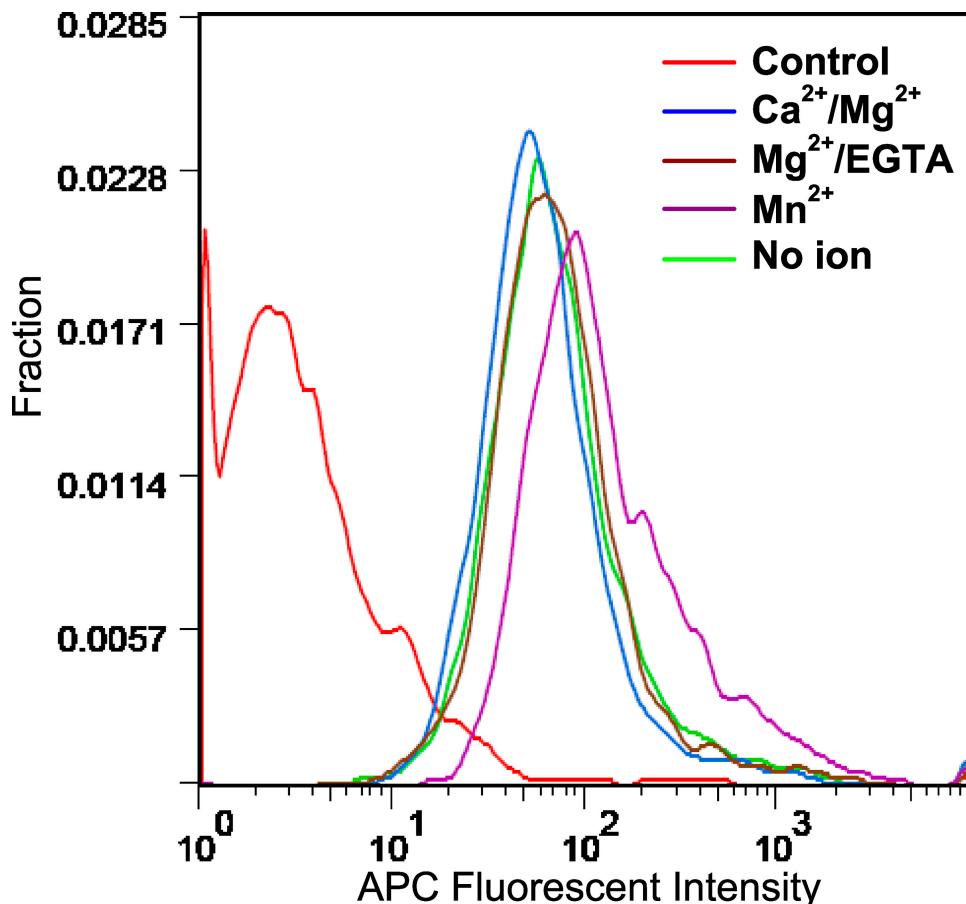
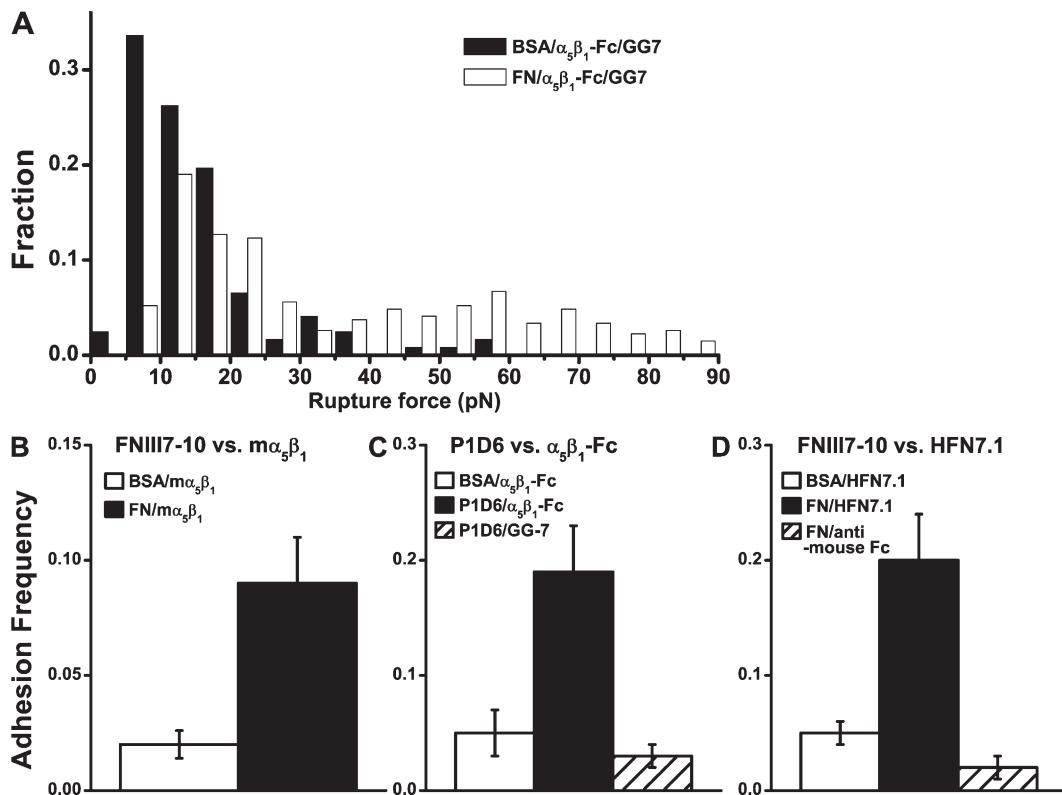


Kong et al., <http://www.jcb.org/cgi/content/full/jcb.200810002/DC1>

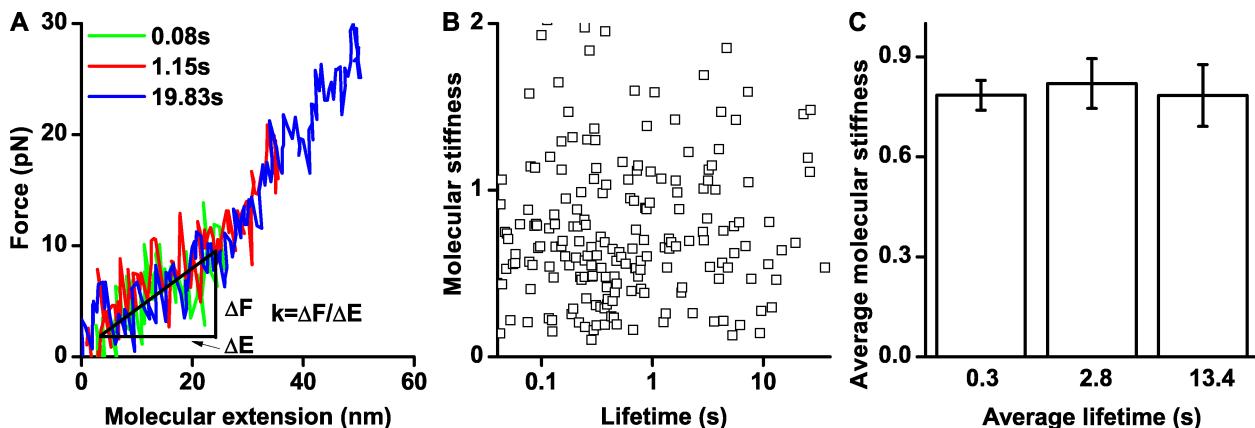


**Figure S1. Expression of 9EG7 epitope by  $\alpha_5\beta_1$ -Fc in different cation conditions.** Binding of allophycocyanin-conjugated anti- $\beta_1$  mAb 9EG7 to  $\alpha_5\beta_1$ -Fc was analyzed by flow cytometry with  $\text{tr}\alpha_5\beta_1$ -Fc that lacks the 9EG7 epitope as control.  $\alpha_5\beta_1$ -Fc or  $\text{tr}\alpha_5\beta_1$ -Fc was captured by anti-Fc mAb GG-7, which was biotinylated and incubated with streptavidin beads in different cation conditions (including no cations). Data are presented as fluorescence intensity histograms.

## ROUGH GALLEY PROOF



**Figure S2. Histograms of rupture forces and additional control for binding specificity.** (A) Histograms of rupture forces of interactions between FNIII<sub>7-10</sub> (open bars)- or BSA (closed bars)-coated cantilever tips and  $\alpha_5\beta_1$ -Fc-functionalized Petri dishes in  $Mn^{2+}$ . (B) Frequency of adhesion between  $\alpha_5\beta_1$  reconstituted into bilayers and streptavidin-coated cantilever tips functionalized with (closed bar) or without (open bar) FNIII<sub>7-10</sub> in  $Mg^{2+}$ /EGTA. (C) Frequency of adhesion between  $\alpha_5\beta_1$ -Fc captured by GG-7 precoated on Petri dishes and P1D6 coated on cantilever tips (closed bar) is compared with that between P1D6-coated tips and GG-7-coated Petri dishes without incubation with  $\alpha_5\beta_1$ -Fc (hatched bar). (D) Frequency of adhesion between anti-FN mAb (HFN7.1) captured by goat anti-mouse Fc antibody preadsorbed on Petri dishes and FNIII<sub>7-10</sub> coated on cantilever tips (closed bar) is compared with that between HFN7.1-functionalized Petri dishes and BSA-coated cantilever tips (open bar) and with that between goat anti-mouse Fc antibody-coated Petri dishes without HFN7.1 incubation and FNIII<sub>7-10</sub>-coated cantilever tips (hatched bar). Error bars indicate SEM.



**Figure S3. Lack of correlation between bond lifetime and molecular stiffness.** (A) The force-scan traces (Fig. 1 D) were transformed to force-extension curves by converting time to molecular extension to allow for measuring molecular stiffness, defined as the force increase divided by the molecular extension increase at the initial linear region of the curve. Force-extension curves from three measurement events that yielded short lifetime at low force (green), intermediate lifetime at intermediate force (red), and long lifetime at high force (blue) are exemplified. (B) Scattered plot of molecular stiffness versus lifetime at forces ranging from 25–40 pN. (C) The data in the scattered plot were grouped into three bins and shown as mean  $\pm$  SEM. These data were obtained for FN- $\alpha_5\beta_1$ -Fc-GG-7 interaction in  $Ca^{2+}/Mg^{2+}$  in 25–40-pN force range, but results measured in other cation conditions and other force ranges were similar.

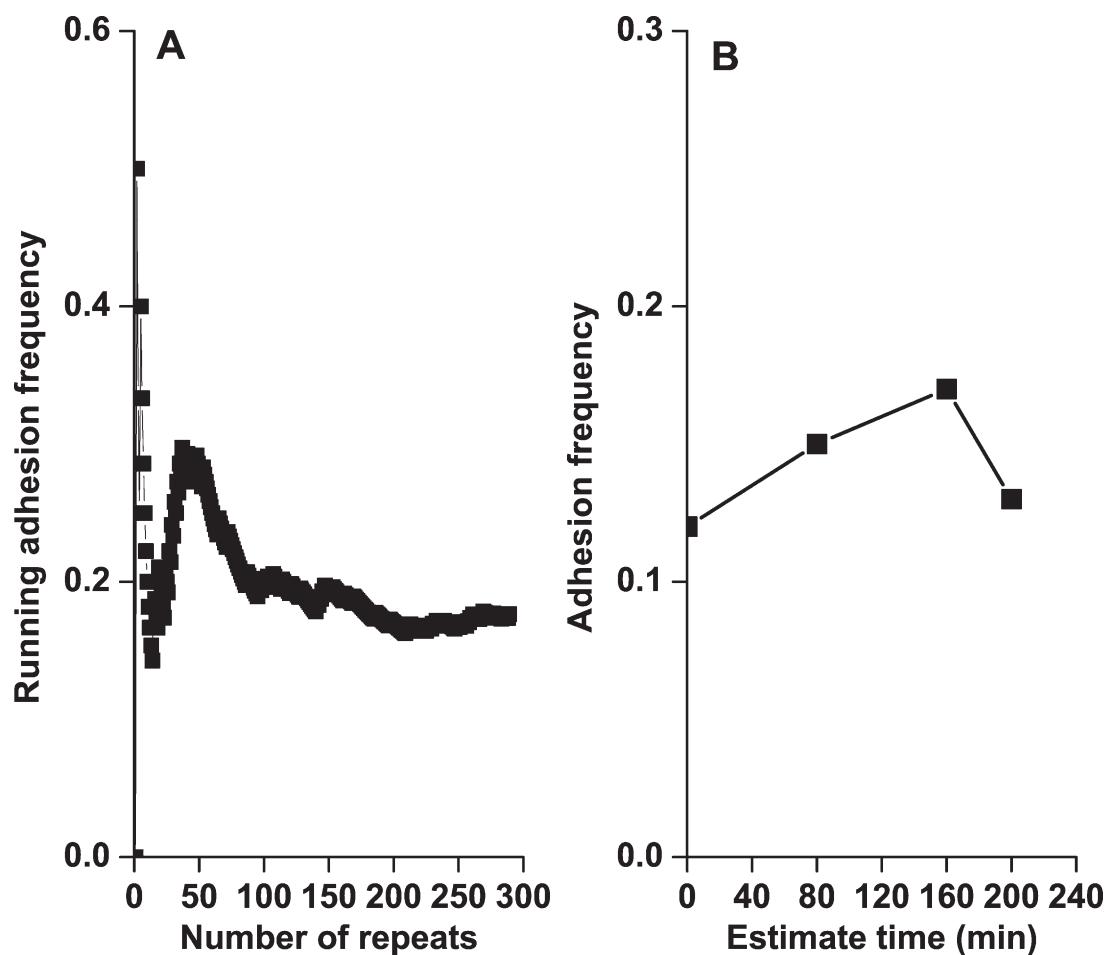


Figure S4. **Functional stability of  $\alpha_5\beta_1$  in supported lipid bilayer.** Measurements were made at the same location on an  $\alpha_5\beta_1$ -reconstituted bilayer and a streptavidin-coated cantilever tip functionalized with FNIII<sub>7-10</sub> in 2 mM Mg<sup>2+</sup>/EGTA. (A) Running frequency, defined as the number of adhesions divided by the number of repeats (both of which are counted up to the most recent contact cycle in a long series of continuing repeated contacts) is plotted versus the repeat number. (B) Four adhesion frequencies each measured from 100 repeated contacts are plotted versus time elapse of a typical experiment.

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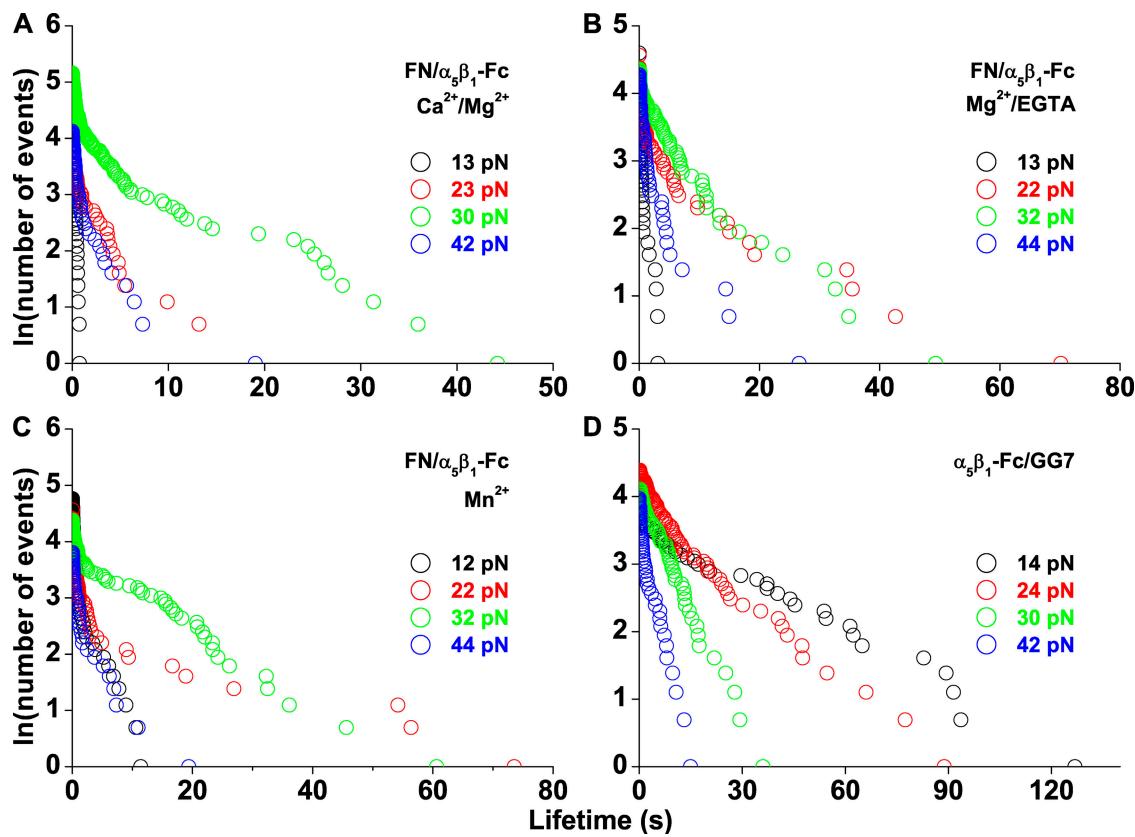


Figure S5. **Lifetime distributions.** (A–D) Natural log (number of measurements with a lifetime  $\geq t$ ) versus  $t$  plots measured at the indicated forces for interactions of FN- $\alpha_5\beta_1$ -Fc-GG-7 in  $\text{Ca}^{2+}/\text{Mg}^{2+}$  (A),  $\text{Mg}^{2+}/\text{EGTA}$  (B), and  $\text{Mn}^{2+}$  (C), as well as for  $\alpha_5\beta_1$ -Fc-GG-7 interaction (D).