a Normal CB CD34+

b anti-FOLR1 / Isotype control

c % FOLR1+

d % Specific Lysis

e Normal CB CD34+
Donor 1 / Donor 2 / Donor 3
No. of colonies (per 6,000 CD34+ input cells)
GEMM / E / GM / M / G
Untreated / Unmodified T / CAR T

f C/G-CB
No. of colonies (per 30,000 CD34+ input cells)
Untreated / Unmodified T / CAR T

Extended Data Fig. 9

Extended Data Figure 9. FORL1-directed CAR T effectively eliminate C/G-CB cells without impacting viability of HSPCs. a. Gating strategy used to identify HPSC subsets from a representative CD34-enriched marrow sample from a healthy donor. Shown is representative of 3 donors. Immunophenotype of the HSPCs is as follows: CD34+CD38-CD90+CD45RA- (hematopoietic stem cell, HSC); CD34+CD38−CD90−CD45RA− (multipotent progenitors, MPP); CD34+CD38−CD90−CD45RA+ multi-lymphoid progenitors, MLP); CD34+CD38+CD10+ (Common lymphoid progenitor, CLP); CD34+CD38+CD10−CD123−CD45RA− (megakaryocyte-erythroid progenitor, MEP); CD34+CD38+CD10−CD123+CD45RA− (common myeloid progenitor, CMP); CD34+CD38+CD10−CD123+CD45RA+ (granulocyte monocyte progenitor, GMP). **b**. Histogram of FOLR1 expression in normal HSPC subsets. **c.** Quantification of percent FOLR1+ in C/G-CB cells (>12 weeks of EC co-culture) and HSPC subsets from three CD34-enriched samples from healthy donors. **d.** Percent specific lysis in C/G-CB cells and the HSPC subsets shown in c following 4-hour incubation with unmodified or FOLR1 CAR T cells at 2:1 E:T ratio. Note that data points for C/G-CB cells are from 2 technical replicates. Only two out of three normal CD34+ samples were used in this experiment. **e, f.** After 4 hours, co-cultures of healthy donor CD34+ or C/G-CB cells with either unmodified or MSLN CAR T cells at 2:1 E:T ratio were transferred to methylcellulose with cytokines for colony-forming cell (CFC) assay. Colonies derived from erythroid (E), granulocyte-macrophage (G, M, and GM) and multipotential granulocyte, erythroid, macrophage, megakaryocyte (GEMM) progenitors were scored and enumerated after 7-10 days (e). Total colonies from C/G-CB cells are tabulated (f). Data are presented as mean +/- SD from 3 technical replicates for each donor. No significant difference in the total number of colonies was detected between cocultures with unmodified T cells versus FOLR1 CAR T cells for normal HSPCs as determined by unpaired Student's t test, assuming unequal variances. Statistical significance was determined by unpaired Student's t test, assuming unequal variances. $p<0.05$ (*), $p<0.005$ (**), $p<0.0005$ (***).