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Supplementary Materials for

Microphysiological model of PIK3CA-driven vascular malformations reveals a role of dysregulated Rac1 and mTORC1/2 in lesion formation

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The PDF file includes:

Figs. S1 to S11
Legends for movies S1 and S2
Uncropped western blots
Tabular format of data from all figures

Other Supplementary Material for this manuscript includes the following:

Movies S1 and S2

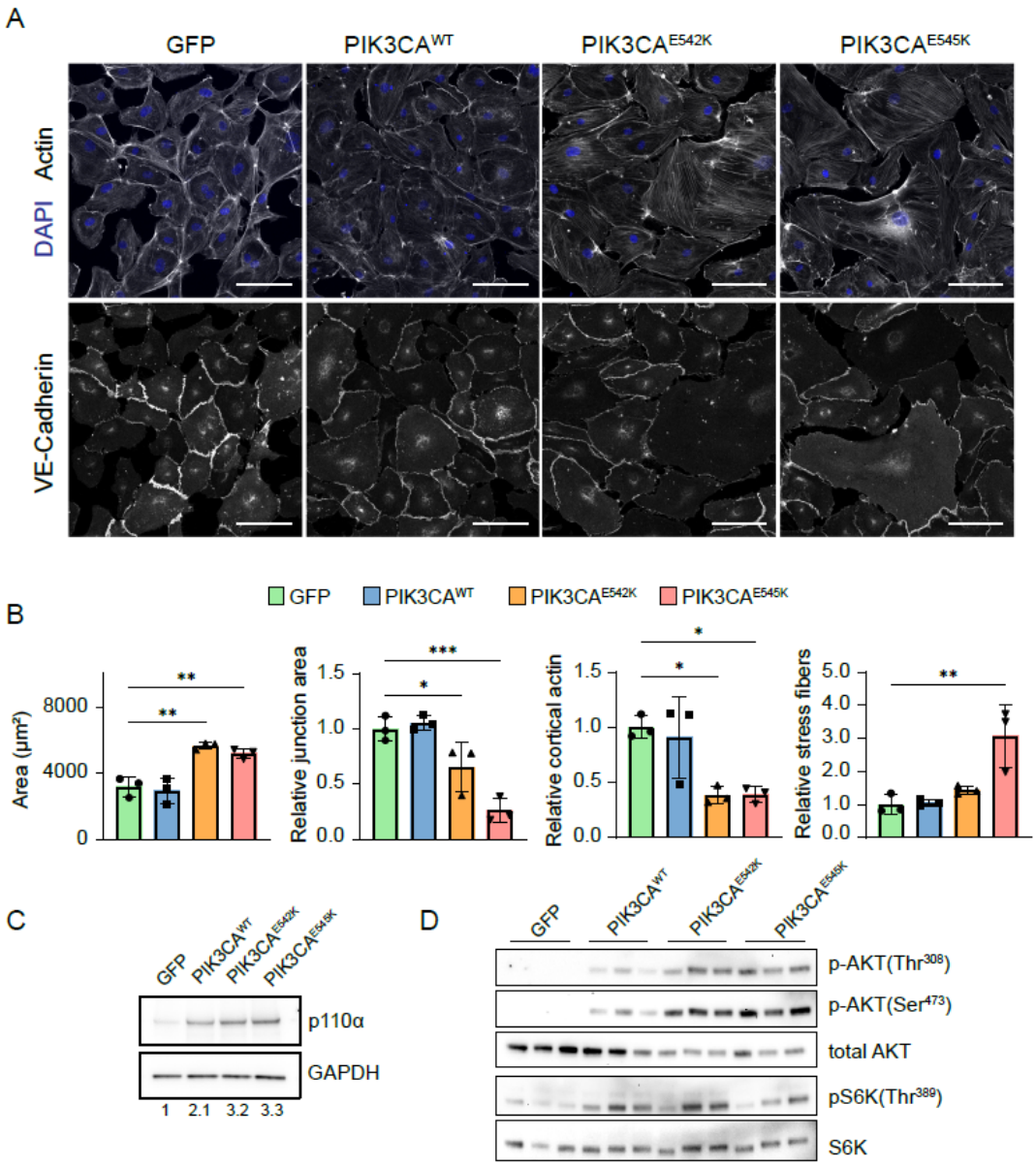


Fig. S1. Increased PI3K/AKT activation and altered cytoskeletal organization in endothelial cells expressing *PIK3CA* activating mutations.

(**A**) Representative confocal images of GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells stained for DAPI, Actin, and VE-Cadherin. Scale bar = 100 μm. (**B**) Quantification of cell area, relative junctional area measured from VE-cadherin staining, relative cortical actin, and stress fiber distributions of GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells. (n = 3; mean ± s.d; One-way ANOVA with Dunnett post-test, *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001). (**C**) Western blot and quantification of p110α expression level in GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells. (**D**) Western blot analysis of relative p-AKT1(Thr308), p-AKT1(Ser473) and p-S6K(Thr389) levels in GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells.

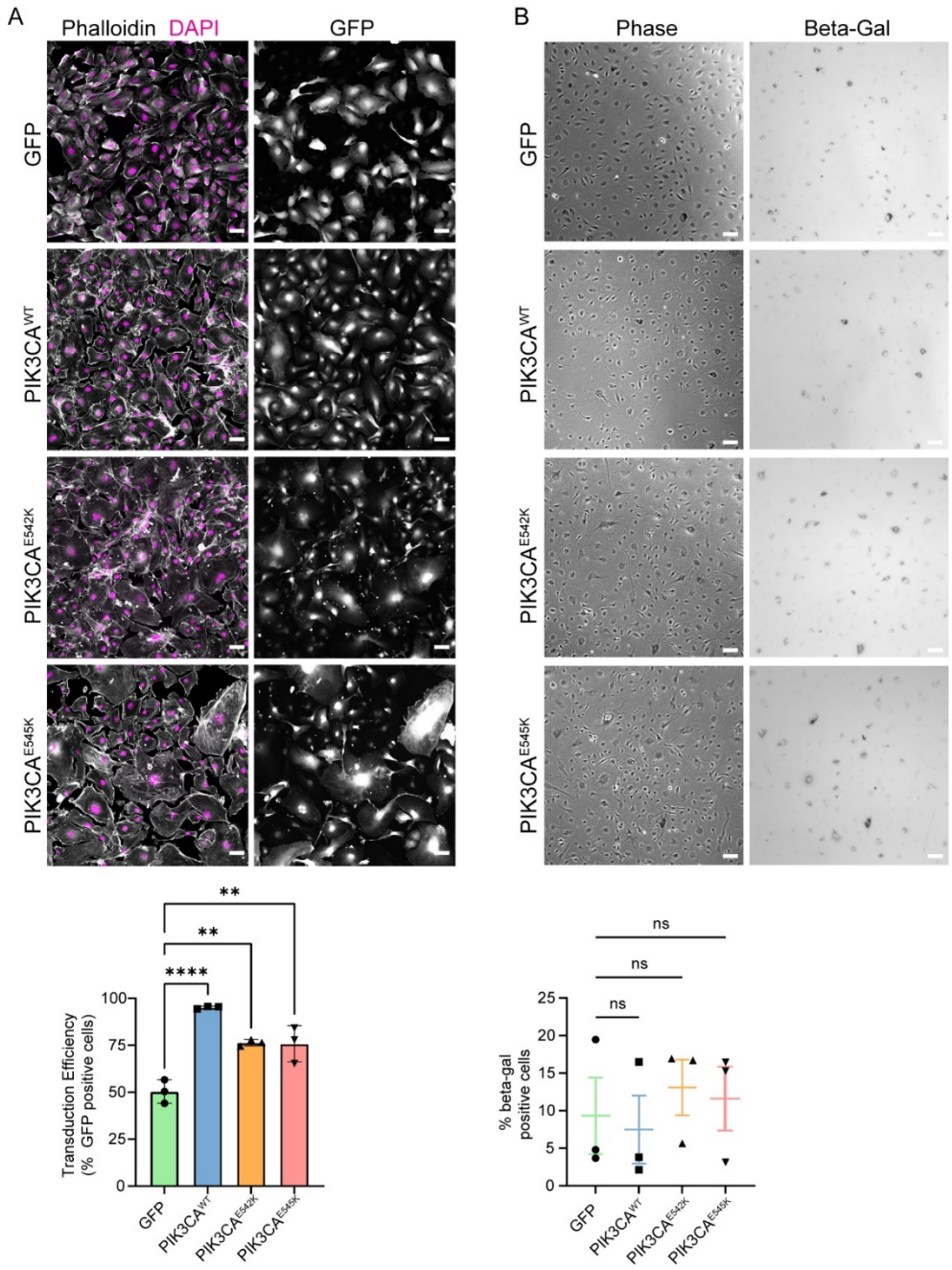


Fig. S2. Characterization of transduction efficiency and senescence-associated beta-galactosidase markers in control and mutant endothelial cells.

(**A**) Confocal images of GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells stained for DAPI, Actin, and GFP. Scale bar = 100 µm. Quantification of transduction efficiency of control and cells expressing PIK3CA activating mutations (n = 3; mean ± s.d; One-way ANOVA with Tukey post test, $^{**}p \leq 0.01$; $^{****}p \leq 0.0001$). (**B**) β-galactosidase staining and quantification for senescent cells. Scale bar = 100 µm (n = 3; mean ± s.e.m; One-way ANOVA with Tukey post-test, ns = not significant).

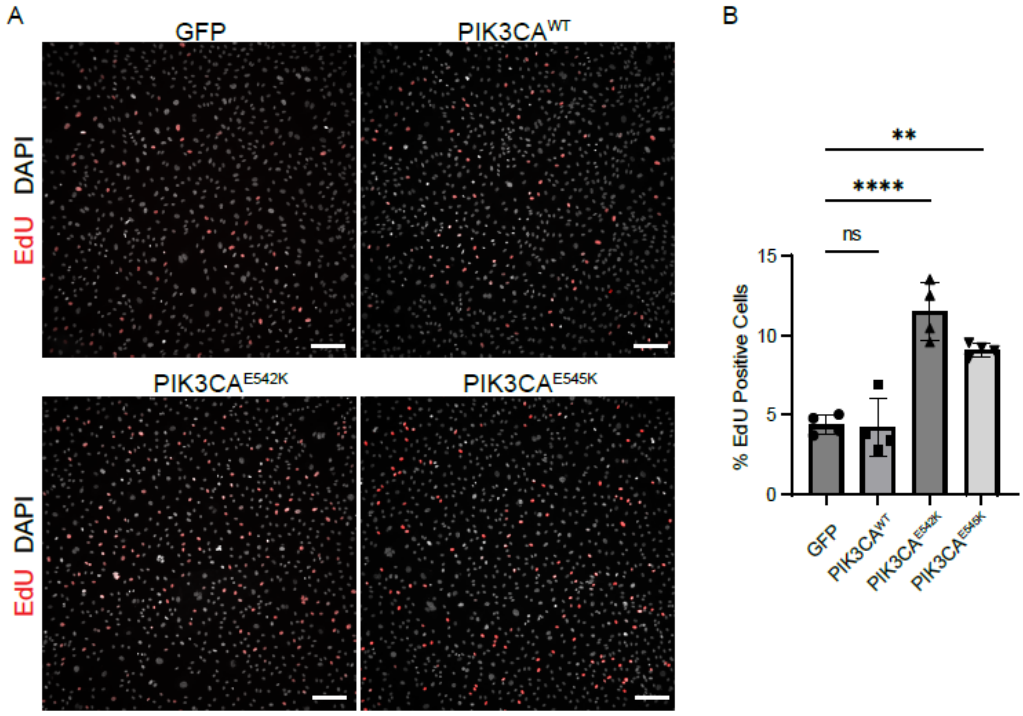


Fig. S3. Elevated cell proliferation in PIK3CA mutant endothelial cells.

(**A**) Representative images of GFP, PIK3CA^{WT}, PIK3CA^{E542K}, and PIK3CA^{E545K} cells stained for DAPI and EdU. Cells were pulse-labeled for 30 minutes with EdU prior to fixation. Scale bar = 100 µm. (**B**) Percentage of Edu positive labelled cells. (n = 4; mean ± s.e.m; One-way ANOVA with Tukey post-test, ns = not significant, **p ≤ 0.01; ****p ≤ 0.0001).

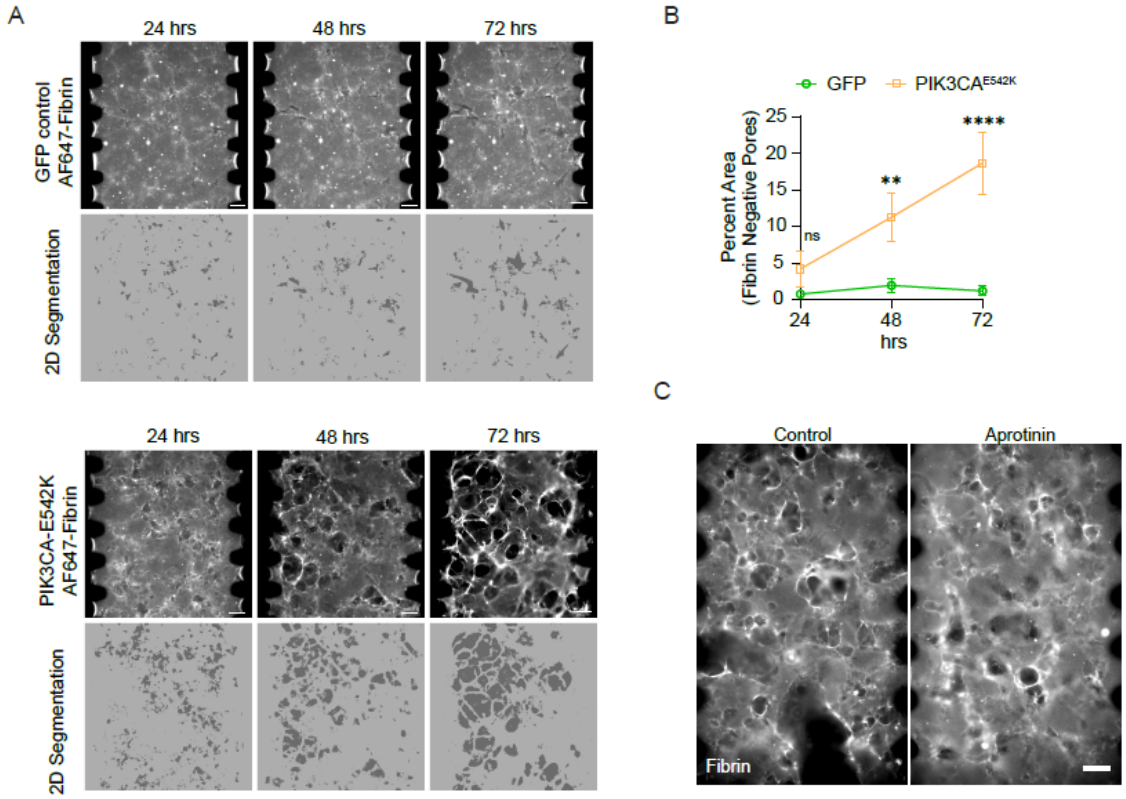


Fig. S4. Dysregulated matrix remodeling and increased fibrinolysis in PIK3CA^{E542K} vascular networks.

(**A**) Wide-field images of control and PIK3CA^{E542K} ECM. Fibrin ECM was fluorescently labeled with AF647-fibrinogen and binary masks for spaces void of fibrin signal were segmented for pore size and percent fibrin negative area quantification. Scale bar = 100 µm. (**B**) Quantification of (A) (n = 3 for GFP control and 6 PIK3CA^{E542K} vascular network; mean ± s.d; One-way ANOVA with Tukey post-test, ns = not significant; $^{**}p \leq 0.01$; $^{****}p \leq 0.0001$). (**C**) Wide-field images of PIK3CAE542K ECM treated with 25µM of Aprotinin, an anti-fibrinolytic agent. Images were captured 24 hours after seeding. Note the decreased fibrin void formation with aprotinin treatment.

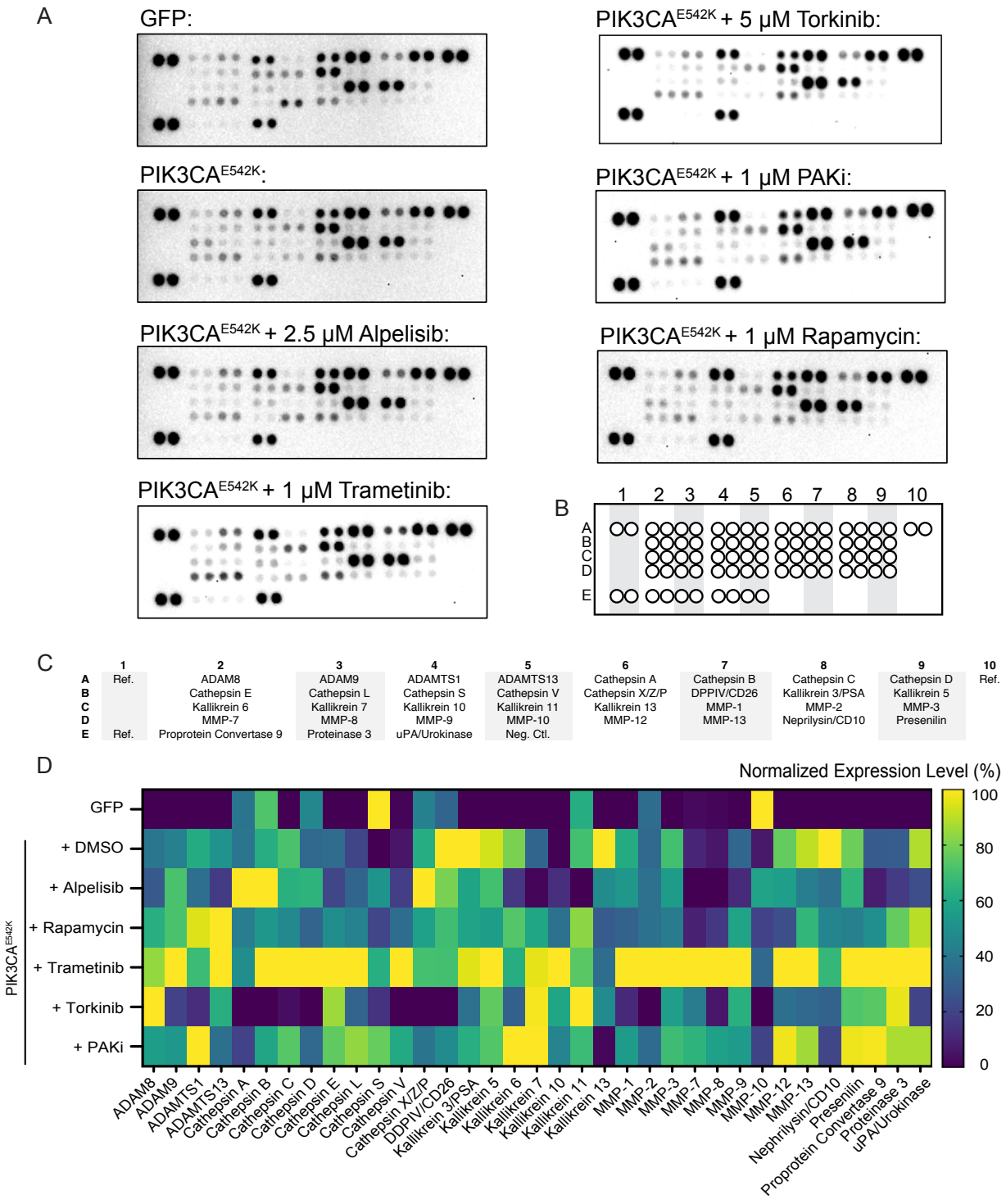


Fig. S5. Expression profile of proteases secreted by GFP control treated with DMSO, PIK3CA^{E542K} treated with DMSO, and PIK3CA^{E542K} cells treated with Alpelisib, Rapamycin, Trametinib, Torkinib, or PAK inhibitor (PF-3758039).

(**A**) Western blot analysis of secreted proteases in conditioned media of endothelial cells cultured overnight with DMSO or respective drug treatments (n = 1). (**B**) Map of spots to indicate

individual proteins as indicated in (**C**). Each protein is measured in duplicate (left and right spots under each labeled column). (**D**) Normalized protease expression profile of (A). The lowest average pixel intensity of each analyte across experimental conditions is defined as 0 %. The highest pixel intensity in each data set was set as 100 %.

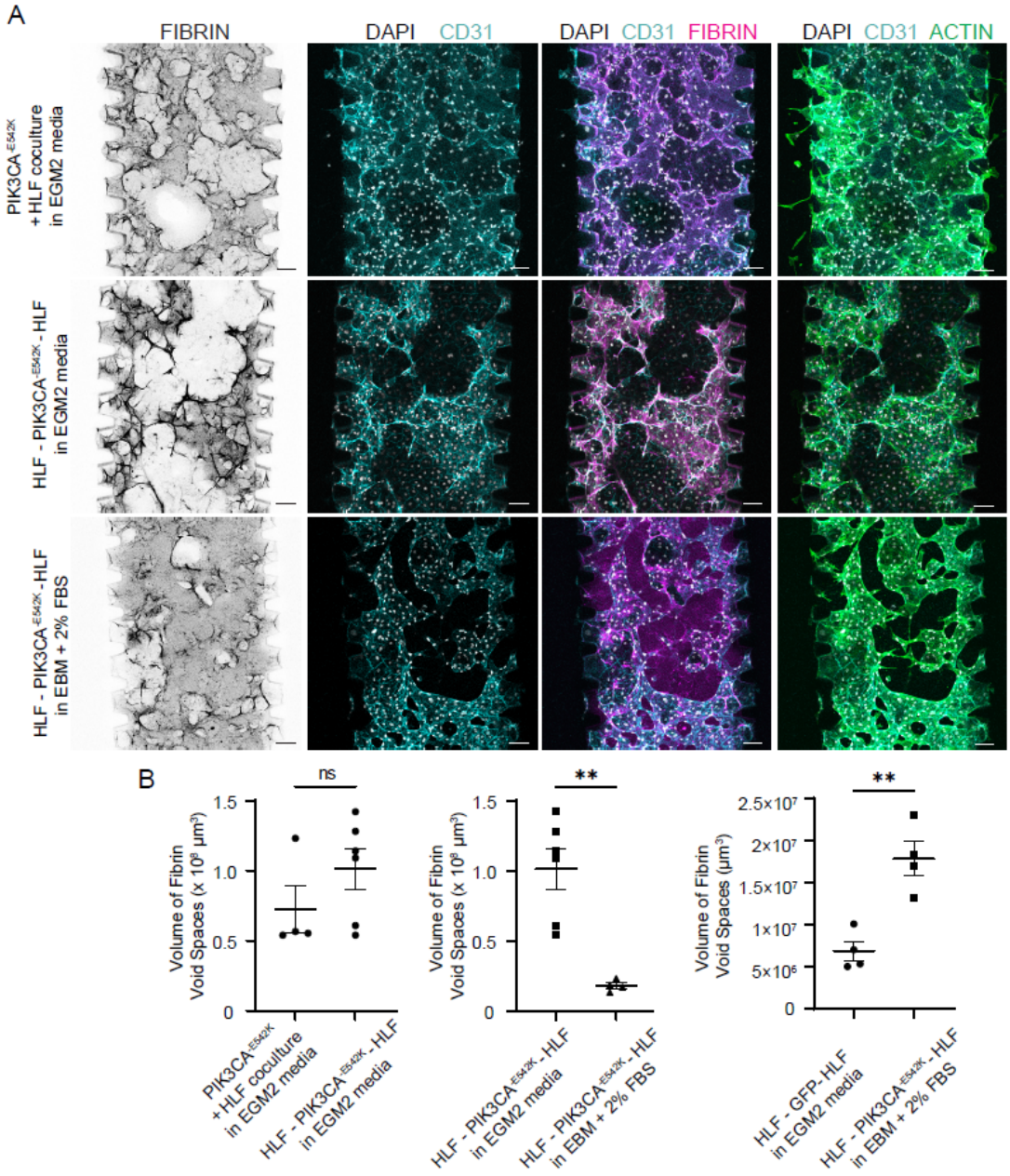


Fig. S6. Characterization of vascular malformation-like phenotype in PIK3CA^{E542K} vascular networks cultured under defined culture conditions.

(**A**) Representative confocal images (maximum intensity projection of the bottom half of vascular networks) of PIK3CA^{E542K} vascular networks labeled with Fibrin (magenta), DAPI (white), CD31 (cyan), and Actin (green). Vascular networks were generated by co-culturing endothelial cells and HLFs in the same compartment (PIK3CA^{E542K} + HLF) or in different spatial compartments (HLF-PIK3CA^{E542K} -HLF). Cells were cultured for 3 days in complete EGM2 media or in reduced growth factors media (EBM + 2% FBS). Scale bar = 100 µm. (**B**) Volumetric quantification of void spaces in fibrin hydrogel of vascular network formed in (A) (n ≥ 3; mean ± s.e.m; two-tailed unpaired Student t-test, ns = not significant; **p ≤ 0.01).

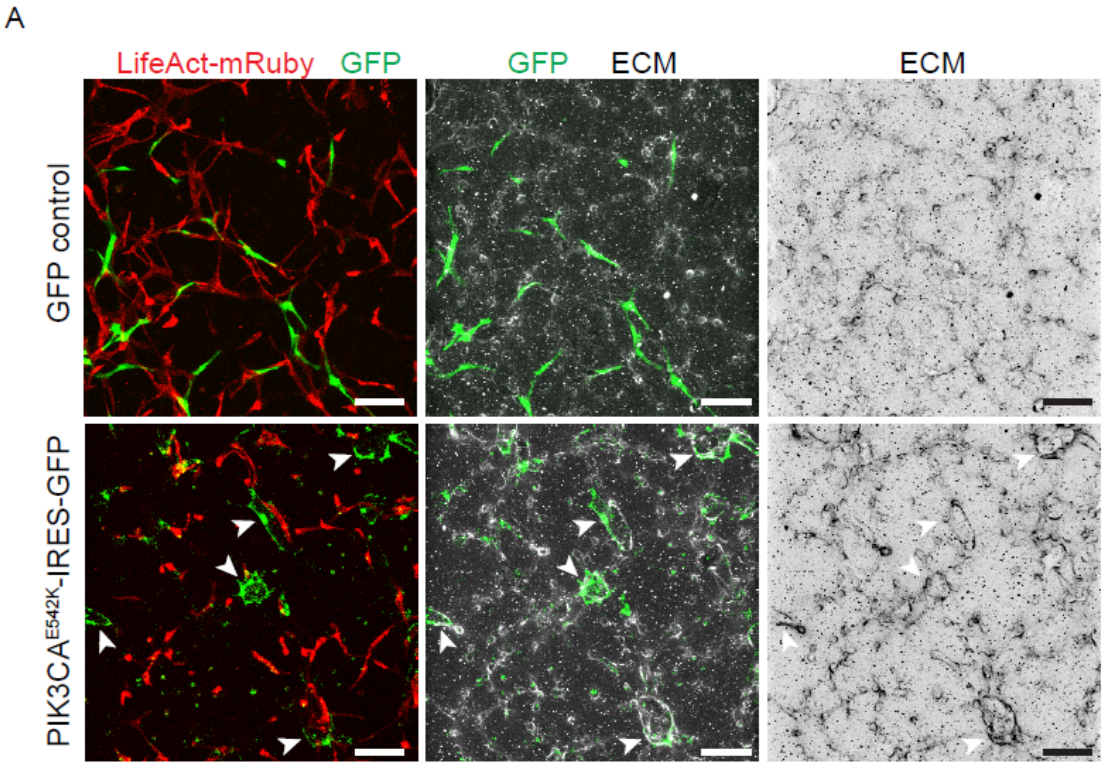


Fig. S7. Localized ECM remodeling and lesion formation by PIK3CA^{E542K} endothelial cells in mosaic vascular network.

(**A**) Representative confocal images of mosaic control and PIK3CA^{E542K} vascular networks. Mosaic vascular networks were generated by co-culturing GFP control(green) or PIK3CA^{E542K} endothelial cells (green, highlighted with arrowhead) with endothelial cells stably expressing LifeAct-mRuby (red) at a ratio of 1:10. Scale bar = 50 μm.

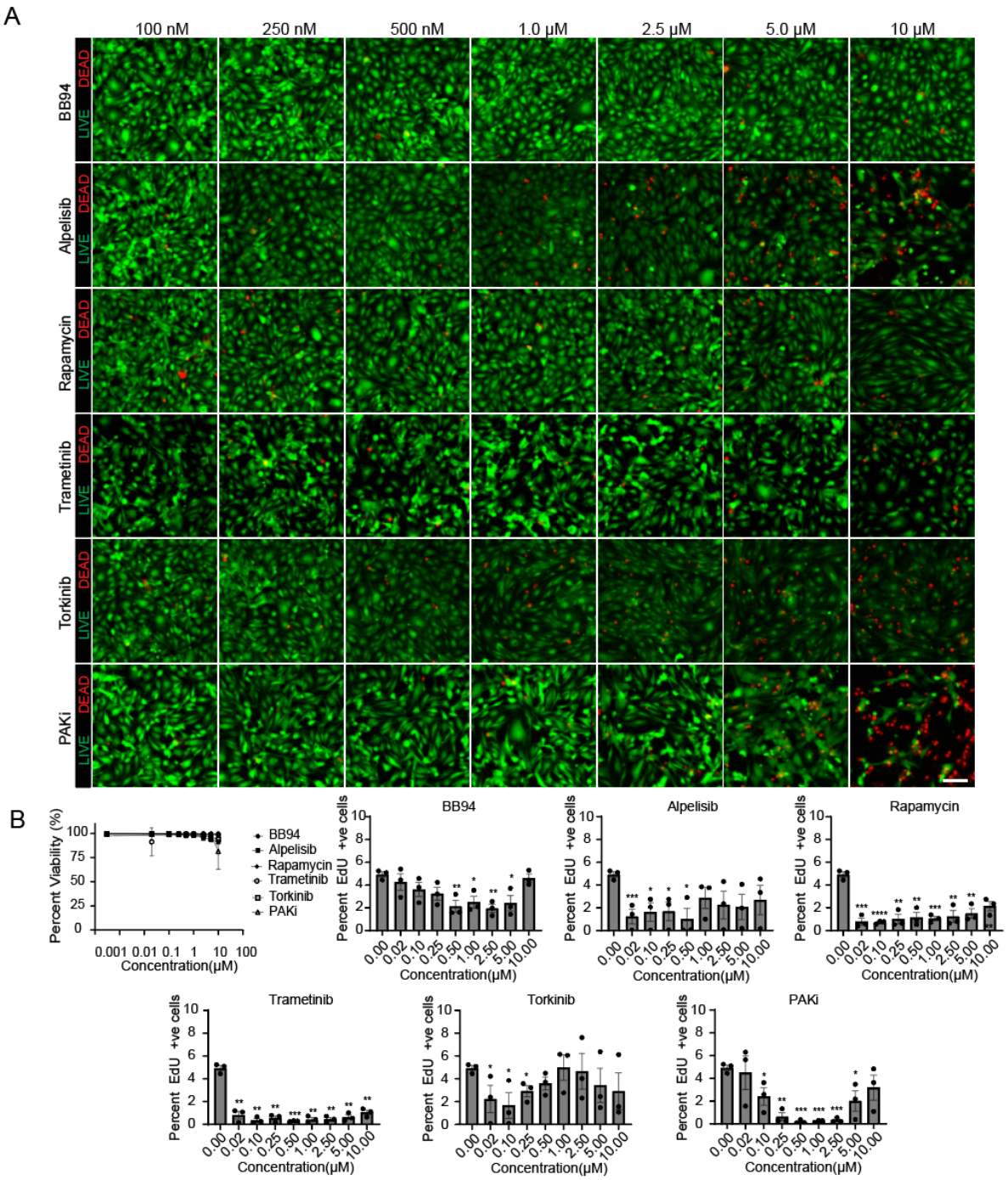


Fig. S8. Cell proliferation and cytotoxicity analysis of drug treatments.

(**A**) Representative images of PIK3CAE542K cells treated with increasing concentration of inhibitors. Live cells were labeled with Calcein AM (green). Dead cells were labeled with Propidium Iodide (red). Scale bar = 100 μm. (**B**) Quantification of live/dead staining in (A) and changes in percent EdU positive cells with drug treatments (n = 3; mean ± s.e.m; two-tailed unpaired Student t-test to DMSO control, *p ≤ 0.05; **p ≤ 0.01; ***p ≤ 0.001, ****p ≤ 0.0001).

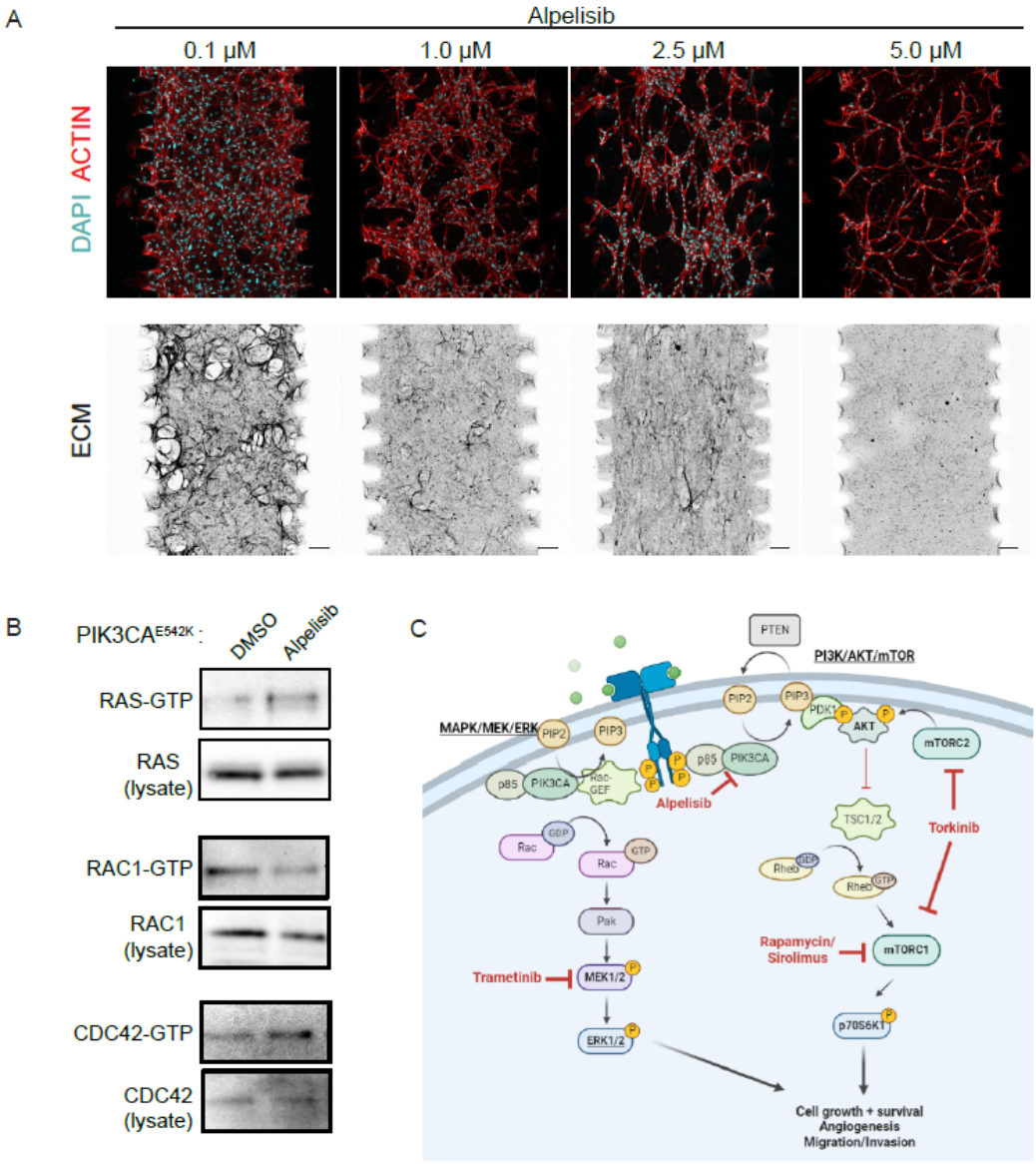


Fig. S9. Effects of Alpelisib treatment on PIK3CA^{E542K} vascular networks.

(**A**) Representative ECM images of PIK3CA^{E542K} vascular networks treated with low to high doses of Alpelisib. Scale bar = 100 μm. (**B**) Active RAS, RAC1, and CDC42 were isolated from PIK3CA^{E542K} cell lysates using GST-Raf-RBD and GST-PBD pull-down. Cells were cultured in a reduced serum condition (0.5 % FBS) and were stimulated with complete EGM2 medium with DMSO or 1.0 μm Alpelisib for 10 minutes before lysis (n = 1). (**C**) Schematic representation of dysregulated PI3K/AKT/mTOR and MEK/ERK signaling in PIK3CA mutant cells. Schematic was created with BioRender.com. Tested inhibitors in this study are highlighted in red.

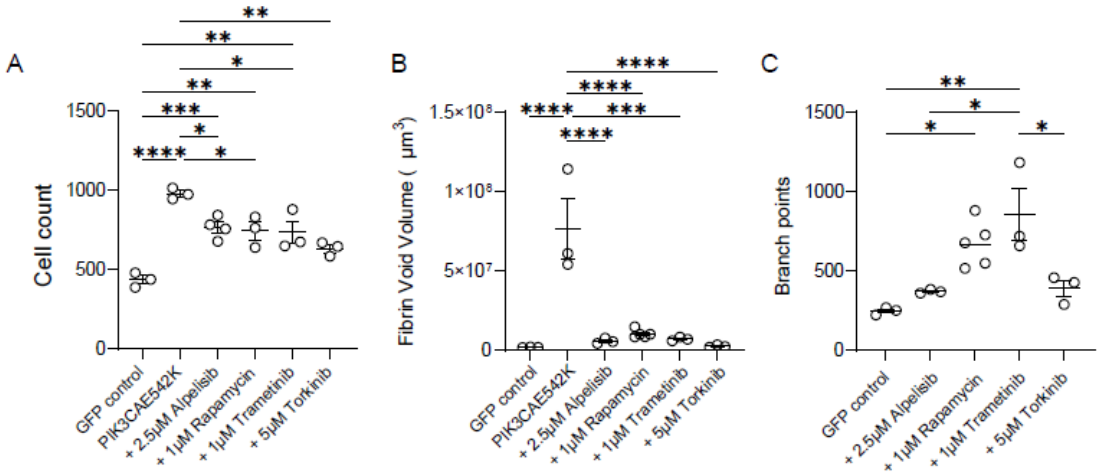


Fig. S10. Effects of Alpelisib, Rapamycin, Trametinib, and Torkinib treatment on PIK3CA^{E542K} vascular network topology.

(**A-C**) Quantification of changes in total cell number (A), matrix (B) and vessel branching morphology (C) with drug inhibitions ($n \geq 3$; mean ± s.e.m; One-way ANOVA with Tukey post-test, *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

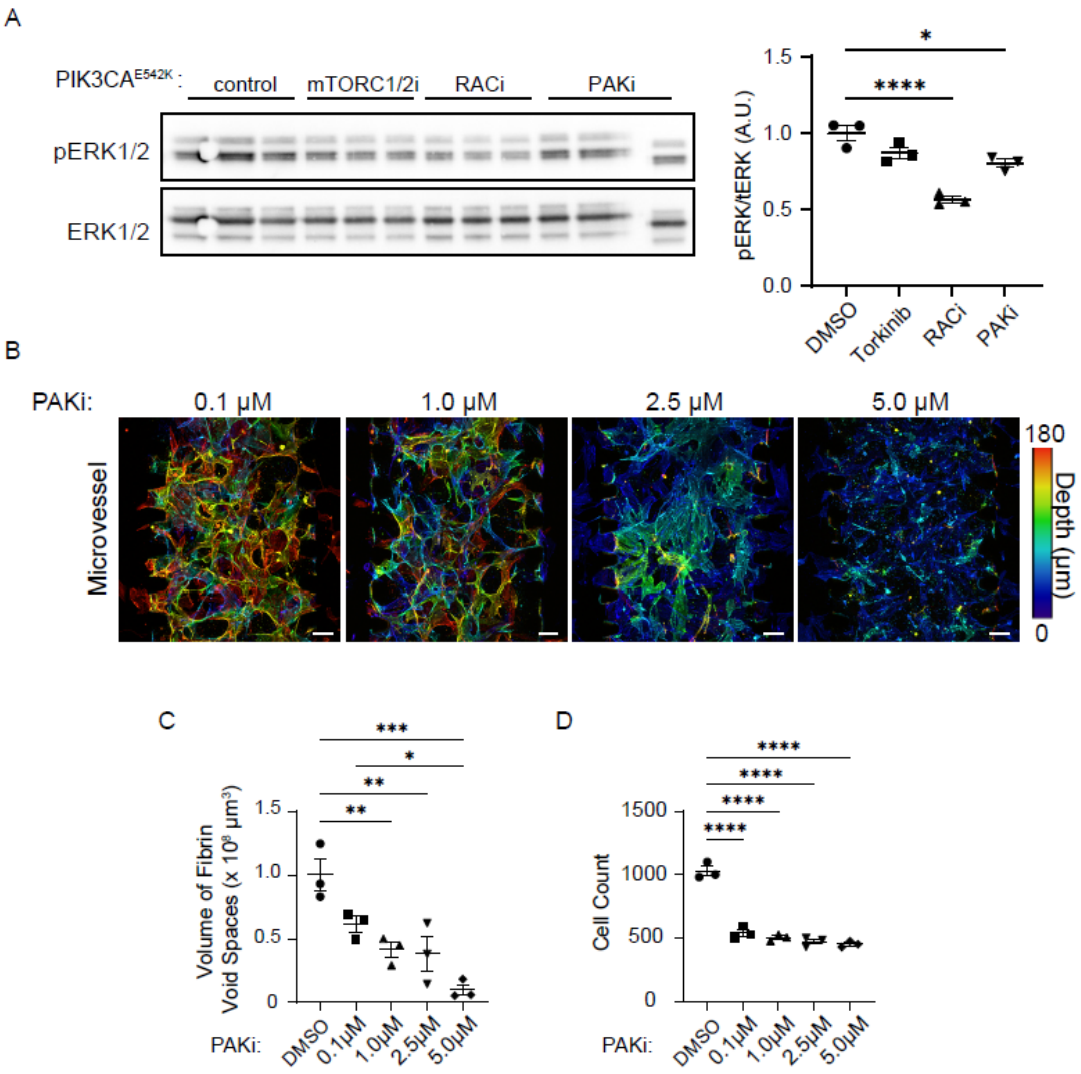


Fig. S11. Effects of Pak inhibition on PIK3CA^{E542K} vascular networks.

(**A**) Western blot analysis and quantification of relative p-ERK1/2(Thr202/Tyr204) in PIK3CA^{E542K} cells treated with DMSO, mTORC1/2 inhibitor (Torkinib PP242, 1 μM), RAC inhibitor (EHT 1864, 1 μM), or PAK inhibitor (PF-3758309, 1 μM) for 24 hours (n = 3; mean ± s.e.m; One-way ANOVA with Dunnett post-test). (**B**) Depth color coded projections of PIK3CA^{E542K} vascular networks treated with 0.1 μM to 5.0 μM concentration of PAK inhibitor, PF-3758309. Scale bar = 100 μm. (**C-D**) Quantification of matrix morphology (C) and cell number (D) in PIK3CA^{E542K} vascular networks treated with PAK inhibitor (n = 3; mean ± s.e.m; One-way ANOVA with Tukey post-test, *$p \leq 0.05$; **$p \leq 0.01$; ***$p \leq 0.001$; ****$p \leq 0.0001$).

Movie S1.
Formation of GFP control and PIK3CA^{E542K} vascular networks over time.

Movie S2.
Example of GFP control and PIK3CA^{E542K} cell membrane ruffling and cell migration.

Uncropped western blots

Figure 5F

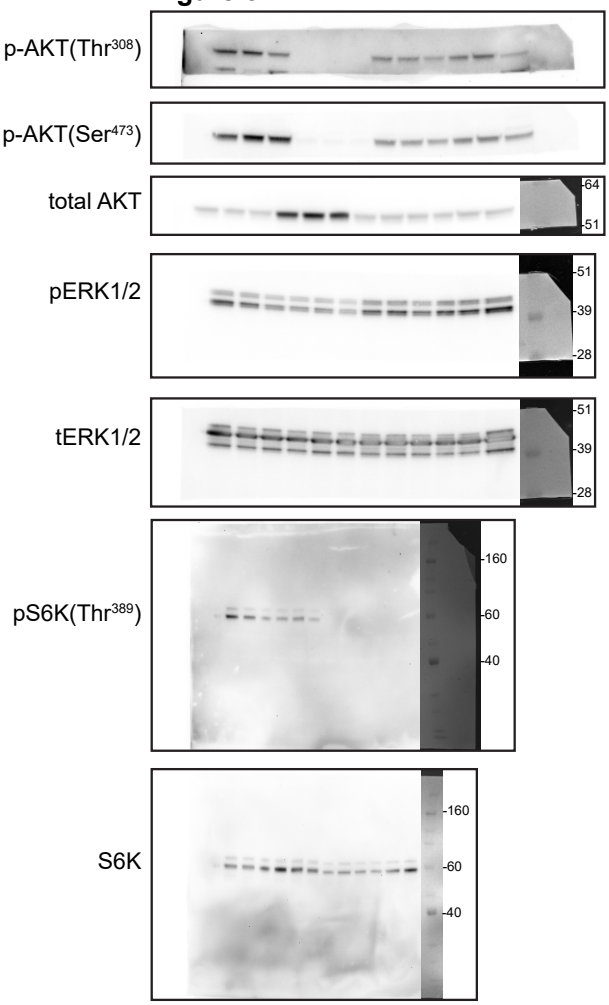


Figure 7A

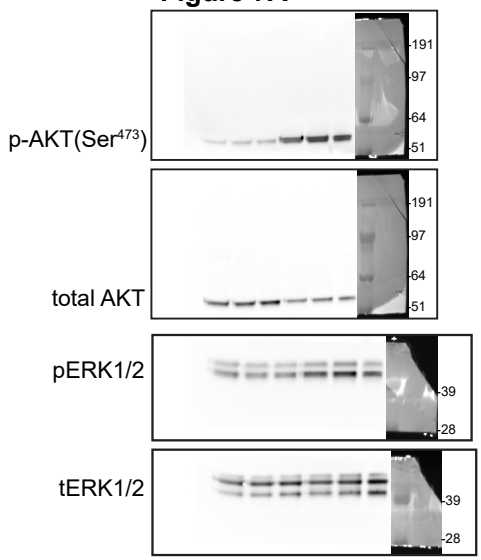


Figure 6A

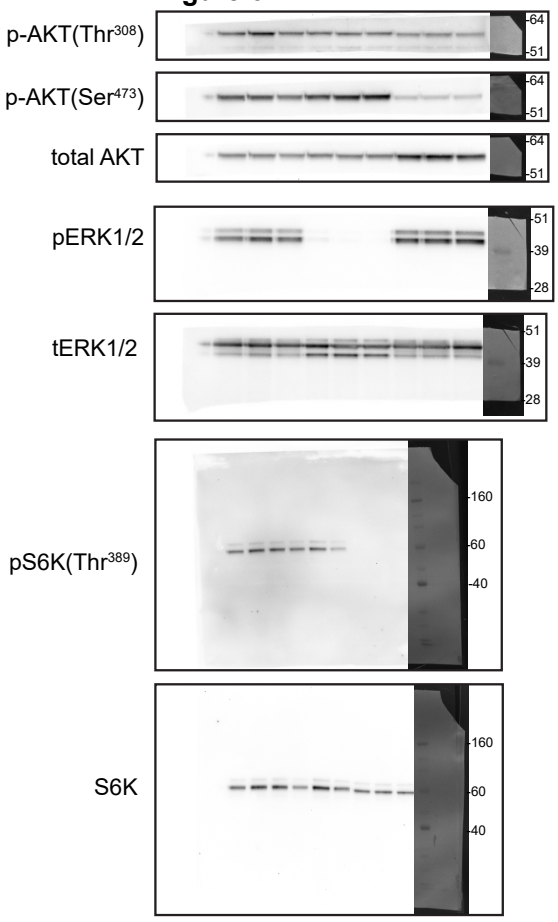


Figure 7E

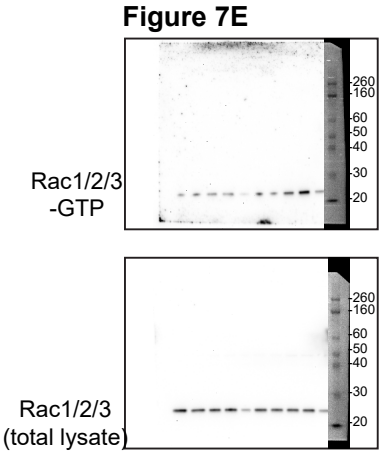


Figure S1C

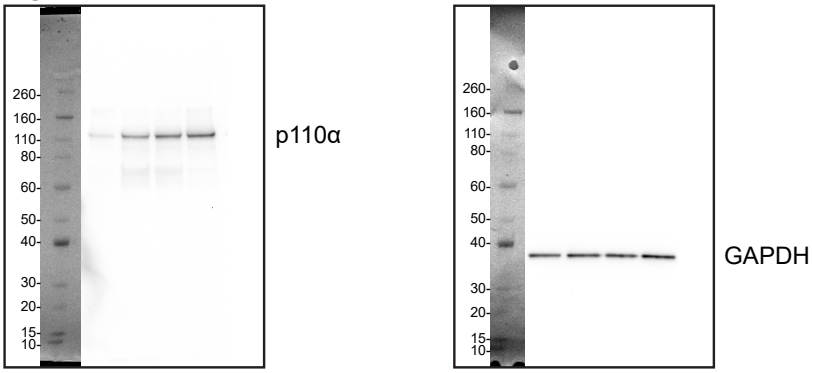


Figure S1D

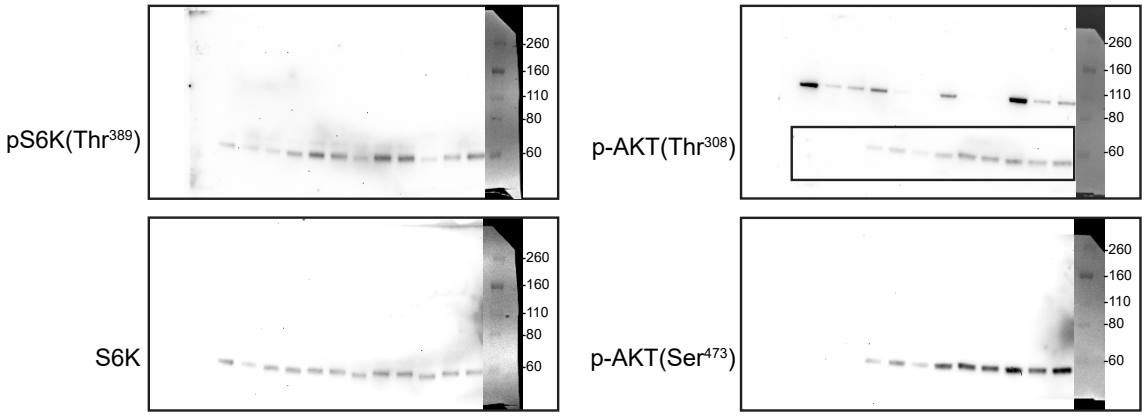


Figure S9B

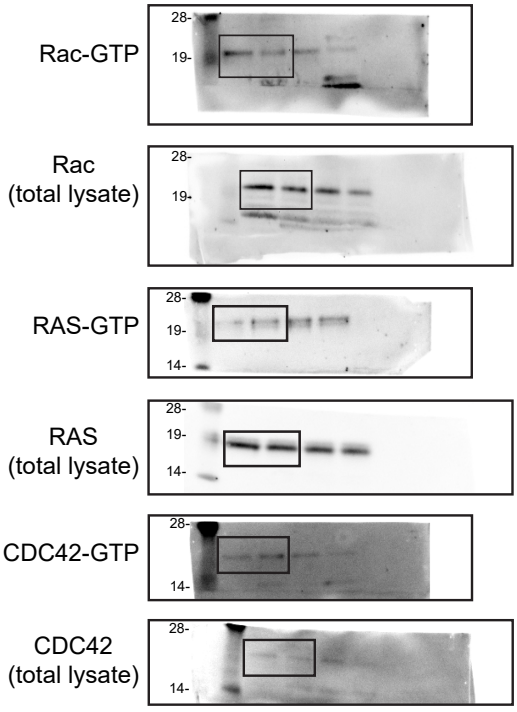
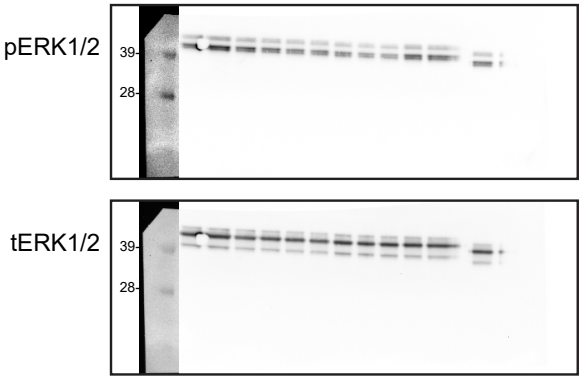


Figure S11A



Tabular data for all figures.

Figure 1d (Area Coverage)

	GFP 24hr	WT 24hr	542 24hr	545 24hr	GFP 168hr	WT 168hr	542 168hr	545 168hr
	30.85	38.98	42.51	63.31	41.33	43.63	65.76	65.83
	33.31	39.29	35.75	64.04	30.55	30.06	63.64	72.27
	37.99	41.88	38.17	65.14	26.91	38.74	66.17	63.17
	25.17	37.12	30.39	71.75	29.21	38.9	55.21	61.61
			35.95	62.52			57.61	53.93
			36.06	69.58			57.64	66.29
Mean	31.83	39.3175	36.47166667	66.05666667	32	37.8325	61.005	63.85
SEM	2.668520189	0.979373737	1.605146757	1.525203083	3.199526007	2.828219036	1.937883295	2.480972927

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP - 24hrs vs. WT - 24hrs	-7.487	-15.39 to 0.4135	No	ns	0.0664
GFP - 24hrs vs. 542 - 24hrs	-4.642	-11.85 to 2.571	No	ns	0.2912
GFP - 24hrs vs. 545 - 24hrs	-34.23	-41.44 to -27.01	Yes	****	<0.0001
WT - 24hrs vs. 542 - 24hrs	2.846	-4.367 to 10.06	No	ns	0.6777
WT - 24hrs vs. 545 - 24hrs	-26.74	-33.95 to -19.53	Yes	****	<0.0001
542 - 24hrs vs. 545 - 24hrs	-29.59	-36.04 to -23.13	Yes	****	<0.0001
GFP - 168 Hrs vs. WT - 168 Hrs	-5.833	-17.32 to 5.658	No	ns	0.4871
GFP - 168 Hrs vs. 542 - 168 Hrs	-29.01	-39.49 to -18.52	Yes	****	<0.0001
GFP - 168 Hrs vs. 545 - 168 Hrs	-31.85	-42.34 to -21.36	Yes	****	<0.0001
WT - 168 Hrs vs. 542 - 168 Hrs	-23.17	-33.66 to -12.68	Yes	****	<0.0001
WT - 168 Hrs vs. 545 - 168 Hrs	-26.02	-36.51 to -15.53	Yes	****	<0.0001
542 - 168 Hrs vs. 545 - 168 Hrs	-2.845	-12.23 to 6.537	No	ns	0.8213

Figure 1d (Norm Network Thickness)

	GFP	WT	542	545
	0.75422159	0.88686131	0.64712932	0.32138577
	1.08168101	0.78725896	0.56623815	0.32138577
	1.01397211	0.78725896	0.88980281	0.35352435
	1.15012529	0.9045103	0.77655518	0.32138577
				0.30531648
				0.30531648
Mean	1	0.8415	0.7199	0.3214
SEM	0.086511867	0.031506777	0.071288327	0.008801513

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. PIK3CA-WT	0.1585	-0.06409 to 0.3811	No	ns	0.2104
GFP vs. PIK3CA-E542K	0.2801	0.05745 to 0.5027	Yes	*	0.0122
GFP vs. PIK3CA-E545K	0.6786	0.4754 to 0.8818	Yes	****	<0.0001
PIK3CA-WT vs. PIK3CA-E542K	0.1215	-0.1011 to 0.3442	No	ns	0.4167
PIK3CA-WT vs. PIK3CA-E545K	0.5201	0.3169 to 0.7233	Yes	****	<0.0001
PIK3CA-E542K vs. PIK3CA-E545K	0.3985	0.1953 to 0.6018	Yes	***	0.0003

Figure 1d (Average Pore Size micron^2)

	GFP 24hr	PIK3CA 24hr	GFP 48hr	PIK3CA 48hr	GFP 72hr	PIK3CA 72hr
	1546.043	2034.924	1381.741	1957.631	1689.239	3771.092
	1219.311	2344.534	1351.683	2471.779	1181.039	4328.325
	1126.589	1091.13	1600.062	1874.401	901.511	2715.362
		1796.101		2485.222		4628.513
		1848.835		2058.654		5917.799
		1801.728		2142.79		5338.183
Mean	1297.314333	1819.542	1444.495333	2165.0795	1257.263	4449.879
SEM	127.2122	168.7518	78.2658	105.8155	230.5692	464.0352

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP 24hr vs. PIK3CA 24hr	-522.2	-1896 to 851.9	No	ns	0.8371
GFP 24hr vs. GFP 48hr	-147.2	-1734 to 1440	No	ns	0.9997
GFP 24hr vs. PIK3CA 48hr	-867.8	-2242 to 506.4	No	ns	0.3879
GFP 24hr vs. GFP 72hr	40.05	-1547 to 1627	No	ns	>0.9999
GFP 24hr vs. PIK3CA 72hr	-3153	-4527 to -1778	Yes	****	<0.0001
PIK3CA 24hr vs. GFP 48hr	375	-999.1 to 1749	No	ns	0.9533
PIK3CA 24hr vs. PIK3CA 48hr	-345.5	-1468 to 776.4	No	ns	0.9244
PIK3CA 24hr vs. GFP 72hr	562.3	-811.9 to 1936	No	ns	0.7922
PIK3CA 24hr vs. PIK3CA 72hr	-2630	-3752 to -1508	Yes	****	<0.0001
GFP 48hr vs. PIK3CA 48hr	-720.6	-2095 to 653.6	No	ns	0.5827
GFP 48hr vs. GFP 72hr	187.2	-1399 to 1774	No	ns	0.999
GFP 48hr vs. PIK3CA 72hr	-3005	-4380 to -1631	Yes	****	<0.0001
PIK3CA 48hr vs. GFP 72hr	907.8	-466.3 to 2282	No	ns	0.3409
PIK3CA 48hr vs. PIK3CA 72hr	-2285	-3407 to -1163	Yes	****	<0.0001
GFP 72hr vs. PIK3CA 72hr	-3193	-4567 to -1818	Yes	****	<0.0001

Figure 2b (Volume of Fibrin Void Space)

	GFP	PIK3CA-E542K	PIK3CA-E542K no HLF
	1378310	59787357	22542606.81
	1776301	51188532	32820259
	1501003	95825764	39937440.14
			47498128.07
MEAN	1551871.3	68933884.3	35699608.5
SEM	117671.7	13673146.9	5311635.2

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. PIK3CA-E542K	-67382013	-102114138 to -32649888	Yes	**	0.0018
GFP control vs. PIK3CA-E542K no HLF	-34147737	-66636666 to -1658809	Yes	*	0.0408
PIK3CA-E542K vs. PIK3CA-E542K no HLF	33234276	745347 to 65723204	Yes	*	0.0456

Figure 4b (Cell count)

	GFP control	DMSO control	mitoC	BB94	Y27632
	439	871	408	847	834
	481	856	395	824	840
	389	786	429	730	882
MEAN	436.3	837.7	410.7	800.3	852
SEM	26.5916	26.1937	9.9051	35.7880	15.0997

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. untreated PIK3CAE542K	-401.3	-515.3 to -287.3	Yes	****	<0.0001
GFP control vs. mitoC	25.67	-88.34 to 139.7	No	ns	0.9416
GFP control vs. 1uM BB94	-364	-478.0 to -250.0	Yes	****	<0.0001
GFP control vs. 10uM Y27362	-415.7	-529.7 to -301.7	Yes	****	<0.0001
DMSO control vs. mitoC	427	313.0 to 541.0	Yes	****	<0.0001
DMSO control vs. 1uM BB94	37.33	-76.68 to 151.3	No	ns	0.8138
DMSO control vs. 10uM Y27362	-14.33	-128.3 to 99.68	No	ns	0.9929
mitoC vs. 1uM BB94	-389.7	-503.7 to -275.7	Yes	****	<0.0001
mitoC vs. 10uM Y27362	-441.3	-555.3 to -327.3	Yes	****	<0.0001
1uM BB94 vs. 10uM Y27362	-51.67	-165.7 to 62.34	No	ns	0.5894

Figure 4b (Volume of Fibrin Void Space)

	GFP control	DMSO control	mitoC	BB94	Y27632
	1378310	59787357	33527609	4564840	60674538
	1776301	51188532	11942848	4757205	54279421
	1501003	95825764	16849576	5556684	41703539
MEAN	1551871.333	68933884.33	20773344.33	4959576.333	52219166
SEM	117671.7178	13673146.9415	6532545.3260	303674.3033	5572497.6077

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. DMSO control	-67382013	-100995412 to -33768614	Yes	***	0.0005
GFP control vs. mitoC	-19221473	-52834872 to 14391926	No	ns	0.3843
GFP control vs. 1uM BB94	-3407705	-37021104 to 30205694	No	ns	0.9969
GFP control vs. 10uM Y27362	-50667295	-84280693 to -17053896	Yes	**	0.004
DMSO control vs. mitoC	48160540	14547141 to 81773939	Yes	**	0.0057
DMSO control vs. 1uM BB94	63974308	30360909 to 97587707	Yes	***	0.0007
DMSO control vs. 10uM Y27362	16714718	-16898680 to 50328117	No	ns	0.5089
mitoC vs. 1uM BB94	15813768	-17799631 to 49427167	No	ns	0.5575
mitoC vs. 10uM Y27362	-31445822	-65059220 to 2167577	No	ns	0.0695
1uM BB94 vs. 10uM Y27362	-47259590	-80872988 to -13646191	Yes	**	0.0065

Figure 4c (Branch Points Count)

	GFP control	mitoC	BB94
	265	821	1210
	248	662	1523
	219	505	2204
MEAN	244	662.6666667	1645.666667
SEM	13.4288	91.2220	293.4248

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. mitoC	-418.7	-1189 to 351.9	No	ns	0.2915
GFP control vs. BB94	-1402	-2172 to -631.1	Yes	**	0.0034
mitoC vs. BB94	-983	-1754 to -212.5	Yes	*	0.0184

Figure 4d (Mean Segment Length)

	GFP control	mitoC 542	BB94 542
	49.73214	39.10894	35.78064
	54.52732	43.8047	33.65594
	53.33863	39.91494	34.84538

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. mitoC	11.59	6.241 to 16.94	Yes	**	0.0014
GFP control vs. BB94	17.77	12.42 to 23.12	Yes	***	0.0001
mitoC vs. BB94	6.182	0.8336 to 11.53	Yes	*	0.0281

Figure 5b (Cell count)

	DMSO	Rapamycin	Alpelisib
	976	641	679
	1015	764	758
	947	833	844
			784
MEAN	979.3333333	746	766.25
SEM	19.7005	56.1516	34.2062

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. Sirolimus	233.3	62.60 to 404.1	Yes	*	0.0122
DMSO vs. Alpelisib	213.1	53.38 to 372.8	Yes	*	0.0137
Rapamycin vs. Alpelisib	-20.25	-180.0 to 139.5	No	ns	0.9268

Figure 5c (Volume of Fibrin Void Spaces)

	DMSO	GFP	Rapamycin	Alpelisib
	114000000	1378310	8182002	5228440
	60849879	1776301	9827182	7490028
	53909663	1501003	14530380	4045672
MEAN	76253180.67	1551871.333	10846521.33	5588046.667
SEM	18979448.7703	117671.7178	1902171.1556	1010426.4413

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. GFP control	74701309	31447470 to 117955148	Yes	**	0.0025
DMSO vs. Rapamycin	65406659	22152820 to 108660498	Yes	**	0.0056
DMSO vs. Alpelisib	70665134	27411295 to 113918973	Yes	**	0.0035
GFP control vs. Rapamycin	-9294650	-52548489 to 33959189	No	ns	0.8988
GFP control vs. Alpelisib	-4036175	-47290014 to 39217664	No	ns	0.99
Rapamycin vs. Alpelisib	5258475	-37995364 to 48512314	No	ns	0.9786

Figure 5d (Mean Segment Length)

	GFP	Rapamycin	Alpelisib
	54.52732	38.68098	47.54549
	49.73214	35.08685	52.42514
	53.33863	38.97669	46.79459
		43.22453	
		37.18176	
MEAN	52.53269667	38.630162	48.92174
SEM	1.4417	1.3395	1.7651

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. Rapamycin	13.9	7.861 to 19.94	Yes	***	0.0004
GFP vs. Alpelisib	3.611	-3.143 to 10.37	No	ns	0.3292
Rapamycin vs. Alpelisib	-10.29	-16.33 to -4.250	Yes	**	0.0031

Figure 5e (Branch Point Count)

	GFP	Rapamycin	Alpelisib
	219	545	366
	265	513	357
	248	878	380
		674	
		724	
MEAN	244	666.8	367.6666667
SEM	13.4288	65.7339	6.6916

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. Rapamycin	-422.8	-641.4 to -204.2	Yes	**	0.0014
GFP vs. Alpelisib	-123.7	-368.0 to 120.7	No	ns	0.3645
Rapamycin vs. Alpelisib	299.1	80.56 to 517.7	Yes	*	0.0111

Fig 5F- pAKT308

	DMSO	Alpelisib	20nM Rapamycin	1uM Rapamycin
	1.266717	0.020655	0.66835	0.587048
	1.141618	0.028965	0.536179	0.474362
	0.591665	0.016542	0.635932	0.380224
MEAN	1	0.022054	0.613487	0.480544667
SEM	0.2073	0.0037	0.0398	0.0598

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO control vs. Alpelisib	0.9779	0.4810 to 1.475	Yes	**	0.001
DMSO control vs. Rapamycin 1uM	0.5195	0.02253 to 1.016	Yes	*	0.0408
DMSO control vs. Rapamycin 20nM	0.3865	-0.1104 to 0.8834	No	ns	0.1361
Alpelisib vs. Rapamycin 1uM	-0.4585	-0.9554 to 0.03843	No	ns	0.0709
Alpelisib vs. Rapamycin 20nM	-0.5914	-1.088 to -0.09451	Yes	*	0.0215
Rapamycin 1uM vs. Rapamycin 20nM	-0.1329	-0.6299 to 0.3640	No	ns	0.8264

Fig 5F- pAKT473

	DMSO	Alpelisib	20nM Rapamycin	1uM Rapamycin	
	0.744361	0.022532	0.467843	0.349861	
	1.23111	0.007306	0.446464	0.304004	
	1.024528	0.012636	0.374178	0.308437	
MEAN	0.999999667	0.014158	0.429495	0.320767333	
SEM	0.1410	0.0045	0.0283	0.0146	

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO control vs. Alpelisib	0.9858	0.6582 to 1.313	Yes	****	<0.0001
DMSO control vs. Rapamycin 1uM	0.6792	0.3516 to 1.007	Yes	***	0.0007
DMSO control vs. Rapamycin 20nM	0.5705	0.2429 to 0.8981	Yes	**	0.0023
Alpelisib vs. Rapamycin 1uM	-0.3066	-0.6342 to 0.02099	No	ns	0.0668
Alpelisib vs. Rapamycin 20nM	-0.4153	-0.7429 to -0.08774	Yes	*	0.0154
Rapamycin 1uM vs. Rapamycin 20nM	-0.1087	-0.4363 to 0.2189	No	ns	0.7199

Fig 5F- pS6K1

	DMSO	Alpelisib	20nM Rapamycin	1uM Rapamycin	
	1.513157	0.281381	0.100334	0.131519	
	1.017351	0.421832	0.060366	0.078915	
	0.469492	0.281487	0.018947	0.041505	
MEAN	1	0.328233333	0.059882333	0.083979667	
SEM	0.3014	0.0468	0.0235	0.0261	

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO control vs. Alpelisib	0.6718	-0.02348 to 1.367	No	ns	0.0582
DMSO control vs. Rapamycin 1uM	0.916	0.2208 to 1.611	Yes	*	0.0124
DMSO control vs. Rapamycin 20nM	0.9401	0.2449 to 1.635	Yes	*	0.0108
Alpelisib vs. Rapamycin 1uM	0.2443	-0.4510 to 0.9395	No	ns	0.6855
Alpelisib vs. Rapamycin 20nM	0.2684	-0.4269 to 0.9636	No	ns	0.6234
Rapamycin 1uM vs. Rapamycin 20nM	0.0241	-0.6711 to 0.7193	No	ns	0.9995

Fig 5F- pERK

	DMSO	Alpelisib	20nM Rapamycin	1uM Rapamycin	
	1.094149	0.784358	1.647677	1.390611	
	1.111776	0.741303	1.70151	1.469345	
	0.794074	0.650274	1.752832	1.243659	
MEAN	0.999999667	0.725311667	1.700673	1.367871667	
SEM	0.1031	0.0395	0.0304	0.0661	

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO control vs. Alpelisib	0.2747	-0.02473 to 0.5741	No	ns	0.0726
DMSO control vs. Rapamycin 1uM	-0.3679	-0.6673 to -0.06845	Yes	*	0.0182
DMSO control vs. Rapamycin 20nM	-0.7007	-1.000 to -0.4013	Yes	***	0.0003
Alpelisib vs. Rapamycin 1uM	-0.6426	-0.9420 to -0.3431	Yes	***	0.0006
Alpelisib vs. Rapamycin 20nM	-0.9754	-1.275 to -0.6759	Yes	****	<0.0001
Rapamycin 1uM vs. Rapamycin 20nM	-0.3328	-0.6322 to -0.03338	Yes	*	0.0304

Figure 6c (pAKT308)

	DMSO	Trametinib	Torkinib		
	0.93727	0.78084	0.306226		
	1.1537	0.702136	0.315915		
	0.909029	0.672963	0.280022		
MEAN	0.999999667	0.718646333	0.300721		
SEM	0.0773	0.0322	0.0107		

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. Trametinib	0.2814	0.06988 to 0.4928	Yes	*	0.0153
DMSO vs. Torkinib	0.6993	0.4878 to 0.9107	Yes	***	0.0001
Trametinib vs. Torkinib	0.4179	0.2065 to 0.6294	Yes	**	0.0022

Figure 6d (pAKT473)

	DMSO	Trametinib	Torkinib		
	1.094114	1.118049	0.19541		
	0.946319	1.240642	0.141743		
	0.959566	1.288045	0.15493		
MEAN	0.999999667	1.215578667	0.164027667		
SEM	0.0472	0.0506	0.0161		

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. Trametinib	-0.2156	-0.3937 to -0.03746	Yes	*	0.0232
DMSO vs. Torkinib	0.836	0.6579 to 1.014	Yes	****	<0.0001
Trametinib vs. Torkinib	1.052	0.8734 to 1.230	Yes	****	<0.0001

Figure 6e (pS6K1)

	DMSO	Trametinib	Torkinib
	1.084068	1.284746	0.061605
	0.972289	0.840975	0.074794
	0.943643	0.576969	0.114293
MEAN	1	0.900896667	0.083564
SEM	0.0428	0.2065	0.0158

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. Trametinib	0.0991	-0.4307 to 0.6289	No	ns	0.8385
DMSO vs. Torkinib	0.9164	0.3866 to 1.446	Yes	**	0.0044
Trametinib vs. Torkinib	0.8173	0.2875 to 1.347	Yes	**	0.0077

Figure 6f (pERK)

	DMSO	Trametinib	Torkinib
	0.891142	0.073555	1.256429
	0.986796	0.026483	1.118652
	1.122062	0.039341	1.154616
MEAN	1	0.046459667	1.176565667
SEM	0.0670	0.0140	0.0413

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. Trametinib	0.9535	0.7533 to 1.154	Yes	****	<0.0001
DMSO vs. Torkinib	-0.1766	-0.3768 to 0.02365	No	ns	0.0786
Trametinib vs. Torkinib	-1.13	-1.330 to -0.9299	Yes	****	<0.0001

Figure 6g (Volume of Fibrin Void Spaces)

	PIK3CAE542K	1uM Trametinib	Torkinib 5uM
	114000000	8052925.491	2041528
	60849879	5698440.969	3180299
	53909663	6640777.851	1631008
MEAN	76253180.67	6797381.437	2284278.333
SEM	18979448.7703	684176.5984	463418.9767

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PIK3CAE542K vs. Trametinib	69455799	21862761 to 117048838	Yes	**	0.01
PIK3CAE542K vs. Torkinib	73968902	26375864 to 121561941	Yes	**	0.0074
Trametinib vs. Torkinib	4513103	-43079935 to 52106141	No	ns	0.9548

Figure 6h (Branch Points)

	GFP control	Trametinib	Torkinib		
	265	1180	424		
	219	656	286		
	248	716	454		
MEAN	244	850.6666667	388		
SEM	13.4288	165.5751	51.7301		
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. Trametinib	-606.7	-1043 to -170.8	Yes	*	0.0124
GFP control vs. Torkinib	-144	-579.9 to 291.9	No	ns	0.5958
Trametinib vs. Torkinib	462.7	26.79 to 898.5	Yes	*	0.0397

Figure 7b (pAKT308)

	GFP control	PIK3CA-E542K	Unpaired t test	
	0.630034	10.2811	P value	<0.0001
	1.150537	10.67841	P value summary	****
	1.21943	9.747855	Significantly different (P < 0.05)?	Yes
			One- or two-tailed P value?	Two-tailed
MEAN	1.000000333	10.23578833	t, df	t=28.20, df=4
SEM	0.1860	0.2696		

Figure 7c (pERK)

	GFP control	PIK3CA-E542K	Unpaired t test	
	1.052544	1.841221	P value	0.0488
	0.961367	1.825541	P value summary	*
	0.986088	1.179302	Significantly different (P < 0.05)?	Yes
			One- or two-tailed P value?	Two-tailed
MEAN	0.999999667	1.615354667	t, df	t=2.800, df=4
SEM	0.0272	0.2181		

Figure 7d (RAC-GTP)

	GFP	PIK3CA-E542K	Unpaired t test	
	0.60667	1.699003	P value	0.0301
	1.183801	1.325983	P value summary	*
	1.234135	3.001577	Significantly different (P < 0.05)?	Yes
	1.29477	5.229291	One- or two-tailed P value?	Two-tailed
	0.680622	2.944057	t, df	t=2.632, df=8
MEAN	0.9999996	2.8399822		
SEM	0.1470	0.6834		

Figure 7g (mean migration speed)

GFP mean speed	PIK3CA E542K mean speed	Unpaired t test	
1.242936	1.48446	P value	0.7103
1.125377	1.112777	P value summary	ns
0.926782	1.658168	Significantly different (P < 0.05)?	No
2.389489	1.549585	One- or two-tailed P value?	Two-tailed
0.782585	2.334734	t, df	t=0.3727, df=91
2.270996	1.22956		
3.688812	1.935047		
0.96708	0.883674		
0.961223	2.078204		
0.662913	2.32318		
1.295655	1.384731		
0.916822	1.15261		
1.310455	1.010856		
1.32088	1.263663		
1.413815	2.505858		
1.977788	1.862949		
1.429767	1.463424		
0.893329	1.40869		
1.570513	0.908823		
2.228927	1.672161		
1.691282	2.167745		
2.322872	0.904641		
2.221058	2.166066		
1.398399	1.536984		
1.940702	1.356907		
1.327047	3.458967		
1.246191	1.7357		
3.1556	2.050681		
3.353328	1.010879		
2.891264	2.11117		
0.552032	1.142557		
1.916015	1.286928		
0.844874	1.915203		
	1.40911		
	2.011025		
	1.333424		
	2.116627		
	1.12967		
	2.353486		
	1.593062		
	1.952379		
	2.964473		

		2.8407		
		1.462967		
		1.570143		
		2.006383		
		1.055159		
		2.512387		
		2.038298		
		1.521894		
		1.017206		
		1.22133		
		1.471854		
		2.58597		
		2.084611		
		1.170011		
		0.89518		
		2.314062		
		1.439992		
		1.671118		
MEAN	1.643539636	1.69683505		
SEM	0.1395	0.0734		

Figure 7J (Confinement Ratio)

	GFP	PIK3CA-E542K	Unpaired t test	
	0.568537	0.026215	P value	0.0392
	0.357595	0.192833	P value summary	*
	0.311344	0.146861	Significantly different (P < 0.05)?	Yes
	0.126196	0.103418	One- or two-tailed P value?	Two-tailed
	0.263133	0.032904	t, df	t=2.085, df=115
	0.177417	0.362387		
	0.09582	0.004014		
	0.265363	0.273343		
	0.050458	0.121916		
	0.166129	0.148491		
	0.587921	0.005269		
	0.133802	0.230708		
	0.126918	0.447934		
	0.229205	0.087062		
	0.390812	0.116264		
	0.192136	0.063975		
	0.454725	0.024115		
	0.32133	0.059032		
	0.198663	0.130742		
	0.204053	0.151452		

0.421097	0.041902
0.041276	0.140625
0.02638	0.110253
0.403703	0.039212
0.174461	0.15203
0.04626	0.13095
0.364325	0.054651
0.29908	0.595514
0.087648	0.050255
0.323123	0.012497
0.266799	0.687332
0.30834	0.046062
0.416041	0.172866
0.020377	0.424647
0.271958	0.042572
0.07381	0.120679
0.133521	0.020377
0.007014	0.271958
0.028539	0.07381
0.160395	0.133521
0.204849	0.007014
0.209783	0.028539
0.124454	0.160395
0.209515	0.204849
0.044449	0.209783
0.177786	0.124454
0.45734	0.209515
0.17998	0.044449
0.171406	0.177786
0.293753	0.45734
0.13467	0.17998
0.11018	0.171406
0.474246	0.293753
0.144179	0.13467
0.059166	0.11018
0.413096	0.474246
0.529503	0.144179
	0.059166
	0.413096
	0.529503

Figure 7J (Confinement Ratio)

GFP	PIK3CA-E542K	Unpaired t test	
0.568537	0.026215	P value	0.0392

0.357595	0.192833	P value summary	*
0.311344	0.146861	Significantly different (P < 0.05)?	Yes
0.126196	0.103418	One- or two-tailed P value?	Two-tailed
0.263133	0.032904	t, df	t=2.085, df=115
0.177417	0.362387		
0.09582	0.004014		
0.265363	0.273343		
0.050458	0.121916		
0.166129	0.148491		
0.587921	0.005269		
0.133802	0.230708		
0.126918	0.447934		
0.229205	0.087062		
0.390812	0.116264		
0.192136	0.063975		
0.454725	0.024115		
0.32133	0.059032		
0.198663	0.130742		
0.204053	0.151452		
0.421097	0.041902		
0.041276	0.140625		
0.02638	0.110253		
0.403703	0.039212		
0.174461	0.15203		
0.04626	0.13095		
0.364325	0.054651		
0.29908	0.595514		
0.087648	0.050255		
0.323123	0.012497		
0.266799	0.687332		
0.30834	0.046062		
0.416041	0.172866		
0.020377	0.424647		
0.271958	0.042572		
0.07381	0.120679		
0.133521	0.020377		
0.007014	0.271958		
0.028539	0.07381		
0.160395	0.133521		
0.204849	0.007014		
0.209783	0.028539		
0.124454	0.160395		
0.209515	0.204849		
0.044449	0.209783		

0.177786	0.124454
0.45734	0.209515
0.17998	0.044449
0.171406	0.177786
0.293753	0.45734
0.13467	0.17998
0.11018	0.171406
0.474246	0.293753
0.144179	0.13467
0.059166	0.11018
0.413096	0.474246
0.529503	0.144179
	0.059166
	0.413096
	0.529503

Supplemental Figure 1b (Area)

	GFP ctrl	PIK3CA WT	PIK3CA E542K	PIK3CA E545K	
	2535.532	2100.992	5807.604	5455.969	
	3401.718	3640.492	5772.96	5298.083	
	3673.089	3106.803	5428.105666	4863.099	
MEAN	3203.446333	2949.429	5669.556555	5205.717	
SEM	343.0222	451.3277	121.1390	177.2685	
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP ctrl vs. PIK3CA WT	254	-1119 to 1627	No	ns	0.9315
GFP ctrl vs. PIK3CA E542K	-2466	-3839 to -1093	Yes	**	0.0019
GFP ctrl vs. PIK3CA E545K	-2002	-3375 to -629.6	Yes	**	0.007
PIK3CA WT vs. PIK3CA E542K	-2720	-4093 to -1347	Yes	**	0.001
PIK3CA WT vs. PIK3CA E545K	-2256	-3629 to -883.6	Yes	**	0.0034
PIK3CA E542K vs. PIK3CA E545K	463.8	-908.8 to 1836	No	ns	0.7093

Supplemental Figure 1b (Relative Junction Area)

	GFP	PIK3CA-WT	PIK3CA-E542K	PIK3CA-E545K	
	0.89699289	1.1272245	0.39640067	0.17647663	
	0.98337379	0.99350516	0.78497523	0.21741322	
	1.11963331	1.03977173	0.77770708	0.38753206	
MEAN	0.999999997	1.053500463	0.65302766	0.26047397	
SEM	0.0648	0.0392	0.1283	0.0646	
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. PIK3CA-WT	-0.0535	-0.3842 to 0.2772	No	ns	0.9357
GFP vs. PIK3CA-E542K	0.347	0.01623 to 0.6777	Yes	*	0.0406
GFP vs. PIK3CA-E545K	0.7395	0.4088 to 1.070	Yes	***	0.0005

Supplemental Figure 1b (Relative Cortical Actin)

	GFP	PIK3CA-WT	PIK3CA-E542K	PIK3CA-E545K
	0.962	1.12	0.322	0.412
	0.922	1.13	0.475	0.447

	1.12	0.483	0.357	0.308	
MEAN	1.001333333	0.911	0.384666667	0.389	
SEM	0.0604	0.2140	0.0463	0.0417	
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. PIK3CA-WT	0.09033	-0.3800 to 0.5606	No	ns	0.9004
GFP vs. PIK3CA-E542K	0.6167	0.1464 to 1.087	Yes	*	0.0137
GFP vs. PIK3CA-E545K	0.6123	0.1420 to 1.083	Yes	*	0.0142

Supplemental Figure 1b (Relative Stress Fibers)

	GFP	PIK3CA-WT	PIK3CA-E542K	PIK3CA-E545K	
	1.34	0.95	1.41	3.74	
	0.812	1.06	1.33	3.5	
	0.847	1.17	1.57	1.98	
MEAN	0.999666667	1.06	1.436666667	3.073333333	
SEM	0.1705	0.0635	0.0706	0.5510	
Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. PIK3CA-WT	-0.06033	-1.251 to 1.130	No	ns	0.9976
GFP vs. PIK3CA-E542K	-0.437	-1.627 to 0.7533	No	ns	0.6081
GFP vs. PIK3CA-E545K	-2.074	-3.264 to -0.8833	Yes	**	0.0027

Supplemental Figure 2a (Transduction Efficiency)

	GFP ctrl	PIK3CA WT	PIK3CA E542K	PIK3CA E545K		
	50.41171	94.3808	76.52157	84.24749		
	56.61351	95.51409	74.62687	65.29764		
	44.13427	95.69614	78.0345	77.87796		
MEAN	50.38649667	95.19701	76.39431333	75.80769667		
SEM	3.6025	0.4115	0.9858	5.5674		
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
GFP ctrl vs. PIK3CA WT	-44.81	-60.02 to -29.60	Yes	****	<0.0001	
GFP ctrl vs. PIK3CA E542K	-26.01	-41.22 to -10.80	Yes	**		0.0026
GFP ctrl vs. PIK3CA E545K	-25.42	-40.63 to -10.21	Yes	**		0.003
PIK3CA WT vs. PIK3CA E542K	18.8	3.593 to 34.01	Yes	*		0.0176
PIK3CA WT vs. PIK3CA E545K	19.39	4.180 to 34.60	Yes	*		0.0149
PIK3CA E542K vs. PIK3CA E545K	0.5866	-14.62 to 15.80	No	ns		0.9993

Supplemental Figure 2b (Percent beta-gal positive cells)

	GFP	PIK3CA-WT	PIK3CA-E542K	PIK3CA-E545K		
	3.683709	2.1398	5.661006	3.139717		
	19.46903	16.49175	16.69516	15.2712		
	4.811715	3.811944	16.91919	16.43836		
MEAN	9.321484667	7.481164667	13.09178533	11.61642567		
SEM	5.0842	4.5311	3.7160	4.2517		
Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value	
GFP vs. PIK3CA-WT	1.84	-18.19 to 21.87	No	ns		0.9905
GFP vs. PIK3CA-E542K	-3.77	-23.80 to 16.26	No	ns		0.9284
GFP vs. PIK3CA-E545K	-2.295	-22.33 to 17.74	No	ns		0.9819
PIK3CA-WT vs. PIK3CA-E542K	-5.611	-25.64 to 14.42	No	ns		0.8069
PIK3CA-WT vs. PIK3CA-E545K	-4.135	-24.17 to 15.90	No	ns		0.9087
PIK3CA-E542K vs. PIK3CA-E545K	1.475	-18.56 to 21.51	No	ns		0.995

Supplemental Figure 3b (% Edu positive cells)

	GFP	PIK3CA-WT	PIK3CA-E542K	PIK3CA-E545K	
	5.026569	3.435865	12.53577	9.539437	
	3.70043	6.911527	13.52887	9.247234	
	4.013955	2.804286	10.46617	8.552941	
	4.788719	3.860612	9.596448	9.062098	
MEAN	4.38241825	4.2530725	11.5318145	9.1004275	
SEM	0.3137	0.9123	0.9073	0.2073	

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP vs. PIK3CA-WT	0.1293	-2.685 to 2.943	No	ns	0.999
GFP vs. PIK3CA-E542K	-7.149	-9.963 to -4.335	Yes	****	<0.0001
GFP vs. PIK3CA-E545K	-4.718	-7.532 to -1.904	Yes	**	0.0016
PIK3CA-WT vs. PIK3CA-E542K	-7.279	-10.09 to -4.465	Yes	****	<0.0001
PIK3CA-WT vs. PIK3CA-E545K	-4.847	-7.661 to -2.033	Yes	**	0.0013
PIK3CA-E542K vs. PIK3CA-E545K	2.431	-0.3827 to 5.245	No	ns	0.0992

Supplemental Figure 4 (% Fibrin negative area micron^2)

	GFP 24hr	PIK3CA 24hr	GFP 48hr	PIK3CA 48jr	GFP 72hr	PIK3CA 72hr
	1.256	1.653	1.626	10.969	1.798	13.312
	0.683	5.518	1.25	14.681	1.29	19.281
	0.347	0.734	2.959	7.142	0.505	13.922
		4.78		15.597		20.748
		6.941		9.69		23.875
		5.704		9.726		21.237
Mean	0.762	4.221666667	1.945	11.30083333	1.197666667	18.72916667
SEM	0.2654	1.0058	0.5185	1.3212	0.3761	1.7282

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP 24hr vs. PIK3CA 24hr	-3.46	-9.849 to 2.930	No	ns	0.5502
GFP 24hr vs. GFP 48hr	-1.183	-8.561 to 6.195	No	ns	0.9956
GFP 24hr vs. PIK3CA 48jr	-10.54	-16.93 to -4.149	Yes	***	0.0005
GFP 24hr vs. GFP 72hr	-0.4357	-7.814 to 6.942	No	ns	>0.9999
GFP 24hr vs. PIK3CA 72hr	-17.97	-24.36 to -11.58	Yes	****	<0.0001
PIK3CA 24hr vs. GFP 48hr	2.277	-4.113 to 8.666	No	ns	0.8699
PIK3CA 24hr vs. PIK3CA 48jr	-7.079	-12.30 to -1.862	Yes	**	0.0042
PIK3CA 24hr vs. GFP 72hr	3.024	-3.366 to 9.414	No	ns	0.6795
PIK3CA 24hr vs. PIK3CA 72hr	-14.51	-19.72 to -9.290	Yes	****	<0.0001
GFP 48hr vs. PIK3CA 48jr	-9.356	-15.75 to -2.966	Yes	**	0.002
GFP 48hr vs. GFP 72hr	0.7473	-6.631 to 8.125	No	ns	0.9995
GFP 48hr vs. PIK3CA 72hr	-16.78	-23.17 to -10.39	Yes	****	<0.0001
PIK3CA 48jr vs. GFP 72hr	10.1	3.714 to 16.49	Yes	***	0.0008
PIK3CA 48jr vs. PIK3CA 72hr	-7.428	-12.65 to -2.211	Yes	**	0.0026
GFP 72hr vs. PIK3CA 72hr	-17.53	-23.92 to -11.14	Yes	****	<0.0001

Supplemental Figure 5

	ADAM8	ADAM9	ADAMTS 1	ADAMTS1 3	Cathepsi n A	Cathepsi n B	Cathepsi n C	Cathepsin D	Cathepsi n E	Cathepsi n L	Cathepsi n S	Cathepsi n V	Cathepsin X/Z/P	DDPIV/CD26	Kallikrein 3/PSA	Kallikrein 5	Kallikrein 6
GFP	265.84	692.83	2254.74	211.765	2930.165	11080.95	925.675	7500.94	71.28	269.475	991.46	915.345	5267.835	759.79	151.375	19.355	-27.98
PIK3CAE542K	365.35	1029.25	6010.12	260.955	2948.825	10364.1	1659.64	6656.4	153.075	386.125	557.065	948.84	6145.165	1005.02	465.205	515.145	816.425
Alpelisib	335.095	1250.22	4430.1	228.375	4949.86	13027.59	1577.52	8722.135	119.785	452.995	780.48	1042.34	7971.46	931.635	333.895	391.405	136.095
Rapamycin	425.585	1248.87	8079.28	307.335	3051.035	9734.87	1409.37	6543.05	186.37	522.305	646.63	1171.07	5888.755	893.275	332.7	313.025	746.29
Trametinib	480.81	1473.815	6438.3	307.525	3260.13	13016.51	1956.74	11056.135	306.38	846.735	829.395	1652.15	6548.18	896.745	450.35	551.34	624.315
Torkinib	521.626	844.528	3008.373	272.048	1682.284	6006.625	956.783	4479.924	272.142	451.069	619.094	910.238	3189.448	643.974	266.186	421.077	289.039
PAKi	406.315	1105.52	8400.465	246.615	2314.825	10124.13	1684.555	6655.59	248.775	753.06	890.005	1357.085	5009.95	815.75	347.58	407.205	1028.84

	Kallikrei n 7	Kallikrein 10	Kallikrein 11	Kallikrein 13	MMP-1	MMP-2	MMP-3	MMP-7	MMP-8	MMP-9	MMP-10	MMP-12	MMP-13	Nephrilysin/CD1 0	Presenilin	Proprotein Convertase 9	Proteinas e 3	uPA/Urokinas e
GFP	72.76	351.2	146.91	578.34	11289.37	7024.955	102.925	521.45	971.68	58.975	1677.2	1084.69	574.395	288.415	-119.85	-78.615	-94.01	3048.39
PIK3CAE542K	169.96	351.28	159.82	909.675	14745.21	6804.395	495.27	611.385	1023.45	174.265	417.105	1614.035	754.37	636.8	257.44	17.54	-6.05	7972.335
Alpelisib	75.85	406.545	50.16	737.435	14273.98	7201.145	410.275	498.3	961.23	100.75	1110.425	1469.53	657.625	416.63	197.745	-52.455	-41.785	4344.54
Rapamycin	253.15	505.46	179.985	671.635	13049.38	7211.165	333.06	596.02	1132.665	264.745	596.055	1248.535	704.87	445.695	89.5	89.575	139.305	8036.72
Trametinib	365.745	767.46	166.55	695.275	16942.8	13802.07	664.3	1624.695	2032.09	413.615	779.765	1779.785	773.4	520.025	366.225	262.775	212.56	8623.865
Torkinib	373.375	597.367	207.057	736.619	11922.75	3355.591	414.649	960.211	1020.259	283.779	334.332	1336.097	640.314	396.242	232.954	174.444	202.016	4153.958
PAKi	377.595	538.815	156.745	582.495	14384.53	6929.33	489.525	1225.825	1561.895	251.795	597.665	1764.335	745.58	359.8	354.54	258.525	174.475	7916.95

Supplemental Figure 6b (PIK3CA-E542K + HLF coculture)

	HLF same channel	HLF separate channel
	123000000	128000000
	55349665	142000000
	56502096	59787357
	53972073	51188532
		95825764
		109000000
Mean	72205958.5	97633608.83
SEM	16939242.3097	14846370.4528
Unpaired t test		
P value	0.2988	
P value summary	ns	
Significantly different (P < 0.05)?	No	
One- or two-tailed P value?	Two-tailed	
t, df	t=1.111, df=8	

Supplemental Figure 6b (GFP control in EGM2 vs PIK3CA-E542K in EBM2 media)

	EGM2 media	EBM2 + 2% serum
	128000000	13232837
	142000000	23002289
	59787357	16946956
	51188532	18296500
	95825764	
	109000000	
Mean	97633608.83	17869645.5
SEM	14846370.4528	2018200.1685
Unpaired t test		
P value	0.0027	
P value summary	**	
Significantly different (P < 0.05)?	Yes	
One- or two-tailed P value?	Two-tailed	
t, df	t=4.282, df=8	

Supplemental Figure 6b (PIK3CA-E542K in EGM2 vs EBM2 media)

	GFP	PIK3CA-E542K
	5040986	13232837
	7034815	18296500
	10096187	23002289
	5342216	16946956
Mean	6878551	17869645.5
SEM	1158827.3609	2018200.1685
Unpaired t test		
P value	0.0032	
P value summary	**	
Significantly different (P < 0.05)?	Yes	
One- or two-tailed P value?	Two-tailed	
t, df	t=4.723, df=6	

Supplemental Figure 8b (Percent viability)

Dosage(micromolar)	BB94			Alpelisib		Rapamycin			Trametinib			Torkinib			PAKi	
10	99.6034	99.5759	99.8658	89.46679	90.91089	92.9866	98.7276	99.4854	99.3253	98.9948	99.4272	97.2549	94.7294	94.0668	92.6890	60.6269	87.2623	96.3414
5	99.2909	99.6414	99.7638	92.73246	91.60176	95.7168	98.9048	99.6907	99.4825	98.8959	99.3961	99.7134	95.6172	94.1000	96.3154	91.9657	95.8498	98.4167
2.5	99.5147	99.7193	99.6833	96.98485	92.14842	97.1404	98.7655	99.2774	99.8086	98.5551	99.4637	99.8452	97.3780	97.4491	98.2411	98.0304	97.934	99.1818
1	99.1546	99.6818	99.7774	98.59994	96.63822	98.7243	99.0093	99.538	99.7903	99.5415	99.3500	99.6381	98.3290	98.3259	99.7267	99.3941	99.0723	99.6990
0.5	99.4979	99.7616	94.9750	96.01594	98.16199	99.2376	99.7982	99.7982	99.2804	99.4531	99.9147	98.5995	98.3973	99.3762	99.4158	99.2387	99.0063	99.7090
0.25	99.6677	99.8090	99.6946	99.03571	98.98461	99.5270	98.9152	99.5137	99.8446	98.9467	99.6118	99.8835	98.8971	98.5257	99.7351	99.2032	99.4257	99.7090
0.1	99.7305	99.7831	99.6744	99.22951	98.09012	99.8711	98.4243	99.7110	99.7666	99.1333	99.6266	99.8665	99.1155	99.383	99.8538	99.1309	99.3972	98.4926
0.02	99.4374	99.5815	99.5111	99.26574	99.59444	99.9080	98.3962	99.8852	99.7746	74.5772	99.4353	99.8249	98.8971	99.5962	99.5597	99.1642	99.6378	99.9172
0.0003	99.3805	99.3784	99.7800	99.38057	99.37845	99.7800	99.3805	99.3784	99.7800	99.3805	99.3784	99.7800	99.3805	99.3784	99.7800	99.3805	99.3784	99.7800

Supplemental Figure 8b (Percent Edu)

BB94 (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		
0.02	5.407969	4.450784	2.923058	0.436223173	ns
0.1	3.79995	4.572987	2.44807	0.120509175	ns
0.25	3.214244	4.229979	2.279671	0.05225582	ns
0.5	1.585869	3.190997	1.622846	0.008782292	**
1	2.1	3.533962	1.850922	0.013824576	*
2.5	1.981755	2.551793	1.31582	0.00237452	**
5	3.703473	2.08306	1.438343	0.02482393	*
10	4.448685	4.042349	5.30245	0.508164272	ns

Alpelisib (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		
0.02	2.219518	0.045527	1.4396	0.005666482	***
0.1	2.066953	0.050916	2.757144	0.017758281	*
0.25	2.744557	0.057945	2.291854	0.020451309	*
0.5	0.0704	0.060076	2.90165	0.016094103	*
1	3.759867	1.009881	3.844314	0.100376972	ns
2.5	2.364076	0.083094	4.303923	0.098386815	ns
5	1.977798	0.19702	4.065947	0.06809619	ns
10	3.342641	0.182592	4.539454	0.166651701	ns

Rapamycin (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		
0.02	1.384818	0.589124	0.404922	0.000443816	***
0.1	0.572457	0.824523	0.792787	8.45152E-05	****
0.25	0.634139	1.848483	0.53813	0.001312558	**
0.5	0.92913	2.019662	0.519494	0.001805299	**
1	1.058981	1.388657	0.739216	0.000237221	***
2.5	0.638314	2.274558	0.810201	0.003049893	**
5	1.147923	2.352929	1.026192	0.002229715	**
10	2.351598	2.742253	1.355934	0.0044836	**

Trametinib (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		

0.02	0.151467	1.080734	1.185539	0.000556382	**
0.1	0.083611	0.665739	0.259651	0.000107365	**
0.25	0.786689	0.707472	0.182089	0.000149115	**
0.5	0.321086	0.31245	0.23474	4.7967E-05	***
1	0.072152	0.737974	0.424846	0.000133541	**
2.5	0.710333	0.455087	0.116617	0.000115836	**
5	1.0127	0.543668	0.323217	0.000176298	**
10	1.36565	1.104444	0.635525	0.000282849	**

Torkinib (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		
0.02	0.060061	4.219016	2.38585	0.092884036	ns
0.1	0.096376	3.853576	1.028349	0.047760487	*
0.25	3.22367	3.580842	1.948369	0.022319788	*
0.5	4.486182	3.703686	2.558824	0.093789107	ns
1	6.14183	6.057751	2.760788	0.956798328	ns
2.5	4.029088	7.621206	2.308258	0.873778659	ns
5	2.443485	6.366542	1.471757	0.38030133	ns
10	1.551715	6.122449	1.072966	0.285487567	ns

PAKi (μM)	N1	N2	N3	P value (unpaired t-test)	P value summary
0	4.436158	5.092271	5.234786		
0.02	6.304357	5.648267	1.572224	0.796862228	ns
0.1	2.605142	3.633267	0.997626	0.035689946	*
0.25	0.299574	1.33067	0.277922	0.000547432	**
0.5	0.088964	0.368569	0.11257	5.50566E-05	***
1	0.319423	0.286686	0.153473	4.92897E-05	***
2.5	0.553411	0.223358	0.208928	7.05048E-05	***
5	0.387318	3.479901	2.188556	0.035482131	*
10	4.838459	3.630557	1.094781	0.199939867	ns

Supplemental Figure 10a (Cell count)

GFP control	DMSO	Alpelisib	Rapamycin	Trametinib	Torkinib
439	976	679	641	880	670
481	1015	758	764	675	647

	389	947	844	833	651	585
			784			
MEAN	436.3333333	979.3333333	766.25	746	735.3333333	634
SEM	26.5916	19.7005	34.2062	56.1516	72.6644	25.3837

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. PIK3CAE542K	-543	-748.8 to -337.2	Yes	****	<0.0001
GFP control vs. Alpelisib 2.5uM	-329.9	-522.4 to -137.4	Yes	***	0.0008
GFP control vs. Sirolimus 1uM	-309.7	-515.4 to -103.9	Yes	**	0.0027
GFP control vs. Trametinib	-299	-504.8 to -93.24	Yes	**	0.0036
GFP control vs. Torkinib 5uM	-197.7	-403.4 to 8.095	No	ns	0.0627
PIK3CAE542K vs. Alpelisib 2.5uM	213.1	20.61 to 405.6	Yes	*	0.0268
PIK3CAE542K vs. Sirolimus 1uM	233.3	27.57 to 439.1	Yes	*	0.0229
PIK3CAE542K vs. Trametinib	244	38.24 to 449.8	Yes	*	0.0169
PIK3CAE542K vs. Torkinib 5uM	345.3	139.6 to 551.1	Yes	**	0.001
Alpelisib 2.5uM vs. Sirolimus 1uM	20.25	-172.2 to 212.7	No	ns	0.9991
Alpelisib 2.5uM vs. Trametinib	30.92	-161.6 to 223.4	No	ns	0.9937
Alpelisib 2.5uM vs. Torkinib 5uM	132.3	-60.22 to 324.7	No	ns	0.2689
Sirolimus 1uM vs. Trametinib	10.67	-195.1 to 216.4	No	ns	>0.9999
Sirolimus 1uM vs. Torkinib 5uM	112	-93.76 to 317.8	No	ns	0.495
Trametinib vs. Torkinib 5uM	101.3	-104.4 to 307.1	No	ns	0.5932

Supplemental Figure 10b (Fibrin Void Volume)

GFP control	DMSO	Alpelisib	Rapamycin	Trametinib	Torkinib
1378310	114000000	5228440	8343005	8052925.491	2041528
1776301	60849879	7490028	9827182	5698440.969	3180299
1501003	53909663	4045672	9865495	6640777.851	1631008
			8182002		
			14530380		

MEAN	1551871.333	76253180.67	5588046.667	10149612.8	6797381.437	2284278.333
SEM	117671.7178	18979448.7703	1010426.4413	1151328.1420	684176.5984	463418.9767

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. PIK3CAE542K	-74701309	-108258342 to -41144277	Yes	****	<0.0001
GFP control vs. Alpelisib 2.5uM	-4036175	-37593208 to 29520857	No	ns	0.9985
GFP control vs. Sirolimus 1uM	-8597741	-38612064 to 21416581	No	ns	0.9293
GFP control vs. 1uM Trametinib	-5245510	-38802543 to 28311523	No	ns	0.9948
GFP control vs. Torkinib 5uM	-732407	-34289440 to 32824626	No	ns	>0.9999
PIK3CAE542K vs. Alpelisib 2.5uM	70665134	37108101 to 104222167	Yes	****	<0.0001
PIK3CAE542K vs. Sirolimus 1uM	66103568	36089245 to 96117890	Yes	****	<0.0001
PIK3CAE542K vs. 1uM Trametinib	69455799	35898767 to 103012832	Yes	***	0.0001
PIK3CAE542K vs. Torkinib 5uM	73968902	40411870 to 107525935	Yes	****	<0.0001
Alpelisib 2.5uM vs. Sirolimus 1uM	-4561566	-34575889 to 25452756	No	ns	0.9954
Alpelisib 2.5uM vs. 1uM Trametinib	-1209335	-34766367 to 32347698	No	ns	>0.9999
Alpelisib 2.5uM vs. Torkinib 5uM	3303768	-30253264 to 36860801	No	ns	0.9994
Sirolimus 1uM vs. 1uM Trametinib	3352231	-26662091 to 33366554	No	ns	0.9989
Sirolimus 1uM vs. Torkinib 5uM	7865334	-22148988 to 37879657	No	ns	0.9502
1uM Trametinib vs. Torkinib 5uM	4513103	-29043930 to 38070136	No	ns	0.9974

Supplemental Figure 10c (Branch Points)

	GFP control	Alpelisib	Rapamycin	Trametinib	Torkinib
	265	366	545	1180	424
	219	357	513	656	286
	248	380	878	716	454
			674		
			724		
MEAN	244	367.6666667	666.8	850.6666667	388
SEM	13.4288	6.6916	65.7339	165.5751	51.7301

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
GFP control vs. Alpelisib 2.5uM	-123.7	-512.8 to 265.5	No	ns	0.8447
GFP control vs. Sirolimus 1uM	-422.8	-770.9 to -74.72	Yes	*	0.0155
GFP control vs. 1uM Trametinib	-606.7	-995.8 to -217.5	Yes	**	0.0024
GFP control vs. Torkinib 5uM	-144	-533.2 to 245.2	No	ns	0.7625
Alpelisib 2.5uM vs. Sirolimus 1uM	-299.1	-647.2 to 48.94	No	ns	0.1056
Alpelisib 2.5uM vs. 1uM Trametinib	-483	-872.2 to -93.84	Yes	*	0.0134
Alpelisib 2.5uM vs. Torkinib 5uM	-20.33	-409.5 to 368.8	No	ns	0.9998
Sirolimus 1uM vs. 1uM Trametinib	-183.9	-531.9 to 164.2	No	ns	0.4778
Sirolimus 1uM vs. Torkinib 5uM	278.8	-69.28 to 626.9	No	ns	0.1424
1uM Trametinib vs. Torkinib 5uM	462.7	73.51 to 851.8	Yes	*	0.0178

Supplemental Figure 11a (Cell count)

	DMSO	Torkinib	Raci	PAKi
	0.902033	0.937367	0.607947	0.842312
	1.047607	0.815698	0.550858	0.814934
	1.050359	0.856639	0.535952	0.751928
MEAN	0.999999667	0.869901333	0.564919	0.803058
SEM	0.0490	0.0357	0.0219	0.0268

Dunnett's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
PIK3CA-E542K vs. torkinib	0.1301	-0.01208 to 0.2723	No	ns	0.0719
PIK3CA-E542K vs. raci	0.4351	0.2929 to 0.5773	Yes	****	<0.0001
PIK3CA-E542K vs. paki	0.1969	0.05477 to 0.3391	Yes	*	0.0102

Supplemental Figure 11c (Volume of void spaces)

	DMSO	0.1 µM PAKi	1 µM PAKi	2.5 µM PAKi	5 µM PAKi
	83330564.1	65571651	50206379	38044204	5906788
	125061809.1	49404196	44938349	14419797	5179676
	93485369	69621863	29044336	62476228	18331890
MEAN	100625914.1	61532570	41396354.67	38313409.67	9806118
SEM	12564695.7809	6175870.9568	6360485.0150	13873349.6782	4268050.4571

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. 0.1 paki	39093344	-4921349 to 83108037	No	ns	0.0882
DMSO vs. 1 paki	59229559	15214867 to 103244252	Yes	**	0.0087
DMSO vs. 2.5 paki	62312504	18297812 to 106327197	Yes	**	0.0062
DMSO vs. 5 paki	90819796	46805103 to 134834489	Yes	***	0.0004
0.1 paki vs. 1 paki	20136215	-23878477 to 64150908	No	ns	0.5814
0.1 paki vs. 2.5 paki	23219160	-20795532 to 67233853	No	ns	0.4562
0.1 paki vs. 5 paki	51726452	7711759 to 95741145	Yes	*	0.0205
1 paki vs. 2.5 paki	3082945	-40931748 to 47097638	No	ns	0.9993
1 paki vs. 5 paki	31590237	-12424456 to 75604929	No	ns	0.203
2.5 paki vs. 5 paki	28507292	-15507401 to 72521984	No	ns	0.2791

Supplemental Figure 11d (Cell count)

	DMSO	0.1 µM PAKi	1 µM PAKi	2.5 µM PAKi	5 µM PAKi
	1100	502	527	502	429
	1000	592	475	431	475
	980	527	506	485	451
MEAN	1026.666667	540.3333333	502.6666667	472.6666667	451.6666667
SEM	37.1184	26.8225	15.1033	21.4035	13.2832

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
DMSO vs. 0.1 paki	486.3	373.1 to 599.6	Yes	****	<0.0001
DMSO vs. 1 paki	524	410.8 to 637.2	Yes	****	<0.0001
DMSO vs. 2.5 paki	554	440.8 to 667.2	Yes	****	<0.0001
DMSO vs. 5 paki	575	461.8 to 688.2	Yes	****	<0.0001
0.1 paki vs. 1 paki	37.67	-75.58 to 150.9	No	ns	0.8056
0.1 paki vs. 2.5 paki	67.67	-45.58 to 180.9	No	ns	0.346
0.1 paki vs. 5 paki	88.67	-24.58 to 201.9	No	ns	0.1487
1 paki vs. 2.5 paki	30	-83.24 to 143.2	No	ns	0.9009
1 paki vs. 5 paki	51	-62.24 to 164.2	No	ns	0.5946
2.5 paki vs. 5 paki	21	-92.24 to 134.2	No	ns	0.9702