Dear Editor,

Please find enclosed the second revision of our manuscript "**Efficient and scalable directed differentiation of clinically compatible corneal limbal epithelial stem cells from human pluripotent stem cells**" by Hongisto and Vattulainen et al. We have revised the text according to the editorial comments. All changes made to the manuscript are tracked with blue shading. The part chosen by the authors to be filmed is highlighted with yellow shading. Responses for the editorial comments are provided below.

We hope that You find the responses given and changes made satisfactory and find our protocol suitable for publication in JoVE.

Sincerely,

Heidi Hongisto, PhD
BioMediTech Institute
Faculty of Medicine and Life Sciences
University of Tampere
Arvo Ylpön katu 34
33520 Tampere
FINLAND
E-mail: heidi.m.hongisto@uta.fi
Phone: +358 40 556 7493

Editorial comments:

Note that some formatting changes have been made, including protocol numbering (e.g., A.1 is now 1.1).

Editorial comment 1. Much of the media names (Essential 8/E8, Knockout/Ko, CnT-30, TrypLE, Defined Trypsin Inhibitor, possibly others) are commercial. Could you change these to generic terms/abbreviations (including in Figure 1A)?

Author response:

We have changed the following names and abbreviations:

"Essential 8 Flex/E8 medium" changed to "hPSC medium" (also in Figure 1A)

"TrypLE Select Enzyme/Tryple Select" changed to "xeno-free trypsin-EDTA"

"Defined Trypsin Inhibitor" changed to "defined trypsin inhibitor"

"XF-Ko-SR medium" changed to "basal induction medium" ("Induction medium" in Figure 1A)

"KnockOut Dulbecco's modified Eagle's medium" changed to "Dulbecco's modified Eagle's medium"

"KnockOut SR XenoFree CTS" changed to "xeno-free serum replacement"

"GlutaMAX" changed to "L-glutamine"

"MEM non-essential amino acids" changed to "non-essential amino acids"

"CnT-30 medium" changed to "differentiation medium" (also in Figure 1A)

As the protocol relies on the use of very specific, commercial hPSC and corneal epithelial differentiation media and reagents, we have mentioned the commercial product names at first use. Additionally we have added a note on this before the protocol section and added a sentence on the matter to the discussion section. If this is not applicable with journal formatting, the commercial names can be removed.

Editorial comment 2. 2.2.3 (formerly A.2.3): How is media replaced?

Author response:

"2.2.3. On the following day (Day 2), replace the medium with **Induction medium 1**." changed to "2.2.3 2.2.3. On the following day (Day 2), remove the medium and replace with 3 mL of basal induction medium supplemented with 10 µM SB-505124 and 50 ng/mL bFGF."

"2.2.4. On the following two days (Days 3-4), replace the medium with Induction medium 2."changed to "2.2.4. On the following two days (Days 3-4), remove the medium and replace with 3 mL of basal induction medium supplemented with 25 ng/mL BMP-4.

Editorial comment 3. 3.3.1.5 (formerly C.3.1.5): What volume of wash buffer?

Author response: Wash buffer volume of 1 mL added.

Editorial comment 4. 3.3.2 (formerly C.3.2): Please provide more detail about this step or a reference. Analyze how, exactly?

Author response: "3.3.2. Analyze the samples with a flow cytometer." changed to "3.3.2. Analyze the samples with a flow cytometer. Use the unstained negative control sample for gating of the correct cell population, and for excluding the fluorescent background signal. Analyze a minimum of 10, 000 p63-α - stained cells. For detailed technical implementation, please refer to the user manual of the given flow cytometer."