IgM regulates airway hyperresponsiveness via modulation of actin associated genes.

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Running Title: IgM regulates airway hyperresponsiveness via modulation of actin associated genes.

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Abstract: Allergic asthma is a disease driven by T helper 2 (Th2) cells, eosinophilia, airway hyperresponsiveness (AHR) and IgE-secreting B cells. Asthma is largely controlled by corticosteroids and β_2 adrenergic receptor agonists that target and relax airway smooth muscle (ASM). Immunoglobulin M (IgM) isotype secreted by naïve B cells is important for class switching but may have other undefined functions. We investigated the role of IgM in a house dust mite (HDM)-induced Th2 allergic asthma model by sensitising wild type (WT) and IgM-deficient (IgM^{-/-}) mice with HDM. We validated our findings using CRISPR and single cell force cytometry in human ASM. We found IgM to be essential in AHR but not Th2 airway inflammation or eosinophilia. RNA sequencing of lung tissue suggested that IgM regulated AHR through modulating brain-specific angiogenesis inhibitor 1-associated protein 2-like protein 1 (*Baiap2l1*) and erythroid differentiation regulator 1 (*Erdr1*). Deletion of *BAIAP2L1* and *ERDR1* reduced human ASM contraction when stimulated with TNF- α . These are unprecedented findings and have implications in future treatment of asthma beyond current therapies.

Summary: IgM regulates bronchoconstriction by interacting with *BAIAP2L1* and *ERDR1* found in airway smooth muscle.

Main text: Immunoglobulin M (IgM) is the first antibody isotype expressed during B cell development and the first humoral antibody responder, conserved across all species from zebrafish to humans (1). IgM can be divided into natural and antigen-induced IgM and can either be membrane bound IgM-type B cell receptor (BCR) or secreted IgM (2, 3). Natural IgM plays multiple roles in homeostasis including scavenging and clearance of apoptotic cell debris in conjunction with phagocytic

macrophages, B cell survival through tonic signals, lymphoid tissue architecture and prevention of autoimmune diseases (4–6). At mucosal sites, both natural and antigen-induced IgM play a role in shaping healthy microbiota and their repertoire, although limited, is also shaped by microbiota (7, 8). Secreted IgM antigen-complexes can connect signals via unique and shared receptors, suggestive of a more pleiotropic role in homeostasis and disease states (9, 10).

Asthma is a T helper 2 (Th2) disease characterised by eosinophilic lung inflammation, mucus production, airway hyperresponsiveness (AHR), Th2 cytokines (interleukin-4 (IL-4), IL-5 and IL-13) and B cells producing IgE (11). IgM is central to class switch recombination that results in IgE class switched B cells. Currently, it is unclear whether natural or secreted IgM plays different roles compared to membrane bound IgM, which is more likely to undergo class switching to IgE. Here, we showed an unexpected function of secreted, and membrane bound IgM; namely, its involvement in modulating airway smooth muscle contraction. We sensitised and challenged IgM-deficient (IgM^{-/-}) and wild type Balb/c (WT) mice with HDM intratracheally (i.t.) and analysed AHR, lung infiltrate and cytokines (Fig. 1, A, Fig. S1, A). We found moderately reduced resistance and elastance in IgM^{-/-} sensitised with a high dose of HDM (100µg) compared to WT mice (fig. S1, B). Similarly, we also observed profound reduction in AHR in IgM^{-/-} mice sensitised with a low dose of HDM (1µg) compared to WT (Fig. 1, B). Interestingly, lung eosinophils were intact at both low dose (Fig. 1, C) and high dose HDM (fig. S1, C-D). We observed similar finding of profound AHR reduction in IgM^{-/-} mice in C57BL/6 background compared to WT mice (fig. S2, A). We also observed no significant changes between IgM^{-/-} and WT mice in mucus production shown by *Muc5a* gene expression (Fig. 1, D) or goblet cells in C57BL/6 mice challenged with HDM (fig. S2, B). Accompanying reduction in AHR in IgM^{-/-} mice was

low titers of total IgE, HDM-specific IgE, and mediastinal lymph node (mLN) B cell surface expression of IgM and IgG1 (Fig. 1, E-F), owing to a lack of class switching. This was also consistent with what we found in IgM^{-/-} mice in C57BL/6 background (fig. S2, C). Interestingly, the numbers of lung B cells were normal (Fig. 1, C) and the proportions of B cell subsets such as follicular, marginal zones and germinal centres (GCs) B cells were not affected by lack of IgM (Fig. 1, F), neither were CD4 T cells and T follicular helper cells (Fig. 2, A). This suggested a normal interaction between B and T cells in GCs, but lack of AID-dependent class switching, despite increased expression of IgD (Fig. 2, D) (12). B1 cells develop in the yolk sac on embryonic day 9, before birth from a common lymphoid progenitor in the foetal liver and seed the peritoneal and pleural cavities (13–19). B1 cells are thought to be the main source of naturally occurring IgM, although spleen and bone marrow (BM) cells can also contribute (20). We checked for natural IgM and antigen-induced IgM in multiple tissues in the absence of IgM. B cells expressing IgD were increased in all tissues including mLNs, peritoneal cavity and spleen (fig. S3, A-C) and pre-B cell subsets were normal in BM (fig. S3, D) in the absence of IgM as previously reported (21). Lack of class switching is in contrast to earlier reports, which suggested that IgD can largely replace IgM for class switching to other isotypes, resulting in delayed neutralising IgG1 against vesicular stomatitis virus (VSV) (21, 22). We could also show that AHR reduction in IgM^{-/-} mice was allergen-independent, as we observed similar findings using ovalbumin (OVA) complexed to alum adjuvant (fig. S4, A-B) and acute papain-induced allergic inflammation (fig. S4, C-D). To investigate whether reduction in AHR was due to reduced Th2 cells and cytokines, we stimulated total mLN and lung cells with anti-CD3 for 5 days or with PMA/ionomycin for 5 hrs in the presence of monensin and measured secreted or intracellular IL-4 and IL-13 expression. We found no

119 differences in secreted or intracellular levels of these cytokines between IgM^{-/-} and WT
120 mice challenged with HDM (Fig. 2, B-C). To investigate what drives this reduced AHR
121 in the absence of IgM between secreted antibodies or the cells, we transferred serum
122 from naïve WT mice into IgM^{-/-} mice a day before sensitization with HDM allergen (Fig.
123 2, D) (23). AHR in IgM^{-/-} was still reduced compared to WT mice even after transfer of
124 WT serum (Fig. 2, E), but levels of total IgE were increased and comparable to those
125 found in WT mice (Fig. 2, F). These findings verified previous studies which showed
126 that IgM is abundant in mouse sera and reconstitution of IgM is difficult (3). We also
127 tried to restore IgM function through adoptive reconstitution with congenic CD45.1 BM
128 or sorted B cells into IgM^{-/-} mice (fig. S5, A). This approach did not replenish IgM B
129 cells to levels observed in WT mice and as a result did not restore AHR, total IgE and
130 IgM in these mice (fig. S5, B-C). In order to understand why we could not reconstitute
131 IgM, we checked for transferred congenic B cells in different organs after reconstitution
132 with BM in IgM^{-/-} mice and found some engraftment in BM and spleen, but poor
133 engraftment to lung and mLNs which may explain our inability to restore AHR in IgM^{-/-}
134 mice challenged with HDM (fig. S5, D). Because we had found no other changes in
135 asthmatic allergic features between WT and IgM^{-/-}, except for profound reduced AHR,
136 we resorted to RNA sequencing to see if there were lung specific factors that
137 influenced AHR regulation in IgM-deficient mice. Principal component analysis (PCA)
138 clustered HDM-treated groups (PC1) separately from PBS control groups (PC2),
139 independent of mouse genotype (Fig. 3A). We found smaller variation in gene
140 expression between WT and IgM^{-/-} in both HDM challenged and PBS control mice (Fig.
141 3, B). We could mainly detect downregulation of genes such as brain-specific
142 angiogenesis inhibitor 1-associated protein 2-like protein 1 (*Baiap2l1*), erythroid
143 differentiation regulatory factor 1 (*Erdr1*), chemokines such as *Ccl8*, *Ccl9*, *Ccl17* and

144 *Ccl22* in IgM^{-/-} mice challenged with HDM (Fig. 3, C-D). Interestingly, *Baiap2l1* and
145 *Erdr1* were also downregulated in IgM^{-/-} saline-treated control mice, suggesting an
146 inflammation independent effect (Fig. 3, C, E). We also found presence of J-chain
147 coding gene in WT mice which was absent in IgM^{-/-} mice, confirming a deletion in
148 genes associated with holding IgM monomers together and thus IgM (24) (Fig. 3, E).
149 Gene set enrichment analyses (GSEA) confirmed that the genes contributing to
150 changes in AHR between WT and IgM^{-/-} were associated with muscle system
151 processes and skeletal muscle contraction (Fig. 3, F). There was also an over
152 representation of gene ratios associated with skeletal muscle development,
153 differentiation and contraction, and suppression of genes associated with chemotaxis
154 and plasma membrane bounded cell projection (fig. S6). We decided to focus on
155 *Baiap2l1* (also known as IRTKS), which has been shown to promote actin
156 polymerisation and microvilli length in the intestinal epithelial cells (25). We also
157 focussed on *Erdr1*, a conserved factor in mice and humans, which has previously been
158 shown to play a role in cancer through activation of natural killer cells via controlling
159 actin rearrangement (26). To understand how *Baiap2l1* and *Erdr1*, genes
160 downregulated in IgM-deficient mice, could influence AHR, we resorted to an *in vitro*
161 model that allowed us to measure contraction using fluorescently labelled elastomeric
162 contractile surface (FLECS) technology (27) (Fig. 4, A). We chose human airway
163 bronchial smooth muscle cell (BSMCs) line as both *BAIAP2L1* and *ERDR1* are
164 expressed in structural cells including muscle and epithelial cells, and actin together
165 with myosin are essential in muscle contraction (28). To this end, we used CRISPR
166 technology to knock down *BAIAP2L1* and *ERDR1* in BSMCs (fig. S7, A-B). BSMCs
167 (160 000/well) were stimulated with 10 μ M acetylcholine (ACh), 10 ng/mL of human
168 TNF- α and IL-13, followed by transfection with ribonucleoprotein (RNP) complexes

containing single guide RNA targeting *BAIAP2L1* and *ERDR1* and Cas9 for 48h (Fig. 4, A). Transfected cells were seeded onto elastomeric patterns, stimulated with cytokines for 3h and fixed in 4% paraformaldehyde, followed by staining with DAPI and Phalloidin before imaging on Stellarvision microscope and quantification (Fig. 4, A). We validated deletion of *BAIAP2L1* by PCR and showed deletion of *BAIAP2L1* in ACh stimulated cells when compared to scramble sgRNA (fig. S7, C). Transfections with sgRNA did not impact cell viability (fig. S7, D). As expected, BSMCs displayed a higher level of contraction in TNF- α or IL-13 stimulated cells compared to unstimulated cells (Fig. 4, B-D). In BSMCs transfected with sgRNA-*BAIAP2L1* and -*ERDR1*, we saw significant reduction in contraction levels in TNF- α stimulated cells when compared to scramble sgRNA transfected and stimulated cells (Fig. 4, B, C). We saw no differences in contraction between scramble sgRNA and sgRNA-*BAIAP2L1* and -*ERDR1* stimulated with IL-13 (Fig. 4, D). Taken together, this data indicates that *BAIAP2L1* and *ERDR1*, two genes downregulated in IgM-deficient mice may have a role in inducing airway contraction of bronchial smooth muscle cells during asthma.

Here, we described an unexpected function of IgM in regulation of bronchoconstriction. We show that in the lung tissue of IgM-deficient mice, there is reduction in genes associated with actin cytoskeleton, a key factor in smooth muscle contraction and AHR. We used single cell force cytometry to validate our findings in human ASM cell line and show as a proof of concept that deletion of *BAIAP2L1* and *ERDR1* reduced muscle contraction. The mechanisms of how IgM, also found at the interface of epithelial-mucosal surface, promotes diverse microbiota remains unclear. We believe these findings report for the first time an independent function of IgM from its natural class switching and non-specific binding to microbial components. We believe that IgM function in regulating ASM may be indirect through other unknown

factors but does not involve microbiota as treatment of mice with mixture of antibiotics did not restore AHR (fig. S8A-B). Early vaccination with bacterial species, such as group *A streptococcus* containing GlcNAc or β -1,3-glucans can protect adult mice against *A. fumigatus*-induced allergic asthma (29). This is mainly through conserved germline-encoded IgM antibodies, which have broad specificities to common allergens containing GlcNAc moieties such as Dermatophytes (8, 30). We, therefore, speculate that in the absence of IgM in all its forms, such broad natural antibodies that recognise and react to environmental factors do not develop at an embryonic stage and therefore never learn to induce bronchoconstriction when seeing these factors at a later stage.

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Author contributions: Conceived and supervised study: SH FB. Performed the experiments: SH AFS JK KJ NM MS AN. Analysed the data: SH AFS KJ JO NM. Wrote

the paper: SH FB. All authors discussed the results and commented on the manuscript.

Competing interests: The authors declare that they have no competing interests.

Data and materials availability: All data are available in the main text or the supplementary materials and have not been deposited in public databases. RNA sequencing data is protected and will require materials transfer agreements (MTAs) until its protected by authors.

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329 FIGURE LEGENDS

Fig. 1. IgM-deficiency leads to reduced airway hyperresponsiveness and class switching to IgE in HDM-induced asthma.

(A) Schematic diagram showing sensitisation and challenge protocol where mice (IgM^{-/-}) and wild type littermate control (WT) were sensitised with HDM 1 µg intra-tracheally on days 0 and challenged with HDM 3 µg on days 8-12. Analysis was done on day 15.

(B) Airway resistance and elastance were measured with increasing doses of acetyl methacholine (0-40 mg/mL).

(C) Total lung eosinophil numbers (live⁺Siglec-F⁺CD11c⁻) and B cells (live⁺B220⁺CD19⁺MHCII⁺) were stained and analysed by Flow cytometry and enumerated from % of live cells.

(D) *Muc5a* gene expression in whole lung tissue.

(E) Total IgE and HDM-specific IgE in serum.

343 (F) IgG1 and IgM surface expression in mediastinal lymph node B cells of WT, IgM^{-/-}
344 and μ MT^{-/-} mice.

345 (G) Marginal Zone (live⁺B220⁺CD19⁺MHCII⁺CD21/CD35⁺CD23⁻), follicular
346 (live⁺B220⁺CD19⁺MHCII⁺CD23⁺CD21/CD35⁺) and Germinal Centre
347 (live⁺B220⁺CD19⁺MHCII⁺GL7⁺FAS⁺) B cells in mediastinal lymph node of WT and IgM^{-/-}
348 mice challenged with HDM.

349 Shown is mean $\pm$ SEM from two pooled experiment (n=10 - 12). Significant differences
350 between groups were performed by Student *t*-test (Mann-Whitney) (C, D, E) or by
351 Two- Way ANOVA with Benforroni post-test (B) and are described as: **p*<0.05,
352 ***p*<0.01, ****p*<0.001, *****p*<0.0001.

353

Figure 2

Fig. 2. IgM-deficiency does not lead to reduced Th2 allergic airway inflammation and serum transfer restores IgE, but not AHR.

(A) Total mediastinal lymph node CD4 T cell numbers (live $^{+}$ CD3 $^{+}$ CD4 $^{+}$) and % of Follicular Helper T cells (live $^{+}$ CD3 $^{+}$ CD4 $^{+}$ PD-1 $^{+}$ CXCR5 $^{+}$) of CD4 T cells were stained and analysed by Flow cytometry and enumerated from % of live cells.

(B) Mediastinal lymph nodes were stimulated with anti-CD3 (10 μ g/mL) for 5 days and supernatants were used to measure levels of IL-4 and IL-13. Cytokines were not detected in unstimulated or HDM (30 μ g) stimulated mLN.

(C) Representative FACS plots and frequencies of lung CD4 T cells (live $^{+}$ CD3 $^{+}$ CD4 $^{+}$) producing IL-4 and IL-13 after 5 hr stimulation with PMA/ionomycin in the presence of monensin.

(D) Schematic diagram showing serum transfer from WT to IgM $^{-/-}$ which were then sensitised as shown in Fig. 1,A.

368 (E) Airway resistance was measured with increasing doses of acetyl methacholine (0
369 -40 mg/mL).

370 (E) Total IgE in serum of mice either transferred with WT serum, IgM^{-/-} serum or no
371 serum.

372 Shown is mean $\pm$ SEM from two pooled experiment (n=10 - 12). Significant differences
373 between groups were performed by Student *t*-test (Mann-Whitney) (C, D, E) or by
374 Two-Way ANOVA with Benforroni post-test (B) and are described as: **p*<0.05,
375 ***p*<0.01, ****p*<0.001, *****p*< 0.0001.

376

377

Figure 3

Fig. 3. Genes associated with muscle contraction are downregulated in IgM-

deficient mice. WT and IgM^{-/-} mice were treated as in Fig. 1A and RNA collected from whole lung for RNA sequencing.

(A) Principal-component (PC) analysis showing variation in the global gene expression profiles across the different groups. PC1 (60%) and PC2 (18%), which capture the greatest variation in gene expression, are shown. Orange colour represents WT HDM, green colour represents WT PBS, Blue colour represents IgM^{-/-} HDM and purple crosses represent IgM^{-/-} PBS. Each dot represents an individual mouse.

(B) Number of differentially expressed genes between groups.

(C) Heatmaps depicting the differentially expressed genes between WT and IgM^{-/-} samples from HDM treated and PBS mice ranked based on hierarchical clustering.

(D-E) Volcano plots: numbers and colour relate to genes that have an adjusted *p* value <0.05. Blue, significantly downregulated; red, significantly up-regulated; grey, non-differentially expressed. *P* values were adjusted for multiple testing using the

394 Benjamini-Hochberg method. (D) represent changes between WT and IgM^{-/-} treated
395 with HDM and (E) represents changes between WT and IgM^{-/-} treated with saline.
396 (F) Gene set enrichment analysis (GSEA) of hallmark gene sets from the Molecular
397 Signatures Database of the Broad Institute, showing the normalized enrichment
398 scores (NES) for lung RNA-Seq data from WT mice.

399

400

Fig. 4. CRISPR-based deletion of *BAIAP2L1* and *ERDR1* leads to reduced smooth muscle contraction at a single cell level.

(A) Bronchial smooth muscle cells (1.6×10^5 cells/well) were transfected with CRISPR-Cas9 single guide RNAs (scramble, *BAIAP2L1* and *ERDR1*), stimulated with recombinant human IL-13 (100 ng/mL) and TNF- α (10 ng/mL) for 48 h. Cells were then transferred to elastomeric micropatterns, stimulated again with rIL-13 and rTNF- α and fixed before imaging on a StellarVision microscope.

(B) Representative images of single BSMCs on micropatterns from scramble, *BAIAP2L1* and *ERDR1* stimulated with 10 ng/mL TNF- α . DNA was stained with DAPI, actin fibers with Phalloidin-565 and elastomeric micropatterns are coated in Fibronectin-488. Merged images are shown on the right.

(C) Violin plots showing contraction of 50-100 cells/condition stimulated with 10 ng/mL TNF- α , individual dots represent a single cell contraction, where blue is scramble sgRNA, red is *BAIAP2L1* sgRNA and green is *ERDR1* is sgRNA.

(D) Violin plots showing contraction of 50-100 cells/condition stimulated with 100

417 ng/mL IL-13, individual dots represent a single cell contraction, where blue is
418 scramble sgRNA, red is *BAIAP2L1* sgRNA and green is *ERDR1* is sgRNA.
419
420 Shown is mean $\pm$ SEMs from two pooled experiment (n=50 - 100). Significant
421 differences between groups were performed by student t-test (Mann-Whitney) and *p*
422 value is shown.
423

424 Supplementary Materials for

425

426 IgM regulates airway hyperresponsiveness via modulation of actin associated genes.

427

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SUPPLEMENTARY MATERIAL AND METHODS

Mice

IgM-deficient homozygotes mice on Balb/C background and on C57BL/6 background were backcrossed up to 10 generations (21). Wild type on Balb/C and C57BL/6 background were used as a littermate control. Mice were housed in independently ventilated cages under specific pathogen-free conditions at the University of Cape Town Animal Facility. All mice were used at eight to 10 weeks of age and animal procedures were performed according to strict recommendation by the South African Veterinary Council and were approved by the University of Cape Town Animal Ethics Committee (Reference number 018/013).

House Dust-mite induced allergic airway disease

A high dose and a low dose treatment schedule were used to induce symptoms of allergic asthma in mice (31). Mice were anaesthetised with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa) and xylazine (Rompun; Bayer, Isando, South Africa). Mice were sensitised intratracheally (i.t.) on day 0 with 1 µg of HDM (Stellergens Greer Laboratories, Lenoir, U.S.A.) and intranasally challenged with 3 µg HDM on days 8, 9, 10, 11 and 12. AHR was measured on day 15. After the procedure, mice were euthanised and tissue samples were collected for analysis.

Adoptive transfer of naïve B cells

Spleens were collected from naïve congenic CD45.1 Balb/C mice and passed through 40µm strainer to obtain single cell suspensions. Cells were stained with FITC-B220 and APC-CD19 for 30min at 4°C. A dead cell exclusion dye (7AAD) was added before sorting on BD FACS Aria I to at least 96% purity. 2-5 x 10⁶ cells were

461 adoptively transferred intravenously (i.v.) into IgM^{-/-} recipient mice a day before HDM
462 sensitisation.

463

464 *Adoptive transfer of naïve serum*

465 Naïve wild type mice were euthanised and blood collected via cardiac puncture
466 before being spun down to collect serum. Serum (200µL) was injected
467 intraperitoneally into IgM-deficient mice, 1 day before sensitisation with HDM.

468

469 *Ovalbumin induced allergic airway inflammation*

470 Mice were sensitised intraperitoneally with (50µg in 200µl) of ovalbumin (OVA)
471 adsorbed to 0.65% alum (Sigma-Aldrich, Aston Manor, South Africa) on days 0, 7,
472 14. On days 23, 24, 25, mice were intranasally challenged with 100µg of OVA under
473 anaesthesia with ketamine (Anaket-V; Centaur Labs, Johannesburg, South Africa)
474 and xylazine (Rompun; Bayer, Isando, South Africa). AHR was measured on day 26.
475 After the procedure, mice were euthanized with halothane and tissue samples
476 collected for analysis.

477

478 *Papain-induced lung inflammation*

479 Mice were sensitised with isoflourane (3L/min) briefly before being challenged with
480 50µL of 25 µg of Papain (Sigma-Aldrich, Aston Manor, South Africa) on days 1, 2
481 and 3 or PBS. AHR was measured on day 4. After the procedure, mice were
482 euthanized with halothane and tissue samples collected for analysis.

483

484

485

486 *Airway Hyperresponsiveness*

487 Airway resistance and elastance of the whole respiratory system (airways, lung chest
488 wall) after intranasal challenge was determined by forced oscillation measurements
489 as described previously (32) with the Flexivent system (SCIREQ, Montreal, Canada)
490 by using the single compartment (“snapshot”) perturbation. Measurements were
491 carried out with increasing doses of acetyl- β -methylcholine (methacholine, Sigma-
492 Aldrich, Aston Manor, South Africa) (0, 5, 10, 20 and 40 mg/mL) for Balb/C or (0, 20,
493 40, 80 160 and 320 mg/mL) for C57BL/6. Differences in the dose-response curves
494 were analysed by repeated-measures Two-way ANOVA with the Bonferroni post-
495 test. Only mice with acceptable measurements for all doses (coefficient of
496 determination >0.90) were included in the analysis.

497

498 *Flow cytometry*

499 Single-cell suspensions were prepared from lymph nodes in Roswell Park Memorial
500 Institute (RPMI) media (Gibco, Paisley, United Kingdom) by passing them through
501 100 μ m strainer. To obtain single cell suspensions from lung tissues, a left lobe was
502 digested for 1 hour at 37°C in RPMI containing 13 mg/mL DNase I (Roche,
503 Randburg, South Africa) and 50 U/mL collagenase IV (Gibco, Waltham,
504 Massachusetts) and passed through 70 μ m strainer. Antibodies used in these
505 experiments included, phycoerythrin (PE)- conjugated anti-Siglec-F (clone, E50-
506 2440), anti-IL-5 (clone, TRFK5), anti-CD44 (clone, KM114), anti- T and B cell
507 activation antigen (clone, GL7), anti-CD43 (clone, S7), FITC- conjugated anti-Ly6G
508 (clone, 1A8), anti-IgD (clone, 11-26C2a), IL-4 (clone, 11B11), anti-PD-1-(clone,
509 29F.1A12), PerCP Cy5.5- conjugated anti-Ly6C (clone, AL-21), -CD45.1 (clone,
510 A20), anti-IL-17 (clone, TC11-18H10), Allophycocyanin (APC)- conjugated anti-

511 CD11c (clone, HL3), anti-CD5 (clone, 53-7.3), BV421 conjugated anti-CD11b (clone,
512 M1/70), anti-CD62L (clone, MEL-14), anti-IgG1 (clone, A110-1), AlexaFlour 700-
513 conjugated anti-CD3 ϵ (clone, 145-2C11) -anti IFN- γ , BV510- anti-CD4 (clone, RM4-
514 5) and anti- B220 (clone, RA3-6B2), APC-Cy7-conjugated anti-CD19 (clone, 1D3)
515 and anti-CD8 (clone, 53-6.7), BV786 conjugated anti-IgE (clone, R35-72) and anti-IL-
516 33R (ST2) (clone, U29-93), biotin-conjugated anti-IgM (clone, AF-78), anti-CD95
517 (clone, Jo2), anti- CD249 (clone, BP-1) were purchased from BD Pharmingen (San
518 Diego, CA). PE-Cyanine7 anti-F4/80 (clone, BM8), anti-IL-13 (clone, eBio13A), anti-
519 CXCR5 (clone, L138D7), AlexaFlouro 700- conjugated anti-MHC II (clone, M5/114),
520 Live/dead Fixable Yellow stain (Qdot605 dead cell exclusion dye) were purchased
521 from eBiosciences. Biotin-labelled antibodies were detected by Texas Red
522 conjugated PE (BD Biosciences). For staining, cells (1×10^6) were stained and
523 washed in PBS, 3% FCS FACS buffer. For intracellular cytokine staining, cells were
524 restimulated with phorbol myristate acetate (Sigma-Aldrich) (50 ng/mL), ionomycin
525 (Sigma-Aldrich) (250ng/mL), and monensin (Sigma-Aldrich) (200mM in IMDM/10%
526 FCS) for 5h at 37°C then fixed in 2% PFA, permeabilised with Foxp3 transcriptional
527 factor staining buffer kit (eBioscience) before intracellular staining with appropriate
528 cytokine antibodies and acquisition through LSR Fortessa machine (BD
529 Immunocytometry system, San Jose, CA, USA) and data was analysed using Flowjo
530 software (Treestar, Ashland, OR, USA).

531

532 *Histology*

533 Left upper lung lobes was fixed in 4% formaldehyde/PBS and embedded in paraffin.
534 Tissue sections were stained with periodic acid-Schiff for mucus secretion, and
535 haematoxylin and eosin (H&E) stain for inflammation. Slides were scanned at 20x

magnification on the virtual slide VS120 microscope (Olympus, Japan). Downstream processing of images was done through Image J (FIJI) for image extraction at series 15 and Ilastik software was used for mucus area quantification on whole lung sections. Data shown is representative of 1 experiment of 3 independent experiments (n = 5-7 mice per experiment).

Antibody and cytokine ELISAs

Antibody ELISAs were carried out as previously described using 10 µg/ml HDM to coat for specific IgGs. Total IgE in serum was measured using anti-mouse IgE (BD Biosciences, 553413) to coat, mouse IgE (κ, anti-TNP, BD Biosciences, 557079) as standard and biotin anti-mouse IgE (BD Biosciences, 553419) as secondary antibody.

For *in vitro* cytokine production analysis, single cell suspensions were prepared from mediastinal lymph nodes of HDM-treated and littermate control mice. Cells (2×10^5 cells, in 200µL) were incubated for 5 days in RPMI/10% FCS (Delta Bioproducts, Kempton Park, South Africa) in 96-well plates. Cells were either stimulated with HDM (30µg/mL) or anti-CD3 (10µg/mL) and supernatants were collected after a 5-day incubation period. Concentrations of IL-4, IL-5 (BD Biosciences) and IL-13 (R&D Systems, Minneapolis, Minn), were measured using ELISA assays according to the manufacturer's protocol.

RNA Extraction

Small lung lobe was frozen in Qiazol (Qiagen, Germany) and stored at -80°C. Total RNA was isolated from the lysate using miRNeasy Mini kit (Qiagen, Germany) according to the manufacturer's instructions. RNA quantity and purity were

561 measured using the ND-1000 NanoDrop spectrophotometer (ThermoScientific, DE,
562 USA).

563

564 *cDNA Synthesis and RT-qPCR*

565 For *Muc5a* gene expression analysis, 100 ng total RNA was reverse-transcribed into
566 cDNA using Transcriptor First Strand cDNA Synthesis Kit (Roche, Germany)
567 according to the manufacturer's instructions. Quantitative real-time PCR (RT-qPCR)
568 was performed using LightCycler® 480 SYBR Green I Master (Roche, Germany)
569 and *Muc5a* primers (IDT, CA, USA). Fold change in gene expression was calculated
570 by the $\Delta\Delta C_t$ method and normalized to β -*actin* which was used as an internal
571 control.

572

573 *Whole lung RNA sequencing*

574 Whole lung RNA was extracted using RNAeasy kit (Qiagen, Germany) according to
575 the manufacturer's instructions. We used Agilent 2100 Bio analyzer (Agilent RNA
576 6000 Nano Kit) to do the total RNA sample QC: RNA concentration, RIN
577 value, 28S/18S and the fragment length distribution. The first step in the workflow
578 involved purifying the poly-A containing mRNA molecules using poly-T oligo attached
579 magnetic beads. Following purification, the mRNA was fragmented into small pieces
580 using divalent cations under elevated temperature. The cleaved RNA fragments
581 were copied into first strand cDNA using reverse transcriptase and random primers.
582 This was followed by second strand cDNA synthesis using DNA Polymerase I and
583 RNase H. These cDNA fragments had addition of a single 'A' base and subsequent
584 ligation of the adapter. The products were then purified and enriched with PCR
585 amplification. PCR yields were quantified by Qubit and pooled samples together to

586 make a single strand DNA circle (ssDNA circle), which gave the final library. DNA
587 nanoballs (DNBs) were generated with the ssDNA circle by rolling circle replication
588 (RCR) to enlarge the fluorescent signals at the sequencing process. The DNBs were
589 loaded into the patterned nanoarrays and pair-end reads of 100 bp were read
590 through on the DNBseq platform for the following data analysis study. For this step,
591 the DNBseq platform combines the DNA nanoball-based nanoarrays and stepwise
592 sequencing using Combinational Probe-Ancor Synthesis Sequencing Method.

593

594 *Bioinformatics workflow*

595 The ribosomal RNA (rRNA) was first removed using SortMeRNA. We then did the
596 Fastq file quality control using Fastqc and multiqc software to assess the quality of
597 the raw reads followed by adapter trimming using Trim galore The reads were then
598 aligned to the mouse reference genome (mm10_UCSC_20180903) using STAR
599 aligner. The map read counts was then extracted using featurecounts. The gene
600 differential analysis was conducted using the DEseq2. The genes with LFC ≥ 2 and
601 adjusted p-value ≤ 0.05 was used to do the gene ontology over-representation
602 analysis done using clusterProfiler(33) Bioconductor package. The Benjamini-
603 Hochnurg method was used for multiple test correction.

604

605 *Human bronchial Airway Smooth Muscle cell culture*

606 Human bronchial smooth muscle cell line (BSMC, Lonza) was cultured in Smooth
607 Muscle Growth Medium (Lonza) with 5% (vol/vol) FCS in a T25 flask (Lonza) until
608 confluence. After two passages in T75 flask, confluent cells were seeded at 1.6×10^5
609 BSMC cells onto 24-well trays and immediately transfected with CRISPR/Cas9
610 single guide RNAs.

611 *CRISPR/Cas9 single guide RNA transfections*

612 single guide RNAs targeting human *BAIAP2L1* (# CD.Cas9.CXVQ6494.AA);
613 *ERDR1*, (# CD.Cas9.YFVV2490.AA); HPRT Negative Control (#Alt-R® CRISPR-
614 Cas9crRNA) were purchased (IDT, CA, USA via WhiteScientific PTY LTD). Single
615 guide RNA (1 µM) and Cas9 enzyme (1 µM) in Opti-MEM medium (Life
616 Technologies™, Carlsbad, CA, USA) was transfected using Lipofectamine RNAi
617 Max 1000 (Thermo Scientific) into BSMC cells (1.6×10^5) per well for 48 hours. Gene
618 deletion was confirmed by DNA extraction (Wizard Genomic DNA Purif. Kit,
619 Promega) and PCR amplification of target genes using *BAIAP2L1* forward
620 (GTCCCGGGGGCCCGA) and reverse (AAGCGCCCAAGAATGTGGGG) primers.

621

622 *Cytotoxic detection Assay*

623 To measure cytotoxicity after single guide RNA transfection, lactate dehydrogenase
624 (LDH) was measured in supernatants collected at 48 hours post transfection using
625 cytotoxicity detection kit ^{PLUS} Assay (CYTODET-RO, Roche) according to
626 manufacturer's instructions and plates were read at 490 nm.

627

628 *Single cell force cytometry using Fluorescently Labelled Elastomeric Contractible*
629 *Surface (FLECS) technology*

630 BSMCs were seeded on Fibronectin-coated elastomeric micropatterns (Forcyte
631 Biotechnologies #F2AX0G03Y, 50 micron, Alexa Fluor 488 - bound Fibrinogen, 24-
632 well plate) at a concentration of 75,000 cells per well in Smooth muscle growth
633 medium (SmGM) (Lonza) and left to adhere and spread for 90 minutes. Unattached
634 cells were then removed and fresh SmGM medium supplemented with rhTNF-α (10
635 ng/mL)/rhIL-13 (100 ng/mL)/ACh (10µM) was added. BSMCs were stimulated for 3

636 hrs before being fixed in pre-warmed to 37°C 4% PFA. Fixed samples were washed
637 then stained with ATTO-565 phalloidin (ATTO-TEC) and DAPI (Life Technologies)
638 and imaged on a StellarVision microscope using Synthetic Aperture Optics
639 technology (Optical Biosystems). All images were analyzed using Fiji software.

640

641 *Statistical analysis*

642 P-values were calculated in GraphPad Prism 6 (GraphPad Software, Inc) by using
643 nonparametric Mann-Whitney Student's t-test or Two-way ANOVA with Bonferroni's
644 post-test for multiple comparisons, and results are presented as standard error of the
645 mean (SEM) or mean of standard deviation (SD). Differences were considered
646 significant if P was <0.05.

647

648

SUPPLEMENTARY FIGURE LEGENDS

Fig. S1.

Supplementary Figure 1

IgM-deficiency leads to reduced airway hyperresponsiveness, but eosinophils and B cells are unaffected.

(A) Schematic diagram showing sensitisation and challenge protocol where mice (IgM^{-/-}) and wild type littermate control (WT) were sensitised with HDM 100 µg intra-tracheally on days 0 and challenged with HDM 10 µg on days 8-12. Analysis was done on day 15.

(B) Airway resistance and elastance were measured with increasing doses of acetylcholine (0-40 mg/mL).

(C) Frequencies of lung and mediastinal lymph node B cells (live⁺B220⁺CD19⁺MHCII⁺) and were stained and analysed by Flow cytometry.

(D) Frequencies of lung eosinophils (live⁺Siglec-F⁺CD11c⁻) were stained and analysed by Flow cytometry.

664 (E) HDM-specific IgM in serum

665 Shown is mean $\pm$ SEMs from two pooled experiment (n= 10-12). Significant
666 differences between groups were performed by Two way ANOVA with Benforroni
667 post-test and are described as: *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001.

668

Fig. S2.

Supplementary Figure 2

Reduced airway hyperresponsiveness in IgM-deficient mice is independent of mouse background in HDM induced asthma.

(A) Mice (IgM^{-/-} and WT) were sensitised and challenged as in Fig. 1, A and Airway resistance and elastance were measured with increasing doses of acetyl methacholine (0 -320 mg/mL).

(B) Histology analyses of lung sections (magnification x20), stained with periodic acid-Schiff. A.U., Arbitrary units.

(C) Total IgE, HDM-specific IgM, HDM-specific IgG1, HDM-specific IgG2a and HDM-specific IgE in serum.

Shown is mean $\pm$ SDs from 1 representative experiment of 2 independent experiments (n= 4-6). Significant differences between groups were performed by student t-test (Mann-Whitney) (C) or Two way ANOVA with Benforroni post-test and are described as: *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001.

Fig. S3.

Supplementary Figure 3

B cell development is not impacted by lack of IgM, however, IgD expression is upregulated in all tissues.

(A) B cell expression of IgM and IgD in IgM^{-/-} and WT mice treated with HDM in mediastinal lymph nodes, **(B)** Peritoneal and **(C)** Spleen.

(D) Bone marrow was flushed out IgM^{-/-} and WT mice treated with HDM and stained for B1 cells (CD5⁺CD43⁺) and pre-B cells (CD43⁺BP-1⁺).

Fig. S4.

Supplementary Figure 4

Reduced airway hyperresponsiveness in IgM-deficient mice is independent of allergen

IgM^{-/-} and wild type (WT) were sensitised intraperitoneally with (50µg in 200µl) of ovalbumin (OVA) adsorbed to 0.65% alum on days 0, 7, 14. On days 23, 24, 25, mice were intranasally challenged with 100µg of OVA. AHR was measured on day 26.

(A) Airway resistance and elastance were measured with increasing doses of acetyl methacholine (0 -40 mg/mL).

(B) Frequencies of lung eosinophils (live⁺Siglec-F⁺CD11c⁻) were stained and analysed by Flow cytometry.

(C) IgM^{-/-} and wild type (WT) were challenged with 25 µg of papain on days 1, 2 and 3 or PBS. AHR was measured on day 4. Airway resistance and elastance were measured with increasing doses of acetyl methacholine (0 -40 mg/mL).

707 (D) Frequencies of lung B cells (live⁺B220⁺CD19⁺MHCII⁺) and eosinophils (live⁺Siglec-
708 F⁺CD11c⁻) were stained and analysed by Flow cytometry.

709 Shown is mean $\pm$ SEMs from two pooled experiment (n= 10-12). Significant
710 differences between groups were performed by Two way ANOVA with Benforroni
711 post-test and are described as: **p<0.01, ***p<0.001, ****p< 0.0001.

Fig. S5.

Supplementary Figure 5

B cell and bone marrow reconstitution does not restore airway

hyperresponsiveness in IgM-deficient mice in HDM induced allergic asthma.

(A) Schematic diagram showing sorted B cells (live⁺B220⁺CD19⁺) (2-5x10⁶) or bone marrow cells (10x10⁶) transferred from congenic WT (CD45.1) to IgM^{-/-} a day before being sensitised as shown in Fig. 1,A.

(B) Airway resistance was measured with increasing doses of acetyl methacholine (0 -40 mg/mL).

(C) Total IgE and IgM in serum of mice reconstituted with WT B cells or bone marrow.

(D) Representative FACS plots of CD45.1 cells in B220 cells (live⁺CD45.1⁺B220⁺) IN spleen, bone marrow, lung and mediastinal lymph nodes.

Shown is mean ±SD from 1 experiment (n= 4-6). Significant differences between groups were performed by Two-way ANOVA with Benforroni post-test and are described as: ***p<0.001.

Fig. S6.

Supplementary Figure 6

Genes associated with muscle contraction are downregulated in IgM-deficient mice. Gene set enrichment analysis (GSEA) showing gene ratio of activated and suppressed pathways from lung RNA-Seq data from WT mice and IgM^{-/-} mice. Related to Fig. 3, F.

Fig. S7.

Supplementary Figure 7

Targeting and validation of BAIAP2L1 deletion by CRISPR.

(A) Schematic of human BAIAP2L1 coding region showing conserved N-terminal IRTKS domain and C-terminal SH3-like domain.

(B) Genomic sequence of show target sgRNA1 region (yellow), sgRNA 2 (blue) and primer 1 regions (green) and primer 2 regions.

(C) DNA gel (1.6%) from PCR showing DNA ladder and a 1000bp product in scramble sgRNA and reduced band in BAIAP2L1 sgRNA using primer 2 region for PCR.

(D) Lactate dehydrogenase (LDH) assay showing absorbance at 490 nm in supernatants from sgRNA transfected cells treated with media alone, TNF-α or acetylcholine. Positive control are dead cells killed by 1% Triton X and control is media alone.

Fig. S8.

Supplementary Figure 9

Reduced airway hyperresponsiveness in IgM-deficient mice is independent of microbial influence in HDM induced asthma.

(A) Schematic diagram showing treatment of IgM^{-/-} and WT mice with antibiotic mixture 3 times a week for 2 weeks via oral gavage. These mice were then sensitised and challenged as in Fig. 1, A.

(B) Airway resistance and elastance were measured with increasing doses of acetylcholine (0 -40 mg/mL).

(C) Total IgE, HDM-specific IgG2a, HDM-specific IgG1, HDM-specific IgE and HDM-specific IgM in serum.

Shown is mean $\pm$ SDs from 1 experiment (n= 4-6). Significant differences between groups were performed by student t-test (Mann-Whitney) and are described as: *p<0.05, **p<0.01, ***p<0.001, ****p< 0.0001.