Supplemental Figure Legends

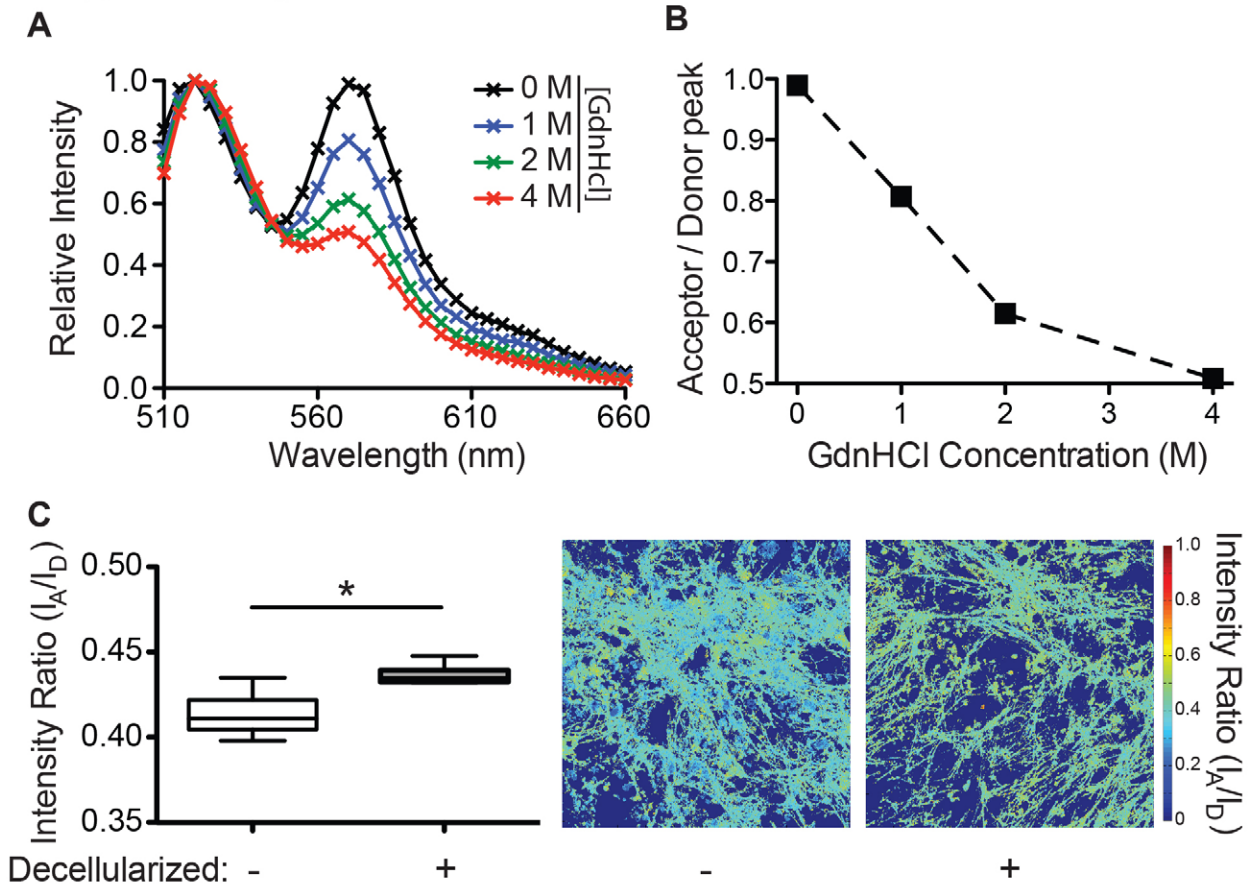
Supplemental Figure 1. Characterization of the FRET-FN probe. A) FRET-FN was denatured in 0-4 M guanidine hydrochloride (GdnHcl) and the emission spectrum of the FRET-FN was measured by fluorescence spectroscopy when excited at 484 nm. B) The maximum intensity of the acceptor peak divided by the maximum intensity of the donor peak was quantified at each concentration of denaturant. C) Fibroblasts were grown for 6 days in fibroblast culture medium supplemented with 5 μg/ml FRET-FN and 45 μg/ml unlabeled fibronectin. Confocal z-stacks of the FRET-FN matrix were captured before and after matrix decellularization (right) and the average FRET intensity ratio of the matrix was quantified (Tukey Boxplots) (left). *$p < 0.05$.

Supplemental Figure 2. SMAD2 phosphorylation in response to definitive endoderm induction medium. CCE and R1 ESCs were grown on decellularized fibroblast-derived extracellular matrix in definitive endoderm or pluripotency induction medium. Blots show cells that were lysed after 2 days and blotted for phospho-SMAD2, SMAD2, and GAPDH.

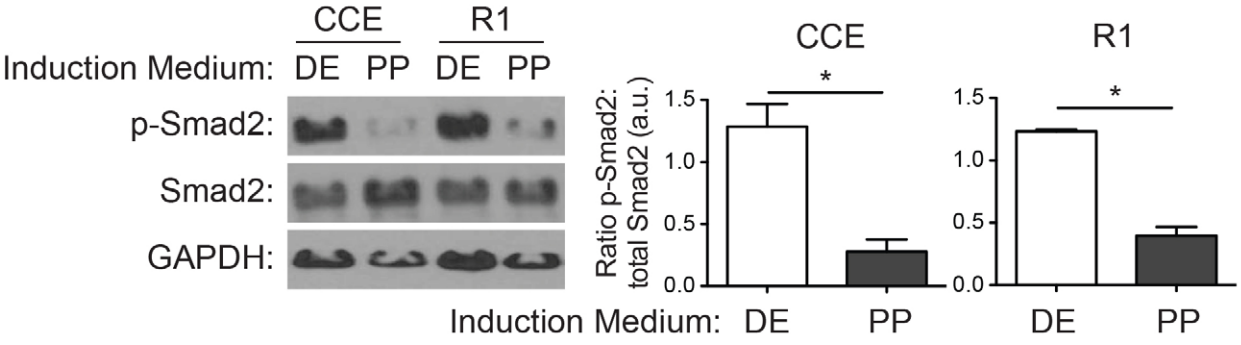
Supplemental Figure 3. R1 ESCs initiate contractility after 2 days of definitive endoderm induction. R1 ESCs were grown in either definitive endoderm (DE) or pluripotency (PP) induction medium on decellularized fibroblast-derived extracellular matrix containing FRET fibronectin. Confocal z-stacks of the FRET fibronectin matrix were captured after 2 days of induction (- Acute Bleb) and the average FRET intensity ratio of the ESC associated matrix was quantified (Tukey Boxplots). FRET intensity ratios of ESC associated matrix were quantified after the ESCs were treated with 50 μM blebbistatin for 1 hour at the end of definitive endoderm or pluripotency induction (+ Acute Bleb).

Supplemental Figure 4. Laminin-binding integrin expression in response to blocking antibodies. A) FACS quantification is shown for the percentages of α3-integrin high and α6-integrin high ESCs in the presence of the indicated blocking antibodies. B) Mouse ESCs labeled without a primary antibody for the indicated integrin (black line) were used to set the $AF488^{High}$ threshold. These data were obtained from mouse ESCs (R1) were grown in definitive endoderm induction medium on decellularized fibroblast-derived extracellular matrix with FRET-FN and 50 μg/ml exogenous laminin. *$p < 0.05$.

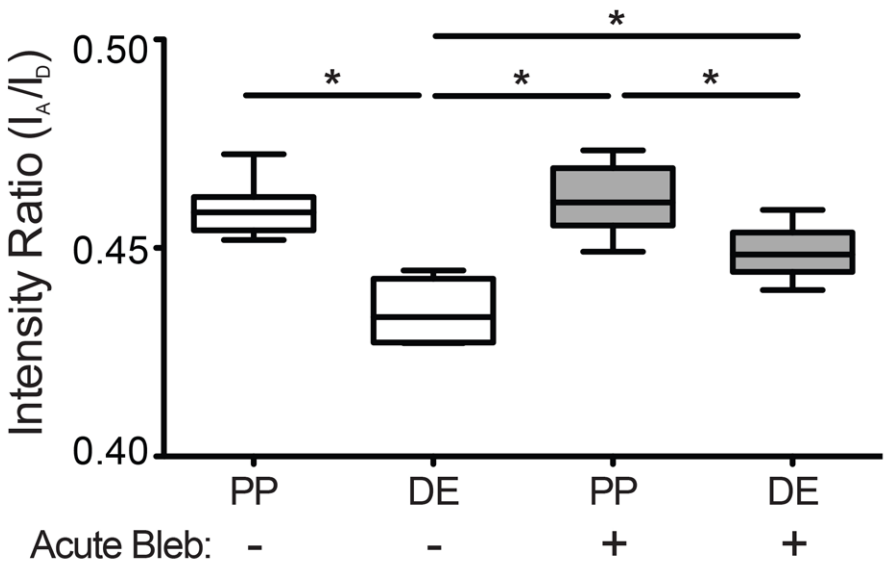
Supplemental Figure 1



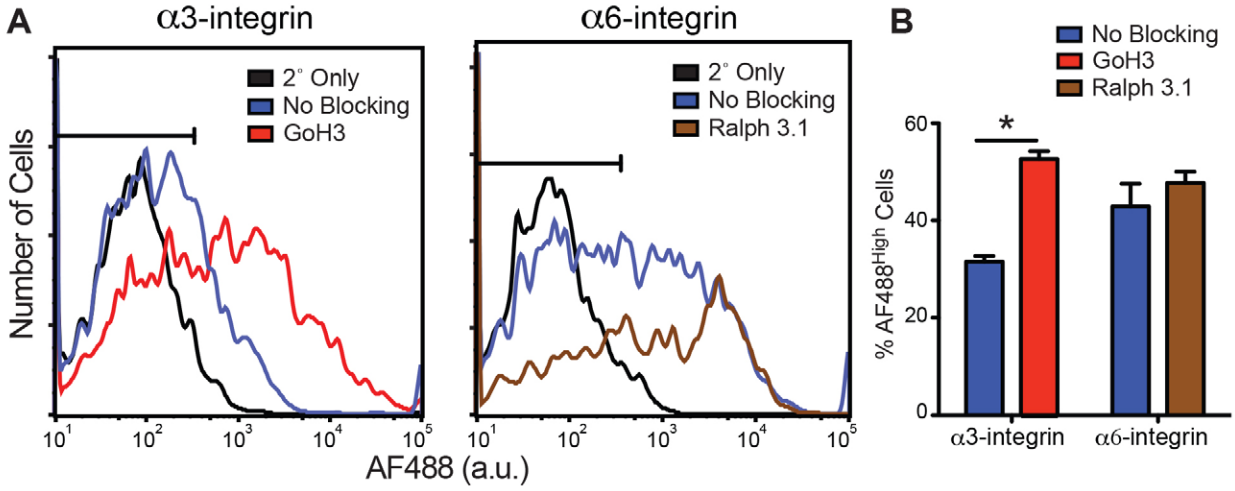
Supplemental Figure 2



Supplemental Figure 3



Supplemental Figure 4



Gene (Accession #)	Primer sequence
GAPDH (NM_008084)	FP: 5'-TCAACAGCAACTCCCACTCTTCCA-3' RP: 5'-ACCACCCTGTTGCTGTACCGTATT-3'
Sox17 (NM_011441.5)	FP: 5'-CTCGCTGTAGAAGAGTGGCTTAGA-3' RP: 5'-GCAGCGGTATCACACTCAAACA-3'
Fibronectin (NM_212482.1)	FP: 5'- AGGCTTGAACCAACCTACGGATGA-3' RP: 5'- GCCTAAGCACTGGCACAACAGTTT-3'

Table S1: List of PCR primer sequences.