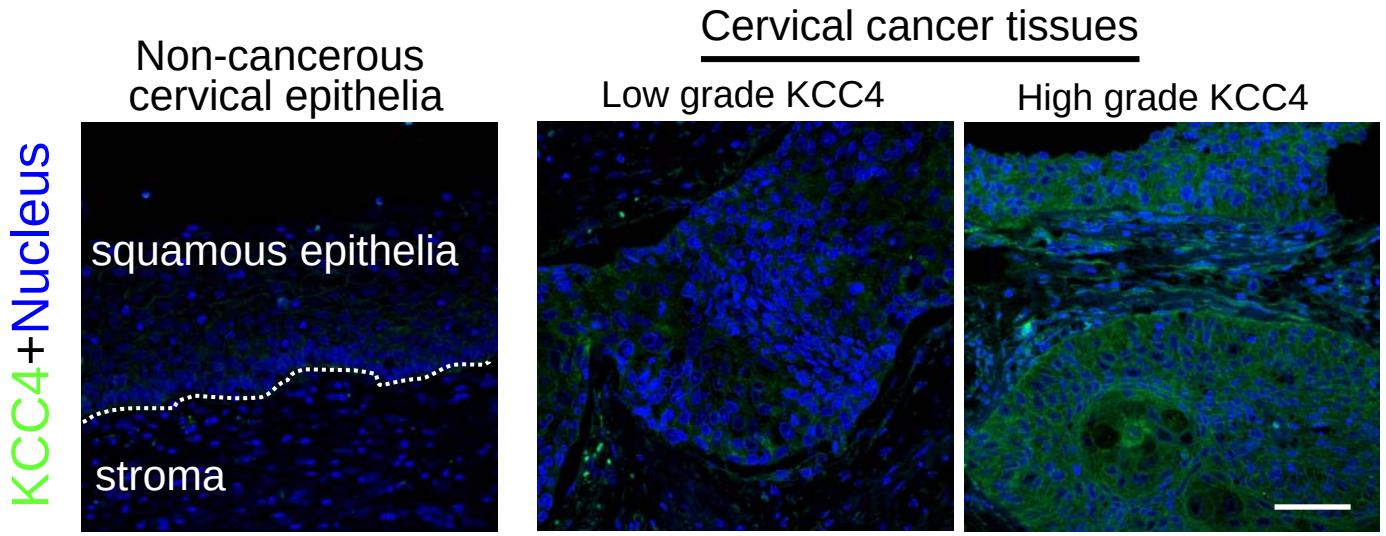
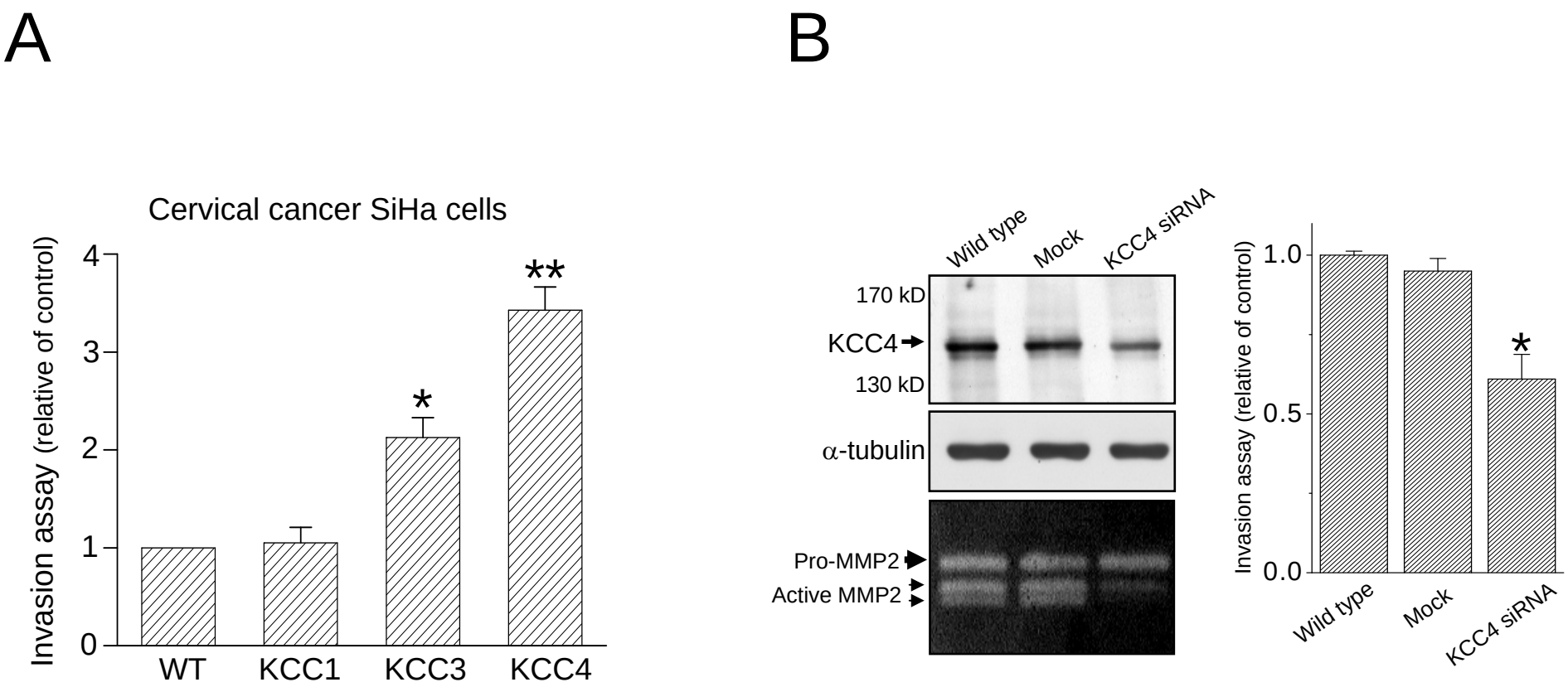
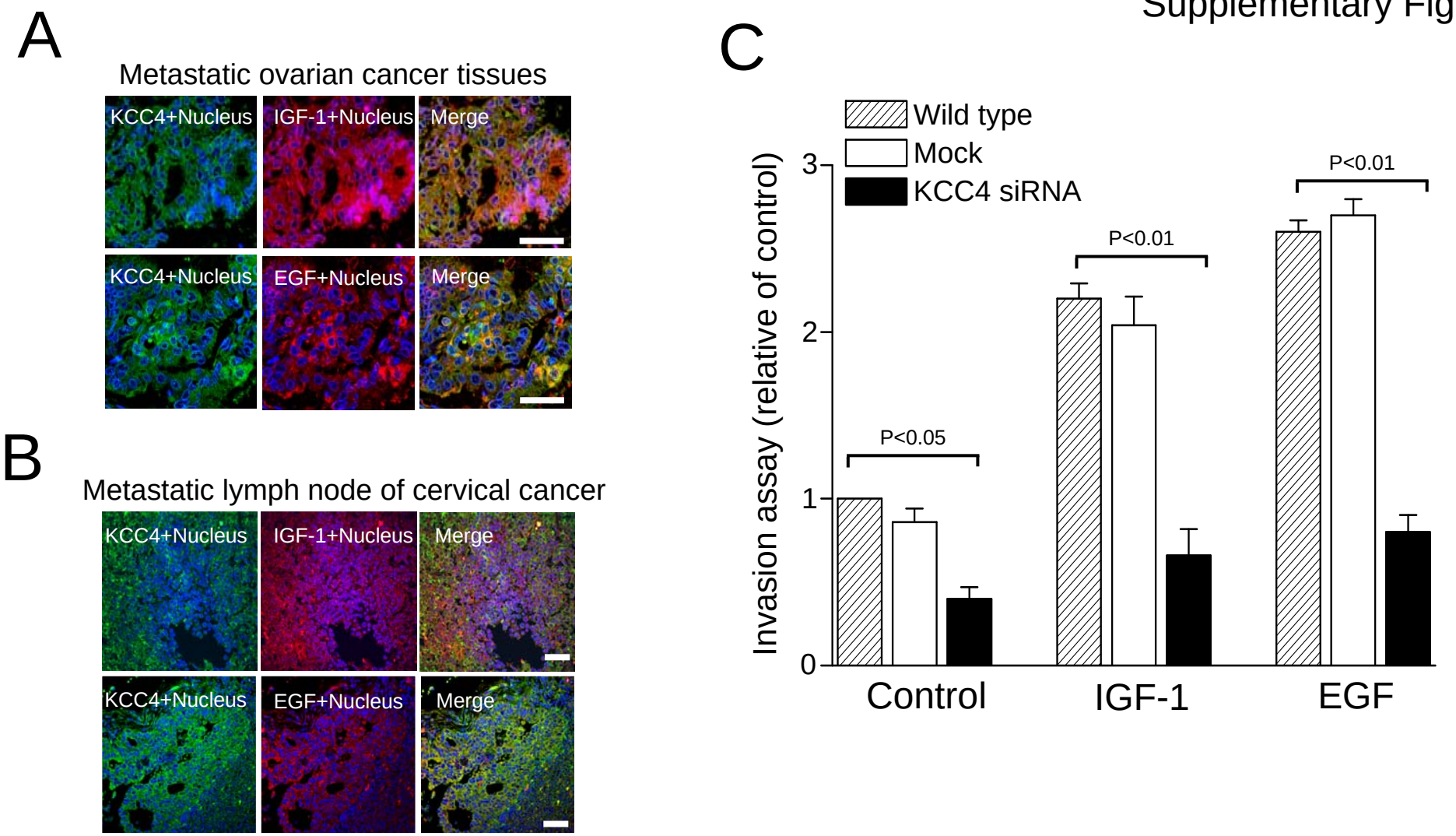


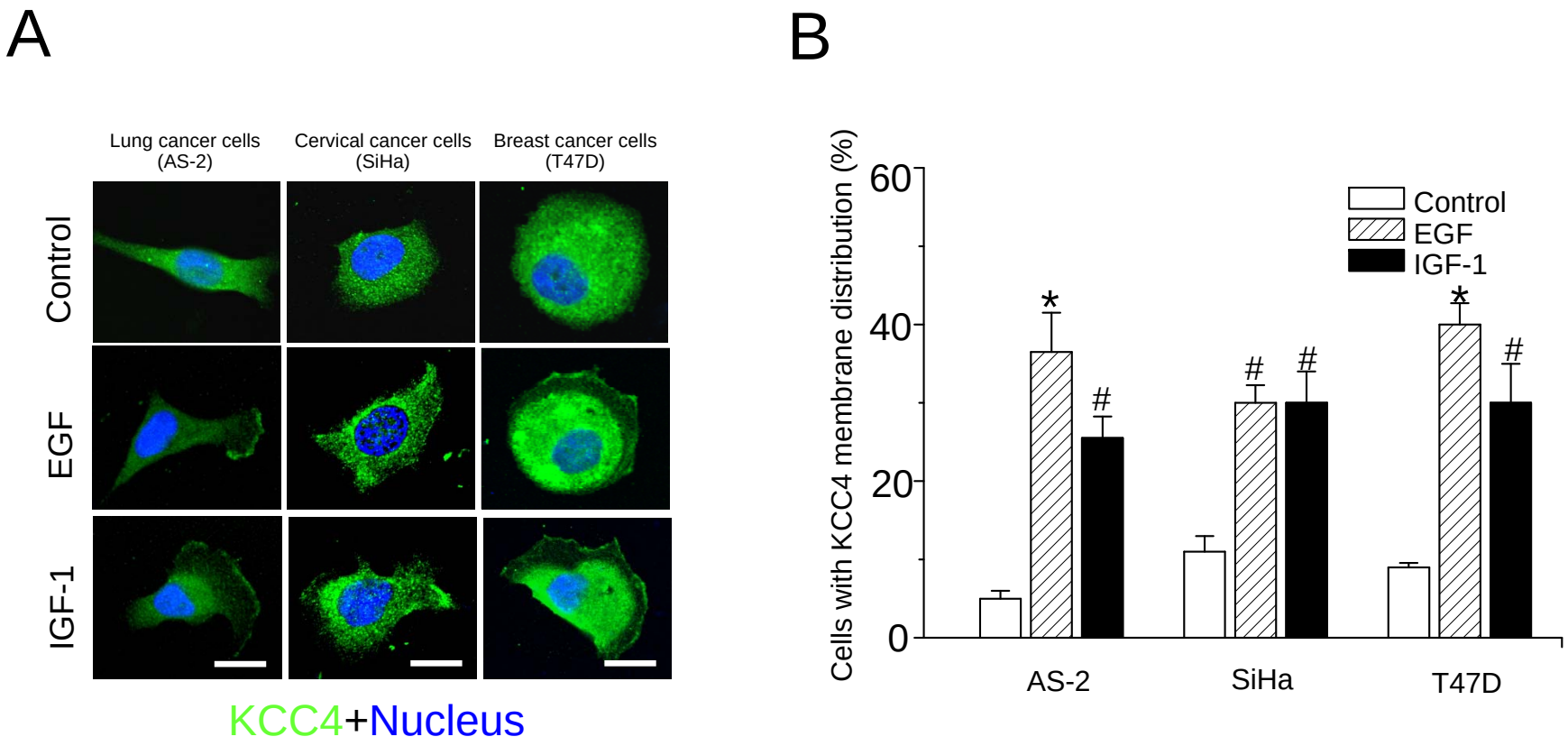
Supplementary Figure 1. Expression patterns of KCC4 in cervical carcinoma. KCC4 protein was scanty in non-cancerous cervical squamous epithelial tissues. In contrast, primary cancerous tissues of cervix clearly expressed KCC4 protein at different levels. The patients (n=80) were grouped by KCC4 grades and the survival data analyzed accordingly. Low grade KCC4 indicates the distribution of KCC4 staining is less than 50% of tumor area. High grade KCC4 indicates the distribution of KCC4 staining is more than 50% of tumor area. Scale bar, 10 μm.



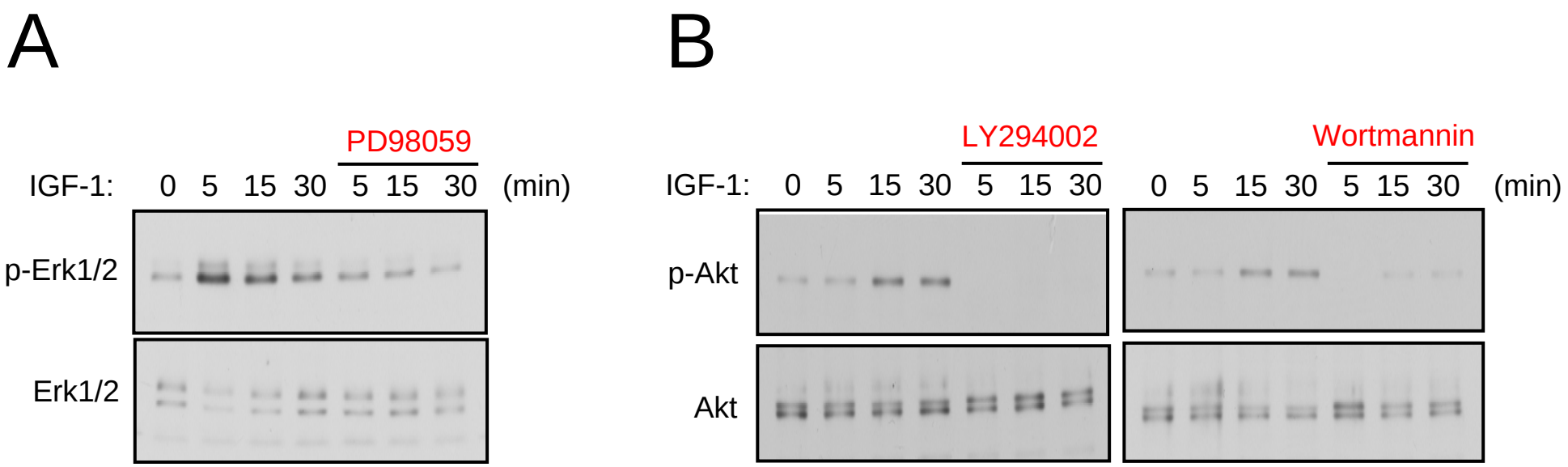
Supplementary Figure 2. KCC4 is associated with cancer cell invasiveness. (A) KCC4 overexpression enhances cervical cancer cell invasiveness. The invasion assays were performed among wild-type, KCC1-, KCC3-, and KCC4-overexpressed human cervical cancer SiHa cell lines. Each column represents mean ± S.E.M. from at least 3 different experiments. The invasive ability of wild-type cells was used as the control. *P<0.01; **P<0.001. **(B)** KCC4 knockdown by siRNA reduced the invasive migration of ovarian cancer OVCAR-3 cells in parallel by decreased activity of MMP-2. Each column represents mean ± S.E.M. from at least 3 different experiments. The invasive ability of wild-type cells was used as the control. *P<0.01.



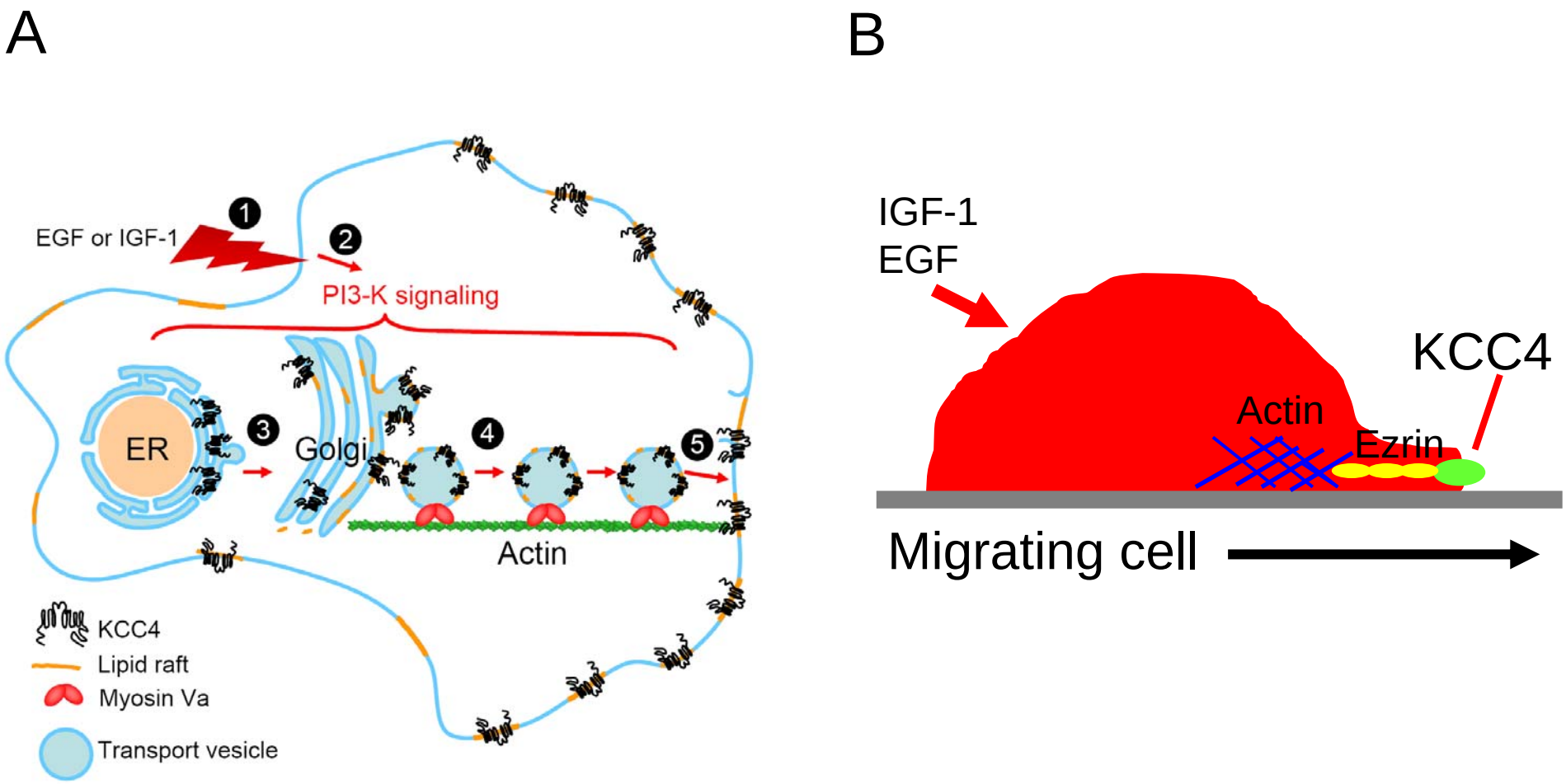
Supplementary Figure 3. KCC4 is important for IGF-1 or EGF-stimulated cancer cell invasiveness. (A) Expression patterns of KCC4, IGF-1 and EGF in surgical specimens of metastatic ovarian cancer. KCC4 (green), IGF-1 or EGF (red), and nucleus (blue). Scale bar, 50 μm. Representative pictures of 15 different cases. **(B)** Expression patterns of KCC4, IGF-1 and EGF in the metastatic lymph node of cervical cancer. The triple-immunofluorescent stain technique was used to identify KCC4 (green), IGF-1 or EGF (red), and nucleus (blue). Scale bar, 10 μm. The representative pictures of 10 different cases. **(C)** Invasive migration was assayed in the Boyden chamber as an index of invasive activity of ovarian cancer OVCAR-3 cells. Each column represents mean + S.E.M. of at least 5 different experiments. EGF, 100 ng/ml; IGF-1, 100 ng/ml.



Supplementary Figure 4. IGF-1 and EGF stimulate KCC4 membrane trafficking of cancer cells. (A) EGF and IGF-1 stimulate the redistribution of KCC4 abundance to the plasma membrane of lamellipodia. Lung cancer AS-2 cells, cervical cancer SiHa cells and breast cancer T47D cells were incubated in the absence (control group) or presence of EGF (100 ng/ml) or IGF-1 (100 ng/ml) for 30 min. Cells were then stained with KCC4 (*green*), and nucleus (*blue*). The represent pictures from 5 different experiments. **(B)** The quantitative analyses of KCC4 membrane trafficking in response to growth-factor stimulation. Positive KCC4 membrane staining was defined as the distribution of KCC4 staining over more than one third of the area of the plasma membrane. Each column represents mean $\pm$ S.E.M. of at least 250 cells. $^{\#}P<0.05$, $^{*}P<0.01$. Scale bar, 5 μm.



Supplementary Figure 5. PI3K and mitogen-activated protein kinase are activated by IGF-1. Ovarian cancer OVCAR-3 cells were pre-incubated with different inhibitors (20 μM LY294002, 100 nM wortmannin or 50 μM PD98059) for 30 min and then exposed to IGF-1 (100 ng/ml) stimulation for 30min. The immunoblots are the representatives of three different experiments.



Supplementary Figure 6. Schematic diagram of KCC4 membrane trafficking. (A) IGF-1 and EGF stimulates the recruitment of KCC4 from the inactive storage pool in the cytosol to the active membranous target pool at the lamellipodia through a mechanism involving PI3K activation and myosin Va-actin trafficking routes. Throughout the process, KCC4 is incorporated into membrane microdomains of lipid rafts. **(B)** KCC4 functions as a plasma membrane scaffold protein to facilitate the modulation of cytoskeletal reorganization via the association with ezrin, that is required for cellular invasive migration.