Stem Cell Reports, Volume *5*

Supplemental Information

Targeted Gene Correction in Osteopetrotic-Induced Pluripotent Stem Cells for the Generation of Functional Osteoclasts

Tui Neri, Sharon Muggeo, Marianna Paulis, Maria Elena Caldana, Laura Crisafulli, Dario Strina, Maria Luisa Focarelli, Francesca Faggioli, Camilla Recordati, Samantha Scaramuzza, Eugenio Scanziani, Stefano Mantero, Chiara Buracchi, Cristina Sobacchi, Angelo Lombardo, Luigi Naldini, Paolo Vezzoni, Anna Villa, and Francesca Ficara

SUPPLEMENTAL FIGURES

Figure S1, relative to Figure 1

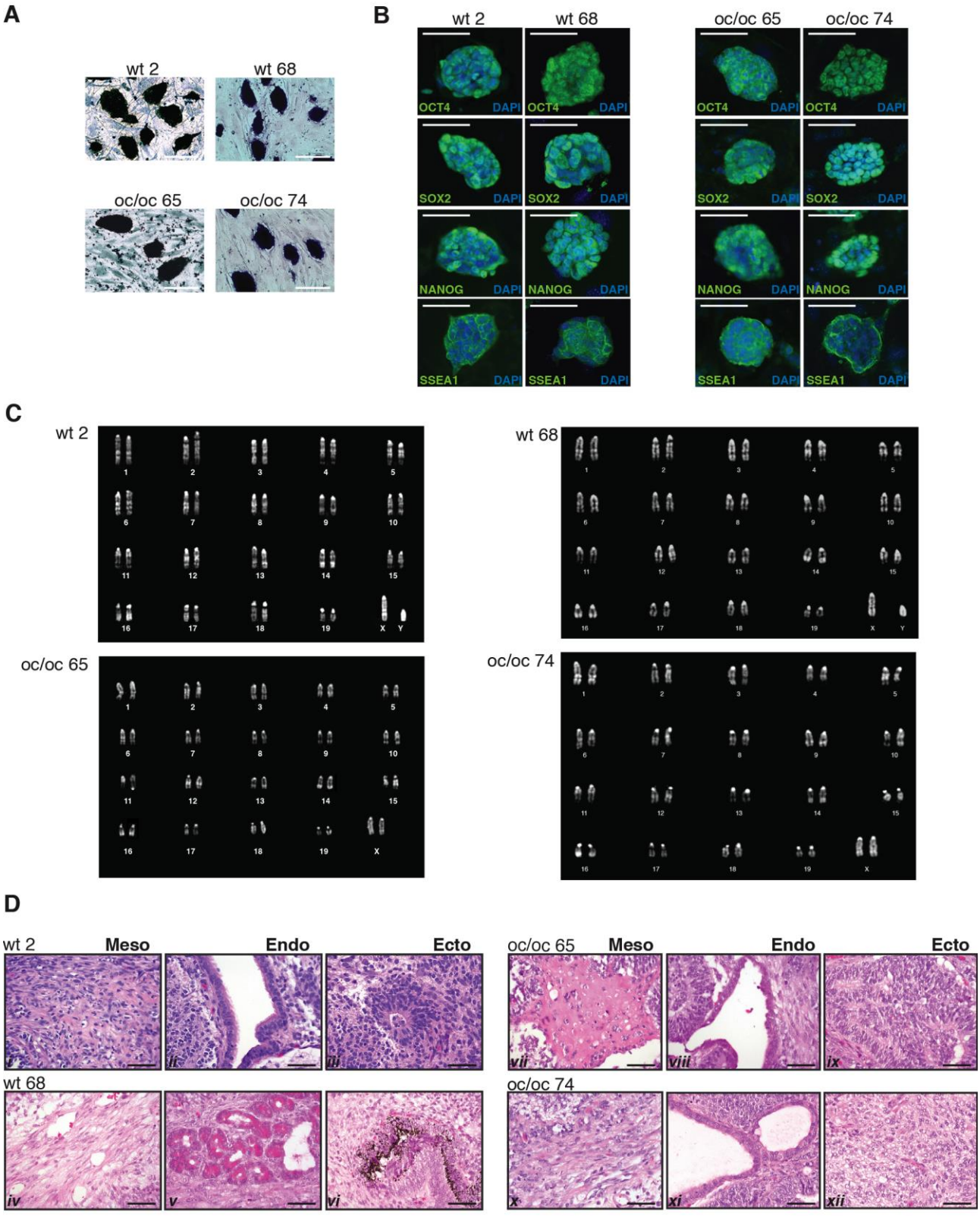
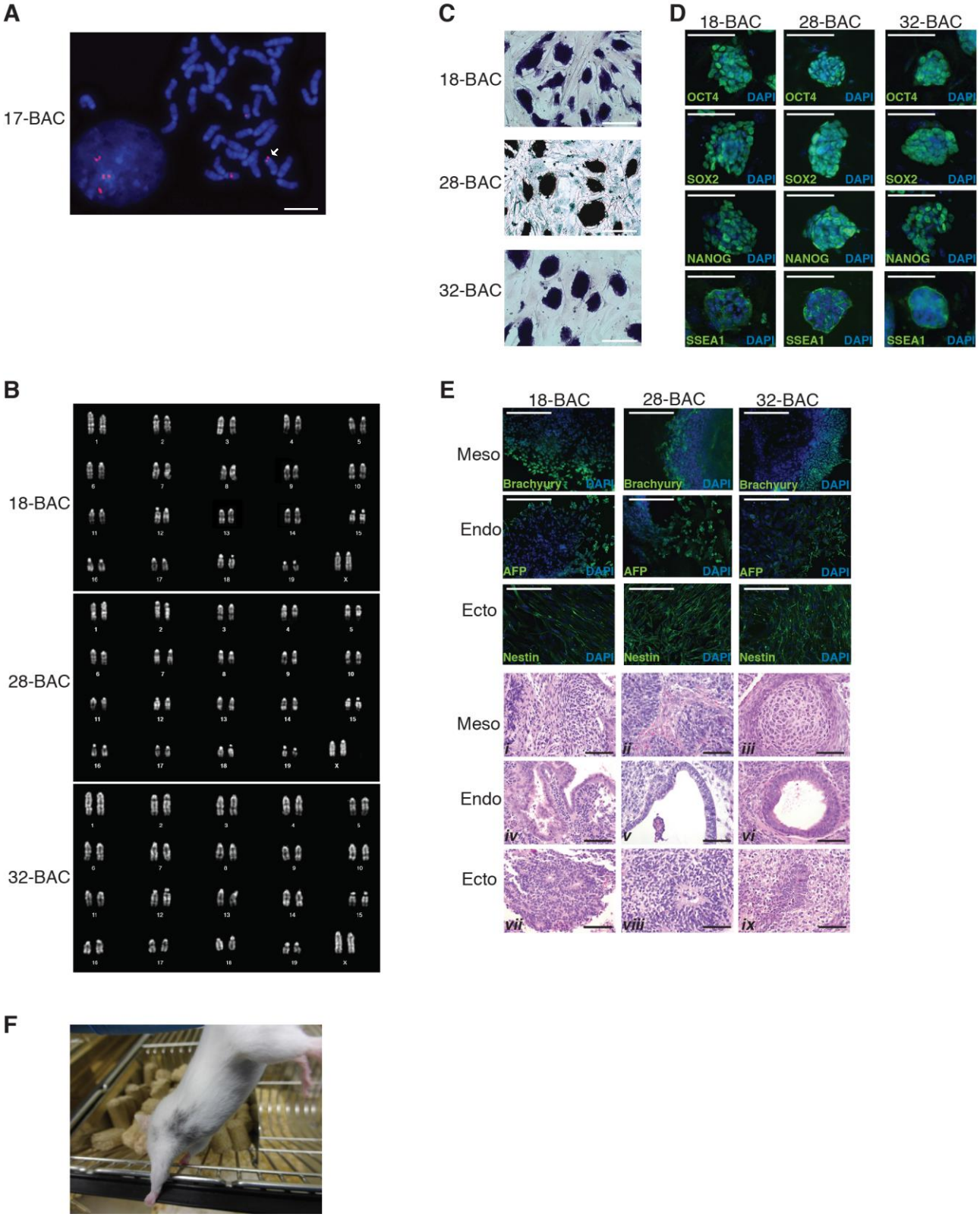


Figure S2, relative to Figure 2



SUPPLEMENTAL FIGURE LEGENDS

Figure S1. Characterization of additional iPSC clones from wt and *oc/oc* mice, Related to Figure 1.

(A) Expression of alkaline phosphatase on wt and oc/oc iPSC colonies (wt 2, wt 68 and *oc/oc* 65, *oc/oc* 74, respectively). Scale bars 120 μm.

(B) Expression of Oct4, Sox2, Nanog and SSEA-1 on wt and oc/oc iPSC revealed by immunofluorescence. Nuclei are stained with DAPI. Scale bars 100 μm.

(C) DAPI-banded karyotypes of wt and *oc/oc* iPSC clones.

(D) H&E staining of teratomas generated after subcutaneous injection of wt and *oc/oc* iPSC clones into NSG mice, demonstrating differentiation into the three germ layer derivatives.

Mesoderm: i, iv and x, fibrous connective tissue; vii, bone-like tissue. Endoderm: ii and viii, columnar ciliated epithelium; v, glandular structures composed of pancreatic acinar-like cells; xi, glandular structures. Ectoderm: iii and ix, primitive neuroepithelium; vi, pigmented neuroepithelium; xii, mature neural tissue. Scale bars 50 μm.

Figure S2. Characterization of gene-corrected iPSC clones, Related to Figure 2.

(A) FISH analysis showing BAC signals (red) on two cells (one interphase, left, and one metaphase, right) stained with DAPI, from 17-BAC iPSC clone that was excluded from the subsequent analysis due to the presence of a third signal with sub-telomeric chromosome localization (white arrow). Scale bar 5 μm.

(B) Representative DAPI-banded karyotypes of BAC-corrected iPSC clones 18-, 28- and 32-BAC.

(C) Expression of alkaline phosphatase by BAC-corrected 18-, 28- and 32-BAC clones. Scale bars 120 μm.

(D) Expression of stemness markers Oct4, Sox2, Nanog and SSEA-1 in BAC-corrected iPSC. Nuclei are stained with DAPI. Scale bars 100 μm.

(E) Upper panels: generation of the three germ layers *in vitro* by differentiated BAC-corrected iPSC revealed by immunofluorescence. Expression of Brachyury, AFP and Nestin indicates formation of mesoderm, endoderm and ectoderm, respectively. Lower panels: H&E staining on teratomas generated after subcutaneous injection of 18-, 28- and 32-BAC clones into NSG mice, demonstrating differentiation into the three germ layer derivatives.

Mesoderm: i, bundles of undifferentiated connective tissue; ii, fibrous connective tissue; iii, early cartilagineous tissue. Endoderm: iv and v, glandular structures; vi, columnar ciliated epithelium. Ectoderm: vii and viii, primitive neuroepithelium; ix, primitive and mature neuroepithelium. Scale bars 50 μm.

(F) Representative chimeric mouse generated after microinjection of the 32-BAC clone into host blastocysts from C57BL/6 albine mice. Black hair derived from iPSC.