Rodríguez et al., http://www.jcb.org/cgi/content/full/jcb.201004067/DC1

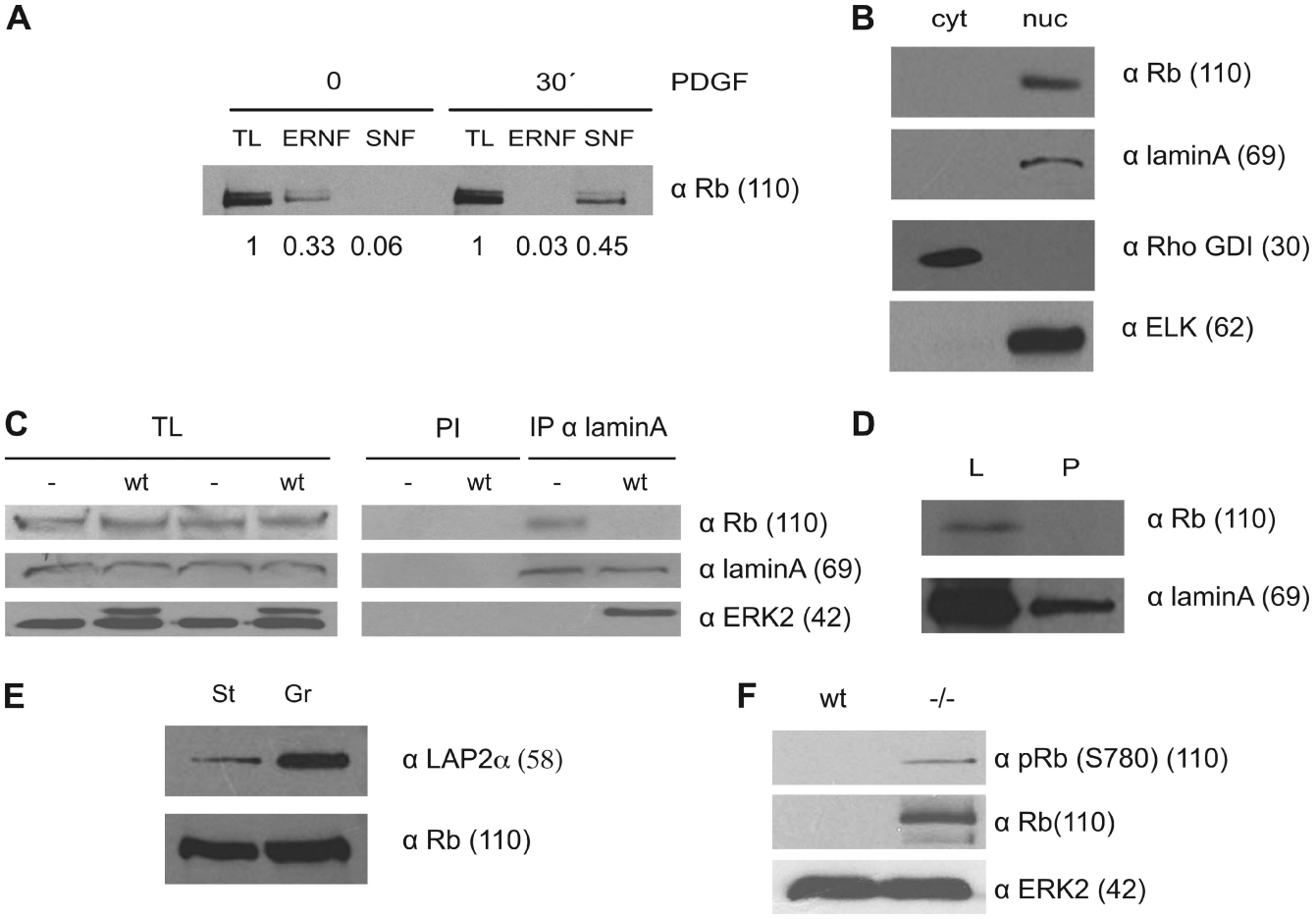

Figure S1. **Control experiments for the expression of key proteins.** (A) Proportions of Rb fluctuating between the SNF and ERNF. The levels of Rb in the SNFs and ERNFs extracted from cells: starved (0) and PDGF stimulated for 30 min (30′) were compared with those found in a total lysate (TL). Numbers show Rb levels relative to those in the total lysate. (B) Control of the purity of the NIH3T3 cell cytoplasmic (cyt) and nuclear (nuc) fractions resulting from lysis in 0.5% NP-40 lysis buffer. Rho GDI and Elk-1 are shown as soluble protein markers for the cytoplasmic and nuclear fractions, respectively. (C) Specificity of the anti–lamin A immunoprecipitations: NIH3T3 cells were transfected with 1 µg of each vector (−) or HA-ERK2-NLS wt (wt), grown until confluence, and kept in 0.5% CS for 18 h. Cellular lysates were immunoprecipitated using lamin A antibodies or preimmune serum (PI). Immunoprecipitates (IP) and the corresponding total lysates were probed by immunoblotting for the indicated proteins (α protein of interest). (D) Control of protein loss resulting from solubilization in 1.5% NP-40 lysis buffer. Rb and lamin A levels in the lysate (L) and in the postcentrifugation pellet solubilized in radio immunoprecipitation assay buffer with strong sonication (P) were compared. (E) LAP2α is down-regulated in serum-starved (St) NIH3T3 cells compared with growing (Gr) cells, in agreement with previous results (Markiewicz et al., 2002). (F) Specificity of the Rb antibodies. Total lysates from exponentially growing MEFs, wt, and $Rb^{-/-}$ (−/−) were probed by immunoblotting using antibodies against total Rb and phospho-Rb (S780). Molecular masses (given in kilodaltons) are shown in parentheses after the protein name.

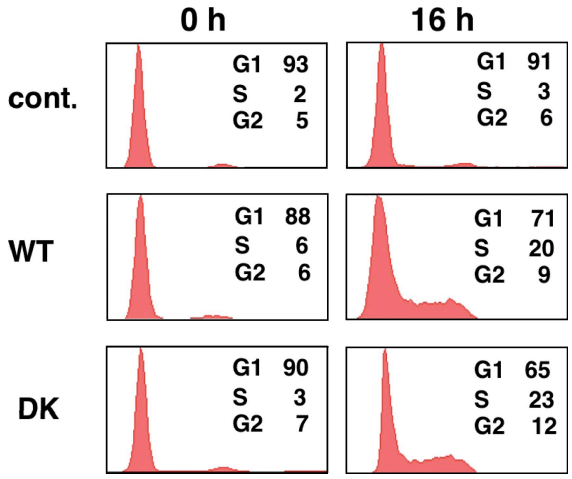

Figure S2. **Cell cycle progression induced by nuclear ERKs in NIH3T3 cells.** NIH3T3 cells were transfected as shown with 1 µg ERK2-NLS forms: wt and dead kinase (DK). Cells were synchronized by aphidicolin block, and cell cycle phases were analyzed by flow cytometry immediately after the block release and after 16 h of stimulation with 0.5% CS. Graphs show a representative experiment out of three independent events.

References

Markiewicz, E., T. Dechat, R. Foisner, R.A. Quinlan, and C.J. Hutchison. 2002. Lamin A/C binding protein LAP2alpha is required for nuclear anchorage of retinoblastoma protein. *Mol. Biol. Cell.* 13:4401–4413. doi:10.1091/mbc.E02-07-0450