Expanded View Figures

Figure EV1. *Huwe1* is an intestinal tumour suppressor gene commonly mutated in human CRC.

A cBioportal OncoPrints of *HUWE1* mutation rate in CRC identified during sequencing projects.

B cBioportal OncoPrints of *HUWE1* mutation rates in a range of human cancers.

C UbcH7 pulldown of HUWE1 HECT domain containing point mutations identified in colorectal cancer sequencing projects.

D Quantification of total tumour numbers per colon in sacrificed *Vil Apc*, *Vil Apc Huwe1*^{het} and *Vil Apc Huwe1*^{hom} mice. Deletion of *Huwe1* led to a significant increase in the number of tumours per colon (Mann–Whitney, $n \geq 3$). Mean and standard deviation are plotted.

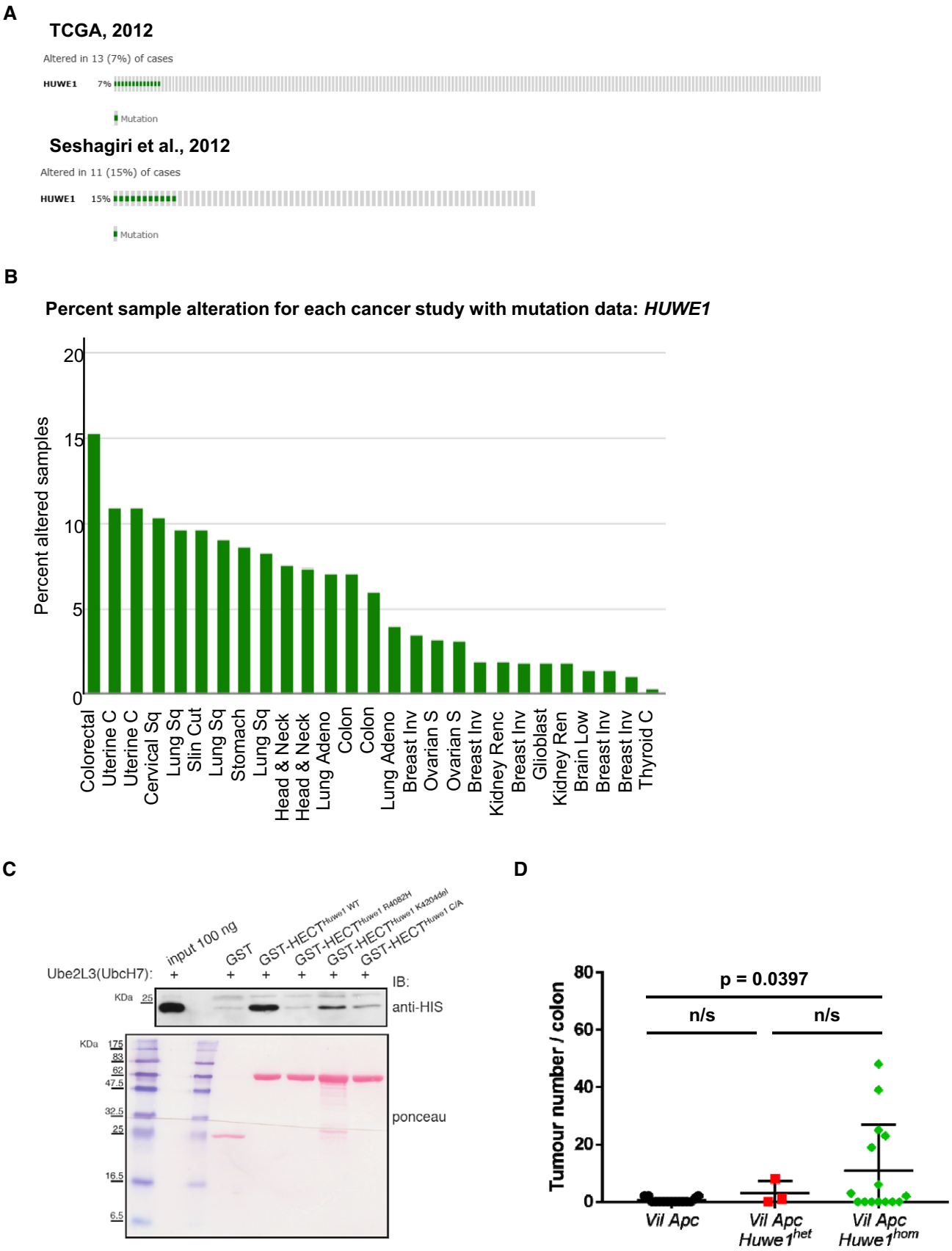


Figure EV1.

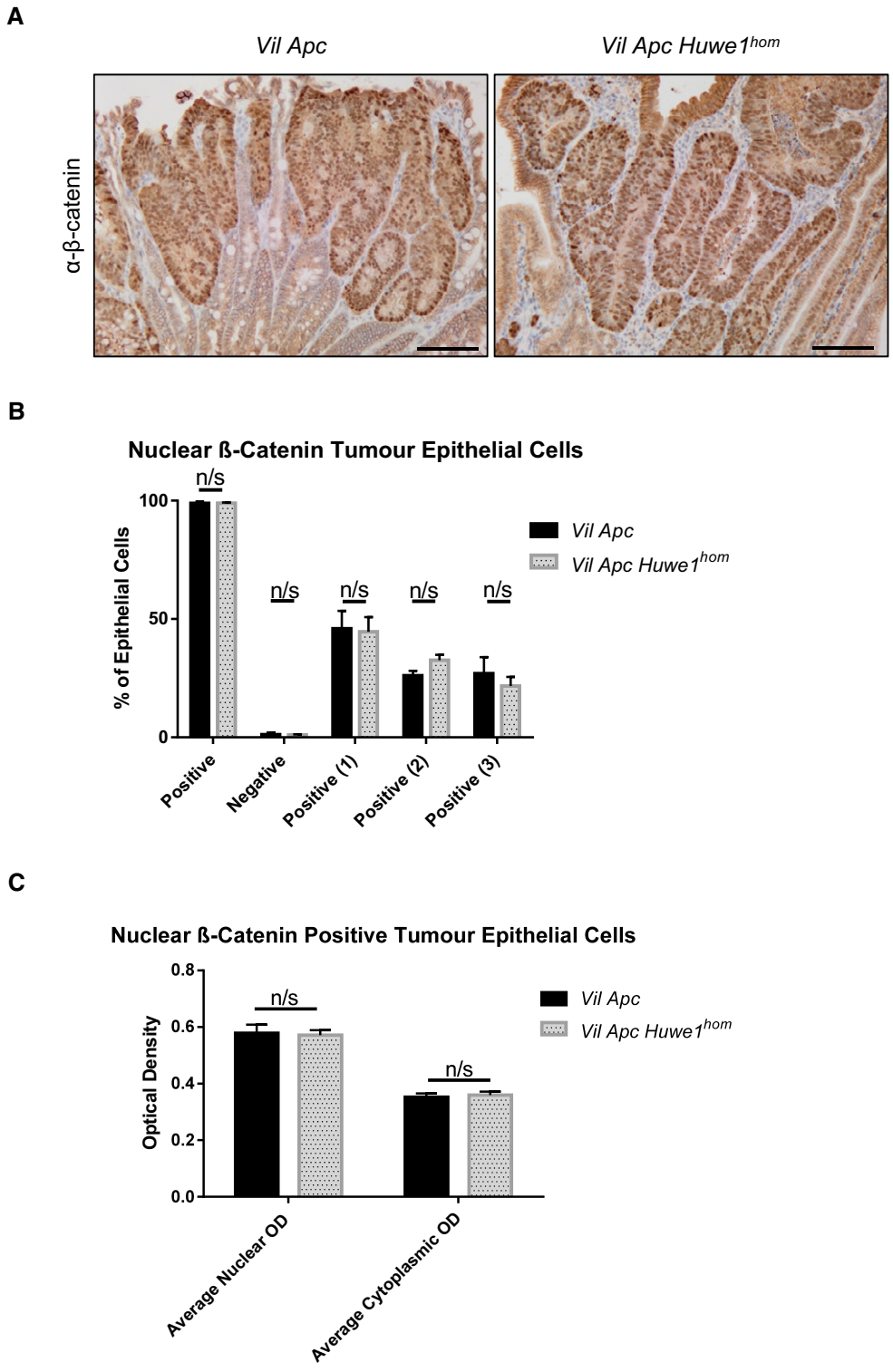


Figure EV2. *Huwe1*-deficient tumours display β-catenin nuclear localisation.

A β-Catenin IHC of *Vil Apc* and *Vil Apc Huwe1*^{hom} adenomas demonstrating nuclear localisation. Scale bars = 100 μm.

B Quantification of percentage of nuclear β-catenin-positive cells in *Vil Apc* and *Vil Apc Huwe1*^{hom} adenomas. Values 1, 2 and 3 represent low, medium and high OD, respectively (Mann–Whitney, n = 3 versus 3). Data are mean and SEM.

C Quantification of β-catenin staining intensity in *Vil Apc* and *Vil Apc Huwe1*^{hom} adenomas (Mann–Whitney, n = 3 versus 3). Data are mean and SEM.

Figure EV3. Intestinal homoeostasis following *Huwe1* deletion.

A Scoring of number of BrdU-positive cells per half-crypt in control and *Huwe1*-deficient small intestines (Mann–Whitney, $n = 4$ versus 4). Mean and SD are plotted.

B Lysozyme IHC demonstrating mislocalised lysozyme expression in *Huwe1*-deficient small intestine. Scale bars = 200 μm. Black arrows indicate mislocalized lysozyme positive staining.

C Quantification of lysozyme-positive cell mislocalisation (per cent of crypts displaying mislocalised lysozyme-positive cells). Significant lysozyme-positive cell mislocalisation observed upon *Huwe1* deletion (Mann–Whitney, $P = 0.0179$, $n = 3$ versus 5). Mean and SD are plotted.

D High-magnification image of lysozyme-positive villus cells in *Huwe1*-deficient intestine. Note typical goblet cell morphology (black arrows). Scale bar = 20 μm.

E qRT–PCR data of WNT target genes in control and *Huwe1*-deleted small intestine (Mann–Whitney, $n = 4$ versus 6, $^{**}P = 0.0048$). Mean and SD are plotted.

F qRT–PCR data of WNT target genes in *Apc* and *Apc Huwe1*-deleted small intestine (Mann–Whitney, $n = 3$ versus 3, $^{*}P = 0.04$). Mean and SD are plotted.

G β-Catenin IHC of control and *Huwe1*-deleted small intestine. Note no gross changes in β-catenin localisation following *Huwe1* deletion. Scale bars = 100 μm.

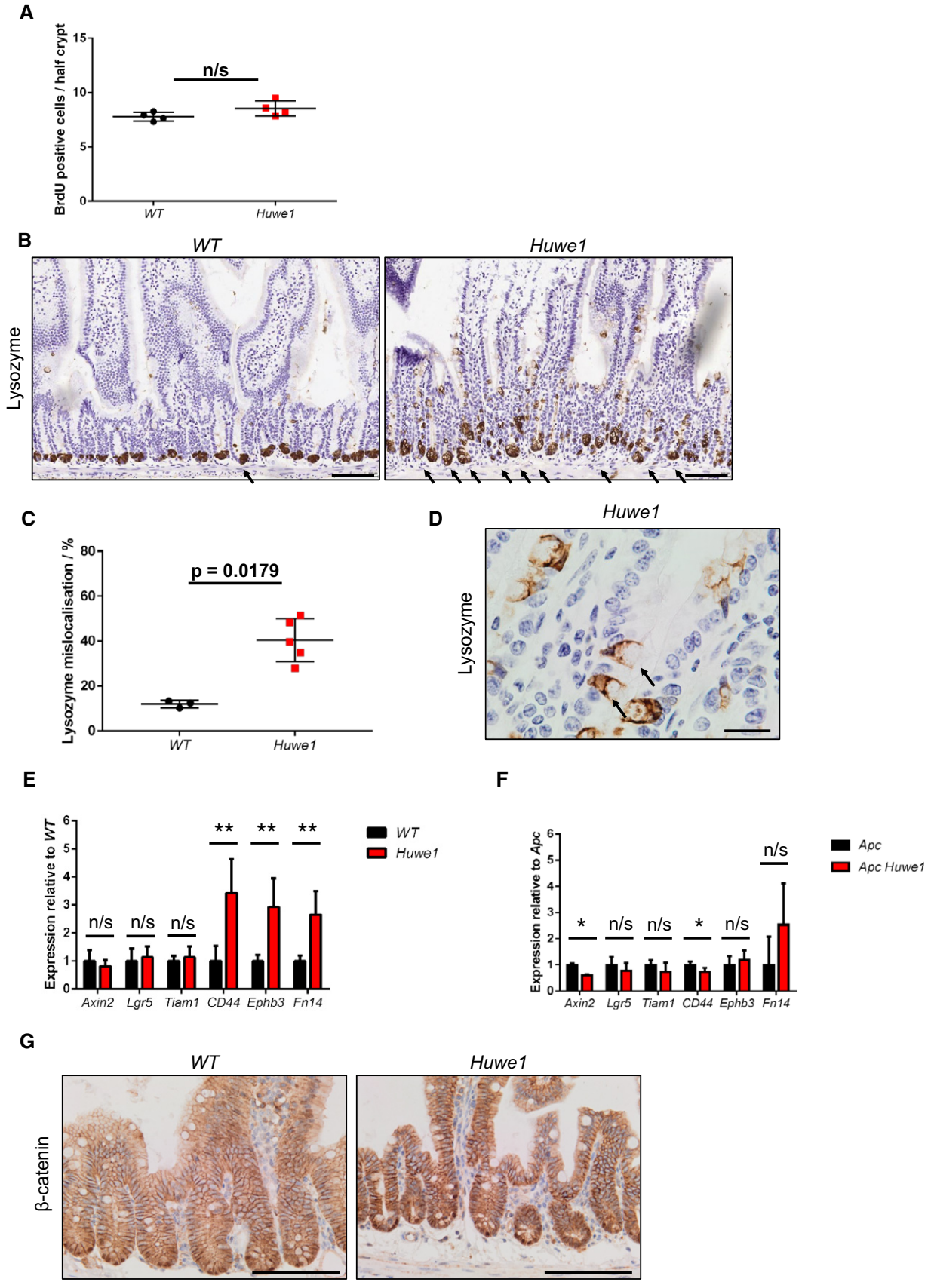


Figure EV3.

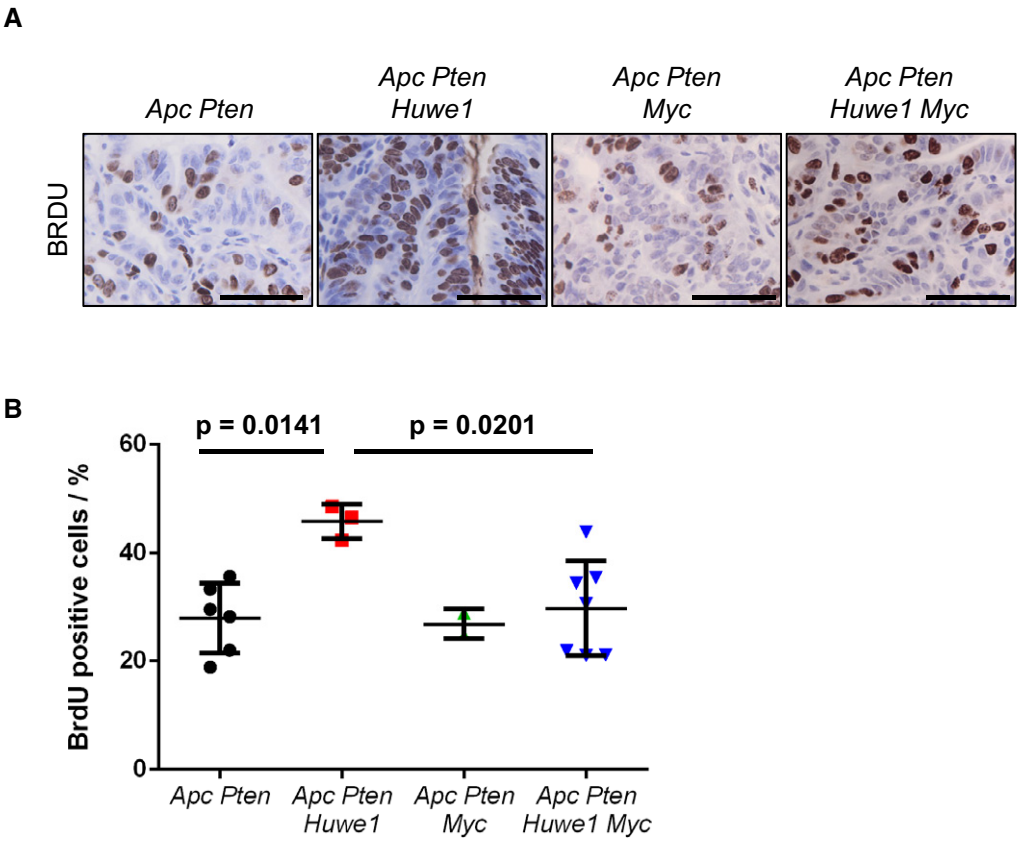


Figure EV4. HUWE1 suppresses tumourigenesis via regulation of MYC.

A BRDU IHC of *Vil Apc Pten*, *Vil Apc Pten Huwe1*, *Vil Apc Pten Myc* and *Vil Apc Pten Huwe1 Myc* tumours. Scale bars = 50 μm.

B Quantification of BrdU IHC in *Vil Apc Pten*, *Vil Apc Pten Huwe1*, *Vil Apc Pten Myc* and *Vil Apc Pten Huwe1 Myc* tumours (Mann–Whitney, $n \geq 3$). Data plotted are mean and SD.

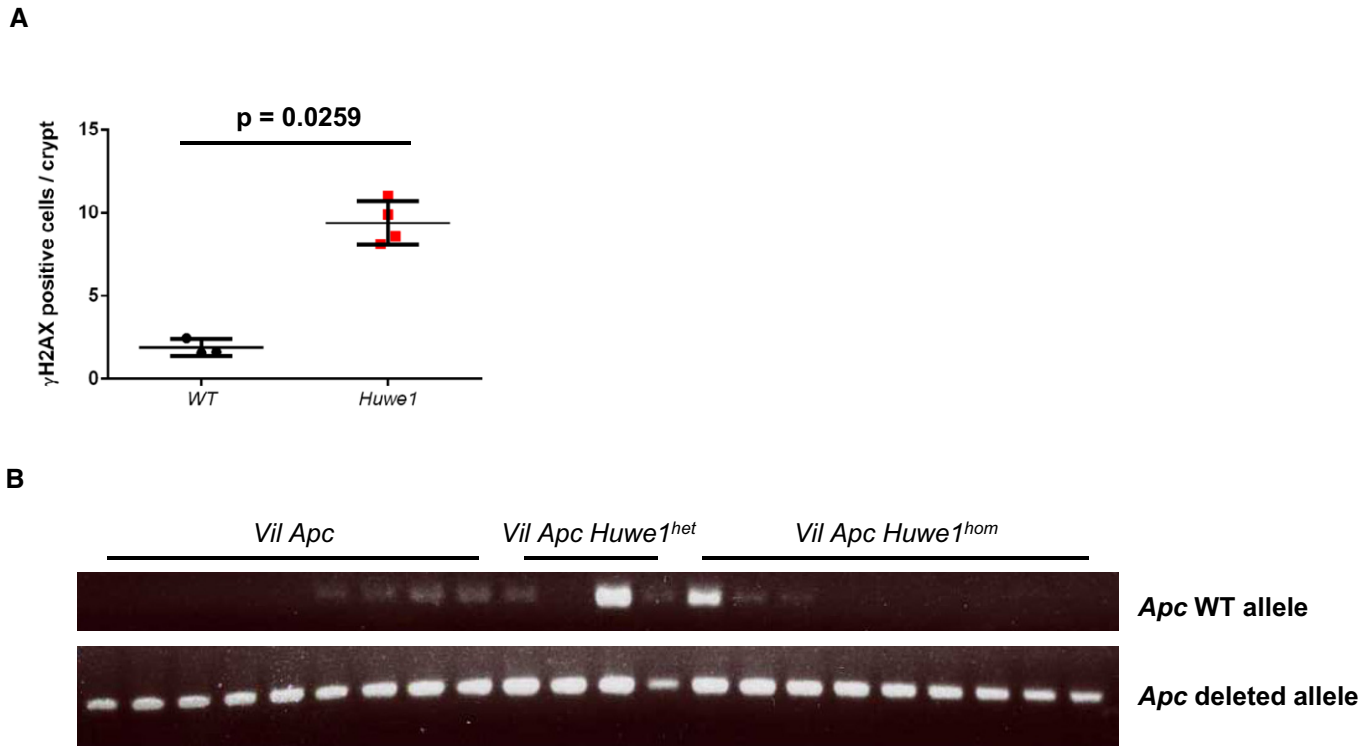


Figure EV5. *Huwe1*-deficient tumours display *Apc* LOH.

A Quantification of γ-H2AX IHC demonstrating increased number of nuclei staining positive following *Huwe1* deletion (Mann–Whitney, $n = 3$ versus 4). Data plotted are mean and SD.

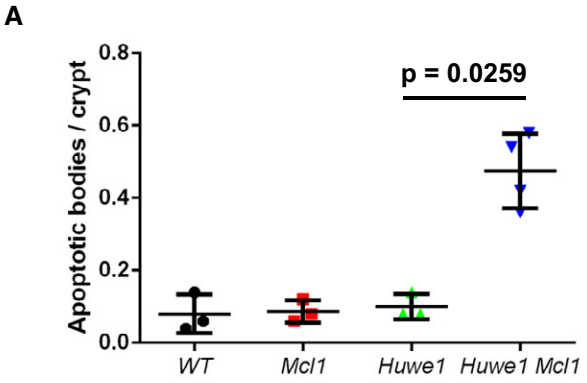
B *Apc* LOH PCR analysis of tumours isolated from *Vil Apc*, *Vil Apc Huwe1*^{het} and *Vil Apc Huwe1*^{hom} mice. The majority of tumours display *Apc* LOH.

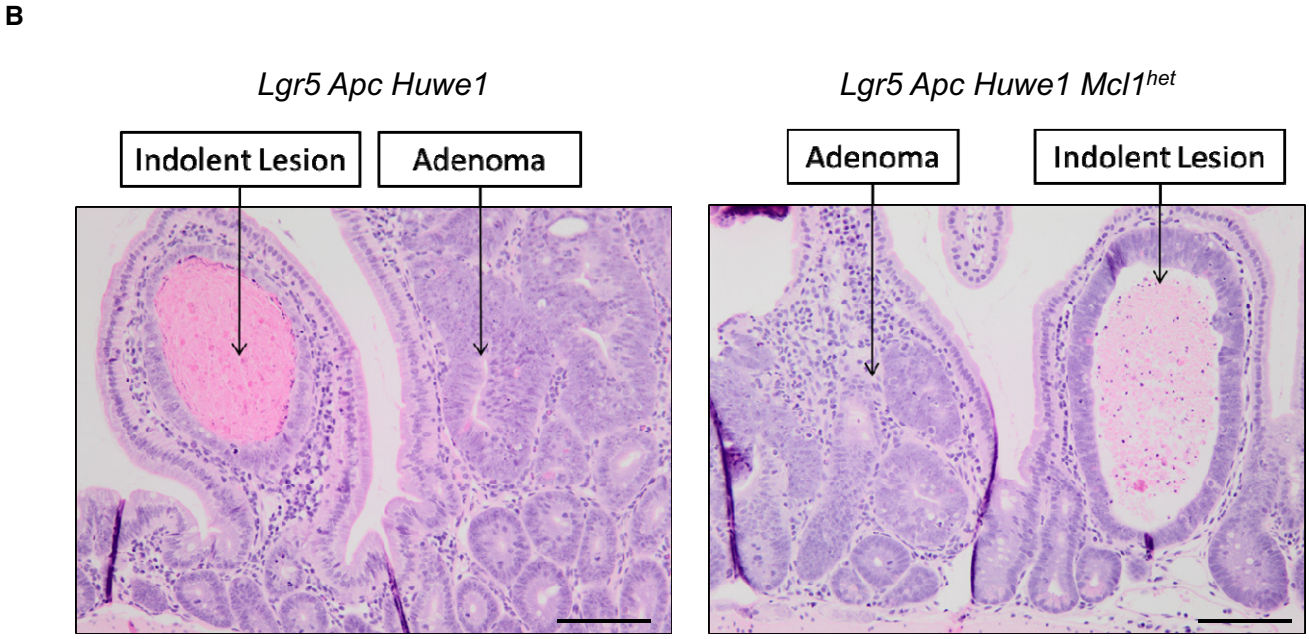
Figure EV6. *MCL1* protects *Huwe1*-deficient tumours from apoptosis.

A Quantification of scoring of apoptotic bodies in control, *Mcl1*$^{fl/+}$, *Huwe1*$^{fl/fl}$ and *Huwe1*$^{fl/fl}$ *Mcl1*$^{fl/+}$ deleted intestines (Mann-Whitney, $P = 0.0259$, $n = 3$ vs 4). Data are mean and SD.

B Classification of adenoma and indolent lesions observed in *Lgr5 Apc Huwe1* (left panel) and *Lgr5 Apc Huwe1 Mcl1*^{het} (right panel) mice. Scale bars = 100 μm.

C Image of intestines from *Lgr5 Apc Huwe1* (left panel) and *Lgr5 Apc Huwe1 Mcl1*^{het} (right panel) mice. Adenomas indicated by red arrows and indolent lesions by black arrows. Note the decreased ration of adenomas/indolent lesions observed in *Mcl1*^{het} intestines. Scale bars = 100 μm.





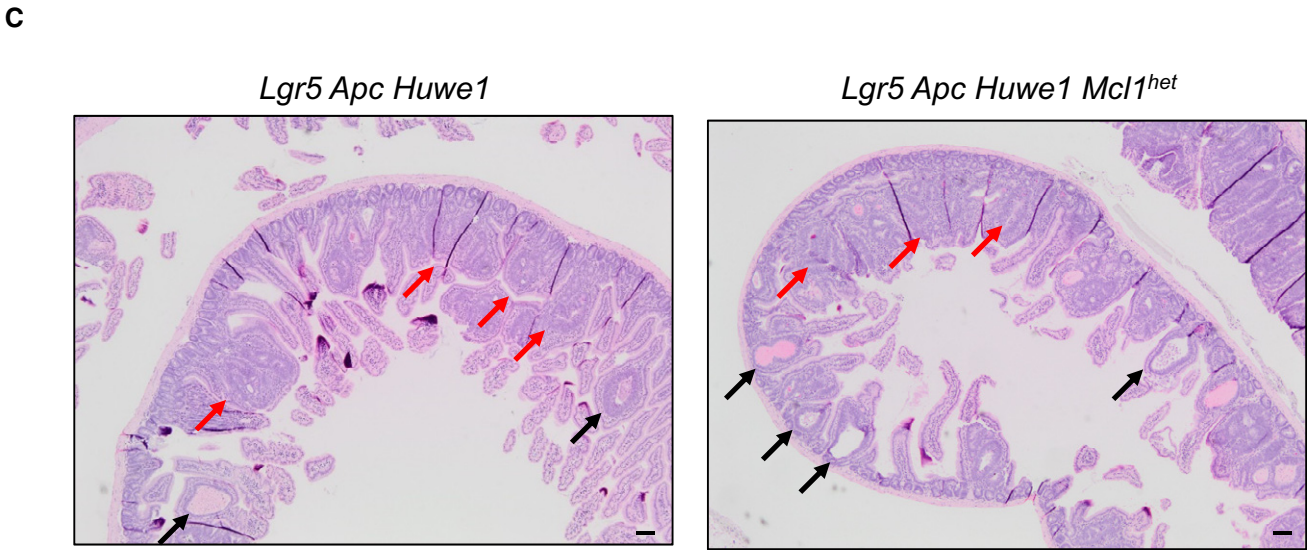


Figure EV6.