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Comparative analysis of the efficiency and specificity of myeloid-Cre deleting strains using ROSA-EYFP reporter mice

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ABSTRACT

Since the first example of conditional gene targeting in mice in 1994, the use of Cre recombinase and loxP flanked sequences has become an invaluable technique to generate tissue and temporal specific gene knockouts. The number of mouse strains expressing floxed-gene sequences, and tissue-specific or temporal-specific Cre-recombinase that have been reported in the literature has grown exponentially. However, increased use of this technology has highlighted several problems that can impact the interpretation of any phenotype observed in these mouse models. In particular, accurate knowledge of the specificity of Cre expression in each strain is critical in order to make conclusions about the role of specific cell types in the phenotypes observed. Cre-mediated deletion specificity and efficiency have been described in many different ways in the literature, making direct comparisons between these Cre strains impossible. Here we report crossing thirteen different myeloid-Cre mouse strains to ROSA-EYFP reporter mice and assaying YFP expression in a variety of naïve unstimulated hematopoietic cells, in parallel. By focusing on myeloid subsets, we directly compare the relative efficiency and specificity of myeloid deletion in these strains under steady-state conditions.

1. Introduction

When Sauer et al. described site-specific DNA recombination using Cre recombinase in 1988, they suggested that this "may be a useful tool for understanding and modulating genome rearrangements in eukaryotes" (Sauer and Henderson, 1988). Directly repeating 34 base pair loxP DNA sequences are placed flanking a target gene ("floxed"). Expression of Cre recombinase in the same cell leads to specific deletion of the floxed sequence. These observations were soon extended to show that site-specific deletion could be achieved in transgenic mice (Lakso et al., 1992; Orban et al., 1992), and by restricting Cre expression to a particular cell type, tissue specific gene deletion was demonstrated (Gu et al., 1994). Twenty five years later, this technology has become an invaluable tool used in laboratories for designing mouse models to answer a variety of research questions, especially in cases where complete gene knockouts cause embryonic or perinatal lethality. Use of inducible promoters that express Cre recombinase upon addition of agents like tamoxifen, tetracycline or type I interferon has enabled temporal analysis of gene function without complications caused by gene deletion during development. Inducible expression of an exogenous gene by inserting a *lox-STOP-lox* sequence upstream of a transgene has further expanded applications of this technology to include methods such as cell type-specific deletion mediated by diphtheria toxin, and lineage tracking mediated by expression of markers such as beta galactosidase or EYFP (Srinivas et al., 2001; Brockschnieder et al., 2006). In addition to the generation of many floxed mouse

Abbreviations: EYFP, enhanced yellow fluorescent protein; HBSS, Hanks balanced salt solution; BAL, bronchioalveolar lavage; BAC, bacterial artificial chromosome; IRES, internal ribosome entry site; DC, dendritic cell; NK, natural killer.

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strains, there has been a huge increase in the generation of Cre-expressing mouse strains, including several large-scale efforts to generate (predominantly neural-focused) new strains and resources to track them, comprehensively reviewed in Smedley et al. (2011) and Murray et al. (2012).

Not surprisingly, as the use of this technology, once described as the "Universal reagent for genome tailoring" (Nagy, 2000), has expanded, several issues have arisen that researchers must be aware of in interpreting results from these mouse models, reviewed in Schmidt-Supprian and Rajewsky (2007). In particular, the specificity of Cre expression is especially important but publications frequently fail to include comprehensive Cre expression profiles across many cell types. There are several methods for generating Cre-expressing strains, using either a transgene that includes a specific promoter or a "knock in" approach that uses endogenous regulatory sequences. Off target effects can arise from unexpected gene deletion caused by ectopic Cre expression or loss of enhancers or repressors that affect promoter activity. Examples of unexpected Cre expression in mice used for lymphoid cell analysis include non hematopoietic cells and germline expression (Schmidt-Supprian and Rajewsky, 2007). Unexpected expression of Cre in the germline can lead to passage of the deleted gene on to subsequent generations, so breeding strategies used for generating these mouse models must be carefully regulated. Use of bacterial artificial chromosomes (BACs) to generate a BAC transgenic that includes more regulatory sequences, or utilizing a neutral docking site that reduces transgene insertion site variation can improve these issues. A knock-in approach using the endogenous locus can be an advantage, although loss of one gene copy can lead to hemizygous effects. Newer lines have incorporated Internal Ribosome Entry Site (IRES)-Cre cassettes, leaving the regulatory gene intact. Although expression of Cre recombinase does not seem to affect mouse development, it has been suggested that at high concentrations, Cre can mediate DNA damage (Schmidt et al., 2000). This might be occurring through pseudo *loxP* sites (Thyagarajan et al., 2000; Semprini et al., 2007). The *RIP-cre* line was found to develop glucose intolerance in the absence of *loxP* targeted genes (Lee et al., 2006) and other examples of Cre toxicity have been reported in the gut and immune cell compartments (Higashi et al., 2009; Huh et al., 2010). Maintaining control of *cre* copy number in transgenic strains when designing breeding strategies may reduce this. Other factors that can influence deletion patterns include the genetic background of the Cre strain, and the sex of the parent contributing the *cre* allele, due to variation in Cre expression between the testes and ovary (Hebert and McConnell, 2000; Heffner et al., 2012). Furthermore, monitoring gene deletion by a PCR-based screen that detects just the *cre* allele can be inaccurate because silencing of this allele has been reported, perhaps due to methylation or other epigenetic changes (Schulz et al., 2007; Long and Rossi, 2009; Huh et al., 2010). Consequently, the presence of the deleted allele should also be monitored. Deletion efficiency using the same Cre strain can also vary depending on the floxed alleles or be inconsistent between littermates. These examples highlight the care that must be taken in analyzing data using these mouse models.

Many Cre strains are reported to be specific for certain cell types and tissues, and data is provided to indicate specificity, but a complete expression pattern is often not reported. As more strains become available, it is useful to be able to compare these different strains in a standard way and determine their relative specificity. Jackson Laboratories has undertaken the development of a Cre portal (www.creportal.org) in order to provide researchers with high throughput data about different Cre strains (Heffner et al., 2012). This work in progress presents a very thorough, histological analysis using beta-galactosidase from four stages of mouse development (E10.5, E15.5, P7 and P56). Our report complements this work, providing a more detailed approach looking at the specificity of Cre expression in myeloid-Cre-driven strains, using a standard protocol against which other myeloid-Cre strains could be compared in the future. We have used a *ROSA-flox-stop-flox*-EYFP reporter mouse to assay the expression patterns of Cre recombinase side-by-side in thirteen myeloid-Cre strains by flow cytometry. Such a parallel comparison using a standardized method has not been reported previously and should provide a useful resource to researchers to guide experimental design.

2. Methods

2.1. Mice and reagents

GE-cre, *LysM-cre*, *MRP8-cre*, *CD11c-cre*, *Vav1-cre*, *F4/80-cre*, *PF4-cre*, *CD11b-cre*, *NKp46-cre*, *CMA1-cre*, *MCPT5-cre*, *Basoph8-*

Table 1
Primers used for PCR genotyping.

Mouse strain	Primers used for genotyping
GE-cre	F = 5′ CAT GAC ACC CCC ACT GTC GTG TCC R (wt) = 5′ CAA TGC CAG TAG CAT GGC AGC CAG R (cre) = 5′ CAG GTA ATC TCT CAC ATC CTC AGG
LysM-cre	F = 5′ CTT GGG CTG CCA GAA TTT CTC R (wt) = 5′ TTA CAG TCG GCC AGG CTG AC R (cre) = 5′ TCA GCT ACA CCA GAG ACG G
MRP8-cre, CD11b-cre and CMA1-cre	F = 5′ CTG CAT TAC CGG TCG ATG CAA C R = 5′ GCA TTG CTG TCA CTT GGT CGT G
CD11c-cre	F = 5′ ACT TGG CAG CTG TCT CCA AG R = 5′ GCG AAC ATC TTC AGG TTC TG
Vav1-cre	F = 5′ AGA TGC CAG GAC ATC AGG AAC CTG R = 5′ ATC AGC CAC ACC AGA CAC AGA GAT C
F4/80-cre	F = 5′ AGA GGA GCA GCC AAA AGC CCC R (wt) = 5′ CTG ATG GTG GCA ACT CAG C R (cre) = 5′ GCG AAC ATC TTC AGG TTC TG
PF4-cre	F = 5′ CCC ATA CAG CAC ACC TTT TG R = 5′ TGC ACA GTC AGC AGG TT
NKp46-cre	F = 5′ GGA ACT GAA GGC AAC TCC TG R (wt) = 5′ TTC CCG GCA ACA TAA AAT AAA R (cre) = 5′ CCC TAG GAA TGC TCG TCA AG
MCPT5-cre	F = 5′ ACA GTG GTA TTC CCG GGG AGT GT R = 5′ GTC AGT GCG TTC AAA GGC CA
Basoph8-cre	F (wt) = 5′ GCT CTT CCA CCT CCT CAG TG F (cre) = 5′ CCA GCC ATC TGT TGT TTG C R = GGG ATG AGG ATG GTT GCT TA
Cx3cr1-cre	F = 5′ ACG TGG ACC TGC TTA CTG CAT G R = 5′ CGG CAA ACG GAC AGA AGC ATT
ROSA26-YFP	F = 5′ AAA GTC GCT CTG AGT TGT TAT R (wt) = 5′ GCG AAG AGT TTG TCC TCA ACC R (KI) = 5′ GGA GCG GGA GAA ATG GAT ATG

cre, *Cx3cr1-cre*, ROSA-EYFP (Gt(ROSA)26Sor$^{tm1(EYFP)Cos}$) and Ai14 (Gt(ROSA)26Sor$^{tm14(CAG-tdTomato)Hze}$) mice have been described (Clausen et al., 1999; Tkalcevic et al., 2000; Srinivas et al., 2001; Schaller et al., 2002; de Boer et al., 2003; Passegue et al., 2004; Ferron and Vacher, 2005; Caton et al., 2007; Tiedt et al., 2007; Musch et al., 2008; Scholten et al., 2008; Madisen et al., 2010; Narni-Mancinelli et al., 2011; Sullivan et al., 2011). All mice were kept in a specific pathogen-free facility at the University of California, San Francisco (UCSF) and cared for in accordance with UCSF institutional guidelines. All mouse strains used were confirmed by PCR genotyping of tail DNA using the primers indicated in Table 1.

The following antibodies were used for flow cytometry: anti-CD11b (M1/70), anti-Gr1 (RB6-8C5), anti-Ly6c (AL-21), anti-CD11c (HL3), anti-B220 (RA3-6B2), anti-CD4 (GK1.5), anti-CD8 (53-6.7), anti-TCRβ (H57-597), anti-CD19 (1D3), anti IgE (02122D), anti-CD3 (145-2C11), anti-SiglecF (E50-2440), anti-CD49b (09795B), anti-NKp46 (29A1.4), anti-c-kit (2B8), anti-NK1.1 (PK136) and anti-CD45 (30 F11) were obtained from either eBiosciences or BD Biosciences; anti-F4/80 (C1: A3-1) was from Serotec; anti-mouse neutrophil antigen (7/4) was from Caltag; and Streptavidin-Pacific orange was from Molecular Probes.

2.2. Cell preparation and flow cytometry

Single cell suspensions of splenocytes were prepared by homogenizing spleens between two frosted microscope slides, followed by passage through a 70 μM cell strainer. Cell numbers were determined by using a Nucleocounter™ (Chemometec). Peripheral blood was collected in microtainer tubes with EDTA (BD Biosciences). Bronchioalveolar lavage (BAL) cells were obtained with five 1 ml flushes of the lungs with ice-cold 5 mM EDTA in PBS. Peritoneal lavage cells were obtained with a 10 ml flush of the peritoneum with ice cold 2% (vol/vol) FCS in PBS. Red blood cells were removed from all above cell suspensions by lysis with ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM EDTA pH 7.2–7.4). Bone marrow cell suspensions were obtained by flushing femurs and tibiae with Hanks-

Table 2
Currently available myeloid-Cre expressing strains. The lines we analyzed in this study are highlighted in red. "Cre-strain" refers to the common name used throughout this report, and the official gene name is listed under "Mouse name". Further information on the Cre-strains can be found by accessing the Mouse Genome Informatics database (www.informatics.jax.org) using the MGI number.

Cre-strain	Mouse name	MGI number	Published specificity	Reference
Vav1-cre	Tg(Vav1-cre)A2Kio	2449949	All hematopoietic cells	de Boer et al. (2003)
GE-cre	Elane$^{tm1(cre)Roes}$	2182177	Neutrophils	Tkalcevic et al. (2000)
LysM-cre	Lyz2$^{tm1(cre)Ifo}$	1934631	Neutrophils and macrophages	Clausen et al. (1999)
MRP8-cre	Tg(S100A8-cre,GFP)1Empa	4415239	Neutrophils	Passegue et al. (2004)
F4/80-cre	Emr1$^{tm1(cre)Kpf}$	2429642	Tissue macrophages	Schaller et al. (2002)
CSF1R-cre	Tg(Csfr-icre)1Jwp	4429470	Macrophages (but deletion in other cell types)	Deng et al. (2010)
Cx3cr1-creER	Cx3cr1$^{tm2.1(cre/ERT)Litt}$	5450813	Tissue macrophages and monocytes	Yona et al. (2013)
	Cx3cr1$^{tm2.1(cre/ERT2)Litt}$	5467985		
Cx3cr1-cre	Tg(Cx3cr1-cre)MW126Gsat	5311737	Tissue macrophages and monocytes	www.gensat.org
Cx3cr1-cre	Cx3cr1$^{tm1.1(cre)Jung}$	5467983	Tissue macrophages and monocytes	Yona et al. (2013)
CD68-creER	Tg(Cd68-creER^{T2})31.11.ICS	(not listed)	(uncharacterized)	www.ics-mci.fr
CD68-cre	(not listed)	(not listed)	Macrophages, but bone marrow and some epithelial cell types	Franke et al. (2013)
CD11c-cre	Tg(Itgax-cre)1-1Reiz	3763248	Dendritic cells	Caton et al. (2007)
CD11c-cre	Tg(Itgax-cre,-EGFP)4097Ach	3720729	Dendritic cells (better in cDCs than pDCs)	Stranges et al. (2007)
Clec9a-cre	Clec9a$^{tm2.1(icre)Crs}$	5502446	Dendritic cells (cDCs)	Schraml et al. (2013)
	Tg(Clec9a-cre)1Crs	5502458		
Langerin/CD207-cre	Tg(CD207-cre)1Dhka	3761645	Langerhans cells	Kaplan et al. (2007)
Langerin/CD207-cre	Cd207$^{tm2.1(cre)Bjec}$	5308064	Langerhans cells	Zahner et al. (2011)
NKp46-cre	Ncr1$^{tm1.1(icre)Viv}$	5308410	NK cells	Narni-Mancinelli et al. (2011)
NKp46-cre	Tg(Ncr1-icre)265Sxl	4941472	NK cells	Eckelhart et al. (2011)
CMA1-cre	Tg(Cma1-cre)6Thhe	3778624	Tissue mast cells (lung and colon)	Musch et al. (2008)
MCPT5-cre	Tg(Cma1-cre)ARoer	3785000	Skin and peritoneal mast cells	Scholten et al. (2008)
Cpa3-cre	Tg(Cpa3-cre)3Glli	5301437	Mast cells, and some basophils, eosinophils, neutrophils and T cells	Lilla et al. (2011)
Cpa3-cre	Cpa3$^{tm3(icre)Hrr}$	3829878	Mast cell ablation	Feyerabend et al. (2009)
Mcpt8-cre	Tg(Mcpt8-cre)1Voeh	4834475	Basophils	Ohnmacht et al. (2010)
Basoph8-cre	Mcpt8$^{tm1(cre)Lky}$	5007937	Basophils	Sullivan et al. (2011)
Eo-cre	Epx$^{tm1.1(cre)Jlee}$	5498555	Eosinophils	Doyle et al. (2013)
PF4-cre	Tg(Pf4-cre)Q3Rsko	3764698	Platelets and megakaryocytes	Tiedt et al. (2007)
Gata1-cre	Tg(Gata1-cre)1Sho	2446599	Erythroid and megakaryocyte lineage	Mao et al. (1999)
	Tg(Gata1-cre)2Bsl	3814186	(germline deletion observed in 2446599)	Jasinski et al. (2001)
	Tg(Gata1-cre)453Sug	3784513		Chisaka et al. (2002)
Cathepsin K-cre	Ctsk$^{tm1(cre)Ska}$	3764465	Osteoclasts	Nakamura et al. (2007)
CD11b-cre	Tg(Itgam-cre)AJva	3577104	Myeloid cells (Transgene on Y chromosome)	Ferron and Vacher (2005)
CD11b-cre	Tg(ITGAM-cre)2781Gkl	3629092	Myeloid cells (Transgene on autosome)	Ferron and Vacher (2005)
C/EBPα-cre	Cebpa$^{tm1.1(cre)Touw}$	4867437	Myeloid cells	Wolfler et al. (2010)
Nur77-cre	Tg(Nr4a1-EGFP/cre)820Khog	5007644	Spleen myeloid cells (some deletion in T/B cells)	Moran et al. (2011)

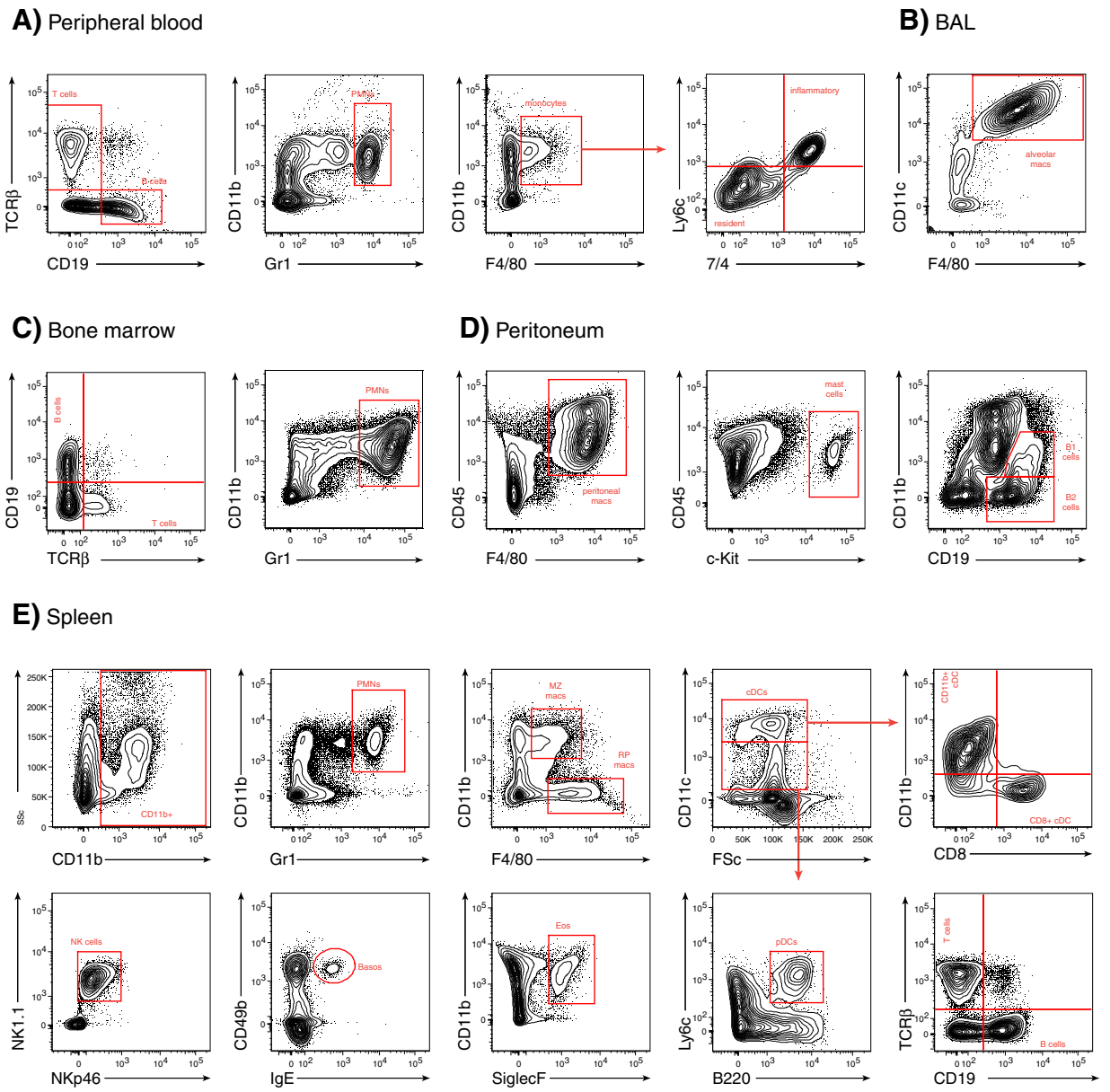

Fig. 1. Gating strategy of myeloid cell types analyzed by flow cytometry. DAPI^{-}autofluorescence^{lo} cells were gated as indicated for all populations, except for macrophages, where the autofluorescence^{hi} population was included and representative FACS plots are shown. (A) In peripheral blood, T cells (TCRβ^{+}; 18.7 ± 2.1%), B cells (CD19^{+}; 32.4 ± 3.7%), neutrophils (CD11b^{+}Gr1^{hi}; 21.6 ± 6.4%) and monocytes (CD11b^{+}F4/80^{+}; 6.16 ± 1.2%) were examined. Monocytes were further subdivided as inflammatory (Ly6c^{hi}7/4^{hi}; 2.4 ± 0.6%) or resident (Ly6c^{lo}7/4^{lo}; 3.3 ± 0.4%). Peripheral blood percentages refer to the percent of total leukocytes. (B) Alveolar macrophages were assayed from the bronchio-alveolar lavage (BAL) and defined as CD11c^{hi}F4/80^{hi} (94 ± 4.2%). (C) In the bone marrow, T cells (0.9 ± 0.14%), B cells (6.3 ± 1.0%) and neutrophils (55.2 ± 1.9%) were defined as for peripheral blood. (D) In the peritoneum, peritoneal macrophages (CD45^{+}F4/80^{+}; 58.9 ± 7.2%), mast cells (CD45^{+}c-Kit^{+}; 2.2 ± 0.4%), B1 B cells (CD19^{+}CD11b^{int}; 8.5 ± 2.5%) and B2 B cells (CD19^{+}CD11b^{-}; 13.9 ± 4.9%) were assayed. (E) In the spleen, CD11b^{+} cells accounted for 17.1 ± 2.4% of total splenocytes. T cells (34.8 ± 2.6%), B cells (43.5 ± 1.8%) and neutrophils (3.7 ± 1.1%) were defined as for peripheral blood. Splenic macrophages were defined as marginal zone (MZ, CD11b^{+}F4/80^{int}; 3.9 ± 0.6%) and red pulp (RP, autofluorescence^{hi}F4/80^{hi}CD11b^{-}; 4.4 ± 0.7%). For the following low abundance populations, a B220^{-}TCRβ^{-} gate was analyzed but the percentage of total splenocytes is given. cDCs (CD11c^{hi}; 1.37 ± 0.3%), further subdivided as "lymphoid" (CD8^{+}; 0.22 ± 0.06%) or "myeloid" (CD11b^{+}; 0.96 ± 0.2%), pDCs (CD11c^{int}Ly6c^{+}B220^{+}; 0.3 ± 0.06%), NK cells (NK1.1^{+}NKp46^{+}; 3.6 ± 1.3%), basophils (CD49b^{+}IgE^{+}; 0.3 ± 0.07%) and eosinophils (SiglecF^{+}CD11b^{+}; 0.9 ± 0.3%) were assayed. Cell frequencies are calculated from the average of 5–6 mice ± StdDev.

balanced salt solution without calcium or magnesium (HBSS) containing 20 mM HEPES. Red blood cells were lysed with a hypotonic NaCl solution and cells were passed through a 70 μM cell strainer. Cells were resuspended in HBSS containing 2% (vol/vol) fetal calf serum, 20 mM HEPES and 1 mM EDTA and maintained at 4 °C. For flow cytometry, 1–2 × 10^{6} cells were

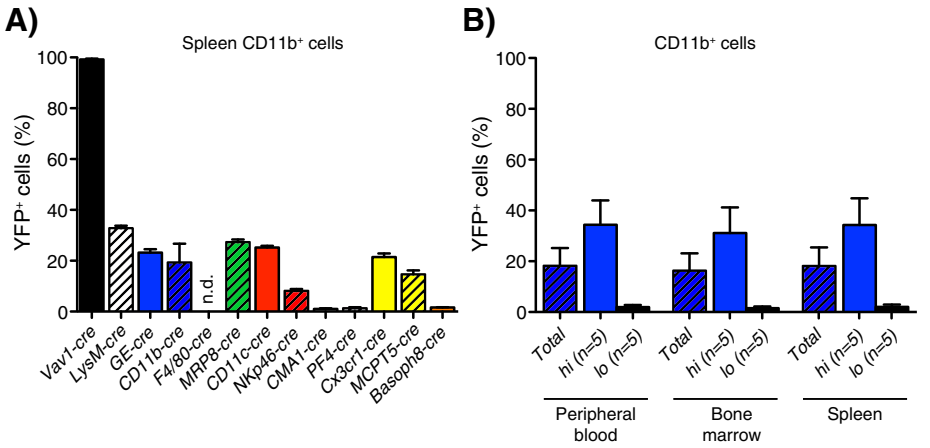

Fig. 2. YFP$^+$ cells from spleen CD11b$^+$ gate. (A) Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM). To illustrate the heterogeneity of deletion seen in the *CD11b-cre* strain, the percentage of YFP$^+$CD11b$^+$ cells from the peripheral blood, bone marrow and spleen were divided into two groups based on mice with higher or lower %YFP + cells (B).

stained. Non-specific binding was blocked by pre-incubation with 0.5 μg anti-CD16/32 antibody (2.4G2, UCSF Immunology Hybridoma Core) and 100 μg murine IgG (Sigma). Live cells were identified using DAPI exclusion. FACS analysis was performed using a Fortessa (BD Biosciences) and analyzed using FlowJo (Treestar), or an ImagestreamX (Amnis) and analyzed using IDEAS (Amnis). Statistical analyses were carried out using GraphPad Prism.

3. Results

In order to assess the efficiency and specificity of Cre-mediated deletion in a variety of mouse strains expressing Cre recombinase under the control of different myeloid-specific promoters, we crossed each Cre strain to *ROSA26-flox-stop-flox*-EYFP reporter (ROSA-EYFP) mice. Many myeloid-Cre strains have been described in the literature, and these are listed in Table 2. The Cre strains used in this study are highlighted in this table. All mice used were on the C57BL/6 background, with the exception of the *Cx3cr1-cre* strain, which was F1 mixed (C57BL/6 and mixed FVB/B6/129/Swiss/CD1). At 6–10 weeks of age, hematopoietic tissues were harvested from these mice, as detailed in the Materials and methods, and the percentage of YFP$^+$ cells was quantified by flow cytometry. The gating strategy for identifying the different myeloid subpopulations in naïve unstimulated mice is shown in Fig. 1.

We compared the deletion specificity in all the populations indicated in Fig. 1 using the myeloid-Cre strains highlighted in Table 2. We used the *Vav1-cre* strain as a marker of deletion in all hematopoietic cell types. In *Vav1-cre*:ROSA-EYFP mice, all hematopoietic cells analyzed are 98-100% YFP$^+$. We do not observe littermate mosaicism as reported in Heffner et al. (2012).

An overview of splenic myeloid cell Cre expression gating on CD11b$^+$ cells is shown in Fig. 2A. The inconsistent results between littermates using the *CD11b-cre* strain appear as low level deletion in all tissues and a larger statistical variance; however, the mice are either good deleters or do not delete at all. In Fig. 2B, we analyzed these two groups separately to illustrate this. This is the only Cre line analyzed where we saw such variability.

Granulocyte populations from spleen, peripheral blood and bone marrow are shown in Fig. 3A–C. *MRP8-cre*:ROSA-EYFP mice show ~80% deletion in neutrophils, with little deletion in macrophages/monocytes, dendritic cells (DCs), natural killer (NK) cells, mast cells, basophils or eosinophils (shown in subsequent figures). *LysM-cre* and *GE-cre*:ROSA-EYFP mice show ~50–70% deletion in neutrophils.

Fig. 4 shows Cre-mediated deletion in peripheral blood monocytes (Fig. 4A), further subdivided into inflammatory and resident phenotypes (Fig. 4B). Fig. 4C confirms that platelets from the *PF4-cre*:ROSA-EYFP mice show efficient deletion. However, we found that ~15% of all peripheral blood leukocytes in the *PF4-cre*:ROSA-EYFP mice are YFP$^+$ (Figs. 3B, 4A and B, 9A and B). It has been well-documented that platelets stick to neutrophils and monocytes, reviewed in Zarbock et al. (2007), so we attributed this YFP$^+$ staining in *PF4-cre*:ROSA-EYFP mice to platelets sticking to the surface of these cell types and not because of non-specific deletion. In order to test this, we analyzed peripheral blood leukocytes using the Imagestream and demonstrated that CD45$^+$YFP$^+$ cells also costain with the platelet marker CD41, and appear in the brightfield image to have bound platelet aggregates (Fig. 5A). More widespread deletion in hematopoietic stem cells using the *PF4-cre* line has been reported but this differs from our results (Calaminus et al., 2012).

Deletion in mature macrophage populations is shown in Fig. 6. *LysM-cre* promotes significant deletion in macrophages, but also in neutrophils. Like *LysM-cre*, *GE-cre* promotes significant deletion in neutrophils and macrophages. *F4/80-cre*:ROSA-EYFP mice show ~50% deletion in peritoneal macrophages, but do not exhibit deletion in other types of macrophages. In contrast to the peripheral blood analysis, Imagestream analysis of peritoneal macrophages from *PF4-cre*:ROSA-EYFP indicated that the YFP signal is not from platelets (Fig. 5B),

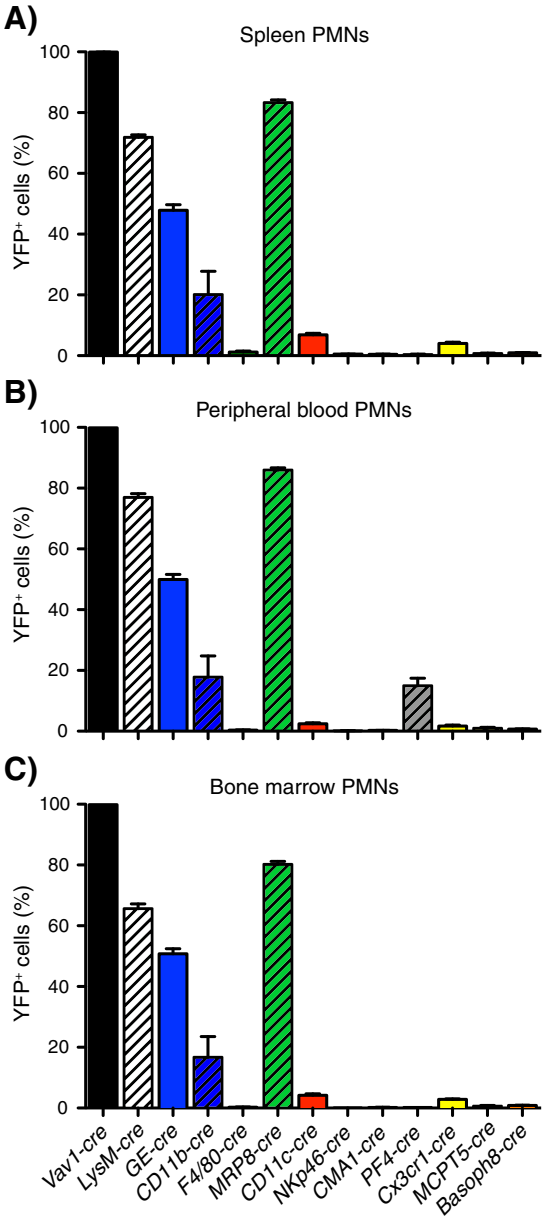

Fig. 3. YFP$^+$ cells from neutrophil populations. Neutrophils were defined as in Fig. 1 and assayed in spleen (A), peripheral blood (B) and bone marrow (C). Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM).

suggesting that this Cre strain does result in significant (~25%) non-specific deletion in peritoneal macrophages.

Deletion in dendritic cell subsets is shown in Fig. 7. *CD11c-cre*:ROSA-EYFP mice show efficient deletion in classical (cDCs) and plasmacytoid (pDCs) dendritic cells, but also in some monocyte/macrophage populations and NK cells. The *Cx3cr1-cre* strain provides deletion that is more specific to CD11b$^+$ "myeloid" DCs versus CD8$^+$ "lymphoid" DCs (Fig. 7C), but deletion is also seen in other tissues using this strain.

Deletion in other myeloid cell subtypes is shown in Fig. 8. The *MCPT5-cre* strain shows good deletion in peritoneal mast cells, but also some deletion in splenic NK cells (Fig. 8A and B). The *NKp46-cre* and *Basoph8-cre* strains of mice are particularly good and specific deleters in NK cells and basophils, respectively (Fig. 8B and C). As the *Basoph8-cre* strain contains an EYFP marker, we also crossed this strain to a *ROSA26-flox-stop-flox*-Ai14 strain to demonstrate that all YFP$^+$ basophils from these mice are also Ai14$^+$ indicating Cre activity in these cells. The *CMA1-cre* strain, which utilizes the baboon *Mcpt5* promoter, reportedly directs deletion in lung mast cells but we were unable to detect any deletion in any cell types we looked at, including lung tissue (data not shown).

Non-specific deletion in lymphoid cells (Fig. 9) was not observed in most strains tested, except for low levels of deletion in T cells present in the *CD11c-cre* strain.

4. Discussion

In general, the currently available strains for Cre-mediated deletion in well-defined, homogenous cell types, such as neutrophils, NK cells, mast cells and basophils, result in specific deletion. Similarly, a recently reported eosinophil-specific Cre crossed to a *floxed-Diphtheria toxin*-containing strain, also demonstrated specific loss of eosinophils but other floxed genes have not yet been tested (Doyle et al., 2013). However, it is clear that deletion in more heterogeneous cell populations such as monocytes, macrophages and dendritic cells is more problematic (Hume, 2011). As we learn how to define these populations more accurately, better cell type specific deletion may be possible.

A "good" macrophage deleter is highly sought after. The *LysM-cre* strain is often used by researchers to ascertain the role of monocytes and macrophages in mouse models but it also deletes in the neutrophil population. The *CD11b-cre* strain appears to be unreliable with inconsistent deletion between littermates and *F4/80-cre* only deletes in a fraction of peritoneal macrophages. The *Cx3cr1-cre* strain deletes in the monocyte/macrophage lineage and not in granulocytes, but deletion is also seen in mast cells and cDCs. The CSF-1R-driven Cre described by Deng *et al.*, hailed as "macrophage-specific", also deletes in neutrophil, dendritic and T-cell lineages (Deng et al., 2010). Two Cre strains utilizing the promoter of CD68 (or macrosialin) have been recently developed. One uses the human *CD68* promoter and reports deletion in monocytes and macrophages, but also some epithelial cells and keratinocytes (Franke et al., 2013). The second uses the mouse *CD68* promoter and is available from the Institut Clinique de la Souris, France but has not been characterized.

CD11c-promoter-driven Cre expression is used widely by researchers to implicate dendritic cells in various models of disease. Two groups independently made *CD11c-cre* strains, and report that they either lead to equal deletion in pDC and cDCs (Caton et al., 2007), or better deletion in the cDC compartment (Stranges et al., 2007). Until these mouse strains are compared side by side in same analysis, it will be hard to know for certain. Analysis of transcription factors distinguishing sub-populations of dendritic cells has suggested new targets for directing Cre expression to dendritic cell subsets, including Zbtb46 and MycL (Satpathy et al., 2012; Wumesh et al., 2014), and led to the development of

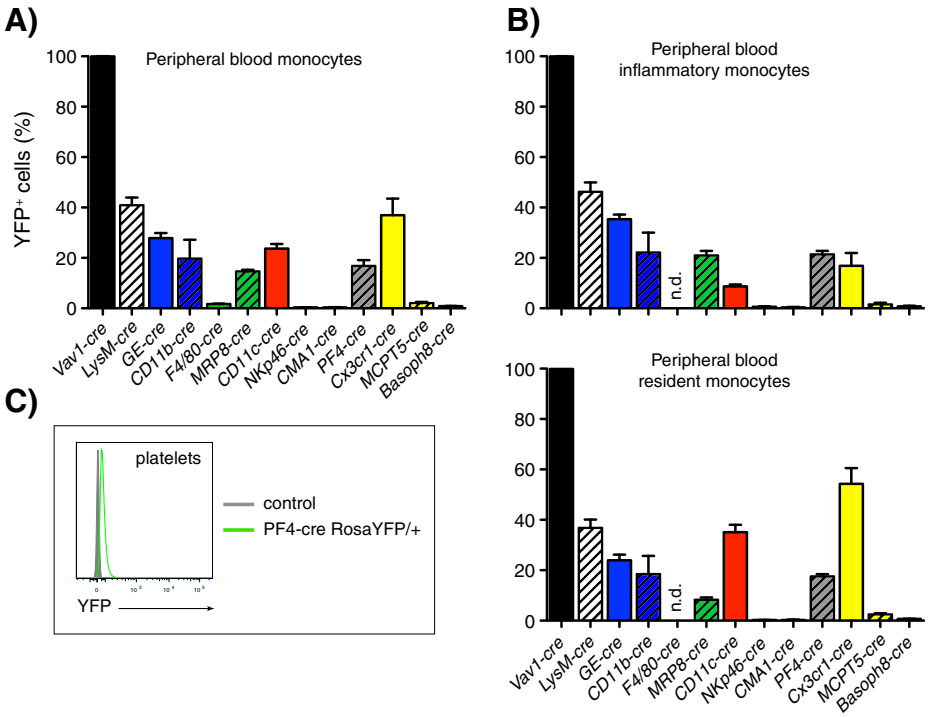

Fig. 4. YFP$^+$ cells from peripheral blood monocyte populations. Total blood monocytes (A) were subdivided into inflammatory and resident monocyte populations as defined in Fig. 1(B). Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM). (C) CD41^{hi} platelets from peripheral blood are YFP$^+$ in the *PF4-cre* strain.

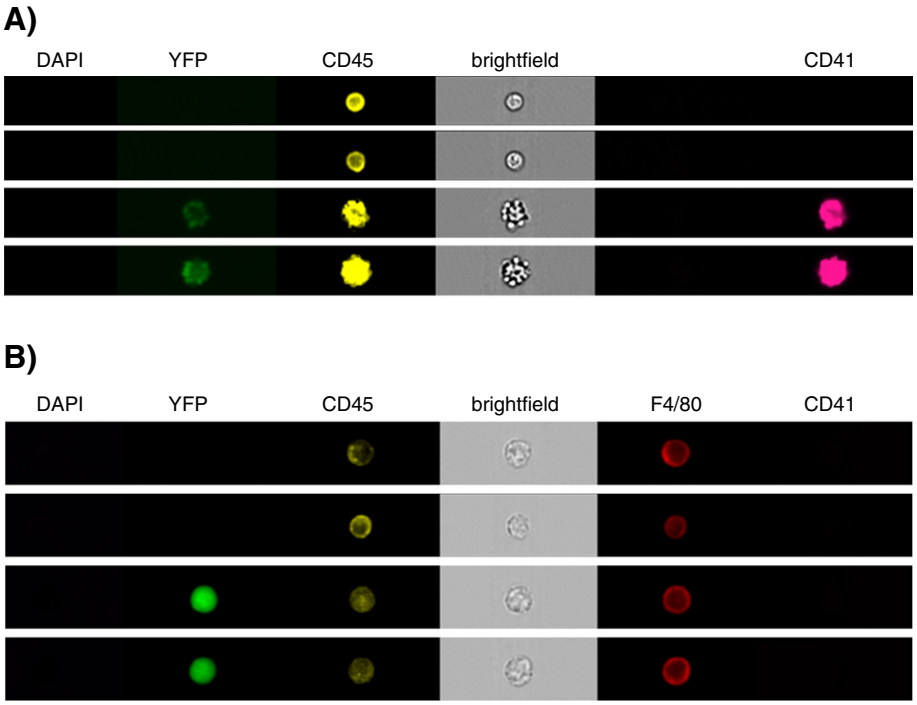

Fig. 5. A fraction of peripheral blood leukocytes in the *PF4-cre* strain appear YFP$^+$ due to platelet adhesion. (A) Cells were examined using the Amnis Imagestream and representative images are shown. (B) YFP$^+$ peritoneal macrophages isolated from the *PF4-cre* strain do not show staining with the platelet marker, CD41.

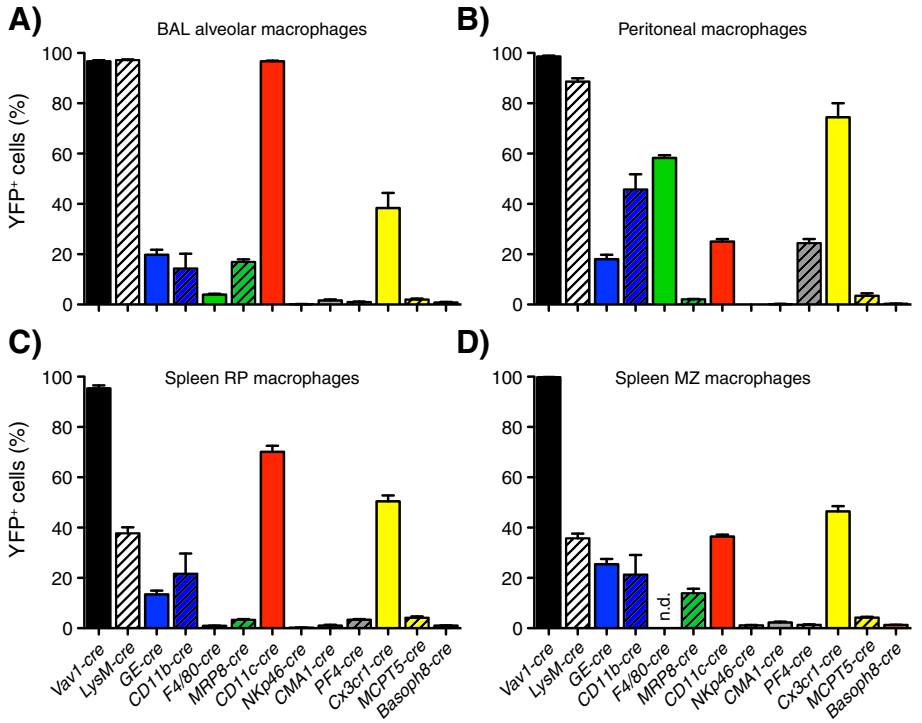

Fig. 6. YFP$^+$ cells from mature macrophage populations. Tissue macrophage populations from (A) bronchio-alveolar lavage, (B) peritoneum and (C, D) spleen as defined in Fig. 1 were assayed. Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM).

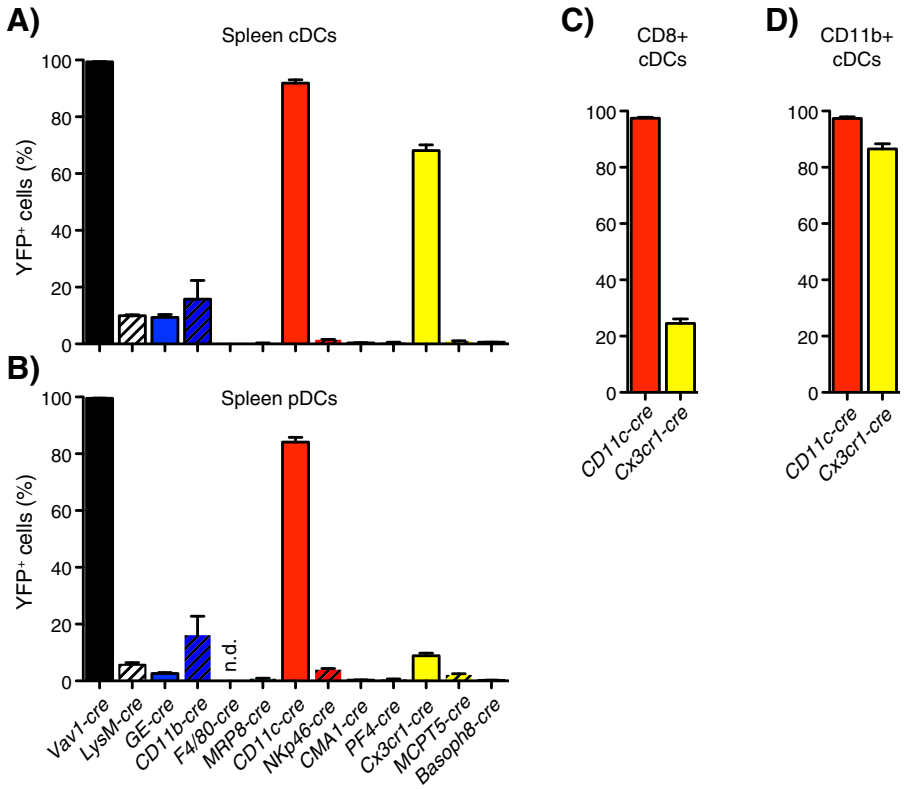

Fig. 7. YFP$^+$ cells from splenic dendritic cell populations. (A) cDCs and (B) pDCs as defined in Fig. 1 were assayed. The cDC population was further subdivided into CD8$^+$ "lymphoid" DCs (C) and CD11b$^+$ "myeloid" DCs (D) to highlight differential Cre expression observed in the *CD11c-cre* and *Cx3cr1-cre* strains. Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM).

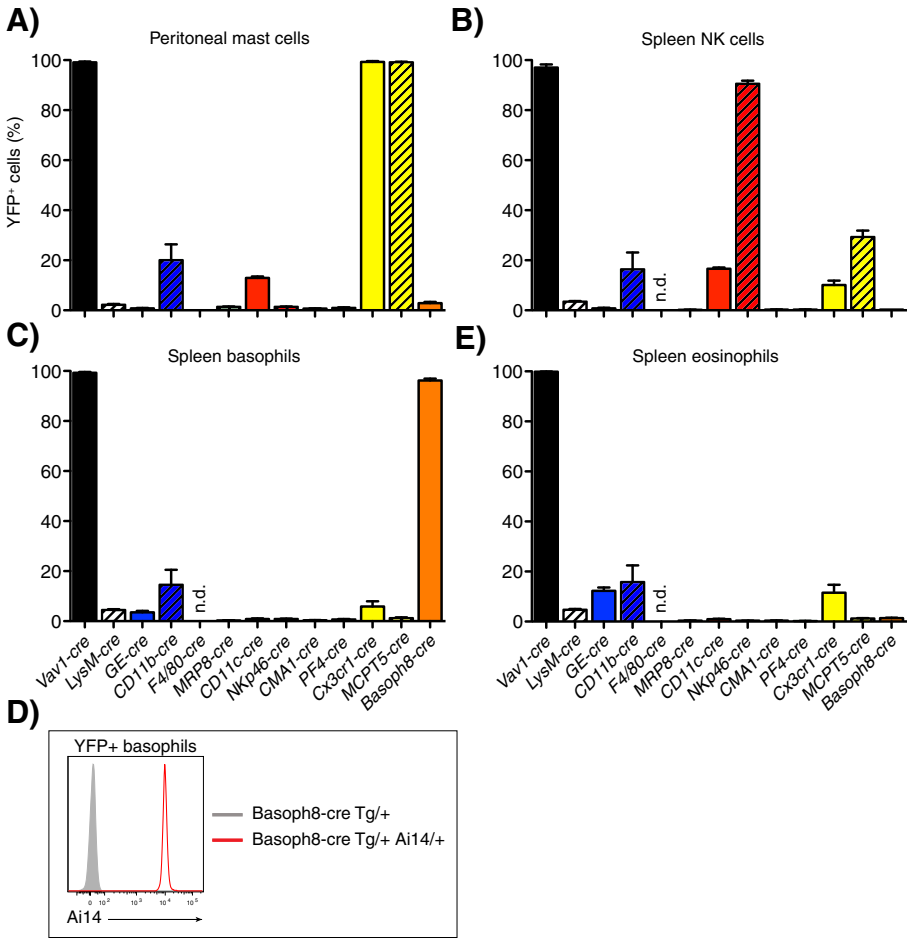

Fig. 8. YFP$^+$ cells from other myeloid cell populations. (A) Peritoneal mast cells, (B) splenic NK cells, (C) splenic basophils and (E) splenic eosinophils as defined in Fig. 1 were assayed. Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM). (D) Cre activity in the YFP$^+$ basophils from Basoph8-cre Tg/+ mice was demonstrated by crossing these mice with an Ai14 reporter strain and confirming that these cells were also Ai14$^+$.

newer Cre lines such as *Clec9A*(*Dngr-1*)*-cre* (Schraml et al., 2013). Other distinguishing genes may be identified from resources such as the Immunological Genome project (www.immgen.org) for use in defining populations of DCs (Miller et al., 2012) and tissue macrophages (Gautier et al., 2012) and leading to the development of new Cre strains. Data from the Immunological Genome project has also been valuable in explaining some of the non-cell-type specific Cre expression seen in our analysis. For example, the Immgen database reports that PF4 is expressed in a population of F4/80$^+$ peritoneal macrophages, which would explain why we observed YFP expression in ~25% of peritoneal macrophages using the *PF4-cre* strain. Furthermore, both Cx3cr1 and MCPT5 are expressed on a fraction of NK cells, and CD11c is expressed much more broadly than just dendritic cells, reflecting our findings with the respective Cre strains.

Our analysis examined the deletion specificity in naïve unstimulated cell populations. It is clear that there are changes in these populations during inflammatory and autoimmune disease settings, which could lead to changes in Cre-mediated deletion efficiency and specificity compared to naïve conditions. Obviously, deletion efficiency and specificity could differ for any individual Cre line in different disease models, for example in pneumonitis versus peritonitis, which precluded us from comparing all these Cre lines in multiple disease models. However, we have observed similar deletion efficiencies of a floxed *Shp1* allele crossed with the *MRP8-cre* and *CD11c-cre* strains, which develop spontaneous inflammation and autoimmune disease respectively, to the *ROSA-EYFP* reporter shown in this report (Abram et al., 2013). The Immgen database shows cell specific promoter activity in some cell types under a limited number of experimental models such as thioglycollate-ellicited peritonitis, arthritis and salmonella infection, but researchers will need to further evaluate Cre-mediated deletion of individual floxed target genes in the specific disease models they are examining.

In conclusion, we feel that our comparison of many different myeloid-Cre strains in parallel will be helpful for researchers considering experimental design and analyzing phenotypes

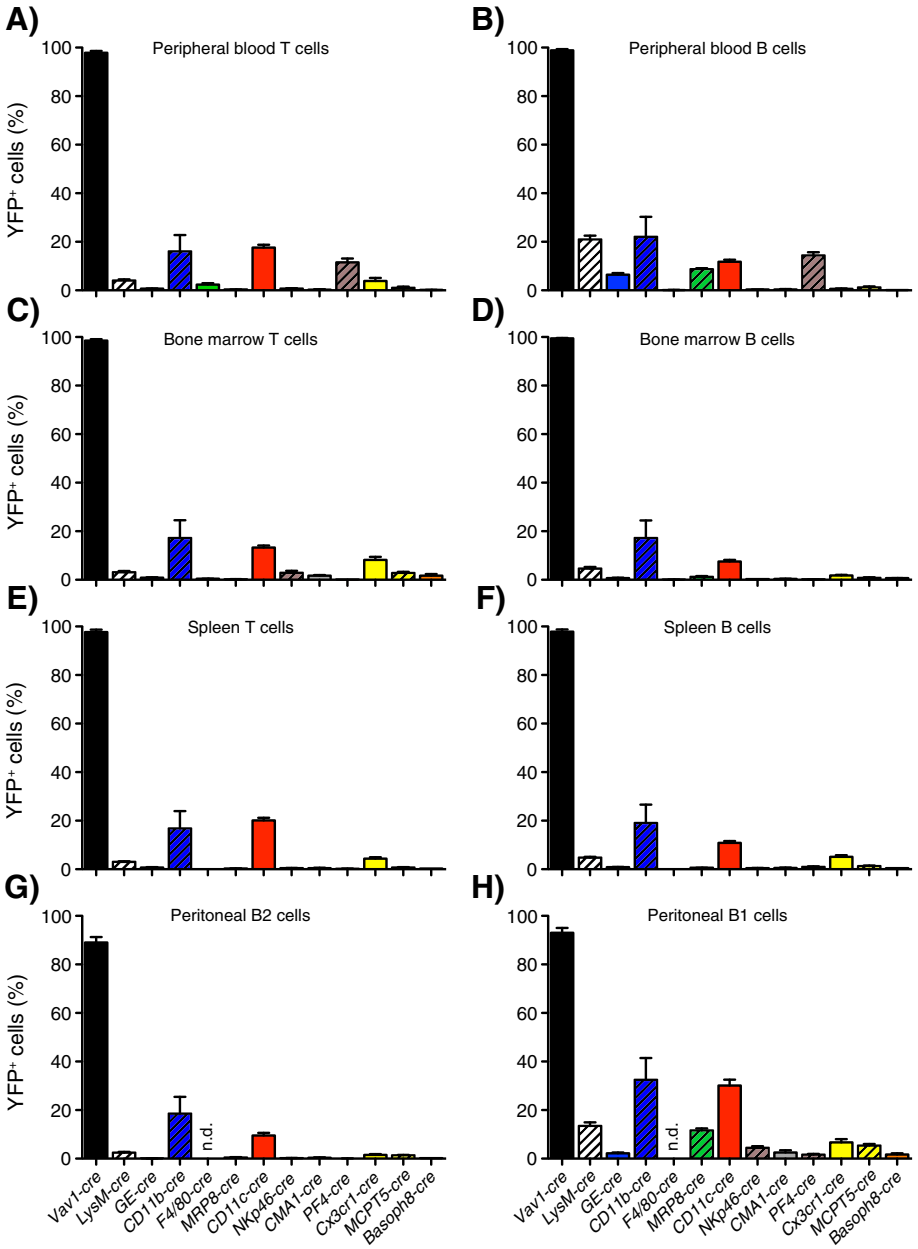

Fig. 9. YFP$^+$ cells from lymphoid populations. T cells (A, C, E), B cells (B, D, F) and peritoneal B1 and B2 cells (G and H) as defined in Fig. 1 were assayed. Bars represent the average of 5–14 mice analyzed per Cre strain (error bars indicate SEM).

observed in mouse models, and establish a framework against which new Cre strains can be compared.

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