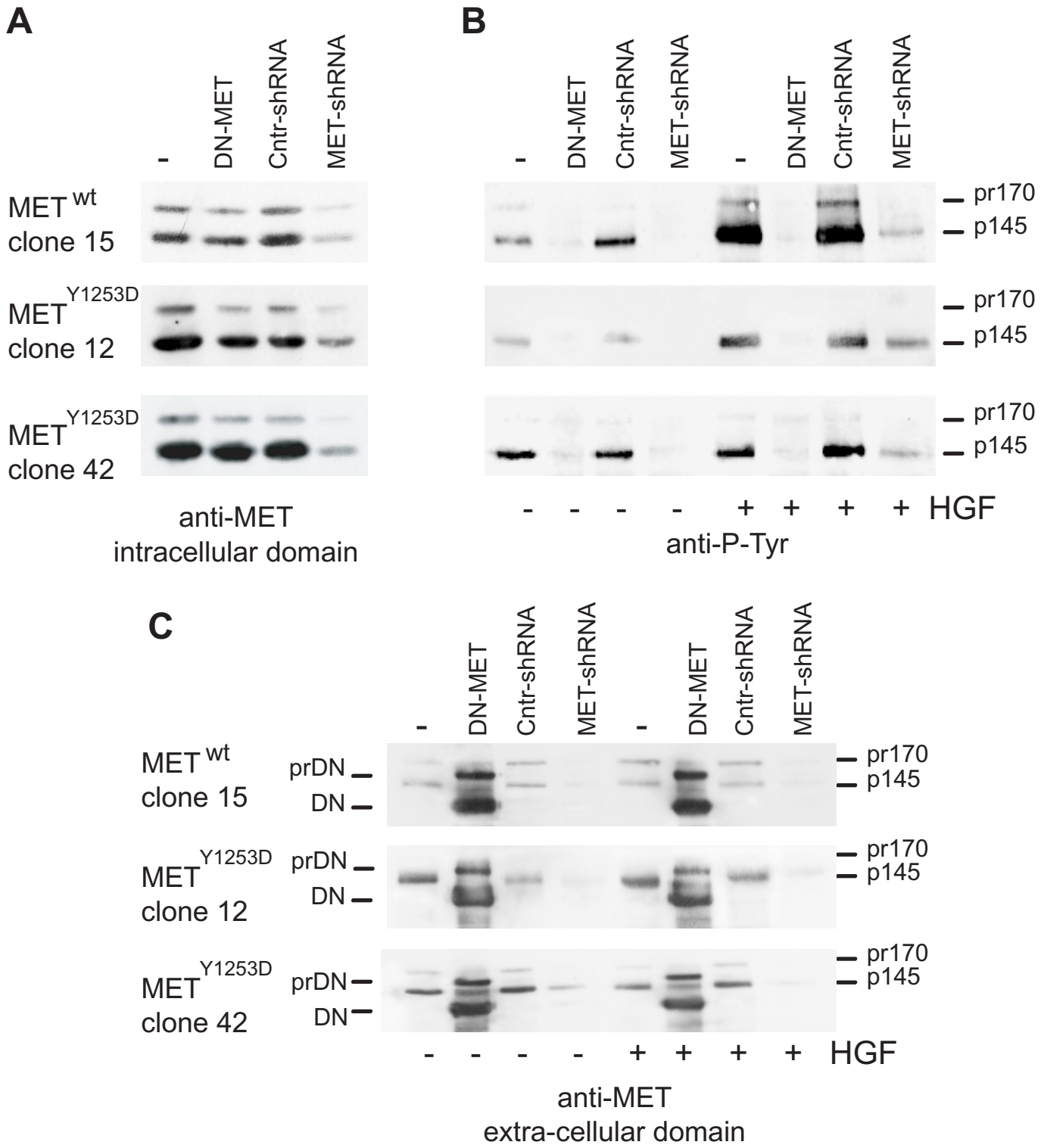

Figure S4

Expression and activation of the MET receptor in MET overexpressing HOB clones after the stable addition of either MET-specific sh-RNA or dominant-negative MET receptor.

Either the MET-shRNA, or a control point mutated shRNA (Cntr-shRNA) or a dominant-negative form of the MET receptor (DN-MET), where the cytoplasmic domain is substituted by the GFP protein, were expressed in the bulk unselected clone populations using LV vectors. After immunoprecipitation with anti-MET antibodies, proteins extracted from clones were labelled with MET antibody against the human COOH terminal peptide of the receptor (A) or anti-phosphotyrosine (P-Tyr) antibodies (B). As expected only the MET-shRNA consistently reduced MET receptor expression. Both DN-MET and MET-shRNA strongly reduced not only the constitutive activation of the MET receptor, but also its enhanced phosphorylation detectable 15 minutes after the addition of 100 ng/ml of its ligand Hepatocyte Growth Factor (HGF). In (C) the expression of DN-MET is demonstrated by re-probing the same blots with a MET monoclonal antibody directed against the MET protein extracellular domain. In DN-MET expressing cells, antibody allowed the visualization of the MET β chain (p145) and its precursor (pr170) expressed thanks to the bicistronic cassette and of the DN-MET β chain (pDN) and its precursor (prDN).

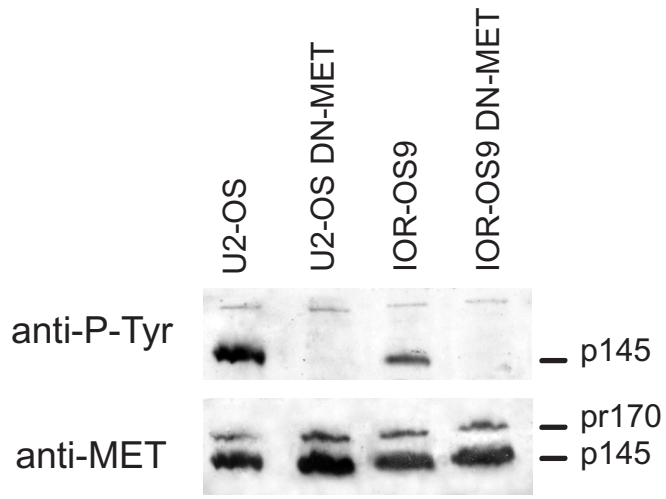

Figure S5

Expression and activation of the MET receptor in MET overexpressing osteosarcoma cell lines (U2-OS and IOR-OS9) after the stable addition of dominant-negative MET receptor.

A dominant-negative form of the MET receptor (DN-MET), where the cytoplasmic domain is substituted by the GFP protein, was expressed in the bulk unselected cell line populations using LV vectors. After immunoprecipitation with anti-MET antibodies, proteins extracted from clones were labelled anti-phosphotyosine (P-Tyr) antibodies (upper panel). The DN-MET abolished the constitutive activation of the MET receptor β chain (p145). The blot was re-probed with MET antibody against the human COOH terminal peptide of the receptor (lower panel) , which allowed the visualization not only of the MET β chain (p145) but also of its precursor (pr170)