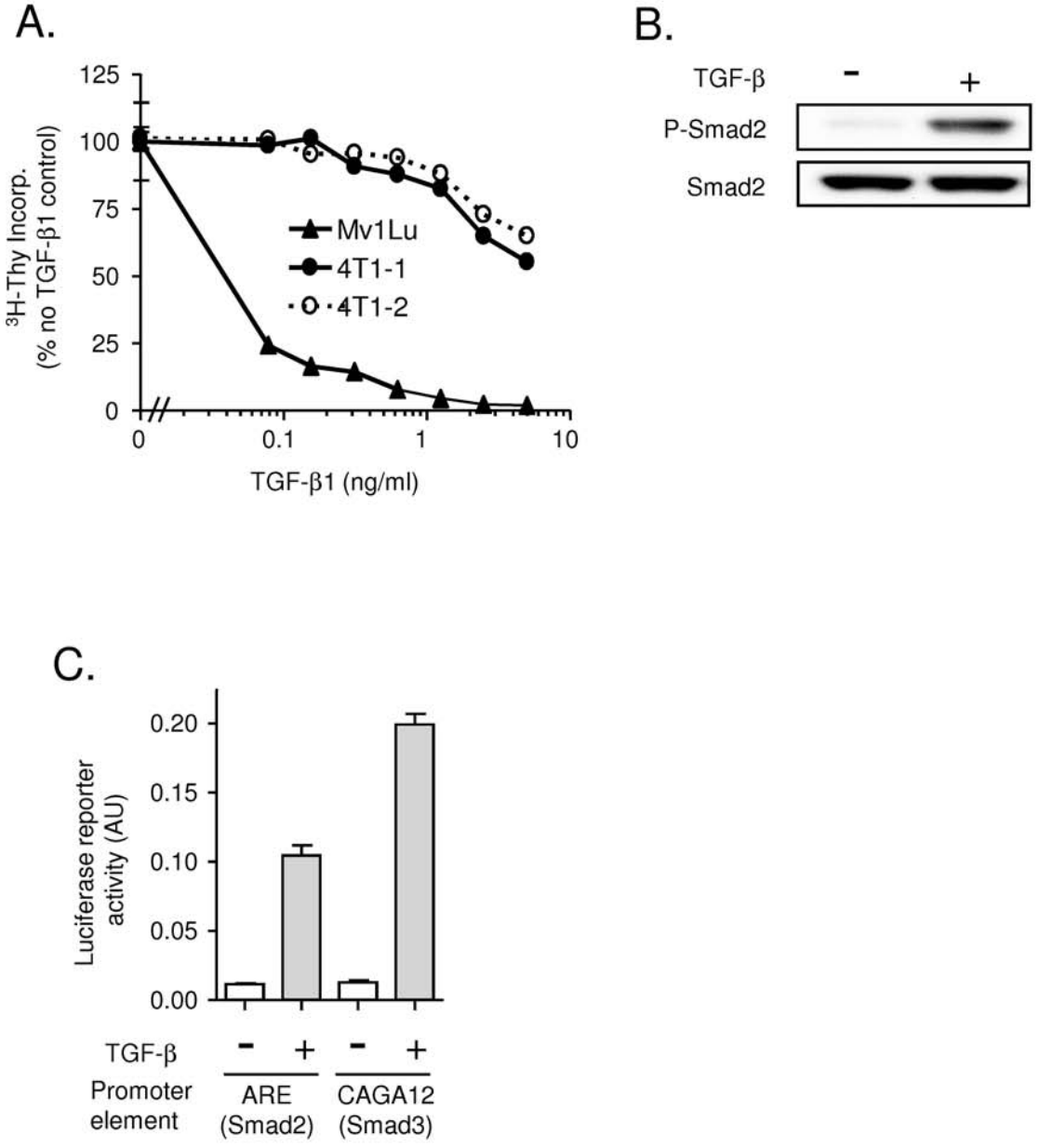
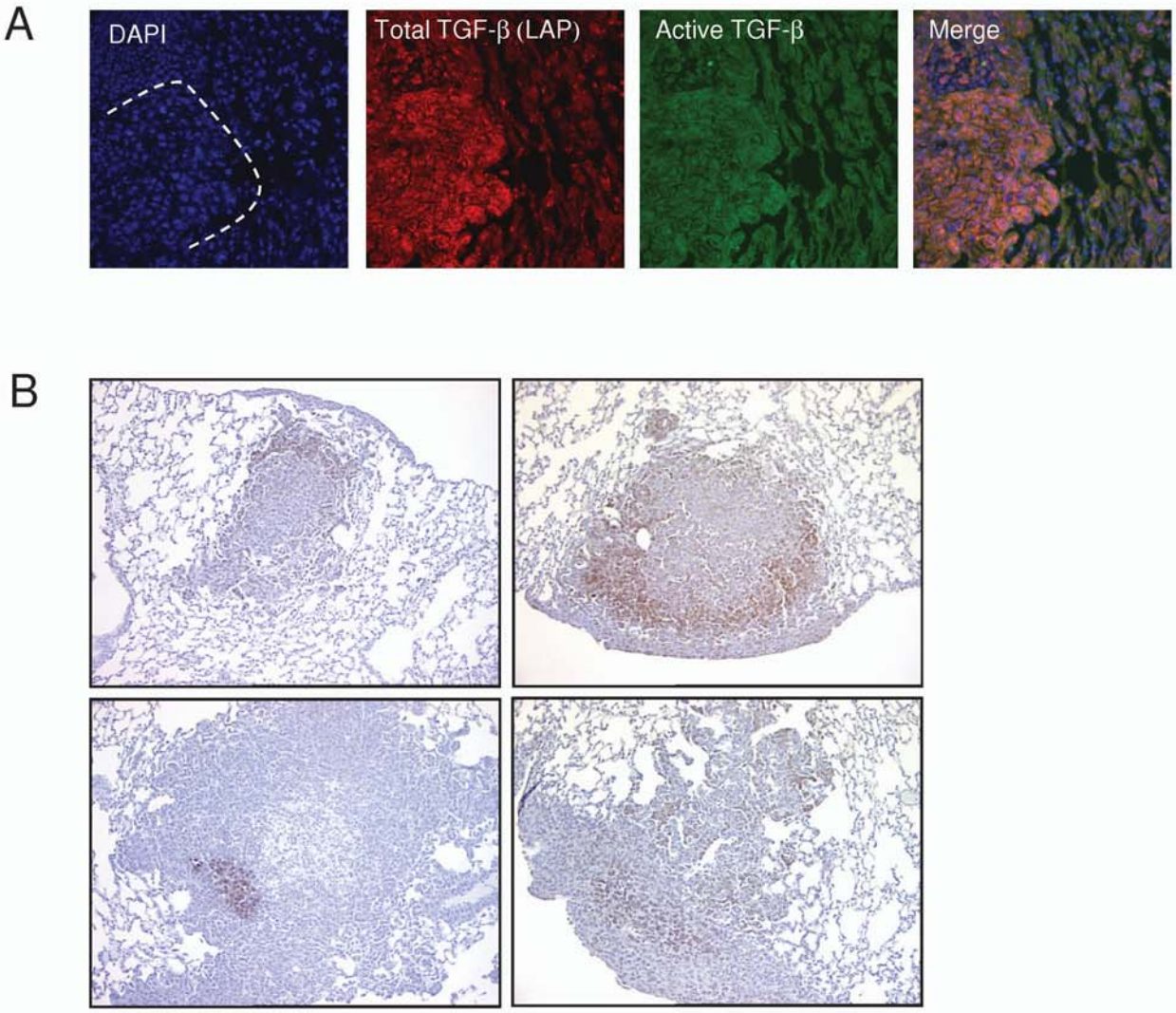
Supplementary Figure 1. *Nam et al.*



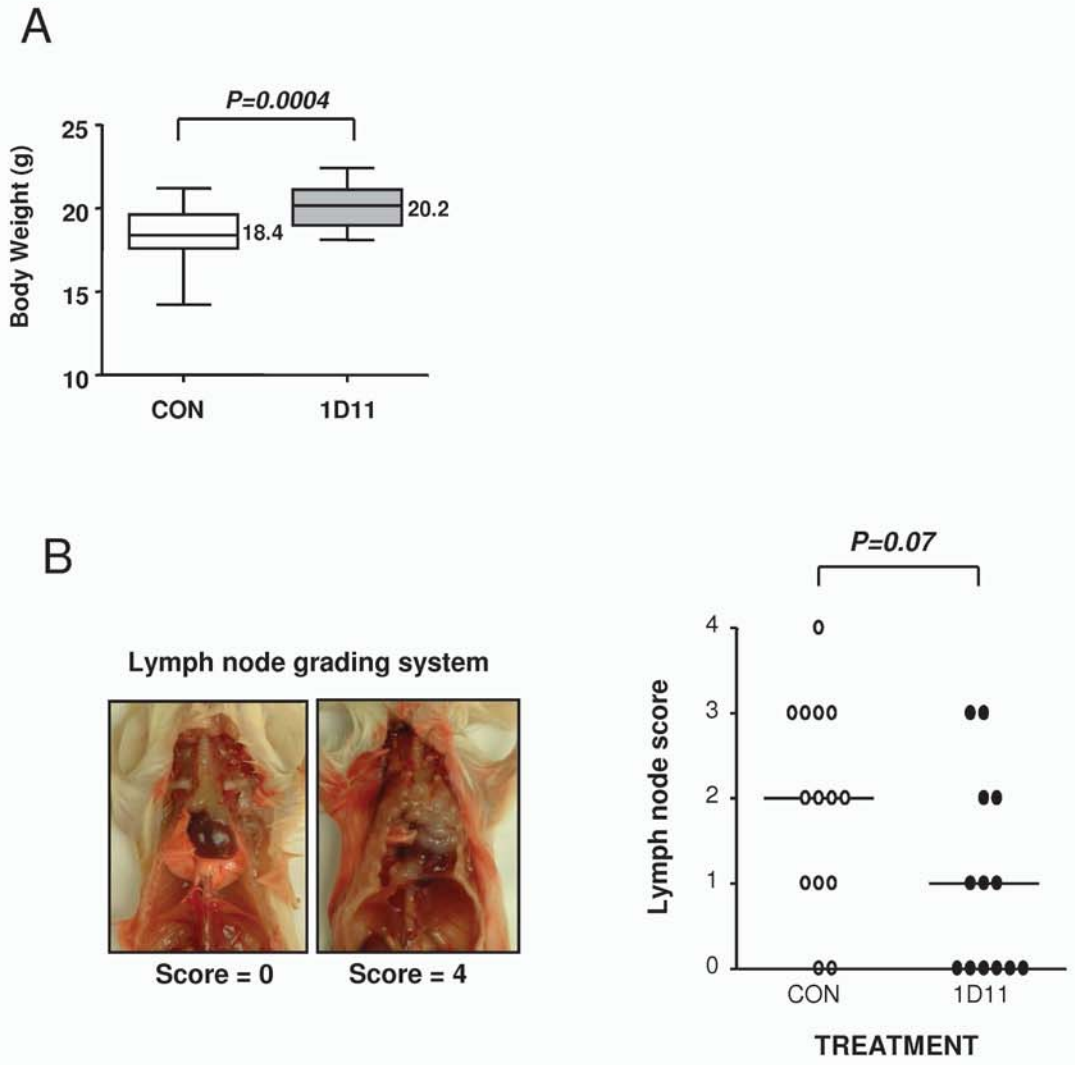
Supplementary Figure 1. TGF-β responses in 4T1 cells *in vitro*. A. Effect of TGF-β on cell proliferation as determined by ^{3}H-Thymidine incorporation (see Methods). All results are normalized to the no TGF-β condition for the particular cell line. 4T1-1 and 4T1-2 represent 4T1 cell stocks maintained in two independent laboratories (Lab of Cancer Biology and Genetics, NCI, and Genzyme Corp). The response of Mv1Lu mink lung epithelial cells is shown for comparison. B. Western blot analysis of Smad2 and phospho-Smad2 levels in 4T1 cells after 24h treatment with TGF-β1 (5ng/ml). C. Effect of TGF-β treatment on transcription from two TGF-β responsive promoter constructs was assessed by measuring luciferase activity in 4T1 cells transiently transfected with pARE-LUC and FAST1 (responds to Smad2) or pCAGA$_{12}$LUC (responds to Smad 3). Cells were treated with TGF-β for 18h prior to assay. Results are mean +/- SD (3 determinations).

Supplementary Fig. 2. *Nam et al.*



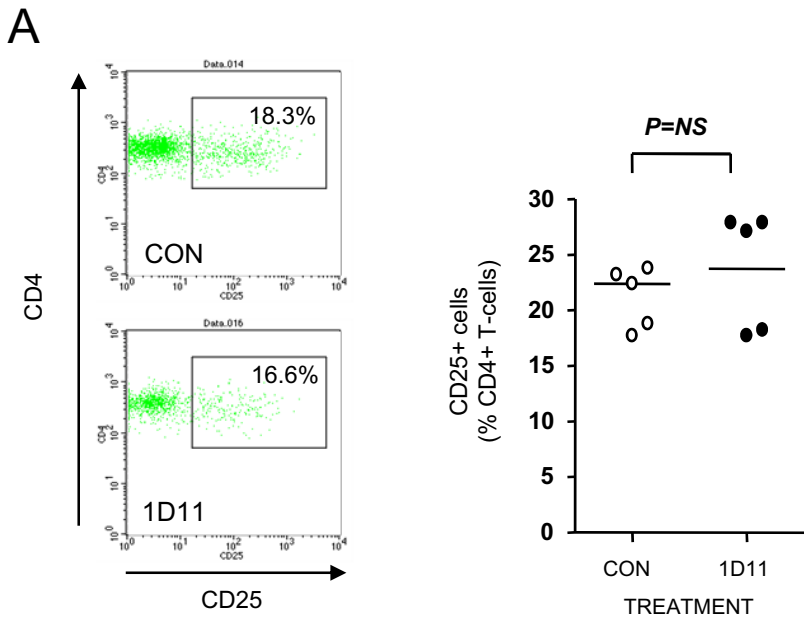
Supplementary Figure 2. TGF-β expression and pathway activation in 4T1 lung metastases. A. Active and total TGF-β in sections from metastasis-bearing lungs were visualized by immunofluorescence on fresh frozen tissue cryosections essentially as described (12). Cryosections (5mm) were thawed, air dried, rehydrated in DPBS, and fixed with 4% neutral buffered paraformaldehyde (10 min, room temperature). After quenching autofluorescence with 0.1M glycine (1h, room temperature) unspecific protein binding was blocked with 0.5% casein in DPBS (1h, room temperature), and specimens were incubated with anti TGF-β (chicken, 1:50, R&D # AF-101-N) and anti LAP (goat, 1:50, R&D # AF-246-NA) in 0.5% casein (4°C, overnight). followed by incubation with anti-chicken IgY ~ FITC (donkey, 1:500, Jackson ImmunoResearch) and anti goat IgG~Alexa 568 (donkey, 1:250, Molecular Probes) in 0.5% casein (1h, room temperature). Specimens were fixed with 4% neutral buffered paraformaldehyde (10min, room temperature), incubated with 0.1M glycine (1h, room temperature), and mounted for viewing under the fluorescence scope. Nuclei were visualized by staining with DAPI. The dotted white line indicates the boundary of a lung metastasis. B. Immunostaining for phosphoSmad2 in 4T1 lung metastases showing the heterogeneity of staining patterns seen among individual metastases. 100X magnification.

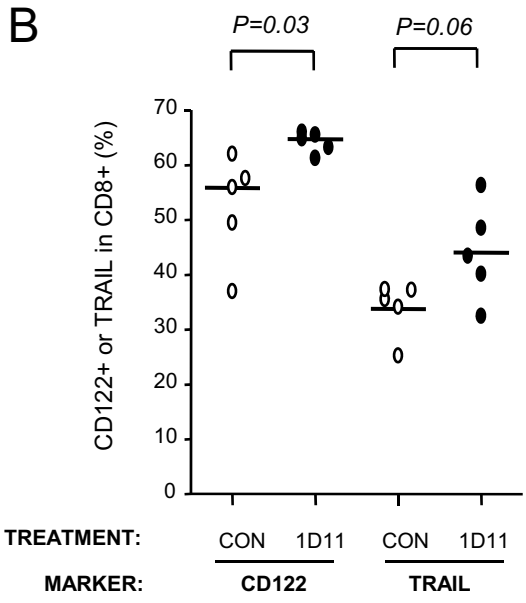
Supplementary Fig. 3. *Nam et al.*



Supplementary Figure 3. Effect of 1D11 treatment on body weight and lymph node involvement in mice bearing 4T1 tumors. 4T1 cells (40,000) were injected into the left thoracic mammary fat pad of 7 week old BALB/c mice. Mice were randomized into 2 treatment groups, receiving anti-TGF-β (1D11) or control antibody (CON) by thrice weekly ip injection (5mg/Kg) starting at day 1 after tumor cell injection. Primary tumors were resected at day10 and mice were euthanized at day 28. A. Effect of 1D11 on mouse body weight at day 28. B. Effect of 1D11 treatment on lymph node grade. A semiquantitative scoring system was established for assessing the degree of expansion of the thoracic lymph node compartment in tumor bearing animals, ranging from 0 (normal) to 4 (lymph nodes enlarged to completely fill thoracic cavity). At the histological level, enlarged lymph nodes were massively infiltrated with tumor cells. Median scores are indicated for 13-14 mice/experimental group.

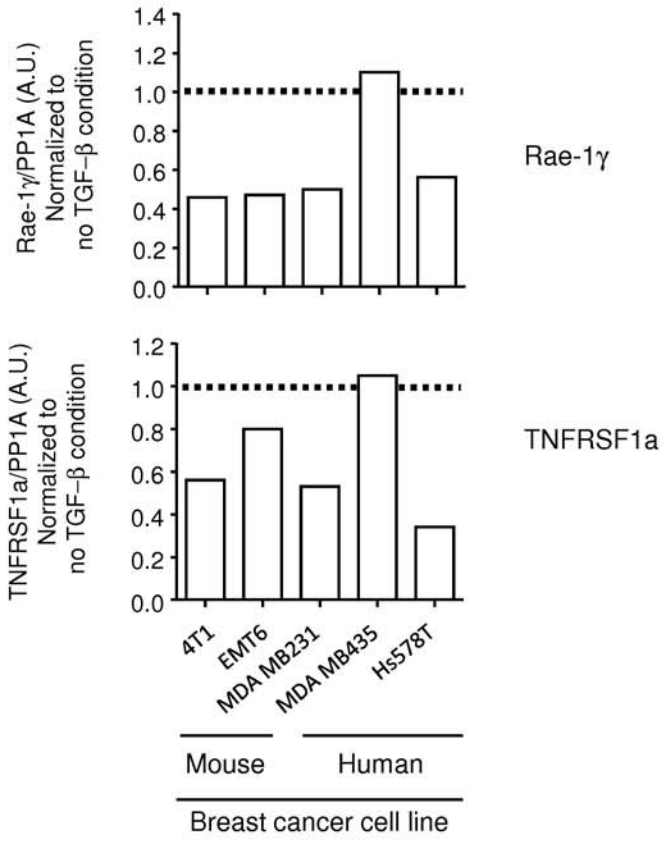
Supplementary Fig. 4. *Nam et al.*





Supplementary Figure 4. Effect of 1D11 treatment on the activation markers on lymphocytes recovered from tumor-bearing lungs. Viable immune cells were recovered from the lungs of mice bearing 4T1 metastases following orthotopic implantation of tumor as described in Methods, and immunophenotyped by FACS. A. Effect of 1D11 on size of the CD25+CD4+ fraction which includes regulatory T-cells. Representative FACS plots for an individual mouse from each treatment group are shown. The scatter plot summarizes data for 5 mice/group treated with either anti-TGF-β (1D11) or control (CON) antibody. B. Scatter plot for expression of CD122 or TRAIL on CD8+ T-cells (5 mice/group)

Supplementary Figure 5. ***Nam et al.***



Supplementary Figure 5. Effect of TGF-β treatment on expression of Rae-1γ and TNFRSF1a mRNA in various mouse and human breast cancer cell lines *in vitro*. Cells were treated with 5ng/ml TGF-β or vehicle alone for 48h prior to determination of Rae-1γ and TNFRSF1 mRNA levels by RTQ-PCR. PP1A mRNA was used as a normalization control for all samples, and then the ratio of expression in the presence of TGF-β to that in the absence of TGF-β was determined for each individual cell line. The dotted line indicates the expected value if TGF-β treatment had no effect.

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Supplementary material: Methods Section

PCR primer sequences.
The primer sets used in this study were as follows:

a) Mouse Primer Sets from Superarray Bioscience Corporation

Description	Gene Symbol	Cat. No.	Refseq no.
CD8 antigen, beta chain 1	Cd8b1	PPM04032A	NM_009858
Granzyme B	Gzmb	PPM05303A	NM_013542
Perforin 1	Prf1	PPM34456A	NM_011073

(b) Additional mouse primer sets

1. Mouse 28s ribosomal RNA –Genebank no. : X00525
i) Name : 28S_F(Start=310)
(5”--) :GGGTGGTAAACTCCATCTAA(--3”)
ii) Name : 28S_R(Start=372)
(5”--) : AGTTCTTTTCAACTTTCCCT(--3”)

2. Rae-1r– Genebank no. : NM_009018 (Rae-1 gamma)
i) Name : Rae-1r_F2 (Start =469 / length=21)
(5”--) : TGATTTATCCGCAAAGCCAGG(--3”)
ii) Name : Rae-1r_R2 (Start=628 / length=21)
(5”--) : AGGTCCCCATCATCGTTCCAT(--3”)

3. TNFRSFIa– Genebank no. : NM_011609
i) Name : TNFRSF1a_F1 (Start =1749 / length=22)
(5”--) : AGCTGGTAGCCACTTCCTTGGT(--3”)
ii) Name : TNFRSF1a_R1 (Start=1894 / length=21)
(5”--) : ACAATCCTGTCTTTGGCACCC(--3”)