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Targeting the human β_c receptor inhibits contact dermatitis in a transgenic mouse model

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[1] Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, South Australia, Australia.

[2] Research and Development, CSL Behring Innovation GmbH, Marburg, Germany.

[3] Research and Development, CSL Limited, Bio21 Institute, Parkville, Victoria, Australia.

[4] Centre for Cancer Biology, SA Pathology and the University of South Australia. Present address: OMNI-Biomarker Development, Genentech Inc., South San Francisco, California, USA.

[5] Faculty of Medicine, University of Adelaide, Adelaide, Australia.

Short title

Blocking human β_c in vivo prevents dermatitis

[#]These authors contributed equally

[*]To whom correspondence should be addressed

Correspondence

Angel F Lopez

Address: Centre for Cancer Biology, SA Pathology and the University of South Australia, Adelaide, SA, Australia

Phone: +61 (08) 830 27915

Email: angel.lopez@sa.gov.au

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ABSTRACT:

Allergic contact dermatitis (ACD) is a prevalent and poorly controlled inflammatory disease caused by skin infiltration of T cells and granulocytes. The β_c cytokines GM-CSF, IL-3 and IL-5 are powerful regulators of granulocyte function that signal through their common receptor subunit β_c, a property that has made β_c an attractive target to simultaneously inhibit these cytokines. However, the species specificity of β_c has precluded testing of inhibitors of human β_c in mouse models. To overcome this problem, we developed a human β_c receptor transgenic (hβ_cTg) mouse strain with hematopoietic cell-specific expression of human β_c instead of mouse β_c. hβ_cTg cells responded to mouse GM-CSF and IL-5 but not IL-3 *in vitro* and developed tissue pathology and cellular inflammation comparable to wild-type mice in a model of ACD. Similarly, *IL-3*$^{-/-}$ mice developed ACD pathology comparable to wild-type mice. Importantly, the blocking anti-human β_c antibody CSL311 strongly suppressed ear pinna thickening and histopathological changes typical of ACD and reduced accumulation of neutrophils, mast cells and eosinophils in the skin. These results show that GM-CSF and IL-5, but not IL-3 are major mediators of ACD and define the hβ_cTg mouse as a unique platform to test inhibitors of β_c *in vivo*.

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INTRODUCTION:

Allergic contact dermatitis (ACD) affects 15 - 20% of the general population with a large proportion of patients exhibiting poorly controlled disease (Martin et al., 2018, Owen et al., 2018). ACD is a type IV hypersensitivity reaction triggered by low-molecular-weight organic chemicals and metal ions called haptens that form complexes with larger carrier proteins enabling them to be recognised by the immune system (Kaplan et al., 2012). Upon hapten exposure, dendritic cells migrate from the skin to the draining lymph nodes where they present the hapten-protein complexes to T cells. Activated T cells then traffic to the site of antigen re-exposure and release inflammatory mediators that initiate the immune response. Release of inflammatory mediators by resident-skin cells, particularly by mast cells, induces further recruitment of leukocytes comprising neutrophils, eosinophils and monocytes that cause pruritic erythema and oedema (Dudeck et al., 2011, Honda et al., 2013, Vocanson et al., 2009).

Cytokines are critical drivers of inflammatory cell accumulation and disease progression during both the afferent and elicitation phases of ACD (Kobayashi et al., 2010, Wang et al., 2003). The β_c cytokine family consists of GM-CSF, IL-3 and IL-5 that are produced largely by activated T cells and mast cells and regulate the development and functional activation of hemopoietic cells (Dougan et al., 2019, Martinez-Moczygemba and Huston, 2003). In humans, GM-CSF, IL-3, and IL-5 each binds a heterodimeric receptor comprising a ligand-specific α chain and a shared beta common (β_c) subunit that mediates signal transduction (Broughton et al., 2012). In contrast, mice have 2 signaling subunits, mouse (m)β_c and m$\beta_{IL\text{-}3}$. Genetic deletion of both these receptors in mice results in no serious hematopoietic abnormalities, indicating the β_c cytokines are not required for steady-state hematopoiesis (Nishinakamura et al., 1995, Scott et al., 2000). The β_c cytokines are therefore regarded as pleiotropic regulators

which play an essential role in emergency hematopoiesis and inflammation (Broughton et al., 2012). While the importance of these cytokines is well demonstrated in allergic airway inflammation (Asquith et al., 2008), their function in ACD is poorly defined. In a contact hypersensitivity (CHS) model, an animal model of human ACD, the CHS response was reported to be diminished in *IL-3*$^{-/-}$ mice (Mach et al., 1998) but not in *GM-CSF*$^{-/-}$ mice (Gillessen et al., 2001), implicating IL-3 rather than GM-CSF as the relevant β_c cytokine in the pathology of ACD.

CSL311 is a fully human monoclonal antibody that binds to the common cytokine binding site of hβ_c (Woodcock et al., 1996, Woodcock et al., 1994) and blocks cytokine binding and signalling (Panousis et al., 2016). Due to the species specificity of CSL311 for hβ_c, it was previously not possible to test the therapeutic potential of CSL311 in pre-clinical disease models such as CHS. We herein show the development and characterisation of a transgenic mouse model engineered to expresses hβ_c under the control of a hematopoietic-specific promoter in $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice and, using this model, we demonstrate that GM-CSF and IL-5 but not IL-3 are the main inflammatory mediators of CHS and provide evidence that targeting hβ_c signalling reduces inflammatory cell accumulation and attenuates the CHS response.

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RESULTS:

Generation and characterisation of hβcTg mice

Human β_c receptor transgenic (hβ_cTg) mice were generated through microinjection of pIC19-HS21/45 expression vector (Ogilvy et al., 1999) containing hβ_c cDNA into pronuclei of C57BL/6N fertilised mouse embryos (**Figure S1a**), followed by crossing with $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice and confirmation of genotype by PCR (**Figure S1b, Table S1**). We performed whole genome sequencing of the hβ_cTg mice and identified the hβ_c cDNA inserted at a single location on chromosome 17, with at least 15 copies of the hβ_c cDNA fused together in a tandem tail-to-head rearrangement at the integration site (**Figure 1a**).

Analysis of peripheral blood from hβ_cTg mice showed that numbers of all major blood cell types were comparable to WT mice (**Table S2**). Tissue-specific expression of hβ_c in lymphoid organs including the spleen, lymph node, thymus and bone marrow showed the highest levels of hβ_c protein staining (**Figure S2**). Cell surface expression of hβ_c was confirmed by flow cytometry using a hβ_c-specific monoclonal antibody 1C1 (Stomski et al., 1996). Expression of hβ_c was detected in different subsets of hematopoietic cell in the spleen (**Figure 1b, Figure S3 for gating strategy**), and the number of cell surface hβ_c receptors on different hematopoietic cell types was quantified using Quantum™ PE beads with the most abundant expression of hβ_c observed on basophils (**Table S3**). Surface expression of the mGMRα, mIL3Rα and mIL5Rα subunits was also examined in these immune cell populations and similar expression levels were determined between hβ_cTg and WT mice (**Figure S4a-c**), indicating that transgenic expression of hβ_c does not affect expression of endogenous cytokine-specific mouse α subunits.

Immune cells from hβcTg mice respond to mGM-CSF and mIL-5, but not to mIL-3

We next analysed the ability of $h\beta_c$ to engage the mouse cytokine-specific α subunits and activate intracellular signalling pathways in response to mouse cytokines. Recognition of the β_c receptor ligands IL-3, IL-5 and GM-CSF results in phosphorylation of STAT5 which induces transcriptional activation of STAT5 target genes (Guthridge et al., 1998). Incubation of splenocytes with mGM-CSF (10ng/mL) or mIL-5 (10ng/mL) for 15 min induced robust STAT5 phosphorylation in monocytes and eosinophils from both WT and $h\beta_c$Tg mice respectively (**Figure 2a-b**). However, while incubation with mIL-3 (10ng/mL) for 15 min stimulated strong STAT5 phosphorylation in basophils, plasmacytoid dendritic cells and monocytes from WT mice, STAT5 phosphorylation was absent in these cell types from $h\beta_c$Tg mice (**Figure 2c**) even when the IL-3 concentration and incubation time increased to 300ng/mL and 60 min (data not shown). While basophils from $h\beta_c$Tg mice did not respond to mIL-3 stimulation, STAT5 phosphorylation in response to mGM-CSF (**Figure S5a**) and mIL-5 (**Figure S5b**) was preserved. IL-3 is critical for mast cell development *in vitro*, however, it is not essential for the generation of mast cells or basophils under physiological conditions (Lantz et al., 1998), and we found that the peritoneal mast cell responses to IgE cross linking were similar between WT and $h\beta_c$Tg mice (**Figure S6**). These data indicate that $h\beta_c$ can functionally replace signalling through $m\beta_c$ by mGM-CSF and mIL-5, but not mIL-3.

We have previously shown that human β_c cytokines engage hβc through a common binding site (Woodcock et al., 1996, Woodcock et al., 1994) that is amenable to inhibition by monoclonal antibodies (Rossjohn et al., 2000, Sun et al., 1999). This led to the development of the similarly $h\beta_c$ blocking antibody CSL311 (Panousis et al., 2016) which we tested for inhibition of signalling by mGM-GSF and mIL-5 in cells from $h\beta_c$Tg mice. Pre-incubation of cells with CSL311 (20μg/mL), prevented mGM-CSF-induced STAT5 activation in monocytes and neutrophils (**Figure 3a**) and mIL-5-induced pSTAT5 signalling in eosinophils

and B1a B cells from hβcTg mice (**Figure 3b**). Importantly, CSL311 had no effect on mGM-CSF or mIL-5 signalling in cells from WT mice, consistent with the specificity of CSL311 for hβc and not the mβc or mβIL-3 subunits.

CSL311 reduces cellular inflammation and tissue pathology in a hβcTg mouse model of contact dermatitis

To establish the therapeutic potential of CSL311 for treatment of ACD, we examined the CHS response to an epicutaneously applied hapten 2,4-dinitrofluorobenzene (DNFB) where mice are sensitised with DNFB on the abdomen and 5 days later DNFB is applied to the ears to elicit CHS (Grimbaldeston et al., 2007). WT mice developed a substantial increase in ear thickness that peaked at 6-8 days after elicitation of CHS and subsided thereafter. The magnitude of the ear swelling response was significantly reduced in $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice that are unable to respond to any of the mβc cytokines (**Figure 4a**). Moreover, we observed no differences in ear swelling responses between WT and *IL-3*$^{-/-}$ mice (**Figure 6a**), indicating that GM-CSF and IL-5 are major contributors to development of CHS *in vivo*.

Mice treated with isotype control antibody developed ear swelling responses similar to WT mice. In contrast, treatment of hβcTg mice with CSL311 significantly reduced ear swelling to a level similar to that of the $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice (**Figure 4a**). Furthermore, CSL311 did not affect CHS responses in either WT, $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ or *IL-3*$^{-/-}$ mice, further indicating the specificity of CSL311 for hβc (**Figure S7**). The DNFB-treated ears of both WT, *IL-3*$^{-/-}$ mice and isotype control antibody-treated hβcTg mice were characterised by substantial dermal oedema, cellular infiltrate, hyperkeratosis and epithelial hyperplasia as shown by H&E staining. These features were largely absent in DNFB-treated ears of both $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice and hβcTg mice

treated with CSL311 (**Figure 4b & c**). These data indicate that blocking GM-CSF and IL-5 signalling with CSL311 may be an effective therapy for CHS.

A significant increase in mast cells, neutrophils, eosinophils and macrophages were detected in DNFB-treated ear tissue at both the resolution phase of the response (day 12) (**Figure 4d, Figure 5b,c & e, Figure S8**) and the peak of the response (day 6) **(Figure S9b, c, e & f)** in WT mice, whereas infiltration of $CD4^+$ T cells was only observed at day 6 (**Figure 5d, Figure S9d**). Absence of β_c cytokine signalling in $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ mice diminished the infiltration of all these cell types except macrophages in the DNFB-treated ears. Notably, immune cell infiltration was similar between WT and *IL-3*$^{-/-}$ mice **(Figure 6b-e)**, further indicating that IL-3 does not affect development of CHS.

Analysis of the ear tissues from hβ_cTg mice treated with the isotype control antibody revealed a significant increase in mast cells, neutrophils and eosinophils in DNFB-treated ears at both the resolution (day 12) and peak (day 6) timepoints, similar to WT mice (**Figure 4d, 5b & c, Figure S9b, c & f)**. $CD8^+$ T cell accumulation in DNFB-treated ears of isotype control treated hβ_cTg mice was also increased at the peak of the response, similar to WT mice but returned to baseline levels by day 12 in hβ_cTg mice while remaining elevated in WT mice **(Figure 5a, Figure S9a)**. The difference in $CD8^+$ T cells during the resolution phase of the response in hβ_cTg mice compared to WT mice will require investigation in future studies. Importantly, we found that treatment of hβ_cTg mice with CSL311 abolished ear tissue accumulation of $CD8^+$ T cells, neutrophils, eosinophils and mast cells at both timepoints (**Figure 4d, 5b & c, Figure S9**) following DNFB treatment. These data show that CSL311 is an effective treatment to block CHS-associated inflammation in the DNFB mouse model.

We also investigated the effect of CSL311 treatment in a well-characterised atopic dermatitis model where daily application of vitamin D3 analogue MC903 (calcipotriol) is used to induce skin inflammation. The resulting phenotype includes epidermal thickening, dermal hyperplasia, and an increased number of inflammatory cells in the skin, most notably mast cells, eosinophils, basophils and T helper type 2 cells (Siracusa et al., 2011). CSL311 significantly reduced ear skin thickening in hβ_cTg mice in this MC903-induced atopic dermatitis model **(Figure S10)**.

Taken together, our data indicate that GM-CSF and IL-5 are major pathogenic β_c cytokines in CHS and that blocking their signalling by CSL311 limits mast cell and granulocyte accumulation and overall pathology in our *in vivo* hβ_cTg model of CHS.

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DISCUSSION

Therapeutic approaches targeting the underlying inflammatory pathways driving skin inflammation may provide effective treatments with fewer side effects than traditional drugs such as corticosteroids. Our data indicate that neutralisation of β_c cytokine signalling may represent a effective therapeutic intervention for ACD. The β_c cytokines GM-CSF, IL-3 and IL-5, individually and cooperatively contribute to pathology in chronic inflammation and provide a link between innate and acquired immunity by virtue of being the product of activated T cells, endothelial cells and mast cells and for their ability to strongly stimulate proliferation, survival, and differentiation of myeloid hematopoietic cells, as well as the effector function of terminally differentiated myeloid cells (Broughton et al., 2012). Their importance in the regulation of inflammation has made these cytokines an attractive target for therapeutic intervention. We previously demonstrated that the human therapeutic antibody, CSL311, inhibited human nasal polyp progression in a humanised mouse xenograft model, showing that targeting the β_c receptor with a therapeutic monoclonal antibody may be an effective strategy for treating chronic rhinosinusitis with nasal polyps (Yip et al., 2020). However, in the absence of human tissue for xenograft models, *in vivo* testing of the therapeutic benefit of CSL311 in mice is not possible due to the species specificity of CSL311 for $h\beta_c$.

To enable preclinical testing of antibodies against the human β_c subunit of the GM-CSF, IL-5 and IL-3 cytokine receptors, we created a $h\beta_c$ receptor transgenic mouse model where the $h\beta_c$ transgene is expressed in hematopoietic cells. Immune cells from the $h\beta_c$Tg mice respond to the mouse β_c cytokines, mGM-CSF and mIL-5, but not mIL-3. The absence of mIL-3 signalling could be due to the low amino acid sequence homology between mouse and human IL3Rα subunits and IL-3, which have 30% and 29% similarity, respectively (Hara and

Miyajima, 1992, Yang et al., 1986). This contrasts with the higher sequence conservation between mouse and human β_c subunits (55% identity) (Guthridge et al., 1998). This diversity between species may reduce the formation or stability of the chimeric receptor complex formed between mIL-3, mIL3Rα and hβ_c, or result in a defective chimeric receptor signalling complex. We herein show that loss of β_c cytokine signals reduced the development of adaptive hapten-specific CHS responses and that the antibody CSL311 was capable of attenuating CHS to a similar extent as complete loss of β_c signalling. The functions of individual β_c cytokines in CHS are not completely defined. Limited published data suggested that IL-3 was the major β_c cytokine driving the development of the hapten-induced CHS 24 hours after hapten exposure and that GM-CSF had a supportive role (Gillessen et al., 2001). Furthermore, IL-5-dependent activation of eosinophils and B-1 cells has been proposed to be involved in the induction and elicitation of CHS in mice (Itakura et al., 2006). In the current study, we found comparable CHS responses in hβ_cTg mice and WT mice and that CHS was reduced in hβ_cTg mice treated with the therapeutic antibody CSL311. These results indicate that GM-CSF and IL-5 play a central pathological role in CHS since hβ_cTg mice lack IL-3 signalling. This finding is further supported by our result showing that CHS pathology was unaffected in *IL-3*$^{-/-}$ mice and indicates that IL-3 may not be required for the development of CHS. This is in contrast to previous reports (Mach et al., 1998) and cannot be ascribed to differences in dendritic cell (Gillessen et al., 2001, Mach et al., 1998) or mast cell numbers (**Figure 4d**).

The β_c cytokines, GM-CSF and IL-5, have been associated with atopic dermatitis (Pastore et al., 1997, Yamamoto et al., 2003). Although dispensable for steady-state myelopoiesis, GM-CSF serves to functionally link tissue-invading lymphocytes and myeloid cells, triggering rapid expansion and differentiation of myeloid cells during inflammation (Becher et al., 2016).

Furthermore, GM-CSF promotes both the maturation and migration of bone marrow precursors to form epidermal Langerhans cells and subsequently the migration of Langerhans cells to draining lymph nodes where they present antigens to T cells (Lutz et al., 2017). Both systemic and locally produced GM-CSF greatly enhances neutrophil and monocyte numbers in circulation and inflamed tissue, upregulating neutrophil adhesion and trafficking, oxidative burst and phagocytosis (Hamilton, 2020). Likewise, many aspects of eosinophil biology, including their development, survival, activation, and migration, are controlled by IL-5 and, to some extent GM-CSF in inflammation. These myeloid cells are thus likely candidate regulators of tissue damage, vascular hyperpermeability and inflammation in CHS. The functional significance of these cytokine changes in skin lesions of chronic skin diseases is supported by elevation of protein (Pastore et al., 1997) and mRNA expression (Jeong et al., 2003) of GM-CSF and IL-5, as well as their corresponding receptors (Taha et al., 1998) in patients with dermatitis. The inhibitory activity of CSL311 in both CHS and MC-903-induced atopic dermatitis models supports the notion that GM-CSF and IL-5 play a major pathogenic role in these mouse models of human disease and establishes the potential of blocking β_c signalling as an effective treatment of patients with poorly controlled dermatitis. Clinical trials are now required to assess the effectiveness of $h\beta_c$ inhibition for the treatment of dermatitis.

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MATERIALS AND METHODS

Extended protocols are described in the Supplementary Materials.

Flow cytometry and intracellular pSTAT5 analysis

Single-cell suspensions were prepared from the mouse spleen and plated at 2×10^6 cells/well in a 96 well round-bottom plate. Fc receptors were blocked with mouse Fc block (1:50 dilution, Miltenyi Biotec) in FACS buffer (PBS with 2% Fetal bovine serum) for 15 min at room temperature and dead cells stained with viability stain 780 (BD Bioscience). Cell were then incubated for 30 min at 4°C with the antibody panels in **Table S4**. For intracellular pSTAT5 analysis, cells were incubated with 10ng/mL mGM-CSF, mIL-5 or mIL-3 for 10 min at 37°C. Cells were incubated with the antibody panels as above for 30 min at 4°C and then washed in PBS and incubated in pre-warmed Cytofix (BD Bioscience) for 10 min at 37°C. Cells were then centrifuged and incubated with cold Perm buffer III (BD Bioscience) for 30 min at 4°C. Cells were washed twice with FACS buffer and incubated with 1:12.5 dilution of anti-pSTAT5-Alexa Fluor®647 (BD Bioscience) for 30 min at 4°C. To assess the blocking action of CSL311, cells were preincubated in 20mg/mL CSL311 or isotype control for 15 min prior to cytokine stimulation. Cells were then washed, resuspended in FACS buffer and data was acquired on a BD LSR Fortessa (BD Bioscience) and analysed with FCS express 5 & 6 (De Novo Software).

Contact hypersensitivity model

Mice were anaesthetised and a $3cm^2$ area shaved on the posterior ventral side. CHS was induced as previously described (Grimbaldeston et al., 2007). Briefly, 30µL of 0.5% 2,4-dinitrofluorobenzene (DNFB, Sigma-Aldrich) in acetone was applied to the abdomen and five days later, baseline ear pinna thickness for both ears was measured using a dial thickness gauge (model-G-1A; Ozaki Manufacturing). Immediately following measurement, 10µL of

0.2% DNFB in acetone was applied to each side of the right ear pinna (20μL total). As a control, 10μL of 100% acetone (vehicle) was applied to each side of the left ear pinna (20μL total). Ear pinna thickness was measured at 24-hours intervals over 6- or 12-days post elicitation. Changes in ear pinna thickness (Δ Ear swelling, mm) were calculated by the formula: (ear thickness, at each day following elicitation) – (baseline ear thickness). CSL311 or isotype mAbs (10 mg/kg) was injected intravenously into the tail vein of hβ$_c$Tg mice at 1, 3, 5, days post elicitation (a total of 3 injections). Mice were humanely killed at the end of the experiment and ear tissues collect for subsequent analyses.

Statistics

Unpaired t-test was used to compare different groups and two-way ANOVA with Bonferroni post-test was used for multiple comparisons. GraphPad Prism Version 5.0d was used. Asterisks above a group indicate the level of significance of that group relative to control, *p<0.05; **p<0.01; ***p<0.001.

Study approval and mice

WT, $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$, hβ$_c$Tg and *IL-3*$^{-/-}$ mice were housed at the Animal Care Facility, UniSA and used at 8-16 weeks old. All experiments were performed in compliance with the ethical guidelines of the National Health and Medical Research Council of Australia and European Convention on Animal Care, and with ethics approval from the UniSA Animal Ethics Committee (U21/18).

Data availability statement

The authors confirm that the data supporting the findings of this study are available within the article and its Supplementary Materials.

ORCIDs

Kwok Ho Yip: https://orcid.org/0000-0002-8453-0118

Duncan McKenzie: https://orcid.org/0000-0003-0961-8846

Hayley S. Ramshaw: https://orcid.org/0000-0001-9909-282X

Jessica Chao: https://orcid.org/0000-0001-9512-6121

Barbara J. McClure: https://orcid.org/0000-0002-5201-4127

Elmar Raquet: https://orcid.org/0000-0002-4909-0735

Timo Kraushaar: https://orcid.org/0000-0003-2904-6208

Joachim Röder: https://orcid.org/0000-0001-7611-1418

Mhairi Maxwell: https://orcid.org/0000-0001-5530-4982

Monther Alhamdoosh: https://orcid.org/0000-0002-2411-1325

Andrew Hammet: https://orcid.org/0000-0003-1330-7303

Jia Hong Fong: https://orcid.org/0000-0003-1722-2294

Kathleen Zeglinski: https://orcid.org/0000-0003-0608-229X

Katherine Monaghan: https://orcid.org/0000-0002-1255-4970

Harshita Pant: https://orcid.org/0000-0002-9388-193X

Michele A. Grimbaldeston: https://orcid.org/0000-0001-6372-8099

Gino Vairo: https://orcid.org/0000-0002-6945-8672

Nicholas J. Wilson: https://orcid.org/0000-0002-5574-9516

Catherine M. Owczarek: https://orcid.org/0000-0003-3576-1881

Timothy R. Hercus: https://orcid.org/0000-0001-5290-9206

Angel F. Lopez: https://orcid.org/0000-0001-7430-0135

Damon J. Tumes: https://orcid.org/0000-0001-5709-857X

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CONFLICT OF INTEREST

K.H.Y, D.M, J.C, H.S.R, B.J.M, H.P., T.R.H and D.J.T, declare no conflict of interest. E.R., T.K., J.R., M.M., M.A., A.H., J.H.F, K.Z., K.M., G.V., N.W. and C.M.O are employees of CSL Limited. M.A.G. is an employee of Genentech Inc (Roche). A.F.L. receives funding from CSL Limited.

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AUTHOR CONTRIBUTIONS

Conceptualisation: A.F.L., K.H.Y., D.J.T.; Data Curation: K.H.Y., D.M., M.M., M.A.; Formal Analysis: K.H.Y., D.M., M.A.; Funding Acquisition: A.F.L.; Investigation: K.H.Y., D.M., J.C., H.S.R., B.J.M., E.R., T.K., J.R., M.M., M.A., A.H., J.H.F., K.Z.; Methodology: K.H.Y., D.M., H.S.R., T.R.H., T.K.; Project Administration: A.F.L,; Resources: H.S.R., K.M., G.V., N.J.W., C.M.O; Supervision: A.F.L., D.J.T; Validation: K.H.Y., D.J.T.; Visualisation: K.H.Y., M.M., M.A.,; Writing - Original Draft Preparation: K.H.Y., A.F.L., D.J.T.; Writing - Review and Editing: K.H.Y., D.M., H.S.R., M.A.G., C.M.O, T.R.H., A.F.L., D.J.T..

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Figure Legends

Figure 1. Expression of hβc receptor in hβcTg mice. (a) Whole-genome sequencing data for the hβcTg mice highlighting sequences around the transgene insertion site and disruption of the endogenous β_c and $\beta_{IL\text{-}3}$ genes. Yellow arrows - the insertion of at least 15 tandem copies of the hβc transgene at the *vav* locus. Orange arrow - the insertion of a hygromycin resistance cassette into the mouse βc gene. Red arrow - the sequence between exon 8 and 14 of the m $\beta_{IL\text{-}3}$ gene is replaced with a neomycin resistance cassette. (b) Cell surface expression of hβc, was examined by flow cytometry on the indicated immune cells from splenocytes of WT, $\beta_c^{-/-}/\beta_{IL\text{-}3}^{-/-}$ and hβcTg mice. Data are representative of at least 3 experiments.

Figure 2. hβcTg mice express functional receptors for mouse (m)GM-CSF and mIL-5 but not mIL-3. Splenocytes were incubated with 10ng/mL of (a) mGM-CSF, (b) mIL-5 or (c) mIL-3 for 15 min prior to measurement of intracellular phosphorylated STAT5 (pSTAT5) by flow cytometry in the indicated cell populations. The left panels show stacked histograms of pSTAT5 in different treatment groups from one representative experiment. The right panels show MFI of pSTAT5 normalised to WT untreated control. Data (Mean + S.E.M.) are pooled from the three independent experiments performed, each of which gave similar results. *p<0.05, **p<0.01, ***p<0.001 Unpaired t-test.

Figure 3. CSL311 suppresses βc-cytokine signalling in immune cells from hβcTg mice. Splenocytes were pre-incubated with CSL311 or isotype control antibodies (20µg/mL) before stimulation with 100ng/mL of (a) mGM-CSF or (b) mIL-5 for 30 min. Intracellular phosphorylated STAT5 (pSTAT5) was detected by flow cytometry in the indicated cell populations. The left panels show stacked histograms of pSTAT5 signals in different treatment groups from one representative experiment. The right panels show MFI of pSTAT5

signals normalised to WT untreated control normalised to isotype control treatment in WT cells. Data (Mean + S.E.M.) are pooled from the four independent experiments performed, each of which gave similar results. **p<0.01, ***p<0.001, Unpaired t-test.

Figure 4. CSL311 represses CHS responses in the hβcTg mouse model. (a) Changes in ear thickness in vehicle- and DNFB-treated ears. (b) Representative H&E stained histological sections of ears from different treatment groups. Images show the entire and high-power magnification images of the ear sections (scale bar = 1mm and 0.2mm). (c, d) Left panel: Epidermal thickness measurement and dermal mast cells number in ears. Right panel: Fold increase in epidermal thickness and mast cell numbers in DNFB-treated ear/vehicle-treated ear Data: (a, b (Right panel), d (Right panel)) mean + S.E.M, (c (Left panel), d (Left panel)) mean; n = 5 to 14 mice with combined results from 4 independent experiments. *p<0.01, **p<0.01, ***p<0.001 Two-way ANOVA with Bonferroni post-test or $^{\#}$p<0.05, $^{\#\#}$p<0.01 unpaired t-test.

Figure 5. CSL311 reduces neutrophil and eosinophil infiltration on day 12 in DNFB-treated ear tissues in hβcTg mice. Flow cytometry on ear immune cell infiltrates (a) $CD8^+$ T cells, (b) neutrophils, (c) eosinophils, (d) $CD4^+$ T cells and (e) macrophages at day 12 following ear application of DNFB or vehicle control. Left panel: The percentage of immune cells in the vehicle and DNFB-treated ears of different treatment groups. Right panel: Fold increase in the percentage of immune cells in DNFB-treated ear/vehicle-treated ear in individual mice. Data: (Left panel) mean and (Right panel) mean + S.E.M ; n = 2 to 10 mice with combined results from 3 independent experiments. *p<0.05, **p<0.01, ***p<0.001 Two-way ANOVA with Bonferroni post-test or $^{\#}$p<0.05, $^{\#\#}$p<0.01, $^{\#\#\#}$p<0.001 unpaired t-test.

Figure 6. IL-3 is dispensable for the DNFB-induced CHS response. (a) Left: Changes in ear thickness in vehicle- and DNFB-treated ears were measured daily for 6 days after DNFB re-challenge. Right: H&E stained histological sections of ears (scale bar = 1mm and 0.2mm). (b) Dermal mast cell numbers in ear tissues at day 6 after treatment were detected with toluidine blue staining. (c – e) Flow cytometry on ear immune cell infiltrates shows the percentage of (c) $CD8^+$ T cells, (d) neutrophils, (e) eosinophils in the vehicle and DNFB-treated ears of different treatment groups at day 6 after treatment. Data: mean + S.E.M ; n = 12 to 13 mice with combined results from 3 independent experiments. *p<0.01, **p<0.01, ***p<0.001 unpaired t-test.

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	Target Gene	5' to 3' sequence of oligonucleotide
Genotyping	$h\beta_c$	F: GCCGCCTCCCACACACCTGAG
		R: CCCACACCTCCCCCTGAACCTG
	$m\beta_c/\beta_{IL-3}$	F: GAGCAGGCTTCTTGCTGAGCAACAG
		R: GCTTCCTGGGTCGGCTAGAAGGAA
		Pgk Poly (A): GAAGGGTGAGAACAGAGTACCTAC

Table S1. PCR primer pairs specific to target genes.

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	RBC (10^3/µL)	WBC (10^3/µL)	Eosinophils (10^3/µL)	Neutrophils (10^3/µL)	Monocytes (10^3/µL)	Lymphocytes (10^3/µL)
WT	7.569 ± 0.194	3.779 ± 0.214	0.065 ± 0.005	0.360 ± 0.078	0.198 ± 0.039	3.137 ± 0.187
hβ$_c$Tg	7.647 ± 0.284	2.785 ± 0.263*	0.053 ± 0.005	0.500 ± 0.142	0.131 ± 0.037	2.065 ± 0.277*

Table S2. ADVIA 2120i analysis of peripheral blood for WT and hβ$_c$Tg mice. Data: mean± S.E.M; n = 12 to 27 mice *p<0.05 Unpaired t test. RBC – red blood cell count; WBC – white blood cell count.

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Cell Type	Receptors per Cell (Blood)	Receptors per Cell (Spleen)	Receptors per Cell (Bone Marrow)
Neutrophils	11127	11002	11183
Monocytes	18068	14109	23549
Lymphocytes	21532	18271	18162
Basophils	36632	35580	*N/A**
Eosinophils	19982	16261	16435

Table S3. hβc receptor numbers on the surface of immune cell subtypes in hβcTg mice.

*Basophils were undetected in bone marrow samples.

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Analysis of mouse splenocytes	**Antibodies**	**Dilution/ concentration**	**Company**	**Catalogue number**
Monocyte and neutrophil populations. Panel (a) in Figure S3	Anti-mouse CD11b FITC	1:50	BD Biosciences	553310
	Anti-mouse Ly6C and Ly6G (Gr-1) BV605	1:50	BioLegend	108439
Basophil, mast cell and pDC populations. Panel (b) in Figure S3	Anti-mouse CD11b FITC	1:50	As above	
	Anti-mouse CD317 PE	1:50	BioLegend	127103
	Anti-mouse CD11c BV421	1:50	BioLegend	117329
	Anti-mouse c-kit PE-Cy7	1:50	BD Biosciences	558163
	Anti-mouse FcεR1α FITC	1:50	Invitrogen	11-5898-82
Eosinophil population. Panel (c) in Figure S3	Anti-mouse CD11b FITC	1:50	As above	
	Anti-mouse SiglecF BV421	1:50	BD Biosciences	562681
B1a B cell population. Panel (d) in Figure S3	Anti-mouse CD3 FITC	1:50	BD Biosciences	553061
	Anti-mouse CD5 PE	1:50	BD Biosciences	553023
	Anti-mouse CD11b BV510	1:50	BioLegend	101245
$h\beta_c$ receptor expression	Anti-human β_c (1C1) antibody	10μg/mL	In house	
	Donkey anti-mouse IgG (H+L), APC	1:200	Invitrogen	17-4013-82
	Goat anti-mouse IgG, PE	1:200	Southern Biotech	1030-09
Mouse GMRα expression	Anti-mouse CD116 APC	10μL/sample	R&D Systems	FAB6130A
Mouse IL3Rα expression	Anti-mouse CD123 APC	1:30	Invitrogen	17-1231-82
Mouse IL5Rα expression	Anti-mouse CD125 PE	1:20	BD Biosciences	558488

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Intracellular pSTAT5 signalling	STAT5 pY694 Alexa Fluor®647	1:12.5	BD Biosciences	562076
Analysis of mouse ear tissues	**Antibodies**	**Dilution/ concentration**	**Company**	**Catalogue number**
Neutrophil, eosinophil, macrophage and mast cell. populations.	Anti-mouse Ly6C and Ly6G (Gr-1) PE	1:200	BD Biosciences	553128
	Anti-mouse F4/80 PE-Cy-7	1:200	Invitrogen	25-4801-82
	Anti-mouse FcεR1α FITC	1:200	Invitrogen	11-5898-82
	Anti-mouse c-kit APC	1:200	BD Biosciences	561118
	Anti-mouse SiglecF BV421	1:200	BD Biosciences	562681
$CD4^+$ and $CD8^+$ T cell populations.	Anti-mouse CD3 FITC	1:200	BD Biosciences	553061
	Anti-mouse CD4 APC	1:200	BD Biosciences	553051
	Anti-mouse CD8b PE	1:200	Invitrogen	12-0083-83

Table S4. Antibodies used for flow cytometry.

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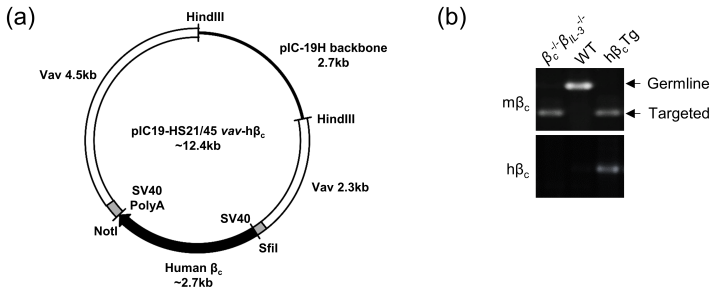


Figure S1.

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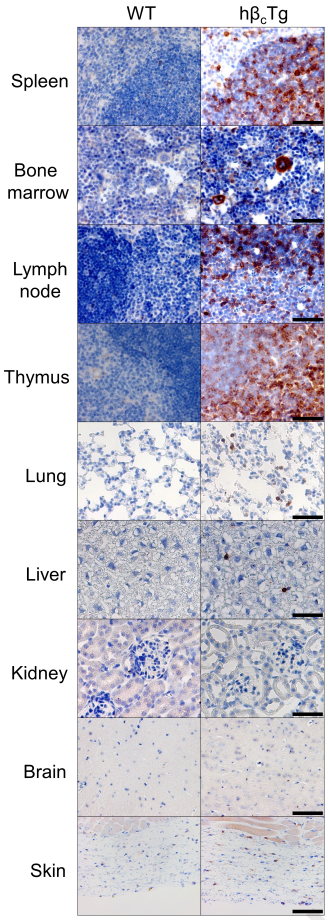


Figure S2.

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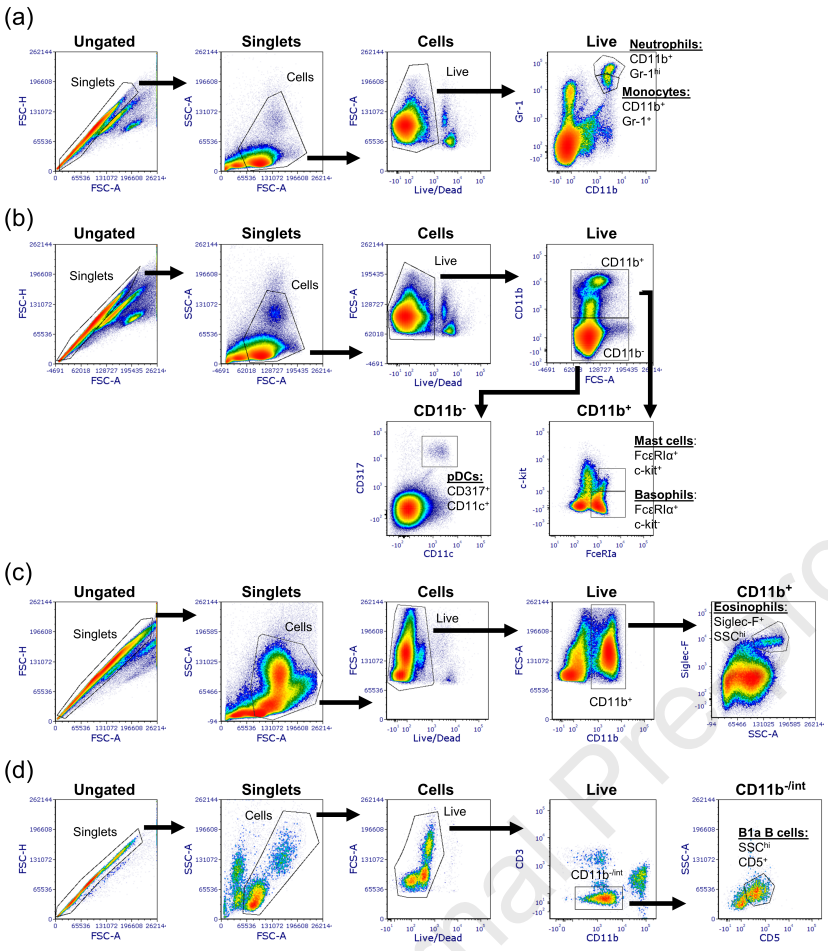


Figure S3.

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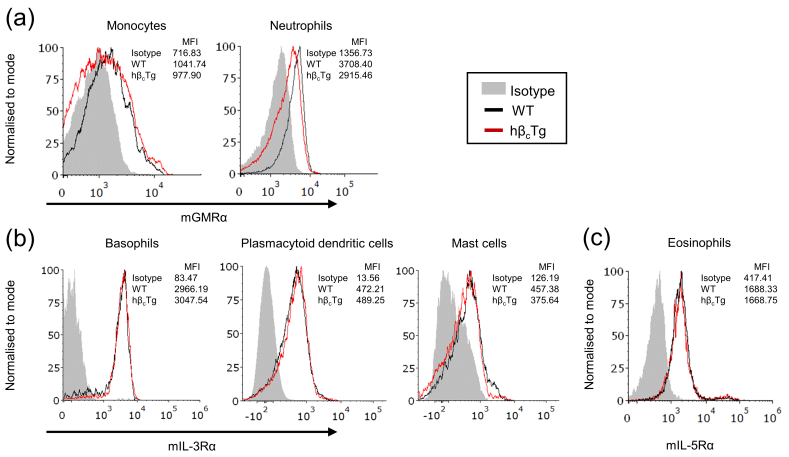


Figure S4.

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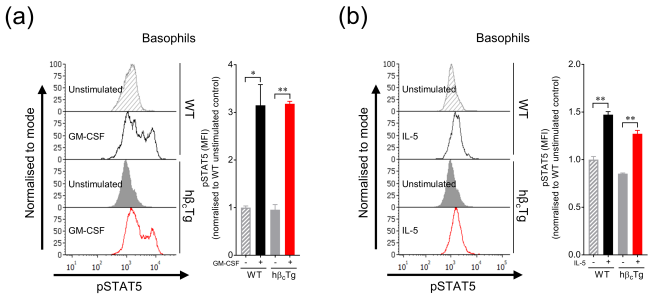


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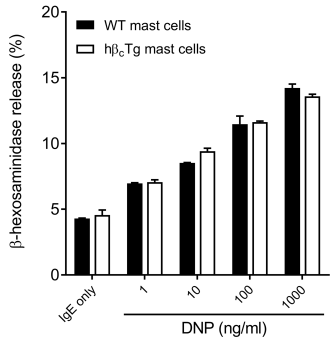


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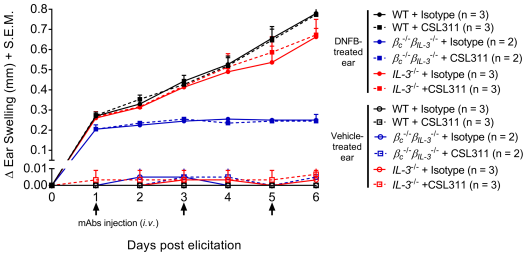


Figure S7.

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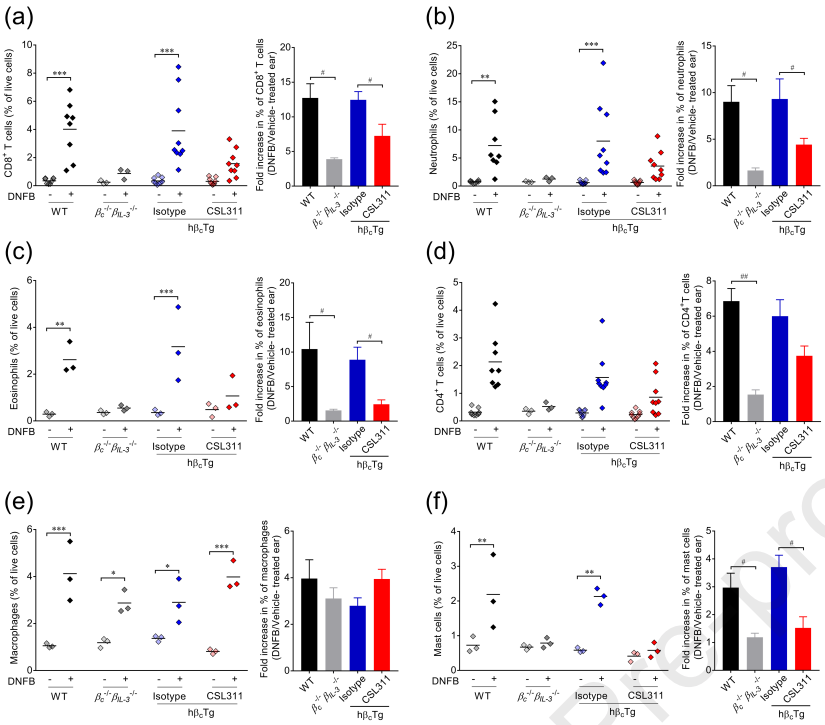


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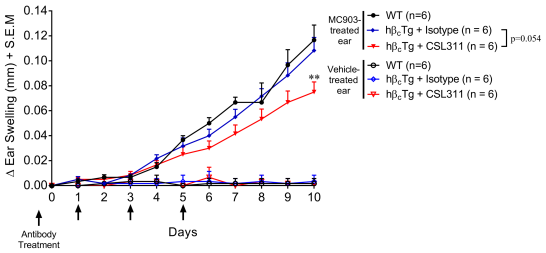


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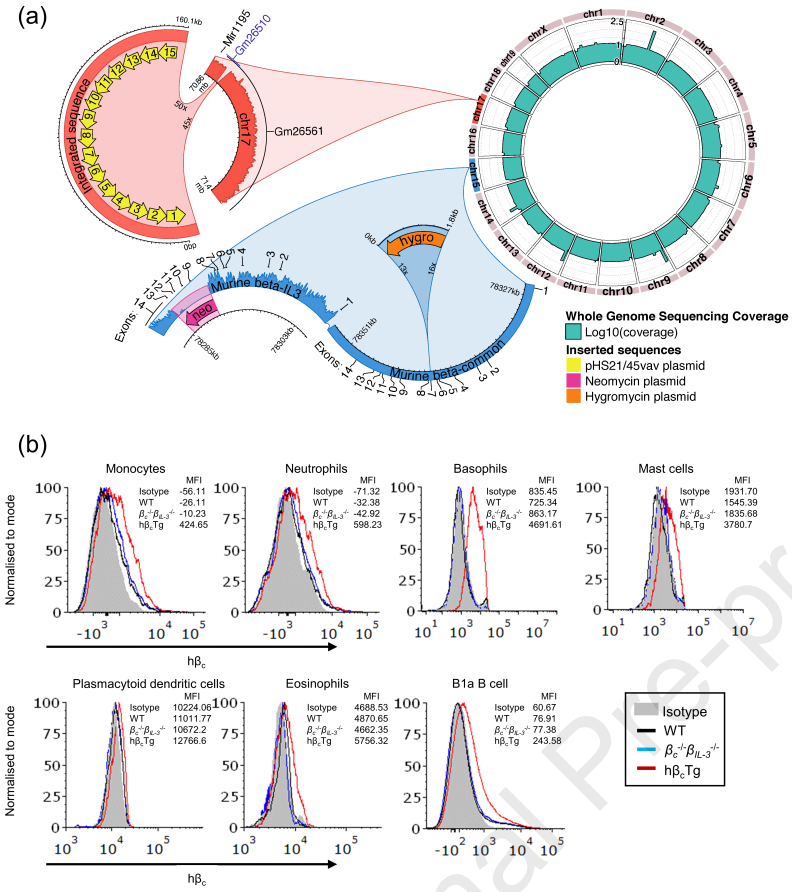


Figure 1.

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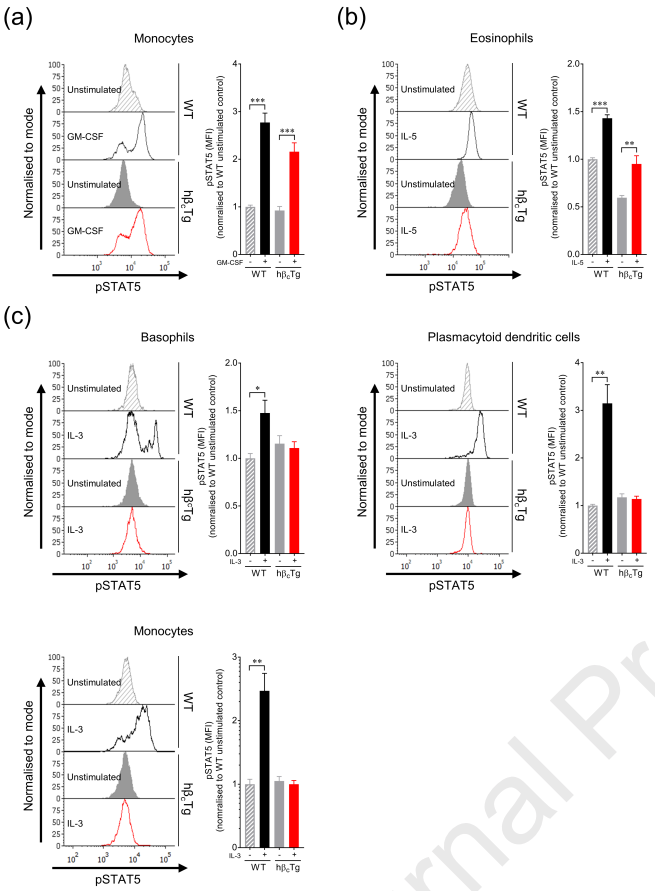


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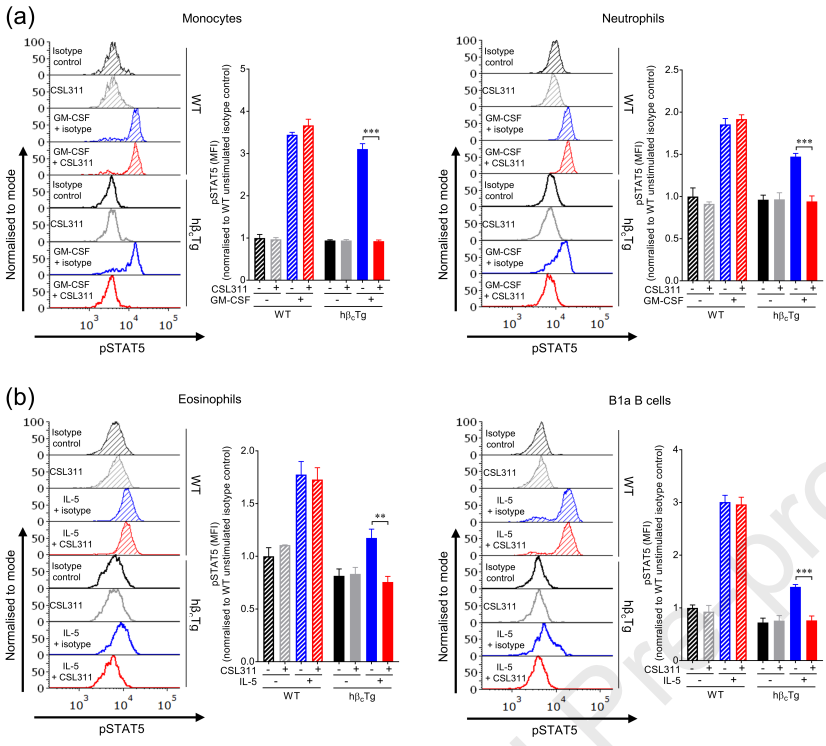


Figure 3.

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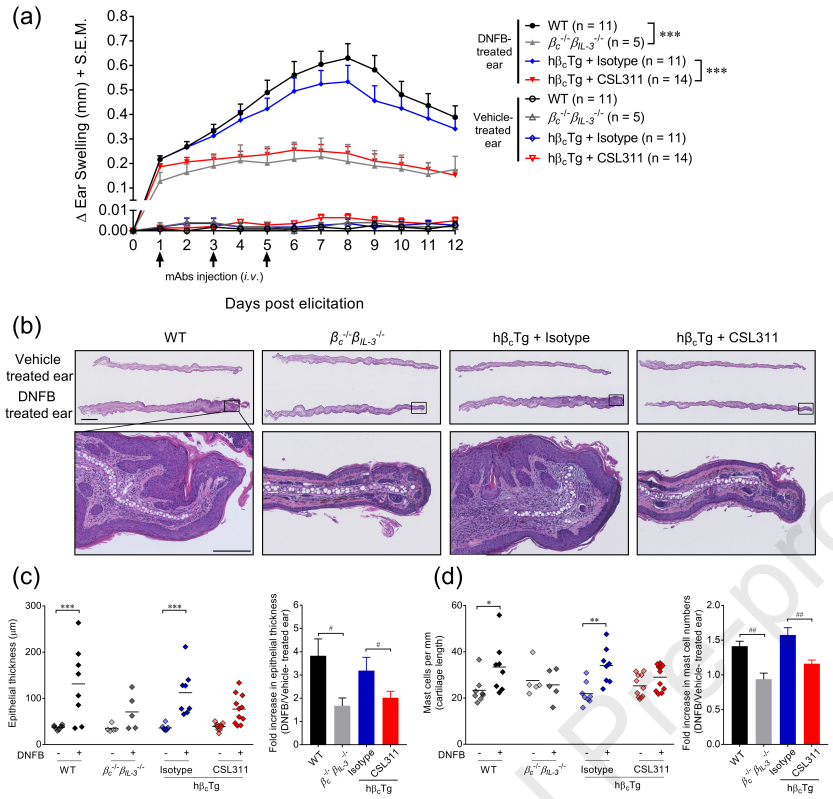


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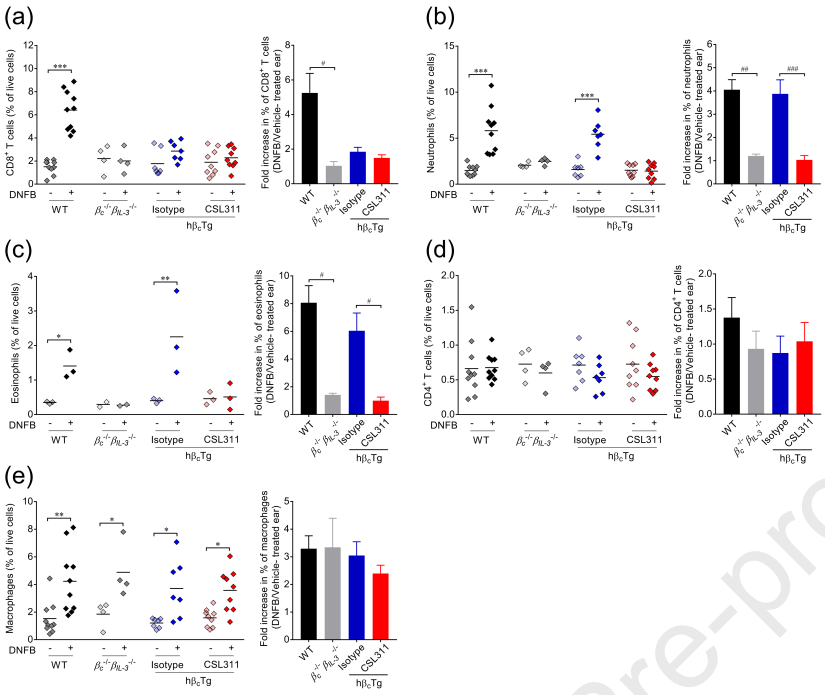


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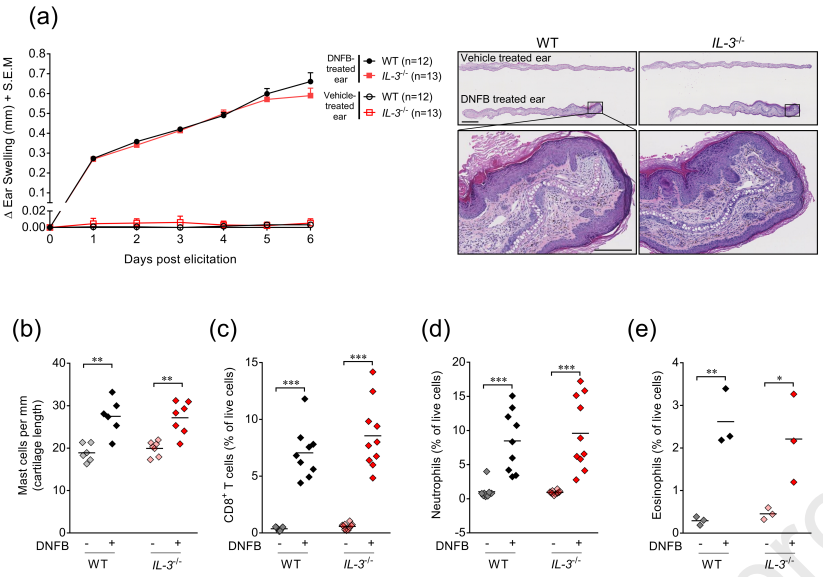


Figure 6.

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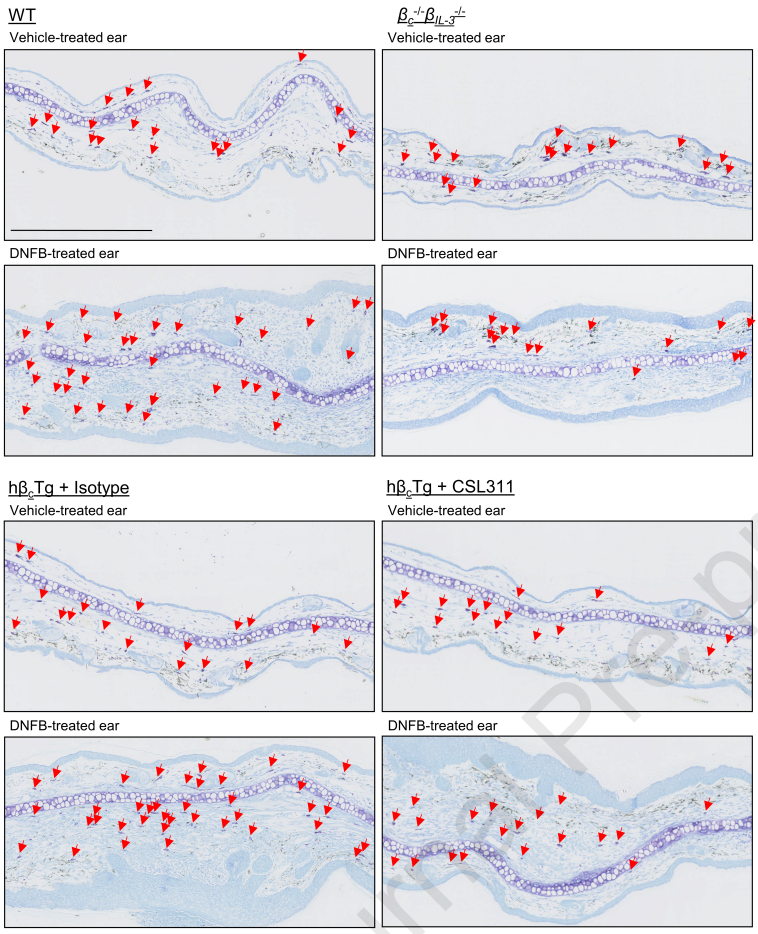


Figure S8.

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