Supplemental Methods:

Cell Lines and Reagents
Ad293 cells were purchased from Cell Biolabs and used for amplifying and titering adenoviruses. Adenoviruses were purified by cesium chloride density ultracentrifugation. MP59 primary mesenchymal progenitor cells were a gift of Dr. William Stanford (University of Toronto, Toronto, ON) and were used from passage 6 to 10. Ad293 cells were maintained in DMEM (Invitrogen) supplemented with 10% FBS (Sigma), and MP59 cells were cultured as detailed (1). Two μg of *PAX3-FKHR* or control pcDNA3.1 plasmid were transiently transfected into $1x10^5$ MP59 cells with Lipofectamine 2000 (Invitrogen), in serum-free Optimem I (Invitrogen). Cleaved caspase-3 antibody for western blotting was purchased from Cell Signaling Technologies.

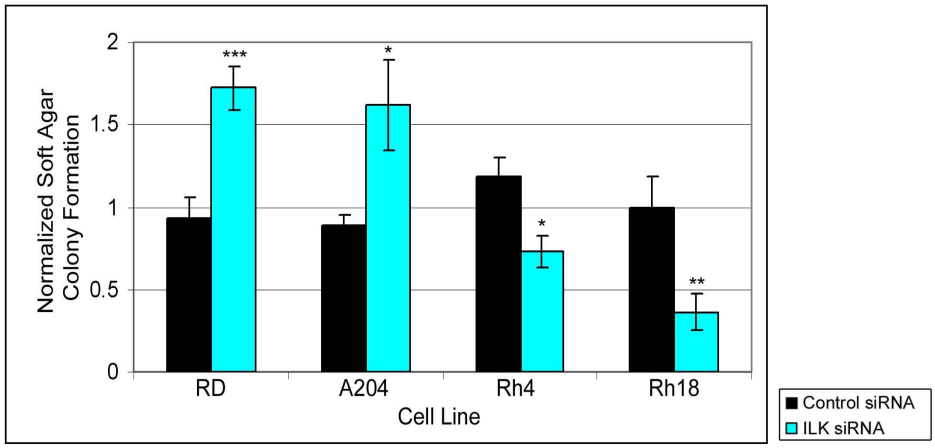
Primers for Real Time PCR
Real time RT-PCR primers to the *JNK* isoforms, *Cyclin A2*, *FasL*, $p21^{CIP1}$, $p14^{ARF}$ and $p16^{INK4A}$ were as in (2-7). Primers to *SDF1α* were forward 5'CCAACGTCAAGCATCTCAAA and reverse 5'CCACTTTAGCTTCGGGTCAA ; *Cyclin D1* were forward 5'CCAGAGGCGGAGGAGAACA and reverse 5'TCTGTGGCACAGAGGGCAA; *PAX3-FKHR* were forward GCAATGGCCTCTCACCTC and reverse 5' TGCACACGAATGAACTTGC; *TBP* were forward 5'CCTAAAGACCATTGCACTTCG and reverse 5'GTTCTTCACTCTTGGCTCCTGT; *HPRT* were forward 5'CCAGTCAACAGGGGACATAAA and reverse 5'CCTGACCAAGGAAAGCAAAG.

Immunohistochemistry
Immunohistochemistry for polyclonal rabbit anti-ILK (Upstate Biotechnology) and monoclonal rabbit anti-Ki67 (Neomarkers) were performed on the Benchmark™ auto-immuno stainer (Ventana Medical Systems) at dilutions of 1:100. Polyclonal rabbit Anti-Factor VIII (Dako) and polyclonal rabbit anti-VEGF (Santa Cruz Biotechnology) were used as indicated, at 1:500 and 1:300, respectively. Sections were enzyme pretreated for antibodies against Factor VIII and VEGF before blocking for endogenous biotin and peroxidase. Immunodetection was performed using the Ventana, i-VIEW™ Open Secondary DAB, LSAB detection System, using a 1:100 dilution of a biotinylated anti-Rabbit IgG (Vector Laboratories) as the secondary antibody. Immunohistochemistry for phosphorylated Thr183/Tyr185 JNK was performed as per the manufacturer's instructions (Cell Signaling Technologies) at a dilution of 1:100 overnight. Sections were subjected to biotinylated secondary anti-rabbit IgG (Vector Laboratories) and counterstained with hematoxylin.

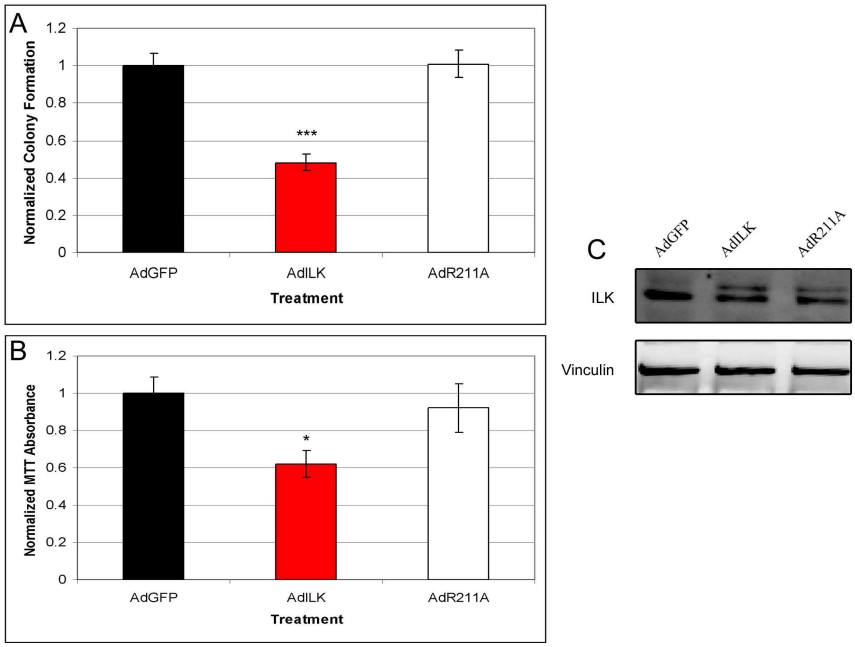
TUNEL
TUNEL assay was adapted to use in an automated *in situ* hybridization instrument, Discovery™ (Ventana Medical Systems). TUNEL was performed with protease I digestion (Ventana Medical Systems) for 12 minutes, followed by treatment with recombinant terminal deoxynucleotidyltransferase (Tdt) (Gibco BRL) and Biotin-16-dUTP (Roche) as a label. Colorimetric visualization was completed with avidin-horseradish peroxidase and DAB, with a hematoxylin counterstain.

Factor VIII Scoring
Factor VIII stained tumor sections were analyzed for vessel density. Four random fields of view of each tumor section was examined at 40X magnification, and the number of Factor VIII positive vessels counted. The sum of these values was averaged over six independent tumor xenografts per treatment to yield a score of vessels/40X field.

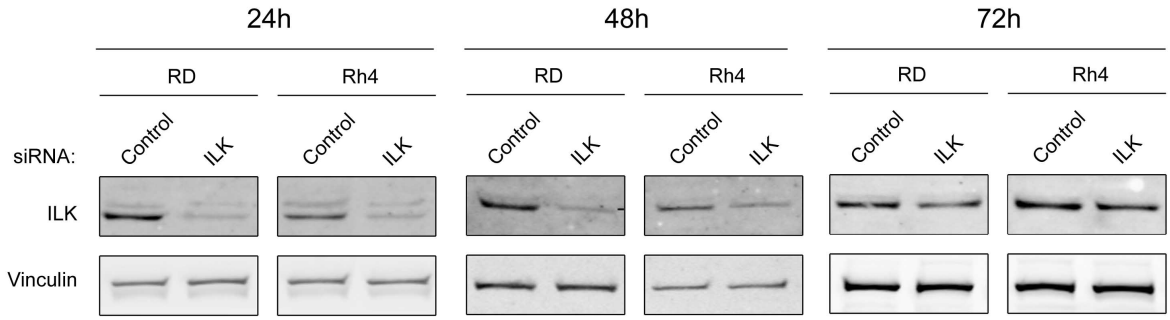
Supplemental Figures:



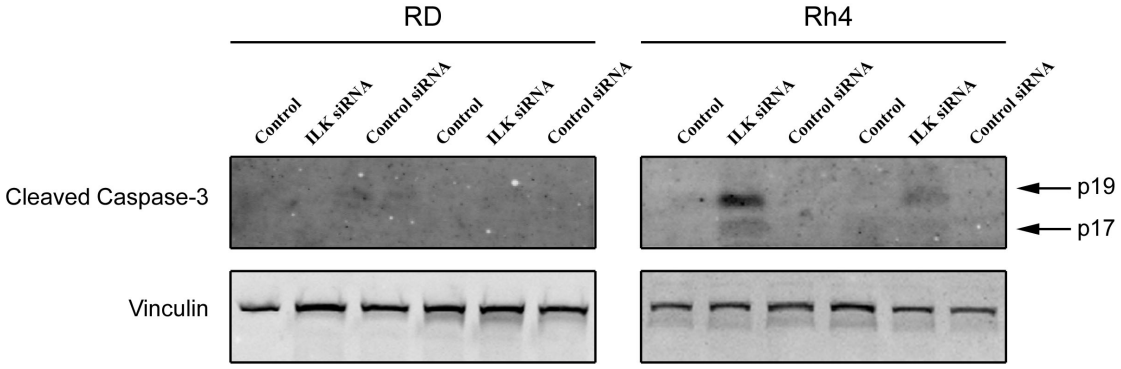
Supplemental Figure 1. ILK siRNA induces opposing effects on RMS cell growth in soft agar. RMS cell lines were treated with ILK or control siRNAs and suspended in soft agar. Twenty-eight days post-suspension, cells forming colonies were counted. Data is normalized against lipofectamine treated controls. n=4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ relative to control and control siRNA.



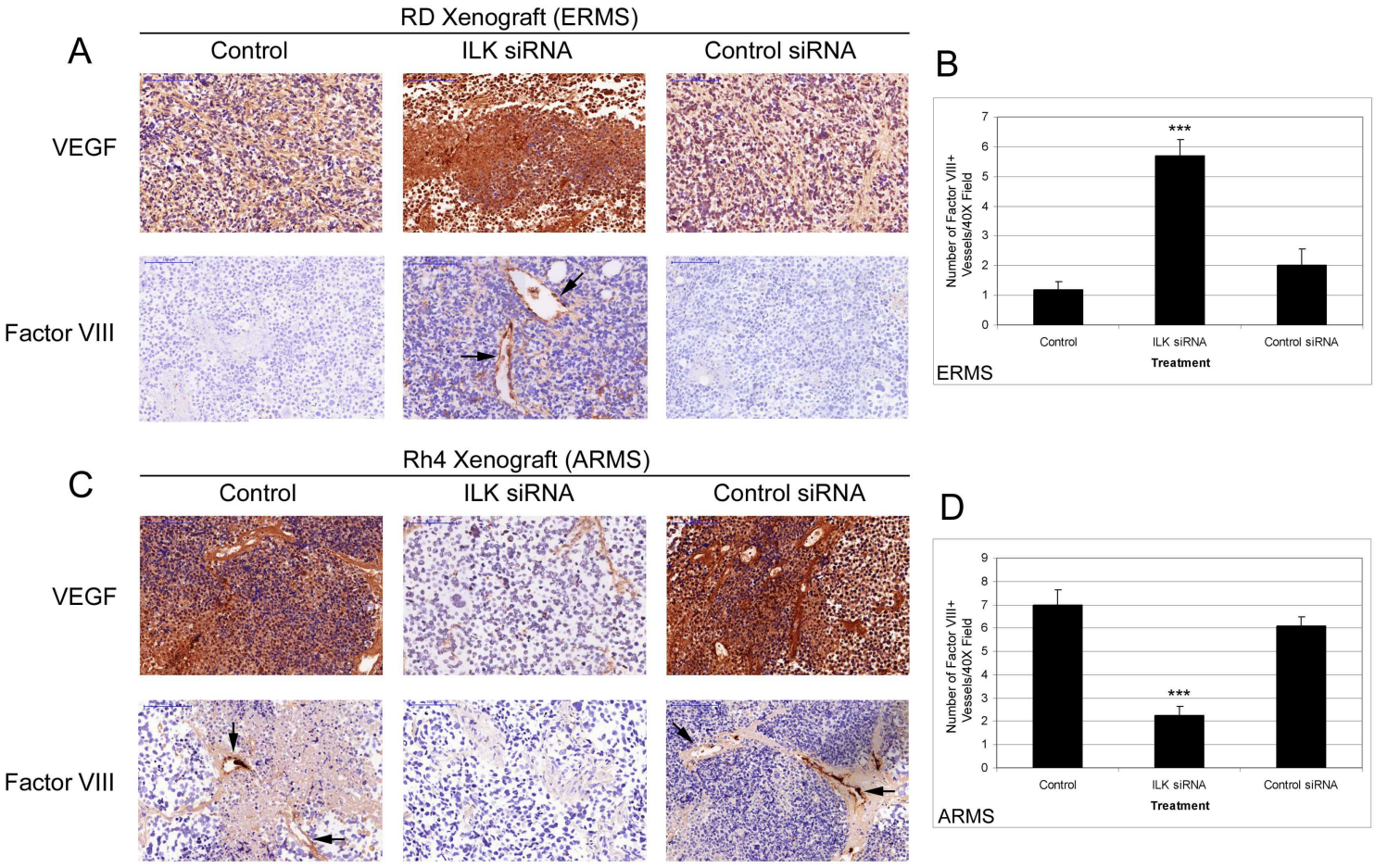
Supplementary Figure 2. ILK is growth suppressive in mesenchymal progenitor cells. MP59 cells were infected with AdGFP, AdILK or AdILK-R211A at 1000 IU. (A) $5x10^3$ treated cells were resuspended for colony assays and incubated for 14 days before processing. (B) $1x10^3$ cells were resuspended and incubated for four days before MTT assay. (C) $5x10^4$ cells were incubated for three days before lysis for western blotting using the noted antibodies. Data is normalized against AdGFP treated controls. ILK doublets are seen in some cell lines endogenously(8) and may represent an effect of antibody recognition of alternative splicing of the *ILK* gene(9). n=4. * $p<0.05$, *** $p<0.001$.



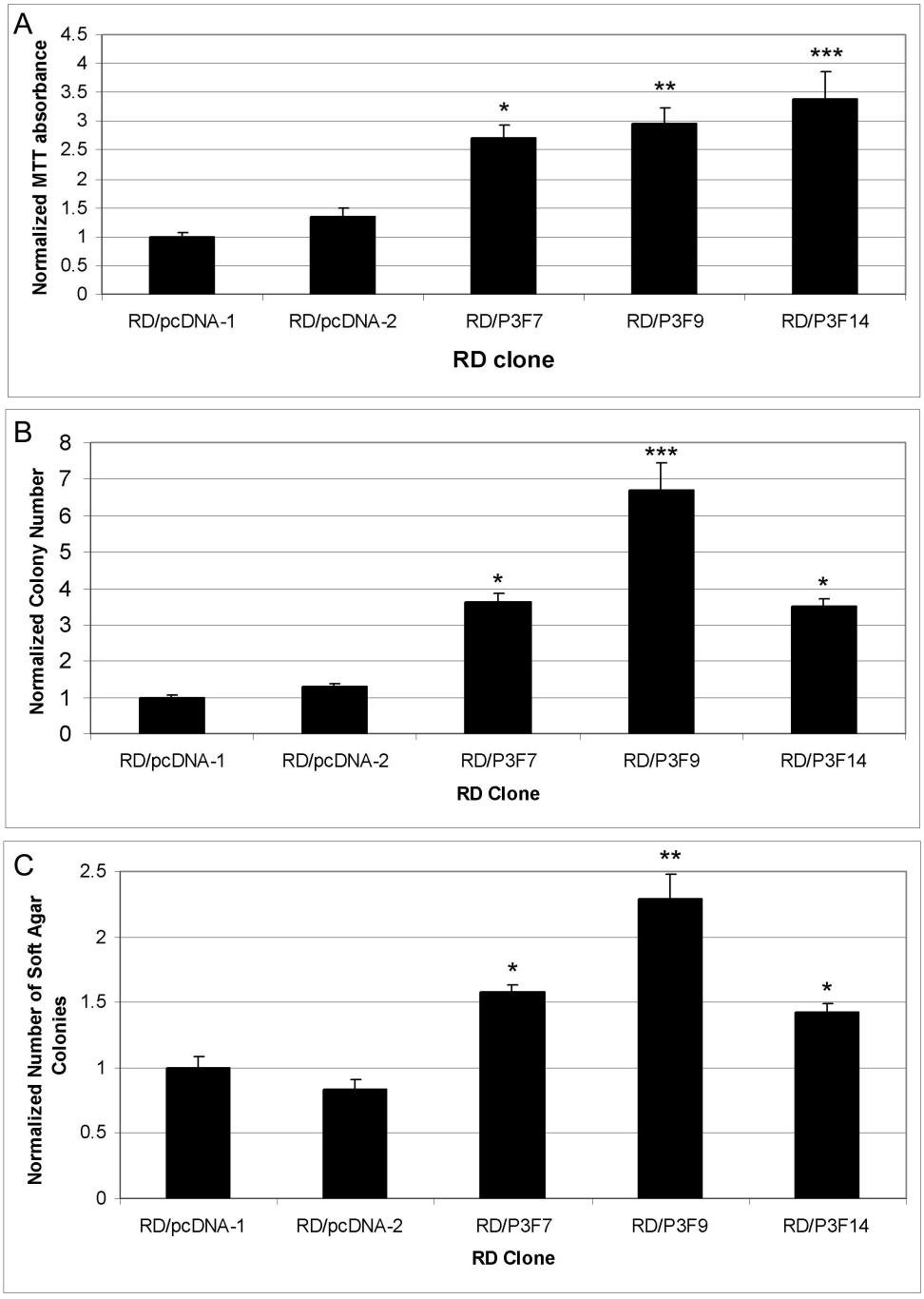
Supplemental Figure 3. ILK siRNA suppresses ILK protein in vivo.
RD and Rh4 cells were transfected with ILK or control siRNAs and cultured in vitro for 3 days. Cells were subcutaneously xenografted, as in the *materials and methods*, and xenografts were recovered 24-72 hours later. Xenografts were lysed for western blotting and analyzed for ILK expression. Data is representative of 3 independent xenografts per cell line, per treatment, per day.



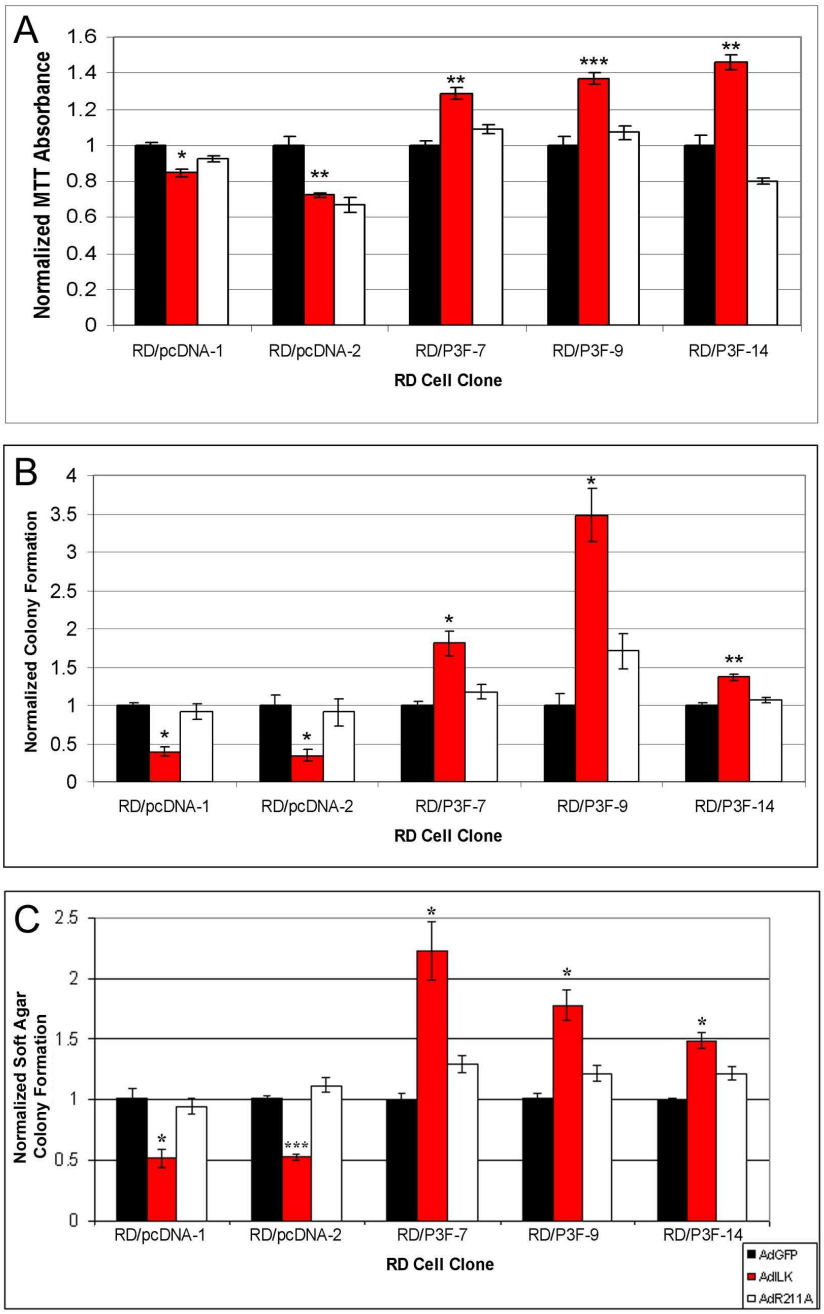
Supplemental Figure 4. ILK siRNA induces caspase cleavage in Rh4 xenografts.
RD and Rh4 xenografts were lysed for western blotting and analyzed using the noted antibodies. Two independent tumor xenografts, per cell line, per condition are shown. Vinculin is shown as a loading control. Data is representative of six independent tumor lysates per treatment group, per cell line. Arrows show cleaved p19 and p17 fragments of caspase-3.



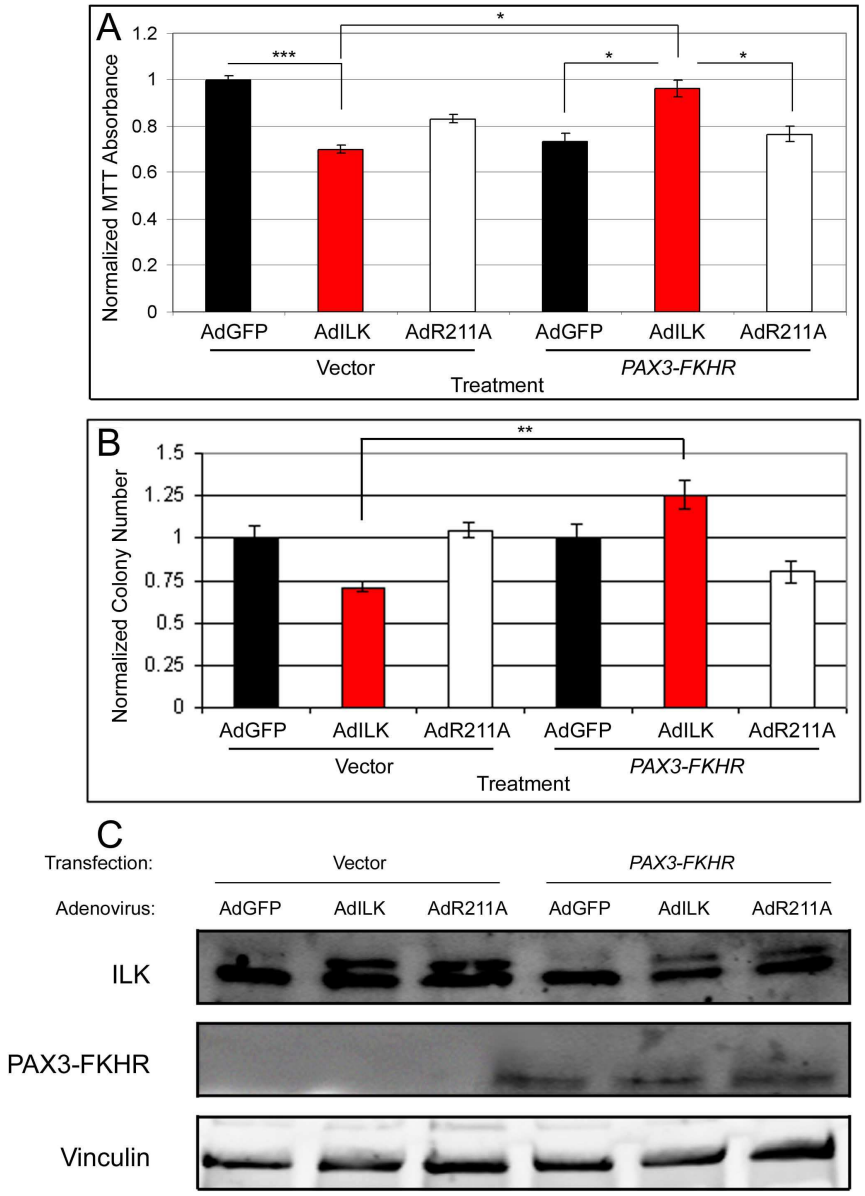
Supplemental Figure 5. ILK siRNA has opposing effects on angiogenesis in RMS xenografts. (A,C) RD and Rh4 xenografts were fixed and analyzed by immunohistochemistry using antibodies against VEGF and Factor VIII to demonstrate mature vasculature. Bar = 100 μm. Arrows show Factor VIII stained vessels. Data is representative of six independent tumors per treatment group, per cell line. (B,D) Average factor VIII positive vessel density of ERMS (B) and ARMS (D) tumor xenografts in response to control or ILK siRNAs, or lipofectamine treatment (control). *** $p<0.001$. n=6.



Supplemental Figure 6. *PAX3-FKHR* induces growth in RD stable clones.
RD vector control (RD/pcDNA-1, RD/pcDNA-2) or *PAX3-FKHR* expressing (RD/P3F-7, RD/P3F-9, RD/P3F-14) clones were analyzed by MTT (A), colony (B) and soft agar (C) assays. Data is normalized to RD/pcDNA-1 cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA. n=20 (MTT), 4 (colony, soft agar).

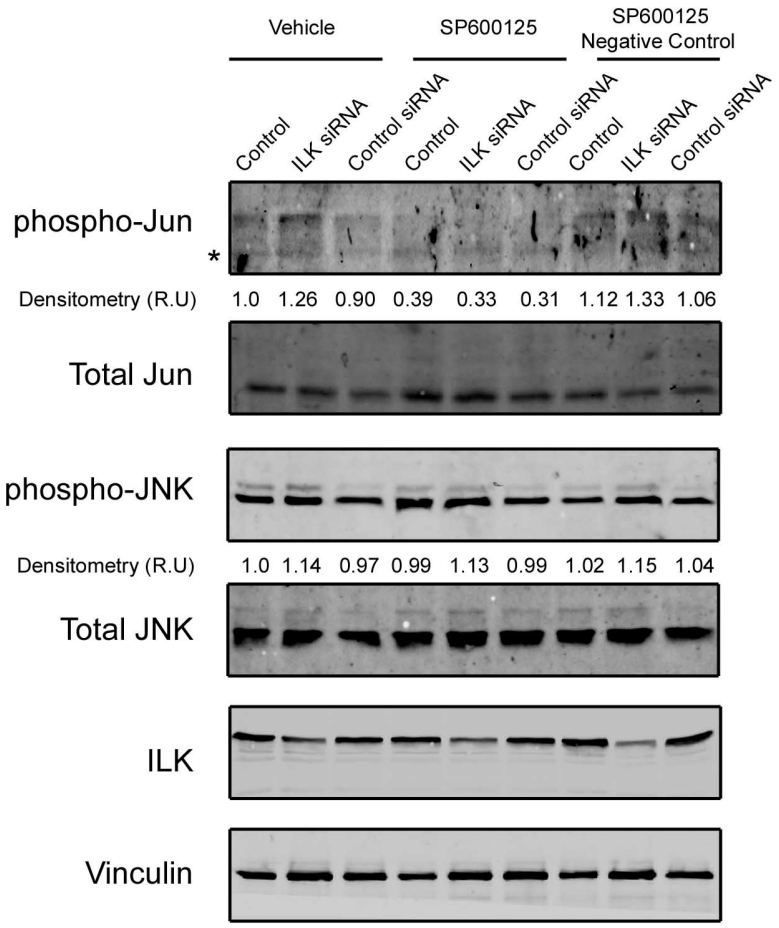


Supplemental Figure 7. *PAX3-FKHR* restores the oncogenic effect of ILK in biological assays. RD vector control (RD/pcDNA-1, RD/pcDNA-2) or *PAX3-FKHR* expressing (RD/P3F-7, RD/P3F-9, RD/P3F-14) clones were infected with 1000 IU AdGFP, AdILK or AdILK-R211A and resuspended for MTT (A), colony (B) and soft agar (C) assays. MTT, colony and soft agar assays were conducted 3, 14 and 28 days post-infection. Data is normalized to AdGFP treated controls. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA to AdGFP. n=4.

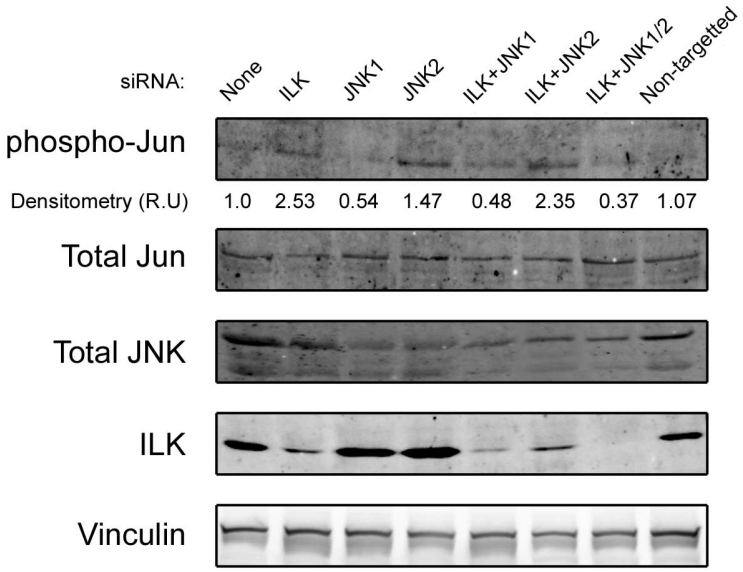


Supplemental Figure 8. ILK growth-suppressive function is sensitive to *PAX3-FKHR* in mesenchymal progenitor cells.

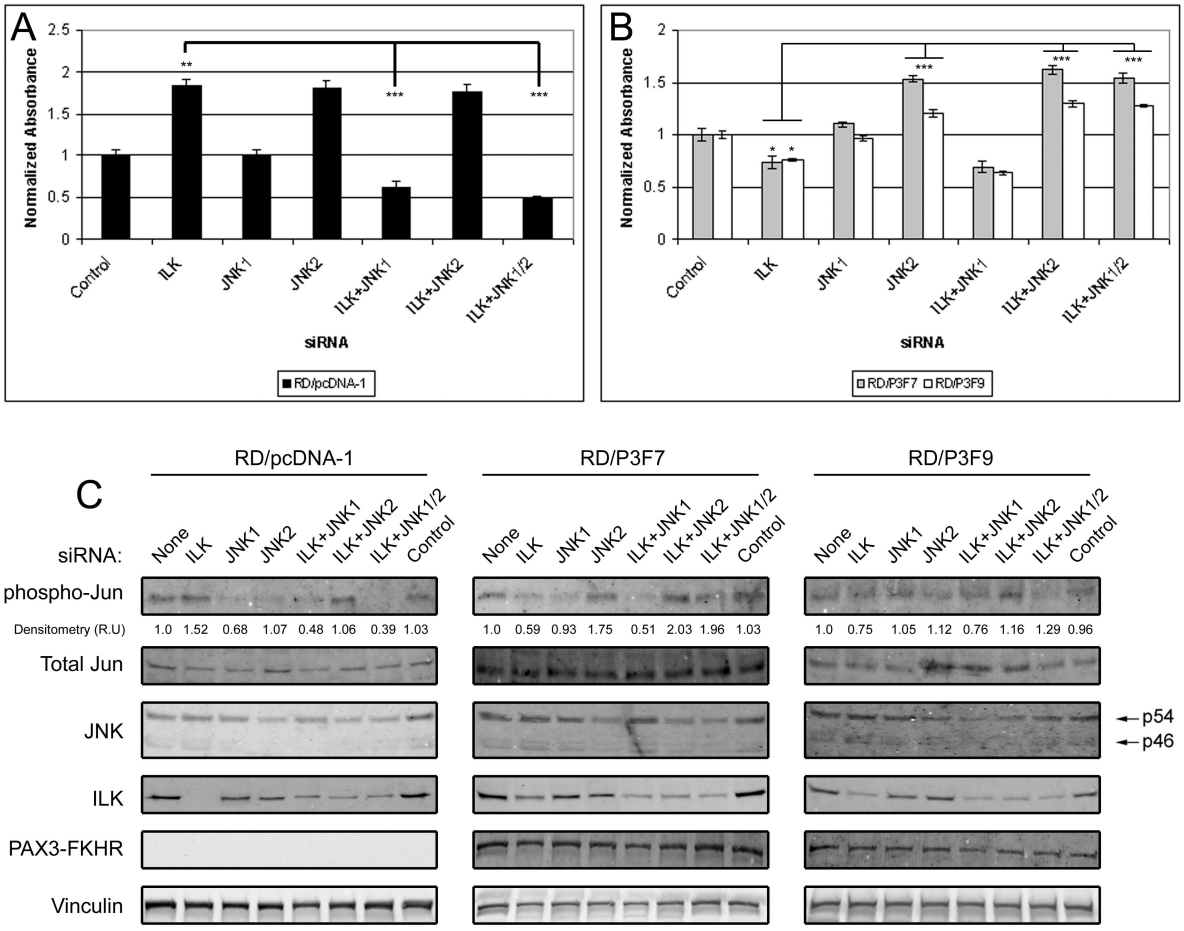
MP59 mesenchymal progenitor cells were transiently transfected with *PAX3-FKHR* or pcDNA3.1 (vector) plasmids alone. Twenty-four hours later, cells were infected with AdGFP, AdILK or AdILK-R211A at 500 IU. Cells were resuspended for MTT (A) and colony assays (B), and protein extraction for western blotting (C), 3 (MTT, protein) and 14 (colony) days post-infection, respectively. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA. n=4.



Supplemental Figure 9. SP600125 reduces c-Jun activation in RD RMS cells.
RD ERMS cells were transfected with ILK or control siRNAs and incubated for 96 hours before exposure to 20 μM SP600125, N^1-Methyl-1,9-pyrazoloanthrone (SP600125 negative control) or equal volumes of DMSO (vehicle) for 1 hour before lysates were prepared for western blotting. Lysates were analyzed using the noted antibodies. Data is representative of 4 independent lysates. * denotes a non-specific band. Arrows show p46 and p54 JNK proteins. Densitometry values represent the average of 4 independent blots and are normalized relative to total protein.

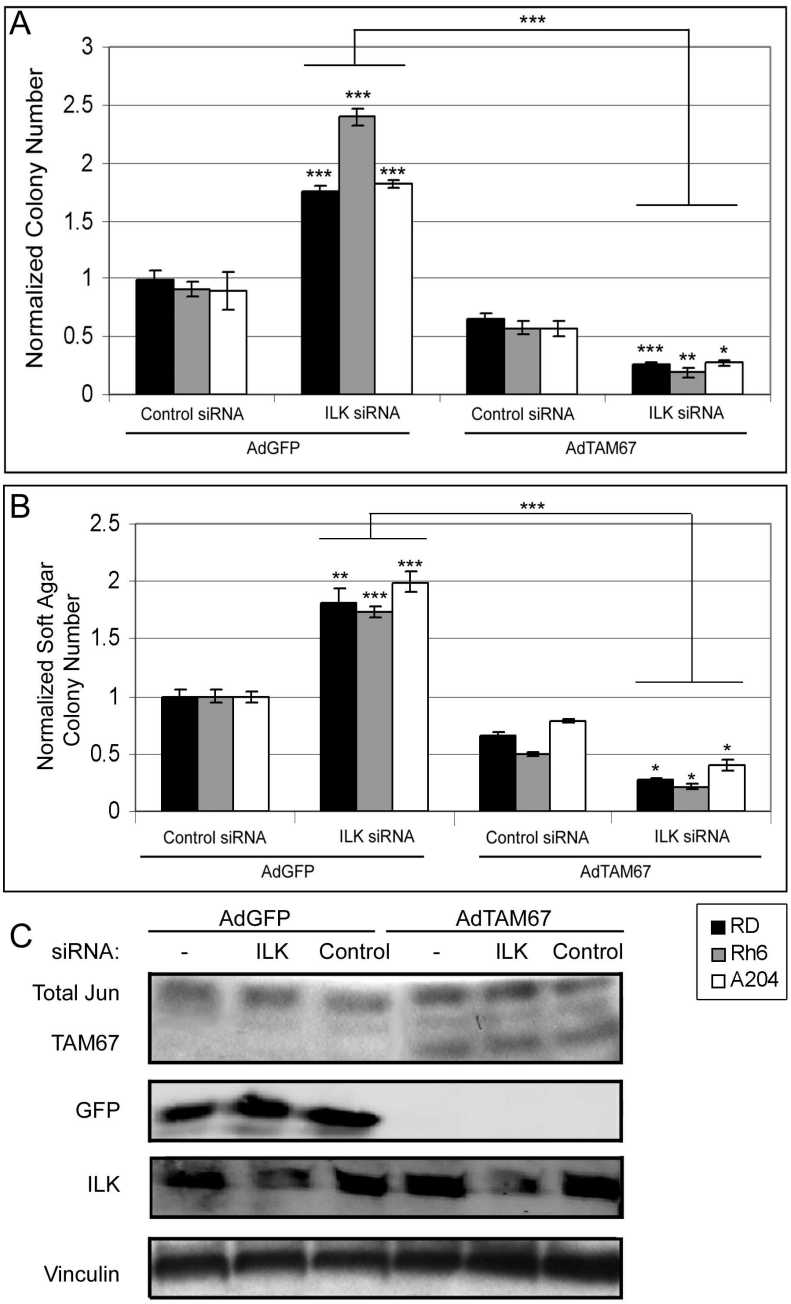


Supplemental Figure 10. JNK1 mediates c-Jun activation in response to ILK siRNA.
RD ERMS cells were transfected with ILK or control siRNAs. Subsequent transfection with JNK2 siRNA and/or JNK1 siRNA was conducted at 48 and 72 hours, respectively. Protein lysates were prepared 96 hours post-initial transfection. Lysates were analyzed using the noted antibodies by western blotting. Data is representative for 4 independent lysates. Arrows show p46 and p54 JNK proteins. Densitometry values represent the average of 4 independent blots and are normalized relative to total protein.



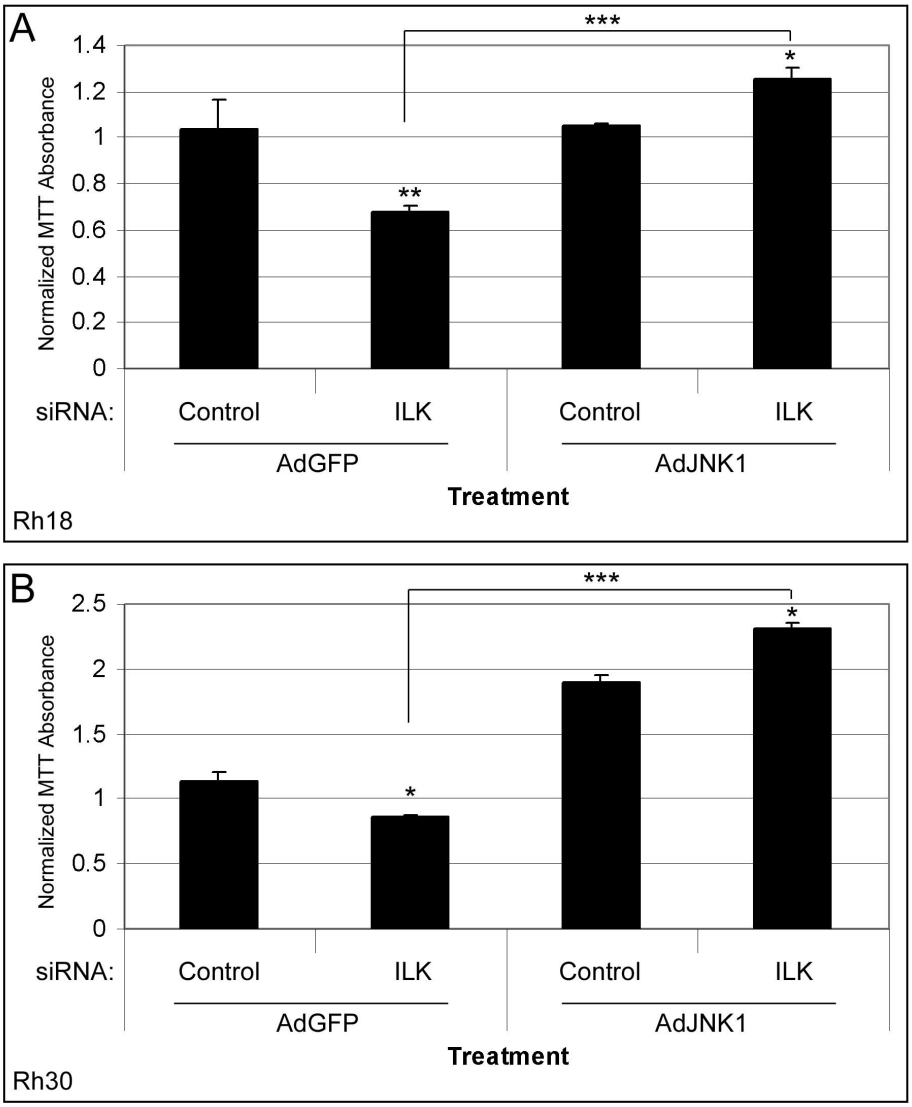
Supplementary Figure 11. JNK1 mediates the ILK growth response in RD vector controls but not *PAX3-FKHR* clones.

(A,B) RD vector (RD/pcDNA-1) and *PAX3-FKHR* (RD/P3F7, RD/P3F9) clones were transfected with combinations of ILK, JNK1 and JNK2-targetted siRNAs, and analyzed by MTT assay. The induction of growth in response to JNK2 siRNA in RD/pcDNA-1 cells is consistent with G418-induced alterations in JNK signaling(10, 11). Data is normalized to cells transfected with control siRNA. n=4. * $p<0.05$, ** $p<0.01$, *** $p<0.001$. (C) Cells were lysed for protein 96-hours post-transfection and analyzed by western blotting using the noted antibodies. Densitometry is the average of four independent blots.



Supplemental Figure 12. ERMS proliferation in response to ILK siRNA can be reduced by disruption of c-Jun/AP-1.

ERMS cells were transfected with nothing (-), ILK or control siRNAs. (A) Forty-eight hours later, cells were infected with AdGFP or AdTAM67, and analyzed by MTT assay at 96 hours. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA. n=4. (B) Cells were resuspended in soft agar and colony formation was assayed 28 days later. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA. n=4. (C) RD ERMS cells were treated as described above and lysates were prepared for western blotting using the noted antibodies at 96 hours post-transfection. Data is representative of four independent lysates. Data in A,B are normalized to levels of AdGFP-treated, lipofectamine transfected cells.



Supplementary Figure 13. JNK1 overexpression restores the growth suppressive effect of ILK in ARMS.

Rh18 (A) and Rh30 (B) ARMS cells were transfected with ILK or control siRNAs. Twenty-four hours later, cells were infected with AdGFP or AdJNK1. Cells were treated with 100 (Rh30) and 500 (Rh18) IU of virus, respectively. At 96 hours post-transfection, MTT assays were conducted. Data is normalized against AdGFP-treated lipofectamine transfected cells. * $p<0.05$, ** $p<0.01$, *** $p<0.001$ by ANOVA. n=4.

Supplemental References:

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