Supplemental figures and tables for **“**Tension stimulation drives tissue formation in scaffold-free systems”

Authors

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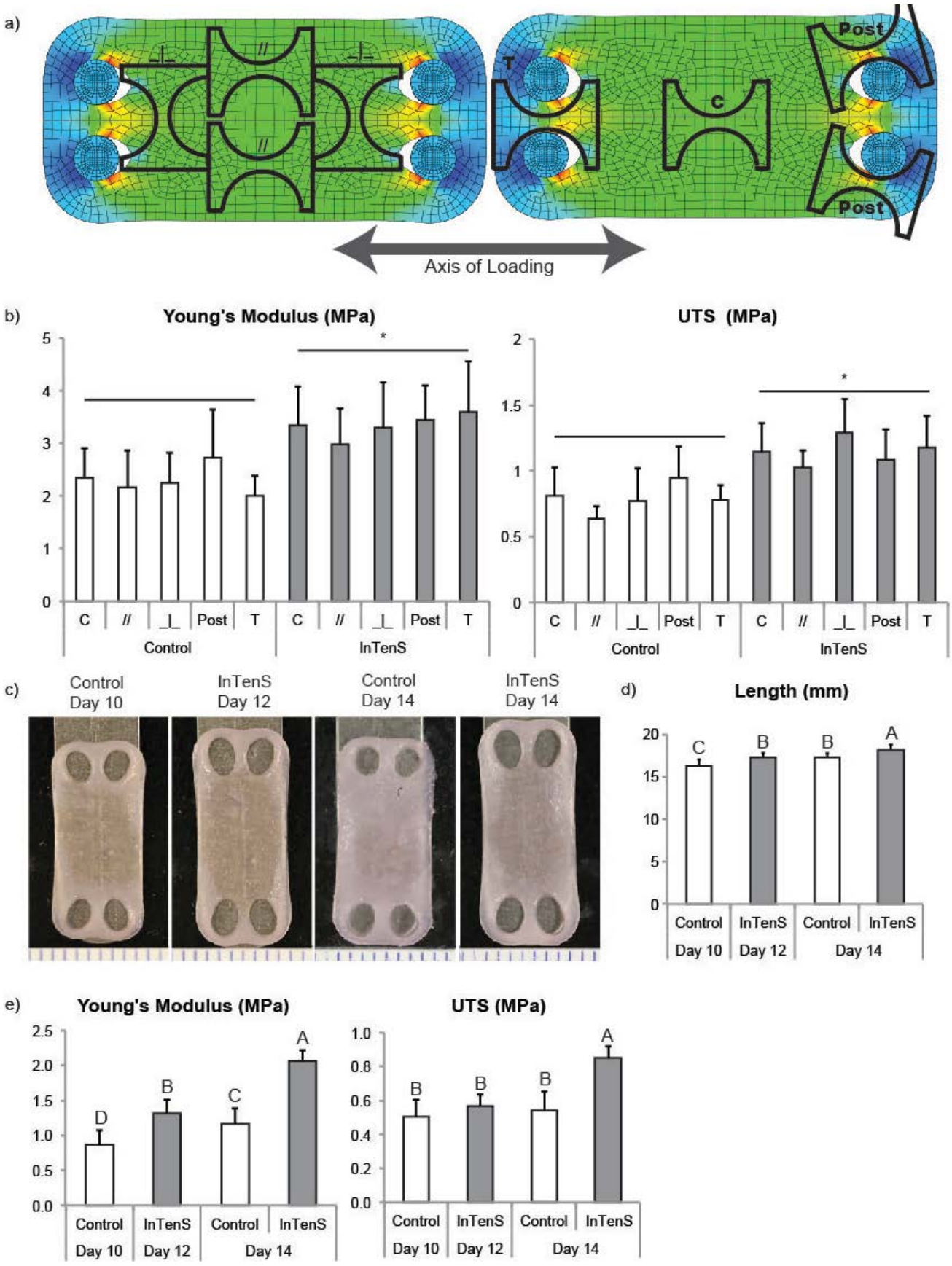
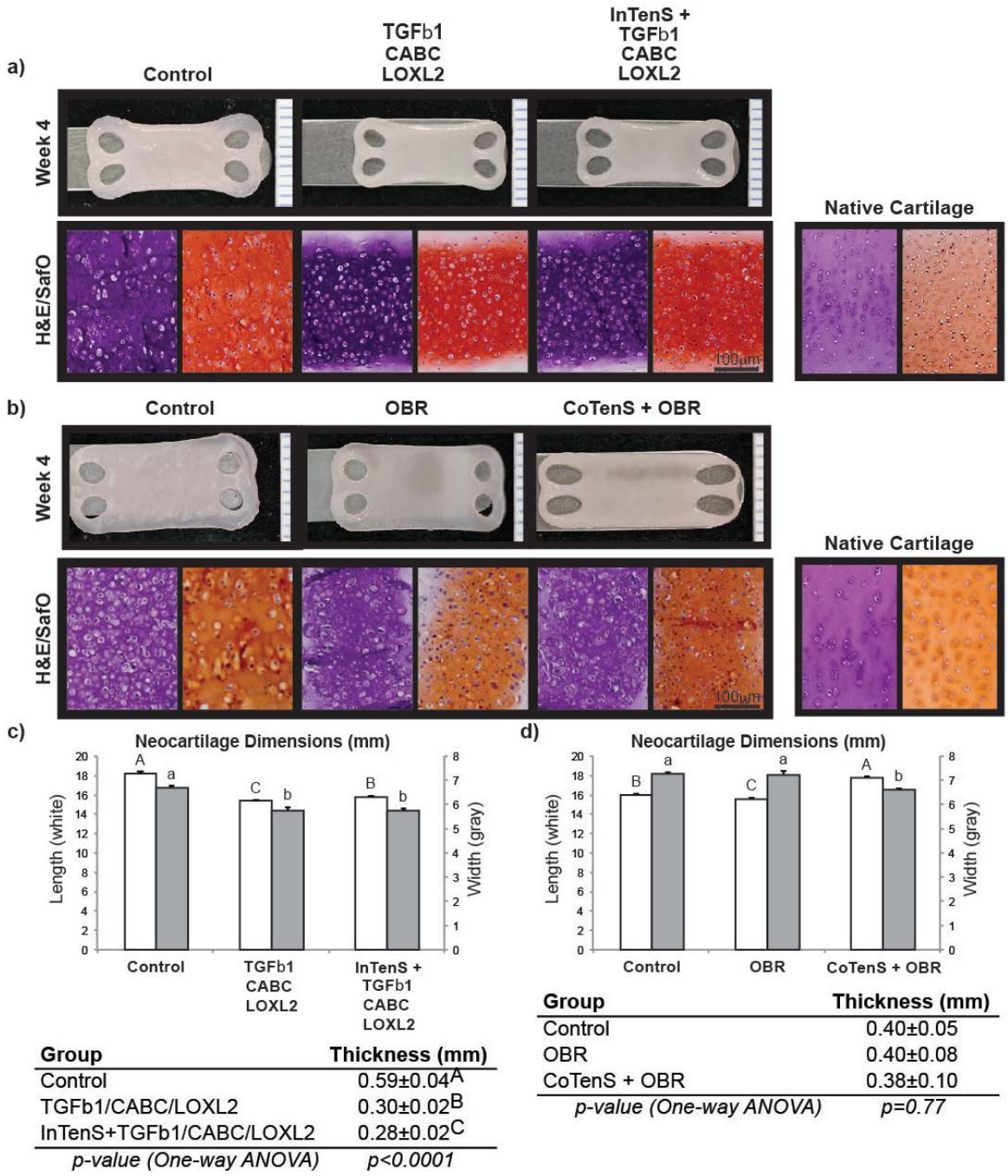


Supplemental Figure 1. Topographical assessment demonstrates tissue uniformity, while time-lapse analysis indicates significant changes by Day 12.

Tensile properties of neocartilage were assessed in various locations, as indicated by the outlines of dog-bone specimens (a), and demonstrated no significant regional differences within the untreated or InTenS-treated groups (b). Student's t-test was performed to compare control and InTenS-treated groups, *p<0.05. X-axis legend matches dog-bones indicated in (a). Across all regions, InTenS led to significantly higher Young's modulus and UTS compared to untreated controls. Neocartilage was assessed on Days 10, 12, and 14 to elucidate the morphological (c,d) and mechanical (e) changes during the InTenS process. By Day 14, InTenS-treated neocartilage constructs were significantly longer than untreated controls. Both Young's modulus and UTS were increased at Day 14 in the InTenS groups over untreated controls. One-way ANOVAs, followed by a Tukey's *post hoc* test, were performed to statistically assess the results. Groups not connected by the same letter are statistically significant. Data are represented as mean±SD.



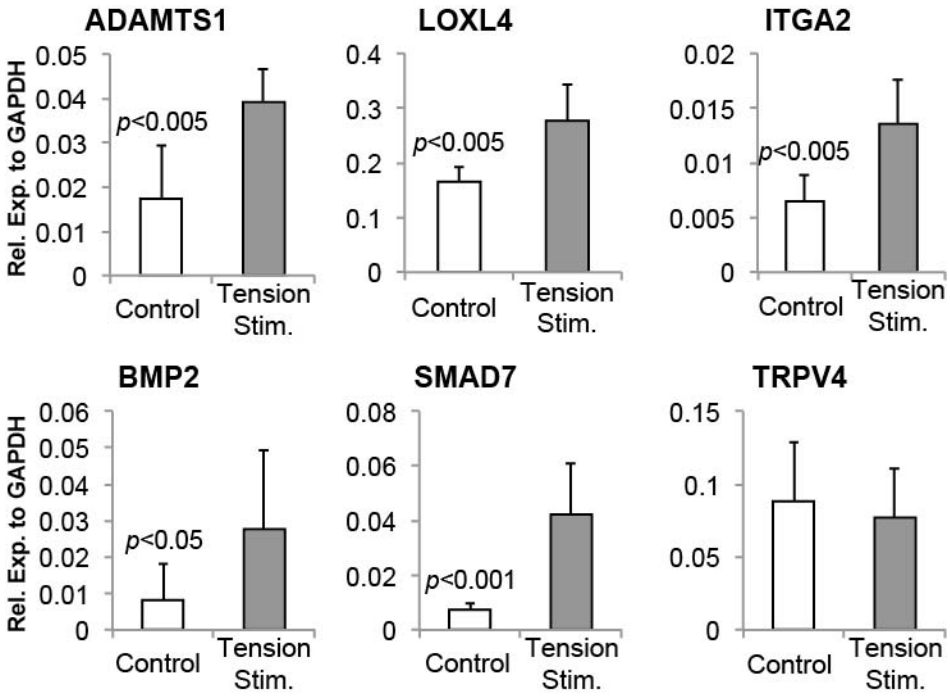
Group	Thickness (mm)
Control	0.59 ± 0.04^{A}
TGFb1/CABC/LOXL2	0.30 ± 0.02^{B}
InTenS+TGFb1/CABC/LOXL2	0.28 ± 0.02^{C}
p-value (One-way ANOVA)	*p<0.0001*

Group	Thickness (mm)
Control	0.40±0.05
OBR	0.40±0.08
CoTenS + OBR	0.38±0.10
p-value (One-way ANOVA)	*p=0.77*

Supplemental Figure 2. Morphological differences are present in week 4 neocartilage prior to *in vivo* implantation, as well as in CoTenS-treated tissues.

Control, InTenS + TGFβ1/CABC/LOXL2-treated, and TGFβ1/CABC/LOXL2-treated neocartilage exhibited a hyaline-like appearance (a). TGFβ1/CABC/LOXL2-treated and InTenS + TGFβ1/CABC/LOXL2-treated groups were markedly smaller than untreated controls. Formalin-fixed tissue sections were stained with H&E and Safranin-O/Fast Green to visualize matrix distribution and GAG deposition. Rich matrix deposition was present in all groups. CoTenS + OBR and OBR alone-treated neocartilage also exhibited a hyaline-like appearance (b). The differences in morphological parameters were confirmed statistically, with tension stimulation leading to significantly longer

neocartilage than those treated with bioactive agents alone (c,d). One-way ANOVAs, followed by a Tukey's *post hoc* test, were used to statistically compare the groups. Groups not connected by the same letter are statistically significant. Data are represented as mean±SD. Tick marks in (a) and (b) represent 1mm.



Supplemental Figure 3. Preliminary gene expression analysis to confirm microarray results. qRT-PCR confirmed up-regulation in genes related to matrix remodeling (i.e., ADAMTS1, LOXL4), cell-matrix interactions (i.e., integrin α2), and the BMP signaling pathway (i.e., BMP2, SMAD7). Tension stimulation did not upregulate TRPV4 expression.

Supplemental Table 1A and 1B. Mechanical and biochemical properties of InTenS-treated neocartilage.

This portion of the work comprised a series of tissue engineering studies evaluating the effect of 1) InTenS alone, 2) InTenS + TGFβ1, 3) InTenS + TGFβ1/LOXL2, and 4) InTenS + TGFβ1/LOXL2/CABC. Combination treatments progressively enhanced the tensile and biochemical properties of engineered neocartilage (1A). Anisotropy of tensile properties in engineered tissues was achieved with InTenS (1B). GAG and collagen contents (mg) were normalized by tissue wet weight (mg). Student's t-test was used for all studies except InTenS + TGFβ1, where a two-way ANOVA was applied, followed by Tukey's *post hoc* test, $p<0.05$. Data are represented as mean±SD.

Supplemental Table 1A: Mechanical and Biochemical Properties of InTenS-Treated Neocartilage

Study	Groups	Tensile Testing		Compressive Testing		Biochemical Properties						
		Young's modulus (MPa)	UTS (MPa)	Aggregate modulus (kPa)	Shear modulus (kPa)	Construct wet weight, WW (mg/mg)	Hydration (%)	Total GAG (mg)	Total collagen (mg)	Cells (10^6)	GAG/WW (mg/mg)	Collagen/WW (mg/mg)
InTenS alone	Control	1.3±0.3	0.3±0.1	113±56	53.1±26.2	82.09±2.53	87.0±0.3	4.50±0.12	1.65±0.10	11.7±1.4	0.055±0.002	0.0204±0.0006
	InTenS	1.8±0.5	0.4±0.1	165±43	68.3±17.4	72.18±4.48	72.2±4.5	4.22±0.27	1.54±0.13	8.4±0.7	0.059±0.002	0.0214±0.0019
	Statistics (±InTenS)	*p=0.003*	*p=0.002*	*p=0.04*	*p=0.2*	*p<0.0001*	*p=0.02*	*p=0.02*	*p=0.07*	*p=0.001*	*p=0.001*	*p=0.2*
InTenS +TGFβ1	Control	$2.6±0.8^{C}$	$0.8±0.3^{C}$	$44±9^{B}$	$15.9±4.3^{B}$	46.11±7.17	$86.2±0.7^{A}$	$2.65±0.50^{AB}$	$0.82±0.17^{B}$	$5.0±0.6^{C}$	$0.063±0.005^{A}$	$0.0194±0.0010^{C}$
	InTenS	$3.5±0.7^{B}$	$1.0±0.2^{BC}$	$72±21^{A}$	$25.1±9.3^{B}$	46.51±3.92	$85.2±0.4^{B}$	$3.15±0.39^{A}$	$1.00±0.10^{AB}$	$5.6±0.6^{BC}$	$0.068±0.004^{A}$	$0.0215±0.0005^{B}$
	TGFβ1	$3.3±0.9^{BC}$	$1.2±0.1^{B}$	$66±18^{AB}$	$25.9±5.8^{AB}$	44.80±2.67	$85.4±0.5^{B}$	$2.26±0.29^{B}$	$1.06±0.15^{AB}$	$6.2±0.4^{AB}$	$0.050±0.004^{B}$	$0.0235±0.0018^{A}$
	InTenS + TGFβ1	$6.2±0.8^{A}$	$2.1±0.2^{A}$	$81±6^{A}$	$35.6±2.9^{A}$	46.76±1.59	$85.4±0.5^{B}$	$2.54±0.14^{B}$	$1.15±0.04^{A}$	$6.7±0.5^{A}$	$0.054±0.002^{B}$	$0.0247±0.0008^{A}$
	Statistics (One-way ANOVA)	*p<0.0001*	*p<0.0001*	*p<0.008*	*p<0.0001*	*p=0.5*	*p=0.005*	*p=0.0006*	*p=0.02*	*p<0.0001*	*p<0.0001*	*p<0.0001*
InTenS + TGFβ1/LOXL2	Control	2.8±0.6	1.0±0.1	69±10	29.0±4.5	54.32±2.33	86.4±0.3	2.74±0.13	0.99±0.11	7.1±0.4	0.051±0.002	0.0177±0.0009
	InTenS + TGβ1	5.6±0.4	2.1±0.3	115±17	46.1±11.3	39.55±0.76	84.7±0.8	1.92±0.07	0.92±0.07	8.0±0.7	0.050±0.001	0.0232±0.0020
	InTenS + TGFβ1/LOXL2	8.0±1.5	3.3±0.5	44±14	10.8±1.7	35.51±1.83	86.6±0.5	0.98±0.13	0.84±0.06	7.3±0.5	0.028±0.003	0.0240±0.0008
	Statistics (±LOXL2)	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p=0.0002*	*p=0.0004*	*p<0.0001*	*p=0.06*	*p=0.06*	*p<0.0001*	*p=0.3*
InTenS + TGFβ1/LOXL2/CABC	Control	1.9±0.4	0.7±0.1	158±70	69.1±21.6	75.52±4.02	88.0±0.5	3.78±0.32	1.25±0.15	5.5±0.4	0.050±0.003	0.0171±0.0011
	InTenS + TGFβ1/LOXL2	5.0±1.1	2.1±0.4	62±15	23.8±5.5	51.04±3.85	88.0±0.4	1.36±0.16	1.12±0.15	3.9±0.5	0.026±0.001	0.0237±0.0026
	InTenS + TGFβ1/LOXL2/CABC	7.6±0.4	2.6±0.1	107±18	38.8±11.0	38.21±2.89	83.7±0.1	1.79±0.14	1.05±0.12	3.3±0.5	0.047±0.003	0.0278±0.0009
	Statistics (±CABC)	*p<0.0001*	*p=0.0002*	*p=0.002*	*p=0.02*	*p<0.0001*	*p<0.0001*	*p<0.0002*	*p=0.3*	*p=0.004*	*p<0.0001*	*p<0.0001*

Supplemental Table 1B: Anisotropy in Tensile Properties		**Young's Modulus (MPa)**		**UTS (MPa)**	
		Parallel	Perpendicular	Parallel	Perpendicular
InTenS + TGFβ1/LOXL2/CABC	Control	1.7±0.5	2.0±0.3	0.6±0.1	0.7±0.2
	InTenS +TGFβ1/LOXL2	5.7±0.6**	3.9±0.4	2.3±0.3*	1.8±0.3
	InTenS +TGFβ1/LOXL2/CABC	8.4±0.9*	7.0±0.7	2.6±0.1	2.6±0.1
	Statistics	Student's t-test, parallel versus perpendicular directions within groups (*p<0.05, **p<0.005)			

Supplemental Table 2: Mechanical and biochemical properties of 4-week-old InTenS-treated neocartilage.

Prior to implantation, neocartilage replicated the results of prior experiments (Supplemental Table 1). Neocartilage treated with only bioactive agents exhibited significantly enhanced mechanical and biochemical properties as compared to untreated controls. Please note that the tensile data presented here are the averages of the parallel and perpendicular directions; for parallel only data showing significant effects of InTenS, please see Figure 4d. As evident in Supplemental Table 3, the effects of CoTenS are even more pronounced. GAG and collagen contents (mg) were normalized by tissue wet weight (mg). One-way ANOVAs, followed by Tukey's *post hoc* test, were used to statistically assess the results. Groups not connected by the same letter are statistically significant. Data are represented as mean±SD.

Supplemental Table 2: Mechanical and Biochemical Properties of 4-week-old InTenS-Treated Neocartilage											
Groups	**Tensile Testing**		**Compressive Testing**		**Biochemical Properties**						
	Young's modulus (MPa)	UTS (MPa)	Aggregate modulus (kPa)	Shear modulus (kPa)	Construct wet weight, WW (mg/mg)	Hydration (%)	Total GAG (mg)	Total collagen (mg)	Cells (10^6)	GAG/WW (mg/mg)	Collagen/WW (mg/mg)
Control	1.4 ± 0.4^{B}	0.4 ± 0.1^{B}	124±46	50.1 ± 13.9^{A}	91.54 ± 3.11^{A}	87.63 ± 0.51^{A}	5.47 ± 0.90^{A}	2.28 ± 0.51^{A}	9.0 ± 0.3^{A}	0.060±0.009	0.0228±0.0016
TGFβ1/LOXL2/CABC	7.5 ± 1.0^{A}	2.6 ± 0.4^{A}	136±54	44.4 ± 10.7^{AB}	37.22 ± 1.32^{B}	83.03 ± 0.39^{B}	2.37 ± 0.16^{B}	0.87 ± 0.08^{B}	4.7 ± 0.6^{B}	0.064±0.004	0.0233±0.0013
InTenS + TGFβ1/LOXL2/CABC	7.2 ± 0.7^{A}	2.6 ± 0.2^{A}	102±36	30.9 ± 11.3^{B}	34.83 ± 0.74^{B}	82.70 ± 0.63^{B}	2.10 ± 0.13^{B}	0.81 ± 0.05^{B}	4.8 ± 0.7^{B}	0.062±0.003	0.0234±0.0015
Statistics (One-way ANOVA)	*p<0.0001*	*p<0.0001*	*p=0.5*	*p=0.04*	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p<0.0001*	*p=0.5*	*p=0.8*

Supplemental Table 3: Mechanical and biochemical properties of neocartilage treated with CoTenS.

To fully examine the effects of tension as a stimulus, CoTenS was applied in the presence of an optimized bioactive regimen (OBR). CoTenS plays a critical role in generating significantly enhanced tensile properties and anisotropy, above and beyond those of OBR alone. GAG and collagen contents (mg) were normalized by tissue wet weight (mg). One-way ANOVA, followed by Tukey's *post hoc* test, was applied to examine the effects of OBR and CoTenS + OBR treatment. Groups not connected by the same letter are statistically significant. Student's t-test was used to assess anisotropic effects of regimens. Data are represented as mean±SD.

Supplemental Table 3: Mechanical and Biochemical Properties of CoTenS-Treated Neocartilage

Groups	Tensile Testing		Compressive Testing		Biochemical Properties						
	Young's modulus (MPa)	UTS (MPa)	Aggregate modulus (kPa)	Shear modulus (kPa)	Construct wet weight, WW (mg/mg)	Hydration (%)	Total GAG (mg)	Total collagen (mg)	Cells (10^6)	GAG/WW (mg/mg)	Collagen/WW (mg/mg)
Control	1.1 ± 0.3^{C}	0.4 ± 0.1^{C}	215 ± 59^{A}	82.2 ± 7.2^{A}	74.34 ± 9.01^{A}	86.3±1.5	4.46 ± 0.88^{A}	1.01±0.12	7.3 ± 0.6^{A}	0.061 ± 0.010^{A}	0.0136 ± 0.0024^{B}
OBR	3.1 ± 1.1^{B}	1.4 ± 0.5^{B}	148 ± 22^{B}	55.4 ± 8.6^{B}	50.61 ± 10.10^{B}	84.2±1.1	2.16 ± 0.21^{B}	0.94±0.19	3.7 ± 0.9^{B}	0.046 ± 0.004^{AB}	0.0185 ± 0.0040^{AB}
CoTenS + OBR	5.1 ± 2.0^{A}	1.8 ± 0.6^{A}	199 ± 33^{AB}	44.3 ± 5.6^{C}	42.54 ± 8.42^{B}	85.9±6.7	1.75 ± 0.83^{B}	0.97±0.19	3.6 ± 1.6^{B}	0.040 ± 0.015^{B}	0.0229 ± 0.0039^{A}
Statistics (One-way ANOVA)	*p<0.0001*	*p<0.0001*	*p=0.03*	*p<0.0001*	*p<0.0001*	*p=0.7*	*p=0.0003*	*p=0.7*	*p<0.0001*	*p=0.03*	*p<0.0001*

Anisotropy	**Young's Modulus (MPa)**		**UTS (MPa)**	
	Parallel	Perpendicular	Parallel	Perpendicular
Control	1.1±0.5	1.0±0.4	0.4±0.2	0.4±0.1
OBR	2.8±1.2	3.3±1.1	1.3±0.6	1.5±0.5
CoTenS + OBR	6.4±2.2**	3.8±0.6	2.3±0.7**	1.4±0.3
Statistics	Student's t-test, parallel versus perpendicular directions within groups (**p<0.005)			

Supplemental Table 4: Mechanical and biochemical properties of human neocartilage treated with CoTenS.

To test the translational potential of tension stimulation, CoTenS was applied to neocartilage generated from a clinically meaningful cell source—human articular chondrocytes. CoTenS significantly enhanced neocartilage properties beyond an optimized bioactive regimen alone, and beyond that of untreated neocartilage. GAG and collagen contents (mg) were normalized by tissue wet weight (mg). One-way ANOVAs, followed by Tukey's *post hoc* test, were used to statistically assess the results. Groups not connected by the same letter are statistically significant. Data are represented as mean±SD.

Supplemental Table 4: Mechanical and Biochemical Properties of Human AC-derived Neocartilage											
Groups	**Tensile Testing**		**Compressive Testing**		**Biochemical Properties**						
	Young's modulus (MPa)	UTS (MPa)	Aggregate modulus (kPa)	Shear modulus (kPa)	Construct wet weight, WW (mg/mg)	Hydration (%)	Total GAG (mg)	Total collagen (mg)	Cells (10^6)	GAG/WW (mg/mg)	Collagen/WW (mg/mg)
Control	0.6 ± 0.1^{B}	0.3 ± 0.1^{B}	22.3 ± 12.5^{B}	9.4 ± 5.8^{B}	18.76 ± 0.90^{C}	86.8±4.3	0.23 ± 0.12^{B}	0.36±0.12	6.7±2.4	0.013±0.006	0.0193±0.0063
OBR	1.2 ± 0.5^{B}	0.5 ± 0.3^{B}	30.4 ± 23.7^{B}	10.9 ± 8.6^{B}	28.57 ± 2.91^{AB}	77.1±10.8	0.57 ± 0.26^{B}	0.80±0.25	9.2±1.8	0.020±0.010	0.0282±0.0084
CoTenS + OBR	2.3 ± 0.7^{A}	1.2 ± 0.5^{A}	67.0 ± 12.1^{A}	33.3 ± 6.2^{A}	31.93 ± 2.19^{A}	86.9±4.6	1.01 ± 0.24^{A}	0.98±0.27	8.8±3.1	0.032±0.009	0.0309±0.0089
Statistics (One-way ANOVA)	*p=0.004*	*p=0.006*	*p=0.0008*	*p<0.0001*	*p<0.0001*	*p=0.1*	*p<0.0005*	*p=0.1*	*p=0.3*	*p=0.1*	*p=0.1*

Supplemental Table 5: Primers used in qRT-PCR.

Forward and reverse primers used for qRT-PCR analysis. Primers were designed using the Primer3 tool.

Supplemental Table 5: Primers used in qRT-PCR		
Gene	**Primer Direction**	**Sequence**
ADAMTS1	Forward	ACACCGAACAGGAACTGGAA
	Reverse	ATGGACTGGTCAGCCACAA
BMP2	Forward	CCCCTACATGCTGGACTTGT
	Reverse	GATTCTTCGTGATGGAAGCTG
GAPDH	Forward	AAGTTCAACGGCACAGTCAA
	Reverse	GATCTCGCTCCTGGAAGATG
ITGA2	Forward	GGTGACCAGATTGGCTCCTA
	Reverse	GTGCACCTACCAAGAGCACA
LOXL4	Forward	CACAGGCACTACCACAGCAT
	Reverse	GAGCCGTTGAGAGTGAGGAG
SMAD7	Forward	GTCAAGAGGCTGTGTTGCTG
	Reverse	GGGGCTAGTTCGCAGAGTC
TRPV4	Forward	ACATCTGGAAGCTGCAGTGG
	Reverse	ATTTCACCGGAGCGGAAG

Supplemental Table 6: Microarray analysis revealed significant up-regulation of genes in response to InTenS.

Microarray results were filtered based on a fold-change >2 and a *p*-value <0.05. Changes in select genes of interest were confirmed via qRT-PCR (Figure 3).

Supplemental Table 6.

Microarray results of changes in gene expression elicited by InTenS

Changes to bold genes were confirmed via PCR.

Fold Change	p-value	Gene Symbol	Description
5.74	0.001506	BHLHE40	basic helix-loop-helix family, member e40
5.55	0.00248	GPRC5A	G protein-coupled receptor, family C, group 5, member A
5.24	0.000119	SKIL	SKI-like oncogene
5.17	**0.00107**	**ADAMTS1**	**ADAM metallopeptidase with thrombospondin type 1 motif, 1**
4.95	0.000125	SERPINE1	serpin peptidase inhibitor, clade E (nexin, plasminogen activator inhibitor type 1), member 1
4.88	0.000212	ERRFI1	ERBB receptor feedback inhibitor 1
4.04	0.002231	PLK2	polo-like kinase 2
3.8	0.000533	SOCS3	suppressor of cytokine signaling 3
3.78	0.002578	HES1	hairy and enhancer of split 1, (Drosophila)
3.74	0.003196	NR4A1	nuclear receptor subfamily 4, group A, member 1
3.6	0.000472	SLC20A1	solute carrier family 20 (phosphate transporter), member 1
3.59	0.000004	SNAI1	snail homolog 1 (Drosophila)
3.57	0.000785	LOC510442	histone-lysine N-methyltransferase, H3 lysine-79 specific-like
3.52	**0.003264**	**SMAD7**	**SMAD family member 7**

			v-ets erythroblastosis virus E26 oncogene homolog 1
3.49	0.000102	ETS1	(avian)
3.45	0.000239	TBX3	T-box transcription factor
3.32	0.135103	MIR125B-1	microRNA mir-125b-1
			nuclear factor of activated T-cells, cytoplasmic,
3.31	0.000259	NFATC2	calcineurin-dependent 2
3.24	0.00011	JUNB	jun B proto-oncogene
		MMP3;	matrix metallopeptidase 3 (stromelysin 1,
3.24	0.017875	BT.18504	progelatinase)
3.19	0.00089	RGS16	regulator of G-protein signaling 16
3.13	0.006479	TRIB1	tribbles homolog 1 (Drosophila)
2.95	0.006842	FOS	FBJ murine osteosarcoma viral oncogene homolog
2.93	0.001188	KIAA1199	KIAA1199 ortholog
			v-ets erythroblastosis virus E26 oncogene homolog 2
2.93	0.003141	ETS2	(avian)
2.9	0.001937	NR4A3	nuclear receptor subfamily 4, group A, member 3
2.8	0.000079	LGALSL	lectin, galactoside-binding-like
2.78	0.000262	IL1R1	interleukin 1 receptor, type I
2.77	0.000392	SERTAD2	SERTA domain containing 2
2.74	0.000087	TXNRD1	thioredoxin reductase 1
2.73	0.00073	FOSB	FBJ murine osteosarcoma viral oncogene homolog B
			nuclear factor of activated T-cells, cytoplasmic,
2.72	0.001385	NFATC1	calcineurin-dependent 1
2.72	0.000404	SPRY2	sprouty homolog 2 (Drosophila)
2.71	0.000975	MT2A	metallothionein 2A
2.7	0.00032	VASN	vasorin
2.7	0.002364	ARC	activity-regulated cytoskeleton-associated protein

2.69	0.000272	TMEM2	transmembrane protein 2
2.68	**0.010509**	**ITGA2**	**integrin, alpha 2 (CD49B, alpha 2 subunit of VLA-2 receptor)**
2.67	0.000202	LMCD1	LIM and cysteine-rich domains 1
2.66	0.000265	TNFRSF11B	tumor necrosis factor receptor superfamily, member 11b
2.59	0.000507	TGIF1	TGFB-induced factor homeobox 1
2.56	0.010229	MIR221	microRNA mir-221
2.51	0.000161	KLF10	Kruppel-like factor 10
2.48	0.000166	BACH1	BTB and CNC homology 1, basic leucine zipper transcription factor 1
2.44	0.000114	JHDM1D	jumonji C domain containing histone demethylase 1 homolog D (S. cerevisiae)
2.41	0.581637	LOC100337093	zinc finger protein 75D-like
2.4	0.001035	SLC2A1	solute carrier family 2 (facilitated glucose transporter), member 1
2.4	0.00028	BAIAP2	BAI1-associated protein 2
2.4	0.017899	ULBP27; ULBP9; LOC783508	UL16-binding protein 27; UL16-binding protein 9; UL16-binding protein 27-like
2.38	0.000425	B3GNT7	UDP-GlcNAc:betaGal beta-1,3-N-acetylglucosaminyltransferase 7
2.37	0.000022	PELI1	pellino homolog 1 (Drosophila)
2.36	0.000684	CSRNP1	cysteine-serine-rich nuclear protein 1
2.35	0.002035	CDA	cytidine deaminase
2.34	**0.006135**	**BMP2**	**bone morphogenetic protein 2**
2.33	0.000063	SGK1	serum/glucocorticoid regulated kinase 1

2.32	0.001482	PHF13	PHD finger protein 13
2.32	0.001809	BCL2L1	BCL2-like 1
2.31	0.079327	ULBP21	UL16 binding protein 21
2.31	0.000638	EPHA2	EPH receptor A2
2.3	0.000191	IER3	immediate early response 3
2.3	0.000797	MAP3K8	mitogen-activated protein kinase kinase kinase 8
2.27	0.006821	FOSL1	FOS-like antigen 1
2.24	0.000809	SDC4	syndecan 4
2.23	0.004424	PLAUR	plasminogen activator, urokinase receptor
2.22	0.000698	SLC2A3	solute carrier family 2 (facilitated glucose transporter), member 3
2.22	0.004464	DUSP5	dual specificity protein phosphatase 5-like
2.22	0.000751	BDKRB2	bradykinin receptor B2
2.21	0.006271	ZFAND2A	zinc finger, AN1-type domain 2A
2.21	0.003645	MT1A	metallothionein-1A
2.21	0.000667	SRXN1	sulfiredoxin 1
2.2	0.031222	MIR23A	microRNA mir-23a
2.18	0.018505	CISH	cytokine inducible SH2-containing protein
2.17	0.000414	TGFB1	transforming growth factor, beta 1
2.16	0.000141	TIPARP	TCDD-inducible poly(ADP-ribose) polymerase
2.15	0.002326	ITGA5	integrin, alpha 5 (fibronectin receptor, alpha polypeptide)
2.14	0.002927	SNCAIP	synuclein, alpha interacting protein
2.14	0.013006	BDKRB1	bradykinin receptor B1
2.14	0.012767	FOSL2	FOS-like antigen 2
2.13	0.000085	NAB2	NGFI-A binding protein 2 (EGR1 binding protein 2)
2.13	0.001151	SLC7A2	solute carrier family 7 (cationic amino acid

			transporter, y+ system), member 2
			splA/ryanodine receptor domain and SOCS box
2.13	0.003142	SPSB1	containing 1
2.12	0.000012	FGF2	fibroblast growth factor 2 (basic)
2.1	0.001182	KLF3	Kruppel-like factor 3 (basic)
2.09	0.000367	ELL2	elongation factor, RNA polymerase II, 2
			chromosome 23 open reading frame, human
2.09	0.001579	PXDC1	C6orf145
2.08	0.001172	DUSP4	dual specificity phosphatase 4
			v-myc myelocytomatosis viral oncogene homolog
2.08	0.000025	MYC	(avian)
			prostaglandin-endoperoxide synthase 2
2.07	0.009514	PTGS2	(prostaglandin G/H synthase and cyclooxygenase)
2.07	0.009874	PMEPA1	prostate transmembrane protein, androgen induced 1
2.06	0.00249	KDM6B	lysine (K)-specific demethylase 6B
2.05	0.001335	EMP1	epithelial membrane protein 1
2.05	0.008185	PKNOX1	PBX/knotted 1 homeobox 1
2.04	0.005312	FMNL3	formin-like 3
			aTP-binding cassette, sub-family C (CFTR/MRP),
2.04	0.185782	LOC100298891	member 4-like
2.02	0.003689	GADD45B	growth arrest and DNA-damage-inducible, beta
2.02	0.003098	ZFP36	zinc finger protein 36, C3H type, homolog (mouse)
2.02	0.000177	EPAS1	endothelial PAS domain protein 1
2.01	0.000746	PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D
2.01	0.000708	RUNX1	runt-related transcription factor 1
1.92	**0.018369**	**TRPV4***	**transient receptor potential cation channel,**

			subfamily V, member 4
1.58	**0.010405**	**LOXL4***	**lysyl oxidase-like 4**
1.13	0.299411	LOXL2*	lysyl oxidase-like 2

*Despite not meeting the 2-fold cutoff, TRPV4 expression was confirmed via PCR, as TRPV4-specific inhibitors were investigated in these studies. Similarly, despite not meeting the 2-fold and $p < 0.05$ cutoffs, LOX protein expression was examined via PCR as LOXL2 was used in neocartilage treatment. The microarray results of these genes are presented here for comparison purposes.

-2	0.033441	CCDC82	coiled-coil domain containing 82
-2.01	0.219315	SKA3	spindle and kinetochore associated complex subunit 3
-2.03	0.053819	MAB21L2	mab-21-like 2 (C. elegans)
-2.05	0.084542	LOC100140123	zinc finger protein 75A-like
-2.05	0.091261	CENPA	centromere protein A
-2.05	0.004352	RBM11	RNA binding motif protein 11
-2.08	0.0093	MAGEF1	melanoma antigen family F, 1
-2.15	0.096388	FAM76A	family with sequence similarity 76, member A
-2.23	0.003164	ZC2HC1A	zinc finger, C2HC-type containing 1A

Supplemental Table 7: Structural properties of tested dumbbell-shaped samples in the parallel direction.

Shown are fold-increases in structural stiffness and structural strength of treated samples over control.

Supplemental Table 7. Structural Properties of Tested Dumbbell-shaped Samples in the Parallel Direction.							
	InTenS studies					CoTenS studies	
CABC	-	-	-	+		+	+
LOXL2	-	-	+	+		+	+
TGFβ1	-	+	+	+		+	+
InTenS	+	+	+	+		-	-
CoTenS	-	-	-	-		-	+
Structural stiffness (fold-changes with respect to control) [(N/mm)/(N/mm)]	1.3	2.2	2.1	2.1		2.5	5.5
Structural strength (fold-changes with respect to control) [N/N]	1.3	1.8	2.1	1.8		3.3	5.5