Supplementary Materials

Molecular Biology of the Cell

Anderson *et al.*

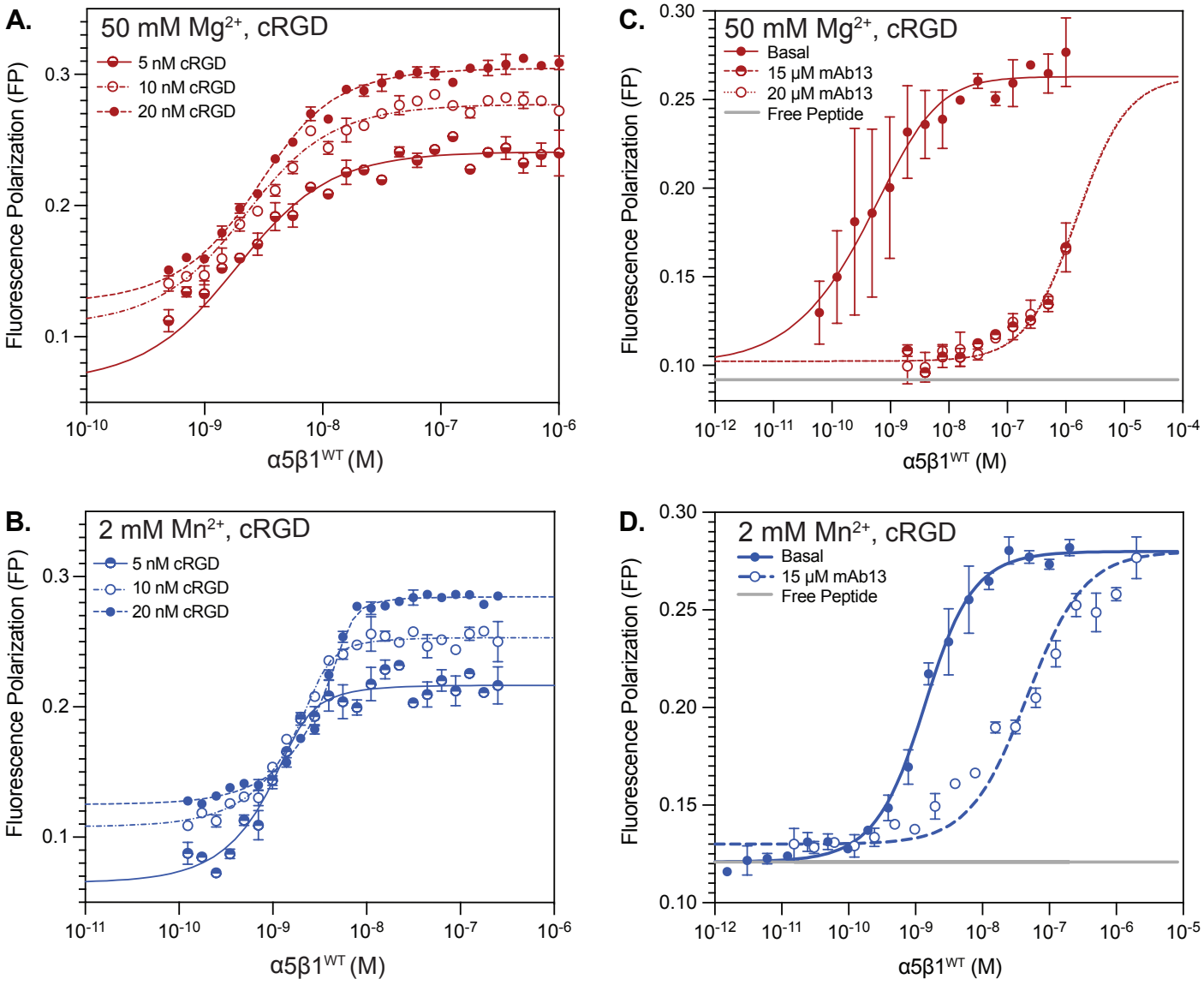
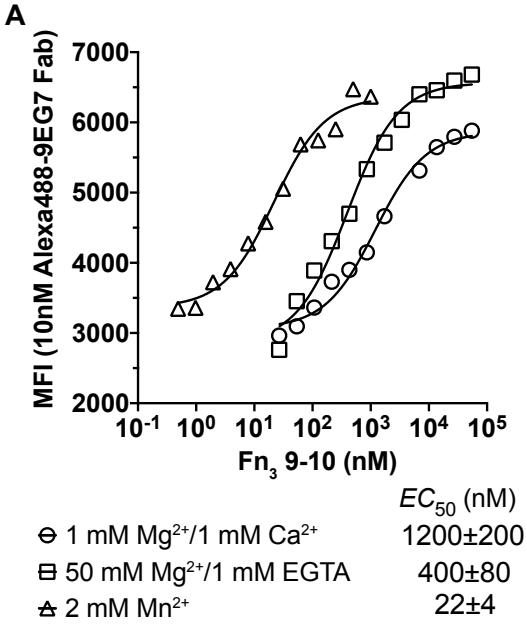
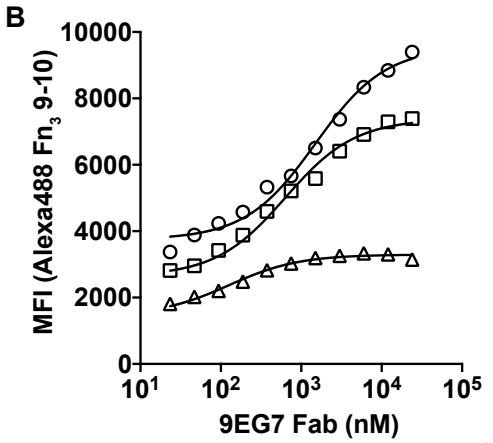


Figure S1. Affinities of α5β1^{WT} for FITC-cRGD. (A-B) α5β1^{WT} was titrated in the presence of FITC-cRGD peptide at three concentrations in (A) 50 mM Mg^{2+} (red) and (B) 2 mM Mn^{2+} (blue). Multiple concentrations of ligand are used to compensate for ligand depletion effects at affinities >1 nM by globally fitting K_d values. (C-D) The closed ensemble (EC+BC) of α5β1^{WT} for ligand FITC-cRGD in (C) 50 mM Mg^{2+} and (D) 2 mM Mn^{2+} was measured by titrating α5β1^{WT} in absence (basal, solid line) and presence of mAb13 fab (15 and 20 μM, dashed and dotted respectively), with 10 nM FITC-cRGD peptide. All titrations contain pH 7.4 TBS. Titrations were done in triplicate and fitted affinities are shown in Table 1.



	EC_{50} (nM)
1 mM Mg^{2+}/1 mM Ca^{2+}	1200±200
50 mM Mg^{2+}/1 mM EGTA	400±80
2 mM Mn^{2+}	22±4



	EC_{50} (nM)
1 mM Mg^{2+}/1 mM Ca^{2+} 10 nM Alexa488-Fn_3 9-10	1500±250
50 mM Mg^{2+}/1 mM EGTA 5 nM Alexa488-Fn_3 9-10	640±100
2 mM Mn^{2+} 0.6 nM Alexa488-Fn_3 9-10	130±20

Figure S2. Titrations of intact α5β1^{WT} on K562 cells with Fn_3 9-10 and Fab 9EG7. Concentrations necessary to saturate intact α5β1 on K562 cells with Fn_3 9-10 and Fab 9EG7, for use in determining the conformational landscape of cell-bound α5β1 in the conditions of 1 mM Mg^{2+}/1 mM Ca^{2+}, 50 mM Mg^{2+}/1 mM EGTA, and 2 mM Mn^{2+}. (A and B) Alexa488-9EG7 Fab (10 nM) (A) or Alexa488-$Fn_3$9-10 (10, 5, or 0.6 nM as indicated) (B) binding to K562 cells (2 × 10^6 cells/ml) was measured by FACS, without washing, in presence of unlabeled Fn_3 9-10 (A) or 9EG7 Fab (B).

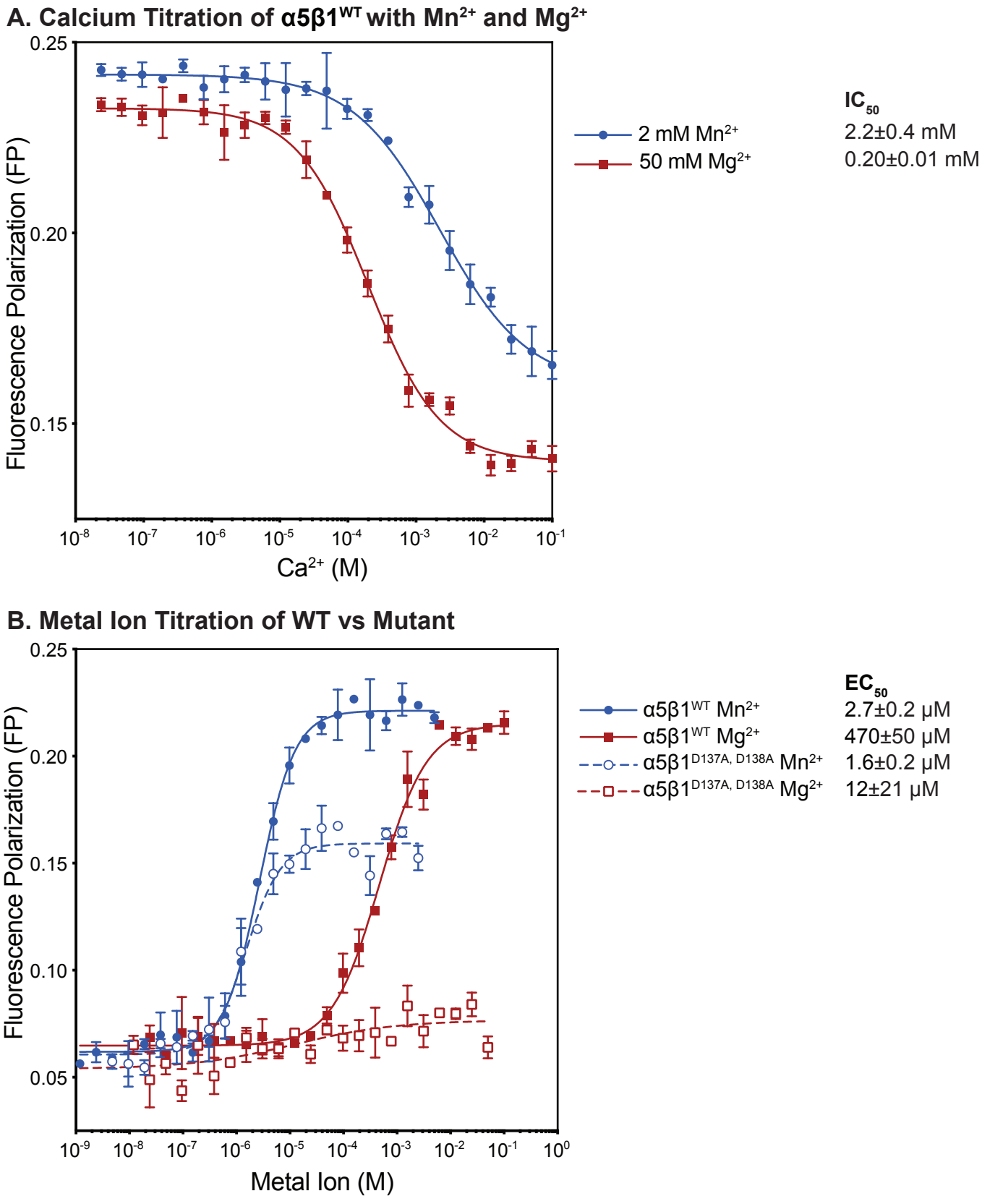


Figure S3. Metal Ion titrations of α5β1. (A) Inhibition by calcium of binding of $\alpha5\beta1^{WT}$ (20 nM) to FITC-cRGD (5 nM) in 2 mM Mn^{2+} (blue) or 50 mM Mg^{2+} (red). (B) $\alpha5\beta1^{D137A,\ D138A}$ shows a reduced FP signal during titration with Mn^{2+} and Mg^{2+} compared to $\alpha5\beta1^{WT}$, because of the lower affinity $\alpha5\beta1^{D137A,\ D138A}$ has for FITC-cRGD. $\alpha51^{WT}$ (solid lines, 20 nM) and $\alpha5\beta1^{D137A,\ D138A}$ (dashed lines, 20 nM) were titrated with Mn^{2+} (blue) or Mg^{2+}(red) in the presence of FITC-cRGD (5 nM). This experiment also shows that 2 mM Mn^{2+} is saturating for both $\alpha5\beta1^{WT}$ and $\alpha5\beta1^{D137A,\ D138A}$. Titrations were done in triplicate.

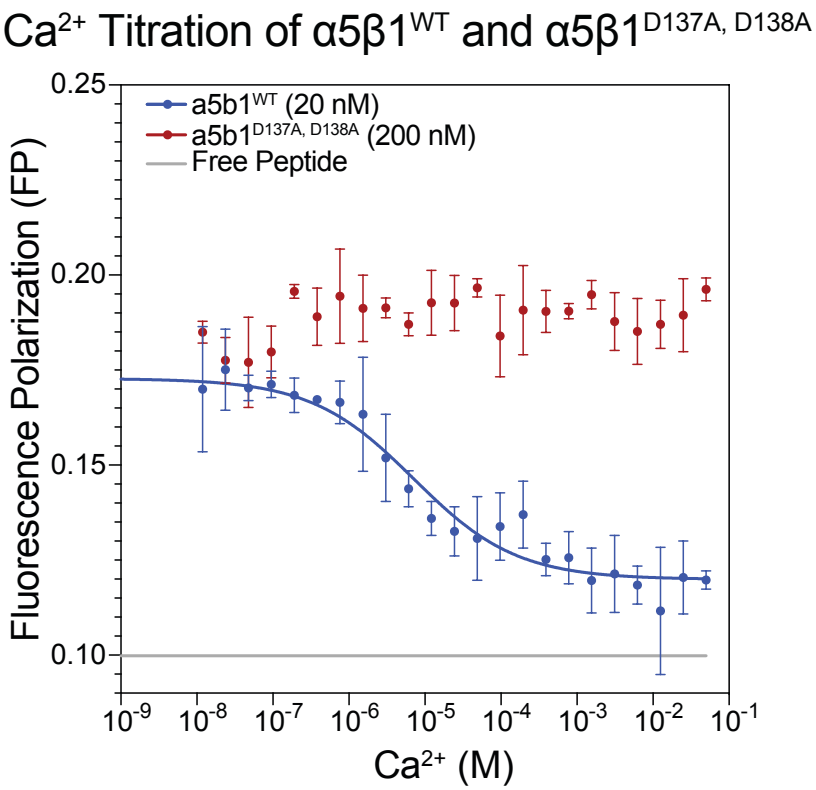


Figure S4. Calcium ion titrations of α5β1^{WT} and α5β1$^{D137A, D138A}$ in 1 mM Mg^{2+}. Similar to Fig. 6, α5β1^{WT} and α5β1$^{D137A, D138A}$ were titrated with Ca^{2+}, in the presence of 1 mM Mg^{2+}. It was found that while binding of α5β1^{WT} to FITC-cRGD (5 nM) can be inhibited by Ca^{2+}, no effect was shown in the ADMIDAS mutant. Titrations were done in triplicate.

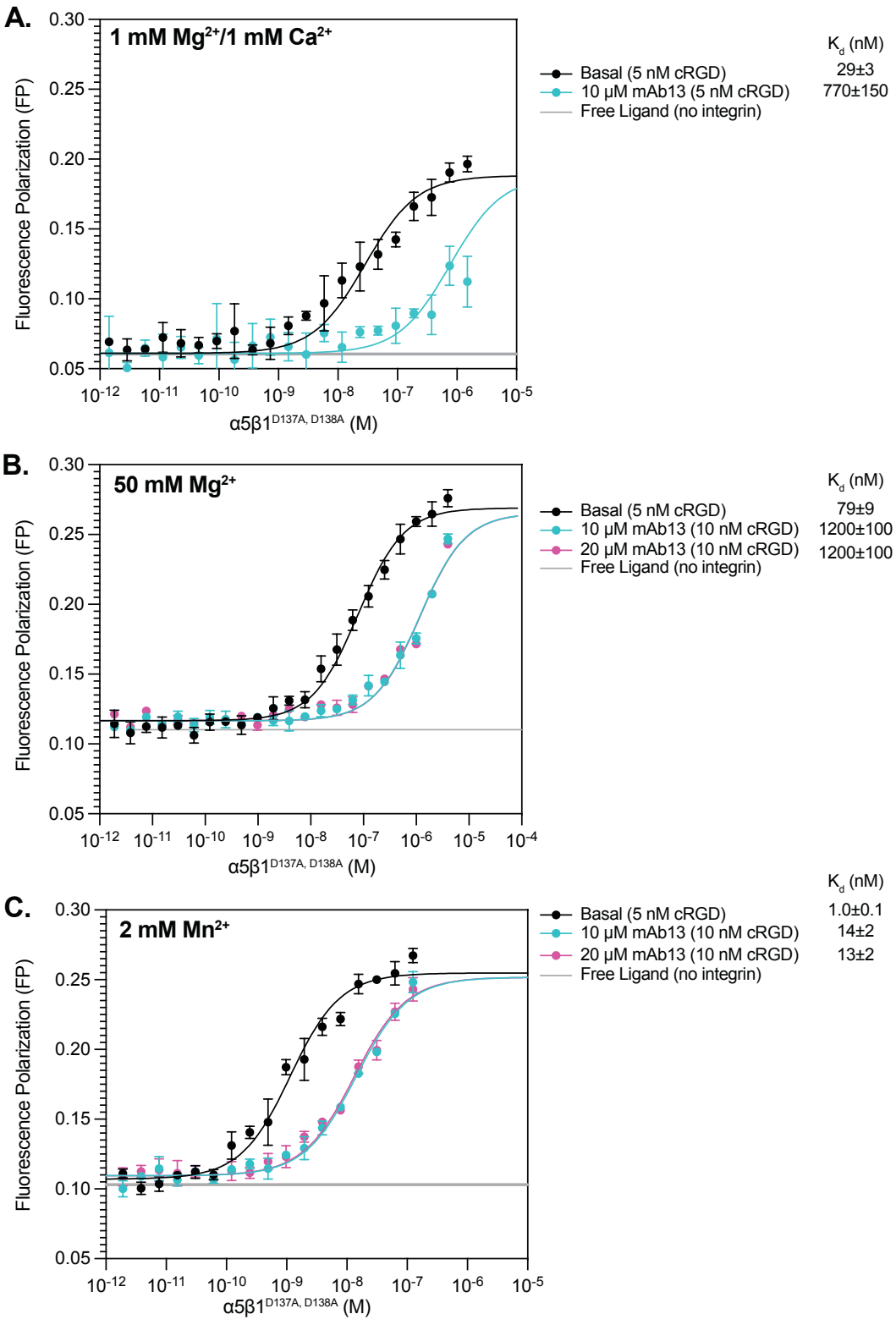


Figure S5. Basal and closed ensemble affinities of α5β1$^{D137A, D138A}$ for FITC-cRGD. (A-C) The basal and closed ensemble affinity were determined in 1 mM Mg^{2+} / 1 mM Ca^{2+} (A), 50 mM Mg^{2+} (B), and 2 mM Mn^{2+} (C), by titrating α5β1$^{D137A, D138A}$ in the absence and presence of Fab mAb13. In the experiments containing 50 mM Mg^{2+} and 2 mM Mn^{2+}, titrations were done in two concentrations of mAb13 to ensure full satuation of the closed ensemble. Titrations were done in triplicate and fitted affinities can be found in Table 1. Basal affinities were measured three (A) and five times (B) and averaged.

Table S1. EC_{50}s of Fab titrations $\alpha5\beta1^{WT}$ and $\alpha5\beta1^{D137A, D138A}$ in different metal ion conditions.
Graphs of the titrations can be found in Figure 3. Concentrations of integrin and ligand can be found in Table S2.

		$\alpha5\beta1^{WT}$			$\alpha5\beta1^{D137A, D138A}$		
		1 mM Mg^{2+} 1 mM Ca^{2+}	50 mM Mg^{2+}	2 mM Mn^{2+}	1 mM Mg^{2+} 1 mM Ca^{2+}	50 mM Mg^{2+}	2 mM Mn^{2+}
●–	**HUTS4** (nM)	20±3	8.5±2.0	-	120±170	120±120	14±6
□–	**12G10** (nM)	1.1±0.2	0.74±0.16	-	0.065±0.015	0.80±0.22	0.22±0.05
▲–	**9EG7** (nM)	3.1±1.4	-	-	-	-	-
▽–	**8E3** (nM)	8.9±3.8	6.3±3.3	-	1.6±1.4	-	-
◆–	**SG/19** (nM)	5.1±1.7	1.5±0.3	-	14±6	10±3	3100±450
○–	**mAb13** (nM)	2.3±1.1	3.5±0.4	-	23±2	18±3	0.98±0.27

Table S2. Concentrations of α5β1 and ligand used in Fab titrations of α5β1^{WT} and α5β1$^{D137A, D138A}$ (Figure 3, Table S1).

		α5β1^{WT}						α5β1$^{D137A, D138A}$					
		1 mM Mg^{2+} 1 mM Ca^{2+}		50 mM Mg^{2+}		2 mM Mn^{2+}		1 mM Mg^{2+} 1 mM Ca^{2+}		50 mM Mg^{2+}		2 mM Mn^{2+}	
		α5β1^{WT}	RGD	α5β1^{WT}	RGD	α5β1^{WT}	RGD	α5β1^{mut}	cRGD	α5β1^{mut}	cRGD	α5β1^{mut}	cRGD
●–	**HUTS4** (nM)	20	5	50	5	5	5	10	10	10	10	10	10
□–	**12G10** (nM)	20	5	50	5	5	5	10	10	10	10	10	10
▲–	**9EG7** (nM)	20	5	50	5	5	5	50	10	50	10	10	10
▽–	**8E3** (nM)	20	5	50	5	5	5	50	10	50	10	10	10
◆–	**SG/19** (nM)	20	5	50	5	20	5	100	10	100	10	10	10
○–	**mAb13** (nM)	20	5	50	5	20	5	100	10	100	10	10	10

Table S3. Fitted K_d values for $\alpha5\beta1^{WT}$ and $\alpha5\beta1^{D137A, D138A}$ in the presence and absence of saturating concentrations of Fabs. Graphs of the titrations can be found in Figure 4, fitted values from panel A can be found in Li et al. {Li, 2017 #24745}.

		$\alpha5\beta1^{WT}$		$\alpha5\beta1^{D137A, D138A}$		
		50 mM Mg^{2+}	**2 mM Mn^{2+}**	**1 mM Mg^{2+} 1 mM Ca^{2+}**	**50 mM Mg^{2+}**	**2 mM Mn^{2+}**
●—	**Basal** (nM)	62±4	2.8±0.3	24000±3600	14000±1300	580±62
—○—	**HUTS4** (nM)	26±2	1.5±0.2	630±54	730±53	51±6
—□—	**12G10** (nM)	-	1.7±0.3	170±14	1000±76	14±2
—▲—	**9EG7** (nM)	53±3	3.3±0.4	-	-	-
—▼—	**8E3** (nM)	54±3	2.2±0.3	-	-	-
—●—	**mAb13,** 15 µM (nM)	48000±8500	3200±130	-	-	-
—●—	**mAb13,** 30 µM (nM)	51000±9500	3500±140	-	-	-