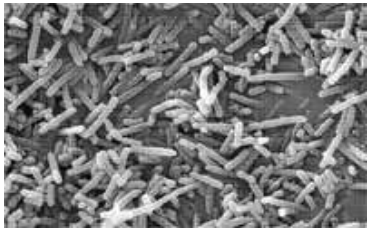
Challenges to Intestinal Immune System

- Vast surface area - 400m^2 (x200 skin)
- Continual antigenic challenge
  - Pathogenic bacteria, viruses, parasites
  - Food proteins - >30kg/year
  - Commensal bacteria - >10^{12}/ml in colon (>1000 species)

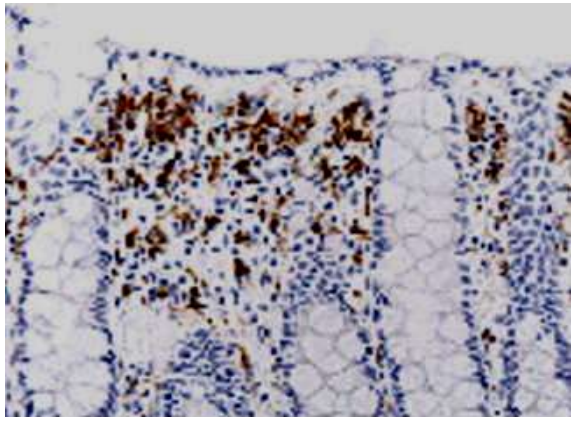


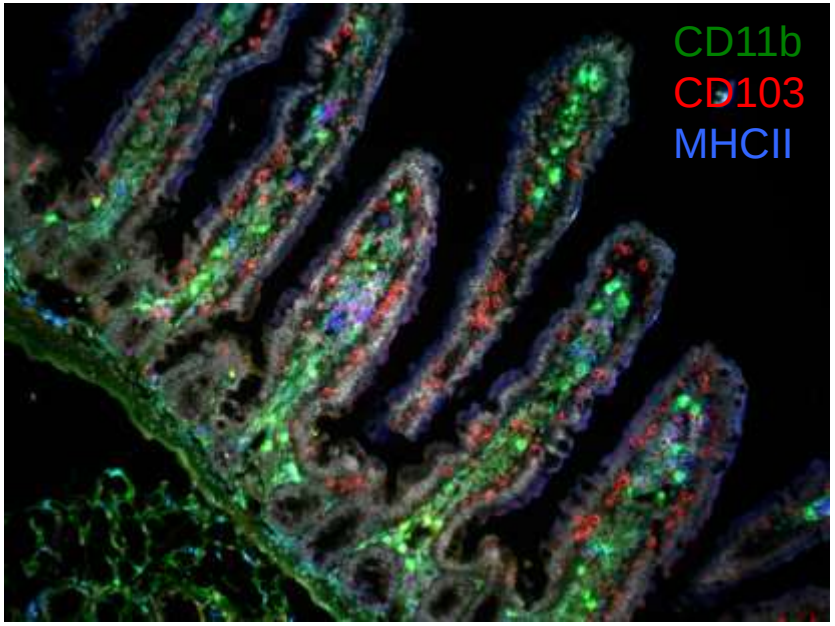
Regulation of Intestinal Immune Responses

- Many materials entering intestine contain specific antigens + PAMPs
- Large resident populations of activated/memory lymphocytes + innate effector cells
- Inappropriate immune responses cause IBD, coeliac disease

What prevents these conditions?

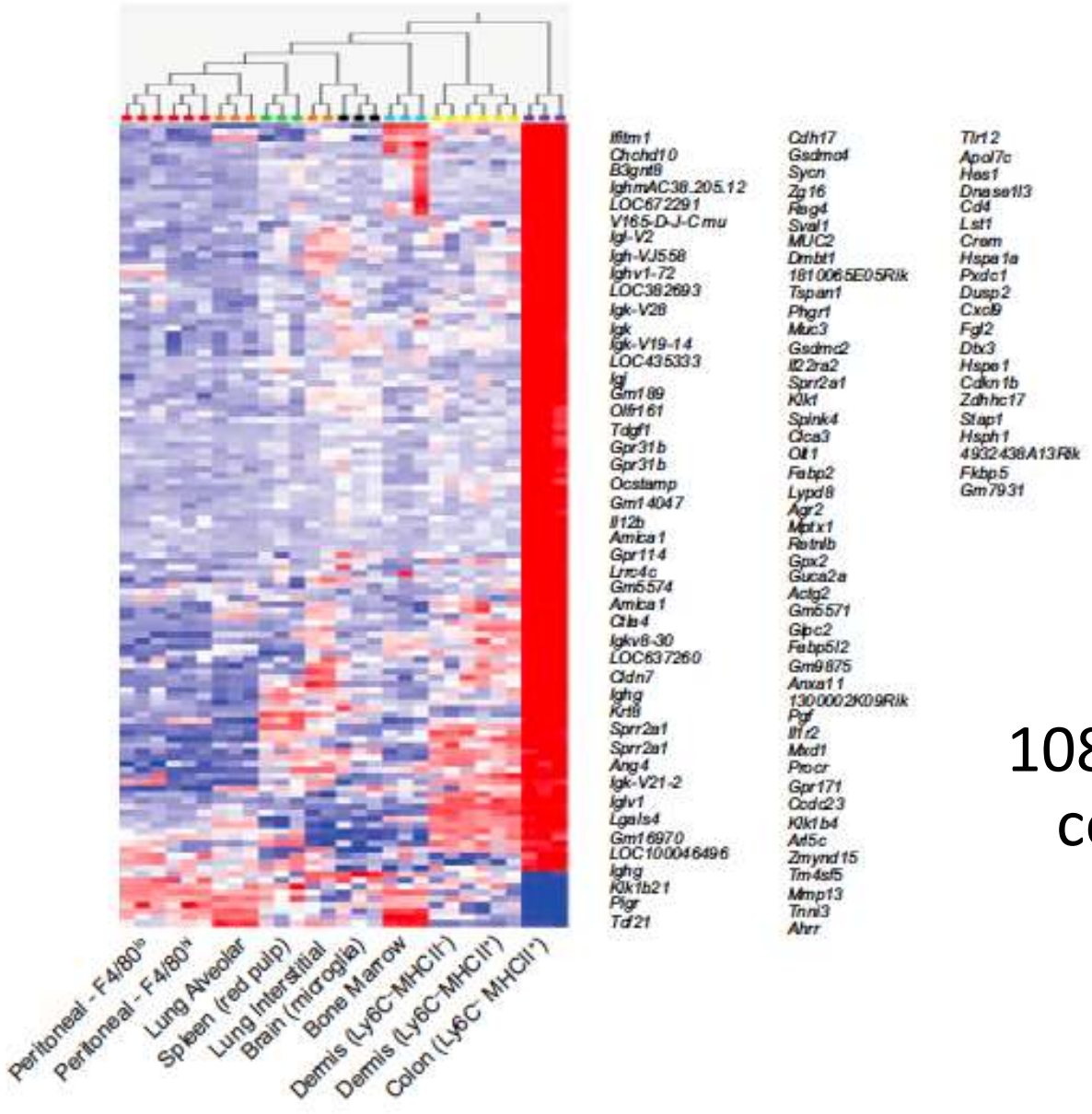
The Intestinal Macrophage Niche





- Complex tissue with unique physiological functions
- Constant need for monitoring and adaptability
- Macrophages numerous in steady state
- Express PRR, look activated, but no response to eg TLR ligands
- Needed to maintain tolerance and tissue homeostasis
- Also important for protective immunity and inflammation
  - How/why are the cells different in the intestine from elsewhere?

Unique Transcriptome of Colonic Macrophages

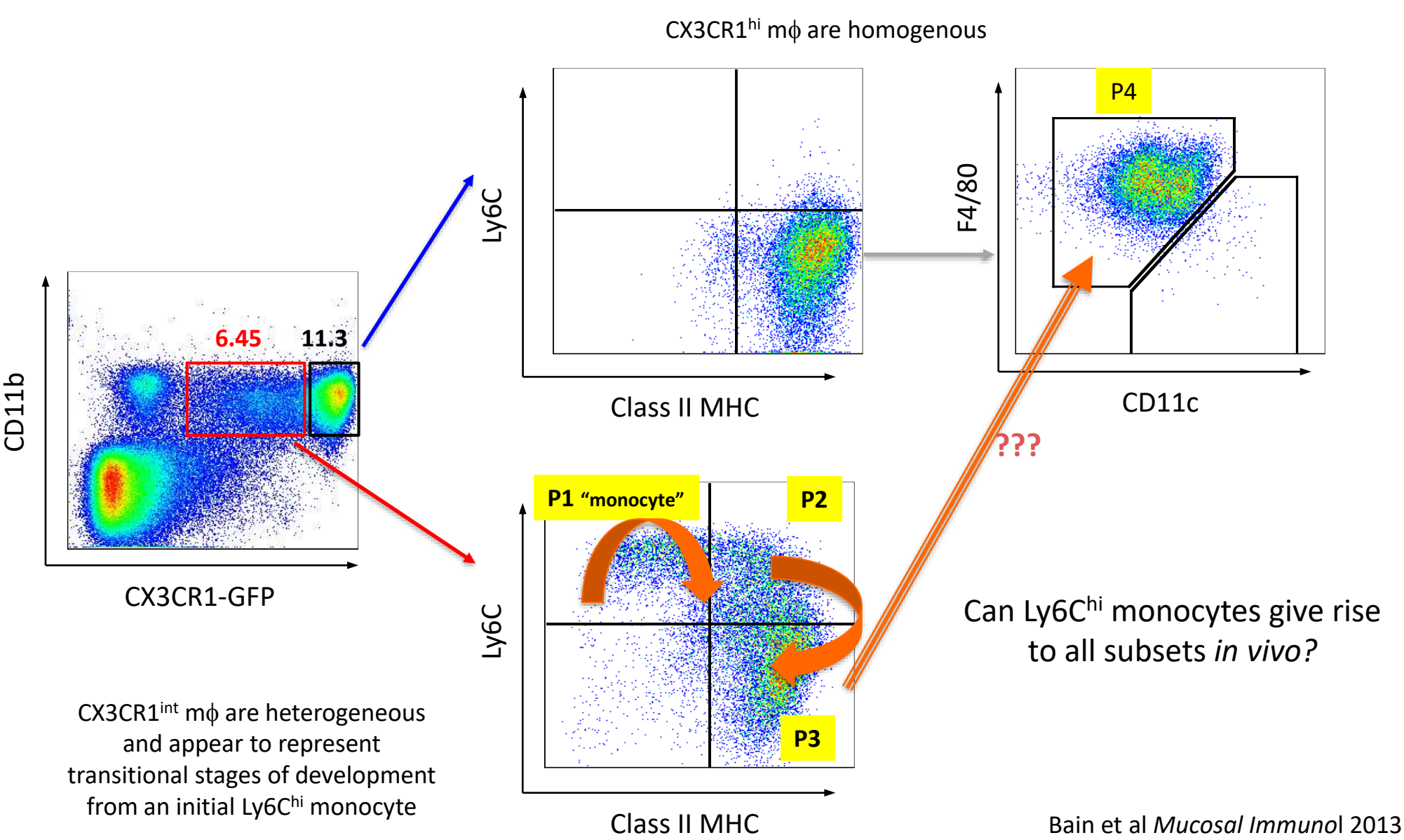


108 genes expressed ≥ x2 in colon vs all other tissues

Schridde, Bain et al *Mucosal Immunol* 2017

Resident Intestinal Macrophages are Derived from Local Differentiation of Monocytes

Monocyte-Macrophage "Waterfall" in Normal Colon

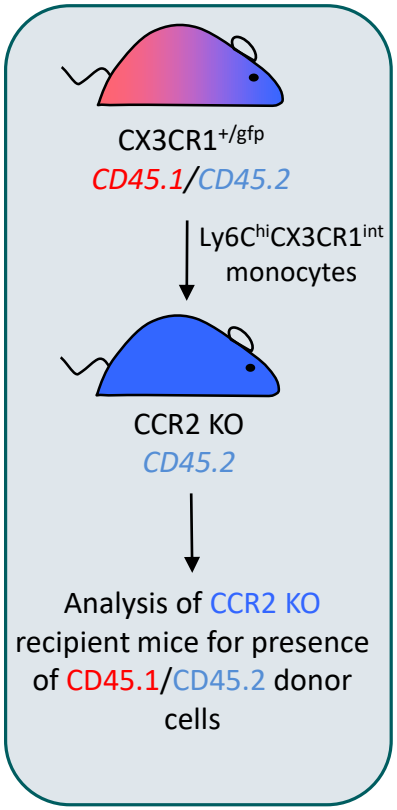


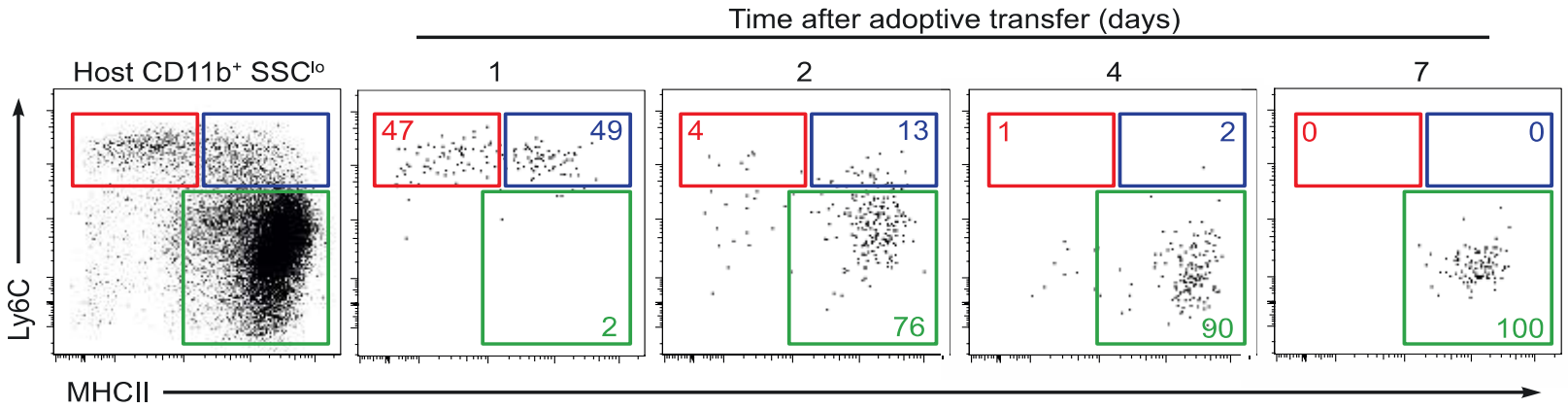
CX3CR1^{int} mϕ are heterogeneous and appear to represent transitional stages of development from an initial Ly6C^{hi} monocyte

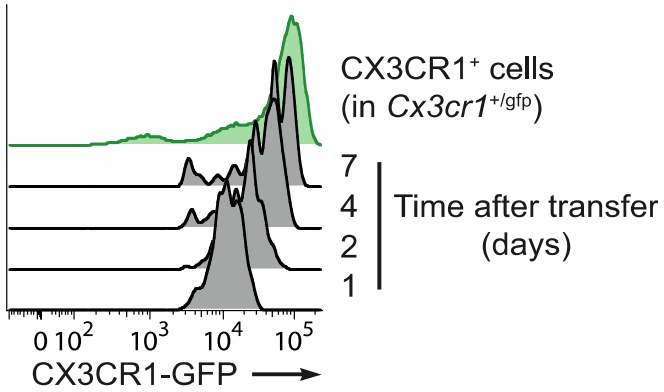
Can Ly6C^{hi} monocytes give rise to all subsets *in vivo?*

Bain et al *Mucosal Immunol* 2013

Classical Monocytes Generate Resident Intestinal Macrophages in Colon





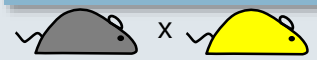


Bain et al *Mucosal Immunol* 2013

Resident Intestinal Macrophages are Derived from Local Differentiation of Monocytes

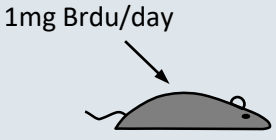
Tamoxifen-inducible CSF1R-reporter Mice

Flt3 Reporter Mice

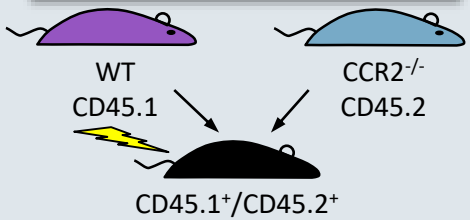


Flt3/CSF-1R-mer-cre x Rosa-LSL-eYFP

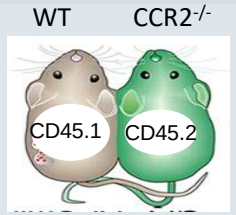
Long Term BrdU



Mixed BM Chimeric Mice



Parabiotic Mice



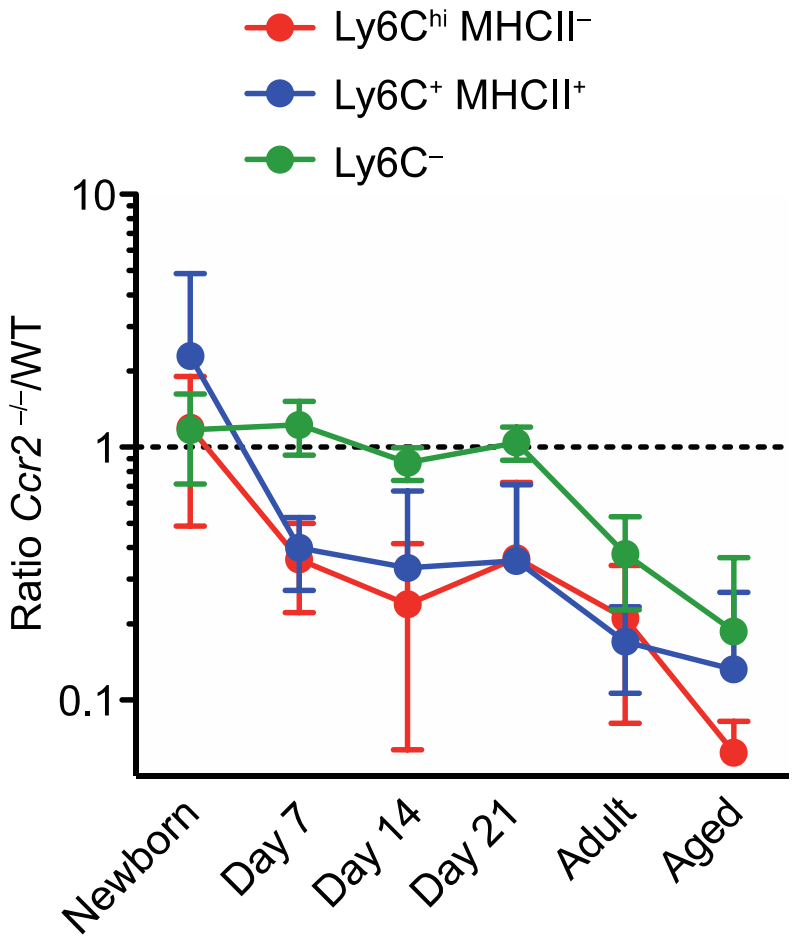
Analysis after 12wks

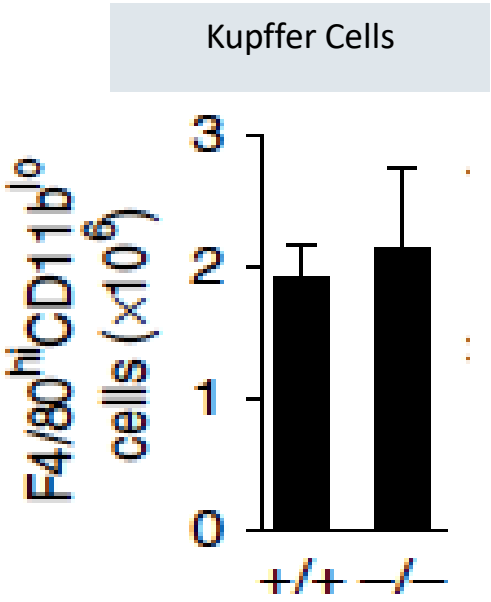
Cell Type	YS Derived	Proliferate *in situ*	Radio-sensitive	BM Derived in Chimeras	Exchange in Parabionts	CCR2 Dependent
Ly6C^{hi} Monocyte	✗	✗	✓	✓	✓	✓
Intestine	✗	✗	✓	✓	✓	✓
Kupffer Cell	✓	✓	✗	✗	✗	✗

Intestinal macrophages behave identically to Ly6C^{hi} monocytes in multiple models

Bain, Bravo Blas et al *Nature Immunology* 2014

Continuous Replenishment of Intestinal Macrophages

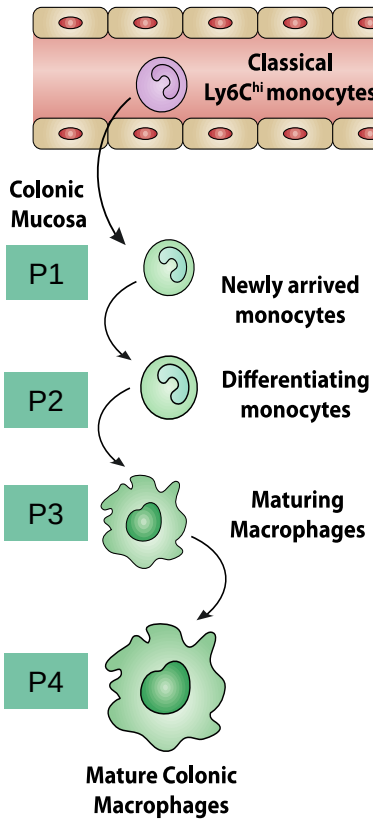




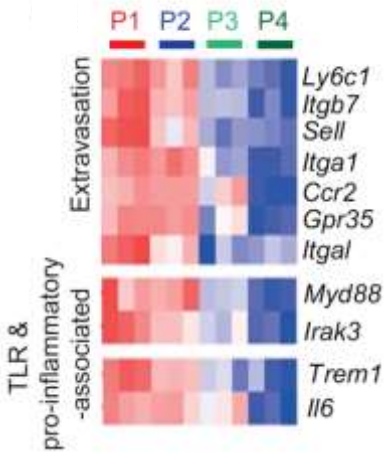
Progressive loss of intestinal macrophages in monocytopenic CCR2KO - unlike other tissues

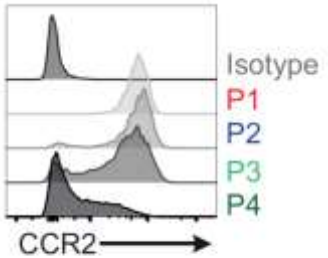
Bain, Bravo Blas et al *Nature Immunology* 2014

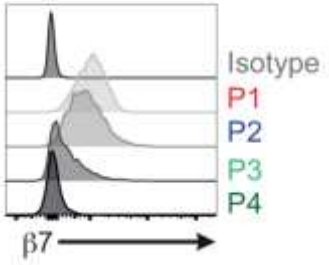
Local Differentiation of Colonic Macrophages

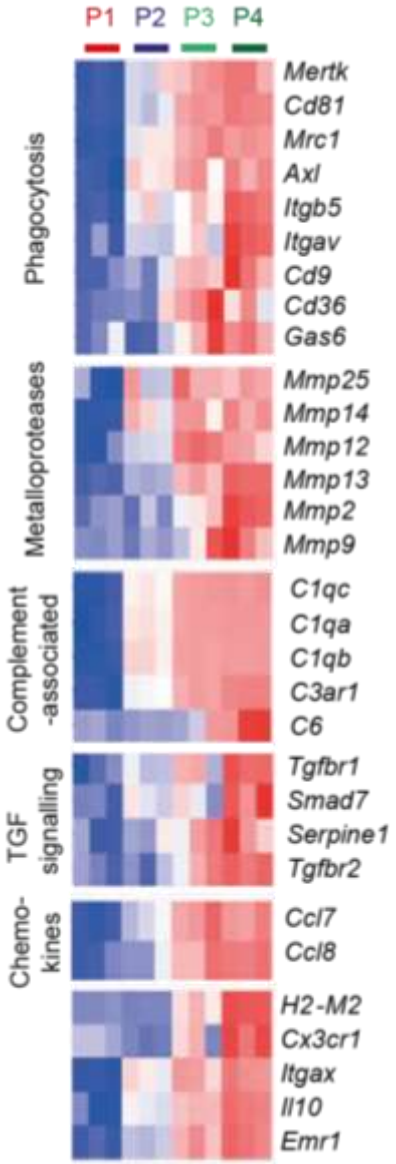


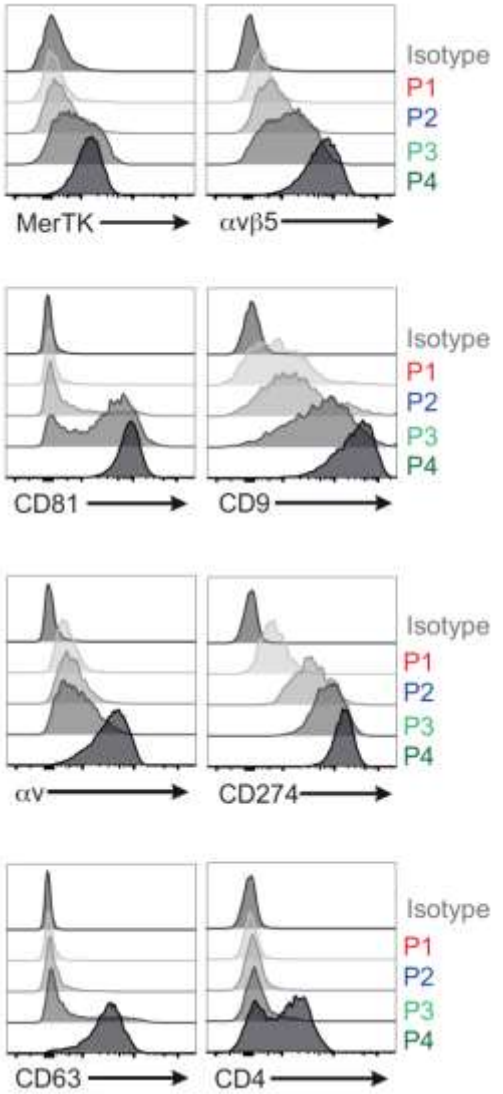
1004 genes change





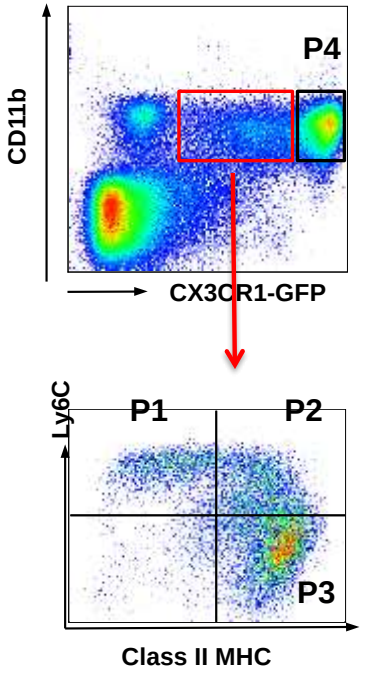


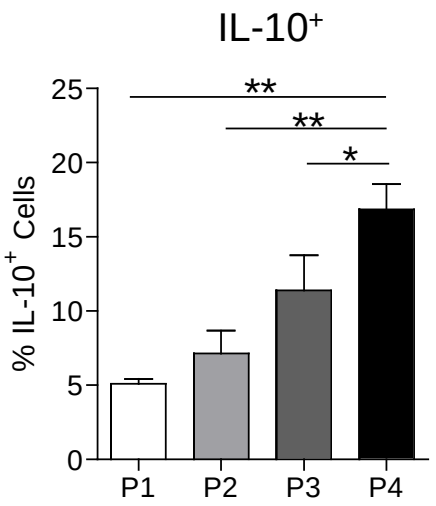




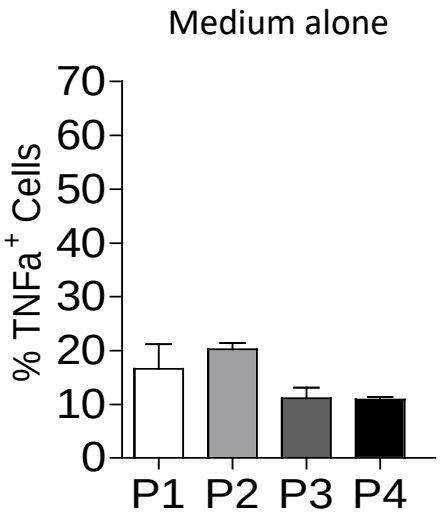
Schridde, Bain et al *Mucosal Immunol* 2017

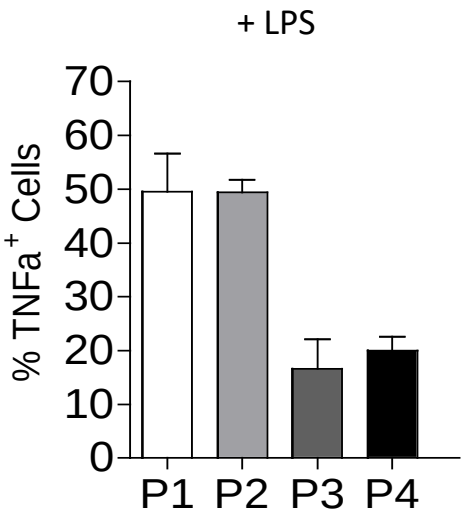
Monocytes Differentiate into Anti-Inflammatory Macrophages in Intestine





Desensitisation to TLR stimulation



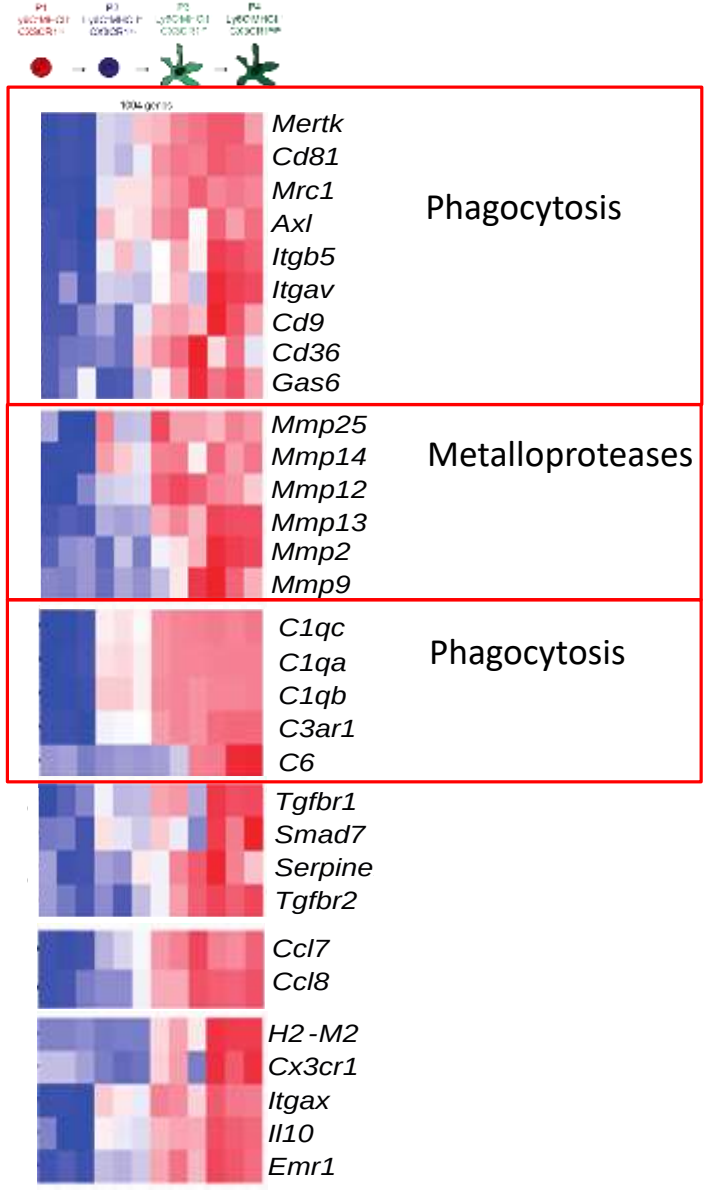


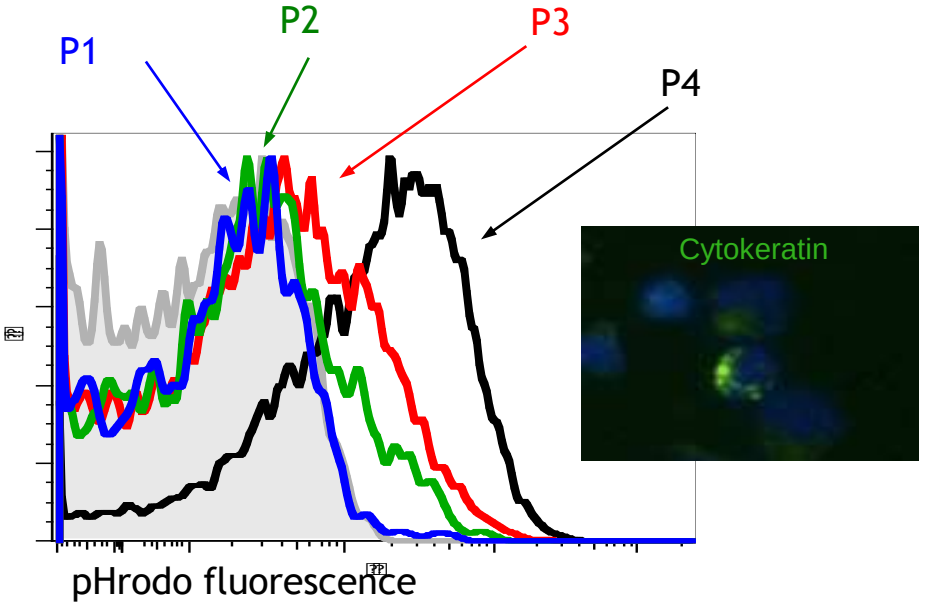
Progressive acquisition of IL10 production and loss of responsiveness to stimulation

Bain et al *Mucosal Immunol* 2013

Monocytes Differentiate into Homeostatic Scavengers in Intestine

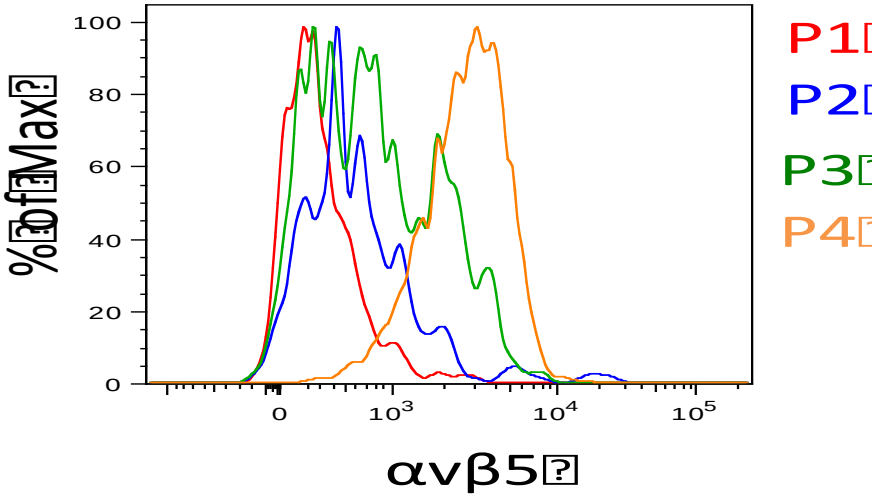
Bain et al *Mucosal Immunol.* 2013
Schridde, Bain et al *Mucosal Immunol* 2017

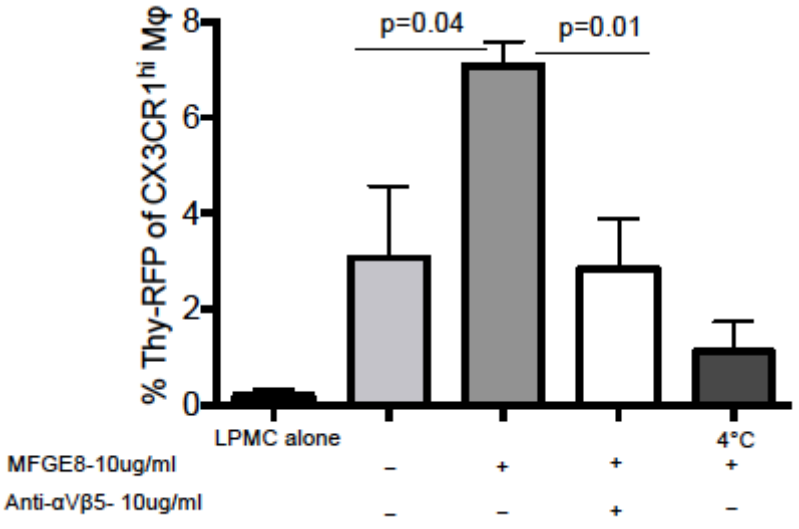




Progressive acquisition of apoptotic cell receptors, phagocytic activity and metalloproteases

$\alpha_v\beta_5$ Integrin Expression and Function in Intestinal Macrophages



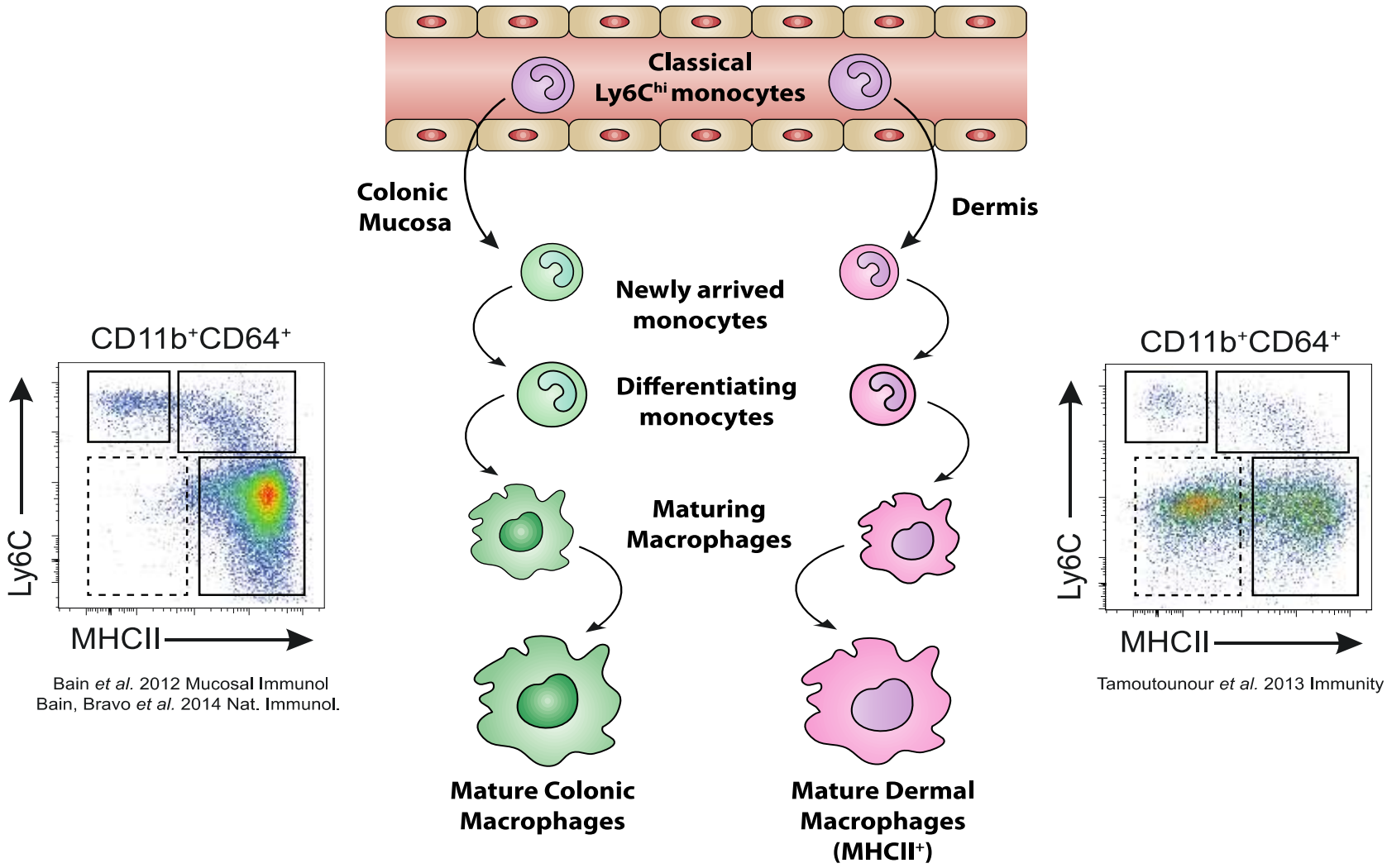


αvβ5 integrin and its ligand mediate uptake of apoptotic thymocytes by colonic macrophages

Kumawat et al *EJI* In Press

Differentiation of monocytes into resident intestinal macrophages is driven by local environment

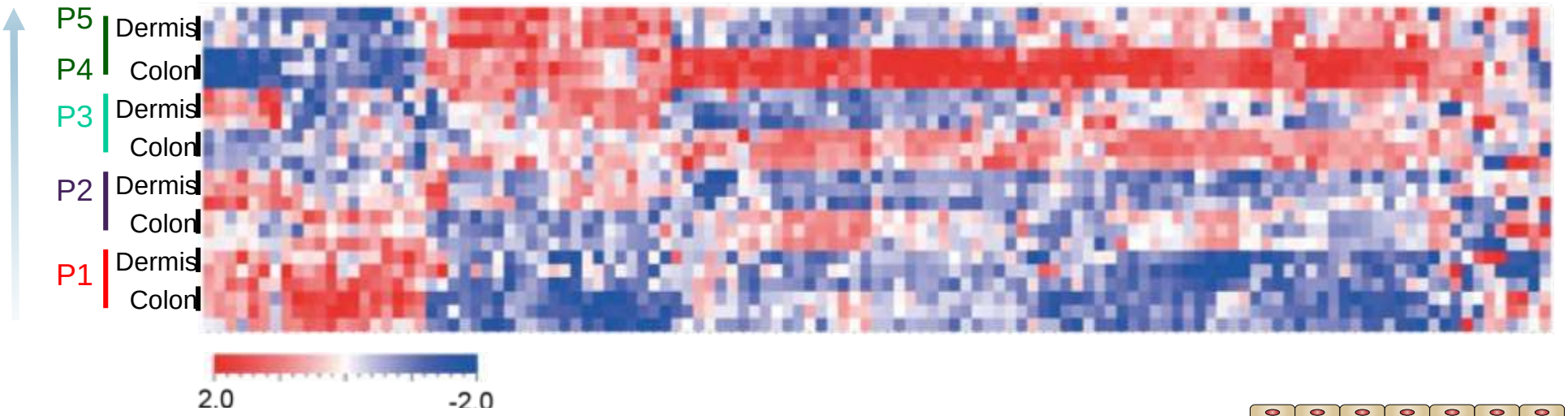
Role of Local Environment In Monocyte-Macrophage Differentiation

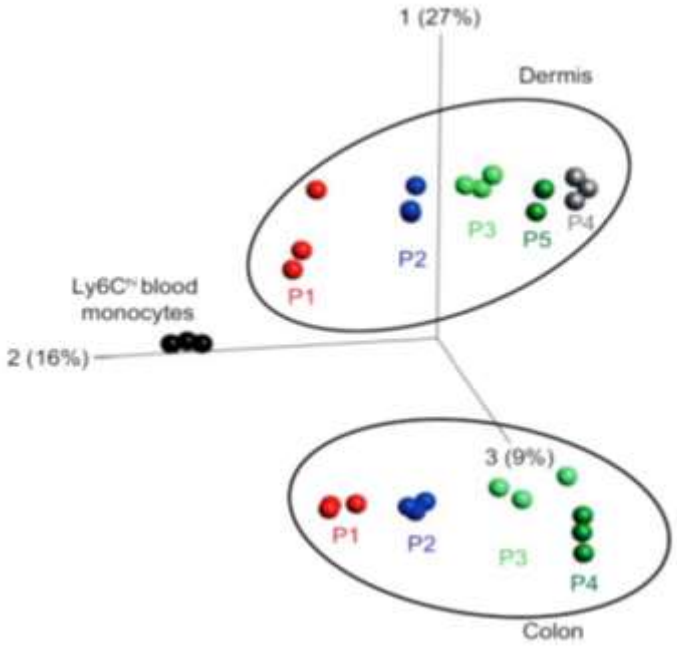


Colon and dermal mϕ share monocyte origin

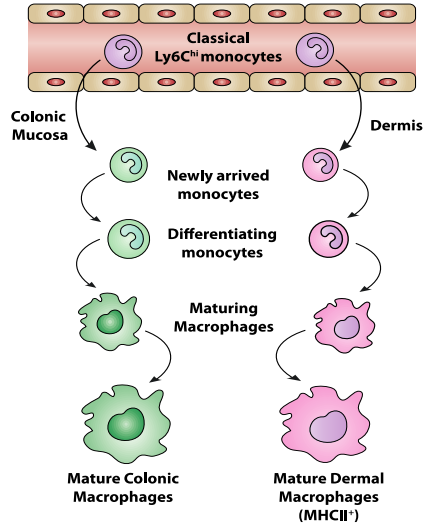
Schridde, Bain et al *Mucosal Immunol* 2017

Local Control of Intestinal Macrophage Development





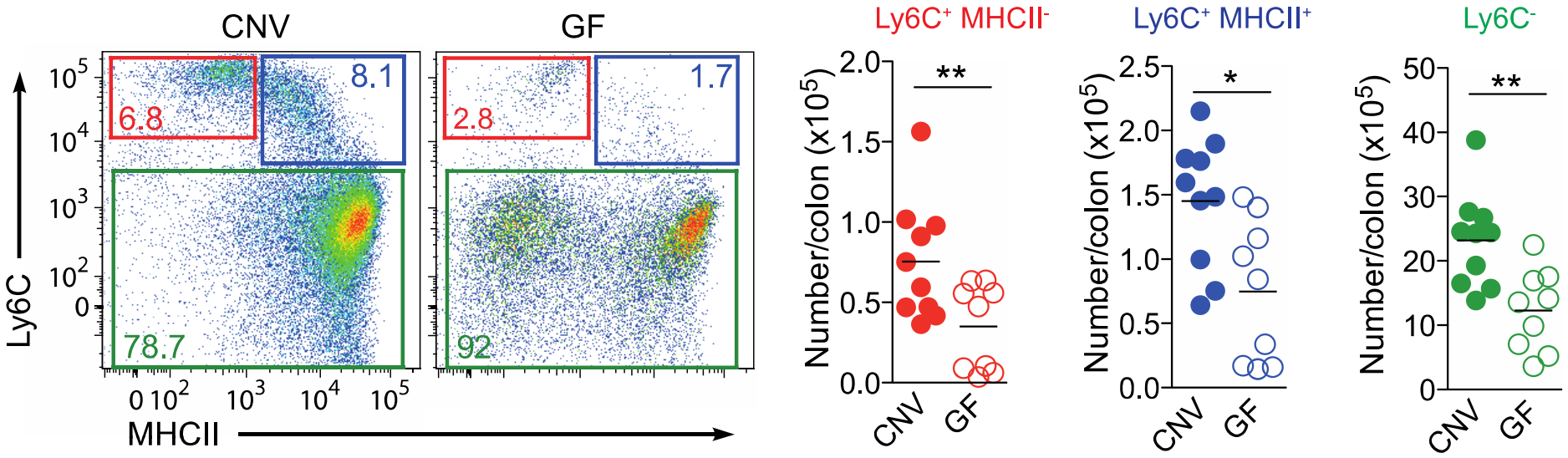
Progressive transcriptional separation between colon and dermal mϕ as they develop from monocytes



Schridde, Bain et al *Mucosal Immunol* 2017
Tamoutounour *Immunity* 2013

What drives local differentiation of monocytes into resident intestinal macrophages?

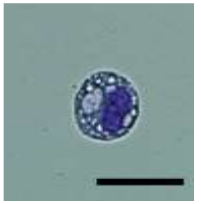
Intestinal Macrophage Development Requires Microbiota



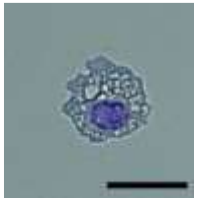
Monocyte and macrophage numbers are reduced in germ free colon – but still present

Bain, Bravo Blas et al *Nature Immunology* 2014

Intestinal Macrophages in the Neonate

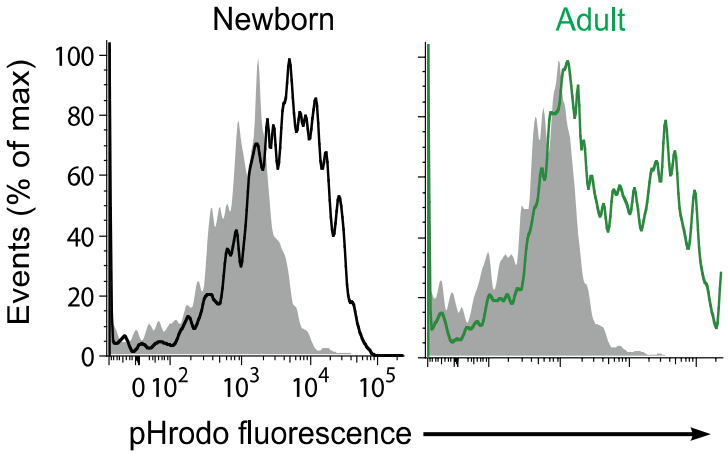


Adult

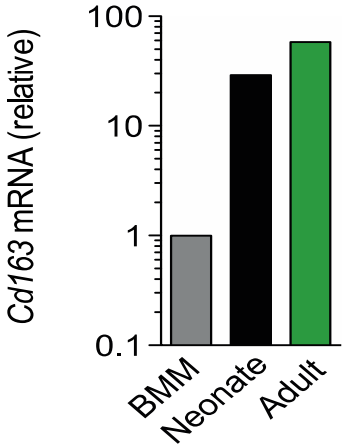


Newborn

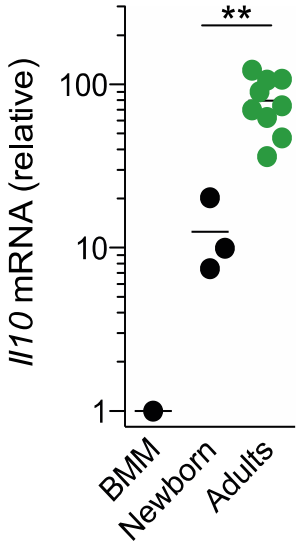
Phagocytic Activity



CD163



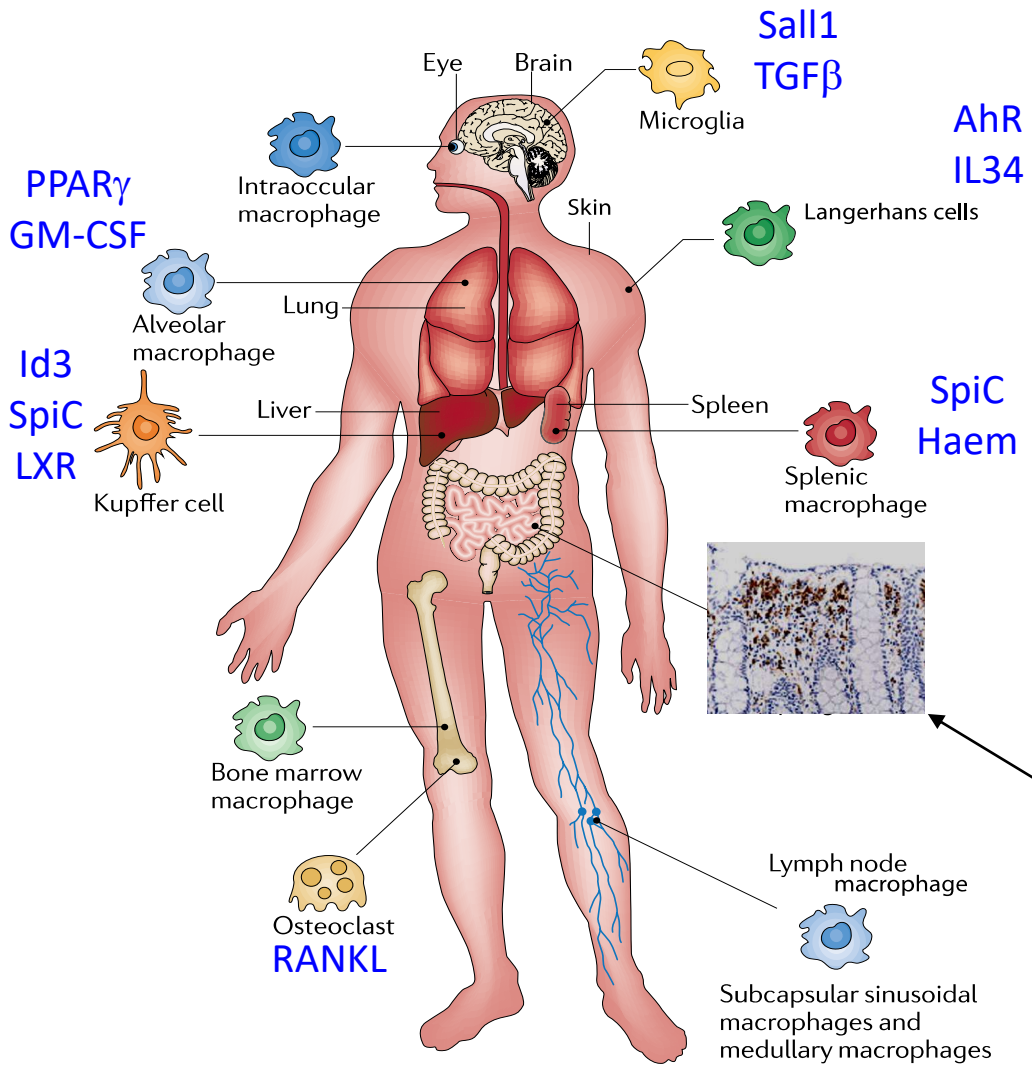
IL10



Mϕ are present in newborn colon – same phenotype and morphology as in adult

Bain, Bravo Blas et al Nature Immunology 2014

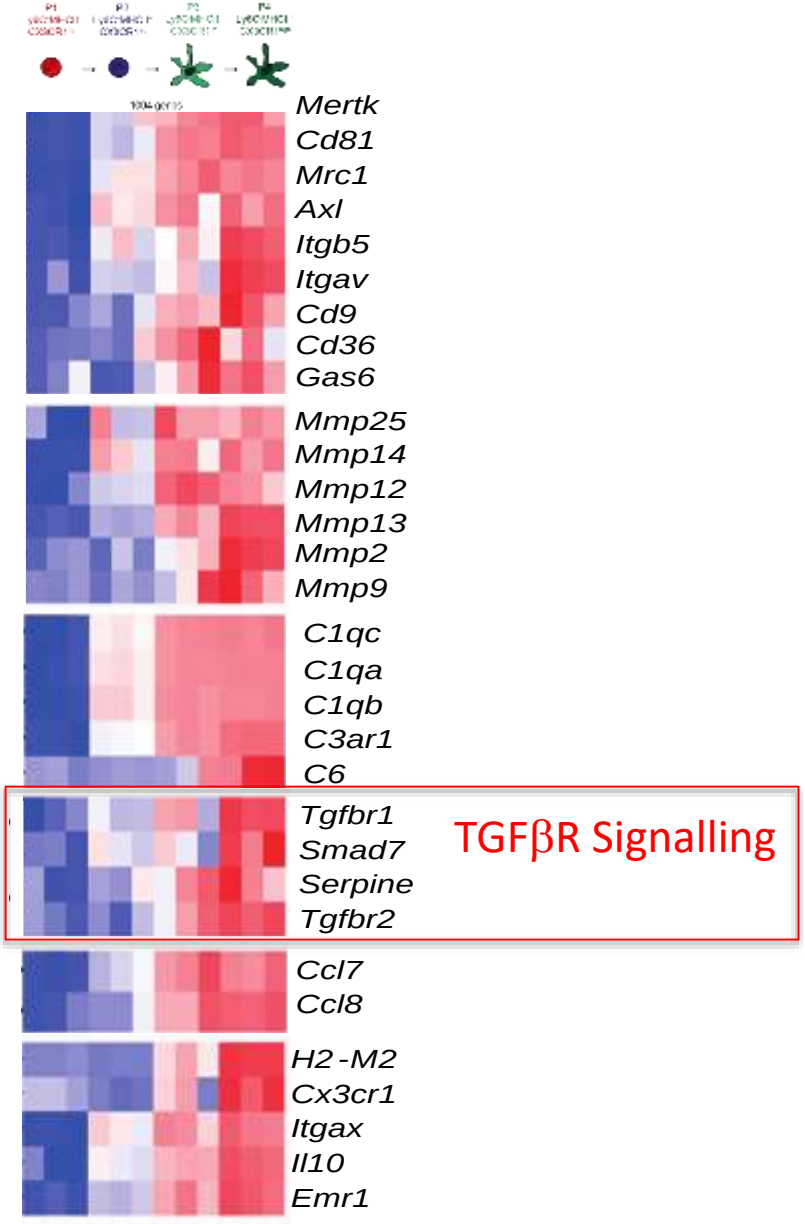
Ontogeny of Tissue Macrophages

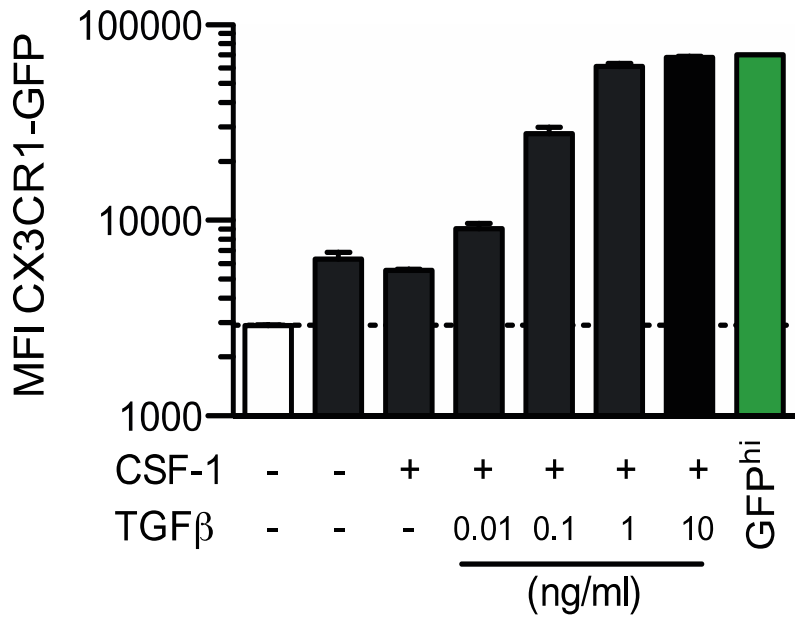


- Resident macrophage functions tailored to location
- Tissue rather than origin specifies behaviour
- Reflects specification early in development via TFs, growth factors

What specifies intestinal macrophage development?

A Role for TGFβ in Intestinal Macrophage Development?

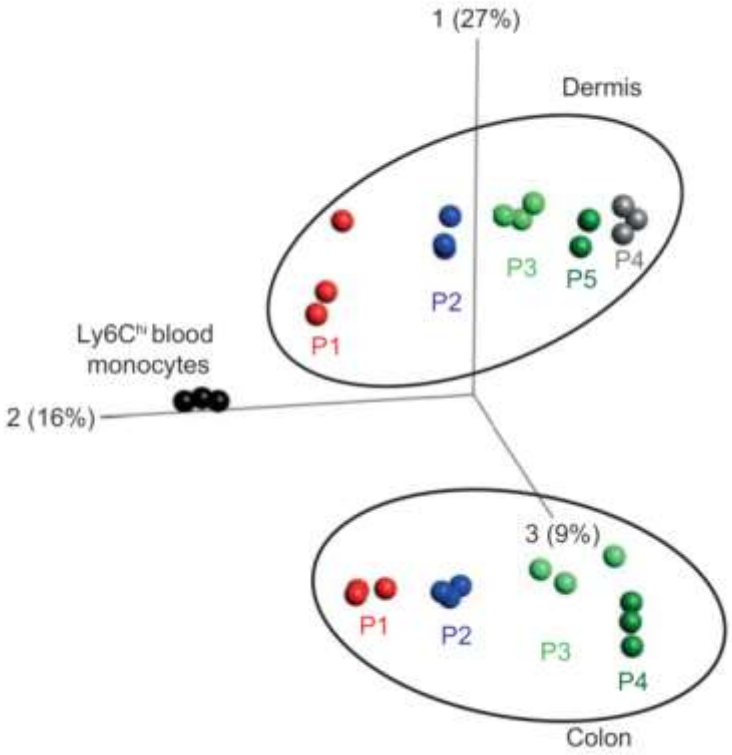


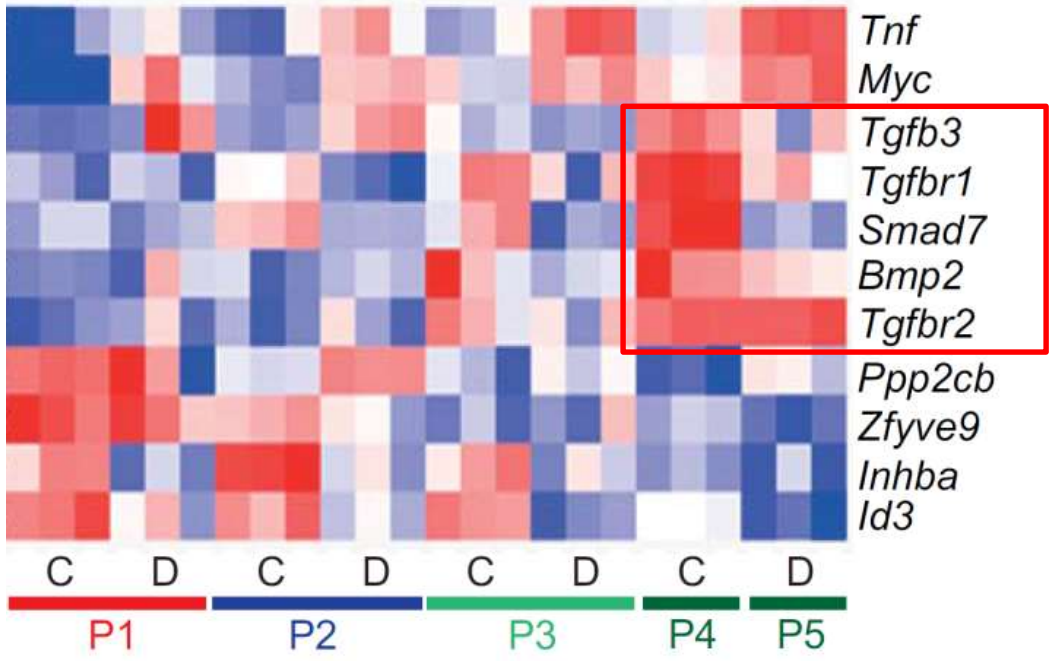


TGFβ induces expression of CX3CR1 on monocytes

Schridde, Bain et al *Mucosal Immunol* 2017

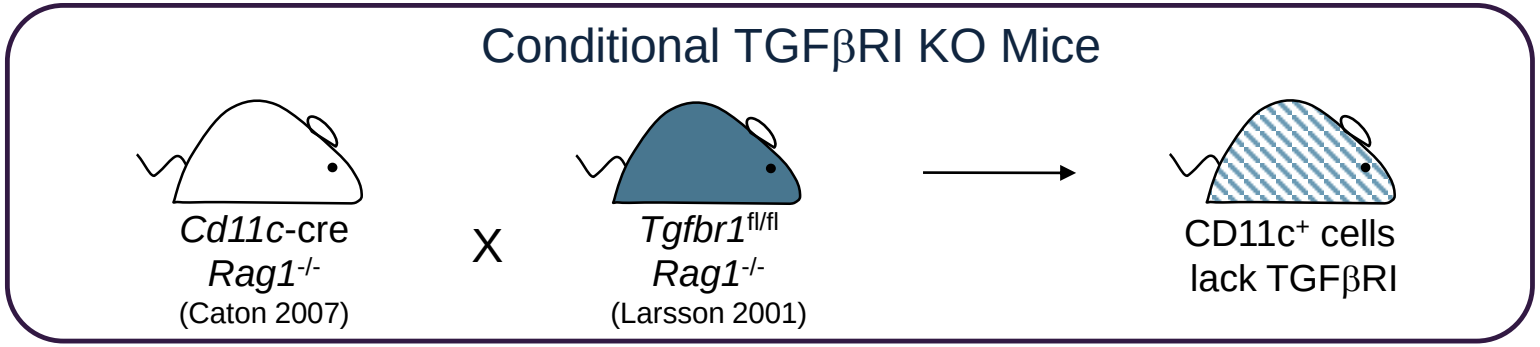
TGFβR Signalling Defines Distinctive Differentiation of Macrophages in Colon and Dermis

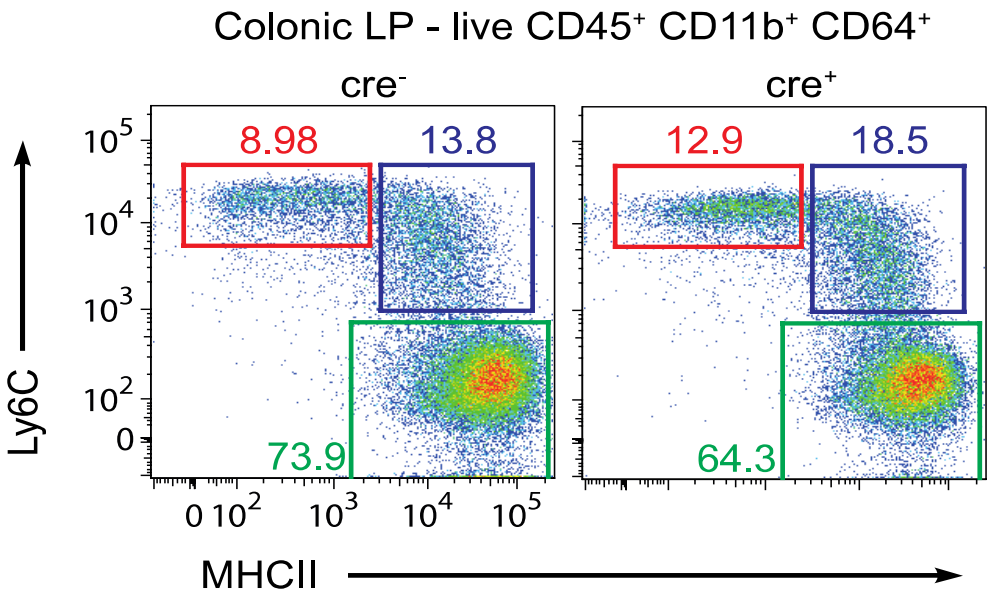


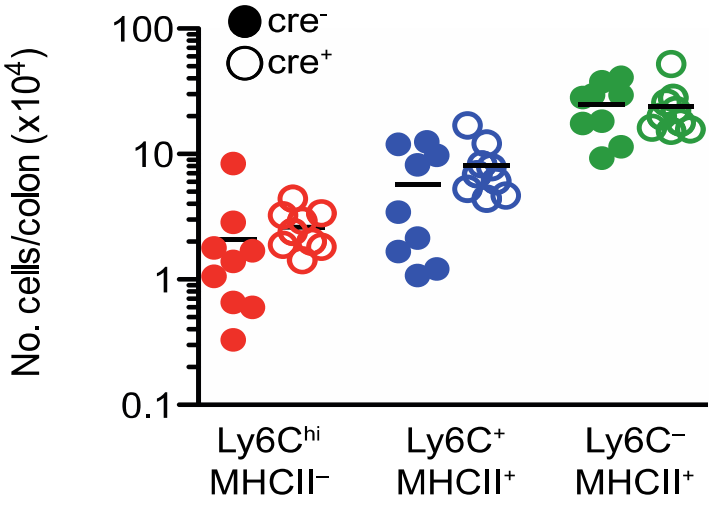


Schridde, Bain et al *Mucosal Immunol* 2017

TGFβ Does not Drive Monocyte Recruitment to Colon

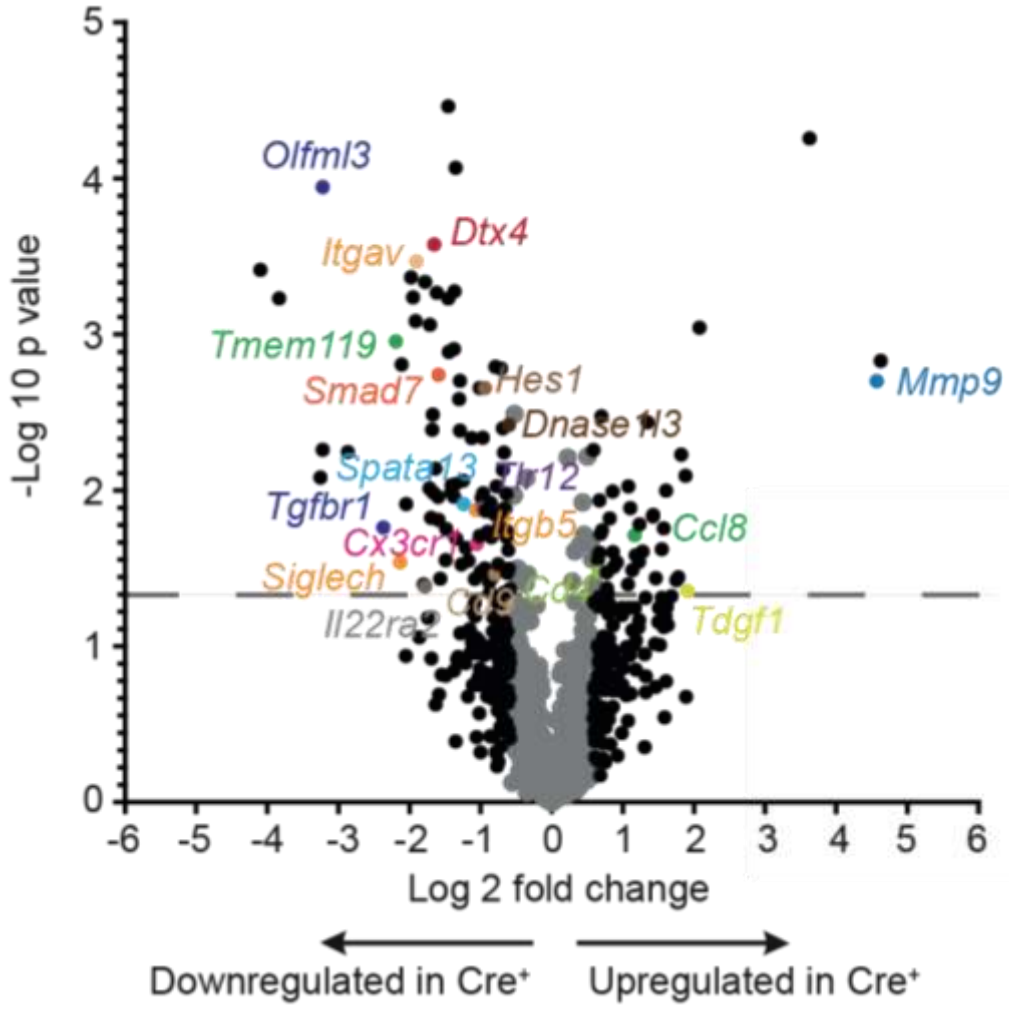


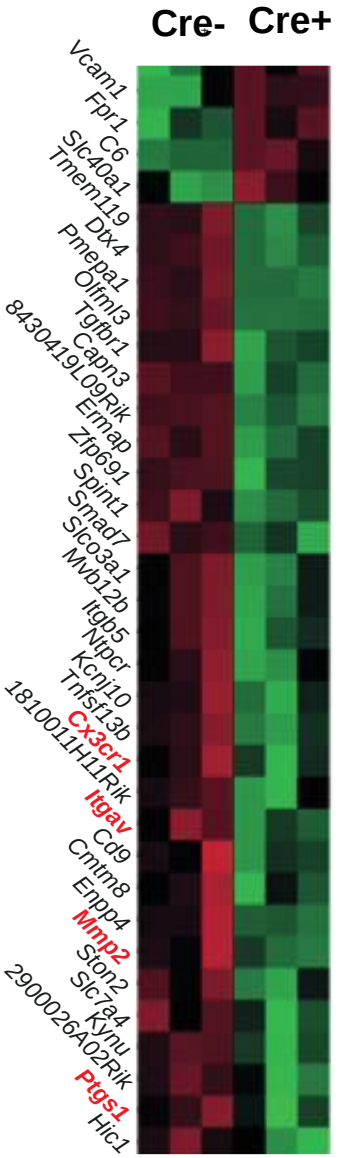


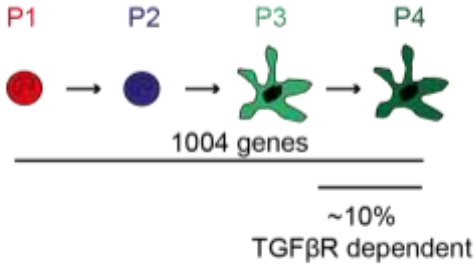


Schridde, Bain et al *Mucosal Immunol* 2017

TGFβR Signalling Regulates Colonic Macrophage Differentiation

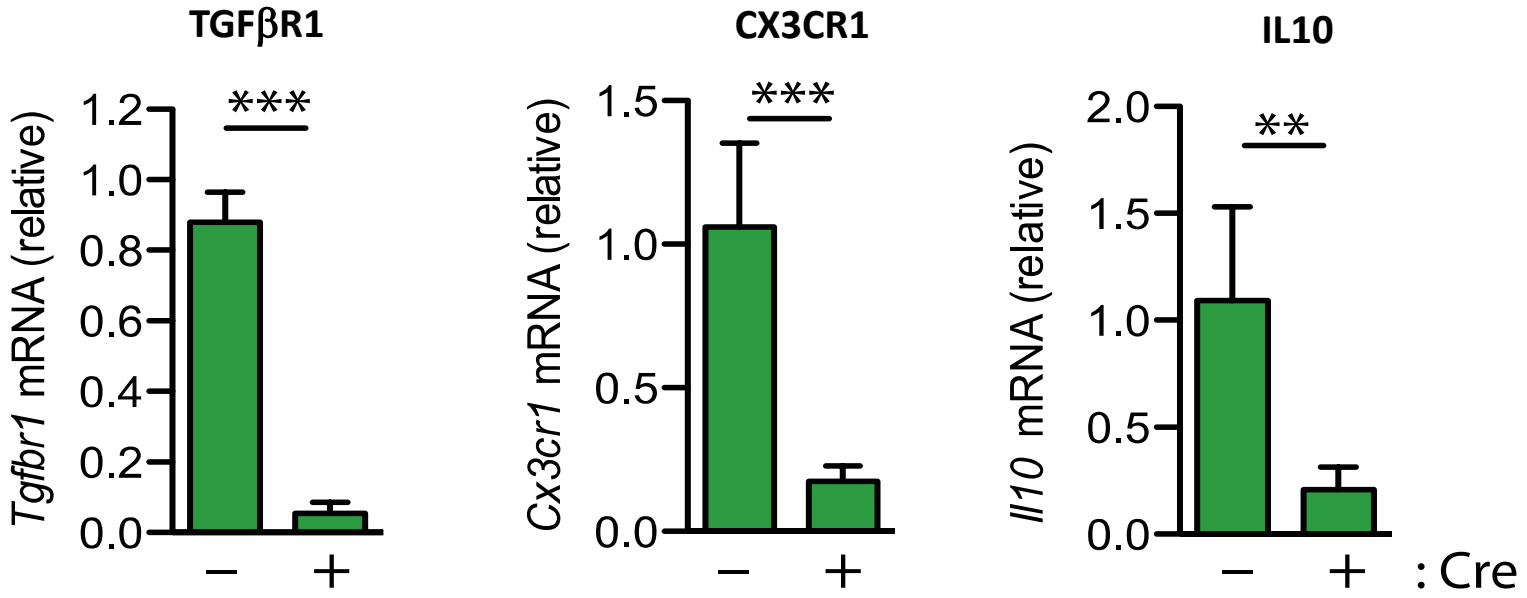


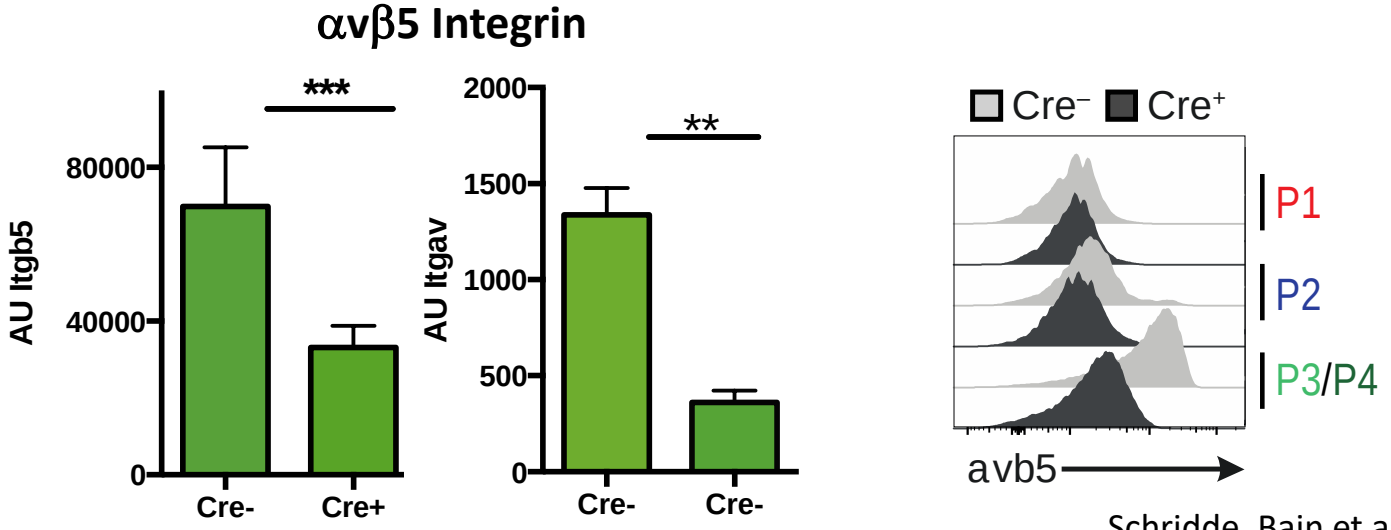




Schridde, Bain et al *Mucosal Immunol* 2017

TGFβR Signalling Regulates Colonic Macrophage Differentiation

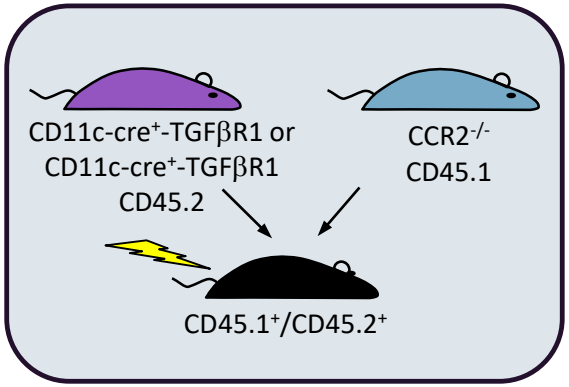




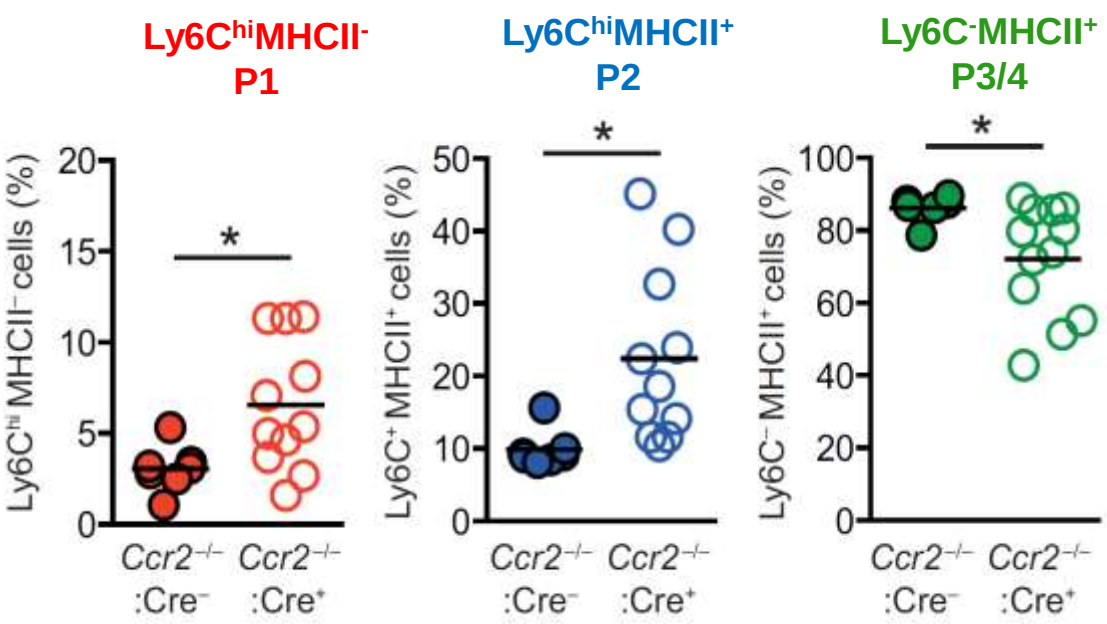
Schridde, Bain et al *Mucosal Immunol* 2017

Loss of TGFβR Signalling in Macrophages Disrupts Monocyte Waterfall

Conditional KO of TGFβR on Mϕ



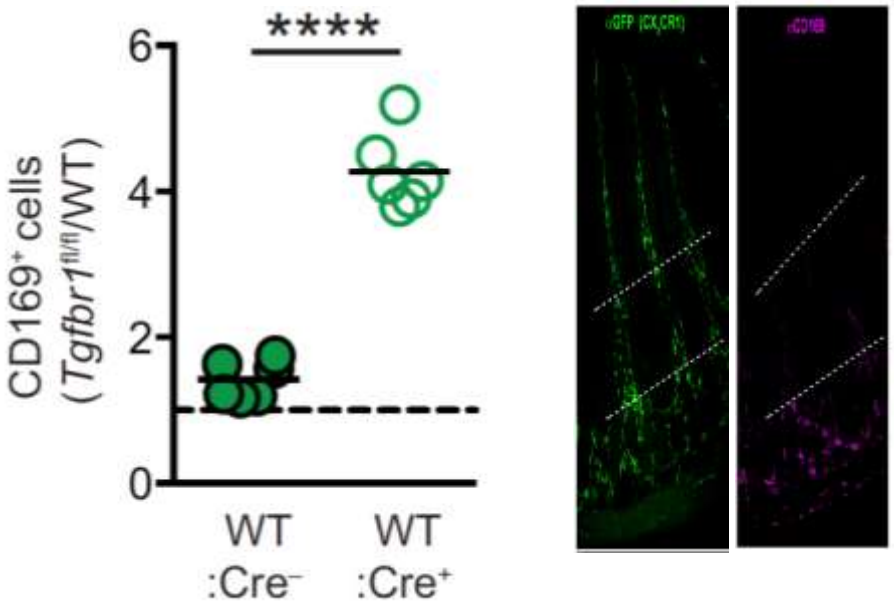
TGFβR$^{fl/fl}$ Derived Populations



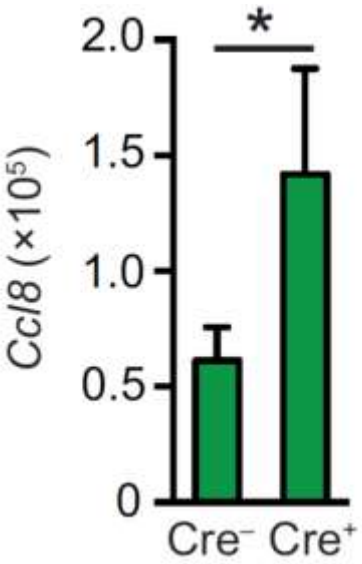
Increased numbers of monocytes and pro-inflammatory early stage macrophages

Schridde, Bain et al *Mucosal Immunol* 2017

TGFβR Signalling in Macrophages Regulates Intestinal Homeostasis



Increased CD169⁺ macrophages in colon in absence of TGFβR signalling



Increased expression of CCL8 by TGFβR deficient macrophages

ARTICLE

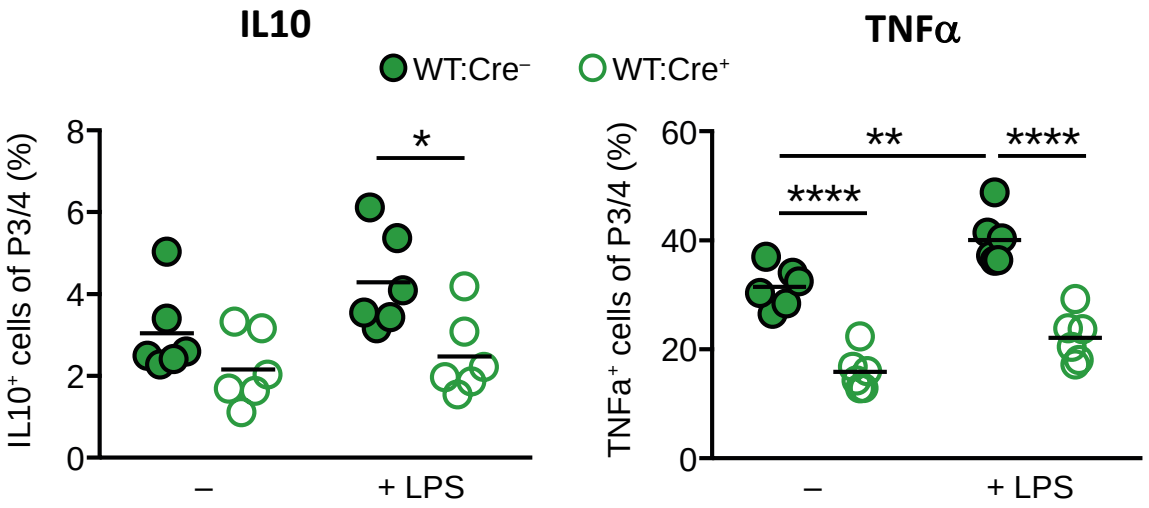
Received 3 Feb 2015 | Accepted 12 Jun 2015 | Published 21 Jul 2015 DOI: 10.1038/ncomms8802 OPEN

Intestinal CD169$^+$ macrophages initiate mucosal inflammation by secreting CCL8 that recruits inflammatory monocytes

Kenichi Asano[1,2], Naomichi Takahashi[1], Mikiko Ushiki[1], Misa Monya[1], Fumiaki Aihara[1], Erika Kuboki[1], Shigetaka Moriyama[1], Mayumi Iida[1], Hiroshi Kitamura[3], Chun-Hong Qiu[4], Takashi Watanabe[5] & Masato Tanaka[1]

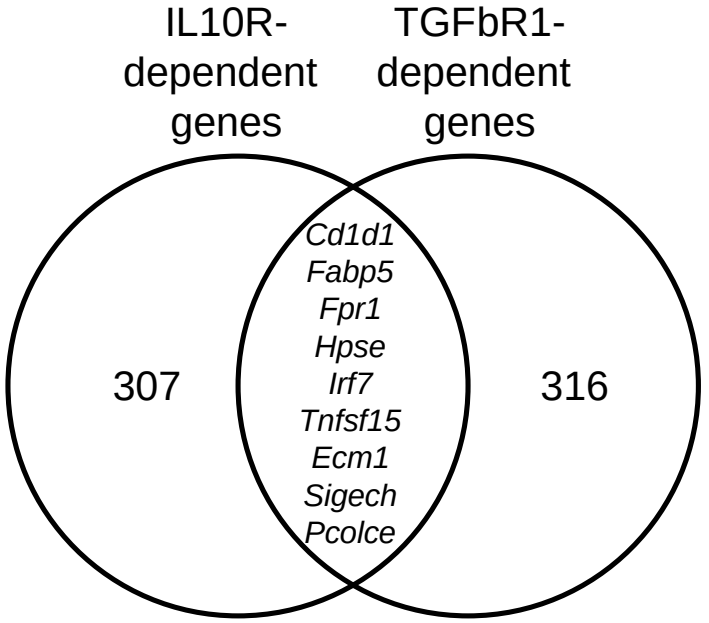
Schridde, Bain et al *Mucosal Immunol* 2017

TGFβ and IL10 Signalling Regulate Intestinal Macrophages by Distinct Pathways



Reduced production of IL10 and TNFα by colon mϕ in mice lacking TGFβR signalling, but no increased responsiveness to LPS

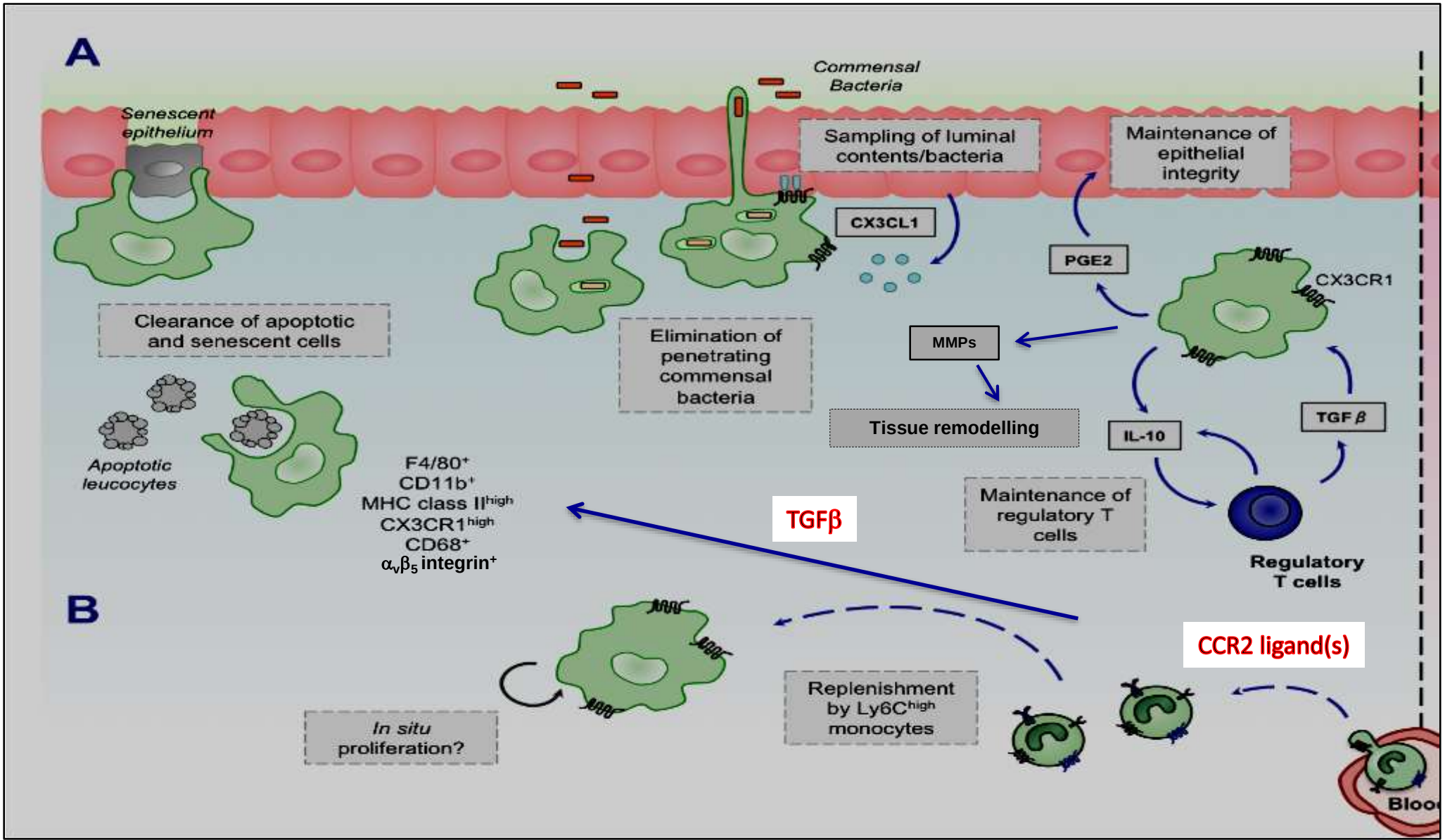
Macrophage-Restricted Interleukin-10 Receptor Deficiency, but Not IL-10 Deficiency, Causes Severe Spontaneous Colitis



Little overlap in effect of deleting IL10R and TGFβR in colon macrophages

Schridde, Bain et al *Mucosal Immunol* 2017

Development and Functions of Intestinal Macrophages



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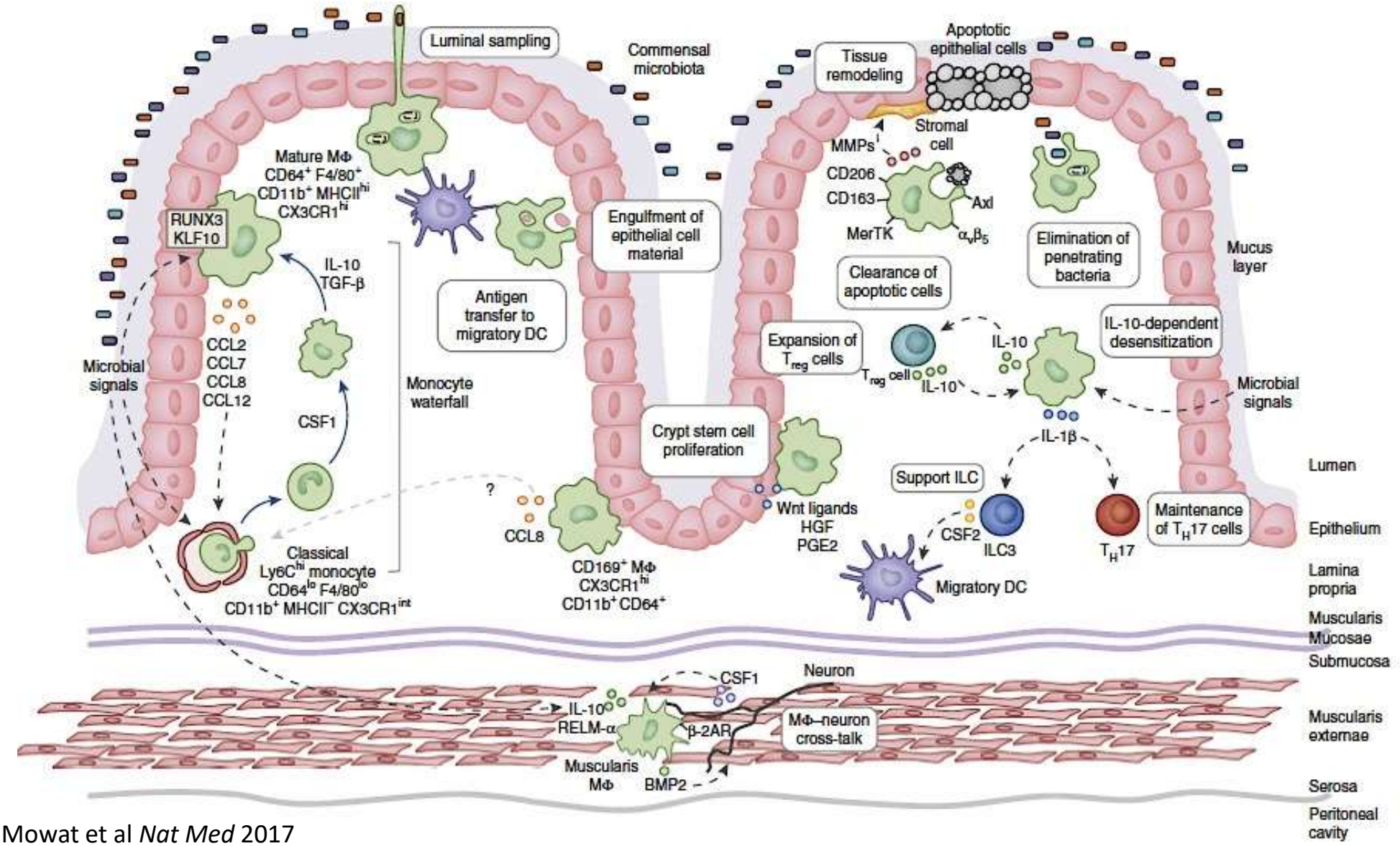
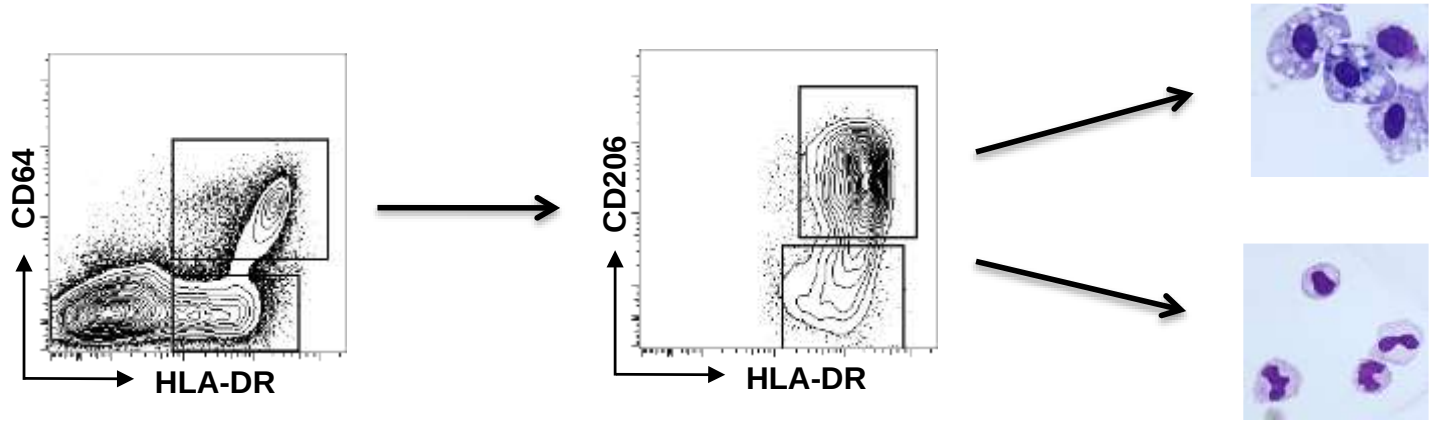
Differentiation and Functions of Intestinal Macrophages

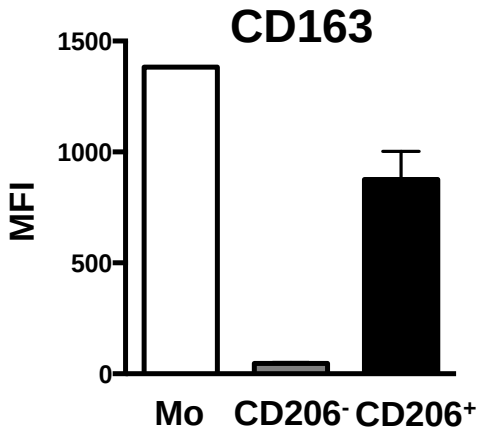


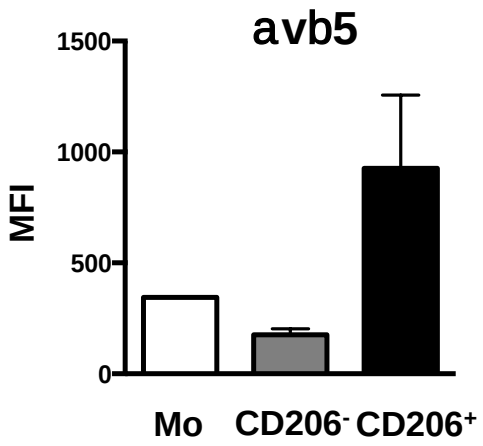
Mowat et al *Nat Med* 2017

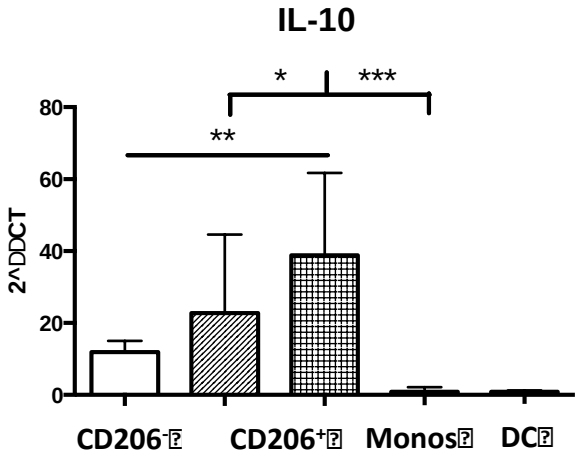
Human Colonic Macrophages

Pamela Wright, Simon Milling



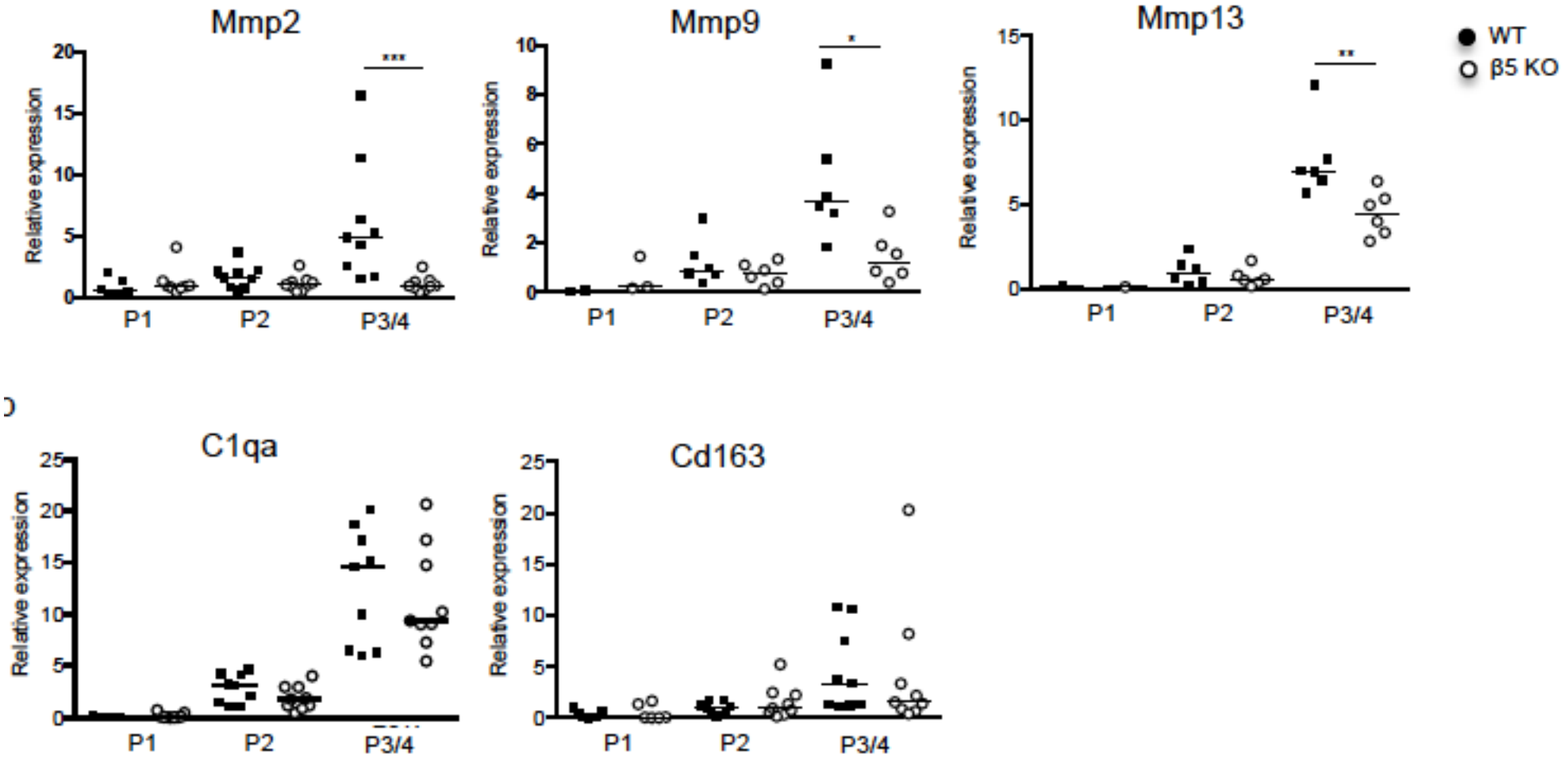






Macrophages from normal human colon show phenotypic and functional heterogeneity

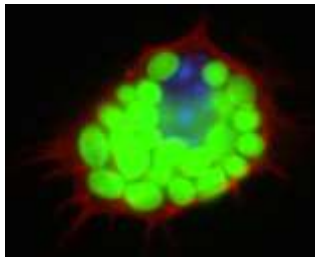
Regulation of Intestinal Macrophages by $\alpha_v\beta_5$ Integrin



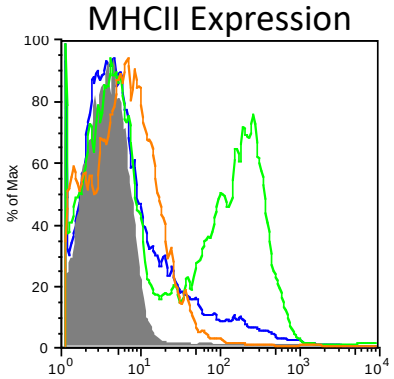
Steady State Intestinal Macrophages

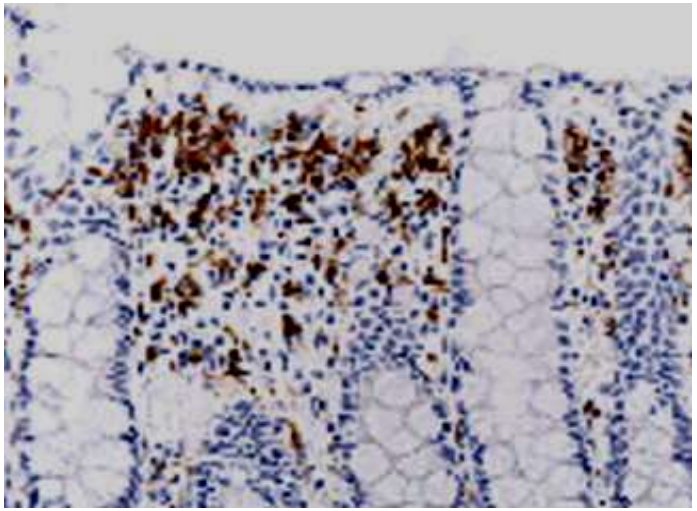
- Resting mϕ partially activated - constitutive production of TNFα balanced by IL10
- Embryonically derived, self renewing mϕ are present early in life, but are overwhelmed at weaning by arrival of classical monocytes
- Continuous replenishment by CCR2 and microbiota dependent monocytes required throughout adult life – $t_{1/2}$ ~ 6-8 weeks?
- Monocytes differentiate *in situ* into anti-inflammatory scavenger mϕ – TGFβ dependent
- Transcriptionally unique - partial activation may allow homeostatic functions
  - Clearance of apopotic epithelial cells
  - Production of epithelial trophic factors
  - Tissue remodelling
  - Clearance of invading commensals
  - IL10 dependent maintenance of Treg

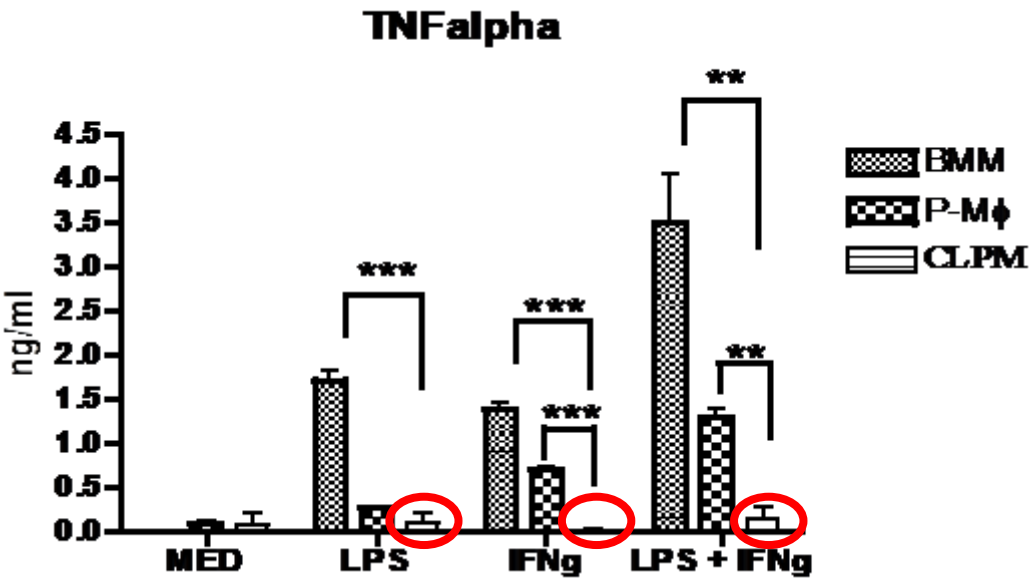
Intestinal Macrophages



Smith et al J Immun 161: 2651 (2001)



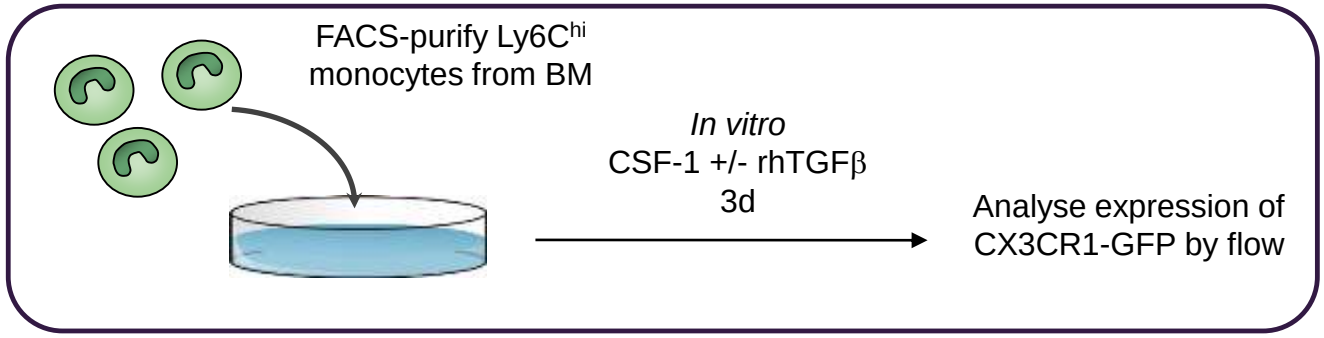


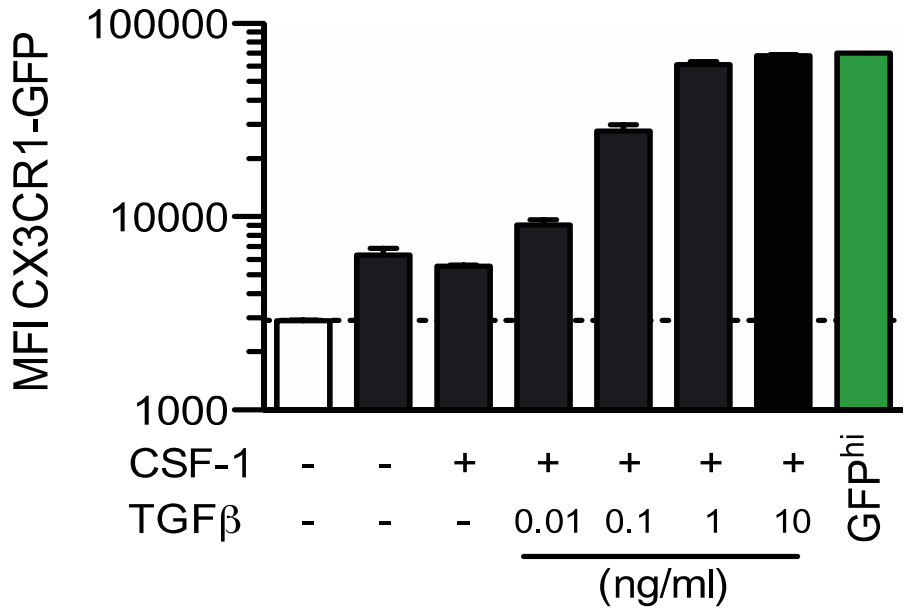


Active appearance, but unresponsive to conventional stimuli

Platt et al J Immunol. 2012

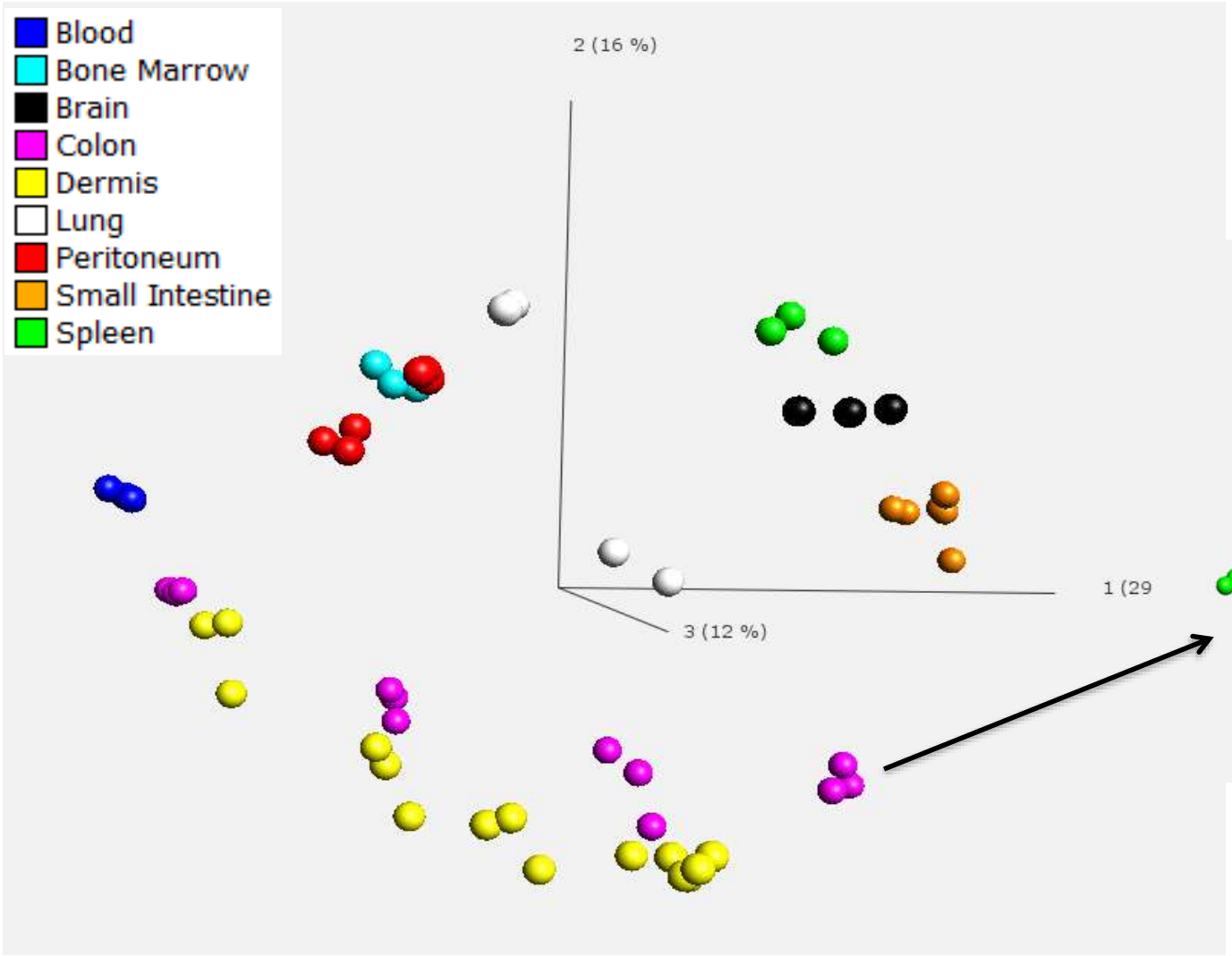
Upregulation of CX3CR1 Expression on Monocytes by TGFβ

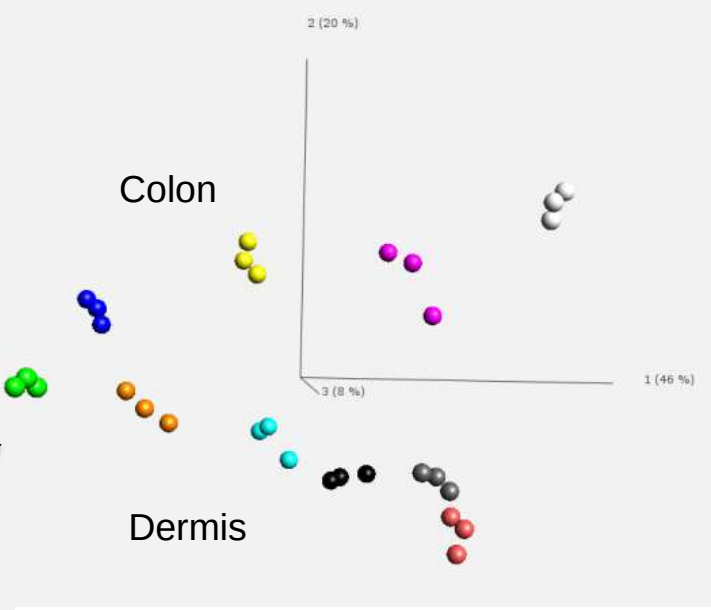




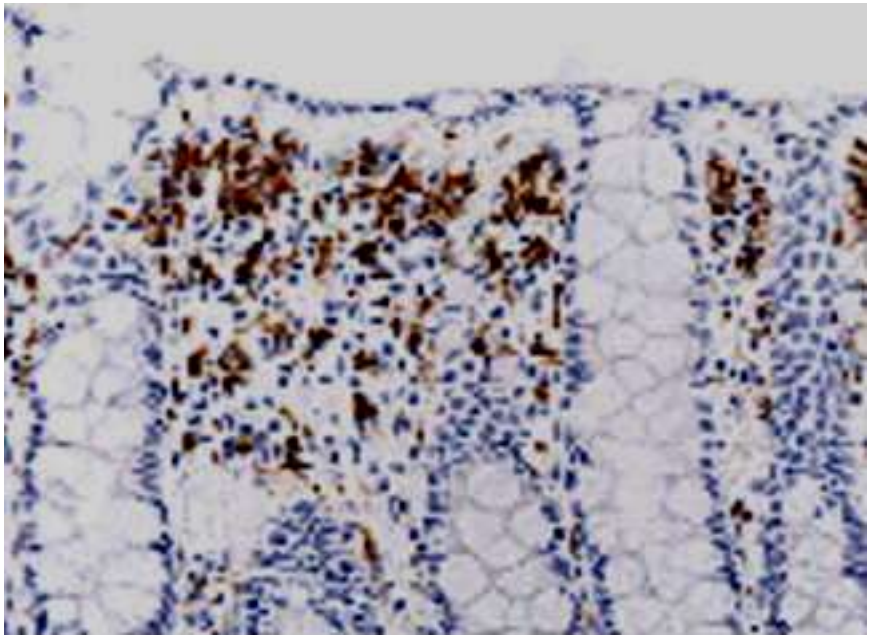
Bain Unpublished

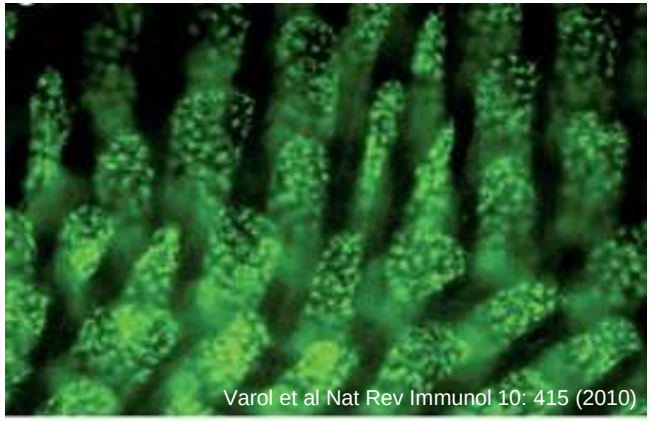
Relationships of Colonic Macrophages





Intestinal Macrophages

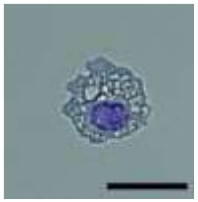




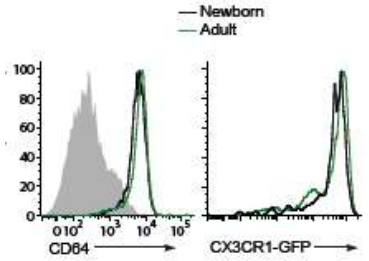
Varol et al Nat Rev Immunol 10: 415 (2010)

- Enormous pool of macrophages in normal mucosa
- In close contact with bacteria
- Highly dynamic tissue – constant cell renewal and remodelling
- Appear activated
- Also major effectors of inflammation
- Often assumed distinct mϕ pools involved in health and inflammation
- What is nature of precursors?
- Mϕ functions in steady state?

Intestinal Mϕ are Derived from Conventional Haematopoiesis



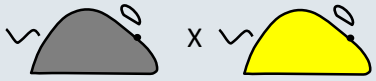
Newborn



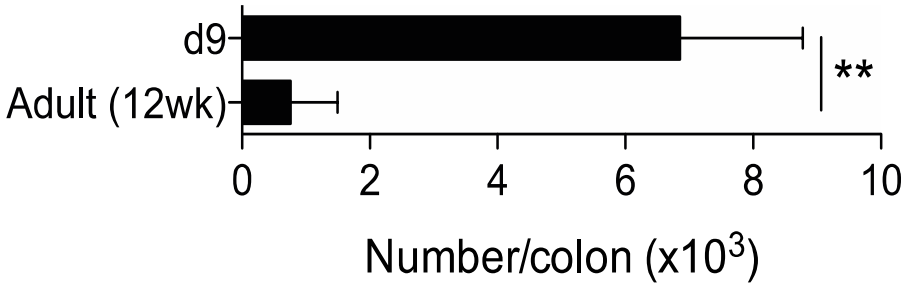
Flt3 Reporter Mice

Flt3-cre x Rosa-LSL-eYFP → Irreversible labelling of Flt3⁺ cells

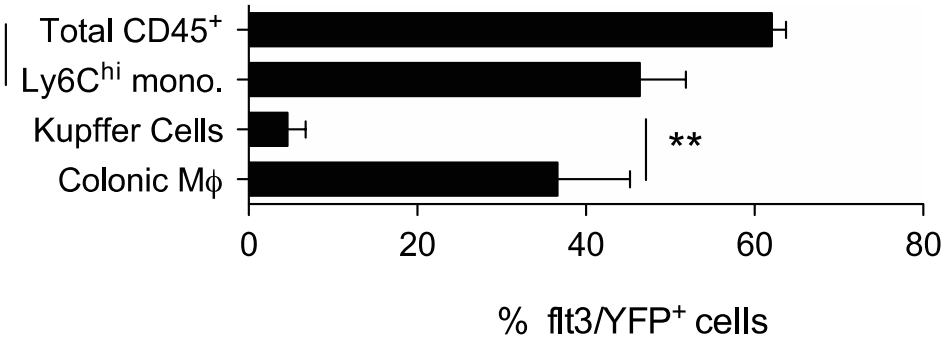
Tamoxifen-inducible CSF1R-reporter Mice



CSF1R-*i*cre x Rosa-eYFP → Pulse pregnant females with tamoxifen at E8.5 → Irreversible labelling of CSF1R⁺ cells present at E8.5



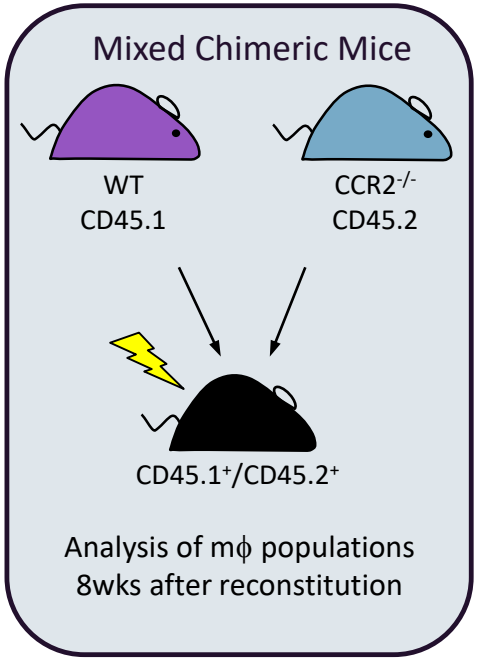
Yolk sac derived mϕ present in newborn intestine, but do not persist into adulthood

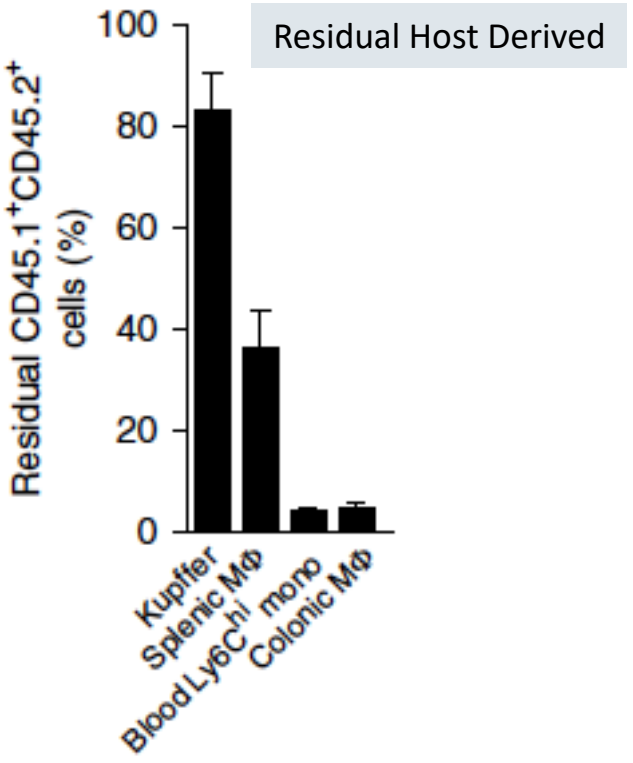


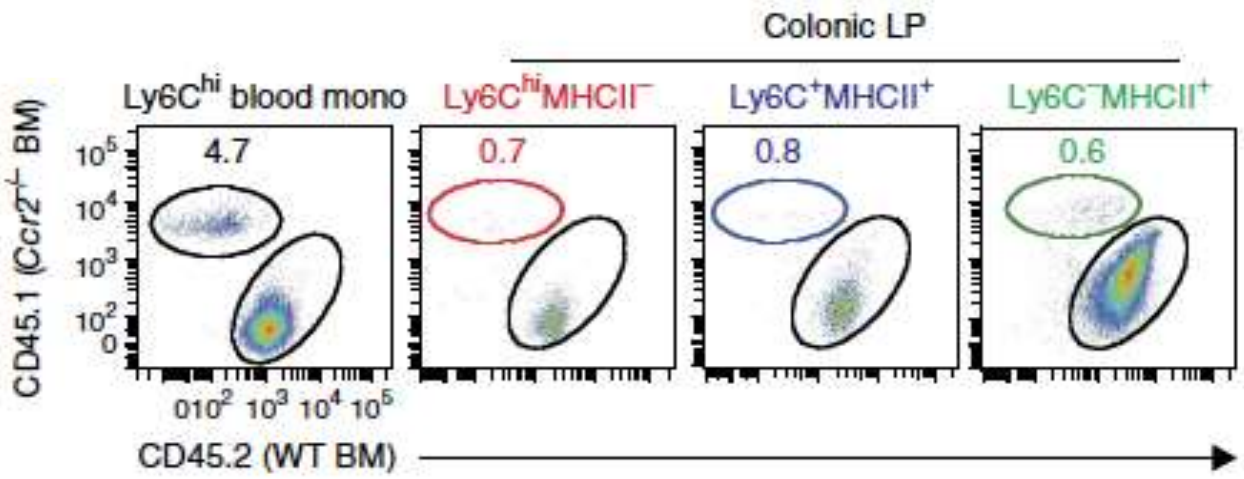
Intestinal mϕ derive from flt3 expressing haematopoietic precursors

Bain, Bravo Blas et al Nature Immunology 2014

CCR2 Dependence of Steady State Intestinal Mϕ



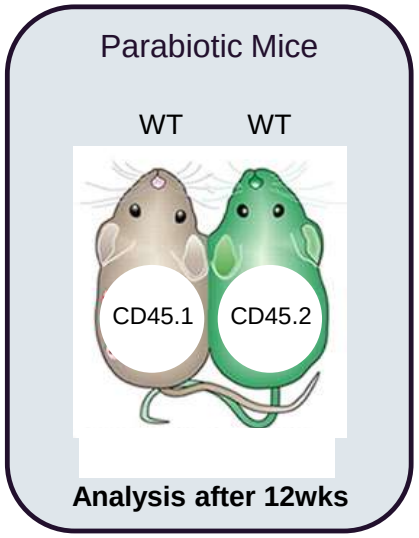


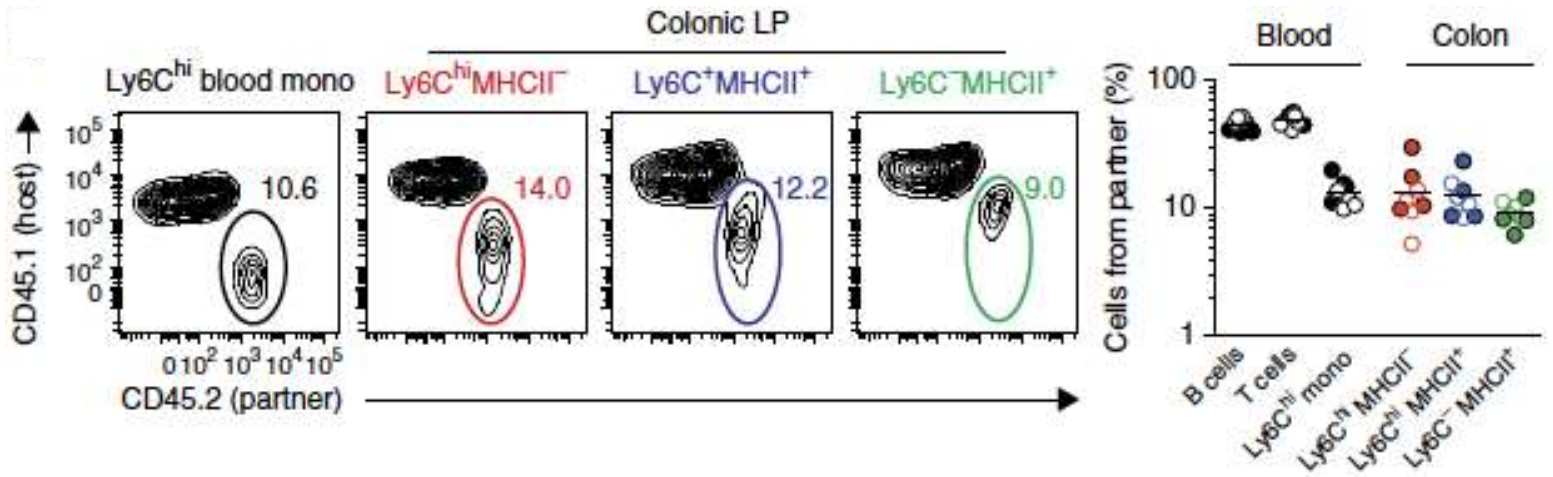


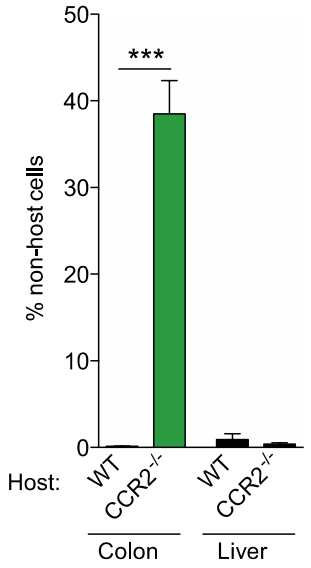
Colonic mϕ are not radio-resistant and replenishment from BM is CCR2 dependent

Bain, Bravo Blas et al Nature Immunology 2014

Intestinal Mϕ in Parabiotic Mice



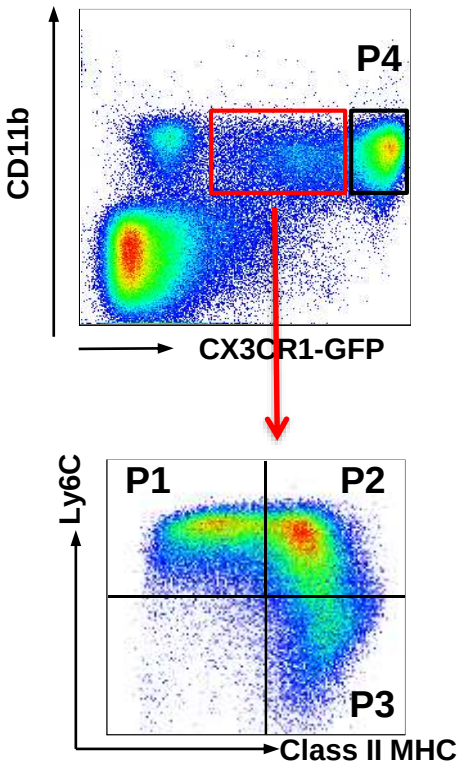


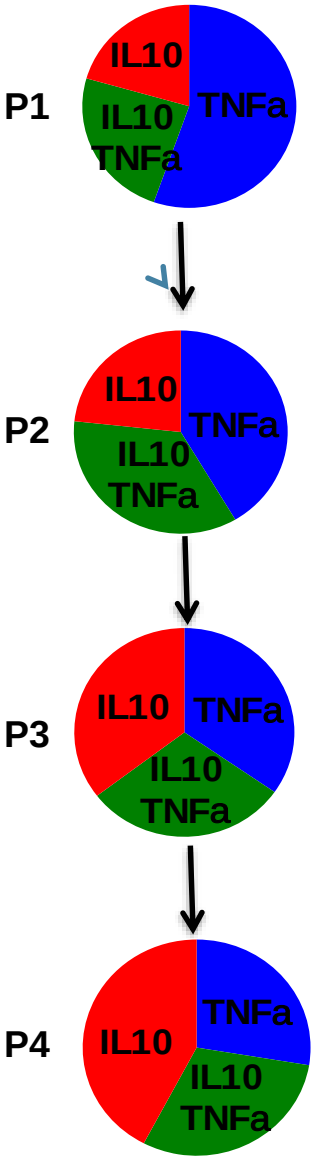


Colon mϕ are replaced to same extent as blood Ly6C^{hi} monocytes – CCR2 dependent and unlike Kupffer cells

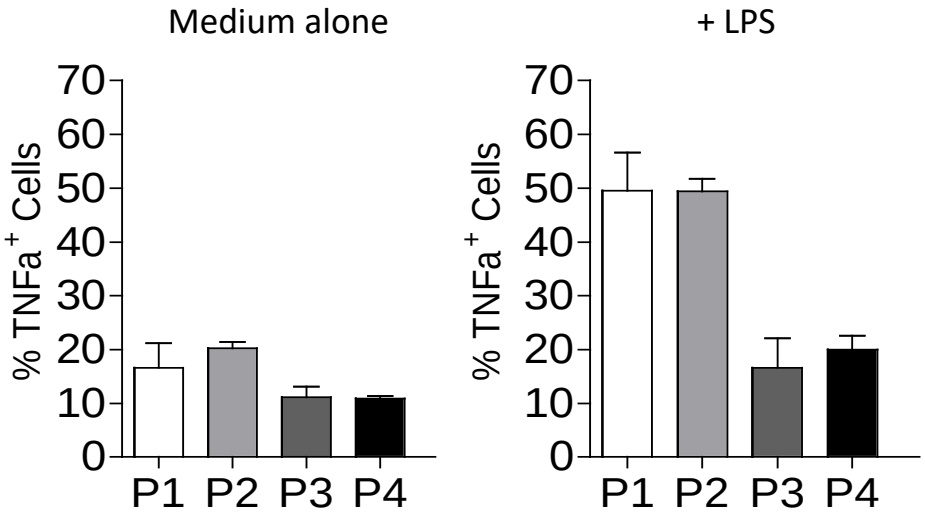
Bain, Bravo Blas et al Nature Immunology 2014

Monocytes Differentiate into Anti-Inflammatory Macrophages in Intestine





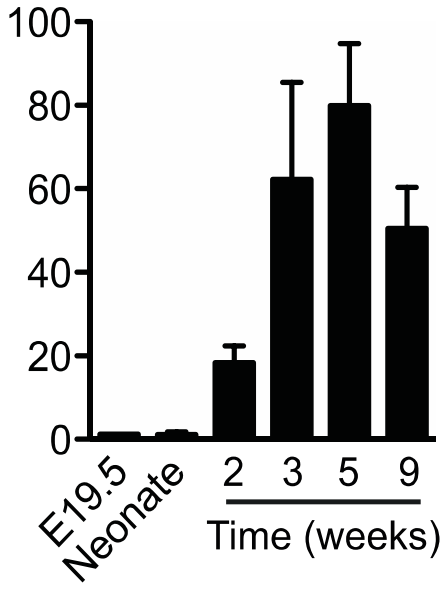
Desensitisation to TLR stimulation



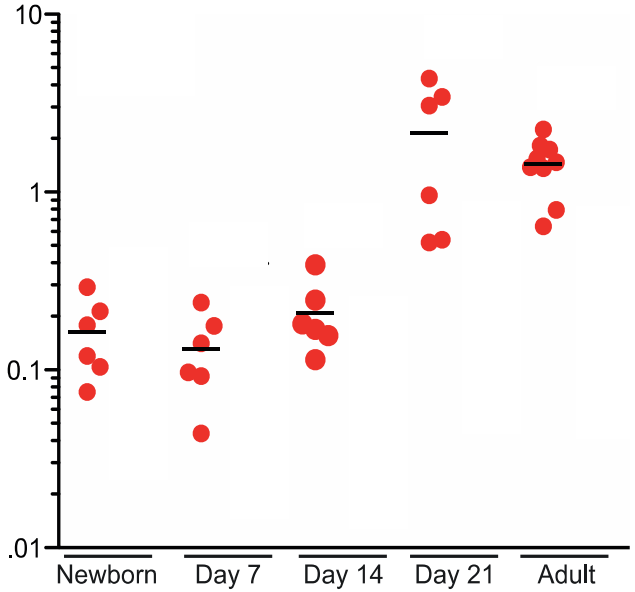
Bain et al Mucosal Immunol. 2013

Development of Intestinal Macrophages

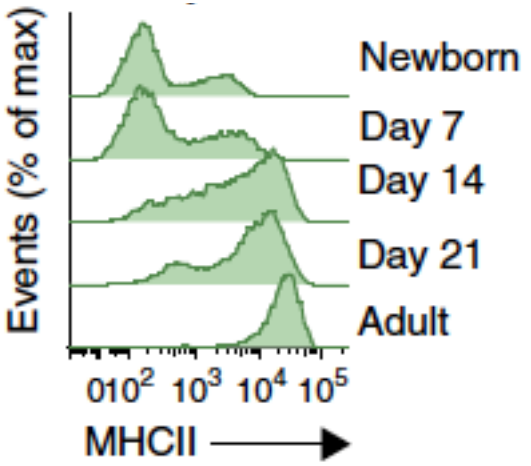
Total Macrophages



Ly6C^{hi} Monocytes



MHCII on Mature Macrophages

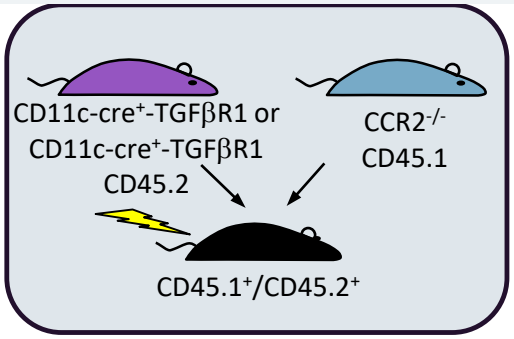


Expansion of macrophage pool associated with burst of monocyte accumulation around weaning, with upregulation of class II MHC - ?? driven by microbiota

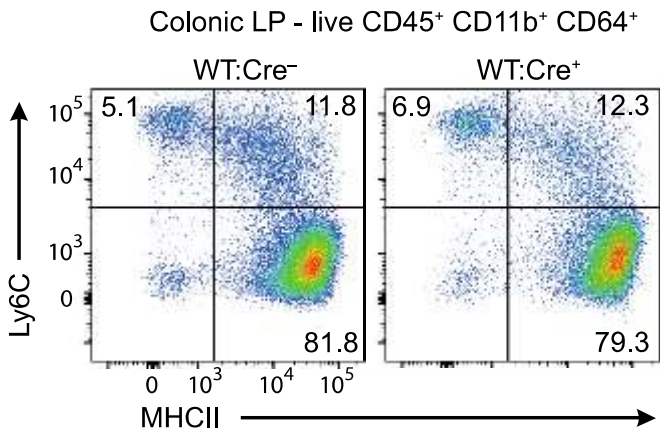
Bain, Bravo Blas et al Nature Immunology 2014

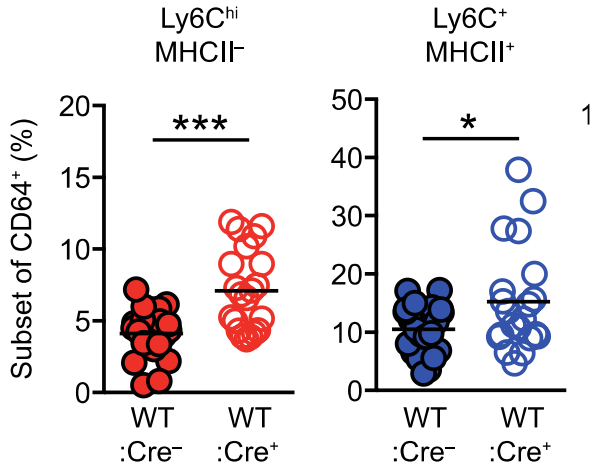
TGFβ Signalling in Macrophages Prevents Intestinal Inflammation?

Conditional KO of TGFβR on Mϕ

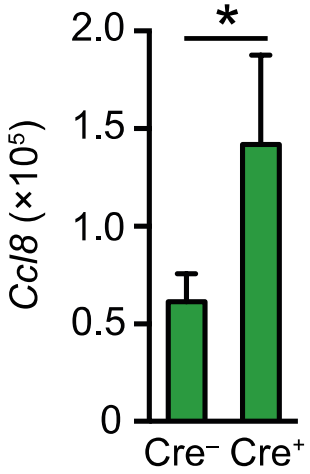


TGFβR$^{fl/fl}$ Derived Populations



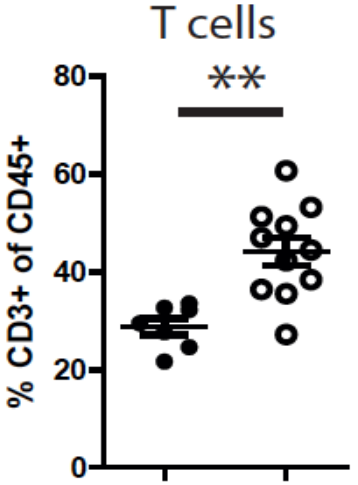


Increased numbers of monocytes and pro-inflammatory early stage macrophages

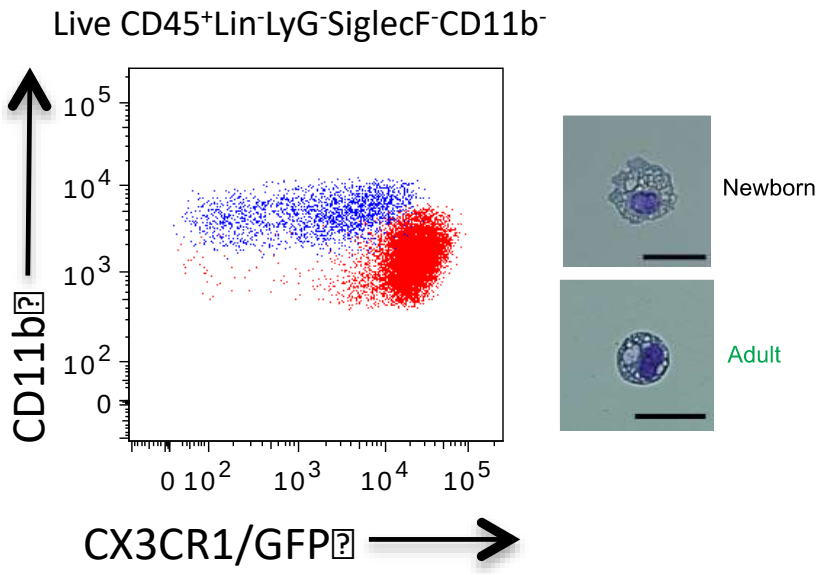


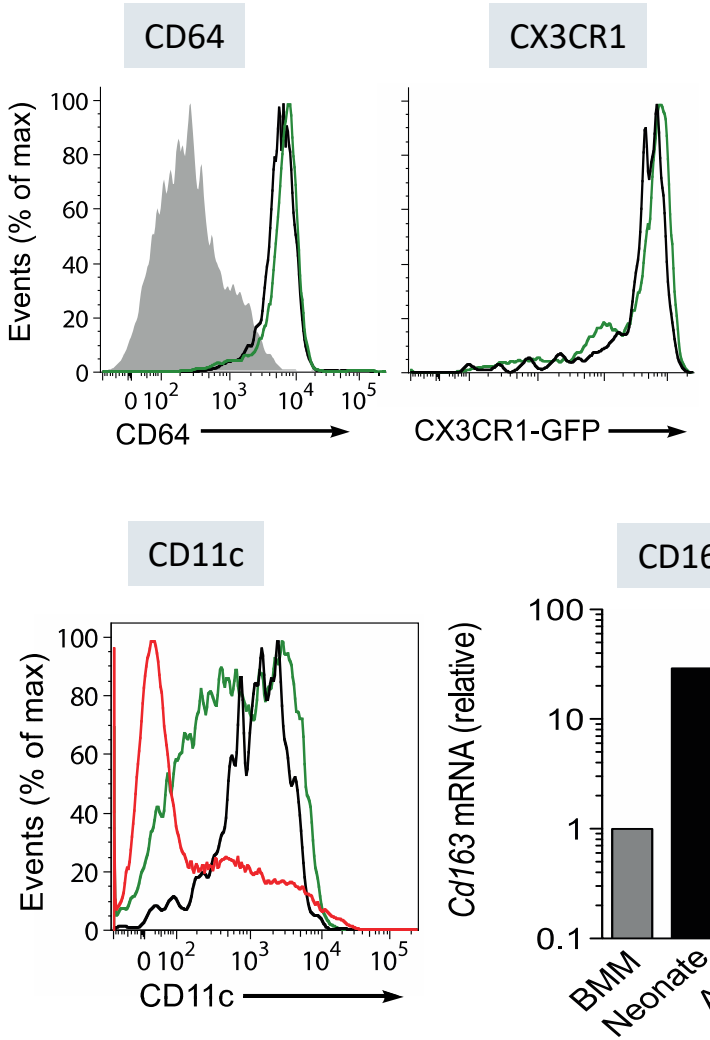
Increased expression of inflammatory chemokine and infiltration by T cells

Bain, Schridde et al Submitted



Intestinal Macrophages in the Neonate

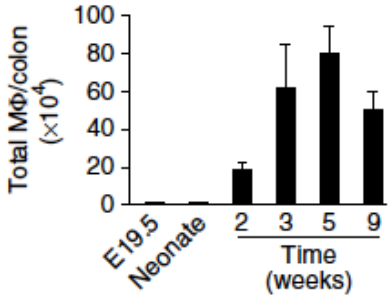


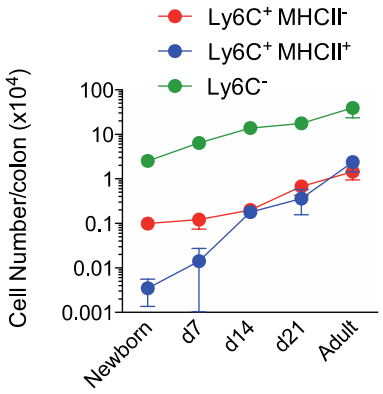


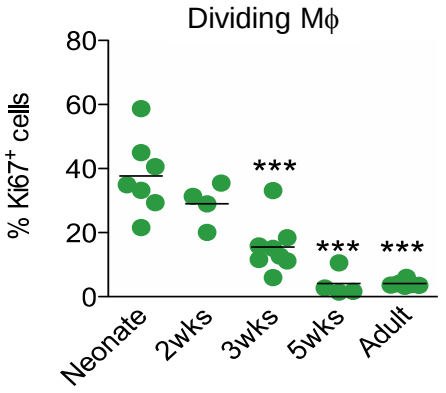
Mϕ are present in newborn colon – same phenotype and morphology as in adult

Bain et al Nature Immunology 2014, 24 August

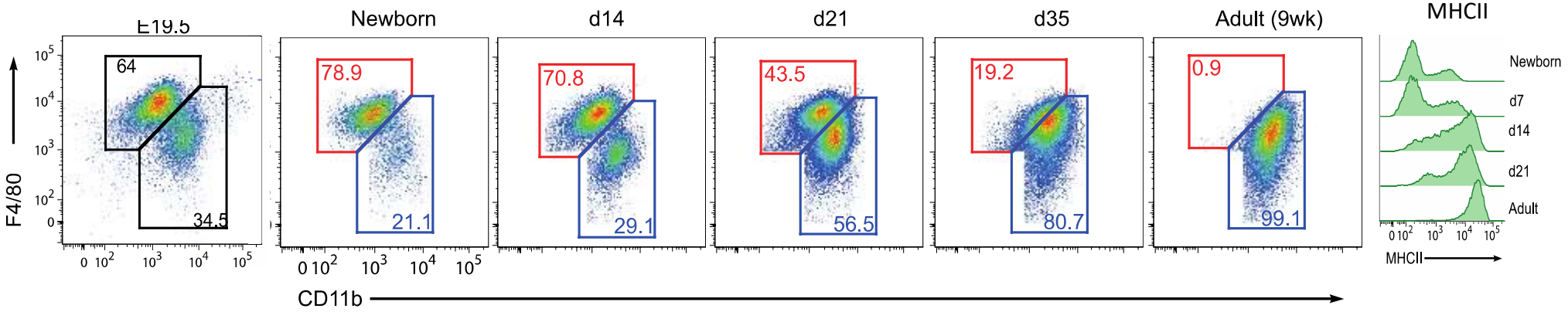
Maturation of Intestinal Macrophages







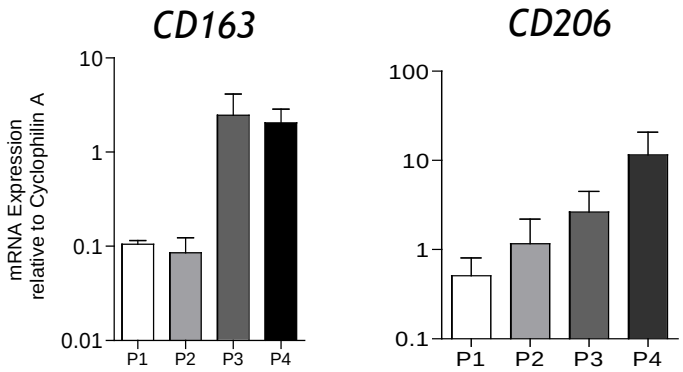
Burst of monocyte accumulation + cessation of self renewal around weaning leads to expansion of mϕ population

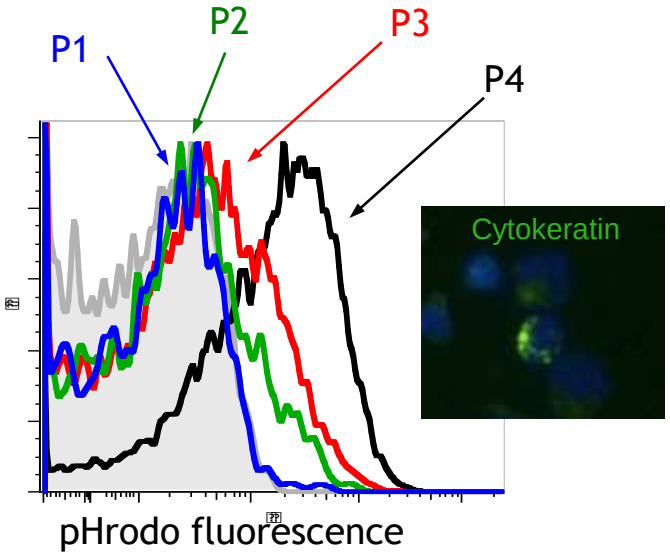


Progressive merging of F4/80^{hi} and CD11b^{hi} populations and acquisition of MHCII

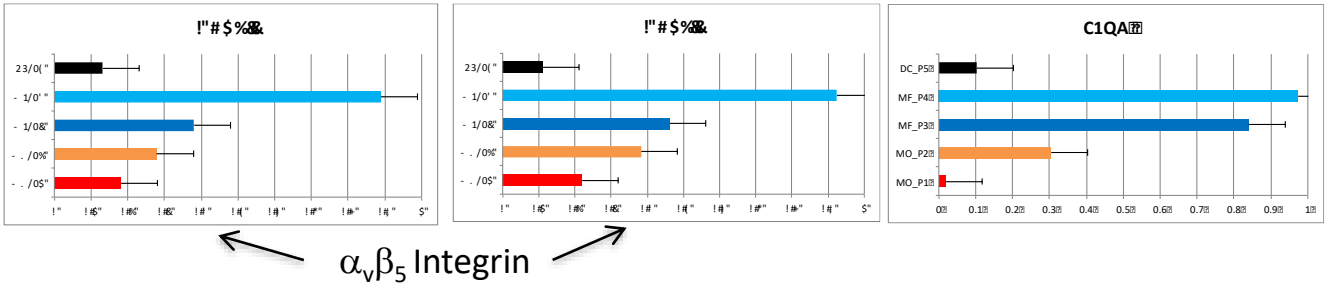
Bain et al Nature Immunology 2014

Monocytes Differentiate into Homeostatic Scavengers in Intestine

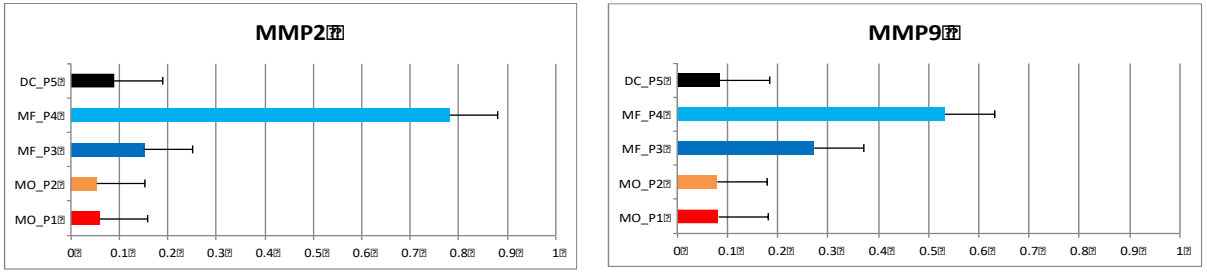




Scavenger Receptors



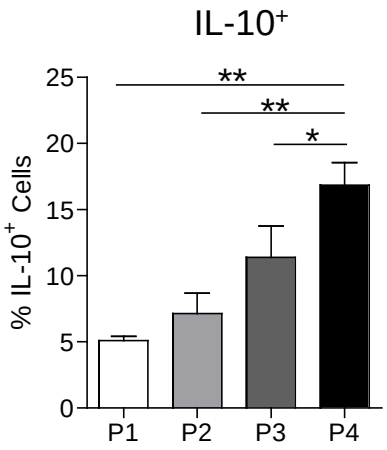
Metalloproteases

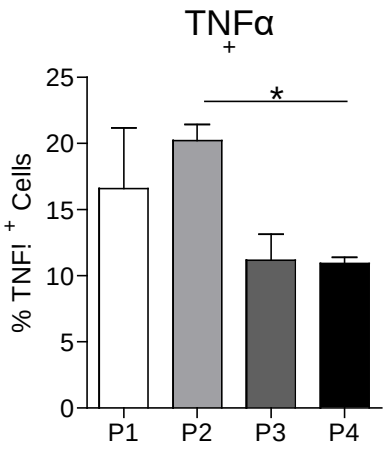


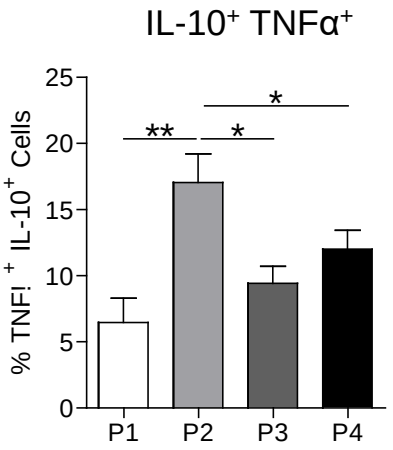
Progressive acquisition of apptotic cell receptors, phagocytic activity and metalloproteases

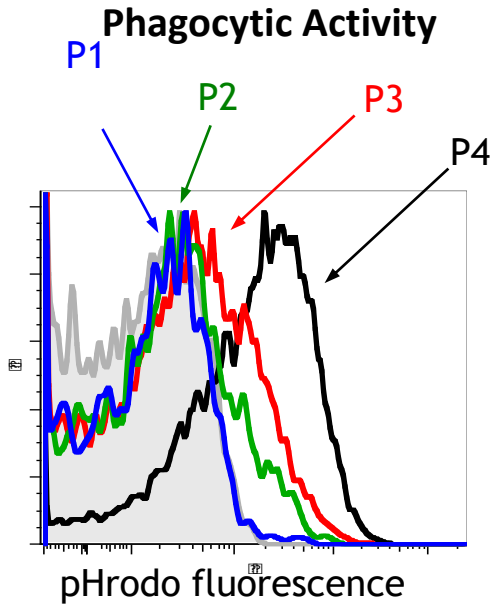
Bain et al Mucosal Immunol. 2013

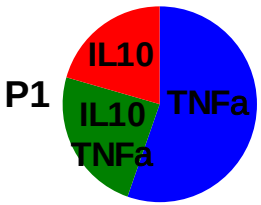
In Situ Maturation of Monocytes into Resident Macrophages

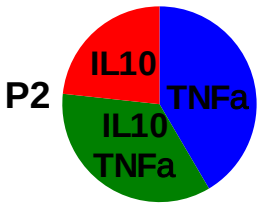


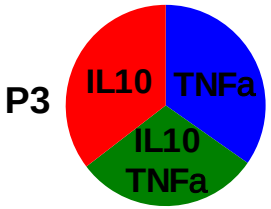


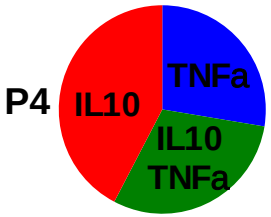


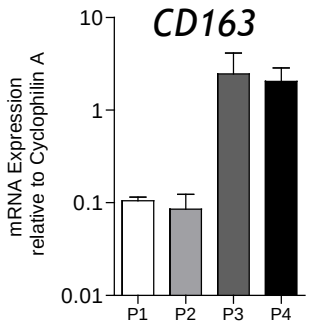


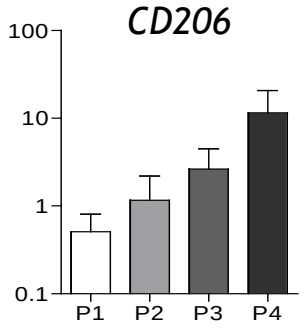


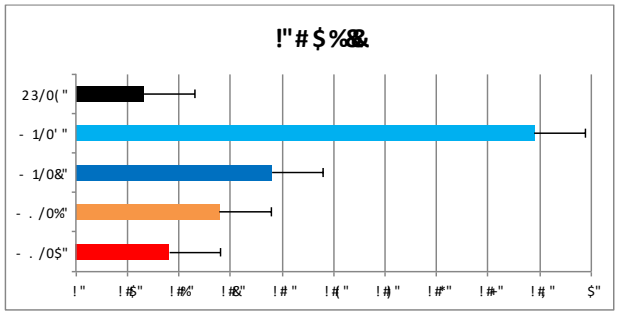


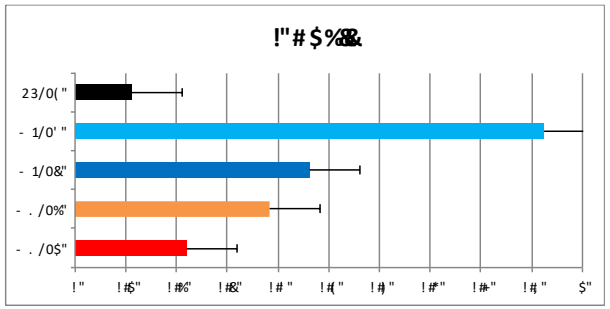










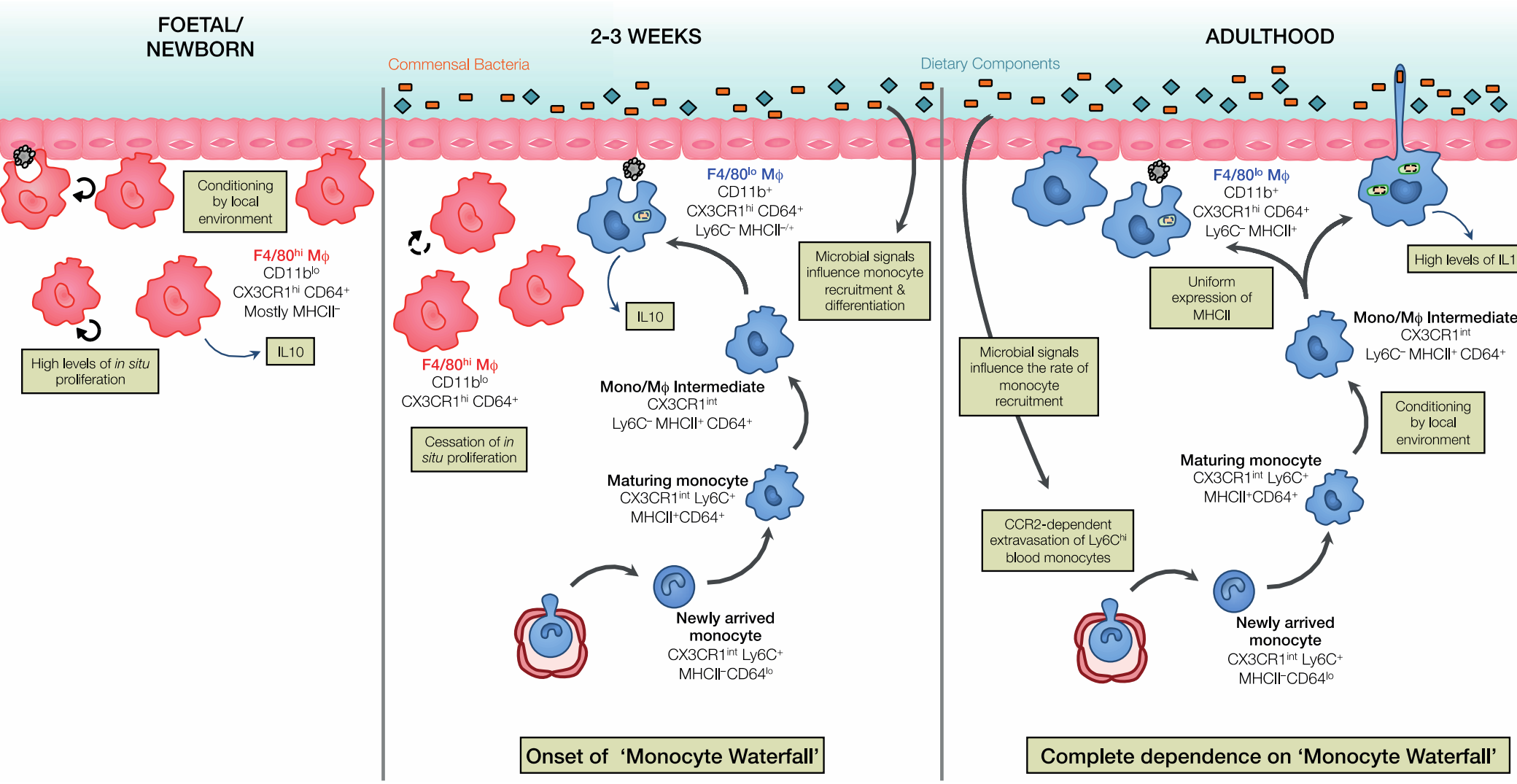


$a_v b_5$ Integrin

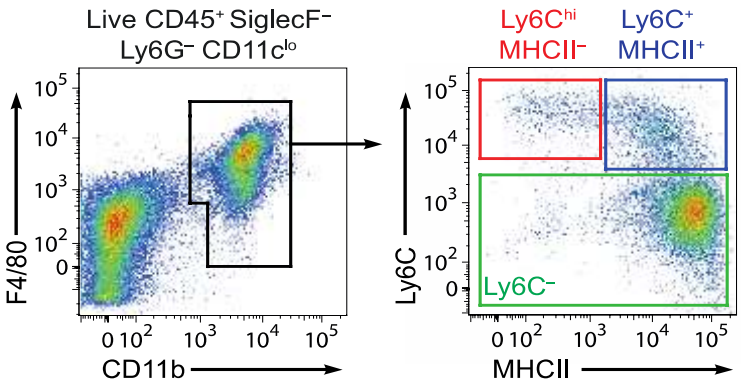
Scavenger Receptors

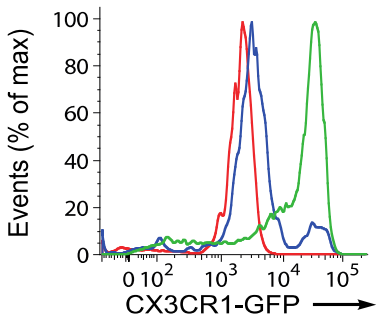
Bain et al Mucosal Immunol. 2013

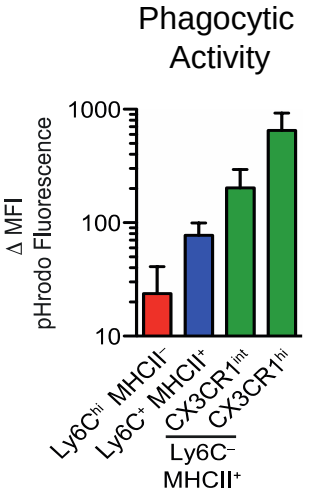
Intestinal Macrophage Development

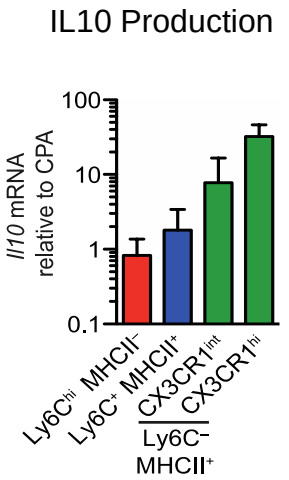


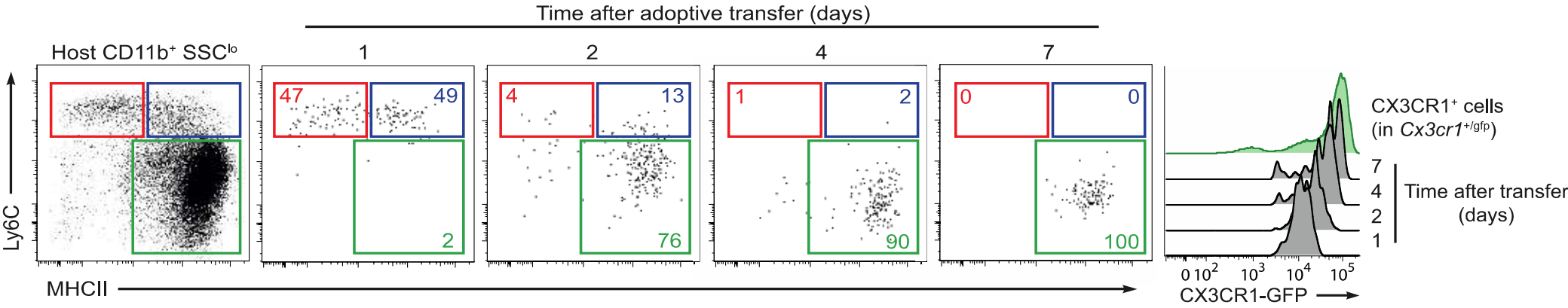
The Colonic Mϕ Compartment Comprises a Monocyte-Mϕ Continuum





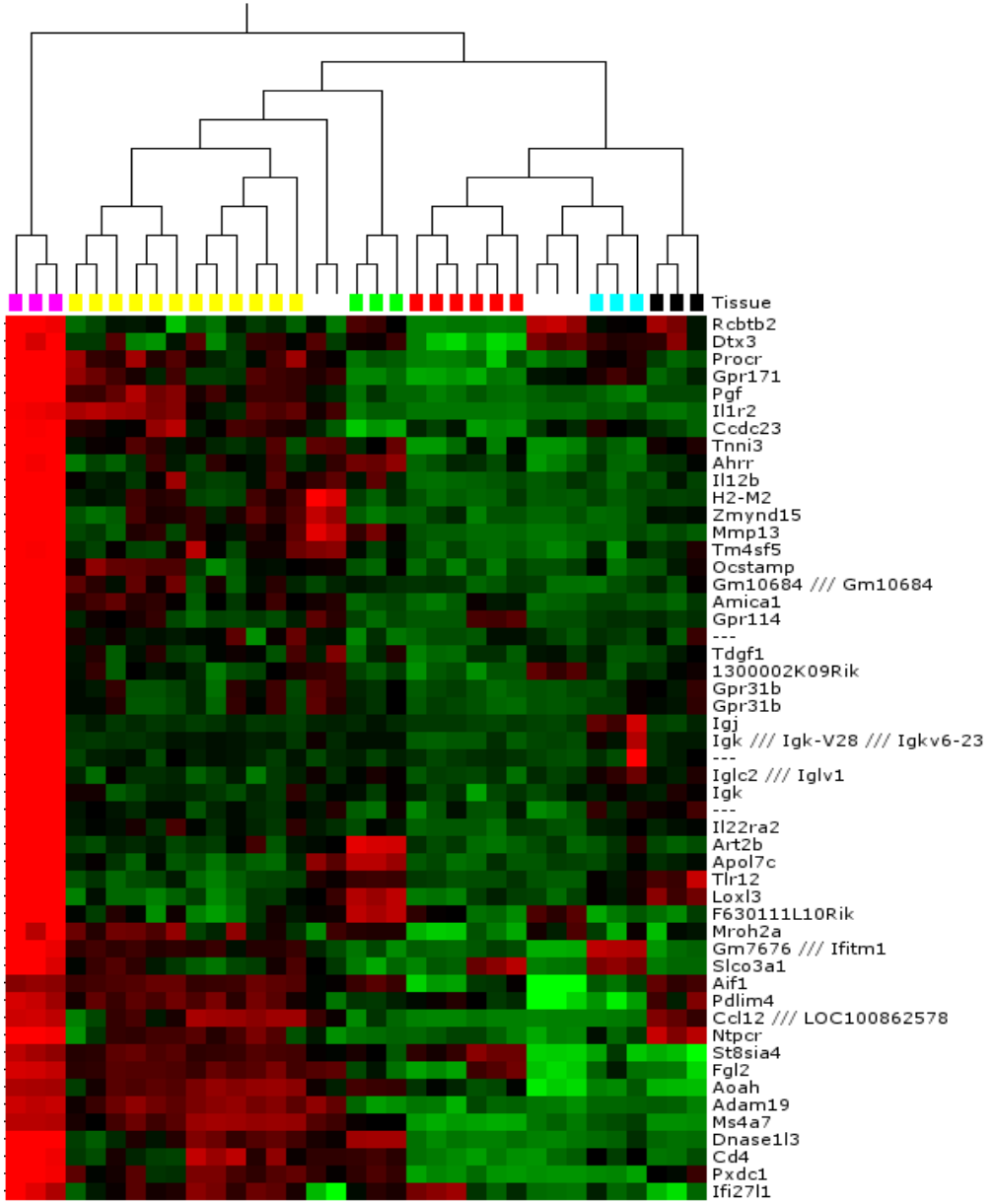




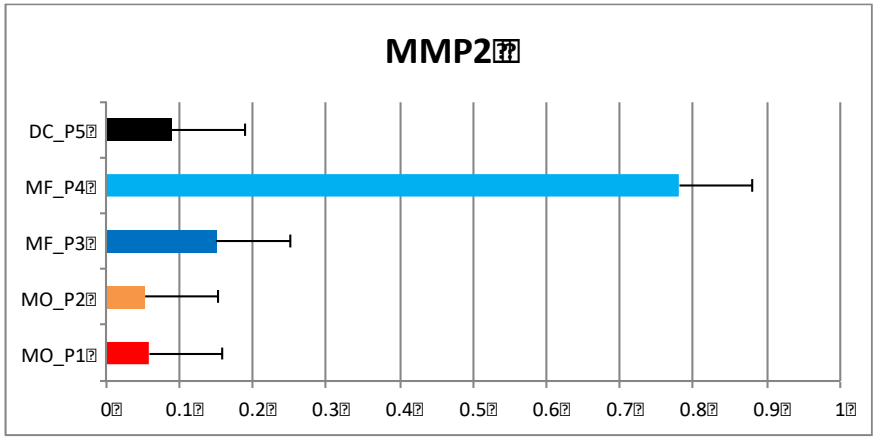


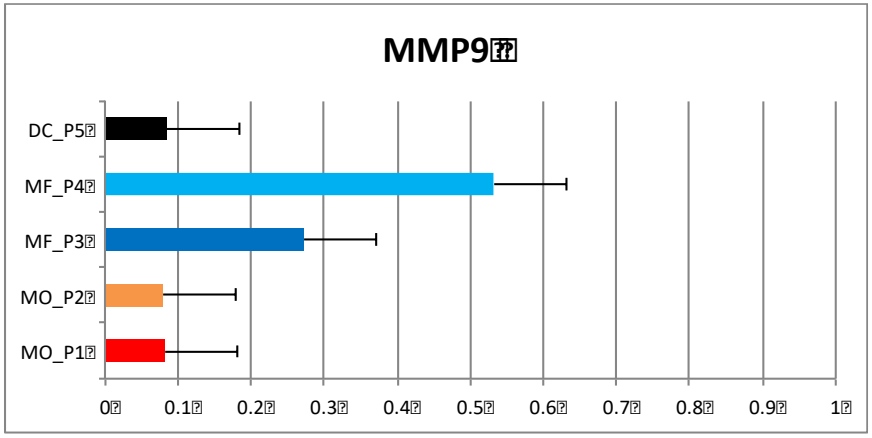
Bain et al. Mucosal Immunol. 2013
Bain, Bravo-Blas et al. Nat. Immunol. 2014

Genetic Signature of Intestinal Macrophages

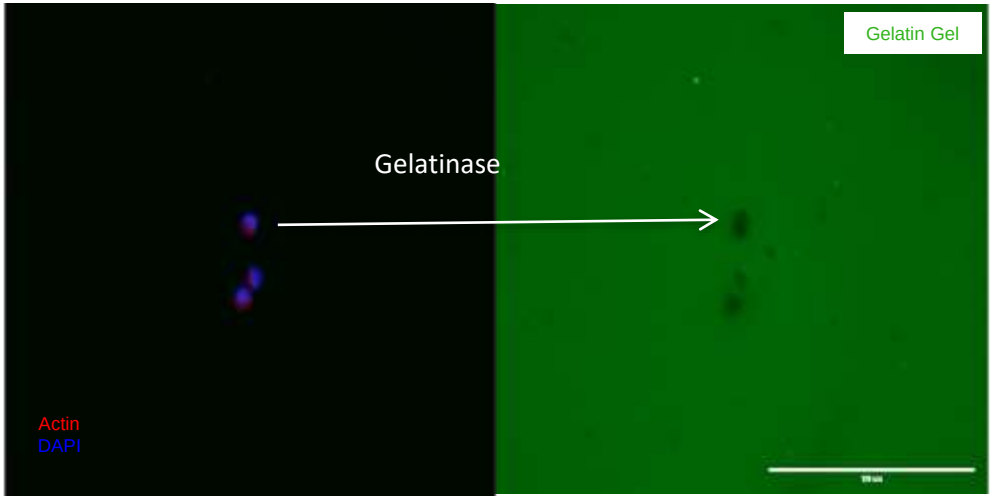


Monocytes Differentiate into Homeostatic Scavengers in Intestine





Sorted F4/80^{hi}MHCII^{hi} colonic macrophages



MMP2 and MMP9 closely related gelatinases

A Role for TGFβ in Intestinal Macrophage Development?

✖ Author's Choice

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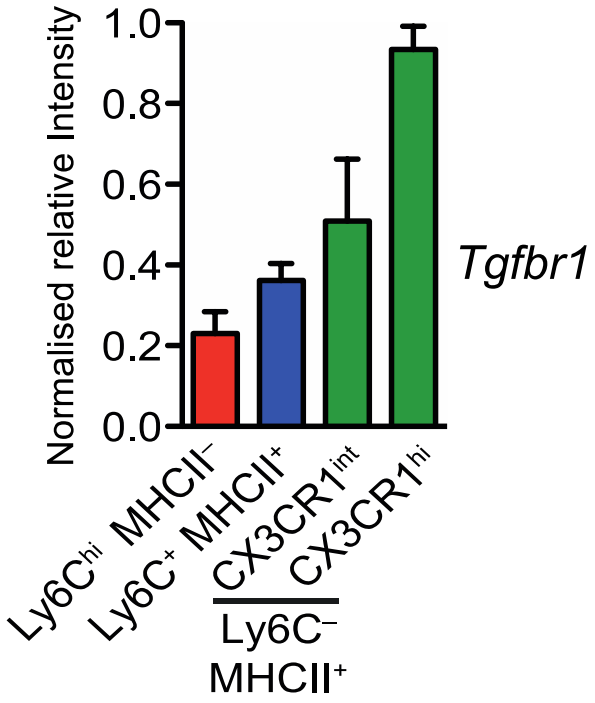
Inflammation Anergy in Human Intestinal Macrophages Is Due to Smad-induced IκBα Expression and NF-κB Inactivation*[S]

Received for publication, September 25, 2009, and in revised form, March 15, 2010 Published, JBC Papers in Press, April 13, 2010, DOI 10.1074/jbc.M109.069955

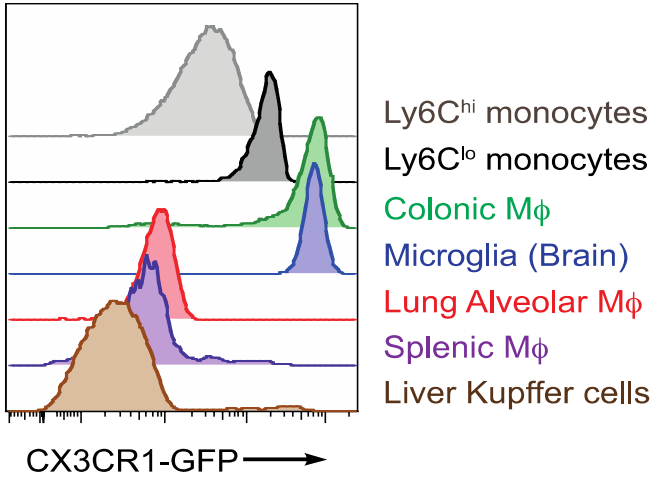
Lesley E. Smythies[‡1], Ruizhong Shen[‡], Diane Bimczok[‡], Lea Novak[§], Ronald H. Clements[¶], Devin E. Eckhoff[‖], Phillipe Bouchard[‡], Michael D. George[**], William K. Hu[**], Satya Dandekar[**], and Phillip D. Smith[‡‡2]



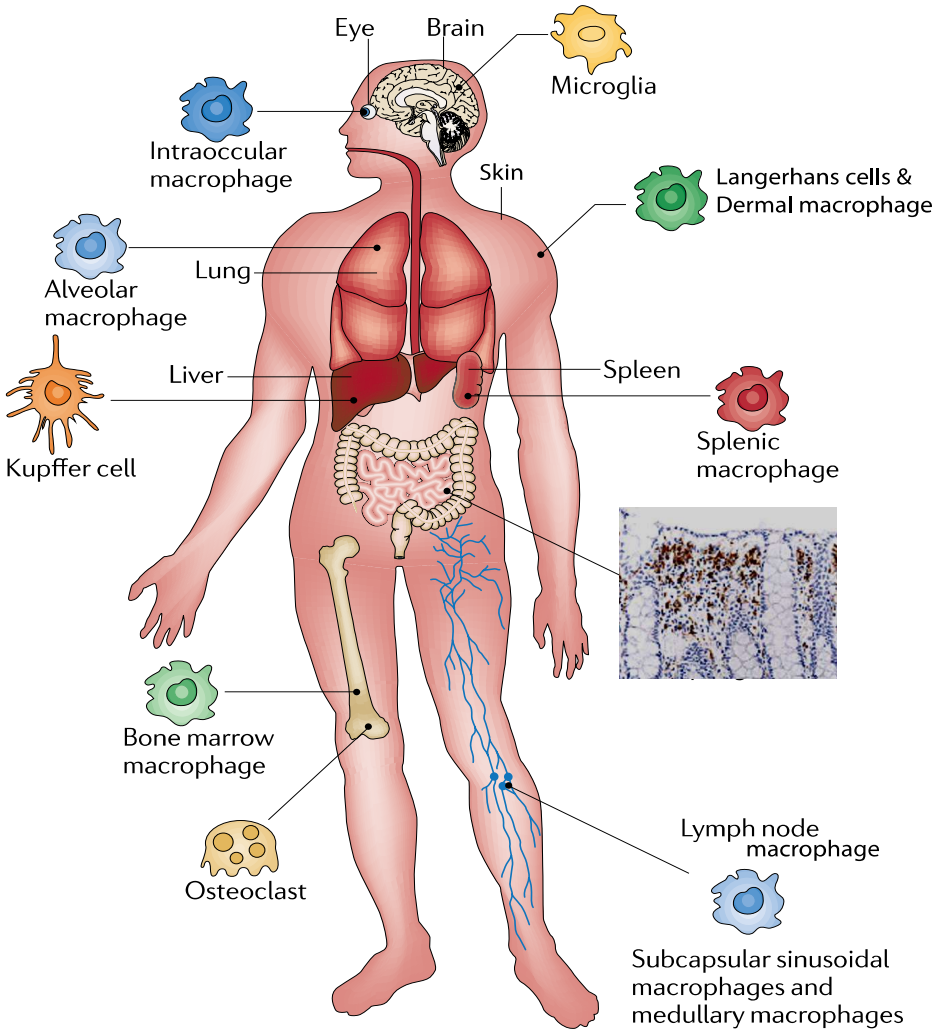
Bain, Scott et al Unpublished

Identification of a unique TGF-β–dependent molecular and functional signature in microglia

Oleg Butovsky[1], Mark P Jedrychowski[2], Craig S Moore[3], Ron Cialic[1], Amanda J Lanser[1], Galina Gabriely[1], Thomas Koeglsperger[1], Ben Dake[1], Pauline M Wu[1], Camille E Doykan[1], Zain Fanek[1], LiPing Liu[4], Zhuoxun Chen[5], Jeffrey D Rothstein[5], Richard M Ransohoff[6], Steven P Gygi[2], Jack P Antel[3] & Howard L Weiner[1]



Ontogeny of Intestinal Macrophages



- Proposed that tissue resident mϕ derived from embryonic precursors
- Intestine has distinct requirements
- Intestinal mϕ are derived from local differentiation of circulating monocytes