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**Supplementary Figure 1. The mutation of AEG-1 palmitoylation site does not affect lymphocytes, neutrophils and**

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**macrophages in mice under normal feeding conditions. (A) The expression levels of AEG-1 in different immune cells were**

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**analyzed using the single-cell sequencing database TISCH. (B) Different immune cells gating strategy in mouse liver. (C)**

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**Absolute number of WBC in mouse livers from WT and AEG-1<sup>C75A</sup> were quantified by Attune NxT volumetric flow cytometer.**

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**(D, E) Comparison of different immune cell frequencies in WBC and absolute number in the livers of WT and AEG-1<sup>C75A</sup> mice.**

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**(F) Macrophage gating strategy in mouse liver. (G) Comparison of macrophage frequency in WBC and absolute number in the**

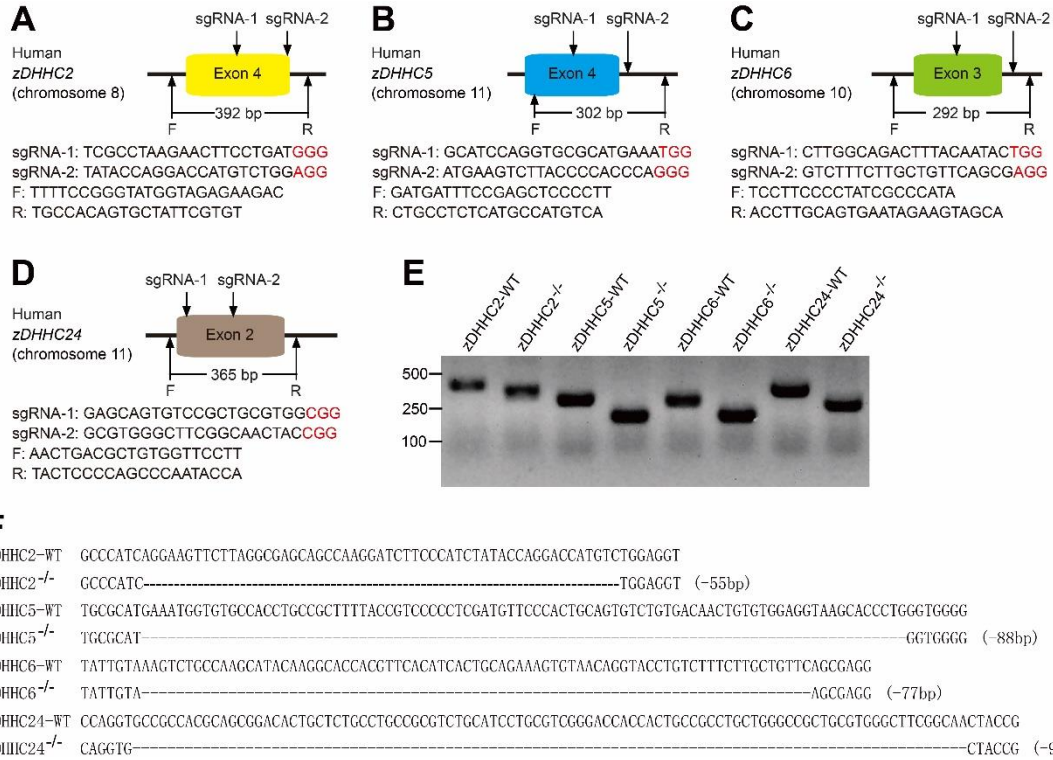
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**livers of WT and AEG-1<sup>C75A</sup> mice (n=8). The data are presented as the mean ± SEM. \*p < 0.05, ns: no significance. Statistical**

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**significance was determined by unpaired two-tailed Student's t-test for comparison between two groups.**

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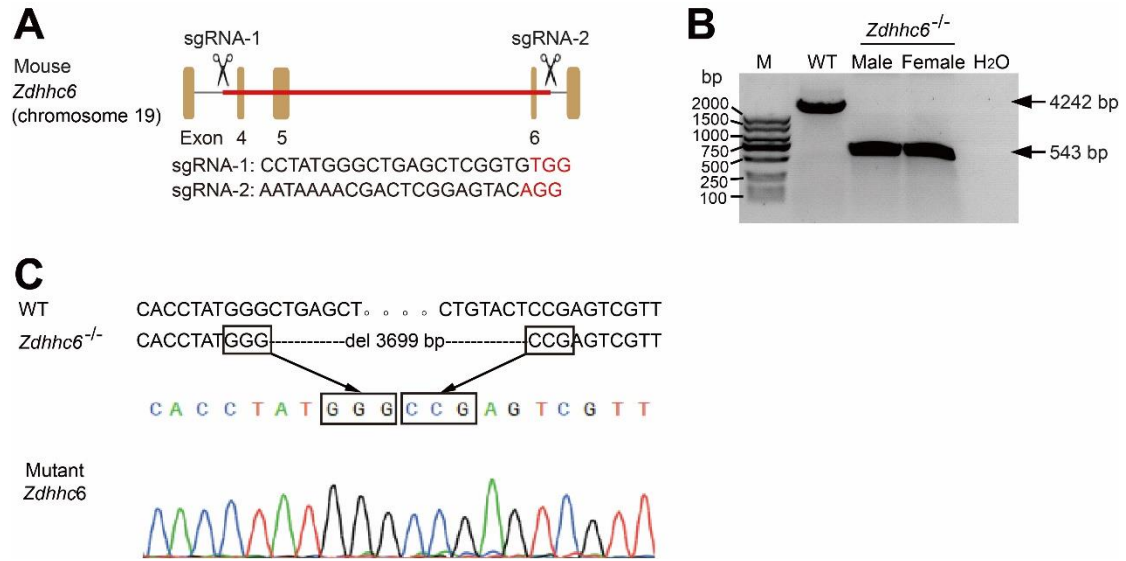
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**Supplementary Figure 2. Generation of HEK293T-*zDHHCs* knockout cell lines. (A-D)** Schematic diagram of the small guide RNA (sgRNA) sequences used to target exons of four *zDHHCs* gene, respectively. Protospacer adjacent motifs (PAMs) are shown in red letters, and PCR detection primers are listed below the sgRNA sequences. **(E, F)** Validation of HEK293T-*zDHHCs* knockout cell lines by agarose gel electrophoresis **(E)** and sequencing **(F)**.



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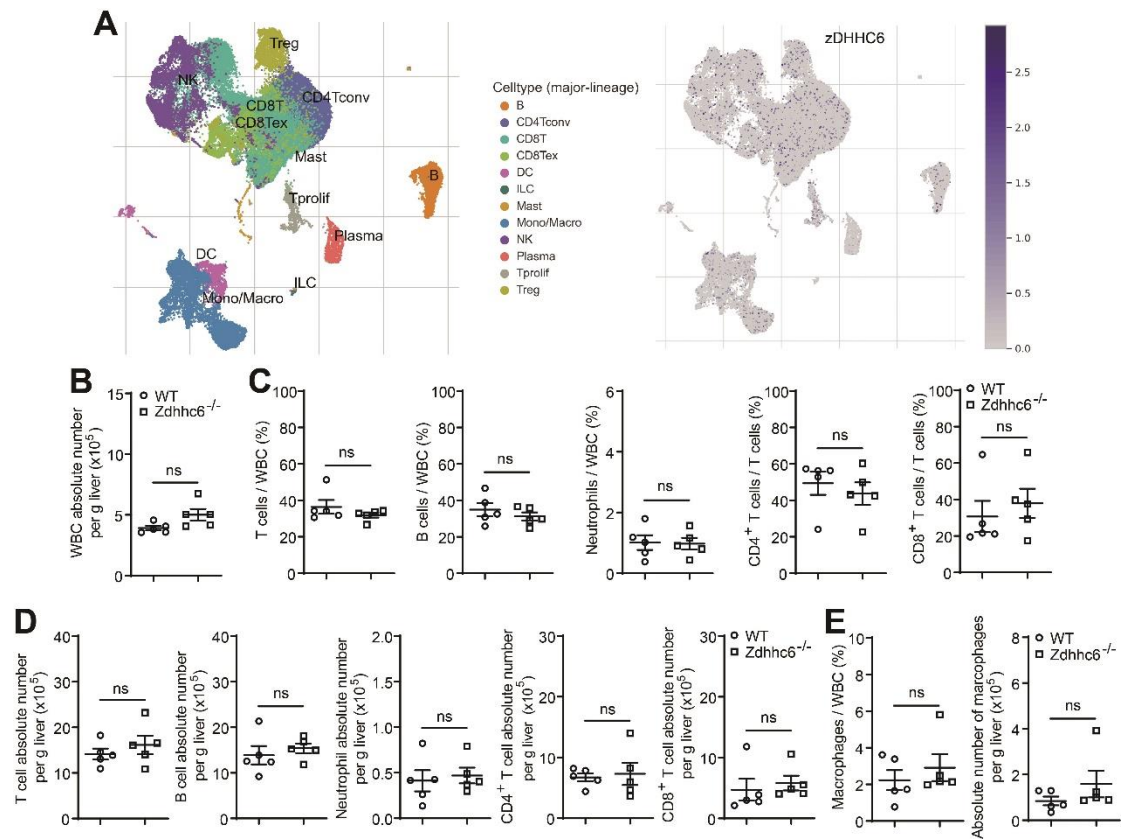
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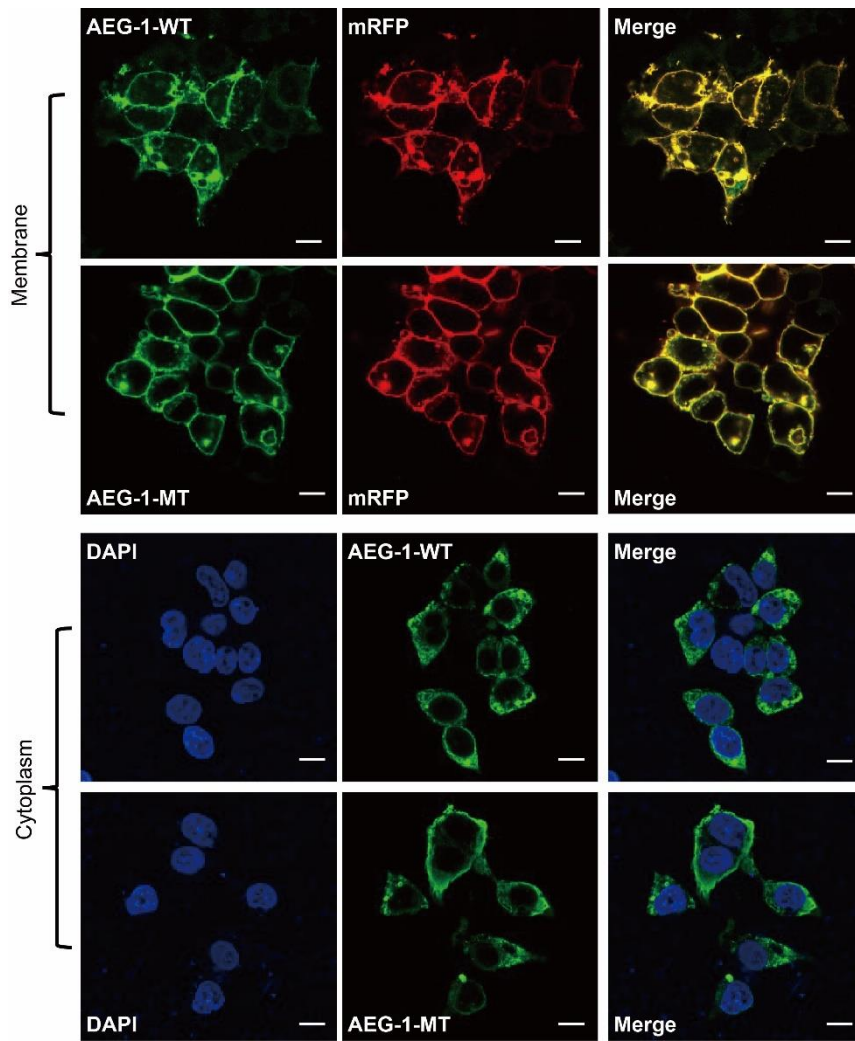
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**Supplementary Figure 3. Generation of *Zdhhc6*<sup>-/-</sup> mice. (A)** Schematic diagram of two sgRNA sequences used to target intron 1 and 4 of *Zdhhc6* gene. PAMs are shown in red letters. **(B, C)** Validation of *Zdhhc6*<sup>-/-</sup> mouse by agarose gel electrophoresis **(B)** and sequencing **(C)**.



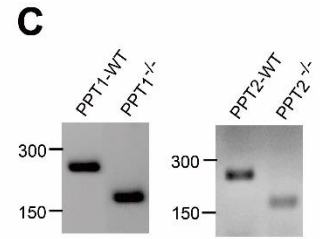
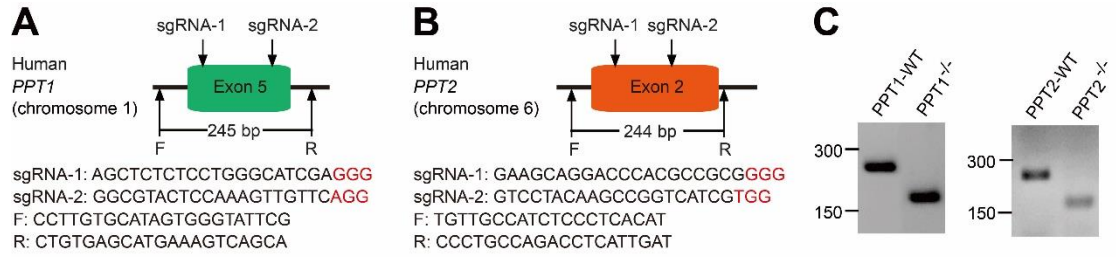
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**Supplementary Figure 4. Knockout of *Zdhhc6* does not affect lymphocytes, neutrophils and macrophages in mice under normal feeding conditions.** (A) The expression levels of *Zdhhc6* in different immune cells were analyzed using the single-cell sequencing database TISCH. (B) Absolute number of WBC in mouse livers from WT and *Zdhhc6*<sup>-/-</sup> were quantified by Attune NxT volumetric flow cytometer. (C, D) Comparison of different immune cell frequencies in WBC and absolute number in the livers of WT and *Zdhhc6*<sup>-/-</sup> mice. (E) Comparison of macrophage frequency in WBC and absolute number in the livers of WT and *Zdhhc6*<sup>-/-</sup> mice (n=5). The data are presented as the mean  $\pm$  SEM. Ns: no significance. Statistical significance was determined by unpaired two-tailed Student's t-test for comparison between two groups.



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**Supplementary Figure 5. Loss of palmitoylation does not affect the subcellular localization of AEG-1.** AEG-1-WT and AEG-1-MT were overexpressed and immunostained for the detection of the subcellular localization of AEG-1-WT and AEG-1-MT in Huh7 cells. mRFP as a membrane marker. Nuclei were stained with DAPI.



**D**

PPT1-WT TCGATGCCAGGAGAGACTCTCACATCTGTGACTTCATCCGAAAACTGAATGCTGGGCGTACTCCAAAGTTGTTTCAGG  
 PPT1<sup>-/-</sup> TCGAT-----TCAGG (-73bp)  
 PPT2-WT CGGGCGTGGGTCTGCTTCTGTTGCCTTCTGCGCGTGTGCTTGCAGCCCCGCGCCACCGCGCTCTACAAGCCGGTCATCGT  
 PPT2<sup>-/-</sup> CGCG-----TCGT (-85bp)

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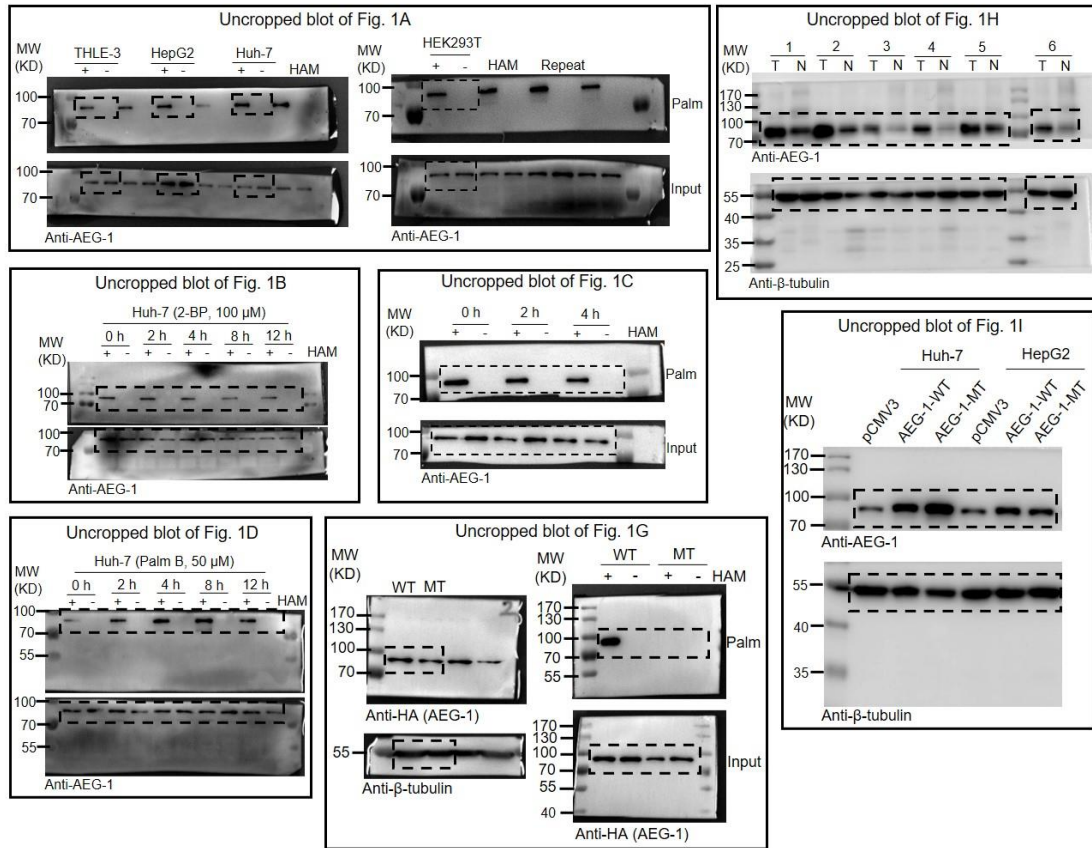
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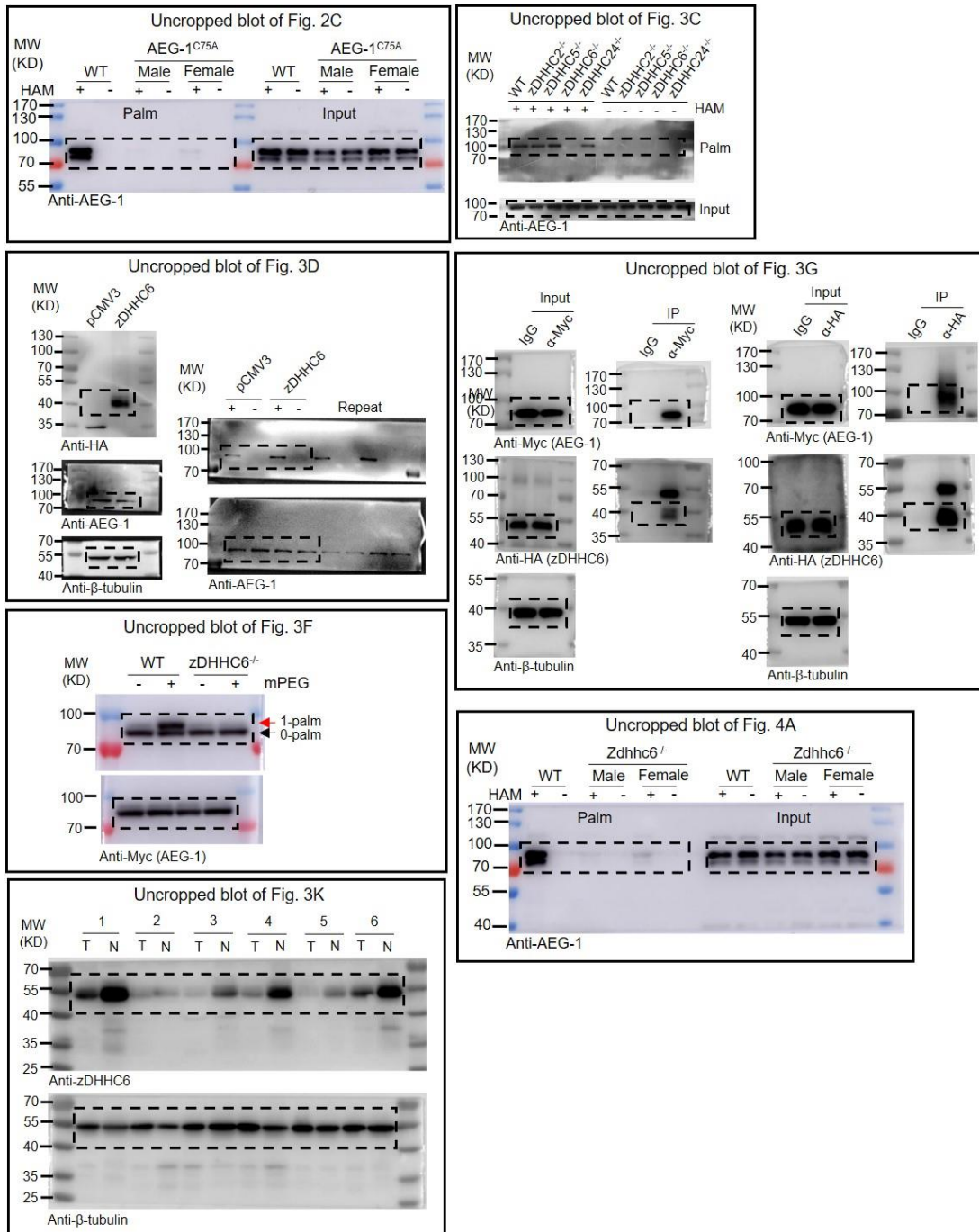
**Supplementary Figure 6. Generation of PPT1 and PPT2 knockout cell lines. (A, B)** Schematic representation of the sgRNA sequences used to target exons of PPT1 and PPT2 genes, respectively. PAMs are shown in red letters, and PCR detection primers are below the sgRNA sequences. **(C, D)** Validation of HEK293T-PPT1<sup>-/-</sup> and HEK293T-PPT2<sup>-/-</sup> cell lines by agarose gel electrophoresis **(C)** and sequencing **(D)**.



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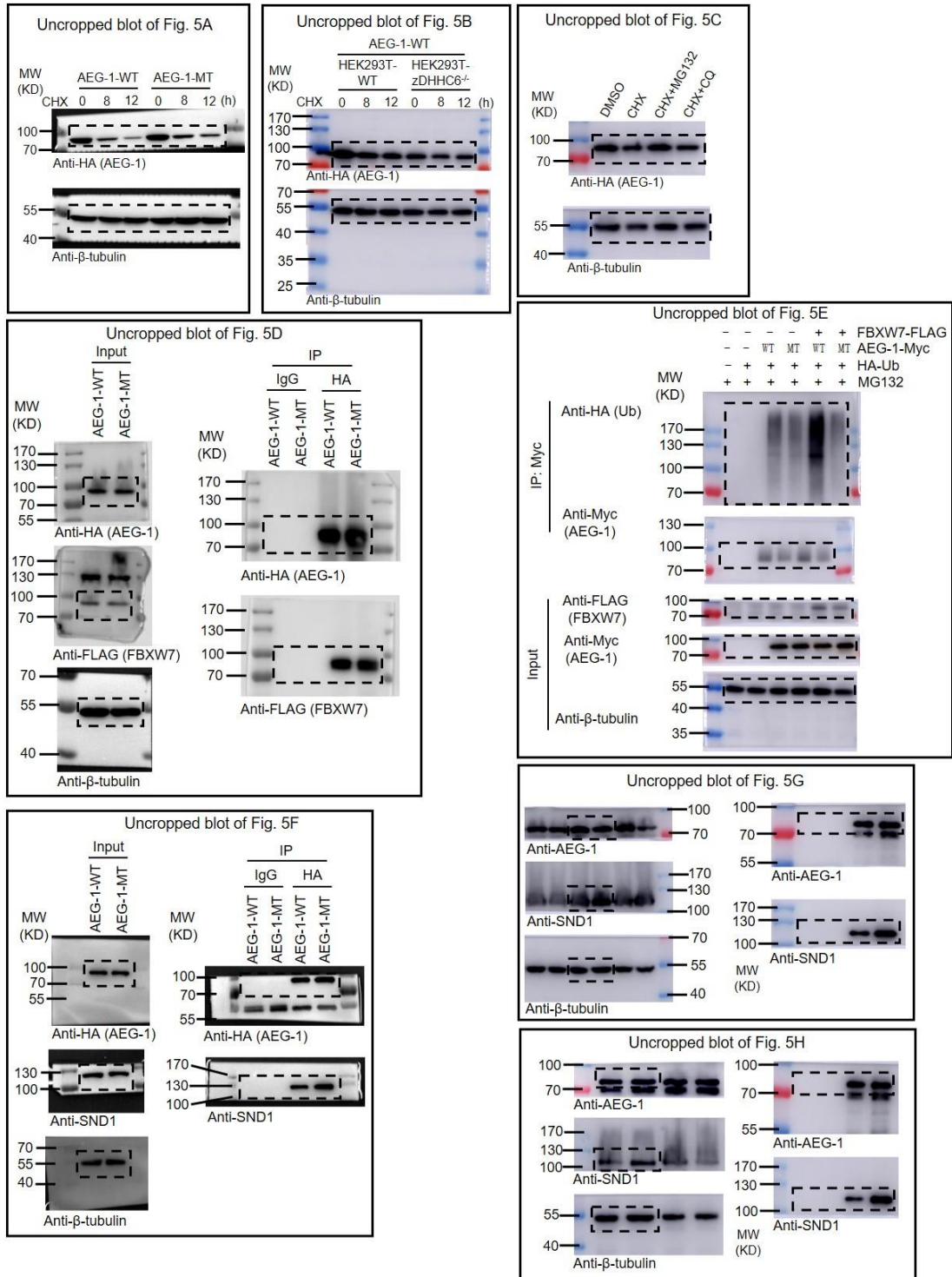
Supplementary Figure 7. Uncropped blot 1.



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Supplementary Figure 8. Uncropped blot 2.

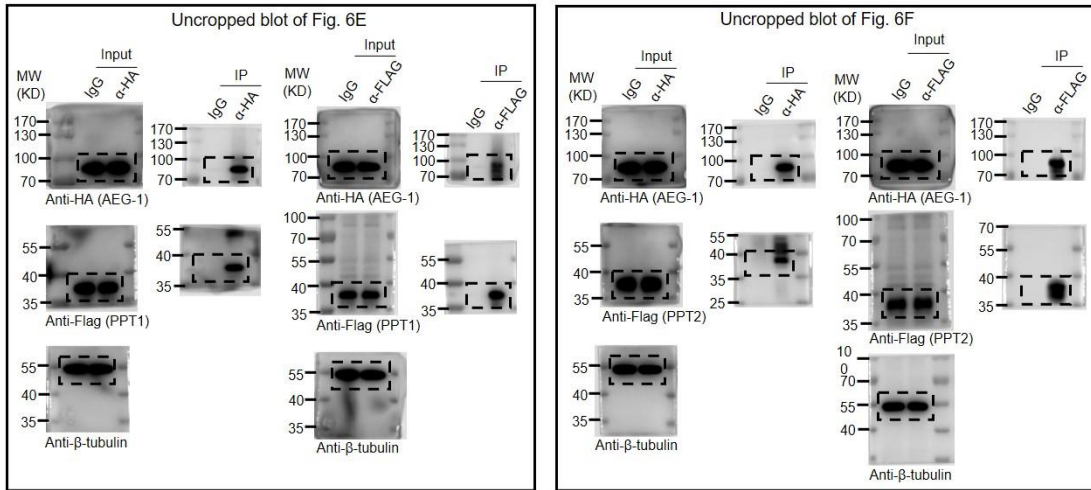
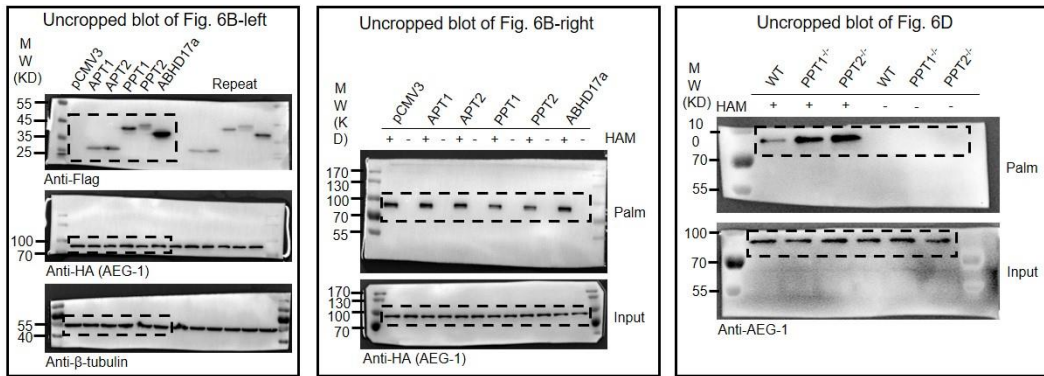




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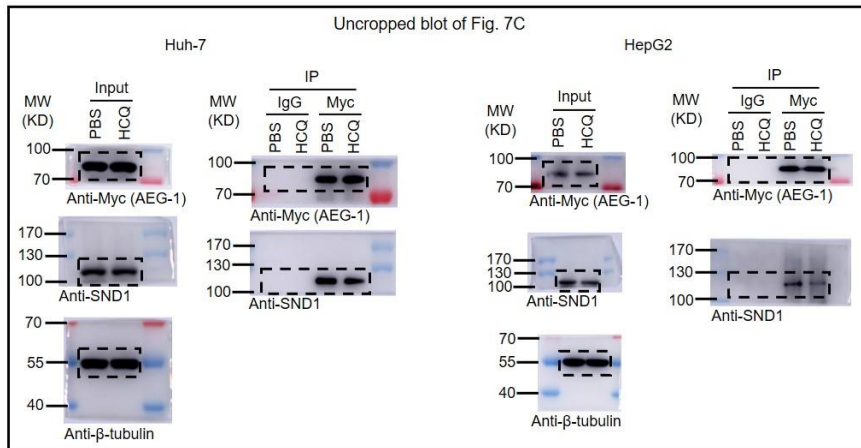
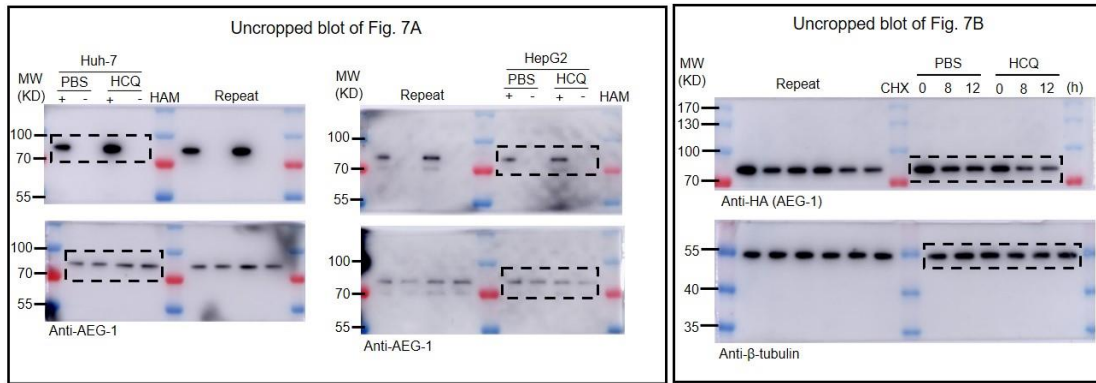
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Supplementary Figure 9. Uncropped blot 3.



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Supplementary Figure 10. Uncropped blot 4.



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Supplementary Figure 11. Uncropped blot 5.

801 **Supplementary Tables:**

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803 **Supplementary Table 1.** Primer pairs of gRNA used for generation of PPT1, PPT2, zDHHC2, zDHHC5, zDHHC6 and  
804 zDHHC24 knockout cell lines

Gene name (human)	Sequence (5' to 3')
PPT1 gRNA #1	CACCGAGCTCTCTCCTGGGCATCGA (F)
	AAACTCGATGCCCAGGAGAGAGCTC (R)
PPT1 gRNA #2	CACCGGCGTACTCCAAAGTTGTTC (F)
	AAACGAACAACCTTTGGAGTACGCC (R)
PPT2 gRNA #1	CACCGAAGCAGGACCCACGCCGCG (F)
	AAACCGCGGCGTGGGTCCTGCTTC (R)
PPT2 gRNA #2	CACCGTCCTACAAGCCGGTCATCG (F)
	AAACCGATGACCGGCTTGTAGGAC (R)
zDHHC2 gRNA #1	CACCGTCGCCTAAGAATTCCTGAT (F)
	AAACATCAGGAAGTTCTTAGGCGAC (R)
zDHHC2 gRNA #2	CACCGTATACCAGGACCATGTCTGG (F)
	AAACCCAGACATGGTCCTGGTATAC (R)
zDHHC5 gRNA #1	CACCGCATGGAGGTGCGCATGAAA (F)
	AAACTTTCATGCGCACCTGGATGC (R)
zDHHC5 gRNA #2	CACCGATGAAGTCTTACCCACCCA (F)
	AAACTGGGTGGGGTAAGACTTCATC (R)
zDHHC6 gRNA #1	CACCGCTTGGCAGACTTTACAATAC (F)
	AAACGTATTGTAAAGTCTGCCAAGC (R)
zDHHC6 gRNA #2	CACCGTCTTTCTTGCTGTTCAAGC (F)
	AAACCGCTGAACAGCAAGAAAGAC (R)
zDHHC24 gRNA #1	CACCGAGCAGTGTCGCTGCGTGG (F)
	AAACCCACGCAGCGGACACTGCTC (R)
zDHHC24 gRNA #2	CACCGCGTGGGCTTCGGCAACTAC (F)
	AAACGTAGTTGCCGAAGCCCACGC (R)

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**Supplementary Table 2.** Primer pairs used for PCR analysis

Gene name (human)	Sequence (5' to 3')	Usage
<i>PPT1-genome</i>	CCTTGTGCATAGTGGGTATTCG (F)	Genomic PCR detection for knockout cell lines
	CTGTGAGCATGAAAGTCAGCA (R)	
<i>PPT2-genome</i>	TGTTGCCATCTCCCTCACAT (F)	
	CCCTGCCAGACCTCATTGAT (R)	
<i>zDHHC2-genome</i>	TTTTCCGGGTATGGTAGAGAAGAC (F)	
	TGCCACAGTGCTATTCGTGT (R)	
<i>zDHHC5-genome</i>	GATGATTTCCGAGCTCCCCTT (F)	
	CTGCCTCTCATGCCATGTCA (R)	
<i>zDHHC6-genome</i>	TCCTTCCCCTATCGCCATA (F)	
	ACCTTGCAGTGAATAGAAGTAGCA (R)	
<i>zDHHC24-genome</i>	AACTGACGCTGTGGTTCCTT (F)	
	TACTCCCCAGCCCAATACCA (R)	

Gene name	Sequence (5' to 3')	Usage
<i>zDHH1</i>	CCGCGGCGCGCAGCTGTTT (F)	qPCR detection for endogenous gene expression levels in human cell lines
	GGGCCGTCTTGTGGAGGGCT (R)	
<i>zDHH2</i>	CCCTCTGCTCGGCTGGTCCT (F)	
	AGGCCATCAGGCACACAACCTGTTT (R)	
<i>zDHH3</i>	GCTGACGGACCCCGTGAGAACCT (F)	
	GGCACTTGCTGCGGCCTGAA (R)	
<i>zDHH4</i>	CTGGCGGTAAGGCCGCTCC (F)	
	GCGGCCCTACACACAAGGGGA (R)	
<i>zDHH5</i>	GCTTCTCTGAGGCAGGACGGCAC (F)	
	ACTGTGTCTACCCGCGAGCCC (R)	
<i>zDHH6</i>	CCCTCTGGGGTGGAAACCGGAAAT (F)	
	CTGCAGTGATGTAACGTGGTGCC (R)	
<i>zDHH7</i>	TGACTGCCGACCATCTGCCTGC (F)	
	ACTGCCCAAGCAGGAGGCTTT (R)	
<i>zDHH8</i>	AGCCCTACACGGCACACGCT (F)	
	CGTGGCACCATGGCGGATGTGT (R)	
<i>zDHH9</i>	GGTCGCTGCTTCCCGACGGA (F)	
	TGCTAGCCCTGGAAGACAATCTCT (R)	
<i>zDHH11</i>	CAGGGGAACTGATGGGCGGTCTG (F)	
	GACCCCGGCCAGAGCCGAGT (R)	
<i>zDHH12</i>	TGGCATGGTCAGGCCTCCGTT (F)	
	GAGCAGGCTGGCCACCAACGA (R)	
<i>zDHH13</i>	CGCCAGCAGGAAGTGGGAGA (F)	
	CATCTCCCCAGGAGCCACA (R)	
<i>zDHH14</i>	CCGGCAGACGGGCGTCTTCTAC (F)	
	AGGTACGGACAGTCGAAGGCGAAG (R)	

Gene name	Sequence (5' to 3')	Usage
	ACAGGGTGGGGAGGAGGCAGAA (F)	
<i>zDHHC15</i>	CGGAAGGGTGATCCGTCAGGCA (R)	
	GTCGGCAGGCGGCAGGTCTT (F)	
<i>zDHHC16</i>	GCTGCTCCACTGCAGGGATGGT (R)	
	TGCCCCCTTCTCCACCCAGAGGAAA (F)	
<i>zDHHC17</i>	GCCTTGACTATGTCCCATGTGCTGT (R)	
	GGCAACTGTGTGGGGAGACGGA (F)	
<i>zDHHC18</i>	TCCCTGAGCGCGCAACGTCA (R)	
	ACCAGGGCTGTGCCAGCAAC (F)	
<i>zDHHC19</i>	CAGGCCCCACCACTCTCTGC (R)	
	GGCGGCGCCTCGGACTTTTG (F)	qPCR detection for
<i>zDHHC20</i>	CGGCGACGGTGACTCGGACG (R)	endogenous gene
	AGCCAGCGGCGACGAATGAA (F)	expression levels in human
<i>zDHHC21</i>	ACTGGCAAAGCAGCGCCTCCAA (R)	cell lines
	CGCCTCAAATGAAAACGCTCTTCGC (F)	
<i>zDHHC22</i>	TACATTCCTGTGTCAGCGGGTCCT (R)	
	CTCCGGGGAAGCCGACCCAT (F)	
<i>zDHHC23</i>	AACCTCCAACGCCTGCCCTCT (R)	
	CACTGTCGGCCCTGCTGCGA (F)	
<i>zDHHC24</i>	GTGCCAGAGACTCTGCCTGTGA (R)	
	TCAACCCCGCTGCCGCCAT (F)	
<i>APT1</i>	GCCCATCCGTGCCAGTATCTCC (R)	
	TGCTGCCACCGTGTCTGGAG (F)	
<i>APT2</i>	GGATGGTGGAGAGGGCGTCA (R)	
	ACCTGCGCTTCTCGGGCGCT (F)	
<i>PPT1</i>	ATTGCAACAGCTGTCTCCCATCCCA (R)	

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Continued

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Gene name	Sequence (5' to 3')	Usage
<i>PPT2</i>	CCGCGTTGTTTCATGGCTGAGGCG (F)	qPCR detection for endogenous gene expression levels in human cell lines
	CCCAGCATGCTCCCGCCAAC (R)	
<i>ABHD17a</i>	TGCGCACCAGGTACGGCATCAG (F)	
	CAGCACCACCGCGGCACACT (R)	
<i>18S</i>	AGGGCACCACCAGGAGTGGA (F)	Reference gene in human
	CCACCACCCACGGAATCGAG (R)	
<i>Pten</i>	GCCAACCGATACTTCTCTCCA (F)	
	GGATCAGAGTCAGTGGTGTC A (R)	
<i>Cdkn1a</i>	GGACAAGAGGCCCAGTACTTC (F)	qPCR detection for tumor suppressor genes in mouse liver
	CTGCGCTTGGAGTGATAGAAA (R)	
<i>Spry2</i>	CGGAGCAGTACAAGGACAAGT (F)	
	TGTAGGCATGCAGACCCAAAT (R)	
<i>Tgfr2</i>	TGGAGAGCATGAAAGACAGTGT (F)	
	CAGCACTCGGTCAAAGTCTCA (R)	
<i>Hprt</i>	CGTCGTGATTAGCGATGATG (F)	Reference gene in mouse
	ACAGAGGGCCACAATGTGAT (R)	
<i>PTEN</i>	GCGTGCAGATAATGACAAGGA (F)	
	TGCTAGCCTCTGGATTTGACG (R)	
<i>CDKN1A</i>	TGTCTTGTACCCTTGTGCCTC (F)	qPCR detection for tumor suppressor genes in HepG2 cells
	CGGCGTTTGGAGTGGTAGAAA (R)	
<i>SPRY2</i>	CAACTGTGCTGACAACCCATG (F)	
	GGCCTGTAAACCCGGTCATAA (R)	
<i>TGFBR2</i>	AACGTGTTGAGAGATCGAGGG (F)	
	CAGCACTCAGTCAACGTCTCA (R)	
<i>GAPDH</i>	CAACAGCGACACCCACTCCT (F)	Reference gene in human
	CACCCTGTTGCTGTAGCCAAA (R)	



**Supplementary Table 4.** Primer pairs used for generation of *Zdhhc6*<sup>-/-</sup> and *Aeg-1* point mutation mice

Gene name (mouse)	Sequence (5' to 3')
Zdhhc6 gRNA #1	CACCGCCTATGGGCTGAGCTCGGTG (F)
	AAACCACCGAGCTCAGCCCATAGG (R)
Zdhhc6 gRNA #2	CACCGAATAAAACGACTCGGAGTAC (F)
	AAACGTACTCCGAGTCGTTTTATT (R)
Aeg-1 point mutation gRNA #1	CACCGGCTGGGCGCGGCTTGCGC (F)
	AAACGCGCAAGCCGCGGCCAGCC (R)
Aeg-1 point mutation gRNA #2	CACCGCGGGCGCCGGCGCAAGCCG (F)
	AAACCGGCTTGCGCCGGCGCCCGC (R)
Zdhhc6-genome	ATTGATTGTGATGTTGACTAGCTTC (F)
	CAGAAGTAGGTGTGACATTCTAAC (R)
Aeg-1-genome	CTCCCACGACTGTTCCAGCGG (F)
	AACCTCCACATGCAGCCTACTTTCT (R)