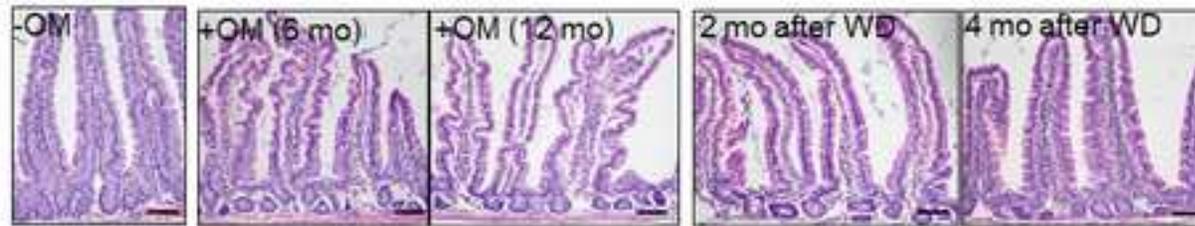
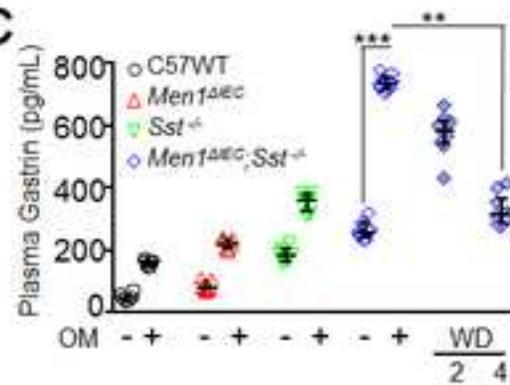


Supplemental Fig. 1

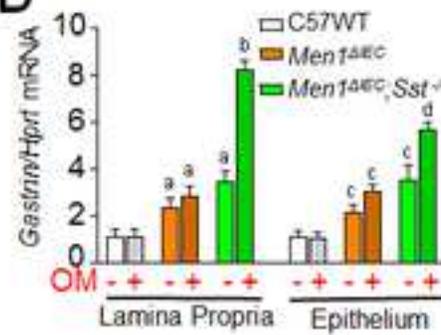
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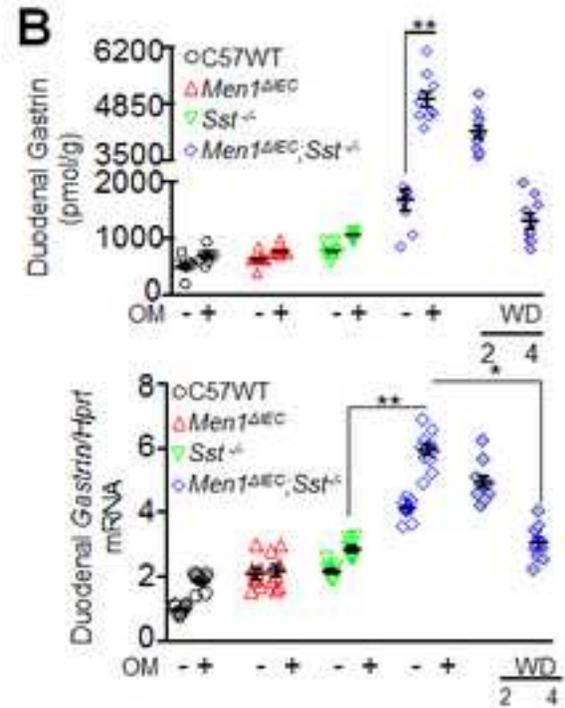
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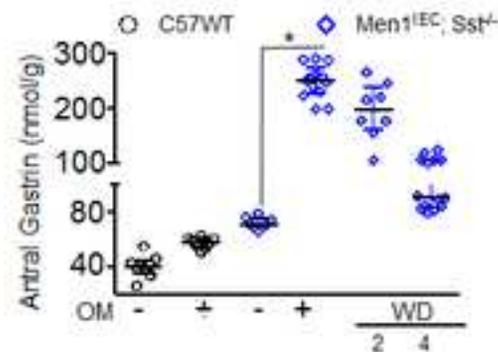
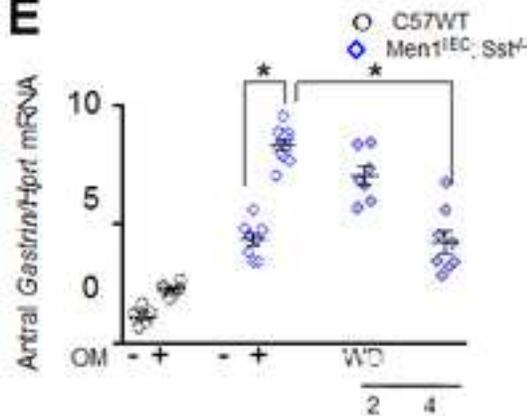
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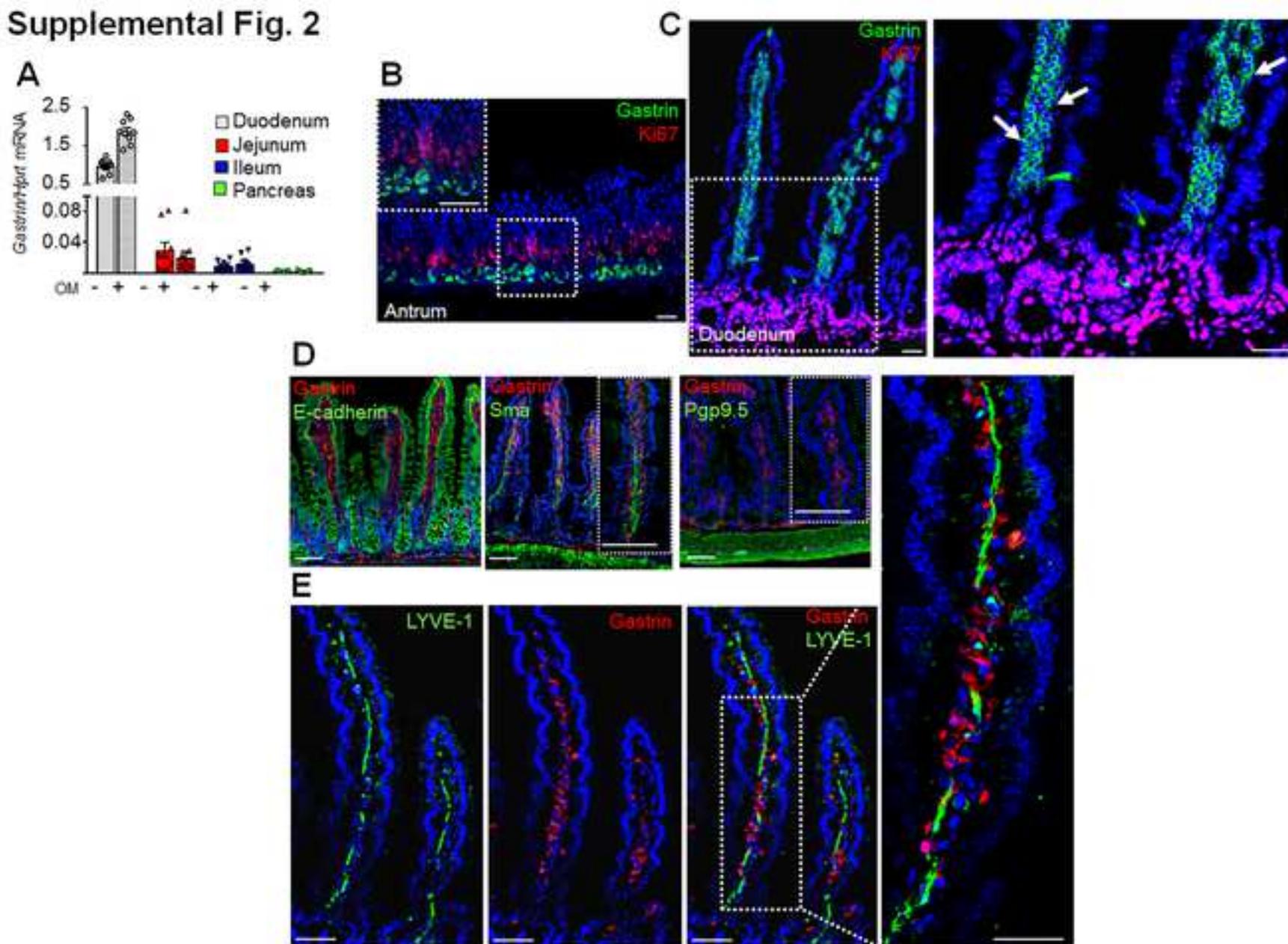
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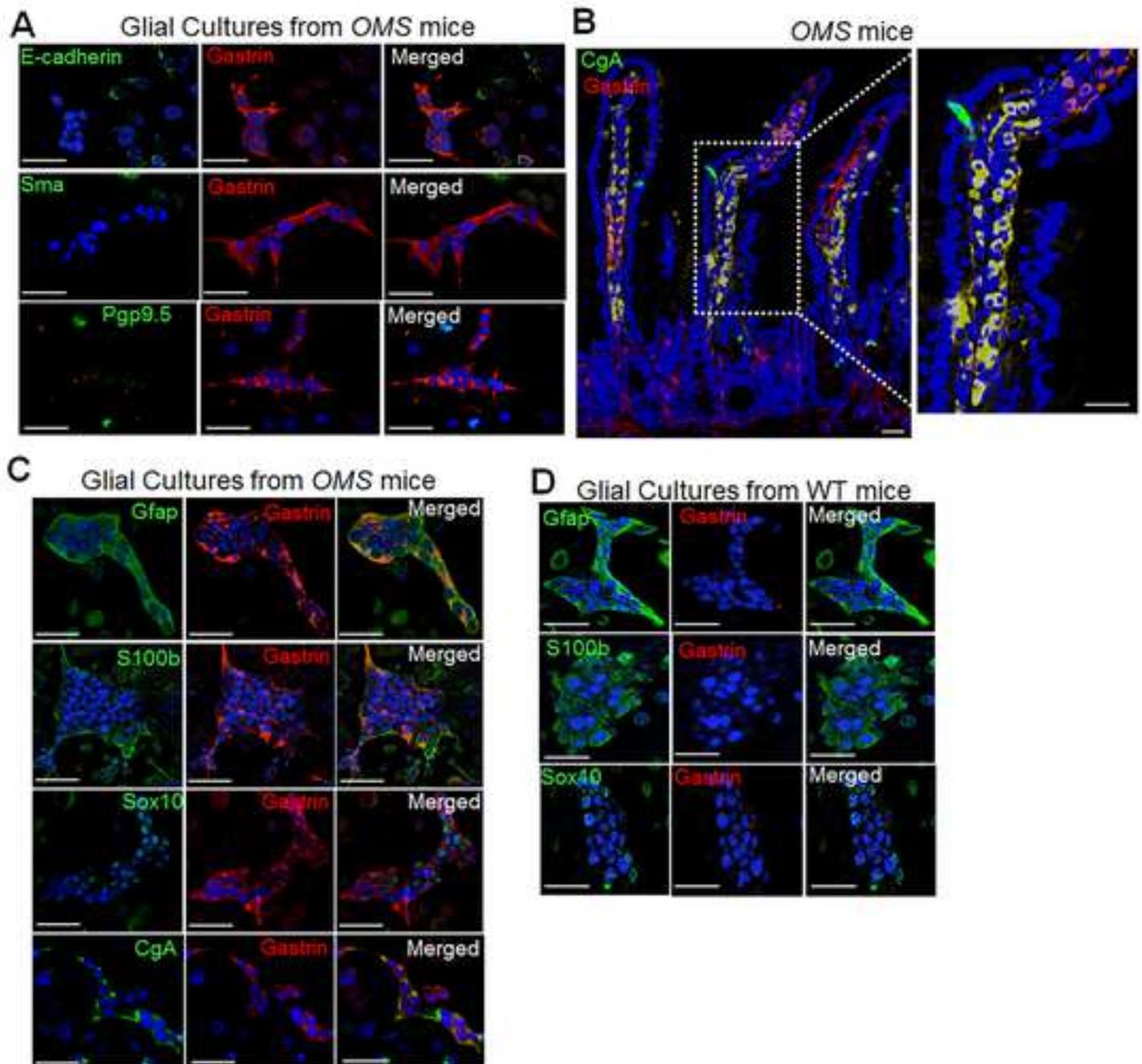
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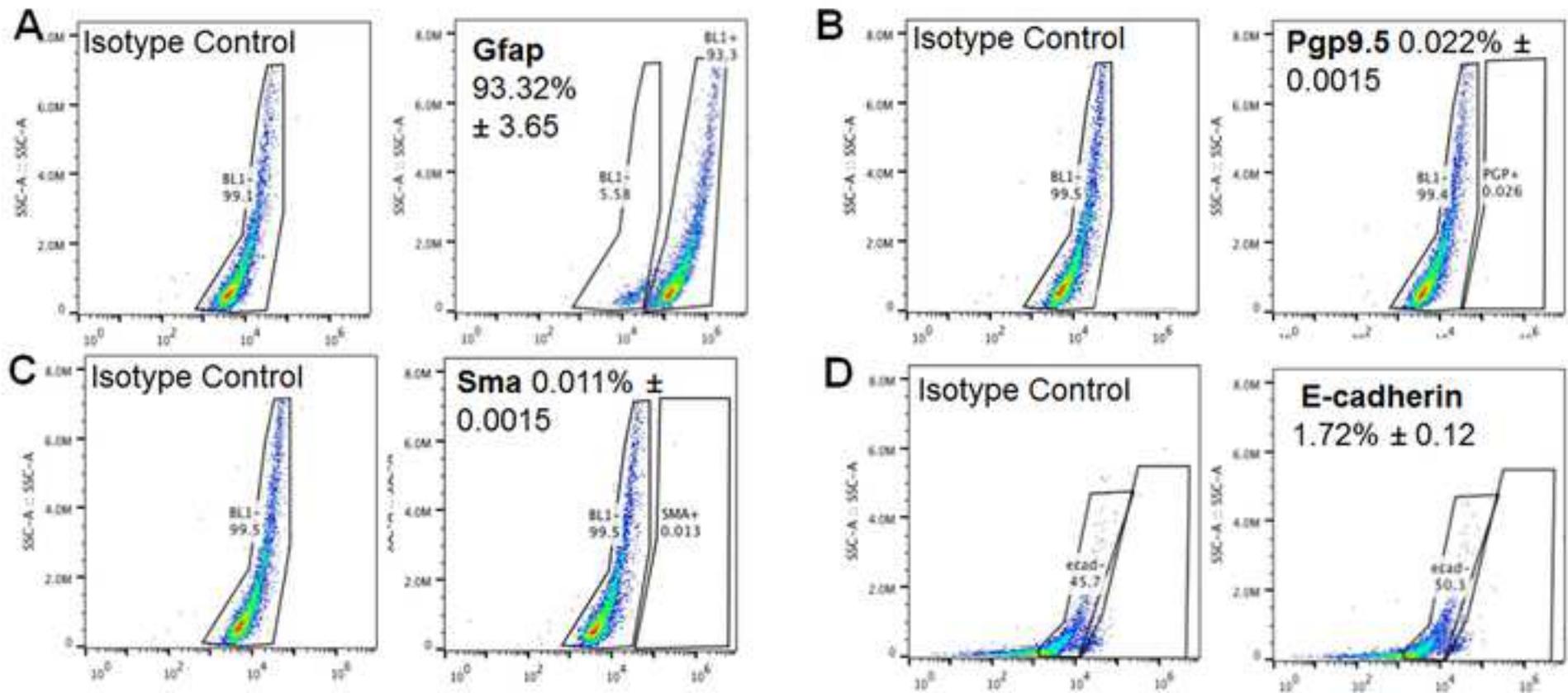
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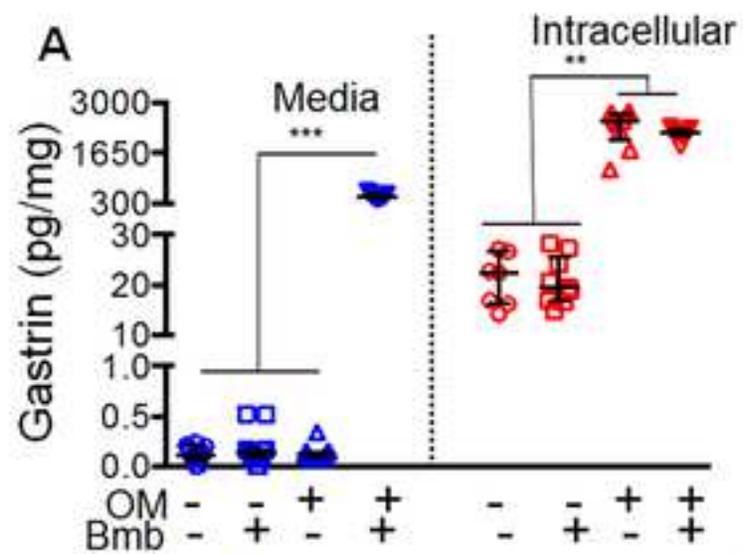
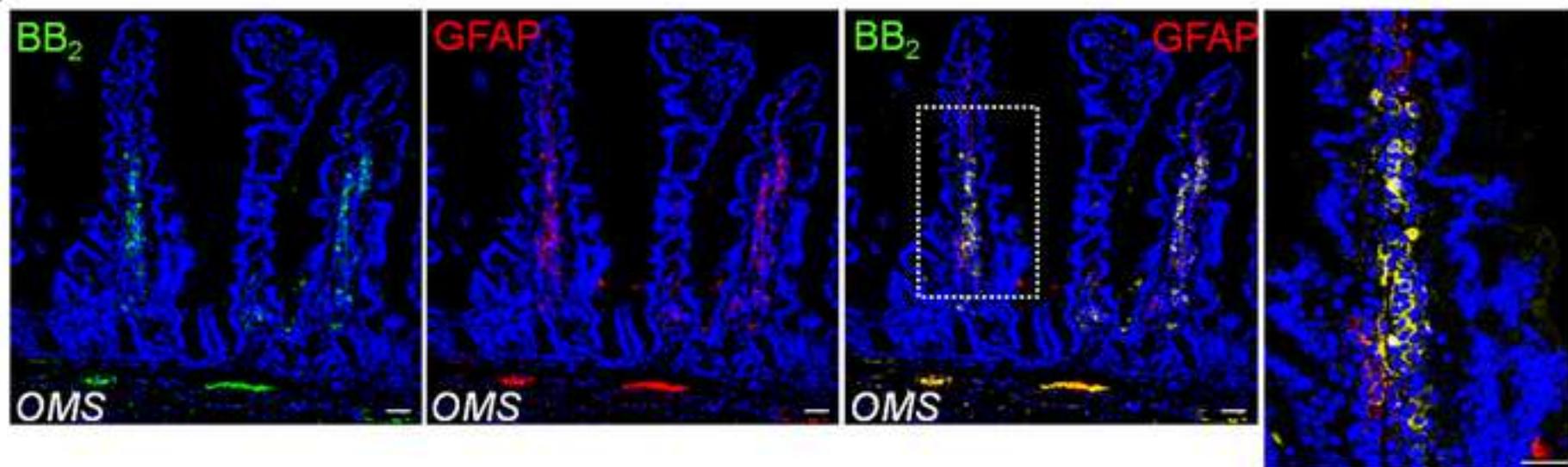
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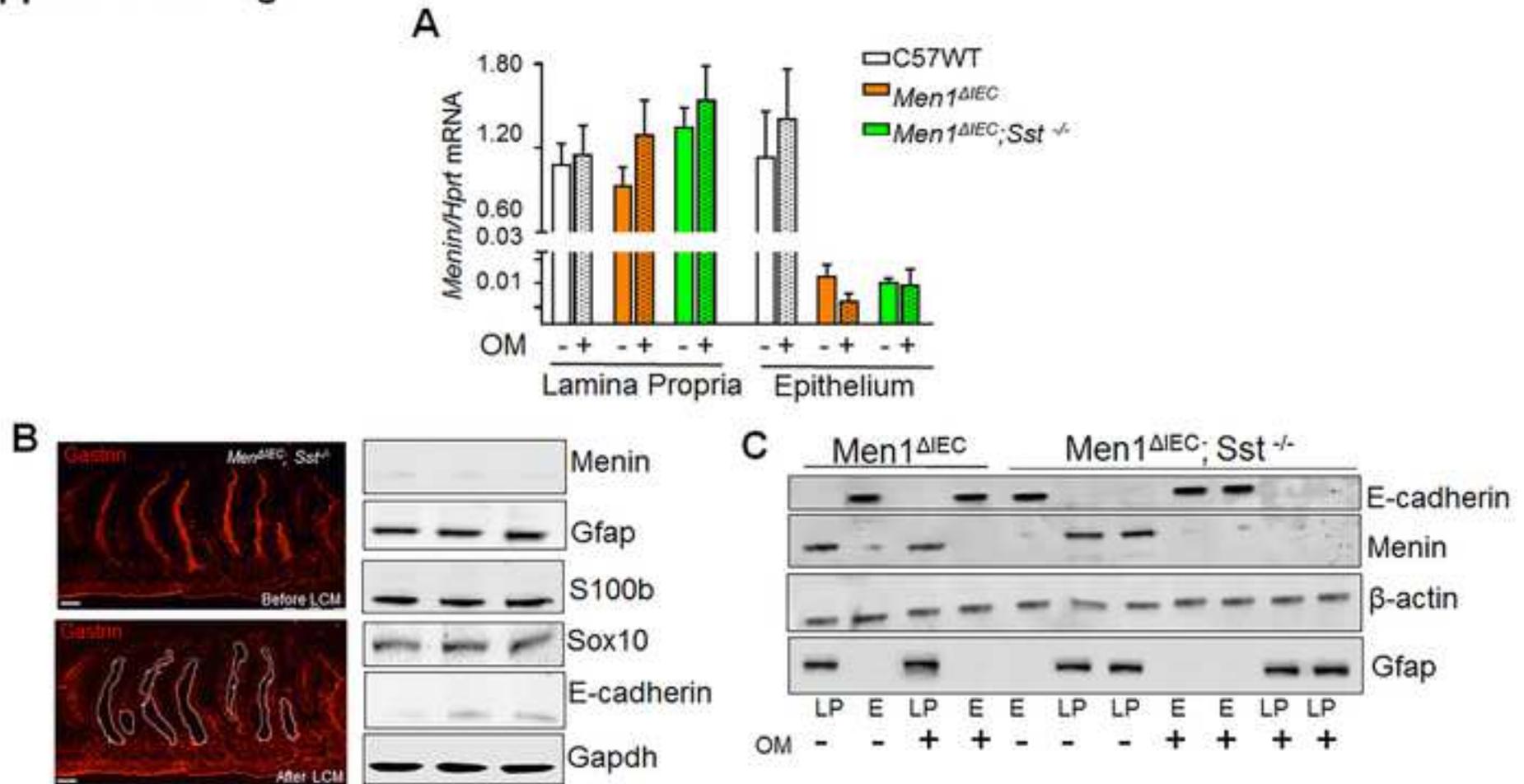
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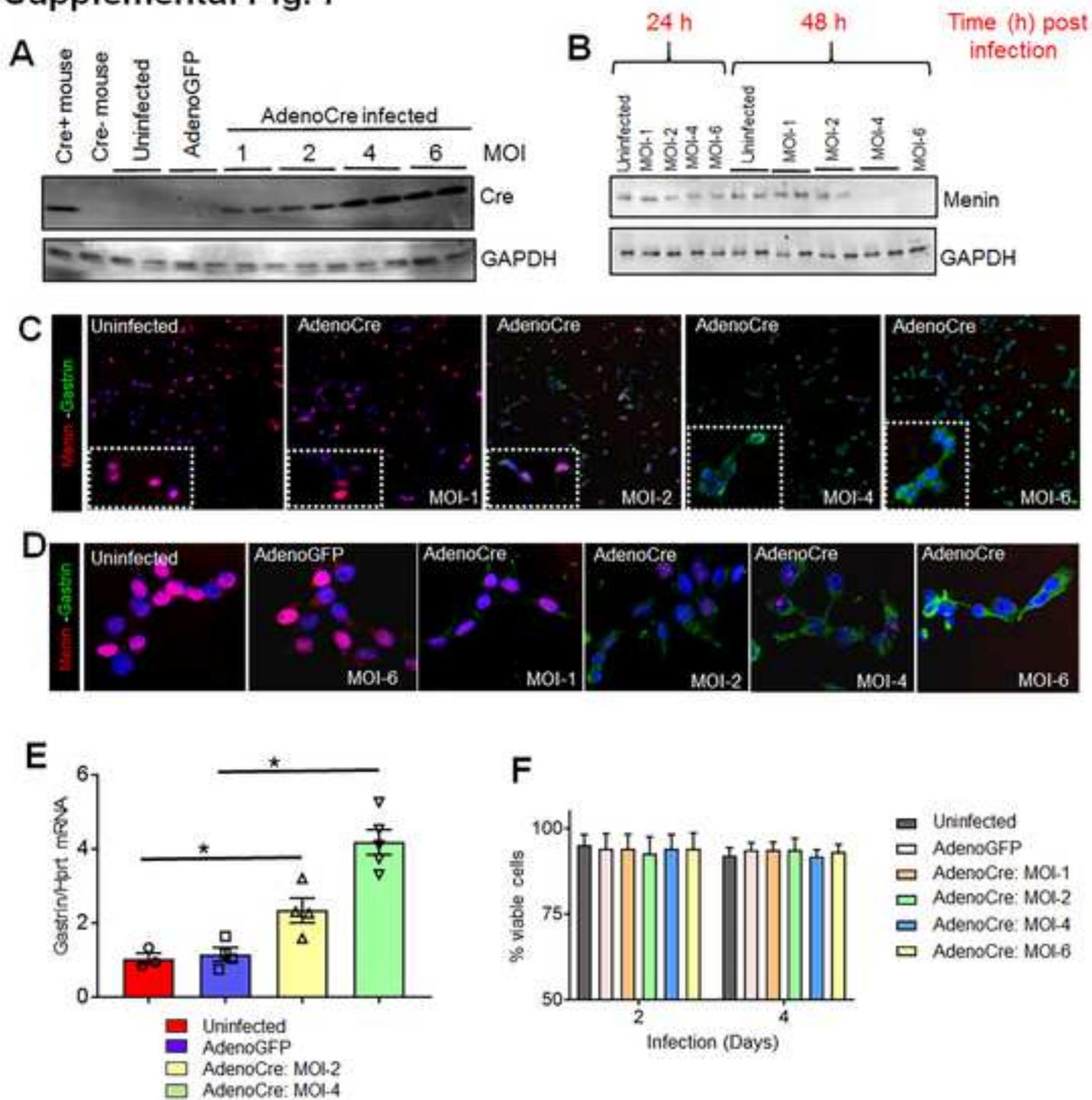
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**B**

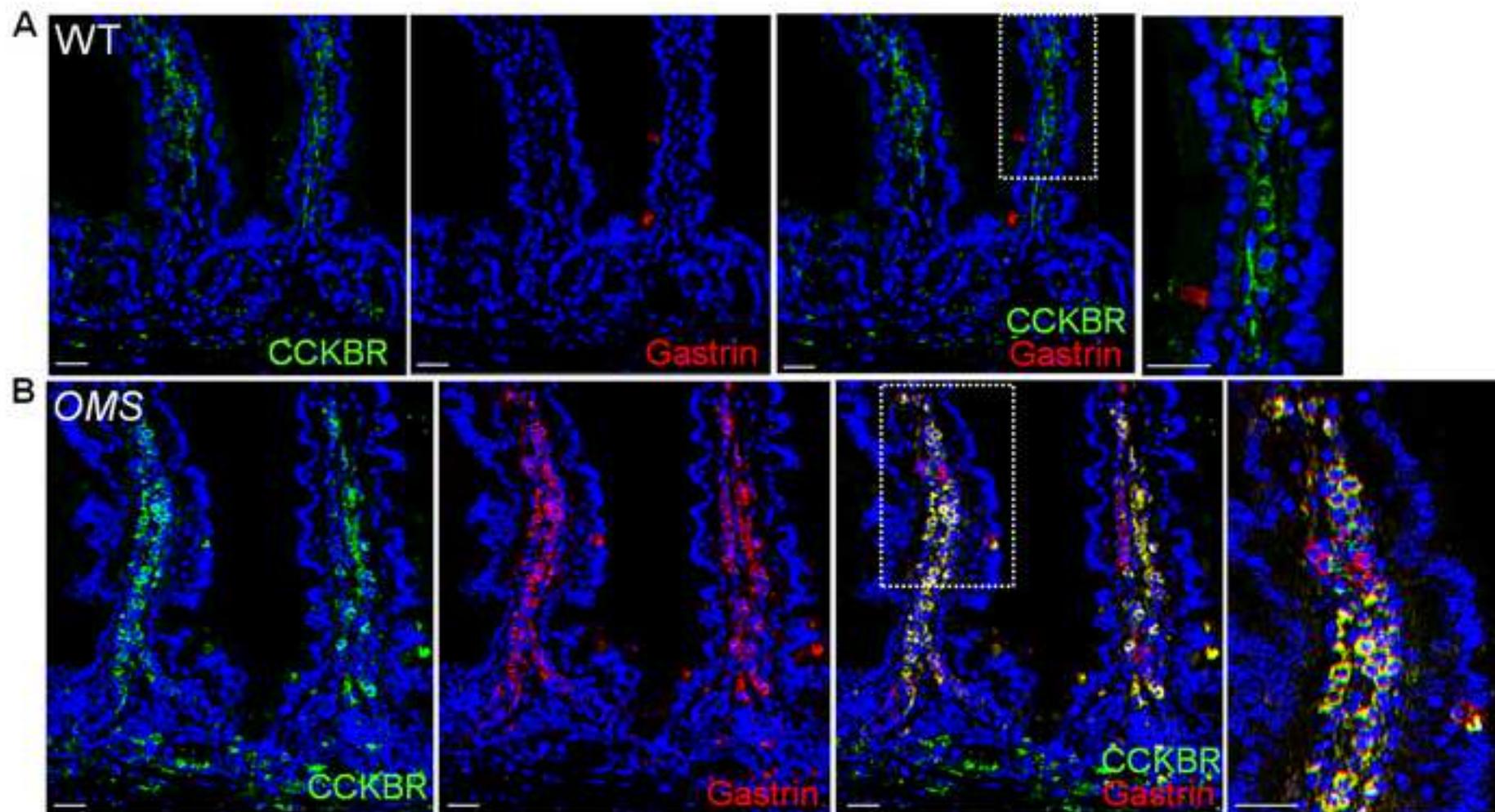
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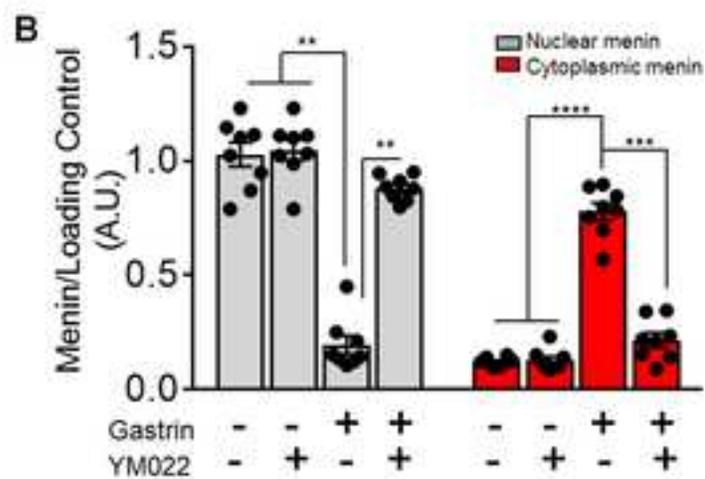
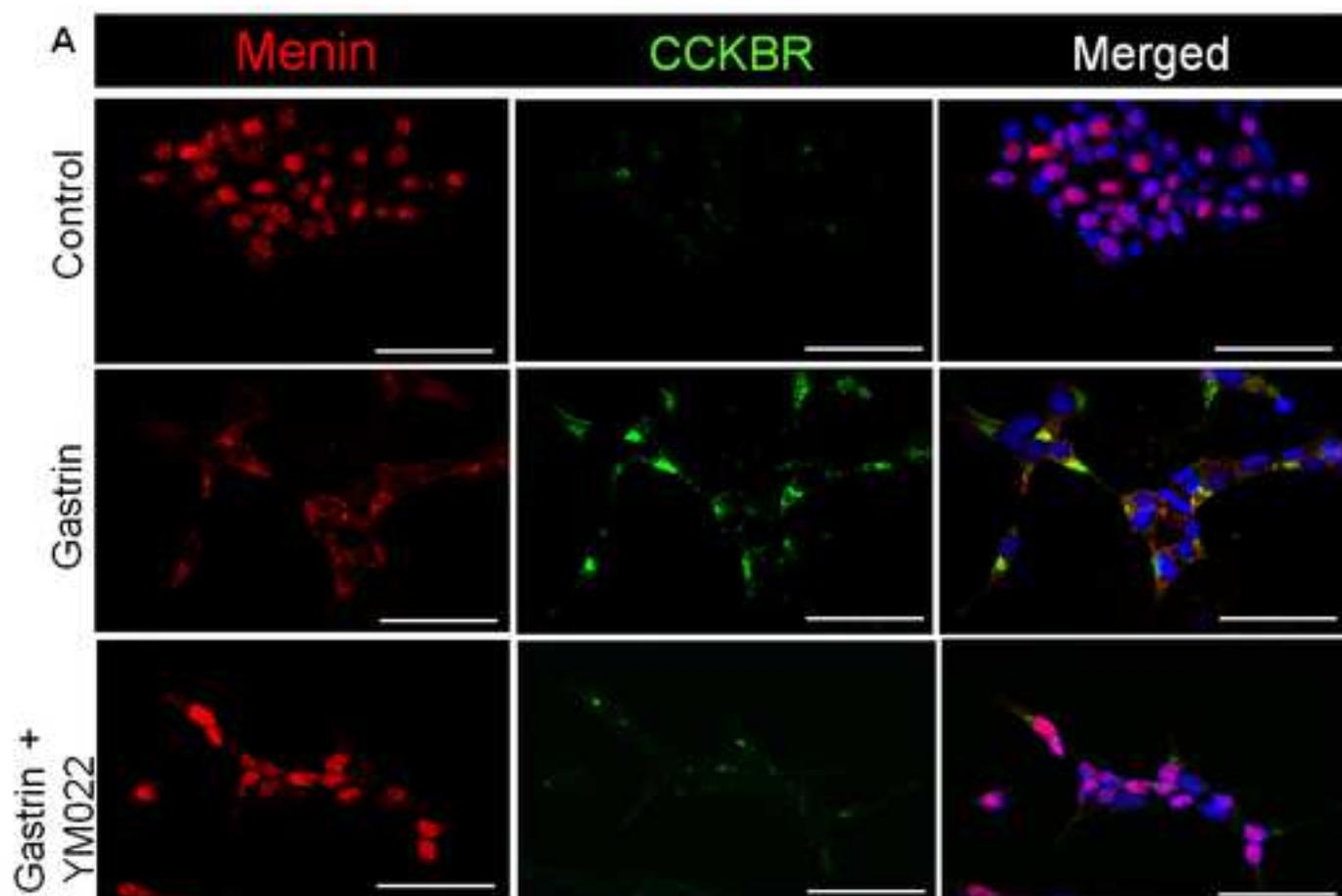
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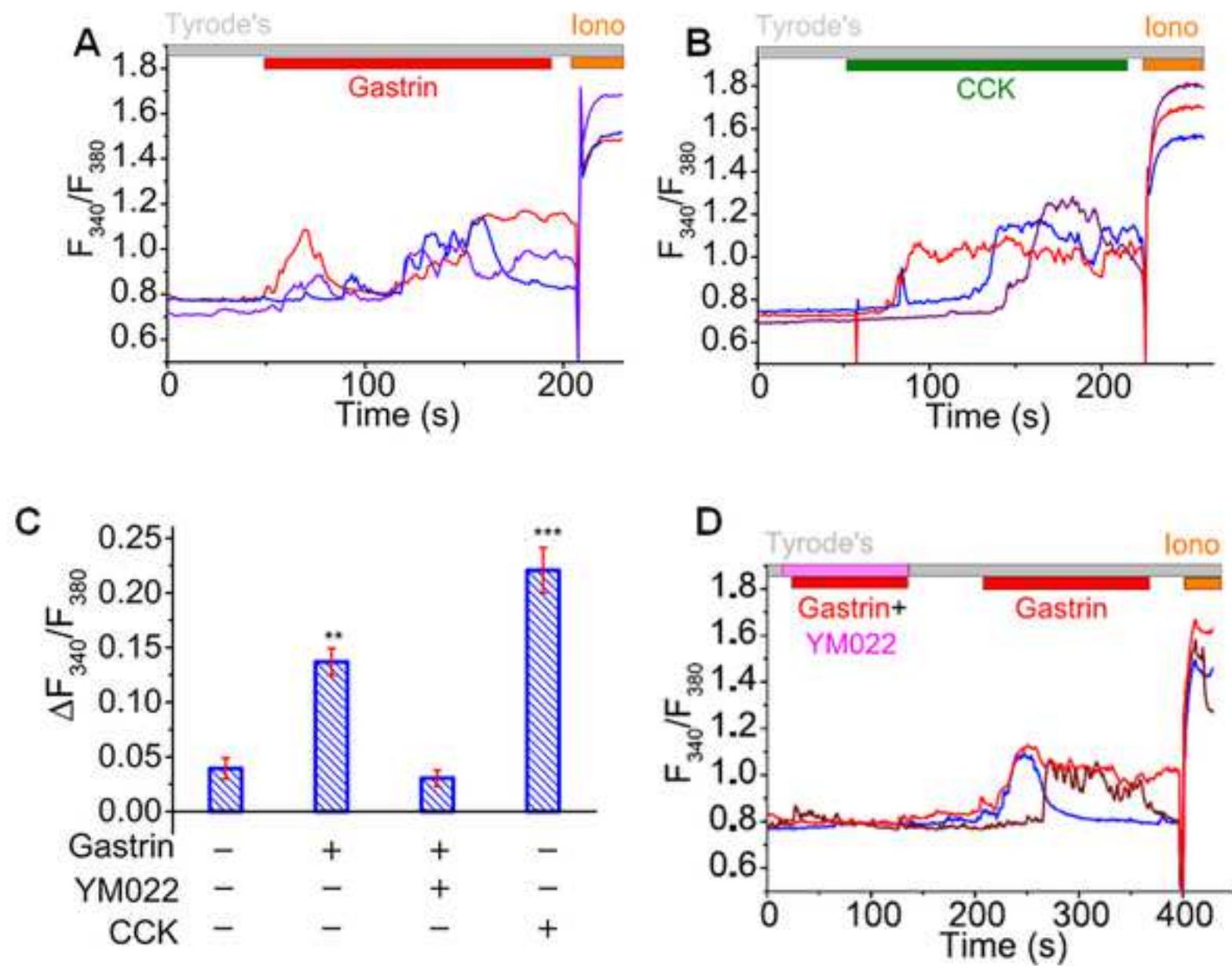
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Supplemental Fig. 9



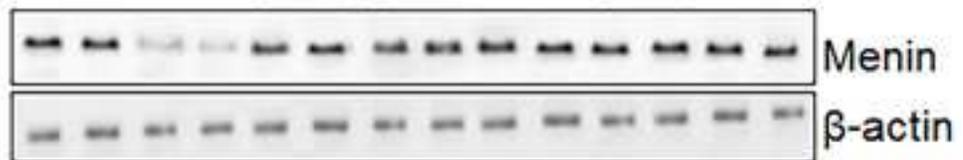
Supplemental Fig. 10



Supplemental Fig. 11

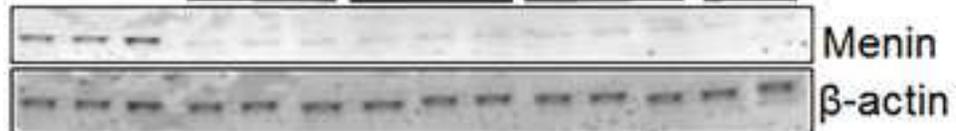
A

Gastrin - - + + - - - - - - - -
 PMA (μM) - - - - 0.02 0.04 0.1 0.2 0.5



B

Gastrin - - - + + + + + + + + +
 Bis IV (μM) - - - 0.01 0.04 0.1 0.2



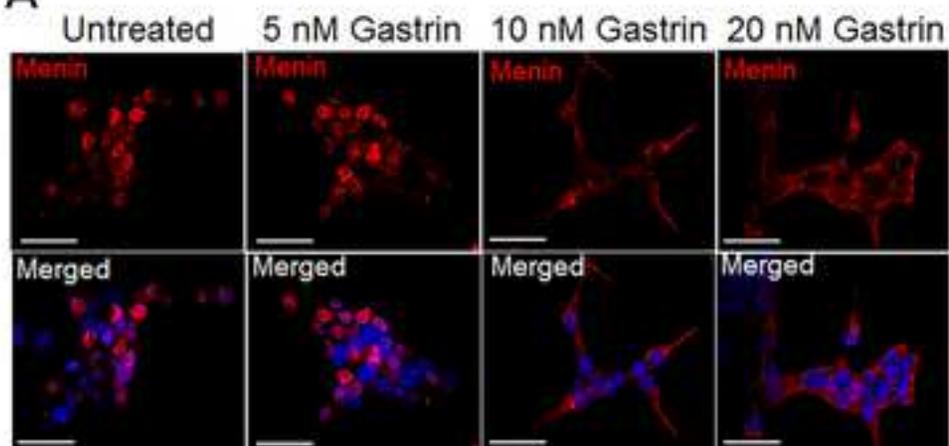
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Gastrin - - - + + + + + + + + +
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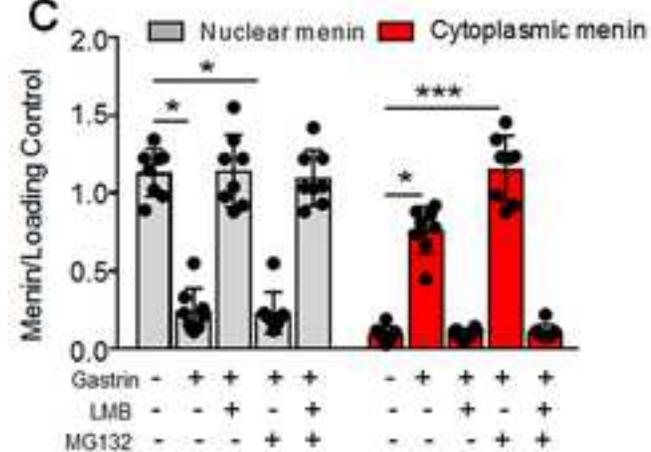


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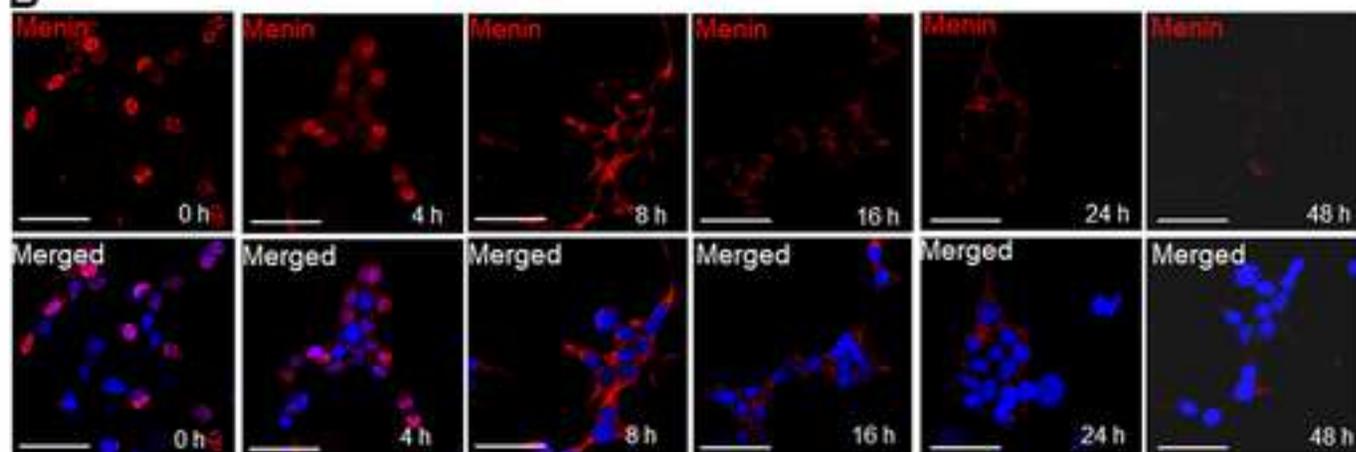
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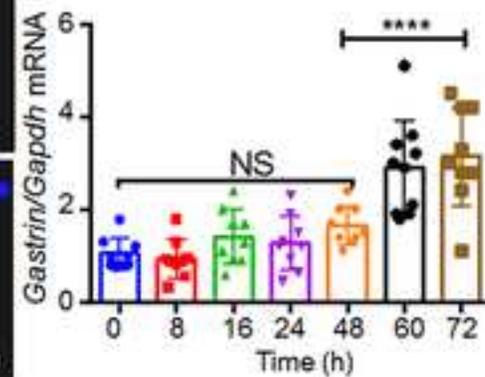
C



B

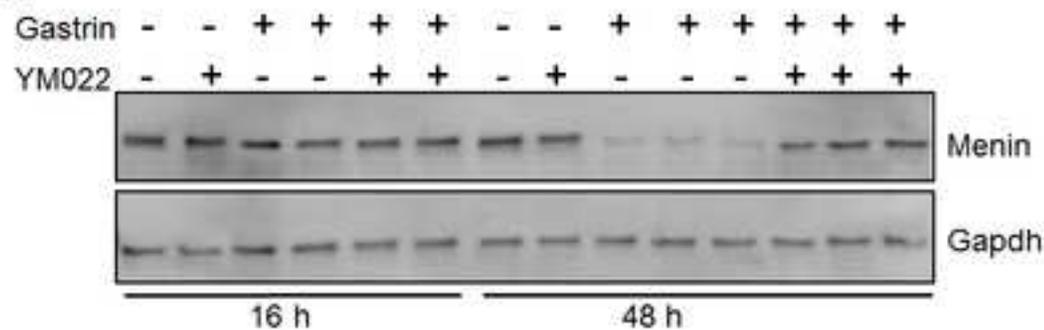


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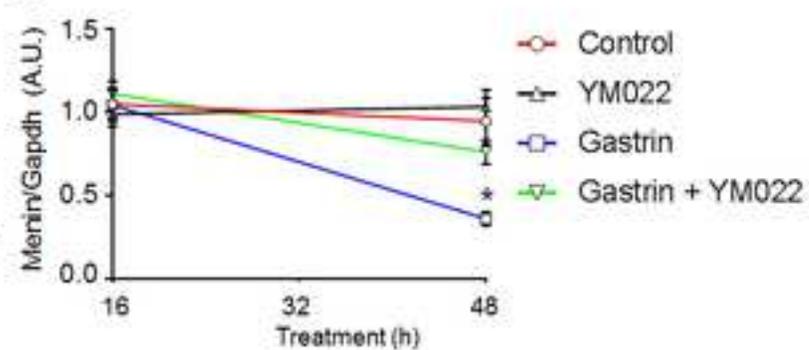


Supplemental Fig. 13

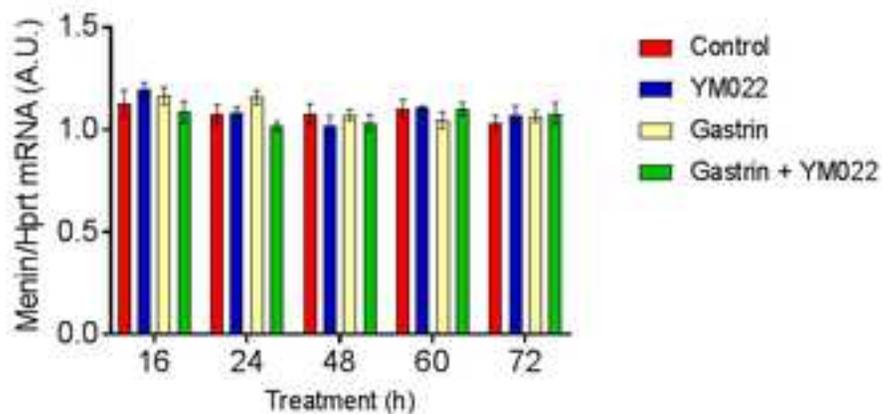
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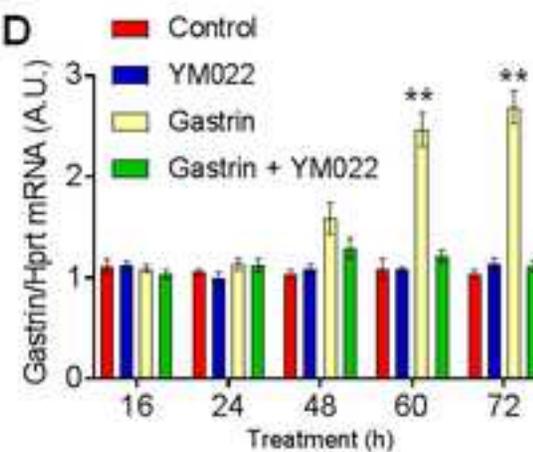
B



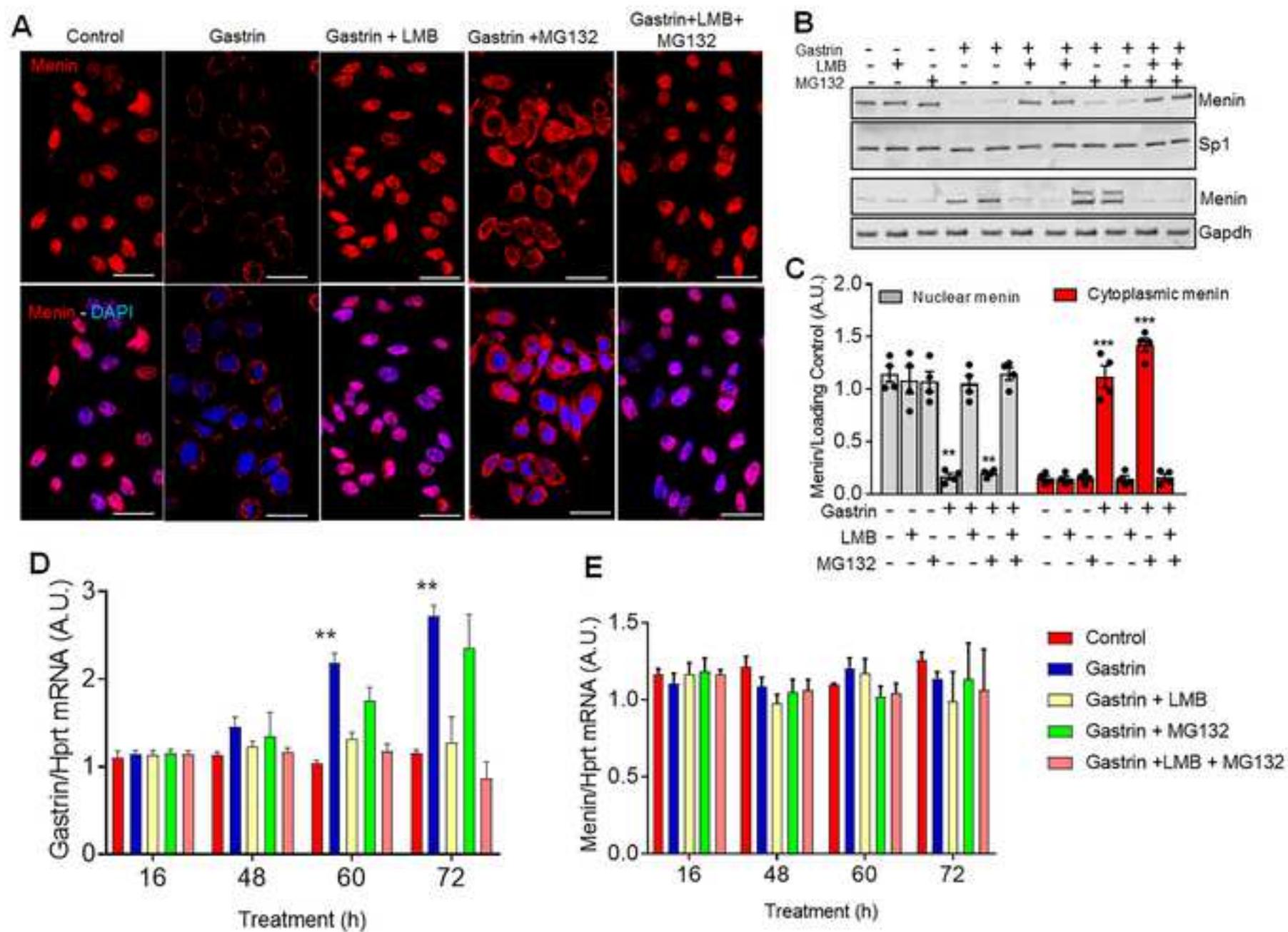
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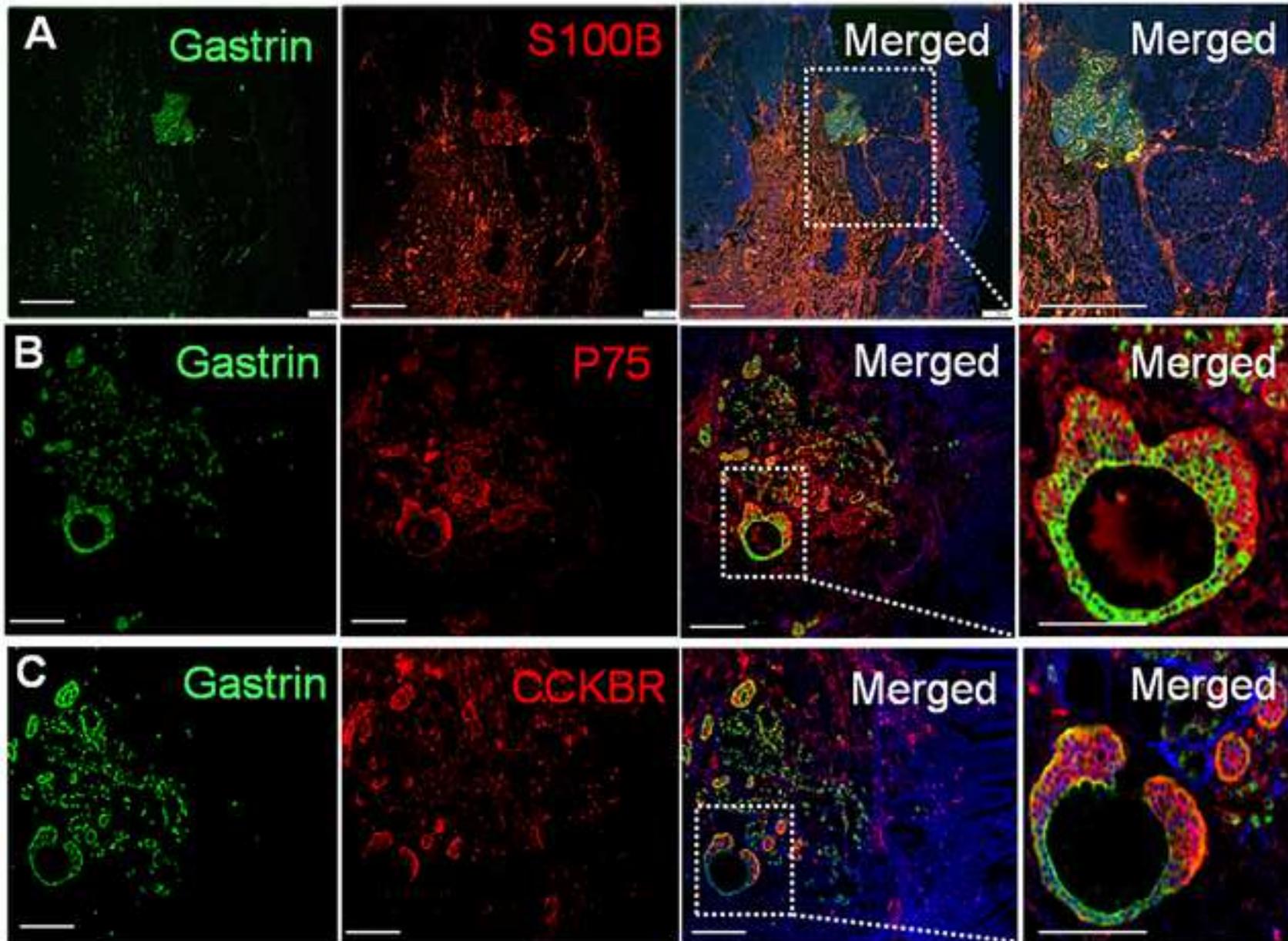
D



Supplemental Fig. 14



Supplemental Fig. 15



Supplementary Table S1: Quantitative PCR Primers

Gene	Forward	Reverse
Hprt (mouse)	AGGACCTCTCGAAGTGTTGGATAC	AACTTGCGCTCATCTTAGGCTTTG
Gastrin (mouse)	ACACAACAGCCAACTATTC	CAAAGTCCATCCATCCGTAG
Gastrin (human)	GCAGCGACTATGTGTGTATGT	CCATCCATAGGCTTCTTCTTCTT
Menin (mouse)	CAGACCCACTCACCCTTTATC	AGTAGCTCCTTCATGCCTTTC
Menin (human)	GTGCCTAGTGTGGGATGTAAG	TGAAGAAGTGGGTCATGGATAAG

Supplementary Table S2: Antibodies

Antibodies	Vendor (Cat#)	Dilution/Application (s)	Antigen Retrieval (for IHC)
Gastrin	Dako Agilent Technologies (A0568) Santa Cruz Biotechnology (sc-7783)	1:1000 (IHC), 1:200 (ICC) 1:400 (IHC)	Tris-EDTA, pH 9
GFAP	Millipore (ab5804)	1:4000 (IHC with TSA), 1:100 (ICC) 1:1000 (WB)	Sodium citrate, pH 6
S100B	Dako Agilent Technologies (Z0311)	1:400 (IHC with TSA), 1:100 (ICC)	Sodium citrate, pH 6
p75	Millipore (MAB5592)	1:10,000 (IF with TSA, mouse sections) 1:2000 (IF with TSA, human sections)	Sodium citrate, pH 6
Sox10	R&D (MAB2864)	1:500 (ICC)	--
E-cadherin	R&D (AF748)	1:400 (IHC), 1:400 (ICC) 1:1000 (WB)	Tris-EDTA, pH 9
CgA	Santa Cruz Biotechnology (sc-1488)	1:400 (IHC), 1:100 (ICC)	Tris-EDTA, pH 9
Pgp9.5	Novus Biologicals (NB600-1160)	1:1000 (IHC), 1:500 (ICC)	Sodium citrate, pH 6
Smooth Muscle actin	Abcam (ab112022)	1:200 (IHC), 1:100 (ICC)	Sodium citrate, pH 6
LYVE-1	R&D (AF2125)	1:400 (IHC), (ICC,1:500)	Tris-EDTA, pH 9

Supplementary Table S2: Antibodies continued

Antibodies	Vendor (Cat#)	Dilution/Application(s)	Antigen Retrieval (For IHC)
Menin	Bethyl Laboratories (A300-105A)	1:10,000 (IHC with TSA, mouse sections) 1:2000 (ICC with TSA, human sections) 1:500 (ICC) 1:1000 (WB)	Sodium citrate, pH 6
CCKBR	Alomone Labs (ACR-042)	2 µg/mg of lysate (IP) 1:500 (IHC with TSA) 1:200 (ICC)	Tris-EDTA, pH 9
GAPDH	Cell Signaling Technology (2118)	1:3000 (WB)	--
β-actin	Santa Cruz Biotechnology (sc-130656)	1:3000 (WB)	--
Sp1	Santa Cruz Biotechnology (sc-14027)	1:2000 (WB)	--
Phospho-PKA substrates	Cell Signaling Technology (9624)	1:1000 (WB)	--
PKA-α	Cell Signaling Technology (4728)	1:1000 (WB)	--
Ubiquitin	Abcam (ab7254)	1:100 (WB) 1:400 (ICC)	--

I HC: Immunohistochemistry; ICC: Immunocytochemistry; WB: Western Blot; IP: Immunoprecipitation; TSA: Tyramide Signal Amplification

Supplemental Figures

Supplementary Figure 1: (A) H&E staining of duodenum from untreated *Men1^{ΔIEC};Sst^{-/-}* mice and OMS mice for 6 and 12 months followed by 2 and 4 months after withdrawal of omeprazole (WD). (B) Duodenal gastrin peptide (*top*) from mice normalized to tissue weight ($n=8-11$), and duodenal *gastrin* mRNA expression (*bottom*) from untreated or OM treated mice, normalized to *Hprt* mRNA ($n=8-11$). (C) Plasma gastrin concentration determined by EIA ($n=8-11$ mice). (D) *Gastrin* mRNA expression in cells isolated from epithelium and lamina propria of mice, expressed after normalization to *Hprt* mRNA ($n=7-8$ mice). (E) Antral *gastrin* mRNA expression (*left panel*) in untreated *Men1^{ΔIEC};Sst^{-/-}* mice, OMS mice, or after OM withdrawal (WD) for 2 and 4 months, measured by RT-qPCR ($n=7-9$ mice) and antral gastrin content (nmol) (*right panel*) from untreated or OM treated mice fasted for 16 hours, normalized to tissue weight ($n=7-12$ mice). Data shown are the median \pm Interquartile Range. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. Scale bars: (A) - 50 μm

Supplementary Figure 2: (A) *Gastrin* mRNA expression in duodenum, jejunum, ileum, and pancreas of untreated *Men1^{ΔIEC};Sst^{-/-}* mice or OMS mice, expressed after normalization to *Hprt* mRNA ($n=9$ mice). (B) IF staining of gastrin and Ki67 in antrum, and (C) duodenum of OMS mice. (D) IF staining for gastrin and E-cadherin, Smooth Muscle Actin, Pgp9.5, and (E) LYVE-1 in duodenal tissue from OMS mice.

Supplementary Figure 3: (A) IF staining for gastrin with E-cadherin, Smooth Muscle Actin, Pgp9.5 in glial cultures isolated from proximal duodenal lamina propria of OMS mice. (B) IF staining for gastrin and CgA in duodenum of OMS mice. (C) IF staining for gastrin with Gfap, S100b, Sox10, and CgA in glial cultures isolated from OMS mice and; (D) gastrin with Gfap, S100b, and Sox10 in glial cultures isolated from untreated C57 WT mice. Scale bars: (A, C, D, inset in B) - 20 μm ; (B) - 50 μm .

Supplementary Figure 4: (A-D) Flow cytometric analysis of primary glial cells isolated from *OMS* mice versus side scatter (SSC). The percentage of **(A)** Gfap⁺, **(B)** Pgp9.5⁺, **(C)** Sma⁺, and **(D)** E-cadherin⁺ cells per mouse is shown as scatter plots., (*n*=5-6 mice).

Supplementary Figure 5: (A) EIA measurement of gastrin secreted into the media by glial cultures from OM-treated or untreated *Men1^{ΔIEC}; Sst^{-/-}* mice after 10 nM Bmb treatment for 3 h, versus intracellular gastrin content. **(B)** IF staining of bombesin receptor 2 (BB₂) and GFAP in duodenum of *OMS* mice. Data shown are the median ± Interquartile Range. ** *p* < 0.01, *** *p* < 0.001. Scale bars: 50 μm; inset - 20 μm.

Supplementary Figure 6: (A) *Menin* mRNA expression in cells isolated from epithelium and lamina propria of mice, expressed after normalization to *Hprt* mRNA (*n*=7-8 mice). **(B)** Frozen duodenal tissue sections from *OMS* mice stained for gastrin and then micro-dissected using laser capture (dissected area outlined by dotted white line). Representative western blot of micro-dissectates from *OMS* mice showing menin, Gfap, S100b, Sox10, E-cadherin and Gapdh protein expressions. **(C)** Representative western blot of micro-dissectates from untreated or omeprazole (OM) treated *Men1^{ΔIEC}*, or *Men1^{ΔIEC}; Sst^{-/-}* mice showing E-cadherin, menin, β-actin, and Gfap expressions in lamina propria (LP) and epithelium (E) (*n*=3 mice). Scale bars: 100 μm.

Supplementary Figure 7: (A) Representative blot showing Cre recombinase expression in glial cultures isolated from *Men1^{ΔIEC}; Sst^{-/-}* mice and infected using adenovirus Cre expression (AdenoCre) for 24 hours at an MOI of 1, 2, 4, and 6. **(B)** Representative blot showing menin expression in glial cultures isolated from *Men1^{ΔIEC}; Sst^{-/-}* mice and infected with AdenoCre for 24 or 48 hours (MOI 1, 2, 4, and 6). **(C)** IF staining of menin and gastrin in glial cultures from *Men1^{ΔIEC}; Sst^{-/-}* mice and infected with AdenoCre or AdenoGFP for 72 hours (MOI 1, 2, 4, and 6). **(D)** High power view of menin and gastrin IF staining in glial cultures from *Men1^{ΔIEC}; Sst^{-/-}* mice and infected with AdenoCre (MOI 1, 2, 4, and 6) or AdenoGFP (MOI 6) for 72 hours. **(E)** *Gastrin* mRNA of glial cultures isolated from *Men1^{ΔIEC}; Sst^{-/-}* mice, and infected with AdenoCre or AdenoGFP (MOI 2, 4)

for 72h were normalized to *Hprt* mRNA ($n=3-5$ mice). **(F)** MTT viability assay for glial cultures from *Men1^{ΔIEC}; Sst^{-/-}* mice infected with AdenoCre, or AdenoGFP (MOI 1, 2, 4, and 6) for 2 and 4 days. Scale bars: (C-100 μm , inset-20 μm); (D) - 20 μm . Data shown are the median \pm Interquartile Range. * $p < 0.05$.

Supplementary Figure 8: Co-IF staining of CCKBR with gastrin in duodenum of **(A)** WT and **(B)** *OMS* mice. Scale bars: 50 μm , insets -20 μm .

Supplementary Figure 9: **(A)** Co-IF staining for menin and CCKBR in glial cultures isolated from C57 WT mice treated without or with 20 nM gastrin in the presence or absence of YM022. **(B)** Quantitation of menin expression in nuclear and cytoplasmic fractions of glial cultures isolated from duodenal lamina propria of C57WT mice and treated with or without gastrin (20 nM, 8 h) in the presence or absence of 10 nM YM022, expressed as integrated band intensities normalized to GAPDH ($n=8-9$ mice).

Supplementary Figure 10: **(A, B)** Representative Ca^{2+} tracings of cultured glial cells from *OMS* mice in response to **(A)** 100 nM CCK, or **(B)** 100 nM gastrin stimulation measured using Fura-2-AM dye. Ionomycin (Iono, 2 μM) was used to detect optimal Fura-2 loading. **(C)** Quantitation of the results shown in A and B, obtained by subtracting basal maximum peak signal (before CCK or gastrin treatment) from CCK or gastrin-induced maximum peak signal. 10 nM YM022 was used to block CCKBR. **(D)** Representative Ca^{2+} tracings of cultured glial cells from *OMS* mice in response to stimulation using 100 nM gastrin and 10 nM YM022, followed by gastrin alone. Data shown are the median \pm Interquartile Range of tracings from at least 40 cells from 3 mice. ** $p < 0.01$, *** $p < 0.001$

Supplementary Figure 11: **(A-C)** Representative blots showing menin expression in total cellular fractions (β -actin loading control) of glial cultures from untreated C57 WT mice treated without or

with 20 nM gastrin in **(A)** the presence or absence of PMA; **(B)** canonical PKC inhibitor Bis-IV or **(C)** the ERK1/2 inhibitor PD98059

Supplemental Fig. 12: **(A)** IF staining of menin in glial cultures from C57 WT mice treated without or with 5-20 nM gastrin for 48 hours. **(B)** IF staining of menin in glial cultures from C57 WT mice treated without or with 20 nM gastrin for the indicated times up to 48 hours. Scale bars: 20 μ m. **(C)** Quantitation of menin expression in nuclear and cytoplasmic fractions of glial cultures isolated from duodenal lamina propria of C57 WT mice and treated with or without 20 nM gastrin for 8 h in the presence or absence of 10 μ M LMB, or 25 μ M MG132, expressed as integrated band intensities normalized to appropriate loading controls ($n=8-9$ mice). **(D)** *Gastrin* mRNA of glial cultures isolated from untreated C57 WT mice and treated with 20 nM gastrin for the indicated times up to 72h expressed after normalization to *Gapdh* mRNA ($n=9$ mice). Data shown are the median \pm Interquartile Range. * $p < 0.05$, *** $p < 0.001$, **** $p < 0.0001$

Supplemental Fig. 13: **(A)** Representative blot showing total menin expression in STC-1 cells treated without or with 20 nM gastrin in the absence or presence of 10 nM YM022 for 16, 24 and 48 h. **(B)** Integrated band intensities of menin normalized to *Gapdh* analyzed using LICOR Odyssey software were plotted as a function of time. **(C)** Menin and **(D)** gastrin mRNA in STC-1 cells treated with or without gastrin for 16-72 h were normalized to *Hprt* ($n =$ triplicates from 3 separate experiments), * $p < 0.05$, ** $p < 0.01$.

Supplemental Fig. 14: **(A)** IF staining of menin in STC-1 cells treated without or with 20nM gastrin for 8h in the presence or absence of 10 μ M Leptomycin b (LMB) or 25 μ M MG132. Scale bars: 20 μ m. **(B)** Representative blots showing menin expression, and **(D)** quantitation of menin expression in nuclear and cytoplasmic fractions of STC-1 cells treated as in **(A)** ($n =$ triplicates from 4 separate experiments). **(E)** Gastrin and **(F)** menin mRNA in STC-1 cells treated with or without gastrin in the presence or absence of LMB for 16-72 h were normalized to *Hprt* mRNA ($n =$ triplicates from 3 separate experiments), ** $p < 0.01$, *** $p < 0.001$.

Supplemental Figure 15. Gastrinomas from *MEN1* patients express glial markers and CCKBR. Co-IF staining of gastrin with **(A)** S100B or **(B)** P75 in duodenal gastrinomas from *MEN1* patients. Co-IF staining of gastrin with **(C)** CCKBR in duodenal gastrinomas from *MEN1* patients. Scale bars: 100 μm , insets - 20 μm .

Supplemental Methods:

Adenovirus Cre infection of primary glial cultures:

Infection was performed as described previously¹ with some modifications. Isolated primary glial cultures were seeded for 2 hours in laminin and poly D-lysine coated 6-wells plate in reduced volume of glial growth media (500 μ L). Cultures were then infected with Adenovirus Cre or AdenoGFP at indicated MOIs for 2 hours, following which they were re-fed with growth media (2.5 mL). Media was changed after 24 hours, and cultures were stained at indicated times.

Ca²⁺ Imaging of primary glial cultures:

Fura-2 Ca²⁺ imaging: Imaging was performed as described previously². Briefly, cultured glial cells were incubated with 3 μ M Fura-2-AM (Invitrogen, F-1221) at 37°C for 60 min. Then CCK peptide (Anaspec, AS-20741), Gastrin peptide (Abbotec, 350332), and CCKBR inhibitor YM022 (Sigma Aldrich, SML0220) were added and Ca²⁺ signal was measured in Tyrode's solution (145 mM NaCl, 5 mM KCl, 2 mM CaCl₂, 1 mM MgCl₂, 10 mM Glucose, and 20 mM HEPES, pH 7.4). Fluorescence was recorded at 340 nm and 380 nm using the EasyRatio Pro system (PTI), and Fura-2 ratio (F340 /F380) was determined to quantitate changes in intracellular [Ca²⁺].

Fluo4 Ca²⁺ imaging: Fluo-4-AM Ca²⁺ imaging was conducted in an Olympus Spinning-Disk Confocal Microscope. Isolated glial cells were incubated with 3 μ M Fluo-4-AM (Invitrogen: Cat# F14201) at 37°C for 30 min. Ca²⁺ release was monitored in Tyrode's solution at an excitation wavelength of 480 nm (F480). Image was analyzed using

MetaMorph Advanced Imaging acquisition software v.7.7.8.0 (Molecular Devices) and Image J (NIH).

RNA extraction and RT-qPCR

Total RNA was isolated from tissues, glial cultures, and cells using the RNeasy Mini kit (Qiagen). About 500 ng of total RNA was used for cDNA synthesis (iScript cDNA synthesis kit, Bio-Rad, Hercules, California, USA). cDNA was diluted 1:5 before use in PCR reactions. Quantitative PCR (qPCRs) was carried out using a thermal cycler (model C1000, Bio-Rad) with Platinum Taq DNA polymerase (Invitrogen) and SYBR Green dye (Molecular Probes, Carlsbad, California, USA). The primers used are listed in the **Supplementary Table S1**. Differences in mRNA expressions were normalized to *Hprt* and then expressed as fold increase over indicated controls.

Western blot

Total cellular proteins were extracted by homogenization in radio-immunoprecipitation assay (RIPA) lysis buffer (Sigma-Aldrich) supplemented with the Complete Protease Inhibitor Cocktail (Roche, Indianapolis, Indiana, USA) and PhosSTOP phosphatase inhibitor cocktail (Roche). Nuclear and cytoplasmic fractions were isolated using the NE-PER kit (Thermo Scientific), as per manufacturer's instructions. Cellular lysates were resolved on Novex 4%–20% Tris-Glycine precast gels (Life Technologies) and resolved proteins were transferred to polyvinylidene difluoride (PVDF) membranes. Membranes were blocked in 5% BSA to prevent non-specific binding and incubated with primary antibodies overnight at 4°C (**Supplementary Table S2**). Following incubation (1 hour, RT) with infrared dye-labeled, IRDye 800CW and

IRDye 680RD (LI-COR) secondary antibodies, target proteins were detected using the Odyssey Infrared System (LI-COR Biosciences, Lincoln, NE). Bands were quantitated using the LI-COR Odyssey software.

Immunohistochemistry

Mice tissues were fixed overnight in 10% formaldehyde solution (4°C), transferred to 70% ethanol and processed before embedding in paraffin (TissueTek). Five micron thick sections were de-paraffinized in xylene and dehydrated by brief sequential incubations in 70%, 90%, and 100% ethanol. Antigen-retrieval was performed by warming the slides in Tris-EDTA (pH 9.0), or sodium citrate (pH 6.0) for 30 min. Non-specific binding was blocked by incubation in 10% donkey serum, following which sections were incubated with primary antibodies (2 h-RT, or overnight at 4°C, **Supplementary Table S2**). For fluorescent detection, sections were incubated (RT) with Alexa Fluor-conjugated secondary antibodies for 30 min (1:400 dilution). Tyramide signal amplification technology (Perkin Elmer, Inc) was used for detection of selected proteins (**Supplementary Table S2**). ProLong Gold antifade reagent with DAPI (Invitrogen) was used for nuclear counterstaining. Images were obtained using a Nikon inverted confocal microscope (Nikon, New York, USA). Primary glial cultures plated on laminin and poly-D- lysine coated coverslips were fixed using 4% paraformaldehyde for 20 min (RT), and then permeabilized with 0.2% Triton X-100/phosphate buffered saline (PBS) before blocking and staining, as described above.

Ubiquitination Assay

Ubiquitinated proteins were pulled down using the Agarose-Tandem Ubiquitin Binding Entities (Agarose-TUBE, Life sensors). Cells were lysed in lysis buffer (50mM Tris-HCl, pH 7.5, 0.15M NaCl, 1mM EDTA, 10 mM NEM, 1% NP-40, and 10% glycerol) supplemented with protease and phosphatase inhibitor cocktail. About 20 μ l of resin in 500 μ l of lysis buffer containing 1-2mg of total protein was used per reaction. Clarified cell lysates incubated with uncoupled agarose (Life sensors) was used as control for non-specific binding. An aliquot of each sample was removed prior to immunoprecipitation and designated "INPUT". The remnant cell lysates were incubated with equilibrated Agarose-TUBEs overnight at 4°C. Beads were collected by low speed centrifugation (3000g, 4°C) for 5 minutes, washed with TBS-T. Bound protein was eluted by boiling for 5 min in Laemmli sample buffer.

Protein Kinase A Activity Assay

Protein Kinase A (PKA) activity in glial cultures were assayed using DetectX PKA activity kit (Arbor Assays, Ann Arbor), as per manufacturer's instructions. The non-linear 4PLC software was used to generate a standard curve and calculate PKA activity for each sample and reported after multiplying by the dilution factor.

Statistical analyses

All reported values are the Median \pm Interquartile range of Media, unless stated otherwise. Statistical analyses of data obtained from mice tissues and primary cultures

were performed using the non-parametric Kruskal–Wallis test (GraphPad Prism 6). Dunn's multiple comparison test was used to identify groups that were significantly different. Data from *in vitro* sample sets was analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test ($p < 0.05$ was considered significant).

Supplementary Table S1: Primers

Supplementary Table S2: Antibodies

References

1. Prost S, Sheahan S, Rannie D, et al. Adenovirus-mediated Cre deletion of floxed sequences in primary mouse cells is an efficient alternative for studies of gene deletion. *Nucleic Acids Res* 2001;29:E80.
2. Sahoo N, Gu M, Zhang X, et al. Gastric Acid Secretion from Parietal Cells Is Mediated by a Ca^{2+} Efflux Channel in the Tubulovesicle. *Dev Cell* 2017;41:262-273 e6.

Figure 2

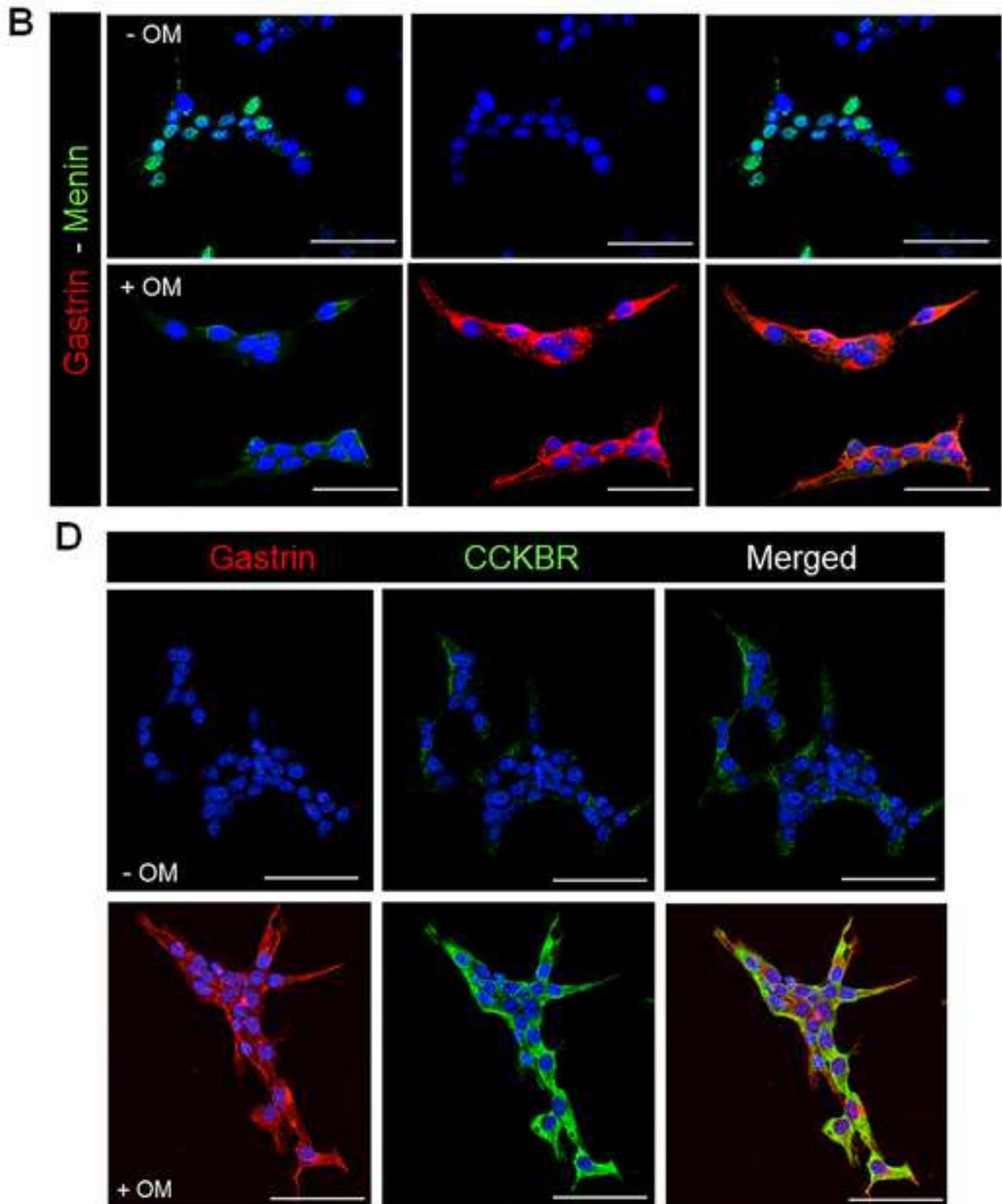


Figure 2. Gastrin induces nuclear export of menin via CCKBR activation. IF staining of menin and gastrin (**B**), gastrin and CCKBR (**D**) in glial cultures isolated from duodenal lamina propria of untreated *Men1^{ΔIEC}; Sst^{-/-}* (-OM) and OMS mice (+OM). Scale bars – 20 μ m.

Figure 3

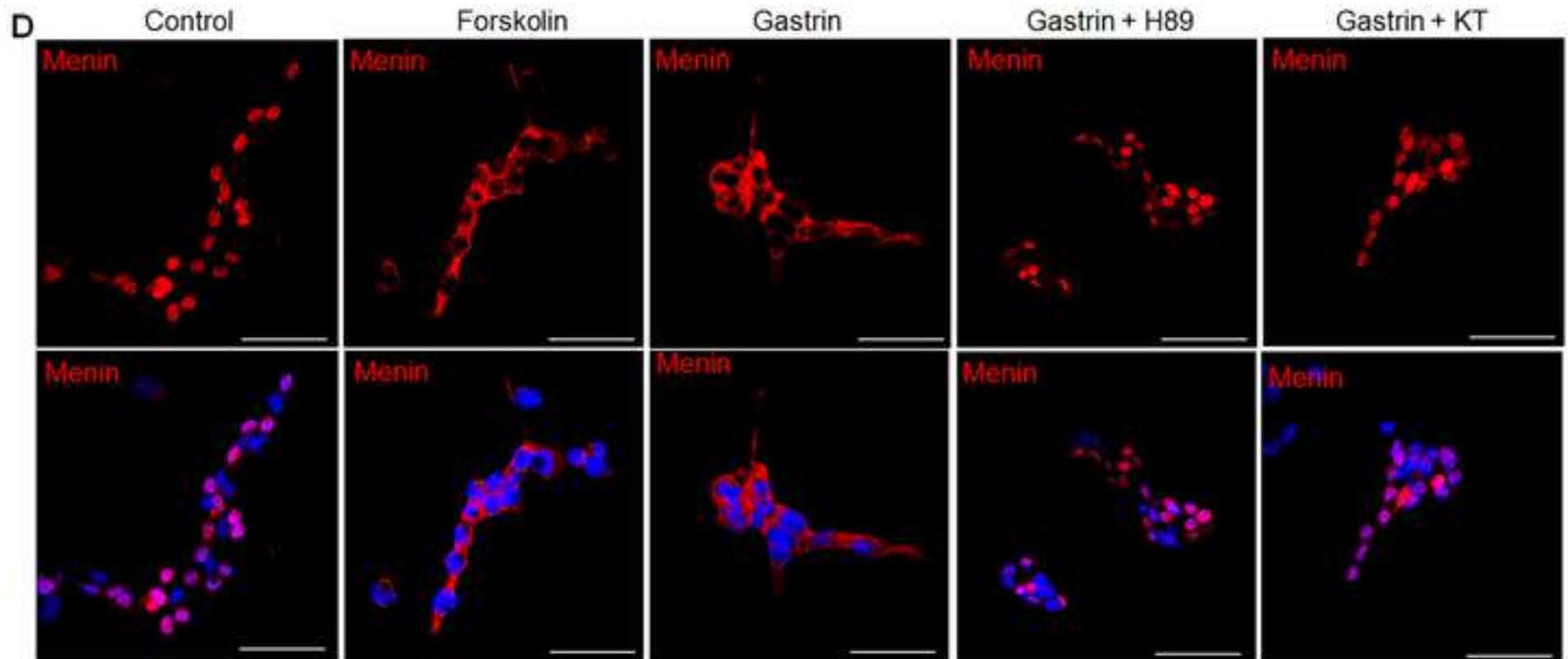


Figure 3. Gastrin mediated nuclear export of menin via CCKBR requires PKA. (D) IF staining of menin in glial cultures isolated from duodenal lamina propria of C57 WT mice and treated with or without gastrin (20 nM, 8 h) in the presence or absence of 10 μ M H-89 and KT5720 (KT), and forskolin (10 μ M, 4 h). Scale bars – 20 μ m

Figure 4

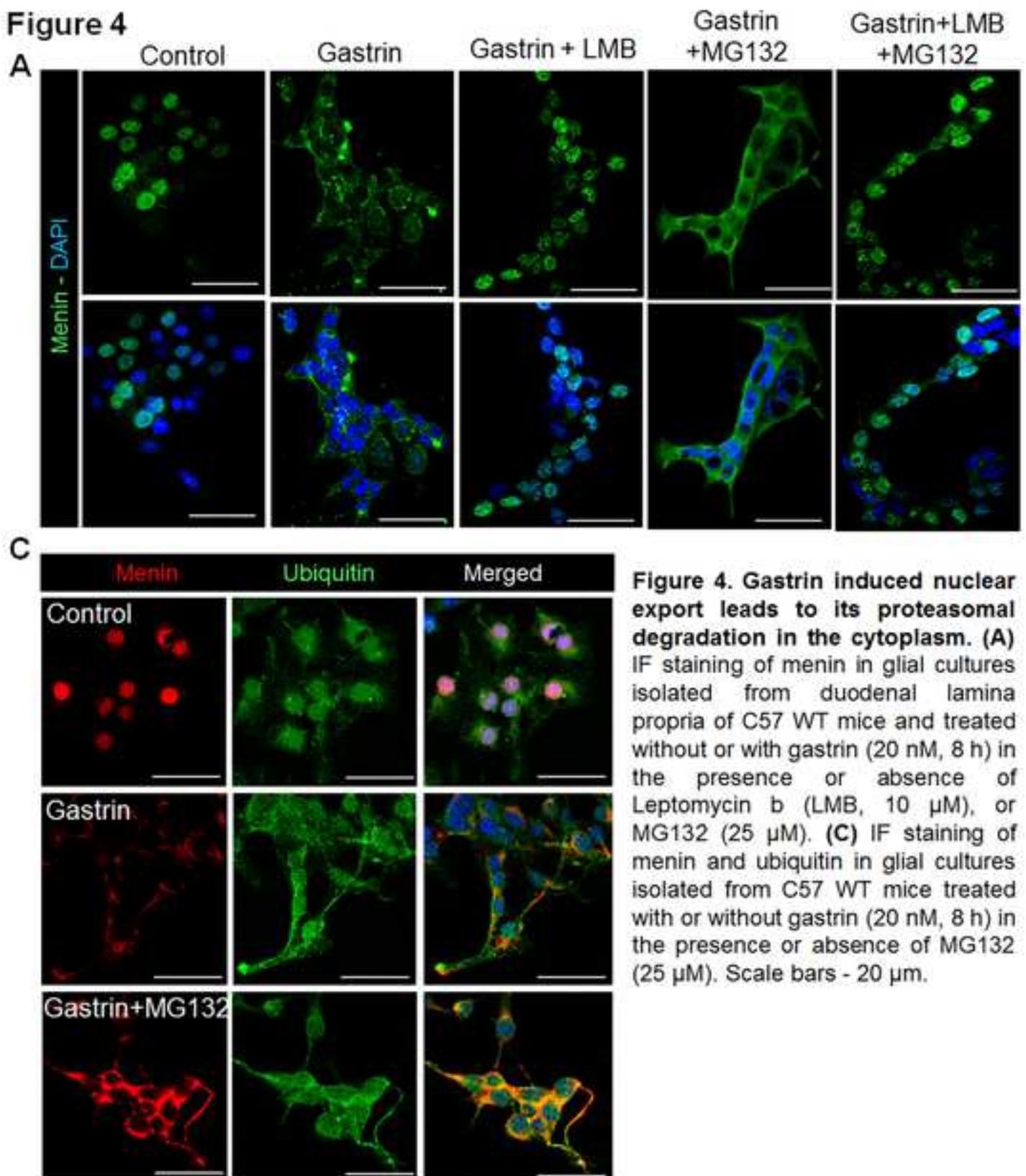


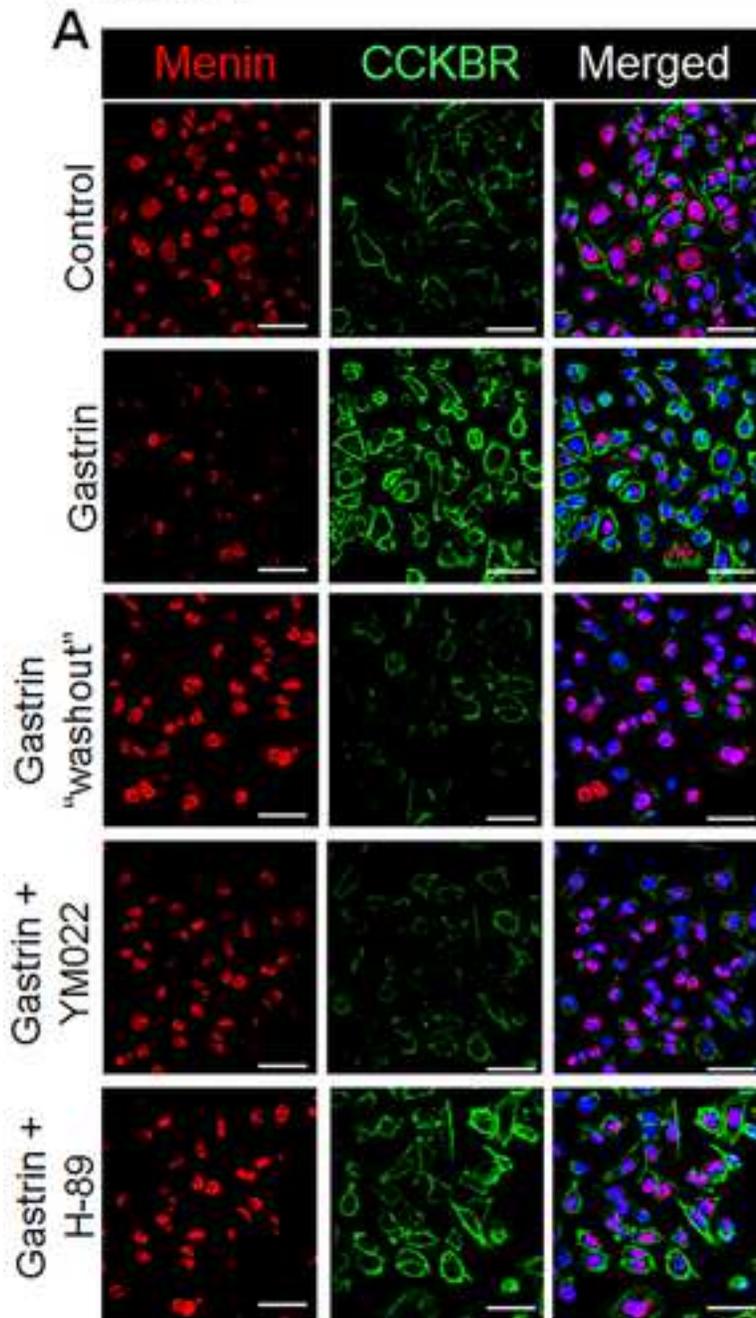
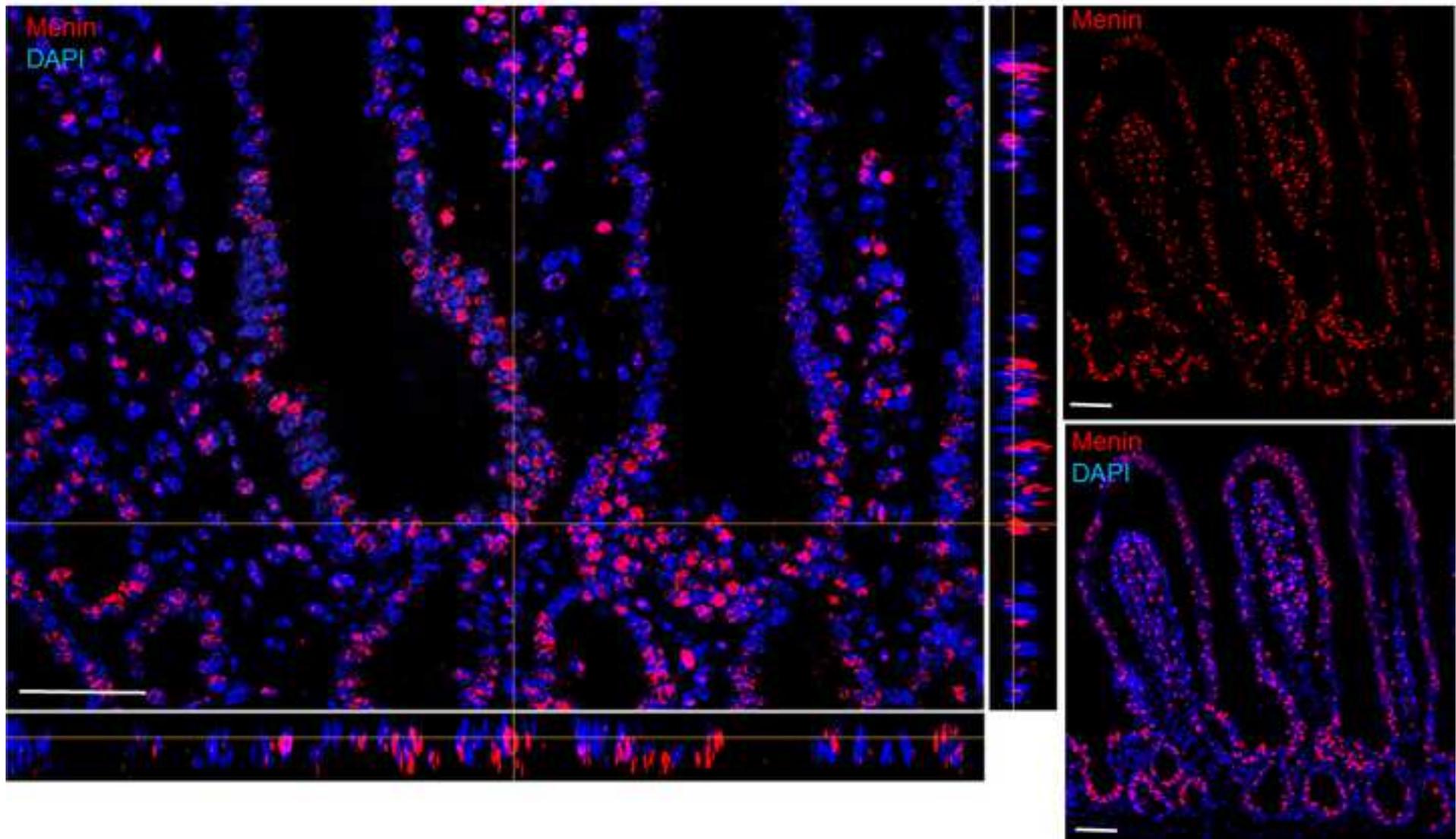
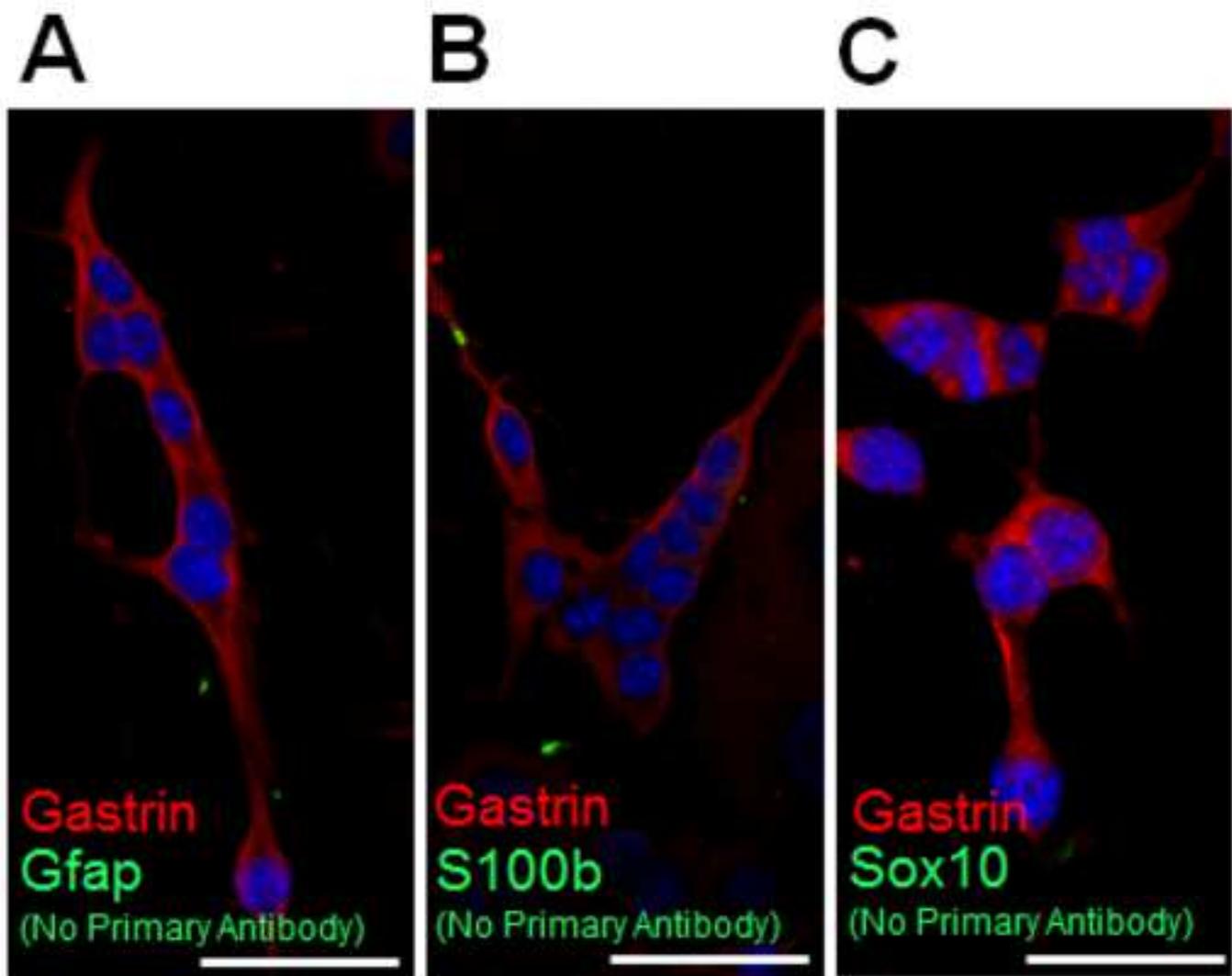
Figure 5

Figure 5. Gastrin stimulation leads to nuclear export of menin in STC-1 cells, and requires activation of CCKBR and PKA. (A) IF staining showing menin and CCKBR expression in STC-1 cells treated with 20 nM gastrin, 8 hrs after gastrin "washout", and gastrin in the presence of YM022, or H-89. Scale bars - 20 μ m.





(A-C) IF staining of “No Primary Antibody” control for Ggap **(A)**, S100b **(B)**, and Sox10 **(C)** in gastrin+ glial cultures isolated from *OMS* mice

