Supplemental Figures

Figure S1. Specific knockdown of ArfGAP1, ArfGAP2 and ArfGAP3 by RNAi. HeLa cells were treated for 120 h with siRNAs indicated, and subjected to immunoblot (A) or immunofluorescence (B) analysis.

Figure S2. Depletion of ArfGAP1 shows no discernible phenotypic change. HeLa cells were treated for 120 h with siRNAs for LacZ (A, control) or ArfGAP1 (B), and doubly stained for ArfGAP1 (a-c) and either β-COP (a'), ERGIC-53 (b') or GM130 (c').

Figure S3. Association of ArfGAP1, ArfGAP2 and ArfGAP3 with the *cis*-Golgi. HeLa cells were left untreated (A-F) or treated with 5 μg/ml nocodazole for 2 h (G-L), and doubly stained for ArfGAP1 (A, D, G and J), ArfGAP2 (B, E, H and K) or ArfGAP3 (C, F, I and L), and GM130 (A-C and G-I) or golgin-245 (D-F and J-L).

Figure S4. Triple ArfGAP- or β-COP does not affect transferrin endocytosis. HeLa cells treated with siRNAs for LacZ (top panels), ArfGAP1+ArfGAP2+ArfGAP3 (middle panels) or β-COP (bottom panels) were incubated with AlexaFluor488-conjugated transferrin for indicated time periods as described under Experimental Procedures and stained for ERGIC-53 to identify cells with stage 1 and stage 2 phenotypes (marked by '1' and '2', respectively).

Figure S5. Two distinct phenotypic stages in cells depleted of COPI. (A) HeLa cells treated with siRNAs for β-COP for 24 h were stained for ERGIC-53. A cell with normal distribution of ERGIC-53, or that with typical stage 1 or stage 2 distribution is shown. (B) HeLa cells treated with siRNAs for β-COP for 12 or 24 h were classified as having those with normal, stage 1 and stage 2 distributions of ERGIC-53, and the number of cells with each distribution was counted.

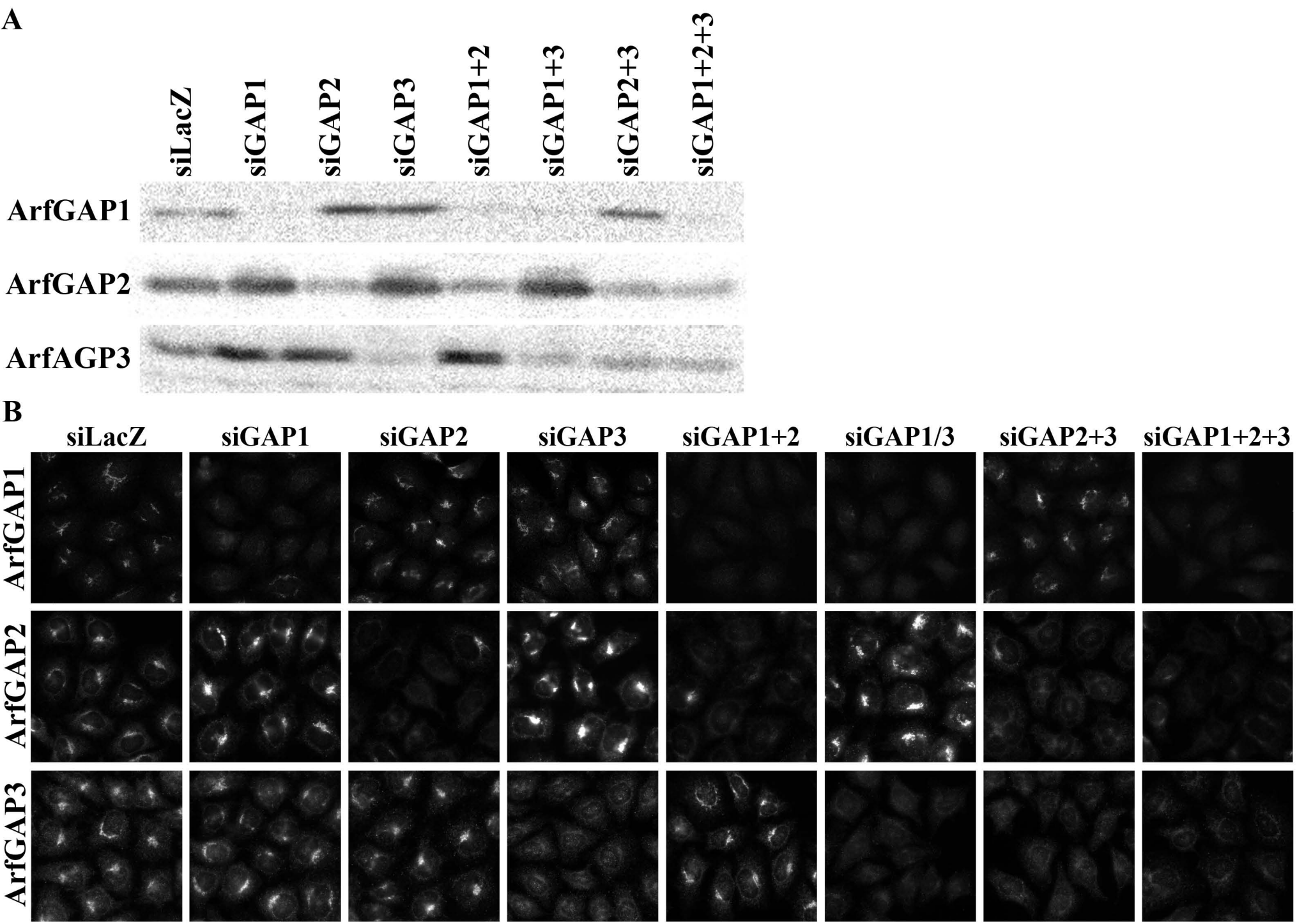

Figure S1 (Saitoh et al.)

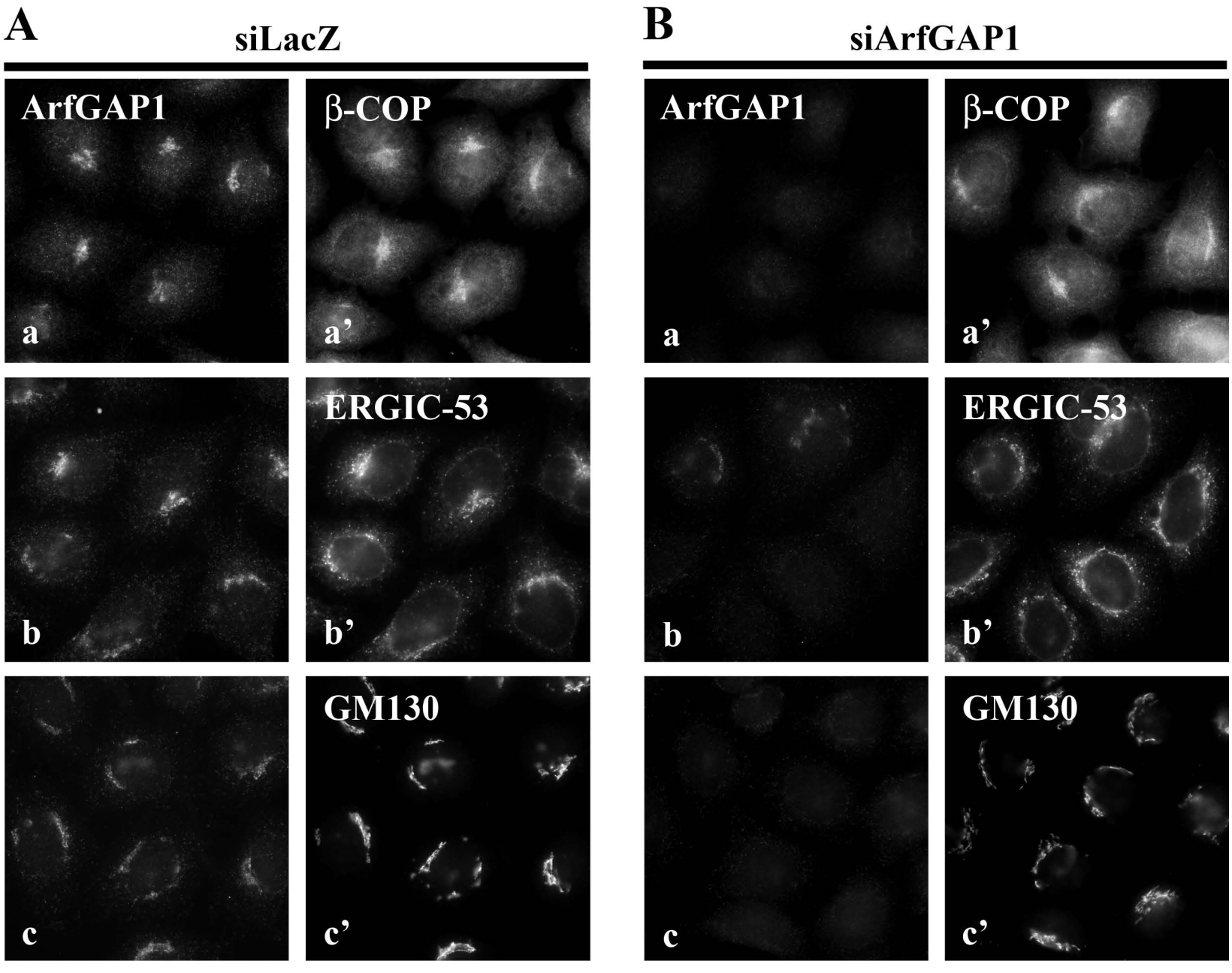

Figure S2 (Saitoh et al.)

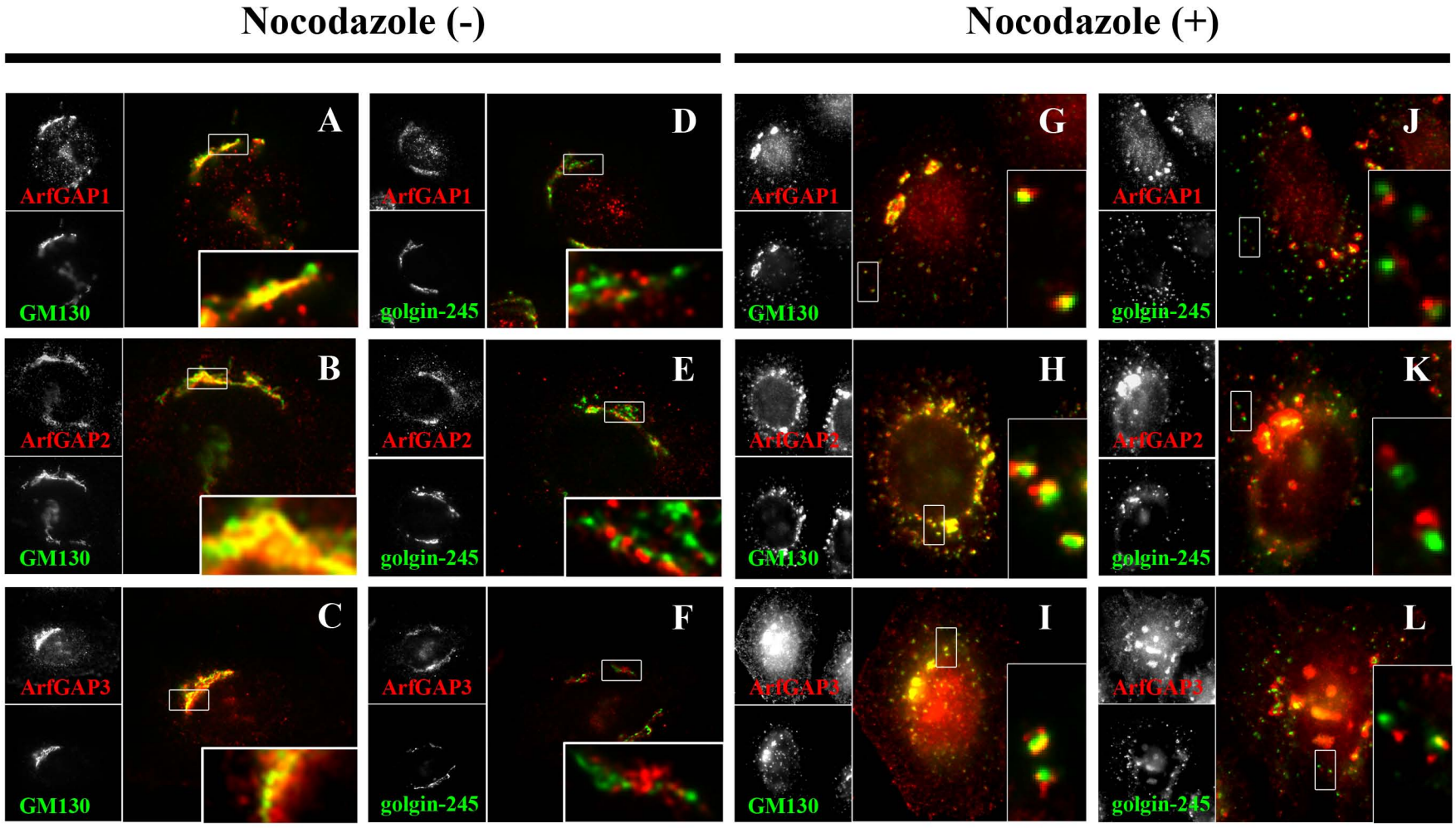

Figure S3 (Saitoh et al.)

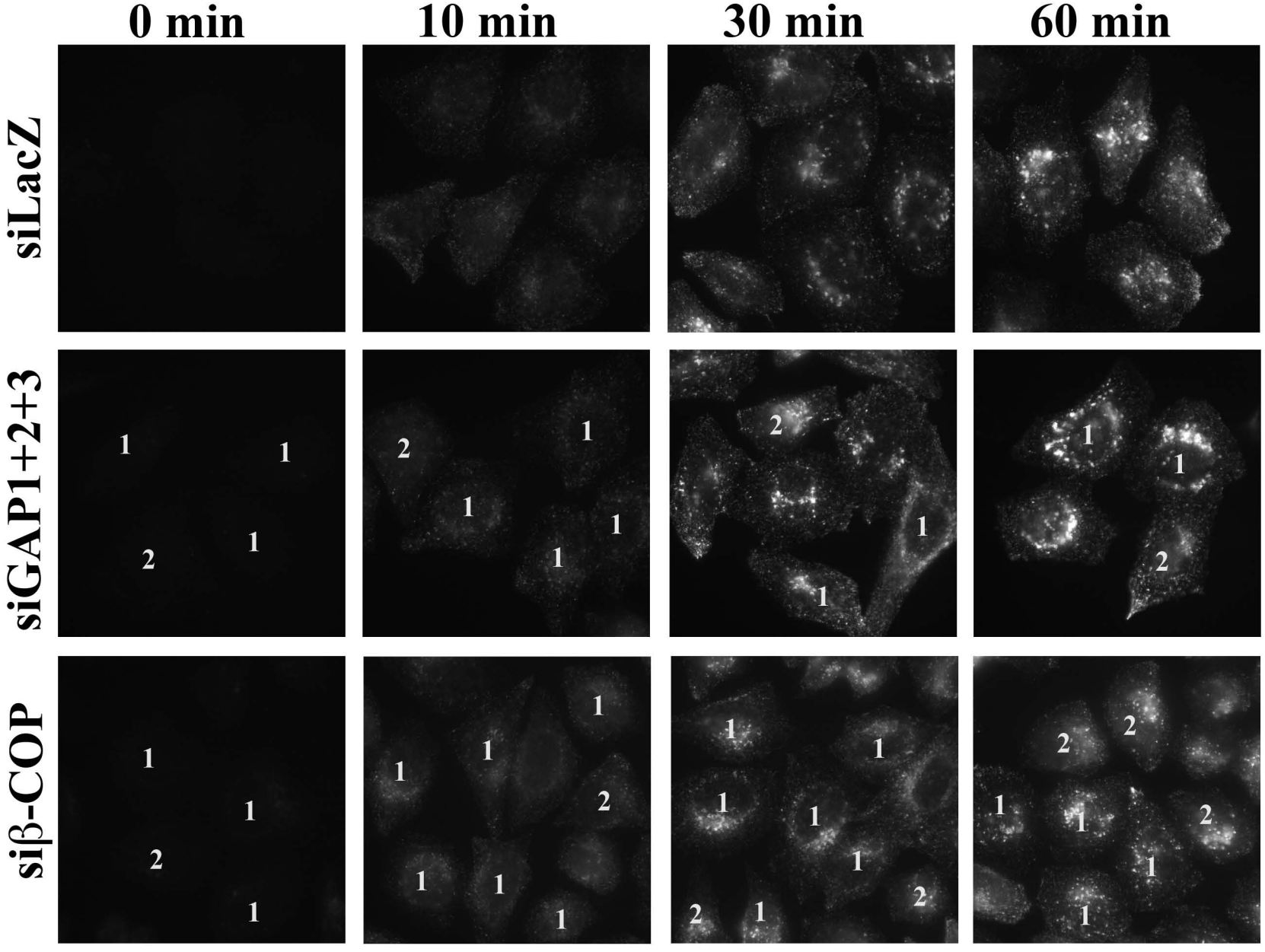

Figure S4 (Saitoh et al.)

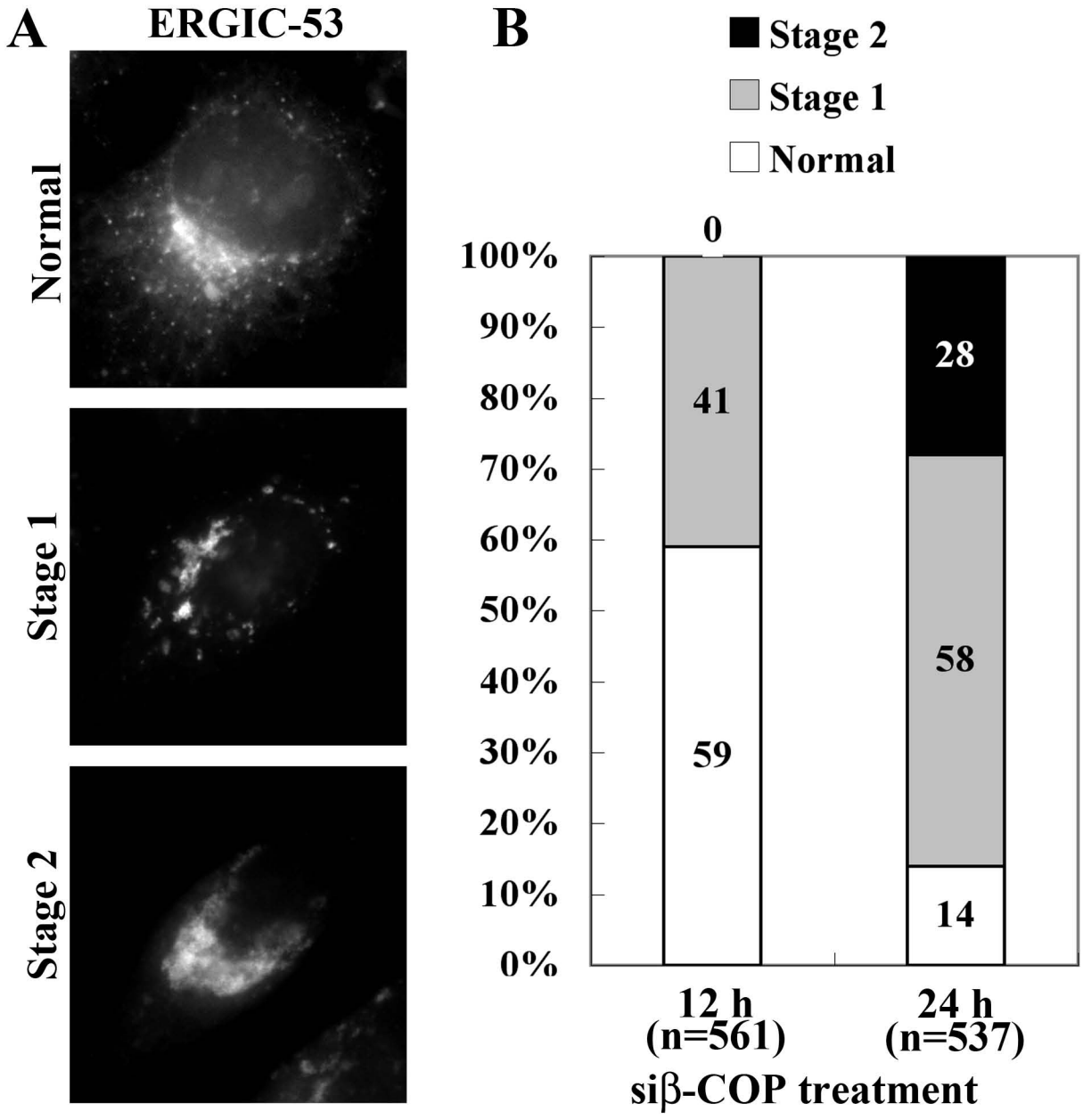

Figure S5 (Saitoh et al.)