SUPPLEMENTARY PROTOCOL FOR IMMUNOSTAINING GFP WITH GBP-MTN

1. Cell culture as described in Section 1.
2. Fixation
   Remove cell culture medium and wash cells with 0.2 M HEPES once. Add 2 mL of 0.1% GA or 4% PFA in 0.2 M HEPES to the 35mm culture dish for 5 min fixation at RT;
3. Neutralization
   Change the solution in the culture dish with 2 mL of 50 mM glycine in 0.2 M HEPES for neutralization at 4 °C overnight; Simply suck away the solution in the dish for next step.
4. Permeabilization
   Permeabilize with 2 mL of 0.05% triton X-100 in 0.2 M HEPES in the culture dish at room temperature for 2 min.
5. Blocking
   Block cells in antibody blocking solution (0.25% cold fish gelatin and 0.1% BSA in 0.2 M HEPES) for 2 h at RT.
6. Immunolabling
   Incubate cells with 2 mL of 50 μg/mL GBP-MTn in blocking solution at 4 °C overnight;
   Wash with 2 mL of 0.2 M HEPES for 5 min x 3;
   Wash with 2 mL of PBS-A for 5 min x 3;

   Ready for the rest standard steps are the same as the above Section 3-6.