Figure 1S. Immature DCs present in bladder cancer tissue. CD1a staining in tumour tissue was used to identify DCs. Representative image of serial paraffin embedded sections that were processed for CD1a immunohistochemical staining. Tumour tissue showed moderate presence of CD1a+ DCs.
Figure 2S. Incubation with mo-DCs increase the proliferation capacity of bladder cancer cell lines. The MCRcont and MCRSTn cells were labelled with CFSE and cultured 24 hours in the presence or absence of mo-DCs. The cells were analysed by flow cytometry with Modfit software, allowing the calculation of the proliferation index, which represents the average number of cells that was originated by a single cell of the parent generation. Mo-DC co-incubation significantly increases the proliferation index of MCRSTn cells ($p=0.007 (**))$. The data are shown as a mean of 3 independent studies.
Figure 3S. Mo-DCs adhere preferentially to STn⁺ MDA-MB-231 breast cancer cell line and show a less mature phenotype. A: Adhesion of mo-DCs to MDA-MB-231 cell lines. Mo-DCs were co-cultured with MDA-MB-231 cell line variants for 2 hours. Non-adherent mo-DCs were washed and the percentage of mo-DCs adherent to MDA-MB-231 cell lines was estimated by flow cytometry, as the total of MHC-II⁺ cells in the coculture, following staining with anti-MHC-II. The mean number of adhering mo-DCs was significantly higher in the co-incubation with MDA-MB-231STn than with MDA-MB-231Cont (p = 0.026 (*), n= 5). B: Analysis of MHC-II expression in mo-DC. Adherent mo-DCs were stained with anti-MHC-II mAb and then analysed by flow cytometry. The expression level was inferred from the mean fluorescence intensity (MFI) of the cells, which was significantly lower in mo-DCs co-incubated with MDA-MB-231 STn than those with MDA-MB-231cont (p= 0.039 (*), n= 5).
Figure 4S. Maturation phenotype of mo-DCs remains unchanged in the presence of STn* cancer cells. A: Immature mo-DCs (imo-DCs) and semi-mature mo-DCs (mmo-DCs, obtained after incubation with LPS) were co-cultured or not with MCR bladder cancer cell lines, stained with anti-MHC-II mAb and then analysed by flow cytometry. The expression level of MHC-II was inferred from the mean fluorescence intensity (MFI) of the cells. As expected, MFI values were significantly lower in imo-DCs than mmo-DCs (*p = 0.0007 (***)). Compared with mmo-DCs alone, the expression of MHC-II was increased in mmo-DCs co-incubated with MCRcont (*p = 0.0341 (*)), while it was unchanged after co-incubation with MCRSTn (n=6). B: Immature mo-DCs were co-cultured or not for 2 hours with MCR bladder cancer cell lines and then incubated with LPS for an additional 24 hours. The co-culture was stained with anti-MHC-II mAb and analysed by flow cytometry. MHC-II expression in the adherent mo-DCs incubated with MCR cells was not significantly changed by LPS stimulation, and was comparable to the expression in the absence of LPS.
Figure 5S. The activation of T cells is also compromised when primed with mo-DCs that adhered to STn+ cancer cells. After adhesion assay, mo-DCs were incubated with a culture enriched in autologous T cells, in the proportion of 1:8. The co-culture was analysed by flow cytometry for the expression of the T cells early activation marker CD69. T cell activation was determined by the percentage of CD69+, within the CD3+ cell population. Mo-DCs co-incubated with MCRSTn cells tended to induce less T cell activation than those with co-incubated with MCRcont cells (n=3).
The expression of short O-glycans (sialyl-Tn, T, sialyl-T antigens) and Lewis antigens, as representative of elongated glycans structures were analysed at the cell surface of MCRcont and MCRSTn cell lines, by flow cytometry. MCRSTn expressed significant higher levels of sialyl-Tn. Moderate lower levels of T, sialyl-T and sialyl-Lewis xa antigens were observed when compared with MCRcont. MCR cells were stained with antibodies against sialyl-Tn (TKH2), sialyl-Lewis xa (SH1), sialyl-Lewis xa (HB80) and Lewis xa (CA3F4) followed by staining with FITC-labeled anti-mouse Ig. T antigen, Neu α2-6Gal and Neu α2-3Gal expression was obtained by FITC-labeled lectins staining, specifically Peanut agglutinin lectin, Sambucus nigra agglutinin and Maackia amurensis agglutinin, respectively. The mean fluorescence intensity was semi-quantified as negative (-), weakly positive (+), positive (++) and strongly positive (+++).