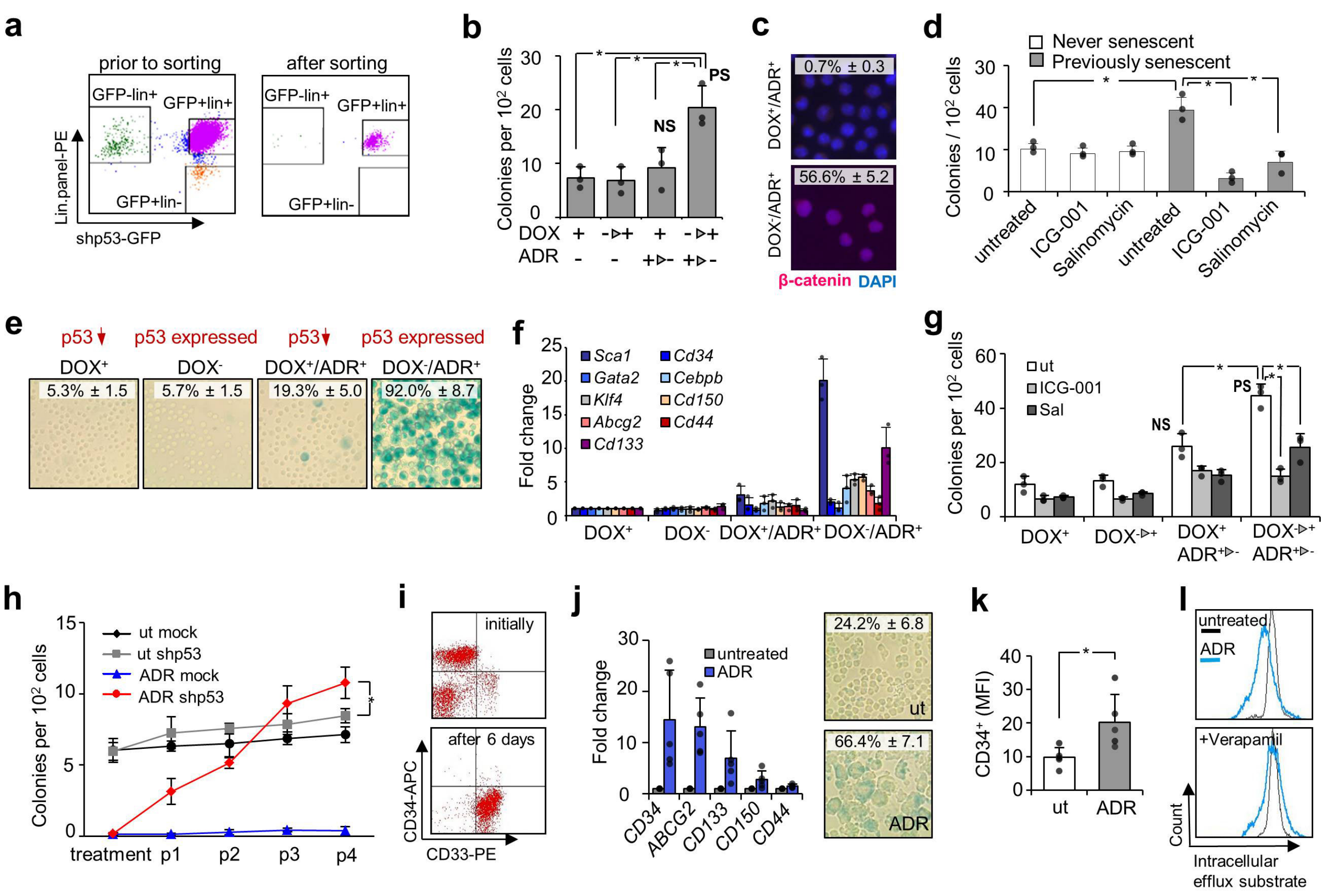

Extended Data Figure 10 | Senescence-associated *de novo* generation of leukaemia stem cells upon depletion of the stem-cell-containing fraction in mouse and human leukaemia samples. **a**, Flow cytometry plots of mouse $Kras^{G12D}$;DOX-on-shp53–GFP-induced T-cell acute lymphoblastic leukaemias (total splenocytes after short-term culture and retroviral *Bcl2* infection), stained with a panel of mouse lineage antibodies before and after flow-based sorting of the $Lin^{+}GFP^{+}$ population. The $Lin^{-}GFP^{+}$ population (including $Kit^{+}Sca1^{+}$ leukaemia stem cells) was used as a positive control. Shown are representative plots ($n = 3$). **b**, Colony formation of mouse $Lin^{+}GFP^{+}$ leukaemia cells as in **a**, pretreated with ADR ± doxycycline (DOX) for five days and subsequently seeded in ADR-free/DOX-supplemented medium, thus producing never senescent and previously senescent cells, respectively. Results represent mean colony counts at passage 2 (each passage reflecting 10 days in culture) ± s.d. ($n = 3$ biologically independent samples). Two-tailed, unpaired *t*-test with Welch's correction. $*P < 0.05$. **c**, Nuclear β-catenin expression by immunofluorescence (in red) in equally five-day-ADR-exposed senescent versus non-senescent settings (that is, DOX^{-} versus DOX^{+}). DAPI was used as a nuclear counterstain (in blue). Numbers represent mean percentages of β-catenin-positive cells ± s.d. ($n = 3$ biologically independent samples). **d**, Colony formation of never senescent and previously senescent leukaemia cells pretreated as in **b** (passage 3) with the addition of the indicated pharmacological Wnt inhibitors (mean colony numbers ± s.d., $n = 3$ biologically independent samples per group). $*P < 0.05$, two-tailed, unpaired *t*-test with Welch's correction. **e**, Senescence induction by SA-β-gal staining in mouse $Nras^{G12D}$;MLL-AF9;DOX-on-shp53;*Bcl2* bulk AML cells ($Lin^{-}Kit^{+}Sca1^{+}$-depleted) after five days of the ADR ± DOX treatment. Numbers reflect mean percentages of SA-β-gal-positive cells ± s.d (experiment performed in triplicate). Notably, viability determined as the percentage of annexin V/PI double-negative cells was typically greater than 80% and comparable between treatment groups. **f**, Stemness-related transcripts by qPCR in conditionally senescent mouse AML cells as in **e**. Graphs represent mean fold induction ± s.d. ($n = 3$ independent experiments). **g**, Colony formation of mouse bulk leukaemia cells pretreated as in **e**, further propagated in ADR-free DOX-containing medium for 14 days, and plated in methylcellulose medium supplemented with the Wnt inhibitors ICG-001 or salinomycin. Colonies were counted after seven days. Previously senescent AML cells, emerging via DOX-mediated p53 knockdown, presented with the highest, Wnt-dependent clonogenicity, which could be attenuated by pharmacological Wnt inhibition. Results represent mean colonies ± s.d. ($n = 3$ independent experiments). Two-tailed, unpaired *t*-test with Welch's correction. $*P < 0.05$. **h**, Colony formation of the $CD34^{+}$ cell-depleted human AML cell line Molm13 (with constitutive retroviral *Bcl2*-expression) exposed to senescence-inducing ADR treatment for five days ('treatment') and subsequently transduced with the lentiviral shp53 or mock construct (p53-knockdown enabling outgrowth from fully established senescence). Results reflect mean colony numbers ± s.d. ($n = 3$ independent experiments). Two-tailed, unpaired *t*-test with Welch's correction. $*P < 0.05$. **i**, Flow cytometric detection of the CD33 myeloid differentiation marker and CD34 stem-cell marker surface expression in samples from patients with AML obtained at diagnosis, before any cell cultivation and after six days of cultivation *in vitro*. Representative plots are shown ($n = 5$ individual patient samples). **j**, Expression of stemness-related transcripts in five-day-ADR-senescent versus untreated, *ex vivo* $CD34^{+}$-depleted primary human AML cells as in **i** (qPCR; average fold induction ± s.d., $n = 5$ individual patient samples, left). Photomicrographs (right) confirm ADR-inducible senescence by SA-β-gal staining (mean percentages of SA-β-gal positive cells ± s.d., representative photomicrographs from five independent samples). **k**, Regained CD34 surface expression upon ADR-induced senescence in $CD34^{+}$-depleted primary human AML cells as presented in **j**. Numbers reflect mean fluorescence intensity detected by flow cytometry ± s.d. ($n = 5$ individual patient samples). Two-tailed, paired *t*-test, $*P < 0.05$. **l**, ABC transporter activity in ADR-senescent versus untreated cells as in **k**. Representative plots are shown ($n = 5$ individual samples).