Integrative Network Analysis Reveals Organizational Principles of the Endocannabinoid System

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Abstract

The endocannabinoid system (ECS) is a dynamic signaling network that regulates diverse physiological processes through coordinated interactions among receptors, enzymes, and lipid-derived ligands. Here we present a systems-level analysis of the ECS that integrates protein–protein and protein–chemical interactions into a unified network framework. Interaction data were obtained from multiple public databases, and networks constructed using NetworkX captured the molecular landscape of ECS signaling. Centrality analyses identified the primary cannabinoid receptors, cannabinoid receptor 1 (CNR1) and cannabinoid receptor 2 (CNR2), as major hubs with extensive connectivity to endogenous and exogenous ligands. In addition to these canonical receptors, transient receptor potential vanilloid 1 (TRPV1) and G-protein coupled receptor 55 (GPR55) emerged as highly ranked nodes across multiple centrality measures, underscoring their integrative roles within the ECS. Community analyses revealed that key nodes, including the primary cannabinoid receptors CNR1 and CNR2, along with major ligands such as the endocannabinoids anandamide (AEA), 2-arachidonoylglycerol (2-AG), and various phytocannabinoids, sustain network connectivity and organize into biologically coherent receptor-centered and metabolic communities. Perturbation analyses showed that removal of highly connected hubs, particularly CNR1, disrupted network integrity and signaling relationships among synthetic cannabinoids and endocannabinoids. This disruption simultaneously increased the influence of other receptors, including CNR2 and GPR55, revealing compensatory mechanisms that preserve network resilience. Together, these findings provide a comprehensive view of ECS organization and adaptability, highlighting the network's structural and functional principles and offering a foundation for exploring cannabinoid signaling and therapeutic intervention.

Introduction

The endocannabinoid system (ECS) is a multifaceted regulatory network that maintains physiological homeostasis across diverse organ systems [1–3]. It is composed of endogenous ligands known as endocannabinoids, their cognate receptors, and the enzymes that mediate their synthesis and degradation [1,4]. Endocannabinoids are cannabinoids produced within the human body, in contrast to phytocannabinoids, which are derived from a number of plants, of which the best known is *Cannabis sativa* [5], and synthetic cannabinoids, which are laboratory-engineered compounds designed to mimic or modulate the effects of natural cannabinoids for research and therapeutic applications [1,4]. Through the coordinated activity of these molecular components, the ECS influences a broad spectrum of biological processes, including pain perception, inflammation, memory, and mood regulation [6–8].

Two primary receptors define much of the functional landscape of the ECS. Cannabinoid receptor 1 (CB1, also called CNR1) is predominantly expressed in the brain and central nervous system,

where it regulates mood, appetite, pain perception, and cognitive processes [1,6–8]. Cannabinoid receptor 2 (CB2, also called CNR2) is primarily located in immune cells and peripheral tissues, where it regulates inflammatory and immune responses [1,6,8]. The ECS also interfaces with intracellular kinase signaling pathways that control cell survival, synaptic plasticity, and metabolic regulation. This extensive interconnectedness underscores how the ECS integrates with broader cellular signaling networks [1–4,6–10]. When the processes governing endocannabinoid synthesis and degradation become dysregulated, the resulting imbalance in "endocannabinoid tone" has been associated with a range of pathological conditions, including chronic pain, neurodegenerative disorders, metabolic syndromes, and cancer [9,10].

The growing acknowledgment of the ECS's role in human physiology has made it a key target for both basic and clinical research [11–14]. Medical cannabis, which contains phytocannabinoids like tetrahydrocannabinol (THC) and cannabidiol (CBD), is used to modulate ECS activity for therapeutic purposes [15–17]. Specifically, CBD-rich formulations with minimal THC content are increasingly studied and prescribed for conditions such as epilepsy, anxiety, and inflammation [15–17]. Despite this rising interest, the ECS remains insufficiently characterized as a networked system, particularly in terms of how its molecular components interact and respond to changes [1]. A systems-level understanding of these interactions is critical for identifying novel therapeutic targets and developing strategies that more precisely modulate ECS function [18].

In this study, we employed a systems biology framework to characterize the ECS as an integrated molecular network. We constructed interaction networks encompassing protein–protein and protein–chemical relationships within the system. Through network analysis, we identified key nodes using multiple centrality measures and performed clustering analyses to delineate groups of molecules with shared functional characteristics. To further examine network dynamics, we introduced perturbations to assess robustness and to explore how the ECS responds to simulated stress. Collectively, this framework provides a systems-level representation of the ECS, offering insight into its organization, stability, and potential applications in therapeutic development.

Methods

Data Collection and Network Construction

To construct a comprehensive representation of the ECS, we compiled proteins, chemicals, protein–protein interactions (PPIs), and protein–chemical interactions (PCIs) from multiple databases (Figure 1). In the resulting networks, nodes represent ECS proteins or chemicals, and edges reflect experimentally supported interactions. All entities were annotated with structural and functional metadata to facilitate reproducibility and public access.

Proteins: A curated set of forty ECS-associated proteins, including receptors CNR1 and CNR2, enzymes fatty acid amide hydrolase (FAAH), monoacylglycerol lipase (MGLL), and N-acyl-phosphatidylethanolamine phospholipase D (NAPEPLD), and signaling partners, was compiled

from prior literature [18] and expanded using UniProt and STRING database queries [19,20]. Core attributes, including amino acid sequence, subcellular localization, and complex membership, were retrieved from UniProt [19]. Structural mappings to the Protein Data Bank were obtained via SIFTS, and protein structures were extracted from RCSB PDB [21,22]. Protein–protein interactions were collected from STRING using a confidence threshold of 0.7 to reduce false positives [20]. STRING confidence scores integrate multiple evidence types to estimate the likelihood of a true interaction, with values approaching one indicating higher confidence.

Chemicals: Forty ECS-relevant chemicals, including endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG), phytocannabinoids THC and CBD, and selected eicosanoids, were curated from prior literature [18] and expanded using PubChem and ChEMBL database queries [23,24]. Chemical metadata, including IUPAC names and SMILES strings, were retrieved from the PubChem database [23]. Molecular descriptors, including molecular weight, partial charges, and topological fingerprints, were calculated using RDKit to serve as metadata [25]. Protein–chemical interactions were obtained from ChEMBL and filtered to retain only interactions involving the predefined ECS proteins, ensuring network specificity [24].

Network Construction and Analysis

Three networks were constructed using NetworkX: a protein–protein only network, a protein–chemical only network, and a combined network containing all interactions [26]. Network topology was quantified using centrality metrics, including betweenness, closeness, degree, and eigenvector. These measures identify nodes that are highly connected, influential, or serve as critical bridges within the ECS, providing insight into the system's organizational hierarchy [27]. Metrics such as in-degree and out-degree centrality [27], which can provide insights into the directional flow of signaling through a network, were not considered in any of our networks, including protein–protein, protein–chemical, and combined, because of the lack of edge directionality information. Similarly, the network does not capture dynamic information, such as the strength or magnitude of a protein's response to a chemical, so these analyses reflect structural connectivity rather than functional activity.

Network Clustering

Functional modules within the ECS network were identified using the Louvain algorithm, which maximizes modularity by clustering nodes with preferential interactions [28]. This approach highlights groups of proteins and chemicals that are likely to share biological functions or participate in coordinated processes.

Network Perturbations

Network robustness was evaluated by targeting the removal of specific nodes. Specifically, the nodes with the highest betweenness and degree centrality, namely the top one and top three nodes from each metric, were systematically removed to simulate the disruption of crucial, highly

connected components. After each removal, the network was reassessed to determine the number of remaining interactions and connections and to evaluate changes in its overall structure. Centrality metrics were recalculated to identify shifts in the relative importance of the remaining nodes and to understand how the network reorganized in response to the loss of these critical elements. This analysis offers insight into the resilience of the ECS network, highlighting the nodes that are essential for maintaining connectivity and communication. Additionally, this approach can guide therapeutic development by pinpointing components whose modulation or targeting could significantly impact ECS function [18,29,30].

Code and Data Availability

All data and code used in this study are available at https://github.com/aanyashridhar/ECS-Network.

Results and Discussion

The combined ECS network, which integrates both protein–protein and protein–chemical interactions, comprised 80 nodes and 218 edges and served as the primary framework for subsequent analyses (Figure 2a, Tables S1-S4). In this network, proteins occupy the central core, while chemicals are positioned toward the periphery. This structural organization reflects the biological architecture of the ECS, where proteins act as molecular hubs mediating signaling and metabolic processes, and chemicals serve as ligands or substrates that connect through these central protein nodes. The resulting topology suggests that the ECS is organized around a dense protein interaction core that enables diverse chemical inputs to converge on shared molecular pathways.

For comparison, the protein–protein and protein–chemical subnetworks, analyzed separately, contained 40 nodes and 92 edges, and 80 nodes and 126 edges, respectively. These subnetworks are shown in Figures S1–S4 and Tables S1–S4, and provide additional insight into the distinct interaction patterns that contribute to the overall connectivity of the ECS.

Network centrality analysis reveals the hierarchical organization of the ECS and provides insight into connectivity and influence patterns.

To understand the structural organization of the ECS network, we quantified key centrality measures that reflect node influence and connectivity (Figure 2b, Tables S11-S12). The analysis revealed distinct patterns in how proteins and chemicals occupy topological positions within the combined network.

The five nodes with the highest betweenness centrality [27] in the combined network were CNR1, transient potential vanilloid receptor 1 (TRPV1), peroxisome proliferator-activated receptor alpha (PPARA), cyclooxygenase-2 (PTGS2), and G-protein coupled receptor 55 (GPR55), in order of

rank (Figure 2b). High betweenness centrality in these proteins indicates that they act as major conduits through which information and signaling flow across the ECS network, underscoring their integrative roles across chemical and protein subnetworks. As one of the two primary cannabinoid receptors, CNR1 connects to numerous cannabinoids and signaling components, serving as a central bridge in the ECS [11,31]. The second-highest ranked node, TRPV1, though not a canonical cannabinoid receptor, is a ligand-gated ion channel that mediates pain perception, inflammation, and thermoregulation [32]. It is strongly activated by cannabinoids, positioning it as a key integrative node within the ECS. The third-ranked PPARA is a nuclear receptor linking cannabinoid signaling to metabolic regulation [33]. The fourth-ranked enzyme, PTGS2, converts endocannabinoids into prostaglandins, thereby integrating the ECS with inflammatory and pain pathways [34]. Finally, GPR55, ranked fifth, is a G-protein coupled receptor that functions as a major non-cannabinoid receptor, further expanding the ECS signaling landscape [35].

The five nodes with the highest closeness centrality [27] in the combined network were GPR55, CBD, tetrahydrocannabivarin (THCV), cannabidivarin (CBDV), and oleoylethanolamide (OEA), in descending order (Figure 2b). High closeness centrality in these compounds suggests that they can efficiently reach many nodes in the network through relatively few interactions, consistent with their broad influence on ECS signaling dynamics. GPR55 interacts with multiple cannabinoids and receptors, resulting in short path lengths across the network [35]. CBD, THCV, and CBDV follow closely, consistent with their established promiscuity across diverse ECS targets, including CNR1, CNR2, TRPV channels, and GPCRs [15]. The fifth-ranked OEA, an endocannabinoid-like lipid, shares biosynthetic and signaling pathways with canonical endocannabinoids but does not bind directly to the cannabinoid receptors CNR1 or CNR2 [36].

Nodes ranked highest by degree centrality [27] in the combined network were CNR1, CNR2, TRPV1, FAAH, and diacylglycerol lipase alpha (DAGLA), in order (Figure 2b). Nodes with high degree centrality function as major hubs within the network, reflecting their broad engagement across ECS components and highlighting their central role in maintaining network connectivity. CNR1 and CNR2, the two canonical cannabinoid receptors, exhibit extensive connectivity across both protein and chemical domains [11,31]. TRPV1, again, emerges as a highly connected integrator of cannabinoid and non-cannabinoid signaling [32]. The fourth-ranked FAAH, a degradation enzyme [37], and the fifth-ranked DAGLA, a biosynthetic enzyme [38], emphasize the essential role of metabolic turnover in regulating ECS tone and signaling plasticity.

Finally, nodes ranked highest by eigenvector centrality [27] in the combined network were CBD, THCV, CBDV, AM251, and GPR55, in descending order (Figure 2b). High eigenvector centrality indicates that these nodes are not only well-connected themselves but also closely linked to other influential nodes, emphasizing their role in shaping highly interactive subnetworks within the ECS. The prominence of CBD, THCV, and CBDV highlights the influence of phytocannabinoids within the network, as they engage several central receptors and enzymes [15]. AM251, a synthetic CB1 antagonist, ranks fourth due to its strong association with the CNR1 receptor, reflecting its frequent

use in receptor-targeting studies [39]. GPR55 reappears among the top five, reinforcing its status as a non-canonical yet highly connected receptor within the ECS [35].

The consistent prominence of GPR55 across multiple centrality measures suggests that this receptor occupies a highly central role within the ECS. In the combined network, which contained a total of 80 nodes, GPR55 ranked among the top nodes for betweenness (5th), closeness (1st), degree (5th), and eigenvector centrality (5th), far surpassing other non-canonical GPCRs such as GPR18 and GPR119, which were frequently unranked or placed much lower across all measures (Table S13). These trends were even more pronounced in the protein–protein network, which contained 40 nodes, where GPR55 achieved the highest closeness and eigenvector centrality values (both 1st) along with high degree centrality (3rd) (Table S13). Its normalized eigenvector centrality score of 0.99 was not only greater than those of GPR18 and GPR119 by 10^{15} and 10^{18} orders of magnitude, respectively, but also the highest observed across all three networks analyzed. High eigenvector centrality scores for GPR55 indicate that it is maximally connected to other highly influential nodes within the ECS. The recurrence of GPR55 as a top-ranked node across multiple centrality measures and network contexts, similar to the canonical receptors CNR1 and CNR2, highlights its structural importance within the ECS. This finding aligns with emerging evidence that GPR55 functions as a cannabinoid-responsive receptor and plays a broad role in modulating cannabinoid signaling and downstream physiological processes [35,40,41].

Across these metrics, distinct patterns emerged. Betweenness and degree centrality tended to elevate proteins such as CNR1 and TRPV1, reflecting their structural and signaling roles, whereas closeness and eigenvector centrality highlighted chemicals like CBD, THCV, and CBDV, emphasizing their widespread interactions and influence (Figure 2b, Table S12). Together, these results demonstrate that ECS organization is driven by a set of highly interconnected receptors, enzymes, and ligands that coordinate information flow across receptor-mediated, metabolic, and signaling pathways. The recurring prominence of both canonical receptors (CNR1 and CNR2) and non-canonical components such as GPR55 and TRPV1 underscores the distributed yet integrated nature of ECS signaling. Overall, the centrality analyses reveal how information flow and functional connectivity are maintained within the ECS, providing a quantitative framework for identifying critical regulatory nodes and potential targets for pharmacological modulation.

Network clustering analysis uncovers the modular architecture of the ECS and identifies biologically meaningful groupings of receptors, enzymes, and ligands.

Clustering analysis identified thirteen distinct communities within the ECS network (Figure 2c, Tables S5-S6). The largest community consisted of six proteins and thirteen chemicals, while the smallest communities included only a single node (Tables S5-S6). Although these isolated nodes were formally classified as communities by the algorithm, they do not represent meaningful biological groupings. The overall modularity of 42.77% (Table S6) falls within the expected range

for biological networks (typically 30–70%) [42], indicating a balance between connectivity and modular organization.

Among the identified clusters, two communities were particularly informative. The first and largest, designated community 0 (Table S5), captured the core signaling architecture of the ECS. This cluster included the two primary cannabinoid receptors (CNR1 [11,31] and CNR2 [31]), PLC enzymes (phospholipase C epsilon 1 [43] and phospholipase C beta 1 [43]), signaling partners (inhibitory G-protein alpha subunits 1–3 (GNAI1–3) [44]), numerous synthetic cannabinoids (WIN55,212-2 [45], WIN55,212-3 [45], JWH-018 [46], JWH-073 [46], JWH-133 [46], CP55,940 [47], SR141716A [48], SR144528 [48], and AM251 [39]), and endocannabinoid AEA [49,50]. The GNAIs represent canonical intracellular signaling mediators in the ECS, while synthetic cannabinoids and AEA illustrate the diversity of ligand–receptor interactions within the system. This cluster can be viewed as the cannabinoid receptor group, showing the cannabinoid receptors and their immediate effectors clustering strongly together. This organization reinforces the biological validity of the network and demonstrates that its modular structure captures known ECS signaling relationships.

The second notable cluster, community 2 (Table S5), which is the fifth largest community, centered on enzymes and molecules involved in the metabolism of endocannabinoids. This community contained degradation enzymes (FAAH [37], MGLL [51], abhydrolase domain-containing protein 6 (ABHD6) [52,53], and abhydrolase domain-containing protein 12 (ABHD12) [52,53]), synthesis enzymes (NAPEPLD [54], DAGLA [38], and diacylglycerol lipase beta (DAGLB) [38]), non-canonical receptor GPR55 [35,55], transporter (fatty acid-binding protein 7 (FABP7) [56]), and endocannabinoid 2-AG [50]. Together, these nodes represent the metabolic group of the ECS, highlighting the tightly coupled nature of endocannabinoid synthesis, degradation, and transport. The inclusion of GPR55 within this community further underscores the functional integration of metabolic and signaling processes that maintain ECS homeostasis.

Taken together, the clustering results reveal a modular yet interconnected architecture of the ECS network (Figure 2c, Figures S3-S4, Tables S5-S10), in which distinct communities correspond to core biological functions such as receptor-mediated signaling and endocannabinoid metabolism. This modularity not only reflects the inherent organization of the ECS but also highlights potential leverage points where pharmacological or genetic perturbations may exert system-wide effects. By linking functional clusters to specific biochemical roles, these results provide a systems-level framework for exploring how modulation at key nodes could rebalance ECS activity in health and disease.

Network perturbation analysis illustrates how changes to key nodes reshape ECS connectivity, revealing dependencies within its signaling network.

A total of four perturbations were performed for each network, including the removal of the top one node by betweenness centrality, the top three nodes by betweenness centrality, the top one node by degree centrality, and the top three nodes by degree centrality. The protein–chemical only network was limited to two perturbations because betweenness centrality relies on shortest path calculations between nodes, which are not well-defined in networks where chemicals only connect to proteins, allowing only degree-based removals. Across all networks, this approach produced a total of ten perturbations (Table 1). After each perturbation, the change in the number of edges before and after node removal was calculated as a high-level indicator of network impact. Perturbation by betweenness centrality in the protein–protein only network produced the most pronounced effect, with a 40.22% reduction in edge count. In general, removing the top one node, regardless of metric, decreased edge count by approximately 14–16%, whereas removing the top three nodes caused a larger reduction of roughly 34–40%. Notably, CNR1 was removed at least once in all networks, regardless of whether it was identified as a top-ranked node based on betweenness or degree centrality, underscoring its structural importance within the ECS. A previous study [18] showed the ECS network to have a scale-free topology, implying that network properties are determined by a relatively small number of highly connected nodes. Our perturbation results also demonstrate that network integrity depends strongly on a subset of highly connected nodes, highlighting the critical influence of nodes in maintaining connectivity and signaling pathways within the ECS.

To evaluate how highly connected and influential nodes contribute to the stability of the ECS network, we performed targeted perturbations informed by network topology. Nodes were selected for removal based on their rankings in betweenness and degree centrality, which capture information flow and connectivity, respectively. For each perturbation, key centrality measures, including betweenness, closeness, degree, and eigenvector centrality, were recalculated to assess changes in network organization and influence distribution. The percent change in each metric was standardized relative to the distribution of changes across all nodes. Perturbations involving the removal of the top one or top three nodes by betweenness centrality (Figure 3) and degree centrality (Figure 4) were used to examine how hub disruption alters ECS structure and signaling balance.

Perturbation analysis revealed that the removal of highly connected or central nodes caused pronounced shifts in network organization, highlighting which nodes and interactions are most critical for ECS function. In particular, in the absence of CNR1 [11,31], CNR2 [31] and GPR55 [35] increased in centrality, reflecting their compensatory relevance within the network (Figures 3a, 3b, 4a, and 4b). CNR2 and GPR55 are important receptors that can partially maintain connectivity and signaling when CNR1 is absent, indicating redundancy and resilience in the ECS. Conversely, degradation enzymes such as DAGLB [38] showed decreases in betweenness centrality by over two standard deviations, suggesting that key signaling or metabolic nodes lose

influence when primary receptors are disrupted (Figures 3a and 4a). Similarly, the endocannabinoid-like lipid linoleoyl ethanolamide (LEA) [36] exhibited a drop in closeness centrality by nearly two standard deviations following the removal of the top-ranked node, indicating that its accessibility to other network nodes and its potential role in modulating signaling are reduced when primary hubs are lost (Figures 3e, 3f, 4d, 4e, and 4f). This observation highlights how perturbations to central nodes can directly affect both receptor-mediated and lipid-mediated pathways, with potential implications for ECS signaling, metabolic regulation, and therapeutic targeting.

Synthetic cannabinoids, including WIN55,212-2 [45], WIN55,212-3 [45], JWH-018 [46], JWH-073 [46], JWH-133 [46], HU-210 [57], HU-211 [57], CP55,940 [47], SR141716A [48], and SR144528 [48], exhibited significant decreases in network metrics following node removal. These effects were most pronounced in Figures 3e and 4e, which depict changes in degree centrality after removing the top one node by betweenness centrality and the top one node by degree centrality, respectively. Several synthetic cannabinoids dropped in degree centrality by nearly two standard deviations, with SR141716A decreasing by over four standard deviations (Figures 3e and 4e). This highlights the critical dependency of these chemicals on central nodes such as CNR1, underscoring how disruption of key receptors can dramatically reduce the functional influence of ligands within the ECS.

Finally, a comparison of eigenvector centrality changes following the removal of the top one versus the top three nodes by degree centrality revealed a dynamic redistribution of influence (Figures 3g, 3h, 4g, and 4h). Removal of a single top node led to substantial decreases in centrality across many nodes, indicating a loss of network cohesion and stability. In contrast, removing the top three nodes produced positive shifts in eigenvector centrality, reflecting a reweighting of influence toward the remaining highly connected and interlinked nodes. This pattern suggests that, in the absence of dominant hubs, the network reorganizes around secondary yet influential nodes, allowing new centers of connectivity to emerge. Such redistribution illustrates the inherent plasticity of the ECS network and its potential for adaptive reconfiguration, maintaining overall structural coherence even when key regulatory nodes are lost.

Overall, these findings map critical nodes and interactions that sustain ECS connectivity and functional influence. Perturbation of nodes such as CNR1 reshapes the relative importance of other receptors, enzymes, and ligands, revealing compensatory pathways, as reflected in the increased centrality of CNR2 and GPR55. Concurrently, changes in network metrics for endocannabinoid-like lipids, such as LEA, illustrate how node loss can reduce accessibility and signaling efficiency, thereby impacting metabolic and receptor-mediated pathways. Understanding the dependencies within these networks offers a framework for predicting how targeting specific receptors, enzymes, or ligands can influence endocannabinoid system signaling, ligand efficacy, or metabolic turnover.

This knowledge can inform strategies for drug design, receptor modulation, and combination therapies, which leverage the resilience of the ECS to achieve specific physiological outcomes.

Conclusion

This study presents a comprehensive systems-level analysis of the ECS by integrating protein–protein and protein–chemical interactions into a combined network. Centrality analyses identified the primary cannabinoid receptors CNR1 and CNR2 as major hubs, reflecting their extensive connectivity with both endogenous and exogenous ligands. In addition to these canonical receptors, other key nodes, including TRPV1 and GPR55, ranked highly across multiple centrality measures, indicating their integrative roles in linking receptor-mediated signaling with metabolic and inflammatory pathways. The prominence of GPR55, in particular, supports emerging evidence [35,40,41] that it functions as a cannabinoid-responsive receptor with broad influence within ECS signaling. Clustering analyses further revealed that key nodes, including the primary cannabinoid receptors CNR1 and CNR2, as well as critical chemical ligands such as the endocannabinoids AEA and 2-AG and various phytocannabinoids, maintain network connectivity and organize into biologically meaningful communities, including receptor-centered and metabolic clusters. Perturbation analysis emphasized the significance of hub nodes in the network. The removal of CNR1 caused substantial disruptions in the network's organization, reducing the importance of dependent synthetic cannabinoids and shifting centrality to other receptors, such as CNR2 and GPR55. This shift revealed compensatory pathways that contribute to the network's resilience. Overall, our study provides a comprehensive analysis of the ECS network and demonstrates how network-based perturbations can yield valuable insights into its organization, connectivity, and function.

These findings demonstrate that the ECS functions as a complex network orchestrating diverse physiological processes, including signaling and metabolic regulation. Characterizing the connectivity of its receptors, enzymes, ligands, and other components provides critical insights for the development of therapeutic strategies and the design of drugs. Future work could expand the network by incorporating newly identified interactions and integrating dynamic or temporal signaling data to capture context-dependent network behavior. Overall, this study highlights the utility of network-based approaches for uncovering the organizational principles, robustness, and vulnerabilities of the ECS, offering mechanistic insight into its physiological functions and therapeutic potential.

Author Contributions

R.G. and S.M.D. conceived the project. S.M.D. designed and supervised the project. A.S. conducted data curation, network construction, computational analyses, and visualizations. A.S. and S.M.D analyzed and interpreted the results and wrote the manuscript. R.G. provided insights on the results, contributed manuscript edits, and supported the research through funding.

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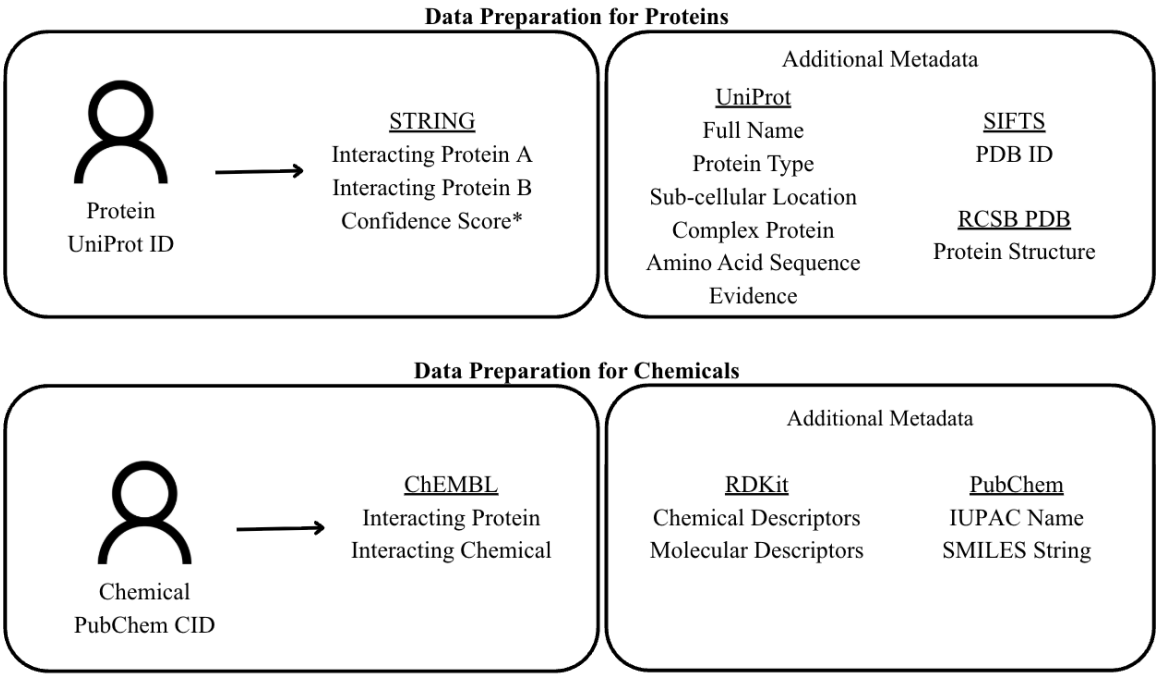
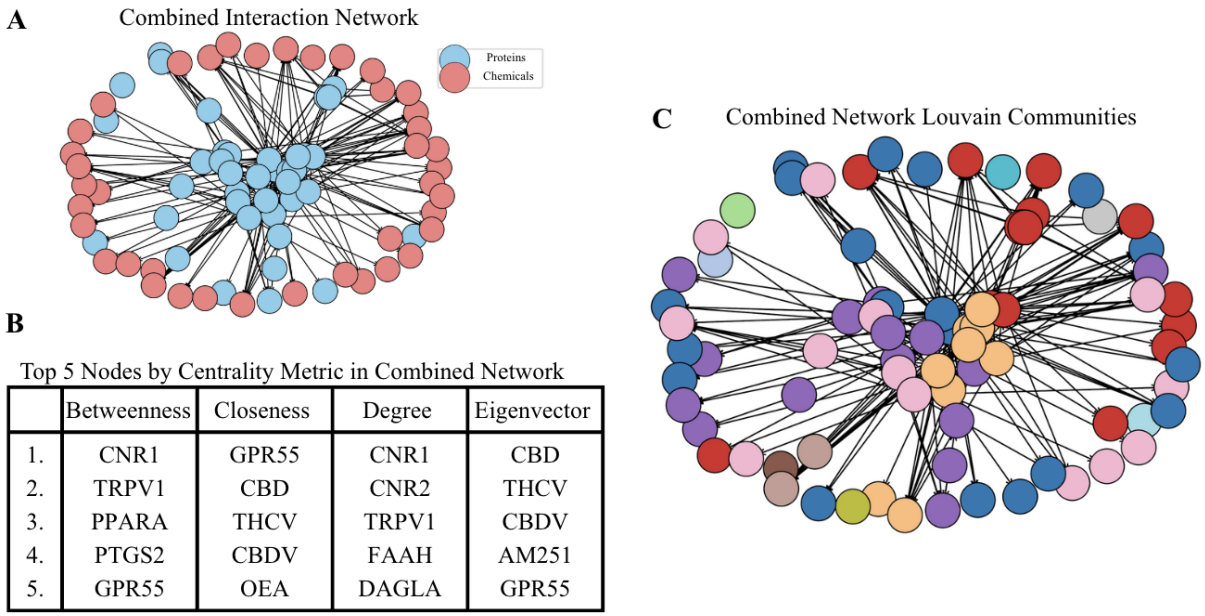


Figure 1: Data preparation pipeline for proteins and chemicals. Schematic depiction of the workflow used to compile protein–level and chemical–level data for network construction. Protein nodes (UniProt IDs) were annotated with interaction data from STRING, including interacting pairs and confidence scores. Additional metadata were integrated from UniProt, SIFTS, and RSCB PDB. Chemical nodes (PubChem CIDs) were annotated with interaction data from ChEMBL. Additional metadata were pulled from PubChem and calculated using RDKit.



B

Top 5 Nodes by Centrality Metric in Combined Network

	Betweenness	Closeness	Degree	Eigenvector
1.	CNR1	GPR55	CNR1	CBD
2.	TRPV1	CBD	CNR2	THCV
3.	PPARA	THCV	TRPV1	CBDV
4.	PTGS2	CBDV	FAAH	AM251
5.	GPR55	OEA	DAGLA	GPR55

Figure 2: Combined interaction network and node characterization. A) Visualization of the integrated network showing proteins (blue) and chemicals (red) connected through documented interactions. The network has 80 nodes and 218 edges. **B)** Top five nodes ranked by centrality measures in the combined network. Results highlight distinct sets of influential nodes across betweenness, closeness, degree, and eigenvector centrality metrics, underscoring multiple dimensions of node importance. Full rankings and centrality metric calculations can be found at https://github.com/aanyashridhar/ECS-Network. **C)** Louvain community detection applied to the combined network, partitioning the nodes (denoted by distinct colors) into modular clusters based on interaction density, which highlights potential functional or mechanistic groupings within the ECS network.

Table 1: Network perturbation analysis across protein–protein, protein–chemical, and combined ECS networks. Summary of robustness simulations following targeted removal of highly central nodes (top one or top three rankings in betweenness or degree centrality). For each perturbation, the table shows the number of original nodes and edges, number of edges after perturbation, and percent change in edge count.

Network	Perturbation Metric	Removal Type	Removed Node(s)	Original # Nodes	Original # Edges	Perturbed # Edges	% Change in Edges
Protein–Protein	Betweenness Centrality	Top 1	CNR1	40	92	77	16.30
Protein–Protein	Betweenness Centrality	Top 3	CNR1, PPARA, PTGS2	40	92	55	40.22
Protein–Protein	Degree Centrality	Top 1	FAAH	40	92	79	14.30
Protein–Protein	Degree Centrality	Top 3	FAAH, CNR1, GPR55	40	92	65	29.35
Protein–Chemical	Degree Centrality	Top 1	CNR1	80	126	105	16.67
Protein–Chemical	Degree Centrality	Top 3	CNR1, CNR2, TRPV1	80	126	82	34.92
Combined	Betweenness Centrality	Top 1	CNR1	80	218	184	15.60
Combined	Betweenness Centrality	Top 3	CNR1, TRPV1, PPARA	80	218	141	35.32
Combined	Degree Centrality	Top 1	CNR1	80	218	184	15.60
Combined	Degree Centrality	Top 3	CNR1, CNR2, TRPV1	80	218	153	29.82

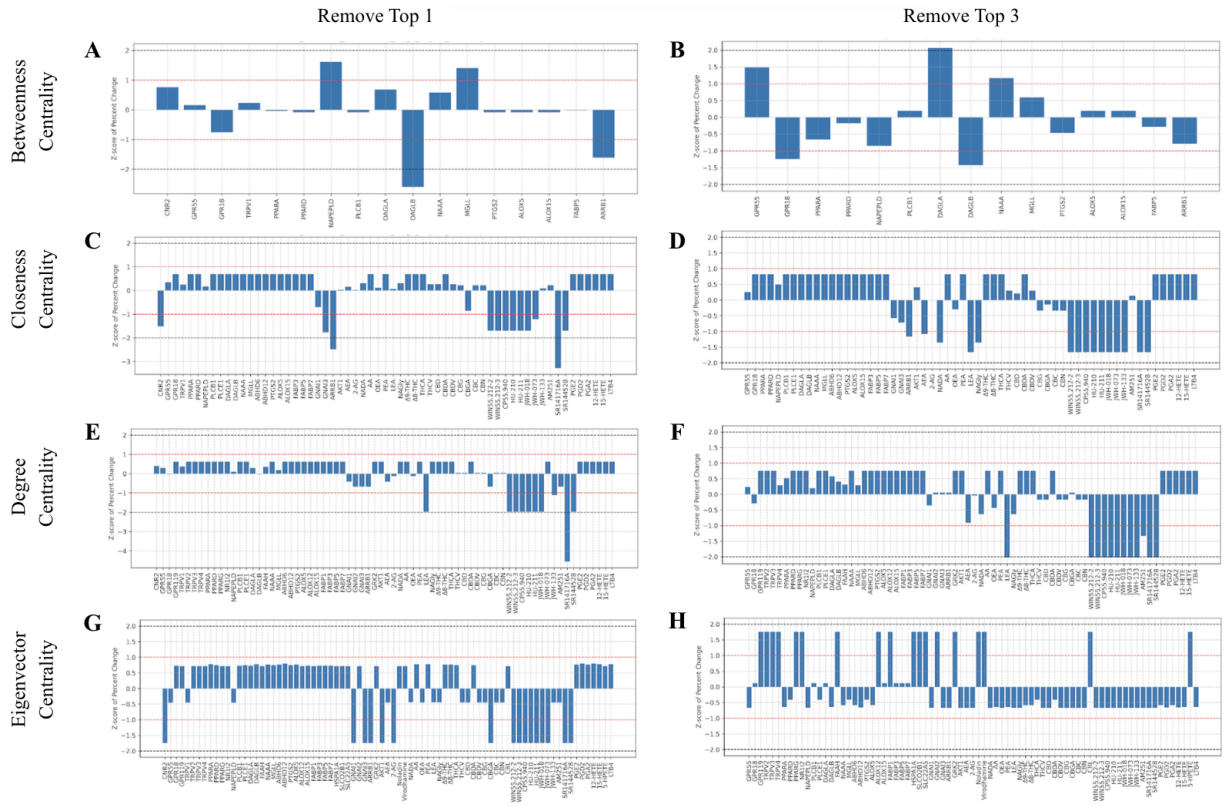


Figure 3: Network perturbation effects on node centrality following removal of high-betweenness nodes. Bar plots depict standardized changes (z-scores) in centrality metrics of the nodes after perturbation. The red and black horizontal dotted lines denote absolute z-scores of 1 and 2, respectively. **A-B)** Betweenness centrality recalculated after removal of the top one **(A)** and top three **(B)** nodes by betweenness. **C-D)** Closeness centrality recalculated after removal of the top one **(C)** and top three **(D)** nodes by betweenness. **E-F)** Degree centrality recalculated after removal of the top one **(E)** and top three **(F)** nodes by betweenness. **G-H)** Eigenvector centrality recalculated after removal of the top one **(G)** and top three **(H)** nodes by betweenness.

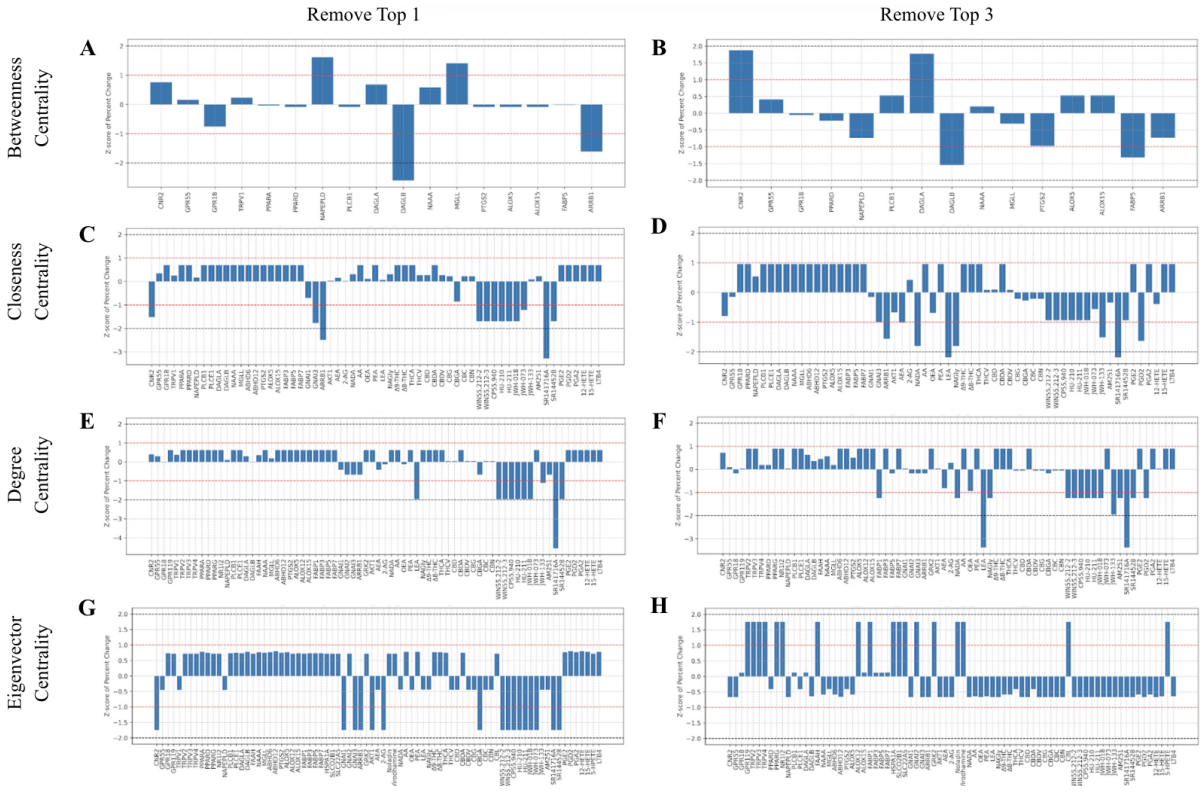


Figure 4: Network perturbation effects on node centrality following removal of high-degree nodes. Bar plots depict standardized changes (z-scores) in centrality metrics of the nodes after perturbation. The red and black horizontal dotted lines denote absolute z-scores of 1 and 2, respectively. **A-B)** Betweenness centrality recalculated after removal of the top one **(A)** and top three **(B)** nodes by degree. **C-D)** Closeness centrality recalculated after removal of the top one **(C)** and top three **(D)** nodes by degree. **E-F)** Degree centrality recalculated after removal of the top one **(E)** and top three **(F)** nodes by degree. **G-H)** Eigenvector centrality recalculated after removal of the top one **(G)** and top three **(H)** nodes by degree.

Table S1: Chemical compounds included in the ECS network. List of chemical nodes (40) with compound names and PubChem CIDs used for integration into the protein–chemical and combined interaction network. Full metadata, including IUPAC names, SMILES strings, and RDKit-calculated descriptors, are provided separately at https://github.com/aanyashridhar/ECS-Network.

Chemical	PubChem CID
AEA	5281969
2-AG	5282280
Noladin	5311103
Virodhamine	6443013
NADA	5311436
AA	5283678
OEA	5286420
PEA	10467
LEA	5283387
NAGly	23667530
Δ9-THC	16078
Δ8-THC	644019
THCA	644017
THCV	644011
CBD	6440190
CBDA	644001
CBDV	92012705
CBG	5281963
CBGA	5281962
CBC	5281965
CBN	65156
CBL	5281447
WIN55,212-2	5311105
WIN55,212-3	5311106
CP55,940	443912
HU-210	5311352

HU-211	146660
JWH-018	588025
JWH-073	9850028
JWH-133	86287562
AM251	6918495
SR141716A	3003824
SR144528	5311135
PGE2	5280360
PGD2	5280896
PGA2	5280895
12-HETE	444899
15-HETE	5280734
5-HPETE	5282820
LTB4	5280934

Table S2: Proteins included in the ECS network. List of protein nodes (40) with protein names and UniProt IDs used for integration into the protein–protein and combined interaction network. Full metadata, including full names, protein types, sub-cellular locations, structural annotations, PDB IDs, amino acid sequences, and evidence codes, are provided separately at https://github.com/aanyashridhar/ECS-Network.

Protein	UniProt ID
CNR1	P21554
CNR2	P34972
GPR55	Q9Y2T6
GPR18	Q14330
GPR119	Q8TDV5
TRPV1	Q8NER1
TRPV2	Q9Y5S1
TRPV3	Q8NET8
TRPV4	Q9HBA0
PPARA	Q07869
PPARD	Q03181
PPARG	P37231
NR1I2	O75469
NAPEPLD	Q6IQ20
PLCB1	Q9NQ66
PLCE1	Q9P212
DAGLA	Q9Y4D2
DAGLB	Q8NCG7
FAAH	O00519
NAAA	Q02083
MGLL	Q99685
ABHD6	Q9BV23
ABHD12	Q8N2K0
PTGS2	P35354
ALOX5	P09917
ALOX12	P18054

ALOX15	P16050
FABP1	P07148
FABP3	P05413
FABP5	Q01469
FABP7	O15540
HSPA1A	P0DMV8
SLCO2B1	O94956
SLC22A5	O76082
GNAI1	P63096
GNAI2	P04899
GNAI3	P08754
ARRB1	P49407
GRK2	P25098
AKT1	P31749

Table S3: Protein–protein interactions included in the ECS network. List of protein–protein interactions (92) with interacting protein pairs and confidence scores (thresholded at 0.7) used for integration into the protein–protein and combined interaction network.

Interacting Protein A	Interacting Protein B	Confidence
GRK2	ARRB1	0.998
CNR1	GNAI1	0.995
GNAI2	CNR1	0.993
GNAI2	GNAI3	0.992
ARRB1	AKT1	0.99
FABP5	PPARD	0.988
GNAI3	GNAI1	0.984
CNR1	GPR55	0.983
CNR1	CNR2	0.981
GNAI2	GNAI1	0.975
FAAH	MGLL	0.974
PTGS2	ALOX5	0.973
FAAH	CNR1	0.972
FABP1	PPARA	0.964
ALOX15	PTGS2	0.963
ABHD6	ABHD12	0.949
ALOX12	PTGS2	0.947
MGLL	CNR1	0.941
CNR1	GNAI3	0.933
FAAH	NAPEPLD	0.931
PLCB1	GNAI3	0.929
FABP1	FABP3	0.929
PLCB1	GNAI1	0.928
GNAI2	PLCB1	0.926
GPR119	GPR55	0.922
TRPV1	GPR55	0.922
MGLL	NAPEPLD	0.92

DAGLA	DAGLB	0.915
ALOX15	ALOX5	0.914
ALOX12	ALOX5	0.914
CNR2	GNAI1	0.909
ALOX12	ALOX15	0.906
MGLL	ABHD6	0.904
FAAH	DAGLA	0.898
DAGLA	NAPEPLD	0.894
FAAH	FABP5	0.891
DAGLB	NAPEPLD	0.89
DAGLA	MGLL	0.887
PPARD	PPARA	0.887
FAAH	CNR2	0.886
FAAH	ABHD6	0.881
GPR119	GPR18	0.868
FAAH	TRPV1	0.855
FAAH	NAAA	0.85
FAAH	GPR55	0.848
FAAH	DAGLB	0.848
MGLL	ABHD12	0.839
FAAH	FABP7	0.839
FAAH	ABHD12	0.831
DAGLA	ABHD6	0.831
MGLL	DAGLB	0.829
PPARG	FABP5	0.828
FAAH	FABP3	0.826
GPR18	CNR2	0.823
ABHD6	NAPEPLD	0.821
DAGLA	CNR1	0.817
GPR18	CNR1	0.816
NAAA	NAPEPLD	0.816

ABHD6	DAGLB	0.812
ABHD12	NAPEPLD	0.803
NAPEPLD	GPR55	0.801
CNR1	NAPEPLD	0.8
GPR18	TRPV1	0.796
CNR2	GPR55	0.793
DAGLA	ABHD12	0.791
PPARG	AKT1	0.79
CNR1	TRPV1	0.781
MGLL	NAAA	0.78
GRK2	AKT1	0.778
MGLL	GPR55	0.776
PTGS2	AKT1	0.775
TRPV4	TRPV1	0.773
NAPEPLD	TRPV1	0.771
GPR18	GPR55	0.767
CNR1	ARRB1	0.757
DAGLB	ABHD12	0.746
PPARA	GPR55	0.746
ARRB1	GPR55	0.744
FAAH	GPR18	0.736
PPARG	PTGS2	0.735
PTGS2	PPARA	0.729
MGLL	TRPV1	0.728
PPARA	TRPV1	0.718
ABHD6	GPR55	0.717
PPARG	PPARD	0.715
FABP5	PPARA	0.708
GPR119	PPARA	0.706
DAGLB	CNR1	0.705
PLCB1	PLCE1	0.705

NAAA	PPARA	0.702
PPARA	AKT1	0.7
DAGLA	GPR55	0.7

Table S4: Protein–chemical interactions included in the ECS network. List of protein–chemical interactions (126) with interacting chemicals and interacting proteins used for integration into the protein–chemical and combined interaction network.

Chemical	Protein
AEA	CNR1
AEA	CNR2
AEA	FAAH
AEA	MGLL
AEA	TRPV1
2-AG	MGLL
2-AG	CNR2
2-AG	CNR1
2-AG	DAGLB
2-AG	DAGLA
2-AG	GPR119
2-AG	FAAH
NADA	TRPV1
NADA	FAAH
AA	PTGS2
AA	ALOX5
OEA	GPR119
OEA	NAAA
OEA	CNR2
OEA	PPARA
OEA	TRPV1
OEA	PPARD
OEA	CNR1
PEA	NAAA
LEA	TRPV1
LEA	CNR1
NAGly	TRPV1

NAGly	FAAH
Δ9-THC	GPR18
Δ9-THC	PPARD
Δ8-THC	GPR18
Δ8-THC	PPARD
THCA	ALOX15
THCV	CNR2
THCV	TRPV1
THCV	TRPV2
THCV	TRPV4
THCV	CNR1
THCV	NAAA
THCV	GPR55
THCV	TRPV3
THCV	DAGLA
CBD	PPARG
CBD	NR1I2
CBD	CNR1
CBD	TRPV1
CBD	PTGS2
CBD	CNR2
CBD	DAGLA
CBD	NAAA
CBD	GPR55
CBDA	ALOX15
CBDV	GPR55
CBDV	CNR2
CBDV	TRPV2
CBDV	DAGLA
CBDV	TRPV1
CBDV	TRPV4

CBDV	NAAA
CBDV	TRPV3
CBDV	CNR1
CBG	PPARG
CBG	TRPV2
CBG	TRPV1
CBG	CNR2
CBG	CNR1
CBG	DAGLA
CBG	TRPV3
CBG	NAAA
CBG	TRPV4
CBGA	DAGLA
CBGA	NAAA
CBGA	CNR1
CBGA	ALOX15
CBC	TRPV3
CBC	TRPV4
CBC	CNR2
CBC	DAGLA
CBC	NAAA
CBC	TRPV2
CBC	PPARG
CBC	TRPV1
CBC	CNR1
CBN	CNR2
CBN	CNR1
CBN	TRPV1
CBN	TRPV2
CBN	NAAA
CBN	TRPV4

CBN	DAGLA
CBN	PPARG
CBN	TRPV3
WIN55,212-2	CNR1
WIN55,212-2	CNR2
WIN55,212-3	CNR1
WIN55,212-3	CNR2
CP55,940	CNR1
CP55,940	CNR2
HU-210	CNR2
HU-210	CNR1
HU-211	CNR1
HU-211	CNR2
JWH-018	CNR2
JWH-018	CNR1
JWH-073	CNR2
JWH-133	CNR2
JWH-133	CNR1
JWH-133	TRPV1
AM251	CNR1
AM251	TRPV1
AM251	GPR55
AM251	CNR2
SR141716A	CNR1
SR144528	CNR2
SR144528	CNR1
PGE2	PTGS2
PGD2	PPARG
PGD2	PPARA
PGA2	PTGS2
12-HETE	ALOX5

12-HETE	ALOX15
12-HETE	ALOX12
12-HETE	PTGS2
12-HETE	PPARA
15-HETE	ALOX5
LTB4	ALOX5

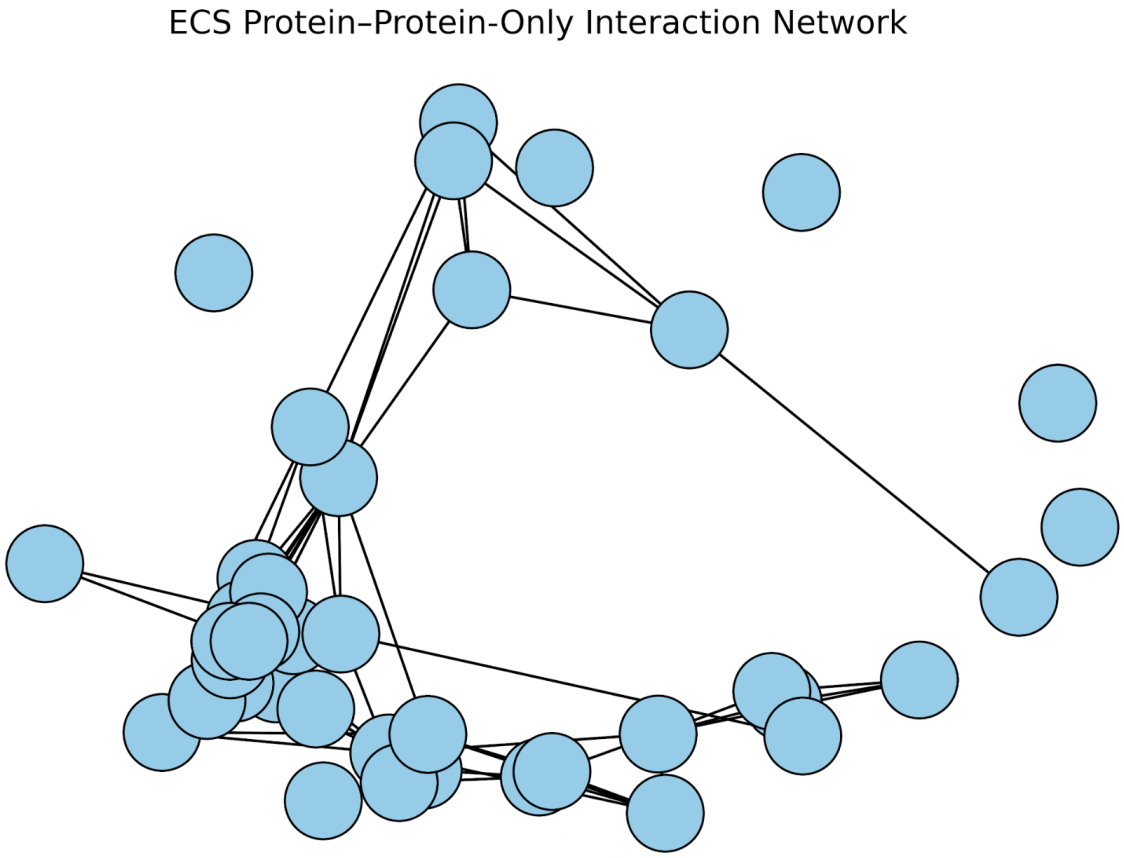


Figure S1: Protein–protein interaction network. Visualization of the protein–protein only network showing proteins connected through documented interactions. The network has 40 nodes and 92 edges.

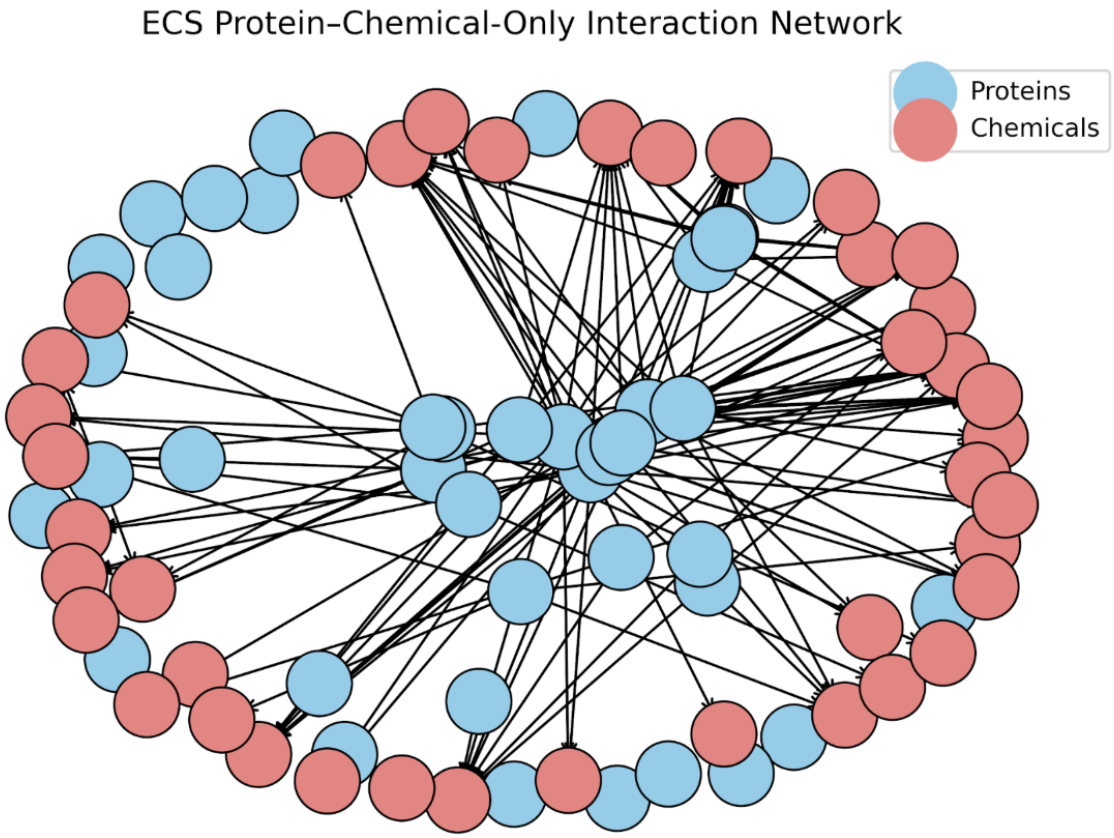


Figure S2: Protein–chemical interaction network. Visualization of the protein–chemical only network (with protein–protein interactions filtered out) showing proteins (blue) and chemicals (red) connected through documented interactions. The network has 40 nodes and 126 edges.

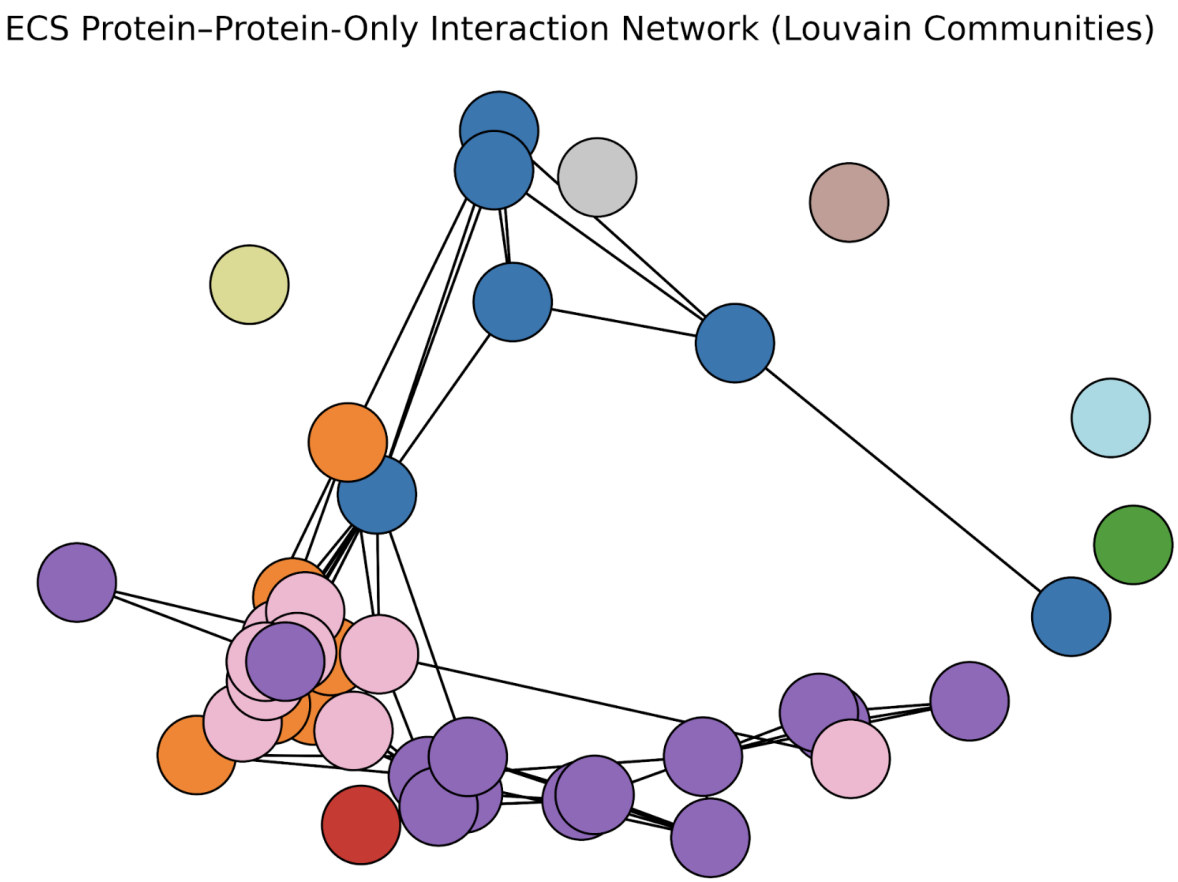


Figure S3: Protein–protein interaction network Louvain communities from clustering analysis. Louvain community detection applied to the protein–protein only network, partitioning the nodes into modular clusters (denoted by distinct colors) based on interaction density, which highlights potential functional or mechanistic groupings within ECS proteins.

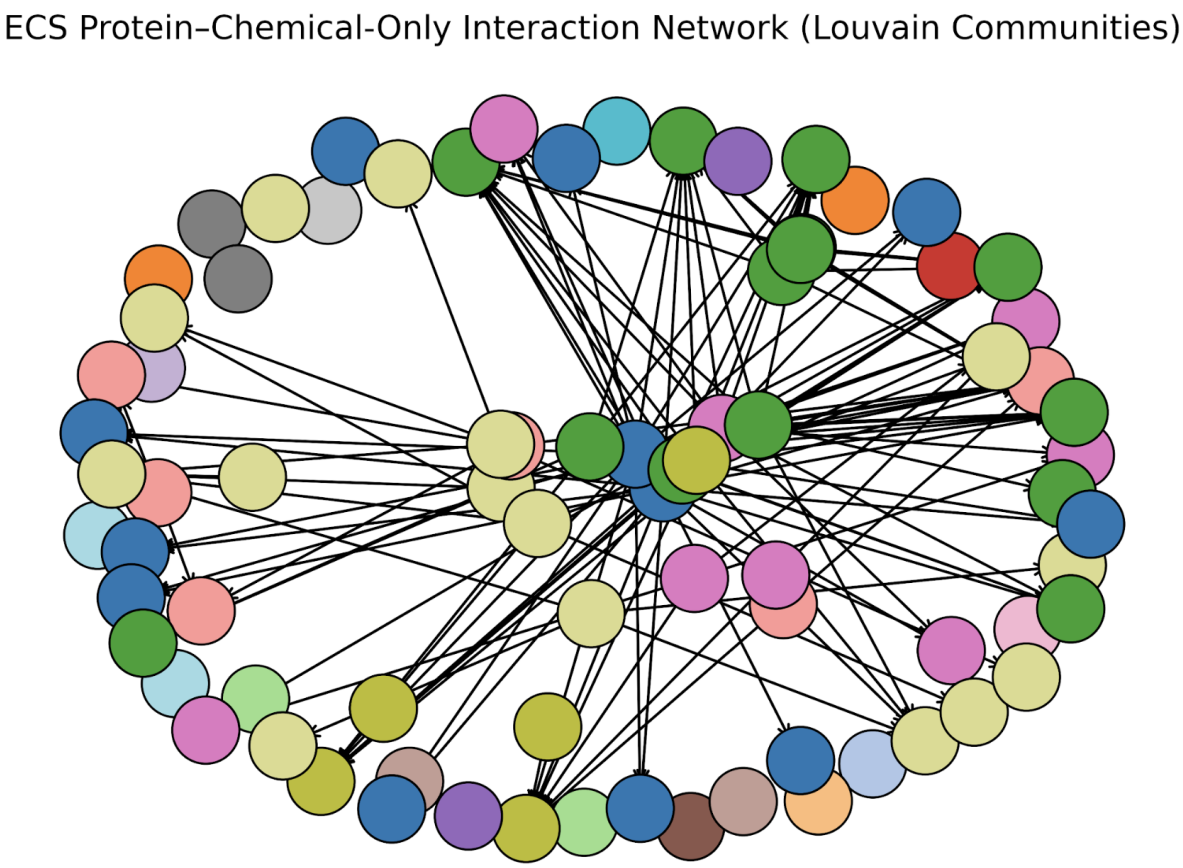


Figure S4: Protein–chemical interaction network Louvain communities from clustering analysis. Louvain community detection applied to the protein–chemical only network (with protein–protein interactions filtered out), partitioning the nodes into modular clusters (denoted by distinct colors) based on interaction density, which highlights potential functional or mechanistic groupings within ECS proteins and chemicals.

Table S5: Louvain community assignments for the combined interaction network. List of proteins and chemicals and their assigned Louvain communities.

Node	Louvain Community
CNR1	0
CNR2	0
PLCE1	0
PLCB1	0
AEA	0
GNAI3	0
GNAI1	0
GNAI2	0
WIN55,212-3	0
WIN55,212-2	0
JWH-133	0
JWH-073	0
JWH-018	0
HU-211	0
HU-210	0
CP55,940	0
SR141716A	0
AM251	0
SR144528	0
SLCO2B1	1
FAAH	2
NAPEPLD	2
DAGLB	2
ABHD12	2
2-AG	2
FABP7	2
ABHD6	2
MGLL	2

GPR55	2
DAGLA	2
SLC22A5	3
THCV	4
CBC	4
CBN	4
CBDV	4
PEA	4
CBG	4
TRPV2	4
TRPV3	4
NADA	4
TRPV4	4
NAAA	4
LEA	4
NAGly	4
TRPV1	4
GRK2	5
Δ8-THC	5
ARRB1	5
AKT1	5
OEA	5
GPR119	5
GPR18	5
PPARA	5
FABP3	5
PGD2	5
Δ9-THC	5
PPARG	5
PPARD	5
FABP1	5

FABP5	5
Noladin	6
CBD	7
NR1I2	7
PTGS2	8
ALOX15	8
ALOX12	8
PGE2	8
THCA	8
AA	8
CBDA	8
ALOX5	8
CBGA	8
12-HETE	8
15-HETE	8
LTB4	8
PGA2	8
Virodhamine	9
CBL	10
5-HPETE	11
HSPA1A	12

Table S6: Louvain community summary metrics for the combined interaction network. Report of overall network modularity, the total number of detected communities, and community size statistics.

Metric	Value
Overall Modularity	0.4277
Number of Communities	13
Largest Community Size	19
Smallest Community Size	1

Table S7: Louvain community assignments for the protein–protein interaction network. List of proteins and their assigned Louvain communities.

Protein	Louvain Community
CNR1	0
PLCB1	0
PLCE1	0
GNAI2	0
GNAI1	0
GNAI3	0
GPR119	1
CNR2	1
TRPV4	1
TRPV1	1
GPR55	1
GPR18	1
TRPV2	2
TRPV3	3
PPARG	4
PPARA	4
FABP1	4
ALOX5	4
FABP5	4
FABP3	4
ALOX12	4
PPARD	4
ALOX15	4
PTGS2	4
ARRB1	4
GRK2	4
AKT1	4
NR1I2	5

ABHD12	6
ABHD6	6
NAPEPLD	6
DAGLA	6
NAAA	6
FABP7	6
FAAH	6
DAGLB	6
MGLL	6
SLC22A5	7
SLCO2B1	8
HSPA1A	9

Table S8: Louvain community summary metrics for the protein–protein interaction network. Report of overall network modularity, the total number of detected communities, and community size statistics.

Metric	Value
Overall Modularity	0.4207
Number of Communities	10
Largest Community Size	13
Smallest Community Size	1

Table S9: Louvain community assignments for the protein–chemical interaction network. List of proteins and chemicals and their assigned Louvain communities.

Node	Louvain Community
CNR1	0
CNR2	0
WIN55,212-3	0
WIN55,212-2	0
JWH-018	0
HU-211	0
HU-210	0
CP55,940	0
SR141716A	0
SR144528	0
JWH-073	0
GNAI1	1
GNAI2	2
GNAI3	3
ARRB1	4
GRK2	5
CBG	6
CBN	6
CBC	6
THCV	6
CBDV	6
TRPV4	6
PPARG	6
PGD2	6
AM251	6
PEA	6
GPR55	6
TRPV3	6

TRPV2	6
NAAA	6
AKT1	7
Noladin	8
Virodhamine	9
PPARD	10
GPR18	10
GPR119	10
Δ8-THC	10
OEA	10
Δ9-THC	10
CBL	11
5-HPETE	12
NAPEPLD	13
PLCB1	14
PLCE1	15
FABP7	16
NADA	17
AEA	17
TRPV1	17
FAAH	17
MGLL	17
NAGly	17
LEA	17
JWH-133	17
HSPA1A	18
SLCO2B1	19
ABHD6	20
ABHD12	21
DAGLB	22
2-AG	22

DAGLA	22
NR1I2	22
CBD	22
ALOX12	23
ALOX15	23
PPARA	23
PTGS2	23
ALOX5	23
PGE2	23
AA	23
CBGA	23
THCA	23
CBDA	23
PGA2	23
12-HETE	23
15-HETE	23
LTB4	23
SLC22A5	24
FABP1	25
FABP3	26
FABP5	27

Table S10: Louvain community summary metrics for the protein–chemical interaction network. Report of overall network modularity, the total number of detected communities, and community size statistics.

Metric	Value
Overall Modularity	0.3969
Number of Communities	28
Largest Community Size	14
Smallest Community Size	1

Table S11: Protein–protein interaction network centrality metrics. Top five nodes ranked by centrality measures in the protein–protein only network. Full rankings and centrality metric calculations can be found at https://github.com/aanyashridhar/ECS-Network.

	Betweenness	Closeness	Degree	Eigenvector
1	CNR1	GPR55	FAAH	GPR55
2	PPARA	TRPV1	CNR1	TRPV1
3	PTGS2	AKT1	GPR55	GNAI1
4	ARRB1	NAPEPLD	NAPEPLD	AKT1
5	GPR18	PPARA	MGLL	NAPEPLD

Table S12: Protein–chemical interaction network centrality metrics. Top five nodes ranked by centrality measures in the protein–chemical only network. Betweenness centrality is excluded in the protein–chemical network as this centrality relies on shortest path calculations between nodes, which are not well-defined in networks where chemicals only connect to proteins. Full rankings and centrality metric calculations can be found at https://github.com/aanyashridhar/ECS-Network.

	Closeness	Degree	Eigenvector
1	CBN	CNR1	CBN
2	CBC	CNR2	CBC
3	CBD	TRPV1	CBD
4	THCV	CBDV	THCV
5	CBG	CBN	CBG

Table S13: Comparative centrality rankings of G-protein coupled proteins, GPR55, GPR18, and GPR119. Rankings of three non-canonical cannabinoid receptors across the combined ECS network and the protein–protein only network showing GPR55 outperforming GPR18 and GPR119 in nearly every metric, with the exception of betweenness centrality in the protein–protein only network. Unranked indicates a centrality value of zero, meaning the node had no measurable contribution for that metric. Full rankings and centrality metric calculations can be found at https://github.com/aanyashridhar/ECS-Network.

Network	Centrality	**GPR55**	**GPR18**	**GPR119**
Combined	Betweenness	5	8	Unranked
Combined	Closeness	1	51	Unranked
Combined	Degree	5	19	29
Combined	Eigenvector	5	56	63
Protein–Protein	Betweenness	Unranked	5	Unranked
Protein–Protein	Closeness	1	18	Unranked
Protein–Protein	Degree	3	11	24
Protein–Protein	Eigenvector	1	20	27