TFF3 interacts with LINGO2 to regulate EGFR activation for protection against colitis and gastrointestinal helminths

Nicole Maloney Belle¹, Yingbiao Ji¹,⁵, Karl Herbine¹,⁵, Yun Wei²,³,⁵, JoonHyung Park¹,⁵, Kelly Zullo¹, Li-Yin Hung¹,², Sriram Srivatsa¹, Tanner Young¹, Taylor Oniskey², Christopher Pastore¹, Wildaliz Nieves⁴, Ma Somsouk⁴ & De’Broski R. Herbert¹,²*

Intestinal epithelial cells (IEC) have important functions in nutrient absorption, barrier integrity, regeneration, pathogen-sensing, and mucus secretion. Goblet cells are a specialized cell type of IEC that secrete Trefoil factor 3 (TFF3) to regulate mucus viscosity and wound healing, but whether TFF3-responsiveness requires a receptor is unclear. Here, we show that leucine rich repeat receptor and nogo-interacting protein 2 (LINGO2) is essential for TFF3-mediated functions. LINGO2 immunoprecipitates with TFF3, co-localizes with TFF3 on the cell membrane of IEC, and allows TFF3 to block apoptosis. We further show that TFF3-LINGO2 interactions disrupt EGFR-LINGO2 complexes resulting in enhanced EGFR signaling. Excessive basal EGFR activation in Lingo2 deficient mice increases disease severity during colitis and augments immunity against helminth infection. Conversely, TFF3 deficiency reduces helminth immunity. Thus, TFF3-LINGO2 interactions de-repress inhibitory LINGO2-EGFR complexes, allowing TFF3 to drive wound healing and immunity.

¹Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, Philadelphia, PA 19140, USA. ²Division of Experimental Medicine, University of California, San Francisco, San Francisco, CA 94110, USA. ³Department of Inflammation and Oncology, Amgen Inc., 1120 Veterans Boulevard, South San Francisco, CA 94080, USA. ⁴Division of Gastroenterology at ZSFG, University of California, San Francisco, San Francisco, CA 94110, USA. ⁵These authors contributed equally: Yingbiao Ji, Karl Herbine, Yun Wei, JoonHyung Park. *email: debroski@vet.upenn.edu

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Gastrointestinal (GI) tissue undergoes regenerative and injury-induced repair through diverse pathways that span arachidonic acid metabolites (e.g., prostaglandins, leukotrienes), growth factors (e.g., KGF, EGF), regulators of development (e.g., Notch, Wnts, Hippo), and reparative cytokines (e.g., Trefoil factor family proteins (TFF1-3))\textsuperscript{1-8}. TFF3 is a small (6 kDa) secreted glycoprotein produced by goblet cells that drives wound healing throughout the respiratory, ocular, biliary, genitourinary and GI mucosa\textsuperscript{9-11}. Despite identification over 30 years ago, and numerous studies implicating TFF3 in human diseases, the existence and/or identity of a bona-fide receptor responsible for TFF3-mediated cellular migration, anti-apoptotic activity, cell adhesion control, and/or immunoregulation remains unknown\textsuperscript{12}. Still, whether a TFF3 receptor exists has been controversial because of evidence that TFF3 (along with TFF2) increase the rheological properties of mucus\textsuperscript{12,20}. Given the implication for TFF3 (and TFF2) as a regulator of GI diseases such as Ulcerative colitis and Crohn’s disease, there is a clear need for a better understanding of TFF biology.

Evidence shows that pathways of tissue repair/regeneration and immunity functionally intersect\textsuperscript{22}. Although TFF3 was previously considered solely a regulator of re-epithelialization, TFF3 has been reported to exert both pro and anti-inflammatory activities\textsuperscript{23-25}. TFF3 undergoes auto-induction at its promoter, which is dependent upon STAT3 activity\textsuperscript{28} and TFF3 treatment can induce both pSTAT3 and pEGFR activation\textsuperscript{14,29,30}. EGFR activation serves an important role in host immunity, particularly against GI helminths such as the rodent hookworms *Heligosomoides polygyrus bakeri* and *Nippostrongylus brasiliensis* (N.b.). Amphiregulin, an EGFR ligand, promotes Type 2 inflammation and worm expulsion through EGFR dependent signals\textsuperscript{31}. Type 2 immune responses can be synergistic with tissue repair, therefore we reasoned that the reparative and immunoregulatory effects of TFF3 would be evident during helminth infection or colitic inflammation.

This work ventures into the major gap of Trefoil factor family biology by searching for a TFF3 receptor. An approach was used called TRICEPS that employs ligand-guided covalent binding and capture of glycoproteins on the cell surface to identify low-affinity ligand-receptor interactions\textsuperscript{32}. This approach led us to discover Leucine-rich repeat and immunoglobulin-like domain-containing nogo receptor-interacting protein 2 (LINGO2), as a critical component of TFF3 cellular responsiveness. LINGO2 is required for TFF3-mediated EGFR and STAT3 activation in intestinal epithelial cells (IEC). Paradoxically, LINGO2 deficiency in mice results in a phenotype opposite of TFF3 deficiency due to repressive EGFR-LINGO2 interactions. In support of this hypothesis, the increased disease severity of DSS colitis in LINGO2 deficient mice and their resistance to *N.b.* infection is both reversed following treatment with the EGFR antagonist Gefitinib. Taken together, these data suggest a model wherein TFF3 binding to LINGO2 reverses tonic inhibition of EGFR by LINGO2, resulting in heightened EGFR activation.

**Results**

**LINGO2 expression facilitates responsiveness to TFF3.** Anti-inflammatory activities of TFF3 suggested that leukocytes may directly respond to TFF3\textsuperscript{25,27}. Given the cell number requirement for the TRICEPS screen (10\textsuperscript{5}/sample) and the apparent lack of success with epithelial cell lines, we used a human macrophage/monocyte cell line (U937) to search for a potential TFF3 receptor. TFF3-induced interleukin 10 (IL-10) production occurred over several orders of magnitude in these cells, but was lost at higher doses of recombinant human TFF3 (rhTFF3) (Fig. 1a). This bi-modal response seemed similar to the activity range for cytokine and growth factor receptors\textsuperscript{33}, implying that TFF3 was acting through a conventional ligand-receptor interaction.

We postulated that TFF3 interacted with its receptor through low-affinity interactions reliant upon carbohydrates because TFF3 glycosylation has been shown critical for biological activity\textsuperscript{34,35}. Therefore U937 cells were subjected to the TRICEPS\textsuperscript{36} protocol as a biochemical screening strategy to identify glycosylated transmembrane protein(s) on the cell surface\textsuperscript{25,26}. As bait, rhTFF3 was covalently linked to the TRICEPS probe and incubated with PMA-treated U937 that had been treated with sodium periodate. Cell pellets were subjected to glycosidase digestion and peptides generated using mass spectrometry. As a positive control to account for enrichment efficiency, recombinant human insulin was used as bait for the insulin receptor. Whereas the TFF3-probe induced an 8-fold enrichment of Lingo2 (LINGO2), the insulin-probe induced a 7.76-fold-enrichment in INSR peptide (Fig. 1b). No other enriched peptides in this screen met this level of enrichment or were derived from proteins that satisfied our selection criterion (e.g., extracellular domain, transmembrane region, cytoplasmic tail).

To further explore functional interactions between TFF3 and LINGO2, experiments were performed that employed anti-human LINGO2 Mab treatment of U937. At high doses, anti-LINGO2mAb blocked TFF3-induced IL-10 production (Fig. 1c) and resulted in a dose-dependent neutralization of TFF3-mediated TNF-alpha suppression (Supplementary Fig. 1A). Next, to ask whether LINGO2 and TFF3 were physical binding partners, we performed co-immunoprecipitation experiments utilizing murine TFF3-Fc vs. empty vector Fc incubated with Flag-tagged LINGO2, which revealed that TFF3 could pull down LINGO2 protein (Fig. 1d), indicating that TFF3 could bind LINGO2.

**LINGO2 and TFF3 interact on the epithelial cell surface.** Epithelial cell lineages are the major source and target of TFF3\textsuperscript{37-39}, therefore we focused on whether LINGO2 could capture TFF3 at the epithelial cell surface. HEK293 cells were double transiently transfected with constructs encoding LINGO2-GFP and TFF3-RFP, fixed, permeabilized, and imaged using confocal microscopy. Whereas TFF3-RFP single transfectants had multiple fluorescent puncta throughout the cytoplasm of the positive cells (Fig. 1e), TFF3-RFP transfectants that co-expressed LINGO2-GFP had clear yellow signal at the cell membrane, suggesting co-localization and resulted in TFF3 red fluorophore and LINGO2-GFP co-localization and resulted in TFF3 red fluorophore and LINGO2-GFP transfectants co-localizing in the extracellular space (Fig. 1f). To confirm the LINGO2 extracellular domain was necessary for the TFF3 interaction, we co-transfected TFF3-RFP with a NH\textsubscript{2}-terminal LINGO2-Flag truncation mutant (Δ350 AA), which abrogated co-localization and resulted in TFF3 red fluorescent puncta, similar to TFF3-RFP single transfectants (Fig. 1g). These data suggested that the leucine-rich-repeat (LRR) ectodomain region of LINGO2 was responsible for ligand binding. As a control and to address reports suggesting that TFF3 may also signal through the SDF-1 receptor CXC\textsubscript{4}R\textsubscript{40}, CXC\textsubscript{4}R-GFP was co-transfected with TFF3-RFP or SDF1-RFP. Data show TFF3-RFP/CXCR4-GFP transfec-

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To ensure that co-localization in our double transfection overexpression HEK system was not due to intracellular interactions,
we changed the approach to a flow cytometry strategy involving exposure of LINGO2-GFP transfectants to soluble rTFF3 labeled with Alexa-Fluor 647 (TFF3-647). A flow cytometry gating strategy was devised (Supplementary Fig. 1C) to determine the portion of cells that were doubly fluorescent for GFP and AF-647 (Fig. 1j). Compared to incubation with PBS, or IL-2-647, (Fig. 1k, l) LINGO2-GFP transfectants had a 12-fold increase in double-positive cells following incubation with TFF3-647 (Fig. 1m).
TFF3-647 did not bind to other LRR receptors such as TLR2 (Fig. 1m) and SDF-1-647 did not bind LINGO2 (Fig. 1o). TFF3 showed moderate binding to CXCR4 transfectants (Fig. 1p).

Next, to determine whether endogenous TFF3 production and LINGO2 expression occurred in a similar intestinal microenvironment to allow biologically relevant interactions in situ, normal human rectal tissues were immunostained with TFF3 and LINGO2 Ab to assess their spatial patterns. Compared to control Ab (Fig. 1q), LINGO2 was expressed in both enterocytes and sparsely along crypt epithelium, whereas TFF3 stained goblet cells located within crypts (Fig. 1r, s). In some instances, patches of cell-free TFF3 localized to the apical surface of LINGO2 positive enterocytes (Fig. 1r). Furthermore, distance between TFF3 and LINGO2 was determined using proximity ligation assay (PLA), which tests whether two Ab-bound proteins are within the 40 nm required for rolling circle amplification to produce fluorescent punctae. Compared to single stained anti-LINGO2 mAb (Supplementary Fig. 1D and F), combined staining with TFF3 and LINGO2 Ab revealed puncta within intestinal lamina propria and crypt epithelium (Supplementary Fig. 1E and G). Collectively, these data suggested that TFF3-LINGO2 interactions occurred within 40 nm on the cell membrane.

**TFF3 requires LINGO2 to activate EGFR and STAT3.** TFF3-mediated cytoprotective functions are associated with EGFR and ERK activation. To determine if TFF3 impacted epithelial EGFR signaling through LINGO2, we used a MC38 cells (murine intestinal adenocarcinoma) that expresses all four LINGO family member genes (Fig. 2a). A Lingo2KO subclone of MC38 (Δ-LINGO2) was generated using CRISPR-mediated insertion of a GFP-H2-K knock in construct followed by FACs sorting (Fig. 2b) showing a 20-fold reduction in LINGO2 mRNA expression (Fig. 2c). To directly test whether TFF3 protected against cytotoxicity in a LINGO2-dependent manner, we compared the parental MC38 vs. Δ-LINGO2 MC38 lines in cell-death assays using the apoptotic agent, staurosporine. Whereas TFF3-Fc pre-treatment significantly reduced cytotoxicity of parental MC38, protection was abrogated in Δ-LINGO2 cells (Fig. 2d). Given the anti-apoptotic role for STAT3 and evidence that TFF3 regulates STAT3 at the promoter level, we asked whether TFF3-driven STAT3 activation required LINGO2. TFF3-Fc (1μg/ml) induced STAT3 phosphorylation (pStat3 705) within 5 min in parental MC38 that slowly dissipated over 30 min (Supplementary Fig. 2A, B). However, TFF3-Fc-treated Δ-LINGO2 cells lacked pSTAT3 to this extent (0.5 fold after 5 min treatment) and showed reduced total STAT3 levels compared to loading controls (Supplementary Fig. 2A, B). Moreover, TFF3-Fc-induced pEGFR within parental MC38, but only a marginal pEGFR increase in Δ-LINGO2 cells (Fig. 2e). Thus, LINGO2 facilitated TFF3 mediated cytoprotection, STAT3 induction, and EGFR activation.

**LINGO2 binds to EGFR and TFF3 reverses that interaction.** Evidence suggesting that LINGO1 (a LINGO2 paralog) binds to and blocks EGFR activation prompted us to investigate whether LINGO2 directly interacted with EGFR. Reciprocal co-immunoprecipitation experiments demonstrated that Flag-LINGO2 and GFP-EGFR bound to each other (Fig. 2f) and confocal microscopy experiments demonstrated that EGFR-GFP and LINGO2-RFP co-localized on GFP transfected cells (Supplementary Fig. 2C). Because TFF3 required LINGO2 to induce pEGFR (Fig. 2e), LINGO2 could pull-down and co-localize with EGFR, and LINGO1 inhibits EGFR, we surmised that a constitutively bound LINGO2-EGFR complex may be disrupted by TFF3 binding to LINGO2 thereby sequestering LINGO2 away from EGFR to facilitate pEGFR activation. Consistent with this hypothesis, Δ-LINGO2 cells expressed levels of the EGFR-responsive genes **Arreg** and **Egfl**, that were 4–5-fold increased compared to parental MC38 following exposure to EGF (Fig. 2g, h). Furthermore EGF-treatment induced stronger pEGFR accumulation in Δ-LINGO2 cells compared to parental MC38 (Fig. 2i).

To directly test whether TFF3 disrupted a constitutively bound LINGO2-EGFR complex, we devised a strategy wherein LINGO2-EGFR protein complexes were co-incubated with either media, an irrelevant cytokine (rIL-2) or rTFF3 in solution. Complexes were immunoprecipitated to detect Flag-LINGO2 and immunoblotted for GFP to detect EGFR. Data show complex formation remained intact following media only or rIL-2 treatment, but rTFF3 reduced the abundance of LINGO2-EGFR complexes (Fig. 2j). Lastly, we noticed the doubling rate of Δ-LINGO2 cells was consistently higher than the MC38 parental line which, if our model was correct, relied upon constitutive pEGFR. Indeed, treatment with the EGFR tyrosine kinase inhibitor Gefitinib significantly reduced the doubling rate of Δ-LINGO2 cells over a three-day period, but had minimal impact on the parental MC38 line (Fig. 2k). Altogether, this indicated a model wherein an otherwise constitutive LINGO2-EGFR complex was disrupted by TFF3, allowing EGFR activation through ligand dependent or independent mechanisms, explaining how TFF3 could drive EGFR activation without being an EGFR ligand.

**LINGO2 deficient mice have excess intestinal EGFR activation.** LINGO2 knockout mice (Lingo2KO) were generated through CRISPR/CAS9 gene-editing using gRNAs for gene disruption through non-homologous end joining (NHEJ). Of the several Lingo2KO founder lines created, we selected one strain with a...
200 bp deletion immediately downstream of the transcriptional start site (Supplementary Fig. 3A). For initial characterization, intestinal tissues were subjected to LINGO2Ab immunostaining which revealed lack of LINGO2 on small intestinal villi in Lingo2KO mice compared to WT C57BL/6 controls and isotype matched Ab (Supplementary Fig. 3B–D).

To test our model that predicted LINGO2 constitutively suppressed EGFR, whole colonic tissues were isolated from WT and Lingo2KO mice and several animals were compared for their total vs. activated pEGFR levels under naive, steady-state conditions. Colonic tissues from Lingo2KO mice had no difference in colon length vs WT (Supplementary Fig. 3E), but higher basal levels of pEGFR compared to WT tissue (Fig. 3a). Consistent with enhanced EGFR activity, despite macroscopically similar appearing colonic crypts and colon lengths (Fig. 3b), quantification of S-phase crypt epithelial cells

**Fig. 2** TFF3 requires LINGO2 to block cytotoxicity, regulate STAT3 and de-repress EGFR activation. a LINGO family mRNA transcript levels in the MC38 mouse intestinal cell line. Mean ± SE of 3–6 replicates are shown. b Gating strategy for FACS-sorting based isolation of CRISPR/CAS9 gene-edited MC38 cells via incorporation of the LINGO2-H2KΔ-KΔ knock-in construct designated as "Δ-LINGO2". c Lingo2 mRNA transcript levels in Δ-LINGO2 MC38 cells as determined by QRT-PCR. Mean ± SE of triplicate wells. d Staurosporine-induced cytotoxicity at 6 h-post exposure of MC38 vs. Δ-LINGO2 cells following exposure to Fc (1 μg/mL) or TFF3-Fc (1 μg/mL or 23.81 nM) in DMEM 1% FBS. Mean ± SE of triplicate wells. e Time-course of phospho-EGFR (Y-1068) vs. total EGFR levels compared to beta-actin loading control in MC38 vs. Δ-LINGO2 cells exposed to Fc vs. TFF3-Fc (1 μg/mL). f Reciprocal co-immunoprecipitation of LINGO2-Flag and EGFR-GFP in co-transfected HEK293 cells. IP was performed with anti-Flag followed by IB with anti-GFP (left) or IP with anti-GFP followed by IB with anti-Flag (right). g mRNA expression levels of Amphiregulin (Areg) and pEGFR compared to Gapdh in parental MC38 vs Δ-LINGO2 cells following exposure to either media alone or rEGF (1 μg/mL) Mean ± SE of triplicate wells shown. h Phospho-EGFR (Y-1068) and total EGFR levels in parental MC38 vs Δ-LINGO2 at baseline or after stimulation with rEGF (1 μg/mL). i Co-IP of LINGO2-Flag and EGFR-GFP complexes in the presence of media only, soluble rIL-2, or rTFF3. j Kinetic analysis of cellular turnover for parental MC38 vs. Δ-LINGO2 cells in the presence or absence of the EGFR inhibitor Gefitinib (5 μM) treatment. Mean ± SE of six replicates are shown. Results of unpaired two-tailed t tests shown as p values in c, d, g, h and two-way ANOVa analyses in k.
following anti-E-cadherin and anti-Ki-67 mAb co-immunostaining revealed a higher basal number of Ki-67 positive cells in the Lingo2KO compared to the WT (Fig. 3c).

Also, Lingo2KO mice displayed impaired mucosal barrier function, demonstrated by 4-fold higher levels of FITC-dextran (4 kDa) in the peripheral blood at 4 h. post intragastric administration as compared to similarly treated WT mice (Fig. 3d). However, 16s-rDNA sequencing to determine fecal microbiome composition did not reveal any substantial differences between strains (Supplementary Fig. 3F). Thus, in mice Lingo2 deficiency resulted in heightened pEGFR activity and increased permeability of the GI tract at the steady-state.
DSS-induced colitis in Lingo2KO mice is EGFR-dependent. To determine the biological consequence of excess EGFR activation on intestinal injury and repair, Lingo2KO and co-housed WT littermates were orally administered dextran sodium sulfate (DSS) to induce transient colitic tissue injury (Fig. 3e). At 5 days following cessation of 2.5% DSS treatment, Lingo2KO mice had severe colitis marked by significantly greater weight loss and disease activity as compared to WT controls (Fig. 3f, g). Colon immunopathology was more severe in Lingo2KO mice, with greater degree of submucosal edema, ulceration, smooth muscle hyperproliferation and inflammatory infiltration as compared to WT controls (Fig. 3h). These experiments were then done with Gefitinib treatment to determine if inhibition of EGFR would reverse colitic disease in Lingo2KO mice. Indeed, weight loss, colon inflammation clinical scores, and colon shortening were all significantly reduced by Gefitinib treatment of Lingo2KO mice with negligible effect in the WT (Fig. 3i–l).

LINGO2 mRNA expression was detected in IEC, but also in myeloid cells, B cells, and CD4+T cells (Fig. 4a), therefore irradiation bone marrow chimeras were generated to test the contribution of hematopoietic vs. non-hematopoietic cells (Supplementary Fig. 4A-D). Compared to WT-WT chimeras, both WT-KO and KO-WT groups showed worse colitis, as defined by weight loss, disease activity, and clinical pathology, yet neither was as severe as the KO-KO group (Fig. 4b–e). There were no differences in colon length (Supplementary Fig. 4E). Surprisingly, despite a report that Tff3KO mice are highly sensitive to (DSS)-induced colitis47, we found that Tff3KO mice were not more susceptible to colitis than WT (Fig. 5a–c). This unexpected outcome was likely due to EGFR activity because Gefitinib treatment of Tff3KO mice, but not of WT mice, exacerbated colitic disease (Fig. 5d–g). Indeed, Egf mRNA transcripts were significantly higher in DSS-treated Tff3KO mice compared to WT or compared to either strain following Gefitinib treatment (Fig. 5h). Collectively, this suggested that EGFR activity, perhaps due to increased availability of EGFR ligand, protected Tff3KO mice from an otherwise increased susceptibility to DSS-induced colitis.

LINGO2 deficiency protects against helminths via EGFR. EGFR signaling drives host protective immune responses against different GI nematode species46. Goblet cells secrete substances toxic to GI helminths including Muc2, Muc5ac, and RELM-beta and also secrete immunoregulatory molecules such as TFF349–52. Mice were inoculated with the infective larval stage (L3) of *Nippostrongylus brasilensis* (*N.b.*). In this system, L3 migrate from the skin to the lung, down the esophagus, and into the jejunum within 3d where they develop into fecund adults that are expelled between 9–12 days post-infection via suppression of Type 1 (IL-12,IFN-γ) and induction of Type 2 cytokine production (e.g., Areg, interleukins 4, 5, 9, 13, 25, and 33)51,53 (Fig. 6a). Tff3 mRNA jejunal transcripts peaked between 5–7d post infection (Fig. 6b). Importantly, *N.b.*-infected Tff3KO mice harbored significantly more adult worms than WT mice at 9d (Fig. 6c). Increased susceptibility in Tff3KO mice was associated with increased IFN-γ production from mesenteric lymph node cells, without differences in Type 2 cytokines (Fig. 6d). At baseline, prior to infection, there were no differences in CD3 stimulated Type 1 vs. Type 2 cytokines between naïve WT and Lingo2KO mice (Supplementary Fig. 4F). Strikingly, *N.b.*-infected Lingo2KO mice had early reduced worm numbers by d4 (Fig. 6e) and reduced fecal egg numbers compared to WT between d7–8 post-infection (Fig. 6f). This protective phenotype occurred regardless of co-housing or the sex of the animals. Curiously, Type 2 cytokines (IL-9 and IL-13) were significantly reduced in Lingo2KO MLN cells compared to WT (Fig. 6g, h). Host resistance in Lingo2KO mice was primarily due to non-hematopoietic cells, as irradiation BM chimeras demonstrated that transfer of WT BM into KO mice resulted in significantly less fecal egg output as compared to the KO−WT chimeras (Fig. 6i). Consistent with the notion that enhanced EGFR activity occurred in the absence of LINGO2, we found higher amounts of pEGFR in intestinal homogenates of Lingo2KO compared to WT (Fig. 6j, k). As our model predicted, Gefitinib treatment during *N.b.* infection reversed resistance in Lingo2KO mice resulting in significantly higher intestinal worms compared to vehicle-treated controls (Fig. 6l). This indicated that *Lingo*2 deficiency protected against helminth infection, at least in part, due to EGFR activity.

**Discussion**

We report a pathway operating at the GI mucosal interface controlled by LINGO2, a leucine rich repeat and Ig domain containing receptor. Our data support a model wherein TFF3 produced by goblet cells can bind the LINGO2 extracellular domain (NH2 terminal 350 AA) to de-repress inhibitory LINGO2-EGFR complexes, thereby allowing TFF3 to help maintain mucosal barrier integrity, suppress colitis, and promote immunity against GI helminths. TFF3-mediated disruption of LINGO2/EGFR complexes seems to operate similar to a rheostat tuning the extent of EGFR activity inasmuch as total LINGO2 deficiency was deleterious due to excessive basal EGFR activity, but loss of TFF3 was somewhat protective against colitis (Fig. 5d, e) and also impaired worm immunity. Inhibition of EGFR in Lingo2KO mice reversed the phenotype in models of colitis and helminth infection. It is likely this EGFR activity was due to activation by other EGF ligands (e.g., Areg, EGFR) or TFF members (i.e., TFF2). While there are certainly other endogenous negative regulators of excessive other EGFR activity such as RALT, LRIG, SOCS4 and SOCS554–56, this is the first demonstration that: (1) TFF family members activate EGFR through de-repression, (2) LINGO2 regulates mucosal repair and immunity, and (3) TFF3 exerts its function(s), at least partially, through LINGO2.

LINGO2 is a 606aa protein with a large extracellular portion (ectodomain), a transmembrane domain, and a short cytoplasmic tail. LINGO2 belongs to the family of leucine-rich repeat and Ig-like domain proteins that includes LRIG157 that is known for ErbB receptor family regulation. LRIG1, a tumor suppressor and stem cell marker, induces proteasomal degradation of EGFR (and its other three ErbB family members) via polyubiquination58. LINGO1 is a component of a heterotrimERIC signaling complex comprised of the Nogo-66 receptor and the p75 neurotrophin receptor that directly binds EGFR45 and inhibits EGFR signaling and axon regeneration59. In oligodendrocytes, LINGO1 suppresses signaling of the ErbB2 receptor by preventing lipid raft translocation where it can be phosphorylated60. Similar to other leucine-rich repeat and Ig-like domain proteins, we observed an ErbB receptor family negative regulatory function for LINGO2. Our data suggest that LINGO2 binds with EGFR constitutively, suppressing its ability to undergo phosphorylation. Whether this occurs through catalytic inhibition or receptor downmodulation remains unclear. Whether LINGO2 operates in a signaling complex similar to LINGO159 is also unknown. Regardless, our data support a mechanism wherein upon injury, TFF3 gains access to LINGO2 in a manner requiring the NH2-terminus, sequestering it away from EGFR, thereby raising the level of EGFR activation at times when the mucosa needs to repair tissue or expel pathogens.

Contrary to the initial report of DSS-treatment of the Tff3KO mouse strain47 and in contrast to the prevailing notion that TFF3
Fig. 4 Non-hematopoietic and hematopoietic cells contribute to LINGO2-mediated protection from DSS colitis. a RT-PCR quantification of Lingo2 mRNA transcript levels in myeloid, B cell, T cell and intestinal epithelial cells isolated using magnetic beads from WT and Lingo2KO mice n = 3/group. Representative of two experiments with results of unpaired two tail t tests shown. b Change in initial body weight and c disease activity index and d clinical pathology inflammation score in colon tissues from irradiation bone marrow chimeras at 4 days following cessation of 2.5% DSS administration for donor-recipient combinations WT → WT, Lingo2KO → Lingo2KO, WT → Lingo2KO, Lingo2KO → WT. Mean ± SE of 4 or 5 mice/group and results of two-way ANOVA analyses are shown. e Representative photomicrograph images at ×20 showing H&E stained colon tissues from irradiation bone marrow chimeras in (b-d). Scale 40 μm
is essential for protection of the GI mucosa\textsuperscript{61}, we did not find that 

tff3KO mice were more susceptible to DSS colitis. There may be several explanations for this discrepancy. The original study used mice on a mixed background (C57BL/6 × 129/SVJ), whereas our strain was backcrossed to C57BL/6 for >8 generations. Also, that report was published before recognition that microbial flora is a major determinant in GI inflammatory disease and did not take into consideration the importance of cohousing, whereas our studies were all completed using cohoused mice. Lastly, our protocol was 6 days on DSS followed by a return to normal drinking water in order to evaluate the wound healing response, whereas that study continuously treated mice with DSS for 9 days\textsuperscript{47}. Notably, IEC-specific EGFR deletion in mice did not lead to any change in colitic disease severity indicating that normally the loss of EGFR on intestinal epithelia is not deleterious\textsuperscript{62}.  

EGFR expression on Th2 cells is critical to their production of Type 2 cytokines that drive worm expulsion\textsuperscript{34}. IEC also serve a host protective role against helminth infection\textsuperscript{49,51,52}. In addition to secretion of toxic factors by IEC, an increased epithelial cell turnover mechanism through sloughing into the lumen\textsuperscript{63}. N.b.-infected tff3KO mice were more susceptible, which was perhaps due to the increased IFN-γ production, known to block immunity against N.b. infection\textsuperscript{64}. TFF3 may serve an additional role as an immunomodulatory cytokine similar to TFF2, which also suppresses Type 1 responses\textsuperscript{65}. 

In conclusion, our results provide supportive evidence for a model that predicts LINGO2 binds to and inhibits EGFR activity and that TFF3 interactions with LINGO2 remove this negative regulation, allowing for enhanced EGFR activity. LINGO2 deficiency basally elevates EGFR activity, susceptibility to colitis and resistance to helminth infection. Genome wide association study (GWAS) data show LINGO2 genetic polymorphisms in human patients with cancer\textsuperscript{66}, asthma\textsuperscript{67}, chronic obstructive pulmonary disease\textsuperscript{68}, Parkinson’s, and Inflammatory bowel disease\textsuperscript{69}. In sum, this complex interplay among TFF3, LINGO2, and EGFR deserves greater attention to decipher how this triad controls mucosal repair and immunity.

**Methods**

**Mice.** All animal procedures were approved by the Institutional Animal Care and Use Committee at University of California, San Francisco and at University of Pennsylvania. Super-ovulated female C57BL/6 mice (4 weeks old) were mated to C57BL/6 stud males. Fertilized zygotes were collected from oviducts and injected with Cas9 protein (30 ng/μl), sgRNA (15 ng/μl) into pronucleus of fertilized zygotes. Injected zygotes were implanted into oviducts of pseudo pregnant CD1 female mice. Animals were housed under specific-pathogen free barriers in vivarium at San Francisco General Hospital or University of Pennsylvania. Irradiation bone marrow chimeras were generated as previously described\textsuperscript{70}. The recipients were irradiated with 10 Gy administered as a split-dose and were retro-orbitally inoculated with 2–5 × 10⁶ total bone marrow cells isolated from the femurs of the donor animals within one hour following the second dose of irradiation. All irradiated recipients were administered 1% enrofloxacin for 2 weeks.
Tff3 mRNA transcript levels in jejunum of WT C57BL/6 mice infected with 750 N. b. L1. Data show mean ± SE of 3 or 4 mice/group. c Intestinal adult worm numbers in WT vs. Tff3KO mice at day 9 post-infection each symbol represents an individual mouse. d Secretion of IL-4, IL-5, IL-13 and IFN-γ levels from WT vs. Tff3KO mesenteric lymph node cells isolated at day 9 post-infection. Cytokine levels in response to media alone, anti-CD3 (1 μg/ml) at 48 h. Experiment performed twice $n = 4–5$ mice/group. e Intestinal worm numbers at day 4 post-infection 9 days post infection with N. brasiliensis in WT versus Lingo2KO mice. Mean ± SE of 5 mice/group are shown Representative of four independent experiments. f Fecal egg numbers, shown as eggs per gram feces (EPG) in WT vs. Lingo2KO mice at days indicated. g IL-9 and h IL-13 production from isolated MLN on day 9 exposed to media or N. brasiliensis antigen extract. i Egg load in feces on day 7 from WT → Lingo2KO versus Lingo2KO → WT BM chimera mice at day 4 post infection. Means ± SE of 3 or 4 mice/group are shown. j Western blot showing intestinal levels of phospho-EGFR and total EGFR in mice at day 9 post-infection. Each represents an individual mouse. k Densitometry for data shown in j. l Adult worm burdens recovered from intestinal lumen at day 9 from WT and Lingo2KO mice treated with Gefitinib or vehicle during infection with N. b. infection. Means ± SE of 9 or 10 mice/group are shown. * $p$ values shown represent the results of unpaired two tail $t$ tests except for $I$ where two-way ANOVA analyses are shown.
adenosine triphosphate (ATP)-binding site of the enzyme. Parasites were maintained on copro-cultures incubated at 25°C. Larvae from 7 to 14-day-old cultures were collected and washed 3× in PBS with 1% Penicillin/Streptomycin. To infect with Nippostrongylus brasiliensis, 650–750 L1 per mouse were injected subcutaneously using procedures previously established. Mice were evaluated between d4–9 for intestinal worm burden using the Baermann technique. On do–d8 fecal pellets were collected from each mouse to assess egg counts. On designated days post-infection, euthanized half of each group and tissues were removed, opened longitudinally, and incubated in PBS for 2 h before collecting and counting adult worms.

**Microbial sequencing.** Genomic DNA was extracted from fecal pellets of mice using Qian DNeasy Power Soil kit. The V4 region of the 16S rRNA gene was amplified using barcoded primers for use on the Illumina platform. Sequencing was performed using paired-end 2 × 150 bp reads. High-quality sequences were trimmed and filtered out with QIIME v. 1.8.4 and used to perform paired-end reads and to perform downstream analyses including taxonomy assignment and heat map generation.

**Cloning/plasmid construction.** The full-length mLingo2 coding region was obtained with KpnI and XhoI double digestion of Lingo2 cdna plasmid (MR215789, Origene) and subcloned into pcDNA3.1GFP vector (Addgene) or pcDNA3.mRFP vector (Addgene) to generate pcDNA3.mLingo2:GFP and pcDNA3.mLingo2:RFP constructs. A series of deletion of Lingo2 functional domains (Ectodomain and cytoplasmic tails) were generated by PCR amplification with the specific primers bearing KpnI and XhoI overhangs, and cloned into pdNA3.mGFP vector (Addgene) to get Lingo2 truncated constructs fused with GFP. The full-length mTFF3 coding region was amplified with mTFF3 forward primer and HindIII overhang and mTFF3 reverse primer with XhoI overhang using mTFF3 cdna plasmid (MR20151, Origene) as the template. The digested TFF3 PCR product with HindIII and XhoI enzymes was cloned into pcDNA3.mRFP vector (Addgene) to generate pcDNA3:mTFF3:RFP construct. Using the similar method, the full-length mLingo2 coding region was also cloned into pINFUSE-mlg2b-2C vector (InvivoGen) to generate pINFUSE-mLingo2:Flag vector. The full-length mSDF1/cXCR4 coding region was amplified with mCXC4R forward primer with HindIII overhang and mCXC4R reverse primer with XhoI overhang using mCXC4R cdna plasmid (OMU2304D, Origene) as the template. The digested CXC4R PCR product with HindIII and XhoI enzymes was cloned into pdNA3.mGFP vector (Addgene) to generate pcDNA3:mouseCXC4R:GFP construct. The full-length sDF1/cXCX12 coding region was amplified with mSDF1 forward primer with EcoRI overhang and mSDF1 reverse primer with XhoI overhang using sDF1 cdna plasmid (MR227229, Origene) as the template. The digested SDF1 PCR product with EcoRI and XhoI enzymes was cloned into the pcDNA3.mRFP vector (Addgene) to generate pcDNA3:sDF1:RFP construct. The sequences of constructs were confirmed by DNA sequencing in UPenn Sequencing facility. HEK293 cells were maintained in DMEM medium (Invitrogen) supplemented with 10% fetal bovine serum (Invitrogen) and 1% penicillin-streptomycin (Invitrogen) at 25°C with 5% CO2. Before transfection, cells (1 × 105 cells/well) were incubated in a well of Lab-Tek Chamber slide (sigma) for overnight culture. 1ug plasmids were transfected in 100 μl serum-free medium (Invitrogen) and 1% penicillin-streptomycin (Invitrogen) at 25°C with 5% CO2. The transfection reagent (Promega) based on manufacturer’s instruction. This model assumes that the measurement error follows statistical ANOVA model. This model assumes that the measurement error follows statistical ANOVA model.
Gaussian distribution and views individual features as replicates of a protein’s abundance and explicitly accounts for this redundancy. It tests each protein for differential abundance in all pairwise comparisons of ligand and control samples and reports the p-values. Next, p-values are adjusted for multiple comparisons to control the experiment-wide false discovery rate (FDR). The adjusted p-value obtained for every protein is plotted against the magnitude of the fold enrichment between the two experimental conditions. The area in the volcano plot that is limited by an enrichment factor of twofold or greater and an FDR-adjusted p-value less than or equal to 0.01 is defined as the receptor candidate space. To allow for statistical analysis, the experiment was done in biochemical triplicates. The samples enriched in glycoproteins were analyzed on a Thermo LTQ Orbitrap XL spectrometer. Peptide identifications were filtered to a false-discovery rate of ≤1% and quantified using an MS1-based label-free approach.

**Histology.** Mice were eviscerated, and colons extracted. A small biopsy of the intact distal colon was excised, fixed overnight at 4 °C in neutral 10% formaldehyde, further dehydrated and embedded in paraffin for histological analysis. Another small biopsy in the distal region was snap frozen in liquid N2 prior to RNA isolation and real-time qPCR. Pathological assessment of colitis was performed blindly by certified pathologists as per established criteria for the Penn Vet Comparative pathology core.

Primers (Forward-Reverse) mTFF3-5′ GCC CTG TGG CTA ATG CTG TT 3′ and 5′ TTG GGA TCA TGG AGT CAA AGC 3′ mLINGO1-5′ GAA CAA CAT GCT CAT CACT GC 3′ and 5′ GAT GTT GAG GAT TGG TAG CCG TAG CC 3′ mLINGO2-5′ GCC GTG AAC CAA GGT GTA GAC-3′ and 5′ TTA AGA GTA ACA CCA CAG CCA GAC 3′ mLINGO3-5′ CTG CAT TTG CTG CTG CT 3′ and 5′ CTC CAG CAT GCG AGA TTA TTC G 3′ mLINGO4-5′ AGA TGC AGC GCC CAA CTT GA 3′ and 5′ GAT GGG ATT GCC CGT AAC CAA GGT GTA GAC-3′ mTTF3-5′ GCC CTC TGG CTA ATG TT 3′ and 5′ TTG GGA TAC TGG

**RNA isolation and real-time qPCR.** Approximately 0.5 cm of duodenum was removed for RNA extraction using NucleoSpin RNA Plus kit (Macherey-Nagel, Düren, Germany). Five hundred nano-grams of total RNA were used to generate cDNA with Super Script II (Invitrogen). Quantitative real-time PCR was performed on the CXF96 platform (Bio-Rad, Hercules, CA). Gene expression levels were normalized to (GAPdh). Intestinal transcripts from colon biopsies were isolated and analyzed as previously described.

**Statistics.** Statistical analyses were performed using GraphPad Prism version 7.0 (GraphPad, La Jolla, CA). A two-tailed Student’s t test or ANOVA was used where appropriate with Kruskal Wallis used for non-parametric data (DAI scores).

**Reporting Summary.** Further information on research design is available in the Nature Research Reporting Summary linked to this article.

**Data availability** The authors declare that the data supporting the findings of this study are available from the authors upon request. The source data underlying Figs. 1a, c, d, 2a, c-k, 3a, d, f-g, i-j, 1, 4a-d, 5a-I, 6b-1 and Supplementary Fig. 2a-b is available as a Source Data file.

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**References**

1. Farrell, C. L. et al. The effects of keratinocyte growth factor in preclinical models of mucositis. *Cancer Chemother. Pharmacol.* 59, 1–10 (2012).

2. Gibson, R. J. et al. The effect of keratinocyte growth factor on tumour growth and small intestinal mucositis after chemotherapy in the rat with breast cancer. *Cancer Chemother. Pharmacol.* 59, 33–58 (2002).

3. Salvati, V. M. et al. Keratinocyte growth factor and coeliac disease. *Gut* 49, 170–181 (2003).

4. Egger, B. et al. Keratinocyte growth factor ameliorates dextran sodium sulphate colitis in mice. *Dig. Dis. Sci.* 44, 836–844 (1999).

5. Miyoshi, H. et al. Prostaglandin E2 promotes intestinal repair through an adaptive cellular response of the epithelium. *EMBO J.* 36, 5–24 (2017).

6. Gong, W. et al. Mesenchymal stem cells stimulate intestinal stem cells to repair radiation-induced intestinal injury. *Cell Death Dis.* 7, e2387 (2016).

7. Cosin-Roger, J. et al. The activation of Wnt signaling by a STAT6-dependent macrophage phenotype promotes mucosal repair in murine IBD. *Mucosal Immunol.* 9, 868–998 (2016).

8. Kovac, S. et al. The Wnt antagonist Dkk1 regulates intestinal epithelial homeostasis and wound repair. *Gastroenterology* 141, 259–268 (2011). 268 251–258.

9. Carrasco, R. et al. Trefoil factor family peptide 3 prevents the development and promotes healing of ischemia-reperfusion injury in weanling rats. *J. Pediatr. Surg.* 39, 1693–1700 (2004).

10. Gereey, M. A., Van Winkle, L. S., Edwards, P. C. & Plopper, C. G. Airway trefoil factor expression during naphthalene inhalation and injury. *Toxicol. Sci.* 113, 453–467 (2010).

11. Jiang, G. X. et al. IL-8/STAT3/TFF3 signaling regulates human biliary epithelial cell migration and wound healing in vitro. *Mol. Biol. Rep.* 37, 3813–3818 (2010).

12. Buda, A., Jepson, M. A. & Pignatelli, M. Regulatory function of trefoil peptides (TFF) on intestinal cell junctional complexes. *Cell Commun. Adhes.* 19, 63–68 (2012).

13. Taupin, D. R., Kinoshita, K. & Podolsky, D. K. Intestinal trefoil factor confers colonic epithelial resistance to apoptosis. *Proc. Natl Acad. Sci. USA* 97, 799–804 (2000).

14. Le, J. et al. ITF promotes migration of intestinal epithelial cells through crosstalk between the ERK and JAK/STAT3 pathways. *Sci. Rep.* 6, 33004 (2016).

15. Liu, D. et al. Phosphorylation of beta-catenin and epithelial growth factor receptor by intestinal trefoil factor. *Lab. Invest.* 77, 557–563 (1997).

16. Esfathouli, J. A. et al. Mutated epithelial cadherin is associated with increased tumorigenicity and loss of adhesion and of responsiveness to the motogenic trefoil factor 2 in colon carcinoma cells. *Proc. Natl Acad. Sci. USA* 96, 2316–2321 (1999).

17. Esfathouli, J. A. et al. Intestinal trefoil factor controls the expression of the adenomatus polyposis coli-catenin and the E-cadherin-catenin complexes in human colon carcinoma cells. *Proc. Natl Acad. Sci. USA* 95, 3122–3127 (1998).

18. Chinery, R. & Playford, R. J. Combined intestinal trefoil factor and epithelial growth factor is prophylactic against indomethacin-induced gastric damage in the rat. *Clin. Sci.* 88, 401–403 (1995).

19. Chinery, R., Bates, P. A., De, A. & Freemont, P. S. Characterisation of the single copy trefoil peptides intestinal trefoil factor and pS2 and their ability to form covalent dimers. *FEBS Lett.* 357, 50–54 (1995).

20. Thim, L., Madsen, F. & Poulsen, S. S. Effect of trefoil factors on the viscoelastic properties of mucus gels. *Eur. J. Clin. Invest.* 32, 519–527 (2002).

21. Landon, H., Poonthoulakis, C., Thim, L., Lynch-Devaney, K. & Podolsky, D. K. Trefoil factor protection of intestinal epithelial barrier function: cooperative interaction with mucin glycoprotein. *Gastroenterology* 109, 516–523 (1995).

22. Biton, M. et al. T helper cell cytokines modulate intestinal stem cell renewal and differentiation. *Cell 175*, 1537–1546 (2018).

23. Cook, G. A., Familiar, M., Thim, L. & Giraud, A. S. The trefoil peptides TFF2 and TFF3 are expressed in rat lymphoid tissues and participate in the immune response. *FEBS Lett.* 456, 155–159 (1999).

24. Zhu, Y. Q. & Tan, X. D. TFF3 modulates NF-kappaB and a novel negative regulatory molecule of NF-κB in intestinal epithelial cells via a mechanism distinct from TNF-α/alpha. *Am. J. Physiol. Cell Physiol.* 289, C1085–C1093 (2005).

25. Barrera, G. J., Sanchez, G. & Gonzalez, J. E. Trefoil factor 3 isolated from human breast milk downregulates cytokines (IL8 and IL6) and promotes human beta defensin (hBD2 and hBD4) expression in intestinal epithelial cells HT-29. *Biosci. Basic Med. Sci.* 12, 256–264 (2012).

26. Teng, X., Xu, L. F., Zhou, P., Sun, H. W. & Sun, M. Effects of trefoil peptide 3 on expression of TNF-alpha, TLR4, and NF-κappaB in trinitrobenzene sulfonic acid induced colitis mice. *Inflammation* 32, 120–129 (2009).

27. Arnold, P. et al. Trefoil factor 3 shows anti-inflammatory effects on activated microglia. *Cell Tissue Res.* 365, 3–11 (2016).

28. Sun, Y., Wang, L., Zhou, Y., Mao, X. & Deng, X. Human Trefoil Factor 3 induces the transcription of its own promoter through STAT3. *Sci. Rep.* 6, 30421 (2016).

29. Rivat, C. et al. Implication of STAT3 signaling in human colonic cancer cells during intestinal trefoil factor 3 (TFF3)- and vascular endothelial growth factor-mediated cellular invasion and tumor growth. *Cancer Res.* 65, 195–202 (2005).

30. antennery, R. & Cox, H. M. Immunoprecipitation and characterization of a binding protein specific for the peptide, intestinal trefoil factor. *Peptides* 16, 749–755 (1995).
31. Minnitti, C. M. et al. Epidermal growth factor receptor expression licenses type-2 helper T cells to function in a T cell receptor-independent fashion. Immunity 47, 726–737 (2017).

32. Frei, A. P., Moest, H., Novy, K. & Wollscheid, B. Ligand-based receptor identification on living cells and tissues using TRÆCÆS. Nat. Protoc. 8, 1321–1336 (2013).

33. Atanassova, M. & Whitty, A. Understanding cytokine and growth factor receptor activation mechanisms. Crit. Rev. Biochem. Mol. Biol. 47, 502–530 (2012).

34. Poulsen, S. S., Kissow, H., Hare, K., Hartmann, B. & Thim, L. Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat. Regul. Pept. 126, 163–171 (2005).

35. Musi, S. S., Kissow, H., Hare, K., Hartmann, B. & Thim, L. Luminal and parenteral TFF2 and TFF3 dimer and monomer in two models of experimental colitis in the rat. Regul. Pept. 126, 163–171 (2005).

36. Hasnain, S. Z. et al. Mucin gene deletion in mice impairs host resistance to experimental an enteric parasitic infection. J. Exp. Med. 189, 2019–2026 (1999).

37. Finkelman, F. D. et al. Effects of interleukin 12 on immune responses and host protection in mice infected with intestinal nematode parasites. J. Exp. Med. 179, 1563–1572 (1994).

38. Pokryski, O. M. et al. Trefoil factor 2 negatively regulates type 2 immunity against Toxoplasma gondii. J. Immunol. 189, 3078–3084 (2012).

39. Li, W. Q. et al. Genetic polymorphisms in the 9p21 region associated with risk of multiple cancers. Carcinogenesis 35, 2698–2705 (2014).

40. Berube, J. C. et al. Identification of susceptibility genes of adult asthma in French Canadian women. Can. Respir. J. 2016, 3564341 (2016).

41. Hansel, N. N. et al. Genome-wide association study identification of novel loci associated with airway responsiveness in chronic obstructive pulmonary disease. Am. J. Respir. Cell Mol. Biol. 53, 226–234 (2015).

42. Anderson, C. A. et al. Meta-analysis identifies 29 additional ulcerative colitis risk loci, increasing the number of confirmed associations to 47. Nat. Genet. 43, 246–252 (2011).

43. Hung, L. Y. et al. Trefoil factor 2 promotes type 2 immunity and lung repair through intrinsic roles in hematopoietic and nonhematopoietic cells. Am. J. Pathol. 188, 1161–1170 (2016).

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Author contributions

N.B., Y.J., Y.W., J.P., L.Y.H., K.Z., T.Y., K.H., T.O., C.P., S.S., and W.N. conducted experiments, M.S. contributed valuable reagents and samples, N.B., Y.W., and J.P. helped to write the manuscript and D.R.H. conceived the study and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Additional information

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Correspondence and requests for materials should be addressed to D.R.H.

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