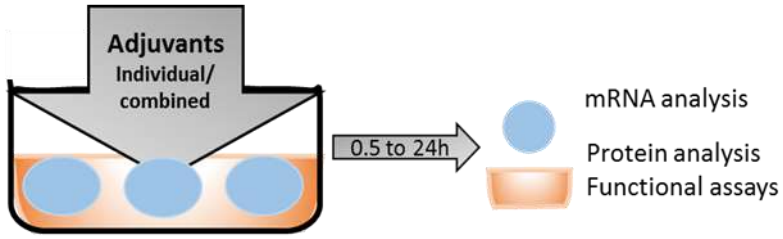
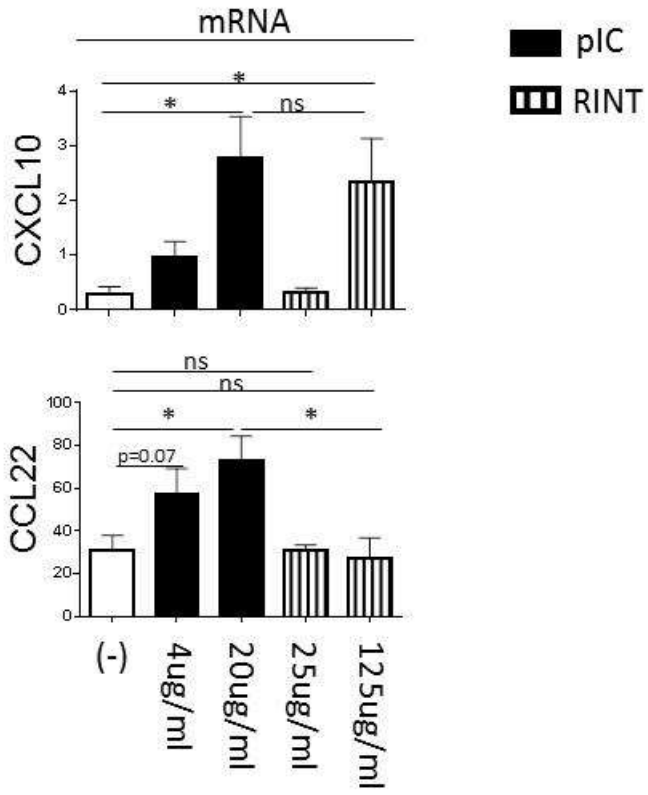
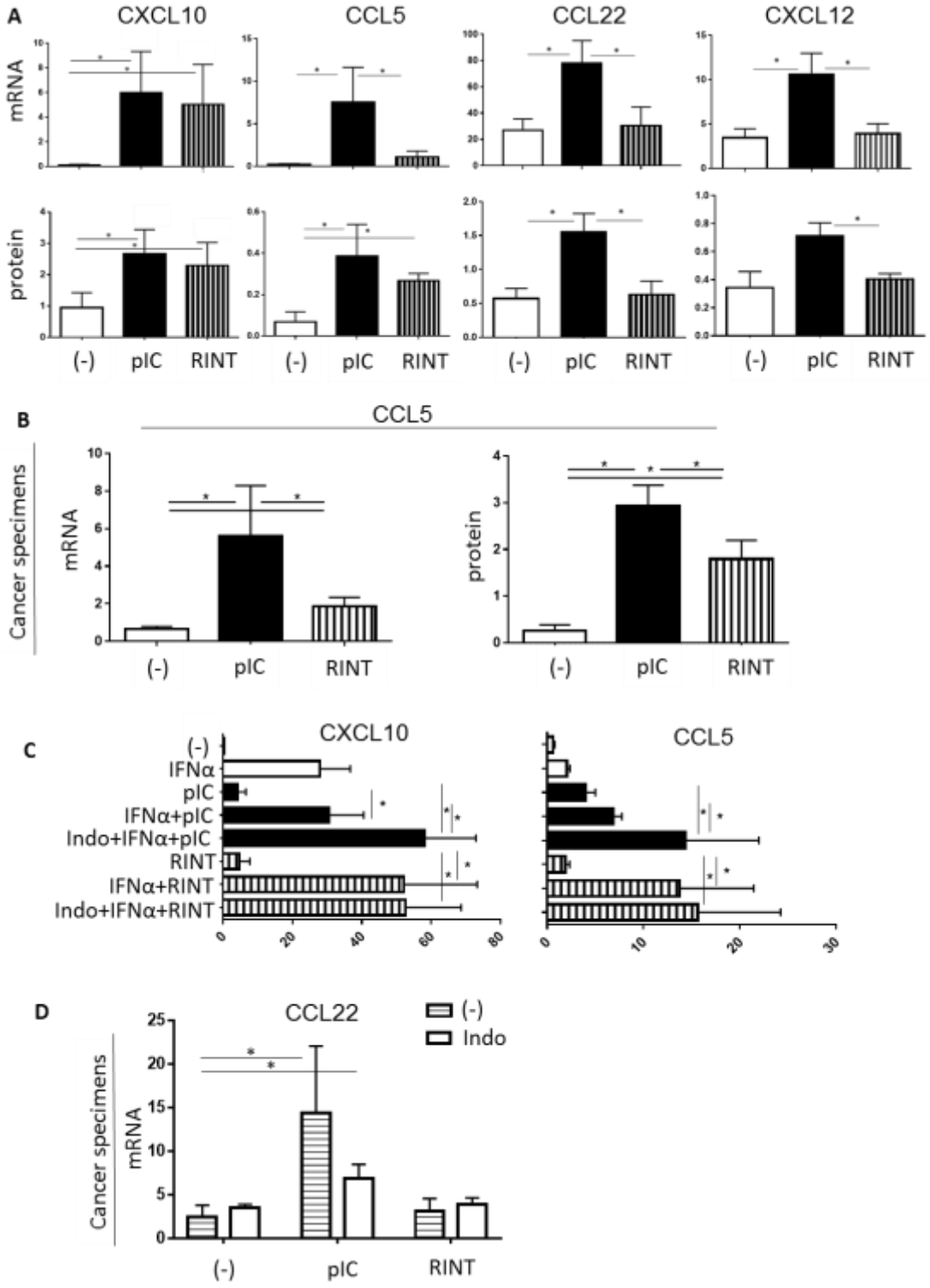


Supplementary Figure 1. *Ex vivo* tumor tissue explant culture system (*see ref 17 for detailed description*).

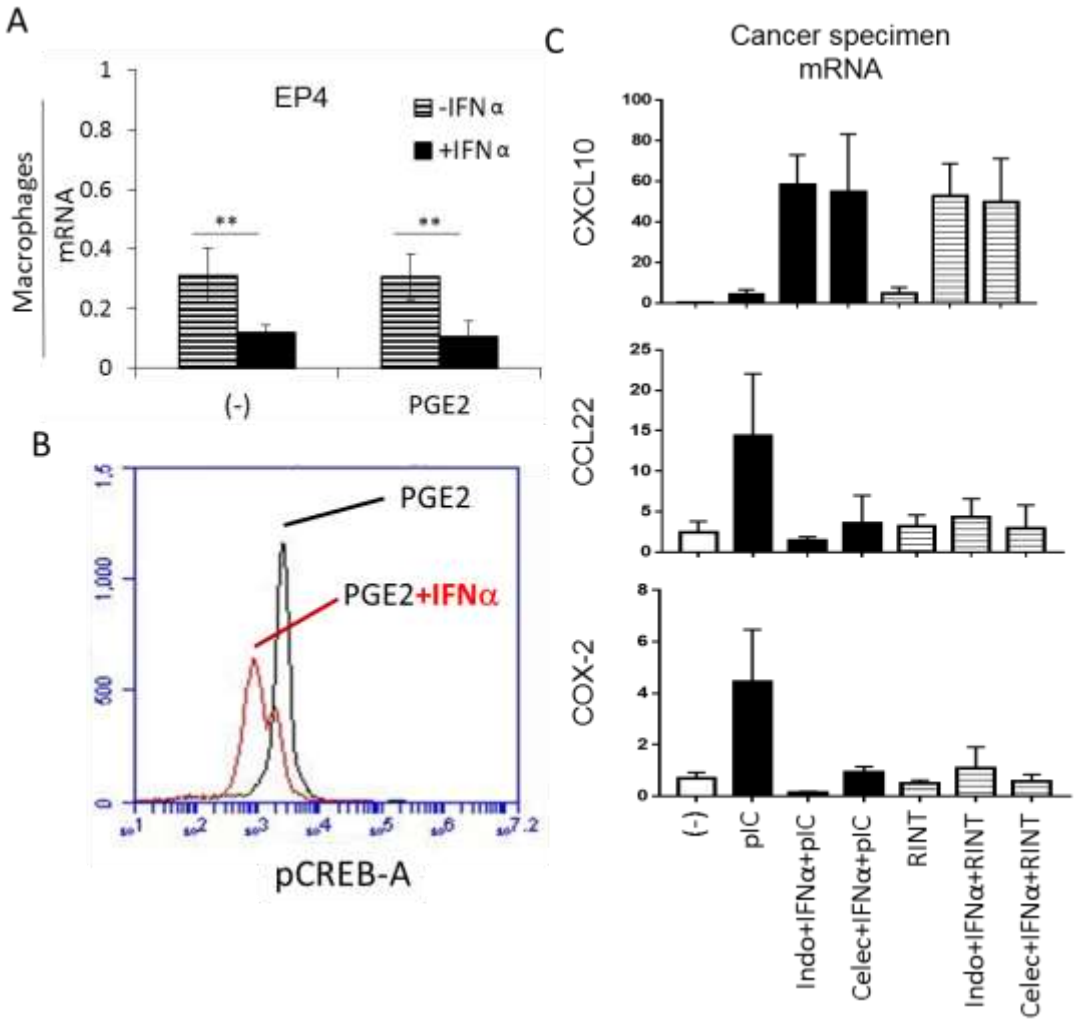


Supplementary Figure 2. Dose-dependent response to rintatolimod (RINT) and poly-I:C (pIC) in macrophage cultures. (n=3). Rintatolimod was used at concentrations of 25ug/ml and 125 ug/ml and poly-I:C at 4ug/ml and 20ug/ml. Note that equivalent levels of CXCL10 were induced by rintatolimod 125ug/ml and poly-I:C 20ug/ml. However, CCL22 was induced only in response to poly-I:C, independently on the concentration used. Results are mean ± SEM, * $p<0.05$ (Wilcoxon signed-rank test).

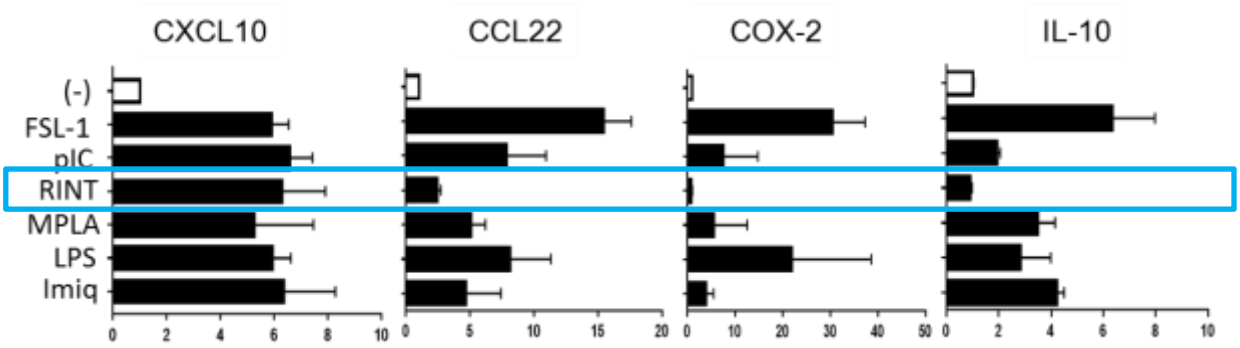


Supplementary Figure 3. IFNα enhances the response to TLR3 triggering by either poly-I:C or rintatolimod, but prostaglandin inhibition is selectively needed to optimize the pattern of inflammation induced by poly-I:C. **A:** Expression and secretion of chemokines in macrophage (n=6) or fibroblast cultures (n=5). Fibroblasts, rather than macrophages were used to evaluate the induction of CXCL12 (stromal-derived Factor-1; SDF-1), which is selectively produced by stromal fibroblasts, rather than myeloid cells. Note similar induction of CXCL10 by both Poly-I:C (pIC) and

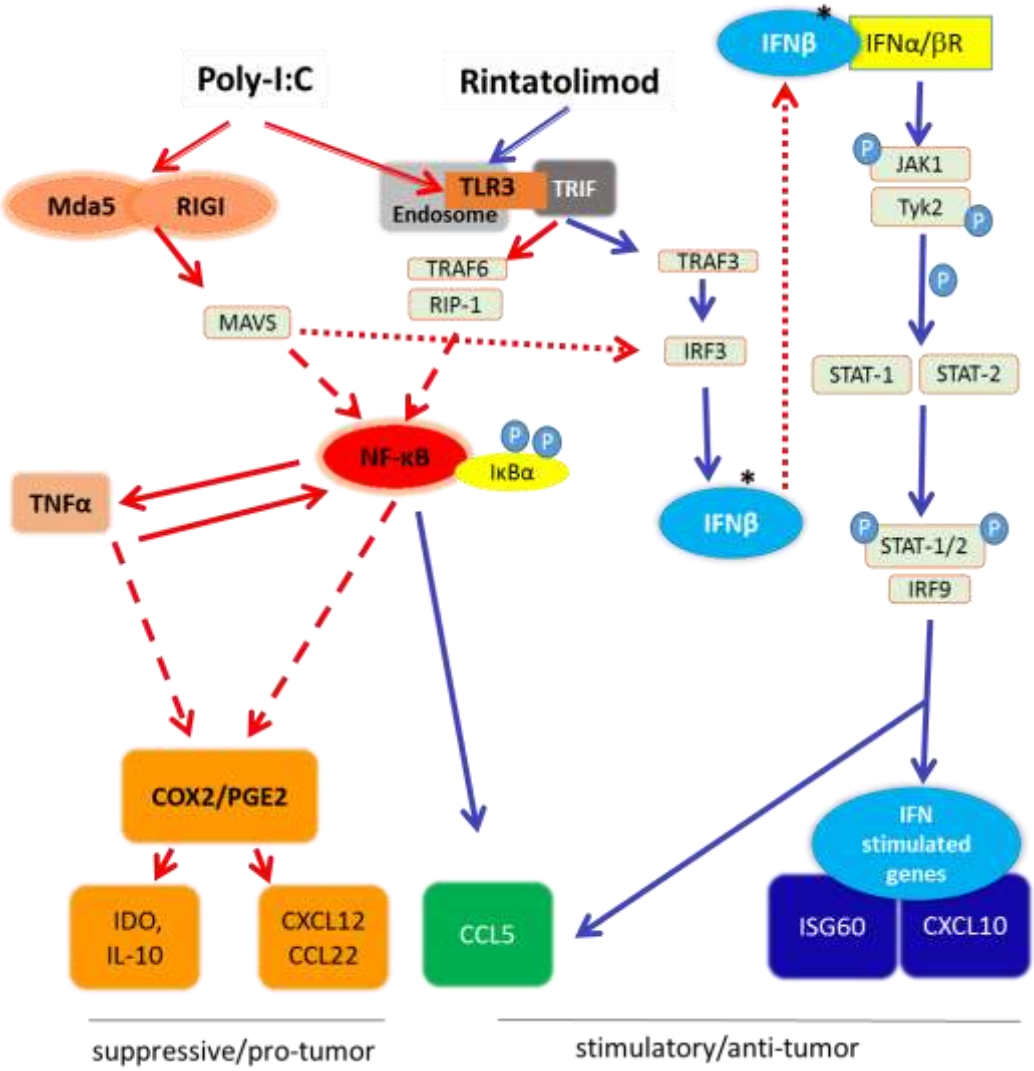
rintatolimod (RINT), but lower induction of CCL5 and no induction of CCL22 by rintatolimod; **B:** CCL5 expression in ovarian cancer specimen (mRNA and protein level, n=7). An intermediate pattern of regulation is visible with Poly-I:C inducing higher levels of CCL5; **C:** Combination of Poly-I:C with IFNα and indomethacin increases induction of CXCL10 and CCL5 in cancer specimens mRNA whereas with rintatolimod increased expression of these chemokines is only by combination with IFNα (n=8); **D**: Indomethacin reduced the levels of poly-I:C-induced CCL22 with no effects on rintatolimod-induced CCL22 production in cancer specimens (mRNA levels, n=5). Results are mean ± SEM, * $p<0.05$ (Wilcoxon signed-rank test).



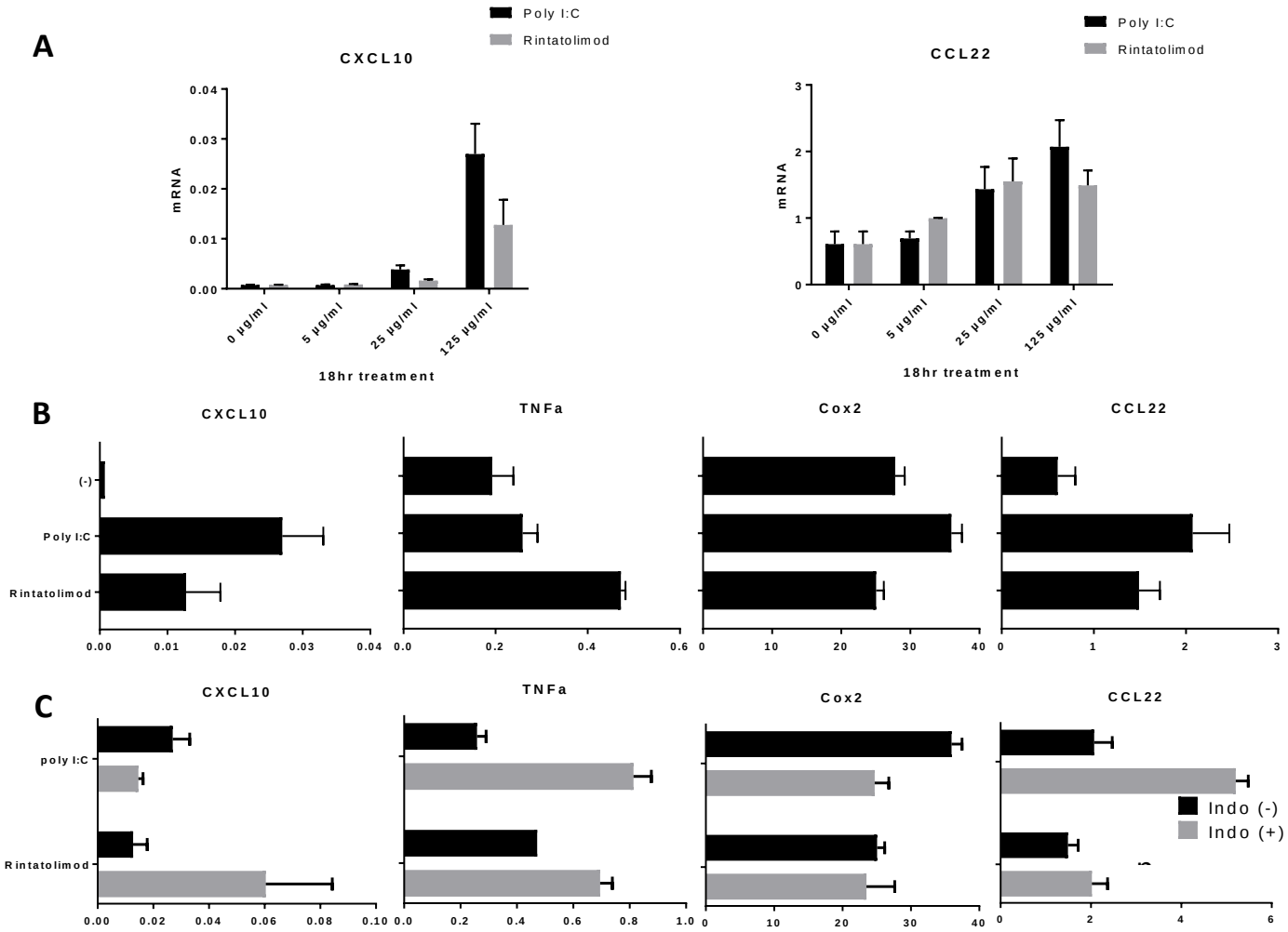
Supplementary Figure 4: Interplay between IFNα and COX-2/PGE2 system in the regulation of and suppressive inflammatory mediators. **A:** In mRNA levels of macrophages treated with IFNα we show that IFNα can suppress the expression of EP4 even if combined with PGE_2 (n=3); **B:** Macrophage cultures treated with PGE2 and IFNα, before membrane permeabilization and detection of phosphorylated CREB by flow cytometry. Note the inhibition of PGE_2 induced phospho-CREB activation after addition of IFNα. **C:** Induction of CXCL10, CCL22 and COX-2 by TLR3 ligands poly-I:C (pIC), rintatolimod (RINT) or by their combinations with Indomethacin (Indo) and IFNα or Celecoxib (Celec) and IFNα. Note the similar effect of Indomethacin and Celecoxib.



Supplementary Figure 5: Uniform activation of immunostimulatory and suppressive inflammatory mediators, including COX-2, by different TLR ligands. **A:** CXCL10, CCL22, COX-2 and IL-10 expression by the indicated TLR ligands in human macrophage cultures (CXCL10 n=3, CCL22 n=6, COX-2 n=6, IL-10, n=6). The ligands for TLR2/6 (FSL-1, 100 ng/ml), TLR3 (poly-I:C, 20 µg/ml and Rintatolimod 125 µg/ml), TLR4 (LPS, 250 ng/ml and MPLA, (1µg/ml), and TLR7/8 (Imiquimod, 10 µg/ml), were used at previously defined standard concentrations which induced similar levels of CXCL10.



Supplementary Figure 6: The model: Molecular separation of pro-inflammatory and suppressive pathways of dsRNA signaling in cancer TME. Type 1 Interferon pathway critical for the induction of high levels of CXCL10 and CCL5 is preferentially induced by the TLR3/TRAF3/IRF3 pathway, triggered by different variants of dsRNA, including both poly-I:C and rintatolimod. In contrast, the induction of suppressive aspects of dsRNA-driven inflammation, COX-2, COX-2-dependent factors and Treg/MDSC-attracting chemokines, IDO, IL10, CCL22 and CXCL12 depends on the induction of the NF-κB/TNFα pathway. This pathway was selectively triggered by poly-I:C, which is able to activate cytosolic helicases and MAVS pathways and this way directly or potentially indirectly activates COX-2 expression, triggering the downstream suppressive events and limiting CTL-promoting aspects of dsRNA signaling (such as production of CCL5, CXCL10 and CTL attraction; see Fig 1B and S3c). * In the absence of COX2/PGE2 induction (which suppresses IFNs induction) TRL3-driven endogenous IFNα/β signaling selectively amplify the production of antitumor factors.



Supplementary Figure 7: dsDNA selectively mobilize CXCL10 production, rather than CCL22 and suppressive component of inflammation in mouse myeloid cells. Mouse bone marrow-derived macrophages (3 cultures per each condition) were treated with the indicated concentrations of poly I:C or rintatolimod. After 18 hours, the cells were lysed and analyzed for the expression of CXCL10, TNFα, COX-2 and CCL22, using Taqman. **A.** Response to increasing concentrations of poly I:C or rintatolimod. **B.** Comparison of responses to poly I:C or rintatolimod at 125 mg/ml. **C.** Modulation of the response pattern by indomethacin. Data are expressed as ratios between the expression of individual genes and HPRT1 (n=3 cultures per each condition).