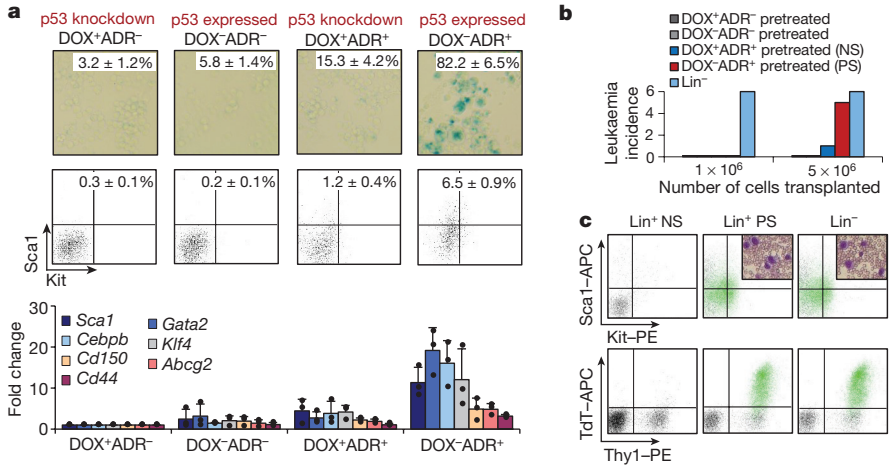

Figure 4 | Cellular senescence catalyses *de novo* reprogramming of non-stem bulk leukaemia cells into leukaemia-initiating cells. a, Stemness-related features in conditionally senescent mouse *Kras*^{G12D};DOX-sh*p53*–GFP;*Bcl2* bulk leukaemia cells (Lin$^-$Kit$^+$Sca1$^+$-depleted) treated for five days with ADR ± doxycycline (DOX)). Senescence induction is demonstrated by SA-β-gal staining (top), expression of stem-cell markers Kit and Sca1 analysed by flow cytometry (middle), and relative expression of the indicated transcripts by qPCR (bottom). Numbers reflect mean percentages of positive cells (top, middle) or average fold induction (bottom) ± s.d. ($n = 3$ biologically independent samples). **b**, Tumour initiation capacity of bulk leukaemia cells pretreated *in vitro* as in **a**, cultivated in ADR-free/DOX-supplemented medium for an additional two passages and transplanted at indicated cell numbers. Lin$^-$ cells were propagated without ADR. Numbers indicate leukaemia-bearing mice out of six animals per group transplanted, within an observation period of up to 100 days ($n = 6$ mice per treatment group). **c**, Flow cytometry plots showing peripheral blood phenotyping of mice transplanted as in **b**. The GFP$^+$ leukaemia cells are depicted in green. The insets show photomicrographs of peripheral blood smears stained with haematoxylin and eosin, showing leukaemic blasts (typically not detectable in never senescent recipients). One representative out of three independent experiments shown.

typically upregulated in senescence[20]—as the cell-autonomous driver of the Wnt program (Extended Data Fig. 6). The implementation of the Wnt program was further promoted by epigenetically permissive remodelling at promoters of stem-cell- and Wnt signalling-related genes in previously senescent as compared to never senescent cells (Fig. 3b). Accordingly, we found that the increased colony-forming potential of previously senescent lymphoma or colon cancer cells was dependent on Wnt signalling, as genetic or pharmacological disruption of the Wnt–β-catenin cascade—without preventing TIS or profoundly affecting cell viability—neutralized the higher clonogenicity of previously senescent cells (Fig. 3c and Extended Data Fig. 7a–d). In contrast to the never senescent cell population, a rarely dividing and strongly β-catenin-expressing subpopulation was detectable in the previously senescent cells only, and maintained at a stable steady state, explaining the lastingly enhanced colony-forming potential of previously senescent compared to never senescent cells (Extended Data Fig. 8). Consistently, the biology of the previously senescent state translated into shortened survival when previously senescent and never senescent cells were propagated in mice, whereas exposure to Wnt inhibitors *in vivo* or stable lymphoma cell transduction with a construct expressing short hairpin RNA (shRNA) against *Ctnnb1* (which encodes β-catenin) improved the poor long-term outcome of mice harbouring previously senescent lymphomas (Fig. 3d and Extended Data Fig. 7b, e, f).

Importantly, cell cycle re-entry out of TIS—as a prerequisite to exert stem-cell potential—is not limited to conditional, switchable systems, but may, as a rare event, spontaneously occur in control;*Bcl2* lymphomas, as demonstrated by the emergence of EdU-co-positive cells out of a solely SA-β-gal-positive senescent cell population (Extended Data Fig. 9). Given their stem-cell potential, we postulated that β-catenin-positive previously senescent cells might be enriched in lymphomas that progressed after chemotherapy. Hence, when comparing primary control;*Bcl2* lymphomas before therapy with the same individual lymphomas that had relapsed after exposure to senescence-inducing cyclophosphamide chemotherapy *in vivo*[8], we found a much higher fraction of cells positive for nuclear β-catenin in relapse lymphomas that also presented with higher expression levels of Wnt target genes (Fig. 3e, f, left). Moreover, longitudinally matched biopsy pairs from the same individual patients diagnosed with diffuse large B-cell lymphoma (DLBCL) before chemotherapy and at disease recurrence revealed significantly more nuclear β-catenin-positive tumour cells in the previously chemotherapy-exposed, re-emerging samples (Fig. 3f, right), further supporting a link between activated Wnt signalling in relapsed tumours and senescence-related tumour cell reprogramming. Taken together, TIS-associated stemness reflects a Wnt-governed capability that is stably maintained in a reprogrammed, hierarchically organized subpopulation of post-senescent tumour cells and critically associated with tumour progression and treatment failure.

As presumably applying to various human tumours including aggressive lymphomas, Eμ-*Myc* transgenic mouse lymphomas do not originate from a distinct fraction of cancer stem cells, because almost all lymphoma cells possess tumour-initiating potential in this model[21]. Consequently, next we asked whether cellular senescence might account for the reprogramming of non-stem tumour cells into cancer stem cells[22], in tumour types in which the tumour-initiating capacity is confined to a rare subpopulation. We isolated a non-self-renewing population of leukaemia cells from a mouse model of T-cell acute lymphoblastic leukaemia (T-ALL) driven by oncogenic *Kras*^{G12D} and conditional inactivation of *p53* via a doxycycline-controlled shRNA (shp53)[23] (Extended Data Fig. 10a). ADR exposure induced senescence in the majority of non-stem leukaemia cells only if p53 expression was not cancelled (Fig. 4a, top). This group exhibited a significant conversion to Kit$^+$Sca1$^+$ cells, indicative of putative leukaemia stem cells ($P = 0.02$, compared to ADR-exposed but p53-deficient cells; Fig. 4a, middle), and higher expression of stem-cell-related transcripts (Fig. 4a, bottom). Upon release from TIS by knockdown of *p53*, these leukaemia cells resumed proliferation (thereby becoming previously senescent cells), and formed significantly more colonies as compared to their equally ADR-treated never senescent leukaemia counterparts that remained *p53*-inactive throughout the experiment (Extended Data Fig. 10b). As reported for TIS lymphomas, cells with nuclear β-catenin expression were almost exclusively detectable in the senescent leukaemia cell population, and Wnt inhibitors completely neutralized the increased colony formation potential of their previously senescent progeny (Extended Data Fig. 10c, d). Most importantly, almost all samples of previously senescent cells—and nearly none of the