Supplementary Methods

AXL Immunohistochemistry

Paraffin embedded tissue slides were deparaffinized with xylene, rehydrated, and unmasked following standard immunohistochemical methods. The AXL primary antibody (RandD Systems, AF154) was used at a 1:500 dilution. Negative controls for all samples were done using the secondary antibody alone. Antigen-antibody complexes were visualized using the VECTASTAIN ABC system (Vector Laboratories) and DAB Substrate Kit for Peroxidase (Vector Laboratories) following the protocols of the manufacturer. Slides were counterstained with hematoxylin. AXL staining on the membrane of tumor cells was scored microscopically according to the percentage of cells positive for AXL expression (0 for absence, 1 for poor quality sample, 2 for < 60%, and 3 for >60%).

Patients and Tissue Microarrays

Ovarian human tissue microarrays were obtained from the Stanford University Pathology Department and US Biomax. A total of 73 paraffin embedded tumor samples were obtained from previously untreated ovarian cancer patients at Stanford Hospital from 1995 to 2001. These primary ovarian tumor samples were assembled into a tissue microarray consisting of two samples per patient. An additional 30 tumor samples from the peritoneum were also evaluated in this microarray. All patients had serous ovarian cancer, and staging information was obtained according to the International Federation of Gynecology and Obstetrics standards. All specimens and their corresponding clinical

information were collected under protocols approved by the institutional review board at Stanford University. An additional commercially available tumor microarray was used to examine 32 metastatic lesions from the omentum (US Biomax).

SKOV3ip.1 Adhesion to Collagen Type I

SKOV3ip.1 shSCRM and shAXL cells were fluorescently labeled with 5um CMFDA (Molecular Probes). Cells were washed and detached using a non-enzymatic cell dissociation buffer (Gibco). Cells (5X10e5) were plated in triplicate into a 96 well plate and precoated with 50ug/ul of collagen type I (BD Bioscience). After a 60-minute incubation at 37C, cells were carefully washed 5 times. Fluorescent activity (excitation, 494 nm; emission, 517nm) was measured using a fluorescent spectrophotometer.

Migration Assays

Cellular migration was examined *in vitro* as previously described (Erler et al., 2006). Briefly, cells were serum-deprived for 24 hr and seeded ($2.5X10^4$ cells) in triplicate onto uncoated inserts (BD Biosciences), moved to chambers containing FBS as chemo-attractant and incubated for 24 hr. After removing the non-invading cells, the cells at the bottom side of the membranes were fixed, stained and counted. Five fields were counted for each membrane. The % migration was determined as follows: (average # of cells migrating in shAXL cells /average # of cells migrating in shSCRM cells) X 100. Experiments were performed in triplicate and repeated three times.

Collagen Invasion Assay

Collagen invasion assays were performed as previously described (22). Invasion through collagen was quantified by calculating the percentage of tumor cells that displayed a branching phenotype per 20X field. Three fields per sample were counted. Experiments were performed in triplicate and repeated 3 times.

Gelatin Substrate Zymography

SKOV3ip.1 shSCRM and shAXL cells were serum starved for 48 hours. 25,000 cells were plated into a 96 well plate and conditioned media was collected 24 hours later. Equal volumes of conditioned media were run under non-reducing conditions on 10% zymogram gels (Invitrogen). After electrophoresis, gels were washed in 2.5% (v/v) Triton X-100 to remove SDS and washed in 50 mM Tris-HCl, 5 mM $CaCl_2$, and 0.1% Triton X-100 (pH 7.8) and incubated overnight at 37°C. Zymograms were stained for 30 min with 0.25% (w/v) Coomassie Brilliant Blue R250 dissolved in 40% methanol and 10% glacial acetic acid. Gels were destained in 40% methanol and 10% glacial acetic acid. Experiments were performed in duplicate and repeated three times.

Cell Proliferation Assays

For monolayer growth curves, cells (50,000) were plated into 60mm dishes in triplicate. Every three days, the cells were trypsinized, counted using a cell counter (coulter counter) and 50,000 cells were replated and counted.

XTT Survival Assay

Cell viability was measured by the XTT assay as previously described (Sutphin et al., 2007). Briefly, serum fed or starved cells (0, 3, 6, and 7 days) were incubated with phenol red–free medium with 0.3 mg/mL XTT and 2.65 μg/mL *N*-methyl dibenzopyrazine methyl sulfate. The 96-well plates were returned to the 37°C incubator for 1 to 2 h. Metabolism of XTT was quantified by measuring the absorbance at 450 nm.

Protein Isolation and Western Blot Analysis

Protein lysates were harvested in 9M Urea, 0.075M Tris buffer (pH 7.6). Protein lysates were quantified using the Bradford assay, and subjected to reducing SDS-PAGE using standard methods. Western blots were probed with antibodies against AXL (RandD Systems), Hsp70 (Affinity BioReagents), AKT (Cell Signaling), phospho-AKT (Cell Signaling), ERK1/2 (Cell Signaling), and phospho-ERK1/2 (Cell Signaling).

Analysis of sAXL expression in the serum of mice was performed by using 1.5 ul of serum from each sample.

For GAS6 stimulation, cells were serum starved for 24 hours. Cells were then treated with 25um of PI3K inhibitor (Ly294002, Bio Mol Research Laboratory) or 100ul of conditioned media containing the AXL Ectodomain for 4 hours before treatment with 400ng/ml of GAS6 for 15 minutes.

RNA and Real Time PCR Analysis

RNA was isolated and Real time PCR was performed as previously described (24). The following primer sets were used to amplify specific target genes: 18S FWD: 5-

GCCCGAAGCGTTTACTTTGA-3 REV: 5-TCCATTATTCCTAGCTGCGGTATC-3; MMP-2 FWD: 5- GCCCCAGACAGGTGATCTTG-3 REV 5-GCTTGCGAGGGAAGAAGTTGT-3; MMP-1 FWD: 5-ACACATCTGACCTACAGGATTGA-3 REV: 5-GTGTGACATTACTCCAGAGTTGG-3; MMP-9 FWD 5-GGGACGCAGACATCGTCATC-3 REV: 5-TCGTCATCGTCGAAATGGGC-3; AXL FWD 5-GTGGGCAACCCAGGGAATATC-3 REV 5-GTACTGTCCCGTGTCGGAAAG-3.

Reporter Assays

The MMP-2 reporter plasmid driven by 1659 bp of the MMP-2 promoter was a gift from Dr. Etty Benveniste (University of Alabama, (20)). Luciferase activity was determined by Dual-Glo Luciferase Assay reagent (Promega) and measured in a Monolight 2010 Luminometer (Analytical Luminescence Laboratory). Firefly luciferase activity was normalized to Renilla activity. Assays were performed in triplicate and were repeated twice.

Supplementary Figure Legends

Supplementary Figure 1. AXL does not affect ovarian tumor cell adhesion or survival. **A.** Percent cell migration of SKOV3ip.1 cells in boyden chamber migration assays towards serum as the chemoattractant. **B.** Analysis of SKOV3ip.1 cellular adhesion to collagen type I (Col I). Error bars represent the standard error of the mean. **C.** Survival analysis of AXL wild type and AXL deficient SKOV3ip.1 tumor cells following serum withdrawal as determined by the XTT assay.

Supplementary Figure 2. AXL does not affect MAPK/ERK signaling in human ovarian cancer cells. Western blot analysis of AXL, ERK1/2 (p44/p42), and phospho-ERK1/2 (p-ERK1/2) in SKOV3ip.1 cells. Heat shock protein-70 (HSP70) was used as a protein loading control.

Supplementary Figure 3. Soluble AXL ectodomain therapy inhibits AXL signaling and invasion in vitro. **A.** Schematic representation of the mechanism for soluble AXL therapy. Soluble AXL (sAXL) functions as a decoy receptor to inhibit endogenous AXL signaling. **B.** Western blot analysis of phospho-AKT Ser473 expression in cells treated with conditioned media containing the soluble AXL receptor (sAXL) or control media (-). All cells were starved for 48 hours and treated with GAS6 (+) or vehicle (-). **C.**

Collagen invasion assay in MDA-231 cells treated with conditioned media containing control vector or sAXL. Photographs are representative of 9 samples per group.

Supplementary Figure 4. Treatment with soluble AXL receptors inhibits metastatic tumor burden in mice with established OVCAR-8 metastases. A. Schematic representation of the soluble AXL receptor treatment study. Nude mice were i.p. injected with 5X10e6 OVCAR-8 cells. Fourteen days after implantation, the presence of macroscopic lesions was verified in mice (shown is a representative photograph of a mouse with peritoneal metastasis at day 14 following injection, metastatic lesions are circled). At day 14, mice were injected with adenoviruses expression the IgG2α-Fc control (Ad-Fc) or soluble AXL receptor (Ad-sAXL). Serum levels of sAXL expression was assessed by western blot analysis. Day 34 following tumor cell implantation tumor burden was assessed in all mice. **B.** Representative photographs of mice treated with adenoviruses expressing Ad-sAXL or Ad-Fc at 28 days following tumor cell injection. Metastatic lesions are circled. Graphs show the average total tumor number and weight for 8 mice per group. Error bars represent the S.E.M.. Note that a statistical difference in tumor number and weight ($p < 0.01$, students t-test) was observed between Ad-Fc and Ad-sAXL treated mice (*).

Supplementary Figure 5. Soluble AXL ectodomain therapy does not induce normal tissue toxicity. H&E staining of liver and kidney tissue collected from mice treated with Fc or sAXL.

Supplementary Figure 6. Soluble AXL ectodomain therapy does not alter blood chemistry in mice.

Supplementary Figure 7. Scanned western blots for Figure 2.

Supplementary Figure 8. Scanned western blots for Figure 4.

Supplementary Figure 9. Scanned western blots for Figure 5.