**Figure S1.** A. Saturation binding of $^{125}$I-TS1/22 Fab to LFA-1 on cultured T lymphocytes activated by CBR LFA-1/2+KIM127+MEM148 Fab. Data are averages±SE from two independent experiments in triplicates. Curve is best fit to specific binding model and derived $K_{D,Fab}$ is shown ±SE. B,D. Competition binding of Hi3-ICAM-1 to freshly isolated naïve T lymphocytes before (B) or after activation by SDF-1α (D). Averages±SE are from triplicate determination. Best fit curves and $K_D$ values with 1 SE intervals are shown. Affinities were compared by F-test with indicated p value. C. Adhesion of K562 transfectants expressing wt LFA-1 or high affinity LFA-1 to ICAM-1 substrate in the presence of 1 mM Ca$^{2+}$/1 mM Mg$^{2+}$ or 10 mM EDTA. Averages±SE are from triplicate determinations.

**Figure S2.** A-C. Hi3-ICAM-1 competition binding to T lymphocytes activated by PMA (A), SDF-1α (B), or T cell receptor crosslinking with OKT3 and anti-IgG2a (C). Data is from Fig. 3E-G. Data analysis was as in Fig. 3, except that the top of the curve ($B_0$) was constrained to 100% during nonlinear regression.

**Figure S3.** The TS1/18 Fab allosterically inhibits LFA-1 adhesiveness induced by CBR LFA-1/2+KIM127. Adhesion of T lymphocytes to ICAM-1 substrate was measured without (left) or after activation by CBR LFA-1/2+KIM127 Fab (right) as a function of concentration of TS1/18 Fab. Averages±SE from triplicates.
**Figure S4.** $^{125}$I-Hi3-ICAM-1 saturation binding to LFA-1 on T lymphocytes activated by Fab combination. Averages±SE are from two independent triplicate experiments. Curve is best fit to specific binding model and calculated $K_D$±SE is shown.

**Figure S5.** Induction of high affinity LFA-1 by KIM127 Fab is not detectable at 0 °C. T lymphocytes were activated by Fab at 37 °C and then transferred to ice for competition binding assays. A. Control LFA-1 has low affinity for ligand. B. KIM127 Fab induces LFA-1 priming in the absence of detectable high affinity receptors, whereas the combination of CBR LFA-1/2 and KIM127 Fab induces high affinity binding of ligand (C). Averages±SE from 1 to 8 experiments, each performed in triplicate.

**Figure S6.** Binding kinetics of TS1/22 Fab. K562 transfectants expressing LFA-1 were incubated with 10 nM $^{125}$I-TS1/22 Fab for various time at 37 °C, and specific binding was determined as described in the experimental section. Averages±SE from triplicates.
Figure S1

A  
CBR LFA-1/2+KIM127+MEM148  
$K_{D,Fab} = 23.6 \pm 3.4 \text{nM}$

B  
Naïve T lymphocytes; control  
$K_D = 40.7 \mu\text{M} (37.3-44.5 \mu\text{M})$

C  
Naïve T lymphocytes; SDF-1α  
$K_D = 24.7 \mu\text{M} (22.3-27.4 \mu\text{M})$  
sign. diff. from control ($p=0.0009$)

D  
Specifically bound TS1/22 Fab  
[% of binding w/o competitor]

log Hi3-ICAM-1 [M]
control: 40.1 µM (36.8-43.7 µM)
PMA: 24.8 µM (23.0-26.7 µM)   
sign. diff. (p<0.0001)

SDF-1α: 24.8 µM (22.5-27.2 µM)   
sign. diff. (p=0.0003)

OKT3: 29.3 µM (26.4-32.6 µM)   
sign. diff. (p=0.022)
Figure S4

CBR LFA-1/2+KIM127+MEM148

$K_D = 36.9 \pm 3.0 \text{ nM}$
Figure S5

**A** control

$K_D=89 \mu M \ (82-96 \mu M)$

**B** KIM127

$K_D=51 \mu M \ (46-57 \mu M)$

**C** CBR LFA-1/2+KIM127

- 67% $K_D=27 \mu M \ (20-36 \mu M)$
- 33% $K_D=42 \text{nM} \ (21-85 \text{nM})$

2 sites (p<0.0001)
Figure S6

Incubation time [min]

Specifically bound $^{125}$I-TS1/22 Fab [cpm/10^5 cells]

0 30 60 90 120 150

0 1,000 2,000 3,000