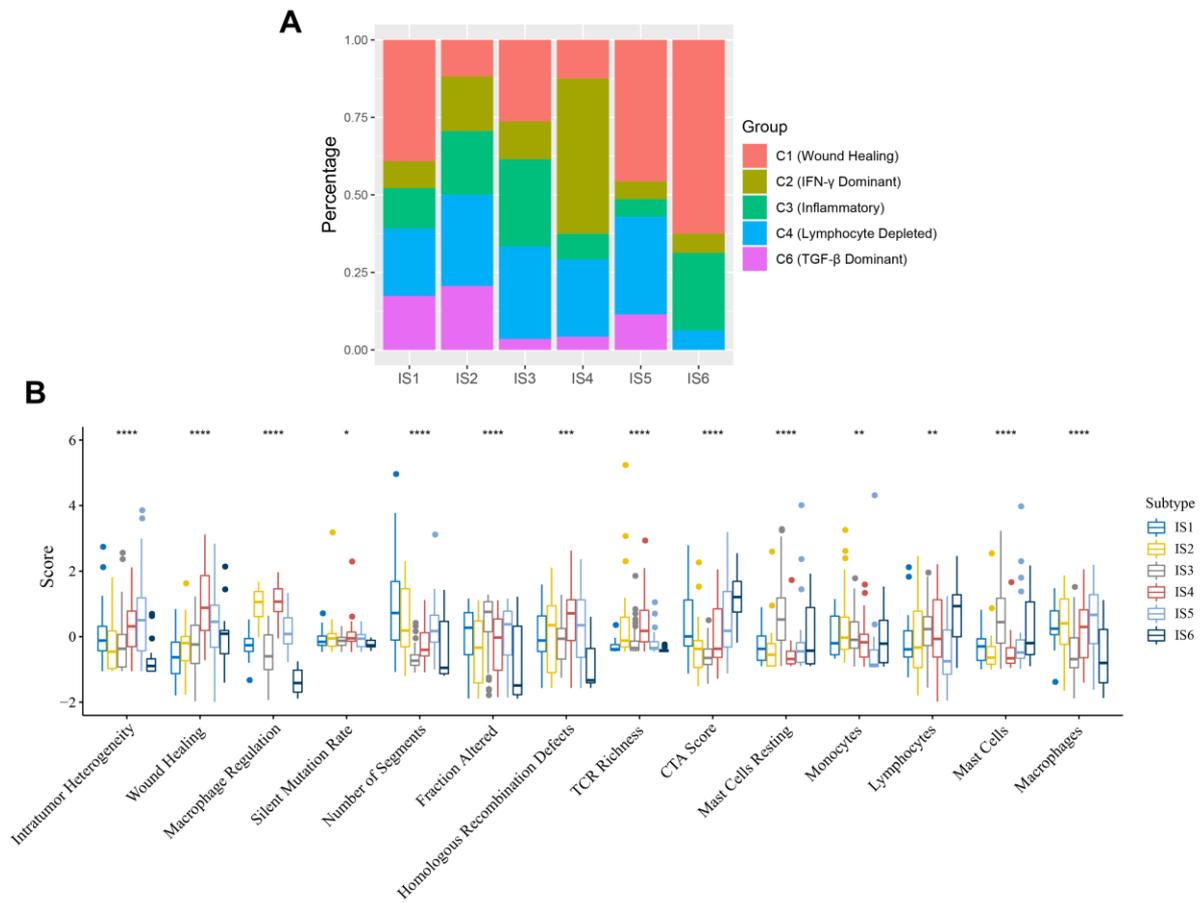


Figure S6. Molecular characteristic of immune subtypes in STS. **A** The distribution of six pan-cancer immune subtypes, defined by Thorsson et al., in the six STS immune subtypes. **B** Immune-related molecular signatures with significant differences among STS immune subtypes. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

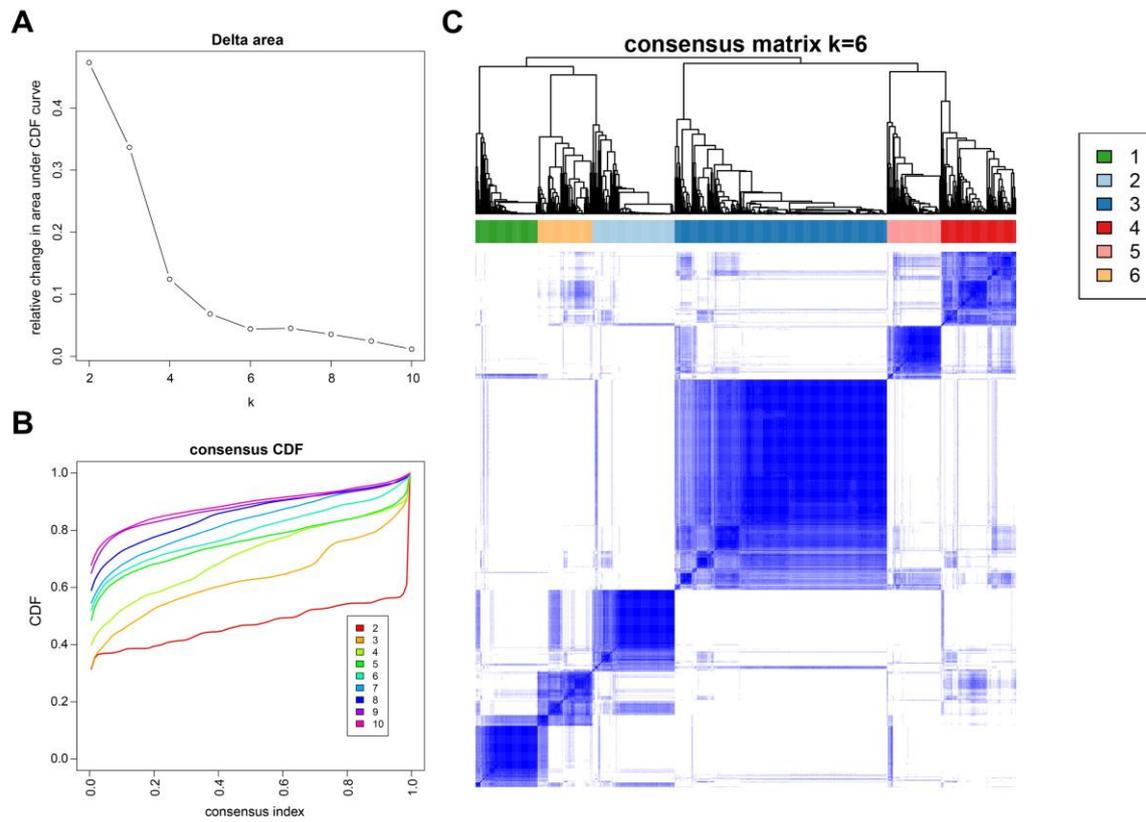


Figure S7. Identification of potential immune-related gene modules in STS. A-C Delta area curve (A), cumulative distribution function curve (B) and consensus heatmap (C) of immune-related gene expression profile in TCGA cohort.

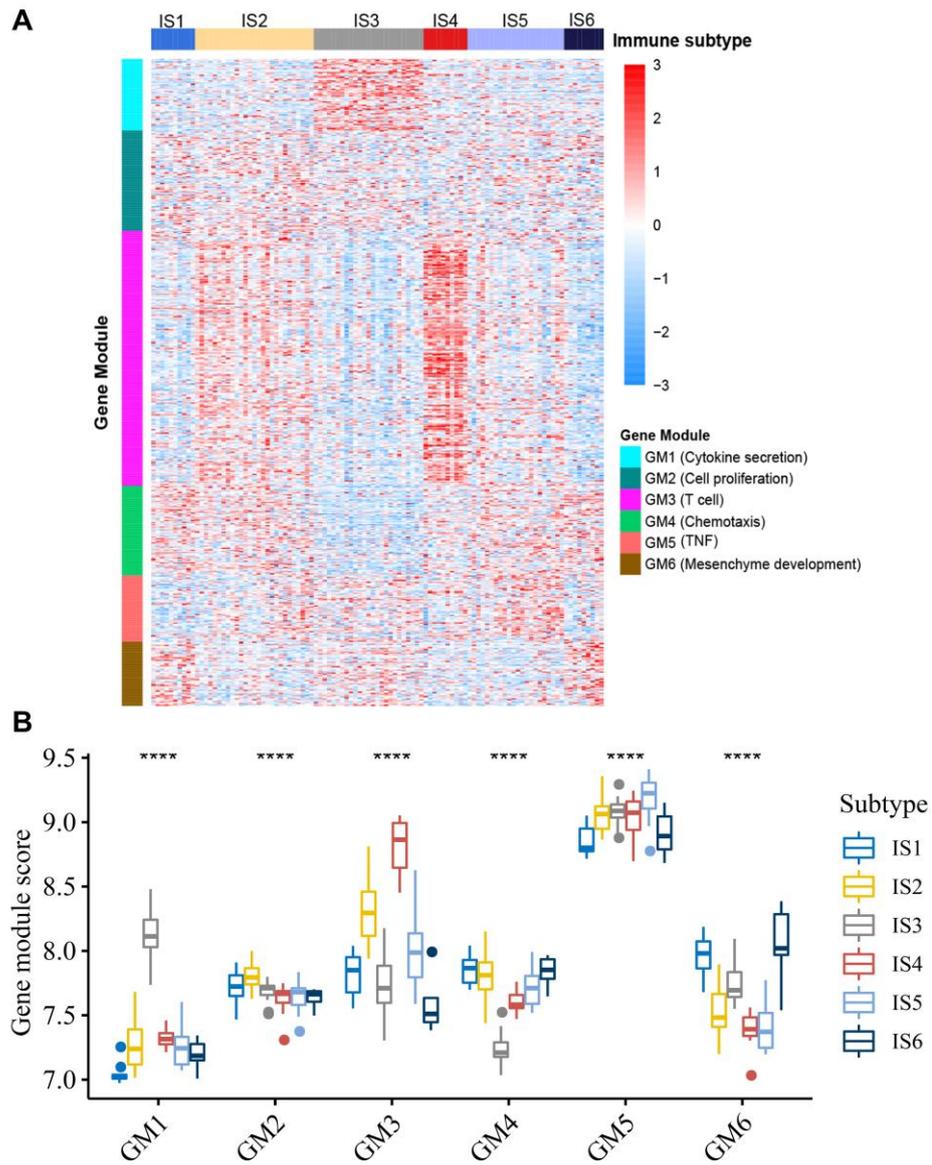


Figure S8. Functional immune genes modules in the GEO cohort. **A** Heatmap of ISs and GMs in the GEO cohort. Genes are ordered based on the GMs, and patients are arranged based on their ISs. **b** Box plots of the GM expression patterns of six immune subtypes in the GEO cohort. **** $p < 0.0001$.

Table S1. Characteristics of STS samples in TCGA and GEO cohorts.

Characteristics	TCGA (n=189)	GEO (n=103)
Histological type		
DDLPS	49 (25.93 %)	46 (44.66 %)
LMS	68 (35.98 %)	26 (25.24 %)
UPS	41 (21.69 %)	NA
MFS	17 (8.99 %)	31 (30.10%)
SS	10 (5.29 %)	NA
MPNST	4 (2.12 %)	NA
Pathologic tumor size		
≤ 10.5 cm	95 (50.26 %)	NA
> 10.5 cm	94 (49.74 %)	NA
Radiotherapy		
Yes	54 (28.57 %)	NA
No	135 (71.43 %)	NA
Pharmaceutical therapy		
Yes	45 (23.81%)	NA
No	144 (76.19%)	NA
FNCLCC grade		
1	10(5.29%)	NA
2	105 (55.56%)	NA
3	74 (39.15%)	NA
Vital status		
Alive	117 (61.90%)	NA
Dead	72 (38.10%)	NA
Relapse status		
Relapse	114 (60.32%)	NA
Non- Relapse	75 (39.68%)	NA

DDLPS: dedifferentiated liposarcoma; LMS: leiomyosarcoma; UPS: undifferentiated pleomorphic sarcoma; MFS: myxofibrosarcoma; SS: synovial sarcoma; MPNST: malignant peripheral nerve sheath tumor; NA: no data or data unavailable.

Table S2. IGP was estimated for each immune subtype in the validation cohort.

Immune subtype	IS1	IS2	IS3	IS4	IS5	IS6
IGP value	0.857	0.625	1.000	0.640	0.588	0.667

Table S3. Functional enrichment analysis of gene modules.

Gene module	Annotation	Top 5 enriched biological processes
GM1	Cytokine secretion	GO:0009755~hormone-mediated signaling pathway
		GO:0007162~negative regulation of cell adhesion
		GO:0030522~intracellular receptor signaling pathway
		GO:0043401~steroid hormone mediated signaling pathway
		GO:0050707 ~regulation of cytokine secretion
GM2	Cell proliferation	GO:0050673~epithelial cell proliferation
		GO:0033002~muscle cell proliferation
		GO:0048638~regulation of developmental growth
		GO:0048015~phosphatidylinositol-mediated signaling
		GO:0048017~inositol lipid-mediated signaling
GM3	T cell	GO:0050900~leukocyte migration
		GO:0042110~T cell activation
		GO:0060326~cell chemotaxis
		GO:0030595~leukocyte chemotaxis
		GO:0034341~response to interferon-gamma
GM4	Chemotaxis	GO:0050920~regulation of chemotaxis
		GO:0061138~morphogenesis of a branching epithelium
		GO:0001763~urogenital system development
		GO:0001763~morphogenesis of a branching structure
		GO:0048608~reproductive structure development
GM5	TNF	GO:0033209~tumor necrosis factor-mediated signaling pathway
		GO:0002479~antigen processing and presentation of exogenous peptide antigen via MHC class I, TAP-dependent
		GO:0042590~antigen processing and presentation of exogenous peptide antigen via MHC class I
		GO:0002833~positive regulation of response to biotic stimulus
		GO:0002223~stimulatory C-type lectin receptor signaling pathway

GM6	Mesenchyme development	GO:0060485~mesenchyme development GO:0048762~mesenchymal cell differentiation GO:0050919~negative chemotaxis GO:0048843~semaphorin-plexin signaling pathway GO:0048863~stem cell differentiation
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Table S4. Univariate analysis of the prognostic value of gene modules in terms of OS/RFS in STS.

Gene Module (GM)	Overall survival			Relapse-free survival		
	HR	95%CI	P-value	HR	95%CI	P-value
GM1 score	0.80	0.63~0.99	0.049	0.92	0.77~1.10	0.368
GM2 score	0.89	0.54~1.46	0.646	0.93	0.62~1.40	0.716
GM3 score	0.79	0.65~0.97	0.027	0.859	0.73~1.01	0.078
GM4 score	1.23	0.91~1.66	0.175	1.01	0.79~1.27	0.966
GM5 score	0.89	0.53~1.53	0.690	0.993	0.65~1.52	0.972
GM6 score	1.22	0.91~1.65	0.184	1.05	0.83~1.32	0.689

Table S5. Multivariate analysis of the prognostic value of gene modules in terms of OS/RFS in STS.

Characteristics	Overall survival			Relapse-free survival		
	HR	95%CI	P-value	HR	95%CI	P-value
Age (Continuous)	1.03	1.01~1.05	0.007	1.01	0.99~1.00	0.390
Gender (Male vs. Female)	0.93	0.55~1.59	0.794	1.24	0.83~1.85	0.301
Pathological tumor size (cm)	1.05	1.04~1.08	<0.001	1.04	1.02~1.14	<0.001
Radiotherapy (Yes vs. No)	0.70	0.36~1.34	0.282	1.04	0.67~1.72	0.894
Pharmaceutical therapy (Yes vs. No)	1.73	0.95~3.13	0.072	1.92	1.21~3.01	0.005
FNCLCC grade (3 vs. 1/2)	1.43	0.79~2.61	0.240	1.592	1.56~2.50	0.064
GM1 score	0.79	0.49~1.25	0.306	0.96	0.67~1.40	0.802
GM2 score	1.60	0.56~4.55	0.379	1.48	0.68~3.21	0.319
GM3 score	0.60	0.41~0.87	0.008	0.70	0.55~1.00	0.053
GM4 score	1.17	0.61~2.22	0.641	0.99	0.59~1.71	0.970
GM5 score	1.70	0.53~5.39	0.369	1.71	0.69~4.32	0.249
GM6 score	0.94	0.57~1.56	0.822	0.93	0.62~1.44	0.727