Supplemental Materials and Methods

Gelatin Zymography

Xenograft tumors from inoculating H460 cell line together with H460-pBMN vector control and H460-pBMN harboring Met, HGF, and HGF/Met co-overexpressing cell lines (clones 4-8 and 4-9) were homogenized in lysis buffer (1% Triton X-100, 10% glycerol, 50 mM HEPES, 150 mM NaCl, 1.5 mM $MgCl_2$, 10 mM sodium pyrophosphate, 100 mM NaF, 10 mM $Na_4P_2O_4$, 1 mM EDTA, 10 mg/ml aprotinin, 10 mg/ml leupeptin, 100 mg/ml phenylmethylsulfonyl fluoride, and 1 mM sodium orthovanadate), and the lysates were cleared by centrifugation. The conditioned media from cell lines were collected after 48 hours of serum starvation of the cells. Proteins (30 μg per sample) were diluted in nonreduced SDS sample buffer and separated by electrophoresis in 10% SDS–polyacrylamide gels copolymerized with 1 mg/ml gelatin (for MMP-2 and MMP-9 activity detection). Gels were washed with 2.5% Triton X-100 for 1 hour and then twice in Tris-HCl (pH 8.0) for 15 minutes at room temperature. The gels were incubated with substrate buffer (50 mM Tris-HCl [pH 8.0] and 10 mM $CaCl_2$) for 18 hours at 37°C. The gels were then stained with Coomassie brilliant blue and destained until the clear bands of lysis appeared. To confirm the lytic bands, gels were treated with 20 mM EDTA (a metalloproteinase inhibitor) in the substrate buffer for 18 hours at 37°C.

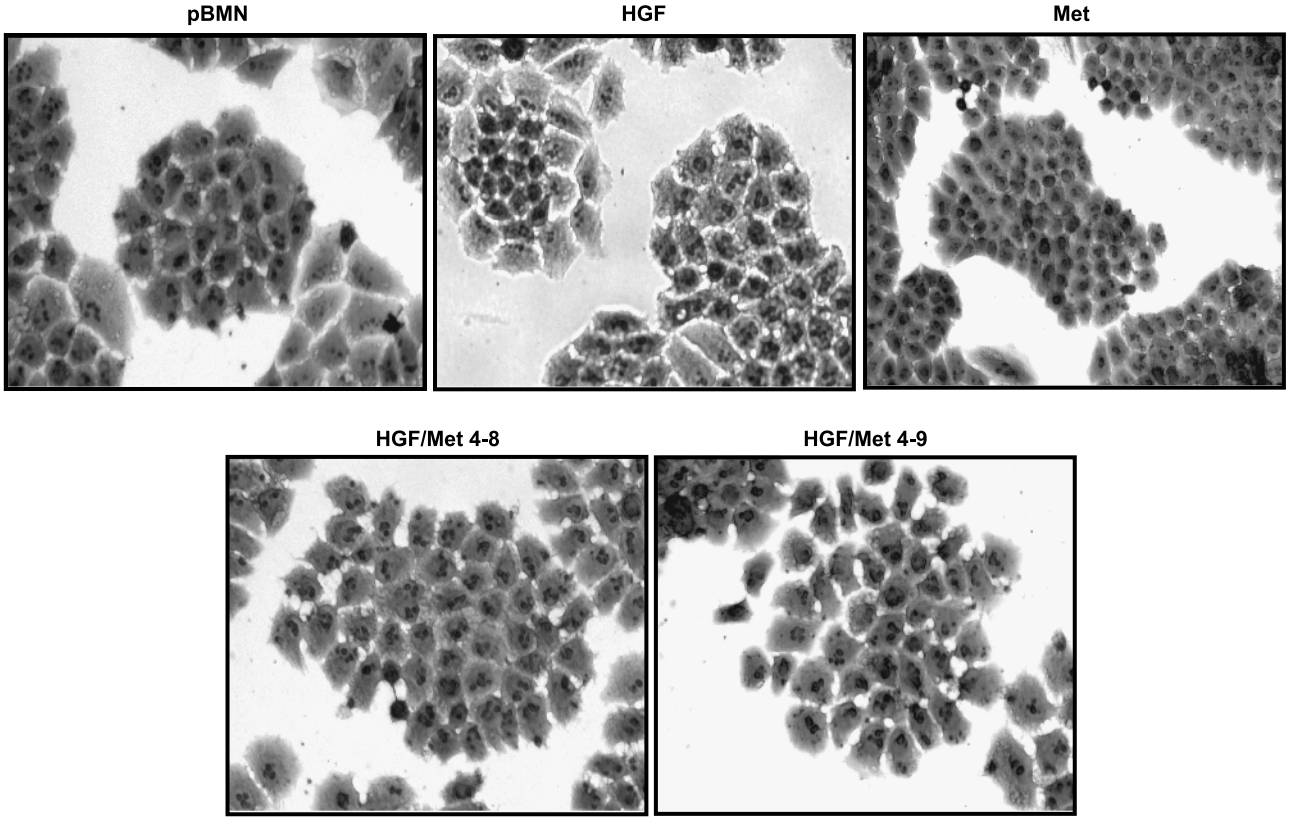

Figure W1. Overexpression of HGF in H460 cell lines had no effect on cell morphology. The morphology of HGF-, Met-, and HGF/Met–overexpressing H460 cell lines were visualized after staining them with crystal violet (0.2%).

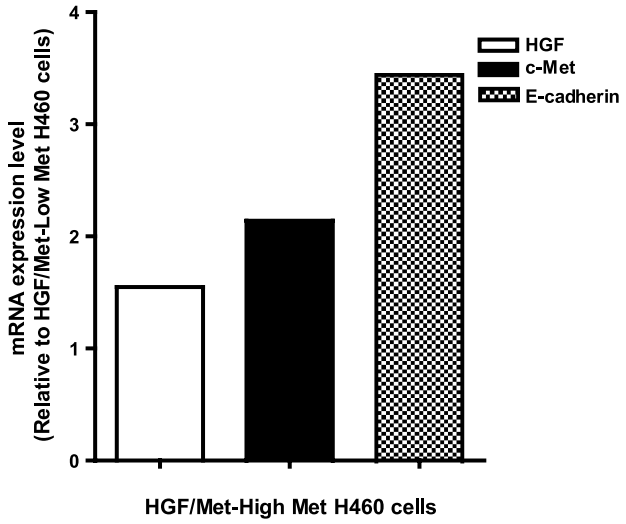

Figure W2. Expression of E-cadherin in high hHGF/Met–expressing cells. The mRNA expressions of hHGF, c-Met, and E-cadherin in HGF/Met 4-9 H460 cell lines with a high expression level of c-Met were evaluated using RT-qPCR and compared with the expression level of the same genes in HGF/Met 4-9 H460 cell lines with a low expression level of c-Met.

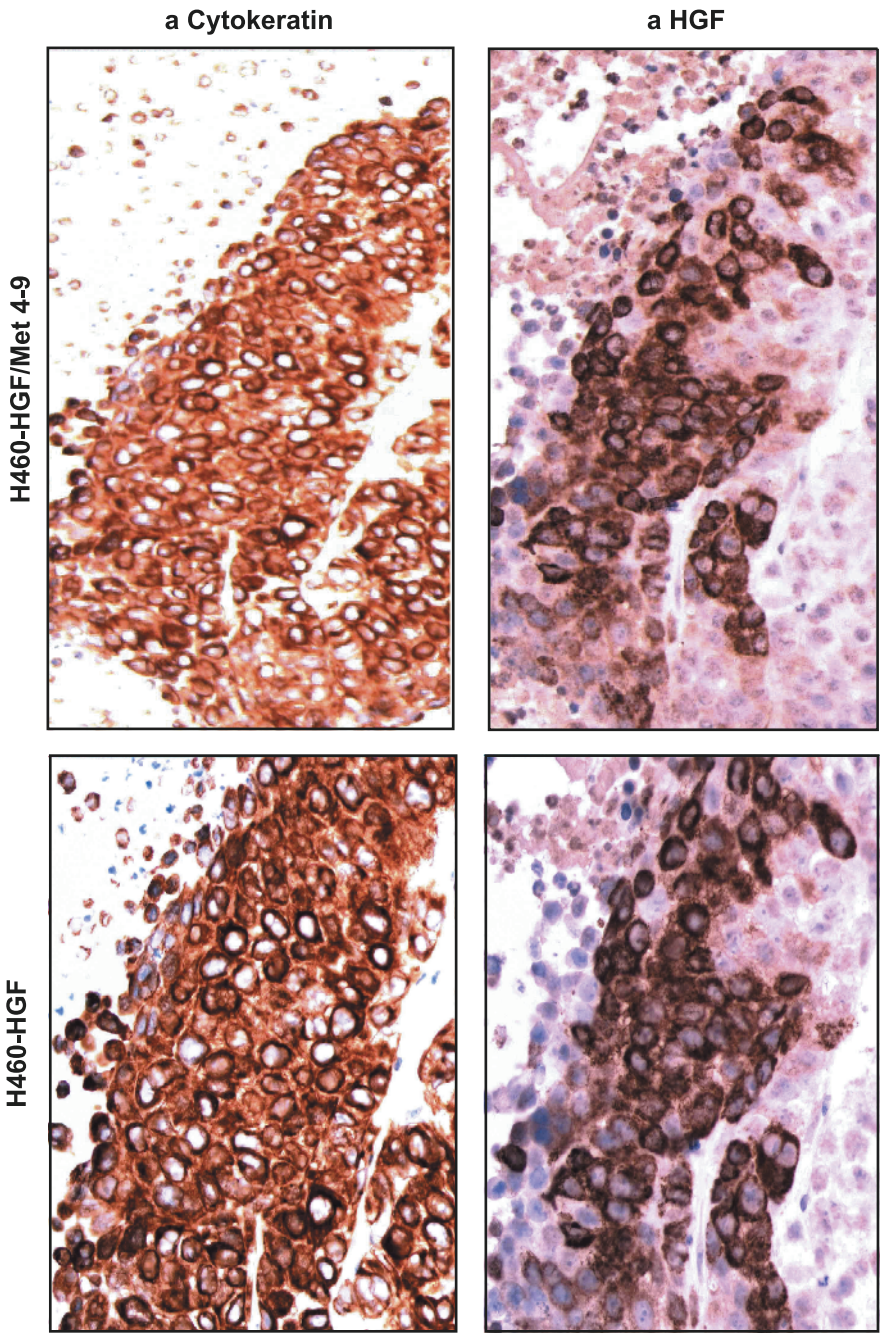

Figure W3. HGF and cytokeratin expression in xenograft tumors appeared to be homogenous, and cells are considered to be epithelial. Xenograft tumors from inoculation of stable HGF- and HGF/Met 4-9–overexpressing H460 cell lines were analyzed by immunohistochemistry for the hHGF (R&D Systems), and human cytokeratin Cam 5.2 (BD Company) specific antibodies at a magnification of ×400.

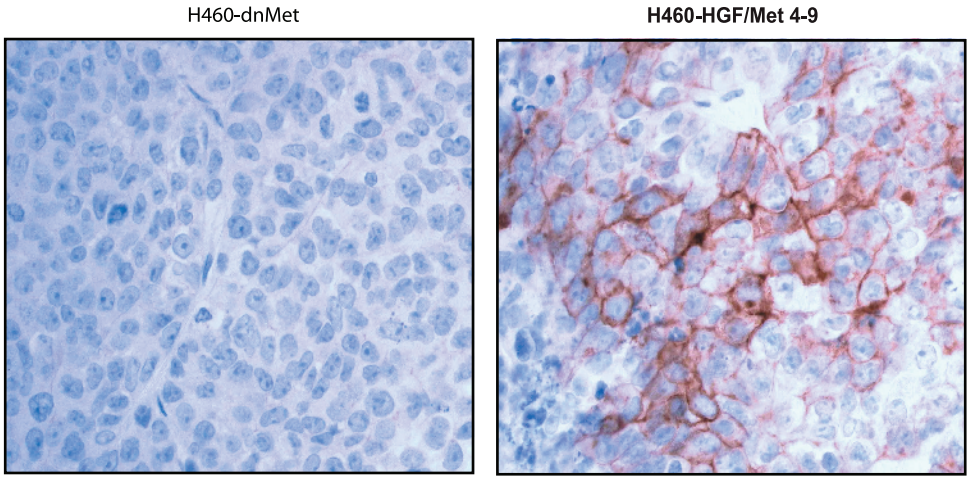

Figure W4. Xenograft tumors from inoculation of stable dnMet and co-overexpressing HGF/Met-H460 (clone 4-9) cell lines were analyzed by immunohistochemistry for the activated receptor using phospho-Met–specific antibody (pMet) at a magnification of ×400.

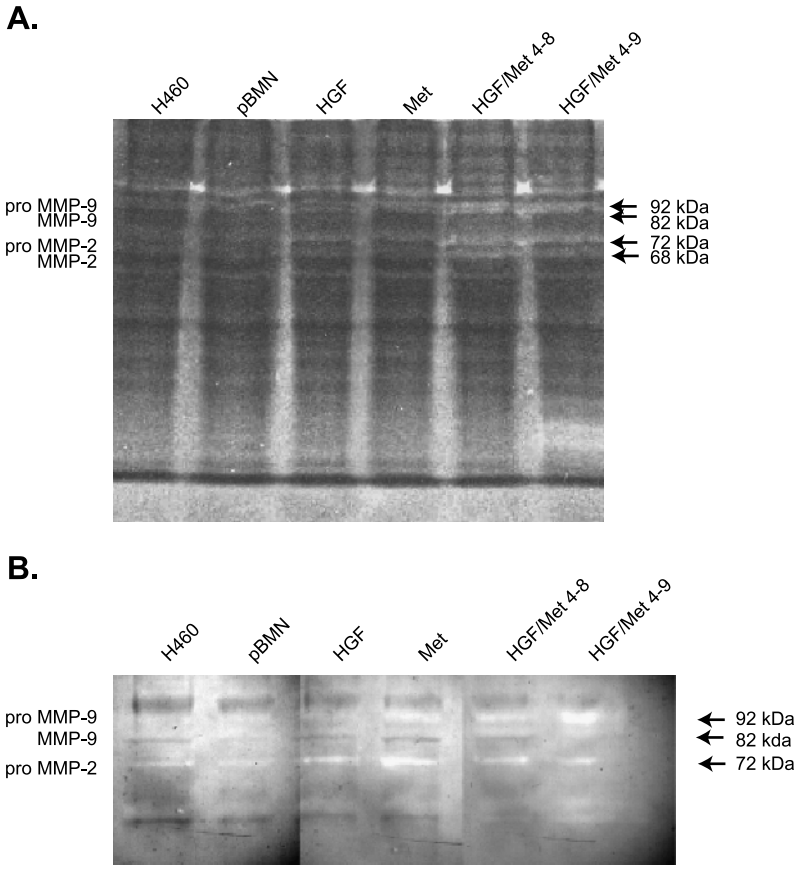

Figure W5. Enhanced activity of MMP-9 and MMP-2 in HGF/Met–overexpressing H460 cell lines. (A and B) Using gelatin zymography in both *in vitro* (A) and *in vivo* (B), we demonstrated higher levels of both proactive and active form of MMP-9 (92 and 82 kDa, respectively) and MMP-2 (72 and 68 kDa, respectively) when both HGF and c-Met were overexpressed compared with pBMN control and parent H460. The bands show the lytic zones.