SUPPLEMENTARY FIGURE LEGENDS:

Supplementary Figure 1. Western blot analysis of protein extracts from different human breast cancer cell lines.

Supplementary Figure 2. Xenograft tumors display solid, poorly differentiated morphology. (A) H&E stained sections from xenograft tumors. Morphology between the tumors generated from different cell lines was not appreciably different. (B) Tumors generated from the ARRDC3 over-expressing line (ARRDC3) showed a decrease in the number of Ki67 positive cells compared the control (Flag). Tumors from the cell lines with repressed ARRDC3 (shARRDC3-A and shARRDC3-C) had an increase in Ki67 positive cells when compared to the control (shCtl). (C) TUNEL staining of tumor samples. Altered ARRDC3 expression did not affect the number of apoptotic cells in the xenograft tumors.

Supplementary Figure 3. The regulation of ITGβ4 by ARRDC3 is not dependent on the lysosome. (A) Western blot comparing MDA-231 cells treated (+/-ARRDC3 over-expression) with 50μM of the lysosome inhibitor chloroquine for 18 hours or left untreated. Inhibition of the lysosome is demonstrated by the accumulation of LC3B. The ARRDC3-mediated decrease of ITGβ4 is not affected by the inhibition of the lysosome. (B) Immunofluorescence of MDA-MB-231 cells infected with an adenovirus expressing ARRDC3 with and without treatment of 50μM chloroquine after 24 hours demonstrate that lysosomes inhibition does not affect the loss of ITGβ4 in ARRDC3 over-expressing cells.

Supplementary Figure 4. Effects of ARRDC3 on in-vitro tumorigenicity is dependent on ITGβ4 Each line was tested at least in triplicate and data bars represent mean +/- SEM. Single asterisk represents $p<0.05$ whereas a double asterisk represents $p<0.001$ as determined by student's t-test. (A) Growth curves from the stable lines demonstrate that expression of ARRDC3 is inversely

correlated to cellular proliferation in ITGβ4-expressing cells with small effects in parental cells (B) Wound assay of mitomycin-C treated cells shows that over-expression of ARRDC3 leads to a decrease in cell migration whereas RNAi-mediated repression of ARRDC3 increases cell migration of ITGβ4-expressing cells with no effects in parental cells. (C, D) Expression of ARRDC3 is inversely correlated to anchorage independent growth both in colony number and colony size for ITGβ4-expressing cells with small effects in parental cells. Dark gray bars represent total colonies (at least 50μm) whereas light gray bars represent colonies larger than 200μm. (E) Matrigel chemo-invasion assay demonstrate that expression of ARRDC3 is inversely correlated to invasion for ITGβ4-expressing cells with small effects in parental cells.