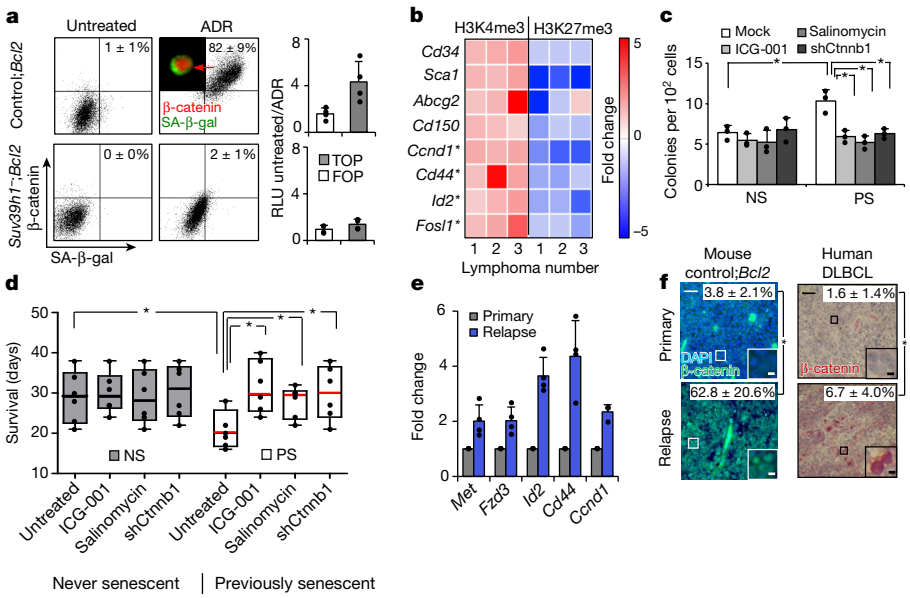

Figure 3 | Canonical Wnt signalling, activated in TIS, is an essential driver of the enhanced tumour initiation capacity exhibited by senescence-released tumour cells. a, Co-expression of the fluorescent SA-β-gal marker and β-catenin in ADR-exposed control;*Bcl2* or TIS-incapable *Suv39h1*$^{-}$;*Bcl2* lymphoma cells (left), and corresponding β-catenin transcriptional activities measured as relative TOPflash T-cell factor (TCF) reporter signals with FOPflash as a TCF-binding site mutant control (right). Mean percentage of double-positive cells or mean relative light units fold change (between ADR-treated and untreated samples) ± s.d., respectively ($n = 4$ biologically independent samples each). The inset shows a representative photomicrograph from four independent experiments. **b**, Colour-coded heat map reflecting fold change (between previously senescent and never senescent cells) of permissive H3K4me3 and repressive H3K27me3 histone marks at the promoters of indicated ATSC- or Wnt-related (asterisks) genes by chromatin immunoprecipitation ($n = 3$ biologically independent samples). **c**, Colony formation of never senescent (NS) versus previously senescent (PS) *Suv39h1*$^{-}$;*Bcl2*;*Suv39h1*-ER^{T2} lymphomas (passage 2, compare with Fig. 2), exposed to the pharmacological Wnt inhibitors (ICG-001, salinomycin) or shRNA against *Ctnnb1* (shCtnnb1) for 7 days. Results reflect mean colony numbers ± s.d. ($n = 3$ biologically independent samples). Two-tailed unpaired *t*-test with Welch's correction, $^{*}P < 0.05$. **d**, Survival of mice transplanted with matched previously senescent or never senescent cells and treated with indicated Wnt inhibitors upon palpable lymphoma formation. Cells with shRNA against *Ctnnb1* were shRNA-infected before transplantation. Boxes frame the 25th to 75th percentile range, with median, minimal and maximal values ($n = 6$ mice per treatment group). Two-tailed, paired *t*-test, $^{*}P < 0.05$. **e**, Expression of Wnt target genes (by qPCR) in matched cases of control;*Bcl2* lymphomas before and after relapse from senescence-inducing cyclophosphamide treatment *in vivo* (mean fold change ± s.d., $n = 4$ biologically independent samples). **f**, Nuclear β-catenin expression by immunostaining of lymph nodes from control;*Bcl2* lymphoma-bearing mice as in **e** (left; $n = 4$ biologically independent samples), and human DLBCL biopsies from the same individual patients at diagnosis and at relapse after first-line induction chemotherapy (right; $n = 5$ independent patients). Mean percentage of positive cells ± s.d.; two-tailed, paired *t*-test, $^{*}P < 0.05$. Representative photomicrographs; scale bars, 100 μm (magnifying inserts, 10 μm).

of the same lymphoma treated with the same dose of chemotherapy, reflecting the now unleashed stemness properties acquired as a latent program during senescence (Fig. 2a, b). The enhanced colony-founding potential of previously senescent cells was stable over an extended observation period of up to 100 days (reflecting 14 serial replatings; Fig. 2b). Similar results were obtained with p53-ER^{TAM} as another inducible senescence gatekeeper; with γ-irradiation as an alternative senescence trigger; with ADR-exposed human lymphoma cell lines; and with colon cancer cells representing a solid, epithelial cancer type (Extended Data Fig. 3a–f). It is noteworthy that previously senescent cells typically retained the ability to re-enter TIS when re-exposed to 4-OHT and ADR, indicating that no selection for senescence-compromising mutations occurred in previously senescent cells (Extended Data Fig. 3g). Previously, an instructive, non-cell-autonomous role has been attributed to the senescence-associated secretory phenotype (SASP; reviewed in ref. 2) in models of inducible reprogramming and tissue regeneration[15,16]; however, our observations, made in pure, homotypic tumour cell populations, even under drastic reduction of SASP factor expression, favour a largely cell-intrinsic mechanism of senescence-associated reprogramming (Extended Data Fig. 4). Although we cannot completely exclude alternative explanations, these and the subsequent data strongly favour senescence-associated stemness as the most compelling and consistent interpretation of the observations presented.

Enrichment assays between matched pairs of never senescent versus previously senescent lymphomas confirmed the higher growth competitiveness of previously senescent lymphomas both *in vitro* and *in vivo* (Extended Data Fig. 2h). Importantly, *in vivo* tumour initiation experiments found previously senescent lymphomas produced malignancies at much lower transplanted cell numbers in immune-competent recipient mice when compared to never senescent lymphomas (Fig. 2c). Taken together, the SAS program exerts its detrimental effect on tumour initiation upon release from TIS, thereby unmasking an unexpected tumour-promoting capability of the senescence program.

To test which key stemness pathways drive SAS, we used GSEA in ADR-exposed control;*Bcl2* versus *Suv39h1*$^{-}$;*Bcl2* lymphomas for numerous gene sets related to Notch, Hedgehog, and canonical and non-canonical Wnt signalling. Canonical Wnt and, to some extent, Notch signalling, appeared to be significantly enriched in TIS (Extended Data Fig. 5a, b). Because Wnt signalling plays a central role in stem-cell renewal in many tissues including the haematopoietic compartment, induces Notch signalling, and is required for cancer stem cell development in haematological malignancies[18,19], we considered activation of the Wnt cascade as the putative driver behind the newly acquired stemness features in TIS lymphomas. Indeed, we detected enhanced, predominantly nuclear expression and transcriptional activation of β-catenin in control;*Bcl2* but not in *Suv39h1*$^{-}$;*Bcl2* lymphomas, as well as in TIS-capable human cancer cell lines after ADR treatment (Fig. 3a, Extended Data Fig. 2b and Extended Data Fig. 5c, d). Independent of Wnt ligand–receptor stimulation, we identified inhibition of the β-catenin degradation-promoting glycogen synthase kinase 3β (GSK3β) via activated MEK–MAPK and PI3K–Akt signalling—which is