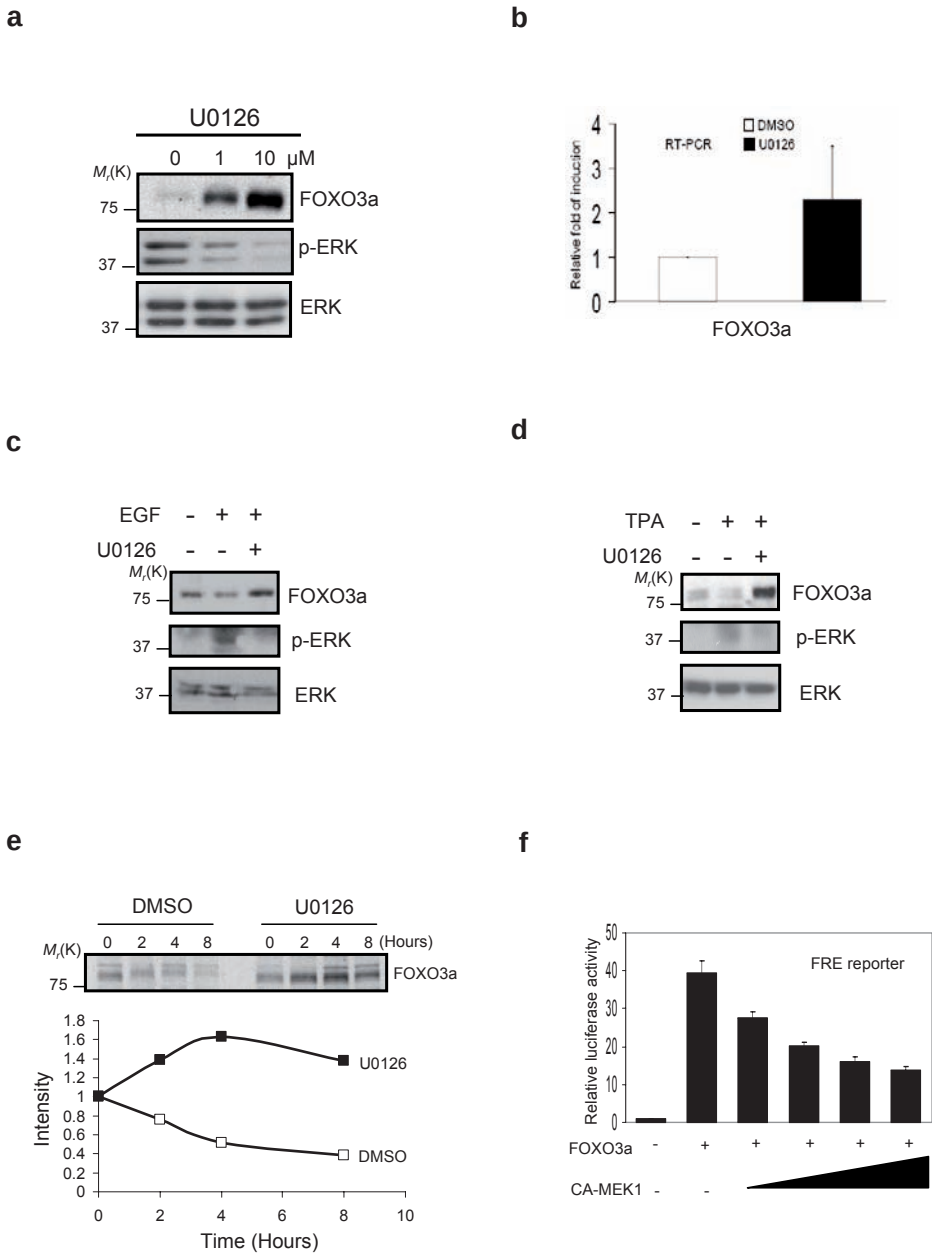


Figure S1 ERK inhibitor increased FOXO3a protein expression and activated ERK inhibited transcriptional activity of FOXO3a-responsive elements driven luciferase reporter. (**a**) Lysates of 293T cells were transfected with wild-type FOXO3a along with different dosages of U0126 and analyzed by immunoblotting. (**b**) Real time PCR transcript products of *FOXO3a* was measured, and the relative fold of induction was calculated and compared between DMSO and U0126 treated cells. (**c**) Serum starved MCF-7 cells were treated with EGF for 8 hrs with or without U0126. Cell lysates were subjected to immunoblotting with the antibodies indicated. (**d**) Serum starved MCF-7 cells were treated with 12-O-tetradecanoylphorbol 13-acetate (TPA) for 8 hrs with or without U0126. Cell lysates were subjected to immunoblotting with the antibodies indicated. (**e**) 293T cells were incubated with methionine and cysteine-free medium overnight. Cells were treated with or without U0126, pulsed with [^{35}S] Met for 30 minutes, and chased for the indicated time intervals. Cell lysates were immunoprecipitated with an anti-FOXO3a antibody and subjected to SDS-PAGE analyses. Gels were fixed, dried, and subjected to autoradiography (with an intensifying screen). (**f**) Lysates of 293T cells co-transfected with marked plasmids were subjected to luciferase assays with FOXO-responsive elements driven luciferase reporter. Graph shows the mean value of the representative results from three experiments (n=3) with s.d. conducted in duplicates for each. All the concentrations and time for treatment were described in the methods.

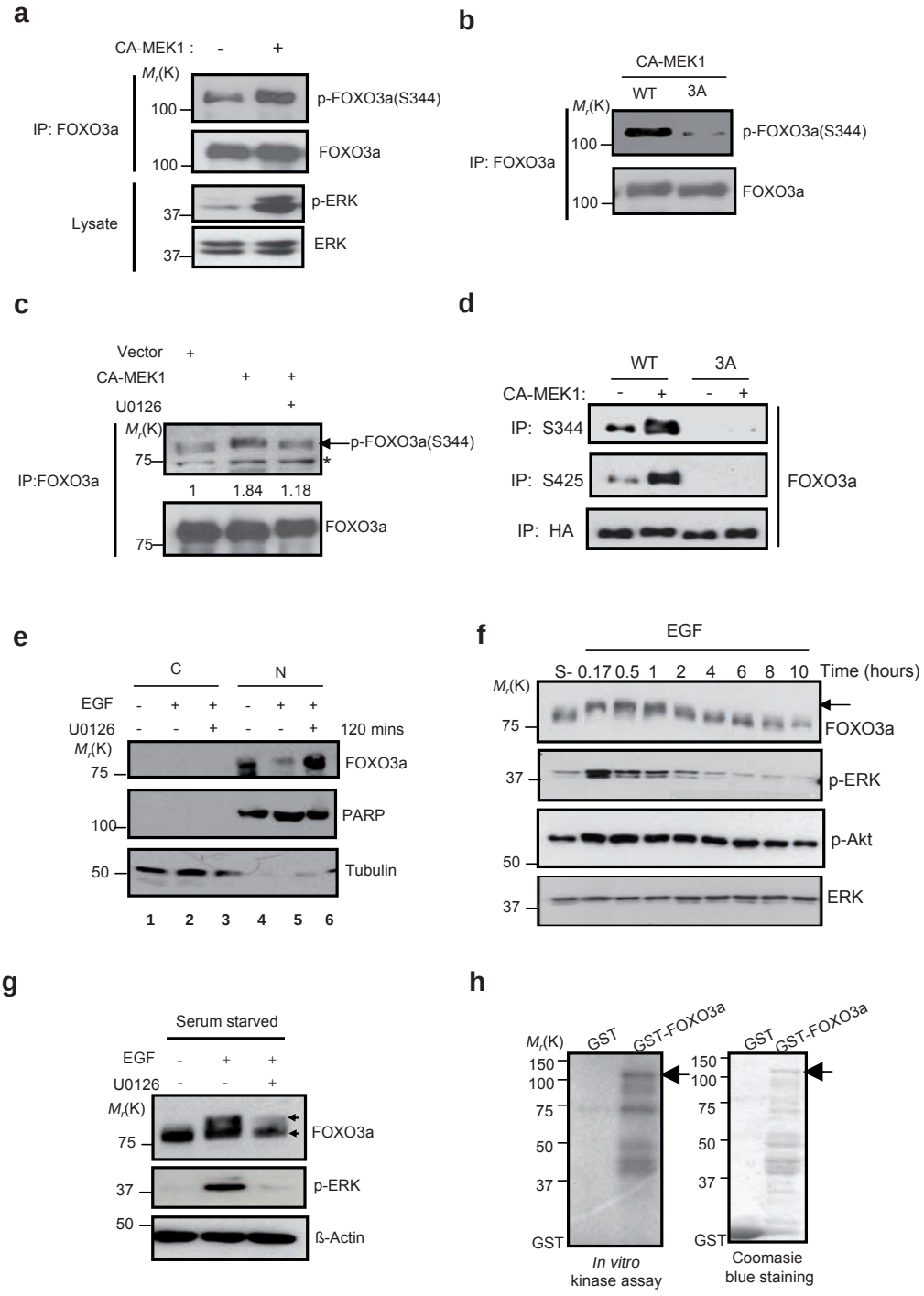


Figure S2 Identification of ERK phosphorylation sites of FOXO3a by specific phospho-antibody targeting p-FOXO3a (S294, S344 and S425). (**a**) Lysates of 293T cells transfected FOXO3a with CA-MEK1 were subjected to immunoprecipitation and immunoblotting with the antibodies indicated. (**b**) Lysates of 293T cells co-transfected FOXO3a or FOXO3a-3A with CA-MEK1 were subjected to immunoprecipitation and immunoblotting (anti-FOXO3a and anti-p-FOXO3a-344). (**c**) Cells treated with PD98059 for 4 hours before the lysates were extracted and subjected to analysis as described in (a) (black arrow). The star represents a nonspecific band for P-FOXO3a antibody. (**d**) Lysates of 293 cells co-transfected with FOXO3a or FOXO3a-3A and CA-MEK1 were subjected to immunoprecipitation and immunoblotting (IP: anti-p-FOXO3a-S344 or S425 and anti-HA; IB: anti-FOXO3a). (**e**) MCF-7 cells, after serum starvation, were treated with EGF for 120 minutes with or without U0126 then cytoplasmic and nuclear fractionations were analyzed by immunoblotting with indicated antibodies. (**f**) MCF-7 cells were extracted at the indicated times after serum starvation and EGF stimulation and then subjected to immunoblotting with the antibodies indicated. (**g**) The FOXO3a band upshift is blocked by ERK inhibitor U0126. MCF-7 cells were starved overnight and stimulated with EGF for 30 minutes. Lysates were left untreated or were treated with U0126 and subjected to immunoblotting. (**h**) ERK directly phosphorylates FOXO3a *in vitro*. *In vitro* kinase assay was performed by incubating recombinant activated ERK2 with full-length GST-FOXO3a.

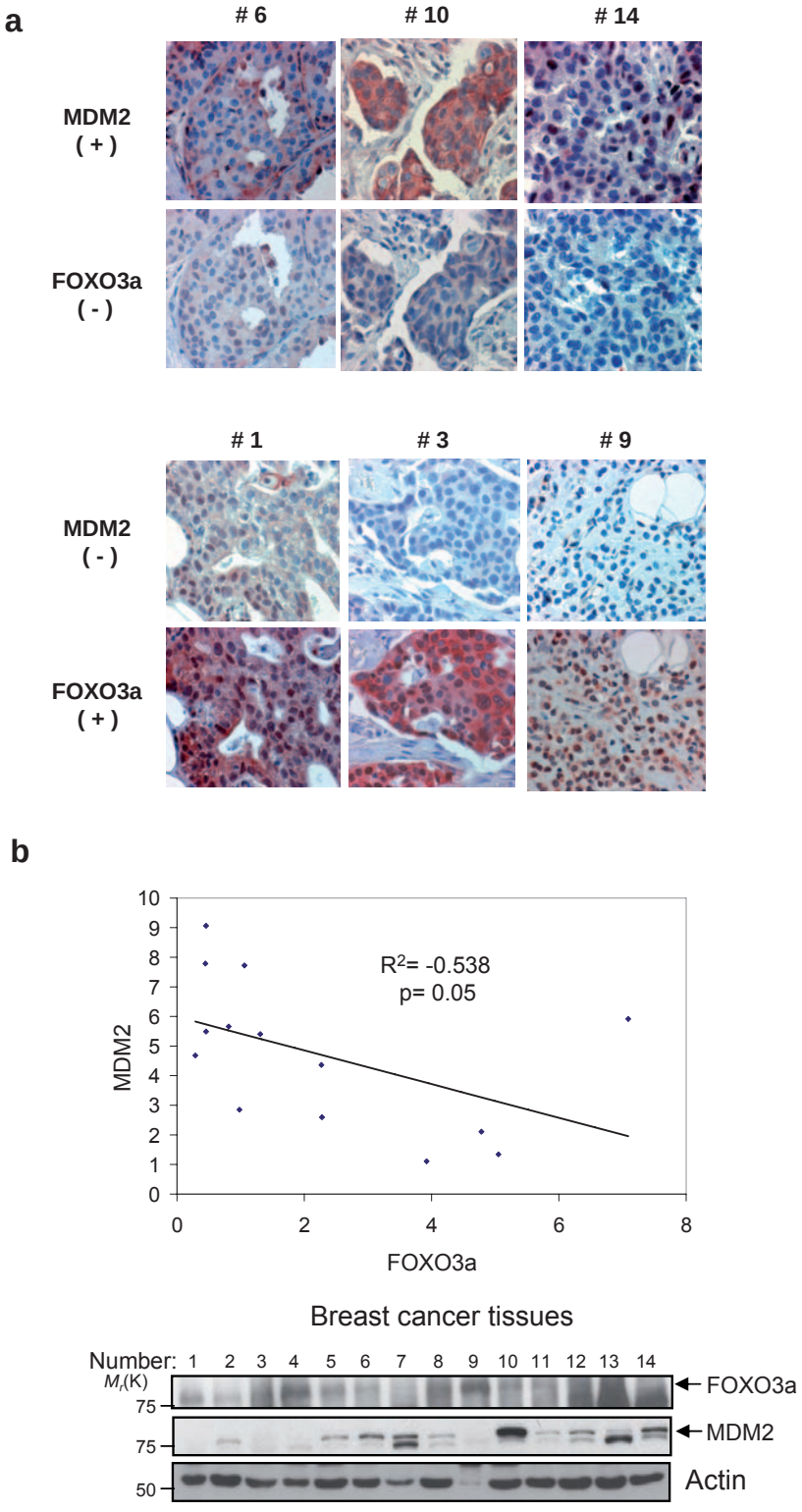


Figure S3 Inverse correlation of FOXO3a and MDM2 expression in human breast cancer patients. (**a**) The IHC levels of MDM2 and FOXO3a from consecutive tumor sections (# 6,10 and 14 represented MDM2+ and FOXO3a- ; # 1,3 and 9 represented MDM2- and FOXO3a+) of six patients. The patient numbers in (**a**) and (**b**) are the same. (**b**) Lysates from fourteen breast cancer patients were subjected to western blotting with indicated antibodies. The linear regression analysis was used to analyze the FOXO3a and MDM2 expression in the lysates of breast cancer patients.

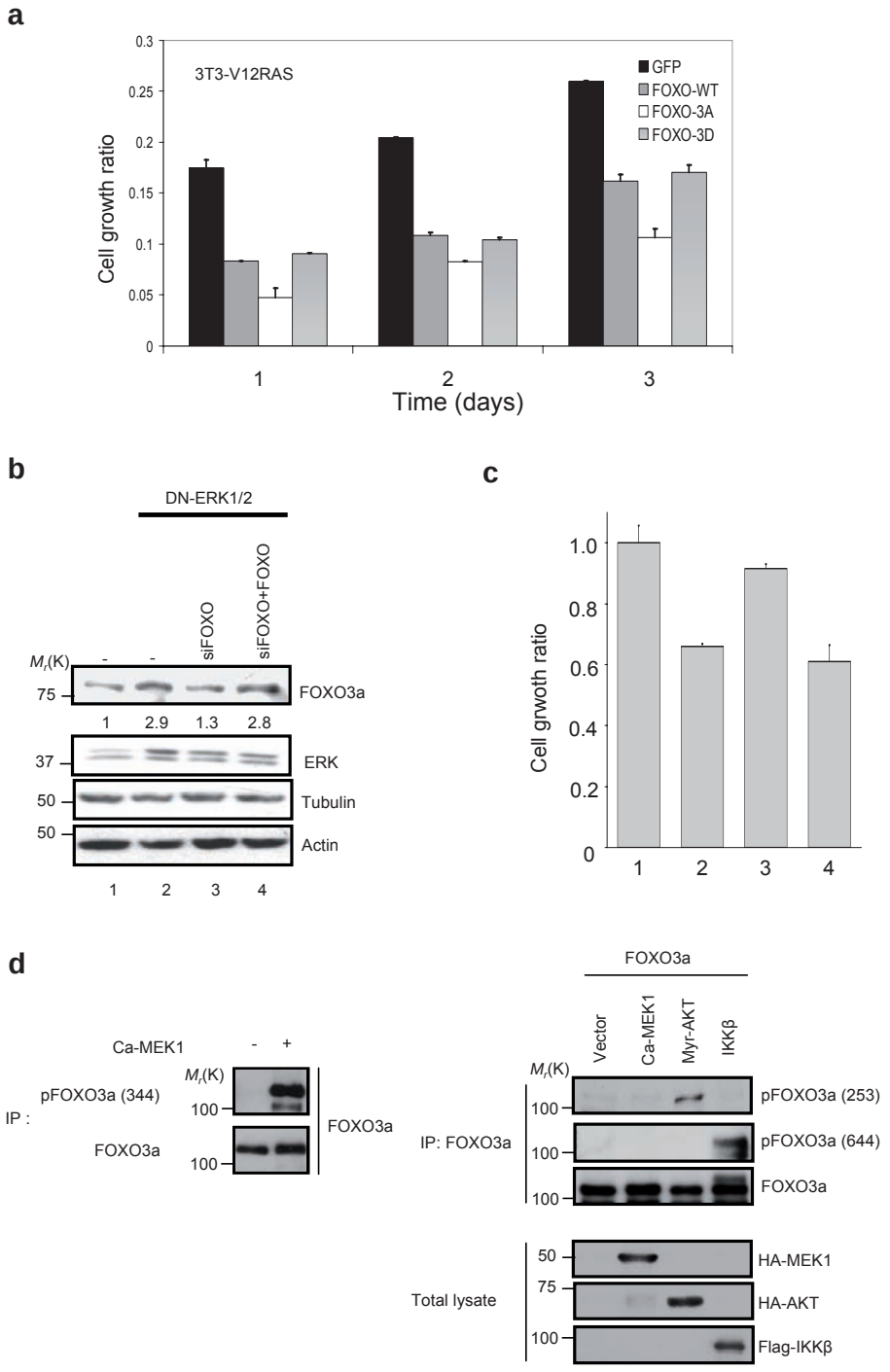


Figure S4 (**a**) FOXO3a-3A mutant, but not FOXO3a-3D, inhibited cell growth. NIH-V12Ras cells were transfected with control vector, wild-type (WT) FOXO3a, and FOXO3a-3A and FOXO3a-3D mutants. After sorting with GFP marker for positive transfection, GFP-positive cells were subjected to MTT assay. Graph shows the mean value of the representative results from three experiments (n=3) conducted in duplicates for each (s.d.) (**b**) (**c**) Forced expressing FOXO3a along with FOXO3a siRNA restores FOXO3a protein level as well as inhibits cell growth. MDA-MB-435 cells were transfected with control vector (lane 1), DN-ERK1 and DN-ERK2 (lane 2), DN-ERK1, DN-ERK2 and pSuper-FOXO3a siRNA (lane 3), DN-ERK1, DN-ERK2 and pSuper-FOXO3a siRNA and FOXO3a (lane 4), and were subjected to (**b**) Western blot and (**c**) MTT assay. MTT assay (**c**) was performed using the same batch of transfected cells as (**b**) and labeled the same as shown in (**b**) Lane1 to lane 4. Graph shows the mean value of the representative results with s.d. from three experiments (n=3) conducted in duplicates for each. (**d**) Lysates of 293T cells co-transfected FOXO3a with CA-MEK1, Myr-AKT or IKKβ were immunoprecipitated with FOXO3a antibody and immunoblotted with the p-FOXO3a-S253, p-FOXO3a-S644 and total FOXO3a antibodies. Total lysates were separately by immnoblotted with indicated antibodies.

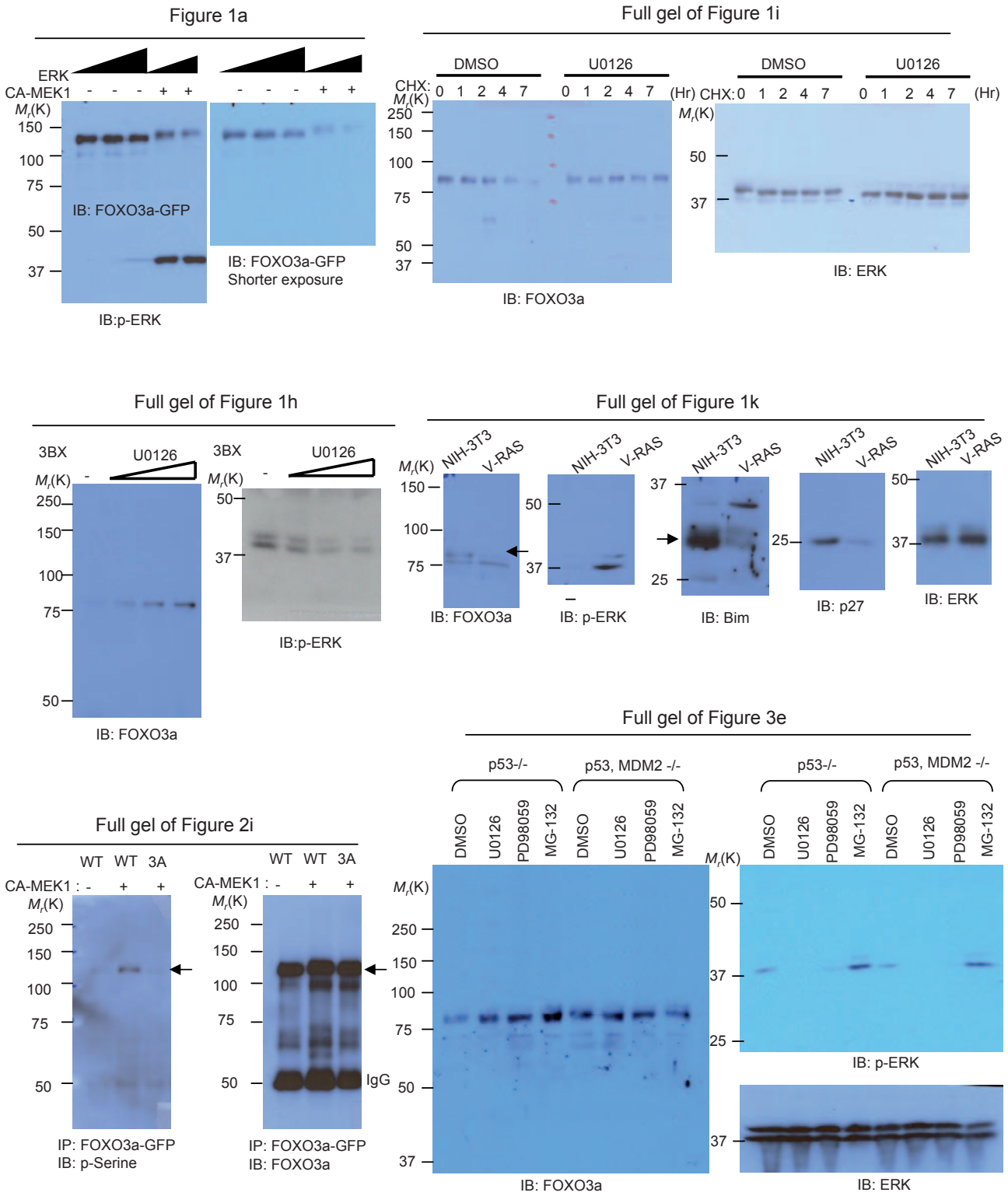
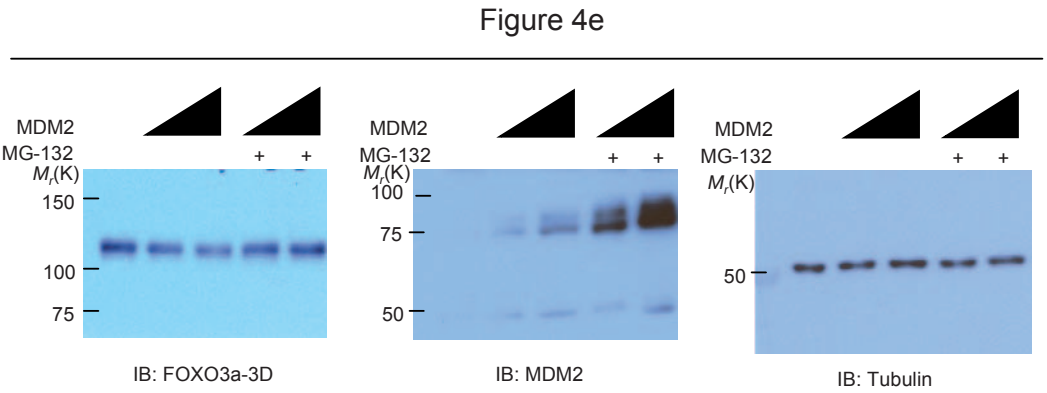
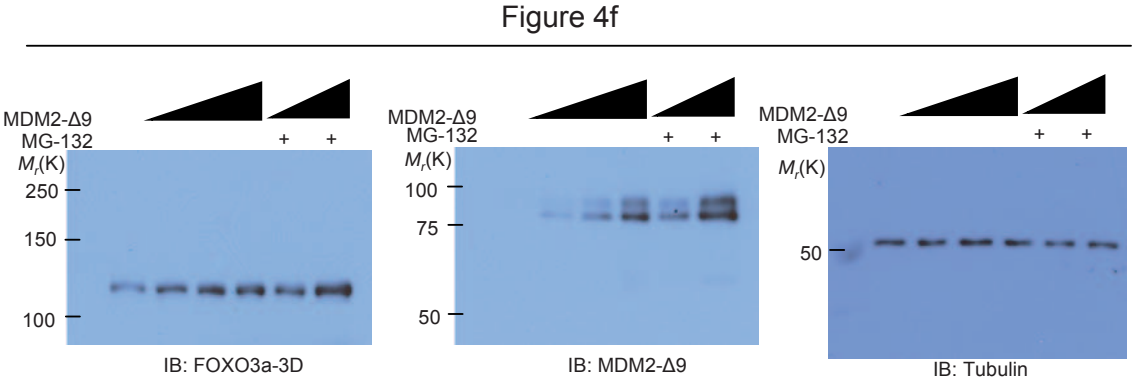


Figure S5 The full panels of primary gels from figures indicated.





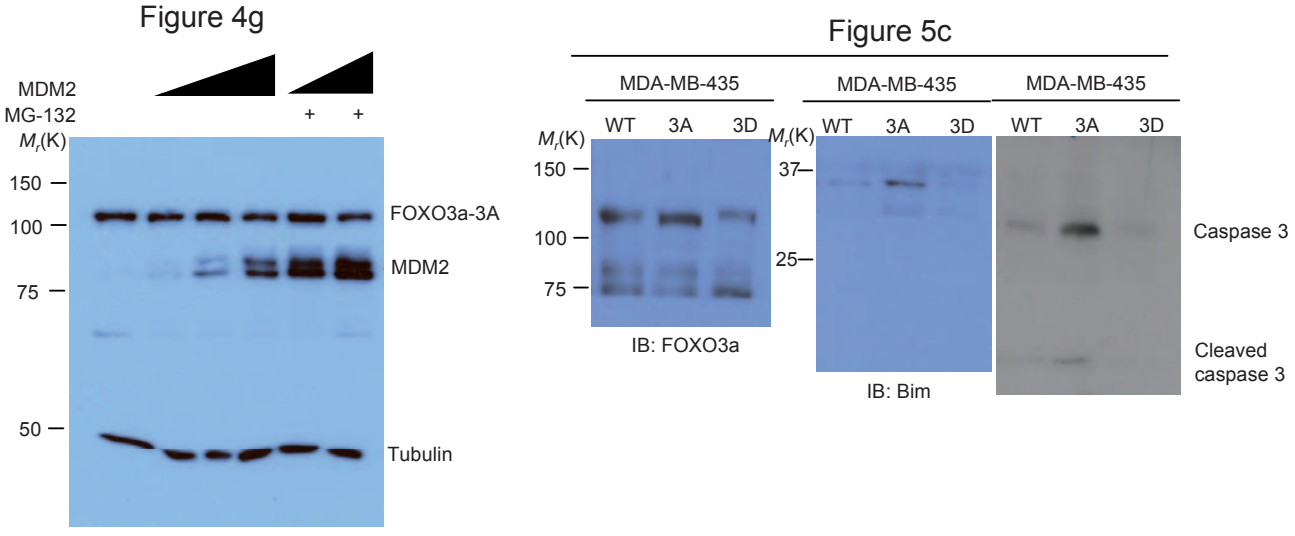


Figure S6. The full panels of primary gels from figures indicated.