E07-07-0658 Lencer

Supplemental figure legends

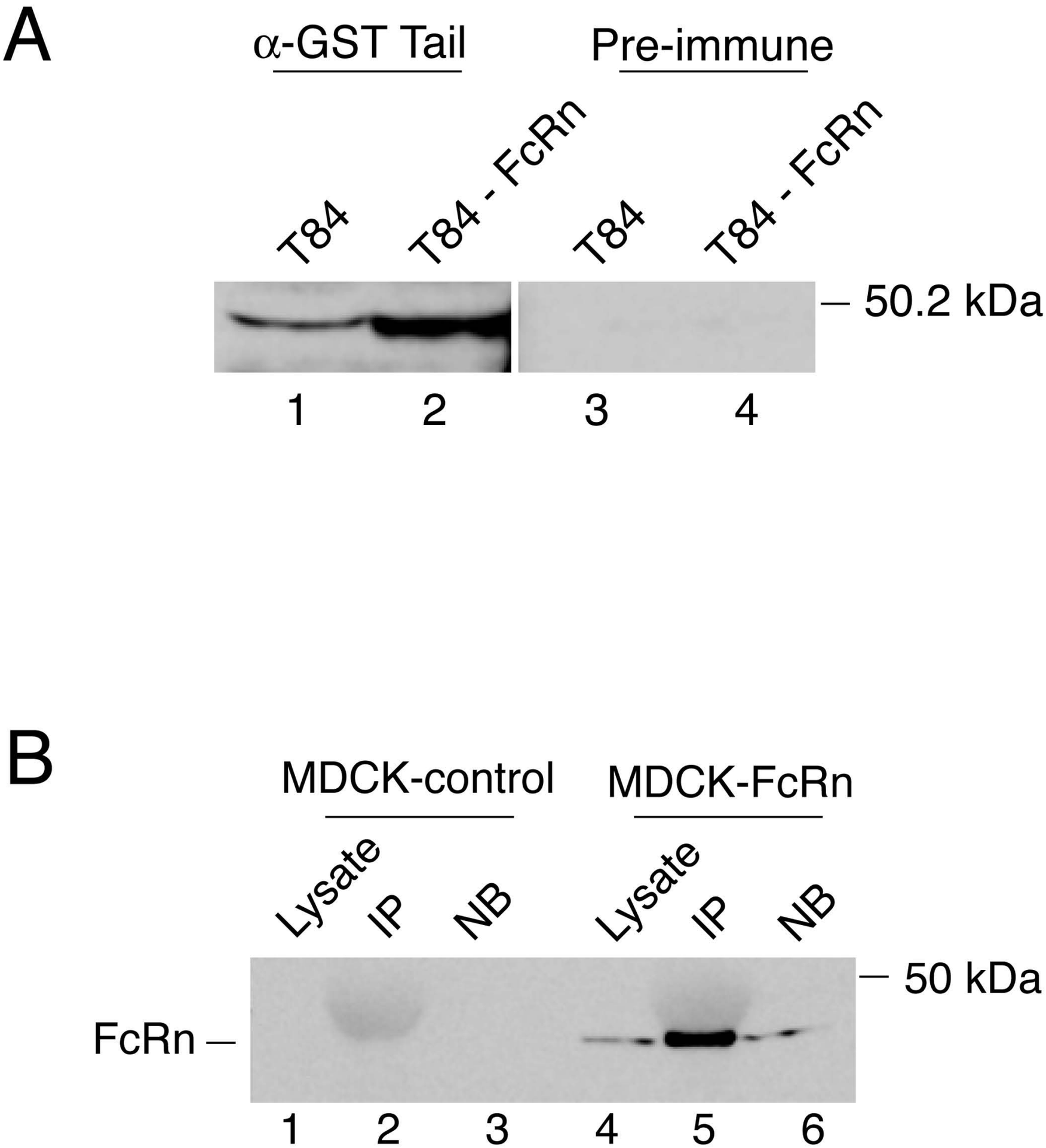
Supplemental figure 1. Rabbit anti-GST-FcRn tail polyclonal antiserum is specific for full-length human FcRn heavy chain expressed in T84 and MDCK cells. (A) To demonstrate specificity for the rabbit anti-GST-FcRn tail polyclonal antiserum, T84 cells expressing endogenous FcRn and T84 cells over-expressing human FcRn heavy chain (clone 5) were lysed and 20μg of lysate was separated by SDS-PAGE and FcRn detected by immunoblot with the antiserum (lanes 1 and 2). Pre-immune serum was used as a negative control (lanes 3 and 4). (B) The rabbit anti-GST-FcRn tail polyclonal antiserum was tested for immunoprecipitation of FcRn heavy chain from MDCK cell lysates. MDCK cells carrying empty expression plasmids (MDCK control; lanes 1-3) or expressing WT FcRn (MDCK-FcRn; lanes 4-6) were lysed and equal quantities of cell lysates (assessed by protein content) were incubated with the antiserum and collected with protein G-sepharose beads. FcRn was detected in whole cell lysates (20μg; lanes 1 and 4), immunoprecipitates (IP; lanes 2 and 5), and the non-binding fractions (NB; lanes 3 and 6) by immunoblot with a rat anti-HA monoclonal antibody.

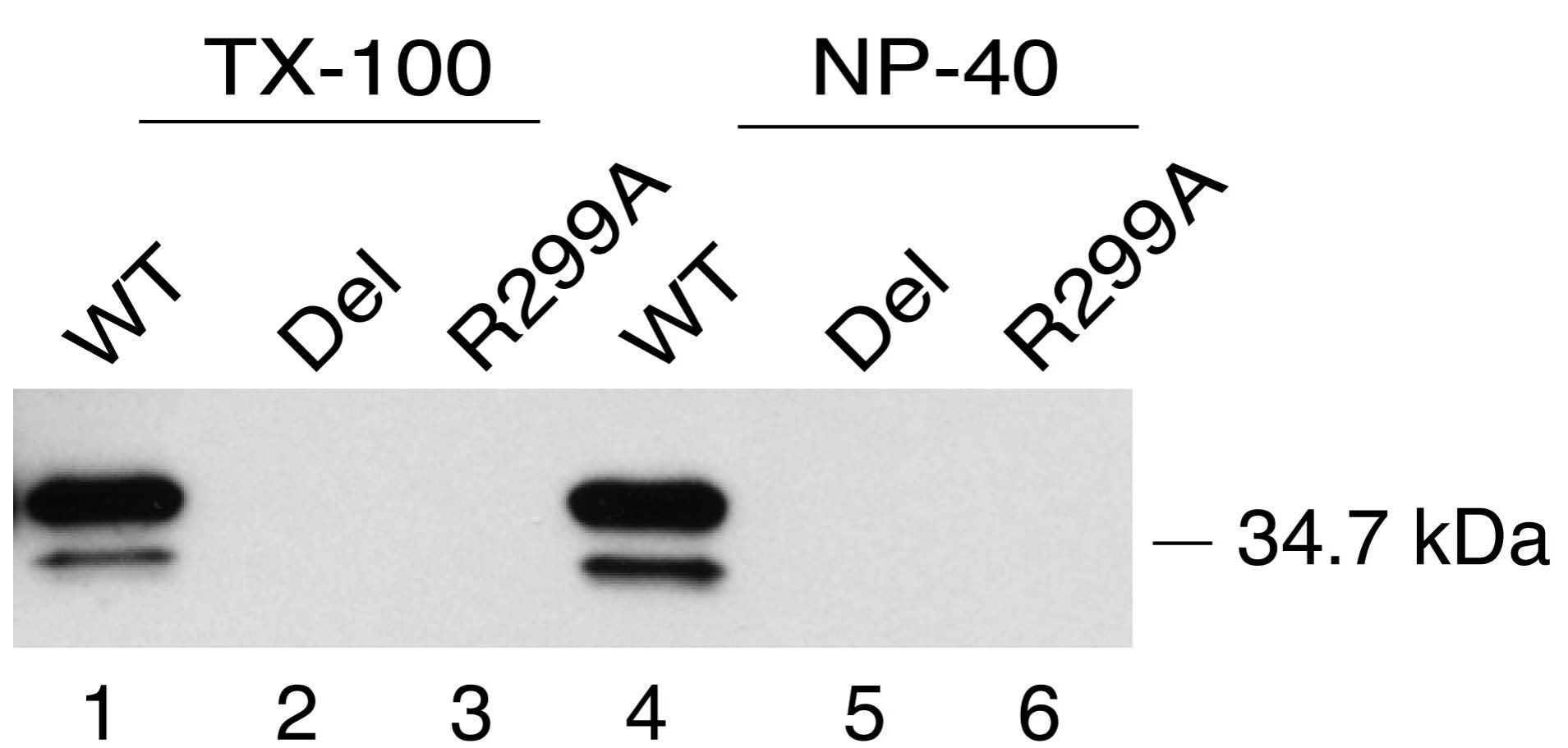
Supplemental Figure 2. Calmodulin binds to the FcRn cytoplasmic tail. The WT, DEL and R299A FcRn tail GST-fusion proteins were incubated with CaM-Seph in Triton X-100 (lanes 1-3) or NP-40 (lanes 4-6) lysis buffer containing calcium and binding was assessed by immunoblot with a rabbit anti-GST-FcRn tail polyclonal antiserum.

Supplemental figure 3. Specificity for FcRn-mediated transcellular transport of IgG. Rabbit IgG blocks Nip-IgG transcytosis across MDCK clones expressing WT and mutant FcRn. (A) MDCK cells cultured on Transwell inserts were pre-treated with buffer or with a 500-fold molar excess of rabbit IgG for 20 min before Nip-IgG (60nM) was added to the apical surface and Nip-IgG transcytosis measured by ELISA. Three Transwells per clone were examined and data were normalized to the mass of FcRn heavy chain present in whole cell lysates relative to the β-actin content (FcRn:β-actin ratio; see B below). Data are expressed as a percentage of Nip-IgG transport by buffer-treated MDCK cells expressing WT FcRn (100%). Data are representative of six independent experiments and expressed as mean ± SEM. (B) Following the experiment, MDCK cells were lysed in RIPA buffer and equal quantities of cell lysates (assessed by protein content) were analyzed for FcRn and β-actin expression by immunoblot. Bands were quantified by densitometry.

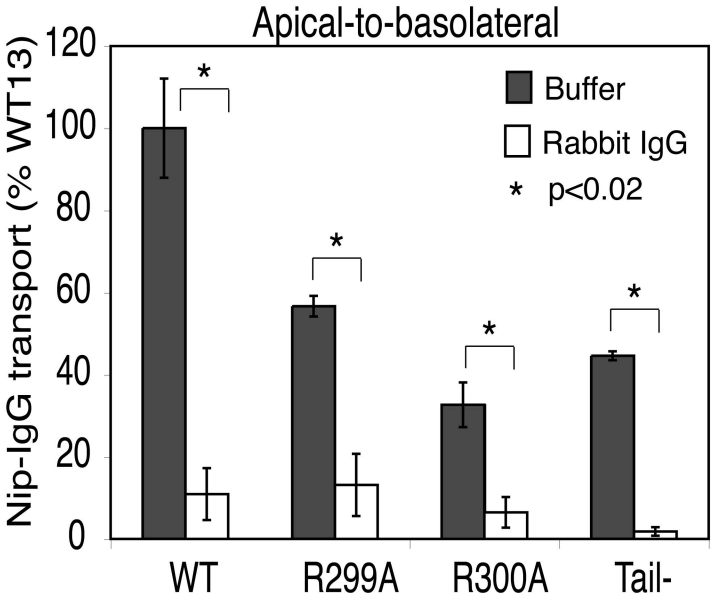
Supplemental figure 4. Calmodulin regulates FcRn-dependent IgG transcytosis in MDCK cells. MDCK cells were plated on Transwell inserts pre-treated with buffer or with a 500-fold molar excess of rabbit IgG, W-5 (50μM), or W-7 (50μM) for 20 min. Nip-IgG (60nM) was then added to the apical surface and transcytosis measured by ELISA. Data were normalized to Nip-IgG transport by buffer-treated control cells (100%) and expressed as the mean ± SEM from triplicate Transwells.

Supplemental figure 5. Calmodulin regulates FcRn half-life. The basolateral membrane of MDCK cell clones cultured on Transwell filters was biotinylated and the cells incubated for 0-24 hours at 37°C. At intervals, biotinylated FcRn was detected by immunblot (A) and quantified by densitometry (B). The mass of biotinylated FcRn is expressed as a percentage of the starting mass of FcRn at time 0 h (100%).

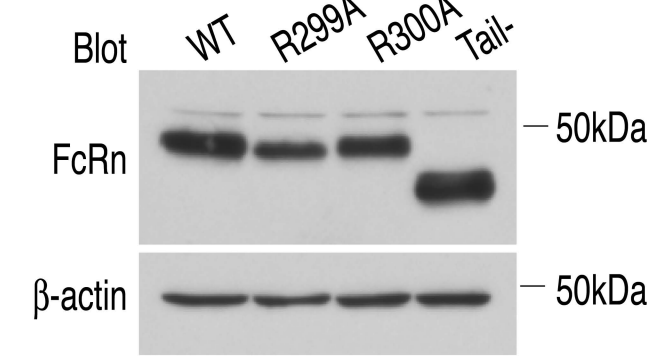

A
α-GST Tail
Pre-immune
T84
T84 - FcRn
T84
T84 - FcRn
50.2 kDa
1
2
3
4
B
MDCK-control
MDCK-FcRn
Lysate
IP
NB
Lysate
IP
NB
50 kDa
FcRn
1
2
3
4
5
6

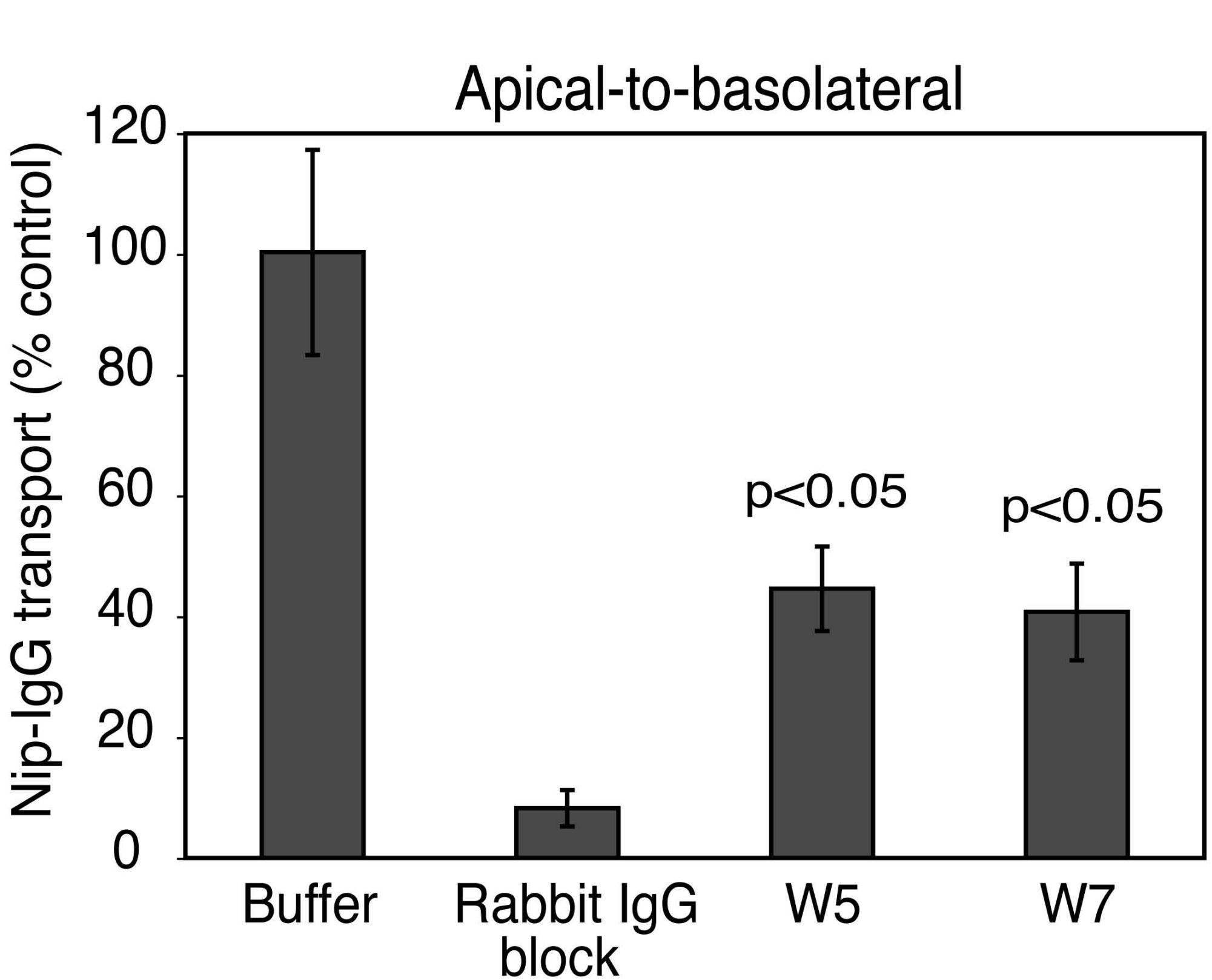

TX-100
NP-40
WT
Del
R299A
WT
Del
R299A
— 34.7 kDa
1
2
3
4
5
6

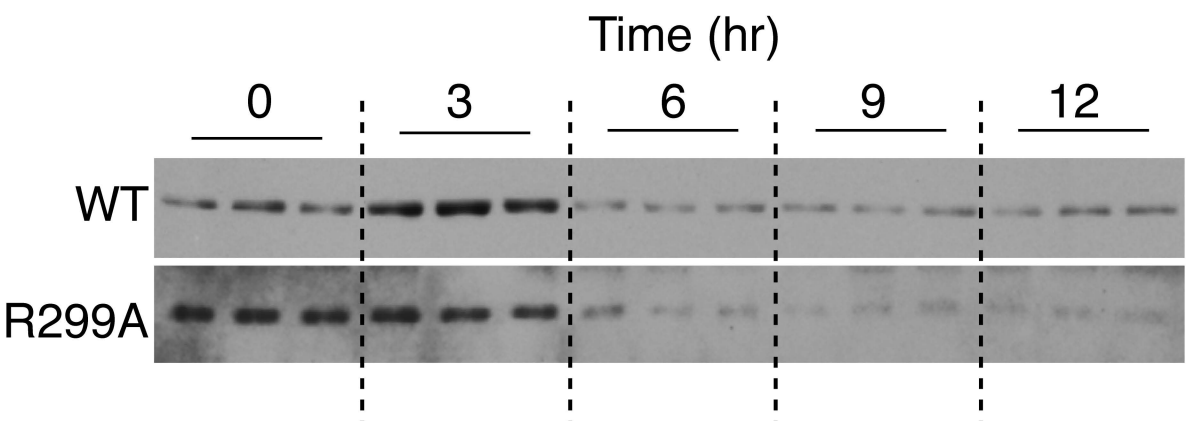
A

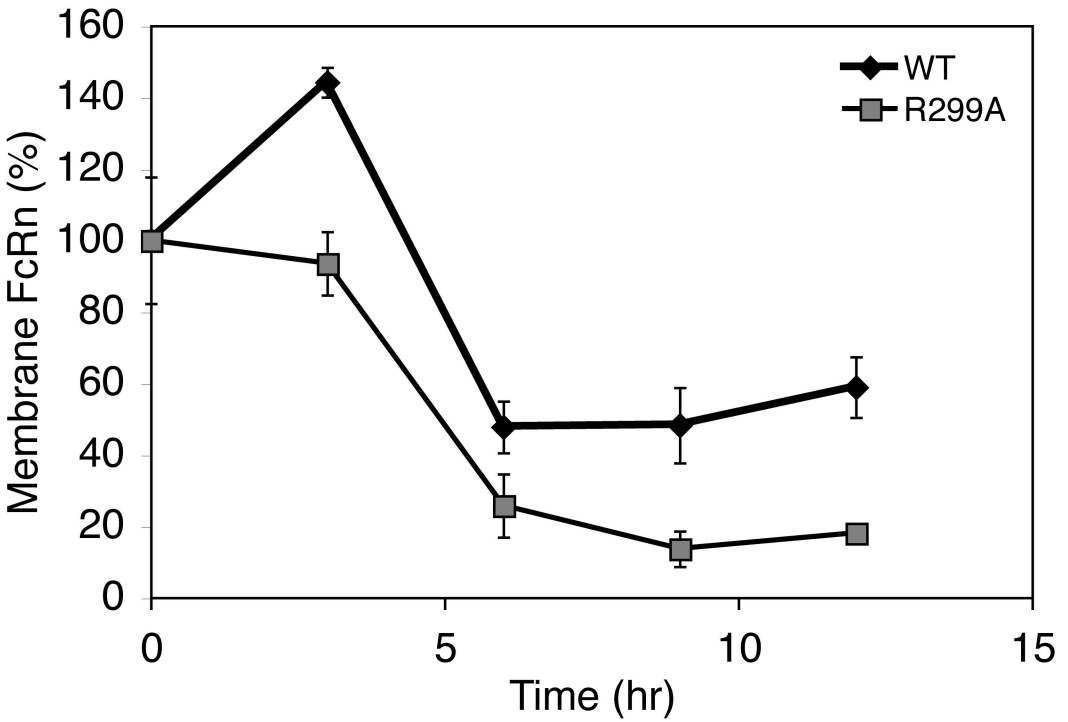

Apical-to-basolateral
Nip-IgG transport (% WT13)
120
100
80
60
40
20
0
Buffer
Rabbit IgG
* p<0.02
*
*
*
*
WT
R299A
R300A
Tail-

B


Blot
WT
R299A
R300A
Tail-
FcRn
50kDa
β-actin
50kDa


Apical-to-basolateral
Nip-IgG transport (% control)
120
100
80
60
40
20
0
p<0.05
p<0.05
Buffer
Rabbit IgG block
W5
W7


Time (hr)
0
3
6
9
12
WT
R299A


160
140
120
100
80
60
40
20
0
Membrane FcRn (%)
WT
R299A
0
5
10
15
Time (hr)