Figure S4. Kinetochore levels of C-Mad2 in the different tethered Mad1 constructs.

A) Cells transfected with the indicated KT-Mad1 proteins and treated with siMad1 were stained for GFP and C-Mad2 as indicated. B) The level of C-Mad2 and GFP was quantified on kinetochores from metaphase-arrested cells using deconvolved images. The intensity from the 3 z-stacks 200 nm apart encompassing the bulk kinetochore signal was used and the C-Mad2 level normalized to that of GFP. Each dot represents a single kinetochore and at least 40 kinetochores from 5 different cells were analyzed. A t-test was used to compare the different conditions (p ≤ 0.0001 (***) , ns: non significant (p > 0.05)) C) Stable HeLa cell lines were treated with control or Mad1 RNAi oligoes and where indicated complemented with RNAi resistant Venus Mad1 or Venus Mad1 F712A/R714A by inducing protein expression by doxycycline. The time from NEBD to anaphase was scored in each condition from time-lapse movies and the red lines indicate the median. A Mann Whitney test was used to compare the samples (p ≤ 0.05 (*), p ≤ 0.0001 (****)). The localization of Mad1 proteins to kinetochores are shown and represents a single frame from the time-lapse analysis.