

Supplementary information

Supplementary Figure S1 – S2 – S3

Extracellular Matrix Rigidity-dependent Sphingosine-1-phosphate Secretion Regulates Metastatic Cancer Cell Invasion and Adhesion

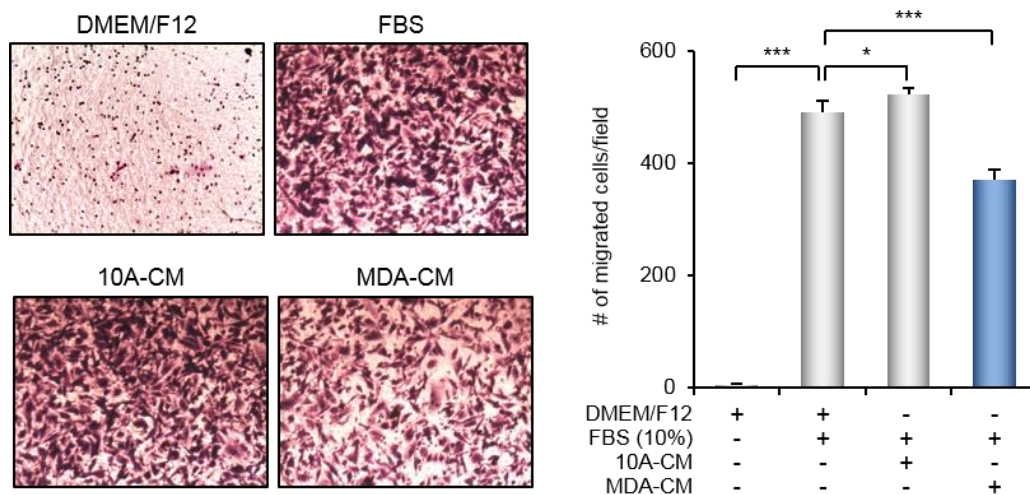
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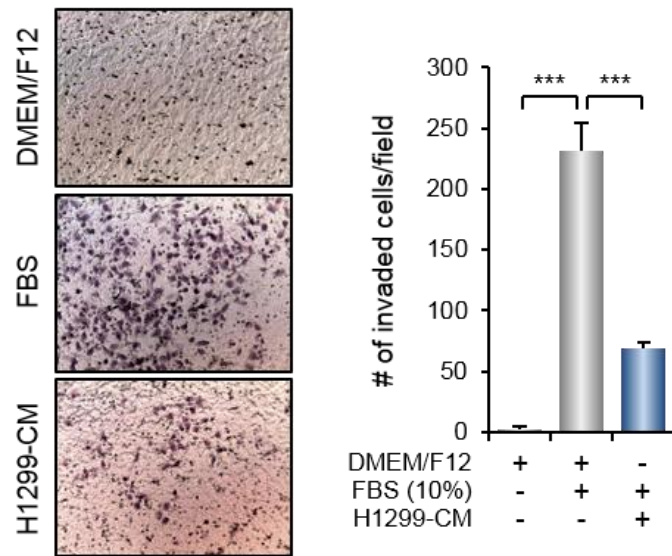
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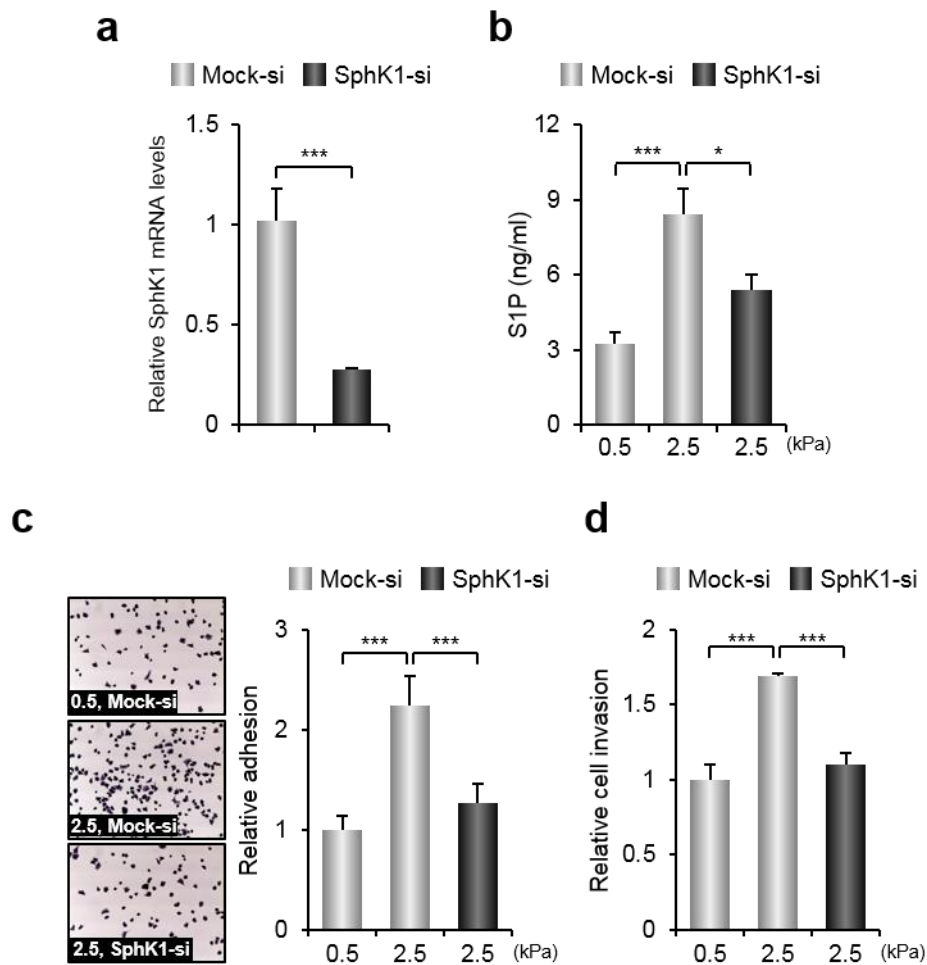
Supplementary Figure S1. MDA-CM inhibits serum-induced chemo-attractive migration.

Migration of MDA-MB-231 cells was examined with MDA- or 10A-CM using a non-collagen-coated Transwell migration assay system for 24 h. Right panels, quantification of the average number of migrated cells per photographic field shown in the left panel. Values represent mean \pm SEM, * $p < 0.05$, *** $p < 0.001$.



Supplementary Figure S2. H1299-CM inhibits serum-induced chemo-attractive invasion.

Invasion of H1299 cells was examined with H1299-CM using a collagen-coated Transwell invasion assay system for 24 h. Right panels, quantification of the average number of invading cells per photographic field shown in the left panel. Values represent mean \pm SEM, *** $p < 0.001$.



Supplementary Figure S3. ECM rigidity-dependent SphK1 expression affects cell adhesion and invasion in H1299. (a) H1299 cells were transfected with a 20 nM of mock or *SphK1* siRNA and incubated for 72 h. Extent of decrease of *SphK1* mRNA was quantified by real-time PCR. Values represent mean \pm SEM. *** $p < 0.001$. (b) Measurement of extracellular S1P from H1299 at 0.5 and 2.5 kPa PAG. (c) Adhesions of H1299 were calculated relative to 0.5 kPa CM. (d) Transwell invasion assays were performed with CM harvest from H1299 cultured at 0.5 and 2.5 kPa. Values represent mean \pm SEM of triplicate experiments * $p < 0.05$, *** $p < 0.01$.