Supplemental Materials and Methods:

Reagents. Concanavalin A, LPS from *Salmonella typhimurium* and diphtheria toxin from *Corynebacterium diphtheriae* were from Sigma. Rho family GTPase inhibitor, Toxin B (TB) from *Clostridium difficile* was from Calbiochem. The recombinant mouse IL-4 and GM-CSF were from Peprotech. Paclitaxel was from Mayne Pharma. Fluorescein-conjugated mAbs to I-A^b, CD86, CD40, CD11c, CD11b, CD4, PDCA1, F4/80, CD205, CD103 and isotype-matched control mAbs were from BD Pharmingen. FITC anti-mouse Ly-6G/Ly-6C (GR-1), APC anti-mouse CD25 Abs and PE anti-mouse FoxP3 Flow Kit were from BioLegend. For MDSC depletion, anti-mouse GR-1(Clone RB6-8C5) Abs were purchased from eBioscience. Anti-*Foxo3* Abs were a gift from Dr. Arthur Hurwitz (NIH, NCI).

Cell cultures and sorting. Murine 3LL (Lewis lung carcinoma) and B16 (melanoma) tumor cell lines were from ATCC. DC were generated from bone marrow hematopoietic precursors isolated from wild type and $TLR4^{-/-}$ mice and cultured in complete medium containing GM-CSF and IL-4 (1000 U/ml) for 7 days as described earlier.[49]. Immature DC were harvested on Day 5. DC phenotype was assessed by flow cytometry (FACSCalibur, BD) and data analysis was performed using FlowJo software.

For the *in vitro* studies, DC were pretreated for 48h with (i) 1nM paclitaxel, (ii) tumor-conditioned medium (TCM) (25% v/v) or (iii) both paclitaxel and TCM. DC treated with TCM were cell sorted (BD FACSAria) and $CD11c^{low}CD11b^{high}$ regDC and $CD11c^{high}CD11b^{low/neg}$ cDC subsets were isolated for the *in vivo* and *in vitro* experiments.

For the *ex vivo* studies, $CD11c^{high}$ cDC and $CD11b^{+}GR\text{-}1^{+}$ MDSC were sorted (FACSAria) from the lungs of tumor-free mice and cultured ($5x10^5$ cells/ml) with 3LL cells ($2x10^4$/ml, inserts 0.2μm pore) for 72h. GM-CSF (50 ng/ml) was added to support cell viability. The cultures were harvested on D3 and analyzed by flow cytometry and in functional assays.

For adaptive transfer of MDSC, to determine MDSC→regDC differentiation *in vivo*, MDSC were sorted from the bone marrow (MDCS Isolation Kit, Miltenyi Biotec), labeled with CFDA SE (Cell Tracer Kit, Invitrogen) and injected i.p. ($10x10^6$/mouse) in 3LL-bearing mice two weeks after inoculation of tumor cells. Cells from the lung and spleen were analyzed for the presence of CFDA SE (FL1) positive regDC by flow cytometry in 3-5 days.

T cell proliferation. Splenocytes ($2x10^6$/ml) from control mice were pre-activated for 5 h with 2.5μg/ml ConA and seeded at $2x10^5$/100μl in 96-well U-bottom plates. Bone-marrow-derived mature and immature syngeneic cDC and $CD11c^{+}$ DC isolated by MACS cell sorting (Miltenyi Biotec) from spleens of tumor-free and 3LL-bearing mice served as controls. cDC and regDC, separated by cell sorting (FACSAria) from cultured 3LL-treated DC or from lungs and spleens of tumor-bearing mice, were added in triplicates at 1:10; 1:30 and 1:100 ratios to pre-activated T cells. T cell proliferation (48h) was measured by uptake of ^{3}H-thymidine (1 μCi/well, 5 Ci/mmol; DuPont-NEN) pulsed for 16-18h using MicroBeta TRILUX counter (WALLAC) and expressed as count per minute (cpm).

Western blot. DC were treated with medium, Toxin B (0.2ng/ml, 30min) and 3LL-TCM, washed, homogenized and centrifuged at 100000*g* for 60min, 4ºC. Cytosolic fractions and membrane pellets were re-suspended in lysis buffer and Laemmli sample buffer. Samples from the same

number of cells ($1x10^6$ cells) were normalized by the protein concentration, resolved by electrophoresis on 12% SDS-PAAG and transferred to PVDF membranes (Invitrogen). The membranes were incubated with a blocking buffer (0.1% Tween-20 and 5% milk) before probing with anti-Cdc42 mAbs (Transduction Laboratories). After incubation with a horseradish peroxidase-conjugated secondary Abs (Pierce), bound Abs were visualized with an enhanced chemiluminescence detection system. β-actin (Sigma) served as a housekeeping marker. Gels were scanned and analyzed by Un-Scan-It Gel software (Silk Scientific, Inc).

IFN-γ assay. T cells ($1x10^6$/ml) isolated from spleens of control and tumor-bearing mice were stimulated with irradiated (300 Gy) 3LL or unrelated tumor cells ($0.2x10^6$/ml). Supernatants were collected 48h later and the levels of IFN-γ were assessed by ELISA (R&D System).

Supplemental Table I. Emergence of immune regulatory cells in the tumor environment *in vivo* in different tumor models

	B16 melanoma						3LL Lewis lung carcinoma					
	Lung			Spleen			Lung			Spleen		
	MDSC	Treg	regDC	MDSC	Treg	regDC	MDSC	Treg	regDC	MDSC	Treg	regDC
Week 1	123.2 ± 17.3	<0.1**	**143.6 ± 19.0**	93.3 ± 2.4	97.3 ± 10.8	88.5 ± 10.1	108.6 ± 6.4	<0.1	**461.7 ± 65.1**	94.9 ± 8.5	105.2 ± 5.2	**170.2 ± 27.2**
Week 2	**136.4 ± 17.5***	<0.1	**147.7 ± 12.7**	98.1 ± 13.3	**129.9 ± 7.4**	**248.9 ± 22.9**	103.5 ± 5.3	<0.1	**1521.2 ± 111.0**	103.3 ± 1.6	93.1 ± 12.1	**640.4 ± 54.6**
Week 3	**316.9 ± 16.7**	<0.1	**188.8 ± 10.2.0**	109.9 ± 6.9	**146.8 ± 12.4**	**402.8 ± 17.6**	**276.6 ± 25.4**	<0.1	**1858.3 ± 200.2**	**544.1 ± 32.1**	102.1 ± 7.2	**1706.7 ± 215.6**

C57BL/6 mice received i.v. injection of B16 melanoma or 3LL lung carcinoma cells as described in M&M. One, two and three weeks later, lungs and spleens were harvested, single cell suspensions were prepared and cells were stained with FITC-, PE- and APC-conjugated antibodies specific for markers associated with MDSC, Treg cells and regDC subsets. Cells were analyzed by flow cytometry. The results are presented as the percentage of $GR1^{+}CD11b^{+}$ (MDSC), $CD4^{+}CD25^{+}FoxP3^{+}$ (Treg) and $CD11c^{low}CD11b^{high}$ (regDC) cells in the lungs and spleens of tumor-bearing mice relative to their percentage in the lungs and spleens of control tumor-free mice. Data are shown as the mean ± SEM. *****, $p<0.05$ (ANOVA, N=4-6) shown in bold. ******, Treg cells were detected in the lungs in neither control nor tumor-bearing mice.