Supplemental information

Figure S1. HGF stimulates the expression of pro-metastatic gene Ezrin and thereby metastasis in 37-7 melanoma cells. (a) The protein levels of Ezrin were analyzed by western blot in 37-7 melanoma cells treated with 30 nM HGF for the indicated time. (b) The Western blot analysis of 37-7 cells stably transfected with HGF (HGF) or HGF plus Met (HGF/Met) expressing vector; c, as empty vector control (top panel); Gross pulmonary metastasis in 37-7 cells stably transfected with HGF (HGF, n=6) or HGF plus Met (HGF/Met, n=6) expressing vector and empty vector control (C, n=6) (bottom panel) . (c, d) Ezrin mediates the regulation of autocrine Met signaling-induced metastasis. (Top panels) Western blot analysis of 37-7 HGF (c) or 37-7 HGF/Met (d) cells transfected with vector (shc), Ezrin shRNA (sh1, sh2) expressing constructs. (Bottom panels) 37-7 HGF (c) (shc, n=6; sh1, n=6; sh2, n=8) or 37-7 HGF/Met (d) (shc, n=6; sh1, n=7; sh2, n=9) cells transfected with vector (shc) and Ezrin shRNAs (sh1, sh2) expressing constructs described in top panels were analyzed with an experimental metastatic assay by tail vein injection to determine gross pulmonary metastasis. (e) Ezrin is required for non-autocrine HGF/Met signaling-induced cell metastasis. (Top panel) Western blot analysis of 37-7 Met cells stably expressing Met transfected with control vector (shc) and Ezrin shRNAs (sh1, sh2) expressing constructs. (Bottom panel) 37-7 Met cells transfected with control vector (shc) or the Ezrin shRNAs (sh1, sh2) expressing constructs described in top panel were analyzed with an experimental metastasis assay employing tail vein injections in wildtype (WT) (shc, n=6; sh1, n=7; sh2, n=9) or HGF transgenic (HGF) (shc, n=6; sh1, n=7; sh2, n=6) syngeneic host mice.

Figure S2. Representation of predicted Sp1 transcription factor binding sites in the Ezrin gene promoter.

Figure S3. Western blot analysis of B16F1-Met cells treated with HGF plus MAPK inhibitor PD89059 or PI3K inhibitor LY294002 for the indicated the short time.