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## Expression and Characterisation of $\alpha v \beta 5$ Integrin on Intestinal Macrophages

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**Key Words:**  $\alpha v \beta 5$  integrin; macrophage; intestine; phagocytosis; homeostasis

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### Abbreviations Used

BM – bone marrow; DSS – dextran sodium sulphate; interleukin-10 – IL-10; LP – lamina propria; MMP – metalloproteinase;  $m\phi$  - macrophage; MF-G8 - milk fat globule EGF-like molecule 8; SI – small intestine; TGF $\beta$  - transforming growth factor  $\beta$ ; WT – wild type

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## Abstract

Macrophages play a crucial role in maintaining homeostasis in the intestine, but the underlying mechanisms have not yet been elucidated fully. Here we show for the first time that mature intestinal macrophages in mouse colon and small intestine express high levels of  $\alpha v\beta 5$  integrin, which acts as a receptor for the uptake of apoptotic cells and can activate molecules involved in several aspects of tissue homeostasis such as angiogenesis and remodelling of the extracellular matrix.  $\alpha v\beta 5$  is not expressed by other immune cells in the intestine, is already present on intestinal macrophages soon after birth, and its expression is not dependent on the microbiota. In adults,  $\alpha v\beta 5$  induces the differentiation of monocytes in response to the local environment and it confers intestinal macrophages with the ability to promote engulfment of apoptotic cells via engagement of the bridging molecule milk fat globule EGF-like molecule 8. In the absence of  $\alpha v\beta 5$ , there are fewer monocytes in the mucosa and mature intestinal macrophages have decreased expression of metalloproteases and interleukin 10. Mice lacking  $\alpha v\beta 5$  on haematopoietic cells show increased susceptibility to chemical colitis and we conclude that  $\alpha v\beta 5$  contributes to the tissue repair by regulating the homeostatic properties of intestinal macrophages.

## Introduction

Macrophages ( $m\phi$ ) are essential for both homeostasis and active immunity in the intestine, as well as playing pathogenic roles in inflammatory disorders such as Crohn's disease (CD) and ulcerative colitis (UC) [1, 2]. Unlike many other tissue  $m\phi$ , those in the intestine require constant replenishment by circulating blood monocytes that then differentiate locally under control of factors present in their environment [3]. This process normally generates  $m\phi$  with high phagocytic activity and expression of scavenger receptors associated with clearance of apoptotic cells, but they lose the ability to produce pro-inflammatory mediators in response to conventional stimuli. Rather, they produce anti-inflammatory cytokine interleukin-10 (IL-10), as well as trophic factors that maintain epithelial integrity and metalloproteinases (MMPs) that are involved in tissue remodelling [4, 5]. Thus resident intestinal  $m\phi$  may contribute to intestinal homeostasis by clearing effete tissue cells and repairing the resulting damage. However the roles of individual scavenger molecules in these processes are unknown.

Here we demonstrate that one of the most significantly upregulated proteins during intestinal  $m\phi$  development is  $\alpha v\beta 5$  integrin, an adhesion molecule crucial for clearance of effete rod and cone photoreceptor outer segment tips in the retina [6]. As mice lacking  $\alpha v\beta 5$  integrin

had dysregulated populations of the monocyte-m $\phi$  lineage in the intestine and were more susceptible to chemical colitis,  $\alpha\beta 5$  may play a crucial role in the regulation of intestinal homeostasis by m $\phi$ .

## Results and Discussion

### $\alpha\beta 5$ Integrin on Mature Intestinal Macrophages Enables Phagocytic Uptake of Apoptotic Cells

Mature m $\phi$  isolated from normal colon contained inclusion bodies that stained for cytokeratin (Fig. 1A, B), suggesting constitutive uptake of apoptotic epithelial cells *in situ*. Consistent with this, as colonic m $\phi$  from CX3CR1<sup>gfp/+</sup> mice differentiated into mature CX3CR1<sup>hi</sup> cells (P4) from Ly6C<sup>hi</sup>MHCII<sup>-</sup>CX3CR1<sup>int</sup> monocytes (P1) via Ly6C<sup>hi</sup>MHCII<sup>+</sup>CX3CR1<sup>int</sup> (P2) and Ly6C<sup>hi</sup>MHCII<sup>+</sup>CX3CR1<sup>int</sup> (P3) intermediaries (Supporting Information Fig. 1), they showed progressive upregulation of mRNA for receptors associated with uptake of apoptotic cells (Fig. 1C, Supporting Information Fig. 2 and [5]).

As well as confirming the significant upregulation of genes encoding proteins involved in phagocytosis (*C1qa*, *Fcrls*, *Cd36*, *Cd163* and *Itgav*), Q-PCR showed this also involved tissue remodelling metalloproteinase enzymes (*Mmp2*, *Mmp9* and *Mmp13*) (Supporting Information Fig. 3). Of the partners for  $\alpha$  integrin, only the mRNA for *Itgb5* (coding for integrin  $\beta 5$ ) was upregulated in colon m $\phi$ , whereas that for  $\beta 1$ ,  $\beta 3$ ,  $\beta 6$  and  $\beta 8$  integrins did not alter (Supporting Information Fig. 3). Mature colonic macrophages expressed high levels of  $\alpha\beta 5$  surface protein (Fig. 1D), as did small intestinal lamina propria (SILP) m $\phi$ , interstitial and alveolar m $\phi$  in the lung and red pulp m $\phi$  in the spleen, but not F4/80<sup>hi</sup> peritoneal m $\phi$  (Figs. 1D, E & F). This staining was specific, as it was absent from SILP m $\phi$  in *Itgb5*<sup>-/-</sup> mice (Fig. 1F).  $\alpha\beta 5$  expression in the colon and SILP was much higher on m $\phi$  compared with T cells, B cells, eosinophils, neutrophils and dendritic cells (Fig. 1G and data not shown). The expression of  $\alpha\beta 5$  was not dependent on the microbiota, as it was already expressed by virtually all colonic CX3CR1<sup>hi</sup>MHCII<sup>+</sup> m $\phi$  and CX3CR1<sup>hi</sup>MHCII<sup>-</sup> m $\phi$  48 hours after birth and throughout the neonatal period, as well as in adult mice treated with broad spectrum antibiotics (Supporting Information Fig. 4A,B and data not shown).  $\alpha\beta 5$  integrin recognises phosphatidylserine on apoptotic cells in cooperation with CD81 and MFG-E8 as a bridging opsonin [7, 8].  $\alpha\beta 5$  integrin appears to have a similar role for intestinal m $\phi$ , as their intrinsic ability to phagocytose apoptotic thymocytes *in vitro* was significantly enhanced in the presence of MFG-E8 and this was significantly reduced by anti- $\alpha\beta 5$  antibody (Fig. 1H, 1I, Supporting Information Fig. 4C).

Thus  $\alpha\beta5$  mediated phagocytosis of apoptotic cells may be one mechanism by which m $\phi$  can contribute to intestinal homeostasis.

### Role of Integrin $\alpha\beta5$ in the Development of Intestinal Immune Cells

To determine the role of  $\alpha\beta5$  in the development and function of intestinal m $\phi$ , we generated chimeric mice in which irradiated CD45.1<sup>+</sup>CD45.2<sup>+</sup> wild type (WT) recipients received bone marrow (BM) cells from CD45.2<sup>+</sup> *Itgb5*<sup>-/-</sup> mice (Fig. 2A). Control chimeras received CD45.2<sup>+</sup> WT BM and in both chimeras, >95% monocytes and m $\phi$  in the colon were of donor origin, as were CD11b<sup>+</sup> monocytes in blood (Supporting Information Fig. 5A,B). *Itgb5*<sup>-/-</sup>→WT chimeric mice developed normally and had no evidence of clinical disease, but they had significantly reduced proportions and numbers of MHCII<sup>-</sup> monocytes (P1) in the colon compared with WT→WT chimeric mice (Fig. 2B). There were no differences in the proportions or numbers of MHCII<sup>+</sup> monocytes (P2) or mature m $\phi$  (P3-4), or in the proportion of Ly6C<sup>hi</sup> monocytes in peripheral blood (Fig. 2B). Total dendritic cells and their subsets based on expression of CD103 and CD11b were also unaltered in *Itgb5*<sup>-/-</sup>→WT chimeric mice (Supporting Information Fig. 5C, data not shown), but the proportions and numbers of eosinophils were significantly higher in the colon of *Itgb5*<sup>-/-</sup>→WT chimeric mice (Supporting Information Fig. 5D). An identical pattern of myeloid cells was found in the SI LP of *Itgb5*<sup>-/-</sup>→WT chimeric mice (Supporting Information Fig. 6A,B). This reflected a lack of  $\beta5$  integrin on the monocyte-m $\phi$  lineage itself, as the effects were replicated in mixed bone marrow chimeras in which CD45.1<sup>+</sup>CD45.2<sup>+</sup> WT recipients were reconstituted with BM cells from CD45.1<sup>+</sup> *Ccr2*<sup>-/-</sup> mice together with BM from CD45.2<sup>+</sup> *Itgb5*<sup>-/-</sup> mice. As intestinal m $\phi$  require constant, CCR2-dependent replenishment (Fig. 2C and [3]), all lack  $\alpha\beta5$  integrin in such chimeras (Fig. 2D). Although *Itgb5*<sup>-/-</sup> BM was more efficient than WT BM in reconstituting colonic monocytes and m $\phi$  in the colon under competitive conditions (Figure 2E), the selective reduction in *Itgb5*<sup>-/-</sup>-derived Ly6C<sup>hi</sup>MHCII<sup>-</sup> monocytes (P1) seen in *Itgb5*<sup>-/-</sup> mice was replicated in the mixed chimeric system (Supporting Information Fig. 6C). There were no differences in the proportions of Ly6C<sup>hi</sup>MHCII<sup>+</sup> monocytes (P2) or mature m $\phi$  (P3-4) that were derived from *Itgb5*<sup>-/-</sup> or WT BM (Supporting Information Fig. 6C).

As the numbers of Ly6C<sup>hi</sup> monocytes were normal in the bloodstream of *Itgb5*<sup>-/-</sup> chimeric mice, the defect in intestinal P1 cells may reflect reduced recruitment. Alternatively, the fact that the mature colonic m $\phi$  population remains unaffected in the absence of  $\alpha\beta5$  could suggest more rapid differentiation of monocytes after their arrival.

## Role of Integrin $\alpha\beta5$ in Homeostatic Function and Colon Inflammation

As the numbers of mature m $\phi$  were not affected by the absence of integrin  $\alpha\beta5$ , we went on to examine whether any of their characteristic properties were altered.

As in intact mice, m $\phi$  sorted from the colon of WT $\rightarrow$ WT BM chimeras showed marked upregulation of mRNA for *Mmp2*, *Mmp9* and *Mmp13* as they matured from monocytes (Fig. 3A). This did not occur with *Itgb5* $^{-/-}$  $\rightarrow$ WT m $\phi$  and although the mechanisms responsible for this are unclear, the results indicate that  $\alpha\beta5$  can influence MMP activity at the level of gene transcription; it can also regulate their enzymatic activity, as has been shown with microglia [9]. There was also a significant defect in the upregulation of *Il10* mRNA in *Itgb5* $^{-/-}$  $\rightarrow$ WT m $\phi$  (Fig. 3A). In contrast, transcripts for the scavenger molecules C1qA and CD163 showed normal upregulation in *Itgb5* $^{-/-}$  $\rightarrow$ WT m $\phi$ , while the expression of mRNA for the chemokine CCL2 was enhanced (Supporting Information Fig. 7A), perhaps reflecting an attempt by these cells to respond to the reduced recruitment of monocytes. Dysregulated production of other chemokines might also help explain the increase in eosinophils that we observed in mice lacking  $\alpha\beta5$ . Although  $\alpha\beta5$  integrin is thought to activate transforming growth factor  $\beta$  (TGF $\beta$ ) [10, 11], colonic m $\phi$  from *Itgb5* $^{-/-}$  $\rightarrow$ WT chimeric mice showed normal upregulation of genes involved in TGF $\beta$  mediated signalling (*Tgfb1*) or whose expression is dependent on TGF $\beta$  (*Il22*) [5] (Supporting Information Fig. 7B). Interestingly, *Itgb5* $^{-/-}$  $\rightarrow$ WT mice showed a marked increase in the proportion of mature m $\phi$  expressing TIM4, another phagocytic receptor that is upregulated during local differentiation from monocytes (Fig. 3B). As Tim4 expression correlates with long term residence of m $\phi$  in tissues [12, 13], our findings could indicate that m $\phi$  differentiating in the absence of  $\alpha\beta5$  may have a longer lifespan than normal, thereby accounting the normal numbers of mature m $\phi$  we observed. The lifespan, proliferative capacity and differentiation kinetics of macrophages in the absence of  $\alpha\beta5$  warrant further investigation.

We examined whether the dysregulated differentiation of colonic m $\phi$  in the absence of  $\alpha\beta5$  had functional consequences *in vivo*. Oral administration of DSS led to clinical colitis and weight loss in all WT $\rightarrow$ WT and *Itgb5* $^{-/-}$  $\rightarrow$ WT chimeric mice, but both parameters progressed more rapidly and showed increased severity in the *Itgb5* $^{-/-}$  $\rightarrow$ WT chimeric mice (Fig. 3C, 3D). Colitis was associated with increased infiltration by P1 and P2 monocytes in both groups, together with a trend towards reduced numbers of mature m $\phi$  (Fig. 3E). The expansion of P2 cells was significantly higher in *Itgb5* $^{-/-}$  $\rightarrow$ WT mice, indicating that the

chemical insult and/or resulting influx of microbes can overcome the intrinsic defect in monocyte accumulation (Supporting Information Fig. 7C). The already expanded population of eosinophils in steady state *Itgb5*<sup>-/-</sup>→WT mice did not increase further after administration of DSS as it did in colitic WT→WT mice (Fig. 3E). These findings that the absence of  $\alpha\beta5$  integrin leads to increased susceptibility to clinical disease and local inflammation in DSS colitis extend previous work showing that MFG-E8 can inhibit colitis and promote mucosal wound healing [14, 15]. Nevertheless, the loss of  $\alpha\beta5$  had only partial effects on colitis development and did not lead to intestinal inflammation under steady state conditions in our conventional animal facility. Thus the requirement for  $\alpha\beta5$  may only become critical with advancing age, as is found for the blindness that occurs in *Itgb5*<sup>-/-</sup> mice [16]. Alternatively, its functions may be compensated for by other receptors for apoptotic cells, such as TAM receptors, GAS6, TREM-2 and CD300f. Although we were not able to examine such molecules in detail, TIM4 was expressed on a significantly increased proportion of mature colonic m $\phi$  in *Itgb5*<sup>-/-</sup> mice. Redundancy of this kind amongst apoptotic receptors is found with the TAM receptor family, where the absence of more than one of Axl, Mer or Tyro3 is needed to reveal autoimmune disease [17].

### Concluding Remarks

Our study reveals for the first time that  $\alpha\beta5$  integrin is highly expressed by mature intestinal m $\phi$  as they differentiate from monocytes in situ in an age and microbiota independent manner. Mice lacking  $\alpha\beta5$  are more susceptible to intestinal inflammation, perhaps reflecting its roles in the clearance of damaged cells and tissue remodelling. However as the absence of  $\alpha\beta5$  had several other effects on the composition and properties of the intestinal environment, the exact mechanisms by which it regulates local homeostasis remain to be explored directly.

### Materials and Methods

#### *In Vivo* Procedures

Wild-type C57Bl/6 (B6) (CD45.2<sup>+</sup>), B6.Ly5.1 (CD45.1<sup>+</sup>.CD45.2<sup>+</sup>), CD2-DsRed (a kind gift of Dr Robert Benson, Institute of Infection, Immunity and Inflammation), *Cx3cr1*<sup>gfp/+</sup>, and *Ccr2*<sup>-/-</sup> mice were bred and maintained under specific pathogen free conditions at the Central Research Facility at the University of Glasgow. *Itgb5*<sup>-/-</sup> mice [18] were bred and maintained in the Animal Research Facility at the Department of Biological Sciences, Fordham University, USA. All experiments were approved by the UK Home Office, or by the Institutional Animal Care Committee at Fordham University. All mice were used at 6-12 weeks of age.

Bone marrow chimeras were generated by reconstitution of lethally irradiated mice with  $2-4 \times 10^6$  BM cells as described [4] and analysed 8 weeks later. Colitis was induced by oral administration of 2% dextran sodium sulphate (DSS) [3] and scored as described in Supplementary Table 1. Mice treated with antibiotics received ampicillin (1g/L), metronidazole (1g/L), neomycin (1g/L), gentamicin (1g/L) and vancomycin (0.5g/L) in the drinking water for 7 days.

### **Preparation of Single Cell Suspensions**

Lamina propria mononuclear cells were isolated from colon and small intestine by enzymatic digestion as described [19]. Spleens were chopped and digested in pre-warmed RPMI 1640 (Gibco, Thermo Fisher Scientific, Paisley, UK) containing 1mg/ml collagenase D (Roche) for 30 minutes in a shaking incubator at 37°C. Lung leukocytes and peritoneal exudate cells were isolated as described [12].

### **Phenotypic and Transcriptional Analysis**

Cells were blocked with anti-CD16/32 (BioLegend, London, UK), stained with the antibodies shown in Supplementary Table 2 and expression compared with fluorescence minus one (FMO) stains as negative controls. Cells were acquired using a FACS Aria I or III cytometer (BD Biosciences) and sorted to a purity of >95%. and analysed using FlowJo software (Tree Star, Ashland, OR). Cytospins were stained with anti-mouse pan-cytokeratin antibody (C-11; Sigma-Aldrich), followed by image analysis using the EVOS Cell Imaging System (Life Technologies).

Gene expression was assessed in sorted cells by quantitative real time PCR (Q-PCR) (Supplementary Table 3) and by microarray analysis as described previously [5] (GEO GSE84764).

### **Phagocytic Uptake of Thymocytes**

Thymocytes from CD2-DsRed mice were irradiated with 30Gy and then were cultured for 4 hr in RPMI 1640 supplemented with 10% foetal calf serum (FCS), 100µg/ml streptomycin/penicillin and 1.25µg/ml Fungizone (Gibco) ('complete medium'). This process yielded 40-45% Annexin V<sup>+</sup> positive cells (data not shown).  $2 \times 10^6$  LPMCs were co-cultured for 2 hours at 37°C with  $4-5 \times 10^6$  apoptotic thymocytes in the presence of 10µg/ml milk fat globule-EGF factor 8 (R&D Systems, UK), with or without 10µg/ml anti- $\alpha_v\beta_5$  integrin antibody. After incubation, cells were washed and analysed by flow cytometry.



## Statistical Analysis

Data were compared using an unpaired Student's t-test or Mann Whitney two-tailed non-parametrical test, while multiple groups were compared using Kruskal–Wallis one-way ANOVA (GraphPad Prism 6, La Jolla, CA).

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## Conflict of Interest

The authors declare no commercial or financial conflict of interest

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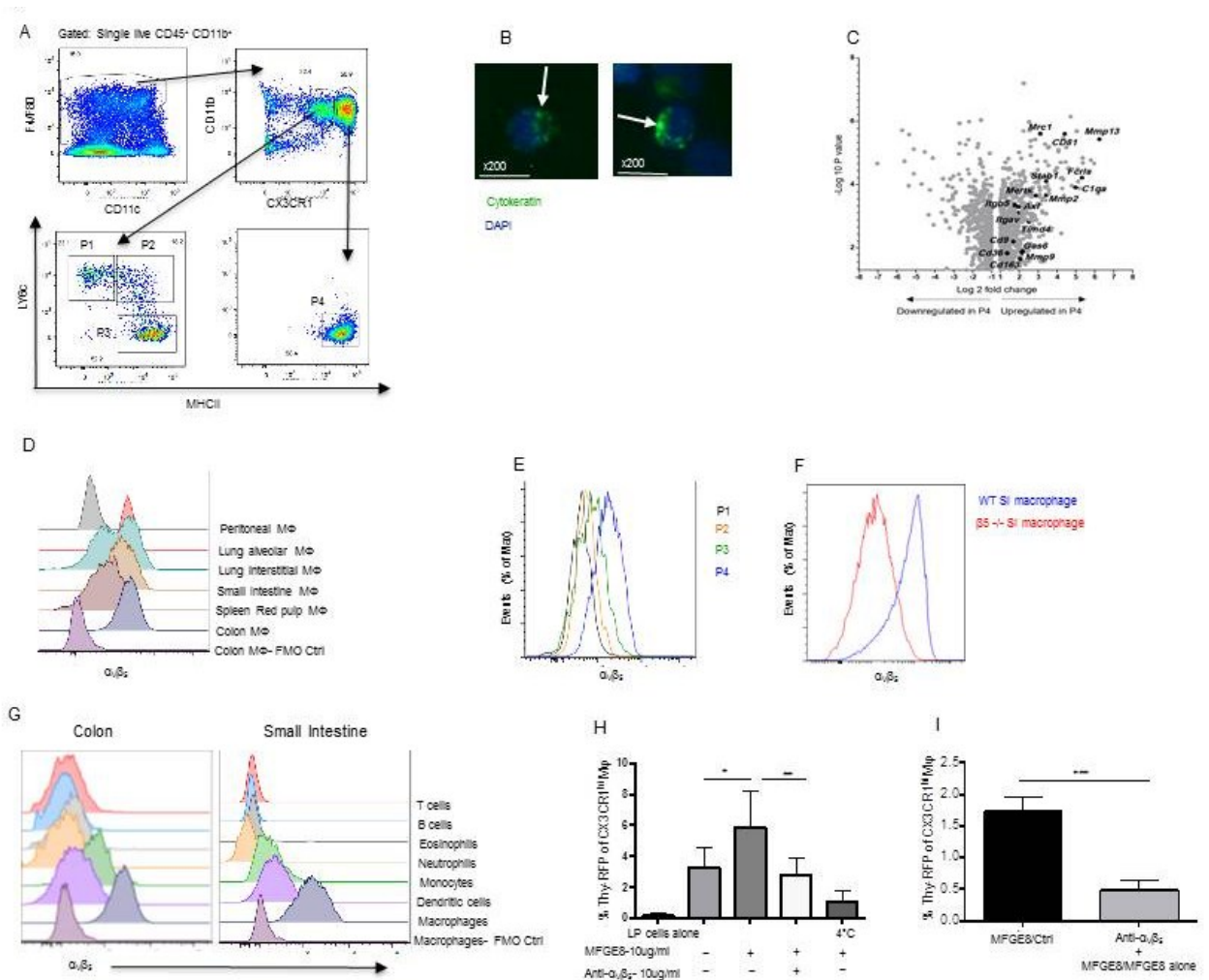
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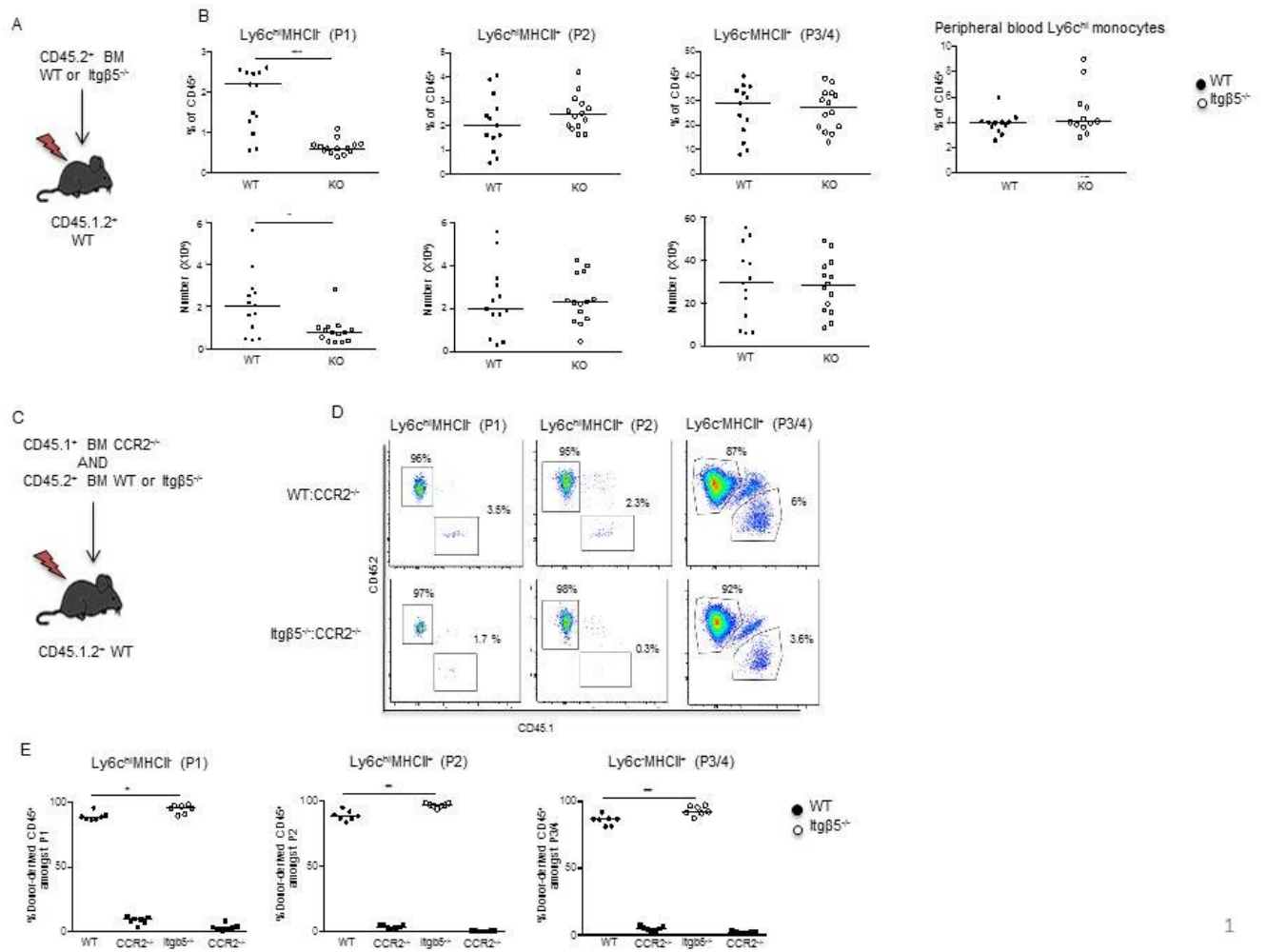
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**Figure 1. Expression of  $\alpha\beta 5$  integrin by mature intestinal macrophages enables phagocytic uptake of apoptotic cells**

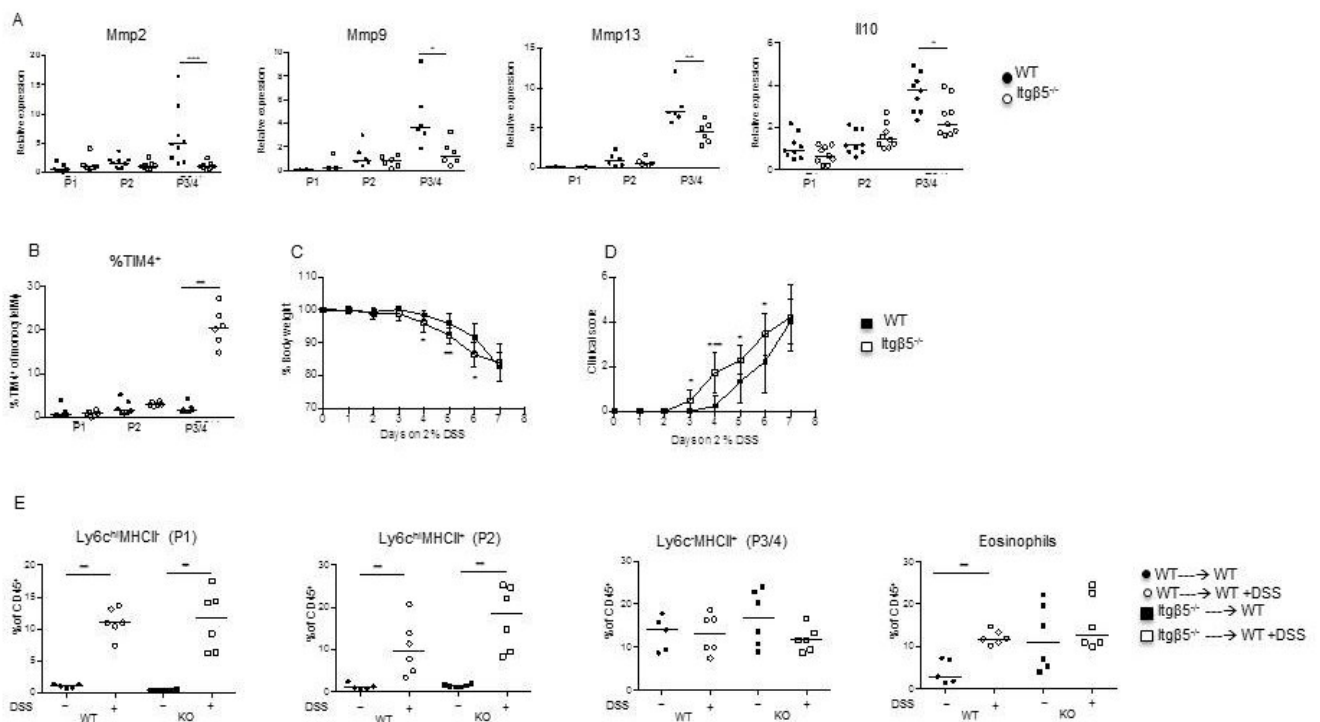
(A) Gating strategy showing the differentiation of  $CX3CR1^{hi}MHCII^{hi}$  colonic macrophages (P4) from  $CX3CR1^{int}Ly6C^{hi}MHCII^{-}$  monocytes (P1) via  $CX3CR1^{int}$  intermediaries (P2 & P3) amongst live  $CD45^{+}CD11b^{+}F4/80^{+}CD11c^{+/-}$  cells in  $CX3CR1^{gfp/+}$  mice. (B) Cytokeratin<sup>+</sup> inclusions (green – arrowed) in sorted  $CX3CR1^{hi}$  macrophages (Final Magnification x200). Image is representative of two independent experiments. (C) Volcano plot showing genes up- or down-regulated in  $CX3CR1^{hi}Ly6C^{i}MHCII^{+}$  colonic macrophages compared with P1 monocytes. Data are from one microarray experiment using 3 biological replicates containing cells pooled from 4-5 mice [5] (GEO GSE84764) (D) Expression of integrin  $\alpha\beta 5$  on  $CD11b^{+}F4/80^{hi}$  peritoneal macrophages, alveolar macrophages ( $CD64^{hi}CD11c^{hi}SiglecF^{hi}MHCII^{lo}CD11b^{-}$ ), lung interstitial macrophages ( $CD64^{lo}CD11b^{hi}MHCII^{+}SiglecF^{lo}CD11c^{lo/+}$ ), splenic red pulp macrophages ( $F4/80^{hi}MHCII^{+}CD11b^{lo/-}CD11c^{-}$ ) and  $CX3CR1^{hi}$  macrophages (P4) from small intestine and colon of  $Cx3cr1^{+/gfp}$  mice. (E) Expression of  $\alpha\beta 5$  on monocyte/macrophage subsets in the small intestine of  $Cx3cr1^{+/gfp}$  mice. (F)  $\alpha\beta 5$  staining of mature macrophages from the small intestine of WT $\rightarrow$ WT and  $Itgb5^{-/-}$  $\rightarrow$ WT bone marrow chimeric mice. (G)  $\alpha\beta 5$  expression on  $CD3^{+}$  T lymphocytes,  $CD19^{+}$  B cells,  $SiglecF^{+}CD11b^{+}$  eosinophils,  $Ly6G^{+}CD11b^{+}$  neutrophils,  $Ly6C^{hi}MHCII^{-}$  monocytes,  $F4/80^{-}CD11c^{+}MHCII^{+}$  dendritic cells and  $F4/80^{hi}CX3CR1^{hi}MHC^{+}$  macrophages from colon and small intestine of  $Cx3cr1^{+/gfp}$  mice, together with the FMO for colon/SI macrophages. Histograms in D-G are representative of three independent experiments with 1-2 mice/experiment. (H, I) Frequencies of colon macrophages taking up apoptotic DS-Red thymocytes in presence of MFG-E8 and anti- $\alpha\beta 5$  antibody. \* $p < 0.05$  vs MFG-E8 ligand alone; \*\* $p < 0.01$  vs MFG-E8 + anti- $\alpha\beta 5$ . \*\*\* $p < 0.001$  vs MFG-E8 ligand + anti- $\alpha\beta 5$  vs MFG-E8 alone, Student's t-test + one-way ANOVA. Data in H, I are means + 1sd for 8 mice/group pooled from three independent experiments.



**Figure 2. Role of integrin  $\alpha\text{v}\beta\text{5}$  in the development of immune cells.** (A) Generation of  $\text{Itgb5}^{-/-} \rightarrow \text{WT}$  bone marrow chimeras. (B) Frequency amongst  $\text{CD45.2}^+$  leukocytes (top panels) and absolute numbers (lower panels) of colonic macrophage subsets, together with monocytes in peripheral blood of  $\text{Itgb5}^{-/-} \rightarrow \text{WT}$  and  $\text{WT} \rightarrow \text{WT}$  chimeric mice, determined by flow cytometry. Horizontal bars represent the medians and each symbol represents an individual mouse. Data pooled from three independent experiments with 4-5 mice/experiment. \* $p < 0.05$ , \*\*\* $p < 0.001$ , two-tailed Mann–Whitney test. (C) Generation of  $\text{CD45.1}^+ \text{Ccr2}^{-/-} : \text{CD45.2}^+ \text{WT}$  and  $\text{CD45.1}^+ \text{Ccr2}^{-/-} : \text{CD45.2}^+ \text{Itgb5}^{-/-}$  mixed bone marrow chimeras. (D) Representative FACS staining of colonic macrophage subsets showing chimerism 8 weeks after reconstitution. (E) Frequencies of donor-derived  $\text{CD45}^+$  leukocytes amongst colonic monocyte and macrophage subsets in  $\text{CD45.1}^+ \text{Ccr2}^{-/-} : \text{CD45.2}^+ \text{WT}$  and  $\text{CD45.1}^+ \text{Ccr2}^{-/-} : \text{CD45.2}^+ \text{Itgb5}^{-/-}$  mixed bone marrow chimeras. Horizontal bars represent the medians and each symbol represents an individual mouse. Data pooled from two independent experiments with 3-4 mice/experiment. \* $p < 0.05$ , \*\* $p < 0.01$ , two-tailed Mann–Whitney test.



**Figure 3. Functional consequences of loss of integrin  $\alpha\beta5$  on macrophages.** Q-PCR analysis of mRNA for (A) *Mmp2*, *Mmp9*, *Mmp13* and *Il10* by FACS sorted colonic monocytes (P1 and P2) and macrophages (P3/4) from *Itgb5*<sup>-/-</sup>→WT and WT→WT chimeric mice. Results show the mean of triplicate assays for each gene relative to cyclophilin A (CPA) calculated by the  $2^{-\Delta\Delta C(t)}$  method. ND: not detected. (B) Proportion of each colonic macrophage subset in *Itgb5*<sup>-/-</sup>→WT and WT→WT chimeric mice expressing TIM4. Horizontal bars in A, B represent the medians and each symbol represents an individual mouse, with data pooled from two independent experiments with 3-5 mice/experiment. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , two-tailed Mann Whitney test. C) Weight loss and D) clinical disease activity during acute DSS colitis in *Itgb5*<sup>-/-</sup>→WT and WT→WT chimeric mice. Data represent means  $\pm$  1sd for 9-11 mice/group pooled from two independent experiments with 4-6 mice/experiment. \* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , Student's t-test. (E) Frequency of colonic monocytes (P1 and P2), macrophages (P3/4) and eosinophils in chimeric mice on day 6 of DSS colitis and in controls. Horizontal bars represent the medians and each symbol represents an individual mouse. Data pooled from 5-6 mice/group from two independent experiments with 2-3 mice/experiment. \* $p < 0.05$ , \*\* $p < 0.01$  two-tailed Mann Whitney test.





### Graphical Abstract Legend

$\alpha\text{v}\beta\text{5}$  integrin acquired by intestinal macrophages as they differentiate locally from classical monocytes confers the ability to phagocytose apoptotic cells via recognition of phosphatidyl serine (PS) and the bridging molecule milk fat globule EGF-like molecule 8 (MFG-E8).

Absence of  $\alpha\text{v}\beta\text{5}$  leads to increased susceptibility to acute colitis, together with decreased numbers of monocytes and reduced expression of metalloproteinases and IL10.

