Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Fabrication of an immunosensor for quantitative detection of breast cancer biomarker UBE2C

VSPK Sankara Aditya Jayanthi, Asim Bikas Das, Urmila Saxena*

Department of Biotechnology, National Institute of Technology Warangal, Warangal-506004, Telangana, India

***Corresponding author**

Dr. Urmila Saxena
Assistant Professor
Department of Biotechnology
National Institute of Technology Warangal
Warangal-506004,
Telangana, India
Mob: +91-8332969442
Email: urmila.saxena@gmail.com, urmila@nitw.ac.in

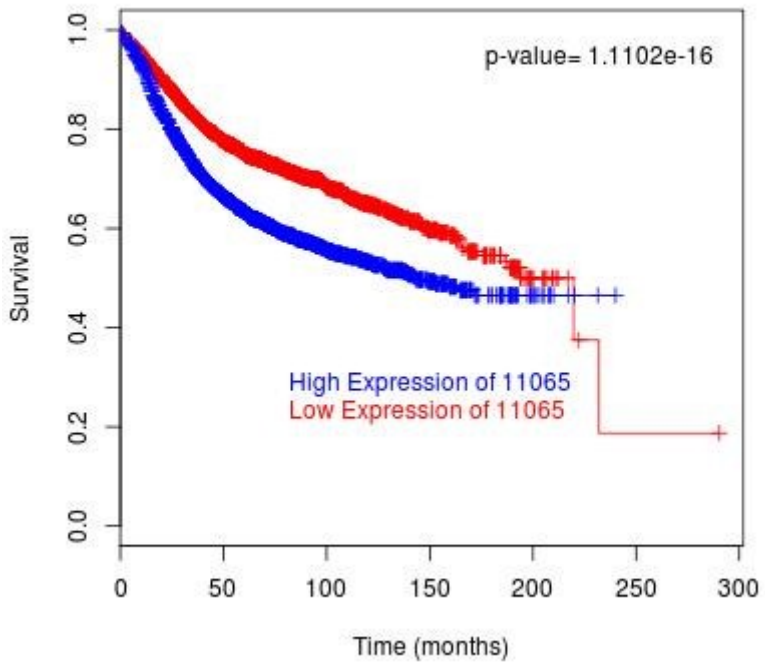

Figure S1. Survival analysis of breast cancer patients with respect to the expression of UBE2C. (n = 4640, Hazard ration = 1.515, P=1.11e^{-16}. Blue and red line represents the high and low expression groups respectively. Higher expression of UBEC is associated with lower patient survival compared to the lower expression.

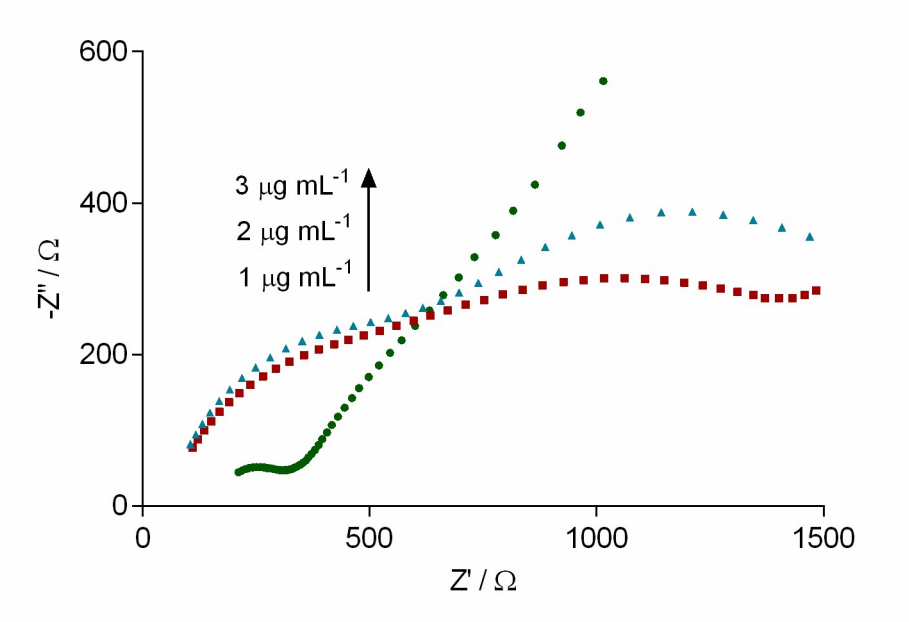

Figure S2. A) Electrochemical impedance spectra obtained towards UBE2C with immunosensors fabricated with varying concentrations of anti-UBE2C antibodies (1, 2, and 3 μg mL^{-1}. EIS measurements were carried out with an alternating wave of 10 mV amplitude in the frequency range between 10,000 and 0.05 Hz. in 0.1 M PBS (pH 7.4) with 5.0 mM [$K_3Fe(CN)_6/K_4Fe(CN)_6$] and 0.1 M KCl.

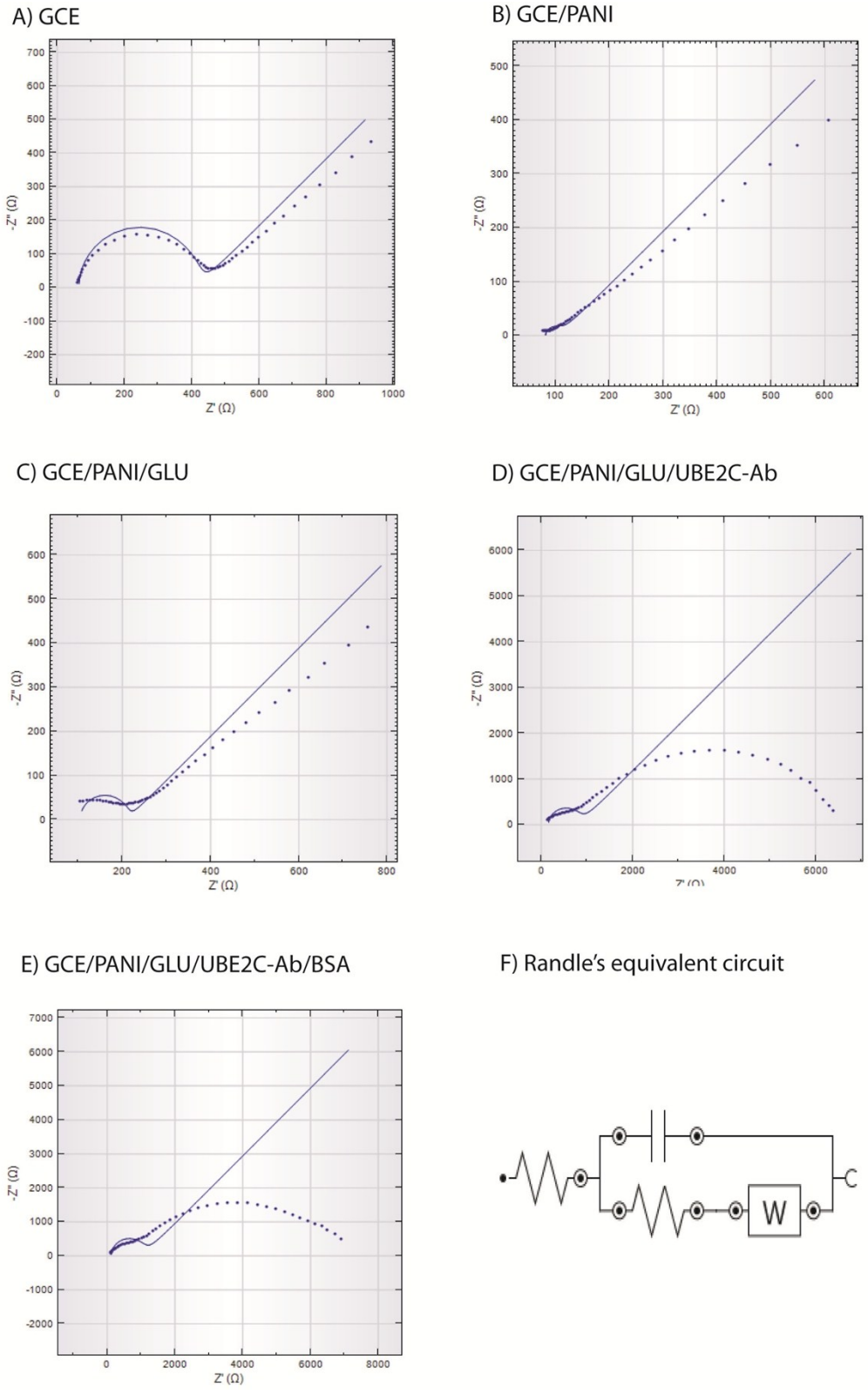

Figure S3. Fitting of electrochemical impedance spectra of A) GCE, B) GCE/PANI, C) GCE/PANI/GLU, D) GCE?PANI?GLU/UBE2C-Ab, and E)GCE/PANI/GLU/UBE2C-Ab/BSA into F) Randle's equivalent circuit with NOVA software.

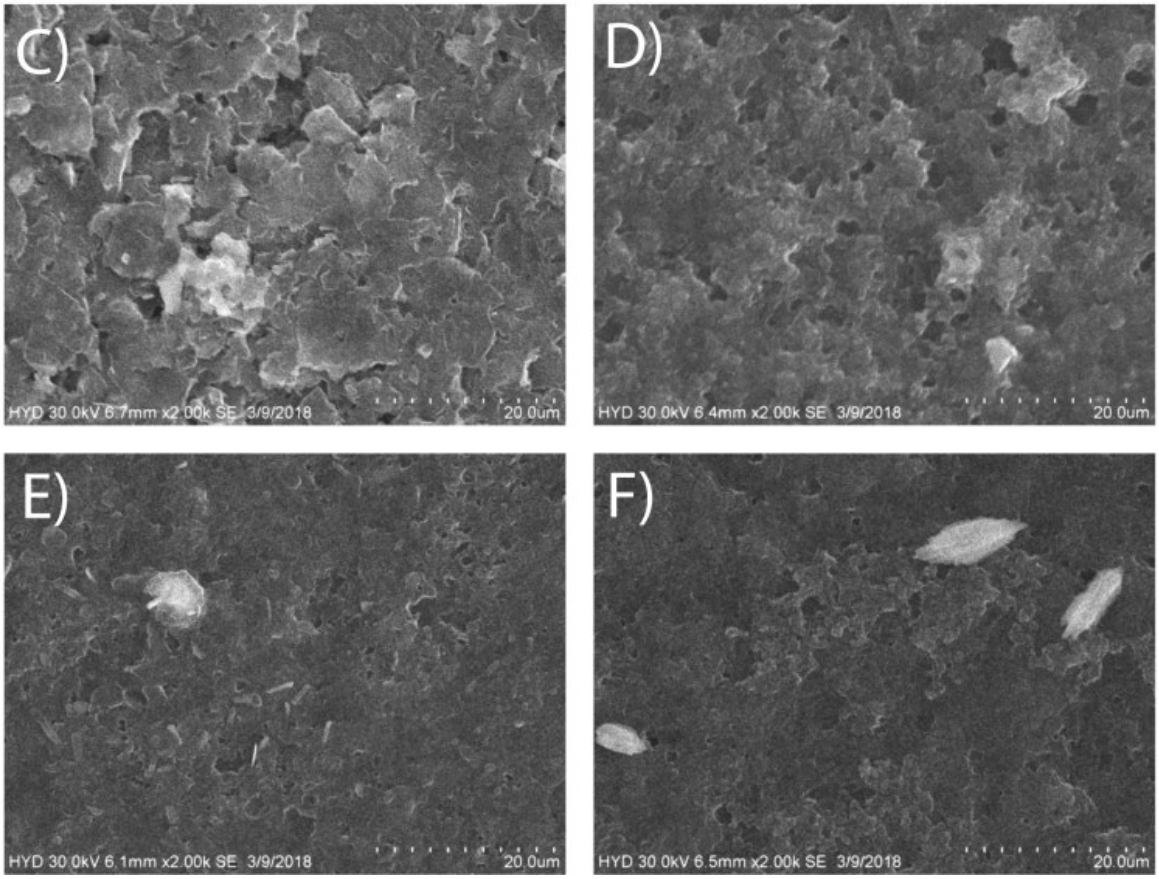

Figure. S4. Enlarged SEM images of figure 2C, D, E and F of the main manuscript.

Table S1: Parameters of fitting the EIS spectra of different electrodes in Randle's equivalent circuit.

Electrodes	**Convergence**	**Number of iterations**
GCE	Yes	46
GCE/PANI	Yes	66
GCE/PANI/GLU	Yes	58
GCE/PANI/GLU/UBE2C-Ab	Yes	82
GCE/PANI/GLU/UBE2C-Ab/BSA	Yes	33

*Convergence:Indication that the data fits the prescribed model, Iterations: the number of iteration used during the fitting of the data.

Table S2: Comparison of performance of the fabricated immunosensor with the electrochemical immunosensors reported in the literature

Biomarker	Immobilization support matrix	Linear range	Detection limit	Ref
Epidermal Growth Factor Receptor	Streptavidin coated magnetic beads,	1- 40 ng mL^{-1}	50 pg mL^{-1}	1
Cytokeratin 19 fragment 21-1 (CYFRA21-1)	Three-dimensional graphene (3D-G), chitosan (CS) and glutaraldehyde (GA) composite	0.1 - 150 ng mL^{-1}	43 pg mL^{-1}	2
Vascular endothelial growth factor(VEGF)	Gold nanoparticles	100-600 pg mL^{-1}	100 pg mL^{-1}	3
IL-13 receptor Rα2 (IL-13Rα2)	Carboxylic acid-modified magnetic microbeads (HOOC-MBs)	3.9-100 ng mL^{-1}	1.2 ng mL^{-1}	4
Prostate Specific Antigen	Gold nanoparticle and chitosan	0 - 100 ng mL^{-1}	7.8 ng mL^{-1}	5
Interleukin-8 (IL-8)	6-phosphonohexanoic acid	0.02 3 pg mL^{-1}	6 fg mL^{-1}	6
Ubiquitin-conjugating enzymes 2C (UBE2C)	Polyaniline	500 pg mL^{-1} – 5 μg mL^{-1}.	7.907 pg mL^{-1}	This work

References

1. H. Ilkhani, M. Sarparast, A. Noori, S. Z. Bathaie and M. F. Mousavia, *Biosens. Bioelectron.,* 2015, **74**, 491-497.

2. Y. Zeng, J. Bao, Y. Zhao, D. Huo, M. Chen, Y. Qi, M. Yang, H. Fa and C. Hou, *Bioelectrochemistry*, 2018, **120**, 183-189.

3. G. I. Kim, K. W. Kim, M. K. Oh and Y. M. Sung, *Biosens. Bioelectron.,* 2010, **25**, 1717-1722.

4. A. Valverde, E. Povedano, V. R. V. Montiel, P. Y. Sedeño, M. G. Asensio□, R. Barderas, S. Campuzano and J. M. Pingarrón, *Biosens. Bioelectron.,* 2018, **117**, 766-772.

5. D. Kiziltan, T. Vural, C. Bayram, S. Ozturk, B. Bozdogan, Y. T. Yaman, S. Abaci and E. B. Denkbas, *Anal. Sci.* 2018, **34**, 789-794.

6. E. B. Aydın and M. K. Sezgintürk, *Anal. Biochem.* 2018, **554**, 44-52.