

SUPPLEMENTAL MATERIAL

Antitumor Effects of Immunotoxins are Enhanced by Lowering *HCK* or Treatment with Src Kinase Inhibitors

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Supplemental Figure Legends

Figure S1. Screening of TK siRNA library.

A431H9 cells were transfected with 88 predesigned smart-pool on-target siRNAs in 96-well format in quadruplet. After 48 hours of transfection, the cells with each quadruplet were treated with 0, 3, 6, and 12 ng/ml of SS1P, respectively, for 72 hours. The cell viability was measured by ATP assay and was calculated as the percentage of relative luminescence of each sample treated with SS1P to no SS1P treatment. We only show data in which there is over a 2-fold difference compared with the nonspecific siRNA control (Con). MSLN is the positive control.

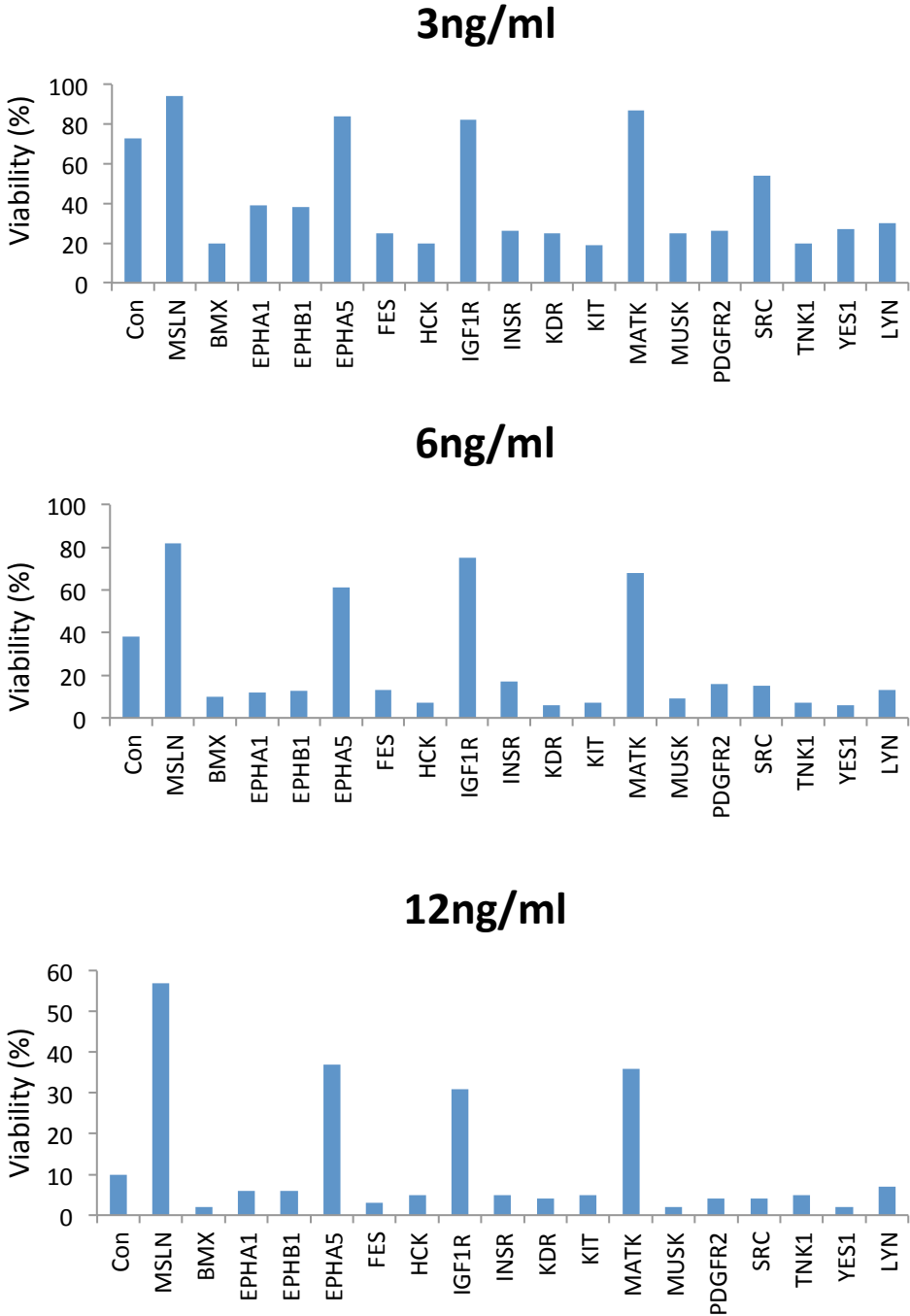
Figure S2. Src knock down increased SS1P toxicity slightly.

A431/H9 (A, B, and C) and KB cells (D, E and F) were transfected with siRNA. RNA expression was analyzed after 48 hours transfection of control siRNA GL2 (siCo) or siSrc (A and D). The relative RNA level was measured by real time PCR and normalized by actin as internal control. B and E: Western blot analysis after 72 hours of transfection of siSrc and siCo. The blot was analyzed by anti-Src and anti-GAPDH (loading control). C and F: Cytotoxicity assay A431/H9 cells (C) or KB (F) were transfected with siRNA for 48 hours, then the cells were treated with SS1P at indicated concentration for 72 hours. Cell viability was measured by ATP assay.

IC₅₀ in A431H9 cells: siCo = 1.7 ng/ml and siSrc = 1.3 ng/ml.

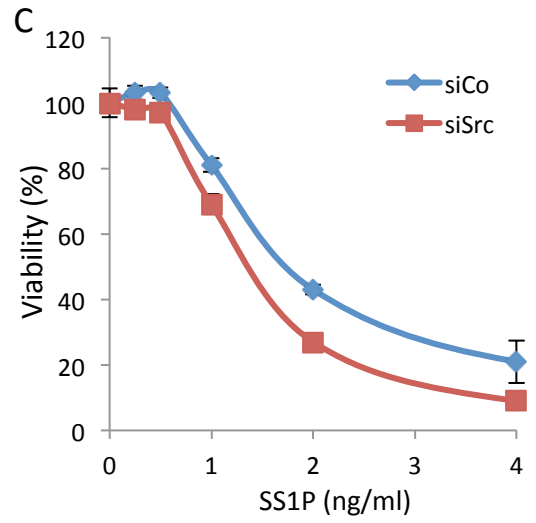
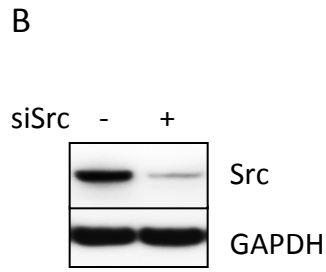
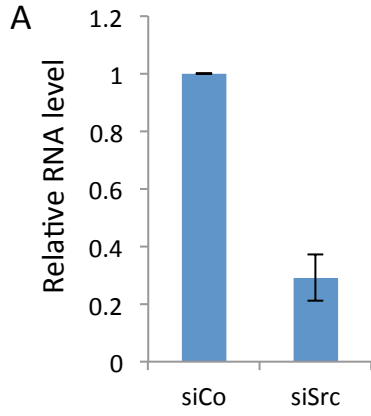
IC₅₀ in KB cells: siCo = 15 ng/ml, siSrc = 11.5 ng/ml.

Supplemental Fig. S1

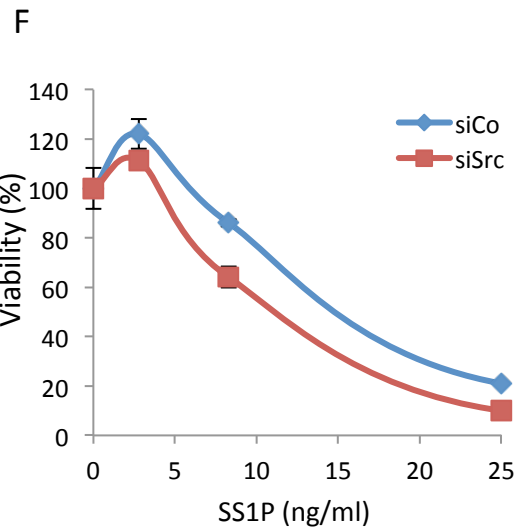
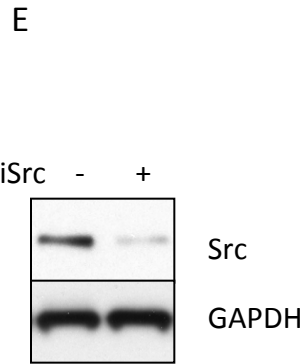
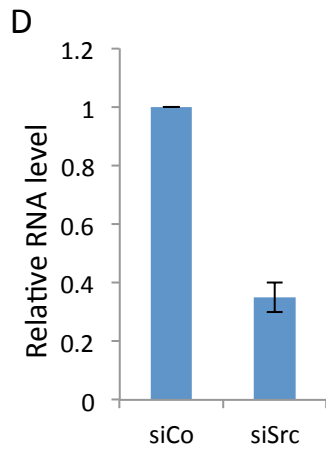


Supplemental Fig. S2

A431/H9



KB



Supplemental Table S1. Oligos used in this study

| <u>Name</u> | <u>purpose</u> | <u>sequence</u> |
|-------------|----------------|------------------------|
| siHCK-1 | siRNA | catcgtggtgccctgat |
| siHCK-3 | siRNA | gtcaaaccctgaagtgatc |
| siHCK-4 | siRNA | ggagataccgtgaaacatt |
| HCK-1497 | QT-PCR | catcaagtggacagctcctgaa |
| HCK-R1650 | QT-PCR | ctccagagctcggatcaactca |
| siSrc | siRNA | ccaagggcctcaacgtgaa |
| Src-325 | QT-PCR | gagcggctccagattgtcaa |
| src-R390 | QT-PCR | ctggggatgtagcctgtctgt |
| Actin-for | QT-PCR | ccggccagccaggtccagac |
| Actin-rev | QT-PCR | ccaaggccaaccgcgagaagat |