27 th BCLF & 30th TBS 2019 ANTALYA/BELEK

Differentiation of Osteopetrotic iPSCs Towards Osteoclasts: Comparision of Osteopetrotic and Healthy Osteoclasts

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Disease Model: Malignant Infantile Osteopetrosis

Osteopetrosis form	Genetic transmission	Gene	Mutation type	Protein
ARO	Autosomal recessive	*TCIRG1*	Loss of function	α3 subunit V-ATPase
		CLCN7	Loss of function	Chloride channel 7
		OSTM1	Loss of function	Osteopetrosis associated transmembrane protein
		PLEKHM1	Loss of function	Pleckstrin homology domain containing family M, member I
		SNX10	Loss of function	Sorting nexin 10
		TNFSF11	Loss of function	Receptor activator for nuclear factor κB ligand
		TNFRSF11A	Loss of function	Receptor activator for nuclear factor κB
IRO	Autosomal recessive	*CAII*	Loss of function	Carbonic anhydrase II
ADO II	Autosomal dominant	*CLCN7*	Dominant negative	Chloride channel 7

International Journal of Endocrinology, Volume 2015, Article ID 372156

Table 1 | Classification, genetics and clinical manifestations of human ARO

Gene*	Age at presentation (years)	Growth retardation	Hypocalcaemia	Impairment		CNS symptom severity and type	Osteoclasts	Life expectancy‡ (years)	Incidence§
				Haematological	Visual				
TCIRG1	<1	Mild to severe	Severe	Severe	Mild to severe	None to moderate (hydrocephalus)	Present, non-functional	0–10	51–53%
CLCN7	<1	Mild to severe	Severe	Mild to severe	Mild to severe	None to severe (hydrocephalus, neurodegeneration)	Present, non-functional	0–3	13–16%
OSTM1	<1	Mild to severe	Moderate	Mild to severe	Mild to severe	Severe (neurodegeneration)	Present, non-functional	0–2	2–6%
SNX10	<1	Mild	Mild	Severe	Severe	None to moderate (hydrocephalus)	Present, non-functional	0–22	4%
PLEKHM1	1–10	None to moderate	None	None	None	None	Present, non-functional	14	2 cases
TNFRSF11A	<1	Moderate	Mild	Mild	Mild to severe	None to moderate (hydrocephalus)	Absent	1–10	<1–4%
TNFSF11	<1	Severe	Mild	Mild	Mild to severe	None	Absent	1–16	<1–3%

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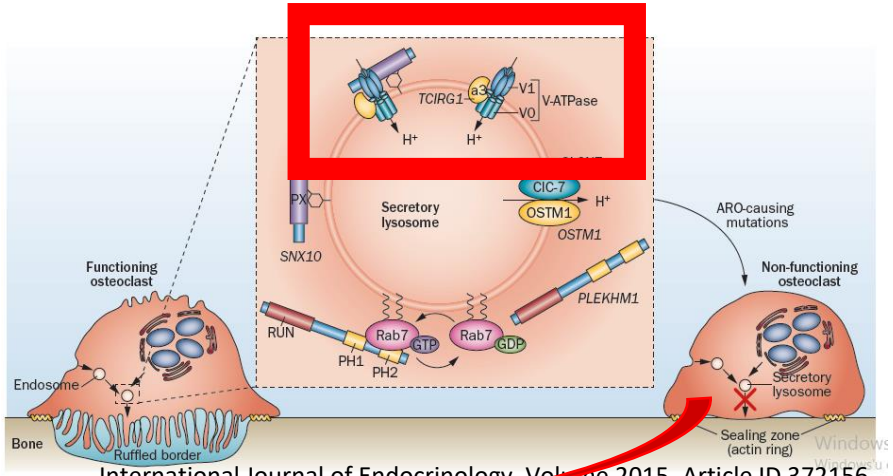
ARO

- **Hydrocephalus,**
- **Bone marrow failure due to reduction of the bone marrow space,**
- **In some patients myelofibrosis (leading to anaemia and thrombocytopaenia with variable leucocyte counts),**
- **Compensatory extramedullary haematopoiesis,**
- **Hepatosplenomegaly and recurrent infections usually in infancy,**
- **Cranial nerve compression (leading to progressive blindness and, in rare cases, deafness),**
- **Choanal stenosis, respiratory and eating difficulties.**



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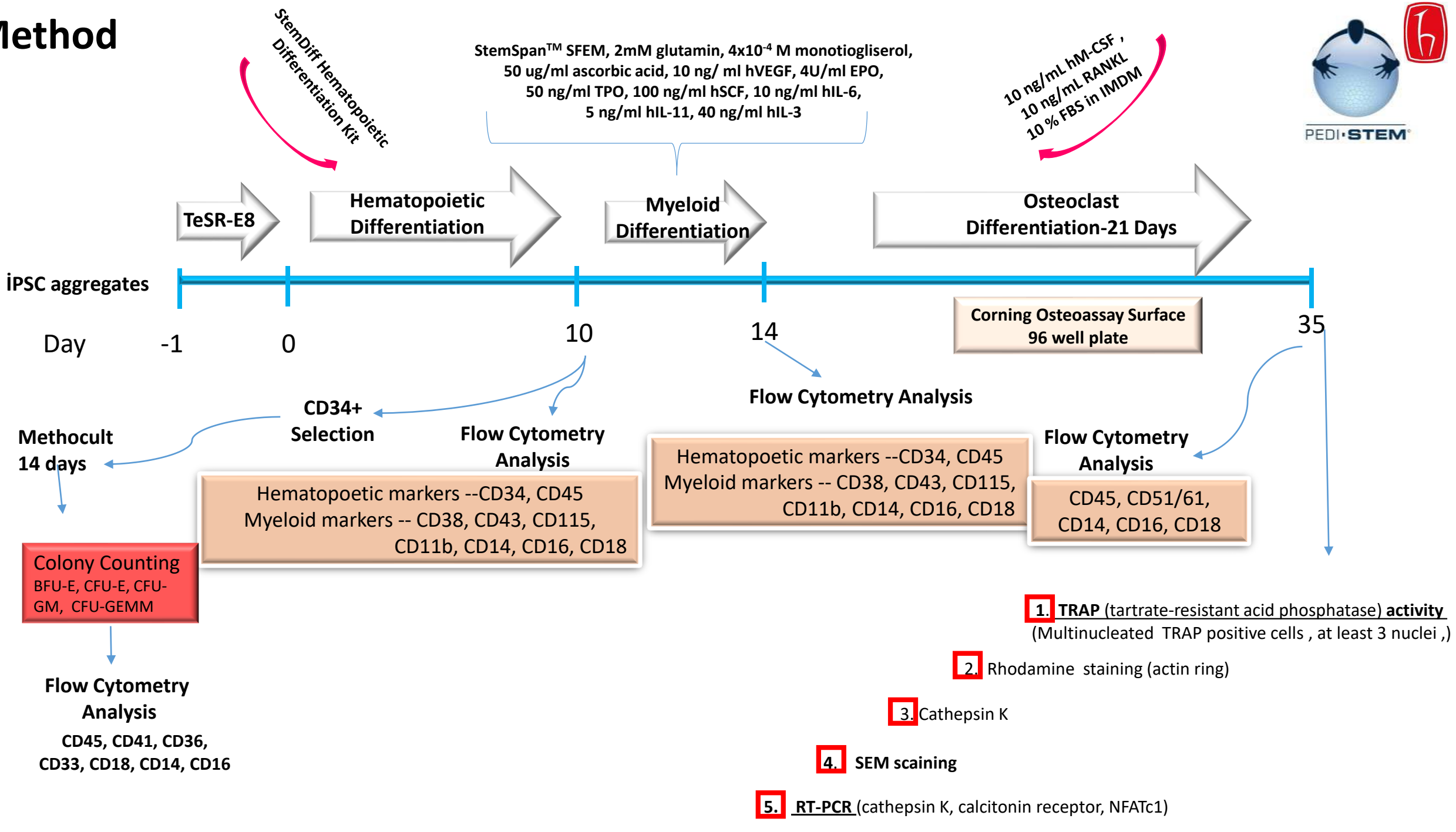
- **Haematopoietic stem cell transplantation (HSCT) is the only curative treatment for ARO**

The main purpose

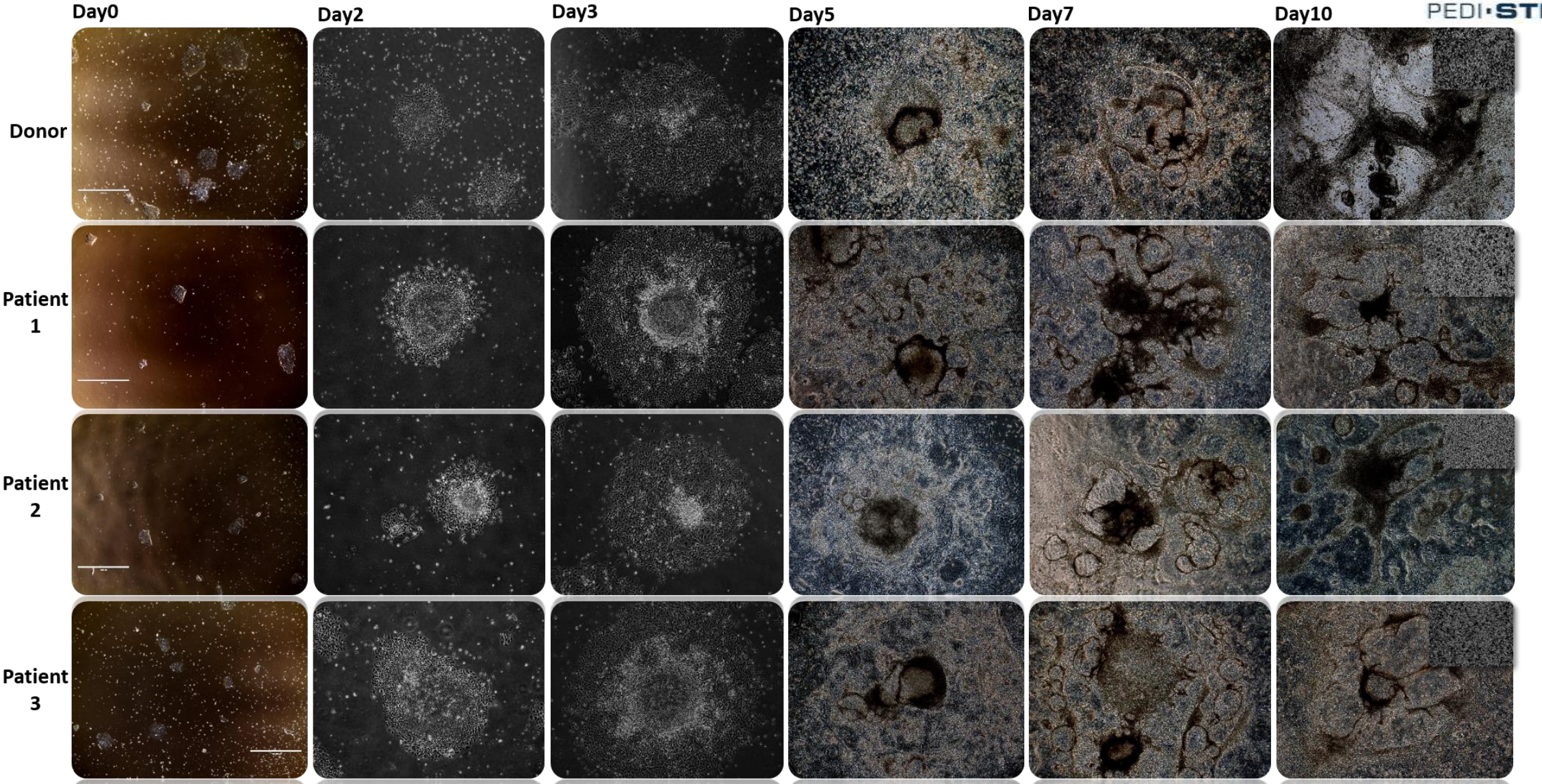
- Directed differentiation of osteopetrotic ipsc cells towards osteoclasts and evaluation of their functionality

Method

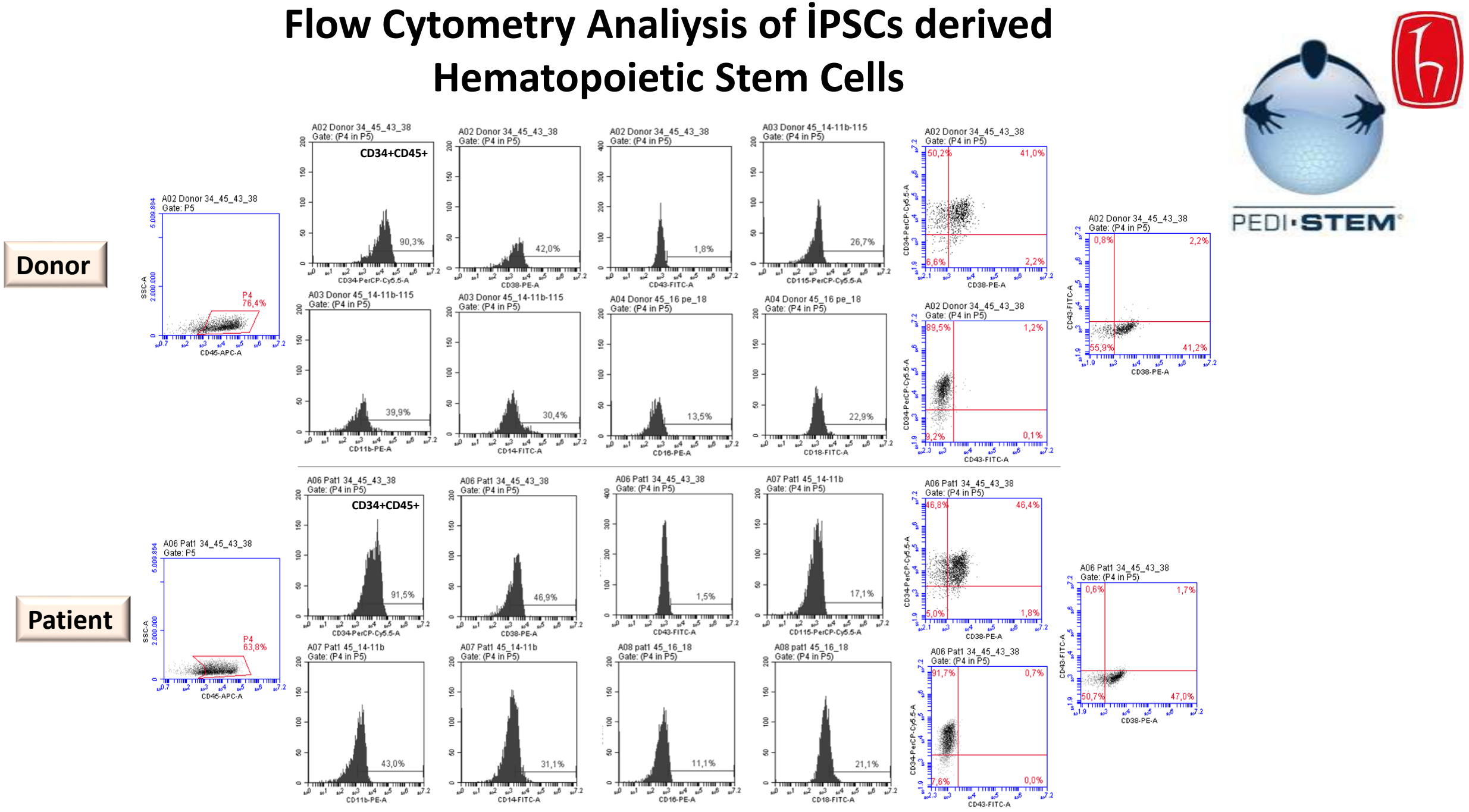

StemDiff Hematopoietic Differentiation Kit
StemSpan™ SFEM, 2mM glutamin, 4x10-4 M monotiogliserol,
50 ug/ml ascorbic acid, 10 ng/ ml hVEGF, 4U/ml EPO,
50 ng/ml TPO, 100 ng/ml hSCF, 10 ng/ml hIL-6,
5 ng/ml hIL-11, 40 ng/ml hIL-3
10 ng/mL hM-CSF ,
10 ng/mL RANKL
10 % FBS in IMDM
PEDI·STEM
TeSR-E8
Hematopoietic Differentiation
Myeloid Differentiation
Osteoclast Differentiation-21 Days
İPSC aggregates
Day
-1
0
10
14
35
Corning Osteoassay Surface 96 well plate
CD34+ Selection
Methocult 14 days
Flow Cytometry Analysis
Flow Cytometry Analysis
Flow Cytometry Analysis
Hematopoetic markers --CD34, CD45
Myeloid markers -- CD38, CD43, CD115, CD11b, CD14, CD16, CD18
Hematopoetic markers --CD34, CD45
Myeloid markers -- CD38, CD43, CD115, CD11b, CD14, CD16, CD18
CD45, CD51/61, CD14, CD16, CD18
Colony Counting
BFU-E, CFU-E, CFU-GM, CFU-GEMM
Flow Cytometry Analysis
CD45, CD41, CD36, CD33, CD18, CD14, CD16
1. TRAP (tartrate-resistant acid phosphatase) activity
(Multinucleated TRAP positive cells , at least 3 nuclei ,)
2. Rhodamine staining (actin ring)
3. Cathepsin K
4. SEM scaining
5. RT-PCR (cathepsin K, calcitonin receptor, NFATc1)

Results

Hematopoietic Differentiation of Osteopetrotic and Healthy Donor derived İPSCs



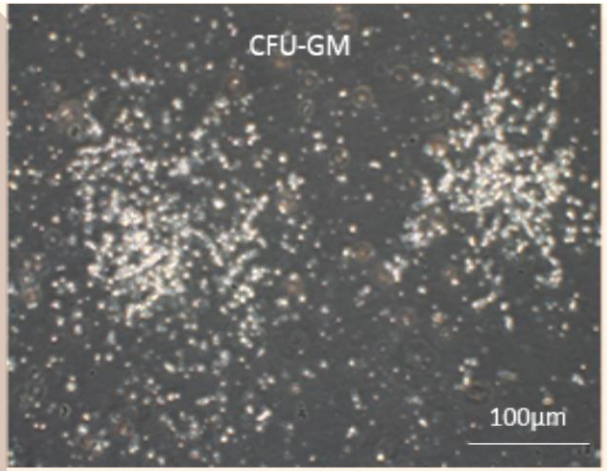
Flow Cytometry Analiysis of İPSCs derived Hematopoietic Stem Cells

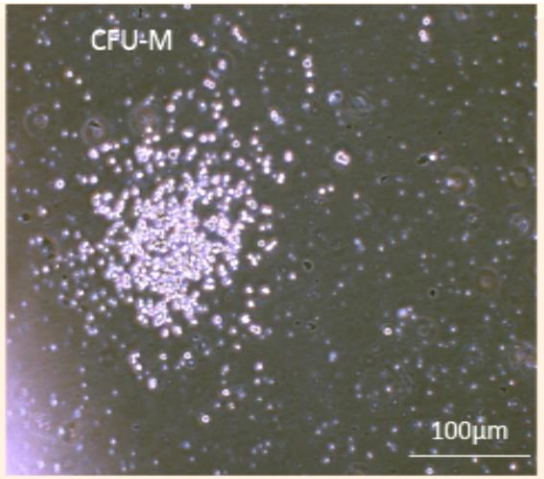


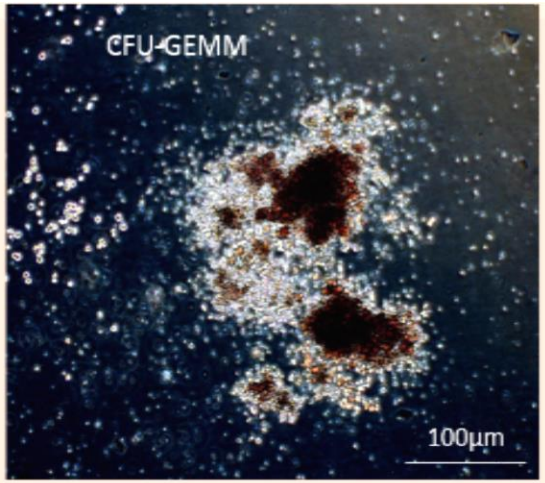
Colony Forming Capacity of Osteopetrotic and Healthy Donor İPSCs derived Hematopoietic Stem Cells

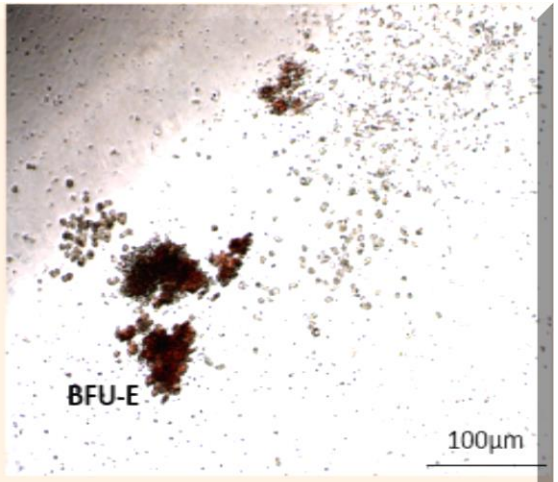
PEDI·STEM

Donor

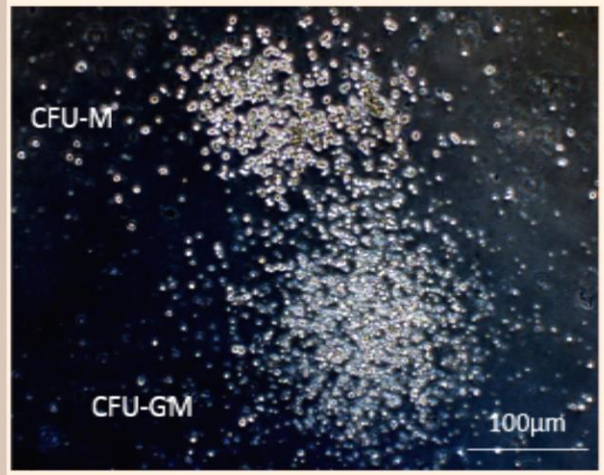


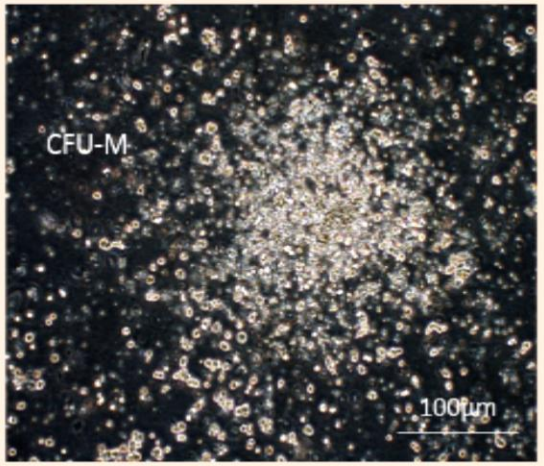


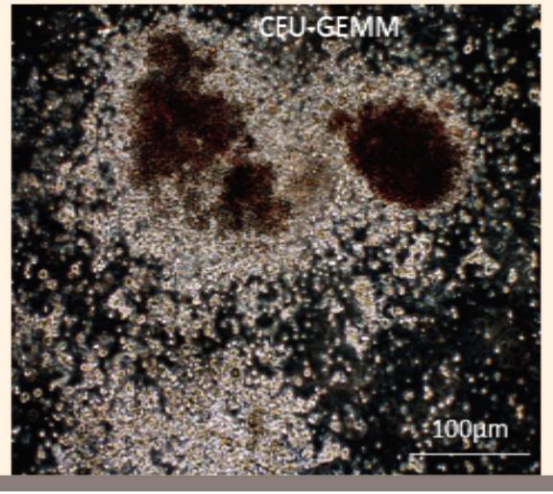


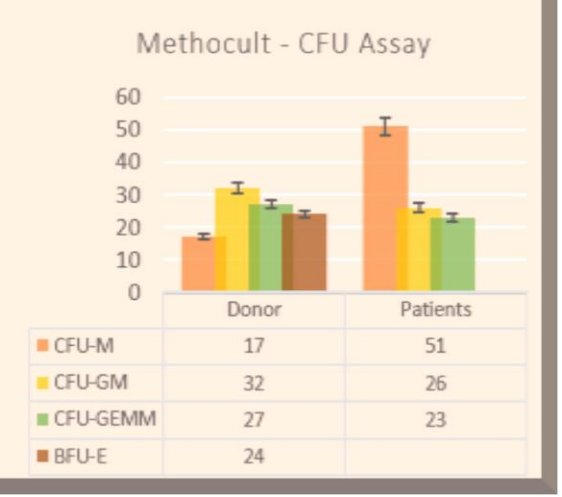


Patient





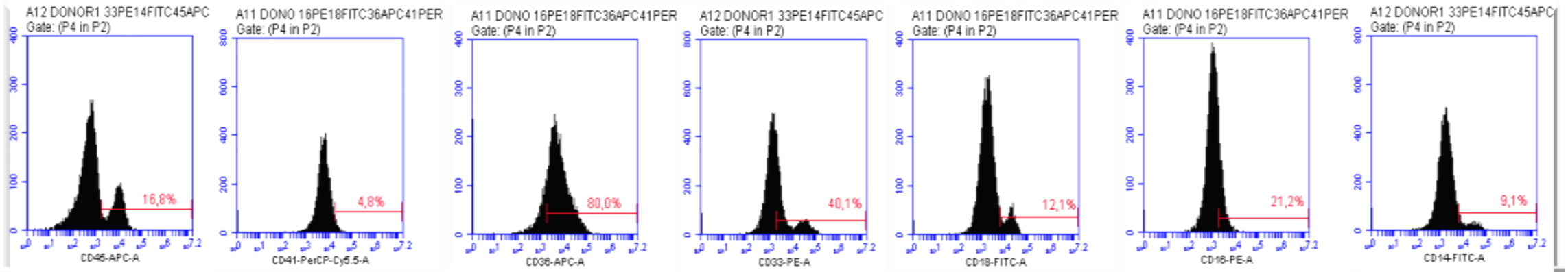




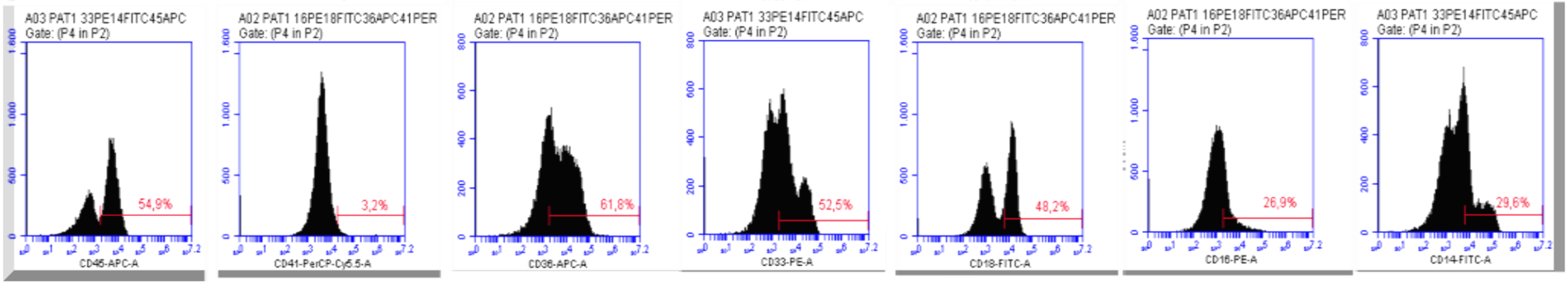
Flow Cytometry Analysis of CFU Colonies

PEDI·STEM

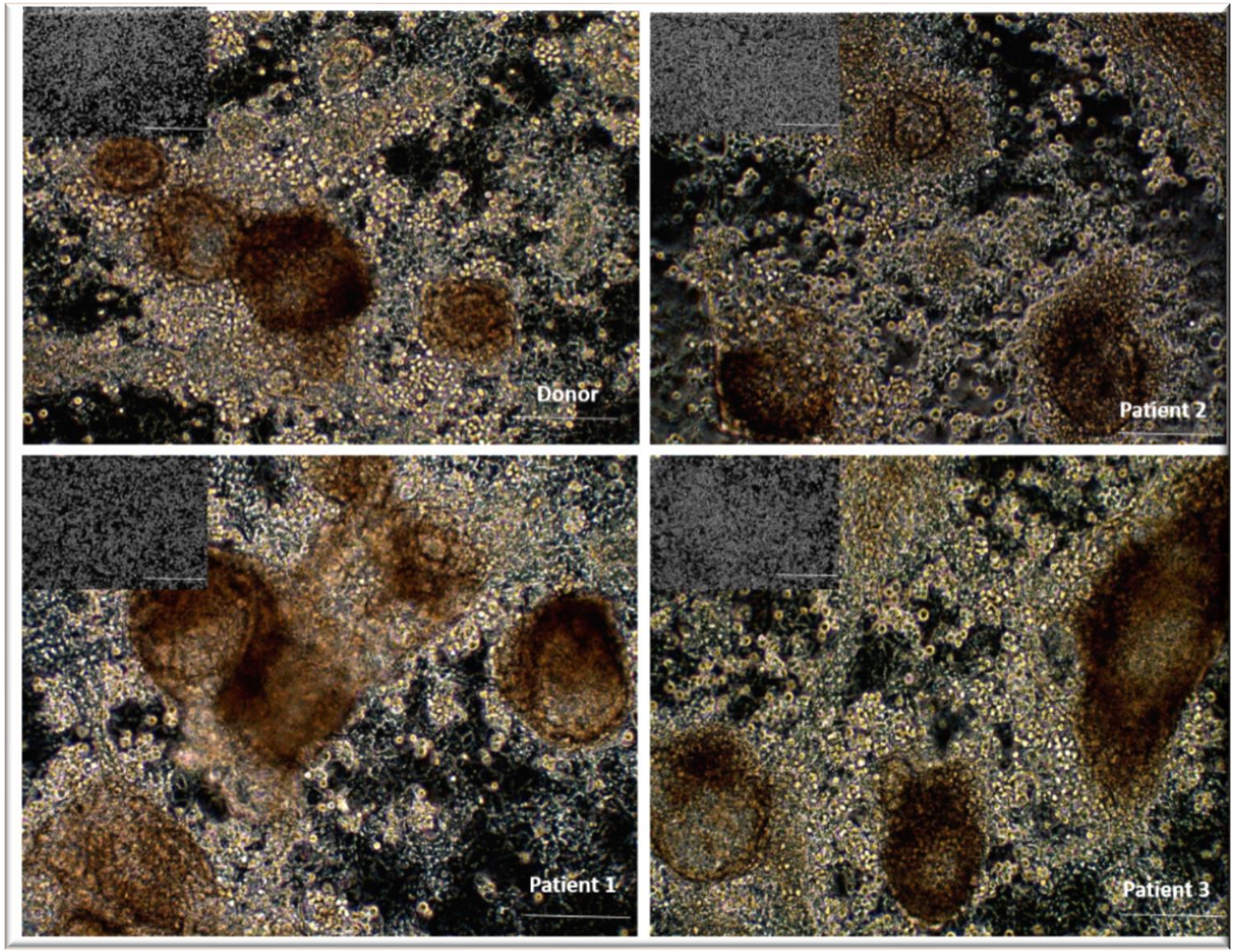
Donor



Patient



Myeloid Differentiation of Osteopetrotic and Healthy Donor derived Hematopoietic Stem Cells

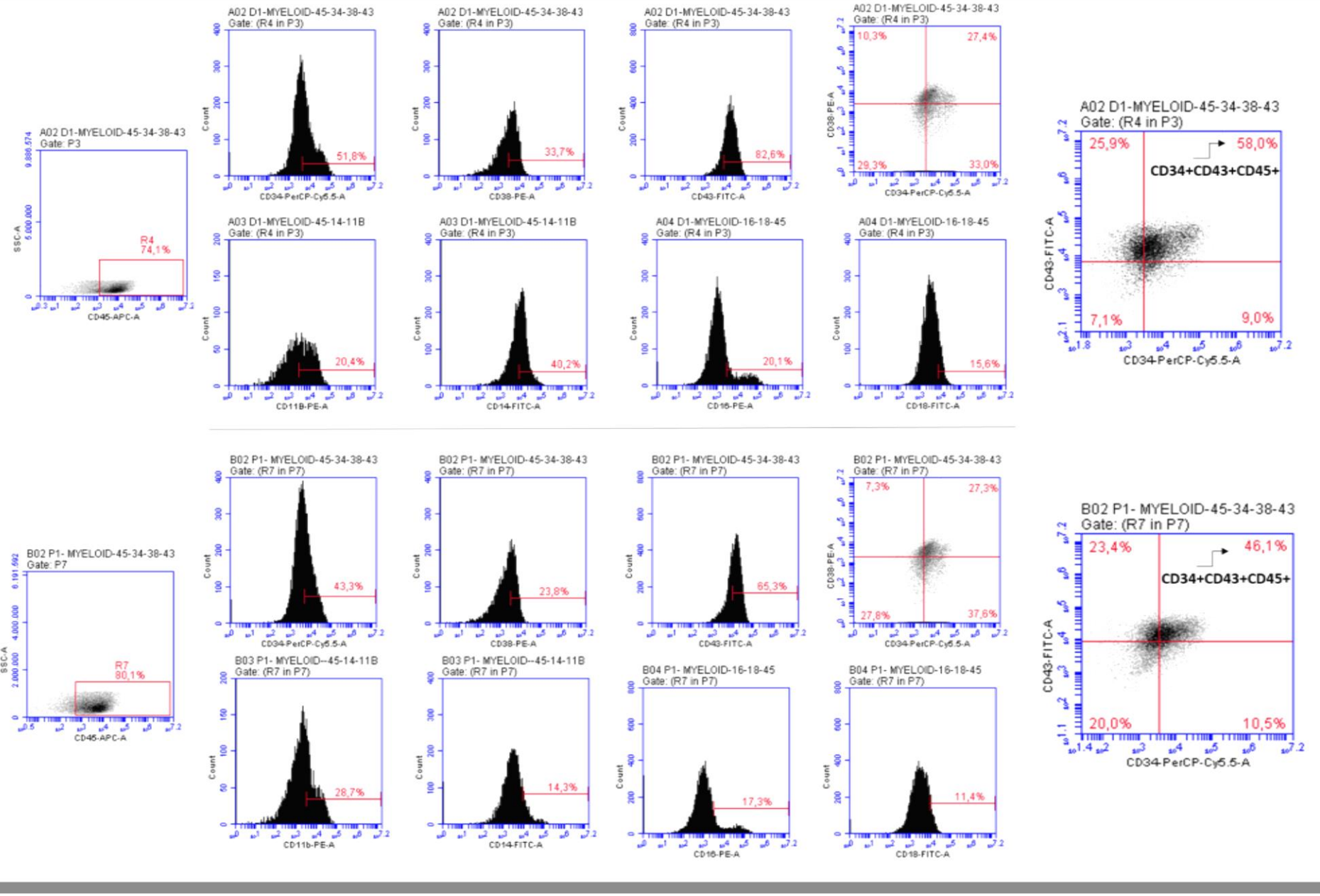


Flow Cytometriy Analysis of Myeloid Differenatiated Cells

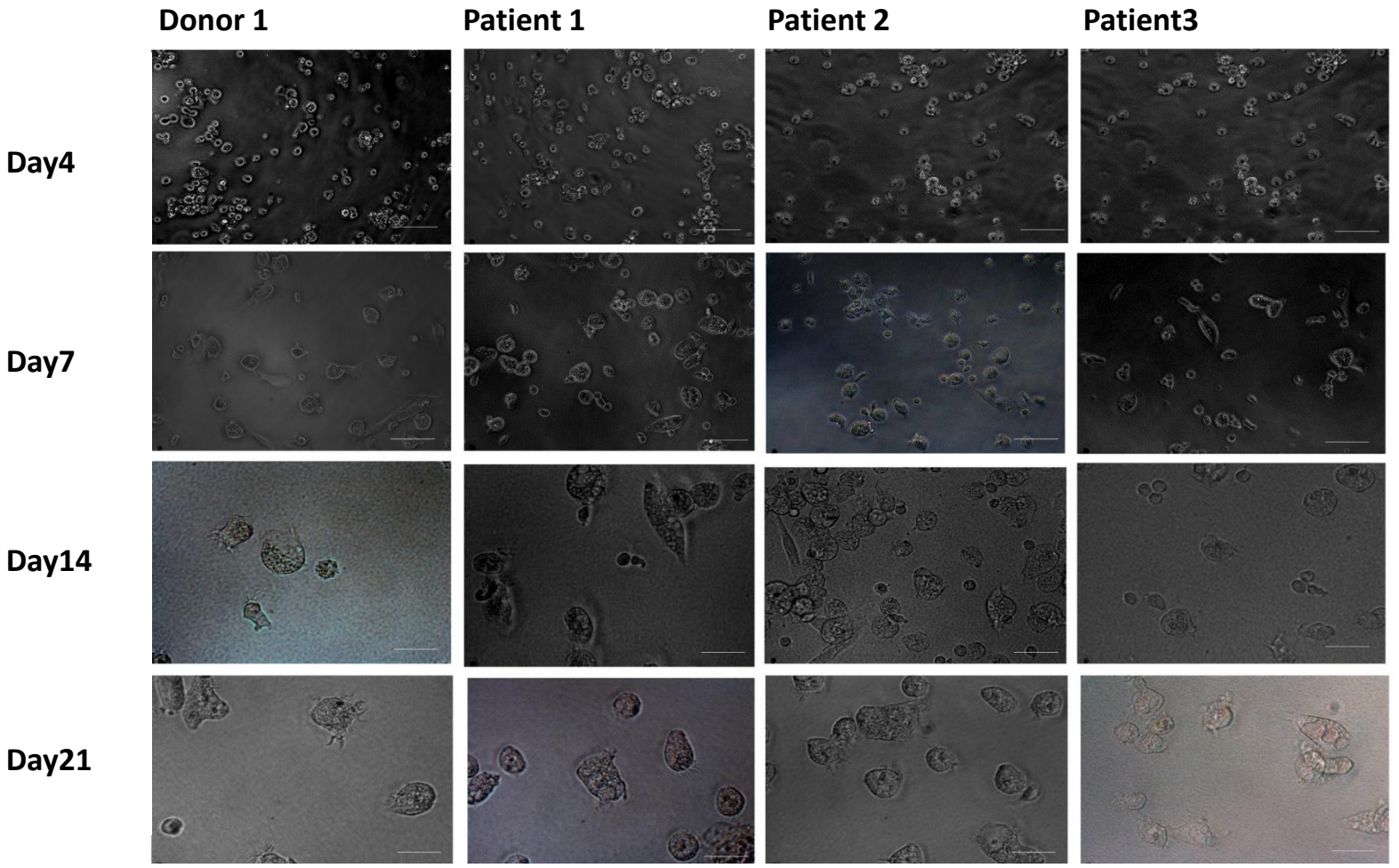
PEDI·STEM

Donor

Patient



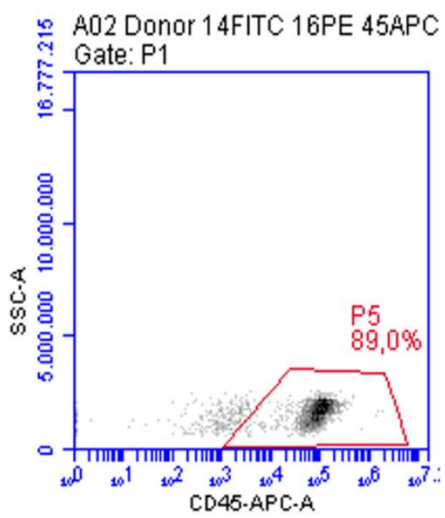
Morphological Evaluation of Osteopetrotic and healthy donor İPSCs derived Osteoclast Cells

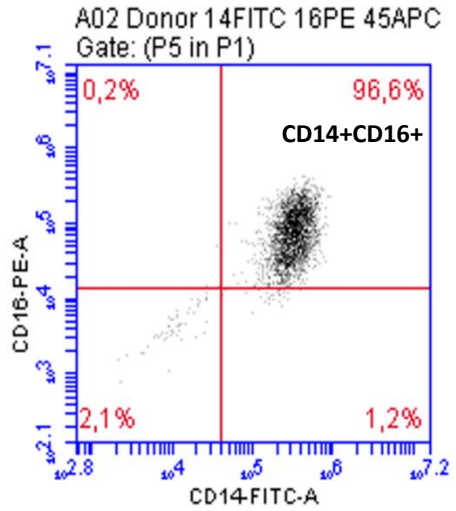


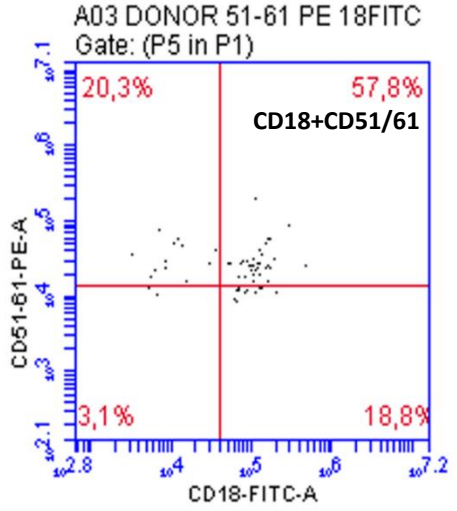
Flow Cytometry Analysis of Osteopetrotic and healthy donor İPSCs derived Osteoclast Cells

PEDI·STEM

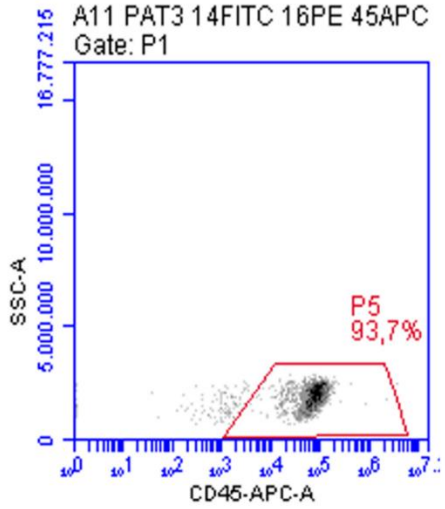
Donor

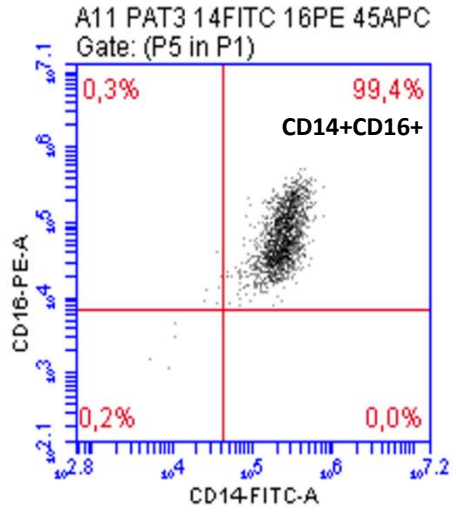


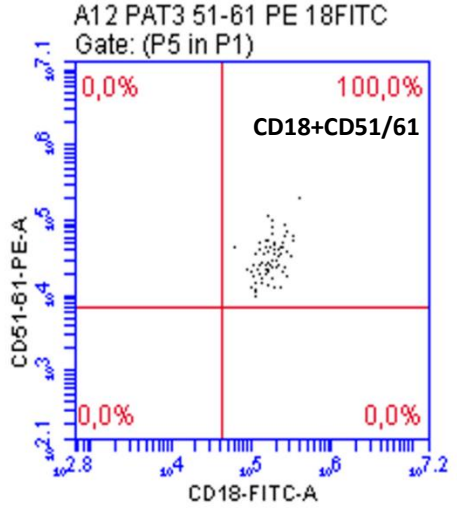




Patient



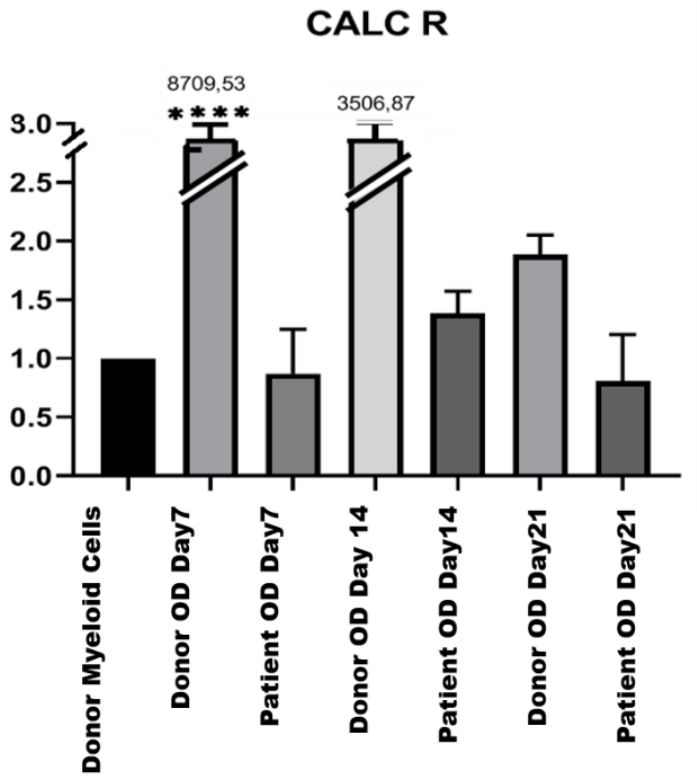


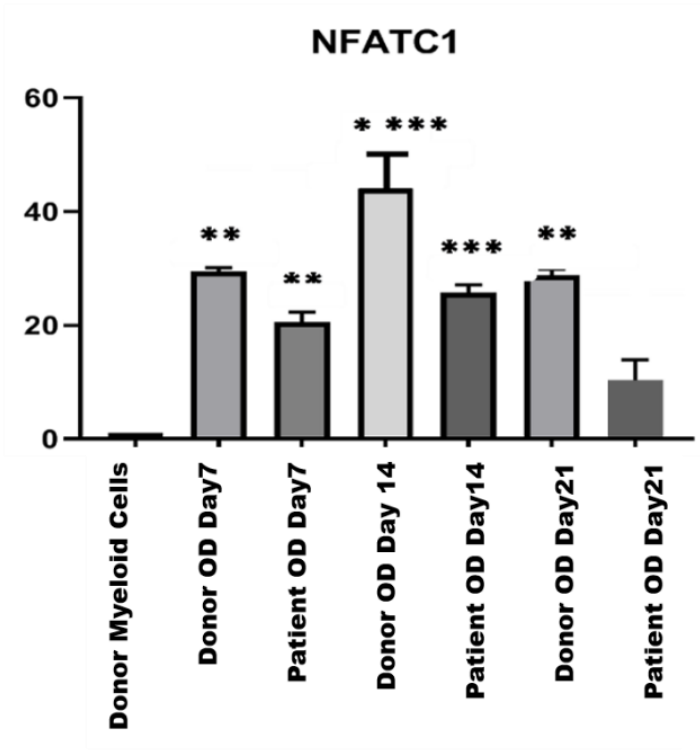


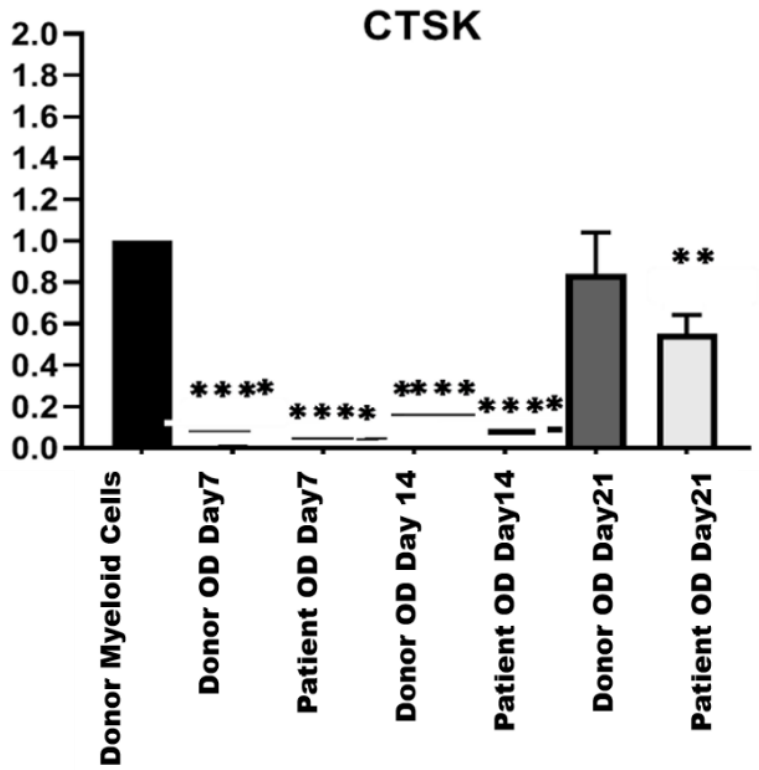
Myeloid marker
CD14, CD16, CD18

Osteoclast marker
CD14+CD16+
CD18+CD51/61+

Gene Expression Profile of İPSCs derived Osteopetrotic and Healthy Donor Osteoclasts



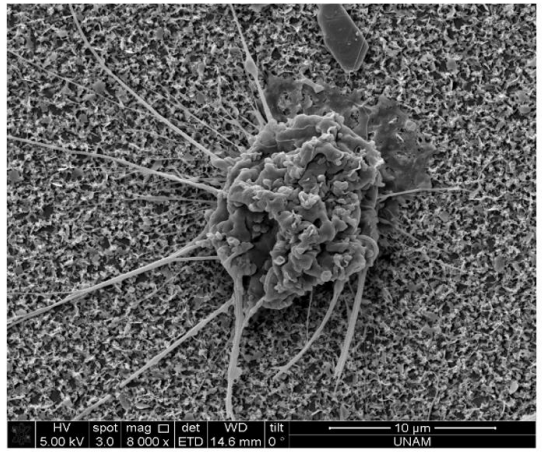
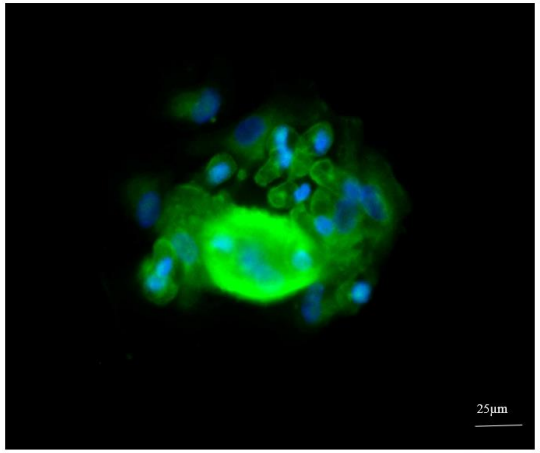
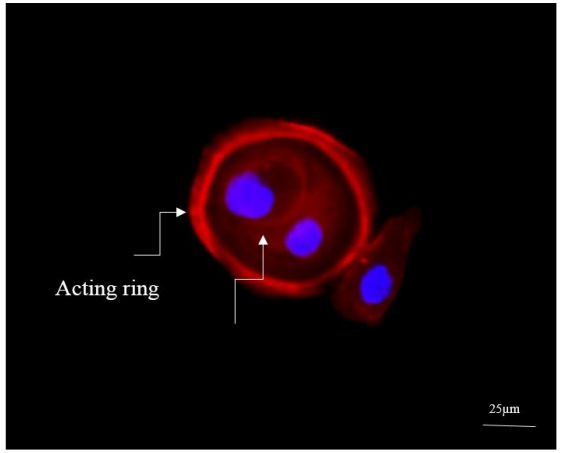
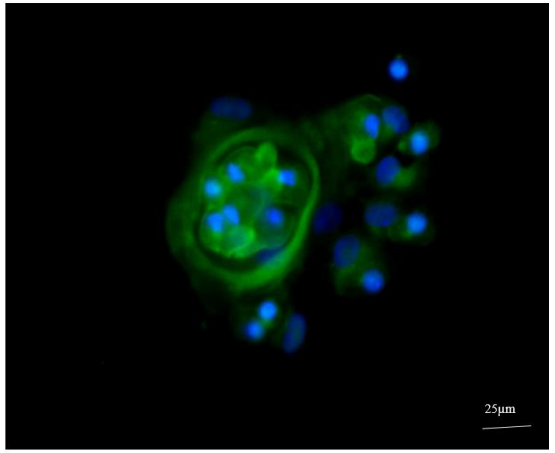
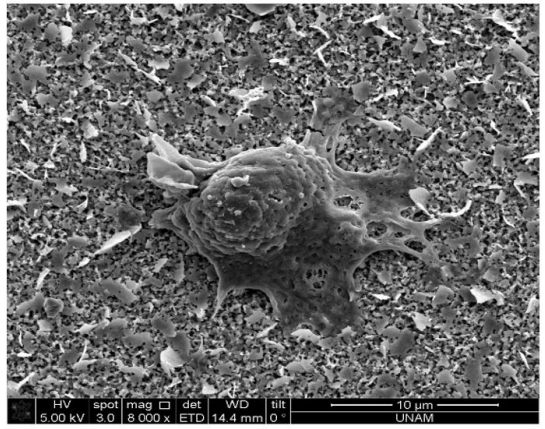
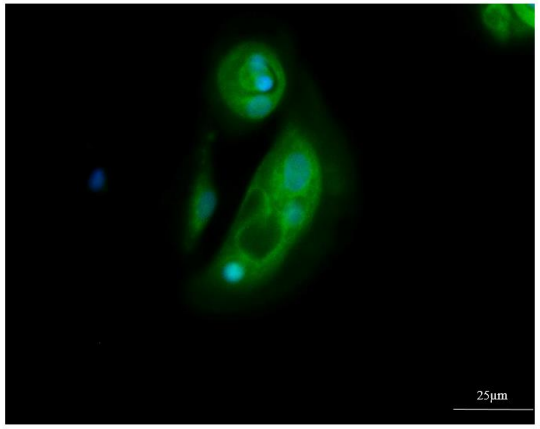
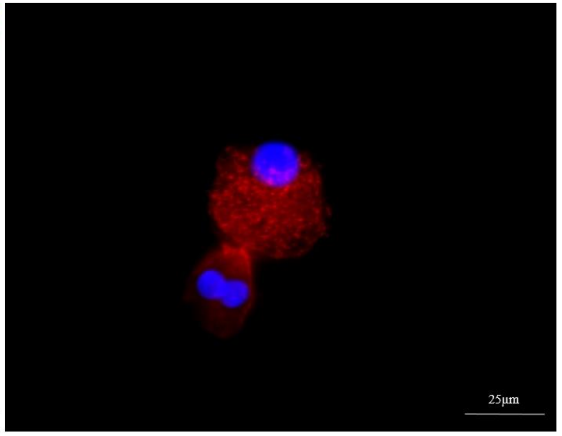
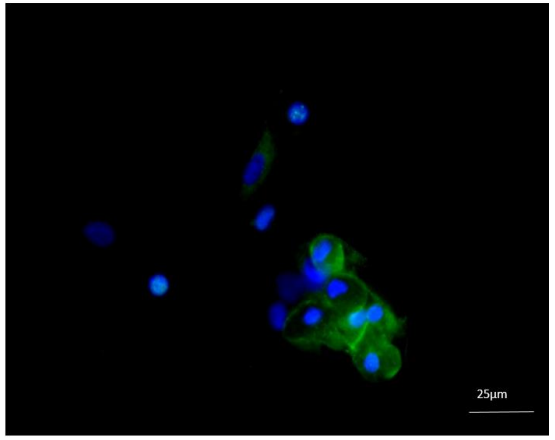




O.D. Osteoclast differentiation

Evaluation of Osteoclast Specific Proteins of Osteopetrotic and healthy donor İPSCs derived Osteoclasts

PEDI·STEM

	SEM	Cathepsin K	Rhodamine	TRAP
Donor				
Patient				

In the future persfective…

- promising tool for investigating mechanisms of osteopetrosis or other osteoclast related disorders.
- increase our knowledge about normal-and osteopetrotic-osteoclastogenesis, but needs to be supported with more detailed functionality-analyses.
- Osteopetrotic niche modelling
- Gene editing…

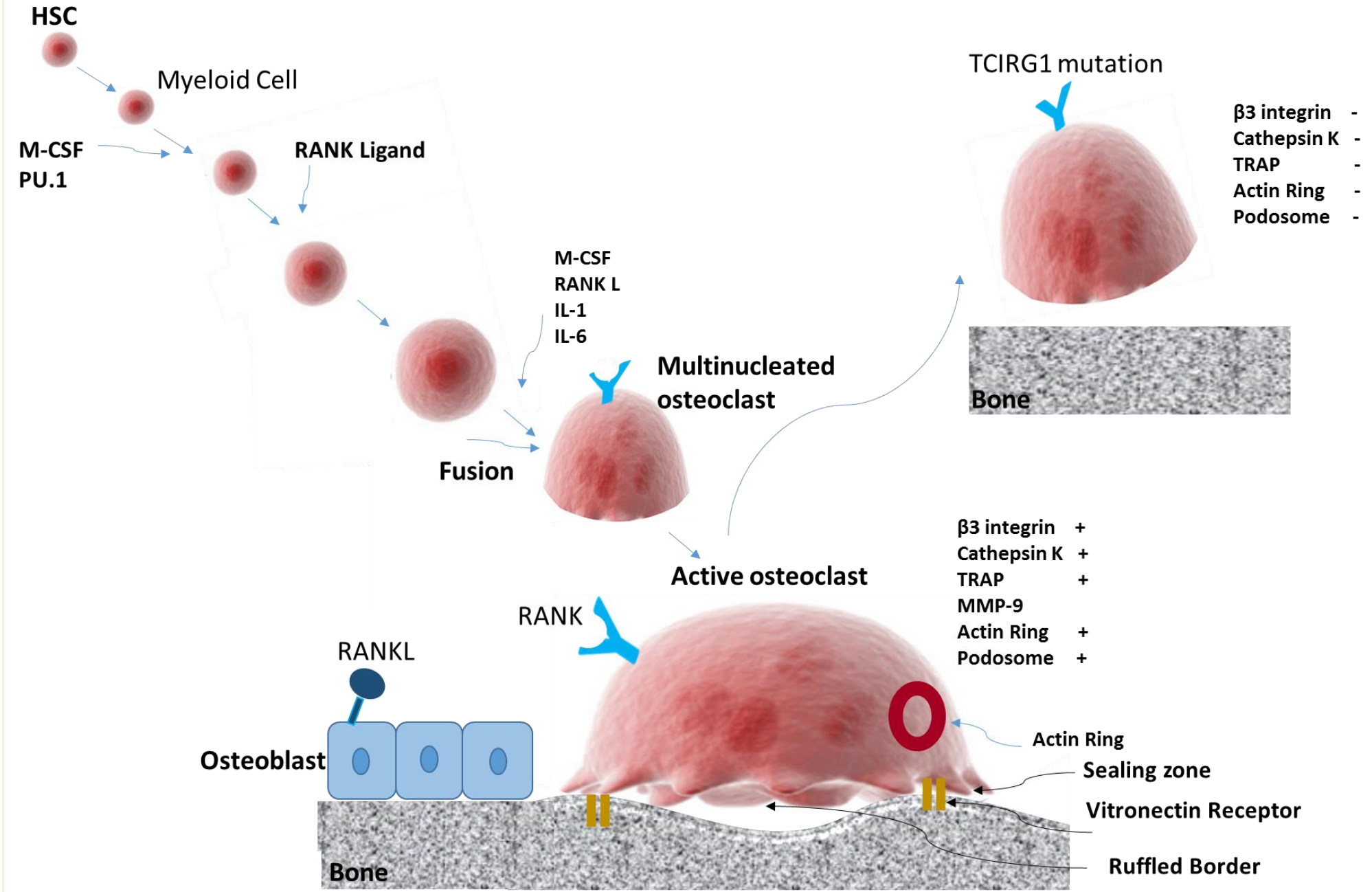
for your attention!

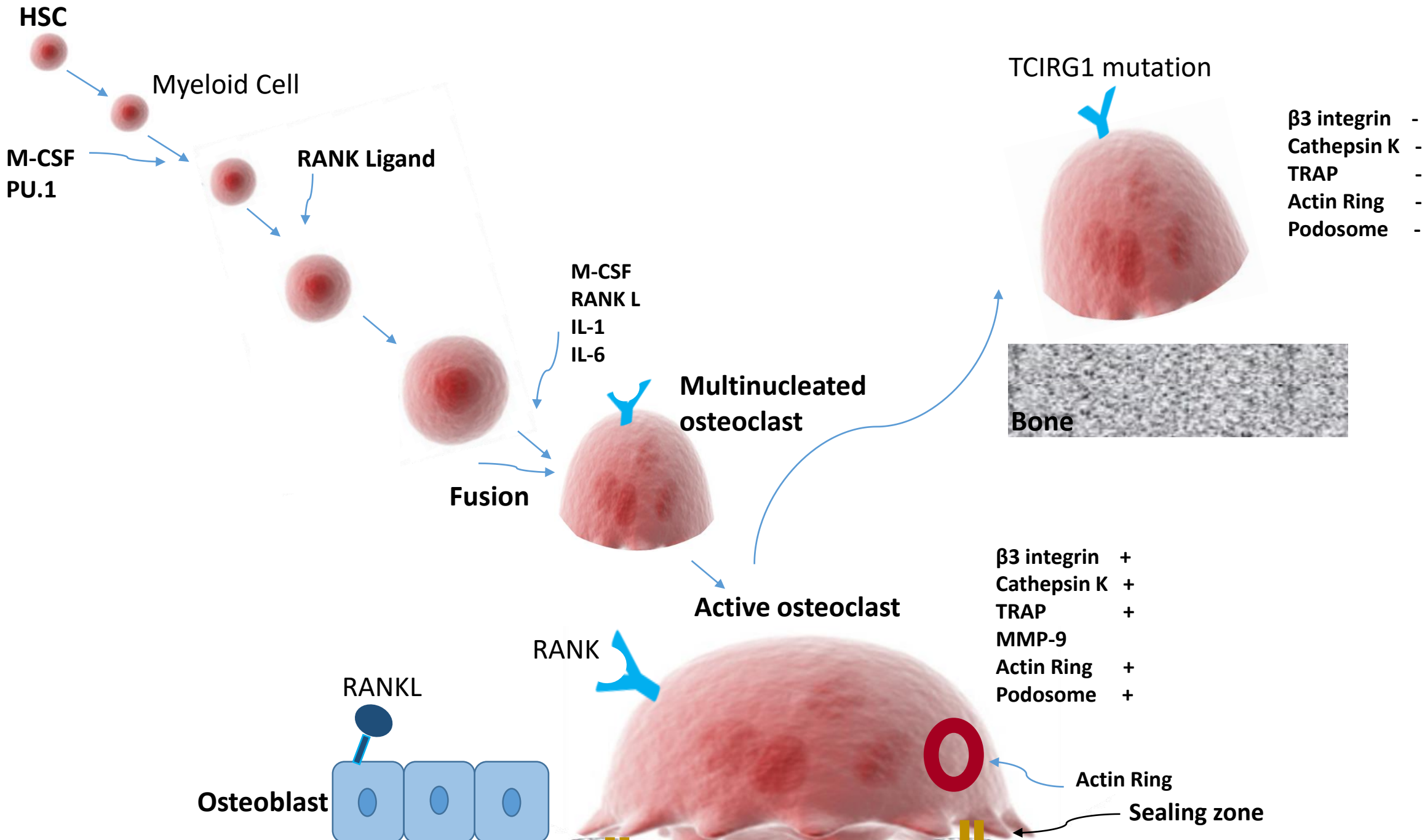
I am on the market for a postdoc position!

Please let me know if you have available positions…

incicevher@gmail.com

Generation of Functional Osteoclast




HSC
Myeloid Cell
M-CSF
PU.1
RANK Ligand
M-CSF
RANK L
IL-1
IL-6
Fusion
Multinucleated
osteoclast
TCIRG1 mutation
β3 integrin -
Cathepsin K -
TRAP -
Actin Ring -
Podosome -
Bone
Active osteoclast
β3 integrin +
Cathepsin K +
TRAP +
MMP-9
Actin Ring +
Podosome +
RANK
RANKL
Osteoblast
Actin Ring
Sealing zone

Mutation Verification of Osteopetrotic İPSCs

Patient	Patient mutations before reprogramming			Patient Derived-İPSC (Passage 20)		
	Gene	Exon	Mutation	Clone name	Mutation	Mutation rate
Patient 1	TCIRG1	5	g.4062G>A	Patient 1-IPS#Sev	IVS5+5G>A (alternative name)	100%
Patient 2	TCIRG1	6	g.4389G>A	Patient 2-IPS#Sev	IVS5-8G>A (alternative name)	100%
Patient 3	TCIRG1	9	g.5212delCinsAA, Leu288Asnfs202X	Patient 3-IPS#Sev	g.5212delCinsAA, Leu288Asnfs202X	100%