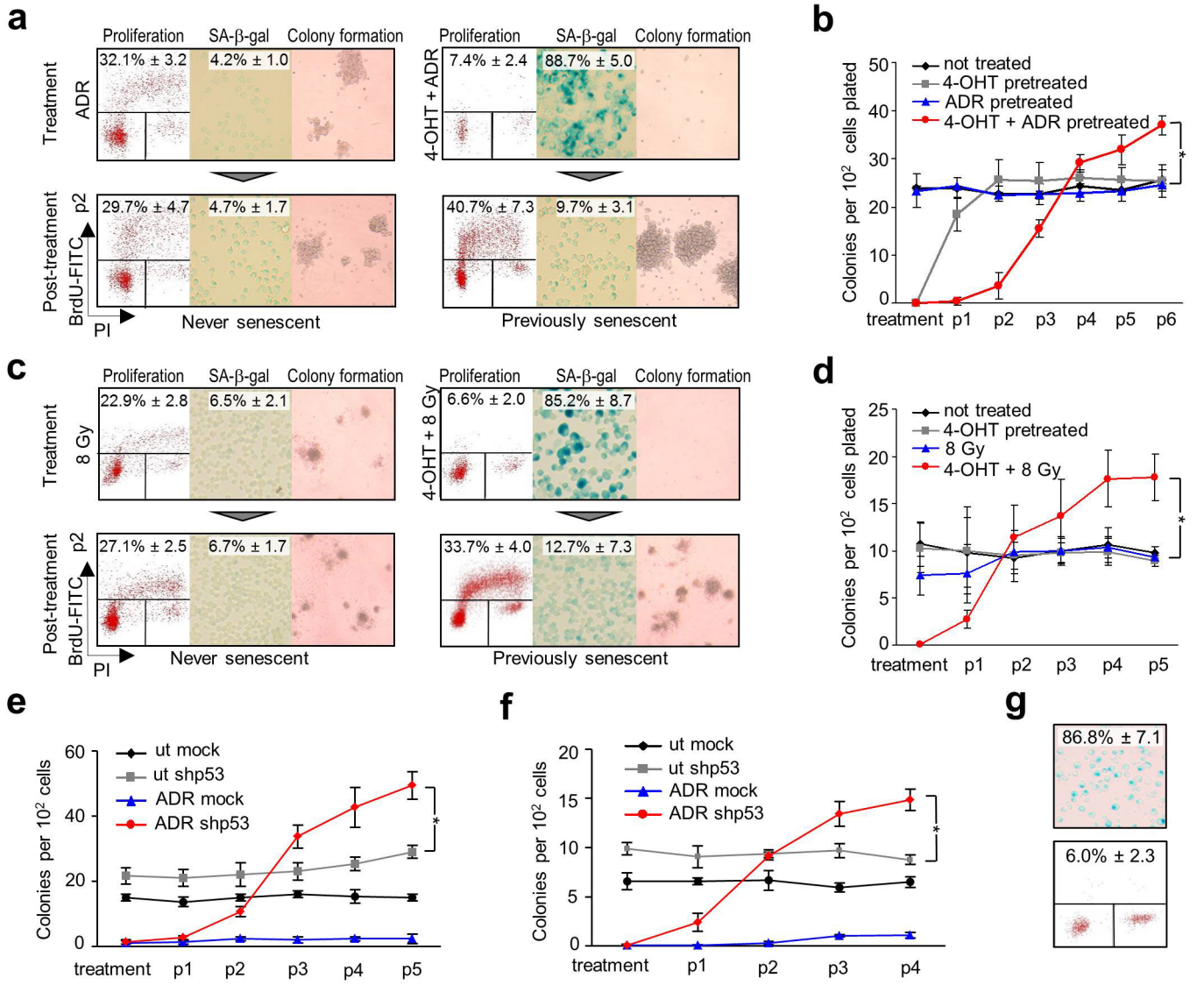

Extended Data Figure 3 | Senescence-released (previously senescent) cancer cells display higher tumour-initiating capacity than their never-senescent counterparts. a–d, Growth properties of conditionally senescent lymphoma cells analysed as in Fig. 2a, b, but using p53-ER^{Tam};*Bcl2* lymphoma cells with ADR ± 4-OHT treatment (**a**, **b**), or *Suv39h1*$^{-}$;*Bcl2*;*Suv39h1*-ER^{T2} lymphoma cells exposed to a single dose of γ-irradiation (8 Gy) instead of ADR, followed by five days of 4-OHT treatment and subsequent passaging in 4-OHT-free medium (**c**, **d**). Results presented as mean positive cells or mean colony numbers ± s.d.; $n = 4$ (**a**, **c**, **d**) or $n = 3$ (**b**) biologically independent samples. Representative photomicrographs from one out of three independent experiments (**a**, **c**). Two-tailed, unpaired *t*-test with Welch's correction, comparing ADR- and 4-OHT+ADR pretreated lymphomas at p6, or 8 Gy- and 4-OHT+8 Gy at p5. *$P < 0.05$ (**b**, **d**). It is noteworthy that the superior growth and clonogenicity of post-senescent cells can be explained neither by rare cells that may simply have bypassed senescence, because the matching never senescent (that is, senescence bypasser) group presented with inferior clonogenicity, nor by an enhanced death rate of non-stem cells in the Suv39h1-proficient aliquot, because no significant differences in viability were observed between never senescent and previously senescent groups throughout these experiments. Viability determined by flow cytometry as thepercentage of annexin V/PI double-negative cells was typically greater than 80% and comparable between never senescent and previously senescent cells (not shown; the same applies for Figs 2a and 4a). Growth-promoting mutations are also unlikely, as senescent cells stopped replicating their DNA. **e**, **f**, Colony formation assay of untreated versus five-day-ADR-senescent human RCK8 lymphoma cells (**e**) or LT174T colon carcinoma cells (**f**) that were exposed to a shp53-lentivirus or mock infection on day five of ADR treatment, with *p53* knockdown enabling outgrowth out of fully established senescence. As observed for mouse lymphoma cells, post-senescent RCK8 and LT174T cells, after just three passages, outperformed the clonogenic potential of tumor cells that were equally exposed to shRNA against *p53* but never experienced senescence. Results represent mean colony numbers at indicated passages (each reflecting seven days in ADR-free methylcellulose medium) ± s.d., $n = 3$ independent experiments. Two-tailed, unpaired *t*-test with Welch's correction, comparing untreated shp53 versus ADR + shp53 at p5 (**e**) or p4 (**f**). *$P < 0.05$. **g**, TIS re-inducibility in *Suv39h1*$^{-}$;*Bcl2*;*Suv39h1*-ER^{T2} previously senescent cells (at passage 2, compare with Fig. 2a) re-exposed to 4-OHT and ADR for five days, as detected by SA-β-gal staining (up) and BrdU/PI incorporation (down). Results represent mean percentages of positive cells ± s.d. ($n = 4$ independent lymphomas).

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