Supplementary Figure S1

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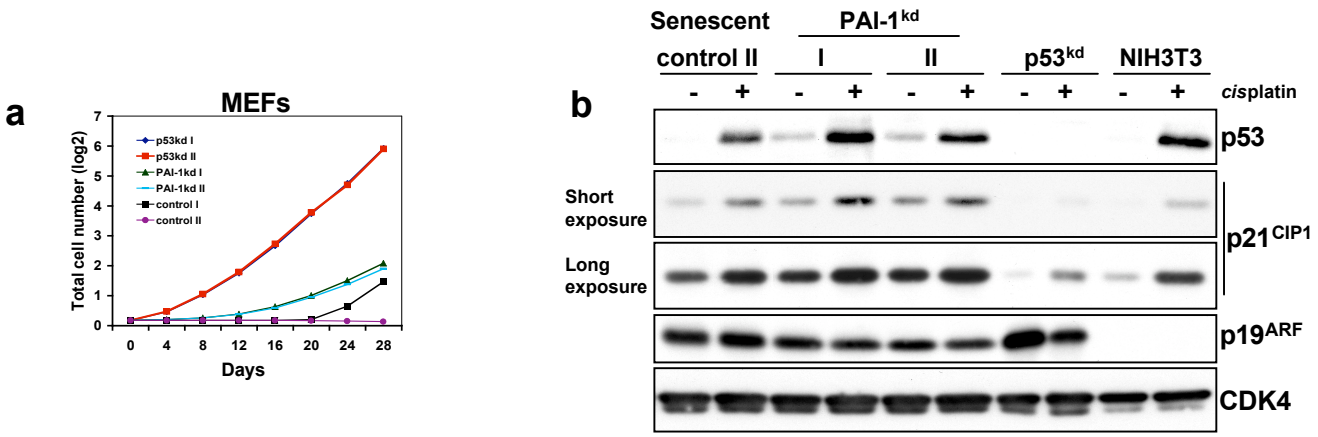

Supplementary Figure S1 PAI-1^{kd} induces senescence-bypass in primary MEFs retaining p19^{ARF}-p53 signalling.
(a) Long-term proliferation curves of p53^{kd}, PAI-1^{kd}, or non-functional shRNA control infected MEFs. Shown are the results of two independent experiments per construct (I, II).
(b) Western blot analysis for p53 and its targets p19^{ARF} and p21^{CIP1} using cell lines depicted in (**a**) after *cis*platin-induced DNA-damage. NIH3T3 cells are immortal p16^{INK4A}/p19^{ARF}-deficient controls.

Supplementary Figure S2

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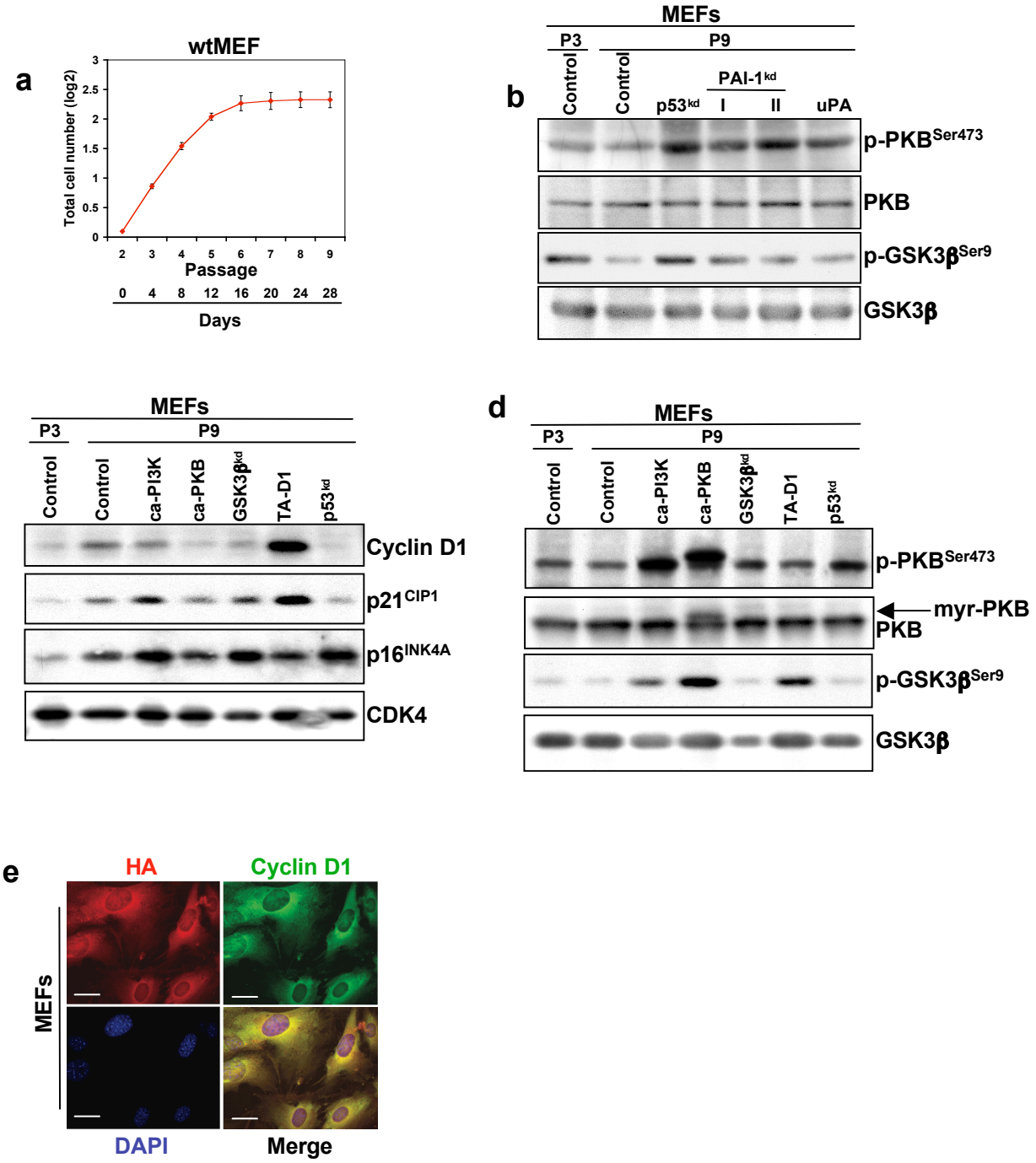

Supplementary Figure S2 Retention or induction of PKB-GSK3β signalling in immortal MEFs.
(a) Proliferation curves of 6 independently isolated cultures of wild-type MEFs. Mean value (+/- SD) is indicated.
(b) Expression of phosphorylated PKB or GSK3β related to unphosphorylated fraction of the same proteins in P3, P9, $p53^{kd}$, $PAI\text{-}1^{kd}$, or uPA over-expressing cells as analyzed by western blot.
(c) Western blots of protein samples of indicated cell lines with antibodies for tumour suppressors $p16^{INK4A}$ or $p21^{CIP1}$, or cyclin D1. CDK4 is a loading control.
(d) Western blots for the same set of cell lines as in (c) for phospho-specific PKB and GSK3β, as compared to unphosphorylated fractions of the same proteins.
(e) Qualitative immunofluorescence analysis for HA-tag (HA) and cyclin D1 in senescent MEFs over-expressing HA-tagged cyclin D1. Bar represents 50 μm.

Supplementary Figure S3

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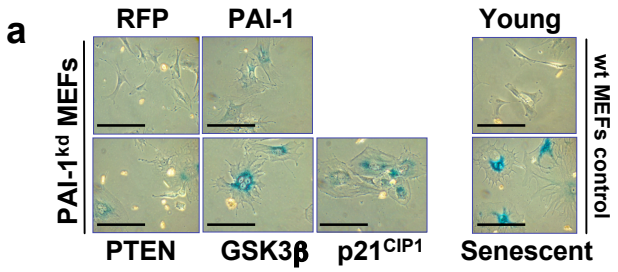

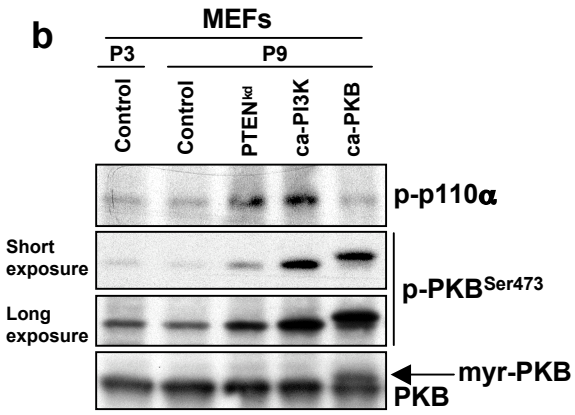

Supplementary Figure S3 PAI-1 is sufficient for induction of senescence in MEFs.
(a) PAI-1, PTEN, GSK3β, or $p21^{CIP1}$ over-expression induces senescence in $PAI\text{-}1^{kd}$ MEFs as indicated by staining for senescence-associated β-galactosidase (SA-β-Gal). Staining controls are young and senescent wild-type MEFs. Scale bar represents 250 μm.
(b) Expression of phosphorylated p110α (catalytic subunit of PI3K) and PKB related to unphosphorylated fraction of the same protein in P3, P9, $PTEN^{kd}$, ca-PI3K or ca-PKB over-expressing cells as analyzed by western blot.

Supplementary Figure S4

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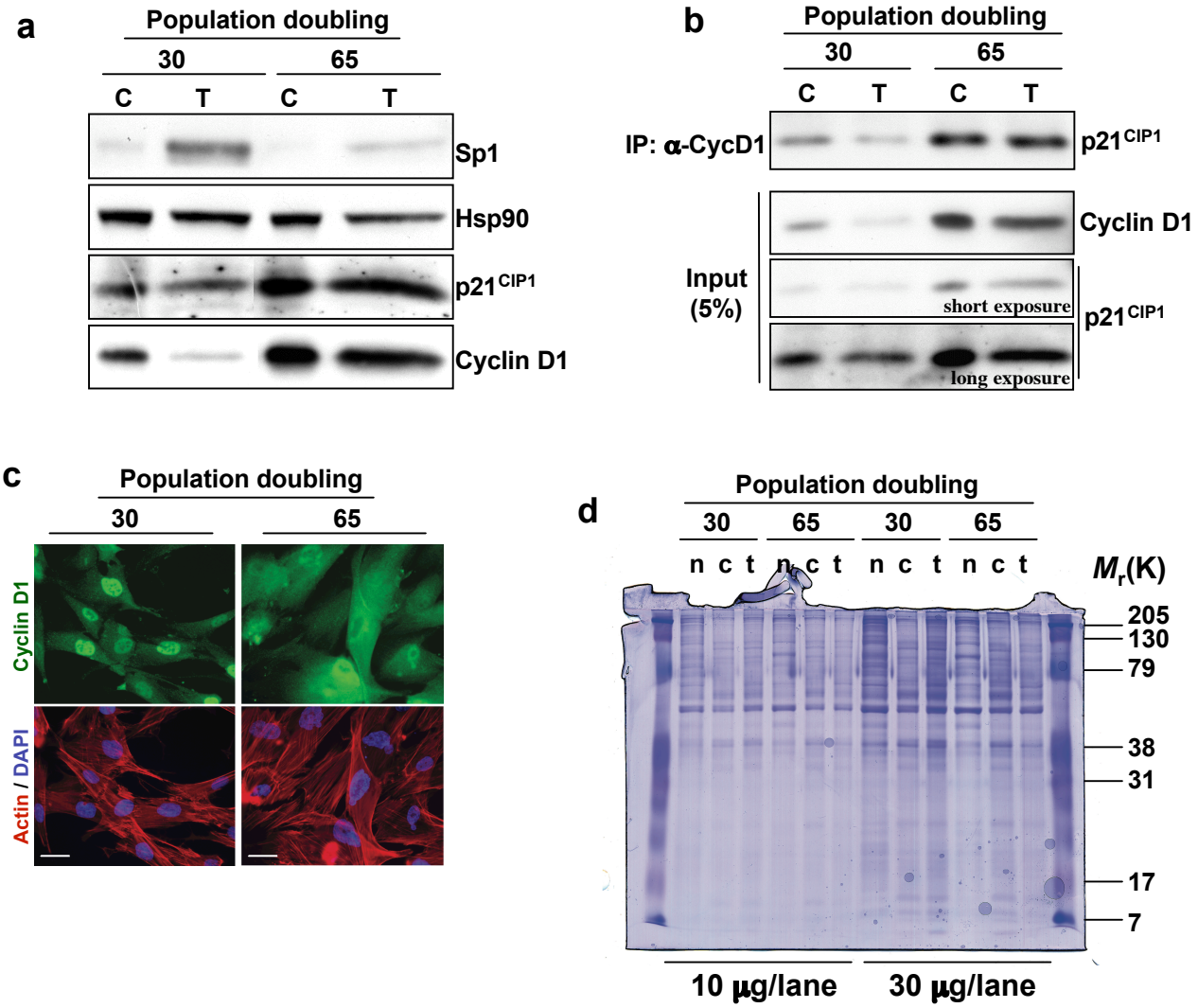
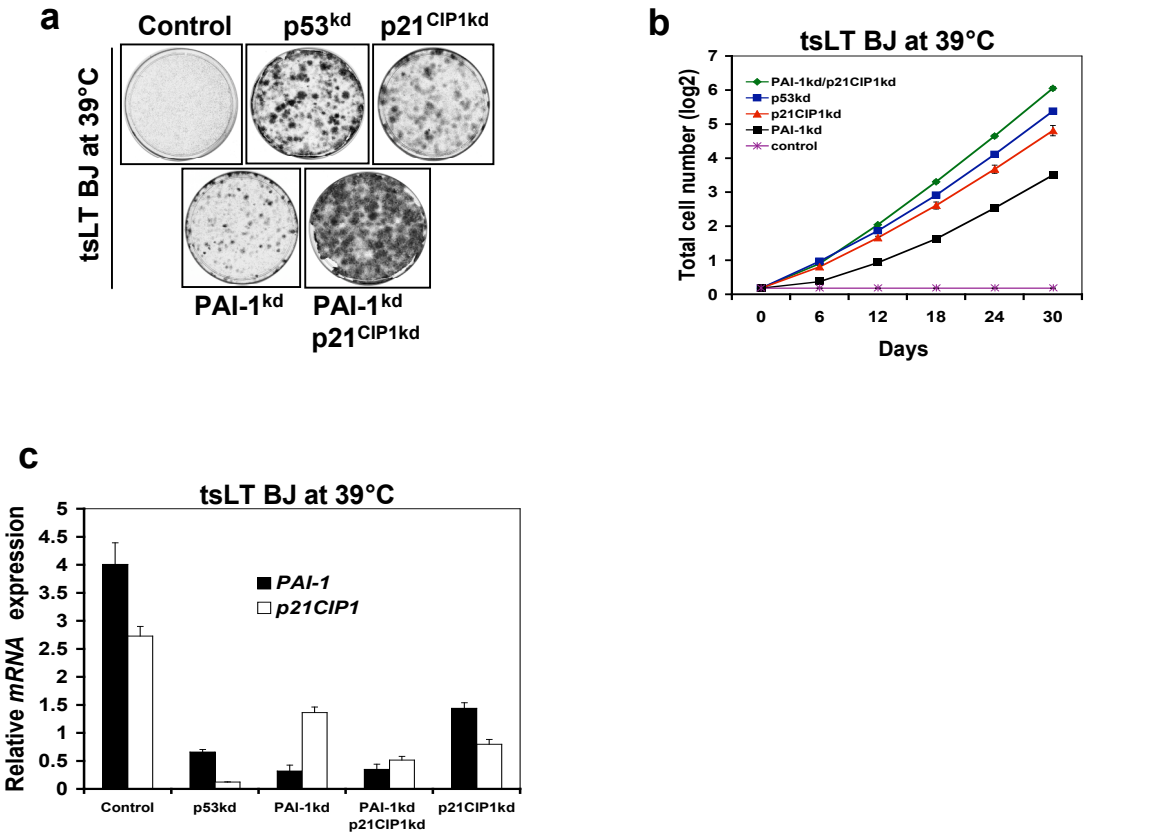


Supplementary Figure S4 Cytoplasmic co-localization of cyclin D1 and p21^{CIP1} in ageing primary BJ fibroblasts.
(a) Western blot of cytoplasmic (C) and total (T) protein lystates from primary young passage doubling 30 and senescent passage doubling 65 BJ fibroblasts probed for indicated proteins. Sp1 is a nuclear protein and Hsp90 a cytoplasmic fraction control.
(b) Immunoprecipitation with cyclin D1 antibody in protein lysates from **(a)** immunoblotted for p21^{CIP1}.
(c) Qualitative immunofluorescence analysis of serially passaged BJs, passage doubling 30 or passage doubling 65, for cyclin D1 expression. Bar represents 50 μm.
(d) Coomassie staining of SDS gel with 10 or 30 μg of nuclear (n), cytoplasmic (c) or total (t) protein from young populatipon doubling (PD) 30 or senescent PD 65 primary BJ fibroblasts, for equal loading.

Supplementary Figure S5

R. Bernards NCB-B07159C



Supplementary Figure S5 PAI-1 and p21^{CIP1} collaborate in senescence response downstream of p53.
(a) Colony formation assay of depicted constructs in conditionally immortalized tsLT hTERT BJ fibroblasts[29]. Control is a non-functional shRNA. These cells enter into a p53-dependent proliferation arrest when shifted to the non-permissive temperature (39°C). Importantly, these cells show virtually exact characteristics as primary BJ fibroblasts when challenged through assays as described in Fig. 5a-e (data not shown).
(b) Long term growth curves at the non-permissive temperature of 39°C of cells over-expressing depicted constructs. Per genotype the mean (+/- SD) of 3 independent cultures is shown. *PAI-1* and *p21^{CIP1}* are both p53 target genes and knockdown of the expression of either gene alone results in a less efficient senescence bypass than seen with knockdown of p53 alone (see also **(a)**).
(c) Quantitative real-time PCR analysis of relative expression of *PAI-1* and *p21^{CIP1}* in cell lines depicted in (**b**). As in primary BJ fibroblasts (see Fig. 5b), we noticed reduction of *PAI-1* mRNA in p21^{CIP1kd} cells and reduction of *p21^{CIP1}* mRNA in PAI-1^{kd} cells, suggesting that loss of either gene influences transcription of the other.