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. *$p<0.05$.

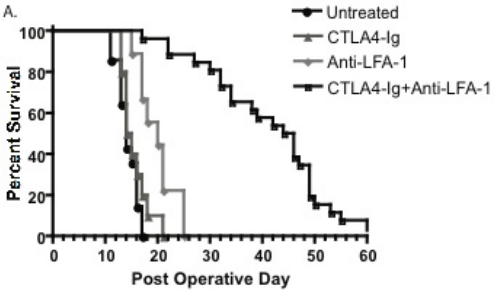
Figure S5. LFA-1 antagonism results in increased $CD4^+$ $CD25^+$ $FoxP3^+$ T-cell frequencies in non-draining nodes but not spleens

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. *$p<0.05$

A.



B.

	UNTREATED	UNTREATED VS. CTLA-4 Ig	UNTREATED VS. ANTI LFA-1	UNTREATED VS. CTLA-4 Ig + ANTI LFA-1
MST (d)	14	14.5	20	44
p value	--	0.222	0.0002	<0.0001

Figure S1

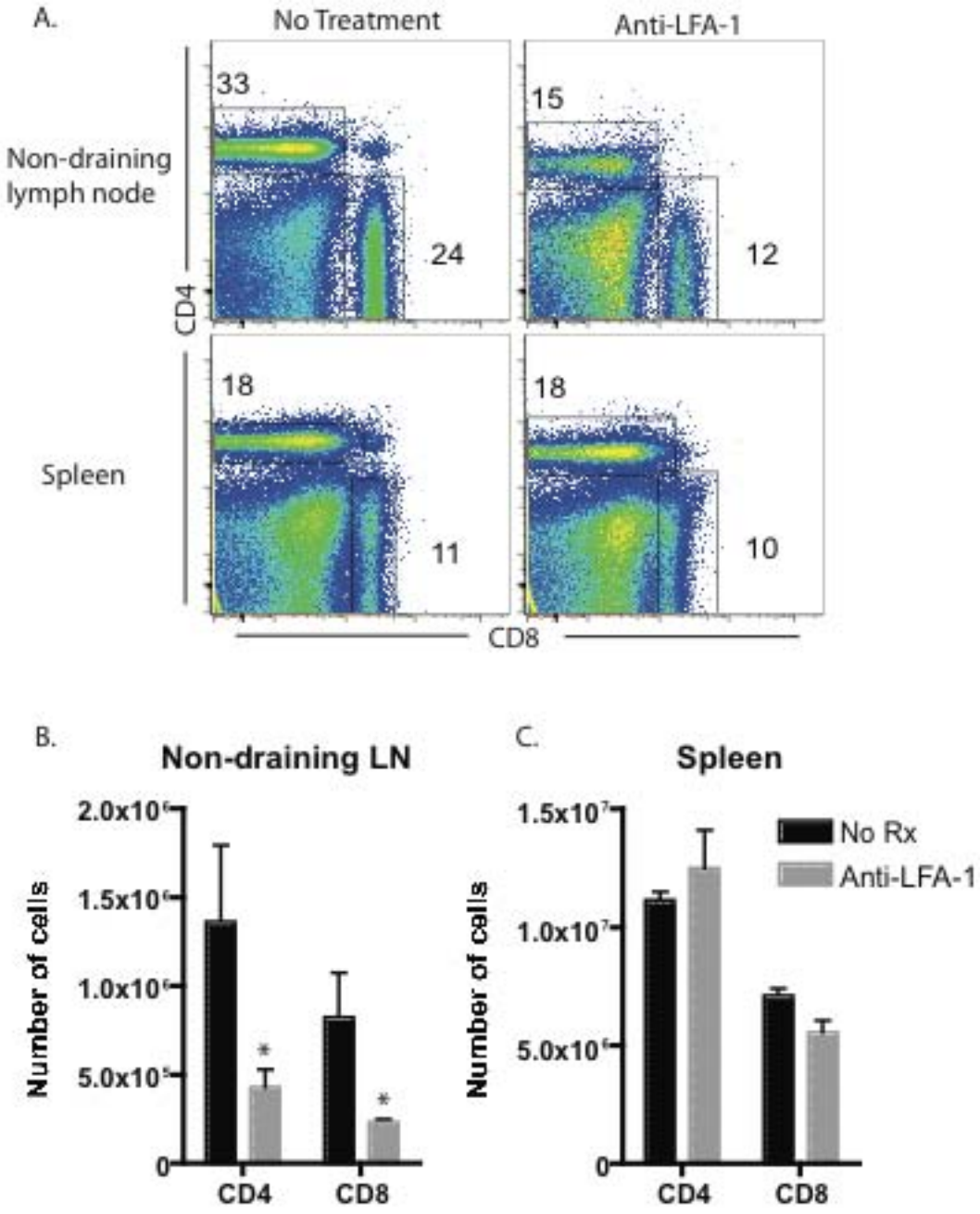

A.
No Treatment
Anti-LFA-1
Non-draining lymph node
Spleen
CD4
CD8
33
24
15
12
18
11
18
10
B.
Non-draining LN
Number of cells
2.0x10⁶
1.5x10⁶
1.0x10⁶
5.0x10⁵
0
CD4
CD8
C.
Spleen
1.5x10⁷
1.0x10⁷
5.0x10⁶
No Rx
Anti-LFA-1

Figure S2

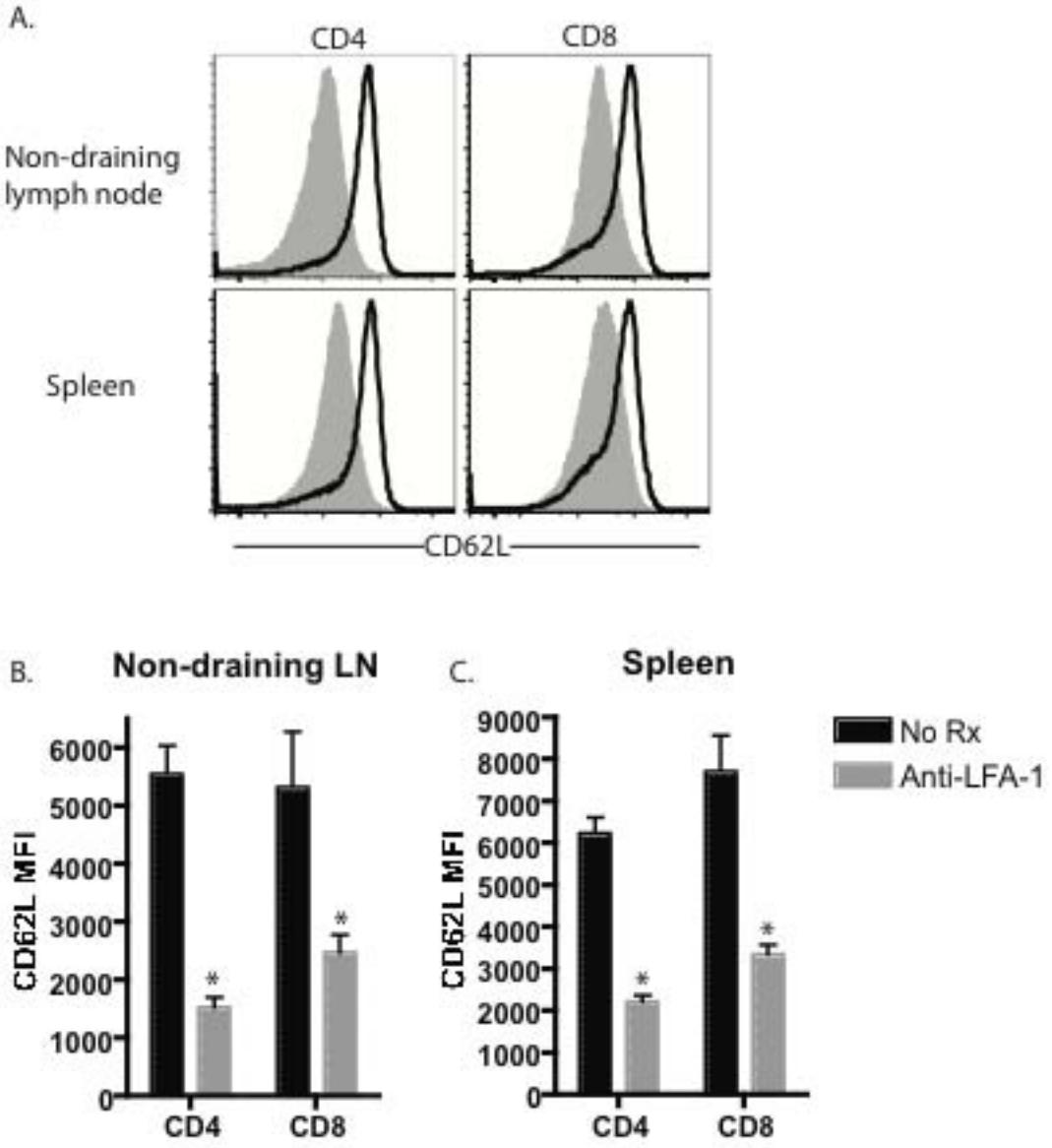

A.
CD4
CD8
Non-draining lymph node
Spleen
CD62L
B.
Non-draining LN
CD62L MFI
6000
5000
4000
3000
2000
1000
0
CD4
CD8
C.
Spleen
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
CD4
CD8
No Rx
Anti-LFA-1

Figure S3

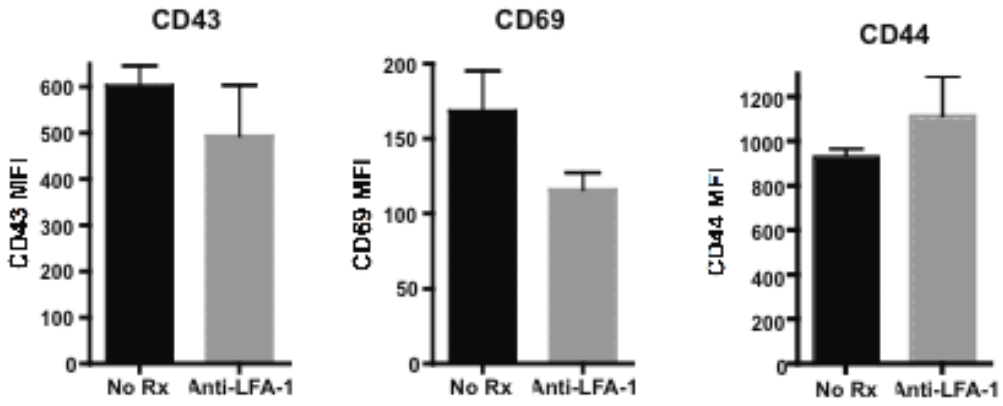

CD43
CD43 MFI
600
500
400
300
200
100
0
No Rx
Anti-LFA-1
CD69
CD69 MFI
200
150
100
50
0
No Rx
Anti-LFA-1
CD44
CD44 MFI
1200
1000
800
600
400
200
0
No Rx
Anti-LFA-1

Figure S4

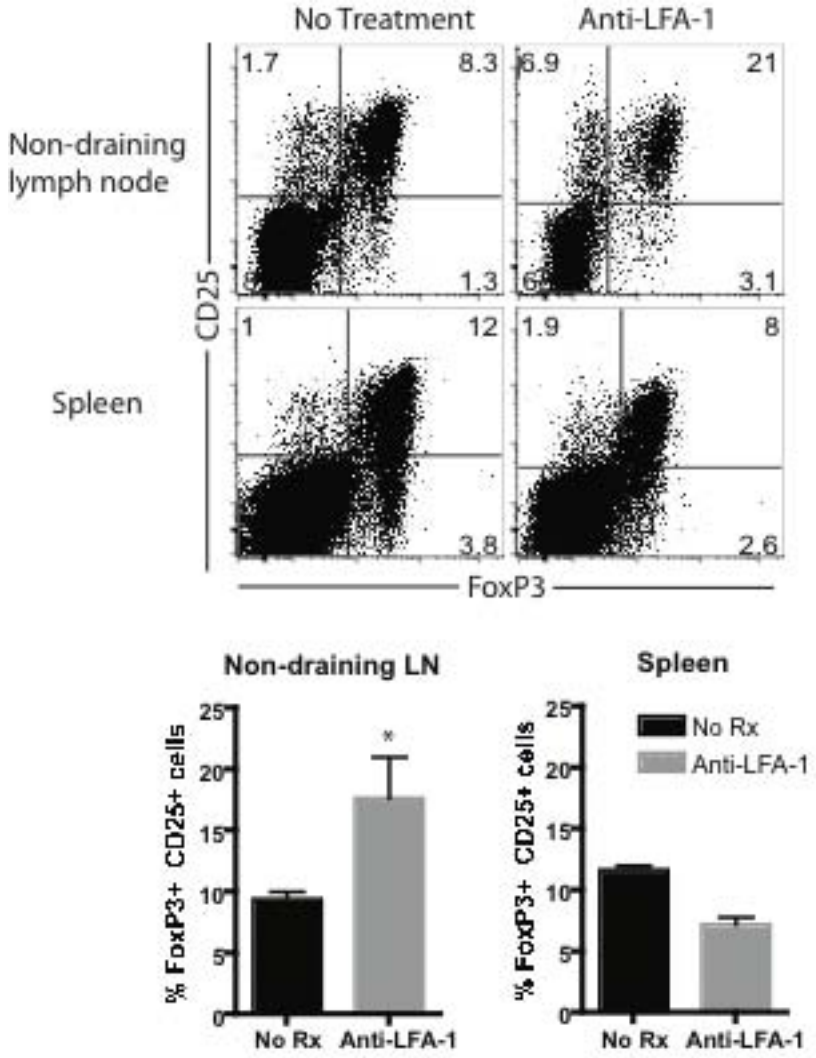

No Treatment
Anti-LFA-1
Non-draining lymph node
Spleen
CD25
FoxP3
1.7
8.3
1.3
6.9
21
3.1
1
12
3.8
1.9
8
2.6
Non-draining LN
Spleen
% FoxP3+ CD25+ cells
No Rx
Anti-LFA-1
*

Figure S5

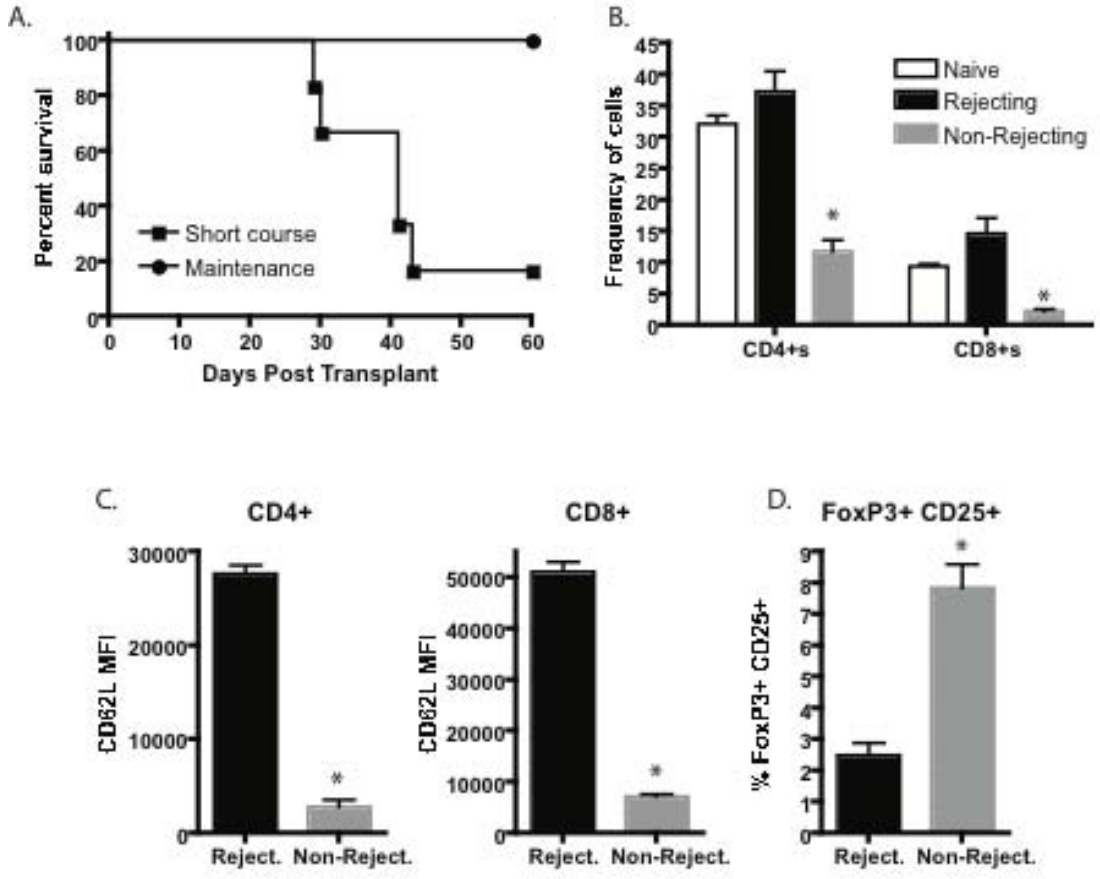

A.
Percent survival
Short course
Maintenance
Days Post Transplant
B.
Frequency of cells
Naive
Rejecting
Non-Rejecting
CD4+s
CD8+s
C.
CD4+
CD62L MFI
Reject.
Non-Reject.
CD8+
CD62L MFI
Reject.
Non-Reject.
D.
FoxP3+ CD25+
% FoxP3+ CD25+
Reject.
Non-Reject.

Figure S6