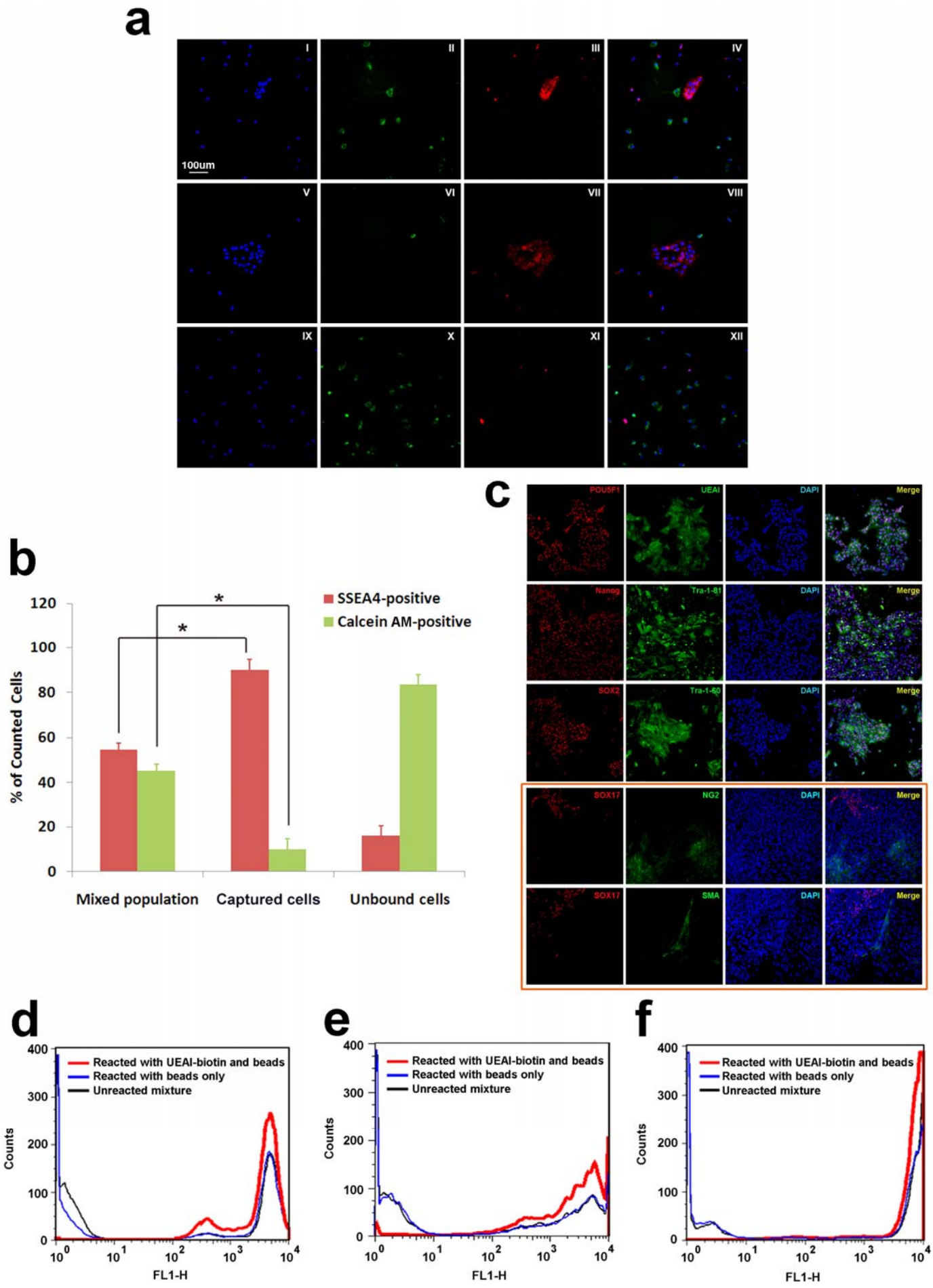

Supplementary information, Figure S3 Isolation of viable hPSCs and purging of hPSCs from mixed cell populations.

(a) HDFs were prelabeled with Calcein AM, which fluoresces green, and then mixed with WA09 cells at a ratio of 1:1.

Biotinylated UEA-I and streptavidin-conjugated magnetic beads were used to separate the WA09 cells from the HDFs in the mixed population. (I, II, III, IV) Cells in the mixed population without separation. (V, VI, VII, VIII) Cells captured by the beads, representing cells carrying glycosylation marks recognized by UEA-I. (IX, X, XI, XII) Cells remaining in the unbound fraction. DAPI (I, V, IX), calcein (II, VI, X), SSEA-4 (III, VII, XI) and merge (IV, VIII, XII) images are shown. Cells recovered from the streptavidin-conjugated beads were viable and capable of reattaching onto culture plates. (b) Quantification of the percentage of pluripotent WA09 cells (SSEA-4-positive) and non-pluripotent HDFs (calcein-positive) in mixed cell populations prior to and after UEA-I-mediated cell separation. More than 250 cells were counted in randomly selected fields from the mixed population and each of the two fractions after separation. Columns, mean of three independent experiments; bars, standard deviation; *P<0.05, t-test. (c) iPS1.HDF51 cells were recovered from the UEA-I magnetic cell separation and replated for two additional rounds of passaging. The cells were positively stained with antibodies against POU5F1, NANOG, SOX2, Tra-1-60 and Tra-1-81, indicating sustained pluripotency in the isolated cells. The EBs were also generated using the iPS1.HDF51 cells recovered from the UEA-I magnetic cell separation. Antibodies against biomarkers for all three germ layers (human NG2: ectoderm, SMA: mesoderm, SOX17: endoderm) were used to stain EBs. The images of EBs were circled by an orange box. (d-f) hESCs (unlabeled) were mixed with different Calcein AM-labeled cell types (green) at a ratio of 1:1. Cells were then incubated with biotinylated UEA-I followed by magnetic submicron beads. The cells captured by the magnetic beads were isolated using magnetic columns and the unbound, eluted cells were analyzed for Calcein AM fluorescence by flow cytometry. Red trace, cell mixture incubated with UEA-I biotinylated beads; blue trace, cell mixture incubated with beads alone, no lectin; black trace, cell mixture incubated without beads and without lectin. (c) WA09 mixed with HDFs. (d) WA09 mixed with HEMd melanocytes. (e) R-Olig2 hESCs mixed with differentiated NSC progeny. Note the dramatic increase in Calcein AM fluorescence when cell mixtures are incubated with biotinylated UEA-I beads, indicating that unlabeled, pluripotent cells have been purged from the population.