SUPPLEMENTARY INFORMATION

Supplementary Discussion

The rapamycin-sensitive mTOR complex 1 (mTORC1) induces protein synthesis[1] and decreased TOR signaling mediates the effects of calorie restriction on lifespan extension in several model organisms[2-5]. It is tempting to speculate that decreased mTORC1 activation due to DR-mediated suppression of PI3K may contribute to the effects of DR in mammals. Conditions that increase the AMP/ATP ratio, such as dietary restriction[6,7], activate AMPK, allowing it to directly phosphorylate and activate FOXO3a, without affecting its cellular localization[8]. Through such a mechanism AMPK may amplify the sensitivity to DR of tumours lacking activated PI3K. SIRT1 is a mammalian homologue of Sir2, an NAD-dependent deacetylase that is required for extension of lifespan by calorie restriction in yeast, worms, and flies[9] and can function as either a tumour suppressor[10] or an oncogene[9]. SIRT1 is linked to the PI3K pathway as it can deacetylate the FOXO factors, leading to an inhibition of their pro-apoptotic function[11,12] and a potentiation of their capacity to promote cell cycle arrest[11].

References

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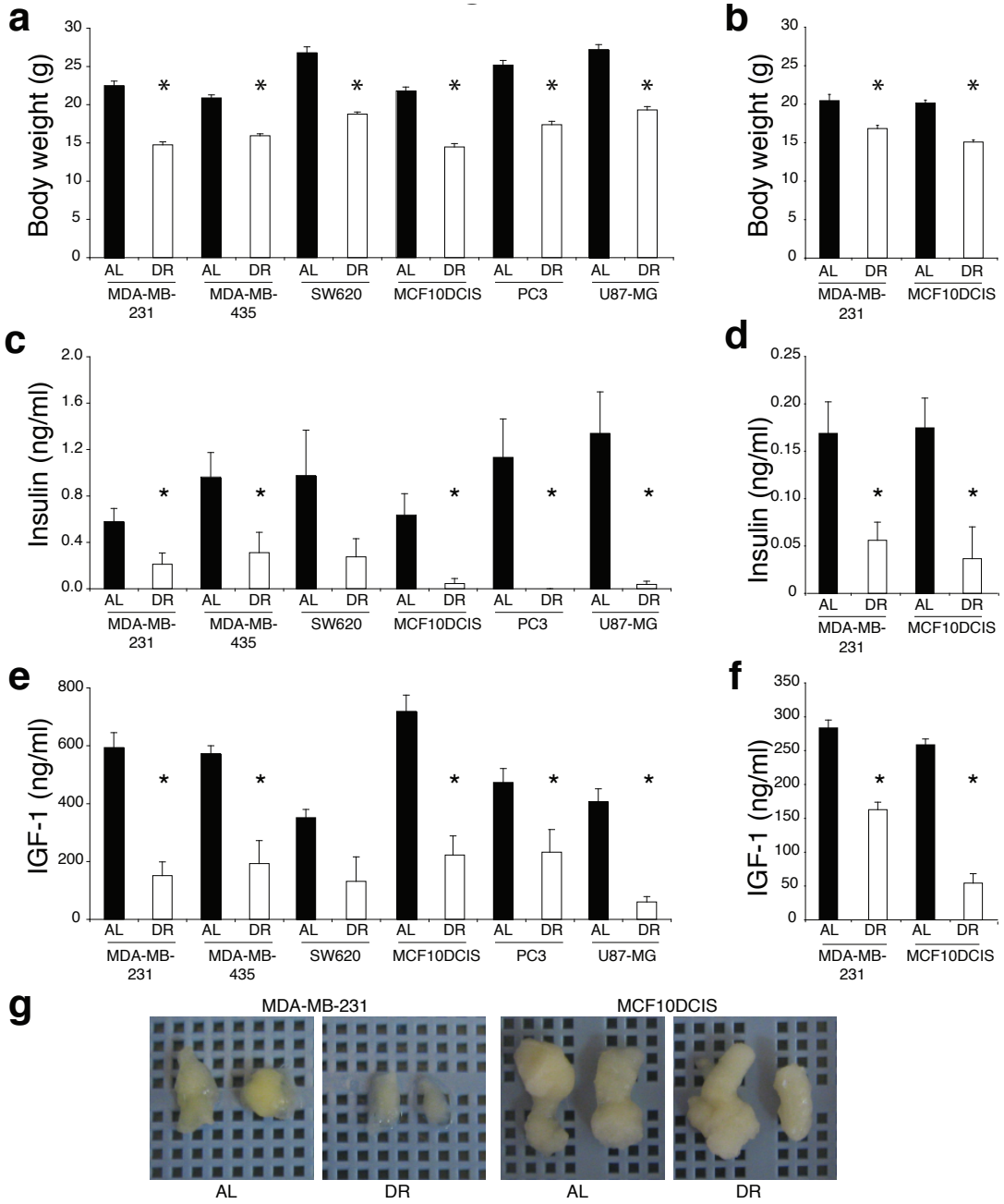
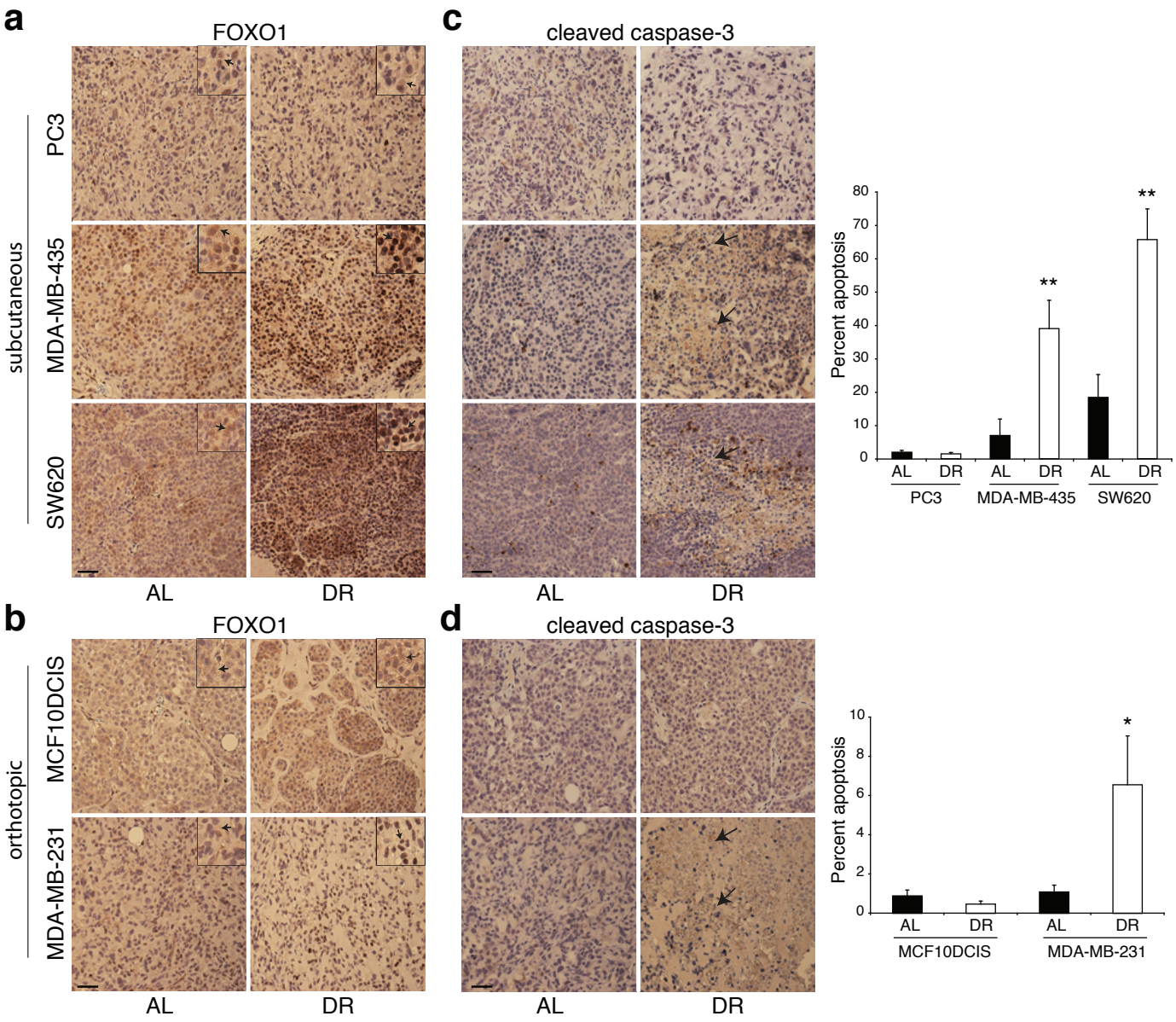
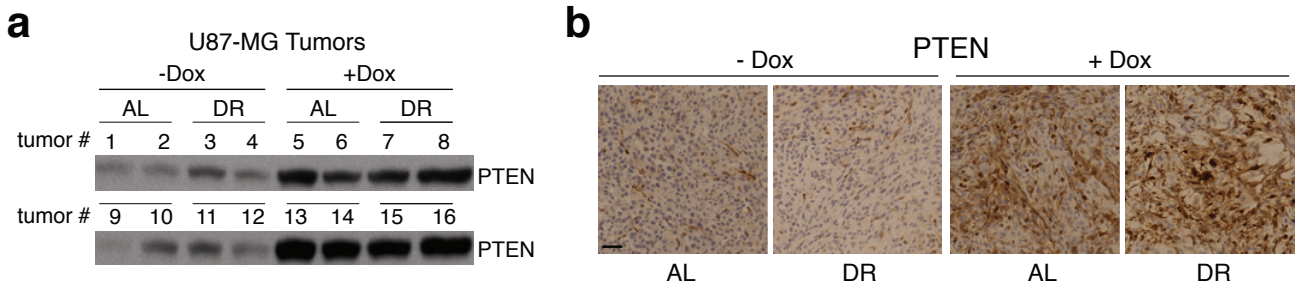


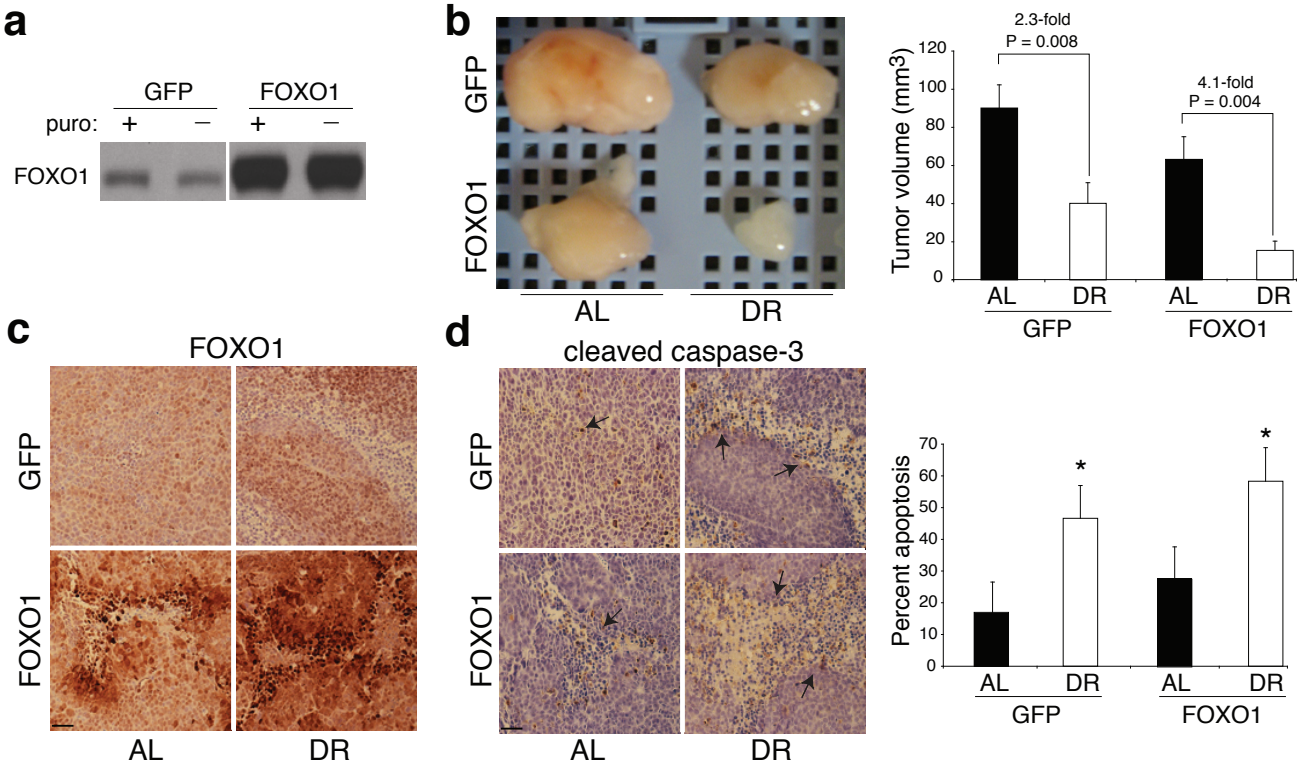
Supplementary Figure S1 | Body weights and plasma insulin and IGF-1 levels of mice with xenografted tumours. Body weights of ad-libitum-fed (AL) and dietary restricted (DR) mice bearing subcutaneous (**a**) or orthotopic (**b**) xenografts of different human cancer cell lines at the end of the dietary restriction studies. All DR groups had similar body weights to their AL counterparts at the beginning of the experiments. **c-f**, Plasma insulin (**c, d**) and IGF-1 (**e, f**) levels in AL or DR xenograft mice bearing subcutaneous (**c, e**) or orthotopic (**d, f**) human tumours that are sensitive (MDA-MB-231, MDA-MB-435 and SW620) or insensitive (MCF10DCIS, PC3 and U87-MG) to DR. Values are means ± s.e.m. * indicates $P < 0.05$. **g**, Images of representative subcutaneous xenografts of the DR-sensitive MDA-MB-231 and DR-resistant MCF10DCIS mammary tumours from AL or DR mice.



Supplementary Figure S2 | FOXO1 nuclear localization and increased apoptosis in tumours in response to DR. **a-d**, Immunohistochemical analyses of FOXO1 (**a, b**), and cleaved caspase-3 (**c, d**), in subcutaneous PC3, MDA-MB-435, and SW620 tumours and orthotopic MCF10DCIS and MDA-MB-231 tumours. Graphs to right of **c** and **d** indicate percent of total cells that are positive for cleaved caspase-3 (mean ± s.e.m. measured in 9 images (1000 nuclei counted per image) from 3 different tumours per group). All images were captured at the same magnification and scale bar = 20 μm. Framed inserts in **a** and **b** are 3.3-fold magnifications of representative areas of the corresponding larger image. Arrows point to immuno-reactivity corresponding to FOXO1 (**a, b**) or cleaved caspase-3 (**c, d**). * indicates P = 0.05 and ** indicates $P \leq 0.01$.



Supplementary Figure S3 | Doxycycline-induced expression of PTEN in tumour xenografts in vivo. a, PTEN expression in lysates (30 mg) of U87-MG tumours from AL and DR mice treated with or without Dox. **b**, Immunohistochemical analysis of PTEN expression in tumours from **a**. All images were captured at the same magnification and scale bar = 20 μm.



Supplementary Figure S4 | Effects of increasing FOXO1 expression on the response of tumours to DR. **a**, FOXO1 expression levels in SW620 cells overexpressing FOXO1 or GFP in the presence or absence of puromycin, the agent used to select cells. **b**, Representative images (left) and volumes (right) of SW620 tumours overexpressing FOXO1 or GFP and harvested from AL or DR mice (n=8). **c**, **d**, Immunohistochemical analysis of FOXO1 (c) and cleaved caspase-3 (d) in tumours from **b**. Arrows point to immunoreactivity for cleaved caspase-3 in **d**. Graph to right of images indicates percent of total cells that are positive for cleaved caspase-3 (mean ± s.e.m, measured in 9 images (1000 nuclei counted per image) from 3 different tumours per group). * indicates $P \leq 0.05$;. All images were acquired at the same magnification and scale bar = 20 μm.