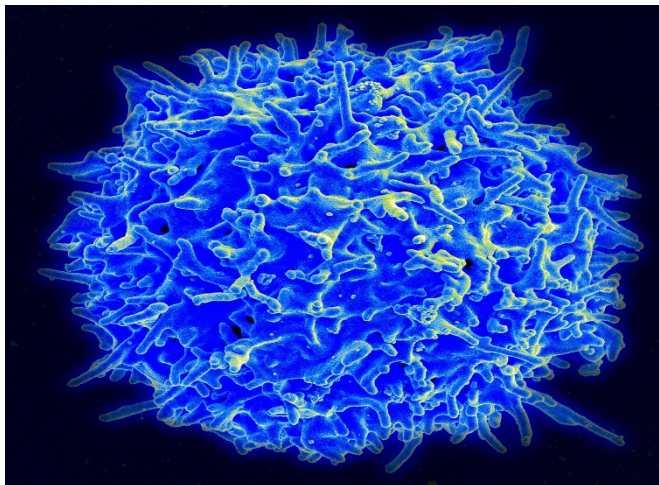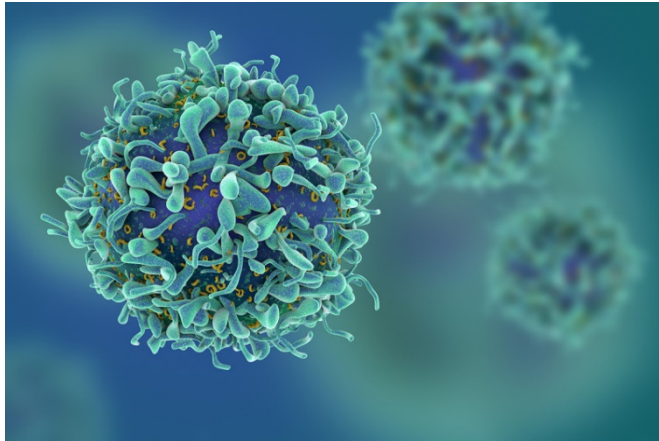# The molecular alteration of T cell during development and differentiation

BioloGist

김도현, 정지훈, 한승엽

2021.06.07

Who **Biolo****G****ist** are?
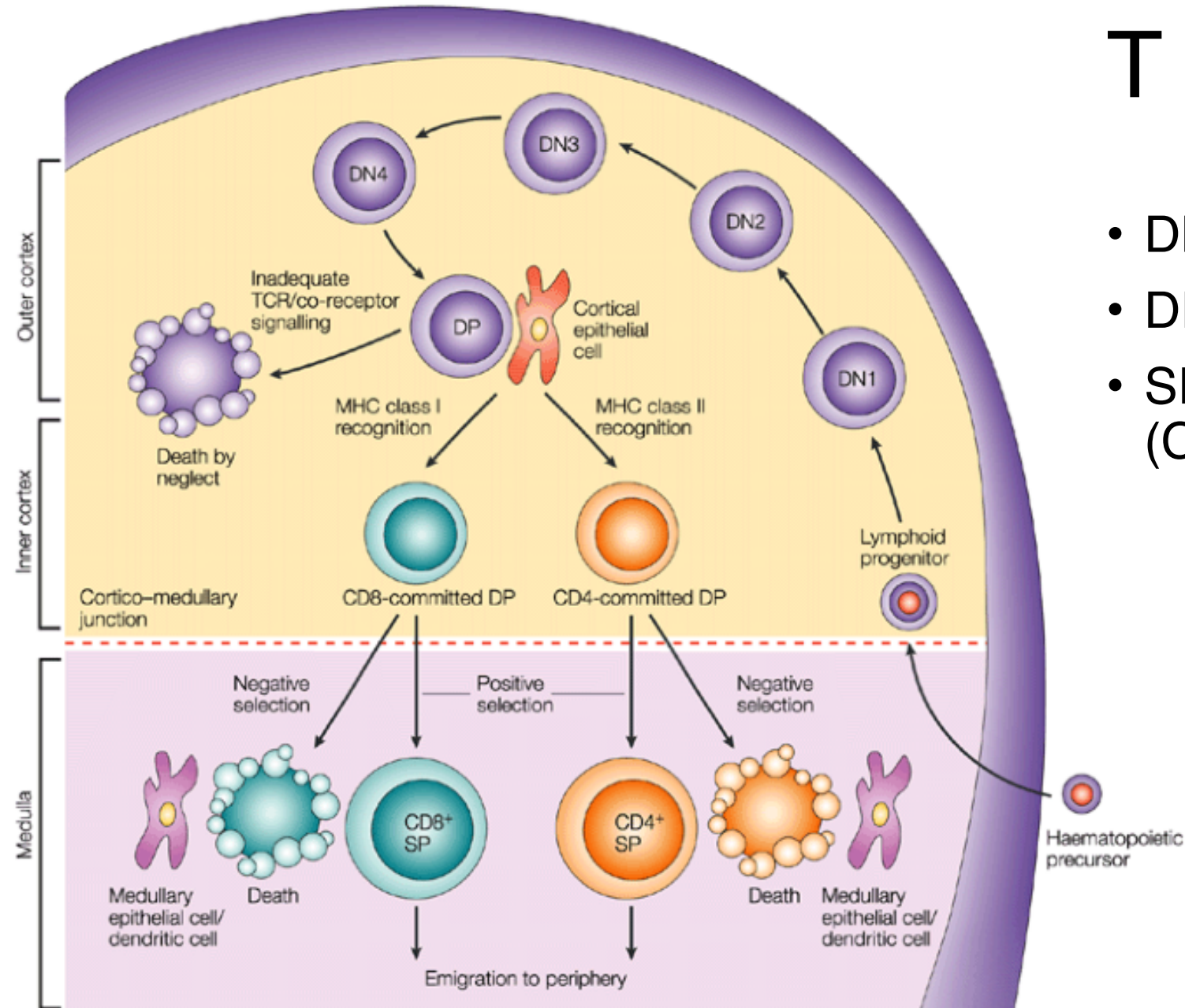

김도현
전기전자컴퓨터 전공

정지훈
생명과학 전공

한승엽
생명과학 전공

T cell development

- DN : double negative (CD4-CD8-)
- DP : double positive(CD4+CD8+)
- SP : single positive (CD4+CD8- or CD4-CD8+)

Germain, R. T-cell development and the CD4–CD8 lineage decision. *Nat Rev Immunol* **2,** 309–322 (2002). https://doi.org/10.1038/nri798

Project Topic

Key Question

- What molecular difference in gene expression level makes T-cell different?

Samples

- development : in vivo, total RNA-seq
- differentiation : in vitro, total RNA-seq

nature
immunology

Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation

Gangqing Hu[1,3], Qingsong Tang[1,3], Suveena Sharma[2,3], Fang Yu[2], Thelma M Escobar[2], Stefan A Muljo[2], Jinfang Zhu[2] & Keji Zhao[1]

Although intergenic long noncoding RNAs (lincRNAs) have been linked to gene regulation in various tissues, little is known about lincRNA transcriptomes in the T cell lineages. Here we identified 1,524 lincRNA clusters in 42 T cell samples, from early T cell progenitors to terminally differentiated helper T cell subsets. Our analysis revealed highly dynamic and cell-specific expression patterns for lincRNAs during T cell differentiation. These lincRNAs were located in genomic regions enriched for genes that encode proteins with immunoregulatory functions. Many were bound and regulated by the key transcription factors T-bet, GATA-3, STAT4 and STAT6. We found that the lincRNA LincR-Ccr2-5′AS, together with GATA-3, was an essential component of a regulatory circuit in gene expression specific to the T_H2 subset of helper T cells and was important for the migration of T_H2 cells.

Samples for T cell **differentiation** (*in vitro*)

	0h		24 hours		72 hours		2 weeks	
	R1	R2	R1	R2	R1	R2	R1	R2
Naïve CD4 T	GSM1169468	GSM1169468						
T_H1			GSM1169465		GSM1169478	GSM1169478	GSM1169470	GSM1169470
T_H2			GSM1169467		GSM1169479	GSM1169479	GSM1169472	GSM1169472
T_H17			GSM1169466		GSM1169480	GSM1169480	GSM1169474	GSM1169474
iT_{reg}			GSM1169481	GSM1169485	GSM1169476	GSM1169477		

Hu, G., Tang, Q., Sharma, S. *et al.* Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat Immunol* **14,** 1190–1198 (2013). https://doi.org/10.1038/ni.2712

1. [GSM1169516: paTh2_StS6KO; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina Genome Analyzer IIx) run: 43.9M spots, 5.3G bases, 3.1Gb downloads
Accession: SRX311751

2. [GSM1169515: paTh2_StS6CT; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina Genome Analyzer IIx) run: 42.5M spots, 5.1G bases, 3.1Gb downloads
Accession: SRX311750

3. [GSM1169514: paTh1_StS4KO; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina Genome Analyzer IIx) run: 39M spots, 4.7G bases, 2.4Gb downloads
Accession: SRX311749

4. [GSM1169513: paTh1_StS4CT; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina Genome Analyzer IIx) run: 36.9M spots, 4.4G bases, 2.3Gb downloads
Accession: SRX311748

5. [GSM1169512: ttTh2_2w_nucl_r2; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina HiSeq 2000) run: 56.2M spots, 2.8G bases, 1.8Gb downloads
Accession: SRX311747

6. [GSM1169511: ttTh2_2w_nucl_r1; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina HiSeq 2000) run: 63.1M spots, 3.2G bases, 2Gb downloads
Accession: SRX311746

7. [GSM1169510: ttTh2_2w_cyto_r2; Mus musculus; RNA-Seq](#)
1 ILLUMINA (Illumina HiSeq 2000) run: 42.6M spots, 2.1G bases, 1.4Gb downloads
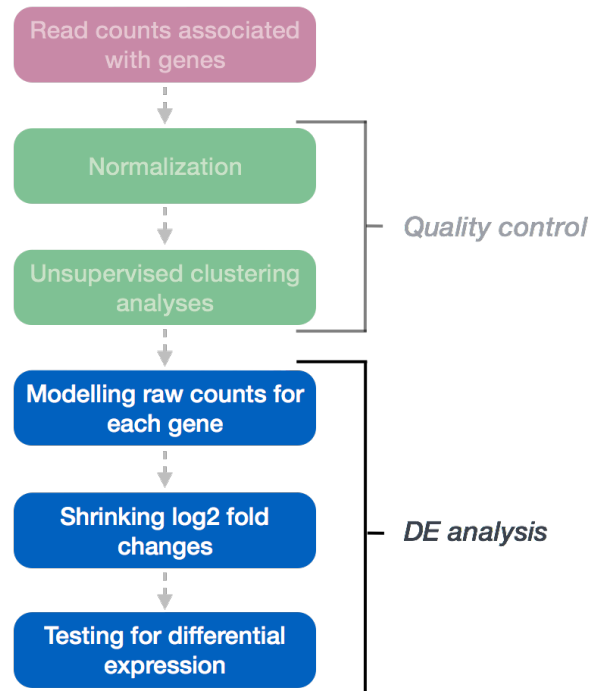Accession: SRX311745

Sequence alignment workflow

https://hbctraining.github.io/GCC-BOSC-2018/lessons/sample_level_QC.html

Differential gene expression analysis

DESeq2 procedure

Differential gene expression list

clustering

sample correlation

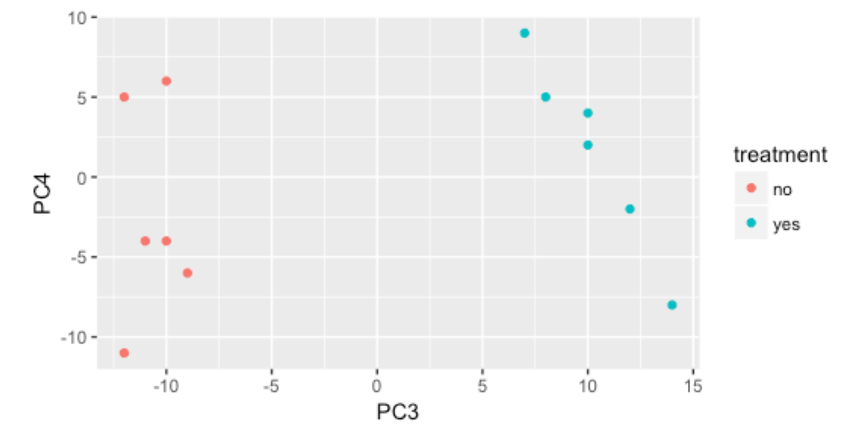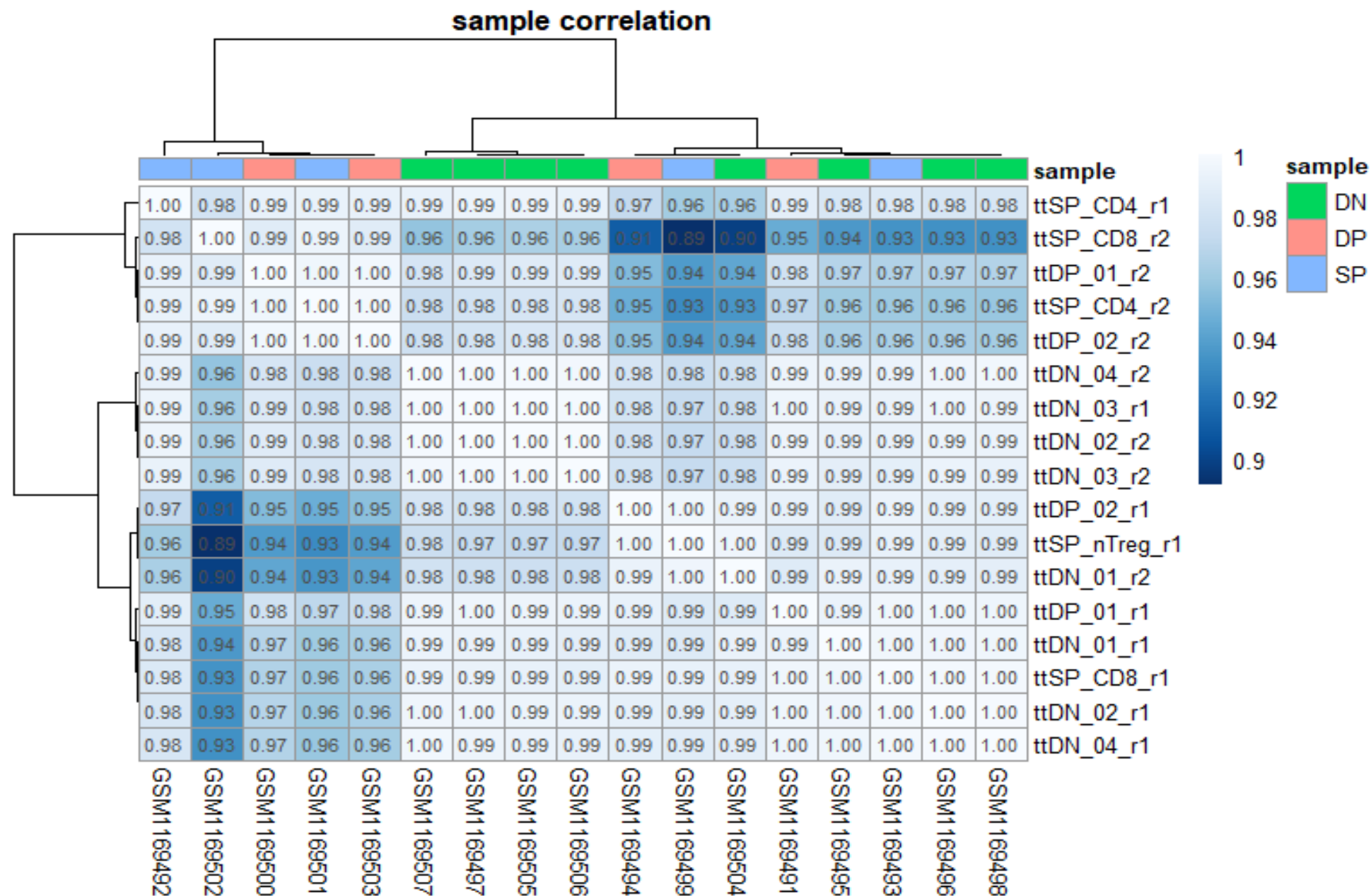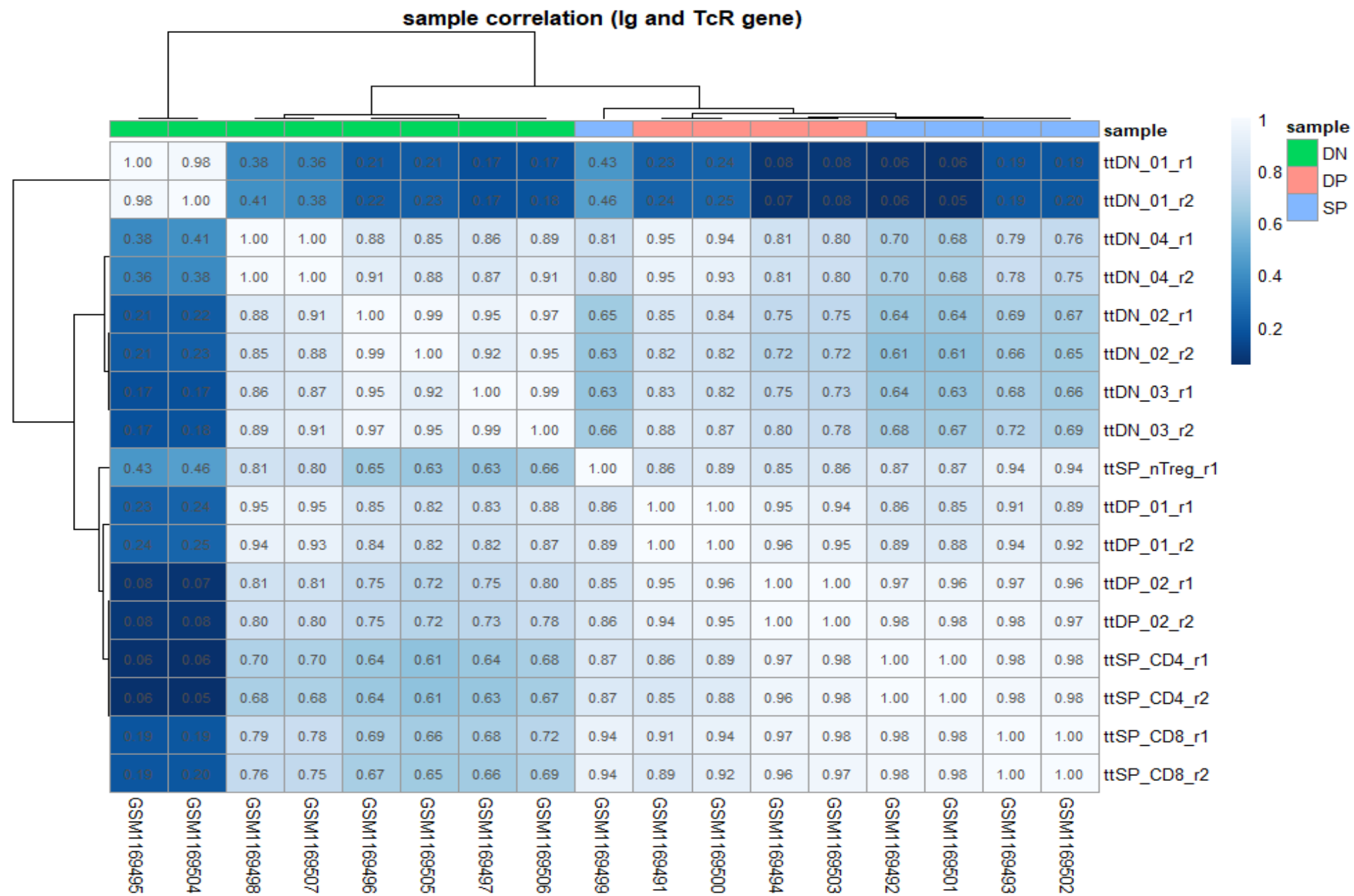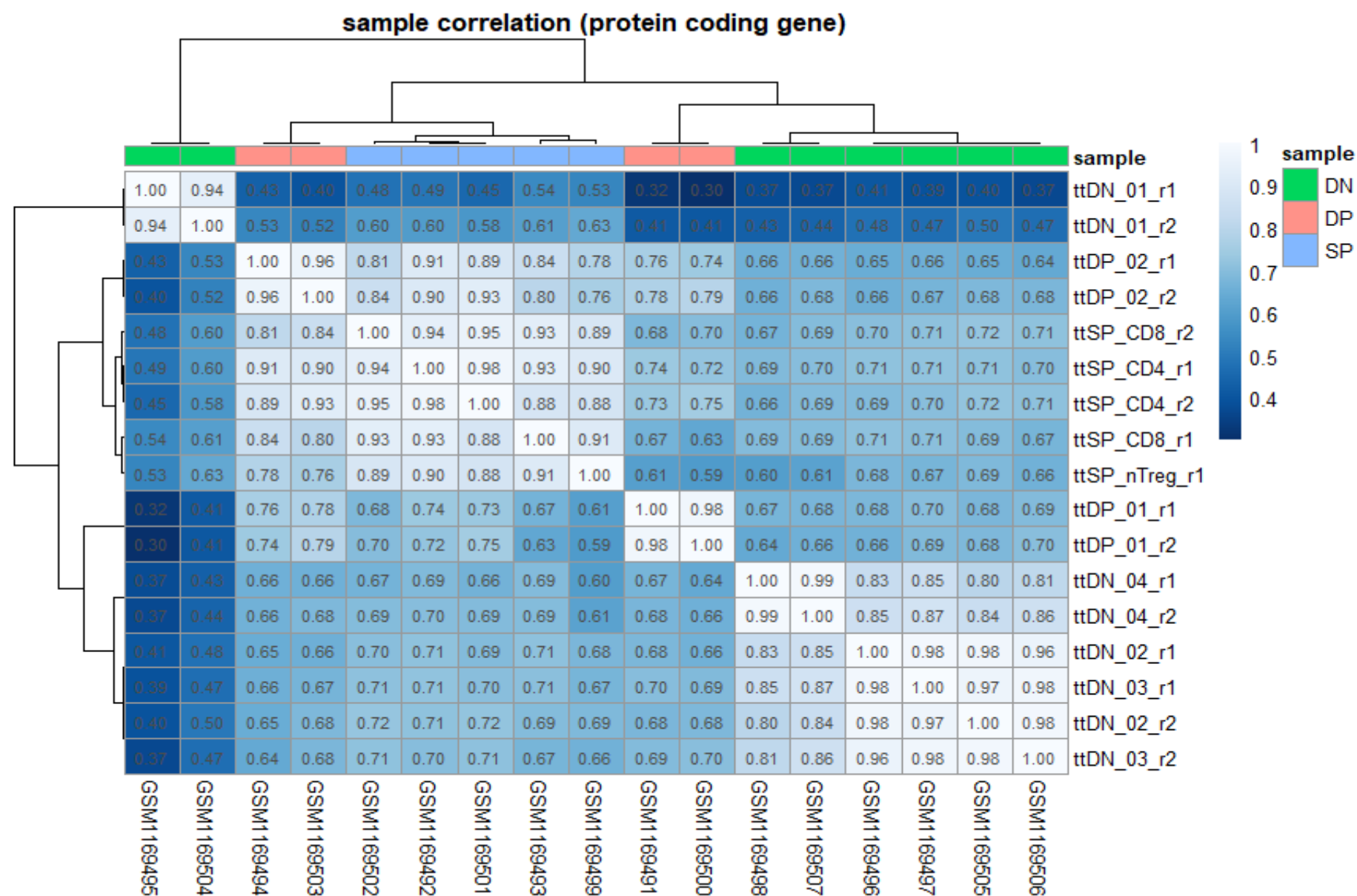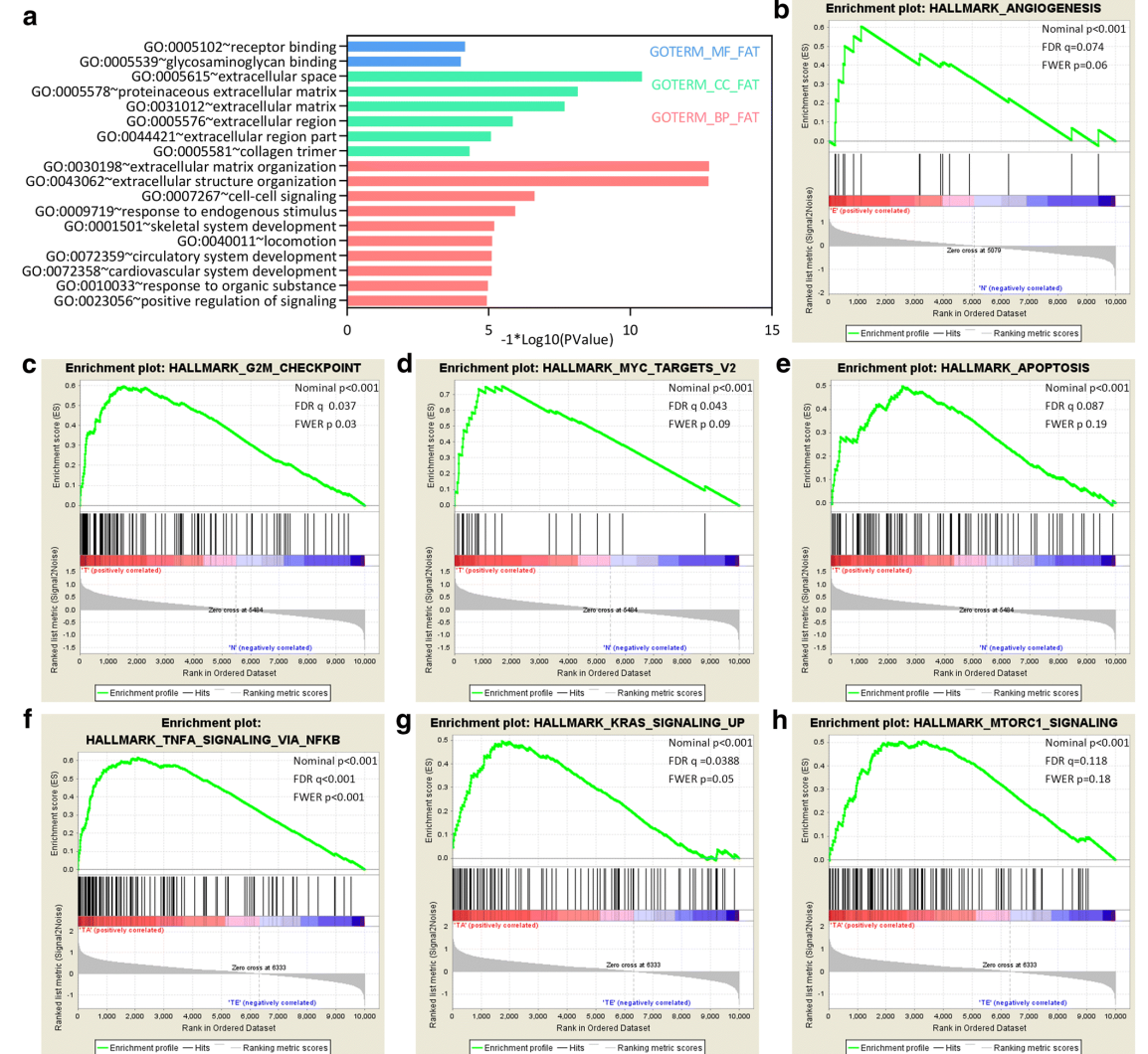|	GSM1169492	GSM1169502	GSM1169500	GSM1169501	GSM1169503	GSM1169507	GSM1169497	GSM1169505	GSM1169506	GSM1169494	GSM1169499	GSM1169504	GSM1169491	GSM1169495	GSM1169493	GSM1169496	GSM1169498	
ttSP_CD4_r1	1.00	0.98	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.97	0.96	0.96	0.99	0.98	0.98	0.98	0.98
ttSP_CD8_r2	0.98	1.00	0.99	0.99	0.99	0.96	0.96	0.96	0.96	0.91	0.89	0.90	0.95	0.94	0.93	0.93	0.93
ttDP_01_r2	0.99	0.99	1.00	1.00	1.00	0.98	0.99	0.99	0.99	0.95	0.94	0.94	0.98	0.97	0.97	0.97	0.97
ttSP_CD4_r2	0.99	0.99	1.00	1.00	1.00	0.98	0.98	0.98	0.98	0.95	0.93	0.93	0.97	0.96	0.96	0.96	0.96
ttDP_02_r2	0.99	0.99	1.00	1.00	1.00	0.98	0.98	0.98	0.98	0.95	0.94	0.94	0.98	0.96	0.96	0.96	0.96
ttDN_04_r2	0.99	0.96	0.98	0.98	0.98	1.00	1.00	1.00	1.00	0.98	0.98	0.98	0.99	0.99	0.99	1.00	1.00
ttDN_03_r1	0.99	0.96	0.99	0.98	0.98	1.00	1.00	1.00	1.00	0.98	0.97	0.98	1.00	0.99	0.99	1.00	0.99
ttDN_02_r2	0.99	0.96	0.99	0.98	0.98	1.00	1.00	1.00	1.00	0.98	0.97	0.98	0.99	0.99	0.99	0.99	0.99
ttDN_03_r2	0.99	0.96	0.99	0.98	0.98	1.00	1.00	1.00	1.00	0.98	0.97	0.98	0.99	0.99	0.99	0.99	0.99
ttDP_02_r1	0.97	0.91	0.95	0.95	0.95	0.98	0.98	0.98	0.98	1.00	1.00	0.99	0.99	0.99	0.99	0.99	0.99
ttSP_nTreg_r1	0.96	0.89	0.94	0.93	0.94	0.98	0.97	0.97	0.97	1.00	1.00	1.00	0.99	0.99	0.99	0.99	0.99
ttDN_01_r2	0.96	0.90	0.94	0.93	0.94	0.98	0.98	0.98	0.98	0.99	1.00	1.00	0.99	0.99	0.99	0.99	0.99
ttDP_01_r1	0.99	0.95	0.98	0.97	0.98	0.99	1.00	0.99	0.99	0.99	0.99	0.99	1.00	0.99	1.00	1.00	1.00
ttDN_01_r1	0.98	0.94	0.97	0.96	0.96	0.99	0.99	0.99	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00	1.00
ttSP_CD8_r1	0.98	0.93	0.97	0.96	0.96	0.99	0.99	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00
ttDN_02_r1	0.98	0.93	0.97	0.96	0.96	1.00	1.00	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00
ttDN_04_r1	0.98	0.93	0.97	0.96	0.96	1.00	0.99	0.99	0.99	0.99	0.99	0.99	1.00	1.00	1.00	1.00	1.00

sample

1
0.98
0.96
0.94
0.92
0.9

sample
- DN
- DP
- SP

sample correlation (Ig and TcR gene)

	GSM1169495	GSM1169504	GSM1169498	GSM1169507	GSM1169496	GSM1169505	GSM1169497	GSM1169506	GSM1169499	GSM1169491	GSM1169500	GSM1169494	GSM1169503	GSM1169492	GSM1169501	GSM1169493	GSM1169502	
1.00	0.98	0.38	0.36	0.21	0.21	0.17	0.17	0.43	0.23	0.24	0.08	0.08	0.06	0.06	0.19	0.19	ttDN_01_r1
0.98	1.00	0.41	0.38	0.22	0.23	0.17	0.18	0.46	0.24	0.25	0.07	0.08	0.06	0.05	0.19	0.20	ttDN_01_r2
0.38	0.41	1.00	1.00	0.88	0.85	0.86	0.89	0.81	0.95	0.94	0.81	0.80	0.70	0.68	0.79	0.76	ttDN_04_r1
0.36	0.38	1.00	1.00	0.91	0.88	0.87	0.91	0.80	0.95	0.93	0.81	0.80	0.70	0.68	0.78	0.75	ttDN_04_r2
0.21	0.22	0.88	0.91	1.00	0.99	0.95	0.97	0.65	0.85	0.84	0.75	0.75	0.64	0.64	0.69	0.67	ttDN_02_r1
0.21	0.23	0.85	0.88	0.99	1.00	0.92	0.95	0.63	0.82	0.82	0.72	0.72	0.61	0.61	0.66	0.65	ttDN_02_r2
0.17	0.17	0.86	0.87	0.95	0.92	1.00	0.99	0.63	0.83	0.82	0.75	0.73	0.64	0.63	0.68	0.66	ttDN_03_r1
0.17	0.18	0.89	0.91	0.97	0.95	0.99	1.00	0.66	0.88	0.87	0.80	0.78	0.68	0.67	0.72	0.69	ttDN_03_r2
0.43	0.46	0.81	0.80	0.65	0.63	0.63	0.66	1.00	0.86	0.89	0.85	0.86	0.87	0.87	0.94	0.94	ttSP_nTreg_r1
0.23	0.24	0.95	0.95	0.85	0.82	0.83	0.88	0.86	1.00	1.00	0.95	0.94	0.86	0.85	0.91	0.89	ttDP_01_r1
0.24	0.25	0.94	0.93	0.84	0.82	0.82	0.87	0.89	1.00	1.00	0.96	0.95	0.89	0.88	0.94	0.92	ttDP_01_r2
0.08	0.07	0.81	0.81	0.75	0.72	0.75	0.80	0.85	0.95	0.96	1.00	1.00	0.97	0.96	0.97	0.96	ttDP_02_r1
0.08	0.08	0.80	0.80	0.75	0.72	0.73	0.78	0.86	0.94	0.95	1.00	1.00	0.98	0.98	0.98	0.97	ttDP_02_r2
0.06	0.06	0.70	0.70	0.64	0.61	0.64	0.68	0.87	0.86	0.89	0.97	0.98	1.00	1.00	0.98	0.98	ttSP_CD4_r1
0.06	0.05	0.68	0.68	0.64	0.61	0.63	0.67	0.87	0.85	0.88	0.96	0.98	1.00	1.00	0.98	0.98	ttSP_CD4_r2
0.19	0.19	0.79	0.78	0.69	0.66	0.68	0.72	0.94	0.91	0.94	0.97	0.98	0.98	0.98	1.00	1.00	ttSP_CD8_r1
0.19	0.20	0.76	0.75	0.67	0.65	0.66	0.69	0.94	0.89	0.92	0.96	0.97	0.98	0.98	1.00	1.00	ttSP_CD8_r2

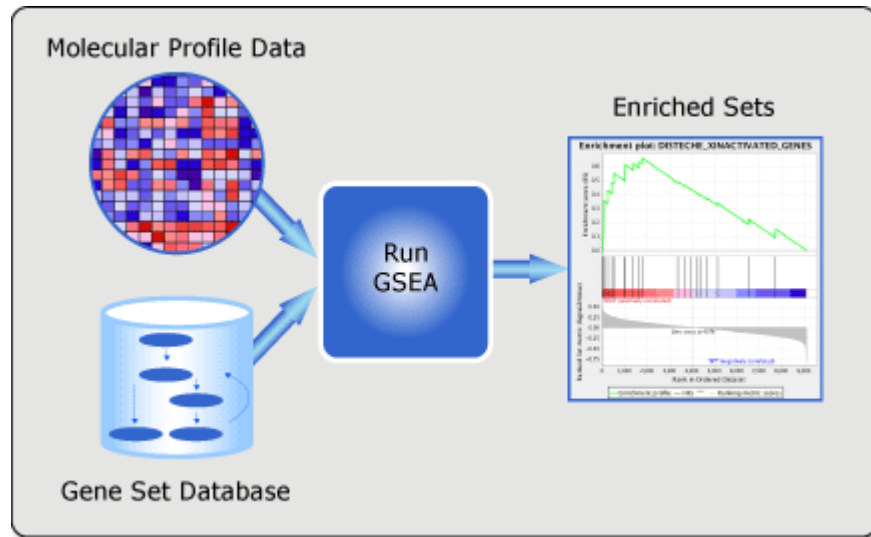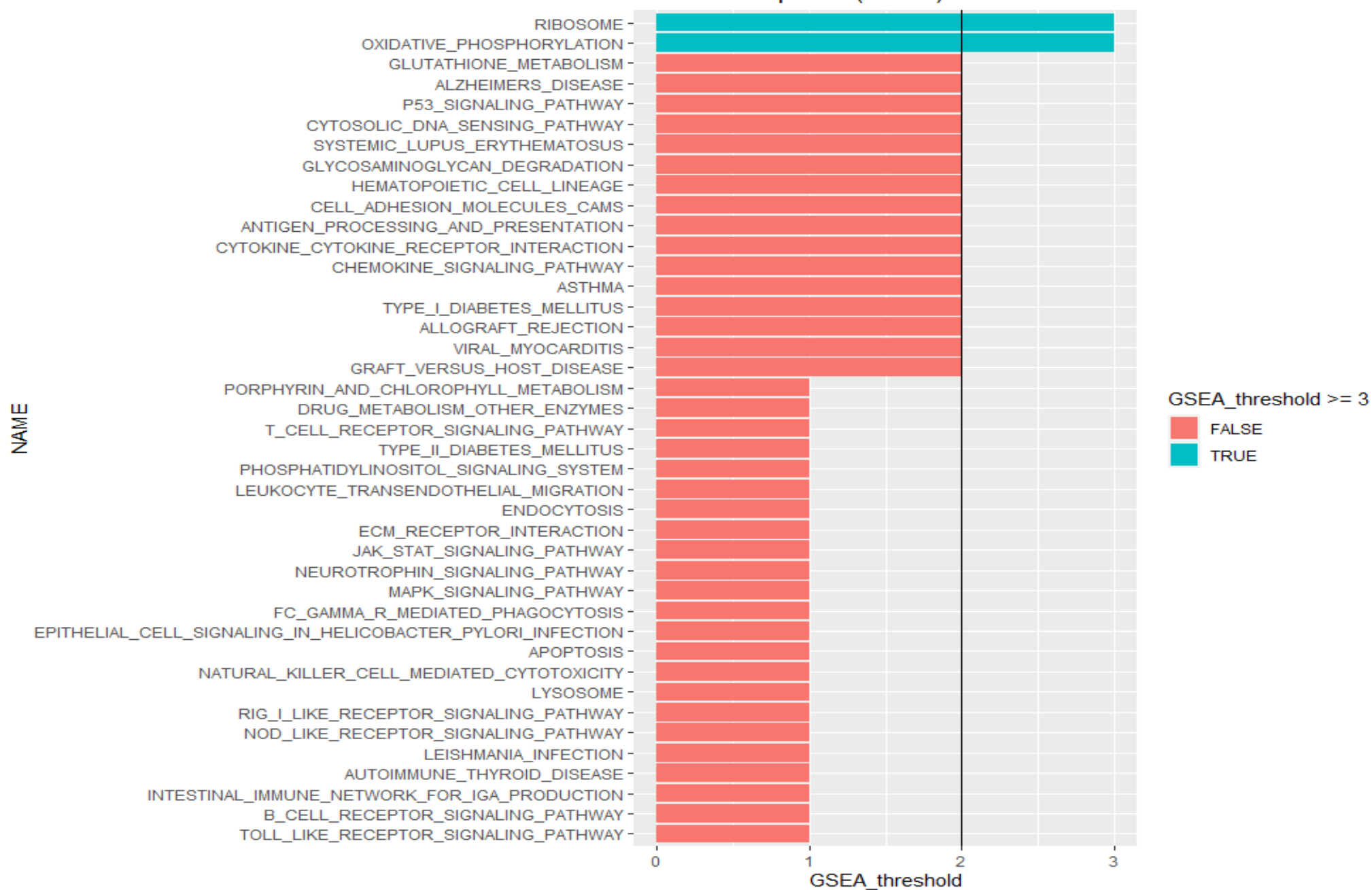sample
DN
DP
SP

sample correlation (protein coding gene)

Gene set enrichment analysis

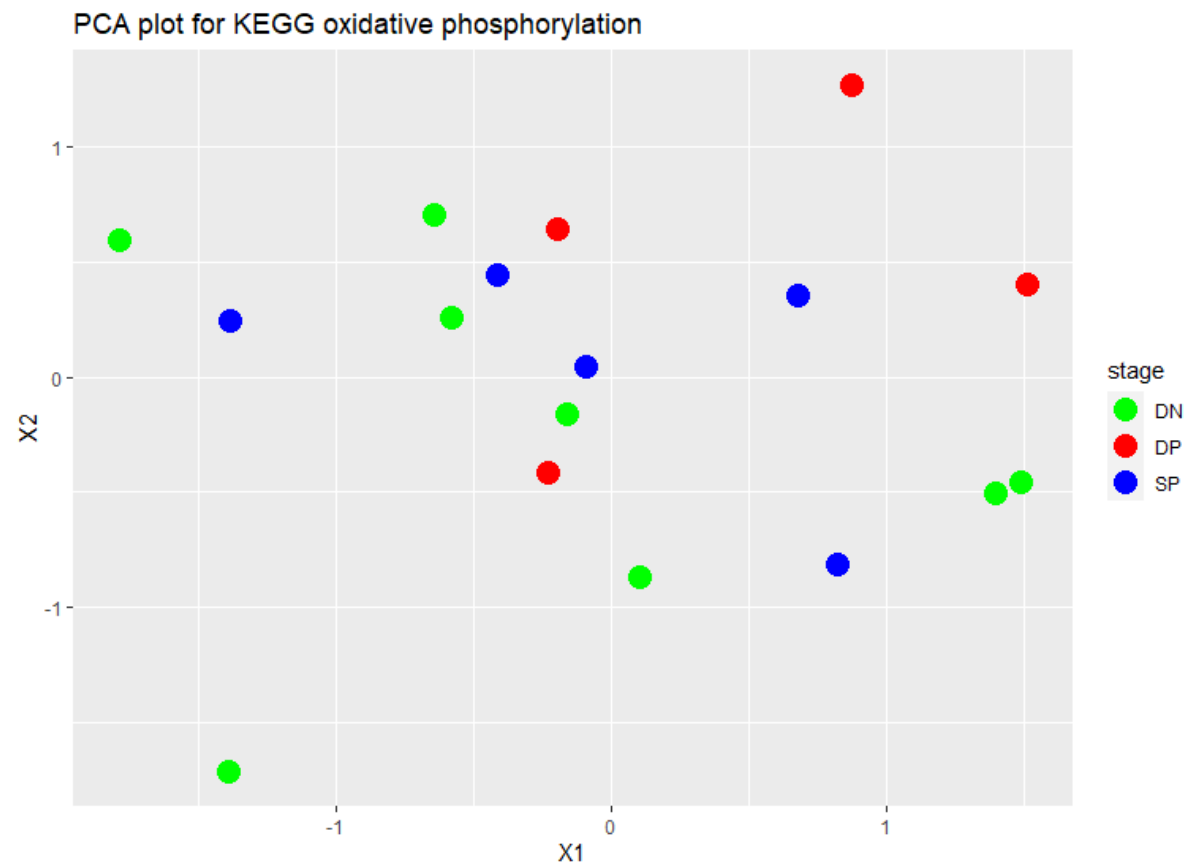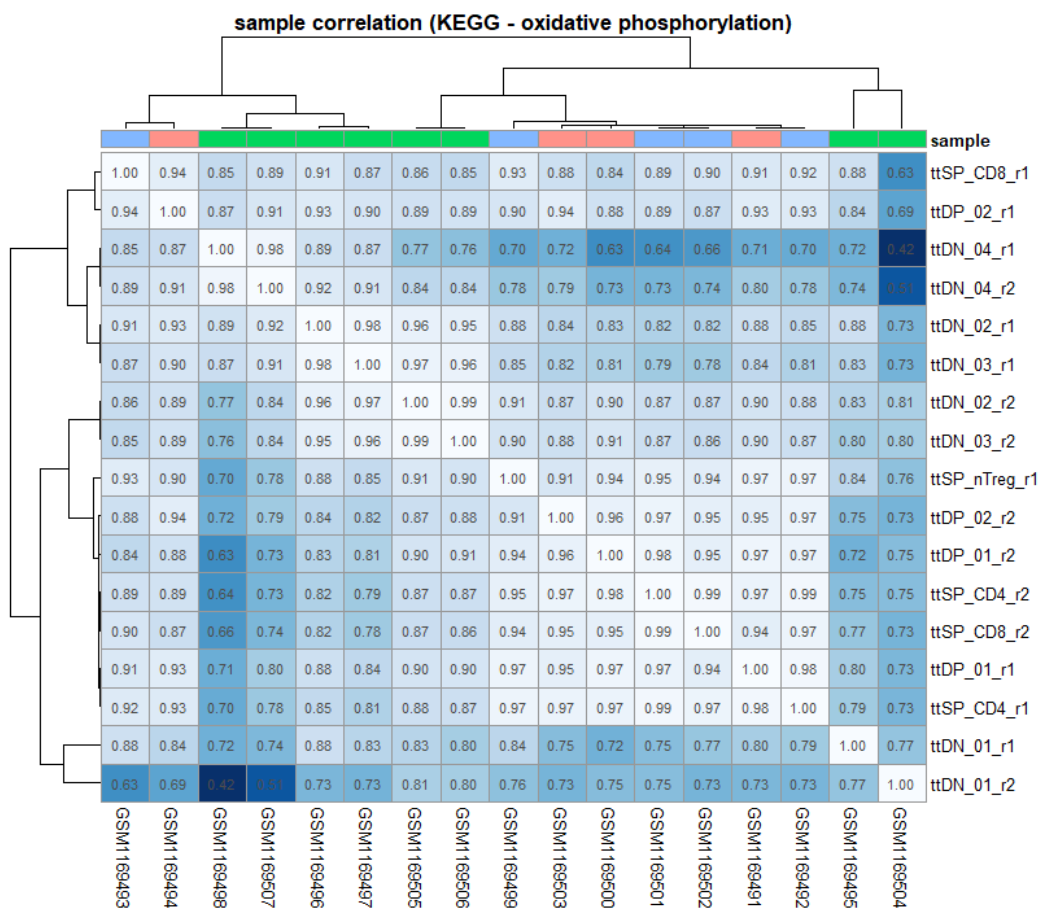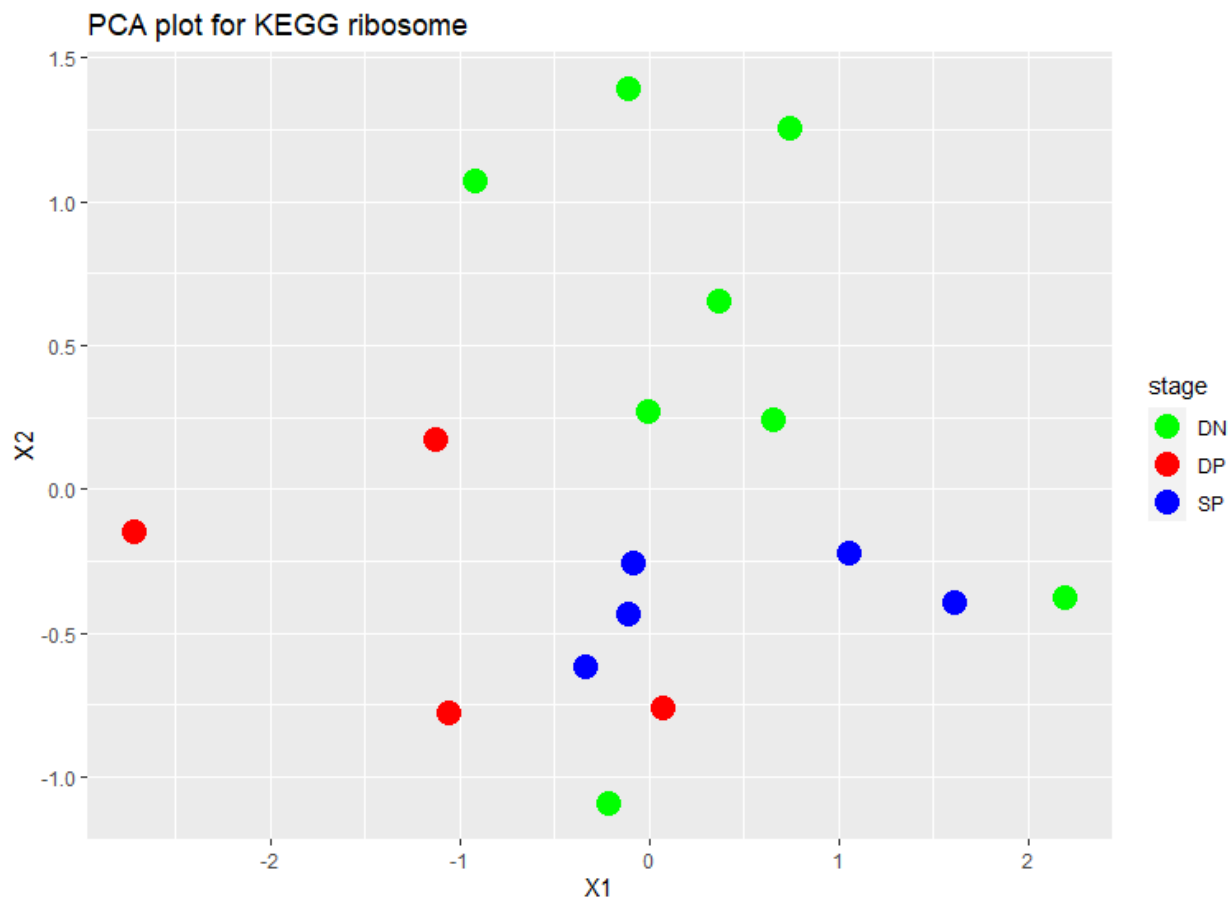- David

- GSEA – Broad Institute
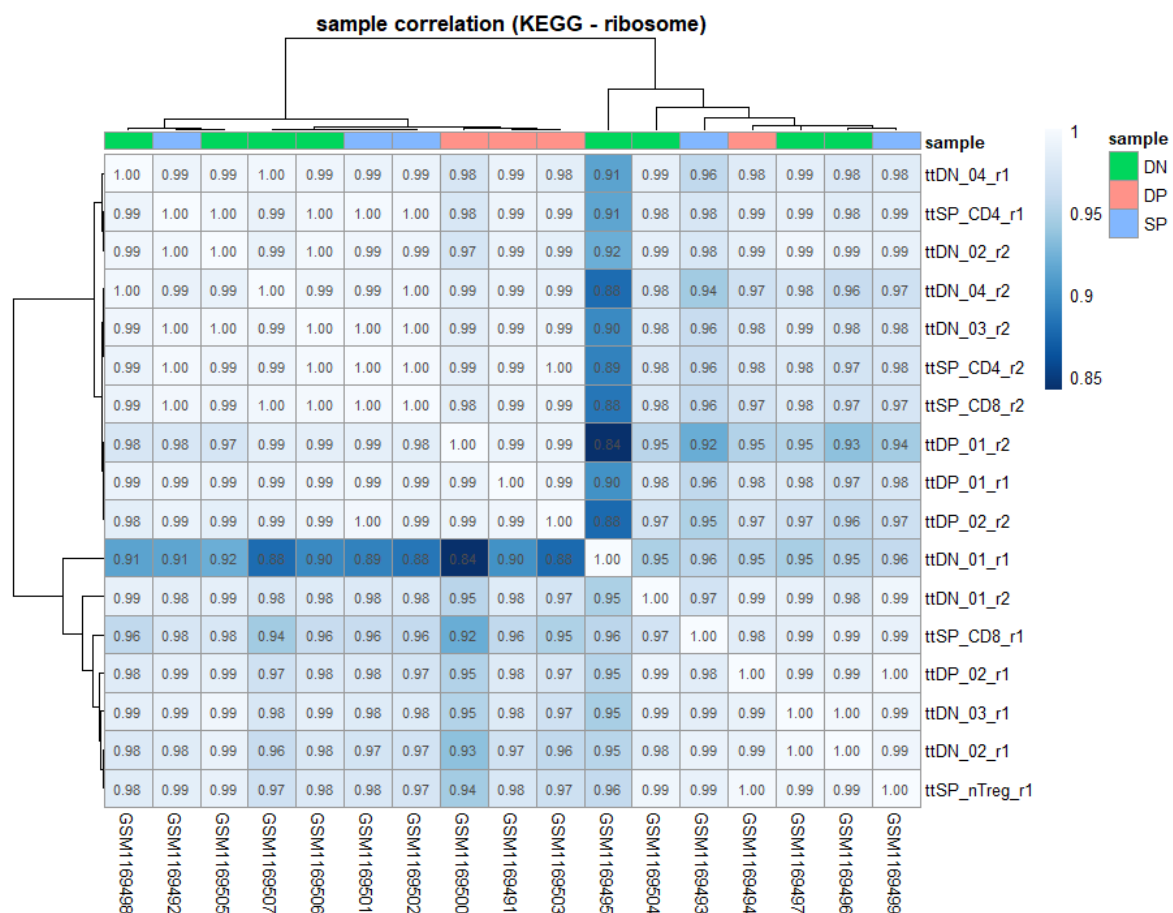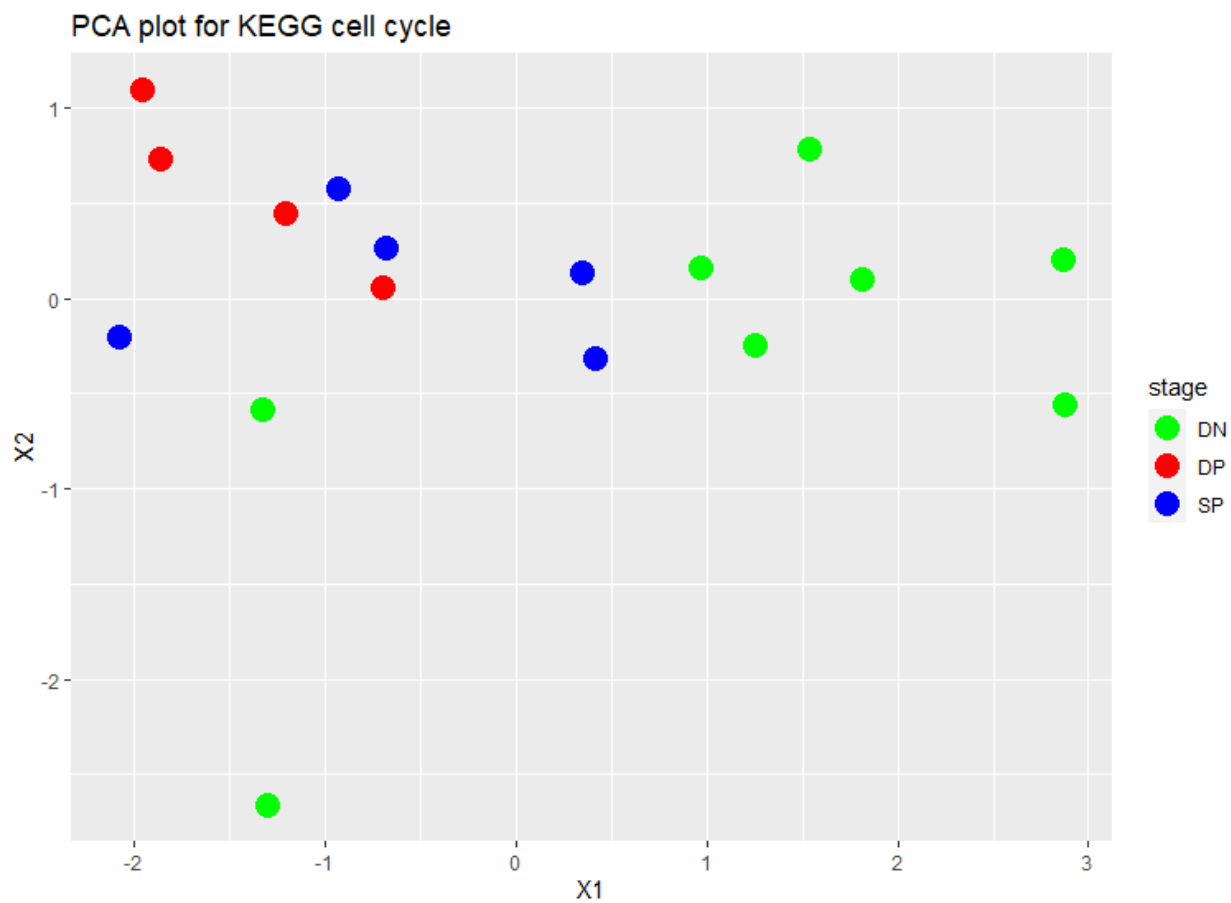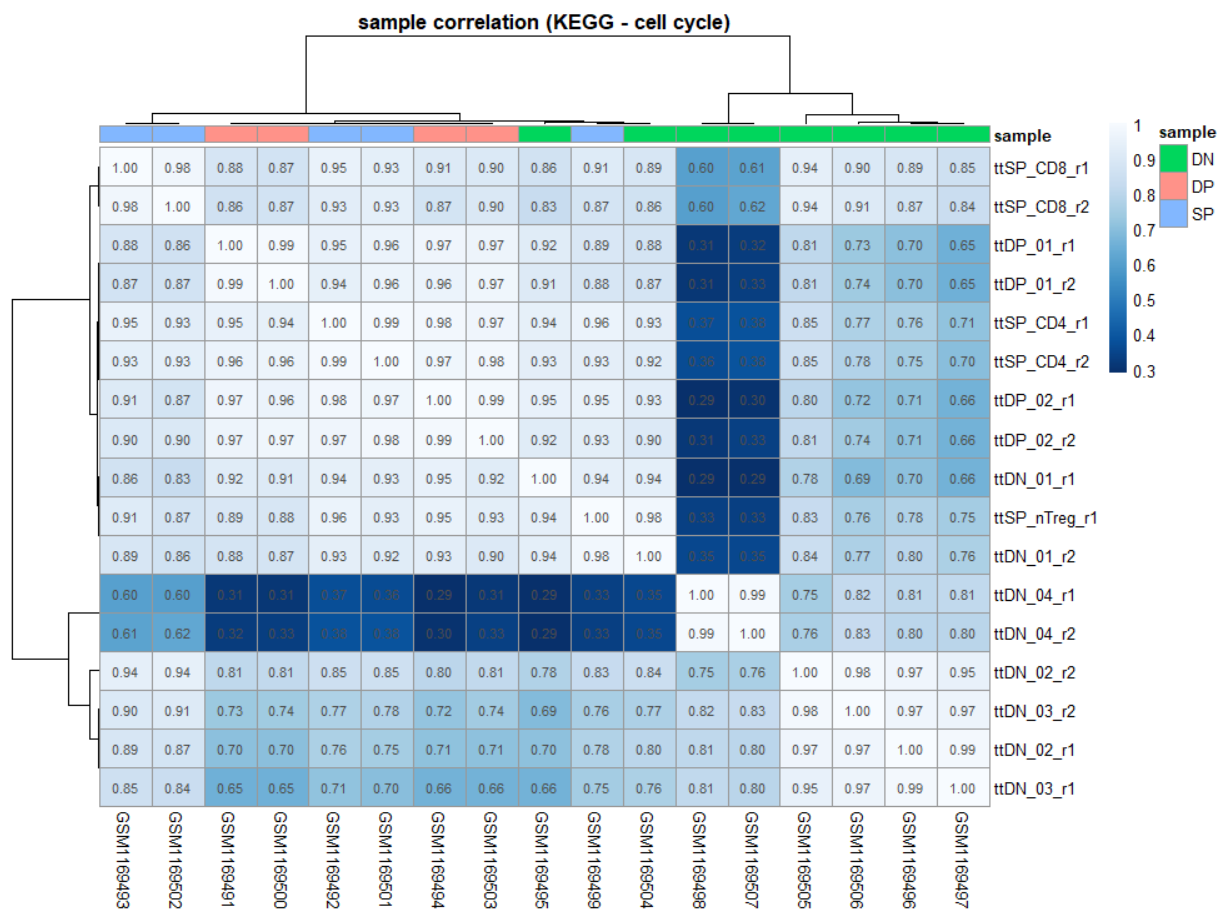
T cell development (in vivo) - KEGG Gene Set

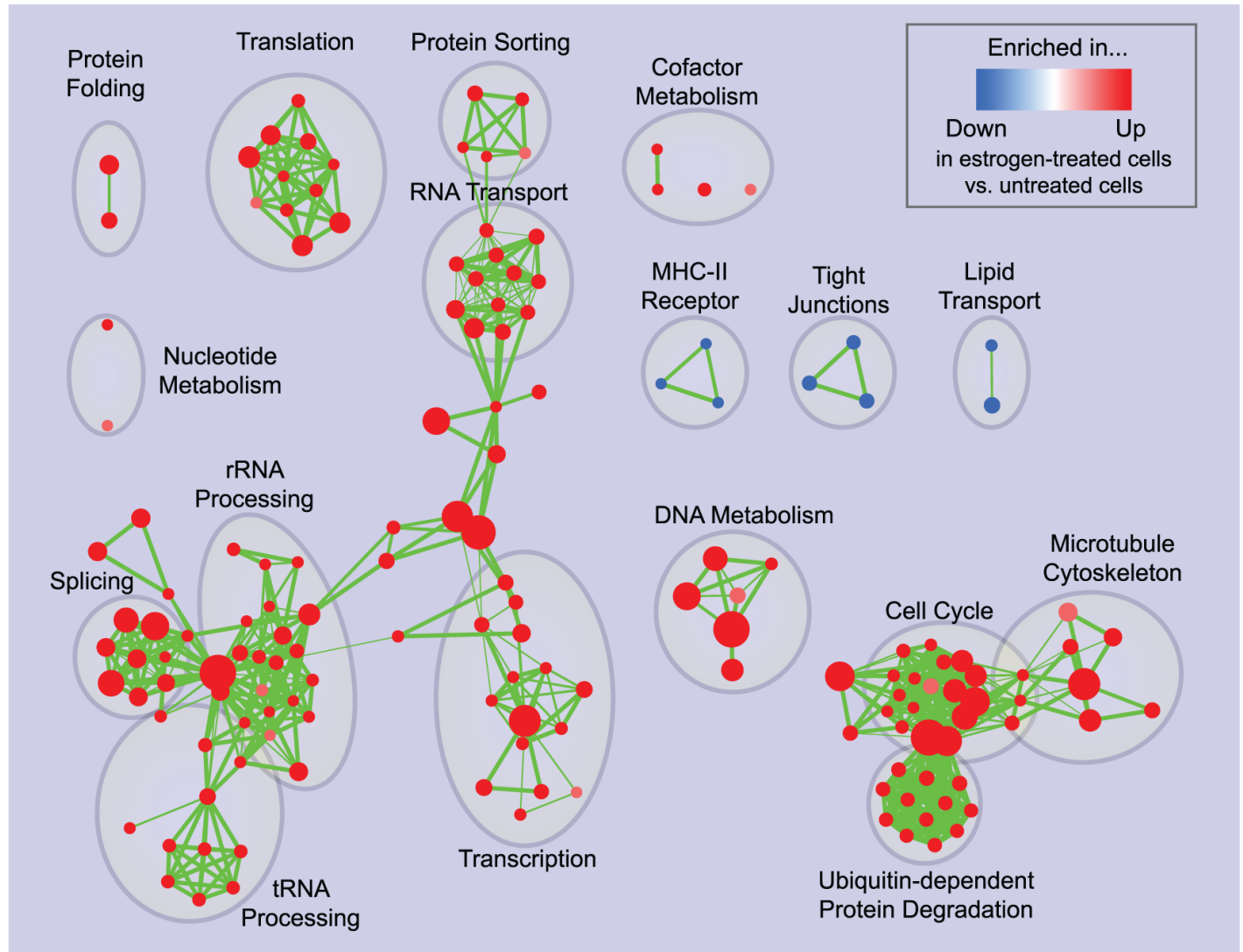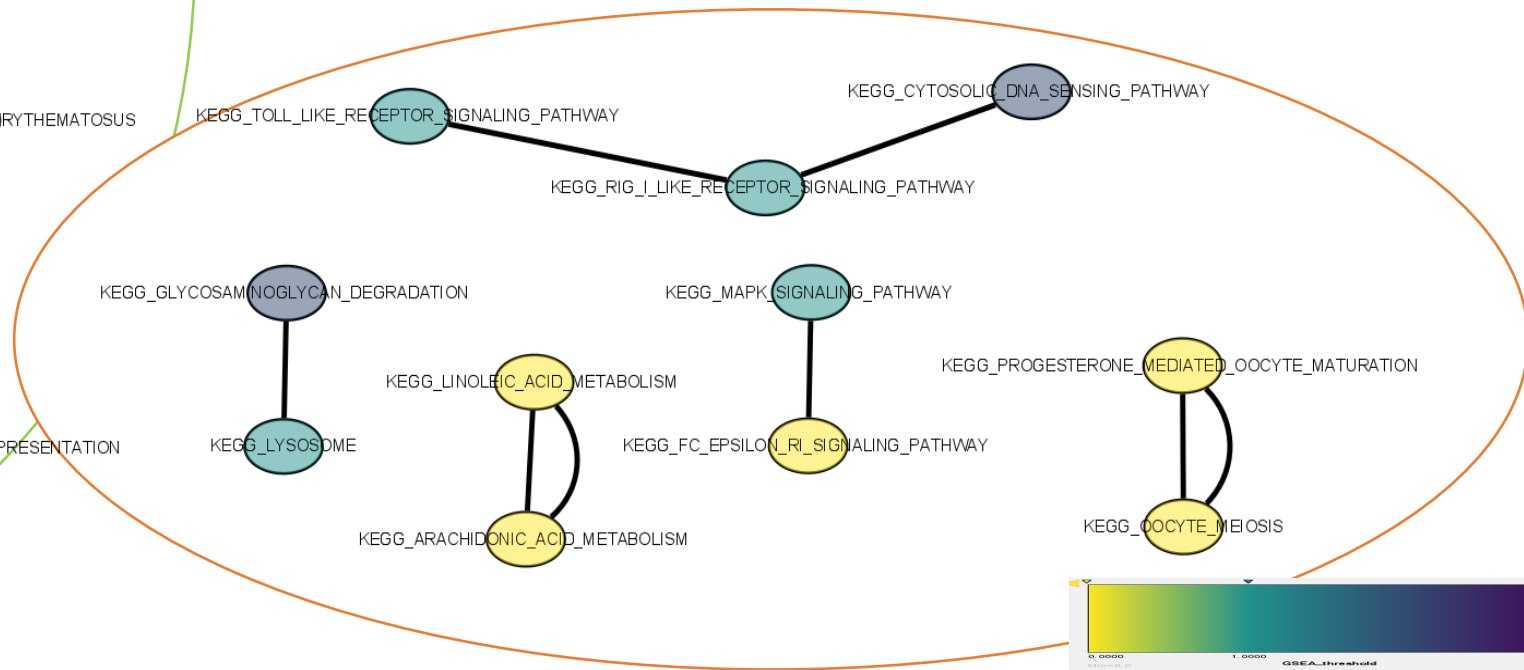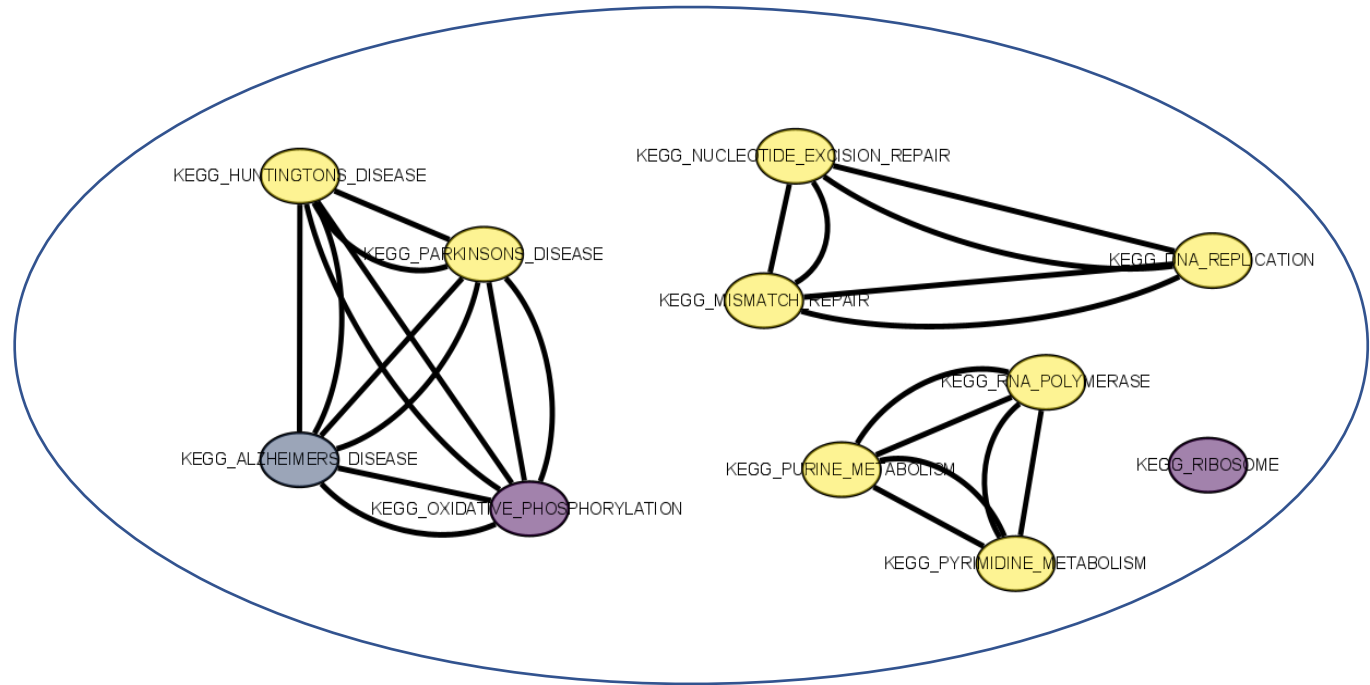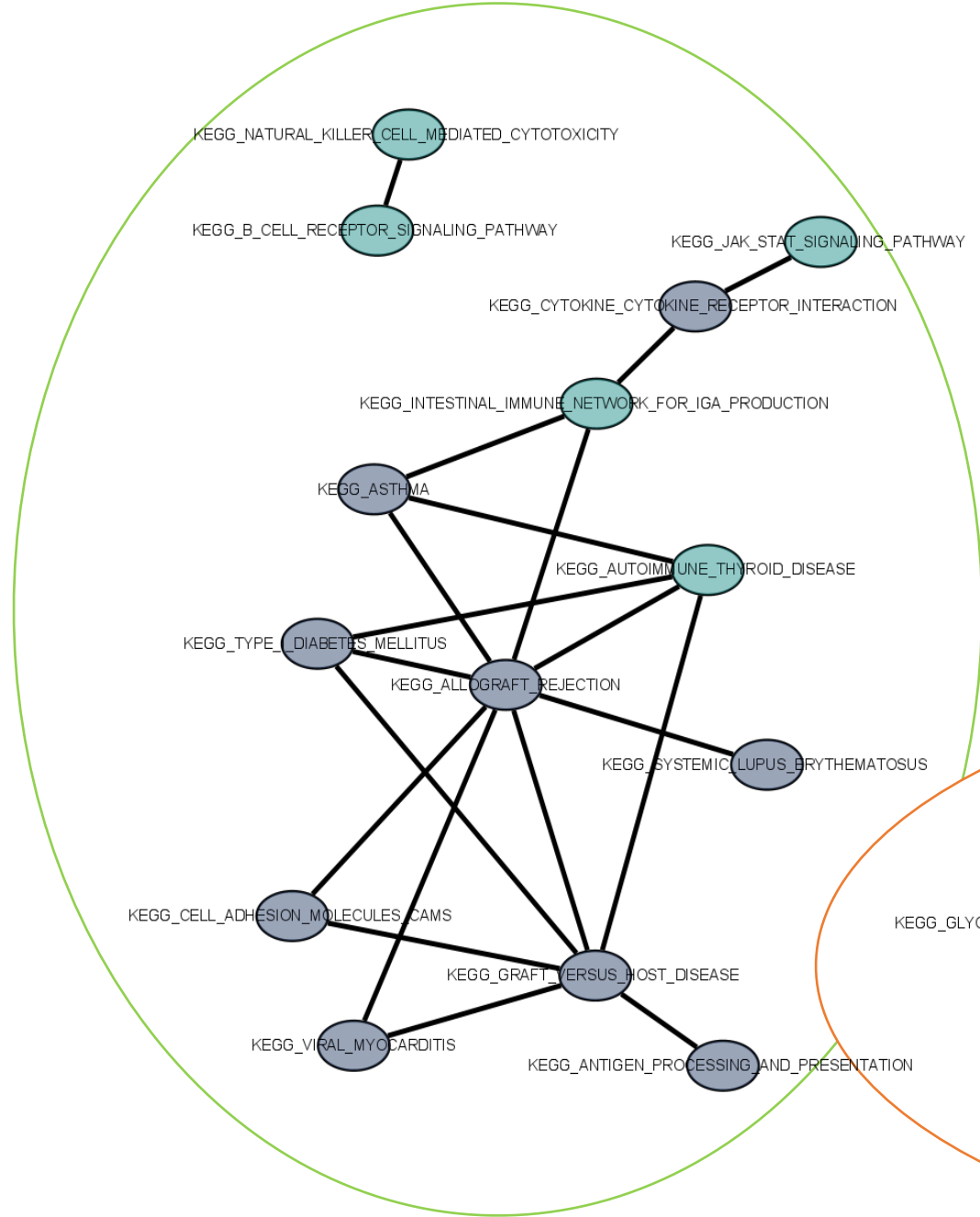# KEGG oxidative phosphorylation

KEGG ribosome

KEGG cell cycle

Network analysis

- Cytoscape enrichment map

Reference

- Hu, G., Tang, Q., Sharma, S. *et al.* Expression and regulation of intergenic long noncoding RNAs during T cell development and differentiation. *Nat Immunol* **14,** 1190–1198 (2013). https://doi.org/10.1038/ni.2712

- Germain, R. T-cell development and the CD4–CD8 lineage decision. *Nat Rev Immunol* **2,** 309–322 (2002). https://doi.org/10.1038/nri798

- Lee, HG., Cho, MZ. & Choi, JM. Bystander CD4^{+} T cells: crossroads between innate and adaptive immunity. *Exp Mol Med* **52,** 1255–1263 (2020). https://doi.org/10.1038/s12276-020-00486-7