SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Allylamine coating - Briefly, electrospun meshes were placed in the reactor chamber. The stainless steel plasma chamber was 50cm in diameter and 60cm long with an internal electrode. Using a rotary pump and a liquid N_2 cold trap, the chamber was evacuated to a base pressure of 1×10^{-3} mbar. Allylamine vapor was flowed through the reactor at $4cm^3\ min^{-1}$ at an operating pressure of 2×10^{-2} mbar. Plasma was excited by a 13.56 MHz radiofrequency generator at a continuous wave power of 5 W for 40 minutes.

XPS analysis - XPS analysis was conducted using a Kratos Axis Ultra with monochromatic Al Kα radiation and an X-ray power of 15 kV, 10 mA. X-ray spot size was approximately 3 mm x 2 mm with an analysis area of 700 μm x 300 μm. Two separate uncoated scaffolds and 7 separate ppAm microfiber meshes were analyzed. Elemental composition of the scaffolds was determined using wide scan analysis at a pass energy of 80 eV. High-resolution core levels of C 1s and O 1s were obtained at a pass energy of 20 eV. Data were analyzed using CasaXPS version 2.3.15dev57. All scans were fitted with a shirley background and charge corrected to the C-H environment at 285.0 eV. For wide scan analysis, elements were identified and quantified using an elemental library and relative sensitivity factors specific to the Kratos Axis Ultra used. High-resolution scans were fitted with Gaussian/Lorentzian ratios between 20 and 30.

Wettability - Contact angle was measured with a Krűss DSA 100B. Five μl droplets of de-ionised H_2O were deposited on the scaffolds surface and the contact angle measured over 15 seconds at one-second intervals.

Detection of functional HS with GAG-BP - Control experiments were conducted with Heparin Binding Plates (BD Bioscience) essentially as described previously (1-4). Modifications of the procedure were necessary for sections of ppAm microfiber and uncoated microfiber scaffolds. Between all steps samples were washed twice with 150 μl pH 6 standard assay buffer (SAB6; 50 mM Na acetate, 100 mM NaCl, 0.2 % (v/v) Tween 20, pH 6.0). In brief, 0.6 cm^2 pieces of microfiber and ppAm microfiber scaffolds were soaked overnight with 100 μl PBS containing 0–5 μg heparin (Iduron, from porcine mucosa); scaffolds were incubated for 90 minutes with 150 μl 1% (w/v) BSA in SAB6 at 37 ˚C for 90 minutes. Samples were then incubated with GAG-BP (2pmol/well) in 150 μl SAB6 for three hours at room temperature (RT), followed by incubation with 200 μl ExtrAvidin Alkaline Phosphatase (Sigma, 1:10,000 dilution in SAB6) for 30 mins after which 200 μl 1 mg/ml p-nitrophenylphosphate in 0.05 M Tris-HCL, 0.1 M NaCl (Sigma) was added. The color development proceeded for 20 minutes before absorbance was read at 405 nm. As the microfiber scaffolds would obscure the reading, 85 μl of the color development solution was removed from each well and transferred to a separate 96 well plate for absorbance to be read.

Phage display ScFv antibody binding - Scaffolds were treated as described for GAG-BP, with the protocol adapted to incorporate the ScFv antibodies. After incubation with heparin/ES cell derived HS, scaffolds were blocked for 90 minutes with 150 μl 1% or 5% (w/v) BSA (for NS4F5/HS4C3 and RB4EA12, respectively) in SAB6 at 37 ˚C. After blocking, samples were incubated with 100 μl ScFv at a 1:10 dilution (HS4C3 or NS4F5) or 1:100 (RB4EA12) dilution in SAB6 for 1 hour at RT. Samples were then incubated with 100 μl biotinylated Rabbit-anti VSV-G (AbCam 1:1000 in SAB6), for 90 minutes at RT. Scaffolds were subsequently treated as described for GAG-BP with the color development proceeding for 20 minutes. As the microfiber scaffolds would obscure the reading, 85 μl of the color development solution was removed at 10 and 20 minutes from each well and transferred to a separate 96 well plate for absorbance to be read at 405 nm.

SEM analysis of cell - Samples were washed 2-times with PBS and fixed with 1.5% (v/v) gluteraldehyde (TAAB) in 0.1 M phosphate buffer, pH 7.3 for 30 minutes at 4 ˚C. Samples were then washed twice with the 0.1 M phosphate buffer. Samples were dehydrated with increasing concentrations of ethanol; 2 x 3 minutes 50% ethanol, 2 x 3 minutes 70% ethanol, 2 x 3 minutes 90% ethanol and 2 x 5 minutes 100% ethanol. Samples were then treated twice with hexamethyldisilazane (HMDS, Sigma) for 5 minutes and allowed to dry. Samples were then gold splutter coated and

visualized using a Zeiss EVO60 VPSEM. Accelerating voltage was set at 5 kV and working distance was approximately 10 cm.

Quantification of neural differentiation - Greyscale images of βIII-tubulin staining were converted to binary using Image J. This process removes pixel intensity by converting images to black and white, enabling the area of staining to be quantified. Images were then analyzed using Image J particle analysis which quantified the area of the image positive for βIII-tubulin, with the results expressed as area fraction.

SUPPLEMENTARY FIGURE LEGENDS

SUPPLEMENTARY TABLE 1. Elemental analysis of uncoated and ppAm microfiber meshes. The ppAm coating resulted in the introduction of nitrogen and a reduction in oxygen at the fiber surface. The data is representative of uncoated (2 separate meshes analyzed) and ppAm (7 separate meshes analyzed) scaffolds.

SUPPLEMENTARY FIGURE 1. Water contact analysis of uncoated- and ppAm-coated meshes. Contact angle was measured over 15 seconds at one second intervals (left). Data points are average values for each time point (microfiber: n=7, ppAm microfiber: n=6) ± S.E.M. Images (right) represent the water droplet after 15 seconds contact. ppAm microfiber meshes had significantly lower initial contact angle of 117^{o} compared to 131^{o} on uncoated scaffolds. Over a 15 second contact period, the water droplet on the surface of uncoated scaffolds remained at $130\text{-}132^{o}$. In comparison, the water contact angle of ppAm scaffolds decreased rapidly as the droplet spread across and into the porous mesh, leveling out at 43^{o}, demonstrating the increased wettability of ppAm surfaces.

SUPPLEMENTARY FIGURE 2: Quantification and disaccharide analysis of HS bound to ppAm microfiber meshes after incubation with different concentrations of HS. a) To quantify the amount of HS bound to ppAm scaffolds, 1 cm^2 sections of scaffold were soaked with 0.5, 1, 2 and 5 μg HS. Bound HS was subsequently removed with 4 M NaCl and the disaccharide composition analyzed using RP-HPLC as previously described (5,6).The total fluorescence of the labeled disaccharides was then compared against a known quantity of HS. Values are an average of replicate experiments (0.5 μg: n=3, 1 μg: n=4, 2 μg: n=4, 5 μg: n=6) ± S.E.M. b) Disaccharide analysis of HS bound at 1, 2 and 5 μg applied HS. Values are an average of replicate experiments (control: n=9, 1 μg: n=5, 2 μg: n=4, 5 μg: n=7) ± S.E.M. At 5 μg applied HS, statistical analysis revealed significant increases in UA-GlcNS6S (P=0.001) and UA2S-GlcNS6S (P=0.016), with significant decreases in UA-GlcNS (P=0.02) and UA-GlcNAc (P=0.000). At 2 μg applied HS, only a significant decrease in UA-GlcNS was observed (P=0.02). *P<0.05 **P<0.01 ***P<0.001.

SUPPLEMENTARY FIGURE 3. Binding of phage display antibodies to heparin immobilized on ppAm-coated scaffolds. To further investigate the presentation of functional sulfation motifs within the heparin chains, phage display HS4C3 and NS4F5 antibodies (that require specific sulfation epitopes for high affinity binding to heparin) were used in a colorimetric assay. Values are the average absorbance from two replicates ± S.E.M.

SUPPLEMENTARY FIGURE 4. Retention of immobilized HS on ppAm-coated scaffolds during incubation in PBS. The retention of bound HS was assessed in PBS over a 10 day period using metabolically labeled ^{3}H HS. ppAm microfiber scaffolds were sampled at day 0, 1, 5 and 10 days of incubation. Uncoated scaffolds demonstrated no binding of ^{3}H HS (results not shown). Data represents the mean of three replicates for each time point (n=3) ± S.E.M.

SUPPLEMENTARY FIGURE 5. SEM images of *Ext1*$^{-/-}$ mES cells at day 8 of neural differentiation on ppAm scaffolds with immobilized HS (+HS). Neural projections can be clearly seen projecting out of large aggregates and extending out across the scaffold surface. White arrows indicate neural

processes. Black arrowheads indicate large aggregates of cells. Scale bars: First column of images 100 μm, second and third column 25 μm, final column 5 μm.

SUPPLEMENTARY FIGURE 6. Quantification of neural differentiation at day 6. Images were converted to binary with βIII-tubulin quantified and expressed as area fraction positive for staining. The data is representative of two separate experiments. Data for +HS+FGF4 and +HS-FGF4 was quantified from two replicate samples. The data for -HS+FGF4 and -HS-FGF4 was quantified from one replicate sample. Data represent averages from separate fields of view (+HS+FGF4 n=7, +HS-FGF4 n=5, -HS+FGF4 n=2, -HS-FGF4 n=3) ± S.E.M.

SUPPLEMENTARY FIGURE 7. Limited neural differentiation observed on scaffolds without HS or supplemented FGF4 can be attributed to heparin/HS present in FCS. a) Representative images of *Ext1*$^{-/-}$ mES cells differentiated on ppAm-coated scaffolds in the absence of HS and FGF. It is important to note that, in these experiments we did observe a low level of βIII-tubulin staining. This limited neural differentiation can be attributed to GAGs present within the standard mES media in which the scaffolds were pre-conditioned and the cells were seeded. Scale bars: 100 μm. b) A HPLC profile of standard mES cell media supplemented with 10% FCS clearly contains peaks relating to heparin/HS disaccharides indicating the presence of heparin/HS within this media. Peaks correspond to: 1) UA 2S GlcNS 6S 2) UA GlcNS 6S 3) UA 2S GlcNS 4) UA-GlcNS 5) UA-GlcNac6S 6) UA-GlcNAc; peak 5 is partially obscured by a contaminant (X) relating to the disaccharide 2-aminoacridone (AMAC) label (5).

SUPPLEMENTARY FIGURE 8. Neural differentiation of *Ext1*$^{-/-}$ mES cells in the presence of soluble Heparin (1 μg/ml) on standard gelatinized TCP (i.e. on 2D surfaces). Cells were stained after 8 days of differentiation for neural differentiation marker βIII-tubulin. Scale bars: 100 μm

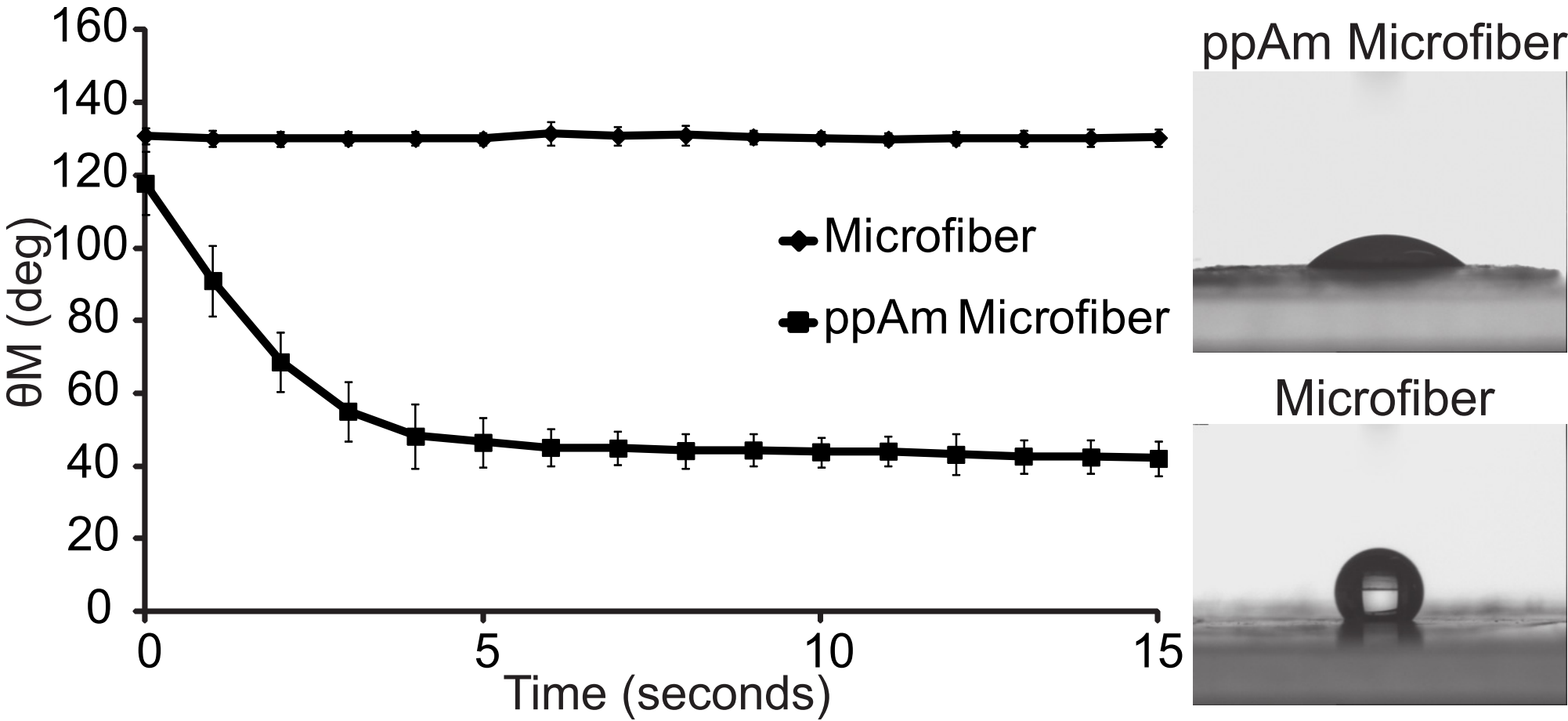
REFERENCES

1. Mahoney, D. J., Whittle, J. D., Milner, C. M., Clark, S. J., Mulloy, B., Buttle, D. J., Jones, G. C., Day, A. J., and Short, R. D. (2004) *Anal Biochem* **330**(1), 123-129
2. Marson, A., Robinson, D. E., Brookes, P. N., Mulloy, B., Wiles, M., Clark, S. J., Fielder, H. L., Collinson, L. J., Cain, S. A., Kielty, C. M., McArthur, S., Buttle, D. J., Short, R. D., Whittle, J. D., and Day, A. J. (2009) *Glycobiology* **19**(12), 1537-1546
3. Robinson, D. E., Buttle, D. J., Short, R. D., McArthur, S. L., Steele, D. A., and Whittle, J. D. (2012) *Biomaterials* **33**(4), 1007-1016
4. Robinson, D. E., Marson, A., Short, R. D., Buttle, D. J., Day, A. J., Parry, K. L., Wiles, M., Highfield, P., Mistry, A., and Whittle, J. D. (2008) *Advanced Materials* **20**, 1166-1169
5. Deakin, J. A., and Lyon, M. (2008) *Glycobiology* **18**(6), 483-491
6. Holley, R. J., Deligny, A., Wei, W., Watson, H. A., Ninonuevo, M. R., Dagalv, A., Leary, J. A., Bigger, B. W., Kjellen, L., and Merry, C. L. (2011) *J Biol Chem* **286**(43), 37515-37524

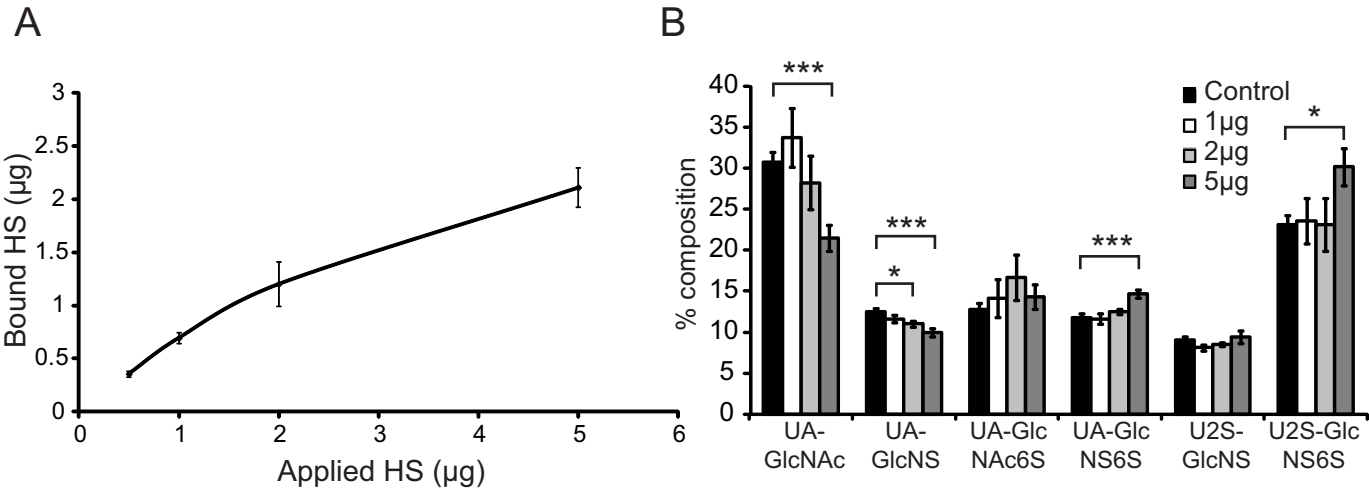
Supplementary Table 1

Elemental Analysis	C 1s	O 1s	N 1s	F 1s
Microfiber	63.19	36.81	---	---
ppAm Microfiber	77.1	5.28	16.24	1.38

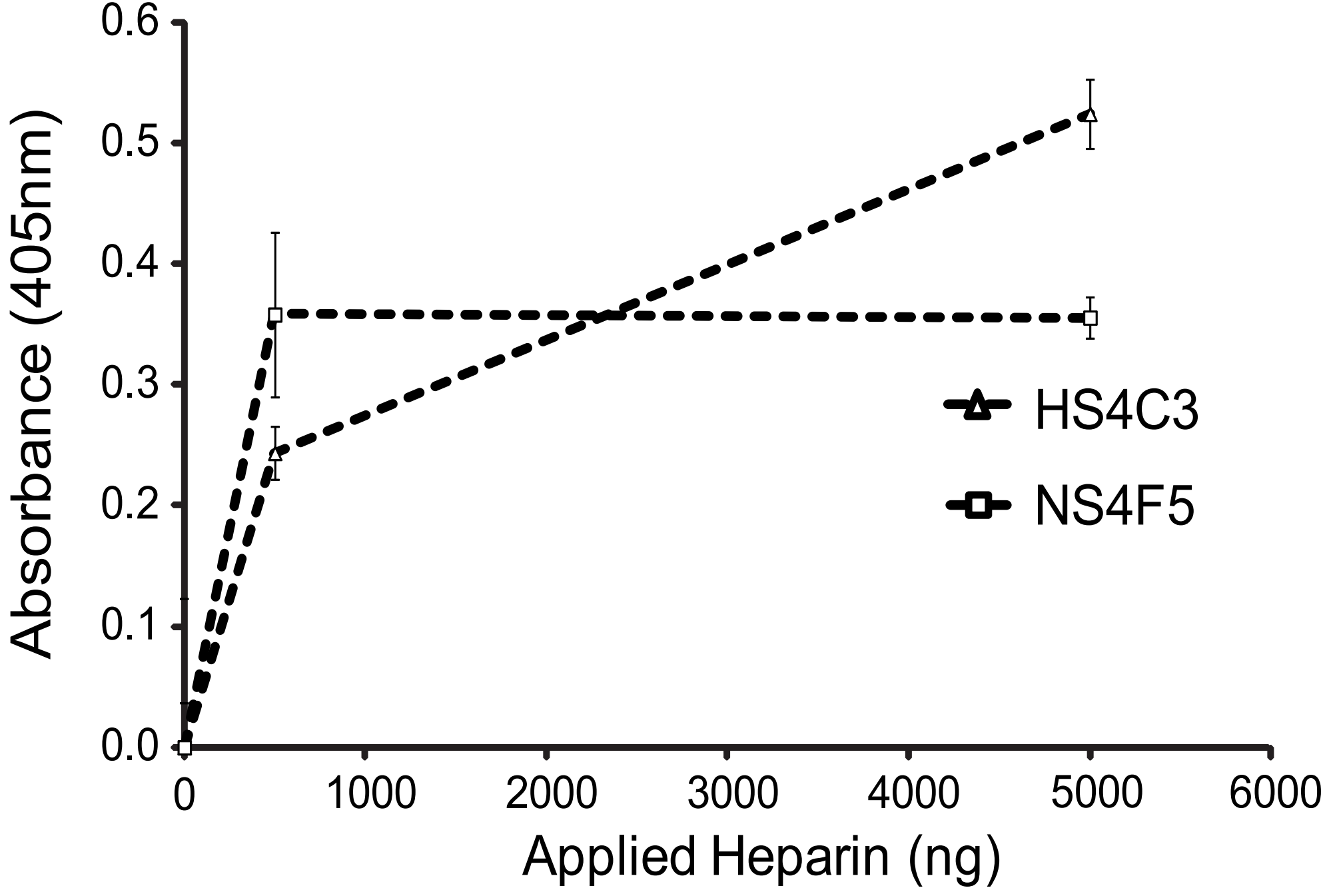
Supplementary Figure 1



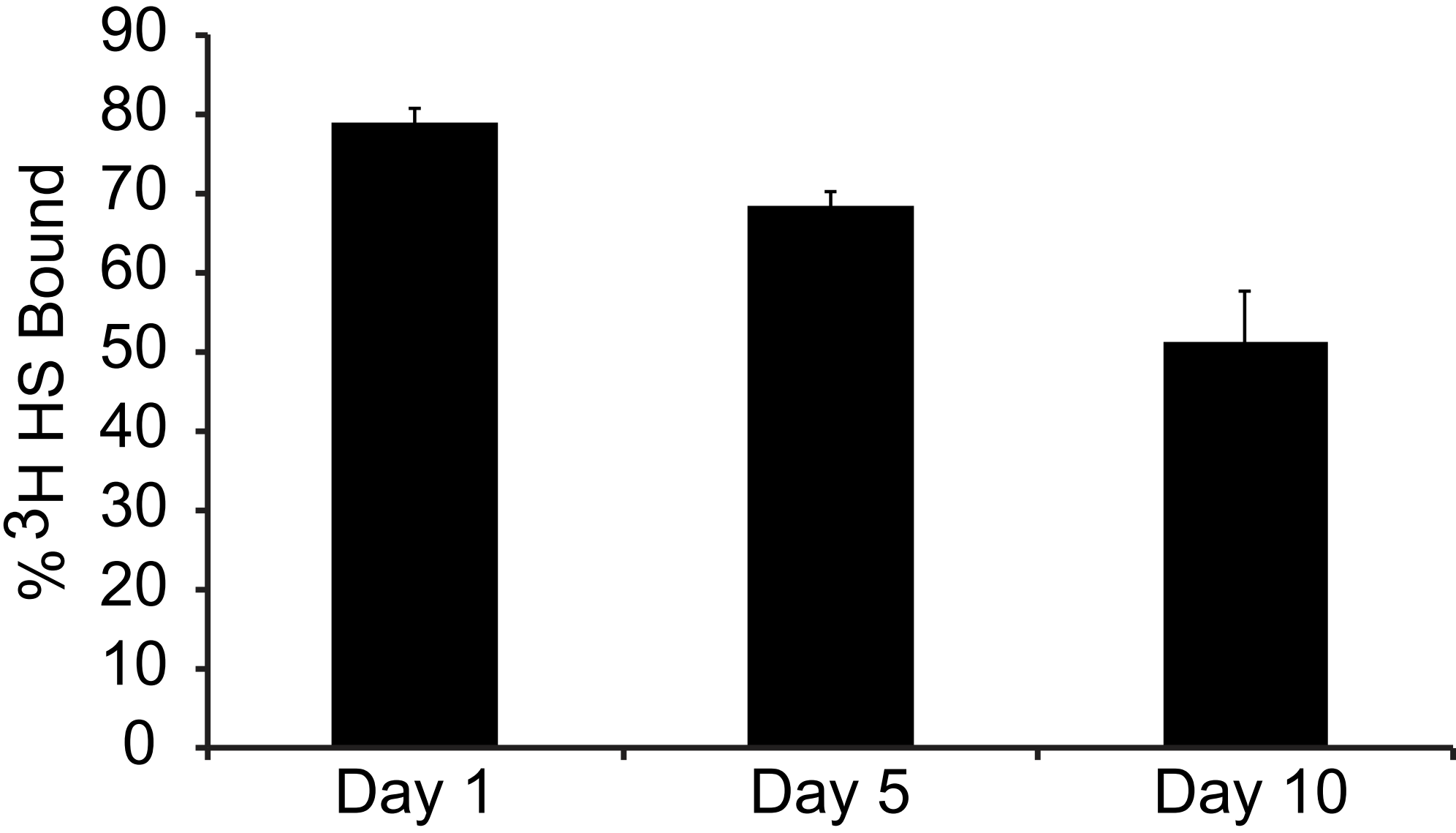
Supplementary Figure 2



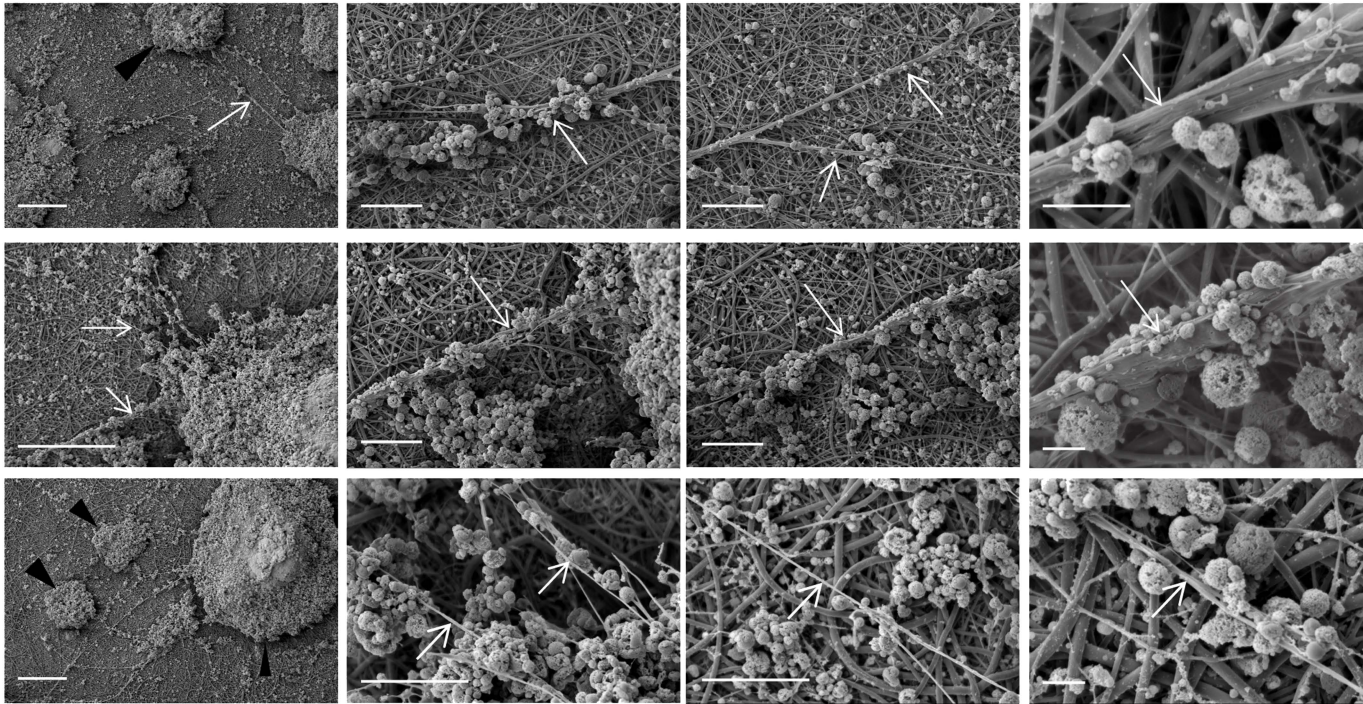
Supplementary Figure 3



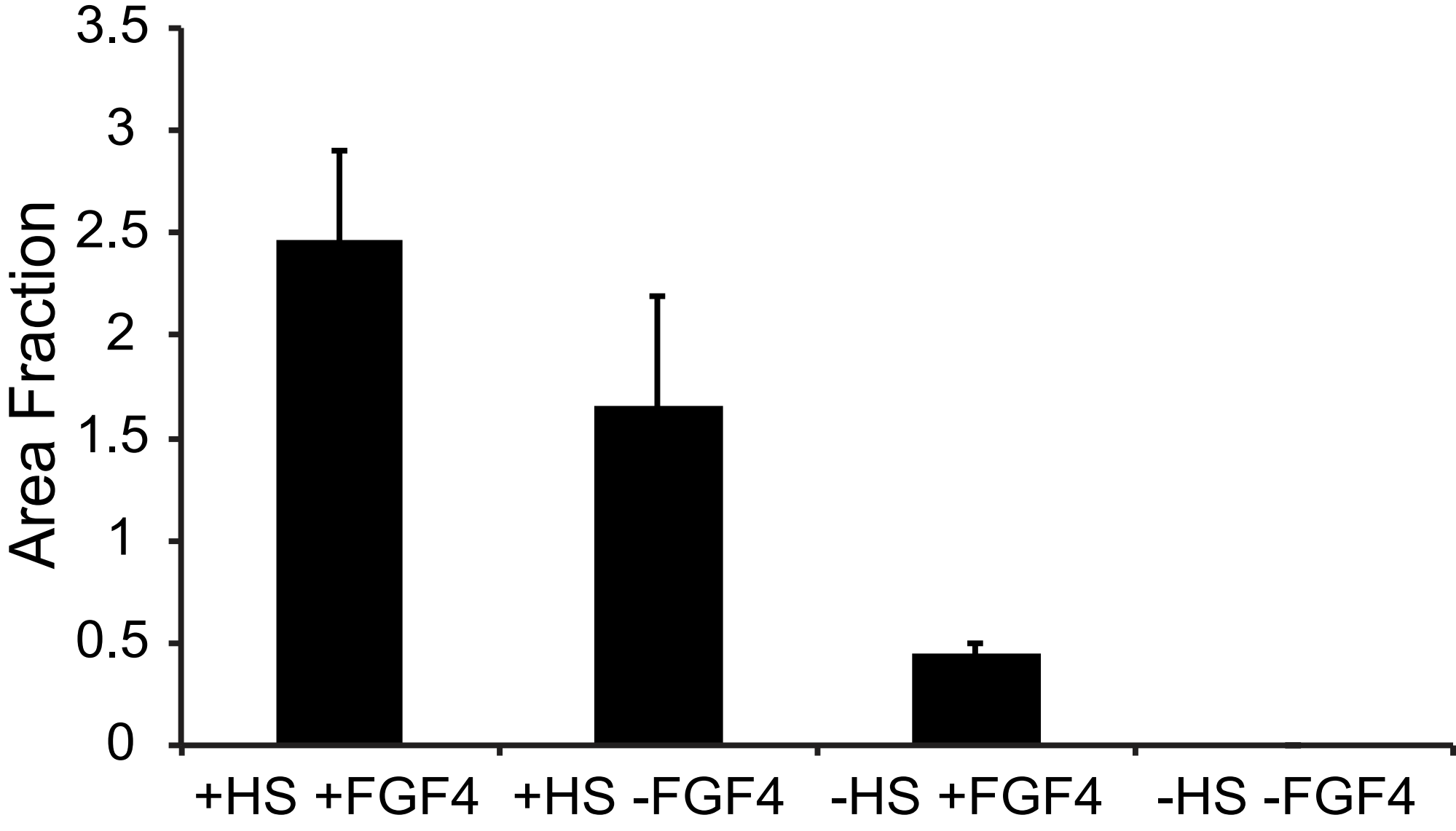
Supplementary Figure 4



Supplementary Figure 5

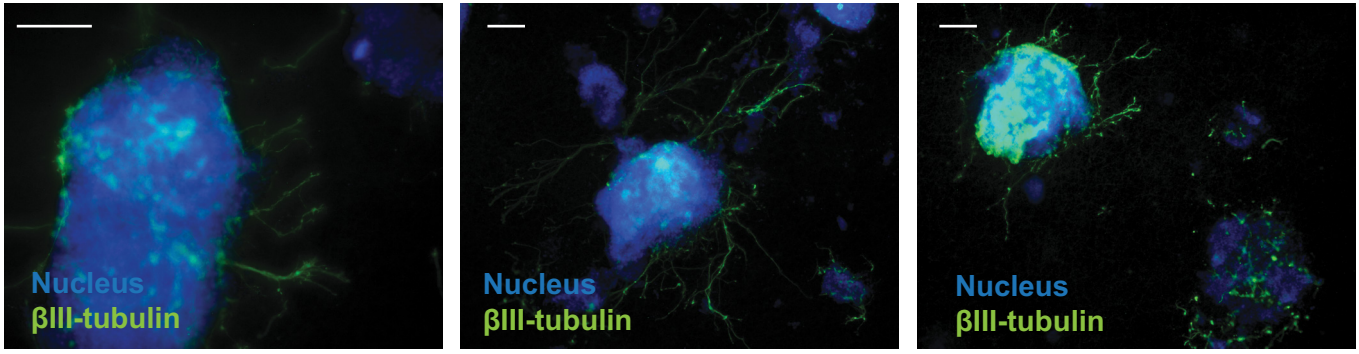


Supplementary Figure 6

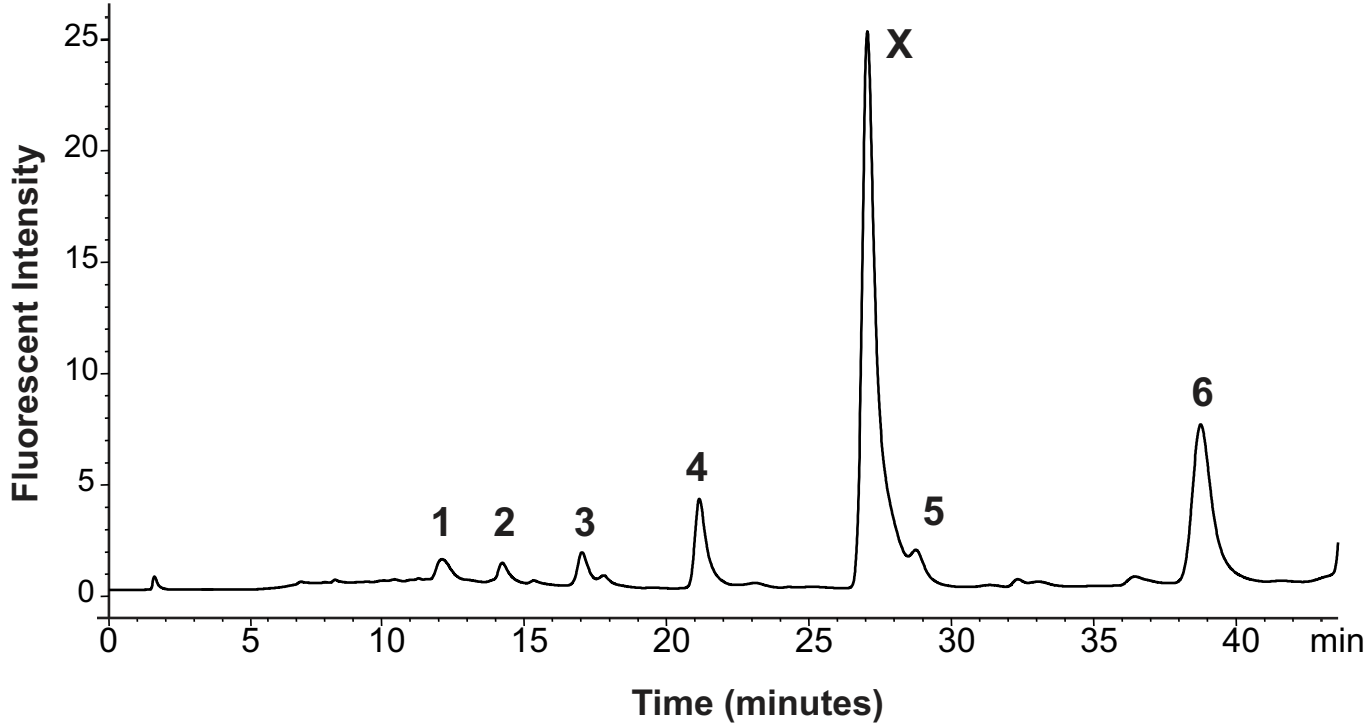


Supplementary Figure 7

A



B



Supplementary Figure 8