Supplementary Data for

KDM5B Promotes Drug Resistance by Regulating Melanoma Propagating Cell Subpopulations

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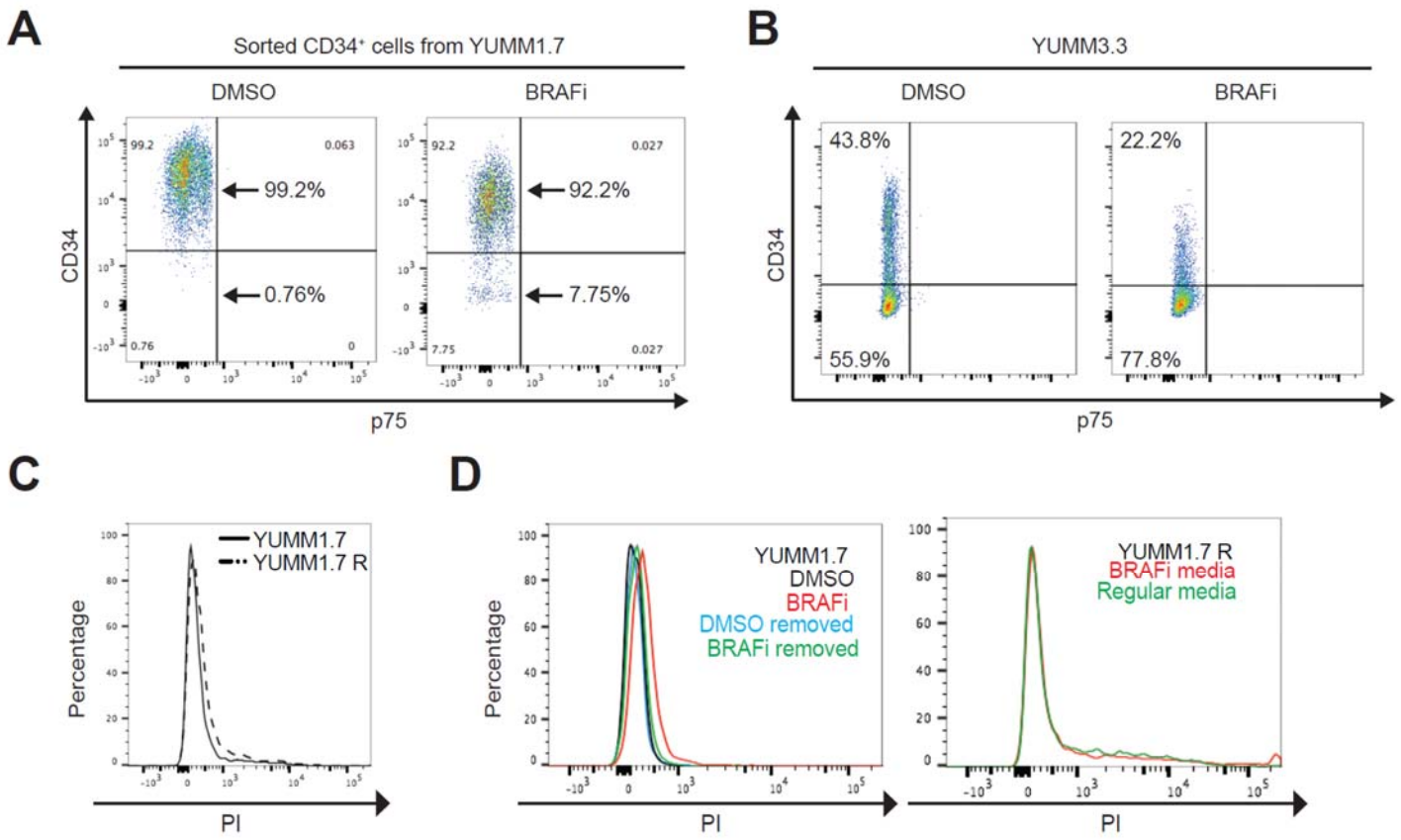

Figure S1. Responses of different YUMM cell lines to BRAFi treatment. (A-B) Flow cytometry analysis of sorted CD34⁺ YUMM1.7 cells (A) or unsorted YUMM3.3 cells (B) treated with DMSO control or 1.5 μM BRAFi for three days. (C-D) There was no significant cell death as shown by propidium iodide (PI) staining in YUMM1.7 and YUMM1.7R cells with or without BRAFi. The plot in C) corresponds to Fig. 1A and the plots in D) correspond to Fig. 2D.

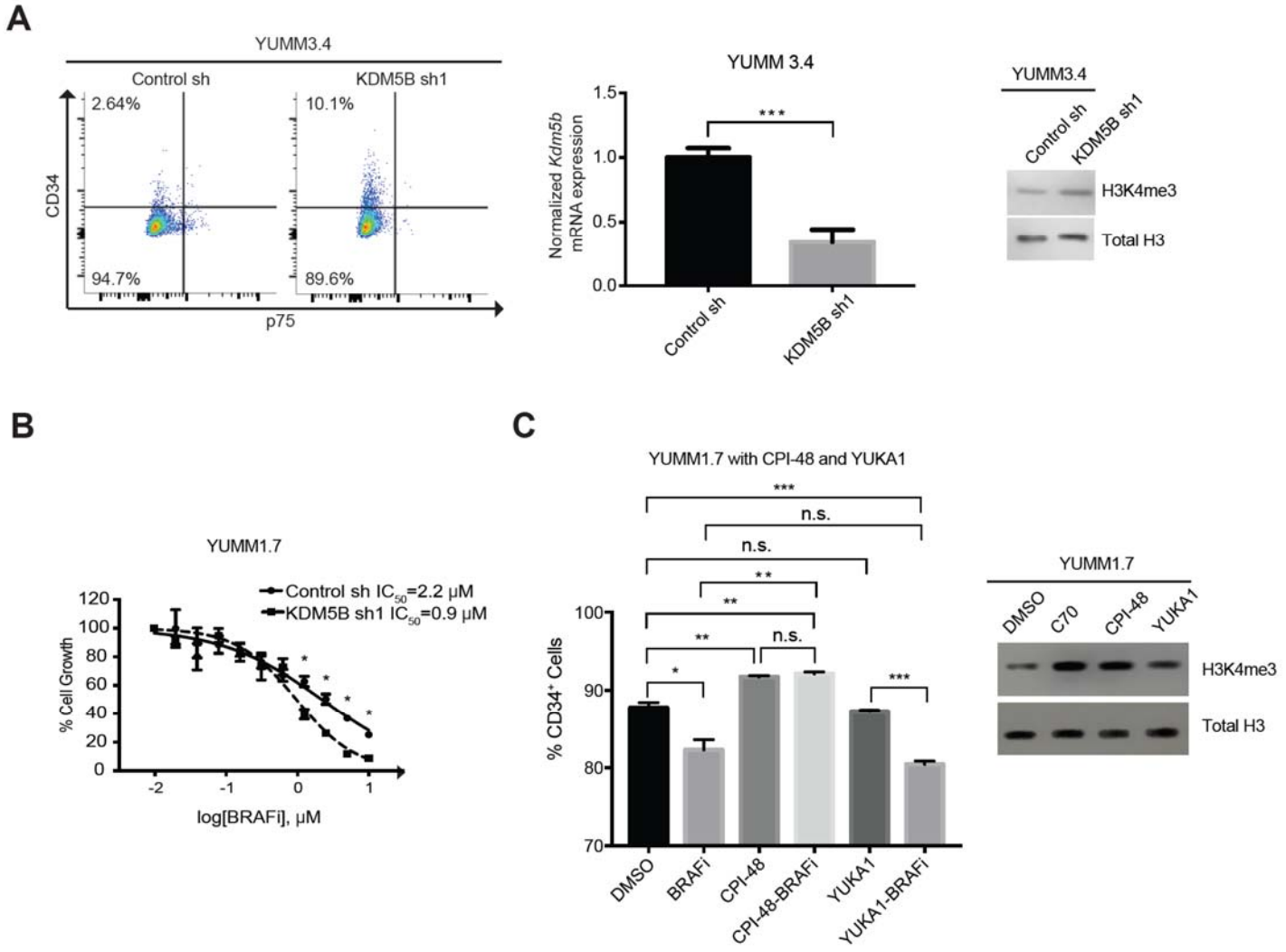

Figure S2. Inhibition of KDM5 family members by shRNA knockdown and pan-KDM5 inhibitors. (A) Knockdown of KDM5B by shRNA in YUMM3.4 led to an increase in the percentage of $CD34^+$ cells by flow cytometry (left). RT-qPCR analysis showed a decrease of *Kdm5b* mRNA level in YUMM3.4 KDM5B knockdown cells compared to YUMM3.4 cells with control shRNA (middle). YUMM3.4 KDM5B knockdown cells also exhibited an increased level of H3K4me3 shown by western blotting (right). (B) Knockdown of KDM5B by shRNA in YUMM1.7 cells decreased the BRAFi IC_{50} value from 2.2 μM to 0.9 μM. (C) 1 μM pan-KDM5i CPI-48 led to an increase in the percentage of $CD34^+$ cells after five days of treatment. Combination treatment of 1.5 μM BRAFi and 1 μM CPI-48 reverted the subpopulation shifting phenotype observed with BRAFi treatment alone. A different KDM5 inhibitor (YUKA1) targeting KDM5A and KDM5C at 100 μM had no effect on MPC subpopulation shifting on YUMM1.7 after eight days of treatment (* p≤0.05, ** p≤0.01, *** p≤0.001, n.s. not significant) (left). Western blotting showed that C70, CPI-48, or YUKA1 treatment increased H3K4me3 level to various degree (right).

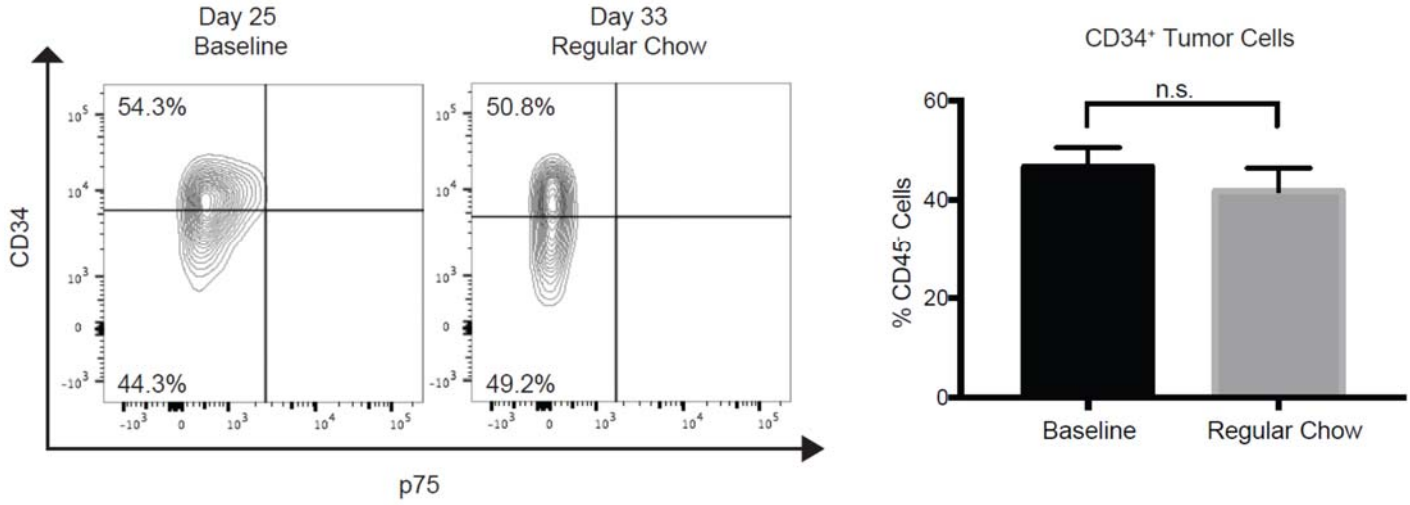

Figure S3. Subpopulation composition in tumors of different volumes. There was no difference in percentage of $CD34^-$ MPC subpopulation between baseline tumors on day 25 and control tumors on day 33 (n.s. not significant, n=3 for each group). Shown are flow cytometry plots (left) and quantification of flow cytometry analysis (right). Flow cytometry plots shown here represented live, non-immune cells gated with negative propidium iodide and CD45 staining.

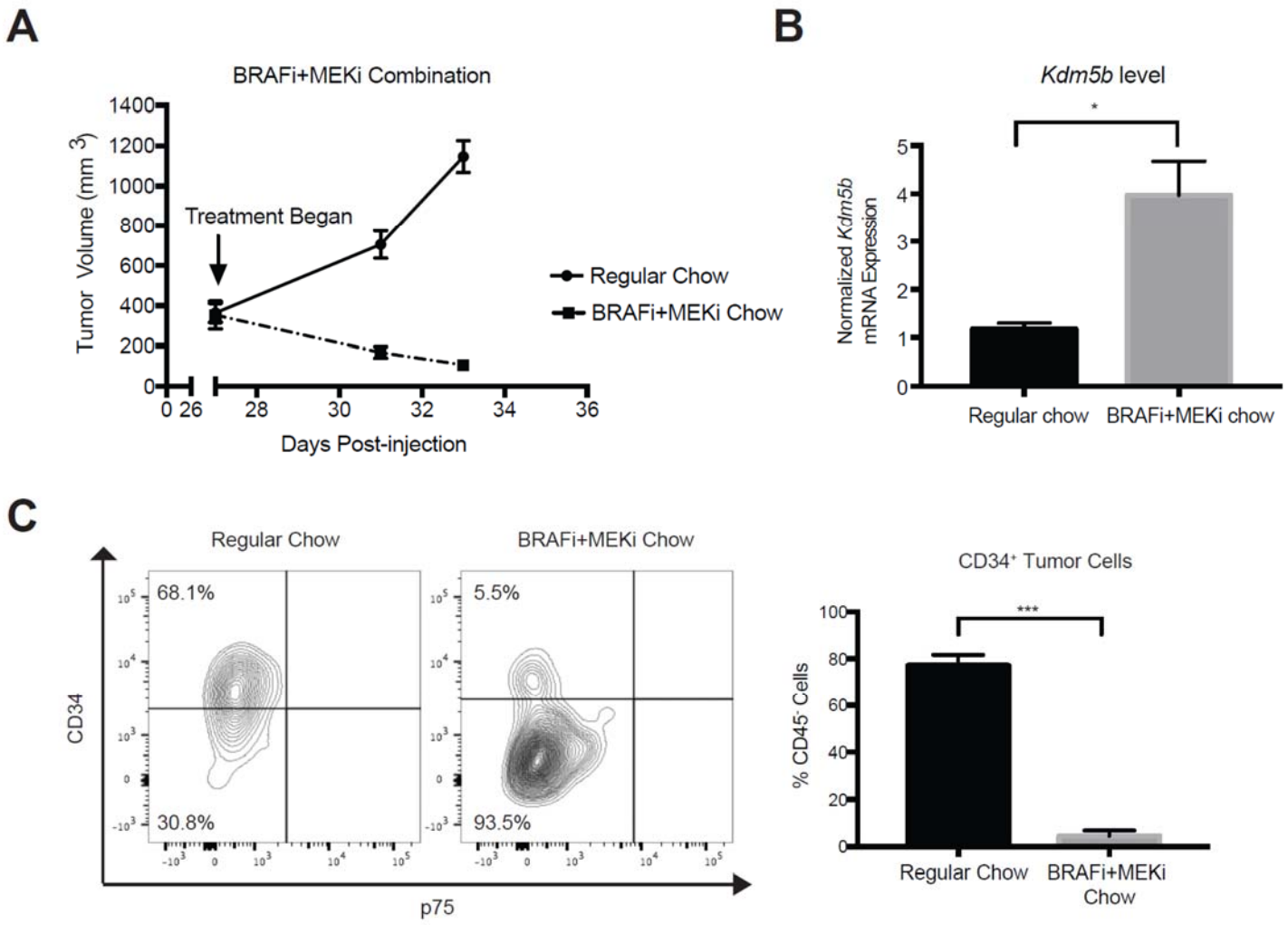

Figure S4. *In vivo* BRAFi/MEKi treatment of grafted YUMM1.7 tumors induced *Kdm5b* expression and increased CD34$^-$ subpopulation. (A) Grafted YUMM1.7 tumors in C57BL/6J tumors were responsive to combination BRAFi/MEKi chow (n=3 for each treatment group). (B) *Kdm5b* level increased post combination treatment (* p≤0.05). (C) The percentage of CD34$^-$ cells increased significantly after combined BRAFi and MEKi treatment (*** p≤0.001). Shown are flow cytometry plots (left) and quantification of flow cytometry analysis (right). Flow cytometry plots shown here represented live, non-immune cells gated with negative propidium iodide and CD45 staining.

Table S1. List of RT-qPCR primers.

H_18s_F	GCCGCTAGAGGTGAAATTCTTG
H_18s_R	CATTCTTGGCAAATGCTTTCG
H-KDM5B-F	AACAACATGCCAGTGATGGA
H-KDM5B-R	TACCAGGTTTTTGGCTCACC
m-GAPDH-F	GTGCTGAGTATGTCGTGGAGT
m-GAPDH-R	TCACACCCATCACAAACATG
m-KDM5B-F	AGAGGCTGAATGAGCTGGAG
m-KDM5B-R	TGGCAATTTTGGTCCATTTT
m-Ypel2-F	TTAGCAACACCCGCTCTTCT
m-Ypel2-R	CACGACGAACTCATTTCCAA
m-Map3k1-F	TCTGCTCCTCTTCATCCTTGA
m-Map3k1-R	TCCAGAAGTTTGTGTCACGC
m-Gdf15-F	GGTTGACGCGGAGTAGCAG
m-Gdf15-R	CCGAGAGGACTCGAACTCAG
m-Ldlr-F	CTAGCGATGCATTTTCCGTC
m-Ldlr-R	GTCATCGCCCTGCTCCTT
m-Slc43a3-F	CAGAGACGTCAGAGCCAGTG
m-Slc43a3-R	ATGGACCGCCTTAAACAGAA

Table S2. Subpopulation composition of different YUMM cell lines. Percentage of $CD34^{-}p75^{+}$ ($p75^{+}$), $CD34^{+}p75^{-}$ ($CD34^{+}$), and $CD34^{-}p75^{-}$ ($CD34^{-}$) subpopulations are determined by FACS analysis.

Genotype	Cell Line	$p75^{+}$	$CD34^{+}$	$CD34^{-}$
$Braf^{V600E}Pten^{-/-}Cdkn2a^{-/-}$	YUMM1.1	76%	0%	24%
	YUMM1.3	12%	0%	88%
	YUMM1.7	0%	75%	25%
	YUMM1.9	0%	6%	94%
$Braf^{V600E}Pten^{-/-}\beta\text{-}cat^{sta/+}$	YUMM2.1	23%	0%	77%
$Braf^{V600E}Cdkn2a^{-/-}$	YUMM3.3	0%	45%	55%
	YUMM3.4	0%	3%	97%
$Braf^{V600E}p53^{-/-}$	YUMM5.1	0%	12%	88%