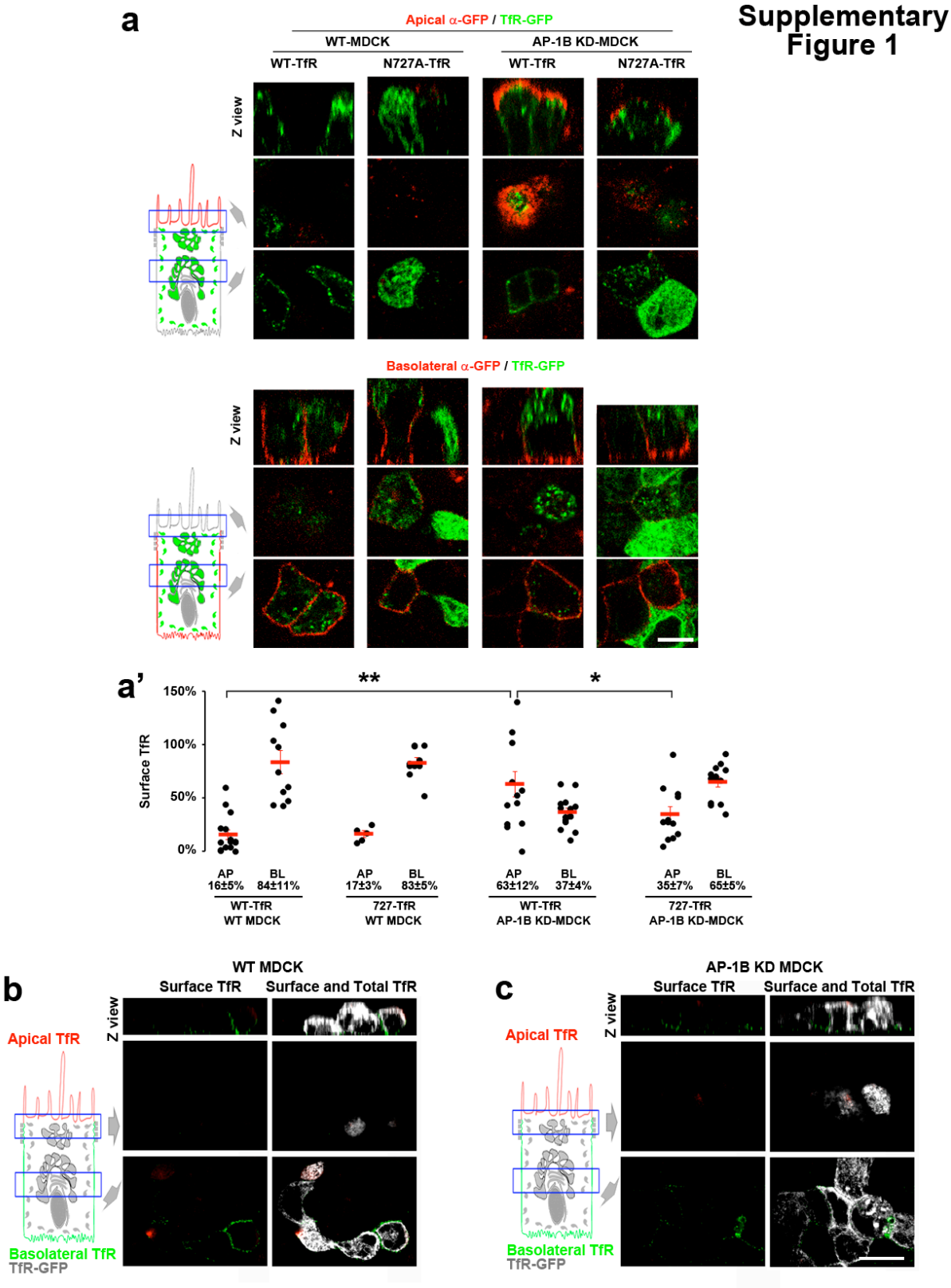
Supplementary material.

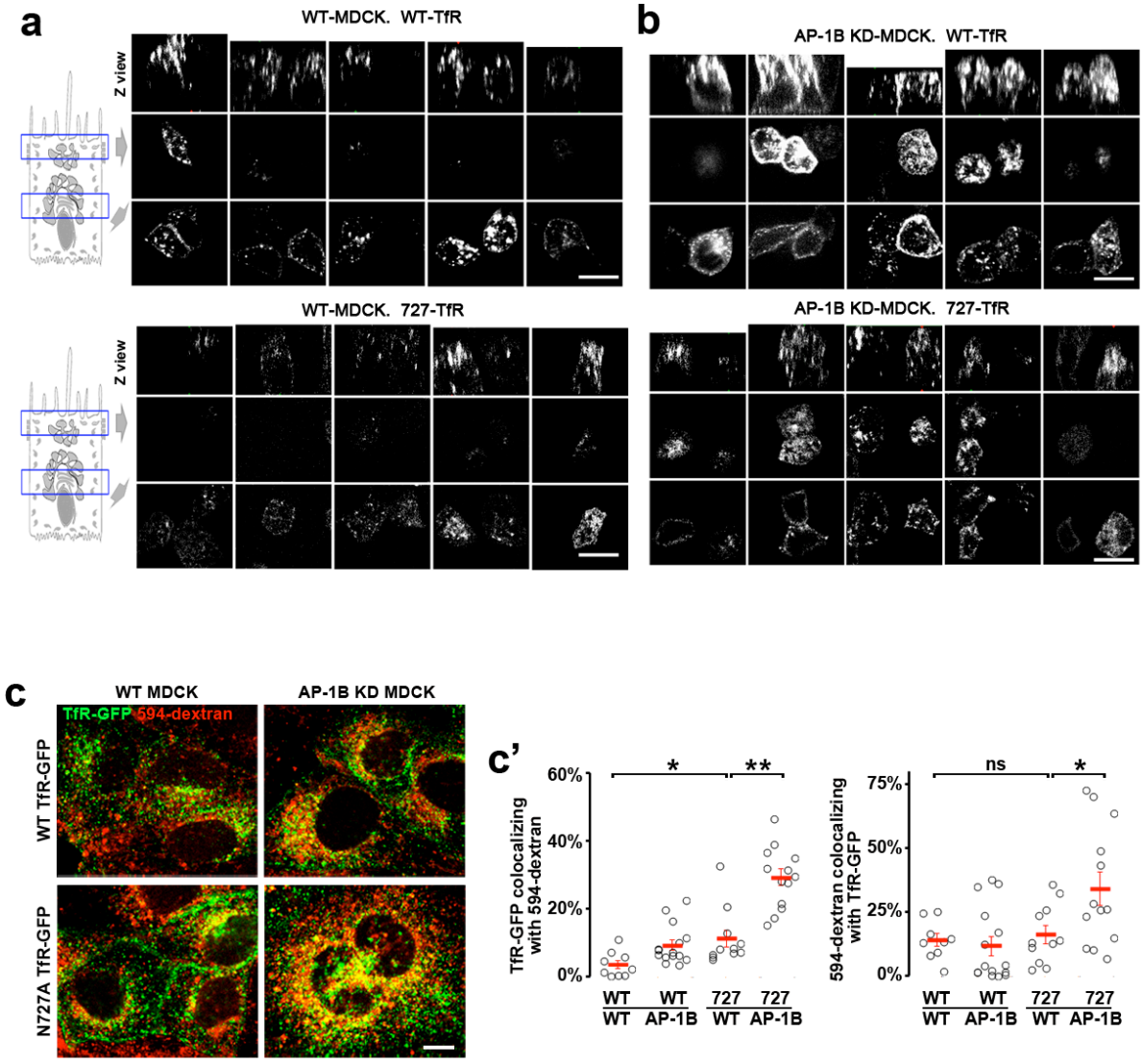
Supplementary Figure 1



Supplementary Figure 1. Mutation of the N727 glycan signal reduces half of apical TfR localization, whereas mutation of all glycosylation sites causes intracellular retention, in AP-1B KD MDCK cells. **(a)** Polarized WT (left) and AP-1B KD (right) MDCK cells were transiently transfected with either WT or N727A TfR-GFP (green). SeTau647-labeled rabbit anti-GFP antibodies (α-GFP, red) were applied either to the apical (top) or basolateral (bottom) chamber of separate transwell filters. **(a')** Cells from experiments represented in (a) were quantified for the apical or basolateral α-GFP signal using the fluorescent signal of each PM domain. Circles correspond to individual cells obtained from different experiments and red lines indicate the mean. *$p<0.05$, ** $p<0.001$.

Polarized WT **(b)** and AP-1B KD **(c)** MDCK cells were transfected with a TfR-GFP construct mutated in all four glycosylation sites of TfR (white). Cells were subjected to surface immunostaining of the apical and basolateral TfR (without permeabilizing the cells) using anti anti-GFP primary antibodies and anti-rabbit-IgG secondary antibodies labeled with Alexa Fluor 568 (red) or 647 (green) in the apical and basolateral chambers, respectively. Note that there is virtually no signal of apical or basolateral mutant TfR-GFP (left panels) although this mutant is highly expressed, as evidenced by the GFP signal (right panels). Scale: 10 μm.

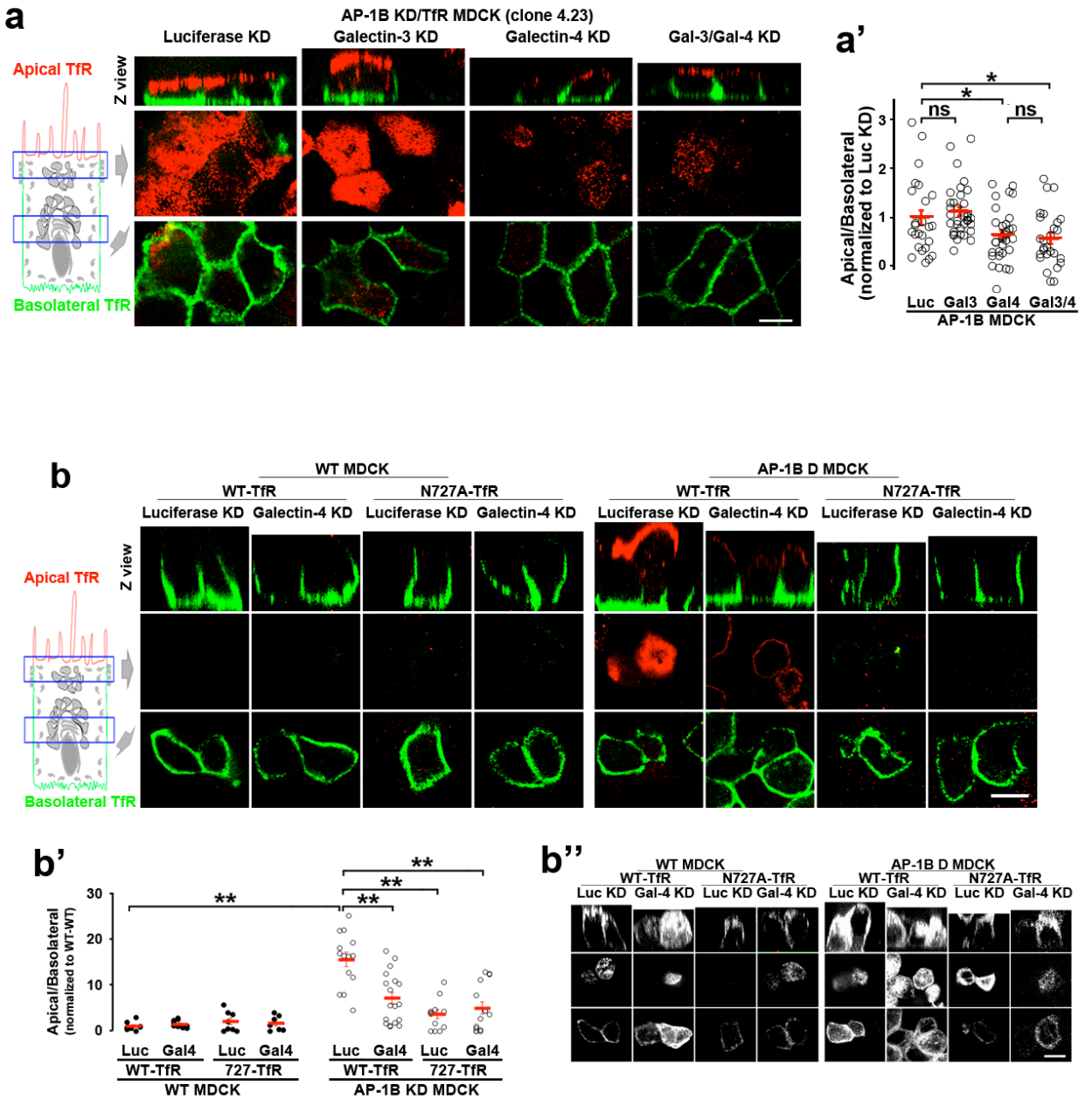
Supplementary Figure 2



Supplementary Figure 2. N727A TfR-GFP is targeted to the lysosomes in non-polarized AP-1B KD MDCK cells. (a and b) GFP signal of the cells displayed in the transcytosis assay of figure 2 confirms

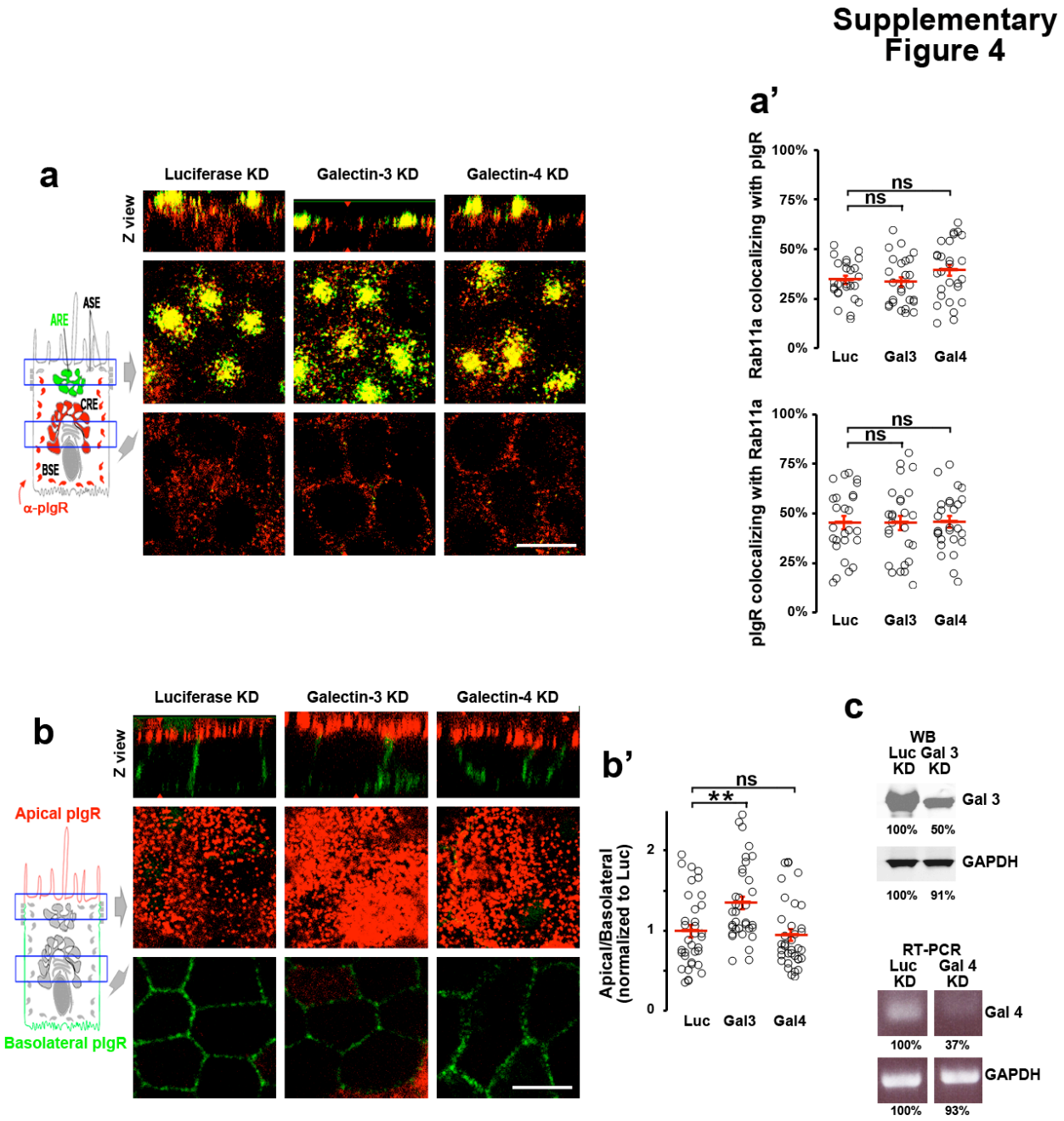
efficient transfection with WT TfR-GFP or N727A TfR-GFP constructs in polarized WT **(a)** and AP-1B KD **(b)** MDCK cells. Comparison of this and figure with figure 2 reveals that only transfected cells bound fluorescent antibodies. Scale: 10 µm. **(c)** Non-polarized WT and AP-1B KD MDCK cells were nucleofected with either WT or N727A TfR-GFP and stained for lysosomes with 60 minutes uptake of Alexa594-labeled 10 kDa dextran (594-dextran, red), followed by 60 minutes chase in dextran-free medium. **(c')** Cells from experiments represented in (c) were quantified for the percentage of pixels of TfR-GFP colocalizing with 594-dextran (left) and the percentage of pixels of 594-dextran colocalizing with TfR-GFP (right). Circles correspond to individual cells obtained from different experiments and red lines indicate the mean. ns not significant, * $p<0.05$, ** $p<0.001$. Scale: 10 µm.

Supplementary Figure 3



Supplementary Figure 3. Galectin-4 mediates TfR apical sorting in different clones of AP-1B KD/TfR MDCK cells, without affecting basolateral polarity of N727A TfR-GFP. (a) A different

clone of human TfR-expressing AP-1B KD MDCK cells to that used in figure 5 was knocked-down for luciferase, galectin-3 and/or galectin-4 and polarized on transwell filters. Apical and basolateral TfR was immunostained (without permeabilizing the cells), using anti-human TfR primary antibodies that recognizes TfR's luminal domain and anti-mouse-IgG secondary antibodies labeled with Alexa Fluor 568 (red) or 647 (green) in the apical and basolateral chambers, respectively. **(a')** Cells from experiments represented in (a were quantified for the apical/basolateral ratio using the fluorescent signal of each PM domain. **(b)** Polarized WT (left) and AP-1B KD (right) MDCK cells were transiently transfected with WT or N727A TfR-GFP and knocked-down for luciferase or galectin-4. Cells were immunostained for surface TfR-GFP (without permeabilizing the cells) using anti-GFP primary antibodies and anti-rabbit-IgG secondary antibodies labeled with Alexa Fluor 568 (red) or 647 (green) in the apical and basolateral chambers, respectively. **(b')** Cells from experiments represented in (b) were quantified for the apical/basolateral ratio using the fluorescent signal of each PM domain. **(b'')** GFP signal of the cells displayed in (b) confirms efficient transfection with WT TfR-GFP or N727A TfR-GFP constructs. Circles correspond to individual cells obtained from different experiments and red lines indicate the mean. ns not significant, * $p<0.05$, ** $p<0.001$. Scale: 10 µm.



Supplementary Figure 4. Galectin-3 or galectin-4 knock-down does not inhibit pIgR apical transcytosis. Polarized T23 cells (MDCK cells stably expressing the pIgR) were knocked-down for

luciferase, galectin-3 or galectin-4 and polarized on transwell filters. **(a)** Cells were incubated basolaterally with sheep anti-pIgR (60 minutes-4°C plus 30 minutes-37°C) and immunostained for the ARE marker rab11a. **(a')** Cells from experiments represented in (a) were quantified for the percentage of pixels of rab11a colocalizing with pIgR (top) and the percentage of pixels of pIgR colocalizing with rab11a (bottom). **(b)** Surface immunostaining of the apical and basolateral pIgR (without permeabilizing the cells) using sheep anti-pIgR primary antibodies and anti-sheep-IgG secondary antibodies labeled with Alexa Fluor 568 (red) or 647 (green) in the apical and basolateral chambers, respectively. **(b')** Cells from experiments represented in (b) were quantified for pIgR apical/basolateral ratio using the fluorescent signal of each PM domain. **(c)** Western Blot analysis of galectin-3 expression (top) and RT-PCR analysis of galectin-4 expression (bottom). Circles correspond to individual cells and red lines indicate the mean. ns not significant, ** $p<0.001$. Scale: 10 µm.