Supplementary Figure 1. Selection of Cas9 expressing organoids. *Trp53-gRNA* was transduced into 4 different AK-Cas9 clones and selected by Nutlin3. Clone 2 and Clone 3 produced several resistant organoids, showing that efficient introduction of *Trp53* mutations were achieved by Cas9 in these clones.

Supplementary Figure 2. FACS analysis of AK-Cas9-C2 organoids and AK-Cas9-C2 organoids transduced with Pool 1-2 gRNAs. GFP positive AK-Cas9-C2 cells are shown in the left panel. Since the gRNA vector carries a BFP expression cassette, AK-Cas9-C2 + gRNA (Pool 1-2) cells are both positive for BFP and GFP. The gRNA transduced organoids were analyzed 2 weeks after puromycin selection by flow cytometry.

Supplementary Figure 3. Macroscopic appearance of Pool 1 AK-Cas9-gRNA organoid-derived tumors from spleen and cecum transplantation. Mice transplanted with Pool 1 AK-Cas9-gRNA organoids into the spleen developed primary splenic tumors (A) in addition to metastatic liver tumors (B). Tumors are GFP positive (right panels of (A) and (B)), showing that they were derived from AK organoids. (C) Mice transplanted with Pool 1 AK-Cas9-gRNA organoids into the cecum developed metastatic liver tumors, which were positive for GFP (C, right panel). Bars; 1 mm for (A) and (B), 5 mm for (C).

Supplementary Figure 4. Parental AK organoids were not metastatic in the orthotopic transplantation model. (A) HE staining of a primary cecum tumor produced in the orthotopic transplantation model. (B) Note that the tumor did not metastasize to the liver. Bars; 1 mm for (A) and (B), 5 mm for (C).

Supplementary Figure 5. Frequencies of over-represented gRNAs in metastatic liver tumors generated in the cecum transplantation model. A mouse transplanted with AK-Cas9-C2 organoids transduced with a pool of gRNAs (Pool1 and Pool2) developed a primary cecum tumor in addition to

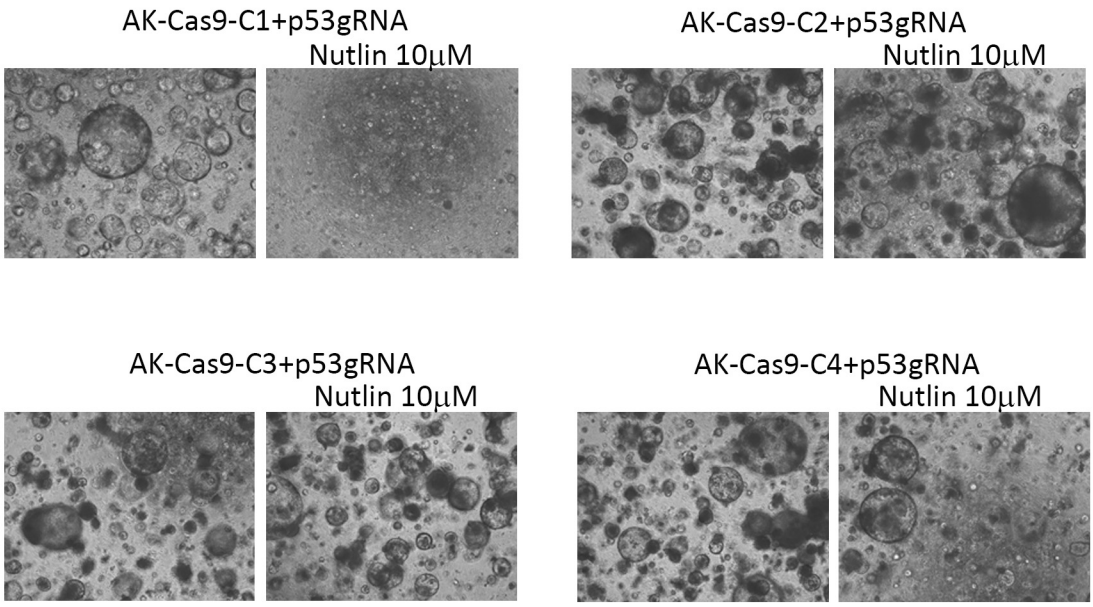
metastatic liver tumors. The over-represented gRNAs in the metastatic liver tumors were composed of gRNAs for *Pten, Trp53, Zfp292* and *Spen.*

Supplementary Figure 6. Selection of high Cas9-expressing organoid clones by qPCR. (A) Comparison of Cas9 mRNA expression in different colon-AK-Cas9 organoid clones. Small intestinal AK-Cas9-C2 organoids were used for a standard in all experiments. Colon-AK-Cas9-C7 was selected for subsequent experiments since AK-Cas9-C4 organoids showed slow cell proliferation. (B) Comparison of Cas9 mRNA expression in different small intestinal AKT-Cas9 organoid clones. AKT-Cas9-C4 was selected for subsequent experiments. (C) Quantification of Cas9 mRNA expression in a colon-AKT-Cas9 organoid clone. (D) Comparison of Cas9 mRNA expression in different human CRC organoid-Cas9 clones. Clone 4 was selected for subsequent experiments.

Supplementary Figure 7. TGF-β superfamily signaling pathways.
Receptors for TGF-β, activin and BMP are composed of type I and type II receptors. Smad2/3 are downstream molecules of TGF-β and activin signaling, whereas Smad1/5/8 are downstream molecules of BMP signaling.

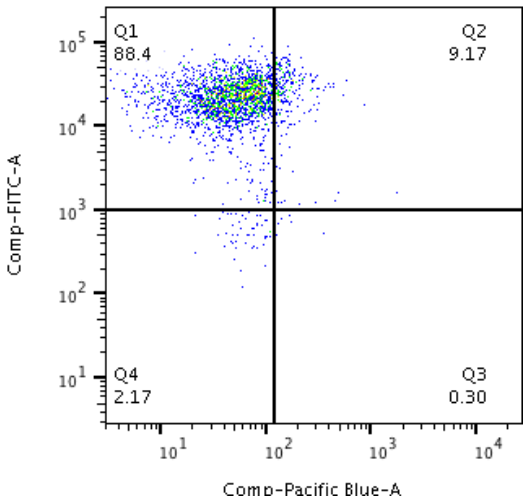
Supplementary Figure 8. Loss of function of *Acvr1b* in colonic AKT organoids enhanced tumor formation.
The graph shows the size distribution of subcutaneous tumors derived from colonic AKT-Cas9 non-T gRNA, AKT-Cas9 Acvr2a gRNA and AKT-Cas9 Acvr1b gRNA organoids. *P<0.05 by t-test.

Supplementary Figure 1

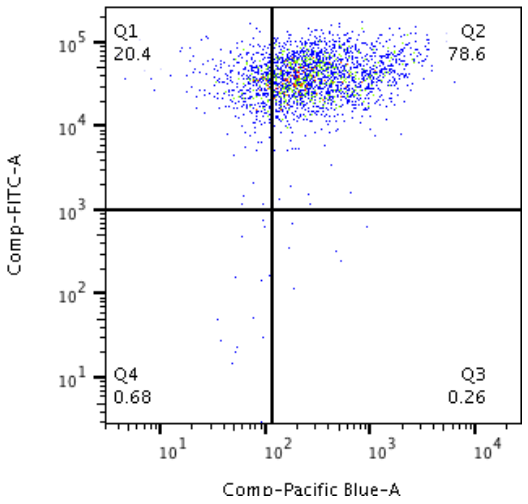


Supplementary Figure 2

AK-Cas9

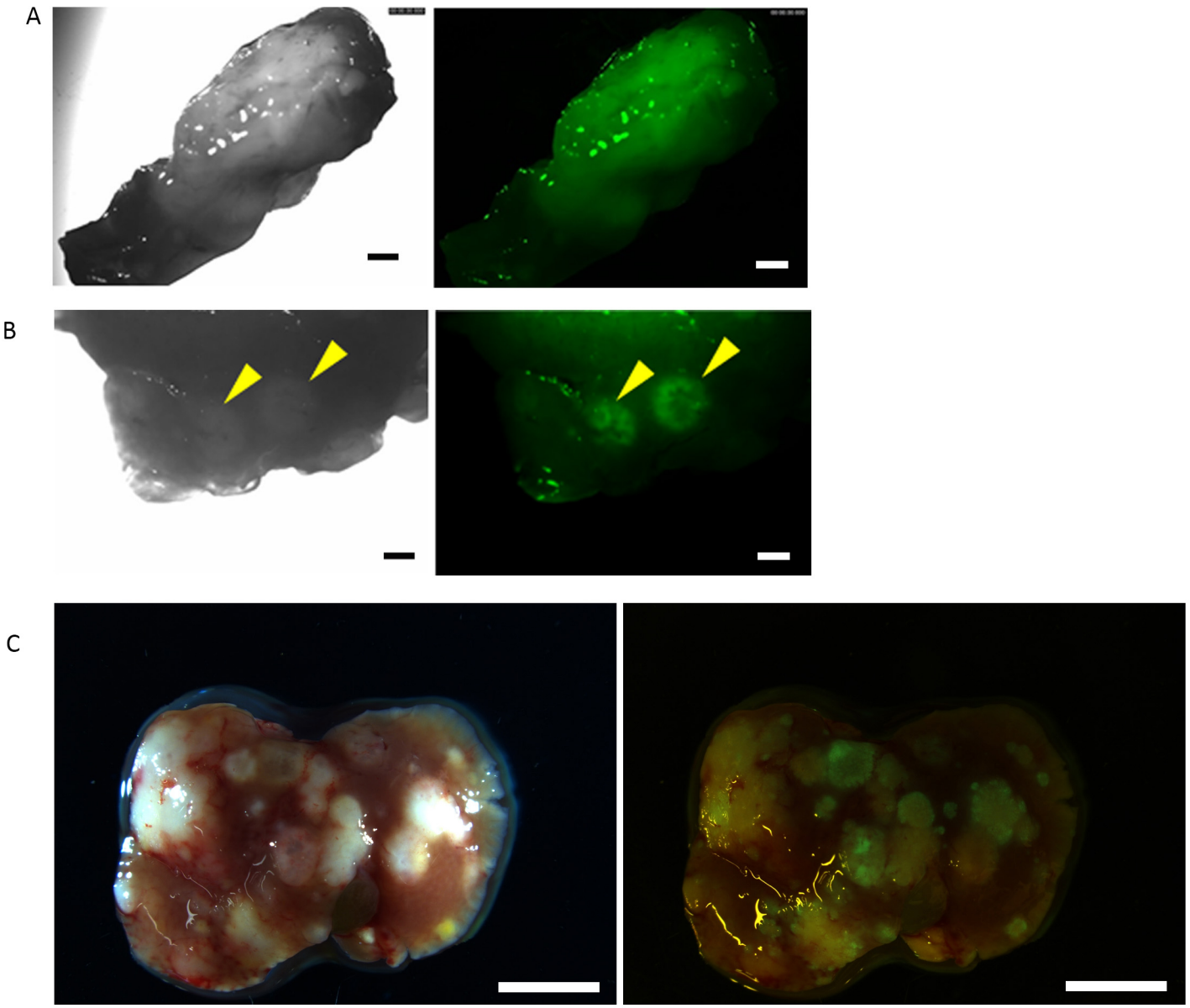


AK-Cas9 + gRNA (Pool 1-2)

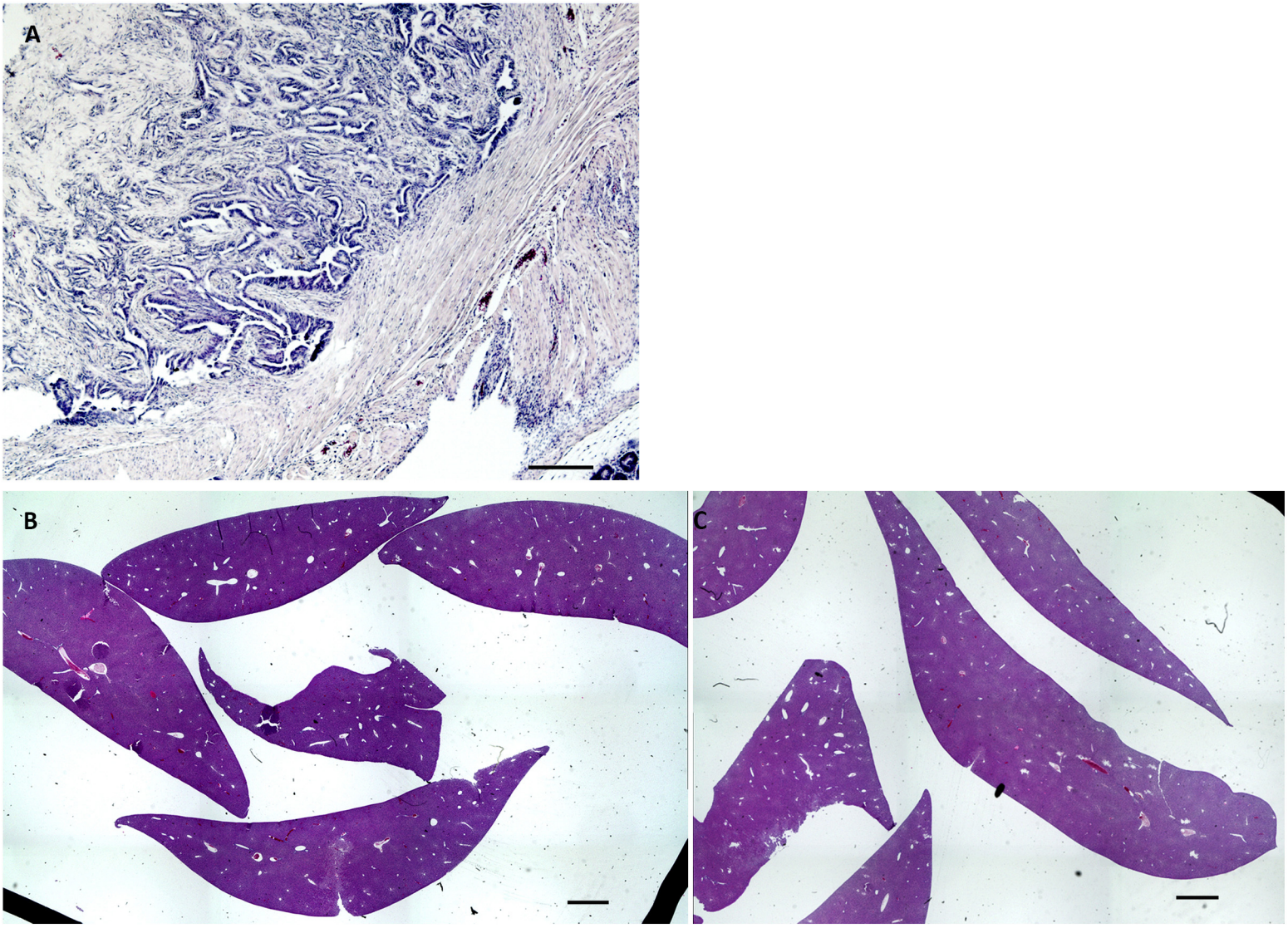


GFP: Cas9
BFP: gRNA

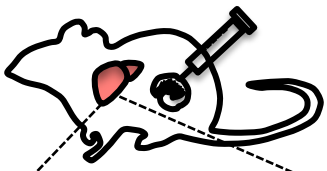
Supplementary Figure 3



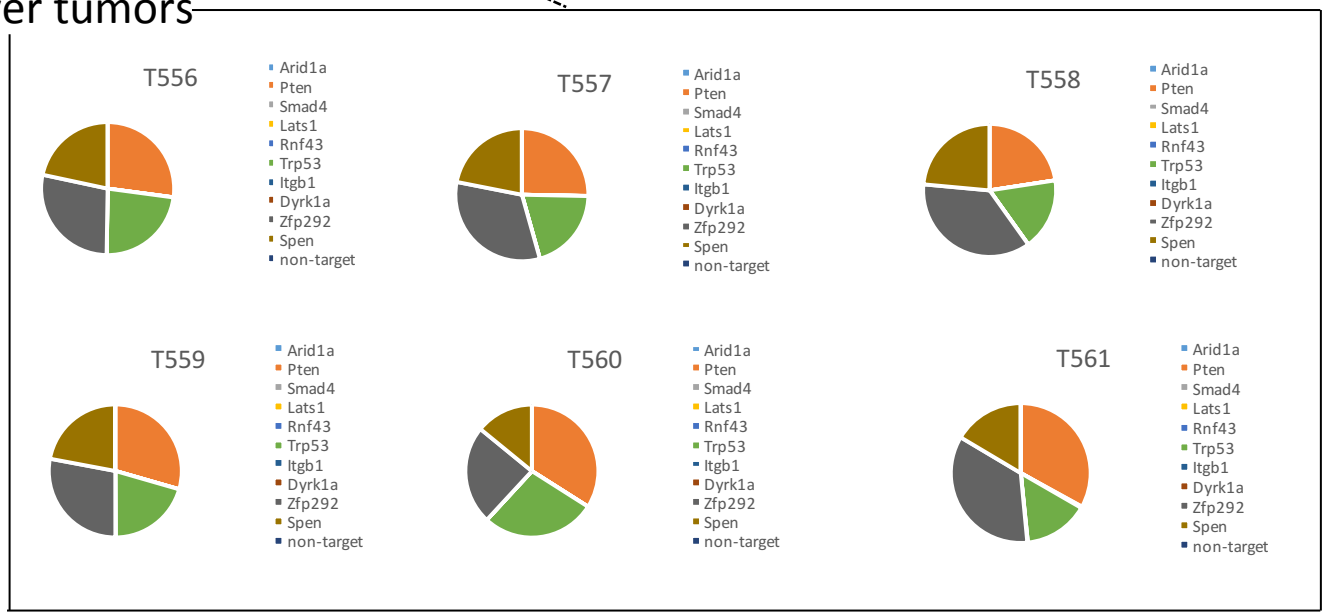
Supplementary Figure 4



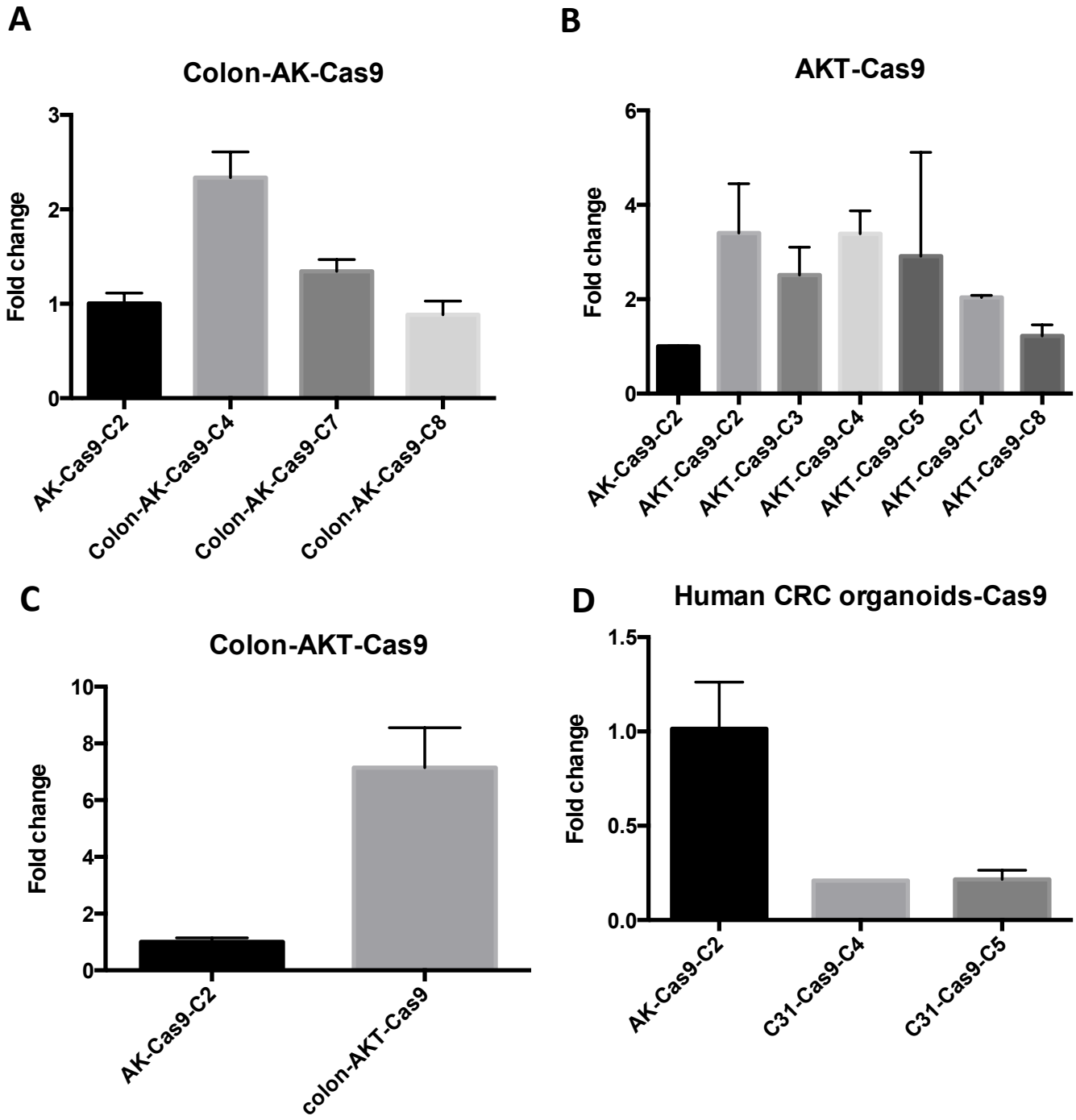
Supplementary Figure 5



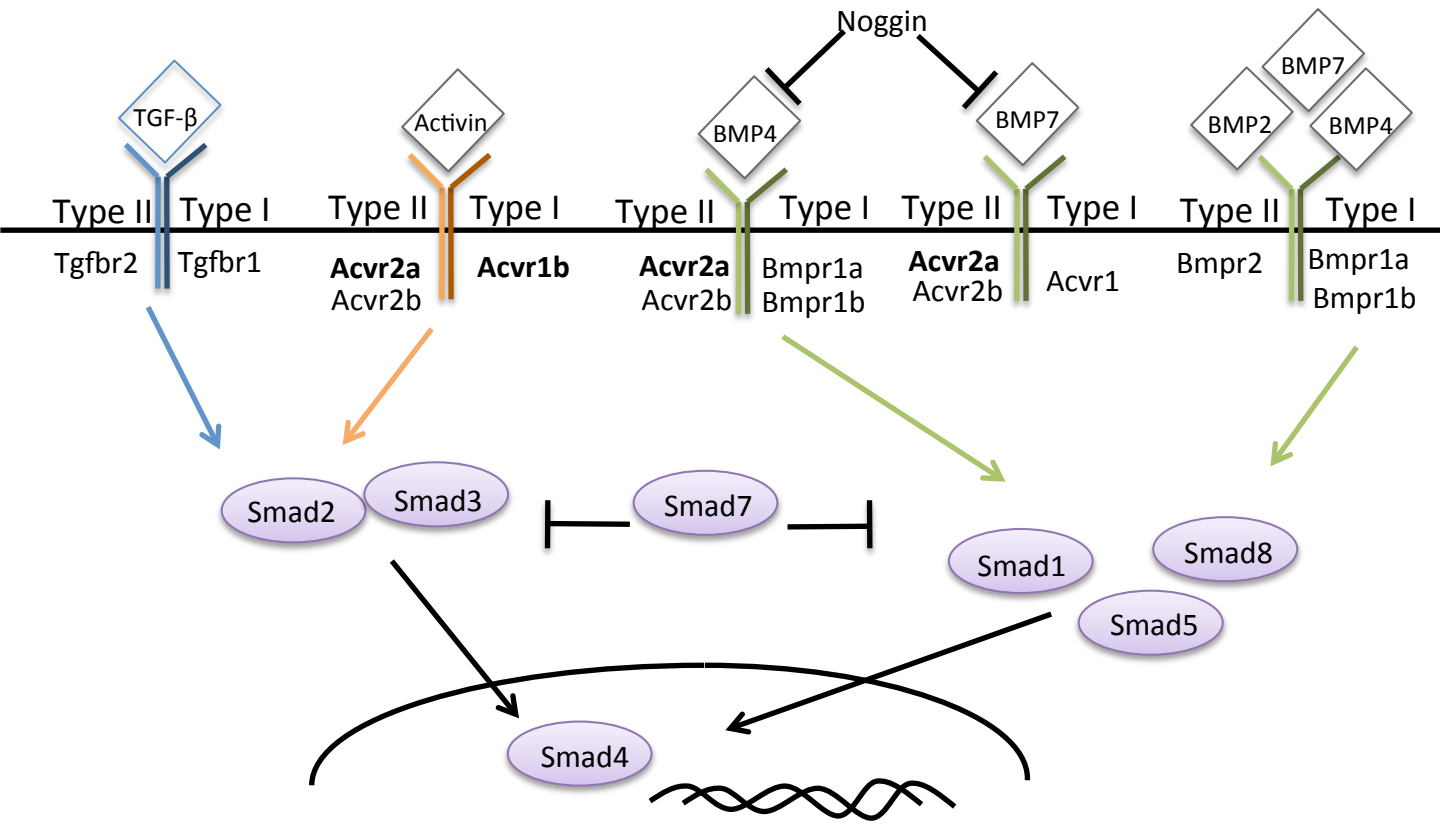
Liver tumors



Supplementary Figure 6



Supplementary Figure 7



Supplementary Figure 8