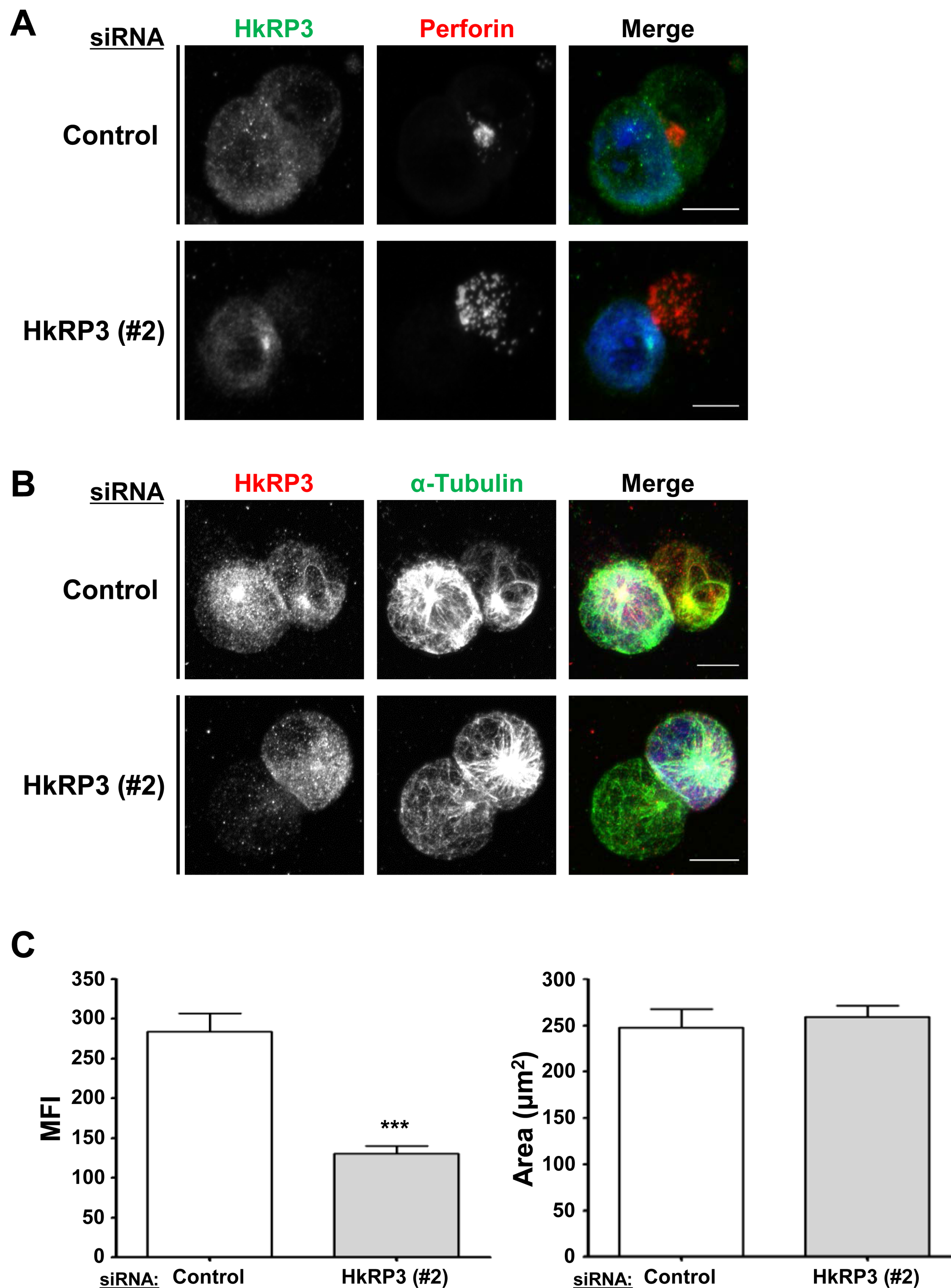
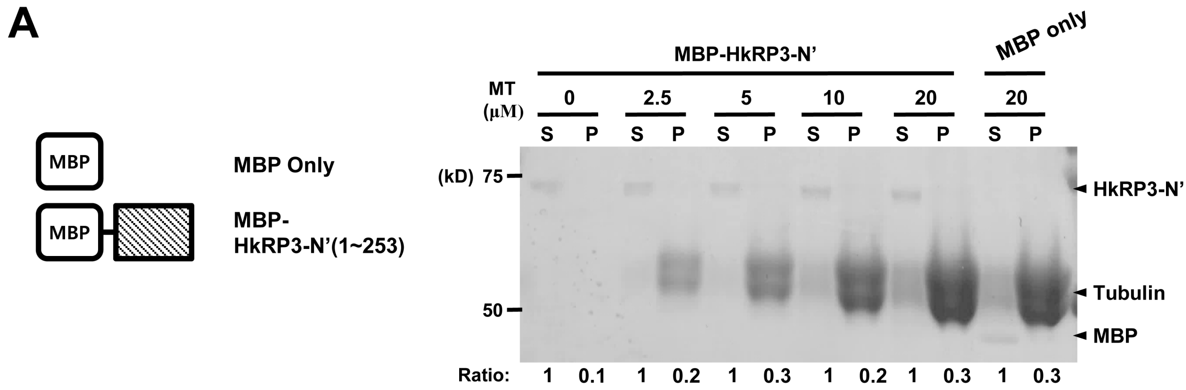
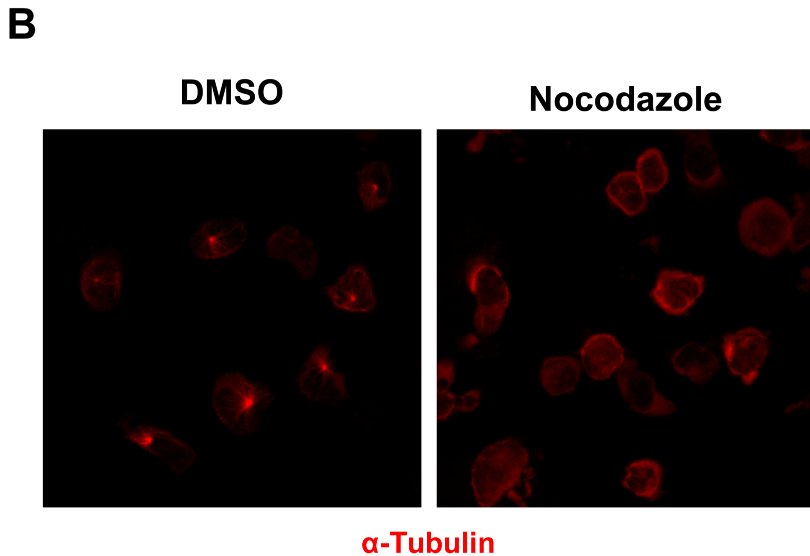


Supplemental Figure 1. Anti-HkRP3 antibody is specific for immunofluorescence staining and HkRP3 suppression does not affect the MT network. YTS cells were transfected with indicated siRNAs. (A and B) At 72 h, these cells were conjugated with CMAC-stained 721.221 target cells (blue), incubated for 25 min at 37 °C, and fixed for immunofluorescence staining as indicated. Data shown are representative of three independent experiments. Bars, 10 μm. (C) 72h of post-transfection, cells were plated on coverslip, and fixed for immunofluorescence staining with anti-HkRP3 antibody and phalloidin. Then, the whole cell area visualized by phalloidin-labelled cortical actin (right) and mean fluorescent intensity (MFI) of HkRP3 inside the area (left) were measured. Data shown are collected and analyzed from 10 single cells of each condition. Error bars indicate SEM. ***$P < 0.005$ compared with control group.

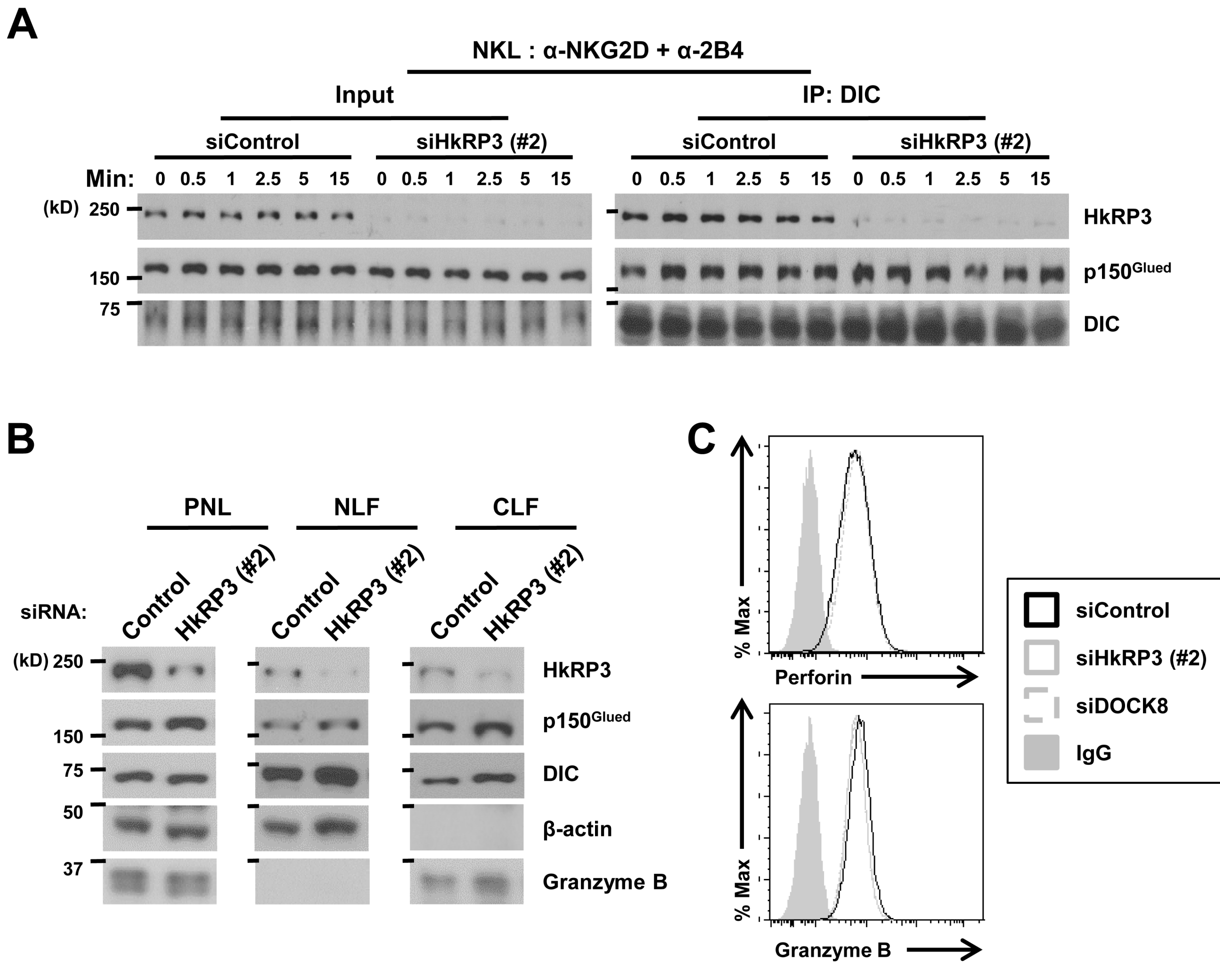
A



B



Supplemental Figure 2. N-terminal region of HkRP3 does not interact with microtubules, and HkRP3 interacts with the dynein complex in a microtubule-independent manner. (A) (Left) Schematic representation of HkRP3 fragments used for microtubule co-pelleting assay. (Right) Microtubule co-pelleting assay. 0.5µM of either MBP or MBP-HkRP3-N' was incubated with MTs of indicated concentrations. After centrifugation, supernatants (S) and pellets (P) were collected and examined for tubulins and fusion proteins by coomassie blue staining. Arrowheads denote band of tubulin and fusion proteins. The numbers beneath the staining image provide densitometric ratio of each signal to signal from supernatant fraction within the same sample group. (B) YTS cells were treated with 10 µM nocodazole or the same volume of DMSO for 1 h at 37 ˚C, and washed. Some cells were fixed for immunofluorescence staining for α-tubulin to confirm disruption of the microtubule network. The remaining cells were lysed and immunoprecipitated using anti-HkRP3 (Fig. 7D).



Supplemental Figure 3. HkRP3 does not affect interaction of dynein intermediate chain with p150^{Glued}, lytic granule contents, or localization of dynein complex relative to lytic granules. (A) At 72 h after siRNA transfection into NKL cells, NK activating receptors (NKG2D and 2B4) were stimulated using antibodies (see Materials and Methods for details). Whole cell lysates and anti-DIC (dynein intermediate chain) immunoprecipitates were examined for HkRP3, p150^{Glued}, and DIC. Data shown are representative of four independent experiments. (B) At 72 h after siRNA transfection into YTS cells, postnuclear lysate (PNL), non-lysosomal fraction (NLF), and crude lysosomal fraction (CLF) were prepared from each group of YTS cells, and examined for HkRP3, p150^{Glued}, DIC, β-actin, and granzyme B by immunoblot. Data shown are representative of three independent experiments. (C) Intracellular levels of perforin (top) and granzyme (bottom) in control or HkRP3-, or DOCK8-depleted YTS cells was determined by flow cytometry. Presented data are representative of three independent experiments.