Expanded View Figures

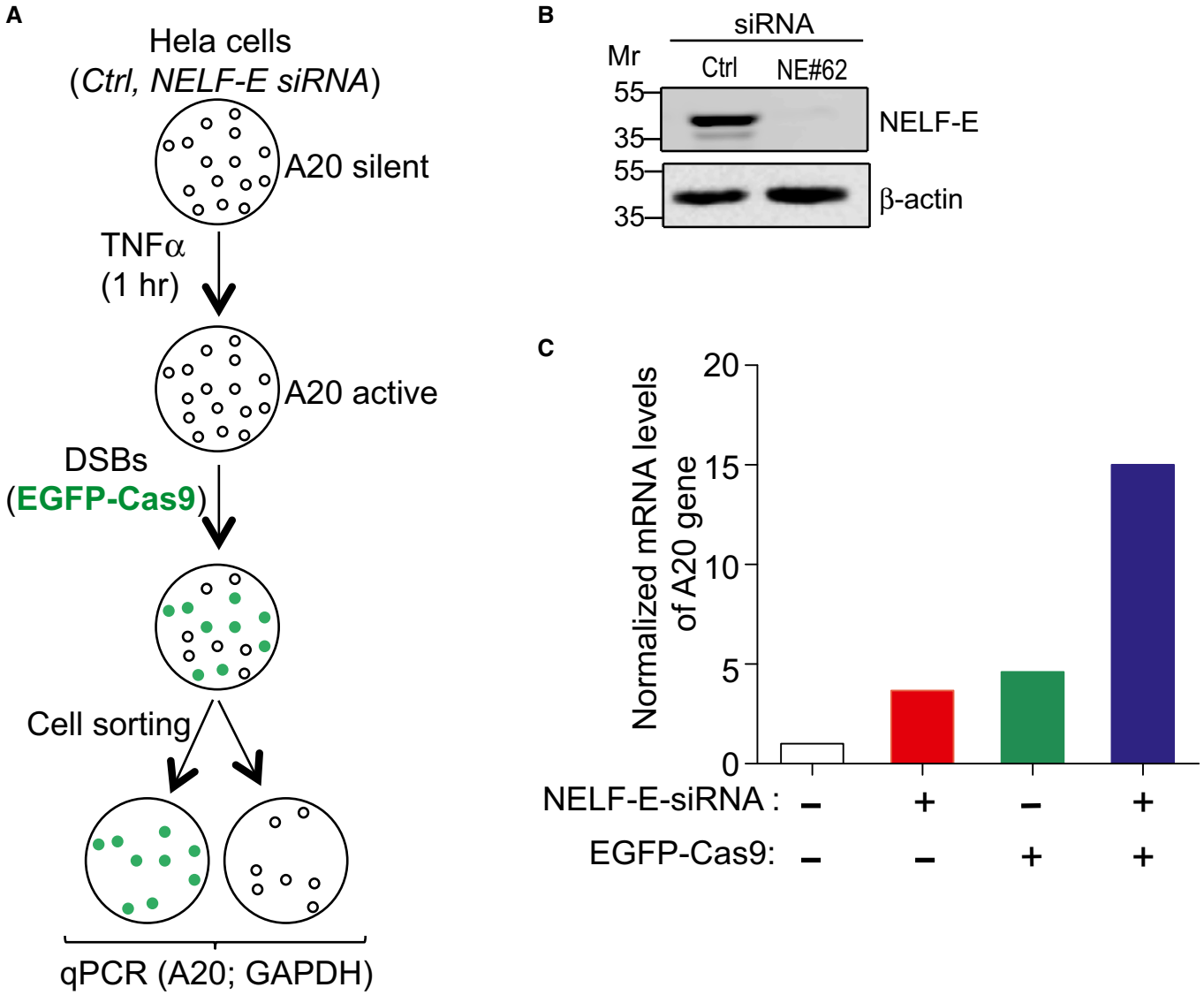

Figure EV1. NELF-E depletion alleviates A20 expression at DSB sites.

A A flowchart depicting the layout of an experiment designed to determine the effect of NELF-E on the transcription of A20 gene following DSB induction.

B Western blot shows NELF-E knockdown in HeLa cells at 72 h after siRNA transfection. β-actin was used as a loading control.

C Graph displays the effect of NELF-E depletion on the transcription activity of A20 gene upon Cas9-induced DSBs. Results show that NELF-E negatively regulates A20 expression at DSB sites. Data represent the mean of two biological repeats.

Figure EV2. The effect of PARP1 and ATM on the expression of the MS2 gene.

A Pharmacological inhibition of PARP1 inhibits NELF-E accumulation at I-SceI-induced DSBs. As in Fig 3B (top panel), except that cells were pretreated for 2 h with 4 μM PARP inhibitor prior to Tam treatment. Error bars indicate SD (n = 3). White arrowheads mark the location of the MS2 reporter cassette. Scale bar, 2 μm.

B PARP inhibition alleviates DSB-induced transcriptional repression of the MS2 gene. As in Fig 1E, except that cells were treated with PARP inhibitor as in (A) prior to Tam treatment. Error bars indicate SD (n = 3). White arrowheads mark the location of the MS2 reporter cassette. Scale bar, 2 μm.

C Graph displays the percentage of cells that show co-localization of YFP-MS2 and Cherry-tTA-ER in U2OS-TRE-I-Sce-19 cells transfected with NELF-E siRNA sequences and/or treated with inhibitor for either PARP and/or ATM kinase. Data represent the mean of 20 cells collected from two biological repeats.

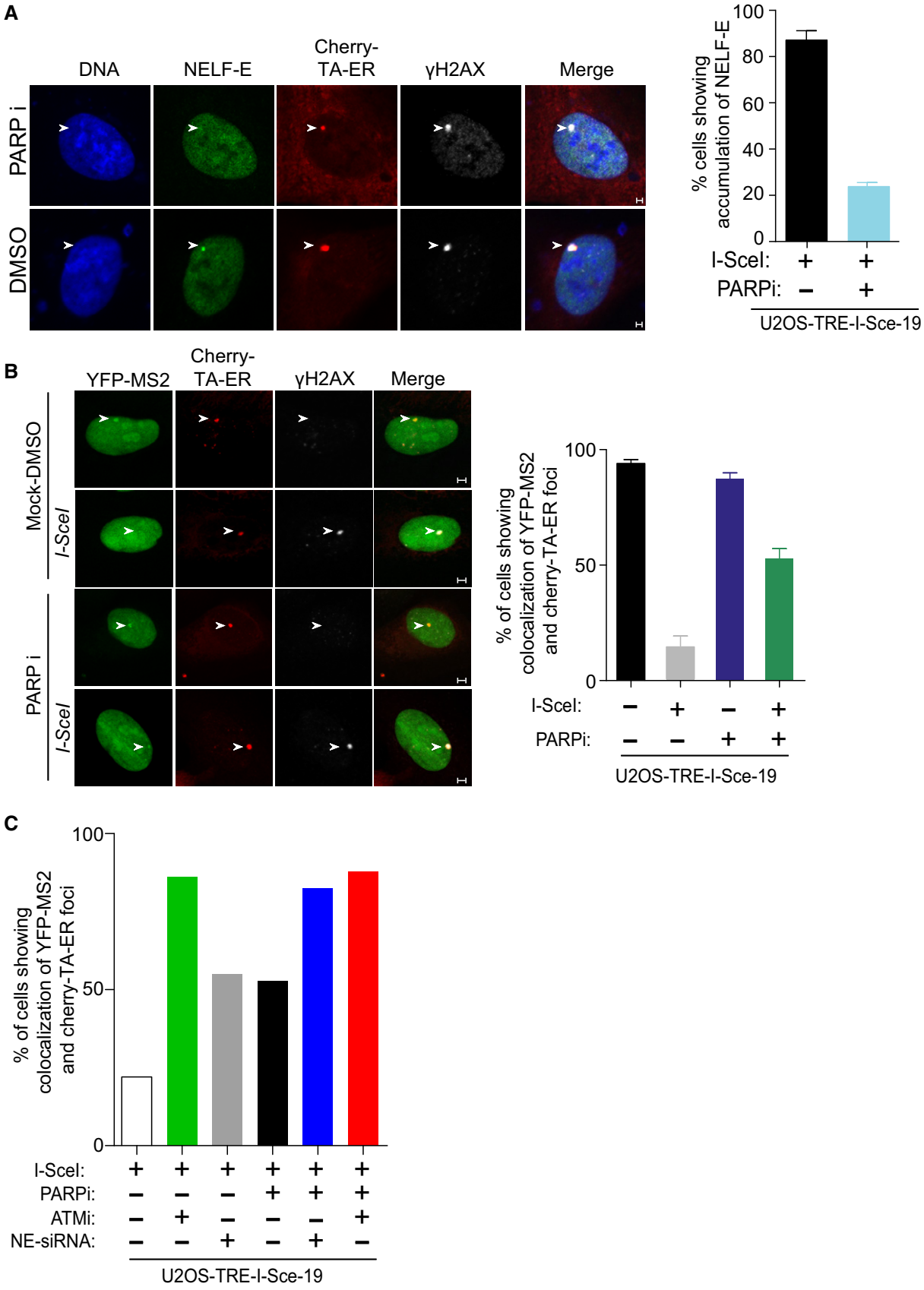
A
DNA
NELF-E
Cherry-TA-ER
γH2AX
Merge
PARP i
DMSO
% cells showing accumulation of NELF-E
100
80
60
40
20
0
I-SceI: + +
PARPi: – +
U2OS-TRE-I-Sce-19
B
YFP-MS2
Cherry-TA-ER
γH2AX
Merge
Mock-DMSO
I-SceI
PARP i
I-SceI
% of cells showing colocalization of YFP-MS2 and cherry-TA-ER foci
100
50
0
I-SceI: – + – +
PARPi: – – + +
U2OS-TRE-I-Sce-19
C
% of cells showing colocalization of YFP-MS2 and cherry-TA-ER foci
100
50
0
I-SceI: + + + + + +
PARPi: – – – + + +
ATMi: – + – – – +
NE-siRNA: – – + – + –
U2OS-TRE-I-Sce-19

Figure EV2.