Legends

Fig. S1. CAF-1 downregulation level in quiescent cells. Expression of MCM2, CAF-1 p60 phosphorylated form (mAb8133), total CAF-1 p60 (anti-p60 poly) and PCNA analyzed in total cell extracts from asynchronous and G0 arrested MCF7 cells by semi-quantitative Western Blot with actin as loading control. The downregulation level in quiescent cells is given in each case. Relative amounts of loaded extracts are indicated above.

Fig. S2. CAF-1 expression in quiescent versus proliferating 1BR3 cells. *A*, Expression of CAF-1 p150 (mAb7655) and p60 (anti-p60 poly) subunits analyzed by immunofluorescence in 1BR3 cells grown with (asynchronous) or without serum (G0) during 4 days. Percentages of p150 and p60 stained cells are indicated below. Bar, 10m. *B***,** Expression of CAF-1 subunits analyzed by semi-quantitative Western Blot in total cell extracts from asynchronous and G0 blocked 1BR3 cells. For simplicity, several analyses with similar actin levels (internal control) are juxtaposed. In each case, a lysate corresponding to 10^5 cells was loaded.

Fig. S3. Expression of CAF-1 upon G0 release in 1BR3 cells. *A*, CAF-1 p150 expression and BrdU incorporation analyzed by immunofluorescence in 1BR3 cells at indicated times after G0 release compared to asynchronous (As.) and G0 arrested cells. Percentages of p150 and BrdU (S phase) stained cells are indicated below. Bar, 10m. *B*, Total extracts from 1BR3 cells made at indicated times after G0 release analyzed by Western Blot (10^5 cells/well) in comparison with asynchronous (As.) and G0 arrested cells as indicated. actin is used as loading control.

Fig. S4. Analysis of p60 pseudogene putative transcript. According to a BLAST search, the p60 gene is present in two copies in human genome: one on chromosome 21 and one pseudogene on chromosome 6 containing several point mutations. Of the two p60 RT-PCR products, the one from the putative pseudogene transcript comprises a PstI restriction site which is not present in the RT-PCR product from the p60 gene on chromosome 21 allowing discrimination between them. P60 specific RT-PCR reactions were performed on total RNA from proliferating and quiescent MCF7 cells. As a positive control, a fragment containing a PstI restriction site in PCRScript plasmid (Stratagene) was amplified using KS and M13 primers (Sigma Genosys). PCR products were digested by PstI enzyme (Ozyme) and digestion products were analyzed on an 8% polyacrylamide gel.

Fig. S5. Specificity of immunocytochemical detection for CAF-1 p60. *A*, Expression of p60 detected by immunocytochemistry on asynchronous (As.) and G0 arrested MCF7 cells using two distinct p60 monoclonal antibodies (mAb8133 from Abcam, mAb96 kindly provided by B.Stillman) and a polyclonal antibody (p60 poly) obtained using a recombinant His-p60 protein produced at our laboratory for rabbit immunization (Agrobio, Villeny, France). For competition experiment, we pre-incubated the p60 polyclonal antibody with a recombinant GST-p60 protein prior to immunostaining, which led to the disappearance of nuclear staining. Percentages of positively stained nuclei indicated below were reproducible throughout all experiments using different antibodies. Magnification is 200x.

B, Comparison of the expression of p60 detected by immunohistochemistry in malignant (high expression) and benign lesions (low expression) using paraffin-embedded breast tissue sections. Antibodies are as indicated. Magnification is 400x.

Fig. S6. Ki-67 and CAF-1 immunodetection in MCF7 cells. Expression of Ki-67 and phosphorylated p60 analyzed by immunofluorescence in MCF7 cells with (+) or without (-) an antigen retrieval step as described in Material and Methods. Bar, 10 m.