

Supplementary Table 1 RNAscope® workflow for mIFISH.

	mIFISH	Dual mRNA mIFISH
De-Paraffinization		
Pre-Treatment 1	10 min at RT	10 min at RT
Pre-Treatment 2	15 min at 98°-102°C	15 min at 98°-102°C
Pre-Treatment 3	15 min at 40°C	15 min at 40°C
Probe Hybridization	2hrs at 40°C	C2 probe 1:50 in C1 2h at 40°C
Amplification 1	30 min at 40°C	30 min at 40°C
Amplification 2	15 min at 40°C	15 min at 40°C
Amplification 3	30 min at 40°C	30 min at 40°C
Amplification 4	15 min at 40°C	15 min at 40°C
Amplification 5	1h at RT	1h at RT
Amplification 6	15 min at RT	15 min at RT
Chromogen or Immunofluorescent signal development	10 min at RT 15 min at RT TSA 1:3000	C2 signal development 15 min at RT TSA 1:3000
Counterstain or continuing with antibody staining		HRP-Blocker 15 min at 40°C
		Amplification 7
		Amplification 8
		Amplification 9
		Amplification 10
		C1 signal development 15 min at RT TSA 1:3000
		continuing with antibody staining

Supplementary Table 2 Secondary antibodies tested for mIFISH compatibility.

Antibody/Target	Species	Reactivity	Conjugate	Isotype	Clone	Cat. Log. #	Vendor	Antibody dilution
Goat anti-mouse AF647	Goat	Mouse	Alexa Fluor® 647	IgG (H+L)	polyclonal	A-21236	ThermoFisher	1:100
Donkey anti-mouse AF488	Donkey	Mouse	Alexa Fluor® 488	IgG (H+L)	polyclonal	A-21202	ThermoFisher	1:100
Donkey anti-rabbit AF488	Donkey	Rabbit	Alexa Fluor® 488	IgG (H+L)	polyclonal	A-21206	ThermoFisher	1:100
Goat anti-rabbit AF647	Goat	Rabbit	Alexa Fluor® 647	IgG (H+L)	polyclonal	A-21245	ThermoFisher	1:100