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1 **Oncostatin M drives intestinal inflammation in mice and its abundance**
2 **predicts response to tumor necrosis factor-neutralizing therapy in**
3 **patients with inflammatory bowel disease**

4

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1 **Abstract**

2 Inflammatory bowel diseases (IBD), including Crohn's disease (CD) and ulcerative colitis (UC),
3 are complex chronic inflammatory conditions of the gastrointestinal tract that are driven by
4 perturbed cytokine pathways. Anti-tumour necrosis factor- α (TNF) antibodies are a mainstay
5 therapeutic approach for IBD. However, up to 40% of patients are non-responsive to anti-TNF
6 agents, and identifying alternative therapeutic targets is a priority. Here we show that expression
7 of the cytokine Oncostatin M (OSM) and its receptor (OSMR) is increased in the inflamed
8 intestine of IBD patients compared to healthy controls, and correlates closely with
9 histopathological disease severity. OSMR is expressed in non-hematopoietic, non-epithelial
10 intestinal stromal cells, which respond to OSM by producing various pro-inflammatory factors
11 including interleukin-6 (IL-6), the leukocyte adhesion factor ICAM-1, and chemokines that
12 attract neutrophils, monocytes, and T cells. In an animal model of anti-TNF refractory intestinal
13 inflammation, genetic deletion or pharmacological blockade of OSM significantly attenuates
14 colitis. Furthermore, high pre-treatment OSM expression is strongly associated with failure of
15 anti-TNF therapy based on analysis of over 200 IBD patients, including two cohorts from phase 3
16 clinical trials of infliximab and golimumab. OSM is thus a potential biomarker and therapeutic
17 target for IBD, with particular relevance for anti-TNF refractory patients.

18

19 **Introduction**

20 IBD is an etiologically complex inflammatory disorder, involving aspects of genetic
21 predisposition, environmental triggers, microbial dysbiosis, and perturbation of immune
22 homeostasis¹⁻⁴. A crucial element of immune dysregulation in IBD is the inappropriate production
23 of diverse pro-inflammatory cytokines, which orchestrate intestinal inflammation and constitute
24 attractive targets for therapeutic development^{5,6}. Indeed, blockade of TNF using monoclonal
25 antibodies (anti-TNF therapy) is now firmly established as an effective therapeutic approach for
26 IBD. Nevertheless, up to 40% of patients with IBD exhibit primary non-responsiveness to anti-

1 TNF therapy, and many patients who are initially responsive develop therapeutic resistance^{7,8}.
2 Various other cytokines have been targeted in clinical trials (including interferon (IFN)- γ , IL-6,
3 and IL-17A) but their blockade has generally resulted in negligible efficacy or, in the case of IL-
4 17A, deleterious side effects^{5,9}. Therefore, we sought to identify novel cytokines that could
5 potentially serve as alternative therapeutic targets to TNF. In a large number of IBD patients, we
6 identified OSM (Oncostatin M) as a highly expressed cytokine that is associated with anti-TNF
7 resistant disease. Furthermore, OSM was found to promote intestinal pathology in an anti-TNF
8 resistant mouse model of IBD. Intriguingly, OSM appears to promote intestinal inflammation by
9 inducing chemokine, cytokine, and adhesion factor expression in gut-resident stromal cells, which
10 express high levels of the OSM receptor- β (OSMR).

11 OSM is part of the IL-6 cytokine family, which shares gp130 as a receptor subunit¹⁰.
12 Depending on the cell type, human OSM can induce signalling via the JAK-STAT pathway
13 (including JAK1, JAK2, STAT1, STAT3, STAT5, and possibly STAT6), the
14 phosphatidylinositol-3-kinase (PI3K)-Akt pathway, and mitogen activated protein kinase
15 (MAPK) cascades via heterodimeric receptors comprised of gp130 and either OSMR or leukemia
16 inhibitory factor receptor- β (LIFR)^{11,12}. In contrast, mouse OSM is thought to mediate similar
17 signal transduction mainly via gp130-OSMR heterodimers^{11,12}. OSM supports diverse
18 homeostatic processes, including liver repair, cardiac tissue remodeling, and
19 osteoclastogenesis^{11,12}. However, overproduction of OSM is thought to promote a variety of
20 pathologies, including skin and lung inflammation, atherosclerosis, and several forms of
21 cancer^{11,12}. Interestingly, a single-nucleotide polymorphism in the human *OSM* locus is strongly
22 associated with risk of developing IBD¹³. Nevertheless, the role of OSM in IBD has remained
23 unclear^{11,14,15}.

24

25

1

2

3 **Results**

4 **OSM and OSMR are highly expressed in IBD**

5 To identify additional cytokines that may promote IBD pathogenesis, we analyzed cytokine
6 mRNA expression in intestinal mucosal biopsies from previously published cohorts of patients
7 with clinically active CD ($n=162$ CD versus $n=42$ non-IBD controls; RNA-seq data) or UC ($n=74$
8 UC versus $n=11$ non-IBD controls; Affymetrix microarray data)^{16,17}. 64 candidate cytokines with
9 data available in both studies were examined. Of these, only 4 were significantly enriched in
10 inflamed tissue in both cohorts compared to non-IBD controls: *IL6*, *IL1A*, *IL1B*, and *OSM* (**Fig.**
11 **1A, Supplementary Table 1**). Among untreated paediatric patients with newly diagnosed CD¹⁶,
12 we found *OSM* to be the most highly and consistently expressed cytokine relative to healthy
13 control mucosa (**Fig. 1b, Supplementary Table 1**). Furthermore, *OSM* was particularly enriched
14 in patients with deep mucosal ulcerations (**Fig. 1c**). While *OSMR* was similarly enriched in IBD
15 mucosa, this was not true of *LIFR* or *IL6ST* (gp130) (**Fig. 1c**).

16 To validate our initial findings, we used quantitative real-time PCR (Q-PCR) to examine
17 *OSM* and *OSMR* expression in freshly isolated biopsies from IBD patients and healthy controls
18 who underwent routine endoscopy at the John Radcliffe Hospital (Oxford). This confirmed high
19 expression of *OSM* and *OSMR* in tissue from IBD patients with active disease (**Fig. 1d**), and also
20 revealed a close correlation between *OSM/OSMR* expression and histopathological disease
21 severity (**Fig. 1e**). No difference in expression of either *OSM* or *OSMR* was observed in patients
22 with CD or UC (**Fig. 1f**). Analysis of transcriptomic data from four different countries¹⁶⁻²⁰ further
23 confirmed that *OSM* and *OSMR* are consistently over-expressed in the intestinal mucosa of
24 patients with active IBD (total control $n=99$, total IBD $n=370$; **Supplementary Table 3**). Neither
25 *OSM* nor *OSMR* expression correlated with standard clinical parameters including gender, age at
26 diagnosis, disease duration, serum c-reactive protein (CRP), peripheral blood leukocyte count, or

1 treatment with pharmacological therapies; however, *OSM* and *OSMR* expression was increased in
2 patients with IBD who required surgery, suggesting an association with treatment-refractory or
3 complicated disease (**Supplementary Fig. 1**).

4 5 **Association of OSM expression with response to TNF-neutralizing therapy**

6 Hierarchical clustering of cytokine and chemokine expression in two IBD cohorts revealed that
7 *OSM* is consistently associated with a discrete module of inflammatory mediators (**Fig. 2a**,
8 **Supplementary Fig. 2**). Although molecular correlates of anti-TNF response have been reported
9 previously, there are currently no clinically accepted predictive biomarkers of anti-TNF response
10 for IBD^{18,21-23}. We therefore asked whether the *OSM*-associated inflammatory module was
11 associated with responsiveness to anti-TNF therapy. Among patients refractory to corticosteroids
12 or other immunosuppressive therapies, unsupervised hierarchical clustering based on expression
13 of *OSM*-associated module genes revealed that high module expression in pre-treatment biopsies
14 was strongly associated with primary non-responsiveness to anti-TNF therapy (**Fig. 1b**,
15 **Supplementary Fig. 3a,b**). Complete mucosal healing (based on endoscopic and histological
16 criteria) following infliximab (Remicade) therapy was achieved by 69–85% of patients with low
17 *OSM* module expression, but was observed in only 10–15% of those with high *OSM* module
18 expression (**Fig. 2c**, **Supplementary Fig. 3b**). Notably, baseline expression of *OSM* and *OSMR*
19 alone was strongly associated with poor primary response to infliximab in three different cohorts
20 (**Fig. 1d**, **Supplementary Fig. 3c,d**)^{18,22,23}. Indeed, at the whole transcriptome level, *OSM* was
21 among the 20 most strongly expressed genes in anti-TNF refractory patients compared to anti-
22 TNF responders (**Supplementary Table 4**). Baseline *OSM* and *OSMR* expression was also
23 elevated in patients who responded initially to infliximab but relapsed by week 30 post-treatment
24 (**Supplementary Fig. 3d**).

25 The association between mucosal *OSM* expression and anti-TNF response was confirmed
26 in two additional prospective patient cohorts from phase 3 clinical trials of moderate-to-severely

1 active UC, one treated with intravenous infliximab (**Fig. 2e–g**), and the other treated with
2 subcutaneous golimumab (Simponi), an alternative anti-TNF agent (**Supplementary Fig. 3e–**
3 **i**)^{24,25}. Unlike the cohorts described above, these trials categorized patients into response groups
4 on the basis of improvement in clinical Mayo scores. This allowed us to assess patients who
5 achieved full remission following therapy, those who partially responded (an improvement in
6 Mayo score, but with remaining disease activity), and those who were completely non-responsive
7 to therapy. While OSM was clearly most highly expressed in the non-responsive group of
8 infliximab-treated patients (**Fig. 2f**), it was significantly elevated in both non-responders and
9 partial responders who received golimumab (**Supplementary Fig. 3f**). Although OSM correlates
10 broadly with inflammation severity in the overall IBD population (**Fig. 1e**), OSM did not
11 correlate substantially with disease severity in these clinical trial cohorts, which were comprised
12 exclusively of patients with strong disease activity (**Fig. 2g, Supplementary Fig. 3h**). In
13 addition, baseline disease activity and clinical biomarker expression in these trials was not
14 significantly associated with treatment response (**Fig. 2e, Supplementary Fig. 3e–g**), suggesting
15 that OSM measurement prior to therapy could yield useful prognostic information that cannot be
16 obtained from conventional clinical assessment. Thus, analysis of five datasets (comprising 227
17 patients) demonstrates that high baseline *OSM* expression in the intestinal mucosa is reproducibly
18 associated with decreased responsiveness to anti-TNF therapy.

19

20 **Human intestinal stromal cells express high amounts of OSMR**

21 To gain insight into the role of OSM in the intestine, we conducted gene ontology network
22 analysis of human IBD transcriptomic data using ClueGo²⁶. Relative to TNF-high tissues (used as
23 a control for generic inflammation), OSM-high and OSMR-high tissues were strongly enriched in
24 genes related to leukocyte chemotaxis, extracellular matrix organization, and mesenchymal
25 development, suggesting that OSM may influence non-hematopoietic stromal cells
26 (**Supplementary Fig. 4**). Indeed, in mucosal biopsies from healthy donors and IBD patients

1 analyzed using Q-PCR, *OSMR* expression correlated closely with the well established fibroblast
2 products *COL1A1* (collagen 1A1) and *FAP* (fibroblast activation protein- α), as well as the
3 lymphoid tissue-like stromal markers *PDPN* (podoplanin/gp38) and *ICAM1* (intercellular
4 adhesion molecule-1; **Fig. 3a**)²⁷⁻³². All of these stromal genes were highly expressed in inflamed
5 IBD specimens (**Fig. 3b**). Furthermore, stromal cells with strong PDPN expression were highly
6 abundant in the colon lamina propria of patients with CD or UC (**Fig. 3c**).

7 Flow cytometry analysis of human intestinal mucosa revealed that OSMR is undetectable
8 in epithelial and hematopoietic cells, expressed in low amounts by endothelial cells, and strongly
9 expressed by the majority of CD45⁺EpCAM⁻CD31⁻ stromal cells (**Fig. 3d-f**). In contrast, OSM
10 was expressed by various hematopoietic populations in human intestinal mucosa, including CD4⁺
11 T cells and HLA-DR⁺ antigen presenting cells from both non-IBD controls and IBD patients
12 (**Supplementary Fig. 5a-d**). Notably, OSMR was expressed more abundantly than the related
13 IL-6 receptor by intestinal stromal cells from both non-IBD control and IBD patients
14 (**Supplementary Fig. 5g**). Consistent with the high amounts of OSMR produced by stromal cells,
15 OSM stimulation elicited phosphorylation of STAT3, STAT1, Akt, and ERK1/2 MAP kinases in
16 CCD18Co cells (primary human intestinal stroma), whereas IL-6 triggered only STAT3
17 phosphorylation (**Fig. 3g**). OSMR expression in different cell populations was equivalent in
18 healthy control and IBD patients in terms of both the frequency of OSMR^{high} cells (**Fig. 3h**) and
19 OSMR expression intensity (**Fig. 3i**). This suggests that the increased *OSMR* expression in
20 biopsies from IBD patients (**Fig. 1**) is due to an accumulation of OSMR-expressing stromal cells
21 in the tissue, not increased OSMR expression per cell. Because stromal cells vastly outnumber
22 endothelial cells in the intestine (**Supplementary Fig. 5h**) and are highly enriched in OSMR,
23 stromal cells appear to be the dominant intestinal OSMR⁺ population. Intriguingly, OSMR^{high}
24 stromal cells co-expressed PDPN and ICAM-1, a phenotype that is similar to fibroblastic reticular
25 cells (FRCs) in secondary lymphoid tissue (**Fig. 3j**)³³.

26

1 **OSM promotes inflammatory activity in the intestinal stroma**

2 To assess the response of intestinal stromal cells to OSM, we treated CCD18Co cells with
3 recombinant human OSM and used Q-PCR to profile expression of the OSM-associated
4 inflammatory module (see **Fig. 2a**). Notably, half of the module members were directly induced
5 by OSM stimulation, including *IL6* and a functionally diverse set of chemokines (**Fig. 4a**). OSM
6 also drove expression of ICAM-1 and PDPN, suggesting that it may enforce the phenotype of
7 OSMR^{high} stroma observed *in vivo* (**Fig. 3j**). The OSM response was strictly dependent on
8 OSMR, but not LIFR (**Supplementary Fig. 5i–j**).

9 To determine if a similar OSM response can be detected *in vivo*, we used fluorescence
10 activated cell sorting (FACS) to purify OSMR^{high} and OSMR^{low} stromal cells from colon tissue of
11 3 donors (**Fig. 4b, Supplementary Fig. 5k**). Q-PCR analysis of the purified cell populations
12 revealed similar expression of classical fibroblast products including collagens, lumican,
13 fibronectin, and CD90 (**Fig. 4c, Supplementary Fig. 5l**). However, relative to OSMR^{low} cells,
14 OSMR^{high} stroma expressed high amounts of several chemokines and cytokines including *IL6*,
15 *CCL2*, *CXCL1*, and *CXCL10*, consistent with the gene induction profile observed in CCD18Co
16 cells (**Fig. 4c**). This suggests that high OSMR expression identifies a more immunostimulatory
17 subset of intestinal fibroblasts.

18 Because OSM is known synergize with other inflammatory cytokines (in breast cancer,
19 for example), we asked whether combined OSM and TNF stimulation have synergistic effects in
20 human intestinal stroma. Indeed, some genes (such as the CXCR3 ligands CXCL9/10/11) were
21 synergistically induced in CCD18Co cells by combined OSM and TNF treatment, while others
22 such as *CCL2* were not (**Fig. 4d, Supplementary Fig. 5m**). Comparable responses were
23 observed in primary *ex vivo* cultures of human colonic stromal cells (**Fig. 4e, Supplementary**
24 **Fig. 5n**). Compared to stromal cells, HUVEC (human umbilical vein endothelial cells) were
25 weakly responsive to OSM, consistent with lower endothelial expression of OSMR
26 (**Supplementary Fig. 5o–p**). Intriguingly, stronger responses to OSM and TNF treatment were

1 observed in primary stromal cultures from IBD patients compared to cultures from non-IBD
2 controls (**Fig. 4f**). Although the mechanism underlying this difference is not clear, it may be
3 related to a similar phenomenon observed in fibroblast-like synoviocytes from rheumatoid
4 arthritis patients, which display imprinted hyperresponsiveness to inflammatory stimuli, possibly
5 due to mutations and/or epigenetic alterations³⁴.

6

7 **The OSM-stromal axis is conserved in mice**

8 To explore the OSM-stromal axis in a relevant pre-clinical setting, we used a model IBD system
9 driven by oral *Helicobacter hepaticus* infection and systemic IL-10 receptor blockade in wild
10 type C57BL/6 mice (*Hh*+ α IL-10R model, **Fig. 5a**)^{35,36}. This causes T cell-dependent pathology
11 that is resistant to anti-TNF therapy (**Supplementary Fig. 6a**)³⁷. Furthermore, this model does
12 not require signaling by IL-6, IL-1 α , or IL-1 β (**Supplementary Fig. 6b–c**). At peak disease
13 severity, colon lamina propria leukocytes increased 10-fold in abundance (**Fig. 5b**). *Osm* and
14 *Osmr* were highly expressed in the colons of colitic mice (**Fig. 5c**), and OSM protein was
15 secreted in abundance by inflamed intestinal explants and detectable in fecal matter (**Fig. 5d**).
16 The *in vivo* expression kinetics of OSM closely mirrored those of IL-6, IL-1 β , and TNF, but
17 differed substantially from IL-23, which is required at early time-points for induction of
18 pathogenic T cell responses (**Supplementary Fig. 6d**)^{35,38}.

19 Like humans, *Osm* displayed a hematopoietic expression pattern with relative enrichment
20 in antigen presenting cells, based on Q-PCR analysis of FACS-sorted colon populations from
21 healthy and colitic mice (**Fig. 5e**, **Supplementary Fig. 6e**). In contrast, *Osmr* expression was
22 restricted to intestinal stromal cells in both healthy and inflamed animals (**Fig. 5e**). The colon
23 stroma from inflamed mice also expressed high amounts of *Il1b* and *Il6*, suggesting that these
24 cells adopt a pro-inflammatory state during colitis (**Fig. 5e**). Furthermore, the colon lamina
25 propria of colitic mice was highly enriched in PDPN⁺ stromal cells, consistent with human IBD
26 (**Fig. 5f**). To determine the location of OSMR-expressing cells in the intestine, we used

1 RNAscope *in situ* hybridization. *Osmr* expression in healthy mouse colon tissue was detected in
2 endothelial and stromal cells, the latter distributed widely within the lamina propria along the
3 entire length of the crypt-villus axis, as well as within lymphoid clusters. No expression was
4 observed in epithelial cells, and a similar expression pattern was observed in the ileum
5 (**Supplementary Fig. 7a**). Consistent with the increased numbers of PDPN⁺ stromal cells and
6 increased OSMR expression in inflamed colon tissue, the number of cells expressing *Osmr* was
7 markedly increased in the lamina propria of colitic mice (**Fig. 5g**). Mouse colon stromal cells
8 responded strongly to OSM in a manner similar to that of human stroma (**Supplementary Fig. 7**).
9 In contrast, substantial OSM responsiveness was not observed in CD45⁺ leukocytes from mouse
10 spleen or colon, or from mouse colonic epithelial organoids (**Supplementary Fig. 7**). Taken
11 together, these data indicate that intestinal OSM biology of healthy and colitic mice is largely
12 consistent with that seen in humans.

13

14 **OSM drives colitis in a pre-clinical model of anti-TNF refractory IBD**

15 To determine if OSM can influence anti-TNF refractory colitis, we compared OSM-deficient
16 mice (*Osm*^{-/-}) to co-housed wild type littermates using the *Hh*+αIL-10R model. At steady state,
17 *Osm*^{-/-} mice showed normal organ histology (**Supplementary Fig. 8a**), normal development of
18 secondary and mucosal-associated lymphoid tissue (**Supplementary Fig. 8b–d**), a normal
19 leukocyte repertoire in lymphoid and intestinal tissue, and normal frequencies of non-
20 hematopoietic cells in the colon (**Supplementary Fig. 9**). At peak disease severity, *Osm*^{-/-} mice
21 displayed reduced colon pathology based on colonoscopy and histological assessment compared
22 to wild type controls, particularly with regard to severe disease features such as crypt abscess
23 formation, submucosal inflammation, and edema (**Fig. 6a–d**). This was not due to differences in
24 *H. hepaticus* colonization (**Supplementary Fig. 9k**).

25 Notably, *Osm*^{-/-} colons displayed normal activation of chemokine and cytokine
26 expression during the first week of colitis, as well as normal accumulation of leukocyte

1 populations in the lamina propria (**Fig. 6e, Supplementary Fig. 10a–d**). However, this response
2 was attenuated during week 2, in parallel with reduced accumulation of CD4⁺ T cells and
3 granulocytes. Reduced proliferation of colonic CD4⁺ T cells (determined by Ki-67 staining) could
4 not explain the differences in T cell abundance, and leukocyte accumulation in *Osm*^{-/-} mesenteric
5 lymph nodes was entirely normal (**Supplementary Fig. 10e–f**). This suggests that OSM has little
6 influence on the early/acute phase of inflammation, but enhances inflammation at later time
7 points by promoting stromal chemokine production and selective recruitment of CD4⁺ T cells and
8 granulocytes. Indeed, to confirm that trafficking of *Osm*^{-/-} leukocytes during acute inflammation
9 is normal, we employed a model of skin inflammation that involves topical application of
10 imiquimod (a toll-like receptor 7 agonist) to mouse ears over 6 days. Consistent with acute colon
11 inflammation, *Osm*^{-/-} and wild type littermates showed equivalent skin thickening and
12 recruitment of monocytes, granulocytes, and T cells to skin and cervical lymph nodes
13 (**Supplementary Fig. 10g–i**). Notably, in the *Hh*+αIL-10R colitis model, *Osm*^{-/-} displayed
14 reduced colon expression of the OSM-associated inflammatory module that is associated with
15 anti-TNF resistance in humans (**Supplementary Fig. 11a**).

16

17 **OSM neutralization suppresses TNF-refractory colitis in mice**

18 To test the therapeutic utility of OSM, we treated wild type C57BL/6 mice with an Fc-tagged
19 soluble OSMR-gp130 fusion protein (OR-Fc; **Fig. 6f, Supplementary Fig. 11b**)³⁹ starting at day
20 7 of the *Hh*+αIL-10R protocol, by which time colitis is readily detectable (**Supplementary Fig.**
21 **11c**). Compared to commercially available polyclonal anti-OSM antibodies, the OR-Fc construct
22 was more efficient at neutralizing OSM in an *ex vivo* mouse intestinal stromal culture assay
23 (**Supplementary Fig. 11b**). OR-Fc treatment significantly reduced colitis severity compared to
24 mock treatment (recombinant Fc protein), demonstrating the potential utility of OSM as a
25 therapeutic target (**Fig. 6g–h, Supplementary Fig. 11d**). Consistent with *Osm*^{-/-} mice, colonic

1 expression of the clinically relevant OSM-associated inflammatory module during colitis was
2 suppressed by therapeutic OSM blockade (**Fig. 6i**).

3

4 **Discussion**

5 IBD is a clinically challenging illness that strikes at a young age and causes life-long morbidity.
6 The high rate of primary and acquired resistance to therapy makes IBD a significant area of
7 unmet medical need, for which alternative therapeutic options and improved strategies for patient
8 stratification are urgently required. Although cytokines are well known to mediate the
9 dysregulated inflammatory state that characterizes IBD, few have proven useful as therapeutic
10 targets⁵. The notable exception is TNF, neutralization of which has been profoundly successful
11 for treating IBD. More recently, the IL-12/IL-23 neutralizing antibody ustekinumab has also
12 shown clinical efficacy for CD⁴⁰. Given that TNF, IL-12, and IL-23 are critical coordinators of
13 immune responses, it is possible that antibodies targeting cytokines such as IFN- γ and IL-17A
14 have failed to show efficacy because they do not hit key “master control” points in the cytokine
15 hierarchy. Thus, identifying cytokines that control an array of downstream inflammatory
16 processes may lead to effective targeted therapies for IBD.

17 In attempting to identify such cytokines, we discovered OSM to be consistently
18 overexpressed in inflamed intestinal tissue of mice and humans. Hematopoietically derived OSM
19 appears to mediate intestinal pathology by promoting inflammatory behavior in gut-resident
20 stromal cells, which constitutes a novel system of leukocyte-stromal cell crosstalk that may have
21 relevance in multiple mucosal tissues. OSM is expressed as part of a core inflammatory cytokine
22 module including IL-6 and IL-1 α/β , with effects that are distinct from the closely related IL-6 and
23 synergistic with those of TNF. Whereas IL-23 is a critical trigger of bacterially driven colitis
24 through its actions on T cells^{35,38}, OSM may act as an inflammatory amplifier and driver of
25 disease chronicity by promoting chemokine, cytokine, and adhesion factor production by
26 intestinal stromal cells (**Supplementary Fig. 11e**). Whether OSM can influence tissue fibrosis via

1 the stromal compartment remains to be determined. Intriguingly, OSM has been shown to bind
2 extracellular matrix components (including collagen, laminin, and fibronectin) in a manner that
3 protects it from proteolytic degradation and maintains biological activity for prolonged periods⁴¹.
4 This system could amplify the biological effects of OSM in chronic inflammation by promoting
5 accumulation of stable OSM protein, particularly in tissues with high amounts of extracellular
6 matrix deposition.

7 The immunological importance of intestinal stromal cells is not well understood; while
8 they can respond to microbial challenges and influence dendritic cell function under steady state
9 conditions^{27,29}, a critical inflammatory role for stromal cells in IBD has not been demonstrated²⁸.
10 Nevertheless, data from other inflammatory diseases such as rheumatoid arthritis support the
11 hypothesis that stromal cells are active contributors to immune pathology³⁴. The high frequency
12 of fibrotic complications in CD is consistent with a pathological role for intestinal stromal cells.
13 However, we found that lesional tissue from both CD and UC patients contains large numbers of
14 PDPN⁺ stromal cells, despite UC not being strongly associated with fibrosis. Similarly, mice
15 subjected to *Hh+αIL-10R* colitis show a substantial expansion of the intestinal stromal cell
16 compartment in the absence of overt intestinal fibrosis. These observations highlight the
17 possibility that intestinal stromal cells may contribute to inflammatory pathology in ways that
18 extend beyond the simple deposition of extracellular matrix components. A major unanswered
19 question regarding intestinal stromal cells is their ontogeny. Although the number of PDPN⁺
20 stromal cells clearly increases during intestinal inflammation in both mice and humans, it is
21 unknown whether they arise from expansion of tissue-resident precursors, are recruited from
22 elsewhere (e.g. via circulating precursors), or if they differentiate from a distinct cell type.
23 Similarly, it is not known if OSMR^{high} intestinal stromal cells represent a distinct mesenchymal
24 lineage, or simply a particular state of activation or differentiation.

25 To assess the role of OSM in a preclinical model of IBD, we chose to employ the
26 *Hh+αIL-10R* system. Although several murine models of IBD exist, we chose this system for the

1 following reasons: (a) it does not require the use of genetically modified mice, which precludes
2 the possibility of confounding developmental defects; (b) similar to current concepts of human
3 IBD etiology, it requires dual triggers in the form of infection with the commensal pathobiont *H.*
4 *hepaticus* and transient immune dysregulation via IL-10R blockade; (c) it is driven by a full
5 spectrum of innate and adaptive immune processes, as occurs in human IBD patients; and (c) it is
6 highly resistant to TNF blockade, making it ideal for investigating alternative drivers of colitis.
7 Nevertheless, we have observed high expression of OSM in various additional mouse models of
8 IBD, including chemically induced colitis and adoptive transfer of naïve CD4⁺ T cells to *Rag*^{-/-}
9 hosts, suggesting that OSM may be relevant beyond the *Hh*+*α*IL-10R model.

10 In addition to alternative therapeutic targets, IBD patients would benefit substantially
11 from improved systems for predicting disease course and response to therapy. In the case of anti-
12 TNF therapy, no biomarkers are currently used for predictive purposes in standard clinical
13 practice, and conventional clinical parameters are insufficient to predict therapeutic response²¹,
14 meaning that caregivers are forced to make treatment decisions with little knowledge of whether a
15 patient is likely to benefit. This places a large number of patients at unnecessary risk of
16 developing anti-TNF related complications (e.g. infections)^{42,43}, and inflates the economic burden
17 of IBD care. However, with a robust degree of reproducibility, we observed that high OSM
18 expression in intestinal mucosa is associated with a high risk of resistance to anti-TNF therapy.
19 Our data thus highlight the potential for developing a robust assay—based on measuring
20 expression of OSM or similar inflammatory factors—that could assist clinicians in determining
21 whether to prescribe anti-TNF antibodies or explore alternative therapeutic options.

22 While OSM can influence tissue remodeling in organs such as the heart and liver⁴⁴⁻⁴⁸,
23 *Osm*^{-/-} mice are viable and healthy, suggesting that therapeutic blockade of OSM may cause
24 minimal side effects. Indeed, OSM has been targeted for rheumatoid arthritis in phase I and II
25 trials using a humanized anti-OSM monoclonal antibody (GSK315234)⁴⁹. Little clinical efficacy
26 was observed, but the drug was well tolerated, with a dose-related decrease in platelet counts

1 being the most notable adverse effect (although all platelet counts remained within the normal
2 reference range). This is consistent with a prior report that OSMR-deficient mice have modestly
3 reduced platelet counts due to a reduction in bone marrow megakaryocyte progenitors⁵⁰. Because
4 *Osm*^{-/-} mice have reduced nociception⁵¹ and OSM has been implicated in other inflammatory
5 disorders such as psoriasis⁵²⁻⁵⁵ and arthritis⁵⁶⁻⁵⁹, which are common comorbidities of IBD⁶⁰, it is
6 tempting to speculate that OSM blockade could also be beneficial in managing extra-intestinal
7 manifestations of IBD. OSM and OSMR are over-expressed in the vast majority of active IBD
8 lesions, particularly in patients with anti-TNF resistant disease. OSM could therefore be a novel
9 predictive biomarker and therapeutic target for this clinically challenging population, and clinical
10 studies to evaluate this hypothesis are warranted.

1 Methods

2 **Human samples and cell isolation.** Intestinal pinch biopsies and surgical resection specimens
3 were obtained from healthy donors or IBD patients attending the John Radcliffe Hospital
4 Gastroenterology Unit (Oxford, UK). Biopsies were collected during routine endoscopy;
5 resections were obtained from patients with IBD undergoing surgery for severe disease,
6 chronically active disease, or complications of disease. Some non-IBD (non-inflamed) control
7 specimens were obtained from normal regions of bowel adjacent to resected colorectal tumors.
8 Informed, written consent was obtained from all donors. Human experimental protocols were
9 approved by the NHS Research Ethics System (Reference numbers: 09/H0606/5 for IBD patients
10 and 11/YH/0020 for controls). Tissues were prepared as previously described with minor
11 modifications⁶¹. In brief, mucosa was dissected and washed in 1mM DTT (dithiothreitol) solution
12 for 15 minutes at room temperature to remove mucus. Specimens were then washed three times in
13 0.75 mM EDTA (ethylenediaminetetraacetic acid) to deplete epithelial crypts and digested
14 overnight in 0.1 mg/ml collagenase A solution (Roche, UK). In some experiments, tissues were
15 rapidly digested for 2 hours using 1 mg/ml collagenase A. For enrichment of mononuclear cells,
16 digests were centrifuged for 30 minutes in a four-layer Percoll gradient and collected at the
17 40%/60% interface. Stromal cells were collected at the 30%/40% interface or were analyzed in
18 unfractionated samples. Stromal cells were cultured *ex vivo* as described²⁷. All solutions used
19 were supplemented with antibiotics (10,000 U/ml penicillin/streptomycin, 40 µg/ml gentamicin,
20 10 µg/ml ciprofloxacin, and 0.025 µg/ml amphotericin B (Sigma Aldrich, UK)).

21

22 **Human mucosal inflammation scoring.** Inflammation severity of human intestinal mucosa was
23 classified by both endoscopic and histological criteria. Endoscopic classification was binarized
24 into either inflamed or uninfamed categories based on assessment by the endoscopist. Where
25 possible, matched biopsies were collected from both active lesions and macroscopically normal
26 tissue at a distance from lesions. Endoscopic assessment was complemented by routine

1 histopathological scoring by a gastrointestinal pathologist. Tissues were classified as quiescent
2 (normal appearance), mildly inflamed, or severely inflamed.

3

4 **Analysis of transcriptomic data.** Whole transcriptome data were downloaded from the Gene
5 Expression Omnibus website (<http://www.ncbi.nlm.nih.gov/geo/>). Before analysis, data for genes
6 of interest were median-normalized and \log_2 transformed. When genes in microarray data were
7 represented by multiple probes, the probe with the greatest interquartile range was selected for
8 analysis. The following publically available datasets were used in this study: GSE57945 (ileal
9 biopsies from pediatric healthy controls and patients with newly diagnosed ileal CD, colonic CD,
10 and UC)¹⁶, GSE59071 (UC)¹⁷, GSE4183 (undefined IBD)¹⁹, GSE38713 (UC)²⁰, GSE16879
11 (matched pre-therapeutic and post-therapeutic biopsies of infliximab treated UC and CD)¹⁸, and
12 GSE12251 (UC before infliximab therapy)²². Unsupervised hierarchical clustering was performed
13 using Gene Cluster 3.0, with complete linkage as the clustering method and Euclidean distance as
14 the similarity metric.

15 In addition to the publically available datasets described above, we analyzed OSM
16 expression using unpublished transcriptomic data from two groups of patients with moderate-to-
17 severe UC who were part of anti-TNF clinical trials (ClinicalTrials.gov Identifiers NCT00207688
18 and NCT00487539). Patients from NCT00207688 were part of the ACT1/2 clinical trials of
19 intravenous infliximab therapy²⁴, and samples were collected and processed as described by
20 Toedter *et al* (2011)²³. Patients from NCT00487539 were part of the PURSUIT trial of
21 subcutaneous golimumab therapy²⁵. Briefly, mucosal colonic biopsies were collected at weeks 0
22 and 6 during endoscopy from a sub-group of PURSUIT patients at 15 to 20 cm from the anal
23 verge. Colon biopsies were also obtained from normal subjects who did not participate in the
24 PURSUIT study to serve as controls. Informed consent was obtained from healthy individuals to
25 undergo additional colonic biopsies for research purposes during colonoscopic procedures
26 performed as part of routine clinical care (such as colorectal neoplasia screening or evaluation of

1 gastrointestinal symptoms). The procedure verified that these individuals did not have
2 inflammatory conditions of the gastrointestinal tract including IBD.. The normal colon samples
3 were obtained from University of Pennsylvania School of Medicine (Philadelphia, PA) and
4 University Hospital Gasthuisberg (Leuven, Belgium). Normal colon sample collection complied
5 with the Principles of the Declaration of Helsinki and had ethics approval by the respective
6 institutional review boards. Following collection, the patient and normal samples were preserved
7 in RNAlater (Applied Biosystems, Foster City, CA). All biopsies were stored at -80°C until
8 RNA isolation was performed, which may have been up to 2 years following collection. RNA
9 was isolated and hybridized to the GeneChip HT HG-U133+ PM Array (Affymetrix, Santa Clara,
10 CA). Expression intensities were obtained from the Robust Multi-array Average (RMA)
11 algorithm. The microarray data were pre-processed and normalized by Robust Multi-array
12 Average using Array Studio software version 4.2 (OmicSoft Corp., St. Morrisville, NC). For
13 analysis of *OSM* expression, Affymetrix probeset 230170_PM_at was used.

14

15 **Definition of response to anti-TNF therapy**

16 The criteria for determining primary responsiveness to anti-TNF therapy are described in the
17 original reports of the GSE12251, GSE16879, and GSE23597 cohorts^{18,22,23}. Briefly, patients with
18 active IBD refractory to corticosteroids and/or immunosuppression underwent colonoscopy (with
19 biopsy collection) within a week prior to anti-TNF therapy. For cohorts GSE12251 and
20 GSE16879, response following treatment was defined as complete mucosal healing by both
21 endoscopic and histological criteria. For GSE23597, treatment response was defined as a
22 reduction from the baseline Mayo score of at least 3 points and at least 30%, with reduction in the
23 rectal bleeding subscore of at least 1 point or an absolute rectal bleeding score of 0 or 1. For the
24 ACT1/2 (NCT00207688)²⁴ and PURSUIT (NCT00487539)²⁵ clinical trials, response categories
25 were defined as follows: clinical remission (post-treatment Mayo score of 0–2); partial clinical
26 response (decrease from baseline Mayo score by $\geq 30\%$ and ≥ 3 points, but post-treatment score

1 ≥ 3); or no clinical response (decrease from baseline Mayo score of $<30\%$ and/or <3 points). It
2 should be noted that the definition of responsiveness in GSE23597 and the clinical trials was less
3 robust than in the GSE12251 and GSE16879 discovery cohorts, where remission was defined
4 strictly by the presence or absence of histologically evident inflammation following therapy.

5

6 **Mice.** Wild type C57BL/6, C57BL/6.*Osm*^{-/-}, and C57BL/6.*Il1r1*^{-/-} mice were bred and
7 maintained under specific pathogen free conditions in accredited animal facilities at the
8 University of Oxford. C57BL/6.*Osm*^{-/-} mice were acquired from the Jackson Laboratory (Maine,
9 USA, stock # 022338) and C57BL/6.*Il1r1*^{-/-} mice were a kind gift of Dr. Vincenzo Cerundolo.
10 All procedures were conducted in accordance with the UK Scientific Procedures Act of 1986.
11 Mice were negative for *Helicobacter* species and other known intestinal pathogens, were age and
12 sex-matched, and more than 6 weeks old when first used. Both male and female mice were used
13 in approximately equal proportions for all experiments. Mice were randomized to different
14 treatments and all treatments were represented in a given cage of animals. In experiments
15 involving *Osm*^{-/-} mice, knockout animals and wild type littermate controls were co-housed.
16 Experiments were replicated in two independent animal facilities within Oxford to control for
17 differences in housing conditions. Minimum sample sizes for individual experiments were
18 determined based not on a statistical method, but on experience with colitis models: $n=3$ for
19 steady state animals and $n=6$ for animals given experimental colitis.

20

21 ***Hh*+ α IL-10R colitis and *in vivo* treatments.** Experimental colitis was induced as described^{35,36}.
22 Briefly, mice were fed 1×10^8 colony forming units (c.f.u.) of *H. hepaticus* by oral gavage
23 delivered with a 22 G curved blunted needle on days 0 and 1 of the experiment. 1 mg of an
24 IL-10R blocking antibody (clone 1B1.2) was administered as an intraperitoneal injection once
25 weekly starting at day 0. In this model, disease severity peaks after 14 to 21 days and slowly

1 resolves thereafter. To neutralize OSM *in vivo*, mice were treated with a previously described
2 OSM receptor fusion protein (OR-Fc)³⁹. For increased *in vivo* stability, this construct was tagged
3 with the Fc region of mouse IgG2A. OR-Fc was administered as a 150 µg intraperitoneal
4 injection every 2 days (equivalent to approximately 6 mg/kg). Molar-equivalent doses of IgG2A-
5 Fc (manufactured under the same conditions as OR-Fc) were used as control treatments. Some
6 mice were also treated with a TNF-neutralizing antibody (clone XT3.11, Bio X Cell, USA) at a
7 total weekly intraperitoneal dose of 1 mg per animal. This dose was found to completely abrogate
8 intestinal pathology in 129SvEv.*Rag*^{-/-} mice infected with *H. hepaticus* (not shown). For
9 experiments involving anti-IL6R treatment and C57BL/6.*Il1r1*^{-/-} mice, anti-IL10R was injected
10 once per week (starting at day 0) and animals were sacrificed after 4 weeks. Similarly, anti-IL6R
11 (clone D7715A7) was administered once per week as a 1 mg intraperitoneal dose starting at day
12 0.

13

14 **Scoring of mouse colitis.** Colonoscopy to assess colitis severity was performed and scored
15 according to the methods of Becker *et al*⁶². Histological assessment of colitis severity was
16 performed as described⁶³. Briefly, formalin-fixed paraffin-embedded cross-sections of proximal,
17 middle, and distal colon were stained with hematoxylin and eosin and graded on a scale of 0 to 3
18 for four parameters: epithelial hyperplasia and goblet cell depletion, leukocyte infiltration, area
19 affected, and features of severe disease activity. Common severity features include crypt abscess
20 formation, submucosal leukocyte infiltration, and interstitial edema. Scores for each criterion are
21 added to give an overall score of 0 to 12 per colon section. Data from the three colon regions are
22 then averaged to give an overall score. Scoring was conducted in a blinded fashion and confirmed
23 by an independent blinded observer. Interobserver Pearson correlation coefficients ranged from
24 0.90 to 0.95.

25

1 **Mouse colon tissue preparation and cell isolation.** Mouse colons were washed with EDTA to
2 remove epithelium and digested with collagenase VIII to liberate cell populations as described⁶⁴.
3 Tissue digests were separated by centrifugation on a 30%/40%/70% percoll gradient. Cells at the
4 30%/40% interface were collected as the stroma/epithelium-enriched fraction, whilst cells at the
5 40%/70% interface were collected as the lamina propria leukocyte enriched fraction. For *ex vivo*
6 stromal culture, stromal fractions were plated and cultured as described³⁶.

7

8 **Colon explant cultures.** Mouse proximal colon segments (0.25 cm²) were cultured overnight in
9 RPMI media with 10% fetal calf serum (FCS) and 10,000 U/ml penicillin/streptomycin. OSM
10 was quantified in the supernatant by enzyme-linked immunosorbent assay (ELISA, R&D
11 Systems, UK) and normalized to explant weight.

12

13 **Stimulation of stromal and endothelial cells.** CCD18Co cells (primary human intestinal
14 fibroblasts; ATCC, not currently listed as misidentified on the ICLAC database) and primary *ex*
15 *vivo* stromal cultures were grown in humidified incubators with 5% CO₂ at 37°C in DMEM
16 media (Sigma) with 10% FCS and 2% human serum (Sigma). HUVEC cells (Gibco) were
17 cultured as above in Medium 200 with low serum growth supplement (Gibco) according to
18 manufacturer instructions. For cytokine stimulation experiments, unless otherwise indicated, all
19 treatments were for two hours, and all cytokines were administered at a concentration of 10
20 ng/ml. Cells were cultured for no more than 8 passages to avoid onset of senescence, phenotypic
21 drift, and contamination (e.g. by mycoplasma). All cytokines were purchased from Peprotech. To
22 knock down OSMR expression in CCD18Co cells, Accel SMARTpool siRNA was used
23 following manufacturer instructions (GE Healthcare Dharmacon, USA), and cells were stimulated
24 to test for OSM sensitivity after 72 hours of transfection.

25

1 **RNA extraction, cDNA synthesis, and qPCR.** Tissues were disrupted using lysis beads and a
2 homogenizer unit (Precellys, UK) in RLT buffer (Qiagen, UK). Sorted or cultured cells were
3 lysed directly in RLT buffer and homogenized by pipetting. RNA was isolated using RNEasy
4 Mini or Micro kits (Qiagen, UK) followed by reverse transcription using random primers
5 (Applied Biosystems, UK). Quantitative PCR (qPCR) was performed using Taqman assays
6 (Applied Biosystems) and PrecisionPlus Mastermix (Primer Design, UK) on a ViiA7 384-well
7 real-time PCR detection system (Applied Biosystems). All expression levels were normalized to
8 an internal house keeping (HK) gene (*RPLP0* for human samples and *Hprt* for mouse samples)
9 and calculated as $2^{-(CT_{HK}-CT_{gene})}$.

10

11 **Flow cytometry and cell sorting.** Mouse cells were stained with combinations of the following
12 monoclonal antibodies according to manufacturer protocols: CD3-PE (UCH-T1), CD4-BV605
13 (RM4-5), CD8-APC (53-6.7), CD11b-PerCP-Cy5.5 (M1/70), CD11c-eFluor450 (N418), CD19-
14 biotin (6D5), CD31-BV605 (390), CD44-V500 (IM7), CD44-AF700 (IM7), CD45-BV650 (30-
15 F11), CD45-AF700 (30-F11), Foxp3-eFluor450 (FJK-16s), PDPN-PE-Cy7 (8.1.1), Gr1-APC
16 (RB6-8C5), ICAM1-PE (YN1/1.7.4), Ly6C-PE-Cy7 (HK1.4), MHCII-AF700 (M5/114.15.2),
17 siglec-f-PE (E50-2440), and TCR β -BV510 (H57-597). Human cells were stained with the
18 following monoclonal antibodies: CD3-BV510 (OKT3), CD4-PE-Dazzle594 (RPA-T4), CD19-
19 BV650 (HIB19), CD31-BV605 (WM59), CD45-AF700 (HI30), CD45RA-PE-Cy7 (HI100),
20 EpCAM-FITC (9C4), PDPN-AF647 (NC-08), HLA-DR-BV711 (L243), ICAM1-BV421 (HA58),
21 OSM-APC (17022), OSMR-PE (AN-V2), TNF-eFluor450 (MAb-11), IFN- γ -FITC (B27), and IL-
22 17A-PE (eBio64DEC17). All antibodies were from eBioscience (UK), Biolegend (UK), Becton
23 Dickinson (UK), or R&D Systems (UK). Dead cells were excluded using eFluor-780 fixable
24 viability dye (eBioscience). Samples were acquired on FACS LSRFortessa and FACS LSRII flow
25 cytometers (Becton Dickinson). Cell sorting was performed using a FACS ARIA III (Becton
26 Dickinson). Data were analyzed using FlowJo (Tree Star, USA). For intracellular cytokine

1 staining, cells were restimulated with PMA (5 ng/ml; Sigma-Aldrich), ionomycin (500 ng/ml;
2 Sigma-Aldrich), and 5 µg/ml brefeldin A (Sigma-Aldrich). After 4 hours, cells were stained with
3 fixable viability dye and surface markers, fixed with 2% formaldehyde (Merck, UK), and stained
4 for intracellular cytokines in permeabilization buffer containing 0.05% saponin (Sigma-Aldrich).
5 For staining Foxp3, cells were stained with fixable viability dye and surface markers prior to
6 fixation and permeabilization using the Foxp3 staining buffer kit (eBioscience) according to
7 manufacturer instructions.

8

9 **OSMR staining.** To stain OSMR in human samples for flow cytometry analysis, cells were
10 labeled with primary OSMR-PE antibody (clone AN-V2, 2 µg/ml) followed by three rounds of
11 amplification with anti-PE-biotin antibody (clone PE001, 2.5 µg/ml (Biolegend, UK)), and
12 streptavidin-PE (0.4 µg/ml (Biolegend, UK)). A separate cell sample was labeled with isotype
13 control antibody (mouse IgG1-PE) and similarly amplified to control for background staining.
14 Specificity of the anti-OSMR antibody was confirmed by siRNA knockdown of OSMR
15 expression in a prior publication⁶⁵.

16

17 **Immunohistochemistry.** Formalin-fixed paraffin-embedded tissues were sectioned at five
18 microns and collected onto Superfrost glass slides. Tissue sections were dewaxed in xylene and
19 rehydrated through graded alcohol to water. Endogenous peroxidase activity was blocked with
20 3% (v/v) hydrogen peroxide before masked antigens were retrieved by microwaving the tissue
21 sections in target retrieval solution (Dako). Endogenous avidin and biotin were blocked (Vector
22 Laboratories) and the tissue sections blocked with 10% (v/v) normal horse serum (Sigma
23 Aldrich). Human tissue sections were incubated overnight at 4°C in a humidified environment
24 with monoclonal mouse anti-PDPN antibody (Clone D2-40; Dako). Primary labelling was
25 detected using biotinylated horse anti-mouse IgG secondary antibody (Vector Laboratories).
26 Tissue sections were then incubated with streptavidin-horseradish peroxidase (Vector

1 Laboratories) and signal detected using diaminobenzidine (Vector Laboratories). Tissue sections
2 were counterstained with Mayer's Haematoxylin (Sigma Aldrich) before being dehydrated
3 through graded alcohol to xylene and mounted with DPX and coverslips applied. Mouse tissues
4 were embedded into paraffin wax, sectioned, and antigens unmasked as above. Tissue sections
5 were labelled with APC-conjugated hamster anti-mouse PDPN (clone 8.1.1; Biolegend) or APC-
6 conjugated IgG isotype control, before being counterstained with Hoechst 33258 and mounted in
7 N-propyl gallate in glycerol-PBS. Images were collected on an Olympus BX51 microscope.
8 PDPN was chosen as a stromal marker for immunohistochemistry for several reasons, including
9 (a) anti-PDPN antibodies display excellent signal to noise ratio on formalin-fixed tissue sections;
10 (b) PDPN staining is clearly specific for stromal and lymphatic endothelial cells in mouse and
11 human based on flow cytometry analysis; and (c) the immunohistochemical performance of anti-
12 PDPN staining can be easily confirmed by observing staining of endothelial vessels.

13 For detection of mouse CD3, B220, and F4/80, tissue samples were fixed in formalin and
14 embedded in paraffin. Paraffin sections were dewaxed and stained with hematoxylin and eosin
15 (H&E) for overview. For immunohistochemistry, the sections were incubated with anti-B220
16 (clone RA3-6B2, eBioscience) followed by incubation with secondary antibody (rabbit anti-rat,
17 Dako). For detection, EnVision+ System-HRP Labelled Polymer Anti-Rabbit (Dako) was used.
18 HRP was visualized with the chromogen diaminobenzidine (Dako). After color development,
19 sections were subjected to a heat-induced epitope retrieval step prior to incubation with anti-
20 CD3 ϵ antibody (clone M-20, Santa Cruz) followed by incubation with biotinylated secondary
21 antibody (Dianova). For detection, alkaline phosphatase-labelled streptavidin and chromogen
22 RED (both Dako) were employed. For the detection of macrophages, sections were subjected to
23 protein-induced epitope retrieval employing protease (Sigma) prior to incubation with anti-F4/80
24 (clone BM8, eBioscience) followed by incubation with biotinylated rabbit anti-rat secondary
25 antibody (Dako). Biotin was detected using alkaline phosphatase-labelled streptavidin (Dako).
26 For visualization of alkaline phosphatase, chromogen RED (Dako) was used. Negative controls

1 were performed by omitting the primary antibody, and nuclei were stained with hematoxylin.
2 Sections were coverslipped with glycerol gelatin (Merck).

3

4 ***Osmr* detection by *in situ* hybridization.** For detection of mouse *Osmr* mRNA, the RNAScope[®]
5 2.5 HD Reagent Kit-RED (ACD Europe SRL) was used. Briefly, paraffin sections were freshly
6 cut, dried for 1 hour at 60°C and dewaxed prior to mild unmasking with Target Retrieval buffer
7 and protease. Pretreated sections were hybridized with specific probes to *Omsr* and *Ppib* (positive
8 control) and irrelevant probe to *dapb* as a negative control. These were accompanied by an
9 additional slide with formalin-fixed and paraffin embedded 3T3 cell line as an additional positive
10 control. After hybridization signal amplification, binding of probes was visualized using FastRed.
11 Nuclei were stained with hematoxylin and sections were coverslipped with Ecomount.
12 Images were acquired using the Axiolmager Z1 microscope (Carl Zeiss MicroImaging). All
13 evaluations were performed in a blinded manner.

14

15 **Total protein extraction and immunoblot analysis.** Total protein extracts were prepared as
16 described⁶⁶. Equal protein amounts were resolved by SDS-PAGE and analyzed with anti-p-
17 STAT3 (D3A7), anti-p-STAT1 (D4A7), anti-p-ERK1/2 (D13.14.4E), anti-p-AKT (D9E), anti-p-
18 p38 (D3F9), and anti-β-actin (13E5). All antibodies were from Cell Signaling, UK.

19

20 **Statistical analysis.** Unless otherwise indicated, all bar charts represent means +/- S.E.M.
21 Parametric and non-parametric analyses were used where appropriate based on testing for a
22 normal distribution using the D'Agostino-Pearson Omnibus normality test. Statistical tests were
23 two-sided and specified in figure legends. Differences were considered to be significant when
24 $p < 0.05$. Multiple testing corrections were applied where appropriate. In rare situations, data
25 points were excluded from analysis only if they were found to be outliers using the ROUT
26 method at Q=1% (pre-determined criteria).

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1 Figure Legends

2

3 **Figure 1. Expression of OSM and OSMR in the inflamed intestinal tissue of patients with**
4 **IBD. (a)** Identification of cytokines associated with intestinal inflammation in CD and UC
5 patients. Data were derived from Gene Expression Omnibus (GEO) datasets GSE57945 ($n=42$
6 controls and $n=162$ CD) and GSE59071 ($n=11$ controls and $n=74$ UC). Briefly, mRNA
7 expression of 64 cytokines was compared in IBD versus healthy control intestinal tissue using t -
8 tests with false discovery rate correction ($Q=1\%$). Significant hits were further selected using a
9 fold difference threshold of ≥ 2 . **(b)** RNA sequencing analysis of 64 cytokine genes in pediatric
10 treatment-naïve CD patients ($n=162$) versus non-IBD controls ($n=42$; GEO #GSE57945). Blue
11 symbols, not statistically significant after t -tests with FDR correction ($Q=1\%$); red symbols,
12 significantly altered cytokines. **(c)** Expression of *OSM*, *OSMR*, *LIFR*, and *IL6ST* (gp130) in the
13 GSE57945 dataset. Statistics: one-way ANOVA with Tukey's multiple comparisons tests
14 ($df=201$). **(d–f)** Q-PCR analysis of *OSM* and *OSMR* in intestinal mucosal biopsies from IBD
15 patients and healthy controls (Oxford cohort, see Supplementary Table 3 for details). **(d)**
16 Specimens categorized by macroscopic evidence of disease activity determined during endoscopy
17 (includes IBD patients with no macroscopic inflammation (uninflamed), uninflamed specimens
18 from patients with inflammation elsewhere in the bowel (uninvolved), and macroscopically
19 inflamed specimens (lesional tissue)). Statistics: one-way ANOVA with Tukey's multiple
20 comparisons tests ($df=88$). **(e)** Samples categorized by inflammation severity, determined by
21 routine clinical histopathological assessment of matched biopsies. Statistics: one-way ANOVA
22 with Tukey's multiple comparisons tests ($df=74$). **(f)** Analysis of inflamed lesions from active CD
23 or UC.

24

25 **Figure 2. Association of OSM with response to anti-TNF therapy. (a)** Identification of an
26 OSM-associated inflammatory module in IBD. Expression of genes encoding chemokines and

1 cytokines was examined in two cohorts, including healthy controls and patients with CD or UC
 2 (see Supplementary Fig. 2). Samples were grouped using unsupervised hierarchical clustering and
 3 21 genes that correlated closely with OSM in both cohorts were identified. **(b)** Unsupervised
 4 hierarchical clustering of OSM-associated module genes in colonic biopsies of UC patients
 5 refractory to corticosteroids or immunosuppression prior to infliximab therapy (GEO
 6 #GSE12251). Patients form two groups after clustering: one with low (blue dendrogram) and one
 7 with high OSM-associated module expression (red dendrogram). Patients with complete
 8 histological and endoscopic disease resolution are indicated in white, while treatment-refractory
 9 patients are indicated in black (see bar below dendrograms). Data are median-normalized and \log_2
 10 transformed. Rates of infliximab response in these patients are shown in panel **(c)**. Statistics:
 11 Fisher's exact test. **(d)** Receiver operator characteristic analysis of *OSM* and *OSMR* expression,
 12 distinguishing infliximab responders and non-responders in the GSE12251 dataset. **(e–g)** An
 13 independent cohort of patients with moderate-to-severe UC treated with infliximab as part of a
 14 long-term safety study (clinical trial NCT00207688). **(e and f)** Mayo scores and colonic *OSM*
 15 expression at baseline and 8 weeks after therapy in different response groups (see Methods for
 16 definition of clinical response). **(g)** Pearson correlation of baseline *OSM* expression and Mayo
 17 score.

18

19 **Figure 3. Non-hematopoietic stromal cells are prevalent in inflamed intestinal tissue and**
 20 **express high levels of OSMR.** **(a)** Spearman correlation of *OSMR* expression with stromal genes
 21 in pooled healthy control and IBD biopsies assessed by Q-PCR (Oxford cohort, $n=73$). **(b)** Mean
 22 mRNA z-scores for the indicated stromal genes (top) and stromal signature expression (bottom)
 23 in intestinal biopsies from healthy controls or IBD patients (uninflamed, no endoscopic evidence
 24 of disease; uninvolved, uninflamed samples from patients with active disease; inflamed, samples
 25 from inflamed lesions). The stromal signature was calculated as the average \log_2 expression of
 26 *COL1A1*, *FAP*, *ICAMI*, and *PDPN*. Statistics: one-way ANOVA with Tukey's multiple

1 comparisons tests (DF=69). (c) Immunohistochemical staining of PDPN in colon tissue from
2 representative non-IBD control, CD, and UC patients (scale bar=250 μ m). (d–f) Flow cytometry
3 analysis of surgically resected human intestinal mucosa ($n=11$ donors). (d) Identification of
4 leukocytes, epithelial cells, endothelial cells, and stroma. (e) OSMR expression and isotype-
5 normalized geometric mean fluorescence intensity (gMFI). Mean (+/- s.e.m.) gMFI of
6 endothelial and stromal cells was compared using t -tests ($t=3.924$, $df=20$). (f) OSMR expression
7 frequencies. (g) Cropped Western blot images of cytokine-stimulated (10 ng/ml, 20 minutes)
8 CCD18Co cell lysates. (h) OSMR expression frequencies in colon mucosal cell populations from
9 non-IBD controls or patients with IBD. (i) Intensity of OSMR expression on colon endothelial
10 and stromal cells from non-IBD controls or patients with IBD. (j) Representative flow cytometry
11 staining of OSMR, ICAM-1, and PDPN on colon stroma, with ICAM-1 and PDPN gMFIs
12 quantified below. Statistics: Mann-Whitney U tests ($n=11$).

13

14 **Figure 4. OSM promotes inflammatory behavior in human intestinal stroma.** (a) Q-PCR
15 analysis of OSM-induced genes in triplicate cultures of CCD18Co cells (normal human colonic
16 stroma), relative to untreated conditions. Results are representative of three independent
17 experiments. (b–c) Analysis of OSMR^{low} and OSMR^{high} stromal cells purified from resected
18 human intestinal tissue using fluorescence-activated cell sorting (FACS). (b) Post-sorting cell
19 purities from a representative non-IBD control. (c) Q-PCR analysis of stromal and inflammatory
20 genes in the purified stromal fractions from $n=3$ donors. (d) Q-PCR analysis of triplicate
21 CCD18Co cultures (representative of three independent experiments). Cells were stimulated with
22 human OSM, TNF, IL-6, or combinations of the three for 2 hours and compared to untreated
23 controls. Statistics: one-way ANOVA with Dunnett's multiple comparisons tests ($df=14$). (e) Q-
24 PCR analysis of primary intestinal stromal cultures from 10 donors, stimulated as in panel (d).
25 Data represent fold changes between matched untreated and cytokine-stimulated samples.
26 Statistics: Wilcoxon signed rank test, versus a theoretical median of 1. (f) Q-PCR analysis of

1 *CXCL9* and *CCL2* expression in cytokine-stimulated stromal cultures from non-IBD controls
 2 ($n=7$) and IBD patients ($n=3$). Data represent fold changes between matched untreated and
 3 cytokine-stimulated samples. Statistics: *t*-tests ($df=8$). For *CXCL9*, $t=3.594$ (OSM), $t=3.493$
 4 (TNF), and $t=8.278$ (OSM+TNF). For *CCL2*, $t=1.928$ (OSM), $t=3.940$ (TNF), and $t=4.87$
 5 (OSM+TNF).

6

7 **Figure 5. The OSM-stromal cell axis is conserved in anti-TNF refractory murine colitis. (a)**

8 Induction of colitis using the *Hh*+ α IL-10R protocol. Mice are sacrificed at day 14 or day 21,

9 which corresponds to peak disease severity. **(b)** Total live CD45⁺ cells in the colon lamina propria

10 at day 14 ($n=8$ steady state and $n=9$ colitic mice, representative of >3 independent experiments).

11 **(c)** Q-PCR analysis of *Osm* and *Osmr* expression in whole-colon tissue from steady state ($n=8$)

12 and colitic mice ($n=15$), representative of >3 independent experiments. **(d)** OSM measured by

13 ELISA in colon explant supernatants and cecal stool extracts from one of two independent

14 experiments (steady state $n=4$, colitis $n=10$). **(e)** Q-PCR gene expression analysis of FACS-

15 purified populations from mouse colon (representative of two independent experiments). Each

16 data point represents lamina propria cells pooled from two mice. Stromal cells were defined as

17 CD45⁺EpCAM⁻CD31⁻. **(f)** Immunofluorescent detection of PDPN⁺ stromal cells in healthy and

18 inflamed mouse colon tissue. Scale bars, 250 μ m (left) and 100 μ m (right). **(g)** Detection of *Osmr*

19 expression in healthy and inflamed mouse colon tissue using *in situ* hybridization (punctate red

20 signal). Tissues were counterstained with hematoxylin. Examples of *Osmr*-expressing cells in

21 healthy tissue are indicated with arrowheads. Scale bars, 250 μ m (top) and 100 μ m (bottom).

22 PDPN and *Osmr* images are representative of 3 different mice per condition.

23

24 **Figure 6. OSM promotes anti-TNF refractory colitis *in vivo*. (a–d) *Hh*+ α IL-10R colitis in**

25 wild type C57BL/6 mice and *Osm*^{-/-} littermates. **(a)** Representative colonoscopy images at day

1 21, with endoscopic pathology scores shown in panel **b**. Data represent one of three independent
2 experiments. **(c)** Representative H&E stained mid-colon cross-sections of healthy mice and colitic
3 animals (day 21). Single arrows, crypt abscesses; double-arrows, submucosal edema. Scale bars:
4 500 μ m (steady state) and 250 μ m (colitic). **(d)** Overall histopathology scores and sub-scores for
5 days 14 and 21. $n \geq 7$ mice per time-point, pooled from three experiments. *P*-values reflect
6 differences between genotypes and are derived from two-way ANOVA. **(e)** Expression of
7 cytokine and chemokine genes in whole colon tissue from mice subjected to *Hh*+ α IL-10R colitis
8 for 4, 9, or 14 days ($n=4-6$ per group). Expression values were averaged for mice within each
9 genotype and timepoint group, and converted to z-scores. **(f)** Therapeutic blockade of OSM in the
10 *Hh*+ α IL-10R model. OR-Fc (150 μ g every two days) or a molar-equivalent dose of Fc control
11 protein were injected intraperitoneally starting at day 7. **(g, h)** Representative H&E stained mid-
12 colon cross-sections of OR-Fc or Fc treated mice and associated histopathology scores ($n=5-13$
13 mice per group, pooled from three experiments). *P*-values reflect differences between treatments
14 and are derived from two-way ANOVA. Single arrows, crypt abscesses; double-arrows,
15 submucosal edema and inflammatory infiltrate. Scale bar=250 μ m. **(i)** Expression of the OSM-
16 associated inflammatory module in colons of mice treated as depicted in panel **f**. Data represent
17 one of three independent experiments.

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8

1 **Competing Interests**

2

3 NRW, ANH, BMJO, and FP are inventors of patents relating to OSM as a therapeutic target and
4 biomarker for inflammatory bowel disease. NRW, ANH, BMJO, SPLT, and FP are shareholders
5 in ImmElpis Pharma Ltd.

6

7 SK has received honoraria and/or research support from Allergan, Abbvie, Astra-Zeneca,
8 ChemoCentryx Inc., Dr Falk Pharma, Ferring, Gilead, GSK, Merck, Mitsubishi-Tanabe Pharma,
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10

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13 Ingelheim, Bristol-Myers Squibb, Celgene, Chemocentryx, Cosmo, Ferring, Giuliani SpA, GSK,
14 Lilly, MSD, Neovacs, NovoNordisk, Norman Collison Foundation, Novartis, NPS
15 Pharmaceuticals, Pfizer, Proximagen, Receptos, Shire, Sigmoid Pharma, Takeda, Topivert, UCB,
16 VHsquared, and Vifor Pharma. SPLT has received speaker fees from Abbvie, Biogen, Ferring,
17 and Takeda.

18

19 BL, FB, CB, and SEP are employees of Janssen Research and Development LLC.