Figure S2. Bub1 and BubR1 localize to kinetochores in KT-Mad2 metaphase arrested cells.

A-B) HeLa cells were transfected with the indicated KT-Mad2 constructs and siRNA oligo. The cells were arrested at metaphase and stained for Bub1 and BubR1. C) ITC measurements using purified antibody and recombinant Mad2 L13A (C-Mad2) or Mad2 V193N (O-Mad2) show specific binding of the C-Mad2 specific antibody to C-Mad2 with a Kd of approximately 5 nM and no binding to O-Mad2. D) Immunofluorescence images of cells stained with the C-Mad2 specific antibody show nuclear envelope staining in interphase and kinetochore staining in prometaphase. E) The C-Mad2 antibody specifically binds Mad2 L13A (C-Mad2) and not Mad2 ΔC (O-Mad2) from cells arrested with nocodazole. Stable cell lines expressing FLAG-tagged versions of Mad2, C-Mad2 or O-Mad2 were used in immunopurifications with control IgG or the C-Mad2 specific antibody.