Reagent table:

Item	Vendor	Catalog #	Dilution
Paraformaldehyde 32%	EMS	15714	-
DPBS (No calcium, No magnesium)	ThermoFisher	SH30028	-
Triton X-100	Sigma		-
Normal Donkey Serum	Millipore	530-100	-
Bovine Serum Albumin (BSA)	Fisher	BP1600	-
Anti-FOXA2	R&D Systems	AF2400	1:500
Anti-TH	PelFreeze	P60101	!:250
Anti-PU.1	Cell SIgnaling Tech	2258	1:300
Anti-Iba1	Abcam	5076	1:800
Anti-CX3CR1	Biolegend	341602	1:100
Anti-P2RY12	Sigma	HPA014518	1:300
Donkey anti-Mouse IgG (HL) Highly Cross Adsorbed Secondary Antibody Alexa Fluor 488	Invitrogen	A-21206	1:500
Donkey anti-Rabbit IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 555	Invitrogen	A-31572	1:200
Donkey anti-Rabbit IgG (HL) Highly Cross Adsorbed Secondary Antibody Alexa Fluor 594	Invitrogen	A-21207	1:500
Donkey anti-Rat IgG (H+L) Highly Cross-Adsorbed Secondary Antibody, Alexa Fluor™ 647	Invitrogen	A-78947	1:200
DAPI	ThermoFisher	D1306	1 ug/ml final
ProLong™ Glass Antifade Mountant	ThermoFisher	P36984	-

Protocol:

Cell fixation:

NOTE1: Cell cultures can be imaged directly after staining in the same plate they were cultured in. For higher quality images, cells should be cultured on coverslips and then mounted onto microscope slides after staining.

NOTE2: At this stage do not shake the plate to avoid cell detachment.

1. Remove the media and gently rinse the cells with 1X PBS. Remove PBS and add 4% PFA in PBS. Incubate the cells at room temperature (RT) for 15-20 min.

2. After fixation, discard the PFA and wash cells with 1xPBS at room temperature for 3 times 10 min each.
3. At this stage, samples may be stored at 4C for up to a week before continuing with the staining process. It is recommended to seal with parafilm to prevent evaporation and store the plate in the dark.

Immunocytochemistry:
NOTE1: Gentle agitation with an orbital shaker may be used after fixation during the washing and staining steps.

NOTE2: Cell permeabilization is required for immunostaining of intracellular proteins and optional for immunostaining of surface proteins using antibodies known to recognize extracellular epitopes.

1. Permeabilization (optional for surface proteins): add 0.3% Triton X-100 in PBS and incubate the cells for 15-20 min at room temperature.

2. Wash the cells three times with 1xPBS at room temperature for 10 min each.

3. Blocking: Add blocking solution (10% serum or 3% BSA in PBS) and incubate for at least 1 h at room temperature. Note: source/species of serum used in blocking solution must be compatible with host species of the primary antibody.

4. Remove the blocking solution and add the primary antibody solution. Primary antibody/ies are diluted in blocking solution to the appropriate concentration.

5. Incubate the plate at 4C overnight.

6. Remove the primary antibody solution and wash the cells with 1xPBS for three times 10 min each at room temperature.

7. Add the secondary antibody/ies diluted in PBS to the appropriate concentration. Incubate for 1 h at room temperature in the dark. Note: all subsequent steps after the addition of the secondary antibody should be performed under limited light exposure of samples to light to prevent fluorescence bleaching.

8. Remove the secondary antibody solution and wash the cells with 1xPBS for three times 10 min each at room temperature.

9. Add DAPI diluted in 1xPBS and incubate the cells at RT for 5-10 min.

10. Rinse the cells three times with 1xPBS. Samples can be stored in the fridge until they are imaged directly from the plate or mounted onto microscope slides for imaging.

11. Mounting: add a drop of ProLong™ Glass Antifade Mountant on a microscope slide and place the coverslip on top avoiding bubble formation. Keep in the dark until mountant has dried and image directly or store in the fridge until use.