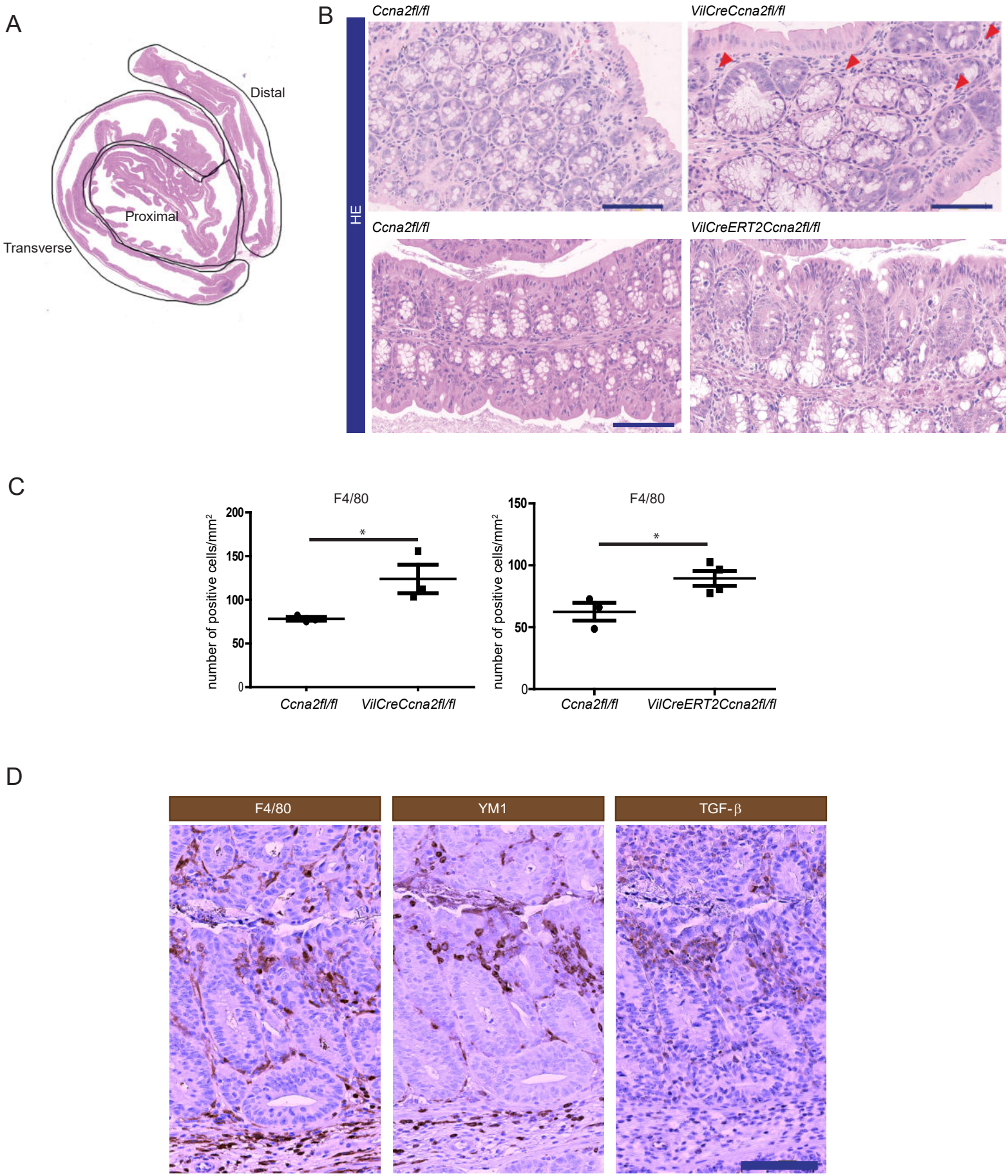
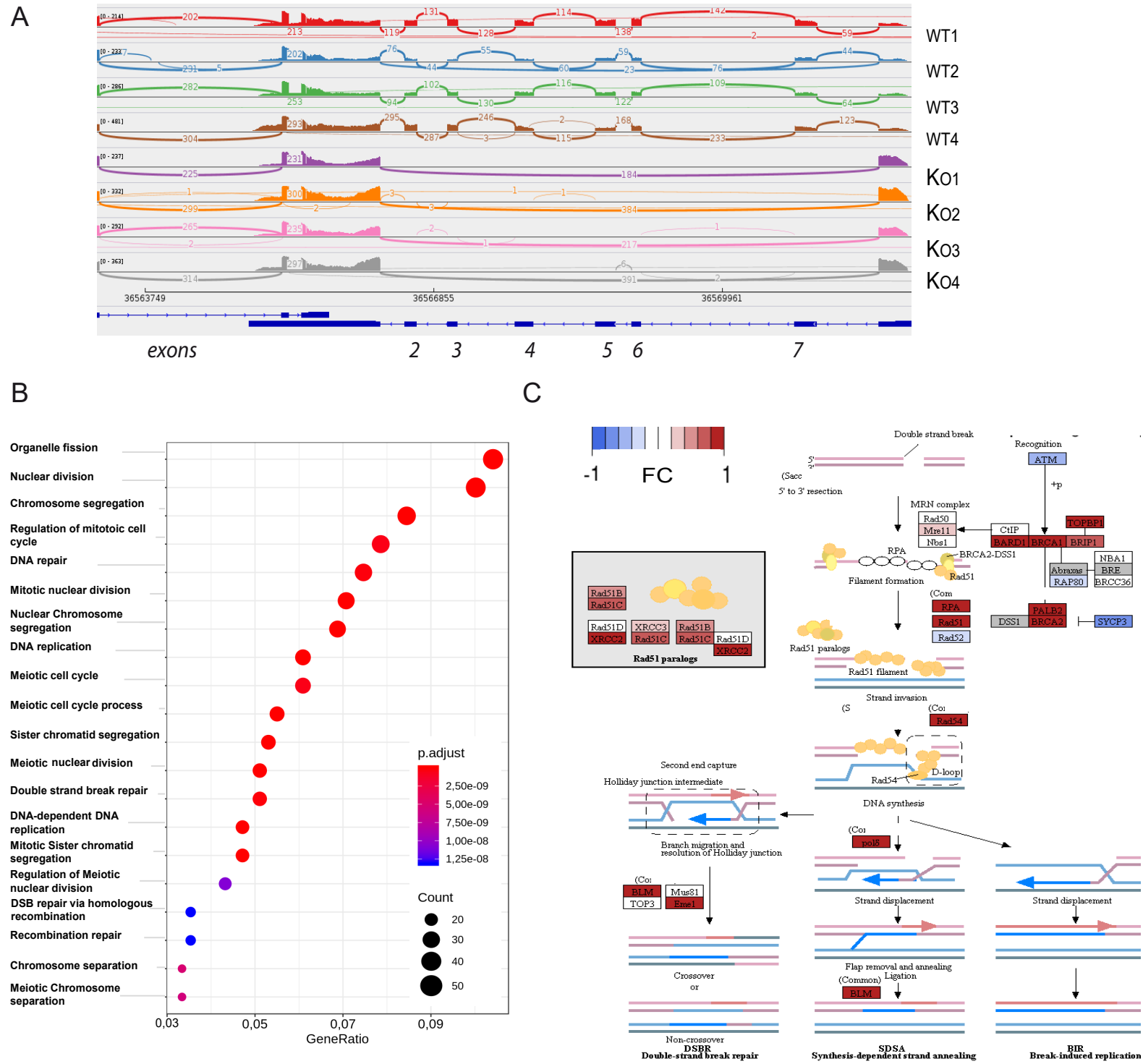
Supplemental Figure 1



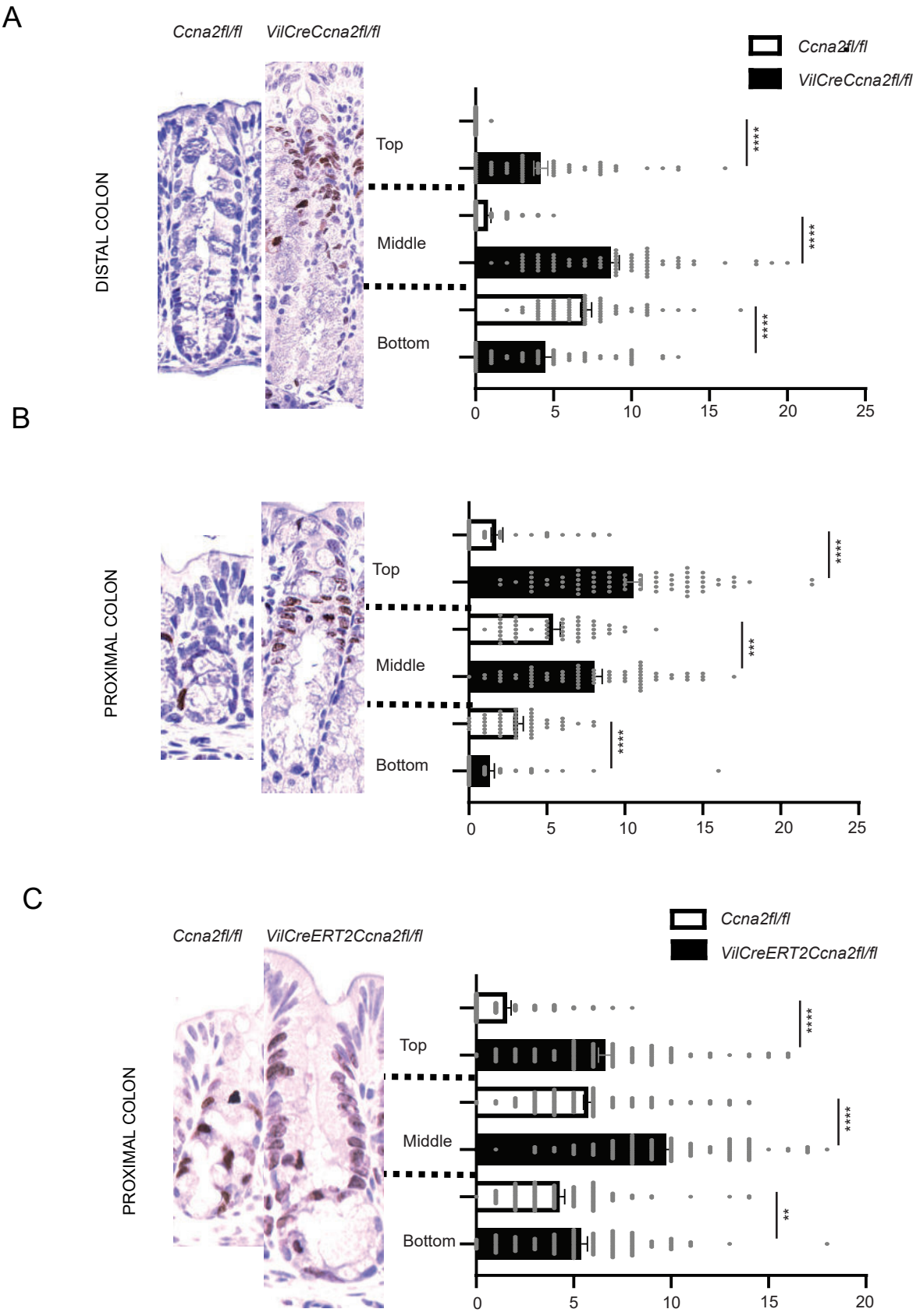
Supplemental Figure 1: Cyclin A2-deficiency in colonic epithelial cells induces architectural changes in the mucosa and inflammation. (**A**). HE staining illustrating the subdivision of the colon in 3 parts for the histological analysis, i.e. the distal part close to the rectum, transverse and proximal part, close to the caecum.(**B**). Representative HE staining of irregular formed crypts from a constitutive and inducible (day8 following tamoxifen injection) knockout mouse by comparison to a control. Please note the immune cell infiltration indicated by arrowheads. Scale bar: 100µm. (**C**). Quantification of the F4/80 staining in control (n=3), constitutive (n=3) and inducible cyclin A2-deficient mice (n=4; expressed as mean ± SEM, * $p<0.05$; two-tailed unpaired t-test) expressed as number of F4/80 positive cells per mm^2 of colon tissue (**D**) Representative images of serial sections from *VilCreERT2Ccna2fl/fl* colons (day8) immunostained for F4/80, YM1 and TGF-β. Scale bar: 100µm.

Supplemental Figure 2



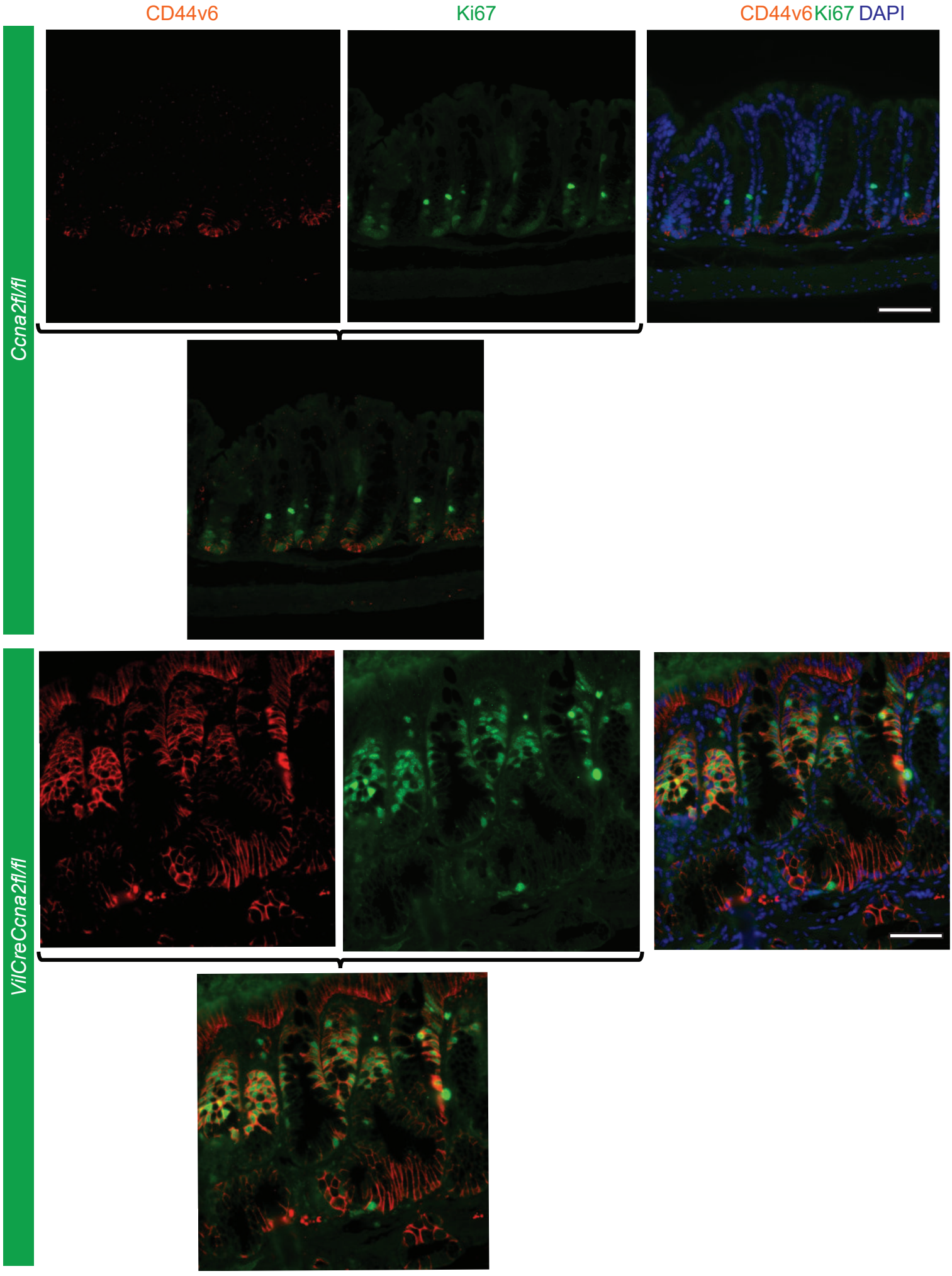
Supplemental Figure 2: RNA-seq analysis of altered expression of genes involved in cell cycle regulation and DNA double-strand break repair in cyclin A2 deficient colonic epithelial cells (CEC). (**A**). Sashimi plot of *Ccna2* reads for each mouse-derived CEC sample analyzed by RNA-seq. Plots show deletion of exons 2 to 7 in transcripts of CEC from *VilCreCcna2fl/fl* mice compared to controls. The coverage for each alignment track is plotted as a bar graph. Arcs representing splice junctions connect exons. Arcs display the number of reads split across the junction (junction depth). Genomic coordinates and the gene annotation track are shown below the junction tracks. (**B**). Gene Ontology over-representation analysis showing the genes up-regulated in *Ccna2 fl/fl* mutant mice compared to controls. The top 20 of Gene Ontology terms (Biological Process) are shown here. The p-values have been corrected for multiple testing by the Benjamini-Hochberg method. In this dot plot, the color represents the adjusted p-values and the dot size represents the number of genes for each term. The gene ratio is shown on the x-axis. (**C**). Alterations in the double-strand break KEGG pathway in cyclin A2 deficient CEC. Genes up-regulated in *VilCreCcna2fl/fl* mice samples are in red, whereas downregulated genes are in blue. The scale indicated on the figure represent the log2 Fold change. In grey are the genes absent from our data. For all analyses, only p-values <0.05 were considered as statistically significant. FC: Fold change; DSB: Double-strand break.

Supplemental Figure 3



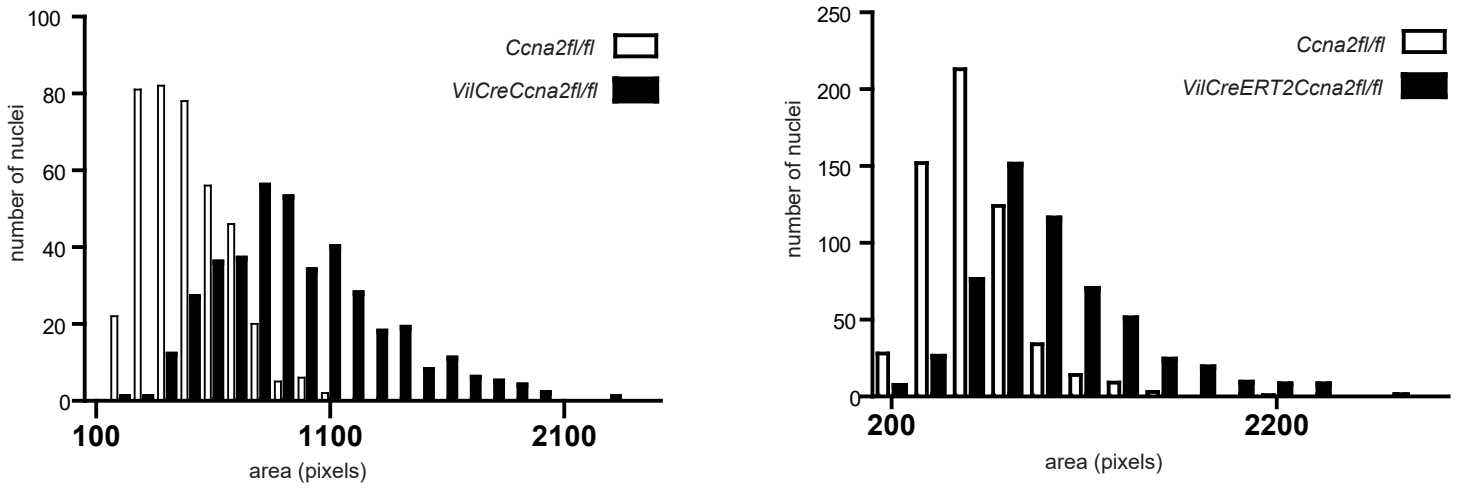
Supplemental Figure 3: Increased proliferation of colonic epithelial cells from cyclin A2- deficient mice. (**A, B, C**). Quantification of Ki67 expression by IHC of the different parts of crypts from the distal and proximal part of the colon from control (n= 66 crypts analyzed from 3 different mice) and constitutive cyclin A2-deficient mice (**A, B**) and proximal part of the colon from induced knockout mice at day 8 following inactivation compared to controls (n=120 from 3 different mice) (**C**). Mean values ±SEM are provided, * p<0,05, ** p<0.01 and *** p<0,001; two-tailed unpaired t-test.

Supplemental Figure 4



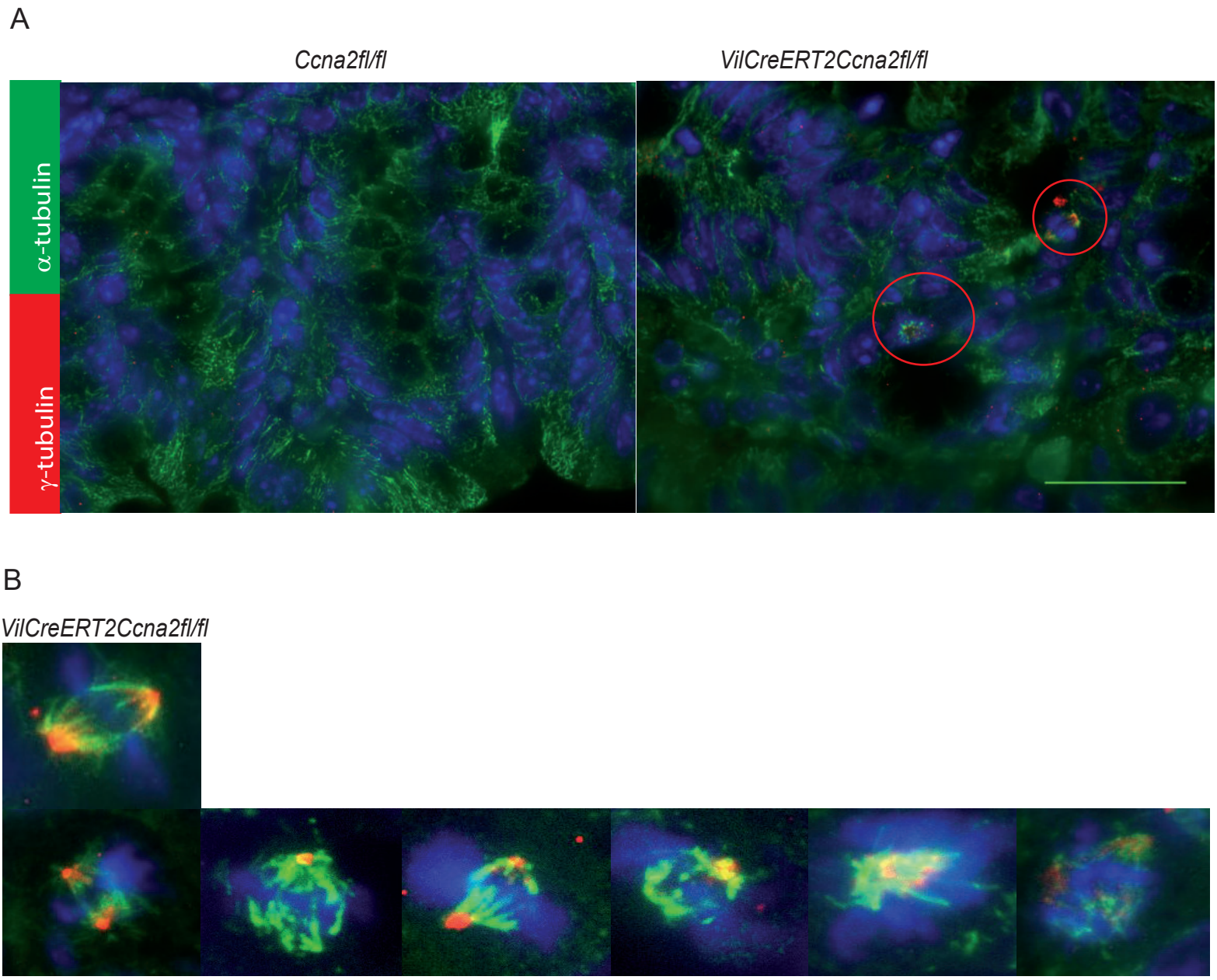
Supplemental Figure 4: Ki67 positive cells express CD44v6. Representative immunostainings for Ki67 (green) and CD44v6 (red). DAPI (blue) was used for nuclei detection on colons of *Ccna2fl/fl* and *VilCreCcna2fl/fl* mice, respectively. Please note the elevated intensity of Ki-67 staining in cyclin A2-deficient colons. Scale bars: 100µm.

Supplemental Figure 5



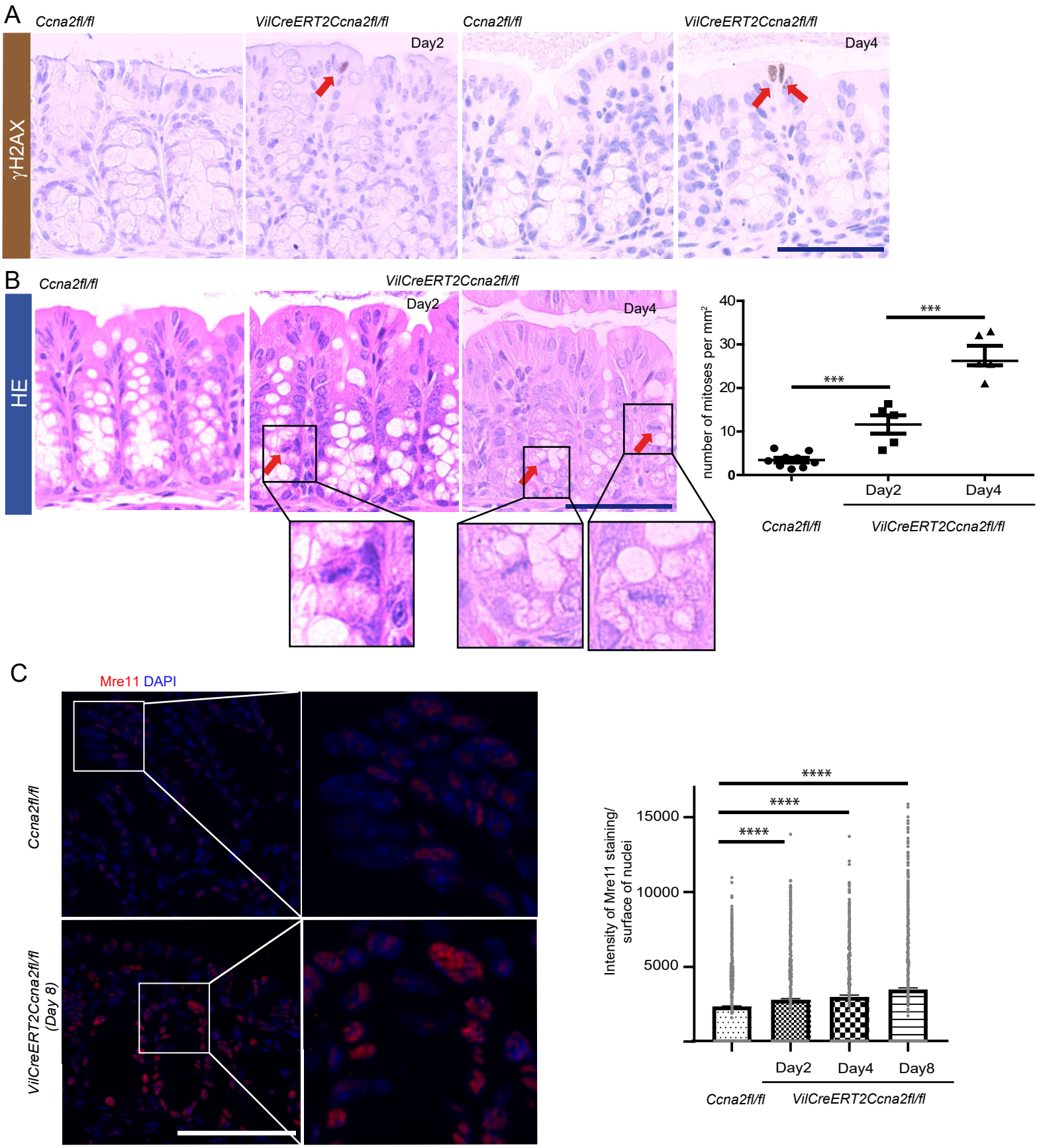
Supplemental Figure 5: Increased nuclear size of colonic epithelial cells in cyclin A2-deficient mice. Distribution curve representing the nuclear size of *Ccna2fl/fl* colonic epithelial cells (white, n=398 for the left panel and 578 for the right panel from 3 mice), *VilCreCcna2fl/fl* (black, left panel, n=400 from 3 mice, p<1.10e-16) and *VilCreERT2Ccna2fl/fl* (black, right panel, n=566, p<1.10e-16) colonic epithelial cells; p-values were determined using Kolmogorov-Smirnov test.

Supplemental Figure 6



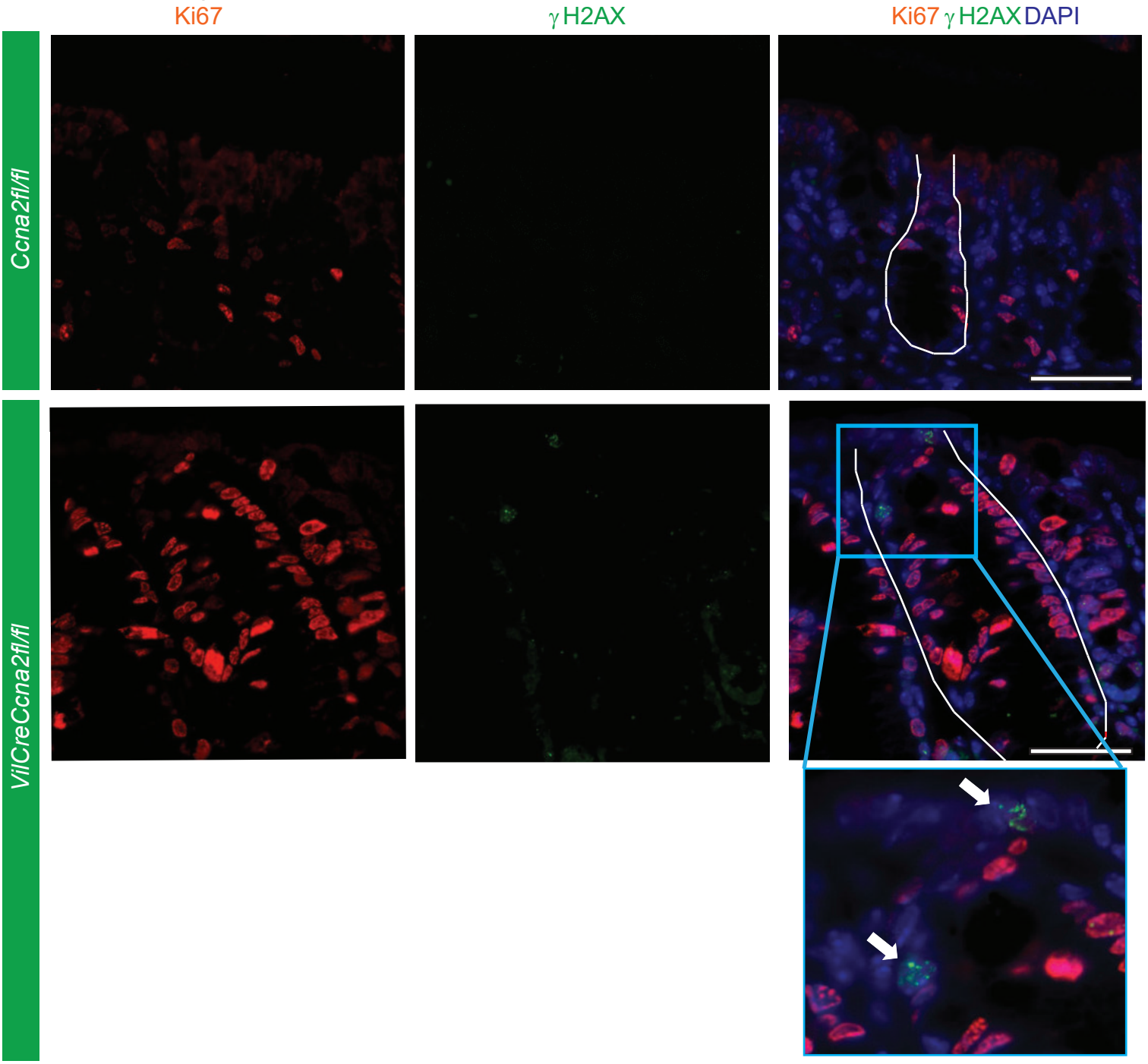
Supplemental Figure 6: Examples of mitoses in colons of cyclin A2-deficient mice. (**A**). Immunofluorescence analysis of mitosis (indicated by red circles) using an anti-α-tubulin antibody (green) to stain for the mitotic spindle in combination with γ-tubulin (centrosome in red) and DAPI for DNA in a colon of *VilCreERT2Ccna2fl/fl* mice at day 8 following inactivation (right panel) by comparison to controls (left panel). Scale bar: 100μm. (**B**). The upper image shows normal mitosis, the lower panel several examples of abnormal mitoses observed in a *Ccna2*-deficient colon. Blow-up: 2.5x.

Supplemental Figure 7



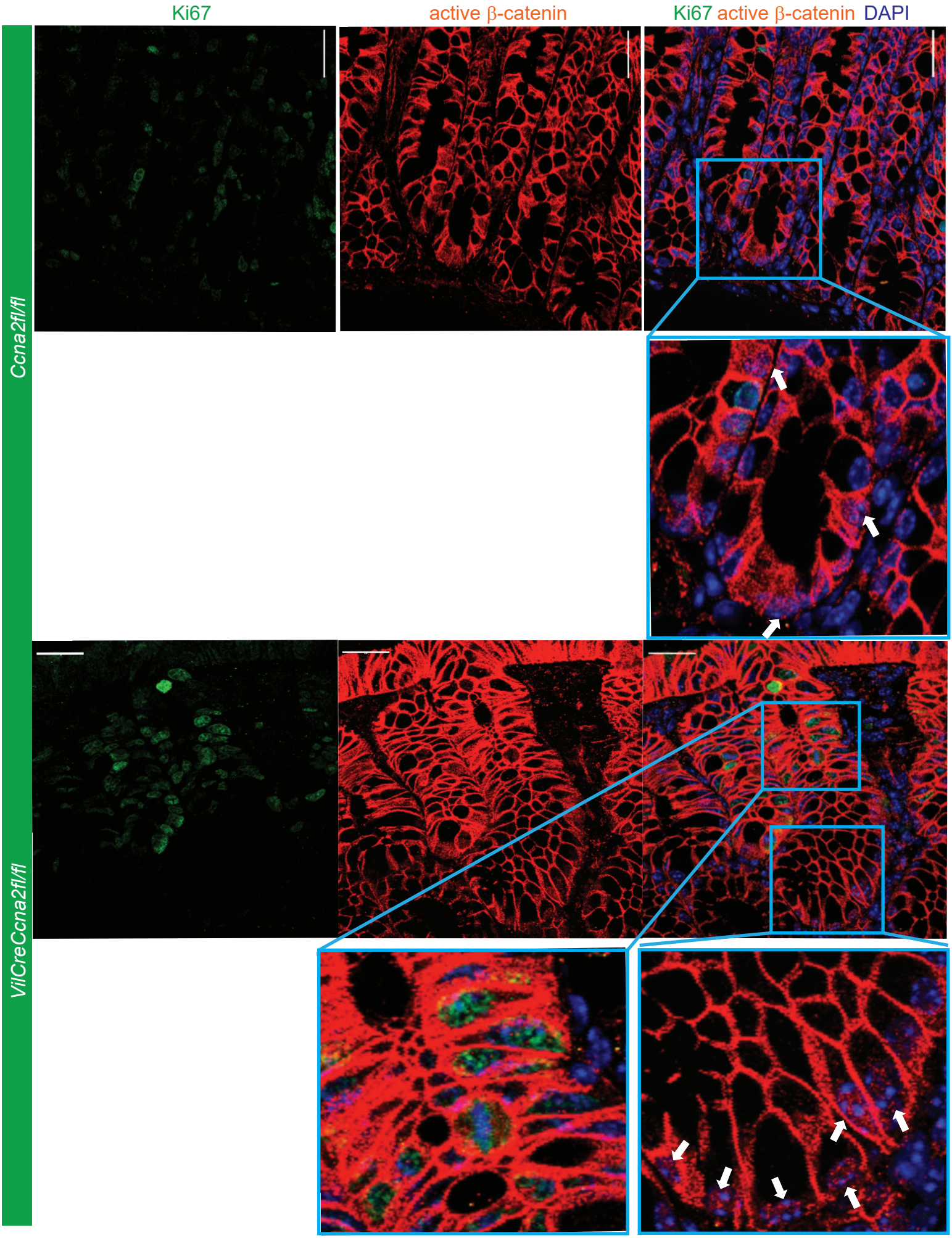
Supplemental Figure 7: Increased mitoses, DNA damage and Mre11 foci formation is already detectable at day2 following cyclin A2 inactivation. (**A**) Representative images of γH2AX staining in *VilCreERT2Ccna2fl/fl* (day 2 and 4) colon and controls (*Ccna2fl/fl*). Red arrows indicate γH2AX staining. Scale bar: 100µm. (**B**) Representative HE staining showing the increased proportion of mitoses in *VilCreERT2Ccna2fl/fl* colons (at day 2 and 4). Red arrows indicate mitosis. The panel on the right shows the corresponding quantification in colons of *Ccna2fl/fl* (n=9), *VilCreERT2Ccna2fl/fl* (day 2, n=5) and *VilCreERT2Ccna2fl/fl* (day 4, n=5) mice. Values are expressed as mean ±SEM. ***p<0.001, two-tailed unpaired t-test). Blow-up: 2x. Scale bar: 100µm (**C**) Left panel: Representative image of Mre11 staining (red) and DAPI (blue) on colons of *Ccna2fl/fl* and *VilCreERT2Ccna2fl/fl* (day 8) mice. Blow-up: 3x. Scale bar: 100µm. Right panel: quantification of Mre11 foci formation in colons of *Ccna2fl/fl* (n=4045 from 3 mice), *VilCreERT2Ccna2fl/fl* day 2 (n=1747 from 3 mice), day 4 (n=709 from 3 mice) and day 8 (n=1167 from 2 mice) mice expressed as intensity of Mre11 staining per surface of nuclei (mean ±SEM, ****p<0.0001; Mann-Whitney test).

Supplemental Figure 8



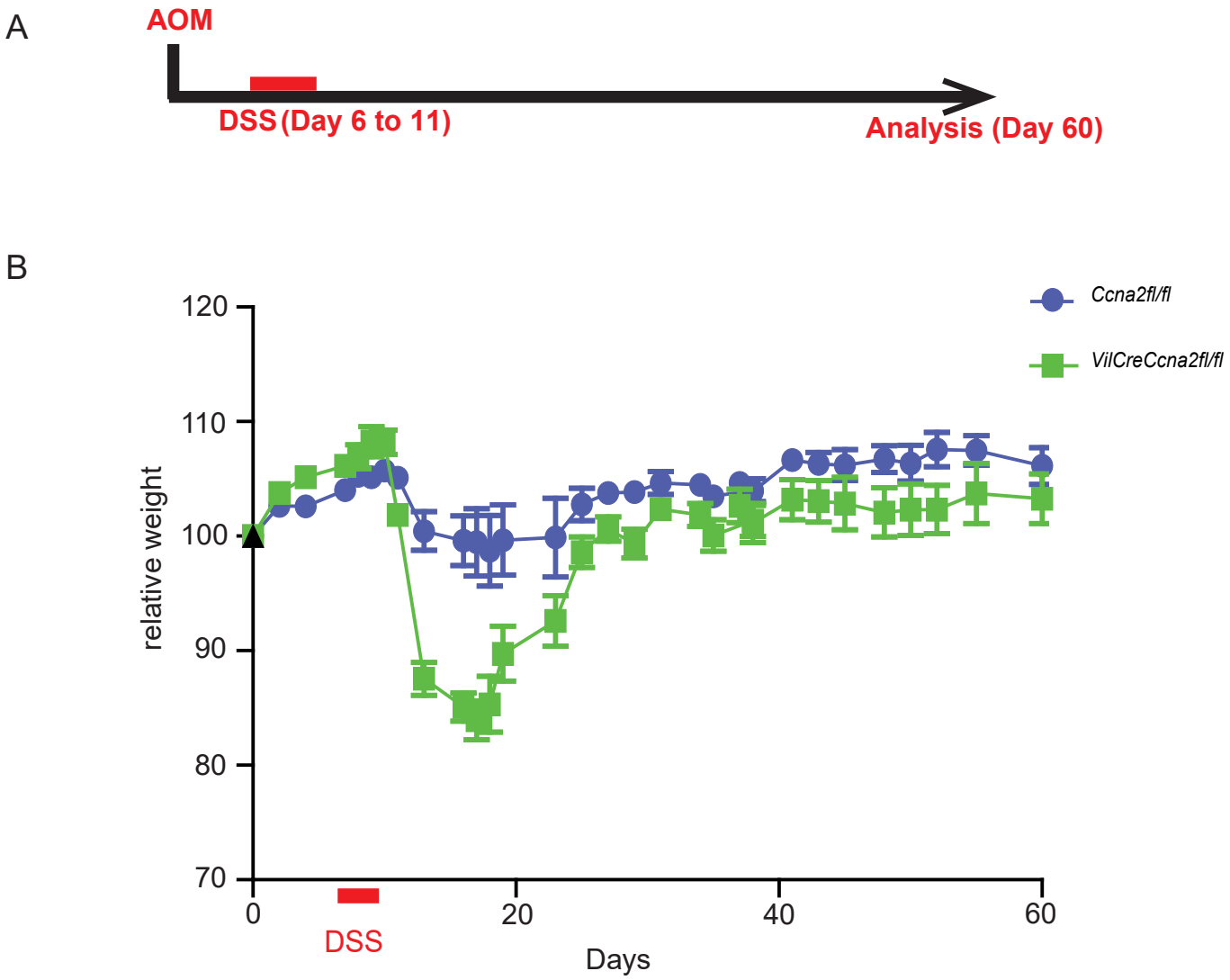
Supplemental Figure 8: γH2AX positive cells do not express Ki67 and localize at the top of the crypts. Immunofluorescence analysis was performed using an anti-Ki67 (red) and γH2AX (green) antibody. DAPI (blue) was used for nuclei detection on colons of *Ccna2fl/fl* and *VilCreCcna2fl/fl* mice. Crypts are depicted in white. Representative images are shown. Blow-up: 2x; white arrows indicate γH2AX positive cells. Scale bars: 100µm.

Supplemental Figure 9



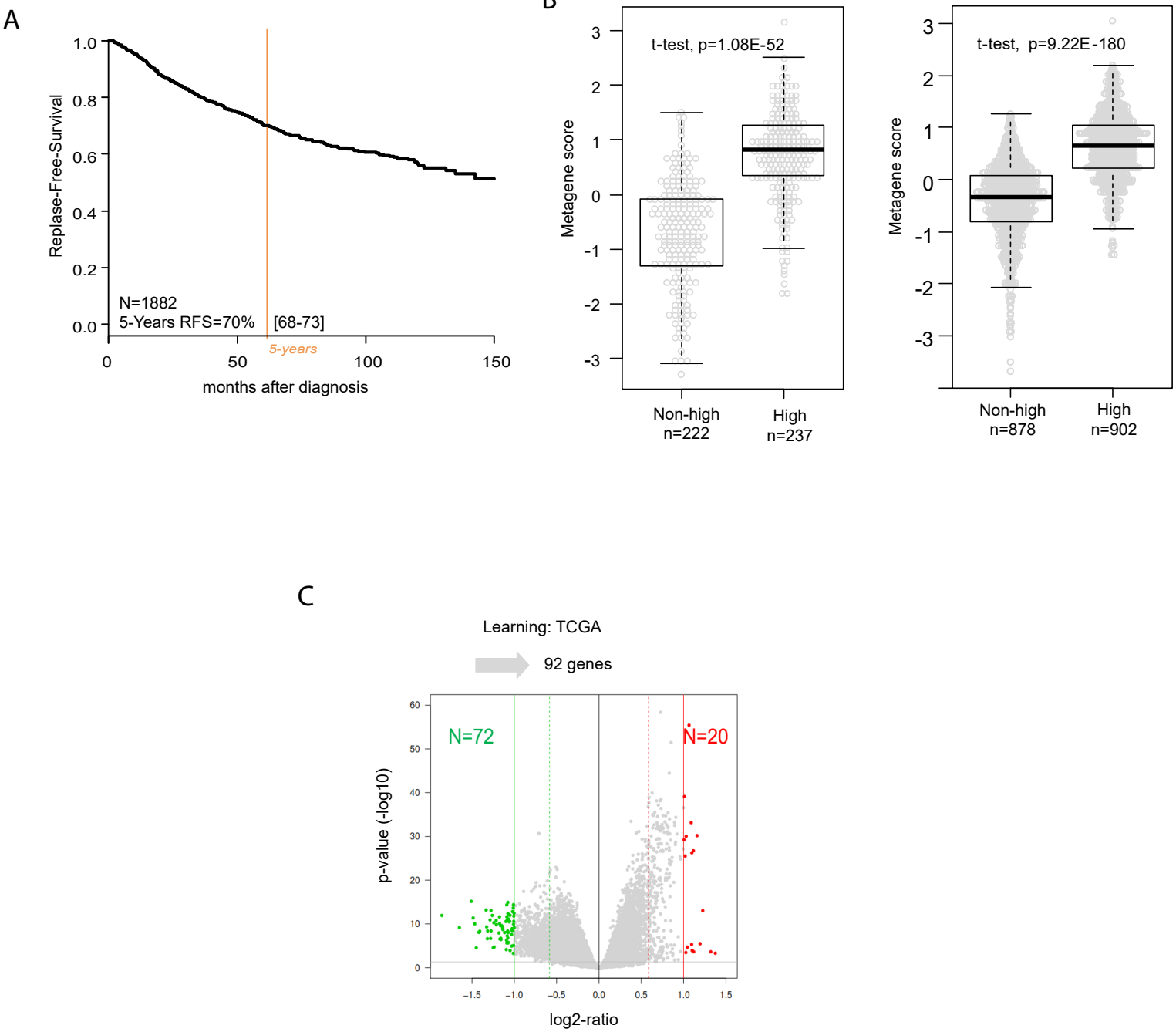
Supplemental Figure 9: Active β-catenin positive cells do not express Ki67 and localize at the bottom of the crypts. Immunofluorescence analysis for-Ki67 (green), active β-catenin (red) and DAPI (blue) for nuclei detection on colons of *Ccna2fl/fl* and *VilCreCcna2fl/fl* mice. Representative images are shown. White arrows indicate nuclear active β-catenin staining. Blow-up: 2.5x. Scale bars: 50 μm.

Supplemental Figure 10



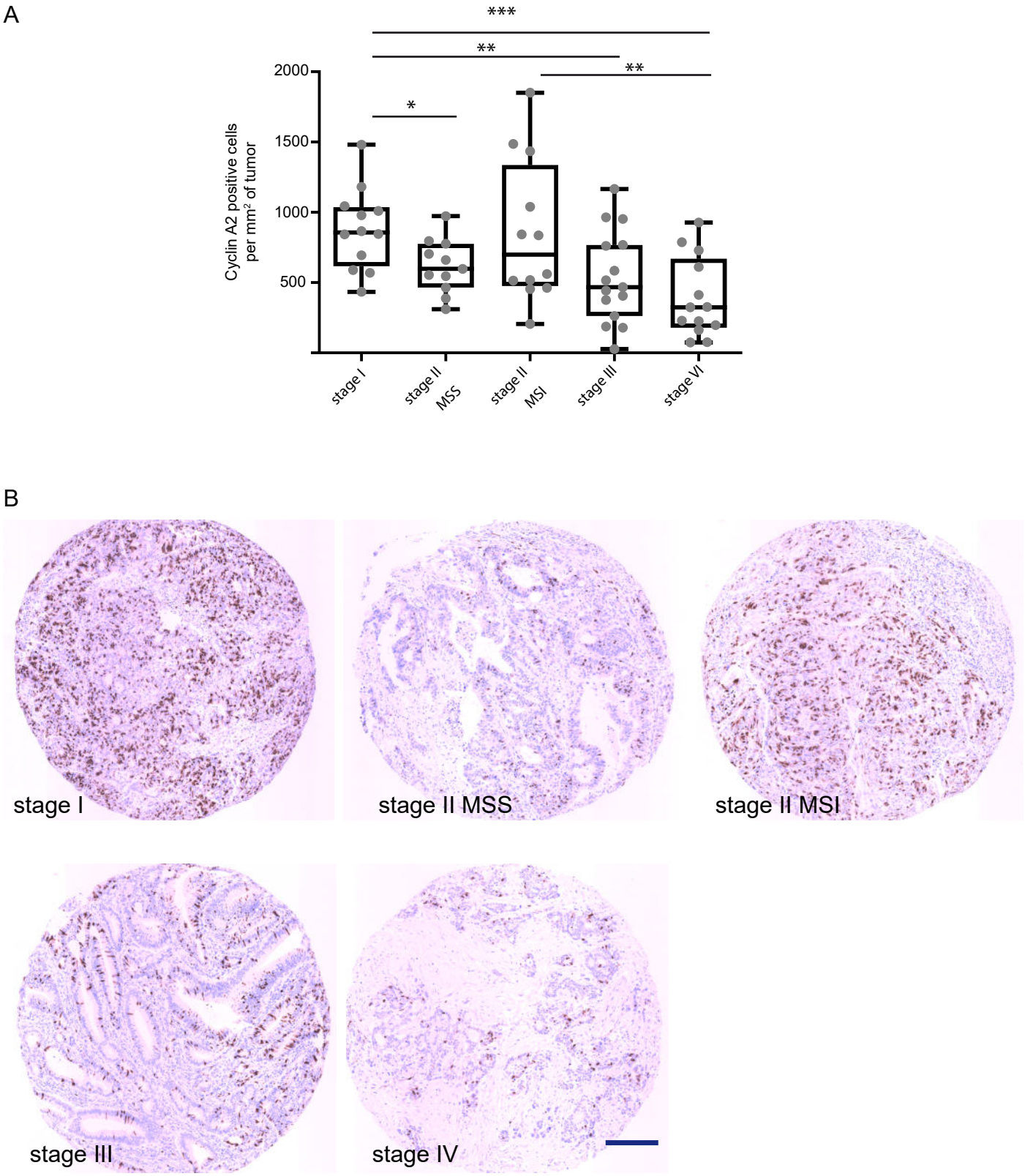
Supplemental Figure 10: Protocol and weight monitoring of the mice during colitis associated carcinogenesis. (**A**). Schematic representation of the modified AOM/DSS protocol applied to *VilCreCcna2fl/fl* (n=7) and control mice (n=6) (see Figs 7 and 8). (**B**). Monitoring of the relative weight (expressed as percentage relative to the weight at the beginning of the protocol) of *VilCreCcna2fl/fl* and control mice during the AOM/DSS protocol.

Supplemental Figure 11



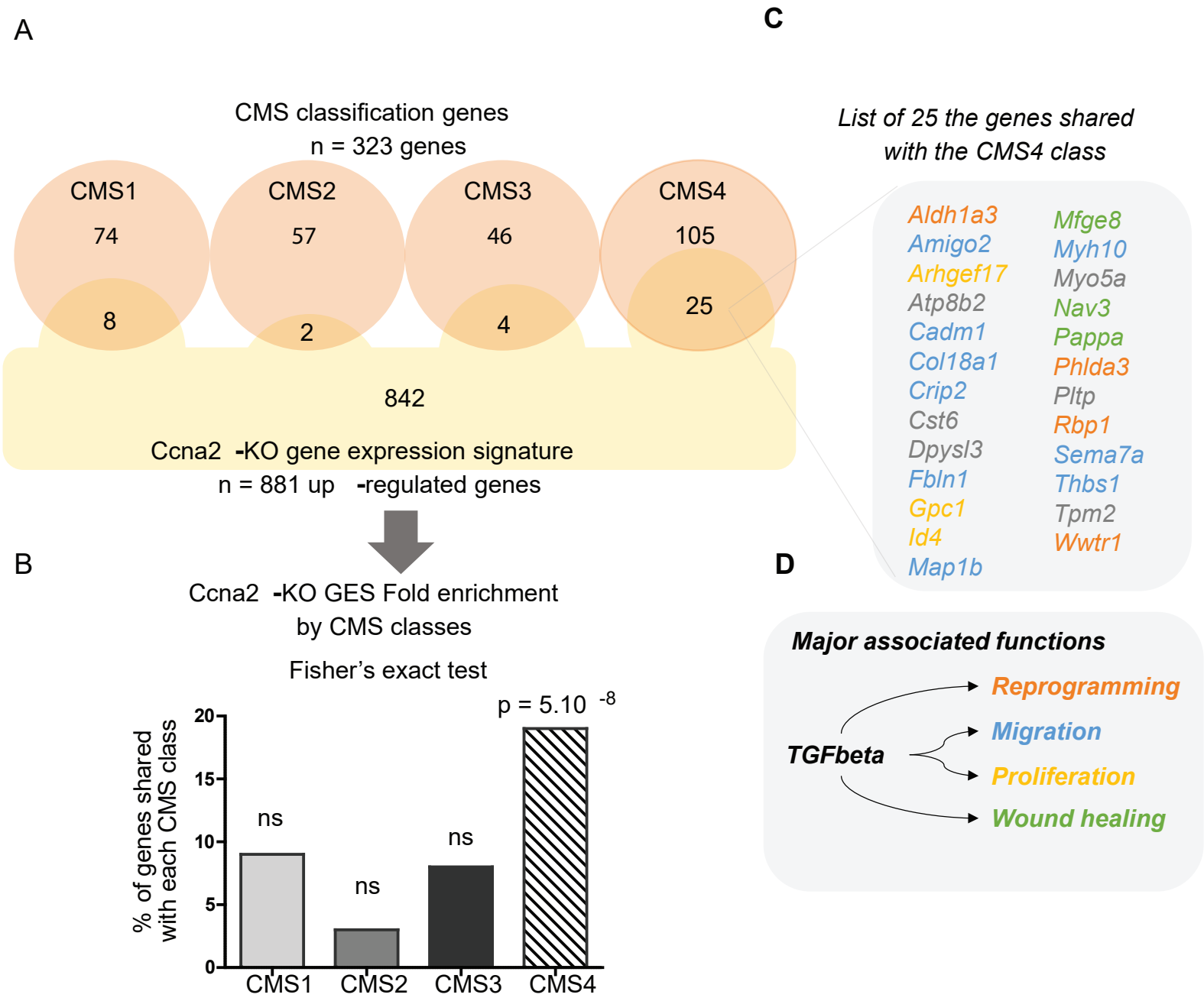
Supplemental Figure 11: Cyclin A2 expression at the mRNA and protein level in CRC patients. **(A)**. Relapse free survival (RFS) curve of the overall patients analyzed for cyclin A2 mRNA expression levels. **(B)**. Metagene-based prediction score of outcome (using Student t-test and expressed as mean ±SD) of the *CCNA2*high samples compared to those of *CCNA2*non-high samples in the learning set (left) and in the independent validation set (right). **(C)**. Volcano plot showing the 92 genes differentially expressed in the learning set (TCGA). Genes up-regulated in the *CCNA2*high samples are colored in red and genes down-regulated in green.

Supplemental Figure 12



Supplemental Figure 12: Cyclin A2 protein expression in CRC tumor samples from different stages. (**A**). Cyclin A2 expression analyzed on the same TMA shown in Figure 10, but using a different anti-cyclin A2 antibody from Novo-castra. (Mean values ±SEM, $p<0.05$ for the analysis between stage I and II-MSS, $p<0.01$ for comparison between stage I and stage III, $p<0.001$ for stage I to IV, $p<0.01$ for stage II-MSI to stage IV, two-tailed unpaired t-test). (**B**). Represen-tative immunostaining of stage I, II-MSS, II-MSI, III and IV tumor samples. Scale bar: 100µm

Supplemental Figure 13



Supplemental Figure 13: Ccna2 gene expression signature in colons of cyclin A2 deficient mice is enriched with genes of the CMS4 subtype. (A) Number of differential and common genes between CCNA2 signatures and CMS classification. **(B)** Fisher's exact test evaluating the significance of the enrichement. (ns = non significant). **(C)** List of the 25 genes shared with the CMS4 class. **(D)** Major associated functions covered by these 25 genes.

Supplemental Table 3. Histological scoring for colitis (sum of scores 1 and 2) according to (40).

Severity	**Extent**	**Score 1**	**Epithelial changes**	**Mucosal architecture**	**Score 2**
Mild	Mucosa	1	Focal erosions		1
Moderate	Mucosa and submucosa	2	Erosions	focal ulcerations	2
Marked	Transmural	3		Extended ulcerations± granulation tissue± pseudopolyps	3

Supplemental Table 6. Patients and tumor characteristics of the TMA cohort (n = 65).

Variable	Value (%)
Age at diagnosis (yr)	
Median	66.26
Range	21-89
Gender	
Male	34 (52.3)
Female	31 (47.7)
Tumor Location	
Colon	65 (100)
Tumor Stage	
I	13(20), n=23 tissue samples
II	23 (35.4), n=48 tissue samples
III	15 (23.1)/5, n=30 tissue samples
IV	14 (21.5)/10, n=26 tissue samples

Supplemental Table 9

Target	Primer	Sequence (5'-3')
***Ccna*$^{flox\ allele}$**	5'	CGCAGCAGAAGCTCAAGACTCGAC
	3'	TCTACATCCTAATGCAATGCCTGG
Ccna Δ	5'	CGCAGCAGAAGCTCAAGACTCGAC
	3'	CACTCACACACTTAGTGTCTCTGG
Cre	5'	CAAGCCTGGCTCGACGGCC
	3'	CGCGAACATCTTCAGGTTCT

Supplemental Table 10: origin and dilution of the antibodies used in the study

Antibody for IHC	**company**	**clone**	**Dilution**	**Antigen retrieval**
Cyclin A2	Abcam, Cambridge, UK	ab181591	1 :1000	Tris 10mM EDTA 1mM pH9
Cyclin A2	Novocastra, Newcastle upon Tyne, UK	NCL-CYCLINA	1:500	Citrate pH6
Ki67	Abcam, Cambridge, UK	ab16667	1 :200	Citrate pH6
Ki67	ThermoFisher Scientific, Waltham, USA	SolA15	1 :1000	Citrate pH6
F4/80	Hycult biotech, Uden, Netherland	HM1066	1 :800	Citrate pH6
YM1	R&D system, Minneapolis, USA		1:100	Citrate pH6
TGF-β	Abcam, Cambridge, UK	ab215715	1:250	Tris 10mM EDTA 1mM pH9
non-phospho (Active) β-catenin	Cell signaling, Danvers, USA	#8814	1 :1000	Citrate pH6
anti-γH2AX	Abcam, Cambridge, UK	ab11174	1 :500	Citrate pH6
anti-IL-6	Novus Biological, Centennial, USA	IL-6/1270	1:100	Tris 10mM EDTA 1mM pH9 or Citrate pH6
anti-TAZ	Novus Biologicals, Centennial, USA	NBP1-85067	1:100	Citrate pH6
anti-CD44v6	ThermoFisher, Waltham, USA	clone 9A4	1:100	Citrate pH6
BrdU	Biolegend, San Diego, USA		1:100	Citrate pH6
Antibodies for IF				
Mre11	Novus Biologicals, Centennial, USA	NB-100-142	1:500	Citrate pH6
alpha-tubulin	Novus Biological, Centennial, USA s	NB-600-506	1:1000	1 mM EDTA
Gamma-tubulin	Sigma, Saint Louis, USA	T3320	1:1000	1 mM EDTA
Antibodies for WB				
Cyclin A2	Abcam, Cambridge, UK	ab181591	1 :1000	
NF-kB p65	Cell signaling Technology, Danvers, USA	#8242	1:1000	
non-phospho (Active) β-catenin	Cell signaling Technology, Danvers, USA	#8814	1:1000	
TFIIB	Biolegend, San Diego, USA		1:2000	
Histone H3	Abcam, Cambridge, UK	ab1791	1:1000	
β-actin	Sigma, Saint Louis, USA		1:5000	
Antibodies for FACS				
EpCAM-APC	Biolegend	118213	1:50	
CD45-PE	Biolegend	103105	1:100	
CD90.2-FITC	Becton Dickinson, Franklin Lakes, USA	553013	1:50	

Supplemental Table 11			
	Product provider	WENR	ENR
Growth factors and supplements		concentrations	concentrations
DMEM/F12	Life technologies, Carlsbad, USA		
Glutamax (100x)	Life technologies	1x	1x
Hepes	Life technologies	10mM	10mM
Penicillin/Streptomycin	Life technologies	1x	1x
B27 (50x)	Life technologies	1x	1x
N2 (100x)	Life technologies	1x	1x
N-acetyl-cystein	Sigma, Saint Louis, USA	1mM	1mM
Mouse EGF	Peprotech, Neuilly sur Seine, France	50ng/ml	50ng/ml
Mouse Noggin	Peprotech	100ng/ml	100ng/ml
Human R-spondin-1	Peprotech	500ng/ml	500ng/ml
Mouse WNt3a	Peprotech	100ng/ml	
Rock Inhibitor (Y27632) only for Day 0 and 1 of culture	Sigma	10nM	
CHIR-99021(only for day 0 and passaging)	Peprotech	3 nM	

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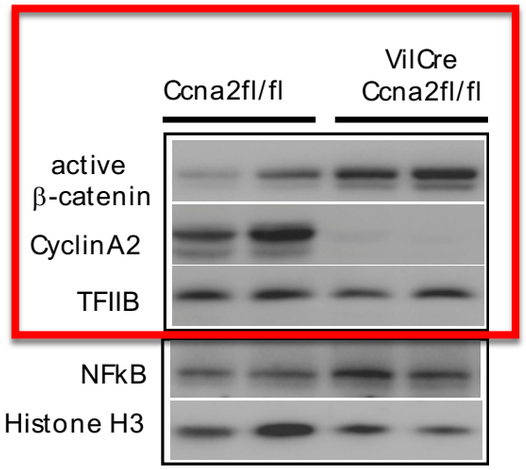
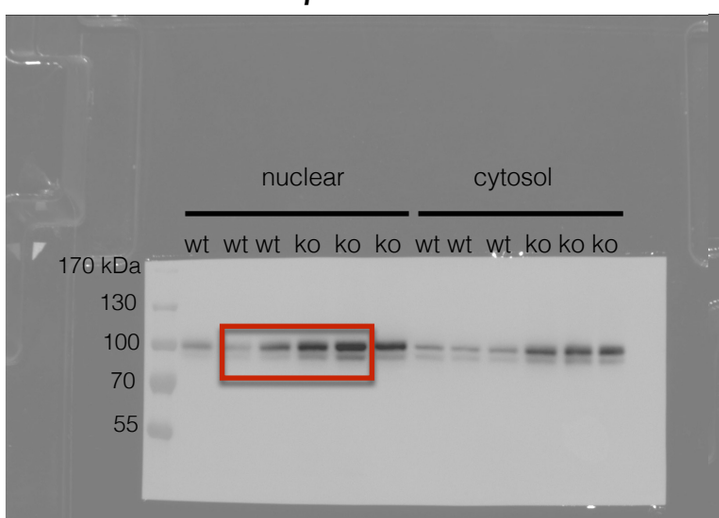
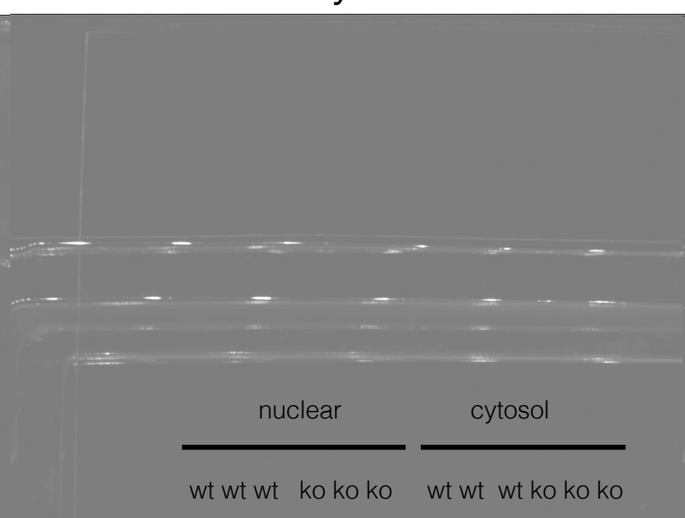


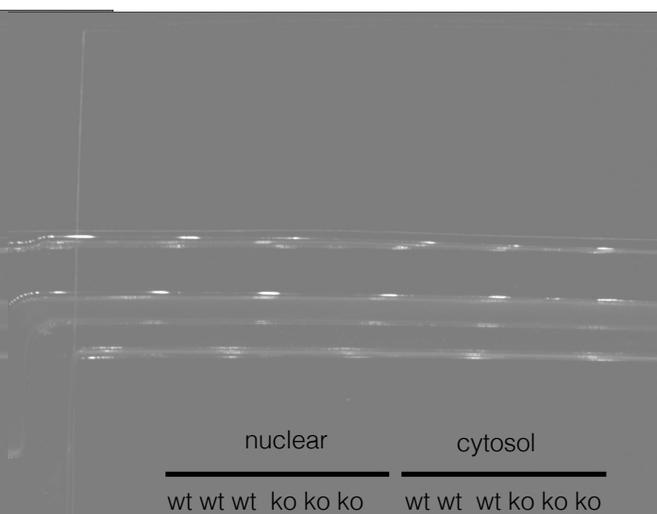
β-catenin



cyclin A2



TFIIB



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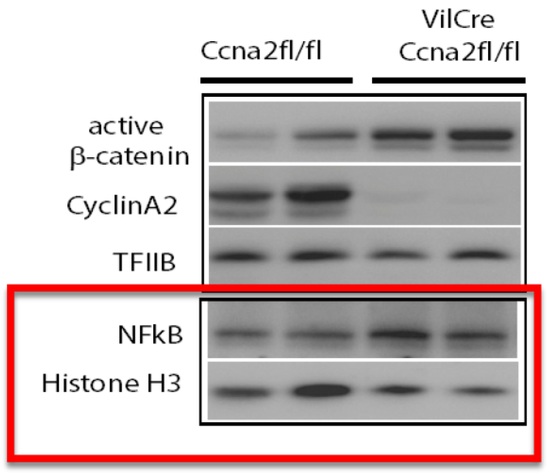
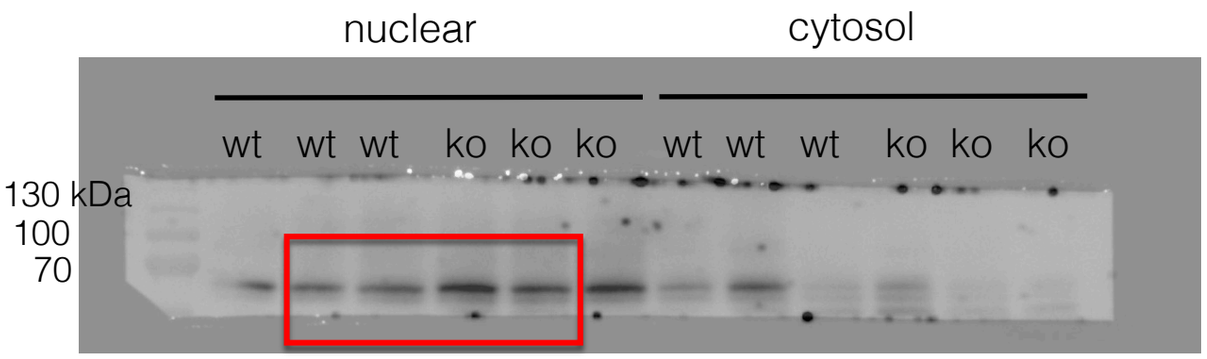
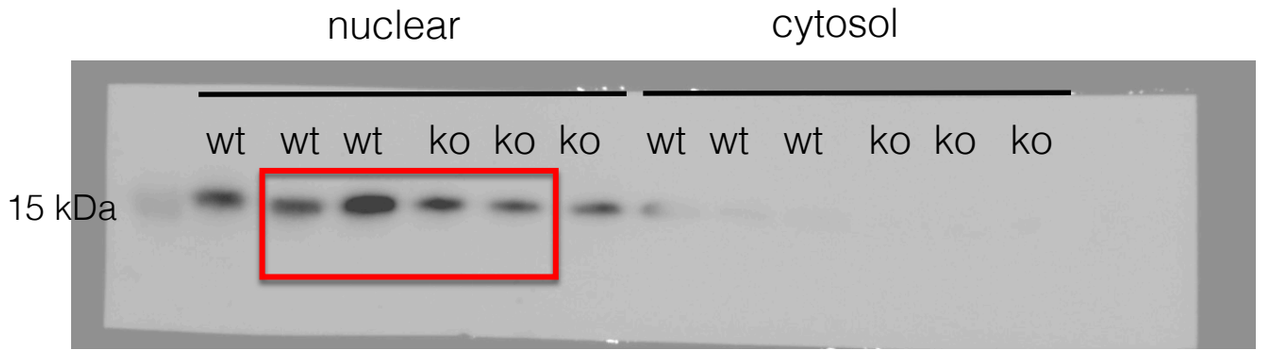


NFκB



Histone H3



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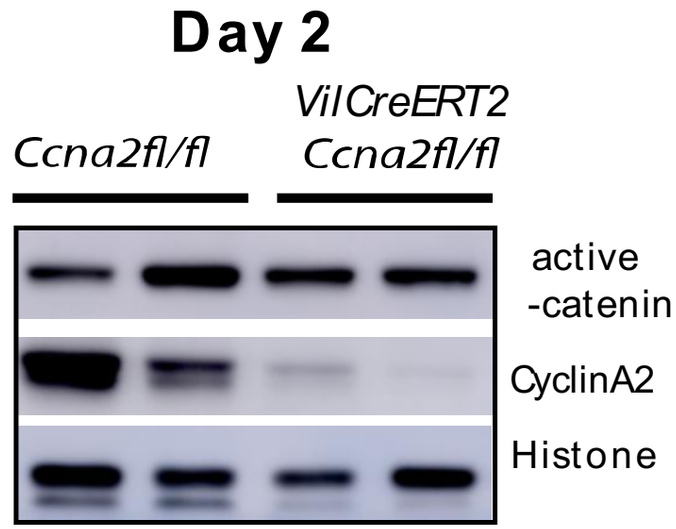

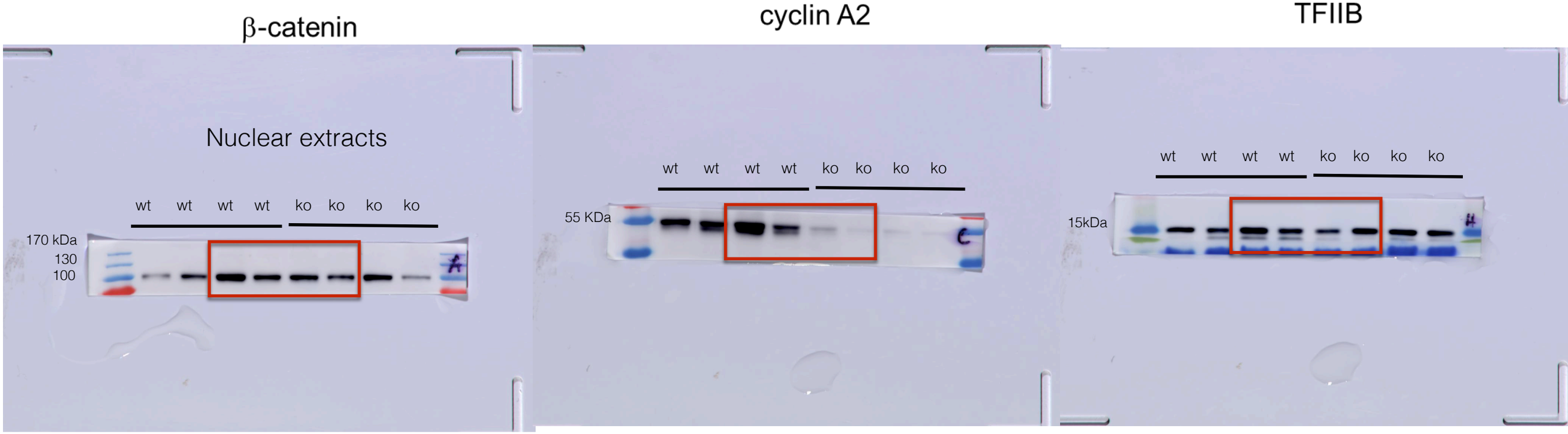

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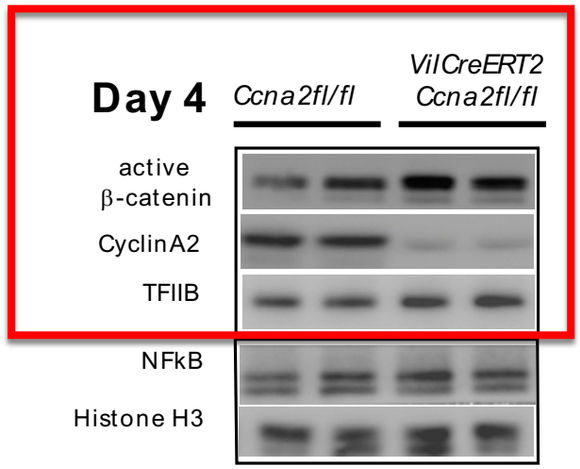


β-catenin

nuclear cytosol

wt wt wt ko ko ko wt wt wt ko ko ko

170 kDa
130
100

cyclin A2

nuclear cytosol

wt wt wt ko ko ko wt wt wt ko ko ko

55 KDa

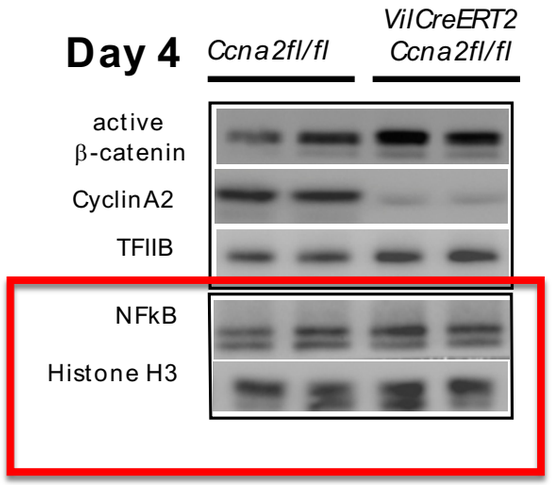
TFIIB

nuclear cytosol

wt wt wt ko ko ko wt wt wt ko ko ko

35 KDa

Full unedited gel for figure5B Day 4 : membrane 2



NFκB

Histone H3