

Enhancing Viability and Function of Electroporated Primary Human Hepatocytes: Implications for In Vitro Evaluation of Oligonucleotide Therapeutics

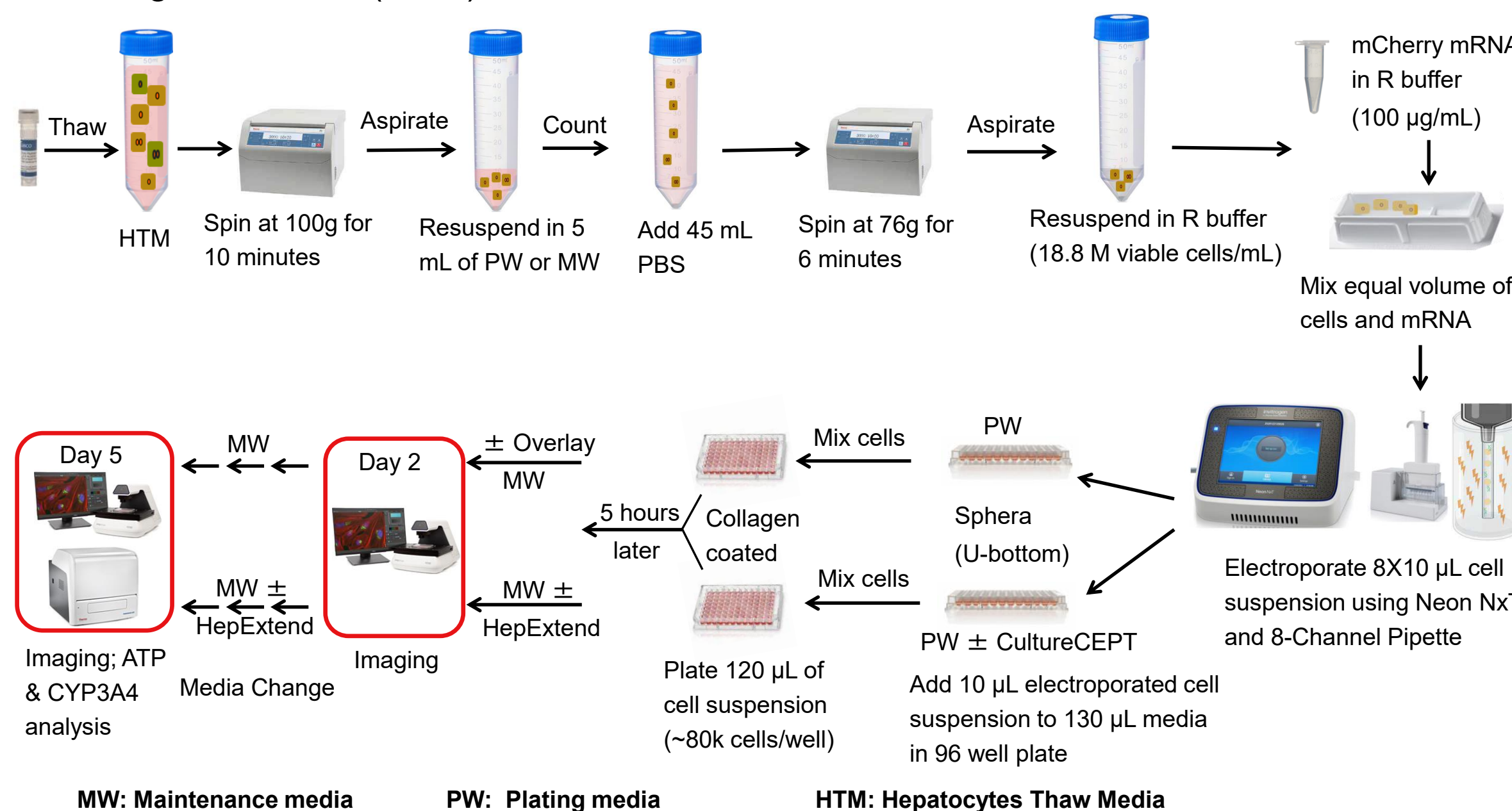
Rohit Jindal, Aaron Spratt, Kevin Pridham, Wadad Waga, Jacquelyn McClenny and David Kuninger
Thermo Fisher Scientific, 7300 Governors Way, Frederick, Maryland, 21704

ABSTRACT

Liver is an important target organ for oligonucleotide therapeutics due to ease of delivery and its involvement in several monogenic disorders. In vitro models assembled from primary human hepatocytes (PHH) facilitate physiologically relevant system for evaluating efficacy, toxicity, pharmacokinetics and any potential off target effect of oligonucleotides. Electroporation of PHH enables relatively flexible route for introducing a wide range of oligonucleotides-based therapeutics in cells and thereby facilitate their independent evaluation not confounded by the effect of delivery vehicle. However, during electroporation PHH are subjected to high voltage pulses, which requires careful selection of voltage program for achieving efficient delivery while maintaining high viability and functionality of the cells. In this work, we utilized NeonTM NxT Electroporation System to assess transfection of mRNA (CleanCap[®] mCherry) in PHH at 24 electroporation programs ranging in voltage from 850 to 1700 V in conjunction with pulse width and frequency varying from 10-40 ms and 1-3, respectively. Fluorescence along with ATP and CYP3A4 levels indicated that the electroporation program comprising of the voltage of 1050 V, 30 ms pulse width and pulse frequency of 2 efficiently transfected mRNA while maintaining high viability and functionality of PHH in sandwich culture. Evaluation of additional donors using this program highlighted the disparate sensitivity of donors to electroporation parameters whereby one of the three donors showed significantly reduced transfection, viability and function. Additional experiments were conducted without the overlay or sandwich configuration as this is the preferred workflow that is more compatible with other in-vivo relevant delivery modalities such as LNPs (lipid nano particles) that may eventually be employed for evaluating the efficacy of fully assembled system. Without the overlay, both viability and metabolic function reduced not only for no-electroporation control but further deteriorated for cells subjected to electroporation. Our previous studies indicate that media supplemented with HepExtend[™] supplement can improve function of PHH in sandwich culture in part by reducing oxidative stress (1). Following on this study, the supplementation of media with HepExtend dramatically enhanced viability and functionality of the electroporated cells cultured without the overlay as well. Finally, using the same electroporation parameters successful editing of HPRT gene was achieved by delivering Cas9-gRNA complex and like mRNA delivery viability and functionality were improved by supplementing the media with HepExtend. In summary, electroporation conditions described above can be used for robust transfection of PHH with some donor-to-donor variability that can be addressed by pre-screening for a donor or perhaps donor specific optimization of electroporation conditions. Additionally, media supplements such as HepExtend can significantly improve performance of electroporated PHH cultured without the overlay. These are important considerations for evaluating oligonucleotide therapeutics in primary human hepatocytes that retain high viability and functionality.

METHODS

The overall workflow for transfection of PHH with mRNA (CleanCap[®] mCherry from TriLink) and subsequent culture under various culture configurations are shown below. Additional details for culturing PHH can be found elsewhere (2). Fluorescence and phase images were acquired using EVOS[™] M7000 Imaging System. ATP and CYP3A4 levels were assessed using CellTiter-Glo[®] 2.0 Cell Viability Assay and P450-Glo[™] CYP3A4 Assay (Promega). For gene editing experiments, gRNA for HPRT gene was complexed with TrueCut[™] Cas9 Protein v2 in R buffer and incubated at RT for 20 minutes. For instance, to 86.4 μ L of the R buffer, 10 μ L of Cas 9 (5 μ g/ μ L) was added and mixed. Next, 3.6 μ L of gRNA (100 μ M) was added followed by mixing and incubation. Next, 100 μ L of cells suspended in R buffer were added to the Cas9-gRNA complex and mixed once using a wide bore pipette tip. The contents were transferred to a reservoir for conducting electroporation as shown in the workflow below. The cells were recovered and analyzed for genome cleavage using the GeneArt[®] Genomic Cleavage Detection (GCD) Kit.



RESULTS

Figure 1. Relative levels of Viability, Metabolic Function and Transfection for different Neon NxT electroporation programs (Sandwich culture)

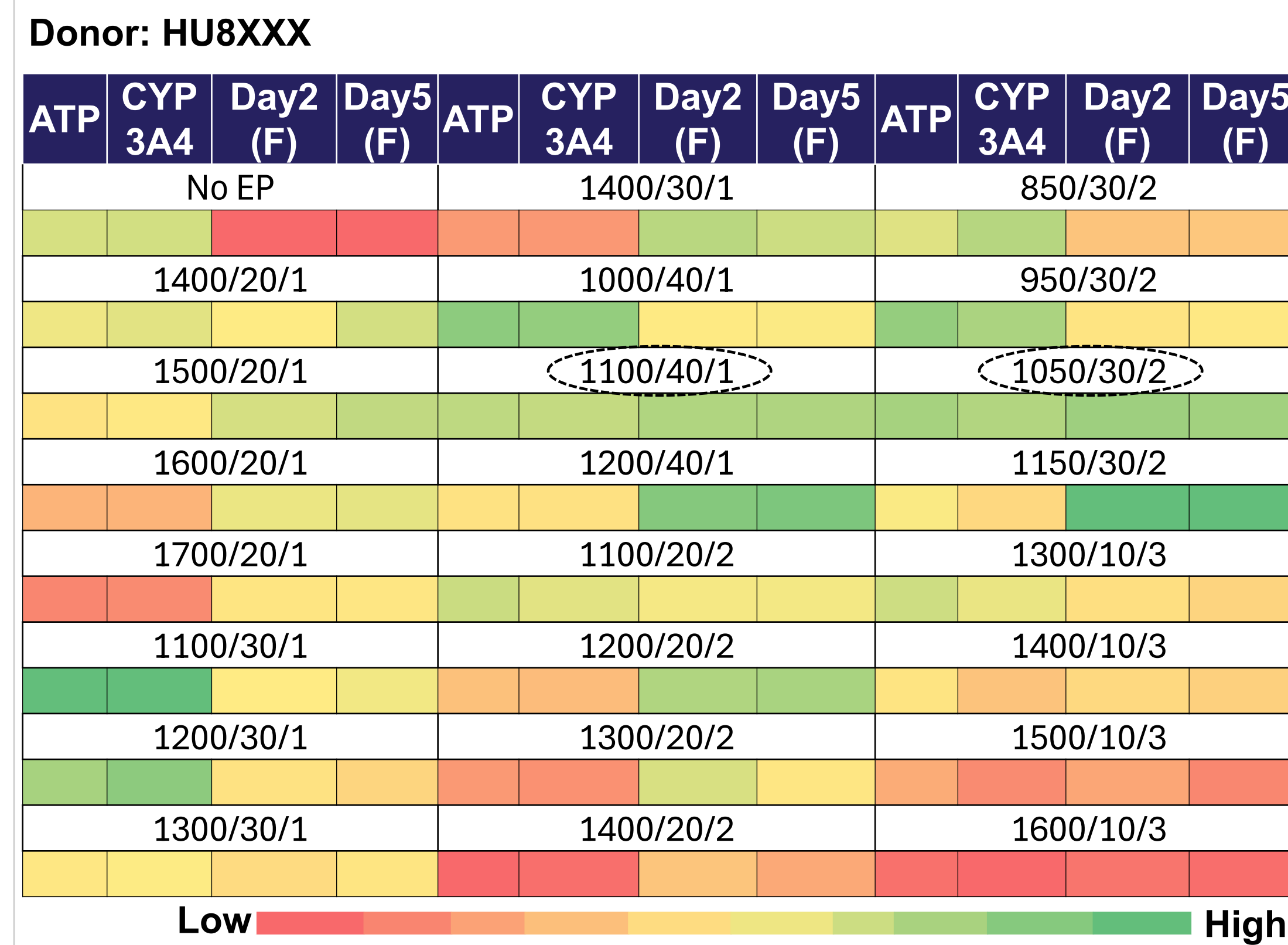
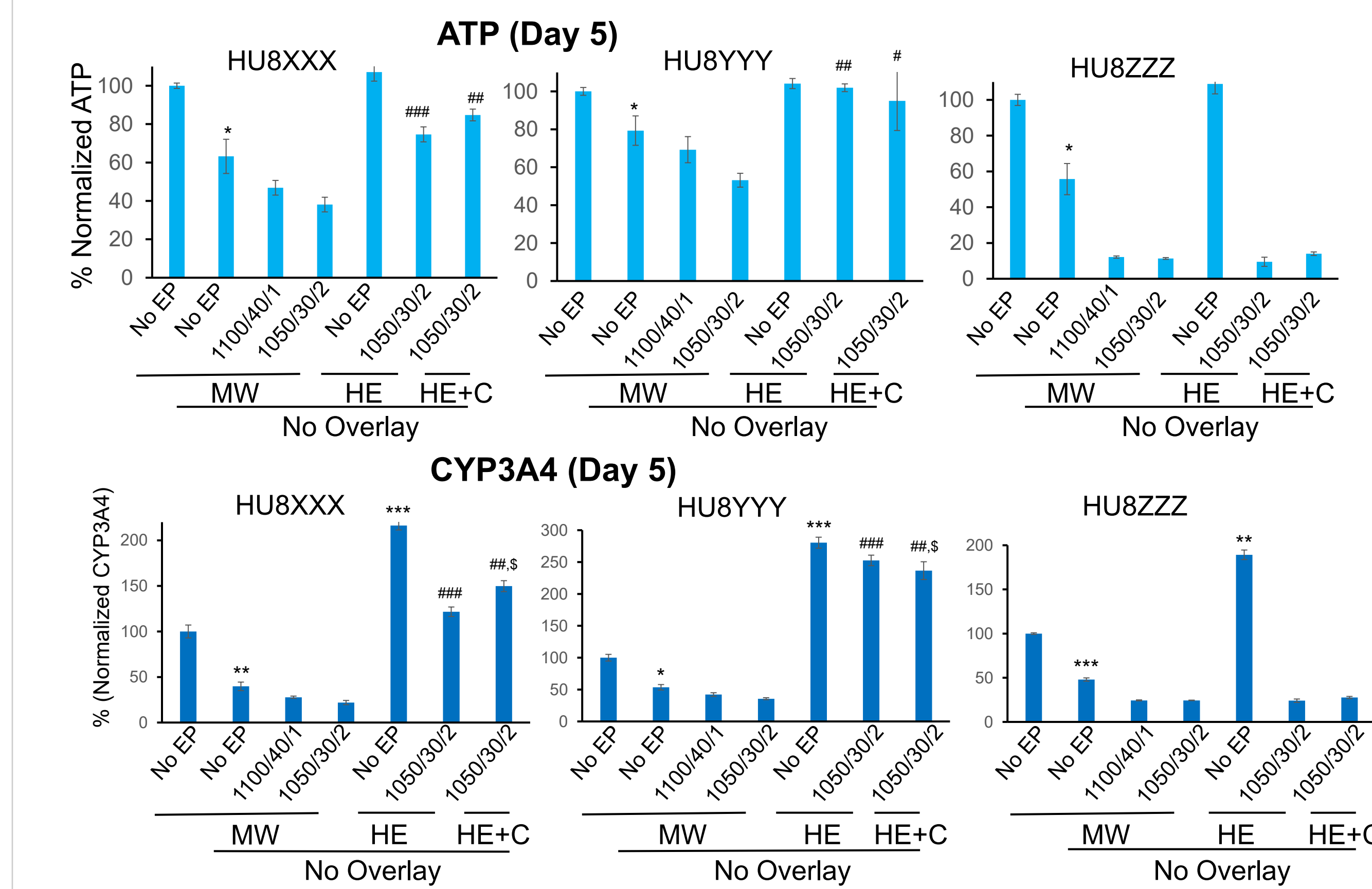


Figure 3. HepExtend better maintains ATP and CYP3A4 for Cultures without Overlay



ATP and CYP3A4 levels normalized to the condition: No-EP cells in sandwich (overlay) culture and maintenance media (MW). HE and C denotes HepExtend supplemented maintenance media and CultureCEPT supplemented plating media, respectively.

Figure 5. Program 1050/30/2 Supports Delivery of Cas9-gRNA RNP for Gene Editing in Cultures without Overlay

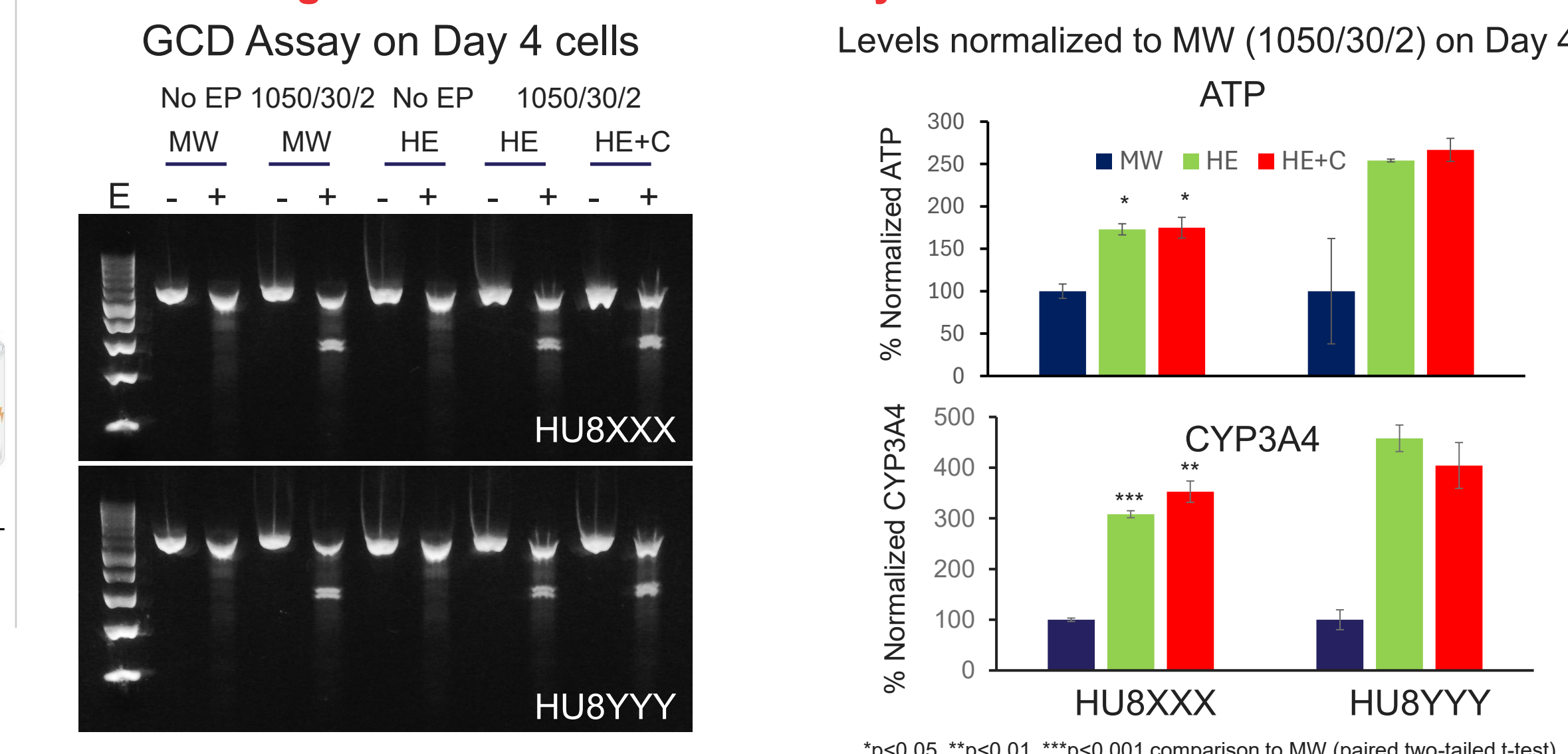


Figure 2. High Transfection Efficiency and Maintenance of ATP and CYP3A4 for Electroporated Cells in Sandwich culture

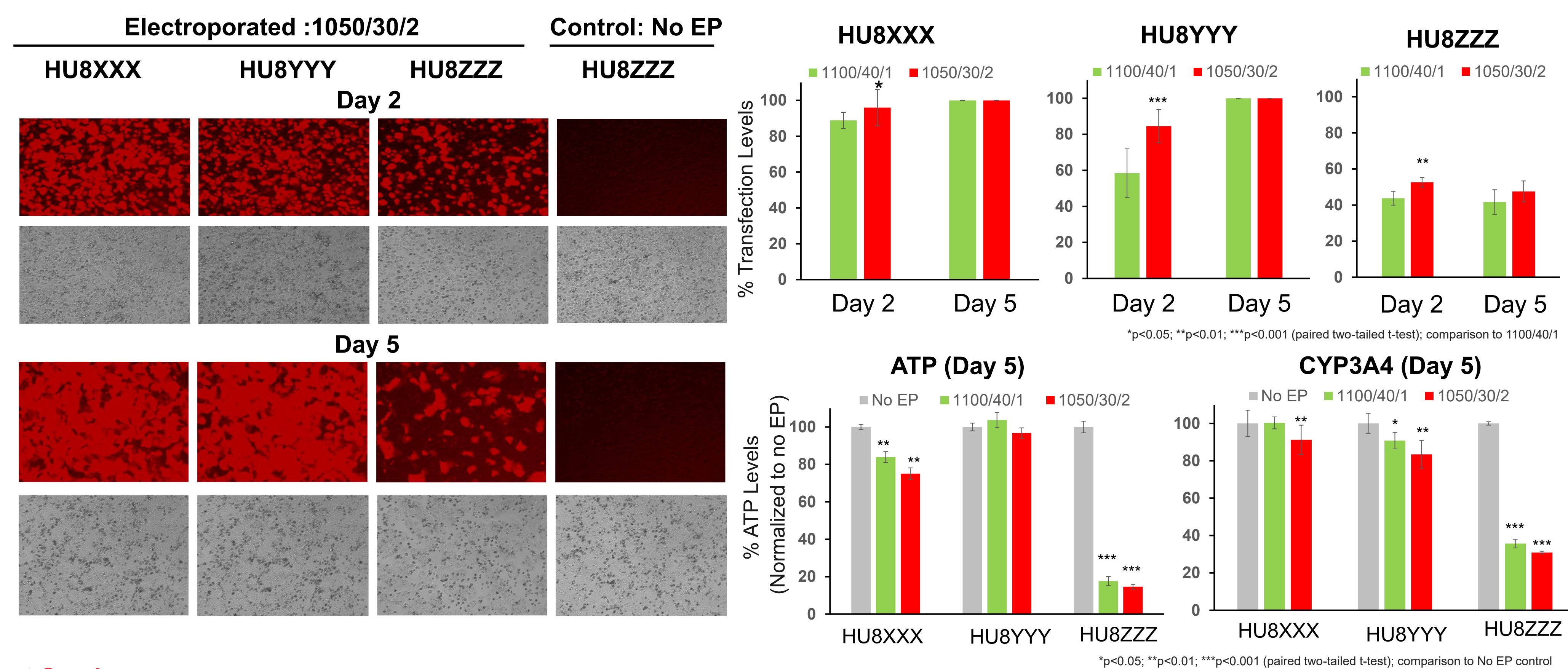
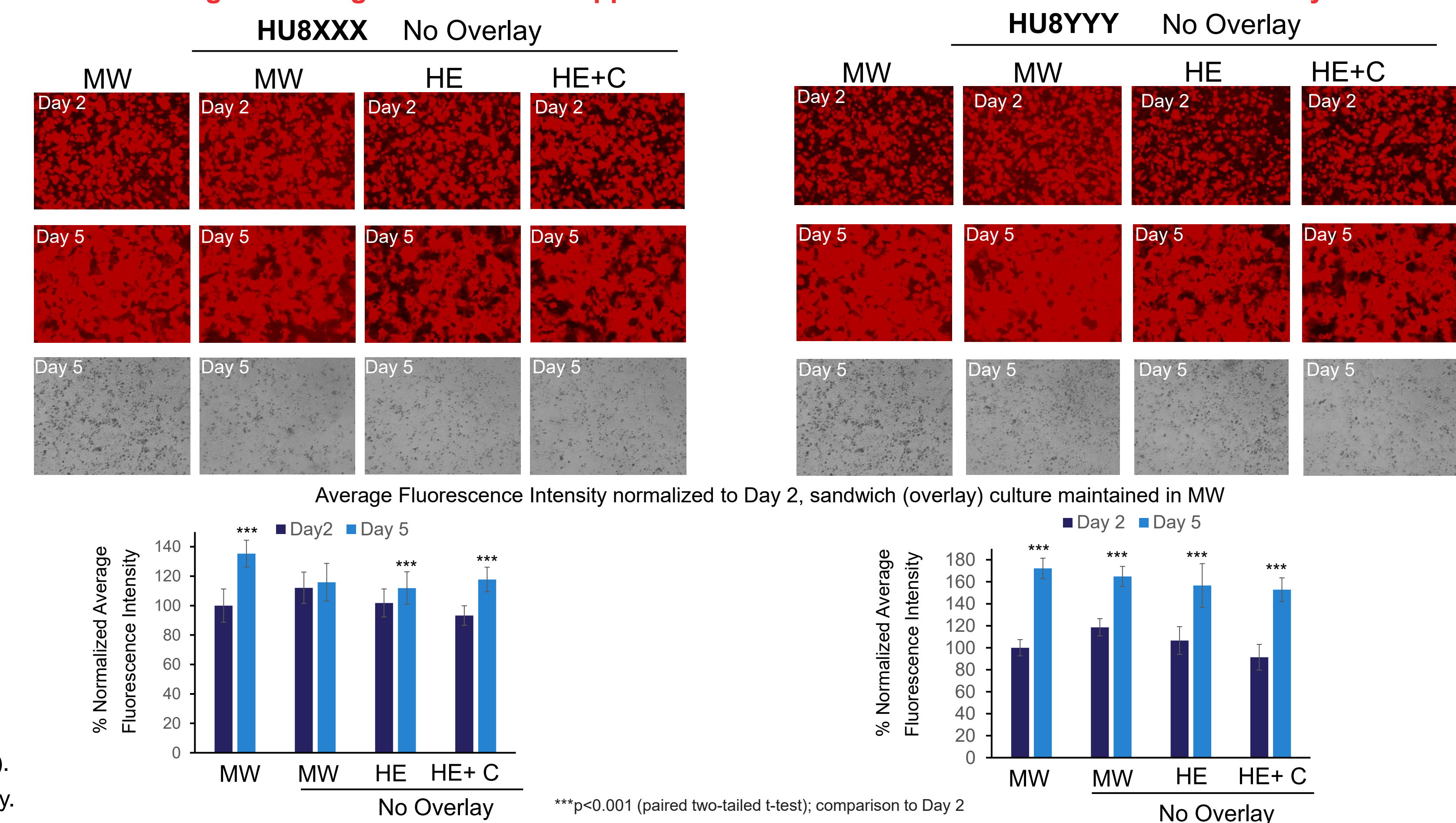


Figure 4. Program 1050/30/2 Supports Robust Transfection in Cultures without Overlay



SUMMARY

- Primary human hepatocytes (PHH) can be robustly transfected with mRNA using NeonNxT program P19 (1050/30/2) while maintaining high viability and functionality in sandwich culture.
 - There is donor variability, which can be addressed by lot selection or perhaps employment of "gentler" program.
- HepExtend supplementation to the maintenance media dramatically improves viability and metabolic function of hepatocytes cultured without overlay.
 - The ATP level recovers to that observed in sandwich culture, while CYP3A4 function increases beyond that.
 - Supplementation of CEPT to the plating media had marginal effect when the cells were subsequently maintained in HepExtend.
- Fluorescence level increases from day 2 to day 5 for hepatocytes transfected with mRNA of mCherry and maintained in culture with and without overlay.
- Using the same program P19 successful editing of HPRT gene was achieved by delivery of Cas9-gRNA RNP.

REFERENCES

- Jindal R, Tritapoe J, Dulany C, Kuninger, D Role of Oxidative Stress in HepExtend Supplement Mediated Improvement in Metabolic & Transporter Function of Primary Human Hepatocytes. ISSX/MDO 2022 meeting abstracts, Supplement 16, No. 1, P75, 76.
- <https://www.thermofisher.com/us/en/home/references/protocols/drug-discovery/adme-tox-protocols/thawing-and-plating-hepatocytes-protocol.html>