

**Cell Reports, Volume 31**

**Supplemental Information**

**mTOR Signaling and SREBP Activity**

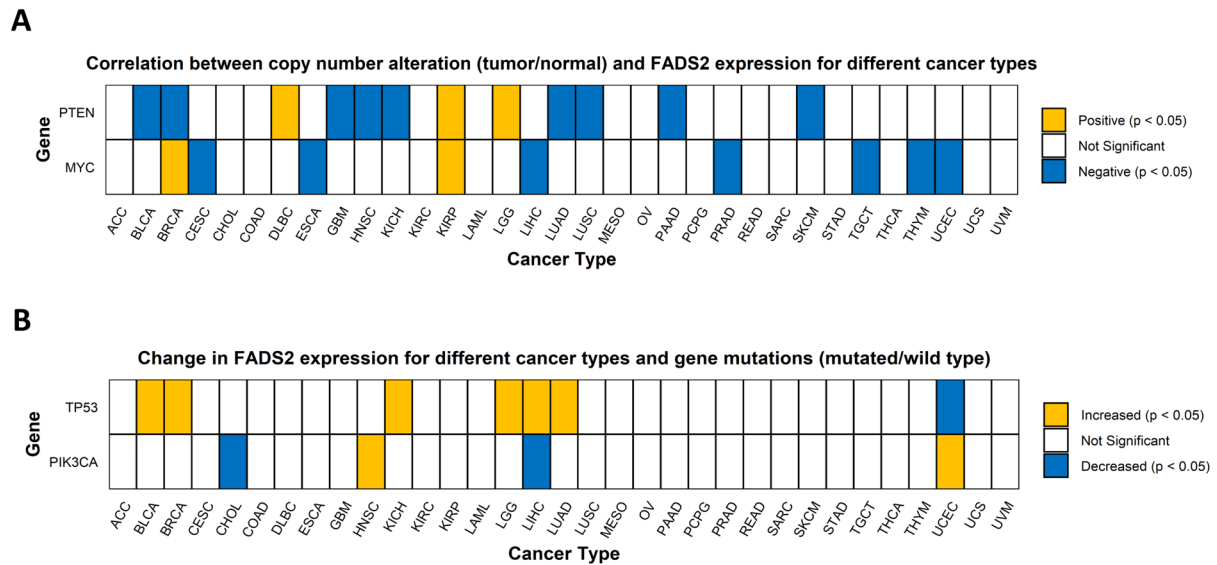
**Increase FADS2 Expression**

**and Can Activate Sapienate Biosynthesis**

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Supplementary figures and tables

Figure S1



**Figure S1:** Correlation of *FADS2* expression with copy number and DNA mutations status of signature genes in TCGA database, Related to **Figure 1** and **Table S4**

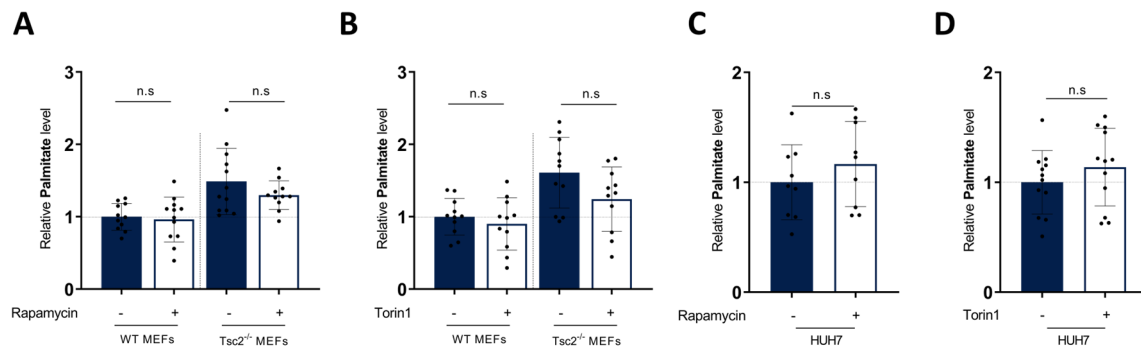
**(A)** Heat map representing the correlation between *FADS2* expression and the average copy number alteration of *PTEN* and *MYC* based on the TCGA data derived from 12,650 patients representing 33 major human cancer type. Positive correlations ( $p < 0.05$ ) are indicated in yellow and negative correlations ( $p < 0.05$ ) are indicated in blue. No significant correlations ( $p > 0.05$ ) are presented in white.

**(B)** Heat map representing the correlation between *FADS2* and the average of *TP53* and *PIK3CA* mutations. TCGA samples representing 33 major human cancer type were divided by mutation status of *TP53* and *PIK3CA* in wild type and mutated (defined as having any type of mutation) and *FADS2* expression was compared between both groups using a t-test. Correlations ( $p < 0.05$ ) between increased *FADS2* expression and the presence of mutations are indicated in yellow; correlations ( $p < 0.05$ ) between decreased *FADS2* expression and the presence of mutations are indicated in blue. No significant correlations ( $p > 0.05$ ) are presented in white.

ACC: Adrenocortical carcinoma, BLCA: Bladder Urothelial Carcinoma, BRCA: Breast invasive carcinoma, CESC: Cervical squamous cell carcinoma and endocervical adenocarcinoma, CHOL: Cholangiocarcinoma, COAD: Colon adenocarcinoma, DLBC: Lymphoid Neoplasm Diffuse Large B-cell Lymphoma, ESCA: Esophageal carcinoma, GBM: Glioblastoma multiforme, HNSC: Head and Neck squamous cell carcinoma, KICH: Kidney Chromophobe, KIRC: Kidney renal clear cell carcinoma, KIRP: Kidney renal papillary cell

carcinoma, LAML: Acute Myeloid Leukemia, LGG: Brain Lower Grade Glioma, LIHC: Liver hepatocellular carcinoma, LUAD: Lung adenocarcinoma, LUSC: Lung squamous cell carcinoma, MESO: Mesothelioma, OV: Ovarian serous cystadenocarcinoma, PAAD: Pancreatic adenocarcinoma, PCPG: Pheochromocytoma and Paranglioma, PRAD: Prostate adenocarcinoma, READ: Rectum adenocarcinoma, SARC: Sarcoma, SKCM: Skin Cutaneous Melanoma, STAD: Stomach adenocarcinoma, TGCT: Testicular Germ Cell Tumors, THCA: Thyroid carcinoma, THYM: Thymoma, UCEC: Uterine Corpus Endometrial Carcinoma, UCS: Uterine Carcinosarcoma, UVM: Uveal Melanoma

**Figure S2**

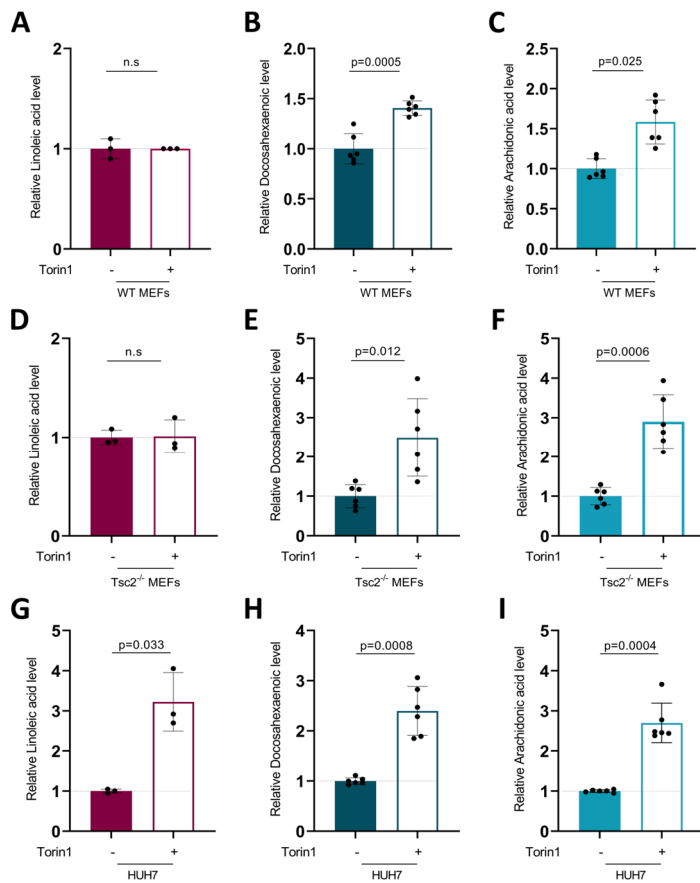


**Figure S2: mTOR inhibition does not alter palmitate levels, Related to Figure 2 and Figure 3**

**(A, B)** Relative palmitate level upon treatment with DMSO (**A**, N=12, **B**, N=11), rapamycin (**A**) (20 nM, 72 h, N=12) or Torin1 (**B**) (40 nM, 72 h, N=11) in WT and *Tsc2*<sup>-/-</sup> MEFs. Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**(C, D)** Relative palmitate level upon treatment with DMSO (**C**, N=9, **D**, N=12), rapamycin (**C**) (20 nM, 72 h, N=9) or Torin1 (**D**) (40 nM, 72 h, N=12) in HUH7 cancer cells. Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**Figure S3**



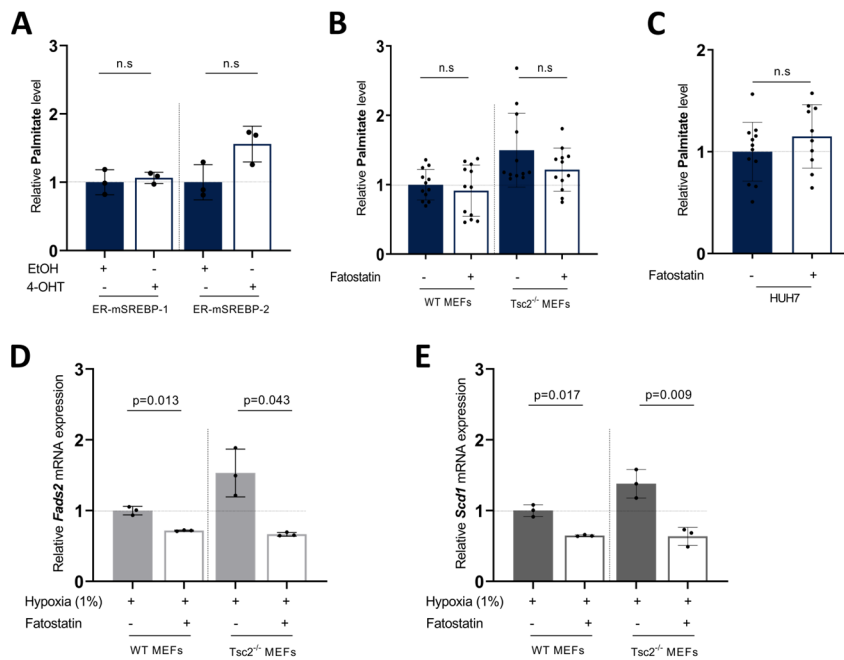
**Figure S3:** mTOR inhibition results in elevated polyunsaturated fatty acid levels, Related to **Figure 2** and **Figure 3**

(A-C) Relative levels of linoleic acid (A), docosahexaenoic acid (B) and arachidonic acid (C) in the wildtype (WT) MEFs upon treatment with DMSO or Torin1 (40 nM, 72 h, A, N=3, B-C, N=6). Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

(D-F) Relative levels of linoleic acid (D), docosahexaenoic acid (E) and arachidonic acid (F) in the Tsc2<sup>-/-</sup> MEFs upon treatment with DMSO or Torin1 (40 nM, 72 h, D, N=3, E-F, N=6). Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

(G-I) Relative levels of linoleic acid (G), docosahexaenoic acid (H) and arachidonic acid (I) in the HUH7 cancer cells upon treatment with DMSO or Torin1 (40 nM, 72 h, G, N=3, H-I, N=6). Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**Figure S4**



**Figure S4:** SREBP modulation does not alter palmitate level and regulates *Fads2* and *Scd1* transcription in hypoxia, Related to **Figure 4**

**(A)** Relative palmitate level was determined in U87 cells stably expressing ER-mSREBP-1 or ER-mSREBP-2. Cells were treated with 100 nM 4-OHT or solvent for 24h in medium containing 10% FBS (N=3). Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**(B, C)** Palmitate level upon treatment with DMSO (N=12) or fatostatin (10  $\mu$ M, 72 h, **B**, N=12, **C**, N=10) in WT and Tsc2<sup>-/-</sup> MEFs (**B**) and in HUH7 cancer cells (**C**). Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**(D, E)** *Fads2* (**D**) and *Scd1* (**E**) mRNA expression in WT and Tsc2<sup>-/-</sup> MEFs cells treated with fatostatin (10  $\mu$ M, 48 h, N=3) combined with hypoxia (1% O<sub>2</sub>). Relative mRNA levels were determined by qPCR. Bar graphs are presented as mean  $\pm$  SD from biological independent samples. Statistical testing was performed by a two-sided unpaired Student's t test.

**Table S5:** qPCR primer sequences list, Related to **KEY RESOURCES TABLE**

	<b>Gene</b>	<b>Forward (5'-3')</b>	<b>Reverse (5'-3')</b>
	<i>FADS2</i>	GACCACGGCAAGAACTCAAAG	GAGGGTAGGAATCCAGCCATT
	<i>SCD1</i>	TCTCTGCTACACTTGGGAGC	GAGCTTTGTAAGAGCGGTGG
<b>Human</b>	<i>FADS2 (Site A)</i>	TCTGCTGATCGCTGTGGAAACT	TCAGCCCTCCCCTATGGACTTT
	<i>FADS2 (Site B)</i>	TGGAGGCAAAAGTCCATAGC	GATCCCTGGCTTCCCAGT
	<i>ACTB</i>	GCCTCGCCTTTGCCGAT	CGCGGCGATATCATCATCC
	<i>RPL19</i>	ACCCCA ATGAGACCA ATG AA	CGCAAAATCCTCATTCTCCT
	<i>Fads2</i>	ATAGTAGCTGATGGCCCAAGC	AGCCCCTTGAGTATGGCAAG
<b>Mouse</b>	<i>Scd1</i>	CGCTCTTTACCCTTTGCTGG	AAGAACTGGAGATCTCTTGGAGC
	<i>Rpl19</i>	CAGGCATATGGGCATAGGGAA	TGCCTTCAGCTTGTGGATGT