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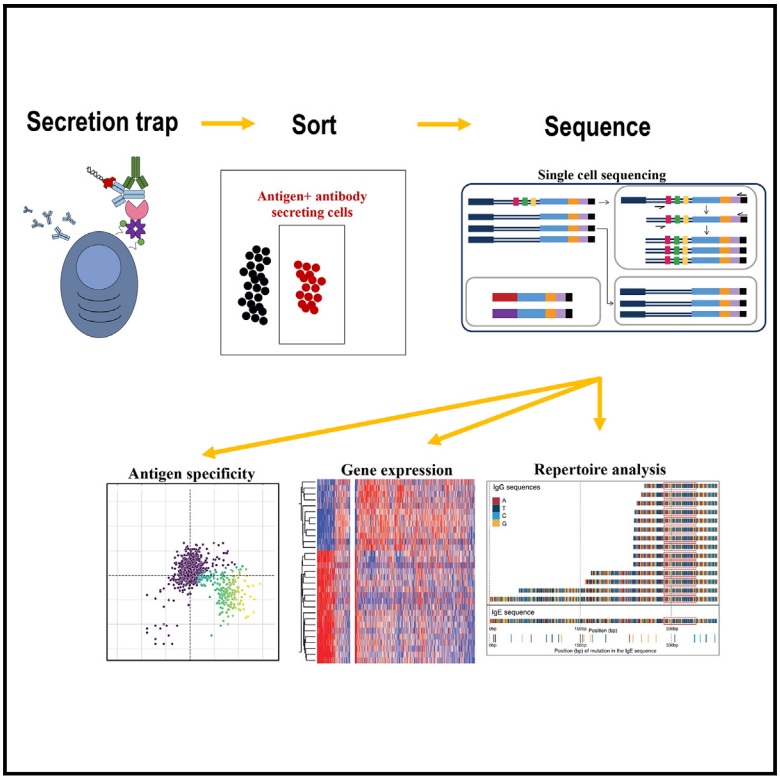
TRAPnSeq allows high-throughput profiling of antigen-specific antibody-secreting cells

Graphical abstract



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In brief

Asrat et al. develop a method, TRAPnSeq, that enables specific isolation and profiling of antigen-specific (Ag^+) antibody-secreting cells. They show the utility of TRAPnSeq in mice and humans by performing high-throughput analysis of Ag^+ IgG and IgE plasma cells.

Highlights

- TRAPnSeq allows antigen specificity mapping through Ig secretion TRAP and Sequencing
- High-throughput profiling of ASCs based on local antibody capture
- TRAPnSeq enables rapid antibody discovery from ASCs and elucidates Ag^+ ASC biology

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TRAPnSeq allows high-throughput profiling of antigen-specific antibody-secreting cells

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MOTIVATION Emerging techniques have enabled high-throughput profiling of antigen-specific (Ag^+) B cells, which has expanded our understanding of the B cell repertoire. A key limitation of existing platforms is profiling of Ag^+ antibody-secreting cells (ASCs) that downregulate their surface BCRs. TRAPnSeq improves on this limitation by incorporating an antibody secretion trap that captures secreted antibodies on the surface of ASCs, which in turn enables rapid and high-throughput profiling of Ag^+ ASCs.

SUMMARY

Following activation by cognate antigen, B cells undergo fine-tuning of their antigen receptors and may ultimately differentiate into antibody-secreting cells (ASCs). While antigen-specific B cells that express surface receptors (B cell receptors [BCRs]) can be readily cloned and sequenced following flow sorting, antigen-specific ASCs that lack surface BCRs cannot be easily profiled. Here, we report an approach, TRAPnSeq (antigen specificity mapping through immunoglobulin [Ig] secretion TRAP and Sequencing), that allows capture of secreted antibodies on the surface of ASCs, which in turn enables high-throughput screening of single ASCs against large antigen panels. This approach incorporates flow cytometry, standard microfluidic platforms, and DNA-barcoding technologies to characterize antigen-specific ASCs through single-cell V(D)J, RNA, and antigen barcode sequencing. We show the utility of TRAPnSeq by profiling antigen-specific IgG and IgE ASCs from both mice and humans and highlight its capacity to accelerate therapeutic antibody discovery from ASCs.

INTRODUCTION

Antibody-secreting cells (ASCs) are crucial in maintaining humoral immune response against pathogens but can also be the source of antibody-mediated pathologies. ASCs are generated via terminal differentiation of activated B cells following engagement with their cognate antigen. Differentiation of ASCs can occur following a germinal center reaction in secondary lymphoid organs after B cells undergo affinity maturation and class switching, resulting in the generation of high-affinity antibodies, or from extrafollicular sources as a way of rapidly responding to antigens at the site of inflammation.[1]

After differentiation, ASCs can stay within secondary lymphoid organs, as mainly short-lived ASCs, or can migrate to a niche, such as the bone marrow (BM), that provides survival signals and supports their longevity.[1] Long-lived plasma cells (LLPCs) are ASCs that are the main source of serological memory following infection or vaccination and produce high quantities of antibodies without the need for further antigen stimulation.[2] In fact, once generated, LLPCs can exist for a lifetime.[3] Many studies have proposed that PCs, in particular T cell-dependent LLPCs, are a source of high-affinity antibodies due to their stringent selection process. Following a germinal center (GC) response, the highest affinity GC B cells are preferentially selected into the PC compartment, ensuring long-term production of high-affinity antibodies against previously encountered antigens.[4–7] While this property of LLPCs is advantageous for protection against pathogen re-infection, it can be deleterious in the case of self-reactive antibodies that contribute to autoimmune disorders. For example, long-lived immunoglobulin G (IgG) PCs have been shown to maintain pathogenic autoantibody production in patients with rheumatoid arthritis or immune thrombocytopenic purpura post-treatment with anti-CD20.[8,9] Recently, long-lived IgE PCs have also been shown to maintain IgE serological memory and are the source of pathogenic IgE that can induce anaphylaxis during allergen exposure.[10]

Despite the crucial role of PCs in health and diseases, much is still unknown about their specificity, heterogeneity, differentiation, and the mechanism that maintains their longevity. Unlike IgA and IgM PCs that retain surface B cell receptor (BCR) post-terminal differentiation,[11] the IgG BCR is downregulated on PCs, making it challenging to isolate and sequence antigen-specific (Ag^+) IgG PCs following immunization or infection. Identification and profiling of IgE PCs has also presented similar challenges due to the lack of known surface markers as well as their low frequency in circulation. Only a few IgE immature plasmablasts have been examined previously with single-cell technologies,[12] leaving IgE-secreting BMPCs largely understudied.

Here, we report a high-throughput method that allows profiling of Ag^+ ASCs along with phenotypic and transcriptional profiling of this population using a method we call TRAPnSeq (antigen specificity mapping through Ig secretion TRAP and Sequencing). We show that this method can easily be coupled with existing B cell profiling/sequencing technologies and demonstrate its versatility by profiling IgG PCs from the BM of immunized mice and IgE PCs from allergen-exposed mice and from the BM of allergic donors. We propose that the application of TRAPnSeq can be used to advance our understanding of PC specificity and heterogeneity, and to inform mechanisms of longevity.

RESULTS

Development of TRAPnSeq for high-throughput profiling of Ag^+ ASCs

To pair secreted antibodies with the ASCs secreting them, we devised an Ig secretion trap that can locally capture secreted antibodies on the surface of ASCs (Figure 1A). To achieve this, the cell surface was biotinylated using NHS-biotin,[2] which has a hydrophilic active group that binds to cell surface primary amines ($-NH_2$). An affinity matrix was then assembled around cells using streptavidin coupled to an Fc-binding reagent (αIgk for IgG and FcεRIα ectodomain for IgE) to capture secreted Igs from ASCs (Figure 1A). To mark antigen specificity of PCs, this method was coupled with barcoded antigens and/or fluorescent-tagged antigen staining (Figure 1A). Single-cell transcriptomes (single-cell RNA sequencing [scRNA-seq]), barcoded antigen signals, and heavy and light chain variable region (VH:VL) sequences were generated using 10× Genomics (Figure 1B).

To determine functionality, sensitivity, and specificity of IgG and IgE secretion traps, ARH-77, an IgG (κ light chain)-secreting cell line,[13] and U266, an IgE (lambda light chain)-secreting myeloma line,[14] were used for proof-of-principle experiments (Figures 1C–1F). We first confirmed the lack of BCR surface expression on both cell lines (Figures S1A and S1B). ARH-77 and U266 cells were biotinylated using NHS-biotin, and an affinity matrix was assembled using streptavidin coupled to anti-human Igk for IgG (StAv-αIgk) and the ectodomain of the high-affinity IgE receptor FcεRIα (StAv-FcεRIα) for IgE. Sensitivity of capture was determined by staining for IgG and IgE (Figures 1C and 1E). While ARH-77 and U266 cells missing any part of the affinity matrix lacked IgG and IgE on their surface, successful assembly of all components of the secretion trap allowed specific detection of secreted IgG and IgE on the surface of the cells that secrete them (Figures 1C and 1E). To further determine the minimal concentration of StAv-αIgk and StAv-FcεRIα required for binding secreted antibodies, we performed dose titration of the traps and observed that StAv-αIgk saturated between 3.75 and 7.5 μg/mL, while StAv-FcεRIα saturated between 15 and 30 μg/mL (Figures 1D and 1F).

To test the specificity of the affinity matrix, IgM-expressing Ramos cells were mixed with U266 cells 1:1 (Figure 1G) or 100:1 (Figure S1C), and an IgE secretion trap was assembled on the mixed population. No binding of secreted IgE was observed on the surface of Ramos cells, suggesting that there is minimal or no crosstalk with neighboring cells at the dilution and culture conditions tested here (Figures 1G and S1C). We confirmed specificity of IgE capture in the presence of other isotype-secreting cells by mixing U266 with ARH77 (Figure S1D) and observed similar findings. It is worth noting that capture conditions require careful optimization and may vary depending on the Ig secretion levels of cells used.

Ag^+ ASCs can be specifically isolated and profiled using TRAPnSeq

After validating successful capture of Ig on ASCs *in vitro*, we determined if TRAPnSeq can be used to isolate and profile Ag^+ ASCs *in vivo* from immunized mice. To detect Ag^+/IgG^+ ASCs, VelocImmune mice[15,16] were immunized with hIL-4Rα monomeric protein via footpad (Figure S2A). Single-cell suspensions of BM cells were generated, and B lineage cells were enriched by negative selection (Figure 2A). BM cells were coated with NHS-biotin and secreted IgG captured with StAv-αIgk as previously described (Figure 1). Following assembly of the biotin-StAv-αIgk complex on the surface of BM cells, enriched ASCs were stained with an antibody mix containing PC-specific markers, αIgG for detection of secreted IgG, barcoded hIL-4Rα for detection of Ag^+ cells, and barcoded hIL-6Rα as a negative control. Spleen and draining lymph nodes (dLNs) from the same mice were pooled and stained directly without using the Ig secretion trap for comparison of VH:VL sequences from BCRs expressing Ag^+ B cells with BMPCs (Figures S2B and S2C). Ag^+/IgG^+ PCs from the BM were sorted using staining controls along with B cells expressing surface BCRs from spleen/dLN (Figures 2B and S2B–S2D).

scRNA-seq was performed on sorted BMPCs and splenic/dLN B cells to quantify antigen specificity, determine transcriptional differences, and identify VH:VL repertoire changes at a single-cell level. From barcoded antigen counts of hIL-4Rα and a negative control antigen hIL-6Rα, Ag^+/IgG^+ cells were verified (Figure 2C). To prioritize specificity and minimize polyreactivity, we developed an antigen specificity score (AgSS) that selects cells with strong specificity for hIL-4Rα and minimal hIL-6Rα binding (described in STAR Methods). Cells with high AgSS were prioritized for hIL-4Rα and had minimal barcoded UMI counts for the control antigen hIL-6Rα (Figure 2C). Combined transcriptional analysis and unbiased clustering indicated that PCs from either spleen or BM clustered together and expressed classical PC markers such as *Slamf7*, *Xbp1*, *Sdc1*, *Prdm1*, and *Slc3a2* (Figures 2D and S3A). Splenic B cells, however, clustered very distinctly from PCs and expressed typical B cell markers such as *Ms4a1* and *Cd79a* (Figure S3A). Additionally, Ag^+ cells with high AgSS (>0.5) from both the BM and spleen generally

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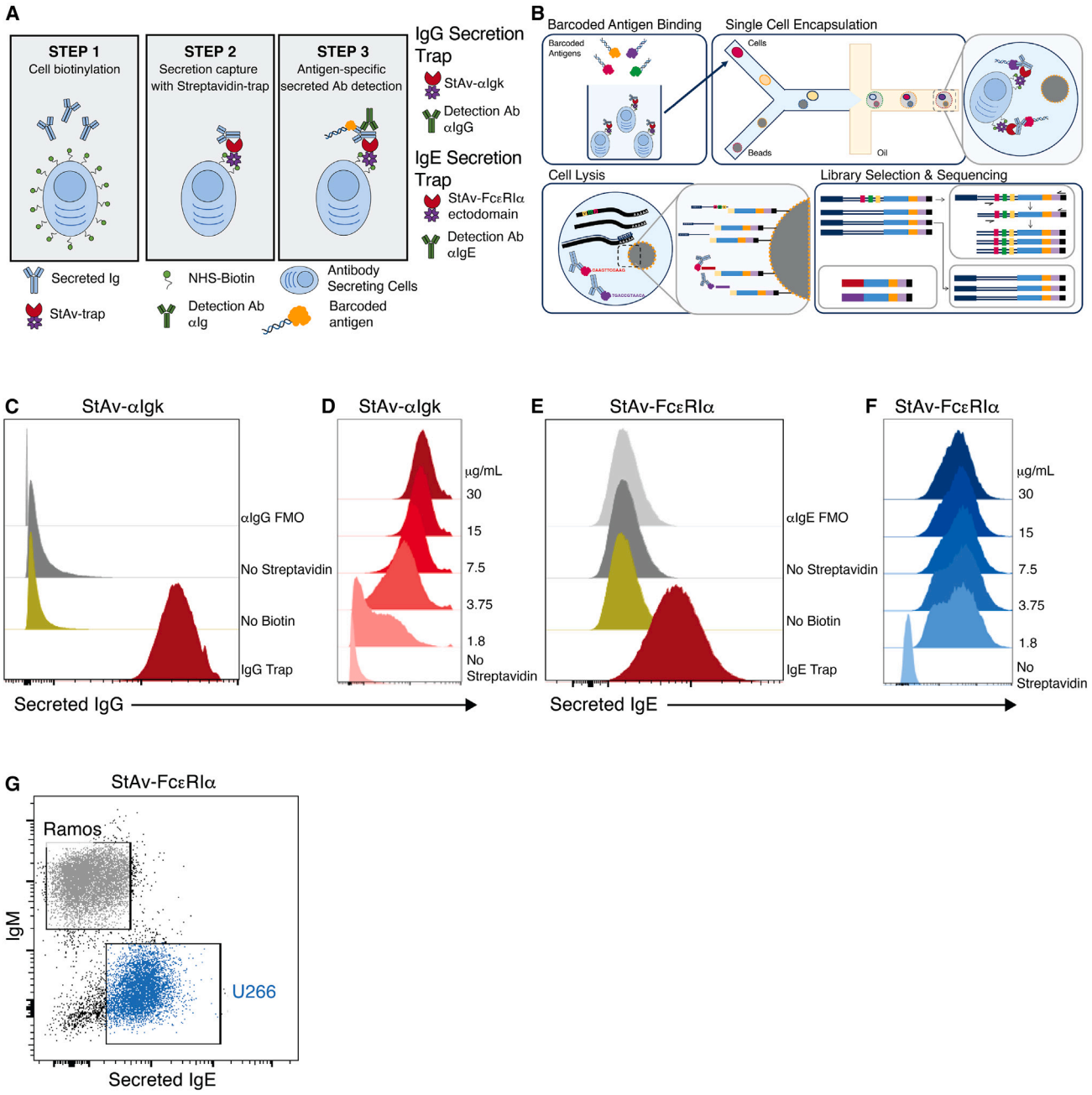


Figure 1. Development of TRAPnSeq for high-throughput profiling of antigen-specific antibody-secreting cells

(A) Schematic of TRAPnSeq technology. Step 1: antibody-secreting cells were biotinylated with amine-reactive NHS-biotin. Step 2: secreted antibodies were captured on the surface of antibody-secreting cells using streptavidin coupled to anti-Fc. αIgk was used for antibodies that express κ light chain or FceRIα ectodomain for specific IgE capture. Step 3: αIgE or αIgG antibodies were used to detect secreted antibodies and oligo-barcoded and fluorescently tagged antigens used to determine antigen specificity.

(B) Fluorescently labeled, oligo-barcoded antigen^{+} antibody-secreting cells were sorted and subjected to single-cell sequencing to determine antigen specificity, VH:VL sequences, and transcriptional profile.

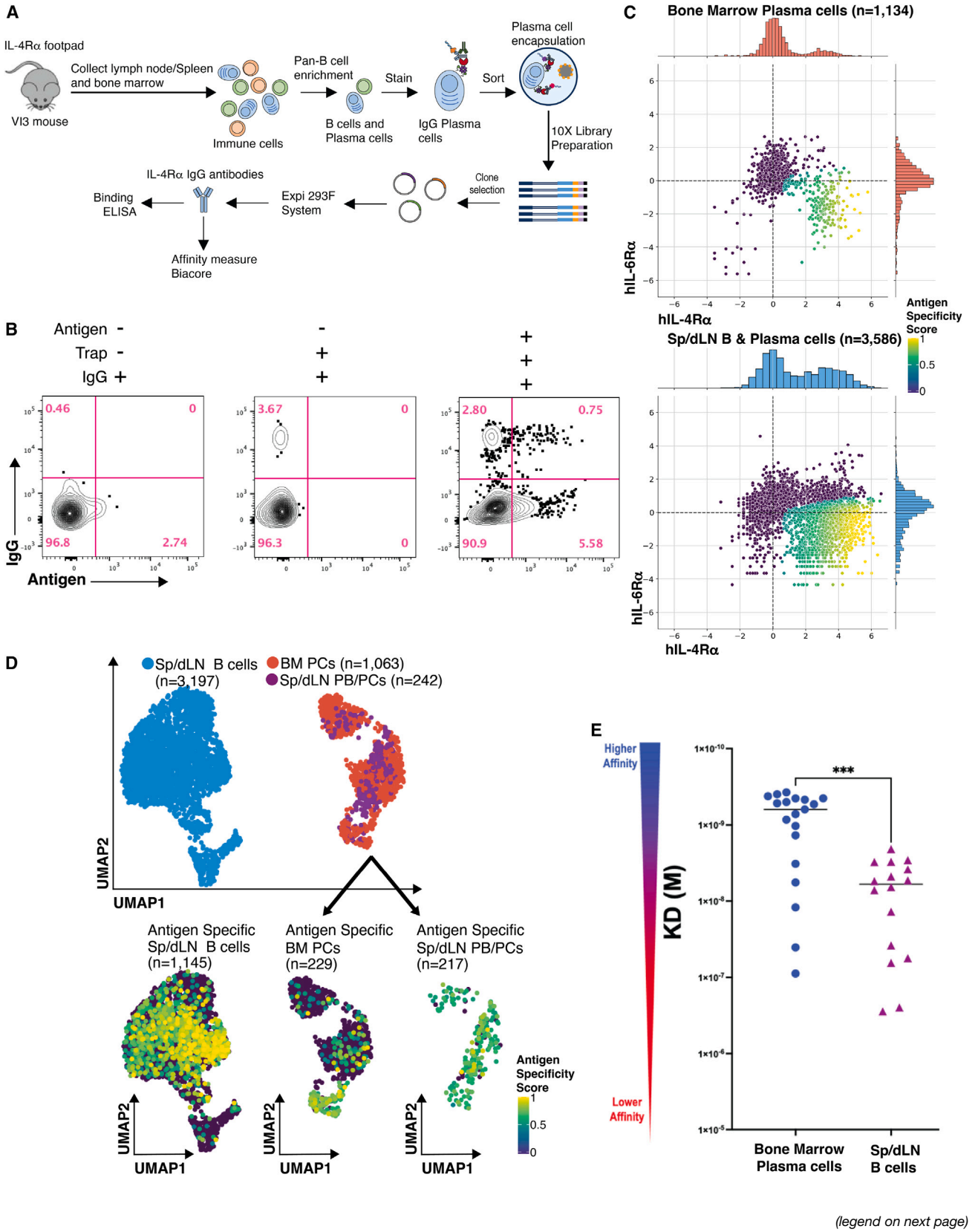
(C) Flow cytometry analysis of IgG captured on the surface of ARH77 (IgG-secreting cells) using Igk secretion trap.

(D) Titration of streptavidin-αIgk (StAv-αIgk) on ARH77 cells.

(E) Flow cytometry validation of IgE secretion trap on U266 (IgE-secreting myeloma cells).

(F) Titration of streptavidin-FceRIα (StAv-FceRIα) on U266 cells.

(G) Flow cytometry analysis of 1:1 mixed U266 and Ramos cells in the presence of StAv-FceRIα secretion trap.



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clustered together (Figure 2D). In BMPCs especially, cells with high AgSS clustered together at the bottom of the uniform manifold approximation and projection (UMAP) (Figure 2D, bottom middle panel).

Ag$^+$ IgG PCs isolated from the BM using TRAPnSeq show higher affinity compared with splenic/dLN B cells

scRNA-seq yielded three distinct cell populations that separate based on transcriptional signatures: BMPCs, spleen/dLN plasmablasts/PC-like cells (Sp/dLN PBs/PCs), and Sp/dLN B cells. To address a key question about affinity differences between BMPCs vs. B cells post-immunization, we focused our comparison on antibody sequences originating from Ag$^+$ BMPCs and Sp/dLN B cells. 20 unique VH:VL sequences from cells with the highest hIL-4Rα barcode signal were selected for cloning and expression analysis from the 2 compartments (Figures S3B and S3C). Heavy and light chain variable sequences were successfully cloned from 20 BMPCs and from 19 Sp/dLN B cells into vectors expressing human IgG4 and human Igk constants (see STAR Methods; Table S1).

Consistent IgG expression was observed across both populations, and hIL-4Rα binding was observed in 95% (19/20) of BMPCs compared with 84% (16/19) of Sp/dLN B cells (Figure 2E; Table S1). Biacore analysis on the supernatants of expressed antibodies showed that antibodies from the BMPCs had the highest median affinity (Figure 2E). Despite the small sample size, these data suggest that antigen-positive and higher affinity clones were preferentially identified in the BMPC pool.

scRNA-seq of IgG cells captured by TRAPnSeq enables deeper biological queries

Since we validated TRAPnSeq as a method to sequence Ag$^+$ ASCs, we investigated the unique BCR sequences obtained by scRNA-seq of the VDJ region (Figure 1B) to compare Ag$^+$ BMPCs with Ag$^+$ B cells from the Sp/dLN. BCR sequences from all cells were mapped to specific V, D, and J gene segments by IgBlast,[17] and the extent of somatic hypermutation was measured by the percentage of similarity to germline V regions. Ag$^+$ cells in both populations exhibited lower percentages of similarity to germline V regions and therefore more frequent mutations than non-Ag$^+$ cells (Figure 3A). We then investigated the clonal similarity of the BCR sequences of Ag$^+$ cells by identifying BCR clonotypes in cells that shared the same heavy and light chain V gene segment and had identical CDR3 amino acid sequences in both the heavy and light chains (Table S2). Ten unique CDR3 sequence pairs were clonally shared among BMPCs, splenic/dLN B cells, and splenic PBs/PCs (Table S2). Interestingly, while the majority of each BCR repertoire was specific to each population (Figure 3B), 40 CDR3 sequences were shared across dLN/B cells and BMPCs, suggesting a common clonal evolution across tissue.

In addition to BCR sequence analysis, we also compared the transcriptomes of Ag$^+$ and non-Ag$^+$ cells to identify gene signatures related to hIL-4Rα specificity. In BMPCs, several gene expression programs differed based on hIL-4Rα specificity (Figures 3C and 3D), despite both hIL-4Rα-specific and non-specific cells showing similar canonical PC marker expression (Figure 3D, top panel). *Tmsbx4*, *Cd24a*, *Tmsb10*, and *Fkbp11* all demonstrated significant upregulation in hIL-4Rα-specific BMPCs, whereas non-Ag$^+$ cells showed increases in *Ly6d*, *Prg2*, *Tmem176a*, and *Tmem176b* (Figures 3C and 3D). Subclustering of BMPCs indicated that *Cd24a*, *Ppib*, *Fkbp11*, and *Ssr2* transcripts were all expressed highly toward the bottom of the UMAP projection (Figure 3E), while gene programs marked by *Tmem176a*, *Tmem176b*, *Ly6d*, and *Cd74* expression clustered in the middle and top regions of the UMAP (Figure 3E). Interestingly, BCR sequences cloned from either of the transcriptionally distinct regions resulted in high-affinity, hIL-4Rα-specific antibodies as measured by negative log10 of the binding K_ds, with a modest bias toward higher affinity in the bottom cluster (Figure S3C). These data highlight the potential of TRAPnSeq to interrogate antigen-specific PC transcriptional profiles in relation to antibody affinity.

1703 IgE PCs sequenced from the BM and LN of HDM-exposed mice using IgE TRAPnSeq

For validation of IgE TRAPnSeq *in vivo*, we used the previously described IgE^{Venus} and Blimp-1^{mCherry} reporter mice (Figure 4A) that were exposed to house dust mite (HDM) extract for 15 weeks,[10,18] and secreted IgE was captured using the StAv-FcεRIα ectodomain as described in Figure 1. Cells were stained with an antibody mix containing PC makers, αIgE for secreted IgE, and oligonucleotide-barcoded HDM allergens, including Der p1, Der p2, and Der f1, and an Olive allergen (Ole e1) as a negative control. PCs were gated using Blimp-1 (Figure 4B, top panel), and IgE PCs were sorted (Figure 4B, bottom panel, black box) along

Figure 2. Antigen-specific IgG plasma cells isolated from the bone marrow using TRAPnSeq show higher affinity compared with splenic/draining lymph node (dLN) B cells

(A) Schematic of the experimental workflow for Igk secretion trap technology. Mice were immunized with hIL-4Rα and single cells prepared from bone marrow (BM), dLN, and spleen. B cells and plasma cells (PCs) were enriched by negative selection. Only BMPCs were cell surface biotinylated and incubated with αIgk secretion trap. All cells were stained with αIgG along with B cell and PC markers and barcoded antigens for detection of antigen-specific B cells and PCs. Antigen$^+$/IgG$^+$ B cells and PCs were sorted, and single-cell sequencing was performed to determine gene expression, VH:VL sequences, and antigen specificity. Selected clones were expressed by transient transfection of 293F cells and antigen binding tested by ELISA and Biacore.

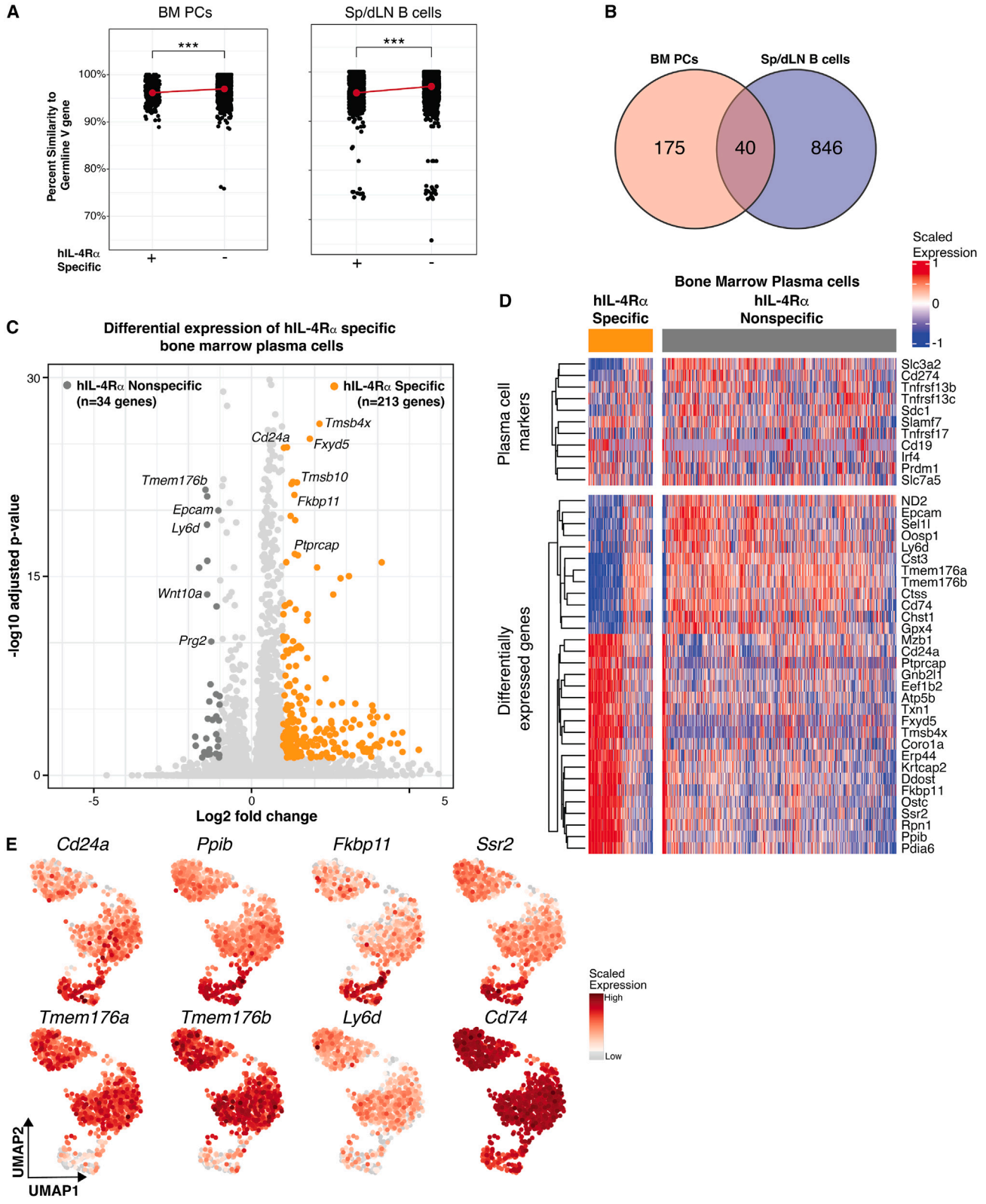
(B) Flow cytometry of antigen-specific IgG that is captured on the surface of PCs in hIL-4Rα-immunized mice.

(C) Visualization of antigen specificity based on barcode signal of hIL-6Rα (non-specific) and hIL-4Rα (specific) in BMPCs (n = 1,134) (top) and spleen/dLN B cells and PCs (n = 3,586) (bottom).

(D) UMAP of B cells and PCs isolated from spleen/dLN and BM of challenged mice shows localization of spleen/dLN B cell cluster (n = 3,197) in blue, BM-PC cluster (n = 1,063) in red, and spleen/dLN-PC cluster (n = 242) in violet (top). UMAP of B cells and PCs shows hIL-4Rα-specific cells in 3 clusters based on antigen specificity score (bottom).

(E) Graph shows binding affinity of antibodies cloned from BMPCs (n = 19) and spleen (Sp)-B cells (n = 16).

In (E), each symbol indicates individual antibodies. Asterisks indicate statistical significance as calculated with Mann-Whitney test. ***p value $\leq$ 0.001.
See also Table S1.



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with non-IgE PCs (Figure 4B, bottom panel, red box) for comparison. Consistent with what was observed for U266 and Ramos (Figure 1G), secreted IgE was only detected on IgE PCs and not on other isotypes (non-IgE PCs), confirming the specificity of the IgE trap in a mixed cell population (Figure 4B).

We merged the single-cell transcriptomic profiles from lung dLN and BM and performed cell clustering to compare IgE and non-IgE PCs. We obtained 12,646 IgG^+ cells, 17,524 IgA^+ cells, 3,503 IgM^+ cells, and 1,703 IgE^+ cells (Figure 4C). From single-cell transcriptomes, we confirmed Ig expression and identified specific gene expression programs for each isotype. IgE PCs expressed high levels of *Ighe*, *Slpi*, *Sdf2l1*, and *Pdia4*. IgG PCs expressed *Ighg1* and modestly higher levels of *Cd74* and *B2m*, while IgA PCs expressed *Igha* and higher levels of *Cd79a* and *Cd69*. IgM PCs expressed *Ighm* and higher levels of *Slc3a2*, *Ctss*, and *Ghg* (Figure 4D). These data show the utility of TRAPnSeq to identify isotype-specific transcriptional profiles.

Using barcoded HDM allergens, we directly mapped IgE PCs to their specificity to Der p1, Der p2, and Der f1 (HDM allergens) and Ole e1 (olive allergen, negative control) and compared their AgSS, which were calculated in the same manner as the IgG secretion trap, except that here the antigens were Der p1, Der p2, and Der f1, and the control for all was Ole e1 (olive antigen) (Figure 4E). A few allergen-specific IgE antibodies were selected and cloned based on AgSS for HDM allergens (Table S3). The relative binding of these antibodies to Der p1, Der p2, and Der f1 as well as to the olive allergen Ole e1 was tested by ELISA. Two IgE antibodies (IgE monoclonal antibody mAb12_2 and mAb 3_1) bound to Der p1 (Figures 4F and S4A), 3 IgEs bound to Der p2 (IgE mAb12_2, mAb8_2 and mAb21_2), and 6 IgEs bound to Der f1 (IgE mAb12_2, mAb1_2, mAb 1_1, mAb3_1, mAb4_1, and mAb 6_1). Interestingly, Ab12_2 bound to Ole e1 in addition to Der p1, Der p2, and Der f1 (Figure 4F) but did not show any binding to Fel d1 (Figure S4B), demonstrating some level of specificity of this antibody.

To determine if the IgEs that bound HDM allergens were able to induce anaphylaxis, we utilized the passive cutaneous anaphylaxis (PCA) mouse model. The PCA model is a functional measure of local mast cell activation-induced vascular permeability in ear tissue upon antigen-induced crosslinking of Ag^+ IgE bound to FcεRIα on tissue mast cells.[19] Mice were sensitized intradermally with a cocktail of Der p1- or Der p2-specific IgEs (Figures 4F and S4A) in their left ear and an irrelevant DNP-IgE in the right ear as a negative control. After 24 h, mice were intravenously challenged with Evans blue containing Der p1 or Der p2 (Figure 4G). Mast cell degranulation was observed only in the ears sensitized with Der p1- or Der p2-specific IgE (left ear), as measured by Evan's blue dye leakage (Figure 4G), demonstrating that the cocktail of IgE antibodies is functional and can induce allergen-specific, IgE-mediated crosslinking of FcεRIα and mast cell degranulation.

Extension of TRAPnSeq to human primary cells: Identification and profiling of IgE PCs from the BM of allergic individuals

To determine if IgE TRAPnSeq could be extended to human primary cells, we performed the IgE secretion trap on the BM of self-reported allergic individuals. B lineage-enriched cells were coated with NHS-biotin, and secreted IgE was captured using the StAv-FcεRIα ectodomain as previously described (Figure 1A). PCs were gated as CD38 high/CD20 low (Figure S5A), and the specificity and functionality of the StAv-FcεRIα secretion trap were confirmed in non-allergic BM that had full Biotin-StAv-FcεRIα assembly (biological control, Figure 5A, middle graph) as well as allergic BM that lacked StAv-FcεRIα (technical control, Figure 5A, right graph). To perform a full validation of human IgE TRAPnSeq, we selected a donor with cat-, timothy grass-, and HDM-reactive IgEs in sera (Figures 5B and S5B), performed the IgE secretion trap without allergen-specific barcodes, and sorted all IgE PCs along with non-IgE PCs from the BM for comparison.

Following sorting, scRNA-seq recovered full transcriptomes and BCR sequences of 5,559 BM PCs from the cat, grass, and HDM allergic individual. We confirmed the specificity of PC capture based on the abundant expression of various Ig genes and PC markers such as *XBP1*, *SLAMF7*, and *CD74* and no observed expression of the B cell markers *MS4A1* and *CD19* (Figure 5C). Ten IgE PCs were identified by VH:VL sequencing and alignment to the human germline database with IgBlast. These IgE PCs correlated very strongly with *IGHE* transcript expression along with a small fraction of cells from IgA and IgG isotypes that also expressed *IGHE*, possibly reflecting sequential class switching between these isotypes or sterile *IGHE* transcription in some IgA and IgG cells[20] (Figures 5C and 5D). This suggests that despite the rarity of IgE PCs in human BM, the TRAPnSeq method enables isolation and sequencing of IgE PCs.

To investigate clonal evolution, VH sequences from all 5,559 cells were clustered based on the amino acid sequence of the heavy-chain CDR3 region allowing for up to 3 amino acid mismatches, deletions, or insertions (see STAR Methods). The 10 IgE PCs clustered uniquely into 5 clonotypes, which we defined as a group of PCs with highly similar heavy-chain amino acid CDR3s (Table S4). Two clonotypes, denoted 753 (n = 4 cells) and 696 (n = 3 cells), were made up exclusively of IgE cells, and each clonotype displayed identical CDR3 sequences,

Figure 3. IgG secretion trap can be coupled with single-cell gene expression and BCR profiling to characterize antigen-specific PCs

(A) Graphs show percentage of similarity to germline V region between hIL-4Rα-specific and non-specific cells isolated from BMPCs and Sp/dLN B cells from challenged mice.

(B) Venn diagram shows clonal overlap based on CDR3 nucleotide sequence from heavy and light chains of clones isolated from BMPCs and Sp/dLN B cells.

(C) Volcano plot comparison of differential gene expression between hIL-4Rα-specific and non-antigen specific BMPCs.

(D) Heatmap of scaled expression shows transcriptional profile of the most differentially expressed genes expressed in hIL-4Rα-specific and non-antigen-specific BMPCs.

(E) UMAP shows scaled gene expression of *Cd24a*, *Ppob*, *Fkbp11*, *Ssr2*, *Tmem176a*, *Tmem176b*, *Ly6d*, and *Cd74* in BMPCs.

In (A), dots represent individual antibodies. Asterisks indicate statistical significance as calculated with Mann-Whitney test. ***p value $\leq$ 0.001.

See also Table S2.

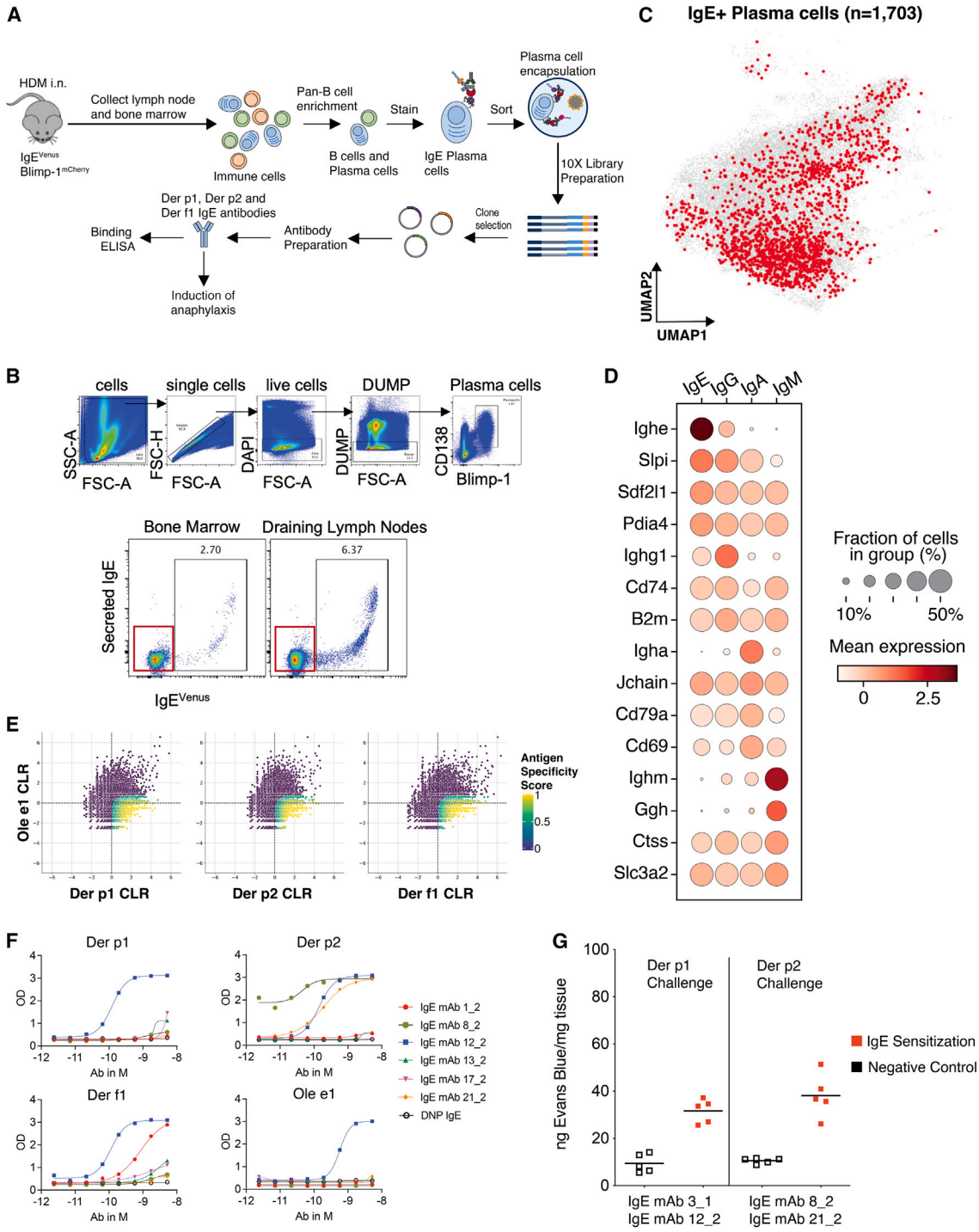

Figure 4. Identification and profiling of allergen-specific IgE PCs in HDM-exposed mice using TRAPnSeq

(A) Schematic of the IgE secretion trap experimental workflow. IgE^{Venus} Blimp-1^{mCherry} mice were exposed to HDM 3× a week for 15 weeks. Single-cell preparations from lung draining lymph nodes (dLN) and BM cells were enriched for B cells and PCs. Cell surface was biotinylated and incubated with FceRIα secretion trap to allow capture of secreted IgE antibody and stained with an antibody mix containing fluorochrome-labeled anti-IgE along with barcoded antigens (Der p1, Der p2, Der f1, and Ole e1) for detection of antigen-specific IgE PCs. Labeled IgE PCs were sorted and single-cell sequencing performed to determine gene expression, VH:VL sequences, and antigen specificity. Monoclonal IgE antibodies were generated from selected clones and tested for antigen binding by ELISA and for functionality *in vivo* using passive cutaneous anaphylaxis assay.

(B) Flow plots of BM and dLN from mice exposed to HDM. PCs were gated as single cells, live, DUMP^{-}, and CD138$^{+/-}$Blimp-1^{+} cells (DUMP gate included CD3, CD11b, IgD, IgM, CD49b, and Ly6g cells). IgE-secreting PCs were sorted as IgE^{-}Venus^{+} cells.

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respectively (Table S4). Two other clonotypes, denoted 1330 and 576, were single-cell clones from individual IgE cells (Table S4). Interestingly, clonotype 21 contained 14 IgG cells and a single IgE (Table S4). Closer inspection of the CDR3 nucleotide sequence of clonotype 21 revealed that the lone IgE sequence contains 3-point mutations within the CDR3 region (red box), while all IgG members of clonotype 21 carry an identical CDR3 region (Figure 5E). Additionally, the IgE sequence harbored somatic hypermutations in the upstream V gene locus (Figure 5E) compared with IgG clonotype members, suggesting additional rounds of affinity maturation.

After selecting the IgE clones, we tested binding of the purified IgEs and a control (αDNP-IgE) to relevant allergens to which the donor was allergic to (Fel d1 for cats, Der p1 for dust, or Can e1 for dog) by ELISA (Figure 5F). One of the IgE mAbs purified bound to Fel d1 but not to Can e1 or Der p1 (Figure 5F). Taken together, these findings suggest that human Ag^+ IgE antibodies could successfully be isolated from allergic BM using IgE TRAPnSeq.

DISCUSSION

PCs are antibody factories that play an important role in maintaining serological memory to infection or vaccination as well as a pathogenic role in allergy and autoimmunity. Despite their importance, the Ag^+ PC repertoire has been poorly studied due to the limitations of current platforms. In contrast, significant advances have been made over the past few years for isolation of therapeutic antibodies from Ag^+ B cells.[21,22] These include high-throughput Ag^+ single B cell sorting (BST) strategies as well as sequencing-based platforms that map BCR sequences to antigen specificity using barcoded antigens (LIBRAseq[22]). Isolation of Ag^+ ASCs, on the other hand, has mainly been restricted to sorting Ag^+ PBs[23] and bulk sorting and RNA-seq of total PCs. Elegant affinity matrix-based methods have been described that allow capture of secreted antibodies on the surface of PCs,[2,24] but these methods are not coupled with high-throughput sequencing of PCs with different specificities, which limits the analysis of clonal diversity and transcriptional signature of antigen- or allergen-specific ASCs. On the other hand, single-cell microfluidic-based platforms have recently been described that allow screening of antibodies from ASCs,[25,26] but these methods rely on custom microfluidics setup and require re-capture of droplets to pair antibody specificity to PC profiling. To overcome these limitations, we designed a method that we called TRAPnSeq, which allows local capture of antibodies on the surface of the ASCs that produce them. We show that this method could easily be integrated with existing B cell profiling platforms for both mice and human PCs and allows high-throughput isolation and characterization of Ag^+ ASCs, thereby pairing secreted antibodies to the ASCs from which they are secreted.

In addition to antibody discovery from PCs, TRAPnSeq also allowed analysis of the putative Ag^+ PC repertoire and transcriptional profile in a high-throughput manner that has previously been challenging. Interestingly, when we compared hIL-4Rα-specific BMPCs with Ag^+ B cells in secondary lymphoid organs, we observed multiple clonally related sequences in these two compartments. Given the enormity of the possible combinations of BCR sequences in mice, this overlap is remarkable and potentially suggests differentiation and migration of hIL-4Rα-specific B cell clones in Sp/dLN to BM. It also highlights the utility of TRAPnSeq to capture and profile potentially rare, but highly relevant, Ag^+ cells. In addition, transcriptional profiling of hIL-4Rα-specific BMPCs showed that Ag^+ PCs have a distinct gene signature and increased somatic hypermutation compared with non-Ag^+ PCs. This suggests that the isolated Ag^+ PCs from the BM are likely selected from a pool of GC or memory B cells that have undergone multiple rounds of affinity maturation compared with non-Ag^+ PCs. Further studies using TRAPnSeq and a larger antigen panel and different immunization protocols are needed to confirm if these findings are applicable to broader Ag^+ PC subsets.

In contrast to other abundant isotypes that express surface BCR, such as IgA, IgE PCs have been notably difficult to study due to their scarcity and lack of known surface markers. Studies using short-term immunization models in mice have shown that short-lived IgE PBs/PCs in secondary lymphoid organs maintain IgE BCR expression,[27] whereas the presence of IgE BCRs on BMPCs in mice or humans has not been clearly demonstrated. To date, isolation of Ag^+ IgE has mainly been limited to sorting IgE^+ PBs from peripheral blood.[12] Using IgE TRAPnSeq, we profiled ~1,700 IgE PCs and demonstrated that Ag^+ IgE PCs can be isolated from tissues (dLN and BM) of chronic (15 weeks) HDM-exposed mice[10] as well as from the BM of allergic donors. IgEs isolated by TRAPnSeq from HDM-exposed mice were specific to the different HDM allergens (Der p1, Der p2, Der f1) and induced crosslinking of Ag^+ IgE bound to FcεRIα on mast cells in a PCA assay. Interestingly, one of the IgEs isolated from dLN using this method bound to Der p1, Der p2, Der f1, and the olive allergen Ole e1, suggesting that there could be a common epitope shared across these allergens. Notably, this IgE failed to bind to the cat allergen Fel d1, which demonstrated that it is not a widely cross-reactive IgE that is non-specifically recognizing allergens. We also showed that IgE TRAPnSeq could be utilized for profiling IgE PCs in allergic individuals, highlighting the interesting biology of IgE PCs, including shared BCR sequences with IgG PCs.

Successful implementation of the TRAPnSeq in the context of ASCs opens the door for novel secretion trap designs for virtually

(C) UMAP of IgE^+ PCs based on VDJ-BCR expression in red over non-IgE PCs in gray.
(D) IgE, IgG, IgA, and IgM PC average scaled gene expression of the top 4 differentially expressed genes from both dLN and BM.
(E) Visualization of antigen specificity based on barcode expression of Der p1, Der p2, and Der f1 vs. the negative control Ole e1.
(F) Antigen specificity validated by ELISA for a subset of IgE antibodies generated from IgE-secreting cells. Representative binding curves of IgE antibodies to Der p1 (top left), Der p2 (top right), Der f1 (bottom left) and to the negative control antigen Ole e1 (bottom right).
(G) PCA (mast cell degranulation) was assayed by intradermal injection of IgE mAb 3_1 and IgE mAb 12_2 for Der p1 and IgE mAb 8_2 and IgE mAb 21_2 for Der p2. After 24 h, the mice were challenged intravenously with Der p1 or Der p2 diluted in 0.5% Evans blue dye. Evans blue dye was extracted from ear tissue and measured spectrophotometrically. Plot shows Evans blue dye extravasation in the tissue quantified as nanograms of Evans blue per milligram of tissue as a measure of local mast cell degranulation. Each mouse is indicated in the plot with a square symbol.
See also Table S3.

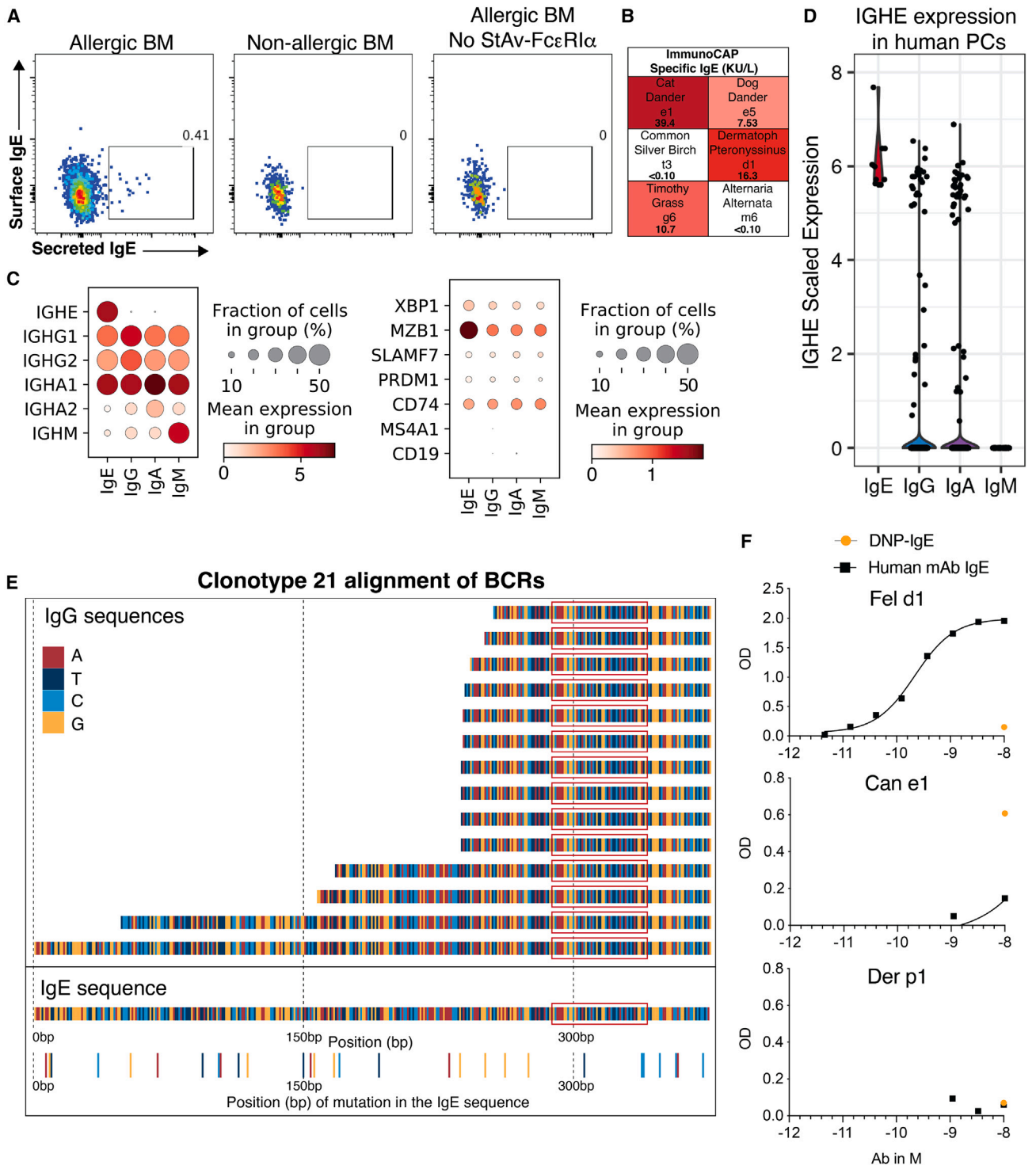

Figure 5. IgE TRAPnSeq can be used to identify and profile IgE PCs from the BM of allergic donors

(A) Flow plots of BMPCs from allergic and non-allergic BM donors. PCs were gated as single cells, live, DUMP^{-}, and CD20^{-}CD38^{++} cells (DUMP includes CD3, CD14, CD16, CD123, IgD, and IgM cells). IgE-secreting PCs were gated as surface IgE^{-} and secreted IgE^{+}. Left plot: BMPCs from an allergic donor with complete IgE secretion trap. Middle plot: Non-allergic BM control donor with complete IgE secretion trap.Right plot: BMPCs from an allergic donor without IgE secretion trap.

(B) ImmunoCap performed on serum of an allergic donor to detect levels of IgE specific to cat dander e1, dog dander e5, common silver birch t3, dermatoph pteronyssinus d1, timothy g6, and alternaria alternata m6.

(legend continued on next page)

any cell whose function involves secreting protein and can serve as a platform for "single-cell secretomics". For example, a secretion trap could be designed to capture interleukin-4 (IL-4)-producing macrophages in the context of an inflammatory response. This is especially helpful to isolate cells with a specific effector function and their effector molecule, here IL-4. The applications for TRAPnSeq complement multimodal single-cell sequencing to provide another layer of information for gene products that undergo extensive regulation between transcription, translation, modification, and eventual secretion.

Overall, we showed that TRAPnSeq is a versatile method that integrates single-cell sequencing from functionally secreting cells and links that to antigen specificity. Along with existing B cell platforms, this method helps facilitate antibody discovery from ASCs and deeper understanding of Ag^+ PC biology.

Limitations of the study

One potential caveat with TRAPnSeq technology is that it may preferentially select cells that secrete larger quantities of antibodies, and high levels of antibody secretion may not necessarily correlate with high affinity. A comparison of affinity from BMPCs that captured high vs. low amounts of antibody would shed more light if this is indeed an issue. Larger-scale antibody purification could also help determine if some of the genes expressed by high-affinity antibody-producing PCs could be used as a general marker to enrich PCs of interest for therapeutic antibody isolation.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

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SUPPLEMENTAL INFORMATION

Supplemental information can be found online at https://doi.org/10.1016/j.crmeth.2023.100522.

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AUTHOR CONTRIBUTIONS

Conceptualization, S.A., J.C.D., A.V., B.K., and I.S.; methodology, S.A., J.C.D., A.V., B.K., D.S., A.R., C.A., and S.P.; formal analysis, J.C.D., I.S., and W.K.L.; writing – original draft, S.A., J.C.D., A.V., and B.K.; writing – review & editing, A.L., J.M.O., G.S.A., and M.A.S.; supervision, A.L., G.S.A., A.J.M., M.A.S., W.K.L., and J.M.O.

DECLARATION OF INTERESTS

This study was sponsored by Regeneron Pharmaceuticals, Inc. All authors are current or former employees of Regeneron and may hold stock options in the company. S.A., J.C.D., A.V., B.K., I.S., G.S.A., M.A.S., W.K.L., and J.M.O. are inventors on a pending US patent application on TRAPnSeq ("Methods of Mapping Antigen Specificity to Antibody-Secreting Cells"). A.J.M. is an inventor on a pending US patent application (#16/363,774; "Humanized Rodents for Testing Therapeutic Agents").

INCLUSION AND DIVERSITY

One or more of the authors of this paper self-identifies as an underrepresented ethnic minority in their field of research or within their geographical location. One or more of the authors of this paper self-identifies as a gender minority in their field of research. One or more of the authors of this paper self-identifies as a member of the LGBTQIA+ community.

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(C) Expression dot plot shows scaled expression of Ig genes (left) and PC and B cell markers (right) within IgE, IgG, IgA, and IgM BMPCs.
(D) Graph shows scaled expression of *IGHE* transcript in IgE, IgG, IgA, and IgM BMPCs.
(E) Alignment plot shows comparison between IgE sequence from an IgE PC with IgG sequences in clonotype 21. Colored lines below indicate mutations where the IgE PC sequence differs from the IgG consensus.
(F) Antigen specificity tested by ELISA for one human IgE antibody isolated by IgE TRAPnSeq from allergic BM. Dose-depedent binding of this human IgE antibody was observed to Fel d1 (top), but not Can e1 (middle)or Der p1 (bottom). αDNP-IgE was included as a negative control (yellow dot).
See also Table S4.

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STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Anti-mouse Fc Block (CD16/32)	TONGO biosciences	Clone 2.4G2; Cat: 70-0161-M001
Anti-mouse B220	BD Biosciences	Clone# RA3-6B2; Cat# 563793; RRID: AB_2738427
Anti-mouse CD138	BD Biosciences	Clone# 281-2; Cat# 563193; RRID: AB_2631190
Anti-mouse IgG1	BD Biosciences	Clone# A85-1; Cat# 740121; RRID: AB_2739879
Anti-mouse IgE	BD Biosciences	Clone# R35-72; Cat# 564207 and 744281; RRID: AB_2738668 and AB_2742118
Anti-mouse IgM	Invitrogen	Clone# II/41; Cat# 47-5790-82; RRID: AB_2573984
Anti-mouse IgD	BD Biosciences	Clone# 11-26c.2a; Cat# 565348; RRID: AB_2739201
Anti-mouse TCRβ	BioLegend	Clone# H57-597; Cat# 109220; RRID: AB_893624
Anti-mouse Ly6G	BioLegend	Clone# 1A8-Ly6g; Cat# 127624; RRID: AB_10640819
Anti-mouse CD49b	BioLegend	Clone# DX5; Cat# 108920; RRID: AB_2561458
Anti-mouse CD11b	BioLegend	Clone# M1/70; Cat# 101226; RRID: AB_830642
Anti-human CD20	BD Biosciences	Clone# 2H7; Cat# 560631; RRID: AB_1727447
Anti-human CD38	BD Biosciences	Clone# HB7; Cat# 562666; RRID: AB_2313578
Anti-human IgE	BD Biosciences	Clone# G7-26; Cat# 566324; RRID: AB_2744487
Anti-human IgD	BD Biosciences	Clone# IA6-2; Cat# 555778; RRID: AB_396113
Anti-human IgM	BioLegend	Clone# MHM88; Cat# 314534; RRID: AB_2566487
Anti-human CD3	BD Biosciences	Clone# UCHT1; Cat# 557694; RRID: AB_396803
Anti-human CD11b	BD Biosciences	Clone# ICRF44; Cat# 562793; RRID: AB_2737798
Anti-human CD14	BioLegend	Clone# M5E2; Cat# 301804; RRID: AB_314186
Anti-human CD16	BioLegend	Clone# 3G8; Cat# 302006; RRID: AB_314206
Anti-human CD123	BD Biosciences	Clone# 7G3; Cat# 558663; RRID: AB_1645485
Goat anti-human IgG	Jackson ImmunoResearch	Polyclonal; Cat# 109-005-098; RRID: AB_2337541
Goat anti-human IgG-HRP	Jackson ImmunoResearch	Polyclonal; Cat# 109-035-098; RRID: AB_2337586
Biological samples		
Human allergic bone marrow	BioIVT	Cat# HMN97138

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Human allergic bone marrow	STEMCELL	Cat# CE0006823
Human non-allergic bone marrow	BioIVT	Cat# HMN97258
Chemicals, peptides, and recombinant proteins		
House Dust Mite extract (HDM)	Greer	Cat# XPB70D3A25
Der p1	IndoorBiotech	Cat# NA-DP1-1
Der p2	IndoorBiotech	Cat# NA-DP2-1
Der f1	IndoorBiotech	Cat# NA-DF1-1
Ole e1	IndoorBiotech	Cat# RP-OE1-1
DAPI	ThermoFisher	Cat# 62247
Human IL-4Rα	Regeneron	N/A
Critical commercial assays		
Mouse IgE ELISA Set	BD Biosciences, OptEIA™	Cat# 555248
EasySep Human Pan-B cell Isolation Kit	StemCell	Cat# 19554
EasySep Mouse Pan-B cell Isolation Kit	StemCell	Cat# 19844A
Streptavidin Conjugation Kit - Lightning-Link	ABCAM	Cat# ab102921
Biotin Conjugation Kit - Lightning-Link	ABCAM	Cat# Ab201795
Deposited data		
scRNA-seq data	This paper	GEO: GSE233507
Experimental models: Cell lines (should be complete)		
U266 cells	Sigma Aldrich	Cat# 85051003-1VL
Ramos cells	ATCC	CRL1923
ARH77 cells	ATCC	CRL1621
Experimental models: Organisms/strains (should be complete)		
IgE^{venus} $Blimp1^{mCherry}$ reporter mice	Regeneron[10]	N/A
VelocImmune® mice	Regeneron[15,16]	N/A
Oligonucleotides		
TotalSeq C0956	BioLegend	Cat# 405283
TotalSeq C0957	BioLegend	Cat# 405285
TotalSeq C0958	BioLegend	Cat# 405293
TotalSeq C0959	BioLegend	Cat# 405159
Chromium® Next GEM Single Cell 5′ Kit, v.2	10x Genomics	Cat# 1000265
Recombinant DNA		
Human IgG4 expression vector	Regeneron	N/A
Human IgK expression vector	Regeneron	N/A
Software and algorithms		
FlowJo v10	FlowJo, LLC	https://www.flowjo.com/
GraphPad Prism 8	GraphPad software, LLC	https://www.graphpad.com/
Python	Python Software Foundation	https://www.python.org/downloads/release/python-360/
R	The R Project for Statistical Computing	https://www.r-project.org/
Other		
Microtainer tubes	BD Biosciences	Cat# 365967
Saline	Sigma	Cat# S8776
BSA	Sigma	Cat# A9576
Normal Rat Serum	StemCell	Cat# 13551
Ficoll	GE Healthcare	Cat# 17-1440-03

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Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
NHS-Biotin	Sigma	Cat# 20311
ELISA Plate	Thermo Scientific	Cat# 80040LE
Carbonate-Biocarbonate coating buffer	Sigma	Cat# C3041-100
100mL Distilled water	Gibco	Cat# 15230-170
Tween™ 20	Sigma	Cat# P1379
Assay Diluent	BD Biosciences, OptEIA™	Cat# 555213
DPBS	Gibco	Cat# 14190
TMB Substrate	BD Biosciences, OptEIA™	Cat# 555214
Stop Solution	BDH VWR ANALYTICAL	Cat# BDH7500-1

RESOURCE AVAILABILITY

Lead contact

Further information and requests for resources and reagents should be directed to and will be fulfilled by the lead contact, Jamie Orengo (jamie.orengo@regeneron.com)

Materials availability

All unique/stable reagents generated in this study are available from the lead contact with a completed Materials Transfer Agreement.

Data and code availability

- Single-cell RNA-seq data have been deposited at GEO under the accession number GSE233507 and is publicly available as of the date of publication.
- Single cell data was processed by standard pipelines and according to the scanpy recommended workflow. No original code was generated.
- Any additional information required to reanalyze the data reported in this paper is available from the lead contact upon request.

EXPERIMENTAL MODEL AND STUDY PARTICIPANT DETAILS

All animal procedures were conducted in compliance with protocols approved by the Institutional Animal Care and Use Committee of Regeneron Pharmaceuticals. All mice were bred in specific pathogen-free conditions. For IgG experiments, 8+ week old male and female VI3/VelocImmune[15,16] mice were used. VI3/VelocImmune[15,16] mice were on a mixed Balb/c C57BL/6 129S6 background and referred to here as VI3 or VelocImmune mice.

For IgE experiments, 8+ week female IgE^{Venus} Blimp-$1^{mCherry}$ mice generated in a hybrid 129S6/C57BL/6 background were used.[10]

Human bone marrow was obtained from allergic and non-allergic donors from BioIVT and STEMCELL using protocols approved by the Institutional Review Board (IRB). BM MNCs were isolated from 37-year-old, male, donor CE0006823 (STEMCELL), self-reported as allergic to Penicillin; from 24-year-old, female, donor HMN97138 (BioIVT), self-reported as allergic to Cat; from 19-year-old, female, donor HMN97258 (BioIVT), self-reported as non-allergic.

For validating human IgE and IgG experiments, U266 cells, ARH77 cells and Ramos cells were used. U266 cells are derived from B lymphocytes isolated from the peripheral blood of a 53-year-old with myeloma and were purchased from Sigma Aldrich. ARH77 cells were derived from B lymphoblasts isolated from the peripheral blood of a 33-year-old with plasma cell leukemia and were purchased from ATCC. Ramos cells were derived from a 3-year-old with Burkitt's Lymphoma and were purchased from ATCC.

METHOD DETAILS

Secretion trap assembly

For secretion trap assembly, cells were washed with PBS/5% BSA and surface Fc was blocked with 1:10 diluted Fc Block (eBioscience) along with detection of surface BCR (for cell line assays) for 15 min at 4°C. Cells were washed 2x with PBS, spun down at 500g for 10 min, resuspended in 1mL of freshly prepared NHS-biotin (Sigma, 0.5 mg/mL in PBS) and incubated at 37°C for 15 min. Cells were washed 3x in cold PBS/5%BSA while changing tubes for every wash step, and incubated with trap reagent (StAv-ectoFceRIα or StAv-αIgk) that was prepared separately (0.03 mg/mL) for 1 h at 37°C. Following secretion trap assembly, cells were washed and stained with antibody mix for detection of secreted IgE or IgG along with a cocktail of barcoded antigens, in PBS/5%BSA for 30 min at 4°C. Cells were washed in MACS buffer, fixed with Cytofix (BD), and acquired on a flow cytometer.

In vivo setup and secretion trap workflow

VI3/VelocImmune mice were immunized via footpad with hIL-4Rα antigen. Mice were immunized once a week for five weeks, rested for one month, then boosted four days prior to takedown. Single cell suspensions of bone marrow, spleen, and lymph nodes were generated, and RBC lysis was performed. Spleen and lymph nodes were pooled, and surface Fc was blocked with 1:10 diluted Fc Block (BD Biosciences) for 15 min at +4°C. Spleen and lymph nodes were then stained using an antibody cocktail to gate for Ag^+/IgG^+ B cells. For bone marrow, B cells and plasma cells were isolated using EasySep mouse pan-B cell isolation kit (STEMCELL) by negative selection to remove unwanted cells (CD4, CD8, CD11c, CD49b, CD90.2, Ly-6C/G (Gr-1), TER119). For the purified cells, after surface Fc was blocked as described above, cells were coated with NHS-biotin as detailed in the previous section. Plasma cells were then incubated with StAv-αIgk for 1 h at 37°C with shaking at 400rpm. Following secretion trap, cells were washed and stained with an antibody mix containing surface markers to gate on plasma cells, anti-IgG FITC (BD Biosciences and Southern Biotech) for detection of secreted IgG, and barcoded hIL-4Rα PE in PBS/5% BSA for 30 min at +4°C. Cells were sorted on a BD Symphony S6.

For IgE TRAPnSeq, IgE^{Venus} $Blimp1^{mCherry}$ mice were exposed to 50 μg of HDM extract (Greer) diluted in 20 μL of saline solution intranasally three times per week for 15 weeks. At the end of the experiment, bone marrow and lung draining lymph nodes were collected and processed. Draining lymph nodes were mashed on 12-well, 70-μm filter plates (Corning Costar) in RPMI 1640 media to generate single cell suspensions. For BM extraction, femurs were cut at both ends, placed in a PCR plate with holes punched at the bottom, and spun down for 3 min at 500g. RBC lysis was performed on single cell suspensions from draining lymph nodes and bone marrow. B cells and plasma cells were isolated using EasySep mouse pan-B cell isolation kit (STEMCELL, cat: 19844) by negative selection to remove unwanted cells (CD4, CD8, CD11c, CD49b, CD90.2, Ly-6C/G (Gr-1), TER119). Purified cells were processed and secretion trap was assembled as described in the previous paragraph. Secreted IgE was captured using StAv-ectoFceRIα for 1 h at 37°C with shaking at 400 rpm. Following IgE secretion trap, cells were washed and stained with an antibody mix containing B220 (RA3-6B2), CD138 (281-2), IgG1 (A85-1), IgE (R35-72), DUMP (IgM (II/41), IgD (11-26c.2a), TCRβ (H57-597), Ly6G (1A8-Ly6g), CD49b (DX5), and CD11b (M1/70)) along with barcoded antigens (Der p1, Der p2, Der f1, and Ole e1). Finally, cells were labeled with DAPI before they were sorted on a BD Symphony S6. Mouse Antibodies used for flow cytometry are reported in Table S2).

Antigens

Antigens for HDM experiment were purchased from Indoor Biotechnology. Der p1 (NA-DP1-1, lot: 44098), Der p2 (NA-DP2-1, lot: 41384), Der f1 (NA-DF1-1, lot: 44001), and Ole e1 (RP-OE1-1, lot: 43138). Antigens for IL-4Rα experiment were made in-house (at Regeneron).

Biotin and streptavidin conjugation of capture reagents

Biotin Conjugation Kit, Lightning-Link (Abcam, cat: ab201795) was used to conjugate antigens to biotin following manufacturer's instructions. α-Igk and FceRIα ectodomain were conjugated to Streptavidin using Streptavidin Conjugation Kit - Lightning-Link (Abcam, cat: ab102921) following manufacturer's instructions.

Fluorescent and barcode labeling of antigens

TotalSeq streptavidin reagents (from Biolegend) with 15bp barcodes and fluorescent tags were used to label biotinylated antigens. The following antigen barcodes were used: GGTAACTCTGGTAGC (Der p1), AGCGTATGTAACTCG (Der p2), TGTGTGAGGCTTGAT (Der f1) ACTCTCACCCATTCC (Ole e1), AACCTTTGCCACTGC (hIL-4Rα) and GTCCGACTAATAGCT (hIL-6Rα).

Antibody expression and purification

All IgE antibodies were synthesized by GenScript. For IgG antibodies, variable regions were cloned into in-house constructs expressing hIgG4 and hIgK constants.

IgG mAbs were expressed in Expi293F mammalian cells (ThermoFisher) by co-transfecting heavy and light chain expression plasmids using the Expi293 Expression System Kit (ThermoFisher). Transfected Expi293F cells were cultured for 5–7 days in serum-free Expi293 Expression Media at 37°C with 8% CO_2 saturation and shaking at 1,500rpm. Cell cultures were then centrifuged at 2000rpm for 5min, clarified using a 0.45μM filter plate, and the resultant supernatants were used for ELISAs (hIL-4Rα and total IgG) and initial Biacore analysis. All confirmed Ag^+ sequences were then transfected for large-scale purification using Expi293F cells. IgG sequences were transfected into Expi293F cells, cultured for 5–7 days in serum-free Expi293 Expression Media at 37°C with 8% CO_2 saturation, then purified using Protein A agarose resin columns. Antibodies were eluted with 100mM Glycine HCl at pH 2.7 directly into a 1:10 volume of 1M Tris-HCl pH 8. Eluted antibodies were buffer exchanged into PBS three times using 10kDa Amicon Ultra centrifugal filter units.

Surface plasmon resonance

All IgG antibodies were assessed for their binding affinity to hIL-4Rα at 25°C and pH 7.4 using Biacore 8K+ (GE Healthcare). Each fully human IgG/IgK antibody was captured using an anti-human Fc (internal antibody) immobilized CM5 sensor chip. Different concentrations (50nM–0.78nM, 4-fold dilutions) of hIL-4Rα were then injected over the flow cell at 30uL/min for 2.5 min followed by a dissociation of 20 min. Binding kinetics parameters were measured by fitting the real time data using 1:1 binding model and using Biacore Insight evaluation.

IgE ELISA

To test antigen-specificity, FceRIα-ecto domain was plated at 2ug/mL overnight at 4°C. The next day, plates were washed three times with PBS supplemented with 0.05% Tween 20 (PBS-T) and coated with (0.5% BSA in PBS) for at least 1 h at room temperature and then washed three times with PBS-T. Primary IgE antibodies were diluted in PBS, starting at 2ug/mL with a serial 1:3 dilution and then added to the plate. The plates were incubated at room temperature for 1 h and washed three times with PBS-T. Then, biotinylated Der p1, Der p2, Der f1 and Ole e1 at 2ug/ml were added to the plate and incubated for 1 h at room temperature. Plates were washed three times with PBS-T and then developed by adding TMB substrate. The plates were incubated at room temperature for 20 min, and then 1 N sulfuric acid was added to stop the reaction. Plates were read at 450 nm.

Passive cutaneous anaphylaxis

PCA was performed by sensitizing ears of naive mice with 10-μL intradermal injection of IgE antibody cocktail containing 25 ng of total IgE (cocktail was made with 2 types of IgE antibodies, with 12.5 ng each). After 24 h, the mice were challenged by intravenous injection of 1 μg of Der p1 or Der p2 (Indoor Biotechnologies) diluted in 0.5% Evans blue dye (Sigma-Aldrich). One hour after allergen challenge, mice were euthanized, and Evans blue dye was extracted from ear tissue and spectrophotometrically quantitated using a standard curve.

Human bone marrow samples

Frozen human allergic and non-allergic BM mononuclear cells (BM MNCs) and matching donor sera were obtained from BioIVT. The allergy status of each donor sera was checked by ImmunoCAP (Phadia, Thermo Fisher Scientific) following the manufacturer's instructions. Frozen BM MNCs were carefully thawed in complete RPMI media (with Penicillin-Streptomycin, Glutamine, 2-Mercaptoethanol, 10% Fetal Bovine Serum and Dnase I), washed 1x and rested at 37 for 1h. To obtain mononuclear cells from fresh allergic BM from STEMCELL, ∼80-120mL of BM aspirate was diluted 7:1 with PBS and 2mM EDTA and loaded onto Ficoll in SepMate tubes (STEMCELL). Samples were spun in SepMate tubes at 1200g for 20 min. The top layer of cells was removed, and cells were washed twice with autoMacs rinsing solution at 300g for 8 min. B cells and antibody secreting cells were isolated using magnetic bead depletion from single cell suspensions of allergic bone marrow cells according to manufacturer's protocol. Human Pan-B cells were blocked using Fc Block (BD) for 15 to 30 min at 4°C, followed by incubation with antibody mix for 30 min at 4°C in PBS with 5% BSA. Antibodies mix included CD20 (2H7), CD38 (HB7), IgE (G7-26), IgD (IA6-2), IgM (MHM88), CD3 (UCHT1), CD11b (ICRF44), CD14 (M5E2), CD16 (3G8), and CD123 (7G3). Finally, cells were labeled with DAPI before they were sorted using BD Symphony. Antibodies used for flow cytometry are reported in Table S2.

Single cell library preparation and sequencing

Cells captured by secretion trap were suspended in PBS with 0.04% w/v BSA and loaded onto a Chromium Controller device (10x Genomics) at 15,000 cells per lane. Partitions of the cells in barcoded beads were formed and the cells lysed. Reverse transcription was performed followed by breakdown of the partition. RNA-seq, Feature Barcode, and V(D)J libraries were prepared using a Chromium Next GEM Single Cell 5′ Kit, v.2 (10x Genomics). After amplification of the libraries, cDNA was split into separate RNA-seq, Feature Barcode, and V(D)J aliquots. To enrich the V(D)J aliquot for BCR sequences, the Chromium Automated Single Cell Mouse and Human BCR Amplification & Library Construction Kit (10x Genomics) was used. Feature Barcode libraries for DNA-barcoded antigens or DNA-barcoded cell surface proteins were prepared using a Chromium 5′ Feature Barcode Library Construction kit (10x Genomics). Paired-end sequencing was performed on an Illumina NovaSeq 6000 sequencing system for RNA-seq, feature barcode, and V(D)J libraries. For RNA-seq and Feature Barcode libraries, the Cell Ranger Single-Cell Software Suite, version 6.1.1 (10x Genomics) was used to perform sample de-multiplexing, alignment, filtering, and UMI counting. The human GRCh38 and mouse mm10 genome assembly and RefSeq gene model for humans and mice, respectively, were used for alignment. For V(D)J libraries, the Cell Ranger software was used to perform sample de-multiplexing, to *de novo* assemble read pairs into contigs, to align, and to annotate contigs against all V(D)J germline reference sequences from the germline mouse and human IMGT reference database.

Single cell data preprocessing and analysis pipeline

The single cell-count matrix was filtered by calculating the ratio of the total number of annotated genes divided by the log10 of the total number of UMIs counted. Cells with a gene to UMI ratio below about 0.1 were filtered out. Cells with more than four times the interquartile range of the total number of UMIs were filtered out. Cells with more than about 80% of reads mapped to a mitochondrial gene were filtered out. High quality cells with a gene to UMI ratio above about 0.1, with less than about four times the interquartile range of the total number of UMIs counted, and with less than 80% of reads mapping to a mitochondrial gene were retained. The count matrices from each individual sample capture were normalized to a total count of 10,000 and batch corrected using the Harmony algorithm to generate a combined uniform manifold approximation and projection (UMAP). Unsupervised clustering by the Leiden algorithm was then used to determine cell type clusters and a Wilcox test was used to identify specific cell type and cluster marker genes. Single cell data was processed using scanpy v1.9 and according to recommended documentation (https://scanpy-tutorials.readthedocs.io/en/latest/pbmc3k.html).

Secretion trap barcoded antigen sequencing and quantification

The barcoded antigen library was mapped by the CellRanger software to a custom short-read reference that contained the DNA tag sequences of the barcoded antigens, namely, hIL-4Rα, hIL-6Rα, Der p1, Der p2, Der f1 and Ole e1 for IgG and IgE secretion trap experiments. These tag sequences were quantified across all cells and were normalized by taking the centered log-ratio (CLR) per barcoded antigen across each sample capture. In addition, background antigen signal for each barcoded antigen was removed by DSB (Denoised and Scaled by Background). Both the CLR and the DSB normalized values were used to quantify the target antigen signals.

Antigen specificity score (*AgSS*) for IgG were calculated by subtracting the quantile of hIL-6Rα barcoded UMI counts multiplied by a penalty factor (x) from the quantile of hIL-4Rα barcoded UMI counts, $AgSS = Q_{hIL4Ra} - Q_{hIL6Ra}{}^{x}$. *AgSS* for IgE were calculated with a similar formula for mouse and human single cell data. For IgE scores the target antigens were either Der p1, Der p2 or Der f1 and the control was always Ole e1.

BCR clustering

Ig BCR sequences were clustered using scirpy v0.10 using the function scirpy.pp.ir_dist based on the amino acid sequence and with a hamming distance of less than 4. Clustered BCRs were collected into clonotypes with the function scirpy.tl.define_clonotypes based on the heavy chain CDR3 amino acid sequence based on the previous criteria of a hamming distance of less than 4.

Scirpy documentation can be referenced at https://scverse.org/scirpy/latest/api.html

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical and graphical analyses were performed using GraphPad Prism software (version 9.0). Normality was determined by Shapiro-Wilk normality test. One-way analysis of variance (ANOVA) or unpaired Student's *t* test was used on normally distributed samples, and Mann-Whitney or Kruskal-Wallis tests were performed on samples that did not pass the normality test. two-way ANOVA was used on experiments that had two independent variables. Results were considered statistically significant at $p < 0.05$. The number of animals used in each experiment is outlined in figure legends.