Inhibition of CXCR2 profoundly suppresses spontaneous and inflammation-driven tumorigenesis in mice

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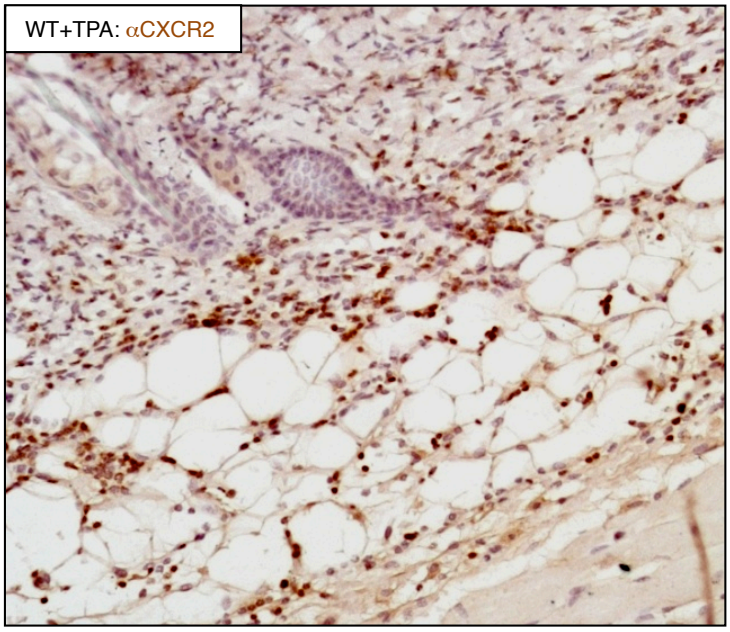
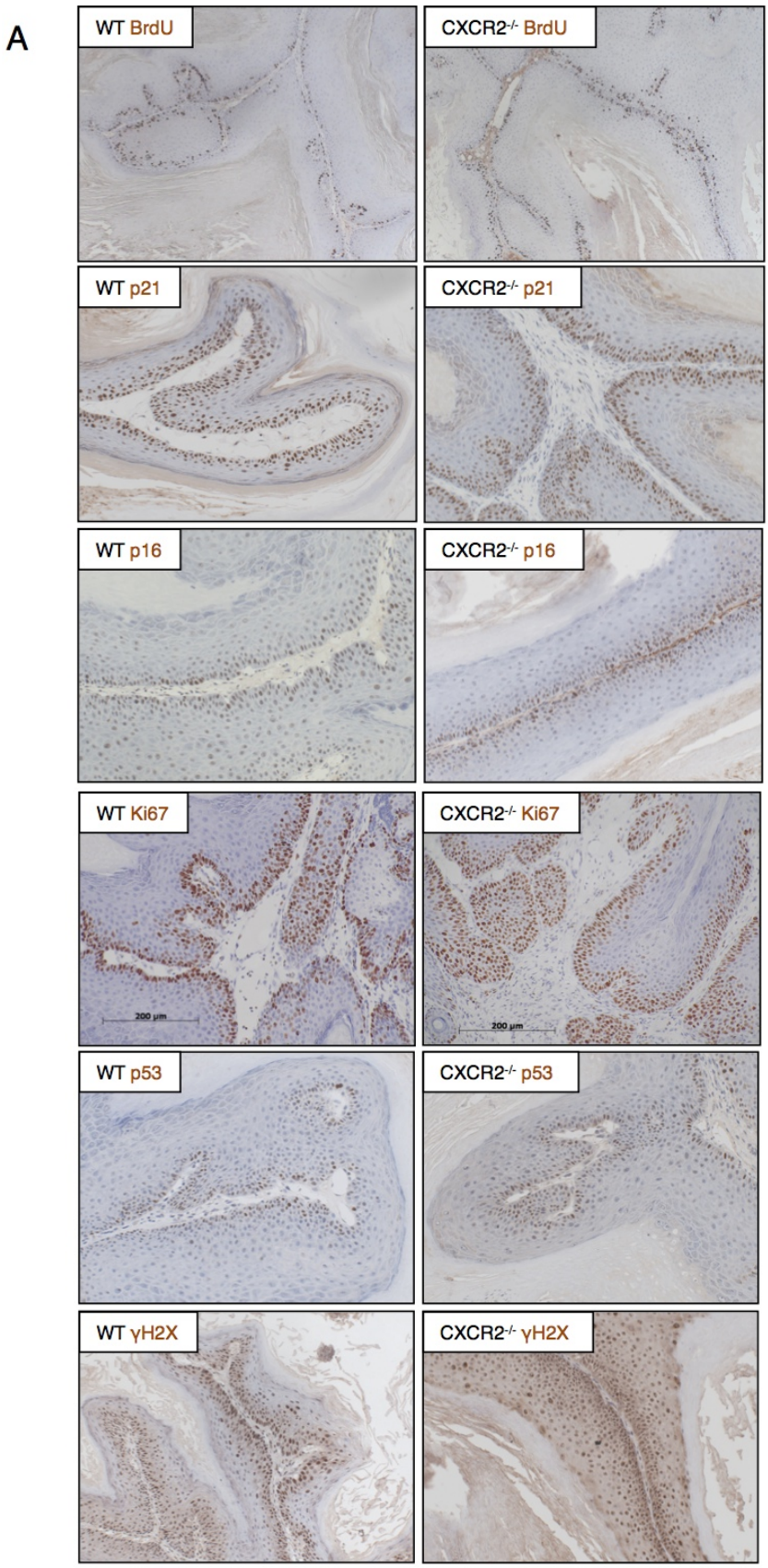
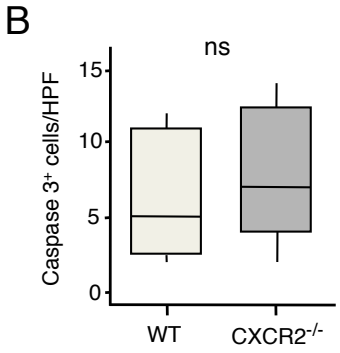
Supplementary Figures



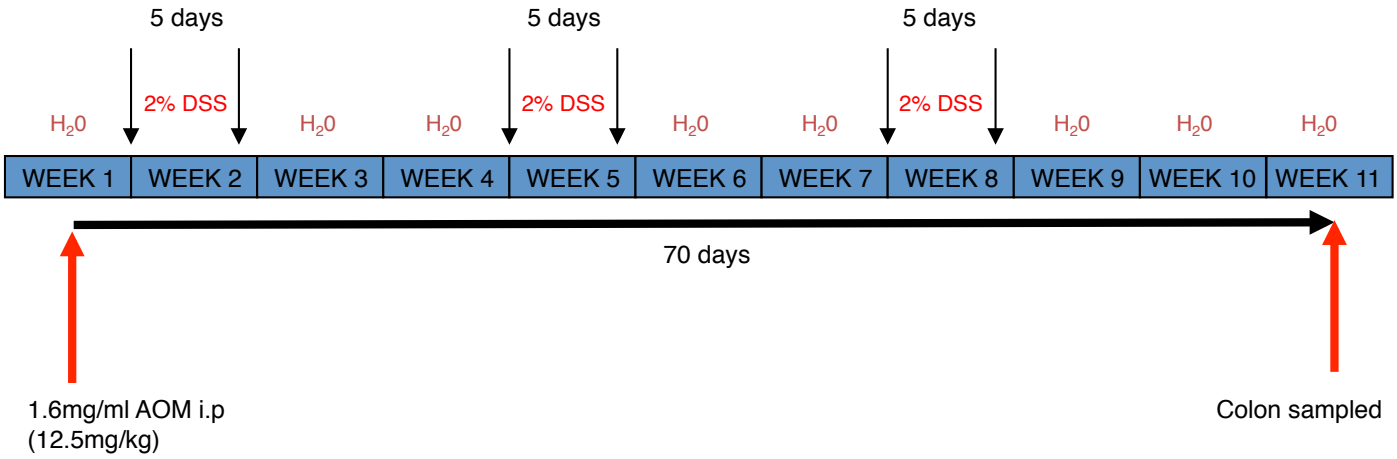
Supplementary Figure 1: TPA-inflamed WT skin immunostained with anti-CXCR2 antibodies. Representative image of a section of anti-CXCR2 immunostained (brown) WT back skin 12 hours after a single TPA application. The section was counterstained with haematoxylin (blue) and visualised by light microscopy.



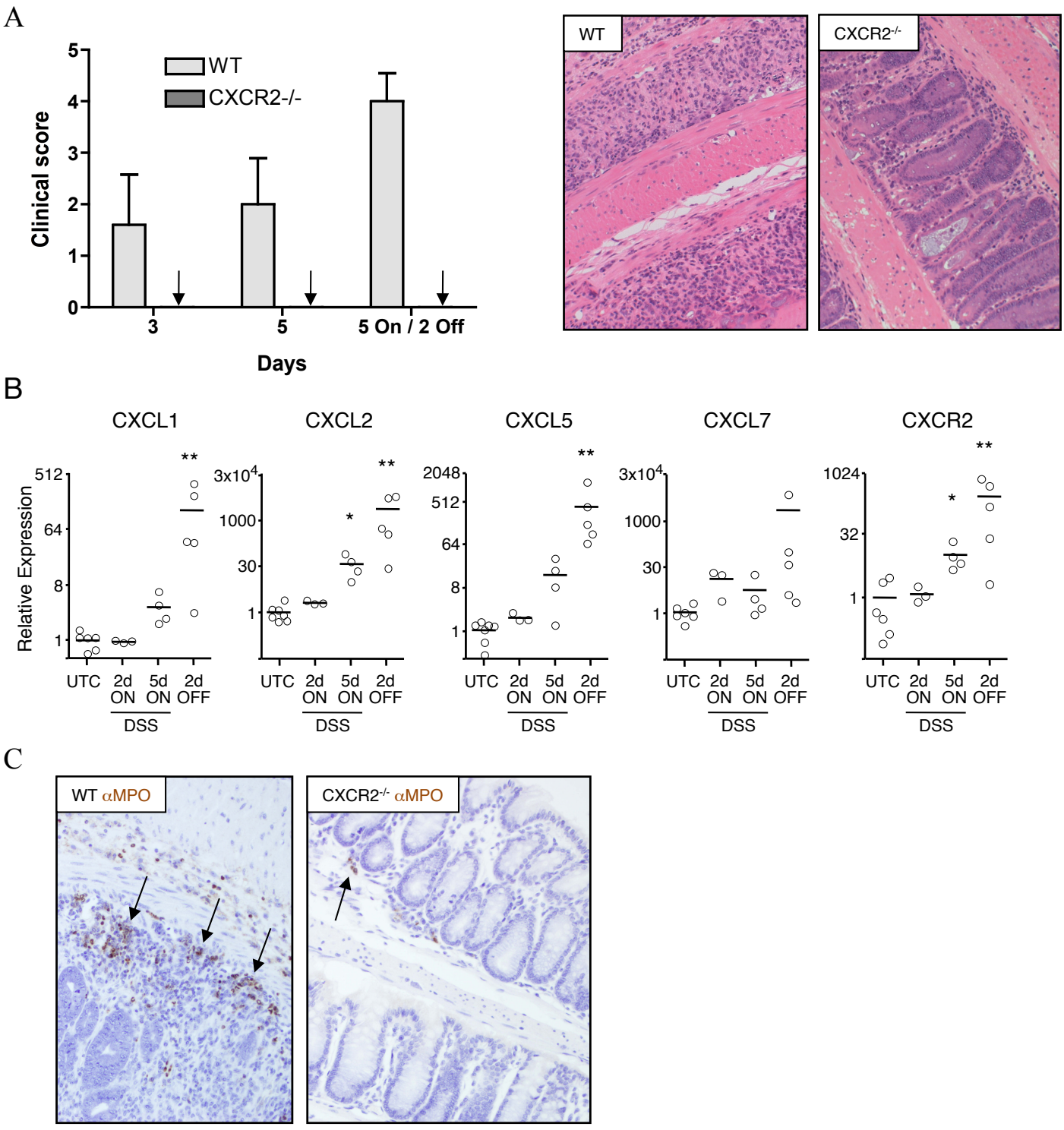
Supplementary Figure 2A: CXCR2 deficiency has no detectable impact on expression of p21, p16, Ki67, p53 or γH2X in papillomas, or ability to incorporate BrdU. Representative images of DMBA/TPA-induced WT and CXCR2-deficient ($CXCR2^{-/-}$) papillomas immunostained with Abs against the indicated proteins, or BrdU (mice were injected i.p. with BrdU 2h before harvest).



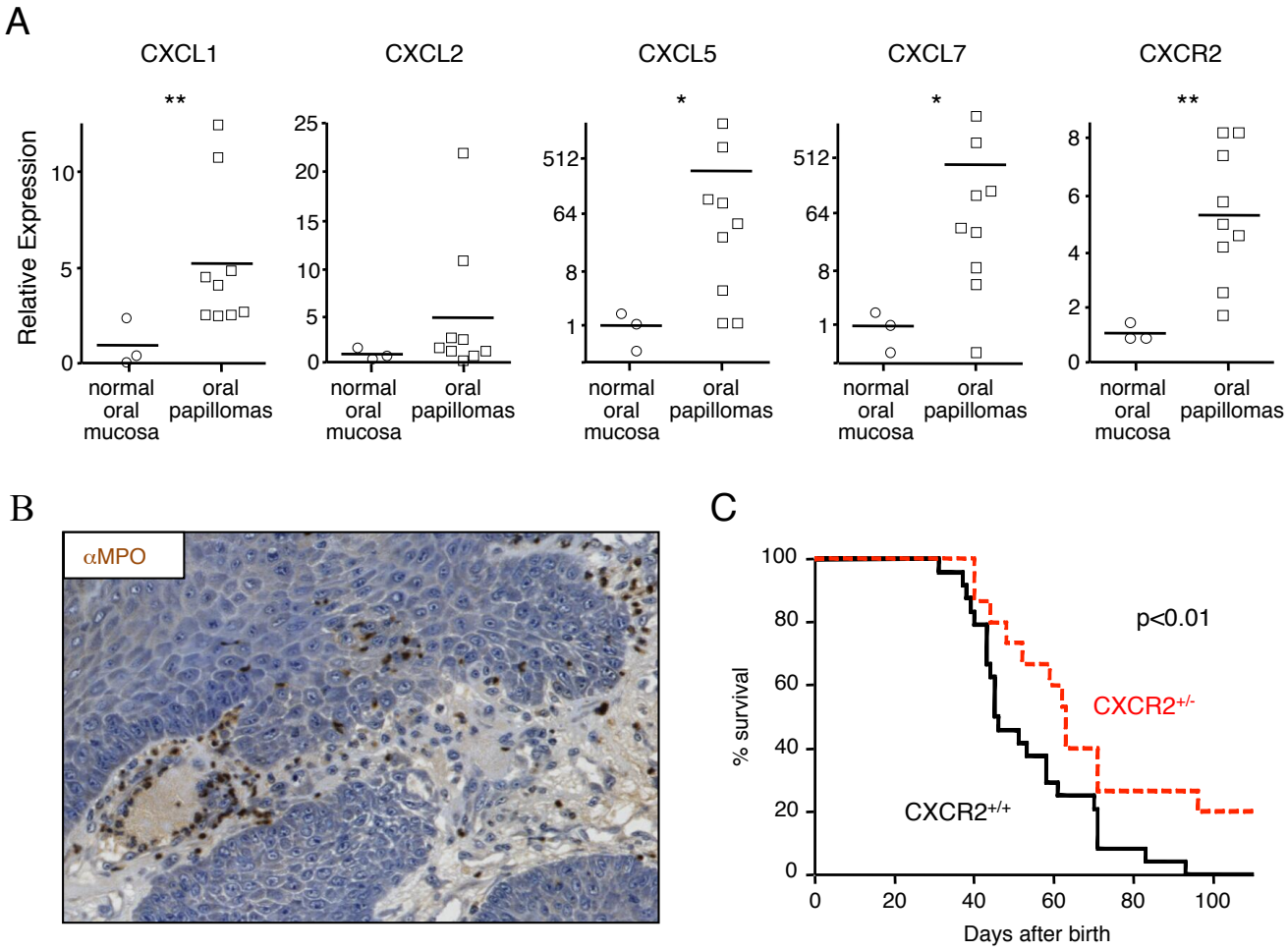
Supplementary Figure 2B: WT and CXCR2 deficient papillomas have similar numbers of tumor cells positive for cleaved caspase 3. Average number of tumor cells positive for cleaved caspase 3 per high-powered field (HPF) in WT and CXCR2 deficient papillomas (n=5 mice per group). ns, not significant (Mann-Whitney).



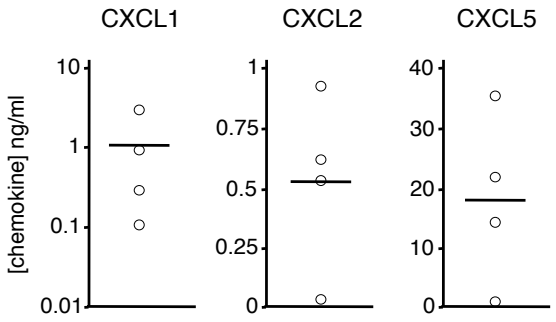
Supplementary Figure 3: Diagrammatic representation of protocol for AOM/DSS induction of colitis-associated adenomas.



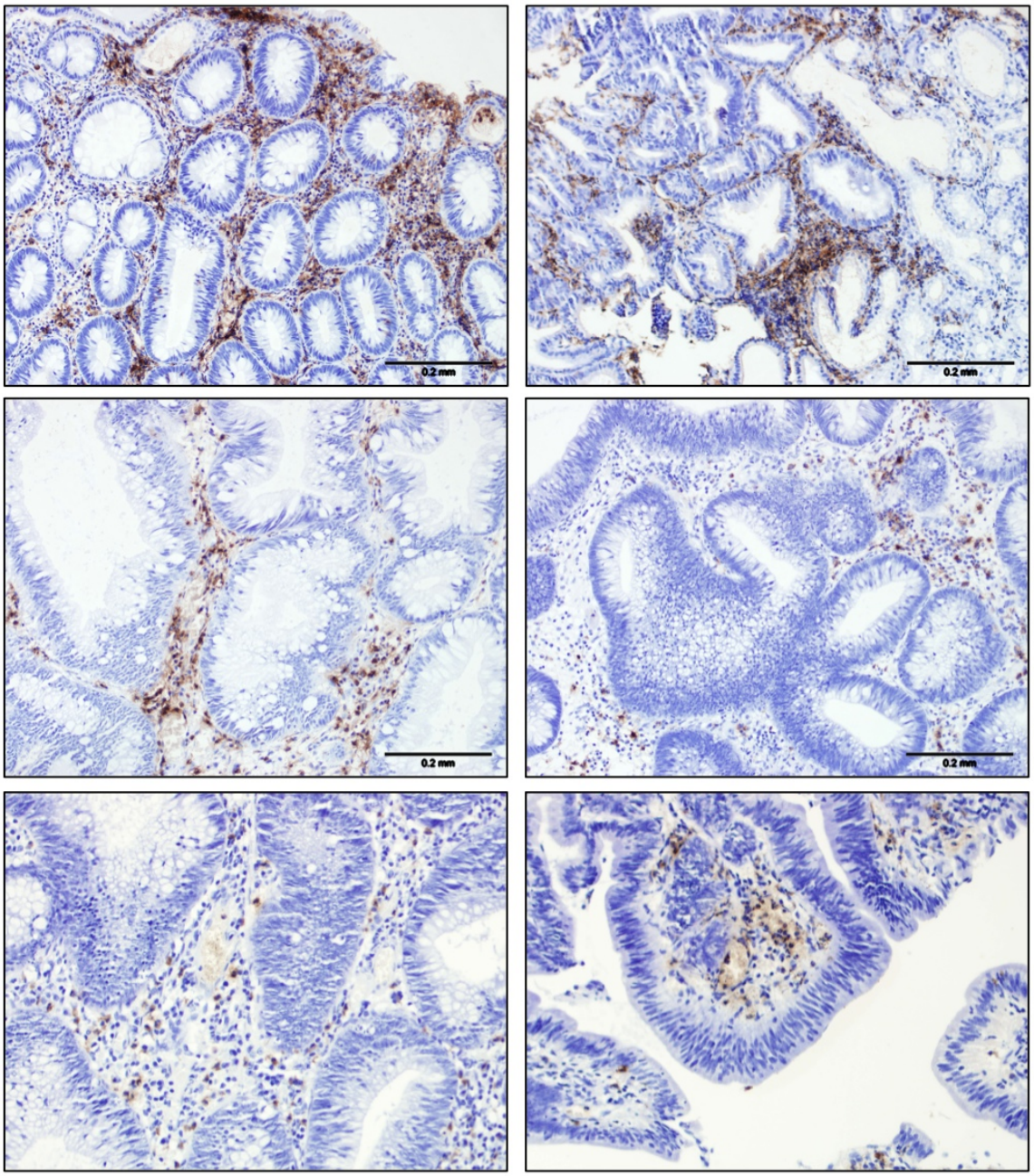
Supplementary Figure 4: CXCR2 deficiency provides protection against colitis induced by 3.5% DSS. WT and CXCR2 deficient (CXCR2$^{-/-}$) mice were fed 3.5% DSS for five days and then normal water for a further 2 days. **A:** The graph shows clinical score of colitis. Arrows indicate that no CXCR2 deficient mice developed any symptoms of colitis. Representative images of H&E stained sections of colons 2 days after being on normal water, after 5 days of 3.5% DSS feeding. **B:** Relative expression of CXCR2 and its ligands in WT Balb/c colon during induction of colitis, as determined by Q-RT-PCR: the mean expression in colon of untreated mice (UTC) is set to 1. 2d ON, 5d ON: 2 and 5 days of 3.5% DSS feeding, respectively; 2d OFF: 2 days after return to normal water after 5 days of 3.5% DSS feeding. *$p<0.05$, **$p<0.01$ vs UTC (one-way ANOVA with multiple comparison post test). **C:** Representative images of sections of colons from WT and CXCR2 deficient (CXCR2$^{-/-}$) mice immunostained with anti-MPO antibodies (brown) 2 days after returning to normal water after 5 days of 3.5% DSS feeding. Sections were counterstained with haematoxylin and visualised by light microscopy.



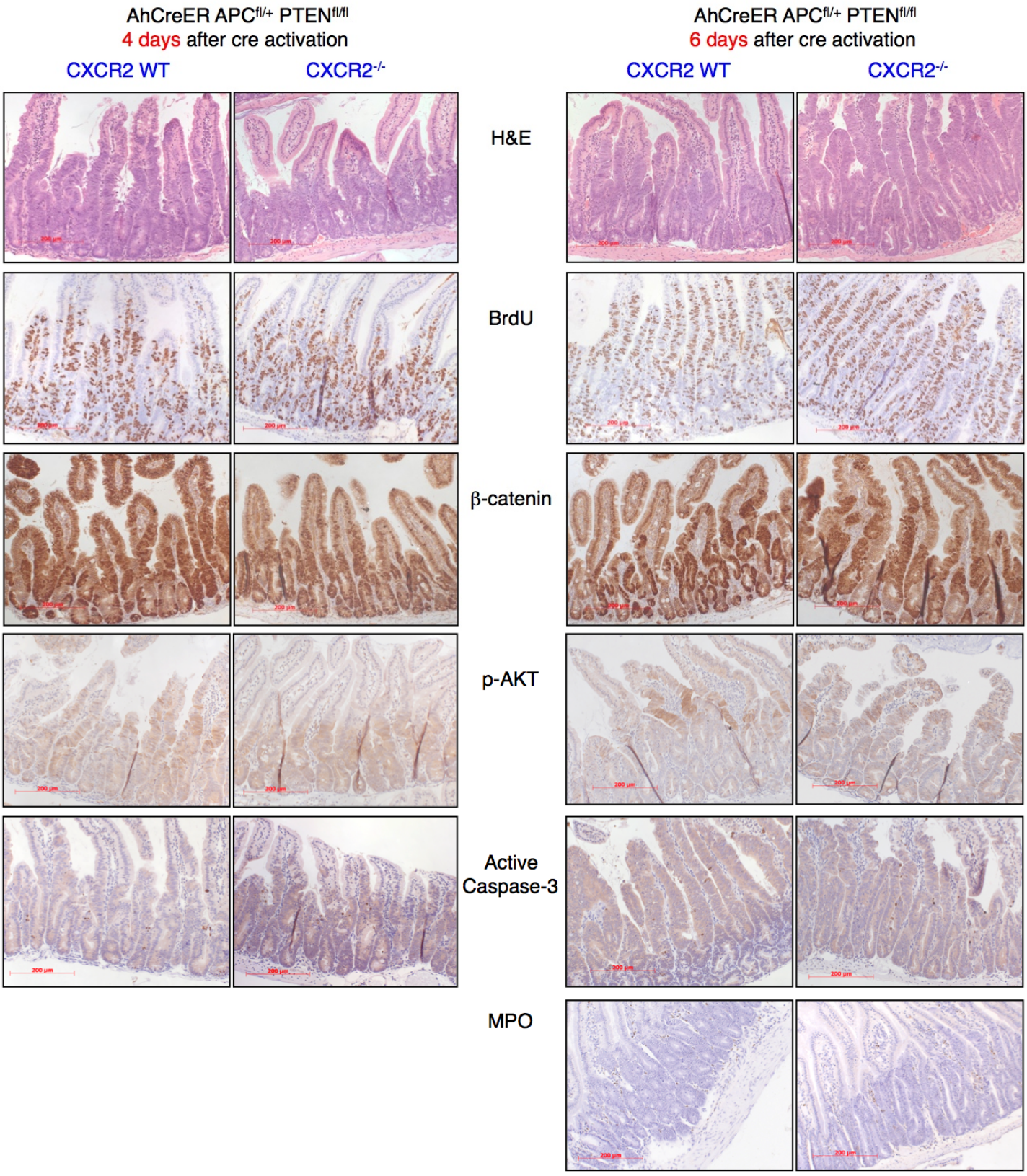
Supplementary Figure 5: CXCR2 contributes to the development of oral papillomas in *K14CreER KRas$^{G12D/+}$* mice. **A:** Relative expression of CXCR2 and its ligands by oral papillomas harvested from 5-8 week old *K14CreER KRas$^{G12D/+}$ CXCR2$^{+/+}$* mice. Data were generated by Q-RT-PCR. The mean expression in normal oral mucosa is set to 1. *p<0.05, **p<0.01 (Mann-Whitney). **B:** Representative image of a section of an oral papilloma from a *K14CreER KRas$^{G12D/+}$* mouse immunostained with anti-MPO antibodies (brown), counterstained with haematoxylin, and visualised by light microscopy. **C:** Survival curve of *K14CreER KRas$^{G12D/+}$ CXCR2$^{+/-}$* and *K14CreER KRas$^{G12D/+}$ CXCR2$^{+/-}$* mice. Data were analysed using Logrank test.



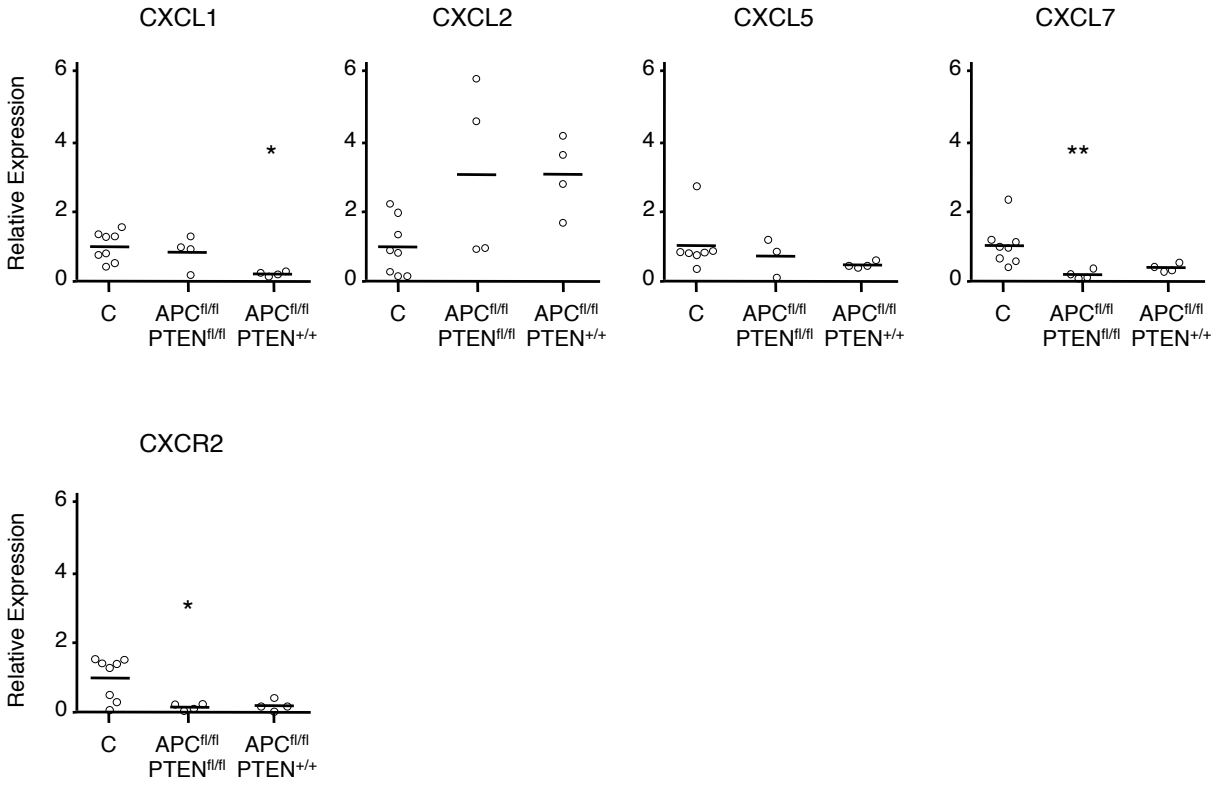
Supplementary Figure 6: CXCL1, CXCL2 and CXCL5 levels in *Apc*$^{Min/+}$ adenoma cultures. The concentration of CXCL1, 2 and 5 protein was quantified by ELISA in conditioned media from four *ex vivo* cultures of *Apc*$^{Min/+}$ adenomas. Conditioned media from WT crypt cultures contained <0.025ng/ml of each chemokine analyzed.



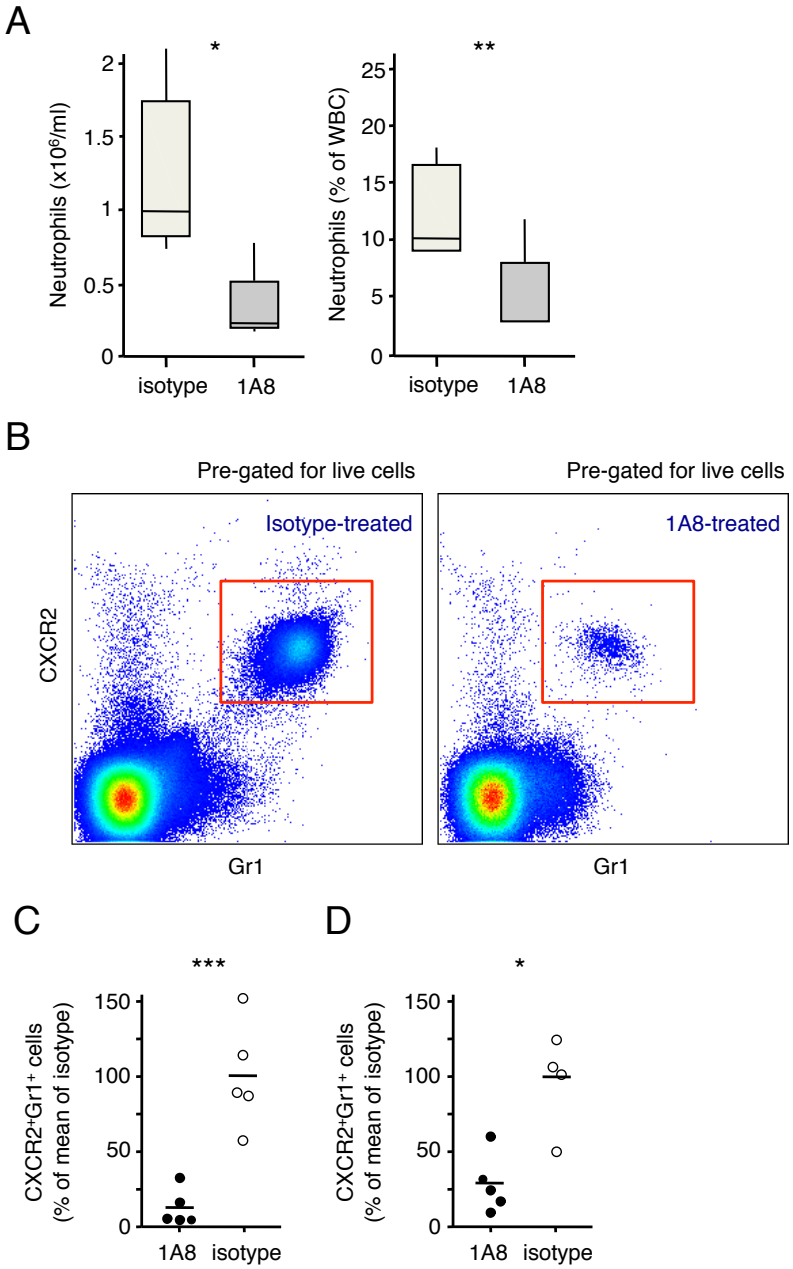
Supplementary Figure 7: MPO$^+$ cells are a common feature of the stroma of human intestinal adenomas. Selected, representative images of sections from six human intestinal adenomas immunostained with anti-MPO antibodies (brown), counterstained with haematoxylin, and visualised by light microscopy.



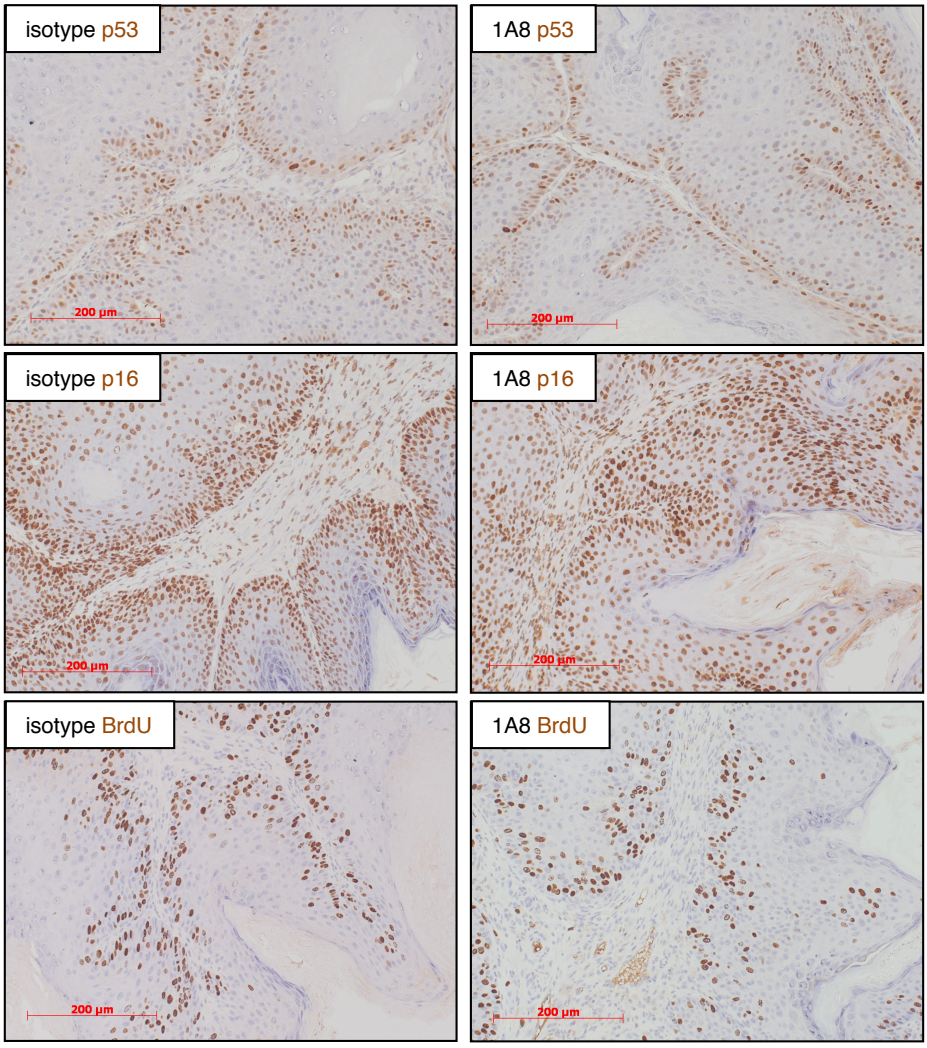
Supplementary Figure 8: CXCR2 deficiency has no detectable impact on early response of intestine to APC and PTEN deletion. *AhCreER APC$^{fl/fl}$ PTEN$^{fl/fl}$ Cxcr2$^{+/+}$* and *AhCreER APC$^{fl/fl}$ PTEN$^{fl/fl}$ Cxcr2$^{-/-}$* mice were treated on four consecutive days i.p. with cre inducers β-napthoflavone and tamoxifen. 4 or 6 days later, 2h after a pulse with BrdU, small intestines were isolated, fixed, sectioned and stained with H&E, or immunostained with antibodies against BrdU, β-catenin, p-AKT, cleaved caspase 3, and MPO and counterstained with haematoxylin. Groups of four mice per genotype were analysed, and representative images are shown.



Supplementary Figure 9: Expression of CXCR2 ligands is not induced in the intestine by cre-mediated PTEN and/or APC deletion. *AhCreER APC$^{fl/fl}$ PTEN$^{fl/fl}$* and *AhCreER APC$^{fl/fl}$ PTEN$^{+/+}$* mice were treated on four consecutive days i.p. with cre inducers β-napthoflavone and tamoxifen. 4 days later intestines were harvested, RNA isolated, and Q-RT-PCR performed for CXCR2 ligands, using non-treated WT intestines as controls (C) (set to 1). *$p<0.05$, **$p<0.01$ vs control (one way ANOVA with multiple comparison post test).



Supplementary Figure 10: 1A8 depletes circulating neutrophils/CXCR2$^+$Gr1$^+$ cells for up to three weeks. Mice were treated with 500µg of 1A8 (anti-Ly6G) or 2A3 (isotype control) once (A and C), or three times a week for three weeks (B and D), and harvested 48 hrs after the last antibody injection. n=4-5 per group. **A:** Circulating neutrophils were enumerated (number/ml of blood or % of WBC (white blood cells)) using an ADVIA 2120 Hematology System. **B:** Representative flow cytometry profiles of live white blood cells from mice after three weeks of treatment with 1A8 or isotype control antibodies. Cells were labelled with fluorescent antibodies against CXCR2 and Gr1, and dead cells excluded using viability dyes. Red boxes highlight live CXCR2$^+$Gr1$^+$ cells. **C-D:** Relative number of live CXCR2$^+$Gr1$^+$ cells retrieved from the blood of mice treated once (C) or for three weeks (D) with 1A8 or isotype control antibodies. Data are presented as % of mean number of CXCR2$^+$Gr1$^+$ cells in isotype-treated mice. *p<0.05; **p<0.01, ***p<0.001 (Mann-Whitney). Additional data on 1A8-treated mice is shown in Figure 7F.



Supplementary Figure 11: Ly6G$^+$ cell depletion does not alter the expression of p53 or p16 by papillomas, or their ability to incorporate BrdU. Representative images of DMBA/TPA-induced papillomas from WT mice (treated with 1A8 or isotype control antibody) immunostained with antibodies against p53 or p16, or incorporated BrdU (mice were injected i.p. with BrdU 2h before harvest).