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Supplemental Material to:

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Overexpression of EVI1 interferes with cytokinesis and leads to accumulation of cells with supernumerary centrosomes in $G_0/_1$ phase

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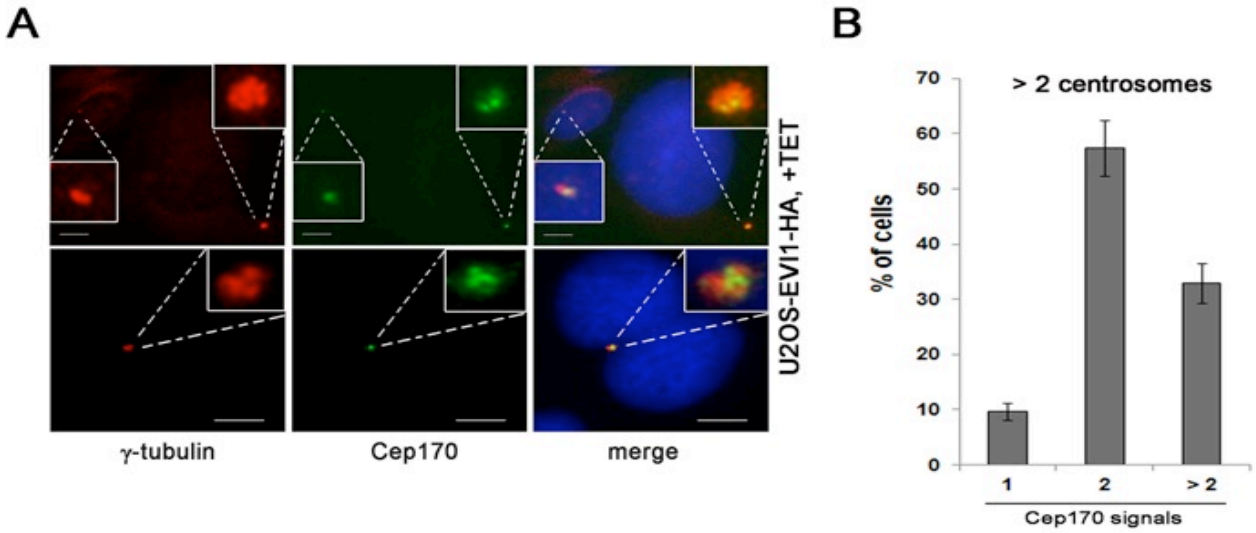

Figure S1. EVI1-induced centrosome amplification is not the result of centrosome overduplication in a single interphase. (A) In U2OS cells conditionally overexpressing EVI1-HA, transgene expression was induced for 72 h. Cells were immunostained for γ-tubulin (red) and Cep170 (green). DNA was counterstained with DAPI (blue). Scale bars represent 10 µm. Insets show centrosomes at higher magnification. Note that the upper panel shows examples of a normal cell with regular centrosome content (upper left) and a cell with enlarged nucleus and supernumerary centrosomes (lower right), while the lower panel shows an example of a binuclear cell with supernumerary centrosomes. (B) The observations shown in (A) were quantified by classifying cells with more than two centrosomes (as determined by the number of γ-tubulin signals) according to their number of Cep170 signals as detailed in the figure. Results are given as mean ± SD of three times 100 cells per data bar.

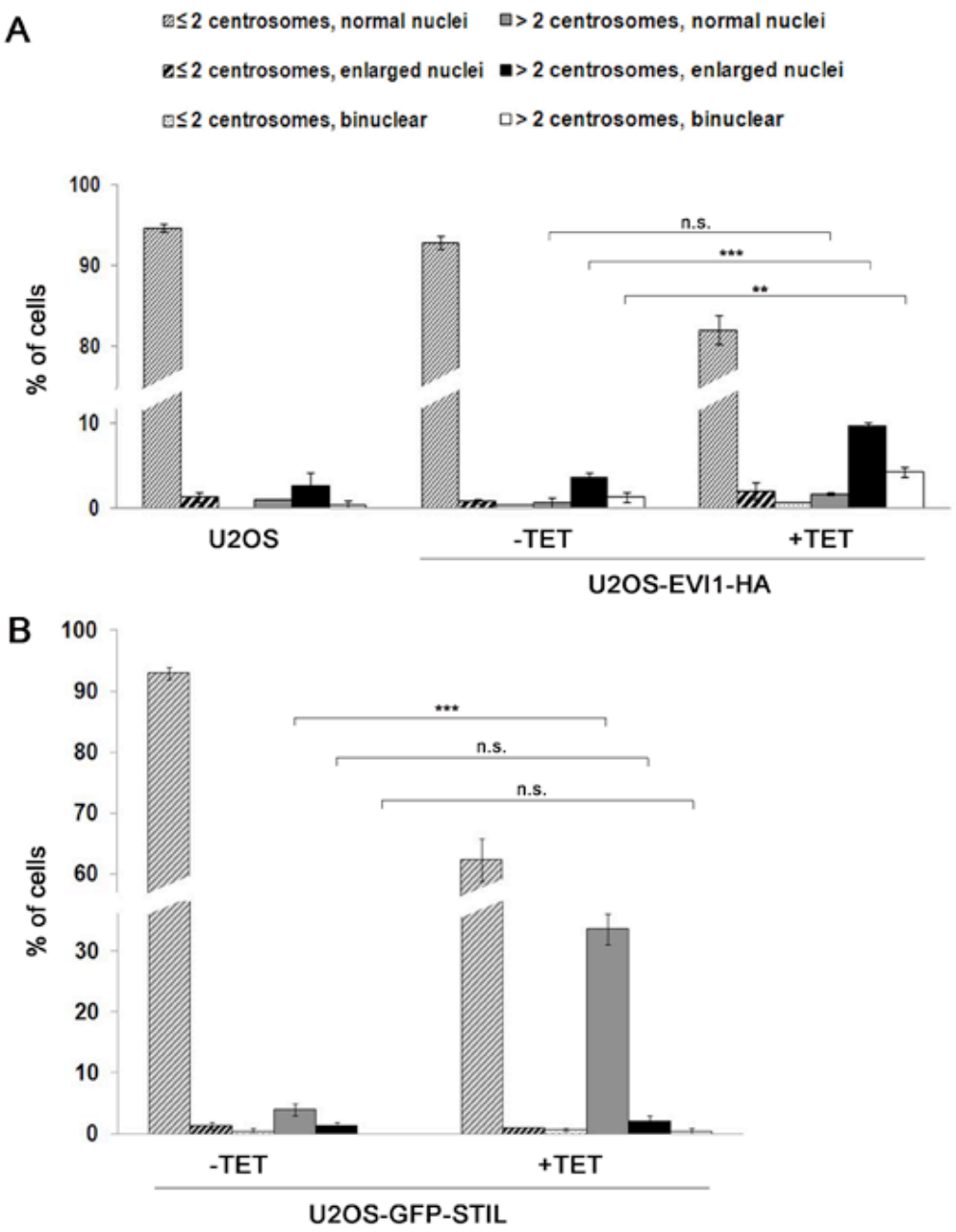

Figure S2. (A) U2OS cells conditionally overexpressing EVI1-HA were left untreated (-TET) or induced to express the transgene for 72 h (+TET). Parental U2OS cells were grown in parallel. Following immunostaining for γ-tubulin and EVI1-HA and DNA counterstaining with DAPI, cells were classified according to their centrosome number and their nuclear morphology as detailed in the figure. Results are given as mean ± SD of three times 100 cells per data bar. ***, this difference is highly significant (P = 0.00021). **, this difference is significant (P = 0.00312). n.s., this difference is not significant (P > 0.05). Note that the source data are identical to the data shown in Figure 2B, where cells with regular centrosome content have been omitted in the presentation. (B) U2OS cells conditionally overexpressing GFP-STIL were left untreated (-TET) or induced to express the transgene for 72 h (+TET). Following immunostaining for γ-tubulin and DNA counterstaining with DAPI, cells were classified according to their centrosome number and their nuclear morphology as detailed in the figure. Results are given as mean ± SD of three times 100 cells per data bar. ***, this difference is highly significant (P = 0.00071). n.s., these differences are not significant (P > 0.05).

Table S1: Cell cycle analysis after knockdown of p53

	Proportion of cells with > 4N DNA (%)	**Proportion of cells with 8N DNA (%)**
U2OS, siluc	1.81	0.09
	1.65	0.18
Mean	1.73	0.14
SD	0.11	0.06
U2OS, sip53	3.46	0.22
	2.78	0.46
Mean	3.12	0.34
SD	0.48	0.17
#1, -TET, siluc	3.51	0.47
#2, -TET, siluc	1.77	0.16
#3, -TET, siluc	1.65	0.13
Mean*	2.31	0.25
SD	1.04	0.19
#1, -TET, sip53	6.74	1.13
#2, -TET, sip53	2.95	0.33
#3, -TET, sip53	3.53	0.42
Mean*	4.41	0.63
SD	2.04	0.44
#1, +TET, siluc	4.53	1.07
#2, +TET, siluc	3.05	0.79
#3, +TET, siluc	4.01	0.79
Mean*	3.86	0.88
SD	0.75	0.16
#1, +TET, sip53	8.11	2.44
#2, +TET, sip53	4.57	0.82
#3, +TET, sip53	8.98	2.40
Mean*	7.22	1.89
SD	2.34	0.92

Abbreviations: #1, U2OS-EVI1-HA cells, passage 3; #2: U2OS-EVI1-HA cells, passage 9; #3, U2OS cells retrovirally transduced to conditionally overexpress EVI1, passage 4; SD, standard deviation; siluc, siRNA targeting luciferase; sip53, siRNA targeting p53; TET, tetracycline

Note: *Since polyploidization frequency and phenotype of U2OS-EVI1-HA cells and U2OS cells retrovirally transduced to conditionally overexpress EVI1 were virtually identical, we combined the respective flow cytometry data.