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Supplemental information

Dynamic regulatory networks of T cell trajectory dissect transcriptional control of T cell state transition

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Table S1: datasets used for constructing dynamic networks.

dataset	Cancer type	#CD8+ T cells	platform	publication
GSE127471	NSCLC	300	10X	Newman AM, et al. Nat Biotechnol 2019
GSE120575	SKCM	6695	Smart-seq2	Sade-Feldman M, et al. Cell 2018
GSE140228	LIHC	16532	10X	Zhang Q, et al. Cell 2019
GSE118056	MCC	756	10X	Paulson KG, et al. Nat Commun 2018
EMTAB6149	NSCLC	12040	10X	Lambrechts D, et al. Nat Med 2018
GSE103322	HNSCC	434	Smart-seq2	Puram SV, et al. Cell 2018
GSE123813	SCC	15560	10X	Yost KE, et al. Nat Med 2019
GSE110686	BRCA	1525	10X	Savas P, et al. Nat Med 2018

Table S2. Curated TFs related to T cell dysfunction.

Table S3. TFs with significantly different regulatory activities in each window.

window	direction	Number	TFs
W1	down	23	ARID4B,ATF6B,ATRX,BACH2,BCL11B,ETS1,FOXP1,HIVEP2,IKZF1,IRF9,KLF2,KLF3,LEF1,MYC,SATB1,TCF7,TRERF1,ZGPAT,ZNF292,ZNF48,ZNF652,ZNF83,ZNF91
W1	up	24	BATF,CREM,DNAJC1,EOMES,FOS,FOSB,HOPX,ID2,IKZF3,IRF1,IRF7,JUNB,KLF6,LYAR,MYBL1,NFATC2,NFKBIA,PHTF2,PRDM1,RBPJ,RUNX3,SP140,ZBTB38,ZNF683
W2	down	19	HOPX,ID2,KLF2,LEF1,LRRFIP1,LYAR,MXD3,MYBL1,MYC,NFKBIA,PRDM1,RUNX2,SATB1,STAT1,TCF7,TRERF1,TSC22D3,ZGPAT,ZNF683
W2	up	13	CREM,EOMES,FOS,IKZF3,KDM2A,NCOR1,NFATC2,RBPJ,TOX,TRPS1,ZBTB38,ZEB2,ZNF48
W3	down	4	EOMES,FOS,TCF7,TSC22D3
W3	up	52	ARID4B,ATRX,BATF,BCL11B,BHLHE40,CREB3L2,CREM,DNAJC1,ETS1,FOXP1,HIF1A,HIVEP3,HMGA1,HOPX,ID2,IKZF1,IKZF3,IRF1,IRF7,IRF9,KAT7,KDM5B,KLF13,KLF6,KMT2A,LRRFIP1,LYAR,MXD3,NCOR1,NFATC2,NFKBIA,NR3C1,PHTF2,PRDM1,RBPJ,RUNX2,RUNX3,SP100,SP140,STAT1,STAT3,STAT4,TRERF1,ZBTB20,ZBTB38,ZEB2,ZGPAT,ZNF276,ZNF292,ZNF48,ZNF652,ZNF683
W4	down	27	ATRX,BCL11B,EOMES,ETS1,FOSB,FOXP1,HOPX,IKZF1,IRF1,JUNB,KLF12,KLF6,KMT2A,LRRFIP1,LYAR,NCOR1,NFATC2,NFKBIA,RUNX3,SP100,STAT4,TRERF1,TSC22D3,ZBTB38,ZGPAT,ZNF276,ZNF91
W4	up	25	ARID4B,BATF,BHLHE40,CREB3L2,CREM,DNAJC1,ETV1,HIF1A,ID2,IKZF3,IRF7,IRF9,MAF,MSC,MXD3,NR3C1,PHTF2,RBPJ,RUNX2,SP140,STAT1,STAT3,TOX,ZNF292,ZNF48

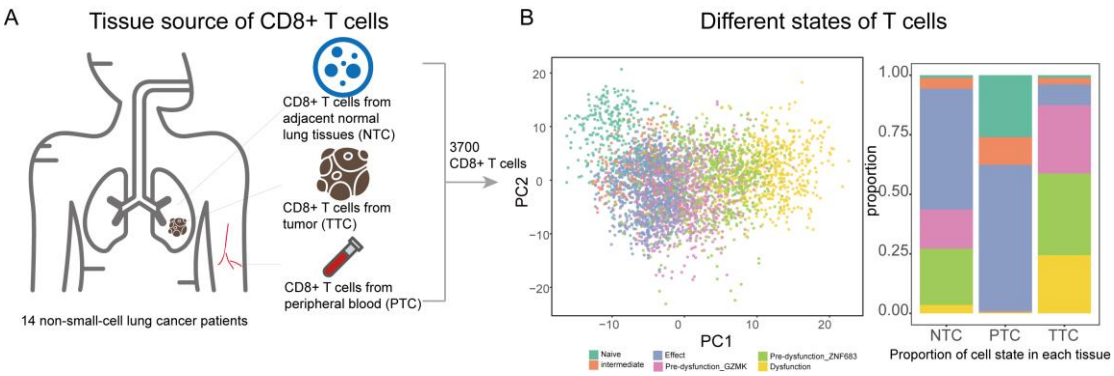

Figure S1. Data collection and statistc information of the NSCLC scRNA-seq.
(A) The detailed information of the NSCLC dataset, including number of patients, tissue sampling. (B) (Left panel) PCA plot for T cells, which colored by their state. (Right panel) The state composition of cells with different origin.

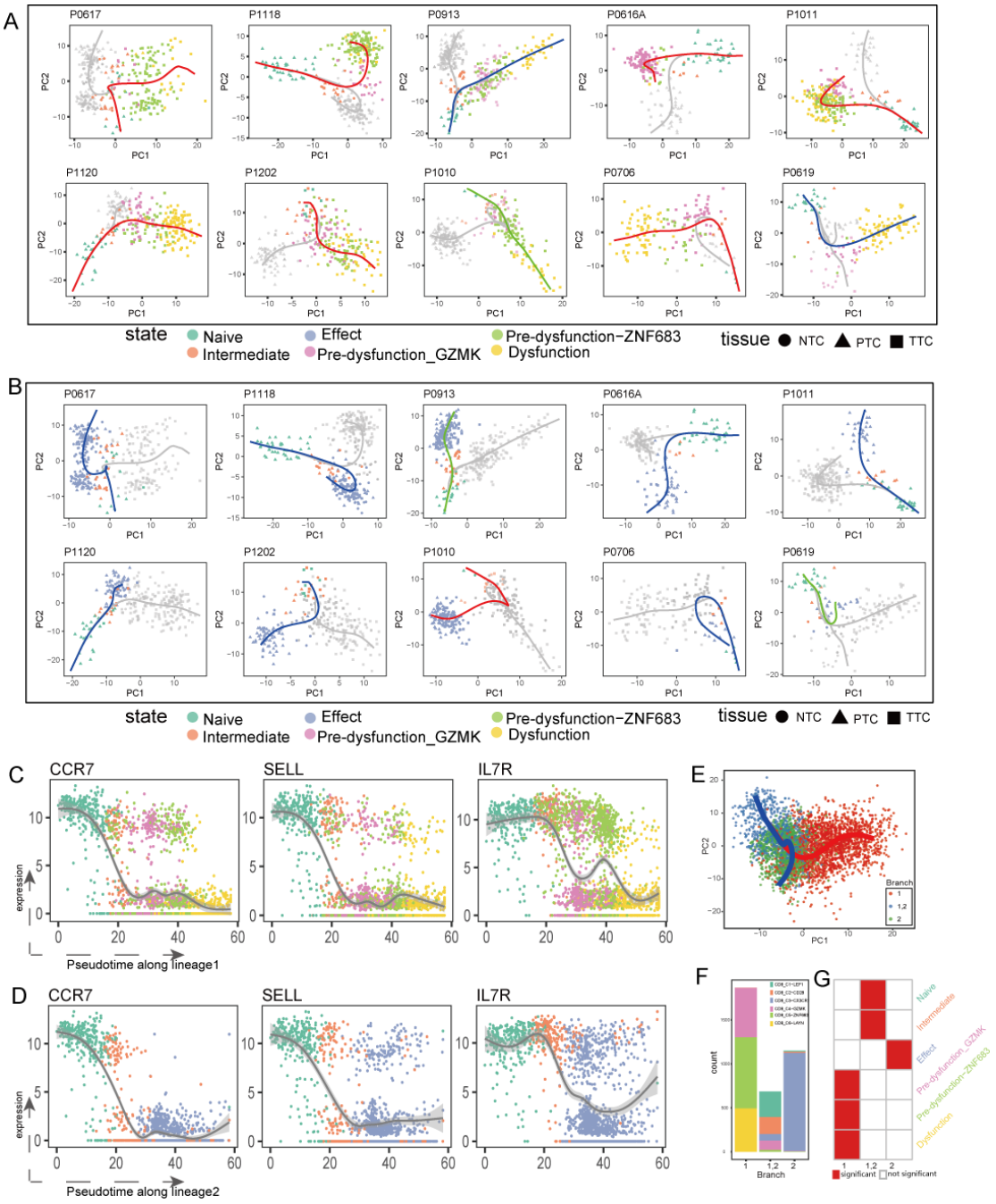

Figure S2. Expression distribution of naive markers along pseudo-time.
(A-B) Pseudo-time analysis of CD8+ T cells for individual patients. The colored lineage was dysfunction lineage (A) and effect lineage (B). Point shape represented by tissues origin. (C-D) Expression distribution of naive markers along pseudo-time on the dysfunction lineage (C) or on the effect lineage (D). (E) T cell trajectory for all cells, colored by branch. (F) Cell state composition on the three branches. (G) Enrichment of cell states in each branch. Boxes filled with red denoted significant results with hypergeometric test p-value < 0.05.

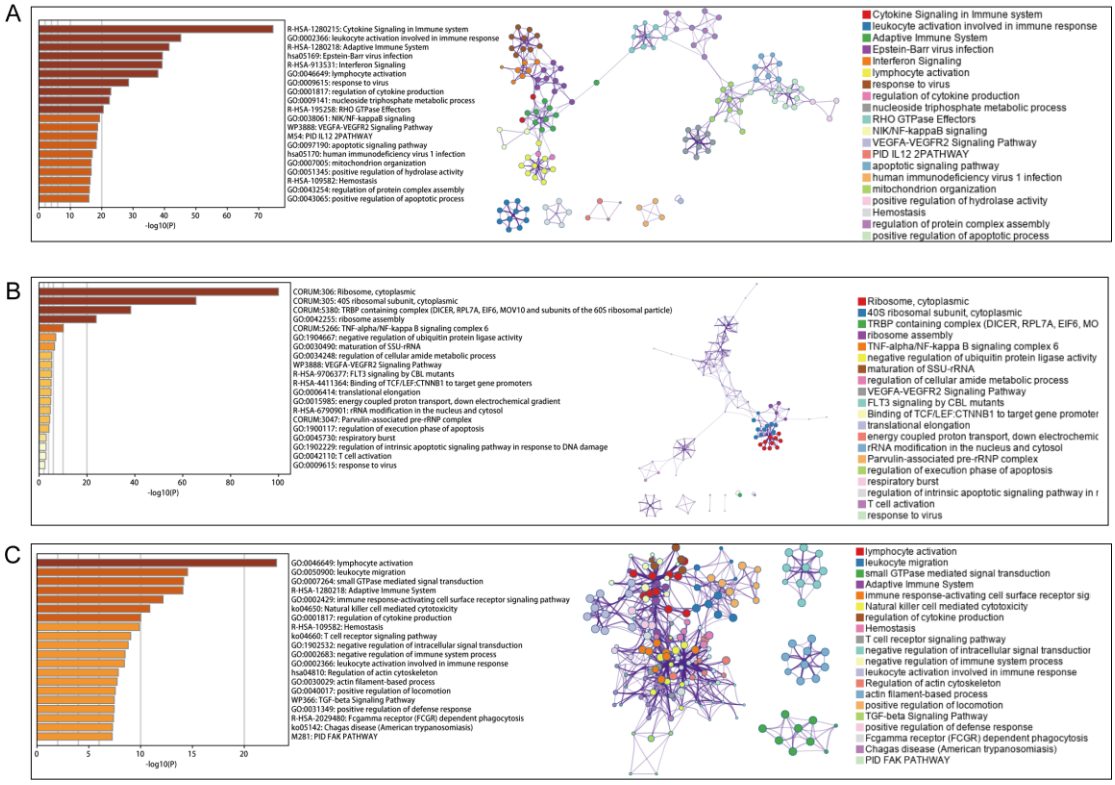

Figure S3. Enriched functional terms for the three dynamic gene clusters. (A-C) Visualization of enrichment results by Metascape for cluster 1, cluster 2 and cluster 3, respectively.

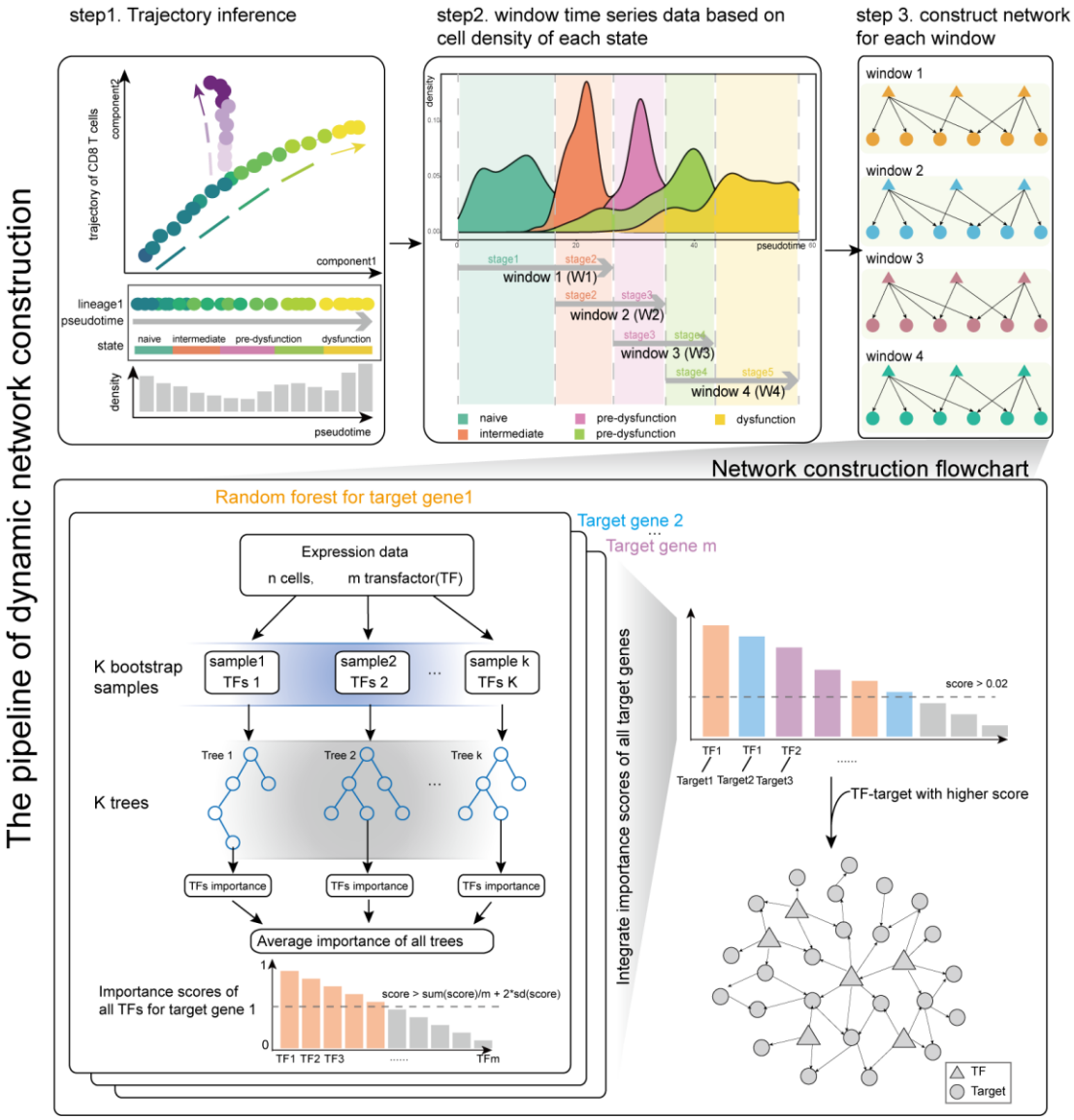

Figure S4. The pipeline of dynamic network construction.

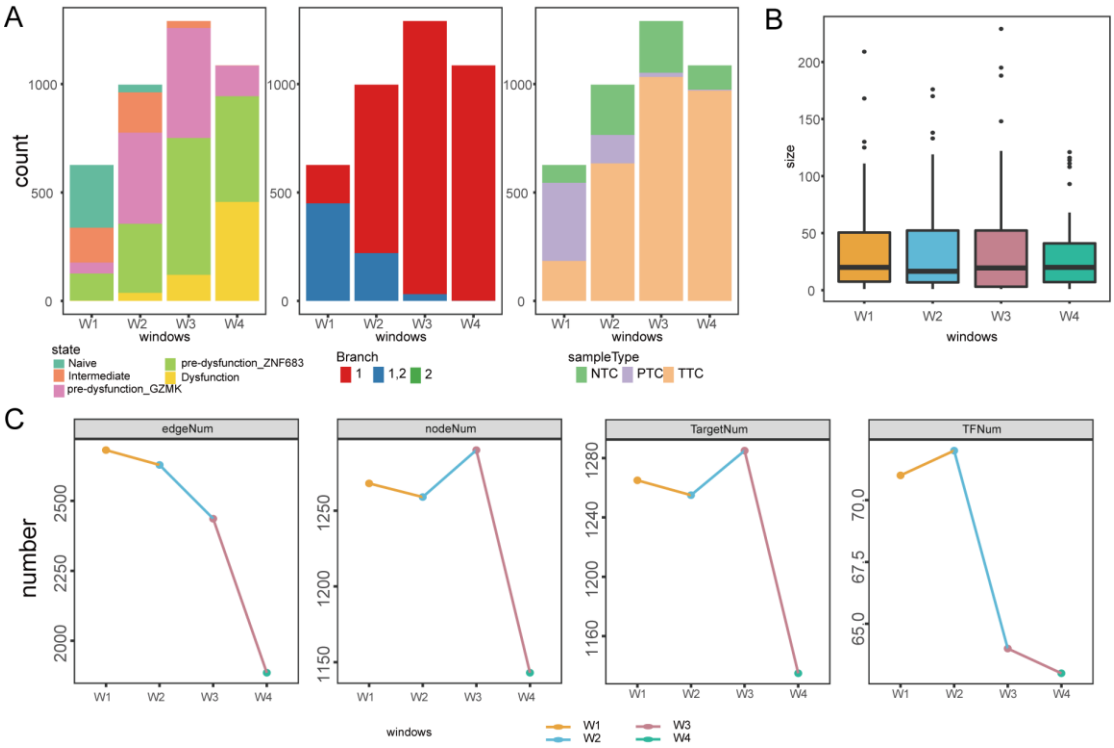

Figure S5. The basic statistics of dynamic networks.

(A) The distribution of cells in the aspect of states, branches and tissue sources in each window. (B) Distributions of regulon size in each network. (C) The number of edges, nodes, targets and TFs in networks of four windows.

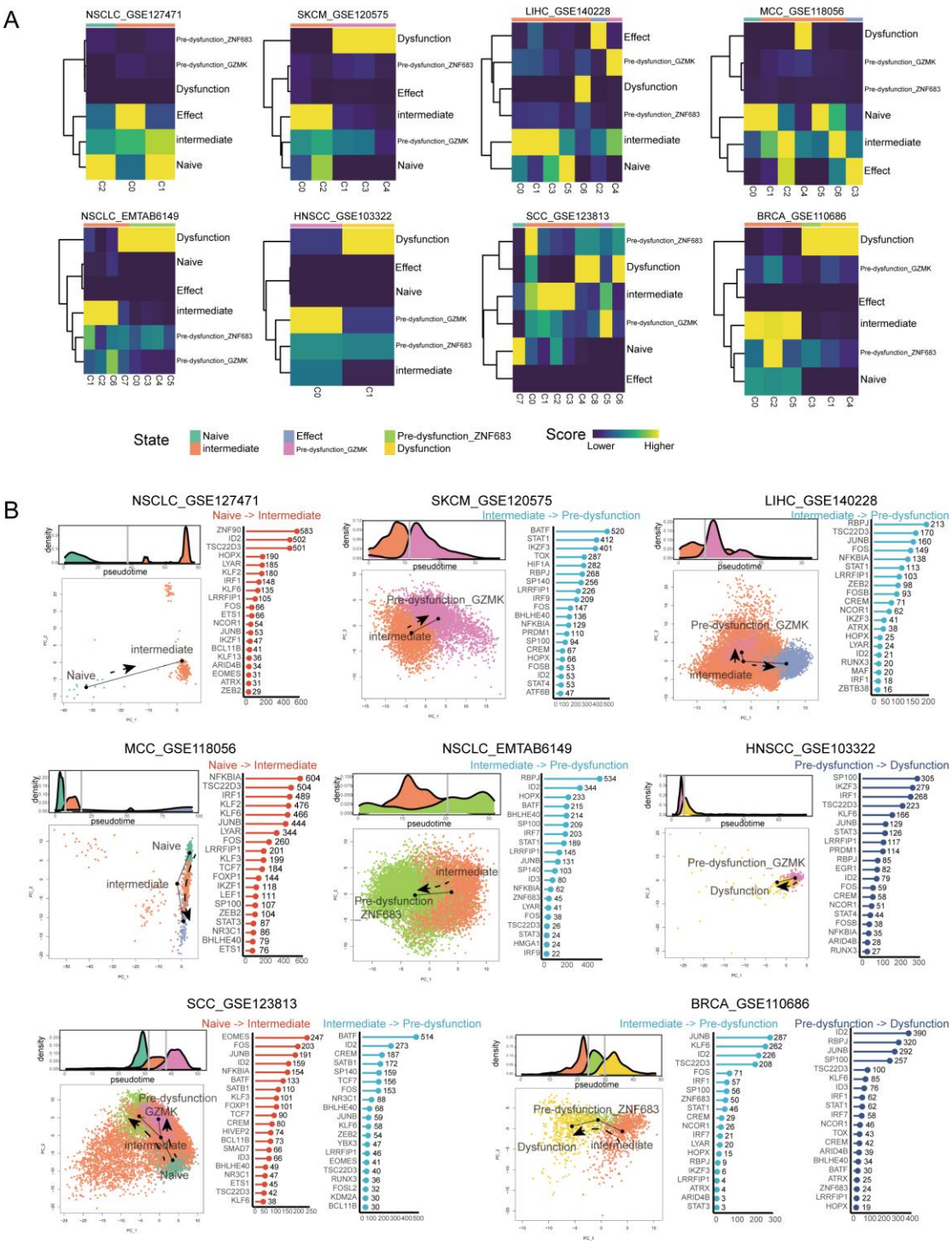

Figure S6. Constructing dynamic networks for independent datasets.
(A) The singleR scores for all clusters across all reference states (CD8_C1-LEF1, CD8_C2-CD28, CD8_C3-CX3CR1, CD8_C4-GZMK, CD8_C5-ZNF683 and CD8_C6-LAYN). (B) States transition trajectory and top 20 regulators for each dataset. The density plot displays the pseudotime distribution of different states. The scatter plot displays the trajectory of states transition. The lollipop plot displays the number of target genes for the top 20 regulators.

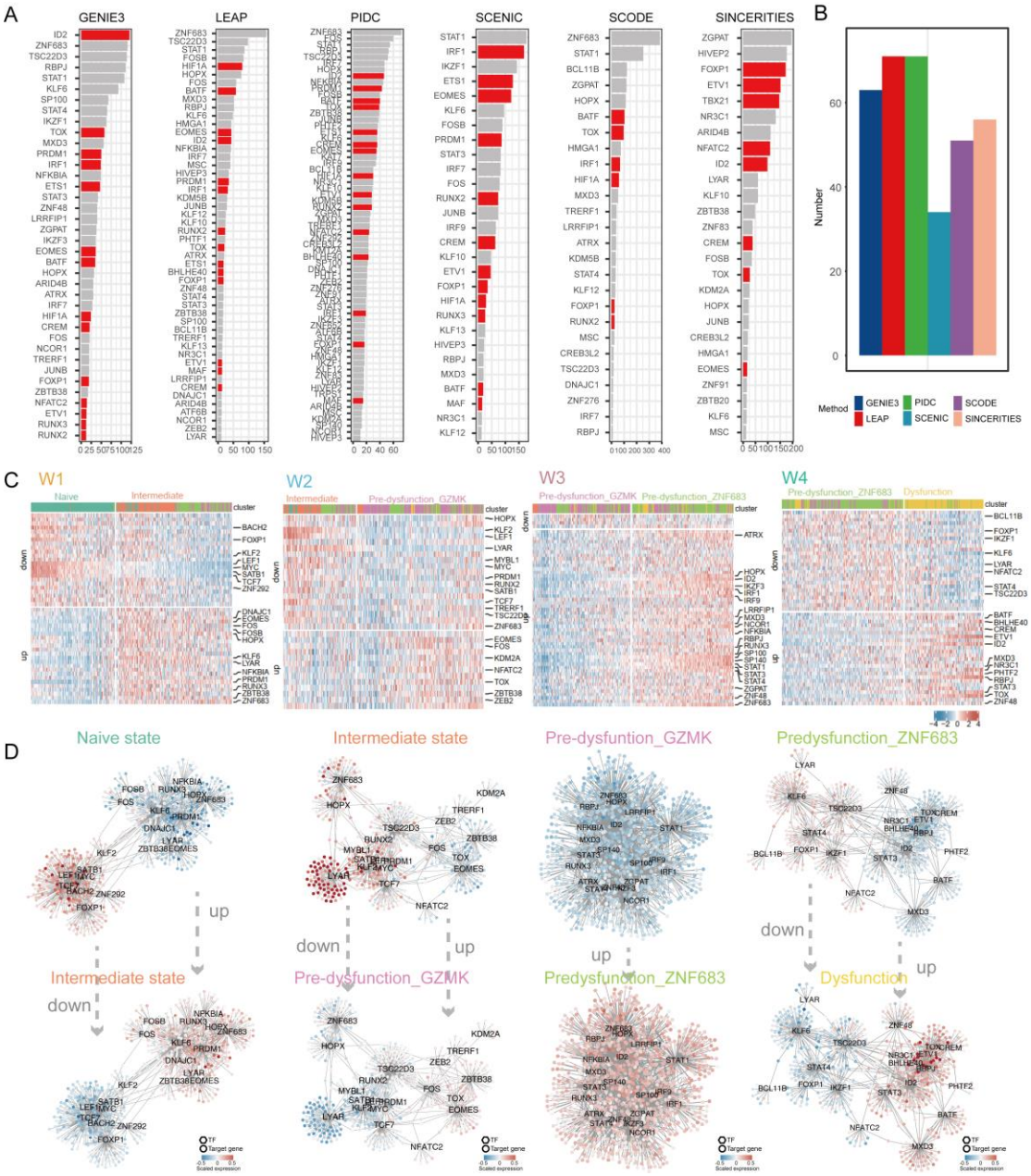

Figure S7. The activities of TFs dynamic changes across different states.

(A) Regulators identified by different method in window W4. The regulators ordered by the number of target genes. The curate TFs related to T cell dysfunction were labeled. (B) The number of regulators identified by different method in window W4. (C) The activities of significantly different TF regulons (Wilcoxon Rank-Sum test, FDR < 0.05) in cells of each window. The top 20 significant TFs were labeled. (D) Expression changes of genes in the sub-network of the top 20 TFs between the corresponding two states in each window. Nodes are colored by the average expression in the corresponding states.

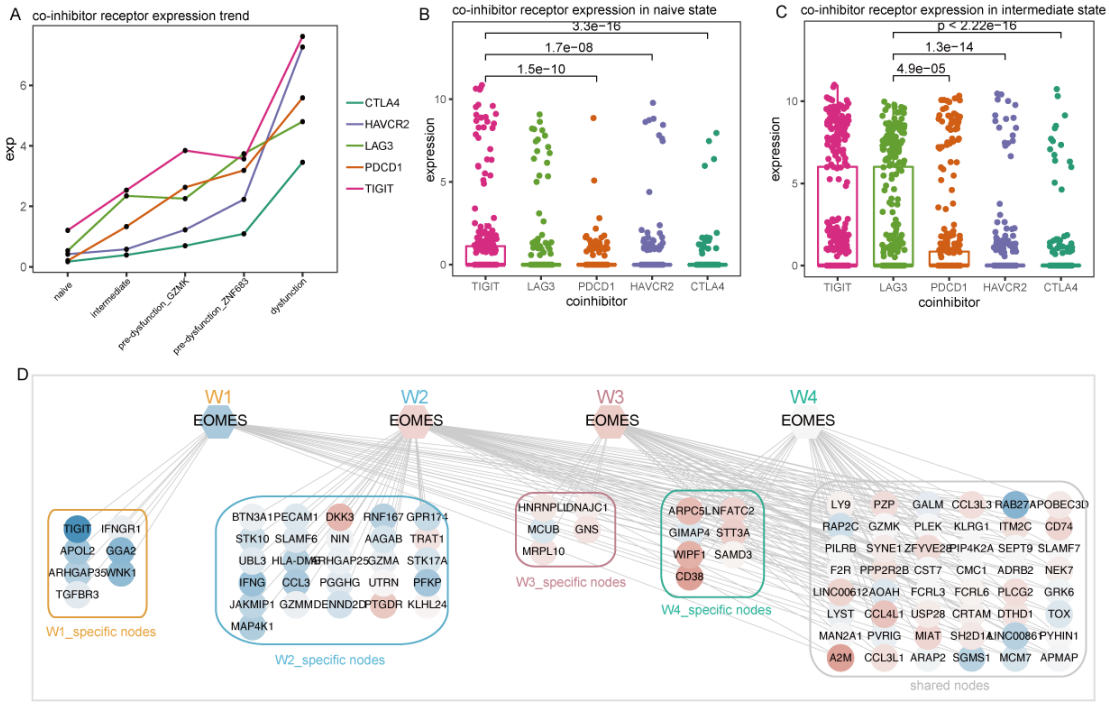

Figure S8. Dynamics of expression for co-inhibitory receptors, and regulatory rewiring of transcription factor EOMES. (A) The average expression of each co-inhibitory receptor in cells from different states. (B-C) The expression of co-inhibitory receptors in naive cells (B) or in intermediate cells (C) within the window W1. (D) The first four groups of target genes represent window-specific targets in W1, W2, W3 and W4, respectively, and the last group of genes contained targets which were shared by at least two windows.

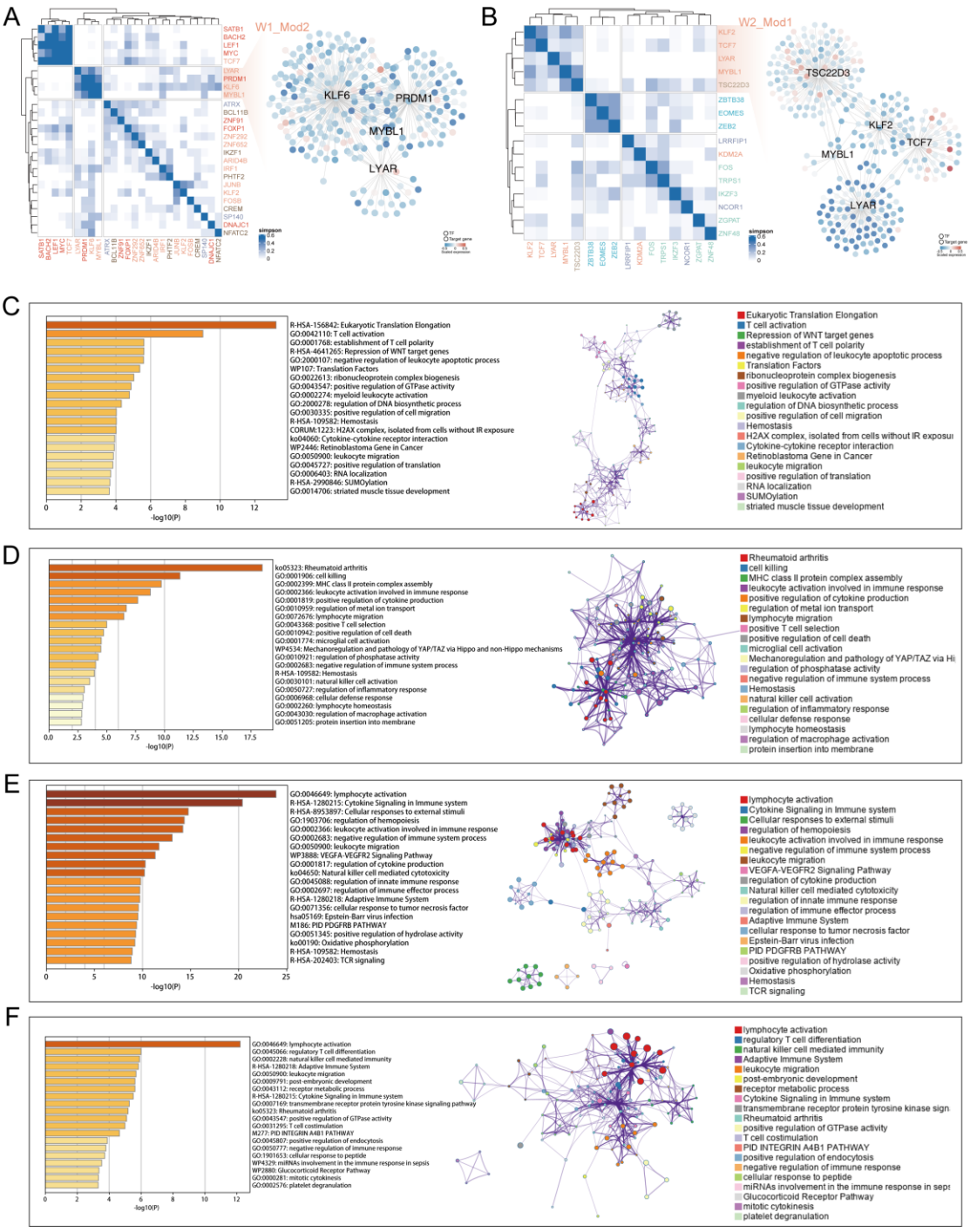

Figure S9. Enriched terms of window-specific regulatory modules. (A) Module M2 in windows W1. Left. Simpson index among TFs in network of W1. Right. Expression of genes in M2. (B) Module M1 in windows W2. (C-F) Visualization of enrichment results by Metascape for module M1 in W1 (C), module M2 in W2 (D), module M1 in W3 (E) and module M1 in W4 (F), respectively.

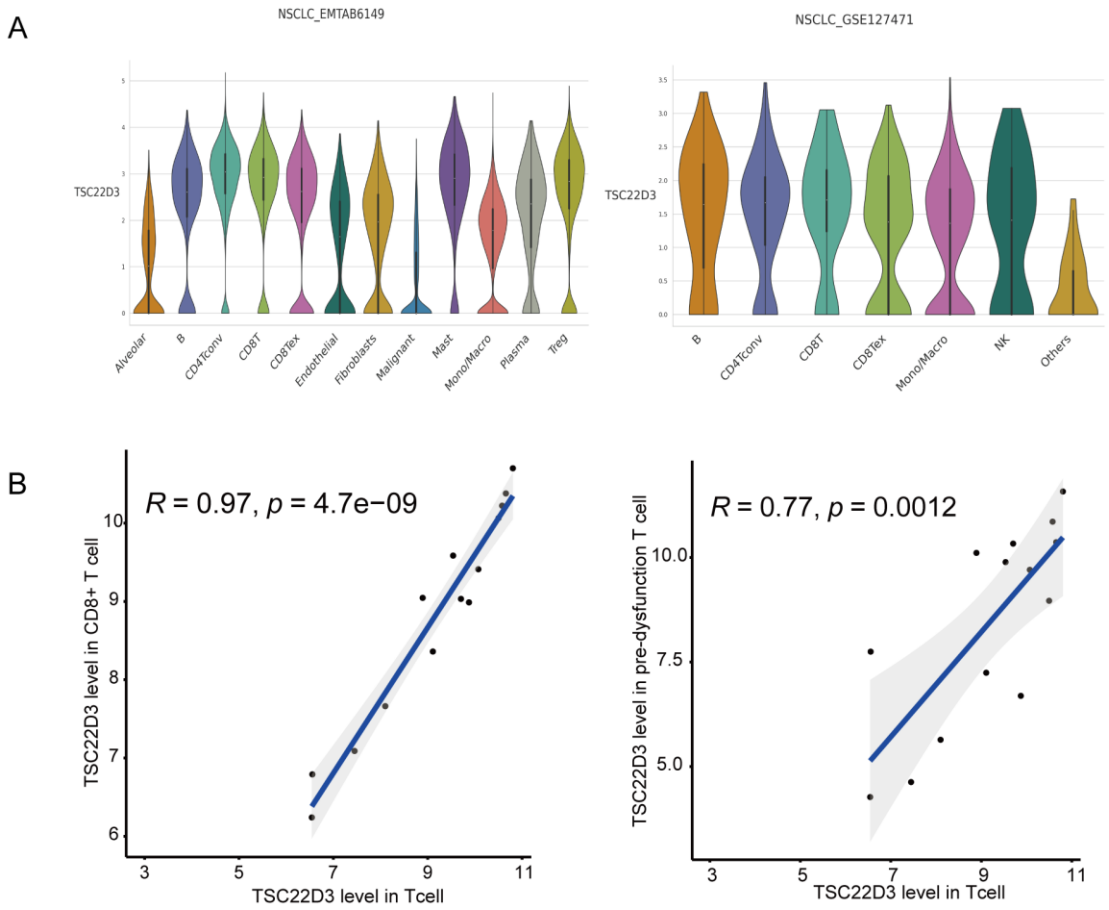

Figure S10. Expression of TSC22D3 in various cell types.

(A) Expression of TSC22D3 across various cell types in dataset EMTAB6149 and GSE127471. (B) In the core dataset GSE99254, correlation between TSC22D3 expression level in the T cells and TSC22D3 expression level in the pre-dysfunction T cells or CD8+ T cells.