Supplemental Material

Movie S1. Utr230-EN dynamics in a live U2OS cell.

Movie S2. Utr230-mEos2-NLS before and after photoconversion by 405 laser in a live U2OS cell. Scale bar = 10 μm.

Table S1. Residue ranges for actin binding domains selected as candidate nuclear actin reporters.

Figure S1. Localization of candidate nuclear actin reporters in transiently transfected U2OS cells: (a) NLS-EGFP-ezrin ERM, (b) alpha-actinin CH1CH2-EN, (c) VASP GAB-EN, (d) JMY WH2b-EN, (e) NLS-EGFP-talin ERM, (f) NLS-EGFP-ABLIM3 headpiece, (g) cortactin FABD-EN, (h) NLS-EGFP-Abl1 FABD, (i-j) Lifeact-EN, (k-l) NLS-EGFP-vinculin FABD.

Figure S2. (a) U2OS cells treated with LatB concentrations between 0.2 and 0.8 μM in medium for 30 minutes at 37°C. (b) U2OS cells stably expressing Utr230-EN treated with cytochalasin D concentrations between 0.2 and 1 μM in medium for 30 minutes at 37°C. Cells were fixed and stained with Alexa Fluor 568-phalloidin and DAPI.

Figure S3. Immunofluorescence in U2OS cells stably expressing Utr230-EN and stained with antibodies for (a) nuclear lamina marker lamin B1, (b) nuclear matrix marker NuMa, (c) nucleoli marker fibrillarin, (d) nuclear speckle marker SC35, (e) PML body marker PML, (f) nuclear matrix marker matrin, (g) nucleoplasm marker hnRNP, (h) small nuclear ribonucleoprotein snRNP, (i) BAF chromatin remodeling complex marker Brg1, (j) SWR1 chromatin remodeling marker p400, (k) telomere marker Rap1 and (l) non-randomly distributed histone H2AZ.

Figure S4. BrdU integrated nuclear intensity distribution in U2OS cells: (a) untransfected, n=103; (b) stably expressing EN at high or moderate levels, n=119; (c) stably expressing Utr230-EN at high or moderate levels, n=108. (d) EU average integrated nuclear intensity in U2OS cells: untransfected (n=311) and stably expressing Utr230-EN at high or moderate levels (n=327). Both BrdU and EU intensity values were normalized by nuclear area.

Figure S5. (a) Length distribution of 10000 nuclear actin filaments, pooled from 109 cells. (b) Nuclear actin filament intensity distributions in three representative cells. (c) Double logarithmic plot of MSD versus time, with average values for all nuclear actin trajectories (red) and for a simulated random walk with a diffusion coefficient 0.07 $\mu m^2/s$ (blue). N=25000 particles for both observed and simulated data. (d) Distribution of particle trajectory lengths for all tracked nuclear actin particles. N=25000 particles.

Domain	NCBI gi	Organism	Residues	References
VASP GABD	66804927	Dictyostelium	195-224	Hansen et al., 2010
JMY WH2b	120659968	Human	521-542	Zuchero et al., 2009
MAL RPEL1	20521918	Human	119-150	Mouilleron et al., 2008
MAL RPEL2	20521918	Human	163-194	Mouilleron et al., 2008
ezrin ERM	46249758	Human	490-586	Saleh et al., 2008
talin ERM	55859707	Human	90-400	Gingras et al., 2008
alpha-actinin CH1CH2	114793788	Human	1-228	Borrego-Diaz et al., 2006
dystrophin CH1CH2	33874355	Human	1-246	Norwood et al., 2000
utrophin CH1CH2	110611228	Human	1-261	Keep et al., 1999
Abl1 FABD	78070527	Human	264-396	Hantschel et al., 2005
cortactin FABD	21707902	Human	80-288	Shvetsov et al., 2009
vinculin FABD	228505	Human	106-293	Janssen et al., 2006
ABLIM3 HP	40788381	Human	652-691	PDB 1UJS (unpublished)
Lifeact	341940078	Synthetic	1-20	Riedl et al., 2008

Table S1.

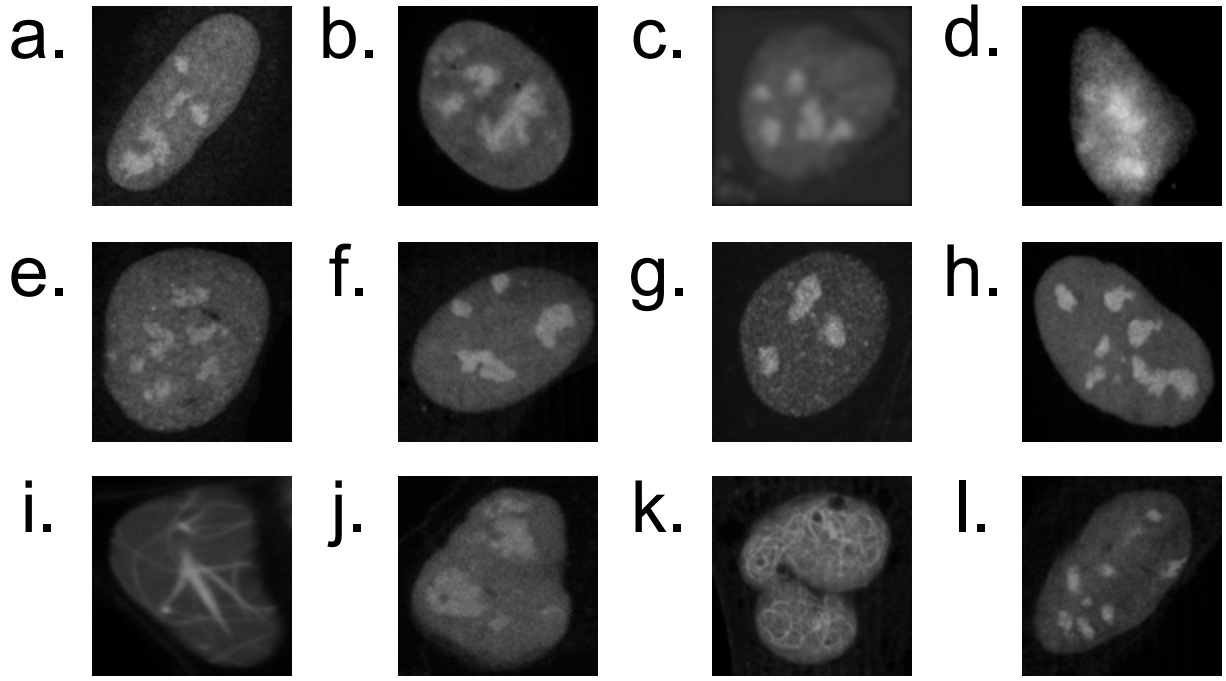

Figure S1.

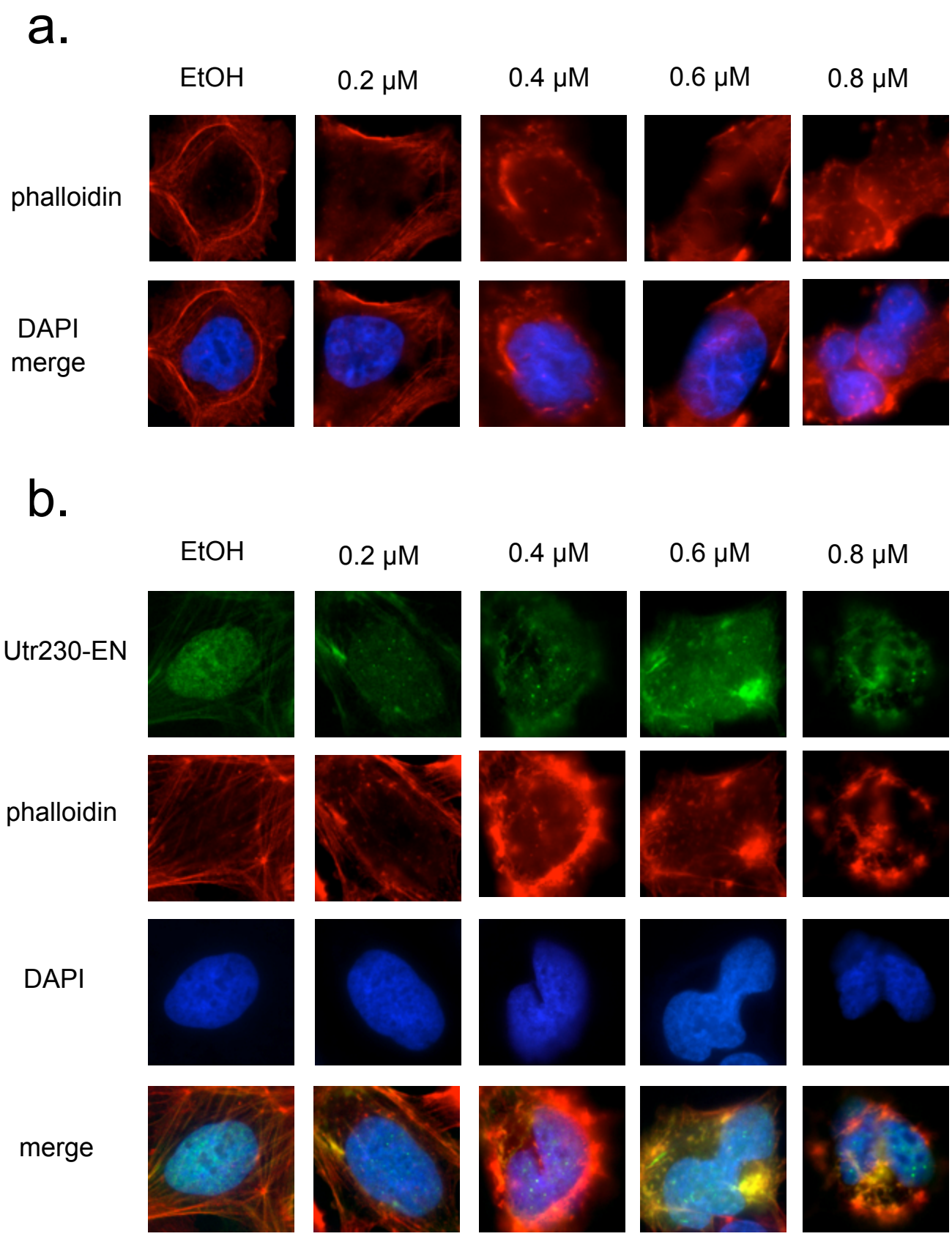
a.
EtOH
0.2 μM
0.4 μM
0.6 μM
0.8 μM
phalloidin
DAPI
merge
b.
EtOH
0.2 μM
0.4 μM
0.6 μM
0.8 μM
Utr230-EN
phalloidin
DAPI
merge

Figure S2.

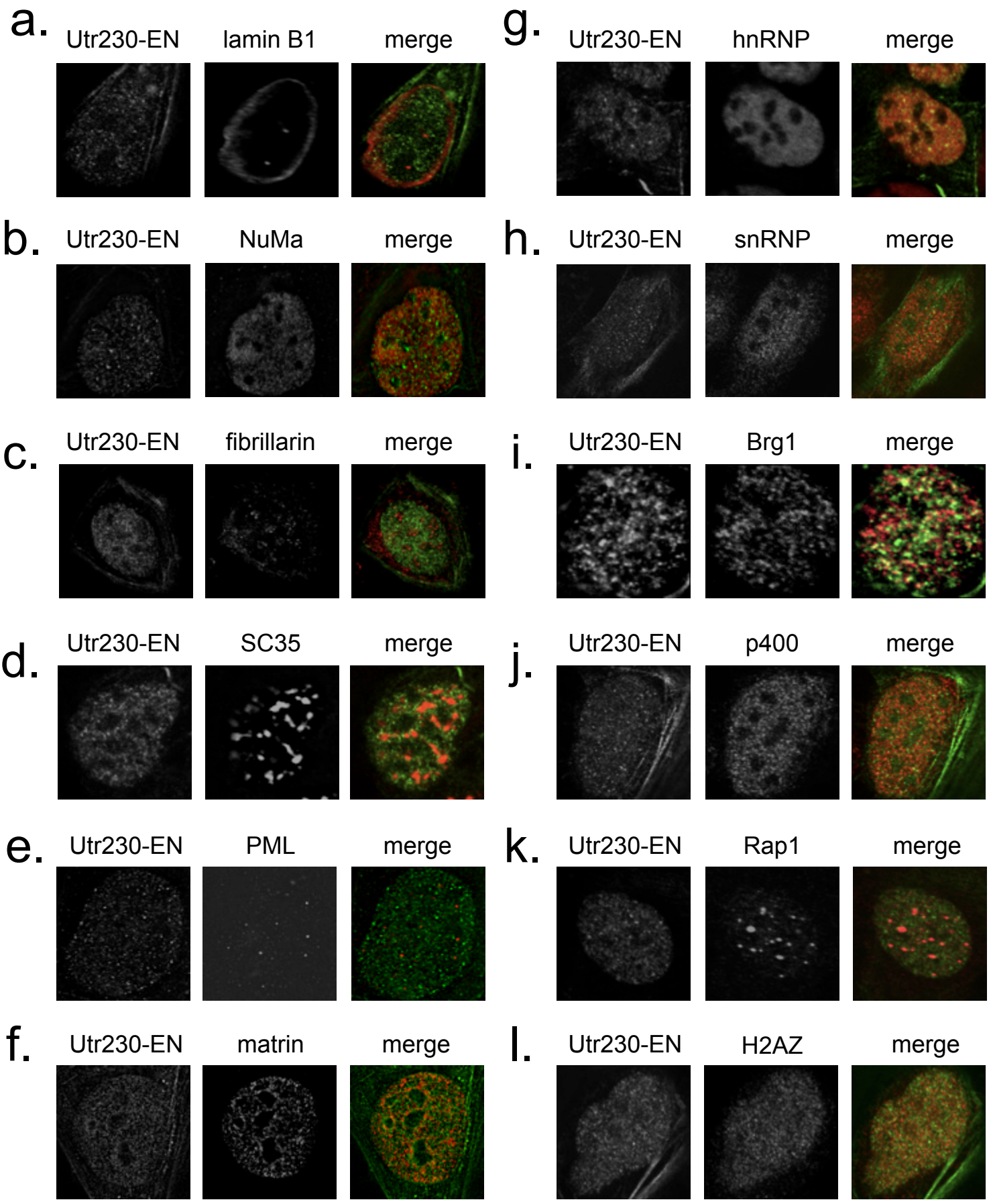
a.
Utr230-EN
lamin B1
merge
b.
Utr230-EN
NuMa
merge
c.
Utr230-EN
fibrillarin
merge
d.
Utr230-EN
SC35
merge
e.
Utr230-EN
PML
merge
f.
Utr230-EN
matrin
merge
g.
Utr230-EN
hnRNP
merge
h.
Utr230-EN
snRNP
merge
i.
Utr230-EN
Brg1
merge
j.
Utr230-EN
p400
merge
k.
Utr230-EN
Rap1
merge
l.
Utr230-EN
H2AZ
merge

Figure S3.

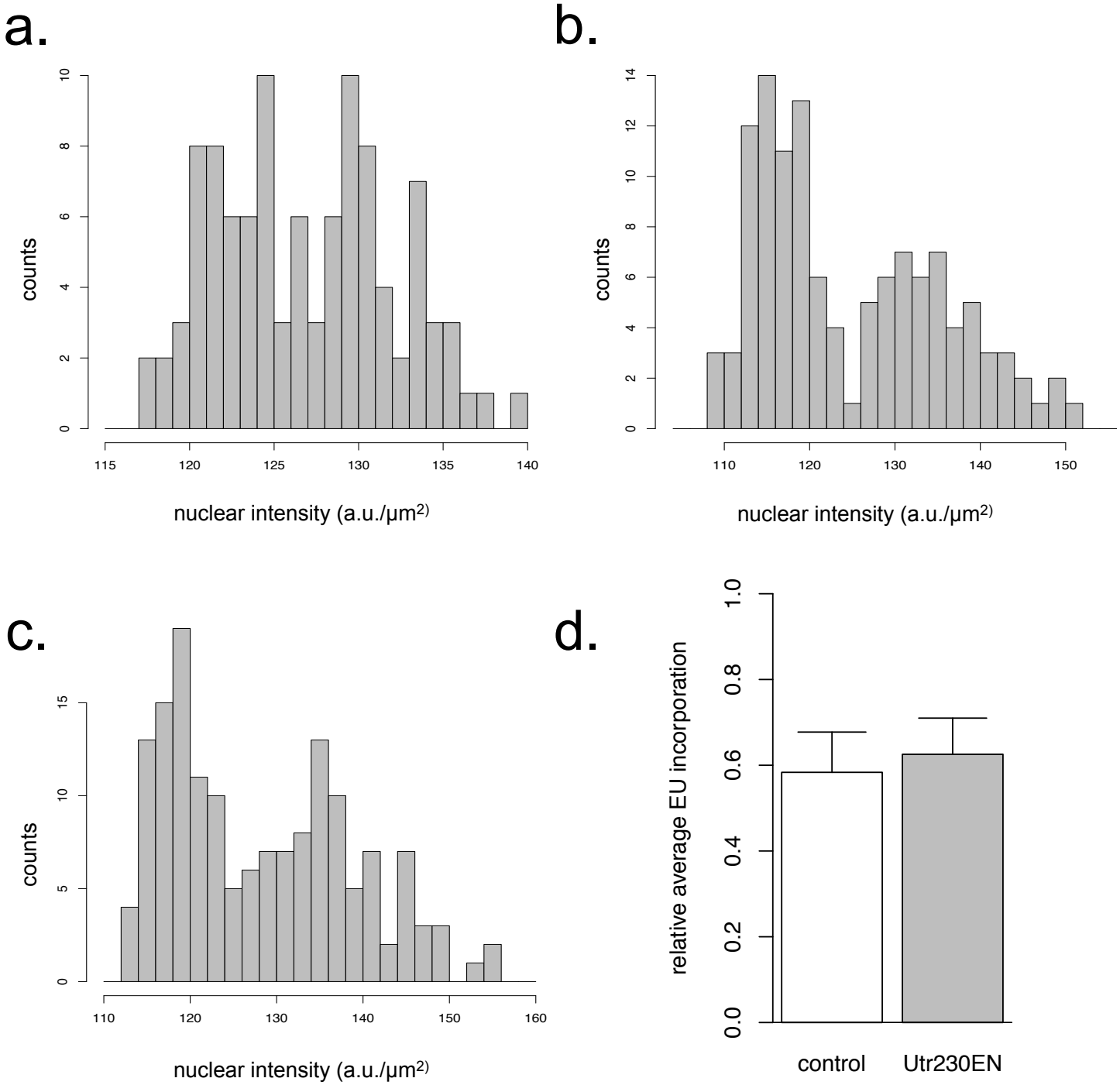
a.
b.
c.
d.
counts
nuclear intensity (a.u./μm2)
relative average EU incorporation
control
Utr230EN

Figure S4.

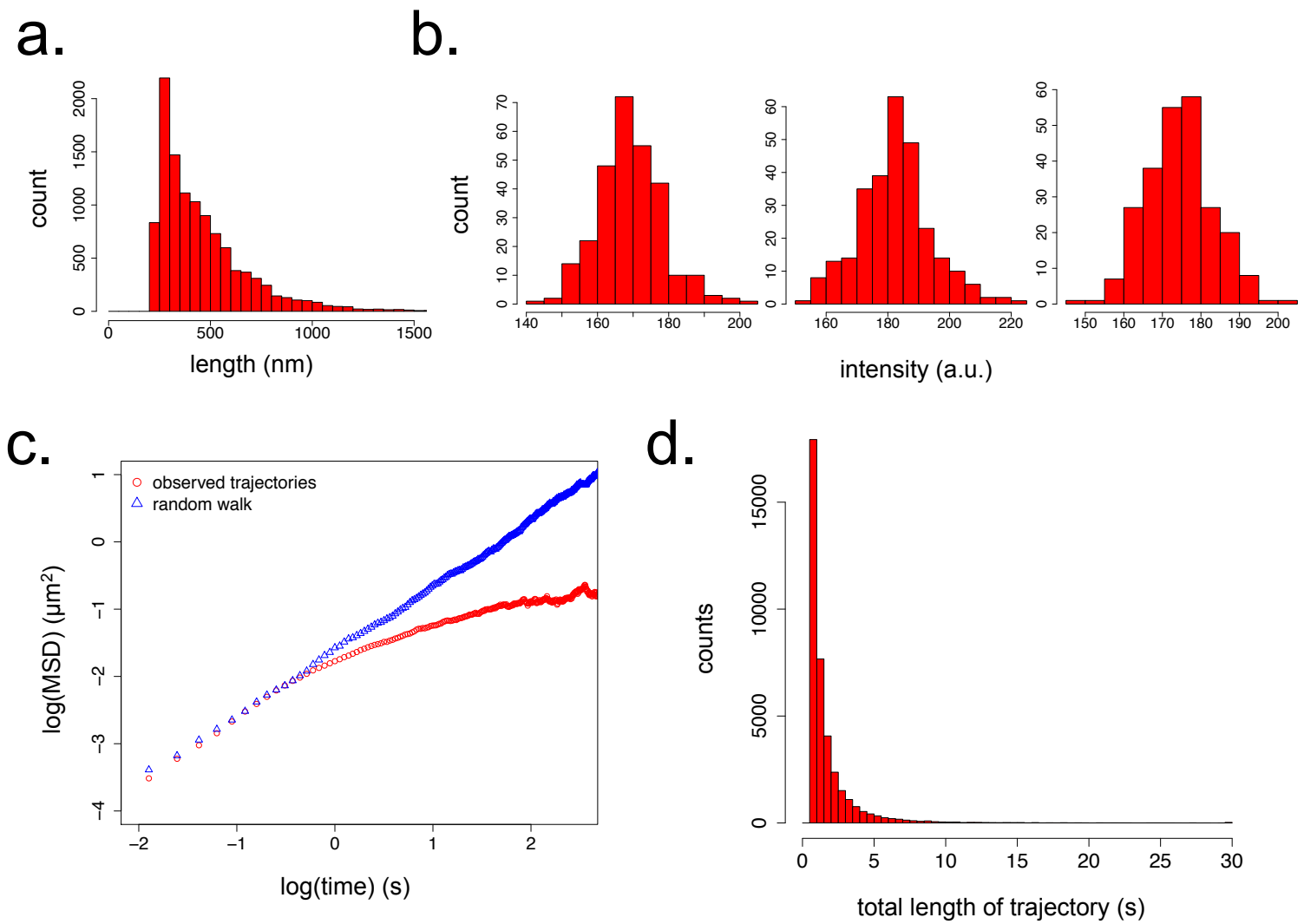
a.
b.
count
length (nm)
intensity (a.u.)
c.
observed trajectories
random walk
log(MSD) (μm²)
log(time) (s)
d.
counts
total length of trajectory (s)

Figure S5.