# Supporting Information

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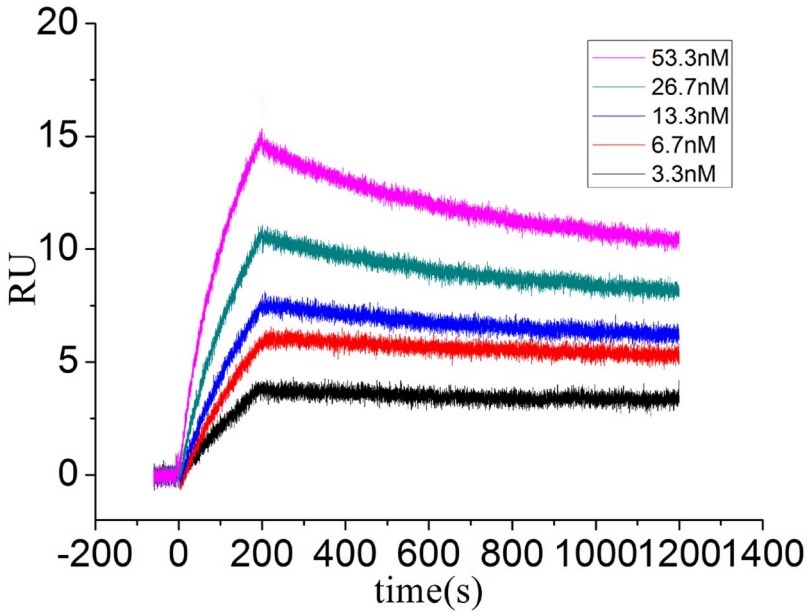


**Fig. S1.** Surface plasmon resonance (SPR) binding analysis of the Efalizumab Fab with the $\alpha_L$ I domain. Surface plasmon resonance experiments were performed by using a BIAcore T-100 instrument (GE Healthcare). The purified $\alpha_L$ I domain was immobilized on a CM-5 chip to a total signal of 20 BIAcore relative units (RU) by amino coupling method. The purified Efalizumab Fab fragment was diluted in PBS buffer (pH 7.4) with 1 mM $Mg^{2+}$ and 2-fold serial dilutions of samples were injected over the test and negative control flow cells at a flow rate of 50 $\mu$L/min at 25 °C. The concentration series for the Fab were 3.3, 6.7, 13.3, 26.7, and 53.3 nM. The data were fitted to 1:1 binding model. The dissociation equilibrium constant ($K_D$) was calculated as $k_{off}/k_{on}$ by using BIAcore T-100 evaluation software 2.0.1. The $K_D$ value is represented as the mean $\pm$ SEM of 3 independent experiments. The binding affinity of the Efalizumab Fab with the I domain ($K_D$) is 2.2 $\pm$ 0.5 nM, which is comparable to that of MHM24 with the I domain ($K_D$ = 1.9 $\pm$ 0.4 nM) [Shimaoka M, et al. (2006) AL-57, a ligand-mimetic antibody to integrin LFA-1, reveals chemokine-induced affinity up-regulation in lymphocytes. *Proc Natl Acad Sci USA* 103:13991–13996.].

**Table S1. Summary of diffraction data and structure refinement statistics of the Efalizumab Fab/I domain complex in crystal form II**

| | Complex of form II |
|---|---|
| Diffraction data | |
| Wavelength (Å) | 1.0000 |
| Space group | $P6_122$ |
| Resolution (Å) | 50.00–3.60 (3.73–3.60)* |
| Cell parameters | |
| *a* (Å) | 111.1 |
| *b* (Å) | 111.1 |
| *c* (Å) | 470.7 |
| Observed reflections | 110,130 (8,862) |
| Unique reflections ($I/\sigma(I) > 0$) | 20,645 (2,014) |
| Average redundancy | 5.3 (4.4) |
| Average $I/\sigma(I)$ | 7.5 (2.0) |
| Completeness (%) | 97.8 (98.9) |
| Rmerge (%)† | 20.3 (62.2) |
| Refinement and structure model | |
| Reflections ($Fo \geq 0\sigma(Fo)$) | |
| Working set | 19,527 |
| Test set | 1,026 |
| R factor‡ | 0.267 |
| Free R factor | 0.333 |
| No. of protein atoms | 9,442 |
| No. of $Zn^{2+}$ atoms | 2 |
| Average B factor (Å$^2$) | |
| All atoms | 97.5 |
| Fab main chain/side chain | 100.3/99.9 |
| I domain main chain/side chain | 90.7/92.2 |
| $Zn^{2+}$ | 99.5 |
| RMS deviations | |
| Bond lengths (Å) | 0.011 |
| Bond angles (°) | 1.7 |
| Ramachandran plot (%) | |
| Most favoured regions | 82.2 |
| Additional allowed regions | 16.7 |
| Generously allowed regions | 1.1 |
| Luzzati atomic positional error (Å) | 0.44 |

*Numbers in parentheses represent the highest resolution shell. †$Rmerge = \Sigma_{hkl}\Sigma_i|I_i(hkl)_i - <I(hkl)>|/\Sigma_{hkl}\Sigma_i I_i(hkl)$. ‡$R = \Sigma_{hkl}||F_o| - |F_c||/\Sigma_{hkl}|F_o|$.

**Table S2. van der Waals contacts between the Efalizumab Fab and the LFA-1 I domain (<4.0 Å)**

| LFA-1 I domain residues | Efalizumab Fab residues |
|---|---|
| Pro144$^{I}$ | Tyr49$^{L}$ (2)*, Phe102$^{H}$ (5), Tyr103$^{H}$ (6) |
| Phe147$^{I}$ | Phe102$^{H}$ (5) |
| Gln148$^{I}$ | His32$^{H}$ (3), Phe102$^{H}$ (1) |
| Leu151$^{I}$ | Phe102$^{H}$ (2) |
| Lys155$^{I}$ | Gly31$^{H}$ (1) |
| Asp191$^{I}$ | Ser54$^{H}$ (3) |
| Asp193$^{I}$ | Thr30$^{H}$ (10), Gly31$^{H}$ (1), His52$^{H}$ (1), Ser54$^{H}$ (1) |
| Ala194$^{I}$ | Ser54$^{H}$ (6), Asp55$^{H}$ (2) |
| Lys197$^{I}$ | Trp33$^{H}$ (7), His52$^{H}$ (7), Glu57$^{H}$ (4), Tyr101$^{H}$ (3) |
| His198$^{I}$ | Glu57$^{H}$ (5), Tyr101$^{H}$ (4) |
| Val199$^{I}$ | Tyr101$^{H}$ (1) |
| Lys200$^{I}$ | Tyr101$^{H}$ (9), Phe102$^{H}$ (3), Gly104$^{H}$ (4), Thr105$^{H}$ (1) |
| His201$^{I}$ | Phe102$^{H}$ (4), Tyr103$^{H}$ (8) |
| Leu203$^{I}$ | Tyr103$^{H}$ (2) |

*Numbers in parentheses refer to the number of van der Waals contacts.

**Table S3. Comparison of the sequential numbering scheme and the Kabat numbering scheme of the amino acid residues of the Efalizumab Fab used in this work**

| Sequential numbering | Kabat numbering |
|---|---|
| $Tyr49^{L}$ | $Tyr49^{L}$ |
| $His91^{L}$ | $His91^{L}$ |
| $Asn92^{L}$ | $Asn92^{L}$ |
| $Tyr94^{L}$ | $Tyr94^{L}$ |
| $Thr30^{H}$ | $Thr30^{H}$ |
| $Gly31^{H}$ | $Gly31^{H}$ |
| $His32^{H}$ | $His32^{H}$ |
| $Trp33^{H}$ | $Trp33^{H}$ |
| $His52^{H}$ | $His52^{H}$ |
| $Ser54^{H}$ | $Ser53^{H}$ |
| $Asp55^{H}$ | $Asp54^{H}$ |
| $Glu57^{H}$ | $Glu56^{H}$ |
| $Gln62^{H}$ | $Gln61^{H}$ |
| $Lys65^{H}$ | $Lys64^{H}$ |
| $Lys74^{H}$ | $Lys73^{H}$ |
| $Tyr101^{H}$ | $Tyr97^{H}$ |
| $Phe102^{H}$ | $Phe98^{H}$ |
| $Tyr103^{H}$ | $Tyr99^{H}$ |
| $Gly104^{H}$ | $Gly100^{H}$ |
| $Thr105^{H}$ | $Thr100A^{H}$ |
| $Tyr107^{H}$ | $Tyr100C^{H}$ |