Development of Extracellular Vesicle-based Liquid Biopsy for MYCN-amplified High-risk Neuroblastoma

Napat Rojsirikulchai,[1,2#] Jirawan Panachan,[3#] Nutkridta Pongsakul,[2] Ladawan Khowawisetsut,[4] Wararat Chiangjong,[2] Pongpak Pongphitcha,[3] Teerapong Siriboonpiputtana,[5] Pitichai Phornsarayuth,[5] Takol Chareonsirisuthigul,[5] Usanarat Anurathapan,[3] Kovit Pattanapanyasat,[6] Suradej Hongeng,[3] and Somchai Chutipongtanate[2,7,8*]

[1] Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand
[2] Pediatric Translational Research Unit, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand
[3] Division of Hematology and Oncology, Department of Pediatrics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand
[4] Department of Parasitology, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10700 Thailand
[5] Department of Pathology, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand
[6] Office for Research and Development, Faculty of Medicine Siriraj Hospital, Mahidol University, Bangkok, 10400 Thailand
[7] Department of Clinical Epidemiology and Biostatistics, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Bangkok, 10400 Thailand
[8] Chakri Naruebodindra Medical Institute, Faculty of Medicine Ramathibodi Hospital, Mahidol University, Samut Prakan, 10540 Thailand
# Share equal contributions
* Correspondence: schuti.rama@gmail.com, somchai.chu@mahidol.edu

Background

MYCN amplification is an important marker for diagnosing high-risk neuroblastoma (NB), an aggressive tumor with a poor prognosis (1-3). However, detecting MYCN amplification needs invasive procedures, e.g., bone marrow aspiration or tumor biopsies (4). Extracellular vesicles (EVs) are particles containing molecular signatures such as DNA, RNA, and protein from originate cells (5). EVs from NB tumor could be an invaluable source for detecting MYCN amplification as a less-invasive liquid biopsy procedures of high-risk NB. This study aimed to establish a method for detecting MYCN amplification status in EVs deriving from NB cell lines.

Methodology

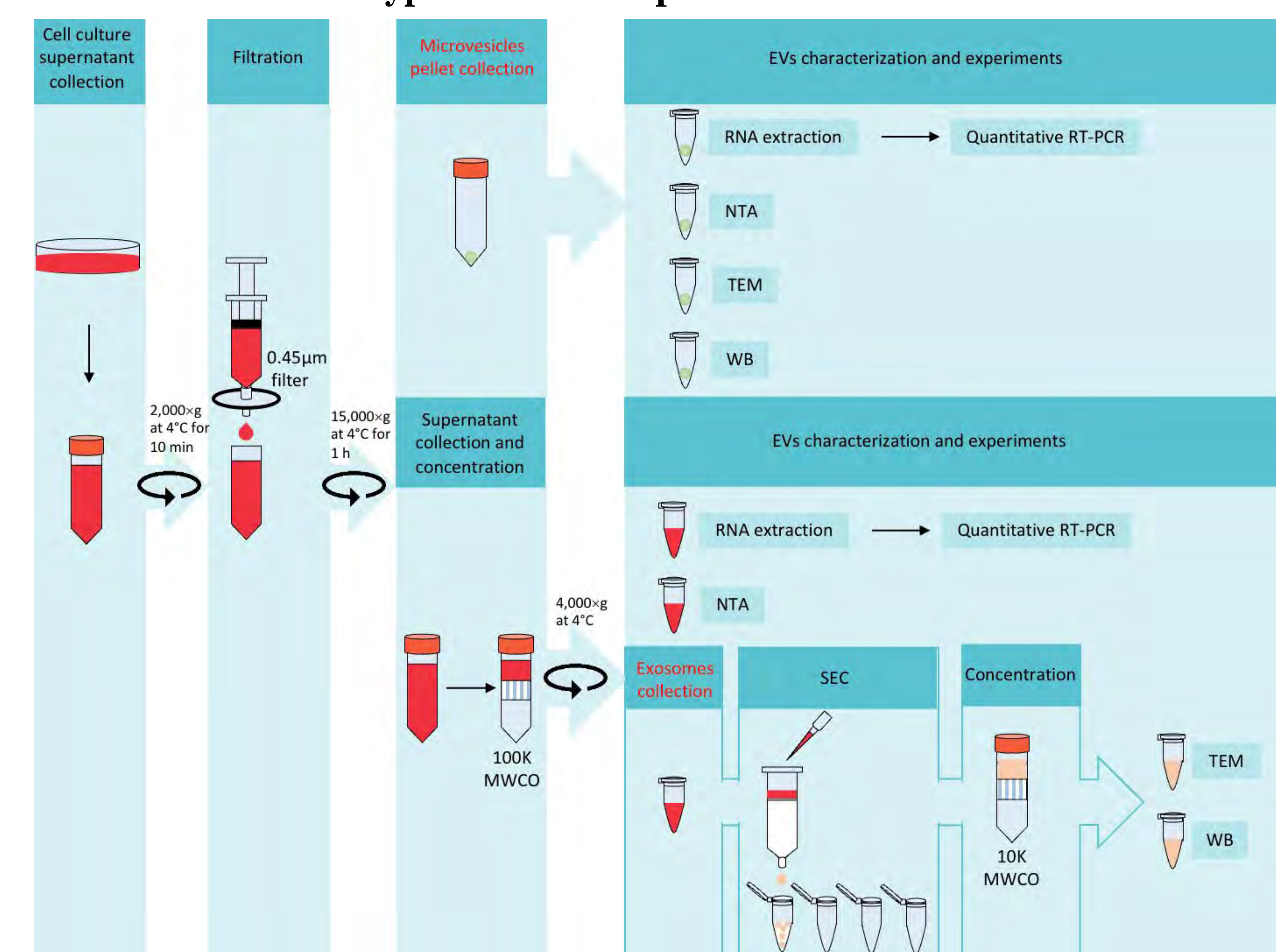### Isolation two EVs subtypes from NB supernatant

Figure 1: The schematic diagram of EVs isolation from NB supernatant and downstream experiments.

Results

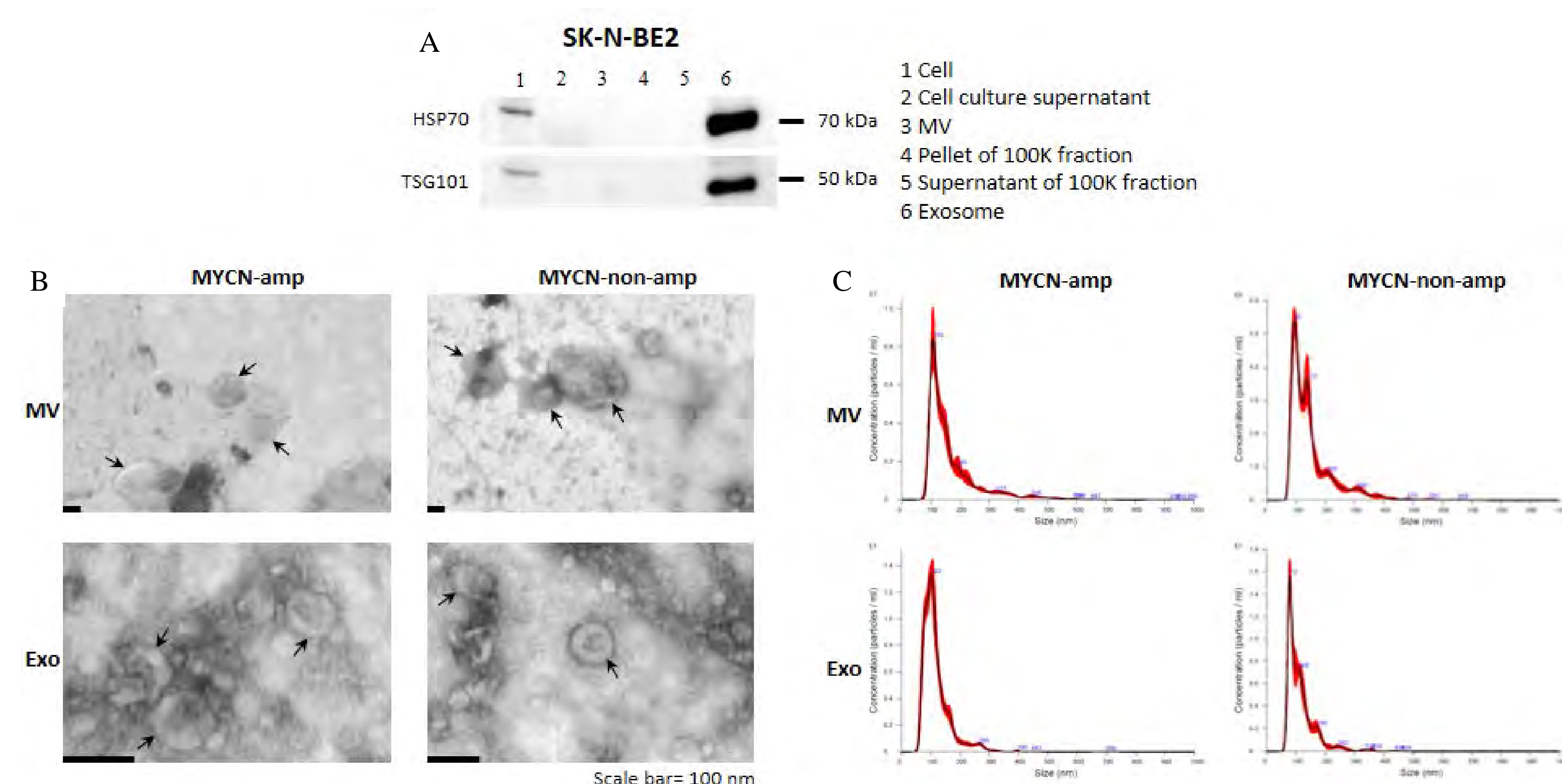### Characterization of EVs subpopulation in NB cell lines

Figure 2: EVs characterization. (A) Western blot analysis of SK-N-BE2-derived EVs protein markers. (B) Particle evidences of MYCN-amplified and MYCN-non-amplified NB cell lines-derived EVs were demonstrated by transmission electron microscopy (TEM). The arrows indicate particle evidences. (C) Size distribution of microvesicles (MV) and exosomes (Exo) of MYCN-amplified and MYCN-non-amplified NB cell lines were shown by nanoparticle tracking analysis (NTA).

Acknowledgements

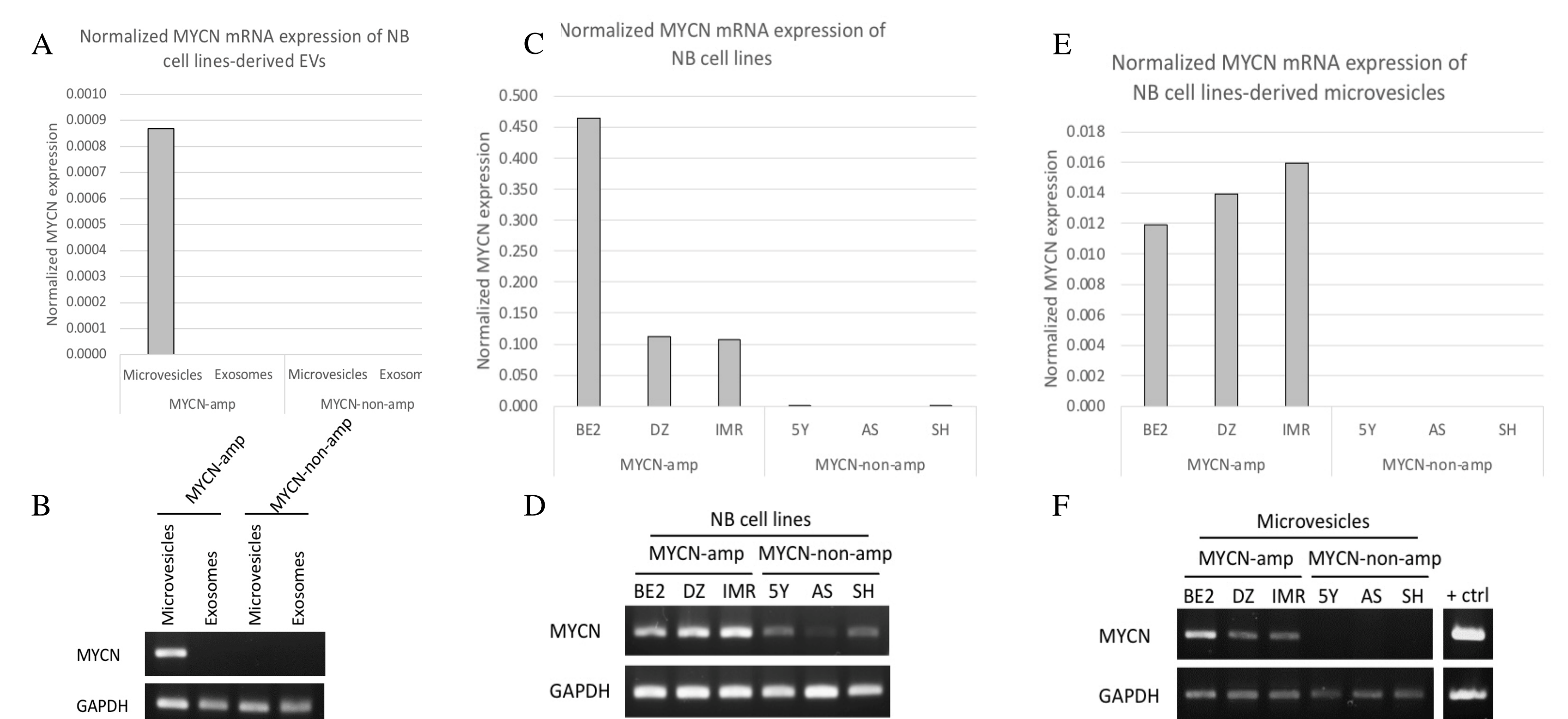### Detecting MYCN amplification status in NB-derived EVs subpopulations and NB cell lines

Figure 3: Detecting MYCN mRNA expressions in EVs subpopulations and cell lines of NB using quantitative RT-PCR (qRT-PCR) with GAPDH normalization. (A) MYCN amplification status solely expressed in microvesicles deriving from MYCN-amplified compared with MYCN-non-amplified NB cell lines. (C) MYCN mRNA expressions in MYCN-amplified (SK-N-BE2, BE2; SK-N-DZ, DZ; IMR-32, IMR) were higher than those in MYCN-non-amplified (SH-SY5Y, 5Y; SK-N-AS, AS; SK-N-SH, SH) NB cell lines. Consistently, (E) MYCN amplification status of NB cell lines-derived microvesicles. (B), (D), and (F) Confirming qRT-PCR products by agrarose gel electrophoresis of (A), (C), and (E), respectively.
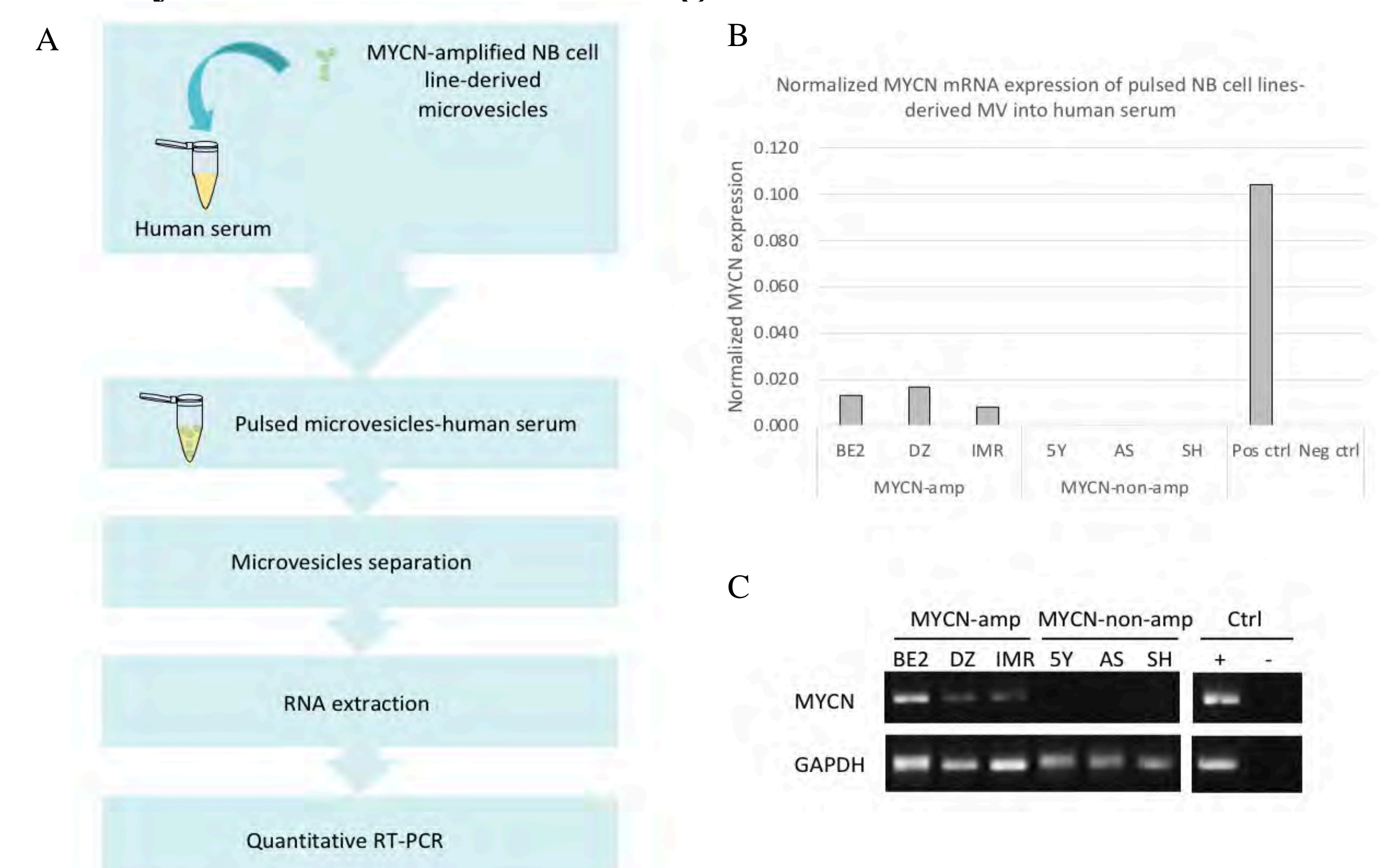
The sensitivity of MV-MYCN detecting workflow

Figure 4: The sensitivity analysis of MV-based detection of MYCN amplification status using the simulated NB serum. (A) The diagram presents the workflow of MV-MYCN mRNA sensitivity analysis performed by pulsing NB-derived MV into the human serum (purchased from Sigma-Aldrich), before MYCN mRNA measurement by qRT-PCR. (B) The MYCN amplification was detected from the isolated MV (MYCN-amplified and MYCN-non-amplified groups) in different-simulated NB serum. (C) qRT-PCR products were shown by agrarose gel electrophoresis, SK-N-BE2 cell line was served as positive control and human serum without MV pulsing was served as the negative condition.

Conclusion

This study established the microvesicle-based liquid biopsy workflow for detecting MYCN-amplification status of NB cell lines. Further validation using clinical specimens serves as less-invasive liquid biopsy procedure for the high-risk NB diagnosis.

References

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Mahidol University
Faculty of Medicine Ramathibodi Hospital