Supplementary figure legends

Figure S1: Chemical structure of the identified enhancers. Chemical structures of compounds #1 to #24 (**a**), #25 to #48 (**b**) and #49 to #50 (**c**).

Figure S2: Determination of cytotoxicity based on cell number. Cell number was determined during the primary screen done in HeLa cells for LNPs (**a**) or Chol-siRNAs (**b**). Cell number was determined during the screen performed in human primary fibroblasts for LNPs (**c**) or Chol-siRNAs (**d**), and in mouse primary hepatocytes for LNPs (**e**) or Chol-siRNAs (**f**). Note that the compounds order in the present graphs are similar to those of the figures 2 and 3.

Figure S3: PD739 and PD740 are transfection reagent. (**a**) Z-score of GFP intensity versus DMSO treated condition. (**b**) Number of cells normalized to DMSO treated condition.

Figure S4: Cytotoxicity determination under compounds dose response. The number of cells from the experiments shown in the figure 2d top panels (increasing doses of compounds) was determined for four enhancers of LNPs (**a**) and of Chol-siRNAs (**b**).

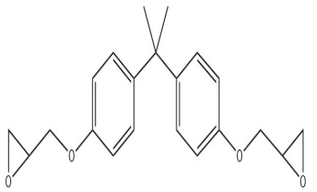
Figure S5: BADGE and CPW1-J18 are both improving silencing by two different mechanisms. (**a**) GFP down-regulation 72 h after transfection with LNP-siRNA (40 nM, top panels) and uptake of LNP-siRNA-alexa647 (40 nM, 5 h incubation, bottom panels) after treated with DMSO (right panels), BADGE (middle panels) or CPW1-J18 (right panels) in HeLa and HeLa GFP cells. (**b**,**c**) Quantification of the uptake of LNP-siRNA-alexa647 (**c**) and of GFP down-regulation (**b**) in HeLa and HeLa GFP cells. Mean ± s.e.m. n=3 (**Pvalue<0.01, ***Pvalue<0.001).

Figure S6: BADGE treatment does not alter the mean number of siRNA-gold particles per LNPs. Quantitative analysis of the number of siRNA-gold per LNPs after treatment with DMSO or BADGE. Mean ± s.e.m. n=3 (Pvalue=0.88).

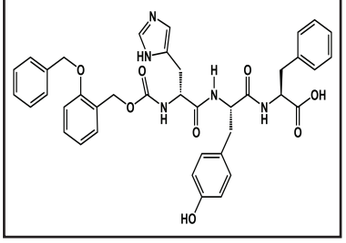
Figure S7: Profile parameters for QMPIA on chemicals impact on EGF and transferrin endocytosis. Description of the different parameters used to drawn the endocytic profiles and exemplified here by hydroxychloroquine.

Figure S8: Proposed lipid crosslinking mediated by reaction of BADGE with lipid head-groups. Description of the proposed reaction between BADGE and the lipid head-groups.

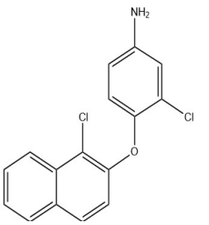
 # 1: BADGE (C21H24O4)
PubChem ID: 2286



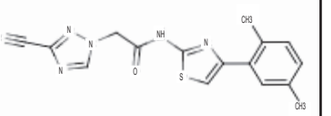
 # 2: CPW1 J18
(C39H39N5O8)



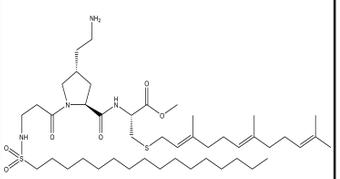
 # 3: CBN47 D17
(C16H11Cl2NO)



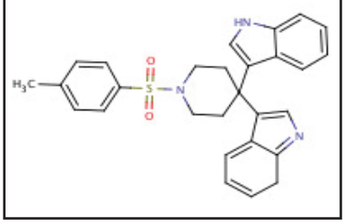
 # 4: ADD31 O11
(C16H14N6OS)



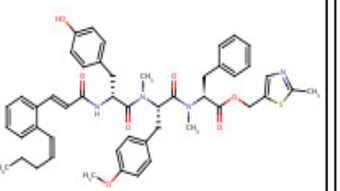
 # 5: PD 740
(C45H82N4O6S2)



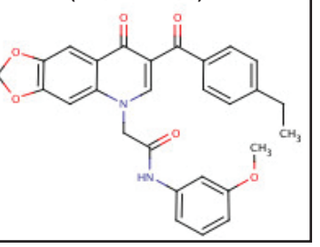
 # 6: CBN40 I13
(C28H27N3O2S)



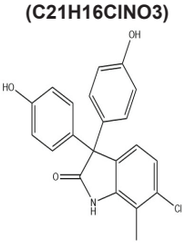
 # 7: CPW1 F10
(C49H54N4O7S)



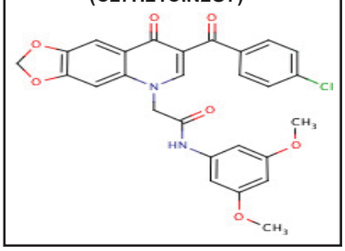
 # 8: CBN40 D12
(C28H24N2O6)



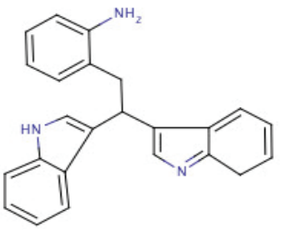
 # 9: ADD25 H20
(C21H16ClNO3)



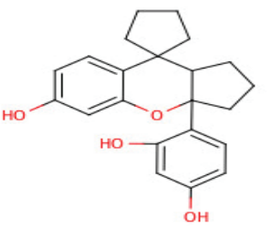
 # 10: CBN40 J8
(C27H21ClN2O7)



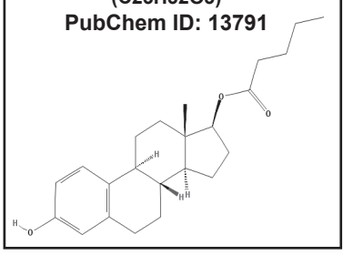
 # 11: CBN7 F7
(C24H21N3)



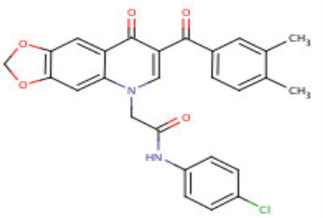
 # 12: CBN2 B19
(C22H24O4)



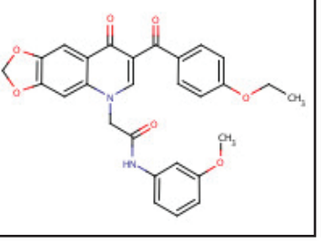
 # 13: Estradiol Valerate
(C23H32O3)
PubChem ID: 13791



 # 14: CBN40 H10
(C27H21ClN2O5)



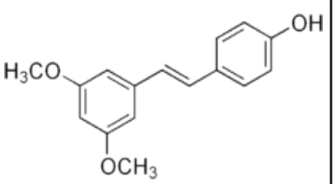
 # 15: CBN40 B8
(C28H24N2O7)



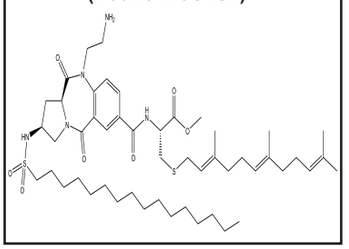
 # 16: JAD#7

Not Provided

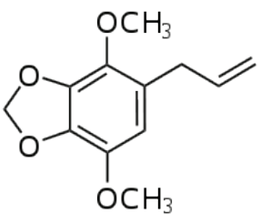
 # 17: Pterostilbene
(C16H16O3)
PubChem ID: 5281727



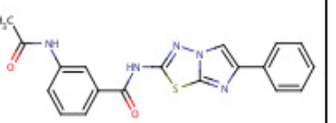
 # 18: PD739
(C50H81N5O7S2)



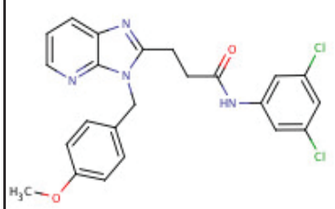
 # 19: Apiole (C12H14O4)
PubChem ID: 10659



 # 20: ADD29 F15
(C19H15N5O2S)



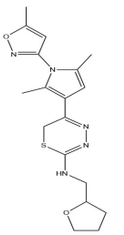
 # 21: CBN40 K7
(C23H20Cl2N4O2)



 # 22: JAD#75

Not Provided

 # 23: ADD42 P18
(C18H23N5O2S)



 # 24: ADD41 D14
(C21H21N3O)

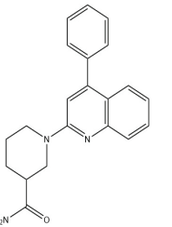
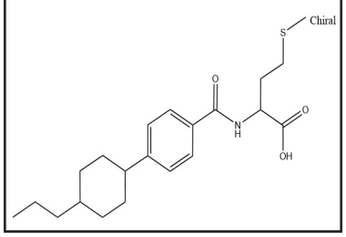


Figure S1a

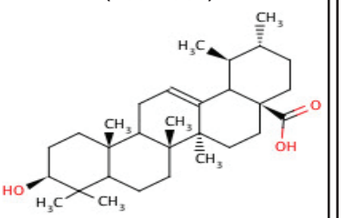
 # 25: CBN35 C21
(C21H31NO3S)



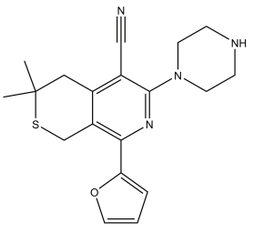
 # 26: JAD001 K11

Not Provided

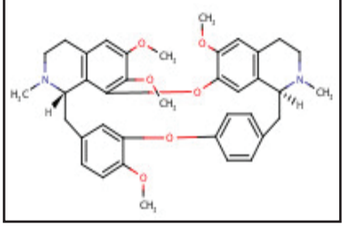
 # 27: Ursolic acid
(C30H48O3)



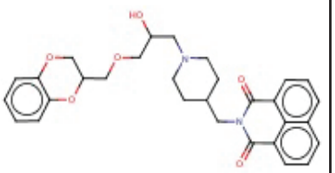
 # 28: ADD51 E07
(C19H22N4OS)



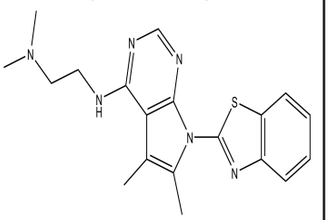
 # 29: Tetrandrine
(C38H42N2O6)



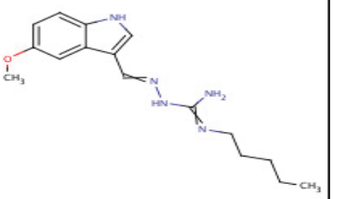
 # 30: CBN052 E20
(C30H32N2O6)



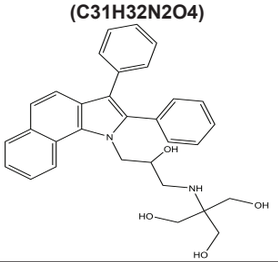
 # 31: ADD054 O05
(C20H23N3O)



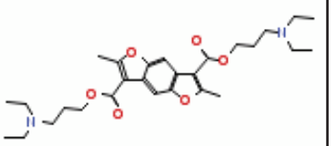
 # 32: Tegaserod maleate
(C16H23N5O)



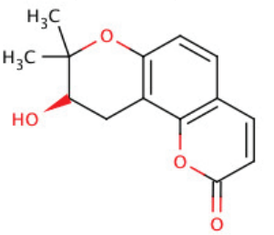
 # 33: CBN024 P13
(C31H32N2O4)



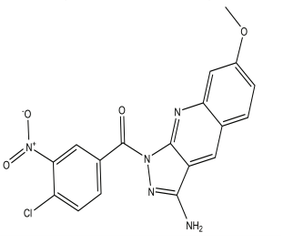
 # 34: CBN026 N14
(C28H40N2O6)



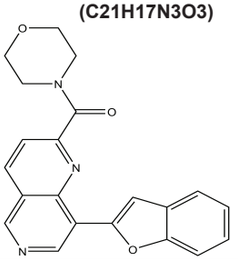
 # 35: Lomatin
(C14H14O4)



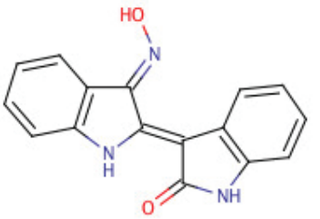
 # 36: CBN 039 P20
(C18H12ClN5O4)



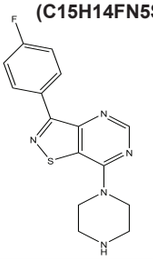
 # 37: ADD004 P08
(C21H17N3O3)



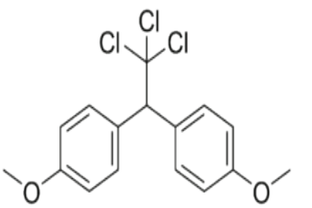
 # 38: Indirubin
(C16H11N3O2)



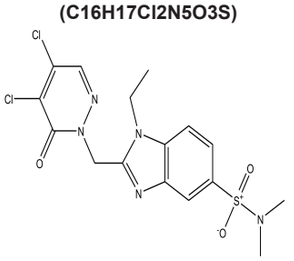
 # 39: ADD017 P20
(C15H14FN5S)



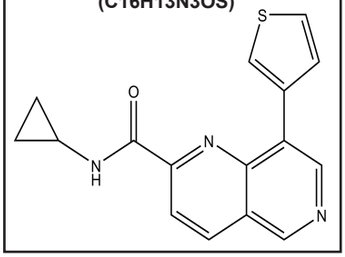
 # 40: Methoxychlor
(C16H15Cl3O2)



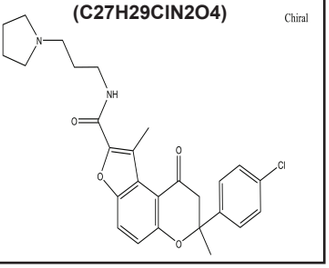
 # 41: ADD044 M12
(C16H17Cl2N5O3S)



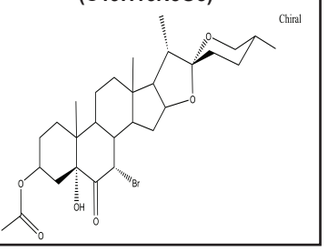
 # 42: ADD004 N08
(C16H13N3OS)



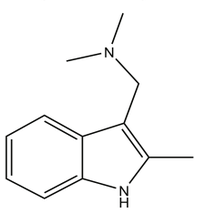
 # 43: CBN059 A12
(C27H29ClN2O4)



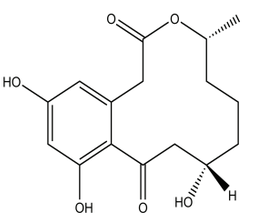
 # 44: CBN007 J18
(C19H16N3O3)



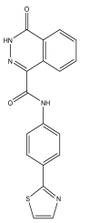
 # 45: Methyl gramine
(C12H16N2)



 # 46: CPW097 A20
(C16H20O6)



 # 47: ADD 029 G19
(C18H12N4O2S)



 # 48: CBN008 N05
(C18H14N4O5)

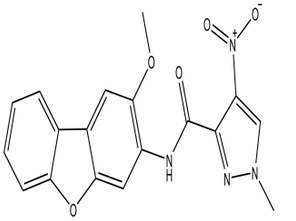


Figure S1b

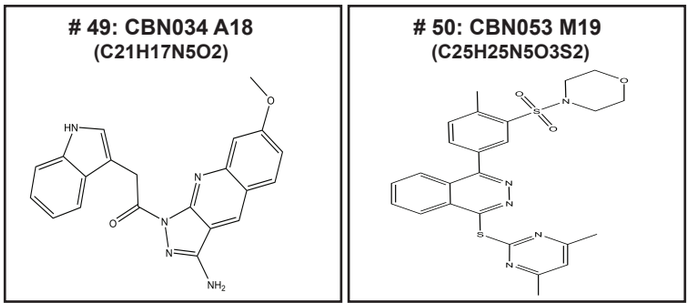

49: CBN034 A18
(C21H17N5O2)
HN
O
N
O
N
N
NH2
50: CBN053 M19
(C25H25N5O3S2)
O
N
S
O
O
N
N
S
N
N

Figure S1c

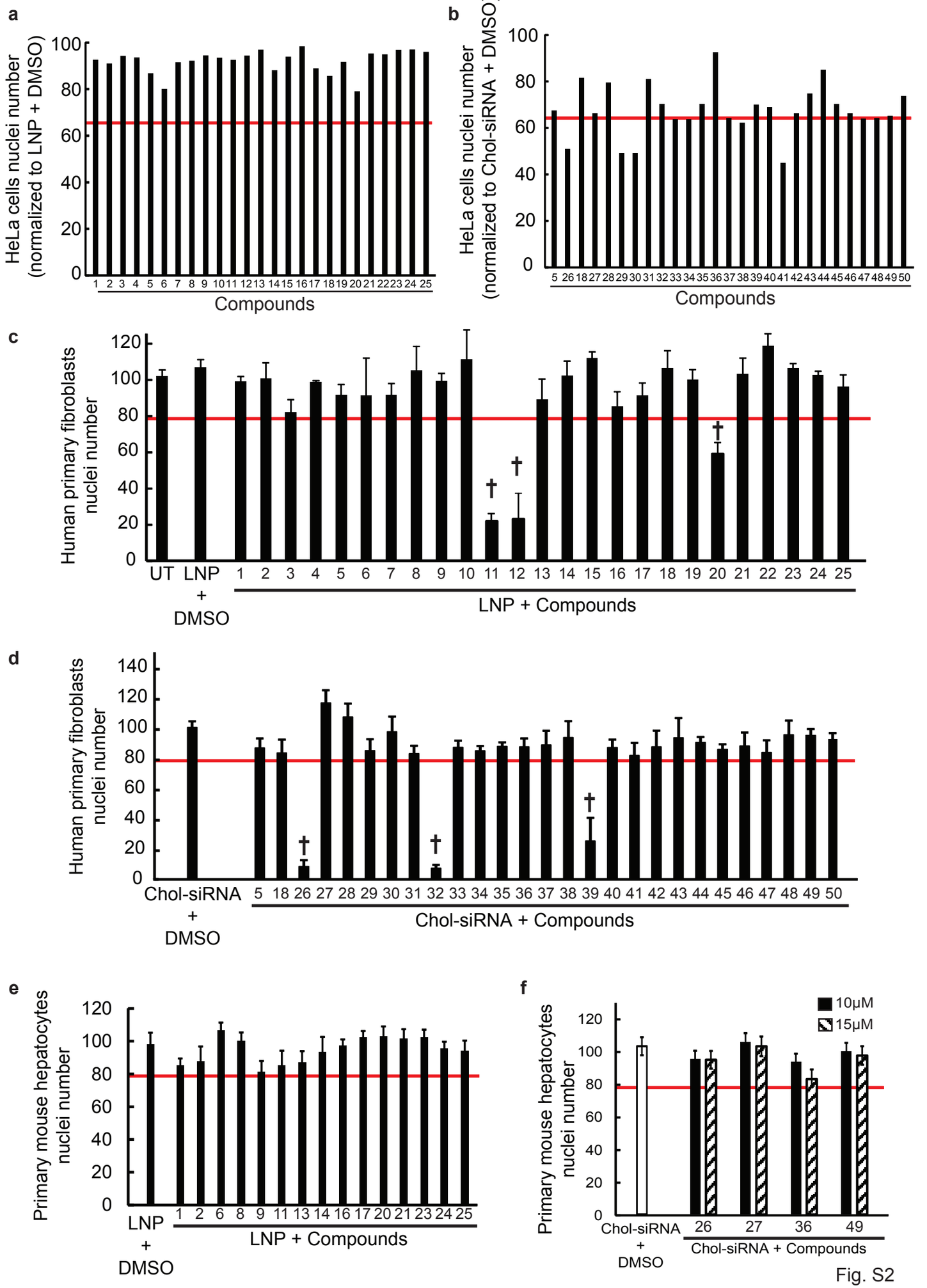

a
HeLa cells nuclei number (normalized to LNP + DMSO)
Compounds
b
HeLa cells nuclei number (normalized to Chol-siRNA + DMSO)
Compounds
c
Human primary fibroblasts nuclei number
UT
LNP + DMSO
LNP + Compounds
d
Human primary fibroblasts nuclei number
Chol-siRNA + DMSO
Chol-siRNA + Compounds
e
Primary mouse hepatocytes nuclei number
LNP + DMSO
LNP + Compounds
f
Primary mouse hepatocytes nuclei number
10μM
15μM
Chol-siRNA + DMSO
Chol-siRNA + Compounds

Fig. S2

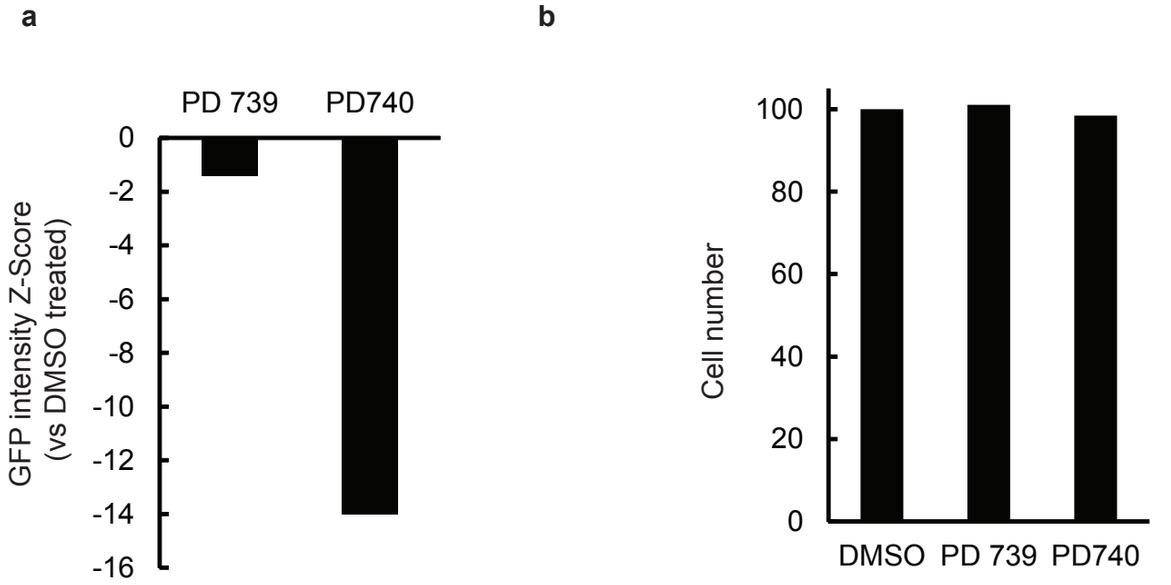
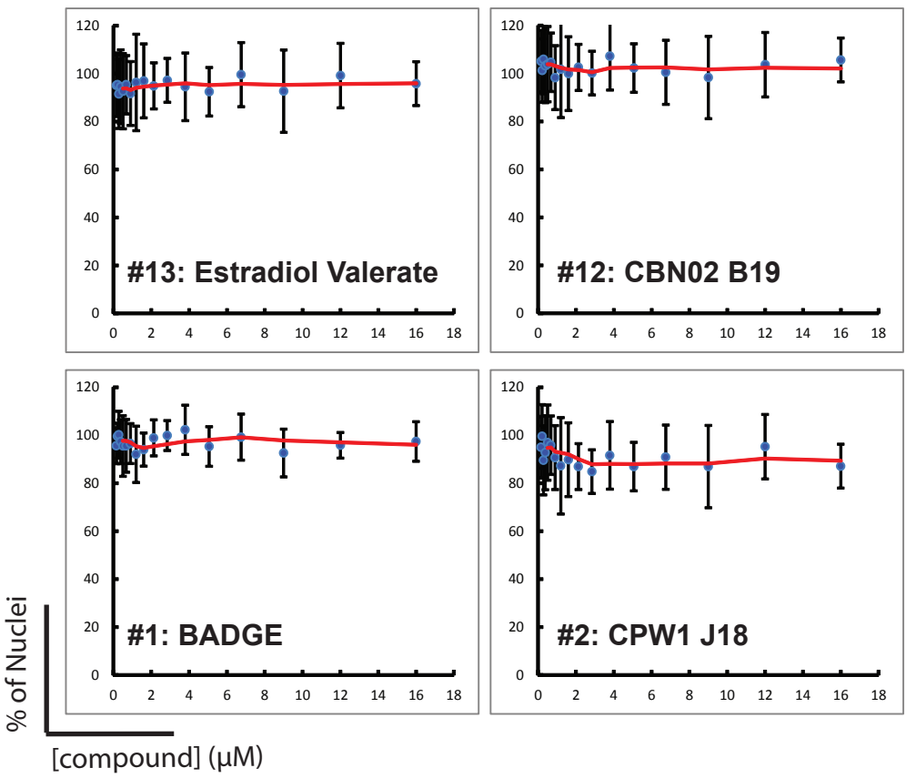

a
PD 739
PD740
0
-2
-4
-6
-8
-10
-12
-14
-16
GFP intensity Z-Score
(vs DMSO treated)
b
100
80
60
40
20
0
Cell number
DMSO
PD 739
PD740

Fig. S3

a


#13: Estradiol Valerate
#12: CBN02 B19
#1: BADGE
#2: CPW1 J18
% of Nuclei
[compound] (μM)

b

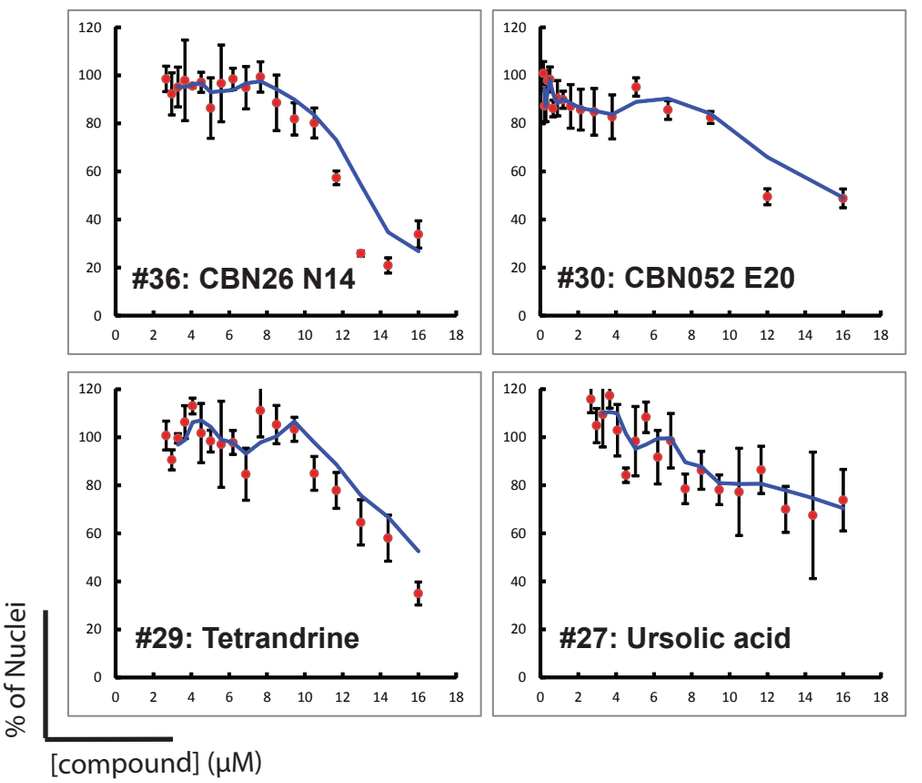

#36: CBN26 N14
#30: CBN052 E20
#29: Tetrandrine
#27: Ursolic acid
% of Nuclei
[compound] (μM)

Fig. S4

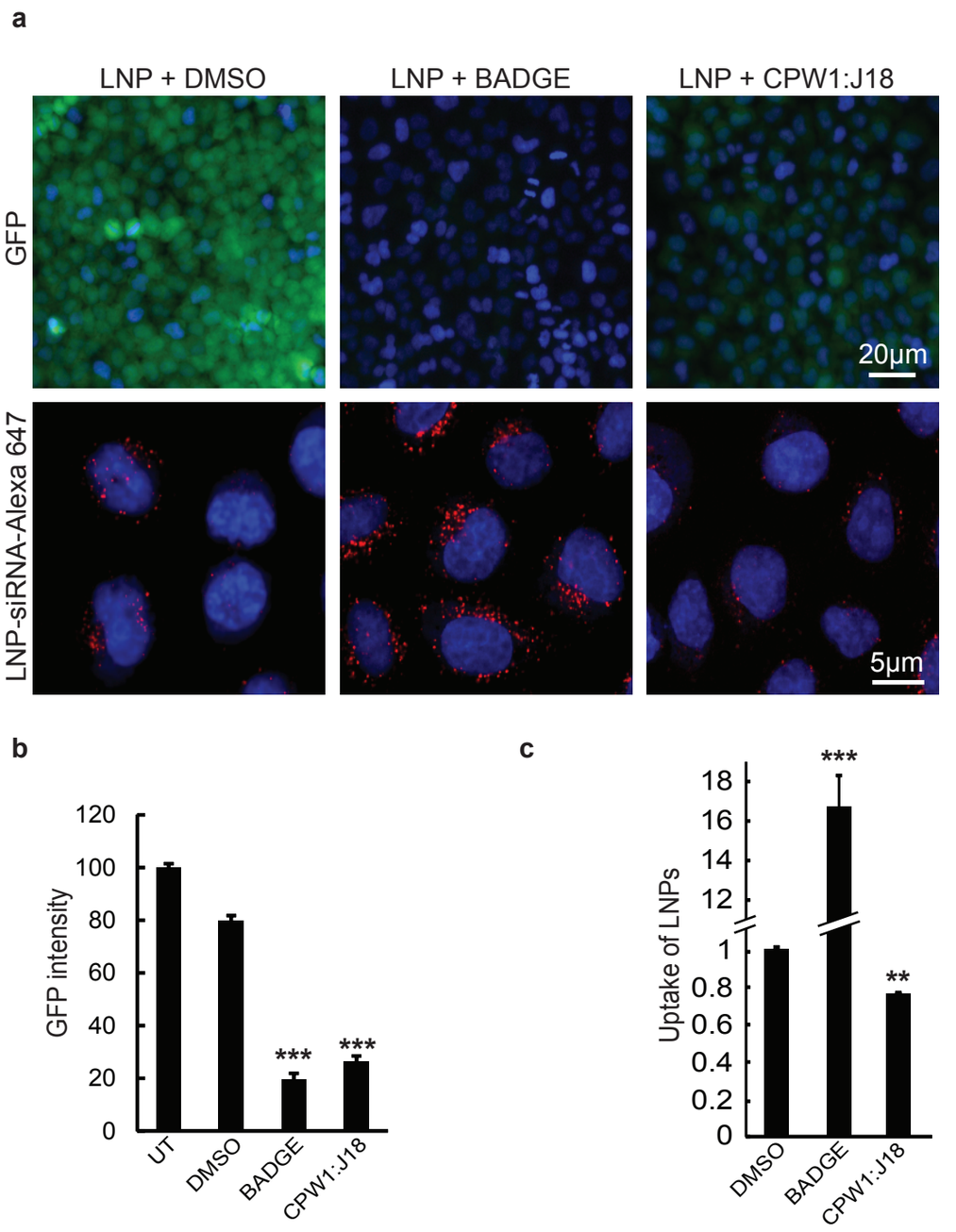

a
LNP + DMSO
LNP + BADGE
LNP + CPW1:J18
GFP
LNP-siRNA-Alexa 647
20μm
5μm
b
GFP intensity
120
100
80
60
40
20
0
***
***
UT
DMSO
BADGE
CPW1:J18
c
Uptake of LNPs
18
16
14
12
1
0.8
0.6
0.4
0.2
0
***
**
DMSO
BADGE
CPW1:J18

Figure S5

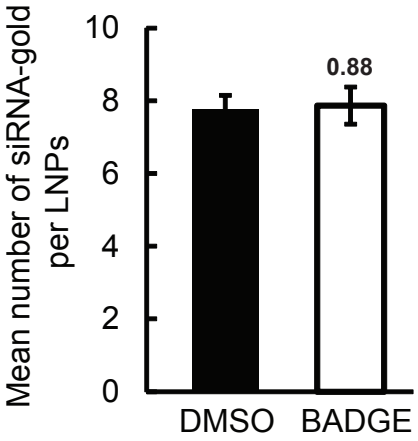


Figure S6

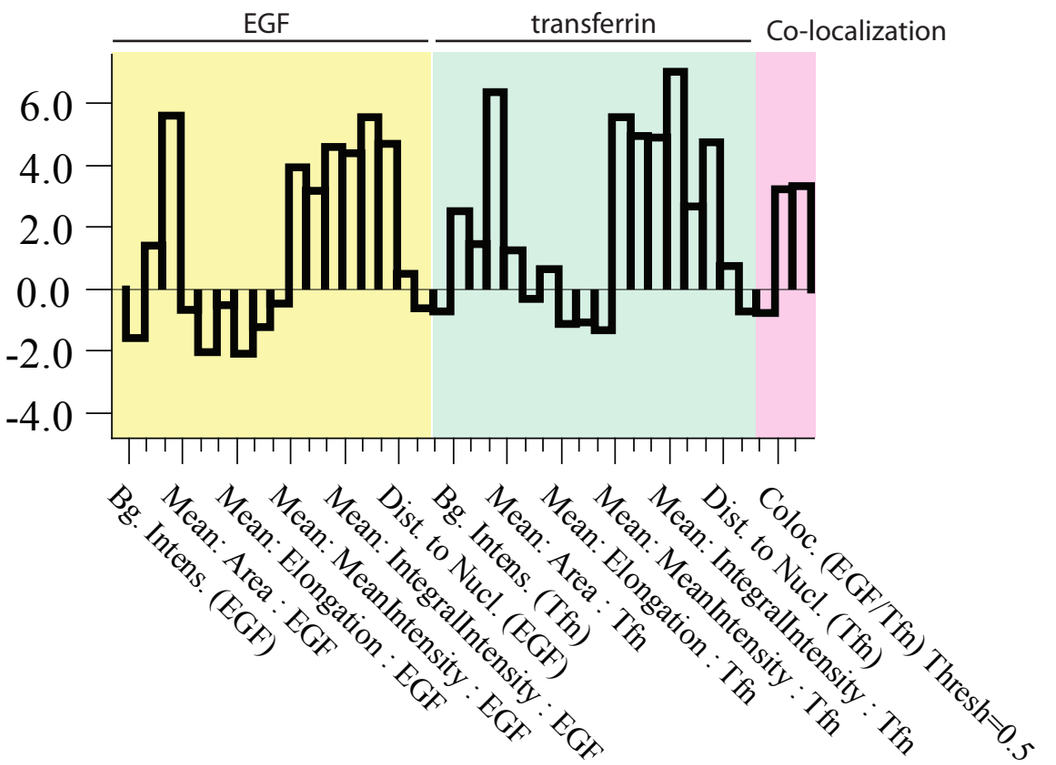

EGF
transferrin
Co-localization
6.0
4.0
2.0
0.0
-2.0
-4.0
Bg. Intens. (EGF)
Mean: Area : EGF
Mean: Elongation : EGF
Mean: MeanIntensity : EGF
Mean: IntegralIntensity : EGF
Dist. to Nucl. (EGF)
Bg. Intens. (Tfn)
Mean: Area : Tfn
Mean: Elongation : Tfn
Mean: MeanIntensity : Tfn
Mean: IntegralIntensity : Tfn
Dist. to Nucl. (Tfn)
Coloc. (EGF/Tfn) Thresh=0.5

Figure S7

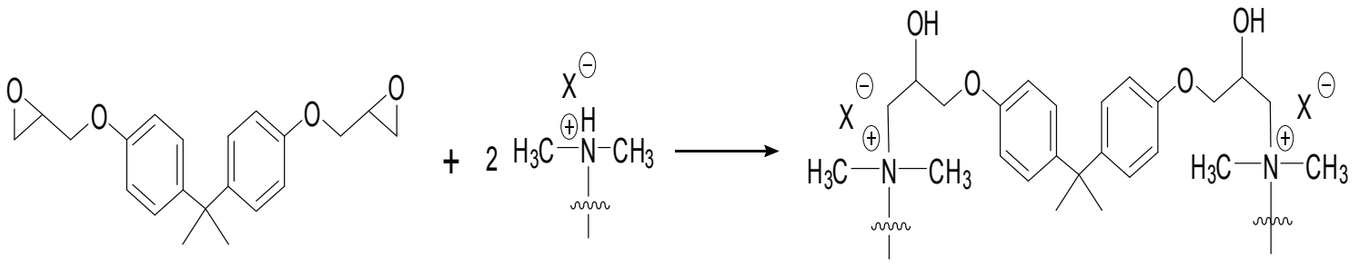


Figure S8