Supplemental material

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High-mannose $\alpha_{\mathbf{4}}\beta_{\mathbf{1}}$ headpiece Α 0.28 Fab EC₅₀ (nM) States Fluorescence Polarization 🛆 12G10 0.2±0.1 0 0.24 ▲ HUTS4 14.4±2.5 0 8E3 C+0 0.20 - No Fab C+O ∇mAb13 0.9±1.0 С 0.16 **V** SG/19 2.3±1.0 Free peptide 0.12 10-2 10 10⁰ 10¹ 10² 10 Fab concentration (nM) **B** 0.28 Unclasped high-mannose $\alpha_{\!\!4}\beta_1$ ectodomain EC₅₀ (nM) States Fab Fluorescence Polarization ▲ 12G10 0.5±0.3 0.24 EO ▲ HUTS4 18.0±5.4 0 8E3 EC+EO 0.20 0 9EG7 No Fab BC+EC+EO 0.16 ∇mAb13 3.8±0.9 BC+EC ▼ SG/19 6.2±2.0 mAb13 with ----- 8.3±1.6 0.12 EC ▼ 1µM 9EG7 - Free peptide 10-2 10 10° 10¹ 10 10³ Fab concentration (nM) С 0.28 Fab EC₅₀ (nM) States **4** 12G10 Fluorescence Polarization EO ▲ HUTS4 0.24 0 8E3 EC+EO 0 9EG7 0.20 No Fab BC+EC+EO ⊽mAb13 8±3 0.16 BC+EC ▼ SG/19 10±2 mAb13 with 33±4 EC 0.12 Δ 1µM 9EG7 Free peptide 10-2 10⁻¹ 10⁰ 10¹ 10² 10^{3} Fab concentration (nM)

Figure S1. *EC*₅₀ of conformation-specific Fabs for soluble $\alpha 4\beta 1$ preparations. Fab titrations were done at 1 nM FITC-LDVP, 2 nM $\alpha 4\beta 1$ high-mannose headpiece (A), 2 nM unclasped high-mannose ectodomain (B), and 2 nM unclasped complex N-glycan ectodomain (C). *EC*₅₀ values were obtained from fitting the FP and Fab concentration data to Eq. S21. Errors are fitting errors from duplicates.



Figure S2. EC_{50} of conformation-specific Fabs for the intact $\alpha 4\beta 1$ on cell surfaces. Fab titrations were done at 1 nM FITC-LDVP for open-stabilizing and extension-stabilizing Fabs, 30 nM for closure-stabilizing Fabs on Jurkat (A), Thp1 (B), and KA4 (C) cells. EC_{50} values were obtained from fitting the MFI and Fab concentration data to Eq. S22. Errors are fitting errors from duplicates.



Figure S3. Lack of sensitivity of the energy landscape on the ensemble affinity of the closed states. Dependence of the stability of BC and EC conformation of the intact $\alpha 4\beta 1$ on cell surfaces and the ectodomain construct on the ensemble affinity of the closed states were plotted according to Eqs. S17 and S18.

	Fab	HUTS4	12G10	8E3	9EG7	SG/19	mAb13	HUTS4 + 9EG7	mAb13 + 9EG7
	Stabilized states	EO	EO	EC + EO	EC + EO	BC + EC	BC + EC	EO	EC
α ₄ β ₁									
High-mannose headpiece	<i>EC</i> ₅₀ (nM)	14.4 ± 2.5	0.2 ± 0.1	NDª	Epitope not present	2.3 ± 1.0	0.9 ± 1.0	ND	ND
	[Fab] _{tot} (nM)	1,000	Unused	1,000	Epitope not present	5,000	5,000	Unused	Unused
	P ^{α4β1·Fab} (%)	98.4	NA	NA	Epitope not present	99.9	100	NA	NA
Unclasped complex <i>N</i> -glycan ectodomain	<i>EC</i> ₅₀ (nM)	ND	ND	ND	ND	10 ± 2	8 ± 3	ND	33 ± 4
	[Fab] _{tot} (nM)	1,000	Unused	1,000	Unused	5,000	5,000	Unused	5,000 + 1,000
	$P^{\alpha 4\beta 1 \cdot Fab}$ (%)	>98.4	NA	>99.9	NA	99.8	99.8		99.3
Unclasped high-mannose ectodomain	<i>EC</i> ₅₀ (nM)	18 ± 5.4	0.5 ± 0.3	ND	ND	6.2 ± 2.0	3.8 ± 0.9	ND	8.3 ± 1.6
	[Fab] (nM)	1.000	120	1.000	1.000	5.000	5.000	Unused	5.000 + 1.000
	$P^{\alpha 4\beta 1}$ Fab (%)	98.0	97.6	>99.9	>99.9	99.9	99.9	NA	99.8
Intact receptor on Iurkat cells	<i>EC</i> ₅₀ (nM)	ND	ND	ND	98 ± 19	8.4 ± 2.5	ND	ND	ND
Jonnan Conto	[Eab]. (nM)	Unused	Unused	Unused	8 000	5 000	Unused	15 + 4000	Unused
	$P\alpha 4\beta 1 \cdot Fab (\%)$	NΔ	NΔ	NΔ	98.8	99.8		ND	NΔ
Intact receptor on Thp1 cells	<i>EC</i> ₅₀ (nM)	ND	ND	ND	205 ± 11	3.5 ± 1.8	ND	ND	ND
	[Eab]. (nM)	Unused	Unused	Unused	8 000	5 000	Unused	100 ± 4000	Unused
	$P_{\alpha 4\beta 1}$ Fab (%)	NA	NA	NA	97 5	99.9	ND	ND	NA
Intact receptor on KA4 cells	EC_{50} (nM)	ND	ND	ND	106 ± 5	17.2 ± 4.6	ND	ND	ND
	[Eab] (nM)	Unused	Unused	Unused	8 000	10.000	Unused	10 ± 4.000	Unused
	$\mathbf{p}_{\alpha 4\beta 1}$ Fab (%)	NA	NIA	NIA	08.7	00 8		ND	NIA
or B b	(70)		1.0.1		/0./	//.0		I I D	
High-mannose headpiece	<i>EC</i> ₅₀ (nM)	2,800 ± 600	28 ± 3	4.3 ± 4.2	Epitope not present	<4.7	<2.3	ND	ND
nouuproco	[Fab] (nM)	Unused	2.000	20.000	Epitope not present	20.000	20.000	Unused	Unused
	$P^{\alpha 5\beta 1}$ Fab (%)	NA	98.2	99.8	Epitope not present	>99.8	>99.9	NA	NA
Unclasped complex N-alvcan ectodomain	<i>EC</i> ₅₀ (nM)	<34	ND	<15	ND	6.9±1.6	ND	ND	ND
rigiyean ecleaonain	[Fab] (nM)	5.000	Unused	2.000	Unused	10.000	Unused	Unused	Unused
	$P^{\alpha 5\beta 1}$ Fab (%)	>99.3	NA	>99.0	NA	99.8	NA	NA	NA
Linclasped high-mannase	(70)	277.0	1.0.1	277.0		//.0		ND	
ectodomain	EC_{50} (nM)	20 ± 3	1.1 ± 0.2	9 ± 4	3.1 ± 1.4	4.7 ± 1.0	2.3 ± 1.0	ND	1.8 ± 0.6
	$[Fdb]_{tot}$ (n/VI) $P^{\alpha 5\beta 1\cdot Fab}$ (%)	5,000 99.6	1,000 99.8	2,000 99.4	2,000 99.8	10,000 99.9	15,000 99.9	NA	13,000 + 10,000 99.9
Intact receptor on K562 cells	<i>EC</i> 50 (nM)	2,900 ± 300	107 ± 5	ND	690 ± 30	ND	ND	21 ± 2	ND
	[Fab] _{tot} (nM) P ^{α5β1·Fab} (%)	Unused NA	2,000 95.0	Unused NA	13,000 95.0	Unused NA	Unused NA	2,000 + 6,000 99.0	Unused NA

lable S1. Fab EC ₅₀ values, concentrations used	in liaand-binding	a attinity measurements	(Fabl), and	probabilities of	t Fab-bound	integrins
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•ND, not determined or titration data not fittable; NA, not applicable. •Data published in Li et al. (2017), Fab concentrations used on Jurkat, Thp1, and KA4 to stabilize certain conformational states, are the same as those listed here for K562 cells.

References

Li, J., Y. Su, W. Xia, Y. Qin, M.J. Humphries, D. Vestweber, C. Cabañas, C. Lu, and T.A. Springer. 2017. Conformational equilibria and intrinsic affinities define integrin activation. *EMBO J.* 36:629–645. https://doi.org/10.15252/embj.201695803