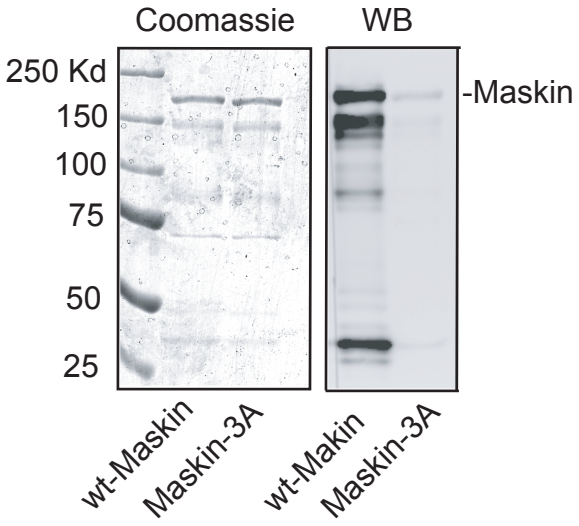
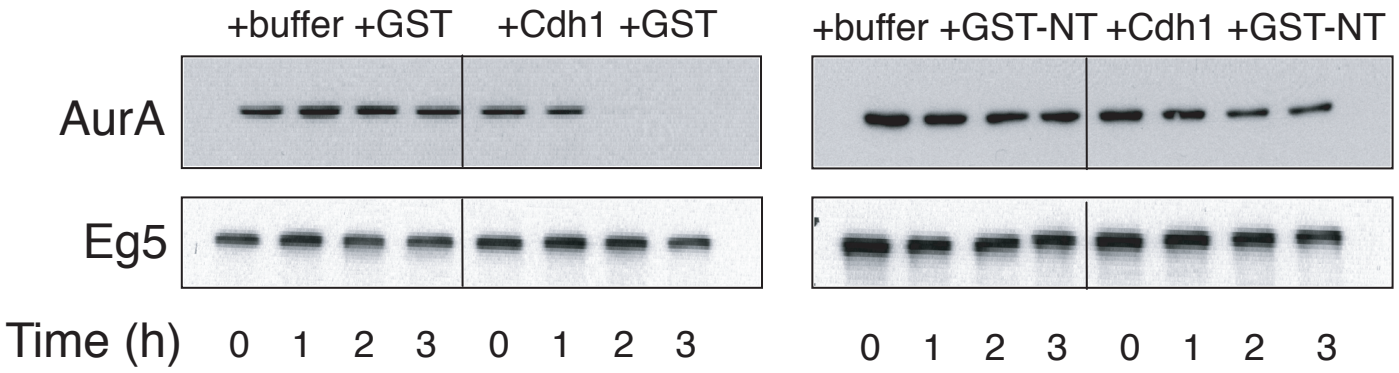


Characterization of phosphospecific anti-Maskin antibody
GST-Maskin (wt-Maskin) or GST-Maskin mutated on Ser33, Ser620 and Ser626 to Ala (Maskin-3A) were incubated with recombinant AurA in the presence of ATP (for kinase assay conditions see Material and methods). The reaction mixtures were loaded onto SDS-PAGE. Proteins were detected in parallel by Coomassie brilliant blue staining (Coomassie) and by Western blotting with the affinity purified anti-phospho-Maskin antibody (see Materials and methods).

Sup. Fig.2 Sardon et al.

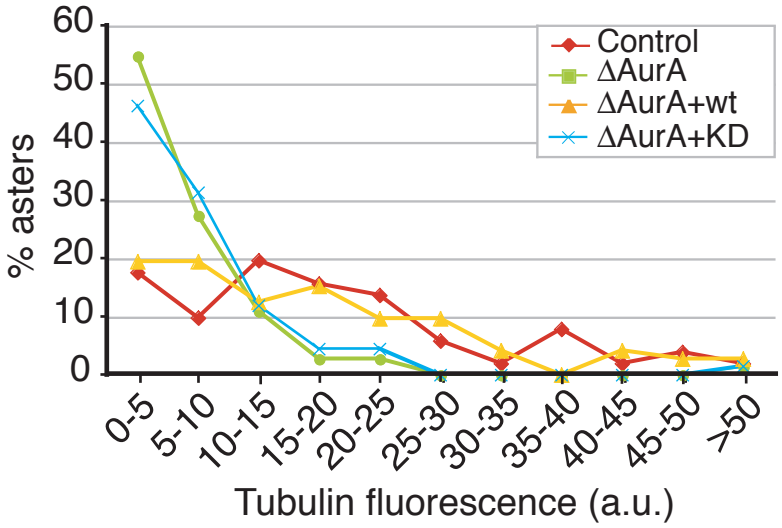


GST-NT impairs the cdh1 dependent degradation of Aur A in the Xenopus egg extract
In humann cells, AurA is degraded in anaphase through the cdh1 activated Anaphase Promoting Complex/Cyclosome (APC). This event is regulated by phosphorylation at Ser53 (Littlepage and Ruderman, 2002), maybe through autophosphorylation (Haydon et al., 2003). As cdh1 is not present in egg extract, AurA protein levels do not fluctuate between M-phase and interphase. However AurA degradation can be monitored in this system by supplementing the egg extract with cdh1 before sending it to interphase with calcium (Castro et al., 2002; Littlepage and Ruderman, 2002).
We wondered whether activation of AurA by GST-NT could interfere with this pathway. CSF-extract supplemented with buffer of cdh1 was sent to interphase by addition of Ca2+ in the presence of GST or GST-NT (2-20 µM). Small aliquots were taken over a period of 4 hours and analyzed by Western blot to monitor AurA levels. The blot was reprobed with anti-Eg5 antibodies (loading control).
The figure shows that in the presence of cdh1 the level of AurA decreases, and the protein is almost undetectable two hours after calcium addition. In contrast, when GST-NT is present, the AurA protein is clearly detectable even after 3 hours of incubation. This result supports the hypothesis that GST-NT, probably by activating AurA, impairs the degradation of the kinase.

Castro, A., Arlot-Bonnemains, Y., Vigneron, S., Labbe, J.C., Prigent, C. and Lorca, T. (2002) APC/Fizzy-Related targets Aurora-A kinase for proteolysis. *EMBO Rep*, **3**, 457-462.

Haydon, C.E., Eyers, P.A., Aveline-Wolf, L.D., Resing, K.A., Maller, J.L. and Ahn, N.G. (2003) Identification of Novel Phosphorylation Sites on Xenopus laevis Aurora A and Analysis of Phosphopeptide Enrichment by Immobilized Metal-affinity Chromatography. *Mol Cell Proteomics*, **2**, 1055-1067.

Littlepage, L.E. and Ruderman, J.V. (2002) Identification of a new APC/C recognition domain, the A box, which is required for the Cdh1-dependent destruction of the kinase Aurora-A during mitotic exit. *Genes Dev*, **16**, 2274-2285.



	Sample	Tubulin fluoresc. (Average ± sd)
◆	Control	18.1±13.3
●	ΔAurA	7.9±10.0
▲	ΔAurA+wt	17.0±16.4
×	ΔAurA+KD	8.2±12.7

Determination of the intrinsic nucleation capacity of centrosomes

The intrinsic nucleation capacity of the centrosomes was measured as described in Carazo-Salas et al. (2001). As reported before, we obtained a wide range of values for the total tubulin fluorescence associated to individual asters in the same sample. To analyze the data we classified the asters into categories comprising an empirically defined equal range of values for the tubulin fluorescence intensity (Carazo-Salas et al., 2001). In this way we obtained a distribution of aster size observed under each experimental condition. Here we show the profiles obtained from a representative experiment and the corresponding average values. Centrosomes reconstituted in control extract nucleated on average more MTs than those reconstituted in AurA depleted extracts (ΔAurA) and accordingly the profiles obtained were very different. We verified that the MT nucleation capacity of centrosomes reconstituted in AurA depleted extract supplemented with GFP-AurA (ΔAurA+wt) was restored to control levels as monitored by the similarity of the profiles and the average values obtained with the control. By contrast, addition of GFP-AurA-KD (ΔAurA+KD) to the depleted extract was unable to rescue the nucleation activity of reconstituted centrosomes. Although the average values obtained from each individual experiment had big standard deviations, the data were highly reproducible. To calculate the average effect from different experiments, control values were set to 100% (Fig.3A and Fig.4).

Carazo-Salas, R.E., Gruss, O.J., Mattaj, I.W. and Karsenti, E. (2001) Ran-GTP coordinates regulation of microtubule nucleation and dynamics during mitotic-spindle assembly. *Nat Cell Biol*, **3**, 228-234.

A

	Number of asters per µl (squash)			Number of asters per field (spin down)	
	Exp. 1	Exp. 2	Exp. 3	Exp. 4	Exp. 5
Control	9832	3841	2340	25.0	41.5
ΔAurA	607	776	1306	12.2	2.0
ΔAurA+wt	4935	3499		19.4	
ΔAurA+KD	1148	1583		9.2	

B

	Asters per µl (squash)	Asters per field (spin down)	
	Exp. 1	Exp. 2	Exp. 3
Control	3012	41.5	39.9
+GST-NT	6976	86.0	51.9

Quantificationt of the RanGTP dependent MT aster formation

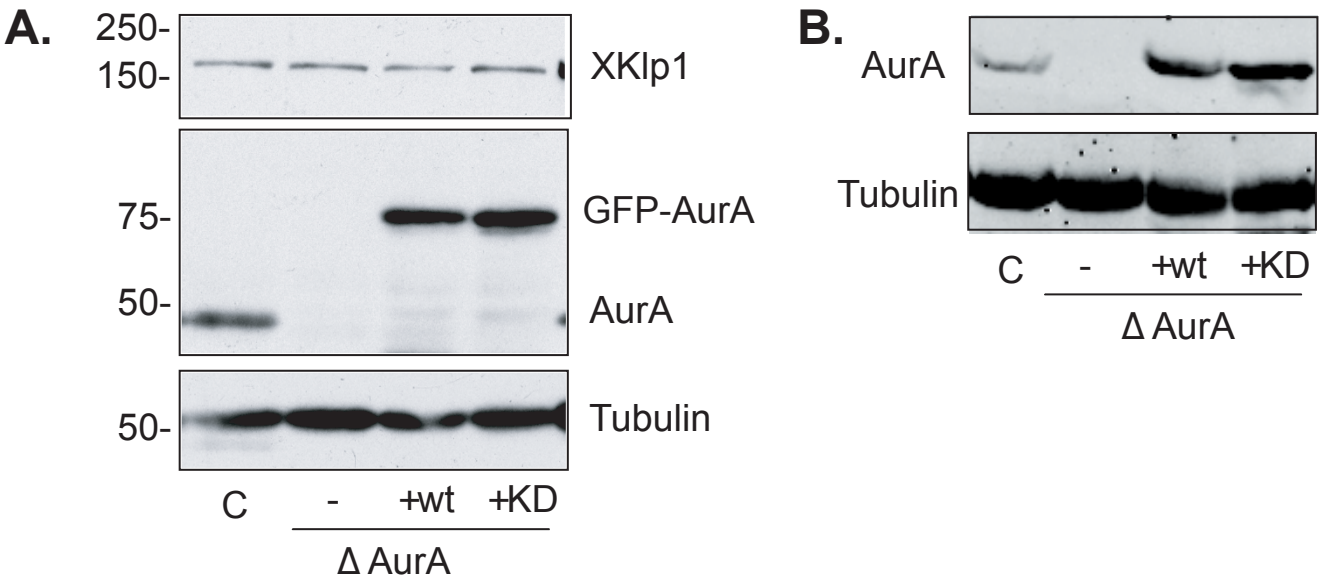
CSF-extract containing rhodamine tubulin was incubated for 20 min at 20°C in the presence of Ran(Q69L)GTP 15 µM. To quantify the efficiency of MT aster formation two alternative methods were used:

(1) 1 µl of extract was squashed and examined. The total number of asters was then determined (number of asters per µl).

(2) The whole extract reaction was fixed and spun onto a coverslips. The number of asters was determined in more than 50 independent microscopy fields (objective x63) and the average calculated.

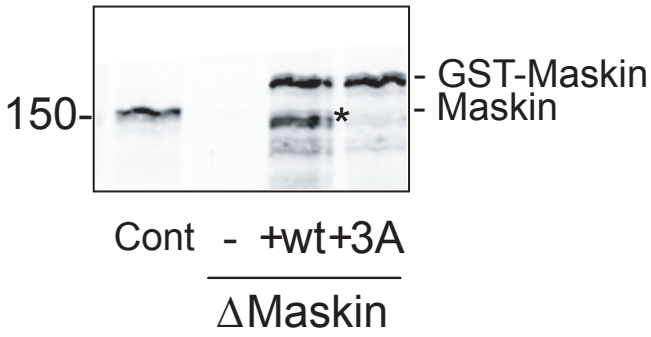
Table above shows the total number of asters counted in several independent experiments by the two methods. Although the number of total asters counted in the controls varies dramatically, the effect on the aster formation of AurA removal (A) or GST-NT addition to the extract (B) is very consistent. Control values were set to 100% and experiments 1, 2, 4, 6, 7 and 8 were considered to calculate the average interexperimental values shown in Fig.5.

1.



1.Western blot analysis of egg extracts used in the depletion and add back experiment. A- Mock (C) and AurA depleted egg extracts (ΔAurA) containing buffer (-) or recombinant GFP-wt (+wt) or GFP-AurA-KD (+KD), probed with anti-AurA antibodies and anti-Xklp1 and anti-α-tubulin (Tubulin) antibodies as loading controls. More than 98% of AurA was depleted. B- Wetern blot of egg extract in the same conditions decrived above but containing His-AurA recombinant proteins, probed with anti-AurA and Anti-α-tubulin antibodies as loading control.

2.



2.Western blot analysis of egg extracts used in Fig.6 probed with the anti-Maskin antibody.
* Degradation product of GST-Maskin