SUPPLEMENTARY INFORMATION:

MATERIALS AND METHODS:

Whole cell lysis and cell fractionation analysis **-** For whole cell lysates, cultures were extracted on ice with RIPA buffer containing protease and phosphatases inhibitors (150mM NaCl, 50mM Tris-HCL pH 7.5, 10mM EDTA, 1% Triton X-100, 10mM NaF, 10μg/ml aprotinin, 10μg/ml leupeptin, 2μg/ml pepstatin, 1mM PMSF, 200μM $NaVO_4$). Protein concentrations were measured using BCA Protein Assay Kit (PIERCE, Rockford, IL) and samples were separated on a 7.5% SDS-PAGE gel and transferred onto nitrocellulose membranes (Bio-Rad, Hercules, CA). Following probing with primary antibodies, membranes were reacted with HRP-conjugated secondary antibodies (Amersham, Piscataway, NJ). Membranes were blotted with anti-GAPDH antibody to verify equal protein loading. For inhibition studies, cultures were treated either with DMSO (50μM), FAK inhibitor Tyrphostin AG1007 (50μM) or Src inhibitor PP2 (1μM) for 4hr prior to whole cell extraction. For cell fractions, cultures were extracted on ice with RIPA buffer containing proteases and phosphatases inhibitors and no Triton X-100. After 5 freeze/thaw cycles lysates were centrifuged at 4°C at 13,000rpm and the supernatant containing the cytosolic fraction was transferred to another tube. The pellet was re-suspended in RIPA buffer containing 1% Triton X-100 with protease and phosphatase inhibitors. Lysates were incubated for 30 min on ice and centrifuged at 4°C at 13,000rpm and the supernatant containing the crude membrane fraction was transferred to a fresh tube and separated from the pellet that is consisting of nuclei and cytoskeletal proteins. Protein concentrations were measured using a modified Lowry assay (Bio-Rad DC Protein Assay kit) and protein samples were separated on 7.5% SDS-PAGE and transferred onto nitrocellulose membrane for IB. The following antibodies were used in

immunoblotting: rabbit anti-FAK (Upstate, Lake Placid, NY), mouse anti-phosphotyrosine (Upstate, Lake Placid, NY), rabbit anti-phospho-FAK (Tyr397, Tyr576/577, Tyr925) (Cell Signaling Technology Inc., Danvers, MA) rabbit anti-Src (Cell Signaling), rabbit anti-phospho Src (Tyr416, Cell Signaling), rabbit anti-Src (Thermo Scientific), mouse anti-E-cadherin (BD Transduction Laboratories, Lexington, KY) and mouse anti-GAPDH (abcam, Cambridge, MA).

Phase-contrast and fluorescence microscopy analysis **-** Phase-contrast images of monolayer cultures were captured with PixeLINK Software 4.5 (Ottawa, Ontario, Canada), using inverted Axiovert 40C Zeiss microscope (Gottingen, Germany) equipped with PixeLINK Camera (PL-A600 Series, Ottawa, Ontario, Canada). 3D tissues or excised tumors were frozen in embedding media in liquid nitrogen vapors and 6μm serial sections were fixed in 4% paraformaldehyde and immunostained for 1hr with mouse anti-E-cadherin (Zymed Laboratories South San Francisco, CA), mouse anti-Ki67 (BD Transduction Laboratories) or mouse anti-K1/K10 (abcam) antibodies (5 Prime, Gaithersburg, MD). Following incubation with Alexa 594™-conjugated goat anti-mouse (Invitrogen, Carlsbad, CA) secondary antibodies, nuclei were counterstained with DAPI in Vectashield mounting medium (Vector Laboratories, Burlingame, CA). For Ki67 staining of paraffin embedded-tissues, sections were steamed in citrate buffer after rehydration, immunoreacted with anti-Ki67 antibodies developed using DAB (Vector) and counterstained with Hematoxylin (Sigma). Images were captured with Spot Advanced version 4.5, using a Nikon Eclipse 80*i* microscope (Nikon Instruments Inc., Melville, NY) equipped with a SPOT RT camera (Diagnostic Instruments, Sterling Heights, MI).

In vitro scrape wounding assay and wound closure measurements **-** 2D monolayer cultures at 80% confluence were scrape-wounded with a 10μl tip. Thereafter, cultures were fed with fresh medium or medium containing DMSO (100μM), Tyrphostin AG1007 (100μM, Sigma, St. Louis, MO) or PP2 (5μM, EMD Biosciences, San Diego, CA) (for inhibition studies). Random areas along the wound gaps were marked and imaged upon wounding, and 24 and 48hr later. At 48hr cultures were washed and re-fed with medium only, and the marked areas were imaged 24hr later. Wound closure measurements analyzed the average change in the width of the wound gaps over time, relative to their initial width. Measurements were performed on 3 captured areas and in each wounded area >50 measurements of the wound gap were performed. At each time point, the mean ± SD of the width of the open wound gaps was calculated. To quantify wound closure over time, the averaged width of the open wound gaps at 24- and 48-hour time points were subtracted from the initial wound gaps measurements. This calculated measurement of wound closure was then divided by the initial width of the wound gaps to determine percentage of wound closure. The experiment was performed in triplicate and was repeated three times.

Real-Time PCR **-** Subconfluent monolayer cultures of sh-Scrambled-, sh-FAK- or sh-Src-H-2K^{d}-Ecad-II-4 cells were washed and total cellular RNA was isolated using RNeasy Plus Mini Kit (Qiagen, Germantown, MD). 1μg of RNA from each cell line was converted into cDNA using iScript (Bio-Rad, Hercules, CA). Real-Time PCR was performed with iQ SYBR Green Supermix and measured on a MyiQ iCycler with MyiQ Software (Bio-Rad). CT values were relative to 18S rRNA. 18S primers were from Ambion (Austin, TX). Other primers used were: FAK 5': CTTGACCCCAACTTGAATCACA; FAK 3': TTCCATACCAGTACCCAGGTG; Src 5': TTGGCAAGATCACCAGACGG; Src 3': GGCACCTTTCGTGGTCTCAC.

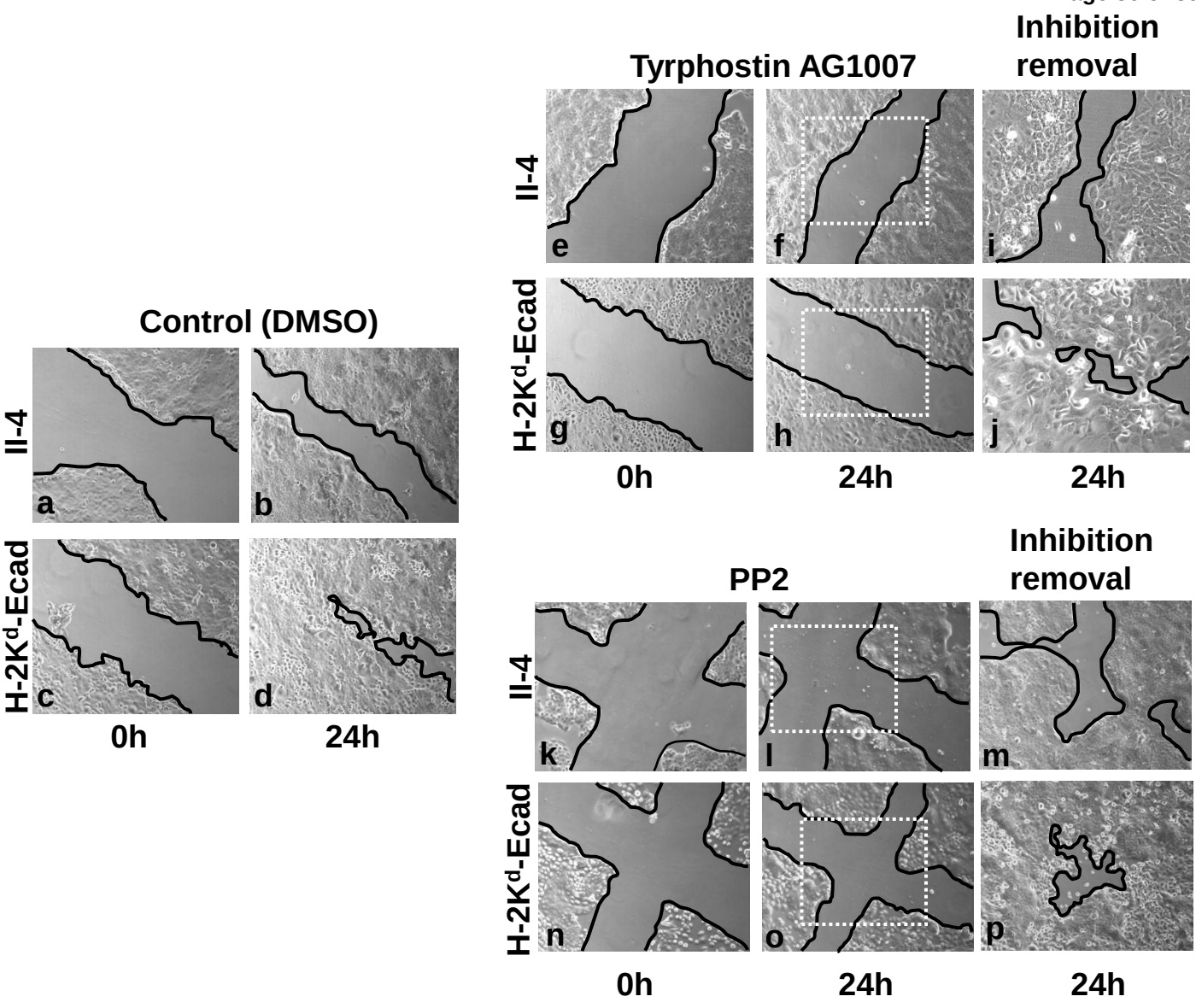

Figure S1 - FAK inhibitor, Tyrphostin AG1007, and Src inhibitor, PP2, inhibits the motility of H-2K^d-Ecad-II-4 cells in response to scrape wounding.

II-4 and H-2K^d-Ecad-II-4 cultures were scrape-wounded, treated with DMSO, Tyrphostin AG1007 or PP2, and imaged at the indicated time points. Wound closure rapidly occurred in DMSO-treated H-2K^d-Ecad-II-4 culture (d) when compared to that seen in II-4 culture (b). Wound closure was inhibited in Tyrphostin-treated H-2K^d-Ecad-II-4 culture (h) and was similar to that seen in Tyrphostin-treated II-4 culture (f). 24hr after inhibition removal (i, j) the accelerated motility of H-2K^d-Ecad-II-4 cells was resumed, and wound closure was similar to that seen in DMSO-treated H-2K^d-Ecad-II-4 culture (d). PP2-treated H-2K^d-Ecad-II-4 cells showed inhibition of cell motility and wound closure (o) similarly to that seen in PP2-treated II-4 cells (l). 24hr after inhibition removal (m, p) wound closure in H-2K^d-Ecad-II-4 culture was similar to that seen in DMSO-treated culture (d). Images i, j, m, p are the areas defined in panels f, h, l, o, respectively. Images were captured at 4x magnification.

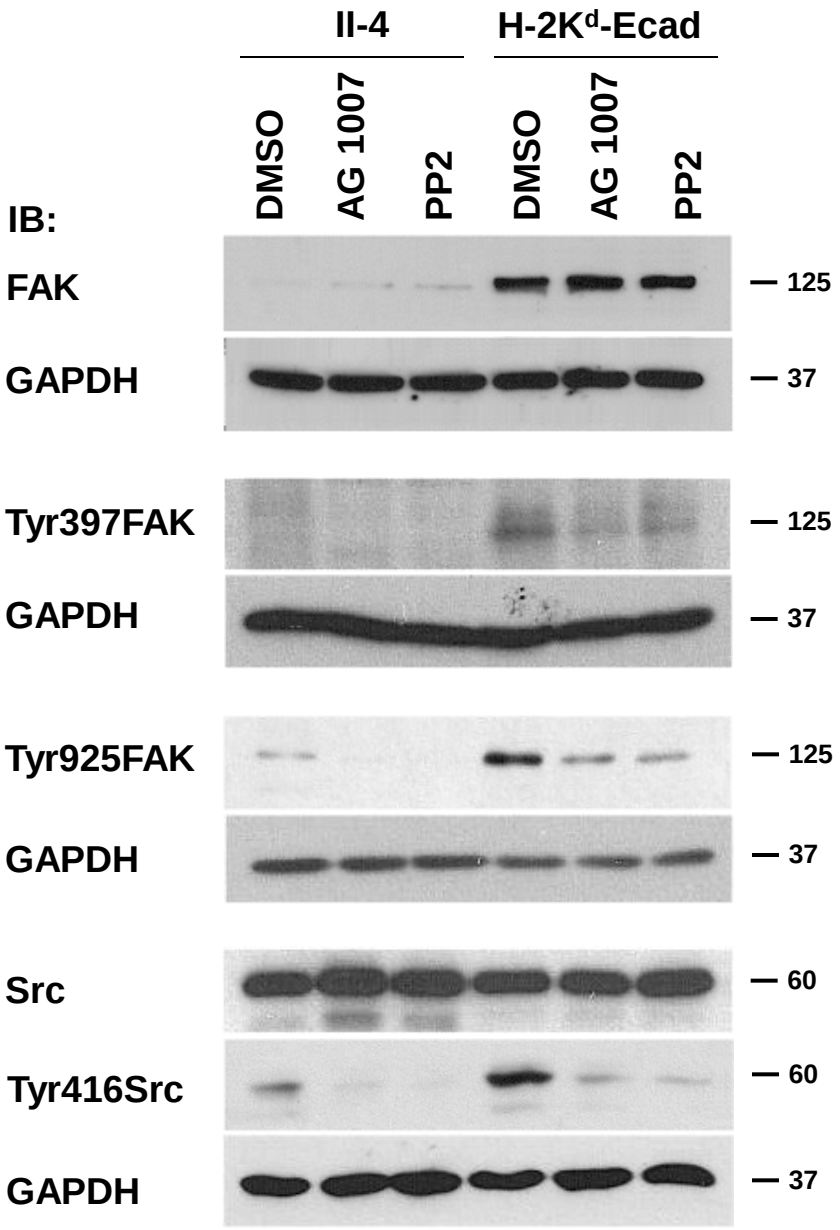

Figure S2 - Inhibition of FAK or Src in H-2K^d-Ecad-II-4 cells is associated with reduced tyrosine phosphorylation of FAK and Src.

Cultures of II-4 cells and H-2K^d-Ecad-II-4 cells were treated for 4h with DMSO, Tyrphostin AG1007 or PP2. WB analysis of whole cell lysates demonstrated that inhibition of FAK and Src did not alter their expression levels in both cell lines. In comparison to DMSO-treated H-2K^d-Ecad-II-4 cells, treating the cultures with Tyrphostin AG1007 or PP2 resulted in decreased phosphorylation on tyrosine Tyr397 and Tyr925 residues of FAK, and Tyr416 of Src.

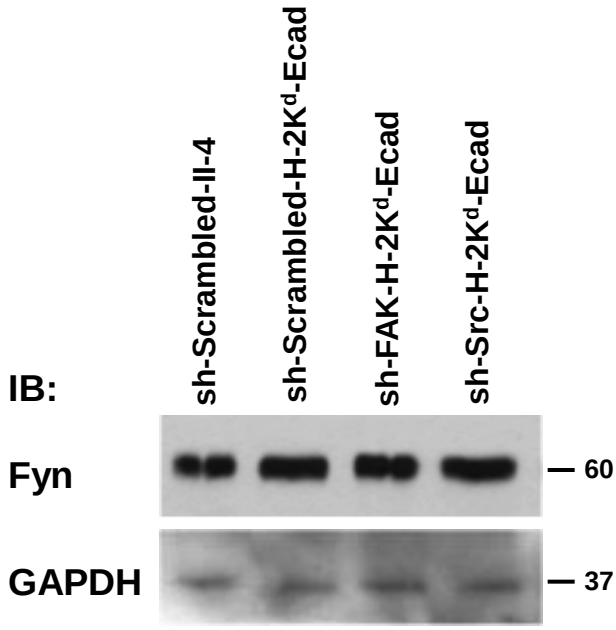

Figure S3 - Depletion of FAK or Src in H-2K^d-Ecad-II-4 cells does not effect the protein expression level of the Src family member Fyn kinase.

WB analysis of whole cell lysates of sh-Scrambled-II-4 cells, and of sh-Scrambled-, sh-FAK- or sh-Src-H-2K^d-Ecad-II-4 cultures demonstrated a similar expression level of Fyn protein.

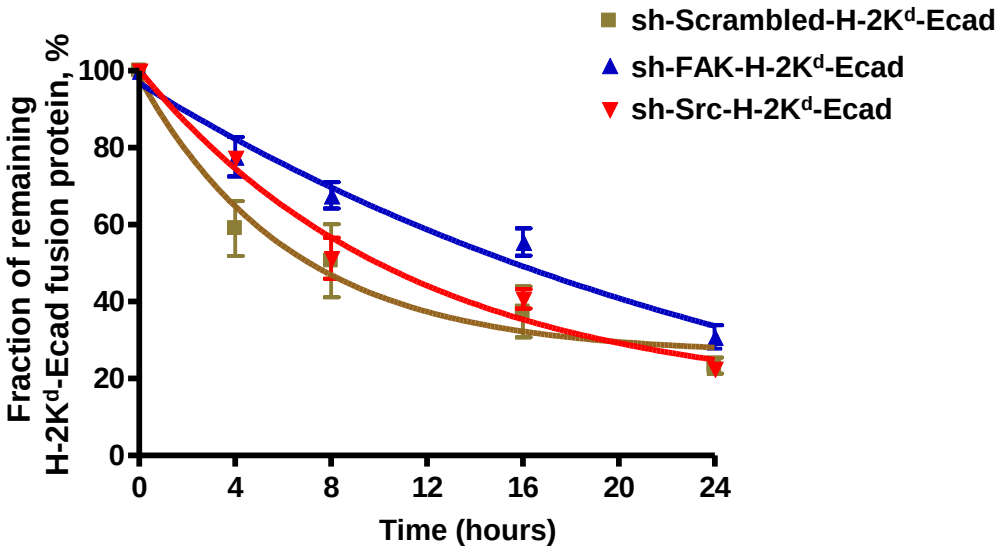

Figure S4 - Knock down of FAK or Src does not alter the degradation rate of the exogenous H-2K^d-Ecad fusion protein in H-2K^d-Ecad-II-4 cells.

Cultures of sh-Scrambled-, sh-FAK-, and sh-Src-H-2K^d-Ecad-II-4 cells were treated for 24 hours with 10µM Cycloheximide. Densitometry analysis of western blots from whole cell lysates demonstrated a similar decrease in the fraction of the remaining exogenous H-2K^d-Ecad fusion protein at the indicated time points. The data represent the mean SD of the remaining H-2K^d-Ecad protein in these cultures in 5 independent experiments.