Li and Springer, https://doi.org/10.1083/jcb.201701169

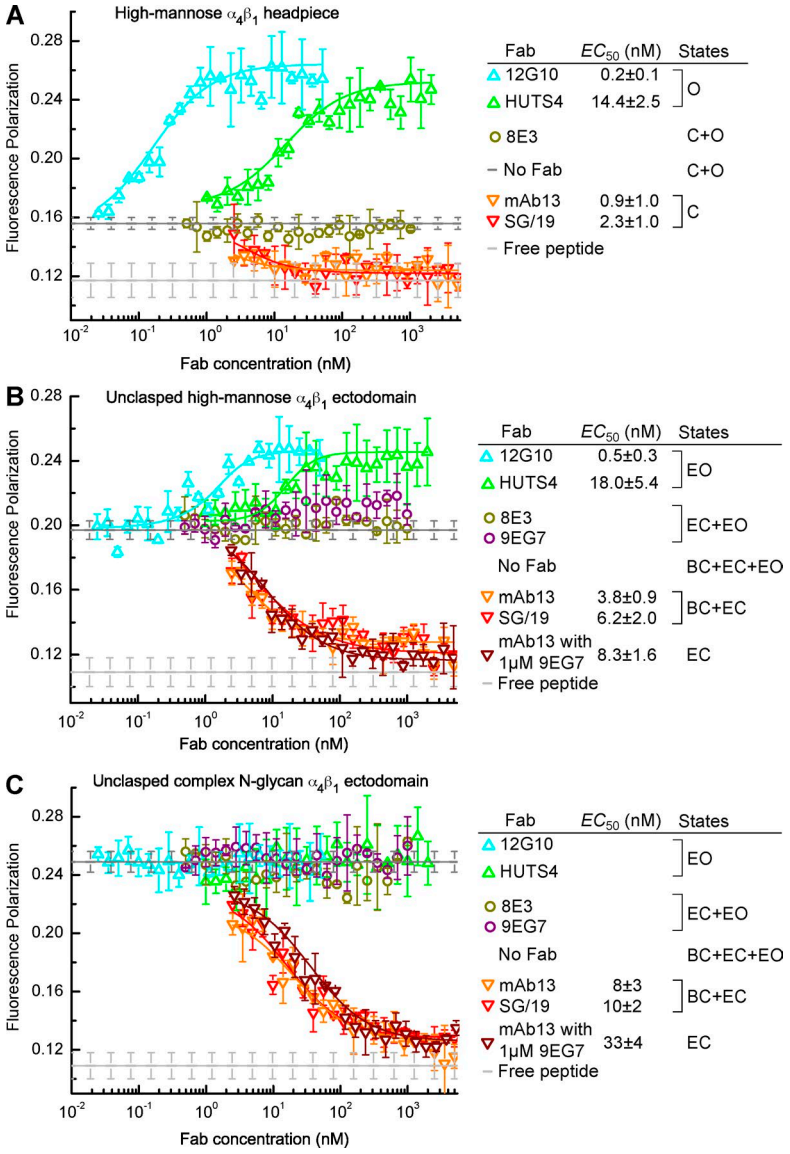

Figure S1. ***EC_{50}* of conformation-specific Fabs for soluble α4β1 preparations.** Fab titrations were done at 1 nM FITC-LDVP, 2 nM α4β1 high-mannose headpiece (A), 2 nM unclasped high-mannose ectodomain (B), and 2 nM unclasped complex *N*-glycan ectodomain (C). EC_{50} values were obtained from fitting the FP and Fab concentration data to Eq. S21. Errors are fitting errors from duplicates.

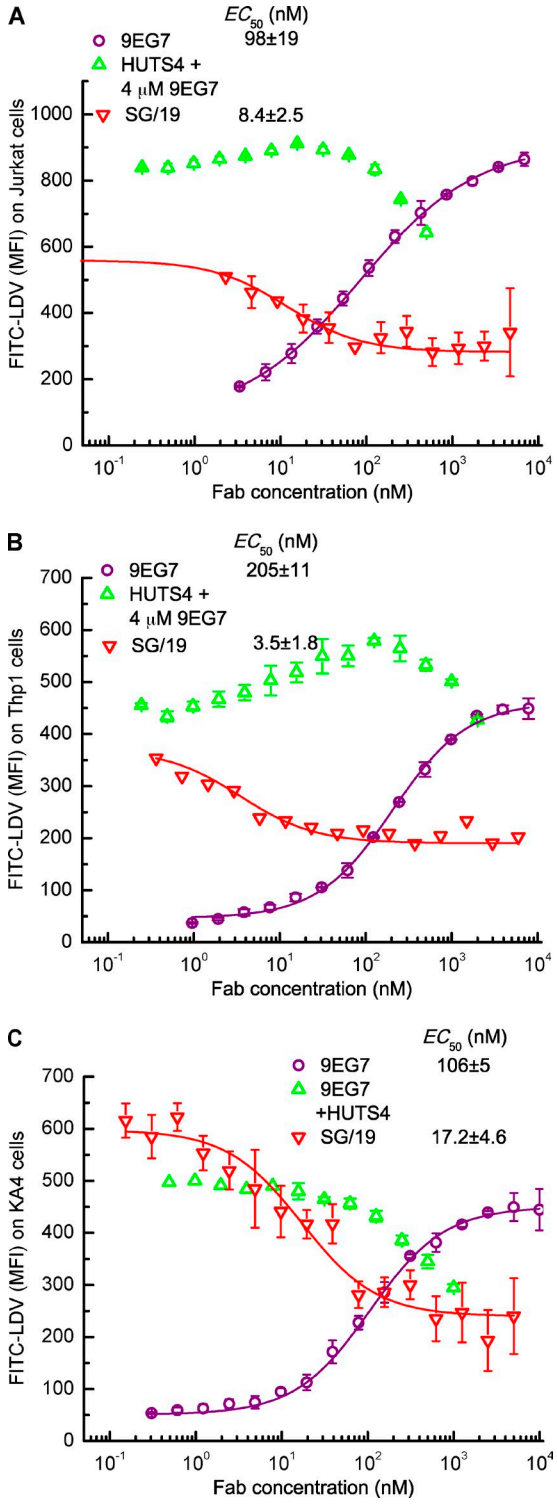

Figure S2. ***EC_{50}* of conformation-specific Fabs for the intact α4β1 on cell surfaces.** Fab titrations were done at 1 nM FITC-LDVP for open-stabilizing and extension-stabilizing Fabs, 30 nM for closure-stabilizing Fabs on Jurkat (A), Thp1 (B), and KA4 (C) cells. *EC_{50}* values were obtained from fitting the MFI and Fab concentration data to Eq. S22. Errors are fitting errors from duplicates.

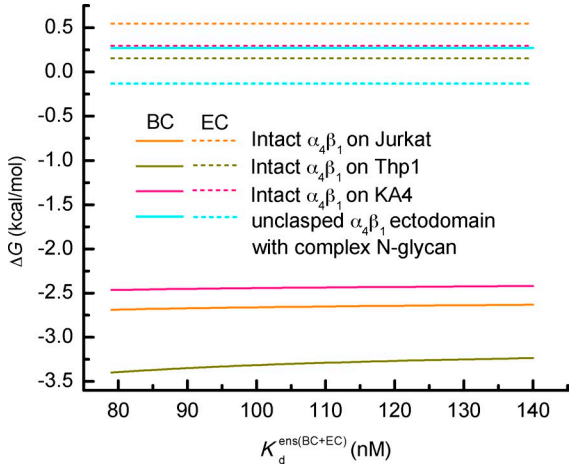

Figure S3. **Lack of sensitivity of the energy landscape on the ensemble affinity of the closed states.** Dependence of the stability of BC and EC conformation of the intact α4β1 on cell surfaces and the ectodomain construct on the ensemble affinity of the closed states were plotted according to Eqs. S17 and S18.

Table S1. **Fab EC_{50} values, concentrations used in ligand-binding affinity measurements ($[Fab]_{tot}$), and probabilities of Fab-bound integrins**

	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**
$\alpha_4\beta_1$									
High-mannose headpiece	EC_{50} (nM)	14.4 ± 2.5	0.2 ± 0.1	ND[a]	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
	$P_{x4\beta1\cdot Fab}$ (%)	98.4	NA	NA	Epitope not present	99.9	100	NA	NA
Unclasped complex *N*-glycan ectodomain	EC_{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
	$P_{x4\beta1\cdot Fab}$ (%)	>98.4	NA	>99.9	NA	99.8	99.8		99.3
Unclasped high-mannose ectodomain	EC_{50} (nM)	18 ± 5.4	0.5 ± 0.3	ND	ND	6.2 ± 2.0	3.8 ± 0.9	ND	8.3 ± 1.6
	$[Fab]_{tot}$ (nM)	1,000	120	1,000	1,000	5,000	5,000	Unused	5,000 + 1,000
	$P_{x4\beta1\cdot Fab}$ (%)	98.0	97.6	>99.9	>99.9	99.9	99.9	NA	99.8
Intact receptor on Jurkat cells	EC_{50} (nM)	ND	ND	ND	98 ± 19	8.4 ± 2.5	ND	ND	ND
	$[Fab]_{tot}$ (nM)	Unused	Unused	Unused	8,000	5,000	Unused	15 + 4,000	Unused
	$P_{x4\beta1\cdot Fab}$ (%)	NA	NA	NA	98.8	99.8	ND	ND	NA
Intact receptor on Thp1 cells	EC_{50} (nM)	ND	ND	ND	205 ± 11	3.5 ± 1.8	ND	ND	ND
	$[Fab]_{tot}$ (nM)	Unused	Unused	Unused	8,000	5,000	Unused	100 + 4,000	Unused
	$P_{x4\beta1\cdot Fab}$ (%)	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
	$[Fab]_{tot}$ (nM)	Unused	Unused	Unused	8,000	10,000	Unused	10 + 4,000	Unused
	$P_{x4\beta1\cdot Fab}$ (%)	NA	NA	NA	98.7	99.8	ND	ND	NA
$\alpha_5\beta_1$[b]									
High-mannose headpiece	EC_{50} (nM)	2,800 ± 600	28 ± 3	4.3 ± 4.2	Epitope not present	<4.7	<2.3	ND	ND
	$[Fab]_{tot}$ (nM)	Unused	2,000	20,000	Epitope not present	20,000	20,000	Unused	Unused
	$P_{x5\beta1\cdot Fab}$ (%)	NA	98.2	99.8	Epitope not present	>99.8	>99.9	NA	NA
Unclasped complex *N*-glycan ectodomain	EC_{50} (nM)	<34	ND	<15	ND	6.9±1.6	ND	ND	ND
	$[Fab]_{tot}$ (nM)	5,000	Unused	2,000	Unused	10,000	Unused	Unused	Unused
	$P_{x5\beta1\cdot Fab}$ (%)	>99.3	NA	>99.0	NA	99.8	NA	NA	NA
Unclasped high-mannose 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
	$[Fab]_{tot}$ (nM)	5,000	1,000	2,000	2,000	10,000	15,000		15,000 + 10,000
	$P_{x5\beta1\cdot Fab}$ (%)	99.6	99.8	99.4	99.8	99.9	99.9	NA	99.9
Intact receptor on K562 cells	EC_{50} (nM)	2,900 ± 300	107 ± 5	ND	690 ± 30	ND	ND	21 ± 2	ND
	$[Fab]_{tot}$ (nM)	Unused	2,000	Unused	13,000	Unused	Unused	2,000 + 6,000	Unused
	$P_{x5\beta1\cdot Fab}$ (%)	NA	95.0	NA	95.0	NA	NA	99.0	NA

[a]ND, not determined or titration data not fittable; NA, not applicable.
[b]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