**A**

Cell number ($\times 10^6$) over days for various cell lines: LNCaP, VCaP, PC3, DU145, C42, and C42b.

**B**

Gene expression and localization:
- **control**: GFP
- **nucARRB1**: GFP, ARR1, NLS
- **wtARRB1**: GFP, ARR1
- **ARRB1Q394L**: GFP, ARR1, NLS

**C**

Relative mRNA expression using primers A+B.

**D**

Relative mRNA expression using primers A+C.

**E**

Immunofluorescence images showing GFP control, nucARRB1, wtARRB1, and endogenous ARR1.

**F**

Western blot analysis showing cytoplasmic and nuclear localization of GFPARRB1 and α-GFP, α-ARRB1, α-tubulin, and α-histone H3.

**G**

Western blot analysis showing cytoplasmic and nuclear localization of endogenous ARR1, α-ARRB1, α-tubulin, and α-histone H3.

**H**

Graphs showing relative fluorescence for SACF, migration, and invasion with controls and ARRB1 shRNA.
**Supplemental Figure S2. Characterization of ARRB1 overexpressing and ARRB1 knock-down (KD) cell-lines used in this study.**

A. Comparison of the growth rates of a panel of prostate cancer cell lines expressing different levels of ARRB1 (see Figure 2A).

B. GFP control, nucARRB1, wtARRB1 and Q394LARRB1 constructs used in the generation of stable cell-lines in this study. The primers (A, B and C) locations used for measuring the expression levels of the various constructs are highlight on the constructs schematics. NLS=nuclear localization signal. Q394LARRB1 mutant construct was previously characterized\textsuperscript{32,33}.

C-D. Expression levels of exogenous ARRB1 constructs (wtARRB1 and nucARRB1) vs control (GFP) and endogenous (scramble control) vs ARRB1 knockdown (shRNA1 and 2) as determined by qRT-PCR using the primer pairs highlighted in B.

E. Confocal sections showing GFP fluorescence in GFP control and GFP-tagged ARRB1 expressing cells (left) and endogenous ARRB1 in parental C4-2s (right, primary=A1CT anti-ARRB, secondary=A488-conjugated anti-rabbit). WIARRB1 localises to the membrane, cytoplasm and nucleus, whereas nucARRB1 is solely nuclear with a pattern very similar to that of the nuclear fraction of the endogenous protein. A488 and GFP=green, DAPI=blue.

F. Immunoblot of the cytoplasmic/nuclear fractions in endogenous and stable ARRB1-overexpressing cell-lines. Tubulin and Histone H3 were used as markers for cytoplasmic and nuclear fractions, respectively.

G. Immunoblot of cytoplasmic/nuclear fractions in control and two different ARRB1 shRNA cell-lines. Tubulin and Histone H3 were used as markers for cytoplasmic and nuclear fractions, respectively.

H. SACF (Soft Agar Colony Formation, i.e. anchorage-independent growth), migration and invasion of nucARRB1 vs control of scramble shRNA vs ARRB1 shRNA. n=6, values are mean ± s.e.m., *=p-value<0.05, **=p-value<0.01.