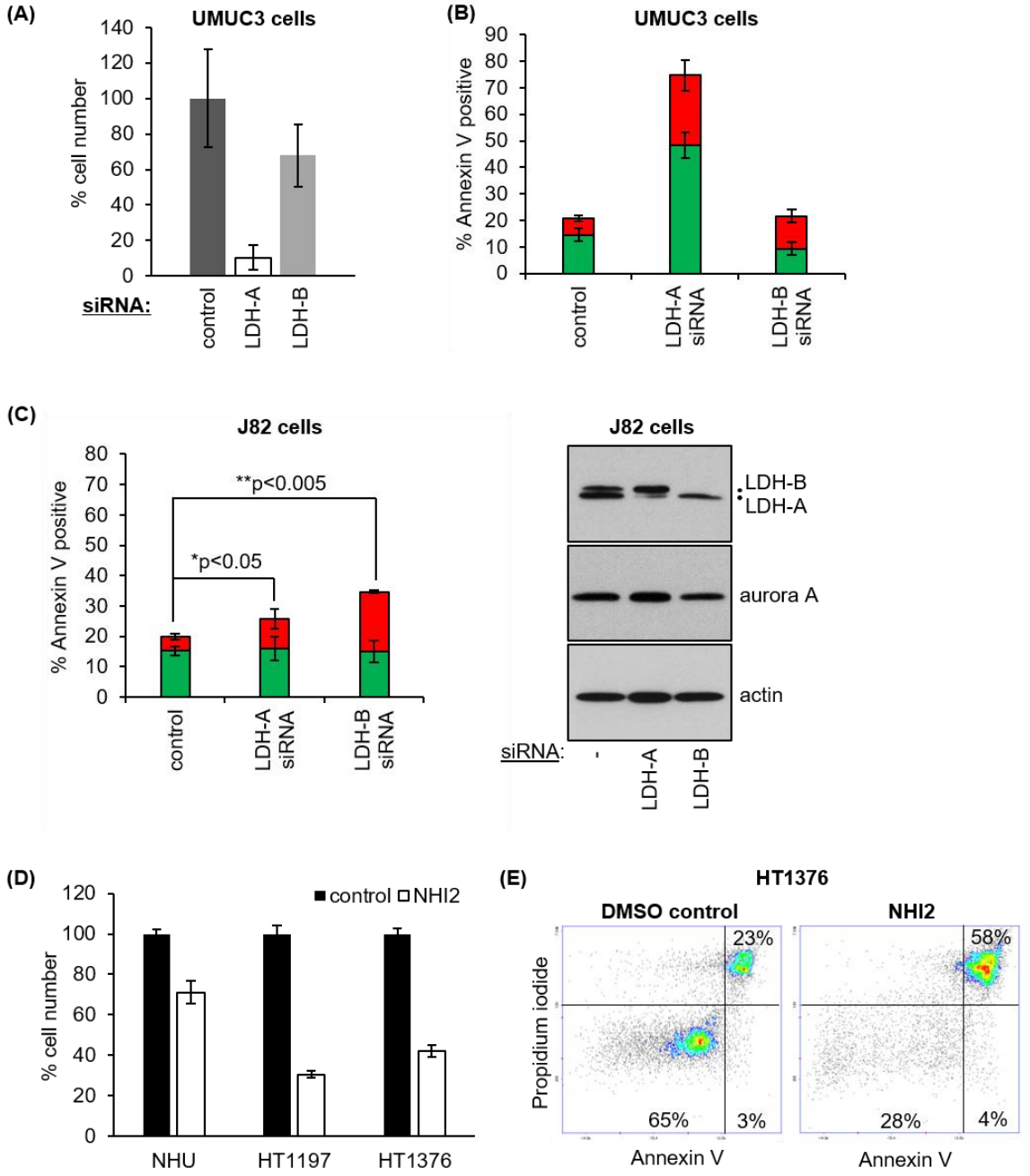

Figure S5. Induction of apoptotic cell death in bladder cancer cell lines via LDH-A knock-down or inhibition.

A, Effect of LDH-A or LDH-B knock-down by RNAi on UMUC3 total cell number 72h following siRNA transfection, cell number is expressed as a percentage relative to that of control transfected cells. Mean ± SD of three independent determinations.

B, Proportion of early and late stage apoptotic UMUC3 bladder cancer cells 72h following siRNA transfection with LDH-A or LDH-B siRNA, mean ± SD of three independent determinations. Green bars indicate early stage apoptotic cells (annexin V positive, propidium iodide (PI) negative), red bars indicate late stage apoptotic cells (annexin V positive, PI positive).

C, Proportion of early and late stage apoptotic J82 bladder cancer cells 72h following siRNA transfection with LDH-A or LDH-B siRNA, mean ± SD of three independent determinations. Green bars indicate early stage apoptotic cells (annexin V positive, PI negative), red bars indicate late stage apoptotic cells (annexin V positive, PI positive). Statistical significance as determined by student's t-test, * $p<0.05$, ** $p<0.005$. Immunoblots shows selective depletion of LDH-A and LDH-B by their respective siRNAs and expression of aurora A kinase in J82 cells.

D, Effect of 48h cell exposure to LDH inhibitor NHI2 (15µM) on normal human urothelial cells (NHUC) and bladder cancer cell lines HT1197 and HT1376. Cell number expressed as a percentage relative to vehicle control treated cells; mean ± SD of three independent determinations.

E, Representative dot plots of NHI2-treated HT1376 cells (48h 15µM NHI2) compared to vehicle control treated cells and following annexin V staining for detection of early and late stage apoptotic cells. Lower left quadrant indicates live cells; lower right quadrant (annexin V positive, PI negative) indicates % of early stage apoptotic cells; upper right quadrant indicates % of late stage apoptotic cells (annexin V positive, PI positive).