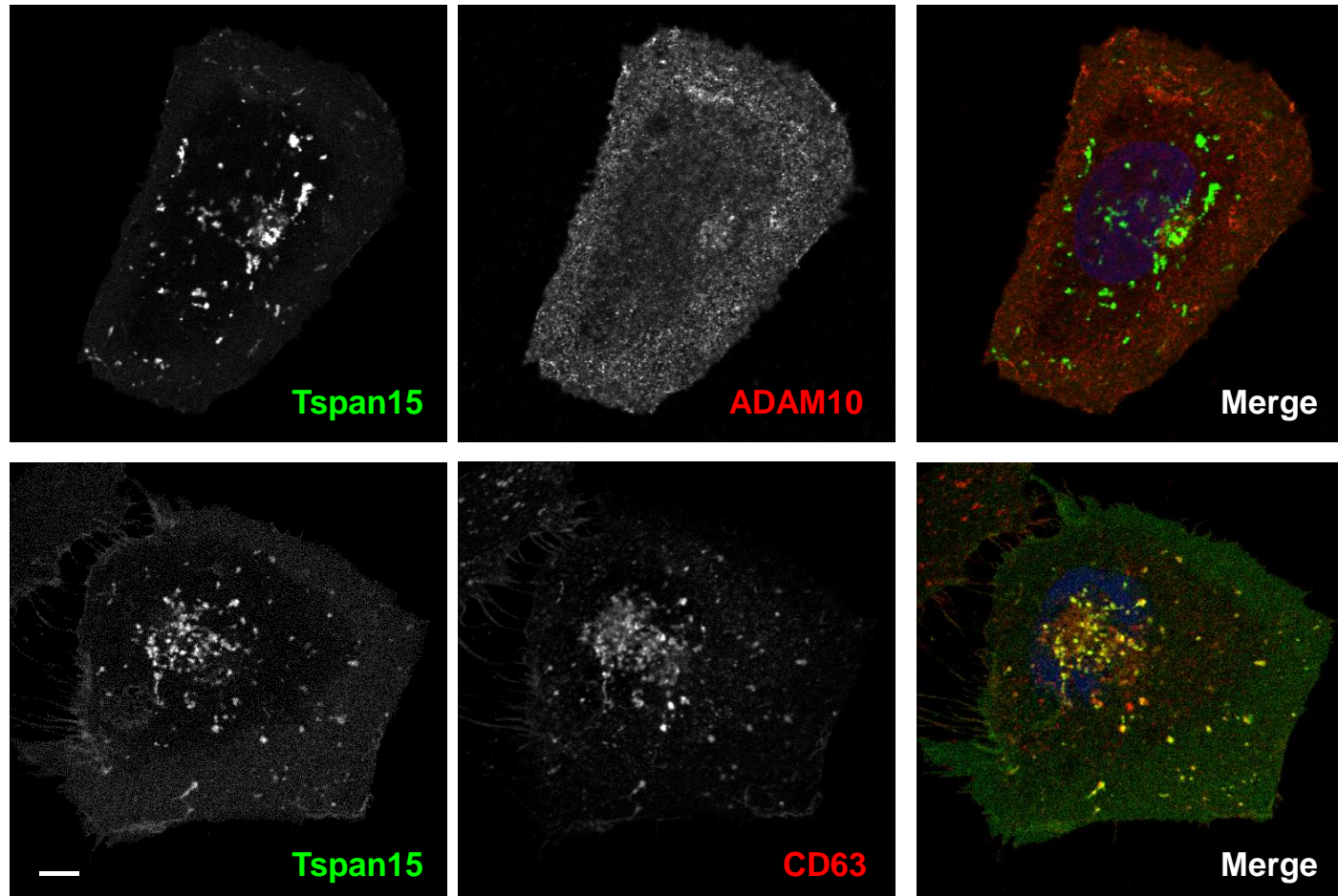
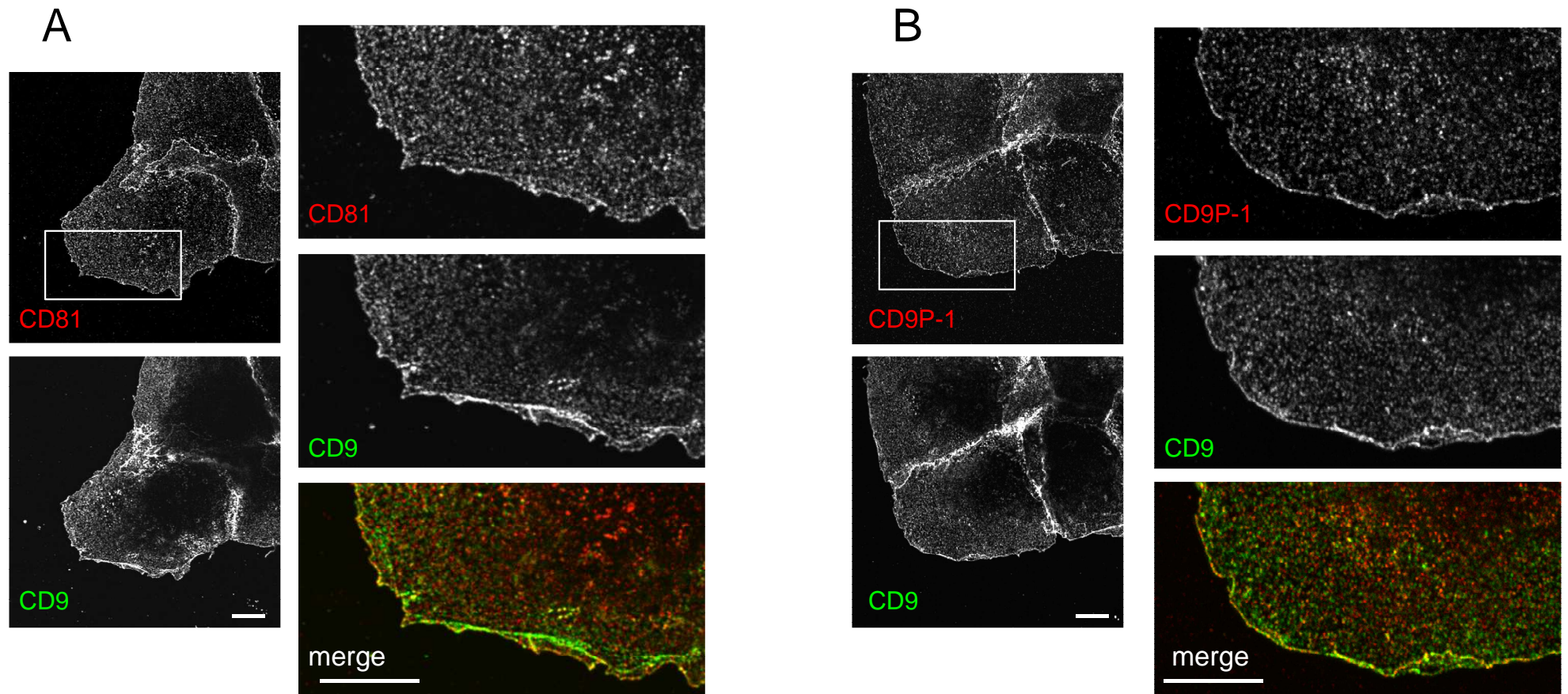


**Fig. S1: Activation of Notch signaling in an independent series of U2OS-N1 cells transfected with the various TspanC8 or CD9**

Notch activity of U2OS-N1 cells stably expressing or not the indicated tetraspanins, measured using a CSL reporter luciferase assay. Notch was activated by incubating the cells with OP9-DLL1 cells for 20-24h. The figure shows the mean $\pm$ SEM of 3 independent experiments in duplicate. In each experiment, the signal obtained is expressed as a percentage of the signal observed for non-transfected U2OS cells.

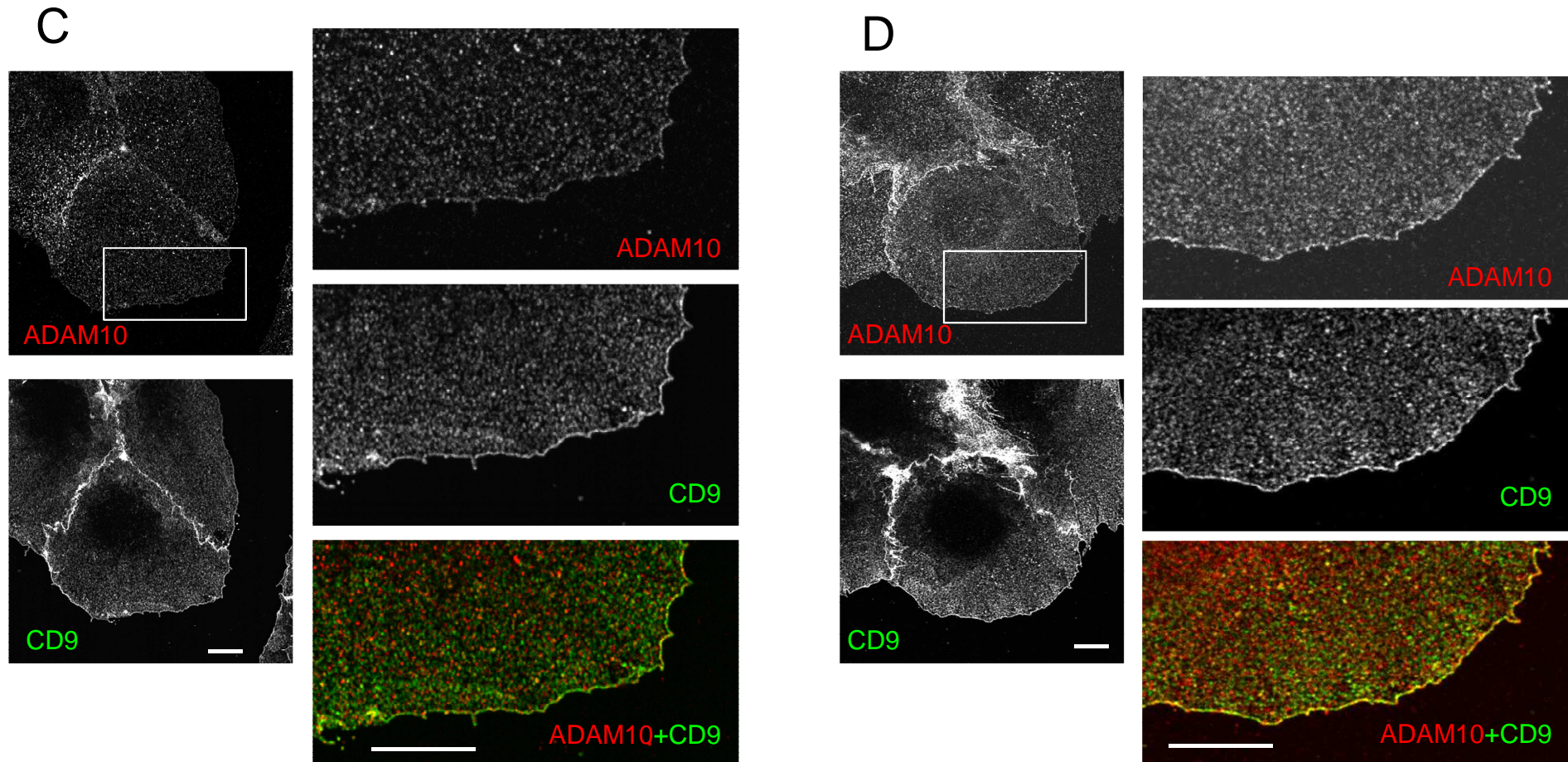


**Fig. S2: Tspan15 but not ADAM10, is enriched in a late endocytic compartment**  
U2OS-N1/tspan15 cells were fixed, permeabilized with saponin, labelled with antibodies to CD63 or ADAM10, and analyzed by confocal microscopy. Bar: 10 $\mu$ m.



**Fig. S3: ADAM10, CD81 and CD9P-1 are enriched at the cell periphery in U2OS cells**  
 The cells were fixed, labelled with antibodies to CD9 and either ADAM10, CD9P-1 or CD81, and analyzed by confocal microscopy at the plane of cell attachment.  
 (A) CD81; (B): CD9P-1; Bar: 10 $\mu$ m.





**Fig. S3 (continued): ADAM10, CD81 and CD9P-1 are enriched at the cell periphery in U2OS cells**

The cells were fixed, labelled with antibodies to CD9 and either ADAM10, CD9P-1 or CD81, and analyzed by confocal microscopy at the plane of cell attachment.

(C) ADAM10; (D): ADAM10 in cells incubated for 15 min with the anti ADAM10 mAb 11G2.

Bar: 10 $\mu$ m.

ADAM10 Apparent Diffusion Coefficients $\pm$ SEM ( $\mu\text{m}^2/\text{s}$ ) / (% of diffusion modes)				
		Cell type		
Trajectories		U2OS-N1	U2OS/Tspan5	U2OS/Tspan15
All	Periphery	0,063 $\pm$ 0,026	0,062 $\pm$ 0,024	0,105 $\pm$ 0,031
	Center	0,075 $\pm$ 0,030	0,079 $\pm$ 0,030	0,103 $\pm$ 0,032
Brownians	Periphery	0,103 $\pm$ 0,027 (54)	0,098 $\pm$ 0,026 (54)	0,137 $\pm$ 0,030 (69)
	Center	0,124 $\pm$ 0,032 (53)	0,123 $\pm$ 0,028 (58)	0,142 $\pm$ 0,030 (68)
Confined	Periphery	0,007 $\pm$ 0,004 (25)	0,010 $\pm$ 0,006 (23)	0,009 $\pm$ 0,006 (16)
	Center	0,007 $\pm$ 0,003 (27)	0,008 $\pm$ 0,005 (24)	0,009 $\pm$ 0,007 (17)
Mixed	Periphery	0,029 $\pm$ 0,012 (21)	0,028 $\pm$ 0,009 (23)	0,045 $\pm$ 0,017 (15)
	Center	0,031 $\pm$ 0,012 (20)	0,032 $\pm$ 0,013 (18)	0,035 $\pm$ 0,014 (15)

**Supplementary Table 2 : Comparison of ADAM10 ADC and distribution of the different modes of diffusion at the cell periphery or center**