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#### Gut

## Figure S1





Supplementary Figure 1. CLDN18-ARHGAP26 fusion induces abnormal organoid morphologies and co-occurrence with P53 inactivation

A, Schematic of CLDN18 fused with ARHGAP26 for the fusion transcript and predicted fusion protein. PH domain: Pleckstrin homology domain; SH3: SRC homology 3 domain; GAP domain: GTPase activating domain. **B**, Confocal immunofluorescence images of  $\alpha$ -SMA in the indicated organoids. Scale bar = 100 µm. (C) phase contrast and (D) H&E and (E) Alcian Blue for gastric organoids with annotated genotypes with P53 knockout. Scale bar = 100 µm. **F**, *In vitro* proliferation (CellTiter-Glo) of *Mist1Cre* and *CLDN18-ARHGAP26* organoids knockout with *Trp53*. Data are mean ± S.D. \*\*\*\**P*<0.0001, two-way ANOVA. **G**, Immunoblotting of proteins involved in cell-to-cell adhesion and maintaining epithelial integrity in *Mist1Cre* and *CLDN18-ARHGAP26* fusion organoids (representative images from 3 independent experiments). **H**, Quantitative real time PCR for epithelial and mesenchymal genes in *Mist1Cre* and *CLDN18-ARHGAP26* organoids (n=3).



# Supplementary Figure 2. CLDN18-ARHGAP26 fusion promotes the RHOA signaling activity.

**A**, Immunoblotting for the RHOA binding of Rhotekin by Rhotekin-pulldown assay with sg-p53. (representative images from 3 independent experiments). **B**, Immunoblotting for p-cofilin in the organoids with annotated genotype (representative images from 3 independent experiments). **C**, Immunoblotting for the RHOA binding of Rhotekin by Rhotekin-pulldown assay with fusion or fusion missense mutation of corresponding to R293A of ARHGAP26 (n= 3 independent experiments). **D**, Immunoblotting for p-cofilin and t-cofilin in the organoids with annotated genotype (representative images from 3 independent experiments).

#### *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup> Organoids



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#### CLDN18-ARHGAP26 Organoids



Trp53<sup>-/-</sup>Kras<sup>G12D/+</sup> Organoids

C
DMSO
Defactinib-1uM
Defactinib-2uM
Defactinib-4uM

Image: Image

DMSO

Defactinib-2uM

D

Е

F











Supplementary Figure 3. FAK inhibition reversed the abnormal morphologies of CLDN18-ARHGAP26.

**A**, Phase contrast of *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup> Organoids knockdown with *sh*Control or *sh*FAK(PTK2). **B**, Representative images of phase contrast of CLDN18-ARHGAP26 organoids treated with DMSO and indicated dose of defactinib for 48h . **C**, Representative images of phase contrast of *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup> organoids treated with DMSO and indicated dose of defactinib for 48h . **R**epresentative images of (**D**) phase contrast and (**E**) H&E and (**F**) Alcian Blue for CLDN18-ARHGAP26 organoids t with *Trp53* knockout. Scale bar = 100 μm. **G**, Immunoblotting *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup>, *Trp53<sup>-/-</sup>* and *CLDN18-ARHGAP26* fusion organoids upon treatment with DMSO, PF-573228 (2 μM) or Defactinib (2 μM) for 48h (representative images from 3 independent experiments).









# Supplementary Figure 4. FAK inhibition reversed the abnormal morphologies of CLDN18-ARHGAP26.

**A,** Immunoblotting for the organoids with annotated genotype with *Trp53* knockout. (representative image from 3 independent experiments). **B,** Immunoblotting for the *CLDN18-ARHGAP26* with *Trp53* knockout organoids treated with DMSO, PF-573228 (2  $\mu$ M) or Defactinib (2  $\mu$ M) for 48h (representative image from 3 independent experiments). **C,** Immunoblotting for the *CLDN18-ARHGAP26* organoids engineered with GFP or YAP-S94A (n=3 independent experiments). **D,** Representative images of phase contrast of CLDN18-ARHGAP26 organoids engineered with GFP or YAP-S94A. Scale bar = 100  $\mu$ m. **E**, Representative images of phase contrast of *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup> organoids engineered with GFP or YAP-S94A. Scale bar = 100  $\mu$ m. **E**, Representative images of phase contrast of *Trp53<sup>-/-</sup>Kras*<sup>G12D/+</sup> organoids engineered with GFP or YAP-S94A. Scale bar = 100  $\mu$ m. **G,** Immunoblotting for the organoids with annotated genotype. Scale bar = 100  $\mu$ m. **G,** Immunoblotting for different cancer pathways upon FAK inhibition in *CLDN18-ARHGAP26* organoids (n=3 independent experiments). **H,** Quantitative real time PCR for YAP target genes upon FAK inhibition in *CLDN18-ARHGAP26* organoids (n=3).



#### Supplementary Figure 5. Synergistic effect of FAK and TEAD inhibition

**A**, Representative images of phase contrast of *CLDN18-ARHGAP26* with *Trp53* knockout organoids treated with DMSO control, TEAD inhibitor VT103 (2  $\mu$ M), Defactinib (1  $\mu$ M) or the combination for 48h. Scale bar = 100  $\mu$ m. **B**, Weigh loss curve for *CLDN18-ARHGAP26* with *Trp53* knockout organoids xenograft tumors (n=8-10) treated with vehicle control, defactinib (50 mg/kg, QD), VT103 (10 mg/kg, QD) or the combination. **C**, Representative p-FAK IHC of xenografts treated with Defactinib, VT103, Combo or DMSO. Scale bar = 100  $\mu$ m . **D**, IHC quantification for pFAK staining in FAKi (Defactinib), VT103, Combo or DMSO xenograft samples (n=4-5).