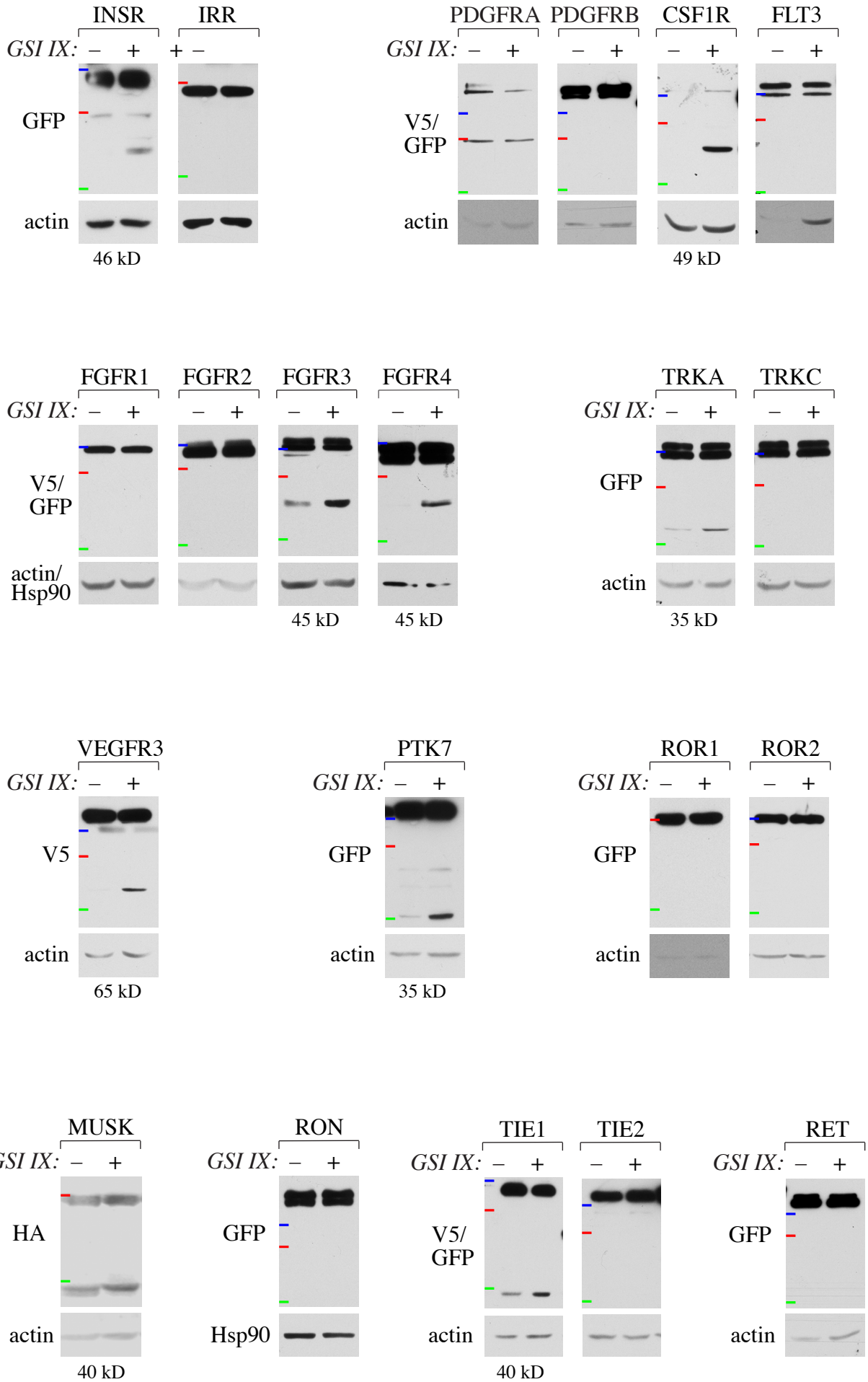
Supplemental Materials

Molecular Biology of the Cell

Merilahti et al.


INSR
IRR
GSI IX: – + + –
GFP
actin
46 kD
PDGFRA
PDGFRB
CSF1R
FLT3
GSI IX: – + – + – + – +
V5/
GFP
actin
49 kD
FGFR1
FGFR2
FGFR3
FGFR4
GSI IX: – + – + – + – +
V5/
GFP
actin/
Hsp90
45 kD
45 kD
TRKA
TRKC
GSI IX: – + – +
GFP
actin
35 kD
VEGFR3
GSI IX: – +
V5
actin
65 kD
PTK7
GSI IX: – +
GFP
actin
35 kD
ROR1
ROR2
GSI IX: – + – +
GFP
actin
MUSK
GSI IX: – +
HA
actin
40 kD
RON
GSI IX: – +
GFP
Hsp90
TIE1
TIE2
GSI IX: – + – +
V5/
GFP
actin
40 kD
RET
GSI IX: – +
GFP
actin

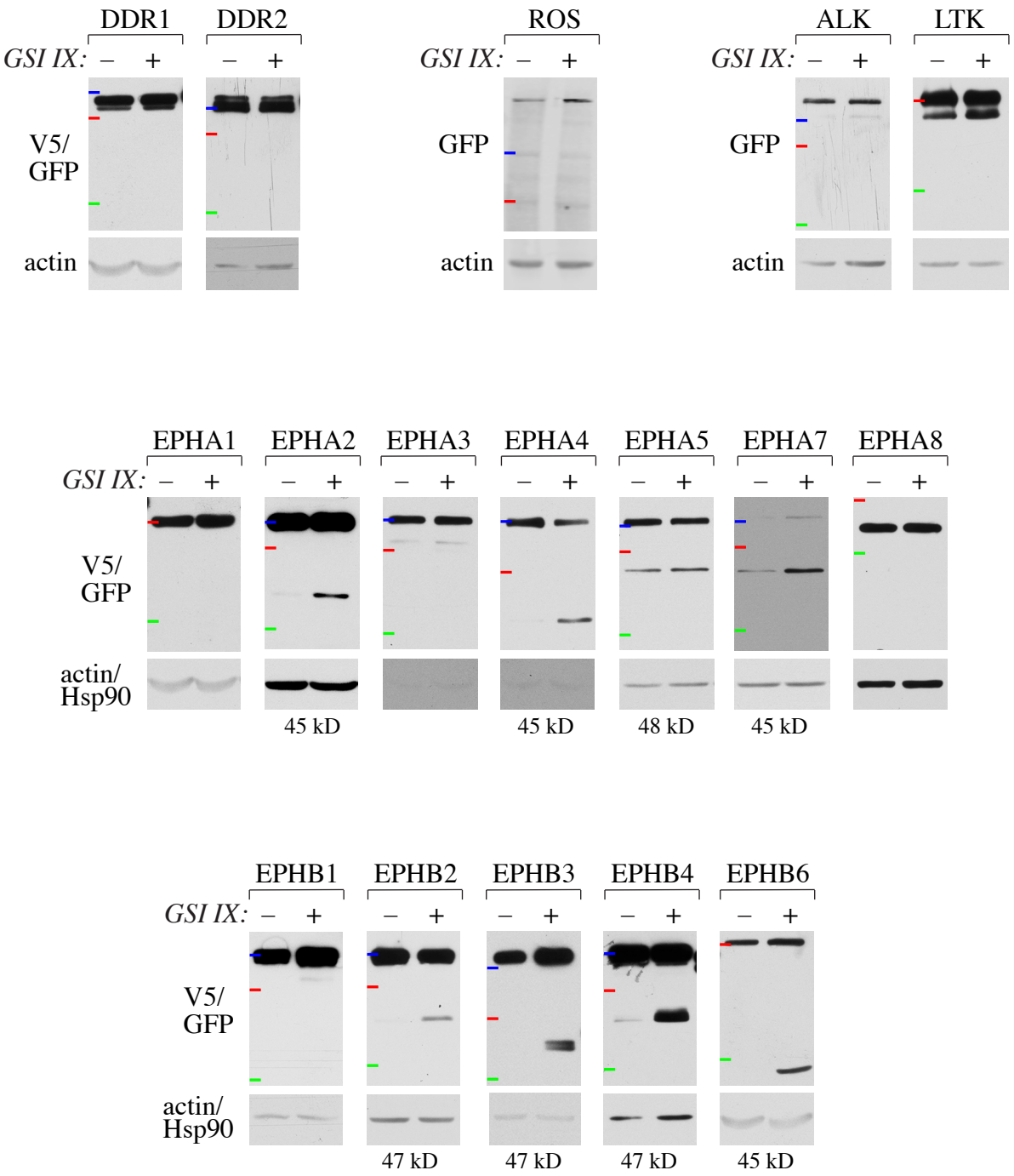


Figure S1. Western analysis of gamma-secretase cleavage of RTKs.
Gamma-secretase cleavage of the RTKs was analyzed in MCF-7 (INSR, IRR, PDGFRA, PDGFRB, CSF1R, FLT3, FGFR1, FGFR2, FGFR3, FGFR4, TRKA, TRKC, VEGFR3, ROR1, ROR2, RON, RET DDR1, DDR2, ALK, LTK, EPHA1, EPHA2, EPHA3, EPHA4, EPHA5, EPHA7 EPHA8, EPHB1, EPHB2, EPHB3, EPHB4, EPHB6) or HEK293 (MUSK, PTK7, TIE1, TIE2, ROS) cells transfected with plasmids encoding carboxy-terminally GFP-, HA- or V5-tagged RTKs. Cells were treated as in Figure 1C. Loading was controlled with anti-actin or anti-Hsp90. The estimated sizes of the cleaved CTFs are indicated below the respective Westerns. The molecular weight markers are indicated by colored horizontal lines: blue, 150 kD; red, 100 kD; green, 50 kD. The ectodomains of FGFR3, TRKA, MUSK, PTK7, TIE1, EPHA5 and EPHA7 were constitutively shed to the extent that CTFs were also visible without GSI IX accumulation. PDGFRA displayed additional, smaller bands in addition to bands representing full length receptor. This RTK was not considered a new gamma-secretase substrate as the bands did not react to gamma-secretase inhibition and the observed size of the band (69 kD) was also different from the predicted size of the CTF (61 kD).

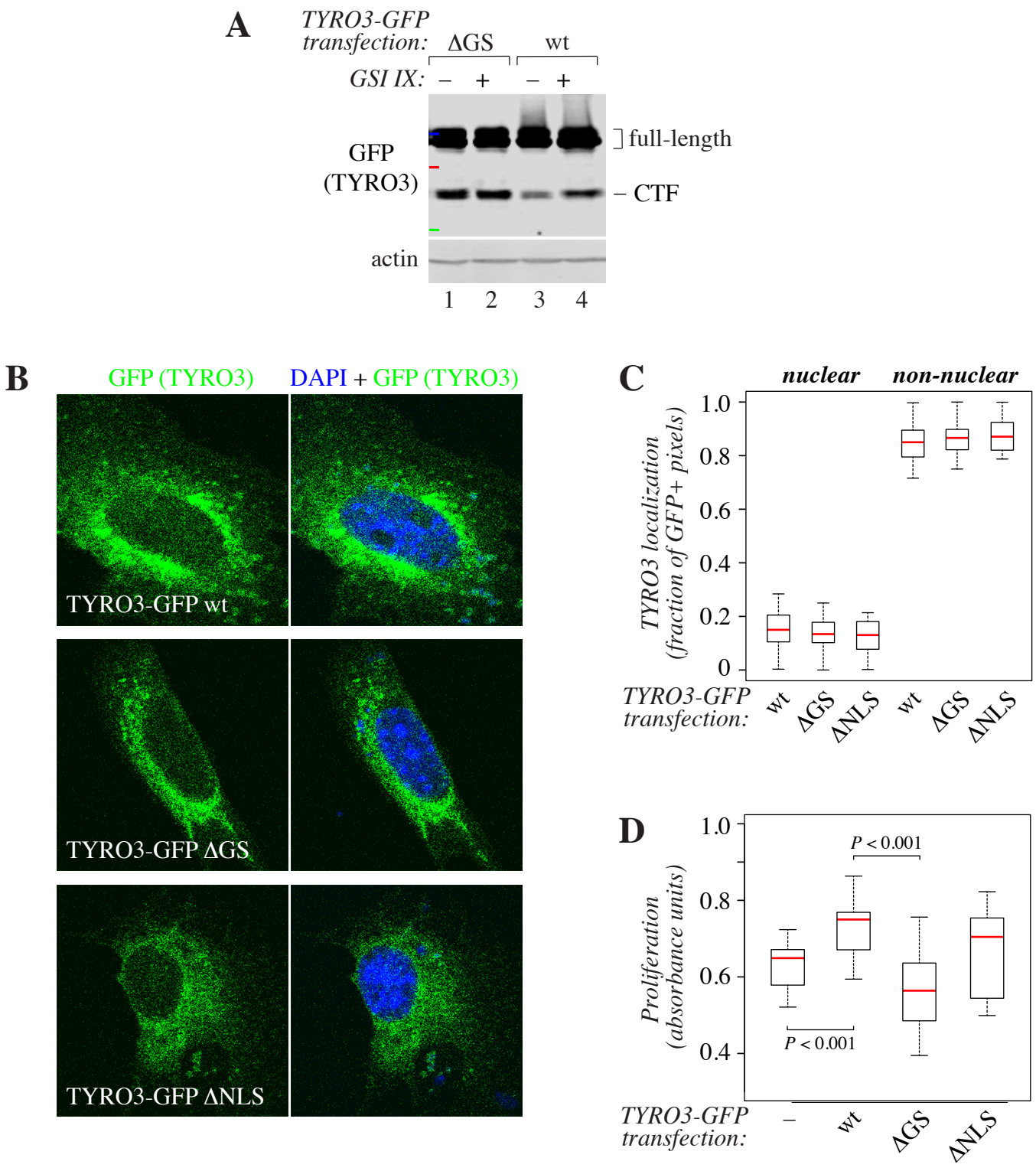


Figure S2. A TYRO3 mutant with defective gamma-secretase cleavage.
(A) Western analysis of gamma-secretase cleavage of TYRO3 in HEK293 transfectants expressing carboxy-terminally GFP-tagged wild-type (wt) TYRO3 or a TYRO3 construct with mutant gamma-secretase cleavage site (ΔGS). The cells were treated with or without 5 μM GSI IX for 4 hours. Loading was controlled with anti-actin. (B) Confocal microscopy analysis of NIH-3T3 cells overexpressing carboxy-terminally GFP-tagged wild-type TYRO3 (wt), TYRO3 with mutated gamma-secretase cleavage site (ΔGS), or TYRO3 with mutated nuclear localization sequence (Δ NLS). (C) Quantification of the confocal immunofluorescence analysis of TYRO3-GFP localization shown in B. Nuclear localization is presented as the percentage of GFP-specific signals colocalizing with DAPI of all GFP-specific signals within the cells. At least 20 cells were analyzed per construct. (D) A 72 hour WST-8 cell proliferation analysis of NIH-3T3 transfectants expressing wild-type TYRO3 (wt), or TYRO3 ΔGS or TYRO3 ΔNLS mutant constructs.