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Supplemental information

Regulation of ERK2 activity by dynamic *S*-acylation

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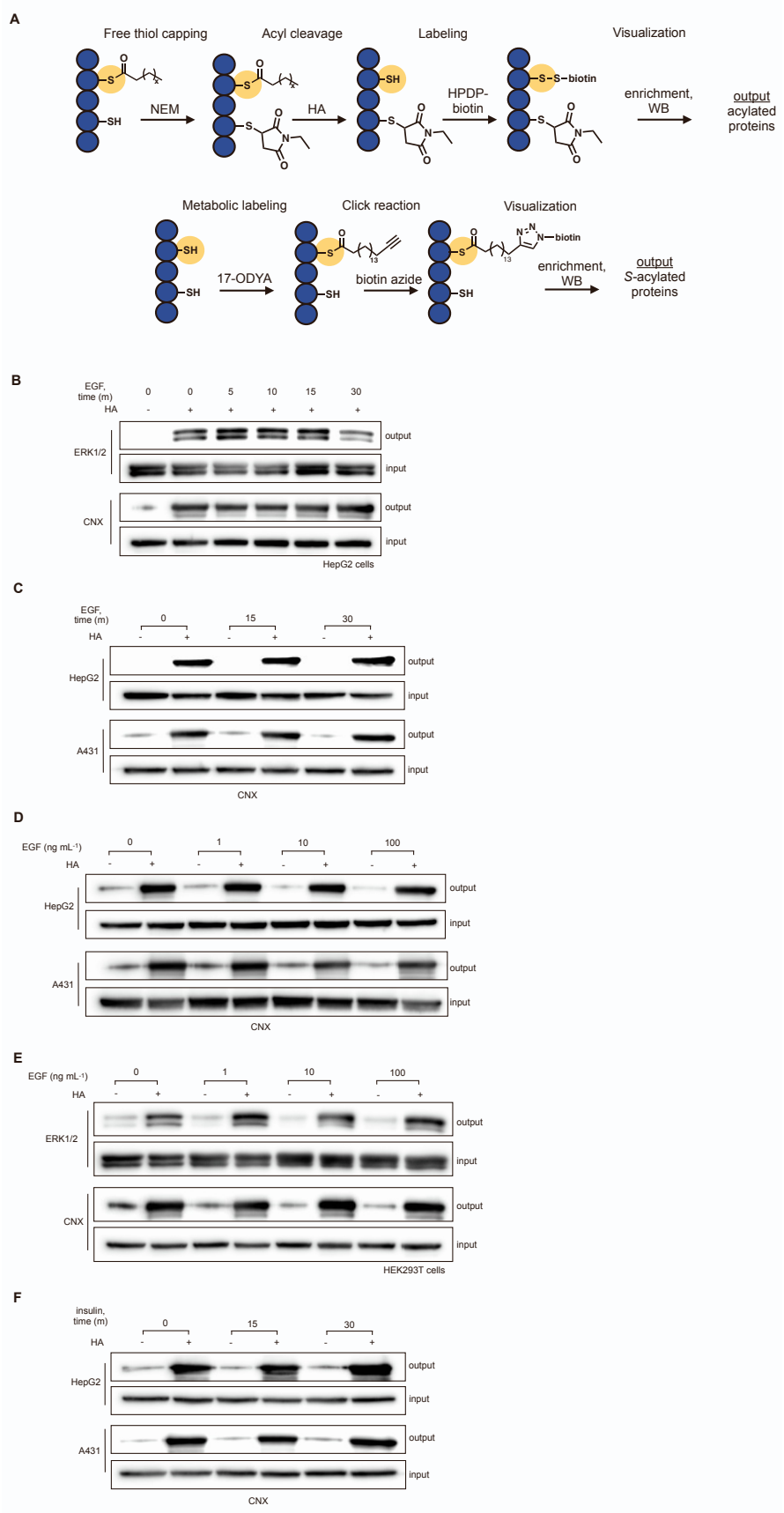

A
Free thiol capping
Acyl cleavage
Labeling
Visualization
NEM
HA
HPDP-biotin
enrichment, WB
output acylated proteins
Metabolic labeling
Click reaction
Visualization
17-ODYA
biotin azide
enrichment, WB
output S-acylated proteins
B
EGF, time (m)
HA
ERK1/2
CNX
output
input
HepG2 cells
C
HepG2
A431
CNX
D
EGF (ng mL-1)
E
HEK293T cells
F
insulin, time (m)

Figure S1. The extracellular signaling regulated kinases (ERK1/2) are dynamically palmitoylated, related to Figure 1.

(A) Schematic representations of two key assays, acyl biotin exchange (ABE, top) and metabolic labeling (bottom) used to visualize protein *S*-acylation.

(B) ABE assay carried out in HepG2 following stimulation with EGF (1 ng mL^{-1}) for 0, 5, 10, 15, and 30 minutes, *n=3.*

(C) Western blots for calnexin (CNX), used as both a loading and assay control for 2A, *n=3.*

(D) Western blots for calnexin (CNX), used as both a loading and assay control for 2B, *n=3.*

(E) ABE assay carried out in HEK293T cells following stimulation with 0, 1, 10, and 100 ng mL^{-1} EGF for 15 minutes, *n=1.*

(F) Western blots for calnexin (CNX), used as both a loading and assay control for 2C.

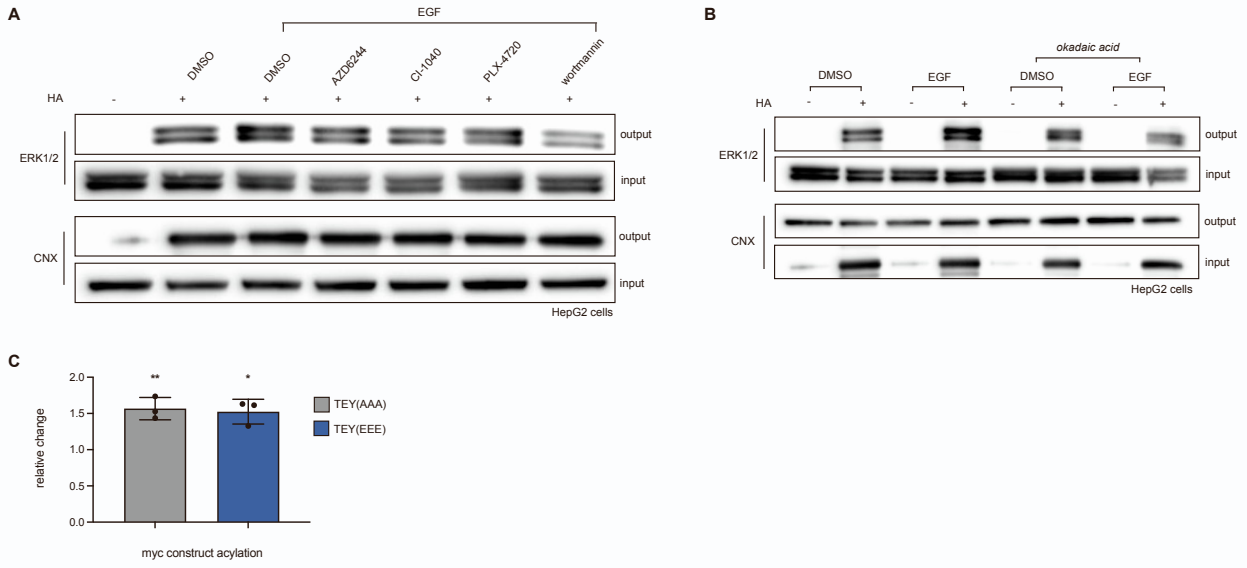


Figure S2. Dynamic S-acylation of ERK1/2 relies on pathway activation, related to Figure 2.
(A) ABE assay on HepG2 cells treated with DMSO or MAPK and PI3K pathway inhibitors, followed by EGF treatment (15 minutes, 1 ng mL^{-1}), *n=2.*
(B) ABE assay carried out on HepG2 cells treated with DMSO or okadaic acid, followed by EGF treatment (15 minutes, 1 ng mL^{-1}), *n*=2.
(C) Quantification of the acylation of the TEY(AAA) and TEY(EEE) ERK2 constructs relative to WT ERK2. Analysis was performed by first determining the ratio of output protein to input protein for each construct and then normalizing to the ratio of WT acylation. * = $p < 0.05$, ** = $p < 0.005$, and *** = $p < 0.0005$.

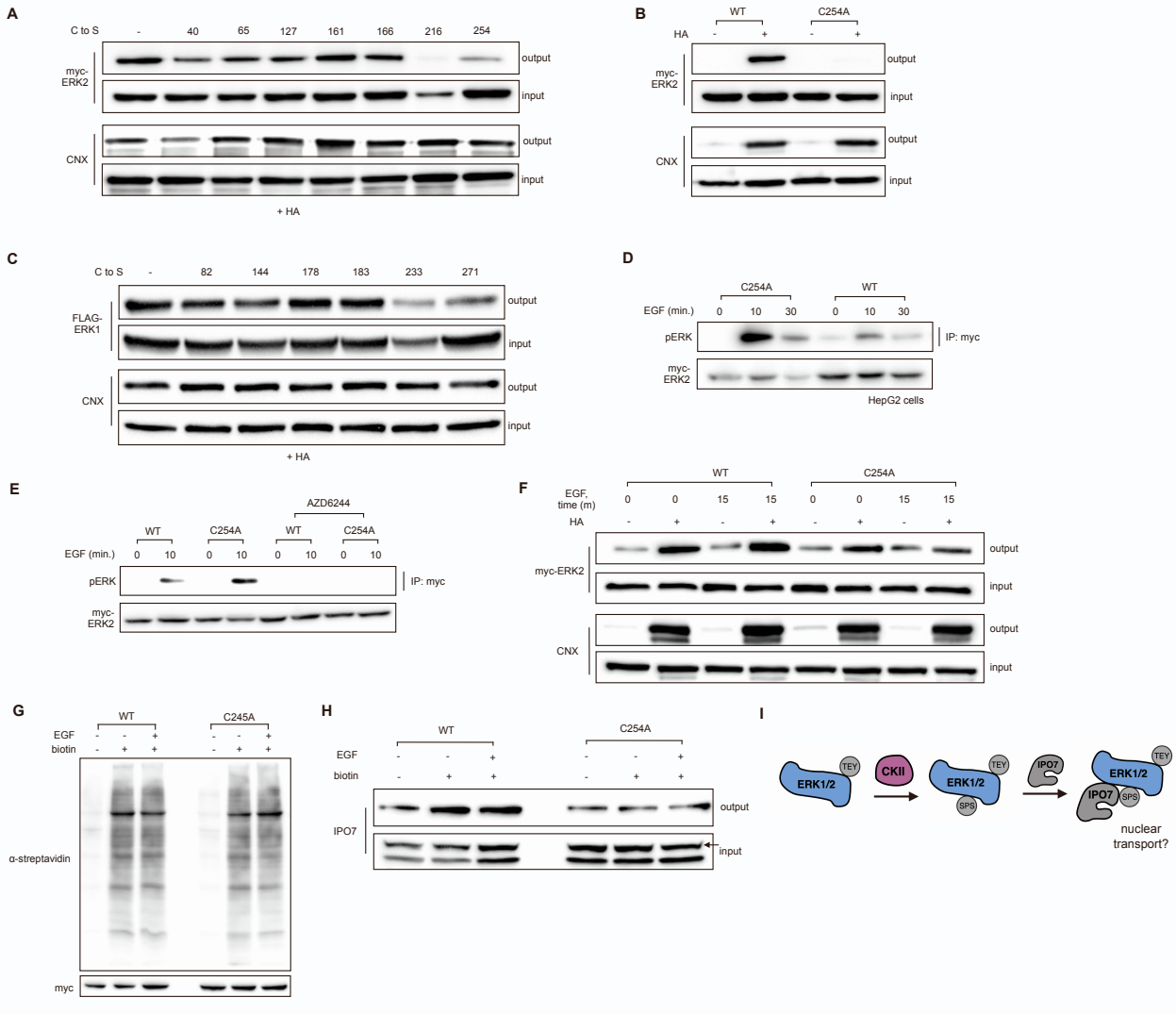


Figure S3. Identification of ERK1/2 acylation sites to probe for effects on regulation, related to Figure 3.

(A) ABE carried out on HEK293T cells overexpressing a panel of myc-ERK2 CΔS mutants, *n*=2.

(B) ABE of HEK293T cells expressing either WT or C254A myc-ERK2, *n*=2.

(C) ABE carried out on HEK293T cells overexpressing a panel of 3xFLAG-ERK1 CΔS mutants, *n*=2.

(D) Immunoprecipitation of WT or palmitoylation-deficient myc-ERK2 in HepG2 cells stimulated with EGF (0, 10, and 30 minutes, 1 ng mL^{-1}), *n*=2.

(E) Immunoprecipitation of WT or palmitoylation-deficient myc-ERK2 in HEK293T cells stimulated with EGF (0, and 10 minutes, 1 ng mL^{-1}), with or without MEK inhibitor AZD6244, *n*=2.

(F) ABE assay carried out in HEK293T cells overexpressing either WT or C254A myc-ERK2 following stimulation with EGF (1 ng mL^{-1}) for 0 and 15 minutes, *n=2.*

(G) Validation of TurboID-tagged ERK2 constructs, with α-streptavidin staining indicating the activity of the biotin ligase and α-myc blotting indicating the expression levels of the constructs*,* *n*=3.

(H) Overexpression of myc-ERK2 tagged with TurboID in HEK293T cells, followed by biotin incubation and streptavidin enrichment of labeled proteins. Enriched proteins were visualized via Western blotting for importin 7 (IPO7), *n=3.*

(I) Schematic of ERK2 nuclear transport as mediated by CK2 serine phosphorylation and subsequent importin 7 (IPO7) binding.

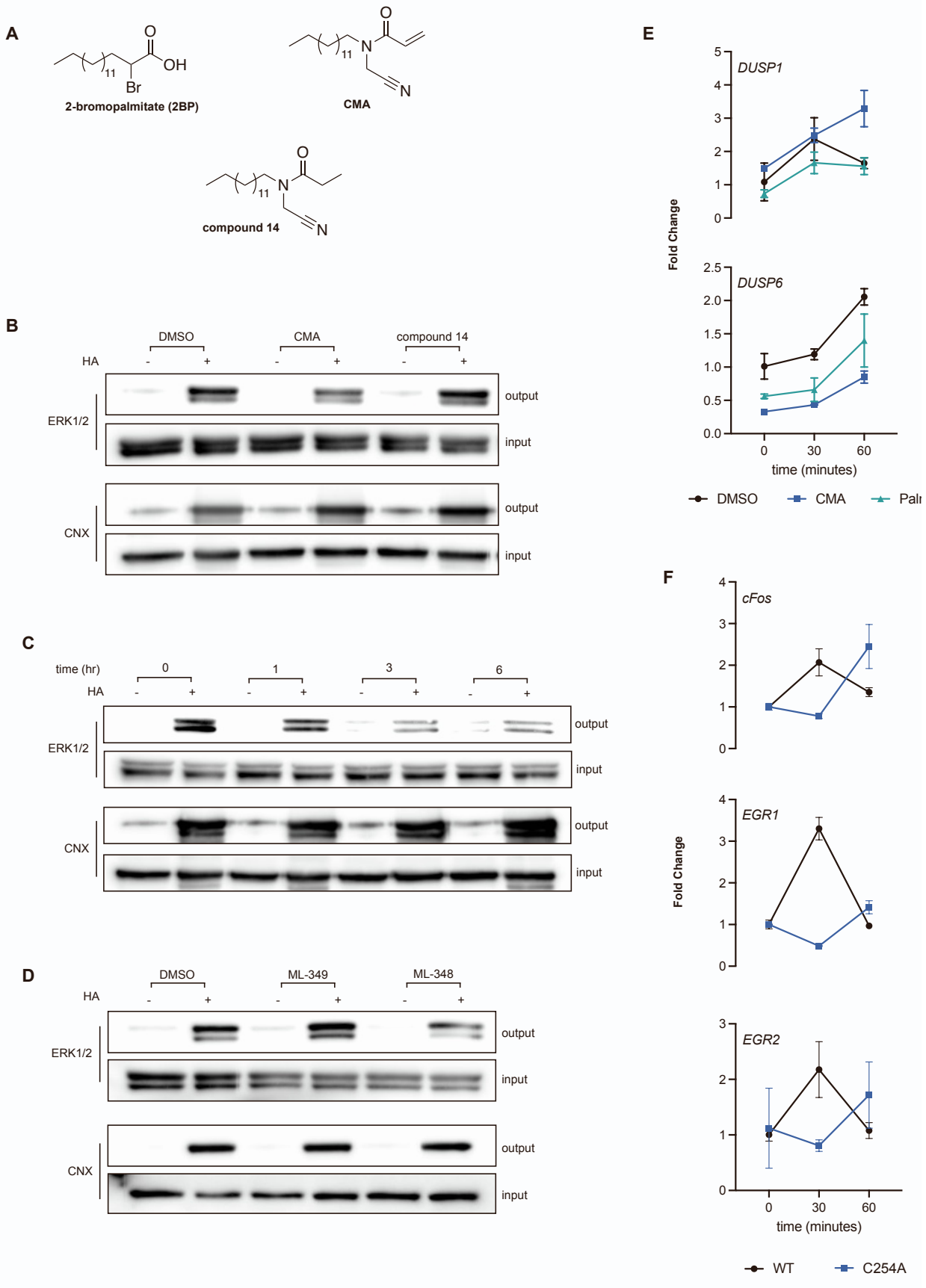
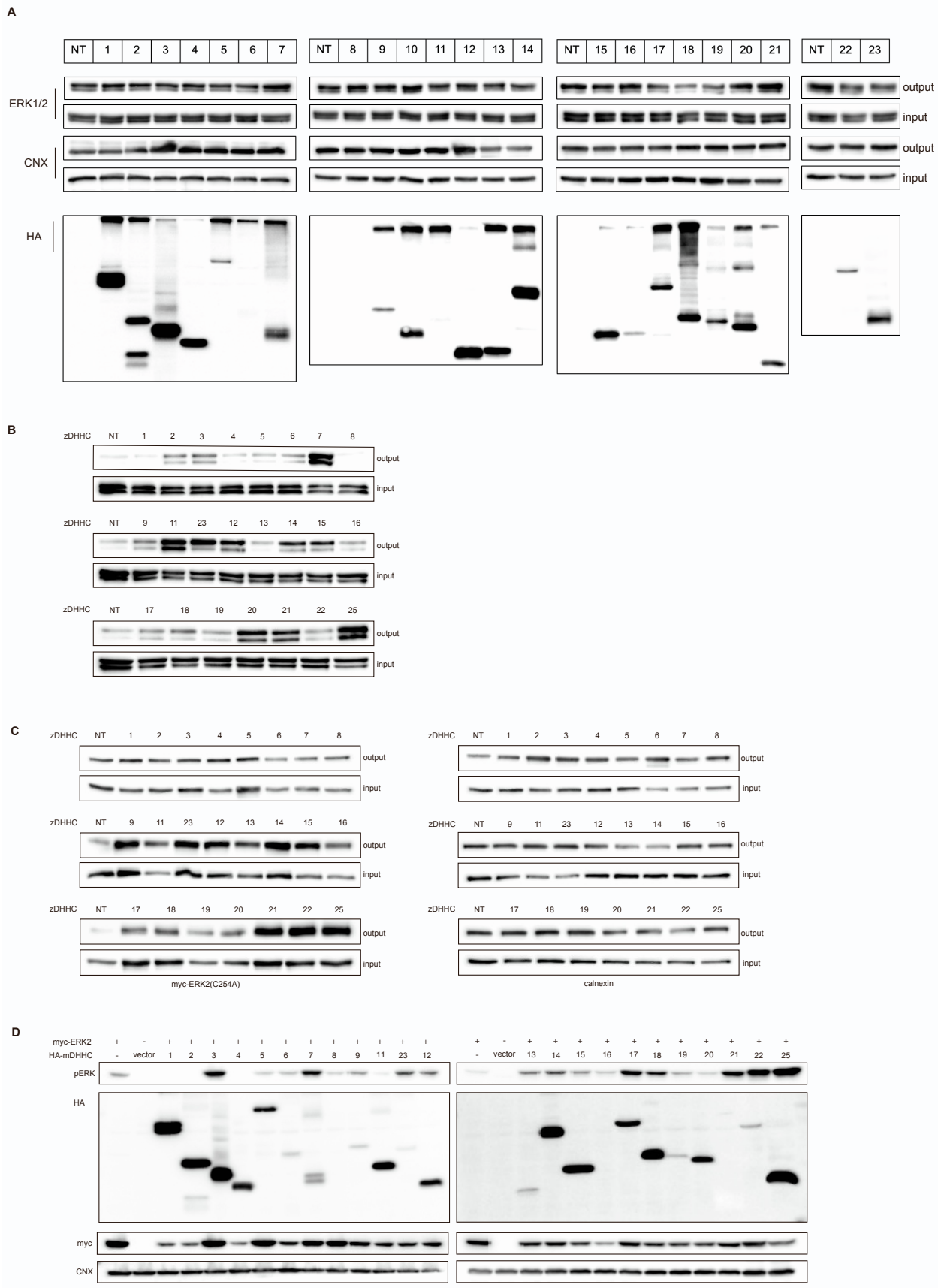

A
2-bromopalmitate (2BP)
CMA
compound 14
B
DMSO
CMA
compound 14
HA
ERK1/2
CNX
output
input
C
time (hr)
0
1
3
6
D
DMSO
ML-349
ML-348
E
DUSP1
DUSP6
Fold Change
time (minutes)
DMSO
CMA
F
cFos
EGR1
EGR2
WT
C254A

Figure S4. **Chemical inhibition of ERK1/2 *S*-acylation, related to Figure 4.**
(A) Structures of small DHHC family inhibitors and controls used in this work.
(B) ABE of HepG2 cells treated with DMSO, CMA (20 μM, 3 hours), or 14 (20 μM, 3 hours), an inactive analogue of CMA, *n*=2.
(C) Timecourse ABE (0, 1, 3, and 6 hours) of HepG2 cells treated with CMA (20 μM), *n*=2.
(D) ABE of HepG2 cells treated with DMSO, ML-348 (20 μM, 3 hours), or ML-349 (20 μM, 3 hours), *n*=2.
(E) RT-qPCR of key transcripts in the EGF-stimulated transcriptional program in HepG2 cells treated with DMSO, CMA (20 μM), or PalmB (20 μM) for three hours, followed by stimulation with EGF (1 ng mL^{-1}) for 0, 30, and 60 minutes, *n=3.*
(F) RT-qPCR of key transcripts in the EGF-stimulated transcriptional program in HepG2 cells overexpressing either WT or C254A myc-ERK2 following stimulation with EGF (1 ng mL^{-1}) for 0, 30, and 60 minutes, *n=3.*


A
NT 1 2 3 4 5 6 7
NT 8 9 10 11 12 13 14
NT 15 16 17 18 19 20 21
NT 22 23
ERK1/2
CNX
HA
output
input
output
input
B
zDHHC NT 1 2 3 4 5 6 7 8
output
input
zDHHC NT 9 11 23 12 13 14 15 16
output
input
zDHHC NT 17 18 19 20 21 22 25
output
input
C
zDHHC NT 1 2 3 4 5 6 7 8
output
input
zDHHC NT 9 11 23 12 13 14 15 16
output
input
zDHHC NT 17 18 19 20 21 22 25
output
input
myc-ERK2(C254A)
calnexin
D
myc-ERK2
HA-mDHHC
- vector 1 2 3 4 5 6 7 8 9 11 23 12
- vector 13 14 15 16 17 18 19 20 21 22 25
pERK
HA
myc
CNX

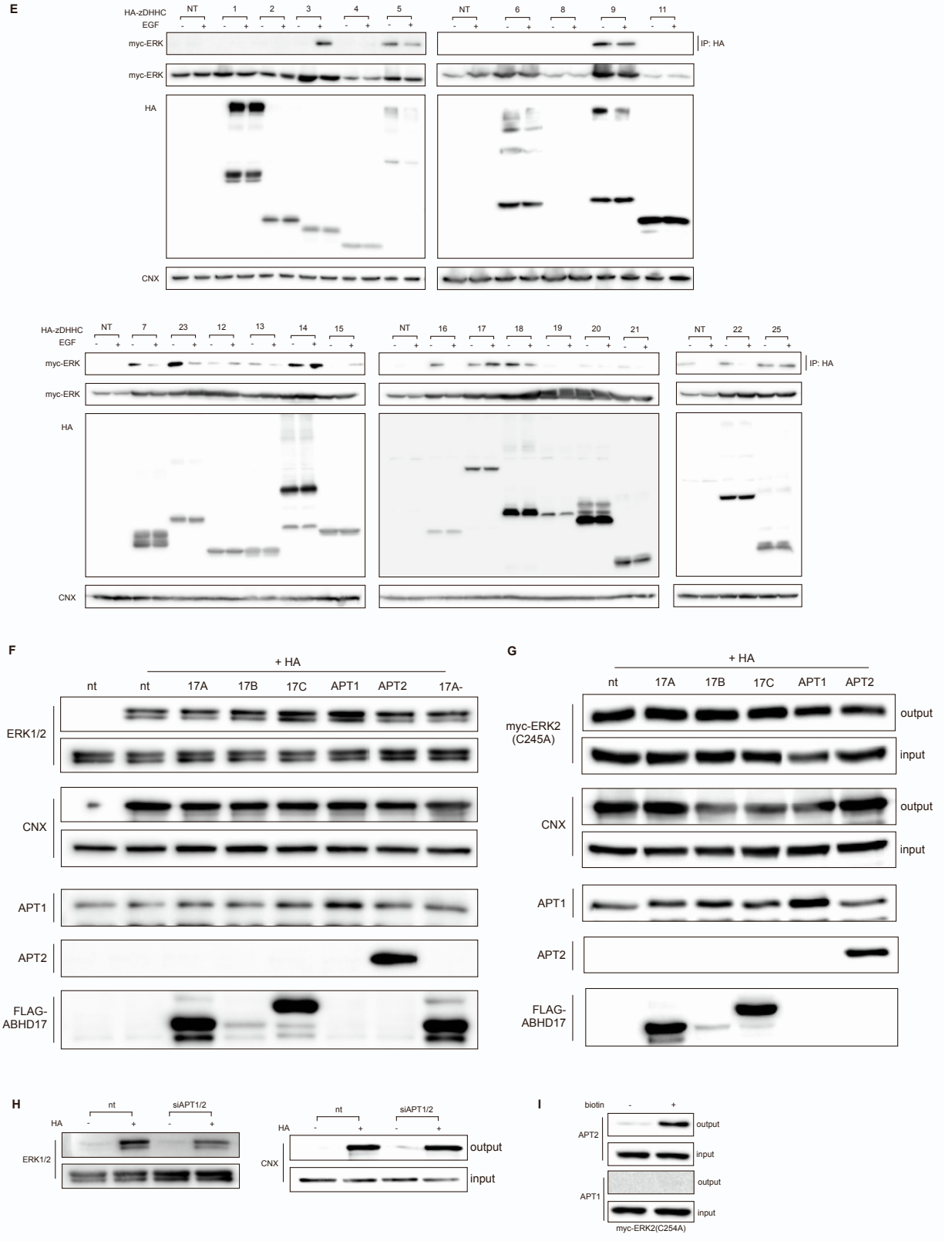

E
HA-zDHHC
EGF
NT
1
2
3
4
5
6
8
9
11
myc-ERK
IP: HA
HA
CNX
7
23
12
13
14
15
16
17
18
19
20
21
22
25
F
+ HA
nt
17A
17B
17C
APT1
APT2
17A-
ERK1/2
FLAG-ABHD17
G
myc-ERK2 (C245A)
output
input
H
siAPT1/2
I
biotin
myc-ERK2(C254A)

Figure S5. **Identification of the writer and eraser enzymes mediating ERK2 *S*-acylation, related to Figure 5.**
(A) ABE analysis of HEK293T cells overexpressing a panel of murine DHHC family proteins, *n*=3.
(B) ERK1/2 metabolic labeling with 17-ODYA in HEK293T cells overexpressing murine DHHC family proteins, *n=2.*
(C) Myc-ERK2(C254A) metabolic labeling with 17-ODYA in HEK293T cells overexpressing murine DHHC family proteins, *n=2.*
(D) Analysis of phospho-ERK(Thr185/Tyr187) in HEK293T cells overexpressing a panel of murine DHHC proteins, following EGF stimulation (10 minutes, 1 ng mL^{-1}), *n*=2.
(E) Immunoprecipitation using an anti-HA antibody in HEK293T cells expressing the indicated HA-tagged DHHC protein with or without EGF stimulation (10 minutes, 1 ng mL^{-1}). Co-immunoprecipitated proteins were visualized via Western blotting for myc-ERK2, *n*=3.
(F) ABE analysis of HEK293T cells overexpressing a panel of known APT enzymes, *n*=2.
(G) ABE analysis of HEK293T cells overexpressing myc-ERK2(C254A) alongside a panel.
(H) ABE in HEK293T cells following siRNA-mediated knockdown of APT1 and APT2, *n*=2.
(I) Overexpression of myc-ERK2(C254A) tagged with TurboID in HEK293T cells, followed by biotin incubation and streptavidin enrichment of labeled proteins. Enriched proteins were visualized via Western blotting for APT2, *n=2.*

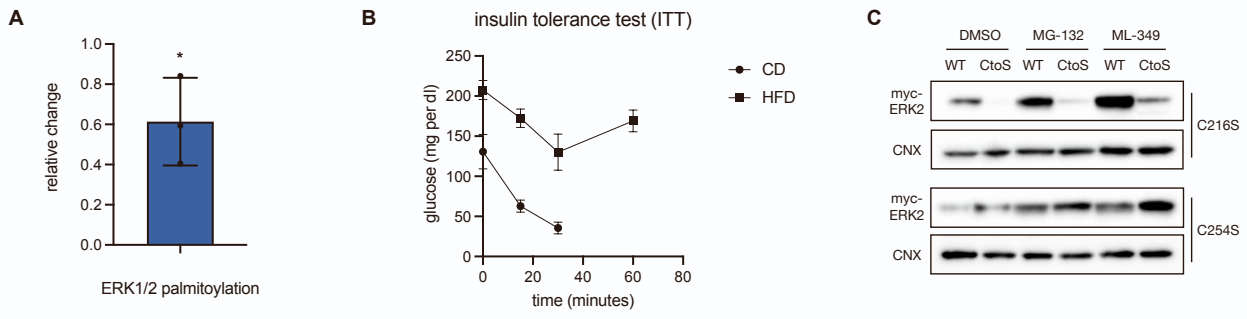


Figure S6. **Characterization of DIO in C57BL/6J mice and characterization of ERK2 expression *in cellulo*, related to Figure 6.**

(A) Quantification of 6A, showing the showing the relative fold change in ERK1/2 *S*-acylation after palmitate bolus. Statistical analysis performed with a two-tailed student's t-test with equal variance, $p < 0.05$.

(B) Insulin tolerance test (ITT) for mice fed either a control diet (CD) or a high-fat diet (HFD). *mean ± std, n=5*.

(C) Overexpression of WT and C254S or C216S ERK2 in HEK293T cells treated with either the proteasome inhibitor MG-132 or the APT2 inhibitor ML-349. CNX was used as a loading control, *n=2.*