## 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<sup>Het</sup> and Vil Apc Huwe1<sup>Hom</sup> mice. Deletion of Huwe1 led to a significant increase in the number of tumours per colon (Mann–Whitney, n ≥ 3). Mean and standard deviation are plotted. |
**A**

TCGA, 2012

Altered in 13 (7%) of cases

Seshagiri et al., 2012

Altered in 11 (15%) of cases

**B**

Percent sample alteration for each cancer study with mutation data: *HUWE1*

![Graph showing percent altered samples for different cancer studies](image)

**C**

![Image of a gel electrophoresis experiment](image)

**D**

![Graph showing tumor number/colon](image)

**Figure EV1.**
**Figure EV2.** *Huwe1*-deficient tumours display β-catenin nuclear localisation.

A. β-Catenin IHC of Vil Apc and Vil Apc Huwe1hom adenomas demonstrating nuclear localisation. Scale bars = 100 μm.

B. Quantification of percentage of nuclear β-catenin-positive cells in Vil Apc and Vil Apc Huwe1hom 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 Huwe1hom 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 \( n = 4 \)). Mean and SD are plotted.

B Lysozyme IHC demonstrating mislocalized lysozyme expression in Huwe1-deficient small intestine. Scale bars = 200 \( \mu \)m. 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 \( n = 5 \)). 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 \( \mu \)m.

E qRT–PCR data of WNT target genes in control and Huwe1-deleted small intestine (Mann–Whitney, \( n = 4 \) versus \( n = 6 \), \( **P = 0.0048 \)). 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 \( n = 3 \), \( *P = 0.04 \)). Mean and SD are plotted.

G b-Catenin IHC of control and Huwe1-deleted small intestine. Note no gross changes in b-catenin localisation following Huwe1 deletion. Scale bars = 100 \( \mu \)m.
**HUWE1 is a critical colonic tumour suppressor**

Kevin B Myant et al

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 ≥ 3). Data plotted are mean and SD.
A

![Graph](image)

\[ p = 0.0259 \]

\[ \gamma H2AX \text{ positive cells / crypt} \]

WT | Huwe1
---|---
0 | 15
5 | 10
10 | 5
15 | 0

Figure EV5. Huwe1-deficient tumours display Apc LOH.

A Quantification of \( \gamma \)-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\(^{\text{het}}\) and Vil Apc Huwe1\(^{\text{hom}}\) mice. The majority of tumours display Apc LOH.

B

Vil Apc | Vil Apc Huwe1\(^{\text{het}}\) | Vil Apc Huwe1\(^{\text{hom}}\)
---|---|---
Apc WT allele | Apc WT allele | Apc WT allele
Apc deleted allele | Apc deleted allele | Apc deleted allele

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

A Quantification of scoring of apoptotic bodies in control, Mcl1\(^{\text{fl/+}}\), Huwe1\(^{\text{fl/+}}\) and Huwe1\(^{\text{fl/+}}\) Mcl1\(^{\text{fl/+}}\) 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\(^{\text{fl/+}}\) (right panel) mice. Scale bars = 100 \( \mu \)m.

C Image of intestines from Lgr5 Apc Huwe1 (left panel) and Lgr5 Apc Huwe1 Mcl1\(^{\text{fl/+}}\) (right panel) mice. Adenomas indicated by red arrows and indolent lesions by black arrows. Note the decreased ratio of adenomas/indolent lesions observed in Mcl1\(^{\text{fl/+}}\) intestines. Scale bars = 100 \( \mu \)m.
**A**

![Graph showing Apoptotic bodies per crypt](image)

- **WT**
- **Mcl1**
- **Huwe1**
- **Huwe1 Mcl1**

$p = 0.0259$

**B**

**Lgr5 Apc Huwe1**

- **Indolent Lesion**
- **Adenoma**

**Lgr5 Apc Huwe1 Mcl1<sup>het</sup>**

- **Adenoma**
- **Indolent Lesion**

**C**

**Lgr5 Apc Huwe1**

- **Indolent Lesion**
- **Adenoma**

**Lgr5 Apc Huwe1 Mcl1<sup>het</sup>**

- **Indolent Lesion**
- **Significant Area**

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**Figure EV6.**