Article

SOURCE DATA | TRANSPARENT PROCESS

THE EMBO JOURNAL

# Dusp5 negatively regulates IL-33-mediated eosinophil survival and function

Derek A Holmes[1], Jung-Hua Yeh[1,†], Donghong Yan[2], Min Xu[2] & Andrew C Chan[1,*]

## Abstract

**Mitogen-activated protein kinase (MAPK) activation controls diverse cellular functions including cellular survival, proliferation, and apoptosis. Tuning of MAPK activation is counter-regulated by a family of dual-specificity phosphatases (DUSPs). IL-33 is a recently described cytokine that initiates Th2 immune responses through binding to a heterodimeric IL-33Rα (ST2L)/IL-1α accessory protein (IL-1RAcP) receptor that coordinates activation of ERK and NF-κB pathways. We demonstrate here that DUSP5 is expressed in eosinophils, is upregulated following IL-33 stimulation and regulates IL-33 signaling. *Dusp5*$^{-/-}$ mice have prolonged eosinophil survival and enhanced eosinophil effector functions following infection with the helminth *Nippostrongylus brasiliensis*. IL-33-activated *Dusp5*$^{-/-}$ eosinophils exhibit increased cellular ERK1/2 activation and BCL-X$_L$ expression that results in enhanced eosinophil survival. In addition, *Dusp5*$^{-/-}$ eosinophils demonstrate enhanced IL-33-mediated activation and effector functions. Together, these data support a role for DUSP5 as a novel negative regulator of IL-33-dependent eosinophil function and survival.**

**Keywords** BCL-X$_L$; eosinophil survival; dual-specificity phosphatase 5
**Subject Categories** Immunology
**DOI** 10.15252/embj.201489456 | Received 6 July 2014 | Revised 3 October 2014 | Accepted 14 October 2014 | Published online 14 November 2014
**The EMBO Journal (2015) 34: 218–235**

## Introduction

Eosinophils comprise a distinct innate immune subset that contributes to host homeostasis, helminth-induced immunity, and allergic diseases. While the role of eosinophils in helminth-induced immunity has been a long-standing area of investigation and accumulation of eosinophils in end-organs has been appreciated to be a common feature of many human diseases involving the respiratory tract (asthma and allergic rhinitis), gastrointestinal tract (gastroenteritis, eosinophilic esophagitis, and inflammatory bowel diseases), and skin (atopic dermatitis) (reviewed in Fulkerson & Rothenberg, 2013; Rosenberg *et al*, 2013), recent studies continue to reveal additional roles for eosinophils in controlling host homeostatic processes including glucose metabolism and tissue regeneration (Wu *et al*, 2011; Goh *et al*, 2013; Heredia *et al*, 2013).

Eosinophils arise in the bone marrow (BM) from CD34$^+$ hematopoietic stem cells, and development is governed by the coordinated actions of GATA-1, PU.1, and C/EBP transcription factors and regulated by a multitude of cytokines (Akuthota & Weller, 2012). GM-CSF and IL-3 direct the early differentiation of granulocytes that include eosinophils, neutrophils, basophils, and mast cells. The latter stages of eosinophil differentiation are controlled by IL-5. *Il5*$^{-/-}$ or *Il5ra*$^{-/-}$ mice have a twofold reduction in eosinophil numbers under homeostatic conditions and are unable to increase BM, blood, or tissue eosinophils following infection with the metacestode parasite *Mesocestoides corti* (Kopf *et al*, 1996) or the nematode *Nippostrongylus brasiliensis* (Knott *et al*, 2007). A similar inability to induce eosinophilia following *N. brasiliensis* infection occurs with administration of a neutralizing anti-IL-5 mAb (Coffman *et al*, 1989). In addition, treatment of allergic asthma patients with anti-IL-5 antibodies reduced numbers of blood, BM, and sputum eosinophils, as well as BM eosinophil myelocytes and metamyelocytes (Menzies-Gow *et al*, 2003). Treatment of patients with an anti-IL-5 mAb has demonstrated a reduction in disease exacerbations in patients with refractory eosinophilic asthma and reduction in corticosteroid use in patients with hypereosinophilic syndrome (Haldar *et al*, 2009; Bel *et al*, 2014; Ortega *et al*, 2014). Hence, IL-5 plays an important role in eosinophil maturation, parasite-induced eosinophilia in rodents, and human eosinophilic diseases.

IL-33, a recently described cytokine of the IL-1 family, has emerged as an important mediator of Th2 biology and fibrosis (Milovanovic *et al*, 2012). Single nucleotide polymorphisms (SNP) in *IL33* and its cognate receptor, *IL1RL1*, are associated with the development of asthma in humans (Moffatt *et al*, 2007; Gudbjartsson *et al*, 2009; Torgerson *et al*, 2011). IL-33 is highly expressed in the airway epithelium of patients with allergic asthma (Sakashita *et al*, 2008; Prefontaine *et al*, 2009) and in intestinal epithelial cells and myofibroblasts of patients with active ulcerative colitis (Kobori *et al*, 2010). Levels of the IL-33 soluble ST2 decoy receptor are elevated in human asthma patients during clinical exacerbation (Oshikawa *et al*, 2001). In addition, *Il33*$^{-/-}$ mice have reduced airway inflammation, while mice overexpressing IL-33 have enhanced

1 Department of Immunology, Genentech, Inc., South San Francisco, CA, USA
2 Department of Translational Immunology, Genentech, Inc., South San Francisco, CA, USA
*Corresponding author. Tel: +1 650 225 8104; E-mail: acc@gene.com
†Present address: Universite d'Alx-Marseille, Marseille, France

airway inflammation (Oboki *et al*, 2010; Zhiguang *et al*, 2010). IL-33 has pleiotropic effects and acts on multiple hematopoietic-derived cell types, including $CD4^+$ Th2 cells, dendritic cells, B1 B cells, mast cells, basophils, neutrophils, macrophages, innate lymphoid type 2 cells (ILC2s), eosinophils, and natural killer (NK) cells, as well as non-hematopoietic epithelial and endothelial cells. The variety of cell types affected underscores the broad evolving biology of IL-33 that includes modulation of cytokines, chemotaxis, cell differentiation, cell proliferation, and fibrosis.

IL-33 has recently been demonstrated to exert a direct effect on eosinophils. IL-33 augments eosinophil differentiation from $CD117^+$ BM hematopoietic progenitor cells (Stolarski *et al*, 2010), triggers production of pro-inflammatory cytokines by mature eosinophils, augments eosinophil-mediated polarization of M2 macrophages (Kurowska-Stolarska *et al*, 2009), and exacerbates eosinophil-mediated airway inflammation. While *Il33*$^{-/-}$ mice have normal eosinophil development, they are unable to mount tissue eosinophilic responses following infection with *Strongyloides venezuelensis* (Yasuda *et al*, 2012) or *N. brasiliensis* (Hung *et al*, 2013).

IL-33 signals through a heterodimeric receptor consisting of the IL-33R α-chain (ST2L) and IL-1α accessory protein (IL-1RAcP). Receptor ligation activates both NF-κB and MAPK pathways (Chow *et al*, 2010) to upregulate cell surface expression of adhesion proteins β2-integrin CD11b and ICAM-1 (Suzukawa *et al*, 2008), induces secretion of pro-inflammatory cytokines including IL-13, IL-6, and IL-8, and provides a potent survival signal for eosinophils (Suzukawa *et al*, 2008).

DUSPs (also known as Mitogen-activated Protein Kinase Phosphatases) include sixteen catalytically active enzymes in mammalian cells (Caunt & Keyse, 2013). Ten of these comprise the 'typical' DUSPs that share a common structure with an amino-terminal domain that regulates binding of specific MAPK isoforms and DUSP subcellular localization and a carboxy-terminal catalytic domain that removes phosphates on tyrosine and serine/threonine residues. These DUSPs are divided into three subfamilies: JNK/p38 selective DUSPs (DUSP8, DUSP10 [MKP-5], and DUSP16 [MKP-7]), ERK selective cytoplasmic MKPs (DUSP6 [MKP-3], DUSP7 [MKP-X], and DUSP9 [MKP-4]), and inducible nuclear MKPs (DUSP1 [MKP-1], DUSP2, DUSP4 [MKP-2], and DUSP5). In immune cells, the functions of DUSPs 1, 2, 4, and 10 have been more extensively studied (reviewed in Lang *et al*, 2006; Jeffrey *et al*, 2007). These DUSPs regulate their cognate MAPK pathways, and overexpression and genetic ablation studies have demonstrated important roles in cytokine secretion and nitric oxide production in macrophages, mast cell apoptosis, and T-cell function.

DUSP5 is a growth-factor-inducible phosphatase that targets and anchors ERK1 and ERK2, but not other MAP kinases, to the nucleus (Mandl *et al*, 2005). DUSP5 is preferentially expressed in immune cells, and overexpression studies have demonstrated a role for DUSP5 in M-CSF signaling during macrophage development (Grasset *et al*, 2010), common gamma chain signaling (Kovanen *et al*, 2003) in T-lymphocyte development/proliferation (Kovanen *et al*, 2008) and plasma cell differentiation (Rui *et al*, 2006). We report here the generation of mice deficient in *Dusp5* and describe a novel mechanistic role for DUSP5 in IL-33-mediated activation of ERK1/2 in eosinophil survival and function.

## Results

### DUSP5 regulates eosinophilia induction during helminth infection

To explore the functions of DUSP5, we analyzed *Dusp5* mRNA from sorted splenic cells from mice. *Dusp5* mRNA was highest in eosinophils and NK cells and, to a lesser extent, $CD4^+$ T lymphocytes (Fig 1A). To better understand the physiologic functions of DUSP5, mice deficient in *Dusp5* were generated (Supplementary Fig S1A). Southern blot analysis confirmed the predicted genomic incorporation (Supplementary Fig S1B). RT–PCR with primers spanning exons 2–4 downstream of the deleted region confirmed the absence of *Dusp5* mRNA (Supplementary Fig S1C). Western blot analysis confirmed the absence of DUSP5 protein (Supplementary Fig S1D). Mice deficient in *Dusp5* were developmentally normal, presented no gross developmental or growth abnormalities, and were fertile.

Since mice expressing a *DUSP5* transgene under the H2-$K^b$ promoter and immunoglobulin heavy chain enhancer demonstrated a block in thymocyte development at the $CD4^+CD8^+$ double- positive (DP) stage (Kovanen *et al*, 2008), we initially characterized the effects of *Dusp5* deficiency on T-cell development. Total thymocyte numbers were normal, though there were modest increases in $CD4^+$ and $CD8^+$ thymocytes in *Dusp5*$^{-/-}$ mice when compared to *Dusp5*$^{+/+}$ mice (Supplementary Fig S2A). No differences in $CD4^+$ or $CD8^+$ T-cell numbers were observed in spleen or lymph nodes (Supplementary Fig S2B and C). As overexpression of DUSP5 also decreased IL-2-augmented T-cell proliferation (Kovanen *et al*, 2008), we examined the function of peripheral $CD4^+$ T cells. Sorted naïve $CD62L^{hi}CD4^+$ T cells from *Dusp5*$^{-/-}$ mice proliferated to a greater degree following activation with anti-CD3 and anti-CD28 mAbs (Supplementary Fig S3A). In contrast, effector/memory $CD62L^{lo}CD4^+$ T cells from *Dusp5*$^{-/-}$ mice proliferated at a rate similar to *Dusp5*$^{+/+}$ cells (Supplementary Fig S3B). These modest differences observed in T cells are consistent with the previously described phenotypes observed with DUSP5 overexpression (Kovanen *et al*, 2008). In addition, we did not observe differences in innate or adaptive cell numbers in the BM, spleen, blood, and inguinal lymph nodes in *Dusp5*$^{-/-}$ mice compared to *Dusp5*$^{+/+}$ mice (Supplementary Table S1 and Supplementary Fig S2B and C).

Given the higher level of *Dusp5* expression in eosinophils, we focused on the effects of *Dusp5* deficiency on eosinophil functions. Because eosinophils regulate host responses to helminthic infections, we analyzed the effects of *Dusp5* deficiency in mice infected with *N. brasiliensis*. Helminth infection evokes a Th2 response characterized by increased systemic eosinophilia (Coffman *et al*, 1989). Since *Dusp5*$^{-/-}$ mice have a modest effect on T-cell functions, we crossed *Dusp5*$^{-/-}$ mice onto a *Rag2*-deficient background to eliminate potential contributions from *Dusp5*$^{-/-}$ T and B lymphocytes. *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice accumulated a greater percentage of circulating eosinophils at days 6 and 13 following *N. brasiliensis* infection when compared to *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Fig 1B). In addition, increased eosinophils were observed in the blood, bronchoalveolar lavage fluid (BALF), spleen, and BM 14 days following *N. brasiliensis* infection (Fig 1C–F). This increased systemic eosinophilia in *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice was only observed following helminth infection, since BM and splenic eosinophil numbers are equivalent in uninfected *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice

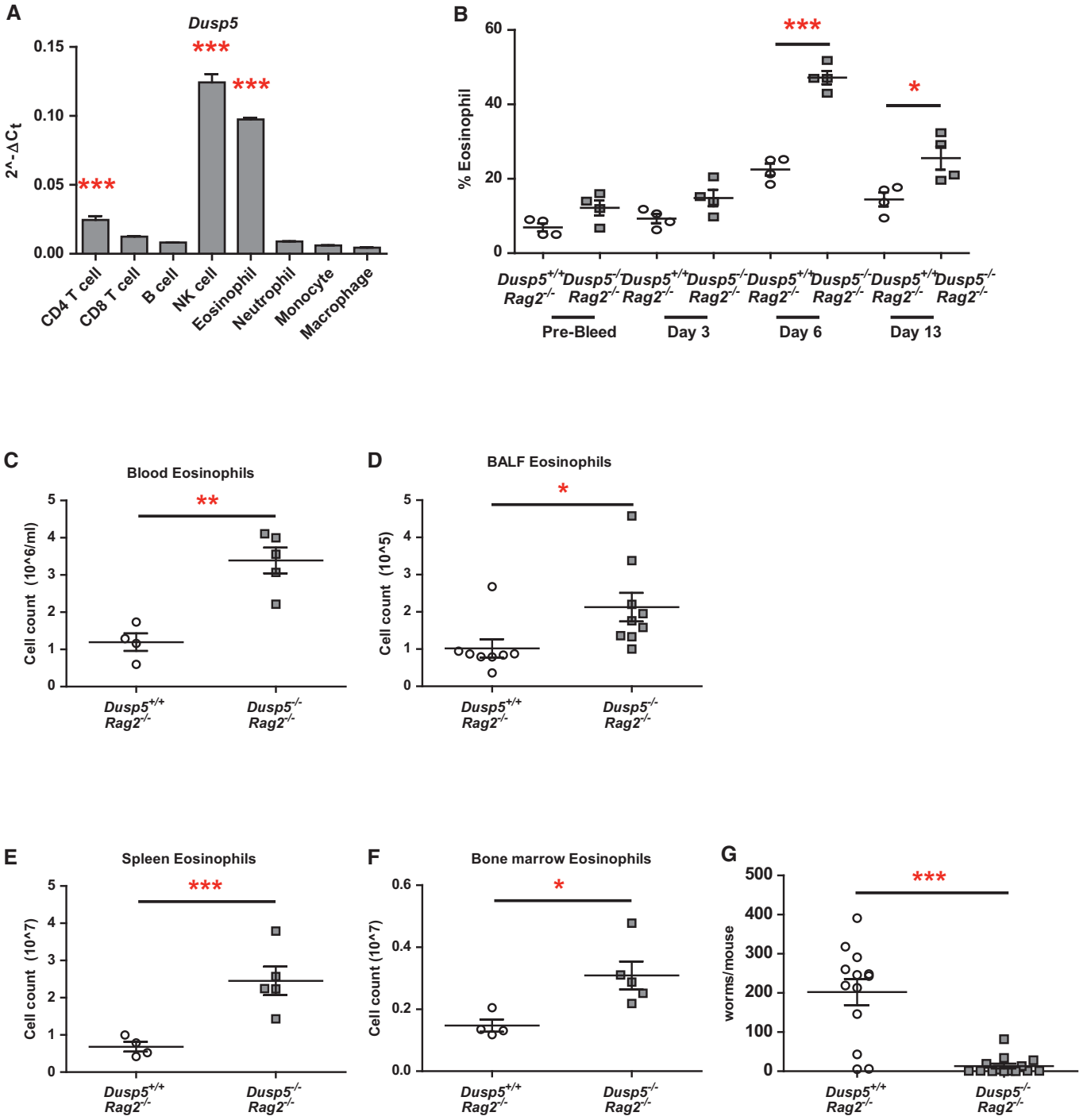


**Figure 1. DUSP5 regulates eosinophilia and immunity following infection with *Nippostrongylus brasiliensis*.**

A Analysis of *Dusp5* mRNA expression by quantitative PCR analysis. Splenic innate and adaptive immune cells were enriched by FACS sorting to > 95% purity and RNA harvested for Taqman gene expression analysis. Gene expression levels were normalized to *Gapdh* using the 2-delta $C_t$ method.

B Percent eosinophils in blood of *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice prior to and at days 3, 6, and 13 following *N. brasiliensis* infection.

C–F Eosinophils numbers in (C) blood, (D) BALF, (E) spleen, and (F) BM of *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice 13 days following infection with *N. brasiliensis*. Results in (C, E, F) are from the same experiment and are representative of three independent experiments. Data in (D) are cumulative of 3 independent experiments.

G Intestinal worm burden 13 days post-infection in *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice. Data are cumulative of three independent experiments.

Data information: Values in graphs represent mean ± SEM. Data presented are representative of at least three independent experiments unless otherwise specified. Statistical analysis is by two-tailed paired Student's *t*-test; *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$.

(Supplementary Table S1). No differences in neutrophil, monocyte, NK, or ILC2 cell numbers were observed (Supplementary Fig S4A–C). Corresponding with increased eosinophils, *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice had a lower worm burden compared to *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Fig 1G). A similar increase in circulating and BALF eosinophils was observed in germline *Dusp5*$^{-/-}$ *Rag2*$^{+/+}$ mice (Supplementary Fig S5A–C). Together, these data suggest that DUSP5 plays a critical role in regulating the eosinophilic response to *N. brasiliensis* infection.

As *Dusp5* expression is also increased in NK cells (Fig 1A), we analyzed whether NK cells contributed to the lower worm burden observed in *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice. *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice treated with an anti-Asialo GM1 antibody (Ab) to deplete NK cells (Supplementary Fig S4E) still maintained a lower worm burden compared to anti-Asialo GM1 Ab-treated *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Supplementary Fig S4D). In addition, eosinophils remained higher in anti-Asialo GM1 Ab-treated *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice when compared to anti-Asialo GM1 Ab-treated *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Supplementary Fig S4F). Hence, despite high levels of DUSP5 expression, NK cells appear dispensable for eosinophilia or worm burden phenotypes observed in *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice.

### DUSP5 functions as a negative regulator of eosinophil survival and activation

To investigate the basis for the increased numbers of *Dusp5*$^{-/-}$ eosinophils following *N. brasiliensis* infection, we analyzed the effects of *Dusp5* deficiency on eosinophil progenitors. In non-infected mice, numbers of BM eosinophil progenitors (defined by Lin$^{-}$ CD34$^{+}$ cKit$^{int}$ Sca-1$^{-}$ IL-5R$\alpha^{+}$ staining) were not affected by *Dusp5* deficiency (Supplementary Fig S6A and B). To analyze the differentiation of eosinophils during *N. brasiliensis* infection, mice were injected intraperitoneally with 1 mg BrdU every 12 h for 24 h and eosinophils analyzed 36 h post-BrdU injection (12 h chase). Similar numbers of BrdU$^{+}$ eosinophils were observed in the BM, blood, and spleen from *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice, consistent with equivalent rates of precursor proliferation (Fig 2A). Since the half-life of mature eosinophils during helminth infection is > 36 h (Ohnmacht *et al*, 2007), we extended the chase period to 60 h to measure the relative turnover of eosinophils during infection. At 84 h post-BrdU injection (60 h chase), a two- to threefold increase in BrdU$^{+}$ eosinophils was observed in BM, blood, and spleen of *N. brasiliensis*-infected *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice compared to *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Fig 2B). We also observed a significant decrease in apoptotic annexin V$^{+}$ propidium iodide (PI)$^{-}$ eosinophils in BM and spleen of infected *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice (Fig 2C). A reduction in annexin V$^{+}$ PI$^{-}$ eosinophils was observed in blood, but was not statistically significant ($P = 0.08$). These findings suggest that the absence of DUSP5 does not affect the proliferation of eosinophil precursors, but prolongs survival of eosinophils following *N. brasiliensis* infection.

To test whether the increased eosinophil survival advantage in *Dusp5*$^{-/-}$ mice was intrinsic to the hematopoietic compartment, we established mixed BM chimeras by reconstituting *Dusp5*$^{+/+}$ or *Dusp5*$^{-/-}$ BM competitively with host BM in a 1:1 ratio (Supplementary Fig S7A). Donor CD45.2$^{+}$ *Dusp5*$^{-/-}$ and *Dusp5*$^{+/+}$ BM reconstituted at comparable rates under homeostatic conditions (Supplementary Fig S7B). Chimeric mice were then infected with *N. brasiliensis* and the relative eosinophil percentage in the blood analyzed 9 days following infection. Donor-derived CD45.2$^{+}$ *Dusp5*$^{-/-}$ eosinophil percentage was increased compared to CD45.2$^{+}$ *Dusp5*$^{+/+}$ eosinophils (Fig 2D). In contrast to the increased *Dusp5*$^{-/-}$ eosinophils, no differences in neutrophil numbers were observed between donor-derived (CD45.2$^{+}$) *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM (Supplementary Fig S7C). Together, these data suggest that the survival advantage of *Dusp5*$^{-/-}$ eosinophils is intrinsic to the hematopoietic compartment.

We next explored the effect of DUSP5 deficiency on eosinophil function during *N. brasiliensis* infection. Eosinophils are generated in the BM from eosinophil progenitors and form a pool of cells capable of replenishing the peripheral population. Following egress from the BM, eosinophils upregulate SiglecF and CD11b, markers associated with functional activation following tissue deposition or response to infection (Voehringer *et al*, 2007). Eosinophils harvested from blood, spleen, and, to a lesser extent, BM of *N. brasiliensis*-infected *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice had higher surface expression of CD11b compared to eosinophils from infected *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ mice (Fig 3A and B). Increased eosinophil activation was intrinsic to the hematopoietic cell compartment, since we also observed increased CD11b surface expression on donor-derived (CD45.2$^{+}$) *Dusp5*$^{-/-}$ blood eosinophils from the mixed BM chimeric mouse experiments following *N. brasiliensis* infection described above (Fig 3C and D), while CD11b surface levels on host-derived (CD45.1$^{+}$) blood eosinophils were similar (Supplementary Fig S7D). These observations are consistent with enhanced eosinophil activation in mice lacking DUSP5.

### DUSP5 negatively regulates IL-33-dependent survival in eosinophils

The coordinated actions of IL-5 and IL-33 regulate eosinophil hematopoiesis (Le *et al*, 2013). Both *Il5*$^{-/-}$ and *Il33*$^{-/-}$ mice develop normally and have minimally reduced and normal eosinophil numbers, respectively, under homeostatic conditions (Kopf *et al*, 1996). However, IL-5 and IL-33 are critical regulators of peripheral eosinophilia following helminthic infection, as both *Il5*$^{-/-}$ and *Il33*$^{-/-}$ mice fail to induce eosinophilia (Knott *et al*, 2007; Hung *et al*, 2013). To test whether *Dusp5* is upregulated in response to these cytokines, BM-derived eosinophils were treated with IL-5 or IL-33 and *Dusp5* mRNA levels analyzed. *Dusp5* mRNA and DUSP5 protein were significantly elevated following treatment with IL-33 (Fig 4A). In contrast, *Dusp5* mRNA levels were unchanged with IL-5 treatment in mouse BM-derived eosinophils (Supplementary Fig S8).

To examine the functional consequences of *Dusp5* deficiency on IL-33-mediated eosinophil survival, we examined the degree of apoptosis in BM-derived eosinophils in the presence of a suboptimal dose of IL-33. IL-33-treated cultures of *Dusp5*$^{-/-}$ eosinophils had less apoptotic (annexin V$^{+}$ 7AAD$^{-}$) and necrotic (annexin V$^{+}$ 7AAD$^{+}$) cells when compared to *Dusp5*$^{+/+}$ eosinophils (Fig 4B). Decreased apoptosis of *Dusp5*$^{-/-}$ BM-derived eosinophils was associated with a reduction in mitochondrial membrane depolarization (Fig 4C) as assessed by the cationic dye JC-1, which selectively enters the mitochondria in response to mitochondrial membrane potential decreases associated with apoptosis. No differences between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils were observed when cells were treated with titrating amounts of IL-5 (Supplementary Fig S9A). *Dusp5*$^{-/-}$ BM-derived eosinophils also had an IL-33-dependent survival advantage in long-term cultures,

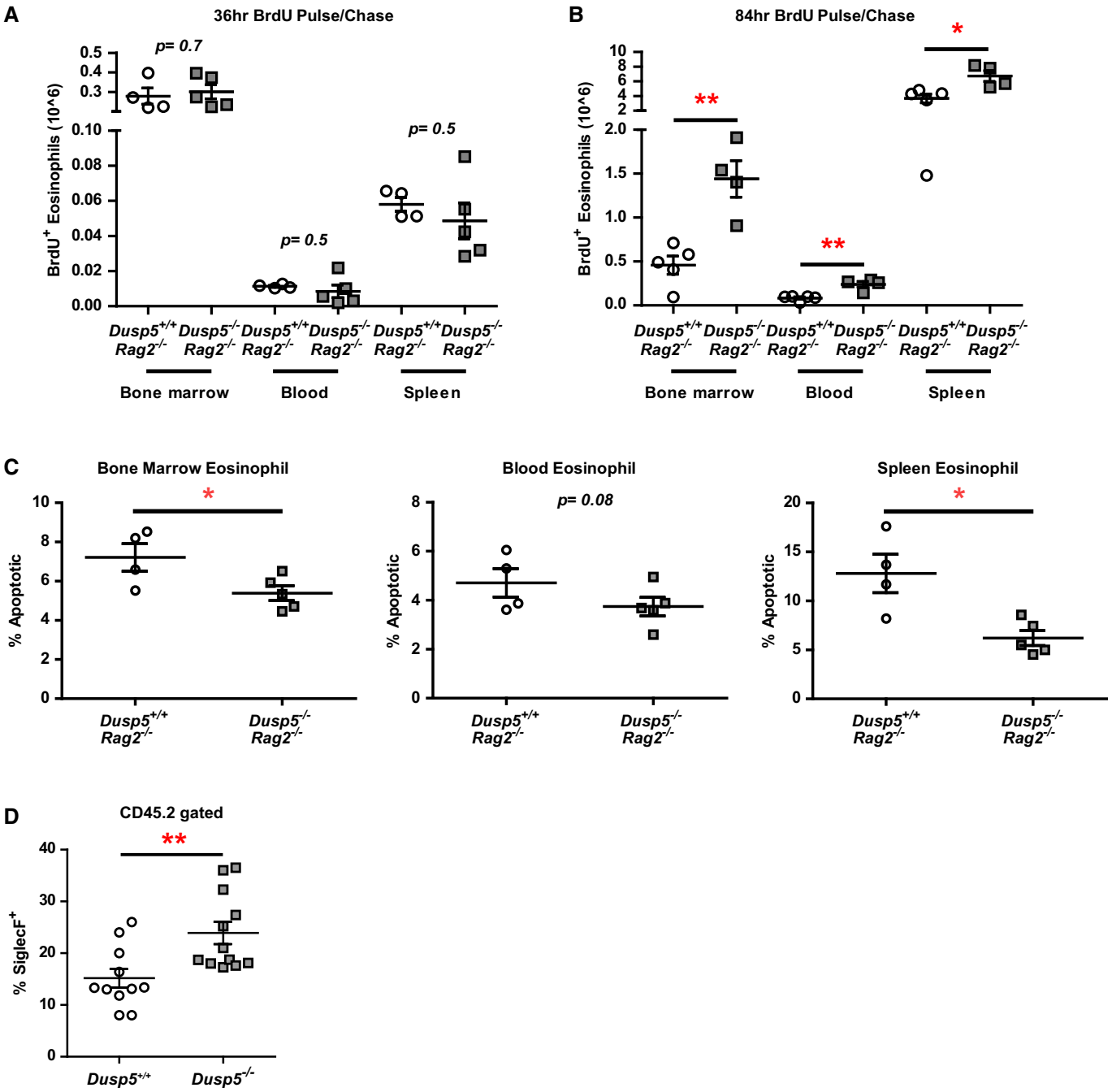


**Figure 2.** ***Dusp5*$^{-/-}$ eosinophils have a survival advantage following *N. brasiliensis* infection.**

A Analysis of eosinophil progenitor proliferation. *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice were injected intraperitoneally (day 10 post-infection) with 1 mg BrdU every 12 h (pulse) for 24 h, followed by a 12-h chase period. BrdU$^{+}$ eosinophils (CD11b$^{+}$ SSC$^{hi}$ SiglecF$^{+}$) in BM, blood, and spleen were quantitated on day 11 after *N. brasiliensis* infection.

B Analysis of eosinophil survival. *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice were injected intraperitoneally (day 8 post-infection) with 1 mg BrdU every 12 h (pulse) for 24 h as in (A), but followed by a 60-h chase. BrdU$^{+}$ eosinophils (CD11b$^{+}$ SSC$^{hi}$ SiglecF$^{+}$) in BM, blood, and spleen were quantitated on day 13 after *N. brasiliensis* infection.

C Analysis of *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) and *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) eosinophil apoptosis 13 days post-infection with *N. brasiliensis*. BM (left), blood (middle), and splenic (right) eosinophils were stained with CD11b and SiglecF along with a non-cell permeable aqua fluorescent reactive dye to discriminate live from apoptotic eosinophils.

D Percent population of eosinophils in BM chimeric mice infected with *N.* brasiliensis and analyzed 8 days following infection. Total blood cells were gated on CD45.2$^{+}$ donor-derived cells, and *Dusp5*$^{+/+}$ (open circles) or *Dusp5*$^{-/-}$ (gray squares) SiglecF$^{+}$ cells were quantitated.

Data information: Data presented in (A–C) are representative of at least three independent experiments. Data in (D) are cumulative of three independent experiments. Statistical analysis is performed by two-tailed paired Student's *t*-test; *$P < 0.05$; **$P < 0.01$.

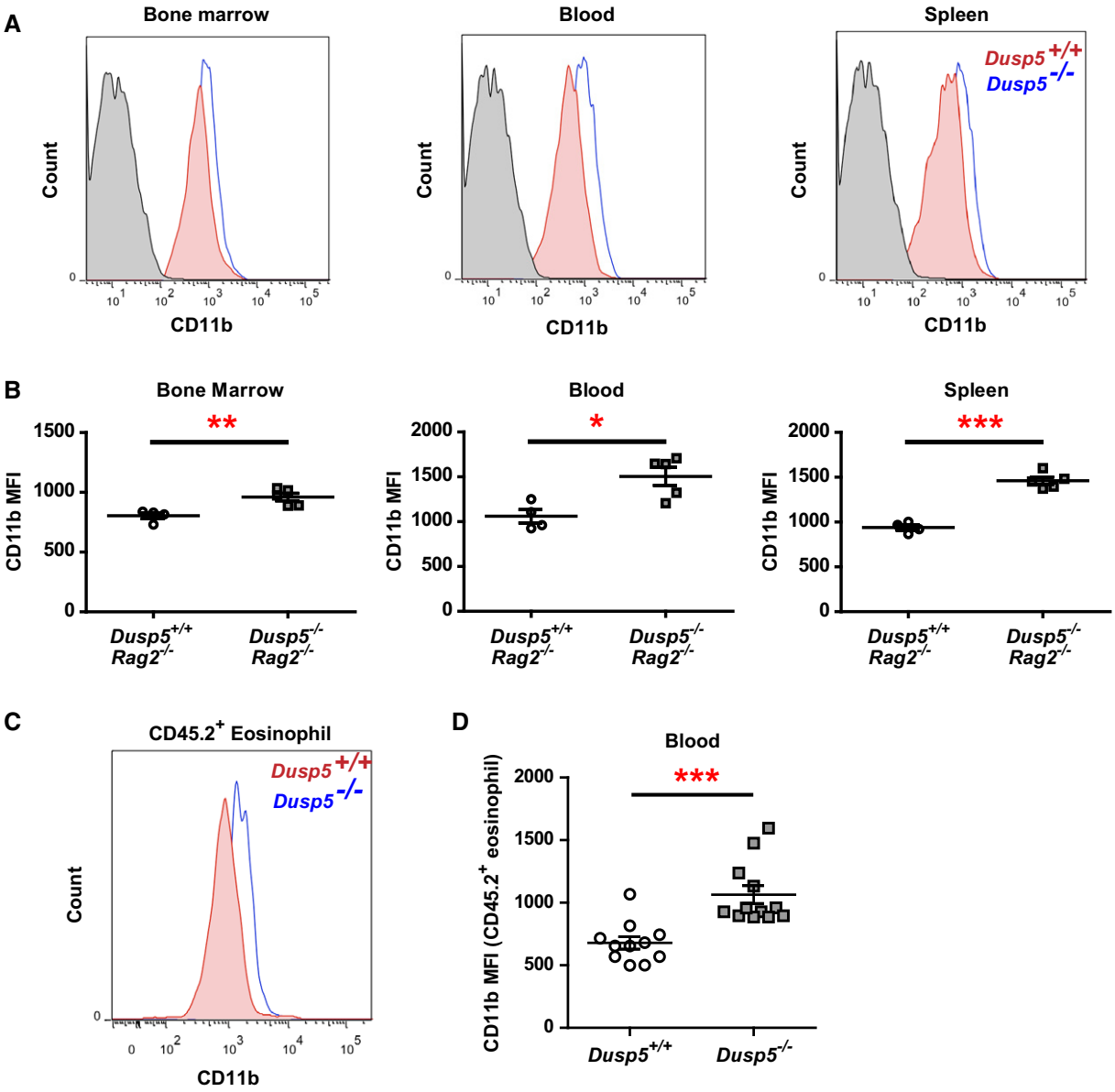


**Figure 3. DUSP5 regulates eosinophil activation during *N. brasiliensis* infection.**

A Cell surface expression of CD11b on BM (left), blood (middle), and splenic (right) eosinophils ($CD11b^+$ $SSC^{hi}$ $SiglecF^+$) 13 days following *N. brasiliensis* infection of $Dusp5^{+/+}$ $Rag2^{-/-}$ (red) and $Dusp5^{-/-}$ $Rag2^{-/-}$ (blue) mice.

B Graphs represent corresponding quantitative analysis of CD11b MFI (geometric mean of fluorescence intensity) on eosinophils from $Dusp5^{+/+}$ $Rag2^{-/-}$ (open circles) and $Dusp5^{-/-}$ $Rag2^{-/-}$ (gray squares) BM (left), blood (middle), and splenic (right) eosinophils as described in (A). Data are graphical representations of the FACS plots in (A) and from multiple experiments.

C Cell surface expression of CD11b of donor-derived ($CD45.2^+$) blood eosinophils during *N. brasiliensis* infection. Histogram of $CD11b^+$ $SiglecF^+$ eosinophils from donor-derived ($CD45.2^+$) $Dusp5^{+/+}$ (red shaded histogram) and $Dusp5^{-/-}$ (blue line) BM chimeric mice.

D Graphical representation of MFI of CD11b cell surface expression on blood eosinophils from *N. brasiliensis*-infected $Dusp5^{+/+}$ (open circles) and $Dusp5^{-/-}$ chimeric mice (gray squares) from donor-derived ($CD45.2^+$) cells shown in (C). Data are a graphical representation of the FACS plots in (C) and from multiple experiments.

Data information: Data from (A, B) are representative of three independent experiments. Data in (D) are cumulative of three independent experiments. Values in graphs represent mean ± SEM. Statistical analysis is by two-tailed paired Student's *t*-test; $*P < 0.05$; $**P < 0.01$; $***P < 0.001$.

while IL-5-treated $Dusp5^{-/-}$ and $Dusp5^{+/+}$ eosinophils demonstrated similar cell survival (Fig 4D). Finally, to confirm the role of DUSP5 in IL-33-mediated survival of eosinophils *in vivo*, recombinant IL-33 was administered intraperitoneally to $Dusp5^{+/+}$ $Rag2^{-/-}$ and $Dusp5^{-/-}$ $Rag2^{-/-}$ mice. Consistent with observations *in vitro* in BM-derived eosinophils (Fig 4B–D) and *in vivo* following *N. brasiliensis* infection (Fig 1B–F), IL-33 induced twice as many peritoneal eosinophils in $Dusp5^{-/-}$ $Rag2^{-/-}$ mice compared to $Dusp5^{+/+}$ $Rag2^{-/-}$ mice (Fig 4E). Together, these data support a role for DUSP5 in IL-33 regulation of eosinophil survival.

IL-5-dependent eosinophil survival is mediated through the upregulation of pro-survival factors such as BCL-$X_L$, MCL-1, and, to a lesser extent, BCL-2 (reviewed in Kankaanranta *et al*, 2005). To date, the mechanism of IL-33-mediated survival in eosinophils has not been studied. Hence, we examined the effects of IL-33 and the consequences of *Dusp5* deficiency on BCL family members. While

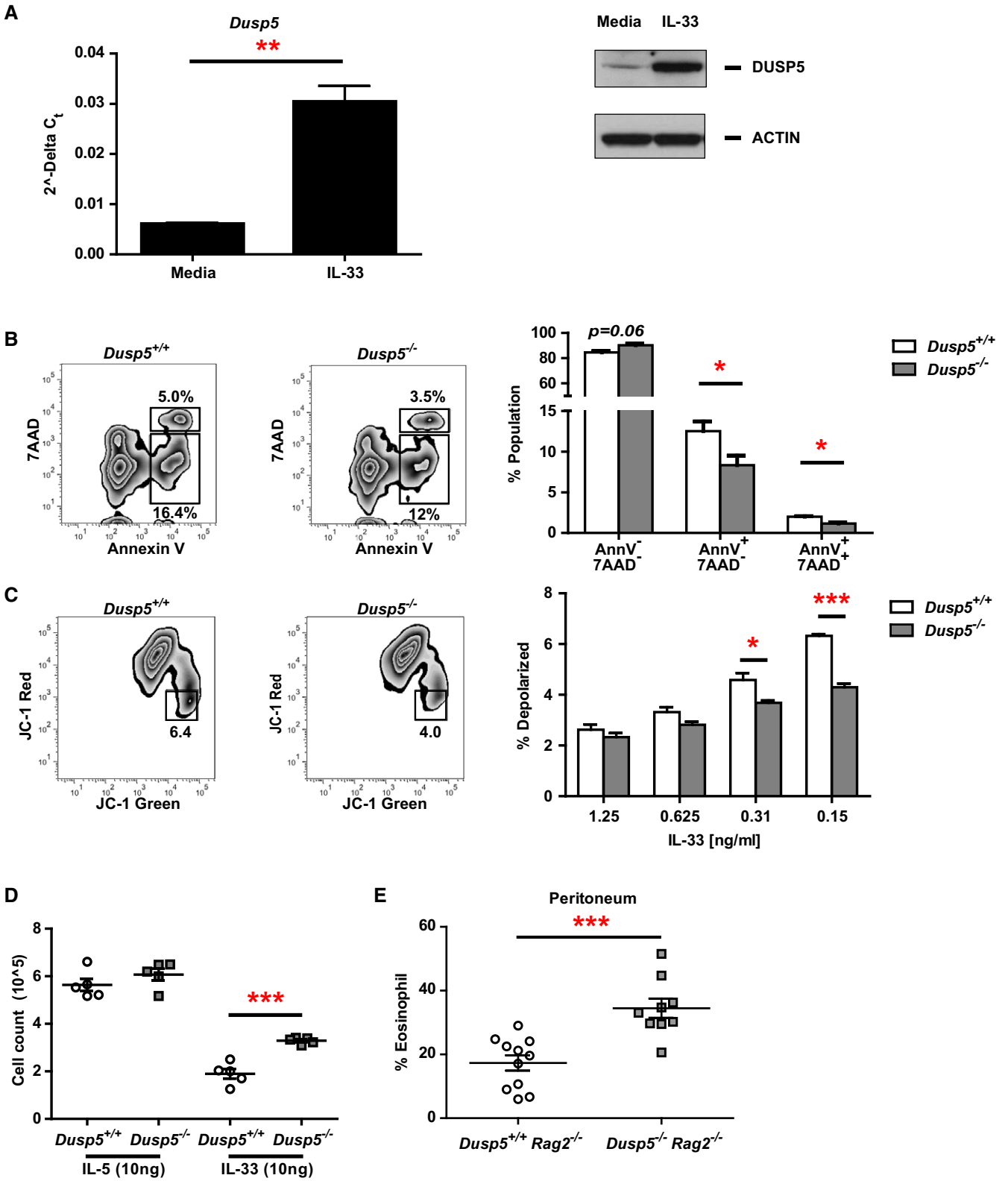


**Figure 4.**

IL-5- and IL-33-treated BM-derived eosinophils had increased levels of cytoplasmic BCL-2 compared to cytokine-starved cells, no differences were observed between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils (Fig 5A, middle row, and 5B). In contrast, IL-33, but not IL-5, increased cytoplasmic BCL-$X_L$ (Fig 5A, top row). Moreover, *Dusp5*$^{-/-}$ BM-derived eosinophils stimulated with IL-33 demonstrated a twofold greater increase in cytoplasmic BCL-$X_L$ than *Dusp5*$^{+/+}$ eosinophils (Fig 5A and B). Increased cytoplasmic BCL-$X_L$ was, in part, due to transcriptional upregulation of *Bclxl*, as *Dusp5*$^{-/-}$ BM-derived eosinophils had higher levels of *Bclxl* mRNA than *Dusp5*$^{+/+}$ (Fig 5C). Consistent with the lack of detectable cytoplasmic BCL-$X_L$ protein following treatment with IL-5 (Fig 5A), *Bclxl* mRNA was not induced by IL-5 (Supplementary Fig S9B).

Membrane fraction-associated BCL-$X_L$ and MCL-1 were similar in *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils treated with IL-5 or IL-33 (Supplementary Fig S9C). Membrane fraction-associated BCL-2 was not detected in unstimulated or cytokine-treated BM-derived eosinophils (Supplementary Fig S9C). Membrane fraction-associated BID was upregulated following IL-5 or IL-33 treatment but did not significantly differ between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils (Supplementary Fig S9D, second row). Membrane-associated BAX levels were comparable between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils (Supplementary Fig S9D, top row). Pro-apoptotic members BIM, BAK, PUMA, and BAD were undetectable in membrane fractions (Supplementary Fig S9D).

Consistent with the growth survival functions of IL-5 and IL-33, cytoplasmic cytochrome c (cyt c) levels and cleaved caspase-3 levels were decreased with IL-5 or IL-33 treatment compared to cytokine-starved cells (Fig 5D and E). However, cytoplasmic cyt c levels differed between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils only when treated with IL-33 (Fig 5E).

### Dusp5 negatively regulates IL-33 signaling in eosinophils

To further characterize the functional consequence of *Dusp5* deficiency on IL-33-treated BM-derived eosinophils, we analyzed global gene expression changes by microarray analysis. A number of genes important in eosinophil function were differentially regulated in IL-33-treated *Dusp5*$^{-/-}$ BM-derived eosinophils compared to *Dusp5*$^{+/+}$ eosinophils (Fig 6A). Genes known to function in eosinophil activation (*Cd69, Spred2*), effector functions (*Il13, Csf2, Il1b*), and transcriptional regulation (*Fosl1*) were significantly upregulated in *Dusp5*$^{-/-}$ eosinophils. Similar differences in *Il4* and *Il13* mRNA, but not *Tnfa*, levels were observed in freshly isolated peritoneal eosinophils elicited with recombinant IL-33-treated *Dusp5*$^{-/-}$ mice (Fig 6B). Consistent with the *in vitro* and *in vivo* mRNA analysis (Fig 6A and B), IL-33-treated *Dusp5*$^{-/-}$ BM-derived eosinophils secreted greater amounts of IL-4 and IL-13 proteins compared to *Dusp5*$^{+/+}$ BM-derived eosinophils, while TNF-α secretion was unchanged (Fig 6C). In contrast, no differences in IL-4, IL-13, or TNF-α levels were observed between supernatants of *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils following IL-5 treatment (Supplementary Fig S10C). Similarly, RT–PCR analysis of IL-33-treated *Dusp5*$^{-/-}$ BM-derived eosinophils confirmed higher levels of *Fosl1* mRNA compared to *Dusp5*$^{+/+}$ eosinophils (Fig 6D). Finally, surface expression of CD11b and CD69 was higher on IL-33-stimulated *Dusp5*$^{-/-}$ BM-derived eosinophils compared to *Dusp5*$^{+/+}$ eosinophils (Fig 6E and F). In contrast, no difference in CD11b was observed following IL-5 treatment and CD69 was not induced by IL-5 treatment (Supplementary Fig S10A and B). Together, these data are consistent with a negative regulatory role of DUSP5 in IL-33-, but not IL-5-, mediated eosinophil activation.

### DUSP5 inhibits IL-33-dependent ERK activation to regulate eosinophil survival and function

As DUSP5 interacts with and regulates ERK1/2, but not JNK or p38, in fibroblasts and T cells (Mandl *et al*, 2005; Kovanen *et al*, 2008), we assessed the activation of ERK1/2 following IL-33 stimulation in BM-derived eosinophils. Phospho-ERK1 (p-ERK1) and phospho-ERK2 (p-ERK2) were increased in IL-33-stimulated *Dusp5*$^{-/-}$ BM-derived eosinophils relative to *Dusp5*$^{+/+}$ eosinophils (Fig 7A), while no difference in p-ERK1 was observed in IL-5-stimulated *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ eosinophils (Supplementary Fig S11A). We did not detect p38 and JNK phosphorylation following IL-33 stimulation (Supplementary Fig S11B), and phosphorylation of the p65 subunit of NF-κB was similar in IL-33-stimulated *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils (Fig 7B). Consistent with this observation, analysis of microarray data from IL-33-treated BM-derived eosinophils showed upregulation of multiple ERK, but not NF-κB, targeted

**Figure 4. DUSP5 negatively regulates IL-33-dependent eosinophil survival.**

A BM-derived eosinophil from *Dusp5*$^{+/+}$ mice were treated with IL-33 (50 ng/ml). Left panel: Total RNA was harvested, and *Dusp5* gene expression was assayed by Taqman real-time PCR. Data are represented as relative gene expression using the 2-delta $C_t$ method normalized to *Gapdh*. Right panel: 250 μg cell lysate was loaded per lane for immunoblotting with anti-DUSP5 mAb (top) and anti-actin antibody (bottom).

B Decreased apoptosis of *Dusp5*$^{-/-}$ BM-derived eosinophils stimulated with IL-33. Representative FACS plots of *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils treated with low dose (0.5 ng/ml) IL-33 for 16 h (left). Apoptosis was assessed by staining for annexin V and 7AAD. Percentage of live (annexin V$^-$ 7AAD$^-$), apoptotic (annexin V$^+$ 7AAD$^-$), and necrotic (annexin V$^+$ 7AAD$^+$) cells are quantitated on the right.

C Decreased mitochondrial membrane depolarization of *Dusp5*$^{-/-}$ BM-derived eosinophils when stimulated with IL-33. *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils were labeled with the mitochondria-permeant dye JC-1 according to manufacturer's recommendations and treated with IL-33 (0.15 ng/ml) for 16 h (FACS plots on the left). Mitochondria depolarization was assessed by a decrease in the red (~590 nm) to green (529 nm) fluorescence ratio (left). Mitochondria depolarization of *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils was quantitated using a range of IL-33 concentrations (right).

D *Dusp5*$^{+/+}$ (open circles) and *Dusp5*$^{-/-}$ (gray squares) BM-derived eosinophils were incubated with IL-5 (10 ng) or IL-33 (10 ng) for 7 days, and remaining live eosinophils were quantitated.

E *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ (open circles) or *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ (gray squares) mice were injected intraperitoneally daily with IL-33 (500 ng) for 6 days and eosinophils quantitated on day 7 following IL-33 administration.

Data information: Data in (A–D) are representative of three independent experiments. Data in (E) are cumulative of 3 independent experiments. Values in graphs represent mean ± SEM. Statistical analysis is by two-tailed paired Student's *t*-test; $*P < 0.05$; $***P < 0.001$.
Source data are available online for this figure.

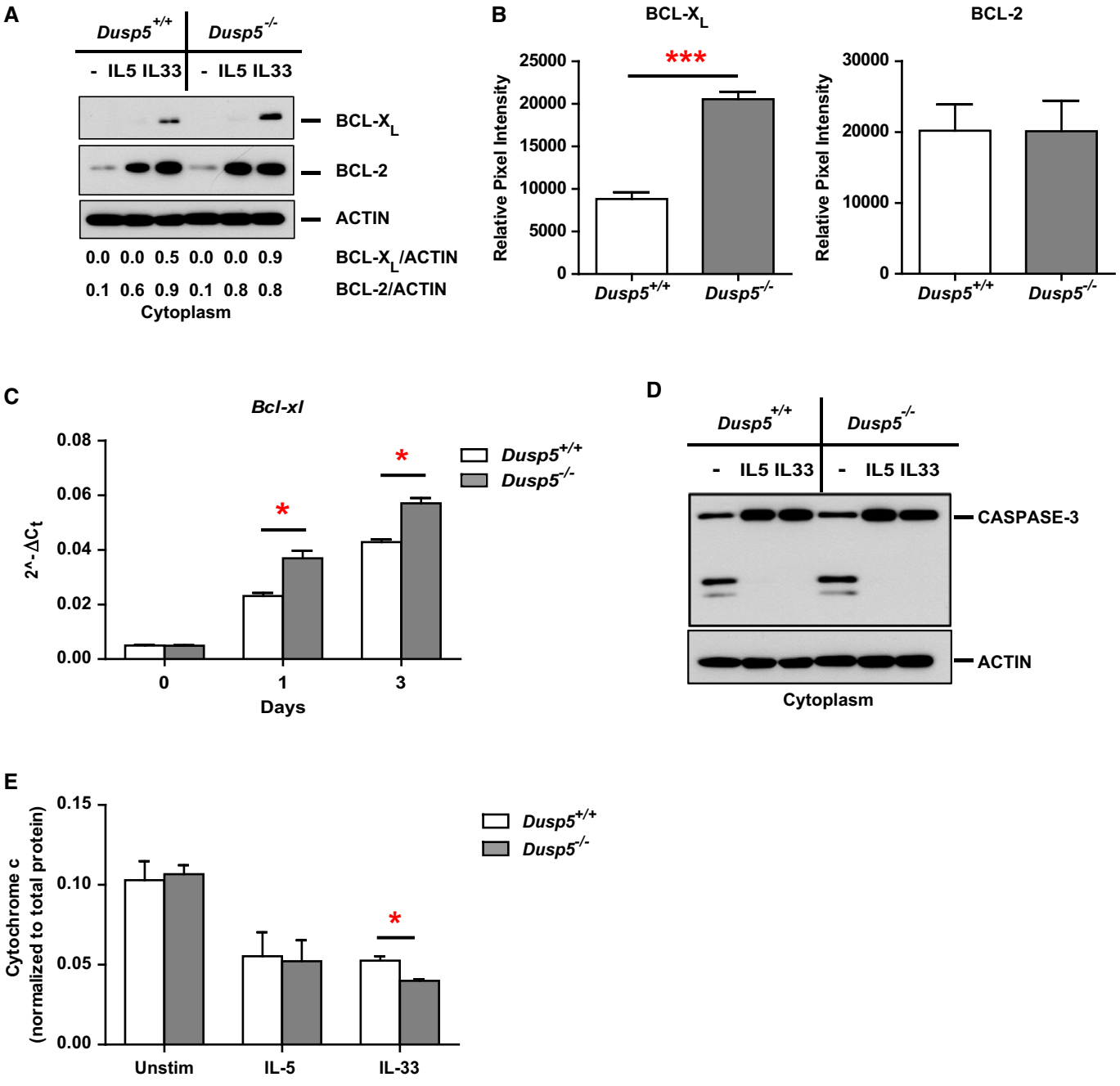


**Figure 5. Regulation of survival and apoptotic pathways in IL-33-treated *Dusp5*$^{-/-}$ eosinophils.**

A Expression of BCL-$X_L$ (top), BCL-2 (middle), and actin (bottom) in *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils. Western blot analysis of cytoplasmic fractions isolated from *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils cultured in the absence or presence of IL-5 (50 ng/ml) or IL-33 (50 ng/ml) for 3 days. Results are representative of four independent experiments. The ratios of BCL-$X_L$ and BCL-2 to actin protein levels are quantified at the bottom of each lane.

B Quantitation of cytoplasmic fraction-associated BCL-$X_L$ and BCL-2 protein from *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils by Western blot analysis from (A). Graphs show the relative pixel intensity of cytoplasmic BCL-$X_L$ and BCL-2 from four independent experiments including (A).

C RT–PCR quantitation of *Bclxl* expression in *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils treated with IL-33 for 0, 1, or 3 days. Results are representative of three independent experiments.

D Cleaved caspase-3 levels of IL-5 (20 ng/ml)-treated, IL-33 (20 ng/ml)-treated, or cytokine-starved *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils as determined by Western blot analysis of cytoplasmic fractions. Results are representative of four independent experiments.

E Cytochrome c levels in cytoplasmic fractions from IL-5 (20 ng/ml)-treated, IL-33 (20 ng/ml)-treated, or cytokine-starved *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils as determined by ELISA. Results are representative of 3 independent experiments.

Data information: Values in graphs represent mean ± SEM. Statistical analysis is by two-tailed paired Student's *t*-test; *$P < 0.05$; ***$P < 0.001$.
Source data are available online for this figure.

**Figure 6. DUSP5 regulates IL-33 signaling to modulate eosinophil activation and function.**

A Microarray analysis of IL-33-treated BM-derived eosinophils. Eosinophils from *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ mice were treated for 4 h with IL-33 (50 ng/ml). Total RNA from independent BM-derived eosinophils cultures derived from 5 mice for each genotype was quantitated by microarray analysis. Genes differentially upregualated (24 genes) or downregulated (62 genes) in *Dusp5*$^{+/+}$ compared to *Dusp5*$^{-/-}$ eosinophils by ≥ 1.5-fold with a *P*-value < 0.05 are highlighted in red or blue, respectively.

B *Dusp5*$^{+/+}$ *Rag2*$^{-/-}$ or *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice were treated with daily intraperitoneal injections with IL-33 (500 ng) for 6 days. Eosinophils were harvested (day 6) 1 h following the final dose of IL-33. Total RNA was harvested, and *Il4*, *Il13*, and *Tnfa* mRNA expression was assayed by Taqman real-time PCR analysis. Data are represented as fold expression of values using the 2-delta $C_t$ method normalized to *Gapdh* mRNA levels. Results are representative of three independent experiments.

C *Dusp5*$^{+/+}$ (open circles) and *Dusp5*$^{-/-}$ (gray squares) BM-derived eosinophils were unstimulated or stimulated with IL-33 (25 ng/ml) for 24 h. Culture supernatants were analyzed for IL-4 (top), IL-13 (middle), and TNF-α (bottom) levels by ELISA. Results are representative of three independent experiments.

D RT–PCR analysis of *Fosl1* expression in *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ BM-derived eosinophils stimulated with IL-33 (50 ng/ml) for 4 h. Results are representative of three independent experiments.

E Cell surface expression of CD11b was assessed on unstimulated or IL-33 (50 ng/ml)-treated *Dusp5*$^{+/+}$ (red) or *Dusp5*$^{-/-}$ (blue) BM-derived eosinophils for 20 h (left). Percentage of CD11b$^{hi}$ surface expression of representative FACS analysis (left) was quantitated (right) from a total of three independent samples.

F Cell surface expression of CD69 was assessed on unstimulated or IL-33 (50 ng/ml)-treated *Dusp5*$^{+/+}$ (red) or *Dusp5*$^{-/-}$ (blue) BM-derived eosinophils for 20 h (left). Quantitation of CD69 surface expression of representative FACS analysis (left) was quantitated (right) from three independent samples.

Data information: Values in graphs represent mean ± SEM. Statistical analysis is by two-tailed paired Student's *t*-test; **P* < 0.05; ***P* < 0.01; ****P* < 0.001.

genes in *Dusp5*$^{-/-}$ eosinophils compared to *Dusp5*$^{+/+}$ eosinophils (Supplementary Table S2).

To link the requirement of ERK signaling to IL-33-mediated survival, we analyzed the effects of ERK inhibition through use of the MEK1/2 inhibitor U0126 (Favata *et al*, 1998). IL-33-mediated upregulation of CD11b was partially inhibited with the addition of U0126 (Fig 7C). Inhibition of MEK1/2 also decreased cytoplasmic BCL-$X_L$ but not BCL-2 following IL-33 treatment (Fig 7D and E). Finally, MEK inhibition reduced IL-33-, but not IL-5-, mediated eosinophil viability (Fig 7F and Supplementary Fig S11C). Together, these data provide a mechanistic link between IL-33-mediated cellular ERK1/2 activation with eosinophil activation and survival.

## Discussion

Our studies here provide the first evidence for a role for DUSPs as a regulator of pro-survival signaling in immune cells. DUSP5 curtails IL-33-mediated survival signals by attenuating the activation of ERK1/2 and controlling BCL-$X_L$ expression and function in eosinophils. DUSPs 1, 2, and 16 have been implicated in controlling cell death processes, but primarily through regulation of pro-apoptotic pathways. Overexpression of DUSP1 in a U937 pro-monocytic cell line inhibits caspase-3-mediated apoptosis (Franklin *et al*, 1998). Overexpression of DUSP16 (MKP7) in Ba/F3 cells or T cells protects from cytokine withdrawal and TCR-mediated apoptosis, respectively. Conversely, knock down of DUSP16 in T cells increases TCR-mediated apoptosis (Hoornaert *et al*, 2003; Kiessling *et al*, 2010). *Dusp2*$^{-/-}$ mast cells upregulate pro-apoptotic genes and undergo increased spontaneous apoptosis in long-term cultures (Jeffrey *et al*, 2007). In addition to DUSPs that serve as negative regulators of apoptosis, DUSP4 is required for TGF-β-induced apoptosis (Ramesh *et al*, 2008). TGF-β induces *Dusp4* expression in a SMAD3-dependent manner and, in turn, DUSP4 curtails ERK1/2 signaling, increases BIM stability, and promotes apoptosis. Our studies here broaden the mechanisms by which DUSPs can regulate cellular survival.

ERK1/2 activation has been critically linked to survival signals in tumorigenesis through transcriptional and post-translational regulation of both pro- and anti-apoptotic BCL family members (Balmanno & Cook, 2009). ERK activation downregulates transcription of pro-apoptotic members *Bim* and *Bad* and can regulate complex assembly of BCL-$X_L$ and MCL-1. Conversely, ERK1/2 activation can induce the transcription of *Mcl1*, *Bcl2*, and *Bclxl* mRNAs through direct binding of AP-1 and CREB to their promoter regions (Ballif & Blenis, 2001; Sevilla *et al*, 2001; McCubrey *et al*, 2007), as well as phosphorylate and stabilize MCL-1 and BCL-2 to promote cell survival (Domina *et al*, 2004). BAX is highly expressed in eosinophils, while other pro-apoptotic (BAK, BID, BIK, and BAD) proteins are absent or expressed at low levels. ERK inactivates BAX in human eosinophils by phosphorylating Thr167 following GM-CSF treatment, retaining it in an 'inactive' form by facilitating interaction with the peptidyl-prolyl isomerase PIN1 (Shen *et al*, 2009). In our studies, we detected high levels of BAX expression, although no differences in expression levels or cellular distribution were observed between *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ eosinophils. Whether PIN1 or its association with BAX is regulated following IL-33 treatment or whether there are any alterations in BAX phosphorylation or BAX association with BCL-$X_L$ in *Dusp5*$^{-/-}$ eosinophils will require further investigation.

IL-33 has recently been shown to provide pro-survival signals in eosinophils and other cells, though the mechanisms have not been elucidated (Cherry *et al*, 2008; Suzukawa *et al*, 2008). Microarray analysis of murine eosinophils has demonstrated that IL-33, but not IL-4, upregulated *Dusp5* mRNA expression (Bouffi *et al*, 2013). In our studies, IL-33, but not IL-5, increased *Dusp5* mRNA expression. In addition, IL-33 induced BCL-$X_L$ but not BCL-2 expression in a MEK-dependent fashion (Fig 7D and E). Consistent with this differential control of BCL-2 and BCL-$X_L$, *Dusp5*$^{-/-}$ eosinophils with increased cellular ERK activation had increased BCL-$X_L$ expression and increased accumulation of cytoplasmic BCL-$X_L$, while BCL-2 expression or localization was unaffected. In ILC2 cells, IL-33 induces expression of BCL-2 and BCL-$X_L$ (Kabata *et al*, 2013). A role for BCL-$X_L$ has recently been demonstrated in the pro-survival effects of IL-33 in human mast cells (Wang *et al*, 2014). In non-immune cells, IL-33 can also provide survival signals for hepatocytes during liver ischemia/reperfusion and Con A-induced hepatitis. In both cases, cytoprotection was associated with BCL-2 upregulation, though BCL-$X_L$ levels were not analyzed (Sakai *et al*, 2012; Volarevic *et al*, 2012). In light of our observations in eosinophils, whether IL-33 also facilitates cell survival in other cell types in a DUSP5/ERK-dependent manner warrants additional investigation.

While IL-5 also induces ERK activation, IL-5-mediated eosinophil survival or activation was not affected by *Dusp5* deficiency. In

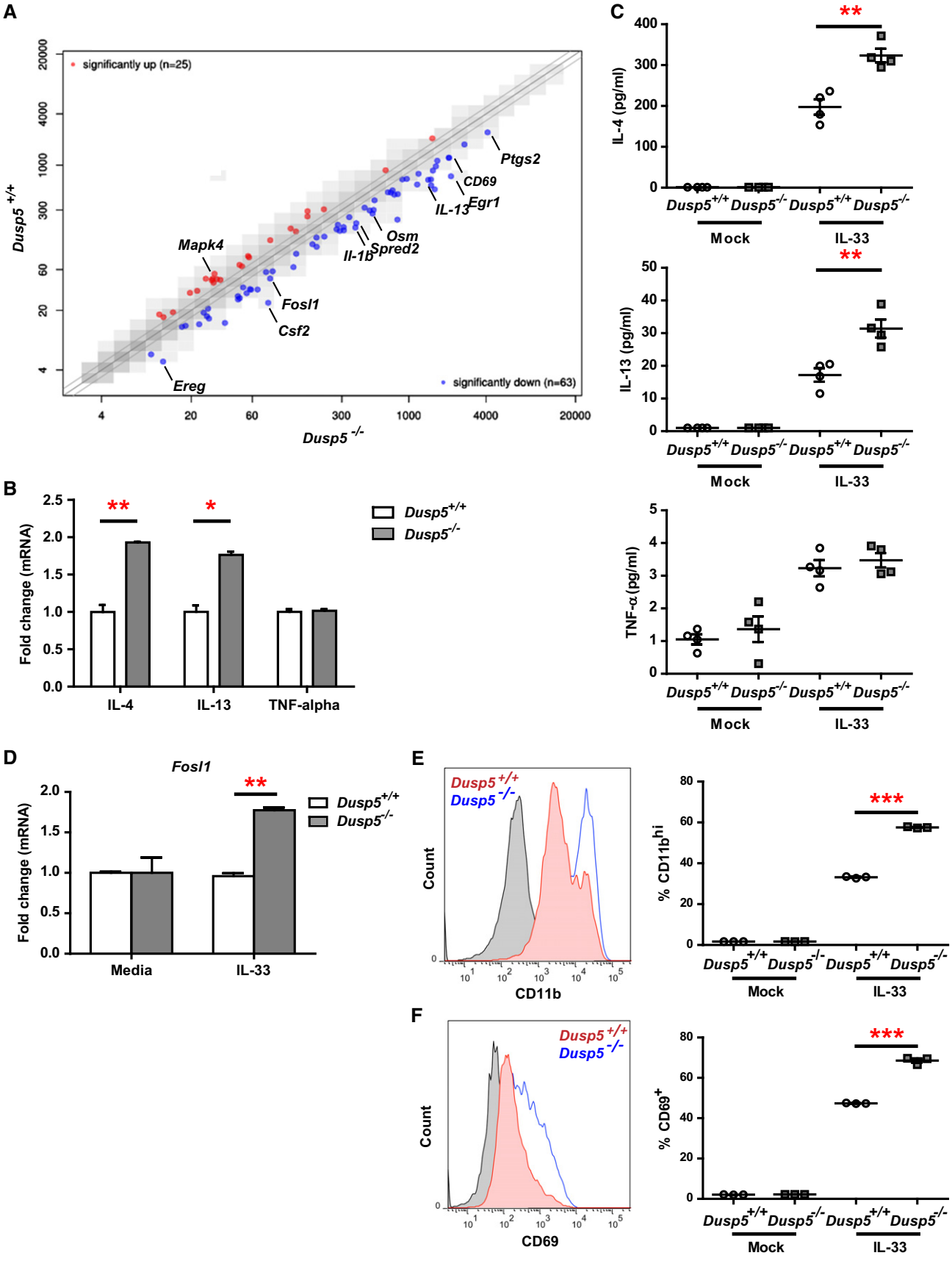


Figure 6.

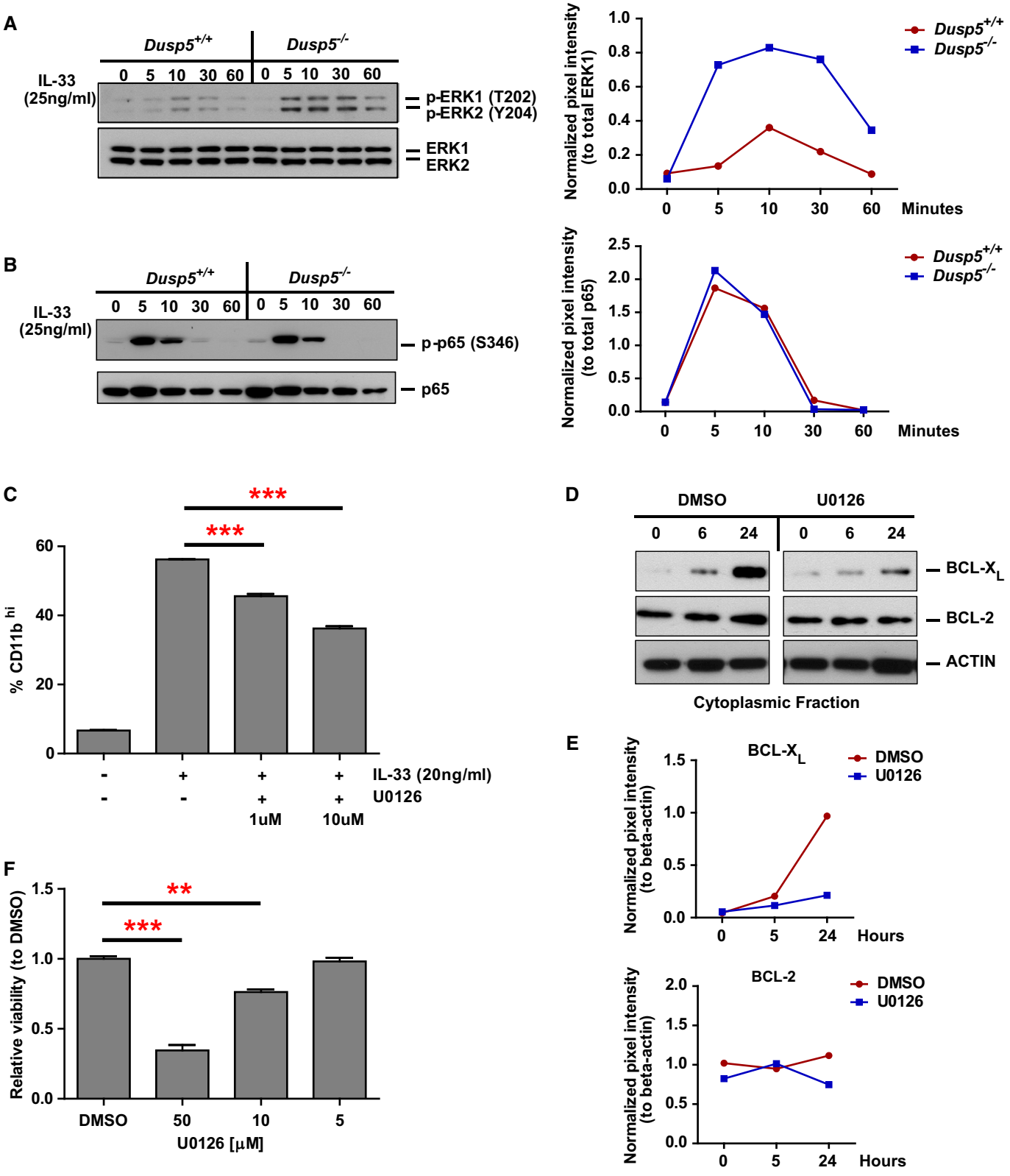


Figure 7.

addition, MEK inhibition did not affect IL-5-mediated survival. We did not observe appreciable levels of cytoplasmic-associated BCL-$X_L$ in IL-5-differentiated BM-derived eosinophils, and treatment with high doses of IL-5 did not induce *Bclxl* expression (Fig 5A and Supplementary Fig S9B). A previous study reported that BCL-$X_L$ expression was downregulated upon cytokine withdrawal and

**Figure 7. DUSP5 is a negative regulator of ERK activation downstream of IL-33 to control eosinophil activation and survival.**

A ERK activation following IL-33 treatment of BM-derived eosinophils. *Dusp5*$^{+/+}$ or *Dusp5*$^{-/-}$ BM-derived eosinophils were stimulated with IL-33 (25 ng/ml) for the indicated times, and total cell lysates were analyzed for p-ERK1/p-ERK2 (top) and total ERK1/2 (bottom) (left panels). Quantitation of p-ERK1 normalized to total ERK1 levels shown on left is graphically depicted on the right.

B NF-κB activation following IL-33 treatment of BM-derived eosinophils. *Dusp5*$^{+/+}$ or *Dusp5*$^{-/-}$ BM-derived eosinophils were treated with IL-33 (25 ng/ml) for the indicated times and cytoplasmic fraction isolated and analyzed for p-p65 (top) and total p65 (bottom) (left panels). Quantitation of p-p65 normalized to total p65 level shown on left is graphically depicted on the right.

C Upregulation of CD11b is partially MEK dependent. BM-derived eosinophils were treated with IL-33 (25 ng/ml) in the presence or absence of indicated concentrations of the MEK small molecule inhibitor U0126. Analysis of cell surface CD11b expression was performed at 4 h.

D Cytoplasmic BCL-$X_L$ accumulation is MEK dependent. BM-derived eosinophils were treated with IL-33 (25 ng/ml) for the indicated times with DMSO or MEK inhibitor U0126 (10 μM). Cytoplasmic fractions were analyzed by Western blotting for BCL-$X_L$ (top) and BCL-2 (bottom).

E Quantitation of BCL-$X_L$ (top) and BCL-2 (bottom) protein levels in cytoplasmic fractions of BM-derived eosinophils treated with IL-33 (25 ng/ml) for indicated times. Protein levels are normalized to actin protein from Western blots shown in (D).

F BM-derived eosinophils were treated with IL-33 (25 ng/ml) and DMSO or the indicated concentrations of MEK inhibitor U0126. U0126 was added to cultures on day 2, and cell viability relative to DMSO-treated cultures was assessed on day 4.

Data information: Results are representative of at least three independent experiments. Values in graphs represent mean ± SEM. Statistical analysis is by two-tailed paired Student's *t*-test; $^{**}P < 0.01$; $^{***}P < 0.001$.
Source data are available online for this figure.

restored with IL-5 (Dibbert *et al*, 1998), though this finding has not been consistently observed (Dewson *et al*, 1999). Our finding that IL-5 does not regulate *Dusp5* mRNA levels in BM-derived eosinophils supports the notion that DUSP5 is a selective regulator of IL-33-dependent functions in mouse eosinophils.

In addition to cell survival, *Dusp5*$^{-/-}$ eosinophils also demonstrated increased cellular activation (CD11b and CD69) and cytokine production in response to IL-33. These augmented IL-33-mediated functions are also MEK dependent and consistent with a previous study demonstrating MEK dependency of IL-33-mediated upregulation of cytokines, chemokines and surface expression of integrins on human eosinophils (Chow *et al*, 2010). Other DUSP family members have also demonstrated requisite or inhibitory roles in immune cell effector functions. *Dusp1*$^{-/-}$ and *Dusp4*$^{-/-}$ macrophages secrete more IL-6 and TNF-α in response to Toll-like receptor (TLR) activation when compared to wild-type cells (Hammer *et al*, 2006; Al-Mutairi *et al*, 2010). A similar heightened response for *Dusp1*$^{-/-}$ macrophages has also been described for glucocorticoid receptor activation (Abraham *et al*, 2006). In contrast to these inhibitory DUSP functions, DUSP2 plays a requisite role in TLR and IgE receptor functions in macrophages and mast cells, respectively (Jeffrey *et al*, 2006). *Dusp2*$^{-/-}$ macrophages and mast cells secrete less TNF-α, IL-6, PGE2, and nitric oxide in response to TLR stimulation and decreased IL-6 and TNF-α production following IgE receptor activation, respectively. Finally, DUSP10 plays both positive and inhibitory roles in cellular functions. *Dusp10*$^{-/-}$ macrophages and T cells demonstrate enhanced production of pro-inflammatory cytokines, while *Dusp10*$^{-/-}$ T cells exhibit a profound defect in T-cell proliferation (Zhang *et al*, 2004).

*Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice were able to more efficiently clear parasite burden following infection with *N. brasiliensis*. Consistent with our working hypothesis that DUSP5 regulates IL-33-mediated effector functions, *IL-33*$^{-/-}$ mice are compromised in their ability to clear *N. brasiliensis* (Hung *et al*, 2013). While *Dusp5* is also expressed in NK cells, these cells do not play a requisite role in parasite clearance (Supplementary Fig S4D). However, since IL-33 has broad effects on many cell types beyond eosinophils, the greater ability of *Dusp5*$^{-/-}$ *Rag2*$^{-/-}$ mice to clear *N. brasiliensis* may still have additional contributing cellular components.

Given the association of SNPs in *IL33* and its cognate receptor *IL1RL1* in human asthma patients, our findings have interesting implications for human diseases. *Dusp5* mRNA is also expressed in human eosinophils and its levels are also increased by IL-33 (Supplementary Fig S13A and B), though there may be differences in *Dusp5* regulation with IL-5 (Temple *et al*, 2001). Investigation of *Dusp5* regulation following treatment of asthma patients with anti-IL-5, anti-IL-13, or anti-IL-33R antibodies should provide additional insights.

In summary, our studies reveal a previously unknown role for DUSP5 in the regulation of IL-33-mediated eosinophil survival and function. While DUSPs have been described to control cell survival through regulation of pro-apoptotic pathways, our studies define a novel mechanism by which DUSP5 regulates IL-33-mediated cell survival through the pro-survival BCL-$X_L$ axis. Given the established and emerging roles of eosinophils and IL-33 in resolution of inflammation and mucosal homeostasis, respectively (Isobe *et al*, 2012; Lopetuso *et al*, 2012), defining how dysregulation of IL-33 or DUSP5-mediated pathways may provide insights into host maintenance and disease pathogenesis.

## Materials and Methods

### Generation of *Dusp5*$^{-/-}$ mice

The construct for targeting the C57BL/6 DUSP5 locus in C57BL/6 ES C2 cells was made using a combination of recombineering and standard molecular cloning techniques. C57BL/6 ES cells were transfected by electroporation with a linearized DUSP5 targeting vector and selected in media containing G418 (200 ng/ml). The targeted ES cells were transfected with a Cre-containing plasmid TNLOX1-3 to remove the neomycin resistance cassette. ES cells were injected into blastocysts using standard techniques, and germline transmission was obtained after crossing resulting chimaeras with C57BL/6 females. Genotyping with primer sets 5′-(WT) CAGCTGCAGAATCTGCAAGGGTGG, 5′-(KO) AAGCTATGCTGGTGCAGCCAGTCC, 3′-TCATTGGTGTTGCTTCTGGGGAGG was used to confirm the generation of *Dusp5*$^{-/-}$ mice.

 

### Mice

B6.SJL-*Ptprc*$^{a}$ *Pepc*$^{b}$/BoyJ mice were purchased from Jackson Laboratories. *Rag2*$^{-/-}$ were purchased from Jackson Laboratories for breeding with *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ mice. All mice were maintained under specific pathogen-free conditions. All animal experimentation protocols were approved by the Laboratory Animal Resources Committee at Genentech, Inc. (S San Francisco, CA).

### Bone marrow chimeras

Eight-week-old (CD45.1$^{+}$) *Dusp5*$^{+/+}$ (B6.SJL-*Ptprc*$^{a}$ *Pepc*$^{b}$/BoyJ) mice were lethally irradiated (2 doses of 500 cGy with 137Cs γ-irradiator) at 3-h intervals ~3 h prior to injection. BM from *Dusp5*$^{+/+}$ (CD45.1$^{+}$) were isolated and mixed at a 1:1 ratio with either *Dusp5*$^{+/+}$ or *Dusp5*$^{-/-}$ (CD45.2$^{+}$) BM in Dulbecco's modification of Eagle's medium. Irradiated mice (CD45.1$^{+}$) were injected in the lateral tail vein with approximately $1 \times 10^{6}$ total BM cells. All irradiated mice were given antibiotic-supplemented water (1.1 g/l neomycin, 2 g/l glucose, and 110 mg/l polymyxin B, Sigma-Aldrich) for 2 weeks.

### *Nippostrongylus brasiliensis* infection

*Dusp5*$^{+/+}$ or *Dusp5*$^{-/-}$ mice were placed under anesthesia and infected subcutaneously on the flank with 500 *N. brasiliensis* L3 larvae in 200 μl of saline. Control and infected animals were placed on polymyxin B and neomycin-supplemented water for 5 days post-infection. Blood, spleen, BM, BALF, and serum were harvested 8 days (*Rag2*$^{+/+}$ mice) or 13 days (*Rag2*$^{-/-}$ mice) after infection. Tissues were harvested for FACS analysis, and worm burden was assessed on day 8–9 (*Rag2*$^{+/+}$ mice and BM chimeric mice) or day 13–14 (*Rag2*$^{-/-}$ mice). Worm burden was determined by counting the numbers of adult worms under a dissection microscope. *In vivo* BrdU labeling was performed by injecting 1 mg of BrdU (BD Biosciences) into the peritoneum. For 36-h pulse/chase experiments, mice were injected on day 10 post-infection three times at 0, 12, and 24 h. Mice were sacrificed, and spleen, blood, and BM were harvested 36 h after initial pulse (12-h chase). For 84-h pulse/chase experiments, mice were injected on day 10 post-infection three times at 0, 12, and 24 h. Spleen, blood, and BM were harvested 84 h after initial pulse (60-h chase) for analysis. BrdU incorporation was analyzed by FACS.

### Flow cytometry

Single-cell suspensions from spleen, blood, BM, or BALF were washed in phosphate-buffered saline (PBS) containing 5 mM ethylenediaminetetraacetic acid and 0.5% bovine serum albumin (BSA). Cells were incubated for 15 min with Fc receptor block (clone 2.4G2, BD Pharmingen) prior to antibody staining. The following antibodies (Ab) were used for FACS staining and sorting: anti-CD3 (145-2C11), CD4 (GK1.5), and Ly6c (HK1.4) Abs were purchased from EBioscience; anti-CD8 (53–6.7), CD43 (S7), B220 (RA3-6B2), NK1.1 (PK136), CD11b (M1/70), SiglecF (E50-2440), CD69 (H1.2f3), and Ly6g (1A8) Abs were purchased from BD Pharmingen; anti-F4/80 (BM8) Ab was purchased from BioLegend. Aqua Fluorescent reactive dye (Life Technologies) was used to discriminate viable from dead cells. Intracellular BrdU was analyzed using the FITC BrdU Flow Kit (BD Biosciences) per manufacturer's protocol. Mitochondrial membrane depolarization was analyzed with the JC-1 dye (Life Technologies) per manufacturer's protocol. Apoptosis was assessed using the Apoptosis Kit – Pacific Blue annexin V/Sytox AADvanced (Invitrogen/Molecular Probes). Cells were sorted for RNA analysis on a BD FACSAria and stained cells visualized on a BD LSR Fortessa (BD Pharmingen). Flow cytometry data were analyzed using FlowJo software (Treestar).

### *In vivo* depletion of NK cells during *Nippostrongylus brasiliensis* infection

Anti-Asialo GM1 antibody was reconstituted in 1 ml water per manufacturer's recommendations (Wako chemicals). 24 h prior to *N. brasiliensis* infection, mice were administered 20 μl antibody or 200 μg isotype control antibody by intraperitoneal injection followed by intraperitoneal injections every 3 days. Worm burden and extent of NK cell depletion was analyzed on day 13 post-infection.

### *In vivo* administration of IL-33

500 ng of recombinant murine IL-33 (Genentech) was injected into the peritoneum of *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ mice for six consecutive days. Peritoneal lavage fluid was harvested and analyzed by FACS or sorted for eosinophils for gene expression analysis on day 7.

### Western blot analysis

BM-derived eosinophils ($5 \times 10^{6}$) were stimulated for indicated times and washed with PBS on ice. For whole-cell lysates, cells were lysed in RIPA lysis buffer (Thermo Scientific) with protease and phosphatase inhibitors. Cells were fractionated using the Pierce cellular fractionation kit according to manufacturer's protocol. Total protein from each fraction was determined by BSA quantification method (Pierce). Nuclear and cytoplasmic fractionation purity was assessed by immunoblotting for HDAC2 (nuclear protein) and HSP90 (cytoplasmic protein) (Supplementary Fig S12). Cell lysates were loaded on a NuPAGE 4–12% Bis-Tris gel (Life Technologies) and transferred to PVDF membrane using an iBlot Gel transfer device (Invitrogen). Membranes were blocked with 5% milk, 2% Tween in PBS. Abs specific for actin, BCL-$X_L$, BCL-2, MCL-1, BAX, BIM, BAD, PUMA, BAK, BID, caspase-3, cytochrome c, ERK, p-ERK, p38, p-p38, JNK, p-JNK, HSP90, HDAC2, p65, and p-p65 were purchased from Cell Signaling. Anti-DUSP5 monoclonal Ab (ab53217) was purchased from Abcam. All Abs were diluted in blocking buffer and incubated overnight at 4°C. Secondary Abs included HRP (Millipore)-conjugated goat anti-rabbit or goat anti-mouse Abs in conjunction with Amersham ECL prime Western blotting detection reagent (GE Healthcare Life Sciences) to visualize bands using X-ray film (Kodak). Bands were measured by densitometry analysis using ImageJ analysis software.

### Cytochrome c ELISA

BM-derived eosinophils were cytokine-starved or stimulated with the indicated cytokines (25 ng/ml) for 3 days. Cells were washed, and

cytoplasmic fraction was isolated (see Western blot method). 20 μl of total cytoplasmic protein was diluted and cyt c levels were analyzed with a cyt c ELISA kit (Invitrogen) per manufacturer's instructions. Total protein content was determined by BSA method, and cyt c levels were normalized to protein levels to derive a relative ratio.

#### Cell cultures

Eosinophils were cultured from total BM as previously described (Dyer *et al*, 2008). Briefly, total BM cells were flushed from femurs from *Dusp5*$^{+/+}$ and *Dusp5*$^{-/-}$ mice. Red blood cells (RBCs) were removed by lysis using ACK Lysing buffer (Gibco). PBS washed cells were cultured at $1 \times 10^7$ cells/ml in RPMI 1640 media supplemented with 20% fetal bovine serum (Hyclone), Pen/Strep, Glutamax, β-mercaptoethanol, and non-essential amino acids (Gibco). Cells were stimulated for 4 days in the presence of rmSCF (R&D) and rmFlt3L (R&D) at 100 ng/ml final concentration. After 4 days in culture, cells were washed and resuspended in complete media supplemented with rmIL-5 (Peprotech) at a concentration of 10 ng/ml for 4 days. On culture day 8, complete media and IL-5 were replenished and cultured until day 11 when cells were > 95% SiglecF$^+$ and SSC$^{hi}$ by FACS analysis. For long-term cultures, day 11–13 BM-derived eosinophils were washed and stimulated with 25–50 ng/ml of IL-5 or IL-33 for 1 day unless specified otherwise and total cell count determined by Guava Viacount Assay on a Guava easyCyte instrument (Millipore). Data are represented in total cell count or relative viability where percent viability of cultures was normalized to control-treated cultures.

#### Real-time quantitative PCR

Total RNA was extracted from cells with RNeasy Mini Plus Kits (Qiagen) and reverse-transcribed with SuperScript III First-Strand synthesis system (Life Technologies), in accordance with the manufacturer's instructions. Primers used to evaluate *Dusp5* expression in activated CD4$^+$ T cells were (forward) 5′-GTCACCACTTGCGGGAGTAT-3′ and (reverse) 5′-GGAGCAGTCACAGGAAATGAC-3′. Taqman probe sets for human *DUSP5* and *GAPDH* or murine *Gapdh, Fosl1*, *Il4*, *Il13*, *Tnfa* and *Dusp5* were mixed with Taqman Universal PCR mastermix (Applied Biosystems), and gene expression was measured on a 7900HT Fast Real-Time PCR System (Applied Biosystems). Sybr Green analysis of gene expression with Quantitect Sybr Green PCR Kit (Qiagen) was used to analyze *Bclxl* normalized to *Actinb*. Primers use for Sybr Green analysis were *Bcl-xl* forward primer, 5′-TGG AGT CAG TTT ACT GAT GTC GAA G-3′, reverse primer, 5′-AGT TTA CTC CAT CCC GAA AGA GTT C-3′; *Actinb* forward primer 5′-TAT TGG CAA CGA GCG GTT C-3′ and reverse primer 5′-CAA TAC CCA AGA AGG AAG GCT-3′. Data were analyzed using the standard $2\Delta C_t$ method, and target genes normalized to housekeeping genes *GAPDH* or *Actinb*.

#### Human cell culture

Buffy coats were obtained through Genentech Health Services from human donors. 50 ml of blood was diluted 1:1 in PBS, layered onto Ficoll, and centrifuged at 800 × *g* at room temperature. Leukocytes were harvested, and B cells, CD4 and CD8 T cells, NK cells, and monocytes were isolated using their respective Miltenyi human isolation kits. RBCs were removed using with Ammonium-Chloride-Potassium lysis buffer (Gibco). Eosinophils were purified using a Miltenyi eosinophil isolation kit per manufacturer's recommendations. Isolated eosinophils were stimulated with 100 ng/ml hIL-33 (Genentech) in RPMI medium (Gibco).

#### Microarray gene expression profiling

Total RNA was extracted from cells using RNeasy Mini Kits (Qiagen) according to the manufacturer's protocol. RNA samples were quantitated with a NanoDrop ND-1000 UV spectrophotometer (Thermo Scientific, West Palm Beach, FL), and RNA quality was assessed with an Agilent 2100 Bioanalyzer (Agilent Technologies, Palo Alto, CA). The quantity of total RNA used in a two-round amplification protocol ranged from 10 ng to 50 ng per sample. First-round amplification and second-round cDNA syntheses were done with the Message Amp II mRNA Amplification Kit (Applied Biosystems, Foster City, CA). Cyanine-5 dye was incorporated with the Quick Amp Labeling kit (Agilent Technologies). Each cyanine-5-labeled test sample (750 ng) was pooled with cyanine-3-labeled Universal Mouse Reference RNA (Stratagene, La Jolla, CA) and hybridized onto Affymetrix Mouse genome 430 2.0 (MOE430V2) arrays as described in the manufacturer's protocol. Data are deposited in the GEO database under accession number GSE62999 (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE62999). Statistical calculations for analysis of gene expression microarray data were performed with the R Project software package, version 2.15.2.

**Supplementary information** for this article is available online: http://emboj.embopress.org


### Acknowledgements

The authors thank Tim Behrens, Eric Brown, Nico Ghilardi, Rajita Pappu, John Monroe, and Menno van Lookeren Campagne for advice and critical review of this manuscript.


### Author contributions

DAH designed experiments, generated and interpreted data, and prepared the manuscript. ACC designed experiments, interpreted data, and prepared the manuscript. DY and MX performed the helminth infection, worm burden analysis and assisted in mouse necropsy. J-HY designed and generated *Dusp5*$^{-/-}$ mice.

### Conflict of interest

The authors are employees of Genentech, Inc.

## References


Abraham SM, Lawrence T, Kleiman A, Warden P, Medghalchi M, Tuckermann J, Saklatvala J, Clark AR (2006) Antiinflammatory effects of dexamethasone are partly dependent on induction of dual specificity phosphatase 1. *J Exp Med* 203: 1883 – 1889

Akuthota P, Weller PF (2012) Eosinophils and disease pathogenesis. *Semin Hematol* 49: 113 – 119

Al-Mutairi MS, Cadalbert LC, McGachy HA, Shweash M, Schroeder J, Kurnik M, Sloss CM, Bryant CE, Alexander J, Plevin R (2010) MAP kinase


 

phosphatase-2 plays a critical role in response to infection by *Leishmania mexicana*. *PLoS Pathog* 6: e1001192

Ballif BA, Blenis J (2001) Molecular mechanisms mediating mammalian mitogen-activated protein kinase (MAPK) kinase (MEK)-MAPK cell survival signals. *Cell Growth Differ* 12: 397 – 408

Balmanno K, Cook SJ (2009) Tumour cell survival signalling by the ERK1/2 pathway. *Cell Death Differ* 16: 368 – 377

Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, Ortega HG, Pavord ID (2014) Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 371: 1189 – 1197

Bouffi C, Rochman M, Zust CB, Stucke EM, Kartashov A, Fulkerson PC, Barski A, Rothenberg ME (2013) IL-33 markedly activates murine eosinophils by an NF-kappaB-dependent mechanism differentially dependent upon an IL-4-driven autoinflammatory loop. *J Immunol* 191: 4317 – 4325

Caunt CJ, Keyse SM (2013) Dual-specificity MAP kinase phosphatases (MKPs): shaping the outcome of MAP kinase signalling. *FEBS J* 280: 489 – 504

Cherry WB, Yoon J, Bartemes KR, Iijima K, Kita H (2008) A novel IL-1 family cytokine, IL-33, potently activates human eosinophils. *J Allergy Clin Immunol* 121: 1484 – 1490

Chow JY, Wong CK, Cheung PF, Lam CW (2010) Intracellular signaling mechanisms regulating the activation of human eosinophils by the novel Th2 cytokine IL-33: implications for allergic inflammation. *Cell Mol Immunol* 7: 26 – 34

Coffman RL, Seymour BW, Hudak S, Jackson J, Rennick D (1989) Antibody to interleukin-5 inhibits helminth-induced eosinophilia in mice. *Science* 245: 308 – 310

Dewson G, Walsh GM, Wardlaw AJ (1999) Expression of Bcl-2 and its homologues in human eosinophils. Modulation by interleukin-5. *Am J Respir Cell Mol Biol* 20: 720 – 728

Dibbert B, Daigle I, Braun D, Schranz C, Weber M, Blaser K, Zangemeister-Wittke U, Akbar AN, Simon HU (1998) Role for Bcl-xL in delayed eosinophil apoptosis mediated by granulocyte-macrophage colony-stimulating factor and interleukin-5. *Blood* 92: 778 – 783

Domina AM, Vrana JA, Gregory MA, Hann SR, Craig RW (2004) MCL1 is phosphorylated in the PEST region and stabilized upon ERK activation in viable cells, and at additional sites with cytotoxic okadaic acid or taxol. *Oncogene* 23: 5301 – 5315

Dyer KD, Moser JM, Czapiga M, Siegel SJ, Percopo CM, Rosenberg HF (2008) Functionally competent eosinophils differentiated *ex vivo* in high purity from normal mouse bone marrow. *J Immunol* 181: 4004 – 4009

Favata MF, Horiuchi KY, Manos EJ, Daulerio AJ, Stradley DA, Feeser WS, Van Dyk DE, Pitts WJ, Earl RA, Hobbs F, Copeland RA, Magolda RL, Scherle PA, Trzaskos JM (1998) Identification of a novel inhibitor of mitogen-activated protein kinase kinase. *J Biol Chem* 273: 18623 – 18632

Franklin CC, Srikanth S, Kraft AS (1998) Conditional expression of mitogen-activated protein kinase phosphatase-1, MKP-1, is cytoprotective against UV-induced apoptosis. *Proc Natl Acad Sci USA* 95: 3014 – 3019

Fulkerson PC, Rothenberg ME (2013) Targeting eosinophils in allergy, inflammation and beyond. *Nat Rev Drug Discov* 12: 117 – 129

Goh YP, Henderson NC, Heredia JE, Red Eagle A, Odegaard JI, Lehwald N, Nguyen KD, Sheppard D, Mukundan L, Locksley RM, Chawla A (2013) Eosinophils secrete IL-4 to facilitate liver regeneration. *Proc Natl Acad Sci USA* 110: 9914 – 9919

Grasset MF, Gobert-Gosse S, Mouchiroud G, Bourette RP (2010) Macrophage differentiation of myeloid progenitor cells in response to M-CSF is regulated by the dual-specificity phosphatase DUSP5. *J Leukoc Biol* 87: 127 – 135

Gudbjartsson DF, Bjornsdottir US, Halapi E, Helgadottir A, Sulem P, Jonsdottir GM, Thorleifsson G, Helgadottir H, Steinthorsdottir V, Stefansson H, Williams C, Hui J, Beilby J, Warrington NM, James A, Palmer LJ, Koppelman GH, Heinzmann A, Krueger M, Boezen HM *et al* (2009) Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. *Nat Genet* 41: 342 – 347

Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, Marshall RP, Bradding P, Green RH, Wardlaw AJ, Pavord ID (2009) Mepolizumab and exacerbations of refractory eosinophilic asthma. *N Engl J Med* 360: 973 – 984

Hammer M, Mages J, Dietrich H, Servatius A, Howells N, Cato AC, Lang R (2006) Dual specificity phosphatase 1 (DUSP1) regulates a subset of LPS-induced genes and protects mice from lethal endotoxin shock. *J Exp Med* 203: 15 – 20

Heredia JE, Mukundan L, Chen FM, Mueller AA, Deo RC, Locksley RM, Rando TA, Chawla A (2013) Type 2 innate signals stimulate fibro/adipogenic progenitors to facilitate muscle regeneration. *Cell* 153: 376 – 388

Hoornaert I, Marynen P, Goris J, Sciot R, Baens M (2003) MAPK phosphatase DUSP16/MKP-7, a candidate tumor suppressor for chromosome region 12p12-13, reduces BCR-ABL-induced transformation. *Oncogene* 22: 7728 – 7736

Hung LY, Lewkowich IP, Dawson LA, Downey J, Yang Y, Smith DE, Herbert DR (2013) IL-33 drives biphasic IL-13 production for noncanonical Type 2 immunity against hookworms. *Proc Natl Acad Sci USA* 110: 282 – 287

Isobe Y, Kato T, Arita M (2012) Emerging roles of eosinophils and eosinophil-derived lipid mediators in the resolution of inflammation. *Front Immunol* 3: 270

Jeffrey KL, Brummer T, Rolph MS, Liu SM, Callejas NA, Grumont RJ, Gillieron C, Mackay F, Grey S, Camps M, Rommel C, Gerondakis SD, Mackay CR (2006) Positive regulation of immune cell function and inflammatory responses by phosphatase PAC-1. *Nat Immunol* 7: 274 – 283

Jeffrey KL, Camps M, Rommel C, Mackay CR (2007) Targeting dual-specificity phosphatases: manipulating MAP kinase signalling and immune responses. *Nat Rev Drug Discov* 6: 391 – 403

Kabata H, Moro K, Fukunaga K, Suzuki Y, Miyata J, Masaki K, Betsuyaku T, Koyasu S, Asano K (2013) Thymic stromal lymphopoietin induces corticosteroid resistance in natural helper cells during airway inflammation. *Nat Commun* 4: 2675

Kankaanranta H, Moilanen E, Zhang X (2005) Pharmacological regulation of human eosinophil apoptosis. *Curr Drug Targets Inflamm Allergy* 4: 433 – 445

Kiessling MK, Linke B, Brechmann M, Suss D, Krammer PH, Gulow K (2010) Inhibition of NF-kappaB induces a switch from CD95L-dependent to CD95L-independent and JNK-mediated apoptosis in T cells. *FEBS Lett* 584: 4679 – 4688

Knott ML, Matthaei KI, Giacomin PR, Wang H, Foster PS, Dent LA (2007) Impaired resistance in early secondary *Nippostrongylus brasiliensis* infections in mice with defective eosinophilopoeisis. *Int J Parasitol* 37: 1367 – 1378

Kobori A, Yagi Y, Imaeda H, Ban H, Bamba S, Tsujikawa T, Saito Y, Fujiyama Y, Andoh A (2010) Interleukin-33 expression is specifically enhanced in inflamed mucosa of ulcerative colitis. *J Gastroenterol* 45: 999 – 1007

Kopf M, Brombacher F, Hodgkin PD, Ramsay AJ, Milbourne EA, Dai WJ, Ovington KS, Behm CA, Kohler G, Young IG, Matthaei KI (1996) IL-5-deficient mice have a developmental defect in CD5+ B-1 cells and lack eosinophilia but have normal antibody and cytotoxic T cell responses. *Immunity* 4: 15 – 24

Kovanen PE, Rosenwald A, Fu J, Hurt EM, Lam LT, Giltnane JM, Wright G, Staudt LM, Leonard WJ (2003) Analysis of gamma c-family cytokine target

genes. Identification of dual-specificity phosphatase 5 (DUSP5) as a regulator of mitogen-activated protein kinase activity in interleukin-2 signaling. *J Biol Chem* 278: 5205 – 5213

Kovanen PE, Bernard J, Al-Shami A, Liu C, Bollenbacher-Reilley J, Young L, Pise-Masison C, Spolski R, Leonard WJ (2008) T-cell development and function are modulated by dual specificity phosphatase DUSP5. *J Biol Chem* 283: 17362 – 17369

Kurowska-Stolarska M, Stolarski B, Kewin P, Murphy G, Corrigan CJ, Ying S, Pitman N, Mirchandani A, Rana B, van Rooijen N, Shepherd M, McSharry C, McInnes IB, Xu D, Liew FY (2009) IL-33 amplifies the polarization of alternatively activated macrophages that contribute to airway inflammation. *J Immunol* 183: 6469 – 6477

Lang R, Hammer M, Mages J (2006) DUSP meet immunology: dual specificity MAPK phosphatases in control of the inflammatory response. *J Immunol* 177: 7497 – 7504

Le H, Kim W, Kim J, Cho HR, Kwon B (2013) Interleukin-33: a mediator of inflammation targeting hematopoietic stem and progenitor cells and their progenies. *Front Immunol* 4: 104

Lopetuso LR, Scaldaferri F, Pizarro TT (2012) Emerging role of the interleukin (IL)-33/ST2 axis in gut mucosal wound healing and fibrosis. *Fibrogenesis Tissue Repair* 5: 18

Mandl M, Slack DN, Keyse SM (2005) Specific inactivation and nuclear anchoring of extracellular signal-regulated kinase 2 by the inducible dual-specificity protein phosphatase DUSP5. *Mol Cell Biol* 25: 1830 – 1845

McCubrey JA, Steelman LS, Franklin RA, Abrams SL, Chappell WH, Wong EW, Lehmann BD, Terrian DM, Basecke J, Stivala F, Libra M, Evangelisti C, Martelli AM (2007) Targeting the RAF/MEK/ERK, PI3K/AKT and p53 pathways in hematopoietic drug resistance. *Adv Enzyme Regul* 47: 64 – 103

Menzies-Gow A, Flood-Page P, Sehmi R, Burman J, Hamid Q, Robinson DS, Kay AB, Denburg J (2003) Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J Allergy Clin Immunol* 111: 714 – 719

Milovanovic M, Volarevic V, Radosavljevic G, Jovanovic I, Pejnovic N, Arsenijevic N, Lukic ML (2012) IL-33/ST2 axis in inflammation and immunopathology. *Immunol Res* 52: 89 – 99

Moffatt MF, Kabesch M, Liang L, Dixon AL, Strachan D, Heath S, Depner M, von Berg A, Bufe A, Rietschel E, Heinzmann A, Simma B, Frischer T, Willis-Owen SA, Wong KC, Illig T, Vogelberg C, Weiland SK, von Mutius E, Abecasis GR *et al* (2007) Genetic variants regulating ORMDL3 expression contribute to the risk of childhood asthma. *Nature* 448: 470 – 473

Oboki K, Ohno T, Kajiwara N, Arae K, Morita H, Ishii A, Nambu A, Abe T, Kiyonari H, Matsumoto K, Sudo K, Okumura K, Saito H, Nakae S (2010) IL-33 is a crucial amplifier of innate rather than acquired immunity. *Proc Natl Acad Sci USA* 107: 18581 – 18586

Ohnmacht C, Pullner A, van Rooijen N, Voehringer D (2007) Analysis of eosinophil turnover *in vivo* reveals their active recruitment to and prolonged survival in the peritoneal cavity. *J Immunol* 179: 4766 – 4774

Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, Humbert M, Katz LE, Keene ON, Yancey SW, Chanez P (2014) Mepolizumab treatment in patients with severe eosinophilic asthma. *N Engl J Med* 371: 1198 – 1207

Oshikawa K, Kuroiwa K, Tago K, Iwahana H, Yanagisawa K, Ohno S, Tominaga SI, Sugiyama Y (2001) Elevated soluble ST2 protein levels in sera of patients with asthma with an acute exacerbation. *Am J Respir Crit Care Med* 164: 277 – 281

Prefontaine D, Lajoie-Kadoch S, Foley S, Audusseau S, Olivenstein R, Halayko AJ, Lemiere C, Martin JG, Hamid Q (2009) Increased expression of IL-33 in severe asthma: evidence of expression by airway smooth muscle cells. *J Immunol* 183: 5094 – 5103

Ramesh S, Qi XJ, Wildey GM, Robinson J, Molkentin J, Letterio J, Howe PH (2008) TGF beta-mediated BIM expression and apoptosis are regulated through SMAD3-dependent expression of the MAPK phosphatase MKP2. *EMBO Rep* 9: 990 – 997

Rosenberg HF, Dyer KD, Foster PS (2013) Eosinophils: changing perspectives in health and disease. *Nat Rev Immunol* 13: 9 – 22

Rui L, Healy JI, Blasioli J, Goodnow CC (2006) ERK signaling is a molecular switch integrating opposing inputs from B cell receptor and T cell cytokines to control TLR4-driven plasma cell differentiation. *J Immunol* 177: 5337 – 5346

Sakai N, Van Sweringen HL, Quillin RC, Schuster R, Blanchard J, Burns JM, Tevar AD, Edwards MJ, Lentsch AB (2012) Interleukin-33 is hepatoprotective during liver ischemia/reperfusion in mice. *Hepatology* 56: 1468 – 1478

Sakashita M, Yoshimoto T, Hirota T, Harada M, Okubo K, Osawa Y, Fujieda S, Nakamura Y, Yasuda K, Nakanishi K, Tamari M (2008) Association of serum interleukin-33 level and the interleukin-33 genetic variant with Japanese cedar pollinosis. *Clin Exp Allergy* 38: 1875 – 1881

Sevilla L, Zaldumbide A, Pognonec P, Boulukos KE (2001) Transcriptional regulation of the bcl-x gene encoding the anti-apoptotic Bcl-xL protein by Ets, Rel/NFkappaB, STAT and AP1 transcription factor families. *Histol Histopathol* 16: 595 – 601

Shen ZJ, Esnault S, Schinzel A, Borner C, Malter JS (2009) The peptidyl-prolyl isomerase Pin1 facilitates cytokine-induced survival of eosinophils by suppressing Bax activation. *Nat Immunol* 10: 257 – 265

Stolarski B, Kurowska-Stolarska M, Kewin P, Xu D, Liew FY (2010) IL-33 exacerbates eosinophil-mediated airway inflammation. *J Immunol* 185: 3472 – 3480

Suzukawa M, Koketsu R, Iikura M, Nakae S, Matsumoto K, Nagase H, Saito H, Matsushima K, Ohta K, Yamamoto K, Yamaguchi M (2008) Interleukin-33 enhances adhesion, CD11b expression and survival in human eosinophils. *Lab Invest* 88: 1245 – 1253

Temple R, Allen E, Fordham J, Phipps S, Schneider HC, Lindauer K, Hayes I, Lockey J, Pollock K, Jupp R (2001) Microarray analysis of eosinophils reveals a number of candidate survival and apoptosis genes. *Am J Respir Cell Mol Biol* 25: 425 – 433

Torgerson DG, Ampleford EJ, Chiu GY, Gauderman WJ, Gignoux CR, Graves PE, Himes BE, Levin AM, Mathias RA, Hancock DB, Baurley JW, Eng C, Stern DA, Celedon JC, Rafaels N, Capurso D, Conti DV, Roth LA, Soto-Quiros M, Togias A *et al* (2011) Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. *Nat Genet* 43: 887 – 892

Voehringer D, van Rooijen N, Locksley RM (2007) Eosinophils develop in distinct stages and are recruited to peripheral sites by alternatively activated macrophages. *J Leukoc Biol* 81: 1434 – 1444

Volarevic V, Mitrovic M, Milovanovic M, Zelen I, Nikolic I, Mitrovic S, Pejnovic N, Arsenijevic N, Lukic ML (2012) Protective role of IL-33/ST2 axis in Con A-induced hepatitis. *J Hepatol* 56: 26 – 33

Wang JX, Kaieda S, Ameri S, Fishgal N, Dwyer D, Dellinger A, Kepley CL, Gurish MF, Nigrovic PA (2014) IL-33/ST2 axis promotes mast cell survival via BCLXL. *Proc Natl Acad Sci USA* 111: 10281 – 10286

Wu D, Molofsky AB, Liang HE, Ricardo-Gonzalez RR, Jouihan HA, Bando JK, Chawla A, Locksley RM (2011) Eosinophils sustain adipose alternatively

 

activated macrophages associated with glucose homeostasis. *Science* 332: 243 – 247

Yasuda K, Muto T, Kawagoe T, Matsumoto M, Sasaki Y, Matsushita K, Taki Y, Futatsugi-Yumikura S, Tsutsui H, Ishii KJ, Yoshimoto T, Akira S, Nakanishi K (2012) Contribution of IL-33-activated type II innate lymphoid cells to pulmonary eosinophilia in intestinal nematode-infected mice. *Proc Natl Acad Sci USA* 109: 3451 – 3456

Zhang Y, Blattman JN, Kennedy NJ, Duong J, Nguyen T, Wang Y, Davis RJ, Greenberg PD, Flavell RA, Dong C (2004) Regulation of innate and adaptive immune responses by MAP kinase phosphatase 5. *Nature* 430: 793 – 797

Zhiguang X, Wei C, Steven R, Wei D, Wei Z, Rong M, Zhanguo L, Lianfeng Z (2010) Over-expression of IL-33 leads to spontaneous pulmonary inflammation in mIL-33 transgenic mice. *Immunol Lett* 131: 159 – 165