

Supplemental Figure 1: Patient-level contributions to single nuclear RNA sequencing atlas. A. Total nuclei contributed per patient. B. Nucleus counts by patient and cell subtype. C. Log-scaled heatmap of patient contributions to each cell subtype. D. Fractional patient contributions to each cell subtype

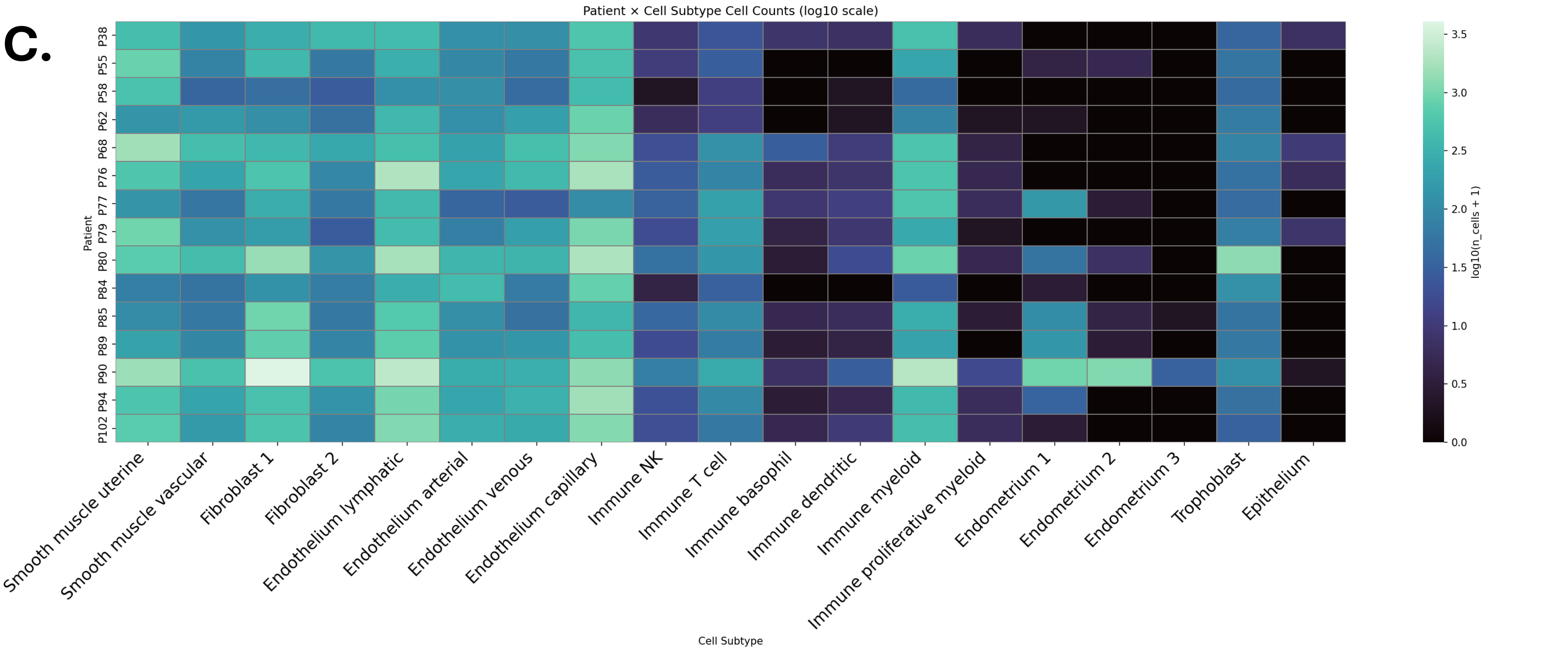
A.

Patient	Nuclei count
P90	15644
P80	9447
P76	6627
P68	5782
P94	5129
P102	4917
P79	3492
P38	3097
P89	3090
P85	2932
P55	2666
P62	2192
P77	2120
P84	2091
P58	1418

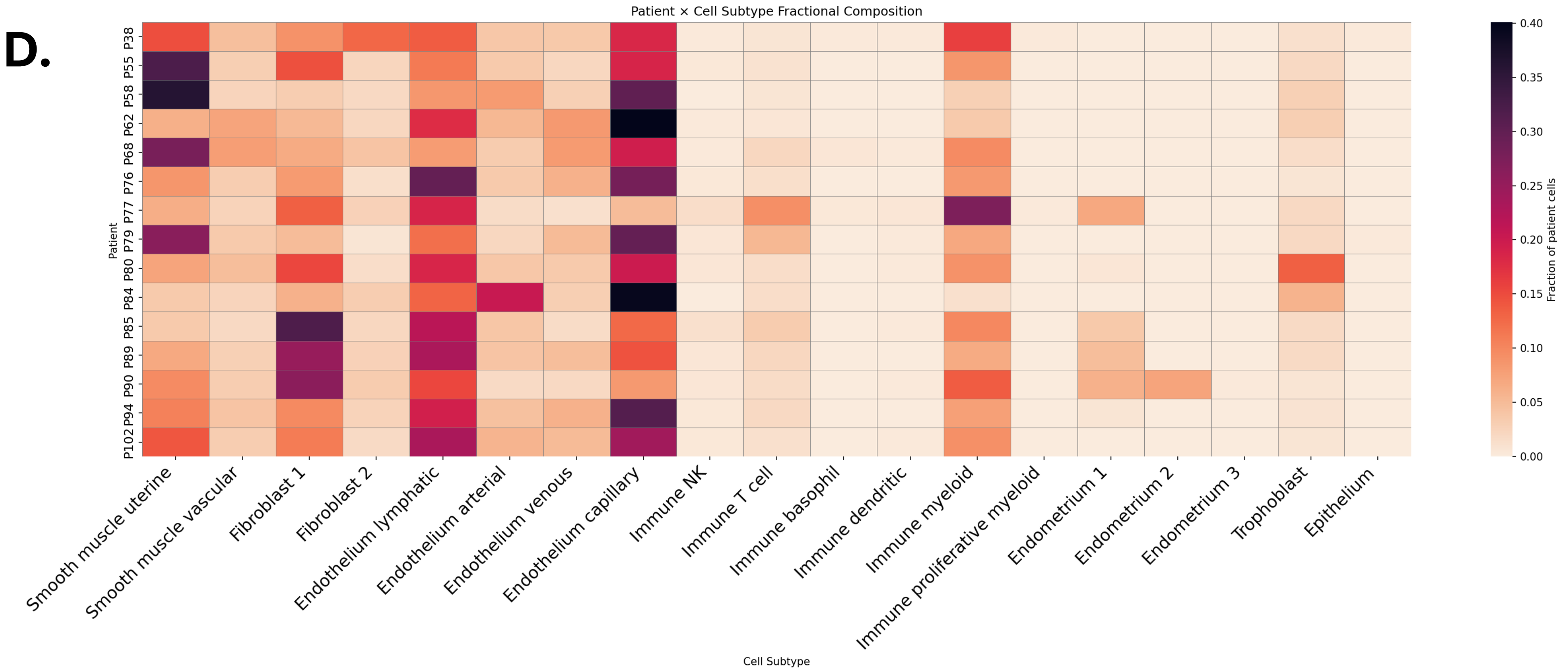
B.

Patient	Smooth muscle uterine	Smooth muscle vascular	Fibroblast 1	Fibroblast 2	Endothelium lymphatic	Endothelium arterial	Endothelium venous	Endothelium capillary	Immune NK	Immune T cell	Immune basophil	Immune dendritic	Immune myeloid	Immune proliferative myeloid	Endometrium 1	Endometrium 2	Endometrium 3	Trophoblast	Epithelium
38	460	143	280	396	426	121	115	571	8	21	7	6	497	5	0	0	0	35	6
55	855	81	390	59	298	94	57	501	10	29	0	0	231	0	3	4	0	54	0
58	512	35	45	27	120	117	41	428	1	11	0	1	40	0	0	0	0	40	0
62	136	160	115	48	388	118	185	879	5	11	0	1	78	1	1	0	0	66	0
68	1605	461	385	240	465	194	476	1128	18	123	28	10	553	3	0	0	0	84	9
76	577	217	544	93	1965	231	398	1863	26	87	5	7	556	4	0	0	0	49	5
77	136	56	283	59	396	34	26	105	32	197	8	11	580	5	149	2	0	41	0
79	918	123	171	27	424	72	180	1035	17	191	3	8	245	1	0	0	0	70	7
80	689	445	1453	136	1748	361	335	1887	49	140	2	16	858	4	50	6	0	1268	0
84	74	51	126	68	275	429	63	823	3	30	0	0	25	0	2	0	0	122	0
85	103	59	935	60	638	117	48	371	36	101	4	5	290	2	107	3	1	52	0
89	209	89	771	87	712	129	146	450	16	66	2	3	204	0	147	2	0	57	0
90	1499	505	4081	527	2409	273	298	1306	74	265	6	28	2143	15	936	1128	31	119	1
94	544	215	505	131	992	225	311	1601	20	97	2	4	397	5	33	0	0	47	0
102	696	161	540	87	1147	281	249	1180	18	57	4	9	450	5	2	0	0	31	0

C.



D.

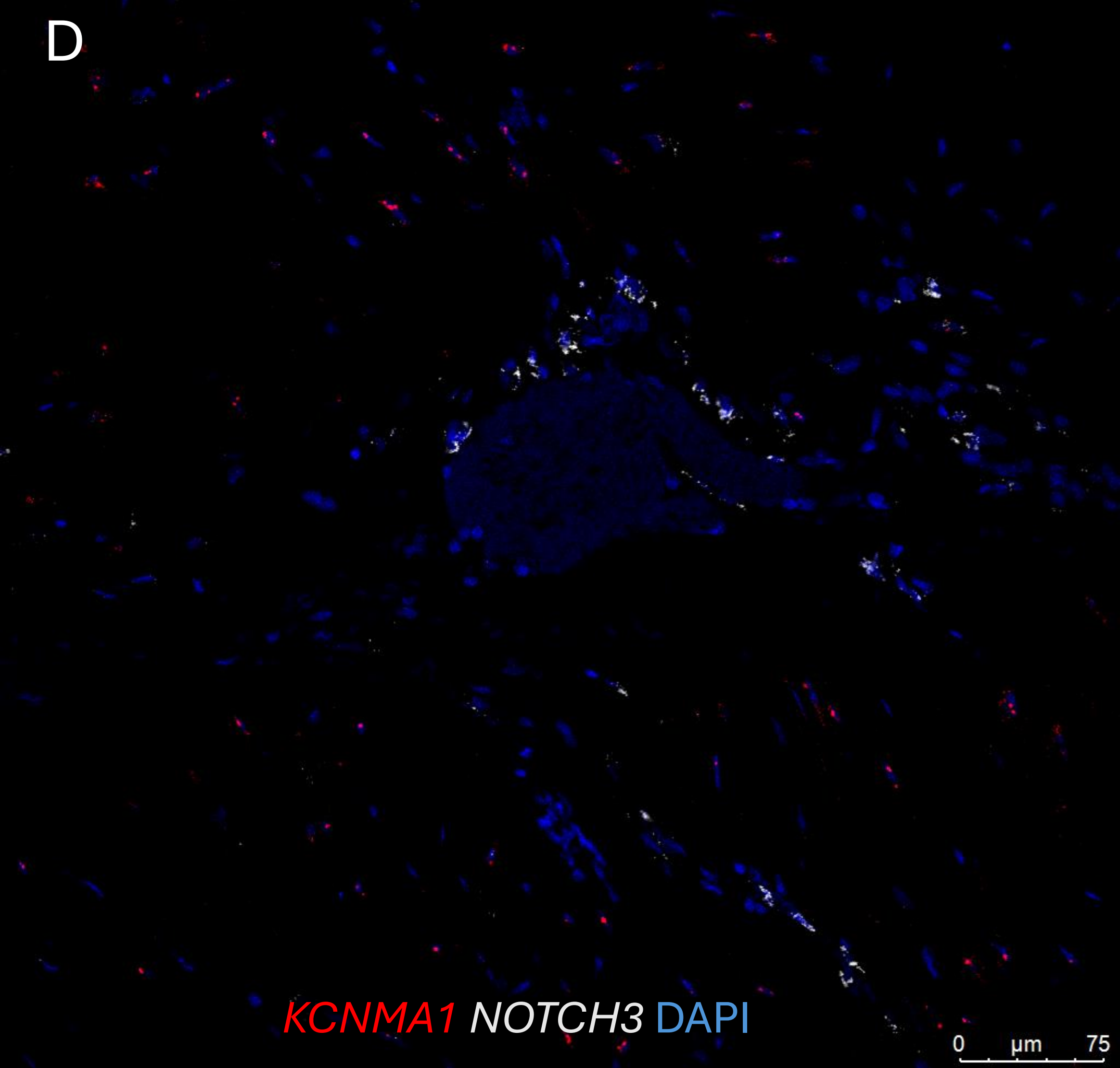
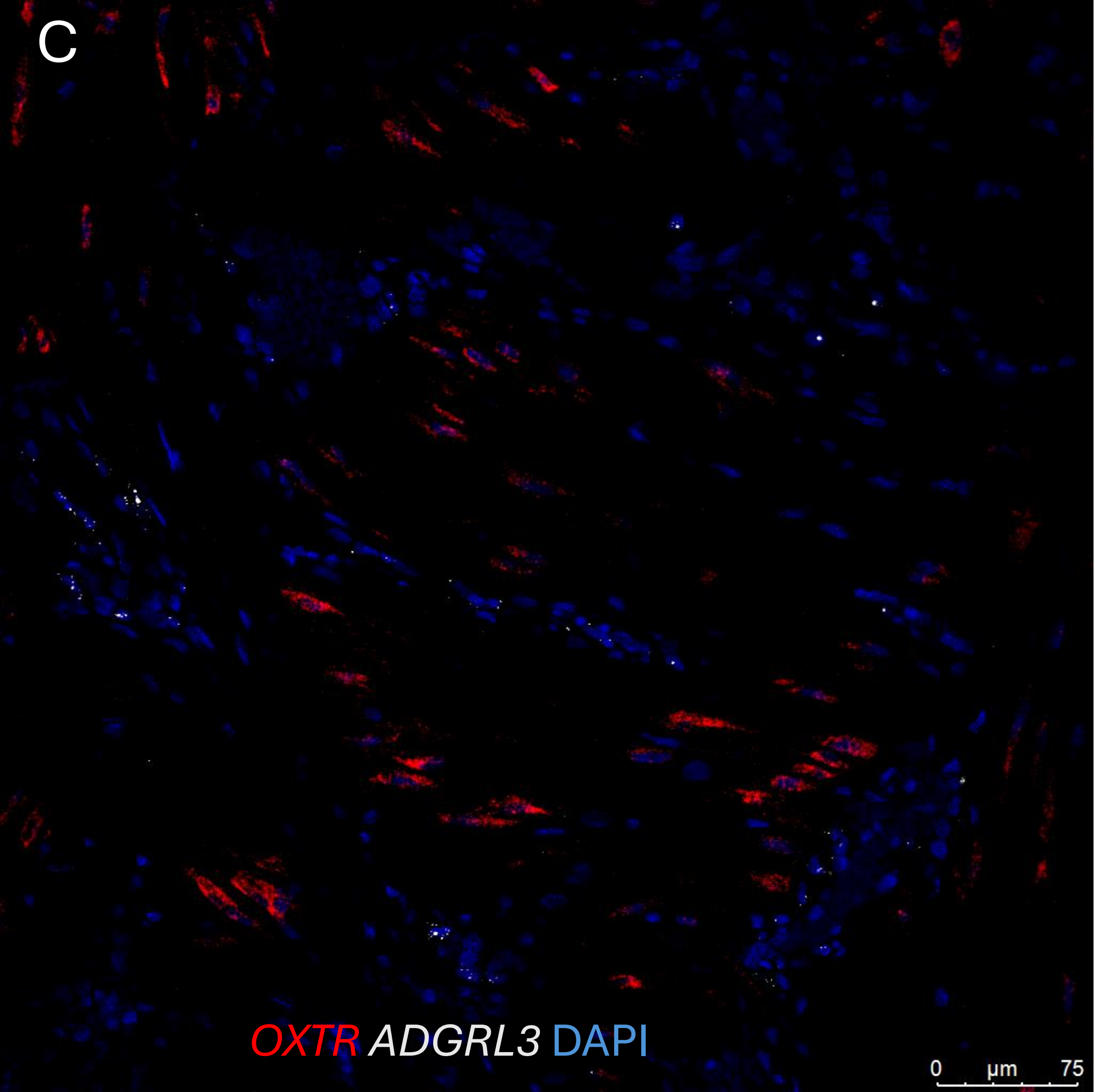
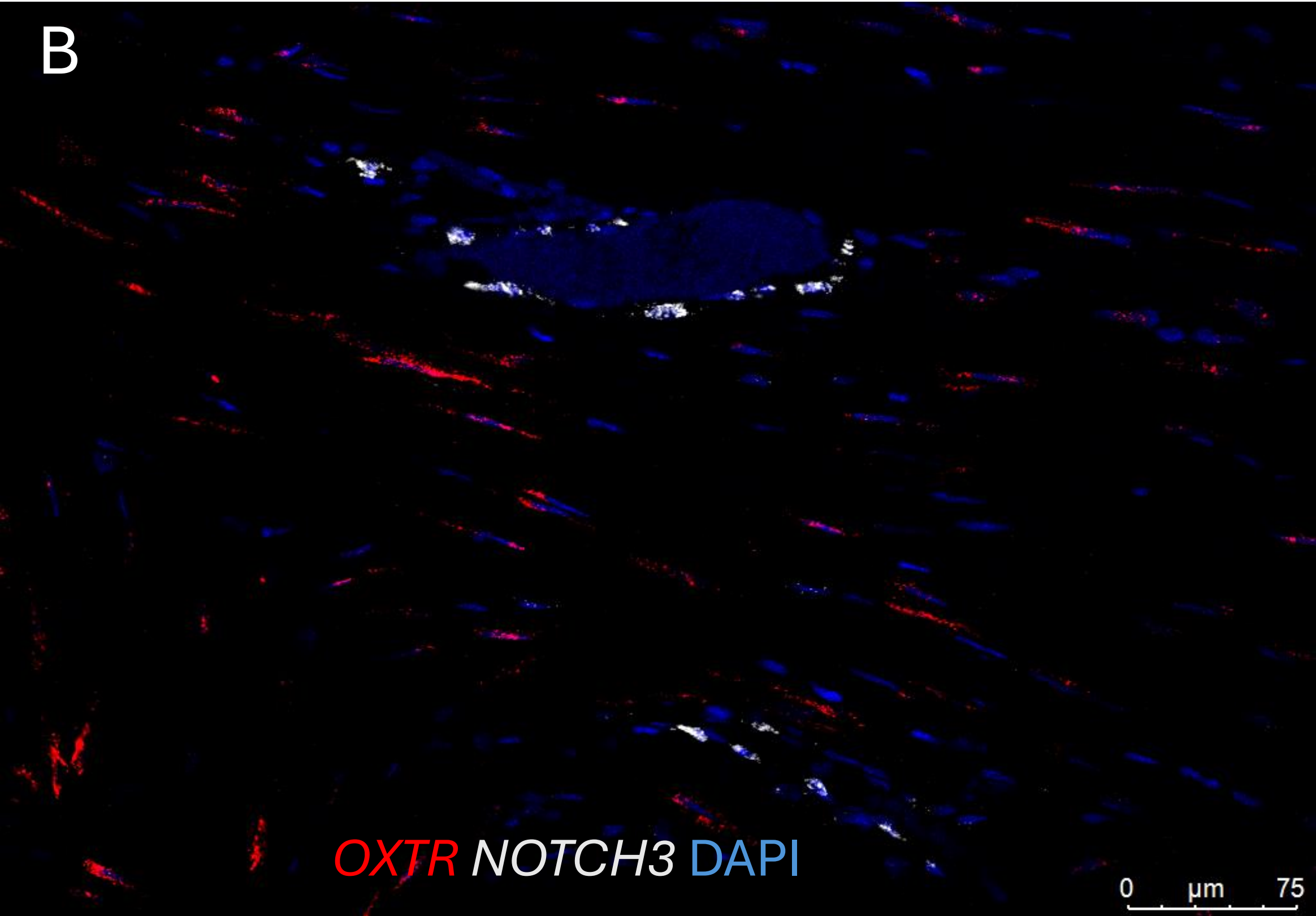
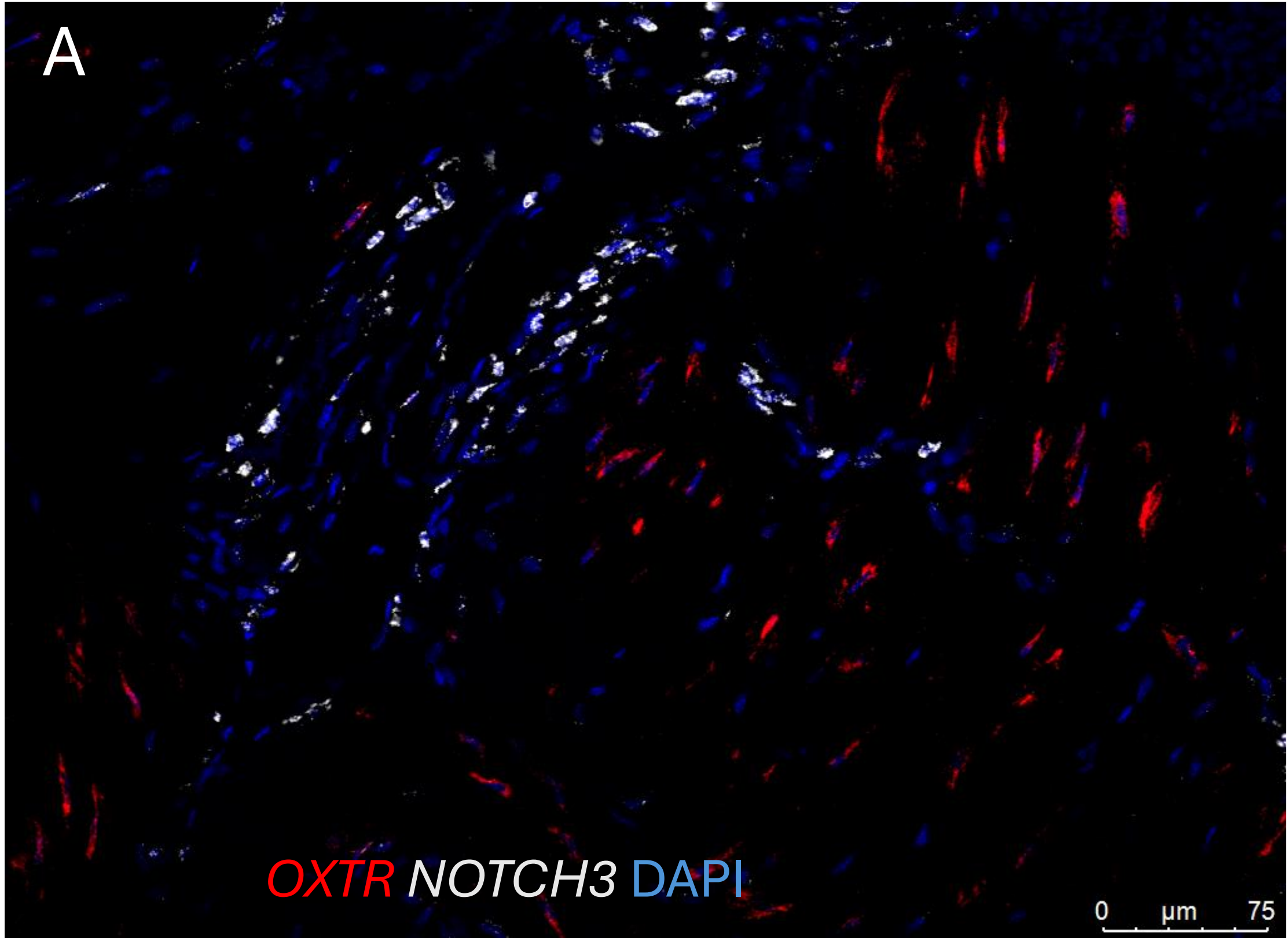


Supplemental Figure 2: Additional Tissue RNAscope validation of distinct uterine and vascular smooth muscle cell populations in the gravid myometrium

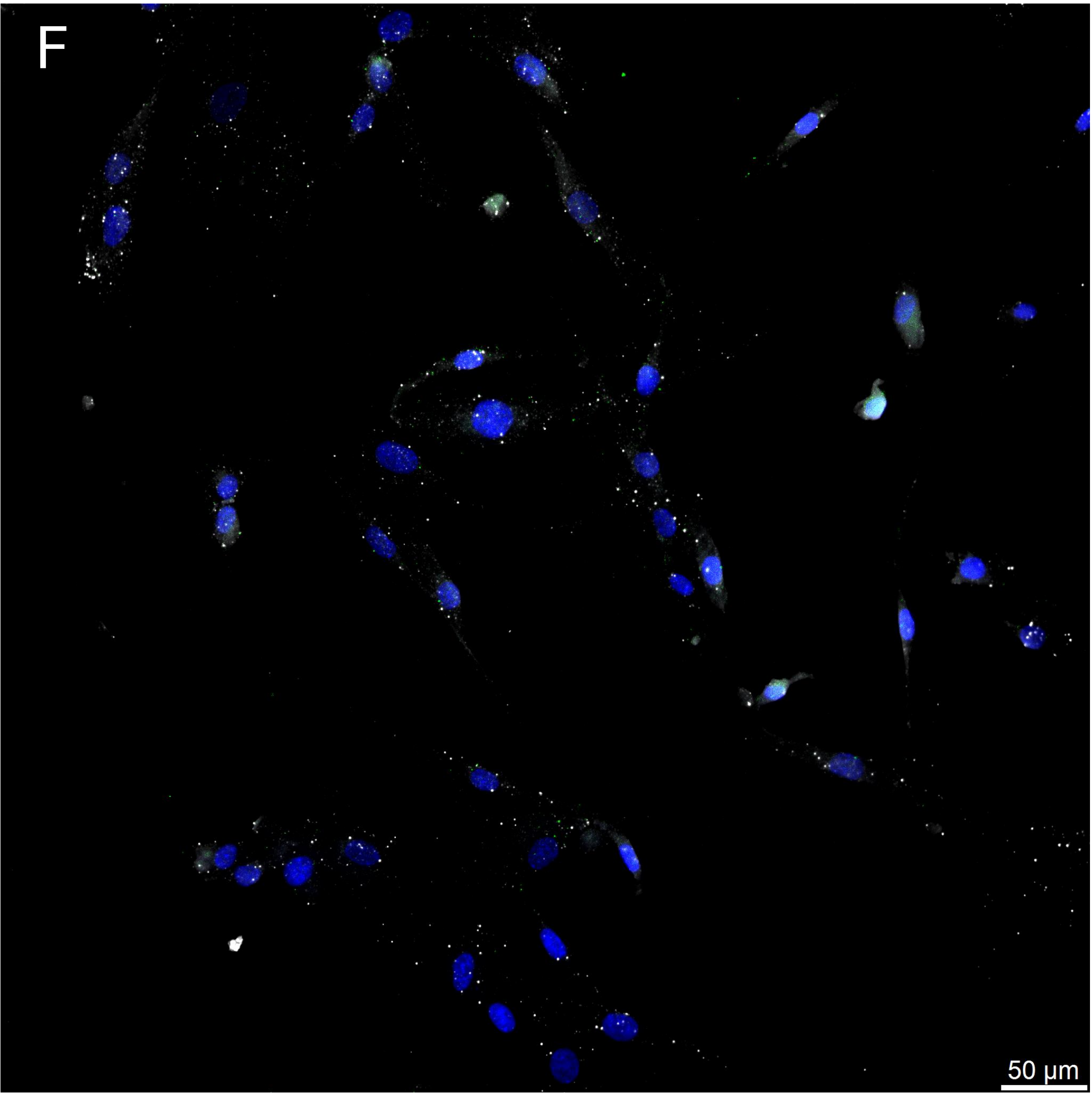
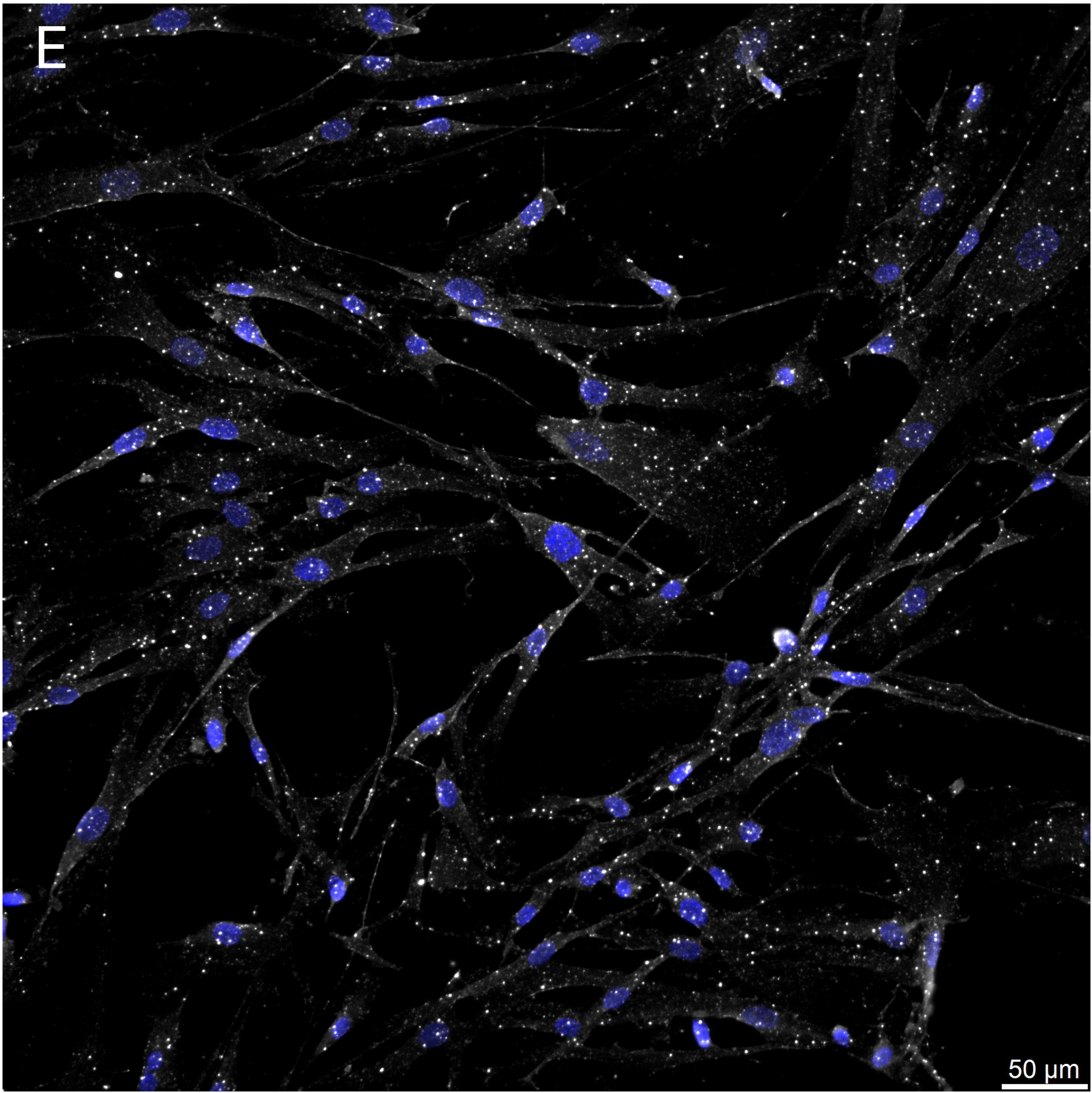
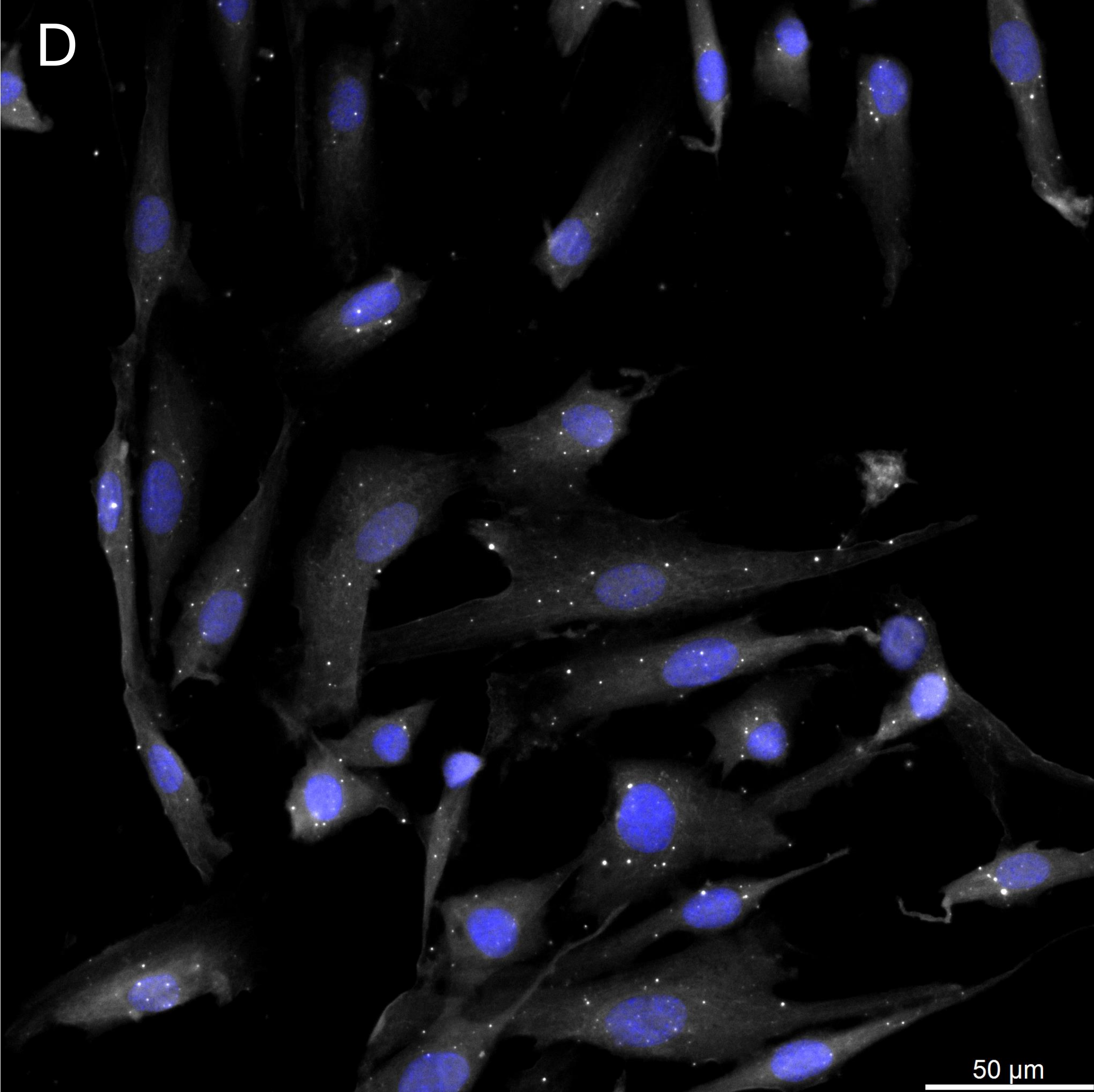
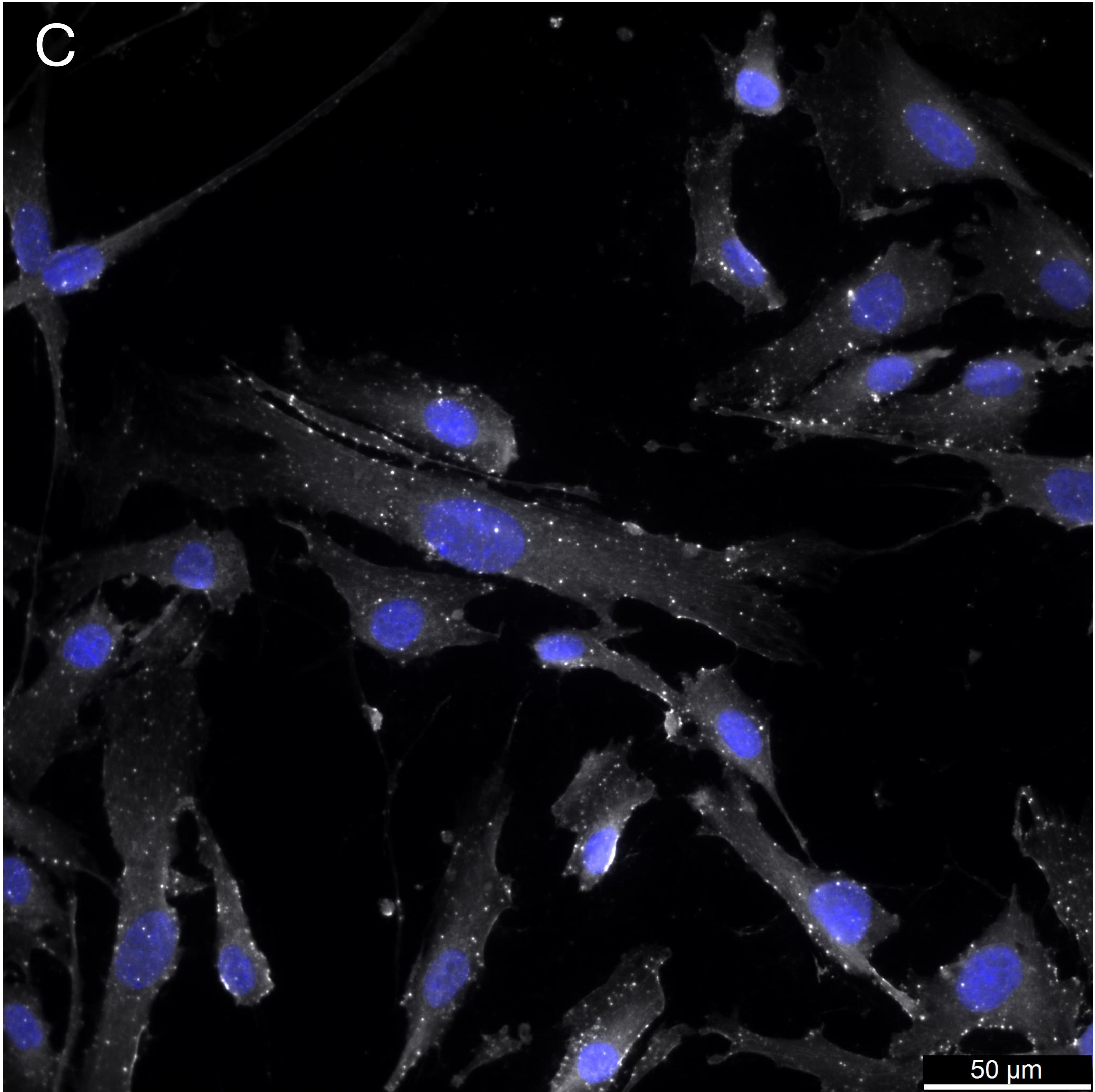
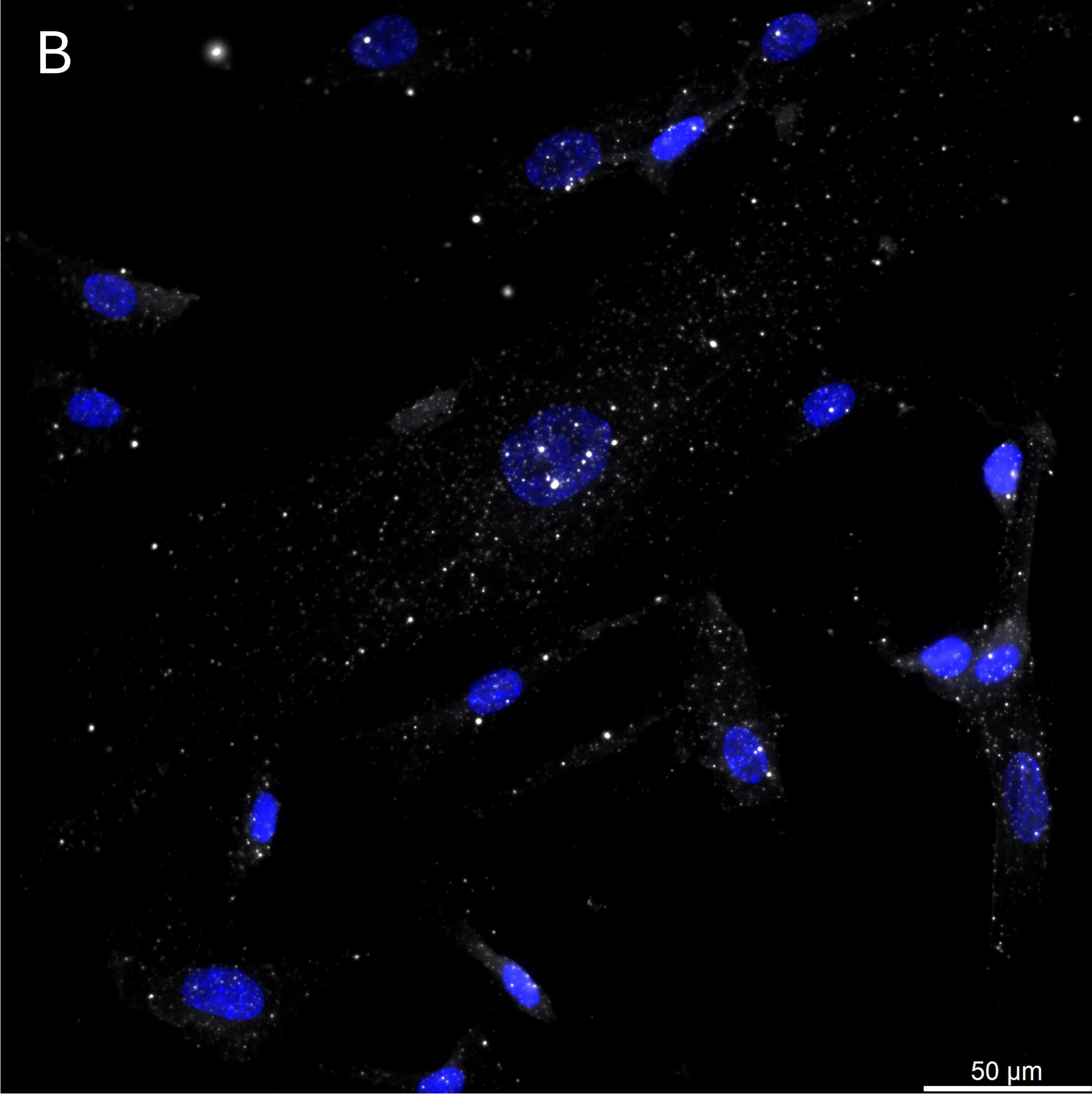
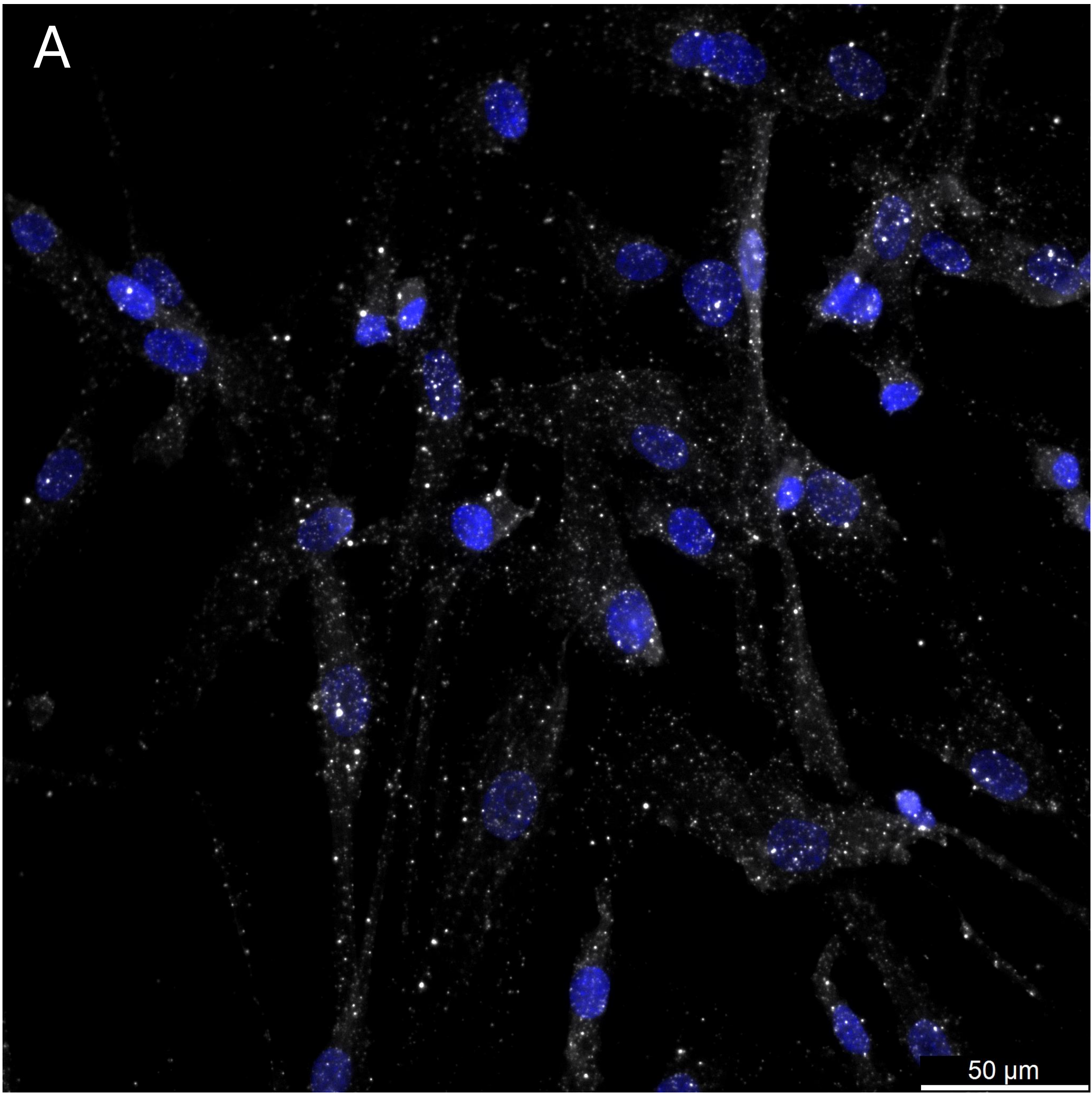
A-B. Additional RNAscope™ validation of uterine vs vascular smooth muscle in myometrial tissue with *OXTR* (uterine smooth muscle, red) versus *NOTCH3* (vascular smooth muscle, white). Nuclei are blue (DAPI)

C. RNAscope™ validation of uterine vs vascular smooth muscle in myometrial tissue with *OXTR* (uterine smooth muscle, red) versus *ADGRL3* (vascular smooth muscle, white).

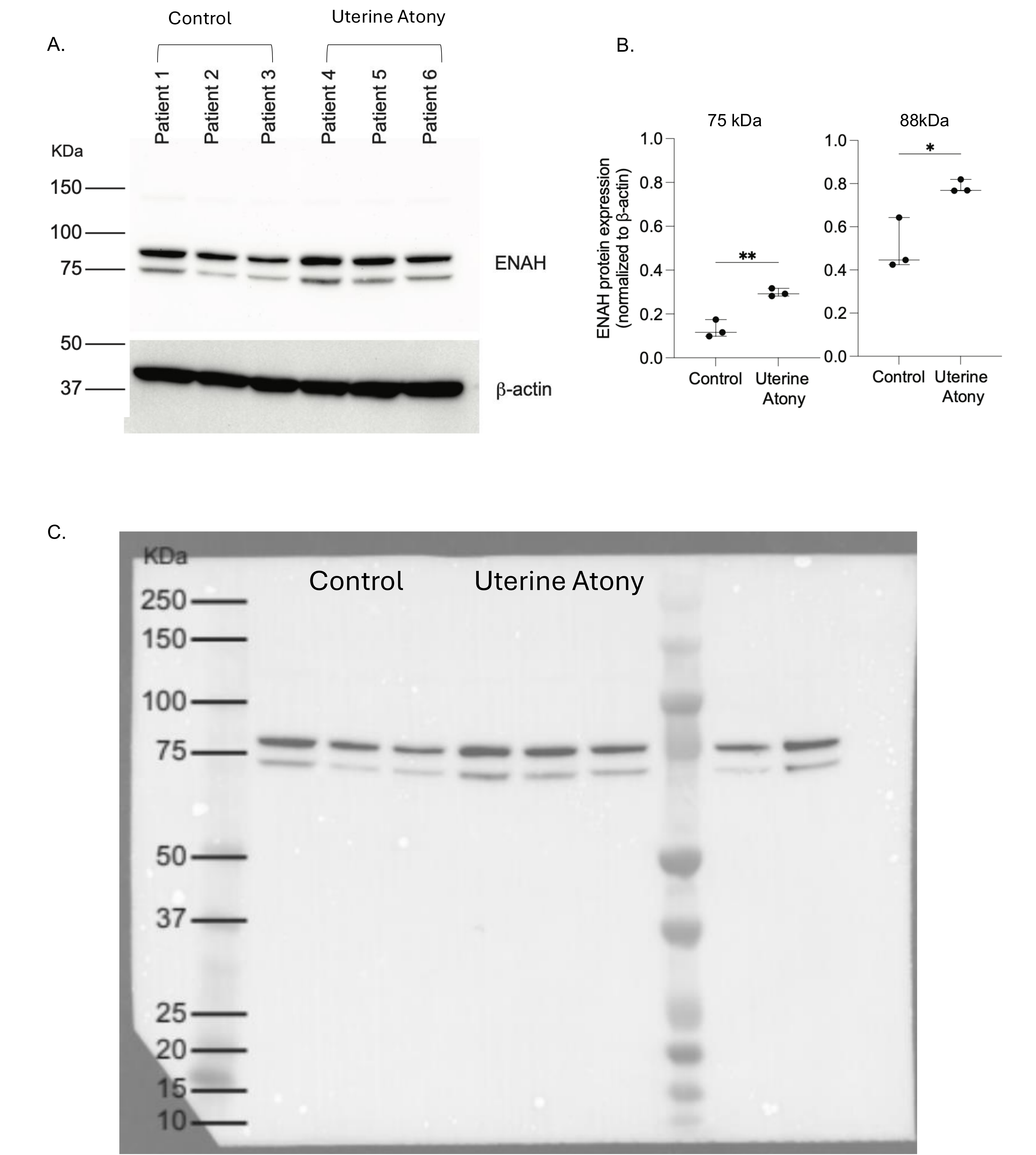
D. RNAscope™ validation of uterine vs vascular smooth muscle in myometrial tissue with *KCNMA1* (uterine smooth muscle, red) versus *NOTCH3* (vascular smooth muscle, white).



Supplemental Figure 3: RNAscope™ of Follicle Stimulating Hormone Receptor transcript (*FSHR*) from n=6 low passage primary human uterine smooth muscle cell cultures from different patient samples. Images A-D are taken at 40X magnification and E and F at 20X magnification.



Supplemental Figure 4: Western blot: ENAH protein in increased in in primary cultured USMC from patient with uterine atony (n=3) versus matched controls (n=3). A. Western blot of ENAH protein in primary cultured USMC from control patients (n=3) and patients with uterine atony (n=3). B. Statistically significant increases in both canonical (~88kDa) and splice variant (~75kDa) forms of ENAH with higher expression in uterine atony Note that though canonical ENAH weighs 67kDa, due to migration properties of the proline rich protein, canonical ENAH is generally observed at about 88kDa (upper band). C. Full Western blot from S5A.



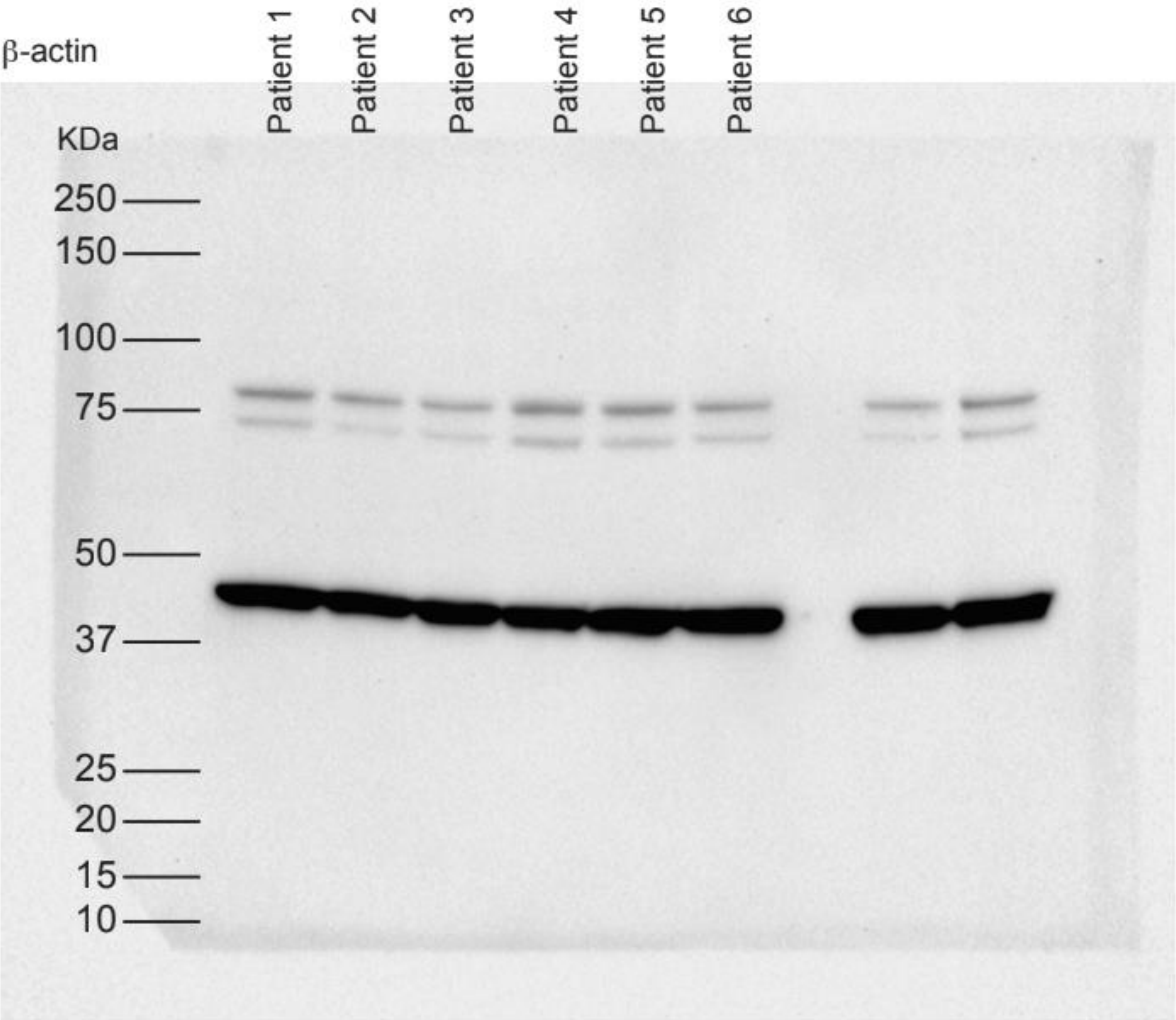
Supplemental Figure 5:

D. Full Western gel with β actin.

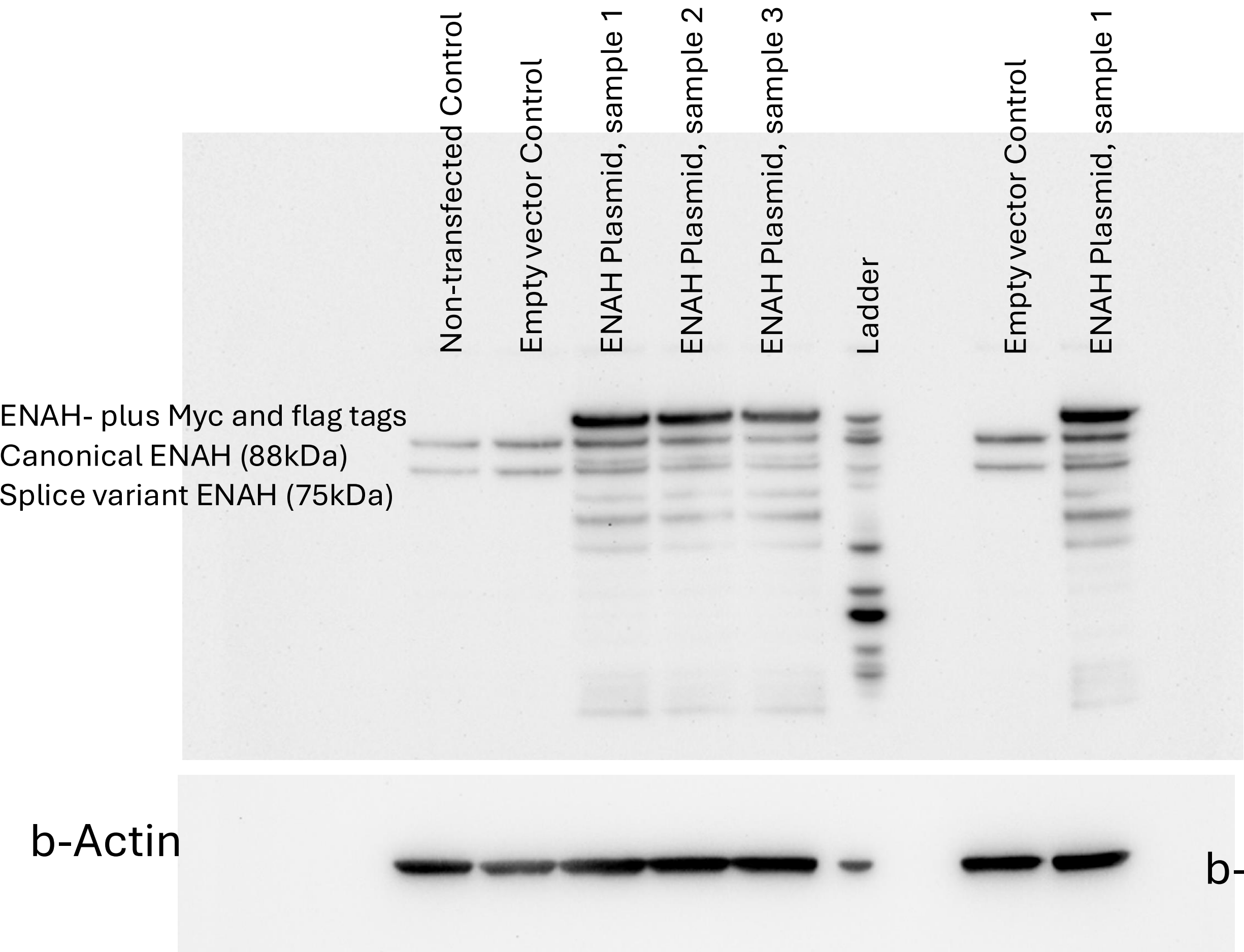
E. Positive control for ENAH Western blot: Plasmid-based overexpression. Canonical ENAH (NM_001008493) was overexpressed in human uterine SMCs using 2 micrograms of ENAH plasmid RC210677 plasmid from Origene and measured at 24 hours post-transfection. Antibody was ENAH from Novus, catalog number NBP-87914 at 1:500. Unfortunately, although this serves as an excellent positive control for our Western blot antibody, ENAH overexpression proved somewhat cytotoxic and could not be used in current form for functional assays, as approximately 20% of transfected USMC died within 48 hours compared to <1% of control plasmid transfected cells.

F. Same samples, but primary antibody shows Myc tag. Antibody from Cell signaling, catalog number 2276, at 1:500.

D.



E. ENAH Antibody



F. Myc-tag Antibody

