Supplemental Materials

Molecular Biology of the Cell

Yin et al.

SUPPLEMENTAL FIGURE LEGENDS

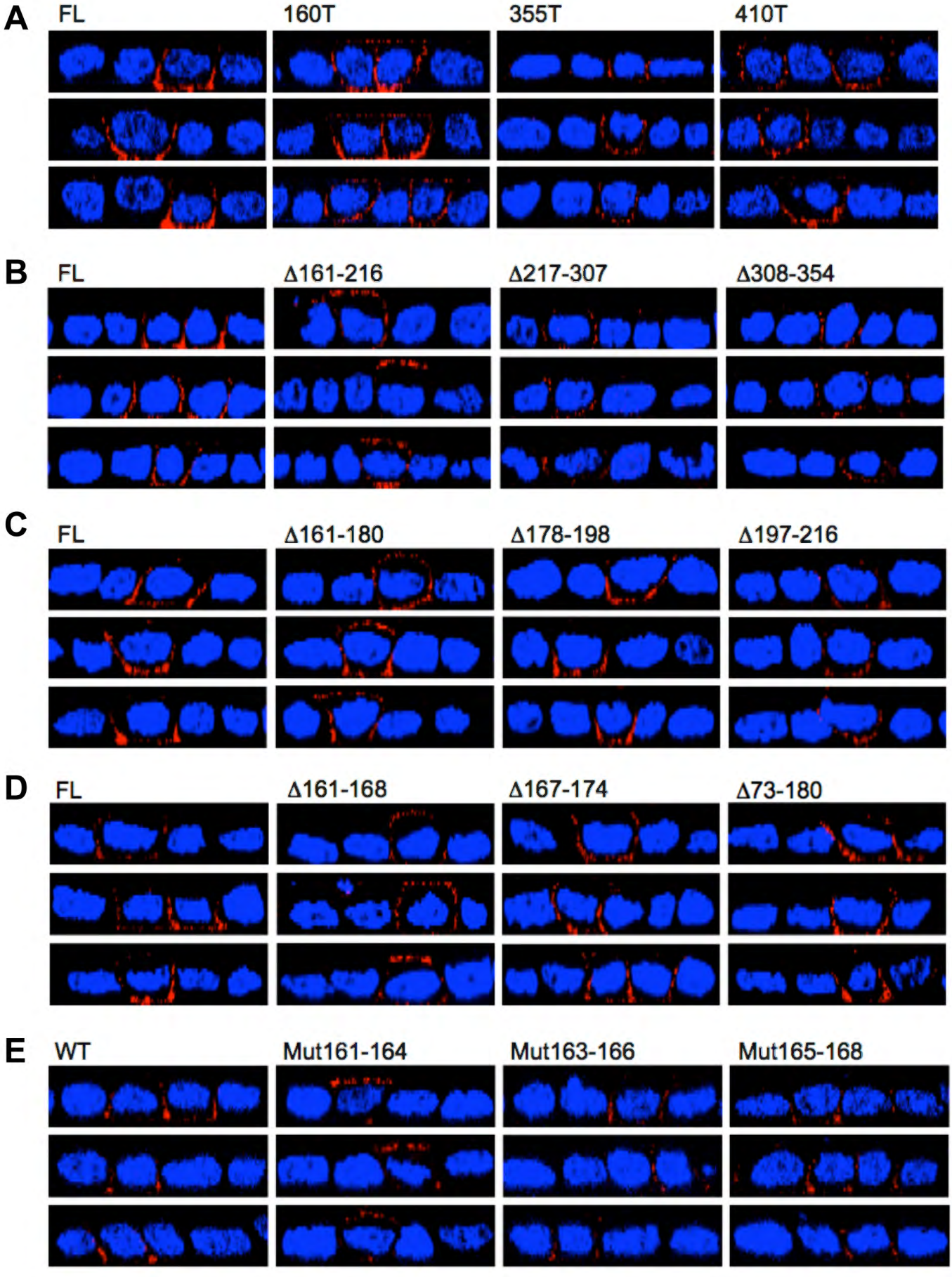
Figure S1. Additional TβRI truncations, deletions, and mutations defining the basolateral targeting signal between amino acids 161-164. (A) Additional images of indicated TβRI truncations as presented in figure 1A. (B-E) Additional images of indicated TβRI deletions and mutations as presented in figure 1B.

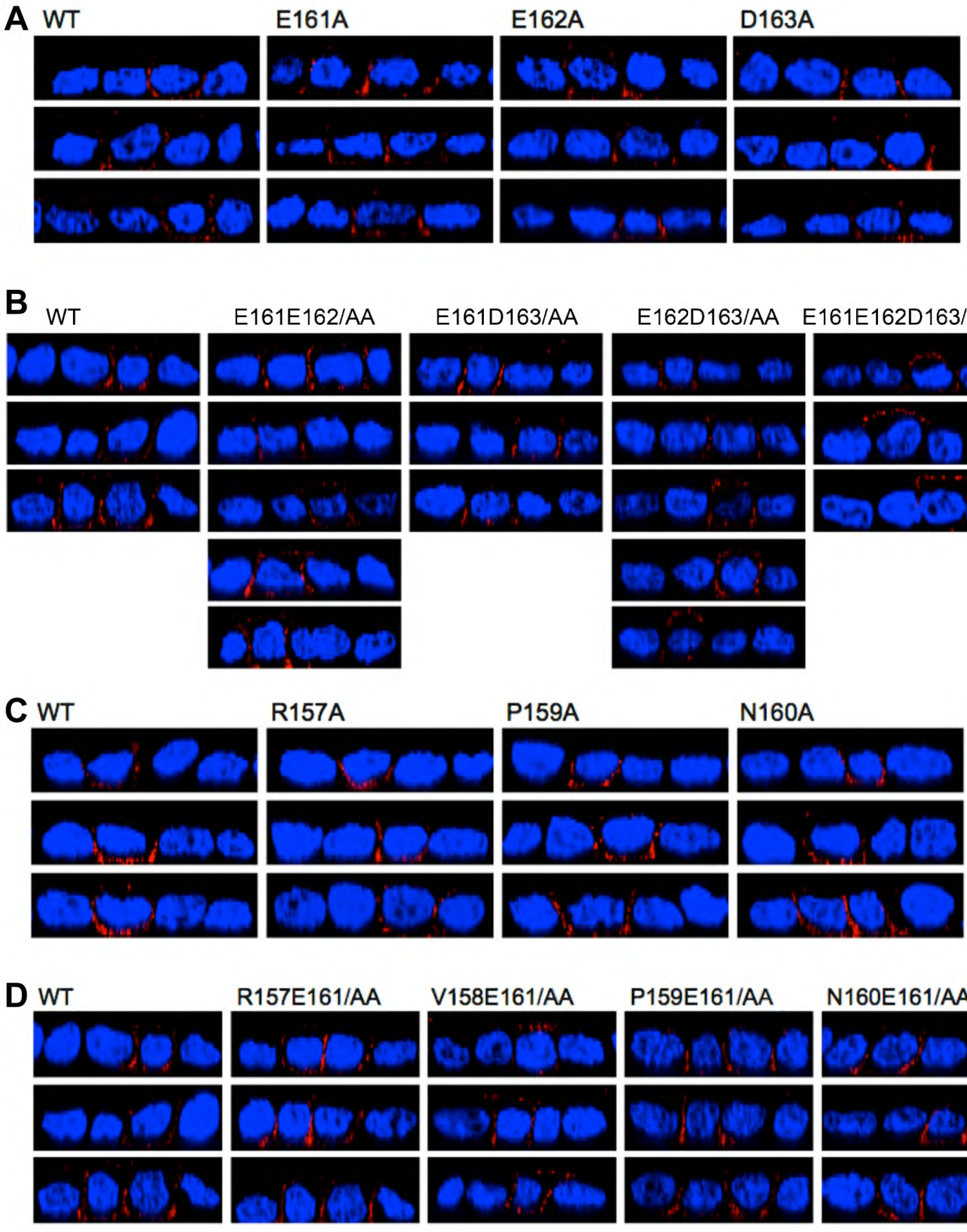
Figure S2. Single and double TβRI point mutations to define the basolateral targeting signal. (A-D) Additional images as in figures 2 and S1 in order to identify the amino acids comprising the TβRI basolateral-targeting element.

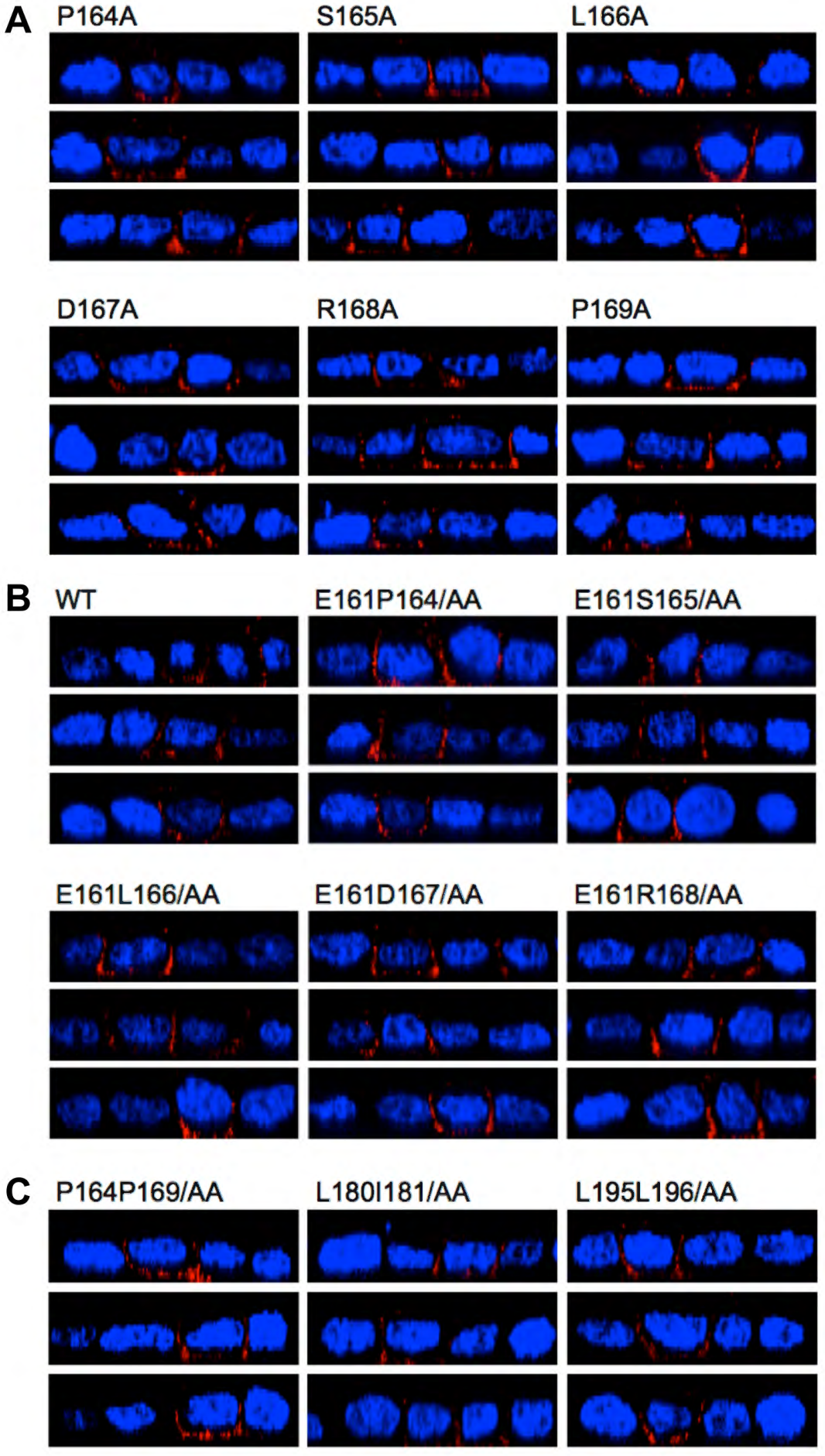
Figure S3. TβRI point mutations tested that did not impact basolateral targeting. (A-C) Images of transiently transfected mutated TβRI constructs as in figure 1 that had no effect on basolateral TβRI targeting.

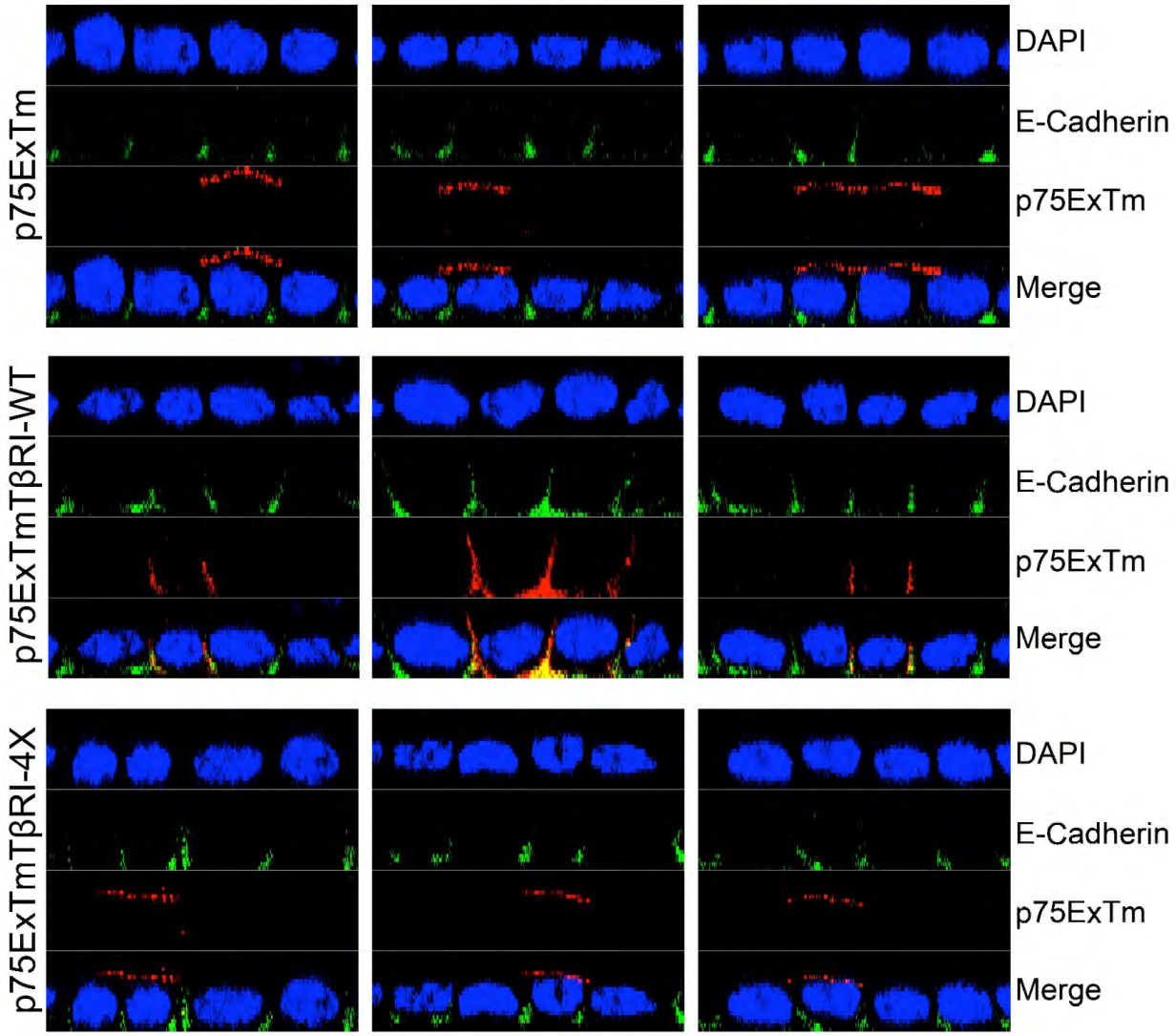
Figure S4. VEED motif in TβRI directs basolateral targeting to the apically expressed nerve growth factor receptor (p75). Additional images as in figure 6 depicting basolateral expression of p75 when the TβRI wild type (WT), but not the ^{158}VxxEED^{163} mutant (^{158}AxxAAA^{163}; 4X), is fused to the extracellular and transmembrane domain (p75ExTm) of the nerve growth factor receptor.

Figure S5. Mutation of the basolateral targeting domain in chimeric type I receptors does not affect ligand-dependent Smad3 phosphorylation. MDCK cells stably expressing wild type chimeric type I and type II TGFβRs (αIβII #7) or a wild type chimeric type II and VEED mutated chimeric type I receptor (αI-4XβII #6) were polarized as in figure 3A and treated with GM-CSF (100 ng/ml; activates chimeric receptors) or TGF-β (10 ng/ml; activates endogenous receptors) from either the apical (AP) or basolateral (BL) transwell chamber. Following 1 hr stimulation, cells were lysed in modified RIPA buffer and phosphorylated (p) or total (t) Smad3 determined as described in Materials and Methods. Blots are representative of 3 separate experiments.


A
FL
160T
355T
410T
B
FL
Δ161-216
Δ217-307
Δ308-354
C
FL
Δ161-180
Δ178-198
Δ197-216
D
FL
Δ161-168
Δ167-174
Δ73-180
E
WT
Mut161-164
Mut163-166
Mut165-168


A
WT
E161A
E162A
D163A
B
WT
E161E162/AA
E161D163/AA
E162D163/AA
E161E162D163/AAA
C
WT
R157A
P159A
N160A
D
WT
R157E161/AA
V158E161/AA
P159E161/AA
N160E161/AA


A
P164A
S165A
L166A
D167A
R168A
P169A
B
WT
E161P164/AA
E161S165/AA
E161L166/AA
E161D167/AA
E161R168/AA
C
P164P169/AA
L180I181/AA
L195L196/AA


p75ExTm
p75ExTmTβRI-WT
p75ExTmTβRI-4X
DAPI
E-Cadherin
p75ExTm
Merge
DAPI
E-Cadherin
p75ExTm
Merge
DAPI
E-Cadherin
p75ExTm
Merge

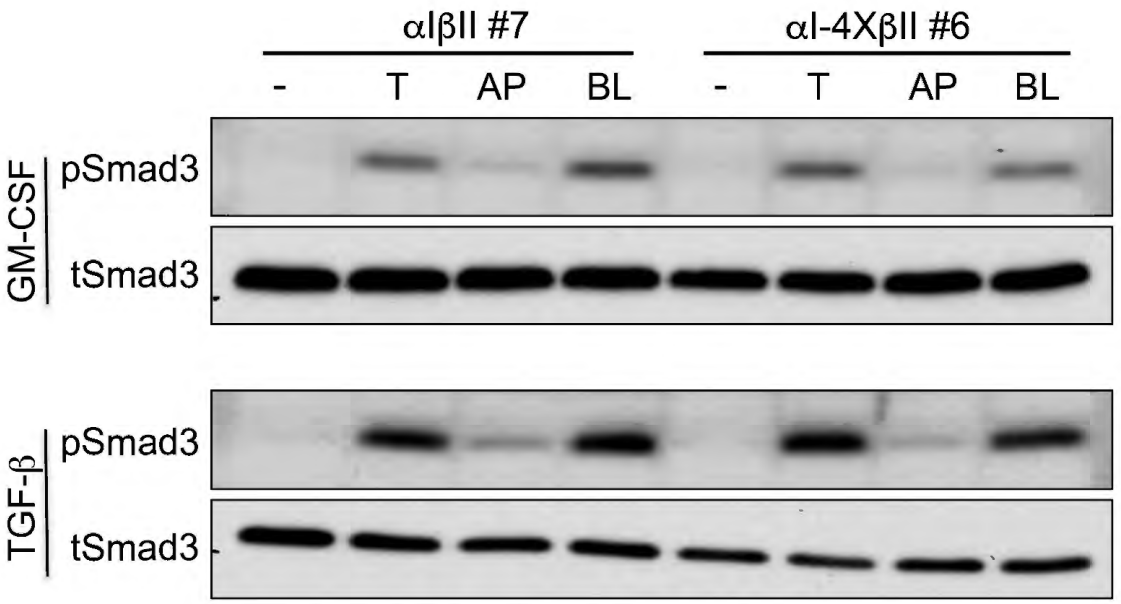

αIβII #7
αI-4XβII #6
-
T
AP
BL
-
T
AP
BL
GM-CSF
pSmad3
tSmad3
TGF-β
pSmad3
tSmad3

Table S1. Antibodies

Antibody	Company	Catalog #	Application	Dilution
E-Cadherin	Sigma	U3254	Immunofluorescence	1:500
GM-CSF α	Santa Cruz	sc-456	Immunofluorescence	1:50
GM-CSF β	Santa Cruz	sc-21765	Immunofluorescence	1:50
IgG (mouse)	Jackson Laboratories	715-165-150	Immunofluorescence	1:250
IgG (rabbit)	Jackson Laboratories	711-165-152	Immunofluorescence	1:250
IgG (rat)	Invitrogen	A21208	Immunofluorescence	1:250
Myc	Cell Signaling	2278	Immunofluorescence	1:100
p75	Santa Cruz	sc-13577	Immunofluorescence	1:100
GAPDH	Millipore	MAB374	Western Blotting	1:10,000
E-Cadherin	BD Biologicals	610181	Western Blotting	1:10,000
GM-CSF α	Santa Cruz	sc-456	Western Blotting	1:1000
GM-CSF β	Santa Cruz	sc-676	Western Blotting	1:1000
IgG (mouse)	Santa Cruz	sc-2005	Western Blotting	1:10,000
IgG (rabbit)	Santa Cruz	sc-2004	Western Blotting	1:10,000
pSmad3	Lab Made (Wilkes *et al.*, 2003)	N/A	Western Blotting	1:5,000
Smad3	Abcam	ab28379	Western Blotting	1:2000
TβRI	Sant Cruz	sc-398	Western Blotting	1:1,000
TβRII	Santa Cruz	sc-220	Western Blotting	1:1,000

Wilkes, M.C., Murphy, S.J., Garamszegi, N., and Leof, E.B. (2003). Cell-type-specific activation of PAK2 by transforming growth factor ß independent of Smad2 and Smad3. Mol Cell Biol *23*, 8878-8889.