

The *Rb1* tumour suppressor gene modifies telomeric chromatin architecture by regulating TERRA expression.

I. Gonzalez-Vasconcellos^a, R. Schneider^b, Natasa Anastasov^c, S. Alonso-Rodriguez^a, B. Sanli-Bonazzi^c, JL Fernández^{a,d}, MJ Atkinson^{c*}

Supplementary 1: **A.** GFP signal in the U2OS cells transfected with the Rb1 knock down vector (shRB1) or the overexpressing vector (Ef1-RB1), 72h post transfection. **B.** Western blot and its quantification performed with the Rb1 antibody form BD to assess the amount of Rb1 protein after transient transfections.

Supplementary 2: Q-PCR quantification showed the endogenous TERRA expression in human U2OS cells modulated by Rb1 expression levels on chromosome XpYp. Control bar is the data for the untransfected U2OS cells. Ef1-RB1 bar shows the data for the U2OS transfected with the RB1 over-expressing vector and the shRB1 bar shows the data for the U2OS transfected with the RB1 knock-down vector. Error bars show the SD of 3 technical replicates. Significance of the experiment was assessed with one-way ANOVA.

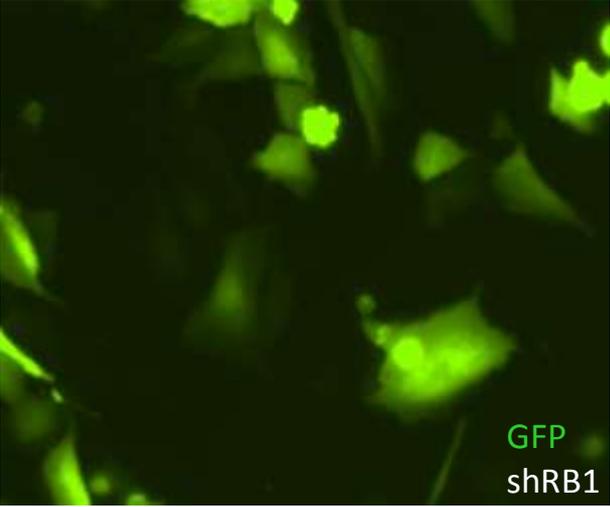
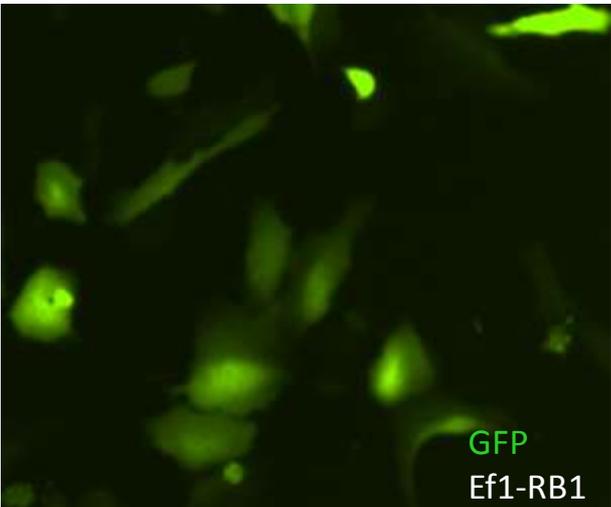
Supplementary 3: Cloning strategy of the TERRA promoter into a luciferase reporter vector (pGI3) and constructs formed. Cloning was performed using the XhoI site (*) and Hind III site (**). The sv40 promoter was removed and replaced with the TERRA promoter predicted sequence (vTERRA). The vTERRA* truncated construct lacking the 3' end was randomly obtained during the cloning process.

Supplementary 4: *In vitro* luciferase expression driven by the sv40 promoter (pGI3) is not modulated by RB1 levels in transient co-transfections with either shRB1 or Ef1-RB1. Error bars show the standard deviation of three technical replicates 72 hours after transfection. RLU stands for relative luciferase units (relative to β -Galactosidase).

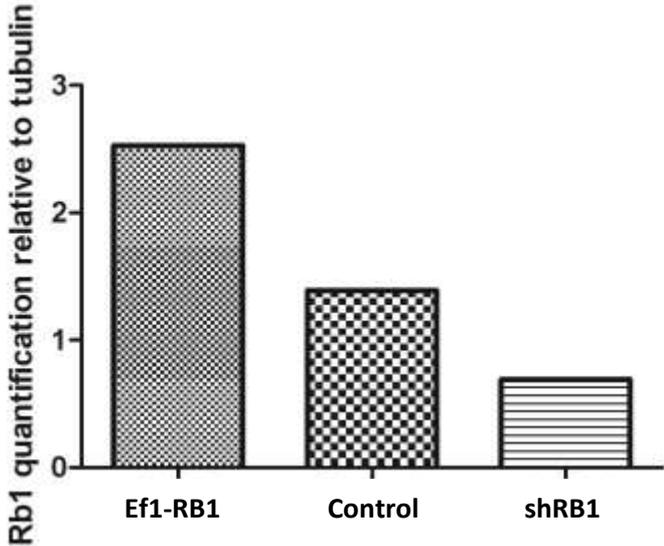
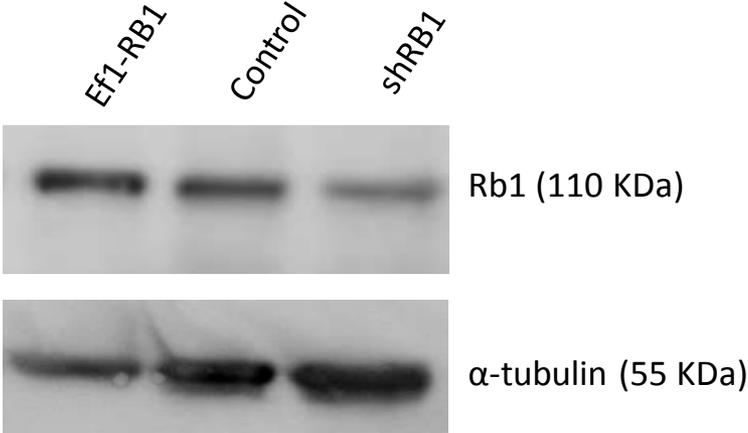
Supplementary 5: All primers used in this study.

Supplementary 1

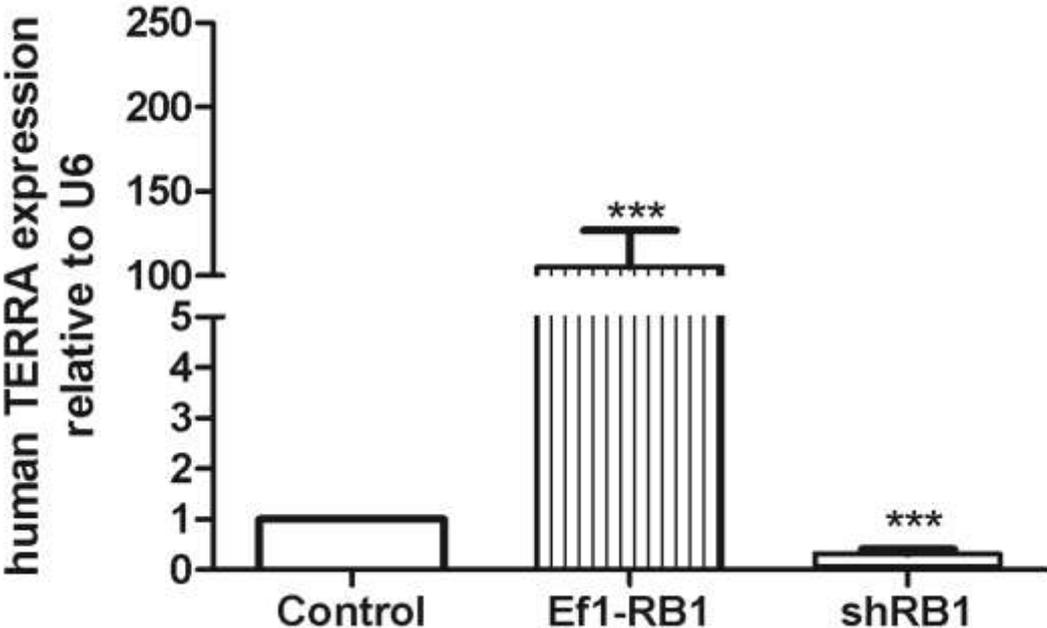
A



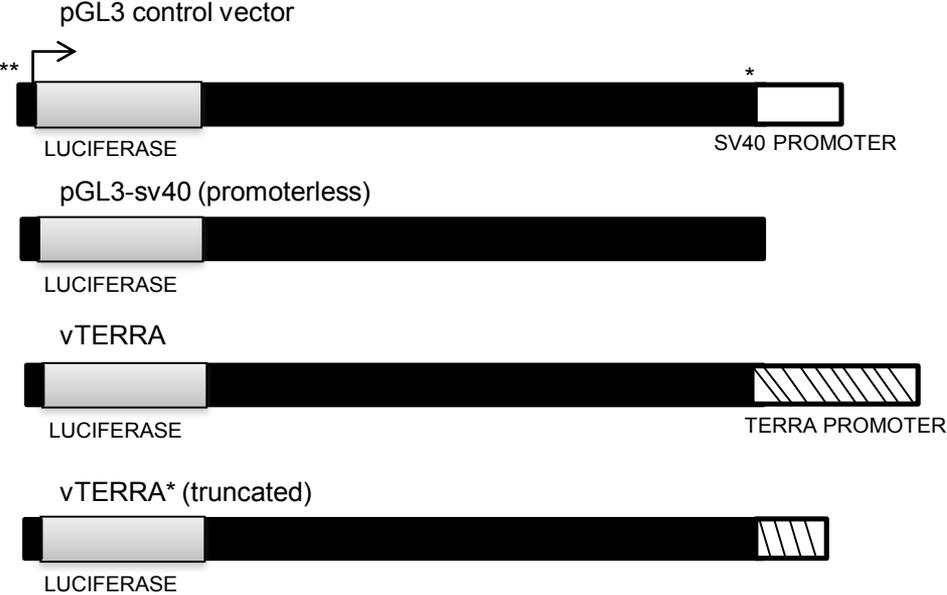
B



Supplementary 2



Supplementary 3



Supplementary 4

