**Stem Cell Reports, Volume *1***

# Supplemental Information

# Derivation and FACS-Mediated Purification of PAX3+/PAX7+ Skeletal Muscle Precursors from Human Pluripotent Stem Cells

Bianca Borchin, Joseph Chen, and Tiziano Barberi

SUPPLEMENTAL INFORMATION INVENTORY

A total of three figures with relative legends and two tables are included.

Figure S1 is related to Figure 1 and provides supporting information about the effect of GSK3β inhibition.

Figure S2 is related to Figure 4 and adds data about the progression of the myogenic program in sorted populations.

Figure S3 is related to Figure 6 and demonstrates the comparable efficiency of myogenic induction across all four hPSC lines.

Table S1 is a list of all the primers used in this study.

Table S2 is a list of all antibodies used in this study.

SUPPLEMENTAL INFORMATION

**Figure S1 related to Figure 1**

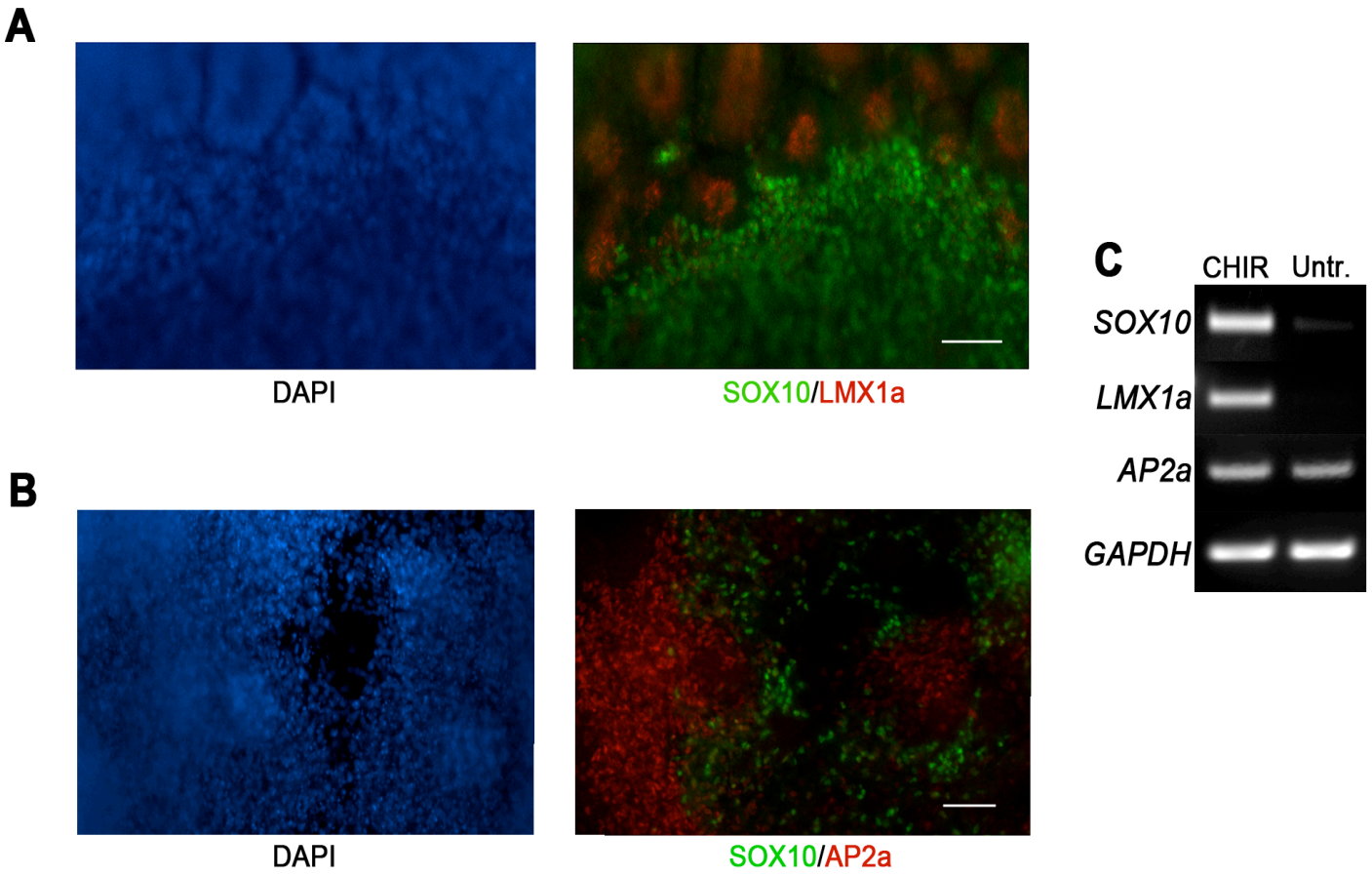

**Figure S2 related to Figure 4**

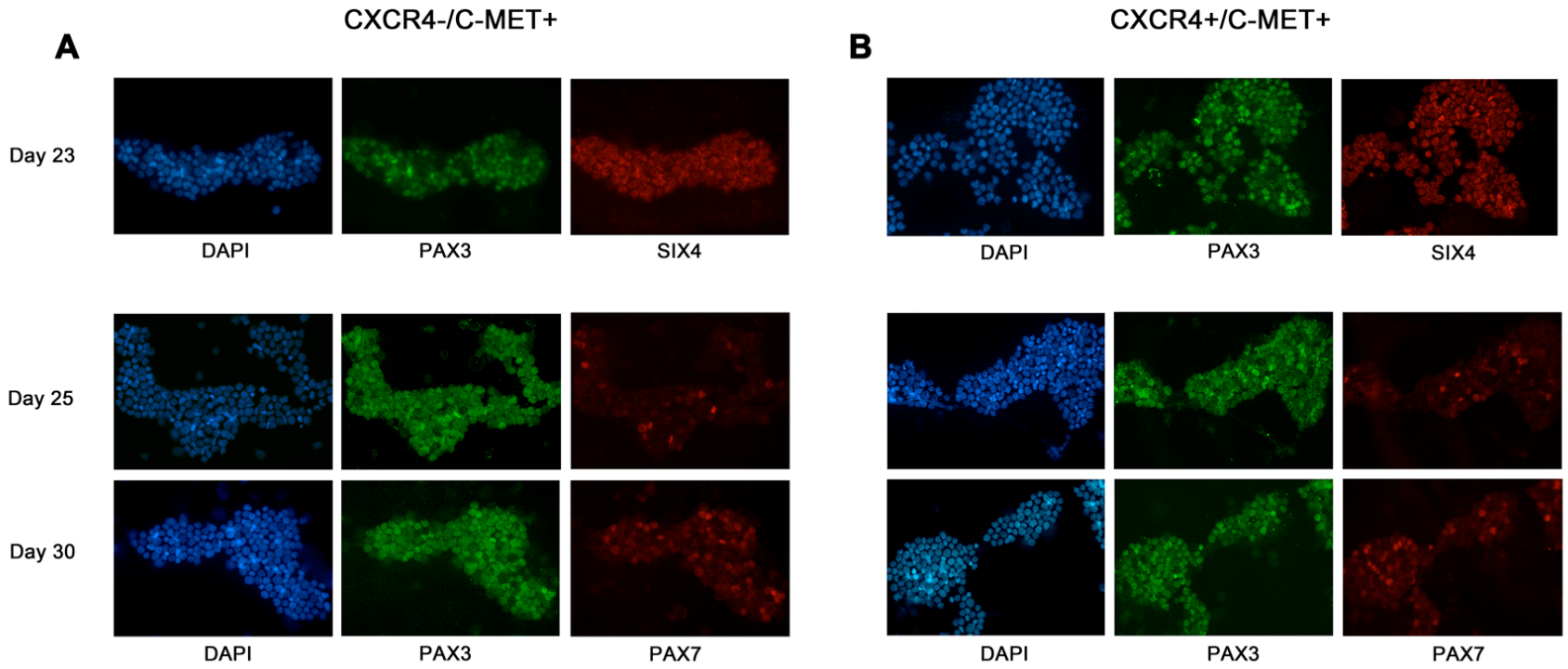

**Figure S3 related to 6**

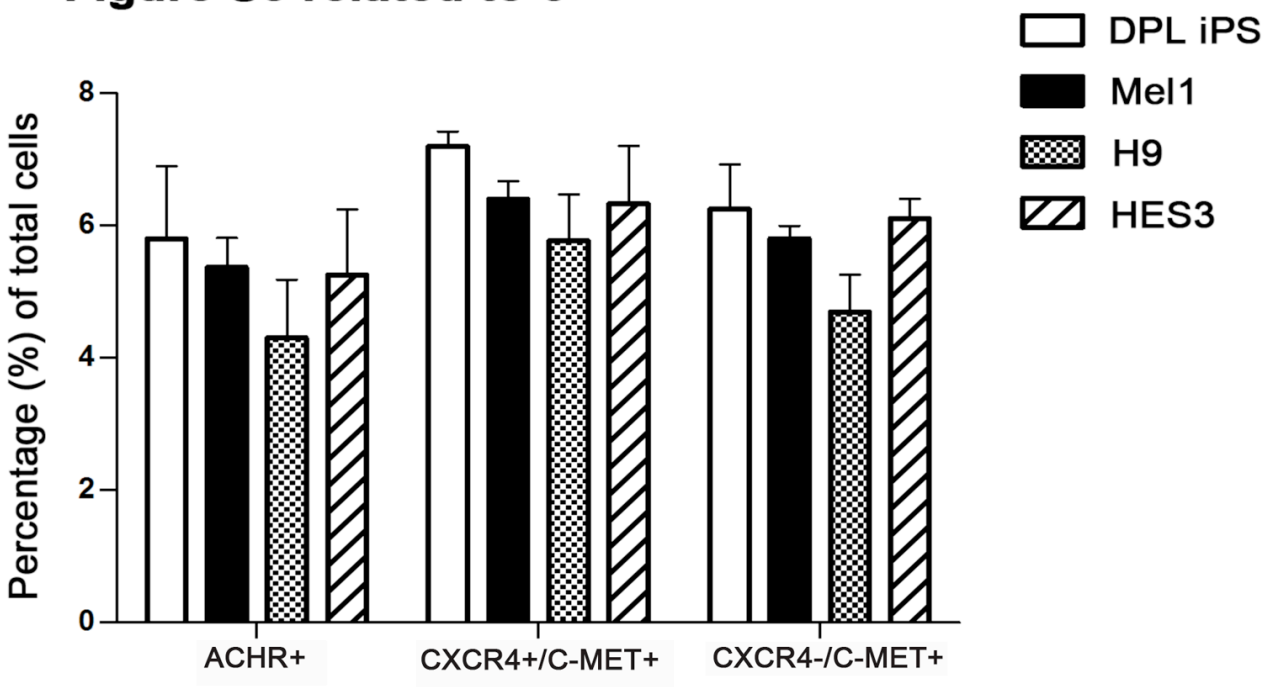

**FIGURE LEGENDS**

**Figure S1. CHIR treatment promotes induction of dorsal tissue in differentiating hPSC.** Immunocytochemical analysis of hESC (H9) at day 12 of differentiation under treatment conditions, showing **(A)** Neural crest marker SOX10 (green), dorsal neural tube/roof plate marker LMX1a (red) and **(B)** SOX10 (green) and non-neural ectoderm marker AP2$\alpha$ (red) Scale bars: 50 μm. **(C)** RT-PCR analysis for *LMX1a*, *SOX10* and *AP2*$\alpha$ expression at day 10 of hESC (HES3) differentiation. *SOX10* and *LMX1a* are expressed only in CHIR treated cells while *AP2*$\alpha$ expression is detected also in untreated cells.

**Figure S2. Developmental progression of hPSC-derived myogenic cell populations.** Representative immunocytochemical analysis on cytospin preparations of progenitor population's CXCR4-/C-MET+ and CXCR4+/C-MET+ at days 23, 25 and 30 of hESC (MEL1) differentiation. Expression of early myogenic regulatory genes SIX4 and PAX3 is detectable as early as day 23, prior to PAX7 expression as expected during myogenic lineage progression. As development proceeds (days 25-30), an increase in PAX7 expression is observed. Scale bars = 50 μm

**Figure S3. Quantification of ACHR+ CXCR4-/C-MET+ and CXCR4+/C-MET+ cell populations derived across independent cell lines.** Percentage of myogenic cell populations isolated at day 35 from four hPSC lines differentiated

under treatment conditions (CHIR+FGF2). Efficiency of myogenic differentiation was similar across all cell lines. Results shown for each cell population represents n = 3 experiments averaged for each of the 4 hPSC lines.

Table S1

| GENE | ORIENTATION | SEQUENCE 5' to 3' | ANNEALING TEMPERATURE °C | AMPLICON LENGTH bp |
|---|---|---|---|---|
| *LBX1** | forward | AAAGTCGCGCACGGCCTTCA | 59 | 249 |
| | reverse | GCCAGCGCCACGATGTCCAT | | |
| *PAX7** | forward | ACCCCTGCCTAACCACATC | 60 | 121 |
| | reverse | GCGGCAAAGAATCTTGGAGAC | | |
| *PAX3** | forward | TACAGGTCTGGTTTAGCAAC | 57 | 183 |
| | reverse | GATCTGACACAGCTTGTGGA | | |
| *MYF5* | forward | TTCTCCCCATCCCTCTCGCT | 59 | 235 |
| | reverse | AGCCTGGTTGACCTTCTTCAG | | |
| *MYH2* | forward | CCGCCCTTGACAAAAAGCAA | 55 | 220 |
| | reverse | GCGCAGGATCTTTCCCTCTT | | |
| *ACHR* | forward | GCTAACCCTCACCAACCTCAT | 59 | 346 |
| | reverse | GGTTGCTGCACTTTGGTCC | | |
| *MYOD** | forward | GCGCGCTCCTGAAACCCGAA | 60 | 166 |
| | reverse | TCGGCGTTGGTGGTCTTGCG | | |
| *TBX6** | forward | TTCCCGGCTCTCACCTCCGT | 60 | 143 |
| | reverse | TGGCCTGCACCAGTGTGTGT | | |
| *MESP1** | forward | CACACCTCGGGCTCGGCATAAA | 60 | 119 |
| | reverse | CAGGCCGCAGAGAGCATCCAG | | |
| *GAPDH** | forward | CCCCTTCATTGACCTCAACTACA | 60 | 342 |
| | reverse | TTGCTGATGATCTTGAGGCTGT | | |
| *SIX4** | forward | CCATGCTGCTGGCTGTGGGAT | 60 | 164 |
| | reverse | AGCAGTACAACACAGGTGCTCTTGC | | |
| *PARAXIS** | forward | AGGGCCACGGAGATGAGCCT | 61 | 120 |

| | | | | |
|---|---|---|---|---|
| | reverse | GGTCCCCCGGTCCCTACACA | | |
| *SIX1** | forward | GTCCAGAACCTCCCCTACTCC | 50 | 101 |
| | reverse | CGAAAACCGGAGTCGGAACTT | | |
| *SOX10* | forward | CCCACACTACACCGACCAG | 59 | 143 |
| | reverse | GGCCATAATAGGGTCCTGAGG | | |
| *MSGN1** | forward | GGAGGCGGAAAGCCAGCGAGA | 60 | 193 |
| | reverse | CTGGGCTCTCTGCCGCGGTTA | | |
| *T** | forward | CGATCCTGGGTGTGCGTAA | 55 | 220 |
| | reverse | GACCAAGACTGTCCCCGCT | | |
| *SOX1* | forward | GAGCTGCAACTTGGCCACGAC | 60 | 271 |
| | reverse | GAGACGGAGAGGAATTCAGAC | | |
| *AP2a* | forward | AGGCAGAGCCAGGAGTCTGGGCT | | |
| | reverse | CGGAGCACTCCGCCCAGCAGCGA | 60 | 464 |
| *LMX1A* | forward | TCCTAGCCTTGGAGAAGCAACT | | |
| | reverse | CAGTGACTGGAGCAGAGAGAA | 59 | 271 |

* Primers used for Quantitative PCR

Table S2

| PRIMARY ANTIBODIES | | | | |
|---|---|---|---|---|
| **ANTIGEN** | **FLUOROCHROME** | **HOST/CLONALITY** | **DILUTION** | **COMPANY** |
| Human HGF/C-MET | APC | mouse IgG1 | 1:50 | R&D Systems |
| CXCR4 (CD184) | Brilliant Violet 421 | Mouse IgG2a | 1:200 | BioLegend |
| Mab 35 (ACHR) | | Mouse IgG1 | 1:100 | DHSB* |
| Human CD57 (HNK-1) | | mouse IgM | 1:200 | Sigma Aldrich |
| PAX3 | | mouse IgG2a | 1:100 | R&D Systems |
| Pax7 | | mouse IgG1 | 1:100 | DHSB |
| Myf5 | | rabbit polyclonal | 1:100 | Santa Cruz Biotechnology |
| Myogenin | | mouse IgG1 | 1:100 | Santa Cruz Biotechnology |
| MyoD | | rabbit polyclonal | 1:100 | Santa Cruz Biotechnology |
| MF20 | | mouse IgG2b | 1:100 | DSHB |
| MYHC2 | | mouse IgG2a | 1:100 | DHSB |
| SOX1 | | goat IgG | 1:100 | R&D Systems |
| PARAXIS | | goat IgG | 1:100 | Santa Cruz Biotechnology |
| Sox10 | | goat polyclonal | 1:100 | R&D Systems |
| AP2a | | mouse IgG2b | 1:100 | DHSB |
| LMX1A | | rabbit polyclonal | 1:200 | Abnova |
| Six4 | | Mouse IgG1 | 1:100 | Abnova |
| **SECONDARY ANTIBODIES** | | | | |
| Anti-rabbit IgG (H+L) | Alexa Fluor 647 | donkey | 1:400 | Molecular Probes (Invitrogen) |
| Anti-goat IgG (H+L) | Alexa Fluor 647 | donkey | 1:400 | Molecular Probes (Invitrogen) |
| Anti-goat IgG (H+L) | Alexa Fluor 488 | donkey | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgG2a | Alexa Fluor 555 | donkey | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgG(H+L) | Alexa Fluor 488 | goat | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgG2b | Alexa Fluor 555 | donkey | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgG1 | Alexa Fluor 647 | goat | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgM | Alexa Fluor 555 | goat | 1:400 | Molecular Probes (Invitrogen) |
| Anti-rabbit IgG (H+L) | Alexa Fluor 488 | goat | 1:400 | Molecular Probes (Invitrogen) |

| | | | | |
|---|---|---|---|---|
| Anti-mouse IgG1 (H+L) | Alexa Fluor 555 | goat | 1:400 | Molecular Probes (Invitrogen) |
| Anti-mouse IgG1 | PE | goat | 1:400 | Molecular Probes (Invitrogen) |

* (Developmental Hybridoma Studies Bank)