

Epithelial damage and tissue $\gamma\delta$ T cells promote a unique tumor-protective IgE response

Greg Crawford¹, Mark David Hayes¹, Rocio Castro Seoane¹, Sophie Ward¹, Tim Dalessandri¹, Chester Lai², Eugene Healy², David Kipling³, Charlotte Proby⁴, Colin Moyes⁵, Kile Green⁶, Katie Best^{6,7}, Muzlifah Haniffa^{6,7}, Marina Botto¹, Deborah Dunn-Walters⁸ and Jessica Strid^{1*}

IgE is an ancient and conserved immunoglobulin isotype with potent immunological function. Nevertheless, the regulation of IgE responses remains an enigma, and evidence of a role for IgE in host defense is limited. Here we report that topical exposure to a common environmental DNA-damaging xenobiotic initiated stress surveillance by $\gamma\delta$ TCR⁺ intraepithelial lymphocytes that resulted in class switching to IgE in B cells and the accumulation of autoreactive IgE. High-throughput antibody sequencing revealed that $\gamma\delta$ T cells shaped the IgE repertoire by supporting specific variable-diversity-joining (VDJ) rearrangements with unique characteristics of the complementarity-determining region CDRH3. This endogenous IgE response, via the IgE receptor Fc ϵ RI, provided protection against epithelial carcinogenesis, and expression of the gene encoding Fc ϵ RI in human squamous-cell carcinoma correlated with good disease prognosis. These data indicate a joint role for immunosurveillance by T cells and by B cells in epithelial tissues and suggest that IgE is part of the host defense against epithelial damage and tumor development.

IgE is an ancient and highly conserved immunoglobulin isotype found in all mammals¹. It is thought that IgE has evolved to provide protection against infection by macroparasites, such as helminths. However, although the abundance of IgE is elevated in both mice and humans with helminth infection, IgE is not critical for protective immunity against helminths, and much of the IgE raised is not parasite specific². An alternative hypothesis suggests that IgE is important for immune responses to environmental toxins such as venoms³, and indeed, published data indicate that IgE can provide protection against bee venom and limit snake-venom toxicity^{4–6}. Furthermore, aberrant IgE responses that cause allergies are frequently directed against environmental irritants and non-replicating agents. A role for IgE in defending the host against immediate danger would be consistent with the very rapid mobilization of its effector functions. Therefore, a broader paradigm proposes that IgE represents an arm of early immunological host defense against xenobiotics or large parasites that threaten tissue integrity⁷. However, what drives these IgE responses in vivo, what they recognize and whether they have a wider role in immunological defense remain unknown.

IgE responses occur most frequently in epithelial tissues such as the skin, lungs and gut, which contain resident IgE-binding cells. These barrier surfaces are continuously exposed to challenges from environmental xenobiotics, and a prominent feature of their response to external insults is the induction of type 2 immunity⁸ and IgE^{9,10}. In mouse skin epithelia, a resident $\gamma\delta$ TCR⁺ population of intraepithelial lymphocytes (IELs) controls tissue homeostasis via the induction of IL-13¹¹, a type 2 cytokine, and also promotes IgE after challenge with protein allergens on stressed tissue¹². These IELs can directly sense epithelial-cell dysregulation via stress-sensing

receptors, such as NKG2D, and this pathway has a key role in promoting the IgE response^{12,13}. The ability of IELs to detect epithelial-cell stress and initiate a restorative response has been called 'lymphoid stress surveillance' and has an important role in the early detection of stressed and pre-malignant cells¹³. Indeed, tissue-specific IELs regulate epithelial tissue integrity and are key to protection of the host against carcinogenesis^{11,13}.

Many toxins and xenobiotics are also carcinogens. Hence, in defending against environmental xenobiotics, IgE might simultaneously confer protection against transformation and against cancer in exposed tissues. Here we report the development of a potent and dominant IgE response following topical exposure to the common environmental xenobiotic and carcinogen DMBA (7,12-dimethylbenz[a]anthracene). This endogenous IgE response was autoreactive and was required for protection against cutaneous carcinogenesis. Furthermore, we found that tissue-resident $\gamma\delta$ TCR⁺ IELs had a unique role in initiating and regulating IgE production, driving an early, innate-like response, which directed a subsequent adaptive response supported by IL-4-producing CD4⁺ $\alpha\beta$ T cells. Deep sequencing of IgE and IgG1 from germinal-center (GC) B cells and plasma cells (PCs) indicated that $\gamma\delta$ TCR⁺ IELs shaped a distinct IgE repertoire that was dependent on the presence of DNA damage in epithelial cells. Thus, lymphoid stress surveillance promoted a unique IgE response that was part of an early host-defense mechanism that provides protection against cancer.

Results

Carcinogen-induced epithelial-cell damage triggers IgE responses. DNA-damaging xenobiotics such as DMBA are

¹Department of Medicine, Imperial College London, London, UK. ²Dermatopharmacology, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; Dermatology, University Hospital Southampton NHS Foundation Trust, Southampton, UK.

³Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, UK. ⁴Division of Cancer Research, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK. ⁵Department of Pathology, Greater Glasgow and Clyde NHS, Queen Elizabeth University Hospital, Glasgow, UK. ⁶Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK. ⁷Department of Dermatology and Newcastle Biomedical Research Centre, Royal Victoria Infirmary, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. ⁸Faculty of Health and Medical Sciences, School of Biosciences and Medicine, University of Surrey, Guildford, Surrey, UK. *e-mail: j.strid@imperial.ac.uk

commonly found in the environment and have been linked to carcinogenesis^{14,15}. We found that a single topical application of DMBA to the back skin of wild-type mice induced an IgE response in serum within 4 d (Fig. 1a). Exposure to DMBA was associated with upregulation of the NKG2D ligand Rae-1 on skin epithelial cells (Supplementary Fig. 1a), indicative of epithelial-cell stress. The number of epithelial cells containing breaks in double-stranded DNA (dsDNA), assessed by staining for the phosphorylated histone H2A variant H2AX (γ H2AX), peaked between 3 d and 7 d after exposure to DMBA (Supplementary Fig. 1b,c). Epidermal Langerhans cells (LCs) must metabolize DMBA to the more mutagenic compound DMBA-trans-3,4-dihydrodiol to cause substantial damage to DNA. Langerin-DTA mice, which do not have LCs (and have transgenic expression of the gene encoding human Langerin, modified to drive expression of diphtheria toxin subunit A (DTA)), showed less epithelial-cell DNA damage following a single dose of DMBA¹⁶ than did their non-transgenic littermates (control mice) (Supplementary Fig. 1d) and failed to show increased serum IgE, in contrast to the control mice (Fig. 1b). However, Langerin-DTA mice generated IgE in response to other topical challenges¹² and, when exposed to DMBA repeatedly, they developed both DNA damage and IgE responses (Supplementary Fig. 1d,e). Other DNA-damaging skin challenges, such as ultraviolet (UV) irradiation, also induced IgE (Supplementary Fig. 1f,g). Once-weekly exposure to DMBA led to the development of papillomas and squamous-cell carcinomas (SCCs) after 8–15 weeks. This was associated with high concentrations of serum IgE, which rose progressively as epithelial DNA damage accumulated (Fig. 1c). When DMBA was given daily for 5 d only, mice developed skin tumors, and their serum IgE showed a progressive increase for 12 weeks (Supplementary Fig. 1h). The systemic IgE responses were paralleled by the infiltration and accumulation of IgE in acutely damaged skin (Fig. 1d) and in skin tumors (Fig. 1d,e). The tissue IgE was carried mainly by basophils expressing Fc ϵ RI, the high-affinity receptor for IgE (Fig. 1d). More mature transcripts encoding IgE were present in the tumor tissue than in the adjacent skin, indicative of some local IgE production, whereas transcripts encoding IgG1 and IgM were lower in abundance in tumors than in adjacent skin (Fig. 1f). These data indicated that the epithelial-cell damage triggered by exposure to DMBA potentially promoted the production of IgE that accumulated in the resulting skin tumors.

Topical exposure to a carcinogen induces class switching in local B cells. To investigate the origin of the DMBA-induced IgE response, we assessed B cells in the skin-draining lymph nodes (LNs) during acute exposure to DMBA (before malignancy). Two applications of DMBA, 3 d apart, on the ear skin induced the enlargement of skin-draining LNs plus the formation of GCs and class switching of GC B cells (Fig. 2a), as well as an increased number of CD138⁺ PCs, which had class switched mainly to IgE and IgG1, with little or no induction of IgG2a (Fig. 2b). There was no induction of GCs or class-switched PCs in the spleen (data not shown), which suggested that the response was localized to the skin-draining LNs. Weekly exposure to DMBA on the back skin for 5 weeks also induced GCs and IgE⁺ and IgG1⁺ PCs. However, under these conditions, the GC structures and the IgG1⁺ PCs diminished from week 3, which resulted in a dominant IgE response (Fig. 2c). By week 5 after exposure, nearly 80% of all CD138⁺ PCs in the LNs had switched to IgE (Fig. 2c). Thus, exposure of the skin to DMBA promoted a local type 2 B cell response with dominant and prevailing production of IgE.

Carcinogen-induced IgE provides protection against carcinogenesis. To explore whether the IgE response induced by DMBA was important for host defense, we assessed skin-tumor formation in mice deficient in the gene encoding the heavy chain of IgE (*Igh-7*; called *Igh7*^{-/-} here), which thus lack IgE. Following once-weekly

exposure to DMBA, *Igh7*^{-/-} mice developed tumors sooner and more rapidly than did their wild-type counterparts, as well as significantly more and larger tumors than those of their wild-type counterparts (Fig. 3a). The IgG1 response in the DMBA-treated *Igh7*^{-/-} mice was identical to that of their wild-type counterparts (Supplementary Fig. 2a). In addition, the number of Fc ϵ RI⁺ effector cells in their tissue was similar to that of wild-type mice (Supplementary Fig. 3a). Mice deficient in the gene encoding Fc ϵ RI (*Fcer1a*^{-/-} mice) also had enhanced susceptibility to DMBA carcinogenesis relative to that of wild-type mice (Fig. 3b), despite the finding of similar concentrations of IgE and of IgG1 in serum from these mice (Supplementary Fig. 2b); this suggested that the protective effect of IgE was mediated by signaling through Fc ϵ RI. CD45^{lo}cKit⁺Fc ϵ RI⁺ basophils represented the main IgE⁺ cell population in the tumors (Fig. 1d). *Cpa3*^{Cre/+} mice, which have sequence encoding Cre recombinase inserted into the locus encoding mast-cell carboxypeptidase (*Cpa3*) and lack skin mast cells, showed susceptibility to tumor development similar to that of their *Cpa3*^{+/+} littermates following DMBA carcinogenesis (Supplementary Fig. 3b), which suggested that most probably, Fc ϵ RI⁺ basophils mediated the protection against carcinogenesis. Splenic CD45^{lo}cKit⁺CD41⁺Fc ϵ RI⁺ basophils from *Igh7*^{-/-} mice produced IL-4 and IL-6 and degranulated equivalent to their wild-type counterparts after stimulation with the phorbol ester PMA and ionomycin; however, the skin-tumor cytokine microenvironment was significantly altered in *Igh7*^{-/-} mice relative to that in wild-type mice (Supplementary Fig. 3c–e).

To investigate whether similar IgE responses occur in humans, we analyzed newly incised skin SSC samples from 12 patients. Fc ϵ RI⁺ cells were present in all tumor and peri-lesional skin samples, varying in frequency from around 1% to over 40% of the tumor infiltrating CD45⁺ leukocytes (Fig. 3c). The Fc ϵ RI⁺ cells were present mainly in the peritumoral infiltrate at the interface between the stroma and the neoplastic keratinocytes, with some entering the tumor and/or hair follicle (average, 11.7 ± 2.9 cells per mm² (mean $\pm$ s.e.m.)) (Supplementary Fig. 4). Fc ϵ RI⁺ cells accumulated more in the skin than in blood samples from the same donor; however, tumor tissue contained fewer Fc ϵ RI⁺ cells than did histopathologically ‘healthy’ peri-lesional skin from the same donor (Fig. 3c). In an additional cohort of 56 patients with SCC, gene-expression analysis revealed that expression of *FCER1A* mRNA was higher in unaffected skin areas than in developed tumors, with the lowest expression of *FCER1A* mRNA detected in high-risk and metastatic SCC (Fig. 3d). A significant linear trend was observed between the expression of *FCER1A* mRNA and the risk of more-advanced SCC (Fig. 3d). Together these data indicated that IgE, via Fc ϵ RI, provided protection against DMBA-induced SCC and that the presence of fewer Fc ϵ RI-expressing leukocytes in human SSC correlated with the risk of more-severe disease.

Carcinogen-induced humoral immunity depends on $\alpha\beta$ T cell-derived IL-4. Conventional and unconventional modes of inducing class switching to IgE have been described⁸. We first explored whether induction of tumor-protective IgE was dependent on $\alpha\beta$ T cells. Mice deficient in the β -chain of the T cell antigen receptor (TCR) (*Tcrb*^{-/-} mice), which lack $\alpha\beta$ T cells, had no enlargement of skin-draining LNs, no GCs and no IgG1⁺ or IgE⁺ PCs following two topical applications of DMBA to the ear skin, in contrast to their wild-type counterparts (Fig. 4a). The response was restored by the transfer of polyclonal CD4⁺ T cells into *Tcrb*^{-/-} mice, but not by the transfer of OT-II CD4⁺ T cells, which have transgenic expression of a major histocompatibility complex class II-restricted (ovalbumin-specific) TCR (Supplementary Fig. 5a); this indicated that it was dependent on a normal TCR repertoire. Furthermore, injection of blocking antibody to the B cell-stimulatory molecule CD40L partially inhibited LN enlargement and repressed GC formation and the generation of IgE⁺ and IgG1⁺ PCs in wild-type mice

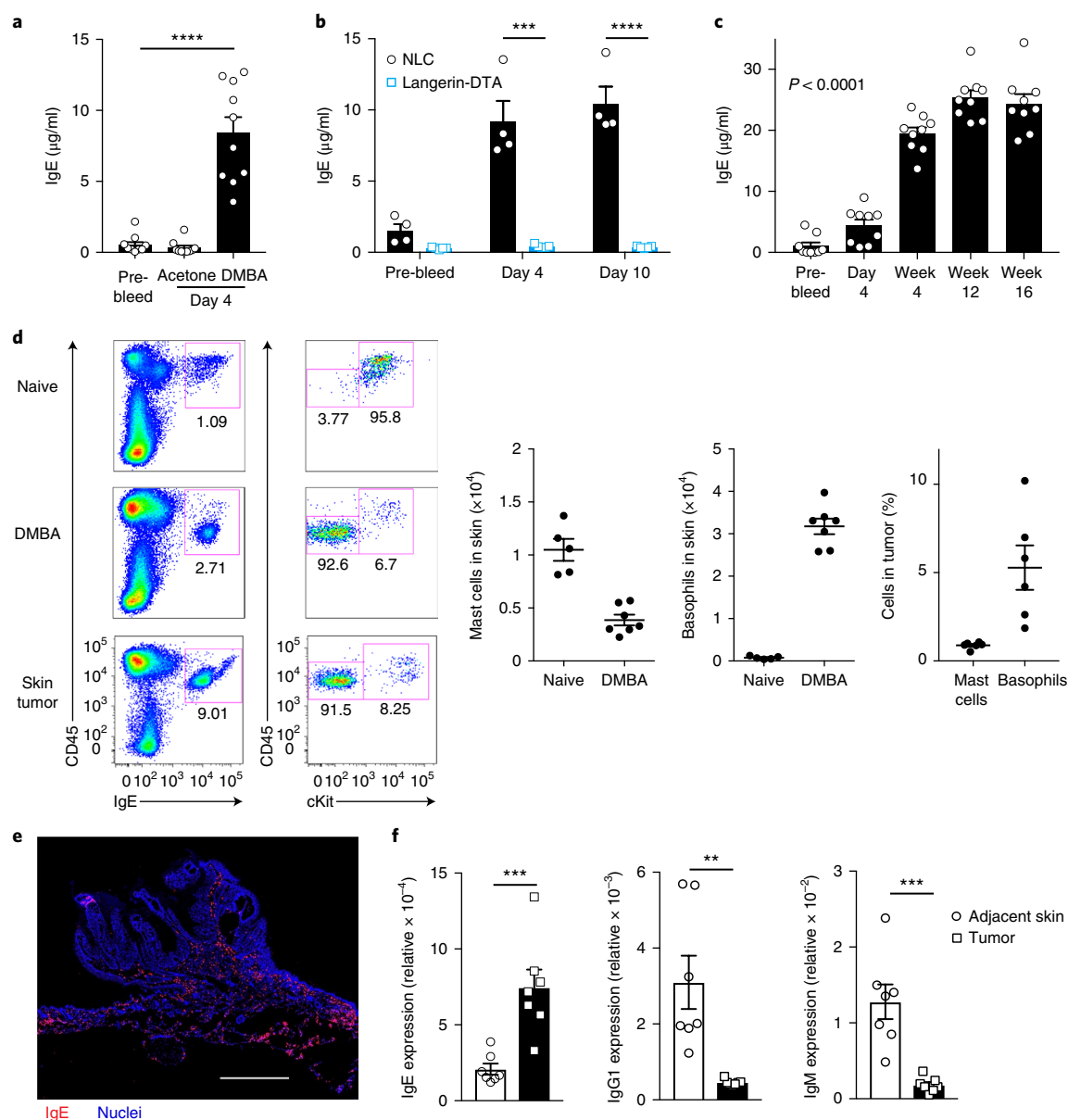


Fig. 1 | Carcinogen-induced epithelial-cell damage triggers a rapid local and systemic IgE response. **a–c**, ELISA of IgE in serum from blood obtained from wild-type FVB mice (n=10) (**a**) or from Langerin-DTA mice (n=5) and their non-transgenic littermates (control mice) (NLC) (n=4) (**b**) before treatment (Pre-bleed) and 4 d after treatment with a single topical dose of 200 nmol DMBA or vehicle control (Acetone) on shaved back skin (**a,b**) and from wild-type FVB mice (n=9 per group) at various times (horizontal axis) before and after topical exposure to 200 nmol DMBA once weekly (**c**). **d**, Flow cytometry (left) of IgE-bearing cells in naive skin, skin 7 d after exposure to DMBA and DMBA-induced skin tumors (left margin), and quantification of those cells from such mice (right); cells in tumor presented as frequency among CD45⁺ cells: n=5 mice (naive skin), n=7 mice (DMBA-treated skin) and n=6 mice (tumors). Numbers adjacent to outlined areas (left half) indicate percent CD45⁺IgE⁺ cells (far left) or CD45^{lo}cKit⁺IgE⁺ cells (basophils) (left gate) and CD45^{hi}cKit⁺IgE⁺ cells (mast cells) (right gate) (middle left). **e**, Microscopy (tile scans) of IgE staining (red) in a DMBA-induced tumor; nuclei are blue. Scale bar, 1 mm. **f**, Quantitative RT-PCR analysis of mature transcripts encoding IgE, IgG1 or IgM in tumors or tumor-adjacent skin (key) in mice (n=7 per group) after DMBA-induced carcinogenesis; results are presented relative to those of the control gene encoding cyclophilin. Each symbol (**a–d,f**) represents an individual mouse; small horizontal lines (**d**) indicate the mean (± s.e.m.). ***P < 0.001, **P < 0.01, ****P < 0.0001 (two-tailed unpaired Student's t-test (**a,f**), multiple t-tests (**b**) or one-way analysis of variance (ANOVA) testing for linear trend of IgE increase with time (**c**)). Data are representative of three (**a,c,f**), two (**b**) or four (**d**) independent experiments with similar results (**a–d,f**; mean ± s.e.m.) or are representative of six independent tumors (**e**).

(Supplementary Fig. 5b). Similar defects in class switching to IgE⁺ and IgG1⁺ PCs were seen *Il4*^{−/−} mice, which cannot produce IL-4, although *Il4*^{−/−} mice showed an enlargement of LNs similar to that of wild-type mice and had some GC B cells (Fig. 4a). *Il4*^{−/−} mice had some class switching to IgG2a, which was never observed in wild-type or in *Tcrb*^{−/−} mice (data not shown). Chimeras generated

by the transfer of wild-type bone marrow into *Il4*^{−/−} host mice (WT→*Il4*^{−/−} chimeras) underwent induction of IgE⁺ and IgG1⁺ PCs similar to the induction of such cells in WT→WT chimeras following topical treatment with DMBA, whereas *Il4*^{−/−}→WT chimeras underwent significantly less induction of IgE⁺ and IgG1⁺ PCs than did WT→*Il4*^{−/−} chimeras (P < 0.0001 and P < 0.01, respec-

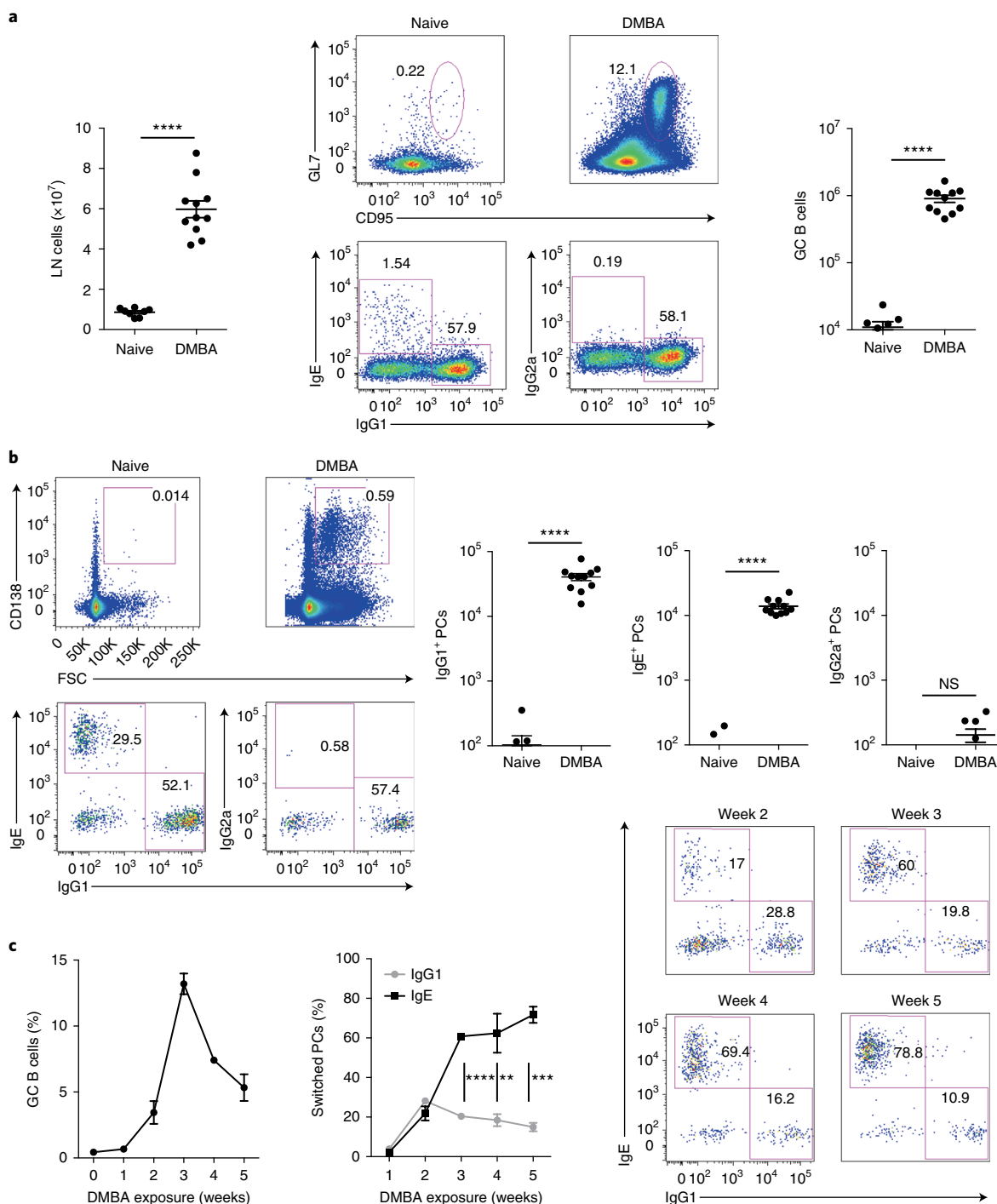


Fig. 2 | Topical exposure to a carcinogen induces local B cell class switching, which results in a dominant IgE response. **a**, Flow cytometry (middle) of GC gated B cells from the skin-draining LNs of wild-type FVB mice left untreated (Naive) or exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart, and analyzed 7 d after the final exposure (DMBA), assessing class switching via intracellular immunoglobulin staining, and quantification of total LN cells (far left) and GC B cells (defined as B220⁺CD95⁺GL7⁺ LN cells throughout) (far right) in such mice ($n=9$ (Naive) or $n=11$ (DMBA)). Numbers adjacent to outlined areas (middle) indicate percent GL7⁺CD95⁺ cells (top row); IgE⁺IgG1⁺ cells (left gate) and IgE⁺IgG1⁺ cells (right gate) (bottom left); or IgG2a⁺IgG1⁺ cells (left gate) and IgG2a⁺IgG1⁺ cells (right gate) (bottom right). **b**, Flow cytometry (left) of PCs in the mice in **a**, with intracellular immunoglobulin staining for analysis of isotype switching, and quantification of IgG1⁺, IgE⁺ or IgG2a⁺ PCs (gated as FSC^{hi}CD95⁺CD138⁺ cells throughout) in such mice (right). Numbers in outlined areas (left) indicate percent CD138⁺FSC^{hi} cells (PCs) (top row) or as in **a** (bottom row). **c**, Flow cytometry (right) of axillary LN B cells from wild-type FVB mice exposed to DMBA once weekly on shaved back skin, assessed at weeks 2–5 after exposure (above plots), and frequency of GC B cells (among B220⁺ lymphocytes) and IgG1⁺ or IgE⁺ (key) class-switched PCs (among CD138⁺ PCs) in such mice ($n=3$ per time point) analyzed weekly for 1–5 weeks (horizontal axis) beginning 7 d after the final exposure (left). Numbers in outlined areas (right), as in **a** (bottom left). Each symbol (**a**, **b**) represents an individual mouse; small horizontal lines indicate the mean ($\pm$ s.e.m.). NS, not significant ($P > 0.05$); * $P < 0.05$, *** $P < 0.001$ and **** $P < 0.0001$ (two-tailed Student's *t*-test for unpaired data). Data are from one experiment representative of five (**a**, **b**) or two (**c**) independent experiments with similar results (mean $\pm$ s.e.m. in **c**).

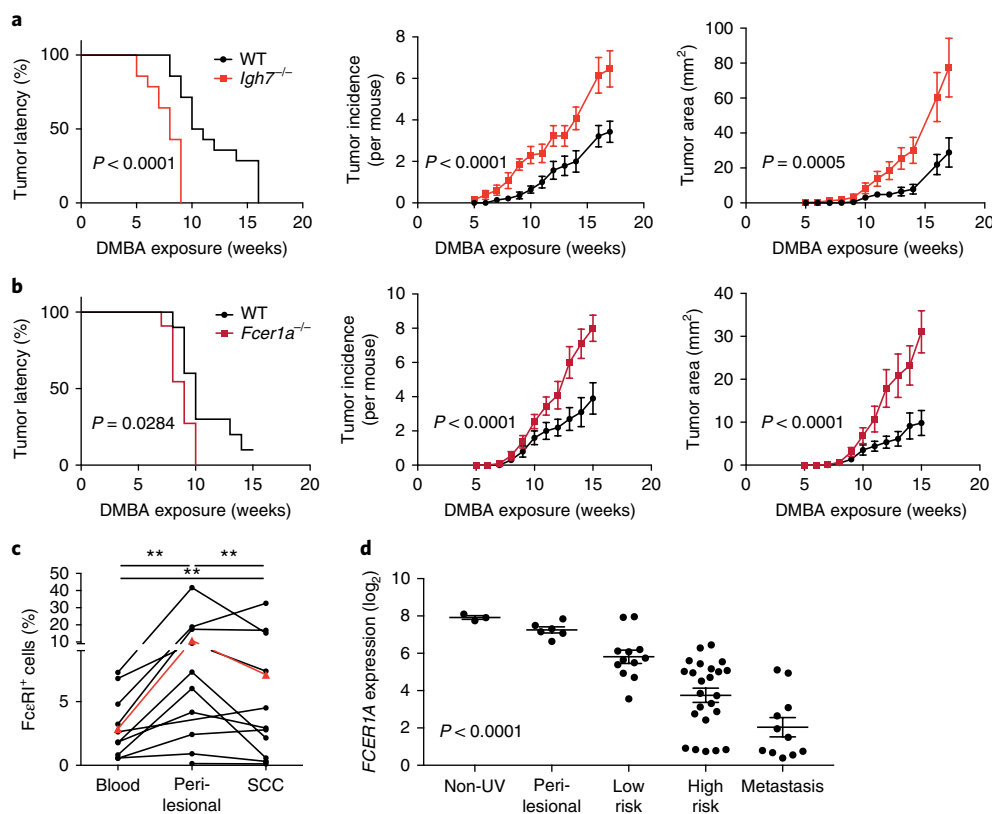


Fig. 3 | Carcinogen-induced IgE provides protection against carcinogenesis. **a, b**, Tumor susceptibility in wild-type (WT) and *Igh7*^{-/-} BALB/c mice (*n* = 14 per group) (**a**) and wild-type (*n* = 10) and *Fcεr1a*^{-/-} (*n* = 11) BALB/c mice (**b**) (key) at various times (horizontal axis) after DMBA-induced carcinogenesis, presented as tumor latency (time to appearance of first tumor; presented as the frequency of tumor-free mice), tumor incidence (average tumors per mouse) and tumor area (average size per mouse). *P* values (in plots), log-rank (Mantel-Cox) test for tumor latency, and linear regression for tumor incidence and area. Data are representative of two independent experiments with similar results (mean ± s.e.m., middle and right). **c**, Frequency of FcεRI⁺ cells among total CD45⁺ leukocyte infiltrates in human SCC tissue (*n* = 12 donors), peri-lesional skin (*n* = 10 donors) and blood samples (each paired with the same tissue and skin donor; *n* = 10 donors) (horizontal axis), assessed by flow cytometry; red triangles, mean proportion of FcεRI⁺ cells in the group; lines connect results for samples from the same donor. ***P* < 0.01 (paired Wilcoxon test). **d**, Expression of *FCER1A* transcripts in human skin and SCC tissue from individual 'risk groups' (assigned scores independently for 'tumor risk' by an experienced dermatopathologist), in unaffected areas (Non-UV; *n* = 3 donors) and peri-lesional tissue (*n* = 6 donors), and from patients with low-risk SCC (*n* = 12 donors) or high-risk SCC (*n* = 24 donors) and metastatic SCC (*n* = 11 donors), assessed with an NanoString nCounter and presented as log₂ values of RNA counts. Each symbol represents an individual donor; small horizontal lines indicate the mean (± s.e.m.). *P* value, one-way ANOVA and testing for linear trend of expression between 'risk groups'.

tively (two-tailed Student's *t*-test for unpaired data); Supplementary Fig. 5c), which indicated that the IL-4 was derived from hematopoietic cells, not from irradiation-resistant skin-resident cells. To determine whether the requirement for αβ T cells in class switching to IgE and/or IgG1 reflected a need for IL-4, we reconstituted *Tcrb*^{-/-} mice with wild-type or *Il4*^{-/-} CD4⁺ T cells 24 h before exposing the host mice to DMBA. Only the wild-type CD4⁺ αβ T cells were able to restore the GC response and the production of IgG1 and/or IgE in the skin-draining LNs of the reconstituted *Tcrb*^{-/-} mice (Fig. 4b). Moreover, *Il4*^{-/-} mice showed a significantly earlier onset of tumor development, a greater incidence of tumors and larger tumors than that of wild-type mice following weekly treatment with DMBA (Fig. 4c), which indicated that IL-4 was required for protection against tumors. Tumor-bearing *Il4*^{-/-} mice did not produce IgE and had diminished production of IgG1, but greater production of IgG2a, in serum than that of their wild-type counterparts (Supplementary Fig. 2c). Thus, αβ T cell-derived IL-4 was critical for IgE production and protection against tumors following exposure to DMBA.

Induction of tumor-protective IgE requires γδTCR⁺ IELs. Skin γδTCR⁺ IELs are key for protective immunity to chemical carcinogenesis and are strong inducers of local type 2 immune responses^{11–13}.

We found that following topical exposure of the ear skin to DMBA, *Tcrd*^{-/-} mice, which lack γδ T cells, had less LN hypertrophy than that of wild-type mice but had a normal number of GC B cells (Fig. 5a). *Tcrd*^{-/-} mice developed IgG1⁺ PCs similar to the development of such cells in wild-type mice but had significantly impaired IgE PC responses compared with those of wild-type mice (Fig. 5a). The decrease in IgE was detected as early as day 4, a time at which the number of IgE⁺ PCs and serum IgE had increased in wild-type mice but remained undetectable in *Tcrd*^{-/-} mice (Fig. 5a,b). GCs were not yet enlarged in wild-type mice at day 4 (Fig. 5a), which suggested that the γδ T cell-dependent IgE response at this time might have been of extrafollicular origin. To explore this, we used CD19^{cre}*Bcl6*^{fl/fl} mice, which have a B cell-specific defect in the transcription factor Bcl-6. CD19^{cre}*Bcl6*^{fl/fl} mice did not develop a GC response following topical application of DMBA, despite LN enlargement, but the number of IgE⁺ and IgG1⁺ PCs in their LNs was equivalent to that in the LNs of their *Bcl6*-heterozygous littermates and wild-type mice (Supplementary Fig. 5d).

We next sought to determine whether the γδ T cells involved in the response were located in the skin itself or in a secondary lymphoid organ such as the draining-LNs. Skin-resident γδTCR⁺ IELs are radiation resistant¹¹, and in *Tcrd*^{-/-}→WT bone-marrow

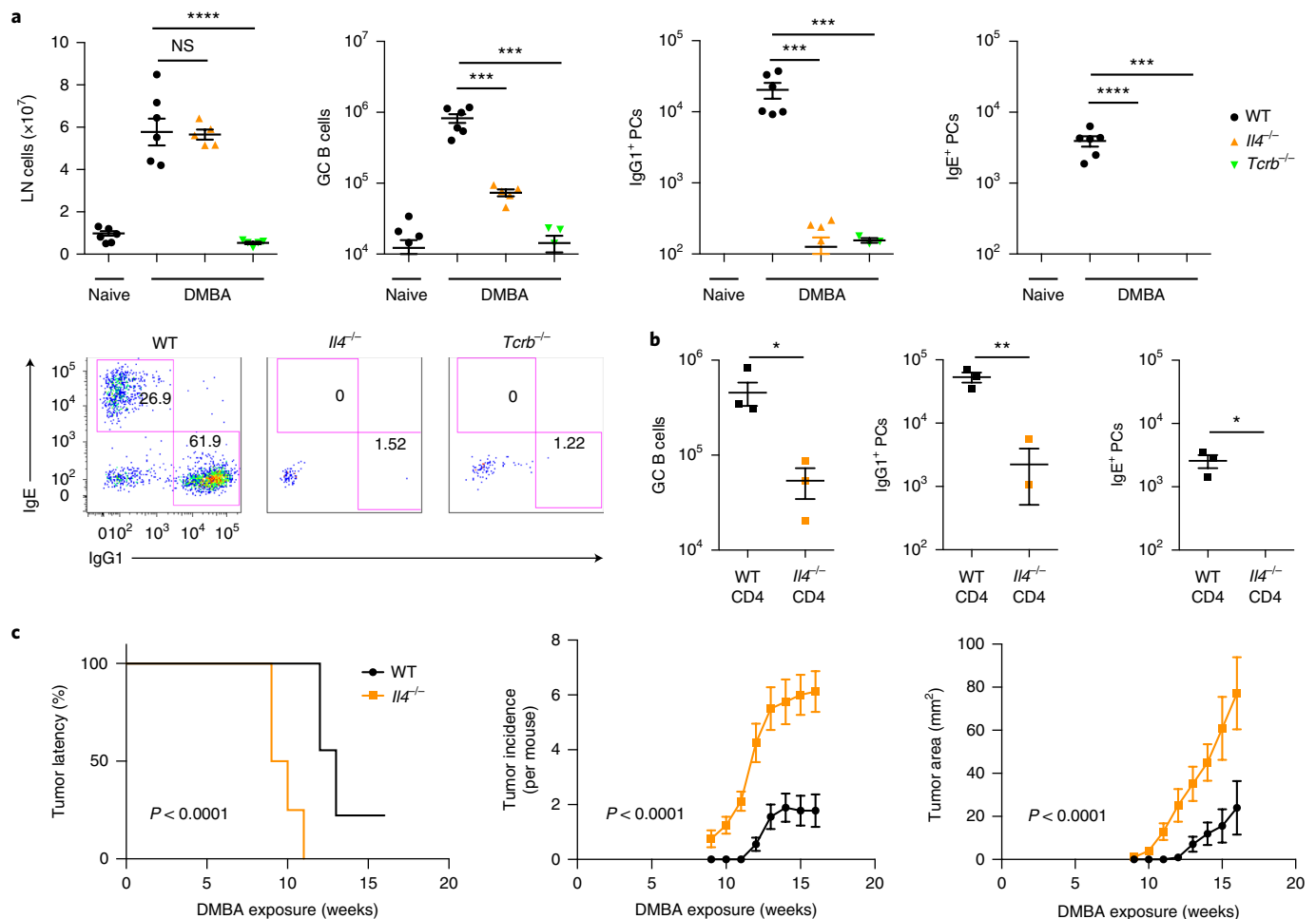


Fig. 4 | Carcinogen-induced humoral immunity depends on $\alpha\beta$ T cell-derived IL-4. **a**, Flow-cytometry analysis of IgE and IgG1 in PCs from the skin-draining LNs of wild-type, $\text{Il4}^{-/-}$ and $\text{Tcrb}^{-/-}$ FVB mice (above plots) exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart, and analyzed 7 d after the final exposure (bottom row), and total LN cells, GC B cells and IgG1^+ or IgE^+ PCs in naive wild-type mice ($n=6$) and such DMBA-treated wild-type FVB mice ($n=6$), $\text{Il4}^{-/-}$ FVB mice ($n=5$) and $\text{Tcrb}^{-/-}$ FVB mice ($n=5$) (key) (top row). Numbers in outlined areas (bottom row) indicate $\text{IgE}^+\text{IgG1}^+$ PCs (top left) or $\text{IgE}^+\text{IgG1}^+$ PCs (bottom right). **b**, Quantification of GC B cells and IgG1^+ or IgE^+ PCs in the skin-draining LNs of $\text{Tcrb}^{-/-}$ mice reconstituted with wild-type CD4^+ $\alpha\beta$ T cells (WT CD4 ; $n=3$ host mice) or $\text{Il4}^{-/-}$ CD4^+ $\alpha\beta$ T cells ($\text{Il4}^{-/-}$ CD4 ; $n=4$ host mice) 1 d before topical exposure of host mice to DMBA as in **a**, assessed by flow cytometry. **c**, Tumor susceptibility of wild-type and $\text{Il4}^{-/-}$ FVB mice ($n=9$ per group) after DMBA-induced carcinogenesis (presented as in Fig. 3a,b). Each symbol (**a,b**) represents an individual mouse; small horizontal lines indicate the mean ($\pm$ s.e.m.). * $P<0.05$, ** $P<0.01$, *** $P<0.001$ and **** $P<0.0001$ (two-tailed Student's *t*-test for unpaired data (**a,b**)), or *P* values (in plots), log-rank (Mantel-Cox) test (tumor latency) or linear regression (tumor incidence and area) (**c**). Data are representative of three independent experiments with similar results (**a,b**); mean $\pm$ s.e.m. (middle and right in **c**).

chimeras or WT $\rightarrow$ $\text{Tcrd}^{-/-}$ bone-marrow chimeras, $\gamma\delta$ T cells were detected only in the skin or LNs, respectively (Fig. 5c). When these chimeras were treated topically with DMBA, IgE was significantly reduced only in WT $\rightarrow$ $\text{Tcrd}^{-/-}$ chimeras, in which $\gamma\delta$ TCR $^+$ IELs were missing from the skin, but $\gamma\delta$ T cells were present in the LNs, compared with the IgE in WT $\rightarrow$ WT chimeras (Fig. 5d). The number of IgG1^+ PCs was similar in $\text{Tcrd}^{-/-}$ $\rightarrow$ WT, WT $\rightarrow$ $\text{Tcrd}^{-/-}$, WT $\rightarrow$ WT and $\text{Tcrd}^{-/-}$ $\rightarrow$ $\text{Tcrd}^{-/-}$ chimeras (Fig. 5d). The abundance of $\text{V}_{\gamma}4^+$ and other 'non- $\text{V}_{\gamma}5^+$ ' $\gamma\delta$ T cells in the skin of $\text{Tcrd}^{-/-}$ $\rightarrow$ WT and WT $\rightarrow$ $\text{Tcrd}^{-/-}$ chimeras was similar to that in the skin of WT $\rightarrow$ WT chimeras (Fig. 5c). In addition, following topical treatment with DMBA, $\text{Tcrd}^{-/-}$ $\rightarrow$ WT mice, which lack only the canonical $\text{V}_{\gamma}5\text{V}_{\delta}1^+$ IELs, had fewer IgE^+ PCs than did wild-type mice (Fig. 5e), which suggested that $\text{V}_{\gamma}5^+$ IELs were most probably the IgE-promoting $\gamma\delta$ T cell subset. These observations indicated that DMBA-induced IgE depended on $\gamma\delta$ TCR $^+$ IELs in the tissue.

$\gamma\delta$ T cells promote a distinct IgE repertoire in response to a carcinogen. To understand the nature of the humoral response produced during epithelial-cell damage, and the role of $\gamma\delta$ T cell immunosurveillance, we sorted B220 $^+$ CD95 $^+$ GL7 $^+$ GC B cells and FSC $^{\text{hi}}$ CD95 $^{\text{hi}}$ CD138 $^+$ PCs from the skin draining LNs of wild-type and $\text{Tcrd}^{-/-}$ mice 7 d after the second of two topical exposures to DMBA. Analysis of the IgG1 and IgE heavy-chain repertoires by high-throughput sequencing revealed that IgE-producing clones had the largest clonal expansion in wild-type mice (Supplementary Fig. 6a). As expected, the average size for IgG1^+ clones was greater among PCs than among GC B cells (Fig. 6a). In contrast, IgE^+ clones among PCs and GC B cells were equivalent and were larger than IgG1^+ clones (Fig. 6a). Furthermore, $\text{Tcrd}^{-/-}$ mice had defective IgE^+ clonal expansion in GC B cells compared with that of wild-type mice, while IgG1^+ clonal expansion was similar (Fig. 6b). The Alakazam tool¹⁷, an analysis framework for the sequencing of adaptive immunoreceptor repertoires, indicated that the IgE^+ PC

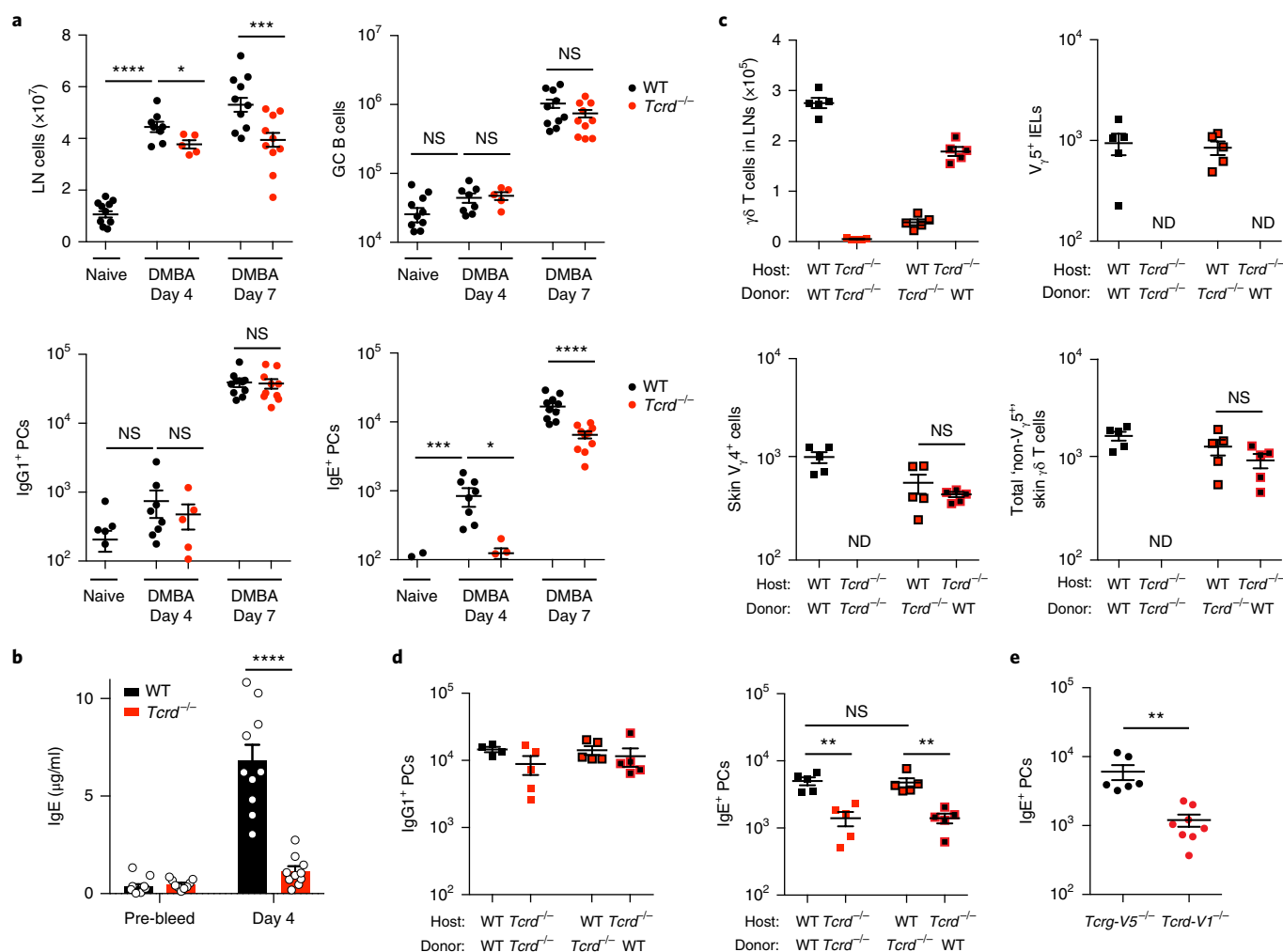


Fig. 5 | Induction of tumor-protective IgE requires $\gamma\delta$ TCR⁺ IELs. **a**, Total LN cells, GC B cells and IgG1⁺ or IgE⁺ PCs in the skin draining LNs of wild-type and *Tcrd*^{-/-} FVB mice (key) left untreated (Naive) or exposed topically to DMBA on the dorsal side of the ears twice, 3 d apart, analyzed by flow cytometry 4 or 7 d after the final exposure (horizontal axis); mice: *n* = 10 (wild-type naive), *n* = 8 (wild-type 4 d), *n* = 10 (wild-type 7 d), *n* = 5 (*Tcrd*^{-/-} 4 d) or *n* = 10 (*Tcrd*^{-/-} 7 d). **b**, ELISA of IgE in serum from wild-type and *Tcrd*^{-/-} mice (key; *n* = 10 per group) topically exposed to DMBA on shaved back skin, with blood obtained before (Pre-bleed) and 4 d after exposure (horizontal axis). **c,d**, Quantification of $\gamma\delta$ T cells in the LNs and V γ 5⁺ IELs, V γ 4⁺ cells and other 'non-V γ 5⁺' $\gamma\delta$ T cells in the skin (**c**) and IgG1⁺ or IgE⁺ PCs in the skin-draining LNs (**d**) of WT $\rightarrow$ WT and *Tcrd*^{-/-} $\rightarrow$ *Tcrd*^{-/-} (control) chimeras and *Tcrd*^{-/-} $\rightarrow$ WT and WT $\rightarrow$ *Tcrd*^{-/-} chimeras (*n* = 5 per group) after exposure to DMBA and analysis (by flow cytometry) at day 7 as in **a**. **e**, Quantification of IgE⁺ PCs in the skin-draining LNs of wild-type FVB mice (*n* = 6) and *Tcrd*^{-/-} *V γ 5⁺* *Tcrd*^{-/-} *V γ 1⁺* FVB mice (*n* = 8) exposed to DMBA and analyzed as in **a**. ND, not detected. Each symbol represents an individual mouse; small horizontal lines (**a**, **c**–**e**) indicate the mean ($\pm$ s.e.m.). **P* < 0.05, ***P* < 0.01, ****P* < 0.001 and *****P* < 0.0001 (one-way ANOVA multiple comparison (**a**–**d**) or two-tailed Student's *t*-test for unpaired data (**e**)). Data are from one experiment representative of three (**a**, **b**) or two (**c**, **d**) independent experiments with similar results (mean $\pm$ s.e.m. in **b**).

repertoire was significantly less diverse in *Tcrd*^{-/-} mice than in wild-type mice in all but the most common clones (Fig. 6c). In contrast, the diversity of the IgG1⁺ PC repertoire in *Tcrd*^{-/-} mice was similar to that of wild-type mice (Fig. 6c).

We also analyzed the physicochemical properties of the complementarity-determining CDRH3 regions by principal-component analysis (PCA) of Kidera factors¹⁸, which are a set of independent factors that encapsulate information from 188 different biophysical characteristics of all 20 amino acids. We observed distinct differences between GC B cells and PCs, as well as between IgG1-switched clones and IgE-switched clones in wild-type mice, in the biophysical characteristics of CDRH3 protein (Fig. 6d), which indicated that the majority of IgE⁺ PC clones were of extrafollicular origin and had switched directly from IgM to IgE. That conclusion was supported by clonal analysis, which indicated that <30% of IgE⁺ PC clones were shared with IgG1⁺ PC clones and only around 30% of IgE⁺ PC

clones were shared with IgE⁺ GC clones (Supplementary Fig. 6b,c). Furthermore, Kidera PCA showed that the properties of IgE and IgG1 in *Tcrd*^{-/-} mice were different from those of IgE and IgG1 in wild-type mice (Fig. 6d), which suggested that immunosurveillance by $\gamma\delta$ T cells had a role in selecting the physicochemical properties of the CDRH3 regions.

Selection events are also expected to shape the B cell repertoire in terms of the use of genes encoding the variable, diversity and joining (VDJ) regions of the immunoglobulin heavy chain. We found that only five main genes of the *Igh*-V family and five main genes of the *Igh*-D family were commonly used, but all four *Igh*-J genes were used, by both IgG1⁺ and IgE⁺ GC B cells and PCs (Supplementary Fig. 6d–f). The third-most-frequent D region-encoding gene in IgE⁺ and IgG1⁺ GC B cells and PCs could not be annotated by the Immunogenetics (IMGT) information system, most probably due to an *Igh*-D gene in the genome of the FVB mouse strain that is

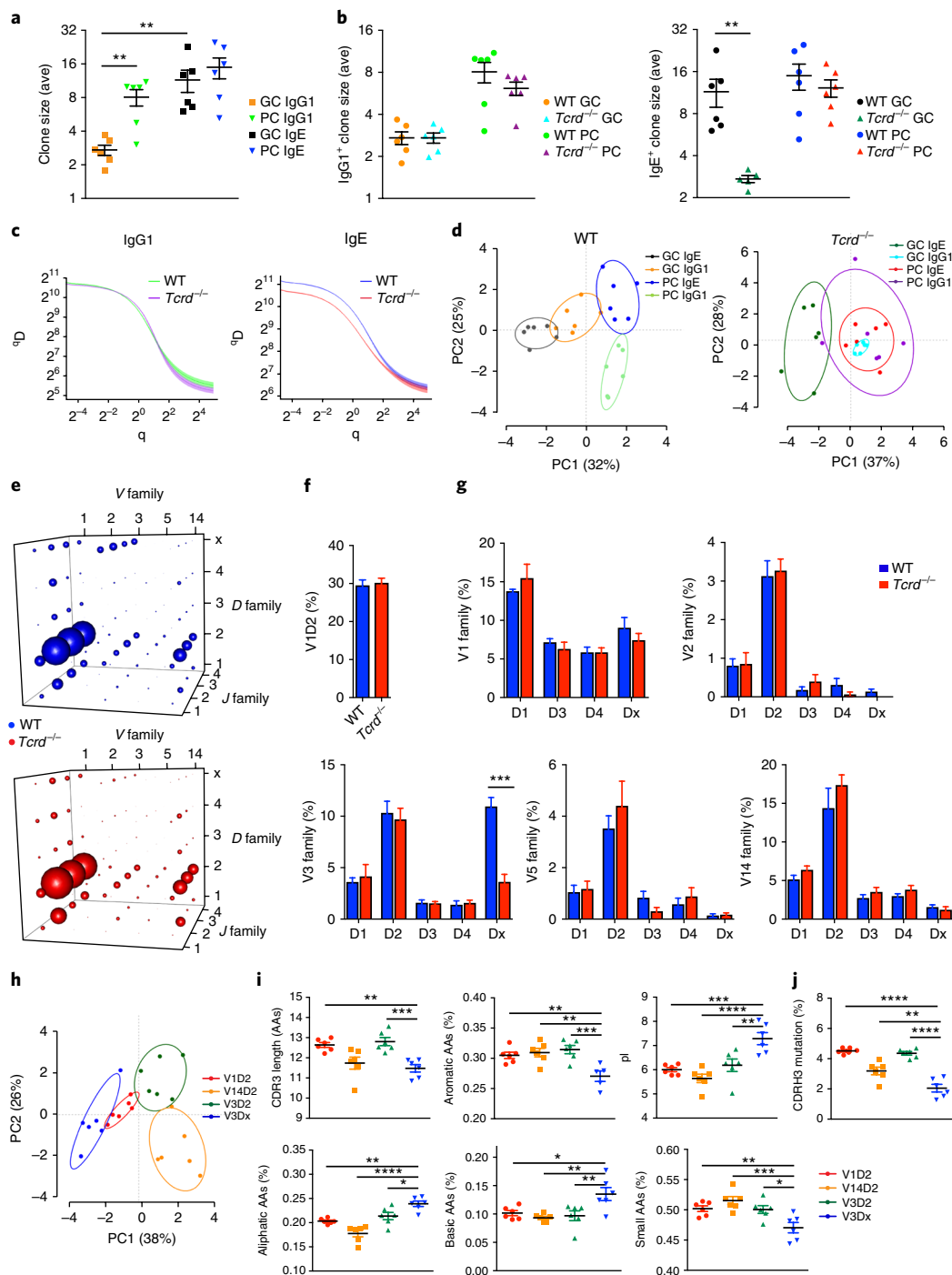


Fig. 6 | $\gamma\delta$ T cells promote a distinct IgE repertoire in response to carcinogen. **a,b, High-throughput sequencing and heavy-chain-repertoire analysis of IgG1 and IgE in sorted GC B cells and PCs from the skin-draining LNs of wild-type mice (**a,b**) and *Tcrd*^{-/-} mice (**b**) ($n=6$ per group) (key) 7 d after the second of two topical exposures to DMBA, presented as clone size (average values (ave)) for IgG1⁺ or IgE⁺ GC B cells (GC) and PCs (key). **c**, Clonal diversity (average values) of the IgG1 and IgE PC repertoires in wild-type and *Tcrd*^{-/-} mice as in **a,b**, presented as the general diversity index (qD) plotted against clonal frequency (q). $P < 0.05$ for IgE from $q < 2^2$. **d**, Kidera-factor PCA of IgG1⁺ or IgE⁺ GC B cells and PCs (key) in wild-type mice (left) and *Tcrd*^{-/-} mice (right) as in **a,b**. **e**, Three-dimensional plot of the use (average values) of VDJ-encoding gene families (along axes) in IgE⁺ PCs in wild-type mice (top) or *Tcrd*^{-/-} mice (bottom) as in **a,b**; V-encoding and D-encoding genes used $< 1\%$ in the repertoire have been removed for clarity. **f,g**, VDJ repertoire of IgE⁺ PCs in wild-type and *Tcrd*^{-/-} mice as in **a,b**, presented as frequency of use of V1D2 (**f**) or of other combinations of V regions (vertical axis) and D regions (horizontal axis) after exclusion of V1D2 (**g**) among the total repertoire. **h-j**, Kidera-factor PCA (**h**), CDRH3 length (in amino acids (AAs)), frequency of aromatic amino acids (phenylalanine, histidine, tryptophan and tyrosine), small amino acids (alanine, cysteine, aspartic acid, glycine, asparagine, proline, serine, threonine and valine), aliphatic amino acids (alanine, isoleucine, leucine and valine) and basic amino acids (histidine, lysine and arginine), and isoelectric point (pI) (**i**), and frequency of mutations in the gene encoding CDRH3, relative to germline mutations (**j**), in V3Dx⁺ clones and other VD families in wild-type IgE⁺ PCs. Each symbol (**a,b,i,j**) represents an individual mouse; small horizontal lines indicate the mean ($\pm$ s.e.m.). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ (two-tailed Student's *t*-test for unpaired data (**a,b,f,g**) or one-way ANOVA multiple comparison (**i,j**)). Data are presented as mean $\pm$ s.e.m. in **f,g**.**

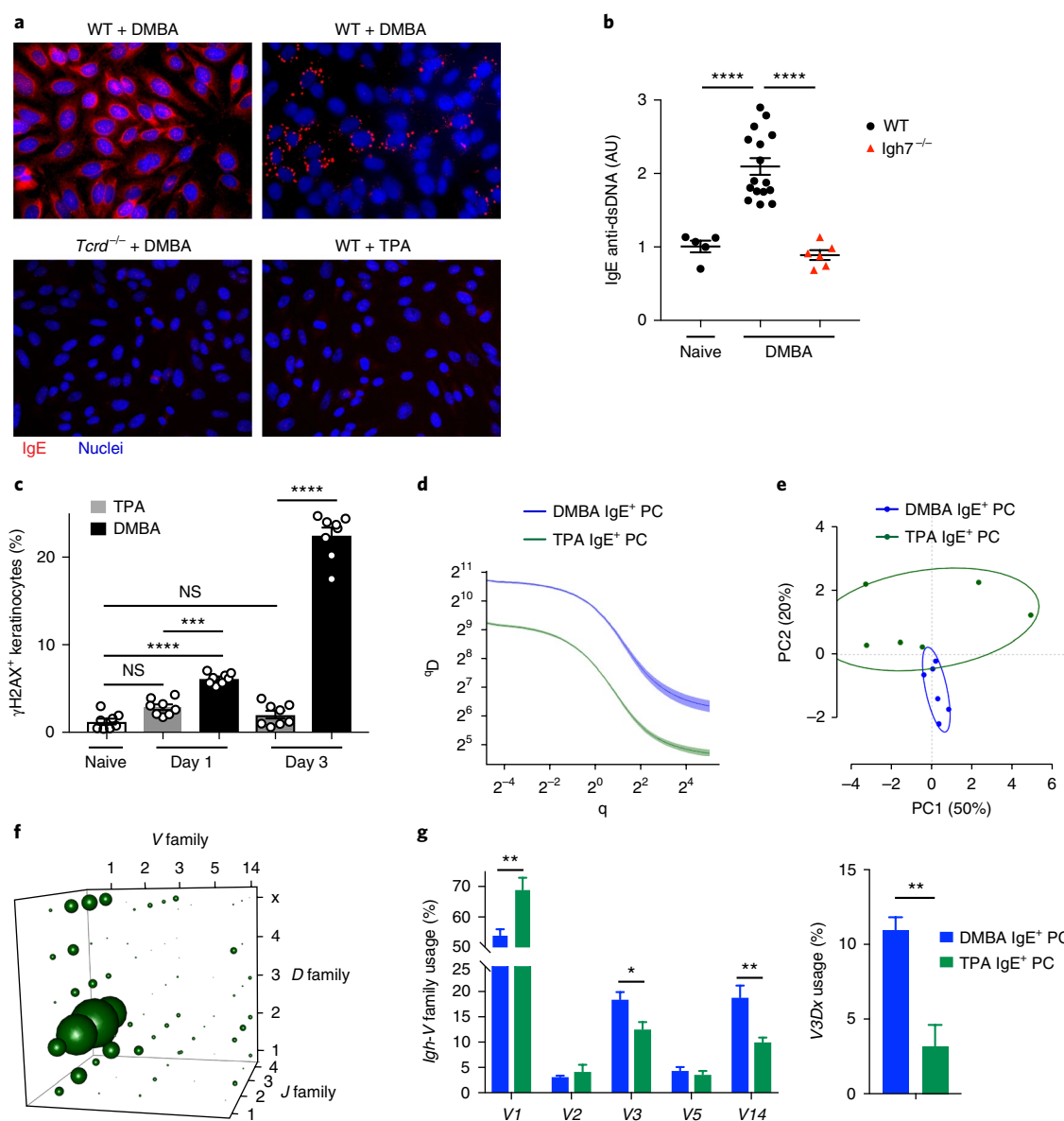


Fig. 7 | DMBA-induced IgE is autoantigenic and differs from TPA-induced IgE. a, Microscopy of the binding of autoreactive IgE (red) to HEp-2 cells in serum from DMBA-treated wild-type or *Tcrd*^{-/-} mice and TPA-treated wild-type mice (above images), with serum diluted 1:25, 1:5 and 1:5, respectively, to match IgE titers; nuclei are in blue. Original magnification, $\times 63$. **b**, ELISA of anti-dsDNA IgE in serum from DMBA-treated wild-type mice ($n=16$) or *Igh7*^{-/-} mice ($n=6$) and naive wild-type mice ($n=5$). **c**, Frequency of keratinocytes positive for γ H2AX (as a measure of dsDNA breaks) among CD45⁺ skin epithelial cells from mice ($n=8$ per group) before (Naive) and 1 or 3 d (horizontal axis) after topical treatment with DMBA or TPA (key), assessed by flow cytometry. **d–g**, High-throughput sequencing and heavy-chain repertoire analysis of IgE in sorted PCs from the skin-draining LNs of wild-type mice ($n=6$ per group) 7 d after topical exposure to DMBA or TPA. **d**, Average clonal diversity throughout the IgE⁺ PC repertoire (presented as in Fig. 6c; $P < 0.05$ throughout the repertoire). **e–g**, Kidera-factor PCA showing CDRH3 properties (**e**), three-dimensional plot of use of VDJ-encoding gene families (average values) in TPA-induced IgE (**f**) and use of the five most common V-region families as a proportion of the total repertoire and frequency of the V3Dx clones (**g**). Each symbol (**b,c**) represents an individual mouse; small horizontal lines (**b**) indicate the mean ($\pm$ s.e.m.). * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$ and **** $P < 0.0001$ (one-way ANOVA multiple comparison (**b,c**) or two-tailed Student's *t*-test for unpaired data (**g**)). Data presented as mean $\pm$ s.e.m. in **g**.

absent from the IMGT genomic data, as the heavy-chain repertoire is highly variable among inbred strains¹⁹. We annotated this newly identified gene as 'Dx'. Three-dimensional analysis of VDJ use in IgE⁺ PCs indicated that V1D2 was the dominant selection (Fig. 6e), consistent with data showing that the immunoglobulin heavy-chain V1 family is most frequently used in mouse immunoglobulins^{19,20}. J-region usage was approximately equal among the four J-region family groups (Fig. 6e). The VDJ analysis also revealed that some VDJ combinations in wild-type mice, particularly the newly identified 'V3Dx' segment, were significantly less selected

in IgE⁺ PCs from *Tcrd*^{-/-} mice than in those from wild-type mice (Fig. 6e–g).

We next compared the physicochemical properties of CDRH3 in the wild-type V3Dx⁺ clones with those of V3D2⁺ clones, which used the same V region-encoding gene but a different D region-encoding gene, V1D2⁺ clones, which were the most common selection, and V14D2⁺ clones, which did not require $\gamma\delta$ T cell selection. Kidera-factor PCA indicated that the V3Dx⁺ clones had unique physicochemical characteristics of their CDRH3 (Fig. 6h) and had a lower frequency of aromatic and small amino acids, but a higher

frequency of aliphatic and basic amino acids, as well as a higher isoelectric point than that of clones of the other VD combinations (Fig. 6i). These characteristics suggested that the antigen-binding site was hydrophobic, with slight positive charge in the loop at blood pH. In addition, the V3Dx-expressing clones had shorter CDRH3 regions than those of the V1D2⁺ or V3D2⁺ clones (Fig. 6i), indicative of antigen selection^{21,22}, and carried fewer mutations (Fig. 6j), which indicated that they were more germline in nature. In sum, these data indicated that in response to DMBA, $\gamma\delta$ TCR⁺ T cells shaped the IgE repertoire by supporting specific VDJ rearrangements, which resulted in unique characteristics of the CDRH3 antigen-binding site, suggestive of polyreactive autologous binding.

DMBA-induced IgE is autoantigenic and differs from TPA-induced IgE. We next explored whether the IgE responses induced by topical DMBA were autoreactive. We screened the binding of IgE from the serum of DMBA-treated wild-type mice to human HEp2 epithelial cells and observed several strong staining patterns (Fig. 7a and Supplementary Fig. 7a) indicative of both autoreactivity of IgE and cross-species reactivity of IgE. The most common staining patterns of IgE on HEp2 cells were ‘nuclear dots’, indicative of anti-nuclear specificity, and ‘cytoplasmic vesicles’ that resembled stress granules (Fig. 7a and Supplementary Fig. 7a). In addition, we detected IgE reactivity to dsDNA (Fig. 7b) and to damaged epithelial cells, frequently around hair follicles, in epidermal sheets isolated from *Fcer1a*^{-/-} mice 24 h after exposure to DMBA (Supplementary Fig. 7f–g). We detected no autoreactivity to HEp2 cells for IgE from the serum of DMBA-treated *Tcrd*^{-/-} mice or IgE from the serum of wild-type mice treated topically with the inflammatory chemical TPA (12-O-tetradecanoylphorbol-13-acetate) (Fig. 7a and Supplementary Fig. 7b,c), which did not elicit DNA damage in epithelial cells (Fig. 7c), but triggers general skin inflammation.

We next sorted FSC^{hi}CD95^{hi}CD138⁺ PCs from the skin-draining LNs of wild-type mice exposed topically to DMBA or TPA and compared their IgE heavy-chain repertoires by high-throughput sequencing. Alakazam analysis indicated that TPA-induced IgE⁺ PCs exhibited significantly less diversity throughout the repertoire than that of DMBA-induced IgE⁺ PCs (Fig. 7d). Kidera-factor PCA indicated more heterogeneity in the CDRH3 regions of TPA-induced IgE than in those of DMBA-induced IgE, with little overlap between the two responses (Fig. 7e). In parallel, use of VDJ-encoding gene families among TPA-induced IgE was dominated by V1-encoding genes, with significantly less selection for V3-encoding genes and V14-encoding genes than that among DMBA-induced IgE (Fig. 7f,g). Notably, TPA also did not generate the V3Dx-expressing clones, which we had found to be promoted by $\gamma\delta$ T cells in response to DMBA-induced DNA damage (Fig. 7g). Together these results indicated that exposure of the skin to DMBA drove a unique, autoreactive IgE repertoire that was distinct from that induced in response to TPA, which induced local acute inflammation.

Discussion

Here we found that IgE was rapidly induced by cutaneous exposure to the carcinogen DMBA and accumulated in the skin and tumors on Fc ϵ RI-expressing basophils. The IgE response required $\gamma\delta$ TCR⁺ IELs, was autoreactive and was protective against epithelial carcinogenesis. Fc ϵ RI-expressing cells also accumulated in human skin SCCs, and the level of Fc ϵ RI expression correlated with disease severity. Together our data indicated that IgE contributed to early tissue immunosurveillance against environmental xenobiotics or toxins and had a role in protection against epithelial tumor development.

Polyaromatic hydrocarbons such as DMBA are ubiquitous and arise from many sources, including car emissions and tobacco smoke^{14,15}. They are the main organic constituent in air pollution,

and their levels correlate with the prevalence and severity of atopic dermatitis^{23,24}. In mice, chronic exposure of the skin to air pollutants can induce atopic dermatitis-like features and IgE production. However, while gene expression in the skin depends on ligation of the aromatic hydrocarbon receptor by polyaromatic hydrocarbons, the increase in IgE does not²⁵. Consistent with that, we found that the initiation of IgE induction after topical application of DMBA depended on epithelial DNA damage, not direct engagement of the aromatic hydrocarbon receptor. Host DNA can selectively induce IgE²⁶, which would suggest that dying cells have unique IgE-adjuvant activity. Furthermore, expression of autologous stress antigens, such as NKG2D ligands, on damaged skin epithelia promotes IgE production¹². Topical application of TPA also induces the expression of NKG2D ligands²⁷, which might partly explain the induction of IgE by this agent. However, sequencing analysis revealed that different types of tissue damage initiated distinct IgE repertoires. In humans, self-reactive IgE has been found in patients with systemic lupus erythematosus^{28,29}, bullous pemphigoid³⁰, atopic dermatitis³¹ or primary immune deficiencies³². Such data suggest that IgE responses are potentially promoted by autologous cell stress and/or damage, which is induced by exposure of the skin to environmental xenobiotics.

IELs provide a first line of defense in epithelial tissues. A large proportion of IELs carry $\gamma\delta$ TCRs and are autoreactive in nature, as they express receptors for tissue-specific stress molecules³³. We found that following DMBA-induced epithelial damage, IELs rapidly elicited IgE in the parafollicular regions and subsequently triggered a conventional adaptive response that required polyclonal $\alpha\beta$ TCR⁺ CD4⁺ T cells that produced IL-4. This mode of inducing IgE differs from the regulation of self-reactive ‘natural IgE’, which is also driven by $\gamma\delta$ T cells but is independent of $\alpha\beta$ T cells and major histocompatibility complex class II^{34,35}. Deep sequencing of IgG1⁺ and IgE⁺ GC B cells and PCs demonstrated that $\gamma\delta$ T cells shaped the repertoire and the nature of the antibodies produced. Thus, antibody deep sequencing revealed a previously unrecognized mode of B cell selection dictated by tissue-resident IELs.

$\gamma\delta$ T cells and tissue-specific $\gamma\delta$ IELs are key participants in host defense against cancer, which is considered to reflect innate properties such as cytotoxicity and cytokine production^{11,13,27}. Here we found that they were able to provide a second line of host defense via the initiation of adaptive autoreactive IgE responses and Fc ϵ RI-expressing effector cells. Our findings that endogenous IgE was able to suppress tumor growth are supported by evidence that immunization with tumor-antigen-specific IgE can eradicate tumors and is more effective than immunization with IgG1^{36,37}. How IgE inhibited tumor growth in our model remains to be determined; however, it required expression of Fc ϵ RI, and mast cells were not essential, which would suggest it might involve soluble factors and/or cytotoxicity mediated by basophils.

In humans, epithelial skin cancers are on the rise^{38,39}. In addition to UV irradiation, environmental chemical carcinogens can also lead to the development of skin SCC, as indicated by the causal link, in chimney sweeps, between scrotal SCC and exposure to soot, which contains polyaromatic hydrocarbons⁴⁰. Moreover, tobacco smokers, who are exposed to large amounts of polyaromatic benz[a]anthracene, have a 50% increased likelihood of developing skin SCC⁴¹. Studies of IgE and cancer risk in humans have shown a negative, albeit weak, correlation between serum IgE and overall cancer risk^{42,43}. In addition, *FCER1A* is reported to be among the top 50 genes associated with positive survival in 39 human malignancies⁴⁴. The presence of intratumoral $\gamma\delta$ T cells is the most favorable prognostic indicator of survival in patients with cancer⁴⁴, while a large number of infiltrating PCs is also strongly associated with good clinical outcome⁴⁴. Consistent with those observations, we found that Fc ϵ RI-expressing cells were abundant in human SCC, and there was an inverse correlation between expression of *FCER1A* and more-advanced disease. Of note, the use of omalizumab, a monoclonal antibody that blocks

IgE, has been associated with more-frequent development of cancer, particularly epithelial and solid-organ cancers⁴⁵.

Together these findings suggest that the IgE–FcεRI axis is part of a tumor-protective immune response and provide support for the ‘toxin hypothesis’, which proposes that IgE is a host defense mechanism against non-infectious cell-damaging environmental xenobiotics.

Methods

Methods, including statements of data availability and any associated accession codes and references, are available at <https://doi.org/10.1038/s41590-018-0161-8>.

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Author contributions

G.C. performed and analyzed the experiments, with help from M.D.H., R.C.S., S.W. and T.D.; C.L. and E.H. provided human blood and SSC samples for flow cytometry; D.K. assisted with sequencing analysis; C.P. and C.M. provided and graded human skin and

tumor samples for Nanostring; K.B. and M.H. generated SCC NanoString data and K.G. analyzed it; M.B. assisted with data interpretation and manuscript preparation; D.D.-W. assisted with sequencing analysis and interpretation; and J.S. performed and analyzed some experiments, directed the study and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Methods

Mice. Genetically altered mice were generated as previously described. *Tcrd*^{-/-} mice⁴⁶, *Tcrb*^{-/-} mice⁴⁷, *Il4*^{-/-} mice⁴⁸, Langerin-DTA (LC-deficient) mice⁴⁹ and *TgV5^{-/-}Tcrd-V1^{-/-}* mice^{50,51} were on the FVB/N background after >10 backcrosses; *Igh-7^{-/-}* mice⁵², *Fcer1a*^{-/-} mice⁵³ and *Cpa3^{Cre/+}* mice⁵⁴ were on the BALB/c background after more than ten backcrosses; and OT-II Tg mice⁵⁵, *Tcrb*^{-/-} mice⁴⁷, CD19-Cre mice⁵⁶ and *Bcl6^{fl/fl}* mice⁵⁷ were on the C57BL/6 background. Strain-matched wild-type control mice were purchased from Charles River. Mice were bred and maintained in individually ventilated cages under specific pathogen-free conditions. Age-matched female mice were used for all experiments at ≥7 weeks of age and were selected at random from a large pool when allocated to experiments. All studies were approved by Imperial College AWERB (Animal Welfare and Ethical Review Body) and by the UK Home Office. Experiments involving cancer studies strictly adhered to the guidelines set out by the National Cancer Research Institute (NCRI) and in the document 'Guidelines for the Welfare and Use of Animals in Cancer Research'⁵⁸. All studies using animals were conducted following the document Animal Research: Reporting In vivo Experiments (ARRIVE)⁵⁹.

Chemical cutaneous damage and carcinogenesis. Chemicals

7,12-dimethylbenz[a]anthracene (DMBA) and 12-0-tetradecanoylphorbol-13-acetate (TPA) were purchased from Sigma and were dissolved in acetone and 100% ethanol, respectively. Acute damage was induced by exposure of the dorsal skin of the ear skin to a single dose or repeated doses of 200 nM DMBA or 2.5 nM of TPA, in 25 µl.

For cutaneous carcinogenesis, age-matched female mice were used at 7 weeks of age. The dorsal back area was shaved with a surgical blade and mice were allowed to 'rest' for 1 week. Applications of chemicals and tumor monitoring were performed as previously described¹⁵. In brief, 200 nM DMBA was carefully and slowly applied by pipette, in a 100-µl volume, to the entire shaved skin area. Mice were allowed to 'rest' for 1 week, and 100 nM DMBA was then applied weekly. Hair regrowth during the experiment was gently removed by clipping with trimmers. Mice were monitored daily, and cutaneous tumors were counted and measured with a caliper once weekly. Back skin and tumors were evaluated by visual inspection by an observer blinded to the experimental groups.

UV irradiation. The back skin of FVB mice was shaved using electrical clippers 3 d before UV irradiation. Mice were exposed to the UV light source in their cages placed under four 100-W UV lamps with the spectral output 270–350 nm (predominantly UVB) (Philips TL12 lamps). The total dose of UVB radiation was monitored during each exposure using an IL-400 A UVB photometer (International Light). Mice were given 100 mJ/cm² three times a week.

Tissue processing. Tissue was cut into small 1-mm² pieces using a scalpel blade and incubated for 2 h in digestion buffer containing 25 µg/ml Liberase (Roche), 250 µg/ml DNaseI (Roche) and 1× DNase buffer (1.21 Tris base, 0.5 g MgCl₂ and 0.073 g CaCl₂) at 37°. Following digestion, tissue was transferred into C-tubes (Miltenyi Biotech) containing RPMI-1640 medium (Thermo Fisher) supplemented with 10% heat-inactivated fetal calf serum, 1% penicillin-streptomycin-glutamine (Thermo Fisher) and was physically disrupted using a Miltenyi cell dissociator. Cell suspensions were then filtered and cells were counted using a CASY cell counter (Roche).

Flow cytometry. Cell suspensions were blocked for non-specific binding using antibody to FcγR (2.4G2, BD) and 2% normal rat serum (Sigma) before any staining protocols. For staining of cell-surface markers, cell suspensions were stained with fluorochrome-conjugated antibodies or the appropriate isotype-matched control antibody with the addition of a fixable, live/dead discrimination dye (Invitrogen) for 25 min and were subsequently washed. Intracellular staining was then carried out using an Intracellular Staining kit according to the manufacturer's instructions (ThermoFisher Scientific). Cells were fixed for 10 min at 4 °C, washed with Perm buffer and stained for intracellular markers for 25 min. For intranuclear staining of γH2AX, cells were fixed and permeabilized in ice-cold 70% ethanol at -20 °C for 2 h, blocked with 2% normal mouse serum (Sigma), Fc-block and 2% FCS for 15 min, followed by 45 min of staining for γH2AX at room temperature. Stained cells were analyzed using a BD FACSVerser and a Fortessa X20 (BD Biosciences). Data analysis was performed using FlowJo 10 for Mac (TreeStar).

Antibodies were sourced from eBioscience unless otherwise stated. The following antibodies were used: anti-CD45 (30-F11), anti-B220 (RA3-6B2), anti-CD38 (90/CD38, BD), anti-CD138 (281-2, BD), anti-CD95 (Jo2), anti-GL7 (GL-7), anti-CD3 (145-2C11, Biolegend), anti-IgE (23G3), anti-IgG1 (M1-14D12), anti-IgG2a (R19-15, BD), anti-TCRβ (H57-597), anti-TCRγδ (eBioGL3), anti-V_H5 (536), anti-V_H4 (UC3-10Ab, Biolegend), anti-CD117 (2B8), anti-CD41 (MWRReg30, Biolegend), anti-FcεRI (MAR-1, Biolegend), anti-IL-4 (11B11), anti-IL-6 (MP5-20F3, Biolegend), anti-CD63 (NVG-2, Biolegend) and anti-γH2AX (JBW301, Millipore).

ELISA. For IgG1 and IgG2a antibodies, NUNC Immune Maxisorp 96-well plates (Thermo Scientific) were coated with 5 µg/ml goat anti-mouse IgH + L (1010-01, Southern Biotech) in borate-buffered saline at 37 °C for 3 h. After washing, plates

were blocked with PBS containing 0.5% BSA for 1 h at room temperature and the appropriately diluted serum was added, followed by incubation overnight at 4 °C. After washing, plates were incubated with alkaline phosphatase-conjugated polyclonal goat anti-mouse IgG1 (1070-04) or IgG2a (1080-04) (also detects IgG2c in C57BL/6 mice) (both from Southern Biotech) for 5 h at 4 °C. Following further washing, the alkaline-phosphatase substrate pNPP (Sigma) was added and absorbance was measured at 405 nm. Total IgE was measured by an IgE-capture method. Serum to be tested and the IgE standard were added to plate wells coated with 1 µg/ml rat monoclonal antibody to mouse IgE (R35-92, PharMingen) and blocked with 1% rat serum. Biotinylated rat monoclonal antibody to mouse IgE (R35-92, PharMingen) at 1 µg/ml was then added, followed by incubation for 2 h at 37 °C. After washing in PBS plus 0.05% Tween, plates were incubated with alkaline phosphatase-conjugated streptavidin (PharMingen) for 1 h at 37 °C. After washing, detection of antibody levels was carried out by addition of the alkaline-phosphatase substrate pNPP (Sigma) and absorbance was measured at 405 nm.

For analysis of IgE autoreactivity to dsDNA, MaxiSorp plates were coated with 1 µg/ml rat monoclonal antibody to mouse IgE (R35-92, PharMingen) and were blocked with 1% rat serum and 3% milk. Serum samples were screened at 1:25 dilution. 100 ng/ml biotinylated herring sperm dsDNA (Promega) was then added, followed by incubation overnight at 4 °C. After washing in PBS plus 0.05% Tween, plates were incubated with alkaline phosphatase-conjugated streptavidin (PharMingen) for 1 h at 37 °C. After washing, detection was carried out by addition of the alkaline-phosphatase substrate pNPP (Sigma) and absorbance was measured at 405 nm. Autoantibody levels are presented in arbitrary ELISA units.

Hep-2 staining. Autoreactivity of IgE was tested by indirect immunofluorescence using Hep-2 cells. Serum samples were diluted appropriately (tested in dilutions between 'neat' and 1:25, depending on IgE titers) and were incubated on Hep-2 slides (Bio-Rad) overnight at 4 °C. After washing in PBS, slides were incubated with purified rat anti-mouse IgE (R35-92, PharMingen) for 2 h, then were washed again and further incubated with the secondary antibody anti-rat 555 (A21434, ThermoFisher) for 1 h at room temperature. As a control, serum from similarly treated IgE-deficient mice was also assessed, as well as serum from naive wild-type mice. Slides were thoroughly washed and mounted with Vectashield containing DAPI (Vectashield) and were visualized with a Leica DM4 fluorescence microscope (Leica).

Immunofluorescent staining of tumor samples. Tumors were removed from the back along with a small piece of adjacent skin and were snap-frozen in OCT on dry ice. 6-µm sections were cut using a Leica JUNG CM1800 cryostat and were stored at -80 °C. For staining, slides were returned to room temperature before fixation with 4% paraformaldehyde for 15 min. Samples were then blocked in 5% goat serum for 1 h at room temperature before staining with rat anti-mouse IgE (R35-92, PharMingen) overnight at 4 °C. Following staining, samples were washed and incubated with Alexa Fluor 555-conjugated goat anti-rat IgG (A21434, ThermoFisher). After extensive washing, samples were mounted in Vectashield containing DAPI (Vectashield) and were visualized with a Leica SP5 confocal laser-scanning microscope (Leica).

Epidermal sheets. Ears were collected, split into dorsal and ventral sides and floated dermis side down in 0.5 M NH₄SCN for 40 min, 37 °C and 5% v/v CO₂. Subsequently, intact epidermal sheets were isolated and washed in PBS. They were then fixed in ice-cold acetone at -20 °C for 15 min, followed by rehydration in PBS. Nonspecific binding to the epidermal tissue was blocked with 2% BSA for 1 h, and then 1:25 dilution of test serum was added, followed by incubation overnight at 4 °C. Any IgE binding to the tissue was visualized by first incubating with rat anti-mouse IgE (R35-92, BD) followed by Alexa Fluor 555-conjugated goat anti-rat IgE (A21434, ThermoFisher), each for 1 h at room temperature. After extensive washing in PBS, samples were mounted in Vectashield containing DAPI (Vectashield) and were visualized with a Leica SP5 confocal laser-scanning microscope (Leica).

Adoptive-transfer experiments. Whole CD4⁺ T cells were isolated from the spleen of donor wild-type and *Il4*^{-/-} mice (using Miltenyi MACS isolation kits). For OT-II transfer experiments, OT-II CD4⁺ T cells were cell sorted using ovalbumin peptide-loaded tetramers (NIH Tetramer Facility). 2 × 10⁶ cells were injected intravenously, mice were allowed to 'rest' for 24 h, and the dorsal ear skin was then exposed to DMBA as described above.

Bone-marrow chimeras. Wild-type, *Tcrd*^{-/-} and *Il4*^{-/-} mice were sublethally irradiated (750 rads) and immediately reconstituted with 5 × 10⁶ donor bone-marrow cells by intravenous injection. Mice were left for 8 weeks for fully reconstitution with the hematopoietic cells, and then they were exposed to DMBA on the skin as described above. Chimerism was assessed by flow-cytometry analysis of peripheral LNs and skin tissue at the end of the experiments.

qRT-PCR and primer sequences. RNA was extracted from ammonium-fixed epidermis or tumor tissue and preserved in RNA-later with an RNeasy Mini kit (Qiagen). RNA was dissolved in nuclease-free water, and yield and purity

were determined. Complementary DNA (cDNA) was synthesized from RNA with an iScript cDNA synthesis kit (Bio-Rad) according to the manufacturer's instructions. cDNA was diluted in nuclease-free double-deionized water for qRT-PCR. All primers were single-stranded DNA oligonucleotides (Sigma) that were intron spanning as verified by NCBI Primer-Blast tool. Real-time PCR products were detected with SYBR Green (Life) measured continuously with a Viia 7 Real-Time PCR system (Applied Biosystems). Ct values for genes of interest were normalized against Ct values of the housekeeping gene encoding cyclophilin (*Cyc*) using the $2^{-\Delta C_t}$ method. The following primers were used: IgE, F (5'-CAAGTCAGCCAAGAACTTCAC-3') and R (5'-GACAGGTCGAACCTAGGATAGTC-3'); IgG1, F (5'-TTGCATATGTACAGTCCCAG-3') and R (5'-GTCAGAGTAATGGTGGAGCAC-3'); IgM, F (5'-AAGACTGGAATAACAGGAAGGA-3') and R (5'-GTTTGTGCACCTCATTGGG-3'); Rael, F (5'-TGGACACTACAAGACCAATG-3') and R (5'-CCCAGGTGGCACTAGGAGT-3'); IL-4, F (5'-CATCGGCATTTTGAACGAG-3') and R (5'-CGAGCTCACTCTCTGTGGTG-3'); IL-6, F (5'-TGATGGATGCTACCAAACTGG-3') and R (5'-TTCATGTACTCCAGGTAGCTATGG-3'); IL-33, F (5'-CACATTGAGCATCCAAGGAA-3') and R (5'-AACAGATTGGTCATTGTATGTACTCAG-3'); and *Cyc*, F (5'-CAAATGCTGGACCAACACAA-3') and R (5'-CCATCCAGCCATTCACTCTTG-3').

Immunoglobulin sequencing and analysis. Mice were treated twice with DMBA or TPA to the dorsal side of the ear skin, 3 d apart. 1 week after the final exposure, draining LNs were collected and GC B cells (B220⁺CD95⁺GL7⁺) and PCs (FSC^{hi}CD95^{hi}CD138⁺) were sorted on a BD FACSAria III (BD Biosciences). RNA was extracted using RNeasy Micro kits (Qiagen) and was reverse transcribed using SuperScript III Reverse Transcriptase (ThermoFisher). Sequences were amplified using Phusion High-Fidelity DNA Polymerase (NewEngland Biolabs) to ensure accuracy and robust performance for large PCR products. PCR was performed with a primer in the constant C_μ region (5'-CTAGGGTCATGGAAGCAGTGC-3') or C_γ1 region (5'-GGGAAATAGCCCTTGACCAGG-3') in combination with a promiscuous V-region primer (binding all V-region heavy-chain genes) (5'-GAGGTGCAGCTGCAGGAGTCTGG-3'). All primers were labeled at either end with multiplex identifiers for multiplexing. PCR thermal cycling was as follows: 98 °C, 30 s; 30 × (98 °C, 30 s; 60 °C, 30 s; 72 °C, 35 s); and 72 °C, 10 min. Amplicons were purified by gel extraction using a QIAquick PCR purification kit (Qiagen) and were deep sequenced by long-read 454 pyrosequencing on the Genome Sequencer FLX system (Roche). Raw sequencing data were presented in FASTA format and unproductive sequences were removed (data cleanup stages as described⁴⁰).

Analysis was performed as previously described⁶⁰. In brief, sequences were assigned to individual samples according to their multiplex identifier. V(D)J gene assignment of individual sequences were annotated using IMGT/HighV-Quest and the mouse database. Mean values of V(D)J gene usage were calculated before three-dimensional bubble plots were created using the R package plot3D. Repertoire diversity was characterized using the Hill diversity index^{61,62} and were plotted using the Alakazam package¹⁷. Physicochemical properties of the CDRH3 region were calculated using the R package Peptides. Physicochemical properties included length, isoelectric point (pI), frequency of amino acid classes in the CDRH3 region, and Kidera factors. Kidera factors are a set of ten independent factors that encapsulate information from ~180 different measurable biophysical characteristics of all 20 amino acids¹⁸. Principal component analysis of Kidera factors was performed using the *prcomp* function in R using Minkowski distances as previously described⁶³.

Human tissue. For flow cytometry, freshly excised SCC tissue and peripheral blood were obtained from 12 patients during surgery at the Dermatology Department of University Hospital Southampton NHS Foundation Trust. Ethics were provided by the South Central Hampshire B NRES Committee (reference number 07/H0504/187). All participants recruited to the study provided informed consent for blood and tissue samples to be used for research purposes. All tumor tissue used in the study was confirmed SCC with a diameter of > 8 mm. Non-lesional skin was histopathologically normal and > 6 mm away from the tumor edge. To obtain single-cell suspensions, tumor samples were cut into small pieces and enzymatically digested with 1 mg/ml collagenase I-A (Sigma) and 10 μg/ml DNase I (Sigma) in RPMI medium at 37 °C for 1.5 h. The resulting suspension was passed through 70-μm cell strainers and centrifuged in Optiprep (Axis-Shield). PBMCs were isolated from venous blood from the patients with SCC by centrifugation with Lymphoprep (Axis-Shield). Single cell suspensions were stained for flow cytometry with antibody to CD45 (2D1) and antibody to FcεR1 (MHE-18) (both from BioLegend). Cells were gated on FSC and SSC and dead cells were excluded using near IR live/dead stain (Invitrogen).

For immunohistochemistry analysis of human SCC tissue, 5-μm tissue sections were cut from formalin-fixed paraffin-embedded samples and were placed

onto 3-aminopropyltriethoxysilane (APES)-coated microscope slides. Sections were deparaffinized in xylene and were rehydrated through graded alcohols. Endogenous peroxidase was inhibited with 0.5% hydrogen peroxide before antigen retrieval by microwaving in 10 mM citrate buffer, pH 6. Endogenous avidin and biotin were blocked (Vector Labs) before the application of Dulbecco's Modified Eagle's Medium (Gibco) containing 1% bovine serum albumin (Sigma) and 20% fetal bovine serum (Gibco) blocking medium. Primary antibody to FcεR1 (9E1, 1:20 dilution (Abcam)) was then left on the sections for 16 h at 4 °C before three washes with Tris-buffered saline (TBS), followed by application of biotinylated goat anti-mouse (1:400 dilution; 115-065-003, Jackson ImmunoResearch) for 30 min. After three washes with TBS, streptavidin-biotin complexes (Vector Labs) were applied, before three washes with TBS and application of diaminobenzidine (DAKO). After washing with TBS, sections were counterstained with Mayer's Hematoxylin (Sigma) and were dehydrated through graded alcohol and xylene. Sections were mounted in DPX mountant (Sigma) and coverslips were applied. Slides were imaged at ×2 and ×20 magnification using an Olympus Dotslide microscope and were analyzed using OlyVIA software.

For gene expression in SSC tumors, fixed frozen paraffin-embedded skin samples were analyzed from 56 patients obtained from the University of Dundee, Tayside NHS Trust and Greater Glasgow and Clyde NHS Trust. The study was conducted according to the Declaration of Helsinki Principles, and all patients donating samples to this study provided written, informed consent in accordance with ethical approval from the East of Scotland Research Ethics Service (EoSRES) REC 1. SSC tumors were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC. In addition, peri-lesional skin (histopathologically normal looking skin > 4 mm away from the tumor edge) and abdominal non-UV-exposed skin were also analyzed. Total RNA was extracted using a Qiagen RNeasy extraction kit and was directly hybridized using the NanoString PanCancer and PanCancer Immune expression panel and analyzed on an nCounter (NanoString).

Statistical evaluation. The statistical significance of difference between experimental groups was determined using two-tailed Student's *t*-test for unpaired data, Wilcoxon test for paired samples, one-way ANOVA or linear regression, where appropriate, with results deemed significant at *P* < 0.05. Asterisk significance correlates as follows: **P* < 0.05; ***P* < 0.01; ****P* < 0.001; and *****P* < 0.0001. Statistics were analyzed with GraphPad Prism 6.00 for Mac (GraphPad).

Reporting Summary. Further information on experimental design is available in the Nature Research Reporting Summary linked to this article.

Data availability statement. The data supporting the findings of this study are available from the corresponding author upon request. RNA sequencing data are available from the public repository on the National Center for Biotechnology Information's Sequence Read Archive in raw format (accession codes: BioProject, PRJNA417372; BioSample, SAMN07985450, SAMN07985451, SAMN07985452, SAMN07985453, SAMN07985454 and SAMN07985455).

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Reporting Summary

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When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

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- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
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- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection BD FACS Diva software version 8.0.1, Quant Studio RT-PCR version 1.3, ELISA Ascents software version 2.6.

Data analysis All software used throughout this work is publicly available: FlowJoV8.8.7/10, ImageJ, Graph Pad Prism 7, Illustrator, Alakazam from the Change-O toolkit (<http://clip.med.yale.edu/changeo/>) and BRepertoire (<http://mabra.biomed.kcl.ac.uk/BRepertoire/>).

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The data supporting the findings of this study are available from the corresponding author upon request. RNA sequencing data is available from the public

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Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. Sample sizes were chosen based on previous experience with the experimental protocols. The antibody responses examined in this work were highly reproducible with little variability. Carcinogenesis experiments have a greater biological variation, however the protocols are well-known, and much previous experience guided us to increase our samples sizes to minimum 10 animals per experimental group to ensure statistical power in these longitudinal studies.
Data exclusions	No data points were excluded from data sets.
Replication	All the experimental data provided are based on biological replicates and are representative of independent experiments. All experiments were reliably reproduced.
Randomization	For animal work, mice of a particular genotype were randomly selected for experiment from a larger cohort - other than that, no randomization was used. For human tissue, individual SSC tumours were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC, as outlined in the materials and methods.
Blinding	For reporting on tumour development following chemically induced carcinogenesis, back skin and tumours were evaluated by an observer blinded to the experimental groups. For most other experiments, the investigators were not blinded to group allocation.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	For all flow cytometry staining, cell suspensions were incubated with 2% Rat Serum (Sigma) and Fc block (clone 93, BD Bioscience) for 30mins on ice, prior to staining. The following antibodies were then used: eBioscience: anti-CD45 (30-F11), B220 (RA3-6B2), , CD95 (Jo2), GL7 (GL-7), IgE (23G3), IgG1 (M1-14D12), TCRβ (H57-597), TCRγδ (eBioGL3), Vγ5 (536), CD117 (2B8), IL-4 (11B11); BD Biosciences: CD38 (90/CD38), CD138 (281-2), IgG2a (R19-15); Biolegend: CD41 (MWRReg30), FcεRI (MAR-1), IL-6 (MP5-20F3), CD63 (NVG-2), Vγ4 (UC3-10Ab), CD3 (145-2C11); Millipore: γH2AX (JBW301). All antibodies were used at a dilution of 1:100-1:200 as per respective manufacturers recommendation. OVA-loaded tetramers were provided by the NIH tetramer facility - Emory University Vaccine Center 954 Gatewood Road Atlanta, GA 30329. Reagents are provided to qualified investigators at no cost.
Validation	All antibodies were validated by the manufacturers and by our own and colleagues' labs where they are used extensively. All antibodies were titrated to determine optimal staining concentration.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Only mice were used in this study and groups were age- and sex-matched for all experiments. In general females mice at 7-9 weeks were used throughout. Tcrd^{-/-}, Tcrb^{-/-}, IL4^{-/-}, Langerin-DTA (LC-deficient) and Vg5Vd1^{-/-} were on the FVB/N background after >10 backcrosses; Igh^{-7/-}, FcεR1a^{-/-} and Cpa3Cre/+ were on the BALB/c background after >10 backcrosses; and OTII Tg, Tcrb^{-/-}, CD19-Cre and Bcl6fl/fl were on the C57BL/6 background. Genetic backgrounds of individual strains and controls are indicated where appropriate. Humoral responses were also examined in male animals and were comparable. All mouse studies were approved by Imperial College AWERB (Animal Welfare and Ethical Review Body) and by the UK Home Office. Experiments involving cancer studies strictly adhered to the guidelines set out by the National Cancer Research Institute (NCRI) and Workman et al. in 'Guidelines for the Welfare and Use of Animals in Cancer Research'. All studies using mice were conducted following the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Wild animals

n/a

Field-collected samples

n/a

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Two cohorts of human research participants were involved in this study:
A: freshly excised SSC tumour tissue and peri-lesional skin for flow cytometric analysis from 12 patients: All tumor tissue used in the study was confirmed SSCs with a diameter > 8mm. Non-lesional skin was histopathologically normal and > 6mm away from the tumor edge.
B: For SSC tumor gene expression, fixed frozen paraffin embedded skin samples from 56 patients obtained from the University of Dundee, Tayside NHS Trust and Greater Glasgow and Clyde NHS Trust were analyzed.

Recruitment

In A: freshly excised SCC tissue and peripheral blood were obtained from 12 patients during surgery at the Dermatology Department, University Hospital Southampton NHS Foundation Trust. Ethics were provided by the South Central Hampshire B NRES Committee (reference number 07/H0504/187). All participants recruited to the study provided informed consent for blood and tissue samples to be used for research purposes.
In B: The study was conducted according to the Declaration of Helsinki Principles and all patients donating samples to this study provided written, informed consent in accordance with ethical approval from the East of Scotland Research Ethics Service (EoSRES) REC 1. SSC tumors were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC. In addition, peri-lesional skin (histopathologically normal looking skin > 4 mm away from the tumor edge) and abdominal non-UV exposed skin were also analyzed.

Flow Cytometry

Plots

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BD FACSVerser and BD LSRFortessa X-20

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For cell sorting: 10,000 to 200,000 cells were collected for each population. The purity of each population was over 95% and was determined by rerunning the sorted population on a FACS.
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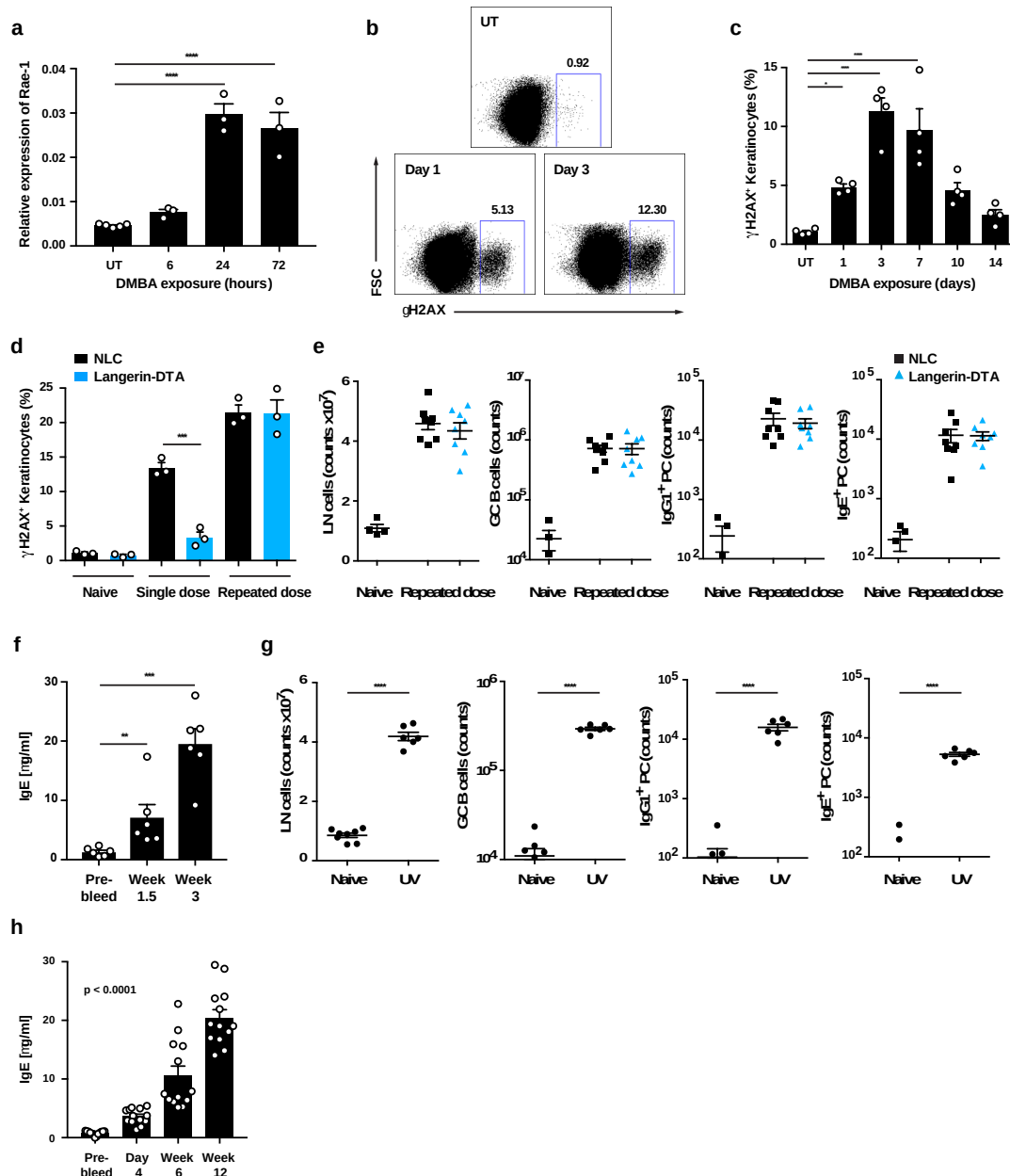
☐ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.

In the format provided by the authors and unedited.

Epithelial damage and tissue $\gamma\delta$ T cells promote a unique tumor-protective IgE response

Greg Crawford¹, Mark David Hayes¹, Rocio Castro Seoane¹, Sophie Ward¹, Tim Dalessandri¹, Chester Lai², Eugene Healy², David Kipling³, Charlotte Proby⁴, Colin Moyes⁵, Kile Green⁶, Katie Best^{6,7}, Muzlifah Haniffa^{6,7}, Marina Botto¹, Deborah Dunn-Walters⁸ and Jessica Strid^{1*}

¹Department of Medicine, Imperial College London, London, UK. ²Dermatopharmacology, Clinical and Experimental Sciences, Faculty of Medicine, University of Southampton, Southampton, United Kingdom; Dermatology, University Hospital Southampton NHS Foundation Trust, Southampton, UK. ³Division of Cancer and Genetics, School of Medicine, Cardiff University, Cardiff, UK. ⁴Division of Cancer Research, School of Medicine, University of Dundee, Ninewells Hospital & Medical School, Dundee, UK. ⁵Department of Pathology, Greater Glasgow and Clyde NHS, Queen Elizabeth University Hospital, Glasgow, UK. ⁶Institute of Cellular Medicine, Newcastle University, Newcastle upon Tyne, UK. ⁷Department of Dermatology and Newcastle Biomedical Research Centre, Royal Victoria Infirmary, Newcastle Hospitals NHS Foundation Trust, Newcastle upon Tyne, UK. ⁸Faculty of Health and Medical Sciences, School of Biosciences and Medicine, University of Surrey, Guildford, Surrey, UK. *e-mail: j.strid@imperial.ac.uk

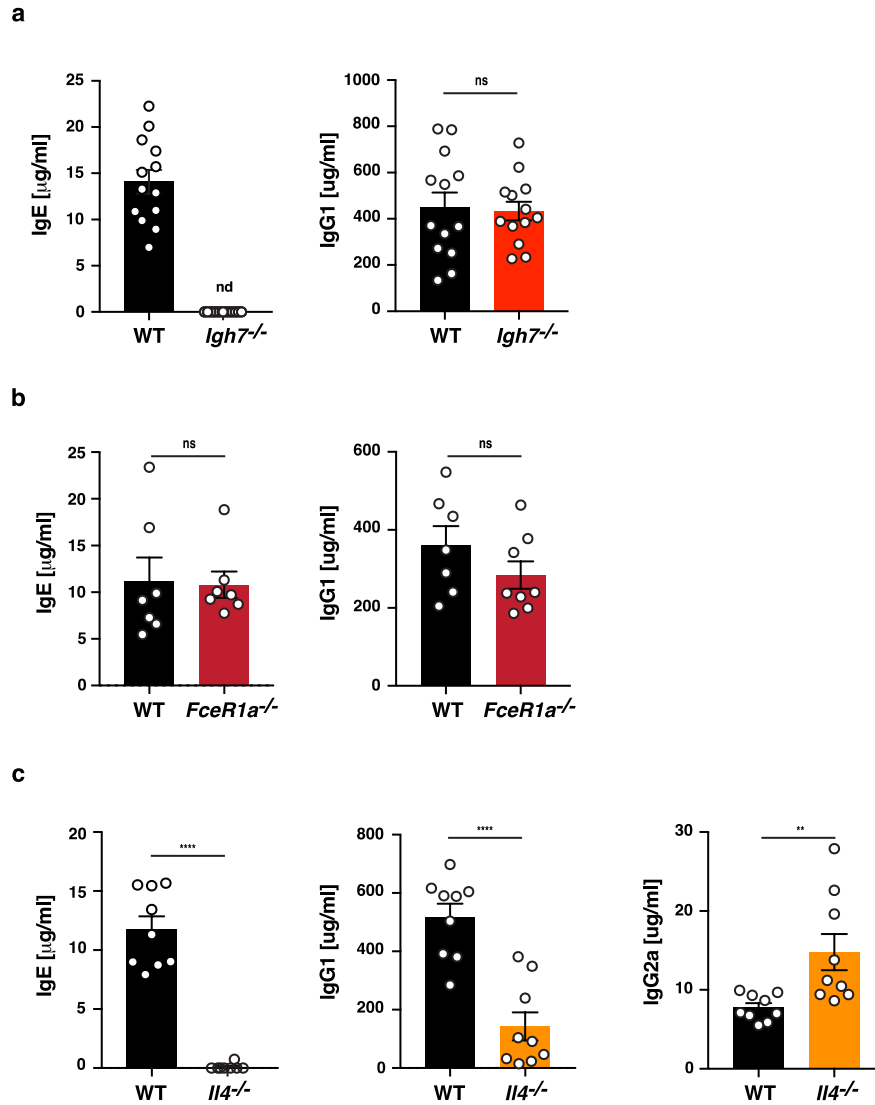


Supplementary Figure 1

Epithelial DNA-damage promote *de novo* IgE production

(a) Quantitative RT-PCR analysis of Rae-1 relative to cyclophylin in skin epithelial cells (n=3) and (b-c) FACS analysis of γ H2AX, as a measure of ds-DNA breaks, in skin epithelial cells (n=4), at indicated time-points after topical exposure to a single dose of DMBA to the dorsal ear skin of wild-type FVB mice. UT = untreated (n=4-5). (b) Representative FACS plots of γ H2AX staining in CD45⁺ epidermal cells. (d) FACS analysis of γ H2AX⁺ CD45⁺ skin epithelial cells from Langerin-DTA mice and non-transgenic littermate controls (NLC) (n=3/group) after a single or repeated topical DMBA treatment, analysed 3 days after last exposure and (e) FACS analysis of humoral immunity in the skin-draining LNs 7 days after the last DMBA exposure. Total LN cells, B220⁺CD95⁺GL7⁺ GC B cells and IgG1⁺ and IgE⁺ FSC^{hi}CD95⁺CD138⁺ PCs were enumerated (n=8/group). (f) ELISA of serum IgE and (g) FACS analysis of humoral immunity as in (e) in wild-type FVB mice exposed to UV light on shaved back skin at 100mJ/cm² 2-3x a week (n=6). Mice were bled after 4 exposures (week 1.5) and again at the end of the experiment (week 3) after 8 exposures. (h) ELISA of serum IgE in wild-type mice exposed to 200nmol DMBA

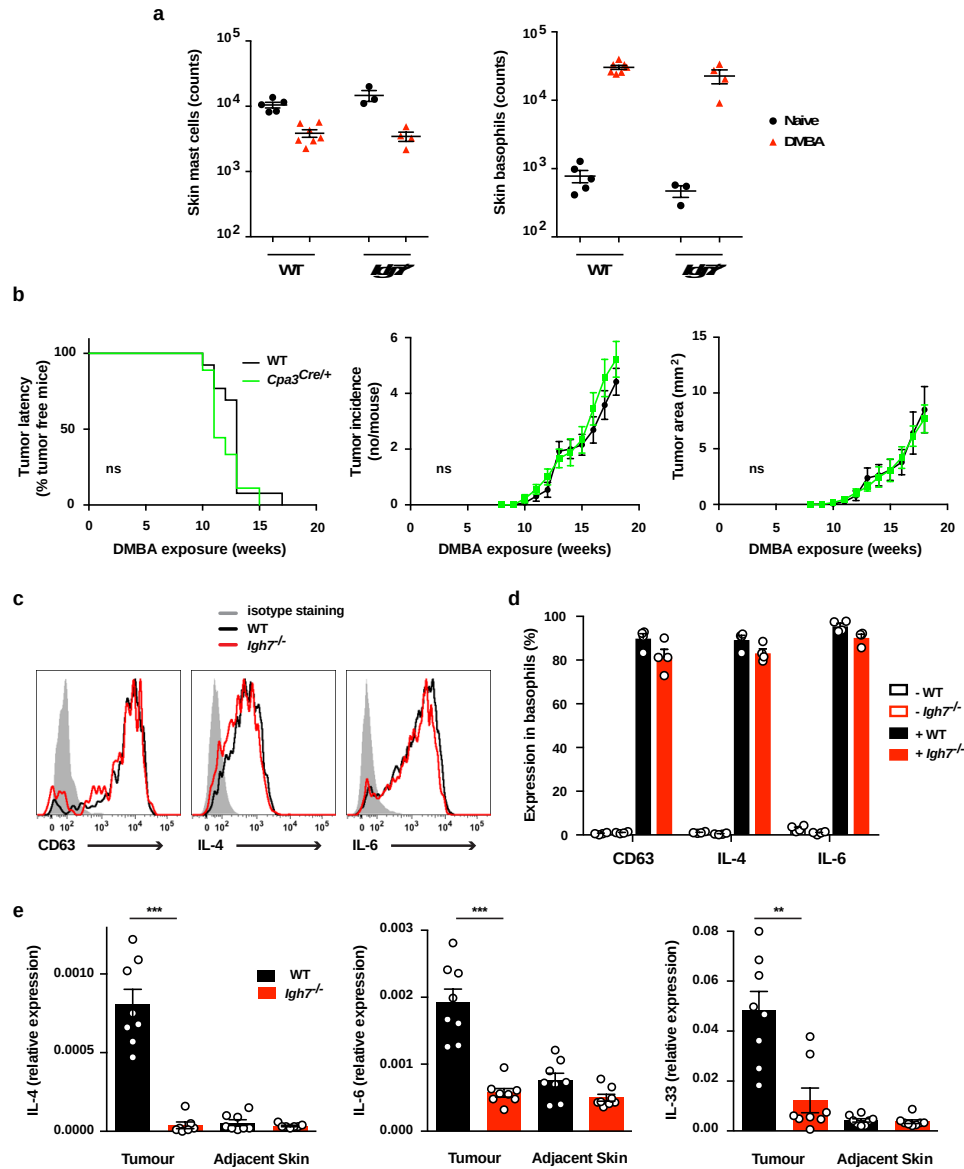
for 5 consecutive days and then left without further exposure. Mice were bled prior to exposure and at indicated time-points after the last DMBA-treatment (n=13). Some mice started to develop tumors around week 6. Statistics by two-tailed Student's t-test for unpaired data (d and g), one-way ANOVA multiple comparison (a, c, f) and one-way ANOVA with testing for linear trend of IgE increase with time (h); **p<0.01, ***p<0.001 and ****p<0.0001. All data are expressed as mean $\pm$ SEM.



Supplementary Figure 2

Antibody levels in wild-type, *Igh7*^{-/-}, *FceR1a*^{-/-} and *Il4*^{-/-} mice following DMBA carcinogenesis

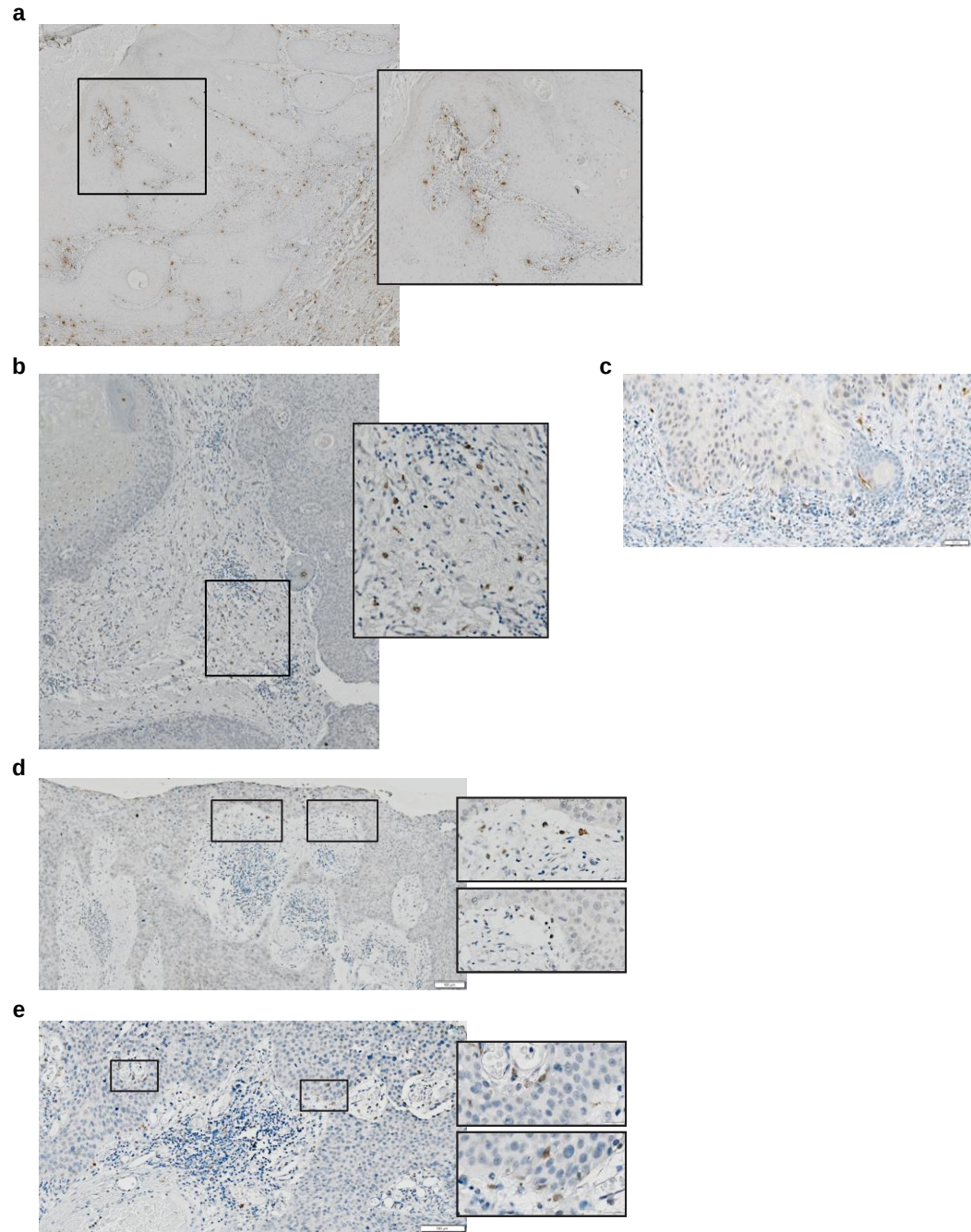
(a-c) ELISA of serum antibodies in mice subjected to DMBA carcinogenesis by once weekly exposure to DMBA on shaved back skin. Mice were bled and sera collected at the end of the carcinogenesis experiment. Data are expressed as mean antibody amount $\pm$ SEM in sera from (a) BALB/c wild-type and *Igh7*^{-/-} mice (n=13/group), (b) BALB/c wild-type and *FceR1a*^{-/-} mice (n=7/group) and (c) FVB wild-type and *Il4*^{-/-} mice (n=9/group). Statistical analysis in (a-c) was determined using two-tailed Student's t-test for unpaired data; **p<0.01 and ****p<0.0001. nd = not detected. ns = not significant. WT = wild-type.



Supplementary Figure 3

FcεRI-signaling in basophils is sufficient to protect against carcinogenesis and alters the tumor microenvironment

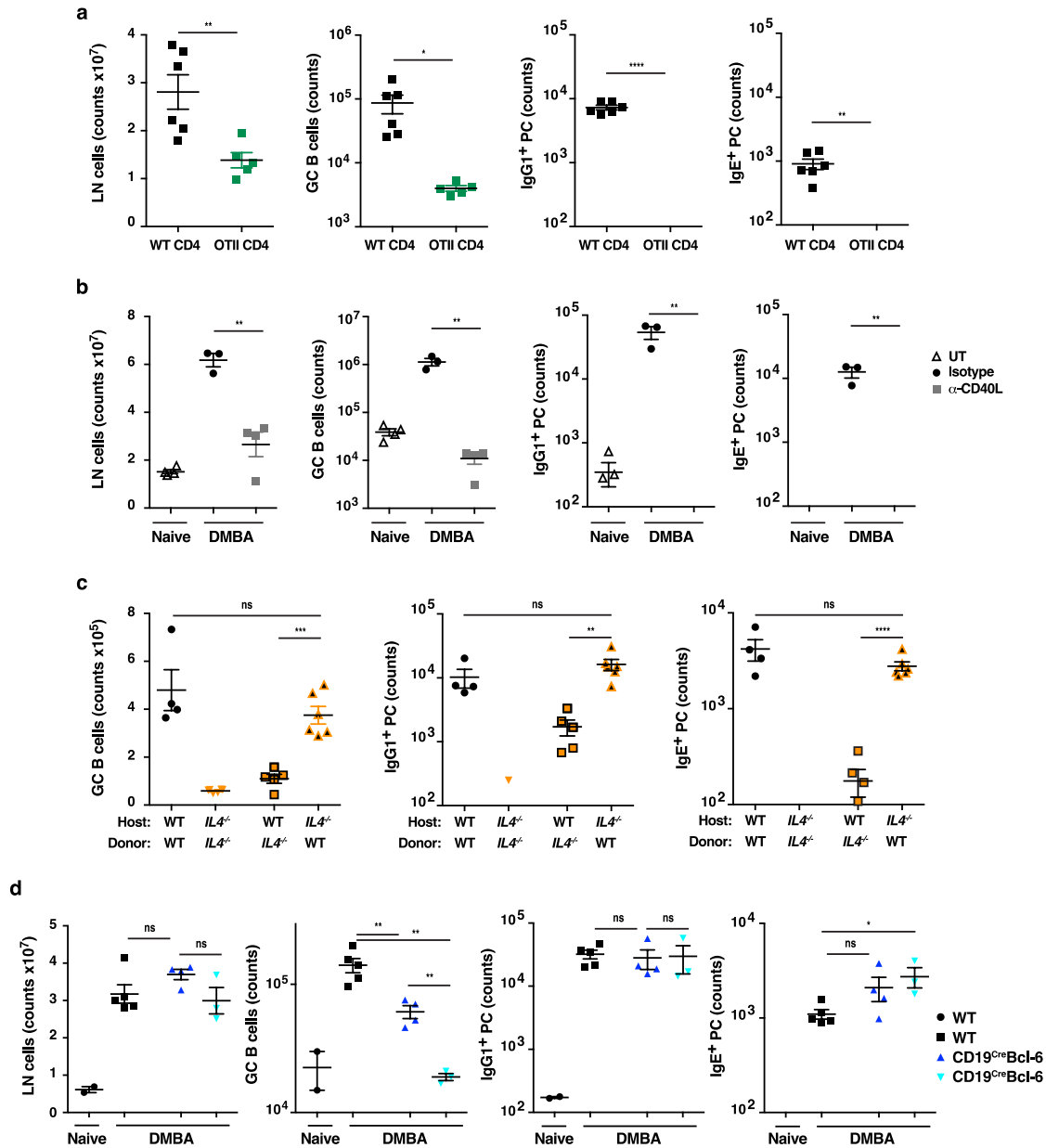
(a) FACS analysis of CD45^{hi}cKit⁺FcεRI⁺ skin mast cells and CD45^{lo}cKit⁺FcεRI⁺ skin basophils in wild-type and *Igh7*^{-/-} mice after twice topically treatment with DMBA compared to naïve mice (wild-type naïve n=6, wild-type DMBA n=7; *Igh7*^{-/-} naïve n=3, *Igh7*^{-/-} DMBA n=4). (b) Tumor susceptibility expressed as tumor latency (time to appearance of first tumor), tumor incidence (average number of tumors per mouse) and tumor area (average tumor size per mouse) in *Cpa3*^{Cre/+} (n=10) and *Cpa3*^{+/-} (wild-type) littermates (n=13) following DMBA-induced carcinogenesis. Data are expressed as mean ± SEM and statistical significance assessed using Log-rank (Mantel-Cox) test for tumor latency and linear regression for tumor incidence and area. ns = not significant. (c-d) FACS analysis of the degranulation marker CD63 and intracellular cytokine staining in splenic CD45⁺cKit⁺CD41⁺FcεRI⁺ basophils from wild-type and *Igh7*^{-/-} mice (n=4/group) left unstimulated (-) or stimulated *ex vivo* with PMA and ionomycin (+). (c) Representative histograms and (d) enumeration of % basophils positive for indicated marker. (e) Quantitative RT-PCR analysis of selected cytokines relative to cyclophilin in tumor tissue and adjacent skin from wild-type and *Igh7*^{-/-} mice (n=8/group) treated topically with DMBA once weekly and analysed at week 17. Data are expressed as mean ± SEM. Statistics by two-tailed Student's t-test for unpaired data; **p<0.01 and ***p<0.001. WT = wild-type.



Supplementary Figure 4

FcεRI⁺ cells in human skin SSCs accumulate at the interface between the stroma and the neoplastic keratinocytes

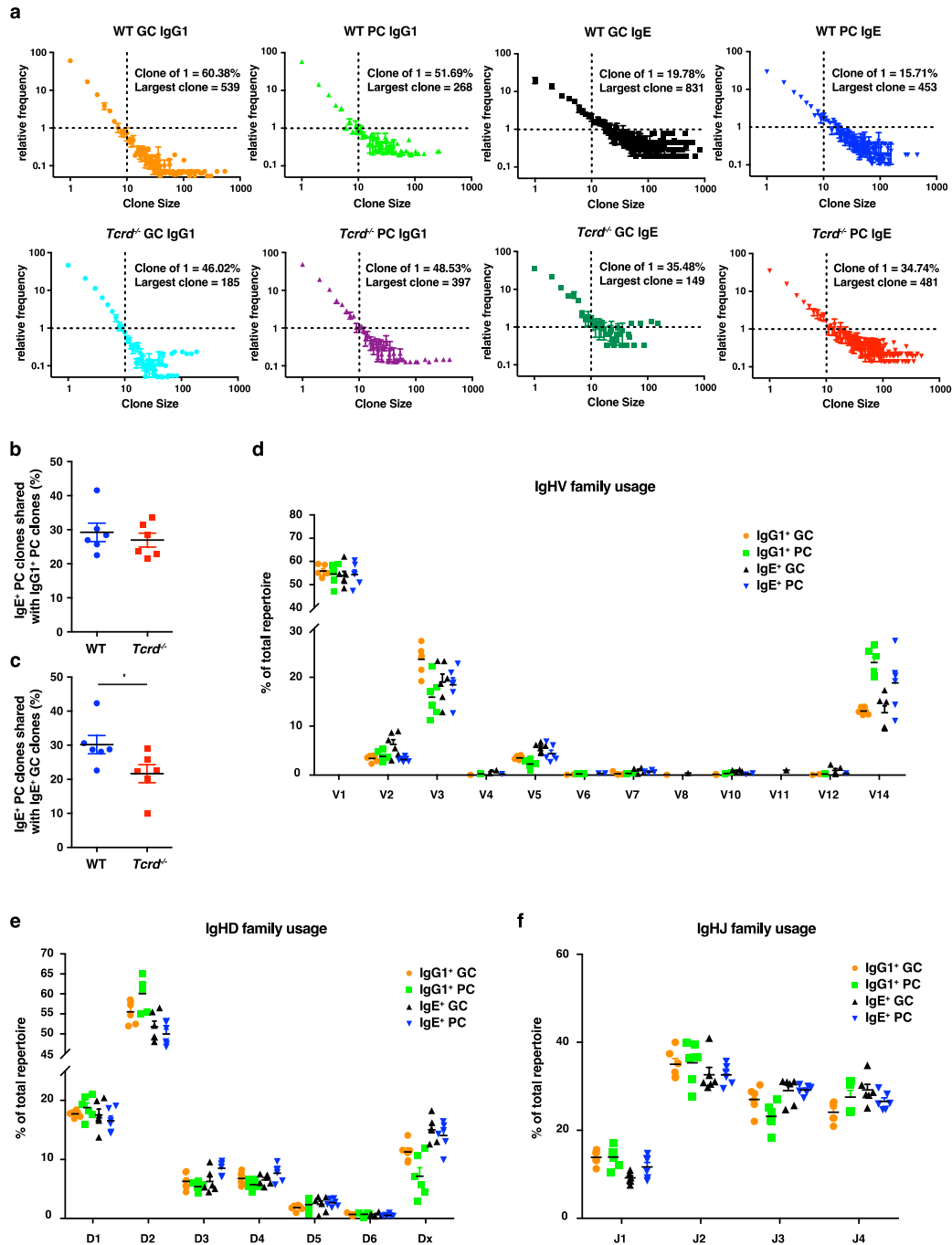
(a-e) Representative SSC histology from 5 patients showing FcεRI staining in brown. 5 μm tissue sections were cut from formalin-fixed paraffin-embedded samples, stained against FcεRI and counterstained with Mayer's Haematoxylin. Slides were imaged at 20x magnification using an Olympus Dotslide microscope and analysed using OlyVIA software. Insets show zoom of outlined areas.



Supplementary Figure 5

Carcinogen-induced antibody responses require TCR signaling, CD40L and LN IL-4 but not GC B cells

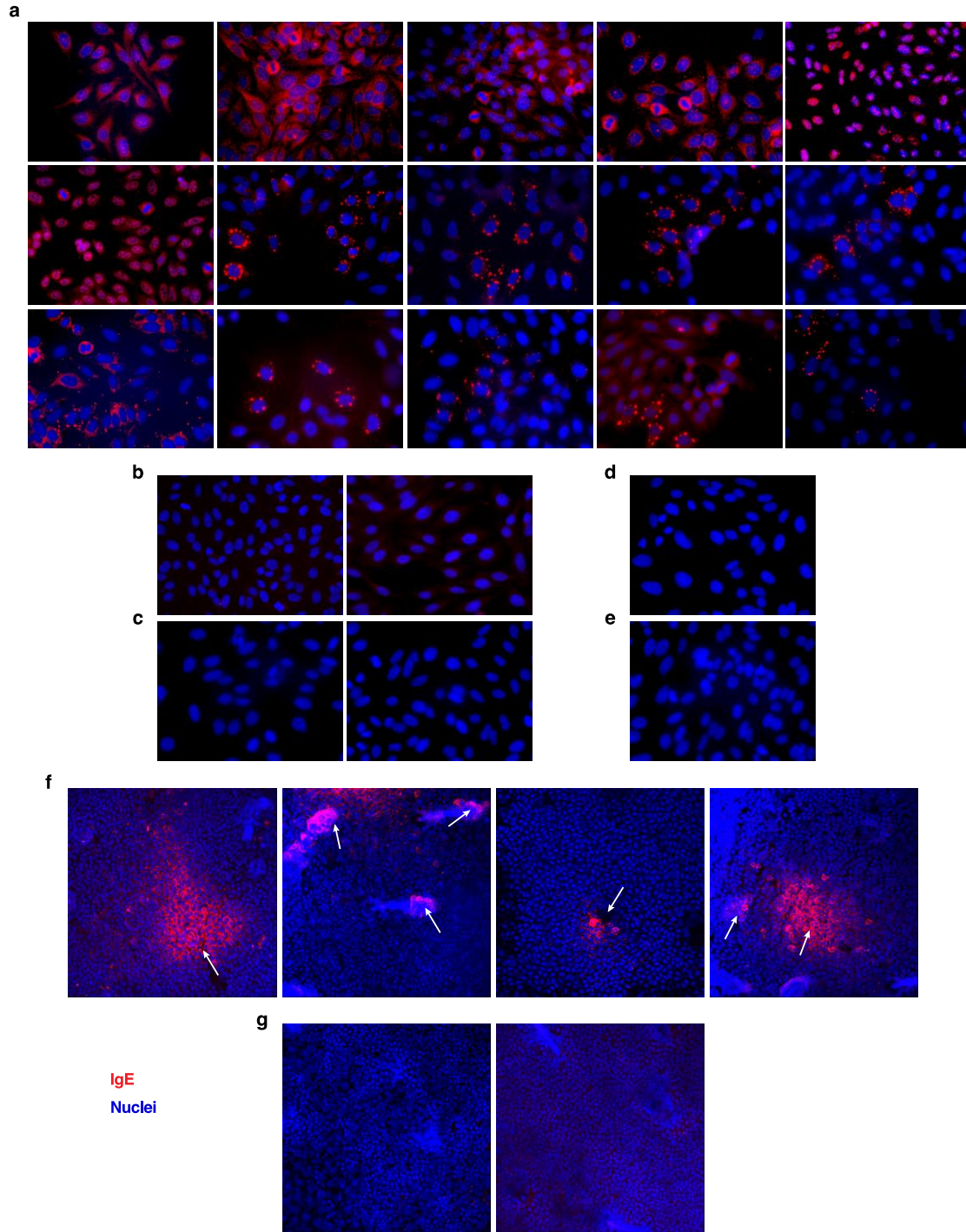
(a-d) FACS analysis of humoral immunity in the skin draining LNs analysed 7 days following twice topical exposure to DMBA on the dorsal ear skin. (a) C57BL/6 *Tcrb*^{-/-} mice were reconstituted with wild-type (n=6) or OTII TCR-restricted CD4⁺ αβ T cells (n=5) 1 day prior to DMBA exposure. (b) Wild-type FVB mice were injected with α-CD40L blocking Ab (MRI clone) (n=4) or isotype control Ab (n=3) prior to DMBA exposure and 3 further times during induction of the response. (c) Lethally irradiated FVB wild-type or *Il4*^{-/-} mice were reconstituted with either wild-type or *Il4*^{-/-} BM cells and exposed to DMBA 8 weeks after BM transplant (n=4-6/group). (d) C57BL/6 wild-type mice and mice with a heterozygous or homozygous deletion of Bcl-6 in B cells (CD19^{Cre}Bcl-6^{fl/wt} and CD19^{Cre}Bcl-6^{fl/fl} respectively) were exposed to topical DMBA (n=3-5/group). Graphs show number of total LN cells, B220⁺CD95⁺GL7⁺ GC B cells and IgG1⁺ or IgE⁺ FSC^{hi}CD95⁺CD138⁺ PCs presented as mean $\pm$ SEM. Statistics by two-tailed Student's t-test for unpaired data; *p<0.05, **p<0.01, ***p<0.001 and ****p<0.0001. ns = not significant. WT = wild-type. UT = untreated.



Supplementary Figure 6

Analysis of the IgG1 and IgE repertoires induced by topical carcinogen exposure on wild-type and *Tcrd*^{-/-} mice

(a-f) High-throughput sequencing and heavy-chain repertoire analysis of IgG1 and IgE in sorted B220⁺CD95⁺GL7⁺ GC B cells and FSC^{hi}CD95^{hi}CD138⁺ PCs from skin draining LNs of wild-type and *Tcrd*^{-/-} mice 7 days after the last of two topical exposures to DMBA (n=6/group). (a) Average frequency of a given clone size in the entire repertoire is shown for wild-type and *Tcrd*^{-/-} IgG1⁺ and IgE⁺ GC B cells and IgG1⁺ and IgE⁺ PCs. (b) Proportion of IgE⁺ PC clones shared with the IgG1⁺ PC clones and (c) the fraction of IgE⁺ PC clones shared with the IgE⁺ GC B cell clones. (d-f) Total IgHV, IgHD and IgHJ family gene usage within the wild-type IgG1⁺ GC B cells and PCs as well as wild-type IgE⁺ GC B cells and PCs. Statistics in (c) by two-tailed Student's t-test for unpaired data; *p<0.05. WT = wild-type.



Supplementary Figure 7

Autoreactivity of IgE in serum from DMBA-treated wild-type mice

(a-e) Examples of autoreactive binding to HEP-2 cells of IgE in serum from (a) DMBA-treated wild-type mice (1:25 dilution), (b) DMBA-treated *Tcrd*^{-/-} mice (1:5 dilution), (c) TPA-treated wild-type mice (1:5 dilution), (d) DMBA-treated *Igh7*^{-/-} mouse (1:5 dilution) and (e) naïve wild-type mouse (1:5 dilution). (f-g) Examples of autoreactive binding of IgE to clusters of epithelial cells in acutely DMBA damaged epidermis from *FceR1a*^{-/-} mice exposed to 200nmol DMBA once on the dorsal ear skin and epidermal sheets isolated 24hr later and stained with serum from (f) DMBA-treated wild-type or (g) *Igh7*^{-/-} mice (1:25 dilution). Arrows point to hair follicles. IgE binding (red) and nuclei (blue). Each image represents an individual mouse.

Reporting Summary

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Statistical parameters

When statistical analyses are reported, confirm that the following items are present in the relevant location (e.g. figure legend, table legend, main text, or Methods section).

n/a Confirmed

- ☐ ☒ The exact sample size (n) for each experimental group/condition, given as a discrete number and unit of measurement
- ☐ ☒ An indication of whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
- ☐ ☒ The statistical test(s) used AND whether they are one- or two-sided
Only common tests should be described solely by name; describe more complex techniques in the Methods section.
- ☐ ☒ A description of all covariates tested
- ☐ ☒ A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
- ☐ ☒ A full description of the statistics including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
- ☒ ☐ For null hypothesis testing, the test statistic (e.g. F , t , r) with confidence intervals, effect sizes, degrees of freedom and P value noted
Give P values as exact values whenever suitable.
- ☒ ☐ For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
- ☒ ☐ For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
- ☒ ☐ Estimates of effect sizes (e.g. Cohen's d , Pearson's r), indicating how they were calculated
- ☐ ☒ Clearly defined error bars
State explicitly what error bars represent (e.g. SD, SE, CI)

Our web collection on [statistics for biologists](#) may be useful.

Software and code

Policy information about [availability of computer code](#)

Data collection

BD FACS Diva software version 8.0.1, Quant Studio RT-PCR version 1.3, ELISA Ascents software version 2.6.

Data analysis

All software used throughout this work is publicly available: FlowJoV8.8.7/10, ImageJ, Graph Pad Prism 7, Illustrator, Alakazam from the Change-O toolkit (<http://clip.med.yale.edu/changeo>.) and BRepertoire (<http://mabra.biomed.kcl.ac.uk/BRepertoire/>).

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers upon request. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research [guidelines for submitting code & software](#) for further information.

Data

Policy information about [availability of data](#)

All manuscripts must include a [data availability statement](#). This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The data supporting the findings of this study are available from the corresponding author upon request. RNA sequencing data is available from the public

Field-specific reporting

Please select the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see [nature.com/authors/policies/ReportingSummary-flat.pdf](https://www.nature.com/authors/policies/ReportingSummary-flat.pdf)

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. Sample sizes were chosen based on previous experience with the experimental protocols. The antibody responses examined in this work were highly reproducible with little variability. Carcinogenesis experiments have a greater biological variation, however the protocols are well-known, and much previous experience guided us to increase our samples sizes to minimum 10 animals per experimental group to ensure statistical power in these longitudinal studies.
Data exclusions	No data points were excluded from data sets.
Replication	All the experimental data provided are based on biological replicates and are representative of independent experiments. All experiments were reliably reproduced.
Randomization	For animal work, mice of a particular genotype were randomly selected for experiment from a larger cohort - other than that, no randomization was used. For human tissue, individual SSC tumours were graded by an experienced dermatopathologist as low risk or high risk based on the Scottish Intercollegiate Guidelines Network for cutaneous SCC, as outlined in the materials and methods.
Blinding	For reporting on tumour development following chemically induced carcinogenesis, back skin and tumours were evaluated by an observer blinded to the experimental groups. For most other experiments, the investigators were not blinded to group allocation.

Reporting for specific materials, systems and methods

Materials & experimental systems

n/a	Involved in the study
☒	☐ Unique biological materials
☐	☒ Antibodies
☒	☐ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☐	☒ Human research participants

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used	For all flow cytometry staining, cell suspensions were incubated with 2% Rat Serum (Sigma) and Fc block (clone 93, BD Bioscience) for 30mins on ice, prior to staining. The following antibodies were then used: eBioscience: anti-CD45 (30-F11), B220 (RA3-6B2), , CD95 (Jo2), GL7 (GL-7), IgE (23G3), IgG1 (M1-14D12), TCRβ (H57-597), TCRγδ (eBioGL3), Vγ5 (536), CD117 (2B8), IL-4 (11B11); BD Biosciences: CD38 (90/CD38), CD138 (281-2), IgG2a (R19-15); Biolegend: CD41 (MWRReg30), FcεRI (MAR-1), IL-6 (MP5-20F3), CD63 (NVG-2), Vγ4 (UC3-10Ab), CD3 (145-2C11); Millipore: γH2AX (JBW301). All antibodies were used at a dilution of 1:100-1:200 as per respective manufacturers recommendation. OVA-loaded tetramers were provided by the NIH tetramer facility - Emory University Vaccine Center 954 Gatewood Road Atlanta, GA 30329. Reagents are provided to qualified investigators at no cost.
Validation	All antibodies were validated by the manufacturers and by our own and colleagues' labs where they are used extensively. All antibodies were titrated to determine optimal staining concentration.

Animals and other organisms

Policy information about [studies involving animals](#); [ARRIVE guidelines](#) recommended for reporting animal research

Laboratory animals

Only mice were used in this study and groups were age- and sex-matched for all experiments. In general females mice at 7-9 weeks were used throughout. Tcrd^{-/-}, Tcrb^{-/-}, IL4^{-/-}, Langerin-DTA (LC-deficient) and Vg5Vd1^{-/-} were on the FVB/N background after >10 backcrosses; Igh^{-7/-}, FcεR1a^{-/-} and Cpa3Cre/+ were on the BALB/c background after >10 backcrosses; and OTII Tg, Tcrb^{-/-}, CD19-Cre and Bcl6fl/fl were on the C57BL/6 background. Genetic backgrounds of individual strains and controls are indicated where appropriate. Humoral responses were also examined in male animals and were comparable. All mouse studies were approved by Imperial College AWERB (Animal Welfare and Ethical Review Body) and by the UK Home Office. Experiments involving cancer studies strictly adhered to the guidelines set out by the National Cancer Research Institute (NCRI) and Workman et al. in 'Guidelines for the Welfare and Use of Animals in Cancer Research'. All studies using mice were conducted following the Animal Research: Reporting In Vivo Experiments (ARRIVE) guidelines.

Wild animals

n/a

Field-collected samples

n/a

Human research participants

Policy information about [studies involving human research participants](#)

Population characteristics

Two cohorts of human research participants were involved in this study:
A: freshly excised SSC tumour tissue and peri-lesional skin for flow cytometric analysis from 12 patients: All tumor tissue used in the study was confirmed SSCs with a diameter > 8mm. Non-lesional skin was histopathologically normal and > 6mm away from the tumor edge.
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