PD04-11

# M2 macrophages differentiated from iPS cells have higher abilities to phagocytose and process calcium oxalate crystals than similarly induced M1 macrophages

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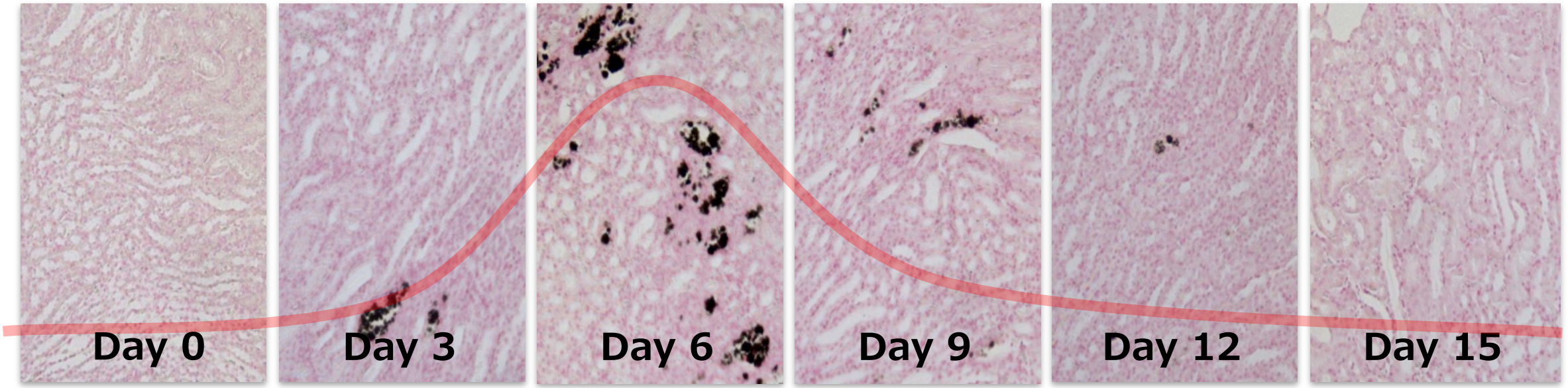
# Backgrounds

## Establishment of kidney stone model mice



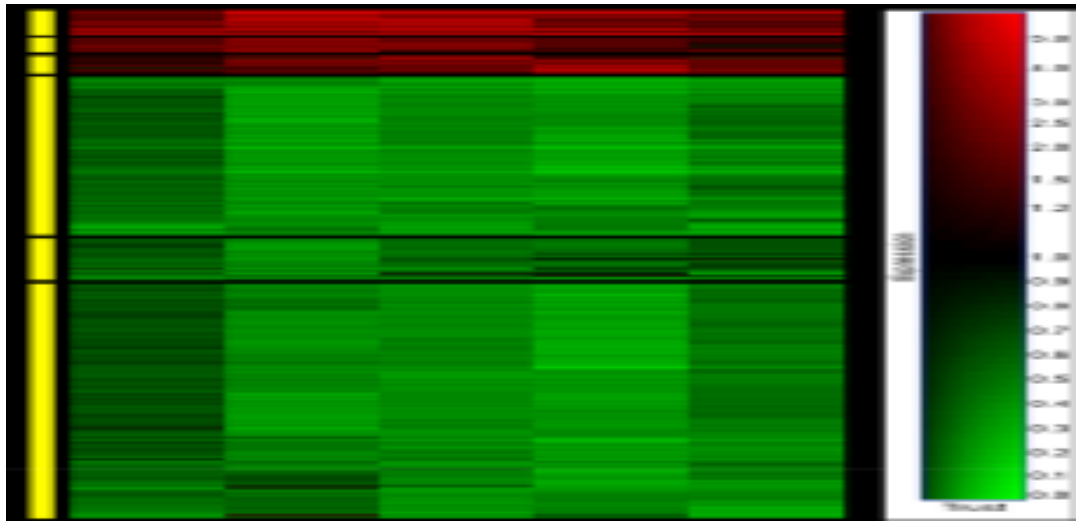

**In this mouse model, we discovered 'spontaneous elimination' of the renal crystal depositions.**

Okada A et al. Urol Res 2007.

# Backgrounds

## Elucidation of the molecular mechanism of crystal elimination

### Microarray analysis

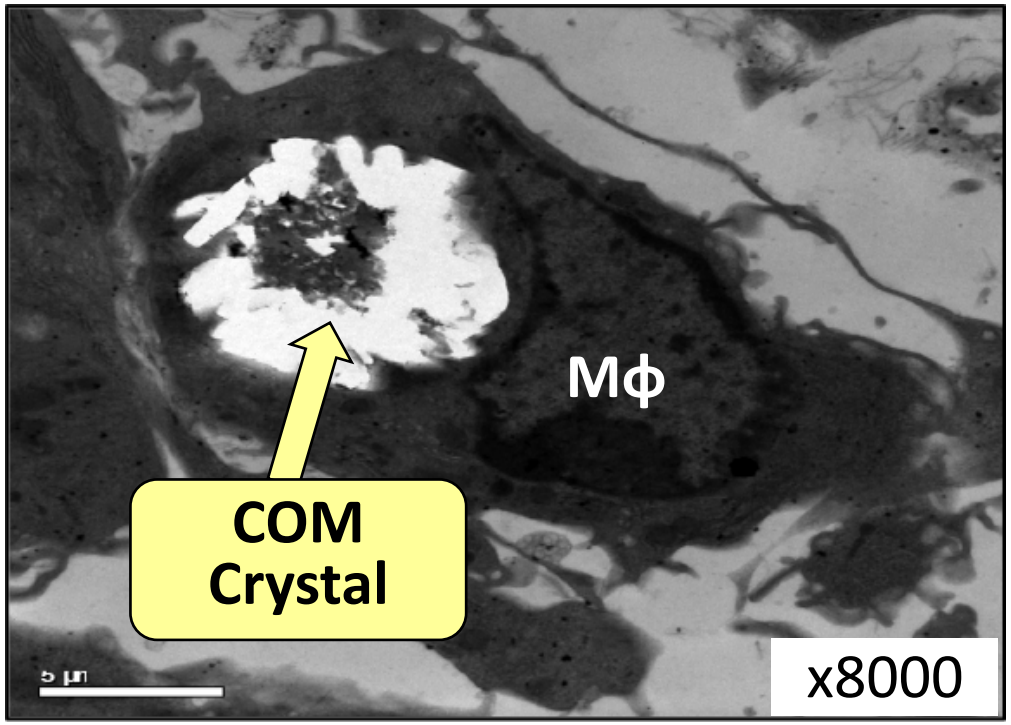


Macrophage-related gene ↑

Antioxidative and lipid metabolism-related gene ↓

Okada A, et al. J Bone Miner Res 2009

### Transmitted electron microscopy



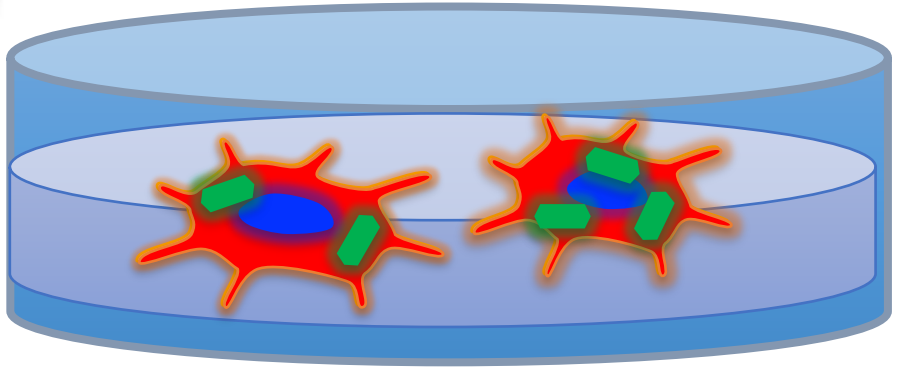

Okada A, et al. J Bone Miner Res 2010

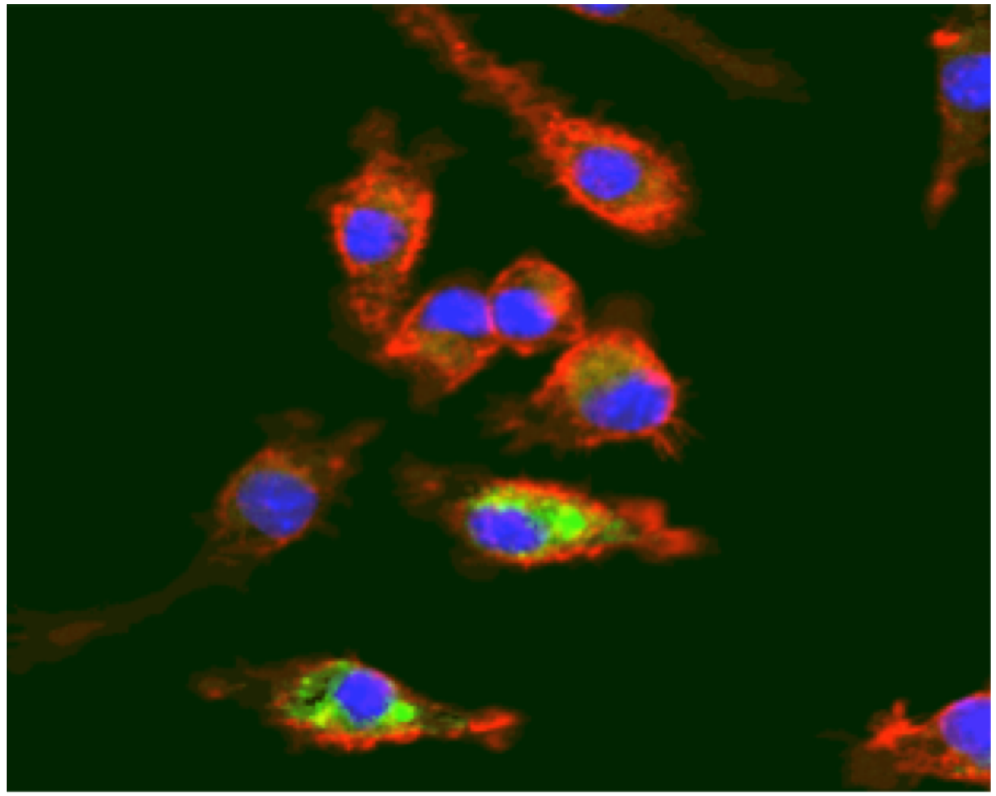
**We captured the images that macrophages englobed crystals**

# Backgrounds

## Establishment of phagocytosis model of fluorescent COM crystals

Imaging cytometer
(IN Cell Analyzer 6000)



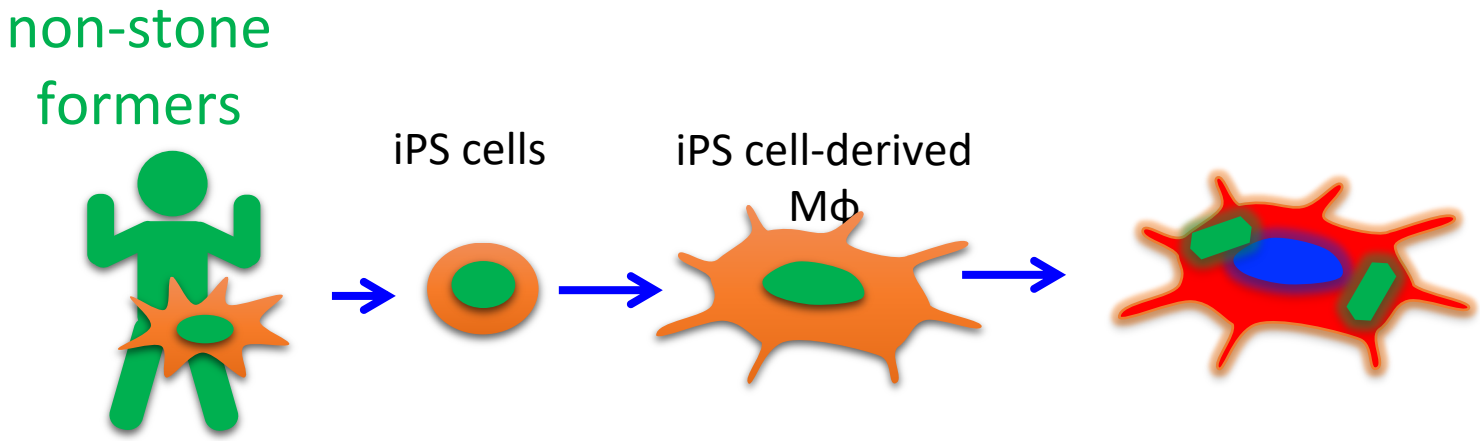


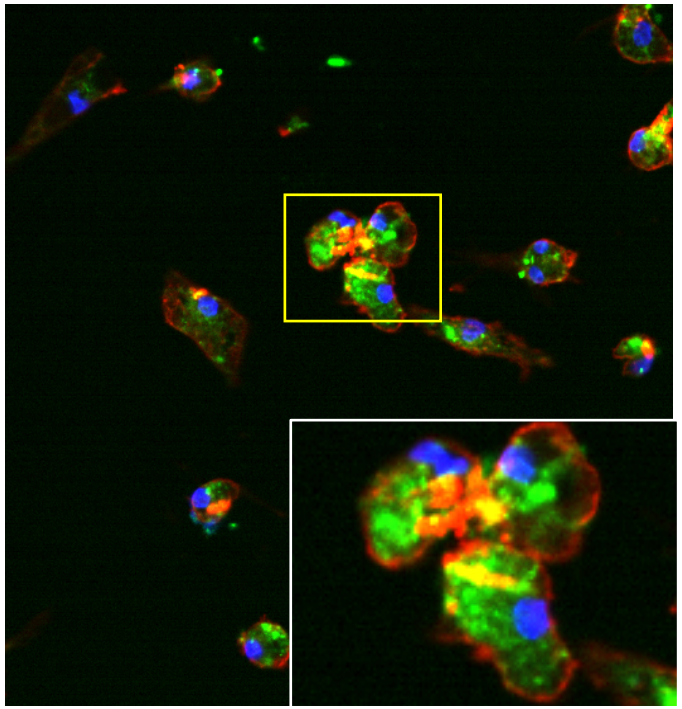
Okada A, et al. AUA annual meeting 2018

**We could quantify crystal phagocytosis rates**

# Backgrounds

**Induction of iPS cell-derived macrophages to phagocytose calcium oxalate crystals for drug screening**



Okada A, et al. AUA annual meeting 2019

- **We generated iPS cell-derive MΦ from the somatic cells of non-stone formers**
- **iPS cell-derive MΦs could phagocytose COM crystals**

# Objectives

**The aim of this study is**
**to differentiate human iPSC-derived Mϕs into inflammatory type (M1) and anti-inflammatory type (M2),**
**and compare their phagocytosis and processing ability of fluorescent calcium oxalate crystals.**

# Methods

## Characteristics of iPS cells used in this study

- 648A1 Origin: cord blood

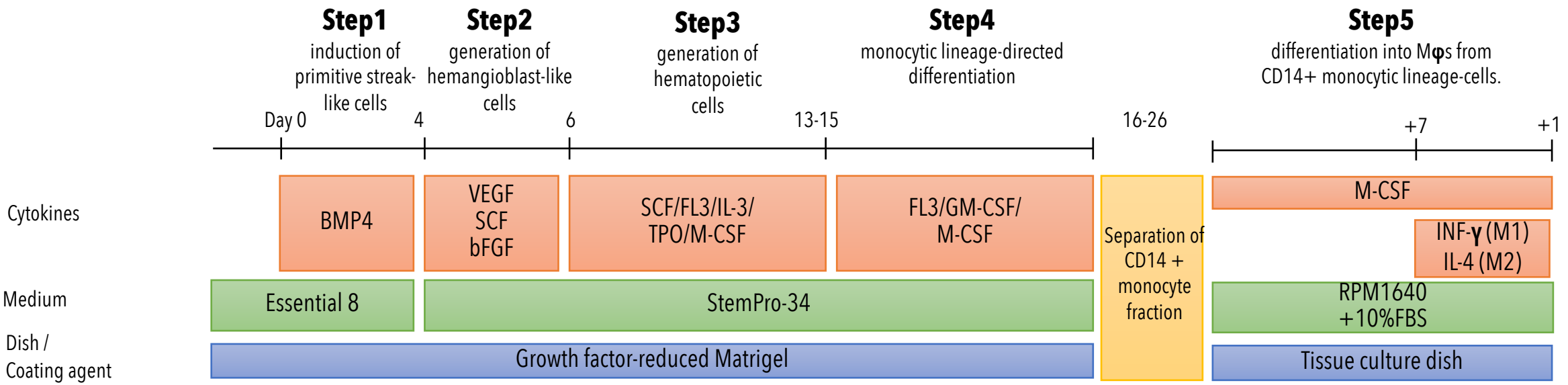
  Transgene: human 6 gene (Oct3/4, Sox2, Klf4, L-Myc, Lin28, shRNA against p53 )

- DO-NSF Origin: peripheral blood (from healthy volunteers)

  Transgene: human 6 gene (Oct4, Sox2, Lin28, L-Myc, Klf4, mp53DD)

# Methods

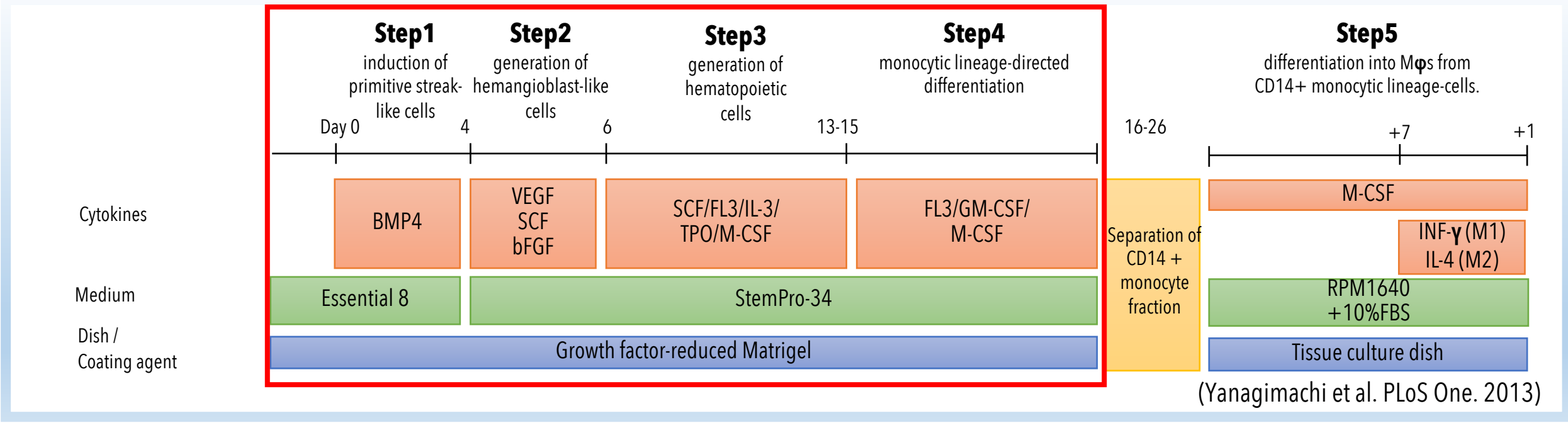
## Differentiation of iPS cells to Mϕ



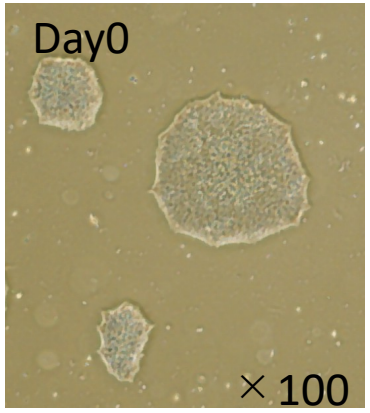

(Yanagimachi et al. PLoS One. 2013)

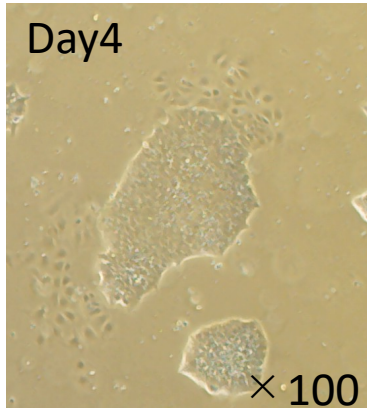
# Methods

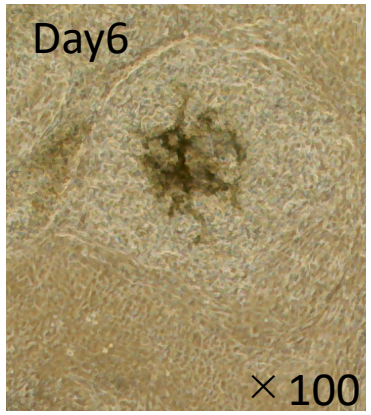
## Differentiation of iPS cells to Mϕ

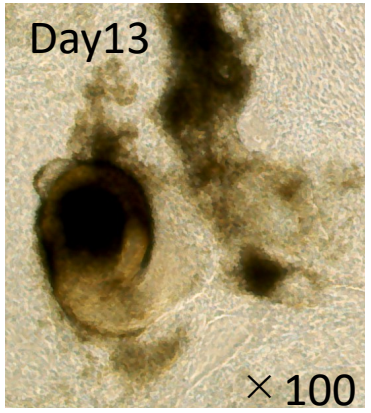


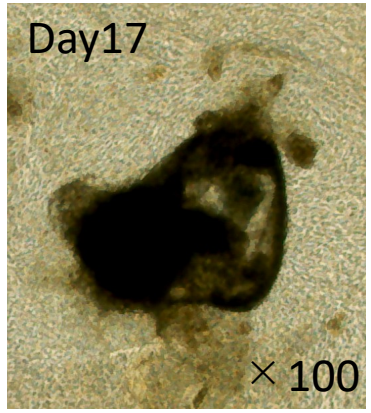

(Yanagimachi et al. PLoS One. 2013)



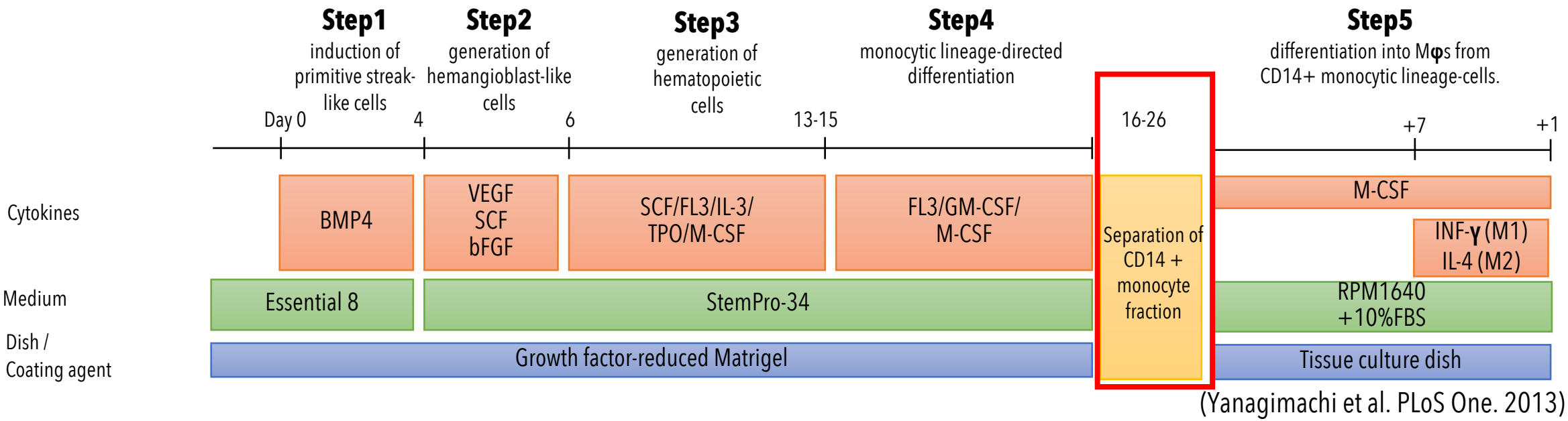



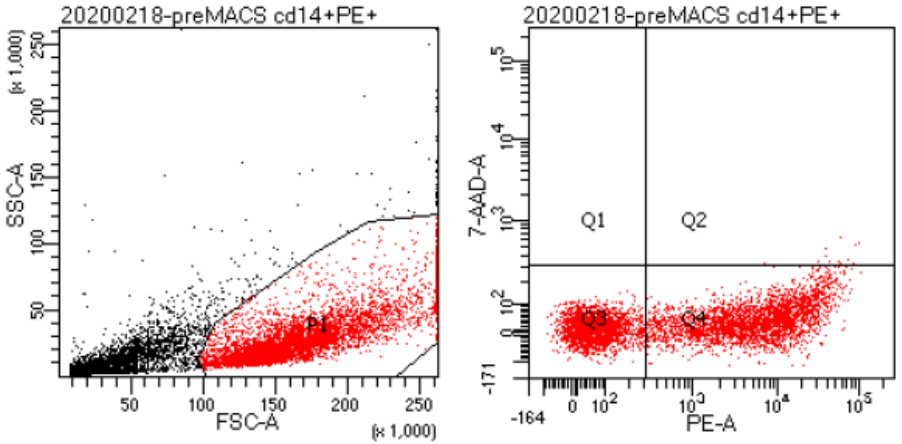

Through the **Step 1-4**, iPS cells formed dense cell clusters, and **floating cells** appeared in about 3 weeks.

# Methods

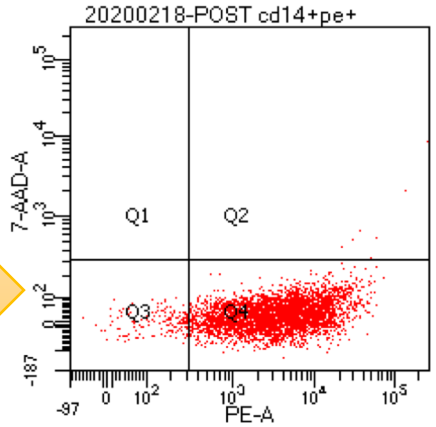
## Differentiation of iPS cells to Mϕ




(Yanagimachi et al. PLoS One. 2013)



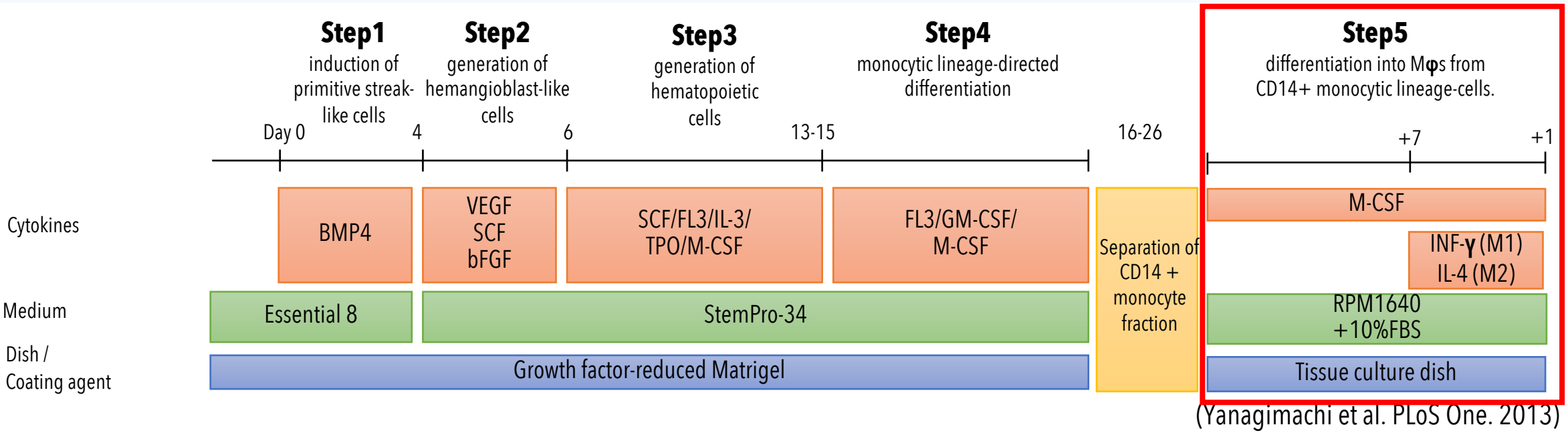




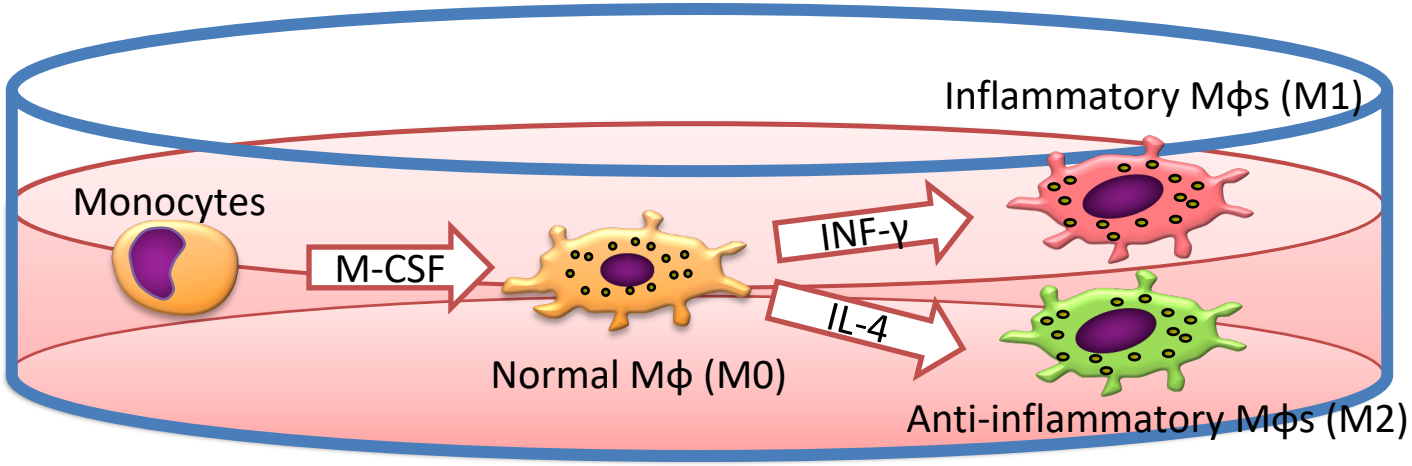

After Step 1-4,
the floating cells were separated into **CD14⁺ cells** using AutoMACS® PRO

# Methods

## Differentiation of iPS cells to Mϕ




(Yanagimachi et al. PLoS One. 2013)

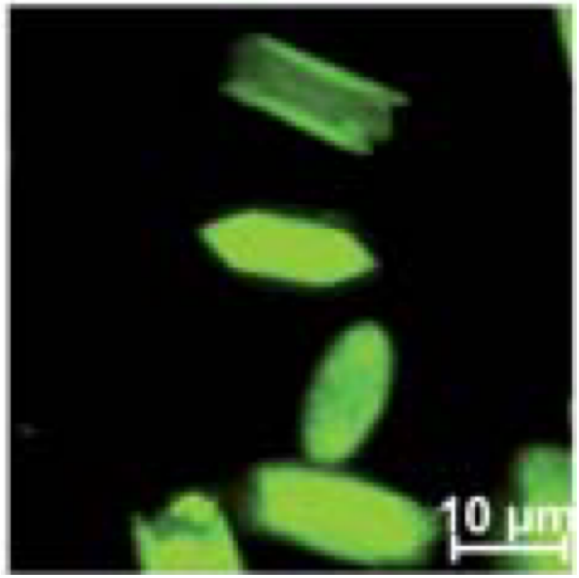



During **Step5**, the iPSC-derived monocytes were differentiated into macrophages for 7days.
Furthermore, they were differentiated into **M1** and **M2** by the addition of INF-γ and IL-4, respectively.

# Methods

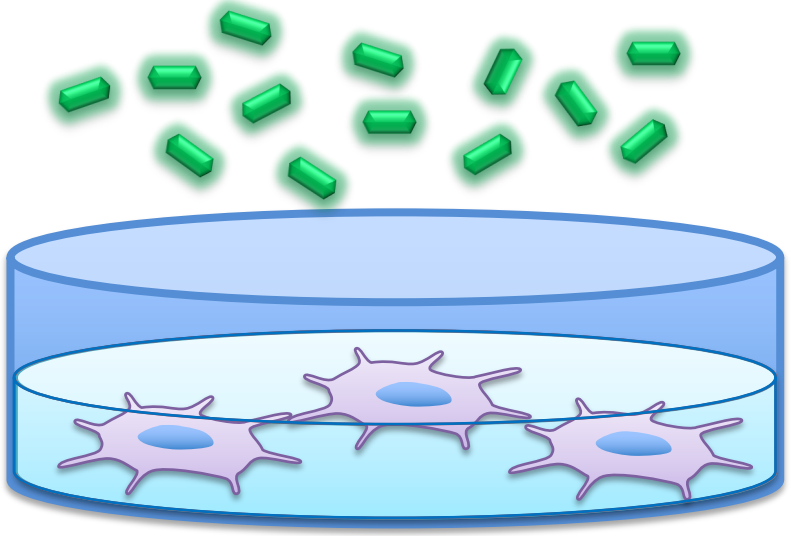
## Imaging analysis using fluorescently labeled calcium oxalate monohydrate (f-COM) crystals

COM crystals stained with AlexaFluor-488

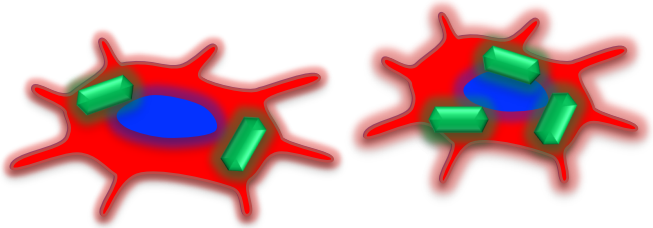



(Chaiyarit S, et al. Anal Methods 2010)

- After differentiation of iPSC-derived Mϕs
- The cytoplasm was stained with **CellTracker™ Orange CMRA** and **DAPI**.
- **f-COM** crystals were exposed

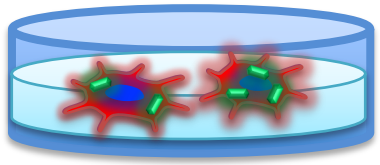


- By using the **imaging cytometer**, we quantified the signal intensity and number of f-COM crystals per cell.



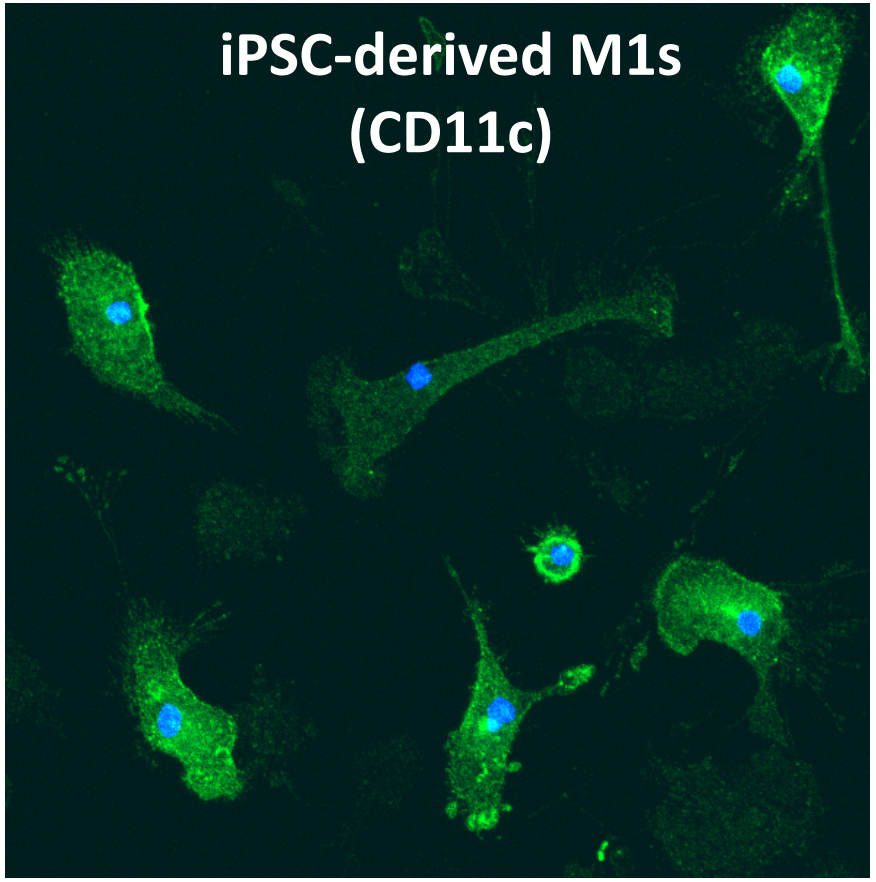
**InCell Analyzer 6000**

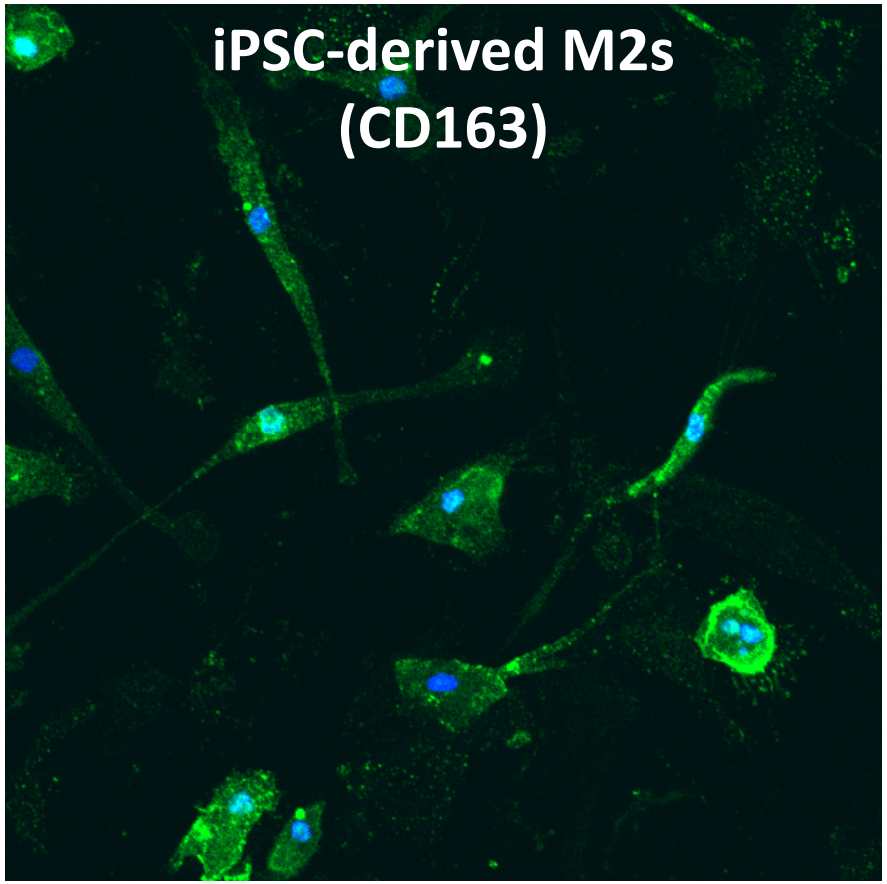
**15 Fields of View /Well**



# Results

## Fluorescent immunostaining of cell surface markers of macrophages

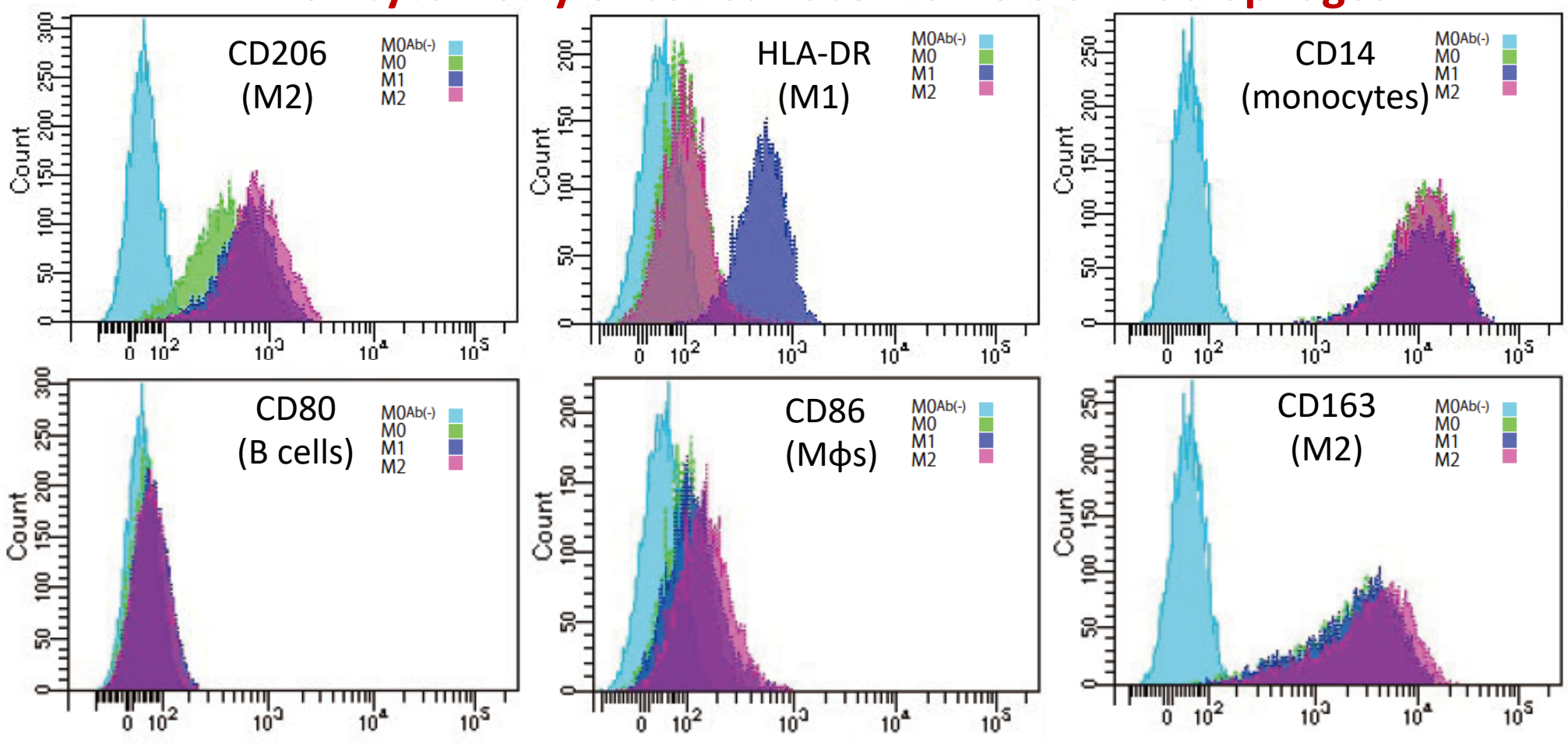



The iPS cell-derived M1 and M2 expressed CD11c and CD163, respectively

# Results

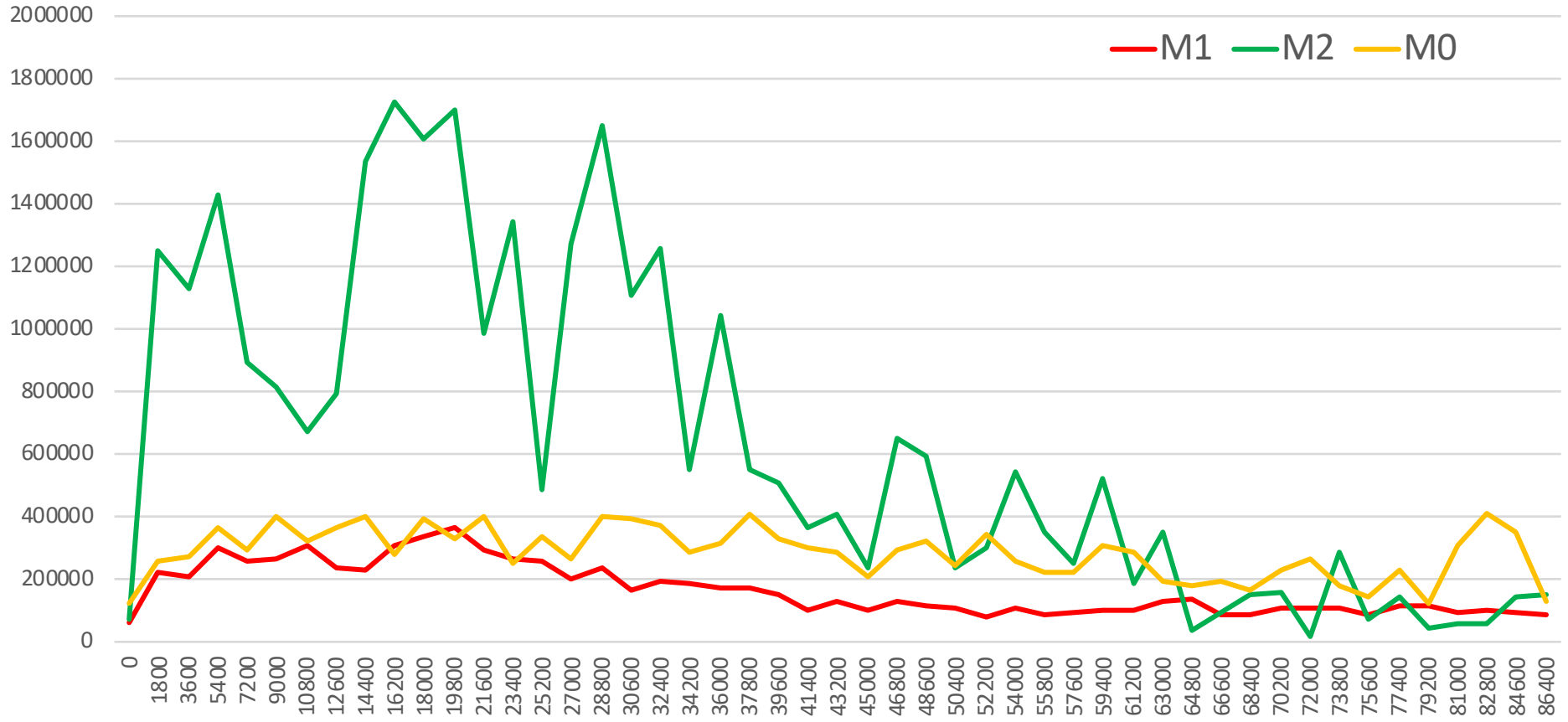
## Flowcytometry of cell surface markers of macrophages




Flowcytometry of the iPS-derived macrophages showed well differentiation of iPS cells into M1 and M2.

# Results

## Chronological changes of fluorescence intensity of COM crystals per cells




iPSC-derived M2 could higher ability of f-COM crystal phagocytosis compared to M0 and M1.

# Discussion

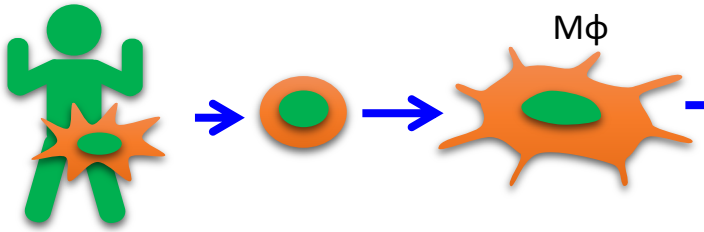
## Concept of drug screening model using iPS cell-derived Mϕs

non-stone formers (NSF)

AUA 2019

iPS cells

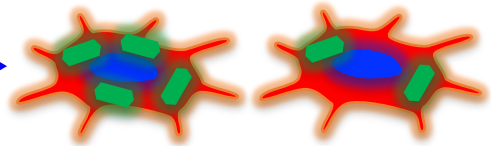
iPS cell-derived Mϕ



- We generated iPS cell-derive Mϕ from the somatic cells of non-stone formers
- iPS cell-derive Mϕs could phagocytose COM crystals

AUA 2020

M2 M1

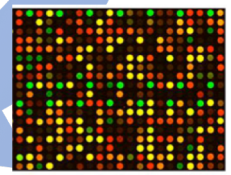


**Phagocytosis rate M2>M1**

In NSF, iPS-derived M2 showed higher property of crystal phagocytosis than M1.

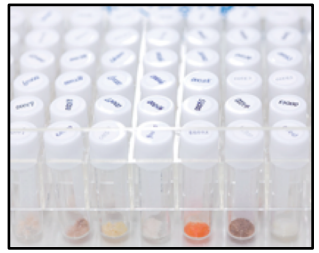
As the next step…

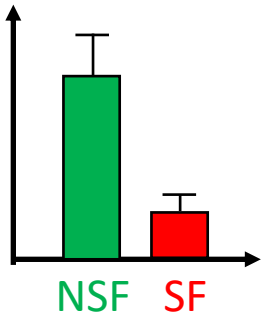
Comparison of the phagocytosis rates and genetic backgrounds between the groups

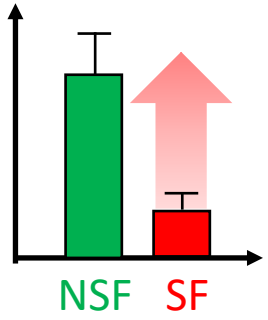


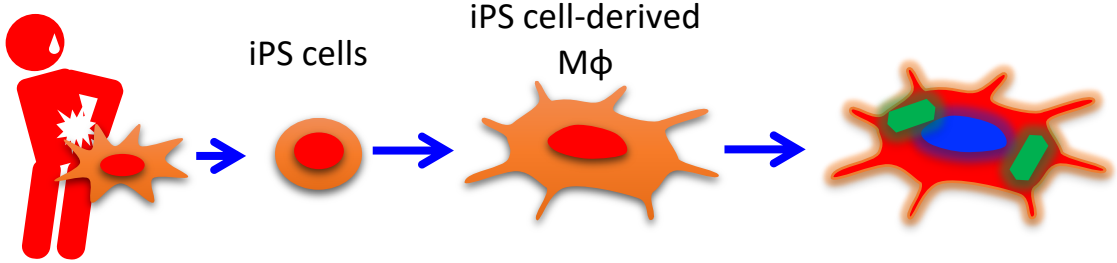
And IF,
we can detect significant differences in phagocytosis rates between NSF and SF

We will project **drug screening using chemical library** for seeking **the drugs that can increase the phagocytosis rates of iPS-derived Mϕ of SFs**





iPS cells

iPS cell-derived Mϕ

?

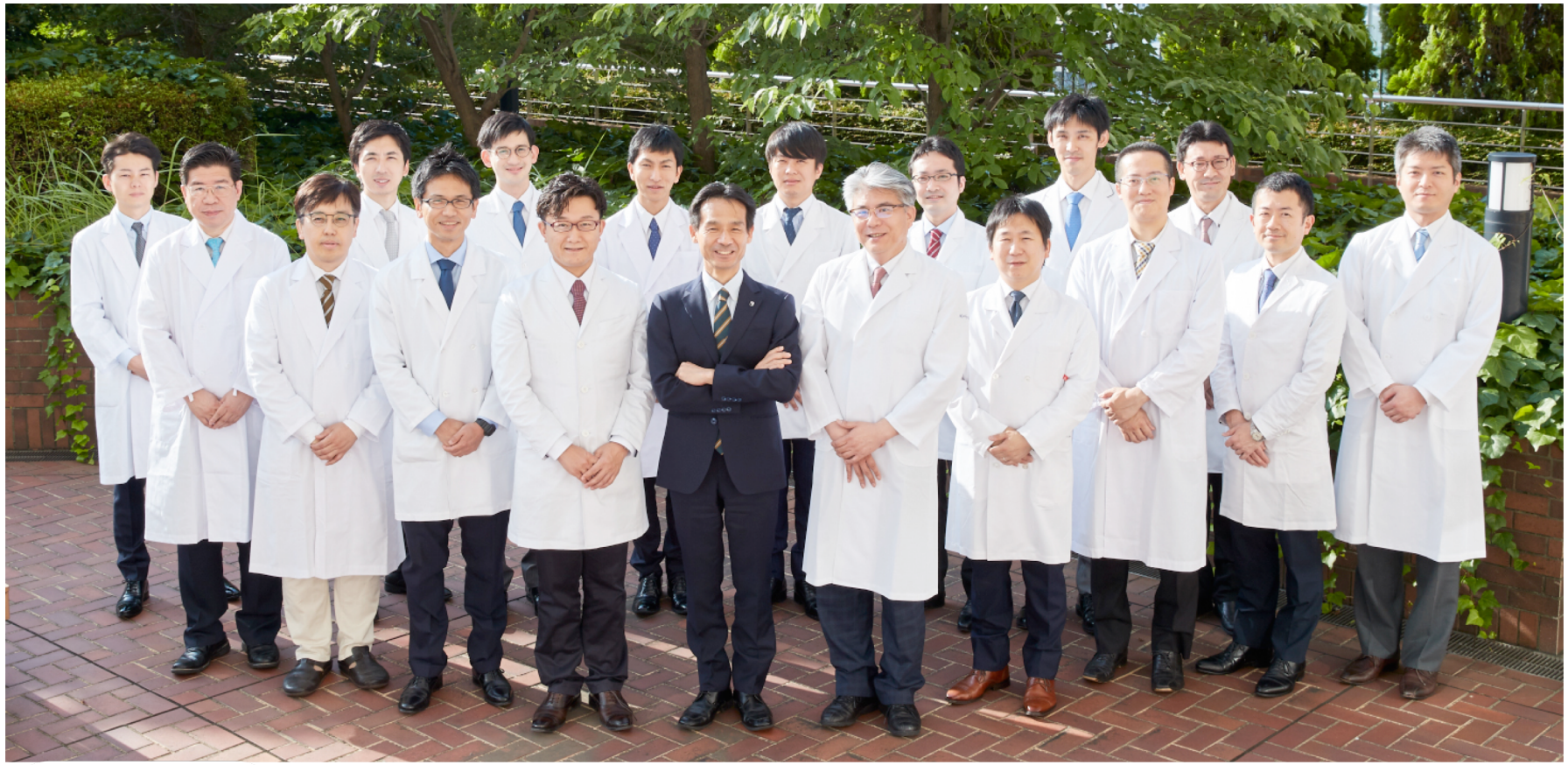
stone formers (SF)

- Now, we are trying to generate the iPS cell- derived Mϕs from stone formers

# Conclusion

- M1s and M2s were differentiated from blood cell line-derived human iPSCs, and a method for analyzing their abilities about not only phagocytosis but also processing of calcium oxalate crystals was established.
- iPS cell-derived M2s was thought to be more capable of phagocytosing and processing crystals than M1s.
- This result showed the possibility that the subtype of M2s differentiated from the human iPSCs contributes to the novel prevention of stone formation.

# Special Thanks



Thank you for your attention.

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