both *Aldh2*$^{-/-}$ (2.9-fold) and *Fancd2*$^{-/-}$ (1.9-fold) mice compared to wild-type controls, but the increase is much larger in *Aldh2*$^{-/-}$ *Fancd2*$^{-/-}$ mice (9.5-fold, Fig. 2b). These micronuclei could represent genomic instability during blood production. We therefore examined cells in metaphase obtained directly from the bone marrow of these mice with multiplex fluorescence *in situ* hybridization (M-FISH). More than 10% of *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ bone-marrow cells carried chromosomal aberrations, encompassing all classes of cytogenetic change (Fig. 2c–e). These aberrations are not clonal events, because each karyotype was unique (Extended Data Fig. 1).

Next, we investigated whether this chromosome damage was exacerbated by exposure to ethanol. As a control, we exposed wild-type or *Fancd2*$^{-/-}$ mice to mitomycin C (Extended Data Fig. 1d). The experimental scheme (outlined in Fig. 2f) shows how we determined the prevalence of micronuclei in reticulocytes and aberrant metaphases following exposure to ethanol. A single dose of ethanol caused a marked increase in the proportion of reticulocytes containing micronuclei in *Aldh2*$^{-/-}$ mice. Notably, this induction was comparable to that observed in wild-type mice following exposure to agents known to induce genome instability, such as ionizing irradiation or vincristine (Extended Data Fig. 1e). However, there was a stronger induction of micronucleus formation in *Aldh2*$^{-/-}$*Fancd2*$^{-/-}$ mice than in controls (Fig. 2g), which was accompanied by a striking increase in the number of abnormal metaphases, with almost 60% of metaphases having damaged chromosomes following ethanol exposure (Fig. 2h, Extended Data Fig. 1g–i). These mice rapidly lost the ability to produce blood and died from bone-marrow failure (Extended Data Fig. 2). These results show that, despite activation of homologous recombination, the Fanconi anaemia crosslink-repair pathway is essential for preventing chromosome breakage and loss of blood homeostasis in response to aldehydes.

Ku70 contributes to repair of aldehyde-induced DSBs

The presence of chromosome breaks and translocations suggests that aldehydes cause double-stranded breaks (DSBs), which could be processed by non-homologous end-joining (NHEJ) repair[17]. Previous studies in cell lines and nematodes have indicated that, in the absence of the Fanconi anaemia pathway, engagement of DSBs by NHEJ leads to further genomic instability[18,19]. Therefore, we investigated whether NHEJ and Fanconi anaemia repair are redundant in resolving endogenous DNA damage in HSCs, and whether there is a role for NHEJ in maintaining resistance to acetaldehyde.

To do this, we crossed mice deficient in the known Fanconi anaemia repair gene *Fanca* with mice lacking the key NHEJ factor Ku70 (encoded by *Xrcc6*, also known as *Ku70*). We failed to obtain *Fanca*$^{-/-}$*Ku70*$^{-/-}$ mice, indicating that there was a synthetic lethal interaction between Ku70-dependent NHEJ and Fanconi anaemia-crosslink repair (Supplementary Information Table 1). To bypass embryonic lethality, we generated blood-specific *Fanca* knockout mice (Extended Data Fig. 3) and crossed them with *Ku70*$^{+/-}$ mice to produce mice that had the double mutation in HSCs and the blood compartment (*Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre). These mice were viable, indicating that the embryonic lethality of *Fanca*$^{-/-}$*Ku70*$^{-/-}$ is not due to failed blood production (Supplementary Information Table 1). However, blood counts show that *Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre mice are anaemic (Fig. 3a) and have fewer HSCs compared to congenic controls (Fig. 3b–e). *Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre mice also display genomic instability, with increased frequency of micronuclei-containing NCEs (Fig. 3f). Finally, we tested whether Ku70 was required to maintain resistance of short-term (ST)-HSCs to aldehydes by exposing bone marrow cells to acetaldehyde *in vitro* before injecting them into lethally irradiated recipients. *Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre ST-HSCs were much more sensitive to acetaldehyde than either of the single mutant ST-HSCs (Fig. 3g). These results indicate that in the mouse haematopoietic system, in the absence of Fanconi anaemia repair, NHEJ is required to provide resistance to endogenous and acetaldehyde-induced DNA damage. This result contrasts with the reported negative impact of active NHEJ on the viability of Fanconi anaemia-deficient chicken DT40 cells and worms[18,19].

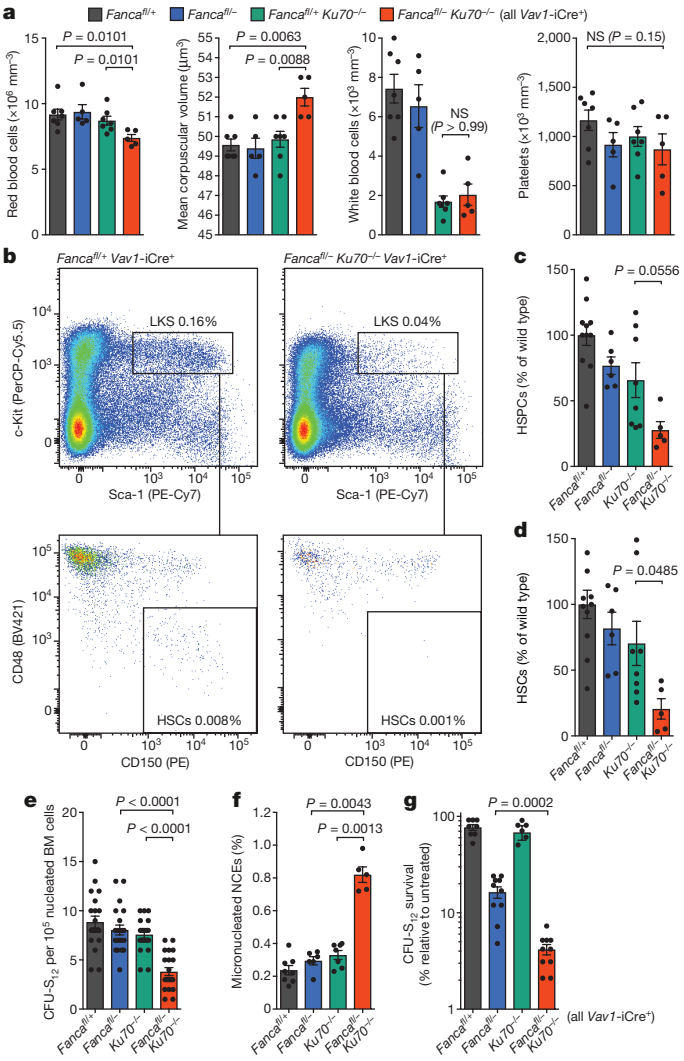

Figure 3 | NHEJ cooperates with the Fanconi anaemia pathway to maintain HSC integrity, genomic stability and cellular resistance to aldehydes. a, Blood parameters of 8- to 12-week old mice (*P* calculated by two-sided Mann–Whitney test; data shown as mean and s.e.m.; $n = 8$, 6, 7 and 5 mice, left to right). **b**, Representative flow cytometry plot of haematopoietic stem and progenitor cells (HSPCs) of *Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre mice and control. LKS, Lin$^-$Kit$^+$Sca-1$^+$. **c**, **d**, Quantification of HSPCs (Lin$^-$Kit$^+$Sca-1$^+$) and HSCs (Lin$^-$Kit$^+$Sca-1$^+$CD48$^-$CD150$^+$) by flow cytometry (*P* calculated by two-tailed Student's *t*-test; data shown as mean and s.e.m.; *n* as in **a**). **e**, Counts of colony-forming unit-spleen (CFU-S) colonies from the bone marrow of *Fanca*$^{fl/-}$*Ku70*$^{-/-}$ *Vav1*-iCre and control mice. Each point represents the number of CFU-S_{12} in a single recipient (*P* calculated by two-sided Mann–Whitney test; data shown as mean and s.e.m.; $n = 20$ mice). **f**, Frequency of Mn-NCE (*P* calculated by two-sided Mann–Whitney test; data shown as mean and s.e.m.; *n* as in **a**). **g**, Survival of CFU-S_{12} after treatment with 4 mM acetaldehyde for 4 h, relative to untreated samples (*P* calculated by two-sided Mann–Whitney test; data shown as mean and s.e.m.; $n = 10$ mice).

Aldehyde-damaged HSCs are functionally compromised

Our results so far indicate that endogenous aldehydes give rise to DSBs in the absence of Fanconi anaemia repair, which are engaged by homologous recombination and NHEJ, but ultimately rearrange chromosomes in bone marrow cells. A key question is whether endogenous DNA damage and subsequent mutations accumulate in the HSC