Supporting Information

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SI Methods

Protein Expression and Purification. Synthesis of recombinant hGBP1 either in its nonfarnesylated (hGBP1NF) or farnesylated forms (hGBP1F) was performed in the E. coli strain BL21 (DE3) (Novagen). Therefore, cells were transformed with pQE80L-hGBP1 only (to obtain hGBP1NF), or according to an established method with both pQE80L-hGBP1 and pRSF-Duet-FTase α/β for coexpression of FTase, which catalyzes farnesylation of hGBP1 in prokaryotic cells (25). Cell cultures were supplemented with ampicillin (100 μg/mL) only (hGBP1NF) or with both ampicillin (100 μg/mL) and kanamycin (25 μg/mL) (hGBP1F) and grown at 37 °C to an OD600 of 0.6-0.8. Expression was induced with 100 μM isopropyl β-D-thiogalactopyranoside at 25 °C for 16-18 h. After harvesting (6,000 x g, 10 min, 4 °C), cells were frozen and stored at −80 °C. For protein extraction, cell pellets were resuspended in buffer A (50 mM Tris-HCl, pH 7.4, 500 mM NaCl, 5 mM MgCl2) containing 1 mM phenylmethylsulfonyl fluoride and disrupted by sonication on ice. Cell debris was removed by centrifugation at 25,000 x g, 4 °C for 1 h and soluble, N-terminally hexahistidine-tagged hGBP1 in the supernatant was purified by affinity chromatography: A 30-mL column with HisPur Cobalt Resin (Thermo Scientific) previously equilibrated with buffer A was loaded with supernatant. After washing of unbound protein with buffer A, specifically bound protein was eluted with an imidazole gradient from 10 mM to 150 mM. Fractions containing the target protein were precipitated with 3 M of (NH4)2SO4 from 10 mM to 150 mM. Fractions containing the target protein and lipid channels of fluorescence (Fig. S7E) using a threshold filter for recognizing the membrane. Second, the script was searching two points of membrane differentiation by a self-designed algorithm (Fig. S7E, red stars) and the contact length L was calculated as a distance between two points with known coordinates.

FRET-Based Tracking of Protein Binding to LUVs. For monitoring hGBP1 binding to LUVs, the mutant Q577C in its farnesylated form (Q577C) was used. Exploiting the additional cysteine residue at the C terminus, labeling with Alexa-488 dye was performed in a similar way as described in material and methods, yielding AF-Q577C with a labeling efficiency of 30-40%. With this construct serving as donor and LUVs with Rhodamine-labeled lipids (Rhodamine-DPPE, Avanti) serving as acceptor, membrane binding of hGBP1 was monitored by FRET on the fluorescence spectrometer LS55 (Perkin-Elmer) (rmax = 495 nm, rmin = 590 nm, and slit width of 2.5/2.5 nm). AF-Q577C was preincubated with LUVs (final lipid concentration, 0.5 mg/mL) and supplemented with the corresponding nucleotide. LUVs were made by extrusion using 0.1-μm membranes if not specified otherwise out of BPL, with the PlotProfile build-in command of ImageJ across a narrow equatorial rectangle (Fig. S7). The fluorescence intensity profiles across a narrow equatorial rectangle taken for protein and lipid channels of fluorescence (Fig. S7D) for the dissociation and tethering assays, the fluorescence signal of the GUV-bound protein was quantified from the video recordings with the same ImageJ command in an automated way by using a javascript for ImageJ.

Quantification of the Contact Length Between a Pair of GUVs. The contact length was calculated as a distance between two edges of shared segment of the membrane for a given pair of GUVs. Coordinates of the edges of the contact line were obtained by running the self-designed javascript in ImageJ in two steps. First, the picture from the lipid channel was transformed into a digital form as shown in Fig. S7E, using a threshold filter for recognizing the membrane. Second, the script was searching two points of membrane differentiation by a self-designed algorithm (Fig. S7E, red stars) and the contact length L was calculated as a distance between two points with known coordinates.

Absorbance-Based Tethering Assay. The absorbance-based tethering assay used for atlastin studies (31) was modified such that the absorbance was monitored at 350 nm instead of 405 nm. GMP, GDP, GppNHp, or GTPyS was added at 400-μM concentration into the cuvette (Hellma Analytics) containing 0.5 mg/mL of LUVs and 30 μM of either hGBP1F or hGBP1NF. LUVs for these experiments were made by extrusion using 0.1-μm membranes if not specified otherwise out of BPL. For LUV preparation as well as for the reactions, buffer C1 (buffer C with 1 mM MgCl2) was used. Absorbance of the samples was measured on a Specord200 UV/Vis spectrophotometer (AnalytikJena), where the cuvette holder temperature was set to 30 °C.

Visual Tethering Assay. Visual tethering assay was modified from the assay used for atlastin studies (31). A total of 30 μM of hGBP1F, hGBP1NF, or R48AF was premixed with liposomes containing ATTO-488-DPPE (ATTO-TEC) (0.5% wt/wt) or Rhodamine-DPPE (Avanti) (0.5% wt/wt) lipids. The vesicles were mixed 1:1 (final lipid concentration 0.8 mg/mL) in a reaction buffer of C1. After addition of the nucleotides, the reaction mixture was incubated for 45 min at 30 °C. Further, the samples of the reaction mixture (15 μL) were diluted 1:20 into buffer C1 and loaded into a newly assembled open homemade observation chamber (55). Fluorescent vesicles were visualized by confocal microscopy. ATTO-488 and Rhodamine dyes were excited with 488- and 561-nm lasers and their emissions were collected with a dichroic mirror (405/488/561) and 525/50-nm and 595/50-nm filters, respectively. Image collection was done using a Nikon Eclipse Ti inverted microscope (Nikon) and NIS software. Image brightness and contrast were adjusted across the entire image, and merged images showing both dyes were generated using Fiji (v1.51e). Fluorescent vesicles were made by extrusion using 0.1-μm membranes.
Fluorescence Microscopy. For immunofluorescence microscopy, cells were fixed in PBS with 3% paraformaldehyde, permeabilized in PBS with 0.2% saponin (Sigma-Aldrich), and blocked in PBS, 3% BSA and 0.2% saponin. Primary and Alexa Fluor-labeled secondary antibodies (Life Technologies) were applied in blocking buffer. Coverslips were embedded in ProLong Gold Antifade (Life Technologies) and examined using a Zeiss Axioplan2 fluorescence microscope (Zeiss). Proteins and epitope tags were detected with the following antibodies against hGBP1 (IB1): Rab5 (3547, Cell Signaling), Rab7 (sc-10767, Santa Cruz Biotechnology), Rab9 (ALX-804–286, ENZO Life Sciences), EEA1 (610456, BD Biosciences), and Lamp1 (611042, BD Biosciences). For transient transfections, the GeneJuice reagent (Novagen) was used according to the manufacturer’s instructions.

Fig. S1. Binding of AF-hGBP1 to GUVs in the presence of GTP. (A) Referred to as the GUV pool, all GUVs in the chamber were screened one after another within a time slot of about 30 min. The experiment was started by addition of 1 mM GTP (t = 0 s) into 2.5 μM AF-hGBP1 premixed with GUVs. Time-dependent protein fluorescence was evaluated in homogeneous surface regions of GUVs. (B) Statistics of GUV phenotypes (as depicted in C–F) identified upon GTP-induced binding of AF-hGBP1 to a pool of vesicles. Representative images of AF-hGBP1-covered GUVs illustrate four different phenotypes: (C) Homogeneous distribution of the protein on the GUV surface, protein amount within clusters was less than 5%. This phenotype accounted for ∼70% of all GUVs. (D) Weak clustering, protein amount within clusters was either tiny or it covered not more than 25% of GUV’s surface. (E) Strong clustering, clusters of AF-hGBP1 covered between 25% and 75% of GUV’s surface, and (F) full clustering, most of GUVs’ surface (>75%) was covered with protein clusters. (Scale bar, 5 μm.) (G) Time lapse of GTP-induced AF-hGBP1 binding to GUVs showed that initial binding was followed by a drop of background fluorescence and appearance of floating protein clusters (only visible in the protein channel of fluorescence and indicated with white arrows). (Scale bar, 5 μm.) a.u., arbitrary units.
Fig. S2. Membrane binding and polymerization properties of hGBP1 mutants. Farnesylated GTPase-deficient mutant R48A (orange) and mutant K76A (blue), which has a slower GTPase activity and a deficient second hydrolysis step leading to a lack of GMP formation, were investigated for their capability for GUV binding and polymerization. (A) Comparison of GTP- (10 min) and GTPγS-dependent GUV binding of Alexa-fluorescent R48A (AF-R48A) and K76A (AF-K76A) to AF-hGBP1. Apo state of AF-hGBP1 is illustrated as representative for all mutants, which likewise failed to associate with membranes in the absence of nucleotide. (Scale bar, 5 μm.) A total of 10 μM of hGBP1 wild type (gray), R48A (orange), and K76A (blue) were investigated for their capability to polymerize. Polymerization was triggered by addition of 1 mM GTP (t = 0 s) in the absence of liposomes. Time courses of absorbance (B) and the fractions of nucleotides GTP (C), GDP (D), and GMP (E) analyzed by HPLC at different time points along the turbidity assay.
Fig. S3. Liposome binding of hGBP1 induced by GTP analogs and their biochemical characterization. (A–C) Binding of AF-Q557C to LUVs labeled with Rhodamine lipids. At time \( t = 0 \) s, indicated nucleotides (arrows) were added into a mixture of 0.5 mg/mL of LUVs and 2.5 \( \mu \)M of protein and an increase of fluorescence intensity reported liposome binding. (A) Addition of 200 \( \mu \)M GTP\( _{\gamma}S \) to LUVs of various sizes, 0.1 \( \mu \)m (orange) compared with 1 \( \mu \)m (gray). After \( \sim 800 \) s, GTP\( _{\gamma}S \) was displaced with 5 mM of GMP, leading to dissociation of the protein from LUVs. Regardless of the LUV sizes, single exponential fits (black lines) yielded similar dissociation rate constants (\( k_{\text{off}} = 0.0028 \text{ s}^{-1} \) orange, \( k_{\text{off}} = 0.0030 \text{ s}^{-1} \) gray). (B) Addition of either 400 \( \mu \)M GppNHp (magenta) or GDP (gray) to 0.1 \( \mu \)m LUVs. (C) Addition of various concentrations of GTP to 0.1 \( \mu \)m LUVs. The fluorescence traces of GTP-dependent liposome binding were interfered with GTP-dependent polymerization events as demonstrated by different concentrations of the substrate (cyan, 50 \( \mu \)M GTP; blue, 100 \( \mu \)M GTP). (D) Farnesyl- and membrane-dependent dissociation kinetics of hGBP1 and GTP were investigated with hGBP1\( _{\text{NF}} \) (blue) and hGBP1\( _{\text{F}} \) either in the absence (orange) or presence of 2 mg/mL LUVs (gray) using the MANT-labeled GTP analog GTP\( _{\gamma}S \) (mGTP\( _{\gamma}S \)) (19). The preformed complex of 1 \( \mu \)M protein and 0.5 \( \mu \)M mGTP\( _{\gamma}S \) was rapidly mixed with 500 \( \mu \)M of nonlabeled GMP to trigger quasi-irreversible displacement of the GTP analog. The decrease of MANT fluorescence (\( \lambda_{\text{ex}} = 366 \text{ nm}, \lambda_{\text{em}} \) detected through 420-nm cutoff filter) reporting dissociation of mGTP\( _{\gamma}S \) was fitted with a single exponential equation (black lines) yielding similar dissociation rate constants under all tested conditions (\( k_{\text{off}} = 1.18 \text{ s}^{-1} \) blue, \( k_{\text{off}} = 1.21 \text{ s}^{-1} \) orange, and \( k_{\text{off}} = 1.34 \text{ s}^{-1} \) gray). (E) GTP\( _{\gamma}S \) hydrolysis catalyzed by hGBP1\( _{\text{NF}} \) or hGBP1\( _{\text{F}} \) was investigated with 500 \( \mu \)M GTP\( _{\gamma}S \) and 10 \( \mu \)M of protein. Kinetics of GTP\( _{\gamma}S \) turnover fitted with linear regression (solid lines) yielded specific activities of 0.037 min\(^{-1}\) (hGBP1\( _{\text{NF}} \)) and 0.0058 min\(^{-1}\) (hGBP1\( _{\text{F}} \)). In the absence of protein (buffer C only), the GTP\( _{\gamma}S \) concentration remained stable over time (dotted line). (F) Binding of the nonhydrolyzable GTP analog GpCpp to hGBP1\( _{\text{F}} \) as tested by its ability to compete with mGTP\( _{\gamma}S \) used as fluorescent reporter of nucleotide binding (\( \lambda_{\text{ex}} = 366 \text{ nm}, \lambda_{\text{em}} = 435 \text{ nm} \)). At \( t = 0 \) s, addition of 0.5 \( \mu \)M hGBP1\( _{\text{F}} \) to 0.25 \( \mu \)M mGTP\( _{\gamma}S \) in the cuvette led to a fast and more than twofold increase of the fluorescence signal, indicating protein/nucleotide complex formation. Addition of 250 \( \mu \)M nonfluorescent GpCpp in contrast to GTP\( _{\gamma}S \) (arrows) did not suffice to displace the preformed protein/nucleotide complex, suggesting that hGBP1\( _{\text{F}} \) does not bind GpCpp.
Fig. S4. EM images of hGBP1. (A) Representative images of either hGBP1<sub>F</sub> or hGBP1<sub>NF</sub> incubated with the indicated nucleotides. Experimental mixture contained 10 μM of hGBP1<sub>F</sub> or hGBP1<sub>NF</sub> and 1 mM of corresponding nucleotide, except for samples with GDP·AlFx, which contained 200 μM GDP, 300 μM AlCl<sub>3</sub>, and 10 mM NaF. Reactions were stopped after ∼10 min (GTP) and after ∼20 min (GTPγS and GDP·AlFx). (Scale bars, 200 nm.) (B) Measures for the identified polymer structures (Fig. 2) of hGBP1<sub>F</sub> in the presence of indicated nucleotides as schematically drawn. The numbers of quantified structures (N in parentheses) as well as obtained mean values with SDs are indicated along each scheme.
Fig. S5. Nucleotide-dependent polymerization of hGBP1. (A and B) Absorbance of the sample with different concentrations of hGBP1F (A) and hGBP1NF (B) after addition of 1 mM GTP (t = 0 s). (C and D) Analysis of polymer formation of 20 μM hGBP1F (C) and hGBP1NF (D) by dynamic light scattering. The particle size, given as the hydrodynamic radius (R_H) in nanometers without nucleotide (blue) and in the presence of 1 mM GTP (gray) or GDP·AlFx (orange) (300 μM AlCl_3, 10 mM NaF, and 200 μM GDP) was monitored over 250–450 s at 25 °C. Farnesylated hGBP1 transiently forms very large polymers during GTP hydrolysis with a radius around 1,000 nm. In contrast, the addition of GDP·AlFx leads to an irreversible formation of polymers with an average R_H of 25 nm. Nucleotide-free hGBP1NF shows a R_H of 5 nm. Complexation with GDP·AlFx leads to a slight irreversible increase to 6.5 nm and addition of GTP to a transient increase to a maximum value of 7.2 nm. (E) Reversibility of GTP-induced hGBP1F polymers investigated by sedimentation. During a typical turbidity experiment (see A, 10 μM hGBP1F and 1 mM GTP added at t = 0 s), samples were taken at indicated time points (arrows), spun down, and the fraction of protein in pellet (P) and supernatant (S), respectively, was analyzed by SDS/PAGE (100-μL samples were taken, centrifuged at 30,000 × g, 4 °C and for 1 min. After removal of the supernatant, the pellet was resuspended in the same volume of buffer, and corresponding fractions of protein in pellet (P) and supernatant (S) were analyzed by SDS/PAGE. Considering that the proportion of protein in the pellet represents protein complexes large enough to sediment, e.g., polymers, SDS/PAGE analysis reflected the same observation as already monitored by time-trace of absorbance: The GTP-induced increase and decrease of the absorbance considered as reversible assembly and disassembly of hGBP1F polymers was accompanied by increase and decrease of protein share in the pellet.

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Fig. S6. Polymerization and GTPase activity of hGBP1 under various lipid and temperature conditions. (A and B) Time courses of hGBP1 polymer absorbance (A) and the obtained background absorbance of liposomes as a function of lipid concentration (B). Experiments were performed with 10 μM hGBP1 in the absence of liposomes (black) and in the presence of 1 mg/mL (gray), 2 mg/mL (blue), and 5 mg/mL (orange) of final lipid concentration. Polymerization was triggered upon addition of 1 mM GTP (t = 0 s). (C and D) Analysis of hGBP1 catalyzed GTP hydrolysis products GDP (C) and GMP (D) for same experiments. (E–H) Nucleotide composition of the GTPase reaction performed either with 2 μM hGBP1F at 25 °C (E) and 37 °C (F) or with 2 μM hGBP1NF at 25 °C (G) and 37 °C (H) in the absence of liposomes (filled symbols) or in the presence of 0.67 mg/mL PIP3 liposomes (open symbols).
Quantification of the fluorescence signal of membrane-bound hGBP1 and calculation of the contact length in the tethering assay. Typical images of GUVs for AF-hGBP1 in the presence of GTPγS for the protein channel of fluorescence (A), the lipid channel of fluorescence (B), or the merge of two fluorescence channels (C) as an example for quantification of the fluorescence signal of membrane-bound hGBP1. (Scale bar, 5 μm.) (D) Superimposed fluorescence intensity profiles across narrow equatorial rectangle as shown in B, taken from A and B. (E) Schematic representation of the image-processing algorithm for contact length calculation in tethering assay. The fluorescent signal from the lipid channel of fluorescence was set to the value of 1 when fluorescent intensity was higher than the threshold value and set to the value of 0 otherwise. The contact length (L) between the two vesicles is indicated. Points of membrane differentiation are indicated as red stars. The gray outlined arrow indicates that the two rectangles correspond to each other.
Fig. S8. Nucleotide-dependent membrane tethering mediated by hGBP1. (A) HGBP1-mediated tethering followed by absorbance-based tethering assay. The increase in particle size was monitored by the absorbance at 350 nm. The initial absorbance value of 0.2 was subtracted. A total of 400 μM of the corresponding nucleotide was added at t = 0 s in the presence of 30 μM of the corresponding protein. Addition of GTPγS was done for LUVs of various sizes, 0.1 μm (black) compared with 1 μm (cyan). A total of 10 mM GMP was added after 45 min (arrow) to dissociate tethered LUVs. Reactions were done at 30 °C. (B) HGBP1-mediated tethering followed by visual tethering assay. LUVs, which also contained either ATTO-488 or Rhodamine-labeled lipids, respectively, were mixed at a 1:1 ratio (total lipid concentration, 0.8 mg/mL) in the presence of 30 μM of either hGBP1 or hGBP1NF. All samples were incubated with 400 μM of the corresponding nucleotide for 45 min at 30 °C. To dissociate tethered LUVs in the presence of GTPγS, one of the reaction mixtures with GTPγS was additionally supplied with GMP after 45 min (final concentration of 36 mM) and incubated for 10 more minutes at 30 °C. All samples were diluted, loaded into the observation chamber, and visualized by confocal microscopy. (Scale bar, 10 μm.) (C) As in B but with the GTPase-deficient farnesylated mutant R48A.
Recruitment of endogenous hGBP1 to phagocytosed latex beads. HeLa cells were IFN-γ induced for 24 h and then incubated for 4 h with non-fluorescent (A and D) or blue-fluorescent (B, C, and E) latex beads with 2-μm (A–D) or 1-μm (E) diameter. The monoclonal 1B1-antibody against hGBP1 and several antibodies against distinct structures of the phagolysosomal pathway were used for costaining. (A) Rab9 (LE/TGN) and Rab5 (EE). (B) LAMP1 (lysosomes) and Rab5 (EE). (C) EEA1 (EE) and Rab7 (LE). (D and E) EEA1 (EE) and phalloidin (filamentous actin). Arrows mark engulfed latex beads within the cytoplasm. (Scale bar, 10 μm.) EE, early endosomes; LE, late endosomes; TGN, trans Golgi network.
Fig. S10. Colocalization of recombinant hGBP1 and marker proteins around latex beads. Unstimulated HeLa cells were cotransfected with mCherry-hGBP1 as well as GFP-tagged marker proteins and incubated with 2 μm latex beads for 4 h to specify distinct stages of the phagocytic pathway. (A) GFP-Rab5. (B) GFP-Rab7. (C) GFP-Rab9. (D) LAMP1-GFP. (Scale bar, 5 μm.) Arrows mark engulfed latex beads within the cytoplasm. (E) Life-cell imaging of recruitment of hGBP1 and Rab5 to latex bead phagosomes. Uninduced HeLa cells transfected with mCherry-hGBP1 and GFP-Rab5 were incubated with blue fluorescent latex beads for 4 h and uptake of beads was followed by life-cell imaging. Arrows indicate a phagocytosed bead, which is attached to Rab5+ (green) and hGBP1+ (red) punctate structures. See also Movie S6. Time scale, hours:minutes:seconds (h:min:s). (Scale bar, 10 μm.)
**Movie S1.** Dissociation of hGBP1 from the surface of GUVs, related to Fig. 1. Observation of AF-hGBP1 and AF-R48A dissociation from the surface of GUVs by color confocal videomicroscopy in the presence of either 400 μM GTP\(_\gamma\)S or 400 μM GTP. Fluorescent proteins were shortly locally injected into the vicinity of observed GUVs from the injection pipette, containing 90 μM of corresponding protein. Left part of the movie is the same field as Fig. 1F. Seven frames per second (fps). Time scale, h:min:s.

**Movie S1**

**Movie S2.** Tethering of GUVs in the presence of farnesylated hGBP1 and GTP\(_\gamma\)S, related to Fig. 4. Observation of the tethering experiment for a pair of GUVs by dual-color confocal videomicroscopy, performed in the presence of 20 μM of nonlabeled hGBP1, and 2.5 μM of AF-hGBP1, upon injection of GTP\(_\gamma\)S. Nucleotide was shortly locally injected into the vicinity of observed pair of GUVs from the injection pipette, containing 400 μM GTP\(_\gamma\)S (same field as Fig. 4A; 7 fps). Green channel corresponds to AF-hGBP1 signal, red channel to BodipyTMR-PIP2. Time scale, h:min:s.

**Movie S2**
Movie S3. Tethering of GUVs in the presence of farnesylated hGBP1 and GTP, related to Fig. 4. Observation of the tethering experiment for a pair of GUVs by dual-color confocal videomicroscopy, performed in the presence of 20 μM of nonlabeled hGBP1 F and 2.5 μM of AF-hGBP1 F upon injection of GTP. Nucleotide was shortly locally injected into the vicinity of the observed pair of GUVs from the injection pipette containing 400 μM GTP (same field as Fig. 4B; 7 fps). Green channel corresponds to AF-hGBP1 F signal, red channel to BodipyTMR-PIP2. Time scale, h:min:s.

Movie S4. Tethering of GUVs in the presence of nonfarnesylated hGBP1 and GTPγS, related to Fig. 4. Observation of the tethering experiment for a pair of GUVs by dual-color confocal videomicroscopy performed in the presence of 20 μM of nonlabeled hGBP1 NF and 2.5 μM of AF-hGBP1 NF upon injection of GTPγS. Nucleotide was shortly locally injected into the vicinity of the observed pair of GUVs from the injection pipette containing 400 μM GTPγS (same field as Fig. 4C; 2 fps). Green channel corresponds to AF-hGBP1 NF signal, red channel to BodipyTMR-PIP2. Time scale, h:min:s.

Movie S5. Tethering of GUVs in the presence of nonfarnesylated hGBP1 and GTP. Observation of the tethering experiment for a pair of GUVs by dual-color confocal videomicroscopy, performed in the presence of 20 μM of nonlabeled hGBP1 NF and 2.5 μM of AF-hGBP1 NF upon injection of GTP. Nucleotide was shortly locally injected into the vicinity of the observed pair of GUVs from the injection pipette containing 400 μM GTP (4 fps). Green channel corresponds to AF-hGBP1 NF signal, red channel to BodipyTMR-PIP2. Time scale, h:min:s.
Movie S6. Life-cell imaging of recruitment of hGBP1 and Rab5 to latex bead phagosomes, related to Fig. S10. Life-cell imaging of recruitment of hGBP1 and Rab5 to latex bead phagosomes. Uninduced WT HeLa cells transfected with mCherry-hGBP1 and GFP-Rab5 were incubated with blue-fluorescent latex beads for 4 h and uptake of beads was followed by life-cell imaging (23 fps). Time scale, h:mins.