(**A**) Self-peeling is initiated by detaching the firmly attached end of the myotube at $t = 0$. Upper insets show cells on micropatterned strips with a typical distribution of FA proteins – here in *mdx* myotubes. Note the end-concentrated FA's, which must be disrupted for the cells to peel under their own tension.
(**B**) Exponential relaxation of control, Pax^+, and blebbistatin-treated myotubes for both single layer and cell-on-cell arrangements. The asymptotic relaxation amplitudes are used to obtain the relaxation attributable to myotube contractility ($L - L_{bleb}$). Pax^+ myotubes are more contractile than controls: 40% moreso for the bottom layer and 60% moreso for the top layer of striated cells (8-10 cells each).
(**C**) Self-relaxation of 6-day old *mdx* myotubes (12 cells) suggests contractility between that of C57 and $\gamma SG^{-/-}$ myotubes (Griffin *et al.*, 2005). After subtraction of the blebbistatin results, *mdx* myotubes relax 16% *versus* 11% for C57 control cells and ~23% for $\gamma SG^{-/-}$ myotubes.
(**D**) AFM probing of cell-on-cell arrangements yields the apparent myotube stiffness E_{app}, which relates to the structural organization and also the intrinsic stress σ or contractility of the myotubes. Representative force-indentation curves are shown for control (CTL), Pax^+ and blebbistatin treated myotubes.
(**E**) Normal myotubes have an elasticity of E_{app} = 12 kPa, but Pax^+ myotubes are significantly stiffer at 22 kPa. Since blebbistatin suppresses contractility, E_{Blebb} = 3 kPa is subtracted from the two other cell systems to yield an intrinsic tension of 9 kPa for control myotubes and 19 kPa for Pax^+ myotubes.
(**F**) EDL muscle was dissected from normal and *mdx* mice and also probed in buffer using AFM. *mdx* muscle have an E_{app} of 18 kPa and appear 1.5 times stiffer than C57 muscle with a mean stiffness of 12 kPa. The relative frequency is normalized to 100 contacts on at least 10 myotubes.

Figure 7. Prednisolone is a mild relaxant but Paxillin is unaffected
Prednisolone (PDN, 1 μM) treatment for 12 hr, softens C2C12 myotubes to ~50% of that achieved with Blebbistatin. AFM measurements were converted to cell tensions per Figure 8D. Immunofluorescence and immunoblotting show that Y31-Paxillin is unaffected by PDN. Scalebar ~10 μm.

Figure 8. Non-linearities in paxillin vs tension and a Signaling Circuit for Anchorage-dependent Contractility and Viability
(A) Non-linear relationship between total paxillin and cell tension, summarizing the various findings.
(B) The underlying Signaling circuit for anchorage dependent contractility and viability**.** Dystroglycan-related defects in the muscular dystrophies leads to upregulation of integrins and associated proteins. Upregulation of FA proteins such as paxillin, vinculin and FAK helps to preserve muscle integrity by maintaining a balance between adhesion and contractility. A role for MAPK proteins in sustained stretch-mediated mechanosignaling emerges as a common pathway in both dystrophies studied.

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SUPPLEMENT

Table S1. Selective profiling of *mdx* vs normal muscle. (**A**) Transcript profile of *mdx* vs normal muscle. Microarray data selected from PERP portal of Children's National Medical Center Washington DC (Bakay M, 2002). (**B**) Increased expression of vinculin protein in *mdx* as determined by Western immunoblot.

Figure S2. Stretch activation of MAPK pathways is generic and sustained, but phospho-Paxillin consistently shows basal levels. Immunoblots of tissue lysates prepared with or without 20 min of 10% muscle extension show sustained activation by 3-10 fold of MAPK's in both normal and dystrophic muscles. Representative blot images are shown with cpm's indicated for phosphopaxillins as illustrative. The blue line-plot in the bottom graph is the simple sum of the top two graphs. Paxillin and FAK phosphorylation are unchanged. The variation for phospho-ERK was (Range/Avg) ≈ 50% (n=2). The $\gamma SG^{-/-}$ dystrophy shows the greatest activation of the four stretch-elevated phosphoproteins. Moreover, $\gamma SG^{-/-}$ muscle, with its slight decreases in DGC mass plus abnormally high FA expression, generates an activation profile remarkably well approximated by adding together the phospho-profiles of wild-type muscle (with a normal DGC level) and *mdx* muscle (with high FA). In addition to underscoring the differences in signaling, this summation also suggests a moderating role for the DGC on normal FA-derived signaling.

Supplementary Figure S3. Co-Localization of Paxillin in Myotubes. Staining of one week old C2C12 myotubes shows paxillin localization along actin stress fibers and on the ends of stable microtubule networks.

Supplementary Figure S4. Blebbistatin effects on tension and (P)-ERK in *ex vivo* Myofibers. Normalized tetanic force in mouse *mdx* EDL muscle shows that active force decreases following blebbistatin treatment.

Supplementary Figure S5. GFP-Paxillin diffusion in myoblasts based on FRAP. **(A)** Focused micro-beam is illustrated by the circle. (**B**) Recovery curves are fitted with single exponentials to obtain the characteristic time constants and the fraction recovery. (**C**) Paxillin is mobile both at focal adhesions and cytoplasm, with time constants of 3 sec and 1 sec respectively. Blebbistatin treatment increases the time constant to 2 sec for cytoplasm. The fraction recovery varies between 75 – 90 % for the three cases. Analysis is for 20 FRAP experiments (Avg. ± S.D.).

Supplementary Figure S6. Paxillin overexpression does not remodel adhesions or contribute to adhesion strength.
(**A**) Vinculin stained images of control and paxillin overexpressed samples are compared to check for changes in adhesion pattern. By thresholding the images and doing a statistical analysis of the number and size of individual focal adhesions for 15 randomly chosen cells, no differences in the distribution of focal adhesion areas are seen. Scalebar ~4 μm.
(**B**) To measure the dynamic adhesive strength of myotubes, cells were forcibly peeled as sketched from their substrate into a large bore micropipette with peeling velocities measured (Griffin *et al.*, 2005). The arrow indicates the direction of cell peeling. The data-points are fitted with $v_{peel} = a \log T + b$, which yields a resting tension T_0 = 6 nN/μm that is independent of paxillin overexpression.

Supplementary Figure S7. Spontaneous relaxation of $\gamma SG^{-/-}$ but not *mdx* myotubes. One week old *mdx* and $\gamma SG^{-/-}$ myotubes cultured on patterns exhibit contrasting morphologies. While the *mdx* myotubes look healthy, the $\gamma SG^{-/-}$ myotubes are bloated in the center due to detachment under excessive contractility.

Supplemental Material and Methods

Tetanic Force and Blebbistatin-induced Relaxation (Figure S3) TA muscles were isolated within 5 min of anesthetizing 8-10 week male *mdx*. For Mouse A (DMSO control) and Mouse B (50μm Blebb) the same protocol was used for each limb.

For the left EDLs:
1. 2 max forces in ringer's solution
2. 1 max force every 5 min for 1 hr in ringer's solution with DMSO or Blebb.
3. passive stretch in ringer's solution with DMSO or Blebb.

For the right EDLs: (lysate 2)
1. 2 max forces in ringer's solution
2. 1 initial max force and 1 max force after 1 hr in ringer's solution with DMSO or Blebb
3. passive stretch in ringer's solution with DMSO or Blebb