**Supplementary information**

# Plk1 phosphorylation of GTSE-1 is essential for p53 inactivation during G2 checkpoint recovery

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## Supplemental Experimental Procedures

### Vector construction and RNAi

To specifically deplete endogenous GTSE-1 in U2OS cells, plasmid pLKOpuro.1-GTSE-1 was constructed. The targeting sequence of human GTSE-1 was AAAUUUGACUUCGAUCUUUCA (Monte *et al*, 2003). Lentivirus targeting p53 were generated as described previously (Liu *et al*, 2006). The targeting sequence used to design siRNA against human Plk1 was: AAGGGCGGCTTTGCCAAGTGCTT (Liu and Erikson, 2002).

### Cell culture and transfection

U2OS and 293T cells were cultured in Dulbecco modified Eagle medium (DMEM) supplemented with 10% (vol/vol) fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin at 37°C in 8% $CO_2$. dsRNA was transfected using transmessenger transfection reagent (QIAGEN), whereas plasmid DNA was transfected using MegaTran (ORIGENE) as described by the manufacturers.

### Immunoprecipitation, immunoblotting (Western blotting) and immunofluorescence staining

For immunoprecipitation (IP), whole cell lysates were incubated with different antibodies indicated in each experiment for 2 hr at 4°C, followed by 1 hr of incubation with protein A/G-Sepharose beads. Immunocomplexes were resolved by SDS-PAGE, and co-immunoprecipitated proteins were detected by immunoblotting using antibodies indicated in the specific experiments. Antibodies against Plk1, Flag, and GFP were used at 1:1000 dilution, and antibody against pS435 was used at 1:200. For immunofluorescence staining, cells were fixed in 4% paraformaldehyde, treated with cold menthol and stained with specific antibodies followed by a fluorescein-conjugated secondary antibody (Invitrogen). Antibody against pS435 was used at 1:500 dilution.

**Supplementary Figure Legends**

**Fig S1 |** Depletion of GTSE-1 by RNAi does not affect normal mitotic progression. (**A**) U2OS cells were transfected with pLKOpuro.1-GTSE-1 or pLKOpuro.1 vector, and the transfection-positive cells were selected by puromycin (10 μg/mL) treatment for two days. GTSE-1-depleted cells were synchronized with the double thymidine block (DTB, 16 h treatment with thymidine, 8 h of release, and a second 16 h block with thymidine) at G1/S boundary. After release from the second thymidine block for different times, the cells were harvested and stained with phospho-histone H3. The mitotic index was determined by counting p-H3-positive cells. (**B**) U2OS and MCF10A cells were transfected with pLKOpuro.1-GTSE-1, and transfection-positive cells were selected by puromycin (10 μg/mL) for two days and harvested for immunoblotting.

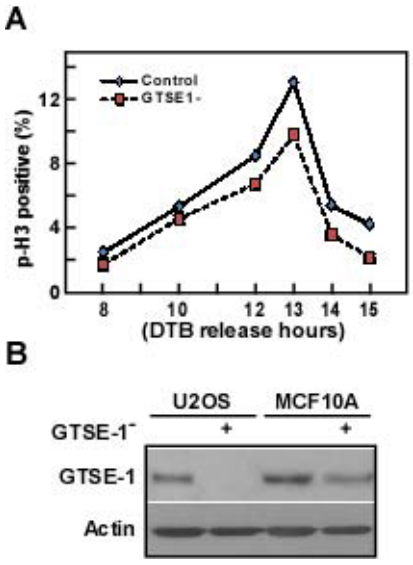
**Fig S2 |** Requirement of GTSE-1 for the G2 checkpoint recovery is p53-depentdent. (**A**) Upon transfection with p53-GFP-WT or p53-GFP-NES (nuclear export signal deficient mutant), U2OS cells were processed as described in Fig 3B. DNA was stained with DAPI to determine the subcellular localization of p53. Scale bar, 10 μm. (**B**) U2OS (p53+/+) and H1299 (p53-/-) cells were processed as described in Fig 3C and immunoblotted.

**Fig S3 |** Plk1 phosphorylation of GTSE-1 does not affect normal cell cycle progression. (**A**) Cell-cycle profiles of U2OS cells stably expressing GFP vector or GFP-GTSE-1 constructs (WT, S435A or S43E). (**B**) U2OS cells stably expressing GFP vector or GFP-GTSE-1 constructs (WT, S435A or S43E) were synchronized with the double thymidine block (DTB) protocol, released for different times, and stained with phospho-histone H3 antibodies. The mitotic index was determined by counting phospho-H3-positive cells.

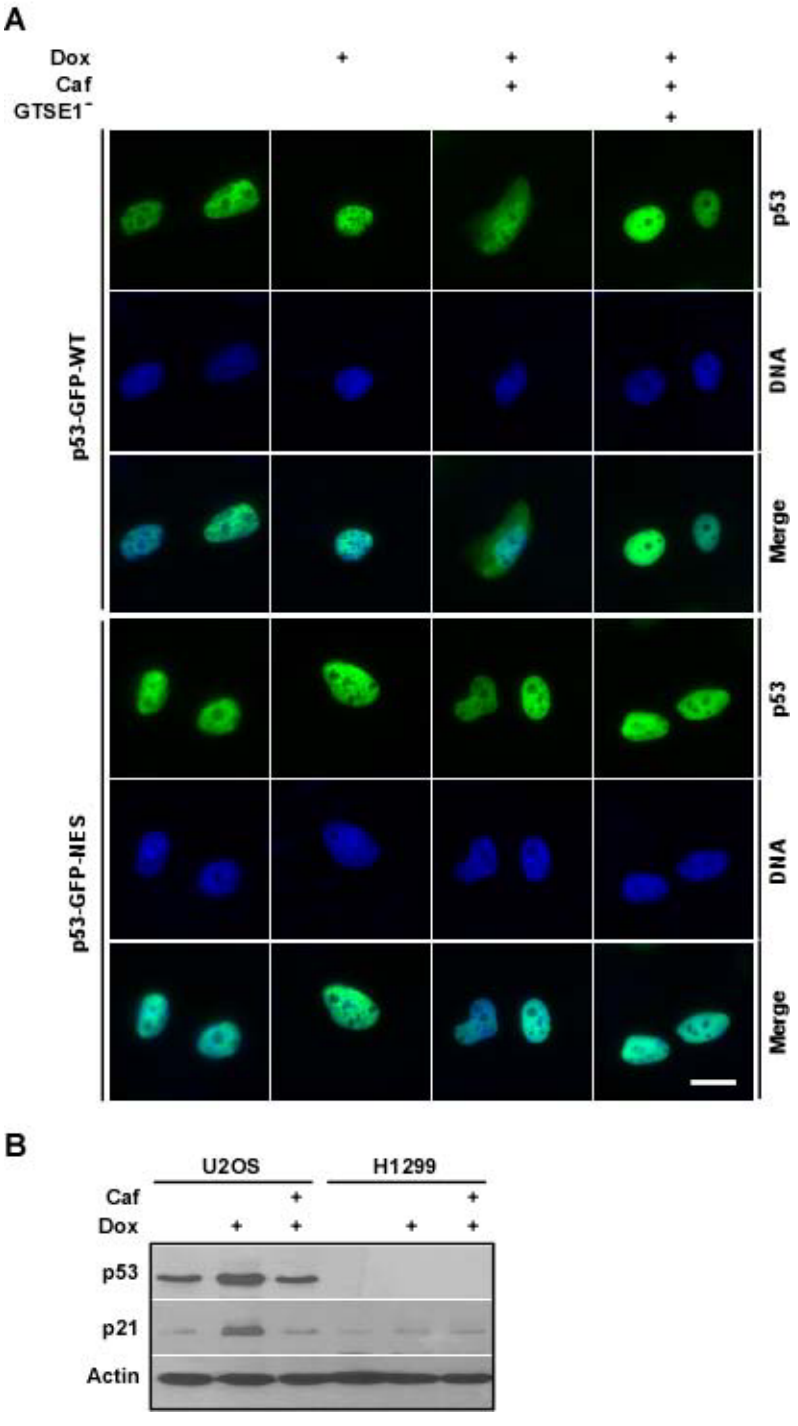
**Fig S4 |** Characterization of GTSE-1 phosphorylation at S435 during normal cell cycle progression and G2 checkpoint recovery. (**A**) U2OS cells were transfected with pLKOpuro.1-GTSE-1 or pLKOpuro.1 vector, and the transfection-positive cells were selected by puromycin (10 μg/mL) for two days. Then the cells were IF stained with pS435 antibodies (red) and DAPI (Blue). (**B**) U2OS cells were transfected with GFP-GTSE-1 for 24 h, and IF stained with pS435 antibodies to determine the subcellular localization of the pS435 epitope during cell cycle progression. (**C**) HeLa cells were synchronized with the DTB protocol, released for 0 h, 8 h, or 13 h, and immunoblotted. (**D**) U2OS cells were synchronized with the DTB protocol and released for 6 h. After the cells were treated with 1.0 μM doxorubicin (Dox) for 1 h, the cells were released into fresh medium containing nocodazole for 6 h, 24 h or 48 h, and immunoblotted. (**E**) U2OS cells were transfected with GFP-GTSE-1 for 24 h and synchronized by the DTB protocol. After release for 6h, the cells were treated with doxorubicin for 1h, released into fresh medium with or without caffeine or BI2536, and IF stained with pS435 antibodies. Scale bars, 10 μm.

**Fig S5 |** Characterization of U2OS cells expressing GFP-GTSE-1-R3A during G2 checkpoint recovery. (**A**) U2OS cells were transfected with GFP-GTSE-1-R3A for 24 h and immunoblotted. (**B**) U2OS cells stably expressing RNAi-resistant GFP-GTSE-1-R3A were processed as in Fig 3C with endogenous GTSE-1 was depleted. (**C**) U2OS cells were transfected with GFP-GTSE-1 constructs (WT or R3A), treated with nocodazole for 14 h, and immunoblotted.

## Supplymentary Figure S1



## Supplymentary Figure S2



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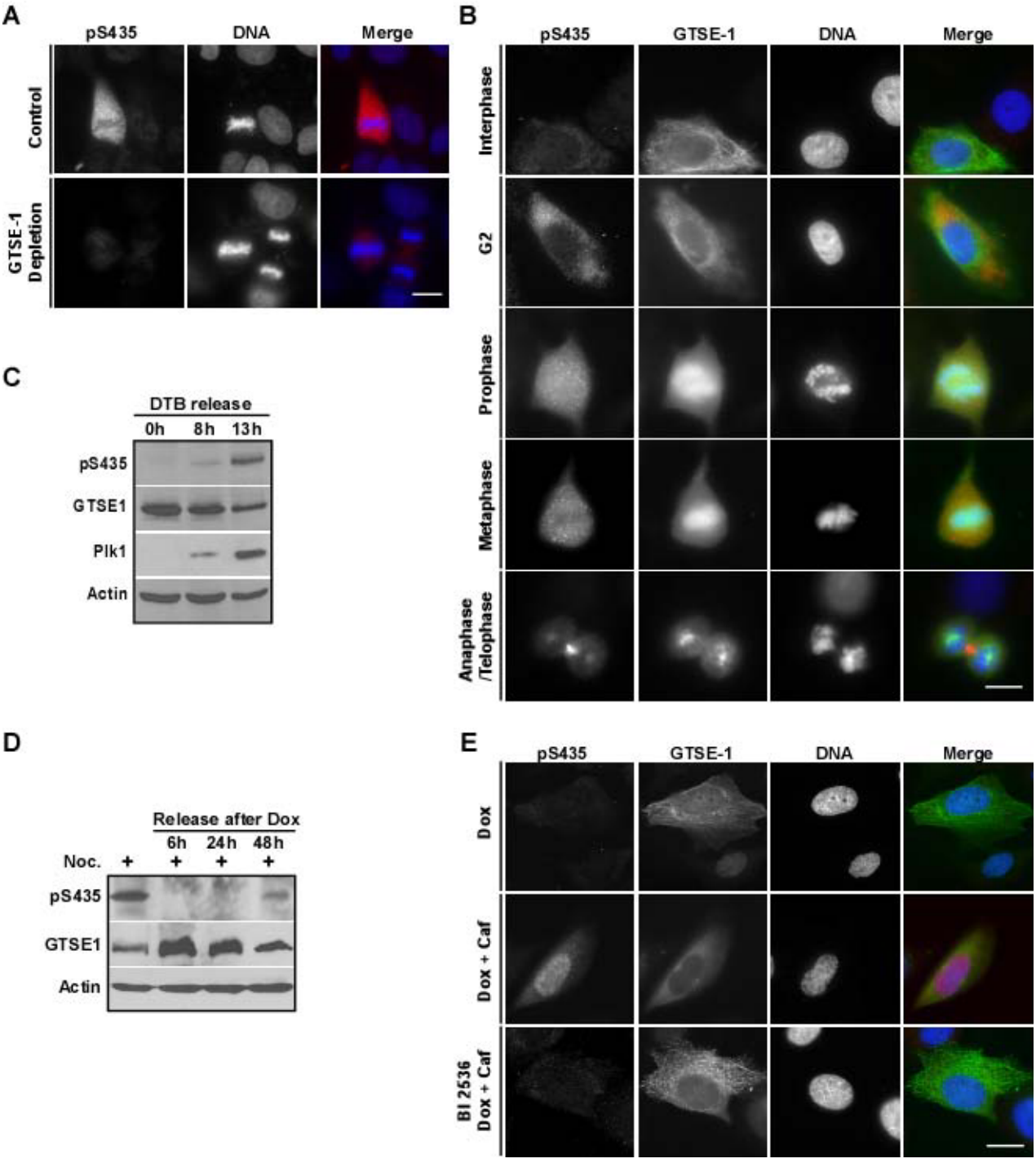

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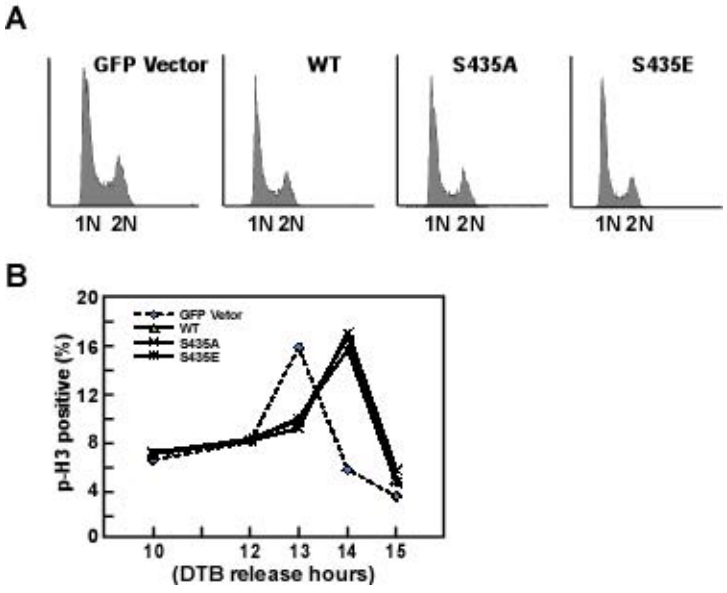

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