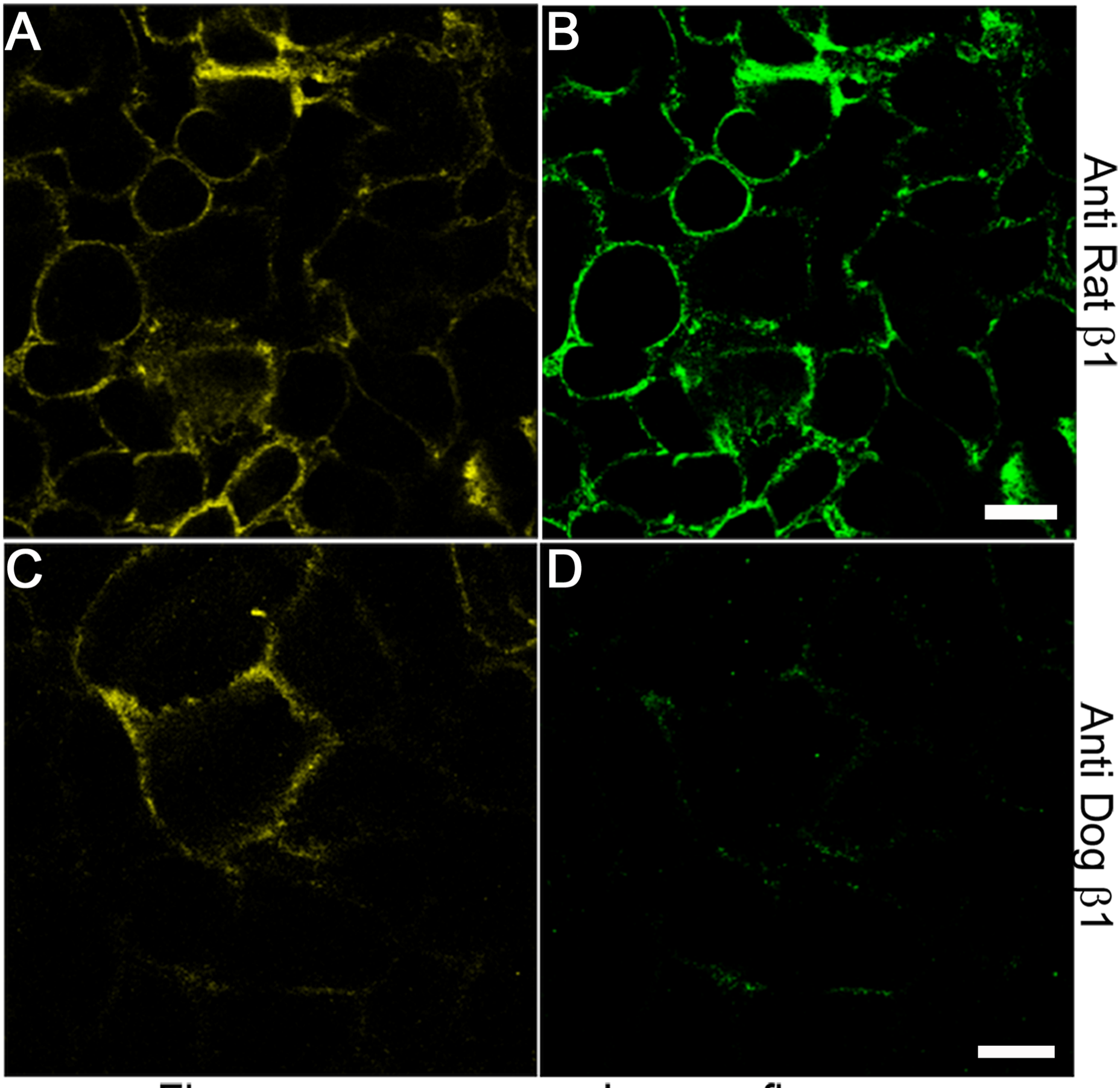
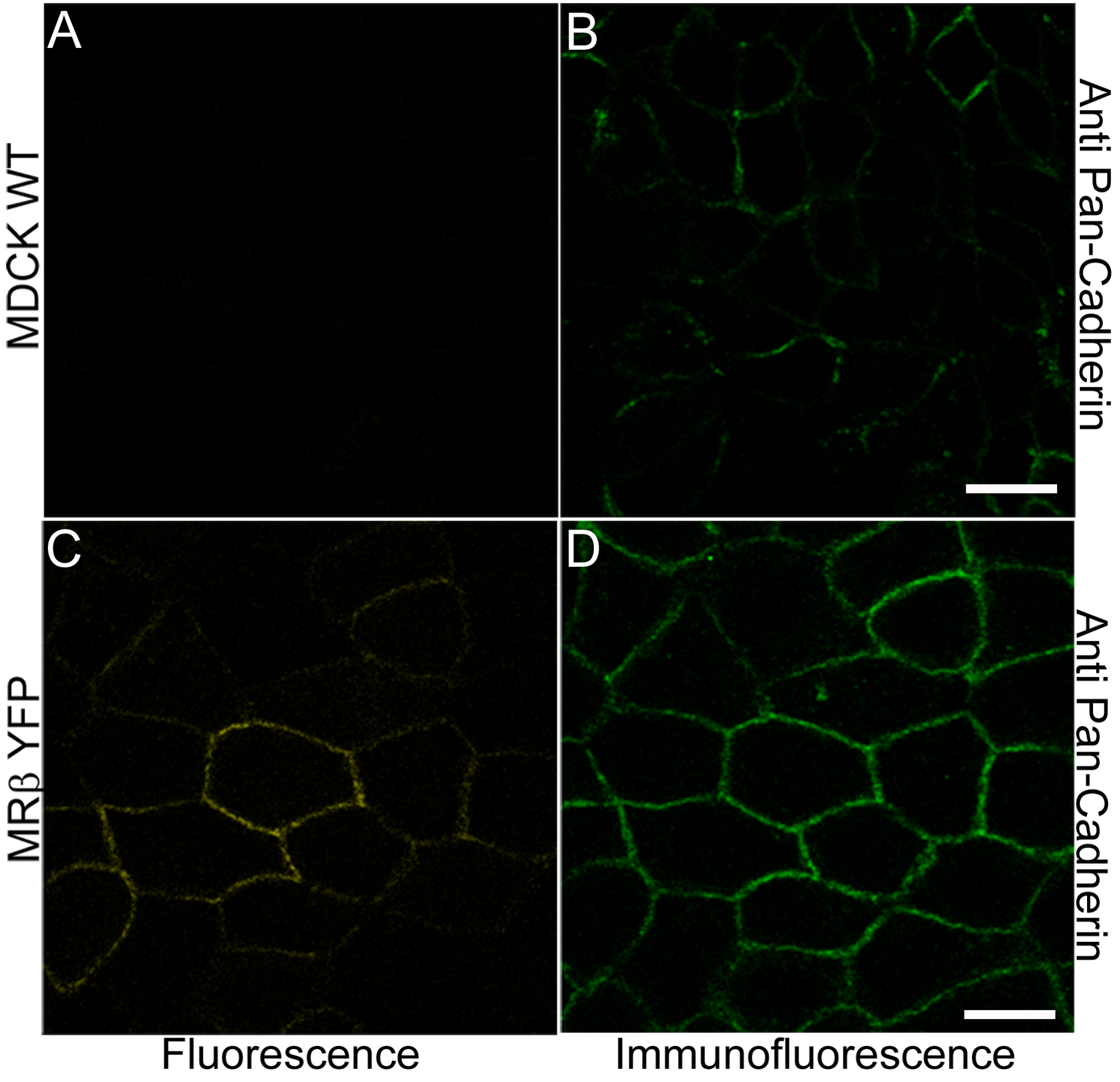
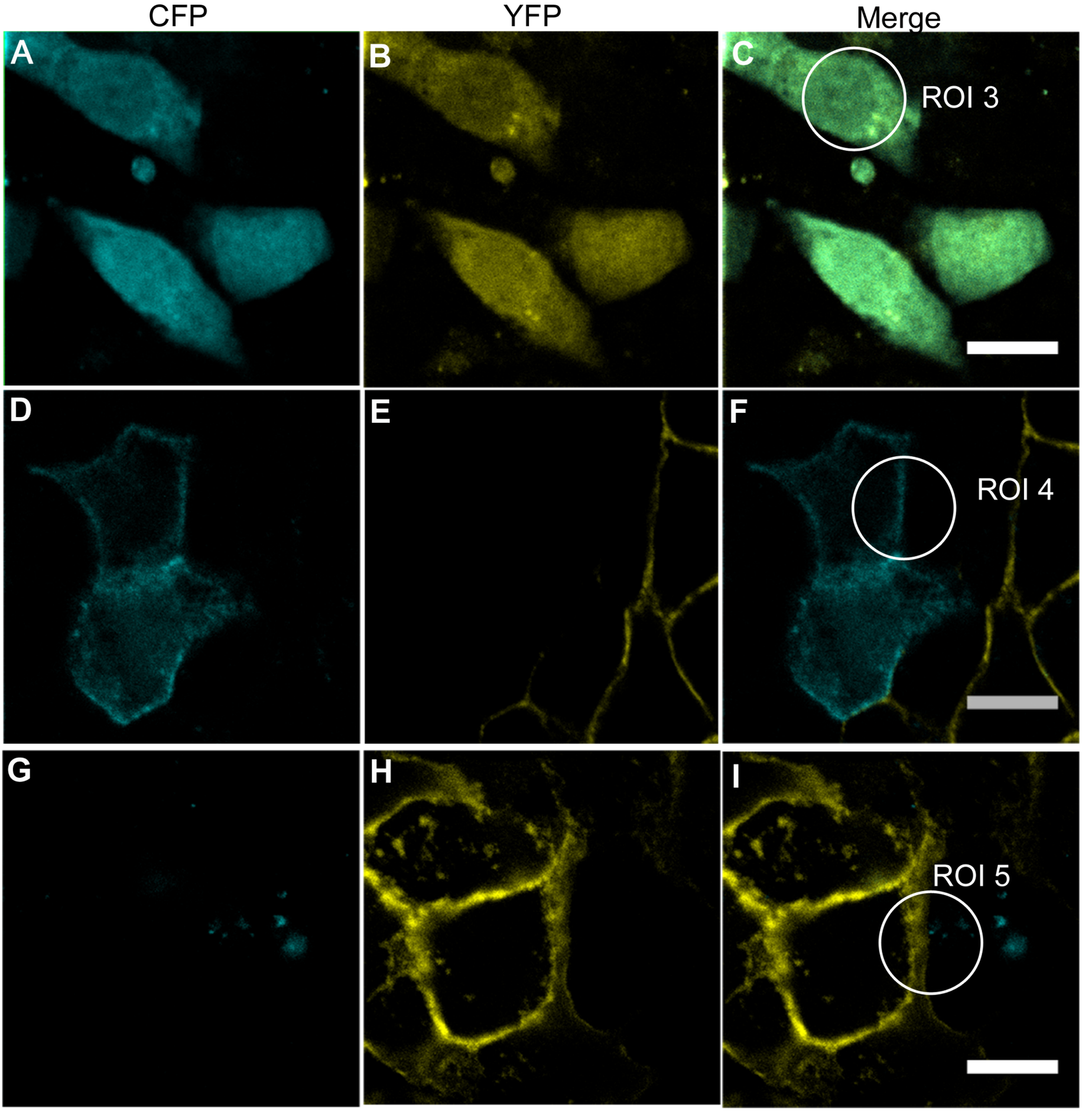
Supplementary Figure 1. Expression of β_1 subunits in transfected MDCK cells. MDCK cells expressing Rβ YFP were fixed and immunostained using antibodies against the rat (A, B) or dog β_1 subunit (C, D) and a Cy5 secondary antibody. (A, C) Representative images of the fluorescence of MRβ YFP cells recorded at 540-575 nm. (B) Immunodetection of the rat β_1 subunit in the same optical section as that in A. Bar: 30 μm. (D) Immunodetection of the dog β_1 subunit in the same optical section as that in C. Bar: 10 μm.

Supplementary Figure 2. Expression of rat β_1 subunits in MDCK cells does not disturb the expression of adherens junction molecules. MDCK WT (A, B) and MRβ YFP (C, D) cells were fixed and immunostained using a pan-cadherin antibody. (A, C) Representative image of the fluorescence produced by excitation with a 425-500 nm laser line and emission recorded 540-575 nm (according to the YFP spectra). (B, D) Typical image of pan-cadherin immunodetection obtained using Cy5 secondary antibodies detected at 650/670 nm. Bars in B and D are 30 and 20 μm, respectively.

Supplementary Figure 3. Representative imaging of the living cells used as controls for the FRET assay. All images were obtained in the same conditions as the β-β interaction FRET after acceptor photobleaching assay. (A, D, G) CFP fluorescence following excitation at 390-425 nm and emission at 475-525 nm. (B, E, H) YFP fluorescence following excitation at 425-500 nm and emission at 540-575 nm. (A-C) MDCK cells transfected with a CFP-YFP fusion construct. ROI 3 was the photobleached region (positive control). (D-F) Co-culture of MRβ CFP and MRβ YFP cells. ROI 4, a membrane domain in which no YFP expression was visible, was the photobleached region. (G-I) Co-culture of MDCK cells expressing CFP alone (cytoplasmic) and MRβ YFP cells. ROI 5 represents a photobleached region in which CFP-containing vesicles were close to the membrane of MRβ YFP. Bars: 15 μm.


MRβ YFP
A
B
C
D
Anti Rat β1
Anti Dog β1
Fluorescence
Immunofluorescence


A
B
C
D
MDCK WT
MRβ YFP
Anti Pan-Cadherin
Anti Pan-Cadherin
Fluorescence
Immunofluorescence


CFP
YFP
Merge
A
B
C
ROI 3
D
E
F
ROI 4
G
H
I
ROI 5

Supplementary Table 1.

Table 1. Fluorescence energy transference in living MDCK cells.

Interaction	D_{PRE} (%)	D_{POST} (% of D_{PRE})	%E $E=((D_{POST}-D_{PRE})/D_{POST})x100$	N
β-β in live cells ROI 1	100	126.6 ± 2.1	21.3 ± 1.4	10
Negative control ROI 2	100	101.0 ± 0.9	0.8 ± 0.6	9
Positive control ROI 3	100	113.4 ± 1.3	11.5 ± 1.1	10
Negative control ROI 4	100	93.5 ± 2.7	0.0 ± 0.0	10
Negative control ROI 5	100	95.4 ± 4.0	0.0 ± 0.0	10

The efficiency of FRET (%E) was calculated from fluorescence recovery after acceptor photobleaching assays. To evaluate β-β interaction in vivo, fluorescence intensity of CFP was measured before (D_{PRE}) and after (D_{POST}) bleaching of YFP in area including the lateral membranes of cells expressing β-YFP and contacting neighboring cells expressing a β-CFP (ROI 1, Fig. 5). D_{pre} was normalized to 100% in all cases. Positive and negative controls were calculated from ROIs described in the corresponding figures. The negative control (ROI 2) is in Fig.5; the positive control (ROI 3) and additional negative controls (ROI 4 and ROI 5) are in SI Fig 4. Means ± S.E.M are for the number of experiments depicted as N.