Supplementary Tables and Figures



Figure S1. The expression of TSP-1 in CD11c⁺ DCs of LN after receiving TSP-1 shRNA. TSP-1 expression levels in CD11c⁺ DCs cells in inguinal LN were examined at day 0 (mice were sacrificed after received shRNA for an hour), day 2, day 3 and day 6 after treatment with TSP-1 shRNA. The expression of TSP-1 and HPRT in CD11c⁺ cells were analyzed by real-time PCR. HPRT served as an internal control. TSP-1 expression was normalized to that in untreated control mice. Experiments were performed three times. Columns and bars represent mean values + SEM (**P* < 0.05; D2 or D3 groups versus scramble group).



Figure S2. Antitumor response was significantly induced by using 10 μ g of TSP-1 shRNA. To evaluate the optimal anti-tumor effects produced by TSP-1 shRNA, three dosages of TSP-1 shRNA were tested in mouse tumor model. MBT-2 tumor-bearing C3H/HeN mice were treatment with 5, 10 and 20 μ g TSP-1 shRNA via skin administration, and the tumor sizes were examined (**P* < 0.05; 10 μ g or 20 μ g groups versus control group).



Figure S3. Expression levels of IDO in CD11c⁺ DCs in inguinal LN after skin administration of TSP-1 shRNA. $CD11c^+$ DCs were isolated from inguinal LN in mice received TSP-1 shRNA for 2 days. The expression of IDO and HPRT in CD11c⁺ cells was analyzed by real-time PCR. HPRT served as an internal control. IDO expression was normalized to that in untreated control mice. Columns and bars represent mean values + SEM. "ns" represents no statistical difference. Experiments were performed three times.



Figure S4. Angiogenesis at the tumor site is not enhanced by skin administration of TSP-1 shRNA. Tumor angiogenesis was analyzed by immunohistochemical staining with anti-CD31 antibody. *Upper panel:* a blood vessel was viewed at a magnification of 200x, and three randomly chosen fields of view were evaluated in each of three samples. *Lower panel:* the number of immunohistochemically positive cells in the cryosection. Experiments were performed three times. Columns and bars represent mean values + SEM . "ns" represents no statistical difference.



Figure S5. Therapeutic effects induced by treating increasing numbers of BMDCs in LL2 tumor-bearing mice. 2.5×10^4 , 1×10^5 and 4×10^5 WT-BMDCs and TSP-1-KO BMDCs were subcutaneously injected into LL2 tumor-bearing mice. Tumor sizes were examined.



Figure S6. TSP-1-KO BMDCs induced cytotoxicity against LL2 tumor cells, but not MBT-2 tumor cells. Mice were implanted with LLC tumor cells, and received with BMDCs isolated from wild-type mice or TSP-1-KO mice. Effector lymphocytes were isolated and cytotoxicity to the indicated targets was measured (a) LL2-luciferase cells were used as target cells (**P < 0.01, BMDC-WT versus BMDC-TSP-1-KO in the ratio 50:1) (b) MBT-2-luciferase tumor cells were used as target cells.



Figure S7. IFN- γ -producing T cells were increased after treatment with TSP-1 shRNA *in vivo*. FACS analysis of CD8⁺ IFN- γ^+ T cells in CD8⁺ T cells in lymph nodes after treatment with TSP-1 shRNA or TSP-2 shRNA. The bar graph represents average + SEM of the percentage of CD8⁺ IFN- γ^+ T cells in CD8⁺ T cells (**P* < 0.05, ****P* < 0.001; n = 3 mice per group).

Table S1. Tumor-infiltrating CD4 T cells, CD8 T cells and NK cells with TSP-1shRNA treatment.

	CD4 ⁺ T cells	CD8 ⁺ T cells	NK cells
Saline	4 ± 1	2 ± 1	1 ± 1
TSP-1 shRNA	18 ± 6 *	17 ± 7 *	4 ± 2
Scramble shRNA	6 ± 12	5 ± 3	3 ± 1

Note: Cell count was performed at magnification x400. Three randomly chosen fileds/sample from three mice were evaluated. Results are expressed as means F standard deviation of immunohistochemically positive cells in the cryosection. *P < 0.05, a statistically significant difference when compared with mice that received the scramble shRNA.

Table S2. Tumor-infiltrating CD4 T cells, CD8 T cells, and NK cells with

	CD4 ⁺ T cells	CD8 ⁺ T cells	NK cells
shTSP-1	15 ± 5	14 ± 5	4 ± 2
Neu	21±4	16 ± 3	3 ± 1
Neu-shTSP-1	37±4*	31 ± 4*	6 ± 1

Neu_shTSP-1 treatment.

Note: Cell count was performed at magnification x400. Three randomly chosen fileds/sample from three mice were evaluated. Results are expressed as means F standard deviation of immunohistochemically positive cells in the cryosection. *P < 0.05, a statistically significant difference when compared with mice that received the shTSP-1shRNA.