SUPPLEMENTARY FIGURES

Supplementary Figure 1

А



Supplementary Figure 1. Confocal microscopy images demonstrating apoptotic tumour cell uptake. (A) Irradiated tumour cell uptake by DCs. Immature DCs were co-cultured with PKH67 labeled irradiated tumour cells (green) at 1:1 ratio for 24h. DCs were stained with CD11c-alexa-647 (red) and visualized by confocal microscopy. Nucleus was stained with DAPI (blue). The white arrows indicate tumour cell material within DCs.

Supplementary Figure 1

В



Supplementary Figure 1. (B) IFN- γ /poly(I:C) treated tumour cell uptake by DCs. Immature DCs were co-cultured with PKH67 labeled IFN- γ /poly(I:C) treated tumour cells (green) at 1:1 ratio for 24h. DCs were stained with CD11c-alexa-647 (red). Nucleus was stained with DAPI (blue). The white arrows indicate tumour cell material within DCs.



Supplementary Figure 2. Immunophenotype of dendritic cells. (A) Typical phenotype of immature monocyte-derived DCs. Immature DCs were generated by GM-CSF and IL-4 and harvested on day 7. Representative figures show the median fluorescence intensity (MFI) of maturation markers on DCs where M1 indicates positive expression of each marker based on the isotype control staining.

В



Supplementary Figure 2. (B) MFI of DC maturation markers expressed as fold increase to non-loaded DCs. Immature DCs were cultured for 24h alone, with untreated, irradiated or IFN- γ /poly(I:C) pulsed MJT3s. The error bars indicate SD of 15 independent experiments. Statistically significant differences are shown (*, p < 0.05; **, p < 0.01; ***, p < 0.001) as determined by paired t-test using GraphPad Prism 5. Data failing normality test was subjected to Wilcoxon signed-rank test.

С



Supplementary Figure 2. (C) DC maturation in the presence of Benzonase nuclease. CD80 and CD86 expression by DCs cultured with IFN- γ /poly(I:C) treated MJT3s or 100µg/ml soluble poly(I:C) in the absence (bold line) or presence of 50U/ml BN (filled histogram). M1 indicates positive expression based on isotype control staining. The numbers indicate the MFI of DCs cultured in the absence/presence of BN.

D



Supplementary Figure 2. (D) DC maturation by IFN- γ pre-treated and poly(I:C) transfected versus pulsed KMs. Immature DC (filled histogram), DC co-cultured with IFN- γ /poly(I:C) pulsed non-apoptotic KMs (dashed line), DC co-cultured with IFN- γ /poly(I:C) transfected KMs (bold line). M1 indicates positive expression.



Supplementary Figure 2. (E) Comparison of DC maturation by IFN- γ treated versus IFN- γ /poly(I:C) pulsed MJT3s. The results show the MFI of 4 independent experiments. Significant differences between IFN- γ treated and IFN- γ /poly(I:C) pulsed MJT3s are shown as determined by paired t test analysis.





Supplementary Figure 3. (A) Cytokine release by naive T cells followed similar patterns in response to MJT3 loaded DCs as PBMCs. Naive CD3+ T cells were isolated by negative magnetic bead selection. Naive T cells were co-cultured with unloaded or tumour cell loaded DCs and cytokines were quantified at day 4 and day 7 by Bio-Plex Cytokine Assay. The graphs show the mean and SD of 3 separate experiments carried out from PBMCs of 3 individuals.





Supplementary Figure 3. (B) Comparison of IFN- γ release by PBMCs stimulated with DCs loaded with IFN- γ /poly(I:C) tumour cells and DCs loaded with tumour cell lysates and matured with conventional maturation cocktail. DCs were loaded with MJT3 lysates (Lys) or IFN- γ /poly(I:C) pulsed MJT3s (IFN/pIC) for 4h followed by 24h incubation in the presence or absence of standard maturation cocktail (mat.co). Non-loaded or loaded DCs were co-cultured with autologous PBMCs and cytokines were quantified in co-culture supernatants at day 4 or 7 by Bio-Plex Cytokine Assay. Mean and SD of 3 independent experiments are shown.