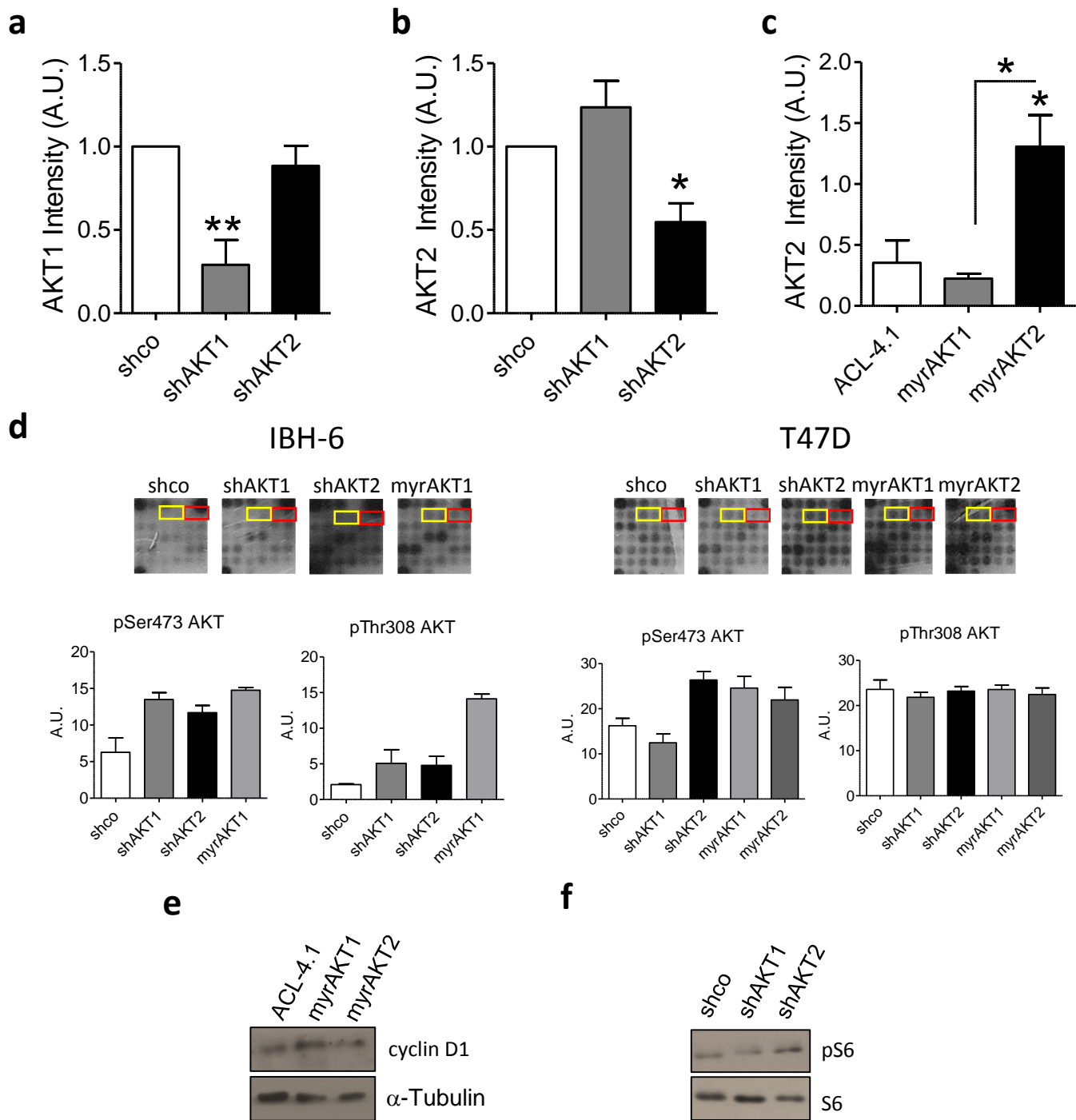


# **AKT1 and AKT2 isoforms play distinct roles during breast cancer progression through the regulation of specific downstream proteins**

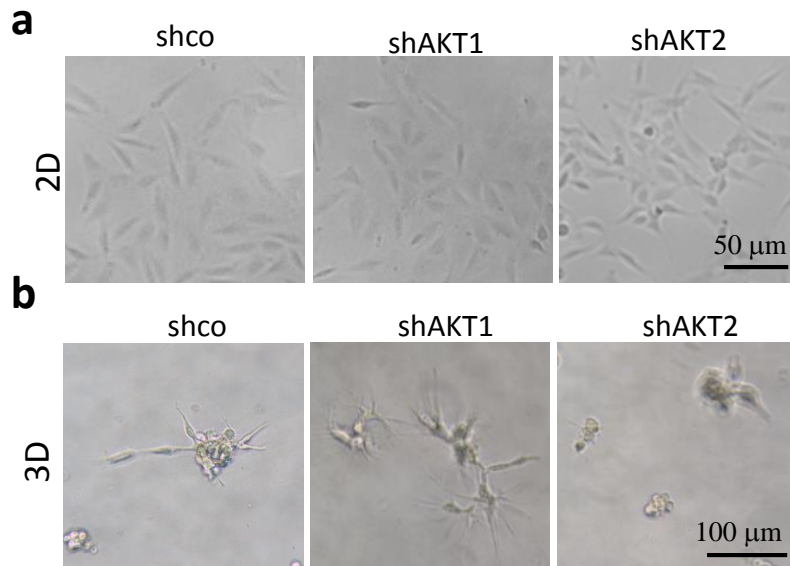
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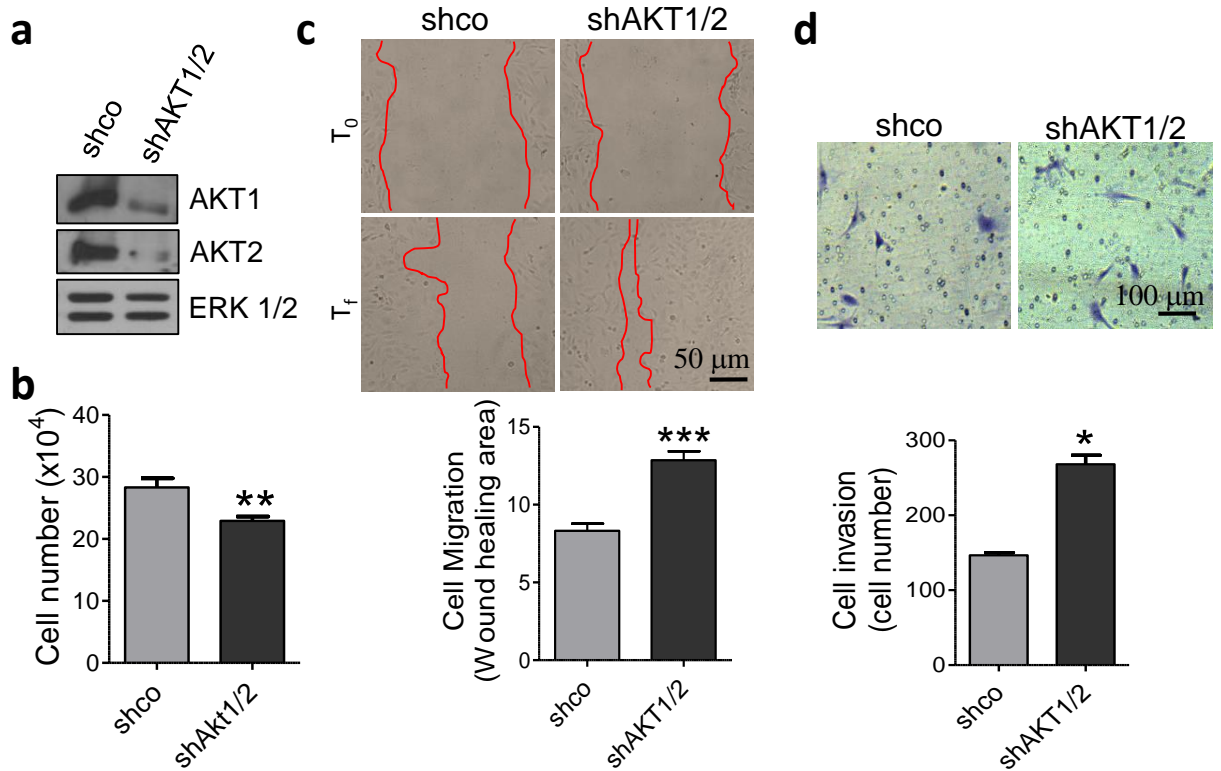
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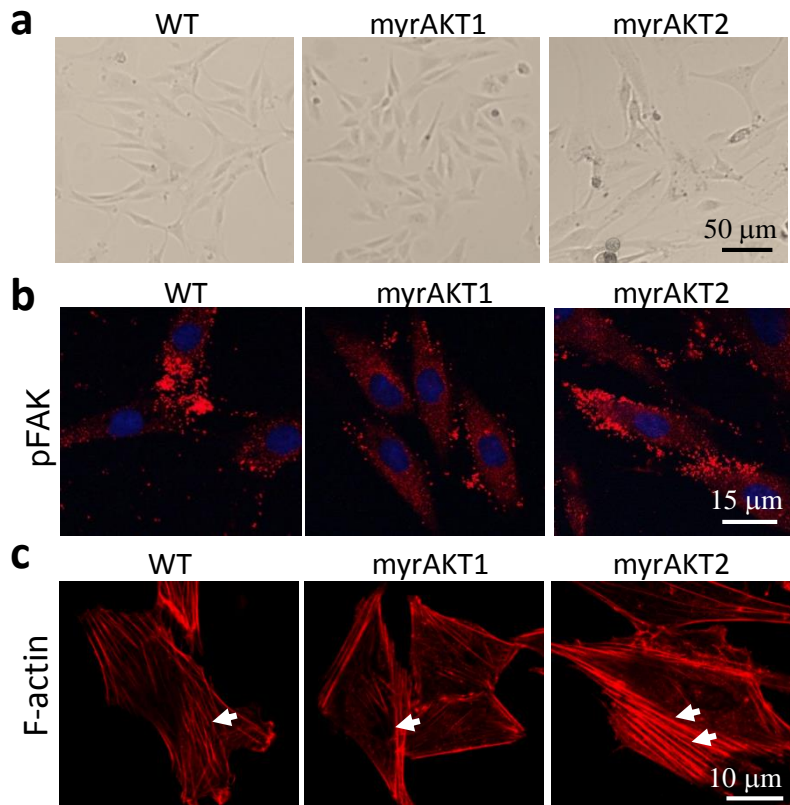
**Supplementary Figure S1: AKT modulation in IBH-6 and T47D cell lines.** WB quantification showing (a) AKT1 intensity and (b) AKT2 intensity in AKT-deficient IBH-6 cells. (c) AKT2 intensity in AKT-overexpressing IBH-6 cells. AKT1 intensity was not quantified in this cells because the myrAKT1 construction has a different molecular weight. (d, upper) PathScan Intracellular Signaling Array Kit for IBH-6 (left) and T47D (right) cells modified to overexpress or downregulate AKT isoforms. Yellow box indicate pThr308 AKT dots and red box indicate pSer473 AKT dots. (d, lower) pSer473 AKT (left) and pThr308 AKT (right) quantification in each cell line. (e) WB showing cyclin D1 expression in T47D AKT-overexpressing cells. (f) WB showing pS6 and S6 expression in T47D AKT-deficient cells.



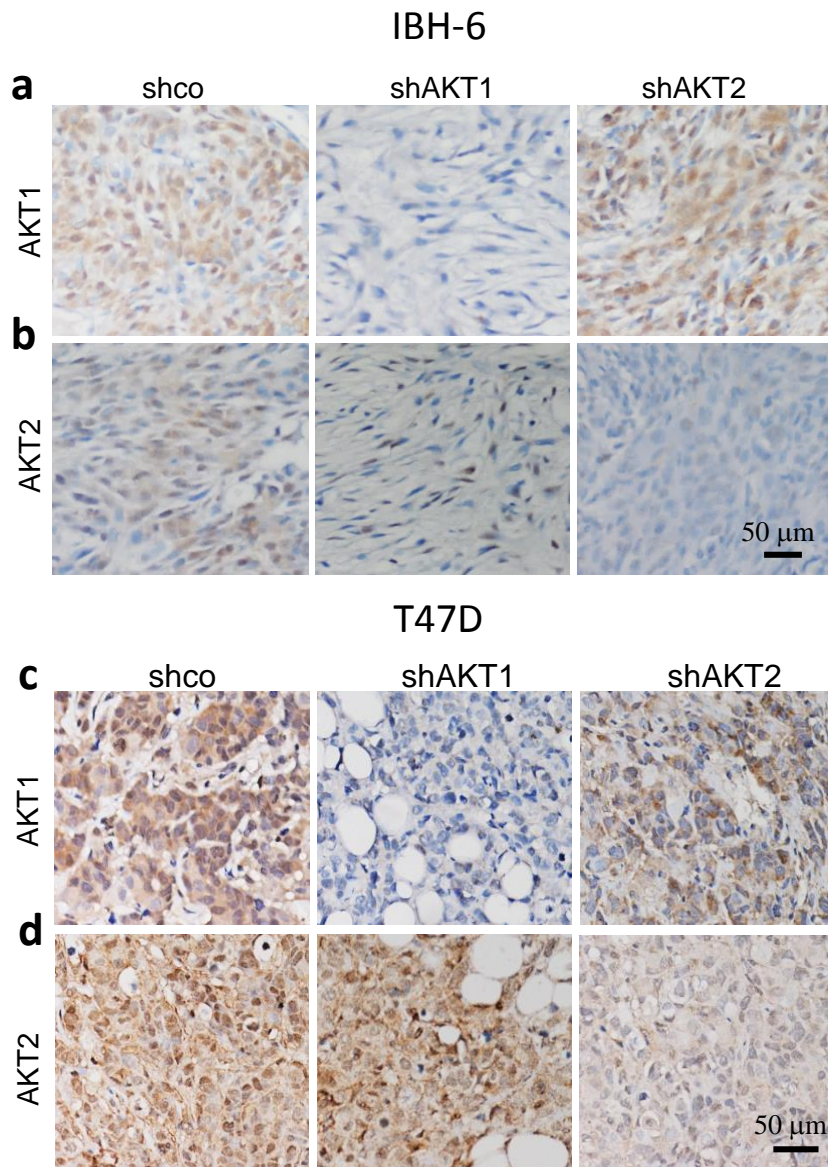
**Supplementary Figure S2: AKT1- and AKT2-regulated cell morphology.** IBH-6 AKT-deficient cells growing on (a) plastic (2D) or (b) tridimensional (3D) Matrigel for 48 hours.



**Supplementary Figure S3: AKT1/AKT2 simultaneous silencing enhances cell migration and invasion.** (a) WB showing simultaneous downregulation of AKT1 and AKT2 isoforms in IBH-6 shAKT1/shAKT2 cells. IBH-6 shAKT1/shAKT2 cells decreased (b) proliferation but increased (c) migration and invasion (d). Representative pictures at  $T_0$  and  $T_f$  of one migration experiment are shown (c, upper). Representative pictures of one transwell invasion assay in shco and shAKT1/2 cells (d, upper). Bar graphs represent the quantification of the migration area (c, bottom) and the average of cells attached on the other side of the insert (d, bottom). \*  $p < 0,05$ ; \*\*  $p < 0,01$ ; \*\*\*  $p < 0,001$ .  $n=3$ .



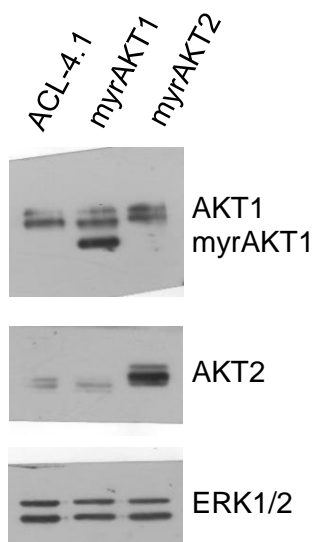
**Supplementary Figure S4: AKT1 and AKT2 overexpression modulate adhesion and cytoskeletal proteins.** (a) Morphology of IBH-6 AKT-overexpressing cells growing on 2D. (b) IF in red showing a decreased pTyr861FAK (pFAK) staining in myrAKT1 cells and (c) an increase in F-actin fibers polymerization (indicated with white arrows) in myrAKT2 cells stained with phalloidin. In (b) nuclei were counterstained in blue with DAPI.



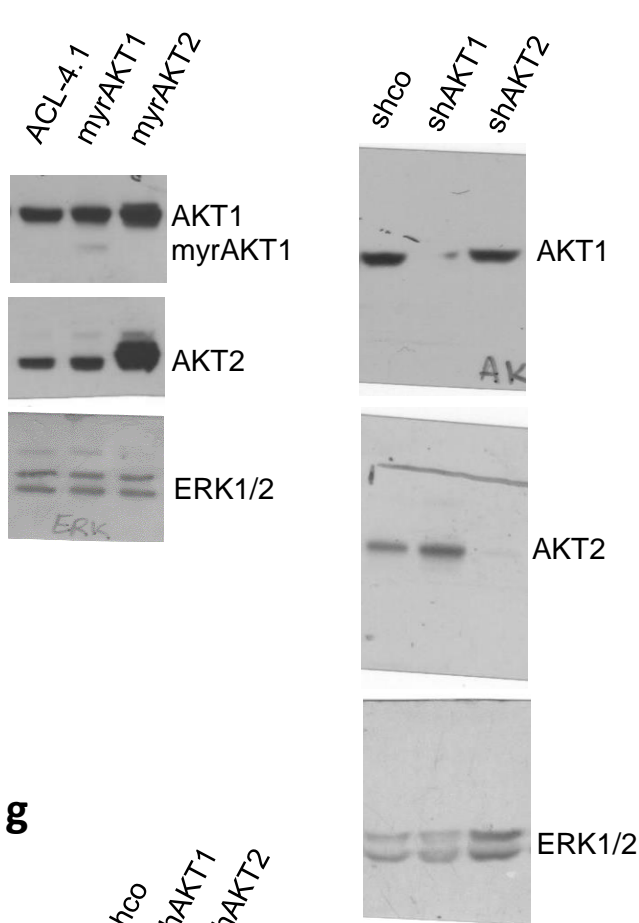
**Supplementary Figure S5: AKT modulation in IBH-6 and T47D xenografts.** IHC for (a and c) AKT1 and (b and d) AKT2 to confirm the specific silencing of each isoform in IBH-6 and T47D xenografts, respectively.

**a**

IBH-6

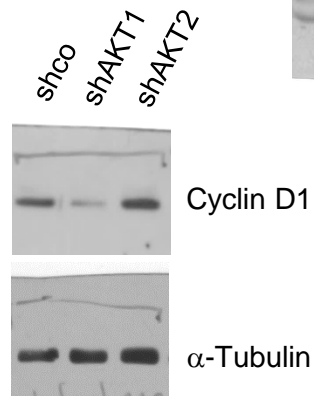
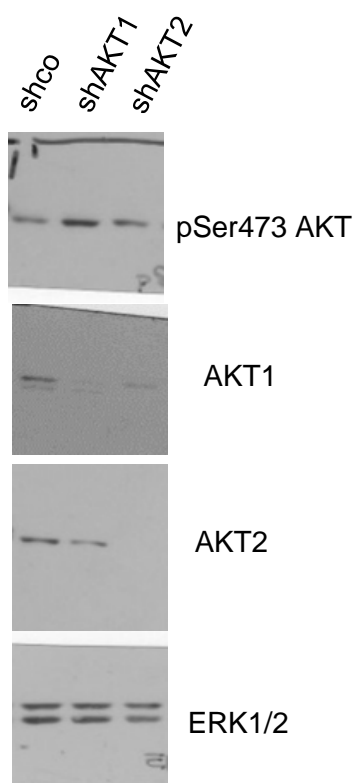
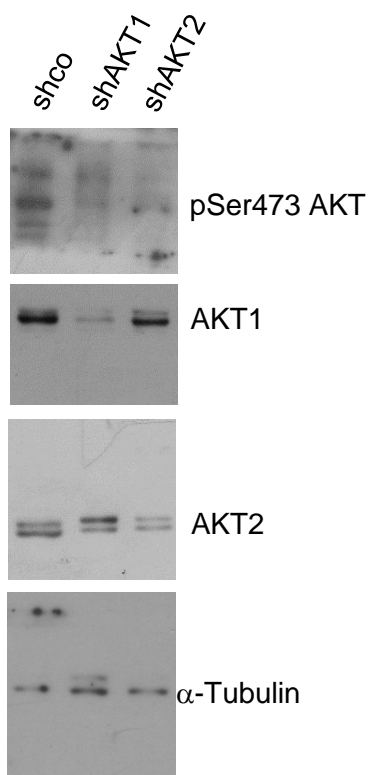
**b**

T47D

**c**

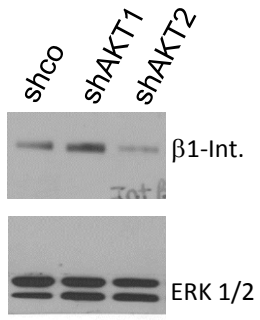
IBH-6

T47D

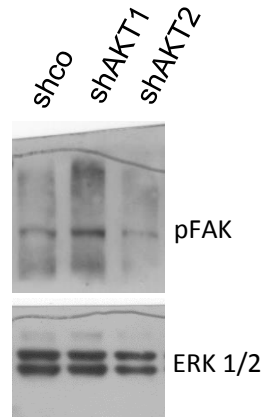
**d**

**Supplementary Figure S6:** Full-length gels from Figure 1.

**d**



**f**



**Supplementary Figure S7:** Full-length gels from Figure 2.