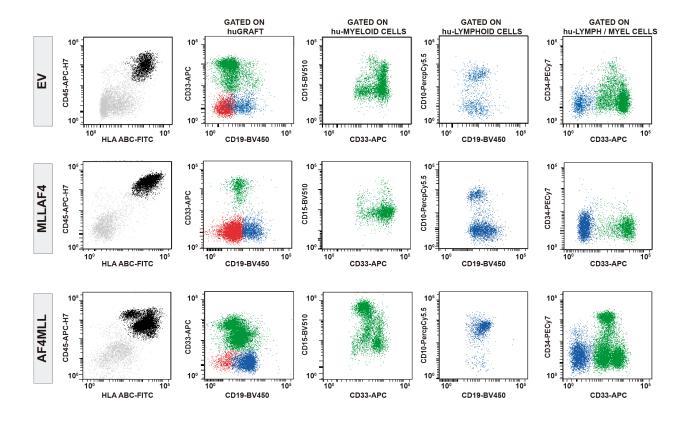
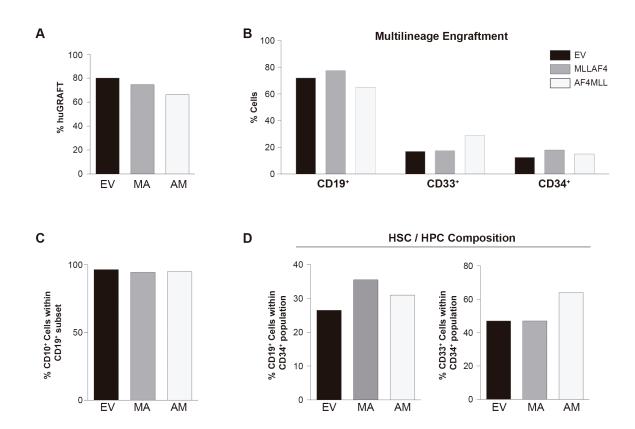
The AF4-MLL fusion transiently augments multilineage hematopoietic engraftment but is not sufficient to initiate leukemia in cord blood CD34+ cells

SUPPLEMENTARY MATERIALS



Supplementary Figure 1: Representative flow cytometry analysis of mice transplanted with EV-, MLL-AF4- or AF4-MLL-expressing CD34⁺ HSPCs. Human engraftment (black) is identified as HLA.ABC⁺ CD45⁺ and includes lymphoid CD19⁺ cells (blue), comprising pre-B (CD10⁺) and pro-B (CD10⁻) fractions, myeloid CD33⁺/CD15⁻ and CD33⁺/CD15⁺ cells (green) and a few CD34⁺ immature cells (either lymphoid- (CD19⁺) or myeloid-committed (CD33⁺)).



Supplementary Figure 2: Twelve-week engraftment composition of mice transplanted with EV-, MLL-AF4- or AF4-MLL-expressing CD34⁺ HSPCs. (A) Total engraftment identified as HLA.ABC⁺ CD45⁺. (B) Multilineage engraftment including CD19⁺ B-lymphoid, CD33⁺ myeloid and CD34⁺+ immature engraftment. (C) Proportion of CD10⁺ pre-B cells within the CD19⁺ B-lymphoid engraftment. (D) Proportion of CD19⁺ (Band CD33⁺ cells within the CD34⁺ immature engraftment).

AF4-MLL (8739 bp)

Red: AF4 exon 1+AF4 exon 2

Blue: MLL exon 10 to 36

ATG:Start Codon

TAA:Stop Codon

ATGGCAGCCCAGTCAAGTTTGTACAATGACGACAGAAACCTGCTTCGAATTAG AGAGAAGGAAGACGCAACCAGGAAGCCCACCAAGAGAAAGAGGCATTTCCT GAAAAGATTCCCCTTTTTGGAGAGCCCTACAAGACAGCAAAAGGTGATGAGCT GTCTAGTCGAATACAGAACATGTTGGGAAACTACGAAGAAGTGAAGGAGTTC CTTAGTACTAAGTCTCACACTCATCGCCTGGATGCTTCTGAAAATAGGTTGGG CCTTCCACACTAGTGTCCACCACCAGTCCATTCACACTCCTGCGTCTGGACCA CTTTCTGTTGGCAACATTAGCCACAATCCAAAGATGGCGCAGCCAAGAACTGA CTGACCCAGGATCGCCTTGGTCAGGAGGGGTTCGGCTCTAGTCATCACAAGA AAGGTGACCGAAGAGCTGACGGAGACCACTGTGCTTCGGTGACAGATTCGG CTCCAGAGAGGGAGCTTTCTCCCTTAATCTCTTTGCCTTCCCCAGTTCCCCCT TTGTCACCTATACATTCCAACCAGCAAACTCTTCCCCGGACGCAAGGAAGCAG CAAGGTTCATGGCAGCAGCAATAACAGTAAAGGCTATTGCCCAGCCAAATCTC CCAAGGACCTAGCAGTGAAAGTCCATGATAAAGAGACCCCTCAAGACAGTTT TCAAAAAGTGTTGCAATGCAGCAGAAGCCCACGGCTTATGTCCGGCCCATGG ATGGTCAAGATCAGGCCCCTAGTGAATCCCCTGAACTGAAACCACTGCCGGA GCCAAGCTCACCAAACTGAAGATGCCTTCTCAGTCAGTTGAGGAGGATTGTG AAGCAGAAAATGTGTGGGAGATGGGAGGCTTAGGAATCTTGACTTCTGTTCCT ATAACACCCAGGGTGGTTTGCTTTCTCTGTGCCAGTAGTGGGCATGTAGAGTT TGTGTATTGCCAAGTCTGTTGTGAGCCCTTCCACAAGTTTTGTTTAGAGGAGA ACGAGCGCCTCTGGAGGACCAGCTGGAAAATTGGTGTTGTCGTCGTTGCAA ATTCTGTCACGTTTGTGGAAGGCAACATCAGGCTACAAAGCAGCTGCTGGAG TGTAATAAGTGCCGAAACAGCTATCACCCTGAGTGCCTGGGACCAAACTACC CCACCAAACCCACAAGAAGAAGAAGTCTGGATCTGTACCAAGTGTGTTCG CTGTAAGAGCTGTGGATCCACAACTCCAGGCAAAGGGTGGGATGCACAGTGG TCTCATGATTTCTCACTGTGTCATGATTGCGCCAAGCTCTTTGCTAAAGGAAA CTTCTGCCCTCTGTGACAAATGTTATGATGATGATGACTATGAGAGTAAGA TGATGCAATGTGGAAAGTGTGATCGCTGGGTCCATTCCAAATGTGAGAATCTT TCAGGTACAGAAGATGAGATGTATGAGATTCTATCTAATCTGCCAGAAAGTGT GGCCTACACTTGTGTGAACTGTACTGAGCGGCACCCTGCAGAGTGGCGACTG GCCCTTGAAAAAGAGCTGCAGATTTCTCTGAAGCAAGTTCTGACAGCTTTGTT GAATTCTCGGACTACCAGCCATTTGCTACGCTACCGGCAGGCTGCCAAGCCT

CCAGACTTAAATCCCGAGACAGAGGAGAGTATACCTTCCCGCAGCTCCCCCG AAGGACCTGATCCACCAGTTCTTACTGAGGTCAGCAAACAGGATGATCAGCA GCCTTTAGATCTAGAAGGAGTCAAGAGGAAGATGGACCAAGGGAATTACACA TCTGTGTTGGAGTTCAGTGATGATATTGTGAAGATCATTCAAGCAGCCATTAAT TCAGATGGAGGACAGCCAGAAATTAAAAAAAGCCAACAGCATGGTCAAGTCCTT CTTCATTCGGCAAATGGAACGTGTTTTTCCATGGTTCAGTGTCAAAAAGTCCA GGTTTTGGGAGCCAAATAAAGTATCAAGCAACAGTGGGATGTTACCAAACGCA GTGCTTCCACCTTCACTTGACCATAATTATGCTCAGTGGCAGGAGCGAGAGG AAAACAGCCACACTGAGCAGCCTCCTTTAATGAAGAAAATCATTCCAGCTCCC AAACCCAAAGGTCCTGGAGAACCAGACTCACCAACTCCTCTGCATCCTAC ACCACCAATTTTGAGTACTGATAGGAGTCGAGAAGACAGTCCAGAGCTGAAC CCACCCCAGGCATAGAAGACAATAGACAGTGTGCGTTATGTTTGACTTATGG TGATGACAGTGCTAATGATGCTGGTCGTTTACTATATATTGGCCAAAATGAGT GGACACATGTAAATTGTGCTTTGTGGTCAGCGGAAGTGTTTGAAGATGATGAC GGATCACTAAAGAATGTGCATATGGCTGTGATCAGGGGCAAGCAGCTGAGAT GTGAATTCTGCCAAAAGCCAGGAGCCACCGTGGGTTGCTGTCTCACATCCTG CACCAGCAACTATCACTTCATGTGTTCCCGAGCCAAGAACTGTGTCTTTCTGG ATGATAAAAAAGTATATTGCCAACGACATCGGGATTTGATCAAAGGCGAAGTG GTTCCTGAGAATGGATTTGAAGTTTTCAGAAGAGTGTTTGTGGACTTTGAAGG AATCAGCTTGAGAAGGAAGTTTCTCAATGGCTTGGAACCAGAAAATATCCACA TGATGATTGGGTCTATGACAATCGACTGCTTAGGAATTCTAAATGATCTCTCC GACTGTGAAGATAAGCTCTTTCCTATTGGATATCAGTGTTCCAGGGTATACTG GAGCACCACAGATGCTCGCAAGCGCTGTGTATATACATGCAAGATAGTGGAG TGCCGTCCTCCAGTCGTAGAGCCGGATATCAACAGCACTGTTGAACATGATG AAAACAGGACCATTGCCCATAGTCCAACATCTTTTACAGAAAGTTCATCAAAA TCATTCACAAACCTCTGGCTCCTGTTATTATCATGTCATCTCAAAGGTCCCCAG GATTCGAACACCCAGTTATTCTCCAACACAGAGATCCCCTGGCTGTCGACCGT TGCCTTCTGCAGGAAGTCCTACCCCAACCACTCATGAAATAGTCACAGTAGGT GATCCTTTACTCTCTGGACTTCGAAGCATTGGCTCCAGGCGTCACAGTAC CTCTTCCTTATCACCCCAGCGGTCCAAACTCCGGATAATGTCTCCAATGAGAA CTGGGAATACTTACTCTAGGAATAATGTTTCCTCAGTCTCCACCACCGGGACC GCTACTGATCTTGAATCAAGTGCCAAAGTAGTTGATCATGTCTTAGGGCCACT GAATTCAAGTACTAGTTTAGGGCAAAACACTTCCACCTCTTCAAATTTGCAAAG CAGAAATGAAGCAGTCCAGTGCTTCAGACTTGGTGTCCAAGAGCTCCTCTTTA AAGGGAGAGACCAAAGTGCTGAGTTCCAAGAGCTCAGAGGGATCTGCAC ATAATGTGGCTTACCCTGGAATTCCTAAACTGGCCCCACAGGTTCATAACACA ACATCTAGAGAACTGAATGTTAGTAAAATCGGCTCCTTTGCTGAACCCTCTTC AGTGTCGTTTTCTTCTAAAGAGGCCCTCTCCTTCCCACACCTCCATTTGAGAG GGCAAAGGAATGATCGAGACCAACACACAGATTCTACCCAATCAGCAAACTC CTCTCCAGATGAAGATACTGAAGTCAAAACCTTGAAGCTATCTGGAATGAGCA ACAGATCATCCATTATCAACGAACATATGGGATCTAGTTCCAGAGATAGGAGA

CAGAAAGGGAAAAATCCTGTAAAGAAACTTTCAAAGAAAAGCATTCCAGTAA CAGAGTTTATGGATGAGGTTTTGACTCCTGAGTATATGGGCCAACGACCATGT AACAATGTTTCTTGATAAGATTGGTGATAAAGGCCTTTCTATGCCAGGAGT CCCCAAAGCTCCACCCATGCAAGTAGAAGGATCTGCCAAGGAATTACAGGCA CCACGGAAACGCACAGTCAAAGTGACACTGACACCTCTAAAAAATGGAAAATGA GAGTCAATCCAAAAATGCCCTGAAAGAAGTAGTCCTGCTTCCCCTTTGCAAA TAGAGTCAACATCTCCCACAGAACCAATTTCAGCCTCTGAAAATCCAGGAGAT GGTCCAGTGGCCCAACCAAGCCCCAATAATACCTCATGCCAGGATTCTCAAA GTAACAACTATCAGAATCTTCCAGTACAGGACAGAAACCTAATGCTTCCAGAT GGCCCCAAACCTCAGGAGGATGGCTCTTTTAAAAGGAGGTATCCCCGTCGCA GTGCCCGTGCACGTTCTAACATGTTTTTTGGGCTTACCCCACTCTATGGAGTA AGATCCTATGGTGAAGAAGACATTCCATTCTACAGCAGCTCAACTGGGAAGAA GCGAGGCAAGAGATCAGCTGAAGGACAGGTGGATGGGGCCGATGACTTAAG CACTTCAGATGAAGACGACTTATACTATTACAACTTCACTAGAACAGTGATTTC TTCAGGTGGAGGACGACTGGCATCCCATAATTTATTTCGGGAGGAGGAA CAGTGTGATCTTCCAAAAATCTCACAGTTGGATGGTGTTGATGATGGGACAGA GAGTGATACTAGTGTCACAGCCACAACAAGGAAAAGCAGCCAGATTCCAAAA AGAAATGGTAAAGAAAATGGAACAGAGAACTTAAAGATTGATAGACCTGAAGA TGCTGGGGAGAAGAACATGTCACTAAGAGTTCTGTTGGCCACAAAAATGAG CCAAAGATGGATAACTGCCATTCTGTAAGCAGAGTTAAAACACAGGGACAAGA TTCCTTGGAAGCTCAGCTCATTGGAGTCAAGCCGCAGAGTCCACACA AGTACCCCCTCCGACAAAAATTTACTGGACACCTATAATACTGAGCTCCTGAA ATCAGATTCAGACAATAACAACAGTGATGACTGTGGGAATATCCTGCCTTCAG ACATTATGGACTTTGTACTAAAGAATACTCCATCCATGCAGGCTTTGGGTGAG AGCCCAGAGTCATCTTCATCAGAACTCCTGAATCTTGGTGAAGGATTGGGTCT TGACAGTAATCGTGAAAAAGACATGGGTCTTTTTGAAGTATTTTCTCAGCAGCT GCCTACAACAGAACCTGTGGATAGTAGTGTCTCTTCCTCTATCTCAGCAGAGG AACAGTTTGAGTTGCCTCTAGAGCTACCATCTGATCTGTCTTGTCTTGACCACC CGGAGTCCCACTGTCCCCAGCCAGAATCCCAGTAGACTAGCTGTTATCTCAG ACTCAGGGGAGAAGAGTAACCATCACAGAAAAATCTGTAGCCTCCTCTGA AAGTGACCCAGCACTGCTGAGCCCAGGAGTAGATCCAACTCCTGAAGGCCAC ATGACTCCTGATCATTTTATCCAAGGACACATGGATGCAGACCACATCTCTAG CCCTCCTTGTGGTTCAGTAGAGCAAGGTCATGGCAACAATCAGGATTTAACTA GGAACAGTAGCACCCCTGGCCTTCAGGTACCTGTTTCCCCAACTGTTCCCAT CCAGAACCAGAAGTATGTGCCCAATTCTACTGATAGTCCTGGCCCGTCTCAGA GAAACTCATAGTTGTTAACCAGAACATGCAGCCACTTTATGTTCTCCAAACTCT TCCAAATGGAGTGACCCAAAAAATCCAATTGACCTCTTCTGTTAGTTCTACACC CAGTGTGATGGAGACAAATACTTCAGTATTGGGACCCATGGGAGGTGGTCTC ACCCTTACCACAGGACTAAATCCAAGCTTGCCAACTTCTCAATCTTTGTTCCCT TCTGCTAGCAAAGGATTGCTACCCATGTCTCATCACCAGCACTTACATTCCTT CCCTGCAGCTACTCAAAGTAGTTTCCCACCAAACATCAGCAATCCTCCTTCAG

GCCTGCTTATTGGGGTTCAGCCTCCTCCGGATCCCCAACTTTTGGTTTCAGAA TCCAGCCAGAGGACAGACCTCAGTACCACAGTAGCCACTCCATCCTCTGGAC TCAAGAAAAGACCCATATCTCGTCTACAGACCCGAAAGAATAAAAAACTTGCT CCCTCTAGTACCCCTTCAAACATTGCCCCTTCTGATGTGGTTTCTAATATGACA TTGATTAACTTCACACCCTCCCAGCTTCCTAATCATCCAAGTCTGTTAGATTTG GGGTCACTTAATACTTCATCTCACCGAACTGTCCCCAACATCATAAAAAGATCT AAATCTAGCATCATGTATTTTGAACCGGCACCCCTGTTACCACAGAGTGTGGG AGGAACTGCTGCCACAGCGCAGGCACATCAACAATAAGCCAGGATACTAGC CACCTCACATCAGGGTCTGTGTCTGGCTTGGCATCCAGTTCCTCTGTCTTGAA TGTTGTATCCATGCAAACTACCACAACCCCTACAAGTAGTGCGTCAGTTCCAG GACACGTCACCTTAACCAACCCAAGGTTGCTTGGTACCCCAGATATTGGCTCA ATAAGCAATCTTTTAATCAAAGCTAGCCAGCAGAGCCTGGGGATTCAGGACCA GCCTGTGGCTTTACCGCCAAGTTCAGGAATGTTTCCACAACTGGGGACATCA CAGACCCCTCTACTGCTGCAATAACAGCGGCATCTAGCATCTGTGTGCTCC CCTCCACTCAGACTACGGGCATAACAGCCGCTTCACCTTCTGGGGAAGCAGA CGAACACTATCAGCTTCAGCATGTGAACCAGCTCCTTGCCAGCAAAACTGGG ATTCATTCTTCCCAGCGTGATCTTGATTCTGCTTCAGGGCCCCAGGTATCCAA CTTTACCCAGACGCTAGACGCTCCTAATAGCATGGGACTGGAGCAGAACAAG GCTTTATCCTCAGCTGTGCAAGCCAGCCCCACCTCTCCTGGGGGTTCTCCAT CCTCTCCATCTTCTGGACAGCGGTCAGCAAGCCCTTCAGTGCCGGGTCCCAC TAAACCCAAACCAAAACCAAACGGTTTCAGCTGCCTCTAGACAAAGGGAATG GCAAGAAGCACAATGTTTCCCATTTGCGGACCAGTTCTTCTGAAGCACACATT CCAGACCAAGAAACGACATCCCTGACCTCAGGCACAGGGACTCCAGGAGCA GAGGCTGAGCAGCAGGATACAGCTAGCGTGGAGCAGTCCTCCCAGAAGGAG TGTGGGCAACCTGCAGGGCAAGTCGCTGTTCTTCCGGAAGTTCAGGTGACCC AAAATCCAGCAAATGAACAAGAAAGTGCAGAACCTAAAACAGTGGAAGAAGA GGAAAGTAATTTCAGCTCCCCACTGATGCTTTGGCTTCAGCAAGAACAAAAGC GGAAGGAAAGCATTACTGAGAAAAACCCAAGAAAGGACTTGTTTTTGAAATT TCCAGTGATGATGGCTTTCAGATCTGTGCAGAAAGTATTGAAGATGCCTGGAA GTCATTGACAGATAAAGTCCAGGAAGCTCGATCAAATGCCCGCCTAAAGCAG CTCTCATTTGCAGGTGTTAACGGTTTGAGGATGCTGGGGATTCTCCATGATGC AGTTGTGTTCCTCATTGAGCAGCTGTCTGGTGCCAAGCACTGTCGAAATTACA AATTCCGTTTCCACAAGCCAGAGGAGGCCAATGAACCCCCCTTGAACCCTCA CGGCTCAGCCAGGGCTGAAGTCCACCTCAGGAAGTCAGCATTTGACATGTTT AACTTCCTGGCTTCTAAACATCGTCAGCCTCCTGAATACAACCCCAATGATGA AGAAGAGGAGGAGGTACAGCTGAAGTCAGCTCGGAGGGCAACTAGCATGGA TCTGCCAATGCCCATGCGCTTCCGGCACTTAAAAAAGACTTCTAAGGAGGCA GTTGGTGTCTACAGGTCTCCCATCCATGGCCGGGGTCTTTTCTGTAAGAGAAA CATTGATGCAGGTGAGATGGTGATTGAGTATGCCGGCAACGTCATCCGCTCC ATCCAGACTGACAAGCGGGAAAAGTATTACGACAGCAAGGGCATTGGTTGCT GCTGCACGCTTCATCAATCACTCGTGTGAGCCTAACTGCTATTCTCGGGTCAT CAATATTGATGGGCAGAAGCACATTGTCATCTTTGCCATGCGTAAGATCTACC

GAGGAGGAACTCACTTACGACTATAAGTTCCCCATTGAGGATGCCAGCAACAGCTGCCCTGCAACTGTGGCGCTAAGAAATGCCGGAAGTTCCTAAAC<mark>TAA</mark>

Supplementary Figure 3: primers sequences.			