

p5RHH nanoparticle-mediated delivery of AXL siRNA inhibits metastasis of ovarian and uterine cancer cells in mouse xenografts

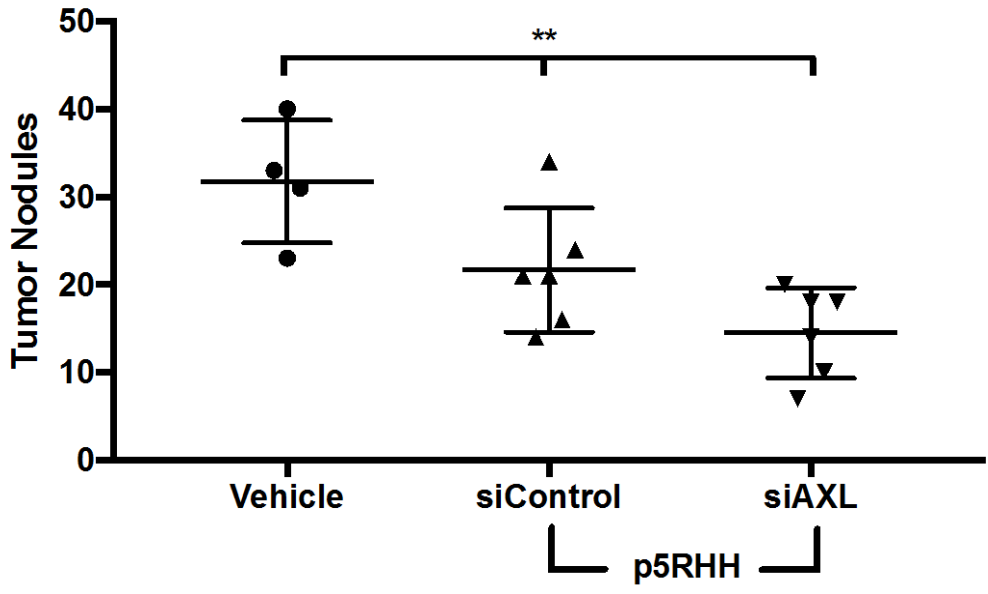
Kathryn A. Mills^{1,2,+}, Jeanne M. Quinn^{1,+}, S. Tanner Roach¹, Marguerite Palisoul^{1,2}, Mai Nguyen¹, Hollie Noia¹, Lei Guo¹, Jawad Fazal³, David G. Mutch², Samuel A. Wickline³, Hua Pan^{3,*}, Katherine C. Fuh^{1,2,*}

¹Center for Reproductive Health Sciences, Department of Obstetrics and Gynecology, Washington University School of Medicine, 425 S. Euclid Avenue, St. Louis, MO 63110

²Division of Gynecologic Oncology, Department of Obstetrics and Gynecology, Washington University School of Medicine, 660 S. Euclid Avenue, St. Louis, MO 63110

³Department of Cardiovascular Sciences, The USF Health Heart Institute, Morsani School of Medicine, University of South Florida, 4202 E. Fowler Avenue, Tampa, FL 33620, USA

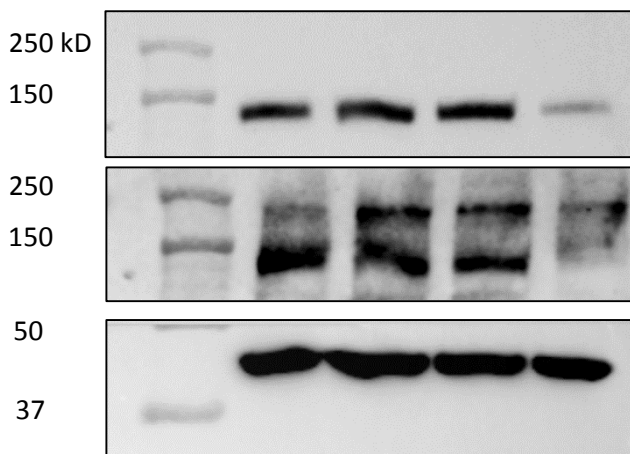
A



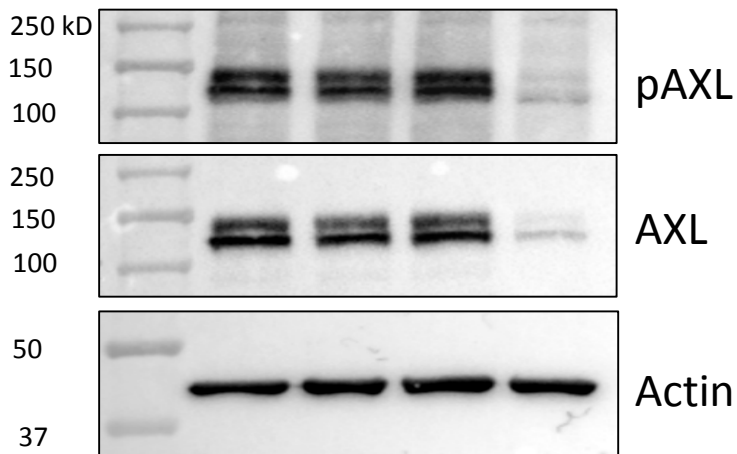
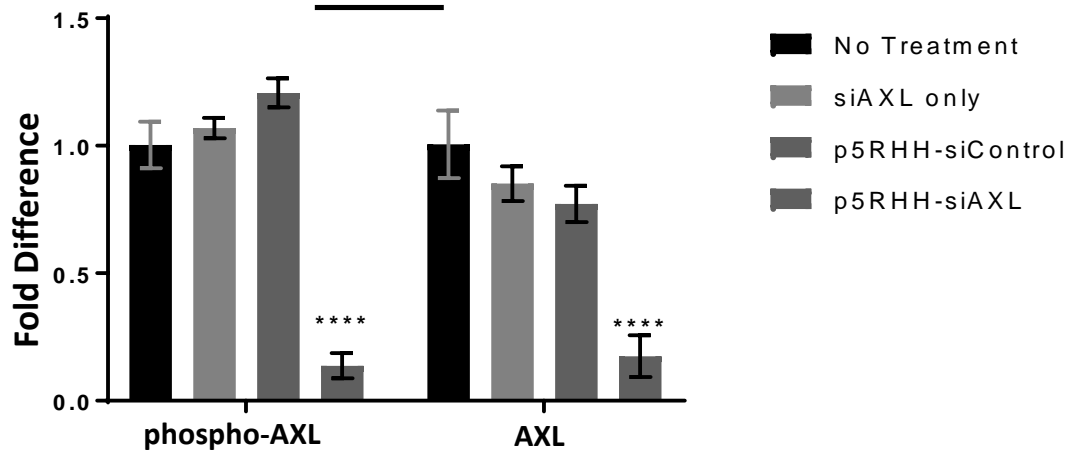
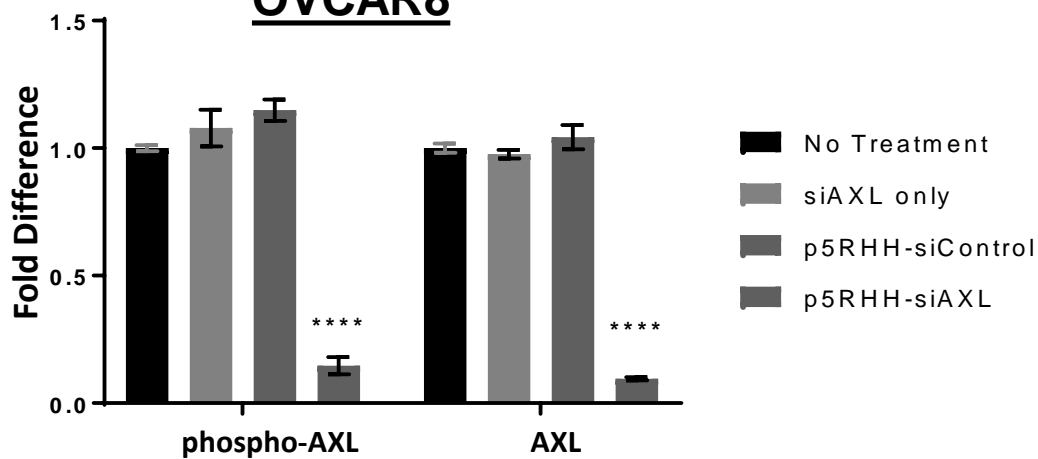
Supplementary Figure S1. Treatment with p5RHH-siAXL reduces OVCAR8 tumor nodules in mouse xenografts. A) Graph depicts the number of peritoneal tumor nodules per mouse treated with vehicle (n=4), p5RHH-siControl (n=6), or p5RHH-siAXL (n=6). Data are presented as mean +/- SD. **** $P < 0.01$** by one-way Anova.

A**ARK1**

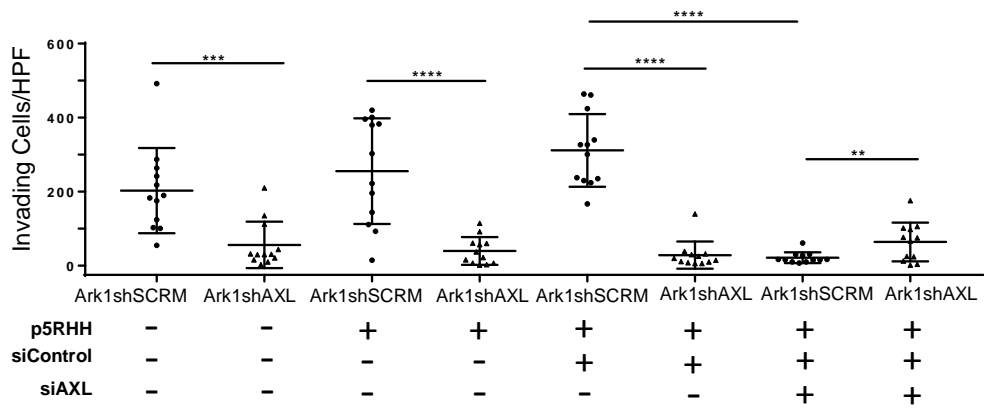
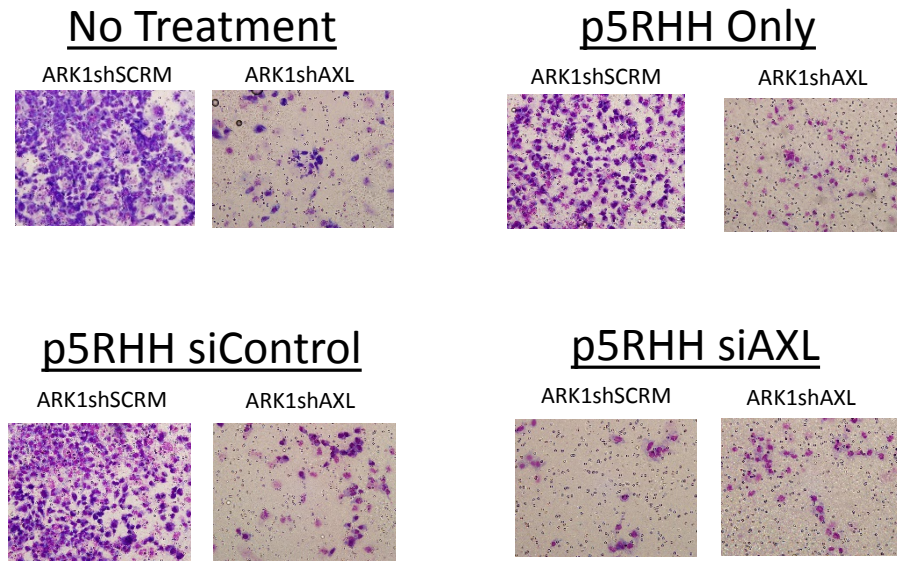
p5RHH	-	-	+	+
siControl	-	-	+	-
siAXL	-	+	-	+

**B****OVCAR8**

p5RHH	-	-	+	+
siControl	-	-	+	-
siAXL	-	+	-	+

**C****ARK1****D****OVCAR8**

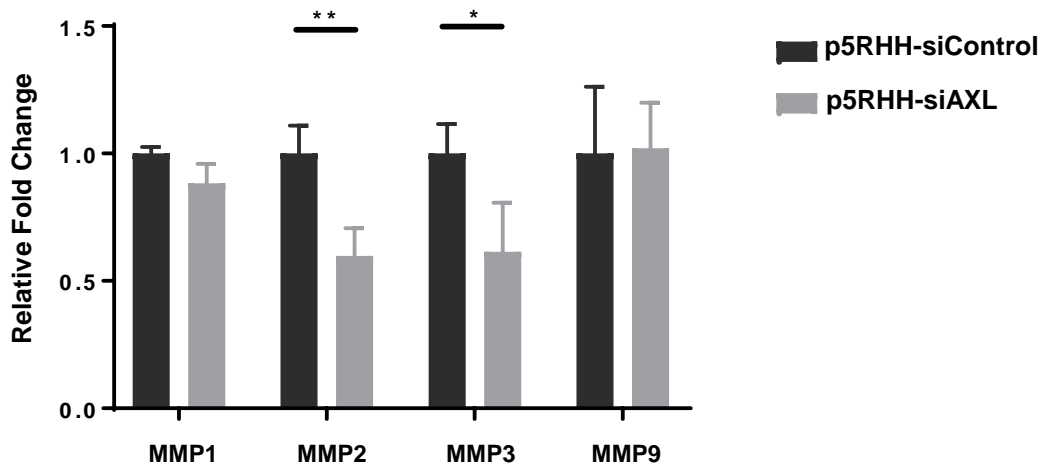
Supplementary Figure S2. A,B) Full length blots of Figure 1A. The red lines indicated the cropping locations. **C,D)** Densitometric analysis of AXL and phospho-AXL expression for blots in Figure 1A

A**B**

Supplementary Figure S3. Ark1shSCRM cell line have reduced invasion after treatment of p5RHH-siAXL in vitro

A) Matrigel Invasion results comparing ark1shSCRM and ark1shAXL no treatment, treatment with p5RHH only, treatment with p5rHH-siControl, and p5rHH-siAXL. Significance was calculated using one-way ANOVA. ** $P < 0.01$; *** $P < 0.001$; **** $P < 0.0001$.

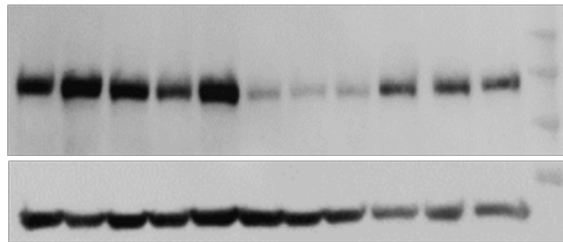
B) Representative images of matrigel invasion, 20x magnification

A

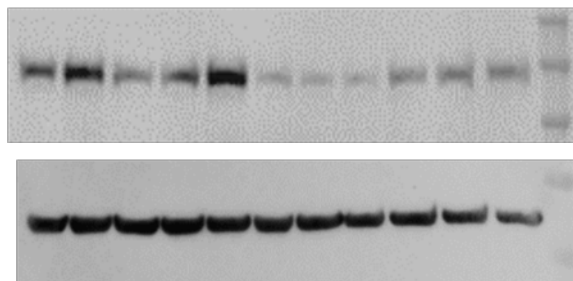
- **Supplementary Figure S4.** qPCR was performed on FFPE tumors treated with p5RHH-siControl or p5RHH-siAXL and phospho-AXL and AXL mRNA expression was assessed. Significance was calculated using SD or DDC_T. Error bars indicate the range of fold change. * P < 0.05; ** P < 0.01.

A

p5RHH	-	-	+	+	+	+	+	+	+	+
siControl	-	-	-	-	-	-	-	-	+	+
siAXL	-	+	-	-	-	+	+	+	-	-

**B**

p5RHH	-	-	+	+	+	+	+	+	+	+
siControl	-	-	-	-	-	-	-	-	+	+
siAXL	-	+	-	-	-	+	+	+	-	-



Supplementary Figure S5. A) Western blot analysis of AXL at 48 hours in Ark1 cells untreated and treated with siAXL alone, p5RHH alone, p5RHH-siAXL, and p5RHH-siControl. **B)** Western blot analysis of AXL at 96 hours in Ark1 cells treated as stated in A.