Supplemental Material

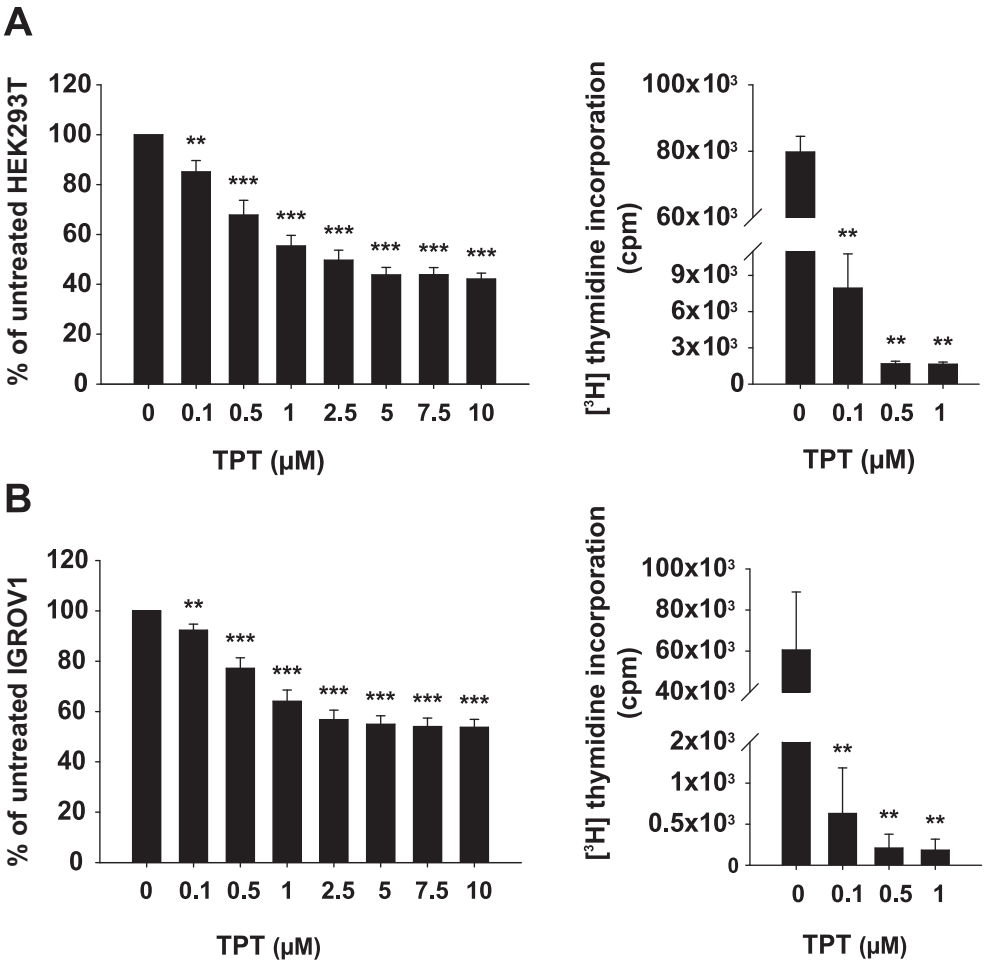

Fig. S1. Viability and proliferation of tumor cell lines are affected by TPT.

(A) HEK293T cells and (B) IGROV1 cells (each $5x10^4$/0.1 ml/well) were treated with the indicated concentrations of TPT for 48 h. (left) Cell viability was monitored by MTT assay. (right) In parallel cultures, cell proliferation was measured by incorporation of [^{3}H] thymidine for the last 16 h of culture. (A, B) Data represent mean ± SEM of two independent experiments each. Statistical significance: * versus untreated control (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$).

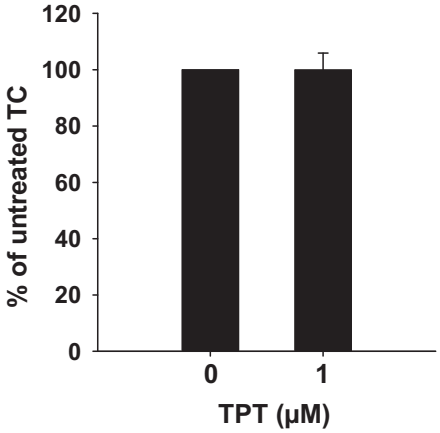

Fig. S2. TPT has no effect on the viability of non-activated T cells.

Aliquots of $CD4^+$ T cells ($5x10^5$/0.1 ml/well), supplemented with rhIL-2 (20 U/ml), were treated with TPT (1 µM) for 48 h, and assayed for viability by MTT assay. Data represent mean ± SEM of two independent experiments.

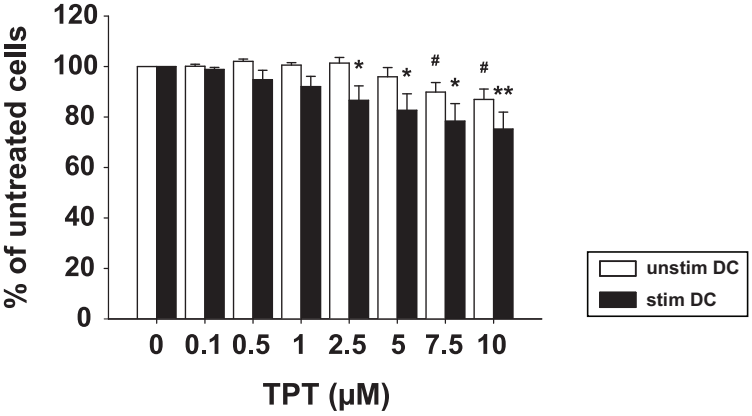

Fig. S3. Cytotoxic effects of TPT on human monocyte-derived DCs.

DCs ($2x10^5$/0.1 ml/well) were treated with TPT at the concentrations indicated for 48 h. Aliquots were cotreated with a stimulation cocktail. DC viability was monitored by MTT assay. Data represent mean ± SEM of five independent experiments. Statistical significance: TPT-treated DCs versus untreated DCs at unstimulated (#), and stimulated (*) state ($^{\#,*}$ $P < 0.05$, ** $P < 0.01$).

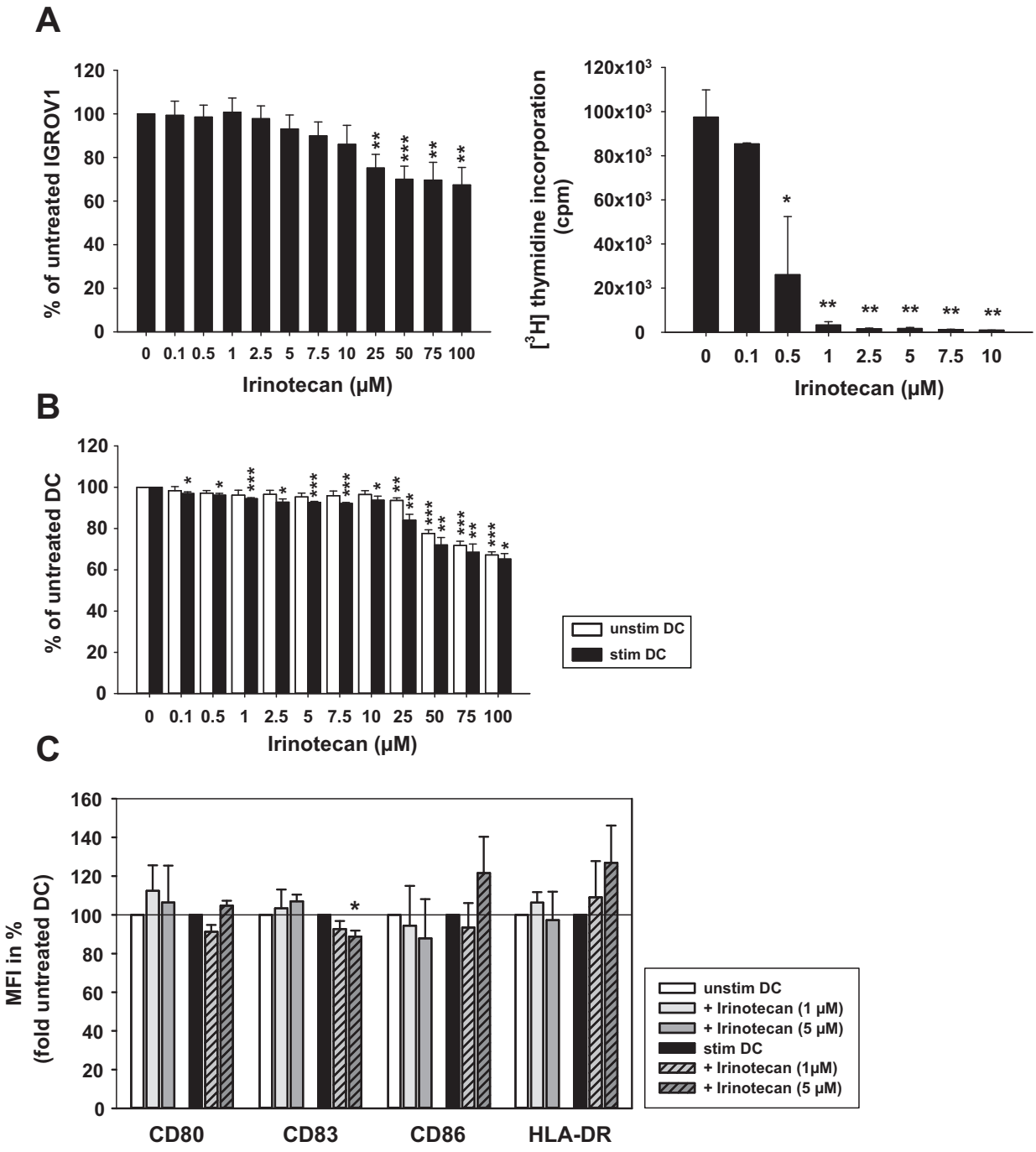

Fig. S4. Irinotecan affects the viability of IGROV1 and DCs in a dose-dependent manner, but has no major effect on the DC phenotype.

(A) IGROV1 cells and (B) DCs were treated with the indicated concentrations of irinotecan for 48 h. (A) Left: Cell viability was monitored by MTT assay. Right: In parallel cultures, cell proliferation was measured by incorporation of [^{3}H] thymidine for the last 16 h of culture.

(A, B) Data represent mean ± SEM of two independent experiments each. Statistical significance: * irinotecan-treated versus untreated control (* $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). (C) DCs were differentiated, and aliquots were treated with irinotecan as indicated for

the last 48 h of culture, and were stimulated as described. The expression levels of DC surface markers were assessed by flow cytometry, and relative changes are given as fold of the MFI of untreated DCs at unstimulated or stimulated state, arbitrarily set to 100 % each. Data represent the means ± SEM of three independent experiments each. Statistical significance: * irinotecan-treated versus untreated DCs at corresponding state of activation (* $P < 0.05$).

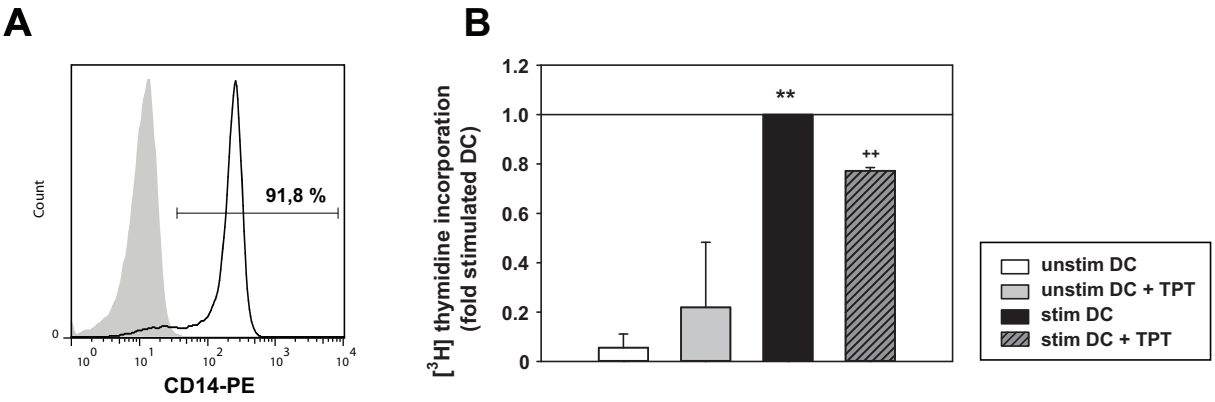

Fig. S5. TPT treatment of DCs enriched from highly purified monocytes affects their allo CD4$^+$ T cell stimulatory capacity.

(A) Monocytes were purified by negative immunomagnetic sorting, and the frequency of CD14$^+$ cells was assessed by flow cytometry. Solid line: specific staining; shaded area: isotype control. The graph is representative for two independent experiments. (B) DCs were differentiated from highly purified CD14$^+$ monocytes, and aliquots were treated with TPT (1 µM), and stimulated as described. DCs ($5x10^3$) were cocultured with immunomagnetically sorted allogenic CD4$^+$ T cells (10^5) in 0.2 ml culture medium in triplicate cultures for 4 days. T cell proliferation was assessed by uptake of [^{3}H] thymidine for the final 16 h of culture. CD4$^+$ T cell proliferation induced by stimulated DCs was arbitrarily set to one. Data represent the mean ± SEM of two independent experiments. Statistical significance: * stimulated vs. unstimulated DCs, and TPT-treated DCs vs. untreated DCs at stimulated state (+) ($^{**,++}$ $P <$ 0.01).

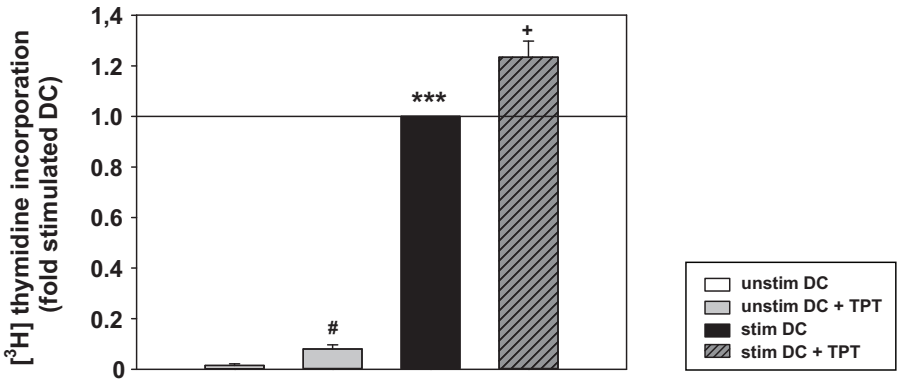

Fig S6. DCs differentiated from monocytes in the presence of TPT displayed a tendency towards higher allo T cell stimulatory capacity than untreated DCs at either state of activation.

DCs were generated and aliquots were treated from beginning (d 0) of the culture with 1 µM TPT. For the last 48 h parts of the DCs were stimulated as described. DCs ($5x10^3$) were cocultured with immunomagnetically sorted allogenic $CD4^+$ T cells (10^5) in 0.2 ml culture medium in triplicate cultures for 4 days. T cell proliferation was assessed by uptake of [^{3}H] thymidine for the final 16 h of culture. $CD4^+$ T cell proliferation induced by stimulated DCs was arbitrarily set to one. Data represent the mean ± SEM of two independent experiments. Statistical significance: * stimulated vs. unstimulated DCs, and TPT-treated DCs vs. untreated DCs at unstimulated (#), and stimulated (+) state ($^{\#,+}$ $P < 0.05$, *** $P < 0.001$).