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Supplemental information

The RNA binding proteins LARP4A and LARP4B promote sarcoma and carcinoma growth and metastasis

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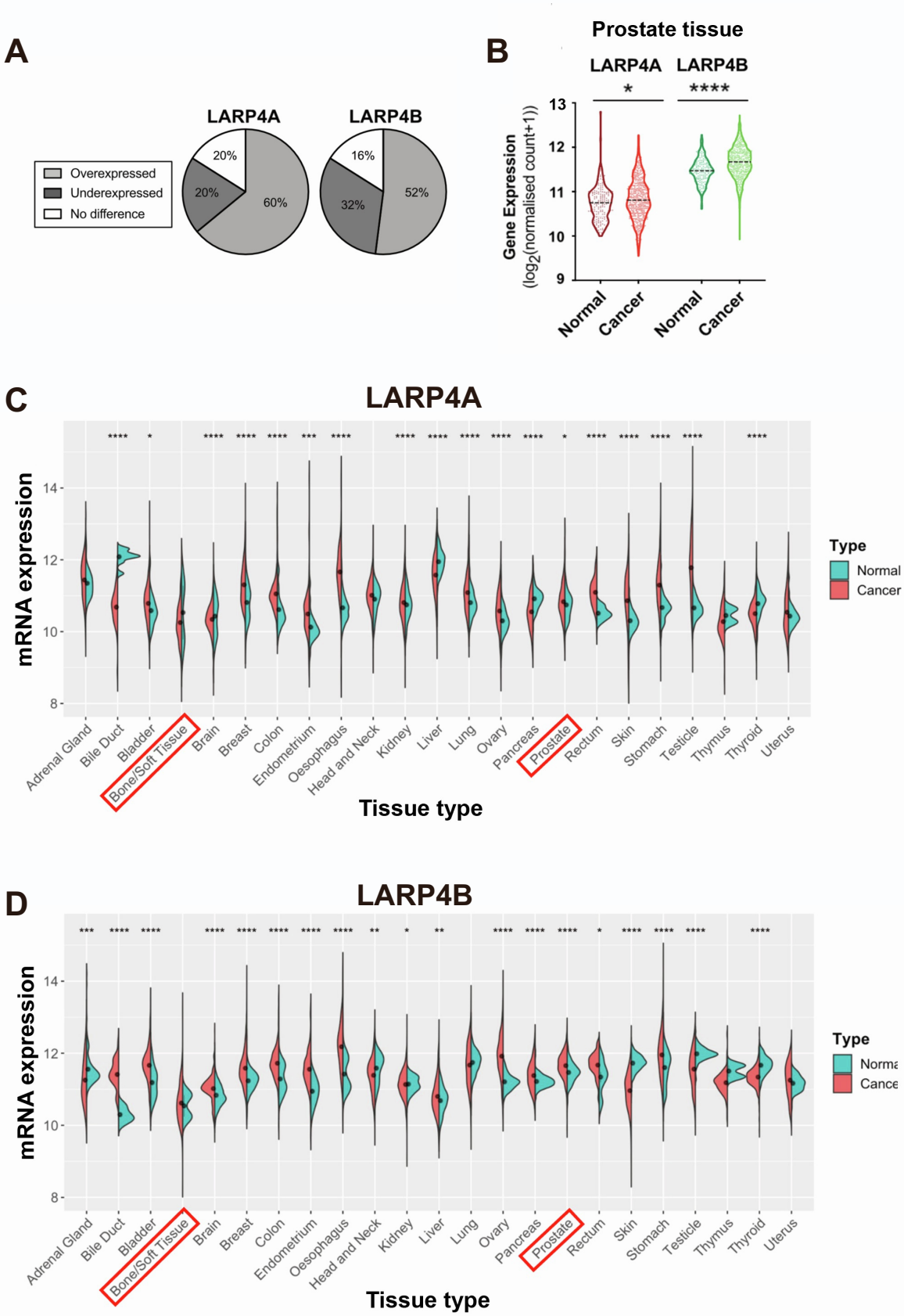

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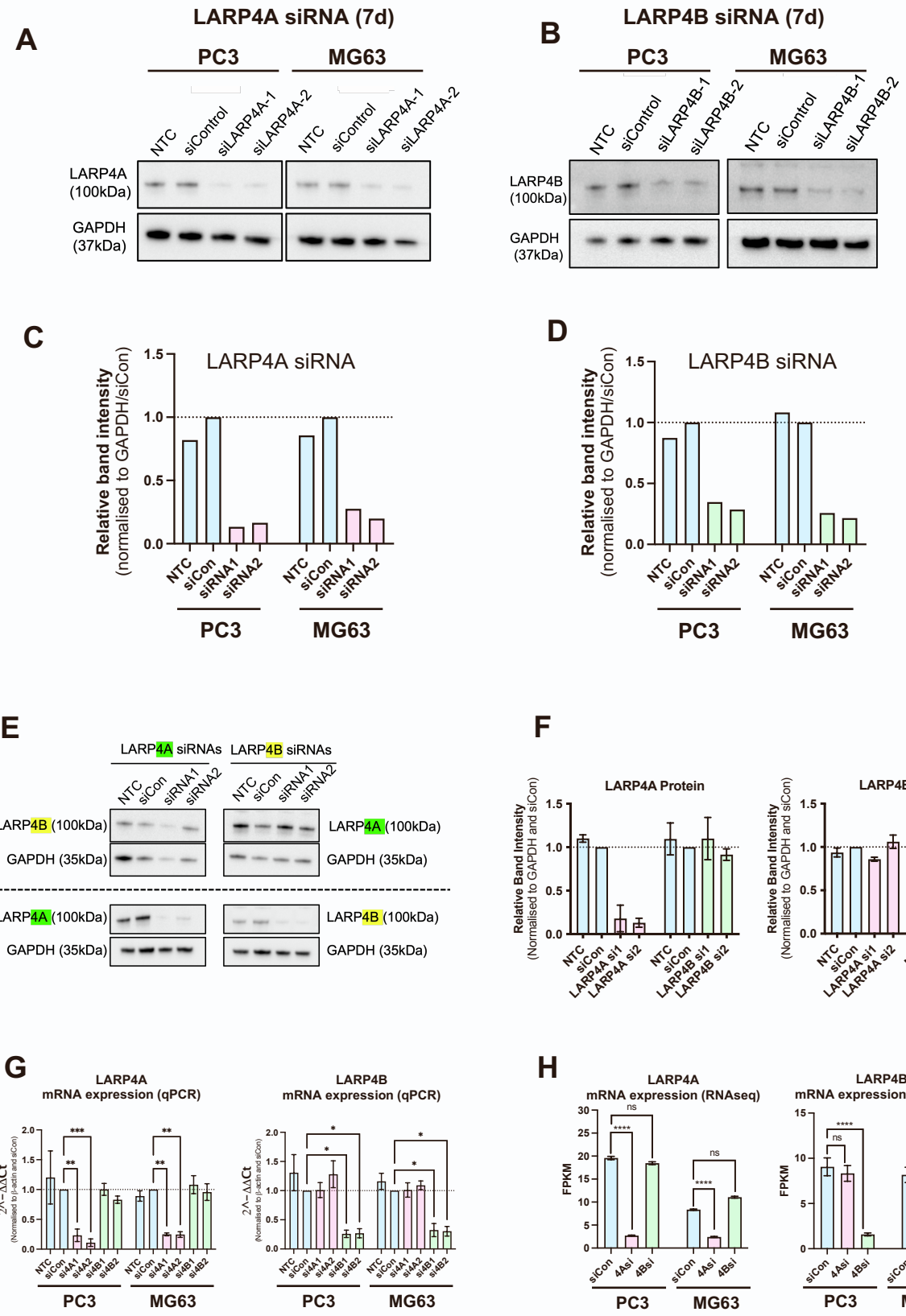

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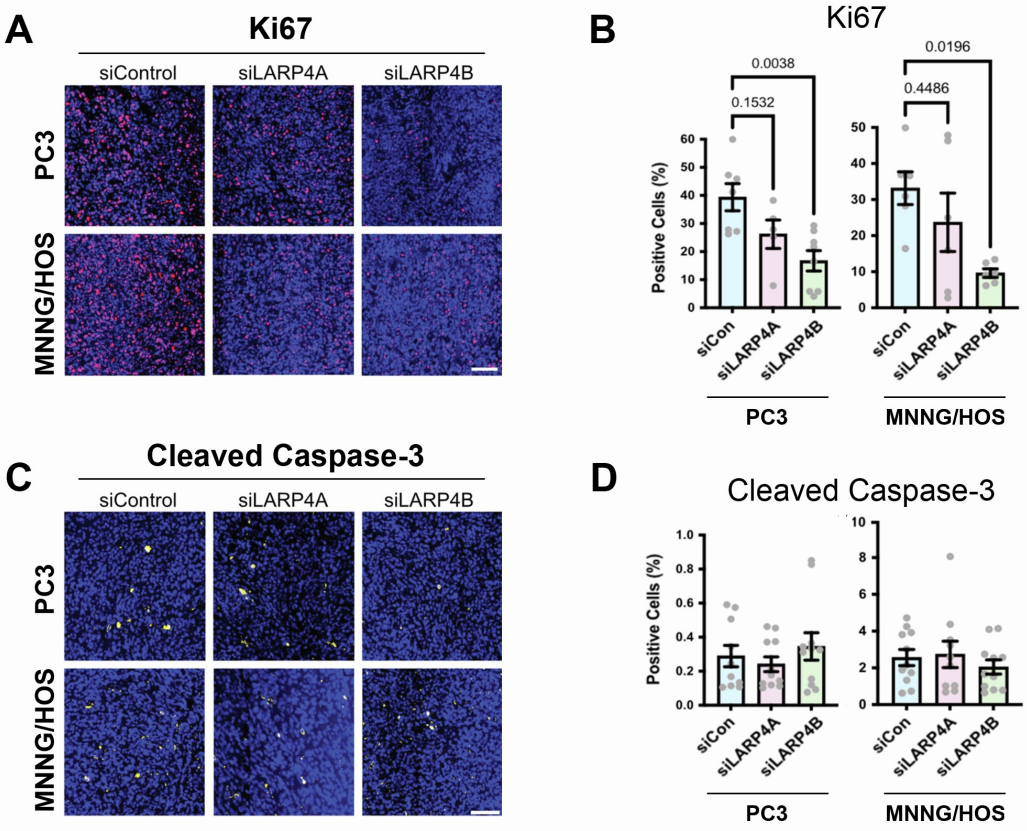

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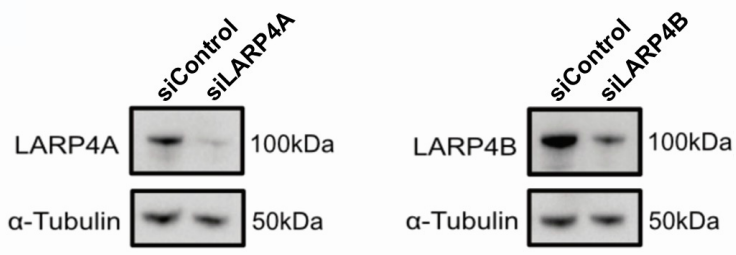

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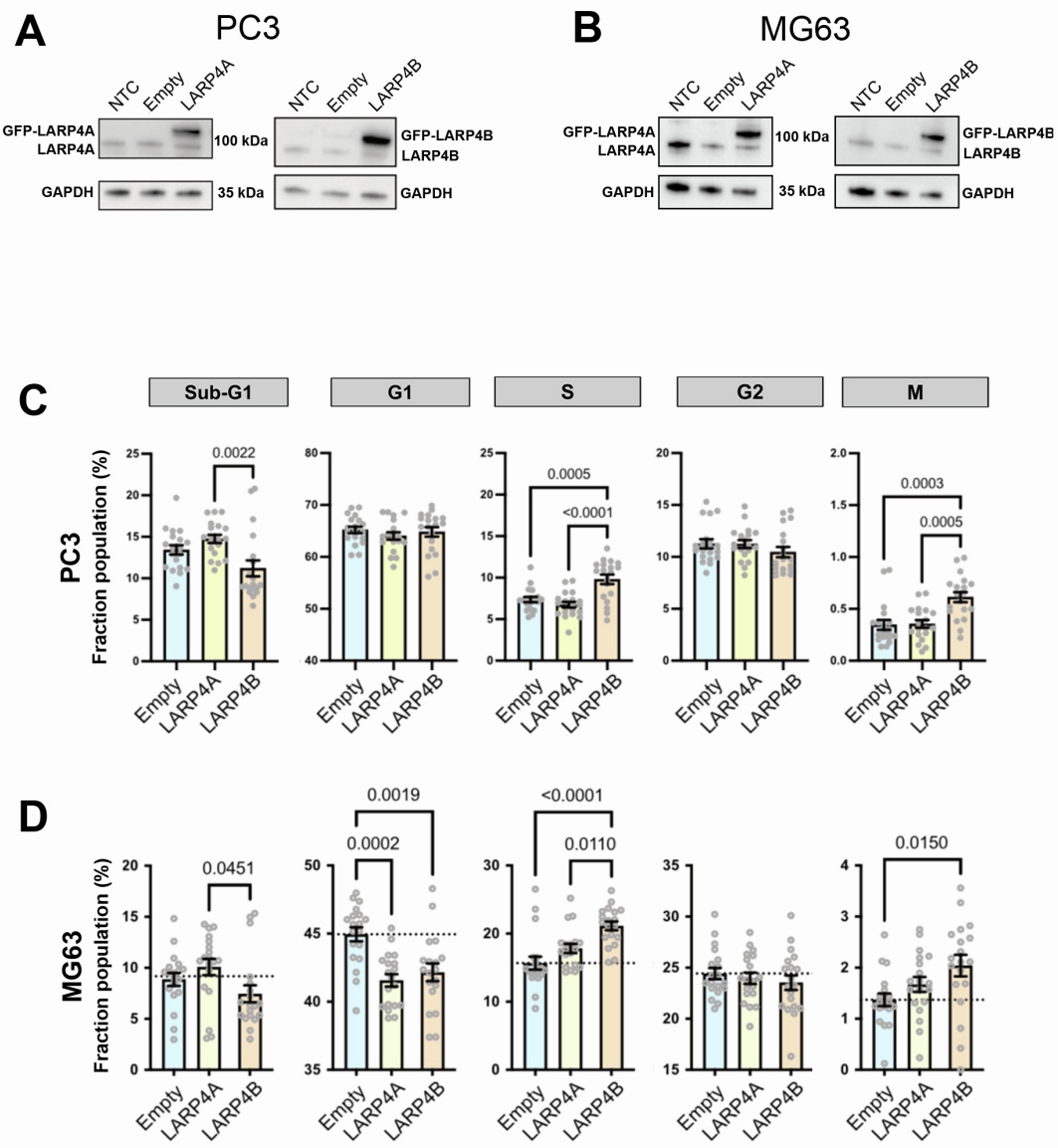

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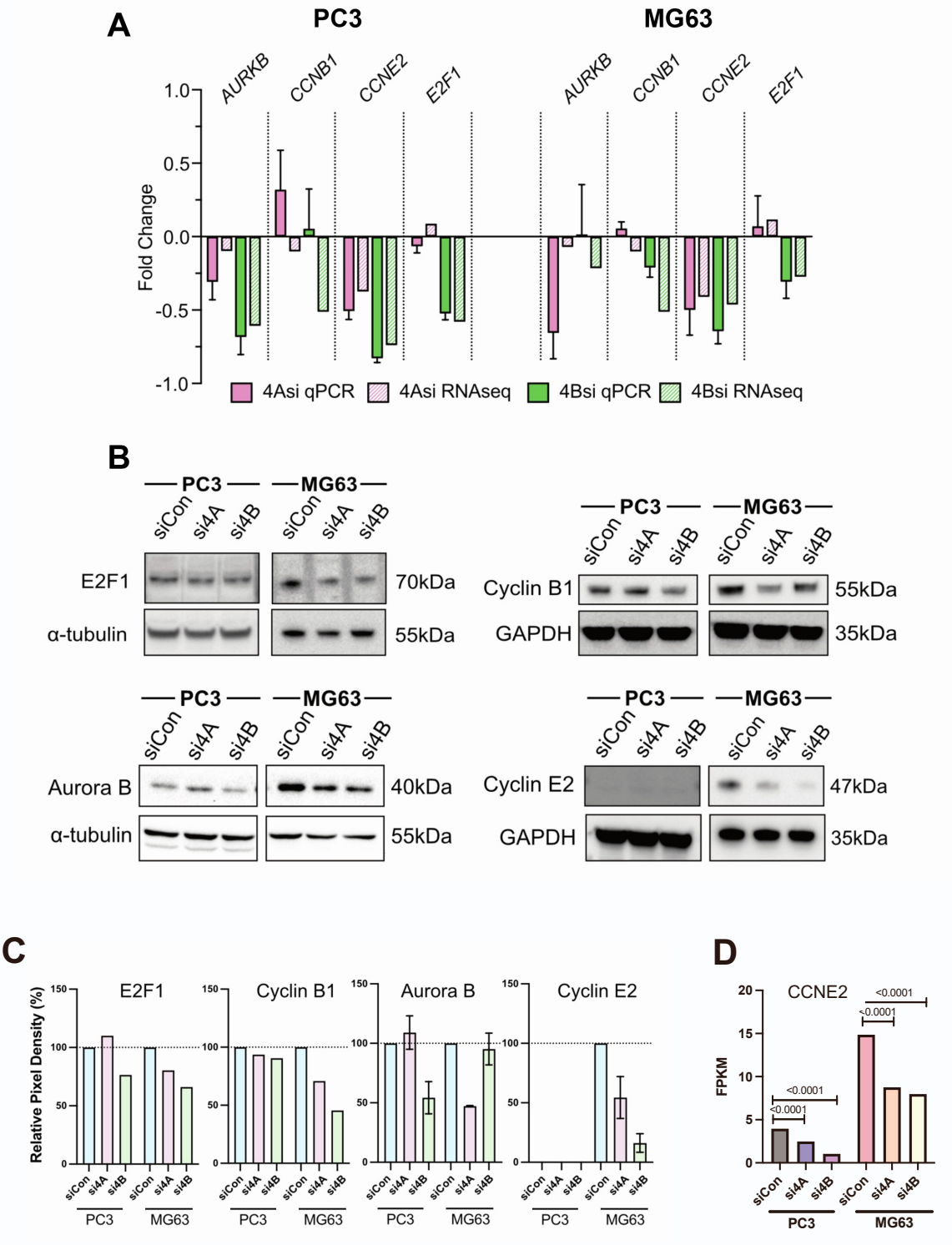

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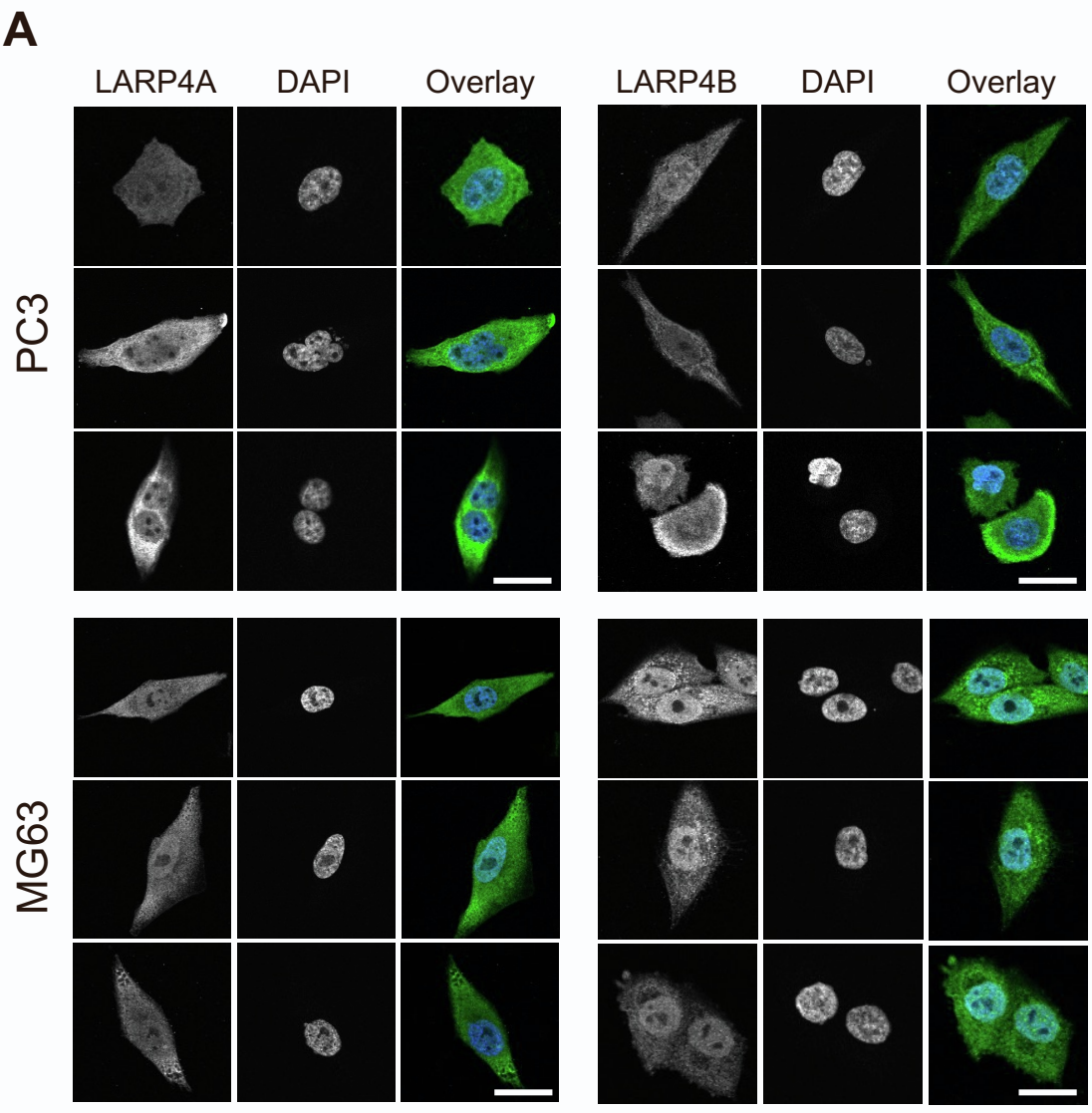

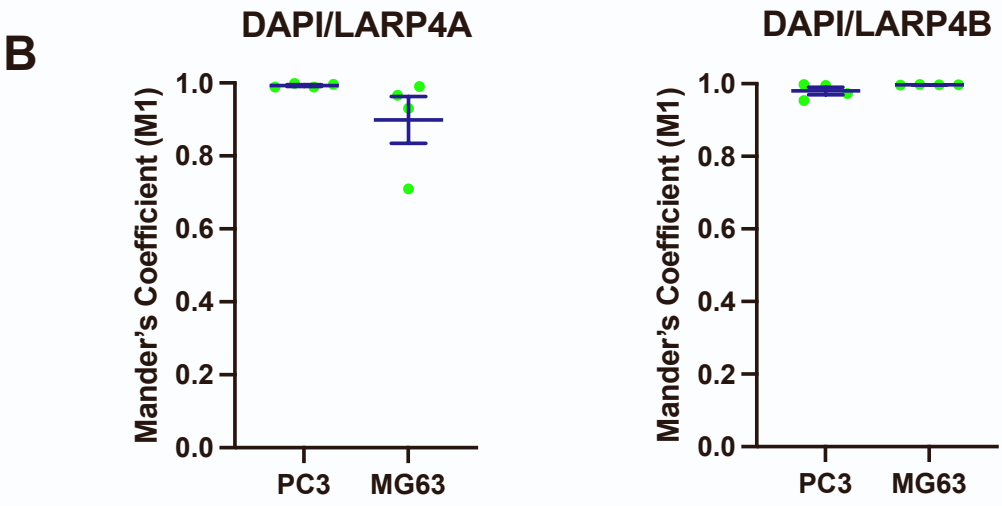

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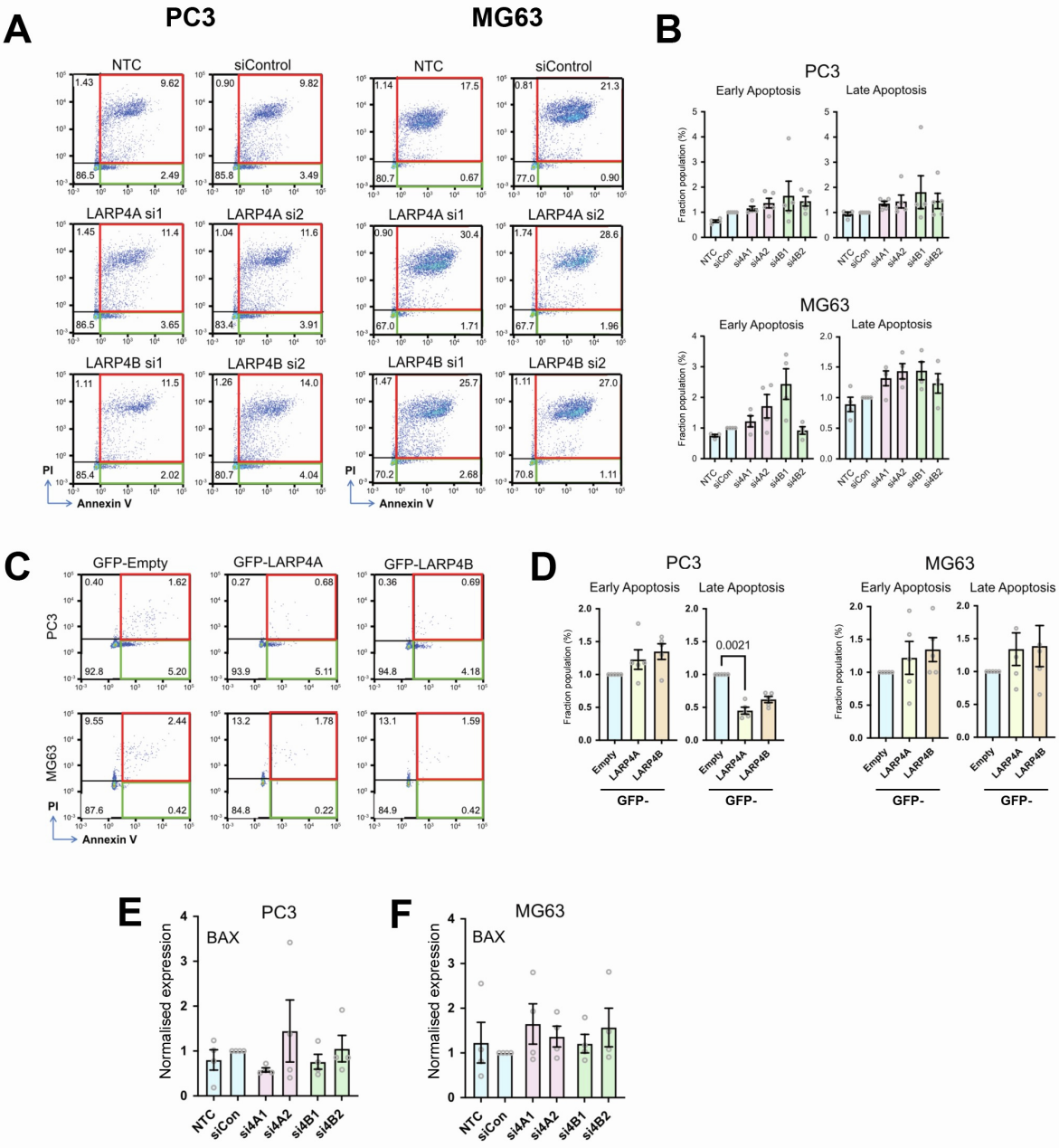

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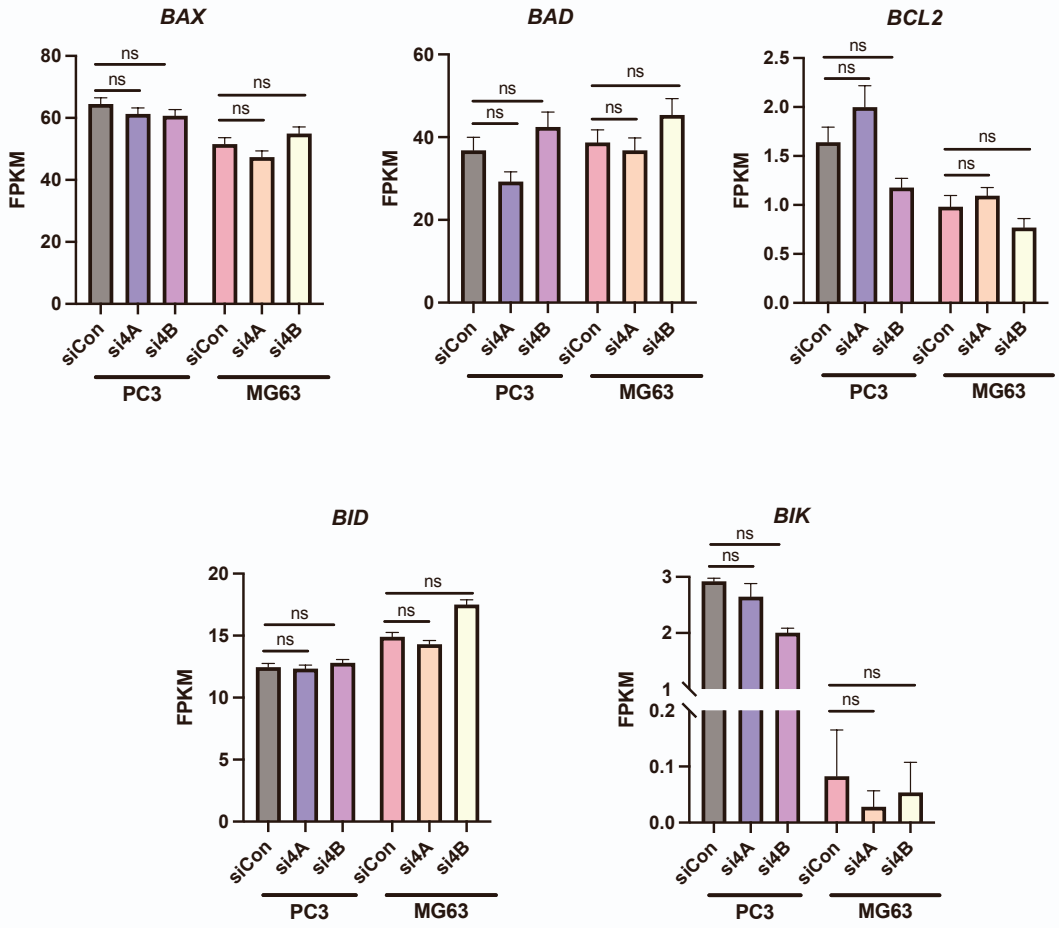

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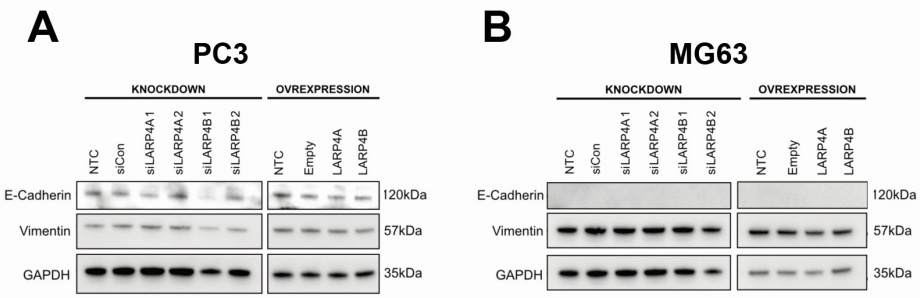

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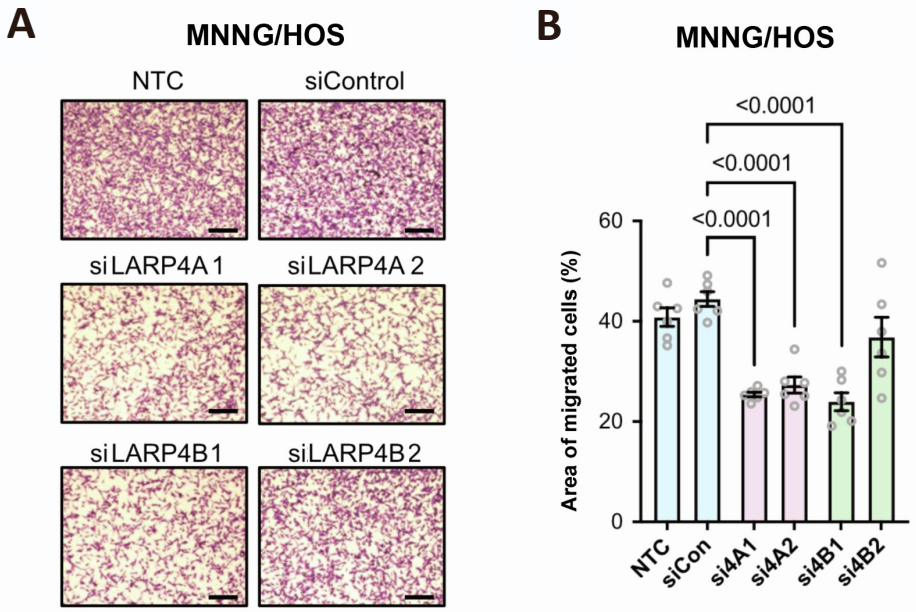

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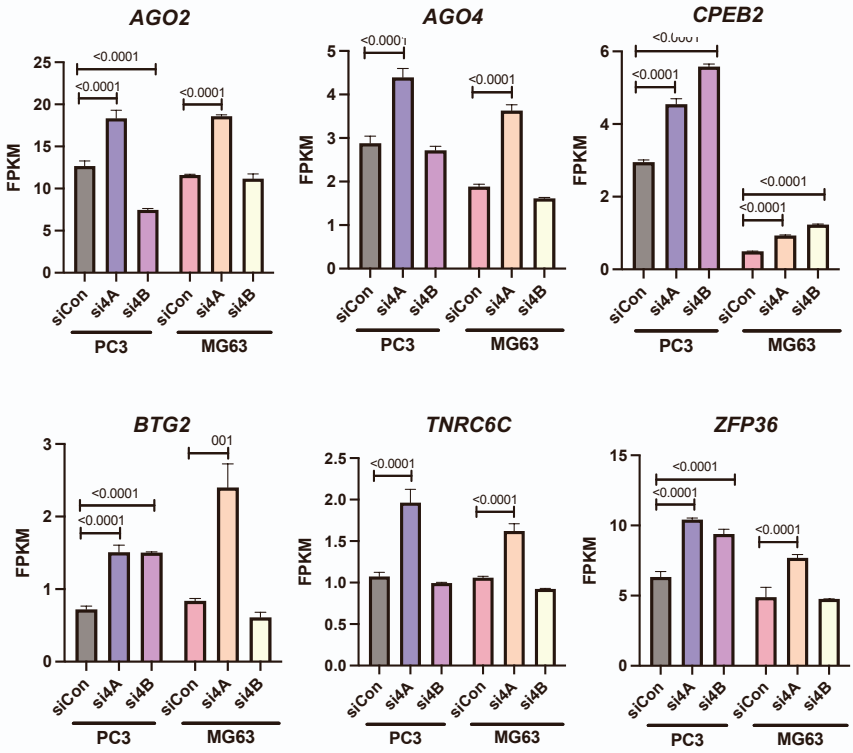

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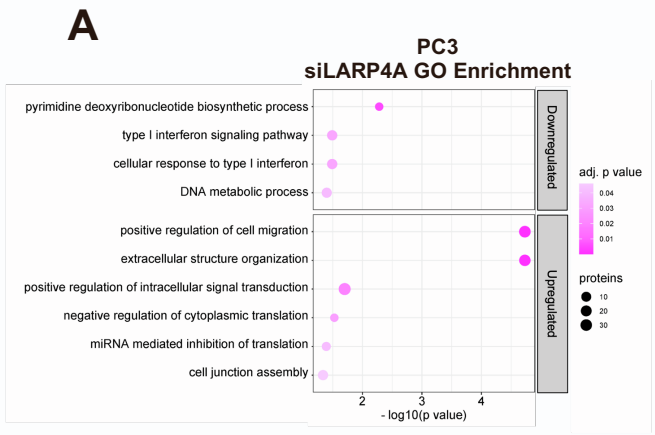

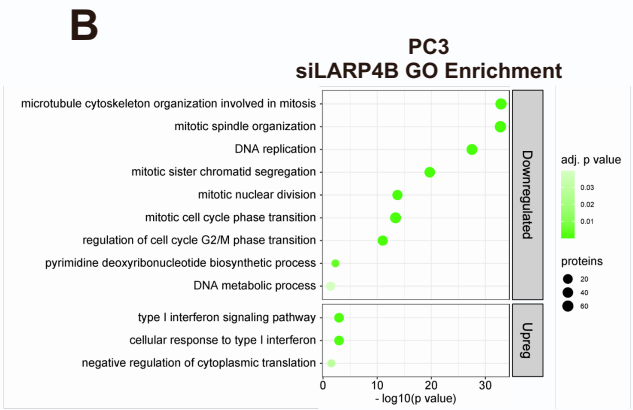

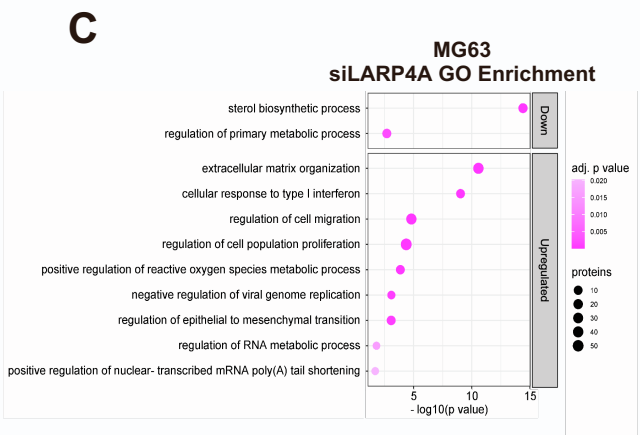

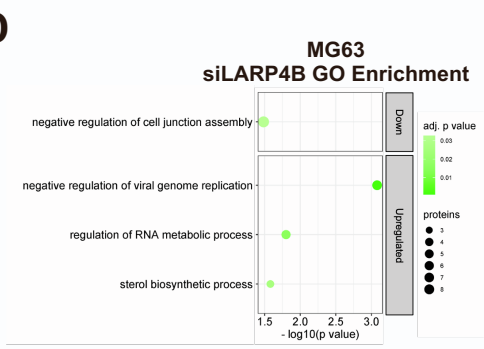

Figure S13, Coleman et al

SUPPLEMENTARY FIGURE LEGENDS

Figure S1. LARP4A and LARP4B expression in different cancers. Related to Figure 2.

(A) Percentage of tumour types where *LARP4A* or *LARP4B* are significantly overexpressed or underexpressed compared to control tissue according to TCGA TARGET GTEx mRNA expression analysis across 23 tissue types. Full statistical analysis across tumour types can be seen in (C,D).

(B) Comparison of *LARP4A* and *LARP4B* mRNA expression in normal prostate and prostate adenocarcinoma samples. Violin plots represent the mean expression in normal (n=152) or cancer (n=496) tissues according to the TGCA TARGET GTEx database. * $p<0.05$, **** $p<0.0001$

(C,D) Analysis of *LARP4A* (C) and *LARP4B* (D) mRNA expression across 23 cancer types. Raw RNAseq data (normalised, RSEM counts) from TCGA TARGET GTEx study, obtained from UCSC Xena browser (https://xena.ucsc.edu). Sample numbers vary between tissue type, ranging from n=2-1555. Bone and prostate tissues are indicated. The statistical significance of differential expression in normal versus cancer tissue in each tissue is shown. T tests were performed to test for statistical significance between normal and cancer tissue types; *$p<0.05$, **$p<0.01$, ***$p<0.001$, ****$p<0.0001$.

Figure S2. Genetic silencing of LARP4A and LARP4B persists for at least 7 days following siRNA treatment, and there is no compensation between LARP4A and LARP4B.
Related to Figure 3.

(A,B) Western blots showing (A) LARP4A and (B) LARP4B expression in PC3 and MG63 cells 7d after treatment with LARP4A or LARP4B targeting siRNAs. Non-transfected (NTC) and non-targeting siRNAs (siControl) were used as controls. GAPDH was used as a loading control. The persistence of knockdown provided the platform for using these siRNAs for short-term *in vivo* studies.

(C,D) Relative band intensities of Western blots 7d following (C) LARP4A and (D) LARP4B knockdown.

(E) Representative Western blot of LARP4A and LARP4B expression showing no compensatory expression of one paralog following siRNA silencing of the other in MG63 cells. Labels are colour-coded for ease of identification of each paralog. GAPDH was used as a loading control.

(F) Confirmation of lack of compensation by quantifiation of relative band intensities from Western blots normalised to GAPDH and siControl. Data represent mean ± SEM from two independent experiments.

(G) Confirmation of lack of compensation by qPCR mRNA expression of LARP4A and LARP4B expression in PC3 and MG63 cells following LARP4A (si4A1/si4A2) or LARP4B knockdown (si4B1/si4B2) compared to non-transfected controls (NTCs) and a non-targeting siRNA control (siCon). Data represent mean ± SEM and are from three independent experiments and β-actin was used as a housekeeping gene. * $p<0.05$, ** $p<0.01$, *** $p<0.001$.

(H) Confirmation of lack of compensation by analysis of bulk RNAseq data from two independent experiments. LARP4A and LARP4B mRNA expression are shown as

FPKM and statistical significance was determined as outlined in the bulk RNAseq Methods. The data represent mean ± SEM from two independent experiments per condition. *** false discovery rate (FDR) <0.0001.

Figure S3. Inhibition of Ki67 and Cleaved Caspase-3 expression following silencing of LARP4A and LARP4B in PC3 and MNNG/HOS cells.
Related to Figure 3.

(A) Representative immunofluorescence images of Ki67 staining in subcutaneous tumours formed by engraftment of PC3 (*top*) or MNNG/HOS cells (*bottom*) transfected with non-targeting siRNA controls (siControl) or siRNAs targeting LARP4A (siLARP4A) or LARP4B (siLARP4B). Scale bar, 100μm.

(B) Quantification of the proportion of cells within PC3 (*left*) and MNNG/HOS (*right*) xenografts expressing Ki67 shown in (A), as a percentage of the total number of cells (DAPI). Bars represent the mean ± SEM per tumour, n=9-16 individual tumours from 6-8 mice. p-values are indicated after testing with one-way ANOVA.

(C) Representative immunofluorescence images of Cleaved Caspase-3 staining in subcutaneous tumours formed by engraftment of PC3 (*top*) or MNNG/HOS cells (*bottom*) transfected with non-targeting siRNA controls (siControl) or siRNAs targeting LARP4A or LARP4B. Scale bar, 100μm.

(D) Quantification of the proportion of cells within PC3 (*left*) and MNNG/HOS (*right*) xenografts expressing Cleaved Caspase-3 shown in (C), as a percentage of the total number of cells (DAPI). Bars represent the mean ± SEM per tumour, n=9-16 individual tumours from 6-8 mice. p-values are indicated.

Figure S4. siRNA silencing of LARP4A and LARP4B for tail vein xenograft studies.
Related to Figure 4.

Western blot analysis confirming knockdown of LARP4A and LARP4B expression in MNNG/HOS cells prior to intravenous injection into the tail vein. α-tubulin was used as a loading control.

Figure S5. Overexpression of LARP4A and LARP4B regulates cell cycle in PC3 and MG63 cells.
Related to Figure 5.

(A,B) Western blot analysis of LARP4A and LARP4B overexpression in PC3 (A) and MG63 (B) cells following transfection with GFP-LARP4A or GFP-LARP4B compared to empty non-transfected controls (NTC). GAPDH is used as a loading control.

(C,D) Multiparametric cell cycle analysis of PC3 (C) and MG63 (D) cells overexpressing LARP4A, LARP4B or empty plasmid control as indicated. The fraction of the population at each cell cycle stage (sub-G1, G1, S, G2 and M phases) for each cell type is depicted. The data represent the mean ± SEM, n=20 from four independent experiments. p-values are indicated

Figure S6. Inhibition of cell cycle targets following silencing of LARP4A and LARP4B in PC3 and MG63 cells.
Related to Figure 6.

(A) RNA expression of mitotic-related genes detected via RNAseq and validated using qPCR. Expression is shown as fold change expression of LARP4A knockdown (si4A) or LARP4B knockdown (si4B) compared to siRNA controls, from 2-ΔΔCt values in qPCR and FPKM in RNAseq. The data represent the mean ± SEM, n=4 (qPCR) or two independent experiments (RNAseq) per condition.

(B) Western blot analysis of E2F1, Cyclin B1, Aurora B and Cyclin E2 protein expression in control (siCon), LARP4A (si4A) and LARP4B (si4B) knockdown PC3 and MG63 cells. α-tubulin or GAPDH are used as loading controls.

(C) Quantification of protein expression by densitometry analysis in immunoblots shown in (B), normalised to pixel intensity of respective controls, bars present show mean ± SEM.

(D) *CCNE2* mRNA expression shown as FPKM and statistical significance determined by bulk RNAseq analysis as outlined in the Methods section. The data represent mean ± SEM from two independent experiments per condition. False discovery rate (FDR) values are denoted.

Figure S7. LARP4A and LARP4B are expressed in PC3 and MG63 cell nuclei (as well as cytoplasm).
Related to Figure 7.

(A) Immunofluorescence of LARP4A and LARP4B overlayed with DAPI in PC3 and MG63 cells. Three independent examples are shown. Scale bar, 25μm.

(B) Mander's M1 coefficient co-localisation analysis showing the proportion of DAPI pixels overlapping with LARP4A or LARP4B. Four fields of view were analysed per condition. Data represent mean ± SEM from three independent experiments

Figure S8. LARP4A or LARP4B silencing does not affect apoptosis of PC3 or MG63 cells.
Related to Figure 5.

(A) Flow cytometry/Annexin V analysis of PC3 and MG63 cells either non-transfected (NTC), or transfected with non-targeting siControl and siRNAs targeting LARP4A (LARP4Asi1, LARP4Asi2) and LARP4B (LARP4Bsi1, LARP4Bsi2) as indicated. Red boxes denote cells in late apoptosis (Annexin V+/PI+), green boxes denote cells in early apoptosis (Annexin V+/PI-). The percentage population in each cell stage are shown within each quadrant.

(B) Quantification of the percentage population of PC3 (*top*) or MG63 (*bottom*) control (NTC, siCon), siLARP4A (si4A1, si4A2) and siLARP4B (si4B1, si4B2) knockdown cells in early and late stages of apoptosis. The data represent the mean ± SEM, n=4 independent experiments

(C) Flow cytometry/Annexin V analysis of PC3 and MG63 cells overexpressing LARP4A or LARP4B following transfection with empty GFP (Empty), GFP-LARP4A or GFP-LARP4B plasmids. Red boxes denote cells in late apoptosis (Annexin V+/PI+), green boxes denote cells in early apoptosis (Annexin V+/PI-). The percentage population in each cell stage are shown within each quadrant.

(D) Quantification of the percentage population of PC3 (*left*) or MG63 (*right*) control (Empty), GFP-LARP4A and GFP-LARP4B overexpressing cells in early and late stages of apoptosis. The data represent the mean ± SEM, n=3 independent experiments

(E,F) Expression of *BAX* mRNA expression in PC3 (E) and MG63 (F) control (NTC, siCon), siLARP4A (si4A1, si4A2) and siLARP4B (si4B1, si4B2) knockdown cells. Relative mRNA expression is normalised to β-actin as a housekeeping gene and to siControl cells. The data represent the mean ± SEM, n=3

Figure S9. Regulators of apoptosis.
Related to Figure 6.

mRNA expression of several apoptosis regulators following knockdown of LARP4A (si4A) or LARP4B (si4B) or transfection with non-targeting siRNA controls (siCon). Expression is shown as FPKM in RNAseq statistical significance determined by bulk RNAseq analysis (EdgeR) as outlined as in the Methods section. These data represent mean ± SEM from two independent experiments per condition. ‘ns’ denotes non significant false discovery rate (FDR) measures.

Figure S10. LARP4A and LARP4B do not regulate EMT in PC3 and MG63 cells. Related to Figure 8.

(A,B) Western blot analysis of EMT markers E-Cadherin and Vimentin in PC3 (A) and MG63 (B) cells following either LARP4A or LARP4B knockdown or overexpression as indicated. Knockdown cells were transfected with either control siRNAs (NTC, siCon) or siRNAs for LARP4A (siLARP4A1, siLARP4A2) and LARP4B (siLARP4B1, siLARP4B2) as indicated. Overexpression cells were transfected with either GFP-empty or GFP-LARP4A and GFP-LARP4B plasmids as indicated. GAPDH used as a loading control. As expected, mesenchymal-type MG63 cells do not express E-cadherin.

Figure S11. LARP4A and LARP4B regulate cell migration and invasion in MNNG-HOS cells.
Related to Figure 9.

(A) Brightfield images of transwells stained with crystal violet following a 24h migration assay of MNNG/HOS control (siControl), siLARP4A (4A1, 4A2) or siLARP4B (4B1, 4B2) knockdown cells. Scale bars, 200μm.

(B) Quantification of migrated MNNG/HOS cells over 24h of either non-transfected cells (NTC, siCon) or cells transfected with siRNAs targeting LARP4A (si4A1, si4A2) or LARP4B (si4B1, si4B2). The data represent the mean ± SEM, n=6 from 3 independent experiments. P values are indicated.

Figure S12. Regulators of translation and poly(A) tail shortening.
Related to Figure 6.

mRNA expression of several regulators of translation and polyadenylated tail shortening following knockdown of LARP4A (si4A) or LARP4B (si4B) or transfection with non-targeting siRNA controls (siCon). Expression is shown as FPKM in RNAseq and statistical significance determined by bulk RNAseq analysis (EdgeR) as outlined as in the Methods section. These data represent mean ± SEM from two independent experiments per condition. False discovery rate (FDR) values are indicated as determined by RNAseq analysis.

Figure S13. Differentially expressed genes (DEGs) following LARP4A and LARP4B knockdown enrich for several biological processes.
Related to Figure 6E.

(A,B) Bubble plots showing gene ontology (GO) of several significantly enriched pathways involving significantly up- or downregulated DEGs when comparing PC3 LARP4A (A) and LARP4B (B) knockdowns to siControls, respectively

(C,D) Bubble plots showing gene ontology (GO) of significantly enriched pathways involving DEGs when comparing MG63 LARP4A (A) and LARP4B (B) knockdowns to siControls.

Enrichment analysis performed by Enrichr and all depicted enriched pathways have an adjusted p value of <0.05. Analysis was performed on statistically significant DEGs, determined as described in the Methods section, from two biological replicates per condition.