The p97-UBXD8 complex modulates ER-Mitochondria contact sites by modulating membrane lipid saturation and composition

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Abstract

The intimate association between the endoplasmic reticulum (ER) and mitochondrial membranes at ER-mitochondria contact sites serves as a platform for several critical cellular processes, in particular lipid synthesis. Enzymes involved in lipid biosynthesis are enriched at contacts and membrane lipid composition at contacts is distinct relative to surrounding membranes. How contacts are remodeled and the subsequent biological consequences of altered contacts such as perturbed lipid metabolism remains poorly understood. Here we show that the p97 AAA-ATPase and its ER-tethered ubiquitin-X domain adaptor 8 (UBXD8) regulate the prevalence of ER-mitochondria contacts. The p97-UBXD8 complex localizes to contacts and loss of this complex increases contacts in a manner that is dependent on p97 catalytic activity. Quantitative proteomics of purified contacts demonstrates alterations in proteins regulating lipid metabolism upon loss of UBXD8. Furthermore, lipidomics studies indicate significant changes in distinct lipid species in UBXD8 knockout cells. We show that loss of p97-UBXD8 results in perturbed contacts due to an increase in membrane lipid saturation via SREBP1 and the lipid desaturase SCD1. Aberrant contacts in p97-UBXD8 loss of function cells can be rescued by supplementation with unsaturated fatty acids or overexpression of SCD1. Perturbation of contacts and inherent lipid synthesis is emerging as a hallmark to a variety of human disorders such as neurodegeneration. Notably, we find that the SREBP1-SCD1 pathway is negatively impacted in the brains of mice with p97 mutations that cause neurodegeneration. Our results suggest that contacts are exquisitely sensitive to alterations to membrane lipid composition and saturation in a manner that is dependent on p97-UBXD8.

45 **Main**

46 Contact sites between the ER and mitochondria allow for compartmentalization of biosynthetic
47 reactions such as lipid synthesis, calcium transport and apoptosis among others[1-3]. ER-
48 mitochondria contact sites (henceforth referred to as contacts unless specified otherwise) form
49 when the membrane of these organelles come into close apposition (observed to be between 5
50 – 100 nm) without fusion[2,4]. Contacts are stabilized by the interaction between tethering proteins
51 that reside in opposing membranes; thus, the ability to regulate transient interactions between
52 these tethers (through their abundance or post-translational modifications) enables the rapid
53 formation and dissolution of contacts to meet cellular needs[5-9].

54 Regulated protein degradation via the ubiquitin proteasome system is an efficient means to
55 modulate contacts as numerous ubiquitin-reliant protein quality control mechanisms surrounding
56 the ER and mitochondria can be co-opted to modulate the contact site proteome[10,11]. The p97
57 AAA-ATPase is an abundant, evolutionarily conserved, ubiquitin-selective unfoldase that
58 regulates multiple protein quality control pathways surrounding the ER and mitochondria. p97 is
59 ideally positioned to regulate contacts by mediating the extraction and degradation of membrane-
60 embedded tethers or contact resident proteins[12-14]. Importantly, specificity in p97-regulated
61 pathways occurs via association with numerous adaptors that recruit p97 to specific
62 substrates[15,16]. We isolated contacts (also known as mitochondria-associated membranes, MAM)
63 from HEK-293T cells and probed for p97 and select adaptor proteins. Percoll gradient
64 centrifugation of crude mitochondria releases associated ER membranes allowing for the
65 purification of ER-mitochondria contacts[17]. Using a known contact site-enriched protein as a
66 marker (fatty acid coenzyme A ligase 4, FACL4) as well as markers for mitochondria (TOMM20)
67 and ER (SEC61β), we found that the ER-localized p97 adaptors UBXD8 and UBXD2 (but not
68 cytosolic UBXN1) are enriched at contacts and that p97 is also present in these fractions (Fig.
69 1a). To determine the role of p97 complexes at contacts we used a split luciferase reporter
70 wherein the N-terminal fragment of luciferase is targeted to mitochondria and the C-terminal

71 fragment to the ER using established targeting sequences[18]. Functional luciferase activity is
72 reconstituted when the two organelles establish close range contacts and luciferase activity can
73 be measured using the substrate, Enduren. We verified the functionality of the reporter system in
74 wildtype cells by over-expressing receptor accessory protein 1 (REEP1)[18], a contact tether and
75 found a robust increase in contacts (Supplementary Fig.1a). To determine what role if any p97-
76 adaptor complexes may have at contacts, cells were transfected with split luciferase cDNAs and
77 siRNAs and luminescence was measured. Loss of p97 and UBXD8 resulted in an increase in
78 contacts, whereas depletion of UBXD2 or another p97 adaptor UBXD7 had no impact (Fig. 1b, c
79 and Supplementary Fig.1b). Hence even though UBXD2 localizes to contacts it does not regulate
80 them and we focus on the role of UBXD8 for the remainder of the study. To verify the specificity
81 of the phenotype, we expressed wildtype p97 or UBXD8 siRNA-resistant cDNAs and were able
82 to rescue the phenotype (Fig. 1b, c). However, individual ATP catalytic site mutants in p97 were
83 unable to rescue (Fig. 1c). To assess the role of individual domains in UBXD8, we expressed
84 point mutants in the ubiquitin associated (UBA) or ubiquitin-X (UBX) domains that serve to bind
85 ubiquitin and p97 respectively, as well as deletion of the UAS domain that has been reported to
86 mediate the oligomerization of UBXD8[19]. Loss of these functional domains in UBXD8 prevented
87 rescue of increased contacts (Fig. 1b, Supplementary Fig. 1c). Two additional assays were used
88 to verify the role of p97-UBXD8 in regulating contacts. We measured contacts by measuring the
89 extent of co-localization between the ER and mitochondria by confocal microscopy
90 (Supplementary Fig. 1d, e), and we performed transmission EM (TEM) studies in wildtype and
91 UBXD8 knockout (KO) cells generated by CRISPR-Cas9 gene editing (Fig. 1d-f). UBXD8 KO
92 cells had a significant increase in ER tubules closely apposed to mitochondria (Fig. 1e, f and
93 Supplementary Fig. 1e). No defects in overall mitochondria number or morphology were apparent
94 in UBXD8 KO cells (Supplementary Fig. 1f).
95

96 The p97-UBXD8 complex is critical for maintaining ER quality control by regulating ERAD and
97 loss of this complex can cause ER stress due to deficits in ERAD[20]. To determine whether altered
98 contacts observed upon depletion of p97-UBXD8 is due to increased ER stress, we treated cells
99 with ER stressors tunicamycin and thapsigargin which cause protein misfolding in the ER.
100 However, no impact on contacts was observed under these conditions (Supplementary Fig. 2a).
101 Furthermore, we probed for the ER chaperone BiP which is induced upon ER stress and found
102 no difference in BiP levels between wildtype and UBXD8 KO cells (Supplementary Fig.2b).
103 Together with our findings suggest that the p97-UBXD8 complex has a novel role in regulating
104 contacts independent of its role in ERAD.

105 To identify pathways altered in UBXD8 KO cells that may contribute to the contact site
106 defect, we isolated contacts (MAMs) from triplicate wildtype and UBXD8 KO HEK-293T cells by
107 biochemical fractionation and performed multiplexed, quantitative proteomics on the post-nuclear
108 supernatant and MAM fractions using tandem mass tags (TMT) (Fig. 2a, b and Supplementary
109 Fig. 3a-f, Supplementary Table 1)[21,22]. We used two filtering criteria for downstream analysis: $|\log_2$
110 WT:KO ratio$|$ > 1.0 and $-\log_{10}$ p value >1.5. The abundance of 23 proteins was enriched and 28
111 proteins was depleted in the MAM fraction of UBXD8 KO cells out of a total of 4499 quantified
112 (Fig. 2b and Supplementary Fig. 3a). Putative contact site proteins identified in previous studies
113 were present in our dataset validating our approach (Fig. 2b and Supplementary Fig. 3b,
114 Supplementary Table 1) [18,23-25]. Furthermore, we identified significant enrichment of known p97-
115 UBXD8 substrates such as squalene monooxygenase (SQLE), and HMG-CoA reductase
116 (HMGCR) in UBXD8 KO cells (Fig. 2b and Supplementary Fig. 3g, h). Interestingly, proteins
117 involved in lipid or cholesterol metabolism and lysosome function were also enriched in the
118 UBXD8 KO contact proteome ($\log_2$ WT:KO ratio < -0.65 and $-\log_{10}$ p value >1.5) (Fig. 2b-d and
119 Supplementary Fig.3c). In summary, our quantitative proteomic studies of the contact proteome
120 in UBXD8 KO cells suggests that perturbation in the abundance of numerous enzymes linked to
121 lipid biosynthesis may underlie the dysregulation in contacts.

122 To better understand how cellular lipid metabolism may be impacted by loss of UBXD8
123 we measured the lipidome of wildtype and UBXD8 KO cells. We identified and quantitatively
124 measured the relative levels of phospholipids (PLs) using liquid-chromatography high-resolution
125 tandem mass spectrometry (LC-MS/MS). Our analyses examined the major classes of PLs found
126 in membranes (and synthesized at contacts) including phosphatidylcholine (PC),
127 phosphatidylethanolamine (PE), phosphatidylserine (PS), and phosphatidylinositol (PI). We also
128 examined one-tailed lysophospholipids, which are metabolic byproducts of phospholipids (e.g.,
129 LPC, LPE, LPS, and LPI). We determined the concentration of 151 PLs in UBXD8 KO cells
130 relative to their concentration in wildtype cells. In general, most PLs were more abundant in KO
131 cells (Fig. 2e, f and Supplementary Fig. 4a-c, Supplementary Table 2). Approximately two-thirds
132 of the two-tailed PC and PE and one-tailed LPC and LPE species were ≥2-fold more abundant in
133 UBXD8 KO cells (Fig. 2e, f Supplementary Fig. 4a-d). The PC species that increased the most in
134 the UBXD8 KO cells contained one or two double bonds among the fatty acyl tails (i.e., PC (44:1),
135 PC (44:2), PC (46:1), PC (46:2), PC (48:2), PC (50:2), and PC (52:2)). MS/MS identification of
136 the individual tails revealed that each of these lipids contained only saturated or monounsaturated
137 fatty acids ranging in size from C16:0 to C32:1 and that lipids with one or fewer double bonds in
138 each tail were increased the most by the loss of UBXD8 (Supplementary Fig. 4f and
139 Supplementary Table 2). Phospholipids are synthesized via a diacylglycerol (DG) intermediate
140 and DGs are metabolized to generate PLs and TGs. We therefore extended our lipidomics studies
141 to measure DG and two additional neutral lipids: triacylglycerol (TG) and cholesteryl esters (CE)
142 that are major components of lipid droplets whose biogenesis at the ER is regulated by UBXD8[26].
143 The relative concentration of most DGs were altered by less than 2-fold (Supplementary Fig. 4e).
144 Of the ten DG species increased by ≥2-fold in UBXD8 KO cells, nine contained only saturated or
145 monounsaturated tails ranging in size from C14:0 to C32:0. Similar to PC and PE, most TG
146 species were ≥2-fold more abundant in KO cells relative to control cells (Supplementary Fig. 4e).
147 The TG species whose abundance was enhanced the most by the loss of UBXD8 were also

148 enriched in saturated and monounsaturated fatty acyl tails from C14:0 to C32:1. In contrast, most
149 CE species was unaltered or slightly depleted by the loss of UBXD8 (Supplementary Fig. 4e). In
150 summary, loss of UBXD8 shifts the lipidome to have a greater abundance of PC, PE, DG, and
151 TG with saturated and monounsaturated fatty acyl tails demonstrating that UBXD8 is necessary
152 for regulating lipid concentrations. The synthesis of these phospholipids occurs at contacts and
153 their altered abundance in UBXD8 KO cells may contribute to defects in contacts[27].

154 The significantly altered lipidome and related alterations in lipid biosynthetic enzymes
155 prompted us to ask if these changes stem from the regulation of the sterol regulatory element-
156 binding proteins 1 and 2 (SREBP1/2) pathway. p97-UBXD8 mediates the activation of ER-
157 localized SREBPs by mediating ubiquitin-dependent membrane extraction and degradation of the
158 SREBP negative regulator insulin induced gene 1 (INSIG1), in a sterol or fatty acid-dependent
159 manner (Supplementary Fig. 5a)[28,29]. A similar pathway for lipid sensing in *S.cerevisiae*, requires
160 Cdc48p and Ubx2p (orthologs of p97 and UBXD8 respectively),[30] and yeast lacking Ubx2p have
161 more saturated cellular membranes due to loss of transcriptional activation of Δ^9 desaturase
162 *ole1*[30]. We found that loss of p97 or UBXD8 resulted in a significant loss of SREBP1 activation
163 and accumulation of the inactive ER-tethered form concomitant with stabilization of INSIG1 (Fig.
164 3a-d). Lipid desaturases are transcriptional targets of SREBP1 and were decreased in abundance
165 at the transcript level in UBXD8 KO cells (Supplementary Fig.5b). This parallels the decreased
166 protein abundance of the best characterized desaturases, SCD1 (Δ^9) and FADS1 (Δ^5) in UBXD8
167 KO or p97 depleted cells (Fig. 3a-d). In contrast, UBXD8 loss did not significantly impact SREBP2
168 activation or its downstream targets although this may be cell type or context specific (Fig.3a, b
169 and Supplementary Fig.5c, d). Previous studies have reported that contacts have lipid raft-like
170 properties and are enriched in cholesterol and sphingolipids[31], thus the localization of cholesterol-
171 sensing proteins to these sub-domains may be advantageous for rapid pathway activation. We
172 find that SREBP1 and SCD1 are enriched at contacts isolated by biochemical fractionation (Fig.

173 3e). Notably, an increase in ER-tethered SREBP1 and a decrease in SCD1 were apparent in the
174 MAM fractions of UBXD8 KO cells (Fig. 3f). Furthermore, over-expression of mature SREBP1a
175 and c and to a lesser extent SREBP2, is sufficient to rescue contacts in p97-UBXD8 depleted
176 cells (Supplementary Fig. 5e, f). Hence, defective SREBP1 activation underlies increased
177 contacts in p97-UBXD8 depleted cells.

178 The decrease in the abundance of lipid desaturases and our finding that UBXD8 KO cells
179 have increased phospholipids with saturated or mono-unsaturated tails, prompted us to determine
180 whether altering membrane lipid saturation perturbed contacts between the ER and mitochondria.
181 FADS1 isoforms are localized to both ER and mitochondria[32], therefore we focused on ER-
182 localized SCD1[33] as it was enriched at contacts (Fig. 3e). We treated wild type HEK-293T cells
183 with the SCD1 inhibitor MF438[34] and found that contacts between the ER and mitochondria
184 increased in a manner that could be rescued by supplementing cells with monounsaturated oleic
185 acid, the product of SCD1, (Supplementary Fig. 5g). We next evaluated whether re-expressing
186 SCD1 in p97 or UBXD8-depleted cells rescued the increased contact site phenotype.
187 Overexpression of wildtype SCD1 rescued ER-mitochondria contacts to wild type levels in p97-
188 UBXD8 depleted cells (Fig. 3g, Supplementary Fig. 5h). In contrast, a catalytically inactive version
189 of SCD1 was unable to rescue the phenotype (Fig. 3g, Supplementary Fig. 5h). Notably,
190 overexpression of SCD1 catalytic mutant in wild type cells resulted in an increase in contacts
191 suggesting that the resulting ordered lipid bilayers may impact contacts (Figure 3g). To further
192 extend these findings, we asked if simply supplementing p97-UBXD8 depleted cells with
193 unsaturated oleic acid (18:1), a precursor for the generation of polyunsaturated fatty acids in cells
194 was sufficient to rescue contacts. Indeed, oleic acid but not saturated palmitic acid (16:0) rescued
195 the contact defect in p97-UBXD8 depleted cells. Strikingly, palmitic acid alone increased contacts
196 in wildtype cells (Fig. 3h).

197 Collectively, these results suggested that contacts are exquisitely sensitive to perturbations in
198 lipid profiles within cellular membranes and that loss of p97-UBXD8 alters membrane lipid

199 composition and saturation. We evaluated whether loss of p97-UBXD8 impacts lipid saturation
200 globally within the cell. We used lipid-binding pyrene probes that insert into both disordered
201 (unsaturated) and ordered (saturated) lipid bilayers throughout the cell and undergo monomer to
202 excimer formation in a manner dependent on local membrane order. We measured the ratio of
203 monomer to excimer fluorescence as an indicator of membrane order in cells treated with the p97
204 inhibitor CB-5083 or in UBXD8 KO cells. Loss of UBXD8 or inhibition of p97 resulted in more
205 ordered cellular membranes (monomer: excimer ratio <1) compared to controls in two different
206 cell lines (Fig.4a, Supplementary Fig.6a). Strikingly this phenotype can be reversed by incubating
207 cells with oleic but not palmitic acid (Fig.4a, Supplementary Fig.6a). Thus, the local degradation
208 of INSIG1 and subsequent activation of SREBP1 at contacts impacts lipid composition and
209 saturation throughout the cell but significantly impact contacts due to their reliance on membranes
210 for association. These findings prompted us to investigate whether the global increase in
211 membrane order impacts other ER-organelle contacts. Analysis of TEM data from wildtype and
212 UBXD8 KO cells demonstrated increased contacts between the ER and plasma membrane (Fig.
213 4b, c). Whether other ER-organelle contacts are similarly perturbed is under investigation.
214 Notably, UBXD8 KO cells have an increase in membranous whorls containing concentric
215 membrane layers, reminiscent of multilamellar bodies that function in lipid storage and secretion
216 (Supplementary Fig. 6b). Interestingly, these membrane rich structures were also identified in
217 yeast strains lacking Ubx2p [30] and may arise due to imbalances in lipid levels.

218 Mutations in p97 cause several primarily neurodegenerative protein aggregation disorders.
219 These include inclusion body myopathy with Paget's disease of the bone and frontotemporal
220 dementia (IBMPFD, also known as multi-system proteinopathy 1, MSP-1)[35], amyotrophic lateral
221 sclerosis (ALS)[36,37], Charcot Marie Type IIB[38], among others. We investigated whether p97
222 disease-associated mutations altered contacts between the ER and mitochondria and perturbed
223 the SREBP1-SCD1 pathway. We measured contacts in mouse embryonic fibroblasts
224 heterozygous or homozygous for p97 R155H (a prevalent mutation observed in patients) and

225 observed that cells with p97 R155H homozygous mutation had a significant increase in contacts
226 that could be rescued with oleic acid but not palmitic acid (Fig. 4d).

227 Next, we evaluated the SREBP-SCD1 pathway in the brains of the two distinct p97 mouse
228 models[39]. A conditional knockout of p97 (p97 cKO) in the cortex and hippocampus has recently
229 been shown to develop cortical atrophy, neuronal loss and TDP43 inclusions reminiscent of
230 frontotemporal dementia[39]. We stained for SREBP1 and SCD1 in the CA1 regions of one-month-
231 old p97 cKO mice before neurodegeneration phenotypes are observed. Strikingly, p97 cKO mice
232 had a significant decrease of SREBP1 and SCD1 immunoreactivity in the CA1 region compared
233 to age-matched controls (Fig. 4e, f). To assess whether these defects were also present in a
234 pathogenic context, we immunoblotted for SREBP1 and SCD1 in brain lysates from 6- and 13-
235 month-old $p97^{R155C/WT}$ mice[39,40]. SREBP1 processing was significantly diminished at 13 months in
236 $p97^{R155C/WT}$ mice relative to controls (Fig. 4g, h). Similarly, loss of SCD1 protein levels was
237 apparent at 6 months and continued to decline at 13 months in $p97^{R155C/WT}$ mice (Fig. 4g, h).
238 Collectively, our findings suggest that p97 mutations that cause disease may also have underlying
239 lipid metabolism deficits that could contribute to disease pathology.

240 **Discussion**

241 Here we have identified an unanticipated role for p97 and its ER-tethered adaptor UBXD8
242 in regulating ER-mitochondria contacts by perturbing membrane composition and fluidity in
243 multiple cell types. We propose that altered lipid bilayers that arise upon loss of UBXD8 impact
244 contacts in multiple ways: (1) preventing their dynamic association and disassociation due to loss
245 of fluidity, and (2) negatively impacting the lateral movement of tethering proteins within
246 membranes. UBXD8 is unique among p97 adaptors in its evolutionarily conserved and
247 multifunctional roles in lipid sensing and metabolism[19,26,30,41-43]. Our proteomic and lipidomic data
248 from UBXD8 KO cells suggests that widespread changes in lipid metabolism is likely due to the
249 inability to mobilize SREBP1 from the ER. Similar to the yeast *ubx2* deletion phenotype, we find
250 decreased abundance of lipid desaturases, particularly SCD1 and complementation of p97-

251 UBXD8 depleted cells with SCD1 rescues contact defects. However, given the significant
252 changes in lipid profiles in UBXD8 KO cells, it is likely that SCD1 regulation is not the only
253 mechanism at play. It remains to be determined whether UBXD8 also facilitates the degradation
254 of contact tethers. We note that UBXD8 is present in pure mitochondrial fractions (Fig. 1a).
255 Previous studies have observed dual localization of UBXD8 to ER and mitochondria[44,45] and
256 recently, the yeast ortholog, Ubx2p was reported to localize to the outer mitochondrial membrane
257 where it associates with the translocon in the outer mitochondrial membrane (TOMM) complex to
258 clear stalled polypeptides in a p97-dependent manner[46]. Further studies are required to determine
259 whether mammalian UBXD8 functions in an analogous manner on mitochondria. Aberrant contact
260 sites are emerging as a common feature in the pathophysiology of a wide spectrum of human
261 diseases ranging from diabetes to neurodegeneration[47-49]. We find that p97 mutations that cause
262 proteinopathies also exhibit increased contacts and display significantly decreased levels of
263 SREBP1 and SCD1. A recent report found that motor neurons from ALS patients with p97
264 mutations exhibited more contacts between the ER and mitochondria relative to controls[50]. Thus,
265 altered organelles contacts and downstream lipid synthesis warrants further investigation in p97
266 associated diseases.
267
268 **Figure Legends**
269
270 **Fig. 1. Loss of p97 and UBXD8 results in increased ER-mitochondria contacts.**
271 **a,** Immunoblot of indicated proteins from subcellular fractionation of HEK-293T cells, PNS: post-
272 nuclear supernatant, MAM: Mitochondria associated membrane (n > 3 biologically independent
273 samples). **b**, Top: Domain organization of UBXD8 and indicated mutations, Middle: Split
274 luciferase assay to measure contacts in HEK-293T cells transfected with siRNAs to UBXD8 and
275 indicated C-HA/FLAG siRNA-resistant rescue constructs, RLU: relative luminescence unit,
276 Bottom: Immunoblot of indicated proteins. UBA: ubiquitin associated, UAS: upstream activating

277 sequence, UBX: ubiquitin X (n > 3 biologically independent samples). **c**, Top: Domain
278 organization of p97 and indicated mutations, Middle: Split luciferase assay to measure contacts
279 in HEK-293T cells transfected with siRNAs to p97 and indicated N-Myc siRNA-resistant rescue
280 constructs, RLU: relative luminescence unit, Bottom: Immunoblot of indicated proteins ($n \geq 3$
281 biologically independent samples). **d**, Immunoblot of UBXD8 in CRISPR-Cas9 edited HEK-293T
282 and HeLa-Flp-IN-T-Rex cells, KO: knockout. **e**, Representative transmission EM micrographs of
283 wildtype and UBXD8 KO HEK-293T cells illustrating contacts between ER and mitochondria. **f**,
284 Quantification of contact length between ER and mitochondria in each genotype from (**e**)
285 (measurements are from $n = 3$ biological replicates with WT = 50 cells from 65 fields and UBXD8
286 KO = 53 cells from 71 fields). OMM: Outer mitochondrial membrane. Data are means ± SEM (*,
287 **, ***$P < 0.05$, 0.01, 0.0001 respectively, One-way ANOVA with Tukey's multiple comparison
288 test). Scale bar, 100 nm.
289
290 **Fig. 2. Quantitative proteomics and lipidomics identifies a role for UBXD8 in regulating**
291 **lipid metabolism at contacts.**
292 **a,** Schematic of tandem mass tag (TMT) proteomic workflow from wildtype and UBXD8 KO HEK-
293 293T cells. PNS: post-nuclear supernatant, MAM: mitochondria associated membrane. **b**,
294 Volcano plot of the ($-\log_{10}$-transformed P value *versus* the $\log_2$-transformed ratio of wildtype/
295 UBXD8 KO) proteins identified from MAM fractions of HEK-293T cells. $n = 3$ biologically
296 independent samples for each genotype. P values were determined by empirical Bayesian
297 statistical methods (adjusted for multiple comparisons) using the *LIMMA* R package; for
298 parameters, individual P values and q values, see Supplementary Table 1. **c,** Network of
299 differentially enriched terms shown as clustered functional ontology categories. Each node
300 represents a functional ontology term enriched in the TMT data (**a, b**) as scored by Metascape[51].
301 Networks were generated using Cytoscape v3.8.2. Size of node represents number of genes
302 identified in each term by gene ontology (GO). Grey and Blue donuts represent percent of genes

303 identified in each GO term in wildtype or UBXD8 KO respectively. Node outline thickness
304 represents $-\log_{10}$-transformed *P* value of each term. The inner circle color of each node indicates
305 the corresponding functional GO cluster. **d**, Bubble plot representing significantly enriched GO
306 clusters identified from TMT proteomics of MAM fractions in wildtype (blue) or UBXD8 KO (green)
307 cells (**a-c**). Size of the circle indicates the number of genes identified in each cluster. **e-f**, Relative
308 levels of Phosphatidylcholine (PC) (**e**) and Phosphatidylethanolamine (PE) (**f**) in HEK-293T WT
309 and UBXD8 KO cells. PLs were measured by LC-MS/MS following normalization by total protein
310 amount. Each dot in the plot represents the level of a PL in UBXD8 KO cells relative to its level in
311 wildtype cells. The dashed line represents a relative level of 1 (e.g., the level in UBXD8 KO cells
312 is equal to the level in wildtype cells). (n = 3 biologically independent experiments were performed,
313 each with duplicate samples). Statistical analysis was performed on the log transformed relative
314 fold change values (UBXD8 KO relative to WT) using independent *t* tests and Benjamini-
315 Hochberg correction in R stats package (p-values are listed in Supplemental Table 1).
316
317 **Fig. 3. Loss of SREBP1 activation and SCD1 expression upon p97-UBXD8 depletion is**
318 **responsible to contact defects.**
319 **a-d** Immunoblot and the corresponding band intensity quantifications of indicated proteins in the
320 SREBP pathway in wildtype and UBXD8 KO HEK-293T cells (**a & b**), or p97-siRNA depleted cells
321 (**c & d**). All samples were transfected with INSIG1-HA/FLAG due to lack of reliable antibodies to
322 the endogenous protein. ($n \geq 3$ biologically independent samples). **e**, Immunoblot of indicated
323 SREBP pathway proteins from subcellular fractionation of HEK-293T cells, PNS: post-nuclear
324 supernatant, MAM: mitochondria associated membrane. (n = 3 biologically independent
325 samples). **f**, Immunoblot of indicated SREBP pathway proteins from subcellular fractionation of
326 wildtype and UBXD8 KO HEK-293T cells, MAMs: mitochondria associated membrane. (n = 3
327 biologically independent samples). Corresponding fold changes (FC: UBXD8 KO *vs* WT) of
328 SREBP1 and SCD1 normalized to FACL4 is shown. **g**, Split luciferase assay in HEK293T cells

329 transfected with indicated siRNAs and wildtype or catalytically dead SCD1. GFP-HA/FLAG was
330 transfected as a negative control. RLU: relative luminescence unit. ($n \geq 3$ independent biological
331 replicates). **h**, Split luciferase assay in HEK293T cells transfected with indicated siRNAs and
332 treated with either monounsaturated oleic acid or saturated palmitic acid. RLU: relative
333 luminescence unit. ($n \geq 3$ independent biological replicates). Data are means ± SEM (*, **,
334 ***$P < 0.05$, 0.01, 0.0001 respectively. Paired *t* test (**b & d**), or One-way ANOVA with Tukey's
335 multiple comparison test (**g & h**).
336
337 **Fig. 4. Supplementation of p97-UBXD8 depleted cells with oleic acid rescues contact**
338 **defects.**
339 **a,** Global membrane fluidity was measured using a pyrene-based lipid probe in wildtype and
340 UBXD8 KO HEK-293T cells. Wildtype cells were also treated with 5 μM of the p97 inhibitor CB-
341 5083 for 4 hours. Cells were supplemented with indicated concentrations of oleic acid and palmitic
342 acid for 4 hours. The fold change ($\text{Treated}_{\text{excimer:monomer}}$ *vs* $\text{Control}_{\text{excimer:monomer}}$) of the ratio of
343 excimer (Em. Max. 460nm) to monomer (Em max. 400nm) fluorescence is indicated. Fold
344 changes < 1 indicate more ordered lipid bilayers relative to wildtype untreated control. ($n \geq 3$
345 biologically independent samples). **b**, Representative transmission EM micrographs of wildtype
346 and UBXD8 KO HEK-293T cells illustrating contacts between ER and plasma membrane. **c**,
347 Quantification of contact length between ER and plasma membrane in each genotype from (**b**)
348 (measurements are from $n = 3$ biological replicates with WT = 50 cells from 65 fields and UBXD8
349 KO = 53 cells from 71 fields). **d**, Split luciferase assay to measure contacts in mouse embryonic
350 fibroblasts with heterozygous or homozygous p97 R155H mutation. Cells were supplemented
351 with indicated concentrations of oleic acid and palmitic acid for 4 hours. ($n \geq 3$ biologically
352 independent samples). **e,** Representative SREBP1 and SCD1 staining from CA1 regions of 1
353 month-old control (CAMK2α) and p97 cKO mice (scale bar is 25 μm). **f,** Quantification of

354 fluorescence intensity of images in (**e**). Individual points represent mean ROI intensity from each
355 mouse, 3 or 4 animals per group. **g,** Representative immunoblot for SREBP1 and SCD1 from
356 cortical brain lysates of 12-month-old control (C57), or 6- and 12-month-old p97$^{R155C/WT}$ mice (n=4
357 for each group). Pan 14-3-3 was used as housekeeping control. **h,** Quantification of (**g**). The ratio
358 of mature SREBP to total SREBP is shown. SCD1 intensities are normalized to 14-3-3 levels in
359 each lane. Individual points represent each mouse, 4 animals per group. Data are means ± SEM
360 (*, **, ***$P < 0.05$, 0.01, 0.0001 respectively. Significance was analyzed by One-way ANOVA with
361 Newman-Keuls multiple comparison test (**a**) or Tukey's multiple comparison test (**c, d**) or
362 Dunnett's multiple comparison (**h**) or Student's *t*-test (**f**).
363
364 **Supplementary Figure Legends**
365
366 **Supplementary Fig. 1. Loss of p97 and UBXD8 results in increased ER-mitochondria**
367 **contacts.**
368 **a,** Split luciferase assay to measure contacts in HEK-293T cells transfected with REEP1 ($n > 3$
369 biologically independent samples). **b**, Left: Split luciferase assay to measure contacts in HEK-
370 293T cells transfected with indicated siRNAs. ($n \geq 3$ biologically independent samples). Right:
371 Immunoblot of HEK 293T cells transfected with indicated siRNAs. **c,** Affinity purification of
372 indicated UBXD8-HA/FLAG constructs transiently expressed in HEK-293T cells. Immunoblots of
373 whole cell lysates and affinity purifications probed with anti-HA, anti-p97, anti-PCNA, and ubiquitin
374 antibodies ($n = 3$ biologically independent samples). **d**, Top: Representative confocal image of
375 HeLa-Flp-IN-TRex cells stably expressing Sec61β-eGFP (green, ER) and stained for endogenous
376 TOMM20, (red, mitochondria). Bottom: ImageJ image analysis pipeline for the quantification of
377 contacts between ER and mitochondria[52]. **e**, Quantification of ER- mitochondria contacts in cells
378 transfected with indicated siRNAs (left and middle panel) or in UBXD8 KO HeLa-Flp-IN-TRex
379 cells (right panel) using assay in (**d**). Bottom panels show immunoblots for knockdown efficiency.

380 (*n* = 3 biologically independent samples) N: numbers of cells analyzed in each condition.
381 (Quartiles represent the upper 75th percentile and the lower 25th percentile. The line inside the
382 box represents the median. Whiskers indicate distribution of data from minimum to maximum in
383 a condition.). **f**, Quantification of number of mitochondria per field from transmission electron
384 microscopy of wildtype and UBXD8 KO cells. (Measurements are from *n* = 3 biological replicates
385 with WT = 50 cells in 65 fields and UBXD8 KO = 53 cells in 71 fields). Data are means ± SEM (*,
386 **, ***$P < 0.05$, 0.01, 0.0001 respectively. One-way ANOVA with Tukey's multiple comparison test
387 (**b & e** (left and middle panel)), Paired *t*-test (**a**) or Unpaired *t* test with Welch's correction (**e** (right
388 panel) & **f**).
389
390 **Supplementary Fig. 2. ER stress does not alter ER-mitochondria contacts.**
391 **a,** Split luciferase assay to measure contacts in HEK-293T cells treated with 2.5 μM Tunicamycin
392 (Tu), 1.5 μM Thapsigargin (Tg), for 2 hours. (*n* = 3 biologically independent samples). Immunoblot
393 of HEK-293T cells treated with Tu and Tg and probed for the indicated proteins. **b**, Immunoblot
394 of HEK-293T wildtype and UBXD8 KO cells treated with 1.5 μM Thapsigargin (Tg) for 2 hours,
395 UT: Untreated. (*n* = 3 biologically independent samples). Data are means ± SEM (ns: not
396 significant). One-way ANOVA with Tukey's multiple comparison test (**a**).
397
398 **Supplementary Fig. 3. Quantitative proteomics of wildtype and UBXD8 KO contact**
399 **proteome.**
400 **a,** Table depicting number of proteins and peptides quantified in post-nuclear supernatant and
401 mitochondria associated membrane fractions identified by proteomics in wildtype and UBXD8 KO
402 cells. Number of proteins up- or downregulated at $\log_2$- fold change (FC) (wildtype/ UBXD8 KO)
403 ± 0.65 and ±1 is indicated. **b**, Venn diagram depicting overlap of our dataset with other putative
404 mitochondria associated membrane proteins identified by proteomics[18,23-25] **c**, Protein-protein
405 interaction network of differentially expressed proteins from MAM fractions of HEK-293T cells

involved in ERAD, cholesterol biosynthesis and lysosome function shown as clustered functional categories. Protein associations were determined using STRING database with score ≥ 0.4. Each node represents a protein belonging to enriched GO clusters as scored by Metascape. Size of node represents $-\log_{10}$-transformed *P* value and color of node represents $\log_2$- fold change (FC) (WT / UBXD8 KO). **d**, Volcano plot of the $-\log_{10}$-transformed *P* value *versus* the $\log_2$-transformed ratio of wildtype/ UBXD8 KO proteins identified in the post-nuclear supernatant of HEK-293T cells. *n* = 3 (each genotype) biologically independent samples. *P* values were computed by empirical Bayesian statistical methods (adjusted for multiple comparisons) available in *Limma* R package; for parameters, individual *P* values and *q* values, see Supplementary Table 1. **e**, Network of differentially enriched functional ontology terms shown as clustered functional ontology categories. Each node represents a functional ontology term enriched in the TMT data (**d**) as scored by Metascape and networks generated using Cytoscape v3.8.2. Size of node represents number of genes identified in each term by gene ontology (GO). Grey and Blue donuts represent percent of genes identified in each GO term in wildtype or UBXD8 KO respectively. Node outline thickness represents $-\log_{10}$-transformed *P* value of each term. The inner circle color of each node indicates the corresponding functional GO cluster. **f,** Bubble plot representing significantly enriched GO clusters identified from TMT proteomics of post-nuclear fractions in wildtype (blue) or UBXD8 KO (green) cells (**d-e**). Size of the circle indicates the number of genes identified in each cluster. **g**,**h**, Squalene epoxidase (SQLE) half-life measurements in wildtype and UBXD8 KO HEK 293T cells. FLAG-SQLE was transiently expressed, and cells were treated with 100 μg/mL cycloheximide for the indicated times. Samples were resolved on SDS-PAGE for immunoblots (**g**) and levels of SQLE were quantified and normalized to loading control PCNA (**h**). (*n* = 3 biologically independent samples). Data are means ± SEM (*, **, *** $P < 0.05$, 0.01, 0.0001 respectively. One-way ANOVA with Tukey's multiple comparison test (**h**).

431 **Supplementary Fig. 4. Loss of UBXD8 alters cellular lipidome with increased abundance**
432 **of saturated fatty acid tail containing phospholipids**

433 **a**, Relative levels of phospholipids (PLs), namely PG, phosphatidylglycerol; PI,
434 phosphatidylinositol; and PS, phosphatidylserine in HEK-293T WT and UBXD8 KO cells. PLs
435 were measured by LC-MS/MS following normalization by total protein amount. Each dot in the
436 plot represents the level of a PL in UBXD8 KO cells relative to its level in wildtype cells. The
437 dashed line represents a relative level of 1 (e.g., the level in UBXD8 KO cells is equal to the level
438 in wildtype cells). (n = 3 biologically independent experiments were performed, each with duplicate
439 samples). **b**, Relative levels of DG, Diacylglycerol; TG, Triacylglycerol in HEK-293T WT and
440 UBXD8 KO cells. The DG and TG is quantified and visualized similarly as in **a**. (n = 3 biologically
441 independent experiments were performed, each with duplicate samples). **c**, Relative levels of CE,
442 Cholesteryl esters in HEK-293T WT and UBXD8 KO cells. The CE is quantified and visualized
443 similarly as in **a**. (*n* = 3 biologically independent samples). **d**-**e**, Changes in the relative levels of
444 PLs (**d**) including lysophopholipids (LPC, Lysophosphatidyl choline; LPE, Lysophosphatidyl
445 ethanolamine; LPS, Lysophosphatidyl Serine; and LPI, Lysophophatidyl Inositol) and (**e**) Neutral
446 lipids (DGs, TGs, and CEs) in HEK-293T WT and UBXD8 KO cells were quantified. The averaged
447 relative fold changes were $\log_2$ transformed and visualized as a heatmap. (*n* = 3 biologically
448 independent experiments were performed, each with duplicate samples). **f**, Dot plots representing
449 fold change of PC and PE lipids in UBDX8 KO cells relative to WT cells and fatty acid tails
450 determined by MS/MS. The two tails of the lipid are shown and organized so that tail 1 (y-axis) is
451 the shorter tail and tail 2 (x-axis) is the longer tail. The labels tail 1 and tail 2 do not represent their
452 stereospecific number (sn). Grey boxes indicate the increase in saturated and mono-unsaturated
453 tails in UBXD8 KO cells. Statistical analysis was performed on the $\log_2$ transformed relative fold
454 change values (UBXD8 KO relative to WT) using independent t tests and Benjamini-Hochberg
455 correction in R stats package (p-values are listed in Supplemental Table S3).
456

457 **Supplementary Fig. 5. Diminished SREBP pathway activation in UBXD8 KO cells**

458 **a,** Schematic of SREBP pathway activation. In cholesterol-replete conditions, SCAP-INSIGs-
459 SREBPs are in an inactive tripartite complex in the ER membrane. Cholesterol depletion triggers
460 a conformational change in SCAP releasing it from INSIGs and enabling the transport of SCAP-
461 SREBPs to the Golgi. Here SREBPs are cleaved sequentially by site 1 and site 2 proteases to
462 release the active transcription factor. INSIGs are ubiquitylated and extracted for degradation
463 from the membrane by p97-UBXD8. **b-c,** Real-time quantitative PCR of SREBP1 target genes
464 including lipid desaturases (**b**), and SREBP2 target genes (**c**). **d,** Band intensity quantifications of
465 mature SREBP2 in wildtype and UBXD8 KO HEK-293T cells corresponding to **Fig 3a** ($n \geq 3$
466 biologically independent samples) **e,** Split luciferase assay to measure contacts in HEK-293T
467 cells transfected with siRNAs to UBXD8 or p97 and indicated 2X-FLAG-tagged mature SREBP1a,
468 1c, and 2 constructs. RLU: relative luminescence unit. ($n = 4$ biologically independent samples).
469 **f**, Immunoblot of indicated proteins in HEK-293T cells transfected with siRNAs to UBXD8 or p97
470 and indicated 2X-FLAG-tagged mature SREBP1a, 1c, and 2 constructs. Immunoblots were
471 probed with antibodies to SREBP1 and 2 to visualize immature and transfected mature forms. **g**,
472 Split luciferase assay to measure contacts in HEK 293T cells treated with SCD1 inhibitor MF438
473 at 1 μM for 4 hours. Cells were also treated with oleic acid for 4 hours as indicated. ($n \geq 3$
474 biologically independent samples). **h,** Immunoblot of indicated proteins in HEK293T cells
475 transfected with indicated siRNAs and wildtype or catalytically dead mutant of SCD1. GFP-
476 HA/FLAG was transfected as a negative control. Related for Fig. **3e**. ($n = 3$ independent biological
477 replicates). Data are means ± SEM (*, **, *** $P < 0.05$, 0.01, 0.0001 respectively. One-way ANOVA
478 with Dunnett's multiple comparison test (**b, c**), Paired *t* test with Welch's correction (**d**) or One-
479 way ANOVA with Tukey's multiple comparison test (**e & g**).

480

481 **Supplementary Fig. 6. Loss of UBXD8 perturbs membrane saturation.**

482 **a,** Global membrane fluidity in cells was measured using pyrene-based lipid probes in wildtype
483 and UBXD8 KO HeLa-Flp-IN-TRex cells. Wildtype cells were also treated with 5 μM of the p97
484 inhibitor CB-5083 for 4 hours. Cells were supplemented with indicated concentrations of oleic acid
485 and palmitic acid for 4 hours. The fold change ($\text{Treated}_{\text{excimer:monomer}}$ *vs* $\text{Control}_{\text{excimer:monomer}}$) of ratio
486 of excimer (Em. Max. 460nm) to monomer (Em max. 400nm) fluorescence is indicated. Fold
487 changes < 1 indicate more ordered lipid bilayers relative to WT untreated control. (n = 3
488 biologically independent samples). **b**, Representative transmission EM micrographs of
489 multilamellar bodies (red arrows) containing membrane whorls in UBXD8 KO HEK-293T cells.
490 Lower panel shows quantification of multilamellar bodies from images in (**c**). (Measurements are
491 from n = 3 biological replicates with WT = 50 cells in 65 fields and UBXD8 KO = 120 cells in 122
492 fields). Data are means ± SEM (*, **, ***P < 0.05, 0.01, 0.0001 respectively, One-way ANOVA with
493 Tukey's multiple comparison test (**a**). Scale bar, 100 nm.
494
495 **Materials and Methods**
496
497 **Cell culture, transfections, immunoprecipitations, and treatments.**
498 HEK293T, HeLa Kyoto Mouse embryonic fibroblasts (MEFs), and HeLa-Flp-IN-TREX
499 (HFTs, gift from Brian Raught, University of Toronto) cells were cultured in Dulbecco's modified
500 Eagle's medium, supplemented with 10% fetal bovine serum (FBS) and 100 units/mL penicillin
501 and streptomycin. Cells were maintained in a humidified, 5 % CO_2 atmosphere at 37°C. HeLa
502 Kyoto wildtype cell line was a generous gift from Ron Kopito (Stanford University).
503 For siRNA transfections, HEK293T cells were trypsinized and reverse transfected with
504 siRNAs. HeLa Kyoto, MEF, and HFT cells were trypsinized and seeded into a 12-well or 6-well
505 dish 24 hours prior to siRNA transfections. In both reverse and forward transfections, the cells
506 were transfected with 20 nM siRNAs using RNAiMax (Invitrogen) according to the manufacturer's
507 protocol. Cells were harvested 48-72 hours post transfection. For DNA transfections, HEK293T

508 cells in 6 well plates were transfected with 0.75 μg pcDNA3-Mit-NRluc91 and pcDNA3-CRluc92-
509 ER, 0.75 μg of UBXD8-C-HA/FLAG constructs, 1 μg of N-Myc-p97 constructs, 1 μg of p97-C-Myc,
510 1 μg of SCD1-C-HA/FLAG constructs, 0.2-0.5 μg of GFP-C-HA/FLAG, 0.75 μg of pCIG construct,
511 0.75 μg of REEP1, or 0.75 μg each of 2X-FLAG-SREBP1a, 2X-FLAG-SREBP1c, 2X-FLAG-
512 SREBP2 using Polyehtylenimine (PEI) at 1:4 DNA:PEI ratio and typically harvested 36-48 hours
513 post transfection. HeLa Kyoto, MEF, and HFT cells were transfected with cDNA using
514 Lipofectamine 2000 (Invitrogen) and the cells were harvested 36-48 hours post transfection. Cells
515 were lysed in mammalian cell lysis buffer (50 mM Tris-Cl, pH 6.8, 150 mM NaCl, 0.5% Nonidet
516 P-40, HALT Protease inhibitors (Pierce) and 1 mM DTT). Cells were incubated at 4°C for 10 min
517 and then centrifuged at 14,000 rpm for 15 min at 4°C. The supernatant was collected, and protein
518 concentration was estimated using the DC protein assay kit (Biorad). Protein G agarose (Pierce,
519 Thermo Fisher scientific) and the indicated antibodies were used for immunoprecipitation at 4°C
520 for 3-5 hours. Beads were washed 3-5 times in 1 ml mammalian cell lysis buffer and resuspended
521 in 2X SDS sample buffer. Cells were treated with 1 μM of Bortezomib, 5 μM CB-5083, 5 μg/ml of
522 puromycin, 1 μM SCD1 inhibitor (MF438), 1 mM Oleic acid, or 0.25 mM Palmitic acid for the
523 indicated times (see figures for details). A full list of constructs used in this study can be found in
524 Supplementary Table 3.
525
526 **Generation of CRISPR cell lines.**
527 The CRISPR-Cas9 gene editing system was used to generate UBXD8 knockout cell lines in
528 HEK293T, and HFT cells. The guide sequence 5' GTTAACCTGCAGGGGTCGTGA 3' was
529 cloned into the pX459 vector carrying the hSpCas9 and transiently transfected into HEK293T and
530 HeLa-Flp-IN-TRex cells using Lipofectamine 3000 (Invitrogen) per the manufacturer's protocol.
531 36 hours post-transfection the cells were selected with 1 μg/ml puromycin for a further 24-36-hrs.

532 The surviving cells were then serially diluted into 96 well plates for clonal selection and expression
533 levels were monitored by immunoblotting.
534
535 **Antibodies, siRNAs and Reagents.**
536 The p97 (10736-1-AP), UBXD8 (16251-1-AP), FACL4 (22401-1-AP), UBXD2 (21052-1-AP),
537 HRD1 (13473-1-AP), Sec61β (51020-2-AP), Calnexin (10427-2-AP), UBXN1 (16135-1-AP),
538 SREBP1 (14088-1-AP), SREBP2 (28212-1-AP), FADS1 (10627-1-AP), anti-GFP (66002-1-AP)
539 and SCD1 (23393-1-AP) antibodies were from Proteintech Inc. The TIMM23 (H-8; sc514463),
540 TOMM20 (F-10; sc17764), TOMM70 (A-8; sc390545), pan-ubiquitin (P4D1; sc8017), c-Myc
541 (9E10; sc40), β-Actin (AC-15; sc69879), GAPDH (O411; sc47724), PCNA (PC10; sc56), UBE2J1
542 (B-6; sc377002), and UBE2S (C-1; sc390917) antibodies were obtained from Santa Cruz
543 Biotechnologies. LC3B (D11; 3868S), and BiP (C50B12; 3177T) were from Cell Signaling
544 Technologies. Derlin1 (A302-849A-T), p97 (A300-589A), and TEB4/MARCHIV (A304-171A-T)
545 were from Bethyl laboratories. The following antibodies UBXD7 (PA5-61972; Invitrogen), anti-HA
546 (16B12; MMS-101P, Covance), anti-FLAG (M2; F3165 Sigma Aldrich), INSIG1 (ab70784;
547 Abcam) were used for immunoblotting. HRP conjugated anti-rabbit (W401B) and anti-mouse
548 (W402B) secondary antibodies were from Promega. CB-5083 was a gift from Cleave Biosciences
549 and Bortezomib was from Selleckchem. Palmitic acid (100905) is from MP Biomedicals and Oleic
550 acid (270290050) is from Acros Organics. MF438 (569406) is from Millipore Sigma. All siRNAs
551 were purchased from Ambion (Thermo Fisher Scientific). UBXD8-0 (s23260), UBXD8-9 (s23259),
552 UBXD7-7 (s24997). UBXD2-1 (D-014184-03), UBXD2-2 (D-014184-04), HRD1-3 (D-007090-03),
553 and HRD1-4 (D-007090-04) were purchased from GE Dharmacon. siControl (SIC001) was from
554 Millipore Sigma. p97 siRNAs (2-HSS111263 and 3-HSS111264) were from Invitrogen (Thermo
555 Fisher Scientific). p97 rescue constructs were previously published [53] and were resistant to siRNA
556 # 2. UBXD8-C-HA/FLAG construct was previously published [53]. The UBXD8 rescue constructs,

557 including UBA* (^{17}LLQF^{20} mutated to ^{17}AAAA^{20}), ΔUAS (deleted amino acids between 122-277),
558 and UBX* (^{407}FPR^{409} mutated to ^{407}AAA^{409}), were cloned using overlap PCR followed by Gibson
559 assembly (NEB) cloning into pHAGE-C-HA/FLAG and were resistant to siRNA # 0. The SCD1-C-
560 HA/FLAG WT and catalytic dead mutant (His^{160} His^{161} and His^{301} His^{302} mutated to Ala^{160} Ala^{161}
561 and Ala^{301} Ala^{302}) constructs were cloned using overlap PCR followed by Gateway cloning
562 (Thermo Fisher Scientific) into pHAGE-C-HA/FLAG.
563
564 **Mitochondria-associated membranes (MAM) fractionation.**
565 MAMs were isolated as previously described [54,55]. Briefly, HEK293T or HeLa-Flp-IN-T-Rex cells
566 were seeded into four 150 mm TC dishes. Cells were lysed in Homogenization buffer (225 mM
567 mannitol, 75 mM sucrose, and 30 mM Tris-Cl, pH 7.4) using a Dounce homogenizer. The lysate
568 was centrifuged three times at 600xg for 5 minutes to remove unlysed cells and nuclei resulting
569 in post-nuclear supernatants (PNS). The cleared lysate was centrifuged at 7000xg to separate
570 crude mitochondrial pellet and supernatant containing microsomes. The supernatant was cleared
571 by centrifugation at 20,000xg for 30 minutes followed by microsome isolation using high-speed
572 centrifugation at 100,000xg for 1 hour. The crude mitochondrial pellet was washed twice in
573 homogenization buffer containing 0.1 mM EGTA at 7000xg and 10,000xg for 10 minutes. MAMs
574 were isolated from crude mitochondria using 30% Percoll gradient centrifugation at 95,000xg for
575 1 hr in a swinging-bucket rotor. The banded MAM fraction was washed once with phosphate-
576 buffered saline (PBS) before lysing in lysis buffer (50 mM Tris-Cl, pH 7.2, 150 mM NaCl, 2%
577 SDS). The pure mitochondrial fractions were resuspended and washed in mitochondrial
578 resuspension buffer (250 mM mannitol, 0.5 mM EGTA, 5 mM HEPES pH7.4). Mitochondrial
579 membranes were solubilized using 0.5% (v/v) Digitonin. Protein concentrations for both soluble
580 and pellet fractions were determined by DC protein assay kit (Biorad).
581
582 **Split luciferase assay to measure ER-Mitochondria contacts.**

583 Cells seeded a day prior in a 12-well plates were co-transfected with 0.75 μg pcDNA3-Mit-
584 NRluc91 and pcDNA3-CRluc92-ER (kind gift from Jeffrey A. Golden, Brigham and Women's
585 Hospital, Boston) using PEI at a 1:4 (DNA:PEI) ratio, or Lipofectamine 2000 (Invitrogen) as per
586 manufacturer's protocol. Media was changed after 6 hour and 18 hour later the cells are split into
587 a clear bottom white 96-well plate with 50-100K cells per well. After 24 hours, 30 μM of live-cell
588 substrate Enduren (Promega) was added to cells and incubated for 2-3 hours in a 37°C incubator.
589 The luminescence was measured using a SpectraMax iD3 multi-well plate reader. The
590 luminescence measurements were normalized to the cell viability in each condition. Cell viability
591 was measured using Cell Titer-Glo (Promega) according to the manufacturers' instructions.
592 Relative luminescence units (RLU) for each cell line were normalized to the DMSO treated
593 samples to derive fold changes in RLU. Mean, standard error of means (SEM) and statistical
594 significance were calculated by one way ANOVA with indicated post-hoc analysis using
595 GraphPad Prism 5.01 (www.graphpad.com).
596
597 **Immunofluorescence and Microscopy.**
598 HFT cells stably expressing Sec61β-eGFP were grown on #1.5 cover slips in a 12 well plate and
599 transfected with indicated siRNAs using RNAiMax. 48 hours post-transfection, cells were washed
600 briefly in PBS and fixed with 4% paraformaldehyde at room temperature for 15 min. Cells were
601 washed in PBS and permeabilized in ice-cold 100% methanol at -20°C for 10 min. Cells were
602 washed three times in PBS and blocked in 2% BSA in PBS with 0.3% Triton X-100 for 1 hour.
603 The coverslips were incubated overnight with the indicated antibodies in a humidified chamber.
604 The cells were washed and incubated for a further hour with appropriate Alexa-Fluor conjugated
605 secondary antibodies (Molecular Probes) for 1 hour in the dark. Cells were washed with PBS,
606 nuclei were stained with Hoechst and mounted on slides. All images were collected using a Nikon
607 A1R scan head with spectral detector and resonant scanners on a Ti-E motorized inverted

608 microscope equipped with 60× Plan Apo NA 1.4 objective lens. The indicated fluorophores were
609 excited with either a 405nm, 488nm or 594nm laser line. Images were analyzed using FIJI
610 (https:/t/imagej.net/Fiji). Using a previously described method[52], co-localized pixel analysis to
611 quantify the ER-mitochondrial contact sites was performed using an ImageJ macro containing
612 tubeness, colocalization highlighter, and isophotcounter plugins (Supplementary fig 2a).
613
614 **Lipid depletion and fatty acid supplementation.**
615 Cells were depleted of or supplemented with fatty acids as previously described[56-58]. Briefly, cells
616 were treated with DMEM containing 0.5% lipid-depleted fetal calf serum (LDFCS; S5394, Sigma-
617 Aldrich) for 24 hours. 500 mM Oleic acid in DMSO was used as a stock solution to prepare a
618 working solution of 1 mM in DMEM containing 0.5% LDFCS. Cells were treated for 4 hr. 500 mM
619 palmitic acid stock solution was prepared in 100% ethanol by heating to 70°C for 20 min. This
620 stock solution, was used to prepare 0.25 mM palmitic acid solution in DMEM containing 0.5%
621 LDFCS which was heated in a water-bath at 50°C for 2 hr. The 0.25 mM palmitic acid solution is
622 cooled down to 37°C before adding to cells. Cell were incubated in palmitic acid solution for 4 hr.
623 All working solutions were prepared immediately prior to treatment.
624
625 **Real time polymerase chain reaction (PCR)**
626 Equal number of HEK293T WT or UBXD8 KO cells were seeded into a 6-well plate. The next day,
627 total RNA was isolated as per manufacturer's instructions using the PureLink RNA Mini kit
628 (Thermo fisher). The purified RNA was quantified and an equal amount of RNA was used for
629 cDNA preparation using iScript cDNA synthesis kit (Biorad). GAPDH was used as a housekeeping
630 gene was used. Primer sequences used in this study can be found in Supplementary Table 3.
631 Real time PCR was performed using the Powerup SyBr green master mix (Thermo Fisher). Data
632 analyses were carried out using the $2^{-\Delta\Delta Ct}$ method.
633

634 **Membrane fluidity measurements**

635 Cells were seeded in clear bottom black 96-well plate and treated with 5 μM CB-5083, lipid
636 depletion or lipid supplementation as described above. Membrane fluidity was measured using a
637 membrane fluidity kit (Abcam, ab189819) as per manufacturer's instructions. The assay uses a
638 lipophilic, membrane embedding pyrenedecanoic acid probe which undergoes a spectral shift in
639 emission from monomer (Em 400nm) to excimer (Em 460nm) based on local membrane fluidity
640 upon excitation at 360nm. The excimer to monomer (Em 460nm / Em 400nm) ratio was calculated
641 for each sample. Then fold changes of ratios ($\text{Treated}_{\text{excimer:monomer}}$ *vs* $\text{Control}_{\text{excimer:monomer}}$) were
642 deduced to provide a relative estimate of membrane fluidity compared to the wildtype untreated
643 control. A fold change less than 1 indicates ordered membranes relative to control.

644

645 **Transmission Electron microscopy.**

646 Cells were fixed in 2.5% glutaraldehyde, 3% paraformaldehyde with 5% sucrose in 0.1 M sodium
647 cacodylate buffer (pH 7.4), pelleted, and post fixed in 1% OsO_4 in veronal-acetate buffer. The
648 cells were stained *en bloc* overnight with 0.5% uranyl acetate in veronal-acetate buffer (pH 6.0),
649 then dehydrated and embedded in Embed-812 resin. Sections were cut on a Leica EM UC7 ultra
650 microtome with a Diatome diamond knife at a thickness setting of 50 nm, stained with 2% uranyl
651 acetate, and lead citrate. The sections were examined using a FEI Tecnai spirit at 80KV and
652 photographed with an AMT CCD camera. The images were analyzed manually for the ER-
653 Mitochondrial and ER-PM contacts using FIJI (https:/t/imagej.net/Fiji). Briefly, the scale of image
654 was set using Set Scale tool on ImageJ. Followed by measuring the length of ER, PM, or
655 perimeter of mitochondria using freehand line tool. The percent of contact length was determined
656 by taking the ratio of the length of ER, or PM (within contact distances of 25-100nm) to the
657 perimeter of mitochondria. The data were analyzed using GraphPad Prism 5.01 for Windows,
658 GraphPad Software, San Diego California USA, (www.graphpad.com).

659

660 **TMT-based proteomics.**

661 **Sample preparation, digestion, and TMT labeling.**

662 The PNS and MAM fractions were isolated from HEK293T WT or UBXD8 KO cells. 100 μg protein
663 from each sample was precipitated using 15% (v/v) Trichloroacetic acid (TCA) followed by 100%
664 Acetone washes. The protein pellets were resuspended in 200 mM N-(2-Hydroxyethyl)piperazine-
665 N′-(3-propanesulfonic acid) (EPPS) (pH 8.5) buffer followed by reduction using 5 mM tris(2-
666 carboxyethyl)phosphine (TCEP), alkylation with 14 mM iodoacetamide and quenched using 5 mM
667 dithiothreitol treatments. The reduced and alkylated protein was precipitated using methanol and
668 chloroform. The protein mixture was digested with LysC (Wako) overnight followed by Trypsin
669 (Pierce) digestion for 6 hours at 37°C. The trypsin was inactivated with 30% (v/v) acetonitrile. The
670 digested peptides were labelled with 0.2 mg per reaction of 6-plex TMT reagents (ThermoFisher
671 scientific) (126, 127N, 127C, 128N, 128C, and 129N) at room temperature for 1 hour. The reaction
672 was quenched using 0.5% (v/v) Hydroxylamine for 15 min. A 2.5 μL aliquot from the labeling
673 reaction was tested for labeling efficiency. TMT-labeled peptides from each sample were pooled
674 together at a 1:1 ratio. The pooled peptide mix was dried under vacuum and resuspended in 5%
675 formic acid for 15 min. The resuspended peptide sample was further purified using C18 solid-
676 phase extraction (SPE) (Sep-Pak, Waters).

677 **Off-line basic pH reverse-phase (BPRP) fractionation.**

678 We fractionated the pooled, labeled peptide sample using BPRP HPLC[59]. We used an Agilent
679 1200 pump equipped with a degasser and a detector (set at 220 and 280 nm wavelength).
680 Peptides were subjected to a 50-min linear gradient from 5% to 35% acetonitrile in 10 mM
681 ammonium bicarbonate pH 8 at a flow rate of 0.6 mL/min over an Agilent 300Extend C18 column
682 (3.5 μm particles, 4.6 mm ID and 220 mm in length). The peptide mixture was fractionated into a
683 total of 96 fractions, which were consolidated into 24 super-fractions[60]. Samples were
684 subsequently acidified with 1% formic acid and vacuum centrifuged to near dryness. Each

685 consolidated fraction was desalted via StageTip, dried again via vacuum centrifugation, and
686 reconstituted in 5% acetonitrile, 5% formic acid for LC-MS/MS processing.

687 **Liquid chromatography and tandem mass spectrometry**.

688 Mass spectrometric data were collected on an Orbitrap Lumos mass spectrometer coupled to a
689 Proxeon NanoLC-1000 UHPLC. The 100 µm capillary column was packed with 35 cm of Accucore
690 150 resin (2.6 µm, 150Å; ThermoFisher Scientific). The scan sequence began with an MS1
691 spectrum (Orbitrap analysis, resolution 120,000, 350−1400 Th, automatic gain control (AGC)
692 target 5 $x10^5$, maximum injection time 50 ms). Data were acquired for 150 minutes per fraction.
693 SPS-MS3 analysis was used to reduce ion interference[61,62]. MS2 analysis consisted of collision-
694 induced dissociation (CID), quadrupole ion trap analysis, automatic gain control (AGC) 1 $x10^4$,
695 NCE (normalized collision energy) 35, q-value 0.25, maximum injection time 60 ms), isolation
696 window at 0.5 Th. Following acquisition of each MS2 spectrum, we collected an MS3 spectrum in
697 which multiple MS2 fragment ions were captured in the MS3 precursor population using isolation
698 waveforms with multiple frequency notches. MS3 precursors were fragmented by HCD and
699 analyzed using the Orbitrap (NCE 65, AGC 3.0 $x10^5$, isolation window 1.3 Th, maximum injection
700 time 150 ms, resolution was 50,000).

701 **Data analysis.**

702 Spectra were converted to mzXML via MSconvert[63]. Database searching included all entries from
703 the Human UniProt Database (downloaded: August 2018). The database was concatenated with
704 one composed of all protein sequences for that database in the reversed order. Searches were
705 performed using a 50-ppm precursor ion tolerance for total protein level profiling. The product ion
706 tolerance was set to 0.9 Da. These wide mass tolerance windows were chosen to maximize
707 sensitivity in conjunction with Comet searches and linear discriminant analysis[64,65]. TMT tags on
708 lysine residues and peptide N-termini (+229.163 Da for TMT) and carbamidomethylation of
709 cysteine residues (+57.021 Da) were set as static modifications, while oxidation of methionine

710 residues (+15.995 Da) was set as a variable modification. Peptide-spectrum matches (PSMs)
711 were adjusted to a 1% false discovery rate (FDR)[66,67]. PSM filtering was performed using a linear
712 discriminant analysis, as described previously[65] and then assembled further to a final protein-level
713 FDR of 1% [67]. Proteins were quantified by summing reporter ion counts across all matching PSMs,
714 also as described previously[68]. Reporter ion intensities were adjusted to correct for the isotopic
715 impurities of the different TMT reagents according to manufacturer specifications. The signal-to-
716 noise (S/N) measurements of peptides assigned to each protein were summed and these values
717 were normalized so that the sum of the signal for all proteins in each channel was equivalent to
718 account for equal protein loading. Finally, each protein abundance measurement was scaled,
719 such that the summed signal-to-noise for that protein across all channels equaled 100, thereby
720 generating a relative abundance (RA) measurement.
721 Downstream data analyses for TMT datasets were carried out using the R statistical package
722 (v4.0.3) and Bioconductor (v3.12; BiocManager 1.30.10). TMT channel intensities were quantile
723 normalized and then the data were log-transformed. The log transformed data were analyzed with
724 limma-based R package where p-values were FDR adjusted using an empirical Bayesian
725 statistical. Differentially expressed proteins were determined using a $\log_2$ (fold change (WT *vs*
726 UBXD8 KO)) threshold of > +/- 0.7.
727 **Gene ontology (GO) functional enrichment analyses of proteomics data.**
728 The differentially expressed proteins were further annotated and GO functional enrichment
729 analysis was performed using Metascape online tool (http://metascape.org)[51]. The GO cluster
730 network and protein-protein interaction network generated by metascape and the STRING
731 database (https://string-db.org/), respectively, were imported into Cytoscape software (v3.8.2) to
732 add required attributes (fold changes, p-values, gene number, and conditions) and prepared for
733 the visualization. Other proteomic data visualizations were performed using the RStudio software
734 (v1.4.1103), including hrbrthemes (v0.8.0), viridis (v0.6.1), dplyr (v.1.0.7), and ggplot2 (v 3.3.5).

735

736 **Lipidomics**

737 **Sample preparation, mass spectrometry, and identification.**

738 For each independent lipidomic experiment, HEK293T WT and UBXD8 KO cells were seeded in
739 triplicate. Two of the three samples for each condition were used for lipidomics (i.e., lipids from
740 duplicate samples were extracted and analyzed in parallel to determine technical variation). The
741 third sample was used to determine the total protein concentration. Cells were washed with PBS,
742 scraped into cold 50% methanol, centrifuged, and the cell pellets were frozen. Next, cells were
743 resuspended in cold 50% methanol and transferred to glass vials. Chloroform was added and the
744 mixture was gently vortexed and centrifuged at 1,000x g for 5 min at 4˚C. Lipids were transferred
745 to a clean glass vial using a glass Hamilton syringe. Lipids were extracted twice using chloroform
746 prior to being dried under nitrogen gas. Samples were normalized according to protein
747 concentration when resuspended in a 1:1:1 solution of methanol:chloroform:isopropanol prior to
748 mass spectrometry (MS) analysis. The samples were stored at 4°C in an autosampler during data
749 collection.

750 Lipids were identified and quantitatively measured using ultra high-performance liquid-
751 chromatography high-resolution tandem MS/MS (UHPLC-MS/MS) as recently described[69,70].
752 Separation of lipids was done by reverse-phase chromatography using a Kinetex 2.6 µm C18
753 column (Phenomenex 00F-4462-AN) at 60°C using a Vanquish UHPLC system (Thermo
754 Scientific) and two solvents: solvent A (40:60 water-methanol plus 10mM ammonium formate and
755 0.1% formic acid) and solvent B (10:90 methanol-isopropanol plus 10mM ammonium formate and
756 0.1% formic acid). UHPLC was performed at a 0.25 ml per min flow rate for 30 min per sample,
757 starting at 25% solvent B and ending at 100% solvent B as described. The column was washed
758 and equilibrated between samples. Samples were run in a semi-random order where WT or
759 UBXD8 KO samples were interspersed with blank samples. Lipids were ionized using a heated
760 electrospray ionization (HESI) source and nitrogen gas and measured using a Q-Exactive Plus

761 mass spectrometer operating at a MS1 resolution of either 70,000 or 140,000 and a MS2
762 resolution of 35,000. MS1 Spectra were collected over a mass range of 200 to 1,600 m/z with an
763 automatic gain control (AGC) setting of 1e6 and transient times of 250 ms (70,000 resolution) or
764 520 ms (140,000 resolution). MS2 spectra were collected using a transient time of 120 ms and
765 an AGC setting of 1e5. Each sample was analyzed using negative and positive ion modes. The
766 mass analyzer was calibrated weekly. SPLASH LIPIDOMIX mass spectrometry standards (Avanti
767 Polar Lipids) were used in determining extraction efficiencies and lipid quantitation. Quality control
768 (QC) samples consisting of lipids extracted from the National Institute of Standards and
769 Technology (NIST) Standard Reference Material 1950 Metabolites in Frozen Human Plasma
770 which contains plasma pooled from 100 healthy donors were used in this study. In parallel to the
771 samples, a control that lacked cells was used to determine any contaminants from the lipid
772 extraction and measurement steps. Any lipids found in the no cell control were removed during
773 analysis steps.
774 Lipids were identified and quantified using MAVEN[71], EI-MAVEN (Elucidata), Xcalibur
775 (ThermoFisher Scientific), and LipidSearch software (ThermoFisher Scientific). UHPLC retention
776 time, MS^1 peaks, and MS^2 fragments were used to identify lipids. The lipid retention time, MS1
777 peak shape, isotopic distribution, and MS^2 fragments were visually confirmed for all lipids reported
778 in this study. Peak area was used to determined lipid abundance. Lipids were included if they
779 were observed in 3-6 samples in both UBXD8 KO and WT cells. Missing values in a sample were
780 not imputed. The fold change of each lipid in UBXD8 KO cells relative to its level in WT cells was
781 used to test for statistical difference between UBXD8 KO and WT cells using independent t-tests
782 and the Benjamini-Hochberg correction method to control for false statistical discovery. The
783 following lipid classes were included in the analysis: cholesteryl esters (CE), diacylglycerol (DG),
784 phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylglycerol (PG),
785 phosphatidylinositol (PI), phosphatidylserine (PS), and triacylglycerol (TG). Guidelines from the
786 Lipidomic Standards Initiative were followed for lipid species identification and quantification,

787 including consideration of isotopic patterns resulting from naturally occurring ^{13}C atoms and
788 isomeric overlap. The following MS^2 information was used to confirm each lipid species: PC
789 fragment of 184.073 (positive mode) and tail identification using formic adduct (negative mode);
790 PE fragment of 196.038 or the tail plus 197.046 (negative mode) and neutral loss (NL) of 141.019
791 (positive mode); PG fragment of 152.996 plus the identification of the FA tails (negative mode)
792 and NL 189.04 of [M+NH4]+ adduct (positive mode); PI fragment of 241.012 (negative) and NL
793 277.056 of [M+NH4]+ adduct (positive mode); PS NL of 87.032 (negative); DG and TG by NL of
794 FA tails (positive mode); and CE fragment of 369.352 or neutral loss of 368.35 (positive).
795

796 **Mouse studies**

797 C57BL/6 (stock No.: 000664) and $p97^{R155H/WT}$ (B6;129S-Vcptm1Itl/J, Stock No: 021968) were
798 purchased from Jackson Laboratory. $p97^{R155C/WT}$ and p97 cKO (VCPFL/FL; CaMKIIa-Cre) were
799 obtained as reported previously[39,40]. All mice utilized in the study were on a C57BL/6 background.
800 Both male and female mice were used in this study. Animal procedures were performed in
801 accordance with protocols approved by the Animal Studies Committee at Washington University
802 School of Medicine.

803 **Immunohistochemistry of tissue sections**

804 Mice were anesthetized in an isoflurane chamber and perfused with PBS containing herapin. The
805 whole brain was removed from the skull and fixed in 4% PFA overnight at 4 °C degrees, cut
806 coronally into 40 micrometer sections, and stored in cryoprotectant solution at 4 °C degree for
807 staining. Sections were first rinsed 3 times with TBS and then blocked with blocking solution for
808 30 minutes (5% normal goat serum with 0.1% Triton X-100 in TBS). Sections were stained with
809 the primary antibody (SREBP1, 1:500, SCD1: 1:250 dilution) in TBS-0.1% Triton X-100 plus 2%
810 normal goat serum at 4 °C overnight, followed by 3 washes with TBS. Sections were then
811 incubated with the Alexa 488 and 555 tagged secondary antibodies (1:1000) for 2 hours at room
812 temperature, followed by counterstaining with DAPI (1:1000) for 20 min. After three washes with

813 TBS, the sections were mounted on the glass slides. True black (Biotium, NC1125051) was
814 incubated with the sections for 5 minutes to quench the auto-fluorescence. Slides were cover-
815 slipped using Prolong Gold mounting medium. Images were acquired using a Nikon Eclipse 80i
816 fluorescence microscope. All images were taken with the same fluorescent settings and
817 subsequently adjusted equally for brightness and contrast to ensure accurate pathology
818 quantification. For CA1 regions, ROIs are drawn according to DAPI staining and the mean
819 intensity was measured in each ROI by ImageJ. Background intensities (from three regions per
820 image were subtracted.

821 **Immunoblot**

822 Mouse cortex was lysed in RIPA buffer with protease inhibitor cocktails (PMSF and PIC) followed
823 by sonication (two cycles of 30 seconds cycles at 50% power). The protein concentration is
824 estimated by the BCA assay. Samples were loaded into 10% gel and transferred into
825 nitrocellulose membrane. The membranes were blocked by 5% milk in PBS-0.2% Tween20 and
826 incubated with the primary antibody in blocking solution overnight at 4 °C degrees. The membrane
827 was then washed three times with PBS-0.2% Tween20 and incubated with a secondary goat anti-
828 rabbit HRP antibody (1:5000) for 1 hour. Blot was rinsed three times with PBS-0.2% Tween20
829 and probed by a fresh mixture of ECL reagents at dark and then exposed by SYNGENE.

830

831 **Statistical analyses.**

832 For all experiments, N ≥ 3 biological replicates for each condition were examined. Fold changes,
833 SEM, and statistical analyses were performed using GraphPad Prism version 5.01 for Windows,
834 GraphPad Software, San Diego California USA (www.graphpad.com). The Statistical tests
835 performed, SEM, and statistical significance values are mentioned in the figures and
836 supplementary tables.

837

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Respective Contributions

R.G and M.R conceived the studies. R.G performed all experiments except for lipidomics studies (that were performed by Y.X. and J.G.P. and analyzed by Y.X., I.K. and J.G.P.) and mouse immunohistochemistry and immunoblotting (that were performed by J.Z). J.P and S.P.G assisted with proteomic studies. M.R wrote the manuscript with input from R.G and J.G.P and C.C.W.

Competing Interests

The authors declare no conflicts of interest.

Request for reagents

Please contact the corresponding author, M.R for reagent requests.

Data availability

All raw proteomic and lipidomic data will be made available on public servers upon acceptance of the manuscript. Any other data is available from the corresponding author upon request.

Code availability

The ImageJ macro to quantify ER-mitochondria contact sites will be made available on Github upon manuscript acceptance.

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Figure 1.

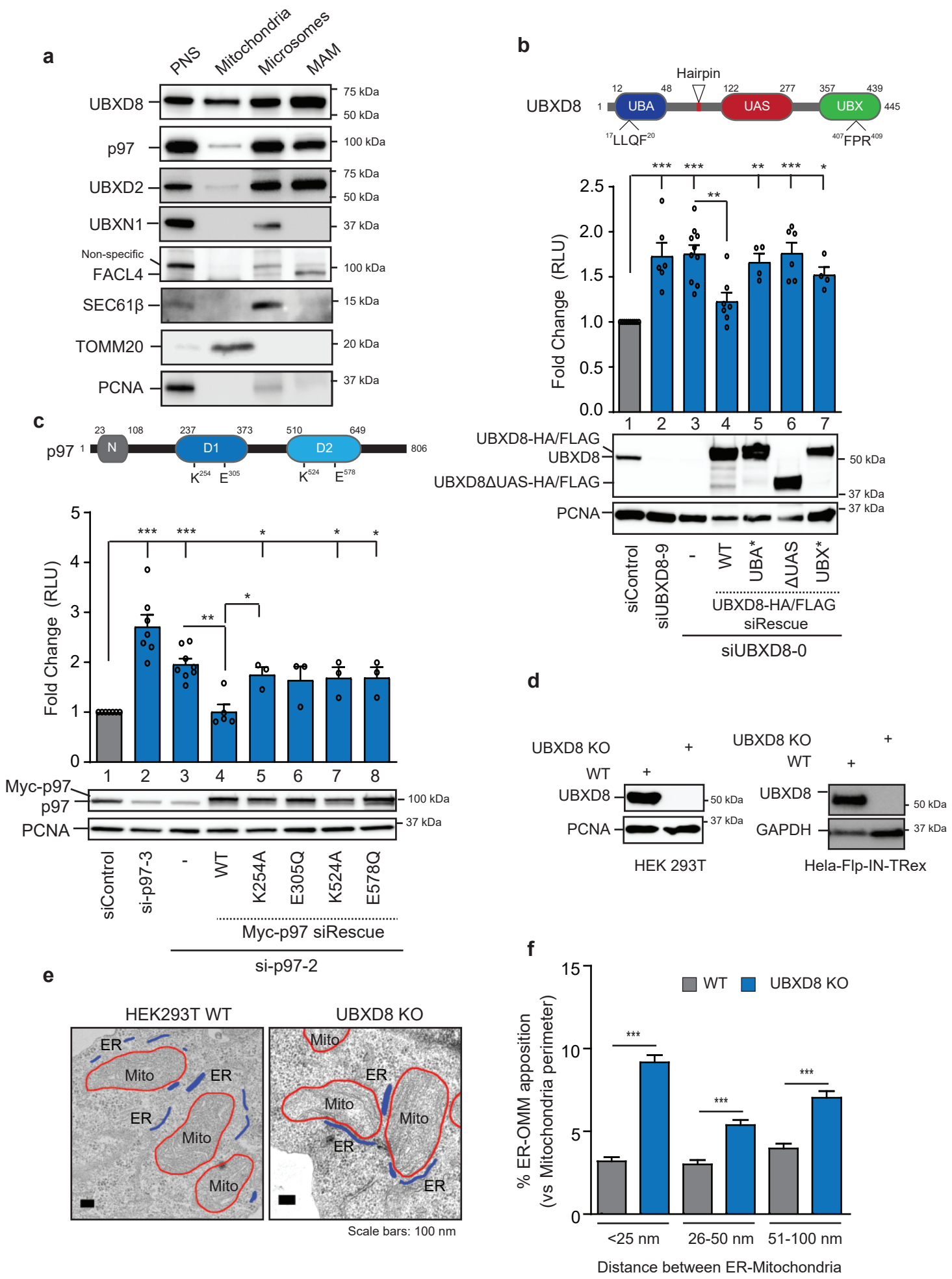

a
PNS
Mitochondria
Microsomes
MAM
UBXD8
p97
UBXD2
UBXN1
Non-specific
FACL4
SEC61β
TOMM20
PCNA
75 kDa
50 kDa
100 kDa
37 kDa
15 kDa
20 kDa
b
Hairpin
UBXD8
UBA
UAS
UBX
LLQF
FPR
Fold Change (RLU)
UBXD8-HA/FLAG
UBXD8
UBXD8ΔUAS-HA/FLAG
PCNA
siControl
siUBXD8-9
WT
UBA*
ΔUAS
UBX*
UBXD8-HA/FLAG siRescue
siUBXD8-0
c
p97
N
D1
D2
K254
E305
K524
E578
Fold Change (RLU)
Myc-p97
p97
PCNA
siControl
si-p97-3
WT
K254A
E305Q
K524A
E578Q
Myc-p97 siRescue
si-p97-2
d
UBXD8 KO
WT
UBXD8
PCNA
GAPDH
HEK 293T
Hela-Flp-IN-TRex
e
HEK293T WT
UBXD8 KO
ER
Mito
Scale bars: 100 nm
f
WT
UBXD8 KO
% ER-OMM apposition (vs Mitochondria perimeter)
<25 nm
26-50 nm
51-100 nm
Distance between ER-Mitochondria

Figure 2

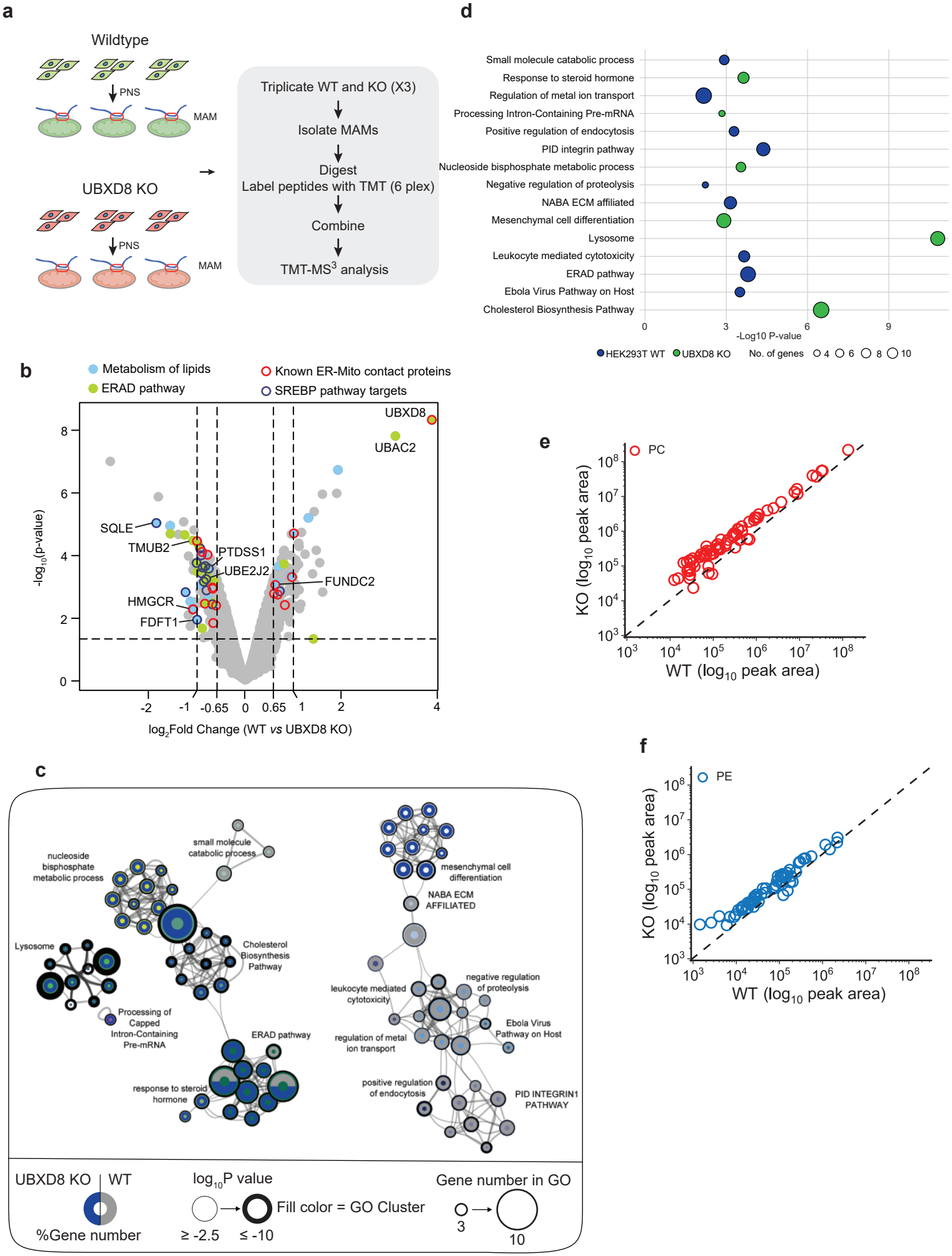

a
Wildtype
PNS
MAM
UBXD8 KO
PNS
MAM
Triplicate WT and KO (X3)
Isolate MAMs
Digest
Label peptides with TMT (6 plex)
Combine
TMT-MS3 analysis
d
Small molecule catabolic process
Response to steroid hormone
Regulation of metal ion transport
Processing Intron-Containing Pre-mRNA
Positive regulation of endocytosis
PID integrin pathway
Nucleoside bisphosphate metabolic process
Negative regulation of proteolysis
NABA ECM affiliated
Mesenchymal cell differentiation
Lysosome
Leukocyte mediated cytotoxicity
ERAD pathway
Ebola Virus Pathway on Host
Cholesterol Biosynthesis Pathway
0
3
6
9
-Log10 P-value
HEK293T WT
UBXD8 KO
No. of genes
4
6
8
10
b
Metabolism of lipids
ERAD pathway
Known ER-Mito contact proteins
SREBP pathway targets
UBXD8
UBAC2
SQLE
TMUB2
PTDSS1
UBE2J2
FUNDC2
HMGCR
FDFT1
-log10(p-value)
log2Fold Change (WT vs UBXD8 KO)
e
PC
KO (log10 peak area)
WT (log10 peak area)
f
PE
KO (log10 peak area)
WT (log10 peak area)
c
nucleoside bisphosphate metabolic process
small molecule catabolic process
mesenchymal cell differentiation
NABA ECM AFFILIATED
Lysosome
Cholesterol Biosynthesis Pathway
negative regulation of proteolysis
leukocyte mediated cytotoxicity
Processing of Capped Intron-Containing Pre-mRNA
Ebola Virus Pathway on Host
regulation of metal ion transport
ERAD pathway
response to steroid hormone
positive regulation of endocytosis
PID INTEGRIN1 PATHWAY
UBXD8 KO
WT
%Gene number
log10P value
≥ -2.5
≤ -10
Fill color = GO Cluster
Gene number in GO
3
10

Figure 3.

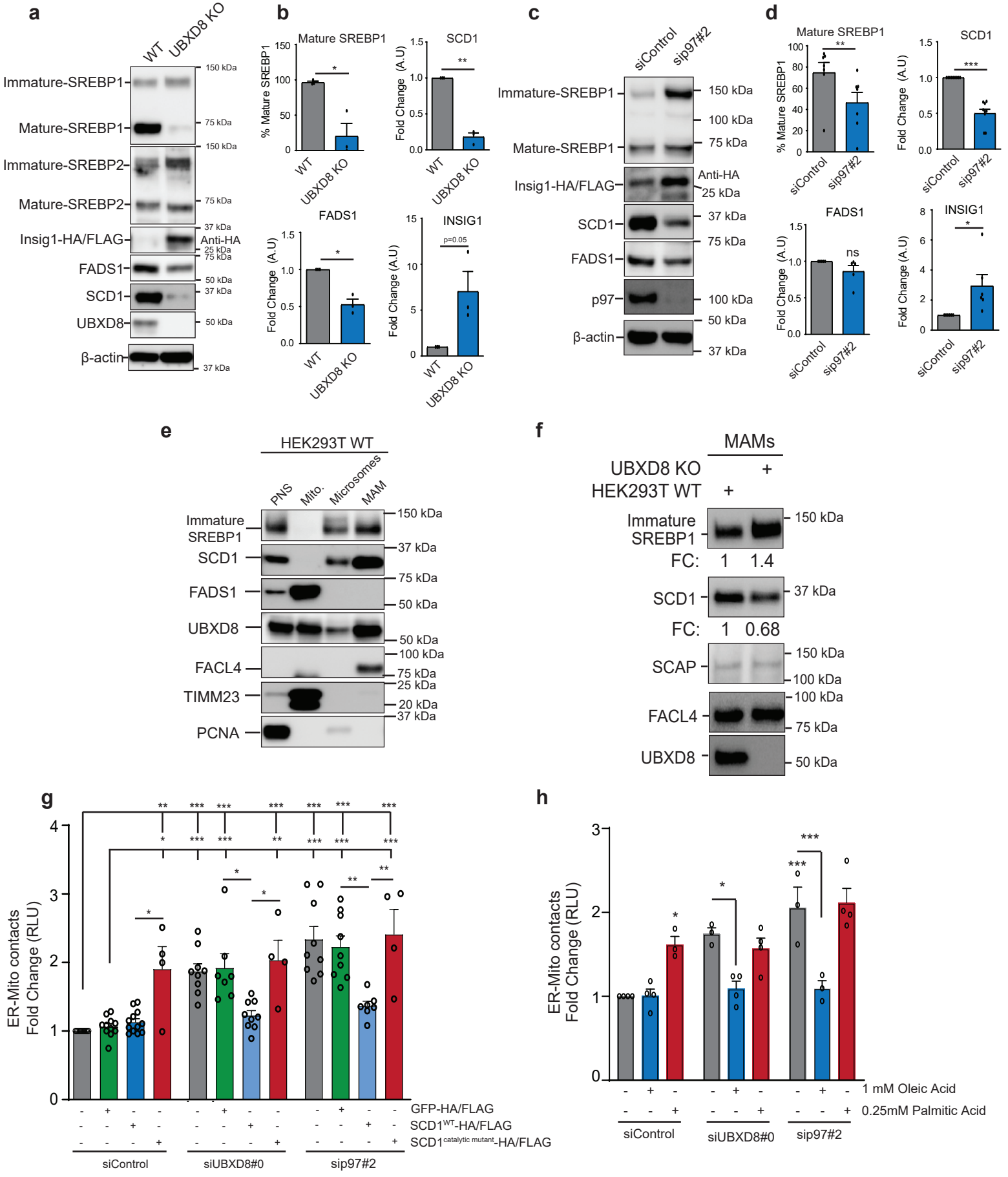
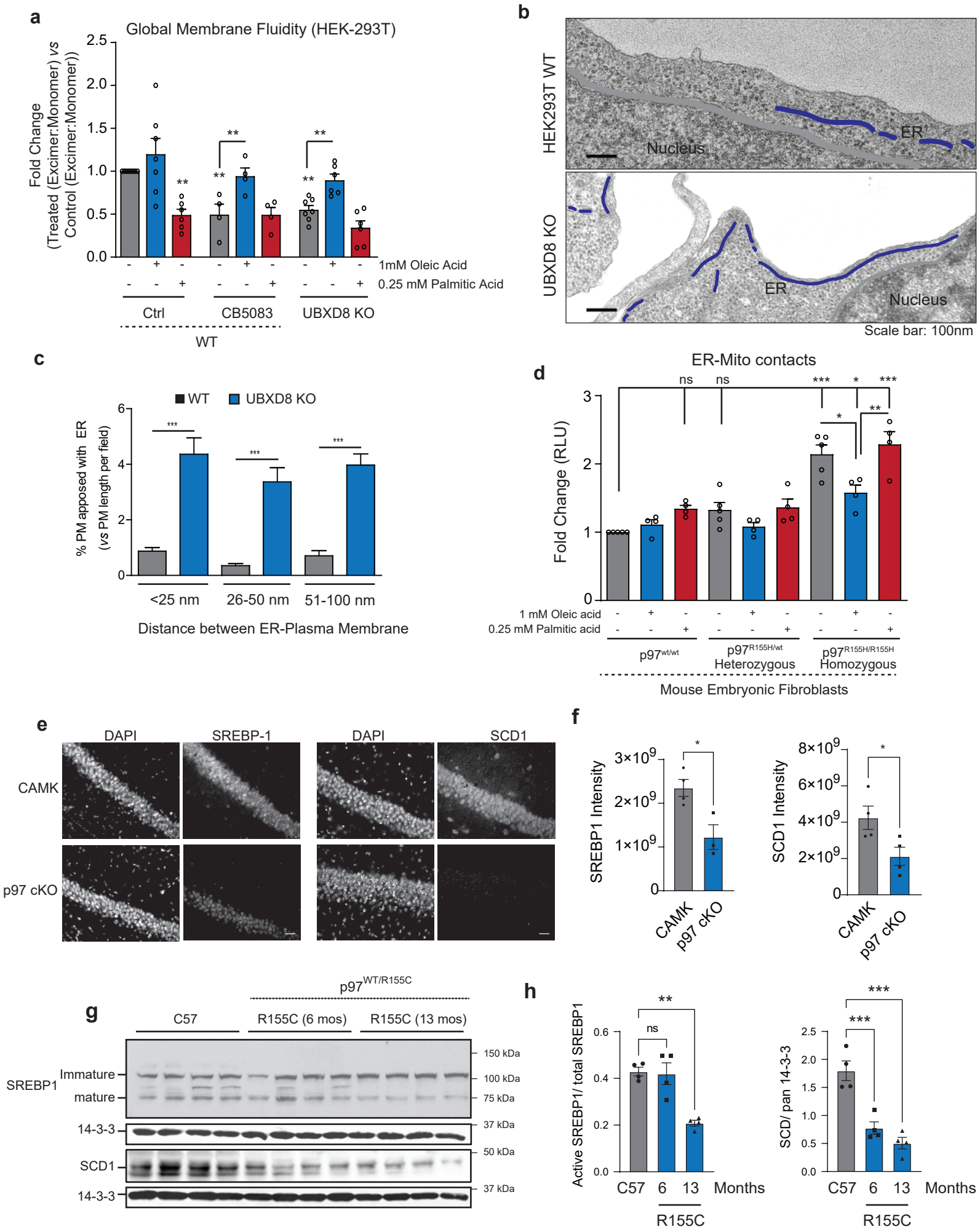
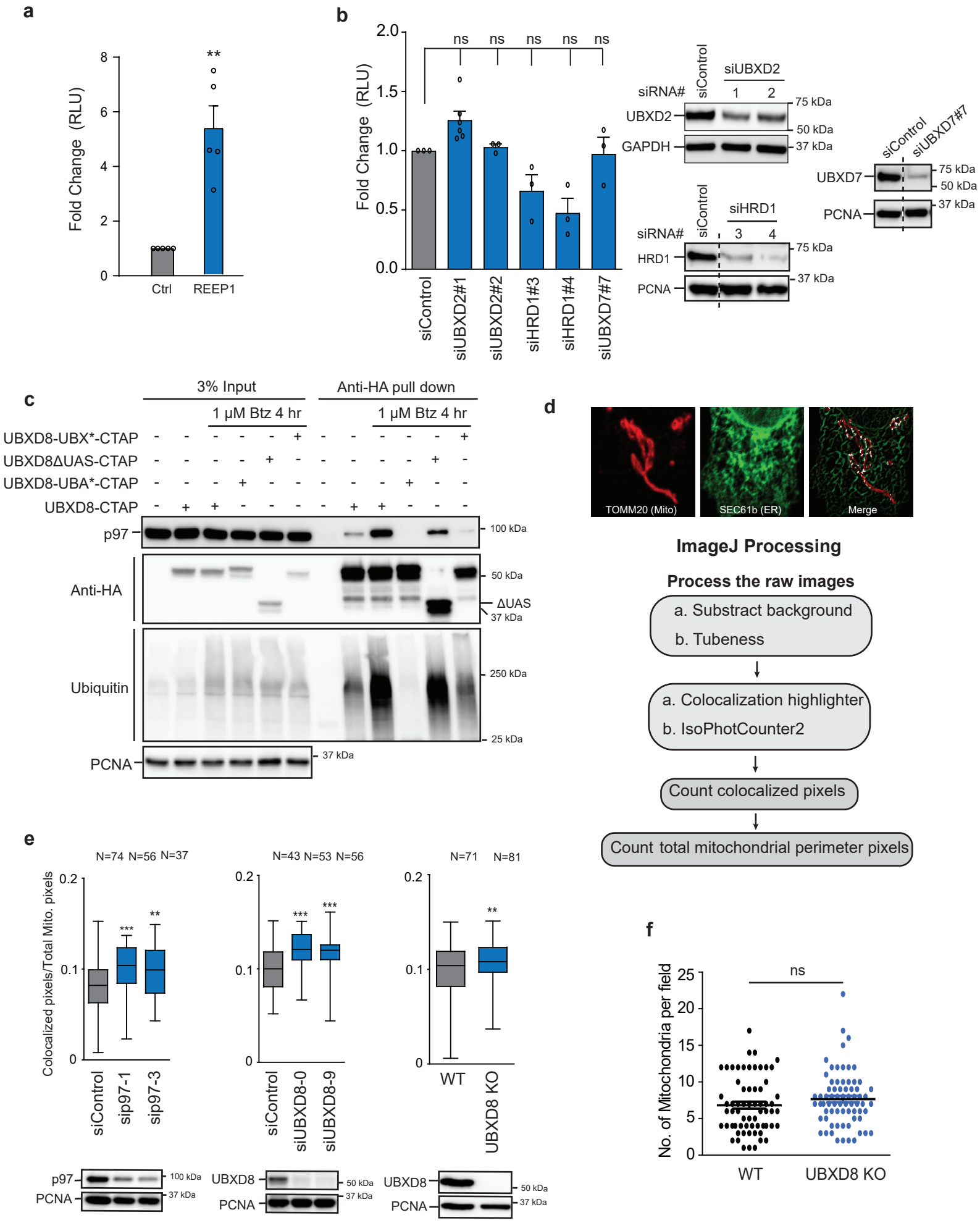

a
WT
UBXD8 KO
Immature-SREBP1
Mature-SREBP1
Immature-SREBP2
Mature-SREBP2
Insig1-HA/FLAG
FADS1
SCD1
UBXD8
β-actin
150 kDa
75 kDa
37 kDa
Anti-HA
25 kDa
50 kDa
b
Mature SREBP1
% Mature SREBP1
SCD1
Fold Change (A.U)
FADS1
INSIG1
p=0.05
c
siControl
sip97#2
p97
d
ns
e
HEK293T WT
PNS
Mito.
Microsomes
MAM
Immature SREBP1
FACL4
TIMM23
PCNA
20 kDa
f
MAMs
UBXD8 KO
HEK293T WT
FC: 1 1.4
FC: 1 0.68
SCAP
g
ER-Mito contacts Fold Change (RLU)
GFP-HA/FLAG
SCD1WT-HA/FLAG
SCD1catalytic mutant-HA/FLAG
siControl
siUBXD8#0
sip97#2
h
1 mM Oleic Acid
0.25mM Palmitic Acid

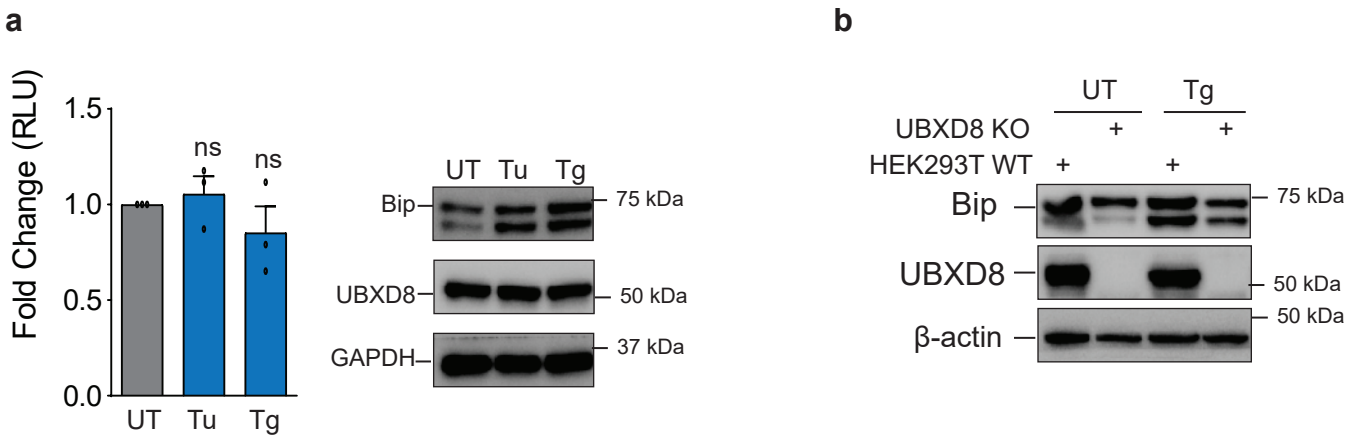
Figure 4.


a
Global Membrane Fluidity (HEK-293T)
Fold Change (Treated (Excimer:Monomer) vs Control (Excimer:Monomer))
1mM Oleic Acid
0.25 mM Palmitic Acid
Ctrl
CB5083
UBXD8 KO
WT
b
HEK293T WT
UBXD8 KO
Nucleus
ER
Scale bar: 100nm
c
WT
UBXD8 KO
% PM apposed with ER (vs PM length per field)
<25 nm
26-50 nm
51-100 nm
Distance between ER-Plasma Membrane
d
ER-Mito contacts
Fold Change (RLU)
1 mM Oleic acid
0.25 mM Palmitic acid
p97wt/wt
p97R155H/wt Heterozygous
p97R155H/R155H Homozygous
Mouse Embryonic Fibroblasts
e
DAPI
SREBP-1
SCD1
CAMK
p97 cKO
f
SREBP1 Intensity
SCD1 Intensity
CAMK
p97 cKO
g
p97WT/R155C
C57
R155C (6 mos)
R155C (13 mos)
SREBP1
Immature
mature
14-3-3
SCD1
150 kDa
100 kDa
75 kDa
37 kDa
50 kDa
h
Active SREBP1/ total SREBP1
SCD/ pan 14-3-3
C57
6
13
Months
R155C

Supplementary Figure 1.



Supplementary Figure 2.

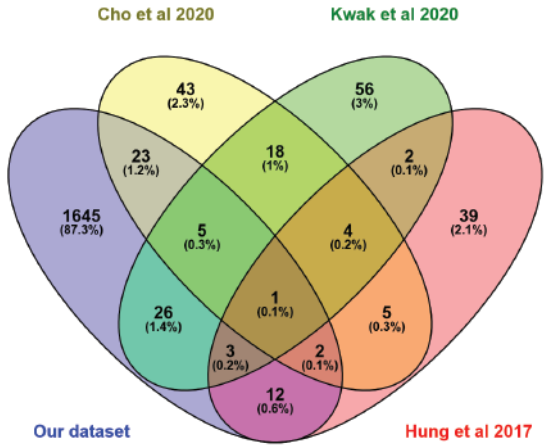


Supplementary Figure 3.

a

		Post nuclear supernatant	Mitochondria-associated membrane
	No. of proteins quantified	7556	4499
	No. of peptides quantified	56738	35371
$Log_2FC=-1$	No. of upregulated proteins in UBXD8 KO	29	23
$Log_2FC=+1$	No. of upregulated proteins in WT	74	28
$Log_2FC=-0.65$	No. of upregulated proteins in UBXD8 KO	123	108
$Log_2FC=+0.65$	No. of upregulated proteins in WT	226	112

b



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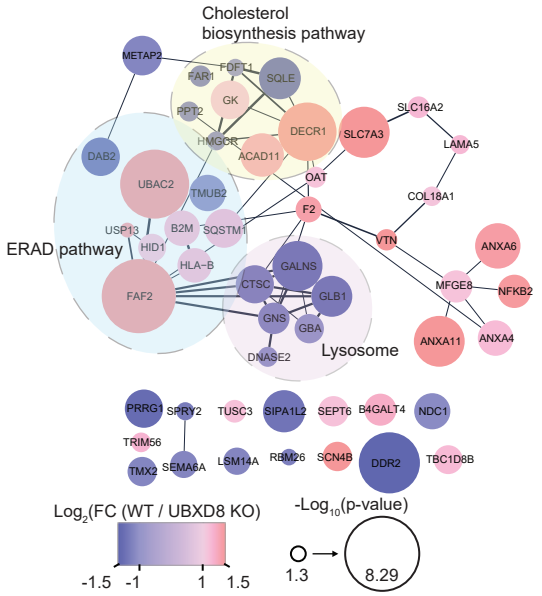

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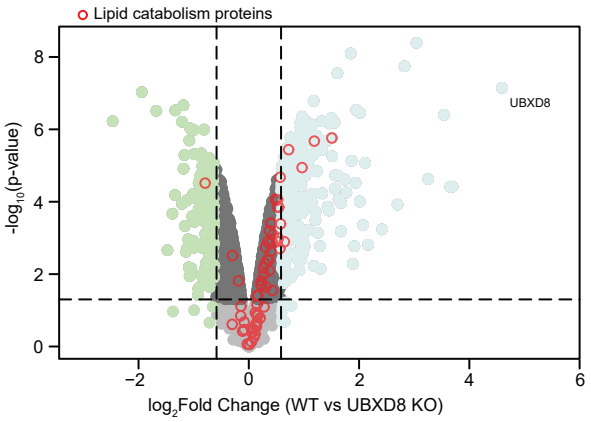

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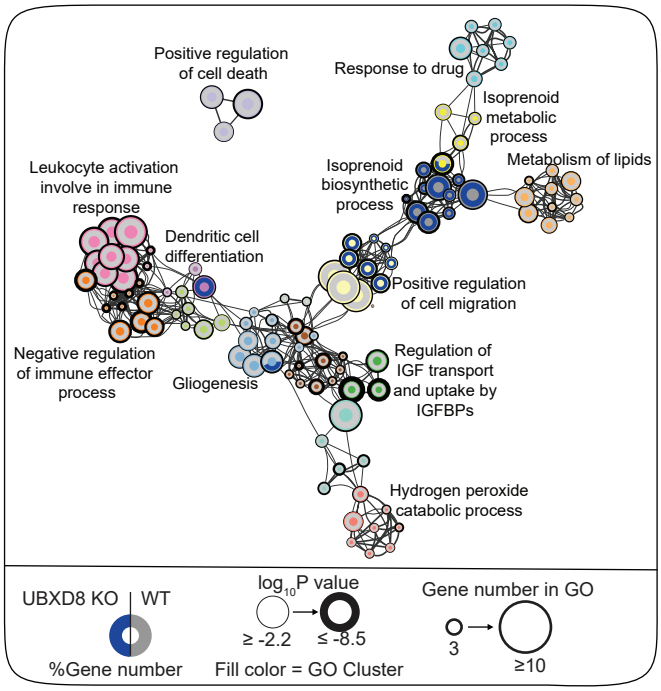

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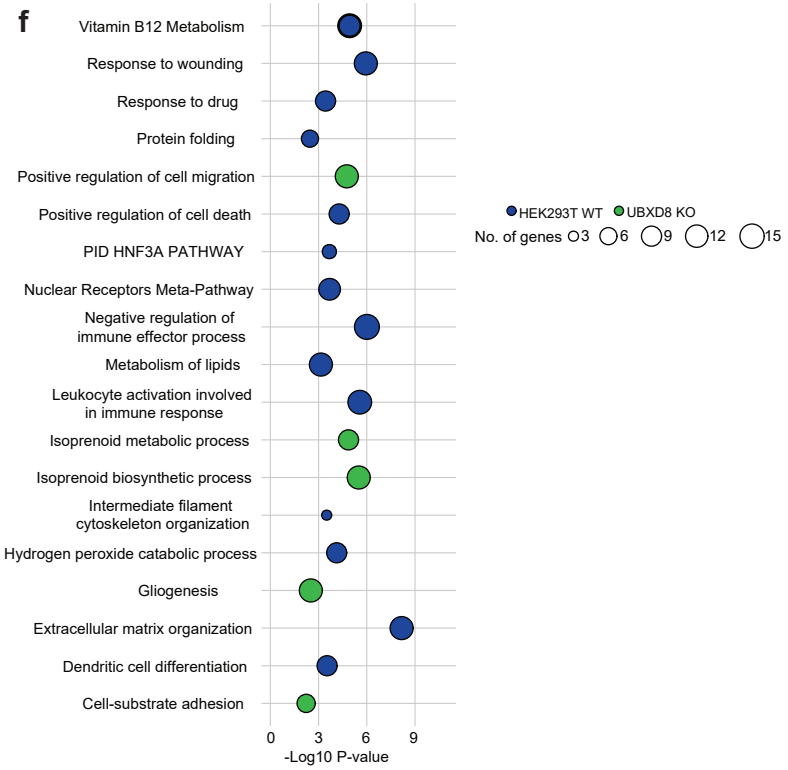
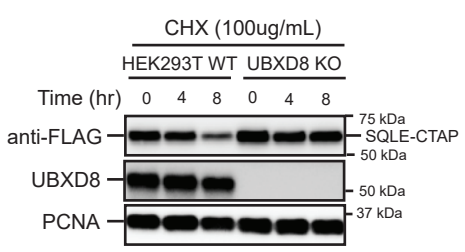
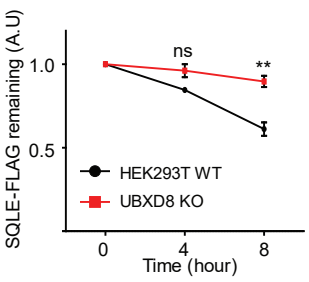


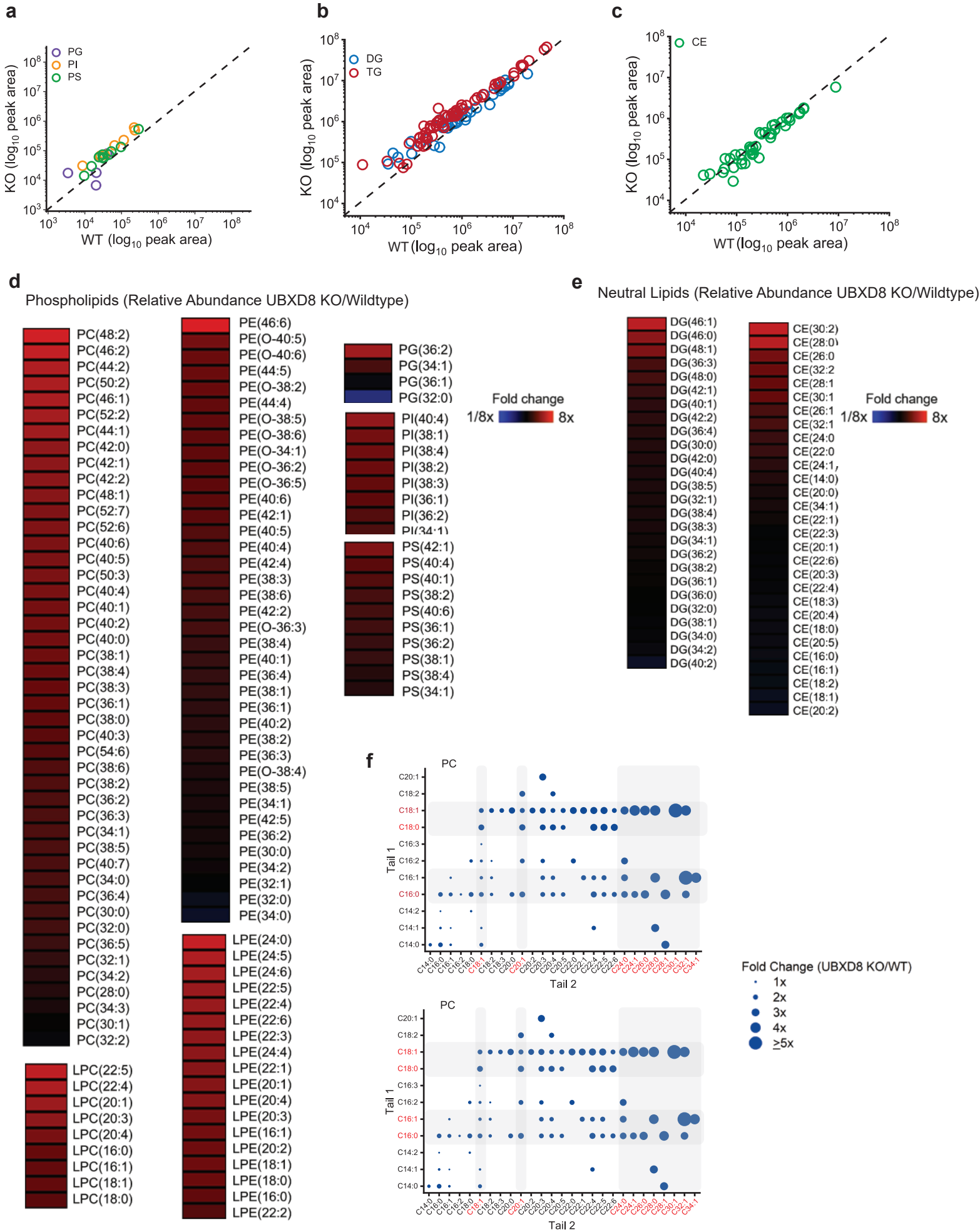
g



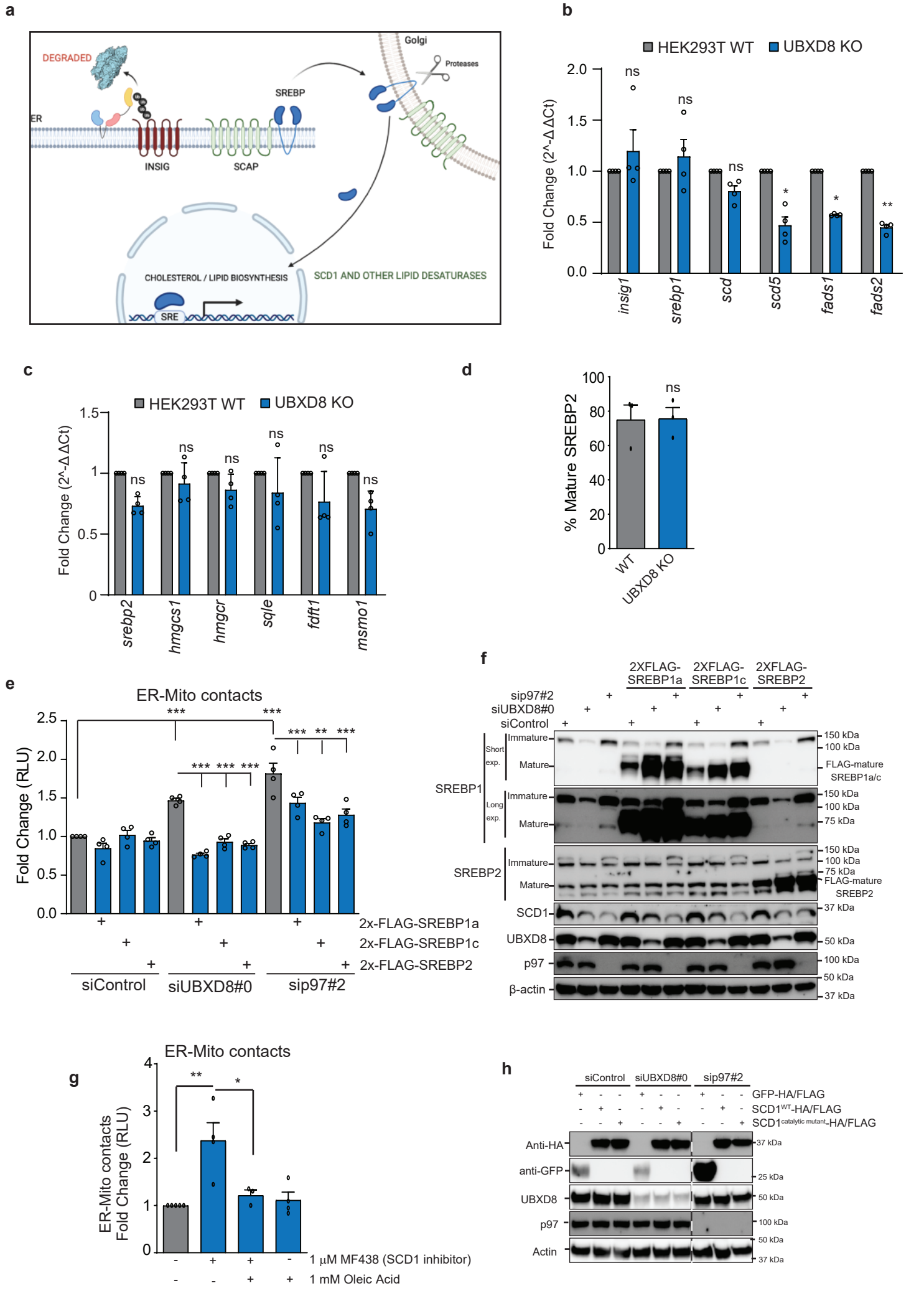
h



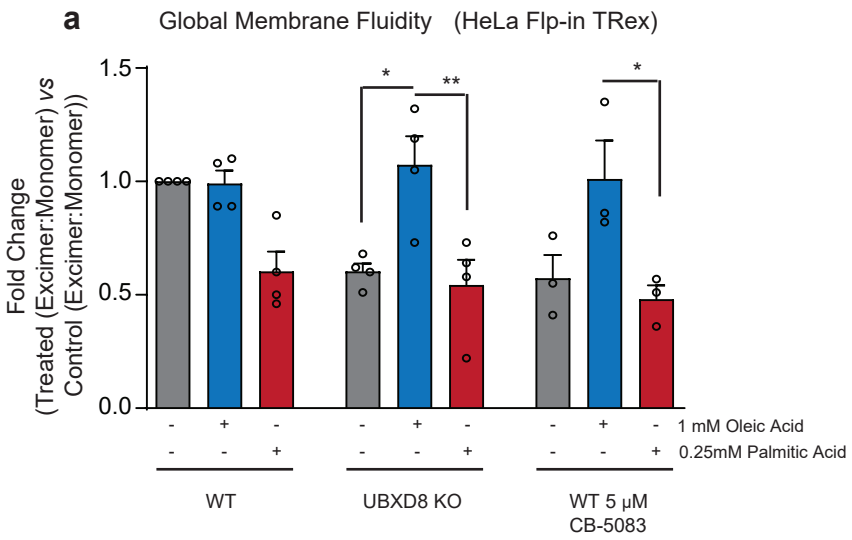
Supplementary Figure 4.

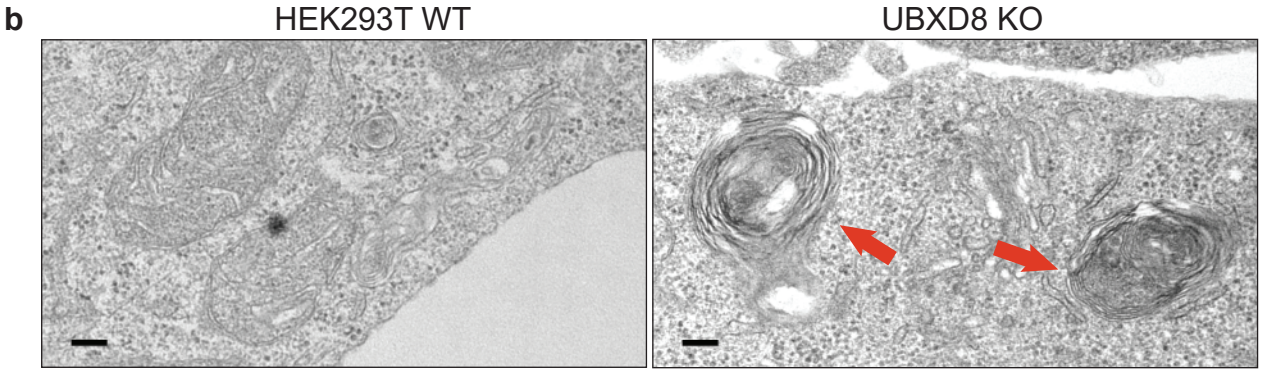


Supplementary Figure 5.



Supplementary Figure 6.





Scale bar: 100nm

	Number of fields used for quantification	Total number of whorls	Percentage
HEK293T WT	65	4	6.15%
UBXD8 KO	122	40	32.7%