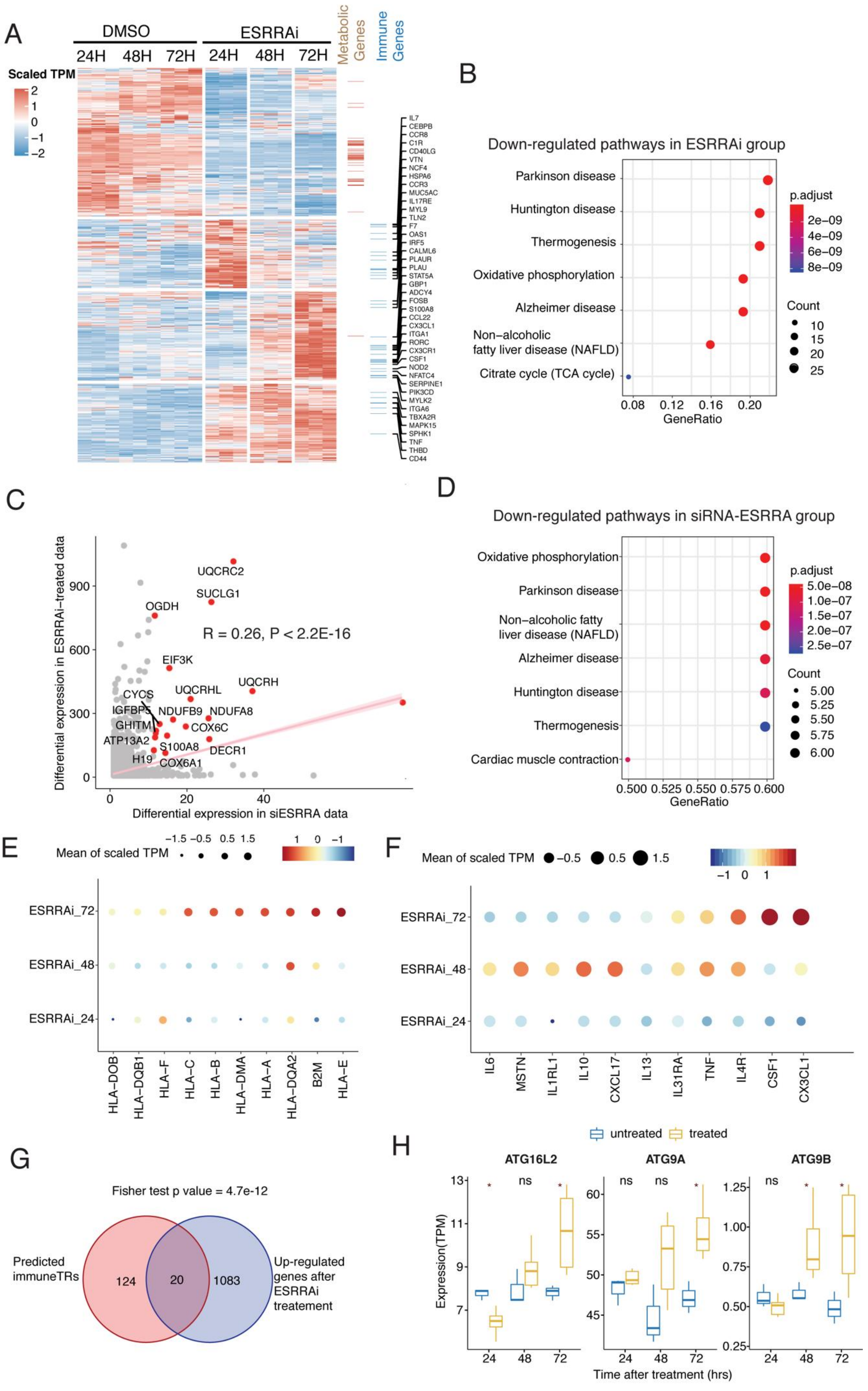
A
DMSO
ESRRAi
24H
48H
72H
24H
48H
72H
Metabolic Genes
Immune Genes
Scaled TPM
2
1
0
−1
−2
IL7
CEBPB
CCR8
C1R
CD40LG
VTN
NCF4
HSPA6
CCR3
MUC5AC
IL17RE
MYL9
TLN2
F7
OAS1
IRF5
CALML6
PLAUR
PLAU
STAT5A
GBP1
ADCY4
FOSB
S100A8
CCL22
CX3CL1
ITGA1
RORC
CX3CR1
CSF1
NOD2
NFATC4
SERPINE1
PIK3CD
MYLK2
ITGA6
TBXA2R
MAPK15
SPHK1
TNF
THBD
CD44
B
Down-regulated pathways in ESRRAi group
Parkinson disease
Huntington disease
Thermogenesis
Oxidative phosphorylation
Alzheimer disease
Non−alcoholic fatty liver disease (NAFLD)
Citrate cycle (TCA cycle)
0.08
0.12
0.16
0.20
GeneRatio
p.adjust
2e−09
4e−09
6e−09
8e−09
Count
10
15
20
25
C
Differential expression in ESRRAi-treated data
Differential expression in siESRRA data
R = 0.26, P < 2.2E-16
UQCRC2
SUCLG1
OGDH
EIF3K
UQCRH
UQCRHL
CYCS
IGFBP5
NDUFB9
NDUFA8
GHITM
COX6C
ATP13A2
S100A8
DECR1
H19
COX6A1
0
300
600
900
0
20
40
D
Down-regulated pathways in siRNA-ESRRA group
Oxidative phosphorylation
Parkinson disease
Non−alcoholic fatty liver disease (NAFLD)
Alzheimer disease
Huntington disease
Thermogenesis
Cardiac muscle contraction
0.500
0.525
0.550
0.575
0.600
GeneRatio
p.adjust
5.0e−08
1.0e−07
1.5e−07
2.0e−07
2.5e−07
Count
5.00
5.25
5.50
5.75
6.00
E
Mean of scaled TPM
−1.5
−0.5
0.5
1.5
1
0
−1
ESRRAi_72
ESRRAi_48
ESRRAi_24
HLA−DOB
HLA−DQB1
HLA−F
HLA−C
HLA−B
HLA−DMA
HLA−A
HLA−DQA2
B2M
HLA−E
F
Mean of scaled TPM
−0.5
0.5
1.5
−1
0
1
ESRRAi_72
ESRRAi_48
ESRRAi_24
IL6
MSTN
IL1RL1
IL10
CXCL17
IL13
IL31RA
TNF
IL4R
CSF1
CX3CL1
G
Fisher test p value = 4.7e-12
Predicted immuneTRs
124
20
1083
Up-regulated genes after ESRRAi treatement
H
untreated
treated
ATG16L2
ATG9A
ATG9B
Expression(TPM)
7
9
11
13
45
50
55
60
0.25
0.50
0.75
1.00
1.25
*
ns
*
ns
ns
*
ns
*
*
24
48
72
24
48
72
24
48
72
Time after treatment (hrs)

Fig. S6. Energy metabolism and immune signaling by targeting ESRRA *in vitro.*

(**A**) Differentially expressed genes between ESRRAi and control in the SKBR3 cell line at three time points. Genes were clustered by K-means. (**B**) Enriched KEGG pathways of downregulated genes in SKBR3 cells treated with ESRRAi. Shown are the significance (color) and the ratio of enriched genes (size). (**C**) The concordance between differential expression induced by siRNA and drug inhibition of ESRRA. Displayed differential expressions were derived using DESeq2 (“stat” value). Labeled are the top genes that were significant in both experiments. (**D**) Same as (B) but inhibition done by siRNA of ESRRA. (**E, F**) The average z-score scaled TPM expression (color and size) of MHC genes (**E**) and M1-polarizing cytokines (**F**) over three-time points (24h, 48h, and 72h) after ESRRAi. (**G**) Overlap of upregulated genes by ESRRAi and immune TFCRs predicted by BipotentR. P-value was calculated by the Fisher test. (**H**) ESRAA inhibition upregulates autophagy genes in cancer cells. Expression of autophagy genes 24h, 48h, and 72h after treatment of ESRRAi.