Supplemental Materials and Methods

Phagocytosis assay

Macrophages were seeded (2.5 x 10^4/well) into 4-well slide chambers. Fluorescein-labeled beads of 1.0-μm diameter (2.5 x 10^6; Invitrogen) were added to each well, followed by a 1 hour incubation. After washing 5 times with PBS, cells were fixed with 4% paraformaldehyde/PBS and the number of the beads incorporated into the cells was counted under a fluorescence microscope.

LPS-responsive TNF-ά production assay

Macrophages were seeded (1 x 10^5/well) onto 96-well plates and stimulated with 100 ng mL^{-1} LPS for 12 hours. Supernatants from the macrophage cultures were collected and TNF-ά levels were measured using the TNF-α ELISA kit (AssayPro).

Supplementary Figure Legends

Figure S1. MT1$^{-/-}$ macrophages are deficient in phagocytosis

(A) Representative photographs of macrophages incorporating FITC-labeled beads by phagocytosis. Note that some beads have accumulated within the cells. **(B)** The number of cells is plotted against the number of beads incorporated per cell. **(C)** Average number of phagocytosed beads per cell is represented as mean ± SEM (N = 90) and was analyzed using the Student's t-test. ** $p < 0.01$.

Results: MT1$^{-/-}$ macrophages incorporated fewer beads than WT cells.

Figure S2. MT1$^{-/-}$ mice are deficient in cytokine production

Macrophages were seeded (1 x 10^5/well) into 96-well plates and stimulated with LPS (100 ng mL^{-1}) for 12 hours. Supernatants of the macrophage cultures were collected and the level of TNF-α was measured using the TNF-α ELISA kit (AssayPro). Following stimulation with LPS, MT1$^{-/-}$ macrophages produced less TNF-α than WT cells. The unpaired Student's t-test was used for analyzing differences between experimental groups. ** $p < 0.01$.

Results: MT1$^{-/-}$ macrophages produced less TNF-α than WT cells.

Figure S3. Lack of MT1-MMP does not affect expression levels of PHD, p300, and CBP in macrophages and MEFs.

(**A** and **B**) Three isoforms of PHDs in macrophages (**A**) or MEFs (**B**) were detected by immunoblotting using PHD-specific antibodies. Actin served as the loading control.

(**C** and **D**) Nuclear extracts were prepared from macrophages (**C**) or MEFs (**D**) and subjected for immunoblot analysis to detect p300 and CBP. Lamin A/C served as the loading control.

Results: Protein levels of PHDs and p300/CBP are not different between $MT1^{-/-}$ and WT cells.

Figure S4. MT1-MMP promotes the association between HIF-1α and p300/CBP in a Mint3 dependent manner in HEK293 cells.

(**A**) Presence or absence of MT1-MMP does not affect the amount of HIF-1α that can bind HRE. Nuclear extracts from HEK293 cells in Fig. 4C were subjected to the plate assay as Fig. 2D. Free HRE or mutant oligonucleotides were used as competitors.

(**B**) The amount of p300/CBP associated with HIF-1α is affected by MT1-MMP in a Mint3

dependent manner. Nuclear extracts from HEK293 cells were subjected to the plate assay as Fig. 2F.

Results: In parallel with the results of reporter assay in Fig. 4C, MT1-MMP promotes the association between HIF-1α and p300/CBP in a Mint3 dependent manner in HEK293 cells.

Figure S5. Domain structure of Mint3

Mint3 consists of an N-terminal (NT) portion that binds FIH-1(1), a phosphotyrosine binding (PTB) domain, and two PDZ domains. Amino acid positions are indicated. The NT portion is sufficient to inhibit FIH-1(1). The PTB domain interacts with APP and furin (2,3).

Figure S6. The expression of MT1-MMP but not dCPT induces binding of Mint3 to FIH-1 in HEK293 cells

FLAG-tagged FIH-1 and MT1-MMP (MT1), the CP deletion mutant of MT1-MMP (dCPT), or the negative control (EGFP) was co-expressed in HEK293 cells. Whole cell lysate (WCL) and anti-FLAG immunoprecipitates (IP) were examined by immunoblotting using antibodies against the proteins indicated. Note that endogenous FIH-1 can be detected as it

forms a homo-dimer with FLAG-tagged FIH-1 (arrow).

Results

HEK293 cells do not express MT1-MMP and no binding of Mint3 to FIH-1 was detected. However, the forced expression of MT1-MMP, but not dCPT, induced binding of Mint3 with FIH-1.

Figure S7. The CPT of MT1-MMP mediates co-localization of FIH-1 with Mint3

The colors of Mint3 and MT1-MMP mutants in Figure 6B were reversed to improve the visibility of MT1-MMP. In addition to the localization of MT1-MMP to the Golgi, a dot-like distribution of MT1-MMP is clearly observed. These dot-like structures might correspond to podosomes of macrophages.

Figure S8. Pull-down assay of Mint3 using the cytoplasmic tail peptide of MT1-MMP and MT5-MMP

(**A**) Amino acid sequences of the CP tail of MT-MMPs are presented. Four amino acids including CP14 (*) are indicated by the box. Underlined amino acids correspond to the PDZ-binding sequences.

(**B**) $(His)_6$-tagged Mint3 was subjected to pull-down assay using GST fusion proteins having the CP tail sequences (GST, GST-MT1CPT, GST-MT5CPT and GST-FIH-1) conjugated to sepharose beads. GST, glutathione S-transferase; GST-MT1CPT, GST fused to the cytoplasmic tail of MT1-MMP; GST-MT5CPT, GST fused with the cytoplasmic tail of MT5-MMP; and GST-FIH-1, GST fused to FIH-1. Mint3 bound to the beads was detected by immunoblotting using anti-$(His)_6$ antibody (upper panel). GST proteins conjugated to the beads were detected using anti-GST antibody.

Results: The amino acid sequences of MT-MMP CP tails are relatively well conserved. In particular, the tyrosine at CP11, arginine at CP14(*) and valine at CP20 are conserved in all members. MT5-MMP is reported to bind Mint3 via the PDZ domain (4) and it did in our assay. However, MT1-MMP CP tail did not bind Mint3. Amino acid sequence at the C-terminus of MT5-MMP (QEWV: CP17-CP20) is conserved in MT2- and MT3-MMPs, but not in MT1-MMP. Sequences upstream of the C-terminal valine (CP20) play a critical role in PDZ-binding specificity (5) and most likely explain differences in the binding activities of MT1-MMP and the other MT-MMP members.

Figure S9. Effect of MT-MMPs on the reporter assay

Upper panel: Effect of MT1-, MT2-, MT3- or MT5-MMP on HIF-1α CAD was evaluated using the reporter assay in HEK293 cells. Luciferase activity relative to that in the control cells (mock) is presented (upper panel). Expression of FLAG-tagged MT-MMP proteins was confirmed by immunoblotting. The data were analyzed using a Student's t-test. **p < 0.01.

Results: Critical importance of the arginine (R) residue in the CP tail for activation of HIF-1α CAD was demonstrated in Figure 3F and for ATP production in macrophages in Figure 3G. The R residue is conserved among the CP tails of MT-MMPs (Figure S8A). However, only MT1-MMP activated HIF-1α CAD. Presumably the surrounding sequences of the R residue are also important for FIH-1 binding and activation of the CAD. Indeed, the four amino acids surrounding the R residue of MT1-MMP (CP13-16) are not identical in the other MT-MMP members (Figure S8A). For example, the Q at CP 13 in MT1-MMP is substituted to a K in all the other family members. In addition, the L at CP16 is also substituted to an M or a V in MT3- and MT5-MMP, respectively.

Supplemental References

1. Sakamoto, T., and Seiki, M. (2009) *J Biol Chem* **284**(44), 30350-30359
2. Han, J., Wang, Y., Wang, S., and Chi, C. (2008) *J Cell Sci* **121**(Pt 13), 2217-2223
3. Tanahashi, H., and Tabira, T. (1999) *Biochem Biophys Res Commun* **255**(3), 663-667
4. Wang, P., Wang, X., and Pei, D. (2004) *J Biol Chem* **279**(19), 20461-20470
5. Hung, A. Y., and Sheng, M. (2002) *J Biol Chem* **277**(8), 5699-5702

Figure S1

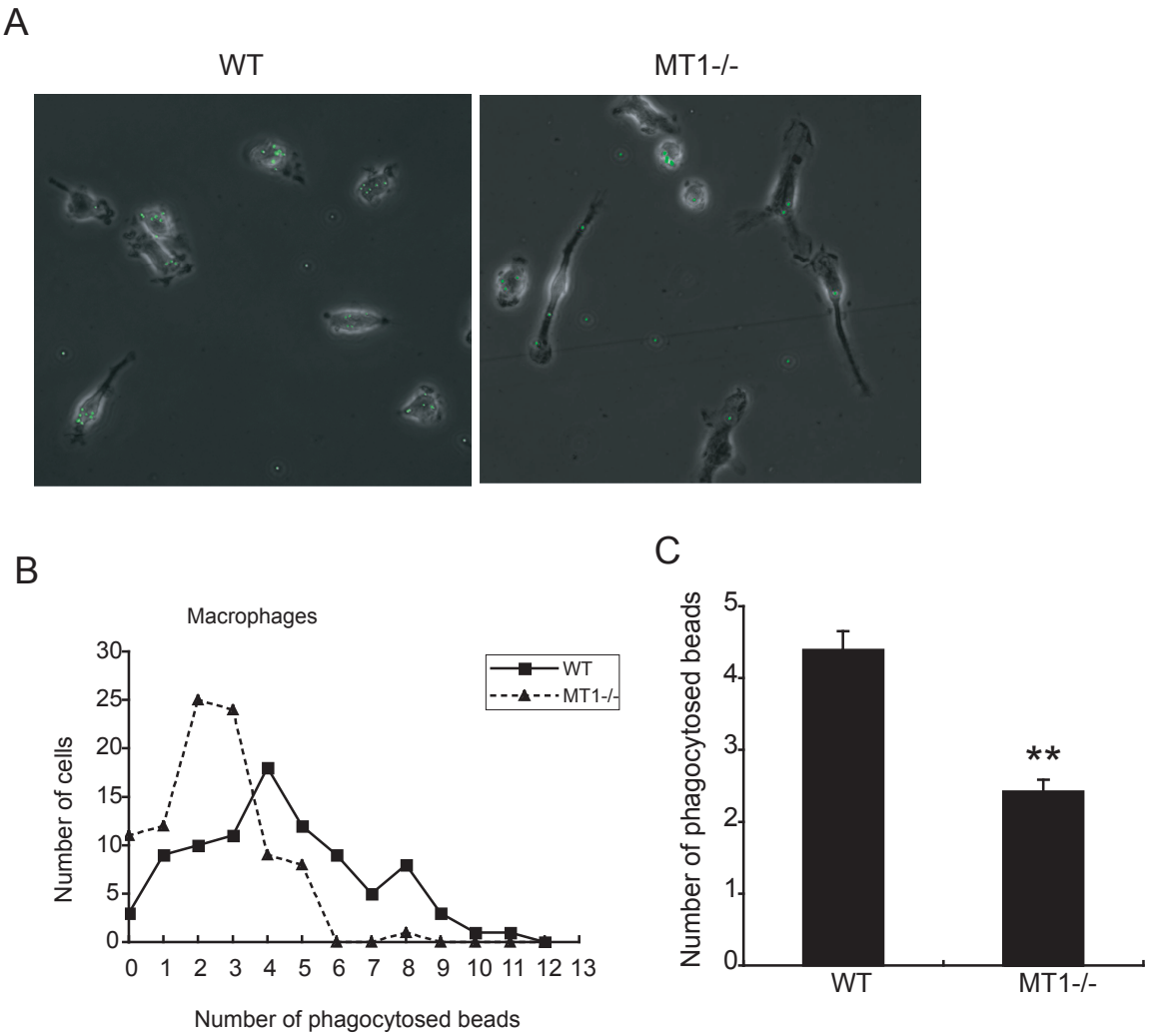

A
WT
MT1-/-
B
Macrophages
Number of cells
Number of phagocytosed beads
WT
MT1-/-
C
Number of phagocytosed beads
**
WT
MT1-/-

Figure S2

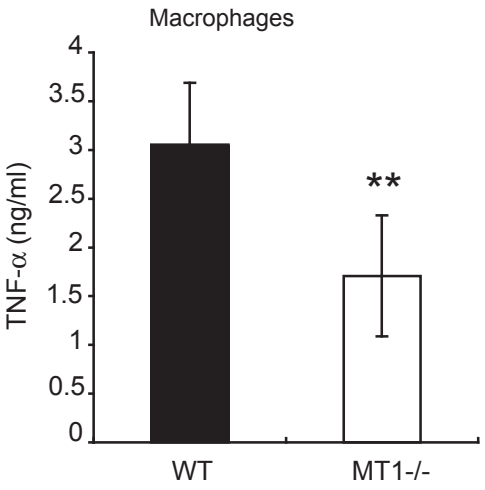


Figure S3

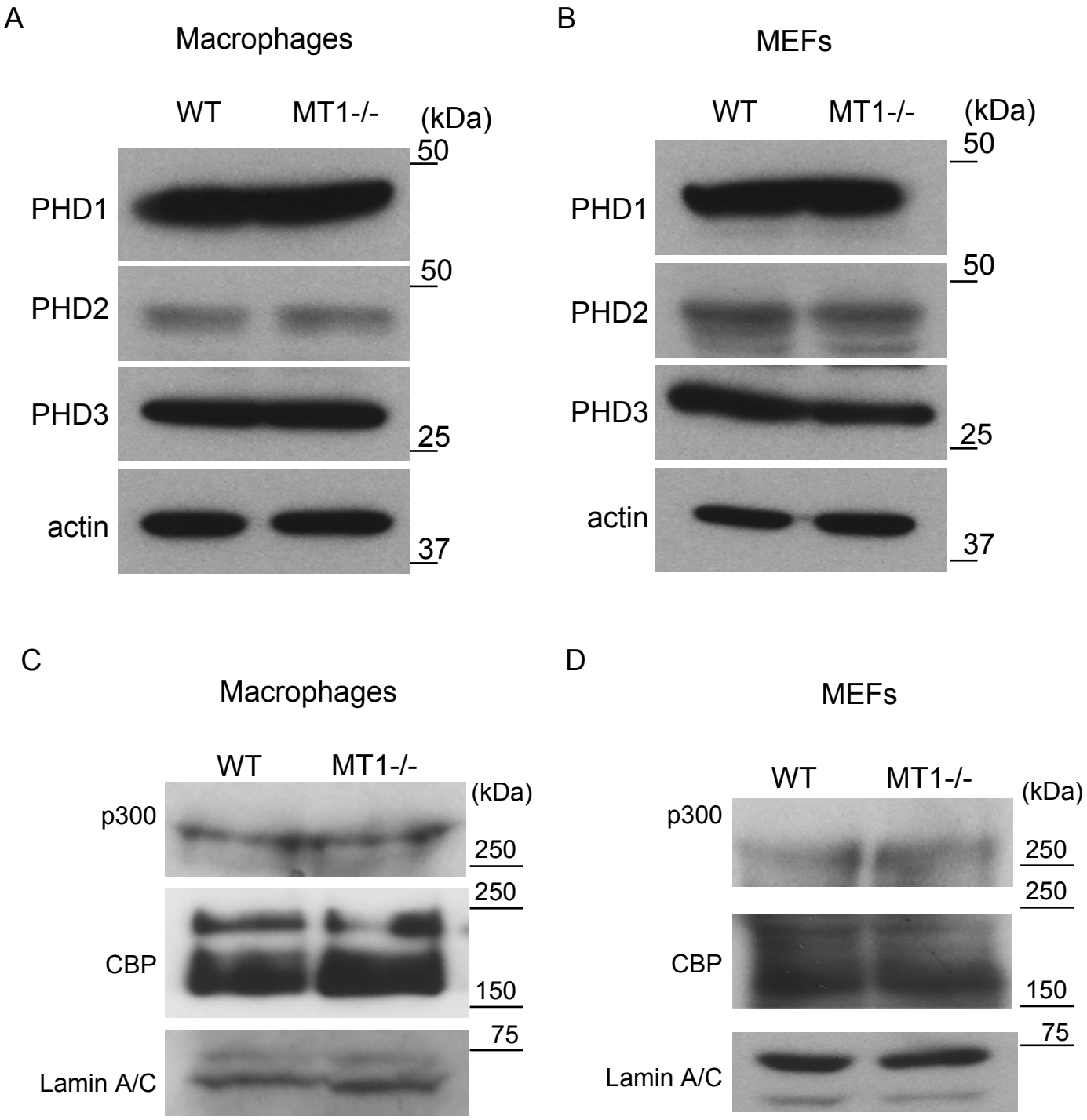
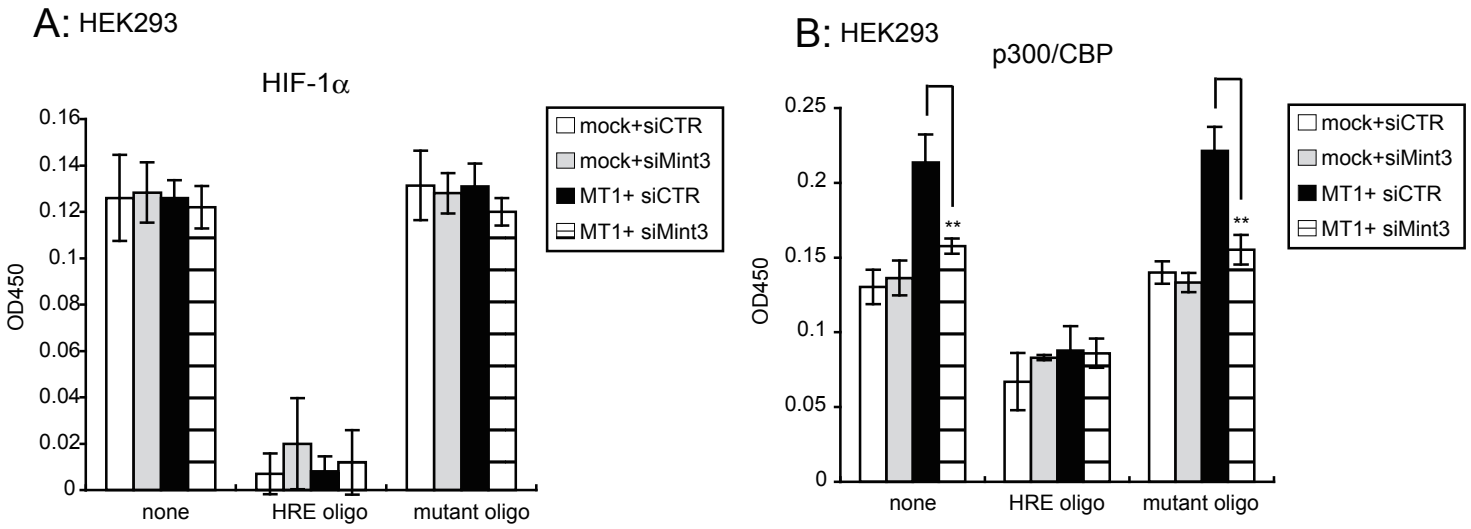

A
Macrophages
WT
MT1-/-
(kDa)
PHD1
PHD2
PHD3
actin
50
50
25
37
B
MEFs
WT
MT1-/-
(kDa)
PHD1
PHD2
PHD3
actin
50
50
25
37
C
Macrophages
WT
MT1-/-
(kDa)
p300
CBP
Lamin A/C
250
250
150
75
D
MEFs
WT
MT1-/-
(kDa)
p300
CBP
Lamin A/C
250
250
150
75

Figure S4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HIF-1α
OD450
0.16
0.14
0.12
0.1
0.08
0.06
0.04
0.02
0
none
HRE oligo
mutant oligo
mock+siCTR
mock+siMint3
MT1+ siCTR
MT1+ siMint3
B: HEK293
p300/CBP
OD450
0.25
0.2
0.15
0.1
0.05
0
**
**
none
HRE oligo
mutant oligo
mock+siCTR
mock+siMint3
MT1+ siCTR
MT1+ siMint3

Figure S5

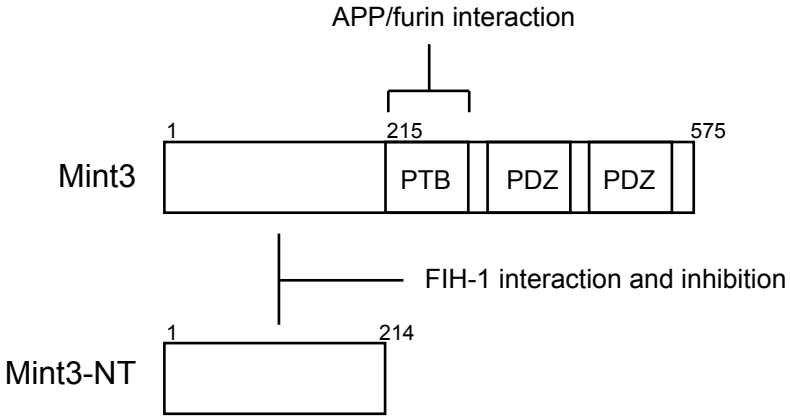
APP/furin interaction
1
215
575
Mint3
PTB
PDZ
PDZ
FIH-1 interaction and inhibition
1
214
Mint3-NT

Figure S6

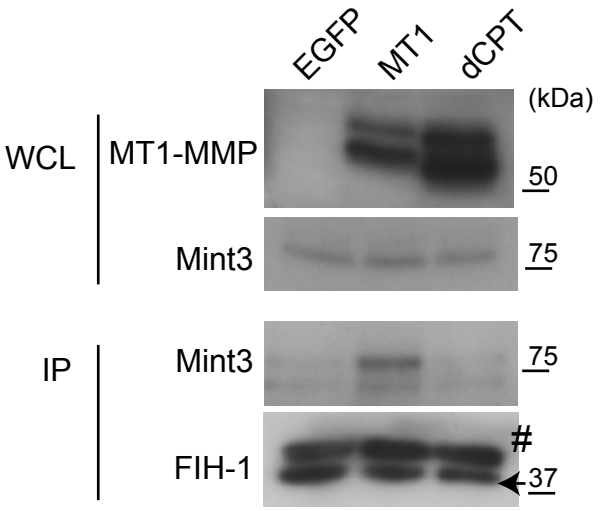

Figure S7

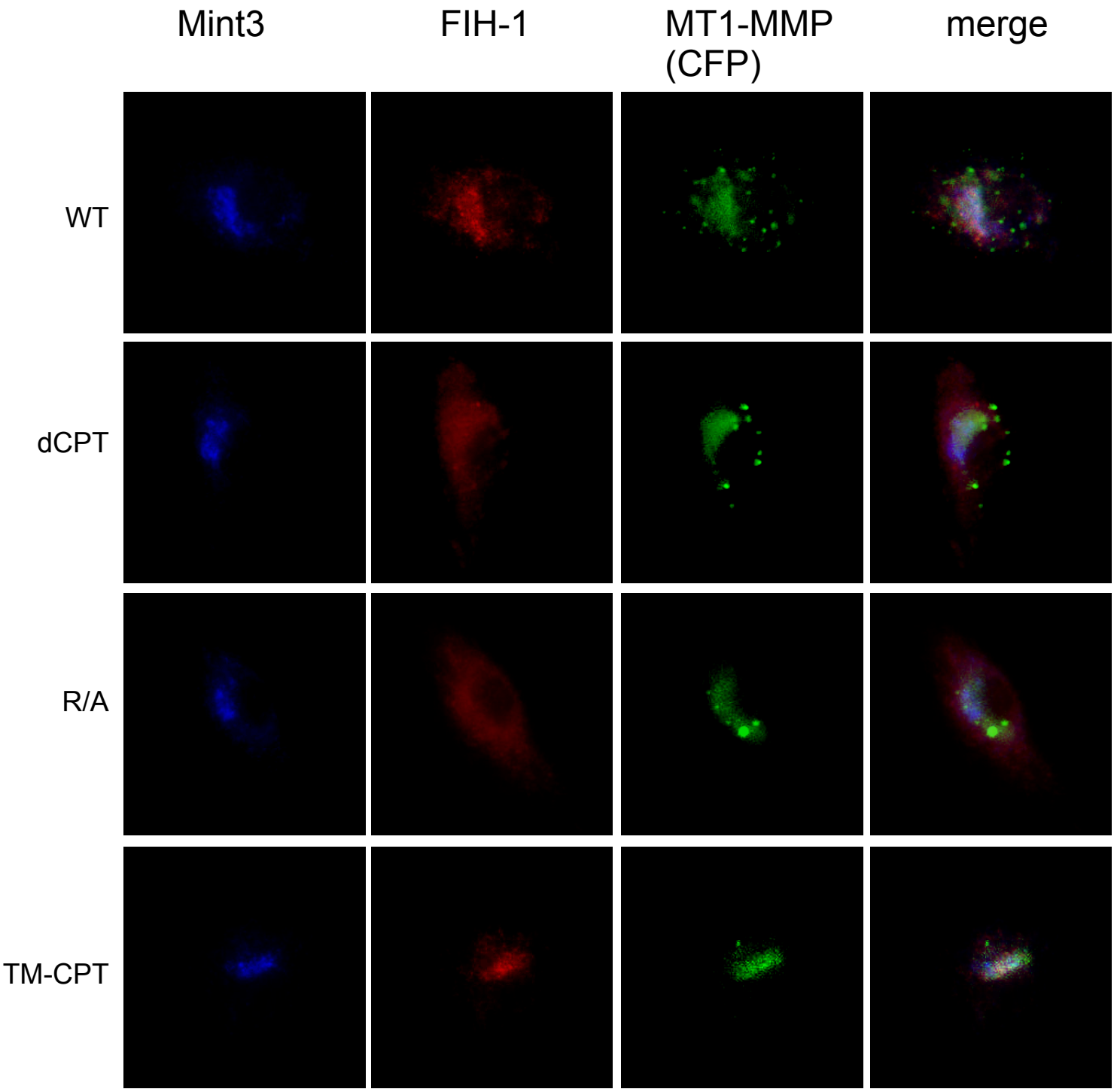

Figure S8

A

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                                  *
MT1-MMP:RRHGTPRRLLYCQRSLLDKV
MT2-MMP:QRKGAPRVLLYCKRSLQEWV
MT3-MMP:KRKGTPRHILYCKRSMQEWV
MT5-MMP:KNKTGPQPVTYYKRPVQEWV
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B

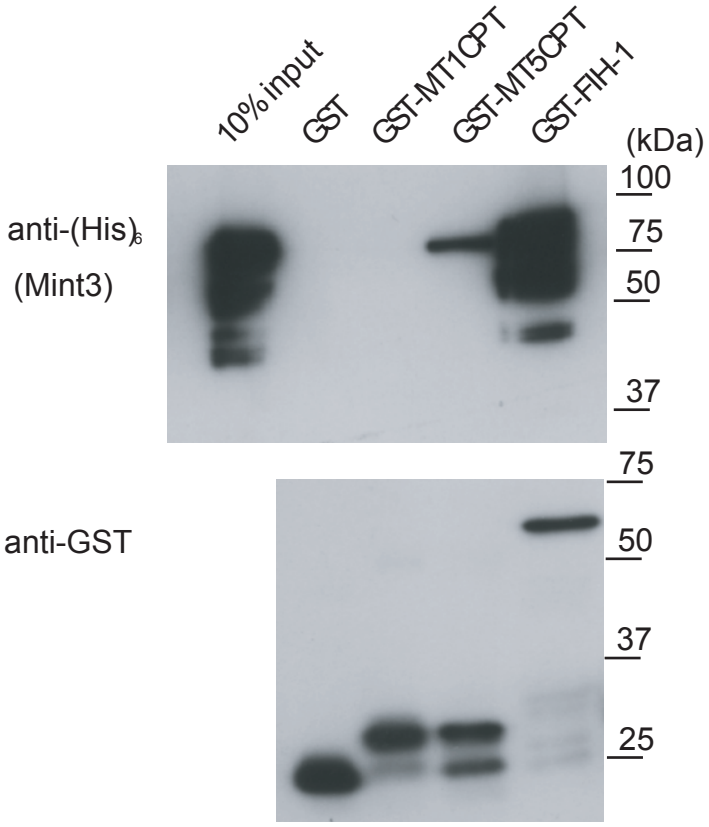
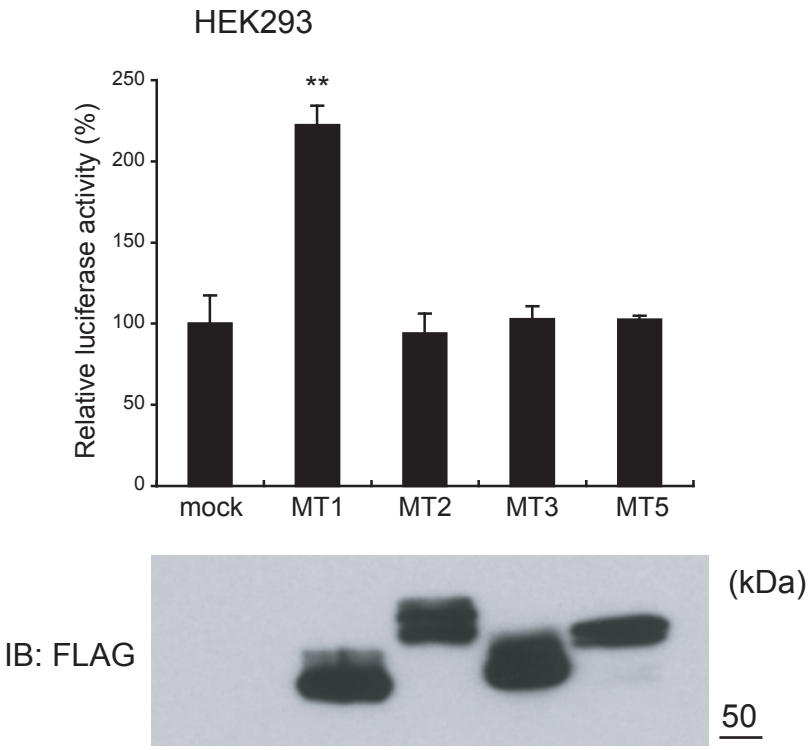


Figure S9


HEK293
Relative luciferase activity (%)
250
200
150
100
50
0
**
mock
MT1
MT2
MT3
MT5
IB: FLAG
(kDa)
50