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**Title: Redox signaling regulates commensal mediated mucosal  
homeostasis and restitution**

**Supplementary Information:**

**This file includes Legends for Supplementary Figures S1 - S5**

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**Supplementary Figure S1** (a) *En face* image of wound beds at day 0 and day 2. Images were captured by a miniature rigid endoscope, a xenon light source, and a triple chip high resolution CCD camera. For histological analysis, wounds were oriented and cut in a proximal to distal manner and sections were prepared in 7- $\mu$ m increments. The sections with the largest area of the wound bed (highlighted by dashed lines) were considered the center of the wound and used for immunofluorescence staining. (b) Transverse image of H& E staining of a biopsy wound bed harvested from wild type mice 15 minutes post injury. The asterisk (\*) indicates the center of the wound bed. (c) Quantification of re-epithelialization on day 2 post wounding. Data shown are mean value of 5 wounds per group. Error bars, s.e.m. and \*  $P < 0.001$  by Student's *t* test. (d) Endoscopic images of colonic wounds in mice (wild type, *Fpr1*<sup>-/-</sup> and *Nox1*<sup>-/-IEC</sup>) at days 1, 2, 4 and 6. Bioptic injuries were inflicted as Seno et al., 2009.  $n=5$  mice/group. Dotted lines outline the depressed area margin of the lesion at each time point.

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**Supplementary Figure S2** Commensal bacteria induce ROS generation in intestinal epithelia. **(a)** CM-H2DCF-DA (5  $\mu$ M)-mediated detection of ROS in SK-CO15 cells treated with different concentrations of fMLF (50 to 5000 nM) or LGG ( $1 \times 10^6$  to  $1.25 \times 10^8$  cfu/ml) over 15 min. Higher magnification and resolution image showing CM-H2DCF-DA (5  $\mu$ M)-mediated detection of ROS in the cytoplasmic and perinuclear area.

**(b-d)** Mice were intrarectally injected with Hydrocyanine 3 followed by wounding of epithelium. **(b)** Mucosa was topically treated with HBSS or LGG ( $2.5 \times 10^9$  cfu) for different times. **(c)** Mucosa was topically treated with HBSS or LGG as indicated for 15 minutes. **(d)** Mucosa was topically treated with HBSS or fMLF as indicated for 15 minutes. Fluorescence was determined by confocal laser scanning microscopy (Zeiss).

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**Supplementary Figure S3** Enteric commensal bacteria-stimulated FAK activation and enterocyte migration in murine colon depends on FPR1 and NOX1. **(a)** Immunoblots analysis of phospho-FAK (pFAK-Y861) and phospho-paxillin in WT mouse colon after intrarectal administration of LGG. Data are representative of two independent experiments with n=3 mice per group. **(b)** A wound bed surrounded by crypts where epithelial cells proliferate in and migrate from to cover the wound bed. Diagram showing experimental scheme to demonstrate microbiota-induced epithelial migration to cover the wound bed. **(c)** Microbiota-stimulated epithelial cell migration requires FPR1. Mice were treated intrarectally with HBSS buffer or LGG followed by I.P. injection of EdU for a chase of 1 or 6 hours before euthanization. Serial sections were stained for EdU positive migrating cells. **(d)** Quantification of epithelial cell migration (represented as % migration and quantified by % position of the EdU positive cell farthest from the bottom of the crypt). Data are representative of two independent experiments.

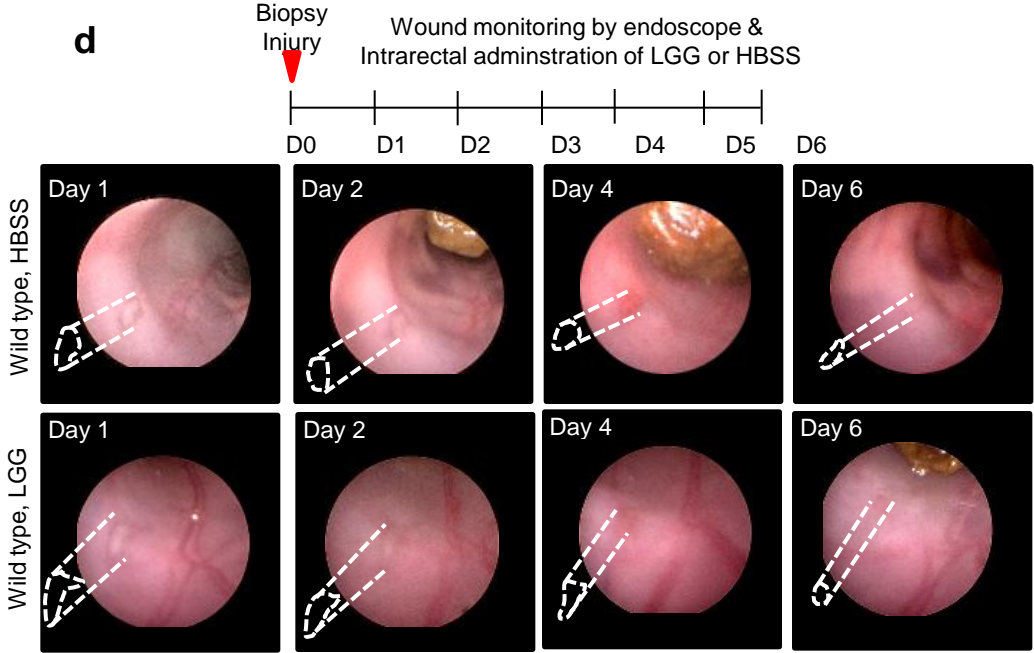
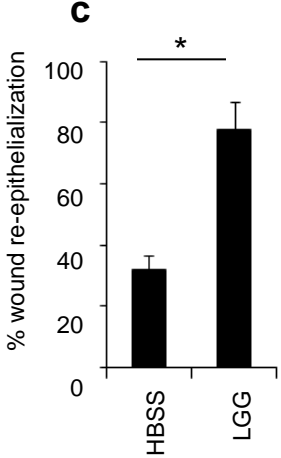
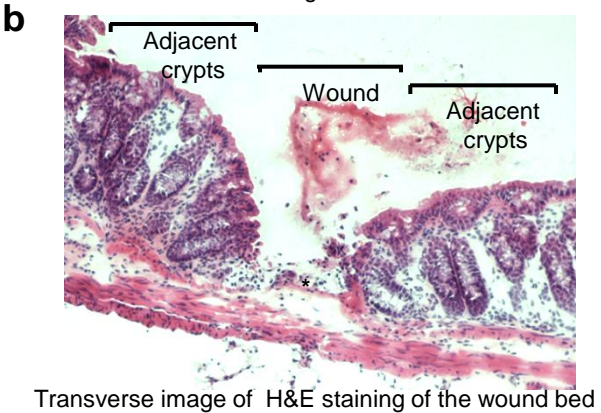
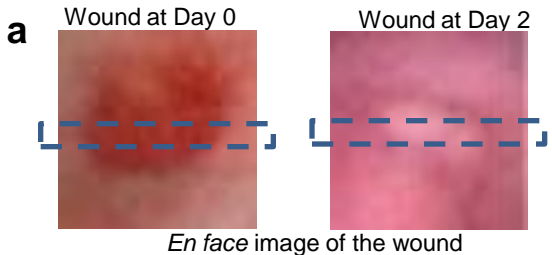
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**Supplementary Figure S4** Commensal bacteria-stimulated ERK activation in murine colon depends on *Fpr1*<sup>-/-</sup> and *Nox1*<sup>-/-IEC</sup>. **(a)** Immunoblot analysis (left) for phospho-ERK in wild type mouse colonic epithelial cell scrapings after *in vivo* treatment with LGG. **(b)** Immunofluorescence analysis of phospho-ERK in colonic tissue of wild type, *Fpr1*<sup>-/-</sup> and *Nox1*<sup>-/-IEC</sup> mice treated *in vivo* with 100  $\mu$ l LGG ( $2.5 \times 10^9$  cfu) of HBSS for 15 min or 60 min. NAC was instilled 30 min prior to LGG treatment. An  $n \geq 3$  for each experimental murine treatment.

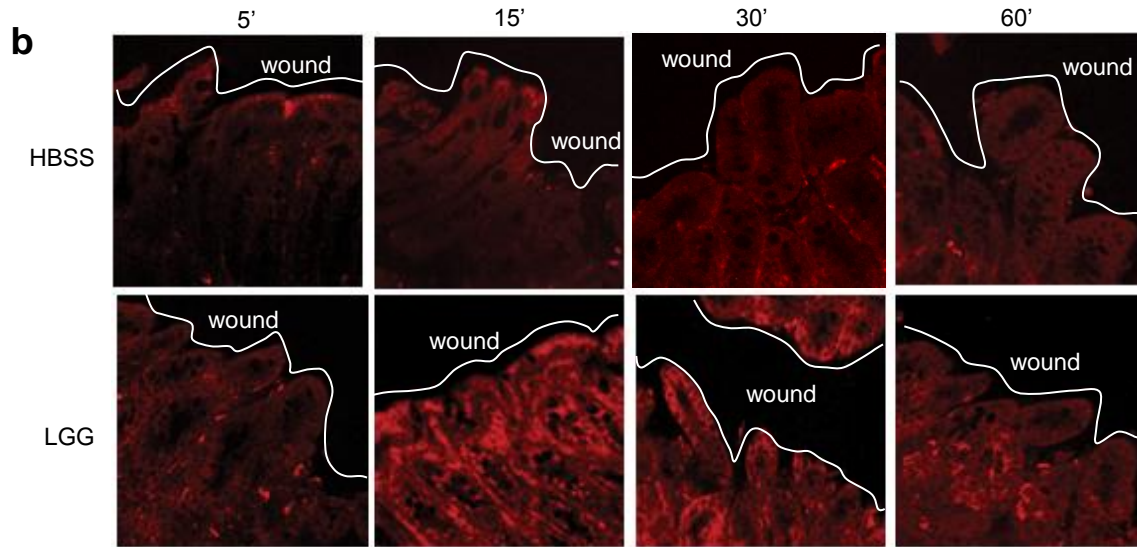
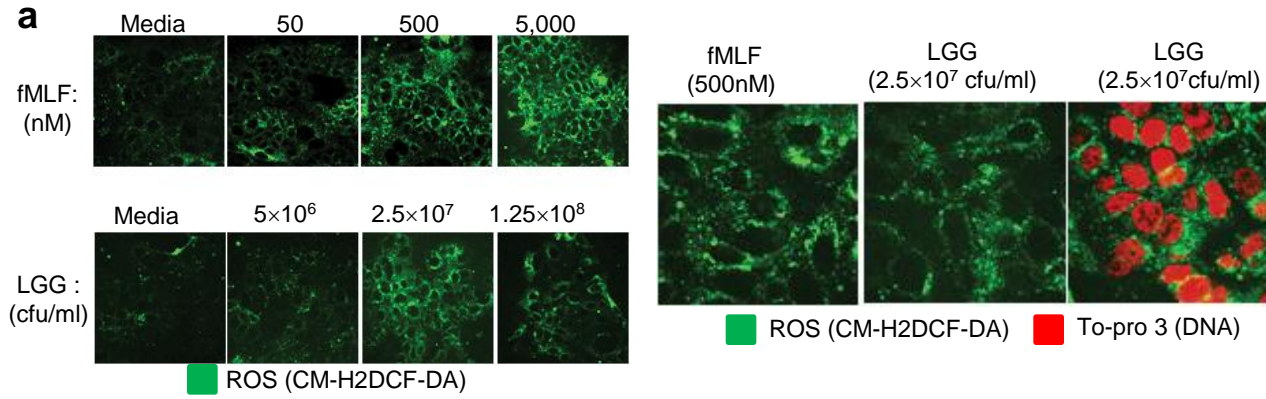
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**Supplementary Figure 5.** Commensal bacteria stimulate enterocyte proliferation in crypts adjoining mucosal wounds. (A) Diagram showing experimental scheme to determine proliferating cells. (B) Determination of enterocyte proliferation in wound adjoining crypts of wild type mice on day one, two and four by detecting EdU in cells at S-phase. Mice were injected I.P. with EdU for a pulse of 2 hours and thin sections of biopsy wound beds were stained for EdU in cells at S-phase. (C) Determination of enterocyte proliferation on day 2 post injury by detecting EdU in cells at S-phase. Mice were treated as described before for 2 days and were injected I.P. with EdU for a pulse of 2 hours. White lines show crypts adjacent to wound.  $n=2$  to 6 lesions per group. (D) Endoscopic biopsy wounds in mouse distal colon were treated with HBSS buffer or *L. rhamnosus* GG for 2 days. Serial sections of wound beds were stained with antibody specific for Ki67 to detect cells in prophase. Scale bar, 50 $\mu$ m.

### Supplementary Figure S1

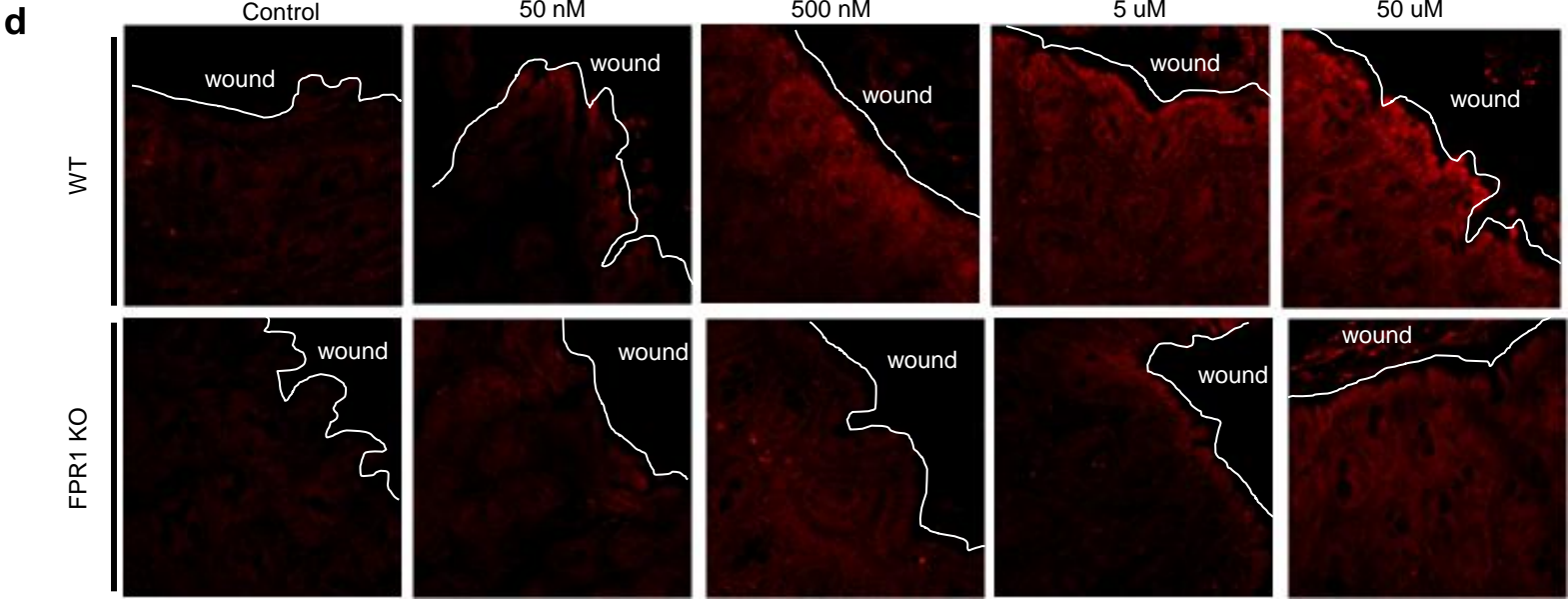
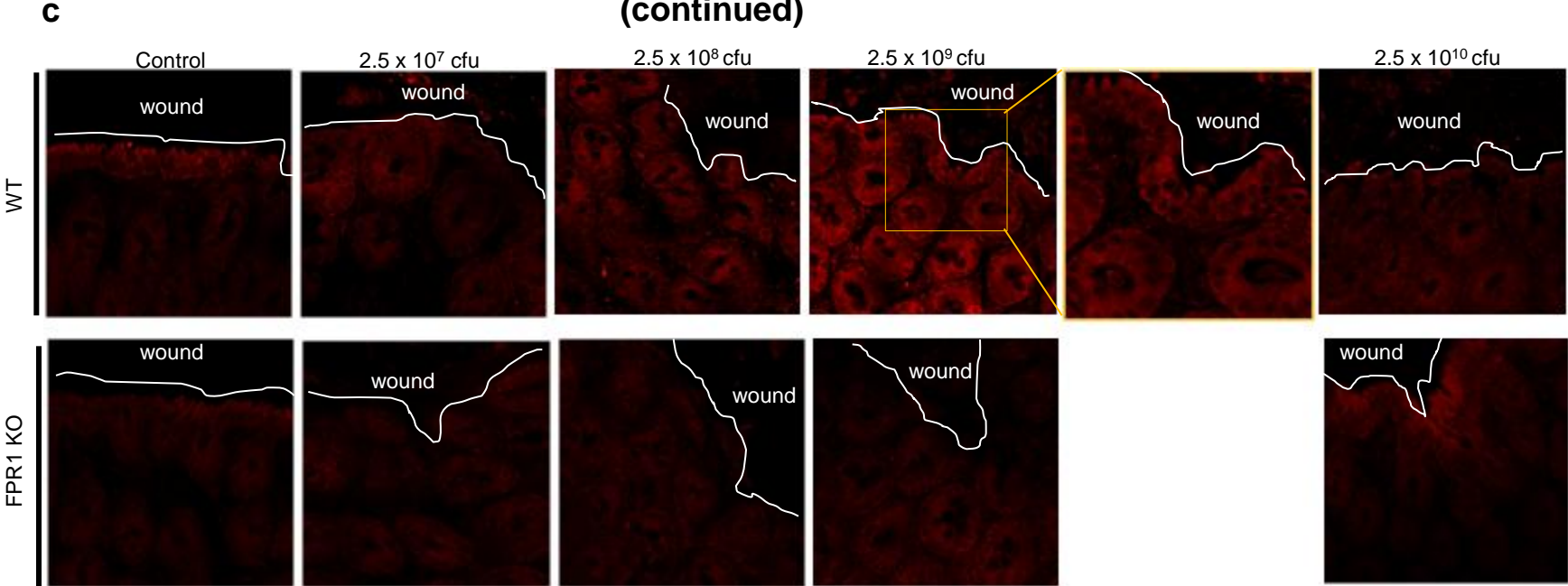


## Supplementary Figure S2

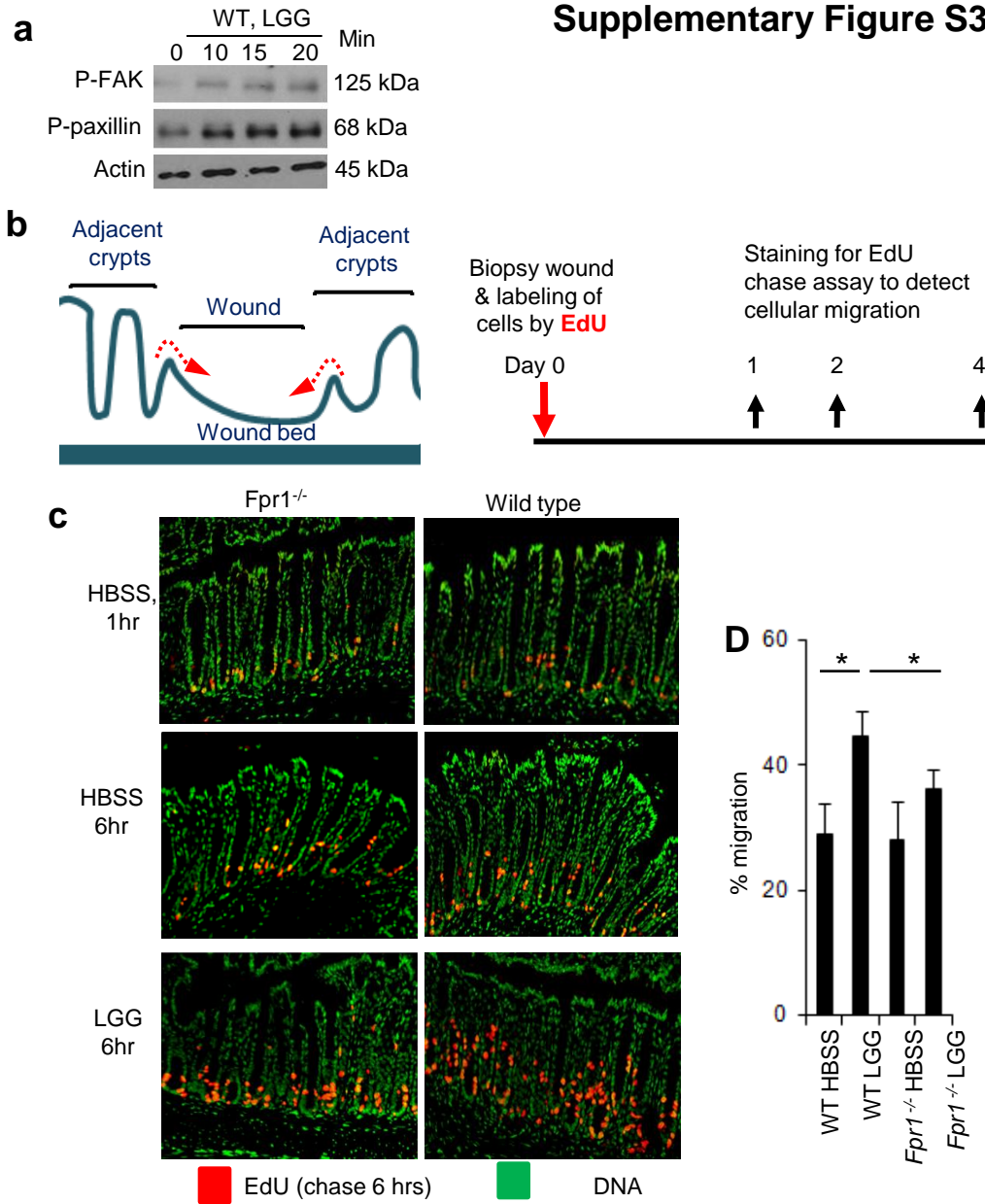




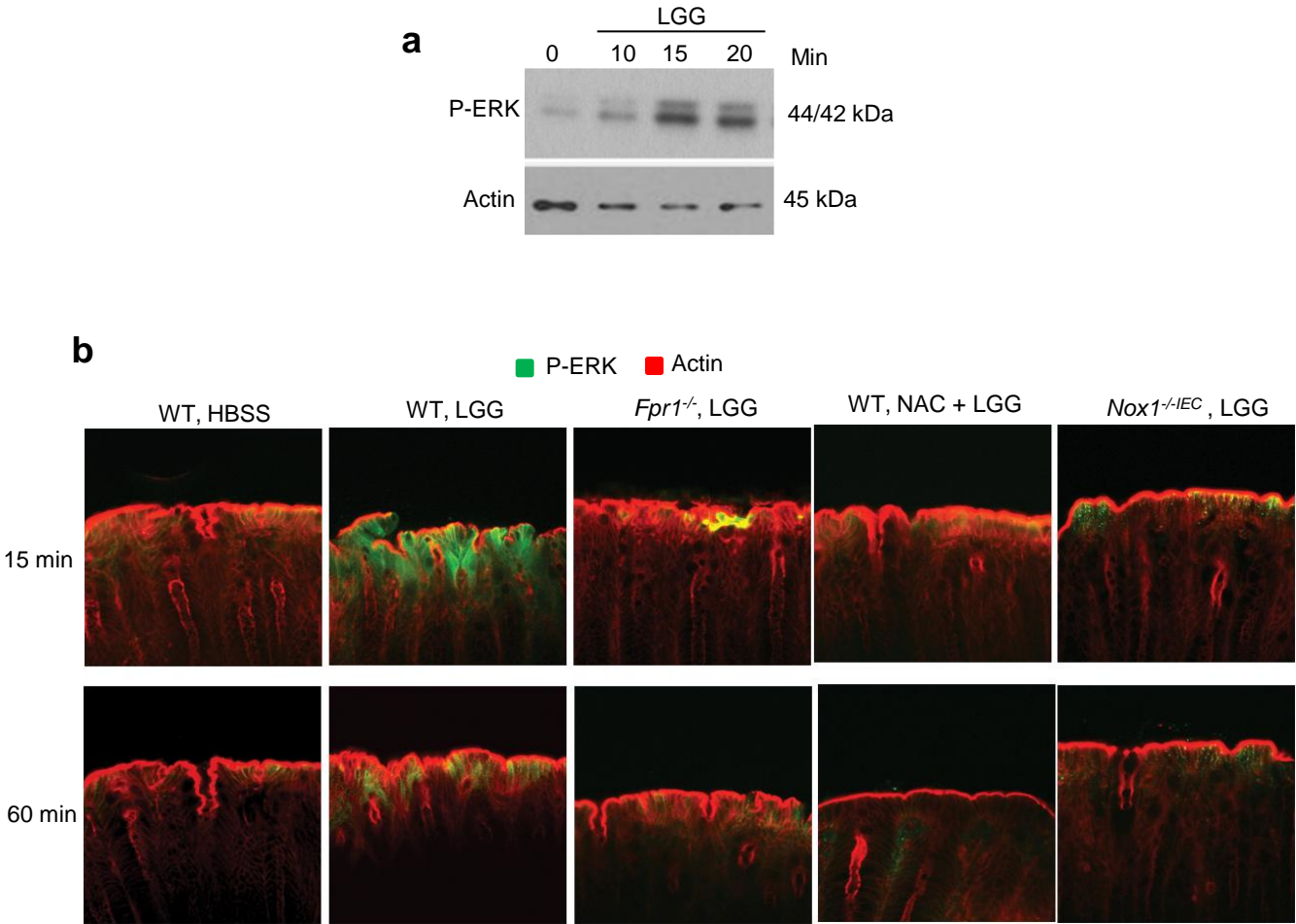
### Supplementary Figure S2 (continued)



## Supplementary Figure S3



### Supplementary Figure S4



## Supplementary Figure S5

