Supplements to

Targeted delivery of an ADP-ribosylating bacterial toxin into cancer cells

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Fig. S1: Cell rounding is induced by PA plus LFN-C3 but not by PA plus LFN-C3(E943A). (PA(F427A), Φclamp) combined with LFN-C3 does not induce cell rounding. OE21 cells were incubated with the following proteins as indicated: PA (10 nM), mPA-EGF (10nM), PA(F427A) (10 nM), LFN-C3 (8 nM), LFN-C3(E943A) (8nM). Photos were taken after 24 h of incubation. A typical result of 2 independent experiments is shown.

Fig. S2: Cell specific cell rounding induced by LFN-C3 mediated by different PA transporters: OE21 and OE33 cells were incubated with the following proteins as indicated: PA (10 nM), mPA-EGF (10 nM), mPA-ZHER2 (10 nM), LFN-C3 (8 nM). Photos were taken after 24 h of incubation. A typical result of 3 independent experiments is shown.

Fig. S3: Uncontrolled actin clustering in esophageal cancer cells induced by LFN-C3 mediated by different PA transporters: OE21 (a) cells and OE33 (b) cells were treated with PA (10 nM), mPA-EGF (10 nM), mPA-ZHER2 (10 nM), LFN-C3 (8 nM) as indicated. After 4 h cells were fixed and stained with Rhodamine phalloidin. The experiment was performed three times with similar results.

Fig. S4: Representative Western blots for each experiment of Fig.6: Post-ADP-ribosylation of cell-lysates (a, b): OE21 cells and OE33 cells were treated with PA (10 nM), mPA-EGF (10 nM), mPA-ZHER2 (10 nM), LFN-C3 (8 nM) for 4 h as indicated, washed and lysed. Lysates were than incubated with LFN-C3 in the presence of radio-labeled NAD^+. Proteins of the lysates were separated by SDS-PAGE. Following drying of the gels labeled protein bands were detected by phosphorimaging and quantified. Labeled actin of an untreated control was set as 100%. Data give the median of three experiments plus standard deviation. Significance was analyzed

using GraphPad Prism 5 (*** : $p < 0,001$, ** : $p < 0,01$, * : $p < 0,05$). Post-ADP-ribosylation of cell-lysates following intoxication as dose response analysis (c): OE21 or OE33 cells were treated with increasing concentrations of mPA-EGF (gray, 0.01 nM to 10 nM) or mPA-ZHER2 (black, 0.01 nM to 10 nM) and a fixed concentration of LFN-C3 (8 nM) for 4 h, as indicated. Lysates were prepared and treated as in a.

Fig. S5: Caspase activity of toxin-treated cells: OE33 cells were treated with PA (10 nM), mPA-EGF (10 nM) or mPA-ZHER2 (10 nM), LFN-C3 (8 nM) or Staurosporine (10 µM) for 24 h, as indicated. Lysates were prepared and tested for caspase 3/7 activity.

Fig. S6: PARP-cleavage detected in OE21 and OE33 cells: Fig. S4: PARP cleavage detected in OE21 and OE33 cells: OE21 and OE33 cells were treated with PA (10 nM), mPA-EGF (10 nM), mPA-ZHER2 (10 nM), LFN-C3 (8 nM) for 24h as indicated. PARP cleavage was then detected by Western blot. The untreated control was set to 1 and PARP cleavage represents X fold of control. The Data give the median of three experiments plus standard deviation. Significance was (*** : $p < 0,001$, ** : $p < 0,01$, * : $p < 0,05$).

Fig. S7: Cell viability of OE21 cells: OE21 cells were treated with PA (10 nM), mPA-EGF (10 nM) or mPA-ZHER2 (10 nM), LFN-C3 (8 nM) or Staurosporine (10 µM) for 24 h, as indicated. Cell viability was measured with CellTiter-Blue kit (Promega).

Fig S1

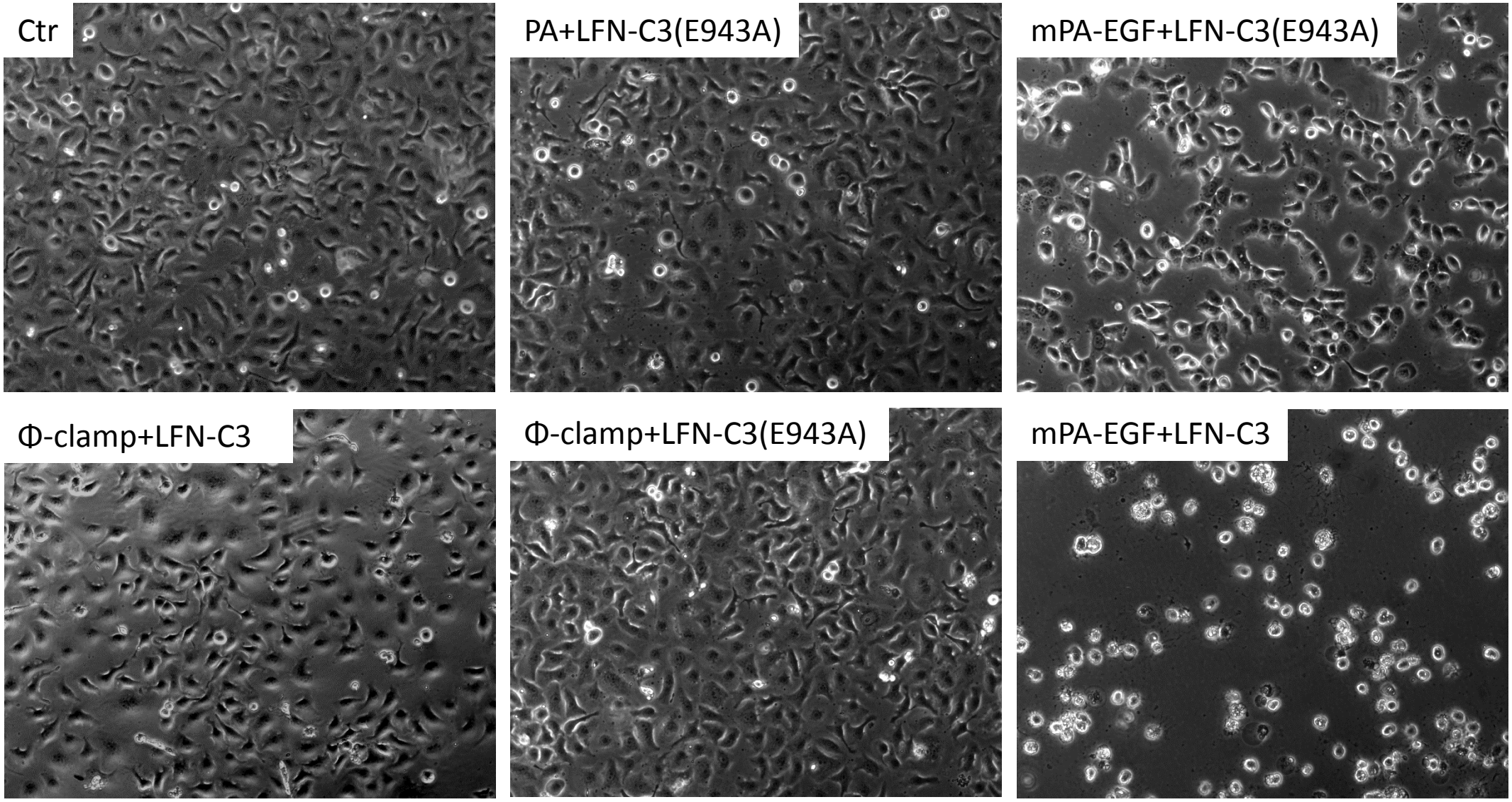

Ctr
PA+LFN-C3(E943A)
mPA-EGF+LFN-C3(E943A)
Φ-clamp+LFN-C3
Φ-clamp+LFN-C3(E943A)
mPA-EGF+LFN-C3

Fig S2

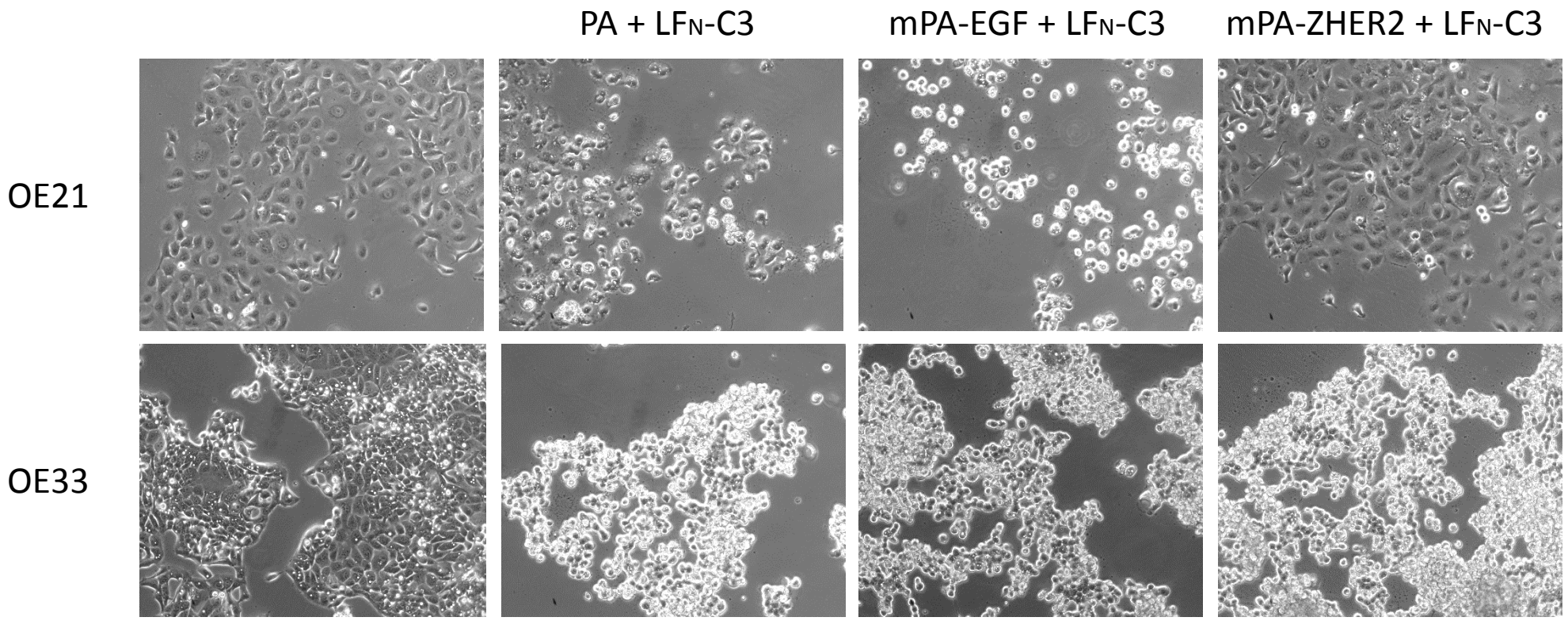


Fig.S3

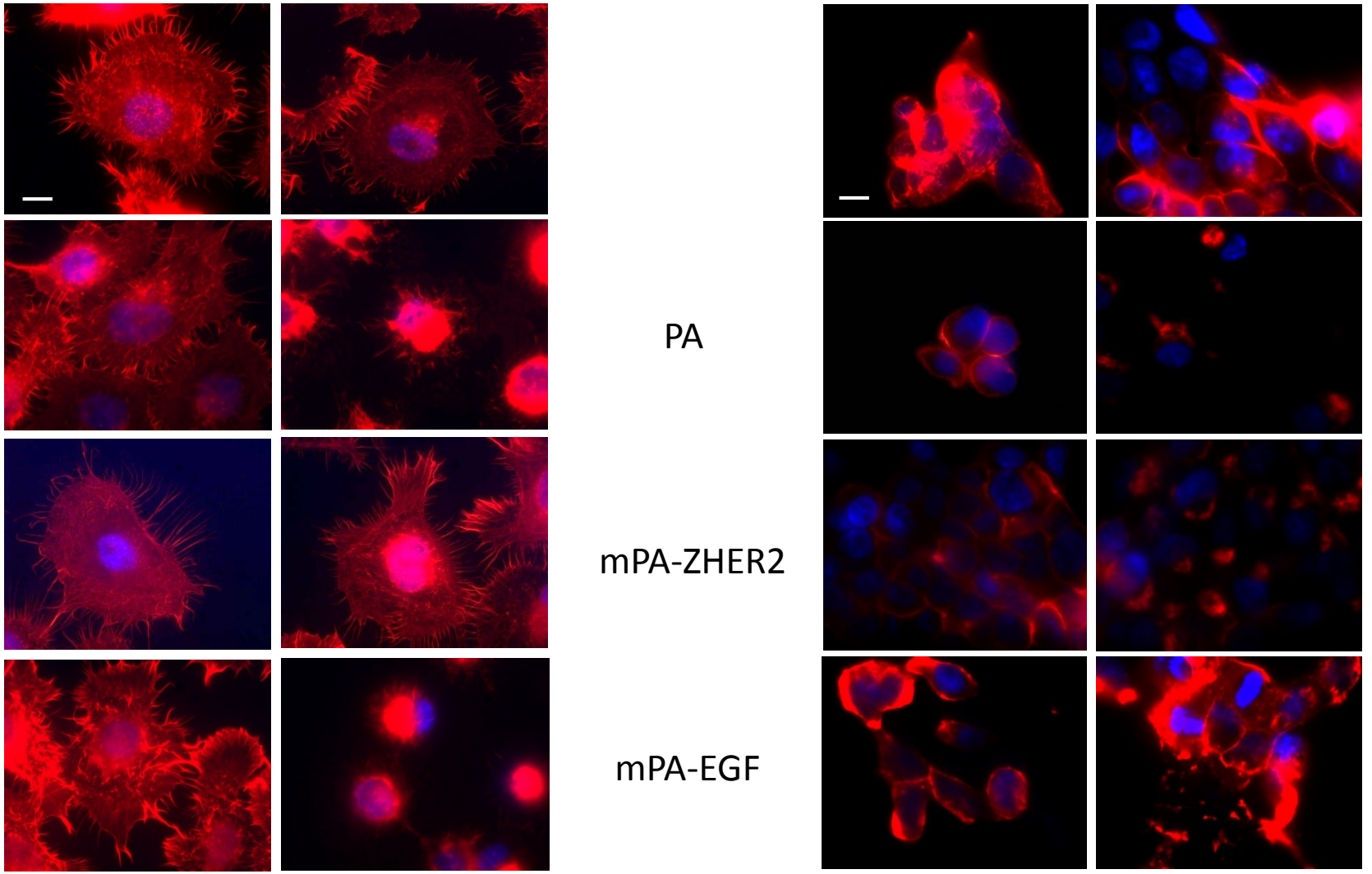
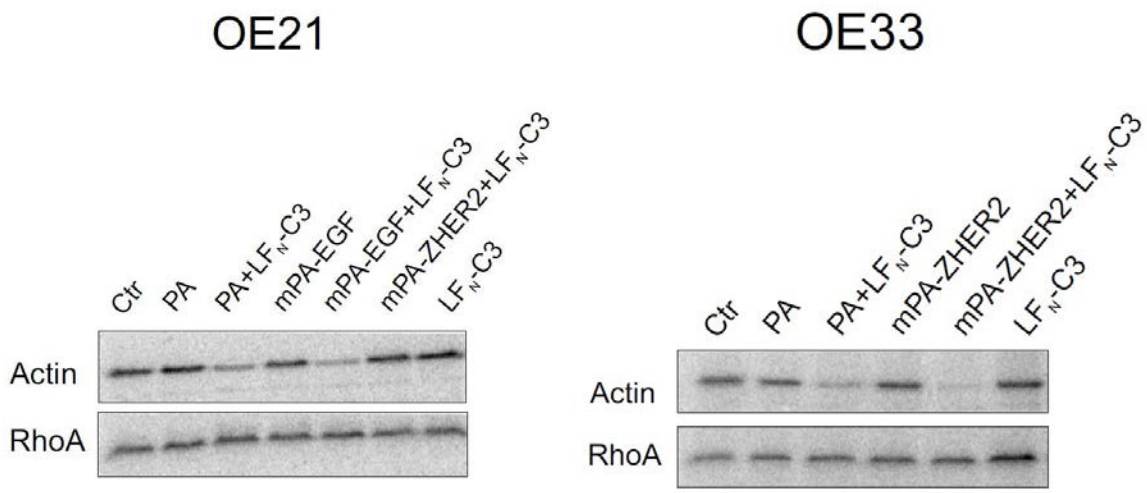
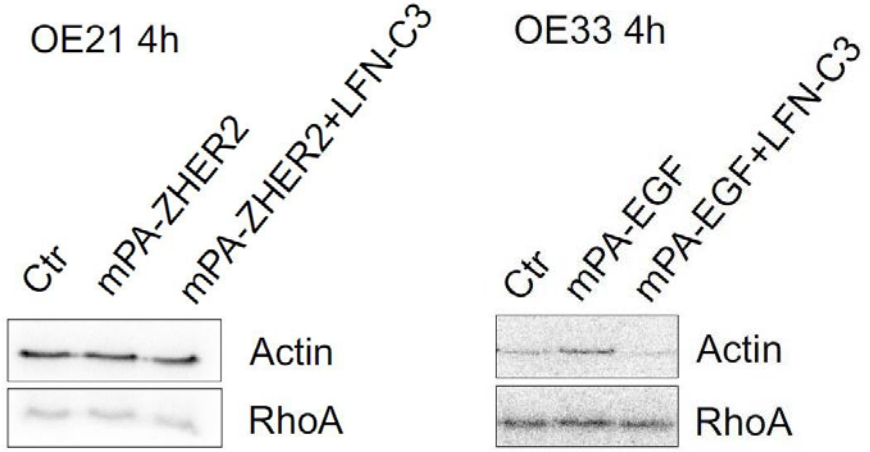


Fig S4

a


OE21
Ctr
PA
PA+LF$_N$-C3
mPA-EGF
mPA-EGF+LF$_N$-C3
mPA-ZHER2+LF$_N$-C3
LF$_N$-C3
Actin
RhoA
OE33
Ctr
PA
PA+LF$_N$-C3
mPA-ZHER2
mPA-ZHER2+LF$_N$-C3
LF$_N$-C3
Actin
RhoA

b


OE21 4h
Ctr
mPA-ZHER2
mPA-ZHER2+LFN-C3
Actin
RhoA
OE33 4h
Ctr
mPA-EGF
mPA-EGF+LFN-C3
Actin
RhoA

c

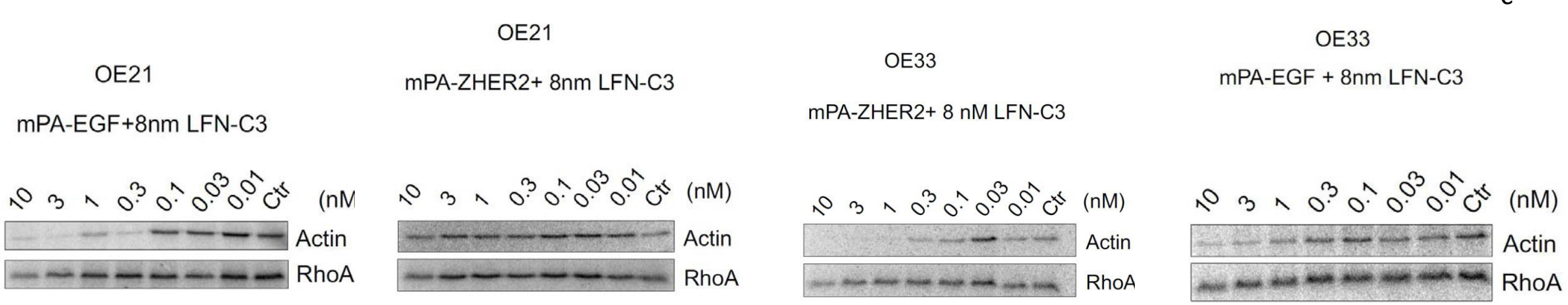
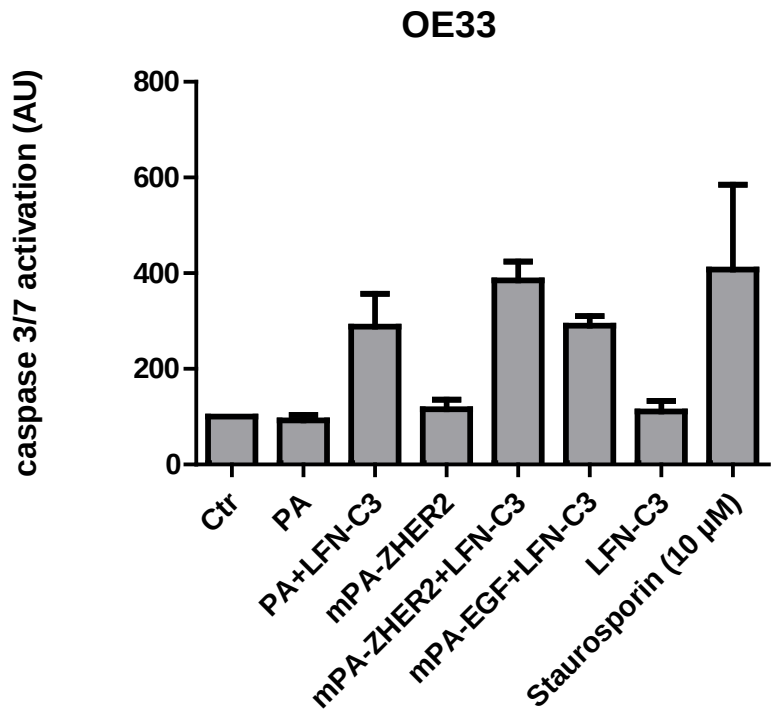
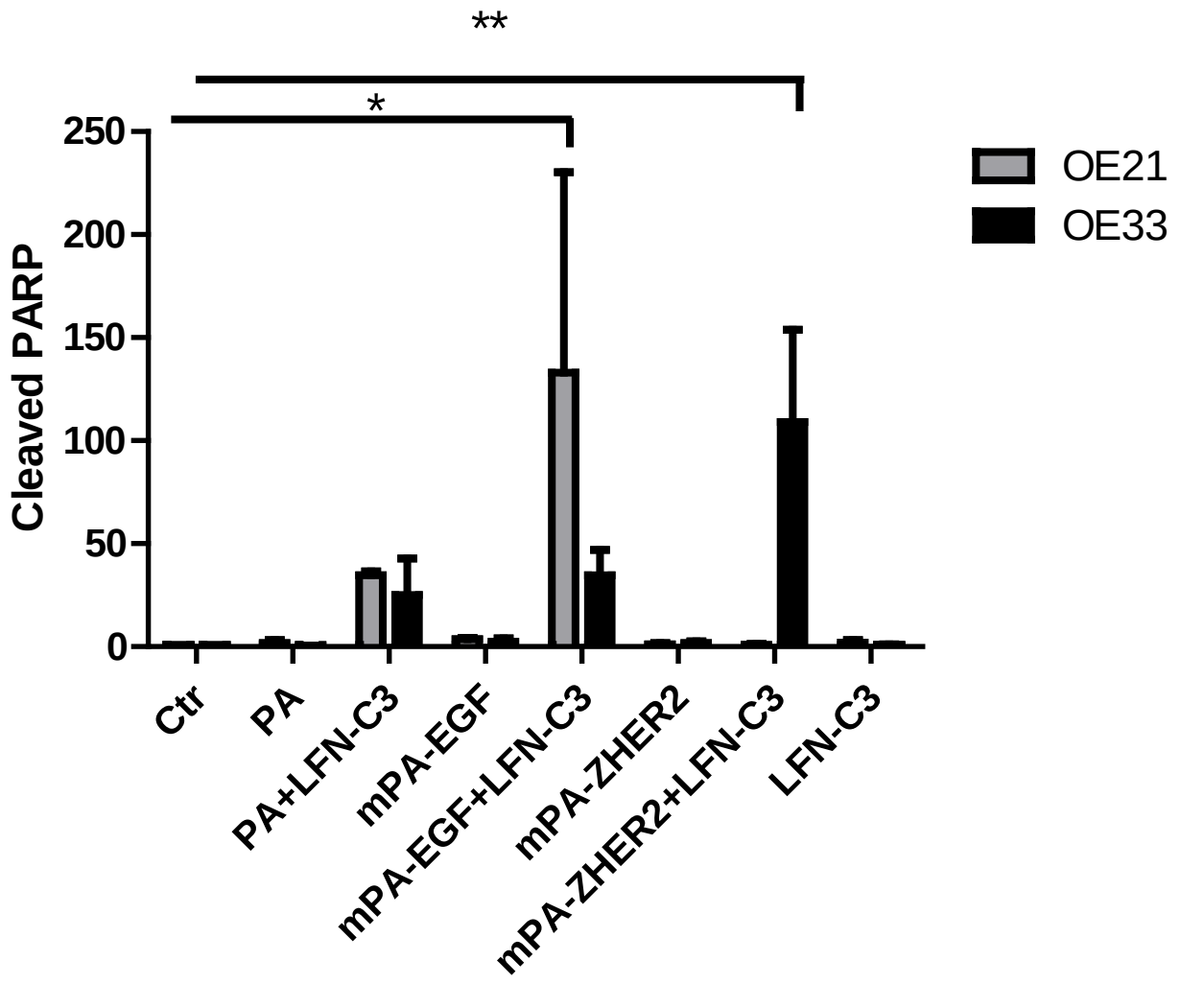
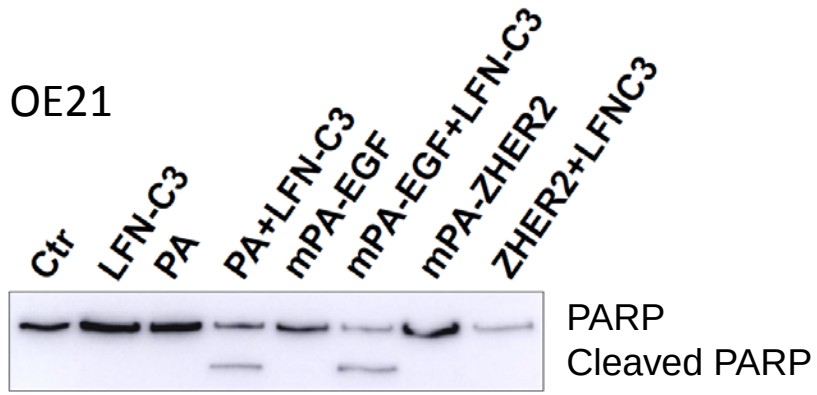

OE21
mPA-EGF+8nm LFN-C3
10
3
1
0.3
0.1
0.03
0.01
Ctr
(nM)
Actin
RhoA
OE21
mPA-ZHER2+ 8nm LFN-C3
10
3
1
0.3
0.1
0.03
0.01
Ctr
(nM)
Actin
RhoA
OE33
mPA-ZHER2+ 8 nM LFN-C3
10
3
1
0.3
0.1
0.03
0.01
Ctr
(nM)
Actin
RhoA
OE33
mPA-EGF + 8nm LFN-C3
10
3
1
0.3
0.1
0.03
0.01
Ctr
(nM)
Actin
RhoA

Fig. S5


OE33
caspase 3/7 activation (AU)
800
600
400
200
0
Ctr
PA
PA+LFN-C3
mPA-ZHER2
mPA-ZHER2+LFN-C3
mPA-EGF+LFN-C3
LFN-C3
Staurosporin (10 µM)


**
*
Cleaved PARP
250
200
150
100
50
0
Ctr
PA
PA+LFN-C3
mPA-EGF
mPA-EGF+LFN-C3
mPA-ZHER2
mPA-ZHER2+LFN-C3
LFN-C3
OE21
OE33


OE21
Ctr
LFN-C3
PA
PA+LFN-C3
mPA-EGF
mPA-EGF+LFN-C3
mPA-ZHER2
ZHER2+LFNC3
PARP
Cleaved PARP

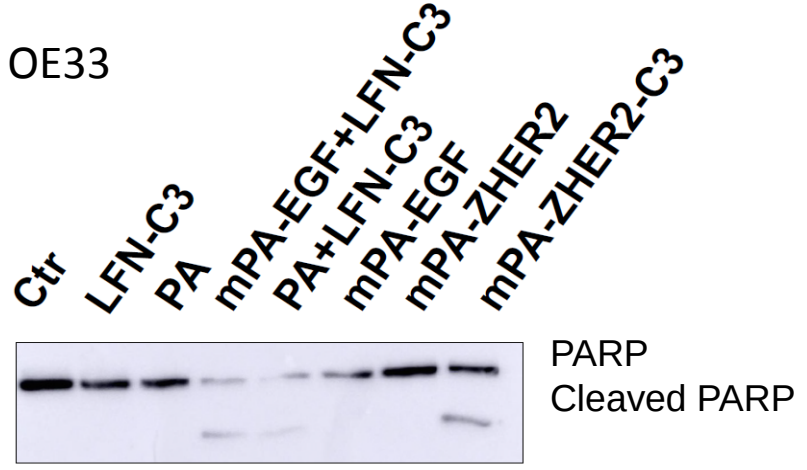
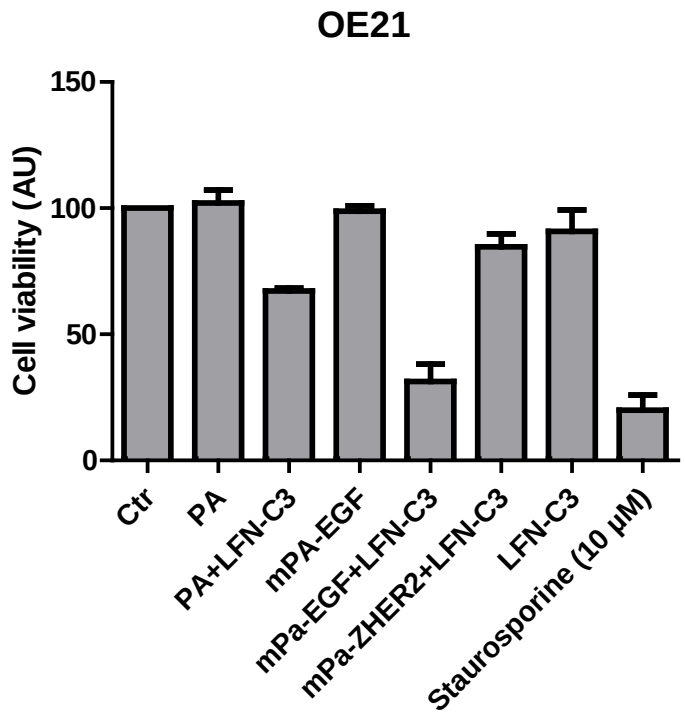

OE33
Ctr
LFN-C3
PA
mPA-EGF+LFN-C3
PA+LFN-C3
mPA-EGF
mPA-ZHER2
mPA-ZHER2-C3
PARP
Cleaved PARP

Fig. S7


OE21
Cell viability (AU)
150
100
50
0
Ctr
PA
PA+LFN-C3
mPA-EGF
mPa-EGF+LFN-C3
mPa-ZHER2+LFN-C3
LFN-C3
Staurosporine (10 µM)