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Supplemental Material to:

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CDK4 inhibition restores G_1-S arrest in MYCN-amplified neuroblastoma cells in the context of doxorubicin-induced DNA damage

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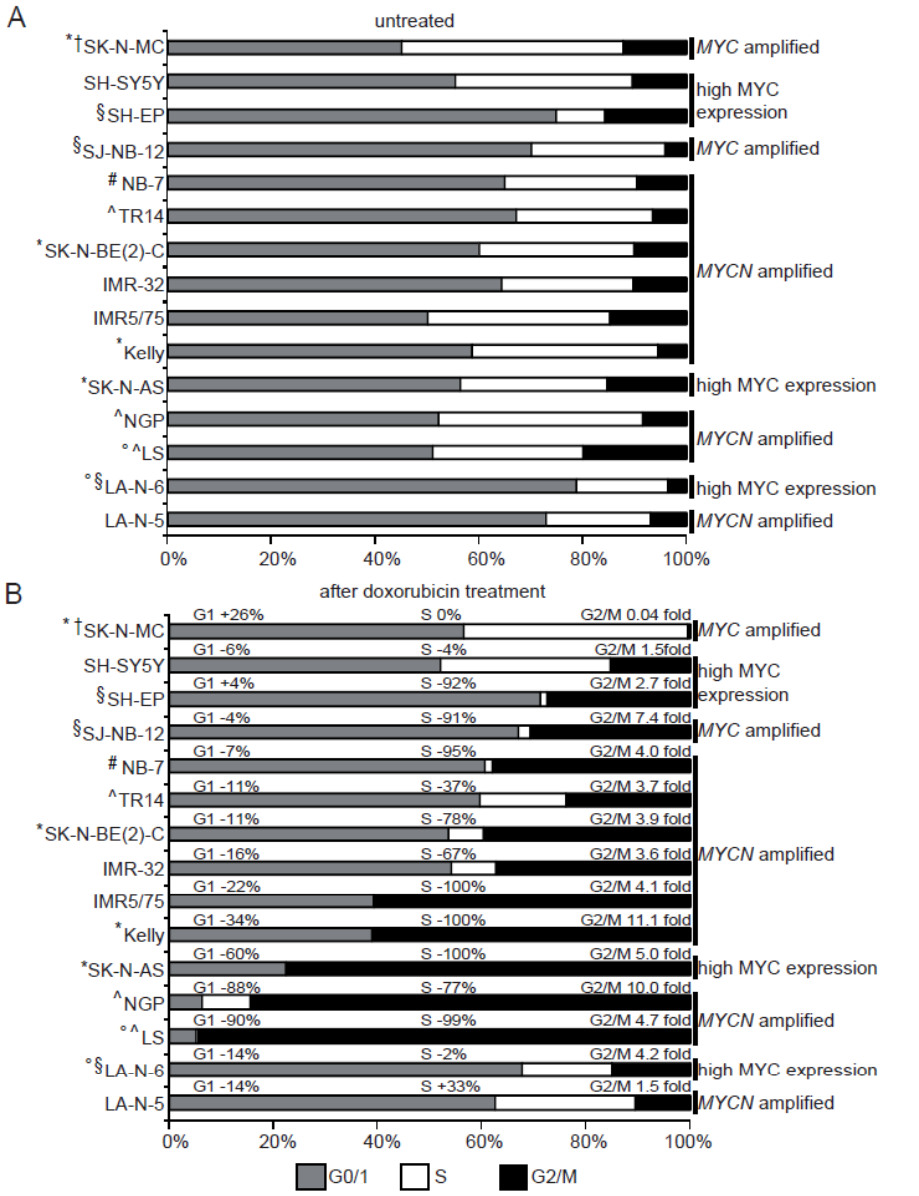

§ *p16INK4A/p14ARF* deletion
° *CCND1* amplification/duplication
* *TP53* mutation
# primary NB
† Ewing sarcoma (*EWS-FLi1* gene fusion)
^ *MDM2/CDK4* co-amplified

% increase/decrease as compared to untreated cells

Figure S1. Impaired drug-induced DNA damage response in neuroblastoma cells. (A and B) Cell cycle distribution 48h after doxo treatment and of control cells (=untreated) using flow cytometry.

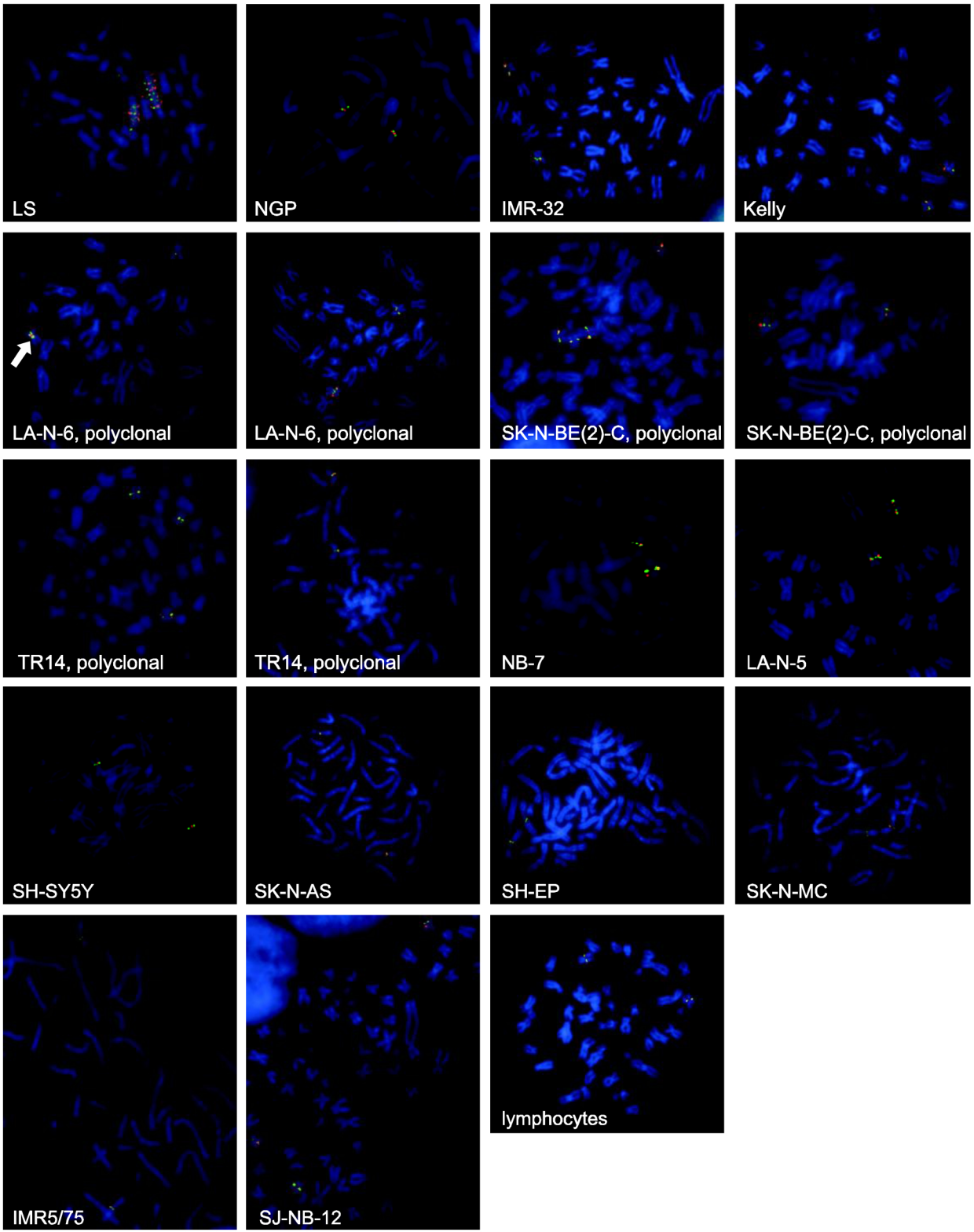

Figure S2. *CCND1* status using FISH. *CCND1* gene status is shown by Cy3-staining (red). FITC (green) fluorescence signal covers the neighboring region to *CCND1;* DNA is stained by DAPI (blue). FISH analysis shows a *CCND1* amplicon in both der(12) chromosomes of LS cells and *CCND1* duplication in polyclonal LA-N-6 (see white arrow). SK-N-BE(2)-C consists of a tetraploid (four chromosomes 11) and diploid (two chromosomes 11) cell clone. TR14 consists of a triploid (three chromosomes 11) and a diploid (two chromosomes 11) cell clone. SJ-NB-12 cells are triploid for chromosome 11. The other cell lines and control lymphocytes harbor two chromosomes 11 and show normal *CCND1* status.

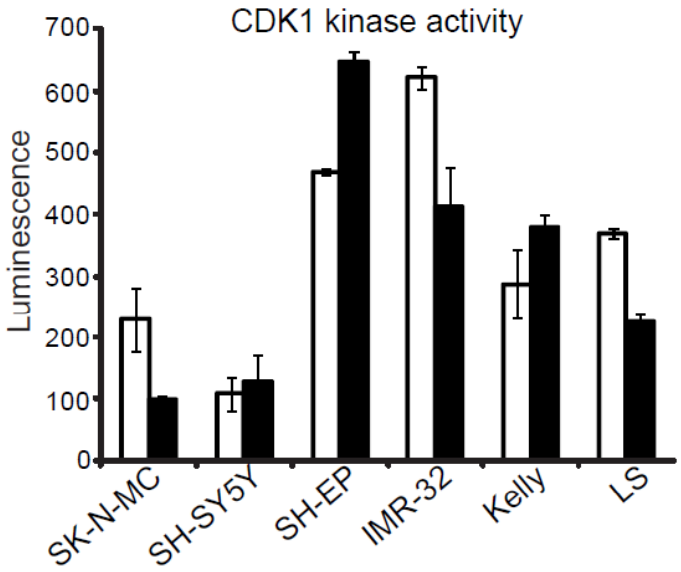

Figure S3. High CDK1 activity after doxorubicin treatment in *MYCN*-amplified cells. CDK1 activity was analyzed 48h after treatment using histone 1 as substrate. Luminescence directly correlates to the amount of produced ADP, indicative for kinase activity. Data are presented as mean ±SD of duplicates.

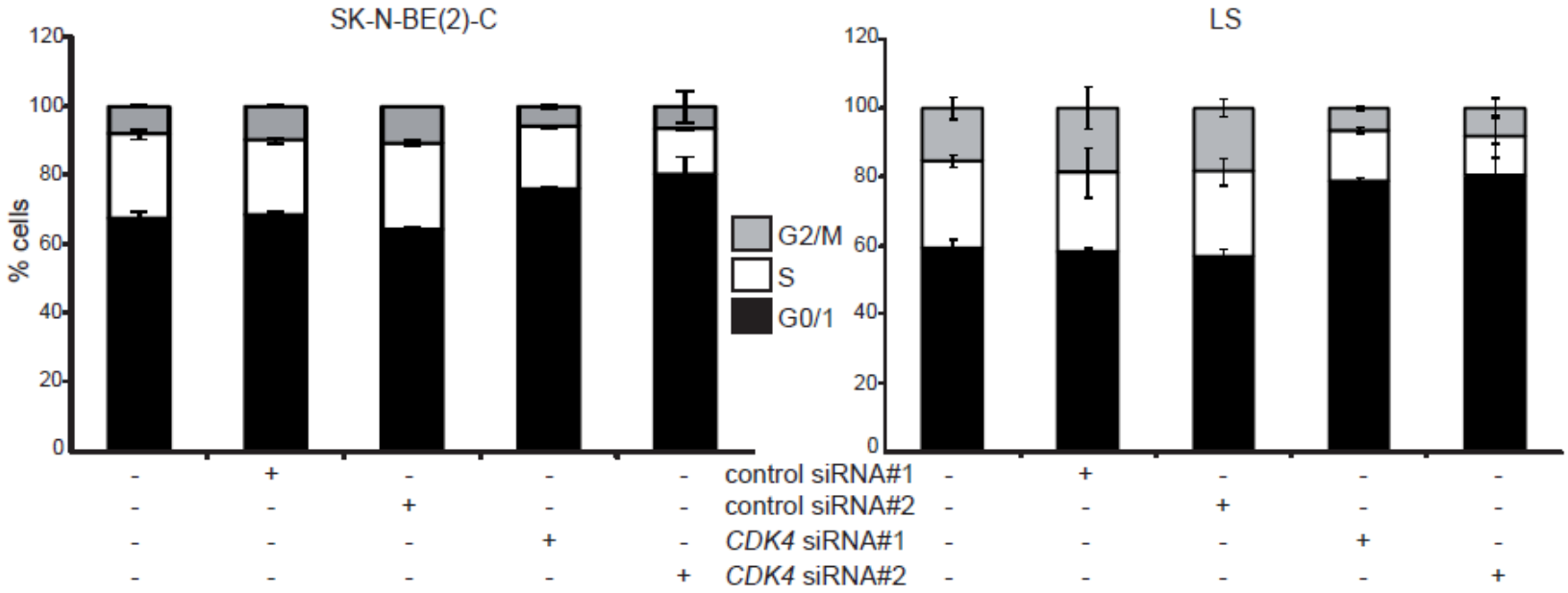

Figure S4. Silencing of *CDK4* by transient siRNA. Control experiment related to CDK4 knockdown in combination with doxo treatment as shown in Figure 3B. Cells were transfected with one of two unrelated control siRNAs or one of four siRNAs targeting *CDK4.* Flow cytometric cell cycle analyses were performed 96h later simultaneously to the doxo counterparts. Data are presented as mean ±SD of triplicates.

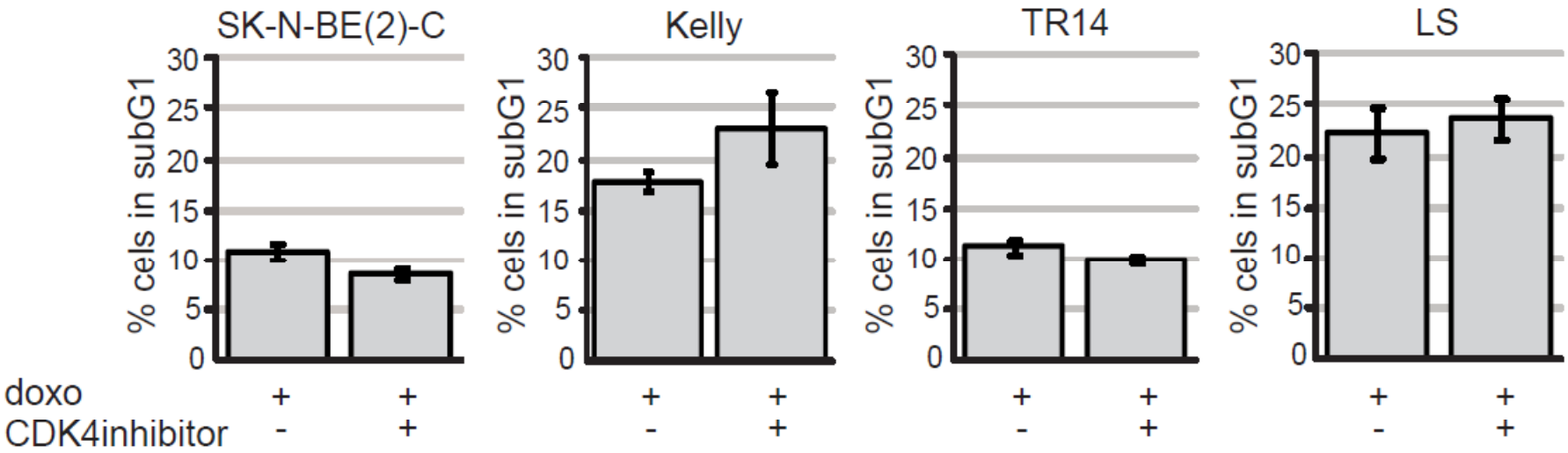

Figure S5. CDK4 inhibition has no cooperative cytotoxic effect with doxorubicin in *MYCN*-amplified cells with non-functional p53. Cell death analysis of four neuroblastoma cell lines harboring mutant *TP53* (SK-N-BE(2)-C and Kelly) or amplified *MDM2* (TR14 and LS) 48h after treatment with doxo alone or the combination of doxo and CDK4 inhibitor RO0505124 using flow cytometry. Data are presented as mean ±SD of triplicates.

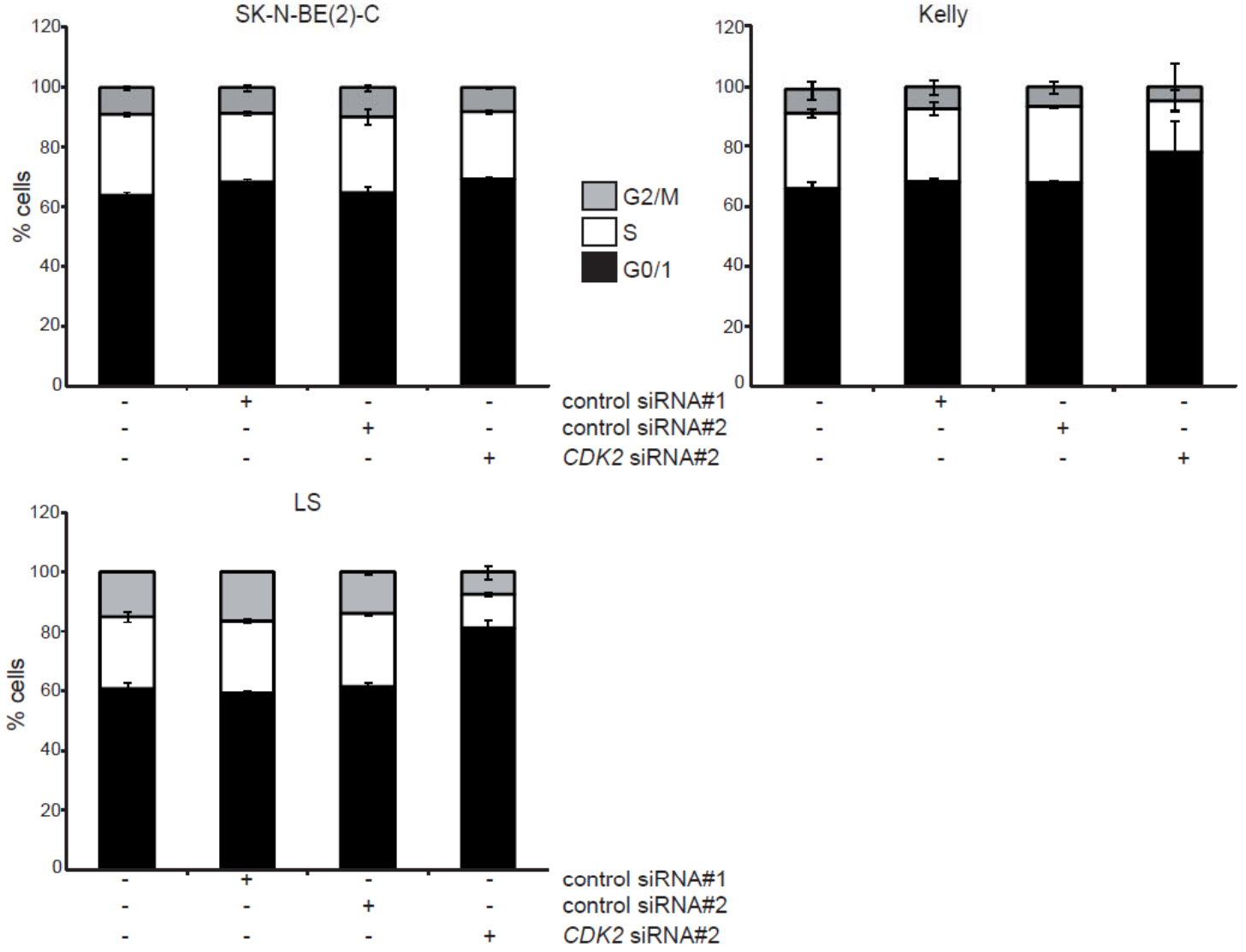

Figure S6. Silencing of *CDK2* by transient siRNA. Control experiment related to CDK4 knockdown in combination with doxo treatment as shown in Figure 4B. Cells were transfected with one of two unrelated control siRNAs or one of four siRNAs targeting *CDK2.* Flow cytometric cell cycle analyses were performed 96h later simultaneously to the doxo counterparts. Data are presented as mean ±SD of triplicates.