Appendix Tables & Figures

Functional Role of Glycosaminoglycans in Decellularized Lung Extracellular Matrix

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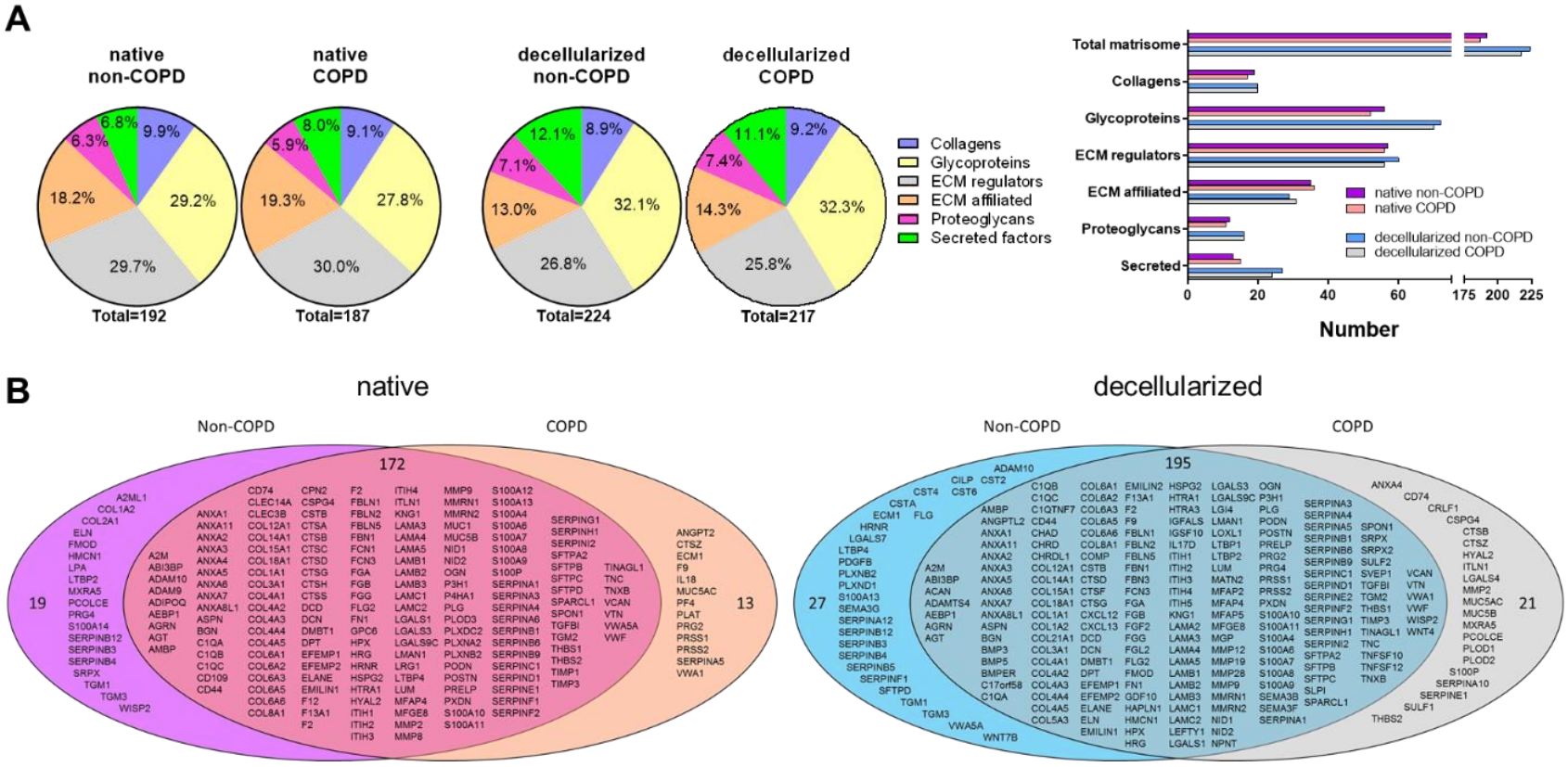
Lung decellularization method

Decellularization was conducted over three days in a sterile environment (cell cultivation hood) using a combined perfusion and physical approach. On day one of the decellularization process a single lobe was dissected from the lung by retaining as much of the main bronchi and vasculature as possible. Lungs were flushed using a roller pump (Stockert Shiley, SOMA Technologies, Bloomfield, CT, USA) at a flow rate of 2 l/min. Wash solutions were infused alternating via trachea/main bronchi and pulmonary artery/main vessel starting with a low flow rate slowly increasing to 2 l/min [2, 14-16, 24, 40, 53] taking care to not under or over inflate the lungs. Typical volumes used per lobe ranged from 2 to 3 L. During the vascular flushing, the trachea/main bronchus was clamped to allow for tissue inflation. Liquid was drained from the tissue by careful manual manipulation as much as possible until the tissue had visibly decreased in size and no liquid could be removed any longer. Lungs were rinsed six times with 1x PBS and six times with de-ionized (DI) water to clear them from blood. Next, the lungs were rinsed once with Triton solution (0.1% Triton X-100 (Sigma) and 5X pen/strep in DI water) infused through both the airways and vasculature. Lungs were filled a second time with Triton solution via both airways and vasculature, submerged in Triton solution, and incubated for 24 h at 4°C on a rocker-shaker (Gene Mate, Bio Express, Kaysville, UT, USA). On day 2, the lungs were removed from the Triton solution and rinsed six times with DI water. The lungs were then rinsed once with sodium deoxycholate (SDC) solution (2% SDC (Sigma) in DI water) and SDC solution was then instilled as previously described for day 1. Lungs were incubated in SDC solution for 24 h at 4°C on the rocker-shaker. The next day, lungs were removed from the SDC solution and rinsed six times with DI water. The lungs were then rinsed once with sodium chloride (NaCl) solution (1 M sodium chloride (NaCl) (USB) and 5X pen/strep in DI water), filled a second time with the NaCl solution and incubated in the NaCl solution for 1 h at room temperature (25°C) on the rocker-shaker. Lungs were removed from the NaCl solution, rinsed six times with DI water as described above. The lungs were then rinsed once with DNAse solution (30 µg/mL porcine pancreatic DNase (Sigma), 2 mM calcium chloride ($CaCl_2$) (Sigma), 1.3 mM magnesium sulfate ($MgSO_4$) (Sigma), and 5X pen/strep in DI water), filled a second time with DNAse solution and incubated for 1 h at room temperature on the rocker-shaker. The lungs were removed from the DNAse solution, rinsed six times with DI water as described above, then rinsed once with peracetic acid (PAA) solution (0.1% (v/v) peracetic acid (Sigma) in 4% (v/v) ethanol solution in DI water), instilled a second time with the PAA solution, and incubated for 1 h at room temperature on the rocker-shaker. Finally, lungs were removed from the PAA solution and rinsed six times with DI water as described above. After six washes with storage solution (5X pen/strep, 50 mg/L gentamicin (Cellgro), 2.5 µg/mL Amphotericin B (Cellgro) in 1X PBS solution) as described for the DI water rinses, lungs were stored in storage solution at 4°C until needed, but at maximum for 3 months prior to use. Samples for GAG analysis were taken after completion of decellularization from three different locations of each lobe, stored at -80°C, and analyzed in batch.

Appendix Table 1: Lung utilization for the different studies. SPR= surface plasmon resonance, IHC= immune histochemistry, MS= mass spectrometry, PCA= principal component analysis.

lung number	DNA amount [ng/mg]	Glycomics analysis (different locations)		Sulfation pattern (different locations)		SPR	cell culture	IHC	MS data (different locations)	PCA
		native	decelled	native	decelled					
non-COPD1	16	3		3					3	X
non-COPD2	145	3	3	3	3				3	X
non-COPD3	22	3	3	3	3				3	X
non-COPD4	12	3	3	3	3				3	X
non-COPD5	12	3	3	3	3				3	X
non-COPD6	22	3	3	3	3				3	X
non-COPD7	4	3	3	3	3				3	X
non-COPD8	n.a.	3		3						X
non-COPD9	n.a.	3		3						X
non-COPD10	6	3	3	3	3	X	X			X
non-COPD11	6	3	3	3	3	X	X			X
non-COPD12	5	3	3	3	3		X			X
non-COPD13	6	3	3	3	3		X			X
non-COPD14	16	3	3	3	3	X	X	X		X
non-COPD15	34	3	3	3	3		X			X
non-COPD16	6	3		3					3	X
non-COPD17	22		3		3					X
COPD1A	67		3		3				3	X
COPD1B	432		3		3				3	X
COPD2	28	3	3	3	3				3	X
COPD3	20	3	3	3	3		X	X		X
COPD4	15	3	3	3	3		X	X		X

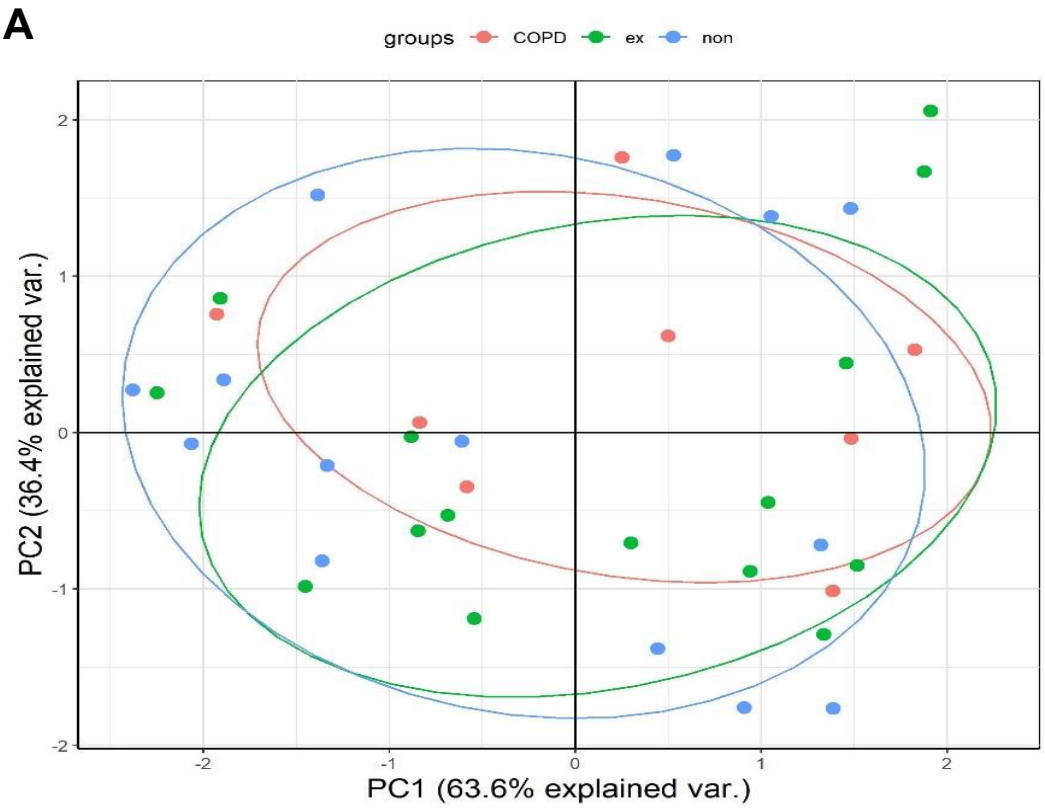


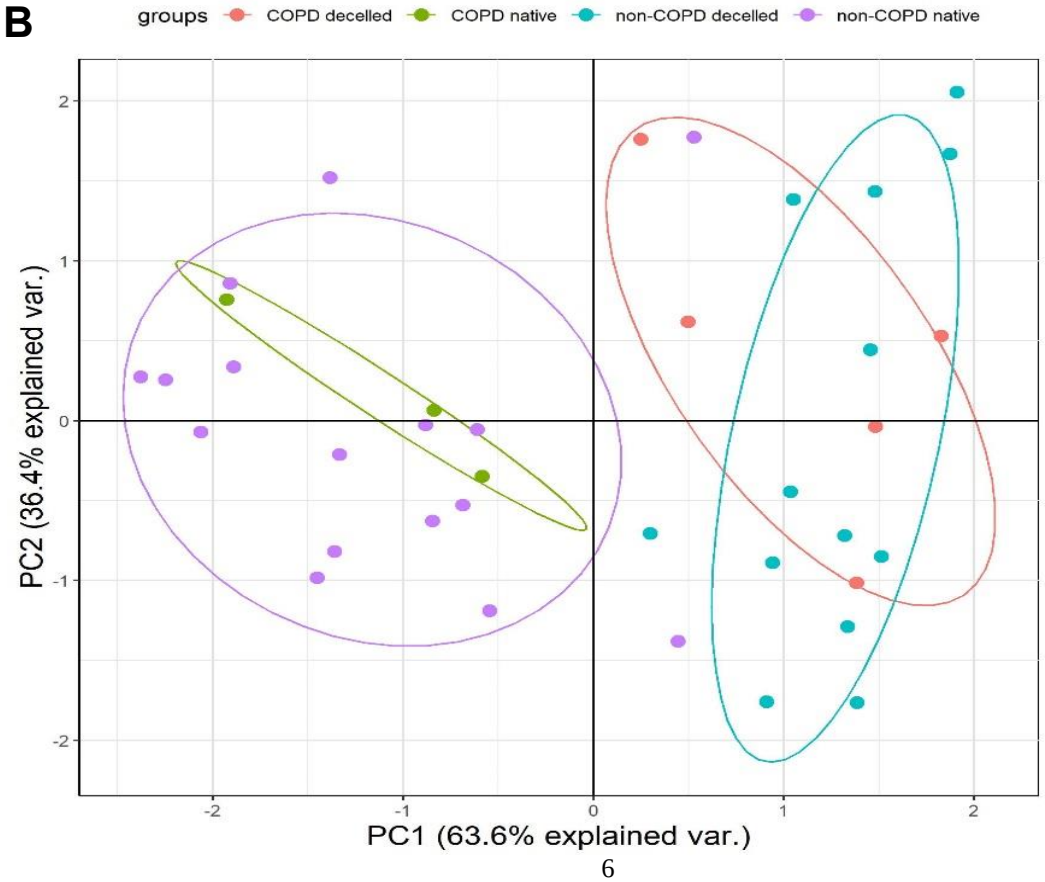
Appendix Figure 1: The matrisome composition of native and decellularized non-COPD and COPD human lungs comparably demonstrate proportionally more glycoproteins, proteoglycans, and secreted factors and less ECM affiliated and ECM regulators in the decellularized scaffolds. A) Percentage and number of matrisome proteins (independent of abundance) detected in native and decellularized lung tissue from non-COPD and COPD patients via MS. B) Overlap of matrisome proteins in native and decellularized tissue from non-COPD and COPD patients.

Appendix Table 2: Summary of binding kinetic data of FGF2, HGF, TGFβ1 to GAG from native lung tissues. The data with (±) in parentheses are the standard deviations (SD) from global fitting of four or five injections. k_a: association rate constant, k_d: dissociation rate constant, K_D: equilibrium dissociation constant, HS: heparan sulfate, CS: chondroitin sulfate, DS: dermatan sulfate, FGF2: fibroblast growth factor, HGF: hepatocyte growth factor, TGFβ1: transforming growth factor beta.

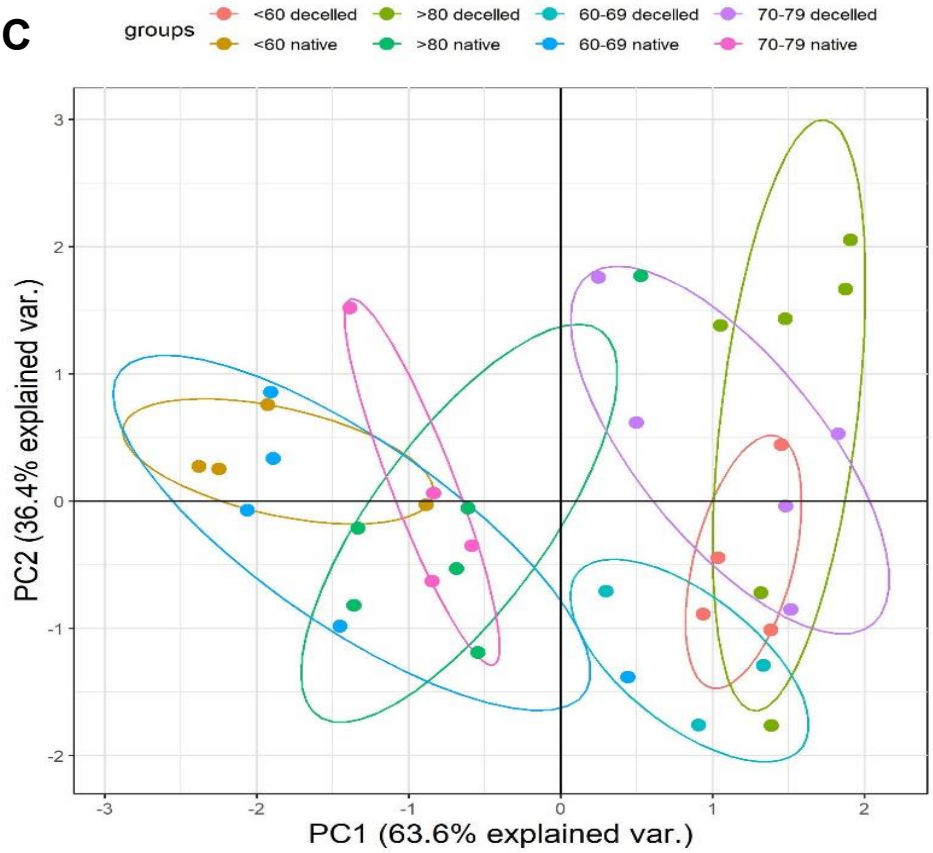
Interaction	**k_a (1/MS)**	**k_d (1/S)**	**K_D (M)**
FGF2/native lung HS	4.50×10^{5} ($\pm4.00\times10^{4}$)	0.213 ($\pm8.00\times10^{-3}$)	4.77×10^{-7}
FGF2/native lung CS	2.70×10^{5} ($\pm2.30\times10^{4}$)	0.332 (±0.01)	1.23×10^{-6}
FGF2/heparin	3.12×10^{5} ($\pm5.20\times10^{3}$)	2.10×10^{-3} ($\pm4.80\times10^{-5}$)	6.62×10^{-9}
HGF/native lung HS	ND*	ND*	ND*
HGF/native lung CS	ND*	ND*	ND*
HGF/heparin	1.00×10^{4} (±342)	9.23×10^{-7} ($\pm1.40\times10^{-7}$)	9.23×10^{-11}
TGFβ1/native lung HS	6.41×10^{5} ($\pm2.54\times10^{4}$)	0.118 ($\pm2.19\times10^{-4}$)	1.84×10^{-7}
TGFβ1/native lung CS	839 (±89.1)	0.050 ($\pm7.75\times10^{-4}$)	5.99×10^{-5}
TGFβ1/heparin	1.01×10^{5} (±342)	9.23×10^{-3} ($\pm4.00\times10^{-5}$)	5.89×10^{-8}

*ND: not detected.

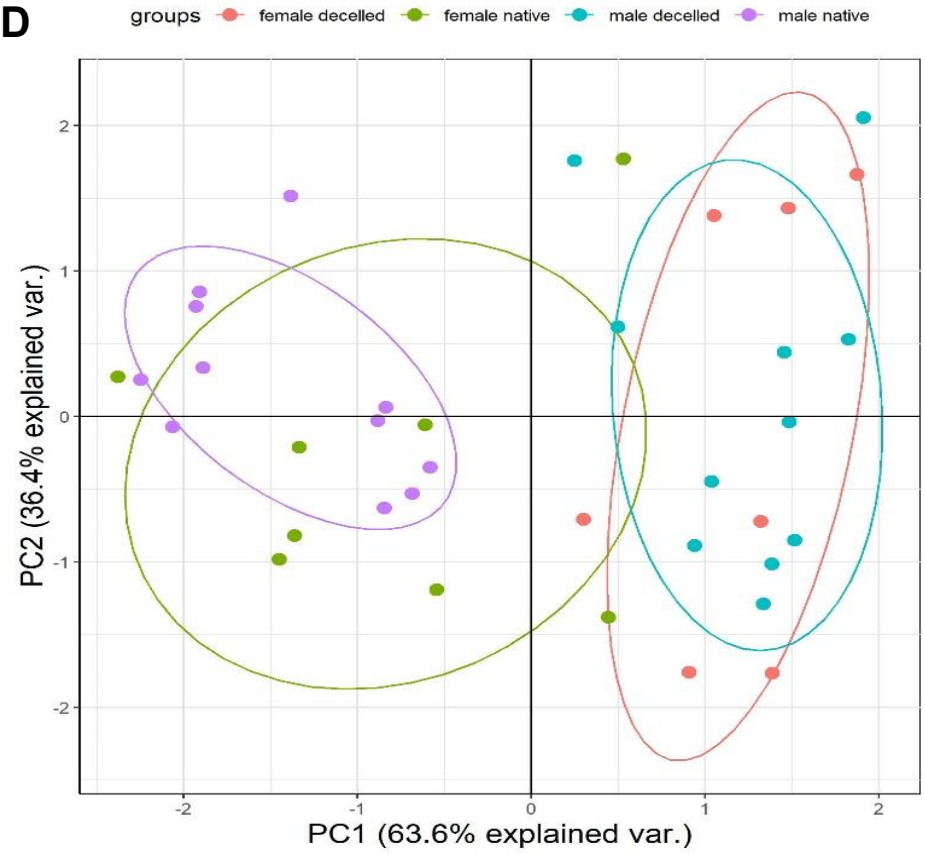

A
groups
COPD
ex
non
PC2 (36.4% explained var.)
PC1 (63.6% explained var.)

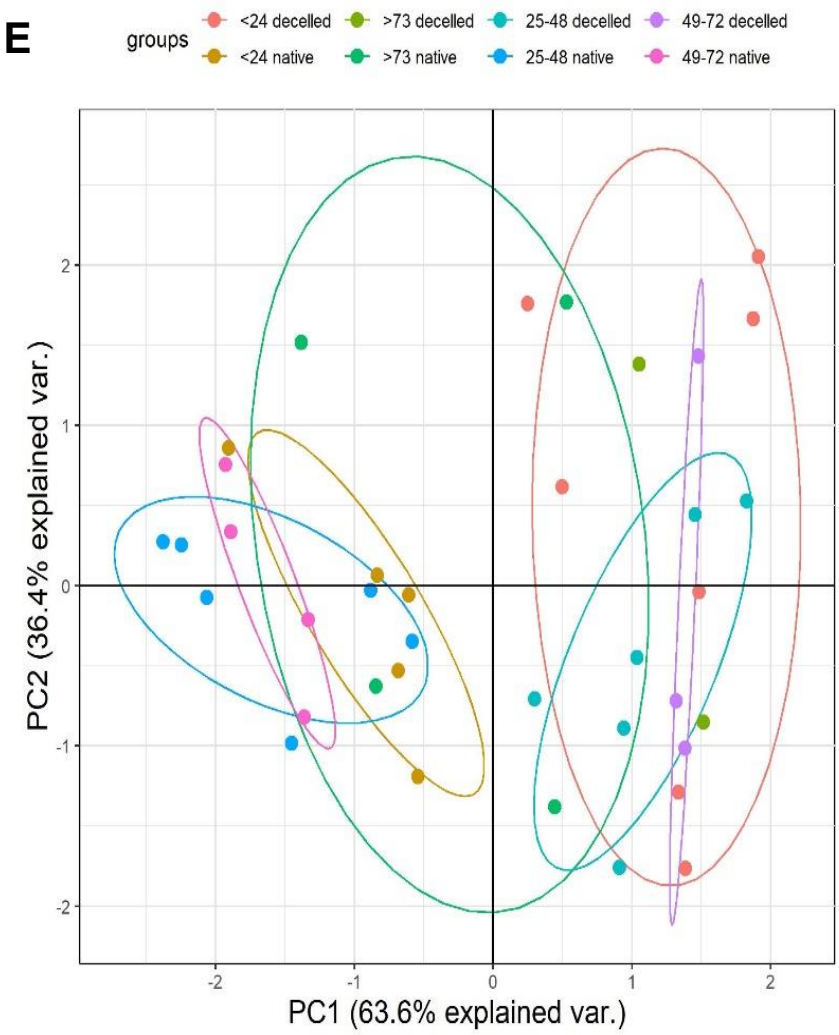

B
groups
COPD decelled
COPD native
non-COPD decelled
non-COPD native
PC2 (36.4% explained var.)
PC1 (63.6% explained var.)

C

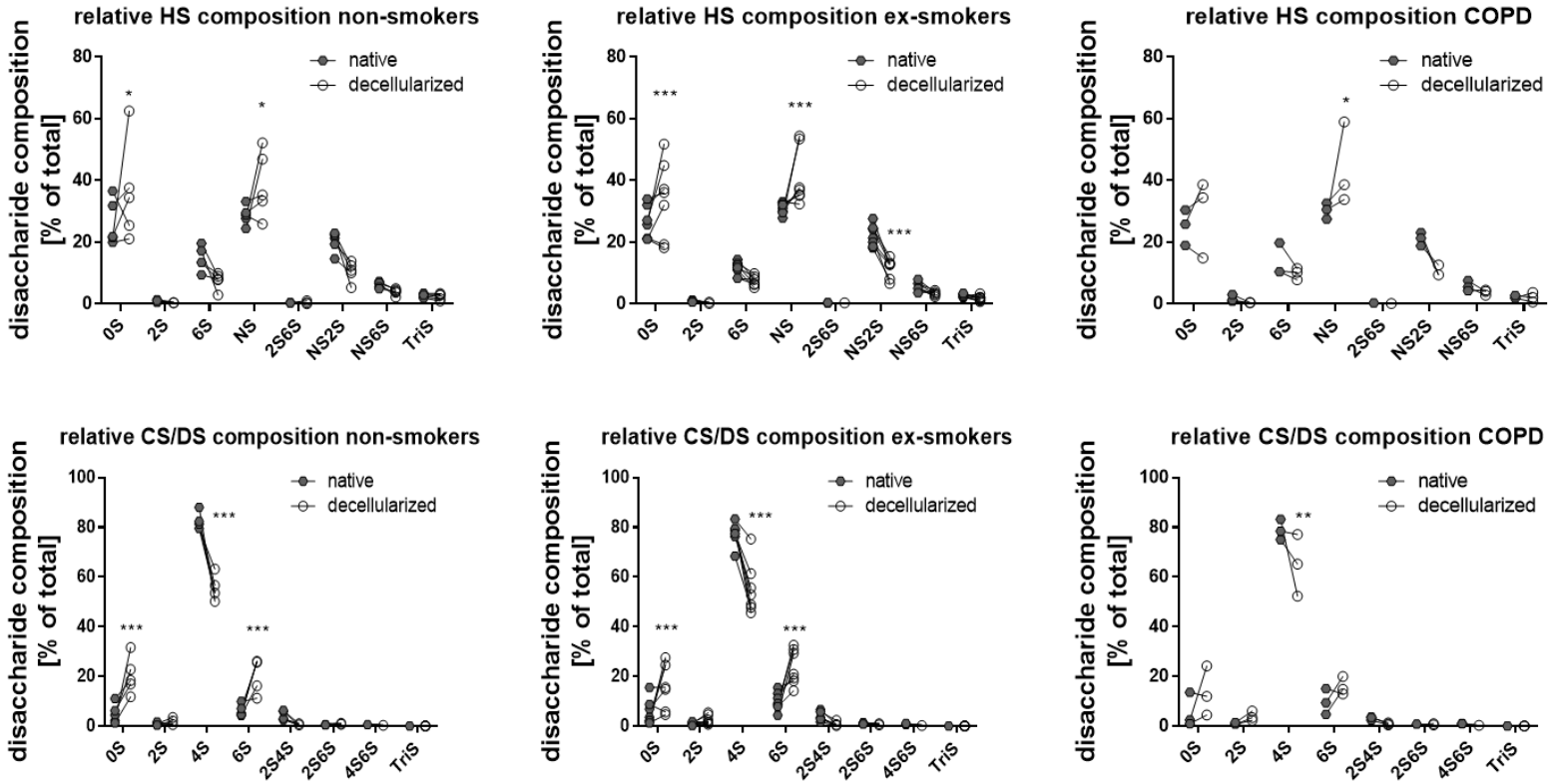

groups
<60 decelled
<60 native
>80 decelled
>80 native
60-69 decelled
60-69 native
70-79 decelled
70-79 native
PC2 (36.4% explained var.)
PC1 (63.6% explained var.)

D


groups
female decelled
female native
male decelled
male native
PC2 (36.4% explained var.)
PC1 (63.6% explained var.)

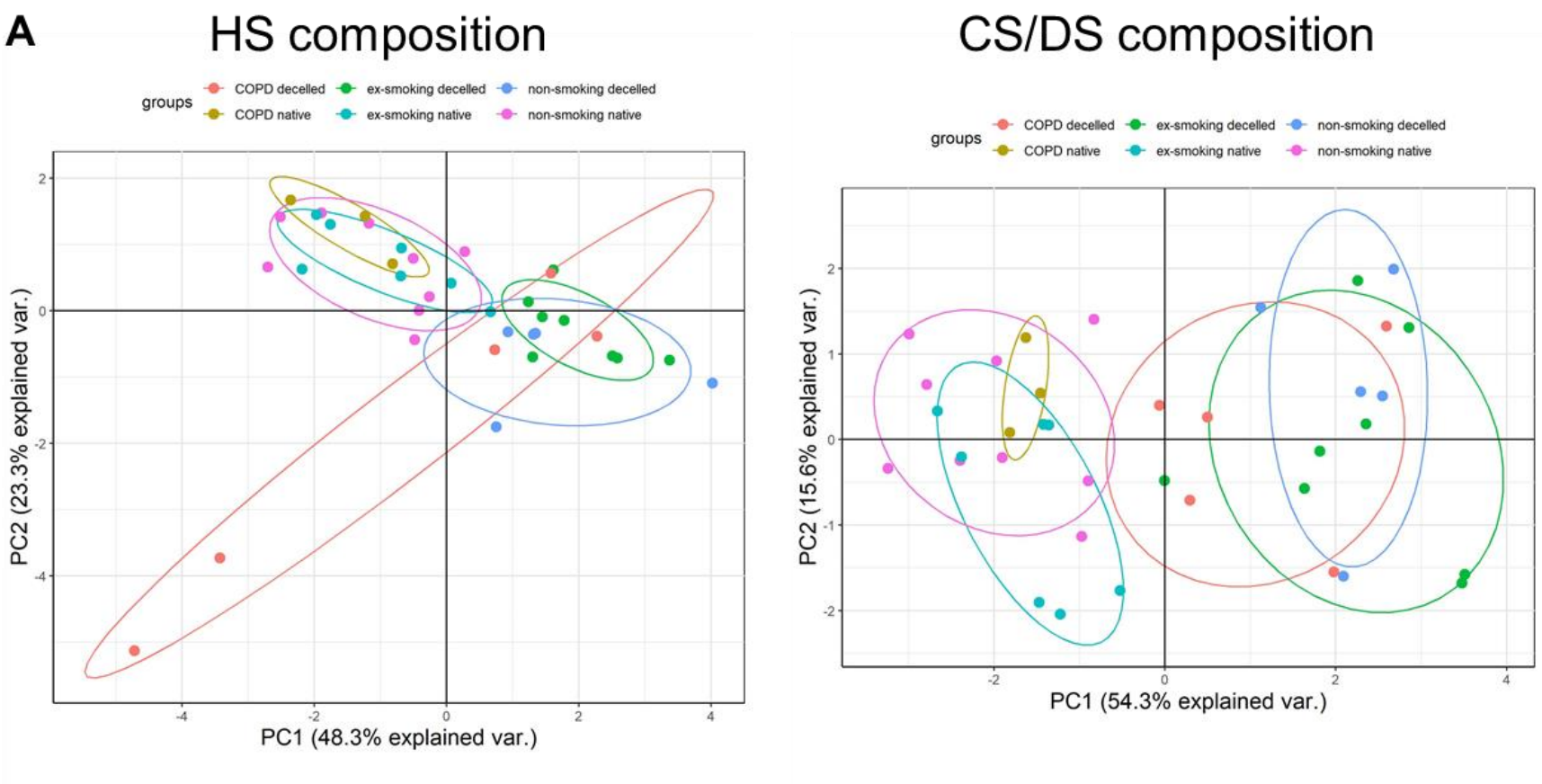


Appendix Figure 2: Principal component analysis of GAG content in native and decellularized (decelled) tissue based on smoking status (A), disease state (B), age (C), gender (D), and time to autopsy (E) before tissue curation and subsequent decelluarization. Results from 5 non-smokers (non), 7 ex-smokers (ex), and 3 COPD (COPD) lungs are depicted.



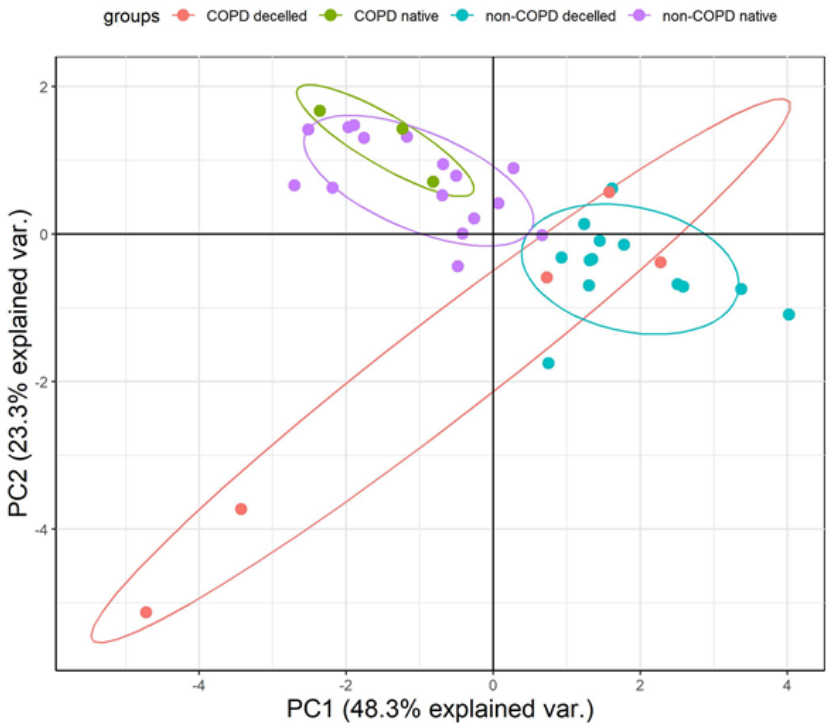
Appendix Figure 3: Quantification of heparan sulfate (HS) and chondroitin sulfate/dermatan sulfate (CS/DS) composition in native and decellularized non-COPD and COPD tissue. Relative amount of HS and CS/DS side chains. Results from 5 non-smokers, 7 ex-smokers, and 3 COPD lungs are depicted. Abbreviations HS graphs: UA-GlcNAc, 0-sulfated (S) = nonsulfated disaccharide, where UA is deoxy-α-L-threo-hex-4-enopyranosyluronic acid and GlcNAc is N-acetylglucosamine; UA2S-GlcNAc, 2S = 2-sulfated disaccharide; UA-GlcNS, NS = N-sulfated disaccharide; UA-GlcNAc6S, 6S = 6-sulfated disaccharide; UA2S-GlcNAc6S, 2S6S = 2, 6-disulfated disaccharide; UA-GlcNS6S, NS6S = N-sulfated, 6-sulfated disaccharide; UA2S-GlcNS, NS2S = N-sulfated 2-sulfated disaccharide; UA2S-GlcNS6S, TriS = trisulfated disaccharide. Abbreviations CS/DS graphs: UA-GalNAc, 0-sulfated (S) = nonsulfated disaccharide, where UA is deoxy-α-L-threo-hex-4-enopyranosyluronic acid and GalNAc is N-acetylgalactosamine; UA2S-GalNAc, 2S = 2-sulfated disaccharide; UA-GalNAc4S, 4S = 4-sulfated disaccharide; UA-GalNAc6S, 6S = 6-sulfated disaccharide; UA-GalNAc4S,6S, 4S6S = 4, 6-disulfated disaccharide; UA2S-GalNAc4S, 2S4S = 2, 4-disulfated disaccharide; UA2S-GalNAc6S, 2S6S = 2, 6-disulfated disaccharide; UA2S-GalNAc4S6S, TriS = trisulfated disaccharide.

A

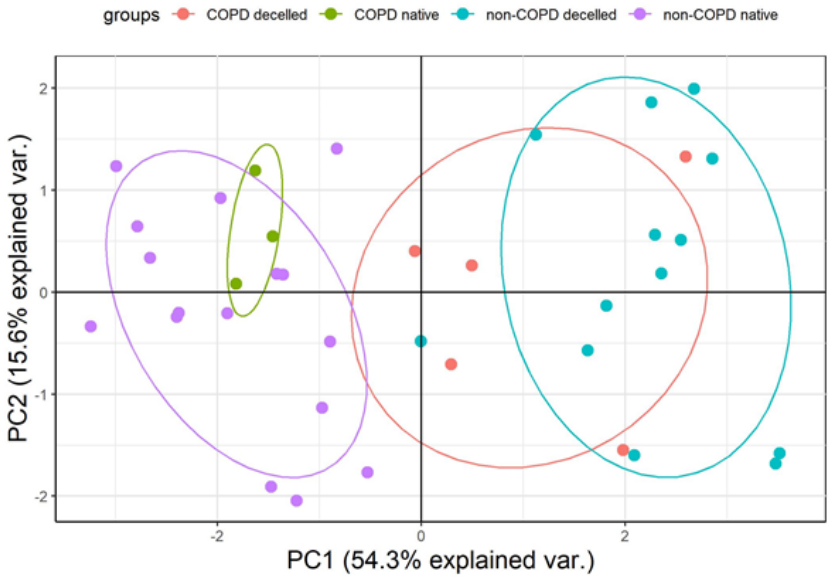

HS composition
CS/DS composition
groups
COPD decelled
COPD native
ex-smoking decelled
ex-smoking native
non-smoking decelled
non-smoking native
PC1 (48.3% explained var.)
PC2 (23.3% explained var.)
PC1 (54.3% explained var.)
PC2 (15.6% explained var.)

B

HS composition

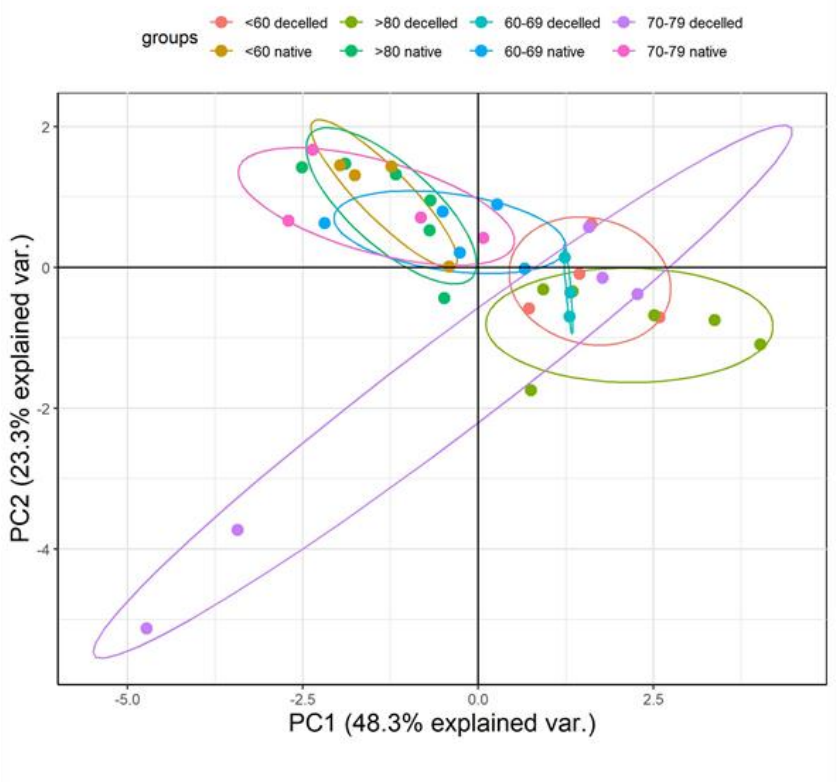


CS/DS composition

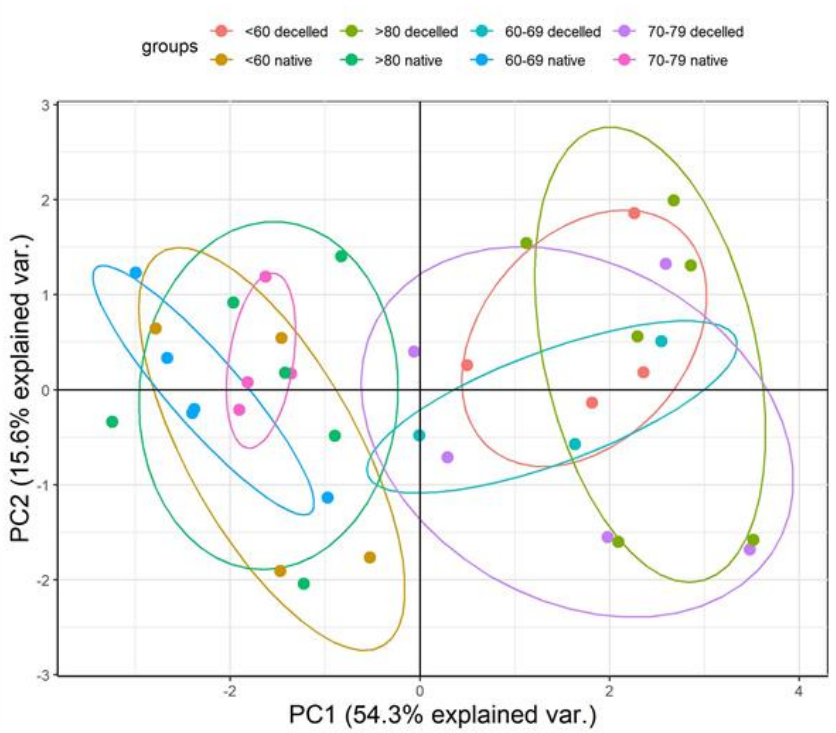


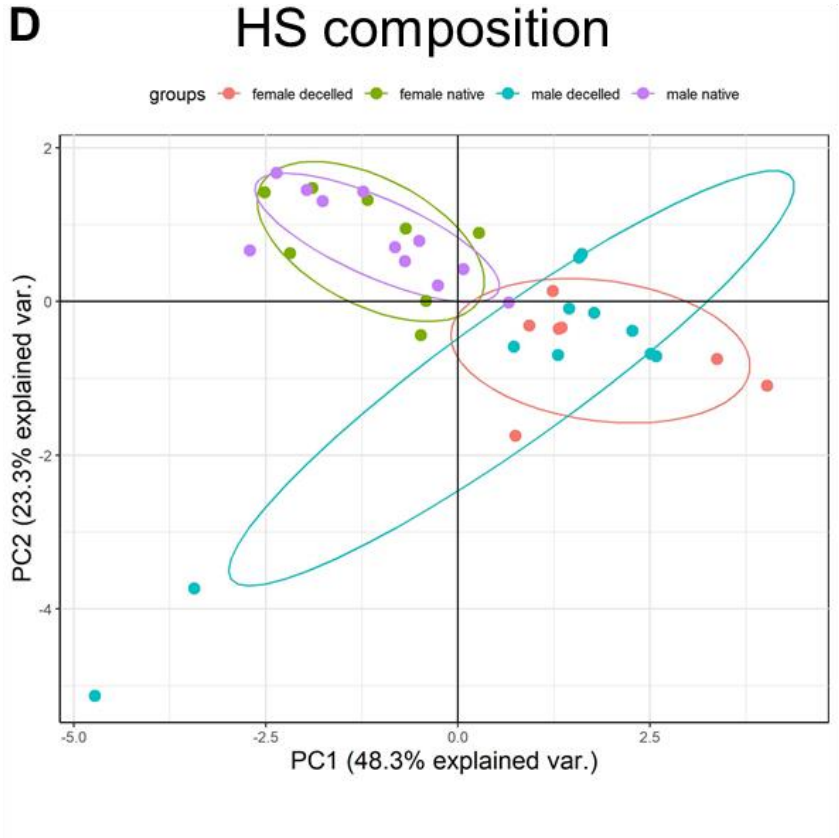
C

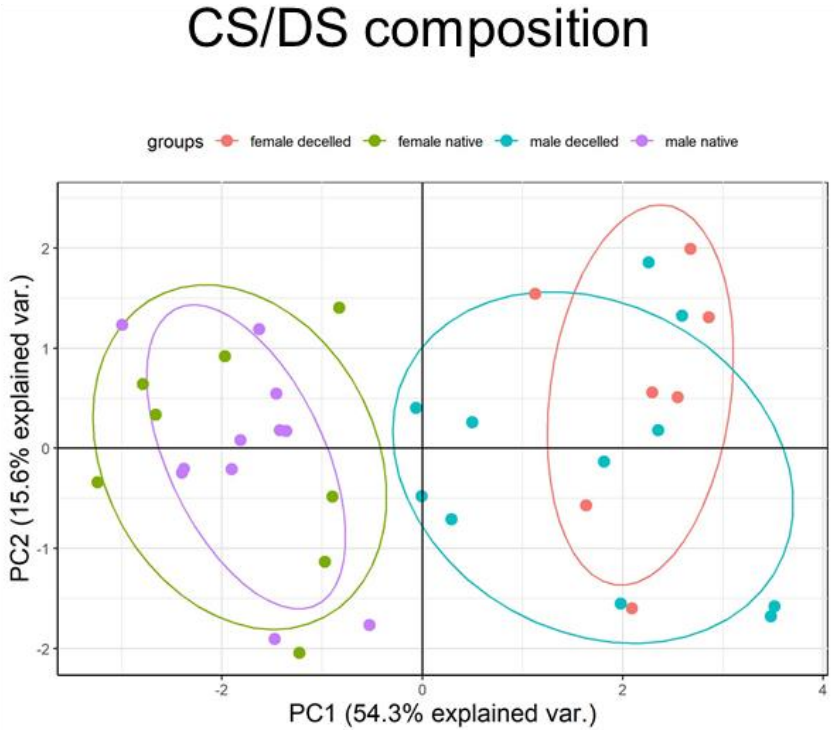
HS composition

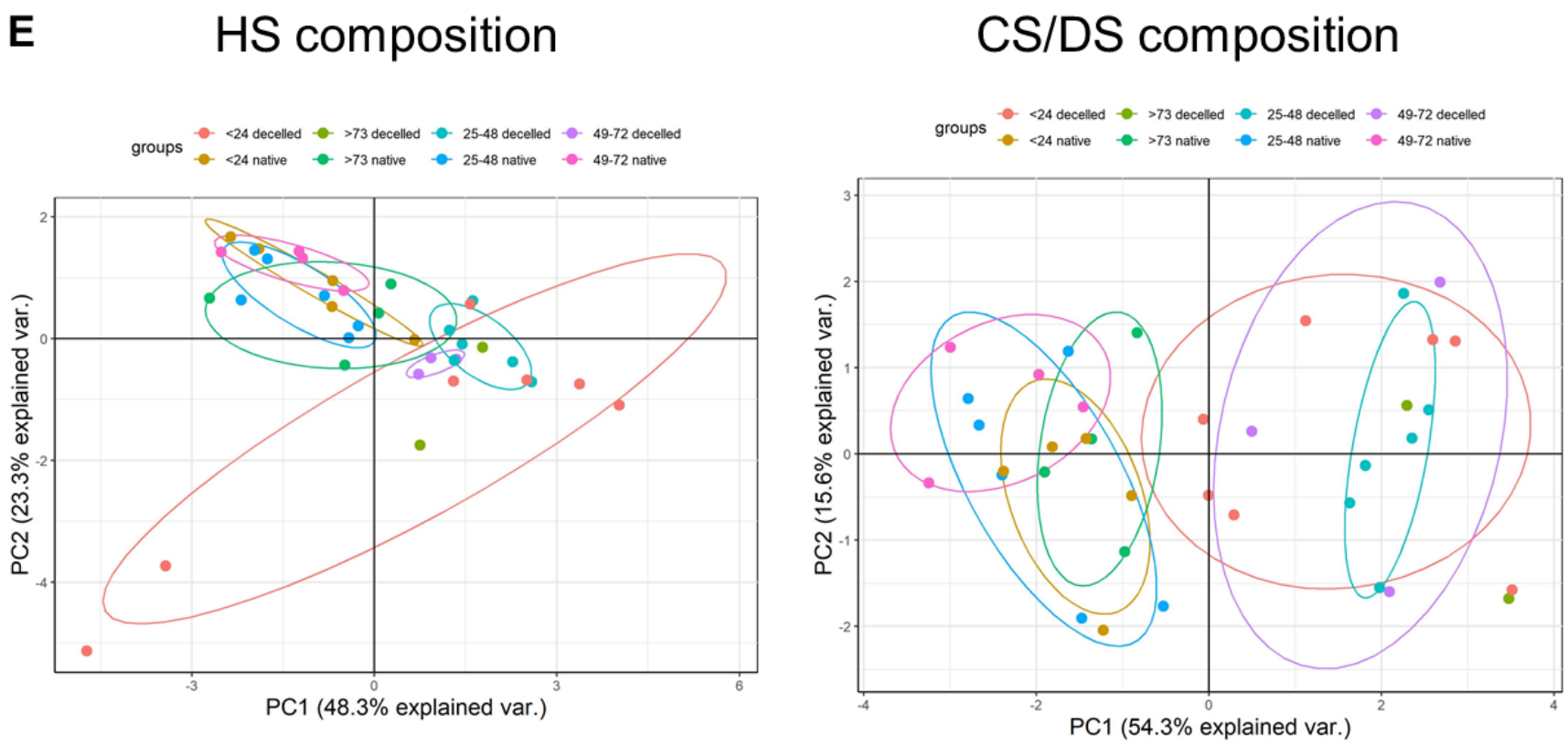


CS/DS composition

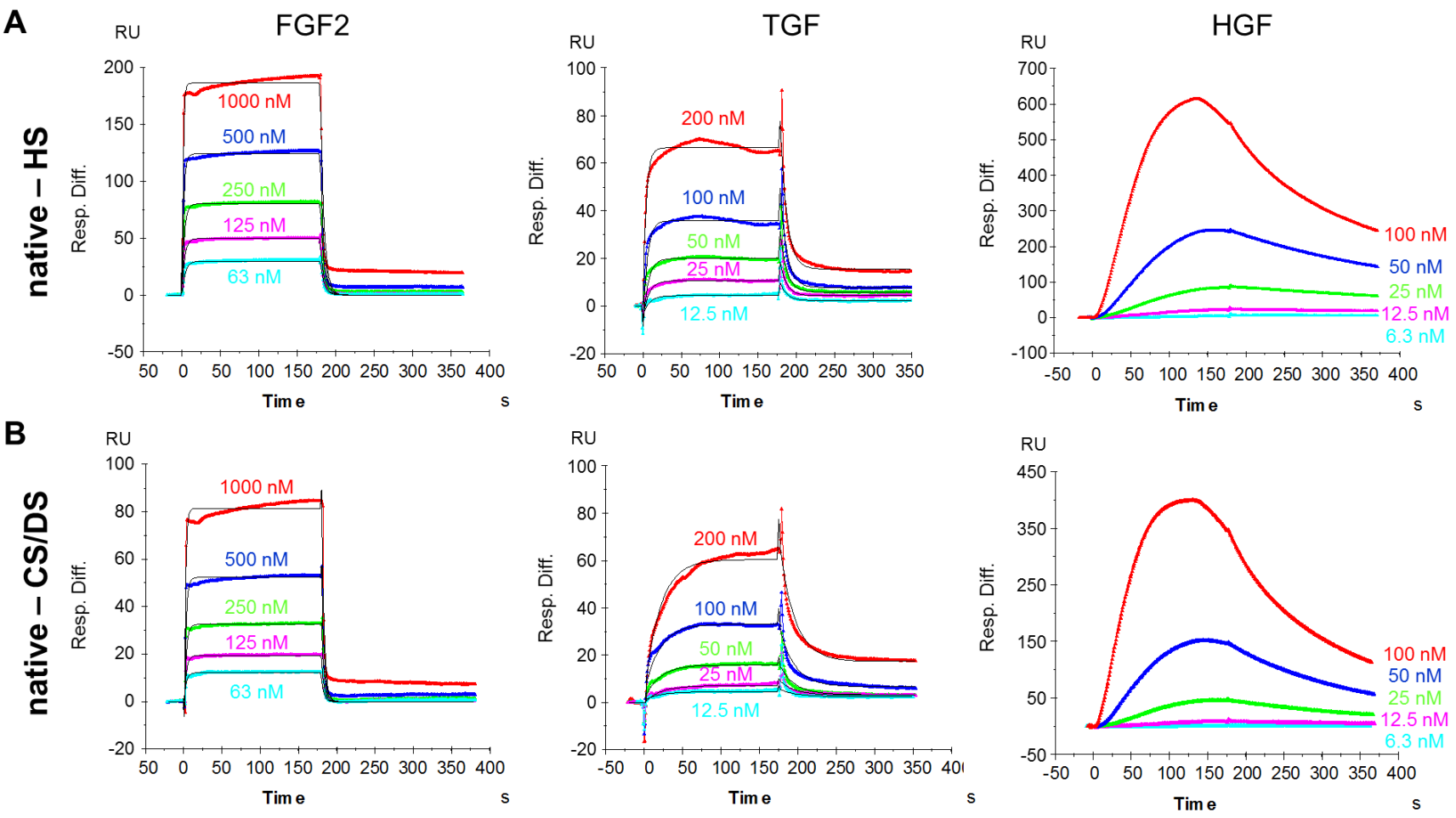


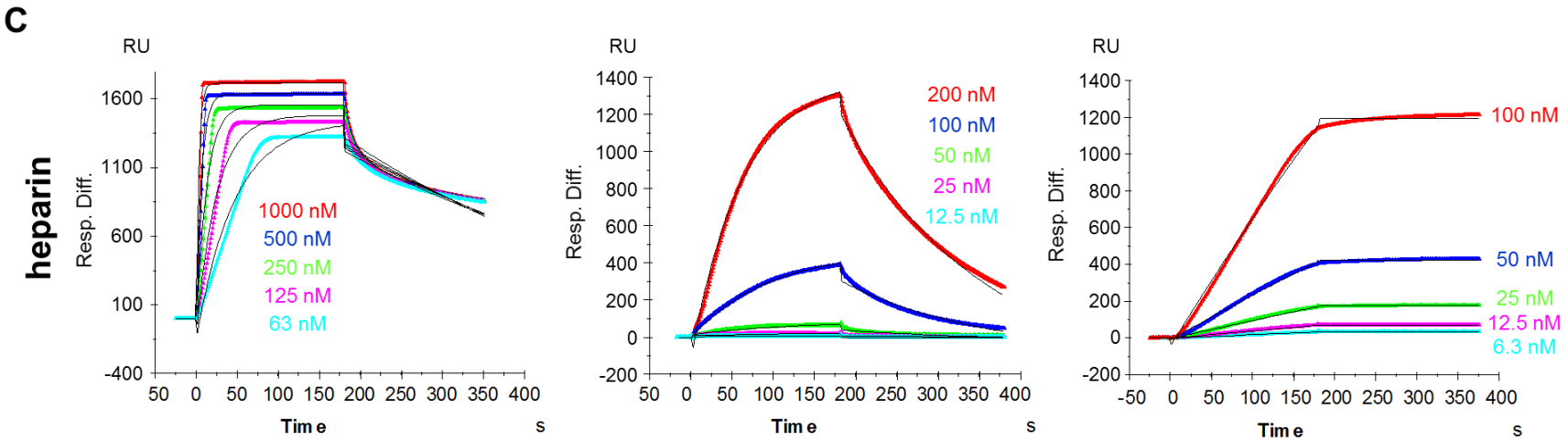

D
HS composition
groups
female decelled
female native
male decelled
male native
PC2 (23.3% explained var.)
PC1 (48.3% explained var.)


CS/DS composition
groups
female decelled
female native
male decelled
male native
PC2 (15.6% explained var.)
PC1 (54.3% explained var.)

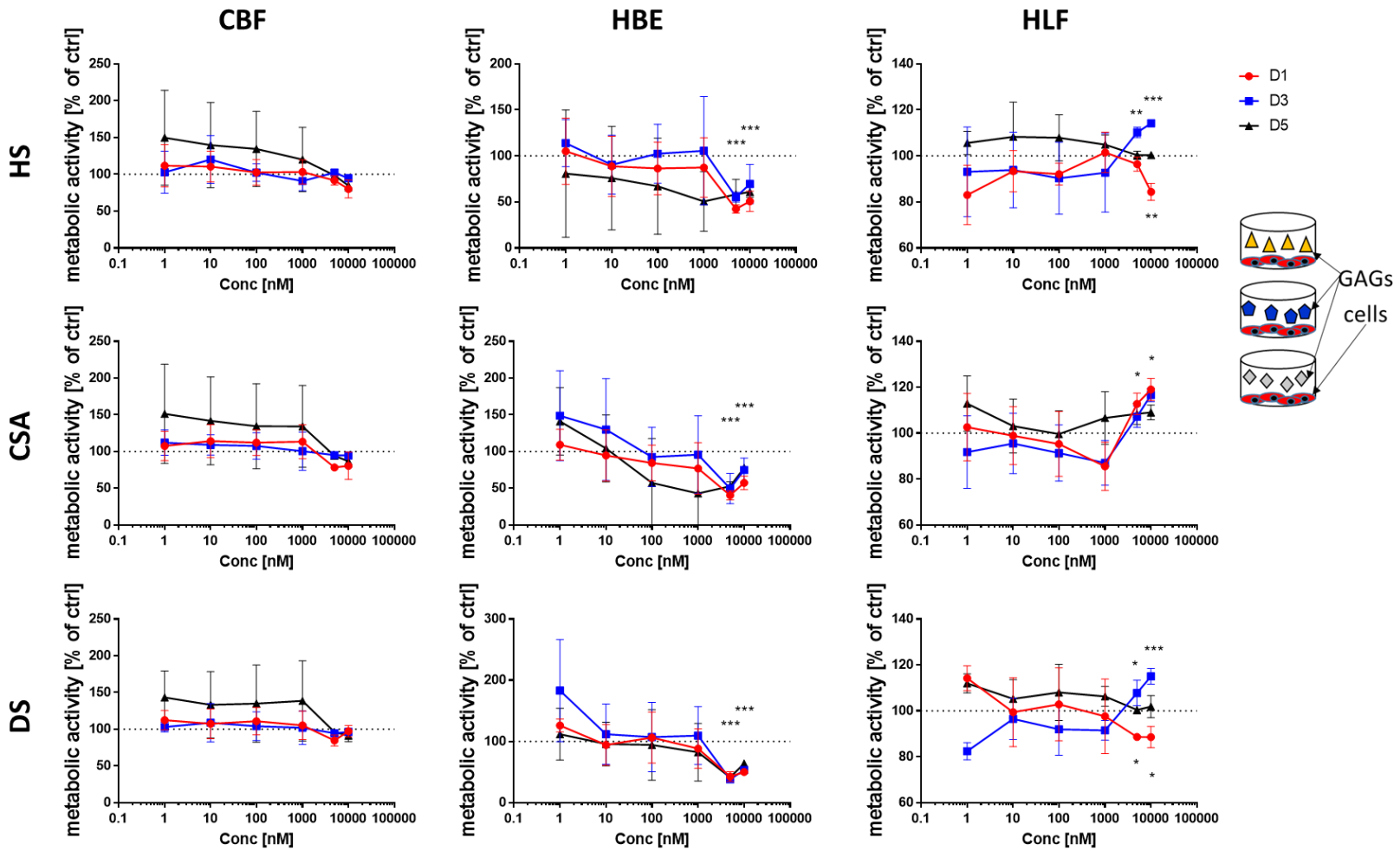


Appendix Figure 4: Principal component analysis of heparan sulfate (HS, left) and chondroitin sulfate/dermatan sulfate (CS/DS, right) composition in native and decellularized (decelled) tissue based on smoking status (A), disease state (B), age (C), gender (D), and time to autopsy (E) before tissue curation and subsequent decelluarization. Results from 5 non-smokers (non), 7 ex-smokers (ex), and 3 COPD (COPD) lungs are depicted.

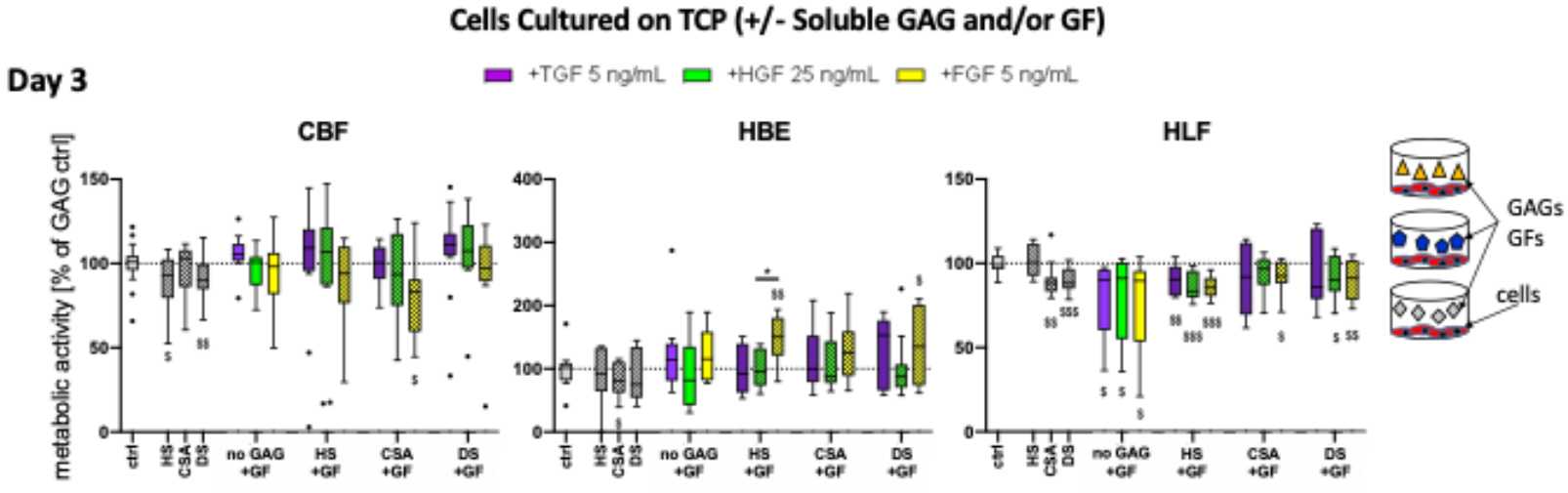

A
native – HS
FGF2
TGF
HGF
RU
Resp. Diff.
Time
s
1000 nM
500 nM
250 nM
125 nM
63 nM
200 nM
100 nM
50 nM
25 nM
12.5 nM
6.3 nM
B
native – CS/DS



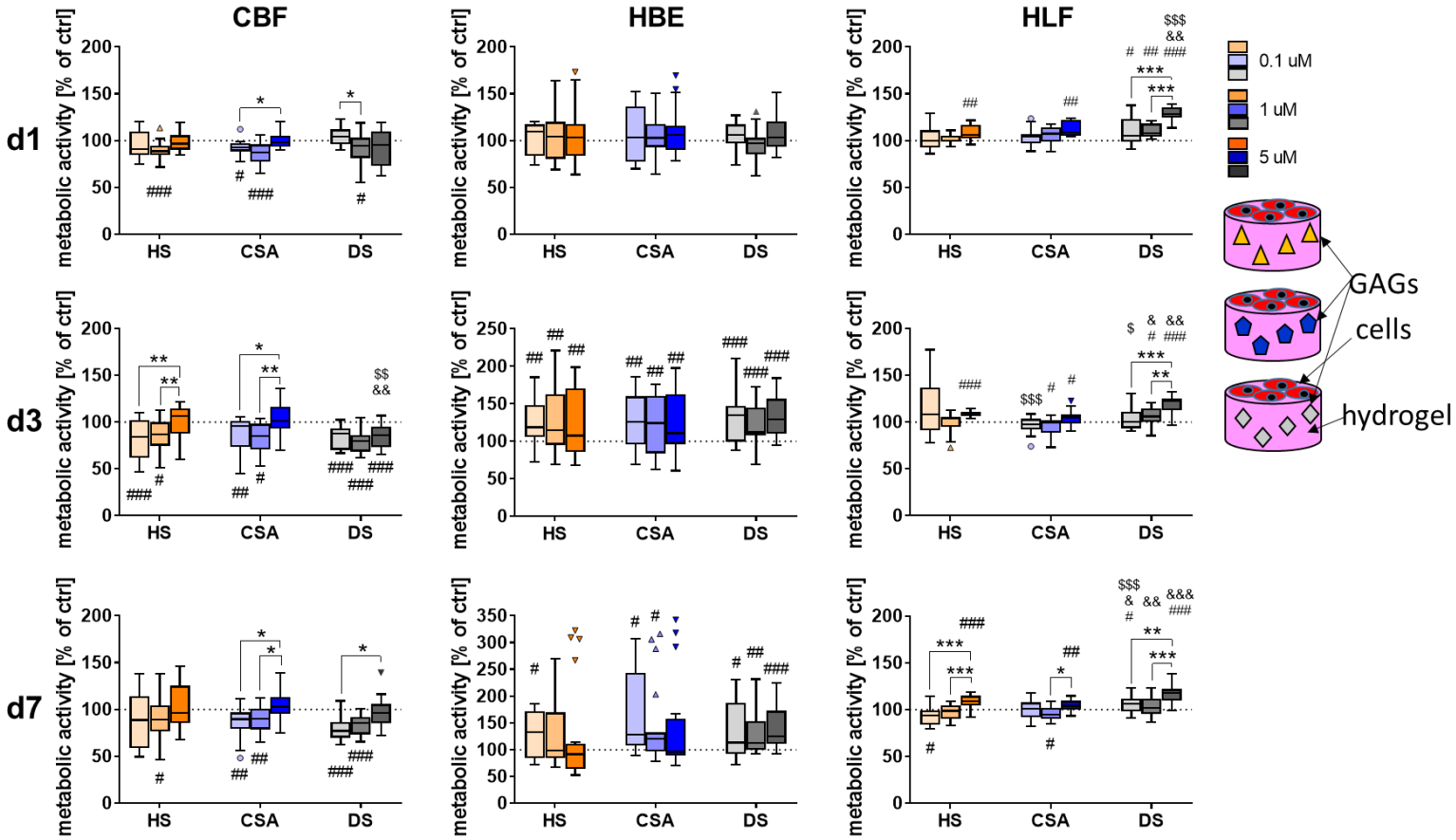
Appendix Figure 5: Binding of key matrix-associated growth factors (fibroblast growth factor (FGF2), hepatocyte growth factor (HGF), and transforming growth factor beta (TGFβ1)) to native lung heparan sulfate (HS, A), chondroitin sulfate/dermatan sulfate (CS/DS, B), and heparin (C, ctrl). Surface plasmon resonance analysis of the binding of FGF2, HGF, and TGFβ1 at different concentrations to HS and CS/DS isolated from 3 native lung tissues. Representative graphs are shown.



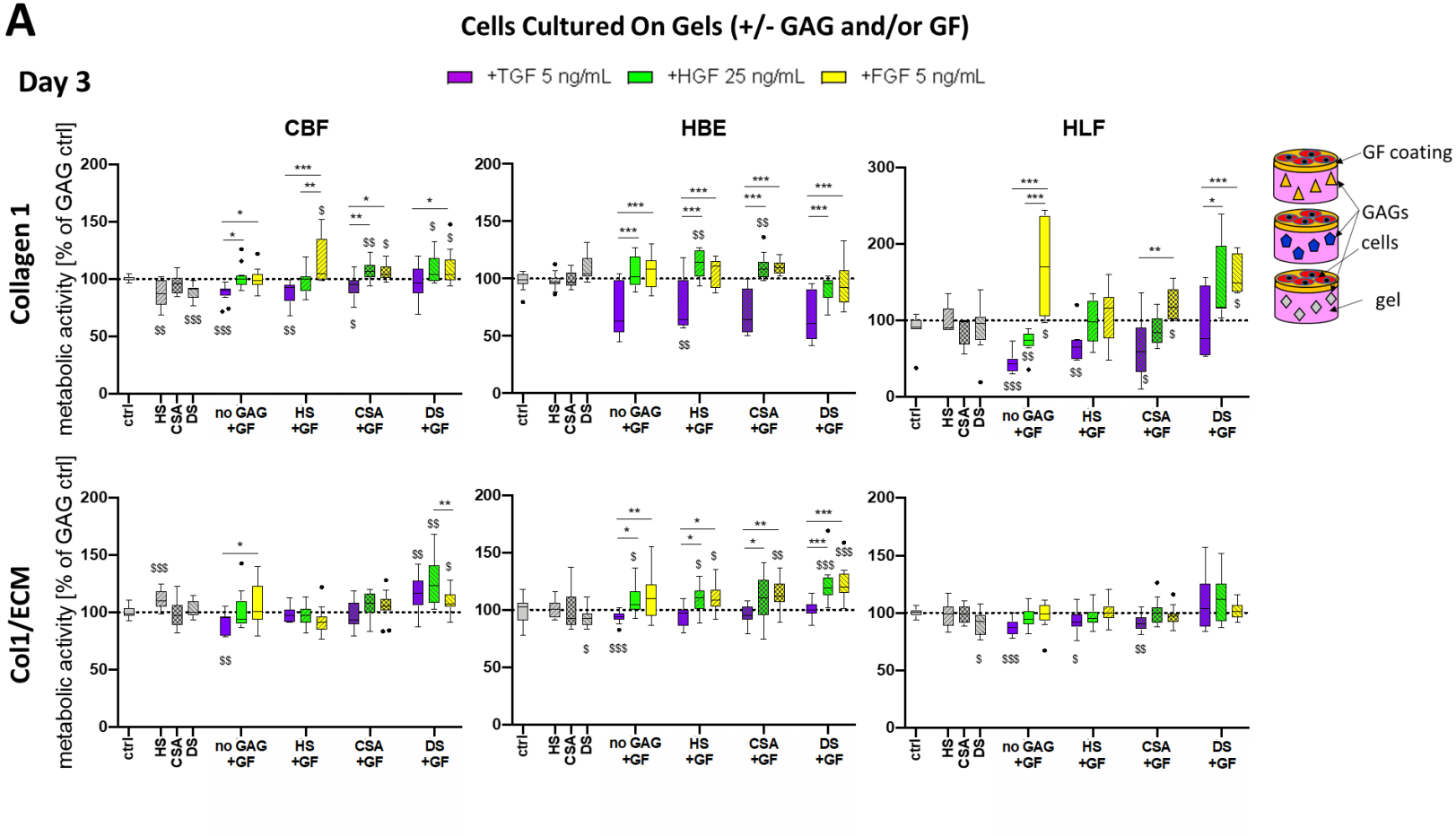
Appendix Figure 6: Metabolic activity of primary human pulmonary vascular endothelial cells (CBF), human bronchial epithelial cells (HBE), and human lung fibroblasts (HLF) incubated with soluble glycosaminoglycans (GAGs) in concentrations between 0.001 – 10 µM after 1 (D1), 3 (D3), and 5 (D5) days of incubation. Data from 3 different experiments with n=4 replicates each. ***=p<0.001, **=p<0.01, *=p<0.05. Data is displayed relative to the metabolic activity of cells on tissue culture plastic without the addition of GAGs (dotted line), HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

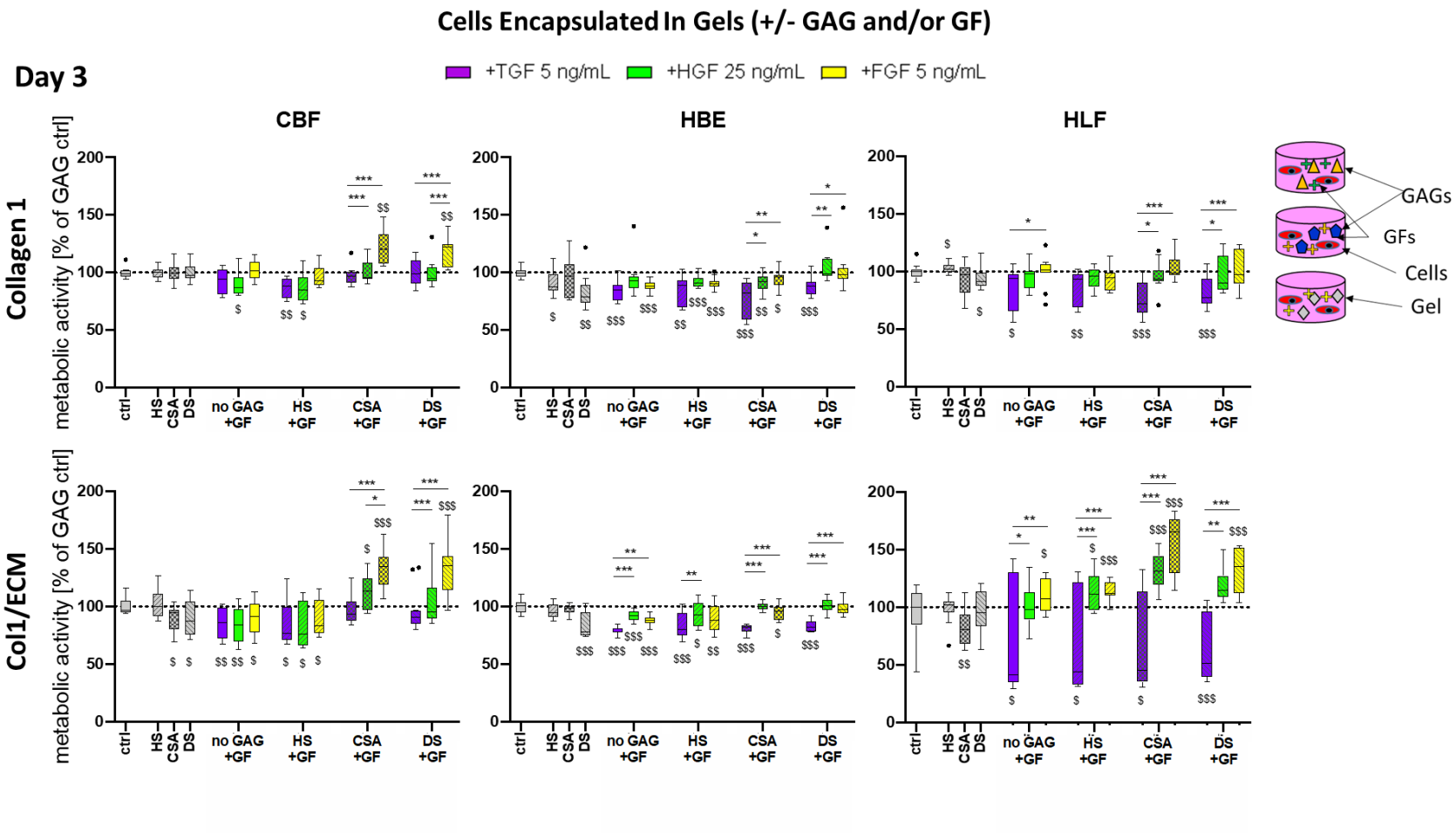


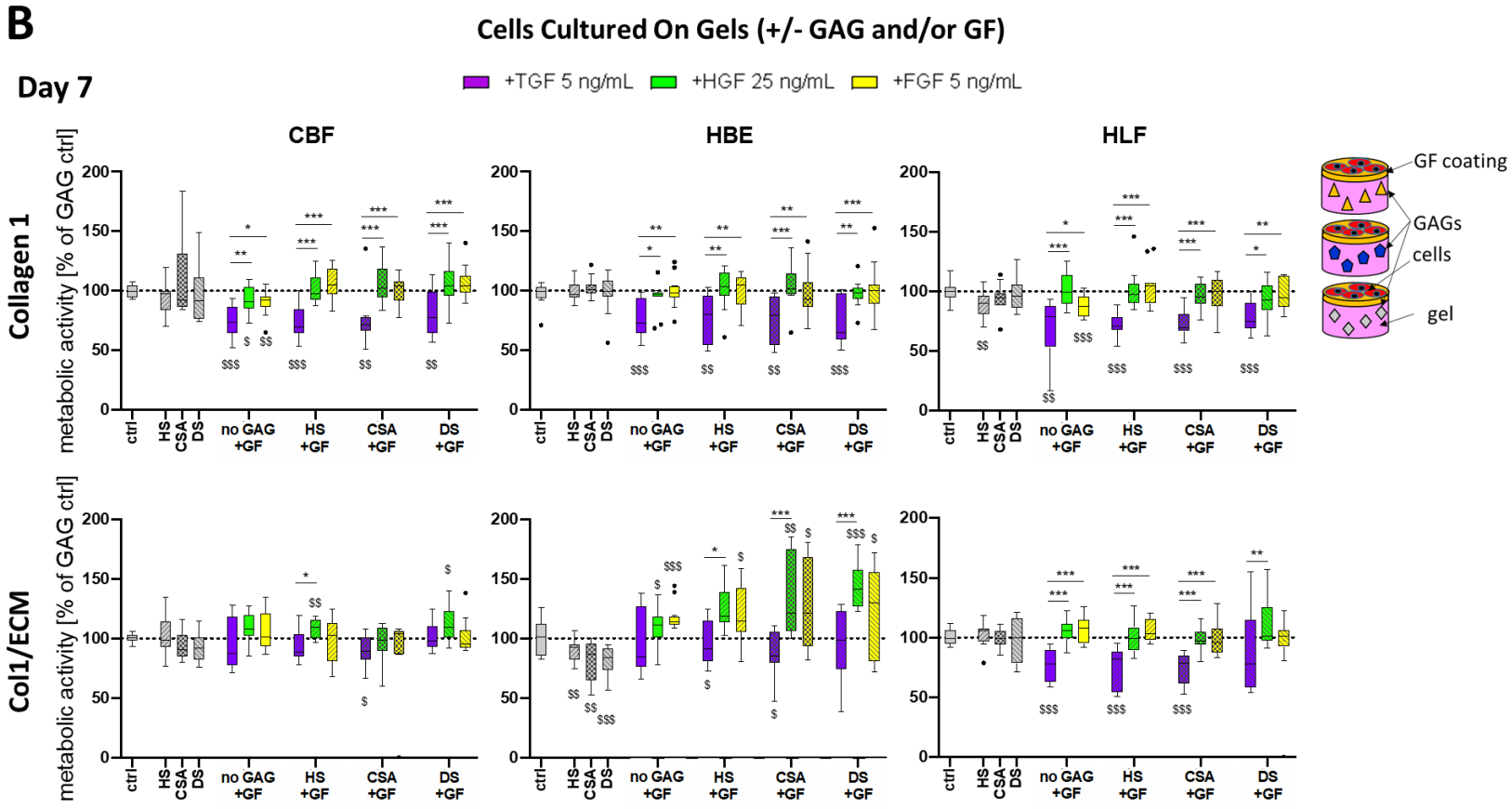
Appendix Figure 7: Soluble glycosaminoglycans (GAGs) in combination with matrix-associated growth factors differentially influences metabolic activity of human pulmonary vascular endothelial cells (CBF), human bronchial epithelial cells (HBE), and human lung fibroblasts (HLF). Metabolic activity of CBF, HBE, and HLF cells grown for 3 days on tissue culture plastic (TCP) with addition of GAGs (1 µM) in combination with different matrix-associated growth factors (GF) (transforming growth factor beta (TGFβ1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF2)) into the cultivation medium. The data from the addition of GAGs only and growth factors only (+HS, +CSA, +DS, +TGF, +HGF, +FGF) was normalized to the control (= medium without GAG or growth factor addition). Metabolic activity of the combination treatments (GAG+GF) was normalized to the respective GAG without addition of GF. $: significant to control (dotted line, one-sample t-test). *: significant within group (One-way ANOVA). $$$,***=p<0.001, $$,**=p<0.01, $,*=p<0.05. Combined data from 3 experiments with n=4 replicates each. HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

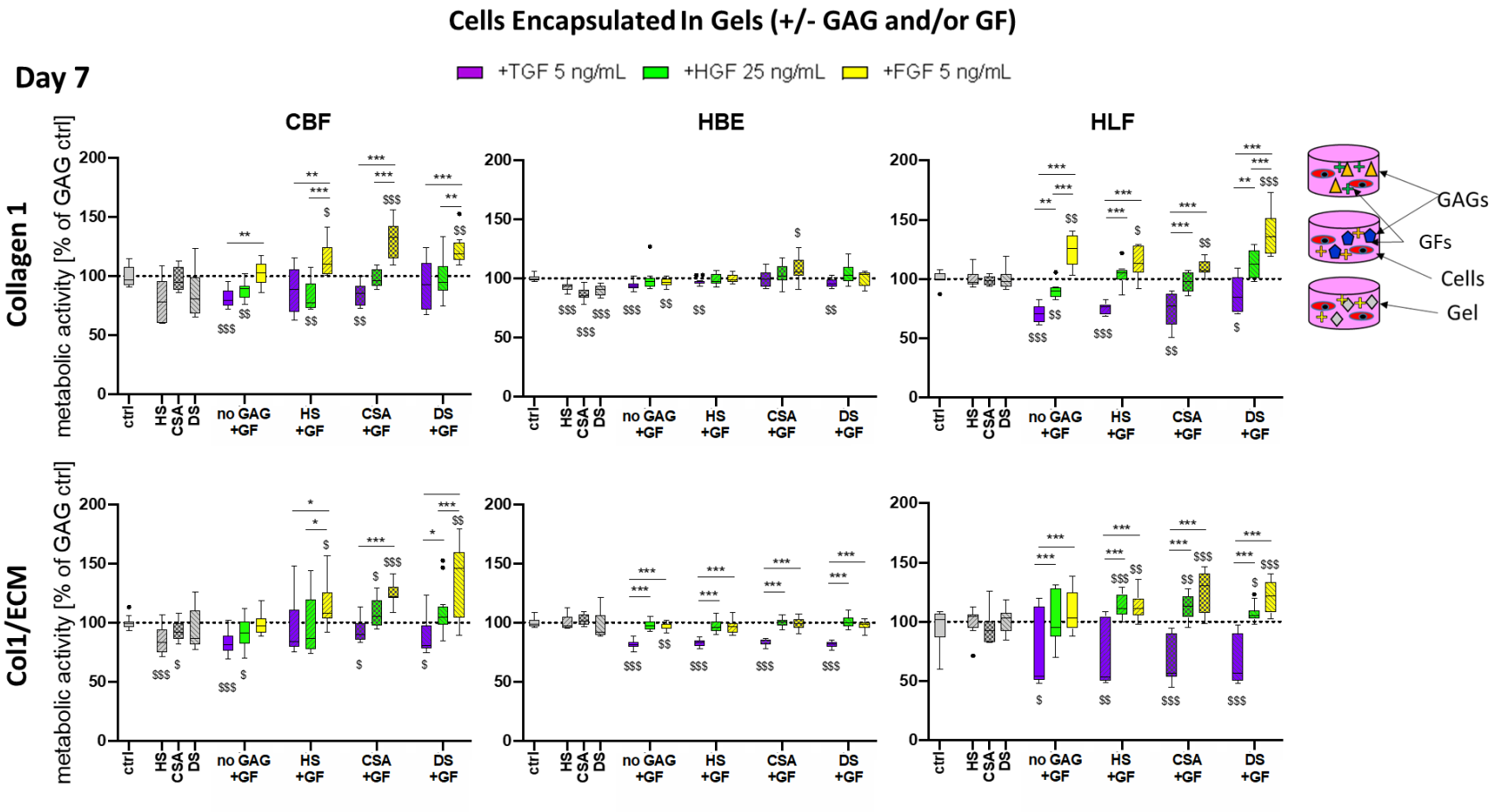


Appendix Figure 8: Addition of GAGs to type I collagen gels influences growth of human pulmonary vascular endothelial cells (CBF) and human lung fibroblasts (HLF) in a dose and time-dependent manner while human lung epithelial cells (HBE) are mostly unaffected when cultured on top of type I collagen gels. Growth of CBF, HBE, and HLF cells on day 1, 3, and 7 on type I collagen gels with addition of heparan sulfate (HS), chondroitin sulfate A (CSA), or dermatan sulfate (DS) in different concentrations. Two-way ANOVA simple multiple comparison with Tukey post-test, $ significant to HS of respective concentration, & significant to CSA of respective concentration, # significant to ctrl. ***= $p<0.001$, **= $p<0.01$, *=$p<0.05$. Combined data from 3-5 experiments per condition with n=4-6 replicates each. The dotted line represents pure gels without addition of GAGs.

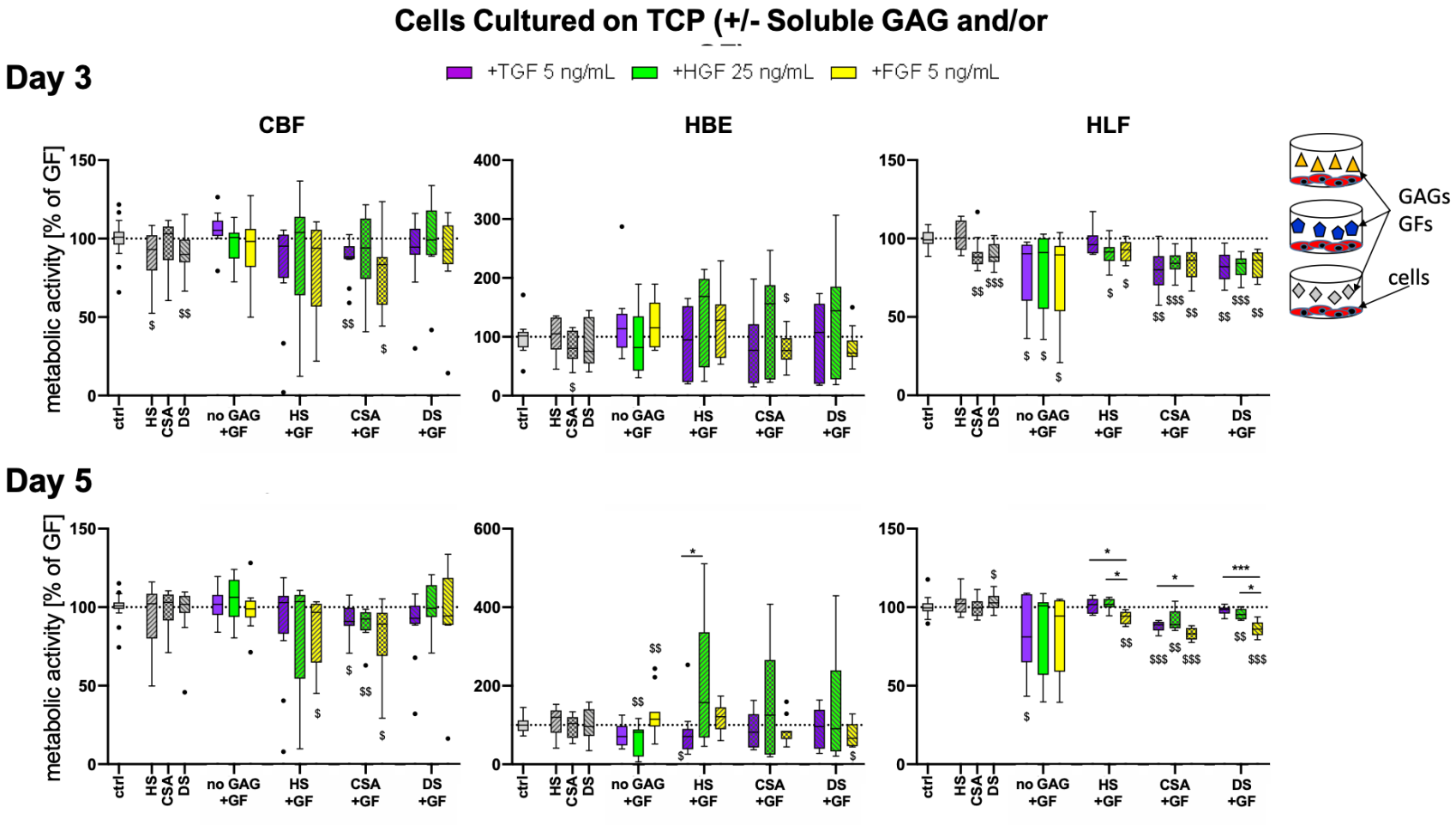

A
Cells Cultured On Gels (+/- GAG and/or GF)
Day 3
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GAG ctrl]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GF coating
GAGs
cells
gel


Cells Encapsulated In Gels (+/- GAG and/or GF)
Day 3
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GAG ctrl]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GAGs
GFs
Cells
Gel

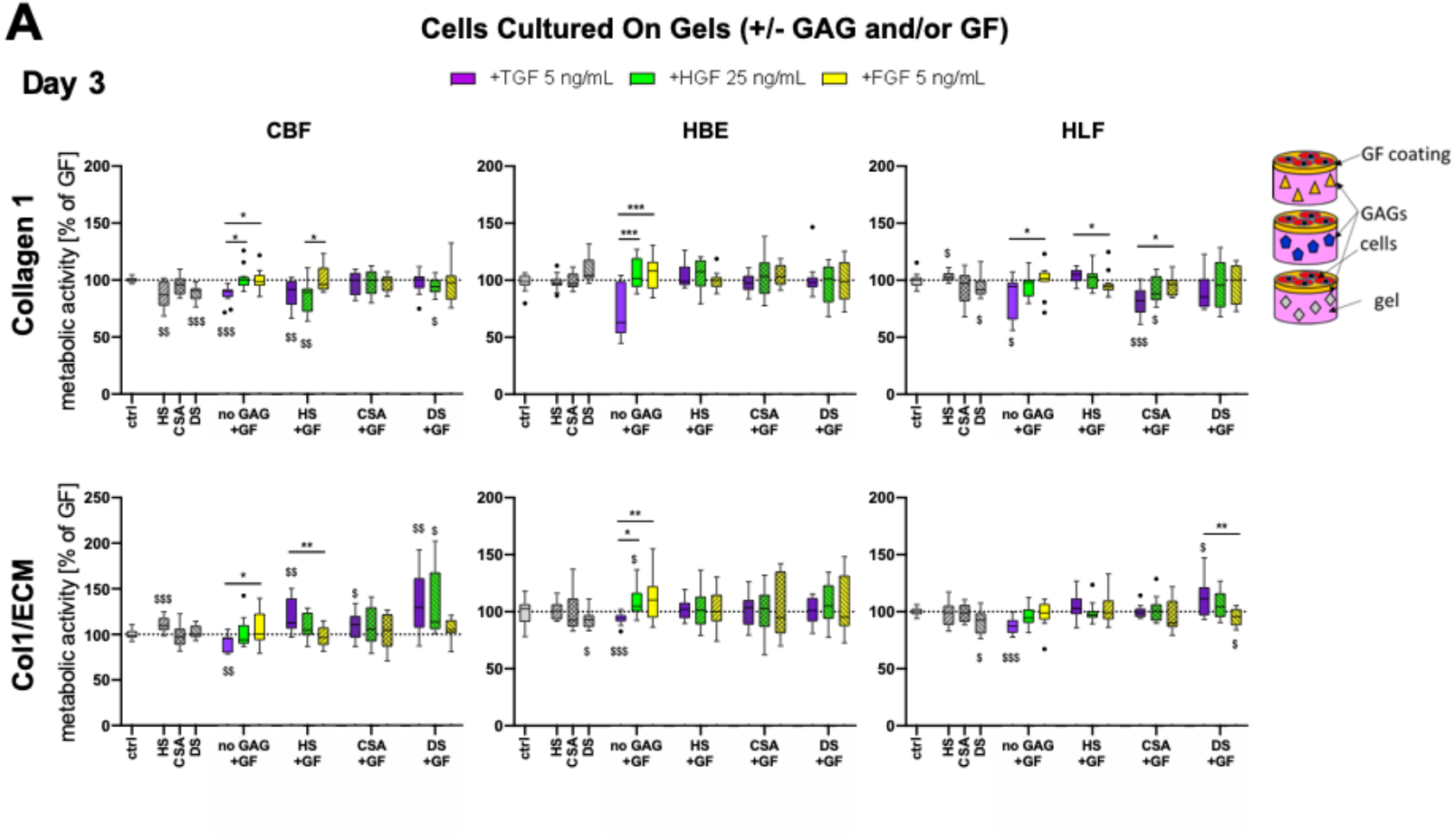

B
Cells Cultured On Gels (+/- GAG and/or GF)
Day 7
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GAG ctrl]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GF coating
GAGs
cells
gel

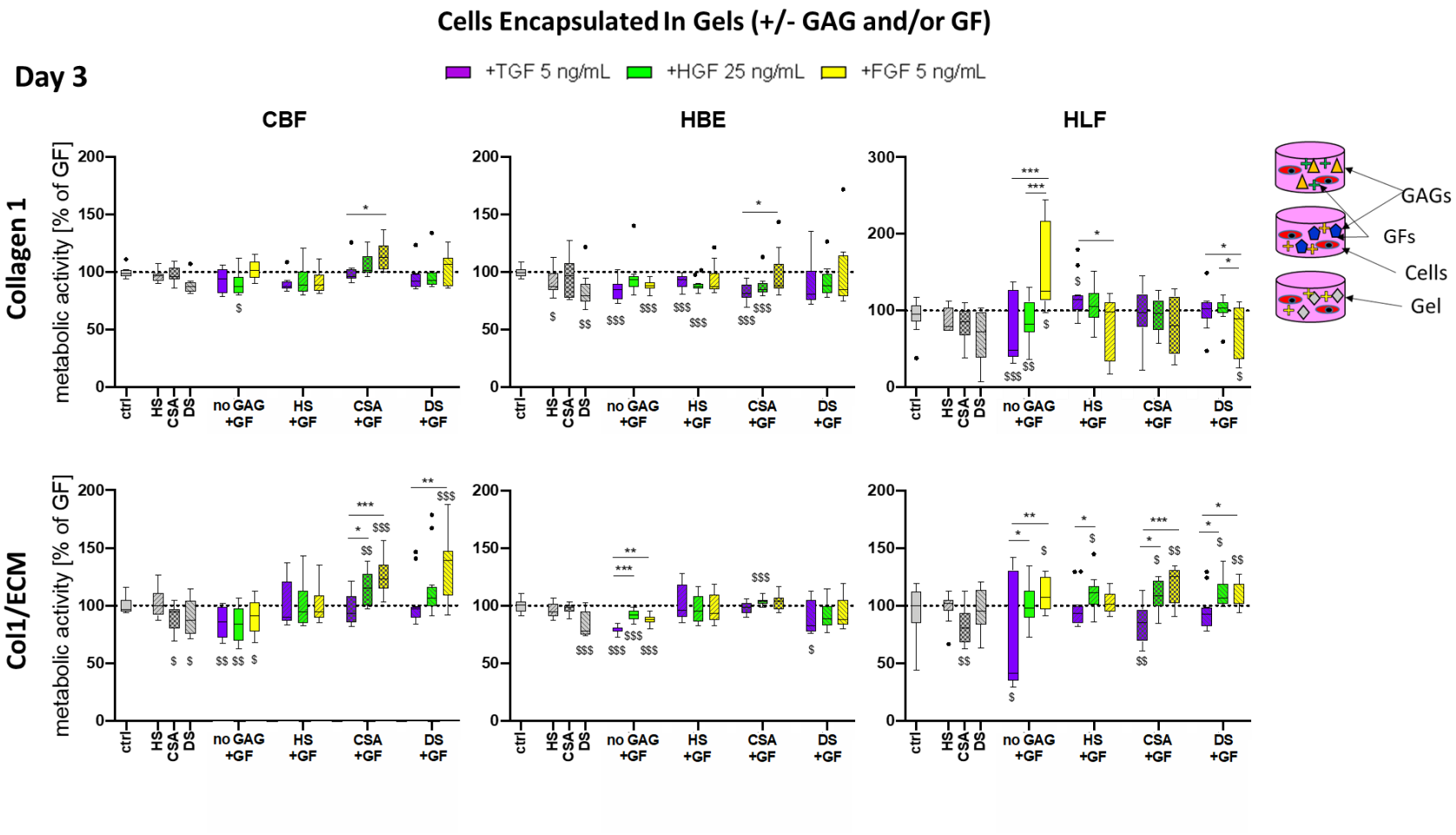


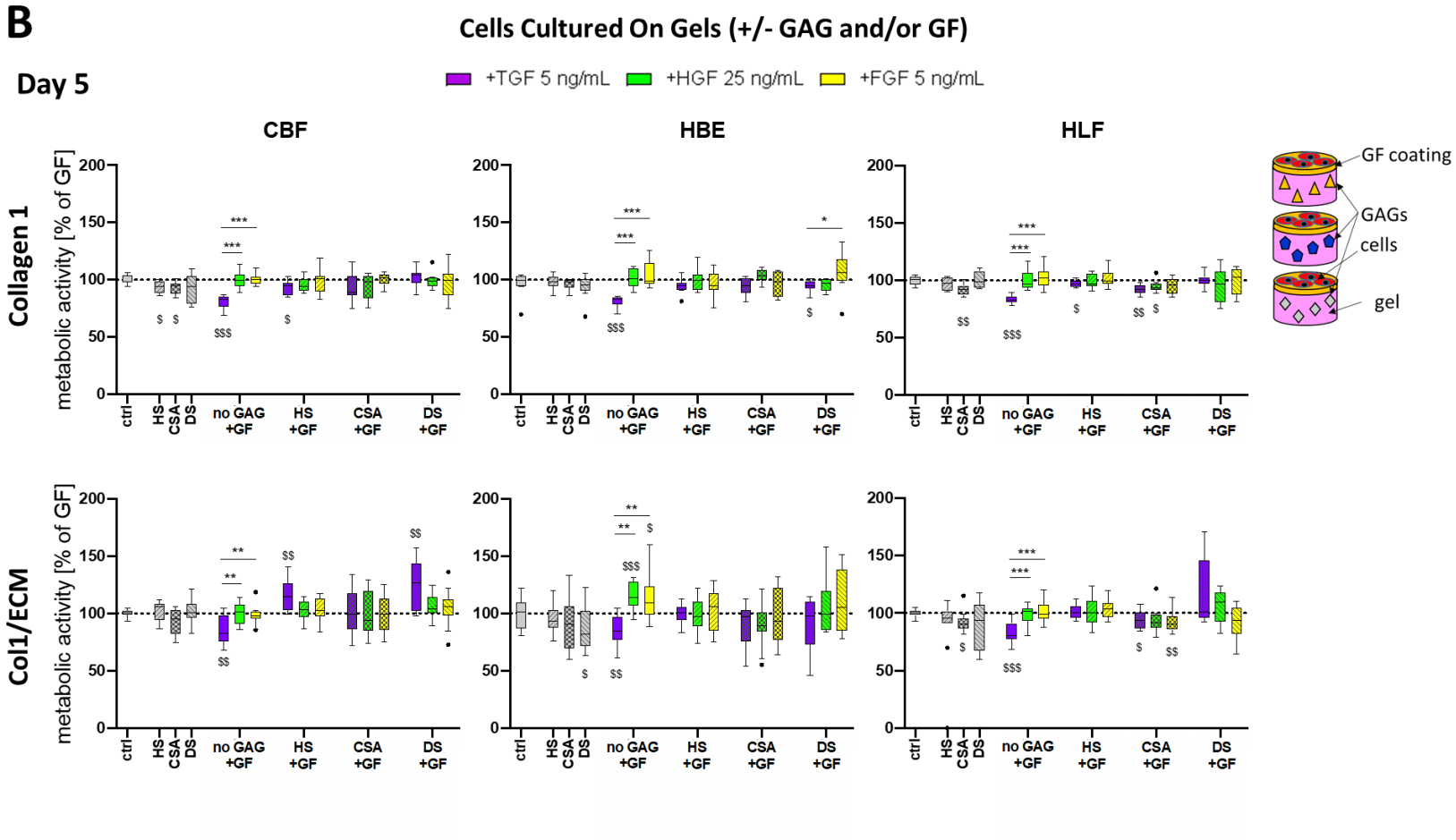
Appendix Figure 9: Glycosaminoglycans (GAGs) in combination with matrix-associated growth factors and method of cultivation differentially influences metabolic activity of human pulmonary vascular endothelial cells (CBF), human bronchial epithelial cells (HBE), and human lung fibroblasts (HLF). Metabolic activity of human pulmonary vascular endothelial cells (CBF), human lung epithelial cells (HBE), and human lung fibroblasts (HLF) on day 3 (A), and day 7 (B) on top of and in type I collagen and human lung ECM/type I collagen gels with addition of heparan sulfate (HS), chondroitin sulfate A (CSA), or dermatan sulfate (DS) at 5 µM after coating with growth factors (GF) (transforming growth factor beta (TGFβ1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF2)). The data from the addition of GAGs only and growth factors only (+HS, +CSA, +DS, +TGF, +HGF, +FGF) was normalized to the control (= medium without GAG or growth factor addition). Metabolic activity of the combination treatments (GAG+GF) was normalized to the respective GAG without addition of GF. $: significant to control (dotted line, one-sample t-test). *: significant within group (One-way ANOVA). $$$,***=p<0.001, $$,**=p<0.01, $,*=p<0.05. Combined data from gels from 3 experiments with n=4 replicates each. The dotted line represents pure gels without addition of GAGs or matrix-associated growth factors. HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

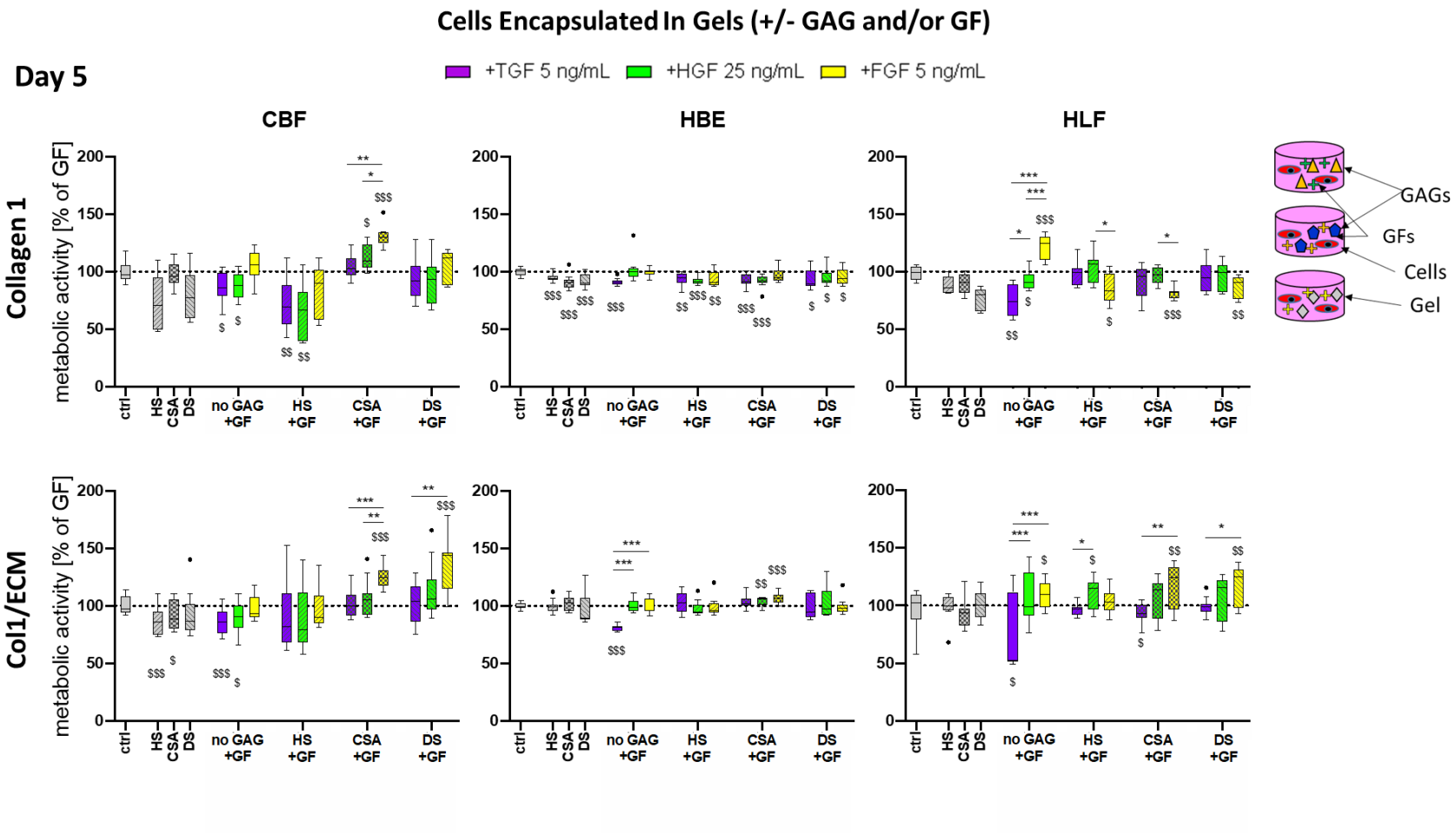


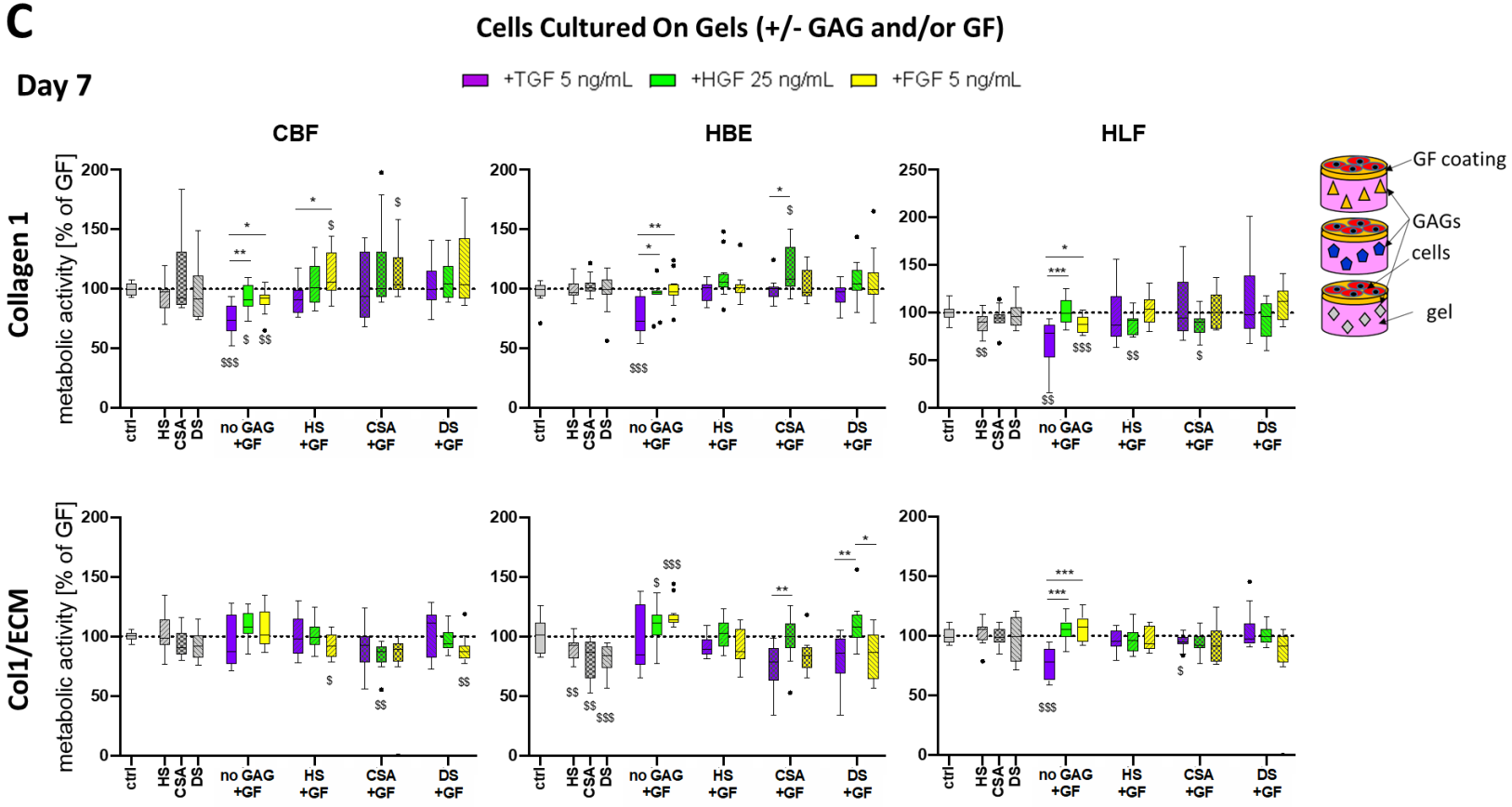
Appendix Figure 10: Soluble glycosaminoglycans (GAGs) in combination with matrix-associated growth factors differentially influences metabolic activity of human pulmonary vascular endothelial cells (CBF), human bronchial epithelial cells (HBE), and human lung fibroblasts (HLF). Metabolic activity of CBF, HBE, and HLF cells grown for 3 and 5 days on tissue culture plastic (TCP) with addition of GAGs (1 µM) in combination with different matrix-associated growth factors (GF) (transforming growth factor beta (TGFβ1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF2)) into the cultivation medium. The data from the addition of GAGs only and growth factors only (+HS, +CSA, +DS, +TGF, +HGF, +FGF) was normalized to the control (= medium without GAG or growth factor addition). Metabolic activity of the combination treatments (GAG+GF) was normalized to the respective GF without addition of GAG. $: significant to control (dotted line, one-sample t-test). *: significant within group (One-way ANOVA). $$$,***=p<0.001, $$,**=p<0.01, $,*=p<0.05. Combined data from 3 experiments with n=4 replicates each. HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

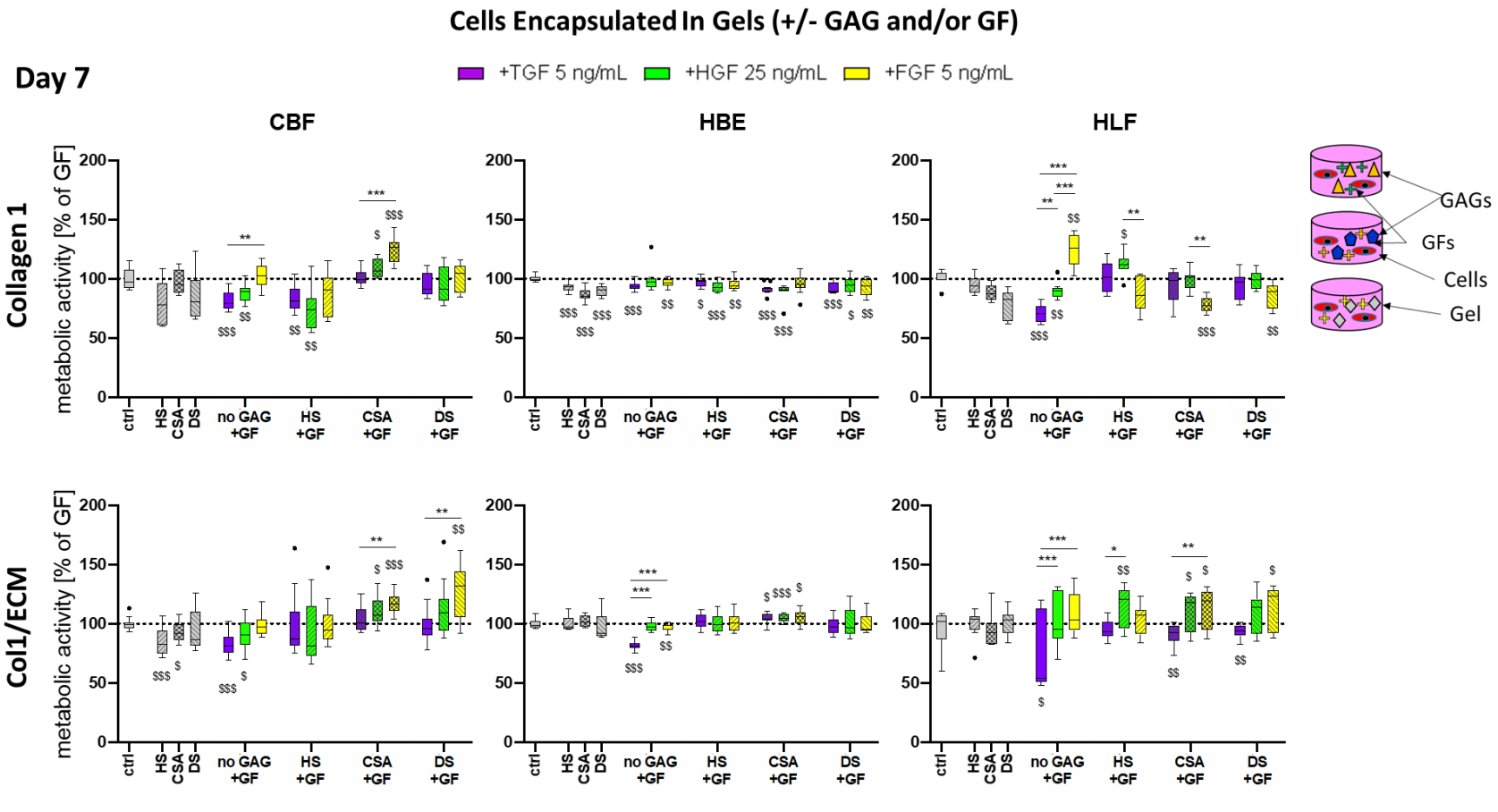

A
Cells Cultured On Gels (+/- GAG and/or GF)
Day 3
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GF]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GF coating
GAGs
cells
gel


Cells Encapsulated In Gels (+/- GAG and/or GF)
Day 3
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GF]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GAGs
GFs
Cells
Gel

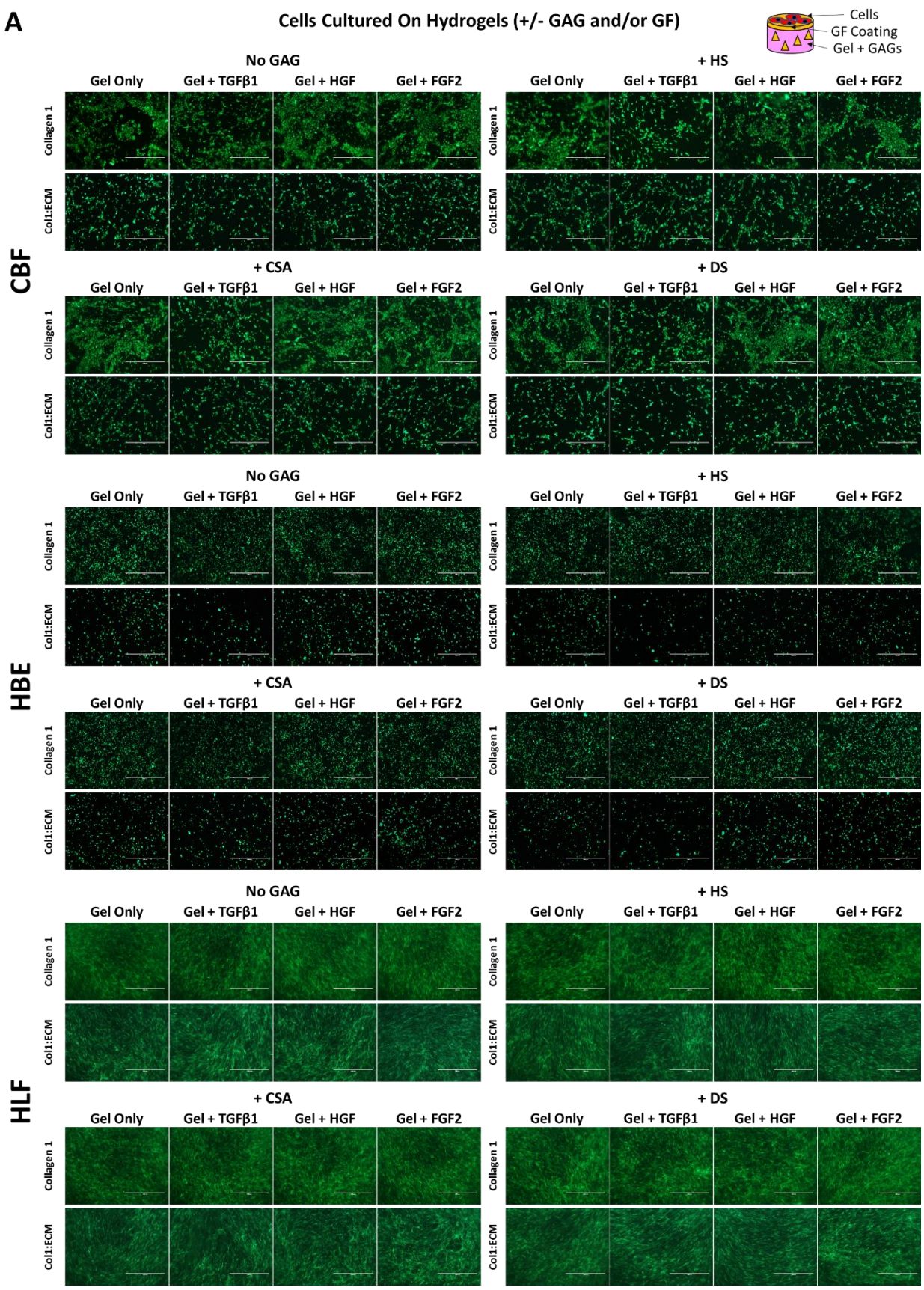

B
Cells Cultured On Gels (+/- GAG and/or GF)
Day 5
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GF]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GF coating
GAGs
cells
gel

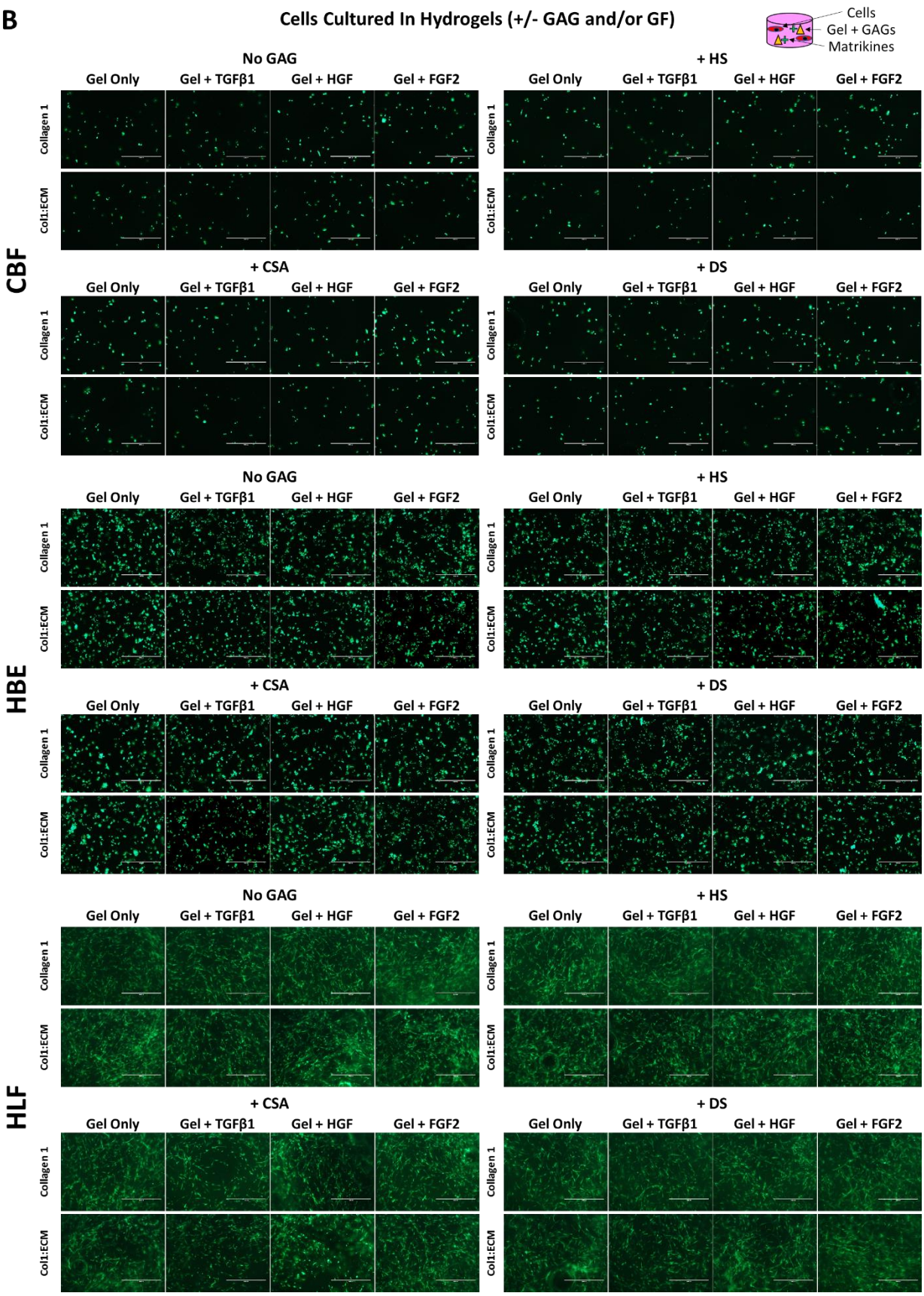

Cells Encapsulated In Gels (+/- GAG and/or GF)
Day 5
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GF]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GAGs
GFs
Cells
Gel

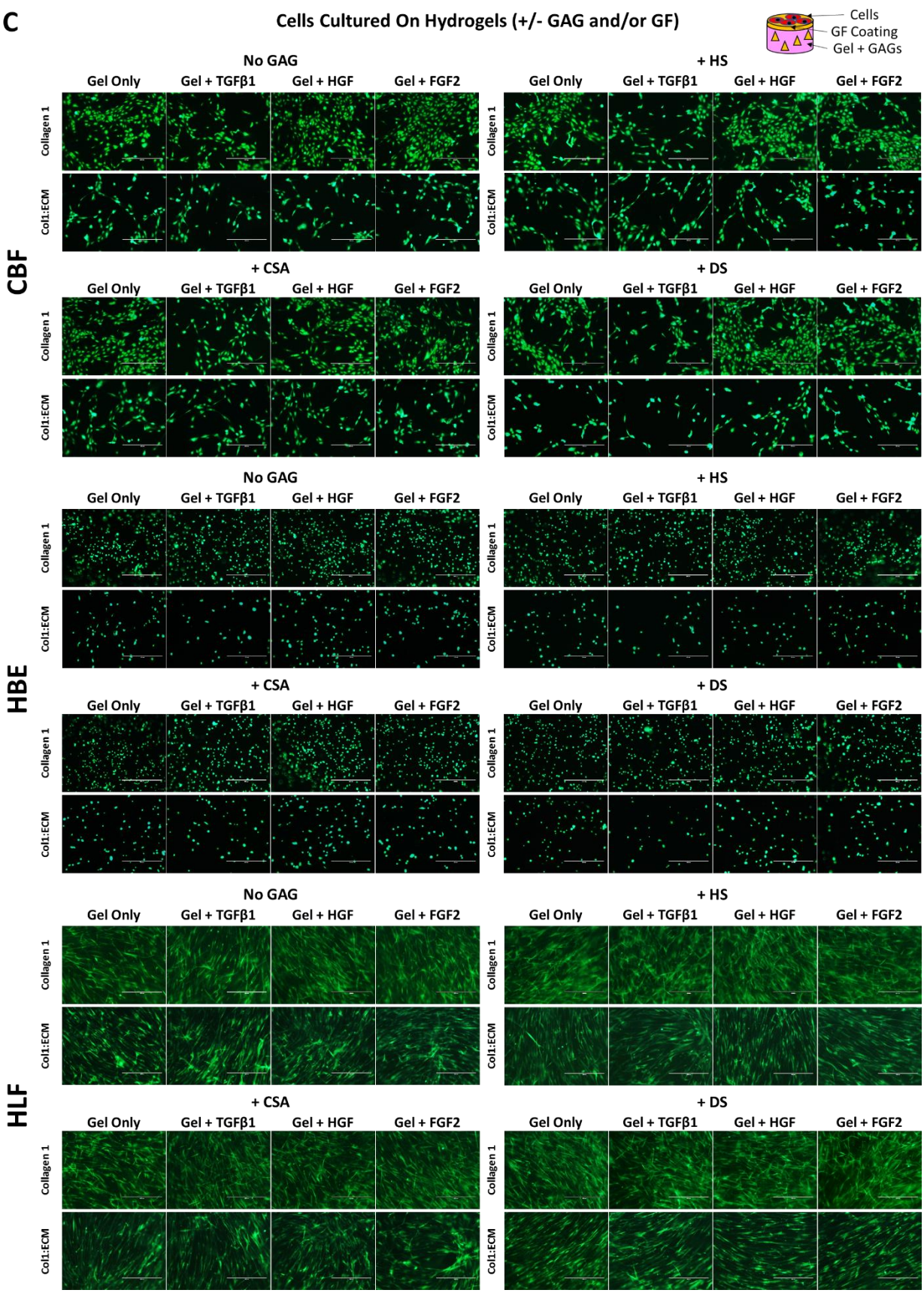

C
Cells Cultured On Gels (+/- GAG and/or GF)
Day 7
+TGF 5 ng/mL
+HGF 25 ng/mL
+FGF 5 ng/mL
CBF
HBE
HLF
Collagen 1
Col1/ECM
metabolic activity [% of GF]
ctrl
HS
CSA
DS
no GAG +GF
HS +GF
CSA +GF
DS +GF
GF coating
GAGs
cells
gel

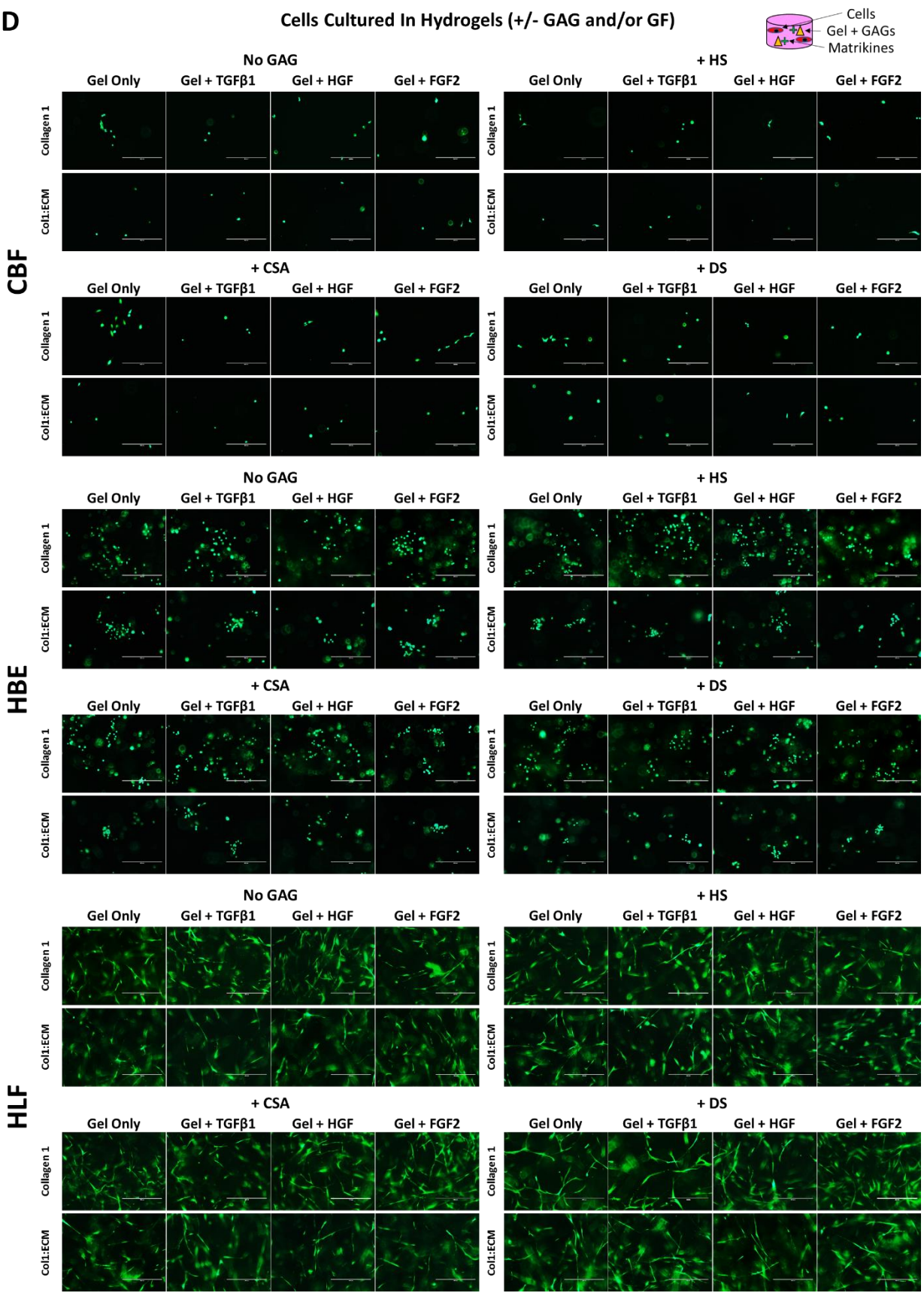


Appendix Figure 11: Glycosaminoglycans (GAGs) in combination with matrix-associated growth factors and method of cultivation differentially influences metabolic activity of human pulmonary vascular endothelial cells (CBF), human bronchial epithelial cells (HBE), and human lung fibroblasts (HLF). Metabolic activity of human pulmonary vascular endothelial cells (CBF), human lung epithelial cells (HBE), and human lung fibroblasts (HLF) on day 3 (A), day 5 (B), and day 7 (C) on top of and in type I collagen and human lung ECM/type I collagen gels with addition of heparan sulfate (HS), chondroitin sulfate A (CSA), or dermatan sulfate (DS) at 5 µM after coating with or addition of growth factors (GF) (transforming growth factor beta (TGFβ1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF2)). The data from the addition of GAGs only and growth factors only (+HS, +CSA, +DS, +TGF, +HGF, +FGF) was normalized to the control (= gel without GAG or growth factor addition). Metabolic activity of the combination treatments (GAG+GF) was normalized to the respective growth factor without addition of GAG. $: significant to control (dotted line, one-sample t-test). *: significant within group (One-way ANOVA). $$$,***=p<0.001, $$,**=p<0.01, $,*=p<0.05. Combined data from gels from 3 experiments with n=4 replicates each. The dotted line represents pure gels without addition of GAGs or matrix-associated growth factors. HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.


A
Cells Cultured On Hydrogels (+/- GAG and/or GF)
Cells
GF Coating
Gel + GAGs
CBF
HBE
HLF
No GAG
+ HS
+ CSA
+ DS
Gel Only
Gel + TGFβ1
Gel + HGF
Gel + FGF2
Collagen 1
Col1:ECM


B
Cells Cultured In Hydrogels (+/- GAG and/or GF)
Cells
Gel + GAGs
Matrikines
CBF
HBE
HLF
No GAG
+ HS
+ CSA
+ DS
Gel Only
Gel + TGFβ1
Gel + HGF
Gel + FGF2
Collagen 1
Col1:ECM


C
Cells Cultured On Hydrogels (+/- GAG and/or GF)
Cells
GF Coating
Gel + GAGs
CBF
HBE
HLF
No GAG
+ HS
+ CSA
+ DS
Gel Only
Gel + TGFβ1
Gel + HGF
Gel + FGF2
Collagen 1
Col1:ECM


D
Cells Cultured In Hydrogels (+/- GAG and/or GF)
Cells
Gel + GAGs
Matrikines
CBF
HBE
HLF
No GAG
+ HS
+ CSA
+ DS
Gel Only
Gel + TGFβ1
Gel + HGF
Gel + FGF2
Collagen 1
Col1:ECM

Appendix Figure 12: Calcein AM Live Cell staining of representative wells after 1 week of cultivation demonstrates different patterns of cell colonization and phenotype. Human pulmonary vascular endothelial cells (CBF), human lung epithelial cells (HBE), and human lung fibroblasts (HLF) (A, C) grown on type I collagen or decellularized human lung ECM/type I collagen gels, with or without encapsulated glycosaminoglycans (GAGs), and/or coated growth factors (GF) (transforming growth factor beta (TGFβ1), hepatocyte growth factor (HGF), fibroblast growth factor (FGF2)), or (B, D) encapsulated within type I collagen or decellularized human lung ECM/type I collagen gels, with or without encapsulated GAGs and/or growth factors. Panel A and B low magnification (4X, scale bars are 1000 μm), while panel C and D high magnification (10X, scale bars are 400 μm). HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

Appendix Table 3: Analysis of glycosaminoglycans (GAGs) used in the cell culture studies. HS: heparan sulfate, CSA: chondroitin sulfate A, DS: dermatan sulfate.

GAG	% of iduronic acid	molecular weight
HS	+ (~20%)	~15 kDa
CSA	-	~20 kDa
DS	++++ (85%)	~20 kDa