Figure S1. Anti LFA-1 in combination with CTLA-4 Ig prolongs graft survival in a fully allogeneic skin graft model
B6 mice were grafted with BALB/c skin grafts and treated with CTLA-4 Ig, anti LFA-1, or a combination of the two as described in Materials and Methods (250 µg of each). (A) CTLA-4 Ig + anti LFA-1 in combination confers a significant survival advantage over each reagent alone. (B) Mice treated with CTLA-4 Ig + anti LFA-1 exhibited an MST of 44 days (p<0.0001 as compared to untreated or singly-treated animals). Data shown include the following numbers of mice: untreated, n=14; CTLA-4 Ig alone, n=10; anti–LFA-1 alone, n=9; CTLA-4 Ig + anti–LFA-1, n=26.

Figure S2. LFA-1 blockade results in reduced frequencies of CD4+ and CD8+ T cells in non-draining lymph nodes but not in spleens
B6 mice were grafted with BALB/c skin grafts and were left untreated or treated with anti LFA-1 as described in Materials and Methods (250 µg of each) (non-draining LN mice did not receive skin grafts). Mice were sacrificed on day 9 and lymph nodes and spleen were harvested. (A) Representative flow panels showing frequencies of CD4+ and CD8+ T cells in non-draining nodes and spleens. Data shown are gated on lymphocytes. (B) Absolute numbers of CD4+ and CD8+ T cells from non-draining node and spleen are summarized from two independent experiments with three mice each per group, and indicated that LFA-1 blockade significantly decreased the number of both CD4+ and CD8+ T cells in non-draining nodes but not in spleens. *p<0.05

Figure S3. LFA-1 blockade results in decreased CD62L expression on both CD4+ and CD8+ T cells in non-draining LN and spleen
B6 mice were grafted with BALB/c skin grafts and were left untreated or treated with anti LFA-1 as described in Materials and Methods (250 µg of each) (non-draining LN mice did not receive skin grafts). Mice were sacrificed on day 9 and lymph nodes and spleen were harvested. (A) Representative flow panels showing CD62L expression on CD4+ and CD8+ T cells in non-draining nodes and spleens. Black line= untreated, shaded histogram=anti–LFA-1 treated. (B) MFI of CD62L expression on CD4+ and CD8+ T cells from non-draining node and spleen are summarized from two independent experiments with three mice each per group, and indicated that LFA-1 blockade resulted in reduced expression of CD62L on both CD4+ and CD8+ T cells in non-draining nodes and spleens. *p<0.05.

Figure S4. LFA-1 antagonism did not impact the activation status of non–graft-specific Thy1.2+ effectors in lymph nodes
mOVA graft-specific OT-I T cells were adoptively transferred into B6 recipients; 48h later mice received an mOVA skin graft and were left untreated or were treated with anti–LFA-1. Draining lymph nodes were harvested on day 10 post-transplant and non-donor reactive endogenous Thy1.2+ T cells were analyzed by flow cytometry for the expression of activation molecules. Results indicated that there was no difference in the expression of CD43, CD69, and CD44 on these cells. Data shown are cumulative from two independent experiments with a total of 8–9 mice per group. *p<0.05.
Figure S5. LFA-1 antagonism results in increased CD4⁺ CD25⁺ FoxP3⁺ T-cell frequencies in non-draining nodes but not spleens
B6 mice were grafted with BALB/c skin grafts and were left untreated or treated with anti LFA-1 as described in Materials and Methods (250 µg of each) (non-draining LN mice did not receive skin grafts). Mice were sacrificed on day 9 and lymph nodes and spleen were harvested. (A) Representative flow panels showing frequencies of CD25⁺ FoxP3⁺ T cells in non-draining nodes and spleens. Data shown are gated on CD4⁺ T cells. (B) Frequencies of CD4⁺ CD25⁺ FoxP3⁺ T cells from non-draining node and spleen are summarized from two independent experiments with three mice each per group, and indicated that LFA-1 blockade significantly increased the frequency of CD4⁺ CD25⁺ FoxP3⁺ T cells in non-draining nodes but not in spleens. *p<0.05

Figure S6. Continuous blockade of the LFA-1 pathway results in long-term graft survival
B6 recipients of fully allogeneic BALB/c skin grafts were treated with either the standard regimen of CTLA-4 Ig and anti–LFA-1 on days 0, 2, 4, and 6 (n=6), or the same regimen of CTLA-4 Ig with an extended course of anti–LFA-1 (days 0, 2, 4, and 6 and weekly thereafter)(n=6). Results indicated that the continuous blockade of LFA-1 resulted in a significant prolongation in graft survival as compared to those animals receiving short course treatment (A, p=0.0045). Mice were sacrificed at day 44 post-transplant and the frequency of CD4⁺ and CD8⁺ T cells in the draining lymph nodes, the expression of CD62L on those cells, and the frequencies of FoxP3⁺ Treg were determined by flow cytometry. *p<0.05
Figure S1

A.

B.

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Figure S2
Figure S3
Figure S5
Figure 56