Electronic Supplementary Material (ESI) for RSC Advances.

Supplementary Information

Wax Patterned Microwells for Stem Cell Fate Study

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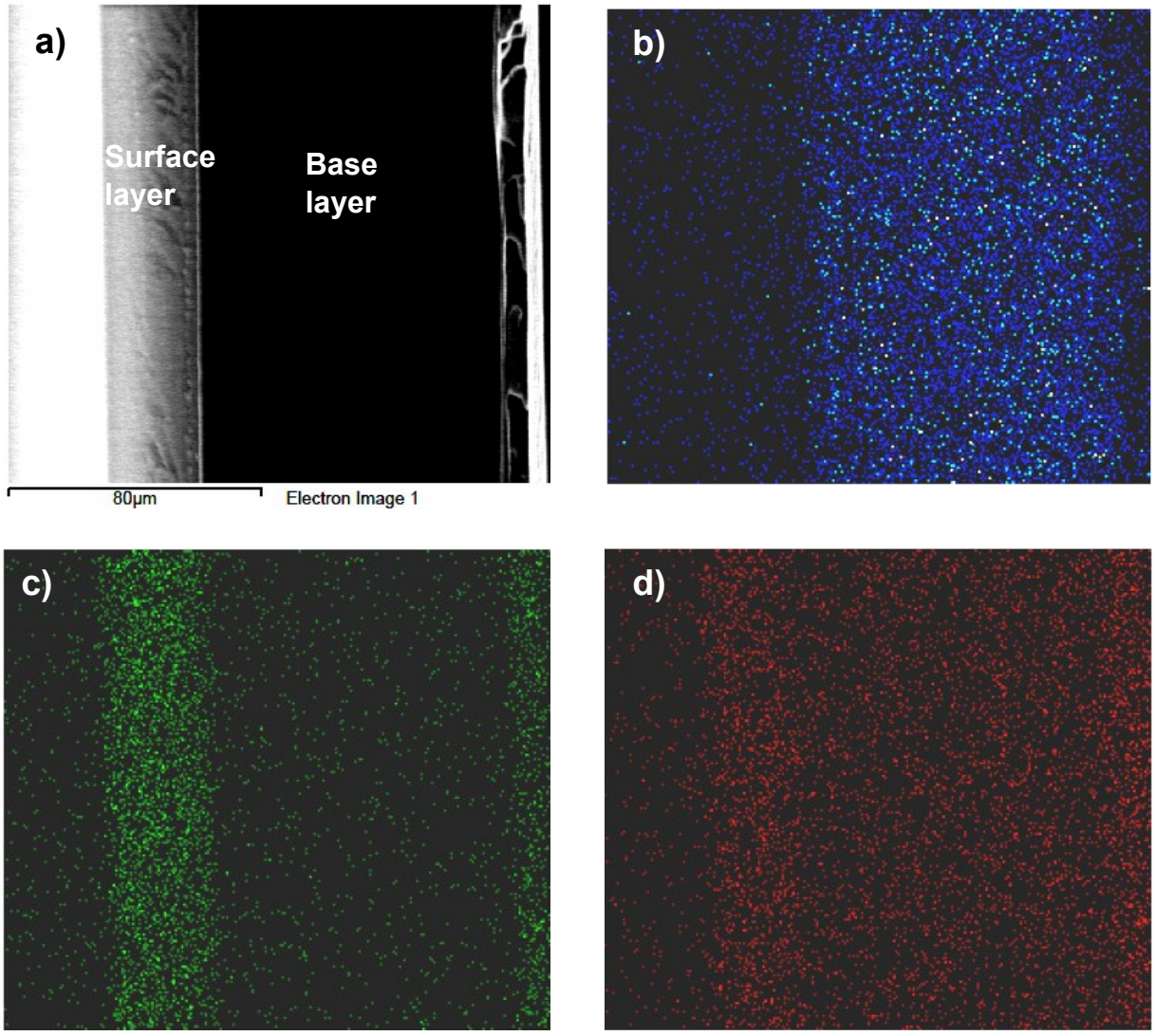

Figure S1. EDS mapping of *m*PET on SEM image. a) SEM image of mPET substrate, b) blue color represents presence of C-atoms, c) green color mapping reflects Si-atoms, and d) red color reflects O-atoms across the two layers of mPET substrate.

Table S1. Elemental composition of surface and base layer of *m*PET.

Element	Surface layer	Base layer
C	10.6%	62.1%
O	52.8%	27.4%
Si	35.2%	4.4%

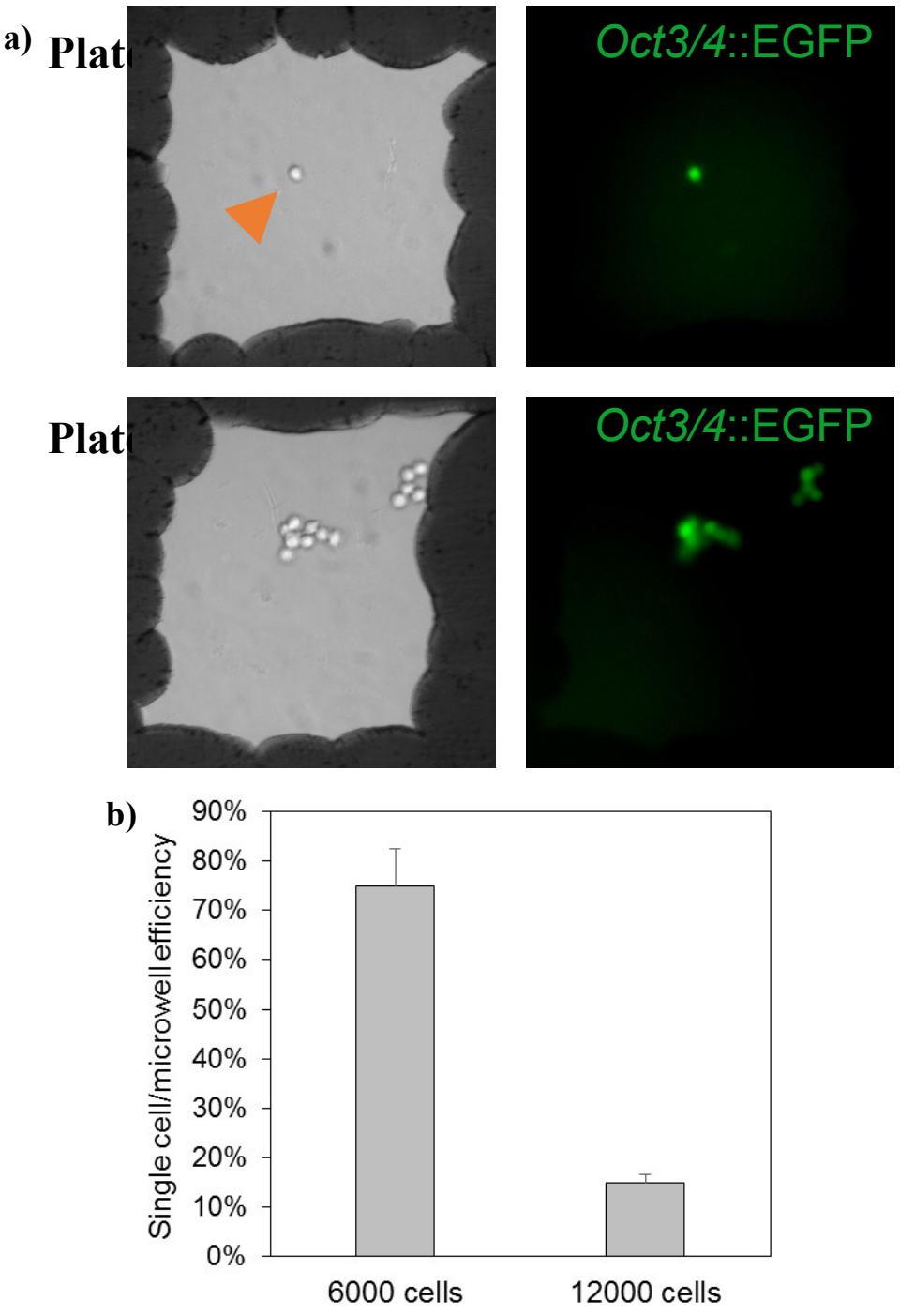

Figure S2. Optimization of cell seeding density for single cell per microwell. Single ES cells at varying cell seeding density were plated on patterned microwells. a) When 6000 or 12000 cells were plated on entire patterned microwells single or multiple cells were housed per microwell. Arrowhead indicates a single cell. b) Further quantification shows 6000 cell density is optimum (~75% of microwells have single cells) for seeding single cell per microwell. Seeding 3000 cells on patterned microwells yield many empty microwells thereby reducing single cell per microwell efficiency (data not shown).

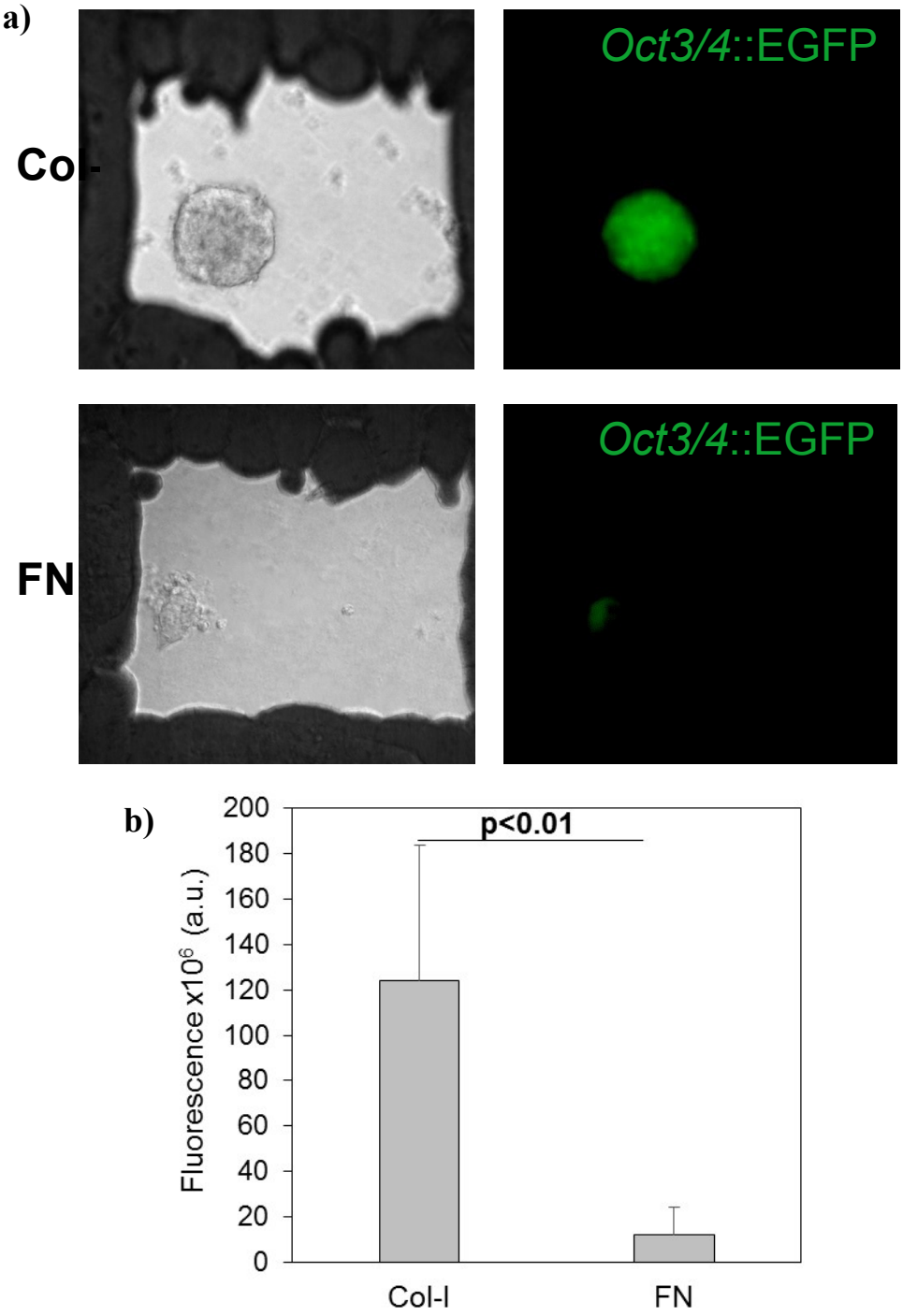

Figure S3. Type I collagen promotes self-renewal while fibronectin induces differentiation in mouse embryonic stem (ES) cells. Microwells were coated with either type I collagen (Col-I) or fibronectin (FN). Single ES cells, expressing Oct3/4::EGFP, were plated in individual microwells and monitored for EGFP expression over 5 days on collagen or fibronectin coated surfaces. Expression level of Oct3/4, a master regulator of pluripotency, was measured by EGFP expression from each microwells. (A) On day 5, representative images of single ES colonies, housed in individual microwells, either on Col-I or FN show high and low Oct3/4 activity respectively. (B) Total EGFP signal quantification from each microwells, housing individual colonies, shows higher total fluorescence signal on Col-I compared to FN coated surfaces indicating self-renewal being promoted on Col-I surfaces. Mean ± S.D. (n=9 for both Col-I and FN).