Science
AAAS

Supplementary Materials for

QRICH1 Dictates the Outcome of ER Stress through Transcriptional Control of Proteostasis

Kwontae You[1], Lingfei Wang[1], Chih-Hung Chou[1], Kai Liu[2,3], Toru Nakata[2,3], Alok Jaiswal[1], Junmei Yao[2,3], Ariel Lefkovith[1], Abdifatah Omar[2,3], Jacqueline G. Perrigoue[4], Jennifer E. Towne[4], Aviv Regev[5,6,*], Daniel B. Graham[1,2,3,*], Ramnik J. Xavier[1,2,3,*]

Correspondence to: xavier@molbio.mgh.harvard.edu

This PDF file includes:

Materials and Methods
References 50-73
Figs. S1 to S7
Table S1 to S4 captions

Other Supplementary Materials for this manuscript include the following:

Table S1 to S4 (Excel)

Materials and Methods

Mouse Colon Organoids Culture

Rosa26-floxed STOP-Cas9 mice (stock 024857, Jackson Laboratories) were crossed with CMV-cre (stock 006054, Jackson Laboratories) to generate a mouse line with constitutive expression of Cas9 in all tissues. Colonic crypts were isolated from Cas9 mice and cultured as previously described (*50*). Briefly, crypts were isolated from mice by incubation of colons in 8 mM EDTA in phosphate buffered saline (PBS) for 60 min at 4℃, followed by manual disruption of the tissue by pipetting. Crypts were plated in 15 ul of Matrigel and maintained in 50% L-WRN conditioned media. Media was replaced every 2 days, and organoids were passaged every 3-4 days.

Mouse Colon Monolayer Culture

Mouse colon monolayer culture has been previously described (*51, 52*). Briefly, day 3 colonic organoids were dissociated into single cells using TrypLE (ThermoFisher) and passed through a 70 mm filter into 50% L-WRN conditioned medium containing 10 mM Y27632. 1.5 x10^5 cells were plated on a 24-well transwell insert (Costar) coated with 1:40 Matrigel diluted in PBS. 150 ul of medium was added to the upper compartment and 600 ul of medium was added to the lower compartment. After 24 h, the media in both compartments was replaced with 50% L-WRN medium. After an additional 24 h, the media was replaced with 5% L-WRN medium to induce differentiation. Media was replaced daily, and day3 monolayers were used for the Tm treatment and scRNA-seq analysis.

Human Colon Organoids Culture

Endoscopic biopsy samples were obtained from healthy donors enrolled in the Prospective Registry in IBD Study at MGH (PRISM). Written informed consent was obtained from study participants and the study protocols were reviewed and approved by the Mass General Brigham Human Research Committee (#2004-P-001067). Human colon spheroids were established and maintained as previously described (*53*) with a slight modification. Briefly, biopsy samples were washed with PBS containing penicillin (100 units/ml), streptomycin (0.1 mg/ml) and gentamicin (50 ug/ml) for 5 min at room temperature. After three washings with PBS, tissues were incubated with 5 mM EDTA in PBS for 45 mins at 4℃. After wash with PBS, several pipetting yielded supernatants enriched in colonic crypts. Crypts were pelleted by centrifugation at 200 g for 5 mins and then plated in 15 ul of Matrigel (Corning) and maintained in 500 ul of 50% L-WRN conditioned media (*50*) supplemented with 10 uM Y-27632 (R&D systems, cat# 1254) and 10 uM SB431542 (Tocris Bioscience, 1614). Medium was changed every 2-3days and organoids were passaged to 1: 3 using TrypLE every 7 days.

Dissociation of monolayer for scRNA-seq

Monolayers were scraped out using a 200 ul pipet tip and incubated in 5 mM DTT and EDTA–PBS on ice for 30 min, shaking every 10 min. Cells were resuspended with TrypLE and incubated for 3 min at 37°C. After inactivating the trypsin, single cells were resuspended in MACS buffer and then passed through a 40 μm filter. Cell number was counted after enriching for live cells using EasySep Dead cell removal Kit (STEMCELL Tech, 17899).

scRNA-seq data processing

Cellranger was used to process the readout separately for each lane. The lanes were aggregated using "cellranger aggr --normalize=mapped". We removed lowly expressed genes, as defined by total UMI read count across all cells less than 300 or proportion of cells with nonzero UMI reads less than 0.2%. Log(CPM+1) was computed for downstream analyses.

scRNA-seq cell clustering and tSNE plot

Each replicate and treatment was regarded as a categorical covariate and the log UMI count as a continuous covariate for each cell, and removed from log(CPM+1). The resulting matrix was reduced to 20 dimensions using principal component analysis, from which cells were clustered using louvain method in SCANPY(*54*) and reduced to 2 dimensions using tSNE for visualization.

scRNA-seq cell-type identification

Cell types of each cluster were determined according to post-covariate-removal expression levels of previously annotated marker genes (*55*). Two clusters were identified as empty droplets based on marker gene expression, mitochondria mapped gene expression, the number of UMIs, and cluster size, and was consequently removed from downstream analyses.

scRNA-seq GO program activity level

For GO categories, we used the average CPM of genes within this GO and all of its sub-categories as its activity level.

scRNA-seq maximal discriminating axes (MDAs)

For a given cell-type, the maximal discriminating axis provides the strongest linear discriminating power between two groups, assuming a normal noise distribution. The average expression level was obtained from log(CPM+1) for each gene in each treatment. The maximal discriminating axes were computed as the differences of the average expression level between treatment pairs. Each cell was then mapped onto the MDAs by inner product. The MDAs and cell coordinates are computed separately for each cell-type and not comparable across cell-types. The complete source code for MDA analysis can be found at Zenodo (*56*).

scRNA-seq transcriptomic profiles for treatments

For each cell type, using the 2-dimensional transcriptional coordinates on the MDAs of every cell, a linear discriminant analysis model was trained against treatment and predicted on the same cells. The predicted treatment class probabilities were visualized in triangular plots, where stronger overlap between treatments indicates less distinguishability between the treatments based on their transcriptomic profiles.

scRNA-seq gene differential expression

Each replicate was regarded as a categorical covariate, and log UMI count as a continuous covariate for each cell. For differential expression between the two treatments, samples were limited to these treatments, covariates were removed from log(CPM+1) of each gene and from the treatment variable, and their Pearson correlation was computed. P-values were obtained empirically with 10^7 random permutations on post-covariate-removal data. LogFC was determined as the coefficient of linear regression of log(CPM+1) on treatment, after covariate removal.

Putative UPR regulators in scRNA-seq

The criteria for putative positive UPR regulators are: induced in Tm-13 h vs. DMSO with p-value < 0.05 and FC > 1.5 in enterocytes and goblet cells and, in Tm-25 h v.s. Tm-13 h, having FC in goblet cells lower than that in enterocytes.

Generation of reporter cell lines using CRISPR knock-In method

Repair template dsDNA was designed to contain the P2A-GFP sequence and the guide RNA sequences targeting the C-terminal region of XBP1s and synthesized it from Integrated DNA Technologies (IDT). 30 pmol of tracrRNA and crRNA were mixed in duplexing buffer (IDT) and incubated at 50℃ for 1 min and 37℃ for 10 min. We mixed 30 pmol of recombinant Cas9 protein (PNA Bio. CP02) with 30 pmol of gRNA duplex and incubated for 10 min at room temperature (RT). Following RNP formation, equimolar amount of repair template DNA was added and incubated for 10 min at RT. We transfected the RNP complex into HT29 cells using the Lonza 4D-nucleofector kit (V4XC-2032) according to the manufacturer's protocol (SF solution with FF-137 program). We cultured HT-29 cells in Glutamax DMEM medium (Invitrogen, 10569044) supplemented with 10% heat-inactivated FBS (Invitrogen), penicillin/streptomycin (100U/ml, Invitrogen,15140163).

XBP1s Screen with CRISPR library

On day 0, the pooled lentiviral library was transduced at an MOI of 0.2 into 40 million HT29 XBP1s-GFP cells, which infected one virus per cell with over 100 coverage of the library. On day 2, transduced cells were selected by adding puromycin (Invitrogen) at 5 ug/ml. On day 5, we re-plated the cells with fresh media.

For the XBP1s-GFP screen, cells were treated with DMSO or 0.25 ug/ml of tunicamycin (Tm) for 14 h. Single cells were filtered (40 um) and stained with DAPI just before the sort to select the live cell fraction. Cells were sorted based on the GFP intensities using FACS (Sony SH800S cell sorter) as shown in Figure S2B, into high or low bins containing 15% of the cells. Approximately 15 million cells were collected per bin (a representation of over 200 coverage of the library).

Preparation of gDNA for Next-generation sequencing

gDNA was extracted from the cells using DNAzol (ThermoFisher, 10503027) according to the manufacturer's instructions and the DNA library was prepared as previously described (*57*). All the gDNAs were used in a total of 3 ug of DNA per PCR reaction, which consisted of 25 cycles using HiFi hotstart polymerase (KAPA Biosystems) with Illumina sequencing-comparable primers. The final PCR product was run on a gel and the right size fragment (354 bp) was gel extracted and sequenced on a NextSeq (Illumina).

CRISPR screen data analysis

bcl2fastq and PoolQ were used to map raw data to gRNA reads. Mageck_nest was used for differential gene analyses (*58*), and beta_1|beta from mageck_nest was treated as logFC. The p-values were converted to relative rankings through signed log p-values. The top and bottom 5% were regarded as significant genes that affect ER homeostasis or UPR activity in DMSO or Tm-treated ER stress conditions, respectively. Essential genes (*59*) were excluded from the significant genes in the Tm-mediated ER stress condition for further analysis.

Lentivirus production

To generate lentiviruses containing individual sgRNAs for all follow-up experiments, 1 ug of the sgRNA lentivector, 1 ug of PAX2 vector and 0.1 ug VSVg vector were transfected into 293T cells using Lipofectamine2000 (ThermoFisher, 11668030) according to the manufacturer's instructions. Cell supernatants were collected at 48 and 72 hours. Media was concentrated using a Lenti-X concentrator (Takara) after removing debris by centrifugation according to the manufacturer's protocol. Viruses were aliquoted and stored at -80°C. Viruses were transduced with MOI less than 0.5.

FACS analysis for cell viability
Single-cell suspensions were obtained by TrypLE treatment. Cells were incubated with 7-AAD and Pacific Blue Annexin V dye (BioLegend) in room temperature 15min for live/dead staining and analyzed using CytoFLEX Cytometer (Beckman).

Quantitative RT-PCR
Cells were harvested and RNA isolated using Direct RNAzol (Zymo Research) according to manufacturers' instructions. cDNA was made using RNA with PrimeScript RT Mix (Clontech) and diluted 10 times with deionized water. PCR samples were prepared by mixing cDNA, specific primers, and iTaq SYBR Green Supermix (Biorad), and quantitative PCR reactions were run on a CFX96 instrument (Biorad).

Immunoblot
Cells were lysed with RIPA lysis buffer containing Protease & Phosphatase Inhibitor Cocktails (Sigma), and Benzonase Nuclease (Sigma). Lysates were incubated on ice for 20 min and centrifuged at 12,000 g x 5 min at 4℃. Supernatants were transferred and mixed with 4x protein loading dye containing DTT. Samples were run on the SDS-PAGE and transferred using I-Blot (Invitrogen). Western blots were performed according to general protocol using the primary antibodies for QRICH1 (Sigma-Aldrich, HPA037677), ATF4 (Cell signaling, #11815), p-eIF2α (Ser51) (Cell signaling, #3398), cleaved CASP3 (Cell signaling, #9664), SRP72 (Abcam, ab200199), SEC61B (Proteintech, 15087-1-AP), TRAM1 (LSbio, LS-C313140), and ACTB for overnight at 4°C. HRP signals from the secondary antibodies were detected using Hyblot Autoradiography Film (Thomas Scientific). Signals on x-ray film were normalized using ImageJ (*60*).

Immunostaining
Cells were fixed in 4% paraformaldehyde followed by permeabilization with 0.3% Triton X-100 for 10 min. After extensive washing with PBS, the ells were incubated in blocking buffer (5% BSA, PBS) for 1 h at RT and then incubated with antibodies for QRICH1, DDIT3 (Cell signaling, #2895s), RTN4 (Abcam, ab47085), FLAG (Sigma, F3165) in blocking buffer at 4ºC overnight. Cells were washed extensively and incubated with Alexa488- or Alexa647-conjugated secondary antibodies in blocking buffer for 1 h at RT. After another round of washing, cells were stained with DAPI for 10 min for confocal microscopy analysis.

Chromatin-immunoprecipitation and sequencing
To induce ER-stressed conditions, wild type or QRICH1 knockout HT29 cells were treated with 0.3 ug/ml of Tm for 14 h and performed chromatin immunoprecipitation as previously described with minor modifications (*61*). Briefly, cells were fixed with 1% formaldehyde and crosslinking

was quenched with glycine. Cell lysates were sonicated using a Branson 450 Ultrasonic 102C sonicator. Lysates were then incubated with anti-QRICH1 antibody (ThermoFisher), anti-ATF4 antibody (Cell signaling), or anti-normal rabbit IgG (Cell signaling) overnight at 4 °C. Protein G dynabeads (Invitrogen) were added for 4 h and subsequent washes were performed with RIPA buffer (0.1% SDS, 0.1% DOC, 1% Triton X-100, 1 mM EDTA, 10 mM Tris-HCl pH 8.1, 150 mM NaCl), high-salt RIPA buffer (RIPA with 500 mM NaCl), LiCl buffer (10 mM Tris-HCl pH 8.1, 250 mM LiCl, 0.5% Triton X-100, 0.5% DOC) and PBS. DNA was incubated overnight at 65 °C to reverse crosslinking and enriched gDNA fragments were extracted after RNase A and Proteinase K treatment. DNA libraries were generated using the NEBNext Ultra II DNA Library Prep Kit for Illumina Sequencing according to the manufacturer's protocol.

ChIP-seq analysis

ChIP-seq data analysis was performed following the ENCODE ChIP-seq Pipeline. Raw sequencing data were aligned to the reference genome (Human GRCh38) using BWA (*62*), SAMtools (*63*) and Picard's MarkDuplicates (*64*) were used for post-alignment filtering, including to remove unmapped, multi-mapped reads, and PCR duplicate reads. The retained high-quality alignment results were used to call peaks by MACS2 (*65*) against an IgG control with a p-value threshold of 0.01. ChIPseeker was used to annotate peak distribution and peaks were visualized using the WashU Epigenome Browser.

Sample preparation for RNA sequencing

Wild type or QRICH1 knockout HT29 cells were treated with DMSO or 0.3 ug/ml of Tm for 14 h and isolated RNA by TCL buffer (QIAGEN) treatment. RNA-seq libraries were generated with three biological replicates according to previously described method (*66*).

RNA-seq analysis

Raw RNA-seq reads were processed by Trimmomatic (*67*) to remove the adapter sequences and low quality bases. Sequencing data was aligned to the reference genome (Human GRCh38) using STAR. Raw read counts from STAR were analyzed using DESeq2 (*68*) with shrinkage estimator apeglm (*69*) to identify differentially expressed genes. Genes with low mean value (≤ 10) were filtered. The genes were defined as differentially expressed if the fold change was greater than or equal to 1.2 with a p value < 0.05. Metascape (*70*) was used to illustrate the biological role of differentially expressed genes.

Metabolic labeling with puromycin

Protein synthesis activity was assessed as previously described (*71*) with a slight modification. For this experiment, we used the Hygromycin selection vector instead of puromycin to prevent the interference by the puromycin resistant gene. Briefy, cells were treated with DMSO or Tm for the indicated time before labeling. Then, the media was replaced with labeling medium containing 10 ug/ml of puromycin for 20 min. Cells were lysed and protein concentration was measured by BCA assay. Equal amounts of protein were loaded in the gel and transferred to the membrane. Signals were detected by anti-puromycin antibody (Sigma, MABE343). Signals on x-ray film were normalized using ImageJ.

Immunofluorescence with tissue array

QRICH1 staining was performed on a tissue array of human colon and liver samples of inflamed and healthy individuals (TMA, US BioMAX, #CO245 and LV20812b) according to the manufacturer's suggestion. After deparaffinization and baking of the tissue sections using the standard protocol, samples were incubated with QRICH1 antibody (1:100) overnight at 4°C, and then incubated with secondary antibodies (1:1000) at room temperature for 1 h. Nuclei were stained with Hoechst 33342 (1: 5000, ThermoFisher). The signal intensities in the nuclei were quantified using ImageJ.

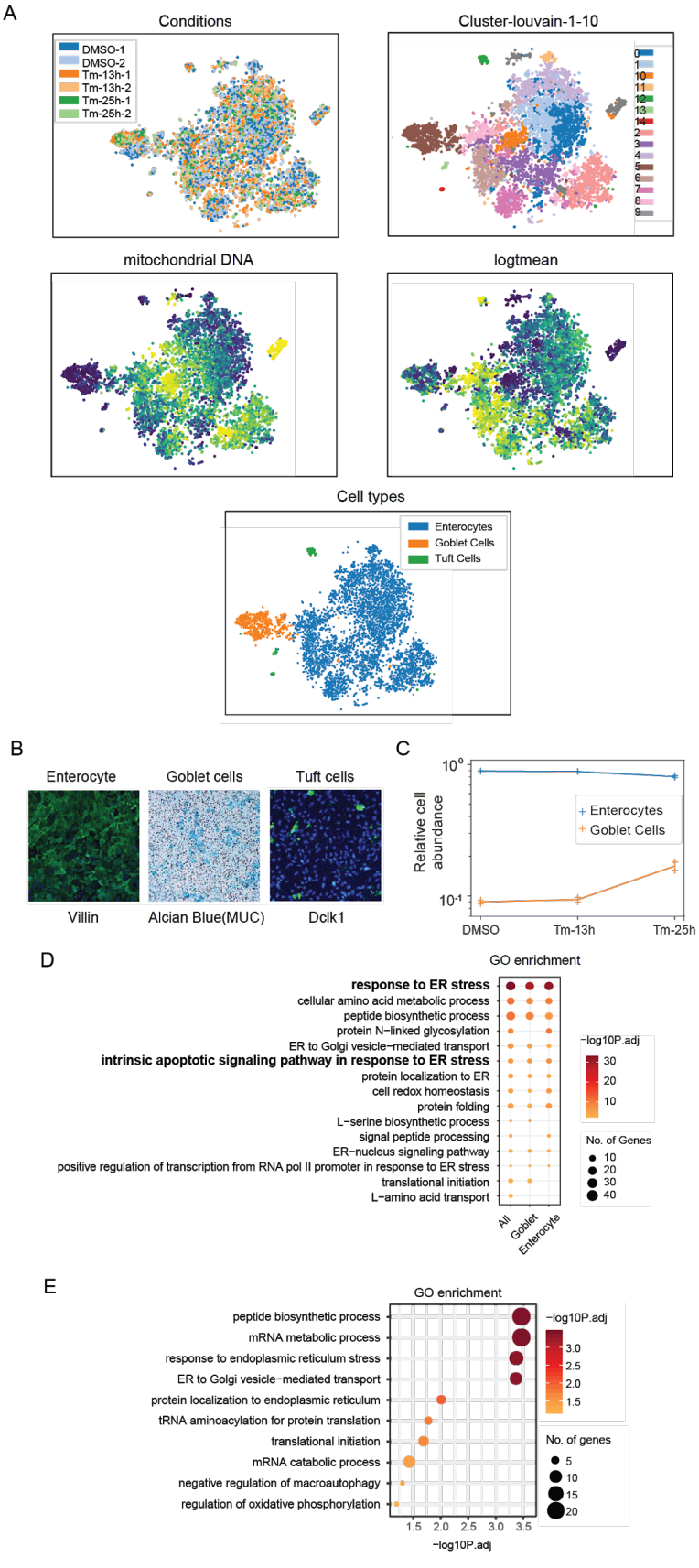
Lentivirus Transduction of Organoids

Mouse organoids from 5 wells were harvested and used for transduction. After washing with PBS, samples were collected, centrifuged at 400 g x 5 min. After removal of the supernatant, cells were suspended with TrypLE and a wash buffer was added (Advanced DMEM F12 containing 10% FBS, GlutaMax and Pen/Strep). Cells were centrifuged at 600 g for 5 min, resuspended with 250 ul of viral suspension and then transferred to a 48-well plate. After centrifugation at 600 g for 90 min at 37℃, samples were incubated at 37℃ for another 2 hours. Cells were collected into a 1.5ml tube and centrifuged at 900 g for 5 min and resuspended with 60 ul of Matrigel. 15 ul of samples were plated each on a 24-well plate. Lentivirus-containing medium was removed 24 h later and replaced with culture medium. 3 days after transduction, organoids were selected for at least two cell passages in puromycin-containing (3 μg/ml) medium. Once the selection was completed, total protein was extracted to confirm knockout of Qrich1.

QRICH1 signature

To identify the minimal number of genes showing QRICH1 effects on translation & protein secretion, we started with the 278 significant QRICH1 target genes from Figure 5A. Of these, we used only 201 genes, which are up-regulated by QRICH1 and enriched in the translation and secretion pathway (**Fig. 5B**). By comparing these with DEGs from Tm treated primary monolayers, we generated a 29 QRICH1-signature geneset. Module scores of QRICH1-signatures were calculated by the average expression levels of QRICH1_signature genes using the AddModuleScore function in Seurat.

Fig. S1.


A
Conditions
DMSO-1
DMSO-2
Tm-13h-1
Tm-13h-2
Tm-25h-1
Tm-25h-2
Cluster-louvain-1-10
mitochondrial DNA
logtmean
Cell types
Enterocytes
Goblet Cells
Tuft Cells
B
Enterocyte
Goblet cells
Tuft cells
Villin
Alcian Blue(MUC)
Dclk1
C
Relative cell abundance
Enterocytes
Goblet Cells
DMSO
Tm-13h
Tm-25h
D
GO enrichment
response to ER stress
cellular amino acid metabolic process
peptide biosynthetic process
protein N−linked glycosylation
ER to Golgi vesicle−mediated transport
intrinsic apoptotic signaling pathway in response to ER stress
protein localization to ER
cell redox homeostasis
protein folding
L−serine biosynthetic process
signal peptide processing
ER−nucleus signaling pathway
positive regulation of transcription from RNA pol II promoter in response to ER stress
translational initiation
L−amino acid transport
All
Goblet
Enterocyte
−log10P.adj
No. of Genes
E
GO enrichment
peptide biosynthetic process
mRNA metabolic process
response to endoplasmic reticulum stress
ER to Golgi vesicle−mediated transport
protein localization to endoplasmic reticulum
tRNA aminoacylation for protein translation
translational initiation
mRNA catabolic process
negative regulation of macroautophagy
regulation of oxidative phosphorylation
−log10P.adj
No. of genes

scRNA-seq of intestinal monolayer cells shows the terminal-UPR signature during ER stress.

(**A**) tSNE plots show the clustering of monolayer cells (colored by conditions, clusters, and cell types).

(**B**) Immunostaining of monolayer cells. Enterocytes, goblet cells, and Tuft cells are detected by Villin, Alcian Blue (Mucin), and Dclk1 staining.

(**C**) Changes in relative abundance of enterocytes and goblet cells after Tm treatment. Two samples were measured per conditions, with + signs indicating percentage of cells for each measurement.

(**D**) Gene set enrichment analysis (GSEA) shows enriched pathways in goblet cells and enterocytes upon 13 h of Tm treatment.

(**E**) GSEA shows the enriched biological processes in the terminal-UPR signature illustrated in Fig. 1H.

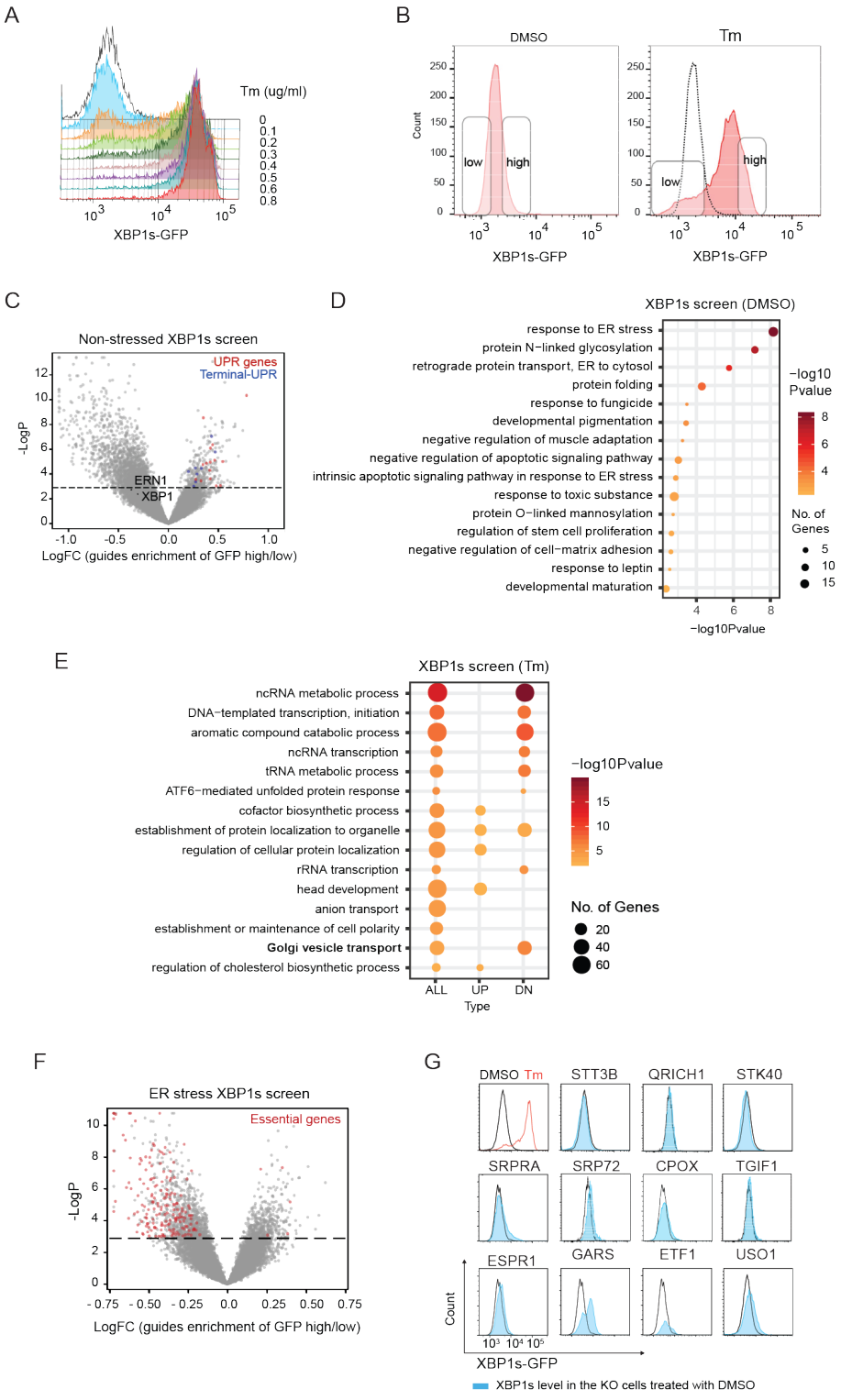

A
Tm (ug/ml)
0
0.1
0.2
0.3
0.4
0.5
0.6
0.8
XBP1s-GFP
B
DMSO
Tm
Count
low
high
XBP1s-GFP
C
Non-stressed XBP1s screen
UPR genes
Terminal-UPR
ERN1
XBP1
-LogP
LogFC (guides enrichment of GFP high/low)
D
XBP1s screen (DMSO)
response to ER stress
protein N−linked glycosylation
retrograde protein transport, ER to cytosol
protein folding
response to fungicide
developmental pigmentation
negative regulation of muscle adaptation
negative regulation of apoptotic signaling pathway
intrinsic apoptotic signaling pathway in response to ER stress
response to toxic substance
protein O−linked mannosylation
regulation of stem cell proliferation
negative regulation of cell−matrix adhesion
response to leptin
developmental maturation
−log10Pvalue
−log10 Pvalue
No. of Genes
E
XBP1s screen (Tm)
ncRNA metabolic process
DNA−templated transcription, initiation
aromatic compound catabolic process
ncRNA transcription
tRNA metabolic process
ATF6−mediated unfolded protein response
cofactor biosynthetic process
establishment of protein localization to organelle
regulation of cellular protein localization
rRNA transcription
head development
anion transport
establishment or maintenance of cell polarity
Golgi vesicle transport
regulation of cholesterol biosynthetic process
ALL
UP
DN
Type
−log10Pvalue
No. of Genes
F
ER stress XBP1s screen
Essential genes
-LogP
LogFC (guides enrichment of GFP high/low)
G
DMSO Tm
STT3B
QRICH1
STK40
SRPRA
SRP72
CPOX
TGIF1
ESPR1
GARS
ETF1
USO1
Count
XBP1s-GFP
XBP1s level in the KO cells treated with DMSO

Fig. S2. XBP1s-GFP reporter screens identify potent regulators of the ER stress-UPR pathway.

(**A**) Dose-dependent response to Tunicamycin (Tm). Quantification of spliced XBP1 (XBP1s) level according to GFP intensity using FACS after 14 h of Tm treatment.

(**B**) Gating strategy. The lowest and highest 15% of cells were sorted out based on the GFP intensities using FACS sorter.

(**C**) Volcano plot shows the average gRNA enrichments in the non-stressed XBP1s screen. The dashed line indicates the p-value cut-off. Known UPR genes and terminal-UPR genes are highlighted in red and blue, respectively.

(**D**) GSEA shows the enriched biological processes in the non-stressed XBP1s screen. Only the list of genes for which KO promotes UPR activity is used for the GO analysis. Many of the known ER homeostasis-related GOs are shown on this screen.

(**E**) GSEA shows enriched biological processes in the stressed XBP1s screen. UP and DN indicate the list of genes for which KO promotes or reduces XBP1s expression in the Tm-treated cells.

(**F**) Volcano plot of ER stress XBP1s screen shows the distribution of essential genes, highlighted in red.

(**G**) Measurement of GFP intensities shows the level of XBP1s in wild-type (dashed black) or KO (filled blue) cells treated with DMSO. The symbol indicates the targeted gene. The dashed red line indicates the GFP intensity in the Tm condition.

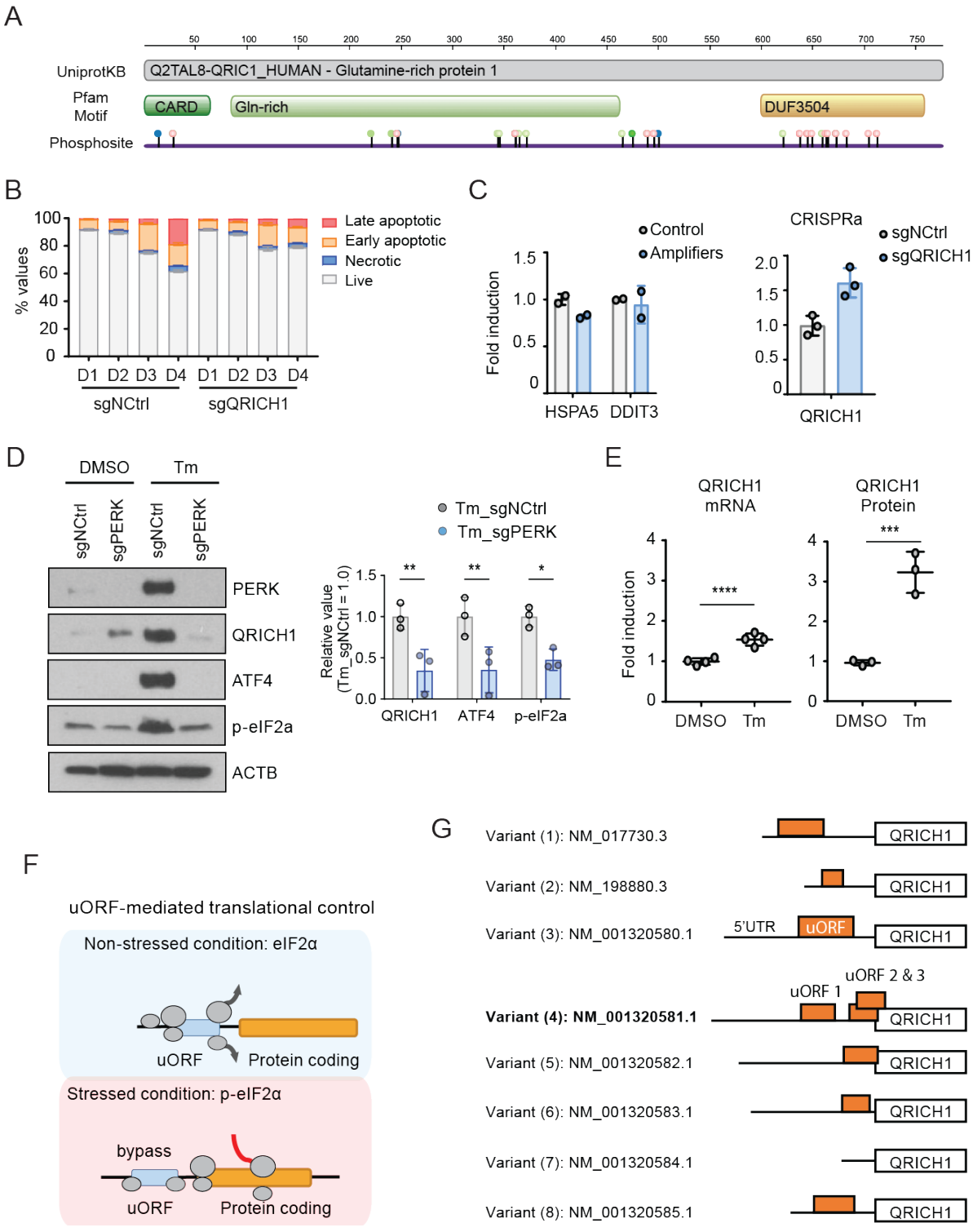

A
UniprotKB
Q2TAL8-QRIC1_HUMAN - Glutamine-rich protein 1
Pfam Motif
CARD
Gln-rich
DUF3504
Phosphosite
B
% values
Late apoptotic
Early apoptotic
Necrotic
Live
D1 D2 D3 D4 D1 D2 D3 D4
sgNCtrl
sgQRICH1
C
Control
Amplifiers
Fold induction
HSPA5
DDIT3
CRISPRa
sgNCtrl
sgQRICH1
QRICH1
D
DMSO
Tm
sgNCtrl
sgPERK
PERK
QRICH1
ATF4
p-eIF2a
ACTB
Tm_sgNCtrl
Tm_sgPERK
Relative value (Tm_sgNCtrl = 1.0)
QRICH1
ATF4
p-eIF2a
E
QRICH1 mRNA
QRICH1 Protein
Fold induction
DMSO
Tm
F
uORF-mediated translational control
Non-stressed condition: eIF2α
uORF
Protein coding
Stressed condition: p-eIF2α
bypass
uORF
Protein coding
G
Variant (1): NM_017730.3
Variant (2): NM_198880.3
Variant (3): NM_001320580.1
5'UTR
uORF
Variant (4): NM_001320581.1
uORF 1
uORF 2 & 3
Variant (5): NM_001320582.1
Variant (6): NM_001320583.1
Variant (7): NM_001320584.1
Variant (8): NM_001320585.1
QRICH1

Fig. S3. **QRICH1 regulates cell viability during ER stress.**

(**A**) Schematic diagram of the domain structure of QRICH1. Figure modified from the RCSB_PDB: Q2TAL8 (http://www.rcsb.org/pdb/protein/Q2TAL8).

(**B**) The proportion of 7-AAD and/or Annexin V positive cells. sgNCtrl or sgQRICH1 transduced cells treated with Tm for the indicated time (n=2).

(**C**) Left panel: Measurement of DDIT3 or HSPA5 in HT29 cells after the transduction of CRISPRa amplifiers by qRT-PCR. Right panel: Transcriptional induction of QRICH1 by CRISPRa. Mean value of three guides. Error bars, mean +/- SD.

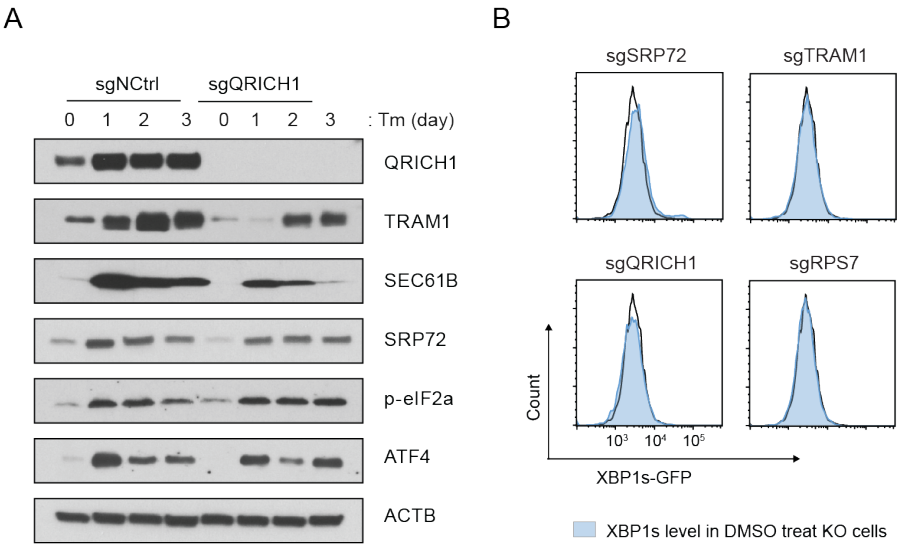
(**D**) Immunoblot shows expression patterns of QRICH1 upon Tm treatment in the sgNCtrl or sgPERK transduced HT29 cells. A representative blot is shown (n=3, two-way ANOVA).

(**E**) Quantification of QRICH1 mRNA (n=4) and protein level (n=3) in cells treated with 0.3ug/ml of Tm for 1 day (multiple *t*-test).

(**F**) Schematic diagram of upstream open reading frame (uORF) mediated translational control depends on the status of eIF2α phosphorylation.

(**G**) Schematic diagram shows QRICH1 mRNA isoforms. All variants encode the same protein — variant 4 was used for QRICH1 reconstitution.

For panels D and E: *p<0.05, **p<0.01, ***p<0.005, ****p<0.0001; error bars, mean +/- SD.


A
sgNCtrl
sgQRICH1
0 1 2 3 0 1 2 3 : Tm (day)
QRICH1
TRAM1
SEC61B
SRP72
p-eIF2a
ATF4
ACTB
B
sgSRP72
sgTRAM1
sgQRICH1
sgRPS7
Count
10^3 10^4 10^5
XBP1s-GFP
XBP1s level in DMSO treat KO cells

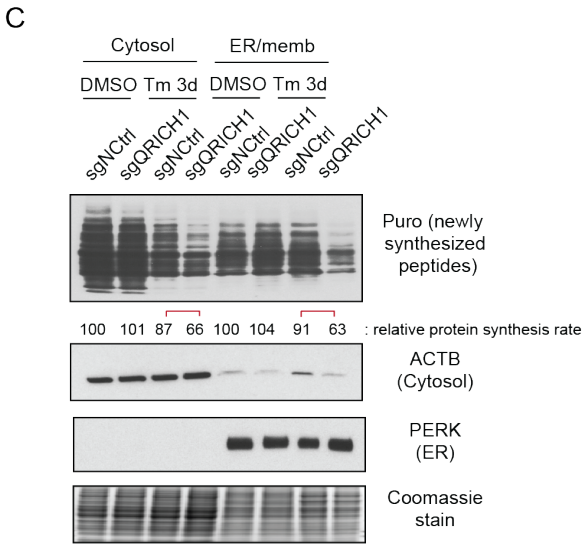

C
Cytosol
ER/memb
DMSO Tm 3d DMSO Tm 3d
sgNCtrl sgQRICH1 sgNCtrl sgQRICH1 sgNCtrl sgQRICH1 sgNCtrl sgQRICH1
Puro (newly synthesized peptides)
100 101 87 66 100 104 91 63 : relative protein synthesis rate
ACTB (Cytosol)
PERK (ER)
Coomassie stain

Fig. S4. QRICH1 facilitates protein secretion during ER stress.

(**A**) Immunoblot shows the kinetics of p-eIF2α, QRICH1, ATF4, and QRICH1-regulated target genes during prolonged ER stress. A representative blot is shown.

(**B**) FACS analysis shows the level of XBP1s in DMSO-treated wild-type (black line) or sgRNA transduced (filled blue) cells, as measured by GFP intensities. The target gene is indicated above each plot.

(**C**) Assessment of protein synthesis rate in the cytosolic or ER/membrane fractions detected using anti-puromycin. WT and QRICH1 KO cells were pulse-labeled with puromycin after Tm treatment for 3 days. The DMSO-sgNCtrl in cytosol and ER/memb were set to 100%, and were used to normalize the data in the cytosol and ER/memb fractions, respectively (n=2). ACTB and PERK were used as markers for cytosol and ER fractions, respectively.

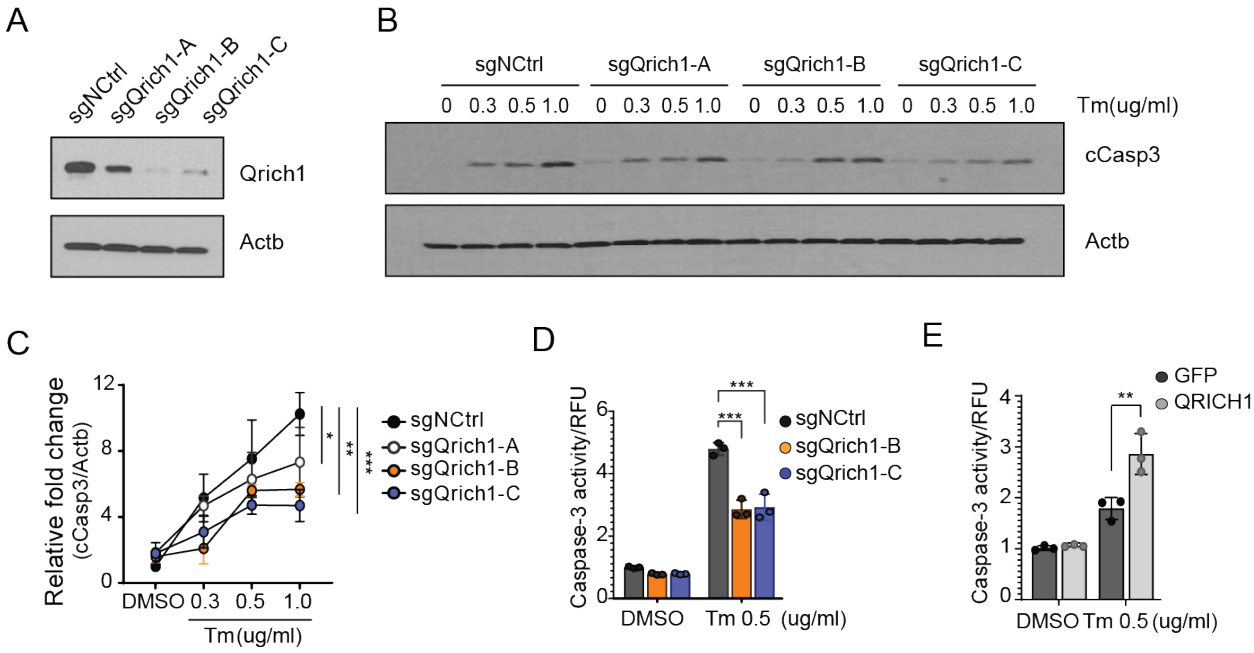

A
sgNCtrl
sgQrich1-A
sgQrich1-B
sgQrich1-C
Qrich1
Actb
B
sgNCtrl
sgQrich1-A
sgQrich1-B
sgQrich1-C
0 0.3 0.5 1.0 0 0.3 0.5 1.0 0 0.3 0.5 1.0 0 0.3 0.5 1.0
Tm(ug/ml)
cCasp3
Actb
C
Relative fold change
(cCasp3/Actb)
12
8
4
0
DMSO 0.3 0.5 1.0
Tm(ug/ml)
*
**
***
sgNCtrl
sgQrich1-A
sgQrich1-B
sgQrich1-C
D
Caspase-3 activity/RFU
6
4
2
0
***
***
sgNCtrl
sgQrich1-B
sgQrich1-C
DMSO Tm 0.5 (ug/ml)
E
Caspase-3 activity/RFU
4
3
2
1
0
**
GFP
QRICH1
DMSO Tm 0.5 (ug/ml)

Fig. S5. QRICH1 regulation during ER stress mediates proteotoxicity in primary mouse intestinal epithelium.

(**A**) Immunoblot analysis of WT and Qrich1 knockout (sgQrich1) mouse intestinal monolayers. Three different Cas9 guides are noted A, B, and C.

(**B** and **C**) WT or Qrich1 KO mouse intestinal monolayer cells were exposed to an indicated dose of Tm for 24 h. Quantification of cleaved Caspase 3 in those cells are measured by ImageJ (n=3, two-way ANOVA).

(**D**) The activity of Caspase 3 was measured by in vitro Caspase 3 assay with fluorogenic Caspase 3 substrate (Z-DEVD-AMC) in WT or Qrich1 KO monolayer cells treated with Tm for 24 h (n=3, two-way ANOVA). RFU, relative fluorescence unit.

(**E**) Caspase 3 activity in the GFP or human QRICH1 transduced mouse intestinal organoids treated with DMSO or Tm for 24 h (n=3, two-way ANOVA).

For panels C-E: *$p<0.05$, **$p<0.01$, ***$p<0.001$, error bars, error bars, mean +/- SD.

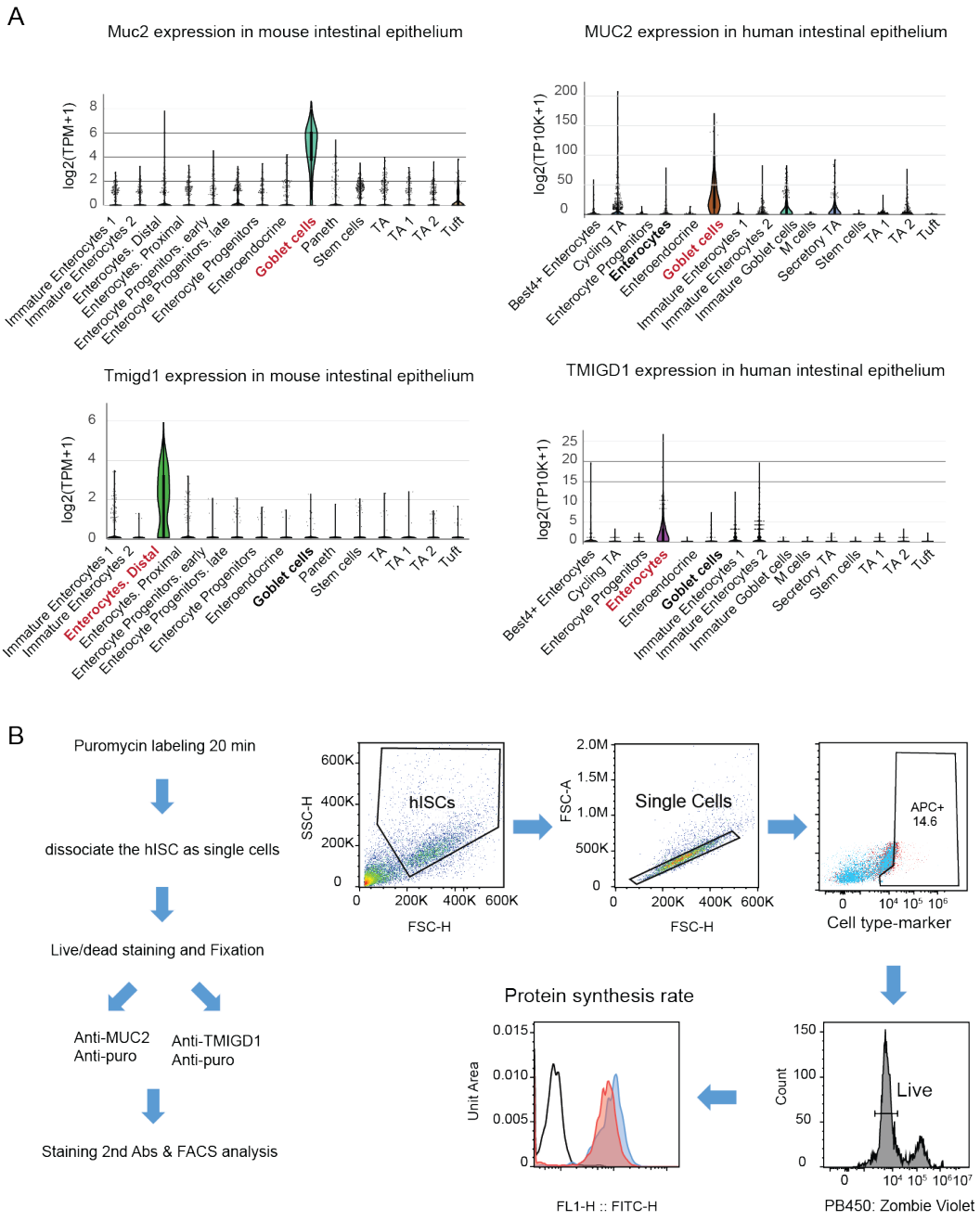
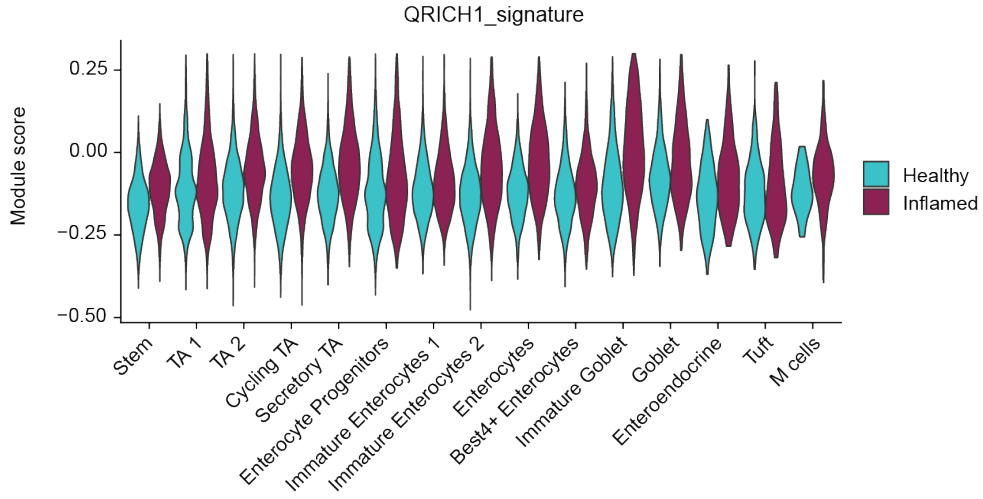

A
Muc2 expression in mouse intestinal epithelium
log2(TPM+1)
Immature Enterocytes 1
Immature Enterocytes 2
Enterocytes. Distal
Enterocytes. Proximal
Enterocyte Progenitors. early
Enterocyte Progenitors. late
Enterocyte Progenitors
Enteroendocrine
Goblet cells
Paneth
Stem cells
TA
TA 1
TA 2
Tuft
MUC2 expression in human intestinal epithelium
log2(TP10K+1)
Best4+ Enterocytes
Cycling TA
Enterocyte Progenitors
Enterocytes
Enteroendocrine
Goblet cells
Immature Enterocytes 1
Immature Enterocytes 2
Immature Goblet cells
M cells
Secretory TA
Stem cells
TA 1
TA 2
Tuft
Tmigd1 expression in mouse intestinal epithelium
TMIGD1 expression in human intestinal epithelium
B
Puromycin labeling 20 min
dissociate the hISC as single cells
Live/dead staining and Fixation
Anti-MUC2
Anti-puro
Anti-TMIGD1
Anti-puro
Staining 2nd Abs & FACS analysis
hISCs
SSC-H
FSC-H
Single Cells
FSC-A
APC+
14.6
Cell type-marker
Protein synthesis rate
Unit Area
FL1-H :: FITC-H
Live
Count
PB450: Zombie Violet

Fig. S6. Experimental scheme to measuring cell type-specific protein synthesis rate in human intestinal organoids during ER stress.

(**A**) Expression patterns of MUC2 and TMIGD1 in the mouse and human intestinal epithelium. (*55, 72*). TPM denotes transcripts per million, TP10K denotes UMI counts normalized by the total number of UMIs per cell and converted to transcripts-per-10,000.

(B) Schematic of the workflow for hISC experiments and FACS analysis.

A


QRICH1_signature
Module score
0.25
0.00
−0.25
−0.50
Healthy
Inflamed
Stem
TA 1
TA 2
Cycling TA
Secretory TA
Enterocyte Progenitors
Immature Enterocytes 1
Immature Enterocytes 2
Enterocytes
Best4+ Enterocytes
Immature Goblet
Goblet
Enteroendocrine
Tuft
M cells

B

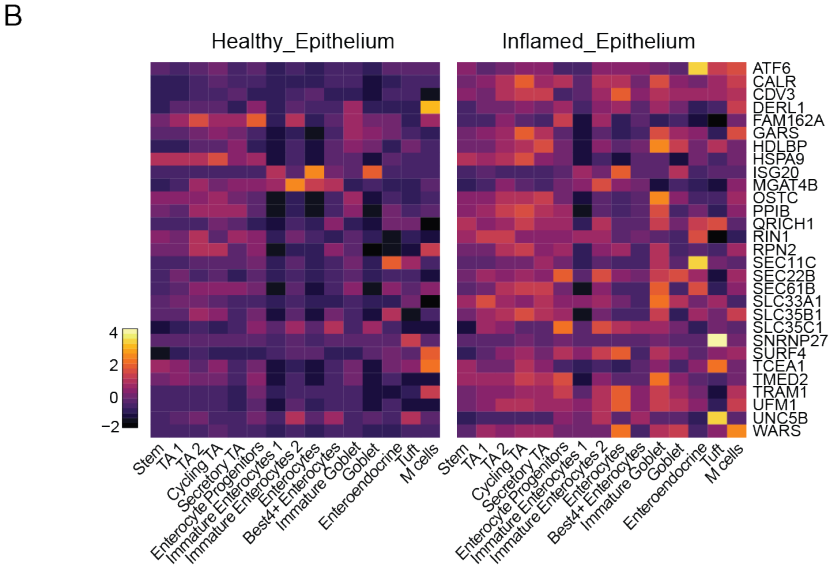

Healthy_Epithelium
Inflamed_Epithelium
4
2
0
−2
ATF6
CALR
CDV3
DERL1
FAM162A
GARS
HDLBP
HSPA9
ISG20
MGAT4B
OSTC
PPIB
QRICH1
RIN1
RPN2
SEC11C
SEC22B
SEC61B
SLC33A1
SLC35B1
SLC35C1
SNRNP27
SURF4
TCEA1
TMED2
TRAM1
UFM1
UNC5B
WARS
Stem
TA 1
TA 2
Cycling TA
Secretory TA
Enterocyte Progenitors
Immature Enterocytes 1
Immature Enterocytes 2
Enterocytes
Best4+ Enterocytes
Immature Goblet
Goblet
Enteroendocrine
Tuft
M cells

Fig. S7. Expression of QRICH1 signature in inflamed conditions.

(A) Module score of the QRICH1_signature mapped to epithelial cells from the colon of healthy individuals and the inflamed colon of UC patients. Cell types are annotated according to previously annotated marker genes (*73*).

(**B**) Heatmap shows the scaled average expression levels of each gene in the QRICH1_signature across the epithelial cell types in the colon of healthy individuals and patients with ulcerative colitis (inflamed regions).

Table S1. (separate Excel file)
Differentially expressed genes for cell subsets and the terminal-UPR geneset from the ER stressed monolayer shown in Fig. 1H.

Table S2. (separate Excel file)
CRISPR-Cas9 Genome-wide screen data for ER stress-UPR pathway using the XBP1s-GFP reporter cells in Fig. 2.

Table S3. (separate Excel file)
ChIP-seq data using anti-QRICH1 and ATF4 and Gene Ontology results illustrated in Fig. 4.

Table S4. (separate Excel file)
Differentially expressed genes between WT and QRICH1 KO cells in response to Tm treatment are listed. Related to Fig. 5A.