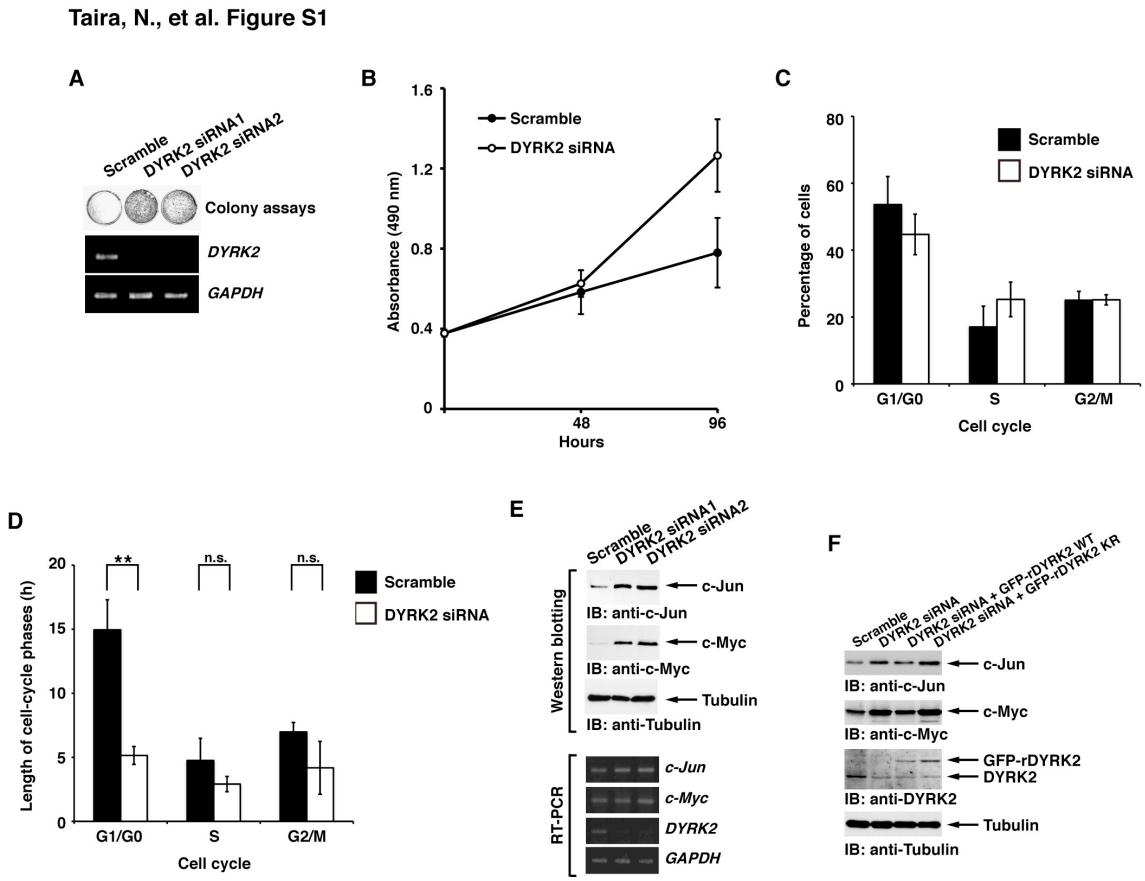

Figure S1. DYRK2 silencing enhances proliferation by shortening G1 phase in U2OS cells. U2OS cells were transfected with scramble siRNA or DYRK2-specific siRNAs. After transfection, colony formation assays were performed. Total RNAs were analyzed by RT-PCR using *DYRK2*-specific or *GAPDH*-specific primers. (B) U2OS cells were transfected with scramble siRNA or DYRK2 siRNA and cell growth was analyzed by MTS assay. (C and D) U2OS cells were transfected with scramble siRNA or DYRK2 siRNA. After transfection, cells were stained with propidium iodide and the cell cycle was analyzed using flow cytometer. ** $P < 0.01$; n.s. 'not significant'. (E) U2OS cells were transfected and analyzed by immunoblotting with anti-c-Jun (top panel), anti-c-Myc (second panel) or anti-tubulin (third panel). Total RNAs were analyzed with *c-Jun*-specific (forth panel), *c-Myc*-specific (fifth panel), *DYRK2*-specific (sixth panel), or *GAPDH*-specific (bottom panel) primers. (F) U2OS cells were co-transfected with scramble siRNA or DYRK2-specific siRNAs and GFP vector, wild type or kinase-dead DYRK2 resistant for DYRK2 siRNA (designated GFP-rDYRK2 WT or GFP-rDYRK2 KR, respectively). Cell lysates were analyzed by immunoblotting

with anti-c-Jun (top panel), anti-c-Myc (second panel), anti-DYRK2 (third panel), or anti-tubulin (bottom panel).

Taira, N., et al. Figure S2

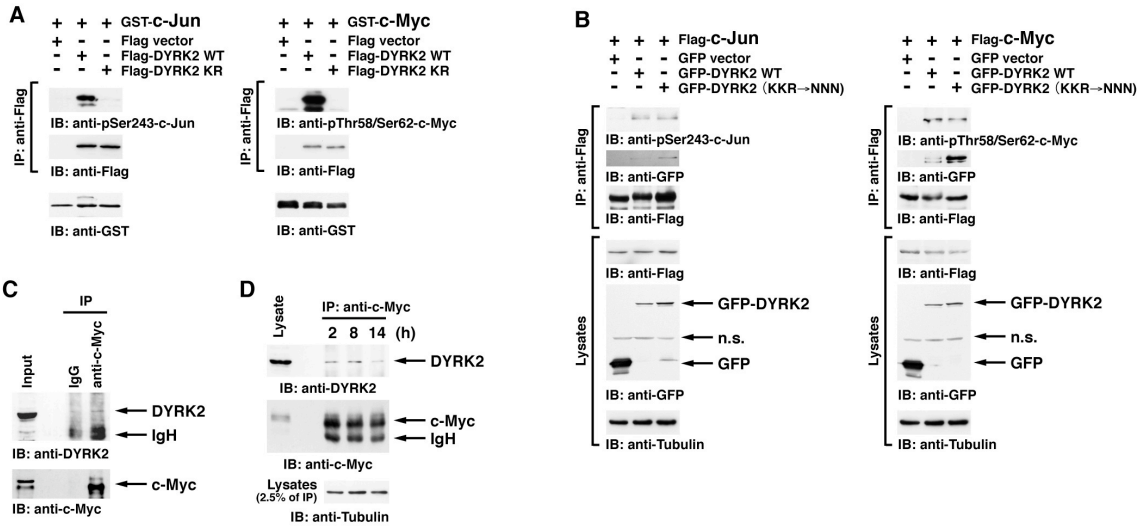

Figure S2. DYRK2 interacts with and phosphorylates c-Jun and c-Myc in the cytoplasm. (A) 293 cells were transfected with Flag vector, Flag-tagged wild-type DYRK2 (Flag-DYRK2 WT) or kinase-dead form (K178R) of DYRK2 (Flag-DYRK2 KR). Cell lysates were immunoprecipitated with anti-Flag agarose. Immunoprecipitates were incubated with GST-c-Jun or GST-c-Myc in the presence of ATP. Reaction products were analyzed by immunoblotting with anti-phospho-c-Jun(Ser243) (left upper panel), anti-phospho-c-Myc(Thr58/Ser62) (right upper panel), anti-Flag (middle panels), or anti-GST (bottom panels). (B) 293 cells were transfected with Flag-tagged c-Jun or c-Myc and GFP vector, GFP-DYRK2 WT or the mutant in which essential amino acid residues in the nuclear localization signal are substituted with asparagine (KKR→NNN). Lysates were immunoprecipitated with anti-Flag agarose and analyzed by immunoblotting with anti-phospho-c-Jun(Ser243) (left top panel), anti-phospho-c-Myc(Thr58/Ser62) (right top panel), anti-GFP (second panels), or anti-Flag (third panels). Cell lysates were also subjected to immunoblot analysis with anti-Flag (fourth panels), anti-GFP (fifth panels), or anti-tubulin (bottom panels). 'n.s.' indicates non-specific bands. (C) Lysates from HeLa cells were immunoprecipitated with anti-c-Myc followed by immunoblot analysis with anti-DYRK2 (upper panel) or anti-c-Myc (lower panel). (D) HeLa cells were subjected to serum-starvation for 24 h followed by serum-stimulation for the indicated times. Lysates were immunoprecipitated with anti-c-Myc followed by immunoblot analysis with anti-DYRK2 (upper panel) or anti-c-Myc (middle panel). Lysates were also analyzed by

immunoblotting with anti-tubulin (lower panel).

Taira, N., et al. Figure S3

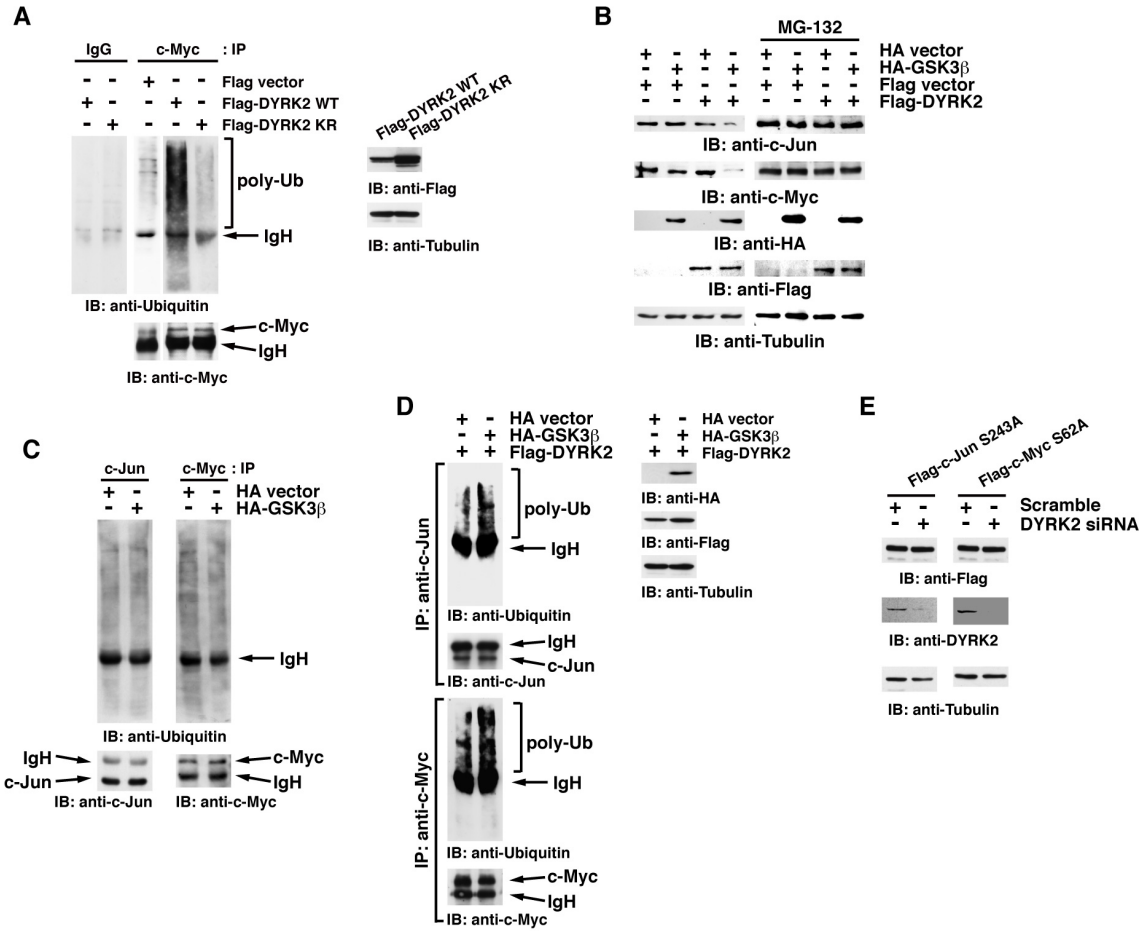

Figure S3. Kinase activity of DYRK2 is required for ubiquitination of c-Myc. (A) U2OS cells were transfected with Flag vector, Flag-DYRK2 WT, or Flag-DYRK2 KR and incubated with 10 μM MG-132 for 4 h. Lysates were immunoprecipitated with IgG or anti-c-Myc followed by immunoblotting with anti-ubiquitin or anti-c-Myc (left panels). Lysates were also analyzed by immunoblotting with indicated antibodies (right panels). (B) U2OS cells were co-transfected with HA vector or HA-GSK3β and Flag vector or Flag-DYRK2 WT in the presence or absence of MG-132. Lysates were subjected to immunoblot analysis with indicated antibodies. (C) U2OS cells were transfected with HA vector or HA-GSK3β and incubated with 10 μM MG-132 for 4 h. Lysates were immunoprecipitated with anti-c-Jun, or anti-c-Myc followed by immunoblotting with anti-ubiquitin, anti-c-Jun, or anti-c-Myc. (D) U2OS cells were co-transfected with Flag-DYRK2 WT and HA vector or HA-GSK3β and incubated with 10 μM MG-132 for 4 h. Lysates were immunoprecipitated with anti-c-Jun, or anti-c-Myc followed by immunoblotting with anti-ubiquitin, anti-c-Jun, or anti-c-Myc (left panels). Lysates were also analyzed by immunoblotting with indicated antibodies (right panels). (E) 293 cells were co-transfected with the Flag-c-Jun S243A mutant or the Flag-c-Myc S62A mutant and scramble siRNA or DYRK2 siRNA. Cell lysates were subjected to immunoblot analysis with anti-Flag (upper panels), anti-DYRK2 (middle panels), or anti-tubulin (lower panels).

Taira, N., et al. Figure S4

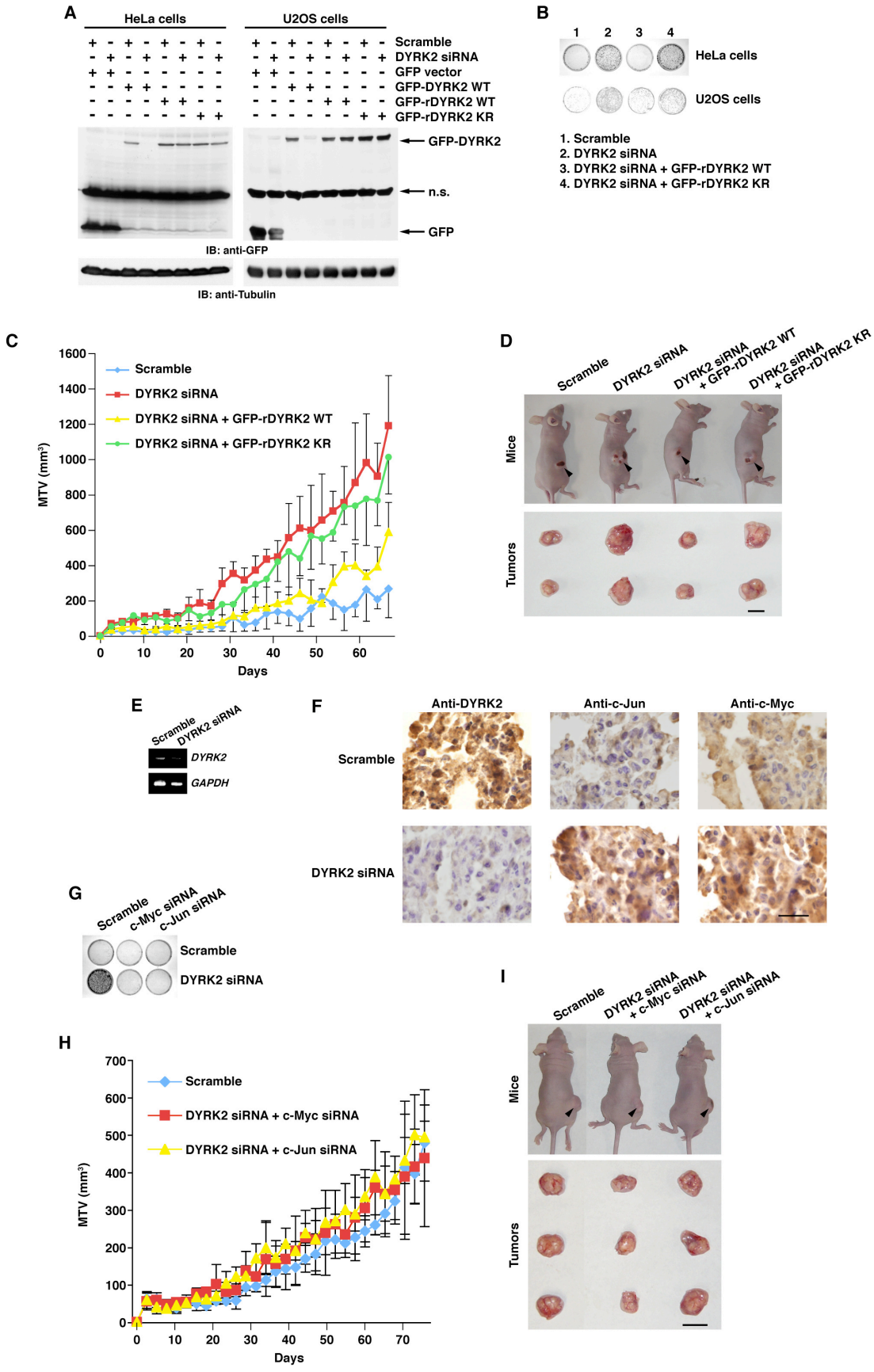

Figure S4. Depletion of DYRK2 accelerates cell proliferation and tumor growth. (A) HeLa cells (left) or U2OS cells (right) were co-transfected with scramble siRNA or DYRK2 siRNAs and GFP vector, GFP-DYRK2 WT, wild type or kinase-dead DYRK2 resistant for DYRK2 siRNA (designated GFP-rDYRK2 WT or GFP-rDYRK2 KR, respectively). Lysates were analyzed with immunoblotting with anti-GFP (upper panel) or anti-tubulin (lower panel). (B) HeLa cells (upper panel) or U2OS cells (lower panel) were transfected with Scramble siRNA or DYRK2 siRNA and GFP vector, GFP-rDYRK2 WT, or GFP-rDYRK2 KR. After transfection, cells were subjected to the colony formation assays. (C and D) HeLa cells were transfected with indicated siRNAs and/or plasmids. After transfection, cells were subcutaneously inoculated into BALB/c nu/nu mice. Tumor size was measured using calipers (n=4) and the data (maximum tumor volume; MTV) indicate the mean ± SD (C). Representative pictures of tumor bearing nude mice (upper panel) and tumors (lower panel), which were taken 10 weeks after inoculation (D). Arrowheads indicate inoculated tumors. The scale bar represents 10 mm. (E and F) Enucleated tumors were subjected to RT-PCR analysis for *DYRK2* expression (E) or immunostaining with anti-DYRK2, anti-c-Jun, or anti-c-Myc (F). The scale bar represents 50 μm. (G) HeLa cells were transfected with scramble siRNA, DYRK2 siRNA, c-Jun-specific siRNA, or c-Myc-specific siRNA. After transfection, cells were subjected to the colony formation assay. (H and I) HeLa cells were transfected with indicated siRNAs. After transfection, cells were subcutaneously inoculated into BALB/c nu/nu mice. Tumor size was measured using calipers (n=5) and the data indicate the mean ± SD (G). Representative pictures of tumor bearing nude mice (upper panel) and tumors (lower panel), which were taken 10 weeks after inoculation (I). Arrowheads indicate inoculated tumors. The scale bar represents 10 mm.

Taira, N., et al. Figure S5

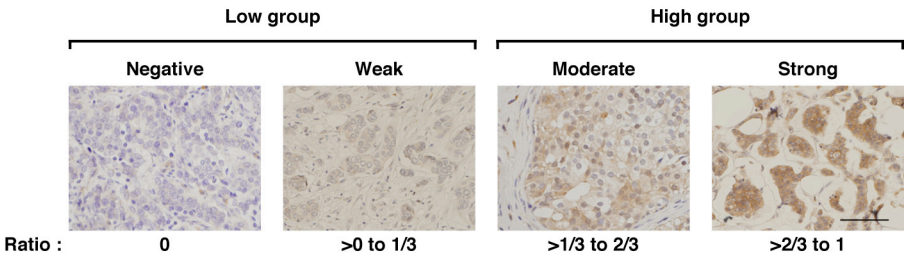

Figure S5. Representative immunohistochemical staining with anti-DYRK2 in the breast cancers. To assess the expression status of DYRK2, the ratio of DYRK2-positve cells were scored to categorize its expression levels into 4 grades, which consist of 'negative' (0), 'weak' (> 0 to 1/3), 'moderate' (> 1/3 to 2/3), and 'strong' (> 2/3 to 1). The expression level of DYRK2 was also defined into two groups, which consist of 'high' expression group (including moderate and strong categories) and 'low' expression group (including negative and weak categories). The scale bar represents 200 μm.