Agenda:

Much appreciation for your patience if the timing on our agenda becomes slightly off.

9:00AM	Welcome **Rachel O'Neill, PhD, Director, Institute for Systems Genomics, UConn**
9:05	Welcome **Lon Cardon, MD, President and CEO of The Jackson Laboratory**
9:10	*The Ethics of Inclusion: Diversity in the Age of Precision Medicine* **Sandra Soo-Jin Lee, PhD** **Professor, Department of Medical Humanities and Ethics** **Chief of the Division of Ethics** **Columbia University**
10:10	Coffee Break Poster Sessions: Set 1 (Bhuiyan, Grady, Khouri-Farah, McDermott, Nargund, Santinello, Zhao)
10:55	Welcome Back
11:00	*Bayesian reconstruction and differential testing of intron excision sequences* **Derek Aguiar, PhD, Assistant Professor, Computer Science and Engineering, UConn**
11:20	*Independent horizontal microbial metallophore gene transfers with replacement in plants* **Bernard Goffinet, PhD, Professor, Ecology and Evolutionary Biology, UConn**
11:40	*Elucidating mitochondrial stress response using CRISPR-based functional genomics* **Xiaoyan Guo, PhD, Assistant Professor, Genetics and Genome Sciences, UConn Health**
12:00PM	*Regulation and function of circRNAs in the brain* **Pedro Miura, PhD, Associate Professor, Genetics and Genome Sciences, UConn Health**
12:20	Lunch Break Poster Sessions: Set 2 (Amjad, Coulter, Kumar, Lee, Novin, Pan, Tandale)
1:40	*Unravelling the endometriosis microenvironment using single cell discovery* **Elise Courtois, PhD, Associate Director, Single Cell Biology, The Jackson Laboratory**
2:00	*Exploring the ethical, legal, social, and policy Implications (ELSI) of voluntary workplace genomic testing (wGT)* **Alyx Vogle, MS, CGC, Clinical Research Project Manager, The Jackson Laboratory**
2:20	*High-throughput functional genomics uncovers cellular phenotypes in hematologic malignancies* **Eric Wang, PhD, Assistant Professor, The Jackson Laboratory**
2:40	Closing Remarks & Poster Awards Announcement **Brenton Graveley, PhD, Associate Director, Institute for System Genomics,UConn Health**

Abstracts:

The Ethics of Inclusion: Diversity in the Age of Precision Medicine

Sandra Soo-Jin Lee, Columbia University

Human genetics has a gap problem. Genetic samples drawn predominantly from individuals of European ancestry have prompted a call for greater diversity aimed at recruiting minoritized populations. Despite the ubiquity of these calls, the meaning of diversity - which dimensions matter, for what outcomes and why - remains strikingly imprecise. Drawing on a multi-sited study of diversity and the recruitment of minoritized groups into precision medicine research, Dr. Lee explores the ethical commitments to bridge the "diversity gap" and the meaning of inclusion for research practice.

Bayesian reconstruction and differential testing of Intron Excision sequences

Derek Aguiar, University of Connecticut, Storrs

Characterizing the differential excision of mRNA is critical for understanding the functional complexity of a cell or tissue, from normal developmental processes to disease pathogenesis. Most transcript reconstruction methods infer full-length transcripts from high-throughput sequencing data. However, this is a challenging task due to incomplete annotations and the differential expression of transcripts across cell-types, tissues, and experimental conditions. Several recent methods circumvent these difficulties by considering local splicing events, but these methods lose transcript-level splicing information and may conflate transcripts. We present a novel hierarchical Bayesian Admixture Model for Intron Excision (BAMIE) sequence reconstruction, which reconciles the transcript and local splicing perspectives. BAMIE achieves higher recall, F1 score, and accuracy for both reconstruction and differential splicing tasks when compared with four state-of-the-art transcript and local splicing methods. Time permitting, I will describe additional problems that our lab is working on and have relevance to the ISG.

Independent horizontal microbial metallophore gene transfers with replacement in plants

Bernard Goffinet, University of Connecticut, Storrs

The origin and subsequent diversification of land plants is marked by major innovations, including ecological interactions with microbes. Beyond their role as critical partners, fungi and bacteria also serve as sources of genetic tools. Analyses of the gene space of land plant model organisms suggest that such transfers are unique and ancient. Complementing available genomic resources with a hundred new genomes spanning the diversity of mosses, we demonstrate that a metallophore-synthesis gene was acquired independently from distinct microbial donors by at least five plant lineages, and that the first ortholog acquired by mosses was later replaced by another fungal copy, transferred to another major moss lineage. Such complex history of acquisition of a gene may reflect a more general pattern of highly dynamic gene exchange across the tree of life.

Elucidating mitochondrial stress response using CRISPR-based functional genomics

Xiaoyan Guo, University of Connecticut School of Medicine

Mitochondria play essential roles in almost all eukaryotic cells, and mitochondrial dysfunction is closely associated with many diseases, including age-related neurodegenerative disorders. One central question of my lab is to study how mammalian cells respond to mitochondrial dysfunction. Previously, leveraging CRISPR-based functional genomics, I identified a novel signaling pathway mediated by OMA1-DELE1-HRI to relay mitochondrial stress to the integrated stress response. Briefly, upon mitochondrial stress, a mitochondrial protease OMA1 is activated, which cleaves DELE1 and generates a DELE1 fragment releasing to the cytosol, where DELE1 interacts and promotes HRI's kinase activity. My lab will continue understanding how OMA1-DELE1-HRI is regulated and how this pathway contributes to mitochondrial dysfunction-associated diseases.

Regulation and function of circRNAs in the brain

Pedro Miura, University of Connecticut School of Medicine

Circular RNAs (CircRNAs) are products of alternative splicing that are largely non-coding in nature. These RNAs have high stability due to the lack of free 5' or 3' ends. CircRNAs are more abundant in brain tissue. This is attributed to 1- the high stability of circRNAs in post-mitotic cells, and 2- regulation by the neural-specific RNA Binding protein Nova2, which we found to enhance back-splicing in mouse cortical neurons (Knupp et al., 2021). Using RNA-Seq analysis, our lab has shown that circRNAs accumulate on a global scale with age in the mouse brain, *Drosophila* neurons, and in *C. elegans* (Gruner et al., 2016, Hall et al., 2017, Cortés-López et al., 2018). We are interested in determining the impact of this age accumulation on lifespan and the aging brain. In recent work in *C. elegans*, we have shown that genome editing of introns flanking a circularizing exon of the *crh-1* gene prevents circRNA expression while not affecting protein-coding *crh-1* mRNA levels. These animals lacking circ-*crh-1* exhibit an extended mean lifespan (Knupp et al., 2022). This suggests that circRNA accumulation might be contribute negatively to the aging process.

Unravelling the endometriosis microenvironment using single cell discovery

Elise Courtois, The Jackson Laboratory

Endometriosis is an inflammatory gynaecological condition that affects 10% of women of reproductive age, with symptoms including pelvic pain and infertility. It is characterized by the presence of endometrium-like tissue outside the uterine cavity (termed lesions), commonly found within the peritoneal cavity, as superficial peritoneal or ovarian lesions. Despite the first description of endometriosis occurring almost a century ago, the exact aetiology and molecular drivers of the disease remain largely unknown which limits early diagnosis and treatment. The lack of non-invasive non-surgical diagnostic tools impede its early detection, resulting in delays of up to 7 years from onset of symptoms to definitive diagnosis.

Advancements in single-cell RNA sequencing (scRNA-seq) and organoid culture systems enable interrogation of the dynamic interactions within the endometrial microenvironment and the cellular complexity and heterogeneity present in endometriosis. Using a combination of scRNA-seq, spatial phenotyping and human-derived organoid model approach, we aim to understand changes within the endometrium and in different types of endometriotic lesions. We uncovered distinct cellular changes in endometriosis endometrium as well as specific subsets of immunomodulatory and immunotolerant myeloid cell populations and vascular changes specific to endometriosis. Our data highlight an unreported endometriosis-specific perivascular population, the presence of tertiary lymphoid structures in some lesions and a progenitor-like epithelial cell population that may be crucial for a deeper understanding of this disease, as well as the niche where lesions establish, evolve and recur.

Exploring the ethical, legal, social, and policy Implications (ELSI) of voluntary workplace genomic testing (wGT)

Alyx Vogle, The Jackson Laboratory

In this study, we employ a mixed-methods approach implemented by an interdisciplinary, multi-site team with expertise in bioethics, law, genetic medicine and counseling, and health psychology to assess the ELSI of wGT. As health-related genomic testing expands, employers have begun offering wGT to employees through workplace wellness programs. wGT can reach populations beyond the traditional medical model of care, and proponents note its potential for improving employee health outcomes and lowering employer costs. Yet wGT also raises important ELSI issues (e.g., genetic discrimination, employees' genetic privacy). Our NHGRI-funded study aims to: (1) Describe employers' current practices and decision-making regarding wGT; (2) Assess how employees view and respond to wGT; and 3) Assess the ELSI of wGT from multiple stakeholder perspectives. We plan to yield data that will inform practice and policy in this emerging area of genetic testing.

High-throughput functional genomics uncovers cellular phenotypes in hematologic malignancies

Eric Wang, The Jackson Laboratory

A hallmark of hematological malignancies are alterations in gene regulatory pathways during normal hematopoiesis. Here, we have applied an integrated cell surface-based CRISPR platform to assess cellular phenotypes associated with various leukemia, including acute myeloid leukemia (AML) and B-cell acute lymphoblastic leukemia (B-ALL). Using this method, we identified the RNA-binding protein ZFP36L2 as a critical regulator of AML maintenance and differentiation. Mechanistically, ZFP36L2 interacts with the 3' untranslated region of key myeloid maturation genes, including the ZFP36 paralogs, to promote their mRNA degradation and suppress terminal myeloid cell differentiation. Moreover, we found the RNA-binding protein, NUDT21, limited expression of CD19 by regulating CD19 messenger RNA polyadenylation and stability. NUDT21 deletion in B-ALL cells increased the expression of CD19 and the sensitivity to CD19-specific CAR-T and blinatumomab.

Posters:

Characterizing the transcriptional landscape of Drosophila melanogaster centromeres

Presenter name: Asna Amjad, PhD Candidate
Affiliated Lab: Mellone Lab, Department of Molecular and Cell Biology, UConn
Abstract: Transposable elements and repetitive regions play a role in genome innovation and genome instability in a variety of species and have been proposed to promote transcription at centromeres. However, the role of transposable elements in promoting centromere transcription is not clear. In Drosophila melanogaster, centromeres consist of islands of complex DNA flanked by satellite repeats. The presence of a non-LTR retroelement Jockey-3 in all D. melanogaster centromeres suggests a conserved role of this element. Previously, low levels of Jockey-3 expression have been observed during embryogenesis by RT-qPCR. To better understand the repetitive elements transcription, we performed PRO-seq to capture nascent transcripts and total RNA-seq in D. melanogaster larval brains and embryos. Nascent transcripts analysis in embryos showed that the Jockey-3 element is active, but using traditional mapping methods to determine centromere derived transcripts has proven to be unfeasible. To overcome the challenges of short read length and repetitive regions of centromeres, we are using a k-mer analysis approach and several mapping methods to characterize the transcriptional landscape of D. melanogaster centromeres. PRO-seq data from embryos showed nascent transcription of Jockey-3 and other retroelements at the centromeres with very little transcription of satellite repeats. In contrast, more satellite repeats transcription is observed in total RNA samples. This suggests that satellite repeats are more stable than retroelements in the genome. We are also using a genome-independent approach to investigate satellite repeat transcripts from centromeric regions. These analyses will help to further explore the function of centromere transcription in D. melanogaster.

Identification of diabetes-associated variants modulating human islet proinflammatory cytokine responses

Presenter: Redwan M. Bhuiyan, Predoctoral Associate
Affiliated Lab: Stitzel Lab, JAX-GM and UConn Health

WHAMM deficiency causes proximal tubule dysfunction in mice and proteostasis defects in cells

Presenter name: Alyssa Coulter, PhD Candidate
Affiliated Lab: Campellone Lab, Department of Molecular and Cell Biology, UConn

Unique Centromeres & Telomeres in Phased Telomere-to-Telomere Assemblies of Male and Female Tammar Wallabies

Presenter name: Patrick G.S. Grady, PhD Candidate
Affiliated Lab: R. O'Neill Lab, Department of Molecular and Cell Biology, UConn
Abstract: Here we present two telomere-to-telomere assemblies and epigenetic profiles of the Tammar Wallaby (Macropus eugenii), the model organism for marsupials. An XX and XY individual were both assembled with Oxford Nanopore (ONT) PromethION long reads, ultra long reads, Hi-C, and short reads for polishing. Based on the principles of the CHM13-T2T consortium, these fully annotated genomes are extremely high quality and complete, with high Merqury QV scores of >40 (99.999% nucleotide accuracy) and gapless chromosomes. These complete chromosome sequences will greatly aid in the understanding of the organization and the evolution of the mammalian genome, given the great similarities and great differences between marsupials and placental mammals. The genomes are deeply annotated, including phased diploid versions, repeat annotations, and tissue-specific transcriptomics. Methylation data derived from ONT reads allow exploration of epigenetics of many previously unknown genome structures. We specifically explore the epigenetics of the centromere and the relationship between CENP-A deposition, sequence similarity, and methylation in this example. These new genome assemblies and accompanying data represents a significantly

improved resource for a deeply studied model organism that will continue to provide insights into mammalian evolution.

Exploring the World of RNA Epitranscriptomics using Mass Spectrometry

Presenter name: Jyotsna Kumar, PostDoctoral Fellow
Affiliated Lab: Fabris Lab, Department of Chemistry, UConn
Abstract: Epitranscriptomics: Gene regulation Chemical modifications on RNA have emerged as a new layer of posttranscriptional gene regulation. Recent progress in identification of these modifications in numerous pathological states including different tumors, viral infection and various cellular process has revealed their significance.
During and after transcription, natural RNAs are covalently modified by enzymes called “writers.” Combined with corresponding “erasers,” these enzymes afford the ability to turn on/off the biological functions of such RNAs. Other factors called “readers” can recognize the modifications to mediate downstream events associated with the parent RNAs. Together, these specific interactions provide the basis for RNA-mediated regulatory mechanisms operating in concert with established genetic and epigenetic regulation of gene expression.
What is the impact on human health? The functions of the most modifications out of >170 known RNA post-transcriptional modifications (rPTMs) are still largely obscure. However, the most extensively studied psuedouridine (Ψ), N6-methyladenosine (m6A), and 5-methylcytosine (m5C) have been implicated in cancer, developmental disorders, and infectious diseases. Our lab has developed methodology to uncover the entire complement of rPTMs expressed by a cell. rPTM profile can be profoundly affected by environmental and pathogenic stressors, thus suggesting that rPTM-based response mechanisms might be more widespread than originally thought. Our lab is engaged in the identification and elucidation of these types of mechanisms in the context of viral infections.

Purkinje Cell Heterogeneity Orchestrates Mammalian Cerebellar Development

Presenter name: Nagham Khouri-Farah, PhD Candidate
Affiliated Lab: Li and Cotney Labs, Department of Genetics and Genome Sciences, UConn Health
Abstract: In the mammalian cerebellum, Purkinje cells (PC) - cerebellum-specific neurons - display transient molecular heterogeneity during development. However, the underpinnings of PC heterogeneity remain poorly understood due to the lack of entry to assess individual PC subtypes by genetic perturbations. Through single-cell RNA sequencing, we identified molecular profiles of PC subtypes in the embryonic mouse cerebellum. Using CyCIF, a highly multiplexed immunofluorescence imaging method, and light-sheet microscopy (LSM), we assigned PC subtypes to their positions and resolved their three-dimensional distribution in the cerebellar cortex, respectively. In utero, different subtypes of PCs form distinct cell clusters coinciding with the anteroposterior and mediolateral patterning of the developing cerebellum. Remarkably, PC subtypes display distinctive combinatorial expression patterns of Foxp1, Foxp2, and Foxp4, which encode a subfamily of the forkhead box transcription factors. We provided evidence that knockouts of Foxp1 and Foxp2 disproportionally disrupt PC subgroups and their organization leading to defect in cerebellar morphogenesis and patterning. Interestingly, FOXP knockouts target cerebellar hemisphere expansion, which is an innovative feature to the mammalian cerebellum.

The Role of Lnc-RHL in Hepatic Cell Lineage Decisions and Proliferation in HepaRG Cells

Presenter name: SooWan Lee, PhD Candidate
Affiliated Lab: Rasmussen Lab, Department of Pharmaceutical Sciences, UConn
Abstract: Long noncoding RNAs (lncRNAs) are functional transcripts with lengths of over 200 nucleotides that do not encode proteins. LncRNAs have received wide attention as key regulators of stem cell proliferation and differentiation, but knowledge of the specific role of lncRNAs in hepatocyte differentiation is still limited. Recently, our group identified and characterized a novel lncRNA, lnc-RHL (regulator of hepatic lineage), which plays a critical role in the differentiation of bipotent hepatoblasts to hepatocytes and cholangiocytes (biliary

epithelial cells). Lnc-RHL maps to human chromosome 11q23.3 in an apolipoprotein (APO) gene cluster and consists of a 670-base pair polyadenylated lncRNA with two exons and an intron. We knocked down lnc-RHL expression with a doxycycline (Dox) inducible shRNA lentiviral vector in HepaRG cells to investigate the role of lnc-RHL. Interestingly, knockdown of lnc-RHL inhibited differentiation to hepatocytes but not to cholangiocytes in HepaRG cells. We observed a significant reduction in the number and size of hepatocyte colonies upon Dox induction. Moreover, knockdown of lnc-RHL downregulated the mRNA levels of hepatocyte markers such as HNF4 and albumin, and many APO genes, including APOA1, APOC3, and APOA5, but upregulated mRNA levels of the cholangiocyte marker, cytokeratin 7 (CK7). We also performed RNA-sequencing analysis and ingenuity pathway analysis (IPA) to further investigate functions of lnc-RHL and the top impacted canonical pathways due to loss of lncRHL during HepaRG cell differentiation. IPA results showed that several cell cycle- and proliferation-associated canonical pathways were significantly regulated. In addition, a key proliferation-associated transcription factor, FOXM1, was markedly upregulated after the loss of lnc-RHL. In summary, this study explores the role and expression of lnc-RHL in HepaRG cell differentiation and shows that it is required for the proper production of hepatocytes in these cells.

Consequences of Activating a Retroelement Enriched at Fruit Fly Centromeres

Presenter name: Tyler McDermott, PhD Candidate
Affiliated Lab: Mellone Lab, Department of Molecular and Cell Biology, UConn
Abstract: Retroelements are a class of selfish genetic elements capable of transposing in the genome via the reverse transcription and re-insertion of their RNA product. Despite the deleterious effects that their activity can have on genomic stability, retroelements have been proposed to play a role in establishing centromere identity. Retroelements have been found enriched at the neocentromere of human cell line 10q25, at every evolutionarily new centromere of the donkey, and at every centromere in Drosophila melanogaster, as well as in other species from across taxa. In Drosophila, every centromere contains at least one copy of the same retroelement, Jockey-3. This association begs questions as to whether this element may be functionally linked to CENP-A, the epigenetic mark for centromeric chromatin. Jockey-3 could preferentially insert within CENP-A chromatin, or provide a favorable sequence for biased deposition of CENP-A. To test these possibilities, we designed a transgenic fly line containing an engineered, full-length copy of Jockey-3 (eJockey-3) that can be activated in progeny by selective mating and whose transposition can be tracked in the genome. Upon activation of this transgenic copy of Jockey-3, lethality and infertility have been observed alongside copy number variation via digital droplet PCR and DNA damage at the centromere via immunofluorescence microscopy. Further experiments are underway to determine if this element is biasing centromeric chromatin.

Why does cancer metastasize in humans and not cows?

Presenter name: Ashkan Novin, PhD Candidate
Affiliated Lab: Kshitiz Lab, Biomedical Engineering, UConn Health

Determining cell type specific pathophysiological differences in type-2 diabetic pancreatic islet cells using single-cell transcriptomic data

Presenter: Siddhi Nargund, Bioinformatics Analyst
Affiliated Lab: Stitzel Lab, JAX-GM
Abstract: Pancreatic islet (dys)function is central to glucose homeostasis and type 2 diabetes pathophysiology. Islets consist of multiple cell types, including discrete endocrine cell types producing distinct hormones (alpha:glucagon, beta:insulin, gamma:pancreatic polypeptide, delta:somatostatin, epsilon:ghrelin) that together modulate glucose mobilization or disposal. Single cell transcriptome profiling studies have dissected human islet cellular heterogeneity to define the molecular repertoire of each islet cell (sub)population. However, precise understanding of cell type-specific differences in healthy vs. disease states is lacking, due in part to the limited number of individuals or cells per individual profiled for comparison. Here, we report a single cell transcriptome atlas of 245,878 islet cells obtained from 48 individuals (17 non-diabetic (ND), 14 pre-diabetic

(PD), and 17 type 2 diabetic (T2D)) matched for sex, age, and ancestry. We identify marker genes that are robustly expressed across disease states for each of the 14 cell types detected and observe a significant decrease in the number of beta cells sampled from T2D individuals. Comparison of aggregated beta cell scRNA-seq profiles revealed 511 differentially expressed genes in T2D vs. ND donors (FDR<5%), including monogenic diabetes and type 2 diabetes effector genes. We describe 8 putative beta cell subpopulations, including 'high functioning' and 'senescent' gene signature subpopulations that significantly increase and decrease in T2D donor islets, respectively. Together, this study provides new and robust, cell type-resolved insights on the cellular and molecular changes in healthy vs. diabetic human islets and represents a substantial resource to the islet biology and type 2 diabetes communities.

Genetics, Genealogy & George Floyd

Presenter name: Cindy Pan, M.P.H. Candidate
Affiliated Lab: UConn Department of Philosophy, UConn

Transcription of a centromeric retroelement in *Drosophila*

Presenter name: Bryce Santinello, PhD Candidate
Affiliated Lab: Mellone Lab, Department of Molecular and Cell Biology, UConn
Abstract: Although the importance of CENP-A in specifying the centromere locus is well known, the function of the underlying DNA remains unclear. We recently identified the organization and linear sequence composition of all endogenous Drosophila melanogaster centromeres. We discovered that the centromeres consist of islands of complex DNA enriched in retroelements flanked by arrays of simple satellites. While each centromere contains different assortments of DNA elements, all of which are also present elsewhere in the genome, all centromeres contain copies of the non-long terminal repeat (non-LTR) retroelement G2/Jockey-3. Jockey-3 is also the most highly enriched repeat in CENP-A chromatin immunoprecipitations. Centromeric DNA satellites are highly divergent even between closely related species, yet we found that Jockey-3 is conserved at the centromeres of three other Drosophila species, suggesting that this element may play a conserved role. Retroelements have been found at the centromeres across taxa, but the functional significance of this association is unclear. To determine if Jockey-3 copies are transcribed, we performed PRO-seq on embryos and found nascent transcripts emanating from centromeric and non-centromeric copies. To determine if these transcripts are associated with the centromere, we performed single-molecule RNA FISH combined with immunofluorescence and found that Jockey-3 transcripts localize to all of the centromeres of mitotic larval brain cells in both D. melanogaster and D. simulans. RNA FISH with probes specific to different Jockey-3 truncations and quantification of RNA FISH signal indicate that centromere transcripts localize in cis rather than in trans. A combination and lack of foci from both Jockey-3 probe sets suggest that both truncated and full-length copies are transcribed. While in metaphase, centromeric RNA FISH signal for G2/Jockey-3 is observed in all cells, only a subset of cells show this localization in interphase. Ongoing experiments are addressing when Jockey-3 localizes to the centromeres and whether there is a difference in Jockey-3 transcription in different cell types and tissue types of Drosophila. Furthermore, experiments using a short hairpin to knock-down G2/Jockey-3 will determine if G2/Jockey-3 transcripts are required for centromere function. Together, this work will shed light on the functional significance of retroelements at D. melanogaster centromeres.

Identifying the role of retroelement-rich islands in centromere function in Drosophila

Presenter name: Prachi Tandale, PhD Candidate
Affiliated Lab: Mellone Lab, Department of Molecular and Cell Biology, UConn

Administration of recombinant FOXN1 protein attenuates Alzheimer's pathology in mice

Presenter name: Jin Zhao, PostDoctoral Fellow
Affiliated Lab: Lai Lab, Department of Allied Health Sciences, UConn

Abstract: Alzheimer's disease (AD) is the most common cause of dementia in the older adult and characterized by progressive loss of memory and cognitive functions that are associated with amyloid-beta (Aβ) plaques. Immune cells play an important role in the clearance of Aβ deposits. T cells are the major component of the immune system. The thymus is the primary organ for T cell generation. T cell development in the thymus depends on thymic epithelial cells (TECs). However, TECs undergo both qualitative and quantitative loss over time. We have previously reported that a recombinant (r) protein containing FOXN1 and a protein transduction domain can increase the number of TECs and subsequently increases the number of T cells in mice. We show here that administration of rFOXN1 into AD mice improves cognitive performance and reduces Aβ plaque load in the brain. This is related to rejuvenating the aged thymic microenvironment, which results in enhanced T cell generation in the thymus, leading to increased number of T cells, especially IFNγ-producing T cells, in the spleen and the choroid plexus (CP), enhanced expression of immune cell trafficking molecules in the CP, and increased migration of monocyte-derived macrophages into the brain. Furthermore, the production of anti-Aβ antibodies in the serum and the macrophage phagocytosis of Aβ are enhanced in rFOXN1-treated AD mice. Our results suggest that rFOXN1 protein has the potential to provide a novel approach to treat AD patients.