

# Subcellular localization of uc.8+ as a prognostic biomarker in bladder cancer tissue

Sara Terreri <sup>a, b, 1</sup>, Sara Mancinelli <sup>a, c, 1</sup>, Matteo Ferro <sup>d, 1</sup>, Maria Concetta Vitale <sup>e</sup>, Sisto Perdonà <sup>f</sup>, Luigi Castaldo <sup>f</sup>, Vincenzo Gigantino <sup>g</sup>, Vincenzo Mercadante <sup>a</sup>, Rossella De Cecio <sup>g</sup>, Gabriella Aquino <sup>g</sup>, Marco Montella <sup>h</sup>, Claudia Angelini <sup>i</sup>, Eugenio Del Prete <sup>i</sup>, Marianna Aprile <sup>a</sup>, Angelo Ciaramella <sup>e</sup>, Giovanna L. Liguori <sup>a</sup>, Valerio Costa <sup>a</sup>, George A. Calin <sup>1</sup>, Evelina La Civita <sup>m</sup>, Daniela Terracciano <sup>m, \*\*\*</sup>, Ferdinando Febbraio <sup>n, 2, \*\*</sup>, Amelia Cimmino <sup>a, 2, \*</sup>

<sup>a</sup> Institute of Genetics and Biophysics "A. Buzzati Traverso", National Research Council (CNR), Naples, Italy

<sup>b</sup> Immunology Research Area, B-cell development Unit, Children Hospital Bambino Gesù, Rome, Italy

<sup>c</sup> IRCCS Humanitas Clinical and Research Center, Rozzano - Milan, Italy

<sup>d</sup> Division of Urology, European Institute of Oncology, Milan, Italy

<sup>e</sup> Department of Science and Technology, University of Naples Parthenope, Naples, Italy

<sup>f</sup> Uro-Gynecological Department, Istituto Nazionale per lo Studio e la Cura dei Tumori, Fondazione "G. Pascale"-IRCCS, Naples, Italy

<sup>g</sup> Pathology Unit, Istituto Nazionale per lo Studio e la Cura dei Tumori, Fondazione "G. Pascale"-IRCCS, Naples, Italy

<sup>h</sup> Department of Physical and Mental Health and Preventive Medicine, Section of Pathology, Università degli studi della Campania "Luigi Vanvitelli", Naples, Italy

<sup>i</sup> Institute for Applied Mathematics "Mauro Picone", National Research Council (CNR), Naples, Italy

<sup>1</sup> Translational Molecular Pathology Department and Leukemia Department, University of Texas MD Anderson Cancer Center, Houston, TX, USA

<sup>m</sup> Department of Translational Medical Sciences, University of Naples "Federico II," Naples, Italy

<sup>n</sup> Institute of Biochemistry and Cell Biology, National Research Council (CNR), Naples, Italy

## Supplementary figures

**Figure S1.** A) Violin plot showing uc.8+ intensity score values across normal surrounding urothelium (NSU), low grade (LG) and high grade (HG) tissues. B) Frequency plot showing the scaled distribution of uc.8+ intensity in NSU, LG and HG according to the uc.8+ intensity. \*\* p-values <0.01.

**Figure S2.** Violin and Frequency plots showing the correlation between the uc.8+ intensity score and clinical traits of patients cohort. (A) Association with sex, (B) age of pts, (C) metastasis and (D) therapy.

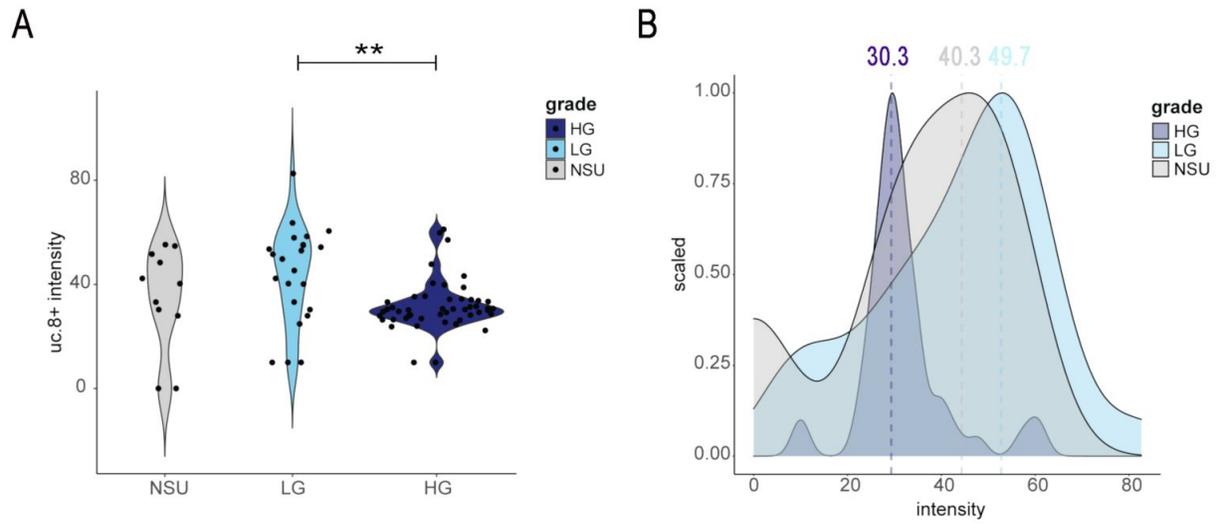
**Figure S3.** Predicted secondary structure representation of the whole uc.8+. Sequences associated with the predicted nuclear (light red) and cytosolic (light blue) localization. In purple the overlapped sequences of both localizations.

**Figure S4.** uc.8+ intensity of sample belonging to HG and LG, with respect to the cellular localization. Boxplots describing the relationship between intensity level of uc.8+ (Y axis) and cellular localization (X axis) in BlCa tissues.

**Figure S5.** Fluorescence microscopy of J82 bladder cancer control cells (first lane, Mock) after transfection with PNA-TO scramble-R8 (second lane, scramble PNA) or with TO-PNA1-R8, complementary to uc.8+ sequence (third lane, uc.8+ PNA). Images were recorded with excitation wavelength (lex)=450–490 nm (DAPI) or lex=510–540 nm (PNA-TO); the superimposition of the images recorded is also reported (Merge). Scale bar, 100 nm. Nuclei of J82 cells were stained with DAPI (blue).

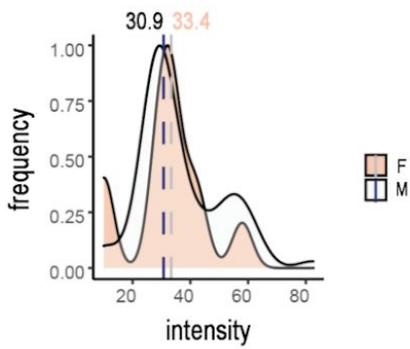
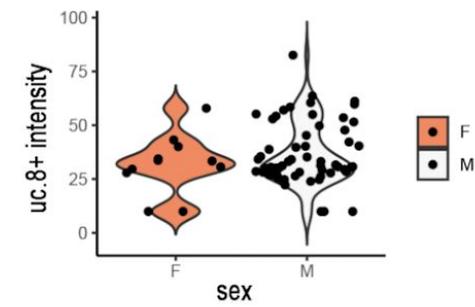
## Supplementary Table

**Table S1.** Subcellular localization of uc.8+ in BlCa patients. Data were reported with respect to the lncRNA intensity expression and patients tumor Grade.

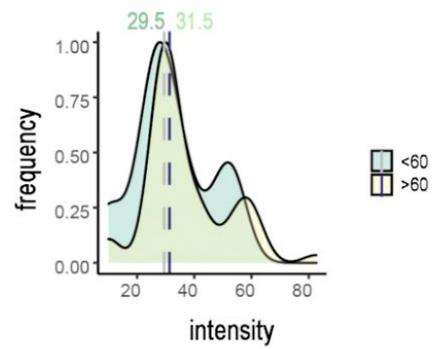
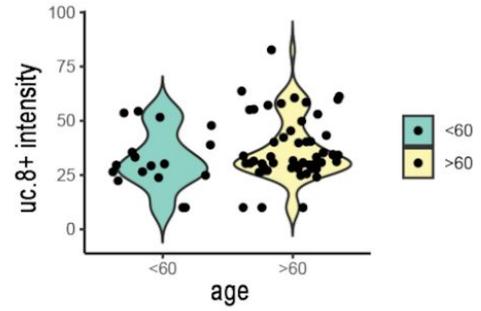


**Figure S1.** A) Violin plot showing uc.8+ intensity score values across normal surrounding urothelium (NSU), low grade (LG) and high grade (HG) tissues. B) Frequency plot showing the scaled distribution of uc.8+ intensity in NSU, LG and HG according to the uc.8+ intensity. \*\* p-values < 0.01.

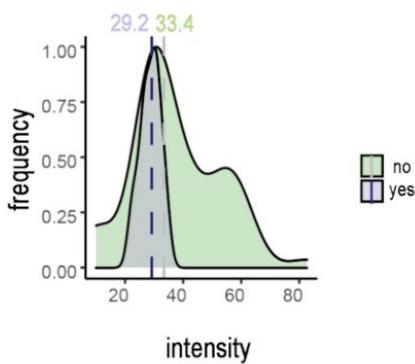
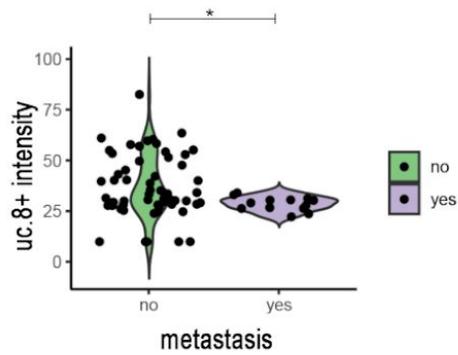
A



B



C



D

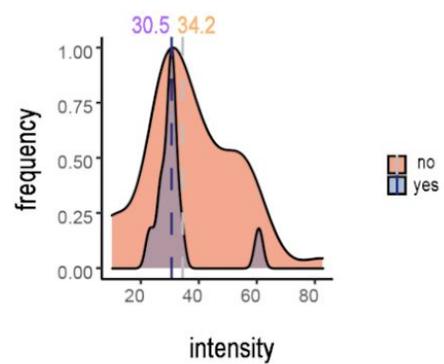
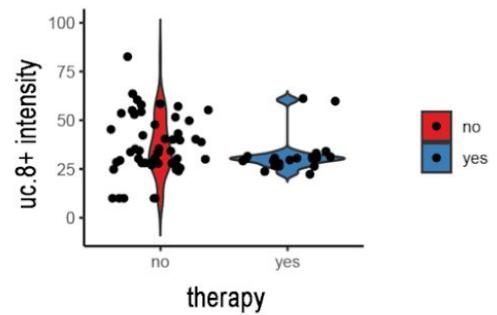
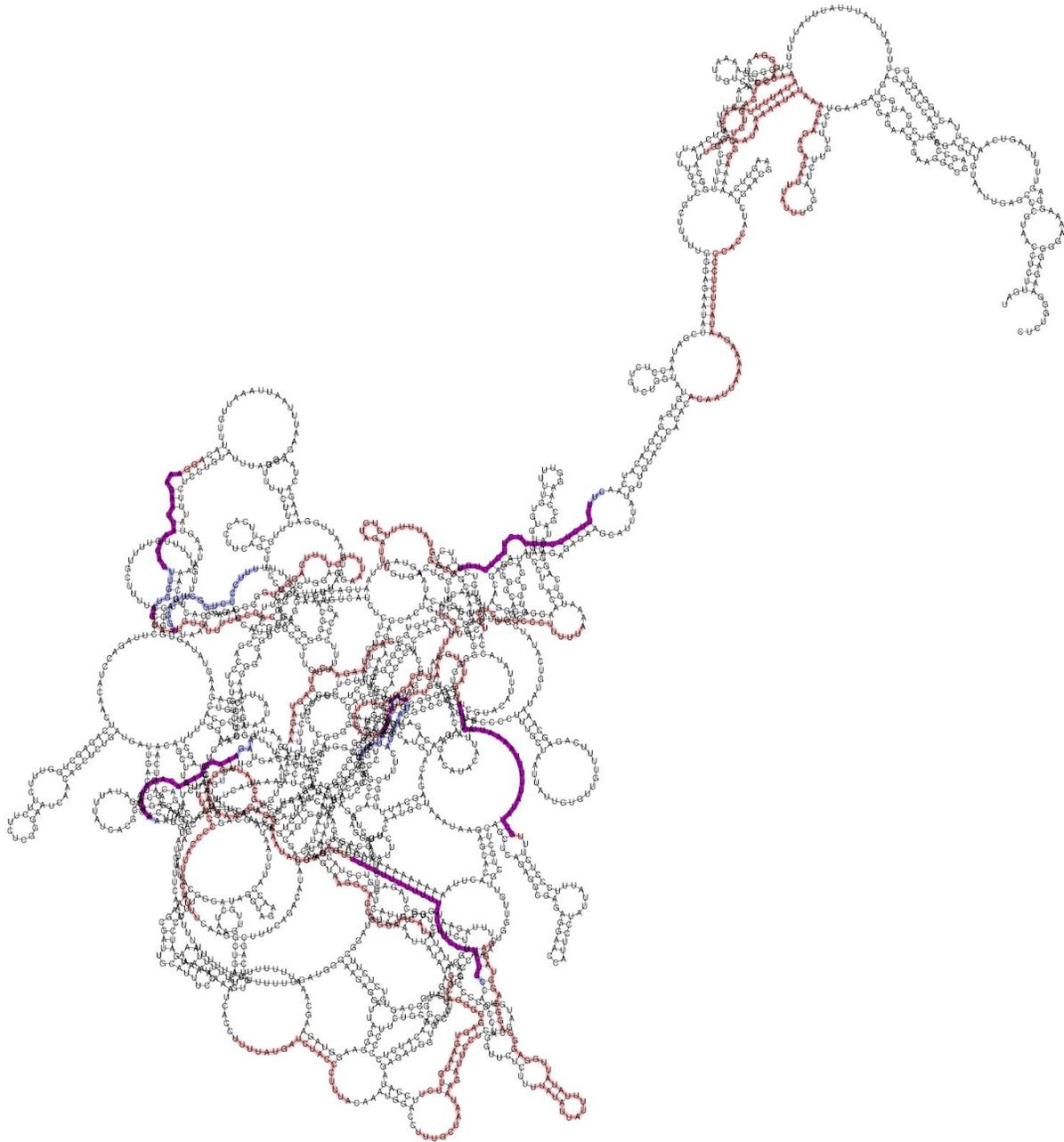
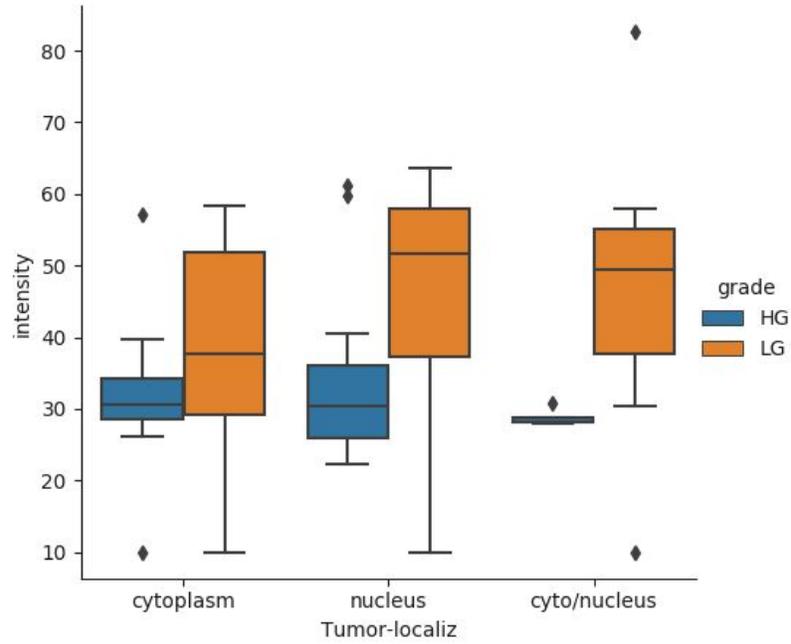


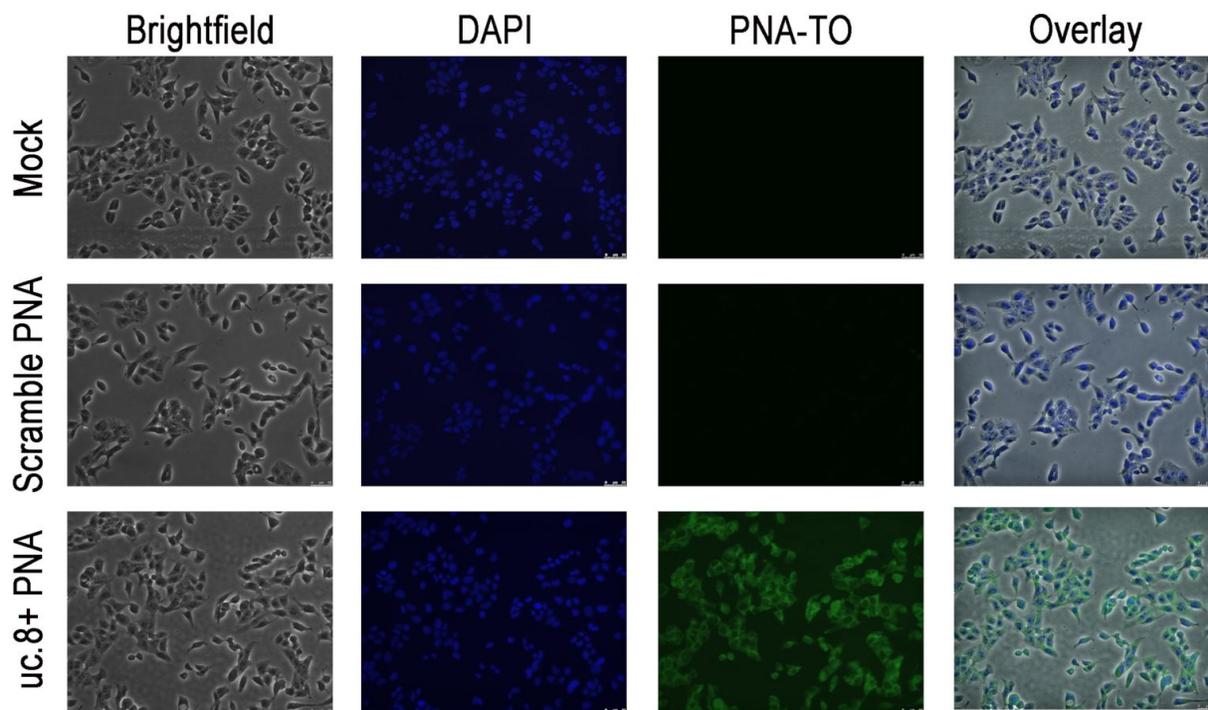
Figure S2. Violin and Frequency plots showing the correlation between the uc.8+ intensity score and clinical traits of patients cohort. (A) Association with sex, (B) age of pts, (C) metastasis and (D) therapy.



**Figure S3. Predicted secondary structure representation of the whole uc.8+.** Sequences associated with the predicted nuclear (light red) and cytosolic (light blue) localization. In purple the overlapped sequences of both localizations.



**Figure S4. uc.8+ intensity of sample belonging to HG and LG, with respect to the cellular localization.** Boxplots describing the relationship between intensity level of uc.8+ (Y axis) and cellular localization (X axis) in BICa tissues.



**Figure S5.** Fluorescence microscopy of J82 bladder cancer control cells (first lane, Mock) after transfection with PNA-TO scramble-R8 (second lane, scramble PNA) or with TO-PNA1-R8, complementary to uc.8+ sequence (third lane, uc.8+ PNA). Images were recorded with excitation wavelength ( $\lambda_{ex}$ )=450–490 nm (DAPI) or  $\lambda_{ex}$ =510–540 nm (PNA-TO); the superimposition of the images recorded is also reported (Merge). Scale bar, 100  $\mu$ m. Nuclei of J82 cells were stained with DAPI (blue).

**Table S1. Subcellular localization of uc.8+ in BICa patients.** Data were reported with respect to the lncRNA intensity expression and patients tumor Grade.

Localization	% of BICa patients			
	Grade		Intensity-score subgroups	
	LG	HG	HI	LI
cytoplasm	26	46	26	49
nucleus	30	38	37	35
nucleus-cytoplasm	44	16	37	16