

Supplementary Figure 1. Phenotypic Characterization of Pluripotency Markers in hiPSCs. (A) hiPSC clones exhibit typical colony morphology and positive alkaline phosphatase (AP) activity, indicative of pluripotency. (B) Immunofluorescence staining of hiPSC colonies reveals robust expression of key pluripotency markers OCT4 and NANOG. (C) Flow cytometry analysis confirms a highly pure stem cell population in hiPSCs, with high levels of SSEA4 expression. (D) PCR analysis of hiPSC colonies demonstrates robust expression of pluripotency markers *OCT4* and *NANOG*, while germline differentiation markers *SOX1* (ectoderm), *TBXT* (mesoderm), and *SOX17* (endoderm) are absent, indicating maintenance of an undifferentiated state. scale bar: 100 $\mu$ m.

Supplementary Figure 2. Electrophysiological properties of motor neurons derived from encapsulated EBs. Representative current-clamp recordings illustrate the diverse firing patterns in response to stepwise depolarizing current injections (0–100 pA), indicating the functional excitability of derived motor neurons. Bar graphs summarize electrophysiological parameters, including action potential amplitude (n = 27), resting membrane potential (n = 21), and spike frequency (n = 10), confirming the acquisition of mature electrophysiological features. Data are presented as mean  $\pm$  SD.