

Expanded View Figures

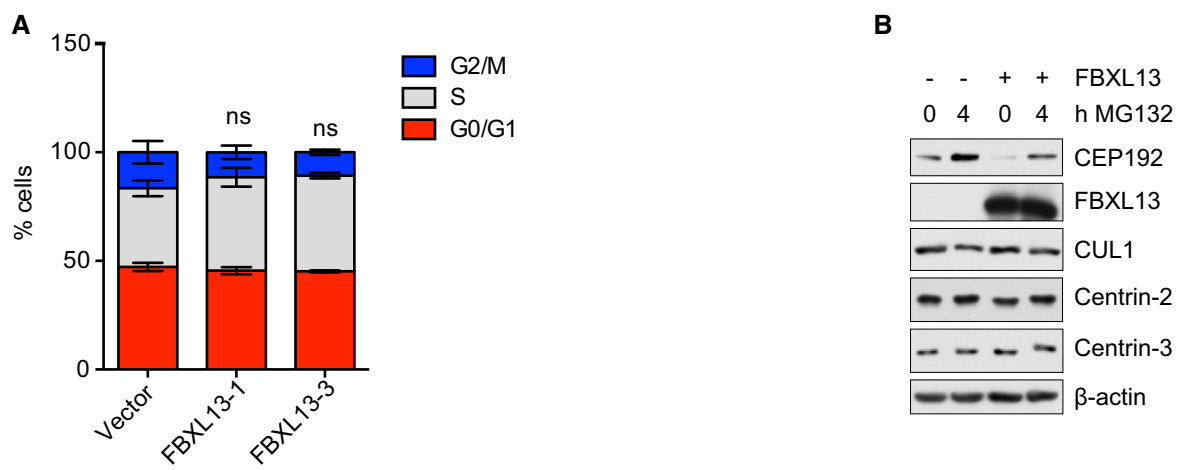


Figure EV1. Impact of FBXL13 on cell cycle distribution and CEP192 expression levels.

- A Quantification of cell cycle profile of HEK293T cells as determined by fluorescence-activated cell sorting (FACS) using propidium iodide staining. HEK293T cells were transfected with FBXL13 isoform 1 (FBXL13-1) or FBXL13 isoform 3 (FBXL13-3). An empty vector (Vector) was used as a negative control (mean \pm SD from three independent experiments; ns, non-significant $P > 0.05$). The statistical method used was a one-sample, unpaired Student's *t*-test with a hypothetical means of 1.0 between G2/M populations of the treatment conditions compared to control.
- B Detection of CEP192, Centrin-2, Centrin-3 and CUL1 after expression of FBXL13 in the presence or absence of MG-132. β -actin was used as a loading control.

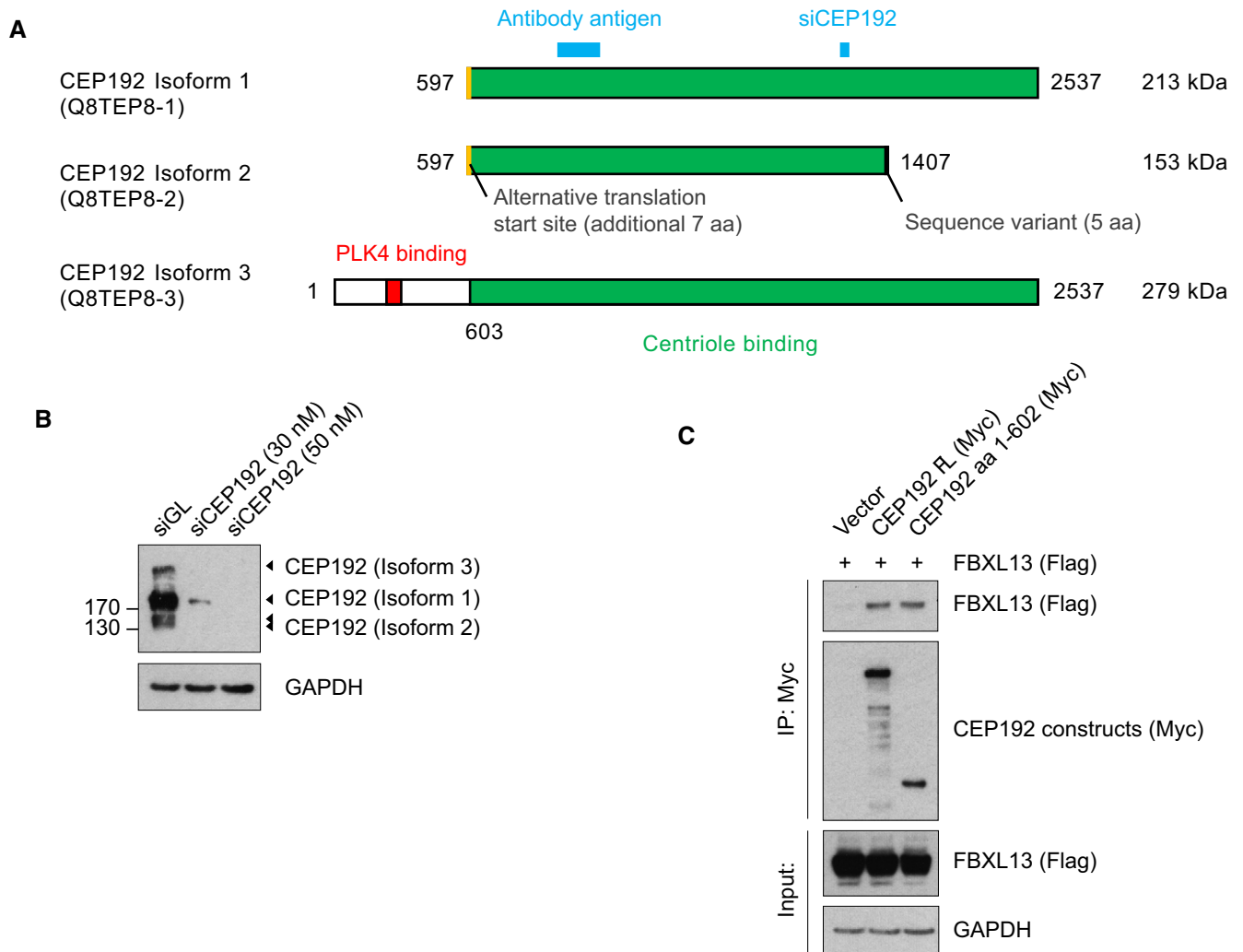


Figure EV2. CEP192 isoform specification and FBXL13-CEP192 interaction mapping.

- A CEP192 (Uniprot ID: Q8TEP8) has three isoforms. The PLK4 binding region is shown in red and the centriole binding region in green. The two shorter isoforms (1 and 2) are generated through an alternative translation start site reported by Sonnen *et al* [8], shown in yellow. CEP192 isoform 2 also contains a sequence variant of 5 amino acids at its C-terminus. The antibody used in this study recognises the antigen shown in blue. The siCEP192 sequence used in this study targets all three isoforms and is also shown in blue.
- B CEP192 protein levels in U2OS cells following transfection with a non-targeting siRNA control (siGL) or an siRNA targeting CEP192.
- C Detection of Flag-FBXL13 binding to immunoprecipitated Myc-tagged CEP192 full length (FL) or a truncation mutant (aa 1–602) specific to CEP192-3. CEP192 and FBXL13 were co-transfected in HEK293T cells as indicated.

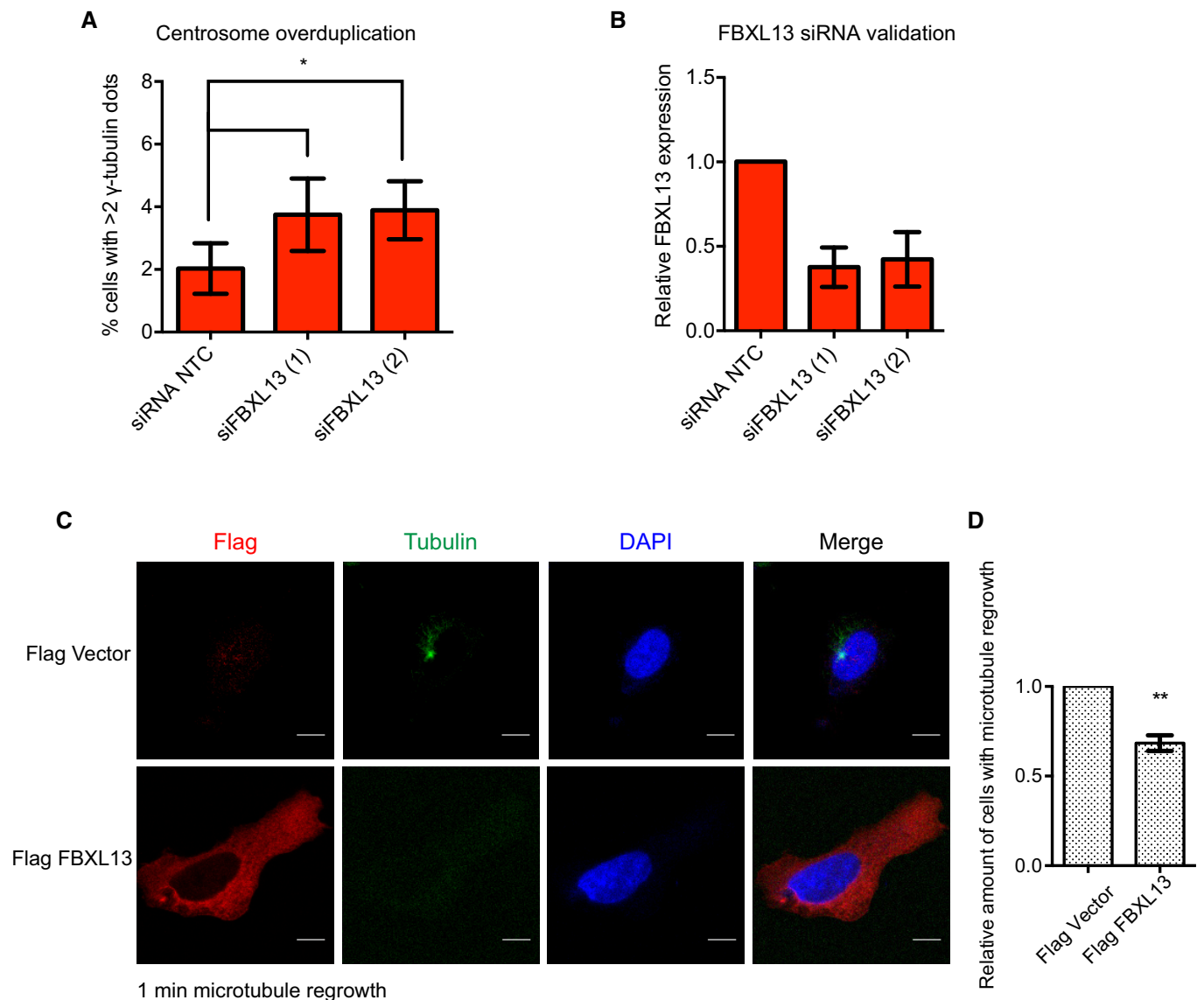


Figure EV3. FBXL13 regulates centrosome duplication and centrosomal microtubule nucleation.

- A** Quantification of centrosome overduplication in U2OS cells transfected with a non-targeting siRNA (siRNA NTC) or two siRNA oligos targeting FBXL13 (1 and 2) (mean \pm SD from three independent experiments, $n > 200$ cells per condition; $*P \leq 0.05$). Statistical analysis was performed by using one-sample *t*-test with hypothetical means of 1.0.
- B** Validation of FBXL13 mRNA level using quantitative real-time PCR (qPCR) in the samples shown in (A) (mean \pm SD from three qPCR triplicates).
- C** Microtubule regrowth assay in U2OS cells transfected with either Flag-FBXL13 or an empty vector control. Microtubules were fully depolymerised by treating cells with 10 μ M nocodazole for 2 h at 4°C. Cells were fixed 1 min after nocodazole washout and stained for Flag (red), α -tubulin (green) and DNA (DAPI, blue). Scale bar, 10 μ m.
- D** Quantification of cells which nucleated centrosomal microtubules after complete depolymerisation shown in (C) (mean \pm SD from three independent experiments, $n = 100$ cells per condition and experiment, $**P \leq 0.01$). Statistical analysis was performed by using one-sample *t*-test with hypothetical means of 1.0.

Figure EV4. FBXL13 depletion impairs cell migration.

- A Representative images from a wound healing assay of U2OS cells transfected with a control siRNA (siGL) or an siRNA targeting FBXL13 (2). The wound edge is shown in white. Scale bar, 100 μm .
- B Quantification of wound closure efficiency shown in (A) as calculated by the percentage of wound area closed (mean \pm SD from three independent experiments, $n > 4$ wounds per condition, $*P \leq 0.05$). The statistical method used was a one-sample, unpaired Student's *t*-test with a hypothetical means of 1.0.
- C Validation of FBXL13 mRNA level in the samples shown in (A) using quantitative real-time PCR (qPCR; mean \pm SD from three qPCR triplicates).
- D Representative images from a wound healing assay of U2OS cells stably overexpressing pBabe FBXL13 or an empty vector control (VEC). Cells were transduced with either a lentiviral vector containing an shRNA targeting FBXL13 or a control shRNA. The wound edge is shown in white. Scale bar, 250 μm .
- E Quantification of wound closure efficiency in (D) (mean \pm SD from three independent experiments, $n > 4$ wounds per condition).
- F Validation of FBXL13 mRNA level in the samples shown in (D) using quantitative real-time PCR (qPCR) (mean \pm SD from three qPCR triplicates).

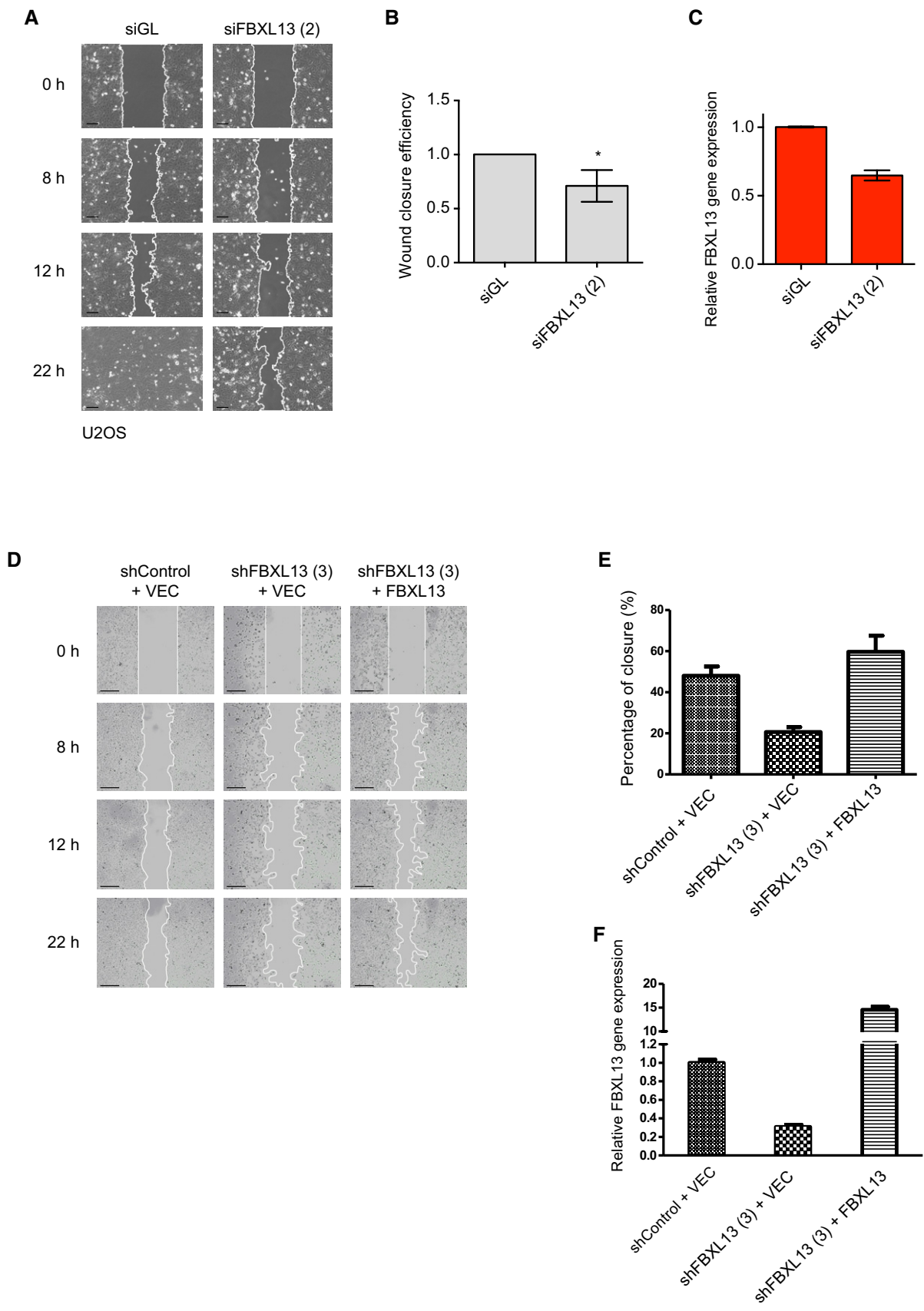


Figure EV4.