Supplementary Materials and Methods (see "Supplementary Figure 3" for results)

1. Osteoinductivity assessment of OI-iPSC constructs

1.1. Osteoinductivity assessment of secreted factors from OI-iPSC constructs

Human bone marrow-derived MSCs sorted by LNGFR$^+$/THY-1$^+$ (LT-MSCs) [Mabuchi Y. *et al.*, *Stem Cell Reports*, 1: 152-65, 2013] were kindly provided by Dr. Y Mabuchi (Tokyo Medical and Dental University, Tokyo, Japan). LT-MSCs were cultured in growth medium (GM) containing Dulbecco's modified Eagle's medium (DMEM with 4.5 g/L glucose and without sodium pyruvate; Nacalai Tesque), 20% FBS (Hyclone), 1% penicillin-streptomycin (Wako Pure Chemical), 10 mM HEPES (Dojindo), and 10 ng/mL recombinant human basic fibroblast growth factor (bFGF; Wako Pure Chemical). LT-MSCs were maintained in GM until the cells reached 80% confluence.

Conditioned medium (CM) was collected from OI-iPSC constructs in the Elp500 condition at day 35. Then, LT-MSCs were prepared in 24-well plates and maintained in GM or OS medium with and without 10 μL/mL CM for 14 days. Half medium change was performed every 3 days. Subsequently, the cells were fixed with 10% neutral-buffered formalin solution (Wako Pure Chemical) prior to staining with von Kossa and methylene blue.

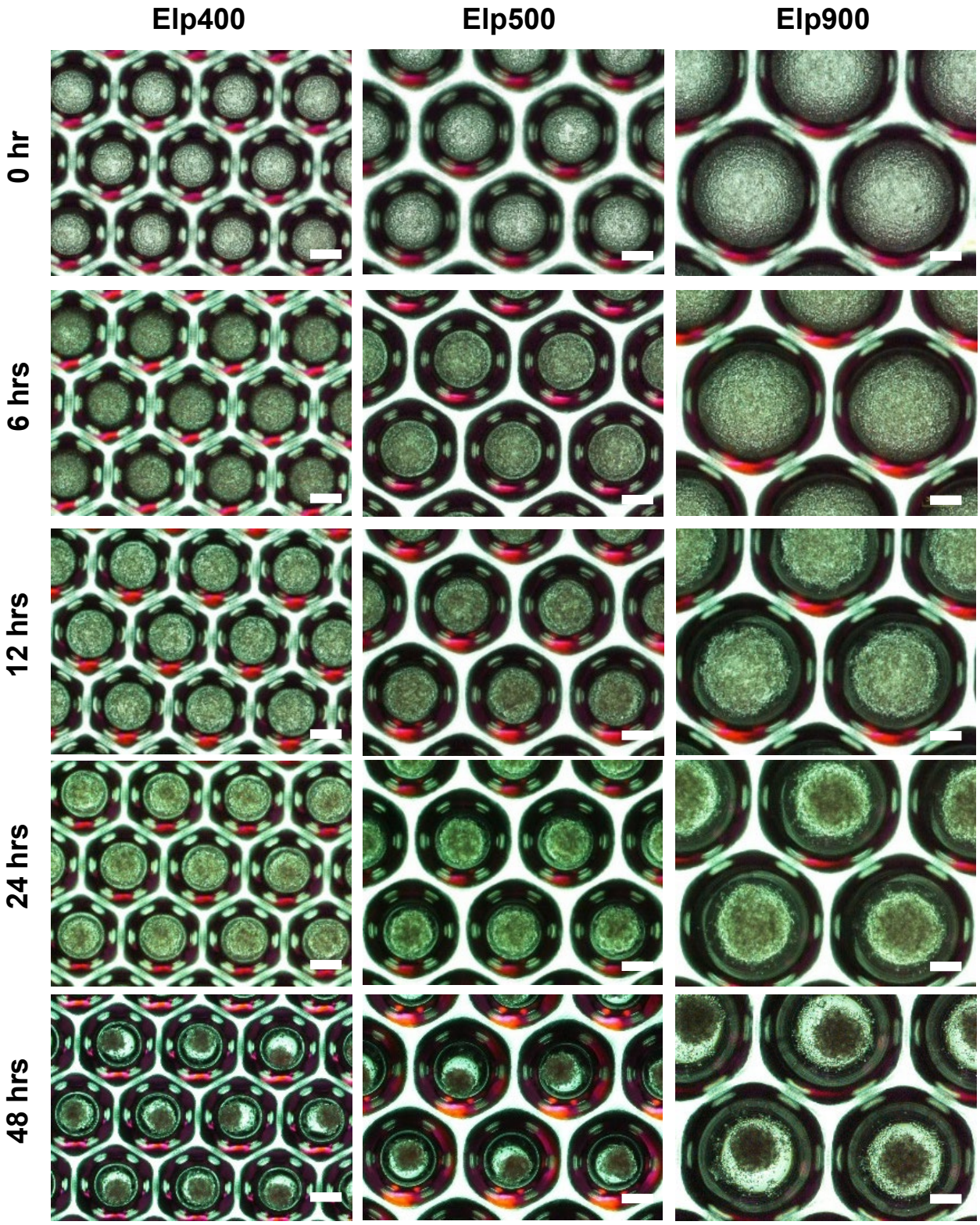
1.2. Osteoinductivity assessment of inactivated (freeze-dried) OI-iPSC constructs

OI-iPSC constructs were collected from Elp500 wells at day 35 and washed twice with PBS (Wako). Next, the constructs were pre-frozen at -80 °C overnight and subsequently transferred to a freeze-drying device (VD-250R; Taitec, Koshigaya, Japan) to inactivate the cells in the constructs at -10 °C under -0.1 MPa overnight.

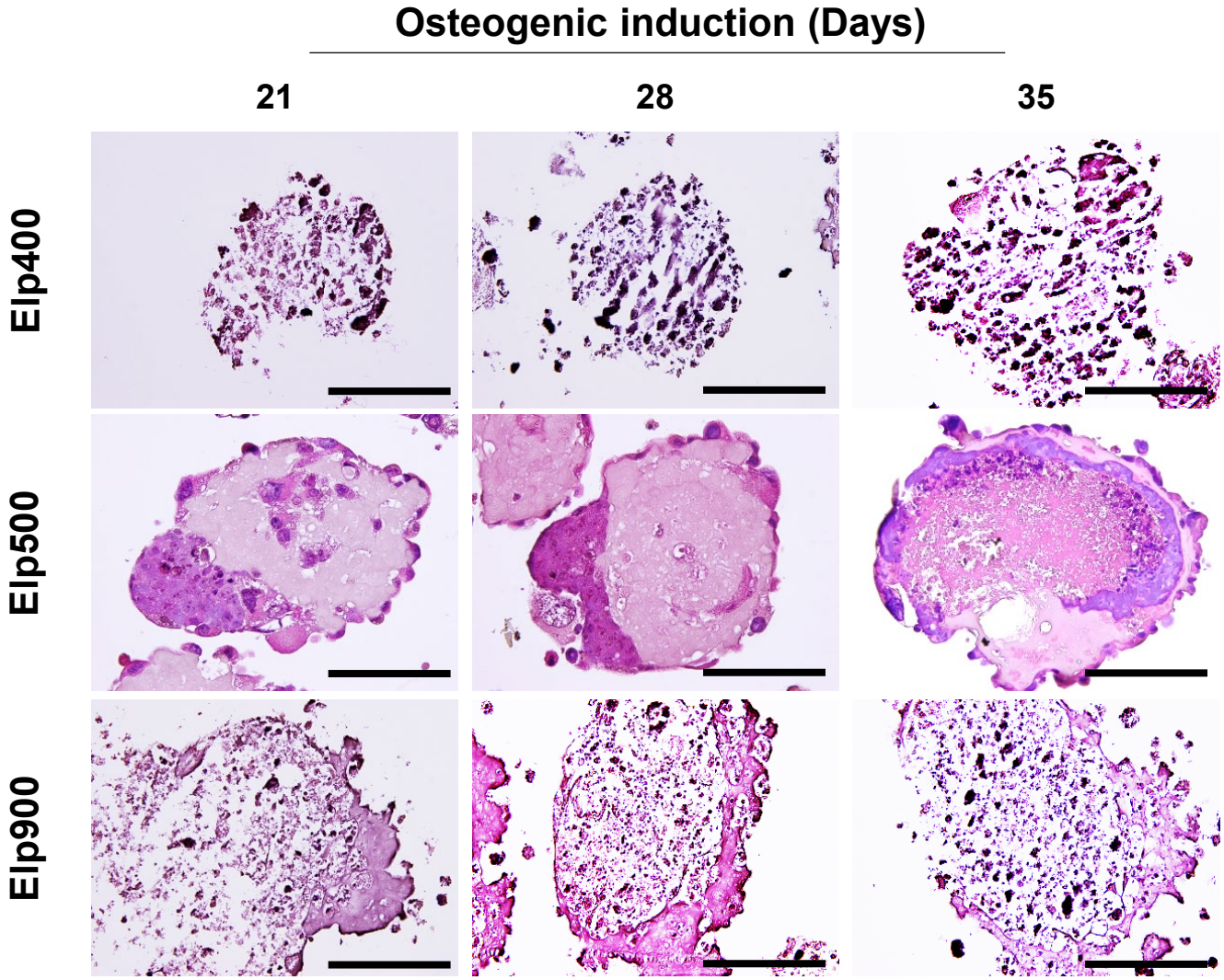
LT-MSCs (2 x 10^4 cells/cm^2) were plated in 6-cm dishes containing freeze-dried OI-iPSC constructs from the Elp500 condition and maintained for 1 day. Next, GM was replaced by osteogenic medium (Zen-Bio, NC, USA) and the culture was maintained for 14 days. Half medium change was performed every 3 days. Then, the cells were fixed in 10% neutral-buffered formalin solution (Wako Pure Chemical) and subsequently stained using Alizarin Red S and von Kossa to detect mineralization nodules.

Supplementary Table 1: Primers used for SYBR Green real-time RT-PCR.

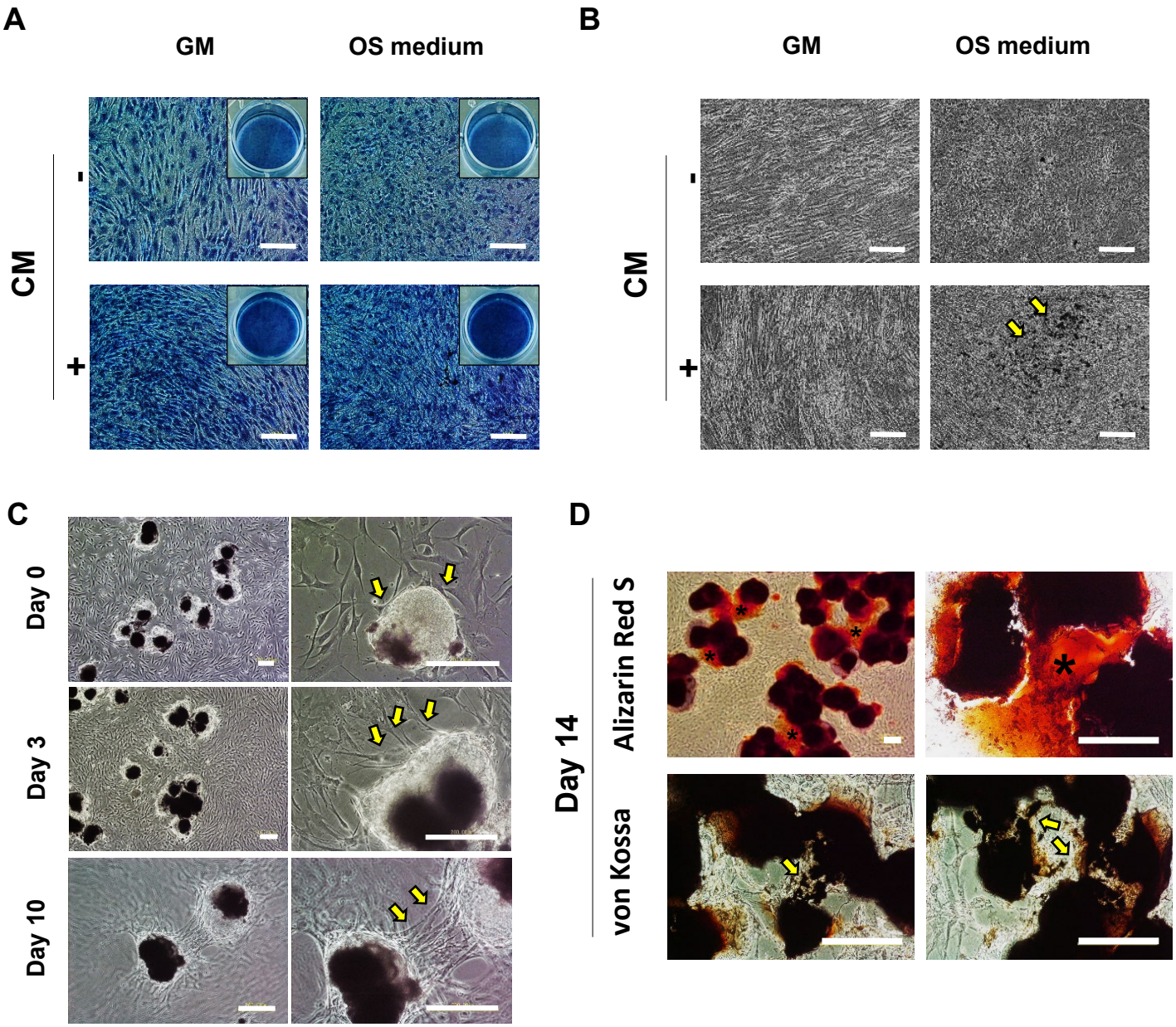
Description (gene name)	Primers (Fw, forward; Rv, reverse)	Product size (bp)	Accession number (reference)
E-cadherin (*CDH1*)	Fw: 5'-GTCTCCTCATGGCTTTGC-3' Rv: 5'-CTTTAGATGCCGCTTCAC-3'	228	NM_009864.3 (Xiang X. et al., *PLoS One*, 7: e50781, 2012)
Runx2 (*Runx2*)	Fw: 5'-CGGGCTACCTGCCATCAC-3' Rv: 5'-GGCCAGAGGCAGAAGTCAGA-3'	78	NM_001146038.2 (Speer M. Y. et al., *J Cell Biochem*, 110: 935–947, 2010)
Osterix (*Sp7*)	Fw: 5'-CTCGTCTGACTGCCTGCCTAG-3' Rv: 5'-GCGTGGATGCCTGCCTTGTA-3'	84	NM_130458.3 (Fowlkes J. L. et al., *Endocrinology*, 149: 1697–1704, 2008)
Collagen 1a1 (*Col1a1*)	Fw: 5'-TGTCCCAACCCCCAAAGAC-3' Rv: 5'-CCCTCGACTCCTACATCTTCTGA-3'	92	NM_007742.3 (Kaback L. A. et al., *J Cell Biochem*, 105: 219–226, 2008)
Osteocalcin (*Bglap*)	Fw: 5'-CCGGGAGCAGTGTGAGCTTA-3' Rv: 5'-AGGCGGTCTTCAAGCCATACT-3'	68	NM_007541.3 (Jadlowiec J. et al., *J Biol Chem*, 279: 53323–53330, 2004)
Osteopontin (*Spp1*)	Fw: 5'-TCTCCTTGCGCCACAGAATG-3' Rv: 5'-TCCTTAGACTCACCGCTCTT-3'	399	NM_001204201.1 (Yang J. et al., *Int J Mol Med*, 39: 1605-1612, 2017)
Bone sialoprotein (*Ibsp*)	Fw: 5'-CGGAGGAGACAACGGAGAAG-3' Rv: 5'-GTAAGTGTCGCCACGAGGCT-3'	295	NM_ 008318.3 (Bouet G. et al., *PLoS One*, 10: e0117402, 2015)
18s rRNA	Fw: 5'-GTAACCCGTTGAACCCCATT-3' Rv: 5'-CCATCCAATCGGTAGTAGCG-3'	151	X00686 (Valamehr B. et al., *Proc Natl Acad Sci U S A.*, 105: 14459-14464, 2008)



Supp Figure 1



Supp Figure 2



Supp Figure 3