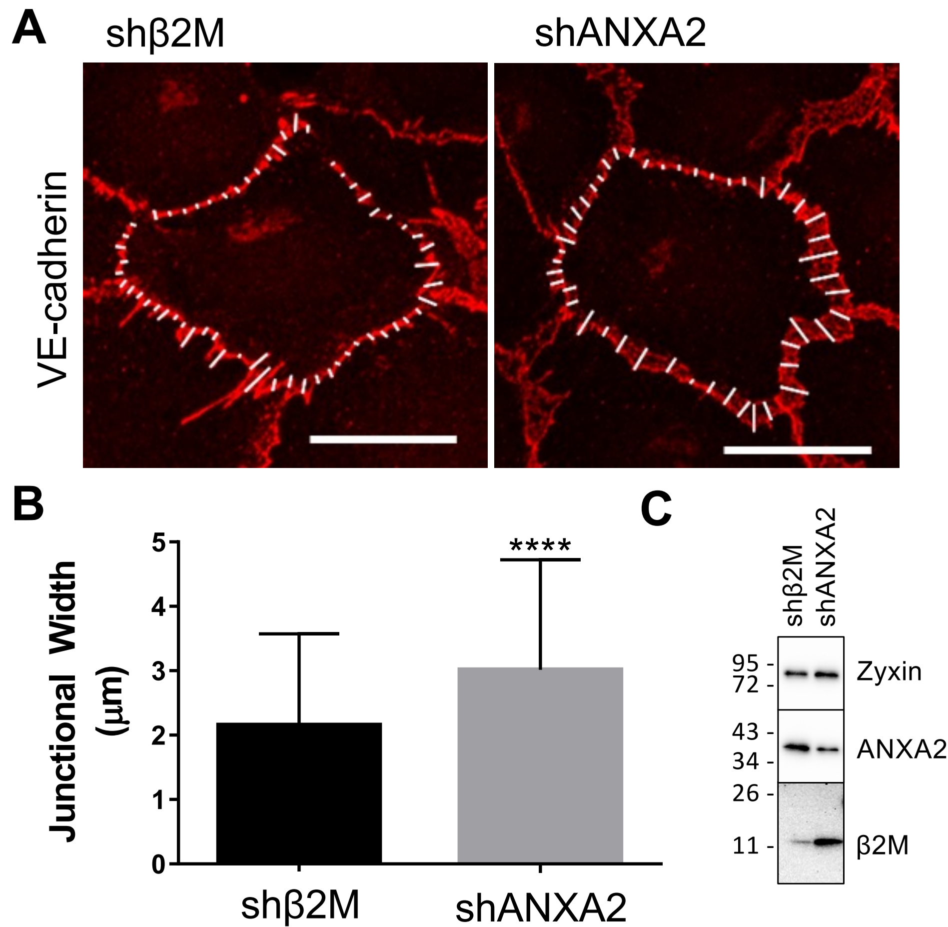
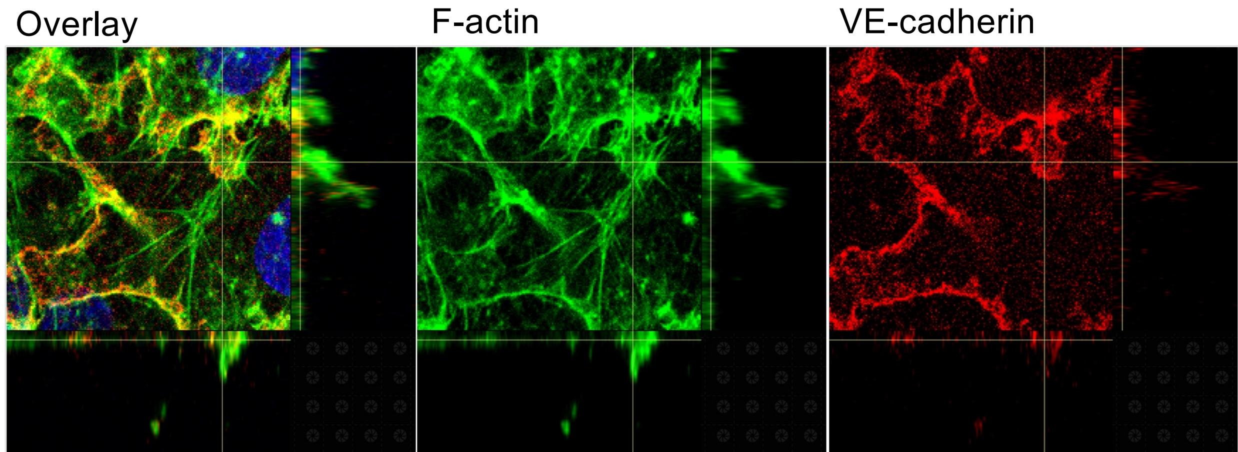
Supplemental Figure 1



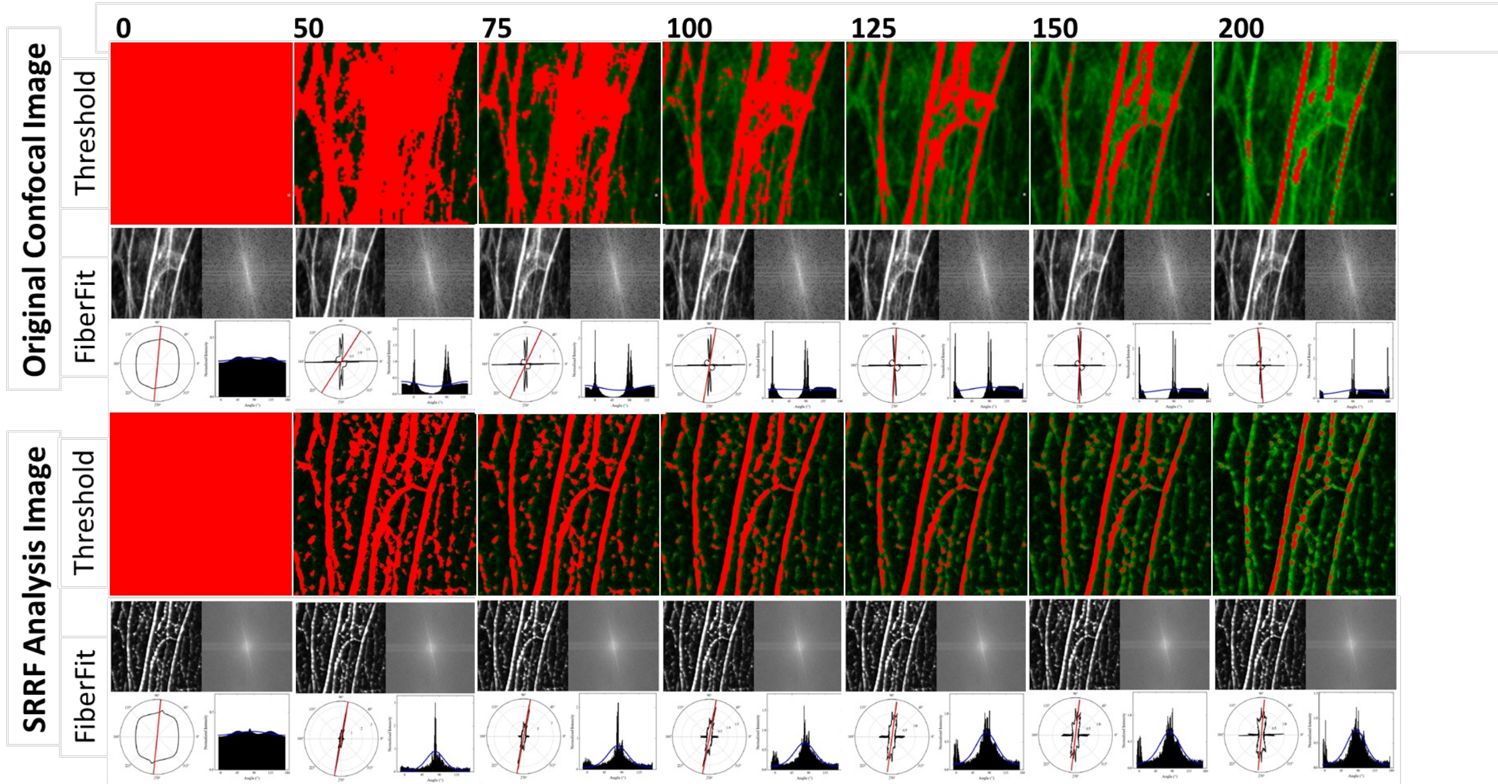
Supplemental Figure S1. Junctional width increases with loss of ANXA2 during one hour of S1P activation. **A)** Immunofluorescence of VE-cadherin in shβ2M and shANXA2 cells, seeded onto collagen-coated coverslips and activated by S1P for one hour. White lines at junctions denote width measurements. Scale bar = 10 µm. **B)** Average junctional width (µm). N = at least 150 points per treatment. Student's t-test: ****, $p < 0.0001$ versus shβ2M. Experiment repeated three times with representative data shown. **C)** Western blot showing confirmation of knock-down of ANXA2 and β2M, along with Zyxin loading control.

Supplemental Figure 2



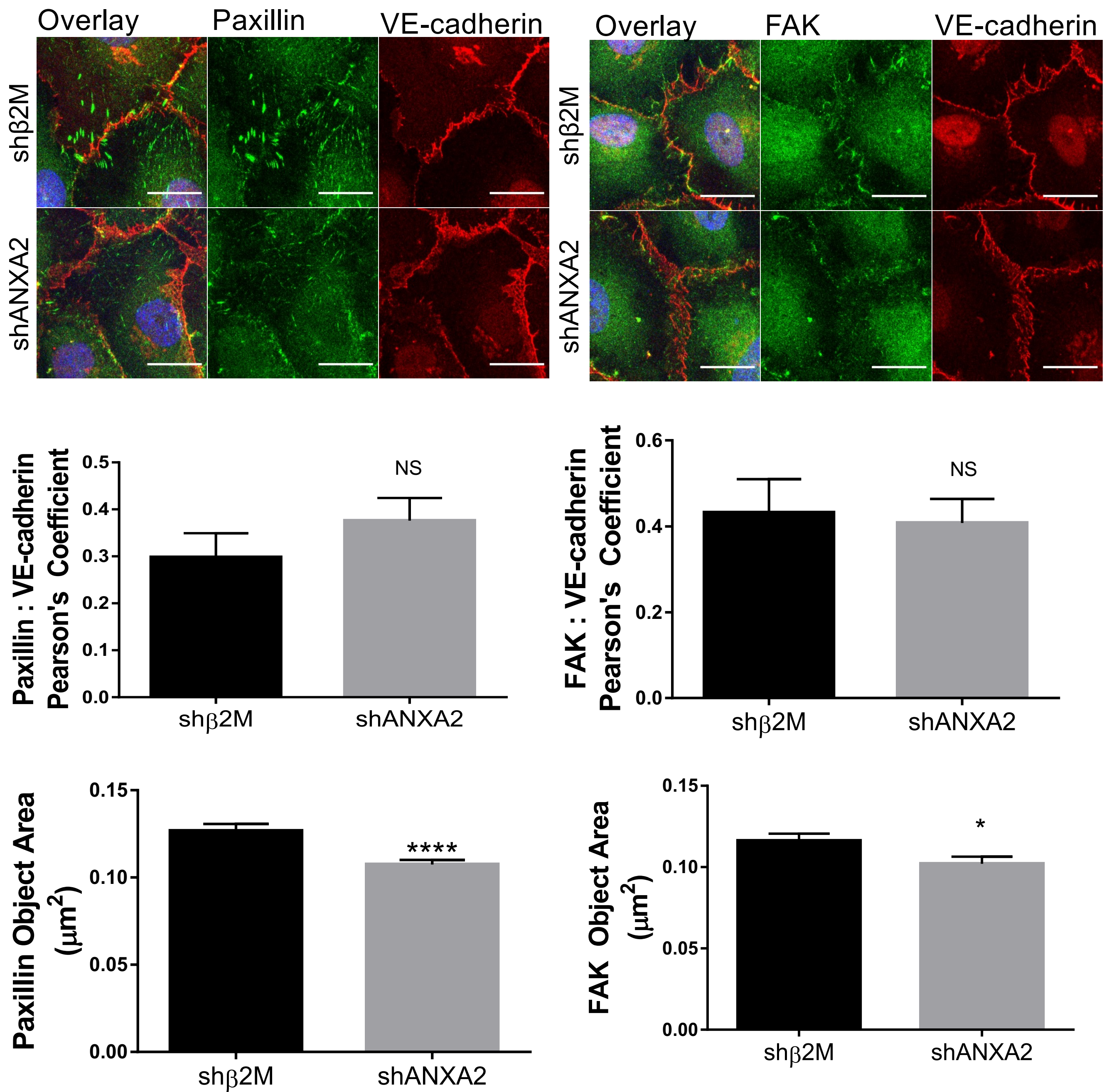
Supplemental Figure S2. Orthogonal view of sprout initiation at VE-cadherin-positive junctions after 1 hour. Samples were stained with F-actin (green), VE-cadherin (red) and DAPI (blue). Yellow lines indicate positions of displayed X and Y slices shown at the bottom and right of each image, respectively. Images were generated using NIS elements.

Supplemental Figure 3



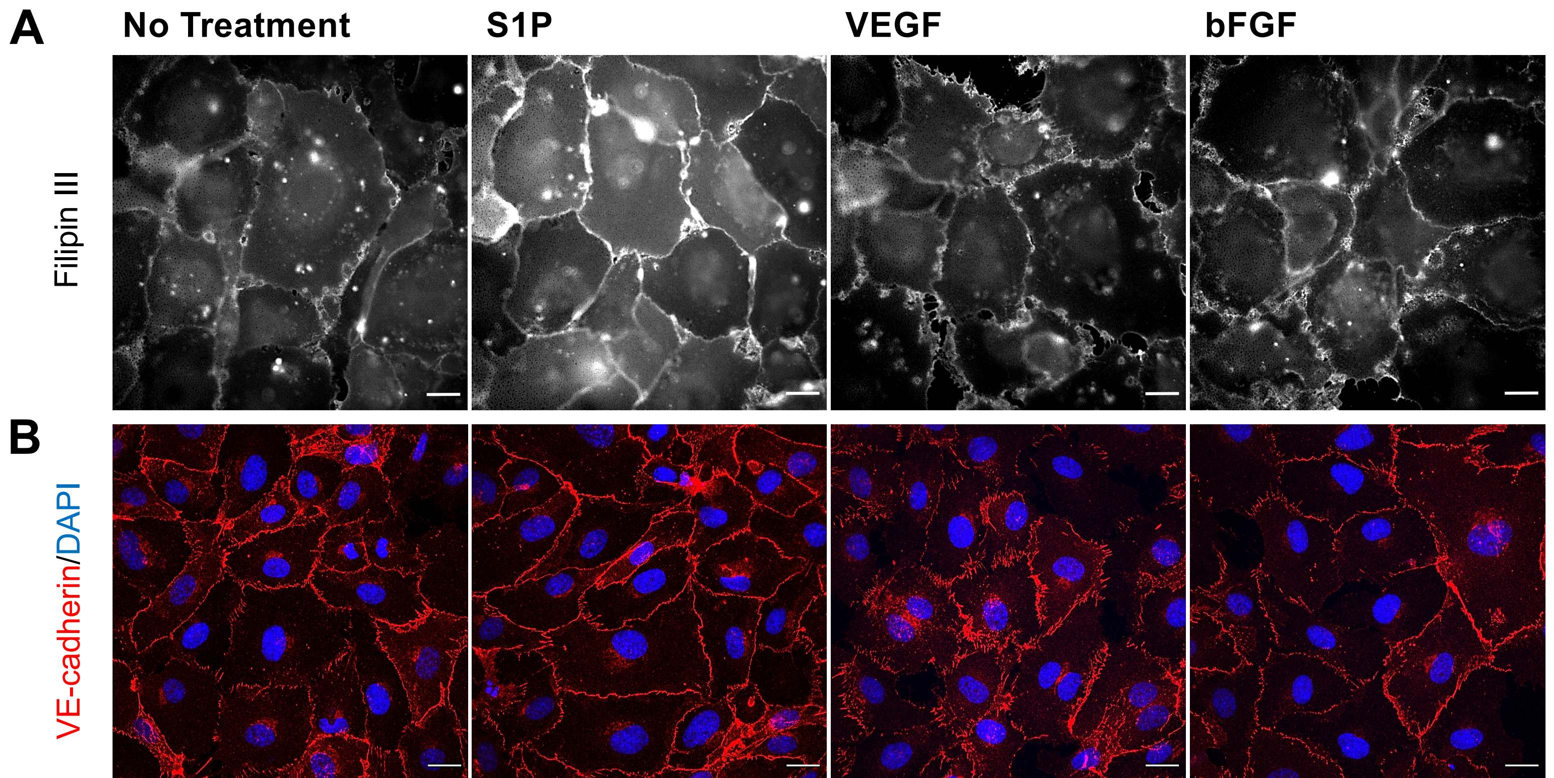
Supplemental Figure S3. Benefits of SRRF analysis prior to FiberFit™. Cropped ROI of 100x confocal image taken at 1024 resolution. **Top)** Original confocal image at a given threshold (red denotes signal included for FiberFit Software™ analysis) and results output of that threshold. **Bottom)** Same field after SRRF analysis with different thresholds. Note: Threshold application with SRRF images provides a more representative output from FiberFit Software™ while preserving signal.

Supplemental Figure 4



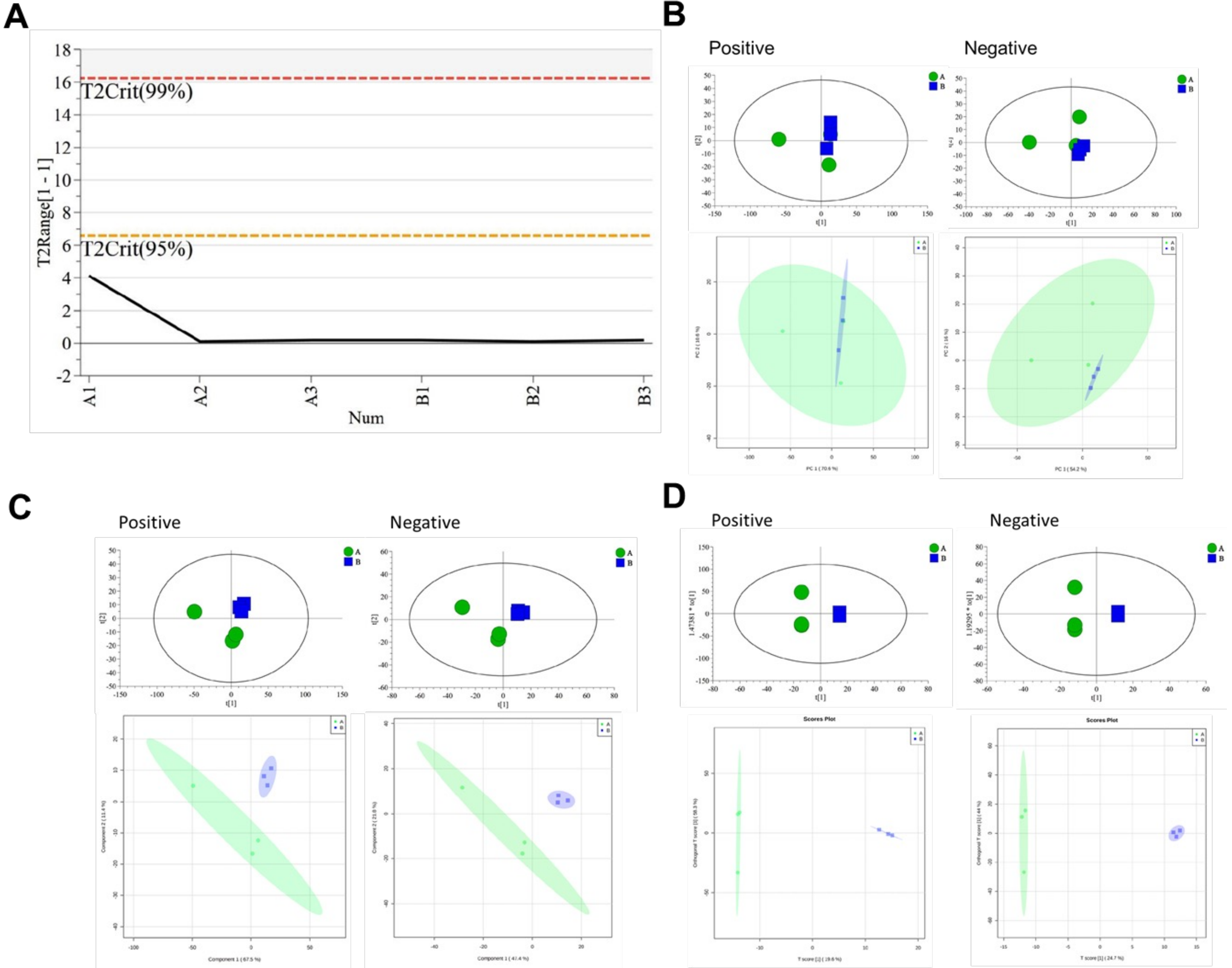
Supplemental Figure S4. Focal adhesion size is reduced with ANXA2 knock-down. **A)** Immunofluorescence of shβ2M and shANXA2 HUVEC after 1 hour S1P, then labelled for Paxillin or FAK (green) and VE-cadherin (red). Blue staining in Overlay panel indicates DAPI staining. Scale bars: 10 µm. **B)** Quantified colocalization between Paxillin and VE-cadherin and FAK and VE-cadherin, as measured by Pearson's Coefficient. n = 5 fields per treatment. Student's t-test: NS = Not significant. **C)** Quantified average object area of Paxillin or FAK signal, denoting focal adhesion size after applying a threshold. n > 2500 focal adhesions per treatment. Student's t-test: ****, $p < 0.0001$ for Paxillin, *, $p < 0.05$ for FAK. All experiments repeated at least three times with representative data shown.

Supplemental Figure 5



Supplemental Figure S5. S1P enhances filipin III labeling of cholesterol at junctions but not VEGF or bFGF. HUVEC were seeded onto collagen-coated coverslips overnight, serum-starved, and treated for one hour with S1P, VEGF, or bFGF. **A)** Cholesterol localization as labeled by filipin III. **B)** Blue signal indicates DAPI staining. VE-cadherin signal defines adherens junctions. Note robust, linear signal at junctions in S1P-treated cells that is not present in cells with no treatment, VEGF, or bFGF treatments. Scale bar: 10 μm.

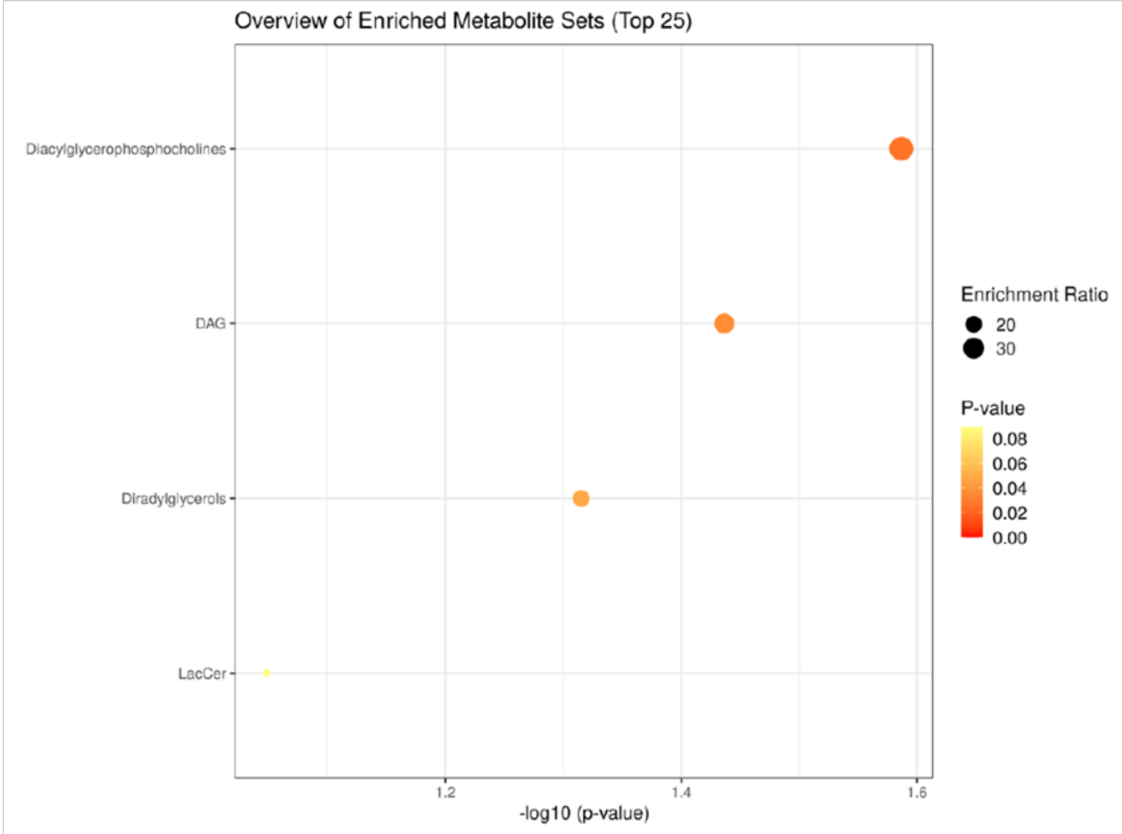
Supplemental Figure 6



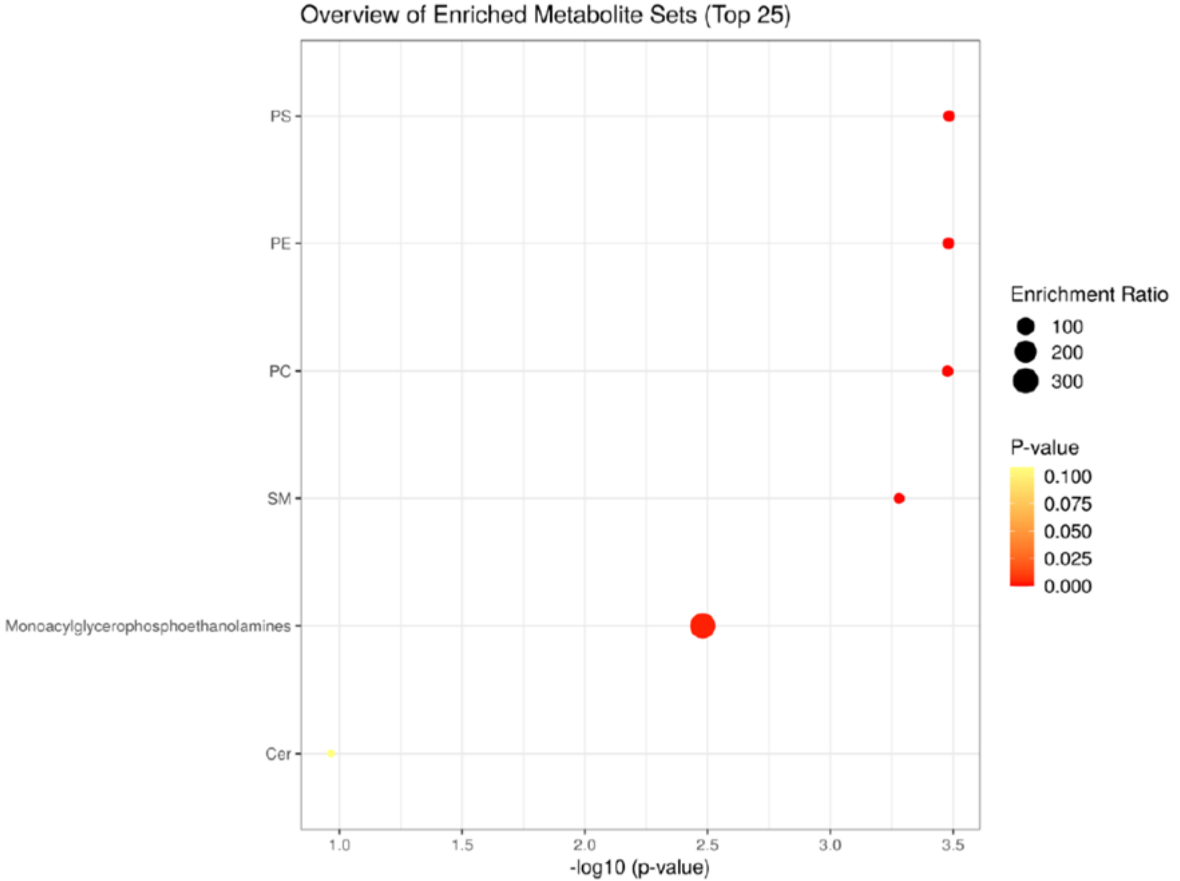
Supplemental Figure S6. Quality control and modeling of LC-MS data via different clustering algorithms. **A)** Quality control of system stability prior to LC-MS analysis. A1-3 denote shANXA2 samples, B1-3 denote shβ2M samples. **B)** Clustering of samples by Principal Component Analysis. **C)** Clustering of samples by Partial Least Squares Discriminant Analysis. **D)** Clustering of samples by Orthogonal Partial Least Squares Discriminant Analysis.

Supplemental Figure 7

Positive

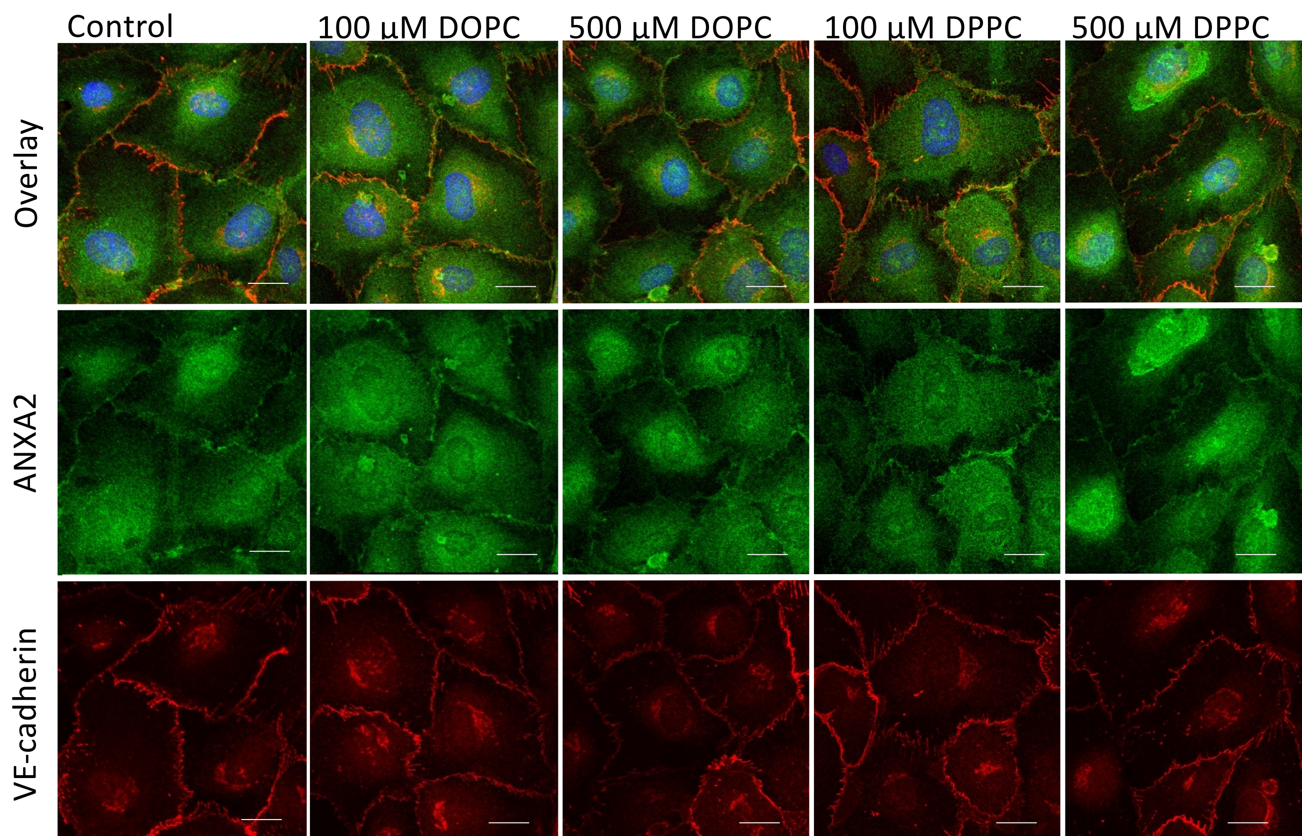


Negative



Supplemental Figure S7. Pathways that were determined to be impacted by loss of ANXA2 using KEGG databases. Note: Plot is shβ2M relative to shANXA2.

Supplemental Figure 8



Supplemental Figure S8. DPPC treatment does not alter ANXA2's ability to localize to junctions with S1P treatment. Primary HUVEC were seeded on coverslips and treated overnight with DPPC or DOPC, then treated with S1P for one hour. Cells were stained with ANXA2 (green) and VE-cadherin (red). Blue staining in Overlay panels indicates DAPI staining. Scale bar: 10 µM. All treatments were repeated independently at least three times with representative data shown.

Supplemental Table 1

Class	FA1	FA2	Fold Change	Log2 Fold Change	T-Test
BisMePA	14:1e	18:0	0.0825	-3.6003	0.0203
Cer	d18:1	24:0	0.2593	-1.9473	0.0413
Cer	d44:2		0.2122	-2.2365	0.0343
Hex2Cer	d29:1		0.2917	-1.7775	0.0494
DG	18:0		0.3826	-1.3862	0.0387
DG	18:1	18:1	0.3700	-1.4343	0.0476
PC	11:0	21:1	0.2169	-2.2048	0.0077
PC	16:0	16:0	2.8089	1.4900	0.0495
PC	17:1	18:1	0.3805	-1.3941	0.0354
PC	18:0	14:1	0.2833	-1.8197	0.0146
PC	18:0	16:1	0.2407	-2.0548	0.0411
PC	18:1	18:3	0.0476	-4.3942	0.0001
PC	19:0	18:2	0.2535	-1.9798	0.0312
PC	20:3	20:4	0.2453	-2.0272	0.0094
PC	40:5		0.1928	-2.3749	0.0003
PC	40:4e		0.2729	-1.8737	0.0491
MePC	37:2		0.1807	-2.4684	0.0002
MePC	37:1e		0.2729	-1.8737	0.0491
PE	16:0	14:0	0.3093	-1.6931	0.0348
PE	16:1	18:1	0.3115	-1.6828	0.0477
PE	18:1	20:5	0.0520	-4.2644	0.0145
PE	18:1	21:1	0.2392	-2.0634	0.0040
PE	18:1	24:0	0.2420	-2.0470	0.0363
PE	28:1	6:0	0.3980	-1.3292	0.0436
PE	16:0p	20:3	0.3698	-1.4350	0.0277
PE	18:0e	20:4	0.2073	-2.2704	0.0106
dMePE	16:0	18:1	0.0868	-3.5267	0.0226
dMePE	18:0	16:0	0.2613	-1.9362	0.0314
dMePE	19:1	18:1	0.0772	-3.6951	0.0000
LdMePE	16:1		0.3042	-1.7167	0.0385
LPE	18:1		0.3112	-1.6841	0.0411
PS	18:0	18:1	0.2725	-1.8757	0.0083
PS	20:1	18:1	0.3636	-1.4596	0.0312
PS	38:1		0.3805	-1.3941	0.0354
PS	42:9		2.5212	1.3341	0.0391
SM	d19:0	15:1	0.3843	-1.3797	0.0452
SM	d19:1	23:0	0.0030	-8.3710	0.0000
SM	d34:0		0.1663	-2.5881	0.0289
SM	d41:2		0.1970	-2.3437	0.0338
SM	d42:1		0.2019	-2.3083	0.0188
SM	t40:1		0.1481	-2.7555	0.0391

Supplemental Table 1. Listed fold change and significance of lipid species affected by loss of ANXA2 after 1 hour of S1P treatment. Lipids that increase with loss of ANXA2 are highlighted yellow.