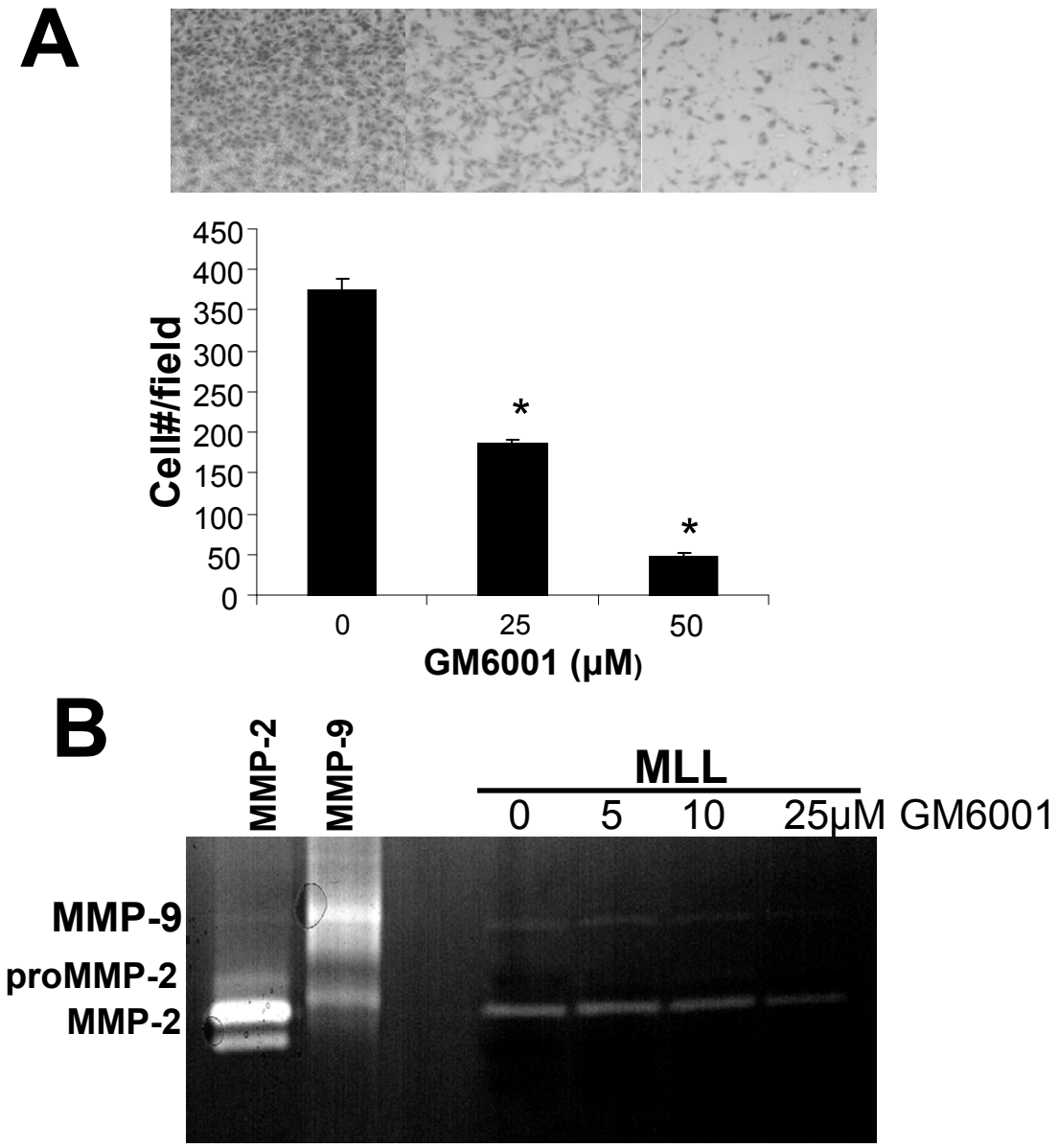


Figure S1. The MMP inhibitor, GM6001 suppresses Matrigel invasion of MLL cells. **A.** *Invasion.* The ability of MLL cells to invade after 24 h through growth factor-reduced Matrigel barriers was assessed. Six separate microscopic fields on the stained membranes (top panels) from duplicate experiments were counted in order to determine the average number of cells/field (bottom panel). Error bar = S.D.; *, $p < 0.01$. **B.** GM6001 inhibits MMP-2/9 secretion. Conditioned media obtained from overnight incubation of MLL cells grown in serum-free DMEM with the different concentrations of GM6001 were subjected to zymography as described in Materials and Methods.

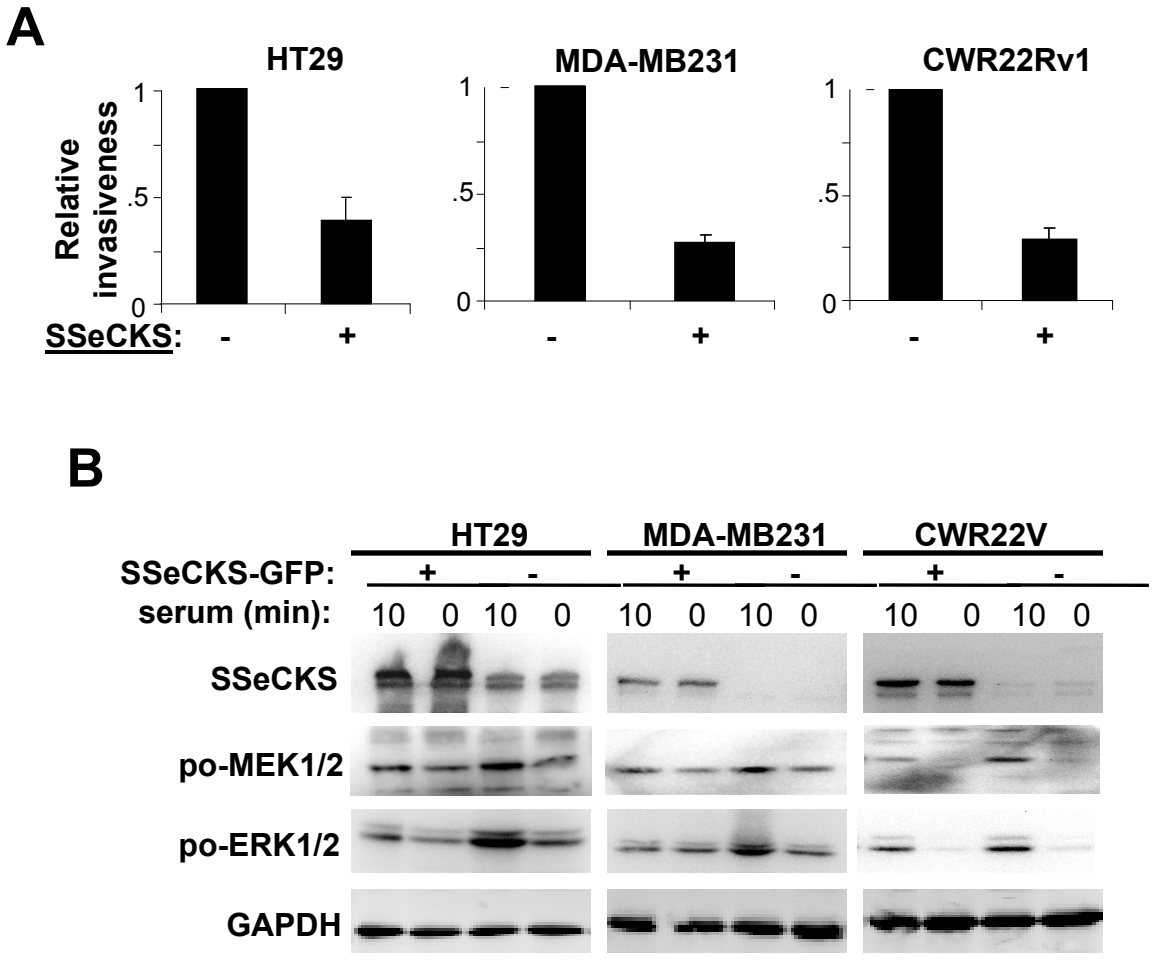


Figure S2. Inhibition of invasiveness by SSeCKS correlates with suppression of serum-induced MEK-1/2 and ERK-1/2 activation in various cancer cells. A SSeCKS-GFP expression vector or pEGFP controlwas transiently transfected into HT29, MDA-MB-231, or CWR22Rv1 cells. GFP-positive cells were isolated by FACS 48 h after transfection, and then analyzed for Matrigel invasiveness (**A**) or relative MEK/ERK activation (**B**). **A.** SSeCKS suppresses Matrigel invasion in various cancer cells. Invasion assays as performed in Fig. 1 from two independent experiments. **B**. SSeCKS decreases serum-induced MEK-1/2 and ERK-1/2 activation. Serum-starved cells were stimulated with 10% BS in DMEM for 10 min, and then RIPA lysates were analyzed by IB for SSeCKS , phospho- forms of MEK1/2 and ERK1/2, and GAPDH (protein loading control) as in Fig. 6.

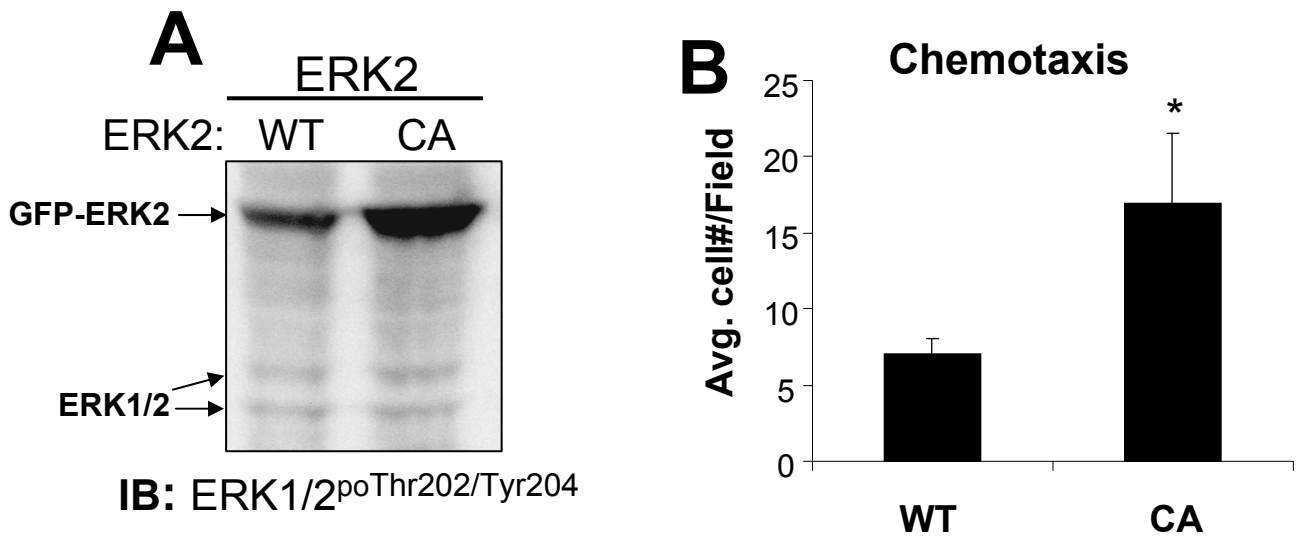


Figure S3. Activated ERK2 rescues SSeCKS-suppressed chemotaxis in MLL/tet-SSeCKS cells. **A.** MLL/tet-SSeCKS stably transfected cells with either GFP-HA-fusion proteins of WT- or CA-ERK2 (R65S) were analyzed by IB using anti-phospho-ERK1/2 Ab. Arrows identify the ectopic GFP-ERK2, showing increased relative phospho-ERK2 levels, as well as the endogenous phospho-ERK1/2 levels. **B**. The WT- or CA-ERK2 expressing cells were tested for chemotactic activity as in Fig. 1. Error bars, S.D. of 6 fields of cells containing >70 cells/field in two independent experiments. *, $p < 0.05$.

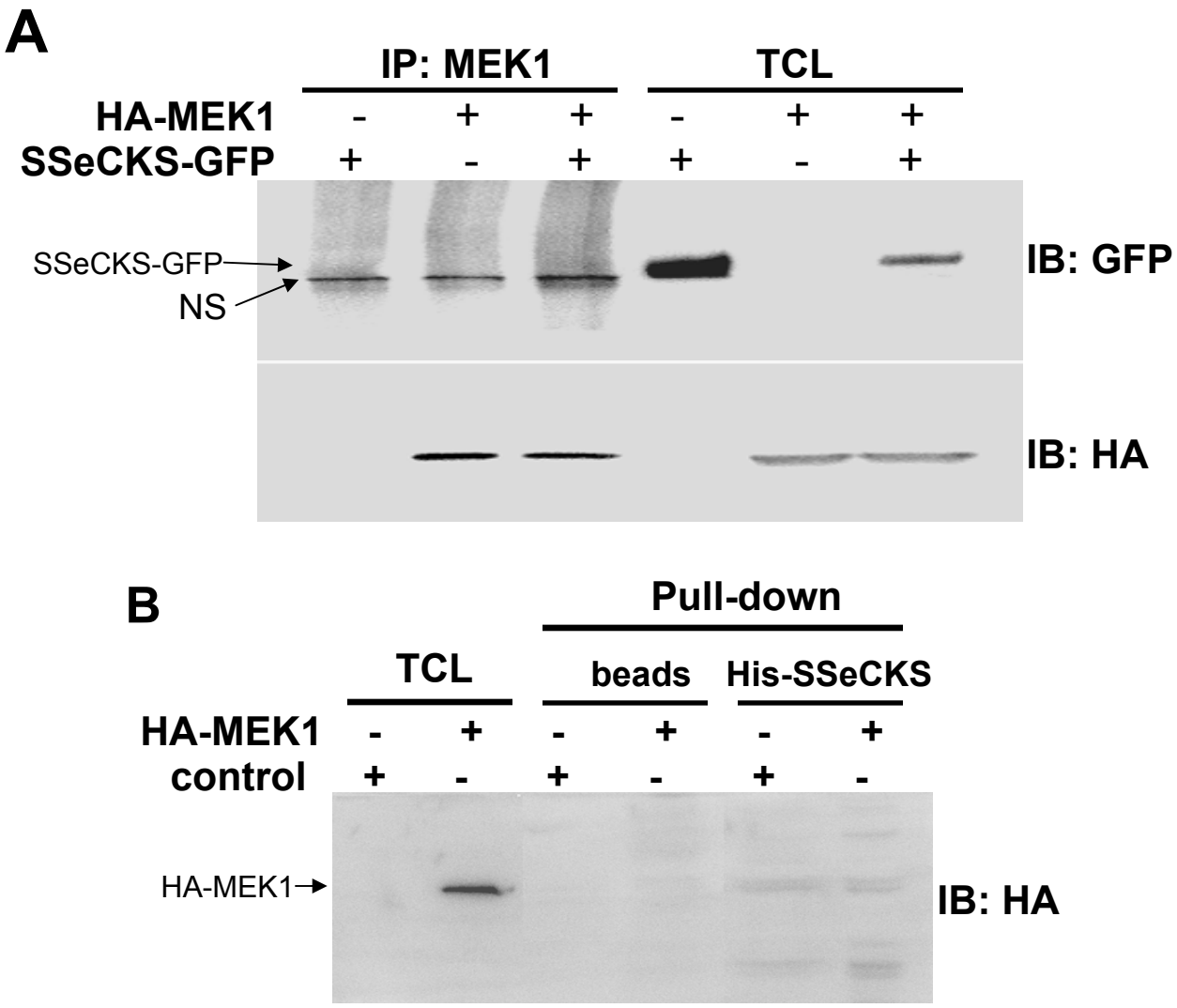
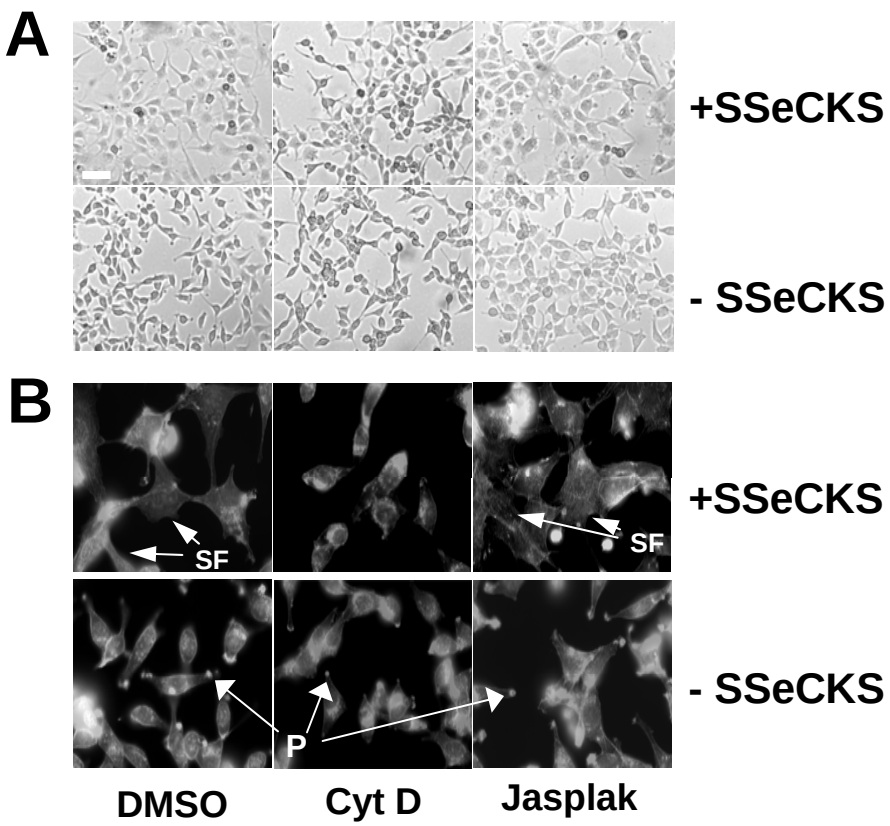


Figure S4. SSeCKS does not associate with MEK directly. **A.** 293T cells were transfected with SSeCKS-GFP, HA-MEK1, or both. After 48 hr, cells were lysed and immunoprecipitation (IP) was performed using anti-MEK1 antibody, followed by immunoblotting (IB) with the indicated Abs. NS, non-specific. TCL, total cell lysates. **B.** Lysates of 293T cells transfected with HA-MEK1 or empty vector ("control") were incubated with Ni^{2+}-beads alone or beads bound to His-TAT-SSeCKS protein. The beads were then subjected to IB analysis and probed with anti-HA Ab.



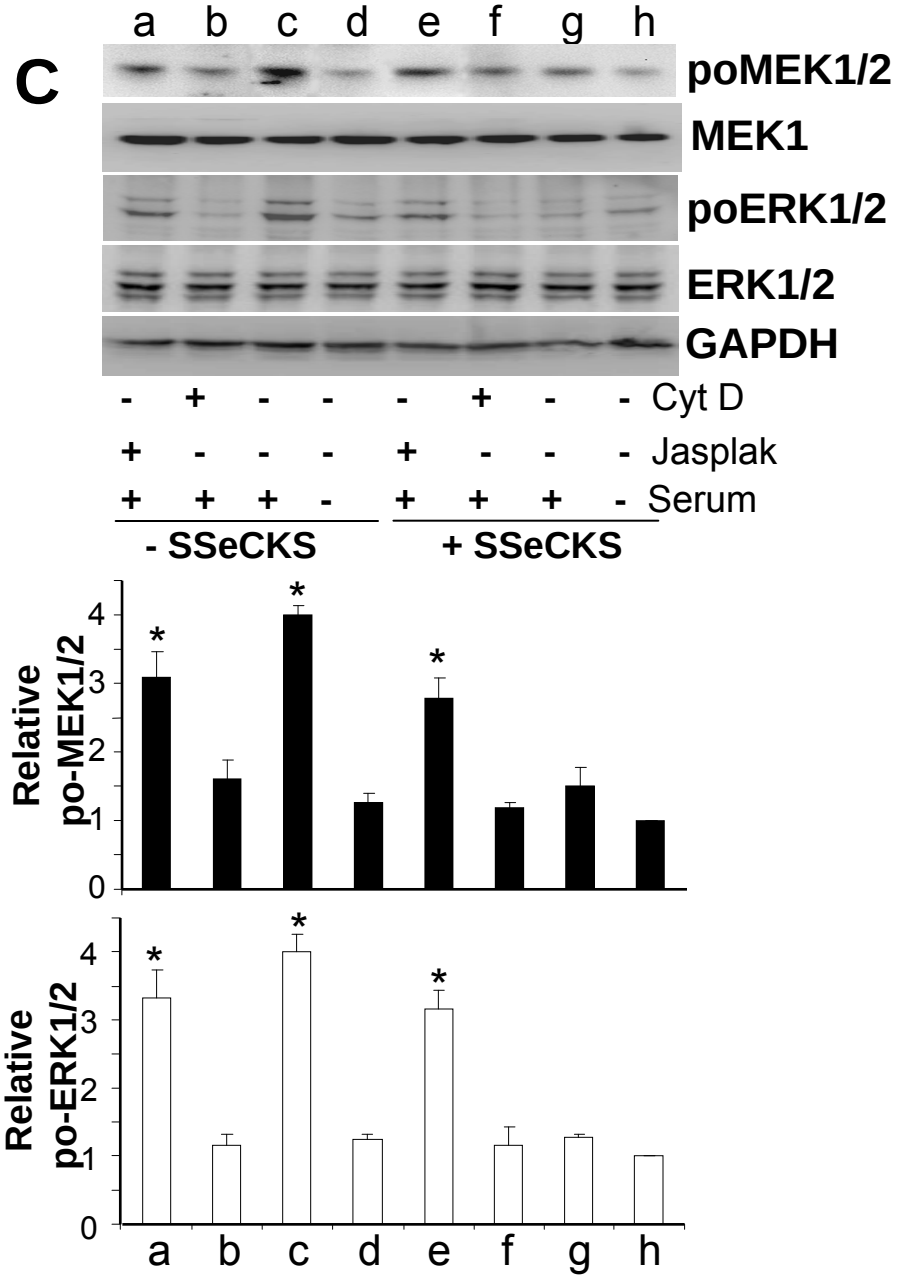


Fig. S5. Actin cytoskeleton-dependent and –independent effects on SSeCKS. **A.** The ability of SSeCKS to flatten MLL cells is reversed by cytochalasin D (Cyt D) but not by jasplakinolide (Jasplak). Size bar, 25 μm. **B.** The re-expression of SSeCKS in MLL cells induces stress fiber (SF) formation that is blocked by Cyt D but not by Jasplak, whereas SSeCKS ability to inhibit podosome (P) formation is not affected by either Cyt D or Jasplak. **C.** Serum induced MEK1/2 and ERK1/2 phosphorylation/activation is blocked by SSeCKS, but this block is sensitive to Jasplak and not to Cyt D. *Top panel*, IB analysis of total and phospho-MEK1/2 and –ERK1/2, and GAPDH. *Middle/Bottom panels,* graphical analyses of relative MEK1/2 and ERK1/2 phosphorylaiton from two independent experiments. Error bars, S.D.; *, p <0.01.