

Figure S1

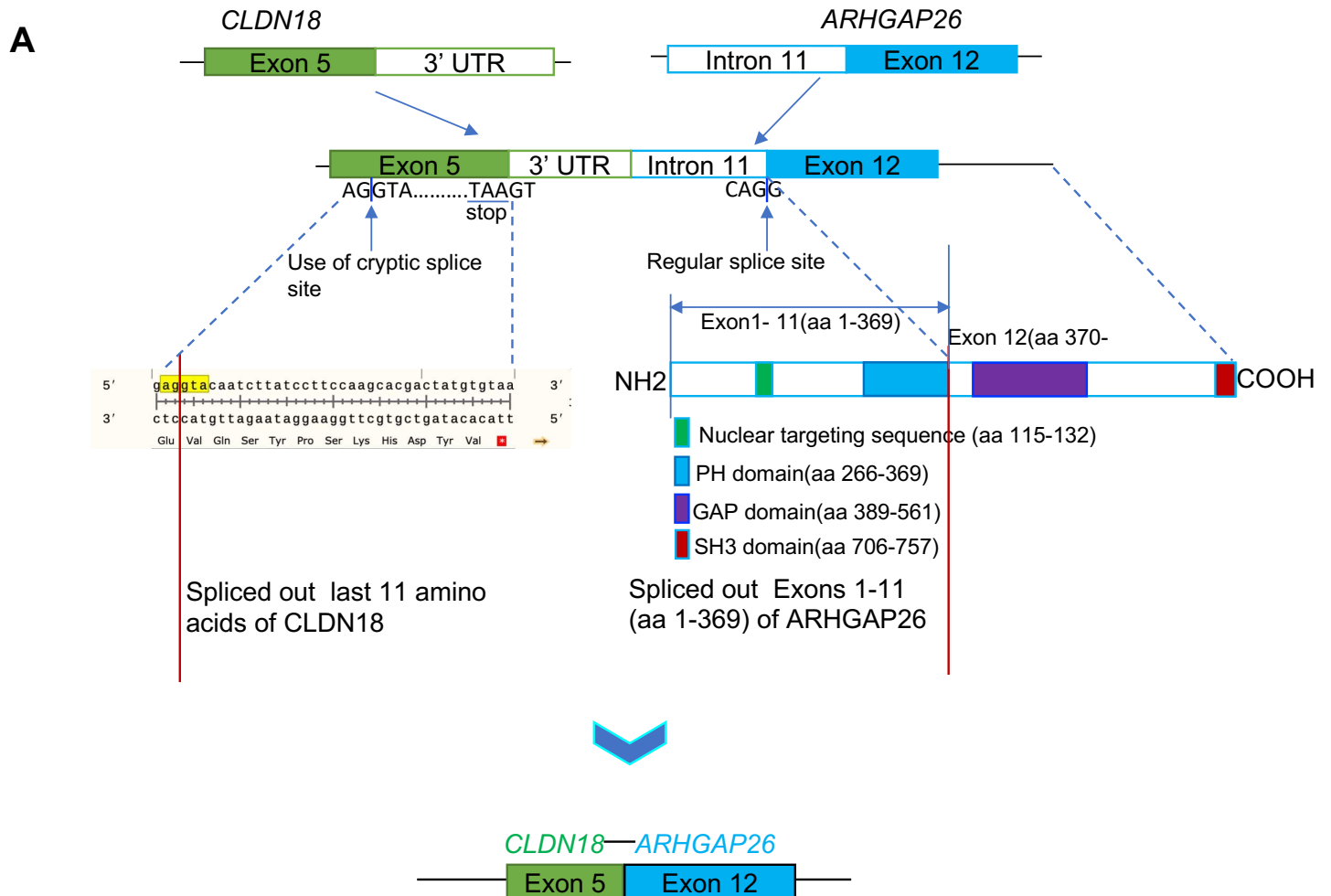
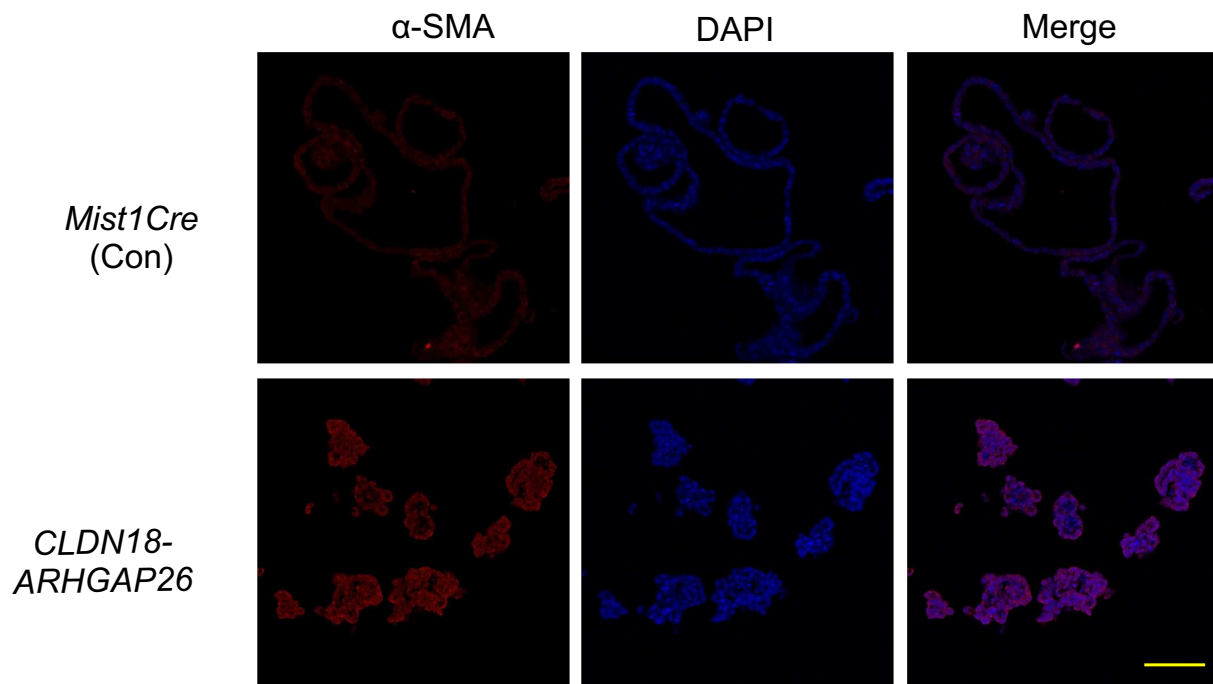
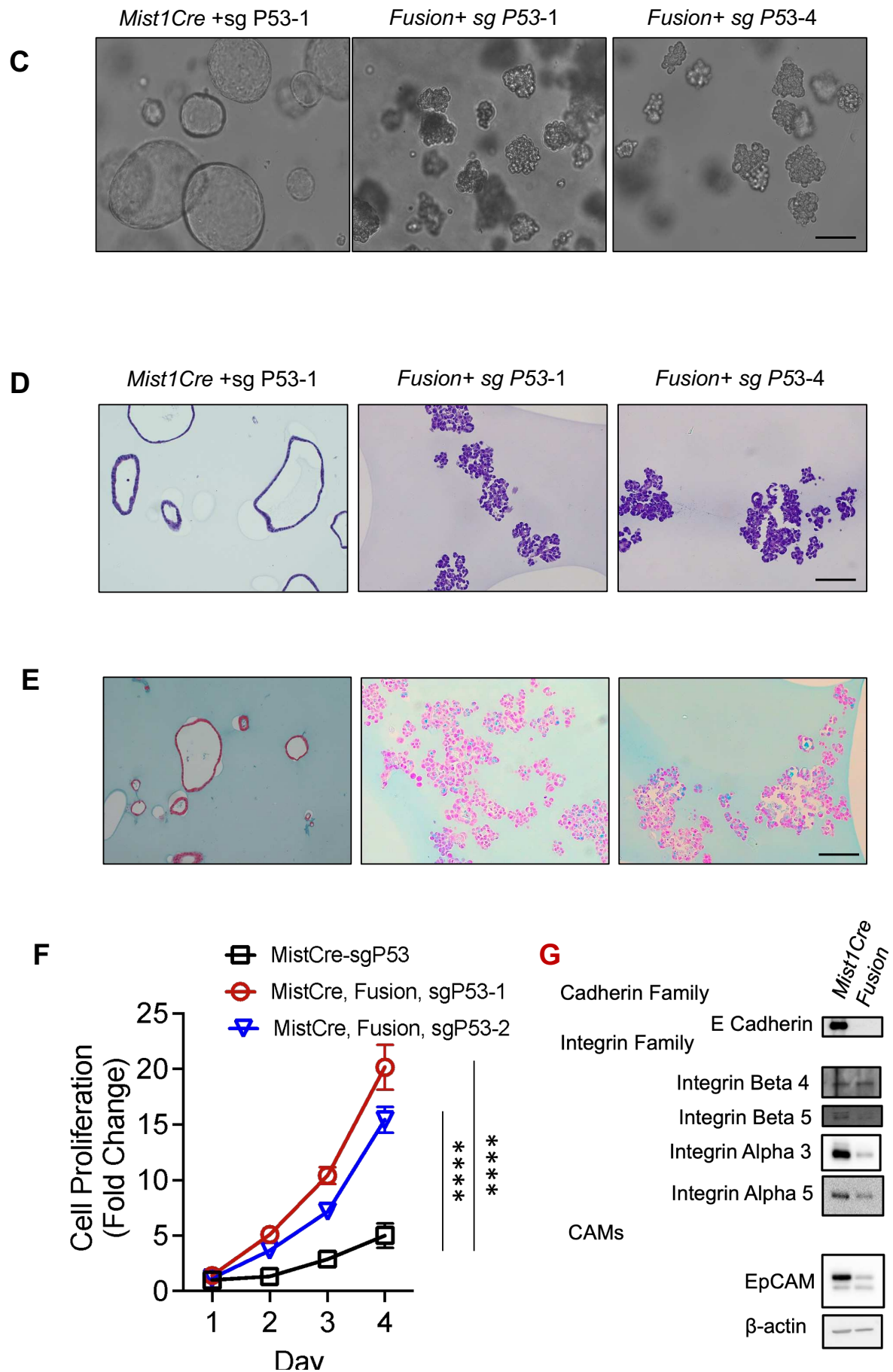
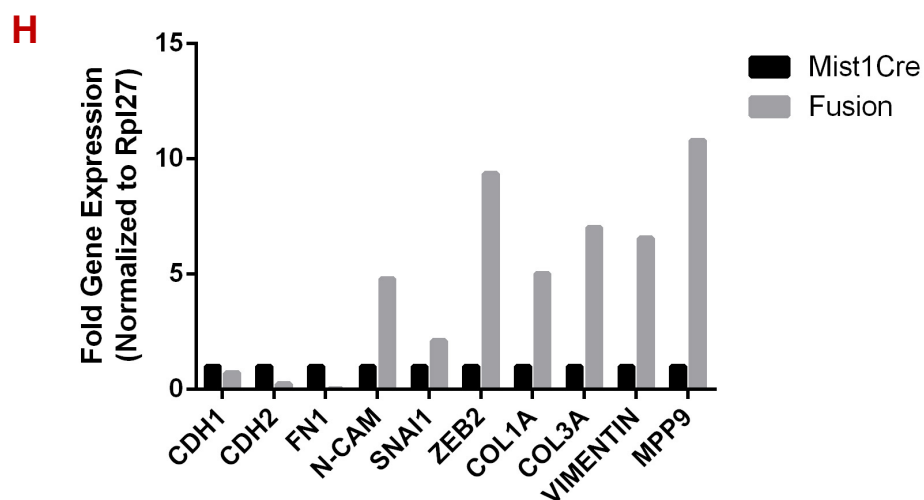
**B**

Figure S1

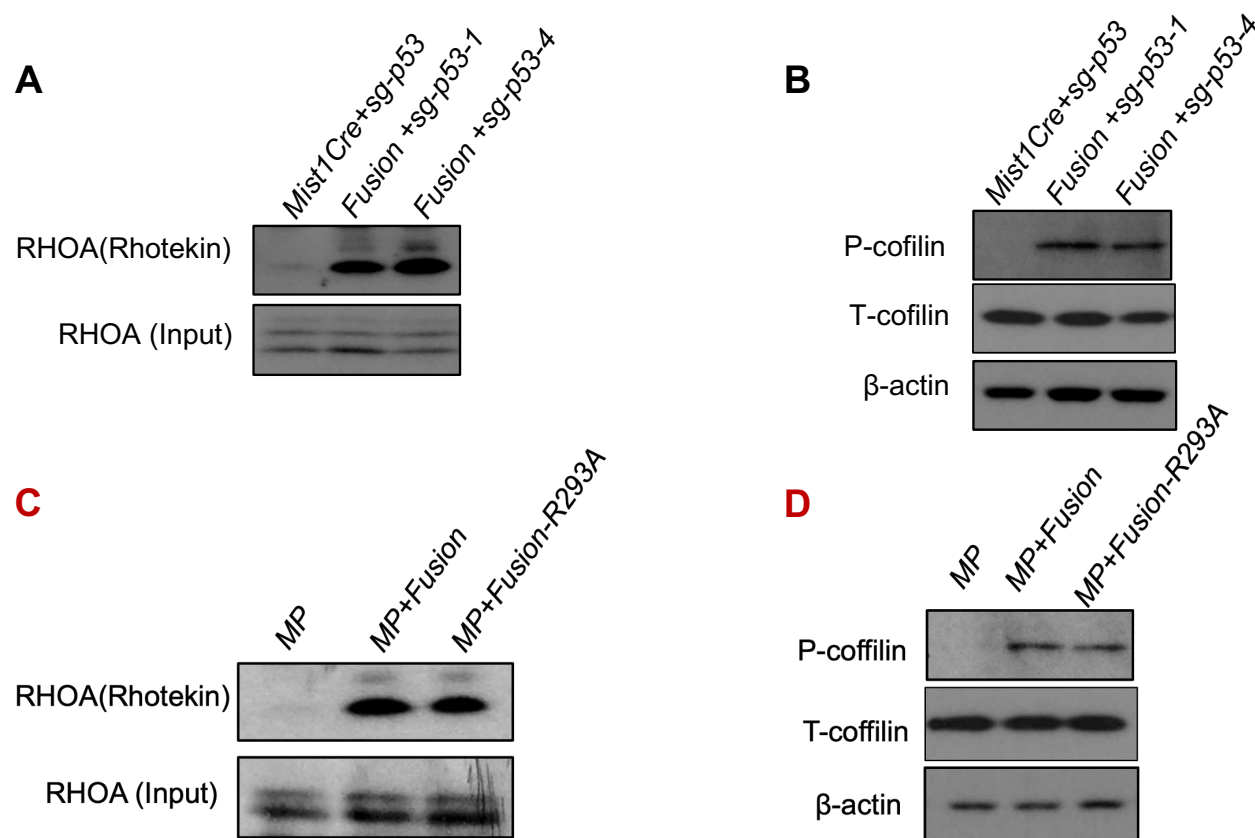




Supplementary Figure 1. CLDN18-ARHGAP26 fusion induces abnormal organoid morphologies and co-occurrence with P53 inactivation

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 α -SMA in the indicated organoids. Scale bar = 100 μ m. **(C)** phase contrast and **(D)** H&E and **(E)** Alcian Blue for gastric organoids with annotated genotypes with P53 knockout. Scale bar = 100 μ m. **F**, *In vitro* proliferation (CellTiter-Glo) of *Mist1Cre* and *CLDN18-ARHGAP26* organoids knockout with *Trp53*. Data are mean \pm 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).

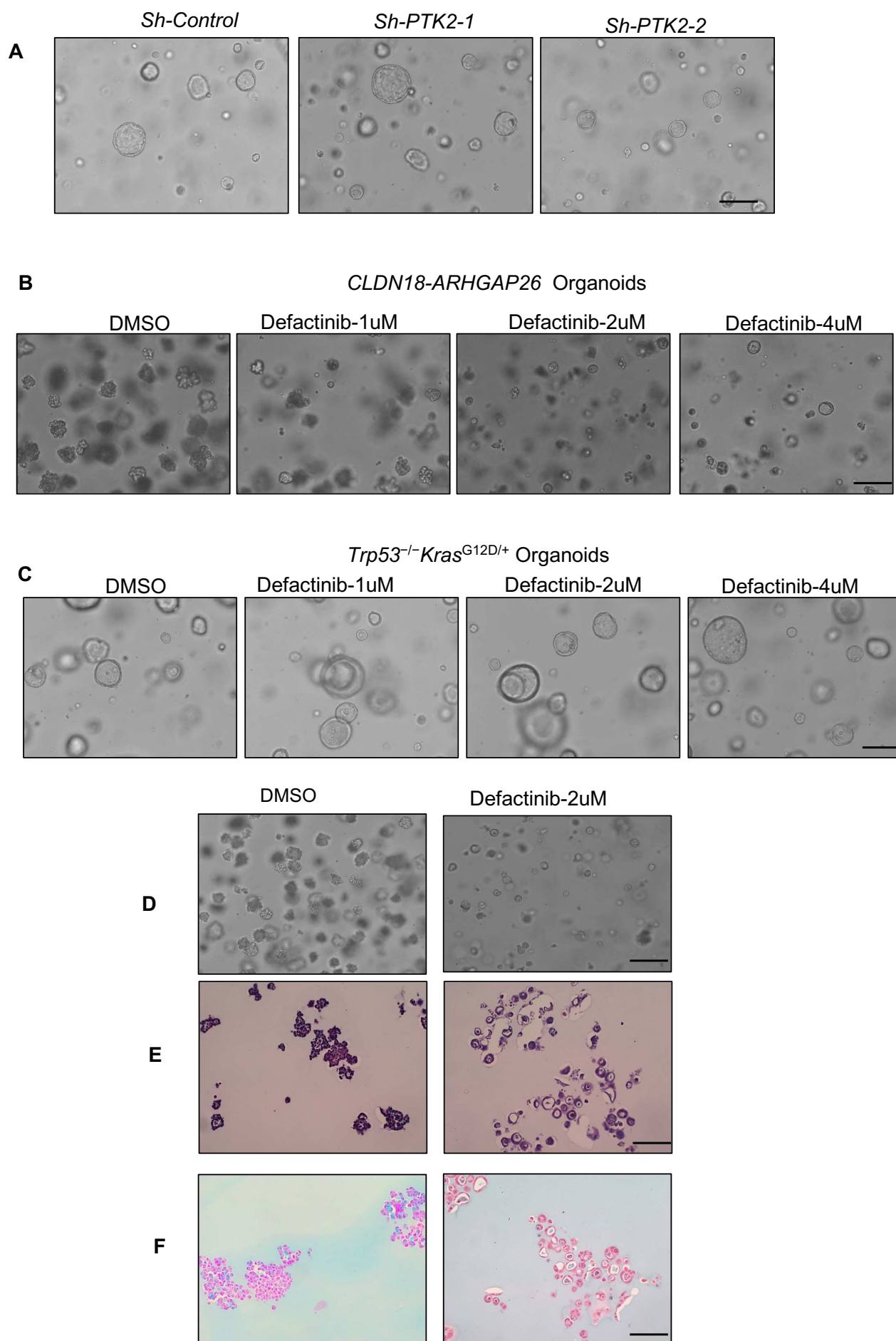
Figure S2

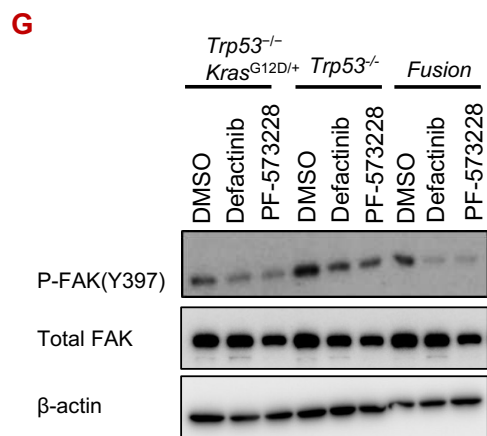


Supplementary Figure 2. CLDN18-ARHGAP26 fusion promotes the RHOA signaling activity.

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).

Figure S3

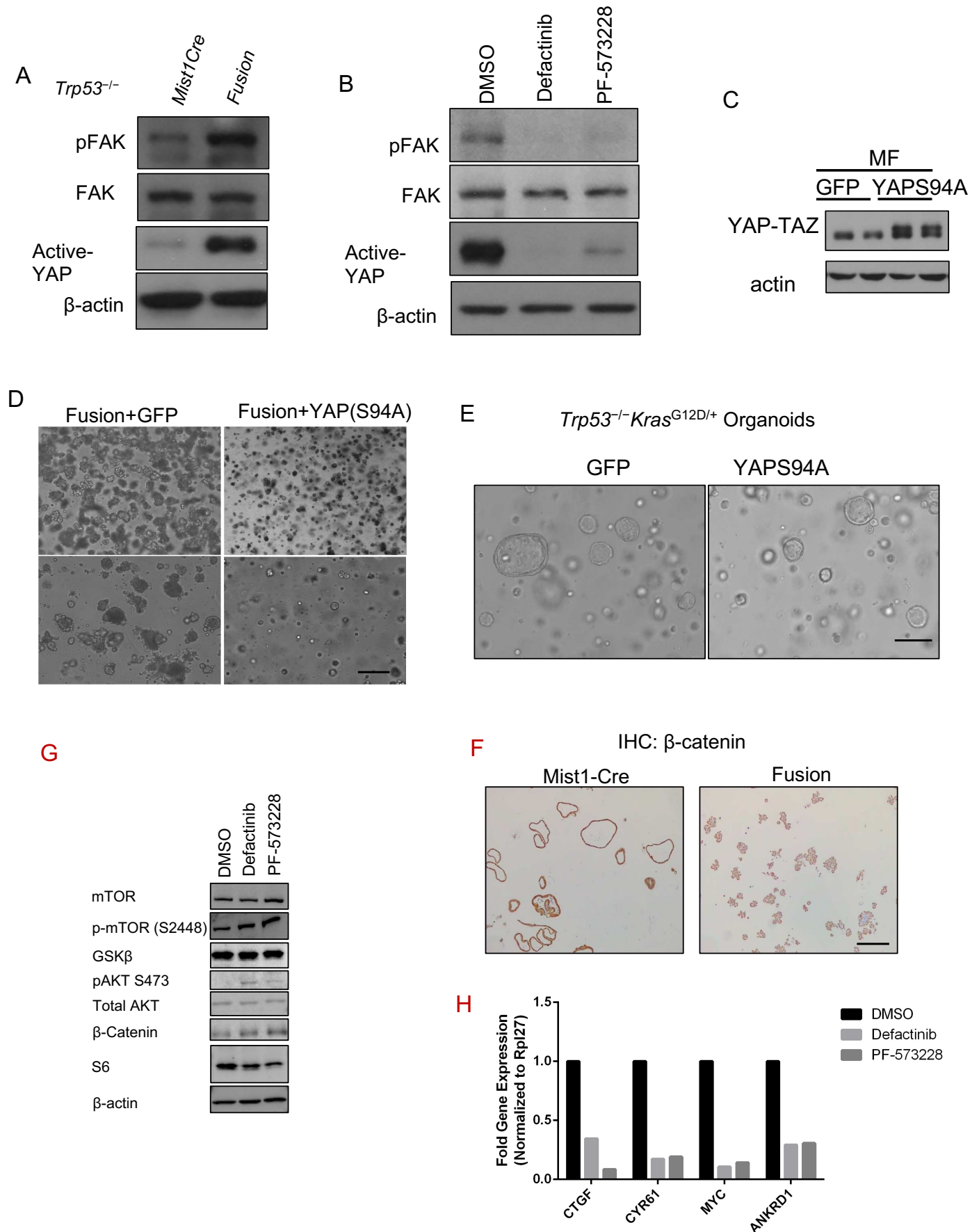
Trp53^{-/-}*Kras*^{G12D/+} Organoids



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

A, Phase contrast of *Trp53^{-/-}Kras^{G12D/+}* Organoids knockdown with *shControl* or *shFAK(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^{-/-}Kras^{G12D/+}* organoids treated with DMSO and indicated dose of defactinib for 48h. Representative 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^{-/-}Kras^{G12D/+}*, *Trp53^{-/-}* 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).

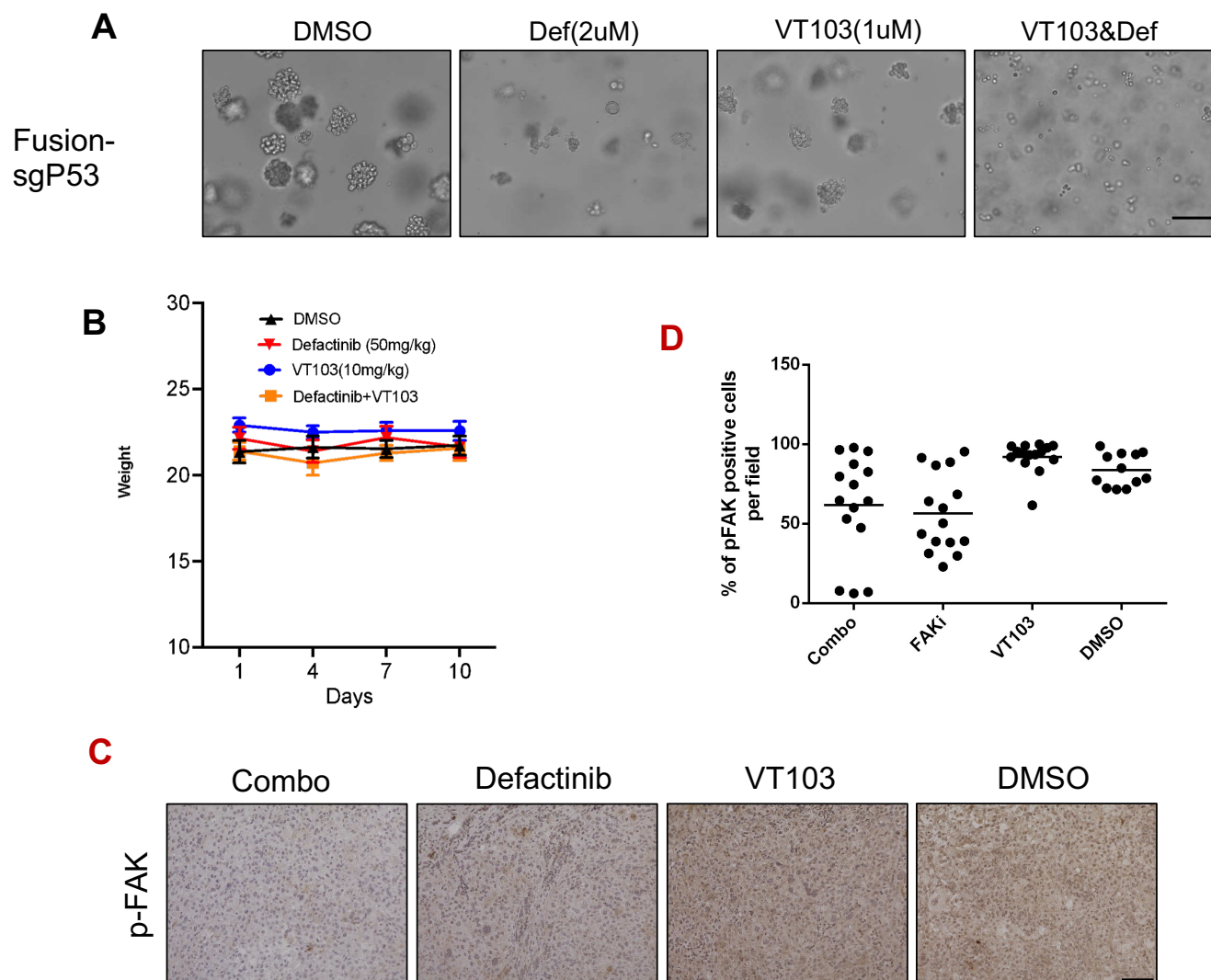
Figure S4



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 μ M) or Defactinib (2 μ 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 μ m. **E**, Representative images of phase contrast of *Trp53*^{-/-}*Kras*^{G12D/+} organoids engineered with GFP or YAP-S94A. Scale bar = 100 μ m. **F**, Representative images of β -catenin staining for the organoids with annotated genotype. Scale bar = 100 μ 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).

Figure S5



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 µM), Defactinib (1 µM) or the combination for 48h. Scale bar = 100 µm. **B**, Weight 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 µm. **D**, IHC quantification for pFAK staining in FAKi (Defactinib), VT103, Combo or DMSO xenograft samples (n=4-5).