Supporting information

Decreasing wound edge stress enhances leader cell formation during collective smooth muscle cell migration

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6 Pages, 3 Figures, and 2 Tables

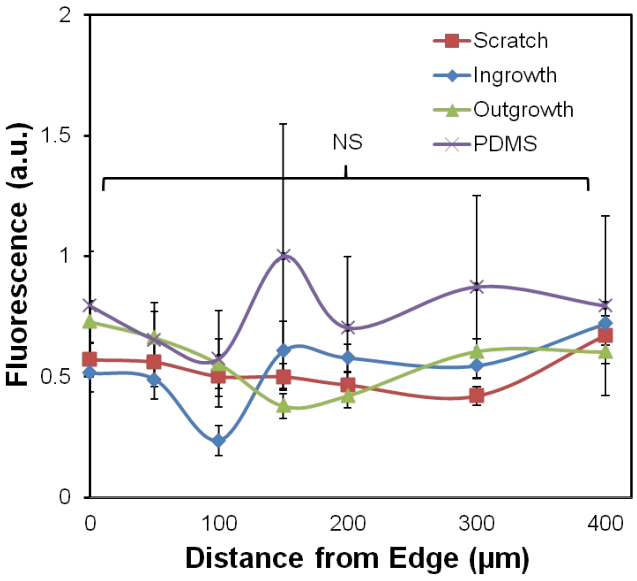

Figure S1. The random probe was transfected into collectively migrating smooth muscle cells. No statistical significance was measured at any point. NS = not significant. One-way ANOVA was performed.

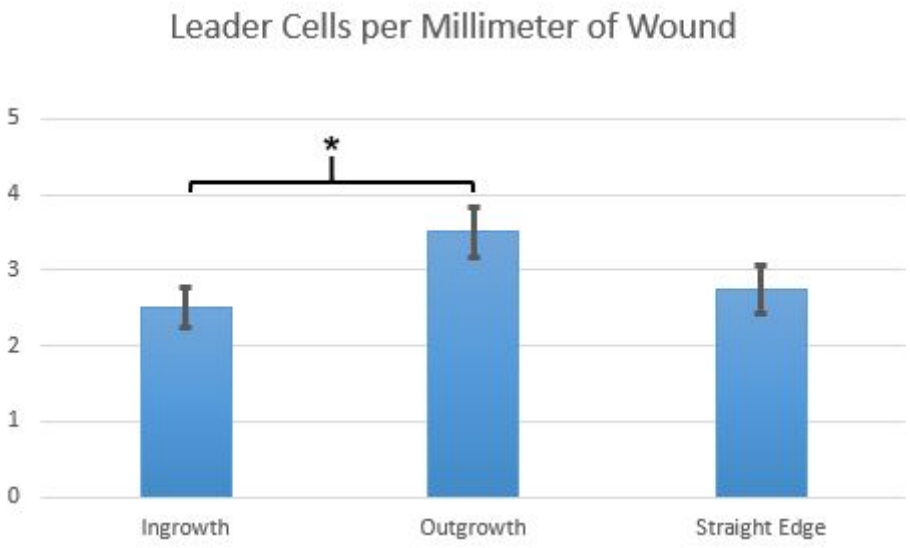

Figure S2. The outgrowth assay increases the density of leader cells. We measured the number of leader cells present over the course of a constant length of 1 mm of starting wound edge in each image. As the outgrowth and ingrowth assays are curved, these measurements took into account the arc length of each assay's circumference when measuring the starting length.

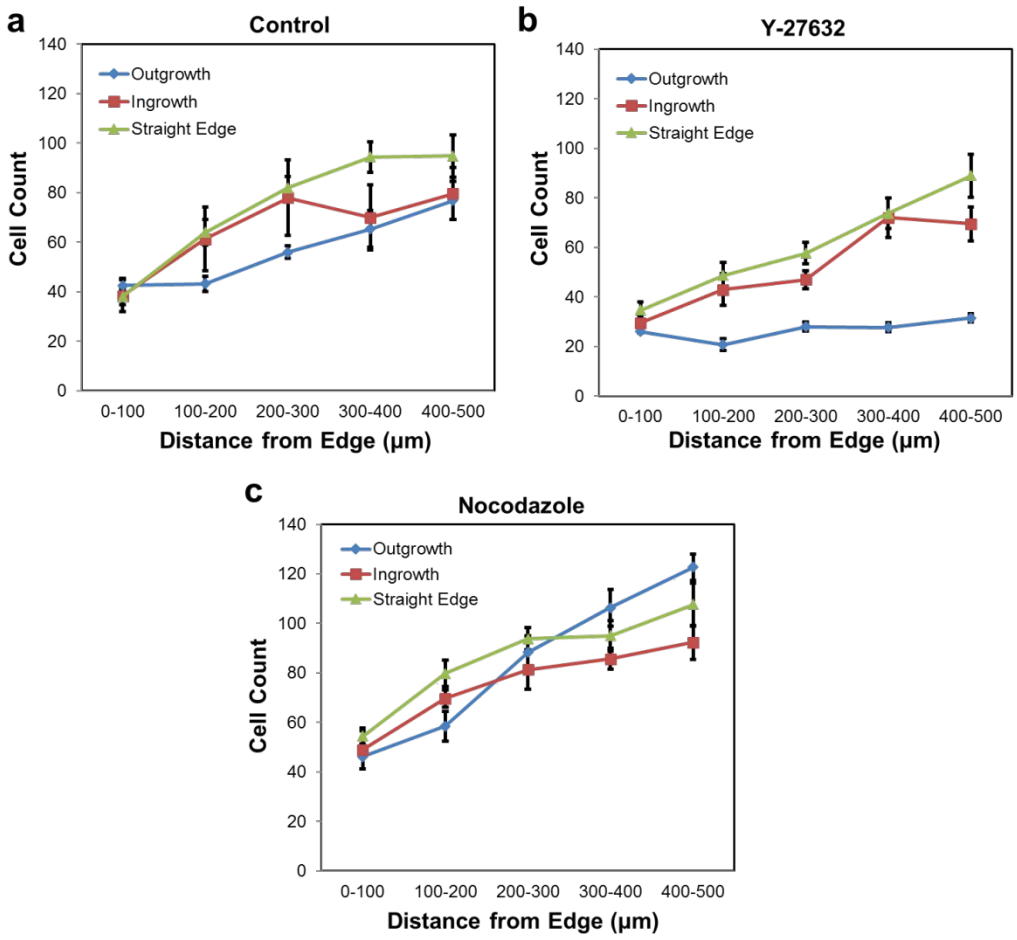

Figure S3. The number of cells versus location in relation to the wound edge was measured. The number of cells was evaluated in (a) control (no drug), (b) Y-27632 addition, and (c) nocodazole. The data is from the same experiments presented in Figure 9. One-way ANOVA analysis with post hoc Tukey's multiple comparison test performed on these data.

Target Gene	Probe Type	Sequence/Fluorophore	Length (nucleotides)	ΔG_{DQ} (kcal/mol)	ΔG_{DT} (kcal/mol)
β3-integrin	Donor (D)	5' /6-FAM/**T**C**C**A**C**T**G**C**T**A**T**A**T**G**A**G**T**C**T**T 3'	20	-10.8	-21.6
	Quencher (Q)	5' **T**A**G**C**A**T**T**G**G**A**/Iowa Black FQ/ 3'	10		
	Target (T)	5' AAGACTCATATAGCATTGGA 3'	20		
β-actin	Donor (D)	5' /6-FAM/**A**G**G**A**A**G**G**A**A**G**G**C**T**G**G**A**A**G**A**G 3'	20	-10.6	-24.9
	Quencher (Q)	5' **C**T**T**C**C**T**T**C**C**T/Iowa Black FQ/ 3'	10		
	Target (T)	5' CTCTTCCAGCCTTCCTTCCT 3'	20		
Dll4	Donor (D)	5' /6-FAM/**A**A**G**G**G**C**A**G**T**T**G**G**A**G**A**G**G**G**T**T 3'	20	-11.8	-25.9
	Quencher (Q)	5' **A**A**C**T**G**C**C**C**T**T/Iowa Black FQ/ 3'	10		
	Target (T)	5' AACCCTCTCCAACTGCCCTT3'	20		
Notch1	Donor (D)	5' /6-FAM/**T**G**C**G**G**T**C**T**G**T**C**T**G**G**T**T**G**T**G**C 3'	20	-12.8	-27.5
	Quencher (Q)	5' **A**C**A**G**A**C**C**G**C**A/Iowa Black FQ/ 3'	10		
	Target (T)	5' GCACAACCAGACAGACCGCA 3'	20		
Random	Donor (D)	5' /6-FAM/**A**C**G**C**G**A**C**A**A**G**C**G**C**A**C**C**G**A**T**A 3'	20	-12.7	-29.4
	Quencher (Q)	5' **C**T**T**G**T**C**G**C**G**T/Iowa Black FQ/ 3'	10		
	Target (T)	5' TATCGGTGCGCTTGTCGCGT 3'	20		

Table S1. The dsLNA probe design for detecting β-actin, β3-integrin, and the random (scrambled) probe. LNA monomers are bolded and underlined, and normal DNA monomers are in regular text. The fluorophore or fluorescence quencher of each probe is bolded and in red.

Outgrowth			Ingrowth			Straight Edge		
Migration straightness			**Migration straightness**			**Migration straightness**		
	Peak 1	**Peak 2**		**Peak 1**	**Peak 2**		**Peak 1**	**Peak 2**
λ	0.672	0.328	λ	0.840	0.160	λ	0.757	0.243
μ	0.485	0.747	μ	0.354	0.745	μ	0.360	0.562
σ	0.150	0.589	σ	0.172	0.075	σ	0.072	0.034
Mean migration speed (μm/hr)			**Mean migration speed (μm/hr)**			**Mean migration speed (μm/hr)**		
	Peak 1	**Peak 2**		**Peak 1**	**Peak 2**		**Peak 1**	**Peak 2**
λ	0.900	0.100	λ	0.816	0.184	λ	0.791	0.209
μ	15.956	24.519	μ	12.078	21.228	μ	12.175	21.156
σ	2.473	1.200	σ	4.885	3.847	σ	2.180	1.880
β-actin dsLNA probe signal			**β-actin dsLNA probe signal**			**β-actin dsLNA probe signal**		
	Peak 1	**Peak 2**		**Peak 1**	**Peak 2**		**Peak 1**	**Peak 2**
λ	0.564	0.436	λ	0.576	0.424	λ	0.592	0.408
μ	126.832	415.310	μ	88.059	238.170	μ	74.345	239.229
σ	58.047	231.705	σ	48.811	118.267	σ	39.670	124.326
Random dsLNA probe signal			**Random dsLNA probe signal**			**Random dsLNA probe signal**		
	Peak 1	**Peak 2**		**Peak 1**	**Peak 2**		**Peak 1**	**Peak 2**
λ	0.843	0.157	λ	0.756	0.244	λ	0.432	0.568
μ	31.064	107.481	μ	16.575	73.482	μ	11.742	50.349
σ	16.859	34.421	σ	15.002	23.025	σ	6.507	29.476

Table S2. A table summarizing the bimodal analyses of the distributions. The overlap (λ), the mean (μ), and the standard deviation (σ) of the peaks are indicated for each wound scenario.