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Mucus Enhances Gut Homeostasis and Oral Tolerance by Delivering Immunoregulatory Signals

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Abstract

A dense mucous layer in the large intestine prevents inflammation by shielding the underlying epithelium from luminal bacteria and food antigens. This mucous barrier is organized around the hyperglycosylated mucin MUC2. Here we show that the small intestine has a porous mucous layer, which permitted the uptake of MUC2 by antigen-sampling dendritic cells (DCs). Glycans

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Supplementary Materials

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Materials and Methods

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associated with MUC2 imprinted DCs with anti-inflammatory properties by assembling a galectin-3-Dectin-1-Fc γ RIIB receptor complex that activated β -catenin. This transcription factor interfered with DC expression of inflammatory but not tolerogenic cytokines by inhibiting gene transcription through nuclear factor- κ B. MUC2 induced additional DC-conditioning signals via intestinal epithelial cells. Thus, mucus does not merely form a nonspecific physical barrier, but also constraints the immunogenicity of gut antigens by delivering tolerogenic signals.

Mechanisms whereby the gut mucosa tolerates commensal bacteria and food antigens without developing inflammation remain elusive. Though traditionally viewed as a nonspecific barrier between the host and the environment, mucus also regulates gut homeostasis. The building block of gut mucus is MUC2, a gel-forming mucin secreted by goblet cells (GCs) (1). In the large intestine (LI), MUC2 prevents inflammation by generating an outer nonattached mucous layer inhabited by the microbiota and an inner mucous layer adherent to intestinal epithelial cells (IECs) and impervious to bacteria (1). The structure and function of mucus in the small intestine (SI) are less well understood.

The SI harbors bacteria that promote homeostasis by inducing Foxp3⁺ T regulatory (Treg) cells and B cell production of immunoglobulin A (IgA) antibodies (Abs) in Peyer's patches (PPs) and mesenteric lymph nodes (MLNs) (2, 3). These responses involve sampling of bacteria by CD11c⁺ dendritic cells (DCs) (4), including macrophage-like CD103⁻CD11b⁺CX3CR1⁺ DCs and myeloid CD103⁺CD11b⁺CX3CR1⁻ DCs (2, 5, 6). In the lamina propria (LP), myeloid DCs sample soluble antigens from GC-associated passages and cooperate with lymphoid CD103⁺CD11b⁻CX3CR1⁻ DCs to generate Treg cells (7–9). Such antigen-sampling activities require a porous mucous barrier, raising questions as to how MUC2 prevents inflammation in the SI.

MUC2 Mitigates Inflammatory Responses in DCs

We first analyzed the structure of gut mucus in wild type (WT) C57BL/6 mice. While LI mucus formed a dense bilayered barrier that segregated bacteria from IECs, SI mucus was less organized and thus permitted the adhesion of bacteria to IECs (fig. S1A–C). Consistent with the presence of some MUC2-coated bacteria on IECs and inside DCs (fig. S2A–C), CX3CR1⁺ DCs from both PPs and SI-LP captured carboxyfluorescein succinimidyl ester (CFSE)-labeled MUC2 bound to fluorescent bacteria (fig. S3A–C). In agreement with recent studies (6), also some CD103⁺ DCs captured MUC2-coated bacteria (fig. S3A–D). Similarly, human monocyte-derived DCs internalized MUC2-bound bacteria across IECs sealed by occludin-containing tight junctions (fig. S4A–D). Moreover, MUC2 was detected in human SI-LP CD103⁺ DCs proximal to GCs (Fig. 1A and movies S1 and S2).

In the presence of MUC2, human DCs exposed to bacteria or LPS across IECs or directly incubated with LPS secreted less interleukin 12 (IL-12) (Fig. 1B and fig. S4E), a cytokine that induces proinflammatory interferon- γ (IFN- γ)-producing CD4⁺ T helper 1 (Th1) cells (2). This effect was comparably induced by human, murine or porcine MUC2 and was not due to elevated endotoxin, impaired DC uptake of bacteria or increased DC apoptosis (figs. S4F and S5A–D). Unlike native MUC2, deglycosylated MUC2, a MUC2 peptide or the mucin-interacting protein trefoil factor 3 did not inhibit LPS-induced IL-12 secretion (fig.

S5E). MUC2 also impaired IL-12 as well as IL-6, IL-8 and tumor necrosis factor (TNF) transcription in response to bacterial Toll-like receptor (TLR) ligands such as LPS and flagellin or cytokines such as TNF (Fig. 1B and fig. S6A–E). MUC2 elicited similar anti-inflammatory effects in monocyte-derived and myeloid CD1c⁺ DCs (Fig. 1B and fig. S6A–E), which include precursors of mucosal DCs (2, 8, 10). Thus, uptake of MUC2 causes carbohydrate-dependent attenuation of proinflammatory cytokine production by DCs.

MUC2 Delivers Tolerogenic Signals to DCs

Next, we established whether MUC2 induces IL-10, an anti-inflammatory cytokine that inhibits IL-12 and IFN- γ (2). Besides sustaining or augmenting IL-10 transcription and secretion in monocyte-derived DCs exposed to LPS or flagellin, MUC2 enhanced IL-10 secretion in LPS-activated myeloid CD1c⁺ DCs (Fig. 1B and fig. S6C–E). MUC2 alone or combined with LPS also increased DC transcription and secretion of transforming growth factor- β 1 (TGF- β 1), a SMAD-signaling cytokine (Fig. 1B and figs. S6E and S7A) that helps the induction of Treg cells by tolerogenic CD103⁺ DCs (2, 7, 11). Moreover, MUC2 augmented DC transcription and activation of retinaldehyde de-hydrogenase (RALDH or ALDH1), a 4-diethylaminobenzaldehyde (DEAB)-sensitive enzyme (Fig. 1B and figs. S6E and S7B) with A1-3 isoforms that help CD103⁺ DCs to induce Treg cells by converting dietary vitamin A into retinoic acid (RA) (2, 7, 11).

Accordingly, human DCs exposed to LPS in the presence of MUC2 decreased CD4⁺ T cell proliferation and IFN- γ production, but increased Foxp3 expression (Fig. 1C and D and fig. S8A). These effects were reversed by neutralizing Abs to IL-10 or TGF- β 1 and by the RA antagonist LE540 (Fig. 1E). In spite of up-regulating the antigen-presenting molecule human leukocyte antigen-DR (HLA-DR), MUC2 down-regulated the T-cell co-stimulatory molecules CD80 and CD86 and the maturation molecule CD83 on LPS-activated DCs (fig. S8B). Consistent with its ability to autonomously induce TGF- β 1 and RA, MUC2 induced regulatory DCs even in the absence of LPS priming (fig. S9A and B). In addition to up-regulating CD103 and CX3CR1 on LPS-activated DCs (Fig. 1F), MUC2 stimulated CD103 expression and Treg cell-inducing signals in DCs undergoing transepithelial sampling of bacteria (fig. S10A–C).

In the presence of MUC2, LPS-primed mouse bone marrow-derived DCs produced less IL-12 but more IL-10 (fig. S11A). When pulsed with the soluble protein ovalbumin (OVA) in the presence of MUC2, these DCs triggered less IFN- γ production by OVA-specific transgenic OT-II CD4⁺ T cells, which concurrently generated more Treg cells (fig. S11B and C). MUC2 also attenuated flagellin-induced TNF but sustained or augmented IL-10 production in SI-LP CD103⁺ and CX3CR1⁺ DCs, respectively (Fig. 2A). Thus, MUC2 elicits tolerogenic IL-10, TGF- β 1 and RA signals that somewhat vary in distinct subsets of DCs.

MUC2 Enhances Gut Homeostasis

The immunoregulatory function of MUC2 was further explored in MUC2-deficient (*Muc2*^{-/-}) mice (12). Compared to WT controls, *Muc2*^{-/-} mice showed more IEC-adherent bacteria and their SI-LP CD103⁺ and CX3CR1⁺ DCs expressed more TNF and IL-12, but

less IL-10, TGF- β 1 and ALDH1A1 and thus induced fewer Treg cells (Fig. 2B and C and fig. S12A). Accordingly, *Muc2*^{-/-} mice had fewer SI-LP Treg cells but more proinflammatory Th1 and IL-17-producing Th17 cells (fig. S12B). These changes were associated with increased bacteria-bound IgA Abs and SI-LP DC expression of B cell-activating factor of the TNF family (BAFF) and a proliferation-inducing ligand (APRIL) (fig. S12C and D), two IgA-inducing cytokines induced by inflammatory signals (3). Compared to WT mice, *Muc2*^{-/-} mice sterilized of gut bacteria normalized SI-LP DC expression of BAFF and APRIL, but neither augmented SI-LP DC expression of IL-10, TGF- β 1 and ALDH1A2 nor increased SI-LP Treg cells (Fig. 2E and fig. S12D and E). Thus, perturbations of gut homeostasis in *Muc2*^{-/-} mice cannot be solely ascribed to increased IEC-adherent bacteria.

We then verified whether MUC2 regulates IECs. Compared to controls, IECs from *Muc2*^{-/-} mice expressed less IL-10, TGF- β 1, ALDH1A1 and thymic stromal lymphopoietin (TSLP) (fig. S13A), which generates tolerogenic DCs (13, 14). Gut sterilization failed to restore WT-like levels of IL-10, ALDH1A1 and TSLP, but did normalize TGF- β 1 and RegIII γ (fig. S13A), an antimicrobial protein induced by bacteria (15). In humans, IECs expressed more TSLP in response to MUC2 (fig. S13A), which confirmed the tolerogenic function of MUC2 on IECs.

The immune and barrier functions of MUC2 were further uncoupled by gavaging *Muc2*^{-/-} mice with MUC2 from WT controls. When exposed to MUC2, SI-LP CD103⁺ and CX3CR1⁺ DCs from *Muc2*^{-/-} mice reduced OVA-specific CD4⁺ T cell proliferation and IFN- γ production as SI-LP DCs from WT mice did (Fig. 2F). Gavaged MUC2 did not restore a visible barrier, but its capture not only augmented IL-10, TGF- β and ALDH1A1 and decreased IL-12 in SI-LP DCs, but also increased SI-LP Treg cells and reduced SI-LP Th1 and Th17 cells (Fig. 3A and B and fig. S14A). Similarly, gavaged MUC2 helped SI-LP CD103⁺ DCs from *Muc2*^{-/-} mice to induce more OVA-specific Treg cells after intra-gastric OVA immunization (Fig. 3C).

Importantly, gavaged MUC2 enhanced the resistance of *Muc2*^{-/-} mice to dextran sodium sulfate (DSS), a colitogenic agent that disrupts the epithelial barrier. Although unable to attenuate inflammation-induced shortening of the LI, gavaged MUC2 ameliorated both clinical symptoms and histological lesions in DSS-treated *Muc2*^{-/-} mice, which showed less weight loss than WT or *Muc2*^{-/-} mice challenged with DSS in the absence of exogenous MUC2 (fig. S14B–D). Thus, MUC2 attenuates bacteria-induced gut inflammation by delivering DC and IEC conditioning signals.

MUC2 Promotes Oral Tolerance

Oral tolerance consists in the attenuation of T and B cell responses to an antigen by prior oral administration of that antigen and involves MLN induction of Treg cells by migratory CD103⁺ DCs (2). WT mice gavaged and later intragastrically immunized with OVA showed decreased gut B cell production of OVA-specific IgG Abs, which correlated with reduced IFN- γ production by OVA-reactive CD4⁺ T cells in response to OVA-pulsed MLN, PP or SI-LP CD103⁺ DCs (Fig. 3D and E and fig. S15A and B).

Tolerization also reduced OVA-induced delayed-type hypersensitivity (DTH) – an inflammatory skin reaction involving Th1 cells – as well as DC-dependent splenic CD4⁺ T cell proliferation and IFN- γ secretion following systemic immunization of *Muc2*^{-/-} mice with OVA (Fig. 3F and G and fig. S15C and D). Unlike WT mice, *Muc2*^{-/-} mice gavaged with OVA developed neither intestinal nor systemic tolerance (Fig. 3D–G and fig. S15A–D). However, *Muc2*^{-/-} mice restored oral tolerance when gavaged with OVA in the presence of MUC2 (Fig. 3D–G and fig. S15A–D). The impairment of tolerance in *Muc2*^{-/-} mice was not merely due to bacteria-induced inflammation, as *Muc2*^{-/-} mice lacking gut bacteria did not attenuate OVA-specific DTH, IgG and IgE responses after gavage with OVA (Fig. 3H–J). Yet, tolerance was restored when OVA was gavaged in combination with MUC2 (Fig. 3H–J). Thus, mucus actively constrains the immunostimulating properties of oral soluble antigens.

MUC2 Binds a Galectin-3-Dectin-1-Fc γ RIIB Receptor Complex on DCs

Carbohydrates account for 80% of the weight of MUC2 (1) and may thus mediate its binding to DCs. Indeed, unlabeled glycosylated MUC2 inhibited the binding of CFSE-labeled native MUC2 to human DCs, whereas unlabeled deglycosylated MUC2 or a MUC2 peptide did not (Fig. 4A and fig. S16A). C-type lectin receptors (CLRs) and soluble galectins form carbohydrate-binding DC platforms with tolerogenic function (16–20). Saturation of CLRs and galectins with mannan and lactose, respectively, attenuated MUC2 binding to DCs (Fig. 4A). DCs express galectin-1, -3 and -9 (21), but only galectin-3 interacted with glycosylated MUC2 and increased its binding to DCs through a process that was inhibited by MUC2 deglycosylation or Ab-mediated blockade of galectin-3 (Fig. 4B–D). In agreement with their ability to bind galectin-3 following its secretion (16, 21), DCs increased both soluble and membrane-bound galectin-3 in response to LPS (fig. S16B). Thus, DCs may form a galectin-3-based MUC2-binding platform after sensing gut microbial signals.

Accordingly, PP and SI-LP CD103⁺ and CX3CR1⁺ DCs produced more galectin-3 than splenic DCs did and showed intracellular galectin-3 co-localized with MUC2 (Fig. 4E and F and fig. S16C–E). SI-LP DCs also displayed more surface galectin-3 (Fig. 4F and fig. S16F), part of which may have an epithelial origin (21). Indeed, galectin-3 was detected in both IECs and GCs and its secretion augmented upon IEC exposure to bacteria (fig. S16G and H). Consequently, DCs acquired more surface galectin-3 after exposure to IEC supernatant or migration across IECs (fig. S16I and J).

We then determined how soluble galectin-3 anchors itself and MUC2 to DCs. Galectin-3 binds Dectin-1, a CLR that induces tolerogenic DCs and enhances gut homeostasis by recognizing fungal carbohydrates (16, 17, 20). Dectin-1 further interacts with the anti-inflammatory receptor Fc γ RIIB in response to glycans from therapeutic IgG Abs (22). In human embryonic kidney 293 cells, MUC2 bound transfected Dectin-1 and Fc γ RIIB in the presence of galectin-3 (fig. S17A and B). In DCs, MUC2 binding correlated with elevated surface levels of galectin-3, Dectin-1 and Fc γ RIIB and caused Dectin-1 recruitment to a pre-formed galectin-3-Fc γ RIIB complex (Fig. 4G and fig. S17C). Accordingly, DCs lacking

galectin-3, Dectin-1 or Fc γ RIIB neither effectively bound MUC2 nor decreased IL-12 production in response to MUC2 (Fig. 4H and I and fig. S17D–G).

In WT mice, SI-LP CD103⁺ DCs expressed less Dectin-1 but more galectin-3 and Fc γ RIIB than CX3CR1⁺ DCs did (Fig. 5A). Compared to *Lgals3* (galectin-3)^{-/-}, *Clec7a* (Dectin-1)^{-/-} or *Fcgr2b* (Fc γ RIIB)^{-/-} mice, WT mice had a comparable SI mucous layer, but showed more SI-LP DCs containing MUC2 (Fig. 5B and fig. S18A and B). Unlike IECs, these SI-LP DCs did not express MUC2 (Fig. 5C), which confirmed that gut DCs acquire MUC2 from the external environment. Compared to controls, *Lgals3*^{-/-}, *Clec7a*^{-/-} or *Fcgr2b*^{-/-} mice had SI-LP DCs that produced more IL-12, but less ALDH1A1 and TGF- β 1 and did not respond to MUC2 (Fig. 5D and fig. S19). These mice also had fewer SI-LP Treg cells, more SI-LP Th1 cells, and impaired tolerogenic T and B cell responses to OVA (Fig. 5E–G). Thus, MUC2 may generate gut homeostasis and tolerance by assembling a signal-transducing Dectin-1-Fc γ RIIB complex on DCs with the help of soluble galectin-3.

MUC2 Tolerizes DCs by Inducing β -Catenin via Dectin-1

How do Dectin-1 and Fc γ RIIB convey tolerogenic signals to DCs exposed to MUC2? Dectin-1 phosphorylates AKT (23) and may thus induce β -catenin, a transcription factor required by gut tolerogenic DCs (24). Indeed, MUC2 alone or combined with LPS phosphorylated AKT and glycogen synthase kinase-3 β (GSK-3 β) (Fig. 6A), two events that cause GSK-3 β inactivation and inhibition of β -catenin degradation (24, 25). Consequently, MUC2 elicited cytoplasmic accumulation and nuclear translocation of β -catenin in WT but not *Clec7a*^{-/-} DCs (Fig. 6A and fig. S20A and B).

In some tumors, β -catenin interacts with nuclear factor κ B (NF- κ B) p65 to impede the binding of p50-p65 complexes to death-inducing genes (25). In DCs, MUC2 alone or coupled with LPS enhanced nuclear β -catenin interaction with NF- κ B p65 and decreased NF- κ B p50-p65 binding to both minimal and *IL12* gene promoters, thus impairing their transcription (Fig. 6B–E and fig. S20C). Accordingly, β -catenin deficiency impeded MUC2-mediated inhibition of LPS-induced IL-12 but not IL-10 transcription and/or secretion, whereas β -catenin overexpression dampened TNF-induced NF- κ B-driven transcription (Fig. 6F and G and fig. S20D–F). Consistent with these data, galectin-3-expressing DCs from PPs contained abundant β -catenin in addition to activated AKT and inactive GSK-3 β (Fig. 6H).

Besides AKT, Dectin-1 phosphorylates SYK, which activates NF- κ B through a pathway mitigated by Fc γ RIIB via SH2 domain-containing inositol 5-phosphatase-1 (SHIP-1) (22, 26). SYK also activates cAMP responsive element binding protein (CREB), a calcium-dependent IL-10-inducing protein that removes the co-activator CREB-binding protein (CBP) from DNA-bound NF- κ B (27, 28). Besides triggering SYK and SHIP-1 phosphorylation, MUC2 alone or combined with LPS decreased NF- κ B p65 but not p50 nuclear translocation and increased galectin-dependent calcium fluxes, phosphorylation of CREB-targeting AKT, ERK1/2 and p38 kinases, phosphorylation and nuclear translocation of CREB, binding of CREB to *IL10* and *IL12* promoters, and loss of CBP from the *IL12* promoter (figs. S20G and S21A–G). Thus, similar to hyperglycosylated IgG Abs used to treat autoimmune disorders (22), MUC2 may recruit SHIP-1 via Fc γ RIIB to constrain

proinflammatory NF- κ B but not tolerogenic CREB signals emanating from Dectin-1 and SYK. In addition to inducing IL-10, CREB may cooperate with dectin-1-induced β -catenin to inhibit IL-12.

Conclusions

We have shown here that MUC2 enhances gut homeostasis and oral tolerance by conditioning DCs and IECs. Antigen-sampling DCs assemble galectin-3, Dectin-1 and Fc γ RIIB to acquire MUC2 across IECs and possibly from GCs (fig. S22A). The MUC2 receptor complex suppresses inflammatory but not tolerogenic DC responses by inhibiting NF- κ B via β -catenin and possibly CREB (fig. S22B). How DCs tune down these signals during infection remains unclear, but pathogen-induced perturbations of MUC2 glycosylation and polymerization patterns may be involved (1). A full understanding of the immunoregulatory function of MUC2 could help to devise better vaccines and treatments against infections and food allergies and to unravel how alterations of MUC2 and its receptors aggravate inflammatory bowel disease (1, 20), thus leading to safer therapies against this disorder.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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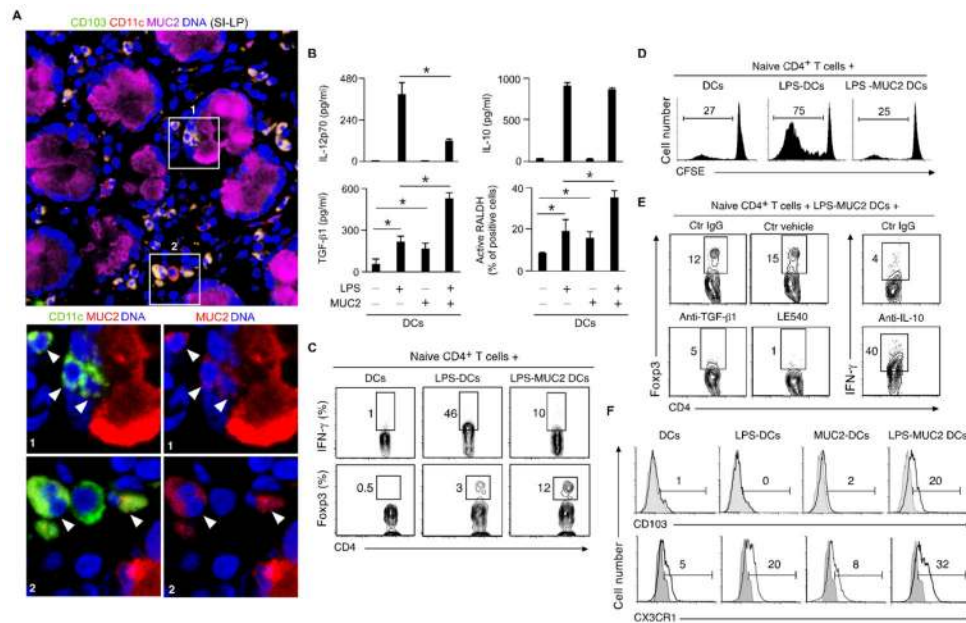


Fig. 1. MUC2 imprints DCs with tolerogenic properties

(A) Confocal microscopy of human SI-LP stained for CD11c, MUC2, CD103 and DNA-capturing 4'-6-diamidino-2-phenylindole (DAPI). Boxes and arrowheads: MUC2⁺CD103⁺ DCs. Original magnification, $\times 63$. (B) Enzyme-linked immunosorbent assay (ELISA) of IL-12p70, IL-10 and TGF- β 1 and flow cytometry (FC) of active RALDH in human DCs cultured for 2 days with or without LPS and/or MUC2. (C–E) FC of IFN- γ , Foxp3, CD4 and CFSE in human naïve CD4⁺ T cells cultured for 4 days with allogeneic DCs stimulated with or without LPS and/or MUC2 for 2 or 5 days in the absence or presence of control (ctr) IgG Ab, ctr vehicle, neutralizing Abs to TGF- β 1 or IL-10 or LE540. (F) FC of CD103 and CX3CR1 on DCs cultured for 2 days with or without LPS and/or MUC2. Data summarize 3 experiments (error bars, s.d.; unpaired *t* test, **P* < 0.05) or show one of 4 experiments with similar results.

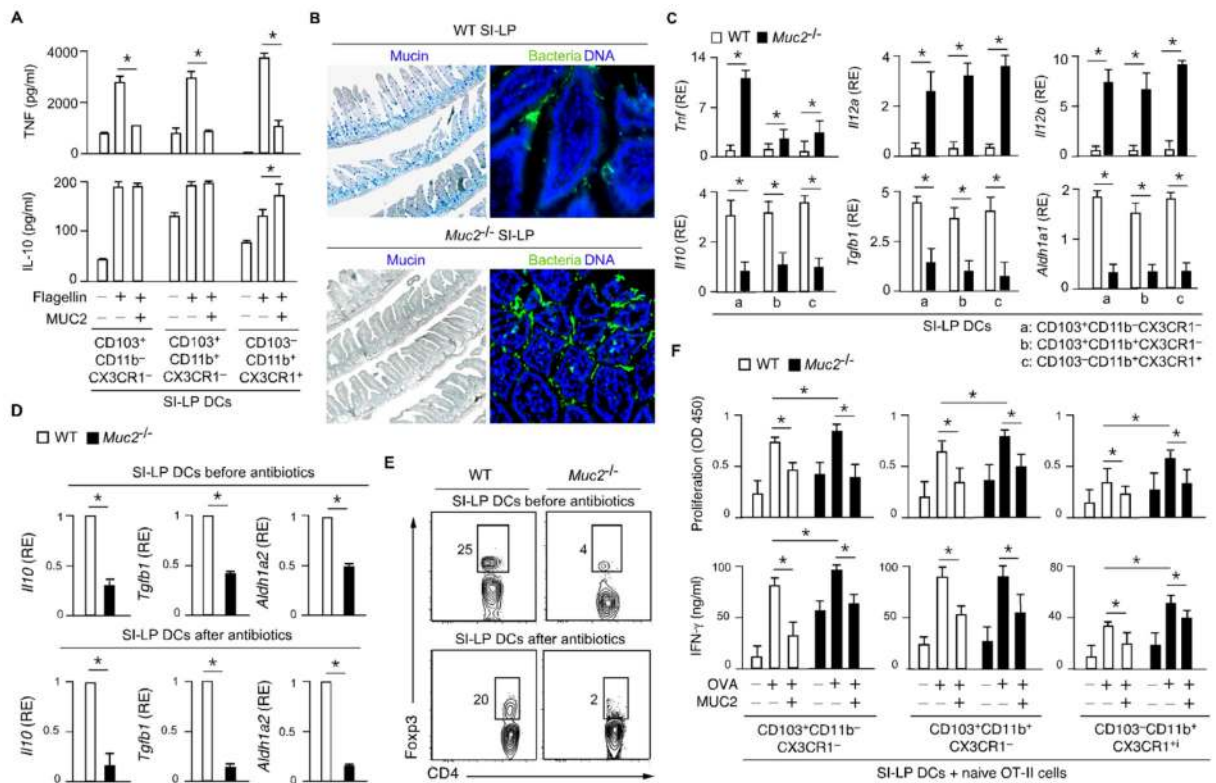


Fig. 2. MUC2 delivers anti-inflammatory signals to gut DCs

(A) ELISA of TNF and IL-10 from mouse SI-LP DCs cultured for 2 days with or without flagellin and/or MUC2. (B) Light microscopy of Alcian blue-stained mucin and fluorescence in situ hybridization (FISH) of bacterial 16S ribosomal RNA in DAPI-stained SI-LP from WT and *Muc2*^{-/-} mice. Original magnification, ×10. (C–E) Quantitative real-time polymerase chain reaction (qRT-PCR) of mRNAs for TNF, IL-12p35 (*Il12a*), IL-12p40 (*Il12b*), IL-10, RALDH1 (*Aldh1a1*) and TGF-β1 in SI-LP DCs and FC of Foxp3 and CD4 on SI-LP T cells from WT and *Muc2*^{-/-} mice before and after oral antibiotics. RE, relative expression compared to *Gapdh* encoding glyceraldehyde 3-phosphate dehydrogenase. (F) ELISA of proliferation-induced bromodeoxyuridine (BrdU) and IFN-γ from OT-II cells activated for 5 days by OVA-pulsed SI-LP DCs from WT and *Muc2*^{-/-} mice with or without MUC2. Data summarize 2 experiments with ≥3 mice/group (error bars, s.d.; unpaired Student's *t* test, **P* < 0.05) or show one of 4 experiments with similar results.

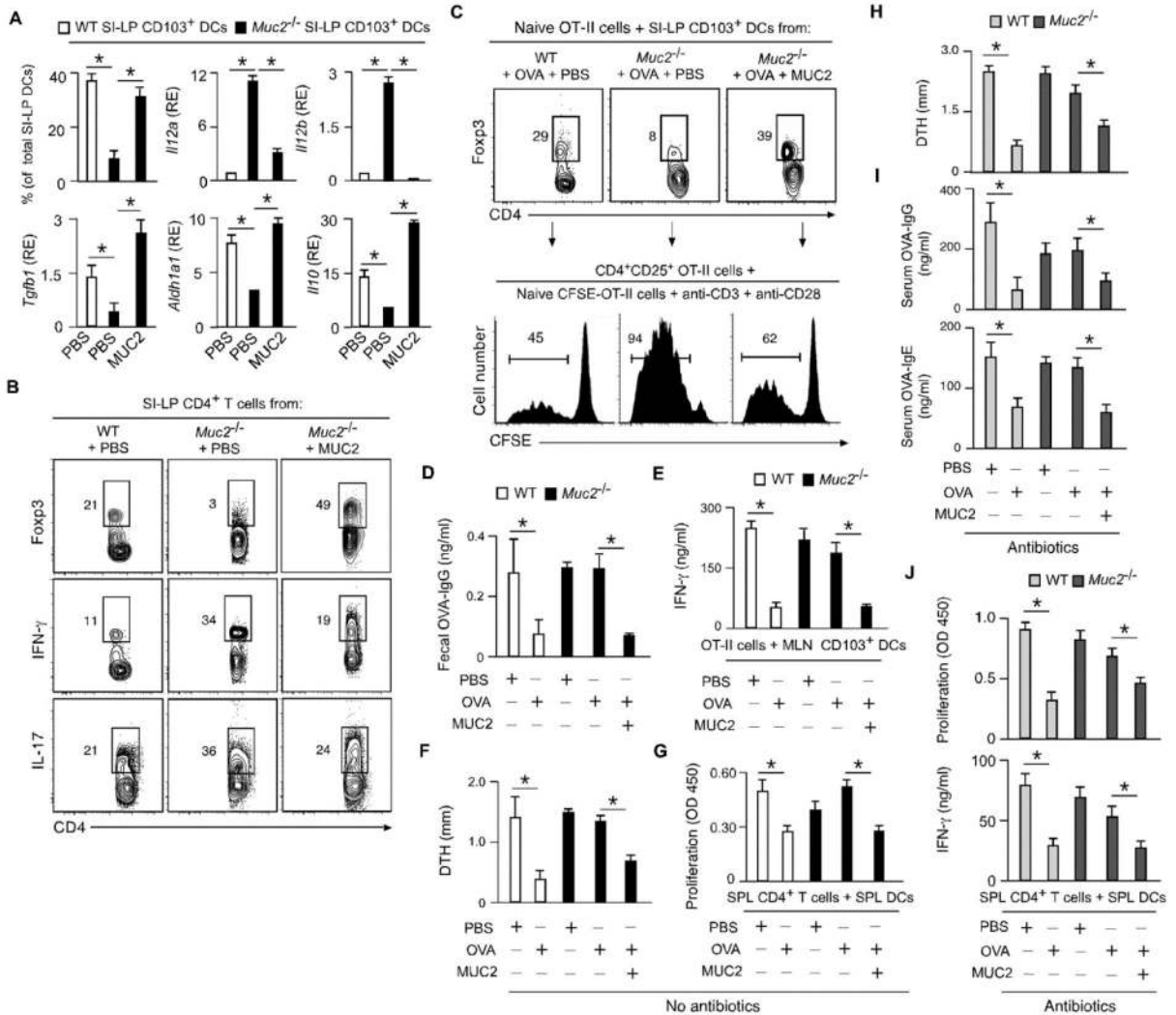


Fig. 3. MUC2 enhances gut homeostasis and oral tolerance

(A) FC of CD103 and qRT-PCR of *Il12a*, *Il12b*, *Il10*, *Aldh1a1* and *Tgfb1* in SI-LP CD103⁺ DCs from WT or *Muc2*^{-/-} mice gavaged for 5 days with phosphate buffer solution (PBS) or MUC2. RE, relative expression compared to *Gapdh*. (B) FC of Foxp3, IFN- γ , IL-17 and CD4 in SI-LP T cells from WT or *Muc2*^{-/-} mice treated as in (A). (C) FC of Foxp3 and CD4 in naïve OT-II cells cultured for 5 days with SI-LP CD103⁺ DCs from WT or *Muc2*^{-/-} mice treated as in (A) and intragastrically immunized with OVA. CD4⁺CD25⁺ OT-II cells from these cultures were incubated for 5 days with CFSE-labeled naïve OT-II cells and Abs to CD3 and CD28; divided CFSElow cells were quantified by FC. (D) ELISA of fecal OVA-specific IgG from WT and *Muc2*^{-/-} mice tolerized with PBS, OVA or OVA plus MUC2 for 5 days and immunized as in (C). (E) ELISA of IFN- γ from OT-II cells incubated for 5 days with MLN CD103⁺ DCs from WT or *Muc2*^{-/-} mice tolerized and immunized as in (D). (F) OVA-induced DTH in WT or *Muc2*^{-/-} mice tolerized as in (C) and subcutaneously immunized with OVA. (G) ELISA of proliferation-induced BrdU from SPL CD4⁺ T cells activated for 5 days with OVA-pulsed SPL DCs from WT or *Muc2*^{-/-} mice tolerized and immunized as in (F). (H–J) DTH, OVA-specific serum IgG and IgE, and SPL CD4⁺ T cell proliferation and IFN- γ secretion in WT or *Muc2*^{-/-} mice immunized and tolerized as in (F) after oral antibiotics. Data summarize 2 experiments with ≥ 4 mice/group (error bars, s.d.; unpaired Student's *t* test, **P* < 0.05) or show one of 4 experiments with similar results.

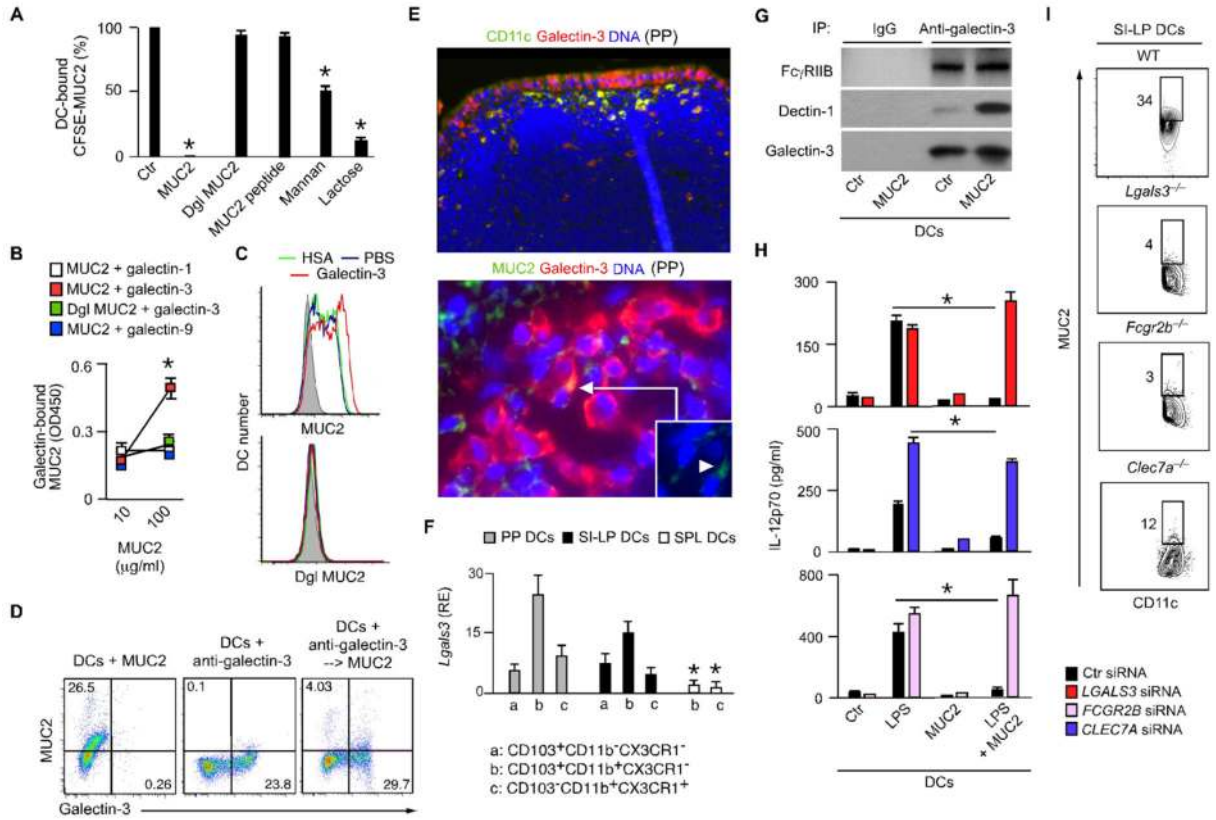


Fig. 4. MUC2 binds galectin-3, dectin-1 and FcγRIIB on DCs

(A) FC of CFSE-MUC2 on human DCs pre-incubated with unlabeled native MUC2, deglycosylated (dgl) MUC2, MUC2 peptide, mannan or lactose. % of MUC2 binding compared to medium alone. (B) ELISA of native or dgl MUC2 interaction with galectins. (C) CFSE-MUC2 or CFSE-dgl MUC2 binding to DCs pre-incubated with PBS, human serum albumin (HSA) or galectin-3. (D) CFSE-MUC2 binding to DCs before and after pre-incubation with a fluorescent Ab to galectin-3. (E) IFA of CD11c, galectin-3, Muc2 and DAPI in mouse PP sections. Original magnification $\times 5$ (upper panel) and $\times 63$ (bottom panel). (F) qRT-PCR of mRNA for galectin-3 in DC subsets from mouse PPs, SI-LP and SPL. RE, relative expression compared to *Gapdh*. (G) Immunoprecipitation (IP) with control or anti-galectin-3 Abs of proteins from human DCs treated without (ctr) or with MUC2 for 30 min, followed by immunoblotting of FcγRIIB, Dectin-1 and galectin-3. (H) ELISA of IL-12p70 from human DCs exposed to scrambled (ctr) or *LGALS3* (galectin-3), *FCGR2B* (FcγRIIB) or *CLEC7A* (Dectin-1) small interfering RNAs (siRNAs) and cultured with or without LPS and/or MUC2 for 2 days. (I) Binding of CFSE-MUC2 to SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7a*^{-/-} or *Fcgr2b*^{-/-} mice. Data summarize experiments with 3 donors or 3 mice from each strain (error bars, s.d.; unpaired *t* test, **P* < 0.05) or show one of 3 experiments with similar results.

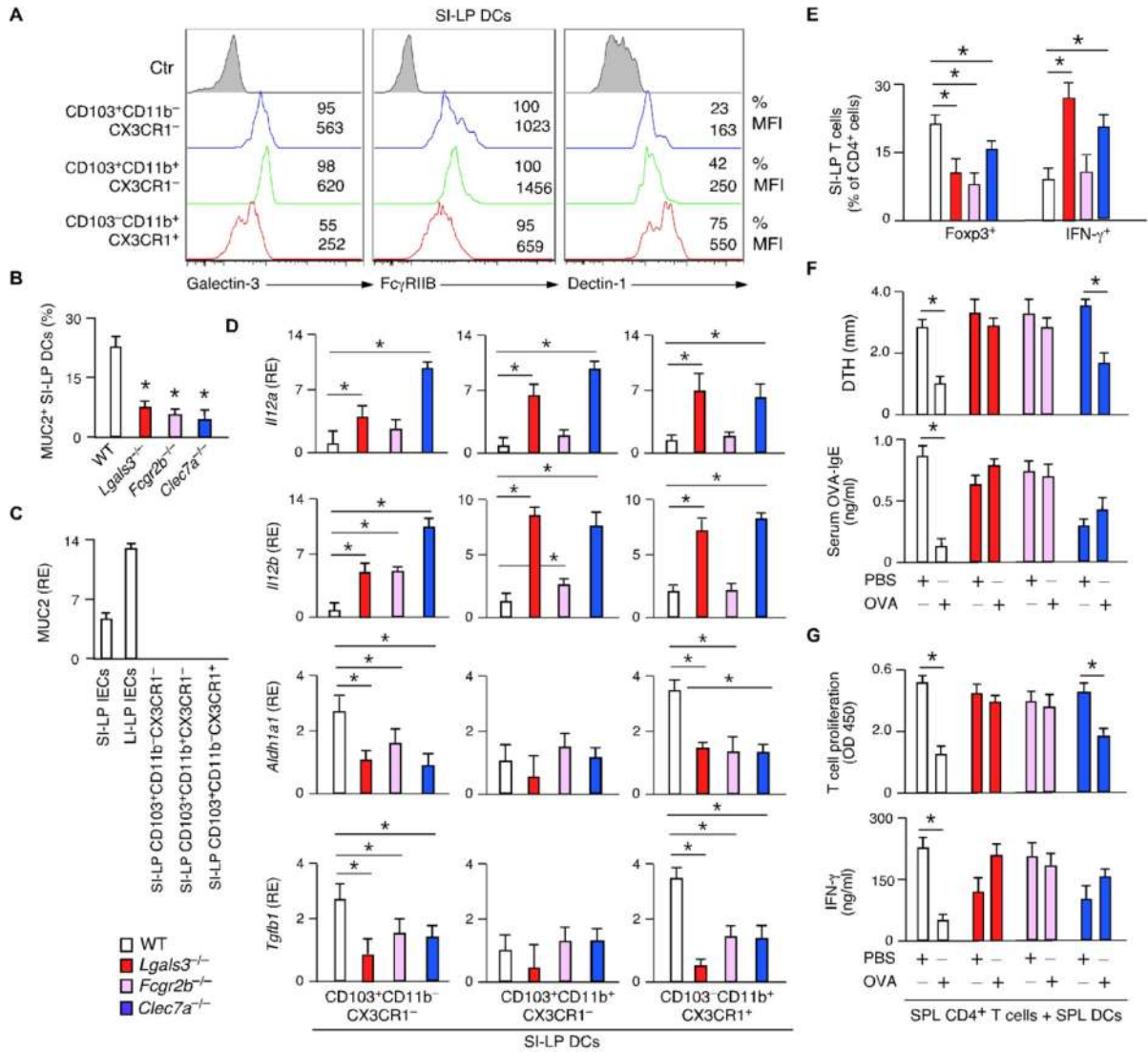


Fig. 5. Galectin-3, Dectin-1 and Fc γ RIIB enhance gut homeostasis and oral tolerance

(A) FC of galectin-3, Fc γ RIIB and Dectin-1 on SI-LP DCs from WT mice. MFI, mean fluorescence intensity. (B) Quantification of MUC2⁺ SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7A*^{-/-} or *Fcgr2b*^{-/-} mice by IFA of 10–12 SI-LP sections/group. (C) qRT-PCR of *Muc2* in IECs and SI-LP DCs from WT mice. RE, relative expression compared to *Gapdh*. (D and E) qRT-PCR of *Il12a*, *Il12b*, *Aldh1a1* and *Tgfb1* in SI-LP DCs and FC of Fc γ RIIB and Dectin-1 on SI-LP DCs from WT, *Lgals3*^{-/-}, *Clec7A*^{-/-} or *Fcgr2b*^{-/-} mice. RE, relative expression compared to *Gapdh*. (F) DTH and ELISA of OVA-specific IgE in WT, *Lgals3*^{-/-}, *Clec7A*^{-/-} or *Fcgr2b*^{-/-} mice intragastrically tolerized with PBS or OVA for 5 days and subcutaneously immunized with OVA. (G) ELISA of proliferation-induced BrdU from SPL CD4⁺ T cells activated for 5 days by OVA-pulsed SPL DCs from WT, *Lgals3*^{-/-}, *Clec7A*^{-/-} or *Fcgr2b*^{-/-} mice tolerized and immunized as in (F). Data summarize 2 experiments with \geq 4 mice/group (error bars, s.d.; unpaired Student's *t* test, **P* < 0.05) or show one of 4 experiments with similar results.

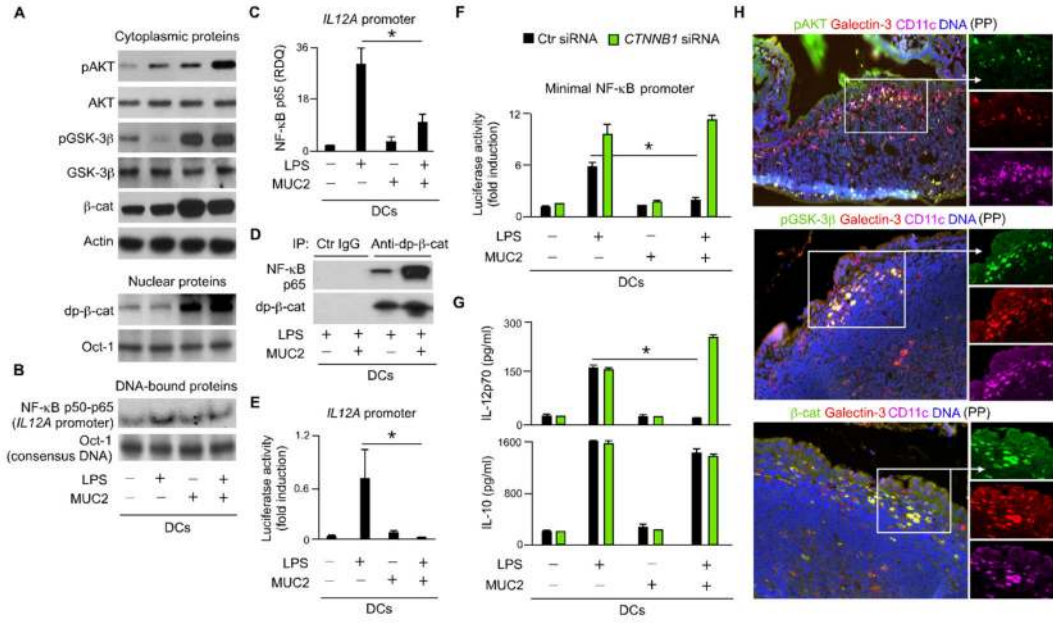


Fig. 6. MUC2 impairs NF-κB-driven inflammatory signals via β-catenin

(A) WB of cytoplasmic or nuclear phospho (p)-AKT, AKT, pGSK-3β, GSK-3β, β-catenin (β-cat), dephospho (dp)-β-cat, actin and octamer-1 (Oct-1) from human DCs cultured with or without LPS and/or MUC2 for 10 min. (B) Electrophoretic mobility gel shift assay of *IL12A*-bound NF-κB p65-p50 and consensus DNA-bound Oct-1 in DCs cultured as in (A) for 3 hours. (C) Chromatin IP (ChIP) of *IL12A*-bound NF-κB p65 in DCs cultured as in (A) for 3 hours. RDQ, relative DNA quantity. (D) IP with control (ctr) IgG Ab or anti-dp-β-cat Ab of nuclear proteins from DCs cultured with or without LPS and/or MUC2 for 10 min, followed by WB of NF-κB p65 and dp-β-cat. (E) *IL12A* transcription in DCs cultured as in (A) for 2 days. (F and G) NF-κB-mediated transcription and ELISA of IL-12p70 and IL-10 in DCs cultured as in (E) in the presence of scrambled (ctr) or *CTNNB1* (β-cat) siRNAs. (H) IFA of pAKT, pGSK-3β, β-cat, galectin-3, CD11c and DAPI in mouse PPs. Original magnification, ×5. Data show one of 3 experiments yielding similar results or summarize 3 experiments (error bars, s.d.; unpaired *t* test, **P* < 0.05).