Figure S2

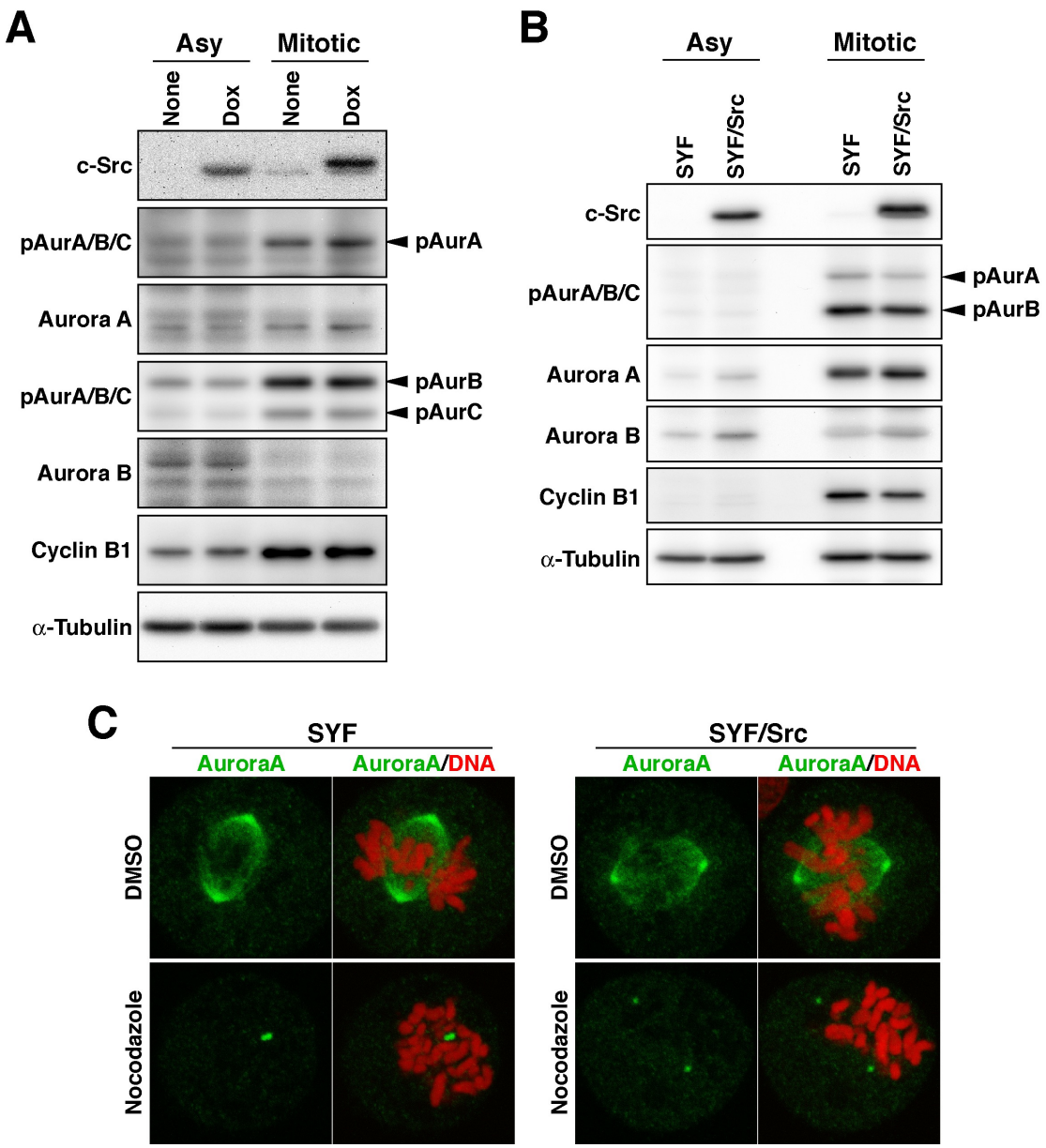

Fig. S2. c-Src does not affect Aurora A activity.

(A) c-Src overexpression was induced in Src45 cells by exposure to 2 μg/ml Dox for 2 days, as described previously (Kasahara et al., 2007b). Cells arrested at the G1/S border by treatment with 2 mM thymidine for 21 h were released into medium containing nocodazole for 12 h to trap cells at prometaphase. Mitotic cells were collected by mitotic shake-off and lysed with SDS-sample buffer. Whole cell lysates were subjected to Western blotting, probed with anti-phospho Aurora A/B/C (pAurA, pAurB, pAurC), anti-Aurora A, anti-Aurora B, anti-Src, anti-cyclin B1, and anti-α-tubulin (loading control) antibodies. **(B)** SYF and SYF/c-Src cells were arrested at late G2 phase by treatment with 8 μM RO-3306 for 20 h and then released into fresh medium for 30 min. Mitotic cells were collected by mitotic shake-off and lysed with SDS-sample buffer. The lysates prepared from asynchronous and mitotic cells were subjected to Western blotting, probed with anti-phospho Aurora A/B/C (pAurA, pAurB, pAurC), anti-Aurora A, anti-Aurora B, anti-Src, anti-cyclin B1, and anti-α-tubulin (loading control) antibodies. **(C)** SYF and SYF/c-Src cells arrested at late G2 phase by 8 μM RO-3306 were released into fresh medium with or without 100 ng/ml nocodazole for 25 min, fixed and stained for Aurora A and DNA.