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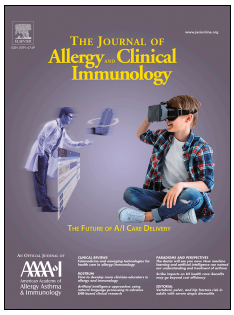

Targeting IgE polyadenylation signal with antisense oligonucleotides decreases IgE secretion and plasma cell viability

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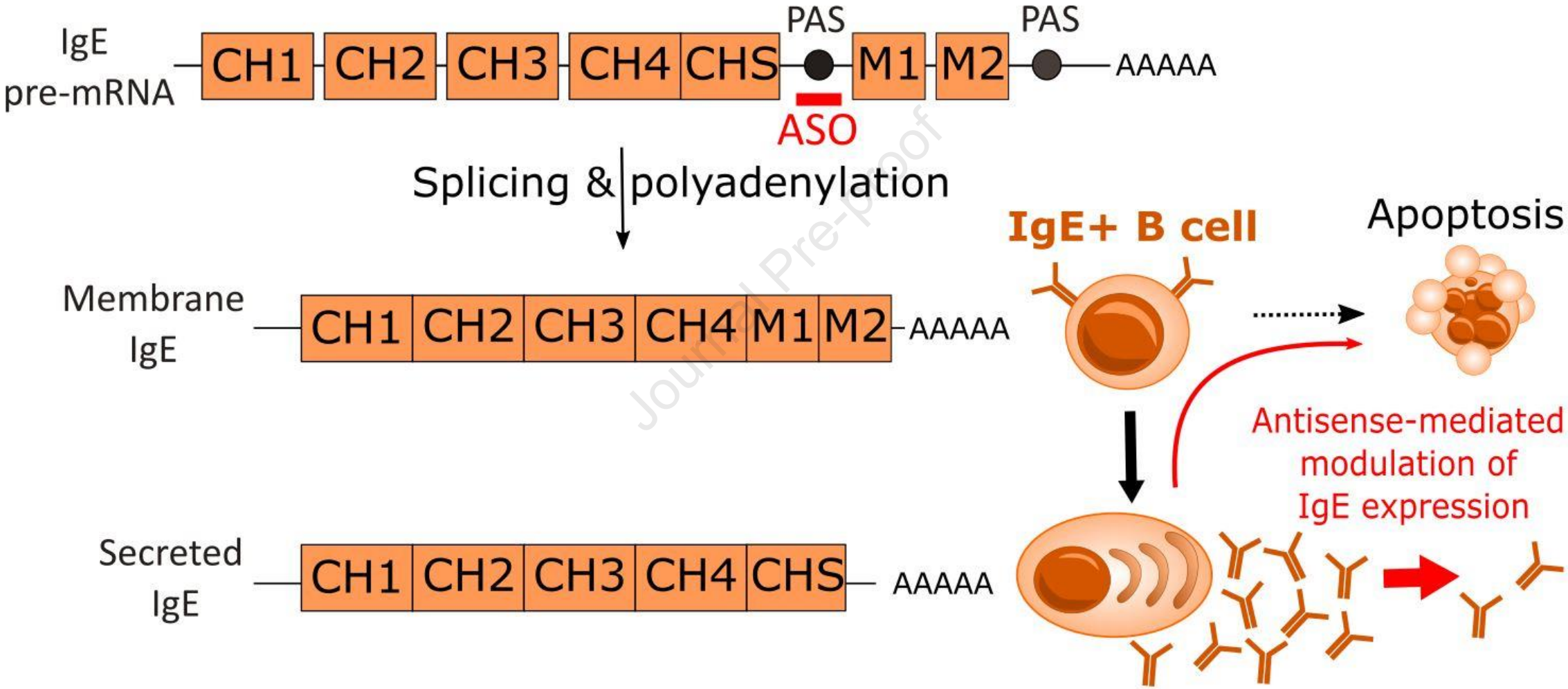
Targeting IgE PAS with ASO decreases IgE secretion and plasma cell viability



ASO : Antisense Oligonucleotide **PAS** : Polyadenylation Signal

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Targeting IgE polyadenylation signal with antisense oligonucleotides decreases IgE secretion and plasma cell viability

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AUTHORS CONTRIBUTION

Anne Marchalot designed and performed experiments, analysed data and wrote the manuscript. Catherine Horiot, Jean-Marie Lambert, Claire Carrion and Christelle Oblet performed experiments and analysed data. Justine Pollet analysed bioinformatics data. Michel Cogné contributed to the initial conception of the project. Jeanne Moreau and Brice Laffleur reviewed the manuscript. Laurent Delpy conceived the project, designed experiments and wrote the manuscript.

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24 **CONFLICT OF INTEREST**

25 The authors declare no conflict of interest.

26 **ABSTRACT**

27 **Background**: Allergy regroups numerous complex and various diseases classified as IgE-dependent or
28 non-dependent hypersensitivities. IgEs are expressed as membrane and secreted forms by B cells and
29 plasma cells, respectively. In IgE-mediated hypersensitivity, IgE secretion and binding to high-affinity
30 FcεRI on effector cells are responsible for the onset of allergic symptoms but in contrast, surface IgE
31 expression as a B-cell receptor (BCR) is barely detectable.

32 **Objective**: To test an innovative antisense approach to reduce IgE secretion.

33 **Methods**: We designed an antisense oligonucleotide (ASO) targeting the polyadenylation signal (PAS)
34 of human secreted IgE in order to redirect IgE transcript polyadenylation from the secreted form to
35 the membrane form. ASO treatments were performed in B cells from transgenic mice expressing
36 humanized IgE (InEps™), as well as human primary B cells and myeloma cells. *In vivo* ASO delivery was
37 tested using the InEps™ model.

38 **Results**: We demonstrated that treatment with morpholino ASO targeting the secreted IgE PAS
39 drastically decreased IgE secretion and inversely increased membrane-IgE mRNA expression. In
40 addition, ASO treatment induced apoptosis of IgE-expressing U266 myeloma cells, and RNA-seq
41 revealed attenuation of their plasma cell phenotype. Remarkably, systemic administration of ASO
42 coupled to Pip6a as an arginine-rich cell-penetrating peptide decreased IgE secretion *in vivo*.
43 **Conclusion:** Altogether, this ASO strategy could be an effective way to decrease IgE secretion and
44 allergic symptoms in patients suffering from IgE-dependent allergies, and could also promote allergen
45 tolerance through apoptosis of IgE+ antibody-secreting cells.

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47 **KEY MESSAGES**

48 • Targeting the secreted IgE polyadenylation signal (PAS) with an antisense oligonucleotide
49 (ASO) results in a switch from expression of secreted IgE mRNA form to the membrane-
50 associated form
51 • ASO targeting secreted IgE PAS are good strategies to decrease the secretion of allergen-
52 specific IgE
53 • Treatment with ASO targeting secreted IgE PAS provokes apoptosis of IgE-expressing plasma
54 cells
55 • *In vivo* experiments demonstrate the effectiveness of this strategy in humanized mice
56

57 **CAPSULE SUMMARY**

58 This study demonstrates that targeting the secreted IgE polyadenylation signal using antisense
59 oligonucleotides strongly reduces IgE secretion and provokes apoptosis of IgE-expressing plasma cells,
60 opening new therapeutic perspectives in patients suffering from IgE-dependent allergic diseases.

61 **KEYWORDS**

62 Immunoglobulin E, Antisense Oligonucleotide, Polyadenylation, Allergy, B Cells, Plasma Cells, Gene
63 Therapy, Cell Viability

64 **ABBREVIATIONS**

65 **BCR** = B cell receptor

66 **ASO** = Antisense Oligonucleotide

67 **Pip** = peptide nucleic acids/Phosphorodiamidate Morholino Oligomer internalization peptide

68 **PAS** = Polyadenylation Signal

69 **APA** = Alternative Polyadenylation

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70 **TEXT**

71 **INTRODUCTION**

72 Immunoglobulin E (IgE) is a well-characterized actor in Type 1 hypersensitivity reactions playing a
73 crucial role in allergy. Upon immunization with an allergen, B cells and plasma cells secrete specific IgE,
74 which binds to the surface of tissue mast cells and basophils through the high-affinity IgE receptor
75 (FcεRI), producing allergic symptoms (1). Omalizumab, an anti-IgE monoclonal antibody, reduces
76 allergic inflammation by depleting circulating free-IgE and preventing its interaction with FcεRI and the
77 low-affinity receptor CD23/FcεRII. In addition, the absence of IgE binding to FcεRI downregulates its
78 expression on the surface of mast cells and basophils (2). IgE is first produced as a membrane-
79 associated form in B lymphocytes, but those cells expressing surface IgE are extremely rare in humans
80 (3). Consistent with the scarcity of IgE+ memory B cells, previous studies have shown that IgE B cell
81 receptor (BCR) expression leads to rapid differentiation into short lived plasma cells and/or apoptosis,
82 preventing IgE memory B cell formation (4–7).

83 Antisense oligonucleotides (ASO) are short single strands of modified DNA, which can be used to
84 modify RNA maturation processes such as splicing or polyadenylation (8,9). Recently, two splice-
85 switching oligonucleotides obtained marketing authorization: eteplirsen and nusinersen, for the
86 treatment of Duchenne's muscular dystrophy and spinal muscular atrophy respectively, rekindling
87 hope for the implementation of new ASO therapeutic strategies in many diseases (10). ASO can also
88 modulate polyadenylation by targeting polyadenylation signals (PAS) or a donor splice site either to
89 reduce gene expression or to direct alternative polyadenylation (APA) and preferably generate one
90 transcript over the other (11–13). Thereby, ASO remain a viable solution for RNA polyadenylation
91 modulation avoiding DNA modifications that can trigger off-target effects (14,15).

92 Here we address a new therapeutic antisense strategy targeting PAS sequences for secreted IgE to
93 decrease IgE secretion while inducing the expression of membrane-associated IgE mRNA isoforms and
94 apoptosis of IgE-secreting plasma cells.

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95 **RESULTS AND DISCUSSION**

96 To evaluate this strategy, we used a Cre tamoxifen-inducible transgenic mouse model called InEps™
97 where human IgM and IgE constant genes have been inserted between Eμ and mouse IgM constant
98 genes. This model produces human IgE upon *in vitro* hydroxytamoxifen treatment of isolated spleen
99 cells (Fig 1, *A*). We found that IgE targeting ASO treated spleen cells were less likely to secrete IgE and
100 less IgE was detected in supernatants compared to irrelevant control ASO (CTRL) (Fig 1, *B*). In addition,
101 secreted IgE mRNA significantly decreased when membrane IgE mRNA increased with ASO treatment
102 (Fig 1, *C*), with strongly diminished secreted / membrane IgE mRNA ratios (Fig 1, *D*). Consistent with
103 this, the frequency of IgE-expressing cells was reduced by ASO treatment (Fig 1, *E*). We next verified
104 this effect on InEps™ mouse hybridoma cells secreting allergen-specific human IgE and found less IgE
105 in supernatants, decreased secreted IgE mRNA, and increased membrane IgE mRNA in ASO treated
106 cells compared to controls (Fig 1, *F,G*).

107 To characterize the effect of IgE polyadenylation switching following ASO treatment on a complete
108 human background, human IgE-secreting myeloma U266 cells were also treated *in vitro*. Similarly to
109 InEps™ transgenic mouse spleen cells, U266 myeloma cells secreted less IgE in supernatants in the
110 presence of IgE ASO compared to cells cultured with control ASO (Fig 2, *A*). Again, secreted IgE mRNA
111 was strongly reduced whereas the expression of the membrane IgE mRNA isoform was increased (Fig
112 2, *B,C*). These results were confirmed by exon coverage analysis of mRNA sequencing reads from which
113 we observed a decrease in CHS reads and an increase in M1 and M2 reads in ASO treated cells
114 compared to control treated cells (Fig 2, *D*). CHS and M1-M2 exons are only found in secreted IgE
115 mRNA and membrane IgE mRNA respectively. Further analysis of total read counts confirmed the
116 significant modulation of CHS/CH4 and M2/CH4 ratios in ASO conditions compared to controls (Fig 2,
117 *E*). Next, apoptosis was examined in U266 cells treated 24h and 48h with ASO. Early and late apoptotic
118 cells were increased in ASO treated cells compared to controls (Fig 2, *F*). Consistent with this increased

119 apoptosis, higher amounts of cleaved PARP proteins were found in ASO treated U266 cells compared
120 to controls (Fig 2, *G*).

121 RNA sequencing of ASO compared to CTRL treated U266 cells revealed 202 differentially expressed
122 genes (Fig 3, *A* and Supplemental Table E1) among which *IGHE* expression was significantly decreased
123 by 1.7 fold. Pathway analysis performed on this list revealed eight significant pathways related to
124 lymphocyte proliferation and activation along with regulation of immunoglobulin production and
125 interleukin 4 and 13 signalling (Fig 3, *B*). *CD80* and *PD-L2* expression, markers of a subtype of memory
126 B cells (16), were increased by almost 2 fold while *MZB1*, an essential gene for plasma cell
127 differentiation (17), was diminished by 2 fold. This transcriptomic analysis suggests that IgE ASO
128 treatment attenuates the plasma cell phenotype of U266 myeloma cells.

129 To address whether IgE ASO treatment displayed a selective effect on IgE secretion, normal human
130 sorted B cells were cultivated and stimulated for IgG1 and IgE class switching *in vitro*. Comparable to
131 other models, secreted IgE mRNA was decreased and membrane IgE mRNA was increased but none of
132 total unswitched IgM or switched IgG1 mRNA were affected by ASO treatment (Fig 4, *A*). IgM and IgG1
133 membrane and secreted mRNA were also checked to verify that no polyadenylation switching effect
134 occurred on those Ig isotypes, regardless of total mRNA amounts (Fig 4, *B*). In agreement with the low
135 surface expression of IgE BCR compared to IgG1 BCR (7), activated B cells displayed much higher
136 secreted / membrane Ig mRNA ratios for ε heavy chains (HCs) (Fig 4, *C*) than for γ1 (Fig 4, *D*) and μ HCs
137 (Fig 4, *E*) in control conditions, albeit the former ratio was strongly diminished by ASO treatment. Our
138 IgE-targeting ASO is therefore specific for the IgE isotype and will not affect synthesis or
139 polyadenylation of other Ig subclasses, thus maintaining a responsive immune system against
140 pathogens.

141 To evaluate the feasibility of our antisense approach *in vivo*, IgE secretion was assessed after treatment
142 with morpholino ASO harboring different cell-penetrating moieties. We compared ASO conjugated to
143 octa-guanidine dendrimer (*i.e.* compounds used throughout this study) with ASO coupled to Pip6a (18),

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144 as an arginine-rich cell-penetrating peptide. Interestingly, IgE concentrations were decreased to similar
145 extent after treatment with ASO and Pip6a-ASO in U266 cell supernatants (Fig 5, *A*). For *in vivo* delivery
146 assessment, Tamoxifen-treated InEps™ mice received 1 intravenous injection (tail vein) of CTRL, ASO
147 and Pip6a-ASO (12.5 mg/kg) and serum IgE concentrations were determined after 24h (Fig 5, *B*).
148 Interestingly, lower IgE amounts were found in serum of ASO and Pip6a-ASO treated mice, with a
149 significant decrease in the later compared to controls (Fig 5, *C*). Thus, these data provide evidence for
150 a systemic effect of Pip6a-ASO targeting the IgE PAS on antibody-secreting cells.

151 IgE+ B cells can be generated either by direct class switching from IgM+ B cells or sequential class
152 switching from IgG1+ B cells (3,19), but exhibit a short lifespan and low levels of BCR IgE expression
153 (7,20). Interestingly, modulation of APA after treatment by IgE targeting ASO induced apoptosis in
154 U266 myeloma cells, yet further studies are needed to identify whether toxic effects are caused by the
155 re-expression of IgE BCR or by a more global attenuation of the PC phenotype with less IgE secretion
156 and *MZB1* expression. Several studies including ours showing that the BCR IgE tonic signal drives
157 apoptosis support the former hypothesis, but conflicting evidence has been also described (7,20). Since
158 the ER-localized cochaperone MZB1 plays a crucial role in protein folding in plasma cells (17,21), its
159 low expression together with disturbed stoichiometric ratio of Ig heavy and light chains induced by
160 ASO treatment could lead to ER stress-associated apoptosis in U266 cells, but this needs further
161 investigation.

162 The herein described proof-of-principle for systemic delivery of ASO targeting IgE PAS could open new
163 avenues for a broad range of IgE-dependent allergies. Nonetheless, the impact of IgE ASO needs to be
164 confirmed with some formulation adjustments to target long-lved plasma cells in bone marrow niches,
165 and conjugation of ASO to monoclonal anti-CD38 antibody used for the treatment of multiple myeloma
166 should be envisioned. Local delivery of ASO could be also advantageous for food allergy since a recent
167 study identified IgE+ B lineage cells in gastrointestinal tract of peanut allergic patients (22). Of note,
168 intradermal injections of a splice-switching oligonucleotide targeting FcRIβ have also demonstrated

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169 positive results in a mouse model of allergic dermatitis (23). In addition, respirable ASO for aerosol
170 delivery has already been developed (24) and such a formulation should be useful to track local IgE
171 production in various inflammatory conditions.

172 We show here that targeting the secreted IgE PAS with an ASO is an effective method to reduce IgE
173 secretion which has already proven to be beneficial for IgE-dependent allergic patients (2) while
174 enhancing apoptosis of IgE-expressing plasma cells. This latter effect could be even more favorable for
175 allergic patient prognosis as it could reduce the IgE plasma cell compartment (4) and, in this manner,
176 decrease IgE production to promote allergen tolerance as other Ig isotypes are not affected.

177 **ACKNOWLEDGMENTS**

178 We thank ArkAb (a division of B Cell Design) for kindly providing InEps™ mice and IgE-specific
179 hybridoma cells. We thank also the staff of our animal facility (University of Limoges, France) and
180 Michael J Gait (Medical Research Council, Laboratory of Molecular Biology, Cambridge, United
181 Kingdom) for helpful discussions regarding the use of Pip6a-ASO for the targeting of antibody-secreting
182 cells *in vivo*.

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239 **FIGURE LEGENDS**

240 **FIG 1. IgE targeting ASO decreased secreted IgE and increased membrane IgE in a human IgE**
241 **transgenic mouse model. A.** IGH locus in InEps™ mouse model. **B,C,D.** Isolated spleen cells treated 48
242 hours with 1µM hydroxytamoxifen and 2µM IgE targeting ASO (ASO) or irrelevant control ASO (CTRL)
243 (n=5). **B.** Total IgE in supernatants measured by ImmunoCap assays. **C,D**. Secreted and membrane IgE
244 mRNA quantified by RT-qPCR. **E**. Intracellular IgE bearing B cells were analyzed by flow cytometry. **F,G.**
245 InEps™ hybridoma cells secreting allergen specific IgE to : penicillin, ovalbumin and wasp venom were
246 treated 48 hours with 3µM ASO or CTRL (n=4). **F.** Total IgE in supernatants measured as in B. **G.**
247 Secreted and membrane IgE mRNA quantified as in C. (*p-value < 0.05, ** < 0.01, *** < 0.001, **** <
248 0.0001).

249 **FIG 2. IgE targeting ASO decreased secreted IgE and promoted apoptosis in a human plasma cell line.**
250 IgE secreting U266 human myeloma cells were treated 48 hours with 6µM ASO or CTRL (n=11). **A.** Total
251 IgE measured by ImmunoCap assays. **B,C**. Secreted and membrane IgE mRNA quantified by RT-qPCR.
252 **D,E**. Exon coverage of RNA sequencing reads on IGHE gene. **F,G.** Apoptosis flow cytometry with
253 Annexin V and TO-PRO-3 labelling. **H,** PARP Western Blot analysis on total extracted protein. (*p-value
254 < 0.05, *** < 0.001, **** < 0.0001).

255 **FIG 3. IgE targeting ASO treatment induces a shifted profile in a human plasma cell line.** IgE secreting
256 U266 human myeloma cells were treated 48 hours with 6µM ASO or CTRL and polyadenylated RNA
257 was sequenced (n=4). **A.** Heatmap of 202 differentially expressed genes by DEseq2 analysis of RNA
258 sequencing results. **B.** Significant Gene Ontology and Reactome pathways performed on differentially
259 expressed genes. Only annotations with a significant false discovery rate (FDR; adjusted p-value of <
260 0.05) are shown.

261 **FIG 4. IgE targeting ASO decreased secreted IgE and increased membrane IgE expression in human**
262 **cells but did not affect other Ig isotypes.** Sorted human B cells stimulated with CD40 ligand and IL-4
263 and treated 72 hours with 2µM ASO or CTRL (n=5). **A.** Secreted and membrane IgE, total IgG1 and total

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264 IgM mRNA and **B.** Secreted and membrane IgG1 and IgM mRNA quantified by RT-qPCR. **C-E.** Secreted
265 vs Membrane IgE (C), IgG1 (D), IgM (E) mRNA ratios. (*ns*: non-significant, **** < 0.0001).

266 **FIG 5. IgE targeting ASO decreased IgE secretion *in vivo*. A.** IgE concentrations assessed in cultured
267 supernatants of U266 cells treated 24h with 6 µM ASO coupled to different cell-penetrating moieties:
268 octa-guanidine dendrimer (ASO) and arginine-rich peptide (Pip6a-ASO) (n=3). **B.** IgE expression was
269 induced in Ineps™ mice (n=3/group) by administration of Tamoxifen (oral gavage) at day 0 and 24h
270 later mice were injected iv (tail vein) with ASO (12.5 mg/kg). **C.** Serum IgE concentrations determined
271 at day 2. (*ns*: non-significant, * < 0.05, *** < 0.001).

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Figure 1

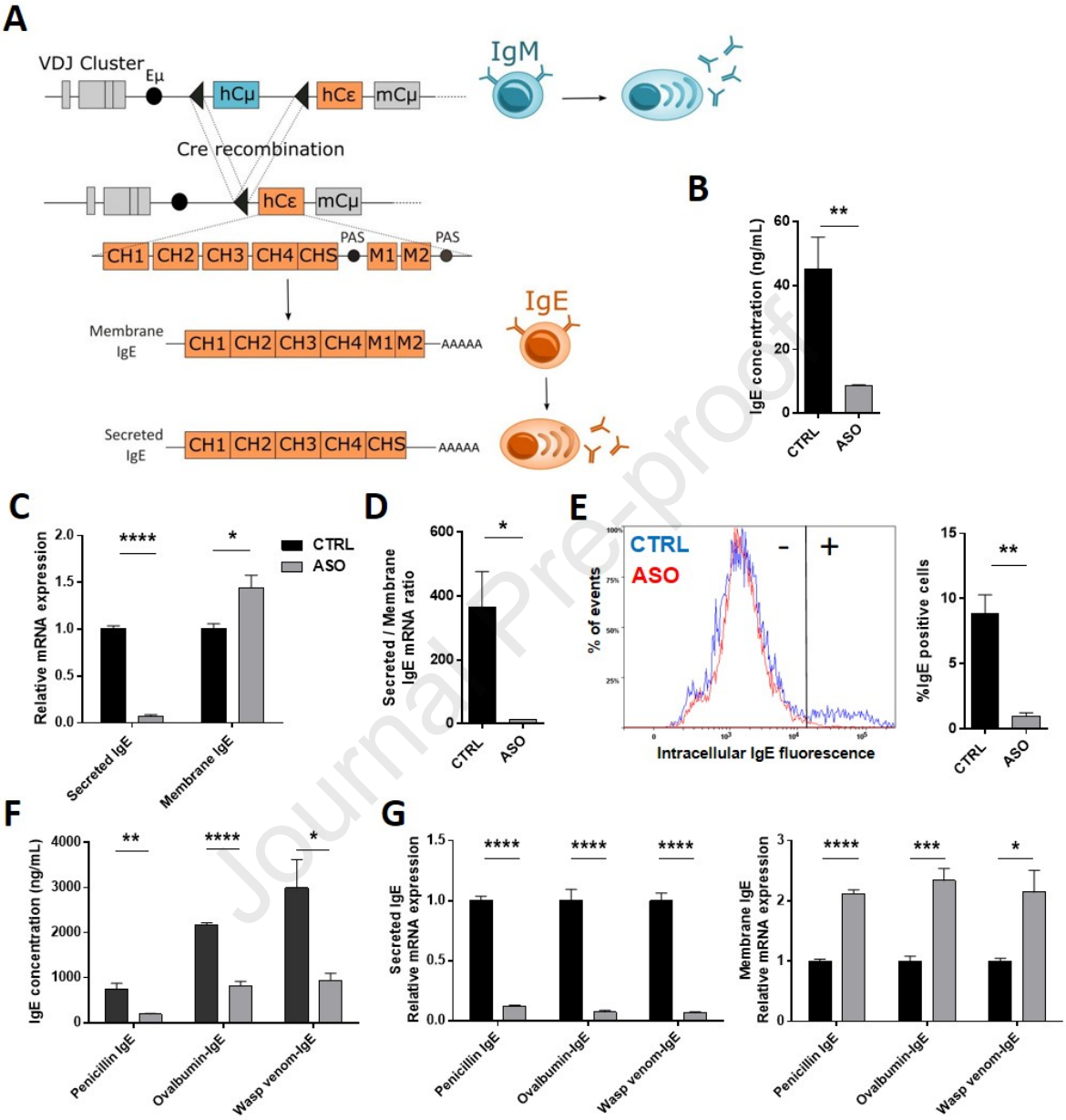

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Figure 2

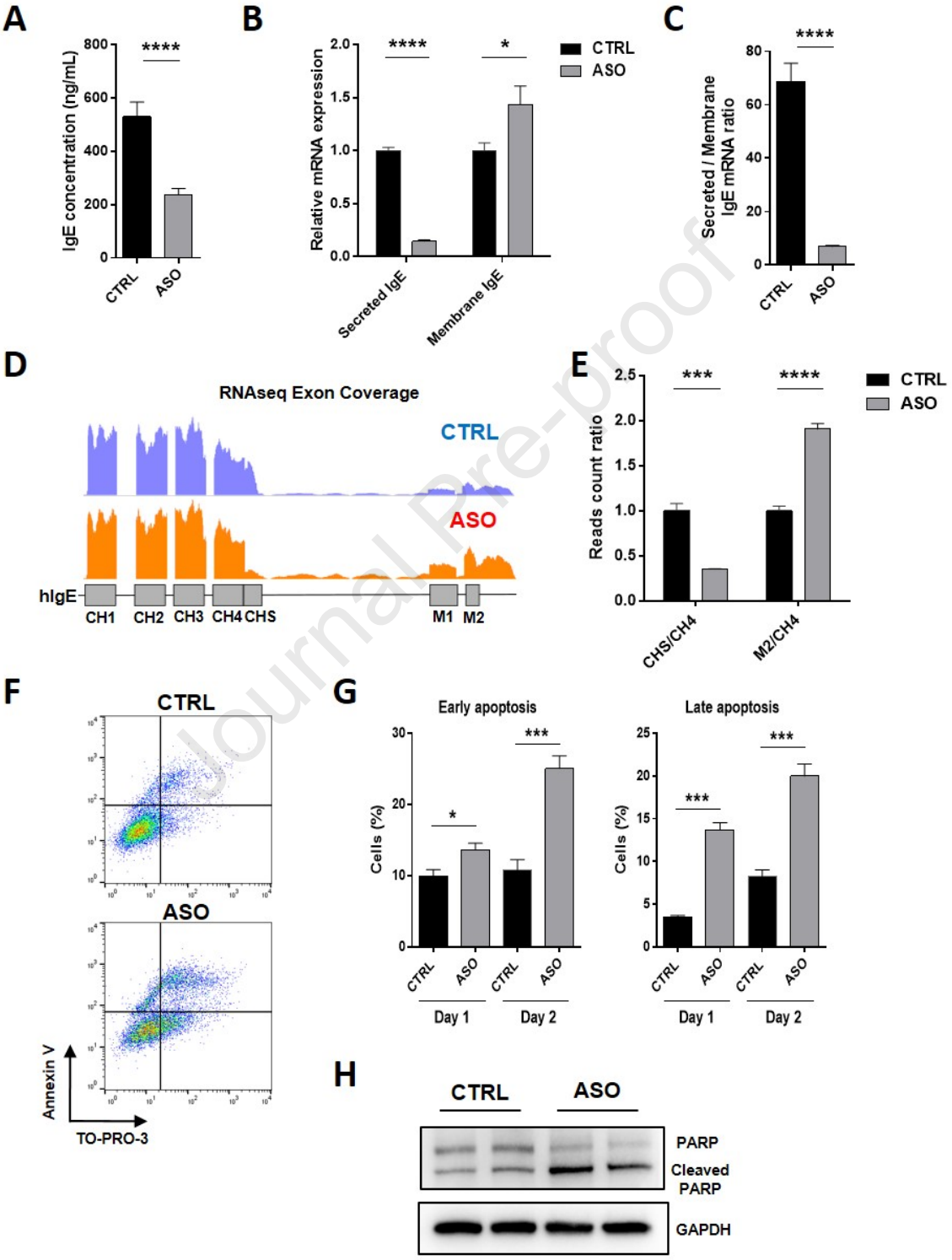

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Figure 3

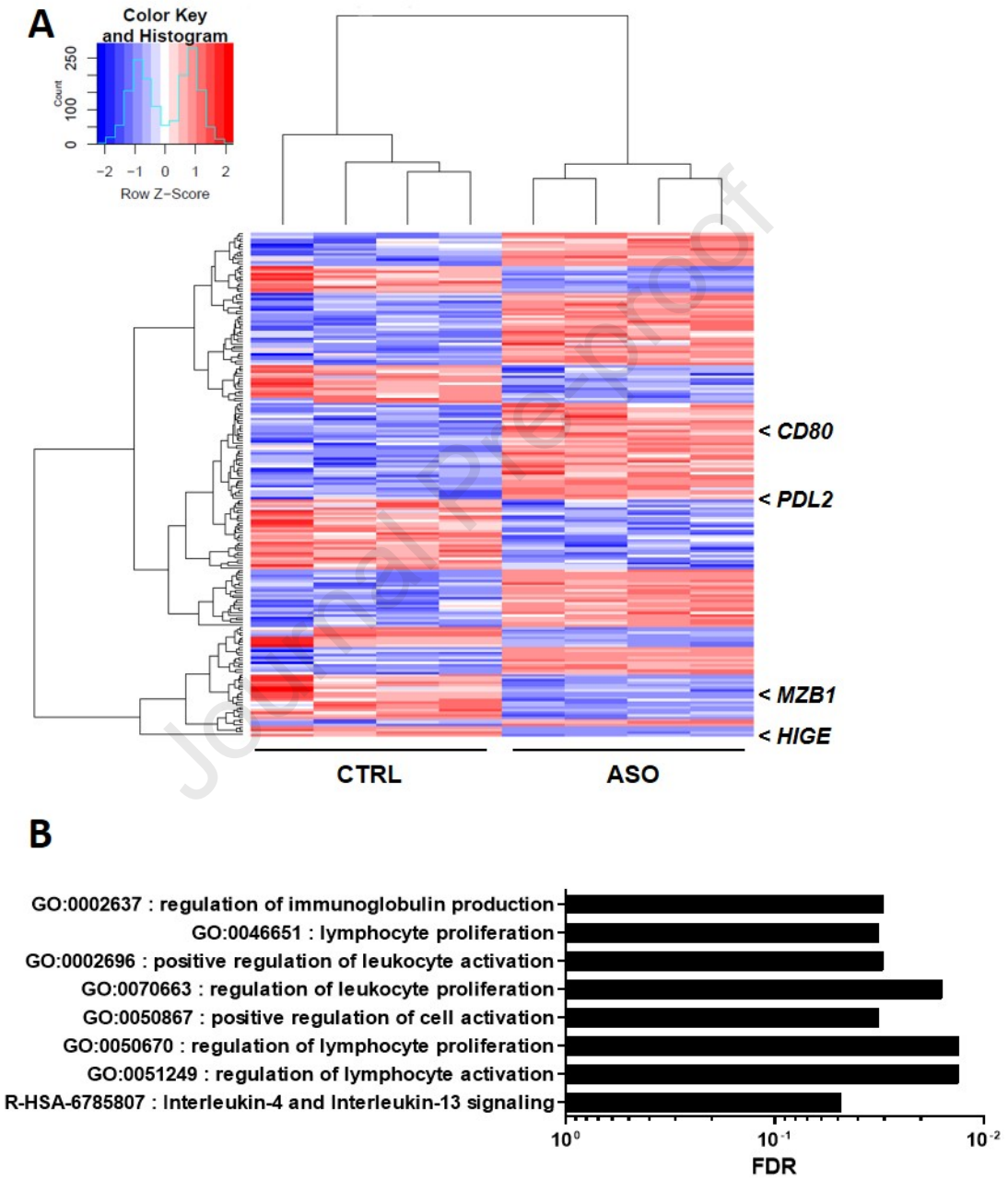

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Figure 4

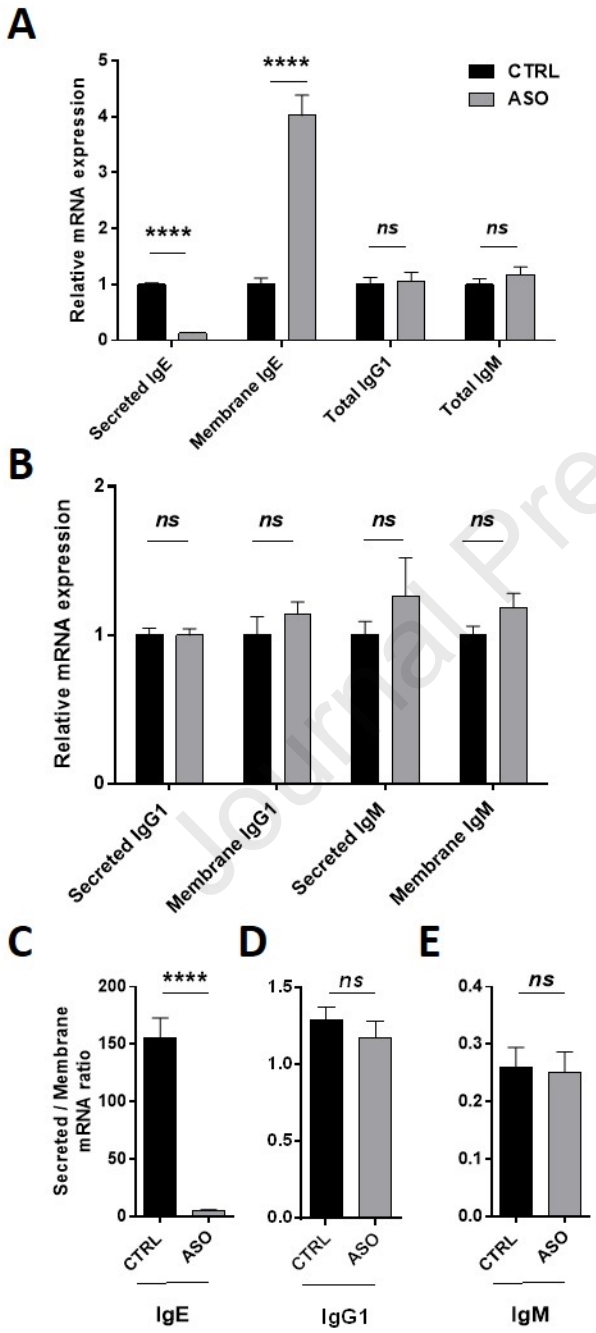

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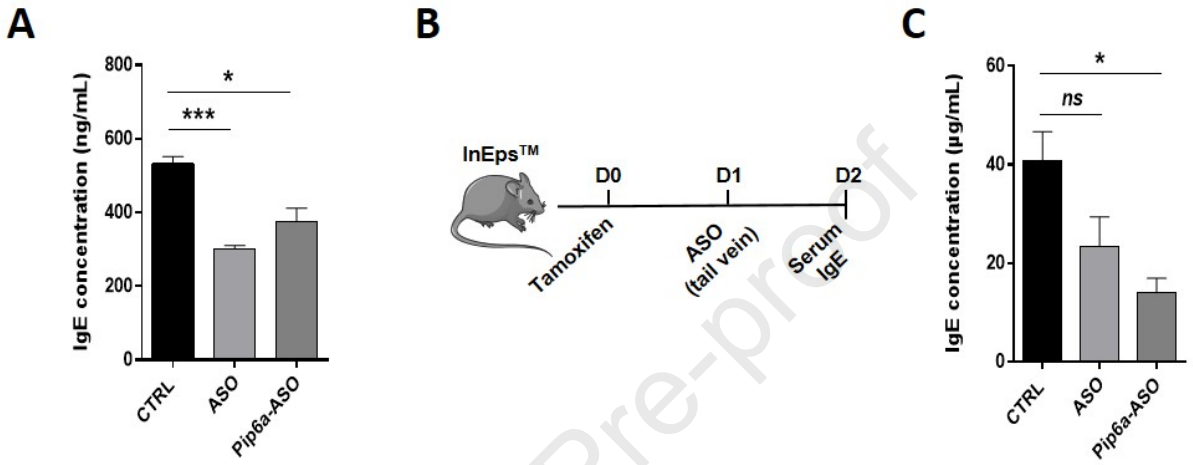
Figure 5



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