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Supplemental Information

**Pluri-IQ: Quantification of Embryonic Stem Cell Pluripotency through
an Image-Based Analysis Software**

Tânia Perestrelo, Weitong Chen, Marcelo Correia, Christopher Le, Sandro Pereira, Ana S. Rodrigues, Maria I. Sousa, João Ramalho-Santos, and Denis Wirtz

Figure S1

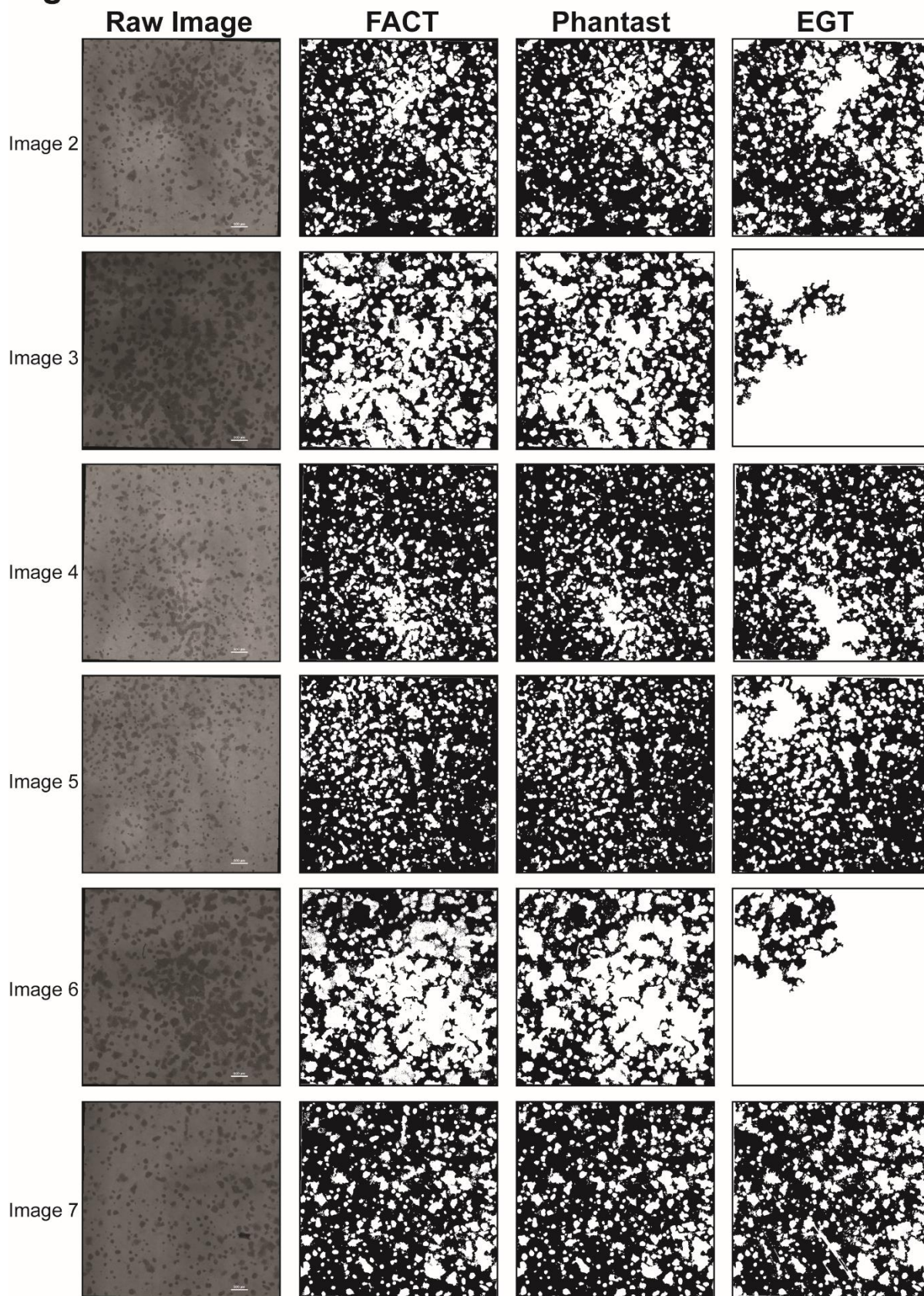


Figure S2

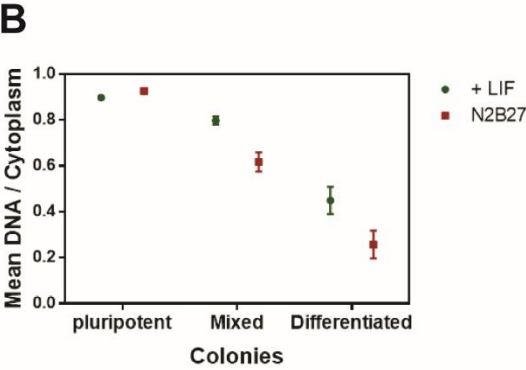
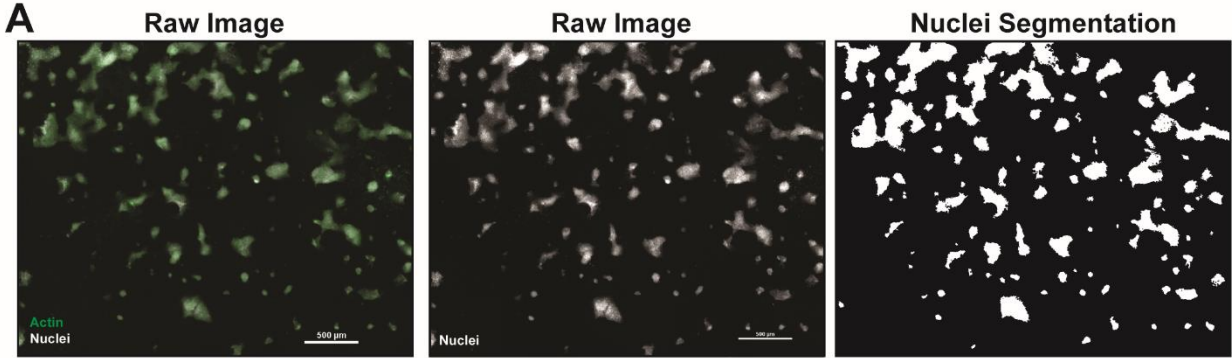


Table S1: Features used to train the random forest classifier and discriminate between pluripotent, mixed and differentiated colonies. Related to Figure 5.

Feature	Feature type
Colony circularity	Morphological
Intensity mode	Intensity
Intensity mean	Intensity
Intensity variance	Texture
Intensity Histogram Skew	Intensity
Variation of intensity histogram peaks	Intensity

Table S2 - Sequences of forward and reverse primer (5' to 3') sets used in this study, related to Figure 6.

<i>Target Gene</i>	<i>Forward</i>	<i>Reverse</i>	<i>Primer Bank ID</i>
<i>Dppa3</i>	GACCCAATGAAGGACCCTGAA	GCTTGACACCGGGGTTTAG	21218416a1
<i>Esrrb</i>	GCACCTGGGCTCTAGTTGC	TACAGTCCTCGTAGCTCTTGC	31542617a1
<i>Fgf5</i>	AAGTAGCGCGACGTTTTCTTC	CTGGAAACTGCTATGTTCCGAG	3721900a1
<i>Klf4</i>	GTGCCCCGACTAACC GTT G	GTCGTTGAACTCCTCGGTCT	6754456a1
<i>Oct4</i>	CGGAAGAGAAAGCGAACTAGC	ATTGGCGATGTGAGTGATCTG	356995852c3
<i>Rex1</i>	CCCTCGACAGACTGACCCTAA	TCGGGGCTAATCTCACTTTCAT	7110739a1
<i>Rplp0</i>	AGATTCGGGATATGCTGTTGGC	TCGGGTCCTAGACCAGTGTTTC	6671569a1
<i>T</i>	GGATTCACATCGTGAGAGTTGG	GTCACAGCTATGAACTGGGTC	118130357c3

Figure S1: Comparison between FACT algorithm segmentation and Phantast and EGT segmentation algorithms. Related to Figure 4.

FACT, Phantast and EGT segmentation results of large images with different degrees of colony density. Binary images, where white is representative of presence of a colony. Scale bar: 500 μm .

Figure S2: Nuclei cytoplasm ratio decreases upon differentiation. Related to Figure 7.

(A) Representative image of the nuclei segmentation through FACT algorithm. Raw image color code: green – actin; white – nuclei. Scale bar: 500 μm . (B) Average nuclei cytoplasm ratio, calculated by Pluri-IQ, of pluripotent, mixed and differentiated cells when cultured in a pluripotent medium (+LIF) and in a neuronal differentiation medium (N2B27). Result is expressed as mean \pm SEM and are derived from two replicates.