N-cadherin is Key to Expression of the Nucleus Pulposus Cell Phenotype under Selective Substrate Culture Conditions

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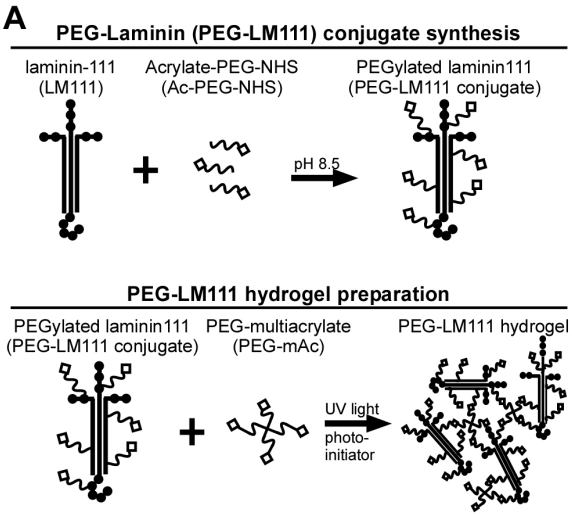
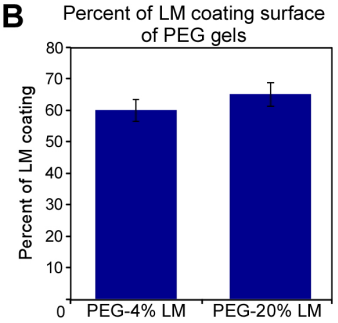
Supplemental Figure 1: Characterization of PEG-LM hydrogels: (A) Synthesis of PEG-LM (adapted from Francisco et al 2013, Biomaterials) **(B)** Percent of laminin coating on hydrogel surface as measured in IMAGEJ **(C)** Uniform pegylated laminin coated on PEG-LM hydrogel surface (representative images displayed) **(D)** Shear modulus of PEG-LM hydrogels formulized as measured via rheometry (adapted from Francisco et al 2013, Biomaterials)

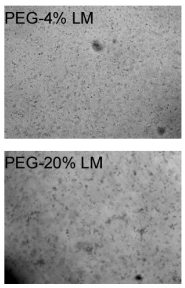
A



B



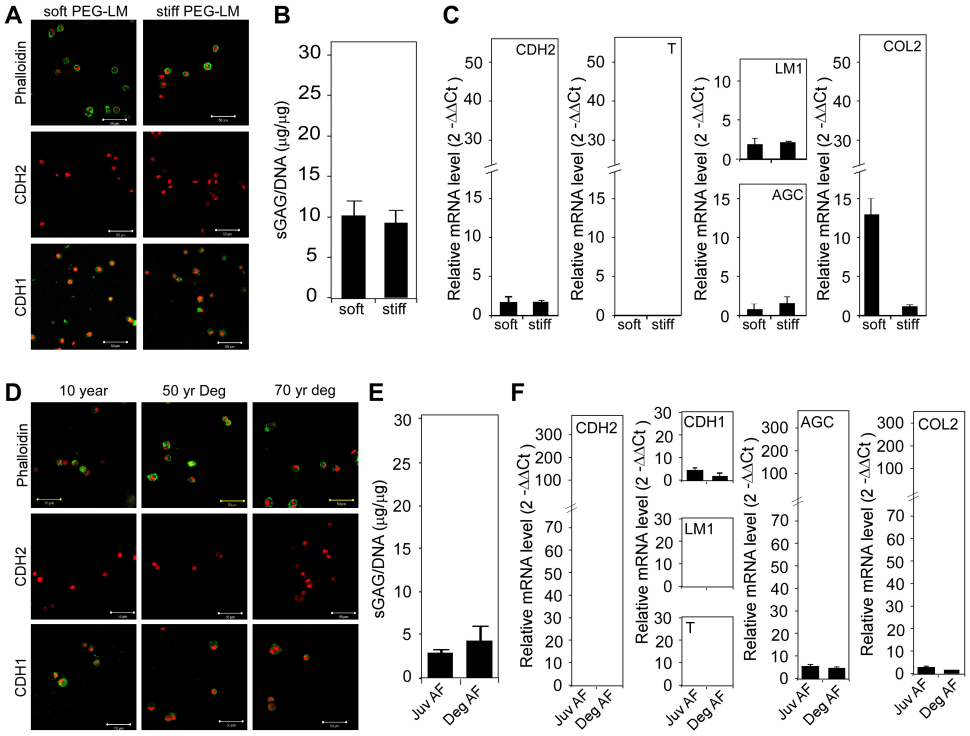
C



D

	PEG-4% LM	PEG-20% LM
Shear Modulus	0.3 kPa	29 kPa

Supplemental Figure 2: Porcine and human AF cells do not form cell clusters on soft, laminin (LM) hydrogels and do not express high levels of juvenile NP markers (A) Representative immunostaining of phalloidin and cadherins for porcine AF cells on soft and stiff PEG-LM (green = protein, red = cell nuclei, scale bar = 50 mm) **(B)** Changes in sGAG production for porcine AF cells on soft and stiff PEG-LM **(C)** Quantification of gene expression for juvenile NP cell phenotype markers in porcine AF cells on soft, relative to AF cells on stiff (CDH2 = N-cadherin, T= brachyury, LM1= Laminin1, AGC = aggrecan, COL2 = type II collagen) **(D)** Representative immunostaining of phalloidin and cadherins for juvenile (juv) and degenerate (deg) human AF cells on soft PEG-LM **(E)** Same as C but with human AF cells on PEG-LM **(F)** Same as D but with human AF cells on PEG-LM (additional marker CDH1 was quantified in human)



Supplemental Figure 3: Matrix synthesis and gene expression of NP markers are not affected by CDH blocking antibody treatment in porcine AF cells (A) Changes in sGAG production for porcine AF cells on soft and stiff PEG-LM after blocking antibody treatment **(B)** Quantification of gene expression for juvenile NP cell phenotype markers in porcine AF cells on soft, relative to AF cells on stiff after blocking antibody treatment (2-way ANOVA with Tukey's post-hoc analysis, *p<0.05) (Key: 1= No treatment conditon, 2= CDH2 blocking antibody treatment, 3 = CDH1 blocking antibody treatment; CDH2 = N-cadherin, T= brachyury, LM1= Laminin1, AGC = aggrecan, COL2 = type II collagen)

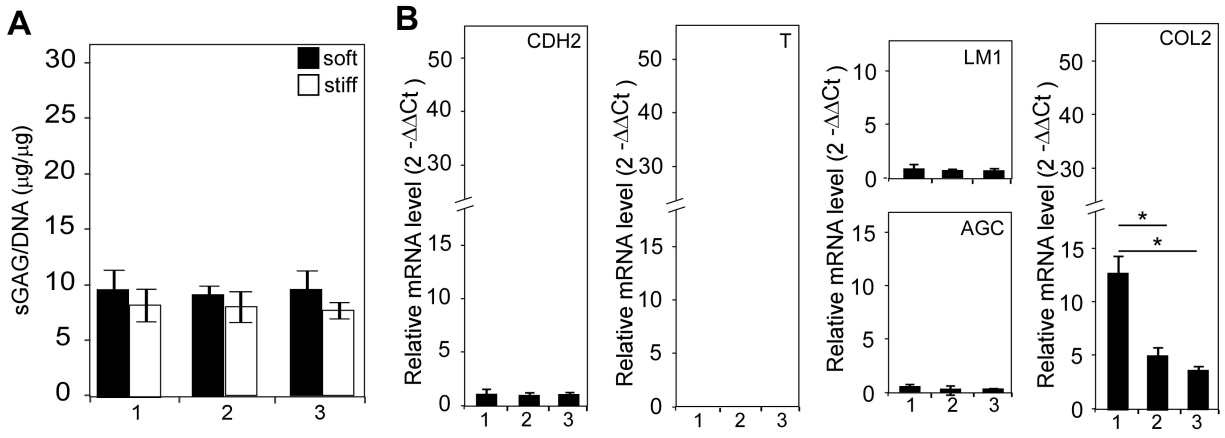
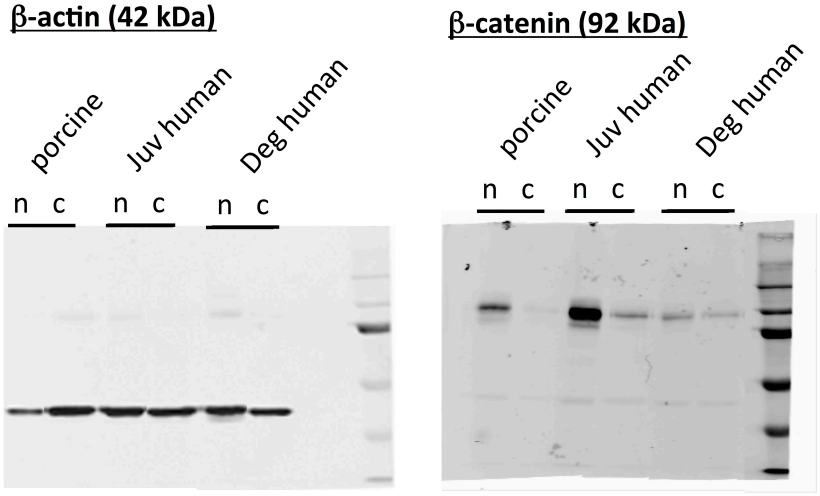
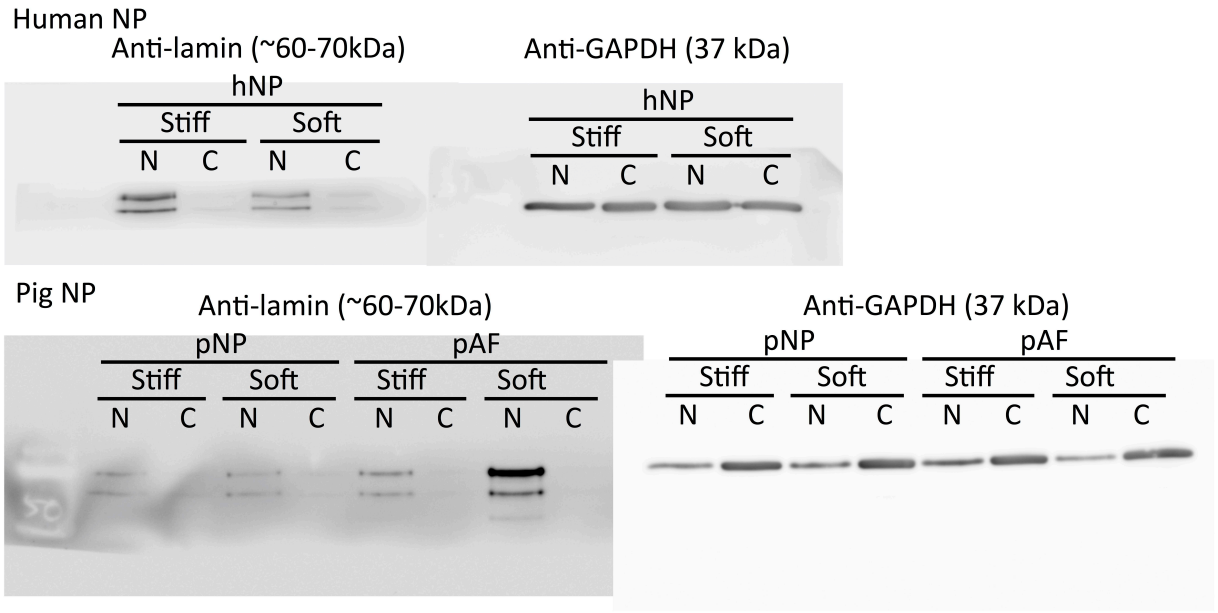


Figure 4: Images of full western blot samples for β-catenin, LaminA/C, and their respective housekeeping markers (from Figure 2)

Western Blot Images for Figure 2G



Western Blot Images for Figure 2H



Supplemental Table 1: Information regarding human samples analyzed and separated into groups (F= Female, M=Male, C= Caucasian, AA=African American, + = N-cadherin positive, - = N-cadherin negative)

	Sample #	Age	Sex	Race	CDH2 (+/-)
Juvenile	1	9	F	C	+
	2	10	M	C	+
	3	7	M	C	+
	4	13	M	C	+
Degenerate	5	67	M	C	+
	6	79	F	C	-
	7	67	F	C	-
	8	63	F	AA	-
	9	66	M	C	-
	10	69	M	C	+
	11	75	M	C	+
	12	65	M	AA	+
	13	73	F	C	-
	14	70	M	AA	+
	15	62	F	C	-
	16	60	M	C	-
	17	68	F	C	+
	18	48	F	C	+
	19	53	M	AA	-
	20	63	M	C	-
	21	50	M	C	+
	22	35	F	C	+