

Gene	Clone	Sequence
Scrambled	1	CCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG
Thbs2	1	GCCCTATTGATGGGTGCTTATCTCGAGATAAGCACCCATCAATAGGGC
	2	GCTGTAGGTTTTCGACGAGTTTTCTCGAGAACTCGTCGAAACCTACAGC
	3	CCATTCTATGAGCAGCTAGAACTCGAGTTCTAGCTGCTCATAGAATGG
	4	CCAAGACAACTGCCCATACATCTCGAGATGTATGGGCAGTTGTCTTGG
	5	CCACGTCAAGGACACTTCATTCTCGAGAATGAAGTGTCTTGACGTGG
	6	GATTCGTGCTGTTCTATAATGCTCGAGCATTATAGAACAGCACGAATC
Dlk1	1	CTCAGGCAACTTCTGTGAGATCTCGAGATCTCACAGAAGTTGCCTGAG
	2	GCTGGTGATGAGGAGATCTAACTCGAGTTAGATCTCCTCATCACCAGC
(clone used in functional assays)	3	GACGGGAAATTCTGCGAAATACTCGAGTATTTTCGAGAATTTCCCGTC
	4	CCATCGTCTTTCTCAACAAGTCTCGAGACTTGTGAGAAAGACGATGG
	5	CATCCTGAAGGTGTCCATGAACTCGAGTTCATGGACACCTTCAGGATG
	6	CCACATGCTTCGCAAGAAGAACTCGAGTTCTTCTTGCGAAGCATGTGG
Bgn	1	CCACTCAGAGACTCCCTATAACTCGAGTTATAGGGAGTCTCTGAGTGG
	2	GCCATCCAATTTGGAATTTATCTCGAGATAATTTCCAAATTGGATGGC
	3	GCCCTGGTCTTGGTAAACAATCTCGAGATTGTTTACCAAGACCAGGGC
	4	GCTATTGAGTTGGAGGACCTACTCGAGTAGGTCTCCAACCTCAATAGC
	5	CTCCCTGGTAGAACTACGAATCTCGAGATTCGTAGTTCTACCAGGGAG
Mmp9	1	CCCTCTGAATAAAGACGACATCTCGAGATGTGCTCTTTATTACAGAGGG
	2	CCAGGATAAACTGTATGGCTTCTCGAGAAGCCATACAGTTTATCCTGG
	3	GAGGCATACTTGTACCGCTATCTCGAGATAGCGGTACAAGTATGCCTC
	4	CCACTTACTATGGAACTCAACTCGAGTTGAGTTTCCATAGTAAGTGG
	5	CAGTACCAAGACAAAGCCTATCTCGAGATAGGCTTGTCTTGGTACTG
Vcam1	1	CCAGATCCTTAATACTGTTTACTCGAGTAAACAGTATTAAGGATCTGG
	2	CCCTTTGACCATCTGGAGATTCTCGAGAATCTCCAGATGGTCAAAGGG
	3	CCAAATTGATTTCTACACTCAACTCGAGTTGAGTGTAGAATCAATTTGG
	4	CGAGGCTGGAATTAGCAGAACTCGAGTTTCTGCTAATTCCAGCCTCG
	5	CCAGATAGACAGCCCACTAACTCGAGTTTAGTGGGCTGTCTATCTGG
Dpt	1	CGTCTCTATTCAAAGATATACTCGAGTATATCTTTGAATAGAGGACG
	2	GAGGAGCATCTTTAGCAAGAACTCGAGTTCTTGCTAAAGATGCTCCTC
(clone used in functional assays)	3	GCGAGGAGCAACAACCACCTTTCTCGAGAAAGTGTTGTTGCTCCTCGC
	4	CTATAGCAAGAGGTGTCCATACTCGAGTATGGACACCTCTTGCTATAG
	5	CTGGATCGTGAGTGGCAATTTCTCGAGAAATTGCCACTCACGATCCAG
Tgfb1	1	GCCTTGGCATGGTTCTGTAAACTCGAGTTTACAGAACCATGCCAAGGC
	2	CGAGTCTTTGTTTATCGAAATCTCGAGATTTGATAAACAAAGACTCG
	3	CCCAATGGGATTGTAACGTGTTCTCGAGAACAGTTACAATCCCATTGGG
	4	CCTCCAGAAGAACTGAACAACTCGAGTTTGTTTCTGTTCTTCTGGAGG
	5	GCCATTGACATCCTCAAACAACTCGAGTTGTTTGAGGATGTCAATGGC
Fap	1	GCCACTTCATATAGGACTTAACTCGAGTTAAGTCCTATATGAAGTGGC
(clone used in functional assays)	2	GCACATTATGAAGCAGAAATTCTCGAGAATTTCTGCTTCATAATGTGC
	3	GCCTATTCTTATTATGGTGATCTCGAGATCACCATAATAAGAATAGGC
	4	CCCTTTGCTAATTCAAGTGTACTCGAGTACACTTGAATTAGCAAAGGG
Col6a3	1	GCCATCAACAAAGTCGTCTATCTCGAGATAGACGACTTTGTTGATGGC
	2	CCAAGGAAGTTTCAATACGAACCTCGAGTTCGTATTGAACTTCCTTGG
	3	CCCTCAGGTATCATAGTATTCTCGAGAATACTATGATAACCTGAGGG
	4	CCTTCTCATATTGTGAACATCTCGAGATGTTTCACAATATGAGAAAGG
Dcn	1	CCTGTCTAAGAACCAACTAACTCGAGTTTAGTTGGTCTTAGACAGG
	2	CGACTTCAATGGACTGAACAACTCGAGTTGTTTCTGTTTCTTGAAGTCCG
	3	GCCTGAAAGGACTGATTAATTCTCGAGAATTAATCAGTCCCTTTCAGGC
	4	GTCCGGTATTGGGAAATCTTTCTCGAGAAAGATTTCCCAATACCGGAC
	5	CCTGAAGGACTTGCATACCTTCTCGAGAAGGTATGCAAGTCCCTTCAGG

Slc38a4	1	GACGCCATGAACAGCCAATTTCTCGAGAAATTGGCTGTTTCATGGCGTC
	2	CGTGCCTACCATCAAATACATCTCGAGATGTATTTGATGGTAGGCACG
	3	CGGAAATCTGACGTTCAACAACCTCGAGTTGTTGAACGTCAGATTTCCG
	4	GCCTGAAGTAATCAGAGCATTCTCGAGAATGCTCTGATTACTTCAGGC
Slco2a1	1	CCACCACTTTGGGAAGTATAACTCGAGTTATACTCCCAAAGTGGTGG
	2	GCCTATGCCAACTTACTCATTCTCGAGAATGAGTAAGTTGGCATAGGC
	3	GCTCGGTCTTCAACAACATTACTCGAGTAATGTTGTTGAAGACCGAG
	4	ACAGGTAATCTACAAGGTCTTCTCGAGAAGACCTTGTAGATTACCTG
Arhgdib	1	CACACATTTTCATCACCAATATCTCGAGATATTGGTGATGAAATGTGTG
	2	CGAGAGTCTAACCAAGTACAACCTCGAGTTGTACTTGGTTAGACTCTCG
	3	GACTGGCATGAGAGTGGATAACTCGAGTTATCCACTCTCATGCCAGTC
	4	AGTGGATAAAGCCACATTCATCTCGAGATGAATGTGGCTTTATCCACT
Igfbp6	1	GCTGTATGTGAAGCAATGAATCTCGAGATTCATTGCTTCACATACAGC
	2	CGGCCCAATCCTGTTCAAGATCTCGAGATCTTGAACAGGATTGGGCCG
	3	CGCAGACACTTGGATTCAAGTACTCGAGTACTGAATCCAAGTGTCTGCG
	4	AGATGGTCAAGGAAGCACTCACTCGAGTGAGTGCTTCCTTGACCATCT
Ptx3	1	CGAGCTCATGTATGTGAATTTCTCGAGAAATTCACATACATGAGCTCG
	2	CTTTGACGAATCATTAGCATTCTCGAGAATGCTAATGATTTCGTCAAAG
	3	CCAATGTTCCATTTGGGAGAACTCGAGTTCTCCCAAATGGAACATTGG
	4	GCATCCTGTGAGACCAATGAACTCGAGTTCATTGGTCTCACAGGATGC
Plaur	1	GCCTCTTAGTTGAGATAATATCTCGAGATATTATCTCAACTAAGAGGC
	2	GGTGCTGGGAAACCGGAGTTATCTCGAGATAACTCCGGTTTCCAGCAC
	3	GAAACCGGAGTTATACCGTAACTCGAGTTACGGTATAACTCCGGTTTC
	4	ACCAACAGGACCATGAGTTACCTCGAGGTAATCATGGTCTGTGG
	5	TGCATGCAGTGTGAGAGTAACCTCGAGGTTACTCTCACACTGCATGCA
Loxl1	1	CTATGACCTCCGAGTGCTATTCTCGAGAATAGCACTCGGAGGTCATAG
	2	ACTGCCAGTGGATCGACATAACTCGAGTTATGTCGATCCACTGGCAGT
	3	TCGCTACGTTTCTACAACAAACTCGAGTTTGTGTAGAAACGTAGCGA
	4	CTTCGTAAACAGTATGAGAACTCGAGTTCTCATACTGGTTTACGAAG
Tm4sf1	1	CTTGTGTCTCATTCAAGTAATCTCGAGATTACTTGAATGAGACACAAG
	2	GCTTCCTGTATTTCACTGTAACCTCGAGTTACAGTGAAATACAGGAAGC
	3	CATTGTGGCATCACTGGGTTTCTCGAGAAACCCAGTGATGCCACAATG
	4	GCTTGCTCTACTTTCCTAATCTCGAGATTAGGAAAGTAGAGCAAAGC

Table S1. List of shRNA sequences against candidate genes. Different colors represent the sense target sequence (red), loop (green) and anti-sense target sequence (blue). Sequences were based on Broad institute online tool (<http://www.broadinstitute.org/rnai/public/>)

Gene	ID	Forward primer	Reverse primer
Dlk1	13386	GGCCATCGTCTTTCTCAACAAGTGC	CCTCGCCGCTGTTATACTGCAAC
Ly6a	17065	TCTTCTTGTGGCCCTACTGTGTG	GGGCAGGTAATTGATGGGCAAG
Slc8a1	20541	GCAAAGAAGAGGAGGAGAGGCGCATTG	ACCACAAGGGCCAGGTTCTGTCT
Npc1	18145	CACGCCTCCGAAAGCTACCTGTTTC	CCCGATGTCTACTTTGTTCACCACCG
Enpp1	18605	TATGGACCTGCTGCTCGGTTGAGAC	GGTAAGGCCCGAAATGCTGGTTTGG
Enpp2	18606	AGGGTTTGTCCGCCCTCCGTTAATC	GCCACAGGACCGCAGTTTCTCAATG
Enpp3	209558	CAGGGTGATGCAGAAGAACGGAGAC	CTCCCGGCTTGAGAACGGTGTATGA
Ly6c	17067	GCTACGAGTGCTATGGAGTGCCAAT	ACACCAGCAGGGCAGAAAGAAAGG
Ly6e	17069	CCCTTCTGGGCATGGAGCAAGTTCAT	CAGGGTGTAGCCAAGGTTGACATTCC
Dpt	56429	CGAGGAGCAACAACCACTTTCTCTGC	GTCGTAGTCAGTCATCCGGCACA
Col5a3	53867	TCCAAGGGACCAACTGGGAAGAGT	CACAGAGGCAGGAAACCCACAGAAG
Col6a3	12835	GCCCATCACCCTCTAACCTCACAG	TTGAGTCTGCGAACGATCCTGCTG
Col7a1	12836	TGGGATGGTGAAGCCGAGATTAAGG	CAGCGCAGGGTATAGGCAGTACA
Col8a1	12837	CCTATGAGATGCCTGCGTTTACTGCC	GGTGAAGATGCCTGTCTGCGGATTG
Col13a1	12817	CCAAAGGGAGAAGCTGGTGTGAT	CGCCTCGTTGATGCTGCCATTA
Tgfb1	21810	GAAATCCTGGTTAGCGGAGGCATCG	CGGCAACAGGCTCCTTATTGACACTC
Matn2	17181	GCAGTATTCCACACAGGTCCGAA	TGGGTCACGGCTTTCTTCATTTC
Fn1	14268	GCTGTGAAAGGGAACAGCAGAGTC	CGGTGTTGTAAGGTGGAATGGAGCG
Thbs2	21826	GTGCTACTAATGCCACCTACCACTGC	CGTCACAAGCATCTCCGATTCCATCC
Svep1	64817	CAAATGCTTGCTCATGCCAGACGG	CACACAGCGCCCACCAATTCAAACAG
Bgn	12111	CTTCAGTGCCATGTGTCTTTTCGG	GTCTAGCAGTGTGGTGTGAGGTGAG
Den	13179	CCAGTGTCTCTTCGAGTGGTGCAGT	GTCTAGCAAGGTTGTGTGCGGGTGGAA
Mmp9	17395	GCAAACCCTGTGTGTTCCCGTTCATC	GTAACCATCCGAGCGGCCTTTAGTG
Ogn	18295	CGACCTGGAATCTGTGCCTCCTAA	CGCTCCCGAATGTAACGAGTGTCA
Ecm1	13601	CCTTGATCTTGGCCTGCTTGGCTCT	TGGCGTCATCTCTCGTGGTCTGAA
Ctsb	13030	ACTTGCTGTGGTATCCAGTGTGGG	GGAGGGATGGTGTATGGTAAGCAGC
Fap	14089	TTGACACCACCTACCCTCACCAC	CACTGCAAGCATACTCGTTCCTG
Adam9	11502	AGGATATGGAGGAAGCGTGGACAGC	GGAAGAAGAAGACCAGAAGCCCGTC
Itgb1	16412	GTAGCAGGCGTGGTTGCTGGAATTG	TTCACCCGTGTCCCCTTGGCATTTC
Itga5	16402	CTACTTGGGATACTCTGTGGCTGTGG	CGTTGTAGAGGGAGTGGATGTCTGAG
Itga6	16403	GCTTCCATAGATGTACCGCTGCTG	GCATCAGAATCCCGGCAAGAACAGC
Shc	20416	GGAGGTCTTACAGTCAATGCGAGCC	CAGGCACAGCTTCACACACCAAAC
Abl1	11350	TTAGTGCGGGAGAGTGAGAGTAGCC	GTAGTGGAGTGTGGTGTGAGGCCA
Pik3r1	18708	CTGAGTACCGAGAGATCGACAAACGCA	CCAGCCACTCGTTCAGCTTCTCTG
Vcam1	22329	GTGACAATGACCTGTTCCAGCGAGG	AAGGTGAGGGTGGCATTTCCTGAGAG
Ncam	17967	CAGGCCAGACAGAGCATCGTGAATG	GCTTCTCGTCATCTTCTCCTCGTTC
Lepr	16847	CTGTGTAGTGTGAGGAGGTACGTGG	AAGCGCCGAGGGAATTGACAG
Lgals3	16854	TATCCTGCTGCTGGCCCTTATGGTG	CCTGTTTGCCTTGGGTTTCACTGTGCC
Lgals8	56048	CTCCATCGGGTTCAGATTCAGCTCG	GCTTCGGGCATTGGTGTCACTTCC
Lgals9	16859	TTGCTTCTGCTGTCAGAGGTCAGAG	TGGTAGGGTACGCGGTGTGGTACT
Nov	18133	CTCTGCATCGTTCGGCCTTGTGAA	GCTTTCAGGGATTCTTGGTGC
Eda	13607	AGCCTTTGGAACCGGGAGAAGATCCAC	CTTATTGCGGCGAACACGCCTACTTCC
Spn	46194	CCTGATGAAGAGGCCACAACCATC	GTAATAAGAGCCCTGGCGAGACTTCC
Cdh11	12552	GGAGAGTACATGCCAAAGACCCAGAT	TGAGCCAGGCAGTTTCTTCCCTAT
Cdh22	104010	TGGCCTCTCAGGTTCCACTACAGTC	TCATGTCCGTGTTCTCGCCACATC
Fzd1	14362	GGACATCGCGTACAACAGACCATC	CGCGTACATGGAGCACAGGAAGAACTG
Lrp6	16974	GCCCACTACTCCCTGAATGCTGACAAC	CCGAAGGCTGTGGATAGGAAGGATGATG
CD82	12521	GTCTACAAACCTCATCCAGCTCGC	GACAAAGTACAGACCCAGCAAGCAGC
Tspan5	56224	TCCAATGCAAGCCGAGAGCGATG	TGGTCAACTTCTGGTTTCTGCCTGG
Tspan6	56496	CTGGAGGGCTGTTATCCACAGAGAGATG	CACGAGACAGGCAGTAGGCAAGAAAG
Tm4sf1	17112	GGCAAGCGATGCTCGATGCTTTCTTC	GTCCCTCAGTGTGTCGCAAGGTGTA
Loxl1	16949	TGGGAGTGGCATAGCTGTACCAAC	TTGTGTCCCTCGGCTACCTTCTTGC
Ptx3	19288	CGTGCATCCTGTGAGACCAATGAAGC	TCTCCTTTCCACCCACCAACACAC
Plaur	18793	CAGAGCTTTCCACCGAATGGCTTCC	CTCCGGTTTCCACGACATCTAAGC
Arhgdib	11857	AGATGTCCCTGTGGTAGCAGACC	TCGAGATCGCCAGTAAGGTCCA
Igfbp6	16012	ACCACAGAGACCGGCAGAAGAATCC	GTCACAGTTTGGCACATAGAGCCAC
Slco2a1	24059	CTTTGTCTCTACCCCTCCACACTTC	TGCTGCTGGTCTCCTTCTGGGTATC
Slc38a4	69354	TGGGCAGTGGGATCTTAGGCTTGTC	GACCTCCTTCTTGGCTGTCTTCA
Adecy6	11512	CAGCATGAGAACCCTCAGCAGGAAC	GCCCTCAATGTCCGCAACAGGATG
Gapdh	14433	CCCCTGAAGGGCATCTTGGGCTAC	GGGTGGGTGGTCCAGGTTTCTTAC

Table S2. List of qRT-PCR primers for candidate gene detection.

Stroma\weeks	4	12	16	20	24	32
AFT024	36.3	53.4	80.5	77.1	74.2	68.8
2018	11.3	6.7	9.1	8.6	11.8	13.8
DLK1^{KD}	29.9	44.3	68.2	43.6	49.4	43.5
DPT^{KD}	12.9	15.7	27.7	10.2	9.9	10.9
FAP^{KD}	31.8	47.9	65.4	52.8	42.7	37.6

Table S3. Percentage of donor contribution of transplanted HSC colonies co-cultured with wildtype AFT024, 2018 or knockdown 2018 stroma.

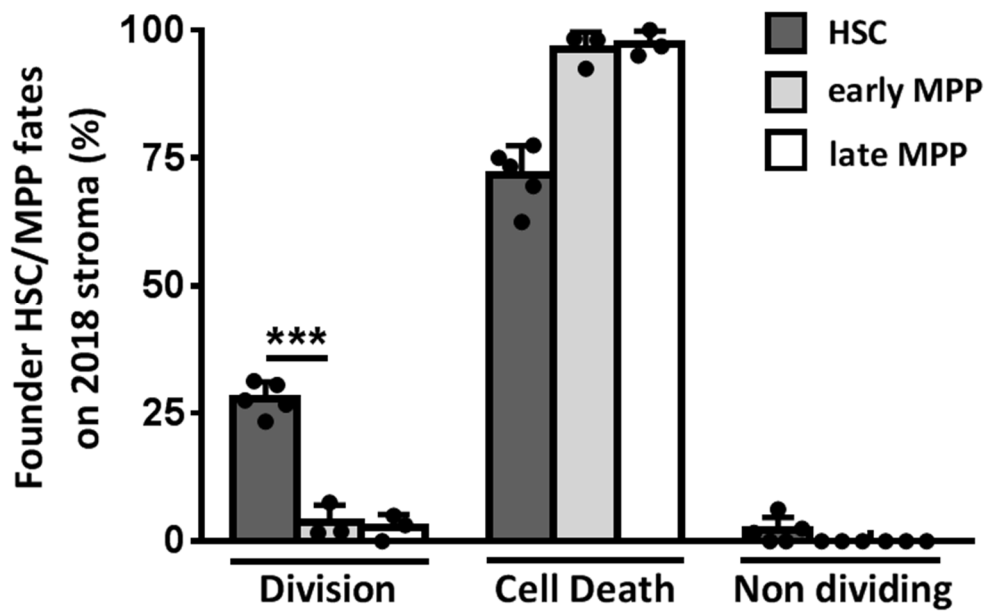


Figure S1 (Related to Figure 2C). Cell-death rates of founder HSC (n=5 independent experiments, 264 trees), early (n=3 independent experiments, 257 trees) and late MPPs (n=3 independent experiments, 184 trees) cultured on 2018 stroma.

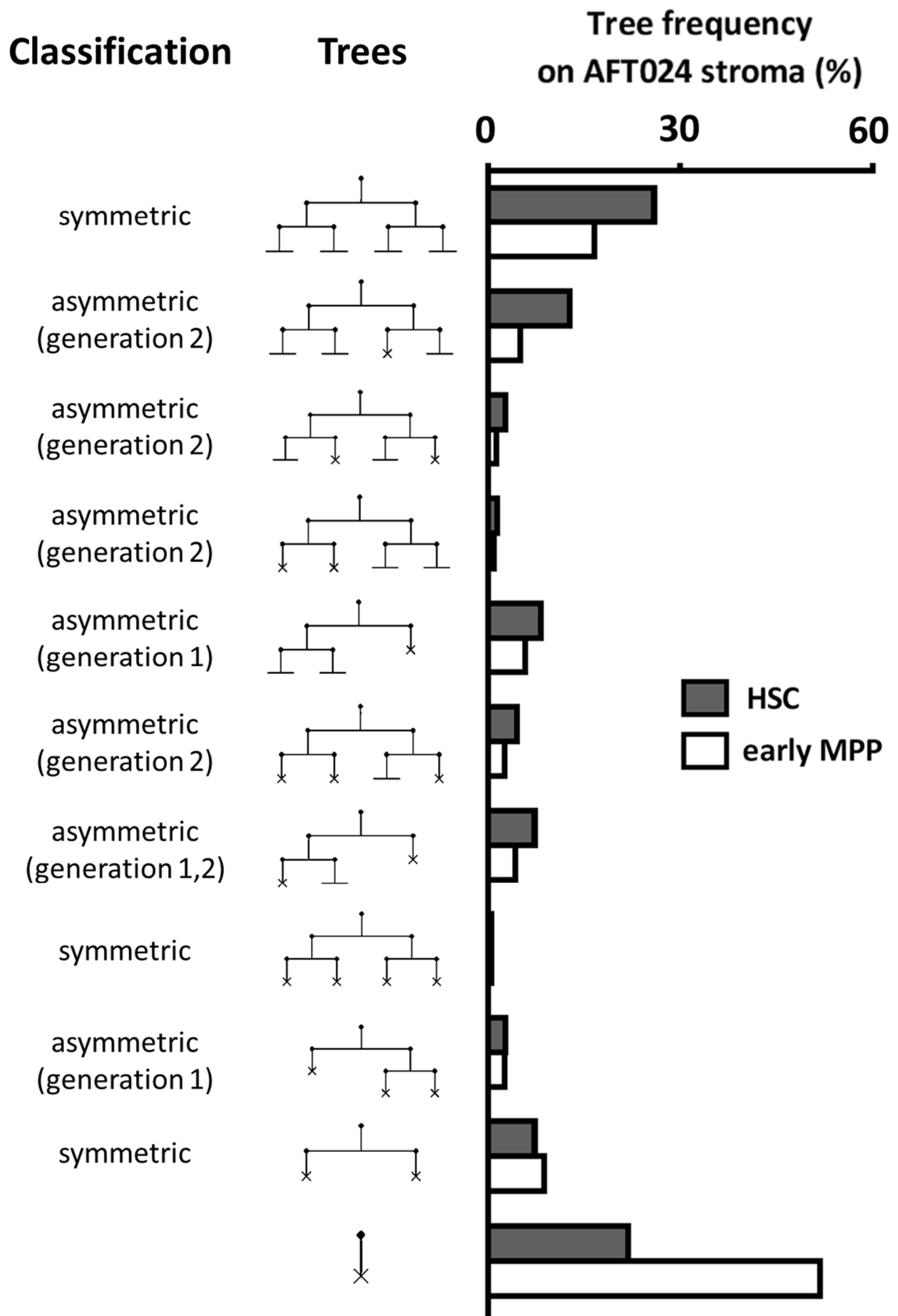


Figure S2 (Related to Figure 2E). Quantification of representative HSC and early MPP tree genealogies cultured on AFT024 stroma (up to generation 2).

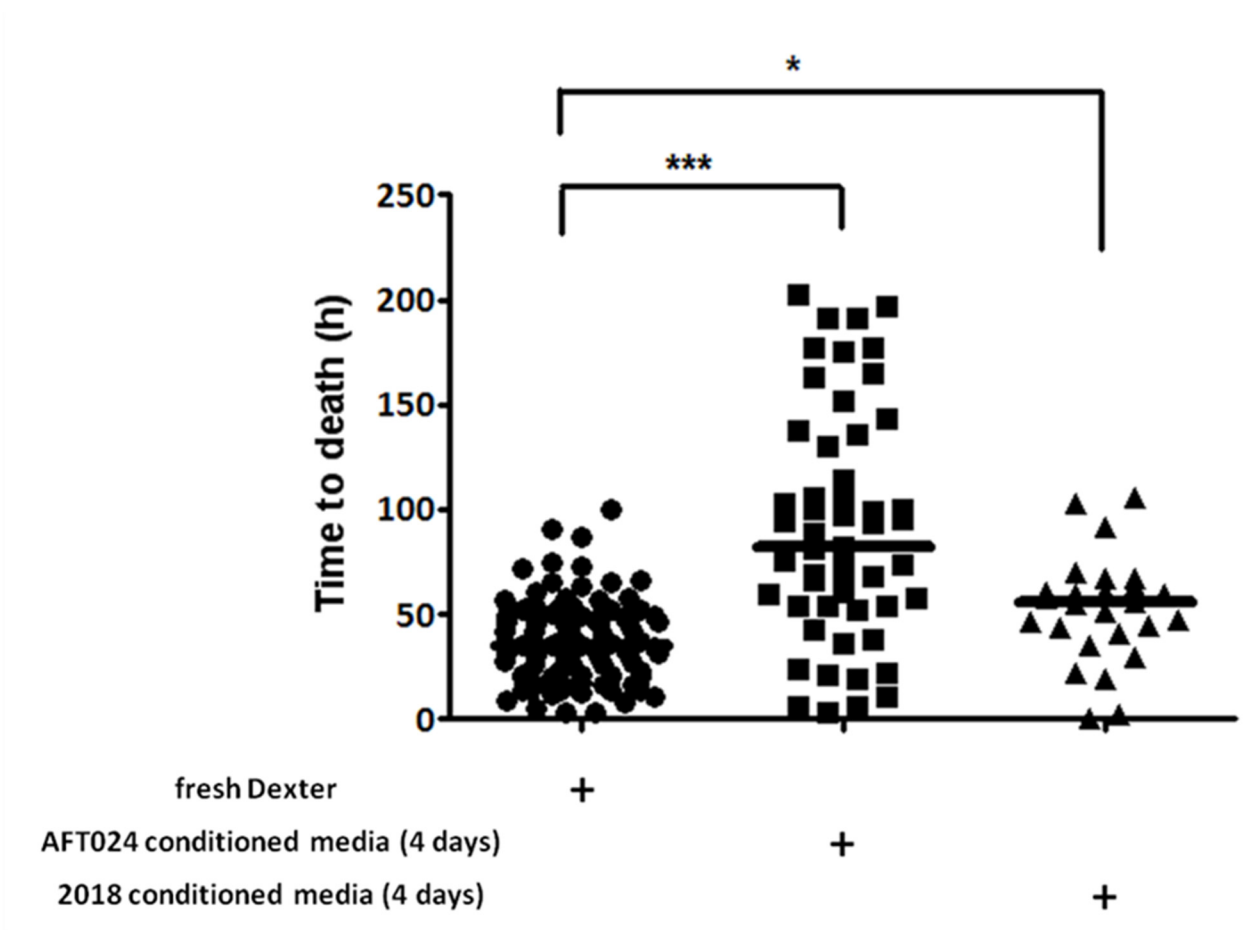


Figure S3 (Related to Figure 3). Dot plot depicting cell lifetime of dying founder HSCs cultured on stroma-free conditions supplemented with fresh Dexter media used for HSC-stroma co-cultures (n=4 independent experiments, 104 trees), 4-day AFT024 conditioned media (n=3 independent experiments, 54 trees) or 4-day 2018 conditioned media (n=3 independent experiments, 27 trees). Black lines represent the median. Data were compared using the rank-based non-parametric Kruskal-Wallis test with Dunn's post-hoc test.

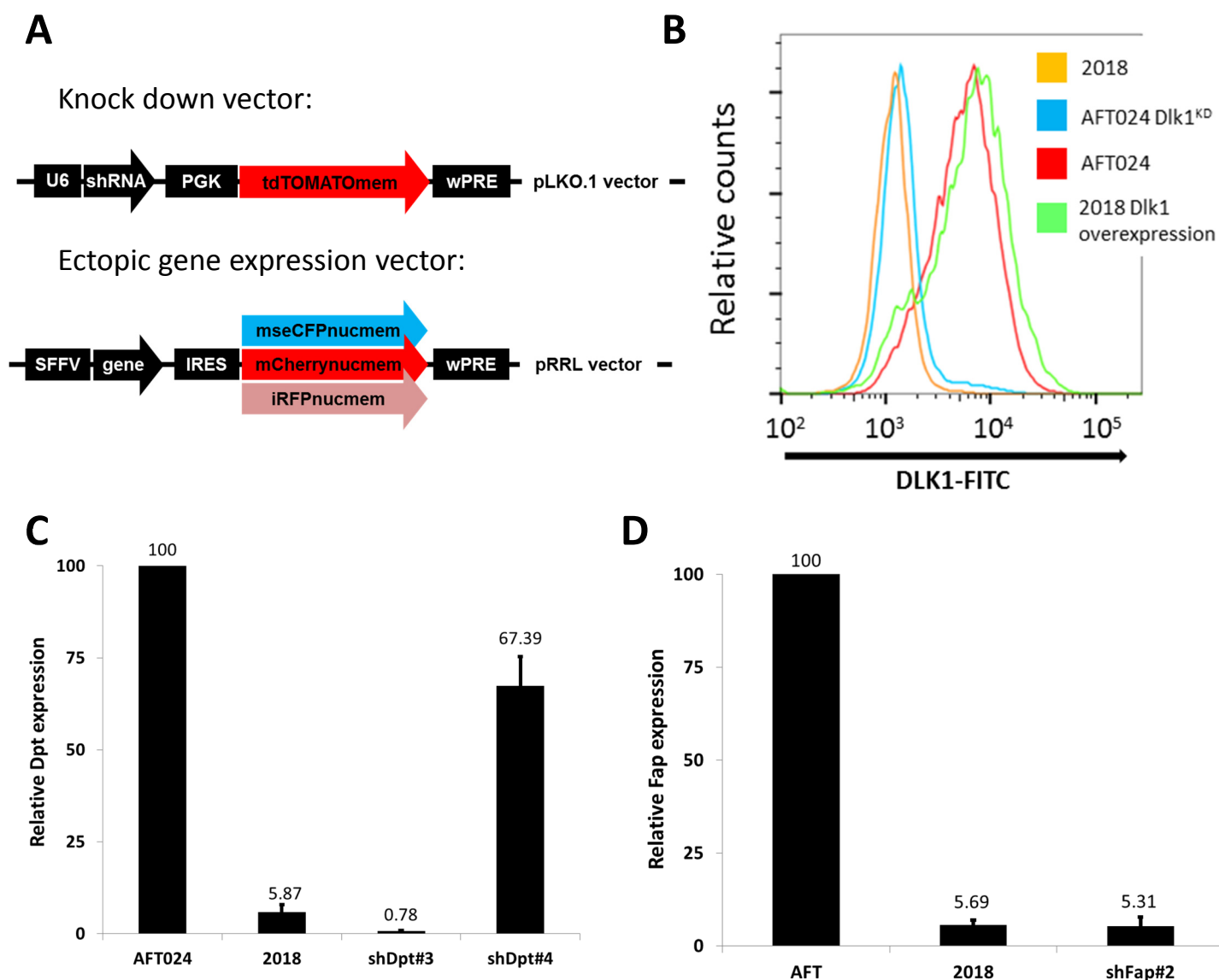


Figure S4 (Related to Figure 4B-E). Quantification of stroma manipulation efficiency upon lentiviral delivery of cDNAs or different shRNA clones against delta-like homolog 1 (*Dlk1*), dermatopontin (*Dpt*) and fibroblast activation protein (*Fap*). (A) Schematic depiction of the viral construct used for shRNA knock down or cDNA overexpression. Fluorescent reporter (tdTOMATO, mseCFP, mCherry or iRFP) expression was used to select transduced cells by flow cytometry. (B) DLK1 protein expression in shDlk1 transduced AFT024 (blue), in *Dlk1* overexpressing 2018 stroma (green) or wildtype, non-transduced AFT024 (red) and 2018 stroma (orange). Gate design was based on isotype controls. Results are representative from 3 independent experiments. (C) Relative *Dpt* expression in AFT024 lines transduced with two different shDpt clones. Since no anti-Dpt antibody worked reliably in our hands, results were analyzed by quantitative RT-PCR. (D) Similar analysis for shFap clones.

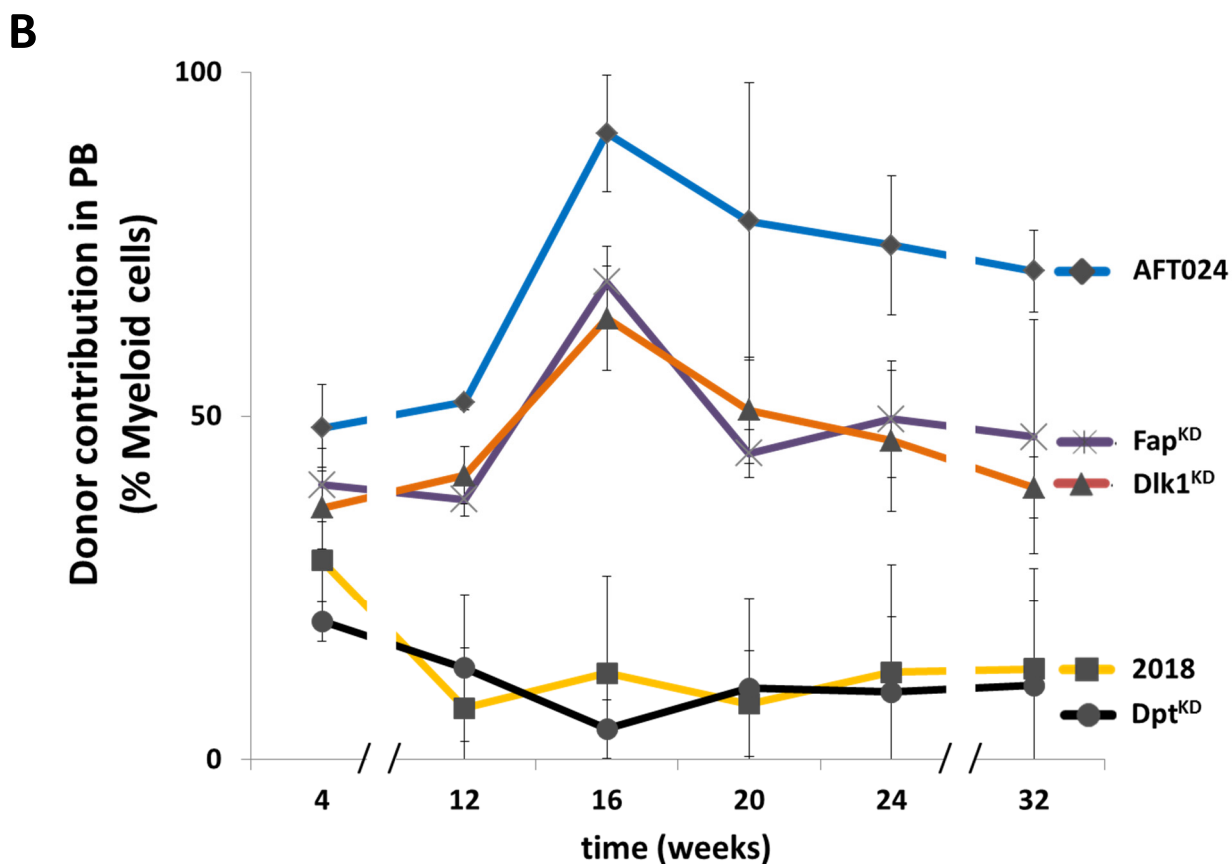
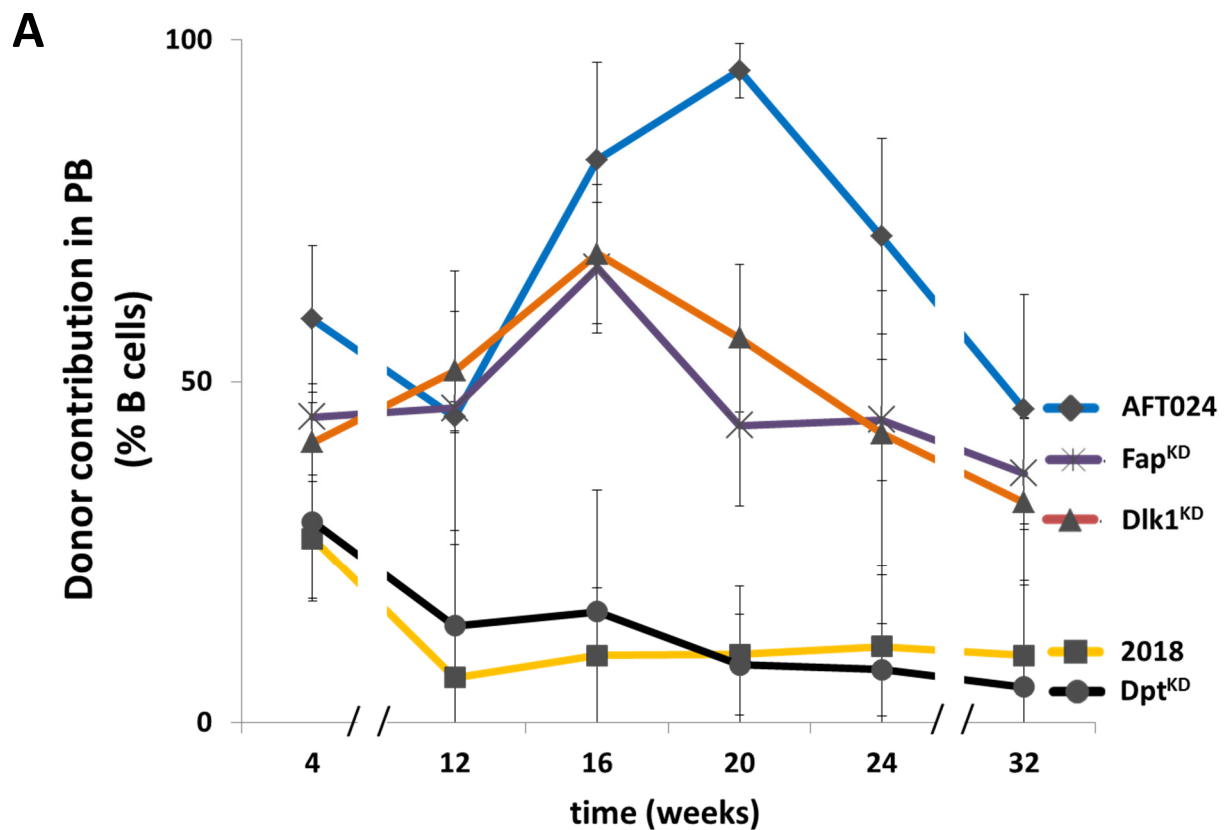


Figure S5 (Related to Figure 4D). Contribution to B cell (A) or myeloid lineage (B) of HSCs cultured different stroma cell lines, including wildtype AFT024, 2018 or knockdown 2018 stroma (Dlk1^{KD}, Dpt^{KD}, Fap^{KD}) at several timepoints post-transplant. B or myeloid-cell contribution was plotted as percentage from total cells of that lineage.

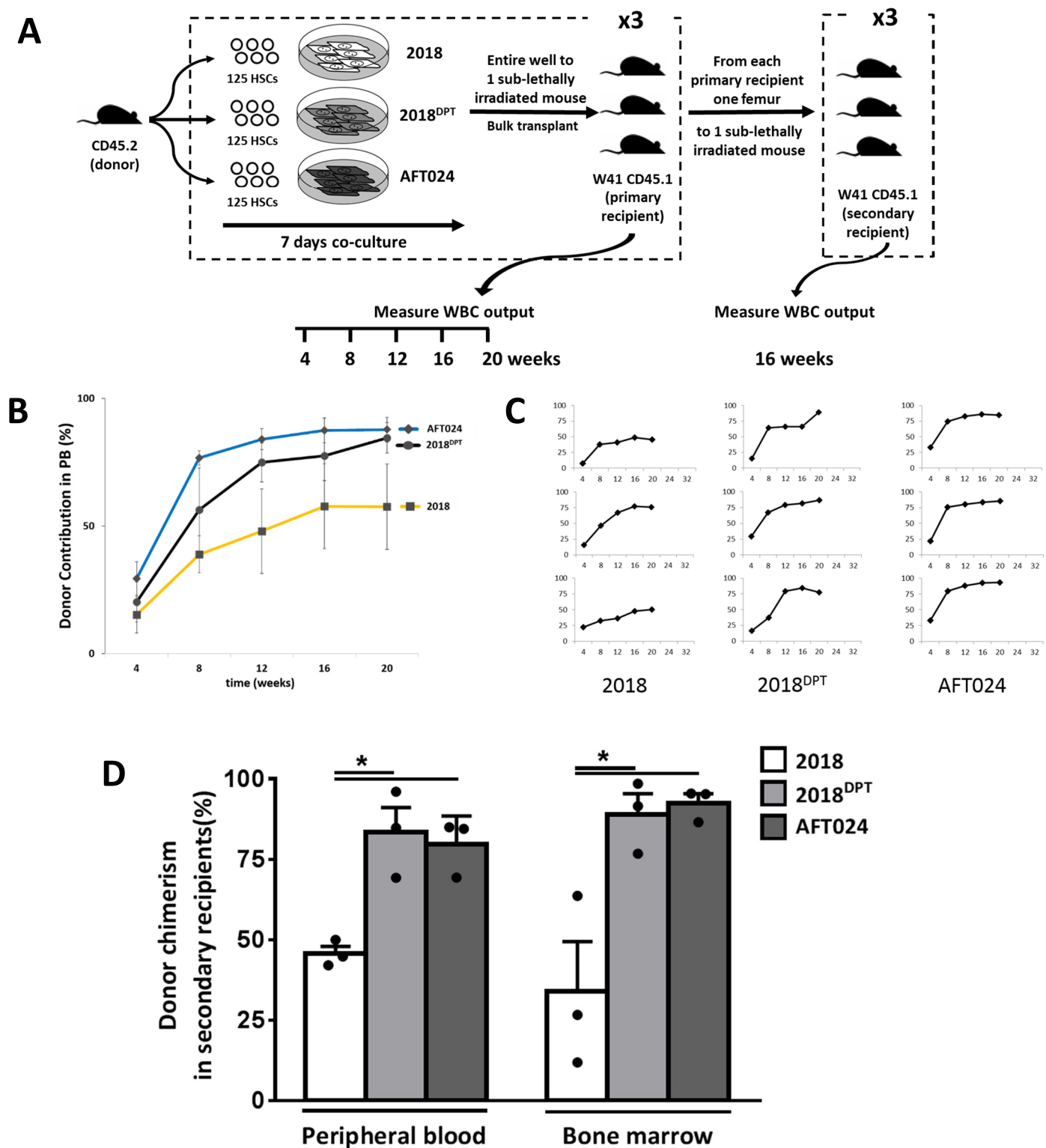


Figure S6 (Related to Figure 5D-F). (A) Experimental approach for in vivo transplantation of sorted HSCs cultured on genetically engineered 2018 stroma ectopically expressing DPT or wildtype supportive (AFT024) and non-supportive stroma lines (2018) for seven days prior to injection into sub-lethally irradiated immunocompromised primary and secondary W41 recipients. Donor contribution in peripheral blood was analyzed at several timepoints up to 20 weeks post transplantation and plotted as the average of all recipients per condition (B) or for each individual recipient separately (C). (D) Donor contribution in secondary recipients 16 weeks after secondary transplantation.

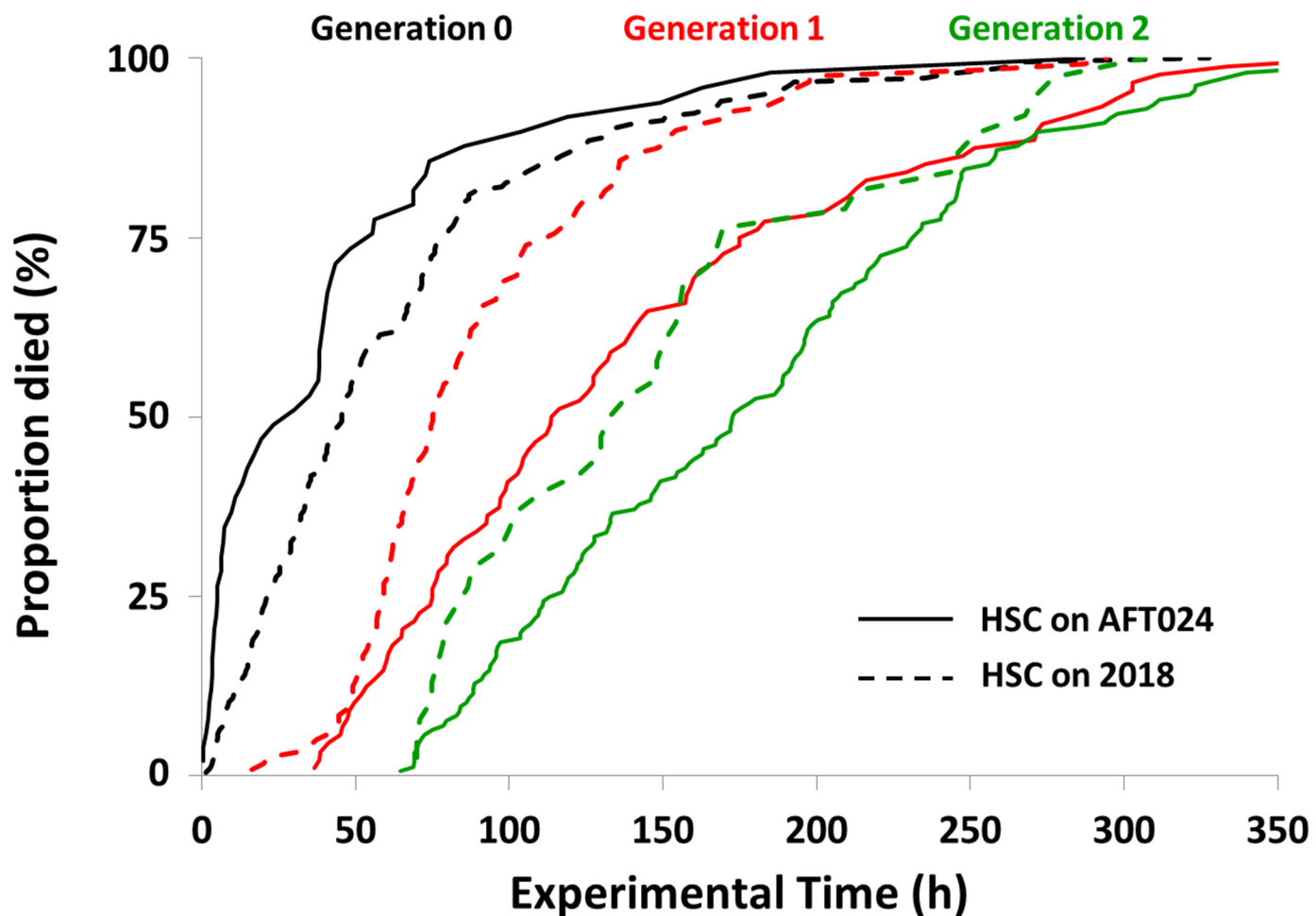


Figure S7 (Related to Figure 3F). Cumulative time curves representing absolute experimental time required for HSC cell death on AFT024 or 2018 stroma.