Dissecting Immune Cell Stat Regulation Network Reveals Biomarkers to Predict ICB Therapy Responders in Melanoma

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Abstract

Background

Immunotherapy is a revolutionary strategy in cancer therapy but the resistance of which is one of the important challenges. Detecting the regulation of immune cells and biomarkers concerning immune checkpoint blockade (ICB) therapy is great significance.

Methods

Here, we investigated the CD45+ immune cell regulation networks and immunotherapy response features by integrating biological pathway data and single cell sequencing data in metastatic melanoma with or without ICB therapy. First, we constructed regulation networks for 11 predefined CD45+ cell stats in context with biology pathway. Furthermore, we identified differently expressed genes between responders and non-responders. Finally, we trained and validated a logistics regression model based on ligands and receptors in the regulation network to predict ICB therapy response.

Results

We discovered the regulation of genes across various immune cell stats. Functional analysis indicated that these stat-specific networks consensually enriched in immune response corrected pathways and highlighted antigen processing and presentation as a core pathway in immune cell regulation. Furthermore, some famous ligands and receptors like SIRPA, CD14, IL2, HLA-G, ITGAM and CD247 were differently expression between cells of responders and non-responders before and (or) during course of treatment. Several gene sets containing differently expressed ligands and receptors performed accuracy prediction with AUCs in validation datasets above 0.7 suggesting that they may be server as biomarkers for predicting immunotherapy response.

Conclusions

In summary, our study provided single cell-based regulation network in context of melanoma model with or without ICB therapy. Our analysis revealed several gene sets consist of therapy response biomarkers which is efficient for construction of ICB therapy response classifier.

Background

Immunotherapy raised a revolutionary weapon against cancer[1–7]. Notably, anti-CTLA4 and anti-PD-1 inheritors, ipilimumab and nivolumab, have achieved great increase in clinical benefit for carcinomas [8–10]. This provided a great sense to investigate mechanisms of cancer-immune cell specific interactions [11]. Therefore, it remains an urgent need to practice regulation analysis and data mining among various immune cell stats under context of on- or post-therapy in melanomas. By profiling of single immune cells in baseline and on- or post-therapy samples in melanoma patients treated with checkpoint therapy, Hacohen et al. has defined CD45 + T cell stats that associated with response to ICB therapy in metastatic

melanoma [12]. These ICB therapy related CD45 + T cell stats lead to another view that concerns regulation networks of different T cell stats and biomarker identification based on the cell stats specific active pathways. Computation of enriched function in immune single cells could perform a systemic insight to understand mechanisms immune cell interaction. Moreover, construction of immune cell stat specific pathways could offer contributions for immunology and ontology, including immune therapy response related research.

Even though oncology is being revolved by the remarkable success of ICB therapies, primary and acquired resistance also obstruct long-term curative-effect in patients with metastatic melanoma [13] since the majority of patients received immune checkpoint blockade (ICB) therapies unfortunately do not benefit from the treatment [13–16]. Currently, primary biomarkers of ICB therapies such as tumor mutational burden (TMB) and programmed death ligand 1 (PD-L1) expression [9, 17] have performed rough immunotherapy selection, and new biomarkers (e.g. eosinophilic count) show associations toward poor or longer survival [18]. There are several studies attempt to inquire into alterations in expression of the PD-1/PD-L1 immune inhibitory axis or tumor microenvironment in patients with melanoma [10, 17], as well as focused on heterogeneities of individual cells by single-cell RNA sequencing [12, 19], for predicting of response to therapy. However, these researches have thus far provided only a limited understanding, and novel potential biomarkers and prediction model for immunotherapy response are urgently needed. Furthermore, the molecular interactions are key factors for immune cell activity and therapy response. Here, we constructed regulation networks based on specific immune cell stat, dissected transcriptome features of multiple single-cell cluster binding pathways and investigated predictive capability of single-cell-based network model. Our observation showed that ligands and receptors in immune cell related regulation networks have selective power of therapy response in patients receiving ICB inheritors.

Materials And Methods

Materials

CD45+ single cell sequencing Immune cell high throughput sequencing matrix of melanoma we used in this study was accessible in GEO database (GSE120575) which profiled 16,291 CD45+ immune cells from 48 tumor samples[12].

Pathways As reference pathways of our networks, BioPAX [20] level 3 integrated pathways were downloaded from https://www.pathwaycommons.org/archives/PC2/v10/. The common pathways contain 2374707 interactions of 32875 participants integrated as 13 types (e.g., controls-expression-of, in-complex-with).

mRNA sequencing of bulk tumor samples mRNA expression of 56 MAGE-A3 checkpoint inhibitor treated melanomas was downloaded from GEO database (GSE35640). And 22 of these patients had complete or partial response for immunotherapy [21].

Others Homo species transcription factor (TF) were downloaded from AnimalTFDB [22] (http://bioinfo.life.hust.edu.cn/AnimalTFDB/#!/). Paired ligands and receptors were obtained from FANTOM [23] (http://fantom.gsc.riken.jp/5/suppl/Ramilowski_et_al_2015/) database.

Methods

Integration of regulation networks across immunity cell cluster specific networks

Each of the 16291 immune cells were previously assigned into one of 11 unsupervised clusters according to the study of Sade-Feldman M et al. [12]. Among those 11 clusters, C1 and C2 tend to be enriched by B cells, C3 and C4 are myeloid clusters and C6 and C9 are T cell clusters [12]. For each cluster, we constructed an immune cell cluster specific network considering following aspects, in other words, two genes were interacted in our networks under three conditions, a) each of the two genes must transcript in more than 1% immunity cells; b) there must be an interaction type in BioPAX pathways between them; c) co-expressed p-value (spearman correlation test) of these two genes must under 0.05(Figure S1).

We combined 11 immunity cell cluster specific networks into regulation networks and annotated it with TF, ligands and receptors (Figure 1, Figure S1).

Topological properties evaluation of cell cluster specific networks and regulation networks

According to Barabási AL etc. [24, 25] and Junpeng Zhang etc. [26], real biological gene interaction lies in scale-free networks, and degree distributions of genes in the network should comply with power law distribution (Eq. 1). We fit liner model for logarithm transformed gene degrees(X) and their distributions(Y) in a derived formula (Eq. 2) where a and b are coefficients to fit.

$$Y = aX^{-b} \qquad \text{[Eq. 1]}$$

$$log_{10}Y = -blog_{10}X + log_{10}a \qquad \text{[Eq. 2]}$$

Identifying differently expressed genes

In each case, we divided cells into four groups: 1) cells from pre-treatment responders; 2) cells from pre-treatment non-responders; 3) cells from on-treatment responders; 4) cells from on-treatment non-responders. Then we calculated two measurement for responder and non-responder groups to access different expression - fold changes and Wilcox test p values. Finally, we used 2-fold and 0.05 significance as thresholds to define differently expressed genes.

Immunotherapy decision classification based on regulation networks

We investigated predictive power of regulation networks in immunotherapy resistance by following steps: 1) fit logistic models - ligands and receptors in regulation networks were used as origin variables for model fitting in train data sets (half of GSE120575 samples selected randomly); 2) choose variables - formula-based model auto-selection were applied to select a subset of ligands and receptors along with their coefficients that can optimally guide therapy outcomes; 3) test precision - variable sets we chosen in step 3 were tested in another half of GSE120575 samples; 4) repeat circulation - resultful step 1 to 3 were repeated 10000 times.

Results

Regulation networks of immunity single cell stats in melanoma

Tumor cell-intrinsic heterogeneity shapes the immune cell infiltration and influence the outcome of immunotherapy [27], and various metabolic pathways orchestrate the behavior of tumor-infiltrating immune cells, which related to enhancing of antitumor immunity and immunotherapy [28]. Moreover, distinct CD45 + cell clusters revealed by single-cell RNA-seq associated with clinical outcome of ICB therapies and reflected by different identifiers [12]. Thus, observation of pathway activities in multiple cell stats could offer both intra- and inter-cellular immunity regulation. Herein, we constructed immunity networks based on BioPAX pathways for previously defined CD45 + cell clusters [12] and combined them into regulation networks (Fig. 1, Figure S2, Method Details). We fitted power law models for degree distributions of cell cluster specific networks and regulation networks (Figure S3, Method Details), and the R squares greater than 0.88 all over these models. Immune networks and regulation networks constructed by our pipeline are scale free and similar to the real biological networks.

In sum, seven hundred and ninety genes and 3048 interactions, including 26 receptors, 26 ligands, and 47 TFs were recognized by the regulation networks. Notably, IL-2 receptor is T cell stimulating cytokine [29], and it not only drives the expansion of T cells and the contraction phase of immune response [30], but also has an effect on cancer stem cells [31, 32]. Most impotently, low dose IL2 combined with other immunotherapy demonstrates benefit in patients with metastatic melanoma [33]. In C10, a memory T cell cluster, IL2 controls expression of KLRK1 which controls state change of ITGAM through CD247(in complex with HLA-C, HLA-E and HLA-G in all 11 cell clusters). IL2 controlling ITGAM, which in complex with CD14 (both are cell surface markers[34], indicted that our framework efficiently highlighted regulation flows of immunity system in distinct and common immune cell stats.

Divergence regulation of overlap genes were revealed by network comparative analysis among multiple networks

Since our study highlighted different interaction flows of cluster specific networks, we accessed expression of genes and interaction pairs in distinct immune cell stats. There are several genes (86) such as major histocompatibility complex (HLA-C, HLA-DMA, HLA-DMB, HLA-DRA, HLA-E, HLA-G and HLA-H),

CD247, cyclin dependent kinase (CDK11B), casein kinase 2 (CSNK2A1 and CSNK2B), FGFR1 oncogene partner (FGFR1OP) etc. that were activated in all 11 predefined T cell stats (Fig. 2A). However, consensus interactions among those common genes are partly observed. On the contrary, obvious inconsistent interaction pattern among common genes were discovered in B cell clusters (C1, C2), myeloid clusters (C3, C4) and CD8 T cell clusters (C6, C9) ([12], Fig. 2B, Figure S4). Even though identifiers of distinct immune cell stat differ from the others, there are common genes, which exercise functions through different flows, that were activated in multi-networks. For instance, mechanistic target of rapamycin kinase (MTOR) state change was controlled by HLA-G in C9 but not in C6, and MTOR controls state change of HSPA1B in C9, TOP1 and PRNP in C6 (Fig. 2C). STAT2 interacts with different genes in C6 (HLA-G) and C9 (MTOR, PIK3CD). Moreover, a specific duplex interaction was observed in C9 (TRAC controls state change of CD247, CD247 in-complex-with TRAC, Fig. 2C). These results provide further evidence for our explanation that common genes from different cell stats can active variety ways in these cells.

Interleukin-2 (IL-2) antigen stimulate memory CD8(+) T cells production, and high relative IL-2 production in T cells of melanoma tend to perform memory CD8(+) T cells phenotype and superior proliferative capacity compared to cells with low IL-2 production [35]. In our research, IL2 and LIME1 specifically activated in a memory T cell cluster (C10). We also calculated different expression of IL2 in C10. IL2 gene significantly expressed higher in cells from responders than cells from non-responders of on-treatment patients. Furthermore, several genes activated distinctly in different networks. For instance, PVRIG specialized in lymphocytes(C5), AGER, BHLHB9, CDK3, GNG8, IL11RA, PKIA, USP50 in regulatory T cells(C7). ARHGAP19, FNIP1 in exhausted/HS CD8 T cells (C9) and DECR2, SESTD1, ZNF775 in exhausted CD8 T cells (C6) etc. Our framework identified different performance of immune related genes in cell cluster specific networks, which may lead a functional nuance.

Consensus functions across immune cell regulation networks

To investigate functional relevance of cell cluster specific networks, we employed online pipeline metascape (http://metascape.org/gp/index.html) for enrichment analysis. We found that gene sets from plural networks enriched in consensus functions, especially in metabolism of RNA, antigen processing and presentation, cell cycle and herpes simplex infection, regardless the different activation flows they presented (Fig. 3). Significantly, all networks partly enriched in several sub-terms of vital functions like T cell activation, cell cycle, cellular responses to stress, apoptotic signaling pathway, cytokine-mediate signaling pathway, herpes simplex infection, metabolism of RNA and so on [36–40]. As for some functions, such as negative regulation of immune system process, are enriched by all networks but in different sub-terms. In addition, exhausted CD8 T cell cluster (C6) and lymphocytes exhausted/cell-cycle (C11) cluster significantly enriched in regulation of complement activation overall situation, yet regulation of complement activation was not enriched by monocytes/macrophage cluster(C3), cytotoxicity (lymphocytes) cluster (C8) and exhausted/HS CD8 T cell cluster (C9). Several sub-terms of negative

regulation of immune system process enriched by different networks despite all eleven networks enriched in negative regulation of immune system process by a significant level.

A core pathway: antigen processing and presentation

We discovered that shared topology of 11 networks covered two major histocompatibility complex union (HLA-C, HLA-E, HLA-G; HLA-DMA, HLA-DRA, HLA-DMB), one ribosomal protein union (RPL10A, RPL9P7, RPL39, RPL12, RPL36A, RPS29, RPS9), one mitochondrially encoded NADH: ubiquinone oxidoreductase core subunit (MT-ND2, MT-ND4L, MT-ND5), one NME/NM23 nucleoside diphosphate kinase union (NME1, NME1-NME2) and one proteasome and proteasome activator subunit (PSME1, PSMB8, PSMB9) (Fig. 4A). Enrichment analysis of these shared topologic structure showed concurrent functions like regulation of expression of SLITs and ROBOs, antigen processing and presentation of exogenous peptide antigen, antigen processing and presentation peptide antigen assembly with MHC class II protein complex and neutrophil deregulation (Fig. 4B, 4C). Especially, subunits of the shared structures lie in two flows of antigen processing and presentation, MHC I and II pathways ([41], Fig. 4D), which play upstream roles of CD8 T cell killing target cells, regulation of NK cell activity (MHC I) and CD4 T cell cytokine production and activation of other immune cells (MHC II). According to Gene Ontology [42] enrichment analysis executed by metascape, three antigen related functions are connected from each other, and the most significant enrichment is antigen processing and presentation.

Key genes from networks were related with immunotherapy response at single cell level

Prior knowledge showed that a large number of genes appeared relevance with not only cancer occurrence but also treatment response, and these appearances came up in the single cell level as well [12, 21, 43–45]. Expectably, there are a batch of genes in our regulation networks that differently expressed between responders and non-responders in both period of treatment (pre- and on-treatment) regardless of immune cell stats (Fig. 5A). Functional analysis showed that genes expressed higher in non-responders from on-treatment samples were enriched in cytokine-mediated singling pathway, transmembrane receptor protein tyrosine kinase signaling pathway and so on, yet different expression genes form pre-treatment samples located in immune response-activating signal transduction, response to toxic substance etc. Interestingly, several ligands and receptors differently expressed between responders and non-responders in pre- or (and) on-treatment samples, especially for a higher-in-non-responder set (CCL7, CCL18, MRC2, FPR2, LILRA3, SIRPA and CD14). Moreover, we detected a ligand, SIRPA, significantly expressed higher in non-responders and in pre-treatment samples. Consequently, SIRPA may play a resistance role in immunotherapy [46–48], and a triple element, which consisted with CAV1, SIRPA and CD14 and expressed higher in non-responder, may potentially support drug antagonism([46–51], Fig. 5B-C).

Although we detected dysregulated genes in the single cell level, it still needs further inspection to uncover transcriptome changes in immune cell stats. Therefore, we next assessed differently expressed

genes from cluster specific networks in corresponding CD45 + immune cell stats (Fig. 5D, Figure S5-6). Our research suggested that ligand integrin subunit alpha M (ITGAM) was significantly upregulated in C3 from non-responders of on-treatment patients, in C1 and C10 from non-responders of pre-treatment patients, as well as in C8 from responders of pre-treatment patients. Regulating cell fate, ITGAM was dysregulated in multiple cell type (T cells, B cells and so on), which may contribute to tumor cell survival in immunotherapy [52]. ligand NTRK1, which expressed higher in pre-treatment non-responders, was dysregulated in C10, and IL2, a type I cytokine which can be associated with durable regression in metastatic melanoma and renal cell carcinoma [53], also was dysregulated in and only in C10 but expressed higher in on-treatment responders. Two major histocompatibility complex, HLA-G and HLA-H, expressed higher in C2 from pre-treatment non-responders. Another major histocompatibility complex, HLA-DMB, expressed higher in both C2 and C10 in non-responders from on-treatment samples. Other famous genes, such as tumor necrosis factor receptor superfamily member 9 (TNFRSF9) and CD247 ligands, was also upregulated in C3 from (non-)responders of on-treatment patients, and another tumor necrosis factor receptor superfamily member (TNFRSF12A) was dysregulated in both C3 and C9. Thus, we have reason to suppose that some ligands and receptors may propose a new insight to understand drug sensitivity and resistance, as well as immunotherapy response.

Ligands and receptors of regulation networks showed robust selective power in immunotherapy response

Some receptors can mediate functions of immune cells through distinct signaling pathways [54]. Changing of these receptors and corresponding ligands may lead unexpected immunotherapy outcomes. Our research also observed massive change of gene expression of these proteins. Hence, we applied logistics regression model to select contribution features from ligands and receptors of regulation networks and access immunotherapy precision of featured gene sets. In the test data sets, medium AUC of 10000 random is 0.7143, and most of the test AUCs are among 0.65 and 0.8 (Fig. 6A). Eventually, we identified 17 genes sets that associated with immunotherapy response. In an independent validation, GSE35640, all 17 gene sets performed AUC greater than 0.7 (Fig. 6B), which suggests robust selective power of ligands and receptors of regulation networks for immunotherapy response. This completed key gene analysis by providing gene sets and their scores which able to construct classification of therapy response.

Several TNF receptor superfamily members are integral to the immune-response regulation by enhancing T-cell growth and dendritic-cell function. These proteins related to modulation of cellular functions, proliferation, survival or deaths [55]. Moreover, TNF receptors controlling TNF receptor signaling, which play its role in inflammation and cell death, could determine the cellular fate [56]. Especially, TNFRSF12A (also known as TWEAK receptor, Fn14, or CD266) correlated with integrin β3 expression, which drives Glut3 expression, associated with clinical outcome and tend to be responsible for inducing cachexia in tumors [57, 58]. We identified one TNF receptor leaded gene set, consisting with TNF receptor superfamily member 9/25/12A (TNFRSF9, TNFRSF25 and TNFRSF12A), sphingosine-1-phosphate receptor 1

(S1PR1), C-C motif chemokine ligand 3 (CCL3), caveolin 1 (CAV1) and major histocompatibility complex, class I, G (HLA-G), that predicted immunotherapy response with AUC up to 0.7433 in independent validation and 0.8824 in GSE12575 (Fig. 6C). Besides, CAV1 catalysis precedes of resistance related gene CD14 [51, 59], as well as supported a firm set predicting immunotherapy response (0.7045 in GSE35640, 0.871 in GSE120575) with CD247, HLA-C, HLA-E, ITGAM and CD14. Thus, TNFRSF12A, which works for 11 of 17 selected gene sets, cooperating with CAV1 and other ligands and receptors are key factors in immunity regulation and flexible immunotherapy response (Fig. 6D).

Additionally, other gene sets with major histocompatibility complex (HLA-C, HLA-E, HLA-G), TNF receptors, ITGAM, CD247 and CD14 performed acceptable precision with AUC around 0.72 in the independent datasets and above 0.85 in GSE12575 as well. Furthermore, ITGAM, which regulate cell fate [52], also works 11 of 17 selected genes sets and HLA-C works in 9 Fig. 6E). These genes not only activate in majority immune cells but also have a quality for prediction of immunotherapy response. Our results indicate that using ligands and receptors in the regulation networks to train decision models could provide a brand-new view for immunotherapy response, and these models could be potential guidance for precision medicine.

Discussion

Great progress has been achieved in ICB therapy, yet therapy resistance must be considered in an actual treatment process. Consequently, it is important to investigate biomarkers of immunotherapy especially in single cell level. In this study, we constructed regulation network across immune single cell types and established that differently expression genes between cells from responders and non-responders. Some differently expression genes coincided with ligands and receptors in immune cell specific pathways. Further analysis of ligands and receptors in regulation networks proposed prediction biomarkers for inhibitor response classifier in metastatic melanoma with ICB therapy. We trained logistics regression models to test prediction accuracy of biomarkers for immunotherapy response. Our results suggested 17 gene sets that could be useful for prognosis of ICB therapy.

We find out that metabolism of RNA, antigen processing and presentation, cell cycle and herpes simplex infection networks were generally activated in all immune cell clusters but with different interaction flows. Our research also dissected that different sub-terms of negative regulation of immune system process were enriched by variety cell cluster specific networks even all the cell stats activated negative regulation of immune system process. Notably, we discovered a common topologic structure of all immune cell specific networks performing antigen processing and presentation function, which connected with antigen processing and presentation of exogenous peptide antigen and peptide antigen assembly with MHC class II protein complex. These three terms were essential for endogenous simulated immunity defense with cell surface MHC molecular carry and display viral peptides [60]. It requires an army of genes to coordinate for immune response and raise a weapon against tumor. MHC class I and class II molecules played global relative role in presentation and processing of the antigen with its high polymorphic [61]. For MHC class I, we detected that HLA-C respectively interacts with HLA-G and HLA-E.

And for MHC class II, we find a triple complex relationship among HLA-DMA HLA-DMB and HLA-DRA. These results indicated that antigen processing and presentation may be a core functional region in immune cell regulation and proposed further explanation of immune response in ICB therapy.

We further revealed some key factors in the immune therapy response. Immune features such as SIRPA, CD14, IL2, ITGAM and CD247 were significantly upregulated in (non-)responders from global or cell cluster specific view. These ligands and receptors tend to associate with drug sensitivity and resistance, as well as immunotherapy response. It is noteworthy that IL2 controls expression of KLRK1 which controls state change of ITGAM through CD247. IL2 controlling ITGAM, which in complex with CD14(both are cell surface markers, [34]), are specific in C10 and this type I cytokine can be associated with durable regression in metastatic melanoma and renal cell carcinoma [53]. In this study, biomarker gene sets detected by CD45 + immune single cells showed robust perspective power in ICB therapy response. Our results suggested that TNF receptors, MHC molecules, ITGAM, CD247 or CD14 leaded gene sets can preciously distinguish the responders for non-responders in patients with melanoma that received ICB therapies. The accuracy of immune cell regulation network-based model may provide helpful guidance for precision medicine, as well as new understanding of immunotherapy response. Specifically, SIRPA and CD14 were upregulated in non-responders of both pre- and on-treatment patients. They both regulate TRIM27, and CD14 contribute as a member of prediction gene sets in the logistics models. CD14 coordinate with ITGAM, CD247 and MHC molecules not only significantly dysregulated between the responders and non-responders of patients with melanoma but also presented accuracy prediction of immunotherapy response. Several famous genes like MHC molecules (HLA-C/E/G), TNF receptor superfamily members (TNFRSF9/12A/25), ITGAM, CD14, CCL3 and CAV1 were all performed dysregulation between responders and non-responders in the global or immune cell stat specific context, and they also provided great independent cooperation in prediction of immunotherapy response. Our research could provide a new view for immunotherapy response prediction, and these models could be potential guidance for translational medicine and precision medicine.

Conclusion

We constructed regulation network across immune single cell types and established that differently expression genes between cells from responders and non-responders. In sum, 790 genes and 3048 interactions, including 26 receptors, 26 ligands, and 47 TFs were recognized by the regulation networks. Some differently expression genes coincided with ligands and receptors in immune cell specific pathways. Further analysis of ligands and receptors in regulation networks proposed prediction biomarkers for inhibitor response classifier in metastatic melanoma with ICB therapy. We trained logistics regression models to test prediction accuracy of biomarkers for immunotherapy response. In summary, our study provided single cell-based regulation network in context of melanoma model with or without ICB therapy and revealed several gene sets consist of therapy response biomarkers which is efficient for construction of ICB therapy response classifier. These results could provide a new view for immunotherapy response prediction, and these models could be potential guidance for translational medicine and precision medicine.

Abbreviations

immune checkpoint blockade (ICB); programmed death ligand 1 (PD-L1); transcription factor (TF); Interleukin-2 (IL-2).

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Availability of data and materials

All data and codes in this study are available under proper request, please contact lixia@hrbmu.edu.cn.

Competing interests

The authors declare no conflict of interest.

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Authors' contributions

Conceptualization, Xia Li, Yanjun Xu and Yunpeng Zhang; Data curation, Jingwen Wang, Xuan Zheng and Chunlong Zhang; Formal analysis, Jingwen Wang and Feng Li; Funding acquisition, Xia Li; Investigation, Jingwen Wang and Feng Li; Methodology, Jingwen Wang and Yanjun Xu; Project administration, Yunpeng Zhang; Supervision, Yanjun Xu, Xia Li and Yunpeng Zhang; Validation, Xuan Zheng, Chunlong

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Figures

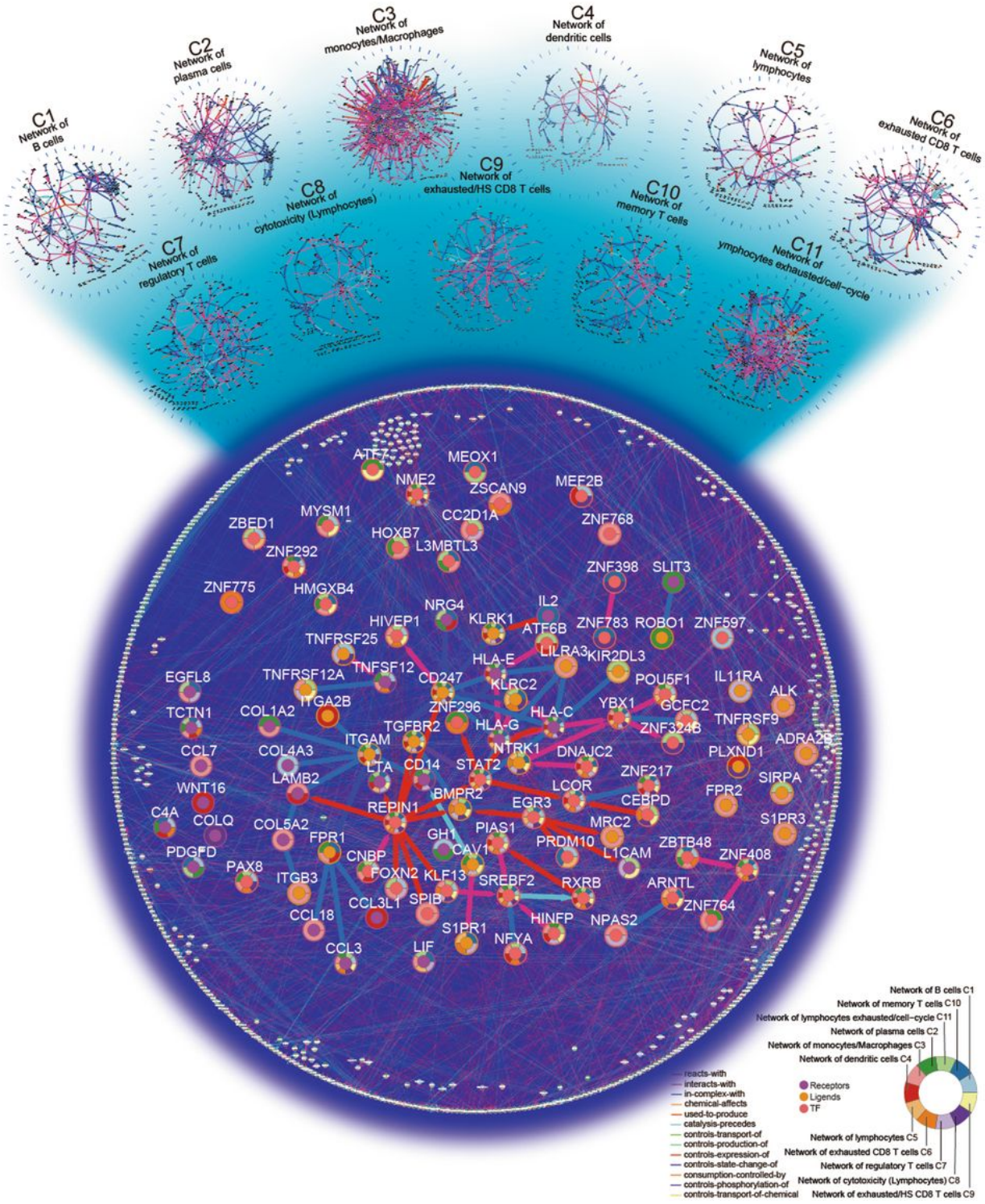
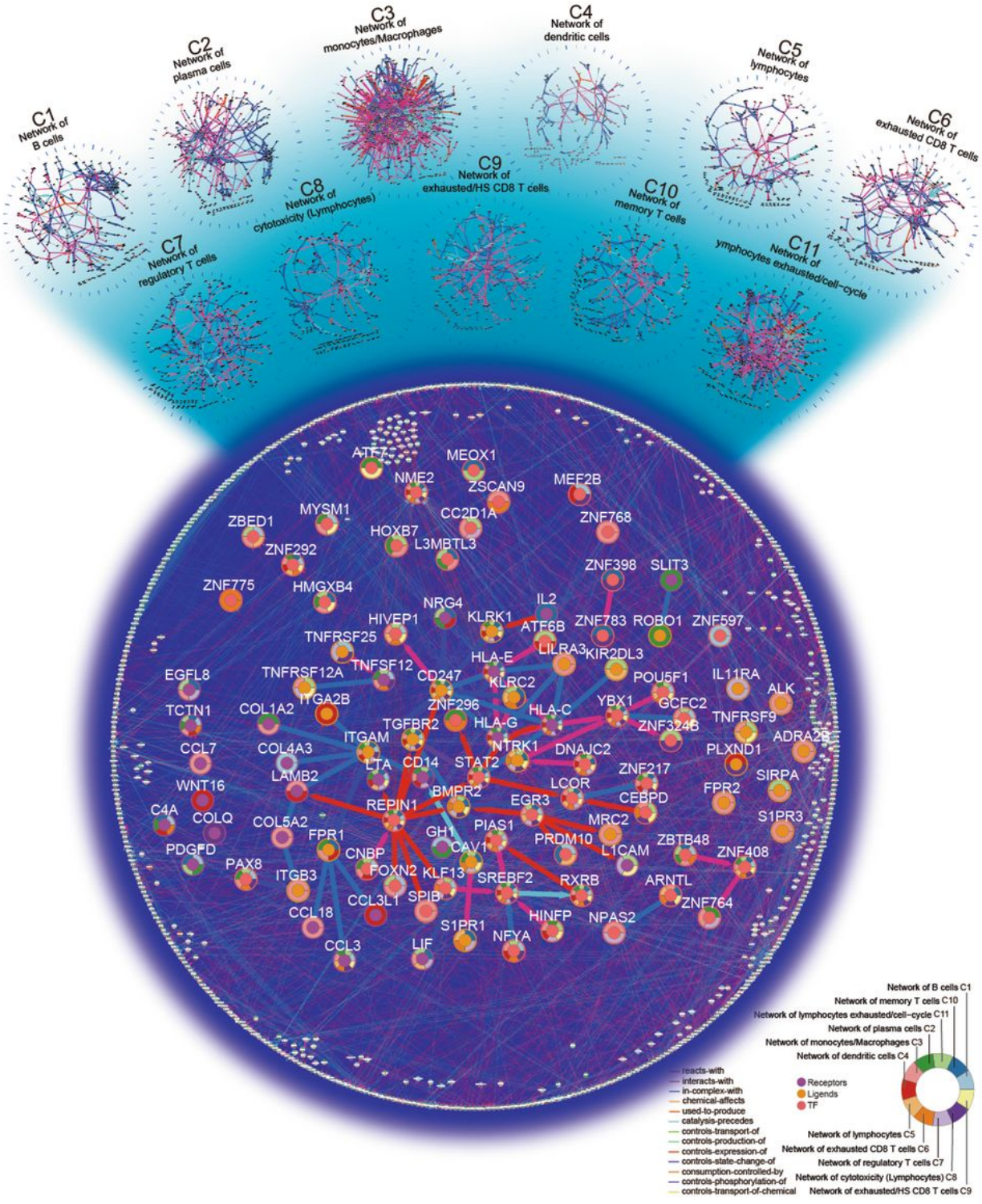


Figure 1

Global pathways of immunity single cell clusters under melanoma. Magnified nodes are TF, ligands and receptors; Bold edges are interaction among them. Float circle charts over nodes are colored by the 11 immuno-networks.



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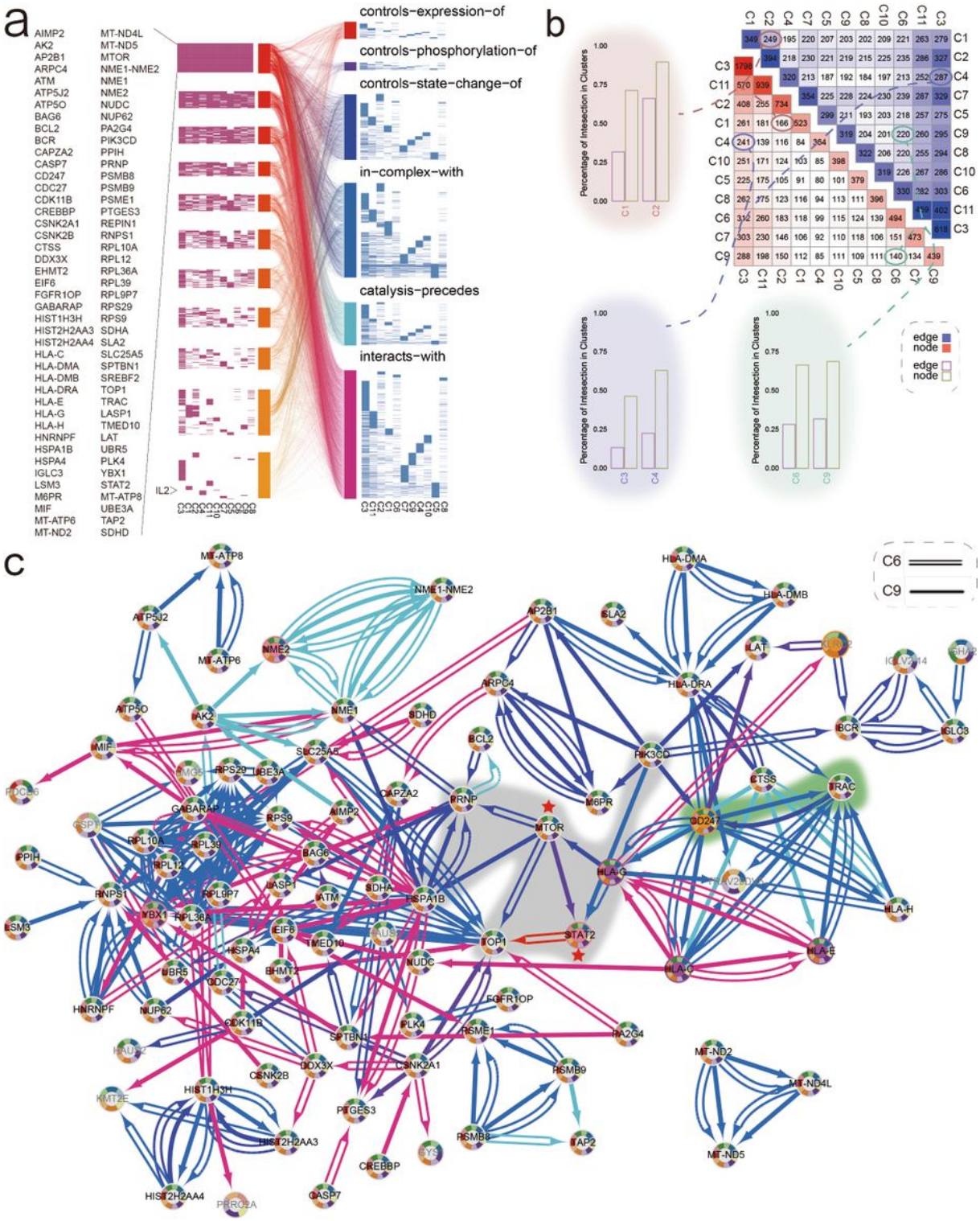
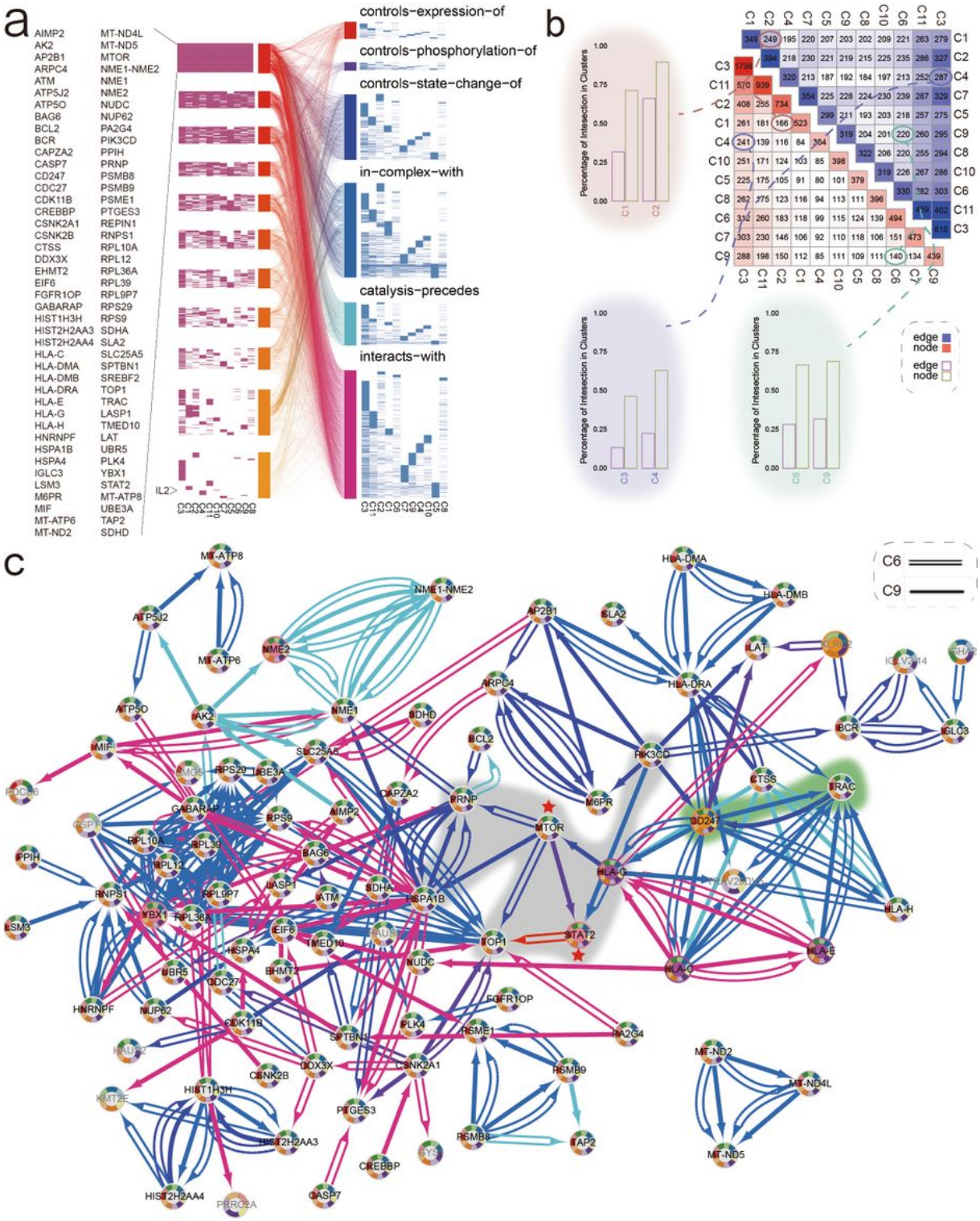


Figure 2

Cross-talk among 11 networks. A) Heatmap showed genes(left) and interactions(right) that were contained by 11 immuno-networks and gaped by degree and interaction types. The middle of two plots showed association between genes and interactions among networks. B) and C) Overlap of edges and nodes among 11 networks. Intersection nodes and edges among networks and percentage of intersection in each network (B); Intersection regulation of networks constructed by cell cluster C6 and C9 (C).



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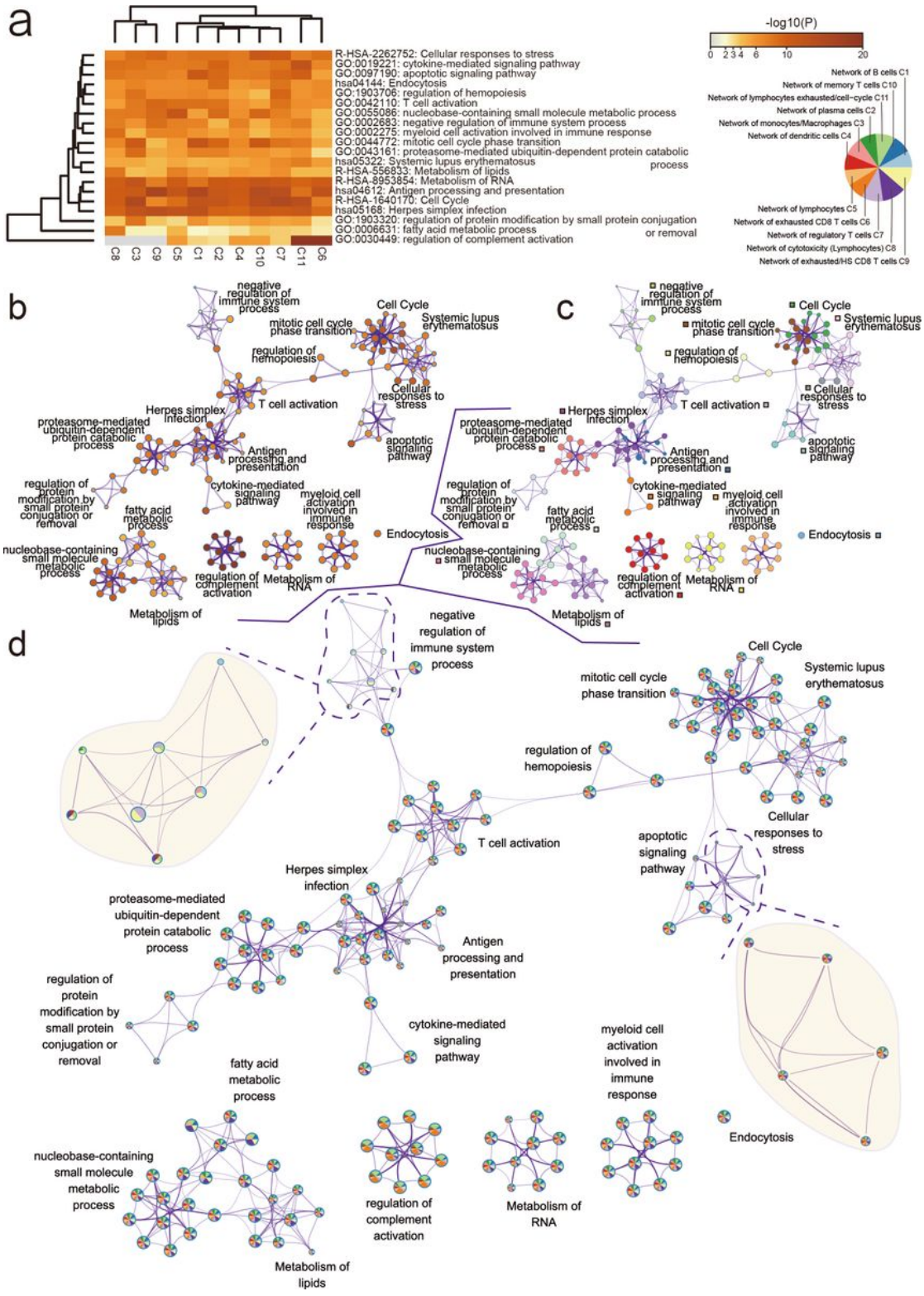
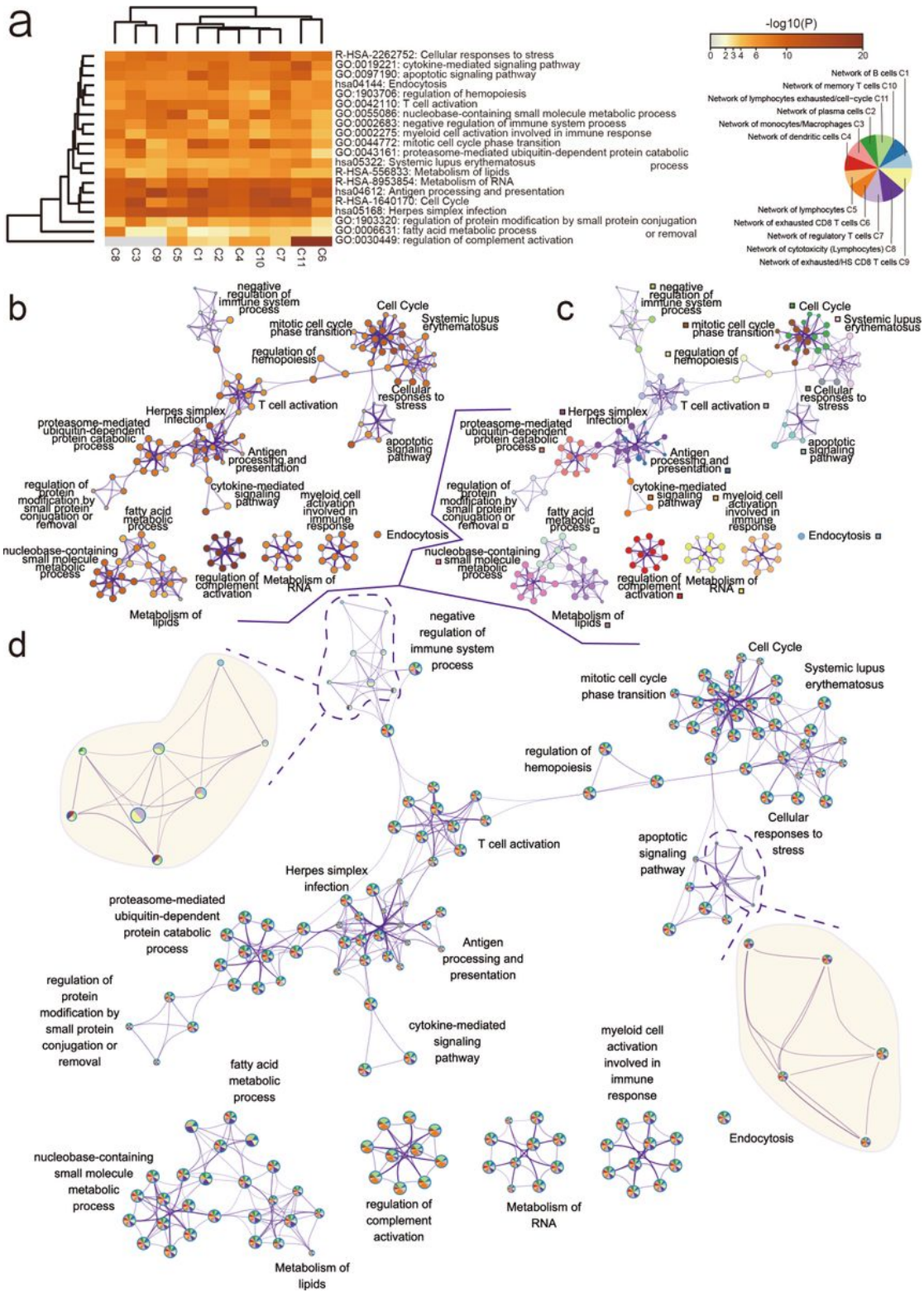


Figure 3

Gene sets functional enrichment of 11 networks. A) Function enrichment of 11 networks and enriched p-values. B), C) and D) Enriched GO-terms colored by p-value (B), cluster (C) and counts (D). Gene sets enrichment analysis are performed by metascape online (http://metascape.org/)



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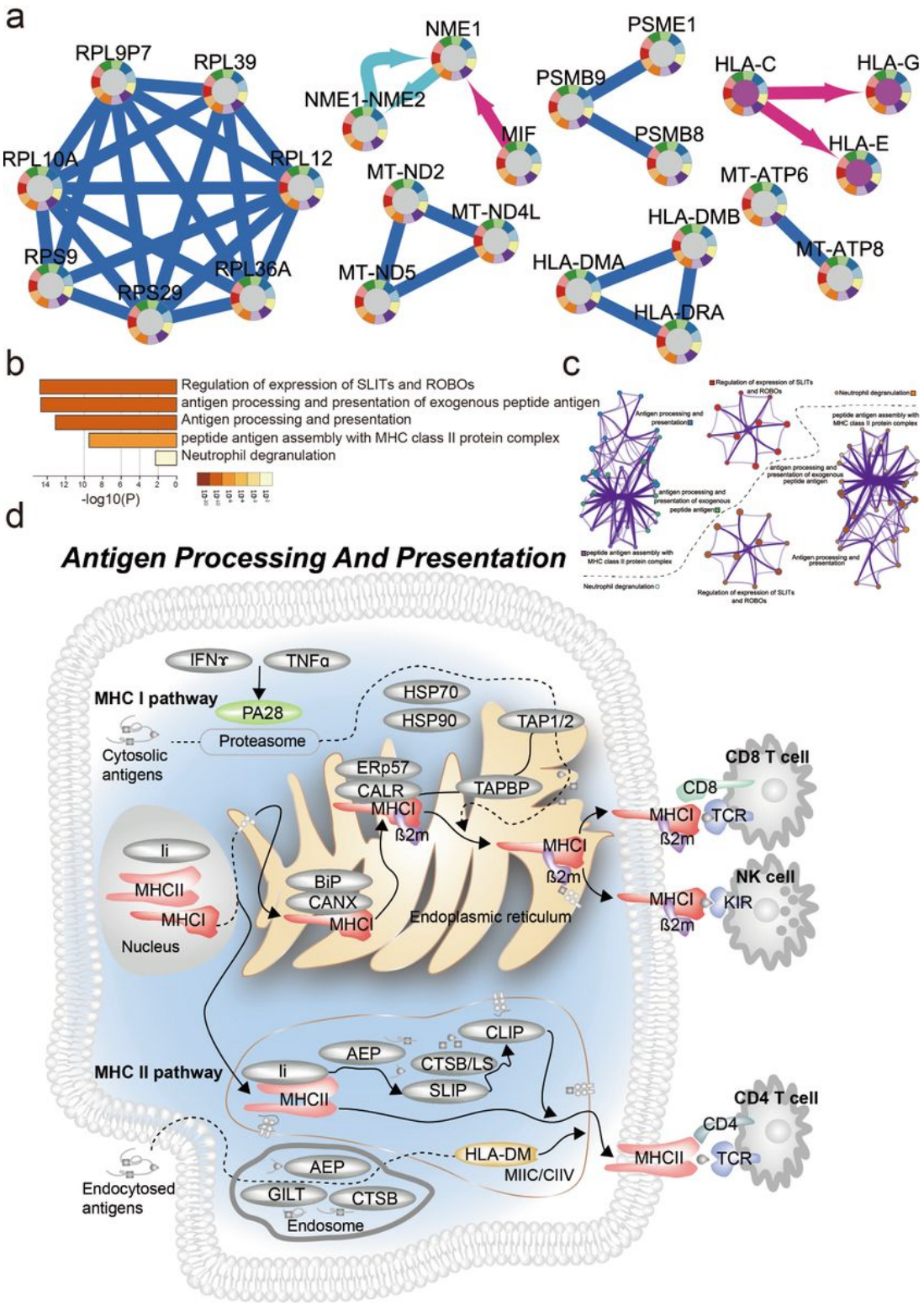

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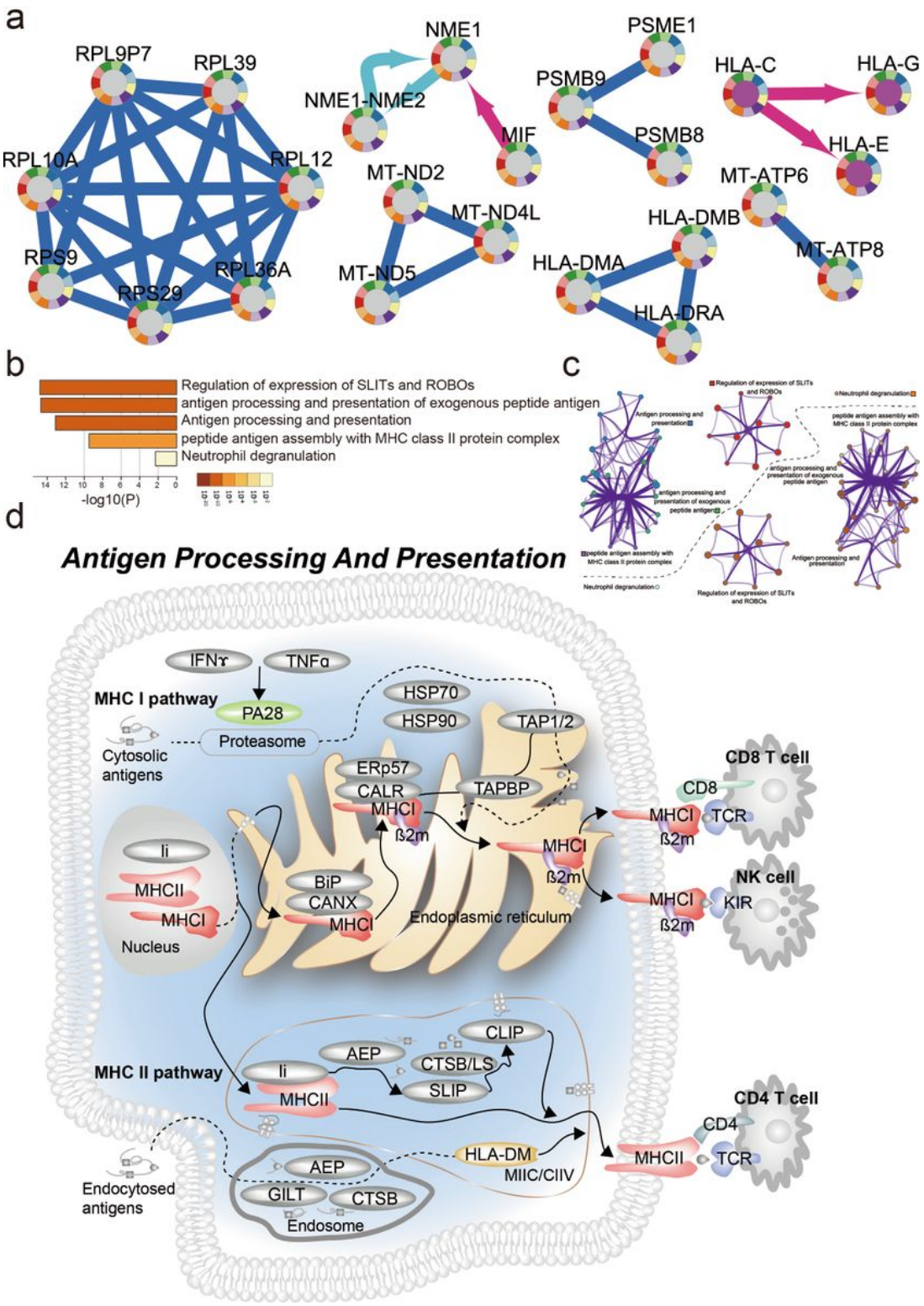
Shared sub-networks of 11 networks. A) Shared interactions of 11 networks. B) and C) Function enrichment of shared genes. Gene sets enrichment analysis are performed by metascape online (http://metascape.org/) D) A core pathway: Antigen Processing and Presentation. Colored nodes are enriched proteins.



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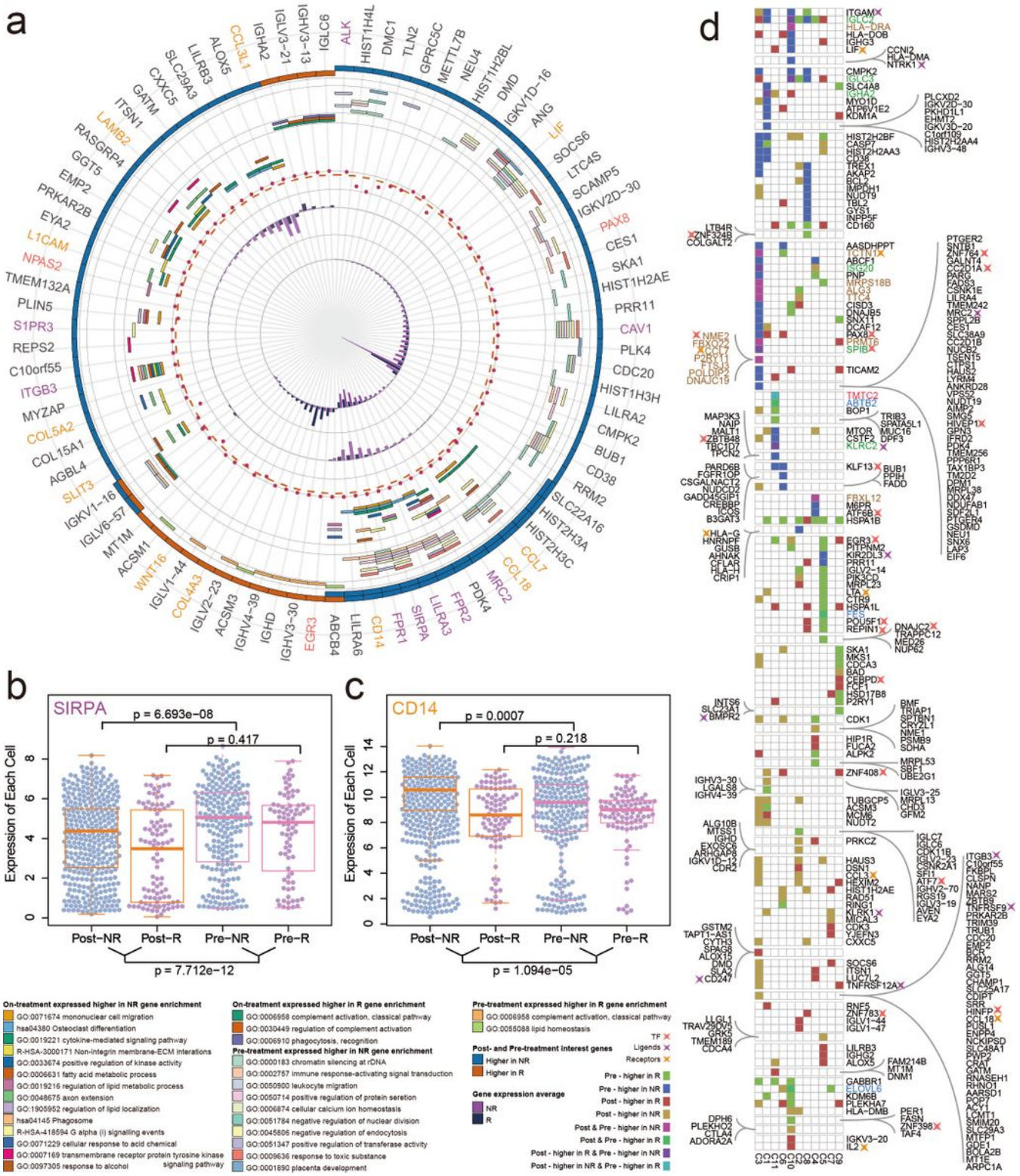
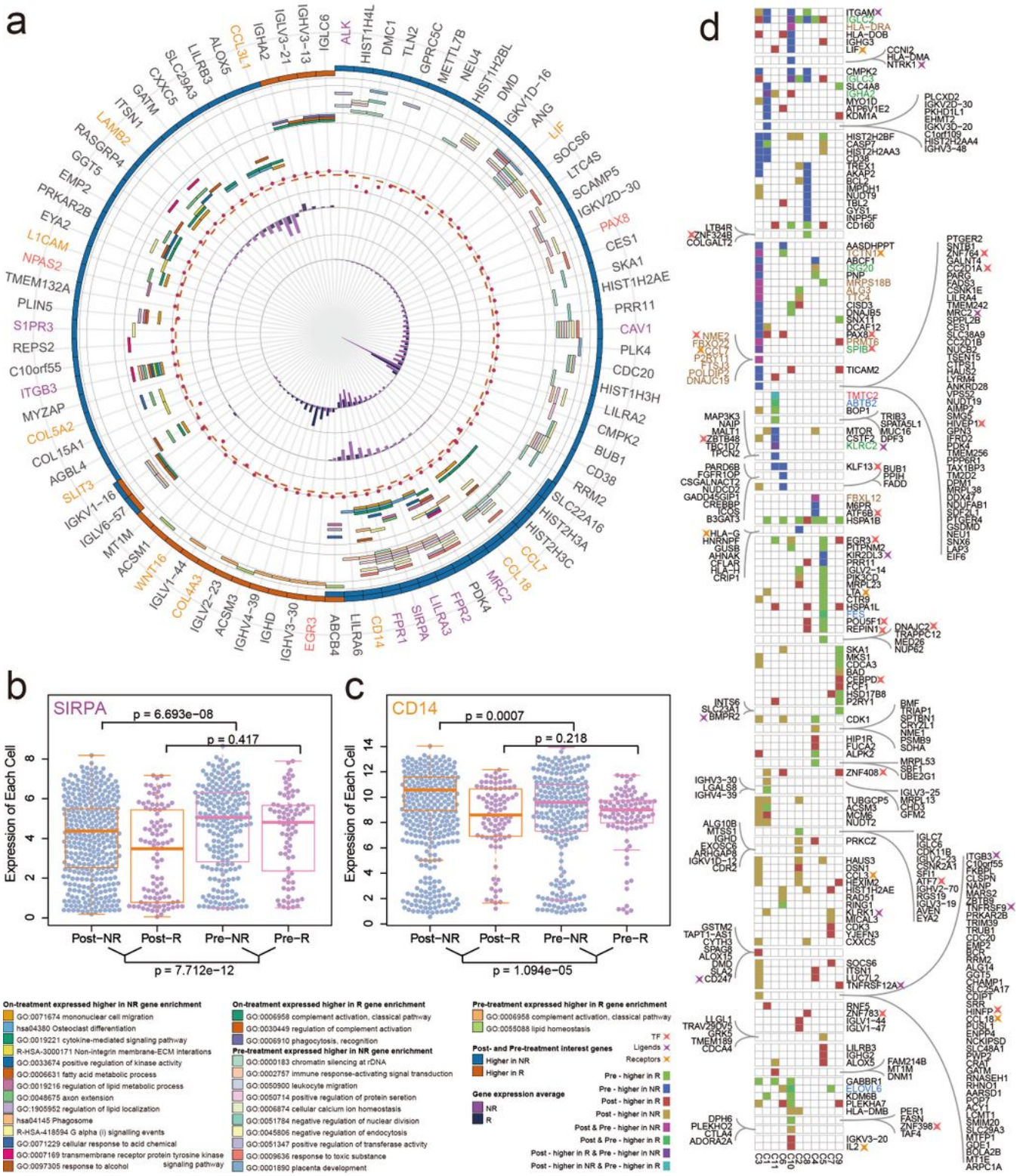


Figure 5

DEGs in global and local networks. A) From the inside out: expression average (pre-treatment NR higher 3-0, pre-treatment R higher 0-1, on-treatment NR higher 1.5-0, and on-treatment R higher 0-1) and p-values (0.05-0) of DEGs. Gene sets function enrichment. on-treatment and pre-treatment DEGs. B) and C) SIRPA and CD14 expression in melanoma immunity single cells. D) DEGs in 11 networks within cell clusters.



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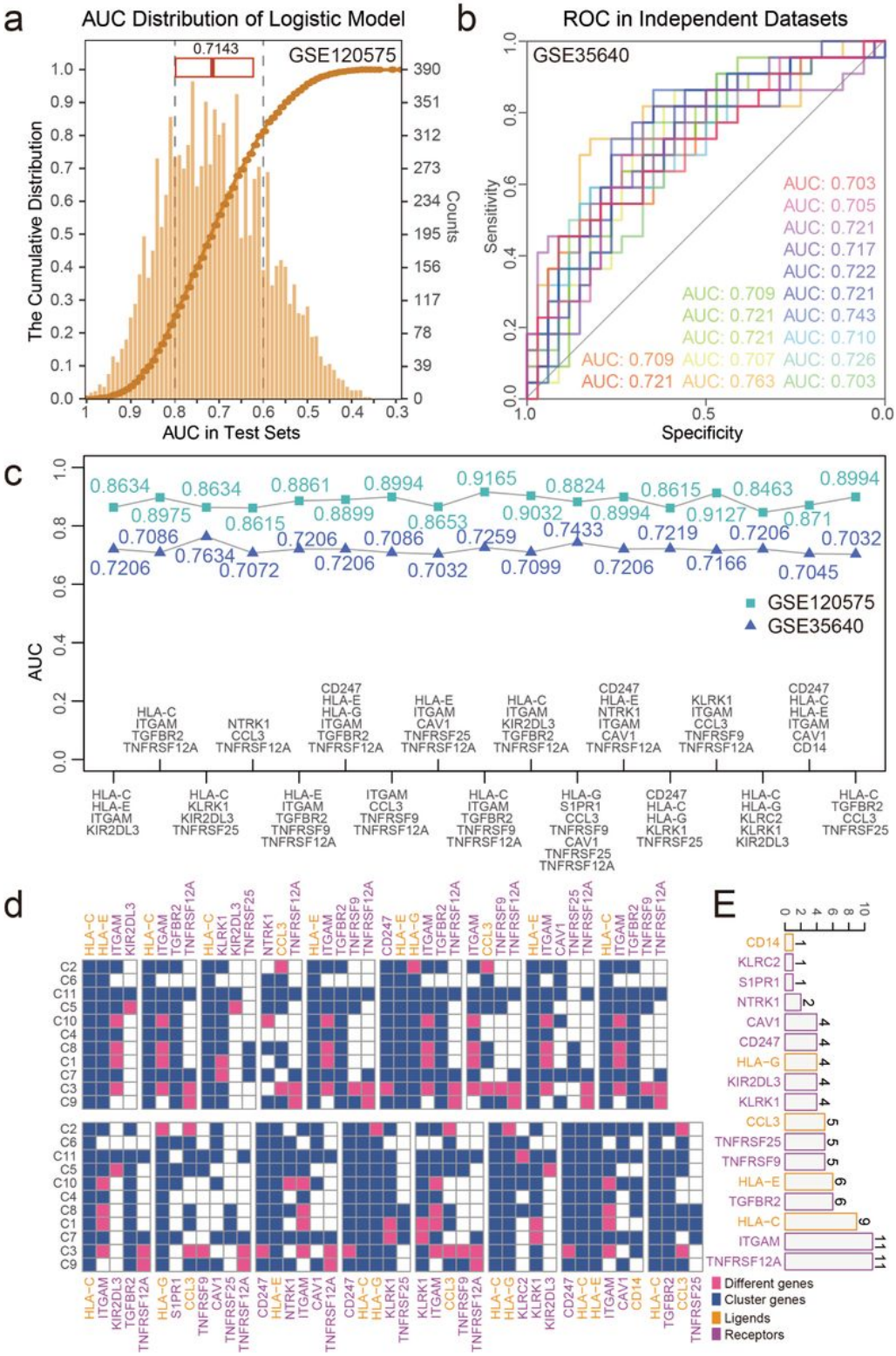

Figure 6

Immunotherapy resistance accuracy of immuno-networks. A) AUCs in test data sets during model construction are showed in form of 1) Boxplot - 0.25, 0.5, and 0.75 quantile; 2) cumulative distribution; and 3) Barplot – counts of AUC values. B) ROCs of 17 promising immunotherapy predictors under independent validation. C) AUCs of test data sets during model construction and independent data sets

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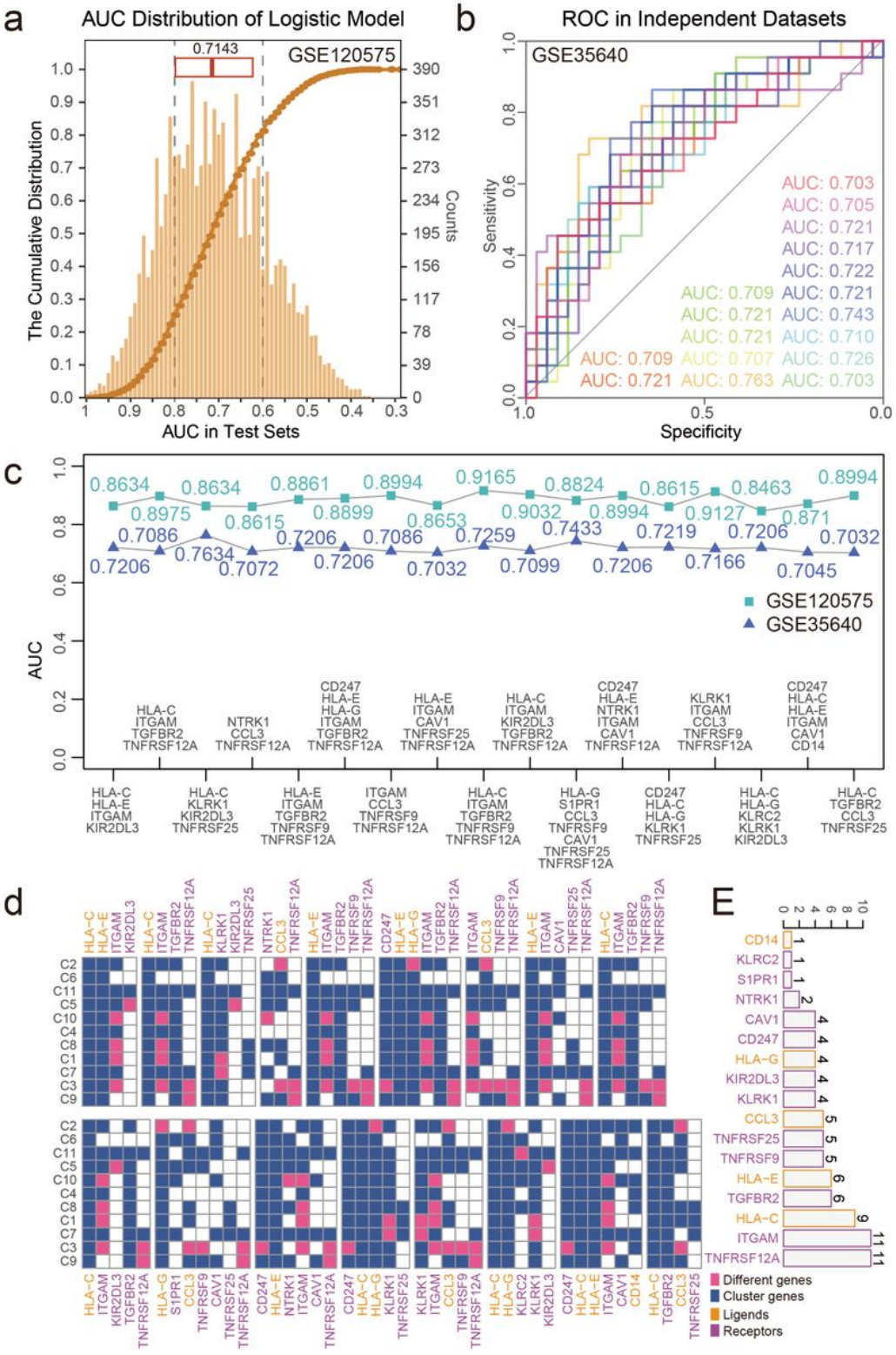

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Supplementary Files

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