Figure S4
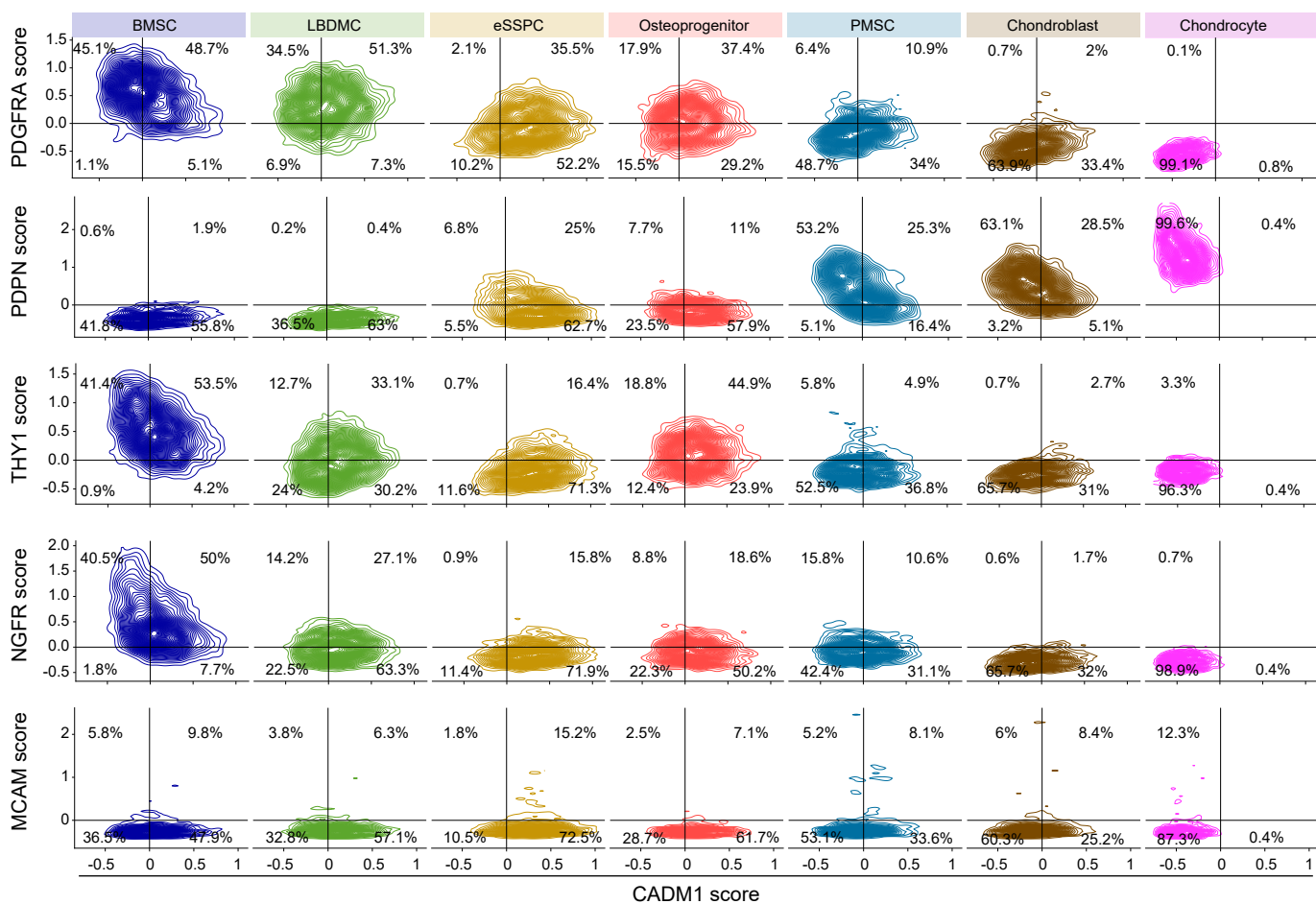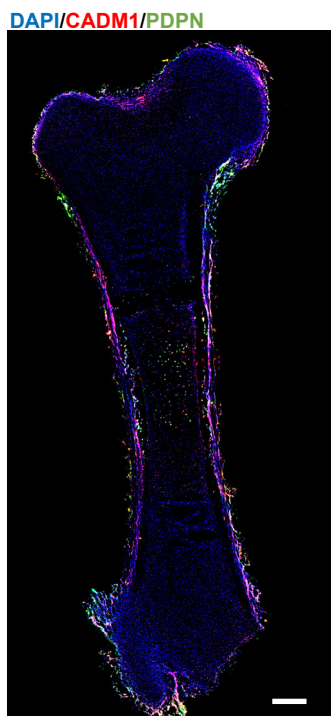
a

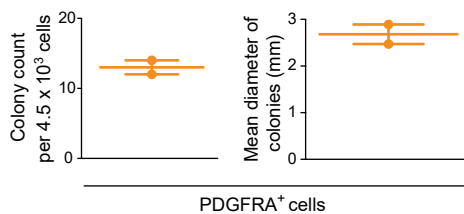### Transcript-averaged cell scoring (TACS)

b

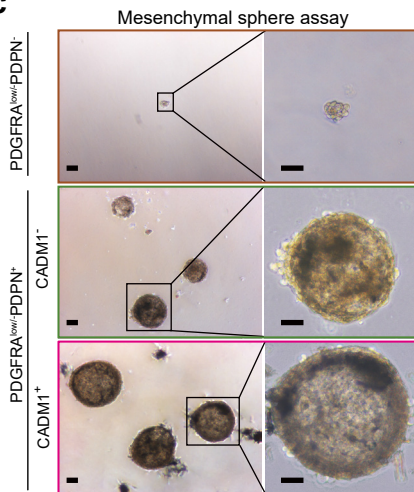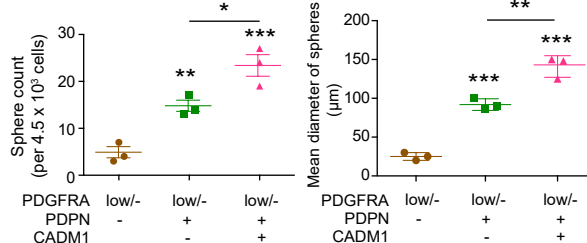DAPI/CADM1/PDPN

c

Mesenchymal sphere assay

d

e

PDGFRA+ cells

f

PDGFRA+ cells

g

Clonal differentiation-Adipogenic

Clonal differentiation-Osteogenic

Clonal differentiation-Chondrogenic

h

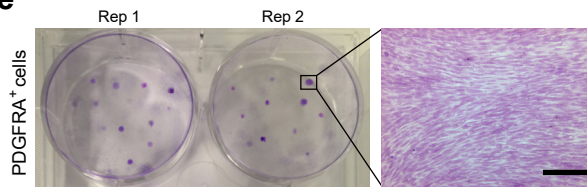Clonal differentiation
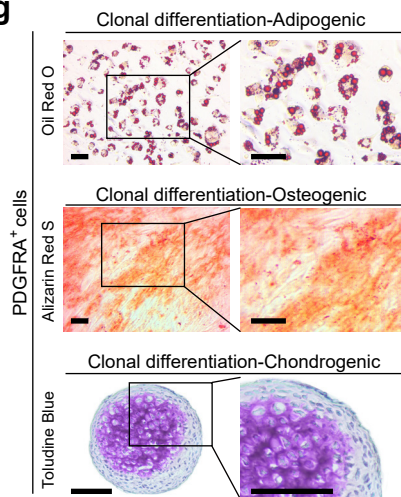
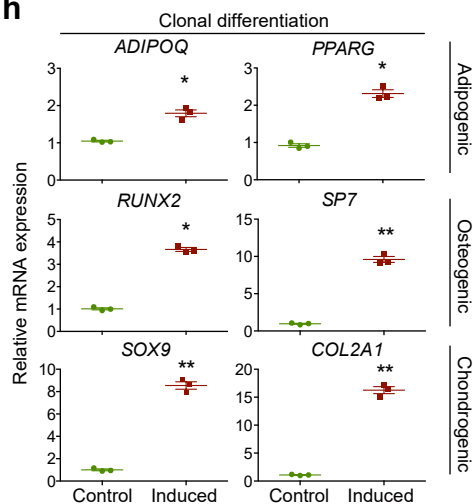ADIPOQ   PPARG   RUNX2   SP7   SOX9   COL2A1

Supplementary Fig. 4. Further in silico and functional analyses of eSSPCs and PDGFRA+ cells

a, TACS plots showing the distribution of each OCLC subset between indicated surface marker pairs. Contours outlined regions of increasingly higher cell density. Cell frequencies were shown on the plots.

b, Representative immunofluorescent image of 8 WPC human femur section stained with DAPI (blue), CADM1 (red) and PDPN (green) (n=2 embryos). Scale bar: 200 μm.

c, Representative images showing the mesenchymal spheres formed by the 3 populations sorted as in Fig. 4c (left), with magnified views (right). Scale bars: 25 μm.

d, Quantifications of the number (left) and mean diameter (right) of mesenchymal spheres (n=3 embryos). The statistical significance of differences was determined using one-way ANOVA with multiple comparison tests (LSD). * $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$. Error bars indicated SEM.

e, Representative crystal violet staining of CFU-F colonies generated by flow cytometrically sorted PDGFRA$^+$ cells from two independent 8 WPC long bones. Magnified images of the boxed areas were shown on the right. Scale bar: 25 μm.

f, Quantifications of the number (left) and mean diameter (right) of CFU-F colonies generated by flow cytometrically sorted PDGFRA$^+$ cells (n=2 embryos). Error bars indicated SEM.

g and **h,** Representative oil red O (top), alizarin red (middle) and toluidine blue (bottom) staining after adipogenic, osteogenic and chondrogenic differentiation of clonally expanded PDGFRA$^+$ cells (**g**) with qPCR quantifications (**h**). Magnified images of the boxed areas were shown on the right (n=2 embryos). Scale bars: 200 μm. The statistical significance of differences was determined using Wilcoxon signed rank test. * $P < 0.05$; ** $P < 0.01$. Error bars indicated SEM.