SUPPLEMENTAL DATA

FIG. S1. Sumoylation of long isoform NFATc1/C is very labile and the short isoform NFATc1/A is only weakly sumoylated. (A) During preparation sumoylated NFATc1/C has to be protected by NEM. As in Fig. 2*A*, except in parallel, a lysate was prepared in the absence of N-ethylmaleimide (NEM). (B) Plasmids encoding NFATc1/A, wild type or K349R, were transfected into 293T HEK cells, either with those expressing Flag-SUMO1 or Flag. After 6 hrs of T/I treatment, immunoprecipitation with anti-Flag and immunoblot by anti-HA were performed for the immunoprecipitated as well as the lysate proteins. (C) A luciferase reporter assay was performed as documented in Fig. 4A, but the short isoform NFATc1/A and NFATc1/AK349R were subjected to analysis.

FIG. S2. Exogenous short, long and K349/702/914R isoforms of NFATc1 exhibit an equal expression. Retrovirally infected A3.01 cells expressing mock-, c1/A-, c1/C- or K349/702/914R-ER (Fig. 3 and 4), were taken to check exogenous NFATc1 expression (anti-ERα) by confocal microscopy and western blot.

FIG. S3. Colocalization with SUMO/PML-bodies depends on its C-terminal modification with SUMO. 293T HEK cells were transfected with plasmids encoding pHA-NFATc1/C or the different ΔSUMO mutants along with pcFlag-SUMO1, and stimulated with T/I+$CaCl_2$ for 1 hr. (A) Nuclear translocation is unaffected by (non-) sumoylation of NFATc1/C. IF was performed with anti-NFATc1 and anti-SUMO1 followed by confocal microscopy. The expression pattern of approximately 100 cells was determined for each DNA transfected. Mean values of three independent experiments are represented as mean +/- SD. (B) Transient transfection is sufficient to sumoylate NFATc1/C and to direct it into PML-nbs. IF was performed with anti-NFATc1, anti-SUMO1, and anti-PML. Triple localization of NFATc1, SUMO1 and PML-nbs was analyzed by confocal microscopy. The scale bar represents 10 μm. (C) The position and amount of sumoylation dictates the degree of colocalization with SUMO-bodies. Experimental setup as in *A*.

FIG. S4. Exogenous Ubc9 does not enhance sumoylation of NFATc1, but exogenous PIAS1 supports recruitment to PML-nb. (A) Cotransfection of NFATc1/C and SUMO1 with Ubc9 in 293 T HEK cells, followed by IP and IB as before. (B) Cotransfection of NFATc1/A, NFATc1/C or the ΔSUMO-mutant and SUMO1 with PIAS1 in 293 T HEK cells, followed by IF as before. The amount of merging with SUMO1 or with PML-nbs was evaluated and given as a graph showing the percentage of cells merging with SUMO and PML, respectively (calculation from three independent experiments).

FIG. S5. Nuclear translocation and function of NFATc1-ER constructs are not regulated properly by TPA/Ionomycin (T/I) alone. (A) Human A3.01 cells, retrovirally infected with NFATc1/C-ER, selected by zeocin, were stimulated by T/I or Tm+T/I for 4 hrs. IF was performed with anti-ERα antibody and DAPI to detect exogenous NFATc1 and nuclei, respectively, followed by laser scanning confocal microscopy. (B) A3.01 cells expressing HA-(mock)-, NFATc1/A-, c1/C- and K349/702/914R-ER and selected by zeocin, were left unstimulated, stimulated with Tm, T/I or Tm+T/I for 16 hours. 5 μg RNA were subjected to RNase protection assay using the hCK1 template set. Out of that panel tested A3.01 cells only express IL2.

FIG. S1

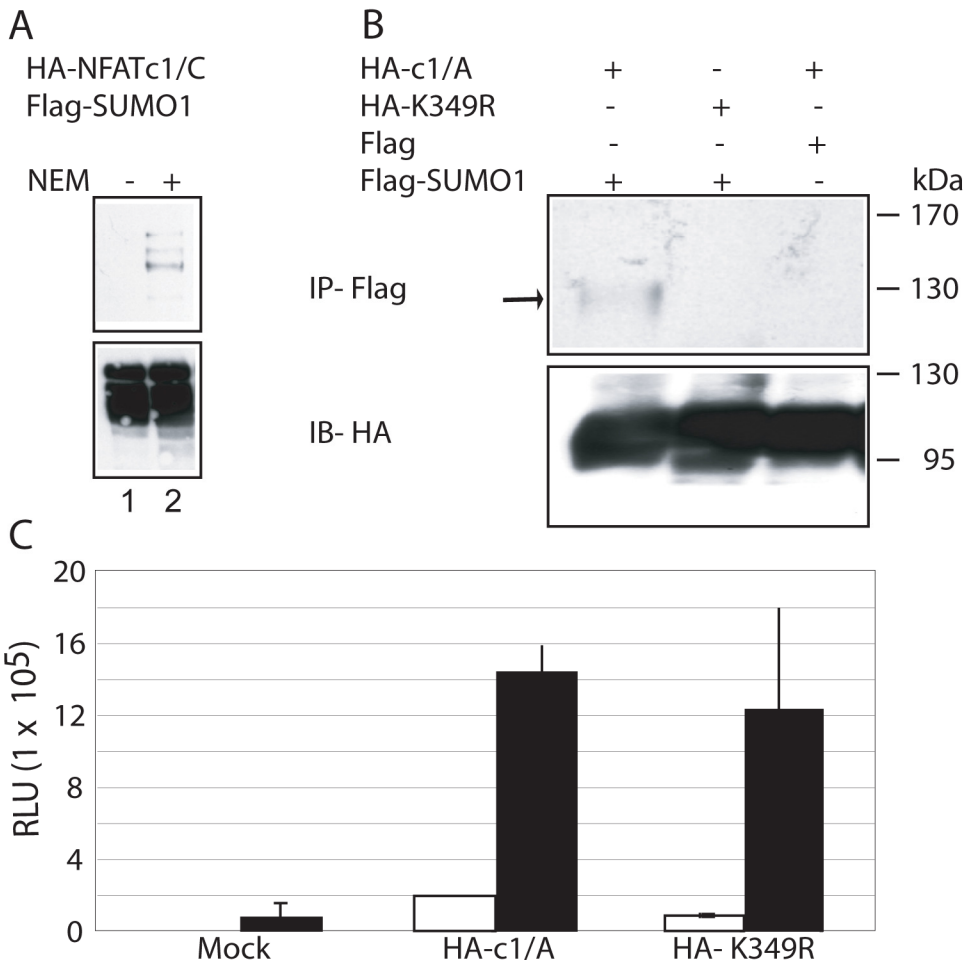

A
HA-NFATc1/C
Flag-SUMO1
NEM - +
1 2
B
HA-c1/A + - +
HA-K349R - + -
Flag - - +
Flag-SUMO1 + + -
kDa
170
130
IP- Flag
130
IB- HA
95
C
RLU (1 x 10^5)
20
16
12
8
4
0
Mock
HA-c1/A
HA- K349R

FIG. S2

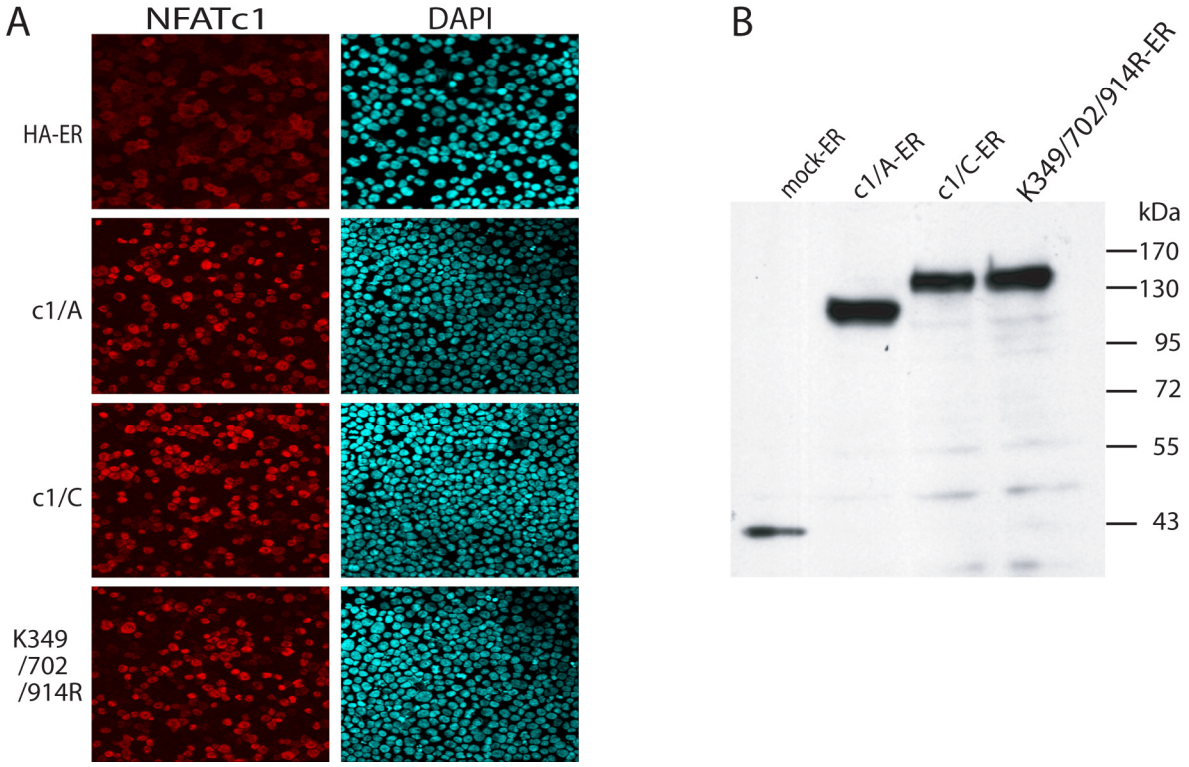

A
NFATc1
DAPI
HA-ER
c1/A
c1/C
K349
/702
/914R
B
mock-ER
c1/A-ER
c1/C-ER
K349/702/914R-ER
kDa
170
130
95
72
55
43

FIG. S3

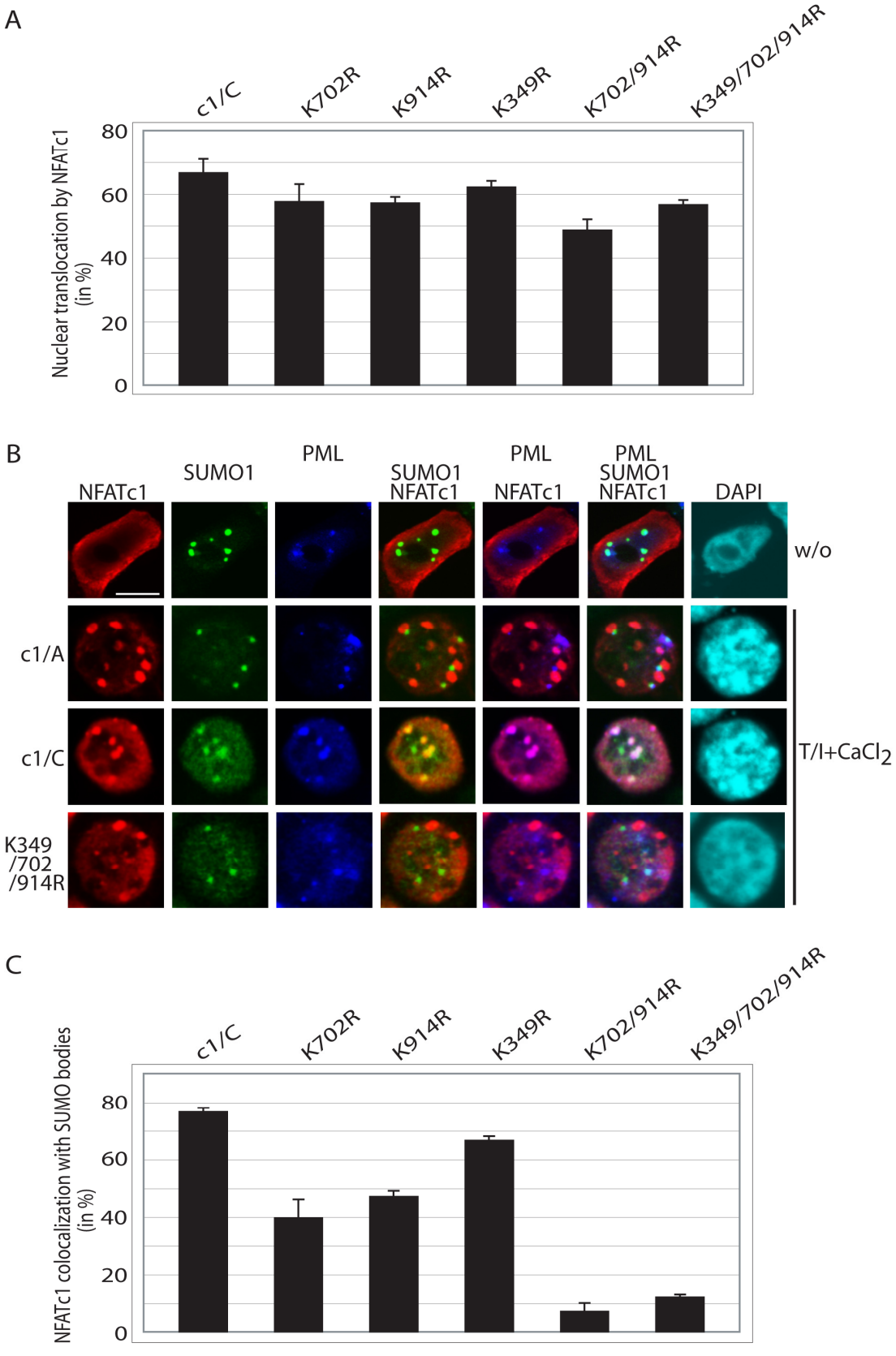

A
c1/C
K702R
K914R
K349R
K702/914R
K349/702/914R
Nuclear translocation by NFATc1 (in %)
80
60
40
20
0
B
NFATc1
SUMO1
PML
SUMO1 NFATc1
PML NFATc1
PML SUMO1 NFATc1
DAPI
w/o
c1/A
c1/C
K349 /702 /914R
T/I+CaCl2
C
c1/C
K702R
K914R
K349R
K702/914R
K349/702/914R
NFATc1 colocalization with SUMO bodies (in %)
80
60
40
20
0

FIG. S4

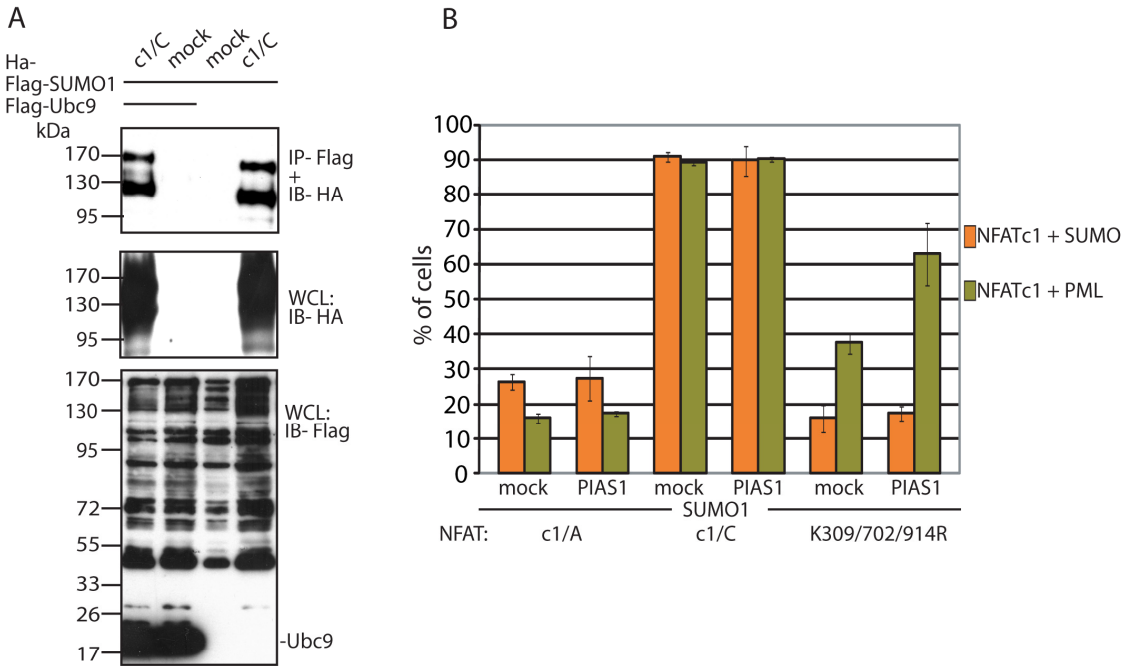
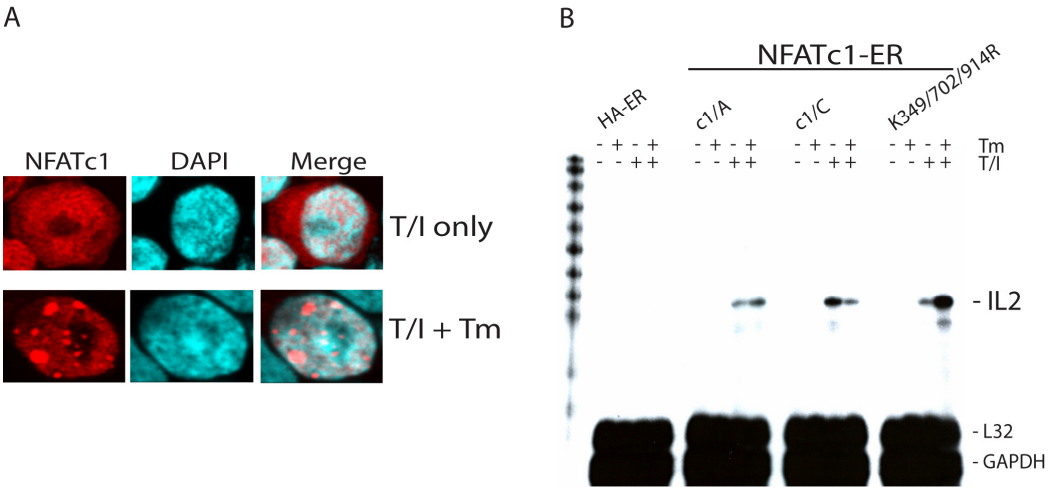

A
Ha-
Flag-SUMO1
Flag-Ubc9
c1/C
mock
mock
c1/C
kDa
170
130
95
IP- Flag
+
IB- HA
170
130
95
WCL:
IB- HA
170
130
95
72
55
33
26
17
WCL:
IB- Flag
-Ubc9
B
100
90
80
70
60
50
40
30
20
10
0
% of cells
NFATc1 + SUMO
NFATc1 + PML
mock
PIAS1
mock
PIAS1
mock
PIAS1
SUMO1
NFAT:
c1/A
c1/C
K309/702/914R

FIG. S5


A
NFATc1
DAPI
Merge
T/I only
T/I + Tm
B
NFATc1-ER
HA-ER
c1/A
c1/C
K349/702/914R
- + - +
- - + +
Tm
T/I
- IL2
- L32
- GAPDH