

## **Heat shock induces rapid resorption of primary cilia**

### *SUPPLEMENTARY FIGURES*

**Figure S1. Dual-immunofluorescent labelling indicates primary cilia are lost in mammalian cells exposed to elevated temperature. (A)** NIH 3T3 cells stained to detect the ciliary axoneme with anti-acetylated tubulin (red) and, Arl13b (green) and nuclei with DAPI (blue). Cells were maintained at 37°C or exposed to 42°C for 5, 15 or 30 minutes. Zoomed panels show colocalisation of acetylated tubulin and Arl13b in individual cilia (indicated by boxes). Scale bar represents 20 µM.

**Figure S2. Heat shock induces cilia loss in IMCD3 and ARPE19 cells.** IMCD3 and ARPE19 cells labelled to detect the ciliary axoneme (green), basal body (red) and nucleus (blue). Cells were maintained at 37°C or exposed to 42°C for 30 minutes. Scale bar represents 20 µM.

**Figure S3. Zebrafish gross development was not affected by exposure to 42°C for 5 minutes.** Development was assessed by measurement of head-trunk angle up to 8 hours after heat shock. Measurements were made in 8 fish at each time point. Error bars represent 2 x SEM. \*  $p < 0.05$ .

**Figure S4. Aurora-A inhibition reduces cilia loss in response to serum addition in NIH 3T3 cells.** NIH 3T3 cells were cultured in media containing the Aurora-A inhibitor PHA-680632 or vehicle (DMSO) for 4 hours, prior to addition of 10% serum. The percentage of cells with primary cilia was quantified after 2 hours. The number of cilia and nuclei were counted in 10 randomly selected fields for each experimental condition. Error bars represent 2 x SEM. \*  $p < 0.05$ .

**Figure S5. HDAC6 and Hsp90 localise to primary cilia.** NIH 3T3 cells were stained to detect the ciliary axoneme with anti-acetylated tubulin (green) and Hsp90 (A) or HDAC6 (B) (both red). Nuclei were detected with DAPI (blue). Two representative cells are shown for each co-staining. Scale bar represents 10  $\mu$ M.

**Figure S6. HDAC6 and Hsp90 show partial colocalisation in primary cilia.** NIH 3T3 cells were stained to detect the ciliary axoneme with anti-acetylated tubulin (magenta). They were co-stained for HDAC (green), Hsp90 (red), and DAPI. Boxes in the top panel are shown enlarged in the lower panel (zoom). Arrows indicate areas where HDAC6 and Hsp90 staining overlap. Scale bar represents 10  $\mu$ M.

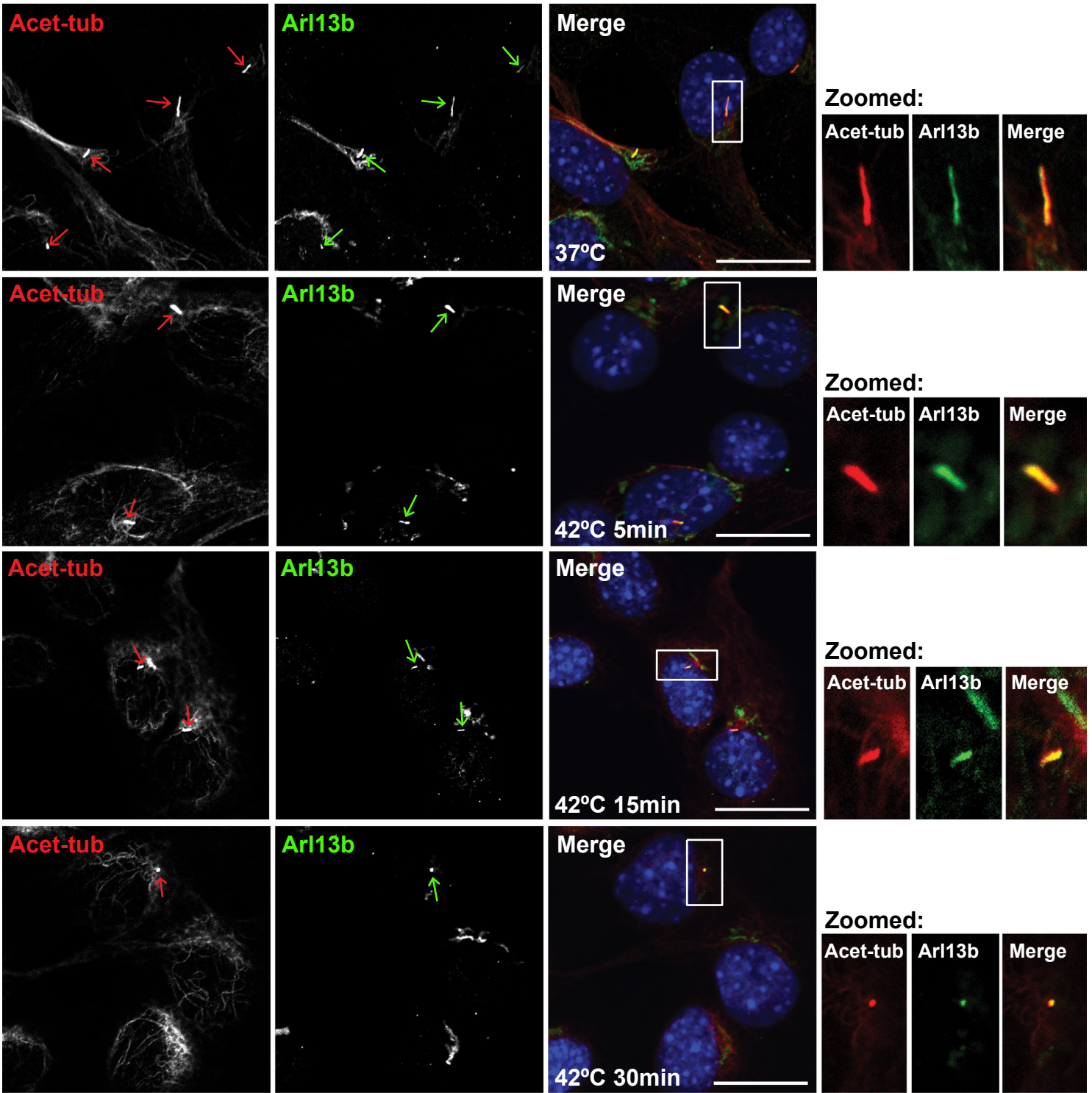


Figure S1

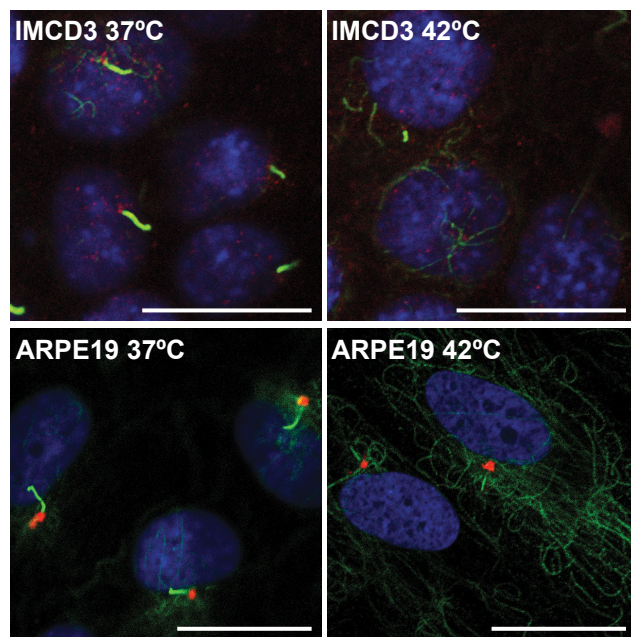


Figure S2



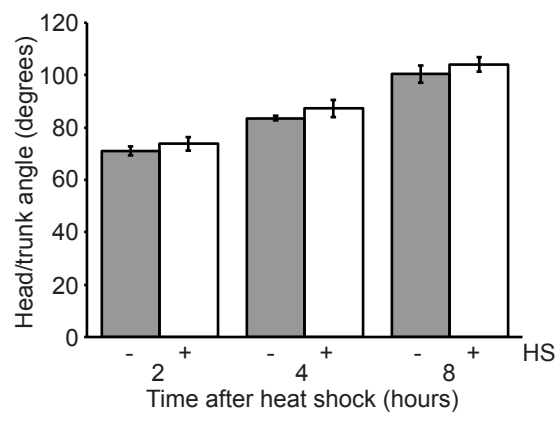


Figure S3

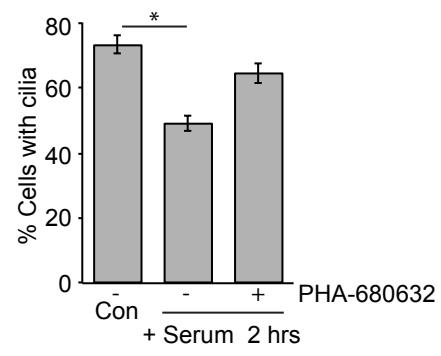


Figure S4

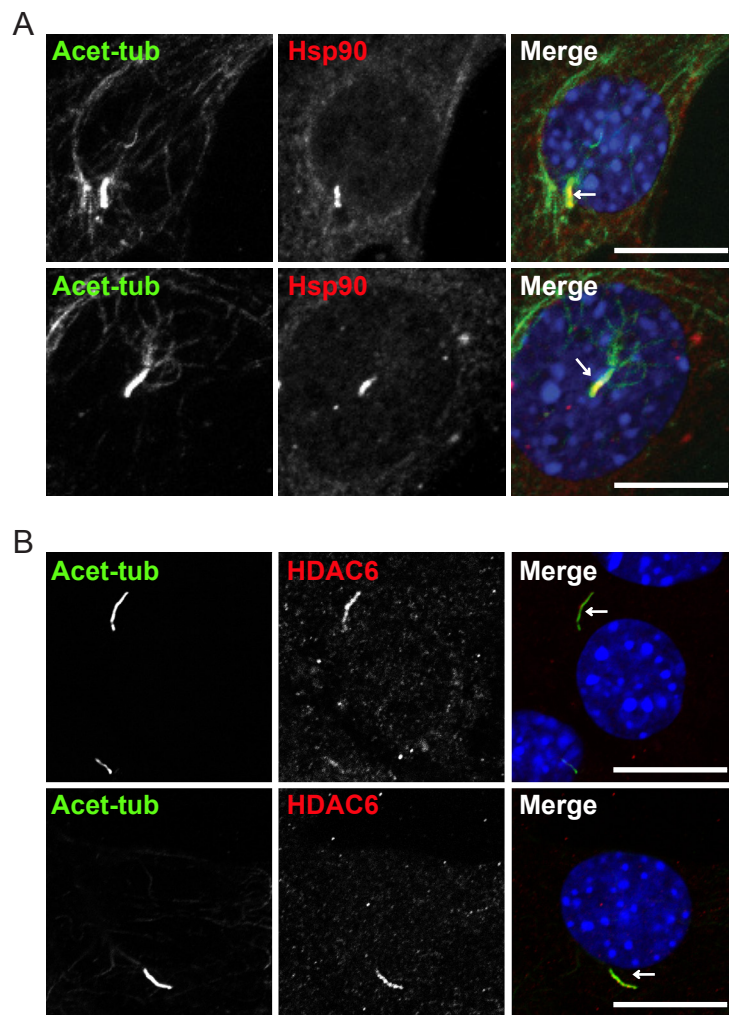


Figure S5

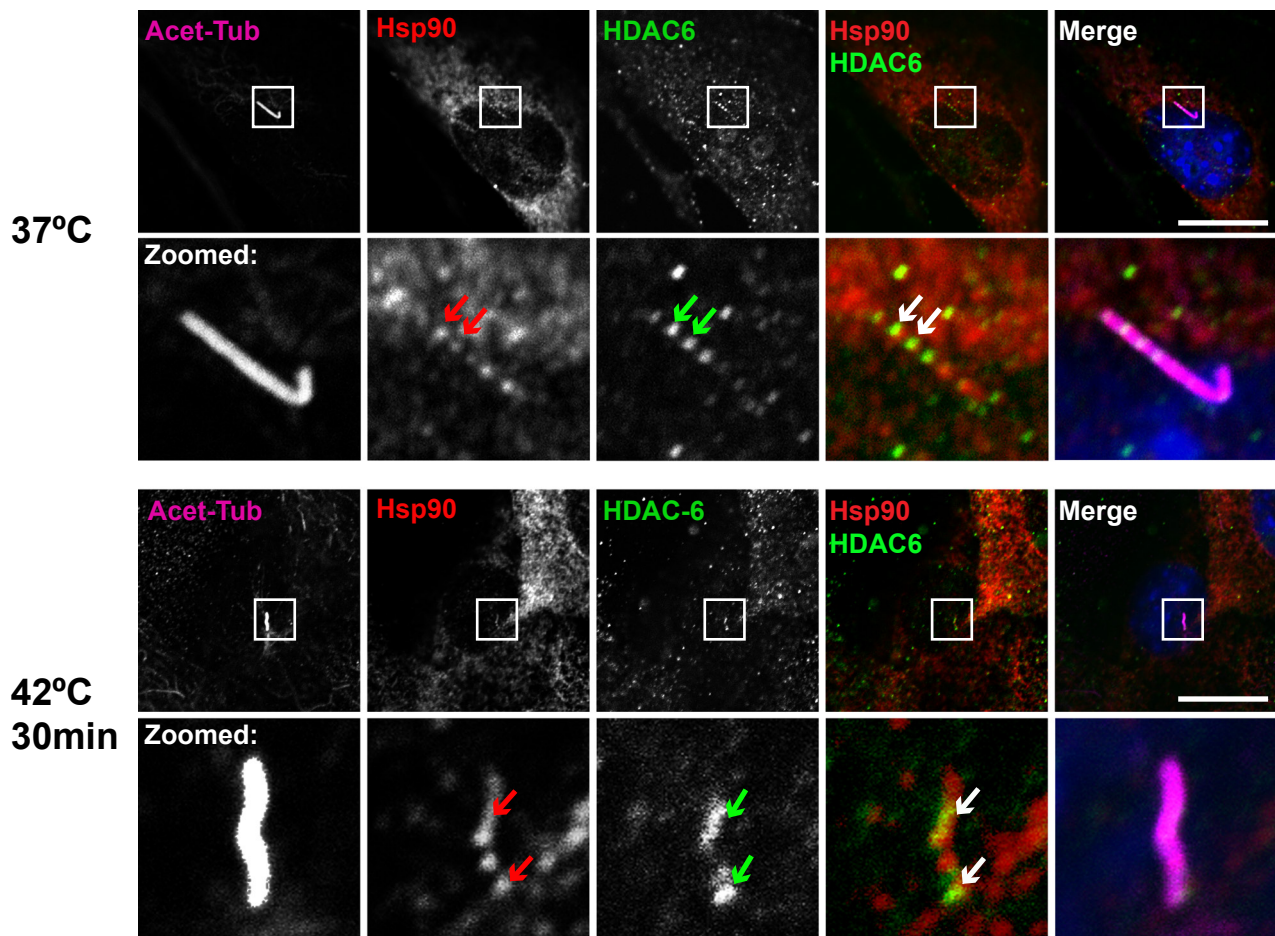


Figure S6