Supplementary data:

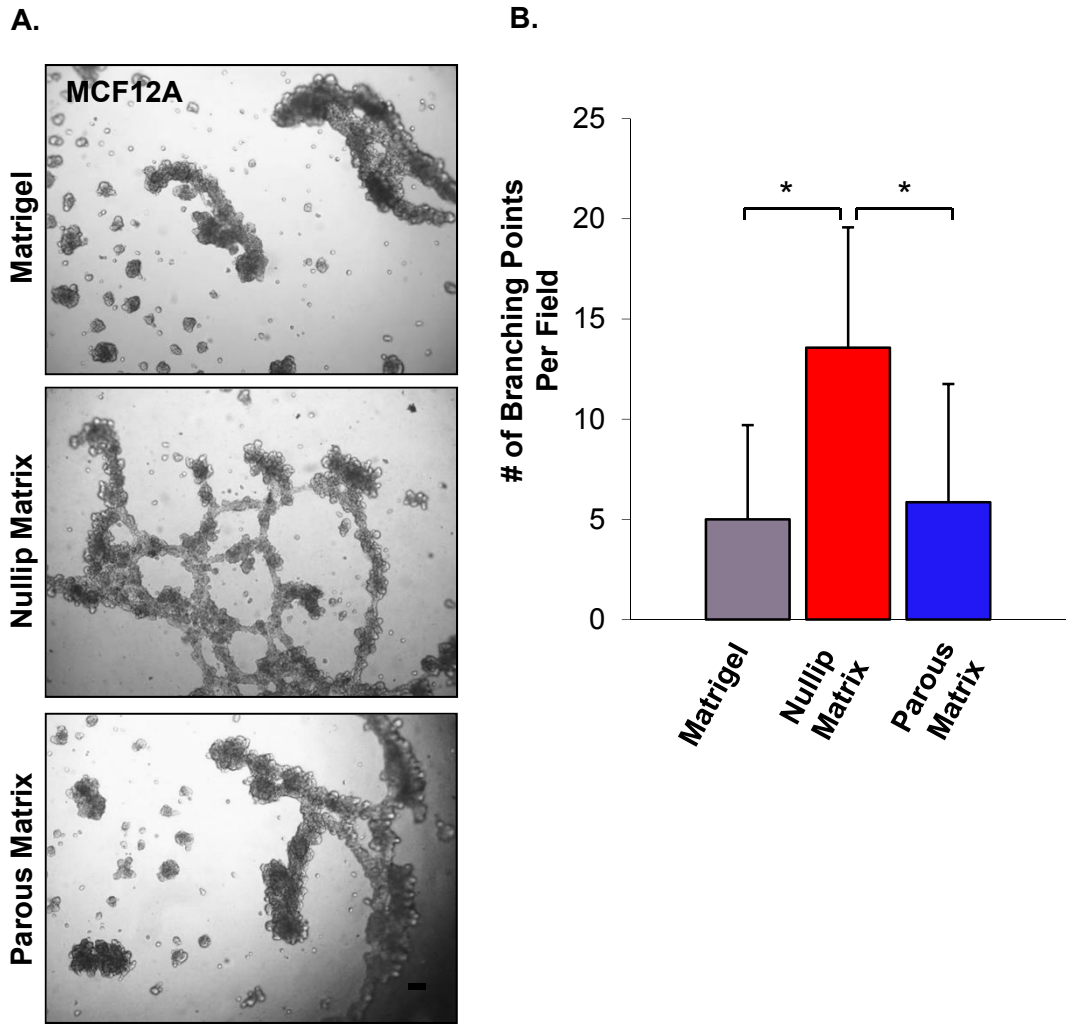

Figure S1
Mammary ECM supports the formation of complex epithelial structures *in vitro*.
(A) Brightfield images of immortalized, non-transformed, human mammary epithelial MCF12A cells mixed with 200µg/mL of Matrigel, or mammary ECM isolated from nulliparous or parous rats and plated on Matrigel pads in 3D cell culture as previously described. Briefly, 45,000 MCF12A cells were plated on 3D 1:1 matrix pad of 200 µg/mL endogenous mammary ECM to 9.7-10mg/mL Matrigel™ for 24-48 hrs as described in Hattar et al. Breast Cancer Research, 2009. The images were taken after 48 hours in culture. Scale bar, 100µm. **(B)** A quantitative analysis of the number of branching points as a measurement of epithelial structure complexity. N=3 wells per condition *$p<0.05$, ANOVA with Bonferroni multiple comparisons posttest.

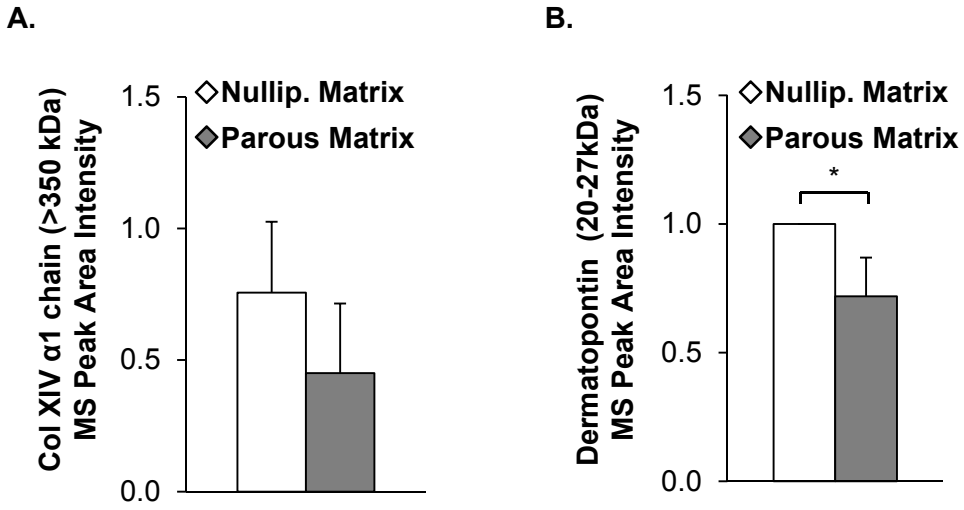

Figure S2

Evidence for parity induced changes in mammary ECM. (A and **B**) Changes in collagen XIV and dermatopontin abundance were assessed in rat nulliparous or parous matrices by MS-based proteomics and label-free quantitative analysis**)**. Means are calculated from an N=3-5. *$p<0.05$, One Sample t-test.

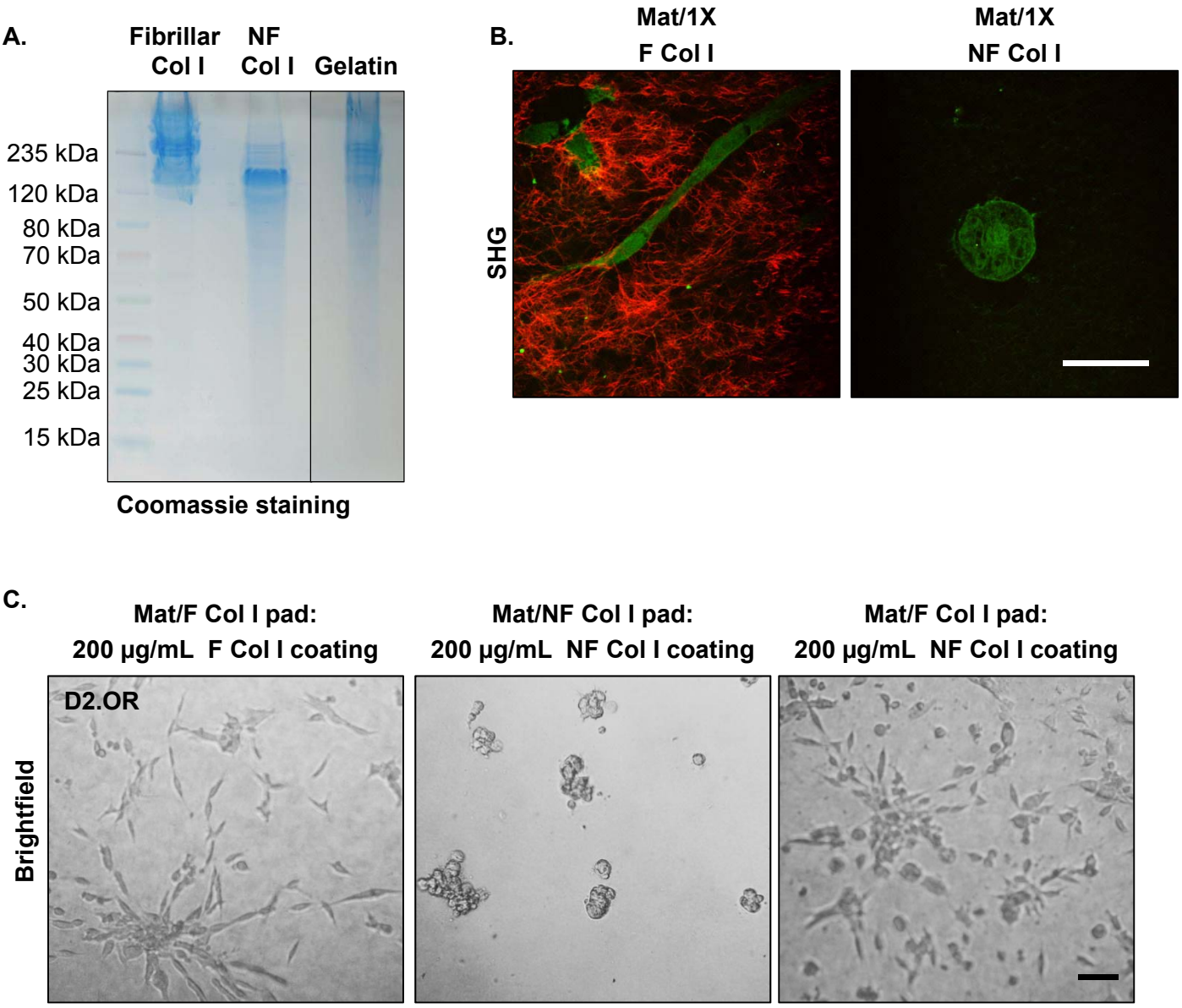

Figure S3

Moderate sonication of collagen I inhibits invasive phenotype. (A) Fibrillar and non-fibrillar (NF) collagen I as well as gelatin were evaluated in non-reduced denatured 4-12% SDS-PAGE gel stained with coomassie. Samples were ran on the same gel. **(B)** D2.OR cells cultured in Mat/ 1X F Col I or Mat/ 1X NF Col I for 6 days, fixed, and then visualized via SHG imaging to assess collagen I organization. Red indicates SHG signal and green is autofluorescence. Scale bar, 25µm. Model #2 was used to perform this 3D cell culture experiment. **(C)** Brightfield images of D2.OR cells coated with fibrillar and NF collagen I and cultured for 4-6 days as previously described. Briefly, 3D matrix pads were prepared from 4 mg/mL Matrigel or 1.5:1 ratio of 4 mg/mL of Matrigel to 4 mg/mL of fibrillar or NF collagen I with a total volume of 100 µL. Then, 2,000 D2.OR cells were mixed with 200 µg/mL fibrillar or NF collagen I and plated on the polymerized 3D matrix pads, as described in Barkan et al. Cancer Research, 2008. Representative images, N=3 wells per condition. Scale bar, 100µm.

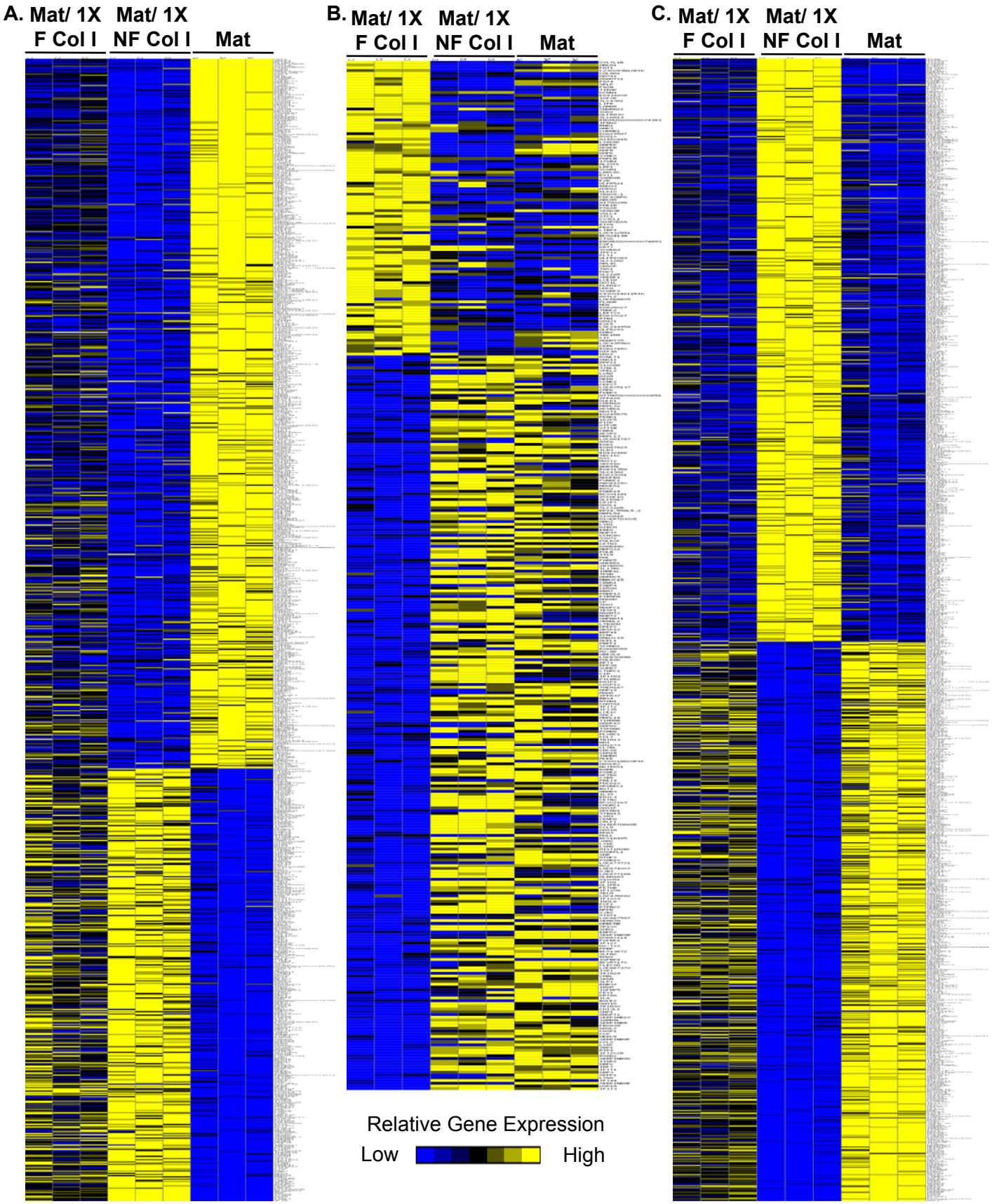

Figure S4

Distinct gene signatures for Matrigel or Matrigel/ Col I conditions. Heat maps for specific gene signatures for D2.OR cells cultured for 4 days in Matrigel **(A)** , Mat/ 1X F Col I **(B)** or Mat/ 1X NF Col I **(C)**.

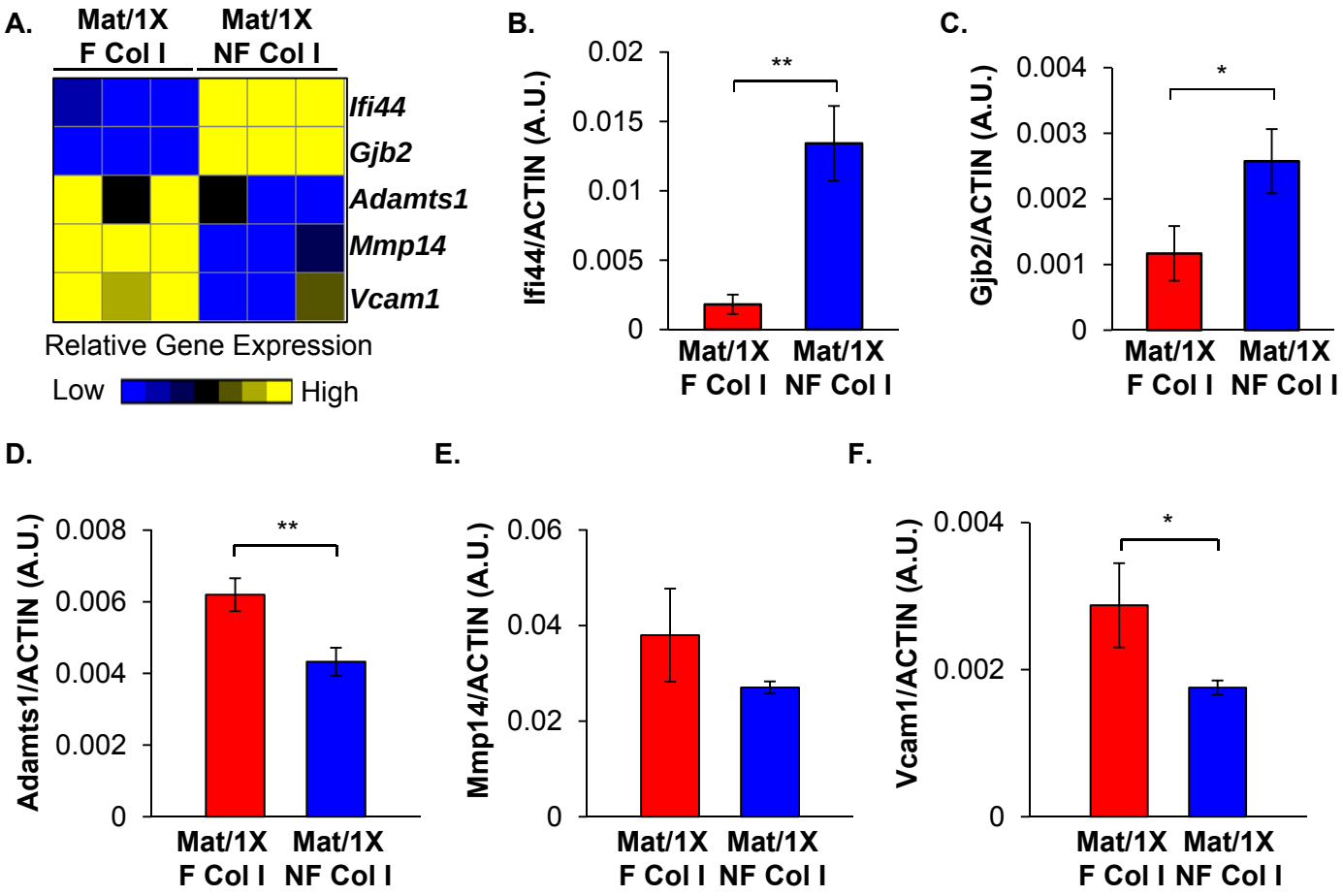

Figure S5

Validation by RT-PCR of differences in expression levels of selected genes identified in the transcriptome microarray analysis. (A) Heat map of selected genes from the transcriptome microarray study. Yellow indicates upregulation and blue downregulation of gene expressions. **(B-F)** Differences in gene expressions identified in **(A)** were validated by quantitative RT-PCR**.** N=3 samples from an independent experiment. *p<0.05, **p<0.01, unpaired t-test. Model #2 was used to perform this 3D cell culture experiment.

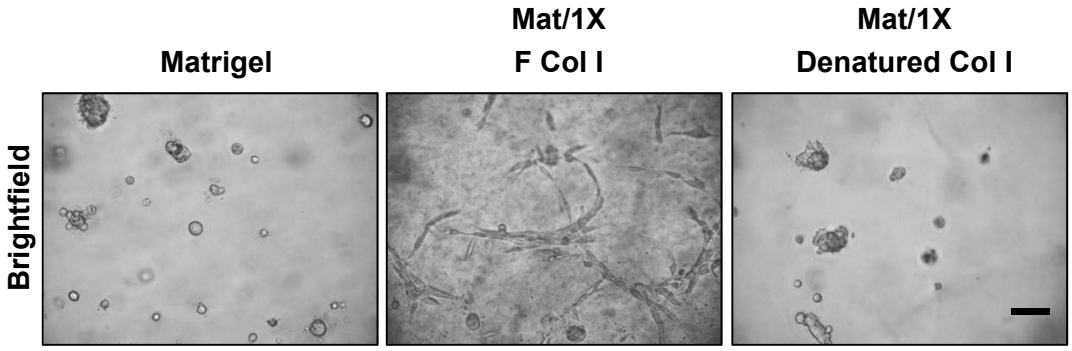

Figure S6

Fibrillar collagen I organization is required for elongated/stellate cell morphology. Brightfield images of D2.OR cells in Matrigel, Mat/ F Col I and Mat/denatured collagen (gelatin). Representative images, N=3 wells per condition Scale bars, 100µm. Model #2 was used to perform this 3D cell culture experiment and Gelatin (Sigma), denatured collagen, was also used at final concentration of 1.6mg/mL.

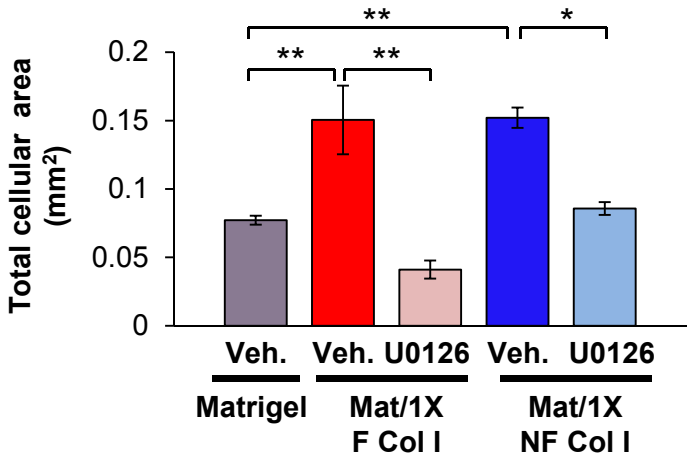

Figure S7

Collagen I promotes ERK1/2-mediated proliferation. D2.OR cells were treated with vehicle (Veh.) or MEK1/2 inhibitor (U0126) and cultured in Matrigel, Mat/ 1X F Col I collagen I or Mat/ 1X NF Col I for 6 days. Total cell areas were assessed using brightfield images and quantified via ImageJ. Total cellular area were quantified from 3-4 images per well and 3 wells per conditions were used. *$p<0.01$, **$p<0.001$, ANOVA with Bonferroni multiple comparisons posttest. Model #2 was used to perform this 3D cell culture experiment.

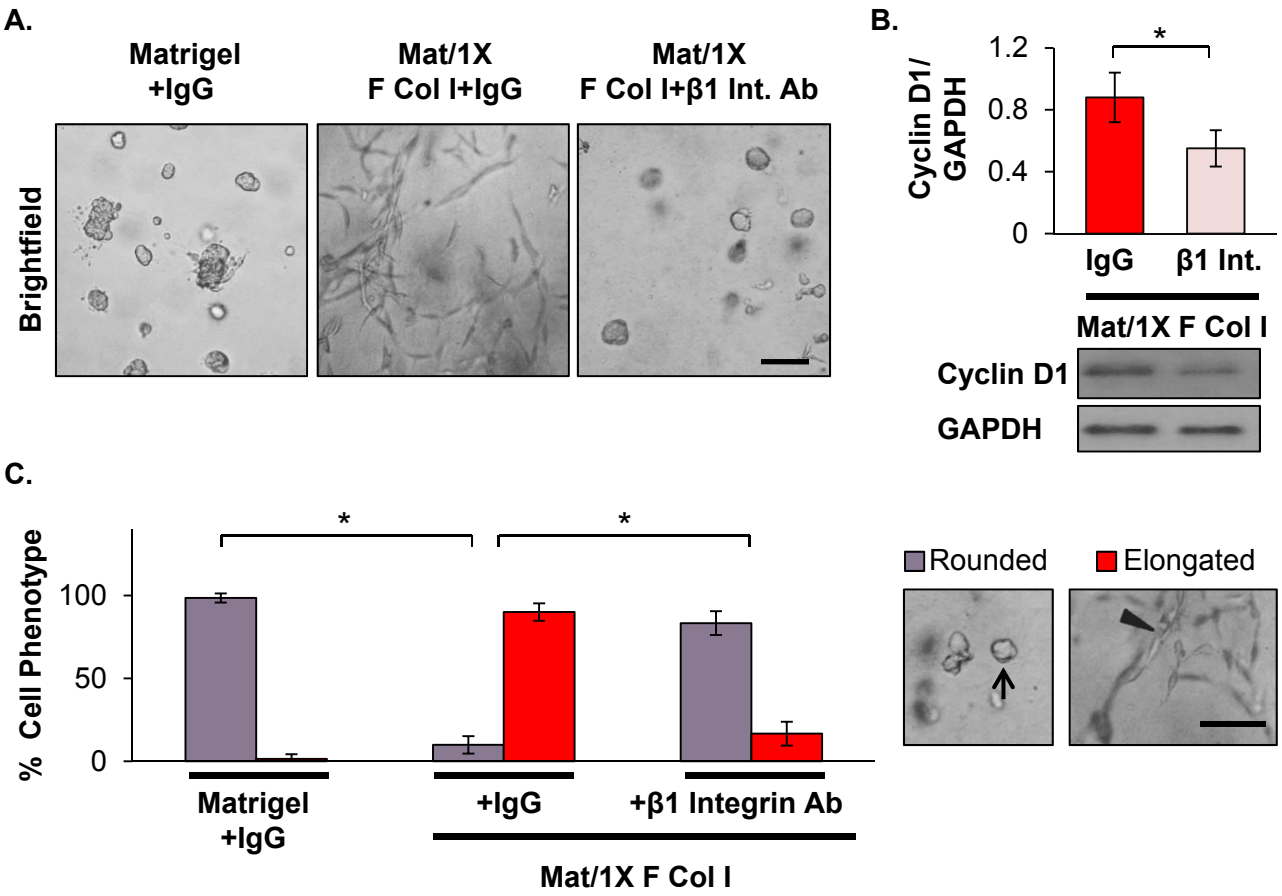

Figure S8

Blocking β1 integrin decreases tumor cell proliferation and reverts elongated cell morphology. **(A)** Representative brightfield images of D2.OR cells treated with IgG or β1 integrin blocking antibody and cultured in Matrigel and Mat/ 1X F Col I until day 4. (**B)** Cyclin D1 levels in IgG and β1 integrin blocking antibody conditions evaluated by IB. N=4 wells per condition, * $p < 0.02$, unpaired t-test. **(C)** Morphological quantitative analyses were performed on brightfield images from conditions described in **(A)**. Arrow and arrowhead point to rounded and elongated structures, respectively. Statistical values are * $p<0.001$, ANOVA with Bonferroni multiple comparisons posttest. Scale bars, 100µm. Model #2 was used to perform this 3D cell culture experiment.

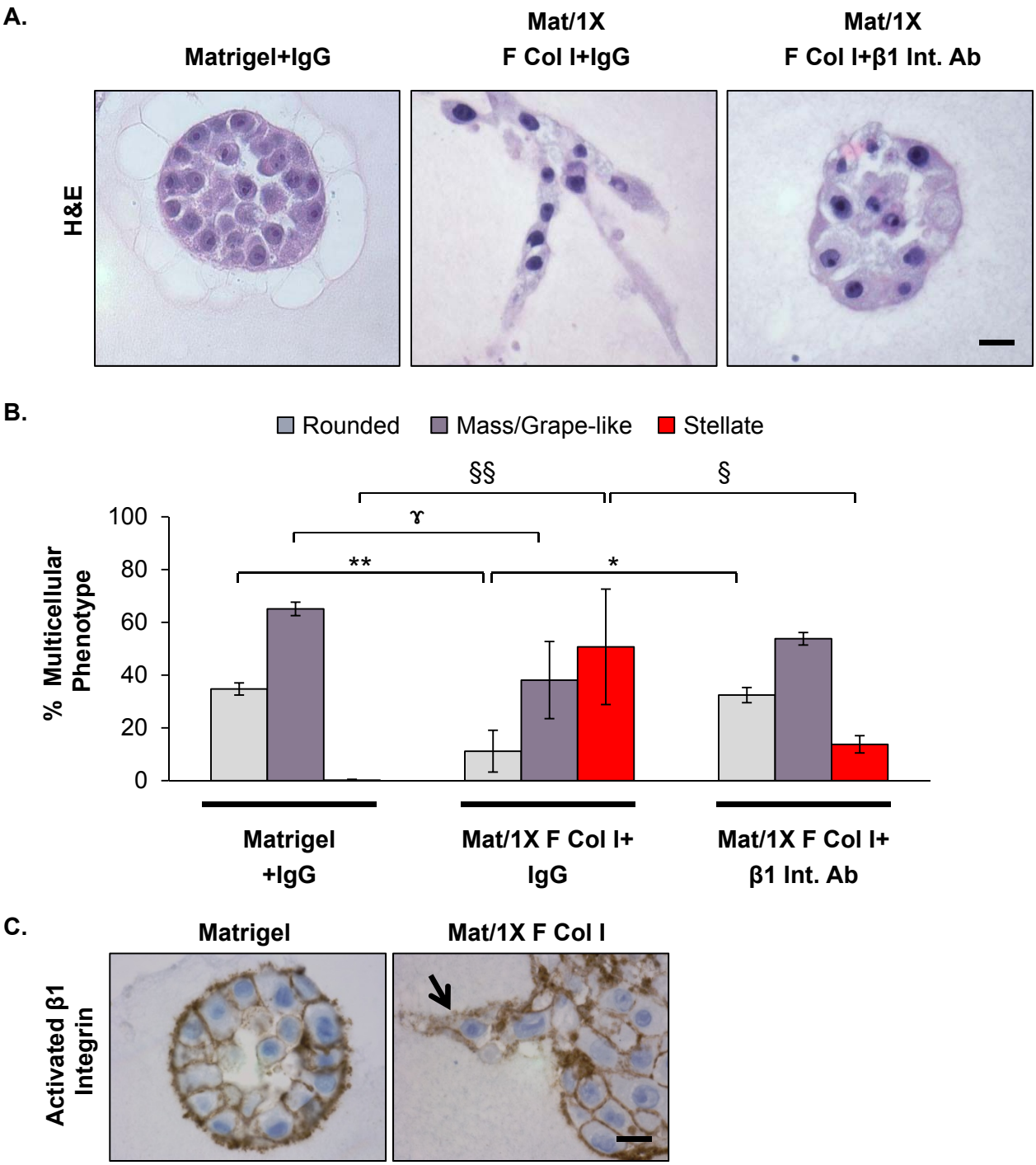

Figure S9

Fibrillar collagen I influences invasive morphology of breast cancer MCF10DCIS cells via β1 integrin signaling. (A) Representative H&E images of MCF10DCIS cells cultured in Matrigel or Mat/ 1X F Col I treated with IgG or β1 integrin blocking antibody. (B) Morphological quantitative analyses were performed on brightfield images from conditions described in **(A).** N=3 well per condition and 3-4 images per well**.** §, ϒ, or *p<0.05, §§ or **p<0.01, ANOVA with Bonferroni multiple comparisons posttest. **(C)** Activated β1 integrin staining on MCF10CIS cells cultured in Matrigel or fibrillar collagen I via IHC. Arrow points to changes in β1 integrin distribution in elanogated tumor cell. Scale bar represents 20μm. Model #2 was used to perform this 3D cell culture experiment.

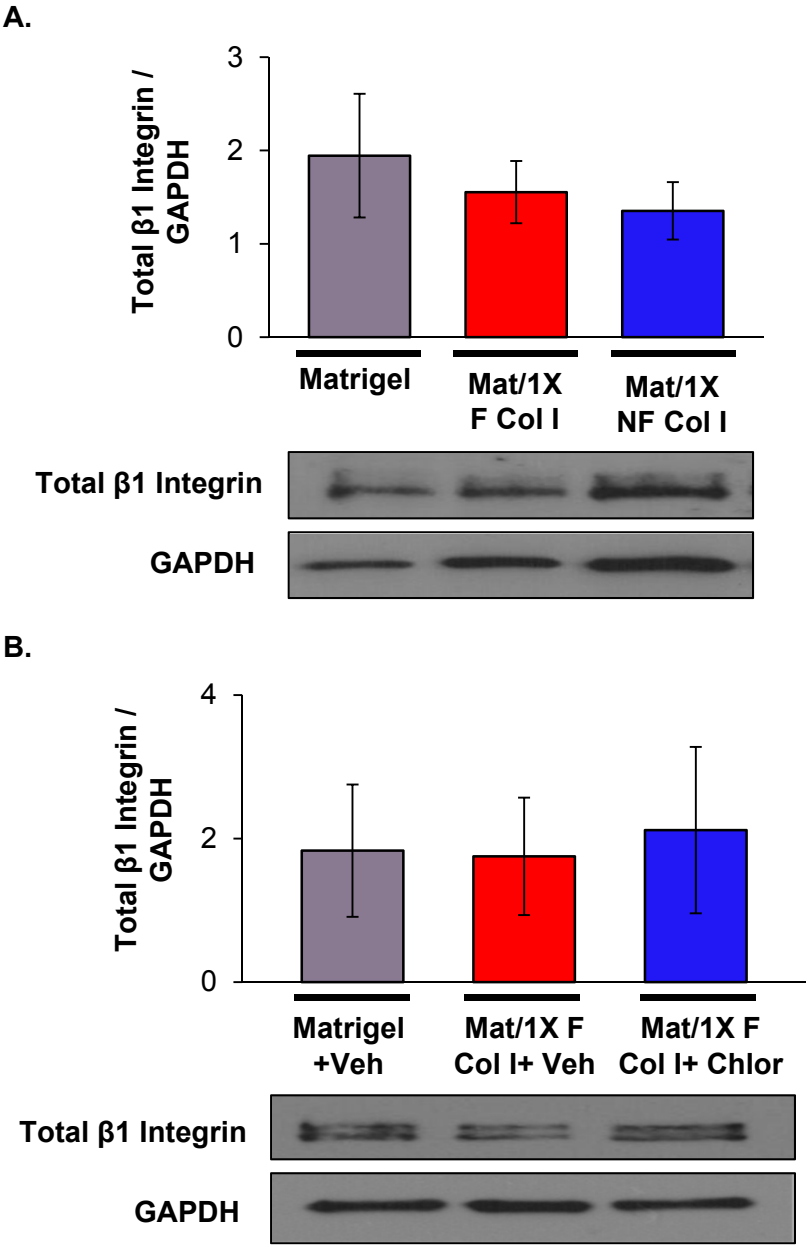

Figure S10

Collagen I organization does not alter protein levels of total β1 integrin. (A and **B)** Total β1 integrin was evaluated by IB from lysates of D2.OR cells cultured in Matrigel, Mat/ 1X F Col I or Mat/ 1X NF Col I for 4 days. N=3 wells per condition, no significance differences were found by statistical evaluation. **(B)** D2.OR cells cultured in Matrigel or fibrillar collagen I treated with vehicle (Veh) or chlorpromazine (Chlor), a clathrin-dependent endocytotic inhibitor. Model #2 was used to perform this 3D cell culture experiment.

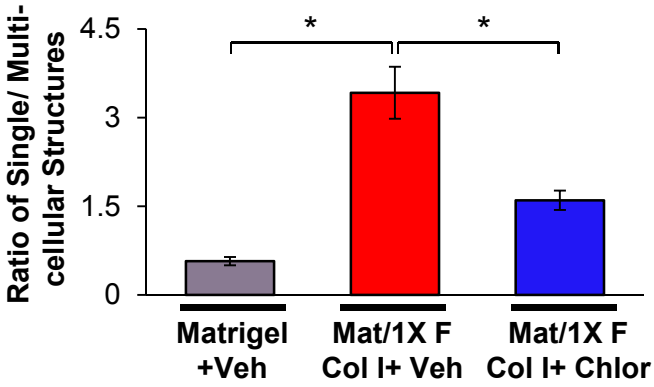

Figure S11

Clathrin-dependent endocytotic inhibition decreases the relative amount of singular cells to multicellular structures. D2.OR cells cultured in Matrigel or Mat/ 1X F Col I treated with vehicle (Veh) or chlorpromazine (Chlor) in 3D cell culture. Evaluating the ratios between singular cell number and multicellular structure number for each condition. N=3 wells per group, *$p<0.001$, ANOVA with Bonferroni multiple comparisons posttest. Model #2 was used to perform this 3D cell culture experiment.

Table S1

Differences in mammary ECM proteomes between nulliparous and parous rats were identified via mass spectrometry-based proteomics and label-free quantitation

Ranking	Identified Proteins	Accession Number	Molecular Weight	Avg TIC	p Value	Spect Counts	p Value
1	Collagen, type I, alpha 1	IPI00188909	138 kDa	1.05	0.998	1.03	0.9454
4	Collagen, type I, alpha 2	IPI00188921	130 kDa	1.07	0.824	1.06	0.7631
13	Collagen, type III, alpha 1	IPI00366944	139 kDa	-1.01	0.808	1.07	0.8604
397	Collagen, type V, alpha 1	IPI00201608	184 kDa	2.66	0.901	1.28	0.8456
441	Collagen, type V, alpha 2	IPI00366945	145 kDa	-1.05	0.419	1.05	0.2554
19	Collagen, type VI, alpha 1	IPI00371853	110 kDa	-1.31	0.162	-1.32	0.0448
30	Collagen, type VI, alpha 2	IPI00372839	110 kDa	-1.20	0.194	-1.34	0.0018
2	Collagen, type VI, alpha 3	IPI00565677	288 kDa	-1.30	0.196	-1.41	0.0238
462	Collagen, type VI, alpha 3	IPI00765205	240 kDa	-2.36	0.250	-1.74	0.5564
423	Collagen, type VI, alpha 6	IPI00778143 (+1)	247 kDa	-1.13	0.770	-1.22	0.8492
330	Collagen, type VII, alpha 1	IPI00767686	295 kDa	6.20	0.986	2.59	0.7002
172	Collagen, type XII alpha 1	IPI00958129 (+1)	333 kDa	3.05	0.642	-1.39	0.3723
5	Collagen, type XIV, alpha 1	IPI00360766 (+1)	192 kDa	-1.09	0.503	-1.16	0.1405
81	Collagen, type XV, alpha 1	IPI00364868	138 kDa	-1.03	0.837	-1.03	0.8609
483	Laminin, alpha 1	IPI00363534	338 kDa	1.35	0.080	-1.36	0.2148
168	Laminin, alpha 2	IPI00361301	343 kDa	1.49	0.483	1.24	0.8619
76	Laminin, alpha 4 (Fragment)	IPI00361106	176 kDa	-1.13	0.601	-1.02	0.8227
431	Laminin, alpha 5	IPI00190577	404 kDa	-3.10	0.001	-1.98	0.4993
109	Laminin beta 1	IPI00365542	203 kDa	-1.05	0.891	1.05	0.7528
44	Laminin, beta 2	IPI00212868	196 kDa	1.14	0.564	-1.03	0.8718
57	Laminin, gamma 1	IPI00363849	177 kDa	-1.07	0.758	-1.06	0.8414
264	Adiponectin	IPI00202515	26 kDa	-1.03	0.519	-1.09	0.5835
86	Asporin	IPI00365784	43 kDa	1.62	0.472	-1.29	0.0755
34	Biglycan	IPI00191090	42 kDa	-1.18	0.148	-1.03	0.8794
214	Bone marrow proteoglycan	IPI00206023	25 kDa	-1.34	0.232	-1.27	0.2984
8	Decorin	IPI00199861	40 kDa	-1.25	0.009	-1.18	0.1362
79	Dermatopontin	IPI00371512	24 kDa	-1.30	0.354	-1.48	0.0482
816	Elastin microfibril interfacer 2	IPI00764937 (+1)	126 kDa	-1.90	0.299	-1.61	0.3764
29	Fibrillin1	IPI00951429	312 kDa	-1.23	0.467	-1.19	0.4367
33	Fibronectin 1	IPI00231984	260 kDa	-1.20	0.375	-1.36	0.0051
588	Fibronectin type III domain	IPI00358887	194 kDa	1.85	0.507	1.06	0.7787
219	Fibulin-5	IPI00326179	50 kDa	-1.01	0.829	-1.06	0.4748

Continued

37	HSPG (Fragment)	IPI00210360	394 kDa	-1.17	0.586	-1.03	0.6026
132	HSPG2, Perlecan (Fragment)	IPI01016479	375 kDa	-1.09	0.827	-1.17	0.4994
11	Lumican	IPI00206403	38 kDa	-1.02	0.957	-1.08	0.7305
535	Matrix Gla protein	IPI00211401	12 kDa	12.00	0.003	6.48	0.0022
65	Nidogen 1	IPI00231136	137 kDa	1.11	0.779	1.09	0.6590
94	Nidogen-2	IPI00372786	153 kDa	1.01	0.998	1.22	0.7589
17	Osteoglycin	IPI00362931	34 kDa	-1.25	0.267	-1.29	0.0480
28	Periostin	IPI00567560	93 kDa	-1.63	0.003	-1.66	0.0004
15	Prolargin	IPI00190287	43 kDa	-1.06	0.722	-1.11	0.6318
88	Tenascin-X	IPI00911228	127 kDa	1.10	0.720	1.14	0.8594
343	Tenascin-X (Fragment)	IPI00948911	23 kDa	1.22	0.641	-1.00	0.8689
924	Versican core protein isoform 1	IPI00957386	367 kDa	1.11	0.905	1.32	0.9146

Ranking refers to ECM protein position in the whole proteome. ECM proteins in parous matrix were quantitated relative to nulliparous matrix using spectral counting and average total ion current (TIC) on MS/MS spectra. N=5 analyses from a single matrix sample per condition. Directions of arrows indicate whether protein abundances are increase (up), decrease (down), or unchanged (straight). For statistical values, yellow dot *p<0.05 and green dot *p<0.01 unpaired t-test.

Table S2

Number of unique peptides from each ECM protein:

		Unique Peptides Used for Quantitation (N=5)	Sequence Coverage
Accession	**Description**	**Avg. (±SD)**	**Avg. (±SD)**
IPI00188909	Collagen alpha-1 (I) chain	179.7 (10.5)	67.3 (5.8)
IPI00188921	Collagen alpha-2 (I) chain	132.3 (16.6)	68.3 (4.0)
IPI00565677	Collagen alpha-3 (VI) chain	121.3 (11.6)	50.3 (8.1)
IPI00366944	Collagen alpha-1 (III) chain	111.7 (6.1)	57.3 (8.0)
IPI00360766	Collagen alpha-1 (XIV) chain	113.7 (9.7)	62.7 (5.0)
IPI00951429	Fibrillin 1	99.0 (14.5)	
IPI00371853	Collagen alpha-1 (VI) chain	45.3 (6.4)	48.0 (17.1)
IPI00200757	Fibronectin	28.7 (15.4)	
IPI00190088	Periostin	41.7 (5.5)	
IPI00372839	Collagen alpha-2 (VI) chain	36.0 (1.7)	41.0 (11.8)
IPI00199861	Decorin	27.0 (1.0)	59.3 (8.4)
IPI00911228	Tenascin X	25.0 (2.0)	
IPI00190287	Prolargin	24.3 (0.6)	
IPI00206403	Lumican	18.0 (2.0)	
IPI00191090	Biglycan	17.7 (0.6)	
IPI00362931	Osteoglycin	17.7 (2.1)	
IPI00371512	Dermatopontin	12.0 (1.0)	
IPI00214859	Collagen alpha-1 (XVIII) chain	12.3 (1.5)	
IPI00326179	Fibulin-5	6.6 (0.6)	
IPI00201608	Collagen alpha-1 (V) chain	3.3 (1.5)	
IPI00366945	Collagen alpha-2 (V) chain	2.0 (1.7)	
IPI00211401	Matrix Gla protein	2.0 (0.0)	

The list includes average number of unique peptides from mammary ECM proteome of both nulliparous and parous rats detected via mass spectrometry. Percent coverage for selected ECM proteins is also included. B=band indicating the sequence coverage for a specific protein at that band only (specific MW range). N=5 analyses from a single matrix sample per group.

Table S3. Mat/1X NF Col I unique gene signature

Download Table S3

Table S4

Custom DNA oligo sequences from IDT.com for RT-PCR

Gene Name	Species		Sequence
Ifi44	Mouse	Forward Sequence (5'->3')	TAC GTC AGA CAT TCG GGA AGC AGT
		Reverse Sequence (5'->3')	AAA GAC AGC CAC TCA GGC GTA TCA
Gjb2	Mouse	Forward Sequence (5'->3')	AGG ATG AGG CAA CCC ATG CTT AGT
		Reverse Sequence (5'->3')	AGA ATT GGG CCT TTG TTT GGG AGC
Adamts1	Mouse	Forward Sequence (5'->3')	TGG ACA CGG GGA ATG TTT GAT
		Reverse Sequence (5'->3')	AGT ACA TGT GCT GGC TGC AT
Mmp14	Mouse	Forward Sequence (5'->3')	GCC CTC TGT CCC AGA TAA GC
		Reverse Sequence (5'->3')	ACC ATC GCT CCT TGA AGA CA
Vcam1	Mouse	Forward Sequence (5'->3')	TGG AGG TCT ACT CAT TCC CTG A
		Reverse Sequence (5'->3')	GAC AGG TCT CCC ATG CAC AA