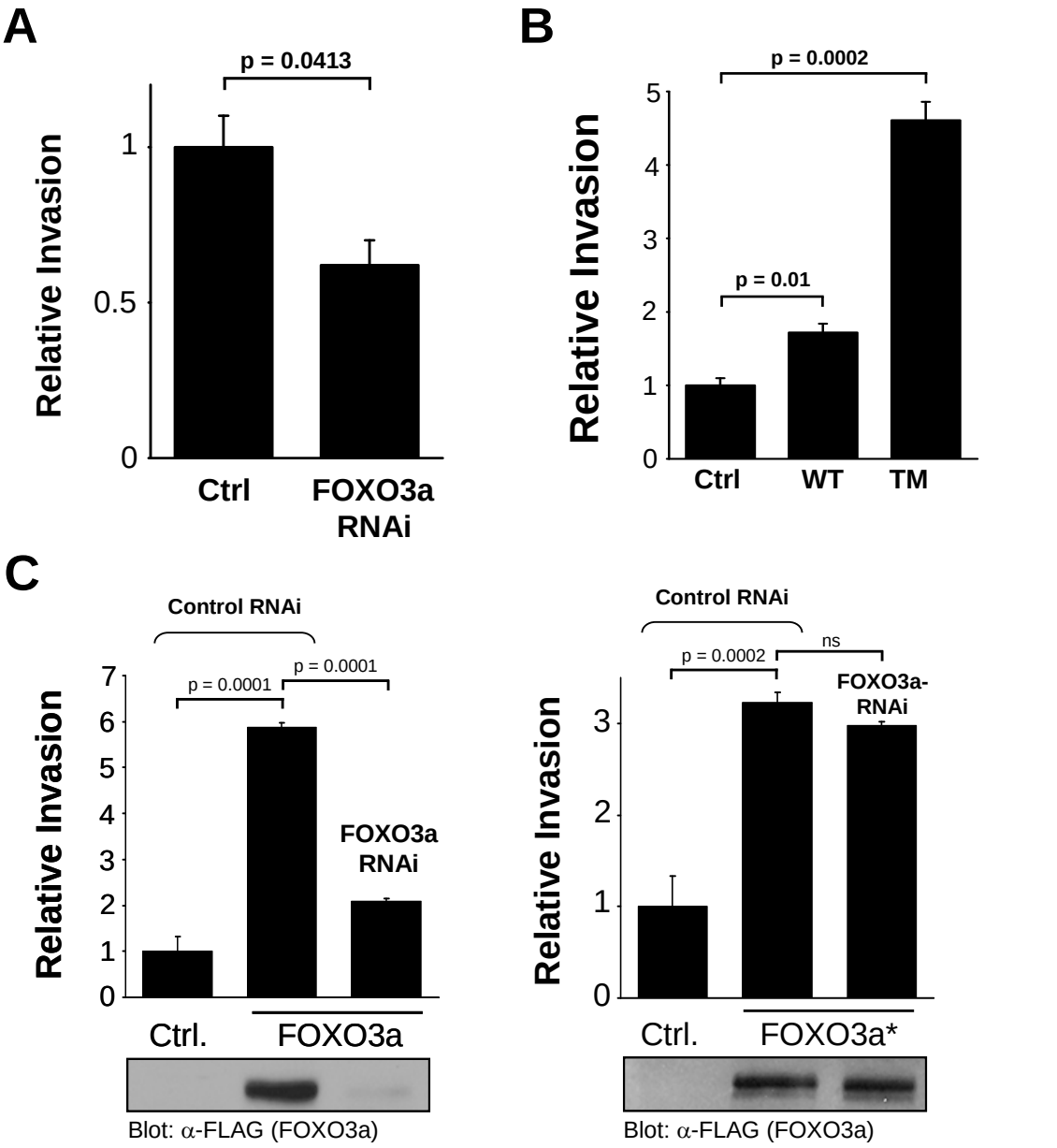
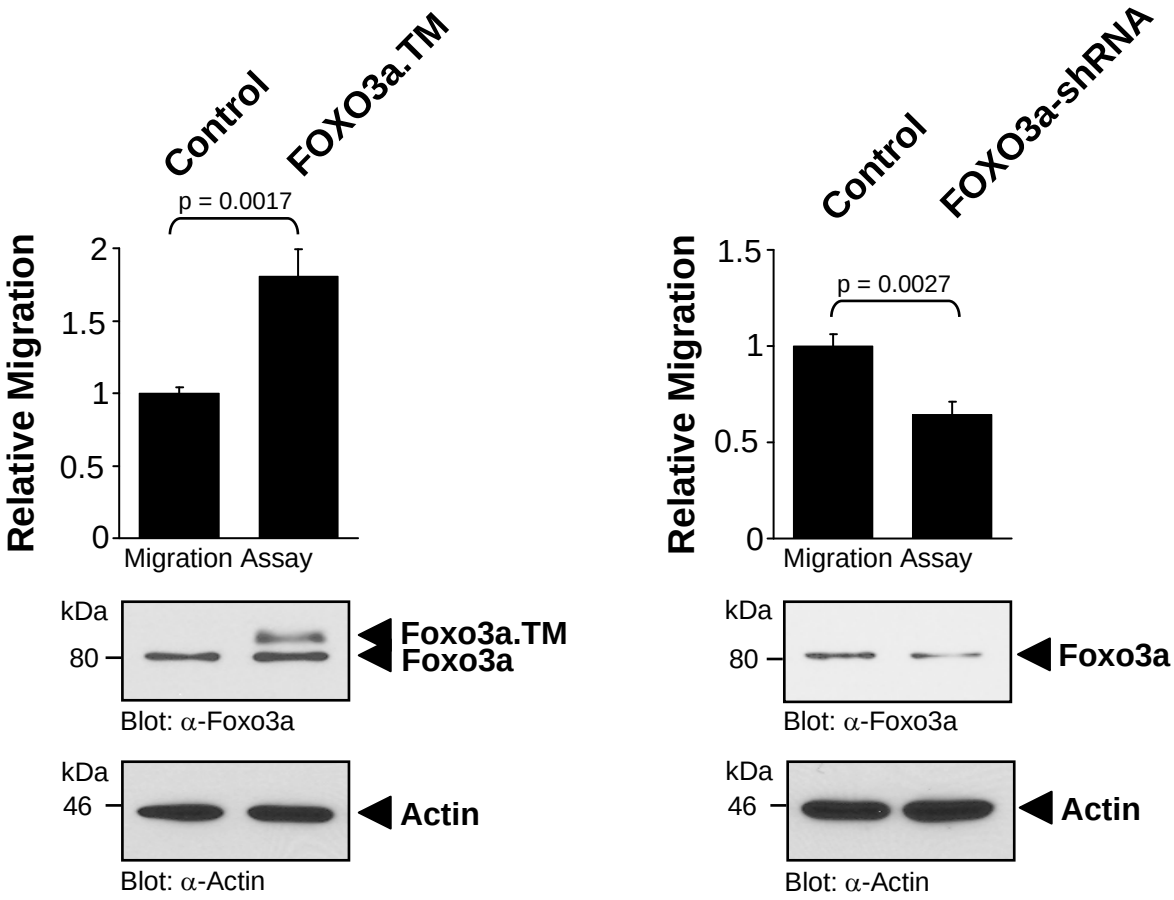
Supplemental Figure S1



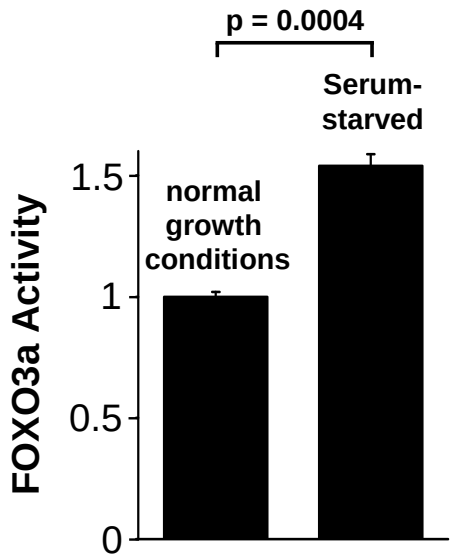
FOXO3a regulates cell invasion. A: HeLa cells were transfected with FOXO3a-RNAi and Matrigel invasion assays were performed. Silencing FOXO3a expression by RNAi was measured using RT-PCR for FOXO3a and actin (control, not shown). **B**, HeLa cells were transfected with vector control, wild-type FOXO3a or FOXO3a.TM and Matrigel invasion assays were performed. In all experiments error bars represent standard deviation. All results are statistically significant as analyzed by Unpaired t-test. Results depicted in A and B are typical of three independent experiments. **C**, HeLa cells were transfected with control RNAi or FOXO3a-RNAi as indicated. In a second transfection wildtype human FOXO3a (left picture) or a human FOXO3a with three silent mutations in the sequence targeted by the FOXO3a-specific RNAi (right picture, FOXO3a*) were transfected. Matrigel invasion assays were performed. Silencing FOXO3a expression was determined by immunoblotting with a-FLAG. In both experiments error bars represent standard deviation. Al results are statistically significant as analyzed by Unpaired t-test. The results are typical of two independent experiments.

Supplemental Figure S2



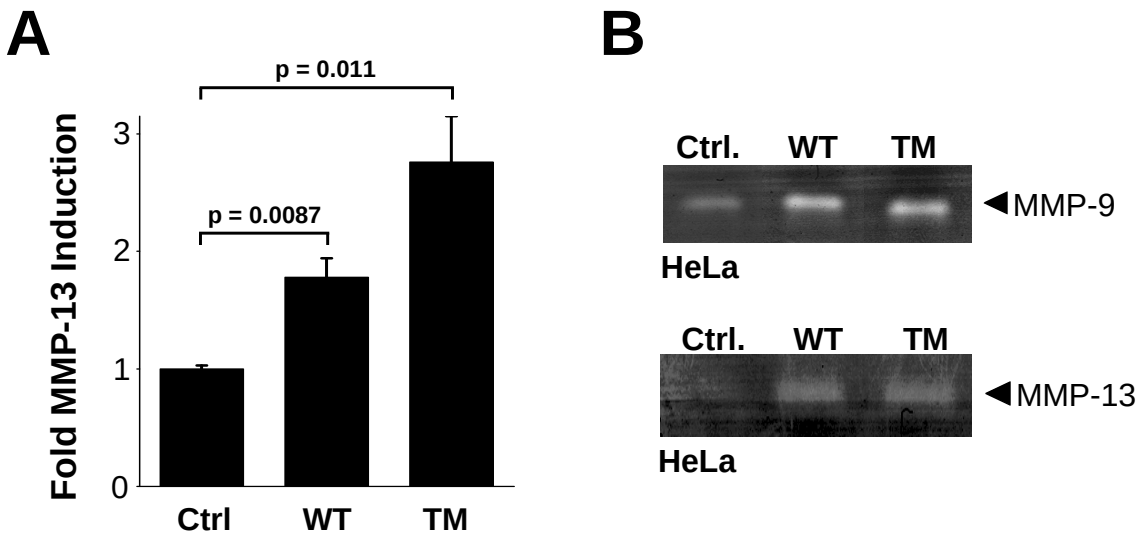
FOXO3a increases cell migration. Left panel: HeLa cells were transfected with vector control or FOXO3a.TM and migration assays using Transwell® chambers were performed. Protein expression was controlled by Western blotting (α-FOXO3a). Western blotting for actin (α-Actin) served as loading control. Right panel: HeLa cells were transfected with control RNAi or FOXO3a-RNAi as indicated and migration assays were performed. Knockdown of Foxo3a was controlled by Western blotting (α-FOXO3a). Western blotting for actin (α-Actin) served as loading control. In all experiments error bars represent standard deviation. All results are statistically significant as analyzed by Unpaired t-test. The results are typical of two independent experiments.

Supplemental Figure S3



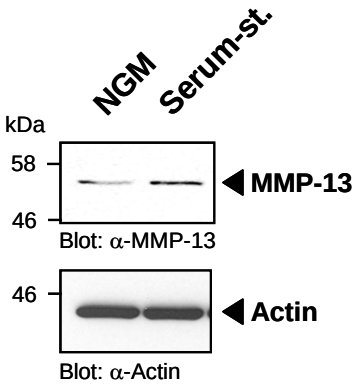
Serum starvation induces FOXO3a activity. HeLa cells were transfected with FHRE-luc and β-gal reporter plasmids. FOXO3a activity was compared under normal and serum-starved conditions. Error bars represent standard deviation. All results are typical of three independent experiments. Statistical significance was analyzed by a Unpaired t-test. This Supplemental Figure shows that reduced serum factors increase the activity of FOXO3a in HeLa cells and supports data generated with MDA-MB-435 cells in Figure 2G of the manuscript.

Supplemental Figure S4



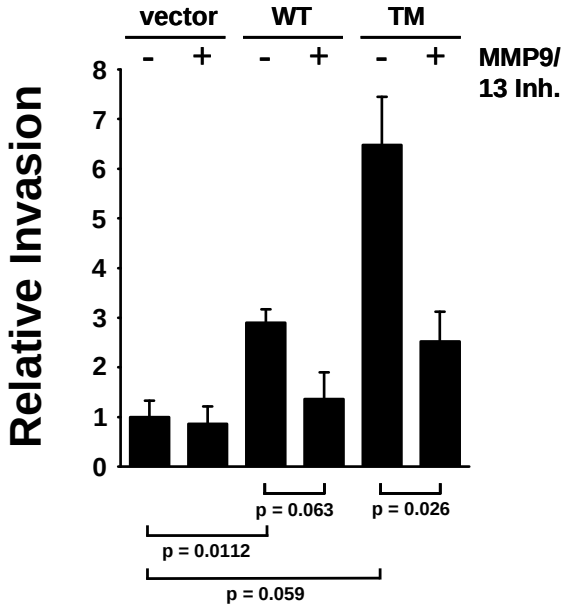
FOXO3a induces expression of active MMP. A: MDA-MB-435 cells were transfected with vector control, wild-type FOXO3a or FOXO3a.TM and MMP-13-luciferase and β-gal reporter plasmids. Reporter assays were performed to measure MMP-13 and β-galactosidase activity. **B.** HeLa cells were transfected with vector control, wild-type FOXO3a or FOXO3a.TM. Supernatants were collected and zymography was performed. All results are typical of three independent experiments. A supports Figure 3 and B supports Figure 4 of the manuscript.

Supplemental Figure S5



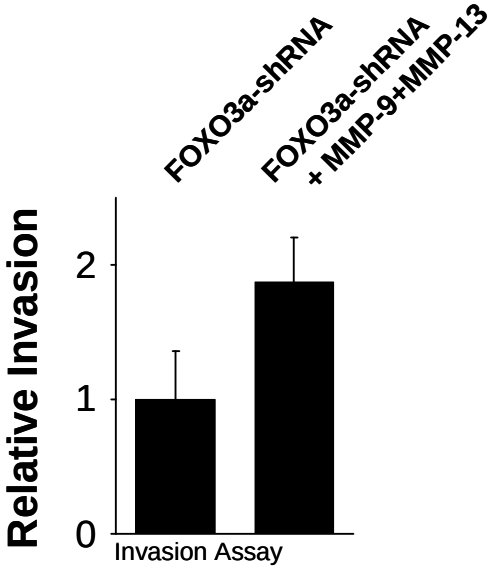
Serum starvation induces MMP-13 expression. HeLa cells were grown under normal conditions (NGM) or in absence of serum. MMP-13 expression was analyzed by Western blotting (α-MMP-13). Actin expression (α-Actin) served as a loading control.

Supplemental Figure S6



Inhibition of MMP9/13 decreases FOXO3a-mediated invasiveness of cells. MDA-MB-435 cells were transfected with vector control, wild-type or FOXO3a.TM and treated with MMP9/13 Inhibitor [5 nM]. Matrigel invasion assays were performed. FOXO3a expression was measured by immunoblot analysis (not shown). In all experiments error bars represent standard deviation. The results are typical of three independent experiments. This Supplemental Figure shows that in MDA-MB-435 cells the inhibition of MMPS decreases FOXO3a-mediated cell invasion. This supports data obtained with HeLa cells depicted in Figure 4D.

Supplemental Figure S7



Ectopic MMP-9 and MMP-13 rescue cell invasion. Cells depleted from FOXO3a were subjected an invasion assay in presence of recombinant MMP-9 and MMP-13.

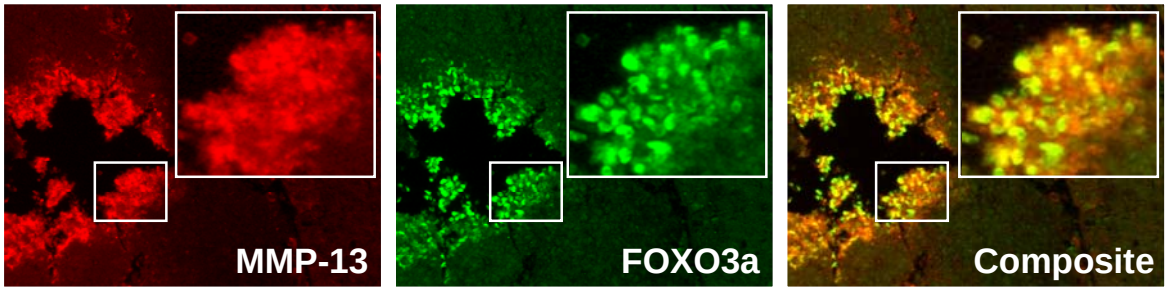
Supplemental Figure S8

HUMAN BREAST CANCER TISSUE	POSITIVE for MMP-13 and FOXO3a	
Infiltrating Duct Carcinoma Stage T1 (n=1)	1	
Infiltrating Duct Carcinoma Stage T2 (n=18)	12	(67%)
Infiltrating Duct Carcinoma Stage T3 (n=10)	6	(60%)
Infiltrating Duct Carcinoma Stage T4 (n=4)	2	(50%)
Infiltrating and Metastatic Carcinoma Stage T1 (n=4)	3	(75%)
Infiltrating and Metastatic Carcinoma Stage T2 (n=21)	15	(71%)
Infiltrating and Metastatic Carcinoma Stage T3 (n=18)	13	(72%)
Infiltrating and Metastatic Carcinoma Stage T4 (n=5)	2	(40%)

FOXO3a and MMP-13 expression in different stages of human breast cancer tissue. Tissue samples of different stages of infiltrating duct and metastatic carcinoma were analyzed for FOXO3a and MMP-13 co-expression using immunohistochemistry. This Supplemental Figure shows that FOXO3a and MMP-13 expression correlates in tissue samples independent of stages.

Supplemental Figure S9

Infiltrating duct carcinoma of the breast (T stage T2)



FOXO3a and MMP-13 Expression Correlate in Human Breast Cancer Tissue. 44 tissue samples of human cancer tissue (35 Infiltrating Duct Carcinoma, 9 Carcinoma in Lymph Node) as well as 5 samples of normal tissue were analyzed for FOXO3a and MMP-13 expression using immunohistochemistry. The immunofluorescence staining shows a representative sample with nuclear expression of FOXO3a (green) and correlating MMP-13 expression (red) in the same tumor region. The large box shows a tumor region enlarged 3x and the small box shows the area enlarged.