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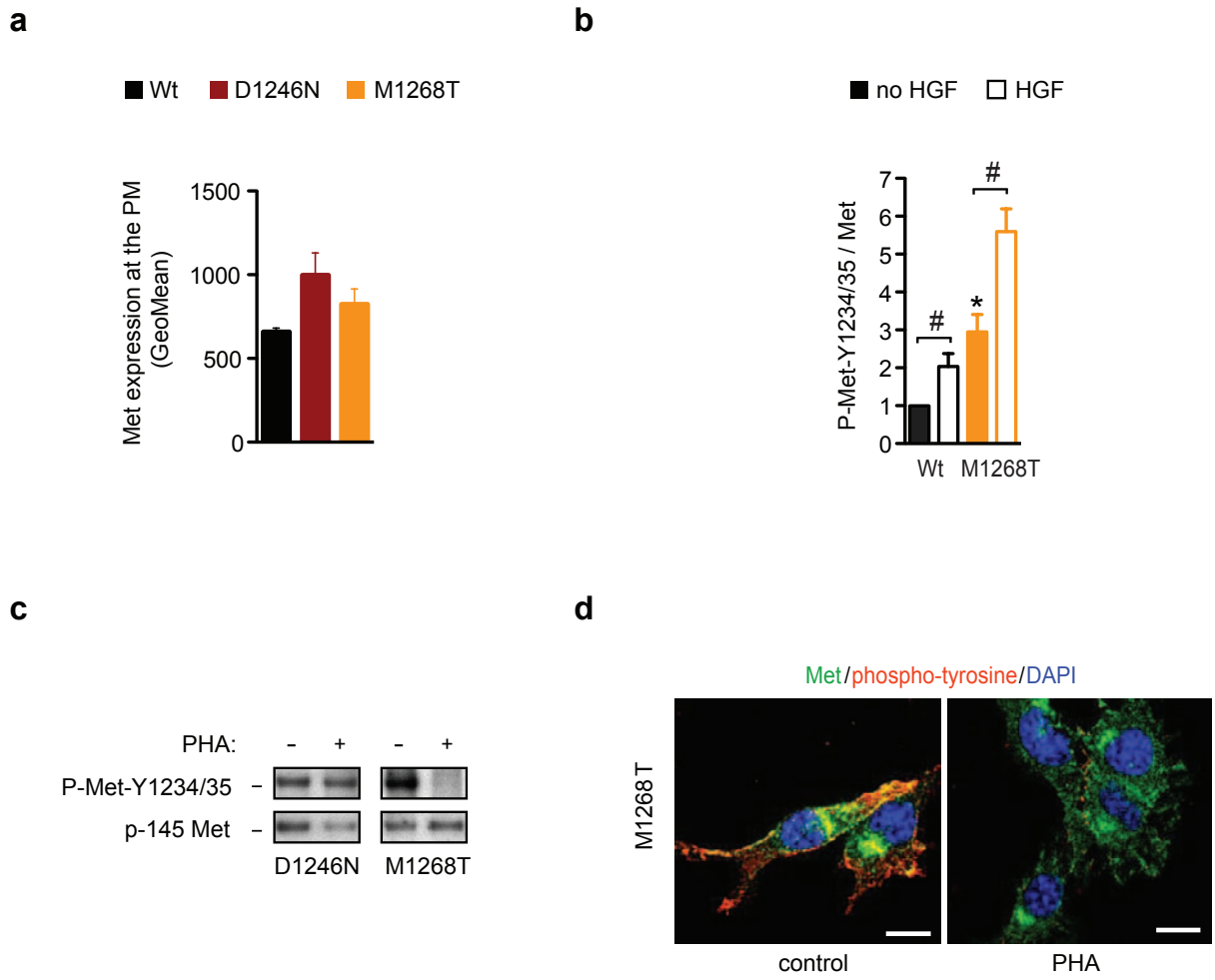

Figure S1 (a) Analysis by flow cytometry of the level of Met expression at the plasma membrane (PM) on the indicated cells. Data are mean ± SEM (n=3). (b) P-Met-Y1234-35 / Met ratios identified by densitometries from Western blots (not shown) in the indicated cells non stimulated (no HGF) or stimulated with HGF (HGF) for 15 min. Data are mean ± SEM represented in arbitrary units (n=3). (c) Western blots for phosphorylated Met (Y1234-35) and Met on cells expressing D1246N or M1268T Met mutants. Cells were treated (+) or not (-) with the specific Met inhibitor, PHA-665752 (PHA) (100nM). (d) Representative confocal sections of M1268T Met expressing cells treated, or not, with PHA (100nM), stained for Met (green), phospho-tyrosine (red) and DAPI (blue). *,# $p<0.05$. Bars: 10 µm.

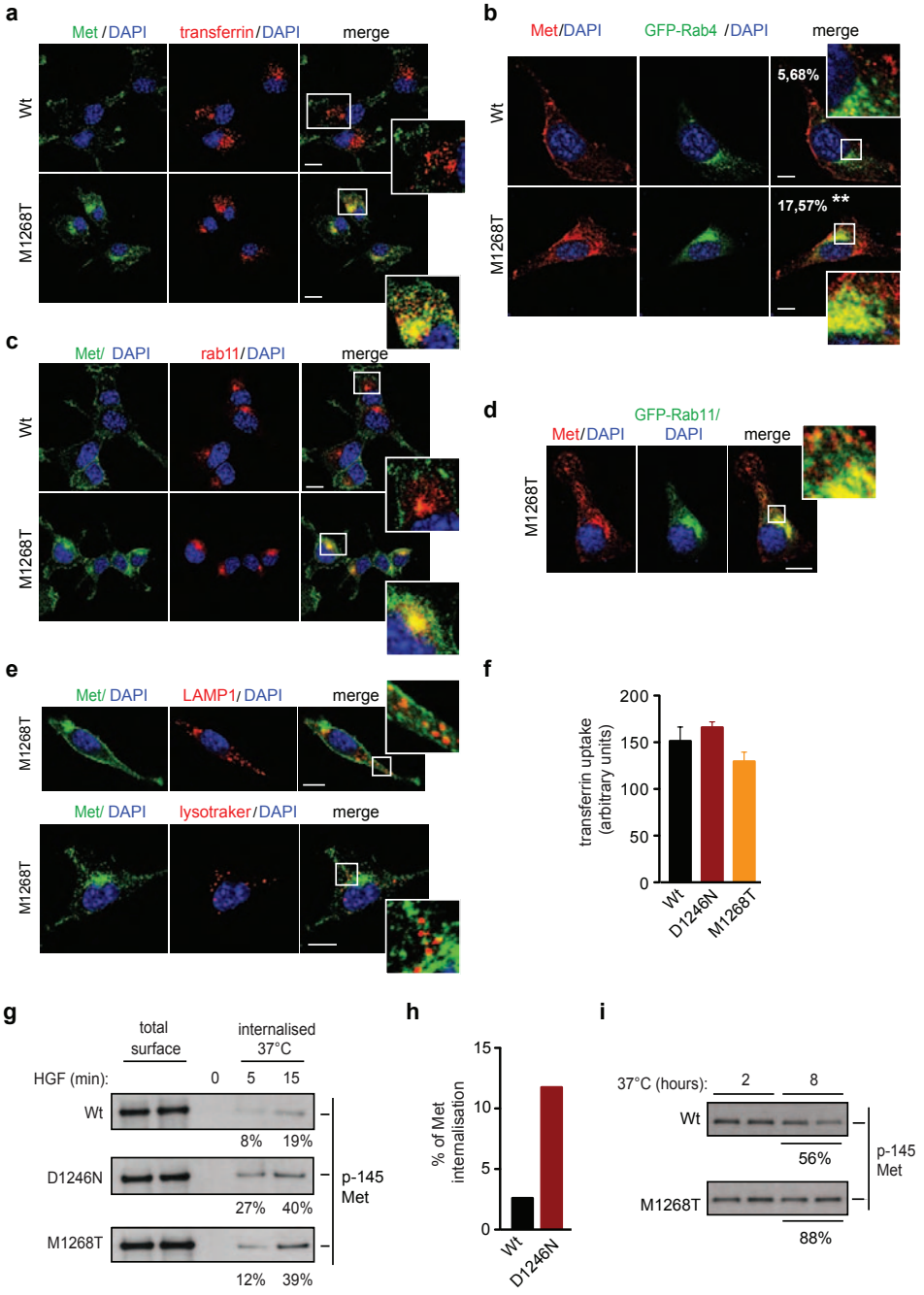

Figure S2 (a) Confocal sections of indicated cells stained for Met (green), transferrin (red) and DAPI (blue). Cells, pre-treated with cycloheximide for 4 hours (50 µg/ml), were incubated with Cy3-transferrin for 30 min before fixation. Bars: 10 µm. (b) Confocal sections of indicated cells transfected with GFP-Rab4 (green) and stained for Met (red), and DAPI (blue). The numbers indicate the % of colocalisation between Met and GFP-Rab4 positive vesicles (mean values, n=3). Bars: 10 µm. (c) Confocal sections of indicated cells stained for Met (green), Rab11 (red) and DAPI (blue). Bars: 10 µm. (d) Confocal sections of indicated Met expressing cells transfected with GFP-Rab11 (green) and stained for Met (red) and DAPI (blue). Bars: 10 µm. (e) Confocal sections of M1268T cells stained for Met (green) and DAPI (blue). Upper panel: cells also were stained for LAMP1 (red); Lower panel: cells were incubated with lysotracker (red) 30 minutes before fixation. Bars: 10 µm. (f) Rate of Cy3-transferrin uptake in the indicated cells. Mean values ± SEM expressed in arbitrary units (n=3). (g) Biotin Internalisation Assay. Cells expressing Wt, D1246N and M1268T Met were surface biotinylated and then incubated at 37°C for 5 or 15 min with HGF (50 ng/ml). The biotin was then cleaved, and the remaining biotinylated Met was analysed by Western blotting with an anti-Met antibody. % of internalisation, calculated from densitometric analysis, is shown. (h) Mean values (n=2) of biotin internalisation assay on cells expressing Wt and D1246N Met. Cells were surface biotinylated and then incubated for 5 min at 37°C. (i) Biotinylation degradation assay. Wt and M1268T Met expressing cells were surface biotinylated and then incubated at 37°C. At the indicated times, cells were lysed and remaining biotinylated Met, after streptavidin pull-down, was analysed by Western blotting with an anti Met antibody. **p<0.01.

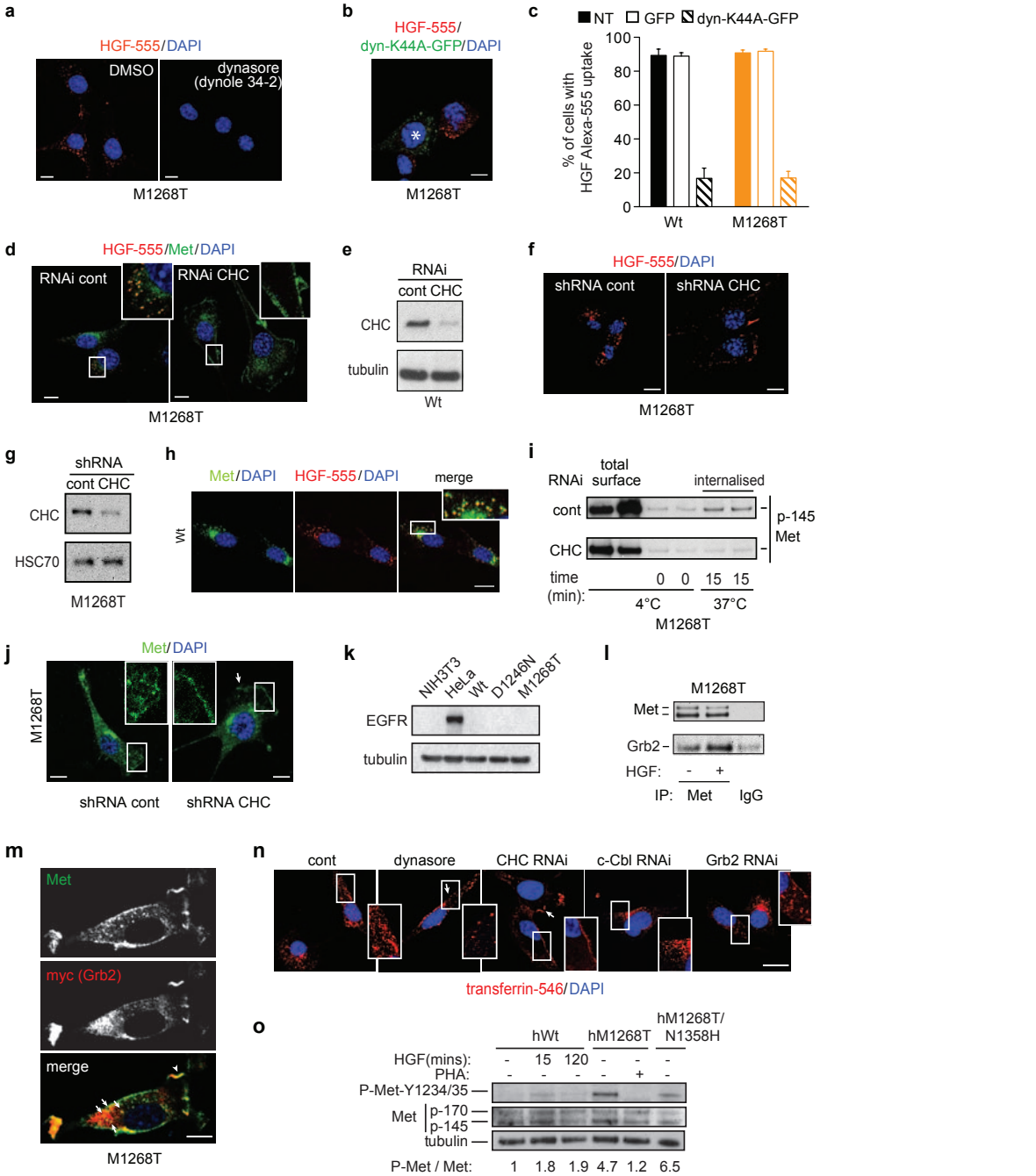

Figure S3 (a,b) Confocal sections of M1268T cells incubated with HGF-Alexa 555 for 15 min and stained for Met (green) and DAPI (blue). Bars: 10 µm. Cells were treated with DMSO, dynasore or dynole 34-2 (a) or transfected with dynamin 2 K44A-GFP (green) (b). (c) % of Wt and M1268T cells with HGF-Alexa 555 uptake when non transfected (NT) or transfected with GFP or dynamin 2 K44A-GFP. Data are mean ± SEM (n=3). (d) Confocal sections of M1268T cells transfected with control or CHC RNAi, stimulated with HGF-Alexa 555 and stained for Met (green) and DAPI (blue). Bars: 10 µm. (e) CHC and tubulin Western blots on Wt Met cells transfected with control or CHC RNAi. (f) Confocal sections of M1268T cells transduced with control or CHC shRNA, stimulated with HGF-Alexa 555, and stained for DAPI (blue). Bars: 10 µm. (g) CHC and HSC70 Western blots on M1268T cells transduced with control or CHC shRNA. (h) Confocal section of Wt cells incubated with HGF-Alexa 555, and stained for Met (green) and DAPI (blue). Bars: 10 µm. (i) Biotin internalisation assay from cells transfected with control or CHC RNAi. (n=1, in duplicate). (j) Confocal sections of cells transduced with control or CHC shRNA stained for Met (green) and DAPI (blue). Bars: 10 µm. (k) Western blots for EGFR and tubulin from the indicated cells. (l) M1268T expressing cells were subjected to Met or control IgG immunoprecipitation (IP). (m) Confocal section of M1268T cells transfected with Grb2-myc Wt and stained for Met (green), myc (red) and DAPI (blue). Grb2-Met colocalisations in vesicles (arrows) and at the plasma membrane (arrowhead). Bars: 10 µm. (n) Confocal sections of M1268T cells transfected with RNAi control, treated with dynasore or transfected with CHC, c-Cbl or Grb2 RNAi, incubated with transferrin-546 and stained for DAPI (blue). Arrows: transferrin retained at the plasma membrane. Bars: 10 µm. (o) P-Met, Met and tubulin Western blots from cells expressing human Met forms, stimulated or not with HGF or PHA. Numbers are densitometric values for P-Met/Met.

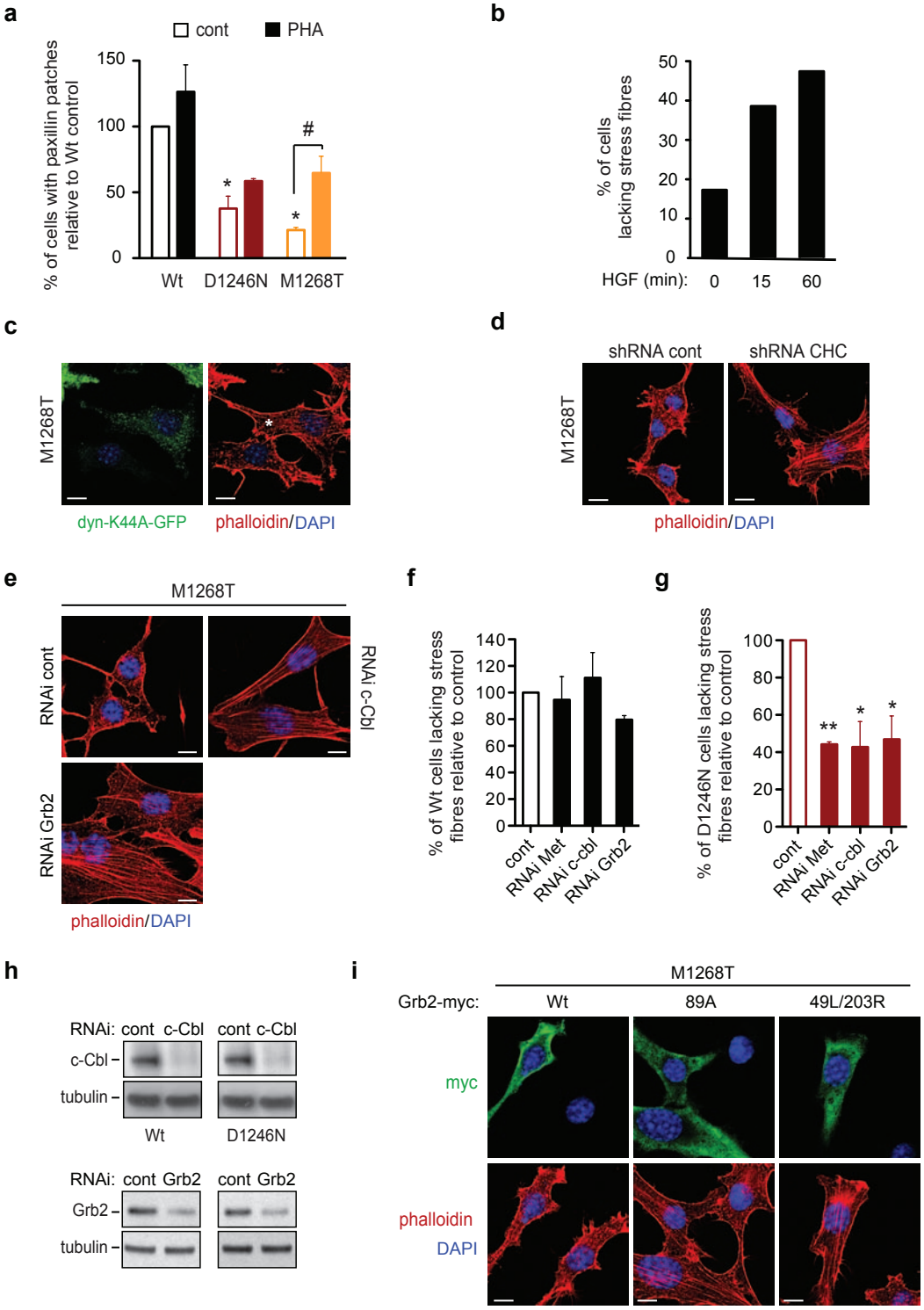

Figure S4 (a) % of cells showing marked patches of paxillin, determined on confocal pictures (not shown), counted in indicated cells treated with DMSO or PHA (100nM) for 60 min. Data are mean ± SEM (n=3). (b) % of Wt cells lacking stress fibres. After stimulation for 15 and 60 min with HGF (50 ng/ml), cells were fixed and stained with Cy3-phalloidin. (c) Confocal sections of M1268T Met expressing cells transfected, or not, for 24 hours with dynamin 2 K44A-GFP dominant-negative mutant (green) and then stained with Cy3-phalloidin (red) and DAPI (blue). * indicates transfected cell. Bars: 10 µm. (d,e) Confocal sections of M1268T Met expressing cells, transduced with control or Clathrin Heavy Chain (CHC) shRNA (d) or transfected with control, c-Cbl or Grb2 RNAis (e), then stained with Cy3-phalloidin (red) and DAPI (blue). Bars: 10 µm. (f-g) % of Wt (f) or D1246N (g) Met expressing cells lacking stress fibres when transfected with Met RNAi, Grb2 RNAi or c-Cbl RNAi. Data are mean ± SEM (n=3) and are normalised to control RNAi. (h) Wt and D1246N Met expressing cells were transfected with Cont, Grb2 or c-Cbl RNAis. Western blots of Grb2, c-Cbl and tubulin are shown. (i) Confocal sections of M1268T Met expressing cells transfected with myc-tagged Grb2 constructs (Wt, dominant negative mutants 89A and 49L/203R) and stained for myc (green), Cy3-phalloidin (red) and DAPI (blue). Bars: 10 µm. #,* p<0.05; **p<0.01.

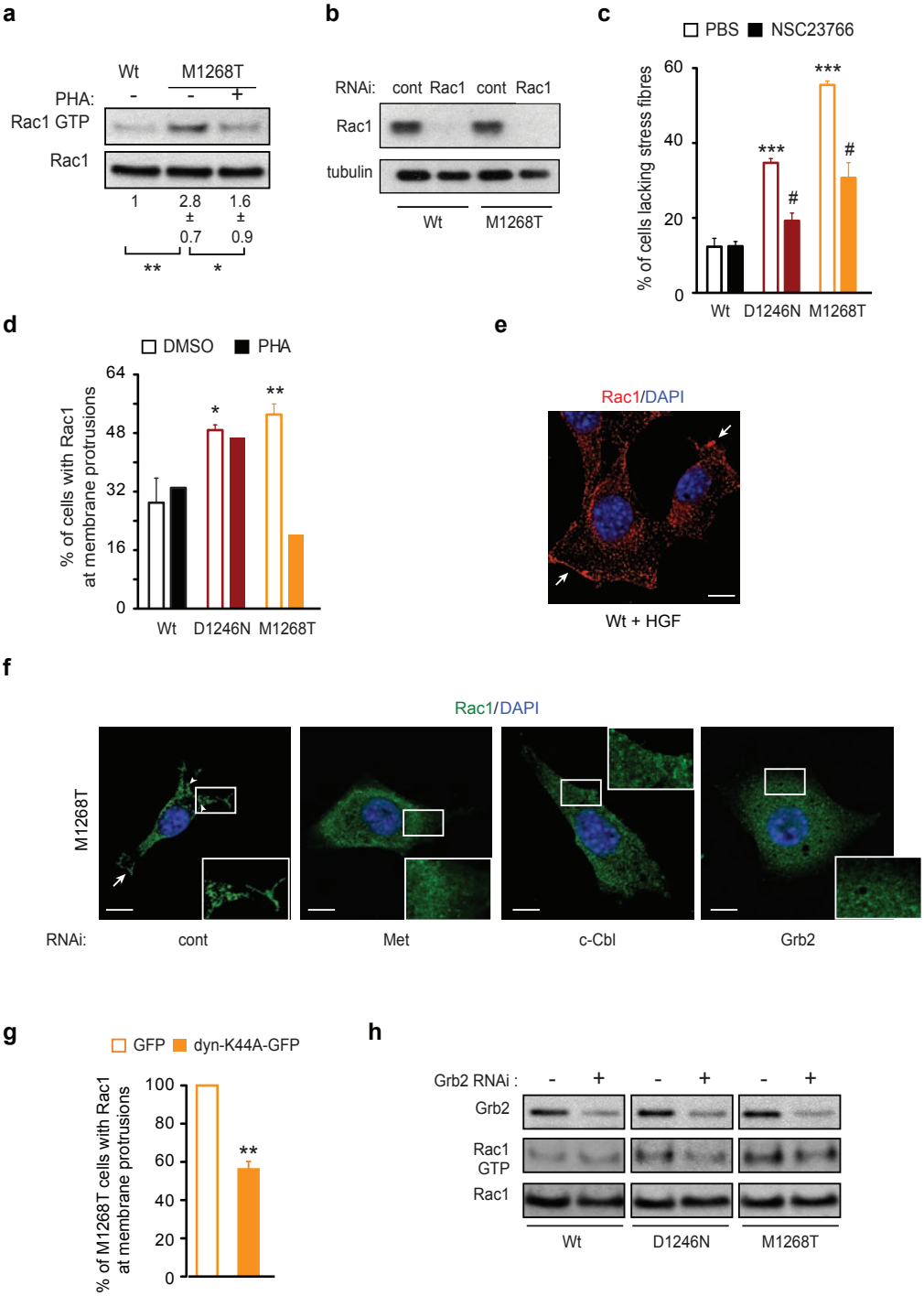

Figure S5 (a) Levels of Rac1-GTP measured by GST-CRIB pull down and of total Rac1 on Wt and M1268T expressing cells treated (+) or not (-) with PHA (100nM) for 90 min. Numbers represent fold increases of Rac1-GTP between M1268T and Wt and are mean ± SEM (n=3). (b) Western blots for Rac1 and tubulin from Wt or M1268T Met expressing cells transfected with control or Rac1 RNAis. (c) % of indicated cells lacking stress fibres after treatment for 60 min with PBS or with the Rac inhibitor NSC23766. Data are mean ± SEM (n=3). (d) Indicated cells were treated with DMSO or PHA (100nM) for 60 min and stained for Rac1 (red) and DAPI (blue). % of cells with marked Rac1 staining present at membrane protrusions. Data are mean ± SEM for DMSO (n=3) and mean for PHA (n=2). (e) Confocal section of Wt Met expressing cells after 30 min of HGF stimulation (50 ng/ml), fixed and stained for Rac1 (red) and DAPI (blue). Arrows indicate Rac1 at membrane protrusions. Bar: 10 µm. (f) Confocal sections of M1268T Met expressing cells transfected with cont, Met, c-Cbl or Grb2 RNAis, fixed and stained for Rac1 (green) and DAPI (blue). The arrow indicates Rac1 at membrane protrusions and the arrowheads Rac1 in vesicles. Bars: 10 µm. (g) % of M1268T Met expressing cells with marked Rac1 staining present at membrane protrusions when transfected with dyn-K44A-GFP. Data are mean ± SEM (n=3). (h) Levels of Rac1-GTP measured by GST-CRIB pull down and of total Rac1 on the indicated cells transfected with control (-) or Grb2 (+) RNAi. Western blots for Grb2, Rac1-GTP and Rac1 total are shown. *,#p<0.05; **p<0.01, ***p<0.001.

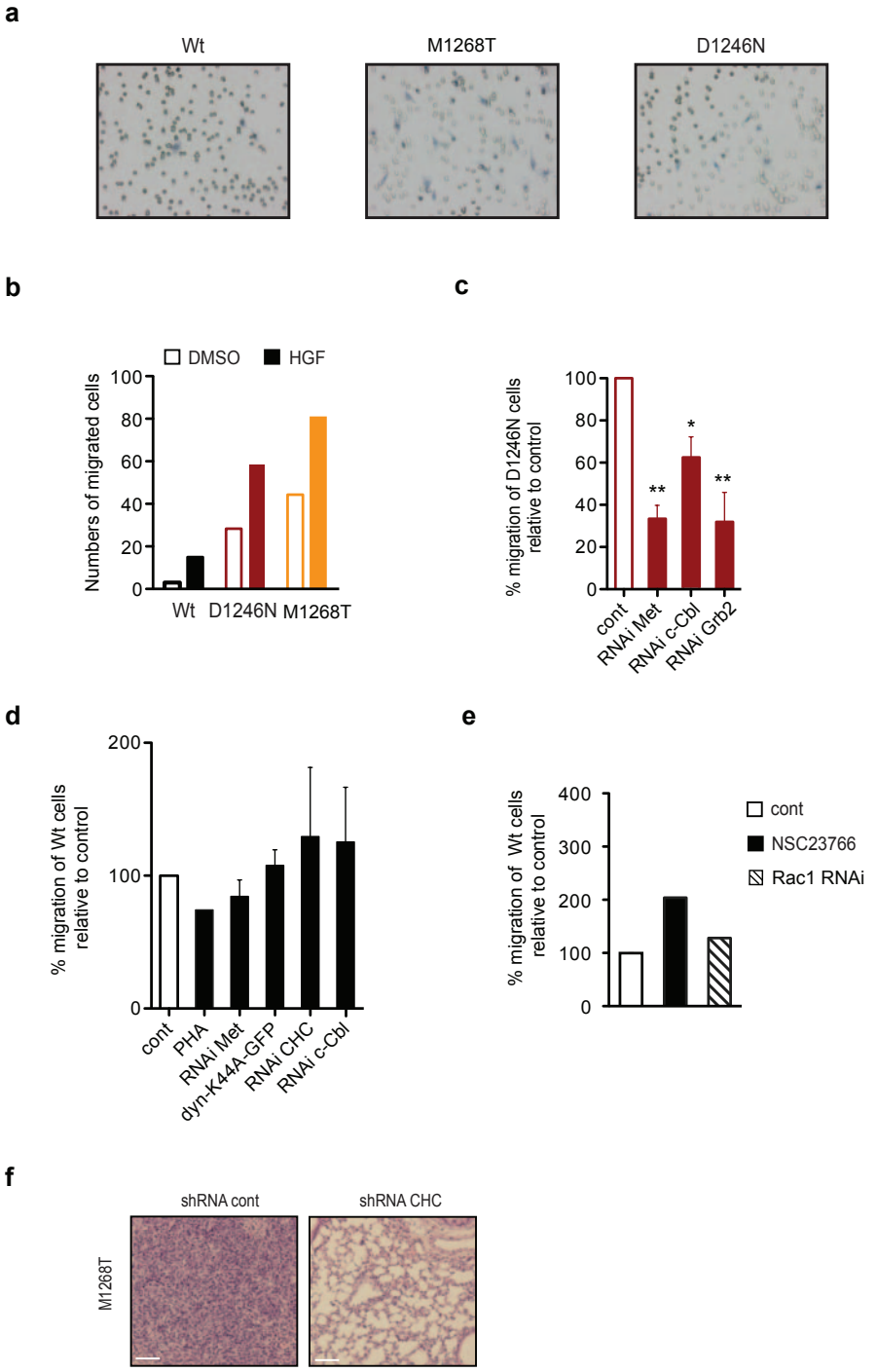

Figure S6 (a) Representative field of migratory cells that went through the membrane after the Transwell assay (see Methods). (b) Average number of cells that have migrated through Transwells over 2 hours of incubation ± HGF gradient (50 ng/ml). Data are mean between triplicates (n=1). (c) % of D1246N Met expressing cells, transfected with control, Met, c-Cbl or Grb2 RNAi, that have migrated through Transwells. Data are mean ± SEM (n=3, experiments done in triplicate). (d) % of Wt Met expressing cells that have migrated in Transwell assays when treated with PHA (100nM) (n=2), transfected with Met RNAi (n=3), dynamin 2 K44A-GFP dominant-negative mutant (n=3), Clathrin Heavy Chain (CHC) (n=3) or c-Cbl RNAis (n=4), over appropriate controls (DMSO, GFP vector, RNAi control respectively). Data are mean ± SEM and mean for PHA. (e) % of Wt Met expressing cells that have migrated in Transwell assays when treated with the Rac inhibitor NSC23766 (100 μM) or transfected with Rac1 RNAi over appropriate controls (DMSO and RNAi control respectively). (n=2, each experiment done in triplicate). (f) Haematoxylin/eosin staining of the paraffin embedded lung sections from mice (from Fig. 6d) 10 days after being injected intravenously with M1268T Met expressing cells transduced with control or CHC shRNA. The lungs from mice grafted with M1268T Met expressing cells transduced with shRNA control were infiltrated with tumour cells and almost no healthy area could be observed. Conversely, lungs from mice grafted with M1268T Met expressing cells transduced with shRNA CHC had no, or very few, tumour foci. Scale bar: 100 μM. *p<0.05; **p<0.01.

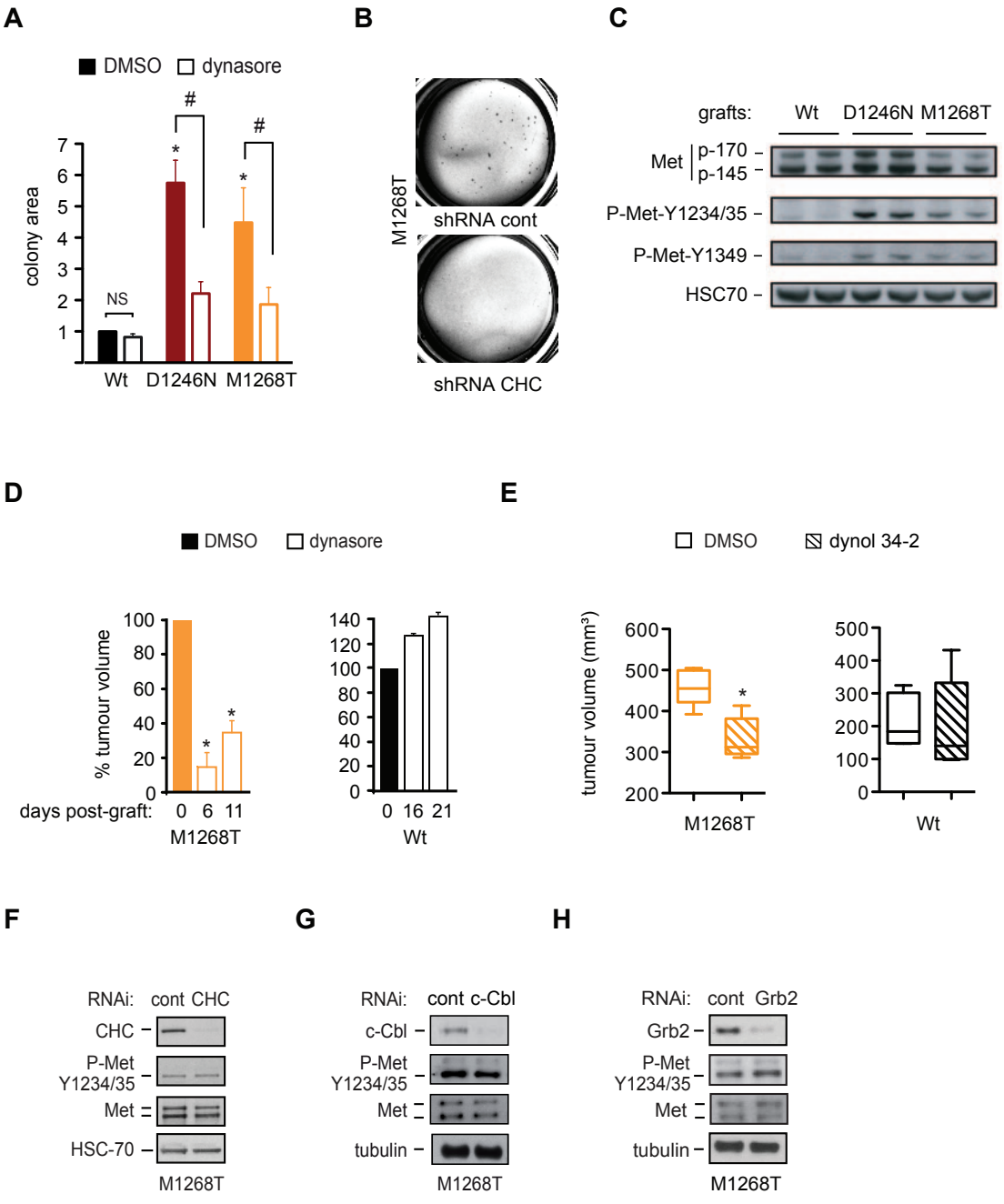

Figure S7 (a) The indicated cells were cultured in soft agar. After day 5, DMSO or dynasore were added daily to the medium. The graph represents the average colony area at day 9 (pictures shown Fig. 1a). Data are mean ± SEM (n=3). (b) M1268T Met expressing cells transduced with control or CHC shRNA. Pictures at day 9. (c) Western blots of lysates from resected tumour tissues (Fig. 7d) for phosphorylated Met (Y1234-35 and Y1349), Met and HSC-70. (d) Wt and M1268T Met expressing cells were injected subcutaneously into the right flank of nude mice. From the following day DMSO, or dynasore, were applied topically over the right flank of the mice, daily. Graphs present % of tumour volume after the indicated time of post-graft. Data are mean ± SEM of n=5 mice. (E) Wt and M1268T Met expressing cells were injected subcutaneously into the right flank of nude mice. When tumours had reached 30-50 mm^3, DMSO or dynole 34-2 (30 μM) were applied topically over the surface of the tumour mass each day for five days. The results are represented as box and whisker plots, a histogram-like method for displaying upper and lower quartiles and maximum and minimum values in addition to median. They represent the tumour volume after seven days of treatment. Data are mean ± SEM of n=5 mice. (f-h) M1268T Met expressing cells were transfected with control, CHC, c-Cbl or Grb2 RNAis. Western blots for CHC (f), c-Cbl (g), Grb2 (h), P-Met-Y1234-35, Met, HSC-70 or tubulin are shown.*,#p<0.05; NS: not significant.

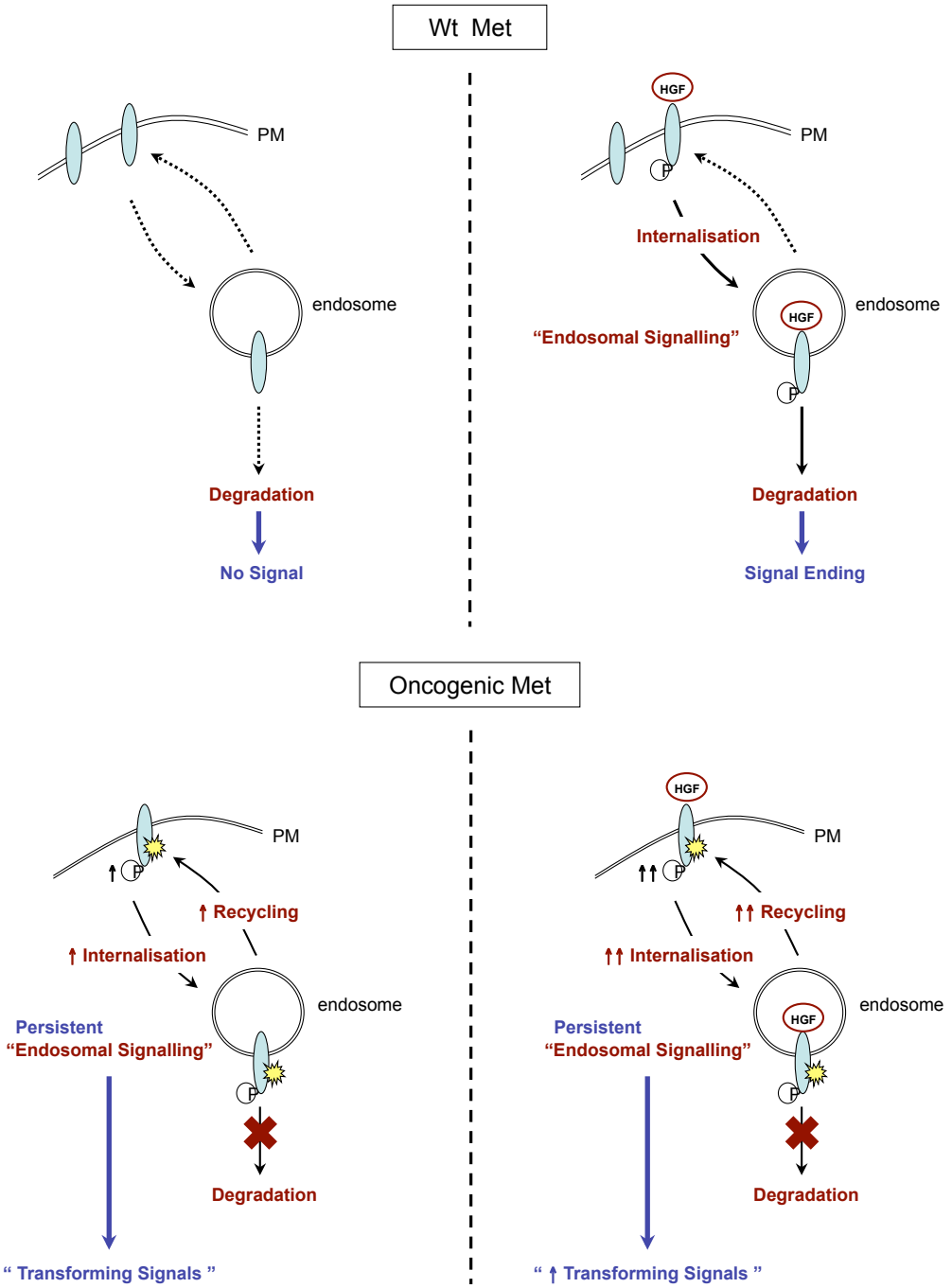

Figure S8 Model of Met Wt and oncogenic D1246N and M1268T mutants signalling. Top left panel: In the absence of ligand (HGF), Wt Met is inactive and is not or poorly internalised. Little or no signal generation occurs. Top right panel: Ligand binding induces Wt Met activation and internalisation followed by recruitment on endosomes. From here it remains active and signals ("endosomal signalling") but Wt Met gets degraded progressively, leading to a restricted period of signal generation and the "signal ending". Lower left panel: Oncogenic D1246N and M1268T mutations (in the kinase domain of Met) lead to constitutive activation on the one hand, and upregulated internalisation on the other hand. In addition, instead of being degraded, the mutants constitutively recycle back to the plasma membrane. These events result in an increased accumulation of Met mutants on endosomes, which leads to "persistent endosomal signalling". We propose that signal specificity (generated through endosomal signalling) and signal persistence (due to the net outcome which results from the mutants' constitutive activation, shuttling between the plasma membrane and endosomes and their impaired degradation) together constitute the "transforming signals" which lead to transformation. Lower right panel: The ligand (HGF), by increasing the activation level of Met mutants while increasing their level of internalisation, further upregulates the level of endosomal signalling, thereby enhancing the "transforming signals". PM, plasma membrane.

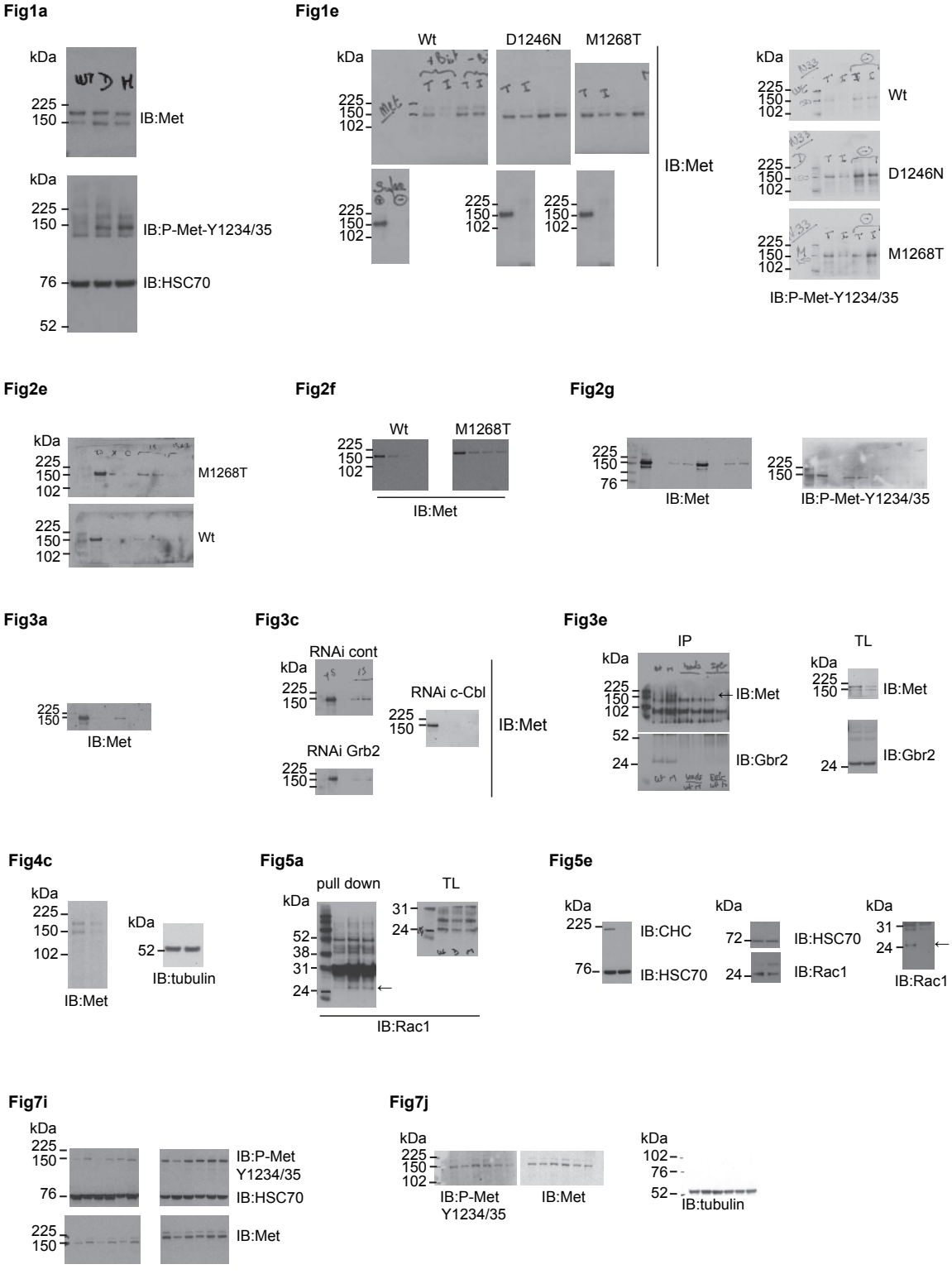

Figure S9 Blots from the indicated figures

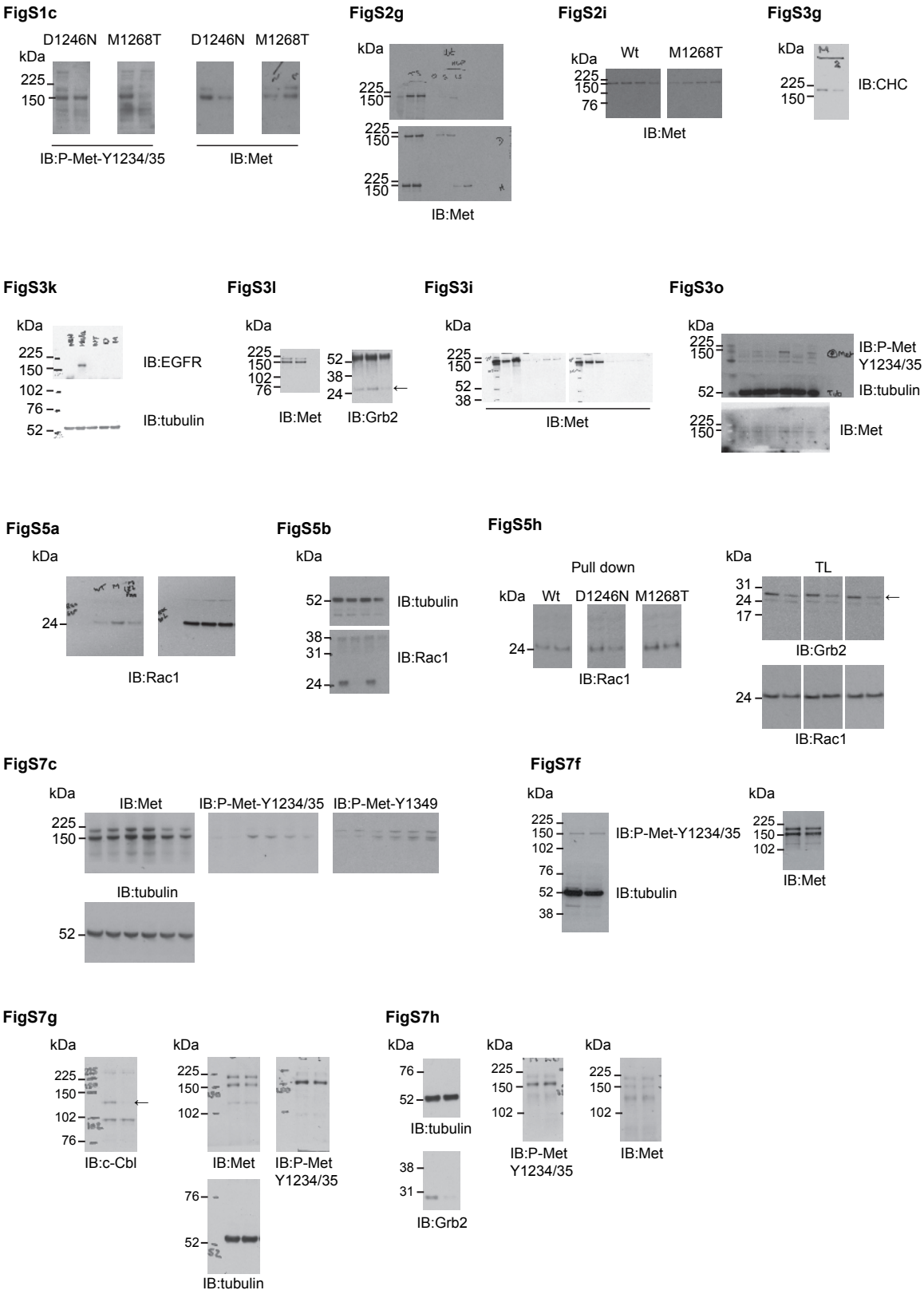

Figure S10 Blots from the indicated supplementary figures