Supplemental Information

Upregulation of acid ceramidase contributes to tumor progression in Tuberous Sclerosis Complex

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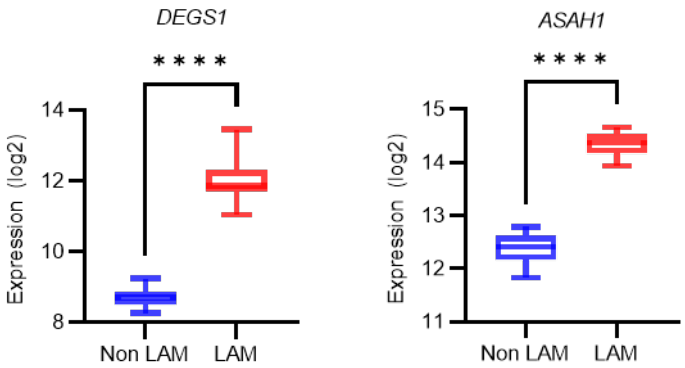
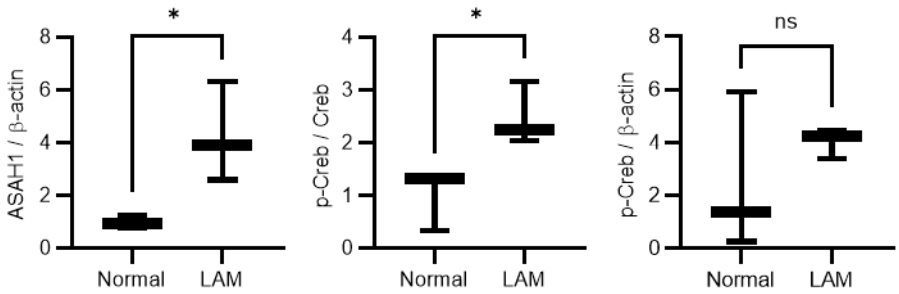
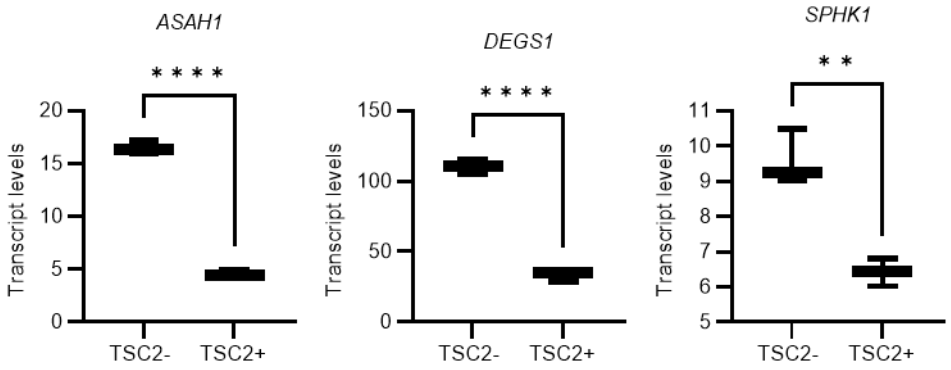


Supplemental Figure S1. Expression of *DEGS1* and *ASAH1* is evident in pulmonary LAM lesions. Box plots with 95% confidence intervals show *DEGS1* and *ASAH1* expression in female non-LAM lungs (n=15 subjects) and laser capture microdissected LAM lesion cells (n=14 subjects). **** P < 0.0001, Mann-Whitney test. (Corresponds to Fig. 2C).



Supplemental Figure S2. Expression of ASAH1 and transcription factor CREB in LAM lungs.

Box plots with 95% confidence intervals show protein levels of ASAH1 and phospho-CREB in LAM (n=3) and control lungs (n=3) quantified by densitometry of ASAH1 and phospho-CREB. * $P < 0.05$, unpaired t test. (Corresponds to Fig. 2F).



Supplemental Figure S3. Expression of sphingolipid biosynthesis pathway genes in TSC2-null cells. Box plots with 95% confidence intervals show mRNA levels of ASAH1, DEGS1, and SPHK1 in TSC2-null (TSC2-) 621-101 cells (n=3) and TSC2-addback (TSC2+) 621-103 cells (n=3). ** P < 0.01, **** P < 0.0001, unpaired t test. (Corresponds to Fig. 3B).

Supplemental Table S1. Statistical analyses and P values in *in vitro* and *in vivo* experiments.

Pairwise comparisons						
Figure panel	**Plot**	**Comparison groups (A vs. B)**	**Unpaired t test P value**	**Mann-Whitney test P value**	**n (A)**	**n (B)**
2C	ASAH1	Normal lung vs. LAM	**<0.0001**	**<0.0001**	15	14
	DEGS1	Normal lung vs. LAM	**<0.0001**	**<0.0001**	15	14
2F	ASAH1 / β-actin	Normal vs. LAM	**0.0411**	0.1	3	3
	p-CREB / CREB	Normal vs. LAM	**0.0377**	0.1	3	3
	p-CREB / β-actin	Normal vs. LAM	0.4387	0.7	3	3
3B	ASAH1	TSC2- vs. TSC2+	**<0.0001**	0.1	3	3
	DEGS1	TSC2- vs. TSC2+	**<0.0001**	0.1	3	3
	SPHK1	TSC2- vs. TSC2+	**0.0034**	0.1	3	3
3D	ASAH1 / β-actin	621-101 vs 621-103, 0% FBS	**0.0051**	0.1	3	3
		621-101 vs 621-103, 10% FBS	**<0.0001**	0.1	3	3
	DEGS1 / β-actin	621-101 vs 621-103, 0% FBS	**0.0063**	0.1	3	3
		621-101 vs 621-103, 10% FBS	**0.0008**	0.1	3	3
4A	Ceramide	TSC2- control vs. TSC2- rapa	0.2374 (0.4748)	0.1797 (0.3594)	6	6
		TSC2- control vs. TSC2+ control	**<0.0001** (**<0.0002**)	**0.0022** (**0.0044**)	6	6
4B	Sphingosine	TSC2- control vs. TSC2- rapa	0.4609 (0.9218)	0.4848 (0.9696)	6	6
		TSC2- control vs. TSC2+ control	**<0.0001** (**<0.0002**)	**0.0022**	6	6
4C	ASAH1 transcript	TSC2- control vs. TSC2- rapa	0.7447 (1.0)	>0.9999 (1.0)	6	6
		TSC2- control vs. TSC2+ control	**<0.0001** (**<0.0002**)	**0.0022** (**0.0044**)	6	6
4F	cAMP levels	TSC2- vs TSC2+	**0.0098**	0.1	3	3
4J	ASAH1 / β-actin	Vehicle vs. AZD6244	**<0.0001** (**<0.0003**)	0.1 (0.3)	3	3
		Vehicle vs. L798106	**<0.0001** (**<0.0003**)	0.1 (0.3)	3	3
		Vehicle vs. PKI	**<0.0001** (**<0.0003**)	0.1 (0.3)	3	3
4K	ASAH1 transcript	Vehicle vs. E2 (10 nM)	**0.0002**	0.1	3	3
4N	ERa / β-actin	Vehicle vs. E2 24h	**0.0014**	0.1	3	3
	ASAH1 / β-actin	Vehicle vs. E2 24h	**0.0019**	0.1	3	3
5A	17a 50 nM	TSC2- vs. TSC2+	**<0.0001**	ND	4	4
	17a 100 nM	TSC2- vs. TSC2+	**<0.0001**	ND	4	4
	17a 200 nM	TSC2- vs. TSC2+	**<0.0001**	ND	4	4
5B	Carmofur 10 μM	TSC2- vs. TSC2+	**0.0038**	**0.0286**	4	4
	Carmofur 20 μM	TSC2- vs. TSC2+	**0.0033**	**0.0286**	4	4
	Carmofur 50 μM	TSC2- vs. TSC2+	**<0.0001**	**0.0286**	4	4
	Carmofur 100 μM	TSC2- vs. TSC2+	**0.0003**	**0.0286**	4	4

5C	Gene expression	siCtrl vs. siASAH1	**0.0019**	**0.0286**	4	4
5E	Cell viability 48 h post	siCtrl vs. siASAH1	**<0.0001**	**<0.0001**	12	12
5G	Apoptotic Cells	siASAH1- vs. siASAH1+	**<0.0001** (**<0.0002**)	0.1 (0.2)	3	3
		siASAH1- vs. siASAH1+ Ceramide	**<0.0001** (**<0.0002**)	0.1 (0.2)	3	3
5I	Cell viability	pLKO.1 vs. shASAH1 #1	**<0.0001** (**<0.0002**)	**<0.0001** (**<0.0002**)	15	16
		pLKO.1 vs. shASAH1 #2	**<0.0001** (**<0.0002**)	**<0.0001** (**<0.0002**)	15	16
5K	Apoptosis	pLKO.1 vs. shASAH1 #1	**0.0001 (0.0002)**	0.1 (0.2)	3	3
		pLKO.1 vs. shASAH1 #2	**<0.0001** (**<0.0002**)	0.1 (0.2)	3	3
5L	Cell death	pLKO.1 vs. shASAH1 #1	**<0.0001** (**<0.0002**)	**<0.0001** (**<0.0002**)	15	16
		pLKO.1 vs. shASAH1 #2	**<0.0001** (**<0.0002**)	**<0.0001** (**<0.0002**)	15	16
6B	Bioluminescence imaging	Vehicle vs. 17a, week 4	**0.0441**	**0.0260**	6	6
6F	Bioluminescence imaging	pLKO.1 vs. shASAH1, week 22	**0.0020**	**0.0357**	3	5
		pLKO.1 vs. shASAH1, week 23	**0.0258**	**0.0357**	3	5
6H	Bioluminescence imaging	Vehicle vs. 17a, 6 hours	**0.0368**	**0.0286**	3	3
		Vehicle vs. 17a, 24 hours	**0.0341**	**0.0286**	3	3
7B	Macroscopic cysts	Control vs. rapa	**0.0345** (0.1035)	**0.0286** (0.0858)	4	4
		Control vs. 17a+rapa	**0.0071** (**0.0213**)	**0.0286** (0.0858)	4	4
		rapa vs. 17a+rapa	**0.0100** (**0.0300**)	**0.0286** (0.0858)	4	4
Ordinary one-way ANOVA with Tukey's multiple comparisons test						
Figure panel	**Plot**	**Comparison groups (A vs. B)**	**Adj. P value**		**n (A)**	**n (B)**
7F	Tumor burden 4 weeks	17a vs. rapa	**0.0011**		5	2
		17a vs. 17a+rapa	**0.0001**		5	5
		rapa vs. 17a+rapa	0.9992		2	5
	Tumor burden 8 weeks	17a vs. rapa	0.1562		4	2
		17a vs. 17a+rapa	**0.0013**		4	5
		rapa vs. 17a+rapa	0.1087		2	5
7H	Percent PCNA	Control vs. Rapamycin	0.0967		5	5
		Control vs. 17a	**0.0035**		5	5
		Control vs. Rapamycin+17a	**<0.0001**		5	5
		Rapamycin vs. 17a	**<0.0001**		5	5
		Rapamycin vs. Rapamycin+17a	**<0.0001**		5	5
		17a vs. Rapamycin+17a	**0.0395**		5	5
Two-way ANOVA with Tukey's multiple comparisons test						
Figure panel	**Plot**	**Comparison groups (A vs. B)**	**Adj. P value**		**n (A)**	**n (B)**
6J	Bioluminescence imaging 6 h	pLKO.1 vs. shASAH1 #1	**0.0013**		4	4

		pLKO.1 vs. shASAH1 #2	**<0.0001**		4	5
		shASAH1 #1 vs. shASA1 #2	0.3537		4	5
	Bioluminescence imaging 24 h	pLKO.1 vs. shASAH1 #1	**0.0414**		4	4
		pLKO.1 vs. shASAH1 #2	**0.0005**		4	5
		pLKO.1 vs. shASAH1 #2	0.2631		4	5
Survival						
Figure panel	**Plot**	**Comparison groups (A vs. B)**	**Log-rank (Mantel-Cox) test P value**			
6E	Tumor-free survival	pLKO.1 vs. shASAH1 #1	**0.0493**			
		pLKO.1 vs. shASAH1 #2	**0.0002**			
		shASAH1 #1 vs. shASAH1 #2	**0.0234**			

Numbers in parenthesis represent P values between comparison groups after Bonferroni multiple comparison adjustment. ND = not determined

Supplemental Table S2. Normality and Lognormality tests for all data groups presented in Fig. 3B.

	ASAH1		*DEGS1*		*SPHK1*	
Test	**TSC2-**	**TSC2+**	**TSC2-**	**TSC2+**	**TSC2-**	**TSC2+**
D'Agostino & Pearson	N too small	N too small	N too small	N too small	N too small	N too small
Anderson-Darling	N too small	N too small	N too small	N too small	N too small	N too small
Shapiro-Wilk	**PASS (P= 0.6805)**	**PASS (P= 0.0587)**	**PASS (P= 0.8759)**	**PASS (P= 0.2738)**	**PASS (P= 0.2720)**	**PASS (P= 0.9511)**
Kolmogorov-Smirnov	N too small	N too small	N too small	N too small	N too small	N too small

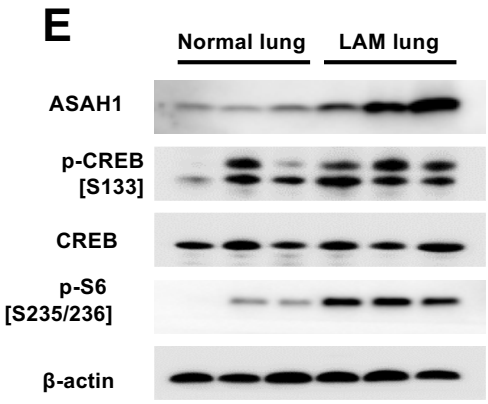
Supplemental Table S3. Normality and Lognormality tests for all data groups presented in Fig. 3D for ASAH1 / β-actin.

Test	**TSC2- 0% FBS**	**TSC2+ 0% FBS**	**TSC2- 10% FBS**	**TSC2+ 10% FBS**
D'Agostino & Pearson	N too small	N too small	N too small	N too small
Anderson-Darling	N too small	N too small	N too small	N too small
Shapiro-Wilk	**PASS (P= 0.2968)**	**PASS (P= 0.3554)**	**PASS (P= 0.4014)**	**PASS (P= 0.1720)**
Kolmogorov-Smirnov	N too small	N too small	N too small	N too small

Supplemental Table S4. Normality and Lognormality tests for all data groups presented in Fig. 3D for DEGS1 / β-actin.

Test	**TSC2- 0% FBS**	**TSC2+ 0% FBS**	**TSC2- 10% FBS**	**TSC2+ 10% FBS**
D'Agostino & Pearson	N too small	N too small	N too small	N too small
Anderson-Darling	N too small	N too small	N too small	N too small
Shapiro-Wilk	**PASS (P= 0.3079)**	**PASS (P= 0.3231)**	**PASS (P= 0.7637)**	**PASS (P= 0.6702)**
Kolmogorov-Smirnov	N too small	N too small	N too small	N too small

Figure 2



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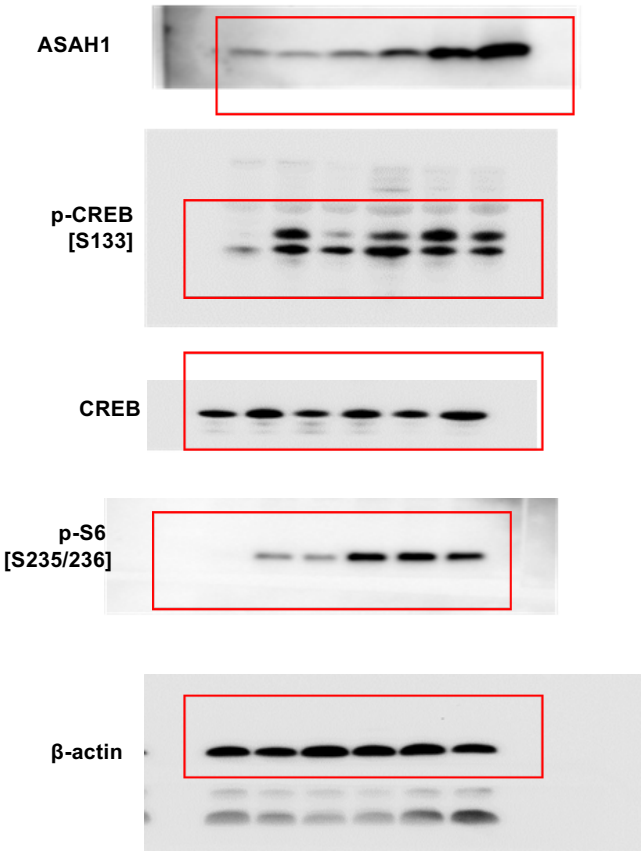

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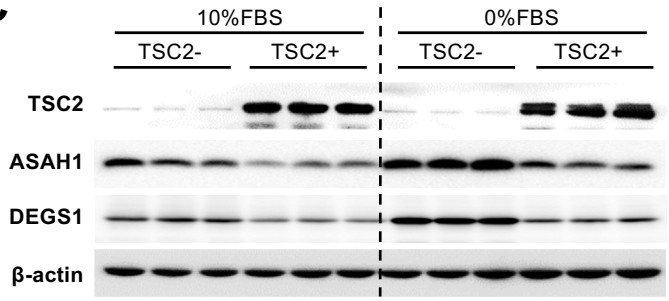

Human renal angiomyolipoma-derived cells

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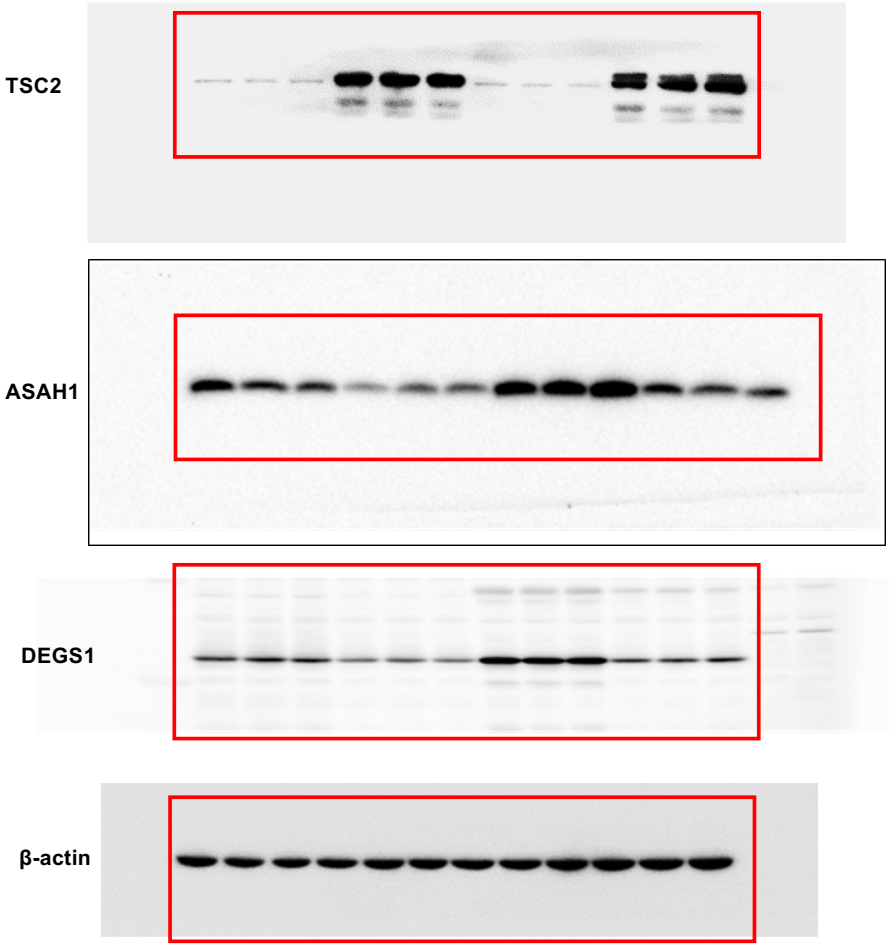

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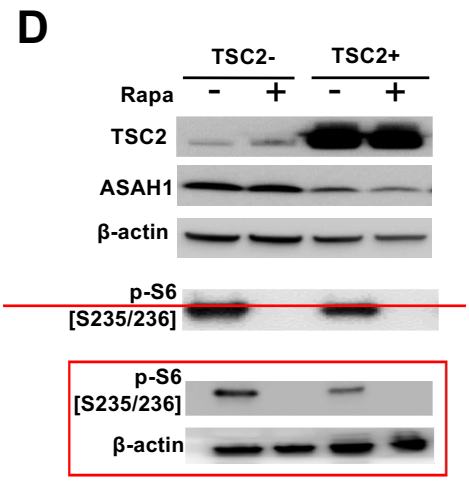

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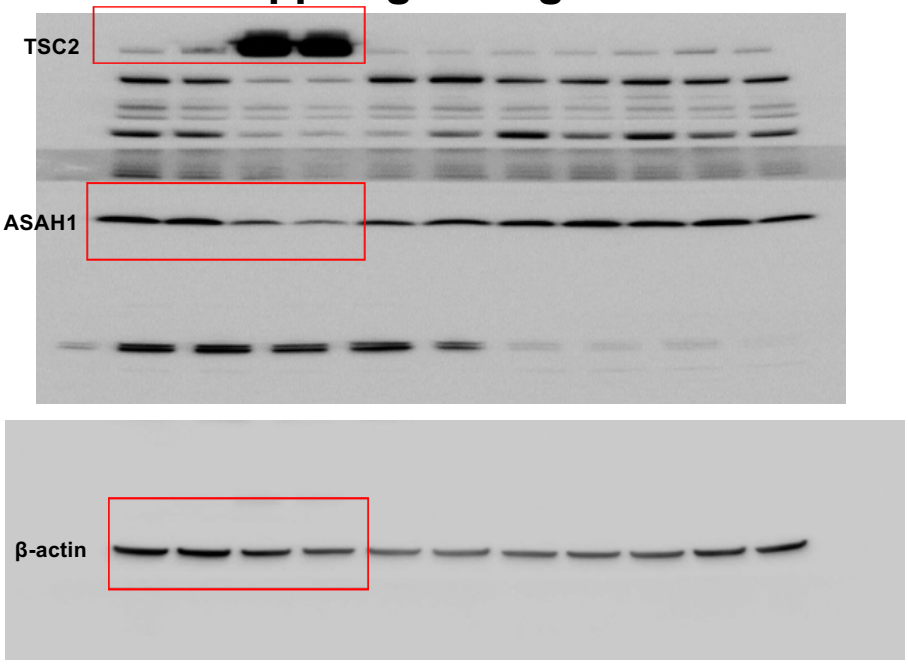

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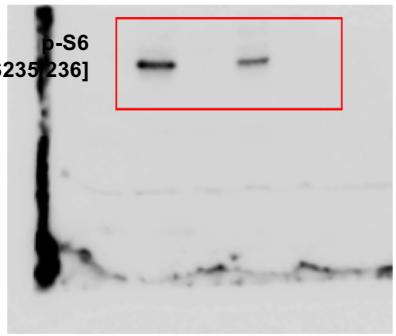

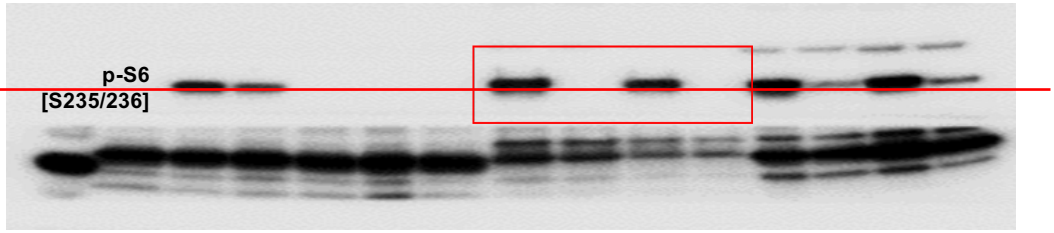

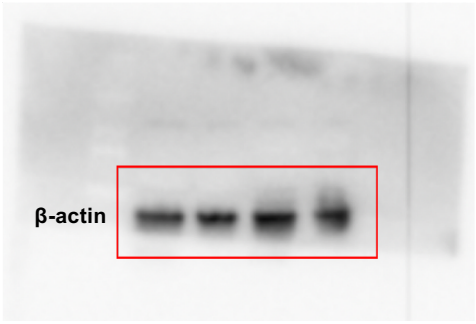

Figure 4

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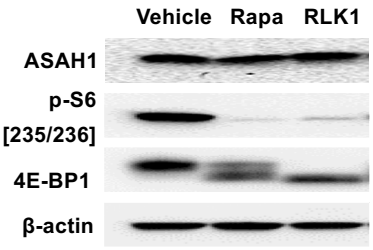
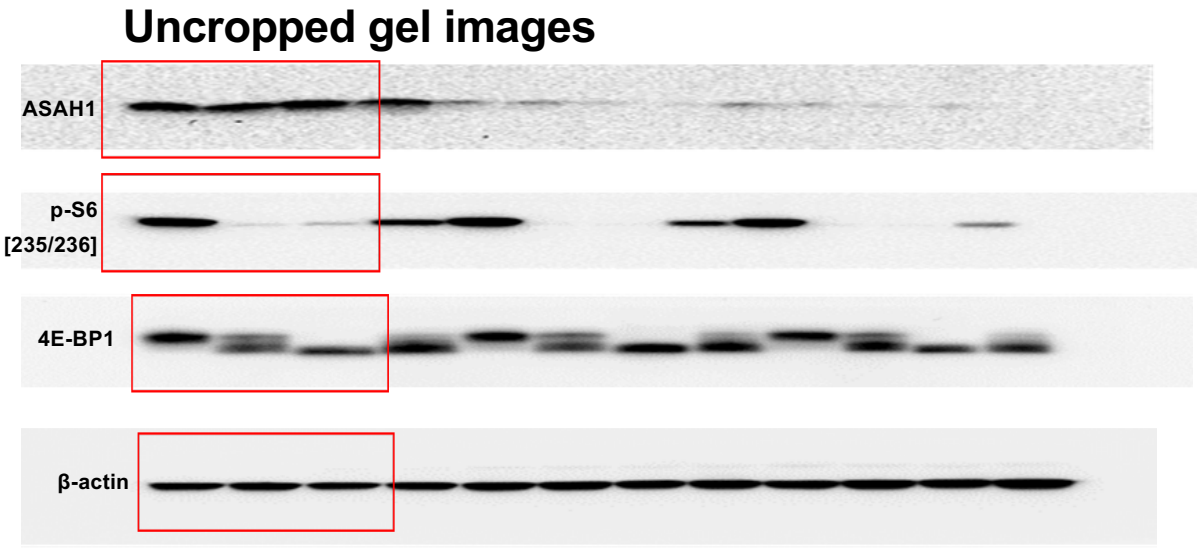

Vehicle
Rapa
RLK1
ASAH1
p-S6
[235/236]
4E-BP1
β-actin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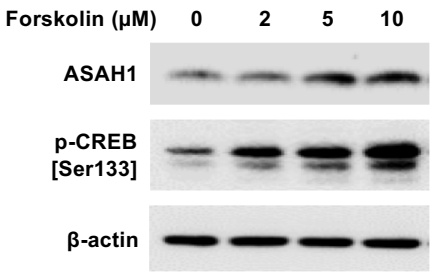
ASAH1
p-S6
[235/236]
4E-BP1
β-actin

Figure 4

H



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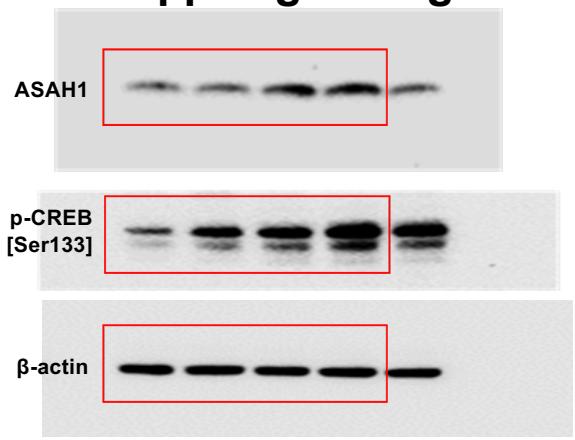

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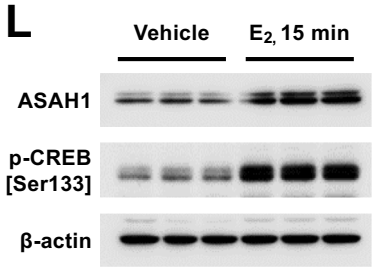

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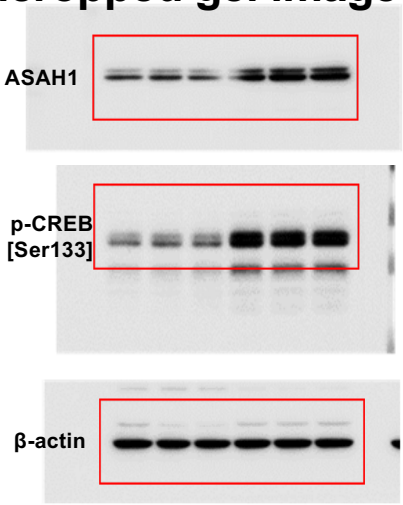

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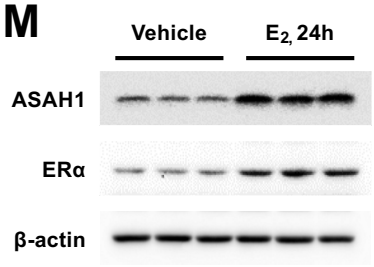

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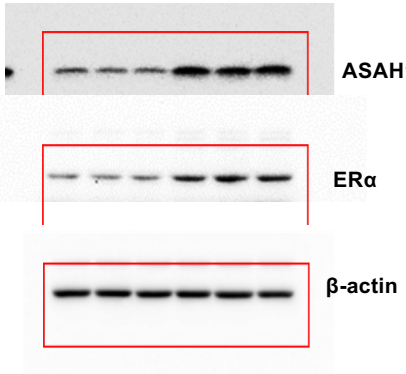
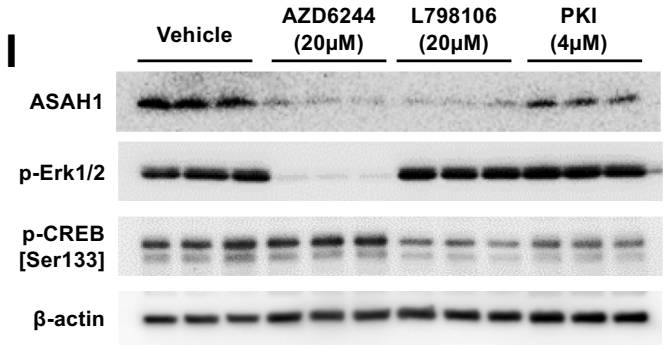


Figure 4



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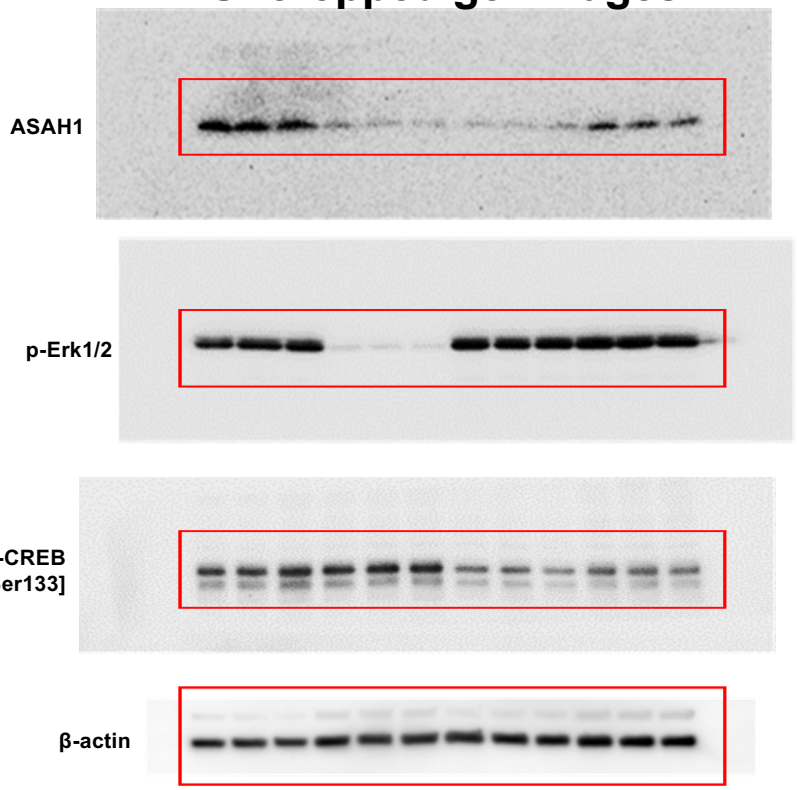

New Figure 5D

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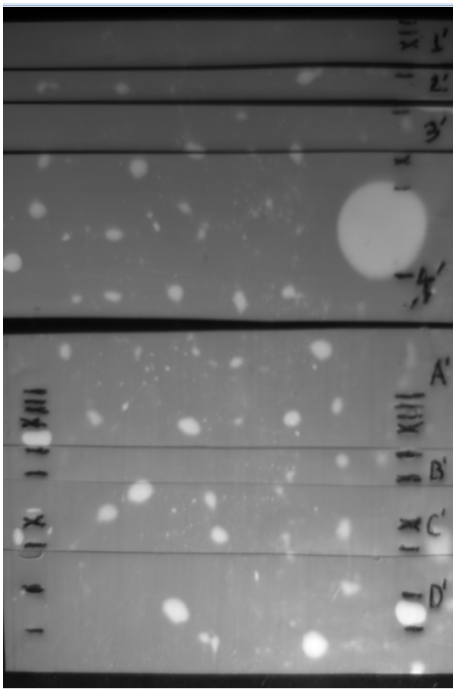

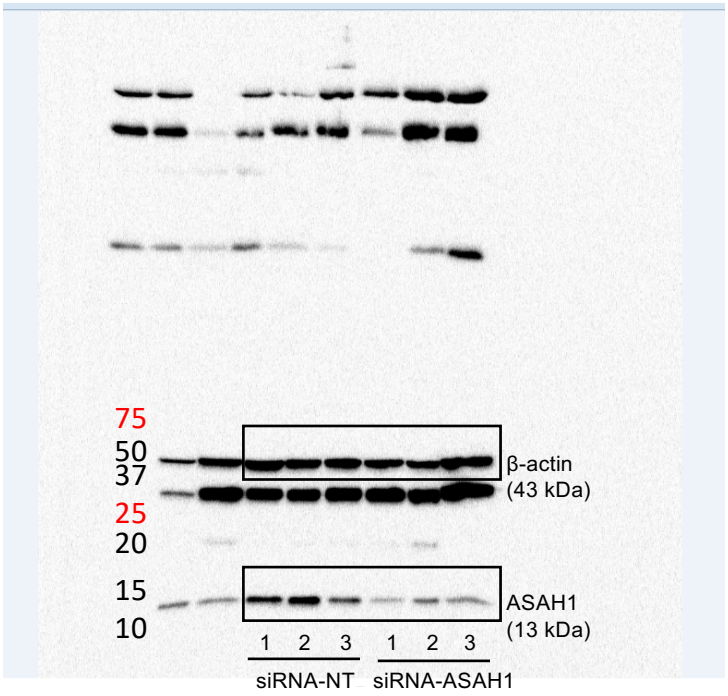

Figure 5

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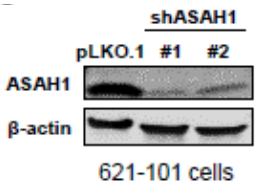

621-101 cells

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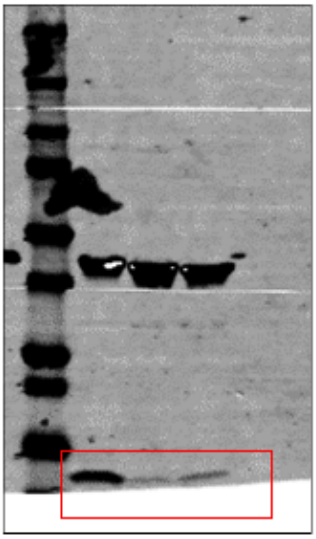


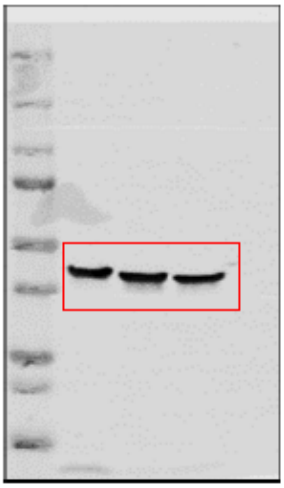
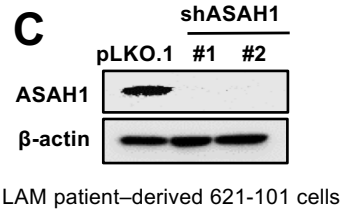
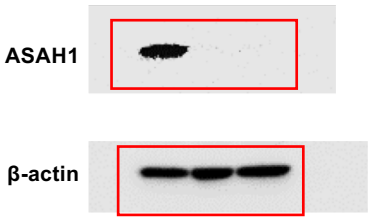


Figure 6



LAM patient–derived 621-101 cells

Uncropped gel images