SUPPORTING INFORMATION – FIGURE LEGENDS

Figure S1. The silencing of *NDRG1* in (A) DU145 cells or (B) Huh7 cells using a second NDRG1 siRNA (NDRG1 II) markedly decreases NDRG1 expression and up-regulates c-Met expression and phosphorylation (Y1003, Y1234/5, Y1349) relative to the non-targeting, negative control siRNA (siControl). DU145 or Huh7 cells were incubated with Opti-MEM media containing the siControl or siNDRG1 II as per the *Experimental Procedures*. The cells were then incubated in normal media alone without hepatocyte growth factor (HGF (-)) or media containing HGF (50 ng/mL; HGF (+)) for 15 min/37 °C. Results are from a representative experiment. **(C-F) Both Dp44mT and DpC down-regulate total c-Met total protein levels in 4 additional tumor cell-types, including: (C) SCC25 oral squamous cell carcinoma; (D) Hep3B hepatoma; (E) PANC-1 pancreatic cancer; and (F) MDA-MB-231 breast cancer cells.** These cells were incubated with Control medium or medium containing Bp2mT (5 μM; negative control), DFO (100 μM), Dp44mT (5 μM), or DpC (5 μM) for 24 h/37 °C. Results are from typical experiments.

Figure S2. The thiosemicarbazones, Dp44mT and DpC, markedly decrease the phosphorylation at Y307 of the downstream c-Met effector Gab1. (A, B) DU145 and **(C, D)** Huh7 cells were incubated with Control medium, or medium containing Bp2mT (5 μM; negative control), DFO (100 μM), Dp44mT (5 μM), or DpC (5 μM), for 24 h/37 °C. These cells were then incubated in media alone without HGF (HGF-) or media containing HGF (50 ng/mL; HGF +) for 15 min/37 °C. Densitometric analyses of western blots for **(B)** DU145 and **(D)** Huh7 cells are mean ± SEM (n = 3) with the results for Gab1 and p-Gab1 (Y307) normalized to the protein-loading control, β-actin. The p-Gab1 (Y307) levels are also presented as a ratio of total Gab1 protein levels. *$p < 0.05$, ***$p < 0.001$ relative to the respective Control. #$p < 0.05$, ##$p < 0.01$ relative to the HGF (-) treated cells.

Figure S3. (A, B) Binding to cellular iron is required for Dp44mT and DpC to down-regulate c-Met protein in DU145 cells. DU145 cells were incubated with Control medium or this medium containing either Bp2mT (5 μM; negative control), DFO (100 μM), Dp44mT (5 μM), or DpC (5 μM), for 24 h/37 °C. Cells were also incubated with Fe(III) (as $FeCl_3$; [Fe] = 2.5 or 100 μM), Bp2mT (5 μM) together with $FeCl_3$ (2.5 μM), or the Fe complexes of the chelators, namely DFO-Fe (1:1), Dp44mT-Fe (1:2) and DpC-Fe (1:2) for 24 h/37 °C. Densitometric analyses of western blots are mean ± SEM (n = 3) normalized to the protein-loading control, β-actin. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ relative to the respective Control. #$p < 0.05$, ##$p < 0.01$ relative to the respective ligands. **(C-F) The anti-oxidant, *N*-acetylcysteine (NAC), has no effect on c-Met down-regulation by Dp44mT and DpC. (C)** DU145 and **(E)** Huh7 cells were incubated with Control medium, or this medium containing Bp2mT (5 μM; negative control), DFO (100 μM), Dp44mT (5 μM), or DpC (5 μM) alone, or in combination with NAC (5 mM) for 24 h/37 °C. Densitometric analyses of western blots for **(D)** DU145 and **(F)** Huh7 cells are mean ± SEM (n = 3) normalized to the protein-loading control, β-actin. *$p < 0.05$, **$p < 0.01$, ***$p <$ 0.001 relative to the Control.

Figure S4. The silencing of *MIG6* in (A, B) DU145 cells or (C, D) Huh7 cells using a second MIG6 siRNA (MIG6 II) markedly decreases MIG6 expression and up-regulates c-Met expression and its phosphorylation at Y1349. DU145 or Huh7 cells were incubated with Opti-MEM media containing non-targeting, negative control siRNA (siControl) or siRNA specific for MIG6 (siMIG6II; **E-H**) in the absence of HGF (see *Experimental Procedures*). Western analysis was then performed. Densitometric analyses of western blots are from a typical experiment and are normalized to the protein-loading control, β-actin. Results are from a typical experiment.

Figure S5. Thiosemicarbazones have no appreciable effect on Gab1 and p-Gab1 (Y307) levels after *NDRG1* or *MIG6* silencing. DU145 or Huh7 cells were incubated with Opti-MEM media containing non-targeting, negative control siRNA (siControl) or siRNA specific for NDRG1 (siNDRG1; **A-D**) or MIG6 (siMIG6; **E-H**) in the absence of HGF. The cells were then incubated with

Control medium, or this medium containing Dp44mT or DpC at 5 μM for 24 h/37 °C. Western analysis was then performed. Densitometric analyses for NDRG1 and MIG6 are mean ± SEM (n = 3) normalized to the protein-loading control, β-actin. *p < 0.05, **p < 0.01, ***p < 0.001 relative to the respective Control. #p < 0.05, ##p < 0.01, ###p < 0.001 relative to siControl samples. For Gab1 and pGab1 (Y307) the densitometry is from typical experiments.

Figure S6. Dp44mT and DpC do not significantly modulate HSP90 protein levels. (A, B) DU145 and **(C, D)** Huh7 cells were incubated with Control medium or this medium containing either: Bp2mT (5 μM; negative control), DFO (100 μM), Dp44mT (5 μM), or DpC (5 μM) for 24 h/37 °C. Densitometric analysis of western blots is shown as the mean ± SEM (n = 3) normalized to β-actin protein **(A-D**).

Figure S7. Incubation of Huh7 cells with lysosomotropic agents does not prevent down-regulation of c-Met expression mediated by Dp44mT and DpC. Huh7 cells were incubated with Control medium or this medium containing either NH_4Cl (15 mM), methylamine (MA; 15 mM), chloroquine (CLQ; 50 μM), Dp44mT (5 μM), or DpC (5 μM) alone, or in combination for 24 h/37 °C, and either: **(A)** confocal immunofluorescence microscopy, or **(B)** Western analysis was performed to determine c-Met protein levels. **(A)** All images were taken with a 63x objective and at the same acquisition settings using Zeiss Zen Blue software. Scale bar represents 10 μm. **(C)** Densitometric analyses of western blots are mean ± SEM (n = 3) normalized to a protein-loading control, β-actin. *p < 0.05, **p < 0.01, ***p < 0.001 relative to the Control.

Figure S8. Dp44mT does not increase c-Met CTF levels when incubated together with the lysosomotropic agents in DU145 cells. (A) DU145 cells were incubated with Control medium or this medium containing either: NH_4Cl (15 mM), methylamine (MA; 15 mM), chloroquine (CLQ; 50 μM), Dp44mT (5 μM), or DpC (5 μM) alone, or in combination for 24 h/37 °C. Results of western blots are from a representative experiment.

Figure S9. Proteasome inhibition prevents degradation of c-Met ICD, and Dp44mT decreases the levels of c-Met ICD in Huh7 cells. (A) Huh7 cells were incubated with Control medium or this medium containing either: Dp44mT (5 μM), MG132 (2.5 or 5 μM), EDTA (1 mM) alone, or these agents in combination for 24 h/37 °C. Three images of blots developed at different exposure times (**Ai-Aiii**) are shown for optimal presentation of different c-Met isoforms, namely c-Met precursor, mature form, c-Met *C*-terminal fragment (CTF) and c-Met intracellular domain (ICD). Densitometric analyses of western analysis for: **(B)** c-Met precursor and mature form and **(C)** 50-kDa c-Met ICD. Results are mean ± SEM (n = 3) normalized to the protein-loading control, β-actin. *p < 0.05 and **p < 0.01 relative to the Control. †p < 0.05, relative to cells incubated with Dp44mT.

Figure S10. **The combined mechanisms of thiosemicarbazones that down-regulate c-Met are demonstrated in multiple cancer cell-types (A)** PANC-1 pancreatic cancer, SCC25 oral squamous cell carcinoma, MDA-MB-231 breast cancer and Hep3B cells were compared to DU145 and Huh7 cells as internal controls and incubated with control medium or this medium containing Dp44mT (5 μM) in the presence or absence of the lysosomotropic agent, CLQ (50 μM/24 h/37°C), to inhibit lysosomal c-Met degradation. **(B)** The cell-types in **(A)** were incubated with control medium or this medium containing Dp44mT in the presence and absence of the broad metalloprotease inhibitor, EDTA (1 mM), for 8 h/37°C. **(C)** The effect of Dp44mT (5 μM) on *NDRG1* and *MET* mRNA levels was assessed in all cell-types described in **(A)** after incubation with Dp44mT or the Control for 24 h/37°C. Results from **(A, B)** western blots and **(C)** RT-PCR were compared relative to β-actin protein and mRNA, respectively, and are from a typical experiment.

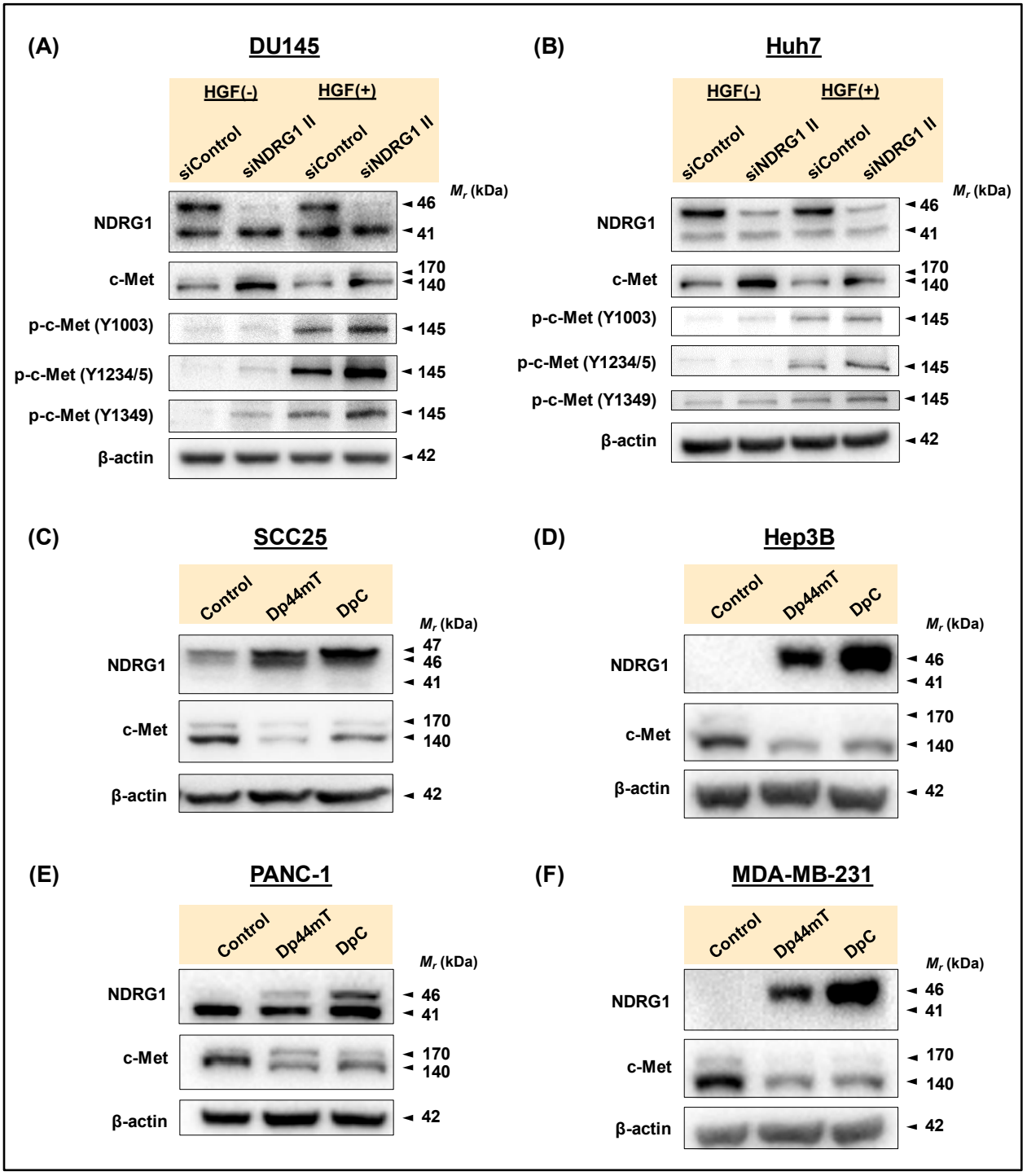
(A)
DU145
HGF(-)
HGF(+)
siControl
siNDRG1 II
siControl
siNDRG1 II
Mr (kDa)
NDRG1
46
41
c-Met
170
140
p-c-Met (Y1003)
145
p-c-Met (Y1234/5)
145
p-c-Met (Y1349)
145
β-actin
42
(B)
Huh7
HGF(-)
HGF(+)
siControl
siNDRG1 II
siControl
siNDRG1 II
Mr (kDa)
NDRG1
46
41
c-Met
170
140
p-c-Met (Y1003)
145
p-c-Met (Y1234/5)
145
p-c-Met (Y1349)
145
β-actin
42
(C)
SCC25
Control
Dp44mT
DpC
Mr (kDa)
NDRG1
47
46
41
c-Met
170
140
β-actin
42
(D)
Hep3B
Control
Dp44mT
DpC
Mr (kDa)
NDRG1
46
41
c-Met
170
140
β-actin
42
(E)
PANC-1
Control
Dp44mT
DpC
Mr (kDa)
NDRG1
46
41
c-Met
170
140
β-actin
42
(F)
MDA-MB-231
Control
Dp44mT
DpC
Mr (kDa)
NDRG1
46
41
c-Met
170
140
β-actin
42

Figure S1

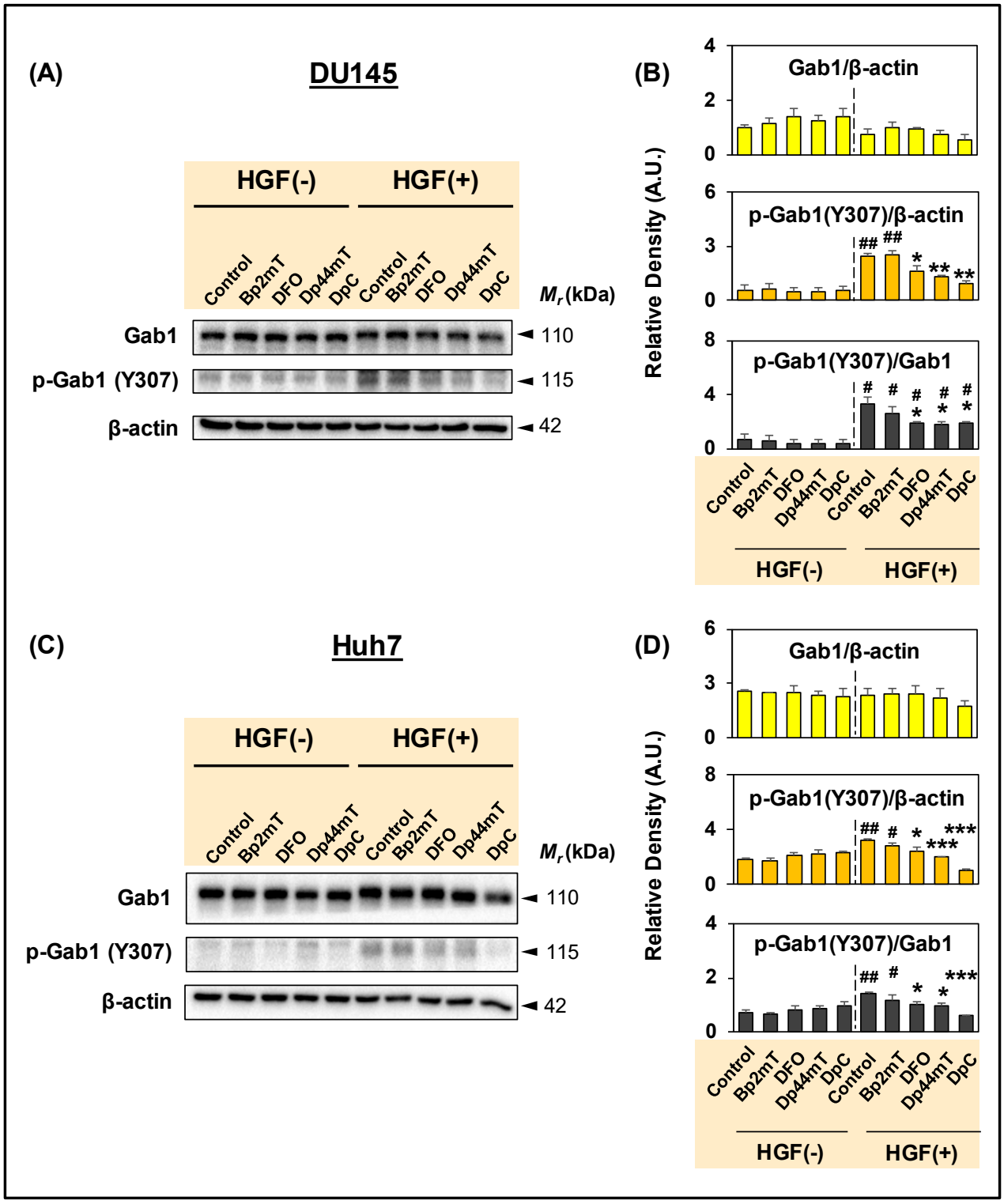
(A)
DU145
HGF(-)
HGF(+)
Control
Bp2mT
DFO
Dp44mT
DpC
M_r(kDa)
Gab1
p-Gab1 (Y307)
β-actin
110
115
42
(B)
Gab1/β-actin
p-Gab1(Y307)/β-actin
p-Gab1(Y307)/Gab1
Relative Density (A.U.)
(C)
Huh7
(D)

Figure S2

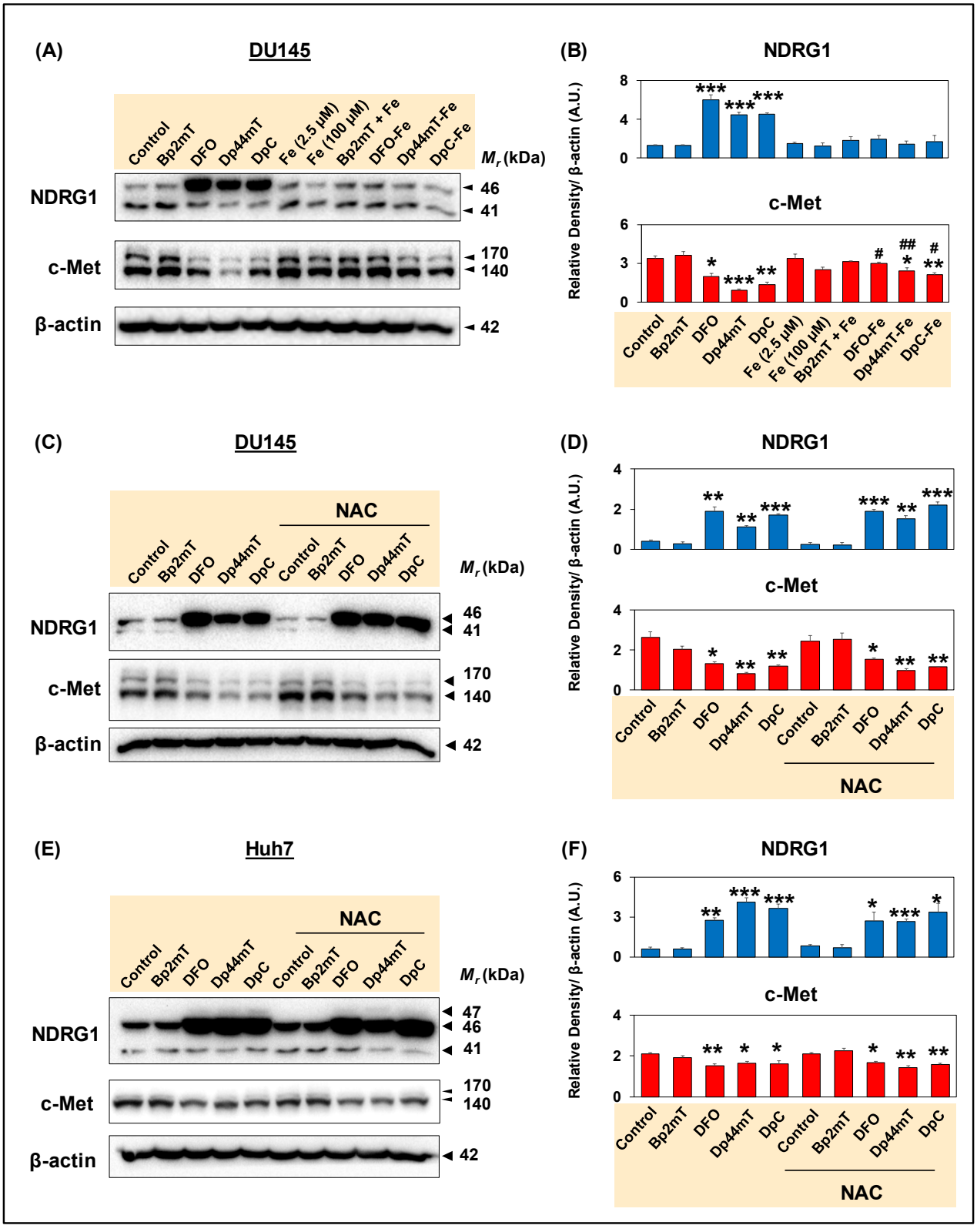
(A)
DU145
Control
Bp2mT
DFO
Dp44mT
DpC
Fe (2.5 μM)
Fe (100 μM)
Bp2mT + Fe
DFO-Fe
Dp44mT-Fe
DpC-Fe
M_r (kDa)
NDRG1
c-Met
β-actin
46
41
170
140
42
(B)
NDRG1
Relative Density/ β-actin (A.U.)
c-Met
(C)
DU145
NAC
(D)
NDRG1
c-Met
NAC
(E)
Huh7
47
(F)
NDRG1
c-Met
NAC

Figure S3

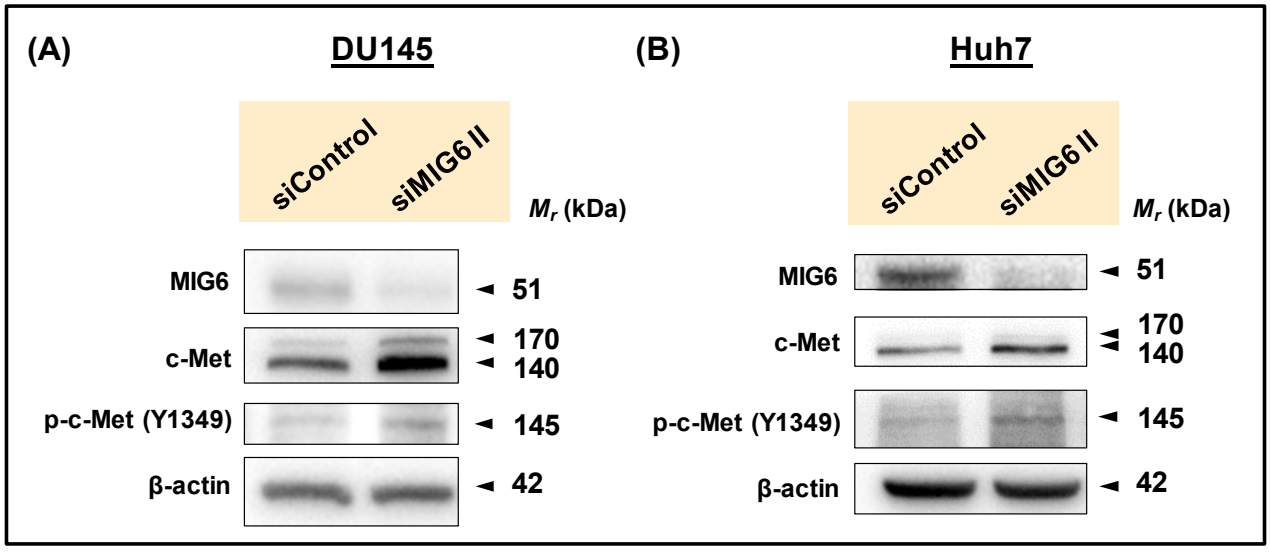
(A)
DU145
siControl
siMIG6 II
M_r (kDa)
MIG6
51
c-Met
170
140
p-c-Met (Y1349)
145
β-actin
42
(B)
Huh7
siControl
siMIG6 II
M_r (kDa)
MIG6
51
c-Met
170
140
p-c-Met (Y1349)
145
β-actin
42

Figure S4

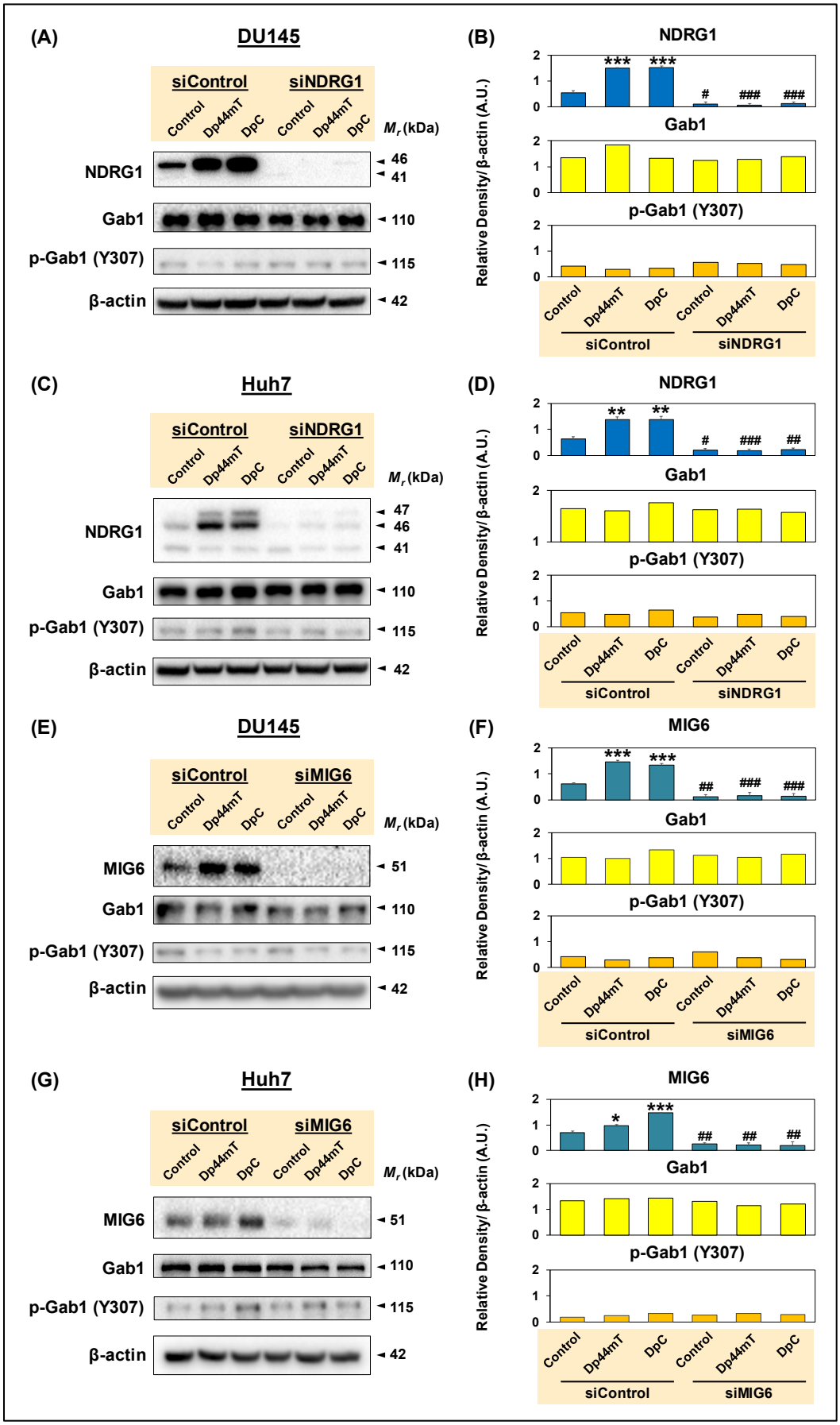
(A)
DU145
siControl
siNDRG1
Control
Dp44mT
DpC
Mr (kDa)
NDRG1
Gab1
p-Gab1 (Y307)
β-actin
46
41
110
115
42
(B)
NDRG1
Gab1
p-Gab1 (Y307)
Relative Density/ β-actin (A.U.)
(C)
Huh7
47
(D)
(E)
siMIG6
MIG6
51
(F)
(G)
(H)

Figure S5

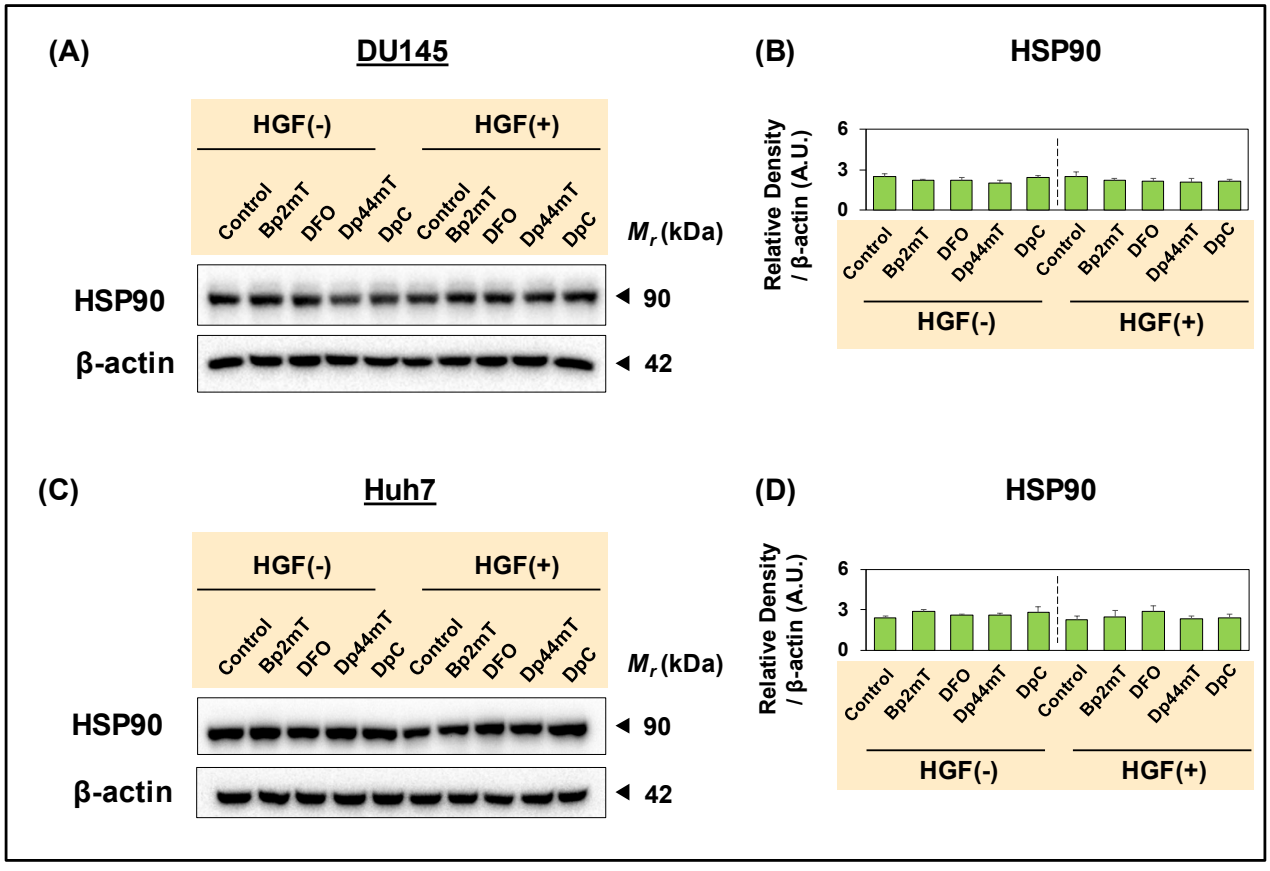
(A)
DU145
HGF(-)
HGF(+)
Control
Bp2mT
DFO
Dp44mT
DpC
Control
Bp2mT
DFO
Dp44mT
DpC
M_r(kDa)
HSP90
β-actin
90
42
(B)
HSP90
Relative Density / β-actin (A.U.)
6
3
0
Control
Bp2mT
DFO
Dp44mT
DpC
Control
Bp2mT
DFO
Dp44mT
DpC
HGF(-)
HGF(+)
(C)
Huh7
HGF(-)
HGF(+)
Control
Bp2mT
DFO
Dp44mT
DpC
Control
Bp2mT
DFO
Dp44mT
DpC
M_r(kDa)
HSP90
β-actin
90
42
(D)
HSP90
Relative Density / β-actin (A.U.)
6
3
0
Control
Bp2mT
DFO
Dp44mT
DpC
Control
Bp2mT
DFO
Dp44mT
DpC
HGF(-)
HGF(+)

Figure S6

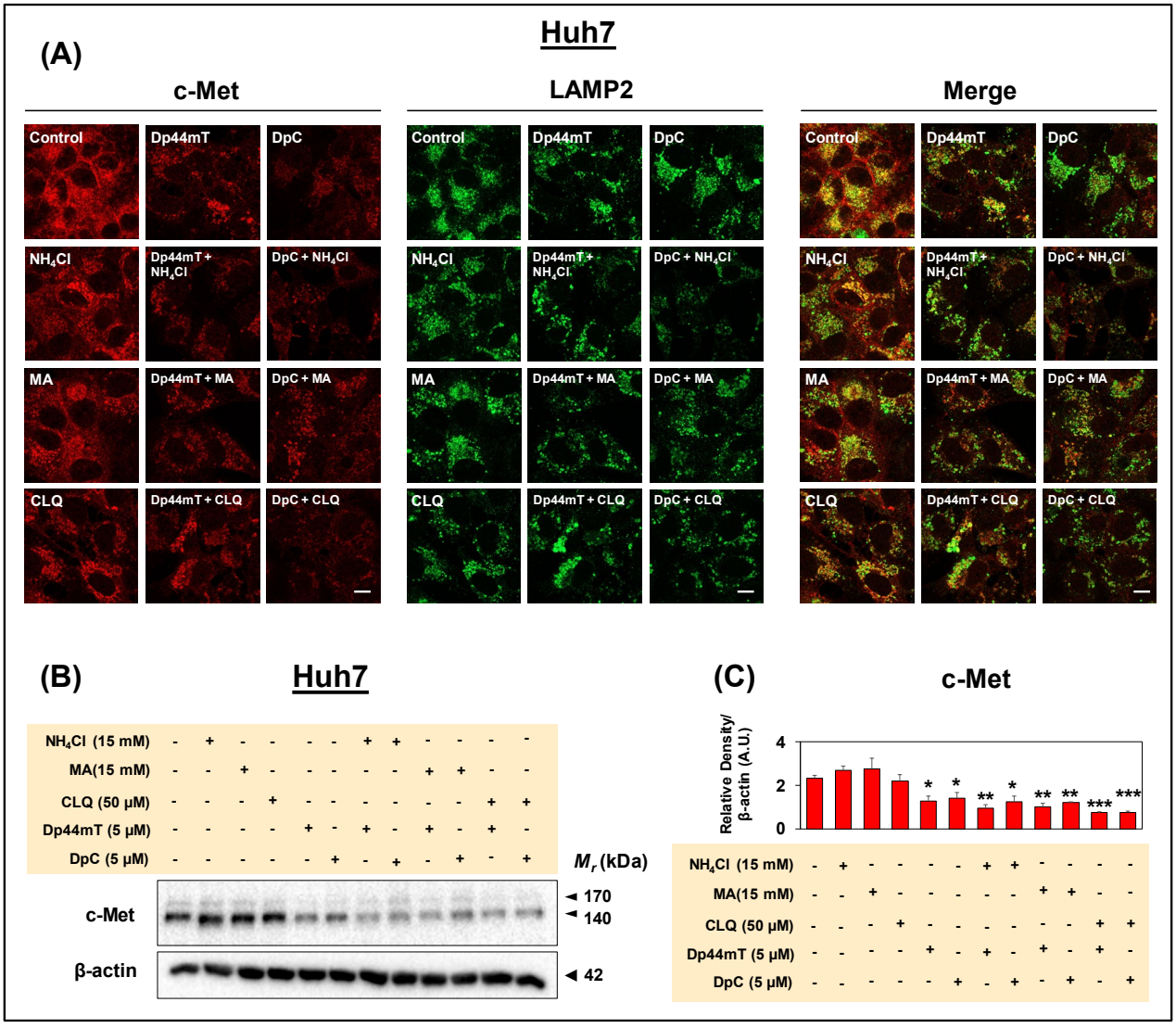
(A)
Huh7
c-Met
LAMP2
Merge
Control
Dp44mT
DpC
NH4Cl
Dp44mT + NH4Cl
DpC + NH4Cl
MA
Dp44mT + MA
DpC + MA
CLQ
Dp44mT + CLQ
DpC + CLQ
(B)
Huh7
NH4Cl (15 mM)
MA(15 mM)
CLQ (50 μM)
Dp44mT (5 μM)
DpC (5 μM)
Mr (kDa)
c-Met
β-actin
170
140
42
(C)
c-Met
Relative Density/ β-actin (A.U.)
4
2
0
*
**
***

Figure S7

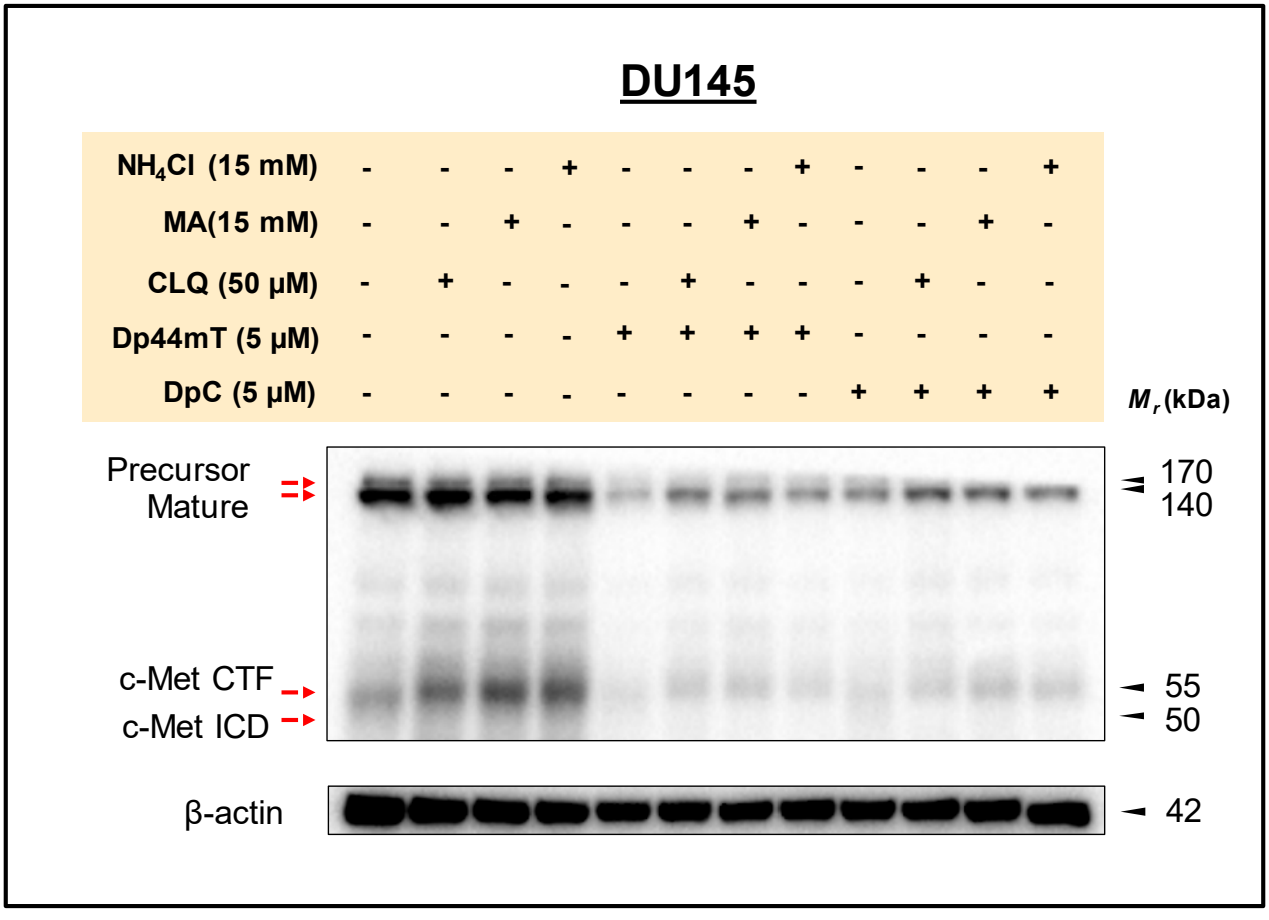
DU145
NH4Cl (15 mM) - - - + - - - + - - - +
MA(15 mM) - - + - - - + - - - + -
CLQ (50 μM) - + - - - + - - - + - -
Dp44mT (5 μM) - - - - + + + + - - - -
DpC (5 μM) - - - - - - - - + + + +
Mr(kDa)
Precursor
Mature
c-Met CTF
c-Met ICD
β-actin
170
140
55
50
42

Figure S8

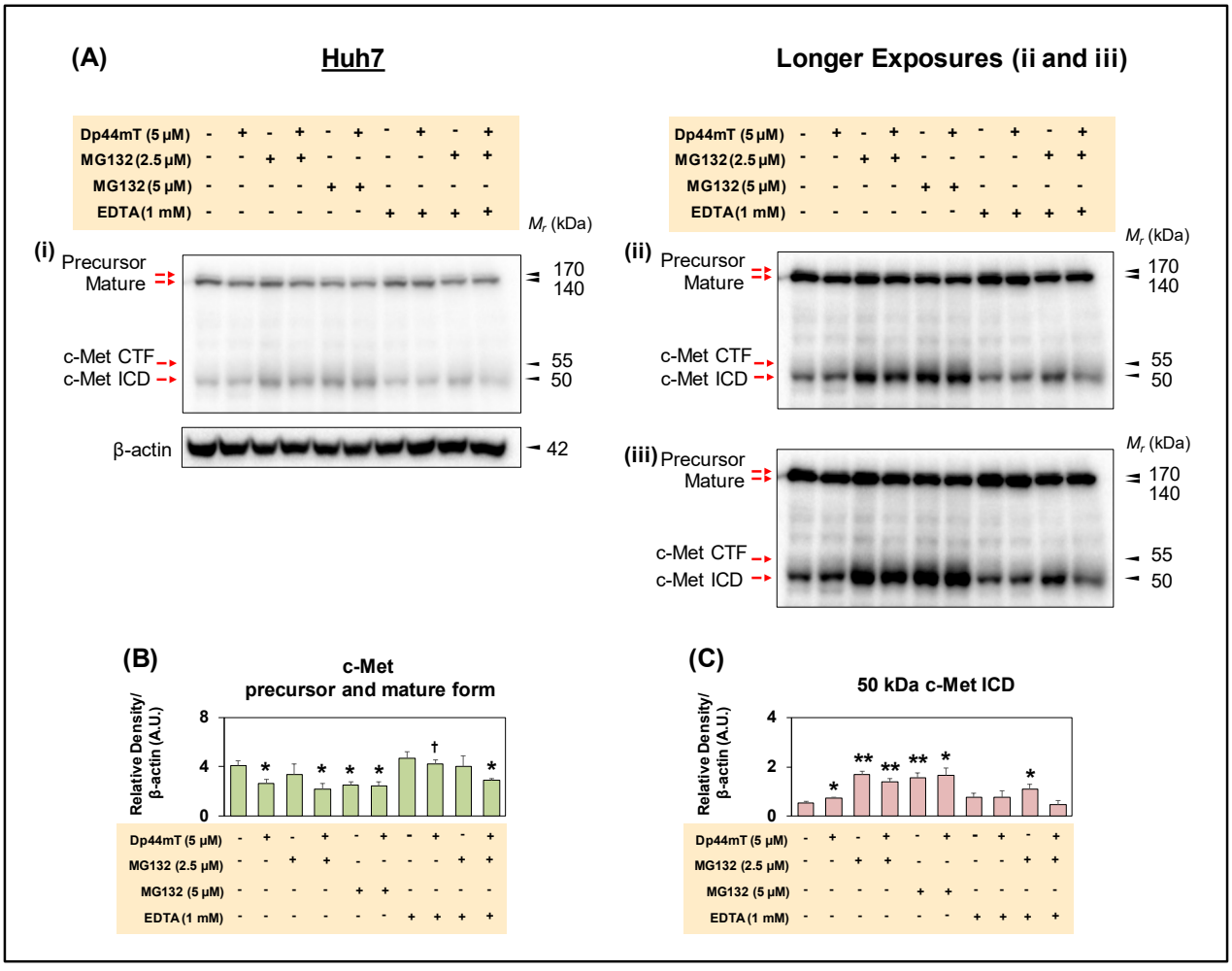
(A)
Huh7
Longer Exposures (ii and iii)
Dp44mT (5 μM)
MG132 (2.5 μM)
MG132 (5 μM)
EDTA (1 mM)
M_r (kDa)
(i)
Precursor
Mature
c-Met CTF
c-Met ICD
β-actin
170
140
55
50
42
(ii)
(iii)
(B)
c-Met
precursor and mature form
Relative Density/
β-actin (A.U.)
(C)
50 kDa c-Met ICD

Figure S9

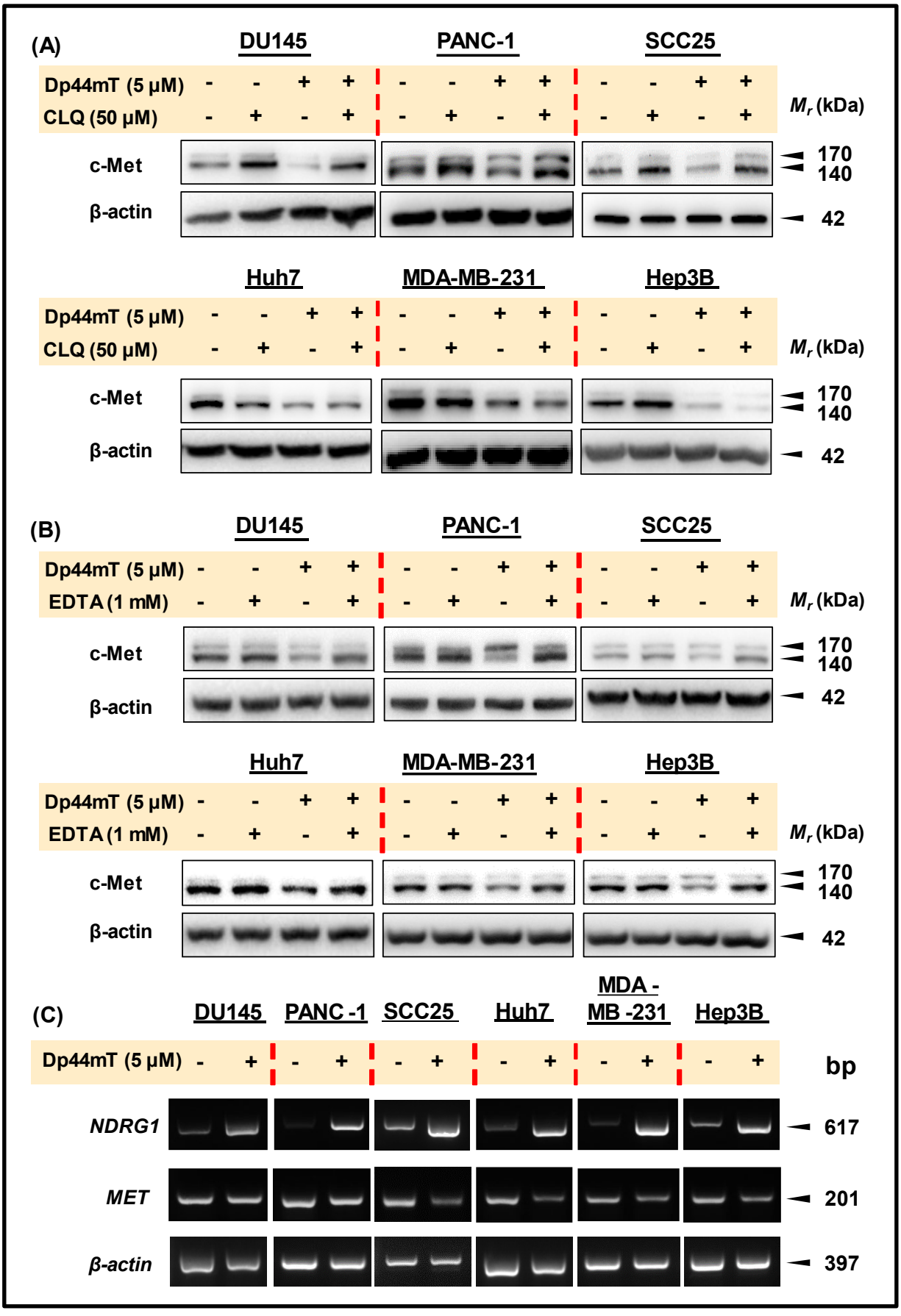
(A)
DU145
PANC-1
SCC25
Dp44mT (5 μM)
CLQ (50 μM)
M_r (kDa)
c-Met
β-actin
170
140
42
Huh7
MDA-MB-231
Hep3B
(B)
EDTA (1 mM)
(C)
MDA -MB -231
bp
NDRG1
MET
β-actin
617
201
397

Figure S10