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Supplemental information

Molecular predictors of response to pembrolizumab in thymic carcinoma

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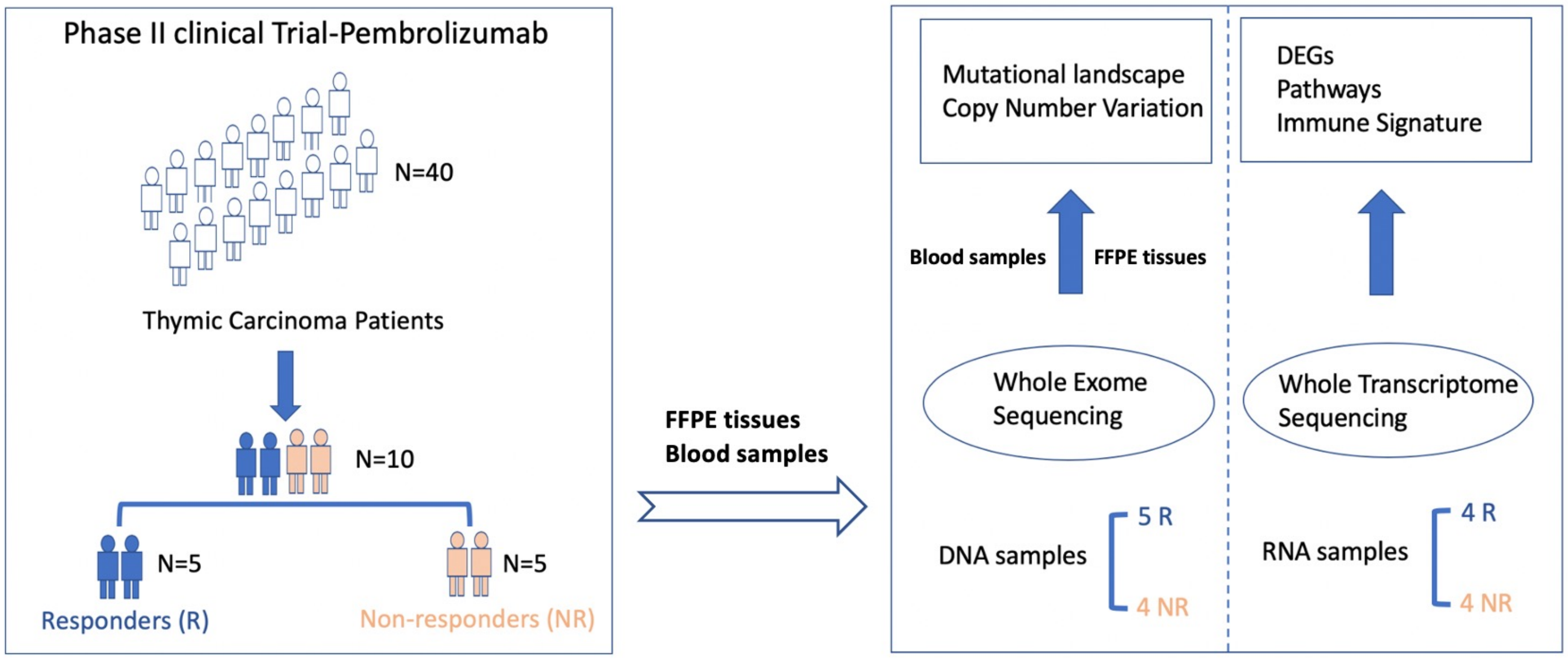


Figure S1. Scheme showing the experimental design. Related to Figure 1. 10 patient samples (5 responders and 5 non-responders) were selected from a single-arm single center phase 2 study of pembrolizumab in patients with recurrent thymic carcinoma. The selection of non-responders was also based on patients who either had progressive disease or short stable disease as best response. Tumor DNA and RNA samples were extracted from formalin-fixed-paraffin-embedded (FFPE) tissue blocks. Control DNAs were extracted from paired patients' peripheral blood samples. The samples that passed quality control were used for whole exome sequencing and whole transcriptome (RNA) sequencing. DNA samples: 5 responders vs 4 non-responders; RNA samples: 4 responders vs 4 non-responders.

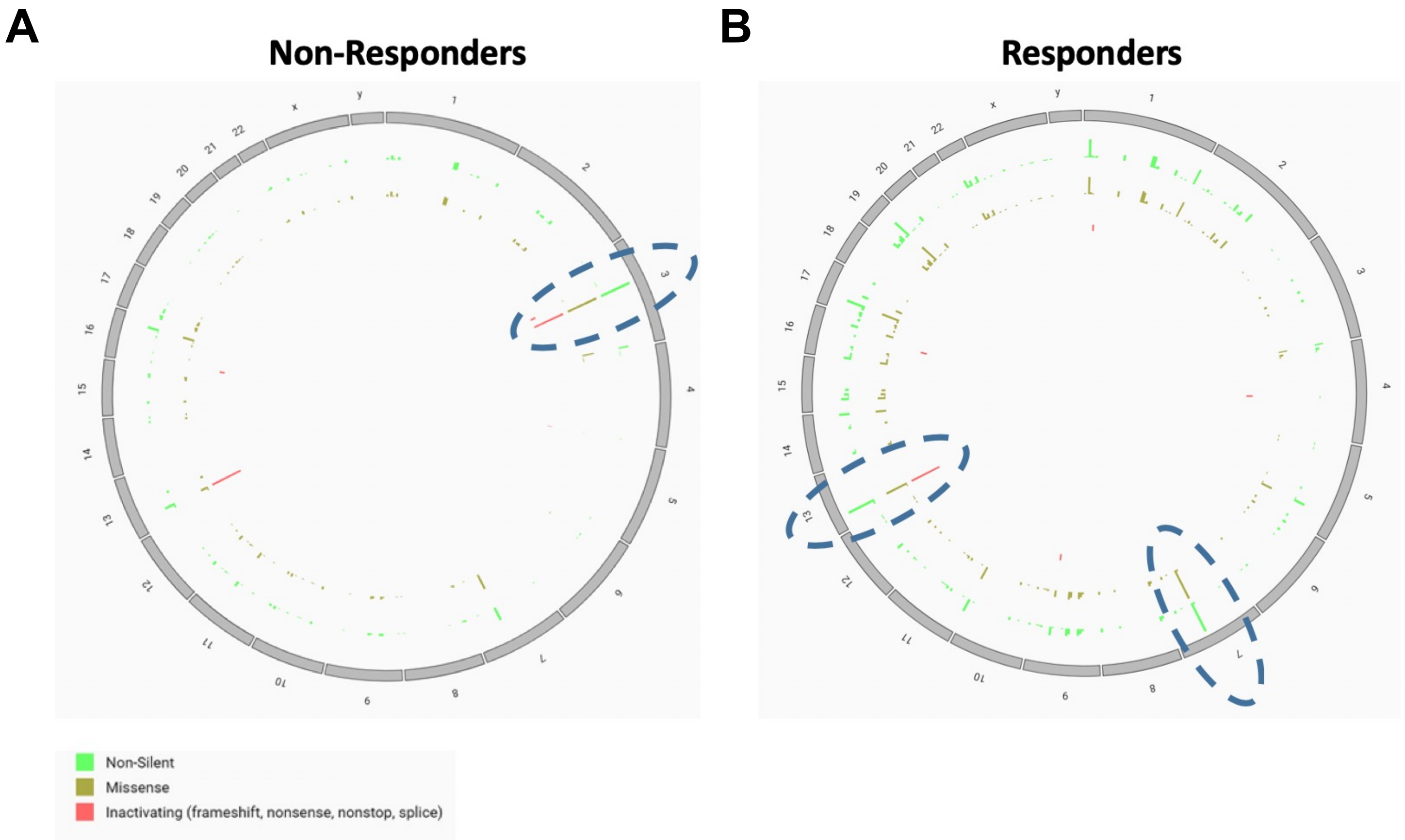
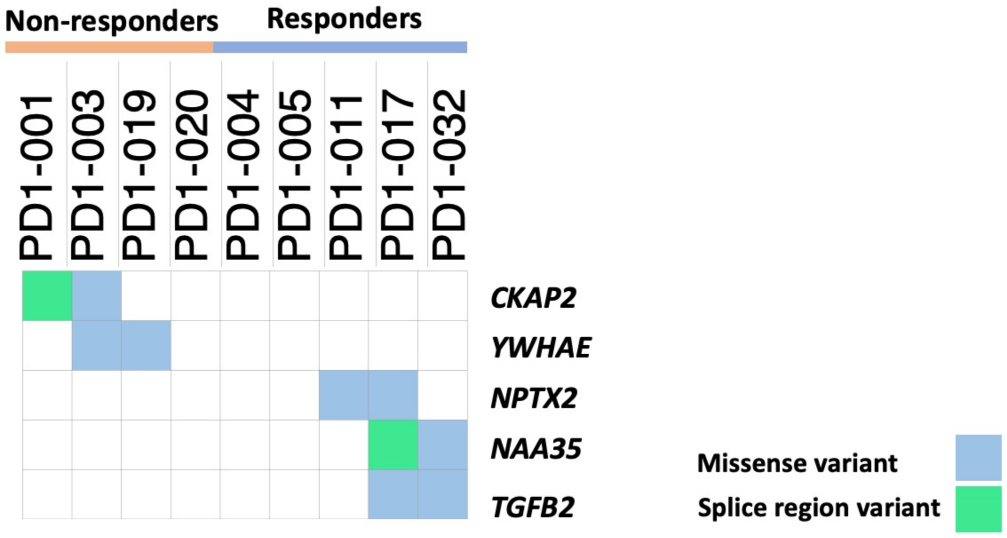


Figure S2. Circos plot showing distribution of mutations across the genome. Related to Figure 1. The Circos plot was generated by Cancer-Related Analysis of Variants Toolkit (CRAVAT)**.**



	PD1-001	PD1-003	PD1-019	PD1-020	PD1-004	PD1-005	PD1-011	PD1-017	PD1-032
CKAP2	🟩	🟦							
YWHAE		🟦	🟦						
NPTX2							🟦	🟦	
NAA35								🟩	🟦
TGFB2								🟦	🟦

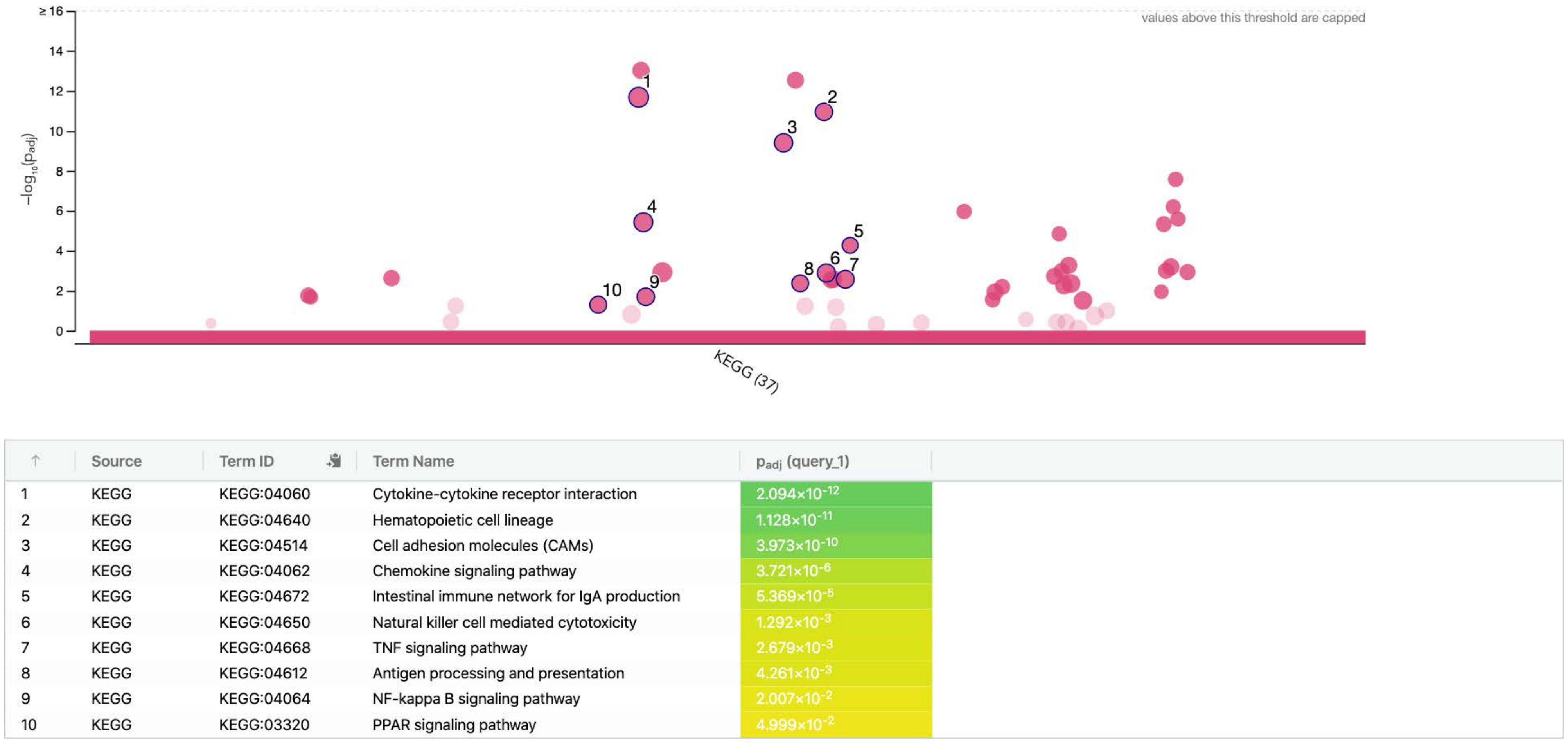
Missense variant 🟦
Splice region variant 🟩

Figure S3. Summary of recurrently mutated genes with unknow significance in the non-responders and responders. Related to Figure 2. Recurrently mutated genes in the non-responders include *CKAP2* and *YWHAE.* Recurrently mutated genes in the responders are *NPTX2*, *NAA35* and *TGFB2*.

A

ID	Source	Term ID	Term Name	p_{adj} (query_1)
1	KEGG	KEGG:04978	Mineral absorption	2.278×10^{-3}
2	KEGG	KEGG:04080	Neuroactive ligand-receptor interaction	2.659×10^{-3}
3	KEGG	KEGG:04971	Gastric acid secretion	8.628×10^{-3}

B



↑	Source	Term ID	Term Name	p_{adj} (query_1)
1	KEGG	KEGG:04060	Cytokine-cytokine receptor interaction	2.094×10^{-12}
2	KEGG	KEGG:04640	Hematopoietic cell lineage	1.128×10^{-11}
3	KEGG	KEGG:04514	Cell adhesion molecules (CAMs)	3.973×10^{-10}
4	KEGG	KEGG:04062	Chemokine signaling pathway	3.721×10^{-6}
5	KEGG	KEGG:04672	Intestinal immune network for IgA production	5.369×10^{-5}
6	KEGG	KEGG:04650	Natural killer cell mediated cytotoxicity	1.292×10^{-3}
7	KEGG	KEGG:04668	TNF signaling pathway	2.679×10^{-3}
8	KEGG	KEGG:04612	Antigen processing and presentation	4.261×10^{-3}
9	KEGG	KEGG:04064	NF-kappa B signaling pathway	2.007×10^{-2}
10	KEGG	KEGG:03320	PPAR signaling pathway	4.999×10^{-2}

Figure S4. Pathway analyses with KEGG database using gProfiler. Related to Figure 3. **A.** Enriched pathways in upregulated DEGs of the non-responders vs the responders. **B.** Enriched pathways in downregulated DEGs of the non-responders vs the responders. The pathways related to immune response and tumorigenesis are highlighted.

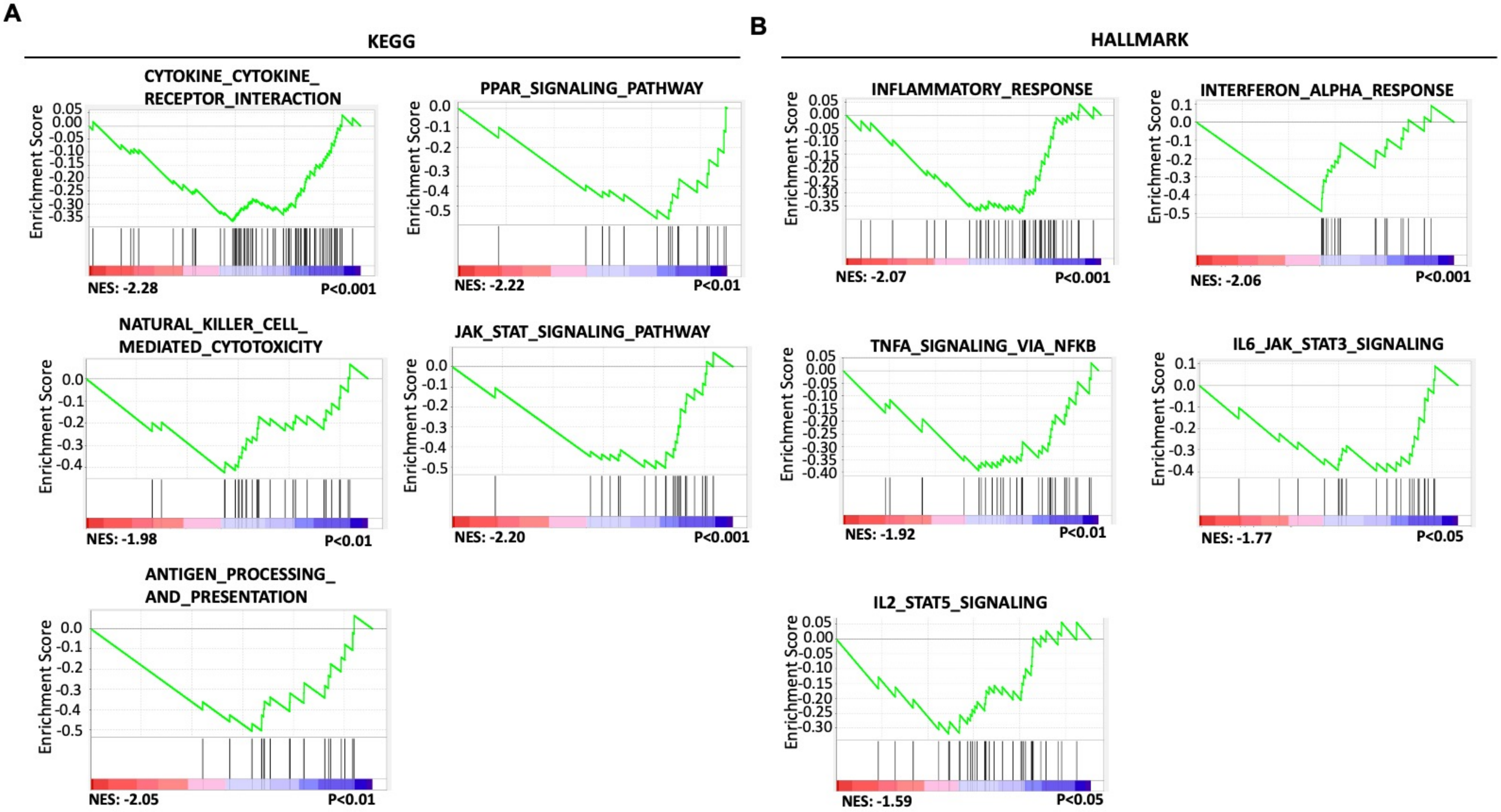


Figure S5. GSEA plots showing negative enrichment of KEGG and Hallmark pathways in pre-ranked DEGs of the non-responders vs the responders. Related to Figure 3. **A.** GSEA plots of enriched KEGG pathways. **B.** GSEA plots of enriched Hallmark pathways.

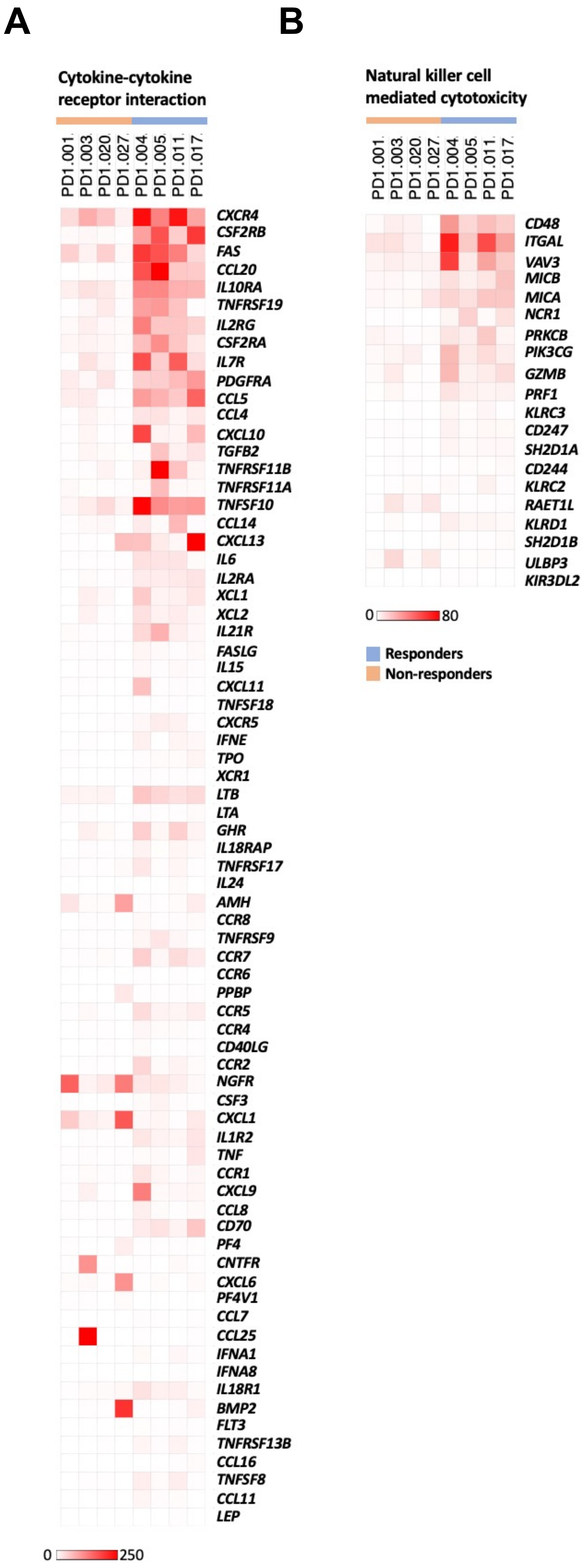
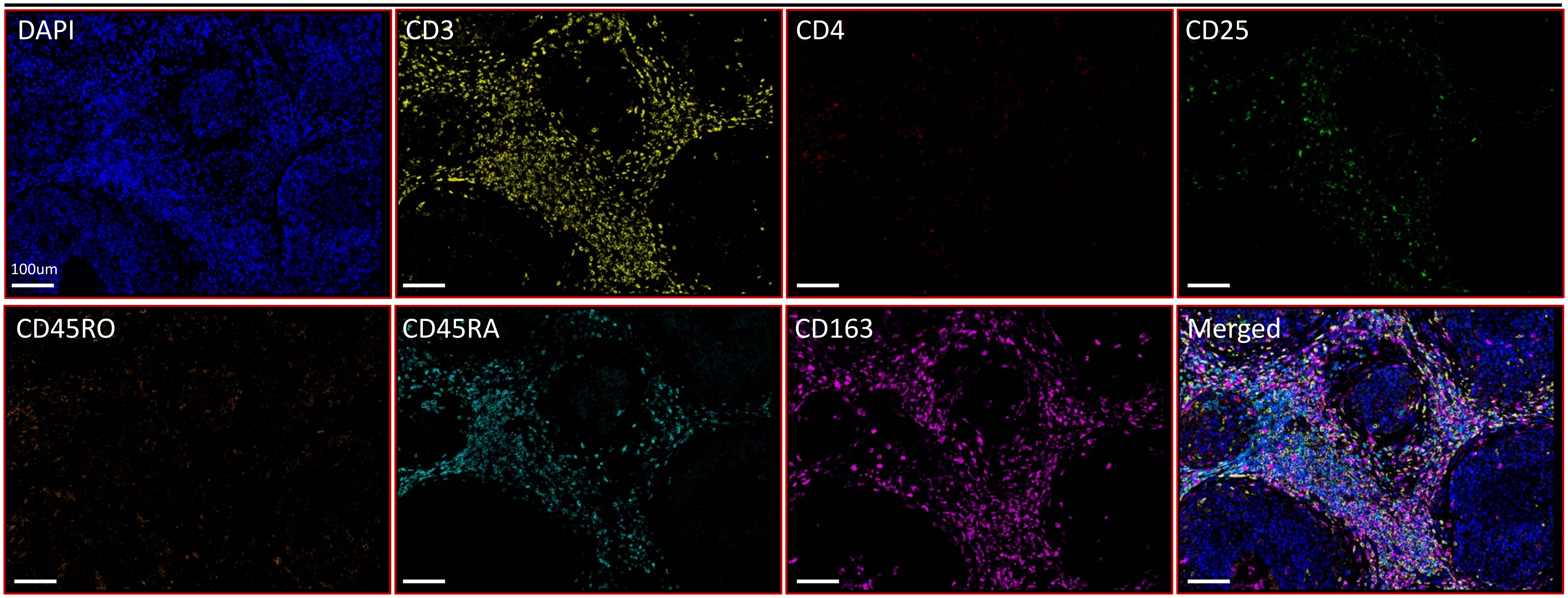


Figure S6. Heatmap of DEGs involved in the indicated pathways. Related to Figure 3. **A.** Heatmap of DEGs involved in cytokine-cytokine receptor interaction. **B.** Heatmap of DEGs involved in Natural killer cell mediated cytotoxicity.

The Non-responder



The responder

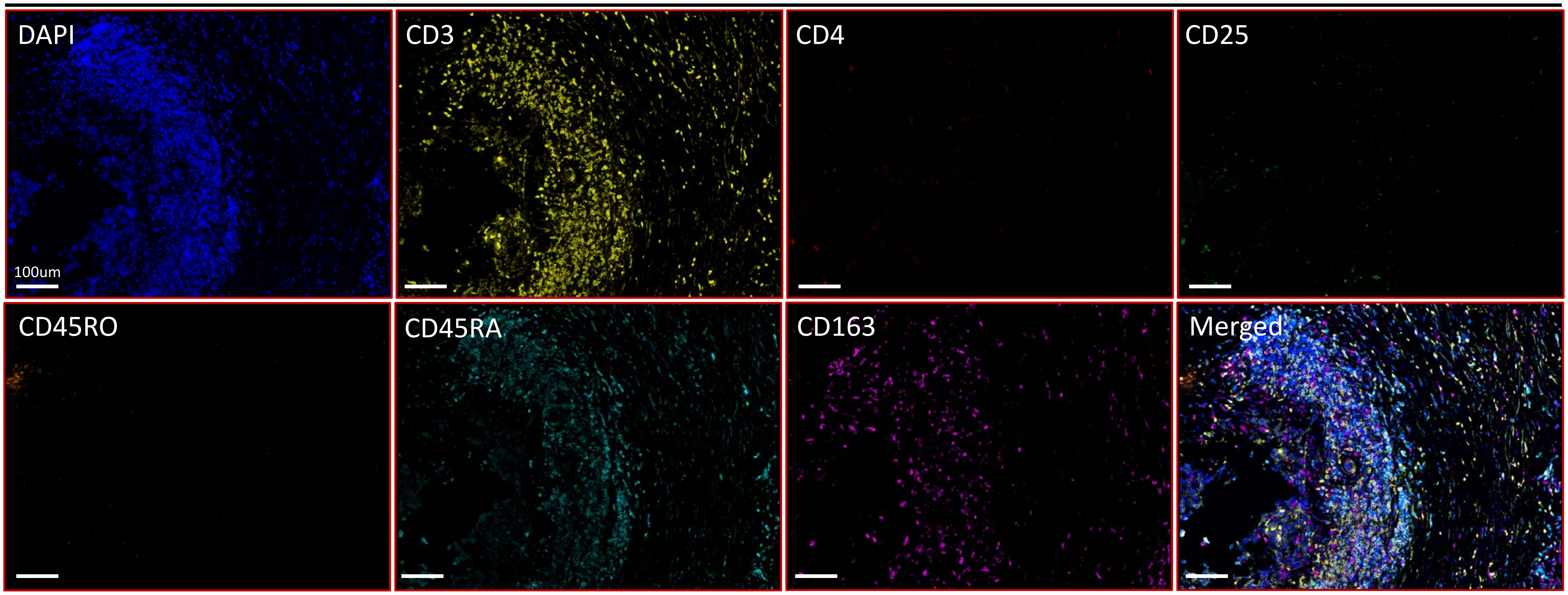


Figure S7. Representative images showing different types of immune cells in thymic carcinoma tissues from the non-responder and the responder. Related to Figure 4. Multiplex immunofluorescent staining was performed with CD3, CD4, CD25, CD45RO, CD45RA and CD163 antibodies, as indicated in the images.