## SUPPLEMENTAL INFORMATION

**Acetylation Targets HSD17B4 for Degradation via the CMA Pathway in Response to Estrone**

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**Inventory of Supplemental Information**

**Figure S1: Linked to Figure 1**

**Figure S2: Linked to Figure 2**

**Figure S3: Linked to Figure 3**

**Figure S4: Linked to Figure 4**

**Figure S5: Linked to Figure 5**

**Figure S6: Linked to Figure 6**

**Figure S7: Linked to Figure 7**

A

B

D

C

C:\Users\Administrator\Desktop\Data 1.tif

**K669Ac antibody**

**+**

**+**

**+**

**-**

**+**

**-**

**-**

**-**

**+**

**-**

**-**

**-**

**HSD17B4 antibody**

**Acetyl-peptide**

**-**

**-**

**+**

**-**

**Unmodified peptide**

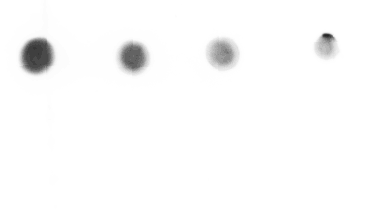
**70 kDa**



**K669Ac**

**ACTB**

**40 kDa**



**Acetyl-K669 peptide**

**200 ng**

**100 ng**

**50 ng**

**20 ng**

**Unmodified peptide**

F

E

**NAM**

**MG132**



**HSD17B4**

**70 kDa**

**HSD17B4:ACTB ratio**



**40 kDa**

**1.0**

**0.5**

**+**

**+**

**-**

**+**

**-**

**+**

**-**

**1.0**

**0.5**

**ACTB**

**-**

**CTNNB1**



**70 kDa**

**NAM**

**HSD17B4**

**70 kDa**

**+**

**+**

**-**

**-**

**HSD17B4: ACTB ratio**

**+**

**+**

**-**

**-**

**ACTB**

**1.0**

**0.9**

**0.4**

**0.9**



**40 kDa**



**BAF**

|  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- |
| **Position** | **Gene Names** | **Mascot Score** | **PTM Score** | **Modified Sequence** | **m/z** | **Mass Error [ppm]** |
| **46** | **HSD17B4; DBP; MFE-2** | **93.8** | **167.3** | **\_DLGGDFK(ac)GVG\_** | **596.33** | **0.33** |
| **139** | **HSD17B4; DBP; MFE-2** | **27.47** | **78.3** | **\_AAWEHMK(ac)K\_** | **521.7** | **-0.21** |
| **565** | **HSD17B4; DBP; MFE-2** | **56.77** | **139.73** | **\_FAK(ac)PVYPGQT\_** | **1033.5** | **-0.12** |
| **669** | **HSD17B4; DBP; MFE-2** | **45.45** | **97.77** | **\_WTIDLK(ac)SGSGK\_** | **617.32** | **-0.89** |
| **702** | **HSD17B4; DBP; MFE-2** | **30.68** | **126.79** | **\_LDPQK(ac)AFFSGR\_** | **654.34** | **-0.14** |

**Figure S1.** HSD17B4 is acetylated at lysine 669 (K669) to promote its degradation via autophagy.(**A**) Identification of acetylated HSD17B4 peptide by mass spectrometry. (**B**) The specificity of the K669 site-specific acetylation antibody was determined by dot blotting assay. (**C**) The K669Ac antibody is specific to HSD17B4 K669 acetylation. The 4 replicate lanes were clipped into four membranes and were exposed to antibodies as indicated, separately. For peptide block, acetylated K669 peptide or nonacetylated K669 peptide were added to the diluted K669Ac antibodies, cultivated at 37℃ for 1 h before used for western blotting. (**D**) NAM treatment decreases HSD17B4 expression at the post-transcriptional level. *HSD17B4* mRNA was determined by qPCR and normalized against *ACTB*. Error bars represent ± SD of triplicate experiments. The two-tailed Student t test was used. NS, no significance. (**E**) Bafilomycin A1 (BAF) inhibits NAM-induced HSD17B4 degradation. HEK293T cells were treated as indicated and HSD17B4 protein was detected by western blotting. (**F**) HSD17B4 is not degraded by the ubiquitin proteasome system (UPS). HEK293T cells were treated as indicated and HSD17B4 protein level was analyzed by western blotting.

**Wortmannin**

**MG132**

**HSD17B4**

**NH4Cl**

**MCF7**

**MDA-MB-231**

**HSD17B4:ACTB ratio**

**-**

**+**

**+**

**-**

**+**

**-**

**-**

**-**

**-**

**-**

**-**

**-**

**-**

**+**

**+**

**-**

**+**

**-**

**-**

**-**

**-**

**-**

**-**

**-**

**ACTB**

**70 kDa**

**40 kDa**



**1.0**

**1.1**

**1.0**

**1.9**

**1.0**

**0.9**

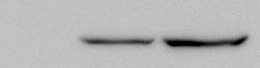
**1.1**

**1.7**

A

C

B



**Flag-HSD17B4**

**HA-HSPA8**

**NAM**

**HA-HSPA8**

**FLAG-HSD17B4**

**IP: FLAG**

**Input**

**HA-HSPA8**

**FLAG-HSD17B4**



**70 kDa**

**70 kDa**

**70 kDa**

**70 kDa**

**-**

**+**

**-**

**+**

**+**

**+**

**+**

**-**

**+**

**70 kDa**

**100 kDa**

**40 kDa**

**sh*LAMP2A***

**-**

**+**

**HSD17B4**



**ACTB**

**LAMP2A**

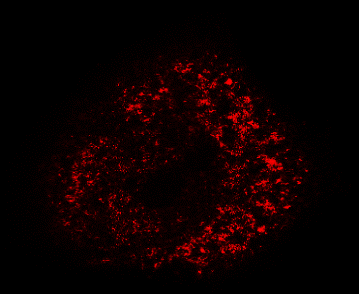
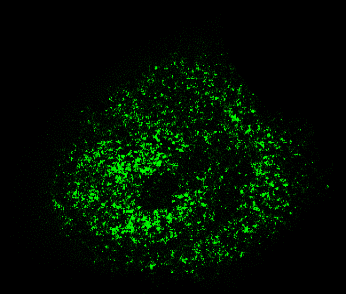
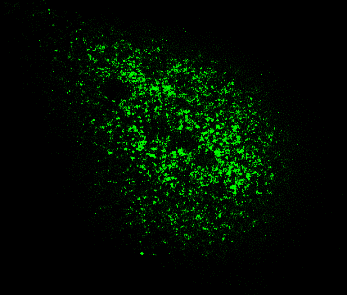
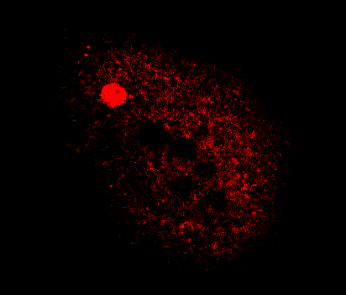
**Figure S2.** HSD17B4 degradation induced by acetylation requires CMA. (**A**) NH4Cl exposure accumulates HSD17B4 protein, while MG132 or wortmannin treatment does not. MCF7 or MDA-MB-231 cells were treated as indicated. The levels of total HSD17B4 protein were determined by western blotting. (**B**) NAM promotes HSD17B4 binding with HSPA8 in HEK293T cells. Ectopically expressed HSD17B4 was cotransfected with HSPA8 into HEK293T cells followed by NAM treatment, the binding between HSD17B4 and HSPA8 was analyzed by western blotting. (**C**) *LAMP2A* knockdown accumulates HSD17B4 protein. *LAMP2A* was stably knocked down in HEK293 cells by shRNA. HSD17B4 and LAMP2A protein levels were determined by western blotting.

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**Figure S3.** E1 increases HSD17B4 acetylation at K669 to promote its degradation via CMA.E1 treatment decreases HSD17B4 at the post-transcriptional level. *HSD17B4* mRNA was determined by qPCR and normalized against *ACTB*. Error bars represent ±SD of triplicate experiments. The two-tailed Student t test was used. NS, no significance.

**Normal condition**

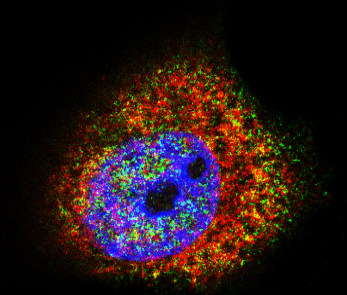
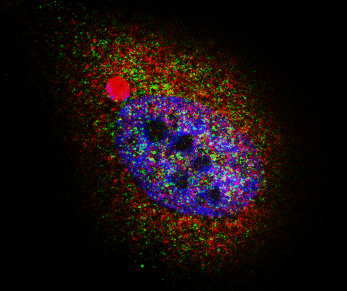
**E1 treatment**



**LAMP2A**

**HSPA8**

**Merge**



A





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B

**Figure S4.** HSD17B4 is a substrate of CMA.(**A and B**) E1 treatment activates CMA. MCF7 cells were cultured with or without E1 for 24 h as indicated, then paraformaldehyde fixed, blocked, and processed for double immunofluorescence with antibodies against LAMP2A (green) and HSPA8 (red). Merged images of both channels are shown on the right. Bar: 5 μm (**A**). Relative LAMP2A colocalization with HSPA8 was calculated by ImageJ software, the ratio was quantified. Mean values were calculated from the individual distributions in 10 cells per condition (**B**).

**FLAG-CREBBP**

**K669Ac:HSD17B4 ratio**

**HSD17B4**

**HSD17B4**

**HSD17B4:ACTB ratio**

**ACTB**

**FLAG-CREBBP**

**1.0**

**1.7**

**1.5**

**2.0**

**+**

**+**

**+**

**-**

**-**

**+**

**-**

**-**

**1.0**

**1.5**

**1.2**

**1.9**

**+**

**+**

**+**

**-**

**-**

**+**

**-**

**-**

**1.0**

**1.3**

**0.4**

**1.9**

**1.0**

**1.9**

**0.6**

**1.4**

**MCF7**

**MDA-MB-231**

**K669Ac**

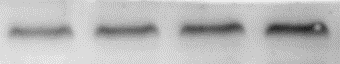
**70 kDa**

**70 kDa**

**70 kDa**

**40 kDa**

**300 kDa**

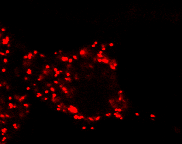
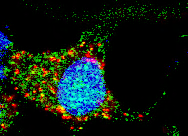


**BAF**

**Figure S5.** CREBBP acetylates HSD17B4 at K669.BAF blocks the reduction of HSD17B4 protein induced by CREBBP overexpression. FLAG-CREBBP was transfected into MCF7 and MDA-MB-231 cells with or without BAF treatment and cell lysates were analyzed by western blotting.



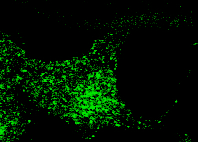
A



**SIRT3**

**HSD17B4**

**Merge**





B

**40 kDa**

**70 kDa**

**NH4Cl**

**SIRT3**

**HSD17B4 antibody**

**IP:HSD17B4**

**SIRT3:HSD17B4 ratio**

**CLQ**



**HSD17B4**

**Input:**



**HSD17B4**

**70 kDa**

**+**

**+**

**+**

**-**

**+**

**-**

**+**

**-**

**-**

**-**

**-**

**-**

**1.0**

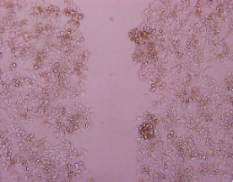
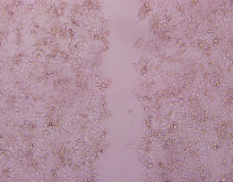
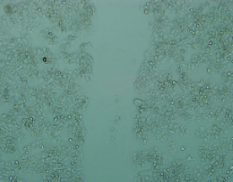
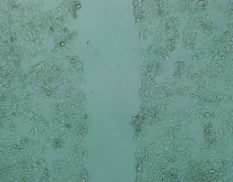
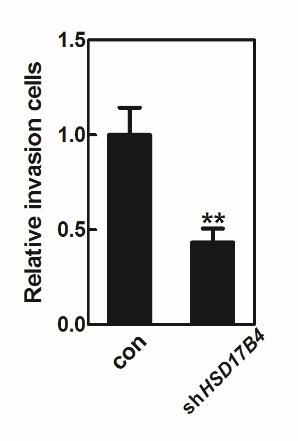
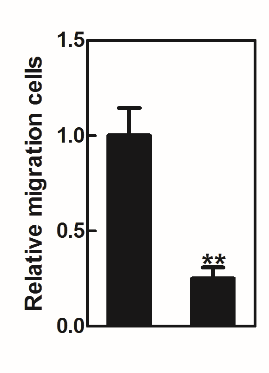
**1.00**

**1.1**

**Figure S6.** SIRT3 deacetylates HSD17B4 at K669.(**A**) SIRT3 colocalizes with HSD17B4. Cultured MCF7 cells were paraformaldehyde fixed, blocked, and processed for double immunofluorescence with antibodies against SIRT3 (green) and HSD17B4 (red). Merged images of both channels are shown on the right. Bar: 5 μm. (**B**) Neither CLQ nor NH4Cl affect the interaction between endogenous HSD17B4 and SIRT3 in MCF7 cells. MCF7 cells were cultured with or without CLQ or NH4Cl for 24 h before harvest. The interaction between endogenous HSD17B4 and SIRT3 was determined by coIP and western blotting.

A

B



**pMKO**

**sh*HSD17B4***

**0 h**

**24 h**

**200 μm**

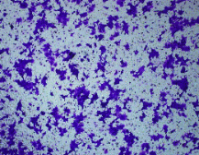
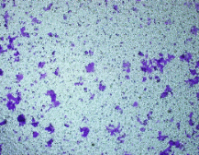
**200 μm**

**200 μm**

**200 μm**

**pMKO**

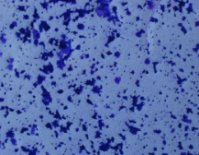
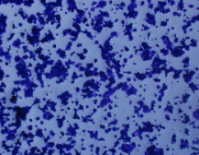
**sh*HSD17B4***



C

**pMKO**

**sh*HSD17B4***



**Figure S7.** K669 mutants increase cell migration and invasion.(**A**) Knockdown of *HSD17B4* inhibits migration of MCF7 cells. MCF7 stable cells treated as indicated were analyzed for migration by a wound-healing assay. Scale bars: 200 μm. (**B**) Knockdown of *HSD17B4* inhibits migration of MCF7 cells. MCF7 stable cells treated as indicated were analyzed by migration assays in 24-well chambers without matrigel. (**C**) Knockdown of *HSD17B4* inhibits invasion of MCF7 cells. MCF7 stable cells treated as indicated were analyzed by invasion assays in 24-well invasion matrigel chambers.