SUPPORTING INFORMATION

Vielemeyer *et al*

SUPPLEMENTARY EXPERIMENTAL PROCEDURES

Antibodies, reagents and mammalian cell lines. The following antibodies were used: mouse-anti myc (9E10); monoclonal mouse-anti poly-His (Sigma-Aldrich); monoclonal mouse anti-GRASP65 (7E10), kindly provided by F.A. Barr (University of Liverpool, Cancer Studies Centre, Liverpool, U.K.); mouse anti FLAG (Sigma-Aldrich), TA10, a monoclonal anti-giantin scFv (1), modified to generate a full, rabbit-Fc-portion containing antibody, rbTA10, (2). Fluorescent secondary antibodies were from Jackson Immuno Research and Molecular Probes; HRP-coupled secondary antibodies were from Jackson Immuno Research. All reagents were from Sigma-Aldrich unless specified otherwise. Greg Winter (MRC, Cambridge, UK) kindly provided the Griffin.1 library. The following mammalian cells lines were used: NRK cells (ATCC CRL-6509), FR3T3 (rat fibroblast cells of the 3T3 type (3)), CHO cells (ATCC CCL-61), HeLa cells (ATCC CCL-2).

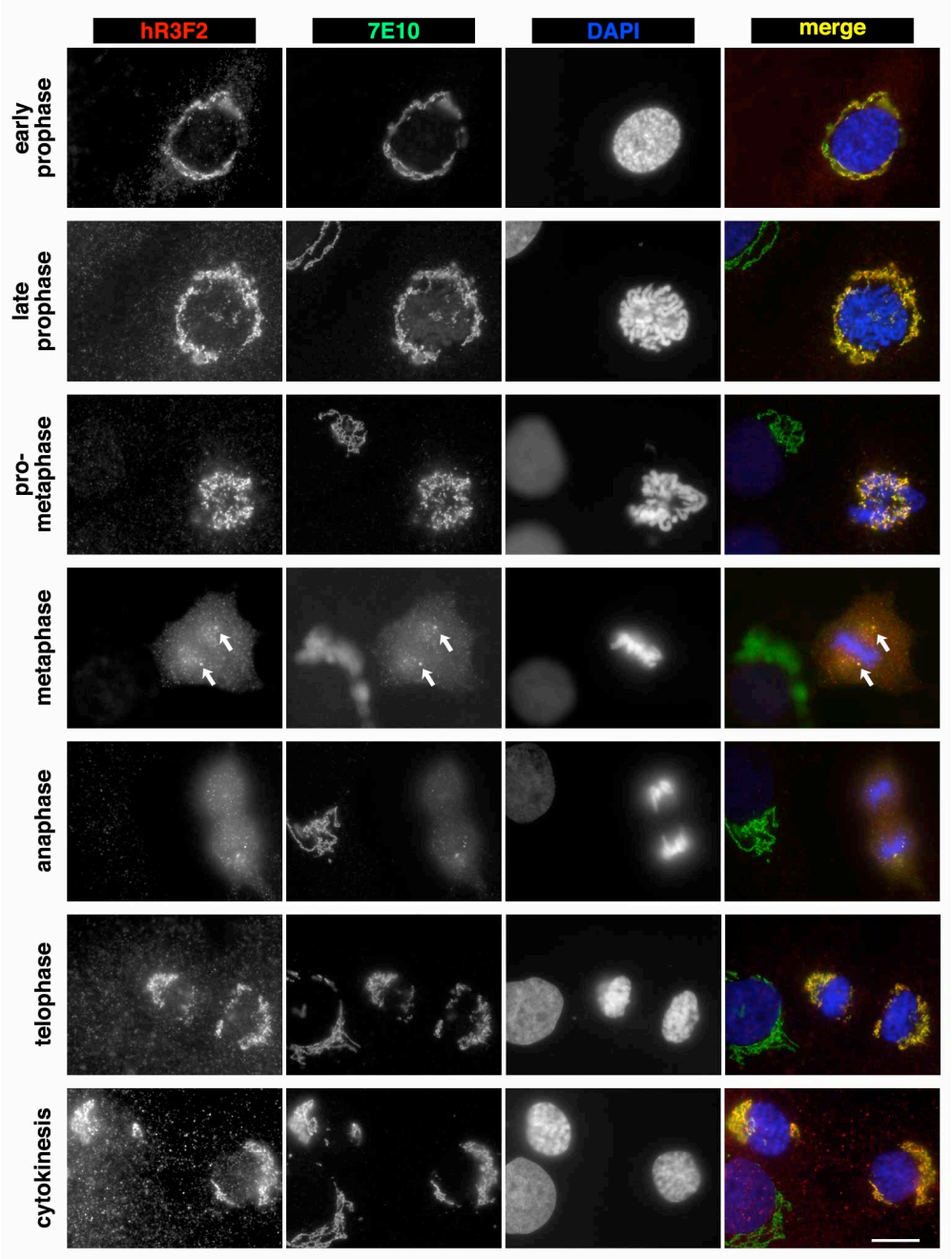
Plasmid constructs, siRNA experiments, selection of stable HeLa cell line clone. Plasmids with GFP-tagged rat GRASP65, either wild type (4) or the GRASP65 8A-mutant (with the following substitutions: T220A, S216A, S217A, S277A, S367A, S372A, S376A, and S400A (5)) were kindly provided by F.A. Barr. The vector expressing fluorescently labeled rat GRASP55 was created by inserting its cDNA into pEGFP-N1 through the use of PCR amplification and XhoI and HindIII restrictions sites. For siRNA experiments two pairs of oligonucleotides, which target the ORF of rat-GRASP65 starting at positions 140 (5'-ggc act gct gaa ggc taa t-3') and 168 (5'-ggc tga aca agg aga atg a-3'), respectively were designed. A mixture of both siRNA was used to transfect either NRK or FR3T3 cells. IF imaging was performed 72 hours after transfection. A FLAG-tagged GRASP65 expression vector was generated by inserting a PCR product, which contained the C-terminus of GRASP65, followed by FLAG (DYKDDDK) and appropriate restriction sites, into the pEGFP-N-GRASP65 plasmid between the restriction sites PasI and BsrGI, thus removing the GFP. HeLa-cell clones stably expressing GRASP65 C-terminally tagged with FLAG were subsequently obtained using G418 and selecting isolated clones.

Cell culture, siRNA, transfection of plasmids. All mammalian cells mentioned above were grown under standard conditions in DMEM containing 10% serum. siRNA experiments were performed using HiPerFect (QIAGEN ®) according to the manufactures instructions. To introduce expression plasmids, HeLa cells were transfected with $CaPO_4$ as described (1).

Immunofluorescence analysis. Cells were imaged on a Leica DMRA microscope equipped with fluorescence filters (Chroma, Leica) and a cooled CCD camera (CoolSnap HP). Images were acquired using Metamorph (Universal Imaging); figures were assembled using Photoshop 11 (Adobe).

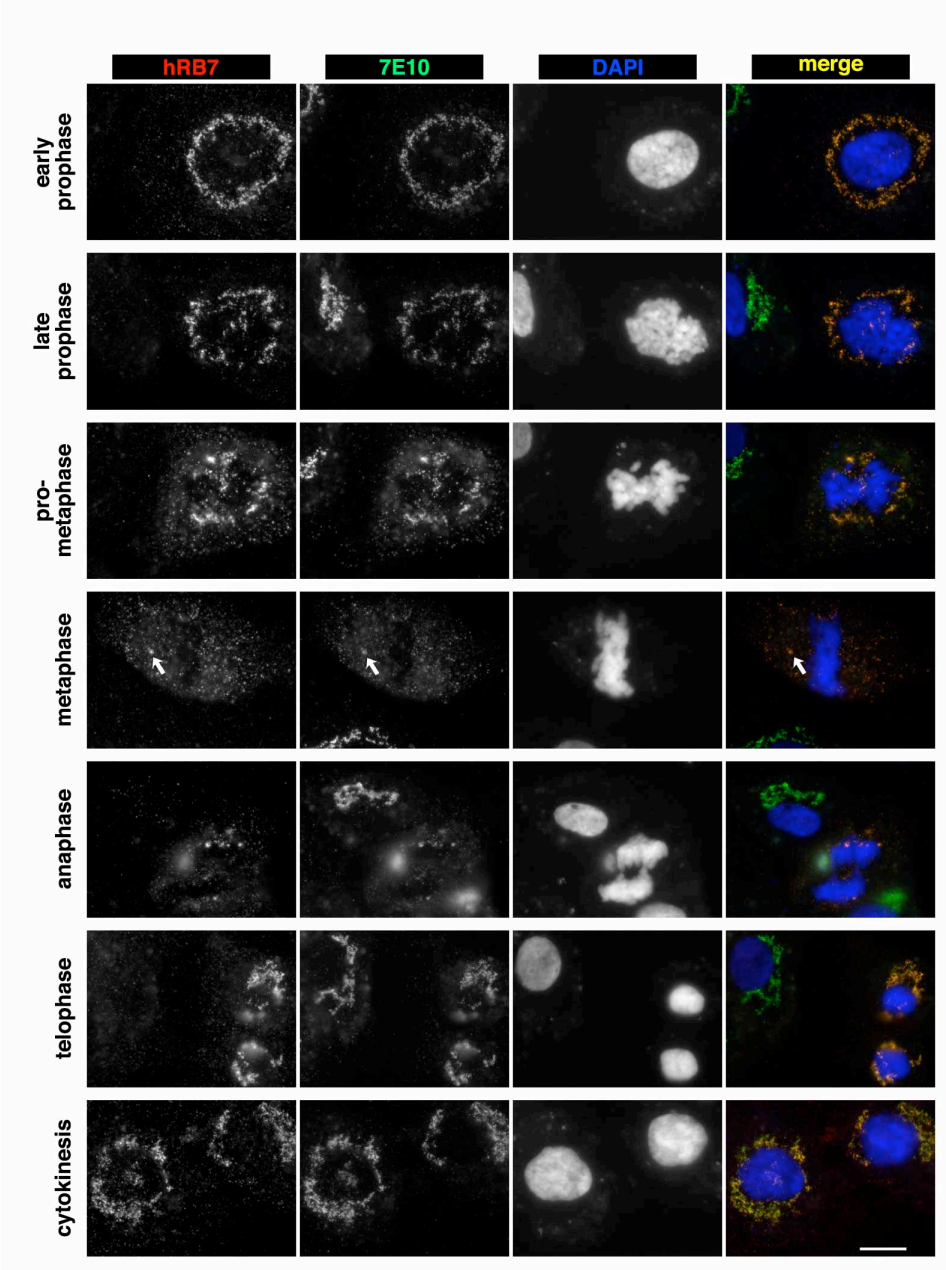
SUPPLEMENTARY FIGURES

SUPPLEMENTARY FIGURE 1



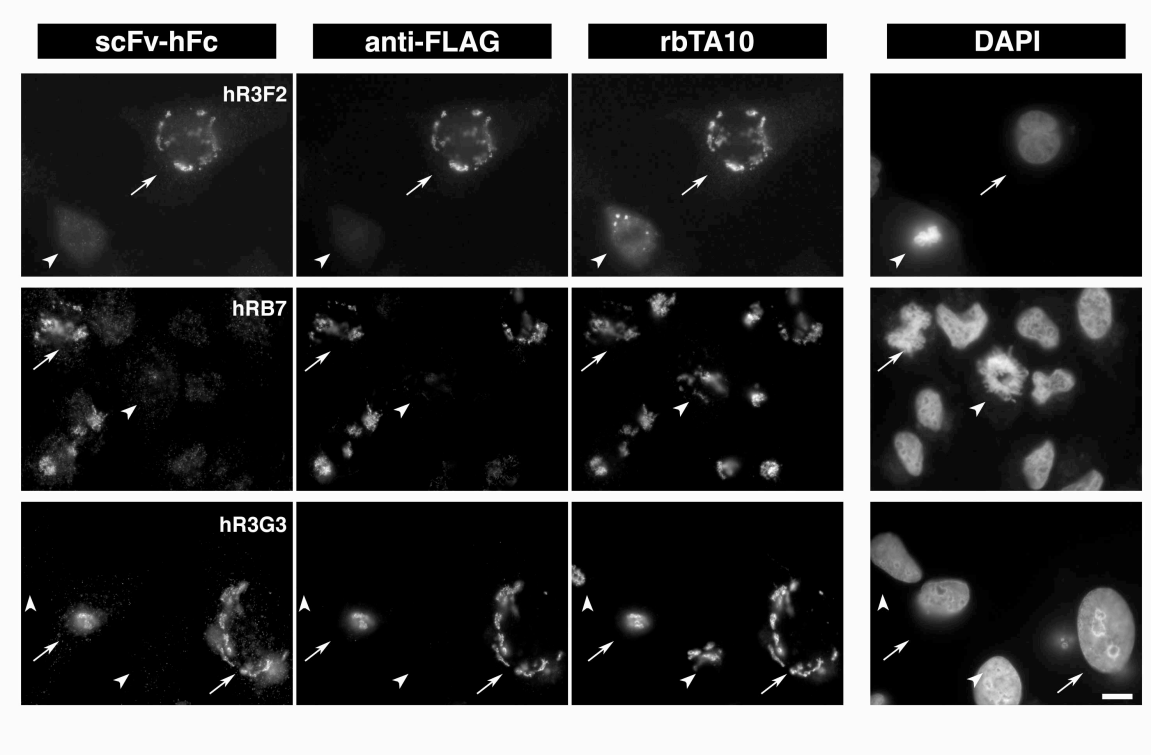
The recombinant antibody hR3F2 stains exclusively mitotic cells. Non-synchronized FR3T3 cells were fixed and stained with hR3F2, the anti-GRASP65 antibody 7E10 and DAPI. Cells in each phase of mitosis were selected and representative images are depicted. Note that Golgi complexes of cells in interphase are only decorated with 7E10 but not with hR3F2. Arrows indicate small visible Golgi cluster remnants in metaphase cells, detected by both antibodies. Bar: 10 µm.

SUPPLEMENTARY FIGURE 2



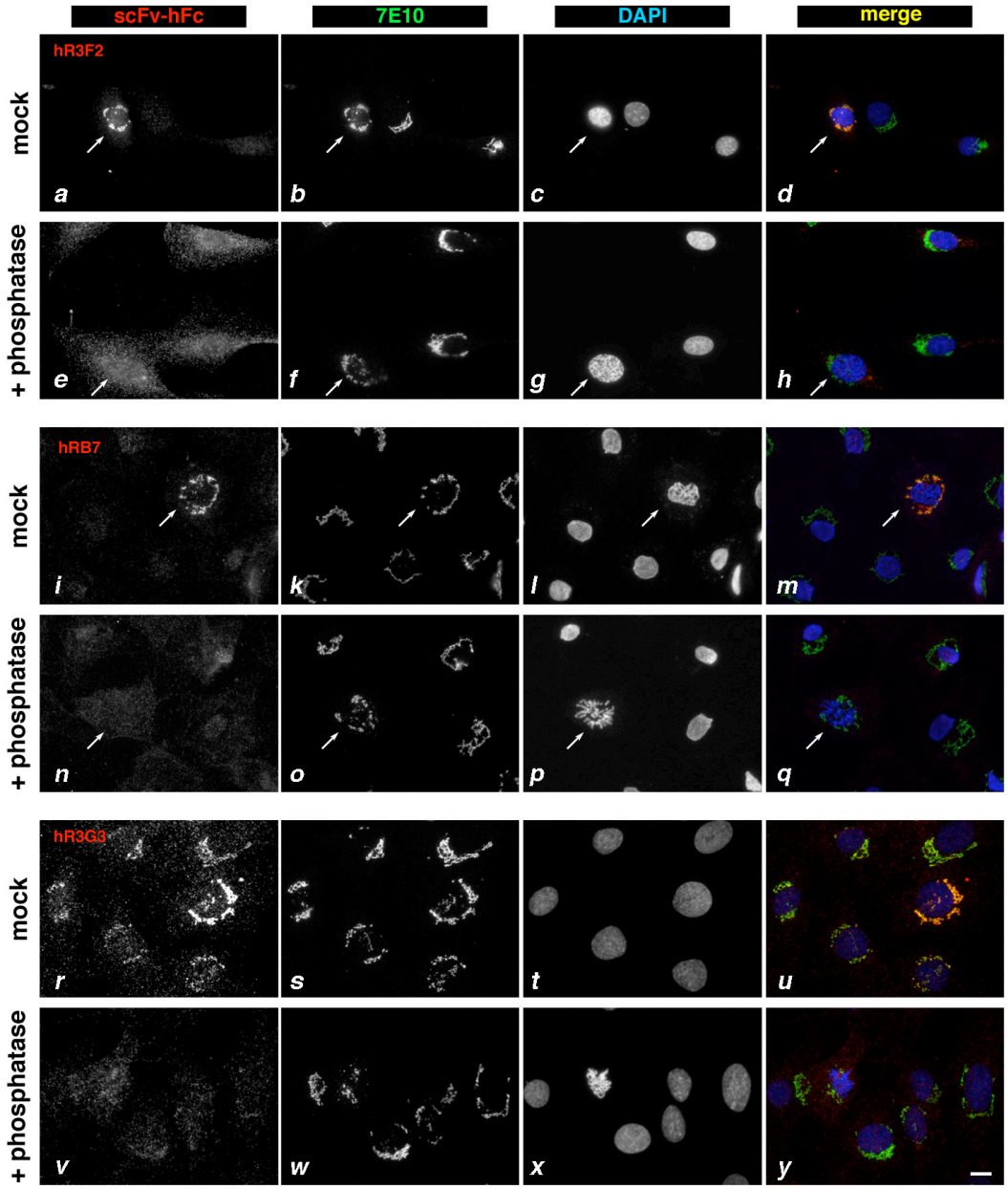
The recombinant antibody hRB7 stains cells only in mitosis. Non-synchronized FR3T3 cells were co-stained with hRB7 and 7E10, DNA was counterstained with DAPI. Cells in each phase of mitosis were selected. Note that Golgi membranes of cells in interphase are only decorated with 7E10 and not hRB7. Arrows indicate a small visible Golgi cluster remnant, which is detected by both antibodies. Bar: 10 µm.

SUPPLEMENTARY FIGURE 3



All three selected antibodies specifically stain rodent but not human GRASP65.
Wild-type HeLa cells were mixed with a HeLa cell clone stably expressing FLAG-tagged full-length rat GRASP65 before preparing them for immunofluorescence. While the recombinant rodent protein is recognized by all three antibodies (arrows), Golgi membranes in non-transfected cells, even when in mitosis (arrowheads), are not stained. The antibody rbTA10 (anti-giantin) stains Golgi membranes of all cells.

SUPPLEMENTARY FIGURE 4



Post-fixation lambda protein phosphatase treatment abolishes target recognition by hR3F2, hRB7 and hR3G3. FR3T3 cells were fixed in paraformaldehyde, permeabilized with Triton X100 and then, prior to performing immunofluorescence analysis, either mock treated (a-d, i-m, r-u), or exposed to λ-phosphatase (e-h, n-q, v-y). While the former had no effect on the labeling by any of the used antibodies, the latter specifically abrogated staining with hR3F2, hRB7 and hR3G3 (e, n, v). GRASP65 remained on Golgi membranes as indicated by staining with the anti-GRASP65 antibody 7E10 (f, o, w). Co-staining with a second scFv, rbTA10, directed against giantin, was likewise unaffected (not depicted). Arrows indicate cells in early mitosis (late prophase). Bar: 10 µm.

SUPPLEMENTARY REFERENCES

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