SUPPLEMENTAL MATERIAL

SUPPLEMENTAL FIGURE LEGENDS

SUPPLEMENTAL FIGURE 1. **Adenoviral-mediated transient expression of FOXC1 increases invasion of MCF10A cells**. A, representative immunoblots demonstrating transient overexpression of FOXC1 in MCF10A cells transduced with control adenovirus (AdGFP) or adenovirus encoding FOXC1 (AdFOXC1). B, relative invasion of MCF10A cells transduced with AdGFP or AdFOXC1. C, relative migration of MCF10A cells transduced with AdGFP or ADFOXC1. D, relative transcript levels of EMT-related genes normalized to AdGFP transduced cells. *GAPDH* was used as an endogenous control. Data represent three independent experiments and are presented as mean +/- SEM. *, $P < 0.05$.

SUPPLEMENTAL FIGURE 2. **Transient expression of FOXC1 increases invasion of MCF12A cells.** A, representative immunoblots demonstrating transient overexpression of FOXC1 in MCF12A cells transfected with empty vector (pcDNA) or vector encoding FOXC1 (FOXC1). B, relative invasion of MCF12A cells transduced with AdGFP as control or AdFOXC1. C, relative migration of MCF12A cells transduced with AdGFP as control or AdFOXC1. D, relative transcript levels of EMT-associated genes normalized to cells transduced with AdGFP with *GAPDH* used as an endogenous control. Data represent three independent experiments and are presented as mean +/- SEM. *, $P < 0.05$.

SUPPLEMENTAL FIGURE 3. **Transient overexpression of FOXC1 by viral transduction slows the growth MCF10A cells and has no significant effect on MCF12A growth**. Graphs illustrate the number of viable MCF10A (A) or MCF12A (B) cells at 24, 48, 72, 96, 120, or 144 hours post-transduction with control adenovirus (AdGFP) or FOXC1 encoding virus (AdFOXC1). Data summarize three independent experiments. Error bars represent SD. *, $P < 0.05$; **, $P < 0.01$.

SUPPLEMENTAL FIGURE 4. **Expression of FOXC1 positively correlates with MMP7 expression in breast cancers and breast cancer cell lines.** Log(2) transformed, median centered data from Ivshina (A), Charaffe (B) and Neve (C) data sets were plotted by *FOXC1* (x-axis) and *MMP7* (y-axis). Data from Scheutz 2 (A) was colored coded according to estrogen receptor (ER) status. D, representative western blots demonstrating FOXC1 (Cell Signaling, #7415) and MMP7 (R&D Systems, AF907) protein expression in a number of breast cancer and non-transformed mammary epithelial cell lines. *P* values less than 0.05 were considered statistically significant.

SUPPLEMENTAL FIGURE 5. **Transient overexpression of FOXC1 in MCF10A or MCF12A cells results in increased expression of MMP7.** A, conditioned media from MCF12A cells transfected with empty pcDNA vector or vector encoding FOXC1 was used in an ELISA for MMP7. The bar graph summarizes the mean +/- SD for three independent experiments. Transcript levels of *MMP7*, *MMP2* and *MMP9* in MCF10A (B) or MCF12A (C) cells transduced with control adenovirus (AdGFP) or adenovirus encoding FOXC1 (AdFOXC1). Data are relative to the respective AdGFP transduced control cells; *GAPDH* was used as endogenous control. Data summarize three independent experiments and are presented as mean +/- SD. D, levels of *FOXC1* (light grey) or *MMP7* (dark grey) transcripts in MCF10A (left panel) or MCF12A (right panel) cells transduced with the indicated multiplicity of infection (MOI) of FOXC1 adenovirus relative to untreated cells (0 MOI). *GAPDH* was used as endogenous control. *, $P < 0.05$.

SUPPLEMENTAL FIGURE 6. **Silencing of FOXC1 in basal-like breast cancer cell lines results in decreased expression of MMP7.** A, *FOXC1* and *MMP7* expression in HCC1187 cells 72h post-transduction with lentivirus encoding control shRNA targeted to luciferase (shluc) or three different shRNAs targeted to FOXC1 (shFOXC1#2, shFOXC1#3, shFOXC1#4). Data summarize three independent experiments and are presented as mean +/- SE. B and C, *FOXC1* and *MMP7* expression in HCC1143 (B) or MDA468 (C) cells 72h post-transduction with lentivirus encoding shRNA targeted to

luciferase (shluc) or FOXC1 (shFOXC1#3, shFOXC1#4). Data are the mean value of triplicate wells from a single experiment. D, *FOXC1* and *MMP7* expression in MDA-MB-468 cells stably expressing shRNAs to luciferase (shluc) or FOXC1 (shFOXC1#3, shFOXC1#4). Cells were selected with puromycin for at least two weeks. RNA was harvest in triplicate from three sequential passages of each cell line. Data are presented as mean +/- SE. *, $P < 0.01$. **, $P < 0.05$. †, $P = 0.053$.

SUPPLEMENTAL FIGURE 7**. Associations between *FOXC1* or *MMP7* expression and overall survival in the Sorlie *et al*. and van de Vijver *et al*. data sets.** Kaplan-Meier curves of overall survival using data from the Sorlie *et al.* (A and B) or van de Vijver *et al.* (C and D) data sets. Samples were stratified by high (upper quartile) or low (lower three quartiles) expression of *FOXC1* (A and C) or *MMP7* (B and D). *P* values less than 0.05 were considered statistically significant.

Supplemental Table 1.

Supplemental Table 1 Gene expression array studies used for co-regulation analysis

Study	Reporter ID *FOXC1*	Reporter ID *MMP7*	Pearson Correlation Coefficient	Affymetrix Array Type	Sample *n*	Reference	GEO or EMBL-EBI Identifier
Bittner	213260_at	204259_at	0.025999963	Human Genome U133 plus 2.0 array	336	Not Published 2005/01/15	GSE2109
Boersma	213260_at	204259_at	-0.037098527	Human Genome U133A array	95	*Int J Cancer*. 2008 Mar 15;122(6):1324-32	GSE5847
Chin	213260_at	204259_at	0.70725274	Human Genome U133A array	118	*Cancer Cell*. 2006 Dec;10(6):529-41.	E-TABM-158
Desmedt	213260_at	204259_at	0.45049435	Human Genome U133A array	198	*Clin Cancer Res*. 2007 Jun 1;13(11):3207-14.	GSE7390
Farmer	213260_at	204259_at	0.40217435	Human Genome U133A array	49	*Oncogene*. 2005 Jul 7;24(29):4660-71.	GSE1561
Ginestier	213260_at	204259_at	0.6364306	Human Genome U133 Plus 2.0 array	55	*Clin Cancer Res*. 2006 Aug 1;12(15):4533-44.	-
Hess	213260_at	204259_at	0.59545517	Human Genome U133A array	133	*J Clin Oncol*. 2006 Sep 10;24(26):4236-44	-
Hoeflich	213260_at	204259_at	0.47088987	Human Genome U133 Plus 2.0 array	30	*Clin Cancer Res*. 2009 Jul 15;15(14):4649-64.	GSE12763
Ivshina	213260_at	204259_at	0.6259974	Human Genome U133A array	289	*Cancer Res*. 2006 Nov 1;66(21):10292-301.	GSE4922
Landemaine	213260_at	204259_at	0.2504087	Human Genome U133 Plus 2.0 array	23	*Cancer Res*. 2008 Aug 1;68(15):6092-9.	GSE11078
Lu	213260_at	204259_at	0.3724028	Human Genome U133 Plus 2.0 array	129	*Breast Cancer Res Treat*. 2008 Mar;108(2):191-201	GSE5460
Minn 2	213260_at	204259_at	0.4808463	Human Genome U133A array	121	*Nature*. 2005 Jul 28;436(7050):518-24.	GSE2603
Poola	213260_at	204259_at	0.51770705	Human Genome U133A array	8	*Nat Med*. 2005 May;11(5):481-3. Epub 2005 May 1.	GSE2429
Richardson 2	213260_at	204259_at	0.06784946	Human Genome U133 Plus 2.0 array	47	*Cancer Cell*. 2006 Feb;9(2):121-32.	GSE3744
Schmidt	213260_at	204259_at	0.41970545	Human Genome U133A array	200	*Cancer Res*. 2008 Jul 1;68(13):5405-13.	GSE11121
Schuetz	213260_at	204259_at	0.40949553	Human Genome U133A Array	10	*Cancer Res*. 2006 May 15;66(10):5278-86	GSE3893
Schuetz 2	213260_at	204259_at	0.79221195	Human Genome U133 Plus 2.0 array	14	Cancer Res. 2006 May 15;66(10):5278-86.	GSE3893
Sotiriou 3	213260_at	204259_at	0.3348717	Human Genome U133A array	189	*J Natl Cancer Inst*. 2006 Feb 15;98(4):262-72.	GSE2990
Turashvili	213260_at	204259_at	0.1501221	Human Genome U133 Plus 2.0 array	30	*BMC Cancer*. 2007 Mar 27;7:55.	GSE5764
Wang	213260_at	204259_at	0.60403466	Human Genome U133A array	286	*Lancet*. 2005 Feb 19-25;365(9460):671-9.	GSE2034

Supplemental Table 2.

Supplemental Table 2 Co-regulation analysis of FOXC1 expression and expression of MMP and TIMP family members

	Pearson Correlation Coefficient																								
Data Set	***MMP7***	***MMP2***	***MMP9***	***MMP1***	***MMP10***	***MMP11***	***MMP12***	***MMP13***	***MMP14***	***MMP15***	***MMP16***	***MMP17***	***MMP19***	***MMP20***	***MMP21***	***MMP24***	***MMP26***	***MMP27***	***MMP28***	***MMP3***	***MMP8***	***TIMP1***	***TIMP2***	***TIMP3***	***TIMP4***
Boersma	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	ND	0.21	-0.04	ND	-0.04	ND	ND	ND	ND	ND	-0.04	-0.04	ND	-0.07	-0.04	-0.04	-0.04
Bittner	0.03	ND	0.03	0.03	-0.04	-0.04	0.03	-0.04	-0.04	0.03	ND	ND	ND	0.10	-0.04	ND	ND	ND	-0.04	-0.04	0.03	ND	ND	ND	-0.04
Richardso	0.07	-0.09	0.07	0.07	-0.09	-0.09	0.07	-0.09	-0.09	0.07	-0.09	ND	ND	0.07	ND	ND	ND	ND	ND	-0.09	ND	ND	-0.09	-0.09	-0.09
Turashvili	0.15	ND	ND	-0.03	ND	-0.03	ND	ND	ND	ND	ND	ND	-0.03	ND	-0.03	ND	ND	ND	-0.03	ND	ND	ND	ND	ND	-0.03
Landemai	0.25	0.55	-0.08	0.25	ND	-0.08	-0.08	-0.08	ND	ND	-0.08	ND	-0.08	ND	ND	ND	ND	ND	ND	0.07	-0.08	-0.08	-0.08	0.10	-0.08
Sotiriou 3	0.33	0.02	0.03	0.10	0.02	0.02	0.03	0.02	-0.22	ND	0.02	ND	ND	ND	ND	ND	ND	ND	ND	-0.22	ND	0.03	ND	0.02	0.02
Lu	0.37	0.00	ND	0.16	-0.02	0.00	0.16	-0.02	ND	ND	0.00	ND	ND	0.00	ND	ND	ND	ND	-0.02	ND	ND	ND	ND	ND	-0.02
Farmer	0.40	-0.03	0.06	0.06	0.06	-0.03	0.06	-0.03	0.13	ND	0.03	ND	ND	0.38	ND	ND	ND	ND	ND	-0.03	ND	0.06	0.06	-0.03	-0.03
Schuetz	0.41	-0.06	-0.19	-0.06	-0.06	-0.06	-0.19	-0.06	ND	-0.19	ND	ND	ND	ND	ND	ND	ND	ND	ND	-0.06	ND	-0.19	-0.06	-0.19	-0.06
Schmidt	0.42	-0.02	0.07	0.07	-0.02	-0.02	0.07	-0.02	-0.02	0.01	-0.01	-0.01	-0.02	-0.01	ND	-0.01	-0.01	ND	-0.02	-0.02	-0.01	ND	-0.02	-0.02	-0.02
Desmedt	0.45	-0.02	0.06	0.07	-0.02	0.00	0.07	-0.02	-0.02	0.01	0.00	0.00	-0.02	0.00	ND	0.00	-0.02	0.00	0.00	-0.02	0.00	ND	0.06	-0.02	-0.02
Hoeflich	0.47	0.15	0.15	0.15	-0.10	0.15	0.07	0.15	0.15	0.23	0.10	ND	ND	0.31	ND	ND	ND	ND	ND	0.15	ND	ND	0.15	0.15	0.15
Minn 2	0.48	0.09	0.09	-0.11	0.09	0.09	0.06	0.09	-0.11	0.09	0.01	0.09	0.09	0.01	ND	-0.11	ND	ND	0.01	0.09	0.06	ND	-0.11	-0.11	-0.11
Poola	0.52	-0.13	-0.13	-0.13	0.00	0.52	-0.13	-0.13	-0.13	-0.13	0.06	-0.13	-0.13	0.16	ND	0.00	-0.13	ND	0.06	-0.13	-0.13	-0.13	-0.13	-0.13	ND
Hess	0.60	0.07	0.11	0.02	0.02	0.07	0.11	ND	-0.19	ND	0.02	ND	-0.19	ND	ND	ND	ND	ND	ND	ND	ND	0.02	0.00	0.02	0.07
Wang	0.60	0.01	0.05	0.01	0.01	0.02	0.05	0.01	0.01	-0.03	0.06	-0.03	0.01	-0.03	ND	-0.03	-0.03	ND	0.01	-0.03	ND	ND	0.01	0.01	0.01
Ivshina	0.63	0.02	0.03	0.03	0.02	0.02	0.03	0.02	0.02	-0.03	0.02	-0.03	0.02	-0.03	ND	ND	ND	ND	0.02	0.02	-0.03	ND	0.03	0.02	0.02
Ginestier	0.64	-0.07	0.06	0.37	-0.07	-0.07	0.37	-0.07	0.19	ND	-0.07	ND	ND	ND	ND	ND	ND	ND	ND	-0.07	ND	ND	-0.07	-0.07	ND
Chin	0.71	-0.04	0.10	0.10	-0.04	-0.04	0.10	-0.04	-0.04	-0.04	-0.04	ND	-0.04	ND	ND	-0.04	ND	ND	ND	0.10	ND	-0.04	-0.04	-0.04	-0.04
Schuetz 2	0.79	0.00	-0.09	0.11	-0.09	0.00	-0.09	0.00	ND	ND	0.00	ND	ND	ND	ND	ND	ND	ND	ND	0.00	ND	ND	0.00	0.00	0.00
***P* value**	**0.0001**	0.954	0.956	0.443	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

ND = no data

NS = not significant at $P < 0.05$

Supplemental Table 3.

Supplemental Table 3 High combined expression of *FOXC1* and *MMP7* is an independent predictor of poor patient outcome in the van de Vijver *et al.* data set

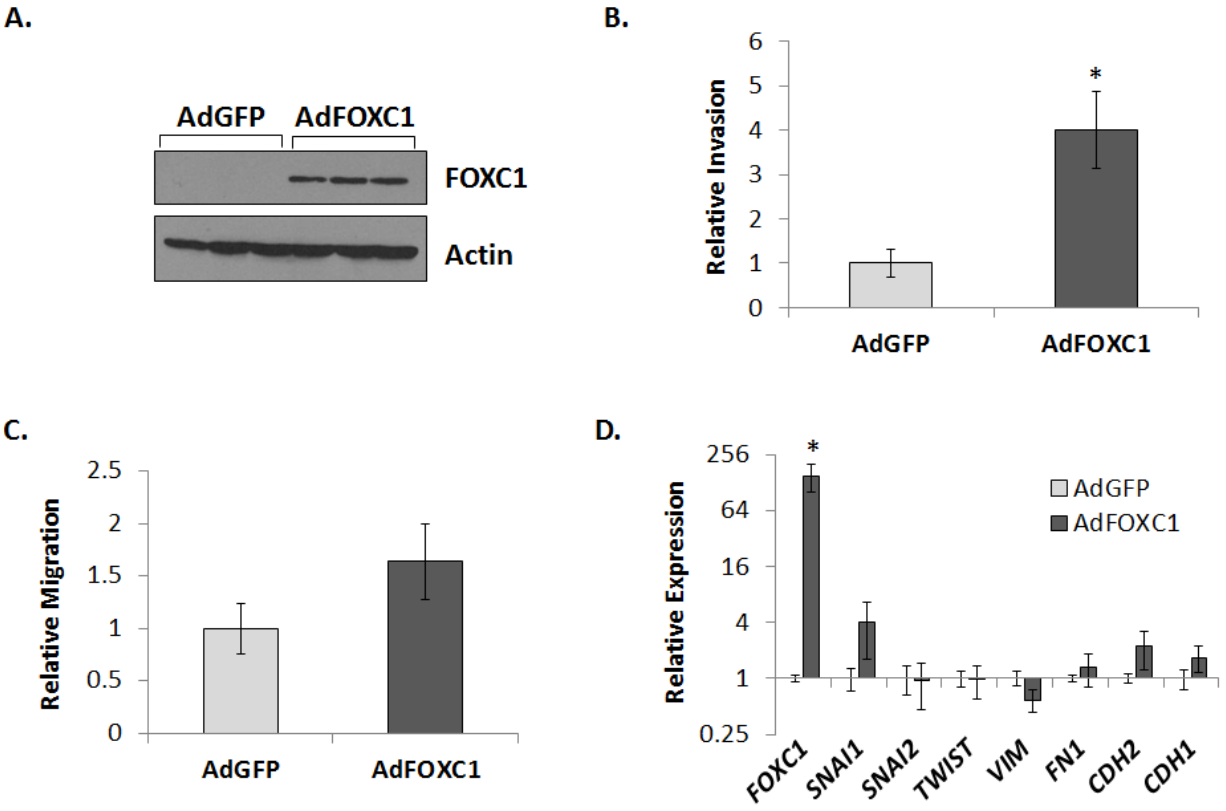
Univariate Analysis

			95% Confidence Interval	
Variable	***P* value**	**Hazard Ratio**	**Lower**	**Upper**
High *FOXC1*	0.0667	1.7393	1.1477	2.3308
High *MMP7*	0.2924	1.3880	0.7777	1.9984
High FOXC1 and MMP7	0.0020	2.6674	2.0448	3.2901
N Stage	0.2840	0.7307	0.1569	1.3046

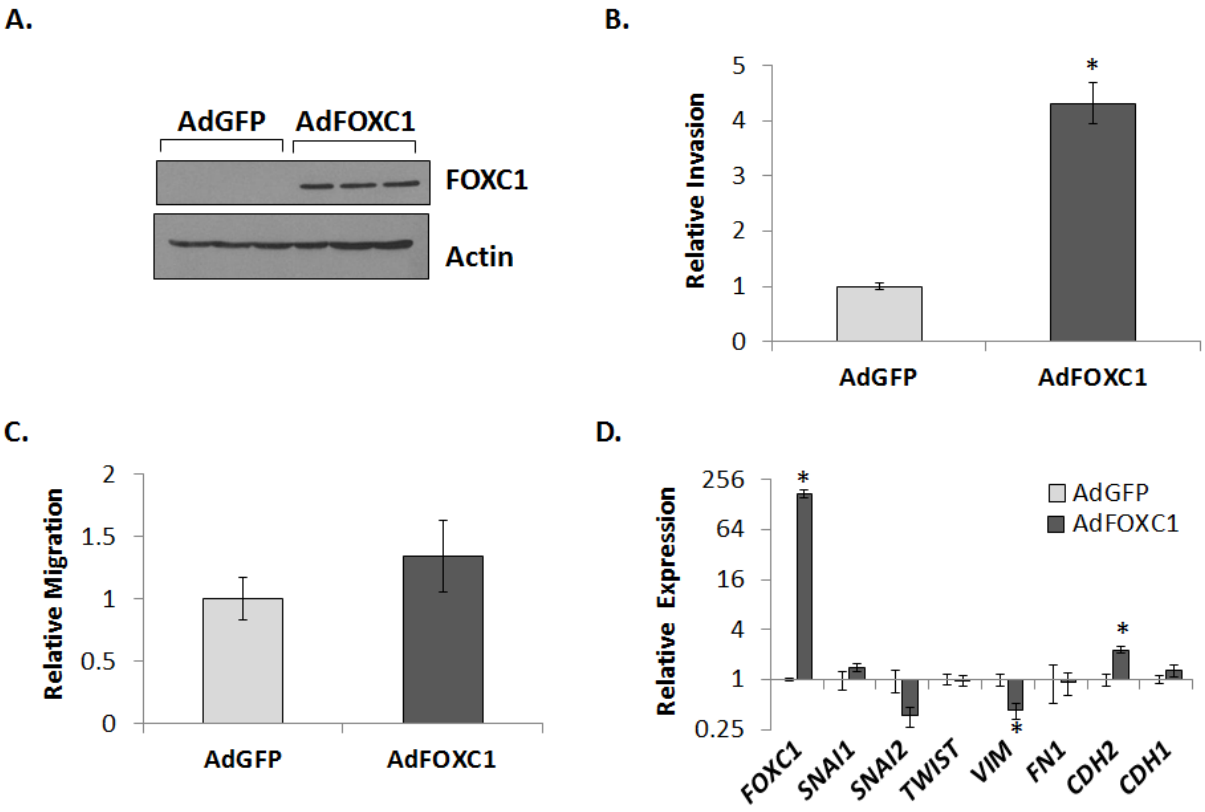
Multivariate Analysis

			95% Confidence Interval	
Variable	***P* value**	**Hazard Ratio**	**Lower**	**Upper**
High FOXC1 and MMP7	0.0020	2.6674	2.0448	3.2901

Supplemental Figure 1.

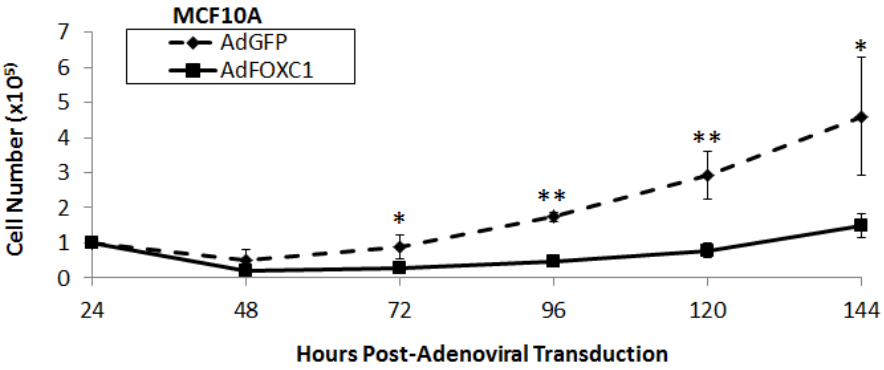


Supplemental Figure 2.

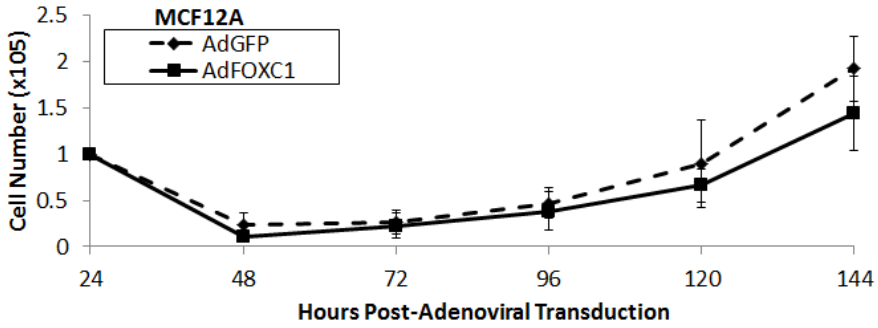


Supplemental Figure 3.

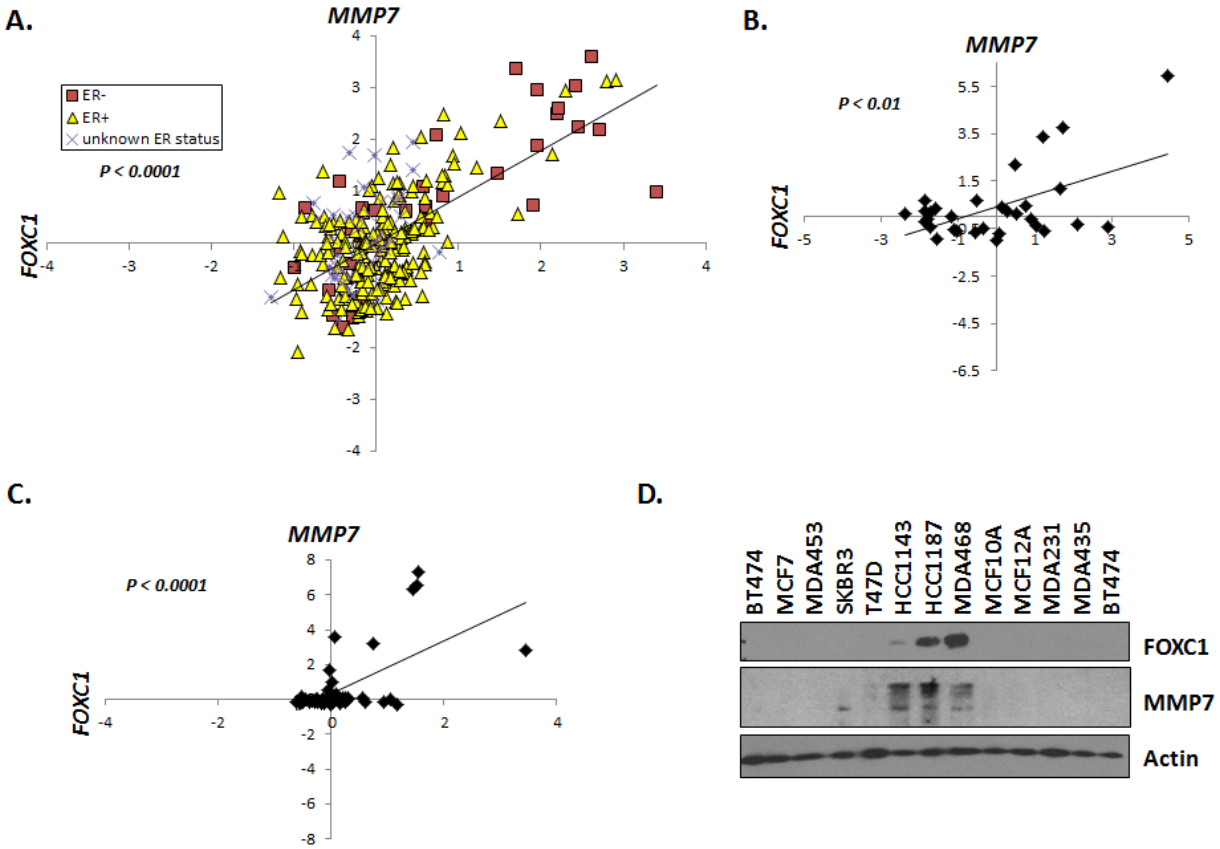
A.



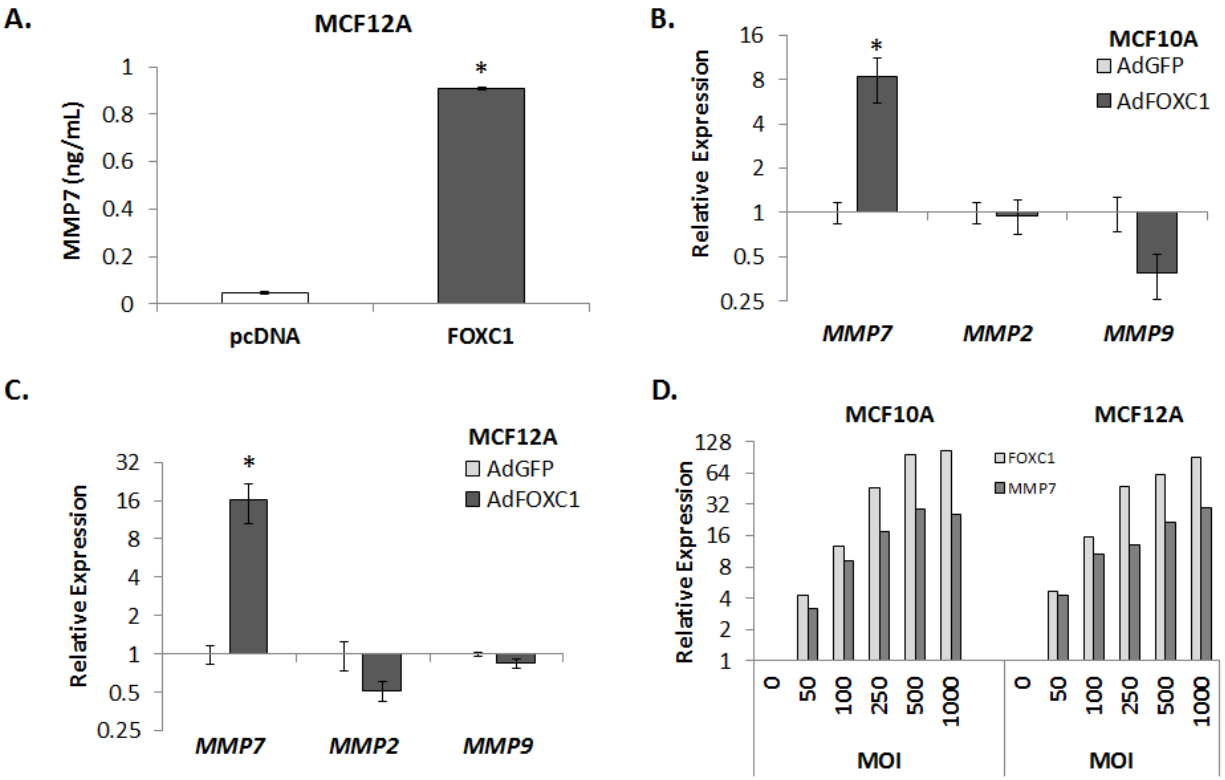
B.



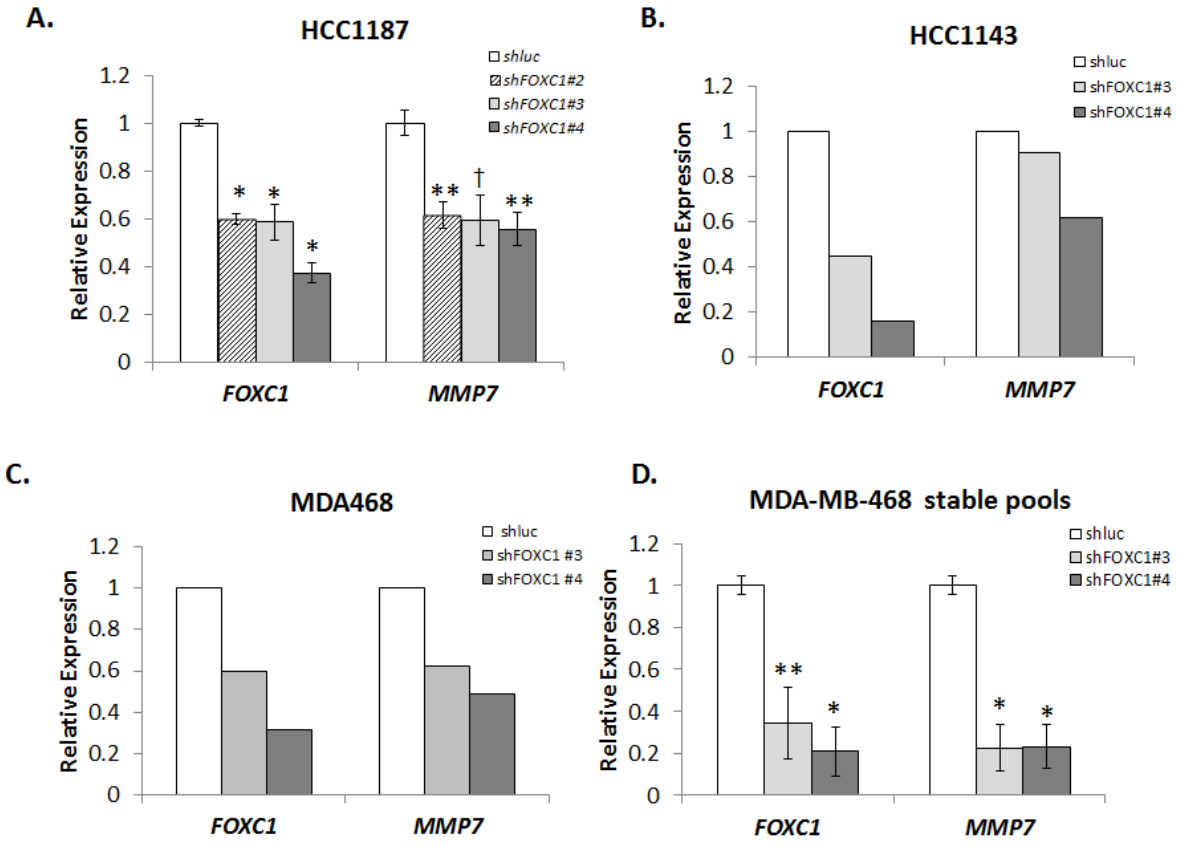
Supplemental Figure 4.



Supplemental Figure 5.



Supplemental Figure 6.



Supplemental Figure 7.