Supplementary Figures

Th17-type cytokines, IL-6 and TNF-α synergistically activate STAT3 and NF-kB to promote colorectal cancer cell growth

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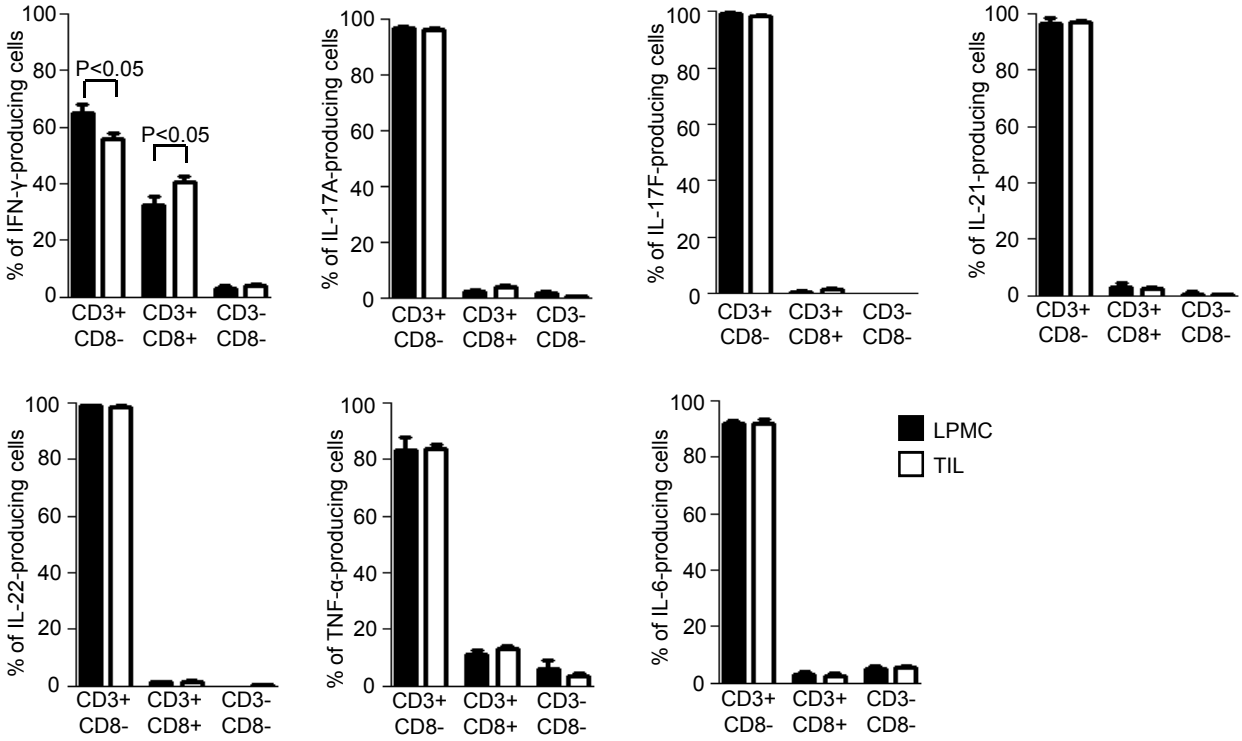
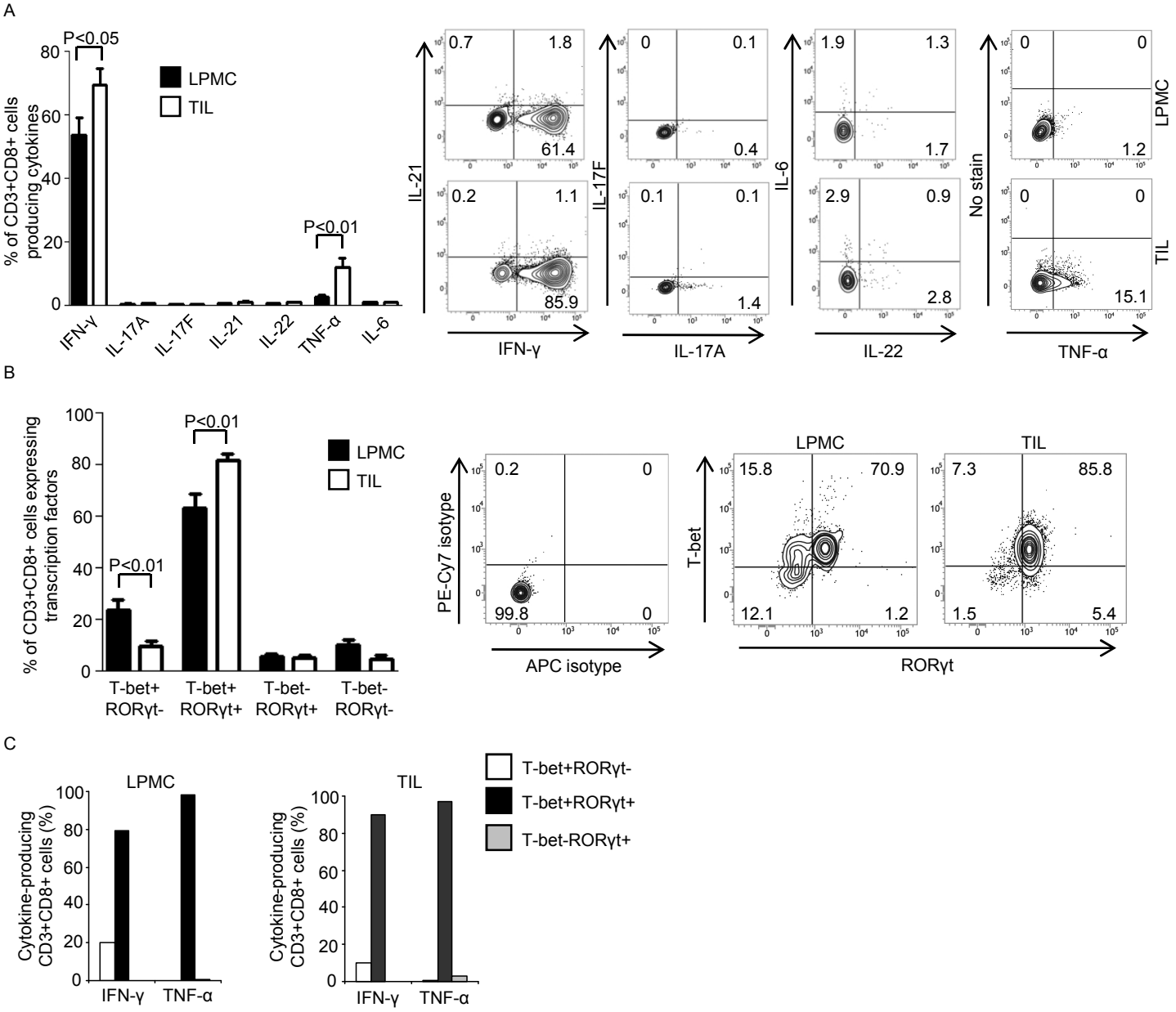
Supplementary figure 1 De Simone et al.

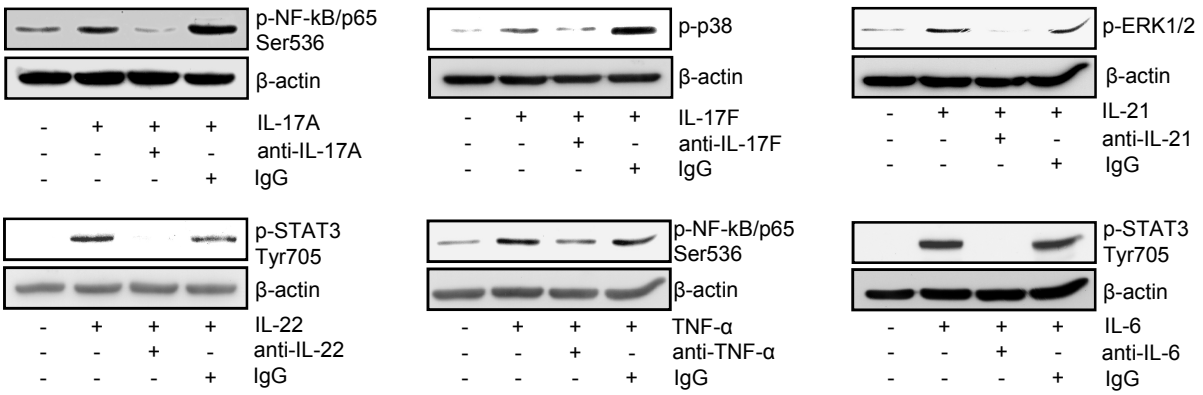


Supplementary figure 1: Characterization of IFN-γ-, Th17-related cytokine-, TNF-α- and IL-6-producing immune cell subsets in LPMC and TIL. Representative histograms showing the percentage of IFN-γ-, Th17-related cytokine-, TNF-α- and IL-6-producing-CD45+ cells in LPMC and TIL isolated from adjacent tumor and non-tumor areas of 14 patients undergoing colectomy for sporadic CRC. IFN-γ-, IL-17A-, IL-17F-, IL-21-, IL-22-, TNF-α- and IL-6-producing CD45+ cells were gated and analyzed for the indicated markers. Data are expressed as mean ± S.E.M. and differences were calculated using the two-tailed Student's t test.

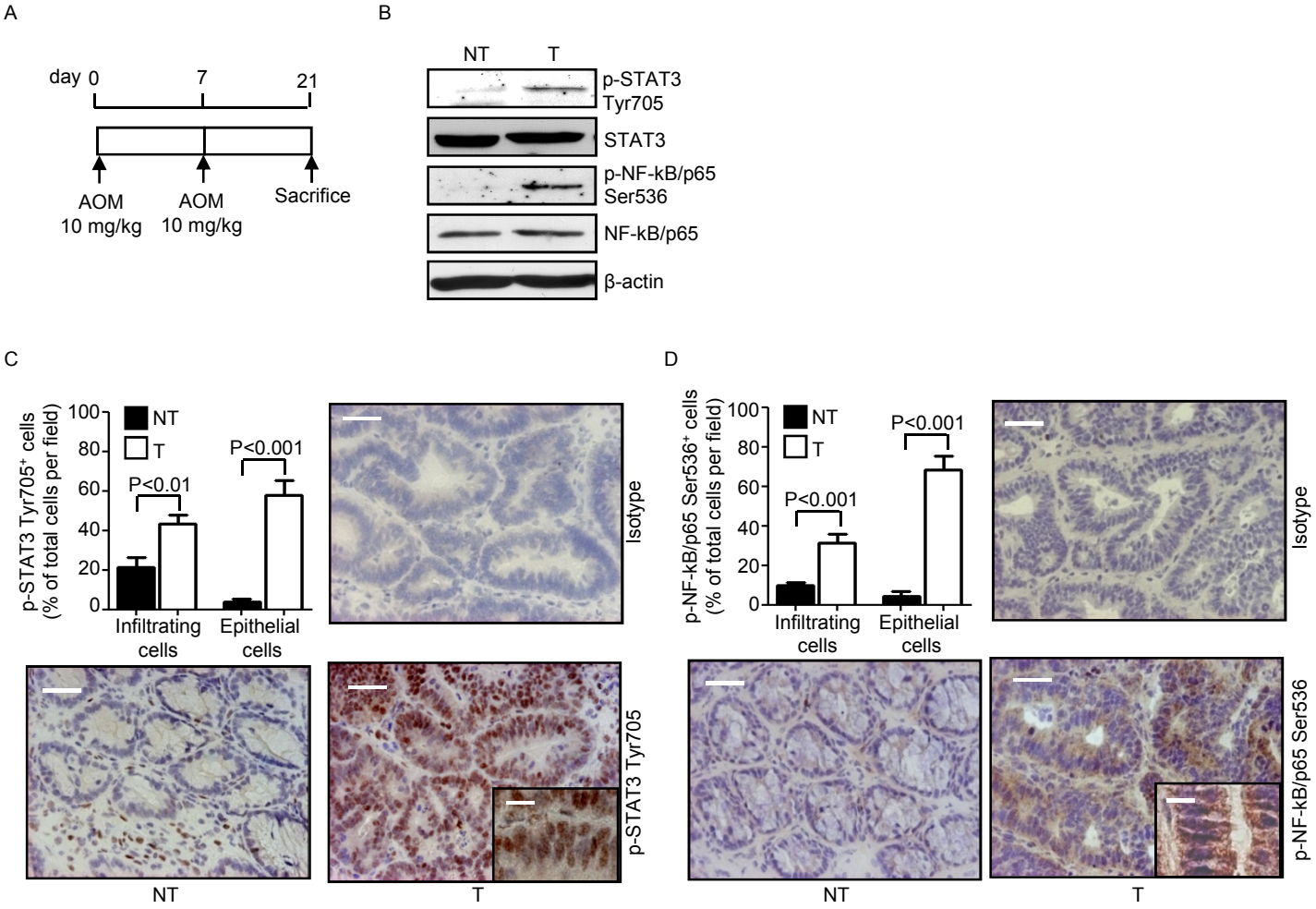
Supplementary figure 2 De Simone et al.



Supplementary figure 2: Analysis of cytokine production and T-bet/RORγt expression in LPMC- and TIL-derived CD3+CD8+ subset. A. Representative histograms showing the fraction of IFN-γ-, IL-17A-, IL-17F-, IL-21-, IL-22-, TNF-α- and IL-6-expressing CD3+CD8+ cells in LPMC and TIL isolated from adjacent tumor and non-tumor areas of 14 patients undergoing colectomy for sporadic CRC. Data are expressed as mean ± S.E.M. and differences were calculated using the two-tailed Student's t test. Right insets. Representative dot plots showing the percentage of IFN-γ- and/or IL-21-producing, IL-17A- and/or IL-17F-producing, IL-22- and/or IL-6-producing, and TNF-α-producing CD3+CD8+ cells in LPMC and TIL. The numbers indicate the percentage of cells in the designated quadrants. B. Representative histograms showing the fraction of T-bet+ and/or Rorγt+ CD3+CD8+ cells in LPMC and TIL isolated from adjacent tumor and non-tumor areas of 14 patients undergoing colectomy for sporadic CRC. Data are expressed as mean ± S.E.M. and differences were calculated using the two-tailed Student's t test. Right insets. Representative dot plots showing the percentage of T-bet+ and/or Rorγt+ CD3+CD8+ cells in LPMC and TIL. The numbers indicate the percentage of cells in the designated quadrants. Staining of LPMC with APC-conjugated and PE-Cy7-conjugated control isotype IgG is also shown. C. Representative histograms showing the percentage of IFN-γ- and TNF-α-producing CD3+CD8+ cells in LPMC and TIL isolated from adjacent tumor and non-tumor areas of one patient undergoing colectomy for sporadic CRC. IFN-γ- and TNF-α-producing CD3+CD8+ cells were gated and analyzed for the indicated markers. The example is representative of ten independent experiments in which cells isolated from ten patients undergoing colectomy for sporadic CRC were analyzed.

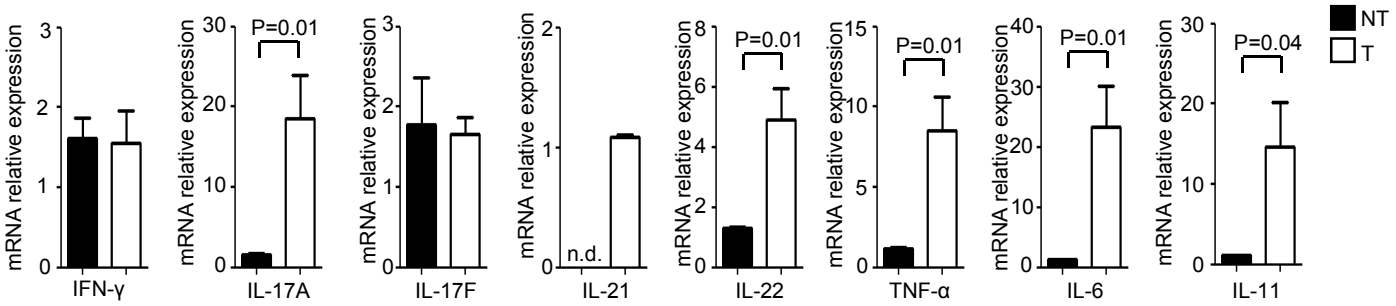


Supplementary figure 3: Representative western blotting showing p-STAT3 Tyr705, p-p38, p-ERK1/2 and p-p65 Ser536 expression in DLD-1 cells stimulated or not with IL-17A, IL-17F, IL-21, IL-22, TNF-α- and IL-6 (all used at 25ng/ml) in the presence or absence of specific antibodies neutralizing IL-17A (anti-IL-17A), IL-17F (anti-IL-17F), IL-21 (anti-IL-21), IL-22 (anti-IL22), TNF-α (anti-TNF-α) and IL-6 (anti-IL-6) (all used at 10μg/ml) as indicated. Normal rabbit or mouse IgG were used as negative controls. β-actin was used as loading control. One of two representative experiments in which similar results were obtained is shown.

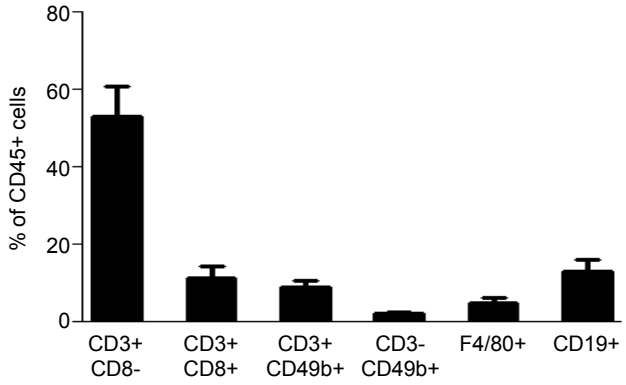


Supplementary figure 4: Activation of STAT3 and NF-kB is seen in early neoplastic lesions of Apcmin/+ mice. A. Experimental protocol used to assess STAT3 and NF-kB activation and cytokine expression in early neoplastic lesions of Apcmin/+ mice. B. Representative western blotting showing p-STAT3 Tyr705, STAT3, p-NF-kB/p65 Ser536 and NF-kB/p65 expression in colonic tissues taken from Apcmin/+ mice treated with azoxymethane (AOM) as indicated in A and sacrificed at day 21. β-actin was used as loading control. One of three representative experiments in which similar results were obtained is shown. NT, non-tumor area, T, tumor area. C. Representative images showing p-STAT3 Tyr705-positive cells in colonic sections taken from Apcmin/+ mice treated with AOM as indicated in A and sacrificed at day 21. Staining with isotype control IgG is also shown. The scale bars are 20μm. The scale bar in the inset is 10μm. One of six representative experiments in which similar results were obtained is shown. Upper left inset. Quantification of p-STAT3 Tyr705-positive infiltrating and epithelial cells in colonic sections taken from Apcmin/+ mice treated with AOM as indicated in A and sacrificed at day 21. Data are presented as mean values of positive cells per high power field (hpf) ± S.E.M. of two independent experiments in which three sections per group were analyzed. Differences were calculated using the two-tailed Student t-test. NT, non-tumor area, T, tumor area. D. Representative images showing p-NF-kB/p65 Ser536-positive cells in colonic sections taken from Apcmin/+ mice treated with AOM as indicated in A and sacrificed at day 21. Staining with isotype control IgG is also shown. The scale bars are 20μm. The scale bar in the inset is 10μm. One of six representative experiments in which similar results were obtained is shown. Upper left inset. Quantification of p-NF-kB/p65 Ser536-positive infiltrating and epithelial cells in colonic sections taken from Apcmin/+ mice treated with AOM as indicated in A and sacrificed at day 21. Data are presented as mean values of positive cells per high power field (hpf) ± S.E.M. of two independent experiments in which three sections per group were analyzed. Differences were calculated using the two-tailed Student t-test. NT, non-tumor area, T, tumor area.

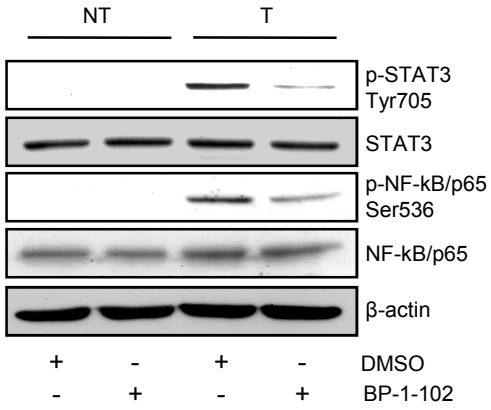
Supplementary figure 5 De Simone et al.



Supplementary figure 5: Up-regulation of IL-17A, IL-21, IL-22, TNF-α, IL-6 and IL-11 is seen in early neoplastic lesions of Apcmin/+ mice. IFN-γ, IL-17A, IL-17F, IL-21, IL-22, IL-6 and IL-11 expression was assessed by real-time PCR in colonic tissues taken from Apcmin/+ mice treated with AOM as indicated in materials and methods and sacrificed at day 21. Values are mean ± S.E.M. of two independent experiments containing at least two mice per group. Differences were calculated using the two-tailed Student t-test. NT, non-tumor area, T, tumor area, n.d., not detectable.



Supplementary figure 6: Representative histograms showing the fraction of T cells, NKT cells, NK cells, macrophages and B cells in TIL isolated from the tumors of Apcmin/+ mice. CD45+ cells were gated and analyzed for the indicated markers. Values are mean ± S.E.M. of two independent experiments containing at least two mice per group.



Supplementary figure 7: Representative western blotting showing p-STAT3 Tyr705, STAT3, p-NF-kB/p65 Ser536 and NF-kB/p65 expression in colonic tissues taken from Apcmin/+ mice treated with either DMSO or BP-1-102 and sacrificed at day 56. β-actin was used as loading control. One of three representative experiments in which similar results were obtained is shown. NT-non-tumor area, T-tumor area.