

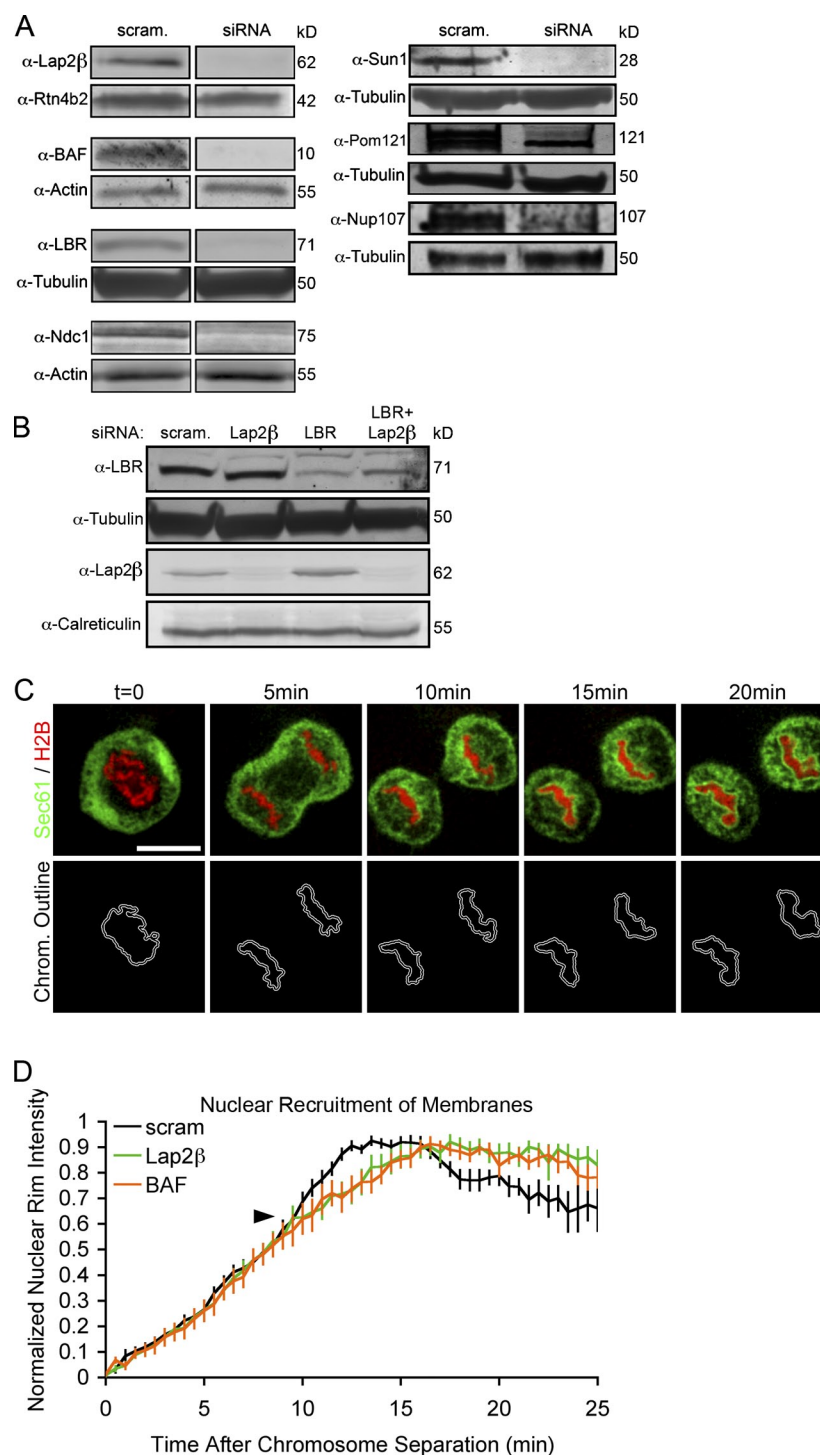
Anderson et al., <http://www.jcb.org/cgi/content/full/jcb.200901106/DC1>

Figure S1. **Reduction of chromatin-binding proteins delays accumulation of membranes at the chromatin surface during NE formation.** (A) Reduction of protein levels by siRNA-mediated gene silencing was measured by Western blot analysis using antibodies that detect endogenous Lap2 β , BAF, LBR, Ndc1, Sun1, Pom121, or Nup107. (B) Reduction of either Lap2 β or LBR by siRNA-mediated knockdown followed by Western blot analysis with antibodies against both Lap2 β and LBR shows levels of both proteins obtained under each knockdown condition. (C) Intensity of Sec61-GFP at the forming NE was quantified for reductions in either Lap2 β or BAF and compared with scrambled siRNA control by measuring fluorescence signal in a border directly around chromatin over time, with $t = 0$ set at the metaphase to anaphase transition. Bar, 20 μ m. (D) Sec61-GFP intensity at the forming NE was measured in cells in which Lap2 β (green) or BAF (orange) protein levels were reduced by siRNA and compared with cells transfected with scrambled (scram) siRNA (black). Arrowhead indicates where curves begin to deviate. Error bars indicate SEM.

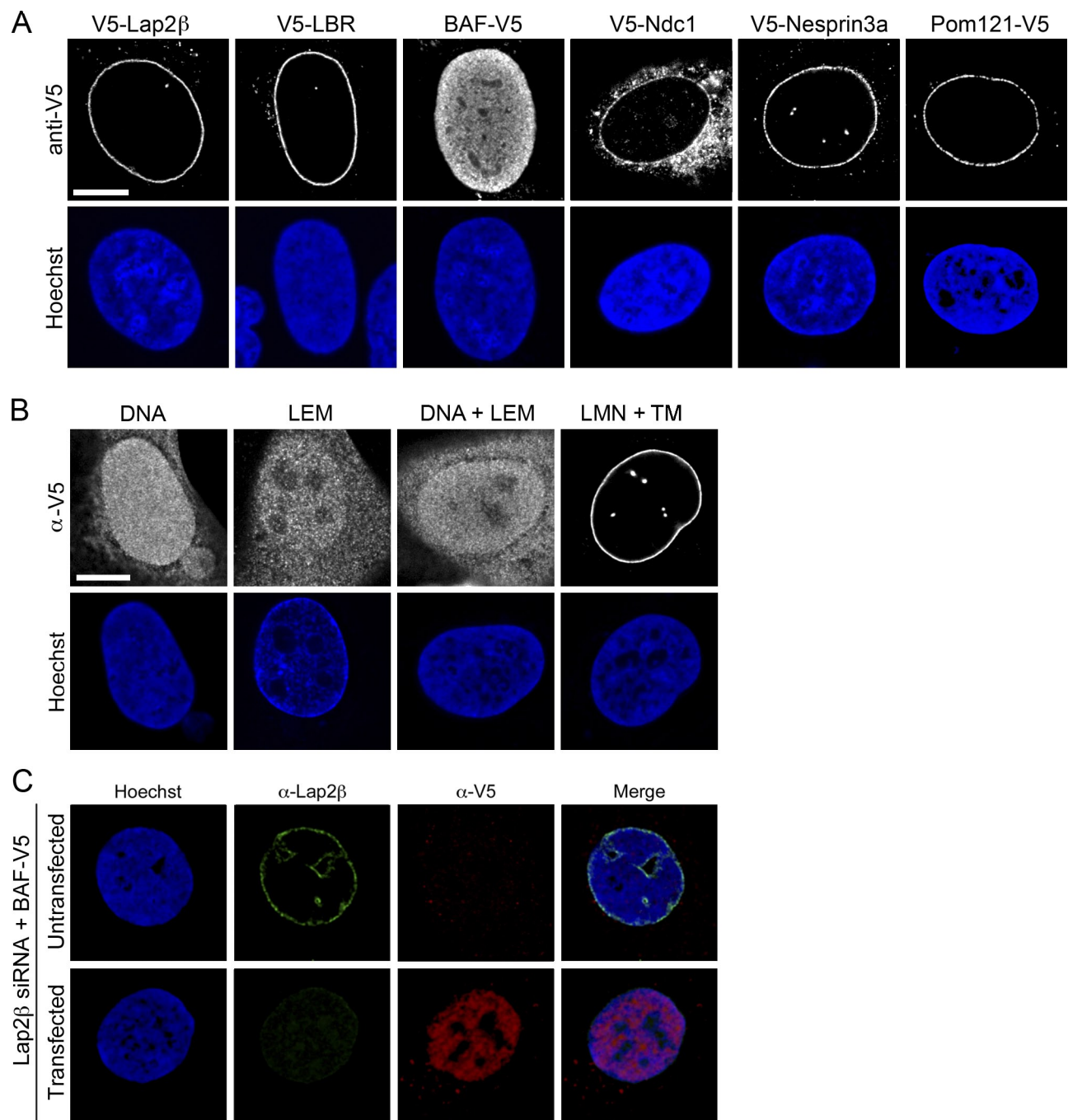


Figure S2. **Localization of V5-tagged constructs.** (A) Localization of V5-tagged Lap2 β , LBR, BAF, Ndc1, and nesprin-3a was characterized by immunofluorescence. (B) Localization of V5-tagged fragments of Lap2 β was characterized by immunofluorescence. (C) Efficiency of simultaneous knockdown and overexpression is shown for U2OS cells reduced with Lap2 β siRNA and transiently transfected with BAF-V5 (bottom) compared with untransfected cells (top) on the same coverslip to ensure similar antibody staining and imaging parameters. Bars, 20 μ m.

Table S1. Average NEF times and statistics for siRNA-mediated knockdown of selected proteins

siRNAs	NEF time	SEM	P-value	n
	<i>min</i>			
Scrambled	11.0500	0.2674	NA	80
LBR	15.6500	0.8930	2.90203E ⁻⁰⁵	20
Lap2b	13.7045	0.5695	9.94983E ⁻⁰⁵	22
MAN1	13.7368	0.8206	0.001606941	38
BAF	18.5208	1.3875	3.58773E ⁻⁰⁵	16
Ndc1	14.6429	1.0618	0.001295758	28
Pom121	14.6000	1.1633	0.003090964	25
Sun1	11.5714	0.6221	0.225576544	14
Nup107	11.3380	0.2067	0.197749598	71

NEF, NE formation; NA, not applicable. P-values are calculated by *t* test compared with scrambled siRNA control.

Table S2. Average NEF times and statistics for transiently increased expression levels of selected proteins

Overexpression constructs	NEF time	SEM	P-value	n
	<i>min</i>			
Control	10.2437	0.1463	NA	145
Lap2β	8.5000	0.2149	1.13863E ⁻⁰⁹	42
LBR	9.1250	0.1449	8.75001E ⁻⁰⁸	68
BAF	8.8409	0.2780	4.21248E ⁻⁰⁵	22
Ndc1	8.8788	0.2656	1.85933E ⁻⁰⁵	33
Pom121	9.3269	0.4132	0.000371853	26
Nes3α	10.7500	0.5775	0.202391077	20

NEF, NE formation; NA, not applicable. P-values are calculated by *t* test compared with reporter-only transfection control.

Table S3. Average NEF times and statistics for combination siRNA and kinetic rescue experiments

Experimental treatments	NEF time	SEM	P-value	n
	<i>min</i>			
Scrambled	11.5000	0.3145	NA	31
Lap2β siRNA	13.3963	0.3695	8.48754E ⁻⁰⁵	82
LBR siRNA	13.6032	0.3436	1.00555E ⁻⁰⁵	63
Lap2β + LBR siRNAs	15.7841	0.5009	0.0002 ^a	44
Lap2β siRNA + BAF-V5	11.7581	0.3809	0.301685638	31
Lap2β siRNA + LBR-V5	12.0000	0.5434	0.215404785	24

NEF, NE formation; NA, not applicable. P-values are calculated by *t* test compared with scrambled siRNA control.

^aP = 0.0002 compared with LBR; P = 0.0001 compared with Lap2β.