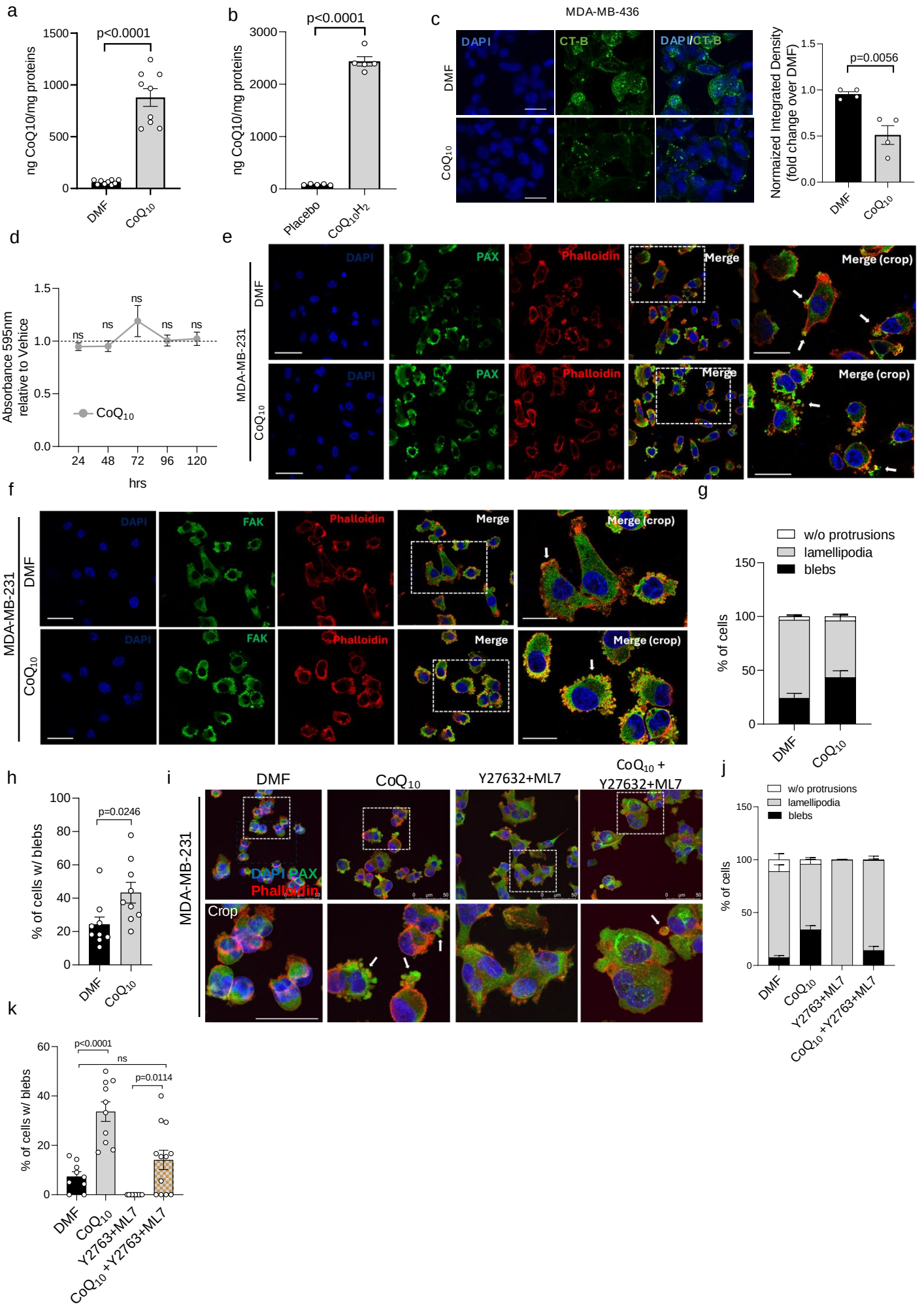
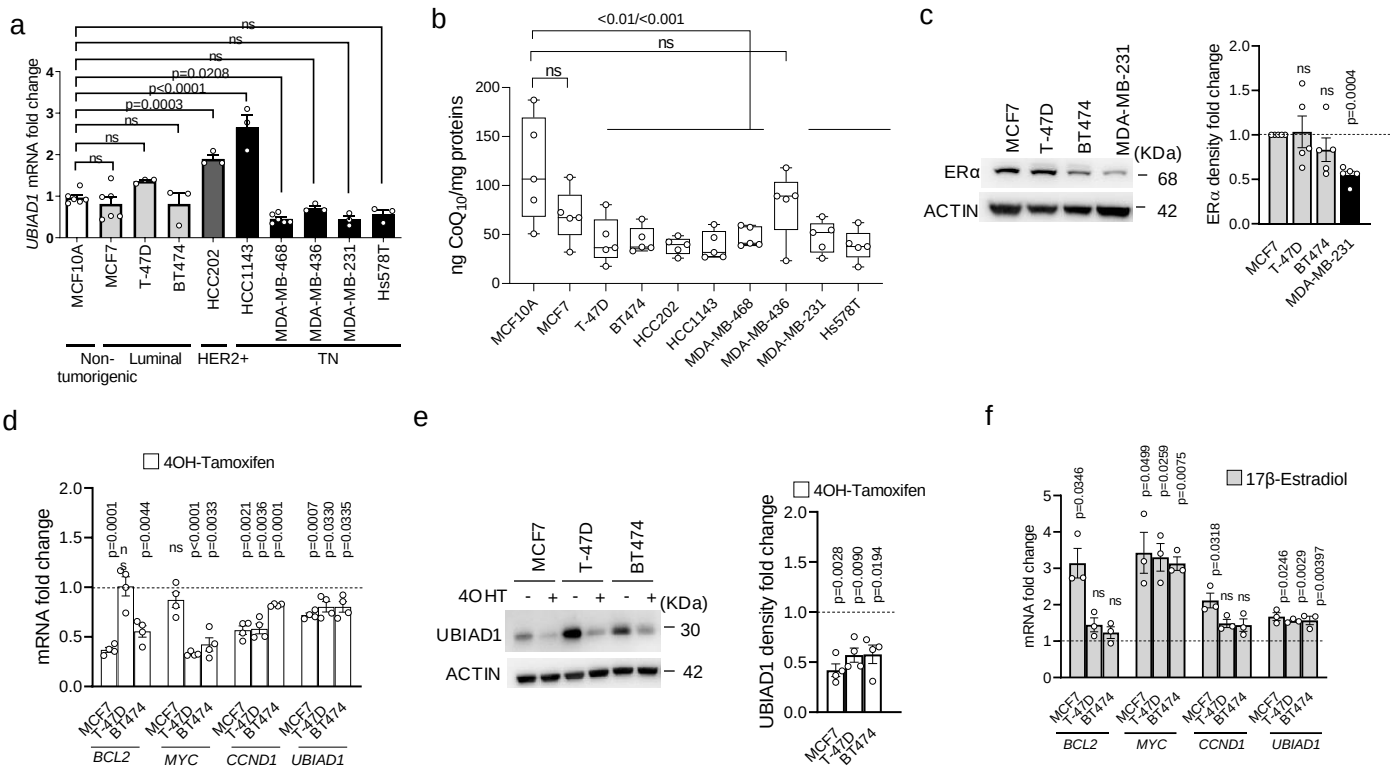

a
p<0.0001
ng CoQ10/mg proteins
DMF
CoQ10
b
p<0.0001
ng CoQ10/mg proteins
Placebo
CoQ10H2
c
MDA-MB-436
DAPI
CT-B
DAPI/CT-B
DMF
CoQ10
Normaized Integrated Density (fold change over DMF)
p=0.0056
d
Absorbance 595nm relative to Vehice
ns
CoQ10
hrs
e
MDA-MB-231
DAPI
PAX
Phalloidin
Merge
Merge (crop)
f
FAK
g
w/o protrusions
lamellipodia
blebs
% of cells
h
p=0.0246
% of cells w/ blebs
i
DMF
CoQ10
Y27632+ML7
CoQ10 + Y27632+ML7
DAPI PAX Phalloidin
Crop
j
k
p<0.0001
ns
p=0.0114

Supplementary Fig. 1: CoQ_{10} alters plasma membrane organization in BC cells. a, b Total CoQ_{10} in MDA-MB-231 treated with 20μM CoQ_{10} or DMF (Vehicle) (**a**) and 20μM $CoQ_{10}H_2$ or Placebo (Vehicle) (**b**). Values were normalized over mg of total proteins. Mean ±SEM of n=9 in **a** and n=5 in **b** independent experiments. P-value from two-tailed un-paired t-test. **c** Lipid rafts (CT-B) in MDA-MB-436 cells treated with 20μM CoQ_{10}. Quantification of Integrated density normalized over cell number. Mean ±SEM of n=4 independent experiments. Data are expressed as fold change over DMF (Vehicle). P-value from two-tailed un-paired t-test. Scale bar, 25μm. DAPI (blue), CT-B (green). **d** MDA-MB-231 cell number at different timepoints upon treatment with Vehicle or 20μM CoQ_{10}. Data are shown as absorbance (595nm) relative to Vehicle. Mean ±SEM of n=3 independent experiments. P-value from one-sample two-tailed t-test comparing the mean of each timepoint with 1.0. ns, not significant. **e** Staining for Paxillin (PAX), DAPI and Phalloidin in MDA-MB-231 treated with DMF (Vehicle) vs. 20μM CoQ_{10} (24h). Arrows: cell adhesions/blebbing structures. Scale bar, 60μm (Crop, 30μm). Cells were analyzed in n=5 independent experiments. **f** Staining for Focal Adhesion Kinase (FAK), DAPI and Phalloidin in MDA-MB-231 treated with DMF (Vehicle) vs. 20μM CoQ_{10} (24h). Arrows: cell adhesions/blebbing structures. Scale bar, 60μm (Crop, 30μm). Cells were analyzed in n=4 independent experiments. **g, h** Percentage of cells without appreciable protrusions, with only lamellipodia or blebs (**g**), and percentage of cells with blebs (**h**). Each dot represents a field of ~20 cells stained for PAX or FAK and Phalloidin as in **e-f**. Mean ±SEM of n=9 independent experiments. P-value from two-tailed un-paired t-test. **i** Staining for Paxillin (PAX) antibody, DAPI and Phalloidin in MDA-MB-231 treated with Vehicle (DMF), 20μM CoQ_{10}, combinations of Y27632 (20μM) and ML7 (20μM) ±CoQ_{10} (24h). Arrows: bleb structures. Scale bar, 50μm. **j, k** Percentage of cells without appreciable protrusions, with only lamellipodia or blebs (**j**), and percentage of cells with blebs (**k**). Each dot represents a field of ~20 cells stained for PAX and Phalloidin as in **i**. Mean ±SEM of n=12 independent experiments. Adjusted p-value from one-way Anova with Tukey's multiple comparisons test.

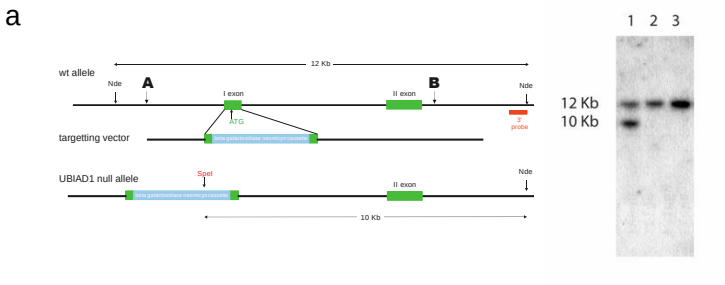
Source data are provided in Source Data file.



Supplementary Fig. 2: Low UBIAD1 expression associates with worse clinical parameters in BC. **a** qPCR analysis of UBIAD1 mRNA expression in a panel of human BC cell lines compared to non-tumorigenic breast cell line MCF10A. BC cell lines are organized according to the molecular subtype. Each dot represents an independent experiment. Data are shown as mean ±SEM. Adjusted p-value from one-way Anova with Dunnett's multiple comparisons test (MCF10A as reference). ns, not significant; HER2+, HER2-positive; TN, triple-negative. **b** Mass spectrometry analysis of total CoQ_{10} levels normalized over mg of proteins in the non-tumorigenic breast line MCF10A and a panel of BC cell lines. Mean ±SEM on n=5 independent experiments. Adjusted p-value from one-way Anova with Tukey's multiple comparisons test. **c** ERα protein expression in luminal (MCF7, T-47D and BT474) and TN (MDA-MB-231) BC cell lines. Densitometry of ERα protein expression: each dot represents an independent experiment (n=5). Data were normalized over housekeeping (ACTIN) and expressed relative to MCF7. Mean ±SEM. P-value from one-sample two-tailed t-test comparing the mean of each sample with 1.0. ns, not significant. **d** qPCR analysis of *BCL2, MYC, CCND1* and *UBIAD1* mRNA expression in ERα+ BC cells treated with 4-Hydroxytamoxifen. Each dot represents an independent experiment (n=4). Data are shown as mean ±SEM of 4OH-Tamoxifen-treated cells over Vehicle. P-value from one-sample two-tailed t-test comparing the mean of each sample with 1.0 (i.e., value set for Vehicle, dashed line). ns, not significant. **e** UBIAD1 protein expression in ERα+ BC cells treated with 4-Hydroxytamoxifen (4OHT). Each dot represents an independent experiment (n=4). Data are shown as mean ±SEM of 4OH-Tamoxifen-treated cells over Vehicle. P-value from one-sample two-tailed t-test comparing the mean of each sample with 1.0 (i.e., value set for Vehicle, dashed line). **f** qPCR analysis of BCL2, MYC, CCND1 and UBIAD1 mRNA expression in ERα+ BC cells stimulated with 17β-Estradiol. Each dot represents an independent experiment (n=3). Data are shown as mean ±SEM of 17β-Estradiol-stimulated cells over Vehicle. P-value from one-sample two-tailed t-test comparing the mean of each sample with 1.0 (i.e., value set for Vehicle, dashed line). ns, not significant.

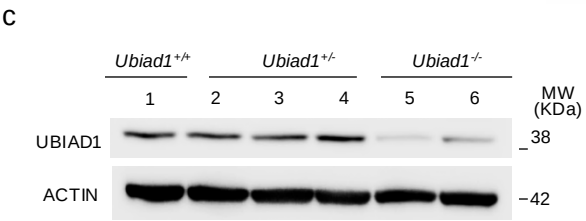
Source data are provided in Source Data file.

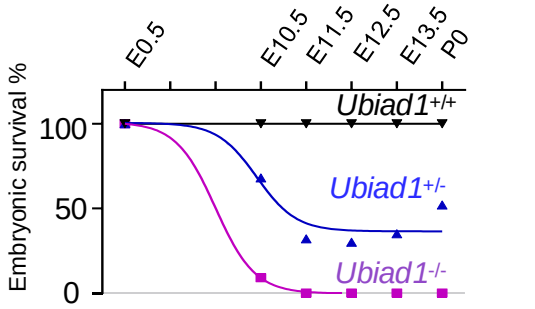
Supplementary Fig. 3: Generation of *Ubiad1* knock-out mice.

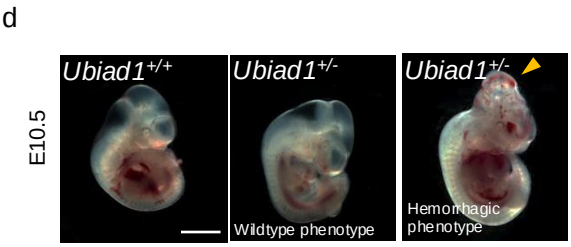


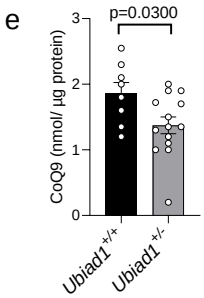
b

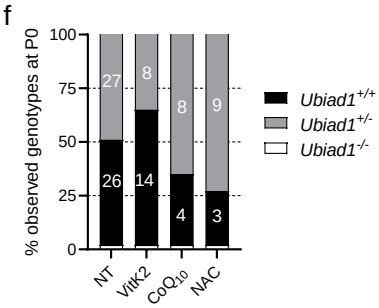
Stage	Total offspring	*Ubiad1*$^{+/+}$	*Ubiad1*$^{+/-}$	*Ubiad1*$^{-/-}$
E9.5	9	1	5	3
E10.5	26	11	15	1
E11.5	23	14	9	n.d.
E12.5	16	10	6	n.d.
E13.5	17	10	7	n.d.
P0	53	26	27	n.d.





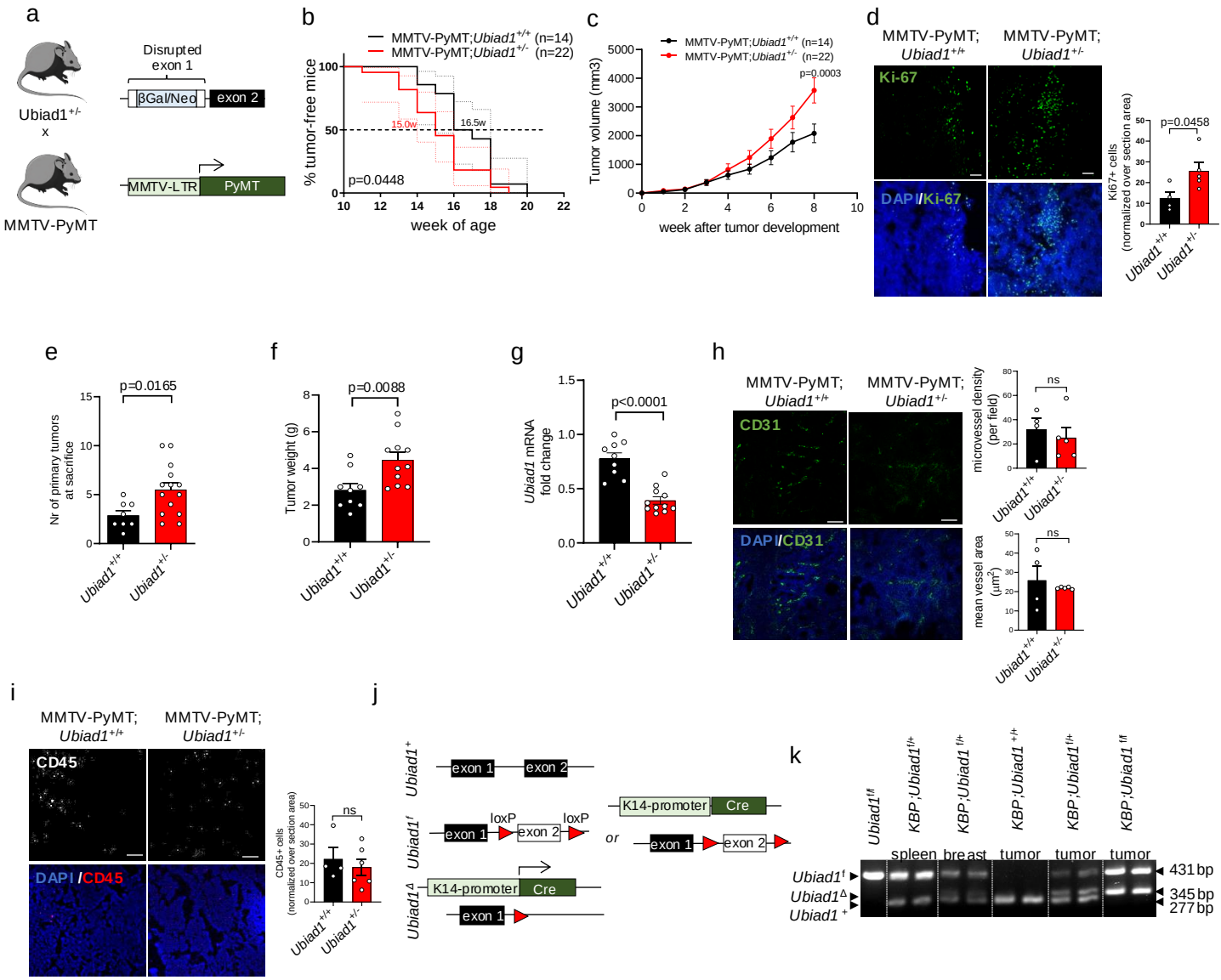






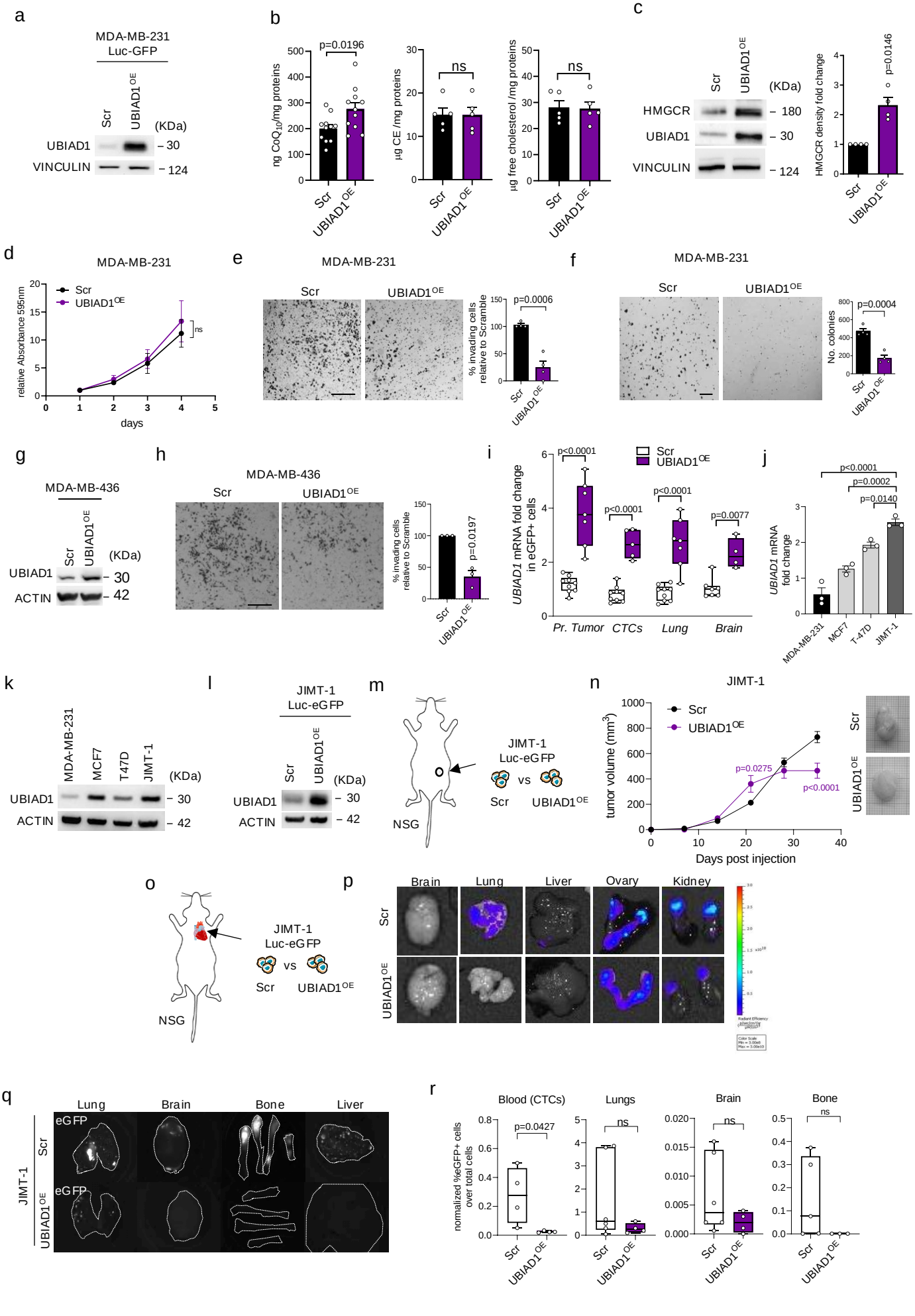
Supplementary Fig. 3: Generation of *Ubiad1* knock-out mice. a Schematic of *Ubiad1* targeting strategy. The start codon in the first exon of the *Ubiad1* gene was disrupted by the insertion of the b-galactosidase-neomycin resistance cassette by homologous recombination in mouse ES cells. A and B indicate the limit of the construct used to electroporate ES cell. SpeI in red, an artificial restriction site not present in wild type allele. Southern blot analysis on genomic DNA extracted from a recombinant (lane 1) and two wild-type clone (lane 2 ,3), digested with Nde-SpeI, and hybridized with the probe shown. **b** Distribution of mouse genotypes at different embryonic stages and after birth (P0). n.d., not detected. Below, Graphical representation of the data in the table, expressed as percentage of surviving embryos. The values were calculated based on the number of observed genotype frequency and theoretical frequency according to Mendelian genetics (25% *Ubiad1*$^{+/+}$, 50% *Ubiad1*$^{+/-}$, 25% *Ubiad1*$^{-/-}$). **c** Western blot analysis of UBIAD1 protein expression in wildtype (*Ubiad1*$^{+/+}$), *Ubiad1*$^{+/-}$ and *Ubiad1*$^{-/-}$ E9.5 embryos. **d** Images of E10.5 *Ubiad1*$^{+/+}$ and *Ubiad1*$^{+/-}$ embryos. About 30% of *Ubiad1*$^{+/-}$ embryos shows a hemorrhagic phenotype with cranial hemorrhages (arrowhead). Scale bar, 1mm. **e** Level of CoQ_9 was measured by Mass Spectrometry on whole E11.5 embryo lysate and normalized to protein concentration. Each dot represents a single embryo. Mean ± SEM. P-value was calculated using two-tailed un-paired t-test. **f** Percentage of genotypes detected in P0 pups after daily injection of Vehicle (NT), Vitamin K2 (VitK2, 150mg/kg), CoQ_{10} (150mg/kg) and N-acetyl Cysteine (NAC, 150mg/kg) in the pregnant mother between day 2.5 and 10.5 of pregnancy. The number of pups obtained for each genotype is reported inside the corresponding box. Mating was set up crossing *Ubiad1*$^{+/-}$ males with *Ubiad1*$^{+/-}$ females.

Source data are provided in Source Data file.



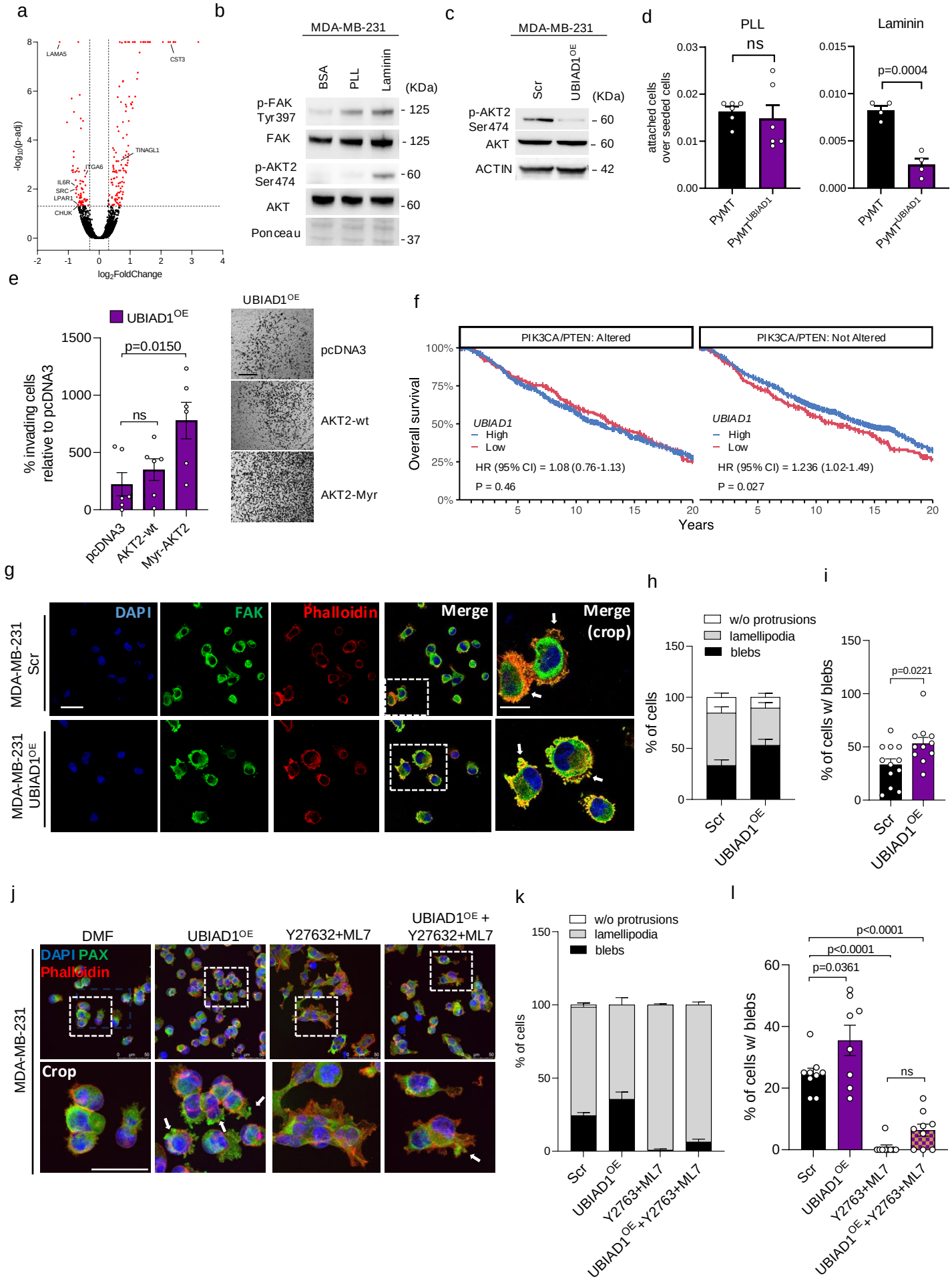
Supplementary Fig. 4: UBIAD1 antagonizes BC development in mouse models. a Generation of MMTV-PyMT spontaneous BC model with disruption of exon 1 of *Ubiad1*. **b** Tumor-free survival from birth until detection of a palpable tumor. MMTV-PyMT;*Ubiad1*$^{+/+}$ and MMTV-PyMT;*Ubiad1*$^{+/-}$ mice. Dashed lines: Confidence Interval. Group mean survival is reported. P-value from Gehan-Breslow-Wilcoxon test. **c** Tumor growth curves from detection of the first palpable tumor (week 0) until experimental endpoint (week 8). Tumor volume indicates the sum of all tumors per mouse. Each dot represents mean ±SEM. Adjusted p-value from two-way Anova test with Sidak's multiple comparisons test. **d** Ki-67 and DAPI immunostaining in tumors from n=4 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=5 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice. Scale bar, 100µm. Quantification shows mean ±SEM. P-value from two-tailed un-paired t-test. **e** Number of mammary primary tumors developed per mouse. Data are shown as mean ±SEM of n=8 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=14 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice. P-value from two-tailed un-paired t-test. **f** Total tumor weight at experimental endpoint. Data are shown as mean ±SEM of n=9 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=11 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice. P-value from two-tailed un-paired t-test. **g** *Ubiad1* mRNA expression in tumor samples from n=9 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=11 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice. Data are shown as mean ±SEM. P-value from two-tailed un-paired t-test. **h** CD31-positive cells in tumors from n=4 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=5 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice, counterstained with DAPI. Scale bar, 100µm. Quantification of micro-vessel density per field and vessel area on the right (at least four fields/sample). Data are shown as mean ±SEM. P-value from two-tailed un-paired t-test. ns, not significant. **i** CD45-positive cells (white/red) in tumors from n=4 MMTV-PyMT;*Ubiad1*$^{+/+}$ and n=6 MMTV-PyMT;*Ubiad1*$^{+/-}$ mice counterstained with DAPI. Scale bar, 100µm. Quantification of CD45+ cells normalized over section area of at least four fields/sample. Data are shown as mean ±SEM. P-value from two-tailed un-paired t-test. ns, not significant. **j** Schematic showing *Ubiad1* wildtype gene "*Ubiad1*$^{+}$"; *Ubiad1* gene without recombination with exon 2 flanked by lox-P sites "*Ubiad1*^{f} " (K14Cre absent or Cre recombinase not expressed); *Ubiad1* gene with deletion of exon 2 "*Ubiad1*$^{\Delta}$ ". **k** PCR of genomic DNA of *Ubiad1*$^{f/f}$, KBP/KBP;*Ubiad1*$^{+/+}$, KBP;*Ubiad1*$^{f/+}$ and KBP;*Ubiad1*$^{f/f}$ mouse tissues. Spleen was used as negative control for the recombination.

Source data are provided in Source Data file.


a
MDA-MB-231
Luc-GFP
Scr
UBIAD1 OE
(KDa)
UBIAD1
VINCULIN
30
124
b
p=0.0196
ng CoQ10/mg proteins
ns
µg CE /mg proteins
µg free cholesterol /mg proteins
c
HMGCR
UBIAD1
VINCULIN
180
30
124
HMGCR density fold change
p=0.0146
d
MDA-MB-231
relative Absorbance 595nm
days
e
MDA-MB-231
% invading cells relative to Scramble
p=0.0006
f
MDA-MB-231
No. colonies
p=0.0004
g
MDA-MB-436
ACTIN
42
h
MDA-MB-436
p=0.0197
i
UBIAD1 mRNA fold change in eGFP+ cells
p<0.0001
p<0.0001
p<0.0001
p=0.0077
Pr. Tumor
CTCs
Lung
Brain
j
UBIAD1 mRNA fold change
p<0.0001
p=0.0002
p=0.0140
MDA-MB-231
MCF7
T-47D
JIMT-1
k
MDA-MB-231
MCF7
T47D
JIMT-1
l
JIMT-1
Luc-eGFP
m
NSG
JIMT-1
Luc-eGFP
vs
n
JIMT-1
tumor volume (mm3)
Days post injection
p=0.0275
p<0.0001
o
NSG
JIMT-1
Luc-eGFP
vs
p
Brain
Lung
Liver
Ovary
Kidney
q
Lung
Brain
Bone
Liver
eGFP
r
Blood (CTCs)
Lungs
Brain
Bone
normalized %eGFP+ cells over total cells
p=0.0427
ns

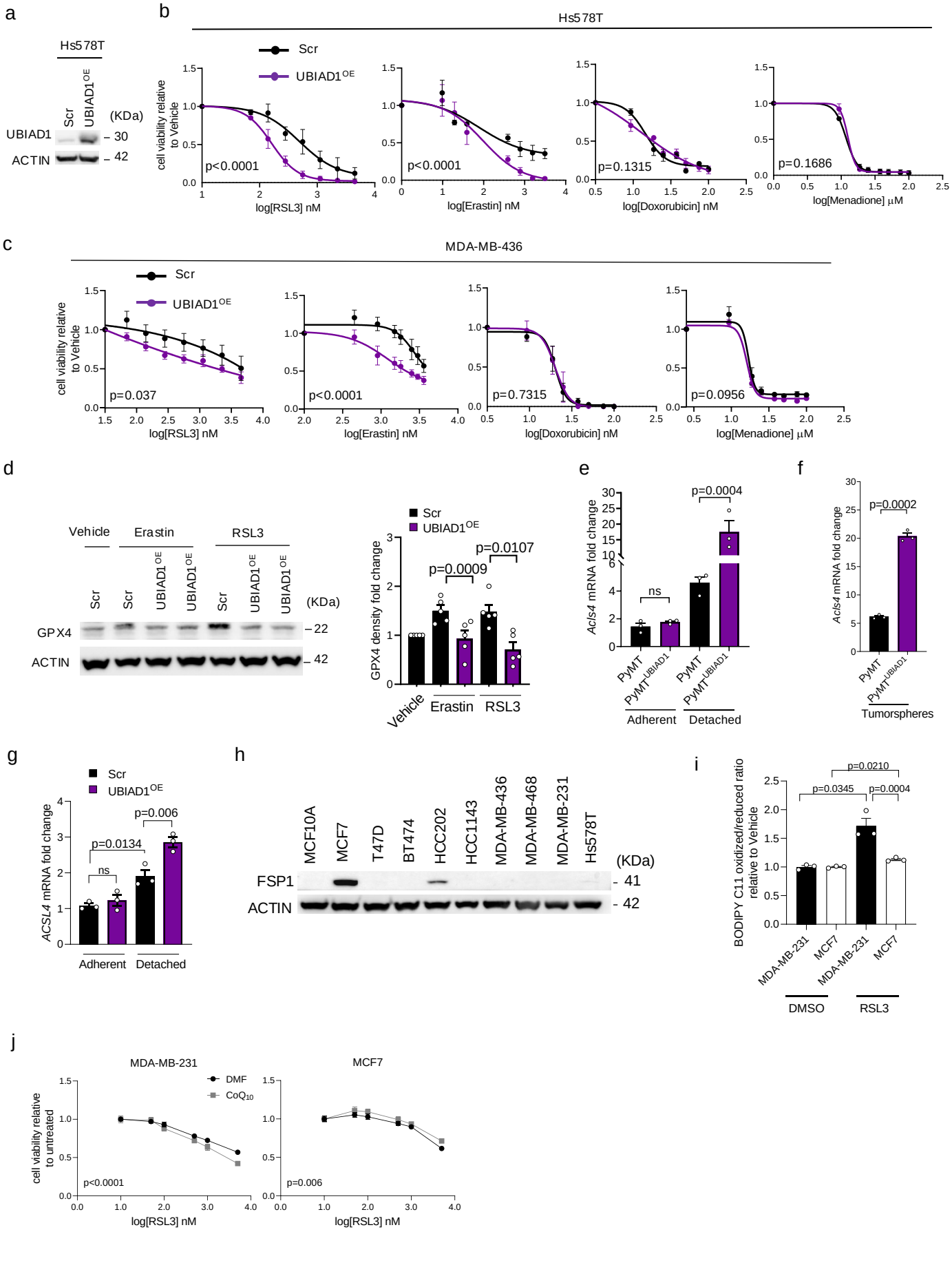
Supplementary Fig. 5: UBIAD1 regulates BC aggressiveness *in vivo*. a UBIAD1 levels in MDA-MB-231 UBIAD1 overexpressing (UBIAD^{OE}) cells compared to control. VINCULIN was used as loading control. N=3 independent experiments. **b** CoQ_{10}, cholesterol esters (CE) and free cholesterol levels. Each dot represents an independent experiment. Mean ±SEM. P-value from two-tailed un-paired t-test. ns, not significant. **c** HMGCR protein expression. Each dot represents an independent experiment. Mean ±SEM. P-value from one-sample two-tailed t-test comparing the mean of UBIAD1^{OE} with 1.0. **d** Cell proliferation assay. Each point represents the mean ±SEM of n=4 independent experiments. Adjusted p-value from two-way Anova. ns, not significant. **e** Invading cells. Scale bar, 300µm. Data are expressed as percentage of invading cells compared to Scr (100%). Data are mean ±SEM of n=4 independent experiments. P-value from two-tailed un-paired t-test. **f** Soft agar assay. Scale bar, 600µm. Data are mean ±SEM of n=4 independent experiments. P-value from two-tailed un-paired t-test. **g** UBIAD1 levels in Scr vs. UBIAD1^{OE} MDA-MB-436 cells. N=3 independent experiments. **h** Invading cells. Scale bar, 300µm. Data are expressed as percentage of invading cells compare to Scr (100%). Data are mean ±SEM of n=3 independent experiments. P-value from one-sample two-tailed t-test comparing the mean of UBIAD1^{OE} with 1.0. **i** *UBIAD1* mRNA expression in eGFP+ cells from whole blood (CTCs), primary tumors (Pr. Tumors), lung and brain of mice injected with MDA-MB-231 Scr and UBIAD1^{OE} cells. Each dot represents a single organ. Data are mean ±SEM. Adjusted p-value from one-way Anova with Sidak's multiple comparisons test. qPCR (**j**) and Western blot (**k**) of UBIAD1 mRNA and protein expression in human BC cell lines. Data are mean ±SEM of n=3 independent experiments. Adjusted p-value from one-way Anova with Dunnett's multiple comparisons test. ACTIN was used as loading control. **l** UBIAD1 levels UBIAD1^{OE} JIMT-1 Luciferase-eGFP+ cells. **m** schematic of injection in the fat pad of the 4th mammary gland of Scr and UBIAD1^{OE} JIMT-1 Luciferase-eGFP cells. **n** Representative growth curve and pictures of tumors from Scr (n=5) vs. UBIAD1^{OE} (n=4) JIMT-1 cells injected. Data are mean ±SEM. Adjusted p-value from two-way Anova with Sidak's multiple comparisons test. **o** schematic of Intracardiac injection. **p,q** eGFP signal of JIMT-1-derived metastasis in distal organs (n=4 mice/group). **r** Percentage of eGFP+ cells over total cells in whole blood (CTCs) and dissociated lungs, brain and bone (Gating: eGFP+ cells from Single_cell_2). Each dot represents a single organ/blood sample. Data are mean ±SEM. P-value from two-tailed un-paired t-test. ns, not significant.

Source data are provided in Source Data file.


a
LAMA5
CST3
TINAGL1
ITGA6
IL6R
SRC
LPAR1
CHUK
-log10(p-adj)
log2FoldChange
b
MDA-MB-231
BSA
PLL
Laminin
(KDa)
p-FAK Tyr397
FAK
p-AKT2 Ser474
AKT
Ponceau
125
125
60
60
37
c
MDA-MB-231
Scr
UBIAD1OE
(KDa)
p-AKT2 Ser474
AKT
ACTIN
60
60
42
d
PLL
ns
Laminin
p=0.0004
attached cells over seeded cells
PyMT
PyMT-UBIAD1
e
UBIAD1OE
p=0.0150
ns
% invading cells relative to pcDNA3
pcDNA3
AKT2-wt
Myr-AKT2
pcDNA3
AKT2-wt
AKT2-Myr
f
PIK3CA/PTEN: Altered
PIK3CA/PTEN: Not Altered
Overall survival
UBIAD1
High
Low
HR (95% CI) = 1.08 (0.76-1.13)
P = 0.46
HR (95% CI) = 1.236 (1.02-1.49)
P = 0.027
Years
g
DAPI
FAK
Phalloidin
Merge
Merge (crop)
MDA-MB-231 Scr
MDA-MB-231 UBIAD1OE
h
w/o protrusions
lamellipodia
blebs
% of cells
Scr
UBIAD1 OE
i
p=0.0221
% of cells w/ blebs
Scr
UBIAD1 OE
j
DMF
UBIAD1OE
Y27632+ML7
UBIAD1OE + Y27632+ML7
DAPI PAX
Phalloidin
Crop
MDA-MB-231
k
w/o protrusions
lamellipodia
blebs
% of cells
Scr
UBIAD1 OE
Y2763+ML7
UBIAD1OE+Y2763+ML7
l
p<0.0001
p<0.0001
p=0.0361
ns
% of cells w/ blebs
Scr
UBIAD1 OE
Y2763+ML7
UBIAD1OE+Y2763+ML7

Supplementary Fig. 6: UBIAD1 expression affects ECM signaling and response in BC cells. a Volcano Plot shows the differentially expressed genes between MDA-MB-231Scr and UBIAD1^{OE} (red). Genes listed in the main text are highlighted. **b** Phospho-FAK (Tyr397) and phospho-AKT2 (Ser474) levels after cell attachment on Poly-L-lysine (PLL), Laminin or not-attached cells (BSA). Ponceau was used as loading control. N=2 independent experiments. **c** Phospho-AKT2 (Ser474) in standard culture conditions. Densitometry of phosphorylated over total protein. ACTIN was used as loading control. N=3 independent experiments. **d** Cell adhesion assay of MMTV-PyMT-derived Scramble (PyMT) and UBIAD1-overexpressing (PyMT^{UBIAD1}) *ex vivo* cultures. PLL, Poly-L-Lysine. Data are shown as ratio of attached over total seeded cells. Each dot represents an independent experiment. Mean ±SEM. P-value from two-tailed un-paired t-test. ns, not significant. **e** Invasion assay of MDA-MB-231 UBIAD1^{OE} cells. Cells were transfected with empty plasmid (pcDNA3), plasmid encoding AKT2-wildtype (AKT2-wt), or plasmid encoding Myristylated-AKT2 (Myr-AKT2). Data are expressed as percentage of invading cells relative to pcDNA3 (100%). Data are mean ±SEM of n=6 independent experiments. Adjusted p-value from one-way Anova with Tukey's multiple comparisons test. Scale bar, 300µm. **f** Kaplan-Meier analysis of overall survival in METABRIC BC patients subgrouped based on PIK3CA and PTEN mutations and stratified according to *UBIAD1* mRNA expression (Low: <25th percentile; High: ≥25th percentile). HR, hazard ratio; CI, confidence intervals. P, Log-rank test p-value. **g** Focal Adhesion Kinase (FAK), DAPI and Phalloidin stainings. Arrows: cell adhesions or blebbing structures. Scale bar, 60µm (Crop, 30µm). **h, i** Percentage of cells without protrusions, with lamellipodia or blebs (**h**), and the percentage of cells with blebs (**i**). Each dot represents a field of ~20 cells stained as in **i**. Mean ±SEM of n=12 independent seedings. P-value from two-tailed un-paired t-test. **j** Paxillin (PAX), DAPI (nuclei) and Phalloidin stainings in cells treated with Vehicle (DMF) or the combination of Y27632 and ML7 inhibitors (24h). Arrows: bleb structures. Scale bar, 50µm. **k, l** Percentage of cells without protrusions, with lamellipodia or blebs (**k**), and the percentage of cells with blebs (**l**). Each dot represents a field of ~20 cells stained as in **j**. Mean ±SEM of n=9 independent seedings. Adjusted p-value from one-way Anova with Tukey's multiple comparisons test.

Source data are provided in Source Data file.


a
Hs578T
Scr
UBIAD1OE
(KDa)
UBIAD1
30
ACTIN
42
b
Hs578T
Scr
UBIAD1OE
cell viability relative to Vehicle
p<0.0001
log[RSL3] nM
p<0.0001
log[Erastin] nM
p=0.1315
log[Doxorubicin] nM
p=0.1686
log[Menadione] μM
c
MDA-MB-436
Scr
UBIAD1OE
cell viability relative to Vehicle
p=0.037
log[RSL3] nM
p<0.0001
log[Erastin] nM
p=0.7315
log[Doxorubicin] nM
p=0.0956
log[Menadione] μM
d
Vehicle
Erastin
RSL3
Scr
Scr
UBIAD1OE
UBIAD1OE
Scr
UBIAD1OE
UBIAD1OE
(KDa)
GPX4
22
ACTIN
42
Scr
UBIAD1OE
GPX4 density fold change
p=0.0009
p=0.0107
Vehicle
Erastin
RSL3
e
Acls4 mRNA fold change
p=0.0004
ns
PyMT
PyMT-UBIAD1
PyMT
PyMT-UBIAD1
Adherent
Detached
f
Acls4 mRNA fold change
p=0.0002
PyMT
PyMT-UBIAD1
Tumorspheres
g
Scr
UBIAD1OE
ACSL4 mRNA fold change
p=0.006
p=0.0134
ns
Adherent
Detached
h
MCF10A
MCF7
T47D
BT474
HCC202
HCC1143
MDA-MB-436
MDA-MB-468
MDA-MB-231
Hs578T
(KDa)
FSP1
41
ACTIN
42
i
BODIPY C11 oxidized/reduced ratio relative to Vehicle
p=0.0210
p=0.0345
p=0.0004
MDA-MB-231
MCF7
MDA-MB-231
MCF7
DMSO
RSL3
j
MDA-MB-231
MCF7
DMF
CoQ10
cell viability relative to untreated
p<0.0001
log[RSL3] nM
p=0.006
log[RSL3] nM

Supplementary Fig. 7: UBIAD1 regulates ferroptosis responses in BC cells. a UBIAD1 expression in Hs578T Scramble (Scr) and UBIAD1-overexpressing ($UBIAD1^{OE}$) cells. ACTIN was used as loading control. **b-c** Sensitivity to RSL3, Erastin, Doxorubicin and Menadione. Cells were treated for 24h with increasing concentrations of each drug; viability was expressed relative to vehicle-treated cells. Data as mean ±SEM of n=5 independent experiments. P-value from two-way Anova. **d** GPX4 protein expression in MDA-MB-231 Scr vs. $UBIAD1^{OE}$ cells treated with vehicle (DMSO), 0.9μM ERASTIN or 0.45μM RSL3 (24h). ACTIN was used as loading control. Densitometry of GPX4 protein expression: data were normalized over housekeeping (ACTIN) and expressed relative to vehicle. Mean ±SEM of n=5 independent experiments. Adjusted p-value from one-way Anova with Sidak's multiple comparisons test. ns, not significant. **e** *Acsl4* mRNA expression in adherent or detached PyMT *ex vivo* cell cultures. Scramble (PyMT) and UBIAD1-overexpressing ($PyMT^{UBIAD1}$). Each dot represents a biological replicate. Mean ±SEM. Adjusted p-value from one-way Anova with Sidak's multiple comparisons test. ns, not significant. **f** qPCR for *Acsl4* mRNA expression in 5-day tumorspheres from PyMT or $PyMT^{UBIAD1}$ *ex vivo* cell cultures. Each dot represents an independent experiment. Mean ±SEM. P-value was calculated using two-tailed un-paired t-test. **g** qPCR for *ACSL4* mRNA expression in adherent or 48h detached MDA-MB-231 Scr vs. $UBIAD1^{OE}$ cells. Mean ±SEM of n=3 independent experiments. Adjusted p-value from one-way Anova with Tukey's multiple comparisons test. ns, not significant. **h** FSP1 protein expression in different BC cell lines and non-tumorigenic MCF10A. ACTIN was used as loading control. **i**, Lipid peroxidation analysis in cells treated with vehicle (DMSO) or with 0.45μM RSL3 (12h). The fluorescent signal was quantified as ratio of oxidized/reduced BODIPY C11 (Gating: P1-Live_Cells-FITC/PE). Data are expressed as fold change over vehicle-treated cells. Mean ±SEM of n=3 independent experiments. Adjusted p-value from one-way Anova with Sidak's multiple comparisons test. **j** Sensitivity to RSL3 in cells treated for 12h. Cell viability was expressed relative to vehicle-treated cells. Data are mean ±SEM of n=6 independent experiments. P-value from two-way Anova.

Source data are provided in Source Data file.

Supplementary Table 1

Variable	ALL	UBIAD1 status		OR (95% CI)	P-value
		High N (% row)	Low N (% row)		
pT					
pT1a/b	46	23 (50%)	23 (50%)	Ref.	
pT1c	278	74 (26.6%)	204 (73.4%)	2.76 (1.46:5.21)	0.0027
pT2	216	56 (25.9%)	160 (74.1%)	2.86 (1.49:5.49)	0.0023
pT3/pT4	26	5 (19.2%)	21 (80.8%)	4.2 (1.35:13.05)	0.0124
Grade					
G1	75	32 (42.7%)	43 (57.3%)	Ref.	
G2	62	69 (30.1%)	160 (69.9%)	1.80 (1.19:2.73)	0.0073
G3	242	50 (20.7%)	192 (79.3%)	2.86 (1.64:4.97	0.0003
ER/PGR					
Not expr.	79	14 (17.7%)	65 (82.3%)	Ref.	
Expr.	487	144 (29.6%)	343 (70.4%)	0.51 (0.28:0.94)	0.031
Ki-67					
<14%	130	49 (37.7%)	81 (62.3%)	Ref.	
≥14%	434	109 (25.1%)	325 (74.9%)	1.80 (1.19:2.73)	0.0073

Association between UBIAD1 status (High, Low) and the clinical and pathological parameters, pT, Grade, ER/PGR, Ki-67. Only significant parameters are shown; see also Figure S1A for the not significant ones. pT, tumor size: pT1a/b <1 cm; pT1c >1 cm and <2 cm; pT2. >2 cm and <3 cm; pT3/4 >3 cm; Grade, grade of differentiation; ER, estrogen receptor; PGR, progesterone receptor; Ki-67, proliferative index. OR, odds ratio. Ref, reference. P-value, Fisher's exact test. The number and percentage of patients in each group are shown.

Supplementary Table 2

Variable	ALL	UBIAD1 status		OR (95% CI)	P-value
		High N (% row)	Low N (% row)		
pN					
pN0	238	69 (29.0%)	169 (71.0%)	Ref.	
pN+	311	82 (26.4%)	229 (73.6%)	1.14 (0.78:1.66)	0.50
HER2					
Negative	484	133 (27.5%)	351 (72.5%)	Ref.	
Positive	72	20 (27.8%)	52 (72.2%)	0.99 (0.57:1.71)	0.99
Age					
<50 years	244	72 (29.5%)	172 (70.5%)	Ref.	
≥50 years	322	86 (26.7%)	236 (73.3%)	1.15 (0.79:1.66)	0.51

Association between UBIAD1 status (High, Low) and molecular subtypes. HER2+, HER2-positive; TN, triple-negative. OR, odds ratio; CI, confidence intervals. P-value, Fisher's exact test. The number and percentage of patients in each group are shown.

Supplementary Table 3

Molecular subtype	High	Low	Total	OR (95% CI)	P-value
Luminal A	550 (79.4%)	143 (20.6%)	693	Ref.	
Luminal B	377 (80.4%)	92 (19.6%)	469	0.94 (0.7-1.26)	0.671
HER2+	184 (79.3%)	48 (20.7%)	232	1.00 (0.7-1.45)	0.986
Basal	176 (58.3%)	126 (41.7%)	302	2.75 (2.05-3.69)	5.75e-12
Other	123 (65.1%)	66 (34.9%)	189	2.06 (1.45-2.93)	4.24e-05
All	1410 (74.8%)	475 (25.2%)	1885		

Association between UBIAD1 mRNA expression levels (High and Low) and PAM50 subtypes in the METABRIC cohort. OR, odds ratio; CI, confidence intervals. P-value, Fisher's exact test. The number and percentage of patients in each group are shown.

Supplementary Table 4

Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways	Genes
0,0067	17	530	3,1	Pathways in cancer	LAMA5 ITGA6 EDN1 HSP90AA1 HES1 EPO EGLN1 TCF7L1 GNAQ IL6R TGFA NKX3-1 NQO1 MMP1 LPAR1 CHUK RASSF5
0,0067	13	354	3,6	PI3K-Akt signaling pathway	LAMA5 ITGA6 NGFR HSP90AA1 EPO THBS1 CREB3L1 IL6R TGFA COL6A3 PRL LPAR1 CHUK
0,0067	7	97	6,0	Fluid shear stress and atherosclerosis	EDN1 HSP90AA1 PLAT BMPR1A NQO1 SRC CHUK
0,0349	6	109	5,4	HIF-1 signaling pathway	EDN1 MKNK2 EPO EGLN1 ALDOA IL6R
0,0349	4	41	9,7	Bladder cancer	HBEGF THBS1 MMP1 SRC

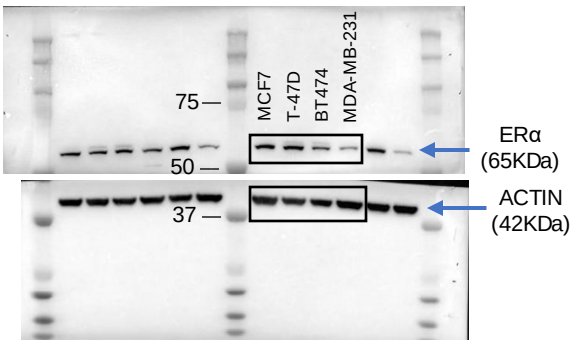
RNA-seq analysis of MDA-MB-231 Scramble (Scr) and UBIAD1-overexpressing (UBIAD1^{OE}) cells. List of genes that have been found significantly altered through KEGG pathway analysis.

Supplementary Table 5

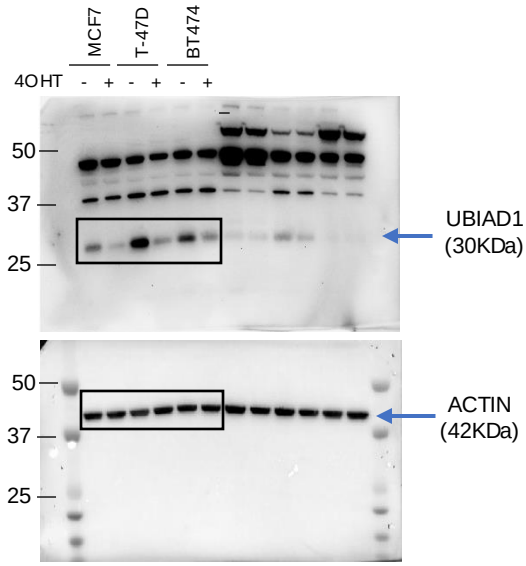
Enrichment FDR	nGenes	Pathway Genes	Fold Enrichment	Pathways	Genes
2,084E-05	19	450	4,5	Extracellular matrix organization	TNFRSF1B TIE1 ITGA6 CCDC80 FERMT1 CST3 COL12A1 COL7A1 LRP1 SOX9 PXDN LAMA5 ADAM19 THBS1 MMP14 CREB3L1 COL6A3 COL18A1 MMP1
7,64409E-06	26	717	3,7	Blood vessel development	NGFR TIE1 TGFBR3 EDN1 NRP1 BMPR1A HES1 EGR1 LRP1 C3 ID1 RAMP1 EGLN1 THBS1 MMP14 CREB3L1 IL6R PLCD3 TGFA ESM1 NKX3-1 PRL COL18A1 NOG TNFAIP2 ECSCR
1,33003E-05	26	748	3,6	Vasculature development	NGFR TIE1 TGFBR3 EDN1 NRP1 BMPR1A HES1 EGR1 LRP1 C3 ID1 RAMP1 EGLN1 THBS1 MMP14 CREB3L1 IL6R PLCD3 TGFA ESM1 NKX3-1 PRL COL18A1 NOG TNFAIP2 ECSCR
7,64409E-06	29	886	3,4	Tube morphogenesis	NGFR TIE1 TGFBR3 EDN1 NRP1 BMPR1A HES1 LRP1 SOX9 C3 ID1 LAMA5 RAMP1 EGLN1 THBS1 MMP14 CREB3L1 PLCD3 TGFA ESM1 NKX3-1 ATOH8 PRL COL18A1 NOG TNFAIP2 SRC TGM2 ECSCR
2,32527E-06	35	1122	3,2	Enzyme linked receptor protein signaling pathway	VIM PTPRU NGFR TIE1 TGFBR3 HSP90AA1 FKBP1A NRP1 LGMN FERMT1 PLAT BMPR1A MTMR4 LPXN HBEGF HES1 EGR1 LRP1 PMEPA1 SOX9 ID1 THBS1 CGN CREB3L1 TGFA ESM1 NKX3-1 IGFBP6 ATOH8 PRL GREM2 NOG ARAP1 SRC ENPP1

RNA-seq analysis of MDA-MB-231 Scramble (Scr) and UBIAD1-overexpressing ($UBIAD1^{OE}$) cells. List of Biological Processes that have been found significantly altered through Gene Ontology enrichment analysis.

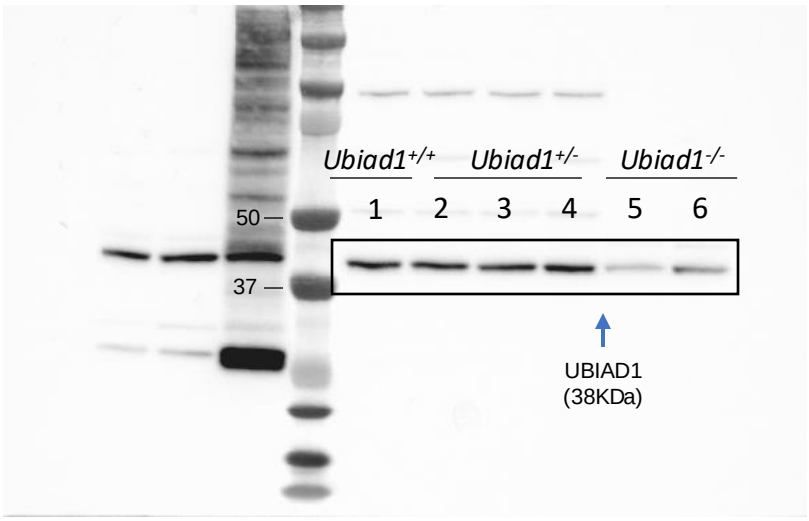
Uncropped blots corresponding to Suppl. Fig. 2f

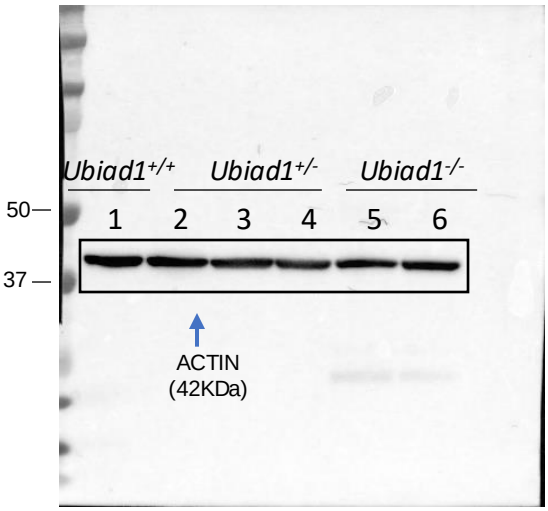


Uncropped blots corresponding to Suppl. Fig. 2h

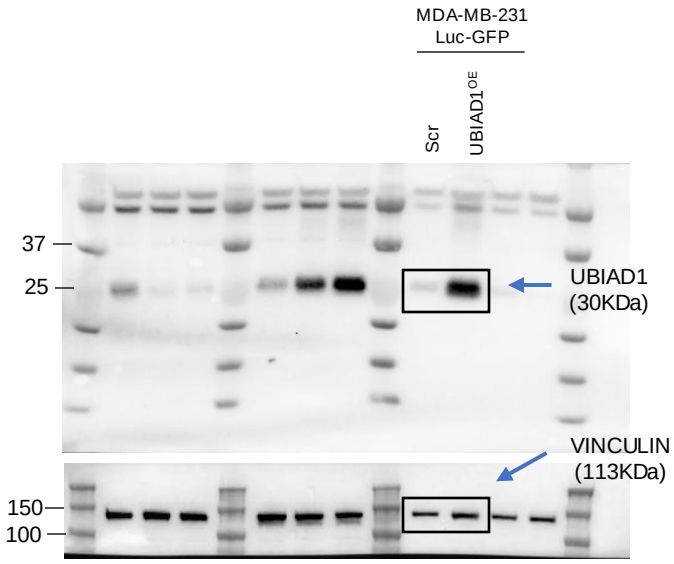


Uncropped blots corresponding to Suppl. Fig. 3c

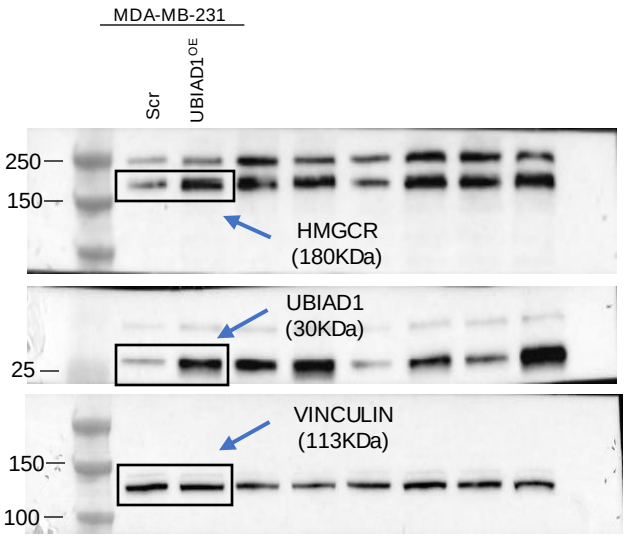




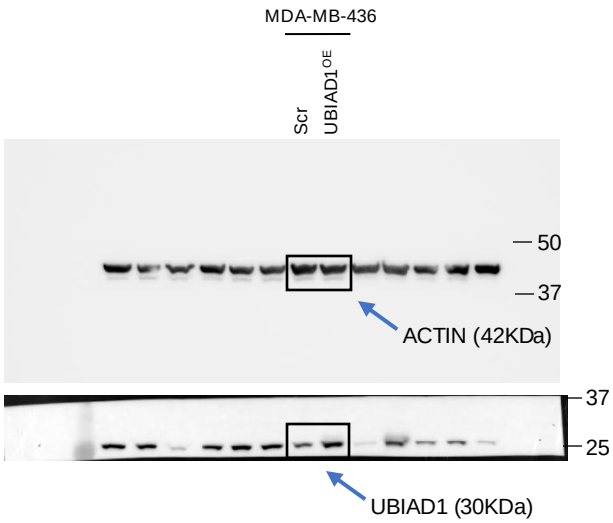
Uncropped blots corresponding to Suppl. Fig. 5a



Uncropped blots corresponding to Suppl. Fig. 5c



Uncropped blots corresponding to Suppl. Fig. 5g



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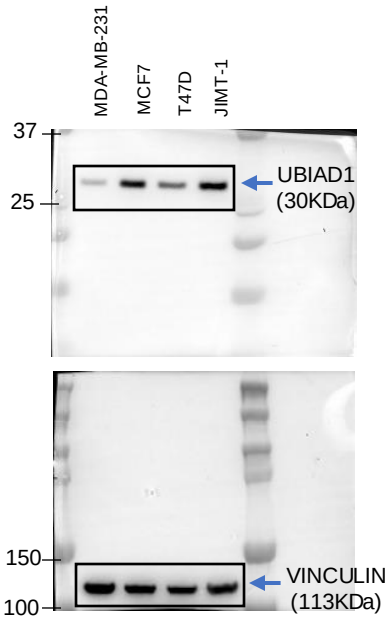

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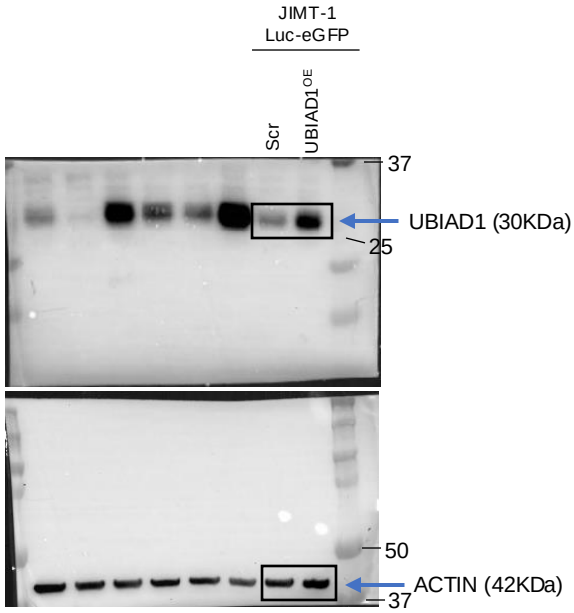
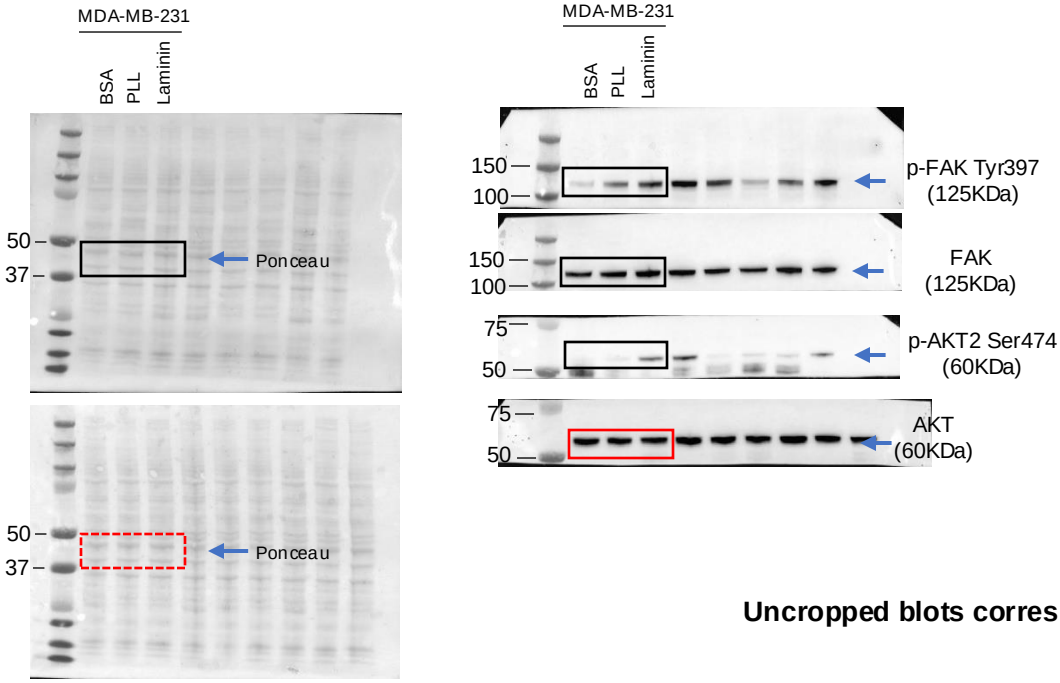
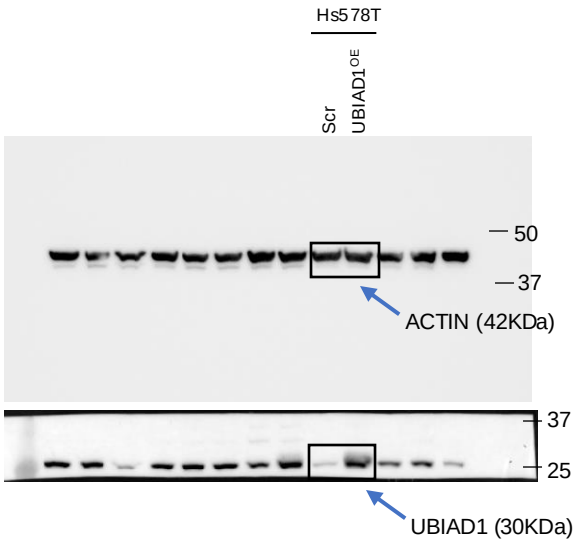


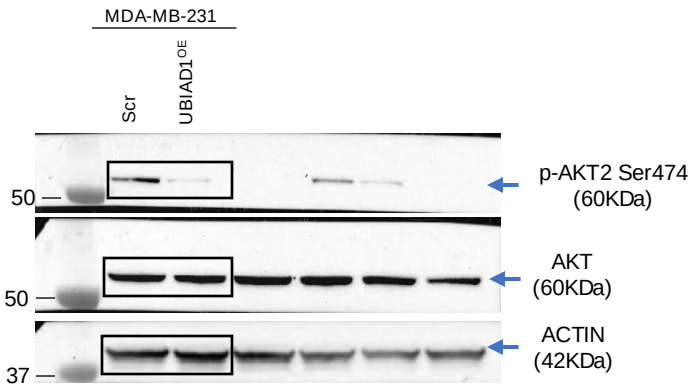
Uncropped blots corresponding to Suppl. Fig. 6b



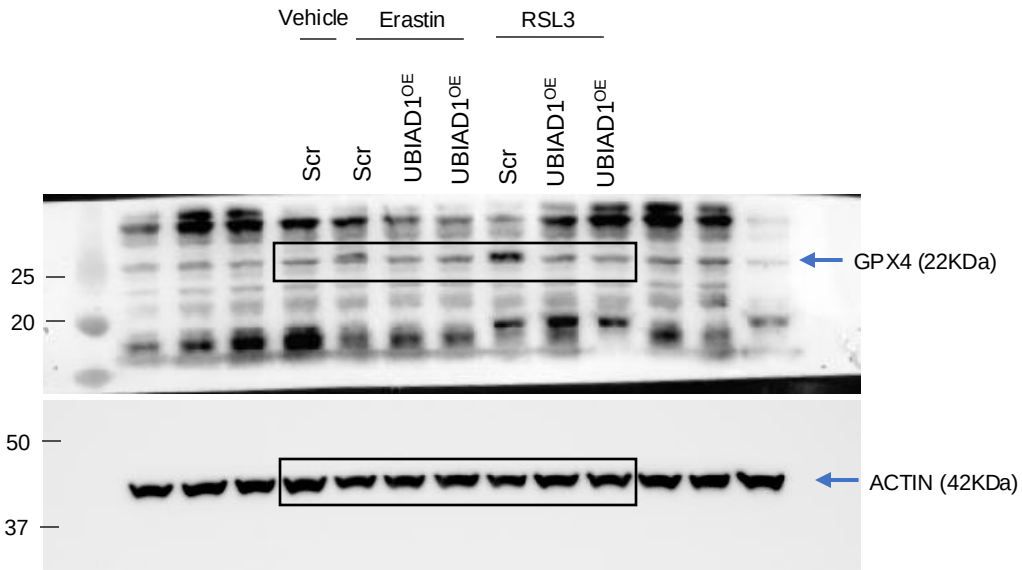
Uncropped blots corresponding to Suppl. Fig. 7a



Uncropped blots corresponding to Suppl. Fig. 6c



Uncropped blots corresponding to Suppl. Fig. 7d



Uncropped blots corresponding to Suppl. Fig. 7h