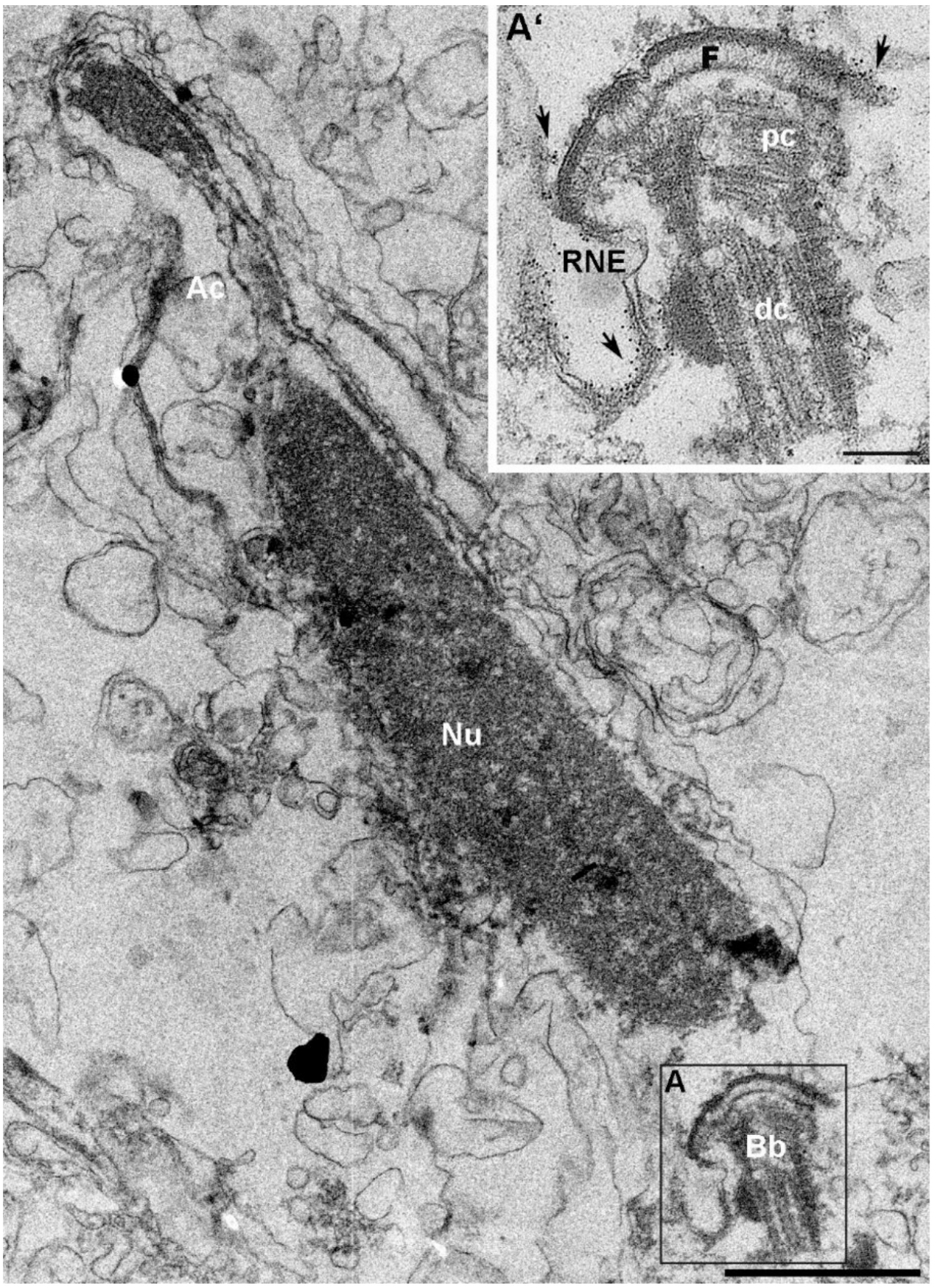

Fig. S1. The SUN4 N-terminal domain is localized at the RNE of elongating spermatids.
Representative electron micrographs of elongating spermatids in native mouse testis cryo-sections with immunogold-labelling (6 nm colloidal gold) of the SUN4 NTD. Area A is enlarged in A'. Gold particles (arrows) are mainly localized along the inner membrane of the redundant nuclear envelope and to both sides of the fossa region, but not directly within the implantation fossa (A'). Scale bars: 1 µm (A) and 200 nm (A'). Bb, basal body; Nu, nucleoplasm; Ac, acrosome; F, implantation fossa; RNE, redundant nuclear envelope, dc, distal centriole; pc, proximal centriole. Image is re-presentative of two experimental repeats.

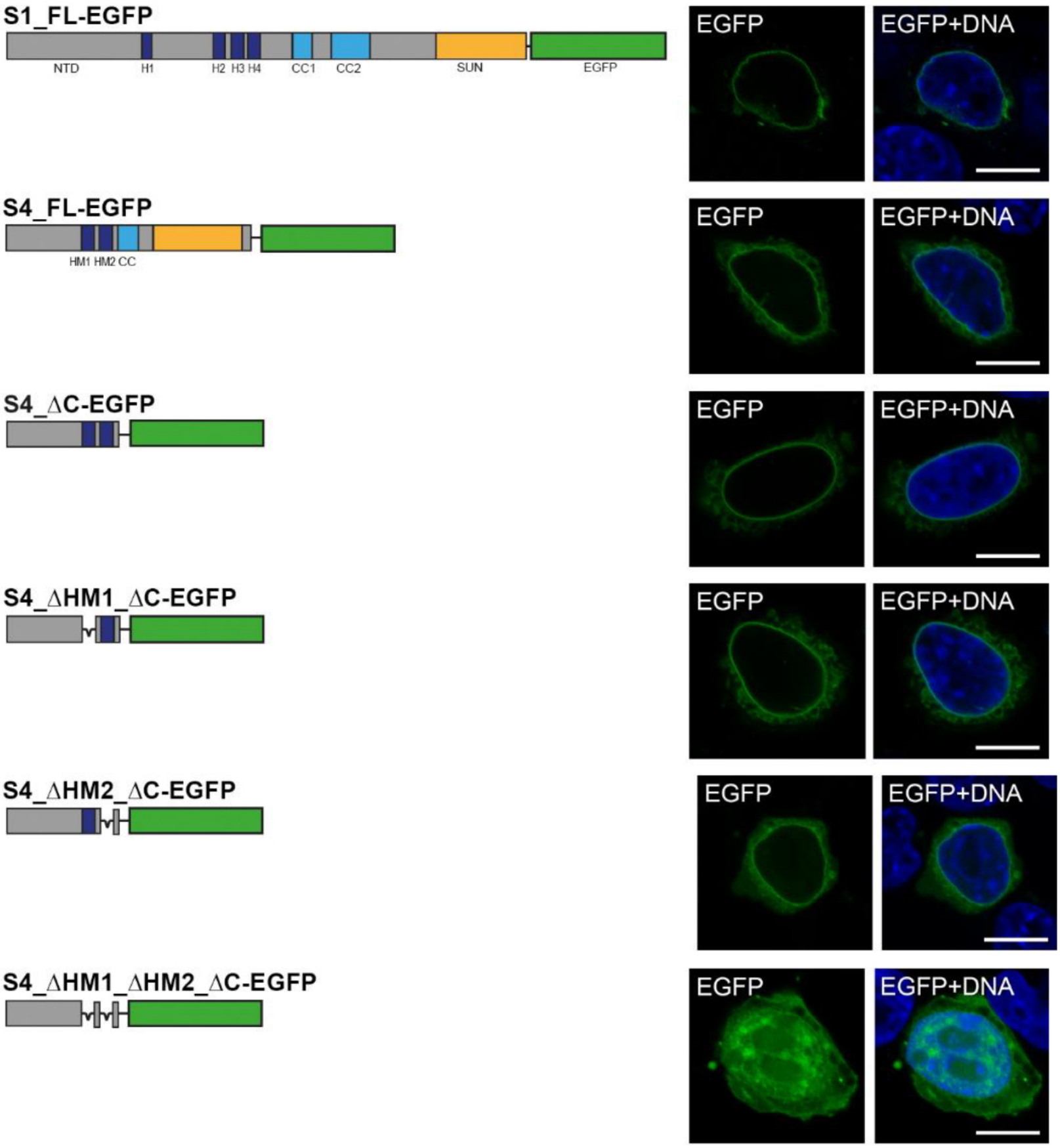

Fig. S2. Subcellular localization of EGFP-tagged SUN1 and SUN4 reporter constructs used for FRAP analysis. EGFP-tagged full-length SUN1 and SUN4, as well as SUN4 constructs with deletion of the C-terminal coiled-coil and SUN domain were transiently transfected into NIH 3T3 cells. Confocal microscopy was used to monitor their expression patterns 48 h after transfection via their EGFP-tag. For each construct, a fluorescence image of one representative cell is shown. DNA was counterstained with Hoechst. Scale bars: 10 µm. Images are reprensentative for at least five independent transfection experiments.

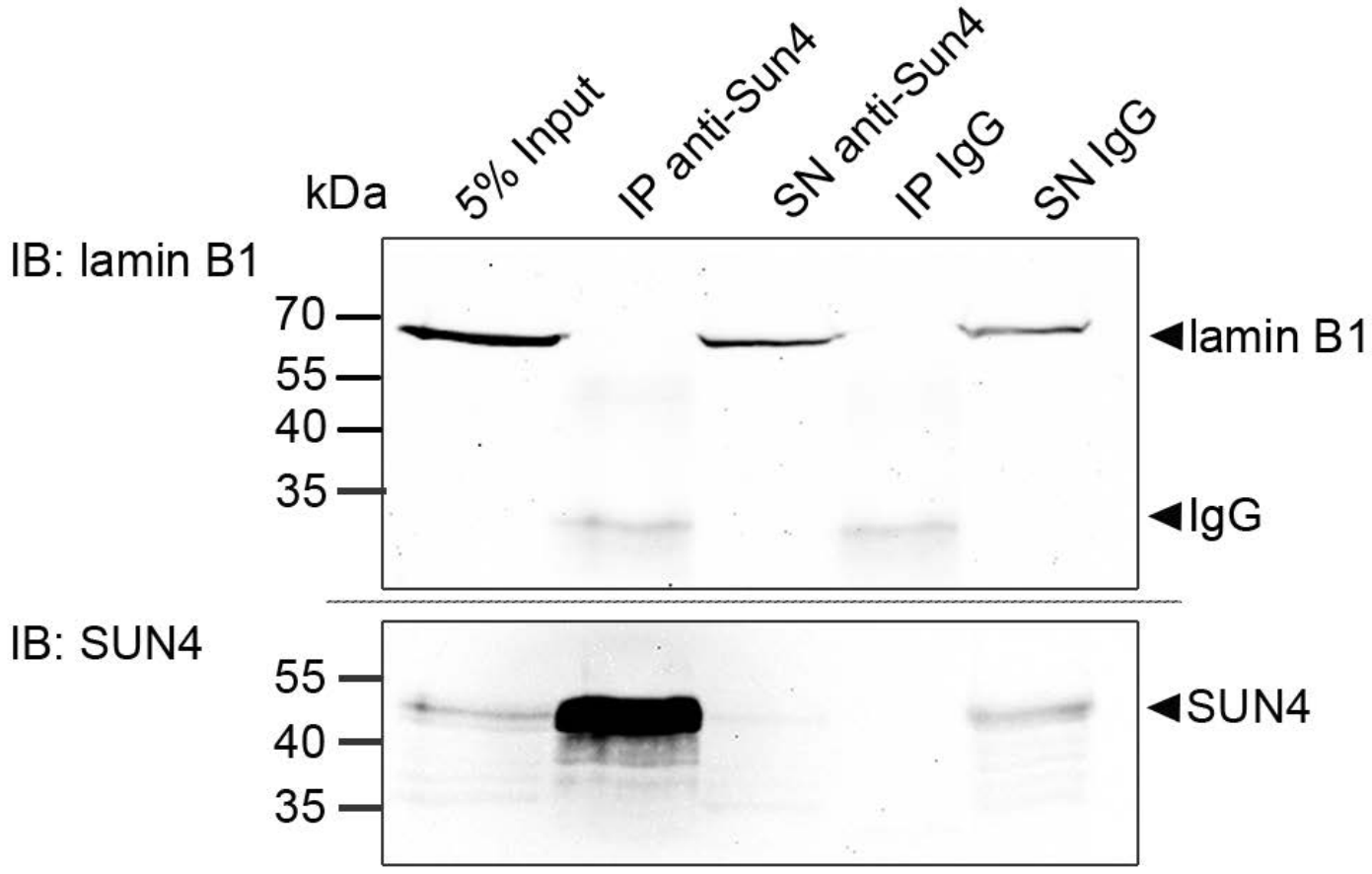

Fig. S3. SUN4 does not appear to bind to lamin B1 *in vivo.* Mouse testicular suspension cells were lysed in RIPA buffer and subjected to co-immunoprecipitation with guinea pig anti-SUN4 NTD antibody and unspecific guinea pig IgG as a negative control. The precipitates (IP) and supernatants containing unbound proteins (SN) were analyzed by immunoblotting (IB) using a rabbit anti-lamin B1 antibody. The SUN4 control presented here is identical to that in Fig. 7, as SUN4-lamin B1 interaction was analysed on the same co-IP samples used to test for SUN4-lamin B3 interaction. It is just shown here again for better visual comparison. Untreated testis cells served as input control. Amounts of loaded cell equivalents: 5% Input=$2x10^6$; IP=$4x10^7$; SN=$5x10^6$. Blot shown is representative for three experimental repeats.

Figure 6A

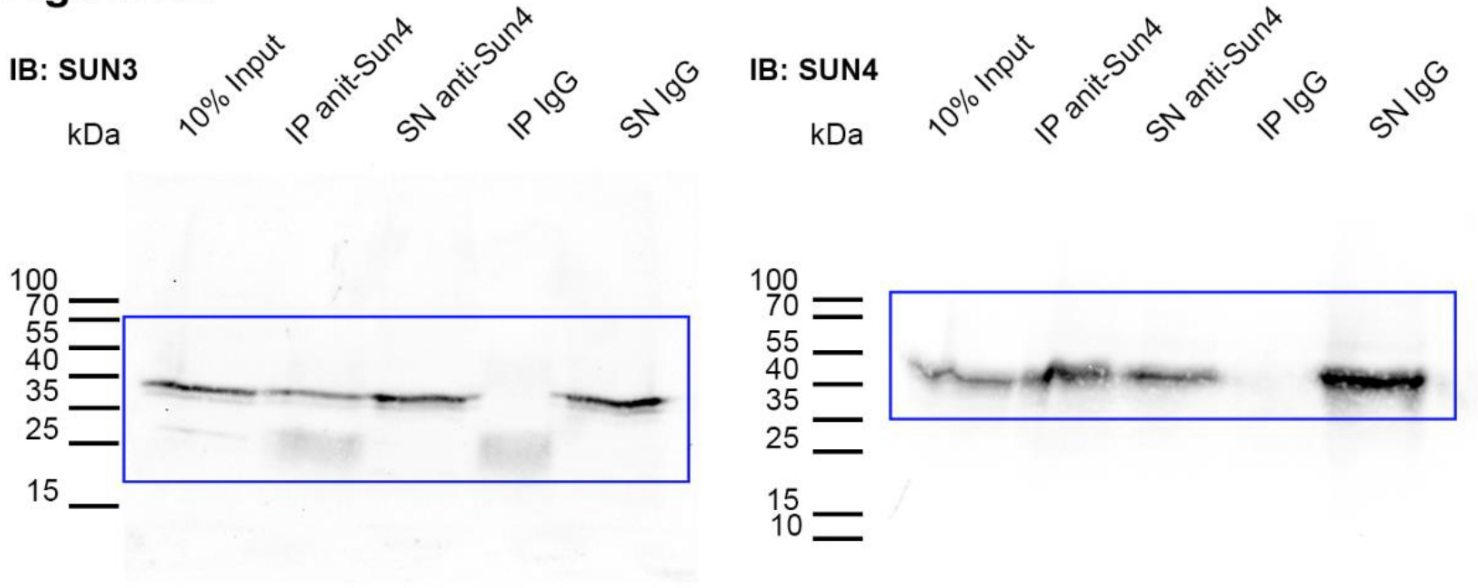

Figure 6B

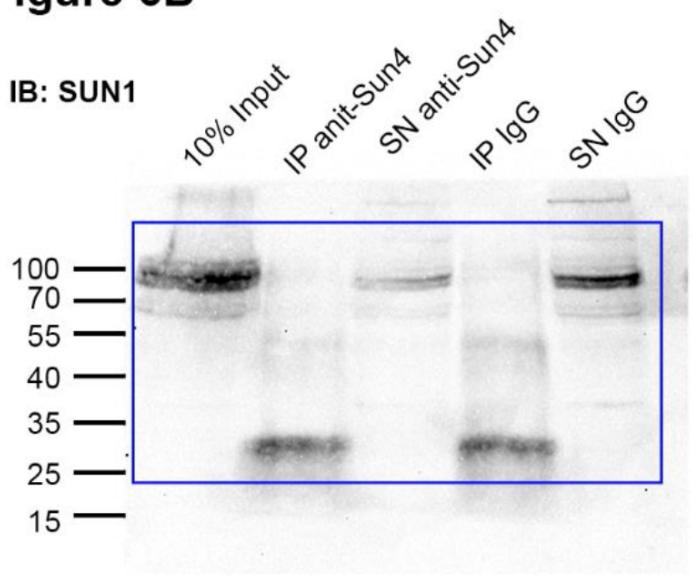

Figure 6C

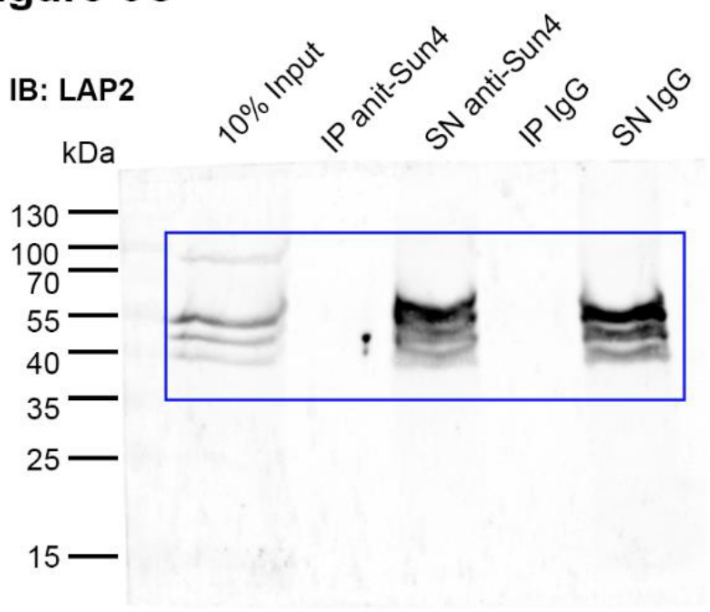

Figure 6D

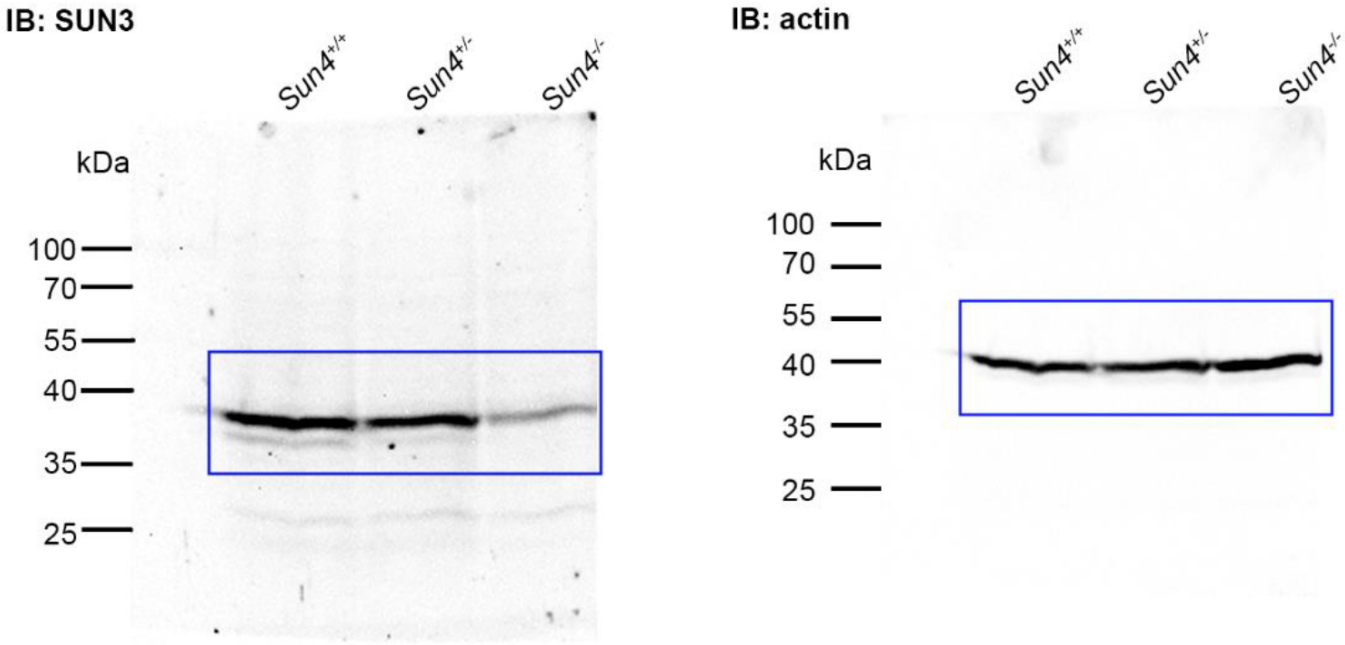

Fig. S4. Blot transparency (continued on the next page)

Figure 7

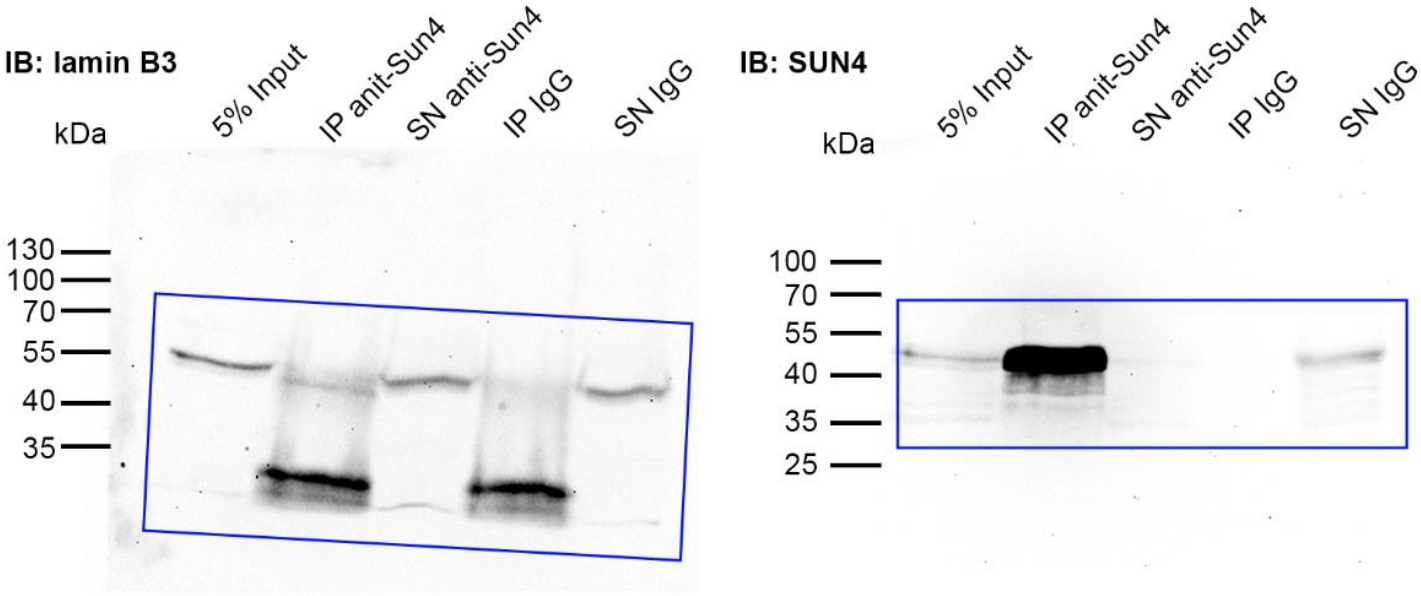

Figure S3

Fig. S4. Blot transparency. Full, uncropped images of all cropped (blue rectangles) Western Blots presented in this study. Reference figure numbers are indicated above the images.

Table S1. Details on statistical analysis of FRAP experiments (Output of GraphPad Prism multiple comparisons analysis)

Ordinary one-way Anova of t50%-values - Multiple Comparisons

Number of families	1
Number of comparisons per family	3
Alpha	0.05

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
S4_ΔC-EGFP vs. S4_ΔHM1_ ΔC-EGFP	2.745	0.8906 to 4.99	Yes	**	0.0024
S4_ΔC-EGFP vs. Sun4_ΔHM2_ ΔC-EGFP	4.903	3.049 to 6.757	Yes	****	<0,0001
S4_ΔHM1_ ΔC-EGFP vs. Sun4_ΔHM2_ ΔC-EGFP	2.158	0.3042 to 4.013	Yes	*	0.0192

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
S4_ΔC-EGFP vs. S4_ΔC_ ΔHM1-EGFP	12.03	9.289	2.745	0.7632	15	15	5.086	42
S4_ΔC-EGFP vs. Sun4_ΔC_ ΔHM2-EGFP	12.03	7.131	4.903	0.7632	15	15	9.086	42
S4_ΔC_ ΔHM1-EGFP vs. Sun4_ΔC_ ΔHM2-EGFP	9.289	7.131	2.158	0.7632	15	15	4	42

Ordinary one-way Anova of fluorescence intensities 30 s post-bleaching - Multiple Comparisons

Number of families	1
Number of comparisons per family	6
Alpha	0.05

Tukey's multiple comparisons test	Mean Diff.	95.00% CI of diff.	Below threshold?	Summary	Adjusted P Value
S4_ΔC-EGFP vs. S4_ΔHM1_ΔC-EGFP	-0.0502	-0.09558 to -0.004812	Yes	*	0.0247
S4_ΔC-EGFP vs. S4_ΔHM2_ΔC-EGFP	-0.05253	-0.09791 to -0.007142	Yes	*	0.0172
S4_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	-0.2849	-0.3330 to -0.2367	Yes	****	<0,0001
S4_ΔHM1_ΔC-EGFP vs. S4_ΔHM2_ΔC-EGFP	-0.00233	-0.04771 to 0.04305	No	ns	0.9991
S4_ΔHM1_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	-0.2347	-0.2828 to -0.1865	Yes	****	<0,0001
S4_ΔHM2_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	-0.2323	-0.2805 to -0.1842	Yes	****	<0,0001

Test details	Mean 1	Mean 2	Mean Diff.	SE of diff.	n1	n2	q	DF
S4_ΔC-EGFP vs. S4_ΔHM1_ΔC-EGFP	0.686	0.7362	-0.0502	0.01711	15	15	4.149	53
S4_ΔC-EGFP vs. S4_ΔHM2_ΔC-EGFP	0.686	0.7385	-0.05253	0.01711	15	15	4.341	53
S4_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	0.686	0.9708	-0.2849	0.01815	15	12	22.2	53
S4_ΔHM1_ΔC-EGFP vs. S4_ΔHM2_ΔC-EGFP	0.7362	0.7385	-0.00233	0.01711	15	15	0.1926	53
S4_ΔHM1_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	0.7362	0.9708	-0.2347	0.01815	15	12	18.29	53
S4_ΔHM2_ΔC-EGFP vs. S4_ΔHM1_ΔHM2_ΔC-EGFP	0.7385	0.9708	-0.2323	0.01815	15	12	18.1	53

Table S2. Oligonucleotides used in this study.

Primer	Sequence	Purpose
Sun4_inc.ATG_5'	5'-AGGTCAGGATGCGGCGGA-3'	5' primer for amplification of *Sun4* and S4_ΔC cDNAs to create entry StrataClone construct
Sun4_SUNdom_3'_woStop	5'-ATGGGGTCCCCCTGTGA-3'	3' primer for amplification of S4_FL cDNA to create StrataClone construct
Sun4_delC-term_3'	5'-CAGCATCTCCGTAGGTTCGTT-3'	3' primer for amplification of S4_ΔC cDNA to create StrataClone construct
Sun4_delTM1_3'	5'-CCTCGGGGTAGGCATCTC-3'	3' primer for deletion of HM1
Sun4_TM2_5'	5'-TGCAGGGAAATCTGCTCC-3'	5' primer for deletion of HM1
Sun4_delTM2_3'	5'-GGAGCAGATTTCCCTGCA-3'	3' primer for deletion of HM2
Sun4_cc_5'	5'-CCTTTGGAGAACGAACCTACG -3'	5' primer for deletion of HM2; 5' primer for *Sun4* RT-PCR expression analysis
Muta_Myc-S4_5'	5'-CCGAATTCGCCCTCAGGTCAGGATGC-3'	5' primer for site-directed mutagenesis to eliminate undesired TAG-codon
Muta_Myc-S4_3'	5'- GCATCCTGACCTGAGGGCGAATTCGG-3'	3' primer for site-directed mutagenesis to eliminate TAG-codon
Sun1_5'	5'-ATGGACTTTTCTCGGCTGCAC-3'	5' primer for amplification of *Sun1* coding sequence
Sun1_3'_woStop	5'-CTGGATGGGCTCTCCGTG-3'	3' primer for amplification of *Sun1* coding sequence
Sun3_RT_5'	5'-ACAGAGTTTCCTCAAAAACCCAG-3'	5' primer for *Sun3* RT-PCR expression analysis
Sun3_RT_3'	5'-AGCCAACTGTATTTGGTCCCCT-3'	3' primer for *Sun3* RT-PCR expression analysis
Sun4_cc_3'	5'-GTCAGGTTTGCGCACGAAGT-3'	3' primer for *Sun4* RT-PCR expression analysis
GAPDH5'	5'-GGGCCCACTTGAAGGGTGGAGC-3'	5' primer for *Gapdh* RT-PCR expression analysis
GAPDH3'	5'-GTCAGATCCACGACGGACACATTGG-3'	3' primer for *Gapdh* RT-PCR expression analysis

Table S3. Primary antibodies used in this study. IF, immunofluorescence; EM, electron microscopy (immunogold labeling); IB, immunoblot; mAb, monoclonal antibody; pAb, polyclonal antibody.

Antibody	Manufacturer (Catalogue number/ Reference)	Host	Working dilution		
			IF	EM	IB
actin	Sigma-Aldrich (A4700)	Mouse mAb			1:500
GFP (B-2)	Santa Cruz Biotechnologies (sc-9996)	Mouse mAb	1:200		1:200
lamin A/C	Santa Cruz (sc-20681)	Rabbit pAb			1:2,000
lamin B1	Sigma-Aldrich (ZRB1143)	Rabbit mAb	1:100		1:5,000
lamin B3	Own synthesis (Schütz et al., 2005)	Rabbit pAb	1:100		
13d4 (LAP2)	Own synthesis (Alsheimer et al., 1998)	Mouse mAb	Undiluted hybridoma cell supernatant		
Myc	Thermo Fisher Scientific (9E10)	Mouse mAb	1:200		1:8,000
PDI RL90	Thermo Fisher Scientific (MA3-019)	Mouse mAb	1:200		1:1,000
SUN1	Own synthesis (Göb et al., 2010)	Guinea pig pAb	1:800		1:5,000
SUN3	Own synthesis (Göb et al., 2010)	Guinea pig pAb	1:1000		1:5,000
SUN4 peptide	Own synthesis (Pasch et al., 2015)	Rabbit pAb			1:5,000
SUN4-NTD	Own synthesis (Pasch et al.,2015)	Guinea pig pAb	1:2000	1:400	1:5,000
SUN4-NTD	Own synthesis (Pasch et al.,2015)	Rabbit pAb		1:2400	
IgG	Jackson ImmunoResearch Laboratories (011-000-003)	Rabbit pAb			
IgG	Jackson ImmunoResearch Laboratories (006-000-003)	Guinea pig pAb			

Table S4. Secondary antibodies used in this study.

Antibody	Manufacturer (catalogue numer)	Host	Antigen	Dilution
6nm Gold	Jackson ImmunoResearch Laboratories (706-195-148)	Donkey	Guinea pig IgG	1:10
6nm Gold	Jackson ImmunoResearch Laboratories (111-195-144)	Goat	Rabbit IgG	1:10
HRP	Jackson ImmunoResearch Laboratories (106-035-003)	Goat	Guinea pig IgG	1:10,000
HRP	Jackson ImmunoResearch Laboratories (706-035-148)	Donkey	Guinea pig IgG Cross-adsorbed	1:10,000
HRP	Jackson ImmunoResearch Laboratories (111-035-003)	Goat	Rabbit IgG	1:10,000
HRP	Jackson ImmunoResearch Laboratories (711-035-152)	Donkey	Rabbit IgG Cross-adsorbed	1:25,000
HRP	Jackson ImmunoResearch Laboratories (115-035-003)	Goat	Mouse IgG	1:10,000
AlexaFluor™ 488	Thermo Fisher Scientific (A-11008)	Goat	Rabbit IgG	1:200
AlexaFluor™ 488	Thermo Fisher Scientific (A-10680)	Goat	Mouse IgG	1:300
Texas Red	Jackson ImmunoResearch Laboratories (115-075-044)	Goat	Mouse IgG	1:150
Texas Red	Jackson ImmunoResearch Laboratories (106-075-003)	Goat	Guinea pig IgG	1:50