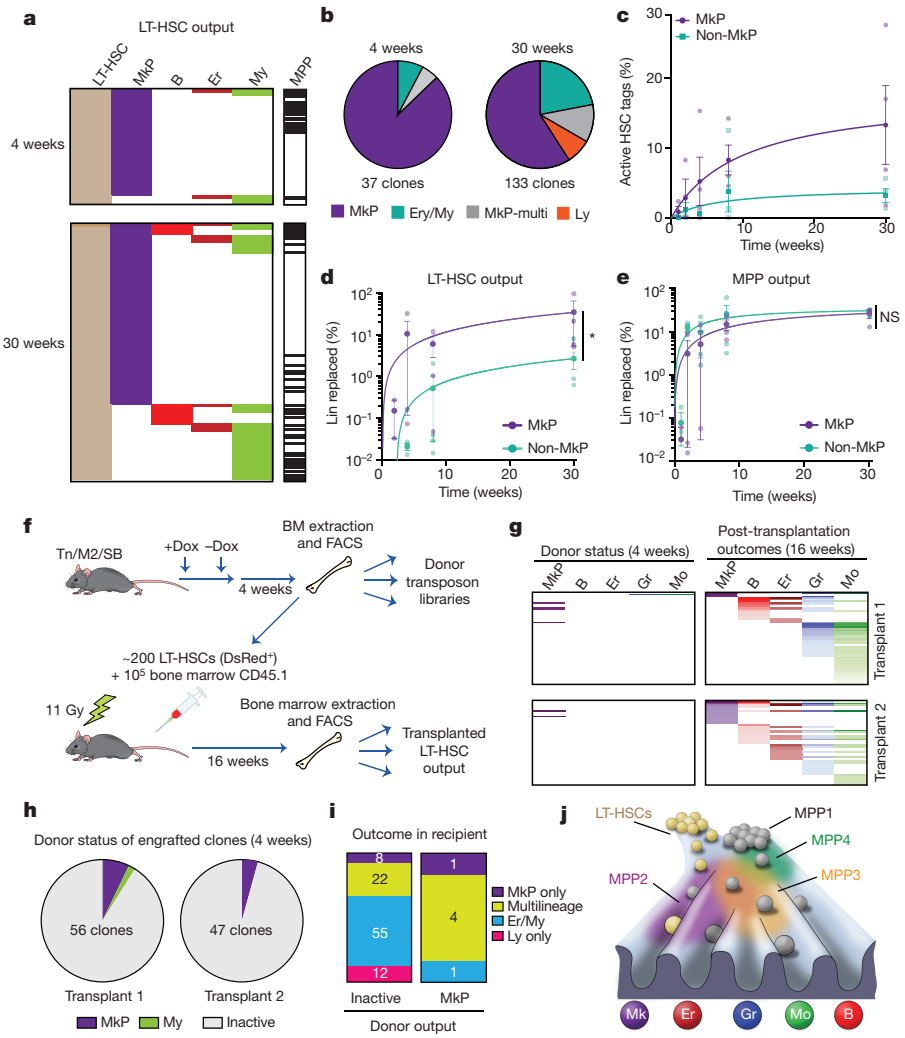

Figure 4 | Steady-state MkP output from bona fide LT-HSCs. a, LT-HSCs, MPPs, and Lin$^+$ cells were purified from bone marrow at 4 and 30 weeks and their transposon tag content was analysed. Only the LT-HSC tags overlapping with detectable Lin$^+$ progeny are shown. Abbreviations as Fig. 1. **b**, Distribution of types of progeny detected from LT-HSCs at 4 weeks and 30 weeks after labelling. Data are pooled from four independent M2/HSB/Tn mice per time point. Ly, lymphocyte. **c**, Percentage of labelled LT-HSC clones producing progeny at 1–8 weeks. Values are mean ± s.e.m. of three or four independent mice. **d**, Dynamics of megakaryocyte versus non-megakaryocyte lineage replacement by LT-HSCs (measured as the percentage of overlapping/total Lin$^+$ reads). Values are mean ± s.e.m. of three or four independent mice. Ratio paired *t*-test, $P = 0.014$. **e**, Dynamics of megakaryocyte versus erythrocyte/granulocyte/monocyte lineage replacement by MPPs (measured as the percentage of overlapping/total Lin$^+$ reads). Values are mean ± s.e.m. of three or four independent mice. Ratio paired *t*-test, $P = 0.599$. **f**, Experimental design for parallel analysis of native versus transplant output of LT-HSC clones. **g**, Alignment of all post-transplantation LT-HSC-derived lineages with unperturbed donor lineage tags. **h**, Pie-chart distribution of successfully engrafted LT-HSC clones by donor behaviour. Only megakaryocyte-restricted and granulocyte/monocyte-restricted output was observed. Inactive means non-detectable output in the donor. **i**, Post-transplantation outcomes comparing donor-inactive versus MkP-producing LT-HSC clones. **j**, Lineage fate landscape of unperturbed haematopoiesis. Self-renewing LT-HSCs preferentially replace the megakaryocyte lineage under steady state, and principally contribute to other blood lineages during transplantation or after injury. By contrast, MPPs take care of most steady-state lymphocyte, erythrocyte, and granulocyte/monocyte blood production. Different MPP sorting gates enrich for heterogeneous collections of lineage-primed and unprimed cell states within a continuum of lineage commitment pathways.

Our single-cell RNA sequencing data also revealed that a subset of marker-defined LT-HSCs exhibited megakaryocyte-lineage priming (Fig. 3c, d and Extended Data Fig. 9). This is in line with previous reports of multipotent, yet platelet-biased, subsets of LT-HSCs in the context of transplantation[10,18–23]. However, the physiological relevance of this observation in native haematopoiesis is unknown. With these precedents, we analysed the Lin$^+$ transposon tag overlap of sorted LT-HSCs. Although only a very small number of LT-HSC clones was active four weeks after labelling (5.5 ± 2.3%), remarkably a large majority of these clones were found exclusively in the MkP population (Fig. 4a, b and Extended Data Fig. 10a). This megakaryocyte-restricted output of LT-HSCs was more pronounced after 30 weeks post-labelling (MkP: 13.3 ± 5.6%; lymphoid/erythroid/myeloid: 3.2 ± 1.0%) (Fig. 4c). Quantitatively, LT-HSCs accounted for replacing at least 31% of the total MkP pool, compared with just 3.8% of granulocyte/monocyte and erythroblast reads (Fig. 4d). Among all MkP that had a detectable tag in primitive populations, approximately half demonstrated overlap with LT-HSCs and the other half with MPPs (where no LT-HSC tag was detected) (Extended Data Fig. 10b). MPP-overlapping clones contributed to the megakaryocyte lineage to a similar extent as LT-HSCs, markedly differing from lympho-erythromyeloid output, which is predominantly MPP-driven (Fig. 4e and Extended Data Fig. 10c). Our analyses also revealed that many LT-HSCs contribute to MkP in the absence of any intermediates in the MPP compartment (Fig. 4a), suggesting that at least a subset of LT-HSCs generates megakaryocyte lineage cells through a 'direct' pathway.

Previous studies have shown that the commonly used LT-HSC gate contains unilineage CD41$^+$ megakaryocyte-restricted progenitors as