

Interaction of sortilin with apolipoprotein E3 enables neurons to use long-chain fatty acids as alternative metabolic fuel

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SUPPLEMENTARY TABLES

Supplementary Table 1: Primary and secondary antibodies used in this study

Antibody	Source	Identifier	Dilution
Total OXPHOS Human WB Antibody Cocktail	Abcam	Cat# ab110411	WB 1:1000
Total OXPHOS Rodent WB Antibody Cocktail	Abcam	Cat# STN-19467	WB 1:250
APOE	Millipore	Cat# AB947	WB 1:1000
ATP5F1	Proteintech	Cat# 68304-1-Ig	WB 1:1000 WB 1:2000 (synaptosomes)
CPT1A (8F6AE9)	Abcam	Cat# ab128568	WB 1:2000
FABP6	Proteintech	Cat# 13781-1-AP	WB 1:1000
FABP7	Sigma-Aldrich	Cat# ABN14	WB 1:3000
GAPDH	Millipore	Cat# 374	WB 1:5000
GFAP	Abcam	Cat# ab53554	IF 1:200
MAP2	Synaptic Systems	Cat# 188 004	IF 1:400
MFN1 (3F11C11)	Proteintech	Cat# # 66776-1-Ig	WB 1:5000 WB 1:1000 (synaptosomes)
MFN2 (7H42L13)	Invitrogen	Cat# 702768	WB 1:2000
MTCO1 (1D6E1A8)	Invitrogen	Cat# 459600	WB 1:1000
NANOG	R&D Systems	Cat# AF1997	IF 1:200
OCT4 (clone 3A2A20)	StemCell	Cat# 60093	IF 1:200
Sortilin	R&D Systems	Cat# AF3154	IF 1:200 (iPSCs) IF 1:400 (iNs)
Sortilin	BD Biosciences	Cat# 612101	WB 1:3000

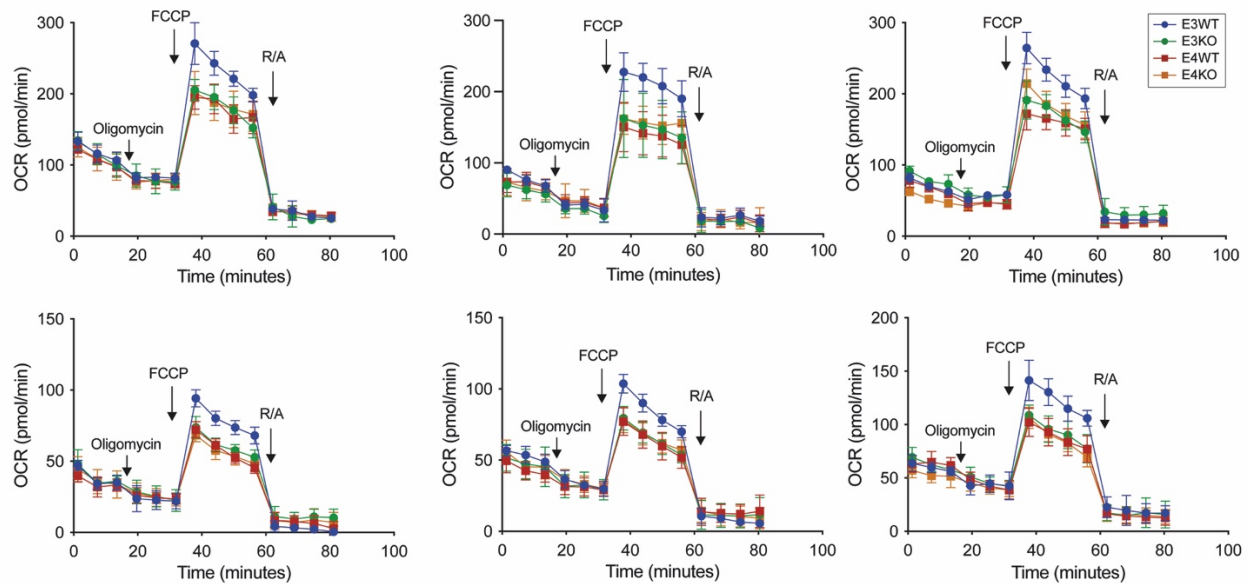
SOX2 (clone 245610)	R&D Systems	Cat# MAB2018	IF 1:200
SSEA4	Abcam	Cat# 16287	IF 1:200

Antibody	Source	Identifier	Dilution
anti-mouse Alexa Fluor 488	Invitrogen	Cat# A21202	IF 1:500 - 1:2000
anti-mouse Alexa Fluor 647	Invitrogen	Cat# A31571	IF 1:500 - 1:2000
anti-mouse igG (HRP-labeled)	Abcam	Cat# ab6728	WB 1:5000
anti-goat Alexa Fluor 488	Invitrogen	Cat# 11055	IF 1:500 - 1:2000
anti-goat Alexa Fluor 555	Invitrogen	Cat# A21432	IF 1:500 - 1:2000
anti-goat Alexa Fluor 568	Invitrogen	Cat# A11057	IF 1:500 - 1:2000
anti-goat igG (HRP-labeled)	Millipore	Cat# AP106P	WB 1:5000
anti-guinea pig Alexa Fluor 647	Jackson ImmunoResearch	Cat# 706-605-158	IF 1:500 - 1:2000
anti-rabbit Alexa Fluor 488	Invitrogen	Cat# A21206	IF 1:500 - 1:2000
anti-rabbit igG (HRP-labeled)	Millipore	Cat# AP132P	WB 1:5000

Supplementary Table 2: Taqman probes used to determine transcript levels of murine and human genes

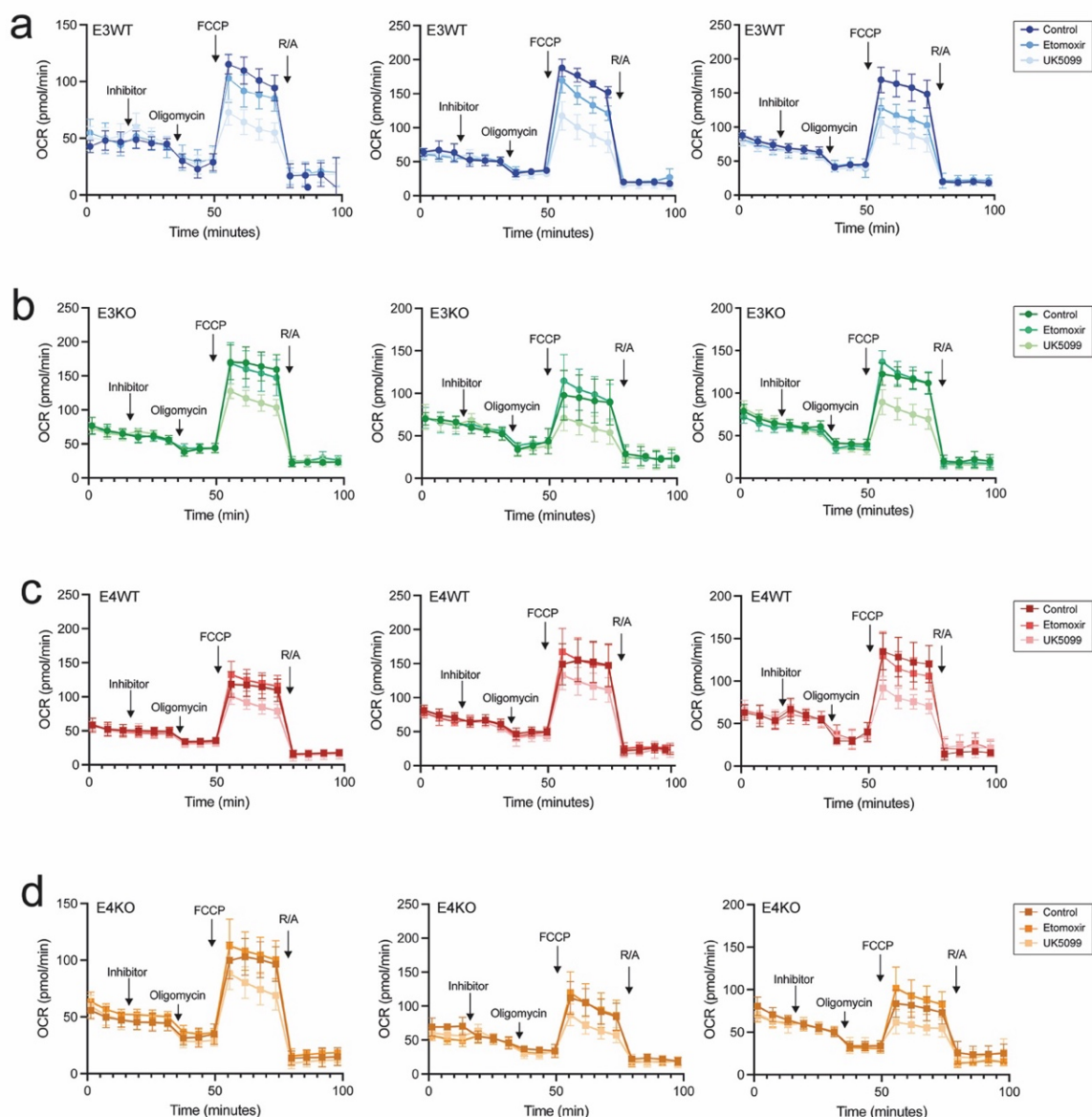
Gene	TaqMan probe identifier	Gene	TaqMan probe identifier
<i>Acadl</i>	Mm00599660_m1	<i>ACADL</i>	Hs00155630_m1
<i>Acadm</i>	Mm00431611_m1	<i>ALDH1L1</i>	Hs00201836_m1
<i>Acadvl</i>	Mm00444293_m1	<i>APOE</i>	Hs00171168_m1
<i>Acsl5</i>	Mm01261083_m1	<i>CD36</i>	Hs00354519_m1
<i>Aldh1l1</i>	Mm03048949_m1	<i>CPT1A</i>	Hs00912671_m1
<i>Baf53b/Actl6b</i>	Mm00504274_m1	<i>FABP3</i>	Hs00997360_m1
<i>Cd36</i>	Mm00432403_m1	<i>FABP5</i>	Hs02339439_g1
<i>Cpt1a</i>	Mm01231183_m1	<i>FABP6</i>	Hs01031183_m1
<i>Cpt1c</i>	Mm00463970_m1	<i>FABP7</i>	Hs00361426_m1
<i>Cpt2</i>	Mm00487202_m1	<i>GAPDH</i>	Hs99999905_m1
<i>Crat</i>	Mm00483985_m1	<i>GFAP</i>	Hs00909233_m1
<i>Gapdh</i>	Mm99999915_m1	<i>MAP2</i>	Hs00258900_m1
<i>Gfap</i>	Mm01253033_m1	<i>NEFL</i>	Hs00196245_m1
<i>NeuN/Rbfox3</i>	Mm01248771_m1	<i>POU5F1</i>	Hs00999632_g1
<i>Slc16a1</i>	Mm01306379_m1	<i>PPARA</i>	Hs00231882_m1
<i>Slc16a3</i>	Mm00446102_m1	<i>PPARG</i>	Hs01115513_m1
<i>Slc16a7</i>	Mm00441442_m1	<i>PPARD</i>	Hs00602622_m1
<i>Slc22a5</i>	Mm00441468_m1	<i>RBFOX3</i>	Hs01370654_m1
<i>Slc27a1</i>	Mm00449511_m1	<i>SI00B</i>	Hs00902901_m1
<i>Slc27a6</i>	Mm01258609_m1	<i>SOX2</i>	Hs04260357_g1
		<i>TUBB3</i>	Hs00964963_g1

SUPPLEMENTARY FIGURES AND LEGENDS



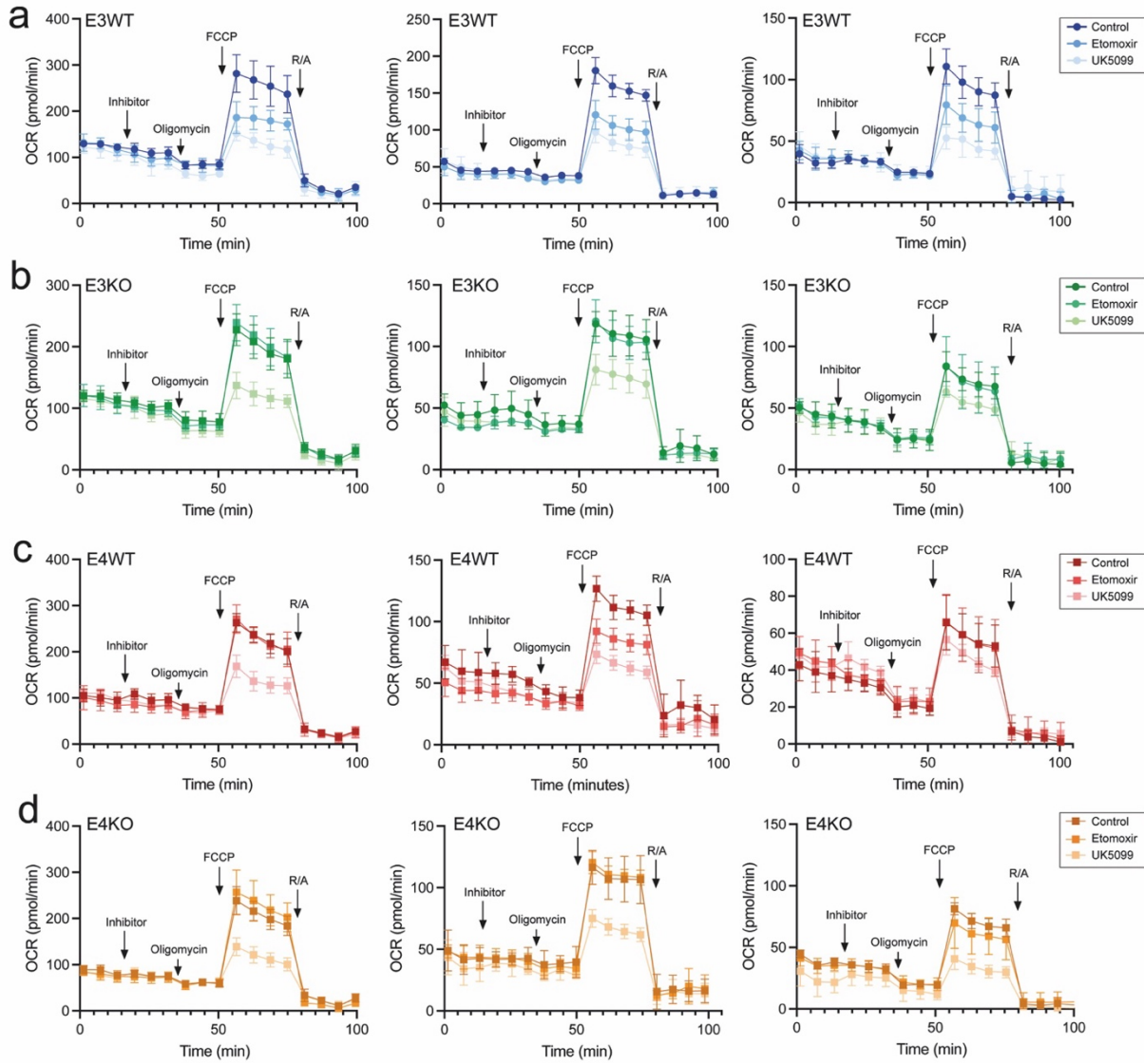
Supplementary figure 1 (related to Fig. 1b-f): Respiration profiles of independent synaptosomal preparations from brains of male mice

Real-time cellular oxygen consumption rates (OCR) in six independent synaptosomal preparations from brain cortices of male mice of the indicated *Sort1* and *APOE* genotypes (12 weeks of age) are given. Each biological replicate data point is the mean of n=18-24 technical replicates. The combined dataset is given in Fig. 1b-f in the main text.



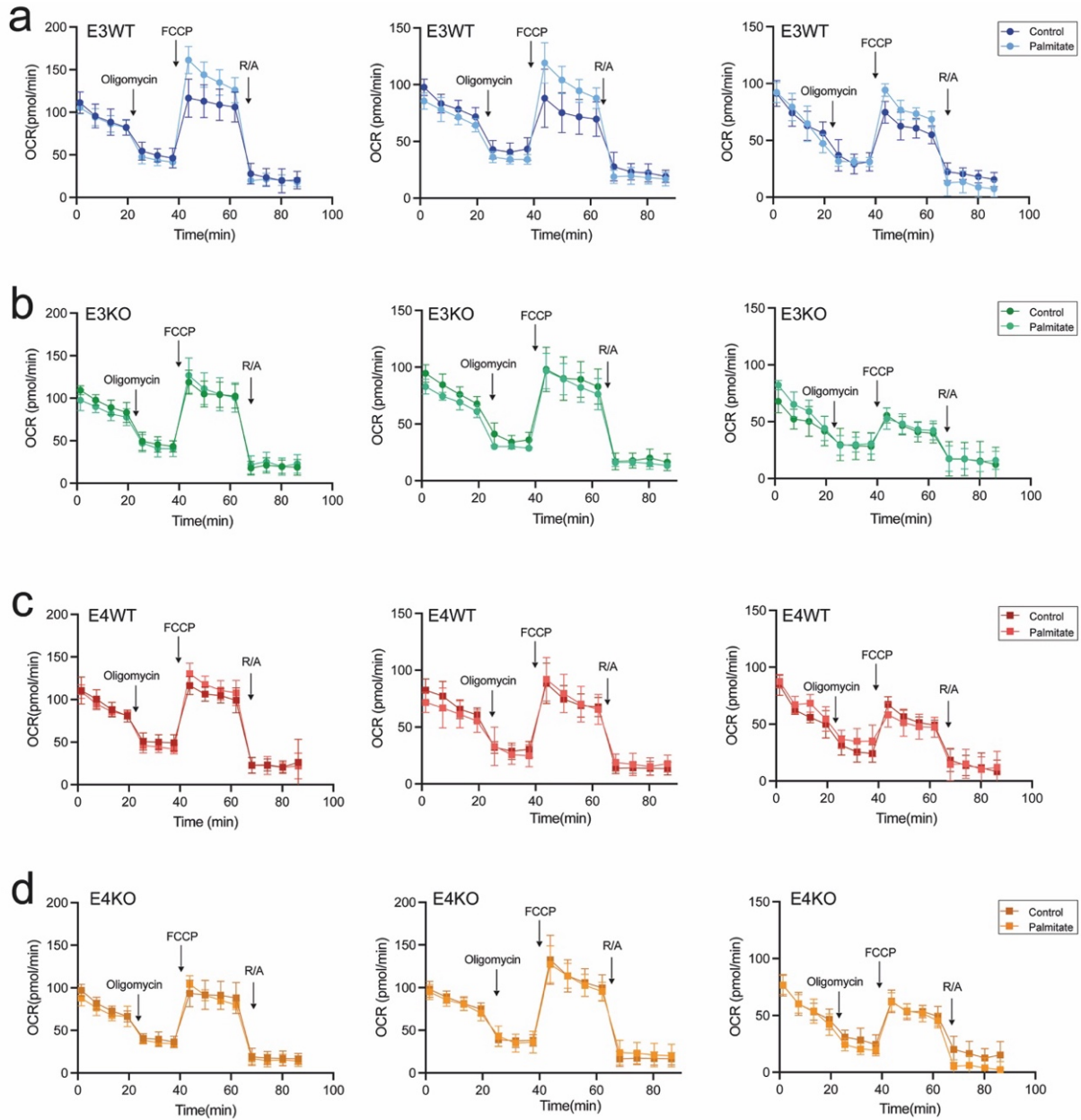
Supplementary figure 2 (related to Fig. 1g-j): Substrate oxidation stress test in independent synaptosomal preparations from brains of male mice

Real-time cellular oxygen consumption rates (OCR) in three independent preparations each of synaptosomes from E3WT (**a**), E3KO (**b**), E4WT (**c**), and E4KO (**d**) are shown. The dependency of mitochondrial respiration on availability of glucose or long-chain fatty acids was assessed in the presence of solvent control buffer (control) or solutions containing 8 μ M UK5099 or 16 μ M etomoxir (inhibitor). Each data point is the mean of n=10-18 technical replicates. The combined datasets are shown in Fig. 1g-j.



Supplementary figure 3 (related to Ext. data figure 2): Substrate oxidation stress test in independent synaptosomal preparations from brains of female mice

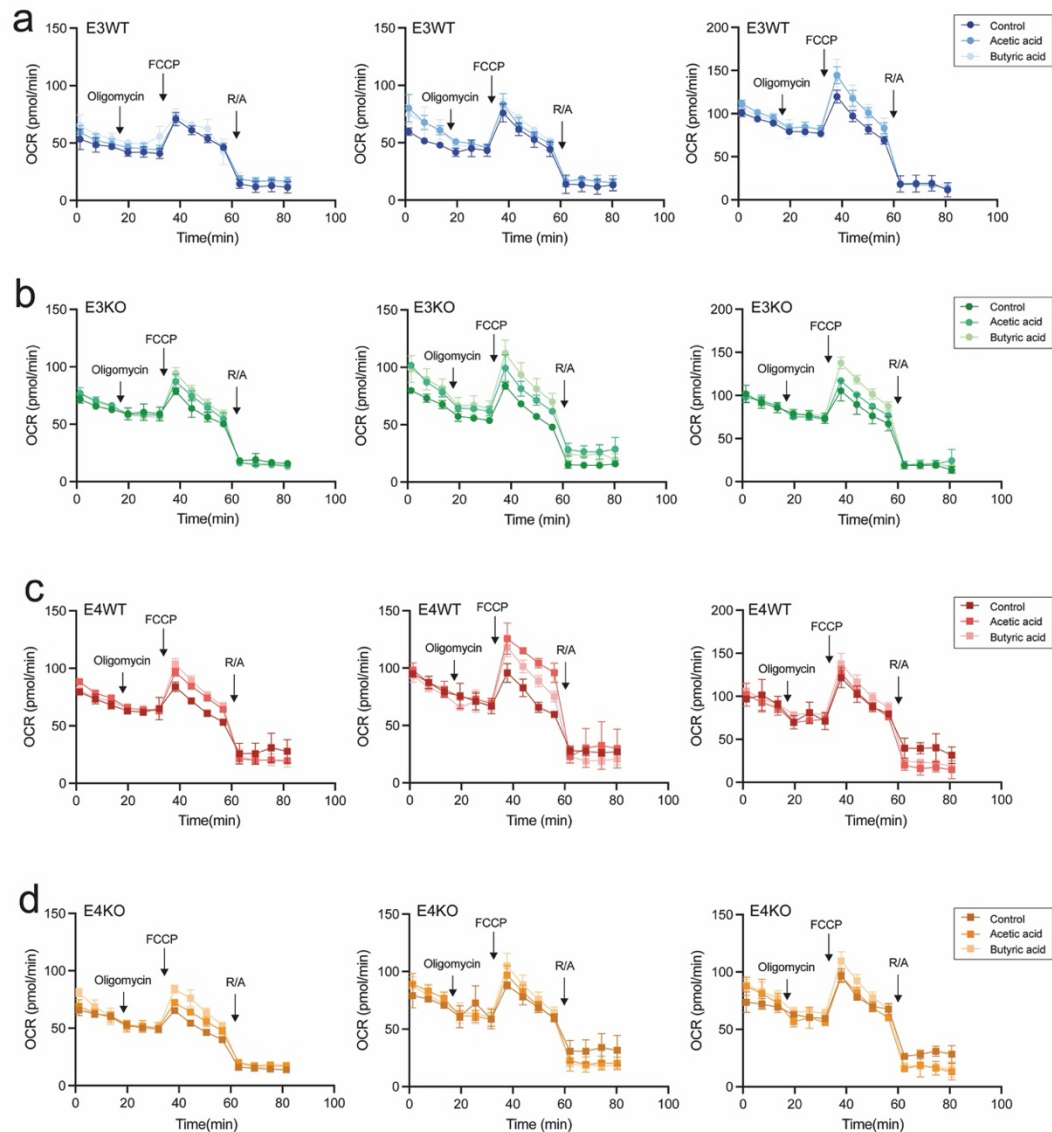
Real-time cellular oxygen consumption rates (OCR) in three independent preparations each of synaptosome from the brain of female E3WT (a), E3KO (b), E4WT (c), and E4KO (d) animals shown. The dependency of mitochondrial respiration on availability of glucose or long-chain fatty acids was assessed in the presence of solvent control buffer (control) or solutions containing 8 μ M UK5099 or 16 μ M etomoxir (inhibitor). Each data point is the mean of n=7-8 technical replicates. The combined datasets are shown in Ext. data figure 2.



Supplementary figure 4 (related to Fig. 2b-e): Respiration profiles of three independent synaptosomal preparations from brains of male mice cultured in the presence of LCFA

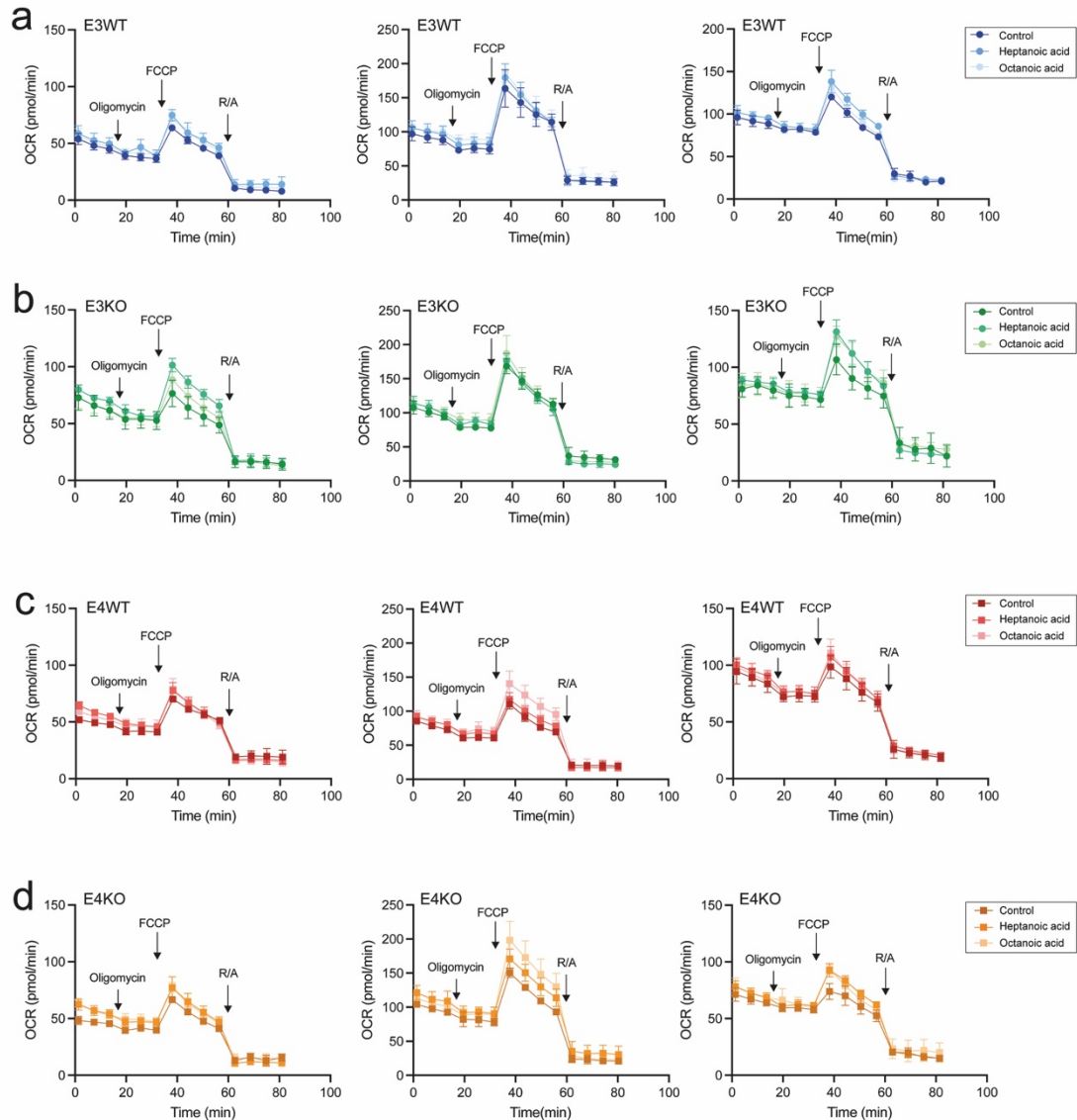
Quantification of real-time cellular oxygen consumption rates (OCR) in three independent preparations each of synaptosomes from brain cortices of male mice, either E3WT (a), E3KO (b), E4WT (c), or E4KO (d). The dependency of mitochondrial respiration on long-chain fatty acid consumption was assessed by pre-treatment of synaptosomes without (control) or with 300 μ M palmitate under low glucose conditions (1 mM sodium pyruvate, 2 mM glucose, 1 mM

glutamine). Each data point represents the mean of 8-15 technical replicates. The combined data set is shown in Fig. 2b-e.



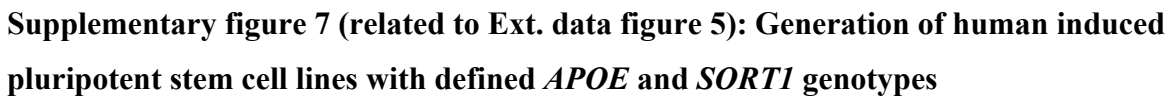
Supplementary figure 5 (related to Fig. 2f-i): Respiration profiles of three independent synaptosomal preparations from male mice cultured in the presence of SCFA

Quantification of real-time cellular oxygen consumption rates (OCR) in three independent preparations each of synaptosomes from brain cortices of male mice (12 weeks of age), either E3WT (a), E3KO (b), E4WT (c), or E4KO (d) are shown. The dependency of mitochondrial respiration on short-chain fatty acids consumption was assessed under low glucose conditions (1 mM sodium pyruvate, 2 mM glucose, 1 mM glutamine) by pre-treatment of synaptosomes without (control) or with 100 μ M acetic acid or 100 μ M butyric acid. Each data point represents the mean of 8-15 technical replicates. The combined data set is shown in Fig. 2f-i.

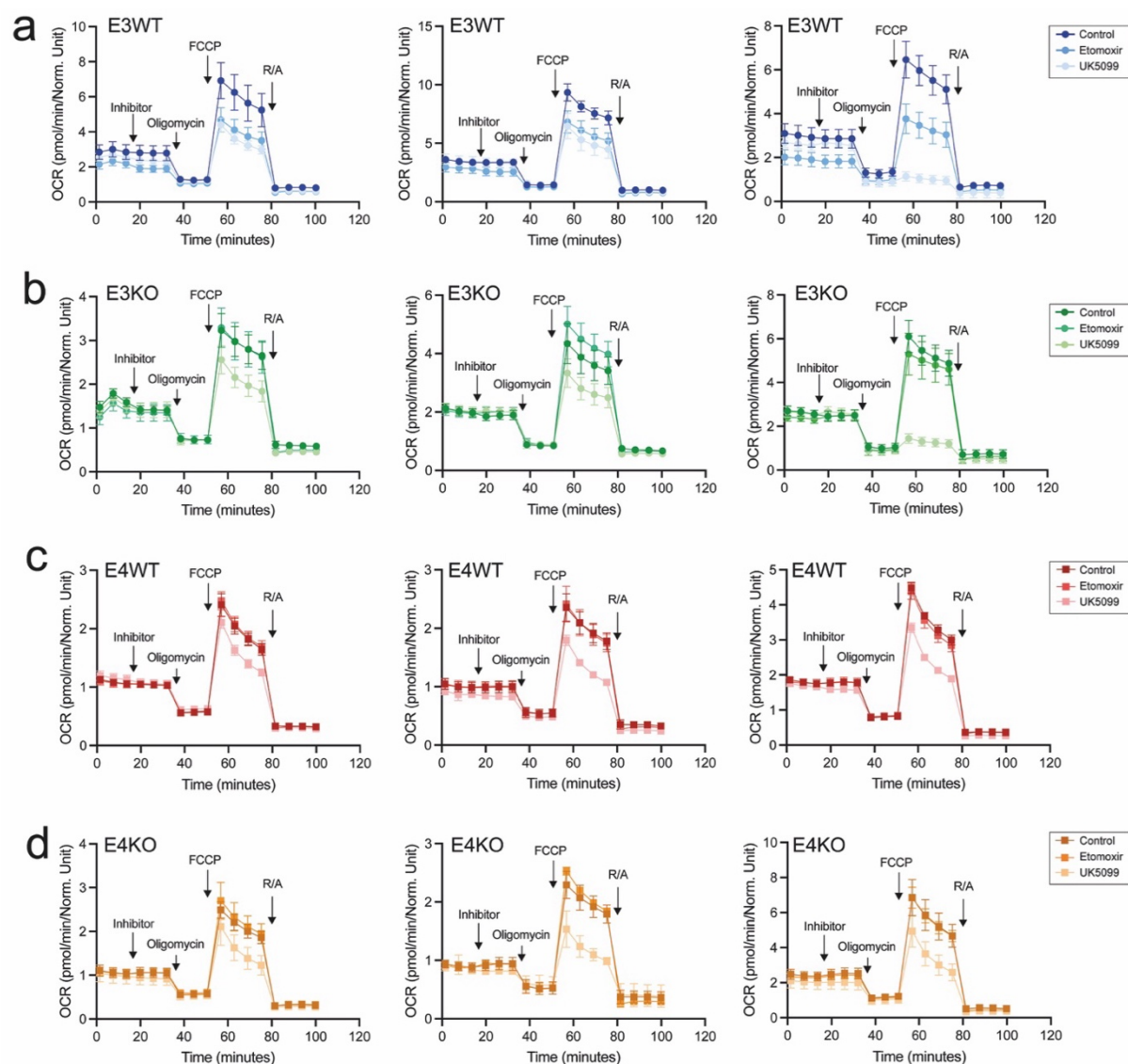


Supplementary figure 6 (related to Fig. 2j-m): Respiration profiles of three independent synaptosomal preparations from brains of male mice cultured in the presence of MCFA

Quantification of real-time cellular oxygen consumption rates (OCR) in three independent preparations each of synaptosomes from brain cortices of male mice (12 weeks of age), either E3WT (a), E3KO (b), E4WT (c), or E4KO (d) are shown. The dependency of mitochondrial respiration on medium-chain fatty acids was assessed under low glucose conditions (1 mM sodium pyruvate, 2 mM glucose, 1 mM glutamine) by pre-treatment of synaptosomes without (control) or with 200 μ M heptanoic acid or 200 μ M octanoic acid. Each data point represents the mean of 8-15 technical replicates. The combined data set is shown in Fig. 2j-m.

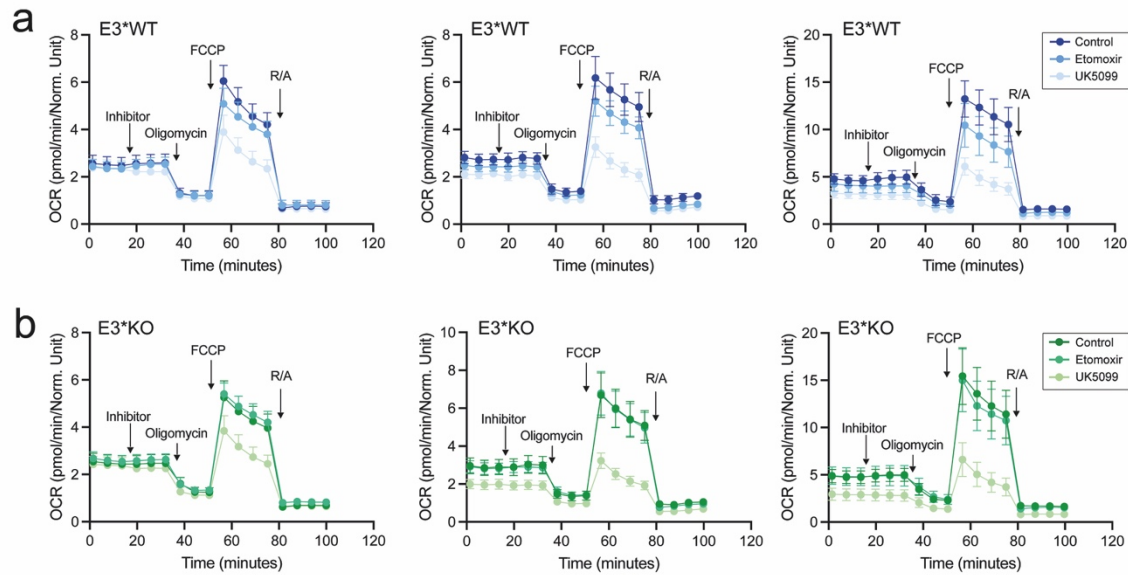


(a) *APOE* genotyping of human iPS cell (hiPSC) lines using quantitative RT-PCR. The scatter plot X- and Y-axes represent allele discrimination for *APOE* ϵ 3 and *APOE* ϵ 4 genotypes. Shown hiPSC lines (colored squares) are *APOE* ϵ 3 (E3) or *APOE* ϵ 4 (E4) and either wildtype (WT) or genetically deficient for *SORT1* (KO). As internal controls, human samples being *APOE* ϵ 3/ ϵ 3 (orange dot), *APOE* ϵ 3/ ϵ 4 (grey dot), or *APOE* ϵ 4/ ϵ 4 (yellow dot) were included in the genotyping PCR. The white dot represents the water-only control. (b) Genome sequence analysis of the *SORT1* gene region in genome edited iPS lines. Cell line E3KO carries a 23 base pair deletion that includes the ATG (green), as compared to the isogenic E3WT control line. Cell line E4KO harbors a one nucleotide insertion (red), resulting in premature stop codon (TGA, yellow), as compared to the isogenic E4WT control. Sequences were aligned to the human *SORT1* reference sequence (NCBI: BC023542.1). (c) Western blot analyses of lysates from the indicated iPS lines, documenting the presence of sortilin in E3WT and E4WT, but absence of protein from lines E3KO and E4KO. Detection of GAPDH served as loading control. (d) Transcript levels of *SOX2* and *OCT4* in iPS lines of the indicated *SORT1* and *APOE* genotypes were tested using qRT-PCR. Data points for n=5-7 independent cell cultures as well as mean \pm SD of the entire data set are given. (e, f) Representative immunofluorescence images of iPS lines of the indicated genotypes stained for pluripotency markers OCT4 (green), NANOG (purple), and SOX2 (red) in e, as well as for SSEA4 (green) and sortilin (red) in f. In f, DAPI (blue) in merge. Scale bars: 200 μ m (e), 50 μ m (f). (g) Transcript levels of 94 genes involved in pluripotency and lineage differentiation potential were quantified in iPS lines of the indicated *SORT1* and *APOE* genotypes using the TaqMan hPSC Scorecard Assay (as detailed in the method section). Expression levels are shown as heatmap with colors correlating to the fold change in expression of the indicated gene relative to the undifferentiated reference set. (h) Box plot depicting transcript levels of genes in the Scorecard Assay panel associated with self-renewal or ectodermal, mesodermal or endodermal differentiation in iPS lines of the indicated genotypes. Sample scores are plotted in color. Gray box and whisker plots represent undifferentiated reference datasets.



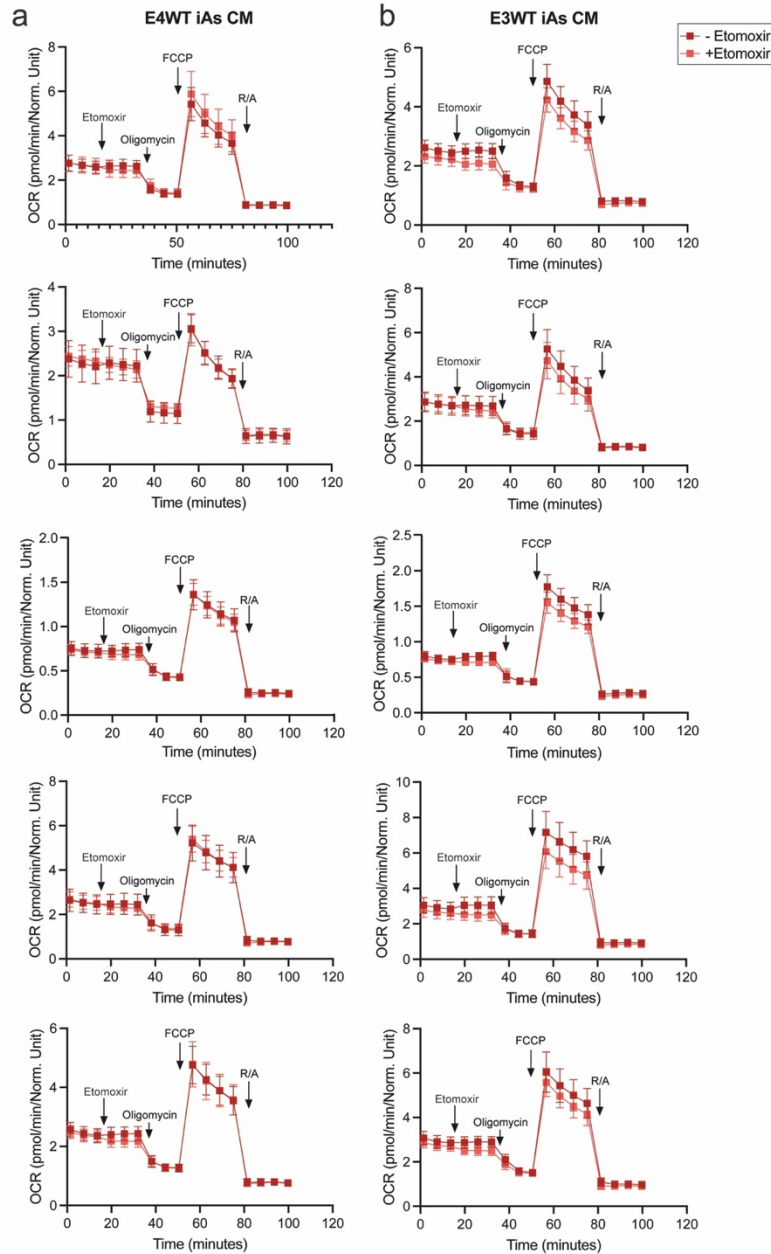
Supplementary figure 8 (related to Fig. 4a-d): Loss of sortilin or the presence of apoE4 causes insensitivity to etomoxir in induced human neurons

Respiration profiles of induced human neurons of the indicated *APOE* and *SORT1* genotypes from three independent differentiation experiments each per genotype. Neurons had been cultured in media conditioned by induced astrocytes of the corresponding genotypes. Realtime oxygen consumption rates (OCR) were measured in the presence of solvent control buffer (control) or solutions containing 8 μ M UK5099 or 16 μ M etomoxir (inhibitor). The combined datasets are given in Fig. 4a-d.



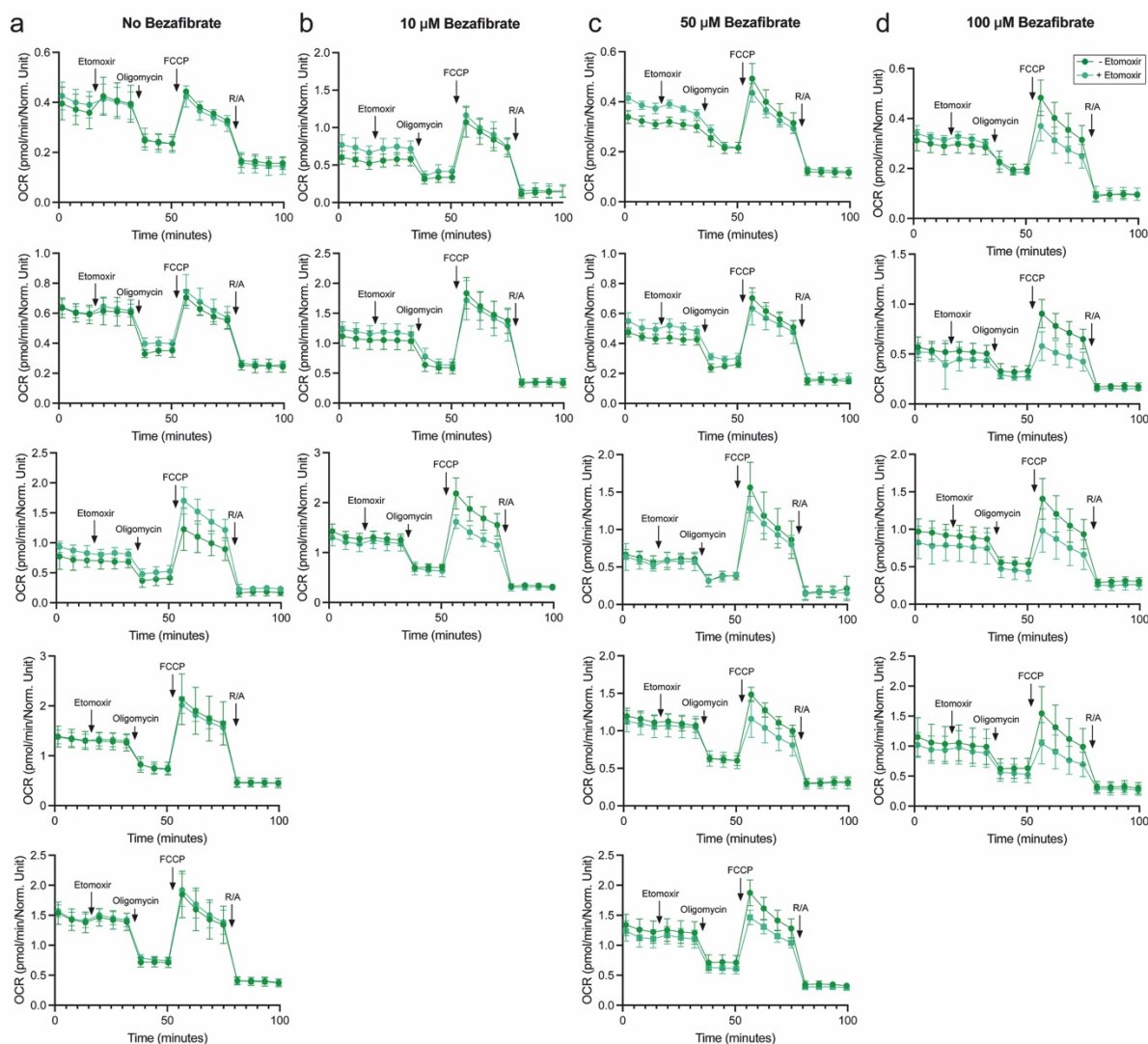
Supplementary figure 9 (related to Ext. data figure 6i-j): Sortilin deficiency causes neuronal insensitivity to etomoxir in a 2nd independent *APOE*ε3 line

Real-time oxygen consumption rates were determined in a 2nd independent *APOE*ε3/ε3 iPSC line, either WT (E3*WT) or KO (E3*KO) for *SORT1* in the presence of solvent control buffer or solutions containing 8 μM UK5099 or 16 μM etomoxir (inhibitor) at the indicated time points. Individual respiration profiles of three independent differentiation experiments for each genotype are shown. The combined datasets are given in Ext. data figure 6i-j.



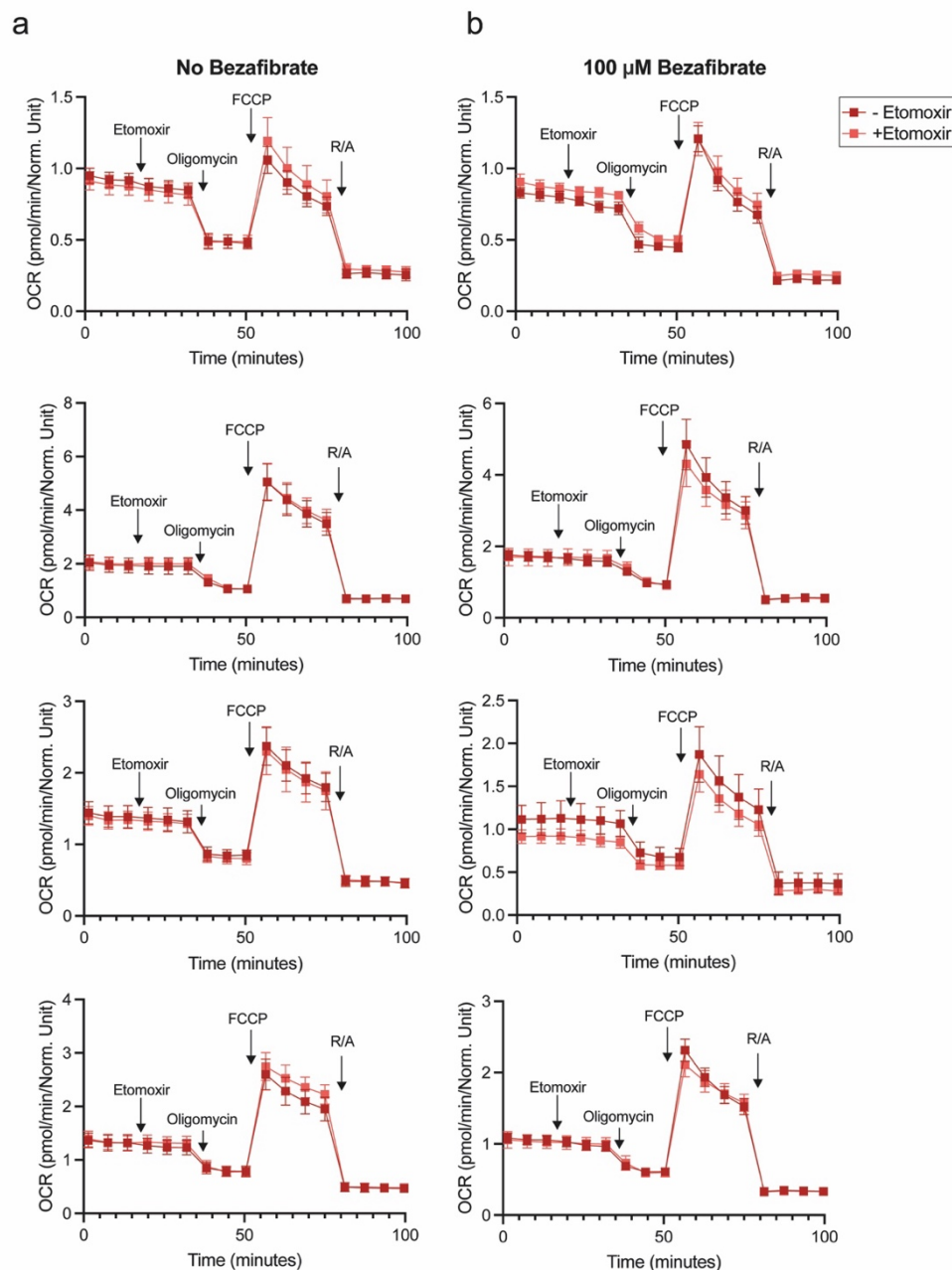
Supplementary figure 10 (related to Fig. 4g): Treatment with conditioned medium from E3WT astrocytes rescues sensitivity of E4WT neurons for etomoxir

Induced human E4WT neurons were cultured in the presence of media conditioned by E3WT astrocytes (E4WT iAs CM; **a**) or E3WT astrocytes (E3WT iAs CM, **b**). Real-time cellular oxygen consumption rates (OCR) were determined in the absence (-) or presence (+) of 16 μ M etomoxir. Individual respiration profiles of five independent differentiation experiments per genotype are shown. The combined datasets are given in Fig. 4g.



Supplementary figure 11 (related to Fig. 7c): Bezafibrate treatment rescues sensitivity of E3KO neurons to etomoxir

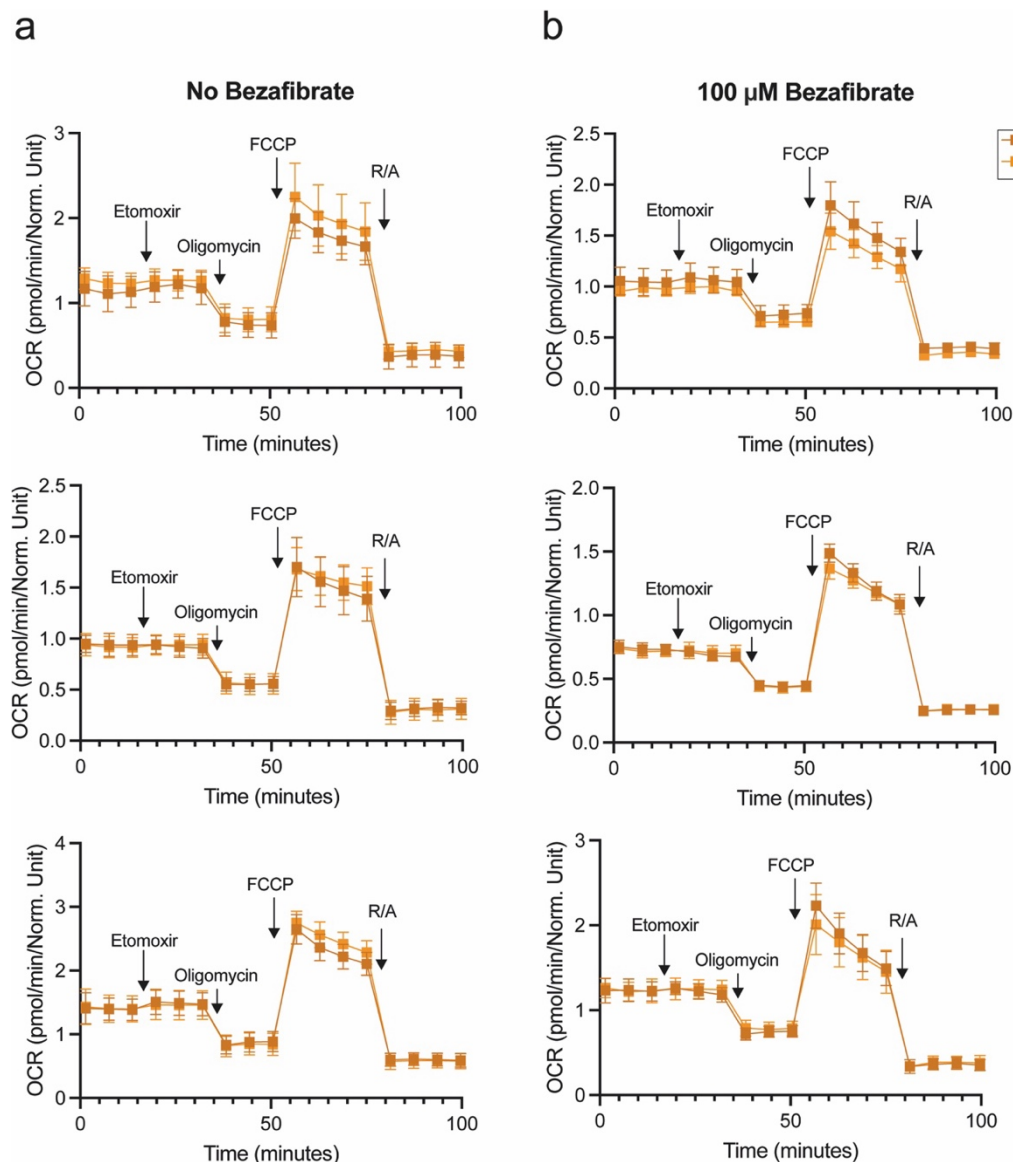
Real-time oxygen consumption rates (OCR) were determined in human induced E3KO neurons in the absence (-) or presence (+) of 16 μ M etomoxir. In addition, cells were treated with buffer (a), 10 μ M (b), 50 μ M (c), or 100 μ M (d) bezafibrate. Each data point is the mean of 10-15 technical replicates. Individual respiration profiles of three to five independent differentiation experiments per genotype group are shown. The combined datasets are given in Fig. 7c.



Supplementary figure 12 (related to Fig. 7f): Bezafibrate treatment rescues sensitivity of induced E4WT neurons to etomoxir

Real-time oxygen consumption rates (OCR) were determined in human induced E4WT neurons in the absence (-) or presence (+) of 16 μ M etomoxir. In addition, cells were treated with buffer (a) or 100 μ M (b) bezafibrate. Each data point is the mean of 8-12 technical replicates.

Individual respiration profiles of three independent differentiation experiments per genotype are shown. The combined datasets are given in Fig. 7f.



Supplementary figure 13 (related to Fig. 7i): Bezafibrate treatment rescues sensitivity of induced E4KO neurons to etomoxir

Real-time oxygen consumption rates (OCR) were determined in human induced E4KO neurons in the absence (-) or presence (+) of 16 μ M etomoxir. In addition, cells were treated with buffer (a) or 100 μ M (b) bezafibrate. Each data point is the mean of 8-12 technical replicates.

Individual respiration profiles of three independent differentiation experiments per genotype are shown. The combined datasets are given in Fig. 7i.

Unprocessed blot supplementary figure 7c

