Landes Bioscience www.landesbioscience.com

Supplemental Material to:

Diego Sisci, Pamela Maris, Maria Grazia Cesario, Wanda Anselmo, Roberta Coroniti, Giovanna Elvi Trombino, Francesco Romeo, Aurora Ferraro, Marilena Lanzino, Saveria Aquila, Marcello Maggiolini, Loredana Mauro, Catia Morelli, and Sebastiano Andò

The estrogen receptor α is the key regulator of the bifunctional role of FoxO3a transcription factor in breast cancer motility and invasiveness

Cell Cycle 2013; 12(21)
http://dx.doi.org/10.4161/cc.26421

http://www.landesbioscience.com/journals/cc/article/26421

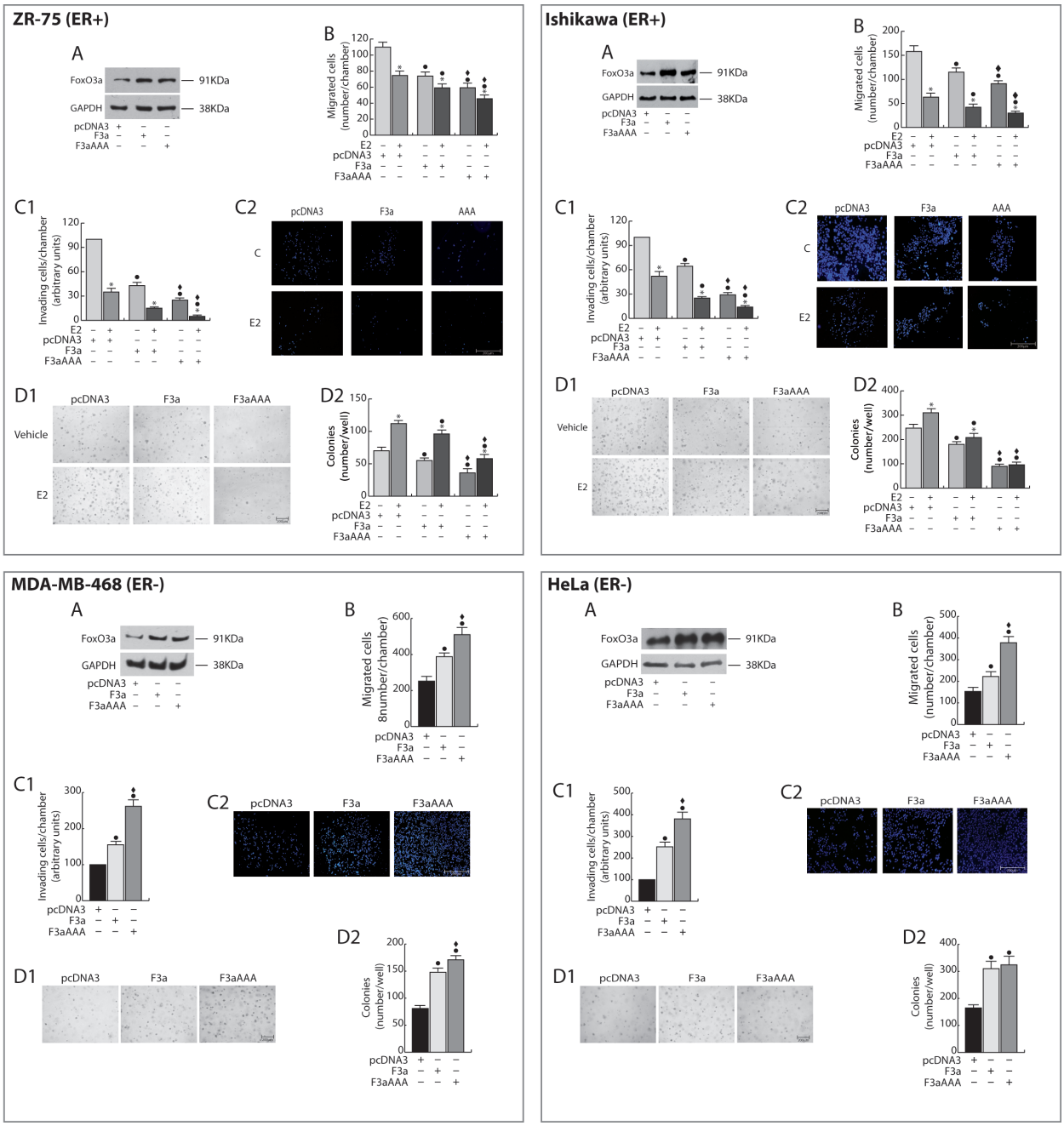
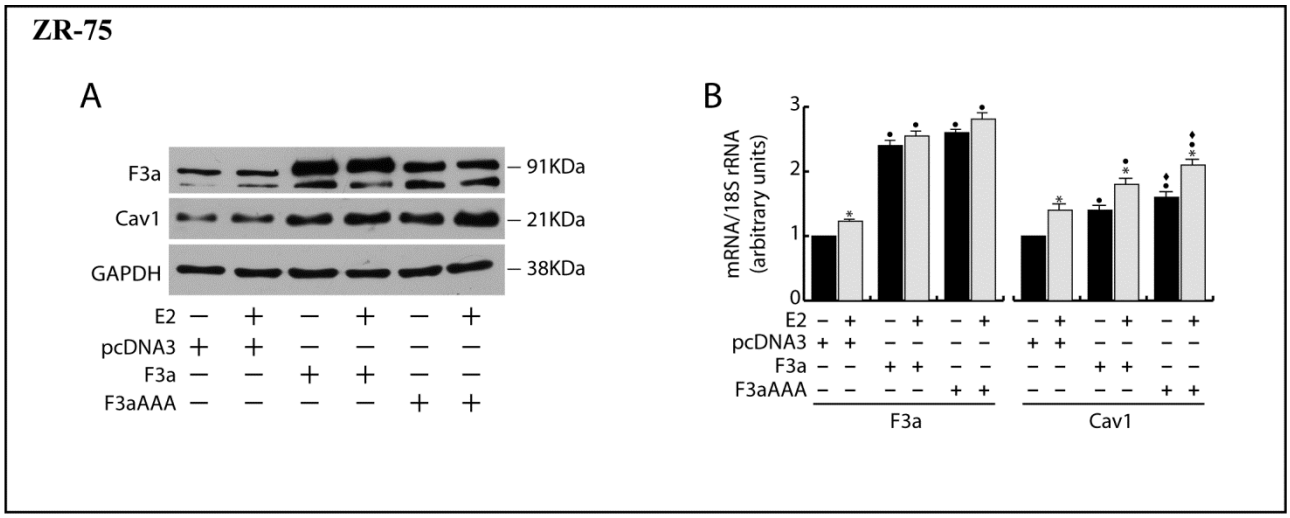


Figure S1 The opposite effects exerted by FoxO3a on migration, invasion and colony formation of ERα+ and ERα- cancer cells is not tissue specific. Double sets of ERα+ (ER+) breast cancer cells (ZR75) and endometrial cancer cells (Ishikawa), and of ERα- (ER-) breast cancer cells (MDA-MB-468) and cervical cancer cells (HeLa) were transiently transfected with F3a, F3aAAA or pcDNA3 as described in Materials and Methods. Following 24h of starvation, one of each double set of cells was harvested and subjected to Migration (B), invasion (C1 and C2) or soft agar assays (D1 and D2), adding 100nM E2 where indicated. Migrated cells were counted after 24h (ZR72 and Ishikawa) or 16h (MDA-MB-468 and HeLa) of incubation. Invading cells were counterstained with DAPI after 48h (MDA-MB-468 and HeLa) or 72h (ZR75, Ishikawa) of incubation (C2), and evaluated by ImageJ software (C1). In soft agar assays, colonies formed after 14 days from plating were photographed at 4x magnification (D1), exposed to MTT and counted under the microscope (D2). The second of each set of cells was used to evaluate transfections efficiency by WB analysis on total protein extract; GAPDH was used as a loading control (A). Results are the mean±s.d. of at least three independent experiments. *, P< 0.01 vs untreated; ●, P < 0.01 vs pcDNA3; ♦, P < 0.01 vs F3a.



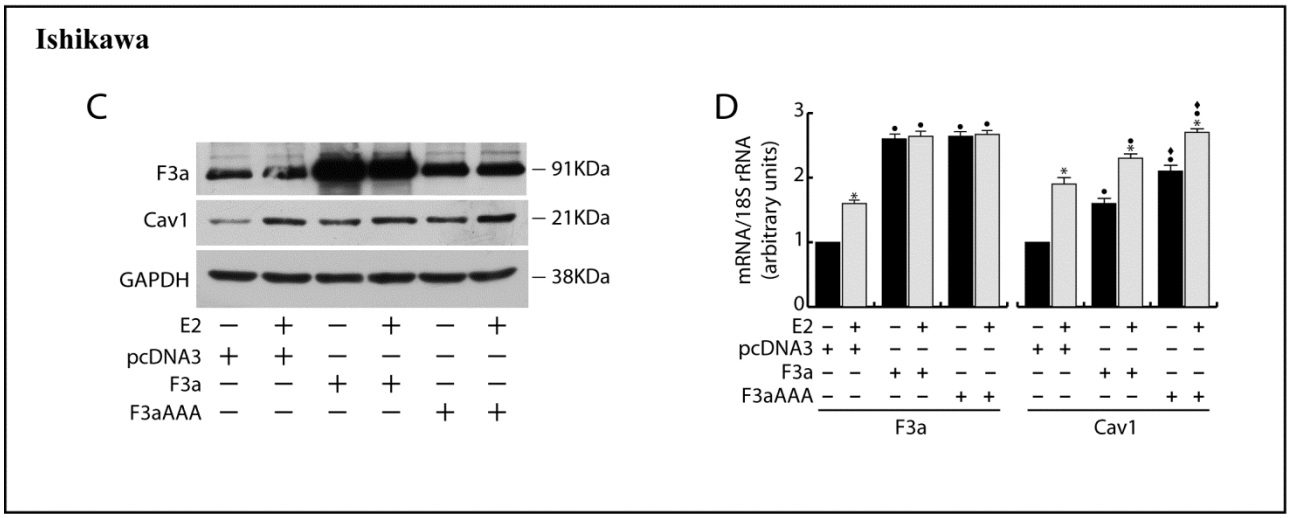


Figure S2 FoxO3a induces Cav-1 expression in ERα+ cancer cells. Double sets of ZR75 and Ishikawa cells were transiently transfected with F3a, F3aAAA or pcDNA3, as described in Materials and Methods. Five hours after transfection, cells were starved for 24h and then treated or not with 100nM E2 for additional 24h. Total proteins (A and C) and RNA (B and D) were extracted and subjected to WB and RT-PCR analysis respectively, to assess FoxO3a and Cav-1 expression. GAPDH was used as loading control in WB analysis. In RT-PCR assays each sample was normalized to its 18S rRNA content. Results are the mean±s.d. of at least three independent experiments. *, $P < 0.01$ vs untreated; ●, $P < 0.01$ vs pcDNA3; ♦, $P < 0.01$ vs corresponding F3a.