Transcriptomic similarities and differences between mouse models and human Alzheimer's Disease

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Abstract

Alzheimer's disease (AD) is a multifactorial pathology responsible for most cases of dementia worldwide. Only a small percentage of AD cases are due to autosomal dominant mutations, while the vast majority have a sporadic presentation. Yet, preclinical research studies relied for decades on animal models that overexpress human genes found in AD autosomal dominant patients. Thus, one could argue that these models do not recapitulate sporadic AD. To avoid human gene overexpression artifacts, knock-in (KI) models have been developed, such as the novel hAβ-KI mouse model, which are still in early phases of characterization. We hypothesize that comparisons at the transcriptomic level may elucidate critical similarities and differences between transgenic/KI models and AD patients. Thus, we aimed at comparing the hippocampal transcriptomic profiling of overexpression (5xFAD and APP/PS1) and KI (hAβ-KI) mouse models with early- (EOAD) and late- (LOAD) onset AD patients. We first evaluated differentially expressed genes (DEGs) and Gene Ontology biological processes (GOBP) overlapping cross-species. After, we explored a network-based strategy to identify master regulators (MR) and the similarities of such elements among models and AD subtypes. A multiple sclerosis (MS) dataset was included to test the molecular specificity of the mouse models to AD. Our analysis revealed that all three mouse models presented more DEGs, GOBP terms and enriched signaling pathways in common with LOAD than with EOAD subjects. Furthermore, semantic similarity of enriched GOBP terms showed mouse model-specific biological alterations, and protein-protein interaction analysis of DEGs identified clusters of genes exclusively shared between hAβ-KI mice and LOAD. Furthermore, we identified 17 transcription factor candidates potentially acting as MR of AD in all three models. Finally, though all mouse models showed transcriptomic similarities to LOAD, hAβ-KI mice presented a remarkable specificity to this AD subtype, which might support the use of the novel hAβ-KI mouse model to advance our understanding of sporadic LOAD.

Keywords: Alzheimer's disease mouse models; humanized Aβ knock-in; APP/PS1; 5xFAD; bioinformatics.

Introduction

Alzheimer's disease (AD) is commonly categorized into early- (EOAD; <65) and late-onset (LOAD; ≥65) based on the age of clinical onset, the latter being the most prevalent form of the disease (Reitz *et al*, 2020). This broad and simplistic definition includes both the mendelian and non-mendelian EOAD, which confers an additional degree of heterogeneity to this group. However, both forms of EOAD seem to present a more prominent brain atrophy, glucose hypometabolism and increased tau PET uptake when compared to LOAD patients (Cho *et al*, 2017; McDade *et al*, 2018; Möller *et al*, 2013; Rabinovici *et al*, 2010; Schöll *et al*, 2017).

Although the autosomal dominant inheritance (mendelian EOAD) due to mutations in APP, PSEN1 and PSEN2 only accounts for ~10% of EOAD cases (Reitz *et al.*, 2020), the genetically driven early-onset forms of the disease represented, for many years, the main target of animal modeling. Indeed, the most used animal models of AD overexpress one or more genes associated with human autosomal dominant AD, such as APP and PSEN1. However, this approach has undergone extensive scrutiny in the past years (Götz *et al*, 2018; Saito *et al*, 2014) due to the fact that these overexpression models do not resemble many important aspects of LOAD. Thus, a major challenge in the field has been the development of new animal models that better recapitulate LOAD. The emergence of new human Aβ knock-in (KI) models, which may better resemble LOAD due to the lack of supra-physiological expression of human transgenes, may avoid some of the pitfalls that overexpression models presented (Baglietto-Vargas *et al*, 2021; Saito *et al.*, 2014; Serneels *et al*, 2020).

In this regard, comparisons between overexpression and KI models with human pathology at the molecular level may help understanding to what extent they can resemble human disease. Additionally, the ability to recapitulate key molecular pathways activated or repressed in AD is crucial for model validity. In this study, we aimed to ascertain the genes and pathways overlapping between two gene overexpression (5xFAD and APP/PS1) and one KI

(hAβ-KI) mouse models, with EOAD and LOAD. With this in mind, we compared the hippocampal transcriptomic profiling of these animal models and AD subtypes, as well as their molecular specificity to the human disease. Finally, we employed regulatory network-based approach to infer and investigate common master regulators (MR) between animal models and AD.

Results

Mouse models exhibit more DEGs overlap with LOAD

DEA between hippocampal region of hAβ-KI, 5xFAD and APP/PS1 mice and their WT controls identified 1537, 3231 and 1768 DEGs, respectively (**Figure 2A, B; Supplemental Figure S1**). We observed that the hAβ-KI animals presented more DEGs overlapping with the 5xFAD mice than with APP/PS1 model [389 (25.3%) *versus* 235 (15.3%) DEGs, respectively; $p < 0.001$; **Figure 2C**]. In addition, the comparison with AD human data demonstrated that hAβ-KI mice exhibited more DEGs in common with LOAD than with EOAD individuals (381 *versus* 164, respectively; $p < 0.001$; **Figure 2D, E**). Despite being mouse models carrying AD familial mutations, APP/PS1 and 5xFAD mice shared more DEGs with LOAD than with EOAD patients (**Figure 2D, E**). Moreover, considering the total of DEGs identified in the mouse models as reference (model-disease overlap), the intersection of DEGs between 5xFAD and EOAD was significantly higher (14%) than the overlap between both hAβ-KI (10.7%; $p = 0.0054$) and APP/PS1 (10.9%; $p = 0.0061$) with EOAD (**Figure 2D - right**). On the other hand, the overlap of LOAD DEGs with 5xFAD (28%) and with APP/PS1 (25.3%) mice were not significantly different from the overlap with hAβ-KI (24.8 %) (**Figure 2E - right**; $p = 0.123$ and $p = 0.063$, respectively). Only seven DEGs were found in common with all the mouse models and human AD. Among them, DEGs related to innate immune response (C1QB, CD33, CD14 and SLC11A1) are consistently upregulated across the groups, while those related with membrane potential regulation and neuropeptide production (KCNK1 and SST) are mostly downregulated. The S100A6 gene, found in neurons and in some populations

of astrocytes, was upregulated in all conditions (**Supplemental Table 2**). Interestingly, the hAβ-KI model shared 212 DEGs exclusively with LOAD, while only 98 with EOAD (**Figure 2A, B**). The PPI network revealed that PTGS3, GNB1, ARIH2, SMURF1, EIF3A are the gene hubs of the four clusters formed according to protein interaction (**Figure 2F**). Considering the DEGs exclusively shared with the hAβ-KI model and EOAD subjects, only 11 remained in the PPI network, with POLR2B being the hub of the single cluster formed (**Figure 2G**). Next, we compared DEGs of each mouse model with a database from MS patients to verify the specificity of these models for AD pathology. Both 5xFAD and APP/PS1 mice presented greater overlap of DEGs with AD than with MS subjects (**Figure 2H, I; Supplemental Figure S2A, B**). Interestingly, no significant differences were observed in DEGs shared between hAβ-KI mice and EOAD (10.7%) or MS patients (9.6%; **Figure 2J; Supplemental Figure S2C**), while the overlap of hAβ-KI DEGs with LOAD was significantly higher than with both EOAD and MS (24.8%; $p < 0.001$; **Figure 2J**; **Supplemental Figure S2C**).

hAβ-KI mice and LOAD patients present higher functional changes similarities

Functional enrichment analysis of gene ontology for biological processes (GOBPs) revealed that ~ 92% of the GOBPs enriched in hAβ-KI mice overlap with enriched terms in LOAD patients (**Figure 3B, E - right**) in the model-disease approach, while the intersection with EOAD was markedly low (around 32%, **Figure 3A, D - right**). The remaining 8% of GOBP terms not shared between hAβ-KI mice and LOAD were related to RNA splicing and protein phosphorylation (**Supplemental Table 3**). Additionally, the hAβ-KI mice presented a higher number of enriched GOBP terms in common with the 5xFAD (79.2%) than with the APP/PS1 (56.1%) model (**Figure 3C**; $p < 0.001$). Unexpectedly, both 5xFAD and APP/PS1 shared more than 75% of their enriched GOBPs with LOAD patients (**Figure 3B, E - right**), an overlap greater than the observed with EOAD (~ 45%) (**Figure 3A, D - right**). Considering the disease-model overlap, the 5xFAD mice was the only one presenting significant higher intersection with both EOAD (55%) and LOAD (46.8%; **Figure 3D - left, E - left**). Specifically, it was observed about 50% of GOBPs overlap between EOAD and LOAD with

5xFAD, indicating a lack of specificity for this model. The comparison among the three models with MS GOBPs demonstrated, however, a great specificity of these mouse models for AD (**Supplemental Figure S2)**. In fact, **Figure 3F-H** shows that all mouse models evaluated presented less than 5% of enriched GOBP terms in common with MS.

hAβ-KI mice present less GOBP terms intersection with EOAD patients in comparison to APP/PS1 and 5xFAD models

The union of enriched GOBP intersecting terms in the mouse models and human AD were generated to better visualize the common biological processes altered in each group. "Regulation of cytokine secretion" (13 nodes), "regulation of catabolic processes" (3 nodes), "NF κ B signaling" (9 nodes) and "intracellular transport and secretion" (21 nodes) were among the GOBP terms enriched in the hippocampus of all three mouse models and EOAD patients (**Figure 4; light gray circles**). Of note, the majority of the GOBP terms in common with EOAD come from the 5xFAD and APP/PS1 models (102 nodes, **orange circles**). These terms are mainly related to cellular response to stressor agents, hormones and cytokines, immune response and calcium homeostasis and transport. Interestingly, GOBP terms related with oxidative phosphorylation found in EOAD only appeared enriched in the APP/PS1 mouse model (**purple circles**).

hAβ-KI mice and LOAD subjects exhibit more overlap among the enriched GOBP terms

Figure 5 shows that hAβ-KI mice GOBP terms are more represented among the GOBPs in common with LOAD (**yellow, purple and pink circles**) than with EOAD. “PI3K and NF κ B signaling pathways” (20 nodes), “regulation of neuronal development” (19 nodes), “regulation of neurotransmitter and hormone secretion” (31 nodes), “immune response” (22 nodes), “antigen processing and presentation” (21 nodes) and “phospholipid and ribose phosphate metabolism” (10 nodes) are among the enriched biological processes shared with all the three mouse models and LOAD (**pink circles**). Interestingly, biological processes as

“glucose, cholesterol and purine metabolism”, “regulation of MAPK cascade” and “calcium homeostasis” only appeared enriched in 5xFAD and APP/PS1 mice (**gray circles**).

The majority of the hAβ-KI enriched KEGGs is shared with LOAD

The most affected pathways related to changes in transcriptome profile were identified using enrichment analysis of KEGG canonical pathways. **Figure 6A, B** shows that among the 10 KEGGs found significantly enriched in the hAβ-KI model, six were also identified in LOAD subjects ("glutamatergic and GABAergic synapse", "calcium signaling", "Rap1 and Ras signaling" and "choline metabolism in cancer"), while only one was enriched in EOAD patients ("amyotrophic lateral sclerosis"; **Supplemental Table 4**). KEGG analysis also revealed that APP/PS1 was the mouse model that presented the highest percentage of pathway overlap with both EOAD (36.7%; **Figure 6C**) and LOAD (73.3%; **Figure 6D**). Interestingly, most of the intersection between APP/PS1 and human AD is represented by KEGGs related to other neurodegenerative diseases (e.g. Parkinson’s disease, Huntington’s disease) or bacterial infection (e.g. *Escherichia coli*, *Salmonella* spp.; **Supplemental Table 4**). Despite PI3K pathway-related GOBPs appeared enriched in LOAD, they were only significantly altered in the hAβ-KI model in the KEGG analysis. “Rap1 and Ras signaling pathways” and “adhesion and apoptosis” were found consistently altered in the APP/PS1 and hAβ-KI models (**Figure 6H, I; Supplemental Table 4**). In addition, the 5xFAD was the mouse model that presented more enriched KEGG terms (**Figure 6G; Supplemental Table 4**). Similar to the GOBP enrichment analysis, 5xFAD presented more pathways shared with LOAD (56.9%) than with EOAD (13.8%; **Figure 6C, D**), which were mainly related to synaptic neurotransmission and insulin regulation (**Supplemental Table 4**). Additionally, alterations in apoptosis- and endocannabinoid system-related pathways were observed in the 5xFAD model and in the human disease (**Supplemental Table 4**). Despite an overlap in KEGGs associated to inflammatory diseases and bacterial infections between 5xFAD and AD, well-studied signaling pathways in the context of inflammation were only observed in the 5xFAD model (e.g. TNF, Toll-like, NFκB).

EOAD and LOAD patients share two enriched MR with AD mouse models

To identify elements located in higher positions of the biological system hierarchy, we performed an MRA to search for transcription factors potentially driving the biological alterations observed in AD. In addition, we asked if these elements were also driving the transcriptional profile changes in the mouse models. Our analysis revealed a total of 95 MR enriched with DEGs that were in at least one experimental group (**Figure 7A, B; Supplemental Table 5**). Interestingly, both 5xFAD and APP/PS1 mouse models presented more MR in common with LOAD than with EOAD, while hAβ-KI mice exhibited a similar overlap of MR with both subtypes of human AD (**Figure 7A, B**). Among the 17 MR identified in ≥ 4 groups, only PARK2 and SOX9 were enriched in all the three models and in the human disease (**Figure 7C**). Next, two-tail GSEA was performed to infer the activation state of each MR candidate. We observed that PARK2 and SOX9 are respectively repressed and activated across the disease/animal model phenotypes evaluated here (**Figure 7D**). In addition, FOXC2 and ZNF461 were identified exclusively in the hAβ-KI mice and LOAD individuals (**Supplemental Table 5)**. The three mouse models share eight enriched MR, most of which involved in the regulation of cell cycle and apoptosis (**Supplemental Table 5**). A different methodological approach to infer activation of transcription factors was also applied and similar results were observed for the 17 MR candidates (**Supplemental Figure S3**). Finally, a comparison with a previously published study that investigated MR associated with AD showed that 11 out of 17 MR identified here were also enriched in that study (**Figure 7E**).

Discussion

Great efforts were made in the last decade to generate mouse models that better recapitulate the sporadic characteristics of LOAD; however, their translational value is still being addressed. Here, we evaluated hippocampal similarities and differences of three mouse models (APP/PS1, 5xFAD and hAβ-KI) at the transcriptional level. A cross-species comparison between these models with EOAD and LOAD subjects was also conducted. Overall, we observed that the hAβ-KI mice presented more similarities with 5xFAD compared to the

APP/PS1 model. Interestingly, all mouse models showed more similarities to LOAD than to EOAD. Finally, the comparison with human AD pointed to a striking specific transcriptomic similarity of hAβ-KI with LOAD.

The specificity of the hAβ-KI model for LOAD was first observed in the DEG analysis, as the hAβ-KI mice presented twice more DEGs exclusively overlapping with LOAD than with EOAD subjects. Further PPI network analysis of these DEGs identified clusters of gene products that physically interact with each other to accomplish certain cellular functions. For example, we found clusters involved in ribosomal RNA processing, inflammatory response and E3-ubiquitin ligase-related immune response, all phenomena well described in AD (Ding *et al*, 2005; Gjoneska *et al*, 2015; Hegde *et al*, 2019). Corroborating, Baglietto-Vargas and colleagues demonstrated that the hAβ-KI mouse presented a decreased production of the anti-inflammatory cytokines IL-2 and IL-10, compared to age-matched WT animals (Baglietto-Vargas *et al.*, 2021). Importantly, a cluster of genes encoding G protein and G protein-coupled receptors (GPCRs) was also evidenced in our study. G proteins act as modulators or transducers in various transmembrane signaling systems and GPCRs are implicated in the pathogenesis of AD, in multiple stages of APP processing, and can be directly or indirectly impacted by Aβ (Thathiah & De Strooper, 2011). Indeed, primary rat neuronal cultures and brain slices treated with a metabotropic, but not ionotropic, glutamate receptor (mGluR) agonist stimulated APP secretion, suggesting that G protein signaling cascade in response to mGluRs activation is linked to APP processing by α-secretase (Lee *et al*, 1995). Interestingly, this process seems to be dependent of the PI3K signaling activation (Ledonne *et al*, 2015), a pathway significantly altered in the hAβ-KI mice. Network-based approaches are powerful strategies to identify elements, pathways or functional modules altered in several diseases (Basu *et al*, 2021; Santiago *et al*, 2017); the clusters identified here might shed light in the understanding of pathogenic mechanisms of LOAD that can be recapitulated by the novel hAβ-KI mouse model, facilitating the search for therapeutic targets.

Interestingly, only seven out of 7868 DEGs identified were shared among all the mouse models, EOAD and LOAD patients, which include the SST, C1QB, CD33, CD14, SLC11A1, KCNK1 and S100A6 genes. Of note, it has already been demonstrated a remarkable decrease in SST RNA levels in the brain of AD patients (Gahete *et al*, 2010). Interestingly, it was recently demonstrated that somatostatin, coded by the SST gene, was the most selectively enriched binder to soluble oligomeric Aβ in the human brain, influencing Aβ aggregation and masking the ability of widely-used antibodies to detect Aβ (Wang *et al*, 2017). In this regard, Nilsson and colleagues demonstrated that the modulation of the somatostatin receptors Sst1 and Sst4 regulates neprilysin, the major Aβ-degrading enzyme. Specifically, they showed that genetic deficiency of Sst1 and Sst4 in the App^{NL-F} KI mice decreased neprisilyn protein levels in the hippocampus, leading to an increase in the $A\beta_{1-42}$ levels (Nilsson *et al*, 2020). The consistent findings observed in our study suggest that SST role in AD pathology seems to be conserved cross-species. Additionally, we found that C1QB, CD33, CD14 and SLC11A1 genes, all associated with immune response, were upregulated in all analyzed mouse models, as well as in EOAD and LOAD patients. Accordingly, Gjoneska and colleagues previously pointed a conserved immunological basis for AD by comparing the CK-p25 mouse model with hippocampal human *post-mortem* tissue (Gjoneska *et al.*, 2015) and a single-nuclei analysis of AD brains already attributed C1QB and CD14 gene expression for microglial population (Mathys *et al*, 2019).

The replication of transcriptomic changes at the pathway level is more consistent than single gene analysis and has the potential to offer insights into the biological processes disturbed in AD. In this sense, we observed that the hAβ-KI mouse presented almost a complete overlap of enriched hippocampal GOBP terms with LOAD, while only about one third was shared with EOAD. This apparent specificity for LOAD seems to be a unique and important feature of this novel KI model, as the 5xFAD and APP/PS1 mice did not discern between AD subtypes. Despite of that, the scanty overlap of GOBP terms among AD mouse models and MS confirmed that these models present transcriptomic features specific to AD rather than general

alterations shared among other neurodegenerative diseases. In addition, this resemblance with AD appears to be a characteristic specifically of rodent models carrying human mutated genes directly related to AD, such as APP and PSEN1, or humanized Aβ. Indeed, Burns and colleagues observed that Tg4510 mice, that resemble tau pathology but also harbor mutations found in familial frontotemporal dementia, presented highest enrichment of genes in common with human amyotrophic lateral sclerosis (ALS) and Huntington's disease rather than with AD (Burns *et al*, 2015).

Compromised calcium (Ca^{2+}) handling by neurons precedes the formation of Aβ plaques and neurofibrillary tangles and has been implicated in virtually all the major molecular alterations underlying the pathogenesis of AD (Tong *et al*, 2018). The association between PS1 mutations and altered Ca^{2+} signaling in neurons has been known for many years (Tong *et al*, 2016). However, the lack of rodent models that resemble more than the AD hallmarks (i.e. Aβ load and cognitive impairment) has been a major limitation in the field (2017). In addition, early imbalance between excitatory/inhibitory (E/I) neurotransmission, with a loss of neuronal network stability, is a well-attested phenomenon in AD (Frere & Slutsky, 2018; Palop & Mucke, 2016). The average incidence of seizure in AD patients is around 15%, which is 7-fold higher when compared to individuals without dementia (Horváth *et al*, 2016; Imfeld *et al*, 2013). Aβ is able to impair the long-term potentiation of synaptic strength, promote depression of synaptic activity and alter brain network (Palop & Mucke, 2016), and affect the E/I balance by impairing the inhibitory activity of the parvalbumin-expressing interneurons (Verret *et al*, 2012). Interestingly, the modulation of interneuron function might improve brain rhythms and cognitive functions in AD (Tong *et al*, 2014; Verret *et al.*, 2012). Our study identified "calcium signaling" and "glutamatergic and GABAergic signaling" to be exclusively altered in the hAβ-KI mice and LOAD, pointing this KI mouse model as an important tool to better understand calcium signaling and the E/I imbalance in AD.

Transcription factors play key roles orchestrating phenotypic determination through the regulation of numerous transcriptional targets that coordinate complex cellular processes (Carro *et al*, 2010; Vargas *et al*, 2018). Based on reverse engineering co-expression regulatory network reconstruction, we identified two transcription factors exclusively altered in the hAβ-KI model and LOAD individuals' hippocampi: ZNF461 and FOXC2. ZNF461 role in brain function is still poorly explored; however, this transcription factor was identified among genes that represent a polygenetic risk for psychiatric disorders and its alteration might contribute to cortical atrophy and changes in functional connectivity (Lee *et al*, 2017). Accordingly, changes in cortical thickness show strong correlation with the clinical stages of AD (Ossenkoppele *et al*, 2019). In the same way, a decreased functional connectivity is not only positively correlated with disease severity in autosomal dominant AD and LOAD patients (Thomas *et al*, 2014), but also predicts cognitive decline in asymptomatic preclinical AD individuals (Buckley *et al*, 2017). On the other hand, FOXC2 function in the brain is implicated in cell proliferation and invasion in glioblastoma (Li *et al*, 2013), in angiogenic processes during fetal brain development (Siegenthaler *et al*, 2013), and is directly regulated by cyclin-dependent kinase 5 (Cdk5) phosphorylation to control peripheral lymphatic vase development (Liebl *et al*, 2015). Interestingly, it has been demonstrated that the deregulation of Cdk5 contributes to AD pathology preceding tau hyperphosphorylation and loss of synaptic proteins (Kurbatskaya *et al*, 2016). On the other hand, Saito and colleagues reported that the App^{NL-F} KI model did not generate p25, necessary for aberrant Cdk5 activation, suggesting that the reports about its relevance in AD might be an artifact due to the overexpression of APP in other mouse models (Saito *et al*, 2016). However, there are several studies using organotypic hippocampal slices and primary neural cells exposed to Aβ show increased p25 generation independently of APP overexpression (Ma *et al*, 2013; Seo *et al*, 2014; Zheng *et al*, 2005), suggesting that experimental validation of FOXC2 and Cdk5 in hAβ-KI mice is needed to understand the potential link among FOXC2, Cdk5 and AD pathology.

Further, in the MRA, we identified SOX9 as a transcription factor consistently activated in the mouse models and human AD. SOX9 is a key factor in the nervous system development, especially for astrocyte and oligodendrocyte cell fate specification (Molofsky *et al*, 2013; Nagao *et al*, 2016; Stolt *et al*, 2003). Recently, Sun and colleagues identified SOX9 as an astrocyte-specific nuclear marker in the adult human and mouse brains, presenting a remarkable expression in murine hippocampus and cortex, when compared to the cerebellum (Sun *et al*, 2017). In addition, the upregulation of SOX9 in mouse models of ALS, spinal cord injury and stroke was already reported in the literature (McKillop *et al*, 2013; McKillop *et al*, 2016; Sun *et al.*, 2017). However, there are no studies to date linking this transcription factor with AD, and our results highlight a promising new target for investigation in AD pathophysiology. In addition, PARK2 gene was also identified in the MRA as a transcription factor consistently repressed among all groups of this study. PARK2 encodes an E3 ubiquitin ligase and its involvement in autosomal recessive parkinsonism is well established. Although less explored, its role in AD has already been demonstrated by computational (Kumar & Kumar, 2019; Vargas *et al.*, 2018) and experimental (Goiran *et al*, 2018; Martín-Maestro *et al*, 2019; Martín-Maestro *et al*, 2016) approaches. Specifically, mitophagy failure, promoted by a repression in PARK2 ability to stimulate PS1, was evidenced in cellular and animal models (Goiran *et al.*, 2018; Martín-Maestro *et al.*, 2019). On the other hand, the overexpression of PARK2 promoted diminished ubiquitinated proteins accumulation, improved its targeting to mitochondria and potentiated autophagic vesicle synthesis (Martín-Maestro *et al.*, 2016). Additionally, a meta-analysis from genome-wide association studies identified PARK2 as a potential risk gene for AD. Our findings highlight the value of hAβ-KI, 5xFAD and APP/PS1 mouse models to better understand these particularly underexplored aspects in AD.

Decades of use of animal models in AD research underline that each one is able to mimic a slightly different aspect of the disease. Therefore, one could argue that animal models of AD should be elected according to the biological aspect aimed for investigation, rather than be seen as a generic model. The clusterization by semantic similarity of enriched GOBP terms

performed here allowed to recognize biological alterations specific of each animal mouse model. For example, several GOBP terms associated to oxidative phosphorylation, purine metabolism and the MAPK pathway were altered in 5xFAD and APP/PS1 mice but not in the hAβ-KI model. On the other hand, inflammatory-related processes seem to be a feature of AD pathology present in all three mouse models. Thus, the comparison of altered biological processes among mouse models and human AD presented here might help to guide future methodological decisions regarding the most appropriate animal model to answer a specific research question. Finally, the transcription factors potentially acting as master regulators of AD observed in this study need to be further explored and validated experimentally, having the potential to greatly aid future research and mechanistic understanding of the disease.

Methods

Mouse Models Data Acquisition

RNA sequencing (RNA-seq) data from 5xFAD [4, 8 and 12 months-old, n = 23 hemizygous; 26 wild-type (WT)] and hAβ-KI (22 months-old, n = 7 homozygous; 8 WT) AD mouse models (**Supplemental Table 1**) were obtained from AMP-AD Knowledge Portal (https://adknowledgeportal.synapse.org/) using synapser (version 0.7.64) and synapserutils (version 0.1.6) packages. Specifically, gene expression information was collected from https://www.synapse.org/#!Synapse:syn16798173 and https://www.synapse.org/#!Synapse:syn18634479 for 5xFAD and hAβ-KI models, respectively. APP/PS1 (8 and 12 months-old, n = 8 APP/PS1; 8 WT) mouse model RNA-seq data (**Supplemental Table 1**) was combined from two Gene Expression Omnibus (GEO) (https://www.ncbi.nlm.nih.gov/geo/) datasets [GSE149661 (Unger *et al*, 2020) and GSE145907] and downloaded through NCBI Sequence Read Archive using SRAToolKit (https://github.com/ncbi/sra-tools). Known phenotypic features of the three mouse models evaluated in this study are depicted in **Figure 1A**.

Human Data Acquisition

Human AD hippocampal processed microarray data of five studies were obtained from GEO repository [GSE28146 (Blalock *et al*, 2011), GSE29378 (Miller *et al*, 2013), GSE36980 (Hokama *et al*, 2014), GSE48350 (Berchtold *et al*, 2008) and GSE84422 (Wang *et al*, 2016)], downloaded using GEOquery package (v2.56.0) (Davis & Meltzer, 2007) and combined under common gene symbol annotations. Afterwards, batch correction was implemented using the sva package (v3.36.0) (Leek *et al*, 2012) and data was split into EOAD (age at death < 65, n = 4 EOAD; 10 control) and LOAD (age at death ≥ 65, n = 59 LOAD; 63 control) for further analyses (**Supplemental Table 1**). AD diagnosis, control definition and exclusion criteria of each GSE study are depicted in **Figure 1B**. Multiple sclerosis (n = 5 MS; 5 control) hippocampal RNA-seq data (**Supplemental Table 1**) was also obtained from GEO under the identifier GSE123496 (Voskuhl *et al*, 2019) and downloaded through NCBI Sequence Read Archive. Sample demographics can be found in **Figure 1C**.

RNA-seq Processing

Raw data for each RNA-seq dataset was downloaded and transcript alignment was performed using Salmon (v1.3.0) (Patro *et al*, 2017). Transcripts were mapped to genome using indexes derived from *Mus musculus* GRCm38 Ensembl build (ftp://ftp.ensembl.org/pub/release-96/fasta/mus_musculus) and *Homo sapiens* GRCH38 Ensembl build (ftp://ftp.ensembl.org/pub/release-96/fasta/homo_sapiens) for the mouse models and human data, respectively. Aligned reads were summarized using tximport (v1.12.3) (Soneson *et al*, 2015) and genes with minimum mean of counts per million cut-off < 2 were filtered out.

Differential Expression Analyses

Differential expression was computed on processed microarray data using the limma package (Ritchie *et al*, 2015) *lmFit* function to fit multiple linear models by generalized least squares. In addition, *eBayes* function was used to compute moderated t-statistics, moderated F-statistic and log-odds of differential expression by empirical Bayes moderation of

the standard errors towards a common value. For RNA-seq datasets, processed expression data from each study was submitted to DESeq2 (v1.28.1) (Love *et al*, 2014) method using previously created tximport Summarized Experiment. Differential expression analysis (DEA) based on the Negative Binomial distribution was computed with the *DESeq* function followed by log fold change shrinkage with the *lfcShrink* function (shrinkage estimator type = “ashr”). Venn diagrams for differentially expressed genes (DEGs) were constructed using VennDiagram package (v1.6.20) (Chen & Boutros, 2011).

Protein-Protein Interaction Network Reconstruction

We used the Search Tool for the Retrieval of Interacting Genes/Proteins (STRING) Consortium to build protein-protein interaction (PPI) networks. STRING is a biological database and web resource of known and predicted PPI which contains information from numerous sources, including experimental data, computational prediction methods and public text collections. The construction of the PPI networks was implemented in R using the STRINGdb (v2.0.2), RedeR (v1.36.0) and igraph (v1.2.6) packages (Castro *et al*, 2012; Mora & Donaldson, 2011; Szklarczyk *et al*, 2019). For the final networks, we retained only the edges with a combined interaction score > 0.7 from all sources and highly connected nodes for the final networks.

Functional Enrichment Analyses

DEGs (p-value < 0.05) from human or mouse model studies were submitted to Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) enrichment analysis using the clusterProfiler package (v3.16.1) *enrichKEGG* and *enrichGO* functions. The GO terms were clustered by semantic similarity using the *mgoSim* function from GOSemSim (v2.14.2) package (Yu *et al*, 2010) (arguments measure = "Wang" and combine = NULL). The resulting similarity matrices were represented as GO networks using the RedeR (v1.36.0) package (Castro *et al.*, 2012) for interactive visualization and manipulation of nested networks. Clusters of GO terms obtained from GOSemSim algorithm were manually named for their

biological interpretation. Venn diagrams for enriched GO/KEGG terms were constructed using VennDiagram (v1.6.20) package (Chen & Boutros, 2011). Nested networks were constructed by maintaining only the intersecting GOBP terms among the mouse models and each human pathology (either EOAD or LOAD). Finally, Jaccard coefficient > 0.7 was used to filter out edges with low gene intersection between terms.

Reverse Engineering of Transcriptional Network

The transcriptional network (TN) centered on transcription factors (TF) and their predicted target genes were inferred using a large cohort of neurologically and neuropathologically normal individuals (n = 122) obtained from GEO under the identifier GSE60862 (Trabzuni *et al*, 2013). Herein, the terms "regulatory unit" or "regulon" are used to describe the groups of inferred genes and their associated TFs. RTN (v2.12.1) package was used to reconstruct and analyze TNs based on the mutual information (MI) using the Algorithm for the Reconstruction of Accurate Cellular Networks (ARACNe) method (Fletcher *et al*, 2013; Margolin *et al*, 2006a; Margolin *et al*, 2006b). In summary, the regulatory structure of the network is derived by mapping significant associations between known TFs and all potential targets. To create a consensus bootstrap network, the interactions below a minimum MI threshold are eliminated by a permutation step and unstable interactions are additionally removed by bootstrap. Finally, data processing inequality algorithm is applied with null tolerance to eliminate interactions that are likely to be mediated by a third TF. The reference hippocampus TN was built using the package's default number of 1000 permutations and 100 bootstraps (p-value < 0.001).

Master Regulators Inference and Two-Tailed Gene Set Enrichment Analysis

Master regulator (MR) analysis (MRA) was employed for the MR inference (Carro *et al.*, 2010). MRA computes the statistical overrepresentation of DEGs (p-value < 0.05) obtained from DEA in the regulatory units of the reference TN. The regulons were considered altered in the disease if they presented (1) statistical enrichment of DEGs, (2) regulon size > 50

and (3) ≥ 80% of the queried case-control studies. Two-tailed Gene Set Enrichment Analysis (GSEA) was also performed using the RTN package (version 2.4.6, p-value < 0.05 and 1000 permutations). Briefly, Pearson's correlation was used to split the regulatory units into positively (A) and negatively (B) associated targets. Afterwards, the phenotype association of each subgroup was tested using the GSEA (Subramanian *et al*, 2005) statistics, resulting in independent enrichment scores (ES) for each subgroup. Finally, we tested the differential enrichment (ESA – ESB) considering the following desirable criteria for clear association: (1) a maximum deviation from zero near opposite extremes and (2) a good separation of the two distributions. Thus, a high negative differential score implies that the regulon is repressed in the disorder phenotype, while a high positive one indicates that the regulon is induced.

Virtual Inference of Protein Activity by Enriched Regulon Analysis

The virtual inference of protein activity by enriched regulon analysis (VIPER) is another regulatory-network based approach to infer protein activity from gene expression profiles. Similar to MRA, VIPER systematically analyze the expression of the regulatory units previously identified by ARACNe algorithm. However, VIPER uses a fully probabilistic, yet efficient enrichment analysis framework based on analytic rank-based enrichment analysis. The analysis was implemented using the viper (v1.22.0) package in R (Alvarez *et al*, 2016).

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Author Contributions

MAB selected the databases and performed transcriptomics analysis. MAB, BB and GCC analyzed and interpreted the data and conceived figures and tables. MAB and BB wrote the manuscript. ERZ conceived and supervised the project. ERZ, GCC, TAP, SF and ACM edited and revised the manuscript for intellectual content.

Conflict of interest

The authors declare that they have no conflicts of interest.

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Figure legends

Figure 1 | Phenotypic features of AD mouse models, AD diagnosis, control group definition, and demographics of human samples. APP/PS1 (top), 5xFAD (middle) and hAβ-KI mice (bottom) timelines showing specific phenotypes of each mouse model across different ages (in months (**A**). The numbers in bold correspond to the time points evaluated in this study. Data for APP/PS1 and 5xFAD mice was available at AlzForum. hAβ-KI information was taken from Baglietto-Vargas et al (Baglietto-Vargas *et al.*, 2021). The subjects were diagnosed with AD according to established clinical criteria and/or neuropathological *post-mortem* evaluation in each selected GSE study **(B)**. Age, sample size and sex information are specified for EOAD, LOAD and MS subjects and their age-matched controls **(C)**. NINCDS-ADRDA 1984 (McKhann *et al*, 1984); CERAD (Mirra *et al*, 1991); NIA-Reagan Institute criteria (Hyman, 1997); Braak staging (Braak & Braak, 1991); NFT = neurofibrillary tangles; MMSE = Mini-Mental State Examination; DLB = Dementia with Lewy-Bodies; PD = Parkinson's disease; MS = Multiple Sclerosis. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease.

Figure 2 | Shared DEGs among EOAD, LOAD and mouse models of AD. Venn diagram showing DEGs overlap between EOAD **(A)** and LOAD **(B)** with 5xFAD, APP/PS1 and hAβ-KI mice. Mosaic plot of hAβ-KI, 5xFAD and APP/PS1 overlap **(C)**. Mosaic plot of EOAD-model (left) and model-EOAD (right) DEGs overlap with 5xFAD, APP/PS1 and hAβ-KI mice **(D)**. Mosaic plot of LOAD-model (left) and model-LOAD (right) DEGs overlap with 5xFAD, APP/PS1 and hAβ-KI mice **(E)**. PPI network of DEGs exclusively shared between hAβ-KI mice and LOAD **(F)** or EOAD **(G)** subjects. Mosaic plot of EOAD, LOAD or MS DEGs overlap with 5xFAD mice **(H)**. Mosaic plot of EOAD, LOAD or MS DEGs overlap with APP/PS1 mice **(I)**. Mosaic plot of EOAD, LOAD or MS DEGs overlap with hAβ-KI mice **(J)**. The size of the red and yellow boxes reflects the proportion of overlapping and non-overlapping DEGs, respectively. Pearson's Chi−squared test with Yates' continuity correction was applied for the

mosaic plot analysis. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure 3 | GOBP overlap among EOAD, LOAD and mouse models of AD. Venn diagram of GOBPs intersections in EOAD **(A)** and LOAD **(B)** with 5xFAD, APP/PS1 and hAβ-KI mice. Mosaic plot of hAβ-KI-5xFAD and -APP/PS1 GOBP overlap **(C)**. Mosaic plot of EOAD-model (left) and model-EOAD (right) GOBP overlap with 5xFAD, APP/PS1 and hAβ-KI mice **(D)**. Mosaic plot of LOAD-model (left) and model-LOAD (right) GOBP overlap with 5xFAD, APP/PS1 and hAβ-KI mice **(E)**. Mosaic plot of EOAD, LOAD or MS GOBP overlap with 5xFAD mice **(F)**. Mosaic plot of EOAD, LOAD or MS GOBP overlap with APP/PS1 mice **(G)**. Mosaic plot of EOAD, LOAD or MS GOBP overlap with hAβ-KI mice **(H)**. The size of the red and yellow boxes reflects the proportion of overlapping and non-overlapping GOBPs, respectively. Pearson's Chi−squared test with Yates' continuity correction was applied for the mosaic plot analysis. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure 4 | Nested networks of enriched GOBP intersections between EOAD and AD mouse models. Blue, purple and orange circles represent the overlap between EOAD and protein overexpression mouse models. Intersection among EOAD and hAβ-KI are represented by circles in shades of gray. Each box represents a cluster of GOBP terms grouped by semantic similarity and named manually according to its main biological role. Circle sizes represent the number of genes enriched in the GO term. EOAD = early-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure 5 | Nested networks of enriched GOBP intersections between LOAD and AD mouse models. Light purple, dark purple and green circles represent all the GOBP terms overlap between LOAD and hAβ-KI. Intersection among LOAD and overexpression protein mouse models are represented by circles in shades of gray. Each box represents a cluster of GOBP terms grouped by semantic similarity and named manually according to its main biological role.

Circle sizes represent the number of genes enriched in the GO term. LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure 6 | Functional enrichment analysis of KEGG terms in mouse models of AD and human disease. Venn diagrams showing the KEGGs overlap among EOAD **(A)** and LOAD **(B)** subjects with 5xFAD, APP/PS1 and hAβ-KI mice. Mosaic plot of 5xFAD, APP/PS1 and hAβ-KI KEGGs overlap with EOAD **(C)** or LOAD **(D)**. Dual-plots showing the up- (red) and down- (blue) regulated KEGGs for EOAD **(E)**, LOAD **(F)**, 5xFAD **(G)**, APP/PS1 **(H)** and hAβ-KI **(I)**. The size of the red and yellow boxes reflects the proportion of overlapping and non-overlapping KEGGs, respectively. Pearson's Chi−squared test with Yates' continuity correction was applied for the mosaic plot analysis. The pathways in the dual-plots are represented in the Y axis; the left and the right part of the X axis corresponds to the number of genes enriched in the KEGG terms and their q-value (grey bars). EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure 7 | Master regulator analysis of mouse models of AD and human disease. Venn diagrams of EOAD **(A)** or LOAD **(B)** with 5xFAD, APP/PS1 and hAβ-KI MRs overlap. Tile-plot of regulons significantly enriched with DEGs in EOAD, LOAD, 5xFAD, APP/PS1 and hAβ-KI (only regulatory units significant in ≥ 4 contexts are represented) **(C)**. Tile-plot showing the activation state of the transcription factors acting as MR in EOAD, LOAD, 5xFAD, APP/PS1 and hAβ-KI **(D)**. Venn diagram showing the overlap between previously MR candidates found by Vargas et al. and the current study **(E)**. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; hAβ-KI = human amyloid-β knock-in; ns = non-significant.

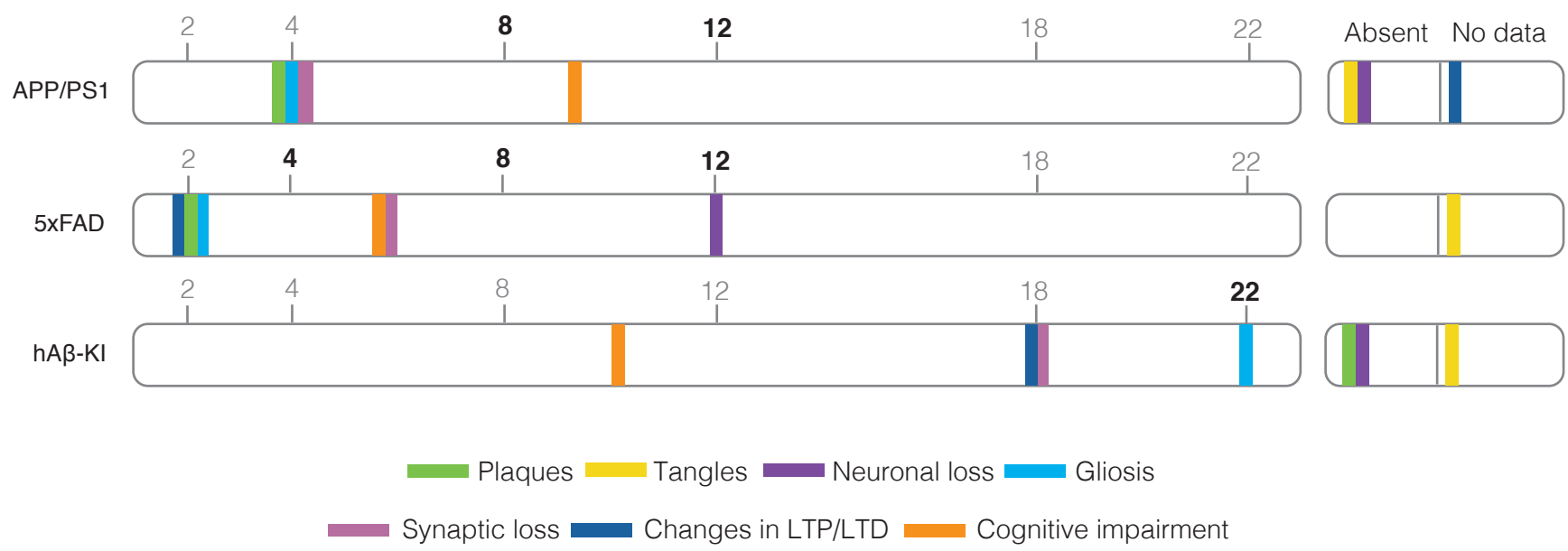
Supplemental Figure Legends

Figure S1 | DEGs in mouse models of AD and human disease. Volcano-plots and pie charts showing the up- and down-regulated genes in 5xFAD **(A)**, APP/PS1 **(B)**, hAβ-KI **(C)**, EOAD **(D)**, LOAD **(E)** and MS subjects **(F)**. The red points in the volcano-plots correspond to a DEG when compared to its control. In the pie charts, the pink and blue colors correspond respectively to the up- and down-regulated DEGs. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure S2 | Shared DEGs and enriched GOBP among mouse models of AD and human disease. Venn diagrams showing the DEGs (left) and GOBP terms (right) overlap among 5xFAD **(A, D)**, APP/PS1 **(B, E)** and hAβ-KI **(C, F)** mice with EOAD, LOAD and MS subjects. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; MS = multiple sclerosis; hAβ-KI = human amyloid-β knock-in.

Figure S3 | VIPER analysis of EOAD, LOAD and AD mouse models. Plot of inferred regulon activity showing the up- (redish) and down- (blueish) regulated transcription factors in each group: EOAD **(A)**, LOAD **(B)**, 5xFAD **(C)**, APP/PS1 **(D)** and hAβ-KI **(E)**. EOAD = early-onset Alzheimer's disease; LOAD = late-onset Alzheimer's disease; hAβ-KI = human amyloid-β knock-in. *p-value < 0.05

A)



B)

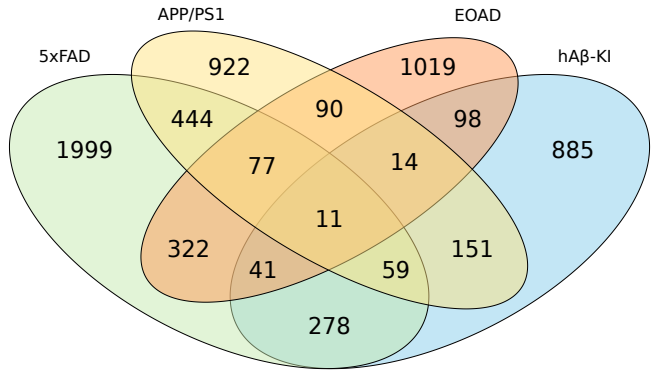
AD diagnosis and control definition			
GSE	AD diagnosis	Control definition	Exclusion criteria
28146	NINCDS-ADRDA + CERAD + NIA-Reagan or NFT count + Braak staging + amyloid plaque count	MMSE > 25	Not specified
29378	NINCDS-ADRDA + Braak staging + amyloid plaque count	Normal cognitive and functional examination	Not specified
36980	CERAD + Braak staging	No dementia	Not specified
84422	CERAD + Braak staging	Not specified	Not specified
48350	CERAD + NIA-Reagan + Braak staging	Cognitively and neurologically normal	DLB, PD, Binswanger's disease, MS, intracerebral hemorrhage

C)

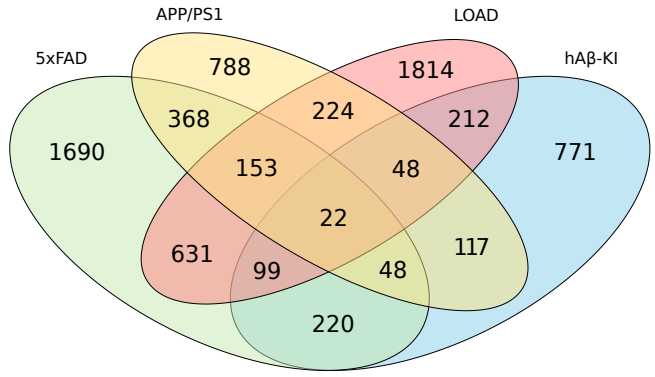
Demographics of human samples						
	Control	EOAD	Control	LOAD	Control	MS
Age (median)	46	61	83	84	Unknown	Unknown
Sample size	10	4	63	63	5	5
Sex (% of females)	50	75	38	44.4	100	100

DIFFERENTIALLY EXPRESSED GENES

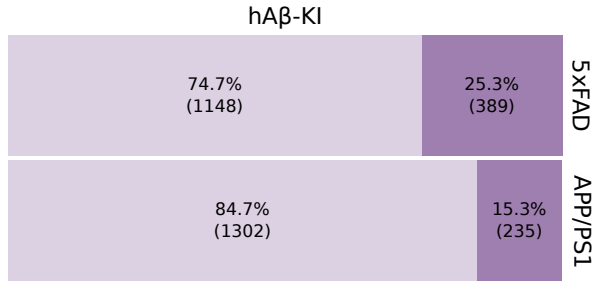
A)



B)

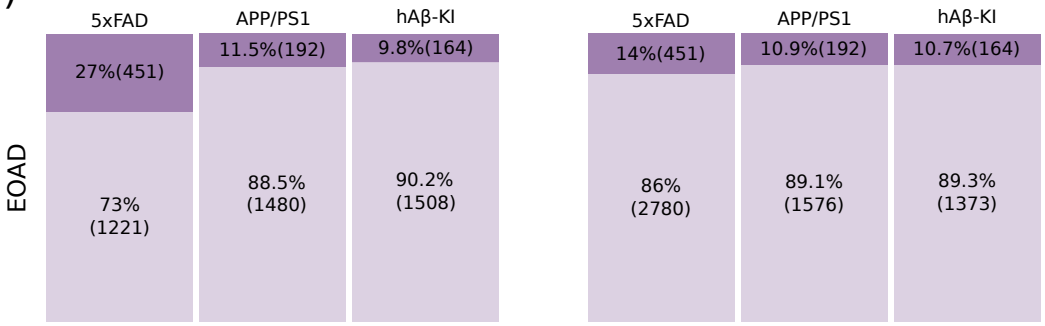


C)



X^2 = 47.07; p−value <0.001

D)



Disease−Model Overlap

X^2 = 221.86; p−value < 0.001

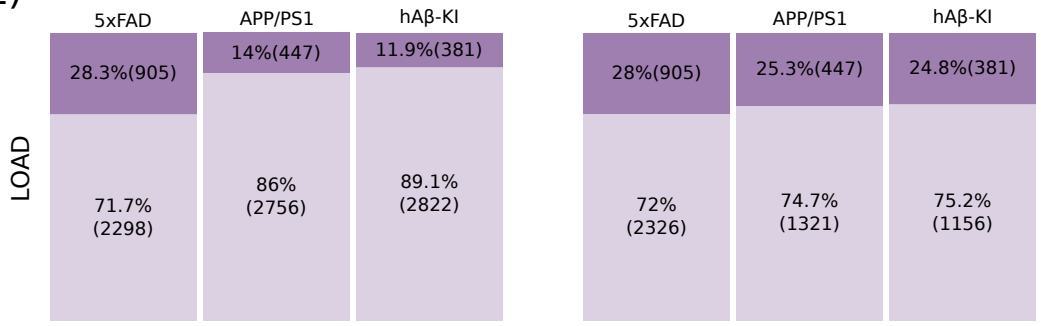
Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	<0.001	<0.001
5xFAD vs hAβ-KI	<0.001	<0.001
APP/PS1 vs hAβ-KI	0.1301	0.3902

Model−Disease Overlap

X^2 = 15.36; p−value < 0.001

Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.002	0.0061
5xFAD vs hAβ-KI	0.0018	0.0054
APP/PS1 vs hAβ-KI	0.9097	1

E)



Disease−Model Overlap

X^2 = 344.04; p−value < 0.001

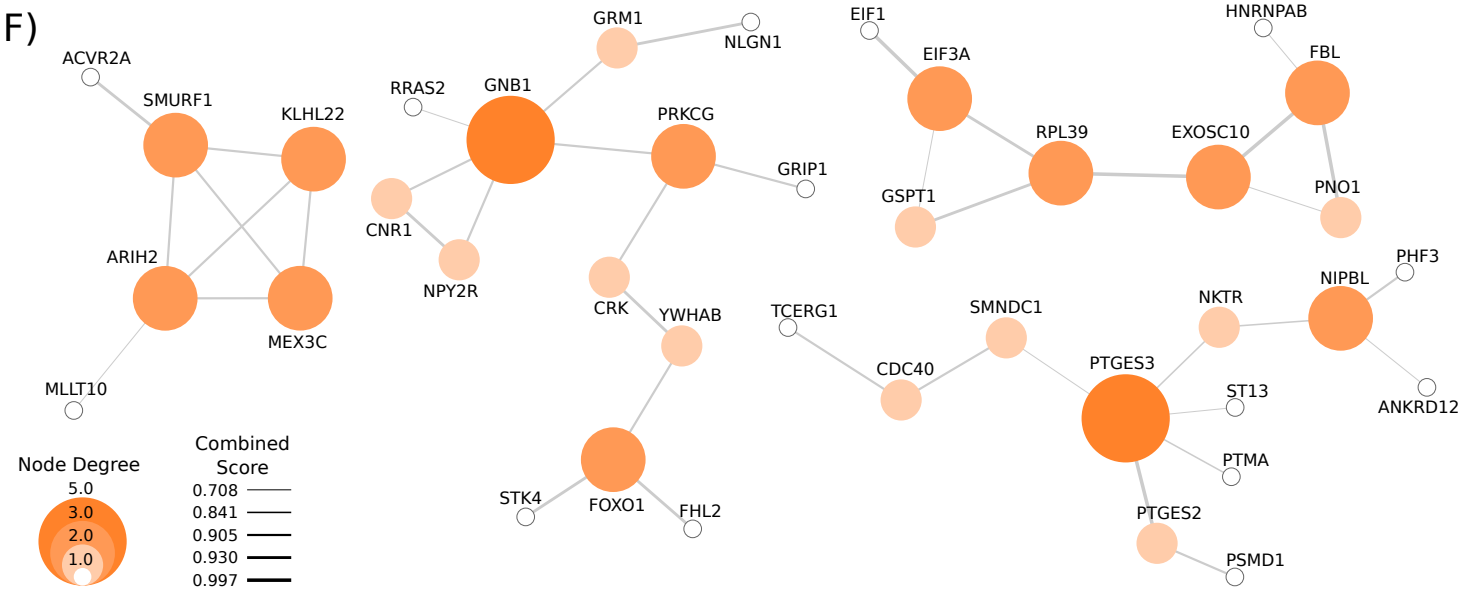
Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	<0.001	<0.001
5xFAD vs hAβ-KI	<0.001	<0.001
APP/PS1 vs hAβ-KI	0.0155	0.0465

Model−Disease Overlap

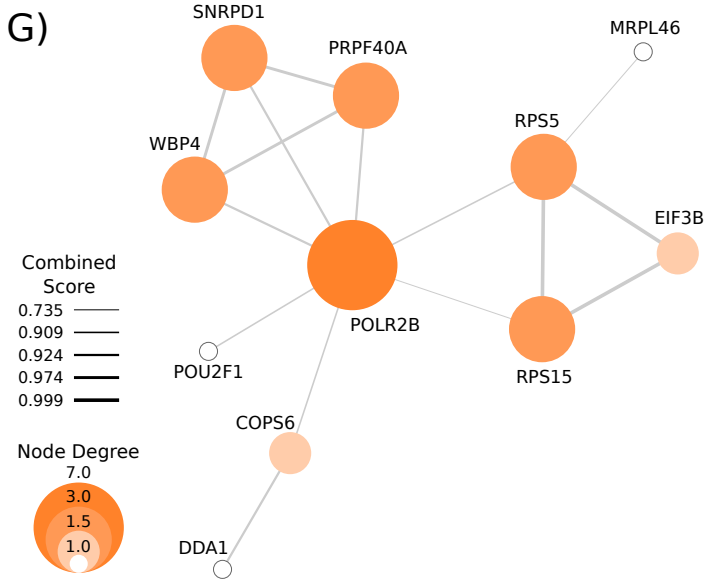
X^2 = 7.43; p−value = 0.0243

Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.0411	0.1234
5xFAD vs hAβ-KI	0.0210	0.0631
APP/PS1 vs hAβ-KI	0.7742	1

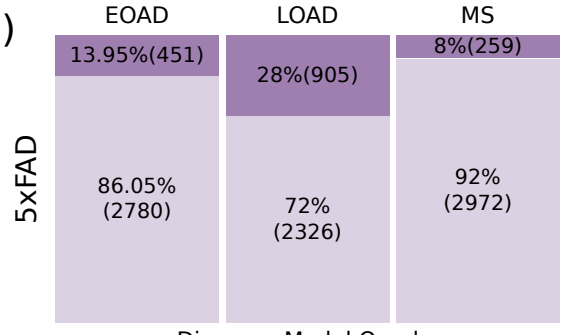
F)



G)



H)

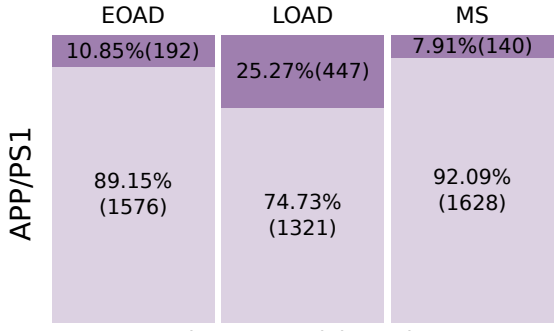


Disease−Model Overlap

X^2 = 490.56; p−value < 0.001

Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	<0.001	<0.001
LOAD vs MS	<0.001	<0.001

I)

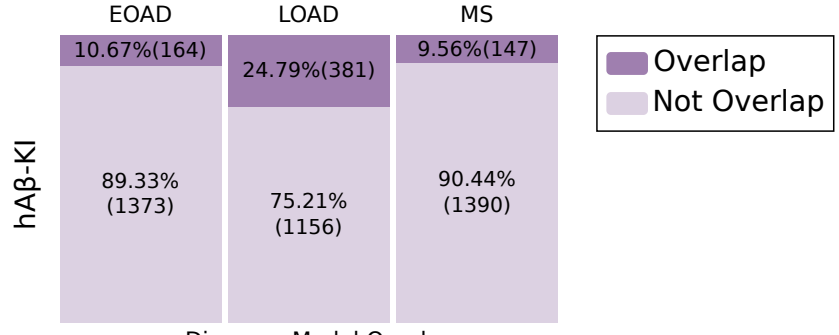


Disease−Model Overlap

X^2 = 243.7; p−value < 0.001

Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	0.0033	0.0098
LOAD vs MS	<0.001	<0.001

J)

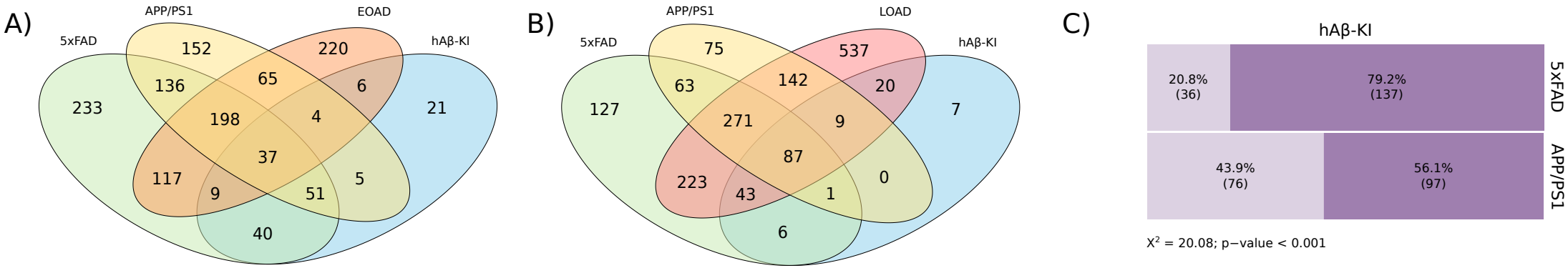


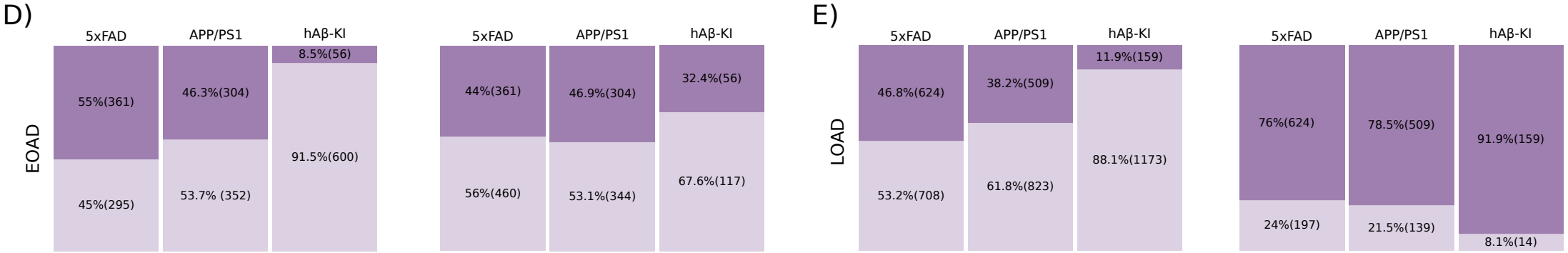
Disease−Model Overlap

X^2 = 173.65; p−value < 0.001

Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	0.3386	1
LOAD vs MS	<0.001	<0.001

GENE ONTOLOGY BIOLOGICAL PROCESS



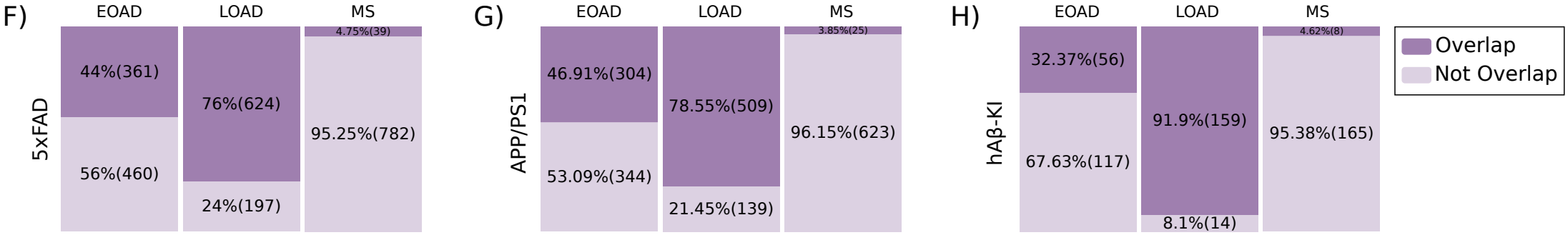


Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.002	0.006
5xFAD vs hAβ-KI	<0.001	<0.001
APP/PS1 vs hAβ-KI	<0.001	<0.001

Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.2836	0.8507
5xFAD vs hAβ-KI	0.0064	0.0193
APP/PS1 vs hAβ-KI	0.0008	0.0025

Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	<0.001	<0.001
5xFAD vs hAβ-KI	<0.001	<0.001
APP/PS1 vs hAβ-KI	<0.001	<0.001

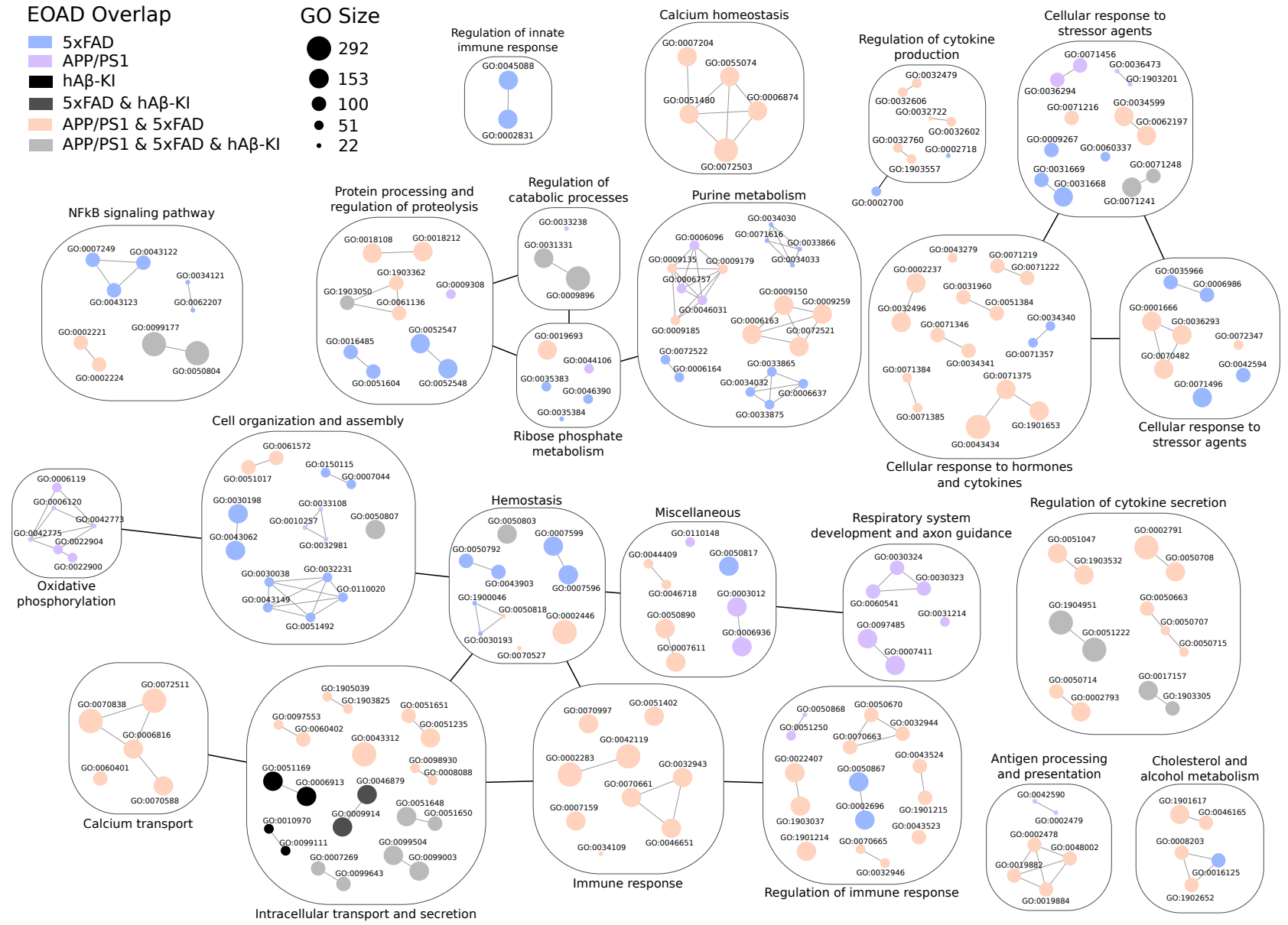
Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.2756	0.8267
5xFAD vs hAβ-KI	<0.001	<0.001
APP/PS1 vs hAβ-KI	0.0001	0.0003



Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	<0.001	<0.001
LOAD vs MS	<0.001	<0.001

Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	<0.001	<0.001
LOAD vs MS	<0.001	<0.001

Pairwise Comparison	raw.p	adj.p
EOAD vs LOAD	<0.001	<0.001
EOAD vs MS	<0.001	<0.001
LOAD vs MS	<0.001	<0.001


EOAD Overlap
5xFAD
APP/PS1
hAβ-KI
5xFAD & hAβ-KI
APP/PS1 & 5xFAD
APP/PS1 & 5xFAD & hAβ-KI
GO Size
292
153
100
51
22
Regulation of innate immune response
GO:0045088
GO:0002831
Calcium homeostasis
GO:0007204
GO:0055074
GO:0051480
GO:0006874
GO:0072503
Regulation of cytokine production
GO:0032479
GO:0032606
GO:0032722
GO:0032602
GO:0032760
GO:0002718
GO:1903557
GO:0002700
Cellular response to stressor agents
GO:0071456
GO:0036473
GO:1903201
GO:0036294
GO:0071216
GO:0034599
GO:0062197
GO:0009267
GO:0060337
GO:0031669
GO:0071248
GO:0031668
GO:0071241
NFkB signaling pathway
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GO:0043122
GO:0034121
GO:0043123
GO:0062207
GO:0002221
GO:0099177
GO:0050804
GO:0002224
Protein processing and regulation of proteolysis
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GO:0018212
GO:1903362
GO:1903050
GO:0009308
GO:0061136
GO:0016485
GO:0052547
GO:0051604
GO:0052548
Regulation of catabolic processes
GO:0033238
GO:0031331
GO:0009896
GO:0019693
GO:0044106
GO:0035383
GO:0046390
GO:0035384
Ribose phosphate metabolism
Purine metabolism
GO:0034030
GO:0006096
GO:0071616
GO:0033866
GO:0009135
GO:0009179
GO:0034033
GO:0006257
GO:0009150
GO:0009259
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GO:0009185
GO:0072521
GO:0072522
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GO:0071346
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GO:0034341
GO:0071384
GO:0071375
GO:0071385
GO:1901653
GO:0043434
Cellular response to hormones and cytokines
GO:0035966
GO:0006986
GO:0001666
GO:0036293
GO:0072347
GO:0070482
GO:0042594
GO:0071496
Cellular response to stressor agents
Cell organization and assembly
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GO:0150115
GO:0051017
GO:0007044
GO:0030198
GO:0033108
GO:0010257
GO:0050807
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GO:0030038
GO:0032231
GO:0110020
GO:0043149
GO:0051492
GO:0006119
GO:0006120
GO:0042773
GO:0042775
GO:0022904
GO:0022900
Oxidative phosphorylation
Hemostasis
GO:0050803
GO:0007599
GO:0050792
GO:0043903
GO:0007596
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GO:0030193
GO:0070527
Miscellaneous
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GO:0050817
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GO:0050890
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GO:0007611
Respiratory system development and axon guidance
GO:0030324
GO:0030323
GO:0060541
GO:0031214
GO:0097485
GO:0007411
Regulation of cytokine secretion
GO:0051047
GO:0002791
GO:1903532
GO:0050708
GO:1904951
GO:0050663
GO:0051222
GO:0050707
GO:0050715
GO:0050714
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GO:0070838
GO:0006816
GO:0060401
GO:0070588
Calcium transport
GO:1905039
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GO:0046879
GO:0051648
GO:0009914
GO:0051650
GO:0010970
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GO:0007269
GO:0099003
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Intracellular transport and secretion
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GO:0002283
GO:0032943
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GO:0046651
Immune response
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GO:0050670
GO:0051250
GO:0032944
GO:0070663
GO:0022407
GO:0043524
GO:0050867
GO:0002696
GO:1901215
GO:1903037
GO:0043523
GO:1901214
GO:0070665
GO:0032946
Regulation of immune response
Antigen processing and presentation
GO:0042590
GO:0002479
GO:0002478
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Cholesterol and alcohol metabolism
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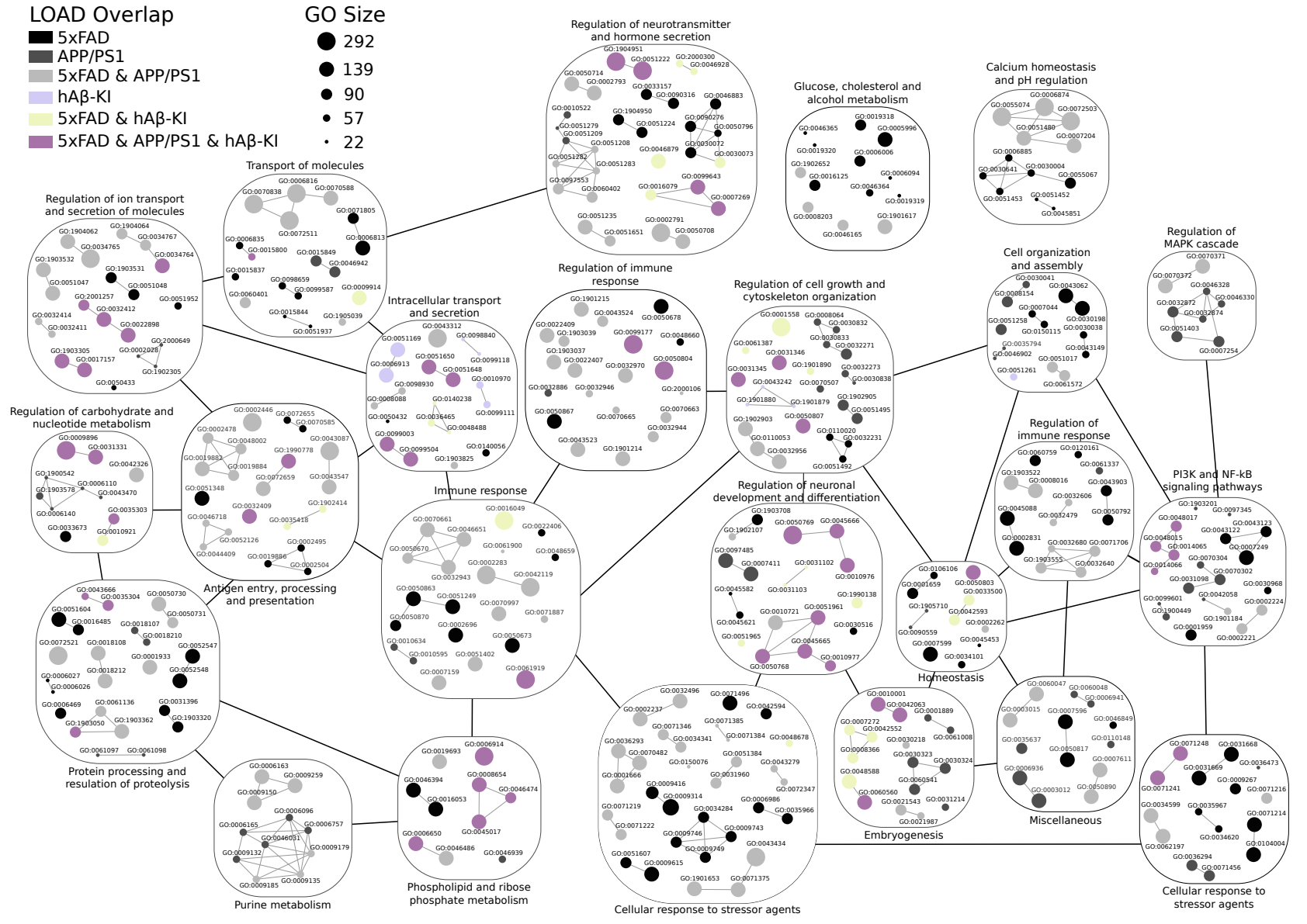
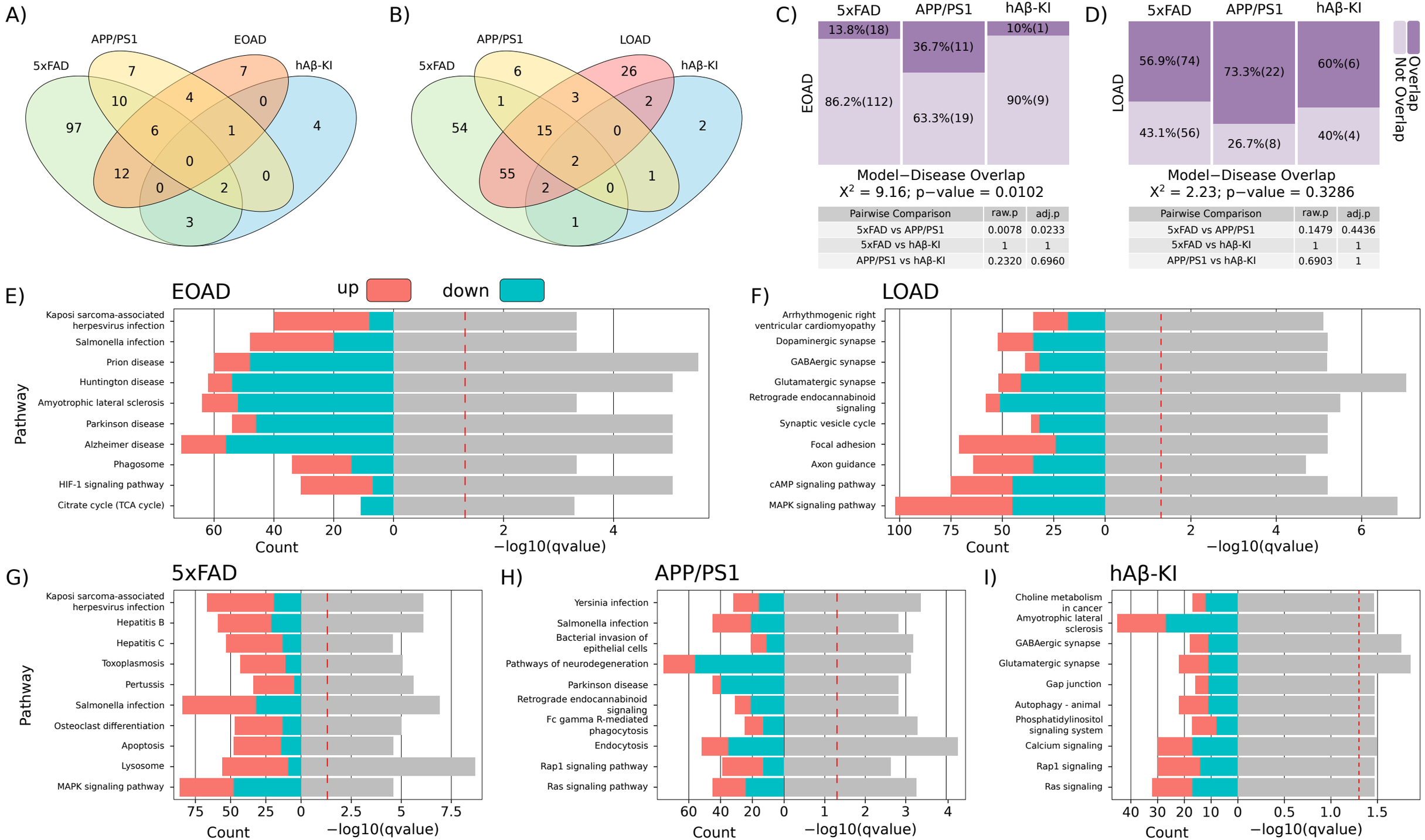
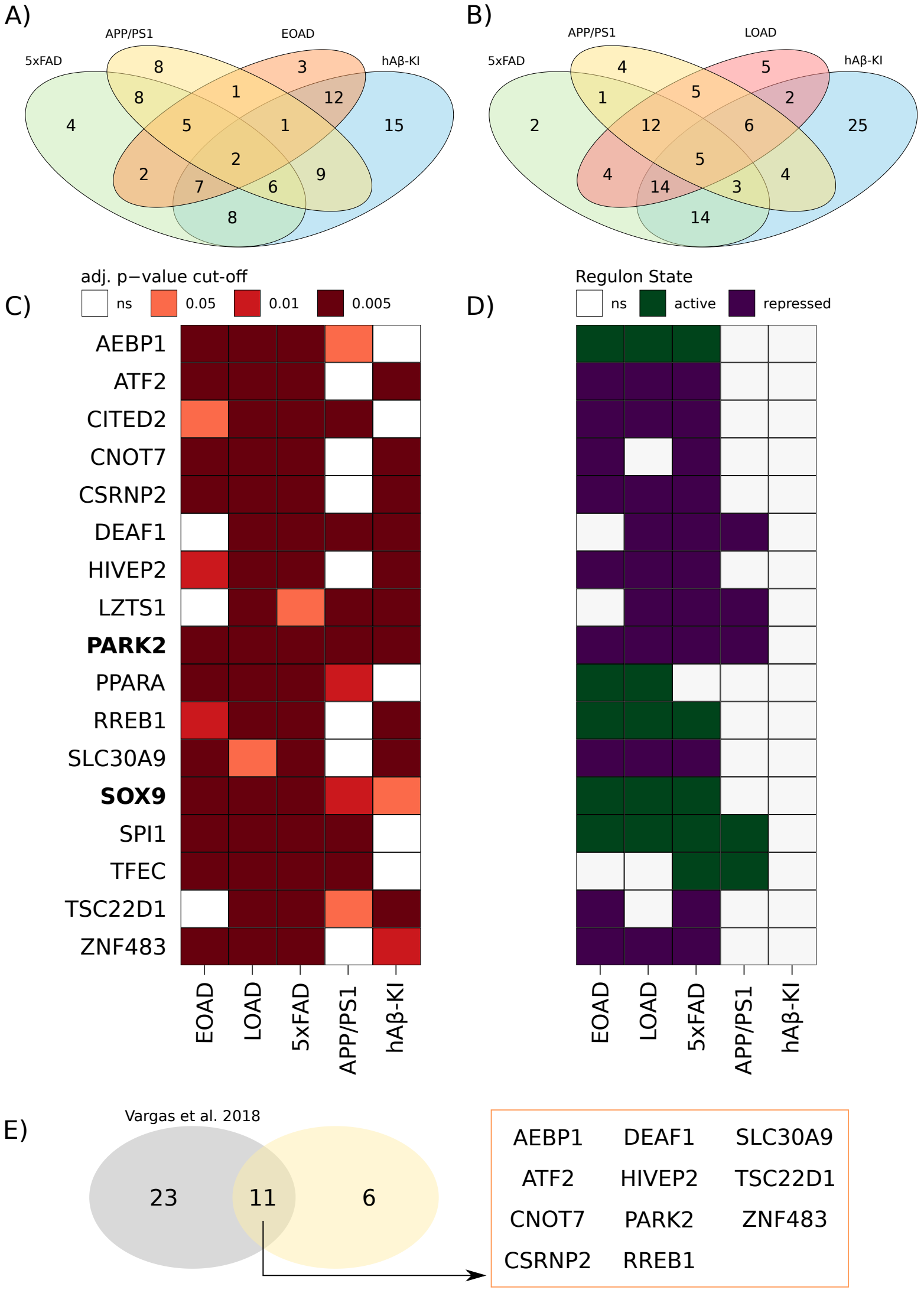

LOAD Overlap
5xFAD
APP/PS1
5xFAD & APP/PS1
hAβ-KI
5xFAD & hAβ-KI
5xFAD & APP/PS1 & hAβ-KI
GO Size
292
139
90
57
22
Regulation of neurotransmitter and hormone secretion
Glucose, cholesterol and alcohol metabolism
Calcium homeostasis and pH regulation
Transport of molecules
Regulation of ion transport and secretion of molecules
Regulation of immune response
Regulation of cell growth and cytoskeleton organization
Cell organization and assembly
Regulation of MAPK cascade
Intracellular transport and secretion
Regulation of carbohydrate and nucleotide metabolism
Regulation of immune response
PI3K and NF-kB signaling pathways
Immune response
Regulation of neuronal development and differentiation
Antigen entry, processing and presentation
Homeostasis
Protein processing and resulation of proteolysis
Embryogenesis
Miscellaneous
Purine metabolism
Phospholipid and ribose phosphate metabolism
Cellular response to stressor agents
Cellular response to stressor agents



Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.0078	0.0233
5xFAD vs hAβ-KI	1	1
APP/PS1 vs hAβ-KI	0.2320	0.6960

Pairwise Comparison	raw.p	adj.p
5xFAD vs APP/PS1	0.1479	0.4436
5xFAD vs hAβ-KI	1	1
APP/PS1 vs hAβ-KI	0.6903	1


A)
APP/PS1
EOAD
5xFAD
hAβ-KI
8
3
8
1
12
4
5
1
15
2
2
7
6
9
8
B)
APP/PS1
LOAD
5xFAD
hAβ-KI
4
5
1
5
2
2
12
6
25
5
4
14
3
4
14
C)
adj. p−value cut-off
ns
0.05
0.01
0.005
AEBP1
ATF2
CITED2
CNOT7
CSRNP2
DEAF1
HIVEP2
LZTS1
PARK2
PPARA
RREB1
SLC30A9
SOX9
SPI1
TFEC
TSC22D1
ZNF483
EOAD
LOAD
5xFAD
APP/PS1
hAβ-KI
D)
Regulon State
ns
active
repressed
EOAD
LOAD
5xFAD
APP/PS1
hAβ-KI
E)
Vargas et al. 2018
23
11
6
AEBP1
ATF2
CNOT7
CSRNP2
DEAF1
HIVEP2
PARK2
RREB1
SLC30A9
TSC22D1
ZNF483