# Supporting Information

## Baeyens et al. 10.1073/pnas.1413725111

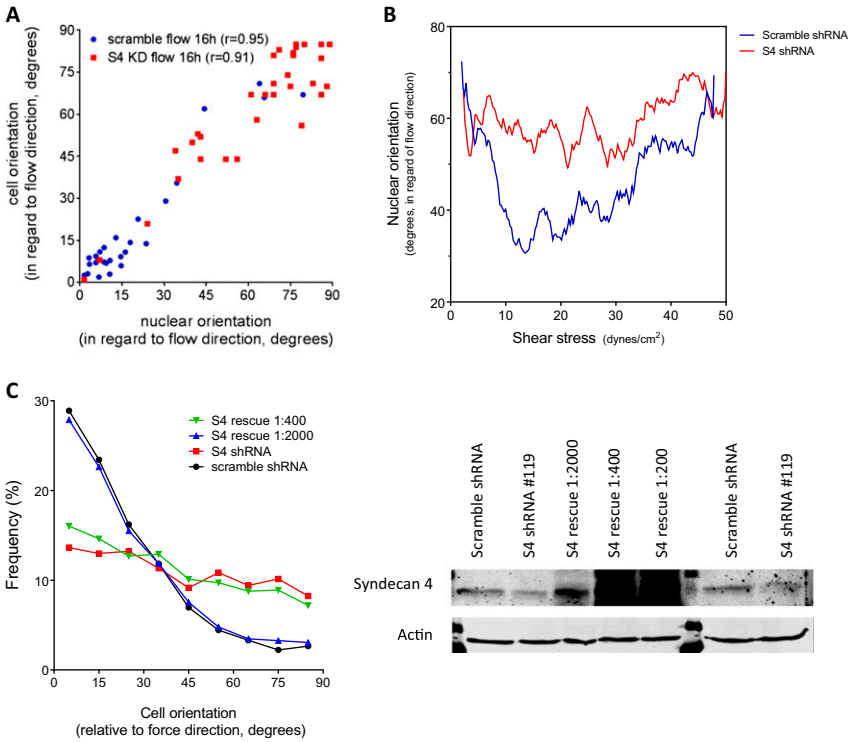


**Fig. S1.** (*A*) Correlation between cell orientation and nucleus orientation in HUVEC cells transfected with scrambled or S4 shRNA. (*B*) A linear gradient of shear stress (2–50 dynes·cm$^{-2}$) was applied for 16 h across slides in a modified parallel plate chamber designed according to Usami and colleagues (1). Adjacent microscopic pictures were taken across the chamber, and the average nuclear orientation was quantified for each picture and reported on the graph. Data were smoothened with a LOWESS fit function. (*C*) Rescue of S4 knockdown (shRNA #121) by adenoviral reexpression of rat S4. Two dilutions of the adenovirus were used: 1/400 and 1/2,000. Nuclear orientation was quantified to characterize cell orientation in regard to flow direction ($n$ > 3,000 cells by condition). Western blot of S4 and actin as a loading control; all samples were sheared for 16 h.

1. Usami S, Chen HH, Zhao Y, Chien S, Skalak R (1993) Design and construction of a linear shear stress flow chamber. *Ann Biomed Eng* 21(1):77–83.

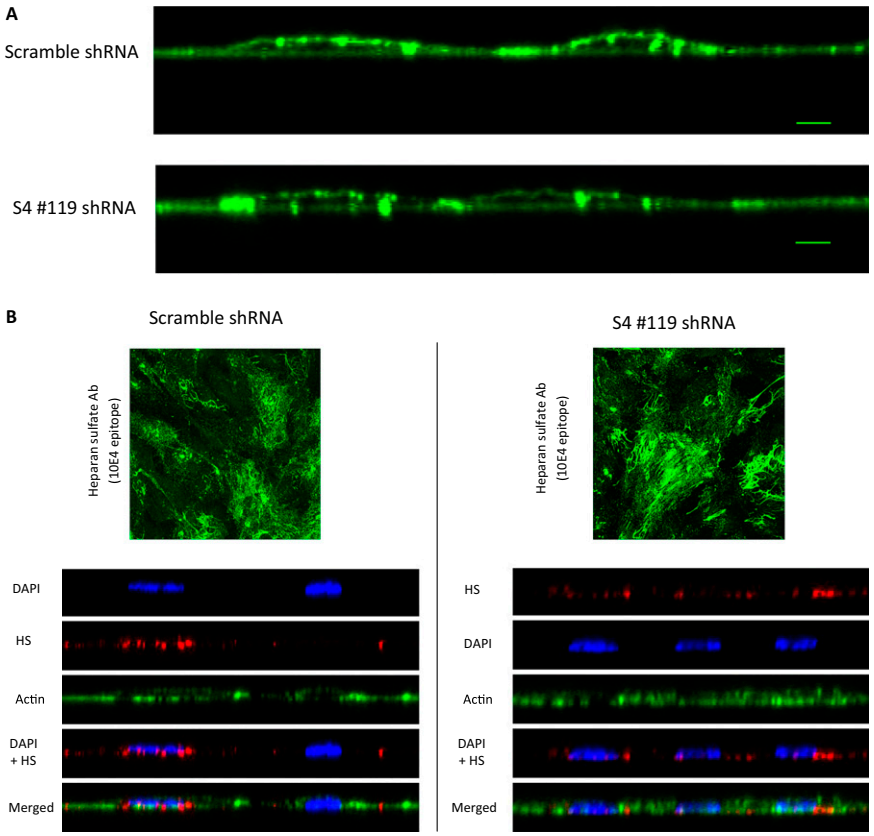


**Fig. S2.** (*A*) X-*z* axis projection of HUVECs expressing scrambled or S4 #119 shRNA and stained with wheat germ agglutinin to label the glycocalyx. Image reconstructed from a Z stack with 0.1-μm steps (Leica SP5 confocal microscope). (Scale bar, 5 μm.) (*B*, *Top*) HUVECs expressing scrambled or S4 #119 shRNA, stained with an antibody against heparan sulfate (10E4 epitope). (*Bottom*) Z plane profile of cells labeled with DAPI, heparan sulfate antibody (HS), and phalloidin (actin) (Leica SP5 confocal microscope, profile from Z stack with 0. 1-μm steps).