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# Synthetic amyloid beta does not induce a robust transcriptional response in innate immune cell culture systems

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## Abstract

**Background:** Alzheimer's disease (AD) is a progressive neurodegenerative disease that impacts nearly 400 million people worldwide. The accumulation of amyloid beta (A $\beta$ ) in the brain has historically been associated with AD, and recent evidence suggests that neuroinflammation plays a central role in its origin and progression. These observations have given rise to the theory that A $\beta$  is the primary trigger of AD, and induces proinflammatory activation of immune brain cells (i.e., microglia), which culminates in neuronal damage and cognitive decline. To test this hypothesis, many in vitro systems have been established to study A $\beta$ -mediated activation of innate immune cells. Nevertheless, the transcriptional resemblance of these models to the microglia in the AD brain has never been comprehensively studied on a genome-wide scale.

**Methods:** We used bulk RNA-seq to assess the transcriptional differences between in vitro cell types used to model neuroinflammation in AD, including several established, primary and iPSC-derived immune cell lines (macrophages, microglia and astrocytes) and their similarities to primary cells in the AD brain. We then analyzed the transcriptional response of these innate immune cells to synthetic A $\beta$  or LPS and INF $\gamma$ .

**Results:** We found that human induced pluripotent stem cell (hiPSC)-derived microglia (IMGL) are the in vitro cell model that best resembles primary microglia. Surprisingly, synthetic A $\beta$  does not trigger a robust transcriptional response in any of the cellular models analyzed, despite testing a wide variety of A $\beta$  formulations, concentrations, and treatment conditions. Finally, we found that bacterial LPS and INF $\gamma$  activate microglia and induce transcriptional changes that resemble many, but not all, aspects of the transcriptomic profiles of disease associated microglia (DAM) present in the AD brain.

**Conclusions:** These results suggest that synthetic A $\beta$  treatment of innate immune cell cultures does not recapitulate transcriptional profiles observed in microglia from AD brains. In contrast, treating IMGL with LPS and INF $\gamma$  induces transcriptional changes similar to those observed in microglia detected in AD brains.

**Keywords:** Microglia, Alzheimer's disease, RNA-seq, Lipopolysaccharide

## Background

Alzheimer's disease (AD) affects about 5.8 million people in the USA and nearly 400 million people worldwide. The estimated cost for health care in the United States is 301 billion dollars and this number is expected to increase to 1.1 trillion dollars by 2050 when AD is projected to

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affect 13.8 million people [1]. To improve diagnosis and treatment options for AD it is imperative to have a better understanding of the molecular mechanisms underlying AD.

The accumulation of Amyloid beta ( $A\beta$ ) in the brain has been associated with AD, since the discovery of this pathology in 1906. This correlation has been supported by a number of findings, including the identification of genetic mutations causing misregulation of  $A\beta$  production and processing as the cause for familial AD [2] and the evidence that identifies  $A\beta$  deposition upstream of Tau tangle formation and neuronal death [3–8]. As a result,  $A\beta$  deposition has been proposed as the main cause for the development of AD, a theory known as the amyloid cascade hypothesis. Nevertheless, recent findings have raised questions about this hypothesis, including the failure of drug trials targeting  $A\beta$  accumulation and the observation of similar patterns of  $A\beta$  deposition in AD and healthy brains [9]. This evidence suggests that although closely associated,  $A\beta$  accumulation might not be the only causal factor for the development of AD and other elements might contribute to a more complex etiology.

Evidence pointing to chronic neuroinflammation as a crucial contributor to AD progression has accumulated over the last decade. Patients with AD have higher levels of neuroinflammation when analyzing inflammatory markers in postmortem AD brains or live neuroimaging [10–15]. Resident brain immune cells from patients with AD express more genes associated with immune pathways and phagocytosis than controls indicating a state of chronic activation [16]. Finally, recent genome wide association studies revealed enrichment of disease-associated variants at monocyte, macrophage, and microglia regulatory loci implicating innate immune cells as key mediators of AD risk [17–19]. This evidence has led to the hypothesis that in the Alzheimer's brain,  $A\beta$  triggers an exacerbated proinflammatory activation of brain innate immune cells, particularly microglia and to a lesser extent astrocytes, resulting in a chronic neuroinflammatory state that causes neuronal damage and cognitive decline.

Several *in vitro* models have been established to further investigate the role of immune cells in AD and the functional consequences of AD risk genes in these cells. Many of these models involve the exposure of mice or human innate immune cell types—either primary cells, established cell lines or human induced pluripotent stem cell (hiPSC)-derived microglia (IMGLs) to synthetic  $A\beta$ . It has been assumed that these *in vitro* scenarios would trigger an inflammatory response similar to the one observed in the human AD brain, but to the best of our knowledge comprehensive profiles of the transcriptional response to this stimulus *in vitro* have not been reported.

Here we assess the transcriptional differences between *in vitro* cell types broadly used to model neuroinflammation—including THP-1 macrophages, human peripheral blood mononuclear cell-derived macrophages (PBMC M $\phi$ ), U-87 Astrocytes, HMC3 microglia-like cells, and IMGLs—and study their transcriptional response to synthetic  $A\beta$ . We found that IMGLs are the *in vitro* cell model that best resembles primary microglia, but that synthetic  $A\beta$  does not trigger a robust transcriptional response in any of the cells analyzed. Finally, we found that treatment of IMGLs with bacterial LPS/INF $\gamma$  induces transcriptional changes that resemble many aspects of the transcriptomic profiles of disease associated microglia present in the AD brain, suggesting the potential suitability of this model to study transcriptional regulation in AD-related neuroinflammation.

## Methods

### Amyloid beta formation

Amyloid-beta ( $A\beta_{1-42}$ ) (AnaSpec; Fremont, CA) was prepared using two different methods as indicated in the figure legends (Methods A or B). When not explicitly indicated,  $A\beta$  generated following Method A was used. In the Method A  $A\beta$  was generated as previously described [20]. Amyloid-beta ( $A\beta_{1-42}$ ) peptide (AnaSpec; Fremont, CA) was dissolved in Hexafluoro-2-propanol (HFIP, Sigma) to 1 mM, incubated for 1 h, then left to evaporate in a vacuum concentrator overnight allowing monomerization. To form oligomerized  $A\beta$  (o $A\beta$ ), the dried film was reconstituted in DMSO (Sigma) to a concentration of 5 mM, and then diluted to 15  $\mu$ M in phosphate buffered saline (PBS) (pH 7.4) and stored at room temperature for 30 min. To form fibrillarized  $A\beta$  (f $A\beta$ ), the dried film was reconstituted in a 40 mM Tris-HCl (pH 8.0) and 250 mM NaCl solution, incubated at room temperature with agitation for 6 days, then centrifuged for 10 min at 15000 g. The resulting pellet was resuspended in PBS to a concentration of 15  $\mu$ M. o $A\beta$  and f $A\beta$  formation were confirmed by western blot as it is described below.  $A\beta$  was thoroughly mixed and diluted in media to the desired concentration prior to cell exposure. f $A\beta$  contained some oligomers as indicated by western blot analysis.

In method B,  $A\beta$  was reconstituted as previously described in Abud, 2017 [21]. Briefly,  $A\beta$  was dissolved in 75  $\mu$ l of  $NH_4OH$  (0.1%) then filled to 1 ml with sterile PBS to achieve a 1 mg/ml concentration.  $A\beta$  was then diluted to 100  $\mu$ g/ml with sterile water, vortexed, and incubated at 37 °C for 30 min to form oligomerized  $A\beta$  (o $A\beta$ ), and 7 days or 5 days to form fibrillarized  $A\beta$  (f $A\beta$ ).  $A\beta$  was thoroughly mixed and diluted in cell culture media to the desired concentration prior to cell exposure. f $A\beta$  contained some oligomers as indicated by western blot analysis (Fig. 2A).

### Characterization of A $\beta$ using western blot

The size of the resulting A $\beta$  preparation after performing each protocol was characterized using SDS-PAGE and western blotting. 120 ng of proteins were separated by electrophoresis in a 12% gel and transferred to a nitrocellulose blotting membrane. After blocking, the membranes were incubated overnight at 4 °C with anti-A $\beta$  primary antibody (6E10 Biolegend, SIG-39320, 1:1000) following incubation with goat anti-mouse secondary antibody (Thermo Scientific, 1:1000) during 1 h. The membranes were subsequently washed and placed in the Radiance Plus Chemiluminescent Substrate (Azure Biosystems, Dublin, CA, USA) and visualized using the Azure c600 gel imaging system (Azure Biosystems).

### Immortalized human cell lines

The immortalized human monocyte cell line THP-1 (ATCC, #:ATCC<sup>®</sup> TIB-202TM, obtained from the Tissue culture facility at UNC Chapel Hill), was cultured in RPMI 1640 with L-Glutamine-Bicarbonate (Sigma-Aldrich # R8758) containing 10% FBS (Thermo Fisher Scientific, Gibco<sup>™</sup> # 10500064), and 1% penicillin/streptomycin (Sigma-Aldrich #P4333) in an atmosphere of 5% CO<sub>2</sub> at 37 °C. To differentiate THP-1 monocytes into macrophages, cells were plated at a density of  $1.0 \times 10^6$  cells/well in 6-well plates and incubated with 25  $\mu$ M PMA (12-O-tetradecanoylphorbol) for 24 h. Macrophages were then allowed to rest for 72 h before A $\beta$  or LPS/INF $\gamma$  treatment as described below. The HMC3 immortalized microglia cell line (obtained from ATCC # CRL-3304) and the astrocyte-like U-87 cell line (ATCC # 30-2003 obtained from the Tissue culture facility at UNC-Chapel Hill) were both cultured in EMEM (ATCC # 30-2003) containing 10% FBS and 1% penicillin/streptomycin in an atmosphere of 5% CO<sub>2</sub> at 37 °C. Cells were treated with 1  $\mu$ M A $\beta$  or 10 ng/ml LPS/20 ng/ml INF $\gamma$  (Sigma-Aldrich # L2630 /Peprotech # 300-02) during 24 h. Treatments were carried on in serum-free media with 1% penicillin/streptomycin (100 U/ml penicillin/100  $\mu$ g/ml streptomycin) unless otherwise stated.

### Generation of peripheral blood mononuclear cell-derived macrophages

Human peripheral blood mononuclear cells (PBMCs) were isolated from deidentified healthy donor blood obtained from the New York Blood Center (Long Island City, NY). Research was approved by the center prior to acquisition. Approximately  $10^8$  PBMCs were isolated using Ficoll–Paque density gradient centrifugation (MilliPore Sigma # GE17-5442-02). Cells were cultured in DMEM 10% FBS and seeded in 6-well ultra-low adhesion plastic culture dishes. Monocytes were allowed to

adhere for a period of 5–7 days in vitro. Media was then exchanged to DMEM containing 15 ng/ml recombinant human granulocyte–macrophage colony stimulating factor (rhGM-CSF; R&D Systems # 215-GM-010/CF). PBMC M $\phi$  were allowed to differentiate for an additional 5 days and were returned to DMEM 10% FBS until stimulation.

### Generation of iPSCs-derived microglia

iPSCs-derived microglia cells were generated as previously described [22]. Briefly, iPSCs (Wicell UCSD021i-3-9) were differentiated into Hematopoietic progenitor cells (HPCs) using the STEMdiff<sup>™</sup> Hematopoietic Kit (Stem Cell Technologies # 05310) according to manufacturer instructions. iPSCs cells were grown in mTeSR1 (STEMCELL technologies Catalog # 85850) and passaged with 0.5 mM EDTA in PBS (–Ca<sup>+2</sup>/Mg<sup>+2</sup>). On day – 1, cells were plated into mTeSR1 medium with 0.5  $\mu$ M Thiazovivin (STEMCELL technologies # 72252) onto hESC-matrigel coated (Corning # 354277) 6-well plates at different densities. On day 0, plates with a density of 10 to 20 aggregates per cm<sup>2</sup> with a size ranging from 0.1 to 0.2  $\mu$ m were selected. A small aggregate density and size was found to be critical at this stage. mTeSR1 medium was replaced with medium A (2 ml per well of a 6-well plate). On day 2, 50% of the medium A (1 ml/well) was removed and replaced with 1 ml of fresh medium A per well. On day 3, all medium was carefully removed, and 2 ml/well medium B was added. On days 5, 7 and 9, 1 ml of medium was removed carefully not to disturb the cells, and 1 ml of fresh medium B was added. On day 12, non-adherent cells, considered hematopoietic progenitor cells (HPCs) were collected carefully to not disturb adherent cells and centrifuged  $300 \times G$  5 min. The differentiation of HPCs into IMGLs was performed using the STEMdiff<sup>™</sup> Microglia Differentiation Kit and the STEMdiff<sup>™</sup> Microglia Maturation Kit (STEMCELL technologies # 100-0019 and #100-0020) according to manufacturer instructions. Briefly, HPCs were plated at a density of  $1-2 \times 10^5$  cells per well in hESC-matrigel coated 6-well plates using 2 ml of Microglia differentiation media. On day 0 until day 24, 1 ml of fresh media was added to each well, except for day 12, in which all the media except for 1 ml was collected and centrifuged  $300 G \times 5$  min, cells were resuspended in 1 ml of fresh media and returned to the well. On day 24 cells were collected, centrifuged  $300 G \times 5$  min and counted. 1 ml of conditioned media per well was left and fresh Microglia Maturation Media was added to achieve  $1 \times 10^6$  cells in 2 ml. Cells were plated (2 ml/well) in fresh hESC-matrigel coated 6-well plates. 1 ml of fresh media was added every other day until cells were used on days 28–30. On day 28–30 cells were semi-adherent, all media except for 1 ml

was collected, centrifuged 300 G × 5 min and cells were resuspended in 1 ml/well of Microglia Maturation media with or without 2 × concentration of A $\beta$  or LPS/IFN $\gamma$  and returned to the well. Cells were collected for RNA extraction after 24 h treatment, a time point commonly used in previous literature [23–25].

#### Cell labeling and A $\beta$ treatment

For live-cell imaging, 50,000 iPSC-microglia or THP-1 macrophages were cultured in a vitronectin-coated eight-chambered cover glass dish (Cellvis, C8-1.5H-N). THP-1 macrophages were labeled with 2  $\mu$ M CellTracker Green CMFDA dye (Invitrogen, C2925) in HBSS containing Ca<sup>++</sup> and Mg<sup>++</sup> (Gibco, 14025-092) for 30 min at 37 °C. Medium was replaced with cell medium containing 2  $\mu$ g/ml  $\beta$ -Amyloid<sub>1-42</sub>-HiLyte Fluor 555 and cells were imaged 1 h following treatment.

#### Live-cell imaging

Confocal images were acquired on an inverted Zeiss 800 laser scanning confocal microscope equipped with 405, 488, 561, and 647 nm diode lasers, gallium arsenide phosphide (GaAsP) detectors, and a transmitted light photomultiplier tube. Images were acquired using a 63X/1.4 NA objective lens at 37 °C and 5% CO<sub>2</sub> (Carl Zeiss, Oberkochen, Germany).

#### RNA-seq

Immediately after cells were harvested, RNA was extracted using RNeasy Mini Kit (QIAGEN) following manufacturer's guidelines. RNA integrity number (RIN) was measured for all samples using Agilent tapestation 4150 system. All sequencing libraries analyzed were generated from RNA samples measuring a RIN score  $\geq$  8.5. RNA-seq libraries were generated using the KAPA RNA HyperPrep with RiboErase kit using 500 ng of isolated mRNA as input and following manufacturer's instructions. Libraries were quantified and normalized using DNA tapestation and sequenced as paired-end 75-base-pair reads in the Illumina Nextseq 500 platform. All RNA-seq experiments were performed in biological duplicate.

#### RNA-seq quantification

RNA-seq libraries were sequenced to an average depth of approximately 55–70 million reads per sample. Low-quality reads and adapters were trimmed using Trim Galore! (v. 0.4.3), and trimmed reads were then mapped to the hg19 transcriptome (GENCODE, release 19) and quantified using Salmon's mapping-based mode (v. 0.8.2) [26, 27]. Both programs were run with default settings, using paired-end inputs. Gene-level quantifications

were summarized from each sample using the R package tximport (v. 1.2.0) [28].

#### Principal component analysis and variance partitioning

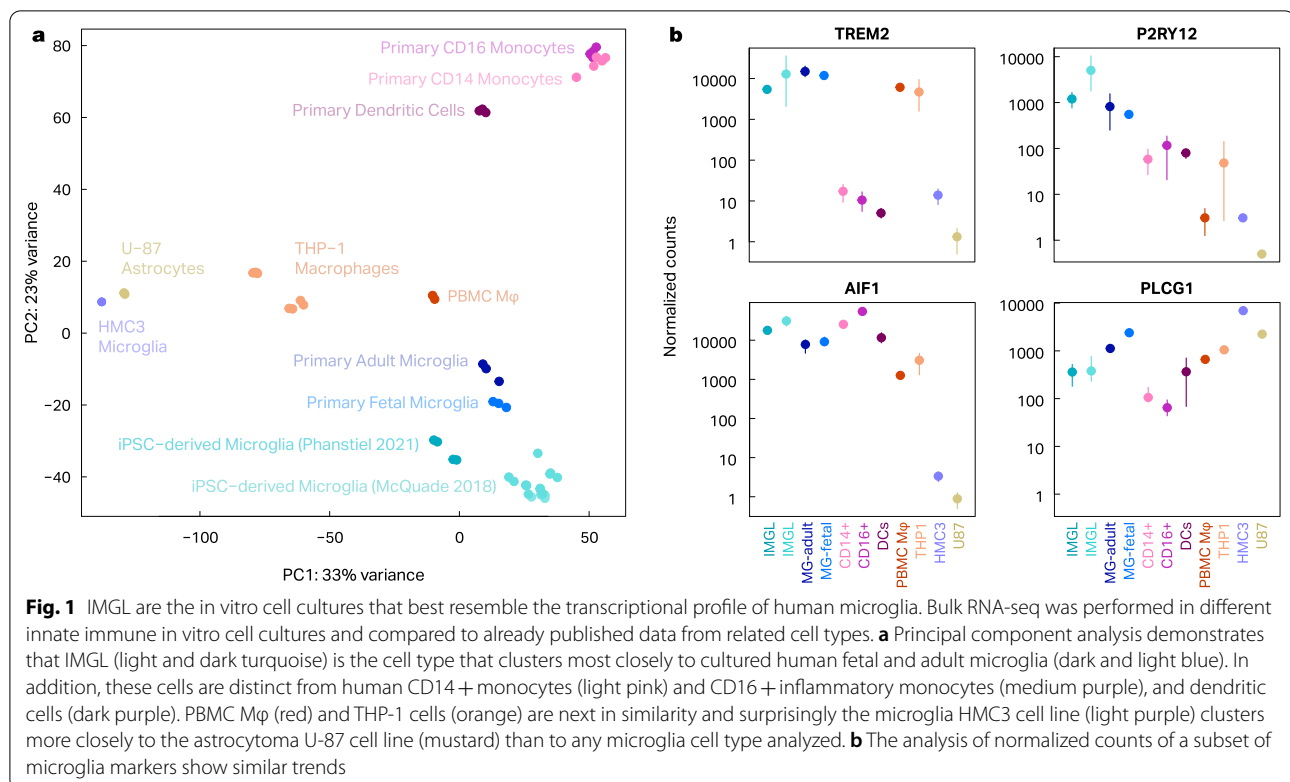
The untreated controls from each experiment were analyzed separately for the purpose of principal component analysis (PCA), shown in Fig. 1, and characterizing drivers of variance. The read counts summarized by tximport were variance-stabilizing transformed (VST) in R using DESeq2 (v. 1.22.2) [29]. Genes were subset for only those with 100 or more transcripts per kilobase million (TPM) in at least one sample (n = 5,192). The VST counts for these genes were used to calculate variance for PC1 and PC2. Variance was characterized using the R package variancePartition (v. 1.22.0) using the same filtered VST counts, and the following formula:  $\sim$  (1|Tech\_Rep) + (1|Bio\_Rep) + (1|Cell\_Type) + (1|Source) [30]. The fitExtractVarPartModel function then fits the VST counts to a linear mixed model and determines the fraction of variance attributable to each covariate in the design formula, with any unexplained variance attributed to residuals. The mean percentage of variation across all genes was reported.

#### Differential RNA-seq analysis

Differential analysis was conducted in R with DESeq2 (v. 1.22.2), using a design adjusting for replicate variability when calculating differences between treatment groups ( $\sim$  replicate + condition). Each experiment was analyzed separately to identify the number of differential genes caused by each treatment relative to untreated controls. Differential genes were defined as genes with an FDR-adjusted *p* value below 0.01 (Wald test, log<sub>2</sub> fold-change threshold of 1) and an absolute fold change greater than 2 when comparing treated samples to their respective controls. Fold changes were shrunken using the "apeglm" method within DESeq2 (v. 1.14.0) [31].

#### Quantitative PCR

THP-1 macrophages were treated with LPS/IFN $\gamma$  during 24 h and RNA was extracted as described above (see RNAseq section). 1  $\mu$ g of RNA from each sample was reverse transcribed into DNA using the ImProm-II reverse transcription system from Promega (A3800). Quantitative PCR (qPCR) was performed in technical duplicate using the Taqman Gene Expression Assays for ACTB (Hs99999903), FOS (Hs04194186), IL1B (Hs01555410), and IL6 (Hs00174131) in the Life Tech QuantStudio 6 Real-Time PCR System. The threshold cycle (Ct) of each gene was normalized to the untreated samples (delta Ct), and reverse log<sub>2</sub>-transformed (2<sup>delta Ct</sup>). The fold-change of each target gene (FOS, IL1B, IL6) was calculated by dividing these normalized values from that of the housekeeping



control (ACTB). These fold-changes were calculated for the average Ct of each gene and sample, as well as for each technical replicate individually, and standard error of the mean (SEM) was used to assess variability in the average fold-change.

#### GO and KEGG enrichment analysis

The “findMotifs.pl” tool in the HOMER software suite (v. 4.10.4) was used on genes that were upregulated in IMGLs upon LPS/INF $\gamma$ -treatment to identify significantly enriched GO terms ( $p < 0.05$ ) and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways ( $p < 0.05$ ).

#### DAM enrichment analysis

IMGL RNASeq data was subset for genes present in DAM gene signatures (DESeq2; > 100 counts per million). Fisher’s exact test was used to determine the enrichment of DAM genes, using all genes expressed in IMGLs as background (DESeq2; > 10 counts per million). Wilcoxon rank sum test was used to determine whether each group was significantly different from zero.

## Results

### Transcriptional comparison of cultured cell types to study AD

To determine which of the currently available and most widely used in vitro immune models best resembles the

transcriptional signature of human microglia, we performed RNA-seq in PBMC Mφ, THP-1 macrophages, HMC3 microglia, U-87 astrocytoma cells, and IMGLs. We used principal component analysis (PCA) to compare the transcriptomic profiles of these cells with already published data from primary human adult and fetal microglia as well as with other related immune cell types [22]. Our results showed that IMGLs cluster closely to both human adult and fetal microglia and further away from other primary immune cells such as monocytes and dendritic cells (Fig. 1A). PBMC Mφ and THP-1 macrophages are the next closest in proximity, while the HMC3 microglia established cell line has the least similarities to primary microglia and, surprisingly, clusters almost perfectly with the U-87 astrocytoma cell line. We used variance partitioning to determine how much of this clustering could be driven by the fact that the data from these cell types was generated by two separate labs [30]. We calculated the percentage of variance explained by cell type, lab of origin, biological replicate, and technical replicate and found that on average 71% of variance was due to differences in cell type, while only 12% was attributable to lab of origin. Investigation of microglia marker genes in each of these cell types further confirmed the similarity between IMGLs and primary microglia (Fig. 1B). IMGLs showed high expression of TREM2, P2RY12, AIF1 (IBA1) and PLCG1, similar to primary adult and fetal microglia.

THP-1 macrophages exhibited the next most similar pattern of expression at most but not all genes, while the HMC3 microglia established cell line exhibited low expression for the majority of these microglia marker genes. These results suggest that, among the cells investigated, IMGLs best resemble primary human microglia at the transcriptomic level and are the most suitable in vitro model available to study transcriptional regulation in the context of neuroinflammation.

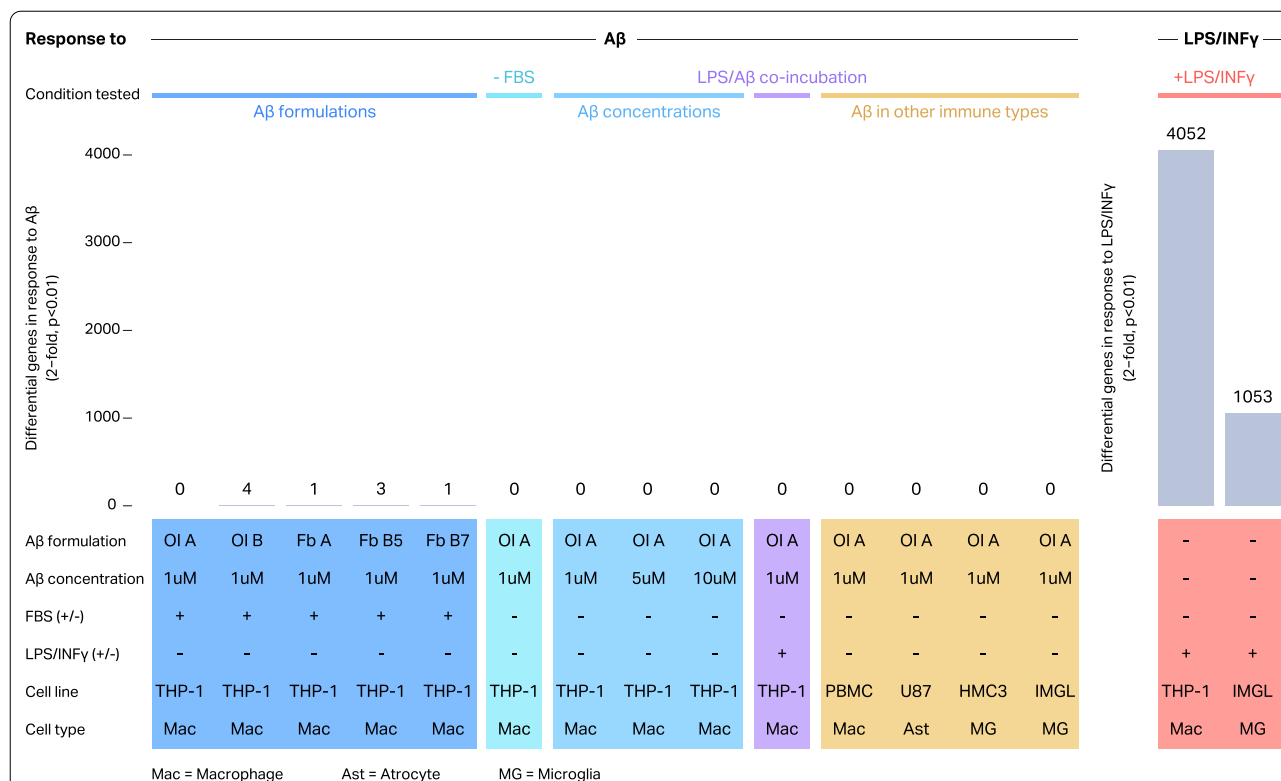
**Synthetic Aβ does not induce a robust transcriptional response**

We next sought to characterize the global transcriptional response of IMGLs to synthetic Aβ to analyze if this in vitro model can be used to study transcriptional regulation in activated microglia. We treated IMGLs with 1 μM oligomeric Aβ for 24 h and performed stranded, paired-end, total RNA-seq. Surprisingly, we observed no significant changes in gene expression in response to Aβ treatment (DESeq2, *p* < 0.01, absolute fold change > 2) (Fig. 2). To explore the degree to which treatment

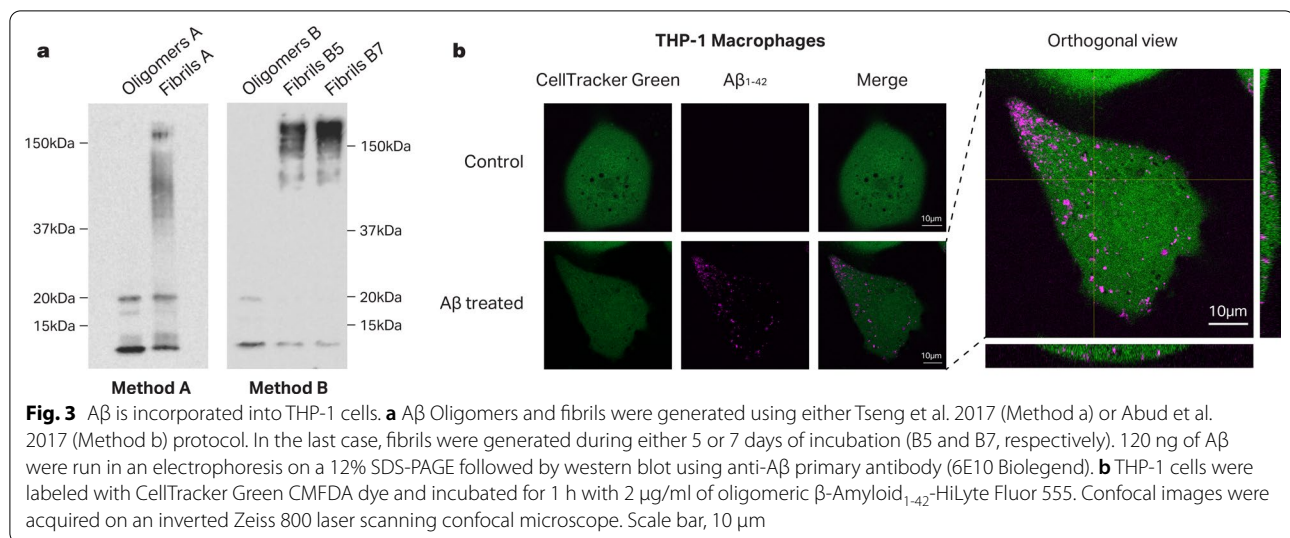
conditions affect these results, we turned to THP-1 cells which are a more tractable system and express many microglia marker genes, such as TREM2, a cell surface receptor known to bind Aβ [32].

We prepared synthetic Aβ in the form of oligomers or fibrils following two different previously published protocols: protocol A described by Tseng et al. [20] or protocol B described by Abud et al. [21]. Successful generation of oligomers and fibrils was confirmed by western blot (Fig. 3A). We also confirmed that both THP-1 cells and IMGLs are capable of phagocytosing synthetic Aβ after 1 h of incubation (Fig. 3B, Additional file 1: Fig. S1).

Next, we proceeded to treat THP-1 cells with Aβ under various conditions and performed RNA-seq to analyze the global transcriptional response. As observed previously in IMGLs, when THP-1 macrophages were treated with the different Aβ formulations (Fig. 2, dark blue), no robust transcriptional response was observed in any condition. From this point forward, we utilized the oligomeric form of Aβ, since it has been previously described as the more “active” form of Aβ [33–35]. We



**Fig. 2** Transcriptional response of immune in vitro cell cultures exposed to different proinflammatory stimuli. Bulk RNA-seq was performed in different innate immune in vitro cell cultures exposed to proinflammatory stimuli during 24 h. THP-1 cells were treated with different Aβ formulations including Aβ oligomers (OI) and Aβ fibrils (Fb) obtained using different protocols (dark blue); Aβ in the absence of FBS (turquoise), with different Aβ concentrations (light blue), or co-incubating with LPS/ INFγ and Aβ together (purple). In addition, other immune cell types were treated with Aβ (orange). Finally, THP-1 cells and IMGLs were treated with 10 ng/ml LPS + 20 ng/ml INFγ (red). Differential genes in response to Aβ or LPS/ INFγ were determined by comparing to condition-matched controls using DESeq2, *p* < 0.01, absolute fold change > 2

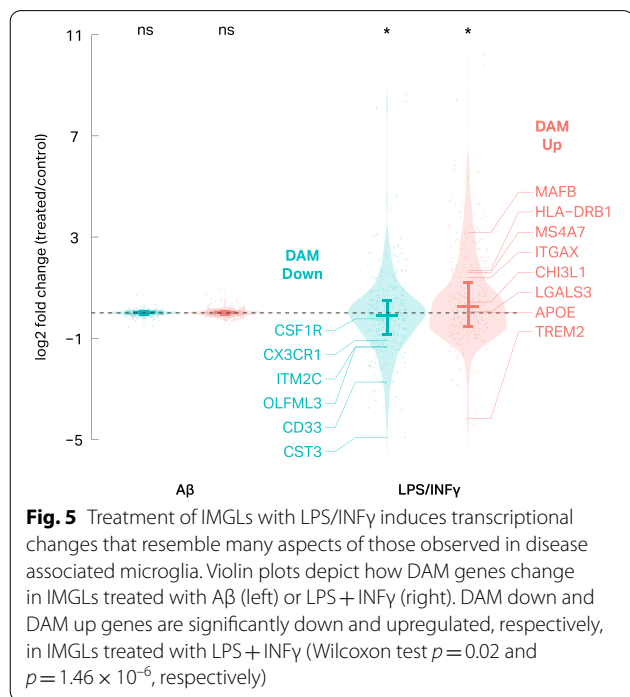
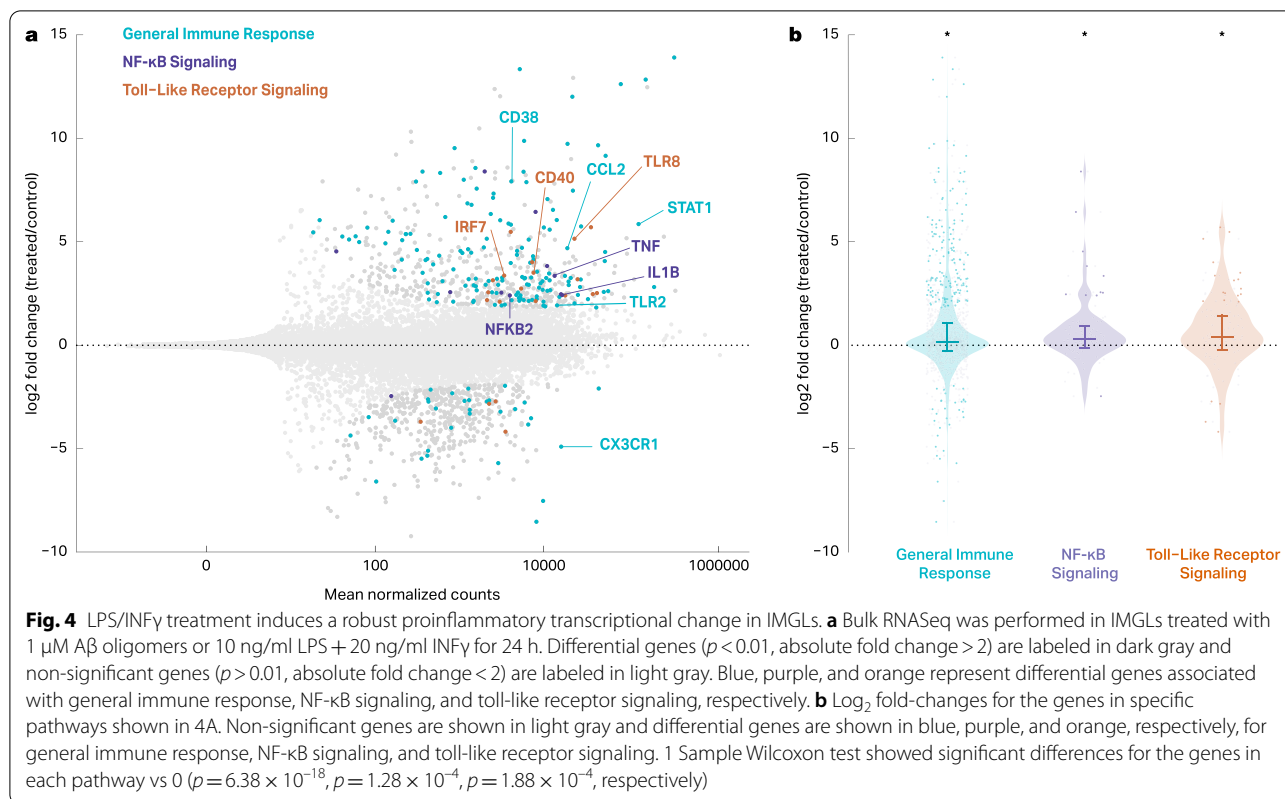


next eliminated the presence of fetal bovine serum (FBS) during the treatment to determine whether it was interfering with the A $\beta$  incorporation, since it has been suggested that the albumin present in the FBS could act by “sequestering” the soluble A $\beta$  in the media, reducing the availability of A $\beta$  capable of binding the cellular receptors [36]. No significant transcriptional response was seen in the absence of FBS during the A $\beta$  treatment. We then decided to test different concentrations of A $\beta$ . Again, no response was seen with any of the A $\beta$  concentrations tested ranging from 1 to 10  $\mu$ M. Following the relatively new hypothesis that microbial presence in the brain could be a contributing factor to the development of AD in addition to A $\beta$  accumulation [37–40], we decided to test whether A $\beta$  has any synergistic effect when the cells were co-incubated with LPS and INF $\gamma$ . We found no significant differences when treating THP-1 cells with this combination compared to LPS/INF $\gamma$  alone. In addition, we tested whether A $\beta$  had a significant effect in any of the other innate immune cell cultures used previously. No significant transcriptional response to A $\beta$  was seen in either PBMC M $\phi$ , U-87, or HMC3 cells. As an alternative method to activate the inflammasome and also as a positive control for proinflammatory activation, we treated THP-1 cells with LPS and INF $\gamma$ . As expected, THP-1 cells treated with this stimulus displayed a robust transcriptional response, (DESeq2, 4053 differential genes,  $p < 0.01$ , absolute fold change  $> 2$ ) (Fig. 3). Quantitative PCR was performed on three genes confirming these results (Additional file 2: Fig. S2). Finally, we treated IMGL with LPS and INF $\gamma$  and observed a robust transcriptional response (DESeq2, 1053 differential genes,  $p < 0.01$ , absolute fold change  $> 2$ ) although smaller to the one observed in THP-1 macrophages.

#### LPS/INF $\gamma$ activation of IMGLs induces a DAM-like transcriptional signature

In contrast to amyloid beta, and in agreement with a recent study [41], LPS/INF $\gamma$  treatment induced a robust transcriptional response in both THP-1 macrophages and IMGLs (Fig. 4A, B). Upregulated genes included key proinflammatory genes IL1B, CD38, and CCL2 and were enriched for classical inflammation pathways including NF- $\kappa$ B and Toll-Like Receptor Signaling (Homer,  $p < 0.05$ ). Because many of these genes and pathways are upregulated in AD microglia and because AD pathogenesis has been associated with viral and bacterial infection [40, 42, 43], some groups have used LPS or LPS/INF $\gamma$  to stimulate innate immune cells either in vitro or by direct injection in animal’s brains as a model of AD-associated neuroinflammation [44, 45].

To assess the validity of LPS/INF $\gamma$ -treated IMGLs as a model of AD, we compared the transcriptional profiles of our LPS/INF $\gamma$ -treated IMGLs to published DAM gene signatures from AD mouse models [46]. DAM genes were significantly enriched among LPS/INF $\gamma$  differentially expressed genes (Fisher’s Exact Test,  $p = 4.17 \times 10^{-15}$ ). The directional effects of our LPS/INF $\gamma$ -treated IMGLs were also concordant with the directional effects of the DAM signature (Fig. 5). Genes upregulated in DAM tended to be upregulated in IMGLs upon treatment with LPS/INF $\gamma$  (median log<sub>2</sub> fold-change = 0.26, Wilcoxon test,  $p = 1.46 \times 10^{-6}$ ), while genes that are downregulated in DAM also tended to be downregulated in IMGLs upon treatment with LPS/INF $\gamma$  albeit to a lesser degree (median log<sub>2</sub> fold-change = -0.09, Wilcoxon test,  $p = 0.02$ ) (Fig. 5). Among the shared upregulated genes are MS4A7, which has been shown to regulate TREM2 protein and to be upregulated in late-onset AD brains



[47, 48], and ITGAX which has been shown to be upregulated in aged microglia [49, 50]. We note that the effect sizes of LPS/INF $\gamma$  on DAM genes are small; however, these effect sizes are comparable to those observed in the mouse models used to identify DAM genes (median log $_2$  fold-change of 0.17 and  $-0.23$  for upregulated and downregulated DAM genes, respectively). It is important to mention that TREM2, which is fundamental for the development of DAM-like microglia [51], was downregulated in response to LPS/INF $\gamma$ . This agrees with previous observations in mouse models [52]. In summary, although not perfect, treating IMGLs with LPS/INF $\gamma$  recapitulates many features of the DAM transcriptome, and may be a useful model to study microglial transcriptional regulation in the context AD.

**Discussion**

Progress to unveil the mechanisms behind the origin and progression of AD has been slow in part due to the difficulty in finding adequate models to mimic this disease in the laboratory. Although mouse models have shown to be useful in many aspects, the genetic and physiological differences between species have limited certain areas of research [53]. Conversely, access to human brain samples is limited and the molecular identity and stability of some cell types, mostly brain immune cells, once



extracted from the brain have been controversial [54, 55]. As a result, the development of human *in vitro* cell culture models, particularly iPSC-derived cellular models, have been broadly used to mimic AD in a dish [56–58]. This alternative has been particularly valuable, since it is amenable to genome engineering, disease modeling, and high-throughput screening techniques unsuitable in primary human microglia.

Here, we analyzed the feasibility of using these *in vitro* cell models, including several established primary and iPSC-derived immune cell lines, to study the transcriptional regulation of AD-associated neuroinflammation using a genome-wide transcriptomic approach. Not surprisingly, we found that primary human microglia expression patterns were most similar to IMGLs (Fig. 1), in agreement with previous observations that also showed high similarity between IMGLs and primary microglia [21, 22].

When the use of IMGLs is not possible due to the complexity and high costs of their obtention, our results also suggested that the use of PBMC M $\phi$  or even macrophages derived from the THP-1 cells are preferable before the use of the HMC3 microglia established cell line, which has fewer similarities with primary microglia. Interestingly, other human and murine cellular models have also shown poor transcriptional similarity to primary microglia [59–61], raising concerns about the established microglia cell lines that are most often selected to study neuroinflammation in neurodegenerative diseases.

The stimulation of different innate immune cells in culture with synthetic A $\beta$  has become a common approach to model AD *in vitro*, as A $\beta$  accumulation in the brain is considered one of the main triggers for the development of AD and immune brain cells have been identified as the main players in AD origin and progression. Although the phagocytosis of this component into *in vitro* microglia-like cell cultures has been previously shown [21, 62], very few studies have shown any kind of pro-inflammatory response after this treatment. In addition, those studies that did investigate inflammation focused on only a handful of proinflammatory genes and proteins quantified via qPCR, microarrays, or western blots [21, 23, 63, 64].

Here we analyzed the effect of synthetic A $\beta$  treatment in innate immune *in vitro* cell cultures using the same genome-wide transcriptomic approach with a variety of experimental conditions. To our surprise, even though A $\beta$  is phagocytosed inside the cells (Fig. 3B), we found that it does not trigger a robust transcriptional response in any of the tested cell types or conditions (Fig. 2).

Several factors could explain why we did not observe robust transcriptional activation in these experiments.

First, the synthetic A $\beta$  used here has known differences in structure, heterogeneity and toxicity compared with the A $\beta$  found in human AD brains. A $\beta$  isolated from AD patients comprises a complex mix of A $\beta$  peptides with heterogeneous lengths and posttranslational modifications [35, 65–71]. The use of A $\beta$  preparations extracted from postmortem human brains using previously published extraction protocols [72] could give more insight into this hypothesis in future studies. Second, the microglia response to A $\beta$  and the consequent neurotoxic effect could be driven uniquely by post-transcriptional events, which would agree with previous reports describing the relevance of this layer of regulation in the control of the innate immune response [73–75] and previous reports of increased proinflammatory cytokine release after A $\beta$  treatment [25, 63]. Third, it is possible that the transcriptional response is only observed at early developmental time points not explored in this study or only after years of exposure, as would be expected in an AD brain. Finally, these monocultured cell lines may not accurately reflect the biology of a human brain which involves a vast array of intertwined cell types. Indeed, studies in animal models have revealed inflammation and microglial activation following injection of synthetic A $\beta$  [76–78]. More work needs to be done to determine if a similar response is observed in brain organoids.

Regardless of these possible explanations, our results suggest that using *in vitro* immune cell cultures in the presence of synthetic A $\beta$  might not be an adequate and/or sufficient model to mimic the microglia response in the context of the AD brain. Moreover, this suggests that A $\beta$  phagocytosis alone is not sufficient evidence of microglia activation, at least at the transcriptional level.

Even though these results do not disprove the hypothesis that in the human brain, A $\beta$  induces an exacerbated inflammatory response driven by brain immune cells, and that this plays a major role in the origin and progression of AD, it adds evidence that supports the current concern about placing the A $\beta$  cascade hypothesis as the sole explanation for the AD etiology [79]. More factors, such as the role of tau, microbial infections, and more complex interactions between different brain cell types should be considered and further investigated to understand the origins of the neuroinflammation state observed in the AD brain.

Finally, in agreement with recent studies [41], we showed that IMGLs exhibit a significant transcriptional response to LPS/INF $\gamma$ . While this response is smaller than the one observed in THP-1 cells, as was also previously reported [61], it resembles many aspects of the transcriptional profile of DAMs in the AD brain.

## Conclusions

Based on our results we identified IMGLs as the most similar in vitro culture to mimic the transcriptional profiles of primary human microglia and that when the use of IMGLs is not possible, PBMC M $\phi$  or even macrophages derived from the THP-1 cells are preferable before the use of the HMC3 microglia established cell line.

We also showed that even though synthetic A $\beta$  is phagocytosed inside immune in vitro cell cultures, it does not trigger a robust transcriptional response. Based on this observation, the use of this in vitro cell model to study AD-related neuroinflammation in a dish should be carefully re-evaluated.

Finally, we found that treating IMGLs with LPS/INF $\gamma$  produces a transcriptional response that resembles many aspects of the transcriptional profile found in DAMs in the AD brain, suggesting that this may be a suitable in vitro platform to study AD-related microglia inflammation when the use of primary microglia or animal models is not possible.

## Abbreviations

A $\beta$ : Amyloid beta; AD: Alzheimer's disease; AIF1: Allograft inflammatory factor 1; CCL2: C–C motif chemokine ligand 2; CD38: Cluster of differentiation 38 molecule; DAM: Disease associated microglia; FBS: Fetal bovine serum; fA $\beta$ : Fibrillarized A $\beta$ ; hPSC: Human induced pluripotent stem cell; HPCs: Hematopoietic progenitor cells; IMGL: Human induced pluripotent stem cell-derived microglia; INF $\gamma$ : Interferon gamma; IL1B: Interleukin 1 beta; ITGAX: Integrin Subunit Alpha X; KEGG: Kyoto encyclopedia of genes and genomes; MS4A7: Membrane spanning 4-domains A7; NF- $\kappa$ B: Nuclear factor kappa B subunit 1; LPS: Lipopolysaccharide; oA $\beta$ : Oligomerized A $\beta$ ; PBMC M $\phi$ : Peripheral blood mononuclear cell-derived macrophages; PBS: Phosphate buffered saline; PCA: Principal component analysis; PLCG1: Phospholipase C gamma 1; PMA: 12-O-tetradecanoylphorbol; qPCR: Quantitative PCR; RIN: RNA integrity number; P2RY12: Purinergic receptor P2Y12; TREM2: Triggering receptor expressed on myeloid cells 2.

## Supplementary Information

The online version contains supplementary material available at <https://doi.org/10.1186/s12974-022-02459-1>.

**Additional file 1: Figure S1.** A $\beta$  is incorporated into IMGLs. IMGLs incubated with 2  $\mu$ g/mL of oligomeric  $\beta$ -Amyloid<sub>1–42</sub>-HiLyte Fluor 555 for 1 h. Confocal images were acquired on an inverted Zeiss 800 laser scanning confocal microscope. Scale bar, 10  $\mu$ m.

**Additional file 2: Figure S2.** qPCR for selected genes confirms RNA-seq results. THP-1 macrophages were treated with 10 ng/ml LPS + 20 ng/ml INF $\gamma$  for 24 h. The fold-changes of 3 genes were measured with qPCR in duplicate and compared to fold-changes detected by RNA-seq.

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## Author contributions

IYQ participated in the acquisition, analysis and interpretation of the experimental data as well as in the draft of the manuscript. AEC participated in the acquisition and analysis of the data. KSMR participated in the analysis of the

data and the draft of the manuscript. MLB participated in the acquisition, analysis and interpretation of the data and the draft of the manuscript. BAE, JHT, and JVR participated in the acquisition of the experimental data. RBM participated in the acquisition of the experimental data and the revision of the manuscript. HW participated in the design of the work, interpretation of the data and the revision of the manuscript. SC participated in the design of the work and interpretation of the data. TJC participated in the design of the work and interpretation of the data. DHP participated in the conception and design of the work, the interpretation of the data and the draft of the manuscript. All authors read and approved the final manuscript.

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## Availability of data and materials

Raw FASTQ files are available on SRA under accession number PRJNA750801. Transcript-level quantifications from Salmon are available on GEO under series GSE181153, in addition to a single gene-level counts table with each sample represented as a column, as generated by tximport. The GEO series page also includes a table describing the samples used in DESeq2 for each column of Fig. 3.

## Declarations

### Ethics approval and consent to participate

Non applicable.

### Consent for publication

Non applicable.

### Competing interests

The authors declare that they have no competing interests.

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