Supplementary Materials

Title: Fluorescent modification of a PSMA-targeting probe reveals preferential uptake by actively dividing cells in metastatic prostate cancer models

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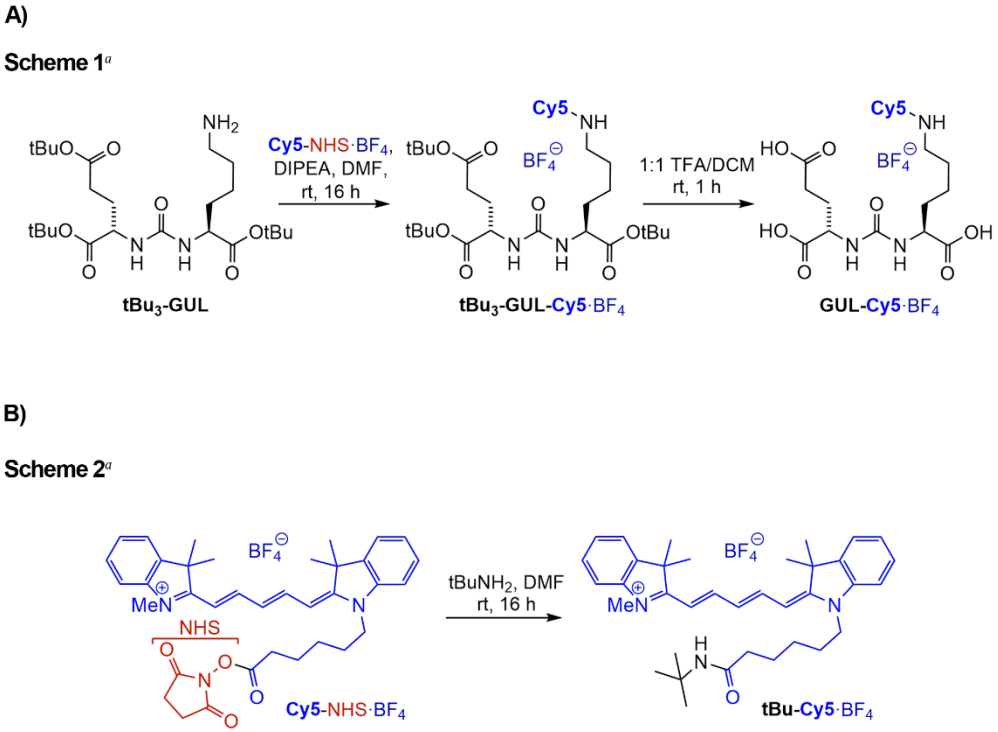
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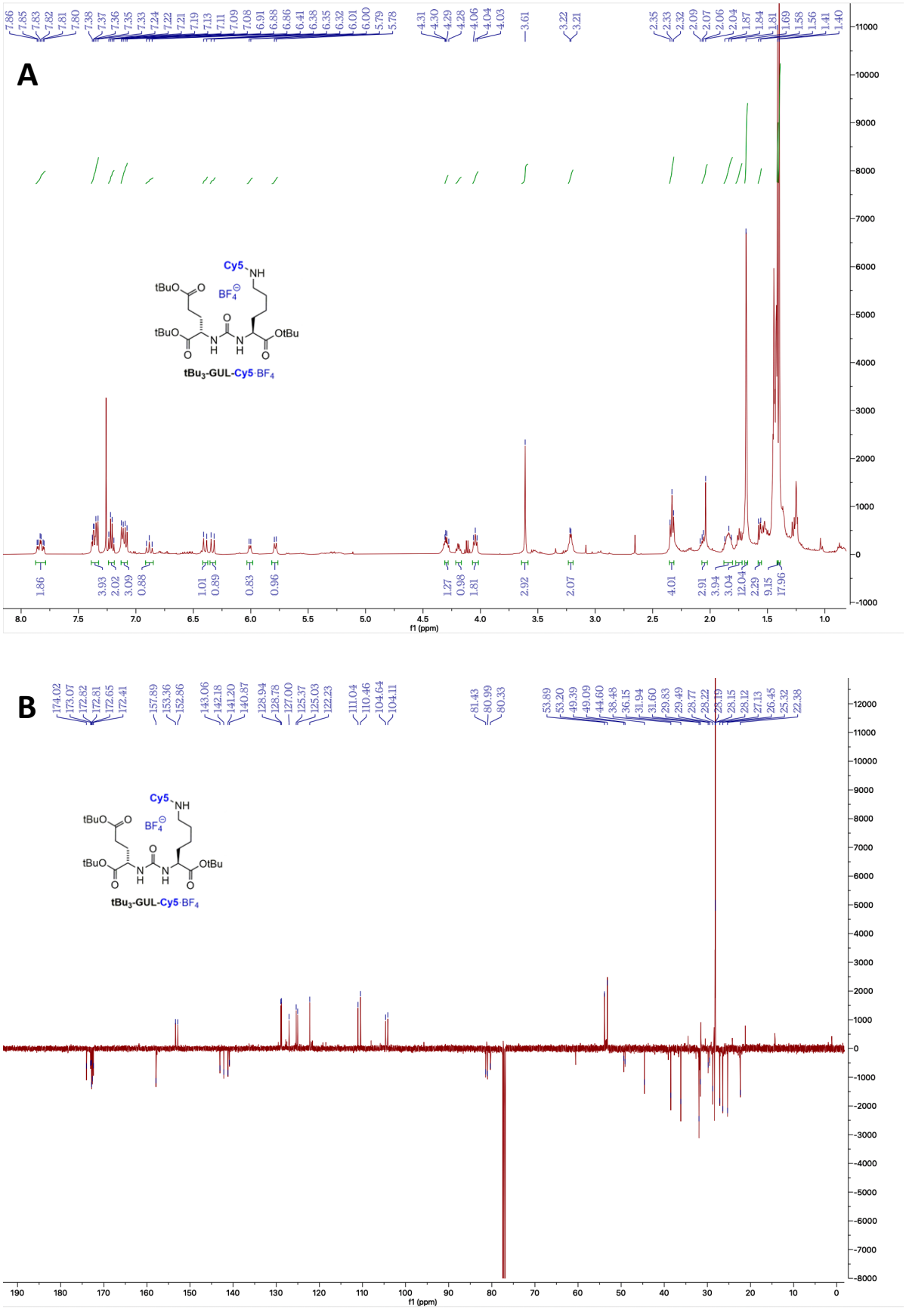
Supplementary Figures S1 to S11

Supplementary Tables S1 to S3

Supplementary Figures and Figure Legends:

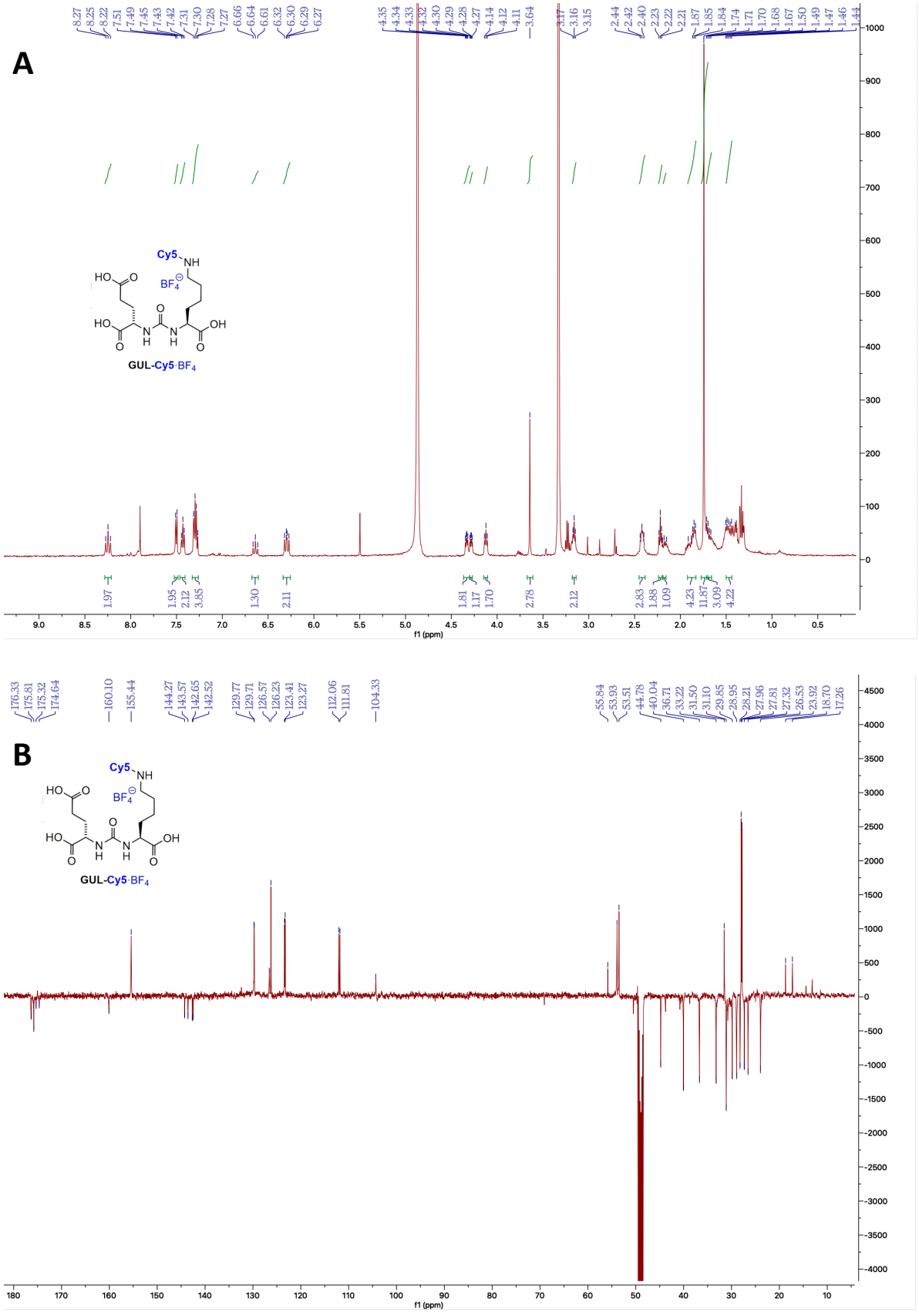


Supplementary Figure S1. Schemes of synthesis for GUL-Cy5 probe and its isotype control (tBu-Cy5). A. Scheme for GUL-Cy5 probe. [a]Reagents and conditions: (a) Cy5-NHS·BF_4, DIPEA, DMF, room temperature, 16 h; (b) 1:1 TFA/DCM, room temperature, 1 h. B. Scheme for tBu-Cy5 probe. [a]Reagents and conditions: (a) tBuNH2, DMF, room temperature, 16 h.

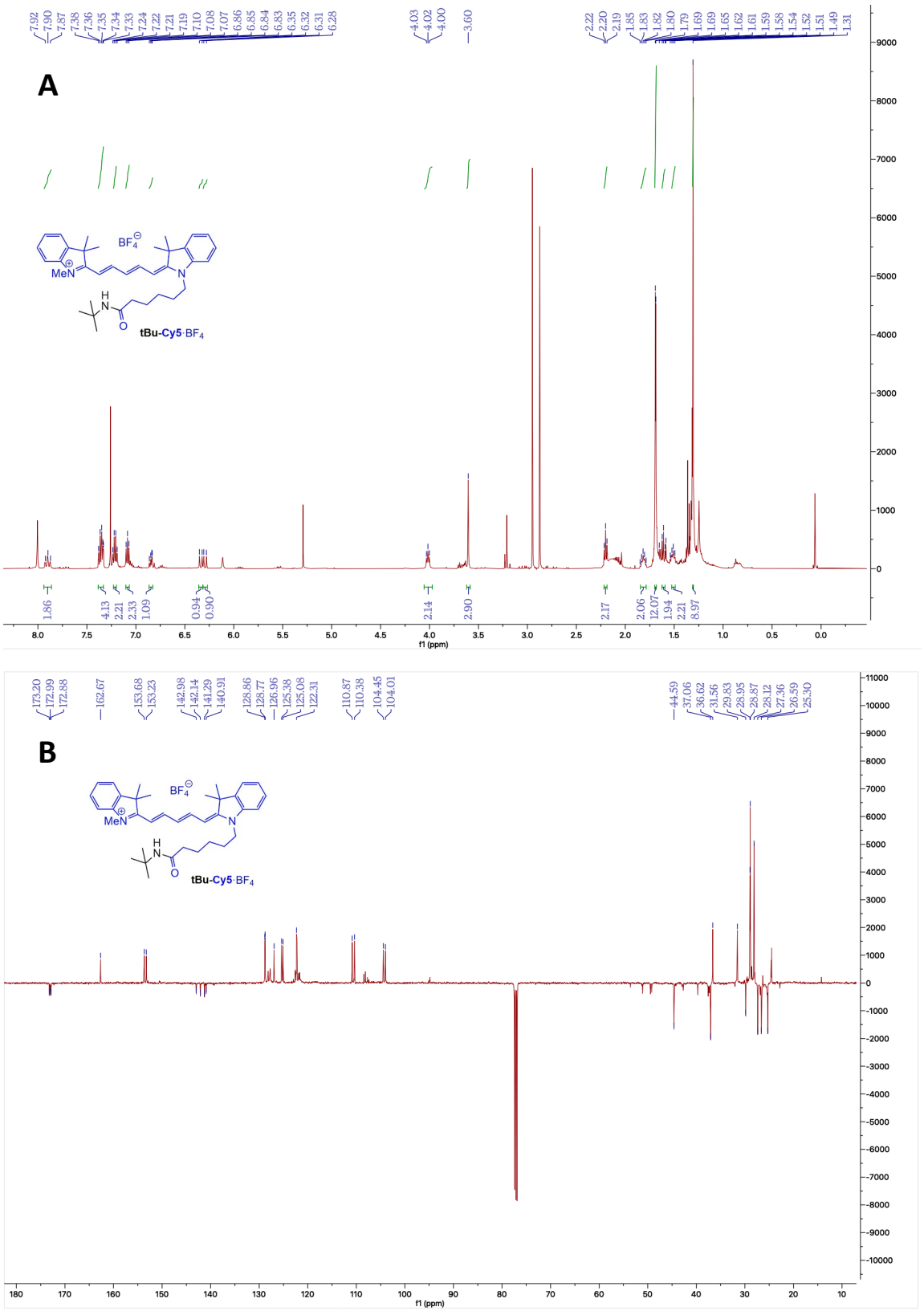


Supplementary Figure S2. A. 1H and B. 13C (DEPT-Q) NMR spectra (500 MHz, 126 MHz

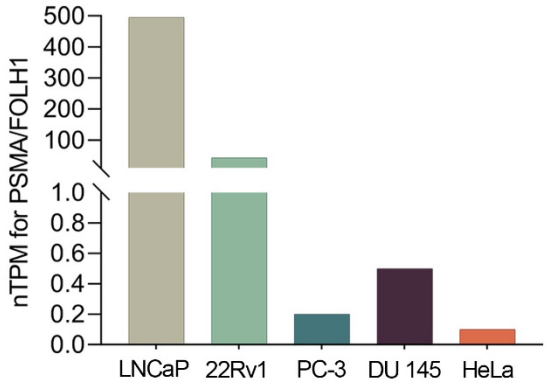
in CDCl3) of tBu3-GUL-Cy5·**BF4**.



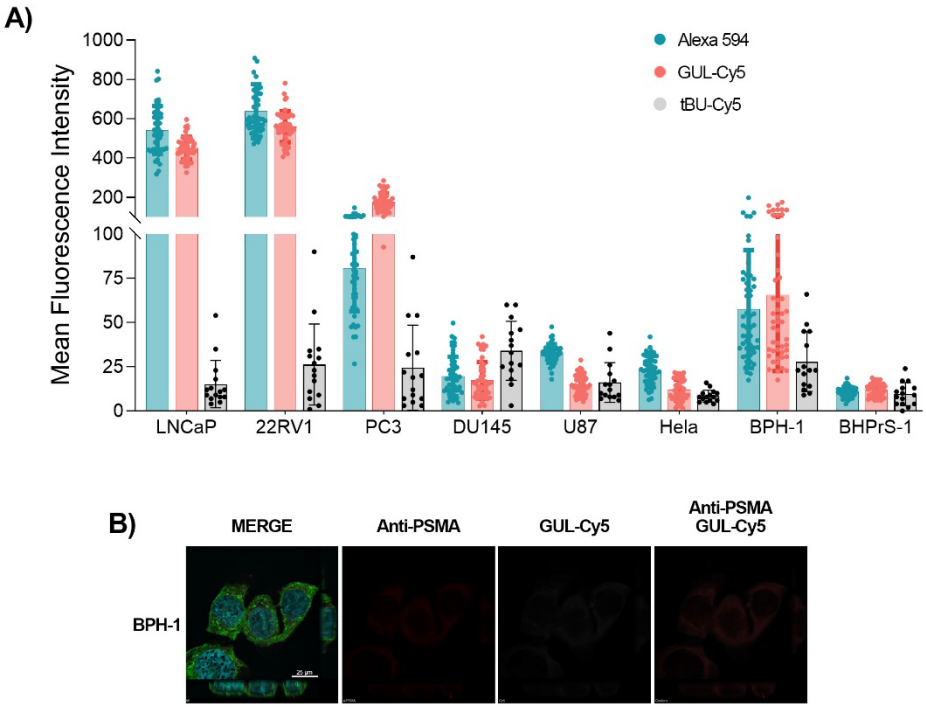
Supplementary Figure S3. A. 1H and B. 13C (DEPT-Q) NMR spectra (500 MHz, 126 MHz in CD3OD) of GUL-Cy5·**BF4**.



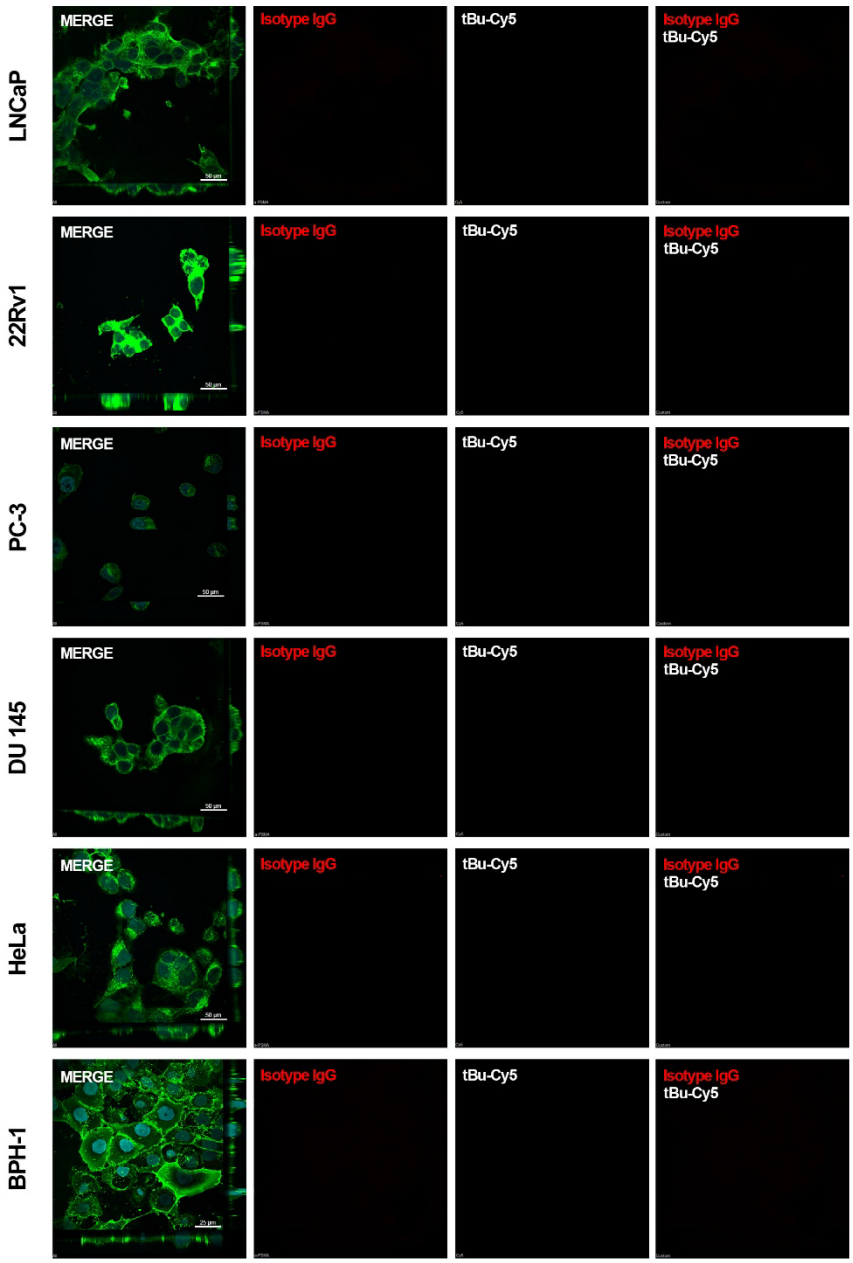
Supplementary Figure S4. A. 1H and B. 13C (DEPT-Q) NMR spectra (500 MHz, 126 MHz in CDCl3) of GUL-Cy5·**BF4**.



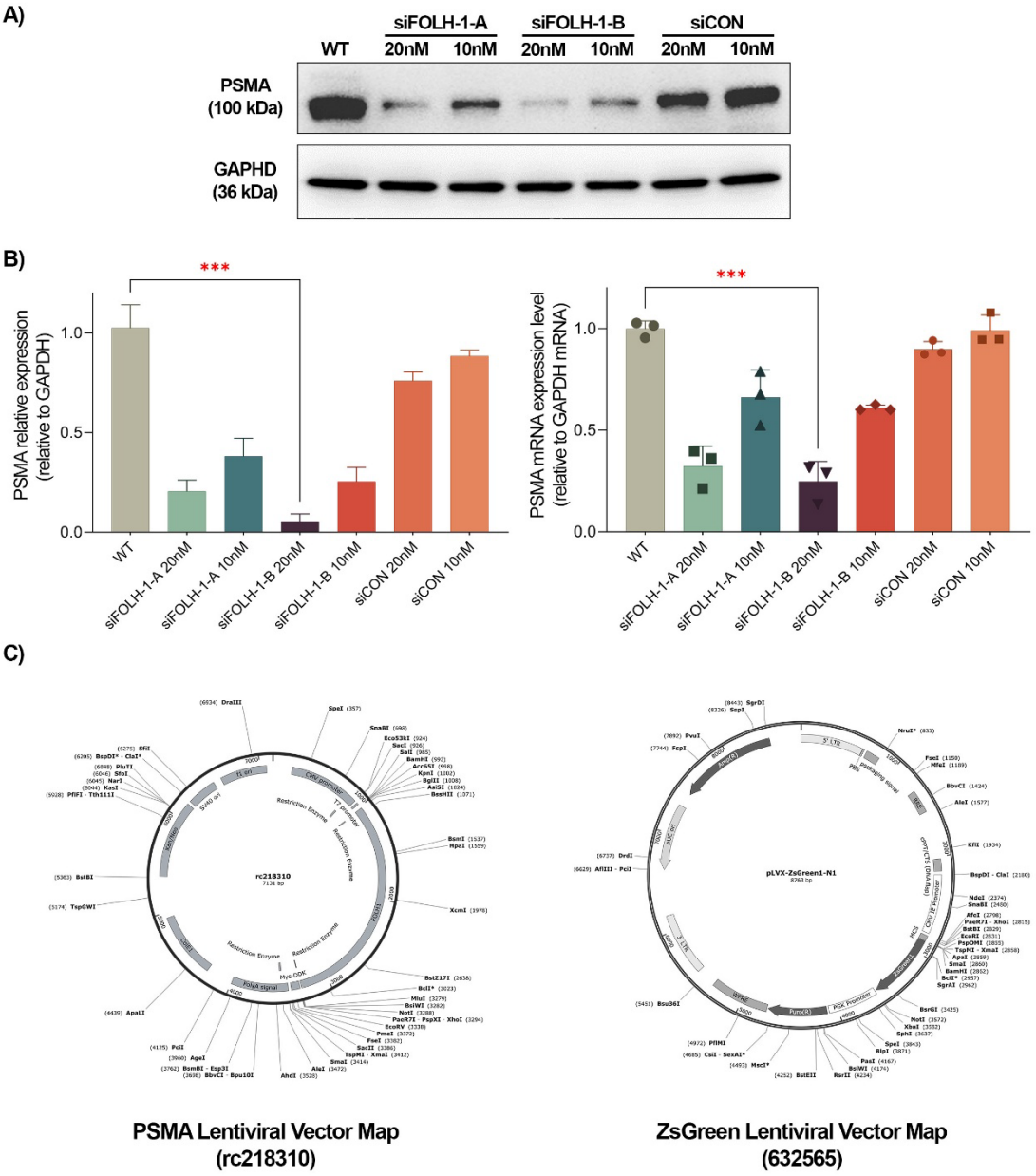
Supplementary Figure S5. FOLH1 mRNA expression levels in cell lines as per Protein Atlas.



Supplementary Figure S6. PSMA expression levels and GUL-Cy5 and tBu-Cy5 binding characteristics in various cell lines and representative images of binding in the BPH-1 cell line. A. Mean fluorescence intensity of 50 cells per cell line in Alexa 594 channel (0.5 µg/mL anti-PSMA mAb, blue columns), GUL-Cy5 channel (0.5 µg/mL, red columns) and tBu-Cy5 (0.5 µg/mL, gray columns). B. Representative images of PSMA and GUL-Cy5 immunoreactivity to the BPH-1 cell line. Scale bars are 50 µm.

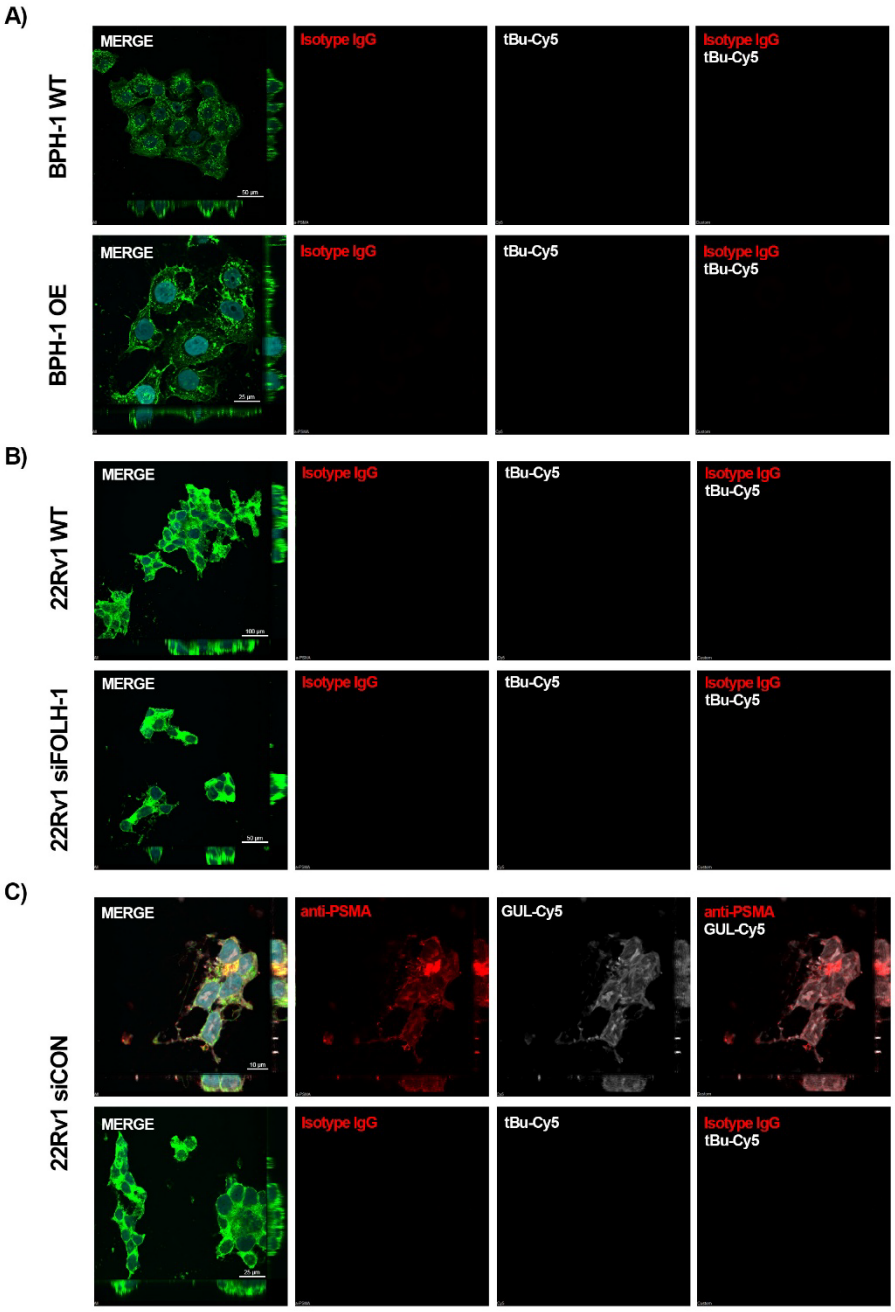


Supplementary Figure S7. tBu-Cy5 probe (isotype control) binding characteristics in PSMA-expressing cell lines. Immunofluorescence images of LNCaP, 22Rv1, PC-3, DU 145, U-87, HeLa, BPH-1 and BHPrS-1 cells immunostained with tBu-Cy5 (0.5 µg/mL) and isotype IgG antibody (0.5 µg/mL). Scale bars are 25 µm and 50 µm.



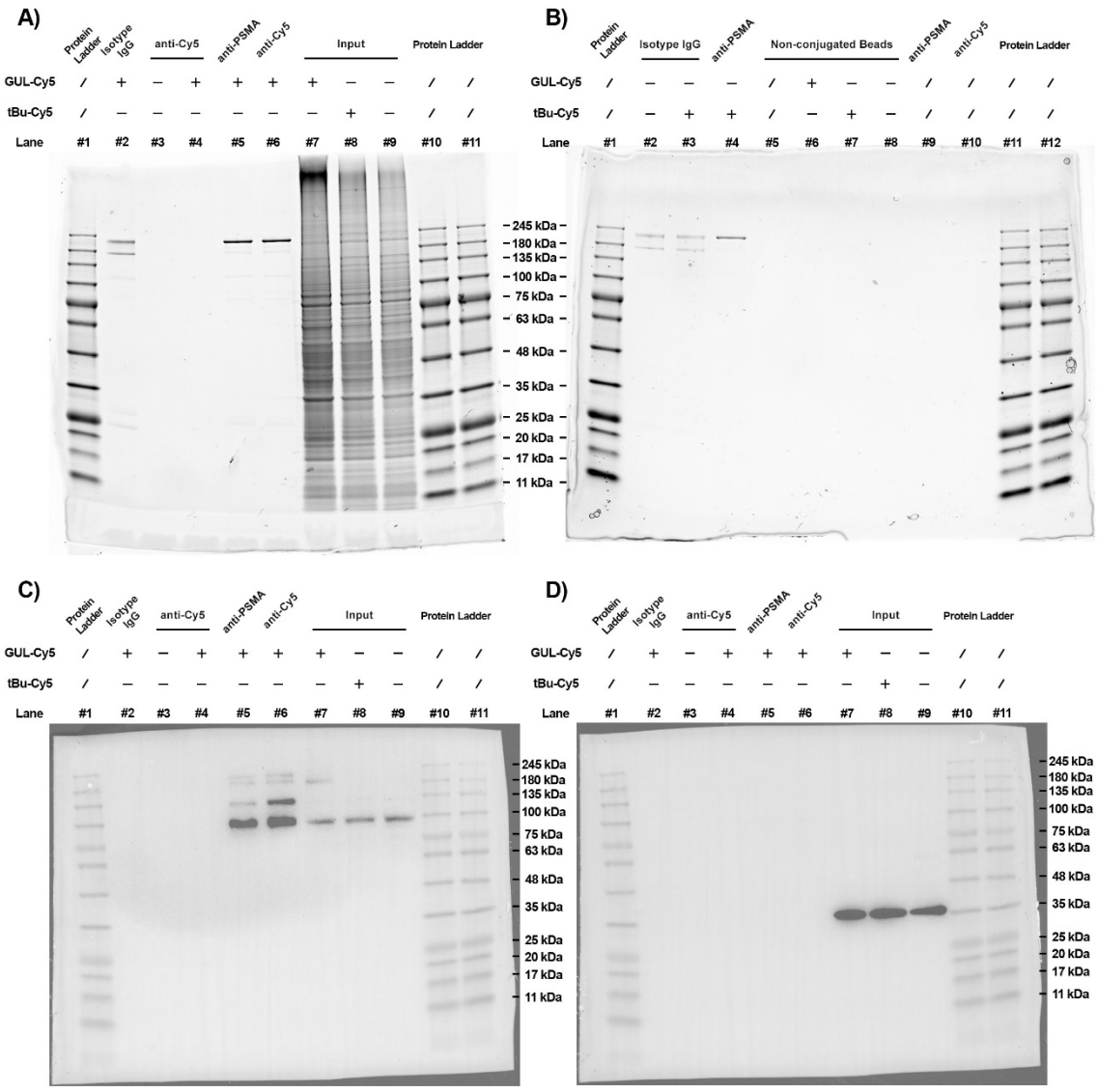
Supplementary Figure S8. Titration and selection of siRNAs of *FOLH1* and vector maps. A. Immunoblot of PSMA and GAPDH expression for titration and selection of siRNAs (siFOLH-1-A and siFOLH-1-B) with controls (WT and siCON). B. Immunoblot analysis (left graph) for titration and selection of siRNAs of *FOLH-1* relative to wild type (WT). * denotes $p < 0.001$, 2-way ANOVA, Bonferroni alpha correction. Quantitative PCR (qPCR) analysis of *FOLH1* mRNA expression (normalized to the housekeeping gene

GAPDH) for titration and selection of siRNAs of *FOLH-1* relative to wild type (WT). * denotes $p < 0.001$, 2-way ANOVA, Bonferroni alpha correction. C. Vector maps for PSMA lentiviral vector (OriGene, cat#: rc218310) and ZsGreen lentiviral vector (Takara Bio, cat#: 632565).



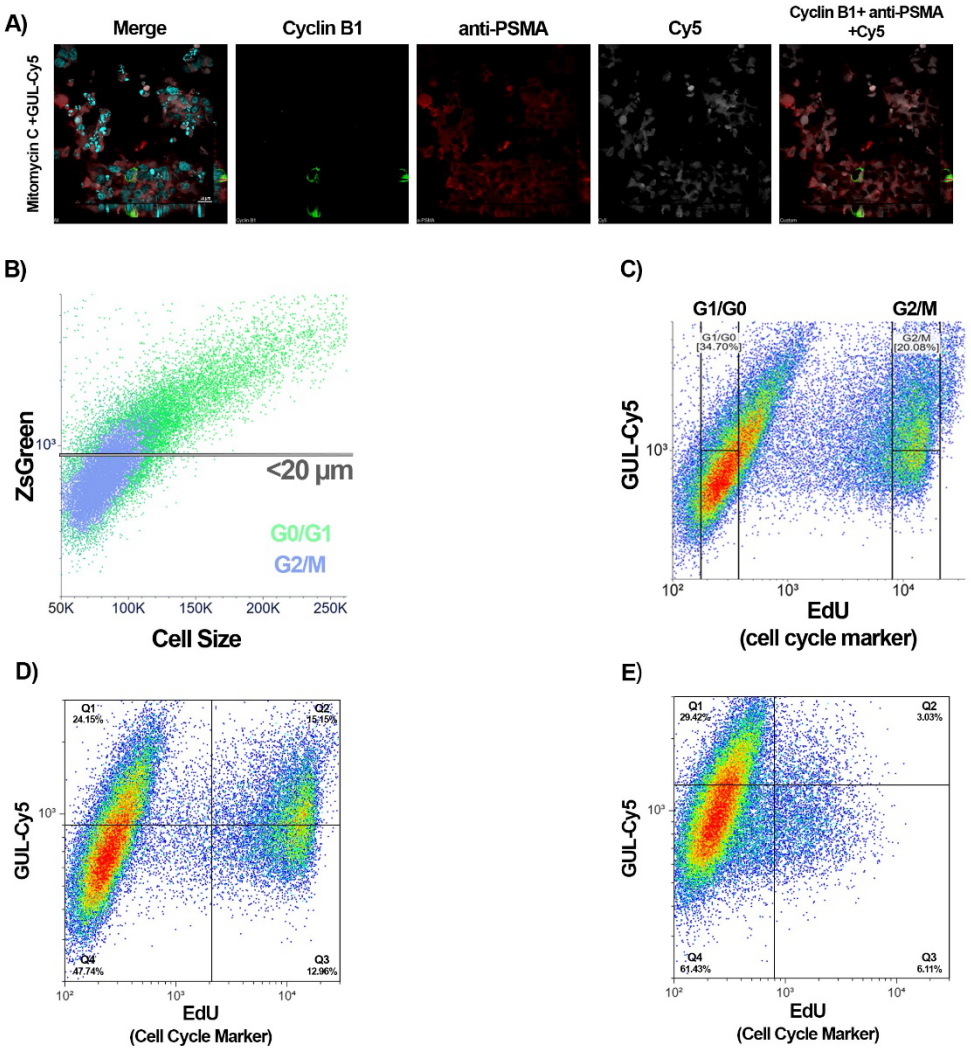
Supplementary Figure S9. Binding specificity of tBu-Cy5 correlated with PSMA expression levels. A. Immunofluorescence images of BPH-1 WT and BPH-1 OE cells immunostained with tBu-Cy5 (0.5 µg/mL) and isotype IgG antibody (0.5 µg/mL). Scale bars are 25 µm and 50 µm. B. Immunofluorescence images of 22Rv1 WT and 22Rv1-siFOLH1 cells immunostained with tBu-Cy5 (0.5 µg/mL) and isotype IgG antibody (0.5 µg/mL). Scale bars are 50 µm and 100 µm. C. Immunofluorescence images of 22Rv1-siCON (knockdown

by Negative Control siRNA) cells immunostained with GUL-Cy5 (0.5 μg/mL) and anti-PSMA mAb (0.5 μg/mL), and immunofluorescence images of same cells immunostained with tBu-Cy5 (0.5 μg/mL) and isotype IgG antibody (0.5 μg/mL). Scale bars are 10 μm and 25 μm.



Supplementary Figure S10. Proteins from immunoprecipitation and controls. A, B. SDS-PAGE and Coomassie blue staining analysis of protein samples obtained from anti-Cy5 antibody-based immunoprecipitation and controls. Protein from immunoprecipitation and three different inputs were separated on a 4%-20% SDS-PAGE and visualized in A with Coomassie Blue. Protein from other controls include isotype controls and beads-based controls were separated on a 4%-20% SDS-PAGE and visualized in B with Coomassie Blue. C, D. Immunoblots transferred from sister SDS-PAGE separating same aforementioned

protein samples to detect PSMA in C using anti-PSMA mAb (Cell Signaling Technology, 12702S) and detect housekeeping protein GAPDH in D using anti-GAPDH HRP-conjugated mAb (ABclonal, AC035).



Supplementary Figure S11. GUL-Cy5 probe uptake preference across stages of the cell cycle. A. Immunofluorescence images of 22Rv1 cells after 2 µM of Mitomycin C treated overnight, immunostained with GUL-Cy5 (0.5 µg/mL) and anti-PSMA mAb (0.5 µg/mL). Scale bars are 50 µm. B. Cell size analysis for 22Rv1 cells in G0/G1 phase (green subpopulation) and G2/M phase (red subpopulation). C. Correlation between the uptake of EdU (cell cycle marker) and GUL-Cy5 in 22Rv1 cells. D, E. Cell cycle analysis of 22Rv1 cells following overnight treatment with 0 µM (D) and 2 µM (E) of Mitomycin C.

Supplementary Tables

Table S1. Nucleotide sequences of lentiviral vector for *FOLH1* gene

Red=Cloning site Blue=ORF Green=Tags(s)
ATGTGGAATCTCCTTCACGAAACCGACTCGGCTGTGGCCACCGCGCGCCGCCCGCGCT
GGCTGTGCGCTGGGGCGCTGGTGCTGGCGGGTGGCTTCTTTCTCCTCGGCTTCCTCTTC
GGGTGGTTTATAAAATCCTCCAATGAAGCTACTAACATTACTCCAAAGCATAATATGA
AAGCATTTTTGGATGAATTGAAAGCTGAGAACATCAAGAAGTTCTTATATAATTTTAC
ACAGATACCACATTTAGCAGGAACAGAACAAAACTTTCAGCTTGCAAAGCAAATTCAA
TCCCAGTGGAAAGAATTTGGCCTGGATTCTGTTGAGCTAGCACATTATGATGTCCTGTT
GTCCTACCCAAATAAGACTCATCCCAACTACATCTCAATAATTAATGAAGATGGAAAT
GAGATTTTCAACACATCATTATTTGAACCACCTCCTCCAGGATATGAAAATGTTTCGGA
TATTGTACCACCTTTCAGTGCTTTCTCTCCTCAAGGAATGCCAGAGGGCGATCTAGTGT
ATGTTAACTATGCACGAACTGAAGACTTCTTTAAATTGGAACGGGACATGAAAATCAA
TTGCTCTGGGAAAATTGTAATTGCCAGATATGGGAAAGTTTTCAGAGGAAATAAGGTT
AAAAATGCCCAGCTGGCAGGGGCCAAAGGAGTCATTCTCTACTCCGACCCTGCTGACT
ACTTTGCTCCTGGGGTGAAGTCCTATCCAGATGGTTGGAATCTTCCTGGAGGTGGTGTC
CAGCGTGGAAATATCCTAAATCTGAATGGTGCAGGAGACCCTCTCACACCAGGTTACC
CAGCAAATGAATATGCTTATAGGCGTGGAATTGCAGAGGCTGTTGGTCTTCCAAGTAT
TCCTGTTCATCCAATTGGATACTATGATGCACAGAAGCTCCTAGAAAAAATGGGTGGC
TCAGCACCACCAGATAGCAGCTGGAGAGGAAGTCTCAAAGTGCCCTACAATGTTGGAC
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CACCGCTGATGTACAGCTTGGTACACAACCTAACAAAAGAGCTGAAAAGCCCTGATGA
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CAAATTCAGCGGCTATCCACTGTATCACAGTGTCTATGAAACATATGAGTTGGTGGAA
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GGTGTTTGAGCTAGCCAATTCCATAGTGCTCCCTTTTGATTGTCGAGATTATGCTGTAG
TTTTAAGAAAGTATGCTGACAAAATCTACAGTATTTCTATGAAACATCCACAGGAAAT
GAAGACATACAGTGTATCATTTGATTCACTTTTTTCTGCAGTAAAGAATTTTACAGAAA
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AAGAATGATGAATGATCAACTCATGTTTCTGGAAAGAGCATTTATTGATCCATTAGGG
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ATGCAGGGGAGTCATTCCCAGGAATTTATGATGCTCTGTTTGATATTGAAAGCAAAGT
GGACCCTTCCAAGGCCTGGGGAGAAGTGAAGAGACAGATTTATGTTGCAGCCTTCACA
GTGCAGGCAGCTGCAGAGACTTTGAGTGAAGTAGCC
ACGCGTACGCGGCCGCTCGAGCAGAAACTCATCTCAGAAGAGGATCTGGCAGCAAAT

GATATCCTGGATTACAAGGATGACGA
Product Information: PSMA (FOLH1) (NM_004476) Human Tagged ORF Clone (OriGene, CAT#: RC218310)

Table S2. Specific small interfering RNAs of *FOLH1* gene

PSMA *(FOLH1*) siRNA Sequences		
siFOLH-1-A	Sense	5' rGrUrArUrGrUrCrArCrUrCrArGrArArArGrArArUrCrGrUAA
	Antisense	5' rUrUrArCrGrArUrUrCrUrUrUrCrUrGrArGrUrGrArCrArUrArCrCrA
siFOLH-1-B	Sense	5' rCrUrArCrUrArArCrArUrUrArCrUrCrCrArArArGrCrArUAA
	Antisense	5' rUrUrArUrGrCrUrUrUrGrGrArGrUrArArUrGrUrUrArGrUrArGrCrU
siCON	Sense	5' rCrGrUrUrArArUrCrGrCrGrUrArUrArArUrArCrGrCrGrUAT
	Antisense	5' rArUrArCrGrCrGrUrArUrUrArUrArCrGrCrGrArUrUrArArCrGrArC
Product information: PSMA (FOLH1) Human siRNA Oligo Duplex (OriGene, CAT#: SR301643) siFOLH-1-A = SR301643A siFOLH-1-B = SR301643B siCON = Control siRNA		

Table S3. Nucleotide sequences of lentiviral vectors for *ZsGreen1* gene, which encodes the ZsGreen1 fluorescent protein.

ZsGreen Lentiviral Vector ORF Nucleotide Sequences
Blue=ORF ATGGCCCAGTCCAAGCACGGCCTGACCAAGGAGATGACCATGAAGTACCGCAT GGAGGGCTGCGTGGACGGCCACAAGTTCGTGATCACCGGCGAGGGCATCGGCT ACCCCTTCAAGGGCAAGCAGGCCATCAACCTGTGCGTGGTGGAGGGCGGCCCC TTGCCCTTCGCCGAGGACATCTTGTCCGCCGCCTTCATGTACGGCAACCGCGTG TTCACCGAGTACCCCCAGGACATCGTCGACTACTTCAAGAACTCCTGCCCCGCC GGCTACACCTGGGACCGCTCCTTCCTGTTCGAGGACGGCGCCGTGTGCATCTGC AACGCCGACATCACCGTGAGCGTGGAGGAGAACTGCATGTACCACGAGTCCAA GTTCTACGGCGTGAACTTCCCCGCCGACGGCCCCGTGATGAAGAAGATGACCG ACAACTGGGAGCCCTCCTGCGAGAAGATCATCCCCGTGCCCAAGCAGGGCATC TTGAAGGGCGACGTGAGCATGTACCTGCTGCTGAAGGACGGTGGCCGCTTGCG CTGCCAGTTCGACACCGTGTACAAGGCCAAGTCCGTGCCCCGCAAGATGCCCG ACTGGCACTTCATCCAGCACAAGCTGACCCGCGAGGACCGCAGCGACGCCAAG AACCAGAAGTGGCACCTGACCGAGCACGCCATCGCCTCCGGCTCCGCCTTGCC CTGA

Product Information:
pLVX-ZsGreen1-N1 Vector (Takara Bio, CAT#: 632565)