

drug  
resistance

**STAT2**

inflammation  
no inflammation

**STAT3**

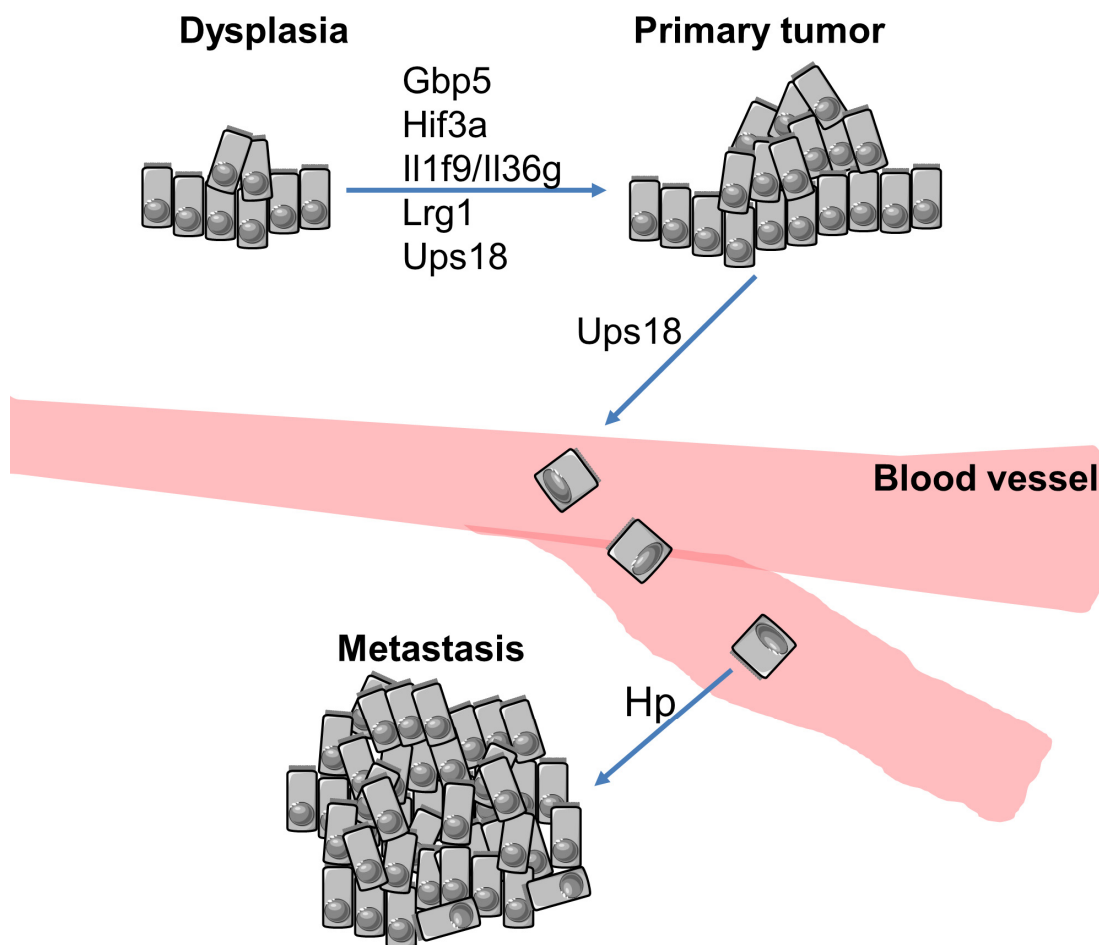
**CRC**

### Therapy failure

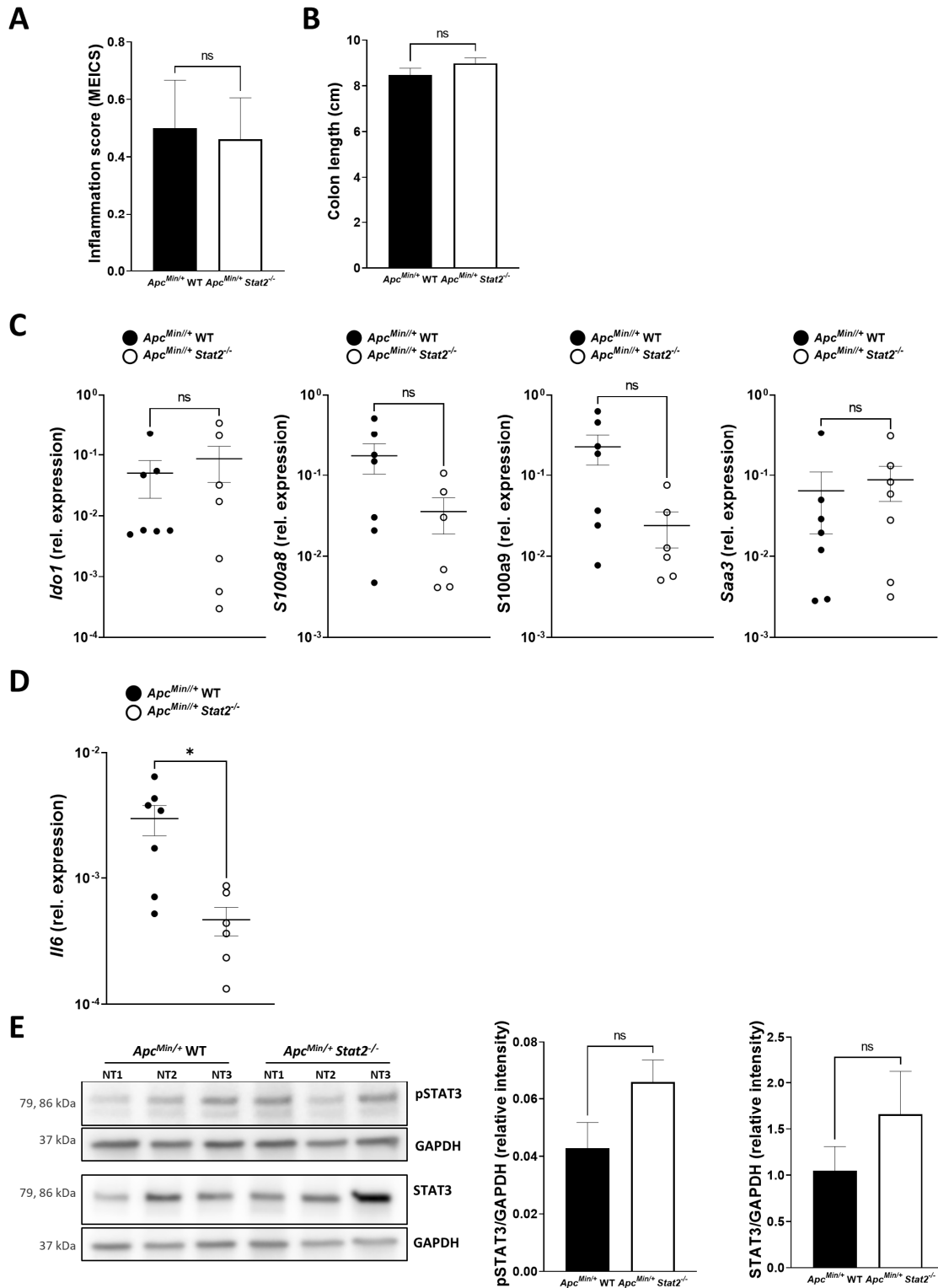
Cd14 (Chen & Wang 2020)  
Cdc20 (Wu et al. 2013)  
Mmp8 (Sirnio et al. 2018)  
Mmp10 (Klupp et al. 2016)

### Cancer progression & metastasis

Gbp5 (Friedman et al. 2016)  
Hif3a (Xue et al. 2016)  
Hp (Marino-Crespo et al. 2019)  
Il1f9/Il36g (Yang et al. 2022)  
Lrg1 (Zhou et al. 2017)  
Ups18 (Zhang et al. 2021)

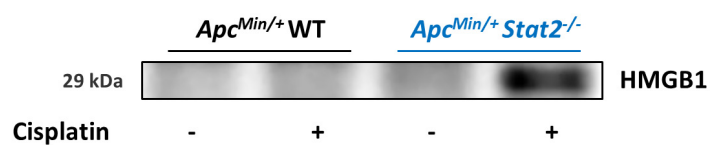


Supplementary Figure S1. Schematic illustration of the possible mechanisms by which STAT2 could control CRC.

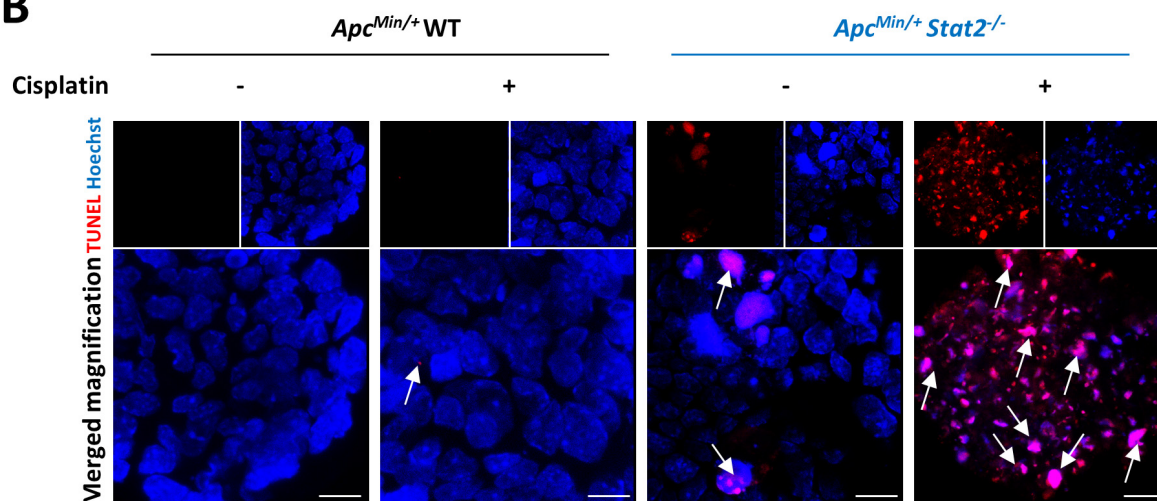


**Supplementary Figure S2. Inflammation-independent role of STAT2 in the *Apc<sup>Min/+</sup>* model *in vivo* and *ex vivo*.** Inflammation in WT and *Stat2*-deficient mice on the *Apc<sup>Min/+</sup>* background was investigated by endoscopy (MEICS score in A) and the length of colons (B). (C) Expression levels of typical inflammation markers in the *Apc<sup>Min/+</sup>* model of CRC were determined by quantitative polymerase chain reaction. (D) The expression levels of the specific NF- $\kappa$ B target gene *Il6* in *Apc<sup>Min/+</sup>* WT and *Apc<sup>Min/+</sup> Stat2<sup>-/-</sup>* mice were investigated by quantitative polymerase chain reaction. (E) Western blot analysis of pSTAT3 and STAT3 levels in non-tumor tissue samples from *Apc<sup>Min/+</sup>* WT and *Apc<sup>Min/+</sup> Stat2<sup>-/-</sup>* mice. GAPDH band intensity was used as a loading control. Quantification of the normalized values. Abbreviation: ns, not significant, NT, non-tumor. Statistics: Welch's *t*-test in A, B, and E and Mann-Whitney in C and in D; mean with SEM is displayed.

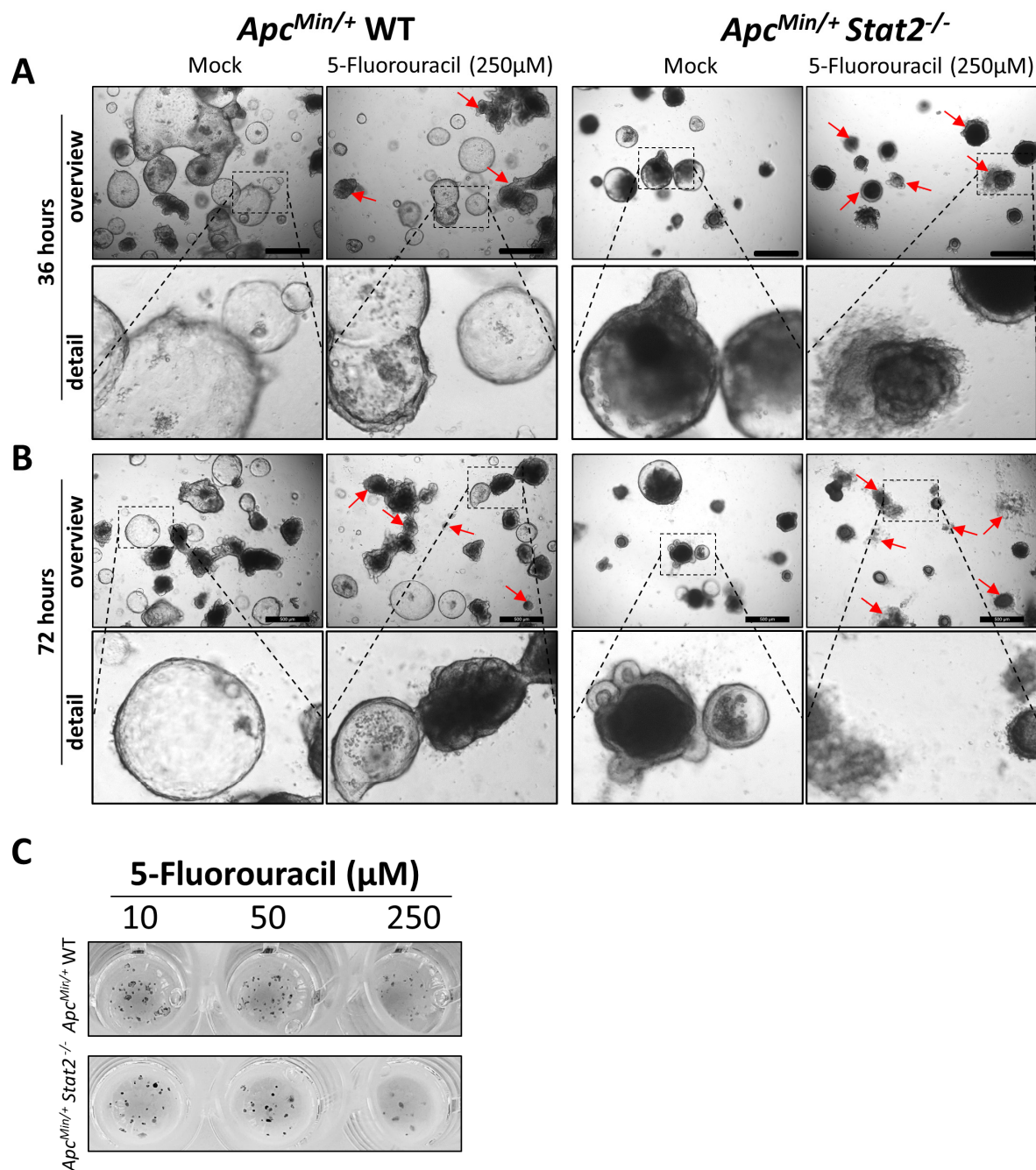
**A**



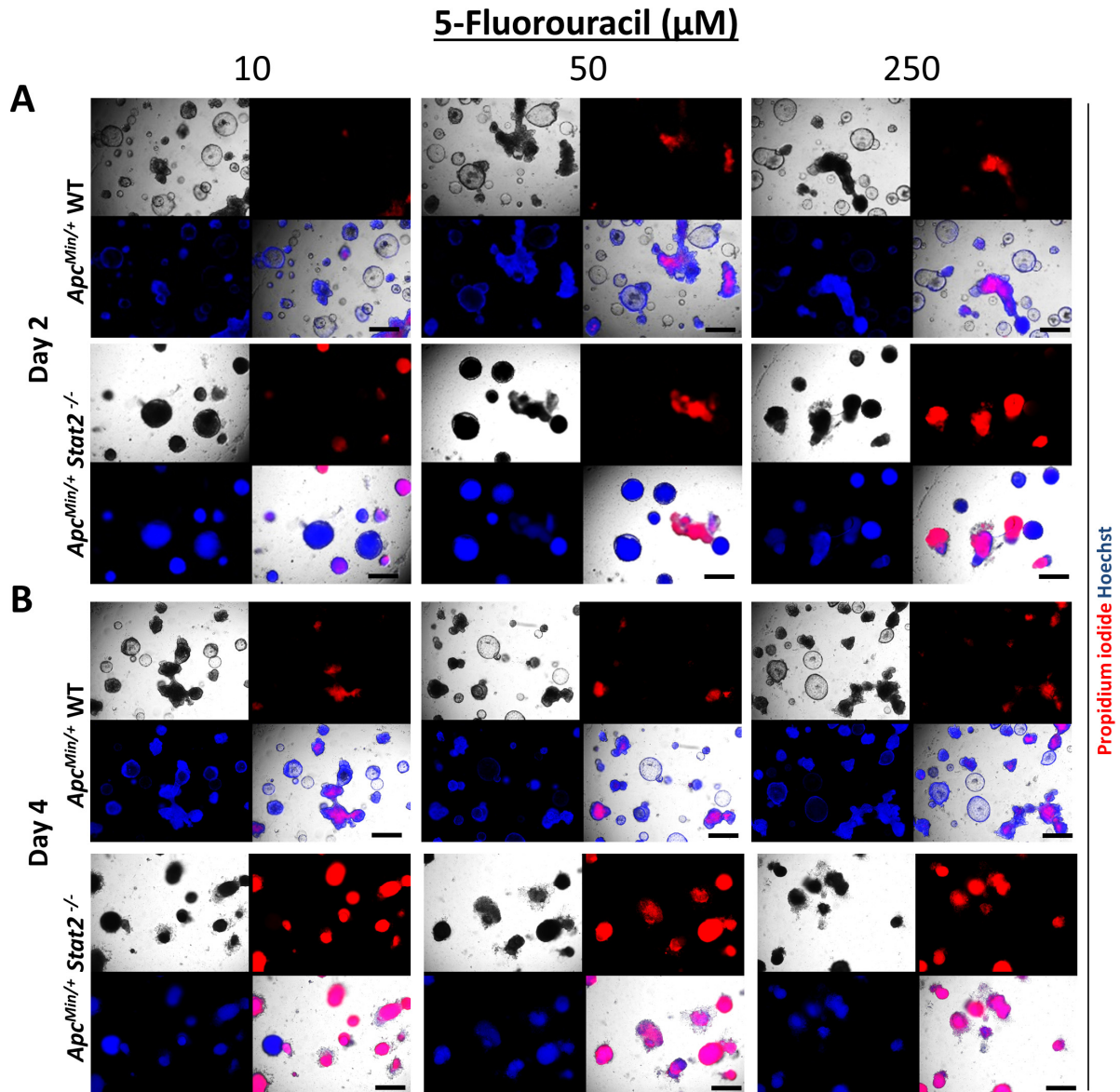
**B**



**Supplementary Figure S3. Effects of Cisplatin on the DNA damage in *Apc<sup>Min/+</sup>* tumoroids.** (A) The levels of extracellularly released HMGB1 were measured by western blot in supernatants of tumoroids cultured in the presence of Cisplatin. Wells receiving only vehicle served as controls. (B) Confocal images of DNA fragmentation evaluated by the TUNEL assay in tumoroids from the Cisplatin stimulation experiments; positive cells are indicated by arrows. Hoechst was used to counterstain nuclei. Scale bars, 10 μm.



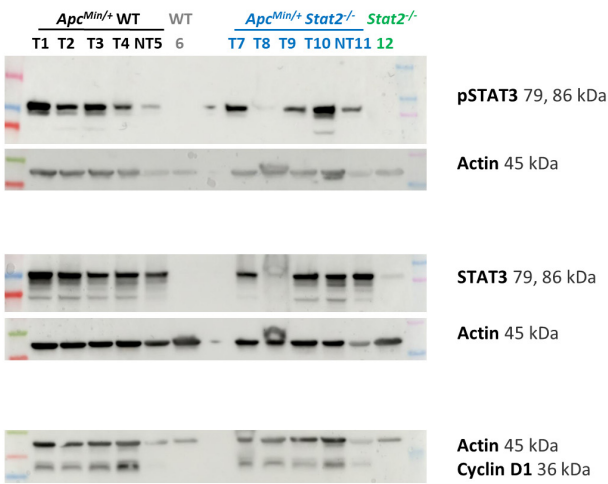
**Supplementary Figure S4. 5-Fluorouracil induces cell death in *Apc*<sup>Min/+</sup> tumoroids in a dose- and time-dependent manner.** (A) Representative light microscopy images from an experiment in which the effect of the highest dose of 5-Fluorouracil used in our study, namely 250μM, was comparatively followed in tumoroids derived from *Apc*<sup>Min/+</sup> WT and *Apc*<sup>Min/+</sup> *Stat2*<sup>-/-</sup> mice after (A) 36 hours and (B) 72 hours. Red arrows in A and B indicate highly differentiated/dead tumoroids. Scale bars, 500μm. (C) Macroscopic images indicating the presence of formazan precipitates as black spots of different sizes in the MTT-formazan assay. The concentrations of the anti-cancer drug used in the assay are indicated above the images of the wells.



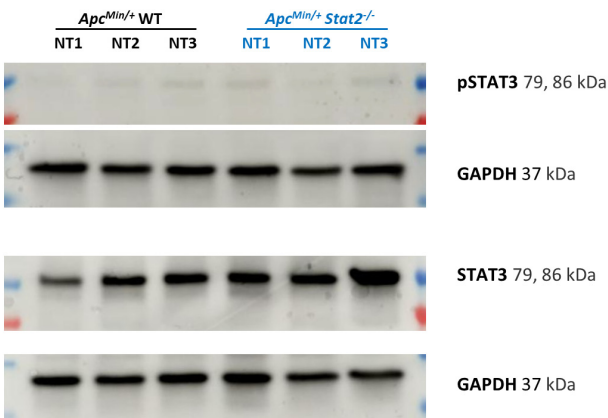
Supplementary Figure S5. *Apc<sup>Min/+</sup>* tumoroids lacking STAT2 have a higher susceptibility to the induction of cell death by 5-Fluorouracil as compared with *Apc<sup>Min/+</sup>* WT tumoroids. (A, B) Induction of cell death by three different doses of 5-Fluorouracil was evaluated by fluorescence microscopy after co-staining of *Apc<sup>Min/+</sup> Stat2<sup>-/-</sup>* and *Apc<sup>Min/+</sup>* WT tumoroids with propidium iodide (red staining - dead cells) and Hoechst (blue staining - all cells) at an early (two days) and a late (four days) time point. Scale bars, 500 $\mu\text{m}$ .

# Source of western blots used in:

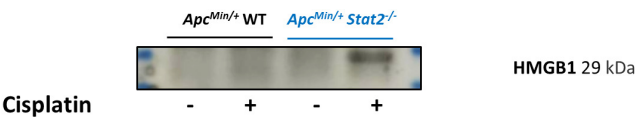
## Figure 2E



## Suppl. Figure 2E



## Suppl. Figure 3A



Supplementary Figure S6. Source of western blots.