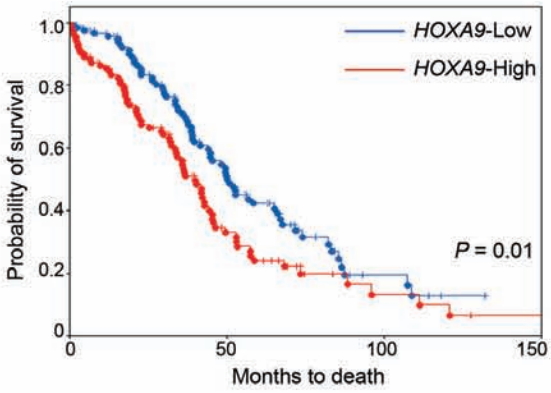
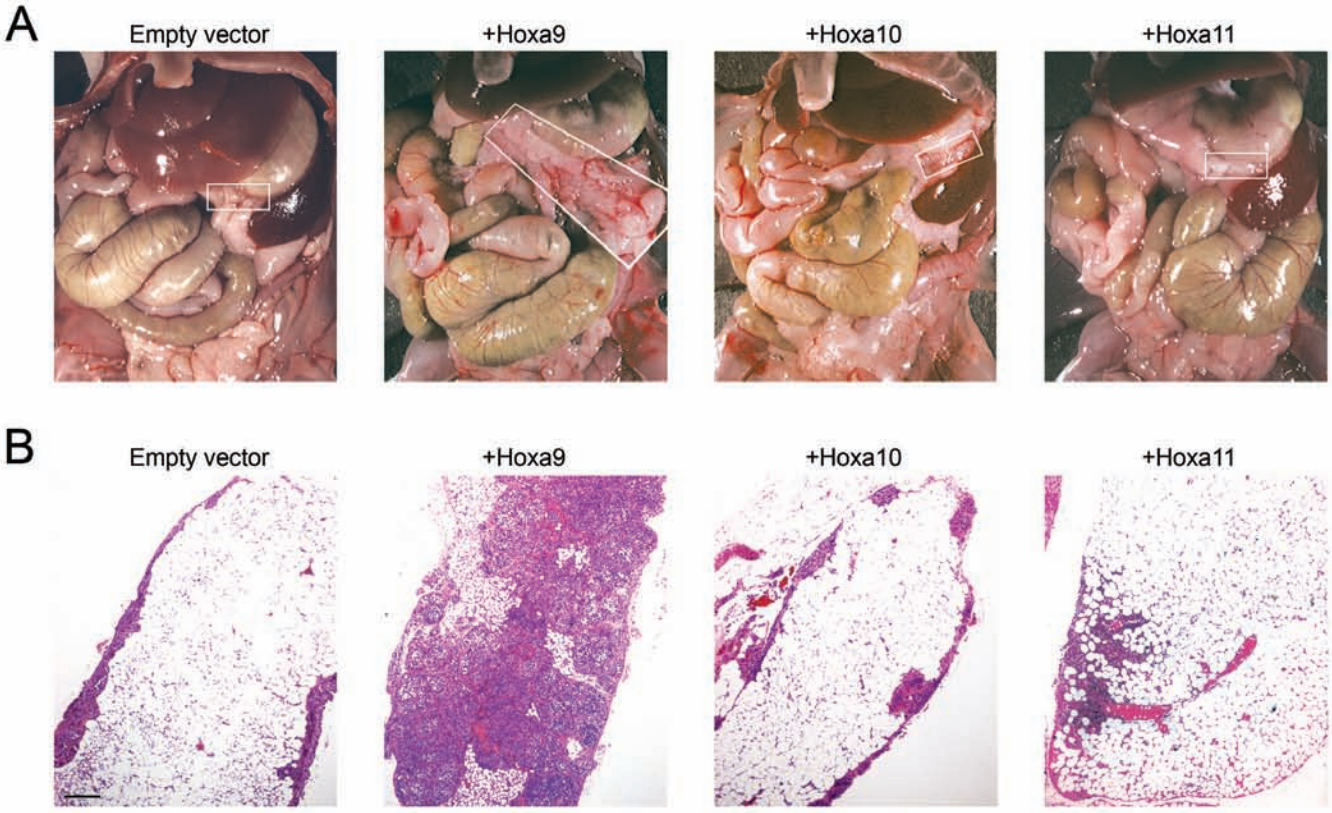
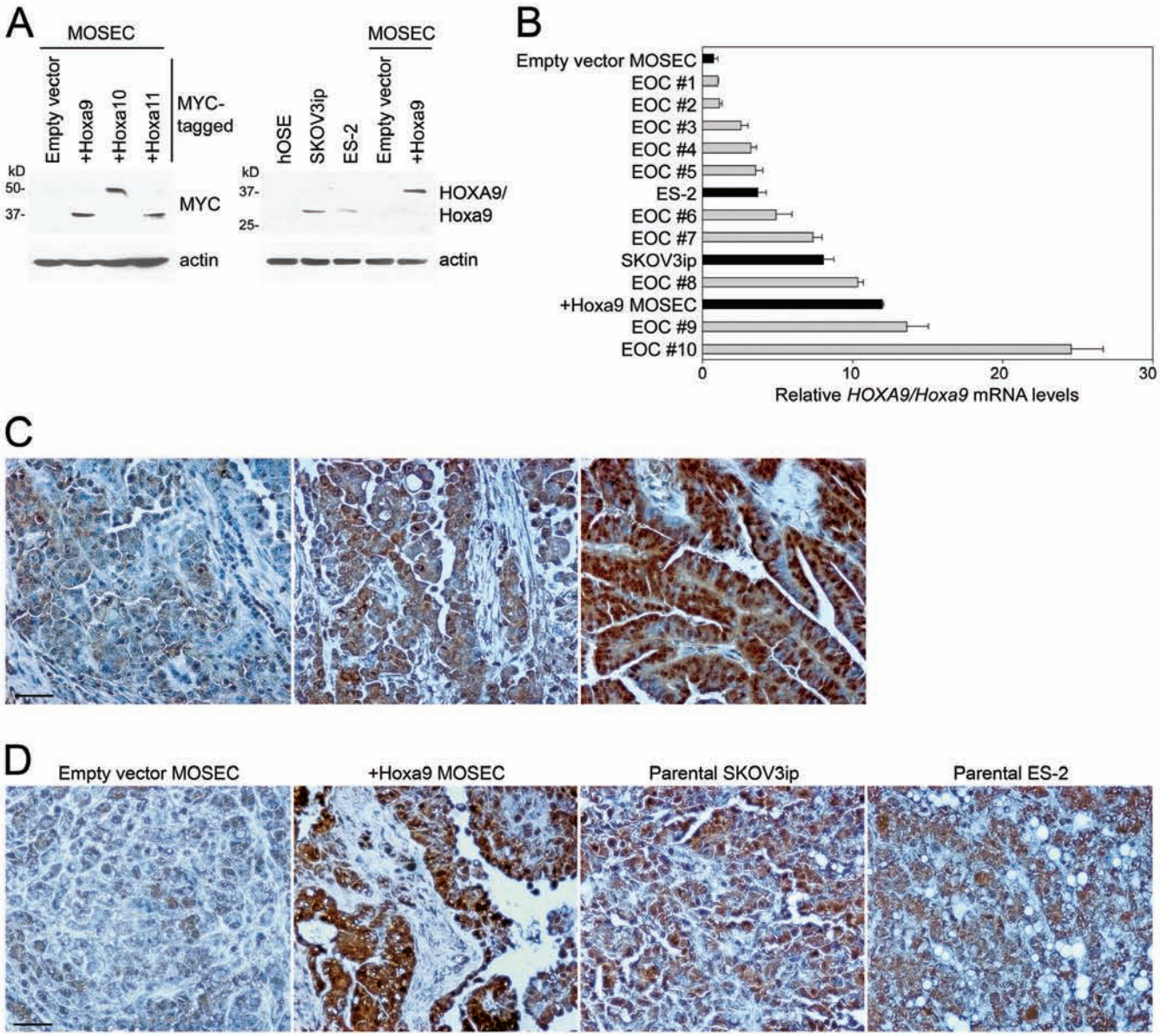


Supplemental Figure 1
Association of high *HOXA9* expression with reduced survival in the TCGA serous EOC dataset.
Kaplan-Meier plot analysis of overall survival times of patients stratified by *HOXA9* expression in tumors, where *HOXA9* transcript levels were defined as High (≥ upper quartile) and Low (≤ lower quartile) (n = 142 cases per group). Significance value was determined by Log-rank test.



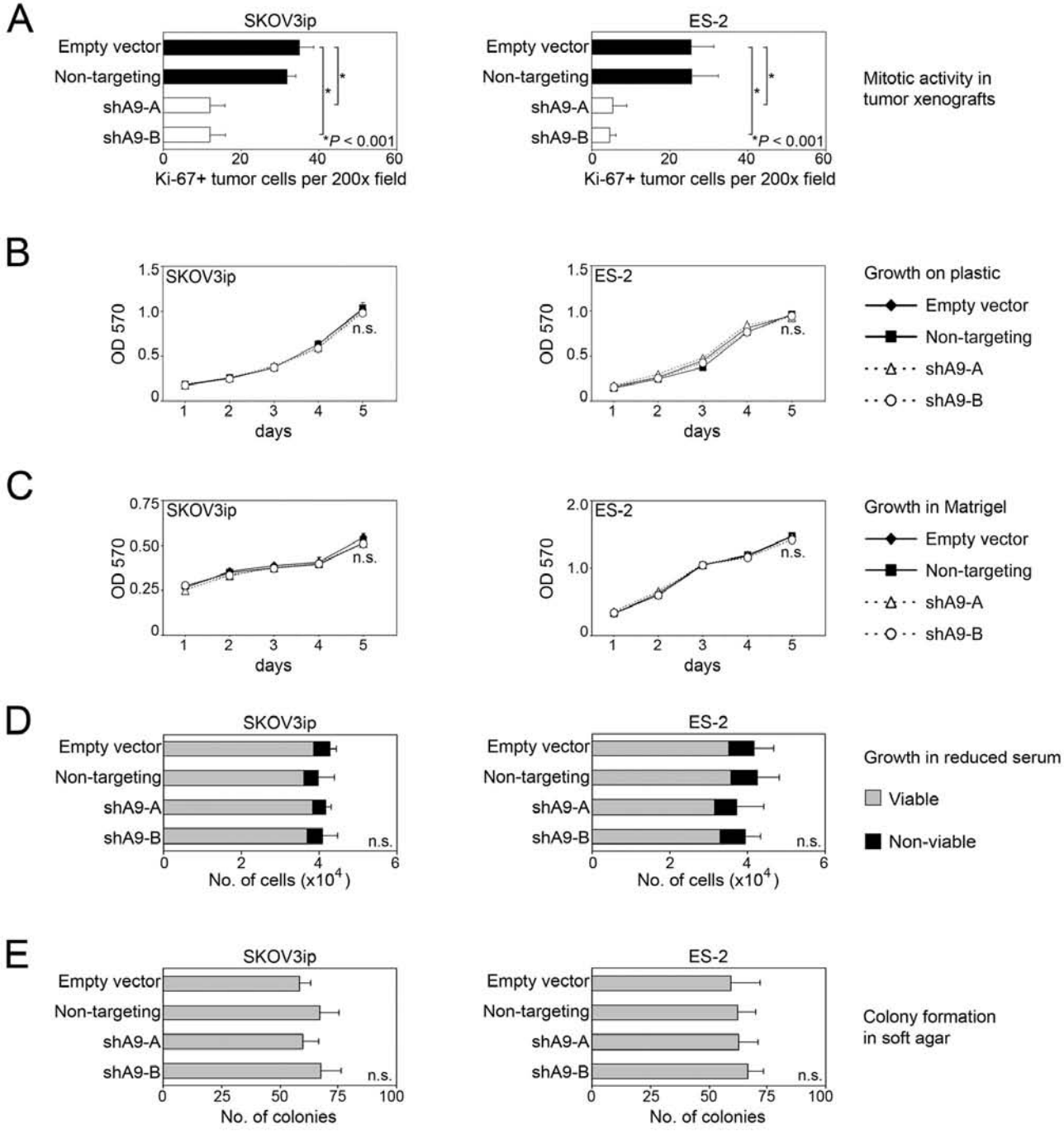
Supplemental Figure 2
Growth of i.p. MOSEC xenografts.
Female nude mice were inoculated i.p. with cells of vector-control and *Hox*-overexpressing MOSEC lines and sacrificed at 2 months thereafter. **(A)** Omental implants are indicated. **(B)** Hematoxylin-eosin-stained tissue sections showing implants on the broad ligament. Bar, 200 μm.



Supplemental Figure 3

HOXA9 expression in EOC lines and clinical specimens.

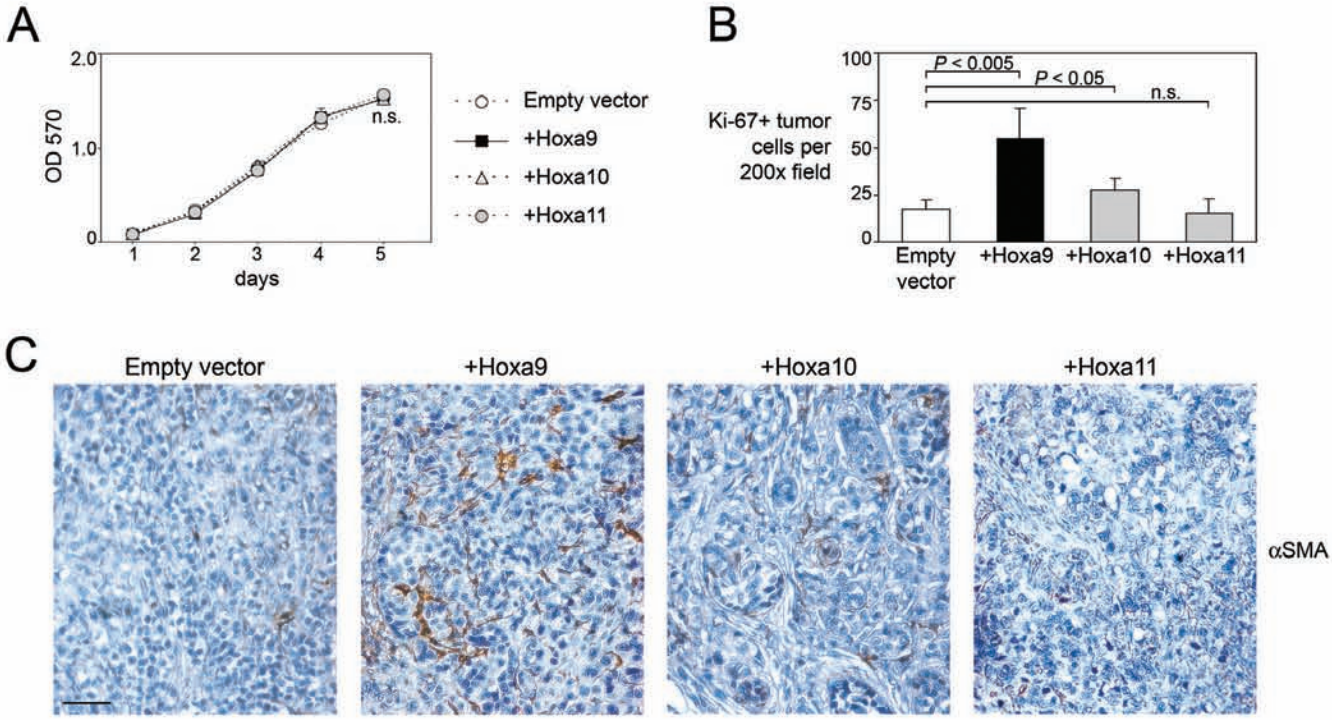
(A) Western blot of MYC-tagged Hox proteins in MOSEC lines, Hoxa9 in MOSEC lines, and HOXA9 in parental SKOV3ip and ES-2 lines and non-tumorigenic human ovarian surface epithelial cells (hOSE). **(B)** qRT-PCR analysis of *Hoxa9* mRNA levels in MOSEC lines and *HOXA9* mRNA levels in parental SKOV3ip and ES-2 lines and clinical specimens of EOC. Primers recognize both mouse *Hoxa9* (MOSEC) and human *HOXA9* (SKOV3ip, ES-2, clinical specimens). **(C)** Representative examples of weak, moderate and strong HOXA9 staining in clinical specimens of EOC. Bar, 50 μm. **(D)** Staining of i.p. xenografts derived from MOSEC lines and parental SKOV3ip and ES-2 lines using Ab recognizing both mouse Hoxa9 and human HOXA9 proteins (as in (A)). Bar, 50 μm.



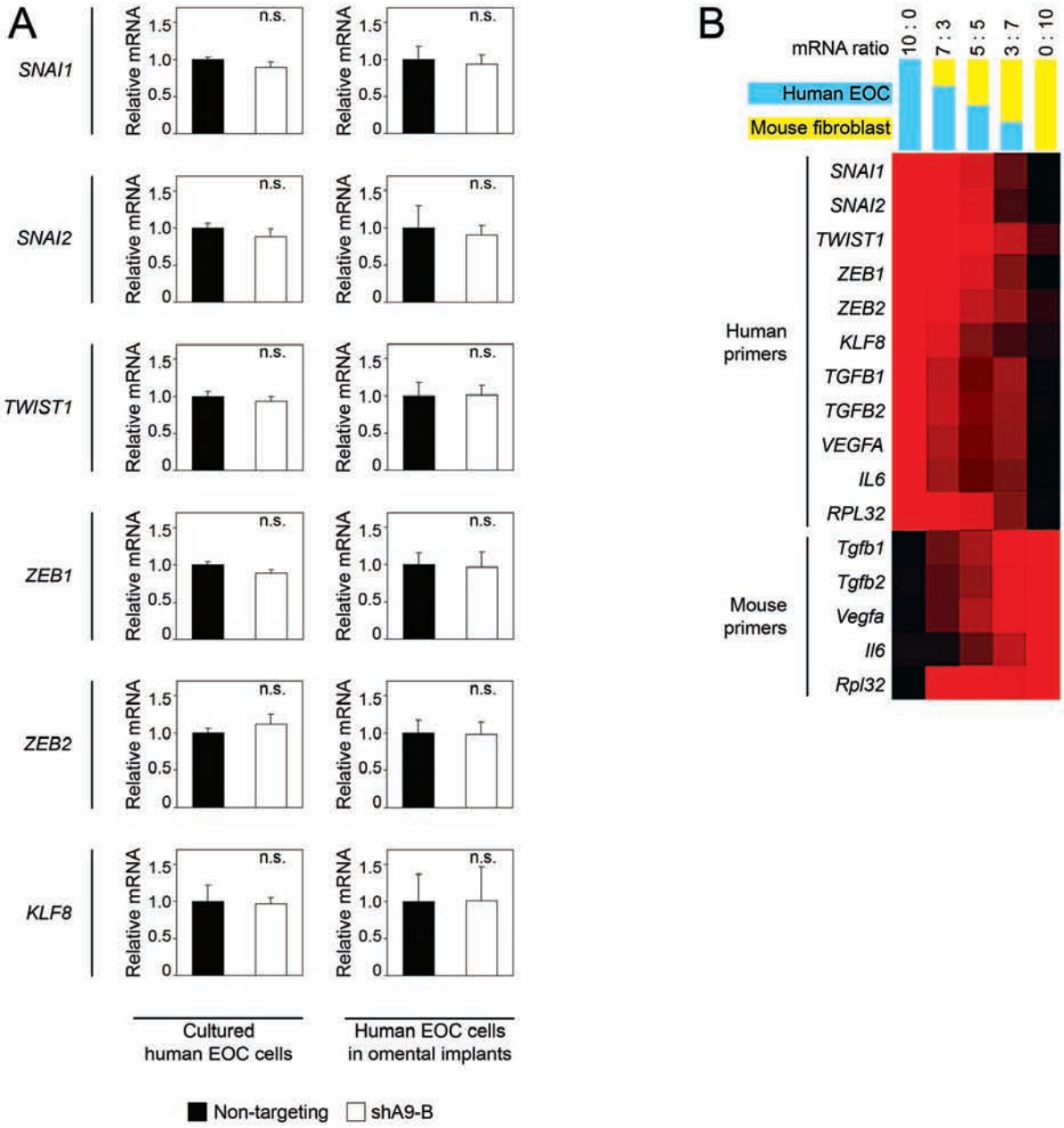
Supplemental Figure 4

Effect of HOXA9-knockdown on EOC cell growth in vivo and in vitro.

(A) Tumor tissues were collected from mice that were inoculated with SKOV3ip and ES-2 lines as described in Figures 2G and 2H. Average numbers of Ki-67+ tumor cells per 200x microscopic field were calculated by scoring five random fields of stained tissue sections of each mouse (n = 5 mice per group). **(B and C)** Growth rates of cell lines cultured **(B)** on plastic and **(C)** in three-dimensional Matrigel cultures were determined by MTT assay. **(D)** Numbers of viable and non-viable cells, determined by exclusion of trypan blue dye, were counted in each well at 3 days after culture in medium with reduced serum. **(E)** Average number of colonies per 40x microscopic field at 2 weeks after culture in soft agar. In **(B-E),** no significant (n.s.) difference was found between cell lines. Shown are average results of three independent experiments.



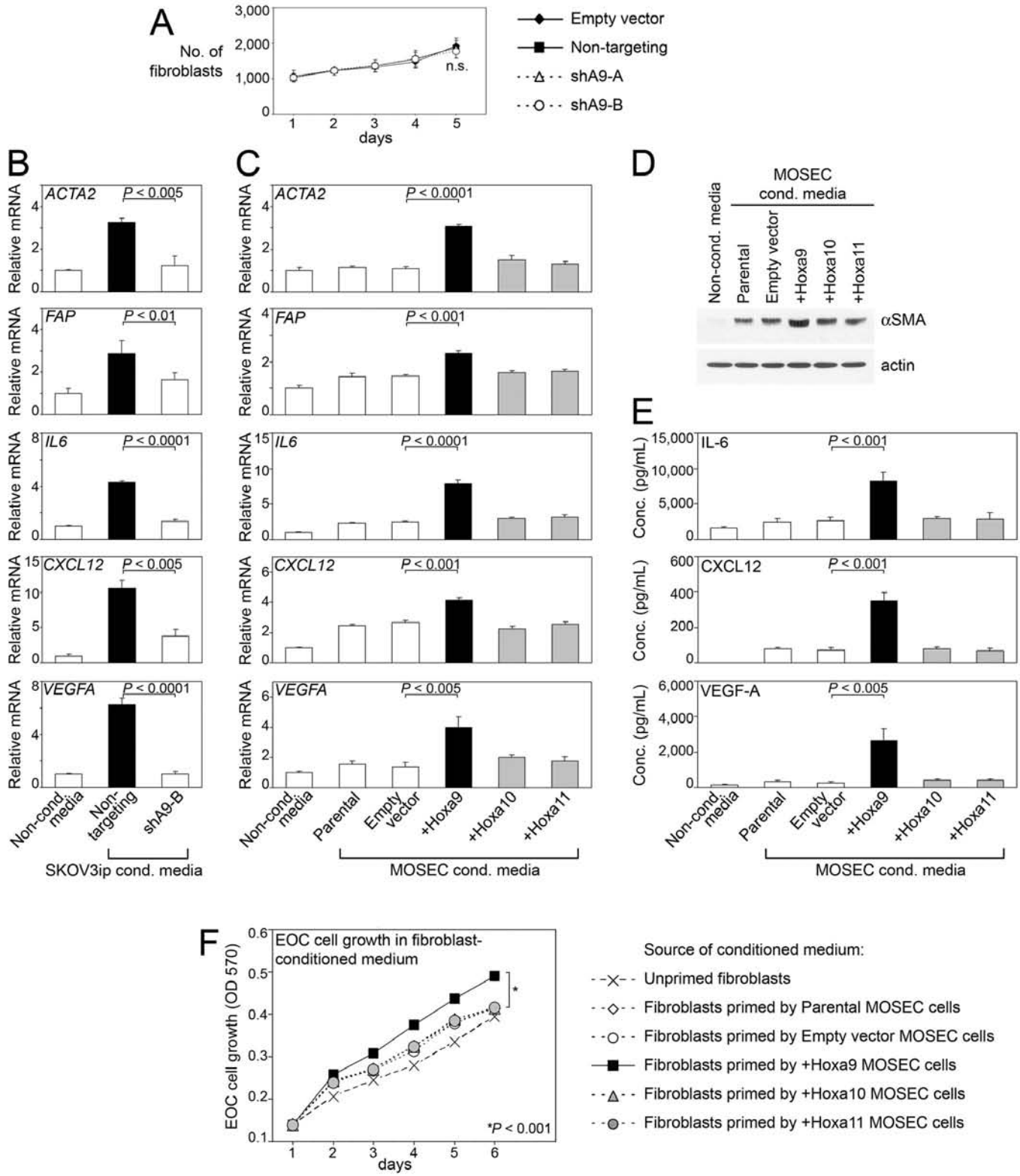
Supplemental Figure 5
Growth characteristics of MOSEC lines.
(A) Growth rates of cultured MOSEC lines were measured by MTT assay. **(B)** Tumor tissues were collected from mice that were inoculated with MOSEC lines as described in Supplemental Figure 2A. Average numbers of Ki-67+ tumor cells per 200x microscopic field were calculated by scoring five random fields of stained tissue sections of each mouse (n = 5 mice per group). **(C)** Staining of αSMA in MOSEC tumors. Bar, 50 μm.



Supplemental Figure 6

Effect of HOXA9 on expression of EMT-associated genes.

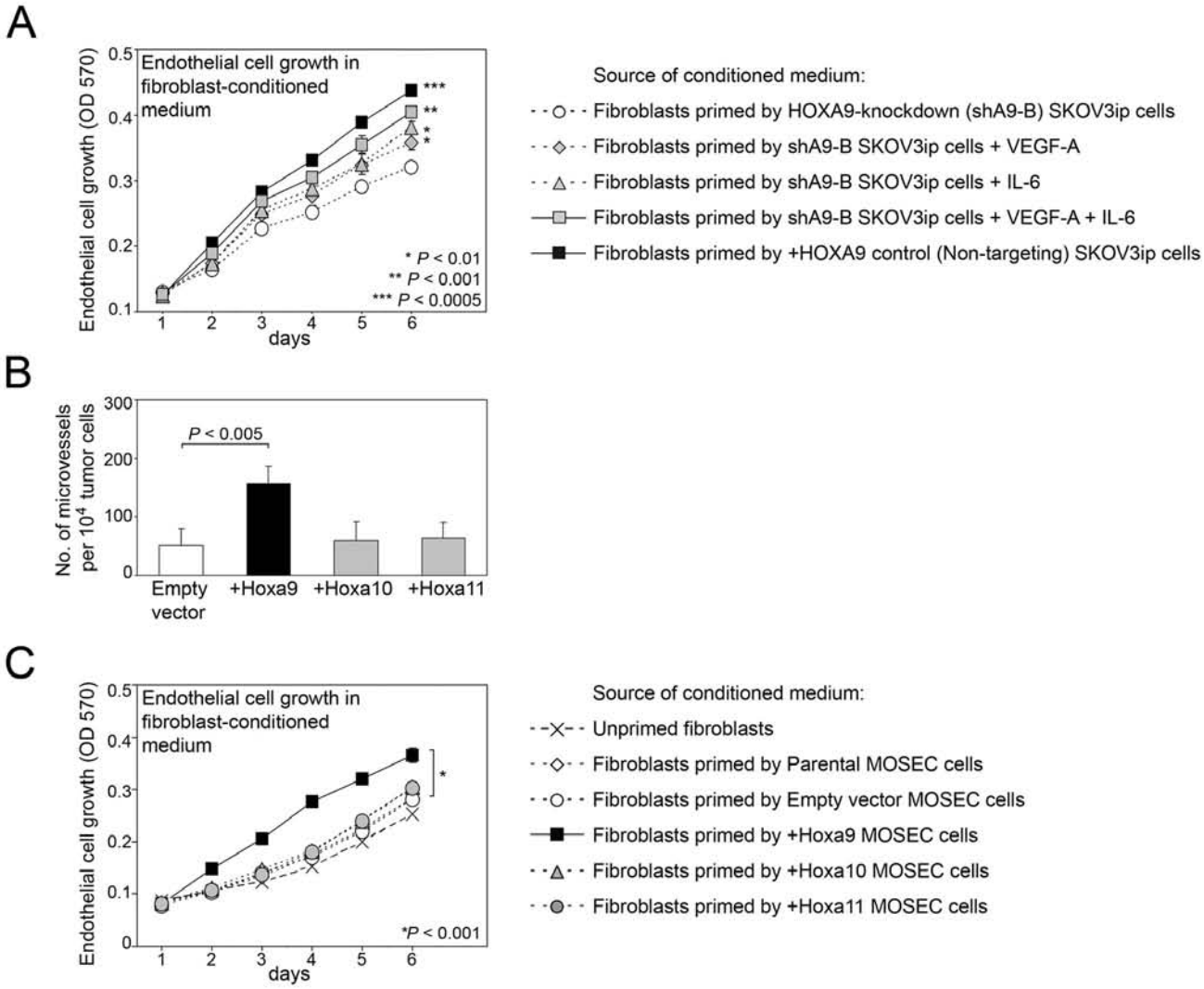
(A) Relative mRNA levels of each indicated gene were assayed by qRT-PCR using human-specific primers in cultured +HOXA9 control (Non-targeting) and HOXA9-knockdown (shA9-B) SKOV3ip lines, and in omental tumors collected from mice that were inoculated with SKOV3ip lines (n = 5 mice per group). **(B)** The ability of human- and mouse- specific primers to discriminate expression of human (tumor) and mouse (stromal) genes was tested using mixtures of RNAs isolated from human EOC cells (SKOV3ip cells) and mouse fibroblasts (L929 cells) at different ratios. Shown is heat map analysis of expression (generated by Tree View software) where the intensity of red color represents the magnitude of expression (log2 scale).



Supplemental Figure 7

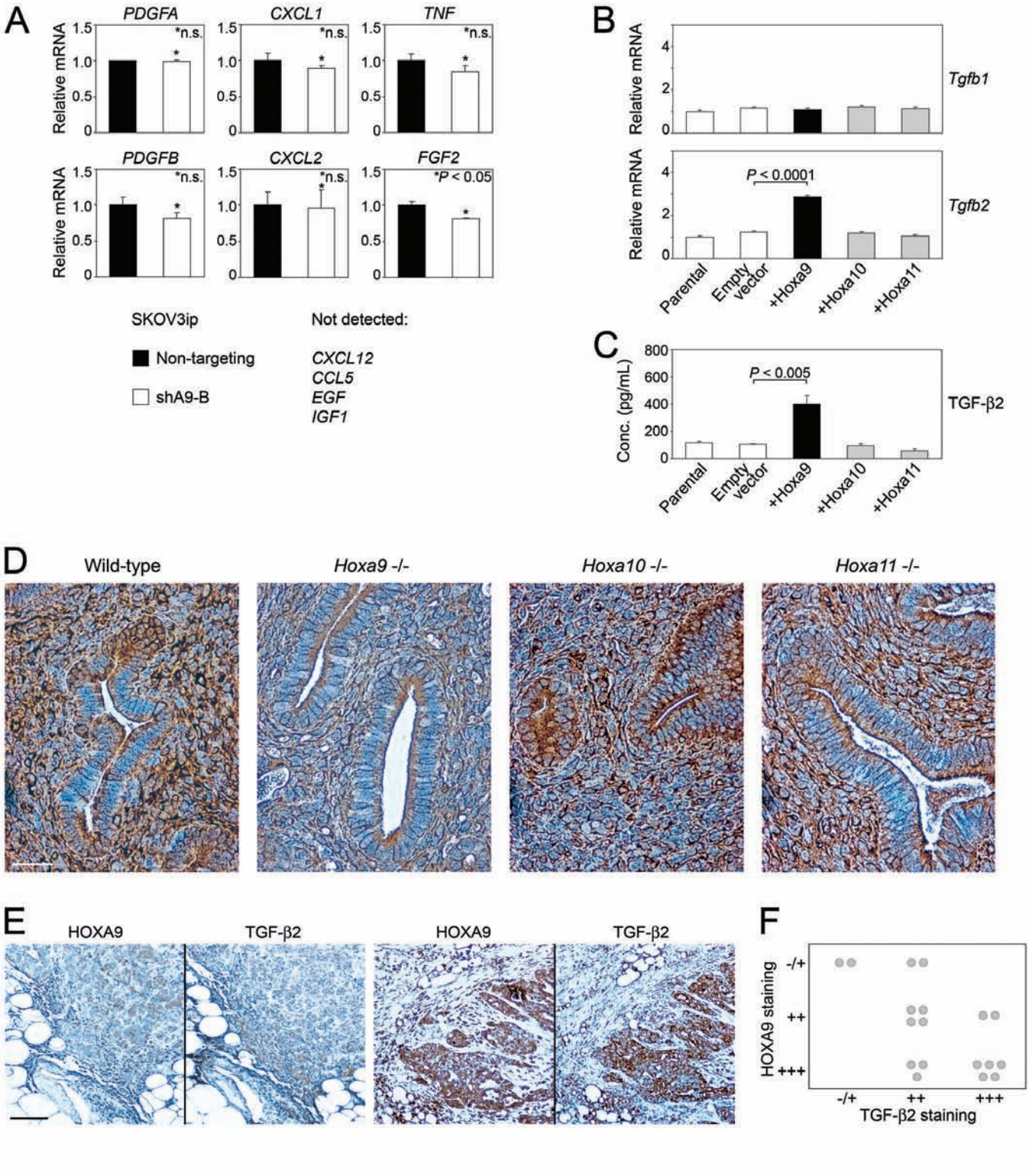
HOXA9 expression in EOC cells induces CAF features in normal omental fibroblasts.

(A) Omental fibroblasts (1,000 per well) were seeded with 1,000 cells of each GFP-expressing SKOV3ip line. Fibroblasts (GFP-negative cells) per well were counted daily. **(B and C)** qRT-PCR analysis of fibroblasts at 5 days after priming in media conditioned by **(B)** +HOXA9 control (Non-targeting) and HOXA9-knockdown (shA9-B) SKOV3ip cells and **(C)** control and Hox-expressing MOSEC cells. The mRNA level of each gene is expressed relative to its level in unprimed fibroblasts (i.e. incubated in non-conditioned medium). Levels of **(D)** αSMA in fibroblasts and **(E)** growth factors released by fibroblasts that were primed by MOSEC-conditioned medium or left unprimed. **(F)** Growth rates of control SKOV3ip cells incubated in medium conditioned by fibroblasts that were primed by MOSEC-conditioned medium or left unprimed. See Figure 4A for assay details. Shown in (B,C,E,F) are average results of assays using three independent sets of each type of conditioned medium.



Supplemental Figure 8

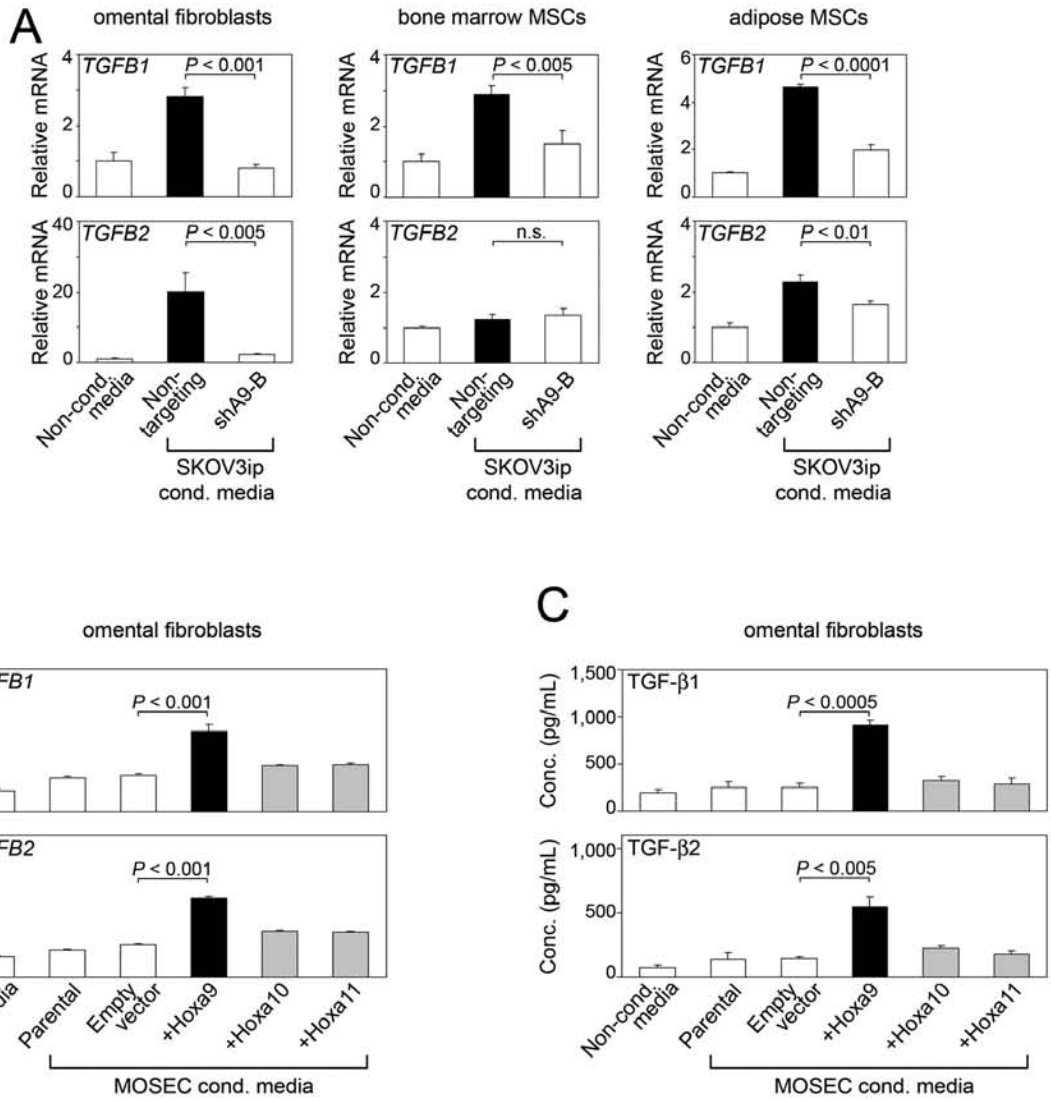
HOXA9 expression in EOC cells promotes the ability of omental fibroblasts to stimulate endothelial cell growth. **(A)** Growth rates of mouse endothelial cells cultured in medium conditioned by omental fibroblasts that were primed by +HOXA9 control (Non-targeting) and HOXA9-knockdown (shA9-B) SKOV3ip cells. See Figure 5D for assays details. Where indicated, recombinant IL-6 and VEGF-A was added to medium conditioned by fibroblasts that were primed by A9-shB SKOV3ip cells to achieve final concentrations of these growth factors that were the same as those detected in medium conditioned by fibroblasts primed by Non-targeting SKOV3ip cells (5,000 pg/mL for IL-6, 800 pg/mL for VEGF-A, see Figure 4C). **(B)** Average numbers of microvessels were calculated in MOSEC-derived tumors by scoring five random fields of CD34-stained tissue sections of each mice (n= 5 mice per group). **(C)** Growth rates of mouse endothelial cells cultured in medium conditioned by omental fibroblasts that were primed by MOSEC-conditioned medium or left unprimed. Shown in (A,C) are average results of MTT assays using three independent sets of each type of fibroblast-conditioned medium.



Supplemental Figure 9

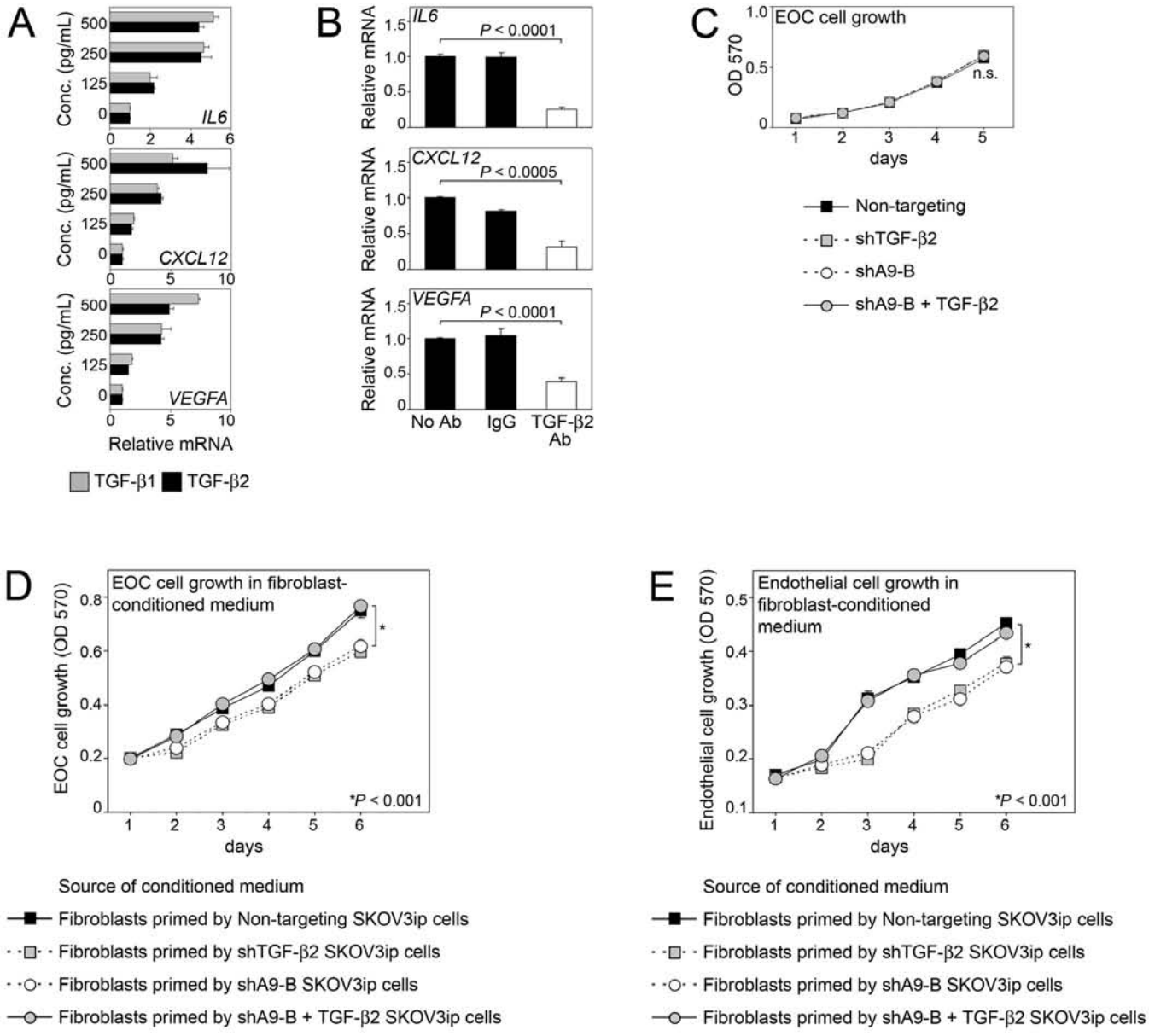
Association of HOXA9 with elevated TGF-β2 expression.

Relative levels of **(A)** transcripts encoding the indicated growth factors in +HOXA9 control (Non-targeting) and HOXA9-knockdown (shA9-B) SKOV3ip lines, and **(B)** *Tgfb1* and *Tgfb2* mRNAs in MOSEC lines. **(C)** Levels of TGF-β2 in media conditioned by MOSEC lines. **(D)** Immunohistochemical staining of TGF-β2 in the posterior uterine segment of adult wild-type and *Hox* knock-out mice. Bar, 20 μm. **(E)** Examples of weak and strong staining of HOXA9 and TGF-β2 in omental tumors of two EOC cases. Bar, 100 μm. **(F)** Staining of HOXA9 and TGF-β2 in sections of omental tumors of 18 EOC cases was scored as nil/weak (-/+), moderate (++) or strong (+++). Each symbol represents an individual case.



Supplemental Figure 10

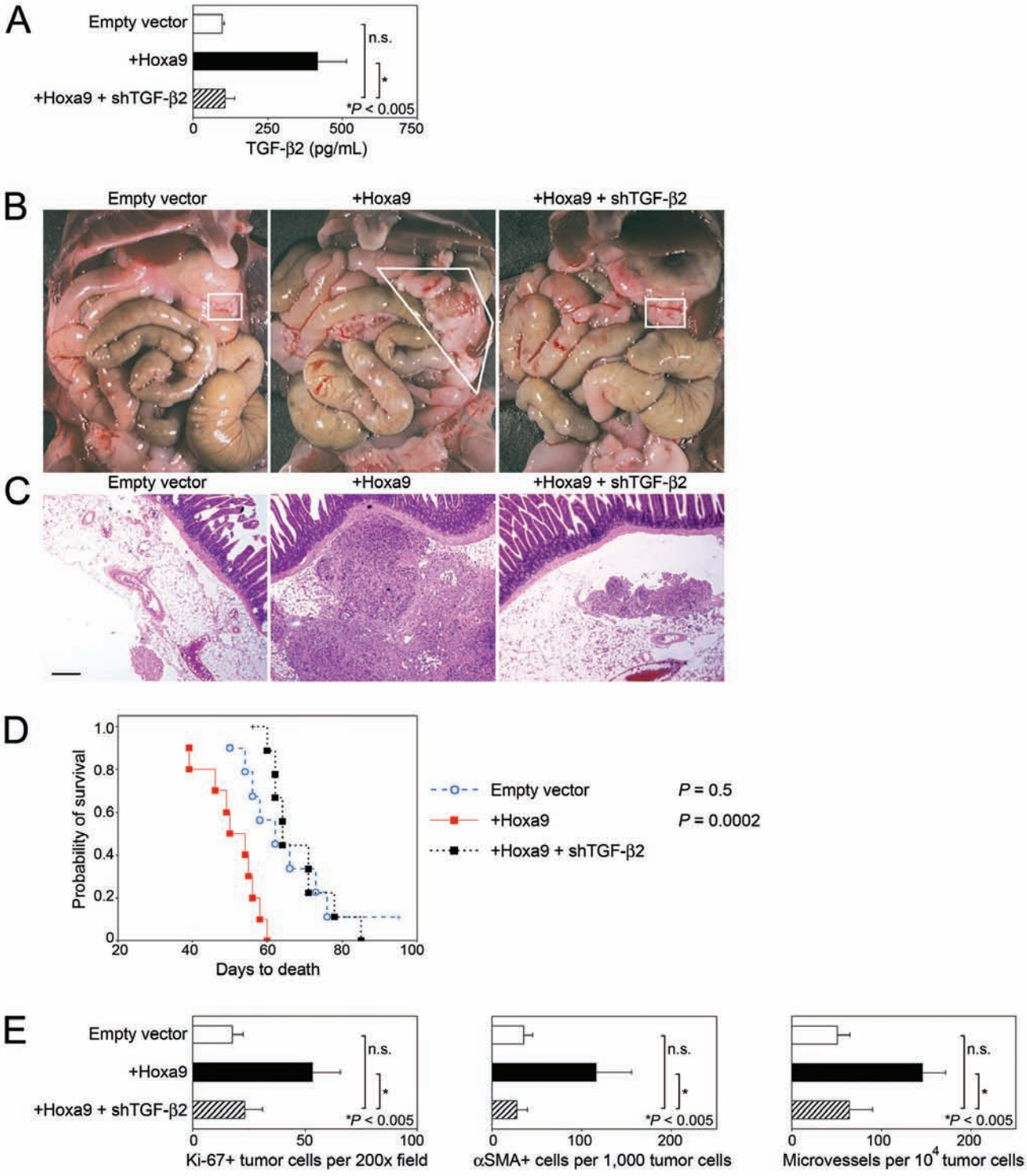
HOXA9 expression in EOC cells induces expression of TGF-β ligands in fibroblasts and MSCs.
(A) *TGFB1* and *TGFB2* mRNA levels were assayed in omental fibroblasts, bone marrow MSCs and adipose MSCs at 5 days after priming in media conditioned by +HOXA9 control and HOXA9-knockdown SKOV3ip cells. The mRNA level of each gene is expressed relative to its level in unprimed fibroblasts and MSCs (i.e. incubated in non-conditioned medium). **(B)** Relative *TGFB1* and *TGFB2* mRNA levels in omental fibroblasts that were primed by MOSEC-conditioned medium or left unprimed. **(C)** Levels of TGF-β1 and TGF-β2 released by primed and unprimed fibroblasts.



Supplemental Figure 11

Effects of HOXA9 on fibroblasts are mediated by its induction of tumor-derived TGF-β2.

(A and B) Relative *IL6, CXCL12* and *VEGFA* mRNA levels in omental fibroblasts at 5 days after incubation with **(A)** recombinant TGF-β ligands at the indicated concentrations, and **(B)** media conditioned by +HOXA9 control SKOV3ip cells, where SKOV3ip-conditioned medium was left untreated, treated with IgG, or depleted by IP with Ab to TGF-β2. **(C)** Growth rates of control (Non-targeting), TGF-β2-knockdown (shTGF-β2), HOXA9-knockdown (shA9-B) SKOV3ip lines and HOXA9-knockdown line stably expressing *TGFB2* (shA9-B + TGF-β2) cultured in non-conditioned medium. **(D and E)** Growth rates of **(D)** control SKOV3ip cells and **(E)** endothelial cells incubated in medium conditioned by omental fibroblasts that were primed by SKOV3ip-conditioned medium or left unprimed (see Figures 4D and 5D for details). Shown are average results of MTT assays using three independent sets of each type of fibroblast-conditioned medium.



Supplemental Figure 12

Inhibition of tumor-derived TGF-β2 blocks stimulatory effects of Hoxa9 on growth of MOSEC xenografts. **(A)** TGF-β2 levels released by vector-control and +Hoxa9 MOSEC cells, and by +Hoxa9 MOSEC cells stably expressing *Tgfb2* shRNA (+Hoxa9 + shTGF-β2). **(B, C)** Female nude mice were inoculated i.p. with MOSEC lines and sacrificed at 2 months thereafter. **(B)** Omental implants are indicated. **(C)** Hematoxylin-eosin-stained tissue sections showing mesenteric implants. Bar, 200 μm. **(D)** Survival rates of mice inoculated i.p. with MOSEC lines, where the +Hoxa9 + shTGF-β2 group was compared to vector-control and +Hoxa9 groups (n = 10 per group). **(E)** Omental tumors were collected from mice described in (B). Average numbers of Ki-67+ tumor cells, αSMA+ cells and microvessels in tumors were calculated by scoring five random fields of stained tissue sections of each mouse (n = 5 mice per group).

Supplemental Table 1:

Differences in *HOX* transcript levels between carcinoma cases in the AOCS dataset grouped by histologic subtype and primary site*

HOXA9	serous ovarian = serous peritoneal	*P* = 0.26
HOXA9	serous ovarian = serous tubal	*P* = 0.31
HOXA9	serous ovarian = endometrioid ovarian	*P* = 0.21
HOXA10	serous ovarian < endometrioid ovarian	*P* = 0.029
HOXA11	serous ovarian < endometrioid ovarian	*P* = 0.005

* determined by Mann-Whitney *U*-test. Cases in the AOCS dataset included serous (n = 204) and endometrioid (n = 20) ovarian carcinoma, and serous tubal (n = 8) and peritoneal (n = 34) carcinoma.

Supplemental Table 2

Human-specific primers for quantitative RT-PCR

ACTA2	Forward	CTATGCCTCTGGACGCACAACT
	Reverse	CAGATCCAGACGCATGATGGCA
FAP	Forward	GGAAGTGCCTGTTCCAGCAATG
	Reverse	TGTCTGCCAGTCTTCCCTGAAG
TGFB1	Forward	GCCCTGGACACCAACTATTG
	Reverse	CGTGTCCAGGCTCCAAATG
TGFB2	Forward	AGAGTGCCTGAACAACGGATT
	Reverse	CCATTCGCCTTCTGCTCTT
VEGFA	Forward	TTGCCTTGCTGCTCTACCTCCA
	Reverse	GATGGCAGTAGCTGCGCTGATA
IL6	Forward	CAATCTGGATTCAATGAGGAGAC
	Reverse	CTCTGGCTTGTTCCTCACTACTC
CXCL12	Forward	CTCAACACTCCAAACTGTGCCC
	Reverse	CTCCAGGTACTCCTGAATCCAC
SNAI1	Forward	GCTGCAGGACTCTAATCCAGAGTT
	Reverse	GACAGAGTCCCAGATGAGCATTG
SNAI2	Forward	ATGAGGAATCTGGCTGCTGT
	Reverse	CAGGAGAAAATGCCTTTGGA
TWIST1	Forward	GGACAAGCTGAGCAAGATTCAGA
	Reverse	TCTGGAGGACCTGGTAGAGGAA
ZEB1	Forward	GGCATACACCTACTCAACTACGG
	Reverse	TGGGCGGTGTAGAATCAGAGTC
ZEB2	Forward	AATGCACAGAGTGTGGCAAGGC
	Reverse	CTGCTGATGTGCGAACTGTAGG
KLF8	Forward	CCTGAAAGCTCACCGCAGAATC
	Reverse	TGCTTGCGGAAATGGCGAGTGA

PDGFA	Forward	CAGCGACTCCTGGAGATAGACT
	Reverse	CGATGCTTCTCTTCCTCCGAATG
PDGFB	Forward	GAGATGCTGAGTGACCACTCGA
	Reverse	GTCATGTTCAGGTCCAACTCGG
CXCL1	Forward	GCCAGTGCTTGCAGACCC
	Reverse	GATGCTCAAACACATTAG
CXCL2	Forward	CGCAGCAGGAGCGCC
	Reverse	TGGATGTTCTTGAGGTGAATTCC
TNF	Forward	CCCAGGGACCTCTCTCTAATCA
	Reverse	GCTACAGGCTTGTCACTCGG
FGF2	Forward	AGCGGCTGTACTGCAAAAACGG
	Reverse	CCTTTGATAGACACAACTCCTCTC
RPL32 control primer	Forward	ACAAAGCACATGCTGCCCAGTG
	Reverse	TTCCACGATGGCTTTGCGGTTC

Mouse-specific primers for quantitative RT-PCR

Tgfb1	Forward	TGATACGCCTGAGTGGCTGTCT
	Reverse	CACAAGAGCAGTGAGCGCTGAA
Tgfb2	Forward	TTGTTGCCCTCCTACAGACTGG
	Reverse	GTAAAGAGGGCGAAGGCAGCAA
Vegfa	Forward	CTGCTGTAACGATGAAGCCCTG
	Reverse	GCTGTAGGAAGCTCATCTCTCC
Il6	Forward	TACCACTTCACAAGTCGGAGGC
	Reverse	CTGCAAGTGCATCATCGTTGTTC
Rpl32 control primer	Forward	GGAGAAGGTTCAAGGGCCAG
	Reverse	TGCTCCCATAACCGATGTTTG

Human/Mouse common primers for quantitative RT-PCR

HOXA9/Hoxa9	Forward	CCCTGACTGACTATGCTTGTGGT
	Reverse	TCTCCGCCGCTCTCATTCTC
RPL32/Rpl32 control primer	Forward	CCTTGTGAAGCCCAAGATCG
	Reverse	TGCCGGATGAACTTCTTGGT

Primers for chromatin immunoprecipitation

Mouse primer set for S1 (-1144bp~-944bp)	Forward	TGTATGCCAGCTATATCATT
	Reverse	TGGCCACGATGACTACACAG
Mouse primer set for S2 (-784bp~-584bp)	Forward	AGTTTGAGCAAGTTGAAGTA
	Reverse	CTCTGTATCACTGCTTTGAA
Mouse primer set for S3 (-524bp~-324bp)	Forward	ACAGGGCTGGATTGTAAACA
	Reverse	AGGCGTGTACACACACACAC
Mouse primer set for S4 (-174bp~+74bp)	Forward	ATGCCAGTCGCCCTCCCTTA
	Reverse	AAACCTGCTGCCAGCAGATA
Mouse primer set for S5 (+832bp~+980bp)	Forward	CAGATCAGCCACTCCGCACCG
	Reverse	GGGGGGAATCTCTCACTTTAAGG
Mouse primer set for *Gapdh*	Forward	CCATTTTGTCTACGGGACGA
	Reverse	GGCCACGCTAATCTCATTTT
Human primer set for S4 (-1007bp~-882bp)	Forward	GGTCTAAGTAACGAGAGGACTTC
	Reverse	CCAGCAGATAACATCACGATC
Human primer set for S5 (+77bp~+227bp)	Forward	CAGATCCGCCACTCCGCACCC
	Reverse	GGGAACCCTGACTTTGGCGAG
Human primer set for *GAPDH*	Forward	TACTAGCGGTTTTACGGGCG
	Reverse	TCGAACAGGAGGAGCAGAGAGCGA

Primers for *Tgfb2* promoter constructs

Primer set for region A	Forward	ATGCCAGTCGCCCTCCCTTA
	Reverse	GGGGGGAATCTCTCACTTTAAGG
Primer set for region B	Forward	AGGAGAAGCTAGCGAAGGGTGC
	Reverse	CGAATTGAAGCTTCCGCGGT
Primer set for region C	Forward	GGTATCGGCTAGCTTGATATCCAC
	Reverse	GACTCGCAAGCTTCCCTAGC
Primer set for region C with mutant S4	Forward	TATGGGATCCCCCCGCCACGTGT
	Reverse	ACGTGGTTTGGGGATCCCGGCTGACGC TCTGCTCC

SUPPLEMENTAL METHODS

Antibodies and growth factors. Sources of Abs were as follows: αSMA (Dako), CD34 (Abcam), Ki-67 (Vector Laboratories), GFP (Molecular Probes), HOXA9 (Millipore, Santa Cruz Biotechnology), TGF-β2, CXCL12, IL-6 (R&D Systems), FLAG, MYC (Sigma-Aldrich), secondary Abs (Invitrogen). Recombinant growth factors were purchased from the following sources: TGF-β1 (Sigma-Aldrich), TGF-β2, VEGF-A and IL-6 (Invitrogen).

Plasmids. pGFP-V-RS *HOXA9, TGFB2, Tgfb2* and non-targeting shRNA plasmids, and *TGFB2* cDNA were purchased from OriGene Technologies.

***Cell culture*.** All culture media were supplemented by addition of 10% FBS and penicillin/ streptomycin unless noted otherwise. Primary cultures of normal human omental fibroblasts were maintained in DMEM medium (Invitrogen) containing non-essential amino acids. Normal human bone marrow MSCs were cultured in α-MEM medium (Invitrogen) containing 20% FBS. Normal human adipose MSCs were cultured in MSC basal medium with growth supplement (ATCC). Immortalized, non-tumorigenic human ovarian surface epithelial cells were cultured in a 1:1 mixture of MCDB 105 medium (Sigma-Aldrich) and Medium 199 (Invitrogen). Immortalized mouse endothelial cells were cultured in DMEM medium. L929 mouse skin fibroblasts were cultured in Eagle's MEM medium (Invitrogen). MOSEC cell lines were cultured in DMEM medium. SKOV3ip and ES-2 cell lines were cultured in McCoy's 5A medium (Invitrogen).

In vitro EOC cell growth assays. EOC cells were seeded either directly on plastic surfaces or embedded in 2% Matrigel (BD Biosciences) in 96-well plates (2,000 per well). Cell proliferation was measured daily by the 3-(4,5-dimethylthiazolyl-2)-2,5-diphenyltetrazolium bromide (MTT) assay (Roche). To assay cell viability under reduced serum conditions, EOC cells were seeded in 12-well plates ($2x10^4$ per well), cultured for 3 days in medium containing 0.2% FBS and stained with trypan blue dye. To assay colony formation, EOC cells were seeded in 6-well plates ($2x10^4$ per well) and cultured for 2 weeks in 0.3% agar. Three independent experiments were performed for each assay.

***Stimulation of cells with conditioned media*.** To generate tumor-conditioned media, EOC cells ($1.5x10^6$) were seeded in 10 cm dishes and cultured in medium containing 1% FBS for 2 days. Tumor-conditioned medium was filtered, assayed by ELISA, and applied to fibroblasts and MSCs. Conditioned medium was replaced every 2 days, and cells analyzed by qRT-PCR and Western blot at 5 days thereafter. For fibroblast-priming assays, fibroblasts were incubated in tumor-conditioned medium for 5 days and then washed. Fresh non-conditioned medium was added and fibroblasts cultured for 2 days. Medium conditioned by fibroblasts was filtered, assayed by ELISA, and applied to EOC cells. EOC cell growth was measured by MTT assay. Endothelial cell growth was measured by MTT assay following culture in media conditioned by EOC cells and by primed fibroblasts. Conditioned media was depleted of CXCL12, IL-6 and TGF-β2 by IP using Abs to these growth factors. Three independent sets of each type of conditioned medium were evaluated in each assay.

***Co-culture assays*.** Fibroblasts or MSCs (1,000) were seeded with 1,000 cells of each GFP-expressing EOC line per well in 96-well plates. Numbers of fibroblasts or MSCs (GFP-negative cells) in each well were counted daily under light and immunofluorescence microscopy. Three independent experiments were performed for each assay.

qRT-PCR. Transcripts were analyzed by using SYBR®Green qPCR Master Mix (SABiosciences) and primers listed in Supplemental Table 2. *RPL32* (human) and *Rpl32* (mouse) transcript levels were used as controls for normalization.

Chromatin IP and reporter assays. Chromatin IP assays were performed using the ChIP Assay kit (Upstate Biotechnology). DNA was purified from precipitated complexes and used to amplify fragments of the *TGFB2* and *Tgfb2* promoters. Fragments of the *Tgfb2* promoter described in the text were also amplified from mouse genomic DNA and cloned into the pGL3 firefly luciferase reporter vector (Promega). pRL-CMV Renilla luciferase reporter plasmid (Promega) was used for normalizing transfection efficiency. Luciferase activities in transfected cells were assayed using the Dual-luciferase reporter assay kit (Promega). Primers used for chromatin IP and subcloning are listed in Supplemental Table 2.

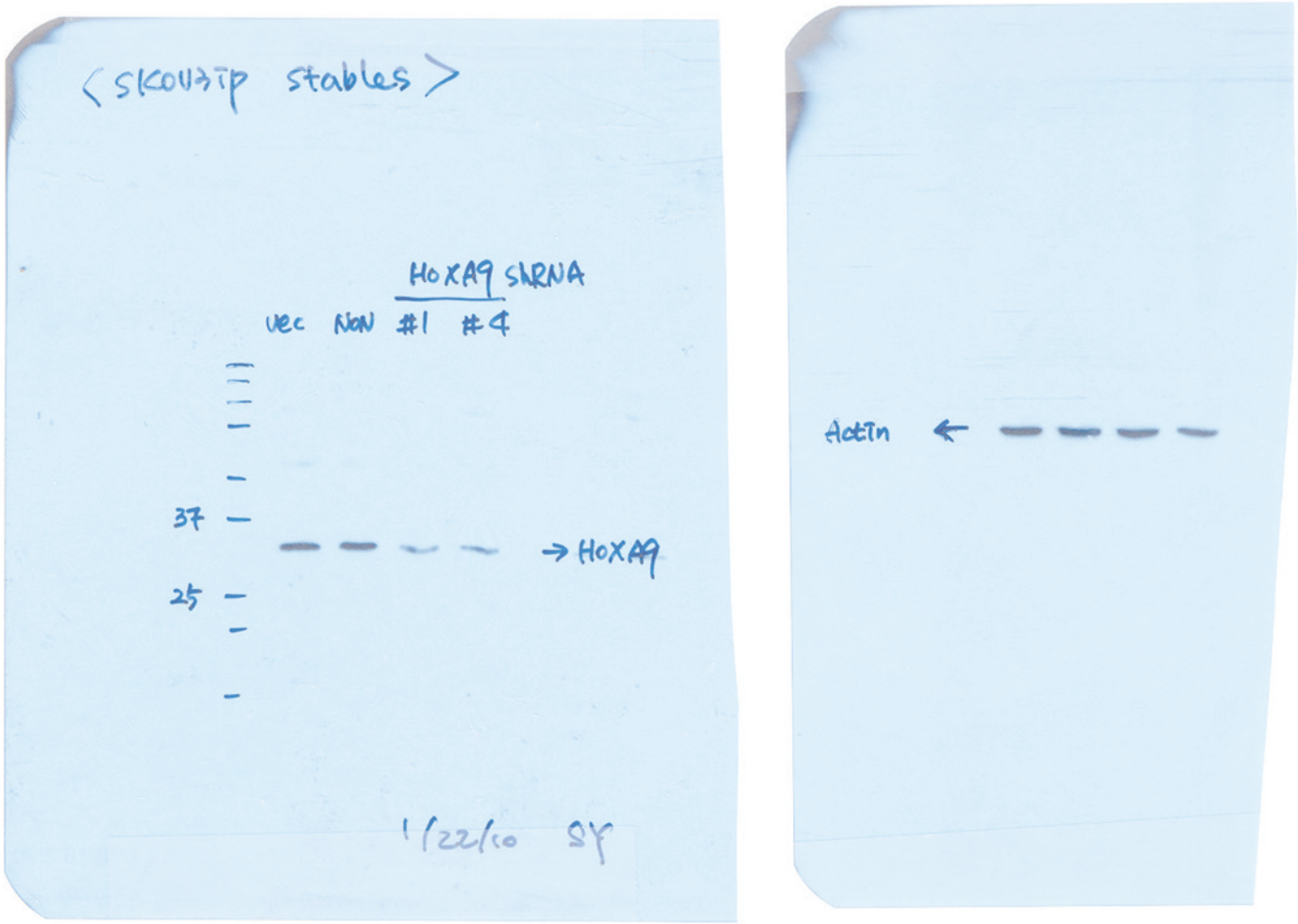
Bioinformatic analysis. Gene expression data from EOC patients at the Peter MacCallum Cancer Center (AOCS study) (GSE9891, n = 285) were downloaded from the National Center for Biotechnology Information Gene Expression Omnibus (GEO) database (http://www.ncbi.nlm.nih.gov/geo). Gene expression data from the TCGA project (n = 567) were downloaded from the TCGA data portal site (http://tcga-data.nci.nih.gov/tcga/). Gene expression data were generated by using Affymetrix microarray platforms (U133 v2.0 for AOCS, U133A for TCGA). All data were normalized by using the robust multi-array average method (59). Where there were multiple probe sets for an individual gene, the mean value for the given gene for each case was used. Patients were stratified according to expression of a given gene, where transcript levels for the given gene were defined as High (≥ upper quartile) and Low (≤ lower quartile).

References

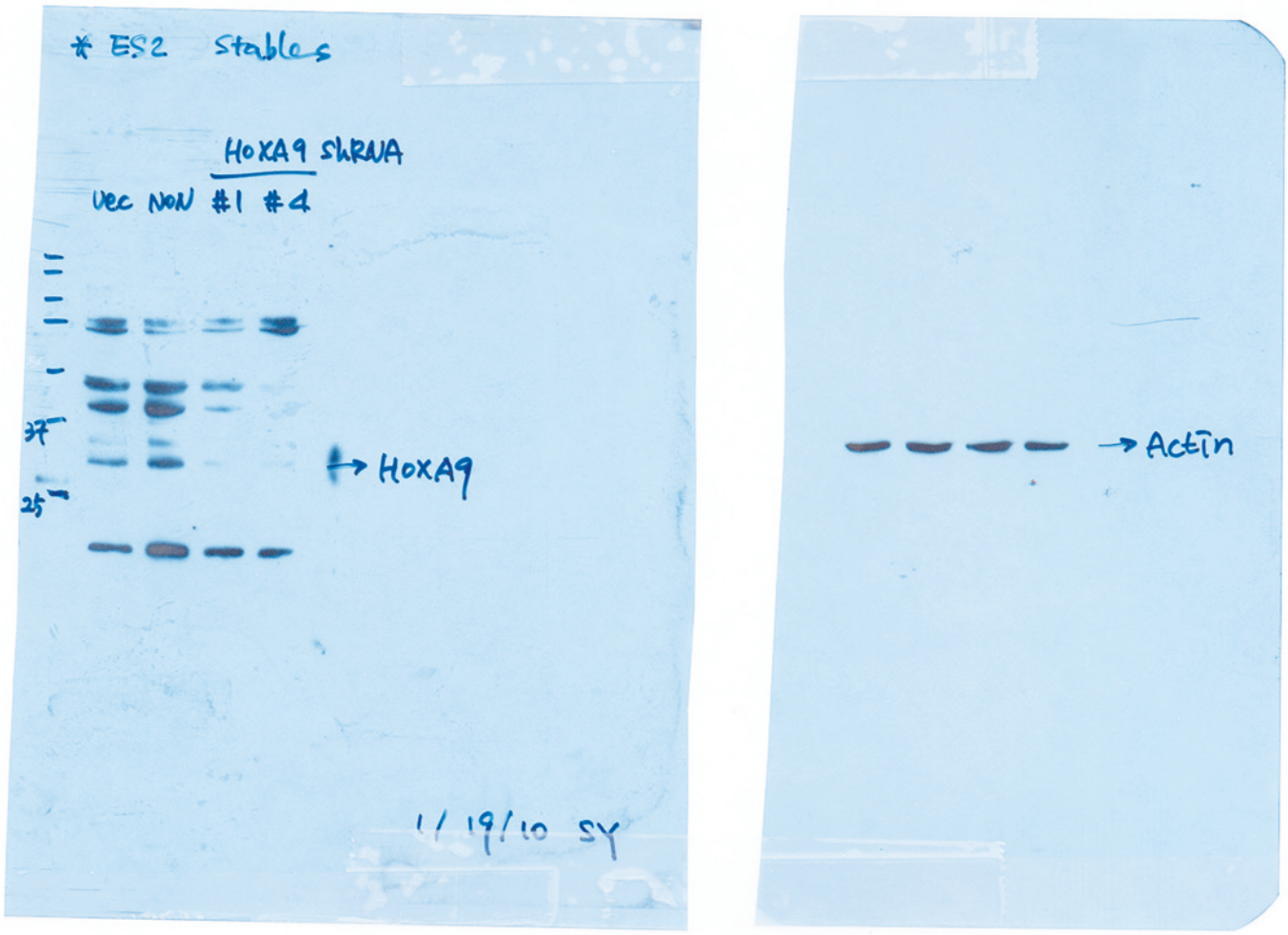
59. Irizarry RA, et al. Exploration, normalization, and summaries of high density oligonucleotide array probe level data. *Biostatistics.* 2003; 4(2):249-264.

Original Western blot for Figure 2, panel D

HOXA9 shRNA (#1 = sh A9-A
#4 = sh A9-B

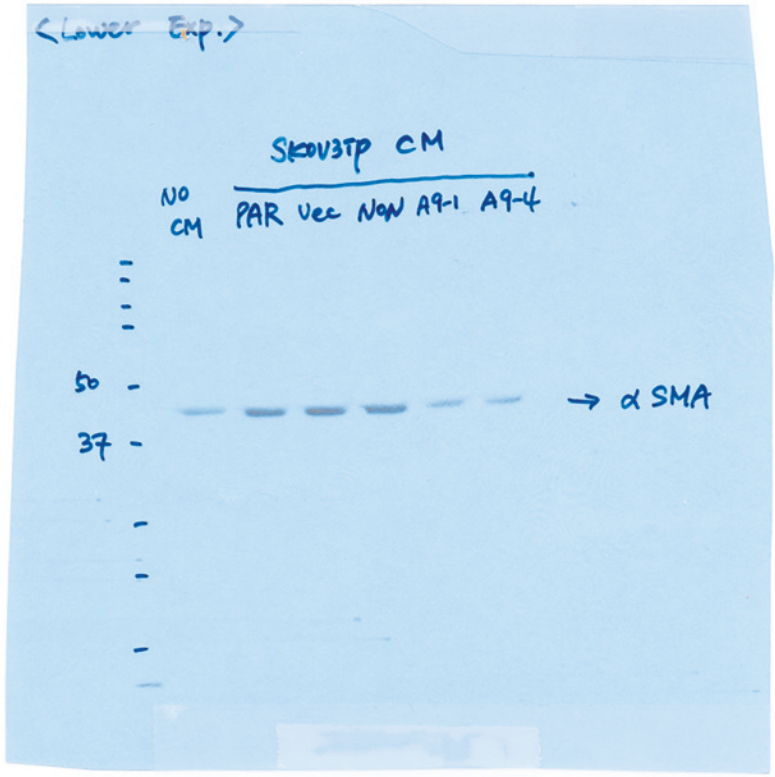


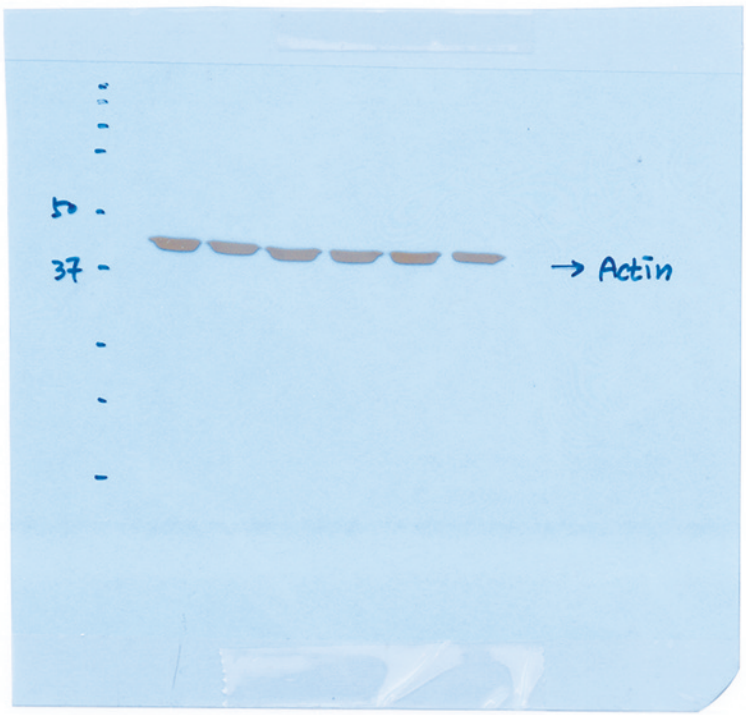
Original Western blot for Figure 2, panel D



Original Western blot for Figure 4, panel B

HPF WB (pretreated w/ SKOV3ip C.M for 5 days)





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< Skoultchi ChIP Result > Original gels of PCR products for Figure 7, panel F

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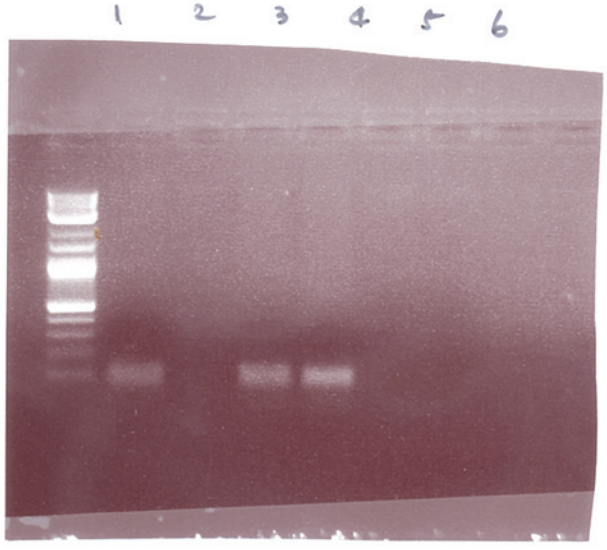

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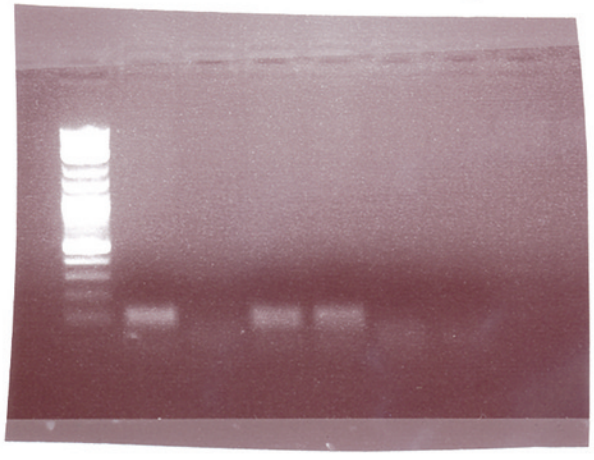
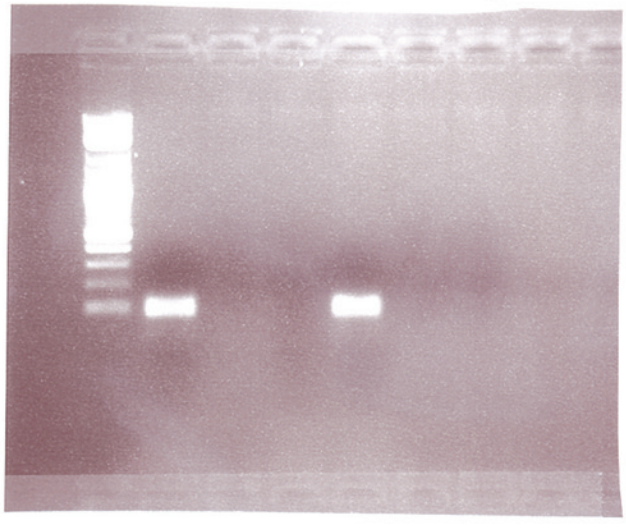


* TGFβ2 Region S5



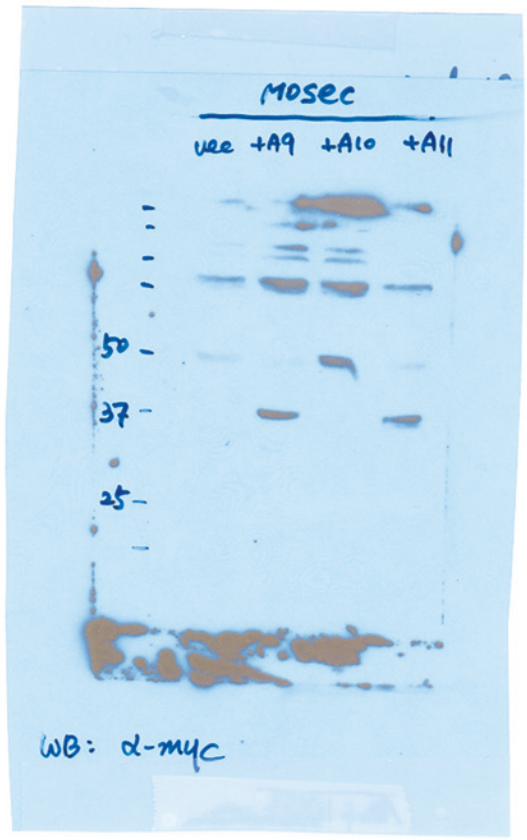
* GAPDH control

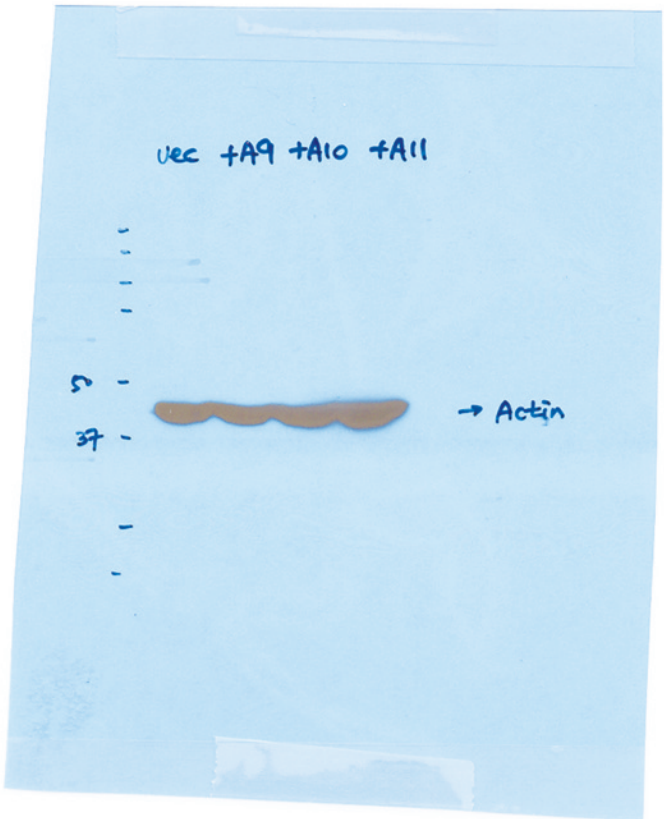
Non-target cells:
- Lane 1 : Input (1%)
- Lane 2 : Normal IgG
- Lane 3 : HoxA9 Ab

HoxA9 SH4 cells:
- Lane 4 : Input
- Lane 5 : Normal IgG
- Lane 6 : HoxA9 Ab

Original Western blot for Supplemental Figure 3, panel A

< HoX Expression level test >





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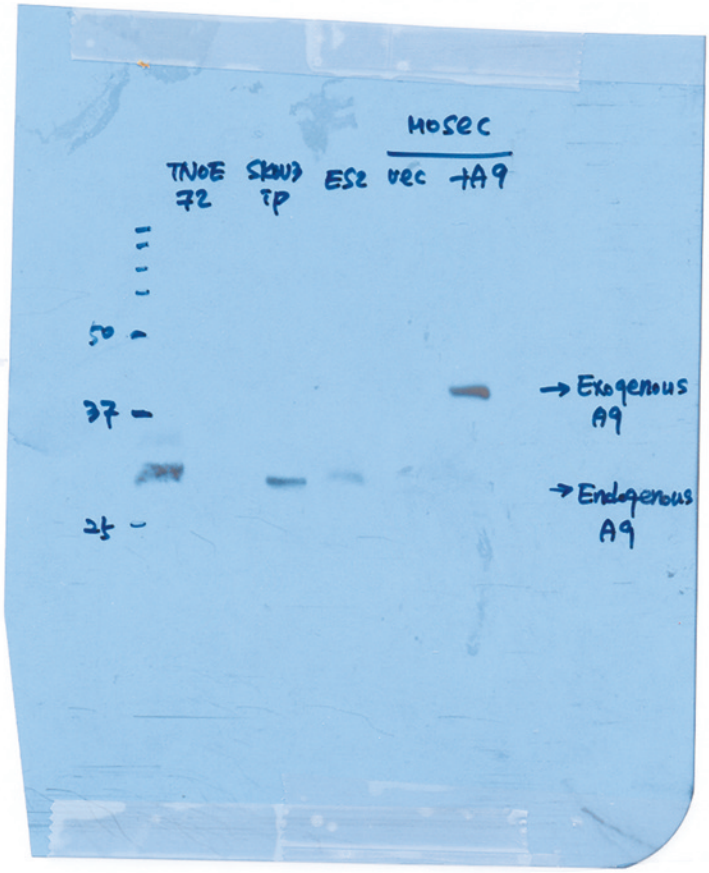
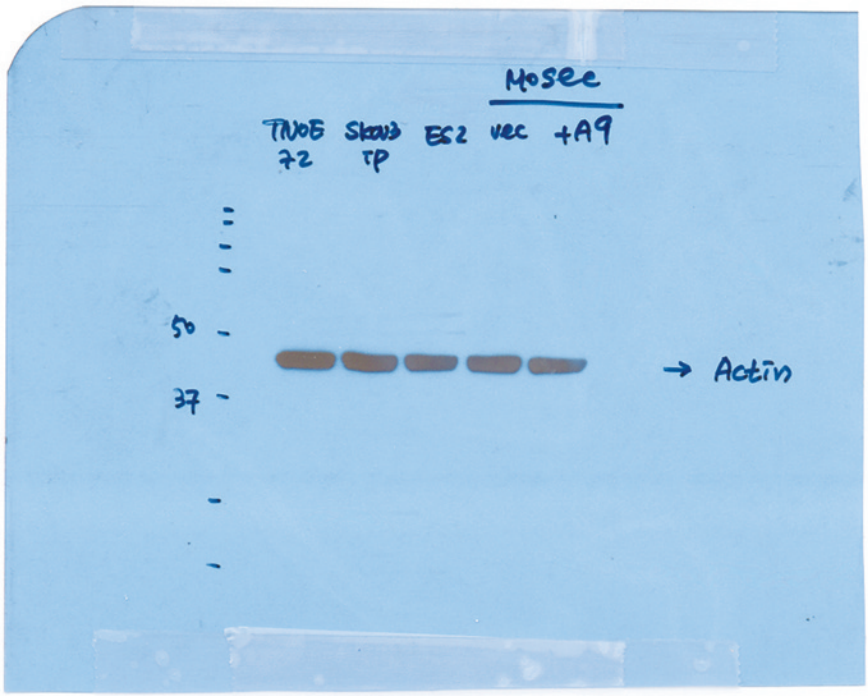
Original Western blot for Supplemental Figure 3, panel A

< HoXA9 Expression level test >





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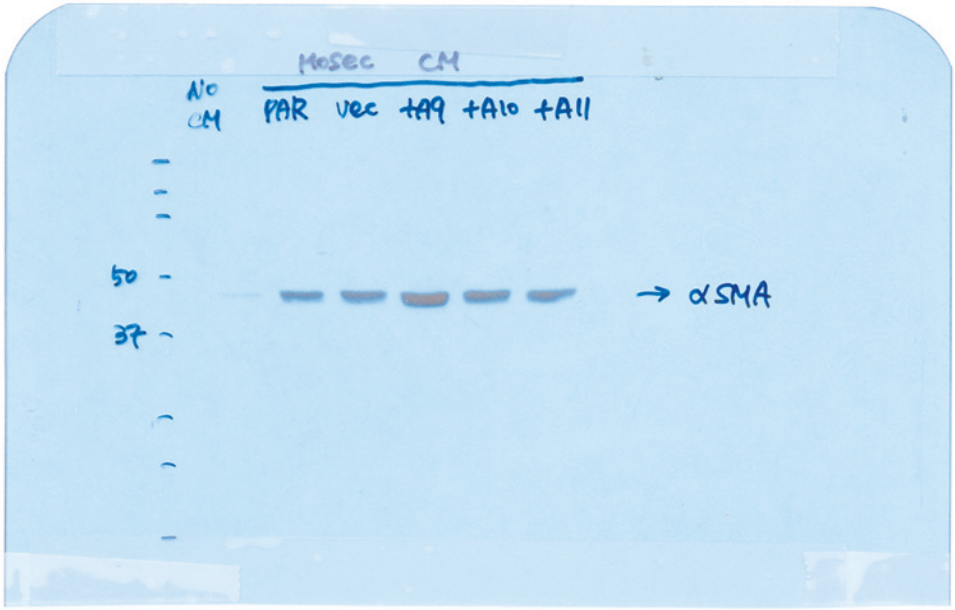
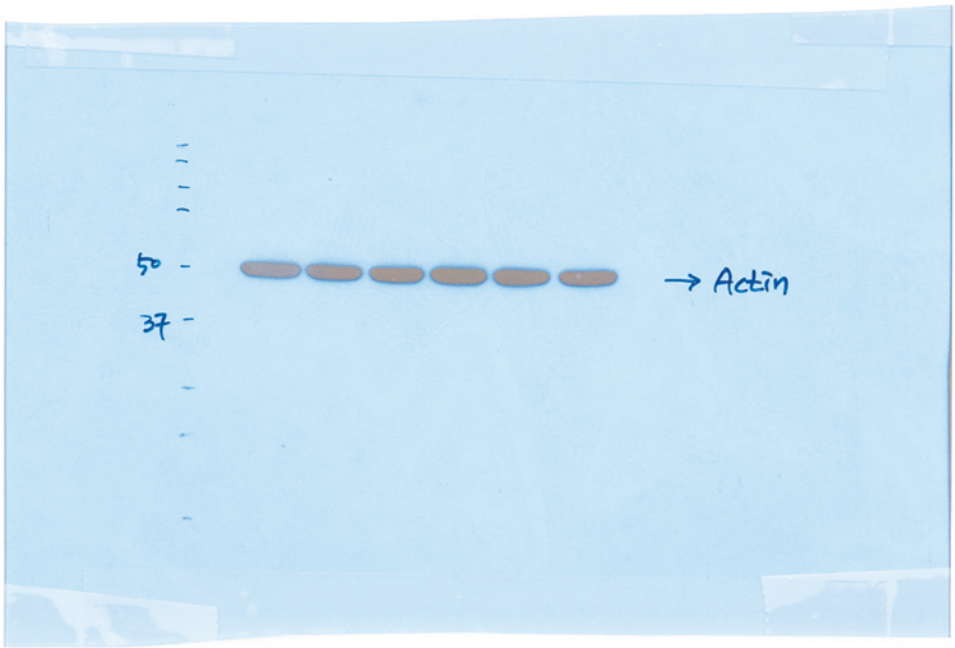
Original Western blot for Supplemental Figure 7, panel D

HPF WB (pretreated w/ MoSec C.M for 5 days)





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