K562 cell lines transfected with Vector-Co, the pMEP-TOGp vector that directs Cd^{2+}-inducible expression of TOGp, or a vector that directs constitutive expression of shRNA-TOGp were generated as in Fig. 1 and Fig. 5. Cell transfected with pMEP-TOGp were analyzed after 20 h of Cd^{2+}-induction of the hMTIIa promotor. Representative confocal sections of methanol fixed cells stained with anti-α-tubulin (green) and rabbit anti-TOGp (red) are shown. To allow comparison of the presented images, the intensity settings are the same.

Analysis of mitotic cells (upper panels) confirms that endogenous TOGp in K562 cells stains the mitotic spindle with a concentration at the spindle poles. Under conditions of overexpression, the localization was similar but TOGp staining of spindles was more intense and uniform. It is also evident that 4 days of shRNA-TOGp expression abolish detectable staining.

Analysis of interphase cells (lower panels) reveals a diffuse staining of endogenous TOGp and an increased intensity of the diffuse staining in TOGp overexpressing cells. It is also evident that, as with mitotic cells, 4 days of shRNA-TOGp expression abolish detectable staining. The absence of clear anti-TOGp staining of microtubules of human interphase cells is consistent with previous reports (Charrasse, et al. 1998, J Cell Sci, 111, 1371-83; Gergely et al. 2003, Genes Dev, 17, 336-41). This contrast to amphibian cells which shows clear staining of interphase microtubules. Species specific differences with respect to the apparent localization of XMAP215/TOGp/Msps during interphase and mitosis are also evident by analysis of the Drosophila homologue Msps, which exhibit a strong localization to the centrosome during both interphase and mitosis but no detectable localization to interphase microtubules (Cullen et al. 1999, Cell Biol.146, 1005-18).