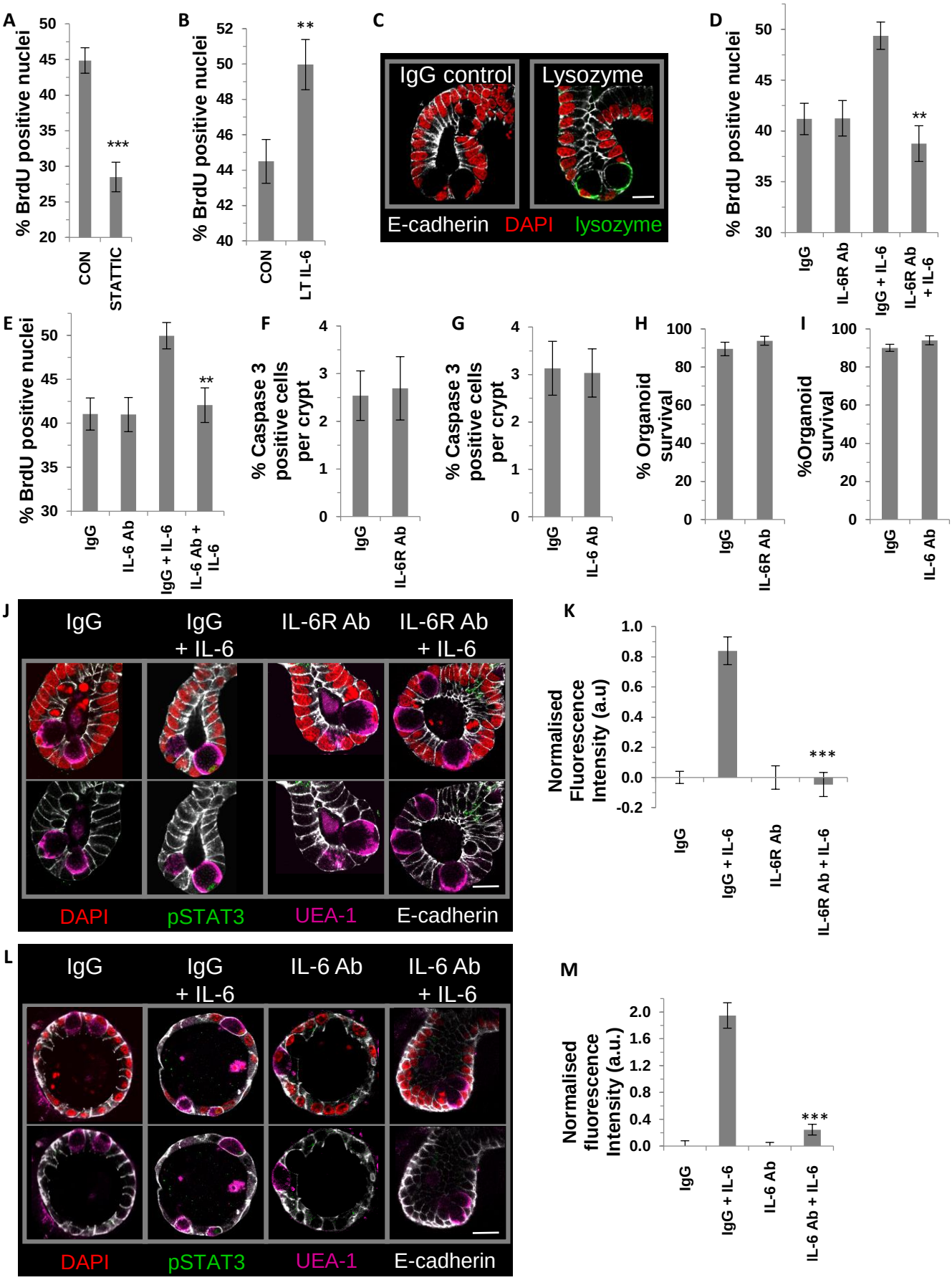
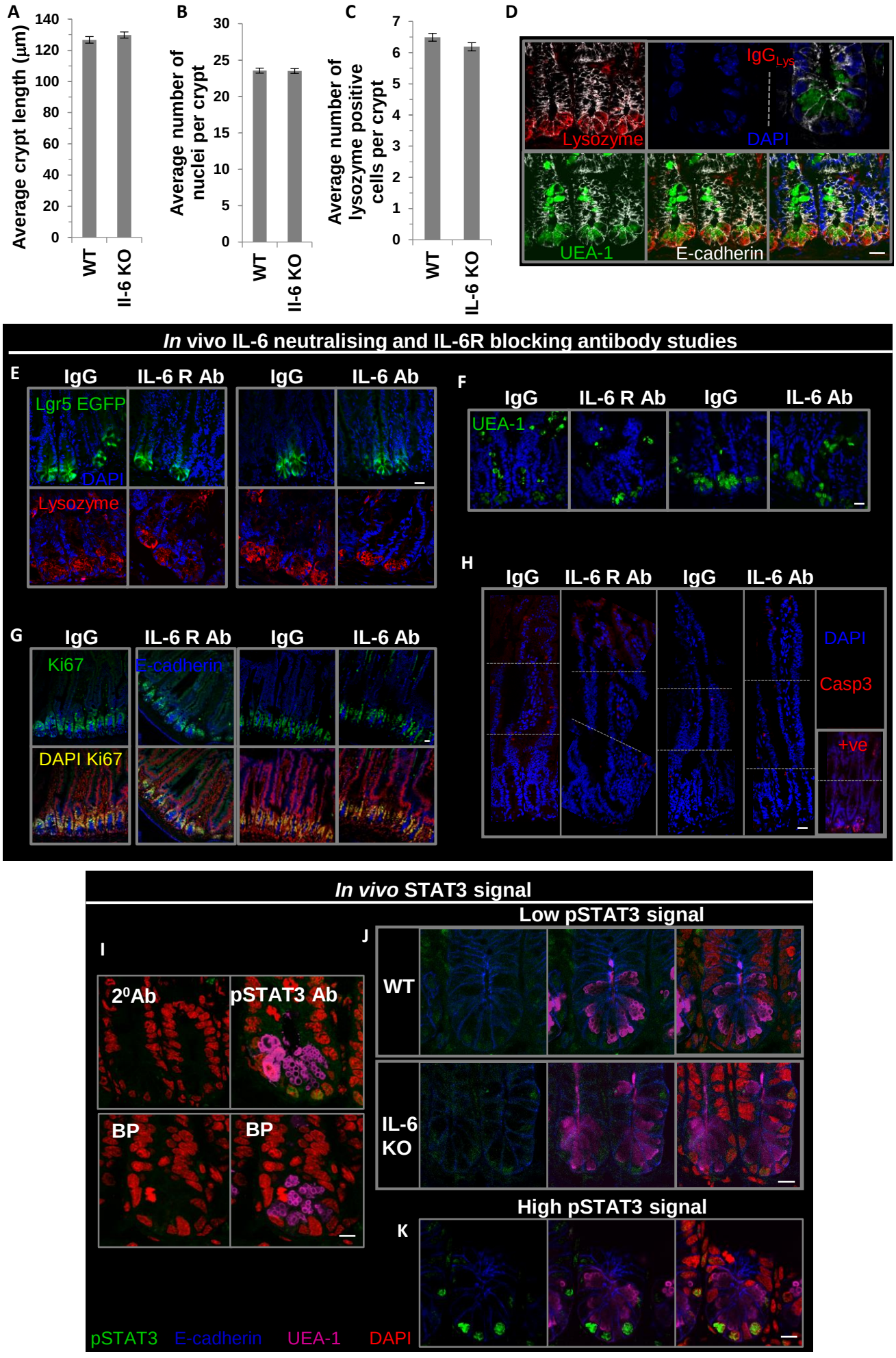
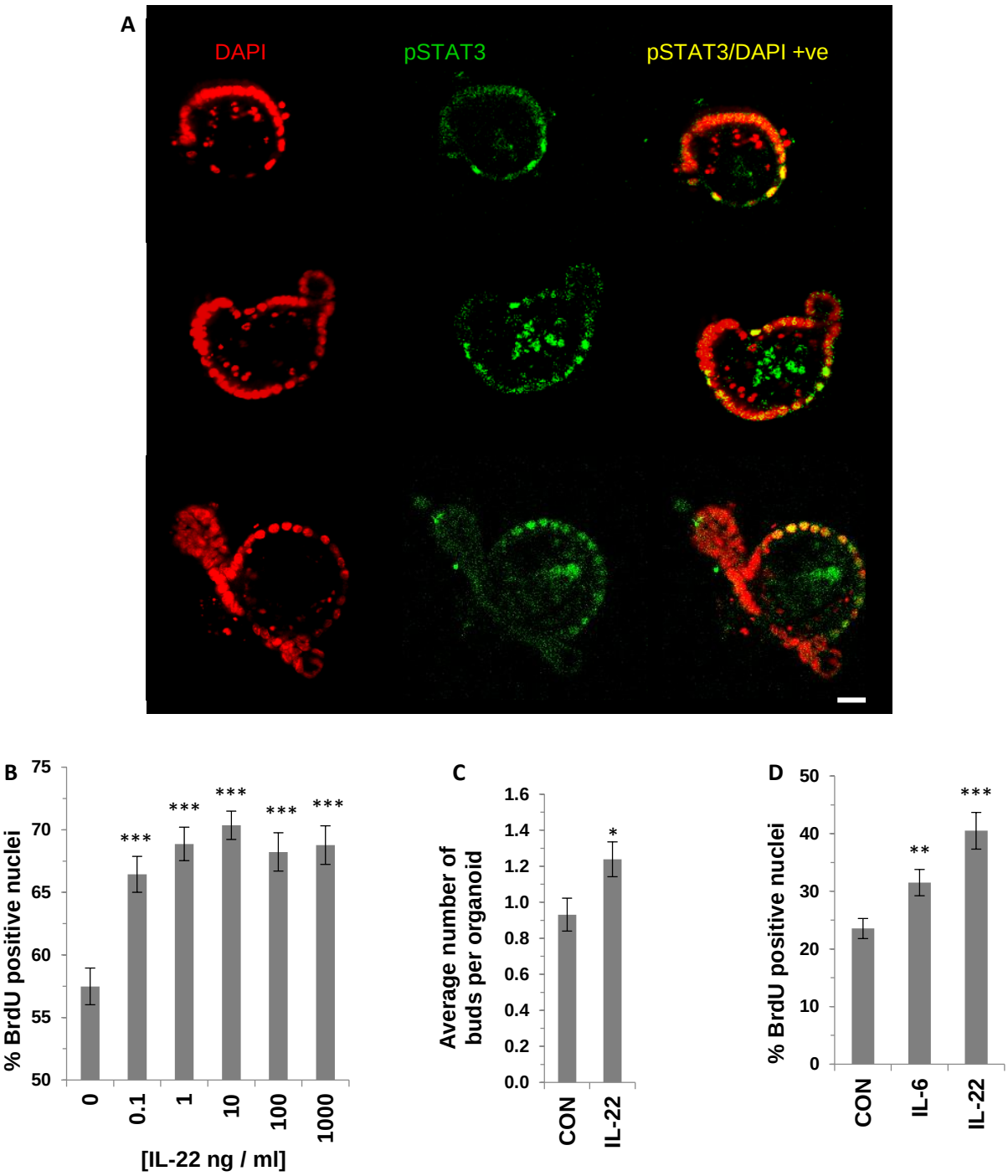


Supplemental Figure 1. Modulation of the STAT3-IL-6 signalling-axis in small intestinal organoids.
(**A**) Histogram showing a significant reduction in the percentage of BrdU positive nuclei in small intestinal organoids following 48 hour culture with STATTIC (20μM) in full growth factor media compared to control organoids (n=3, ***P<0.001). (**B**) Histogram showing a significant increase in BrdU incorporation following exposure to IL-6 (100ng / ml) for 5 days, media was changed every 2 days. BrdU was added for the last 18 hours of culture (n=3, **P<0.01) (**C**) Representative confocal images showing labelling of lysozyme (green) with a primary mouse anti-lysozyme antibody followed by a secondary donkey anti-mouse Alexafluor 568 antibody and absence of labelling with the mouse IgG antibody control. E-cadherin white. (**D-E**) Histograms showing a significant reduction in the percentage of BrdU positive nuclei in small intestinal organoids treated for 18 hours with IL-6 (100 ng / ml) in the presence of (**D**) an IL-6 receptor blocking (n=4, **P<0.01) or (**E**) an IL-6 neutralising antibody compared to respective IgG + IL-6 treated crypts (n=3, **P<0.01). (**F**) Histogram showing the percentage of caspase 3 positive cells in organoids following culture with an IL-6 receptor antibody or a (**G**) IL-6 neutralising antibody compared to respective IgG controls (n=3). Histograms showing the organoid survival after treatment with (**H**) an IL-6 receptor antibody or (**I**) IL-6 neutralising antibody compared to respective IgG controls (n=3) (**J**) Representative confocal images showing abrogation of IL-6-induced pSTAT3 labelling (green) in the nucleus (DAPI-red) of UEA-1 positive cells (pink) in organoids treated with an IL-6 receptor blocking antibody compared to IL-6 + IgG treated crypts. E-cadherin white. (n=3). (**K**) Histogram showing a significant reduction in IL-6-induced pSTAT3 fluorescence intensity (a.u.) in organoids treated with an IL-6 receptor blocking antibody compared to IL-6 + IgG treated crypts. Fluorescence intensity was normalised to control (n=3, ***p<0.001). (**L**) Representative confocal images showing abrogation of IL-6-induced pSTAT3 labelling (green) in the nucleus (DAPI-red) of UEA-1 positive cells (pink) in organoids treated with an IL-6 neutralising antibody compared to IL-6 + IgG treated crypts. E-cadherin white. (n=3). (**M**) Histogram showing a significant reduction in IL-6-induced pSTAT3 fluorescence intensity (a.u.) in organoids treated with an IL-6 neutralising antibody compared to IgG + IL-6 treated crypts. Fluorescence intensity was normalised to control (n=3, ***p<0.001). Data are represented as mean +/- SEM. Scale bar 10μm.

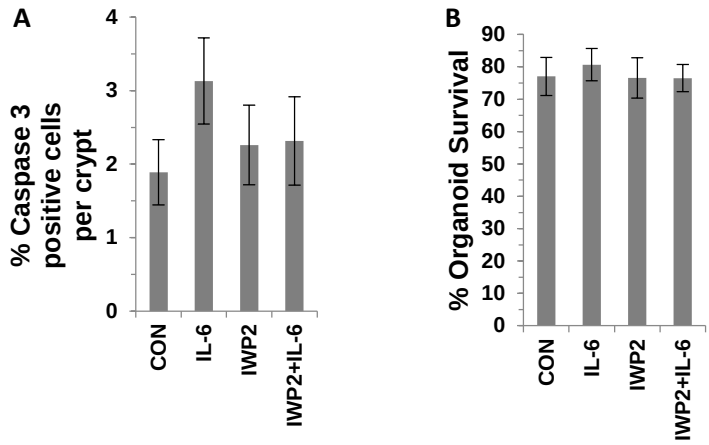


Supplemental Figure 2. Crypt morphology and pSTAT3 labelling of IL6KO tissue is the same as WT. IL-6 receptor blocking or neutralising antibodies alter crypt stem cell and Paneth cell status.

Histogram showing (**A**) the average crypt length (**B**) the average number of crypt nuclei and (**C**) the average number of lysozyme positive cells per crypt in the small intestine of IL-6 knockout mice and WT (n=3). Data are represented as mean +/- SEM. (**D**) Representative confocal images demonstrating *in vivo* labelling of lysozyme (red) and absence of labelling with a corresponding IgG to the mouse lysozyme antibody followed by a secondary anti-mouse IgG Alexafluor 568 antibody (red). Lysozyme positive cells (red) were also UEA-1 positive (green). E-cadherin; white and DAPI / nuclei blue. (**E-H**) Sections from IL-6 neutralising antibody, IL-6 receptor antibody and IgG treated mice. Representative confocal images showing a decrease in (**E**) Lgr5EGFP (green) , lysozyme (red) and (**F**) UEA-1 (green) positive cells in antibody treated mice compared to IgG controls. (**G**) Representative confocal images of Ki67 labelling (green). DAPI / nuclei-red, E-cadherin-blue. (**H**) Caspase 3 (red) labelling (+ve; caspase 3 positive control tissue) DAPI / nuclei-blue. (**I-K**) Representative confocal images of *in vivo* WT and IL-6KO small intestine. (**I**) A blocking peptide (BP) to pSTAT3 pre-incubated with the pSTAT3 antibody abrogated the pSTAT3 labelling in the nucleus of UEA-1 positive cells (pink) compared to tissue treated with pSTAT3 antibody (green) alone. No nuclear labelling was observed utilizing the secondary antibody (2^0Ab) alone. (**J**) Representative confocal images demonstrating low level pSTAT3 (green) labelling in UEA-1 (pink) positive cells of both WT and IL-6 KO mice. Images are projections of several focal planes formed using image analysis software. (**K**) Representative confocal image of high pSTAT3 fluorescent labelling observed in the mouse small intestine. Experiments were repeated on three different mice. Scale bar 10µm.



Supplemental Figure 3. IL-22 induces global nuclear pSTAT3 activation, BrdU incorporation and crypt budding in small intestinal organoids. IL-6 and IL-22 induces BrdU incorporation in colonic organoids.
(**A**) Representative confocal images showing the presence of pSTAT3 immunofluorescent labelling (green) in the nuclei (red) of crypt organoids following 1 hour IL-22 (100 ng / ml) stimulation. Scale bar 50 μm. (**B**) Histogram showing the percentage of BrdU positive nuclei in small intestinal organoids following 24 hours stimulation with IL-22 (0.1- 1000 ng / ml) compared to control (n=3,***P<0.001). (**C**) Histogram showing the average number buds per small intestinal organoid following IL-22 (10 ng / ml) treatment (n=3, *P<0.05) compared to control. (**D**) Histogram showing the percentage of BrdU positive nuclei in colonic organoids following 24 hours stimulation with IL-6 (100 ng / ml) or IL-22 (100 ng / ml) compared to control (n=3,**P<0.01; ***P<0.001).



Supplemental Figure 4. IWP2 has no effect on cell death and survival of small intestinal organoids.
Histogram showing no change in (**A**) the percentage of caspase 3 positive cells per crypt or (**B**) the percentage survival of organoids, following 17h culture with IWP2 (5μM) +/- IL-6 (100 ng / ml) compared to control (DMSO).