Supplementary Information

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A Stapled BIM BH3 Helix Overcomes the Apoptotic Resistance of Refractory Hematologic Cancers

Supplementary Figure Legends

Supplementary Table 1

Sequence composition of SAHB peptides. 'X' denotes the positions of the non-natural amino acids within the peptide sequences. (M+3)/3 is the triply-charged molecular ion of each peptide detected during LC/MS purification and corresponds to the predicted molecular weight (MW) of the peptides.

Supplementary Figure 1

Circular dichroism of BIM BH3 peptides. BIM SAHB$_A$s exhibited marked enhancement of α-helical structure compared to the corresponding unmodified peptide, as evidenced by the shift in spectral contour from a random coil (single minimum at 195 nm) to an α-helical pattern (double minima at 208 and 222 nm). [θ], mean residue ellipticity, deg cm^2 dmol^{-1}.

Supplementary Figure 2

BIM SAHB$_A$-triggered, BAX-mediated liposomal release is inhibited by the addition of recombinant anti-apoptotic proteins (A) BCL-X$_L\Delta$C, (B) MCL-1ΔNΔC,

(C) BCL-wΔC, and (D) BFL-1/A1ΔC (1:1:1 BAX:BIM SAHB$_A$:anti-apoptotics, 400 nM). Liposomal release assays were performed in triplicate with similar results.

Supplementary Figure 3

Solubilized BIM SAHB$_A$ peptides elute as monomers by gel filtration chromatography. Aprotinin, 6.5 kD.

Supplementary Figure 4

Uptake of BIM SAHB$_A$ by human and murine fibroblasts. Cultured WS1 and WT and DKO MEFs were exposed to FITC-BIM SAHB$_A$ (1 μM) for 2 h, followed by PBS washing, trypsinization, repeat PBS washing, lysate preparation, electrophoresis, and fluorescence detection. FITC-BIM SAHB$_A$ is present in the lysates of all three fibroblast cell lines.

Supplementary Figure 5

BIM SAHB$_A$ treatment of fibroblasts at escalated dosing. **(A)** WS1 human fibroblasts treated with 20 μM BIM SAHB$_A$, but not vehicle or 20 μM BIM SAHB$_A$(R153D), manifest modestly reduced viability at 24 h with caspase 3/7 activation evident at 6 h. Adherent wild-type **(B)** and DKO **(C)** MEFs show no viability response to vehicle, BIM SAHB$_A$, or BIM SAHB$_A$(R153D) (20 μM) treatment, although measurable caspase 3/7 activation is detected in wild-type, but not DKO, MEFs upon treatment with 20 μM BIM SAHB$_A$. Data are mean ±

SEM for experiments performed in at least triplicate. RLU, relative luminescence units

Supplementary Figure 6

Peripheral white blood cell counts from treated cohorts of OCI-AML3 xenograft mice (Figure 7C). Data are mean ± SD (n=8 per arm).

Supplementary Figure 7

B-cell infiltrative disease of *Bim*$^{-/-}$-marrow reconstituted *Rag2*$^{-/-}\gamma c^{-/-}$ mice. (**A**) Like native *Bim*$^{-/-}$ mice, *Bim*$^{-/-}$-marrow reconstituted *Rag2*$^{-/-}\gamma c^{-/-}$ animals manifest a pervasive hypergammaglobulinemia, as measured by Ig ELISA. (**B**) *Bim*$^{-/-}$-marrow reconstituted *Rag2*$^{-/-}\gamma c^{-/-}$ mice likewise exhibit marked splenomegaly as measured by microultrasound.

Supplementary Figure 8

Spleen weights of treated *Bim*$^{-/-}$-marrow reconstituted *Rag2*$^{-/-}\gamma c^{-/-}$ and wild-type B6/129 mice. Mice were treated intravenously for 5 days with vehicle (5% DMSO/D5W), BIM SAHB$_A$, or BIM SAHB$_A$(R153D) (10mg/kg/day), and animals and harvested spleens weighed upon sacrifice on day 6. BIM SAHB$_A$-treated *Bim*$^{-/-}$-marrow reconstituted *Rag2*$^{-/-}\gamma c^{-/-}$ mice manifested a trend toward decreased spleen weight (p=0.06), which was not observed for vehicle- or BIM SAHB$_A$(R153D)-treated animals **(A, B)**. No differences in spleen weights were

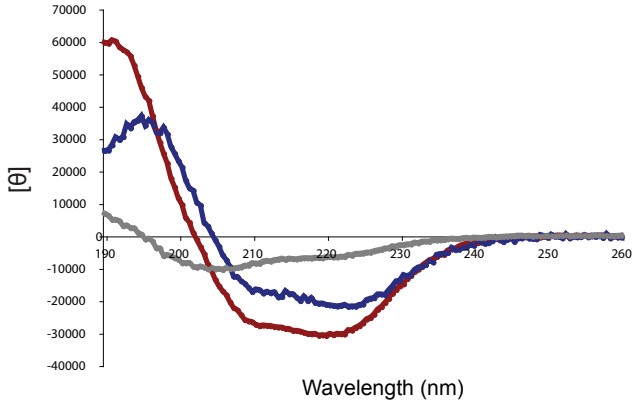
observed among the treatment groups of B6/129 mice **(C, D)**. Data are mean ± SD (n =4 per arm).

Supplementary Figure 9

Pre- and post-treatment peripheral white blood cell and splenic lymphocyte counts from treated cohorts of wild-type B6/129 mice. **(A)** Absolute white blood cell counts and differential from wild-type B6/129 mice prior to and after 5 day intravenous treatment with vehicle (5% DMSO/D5W), BIM $SAHB_A$, or BIM $SAHB_A$(R153D) (10 mg/kg/day). Post-treatment peripheral **(B)** and splenocyte **(C)** CD4+ CD8+, and B220+ lymphocyte counts from treated wild-type B6/129 mice. Data are mean ± SD (n =4 per arm).

Peptide	Sequence	Species	N-terminus	(MW)	(M+3)/3
BIM BH3	IWIAQELRRIGDEFNAYYARR	Human	FITC-β-Ala-	3098	1034.2
BIM SAHB$_A$	IWIAQELR**X**IGD**X**FNAYYARR	Human	Ac	2645	883.0
BIM SAHB$_A$	IWIAQELR**X**IGD**X**FNAYYARR	Human	FITC-β-Ala-	3063	1022.5
BIM SAHB$_A$(R153D)	IWIAQELD**X**IGD**X**FNAYYARR	Human	Ac	2604	869.3
BIM SAHB$_A$(R153D)	IWIAQELD**X**IGD**X**FNAYYARR	Human	FITC-β-Ala-	3022	1008.8
BIM SAHB$_A$	IRIAQELR**X**IGD**X**FNETYTRR	Murine	Ac	2641	881.7
BIM SAHB$_A$(R153D)	IRIAQELD**X**IGD**X**FNETYTRR	Murine	Ac	2600	868.0
BAX SAHB$_A$	ASTKKLSESLK**X**IGD**X**LDSN	Human	FITC-β-Ala-	2614	872.7
BAK SAHB$_A$	QVGRQLA**X**IGD**X**INRRYD	Human	FITC-β-Ala-	2582	862.0

Supplement Table 1



Supplementary Figure 1
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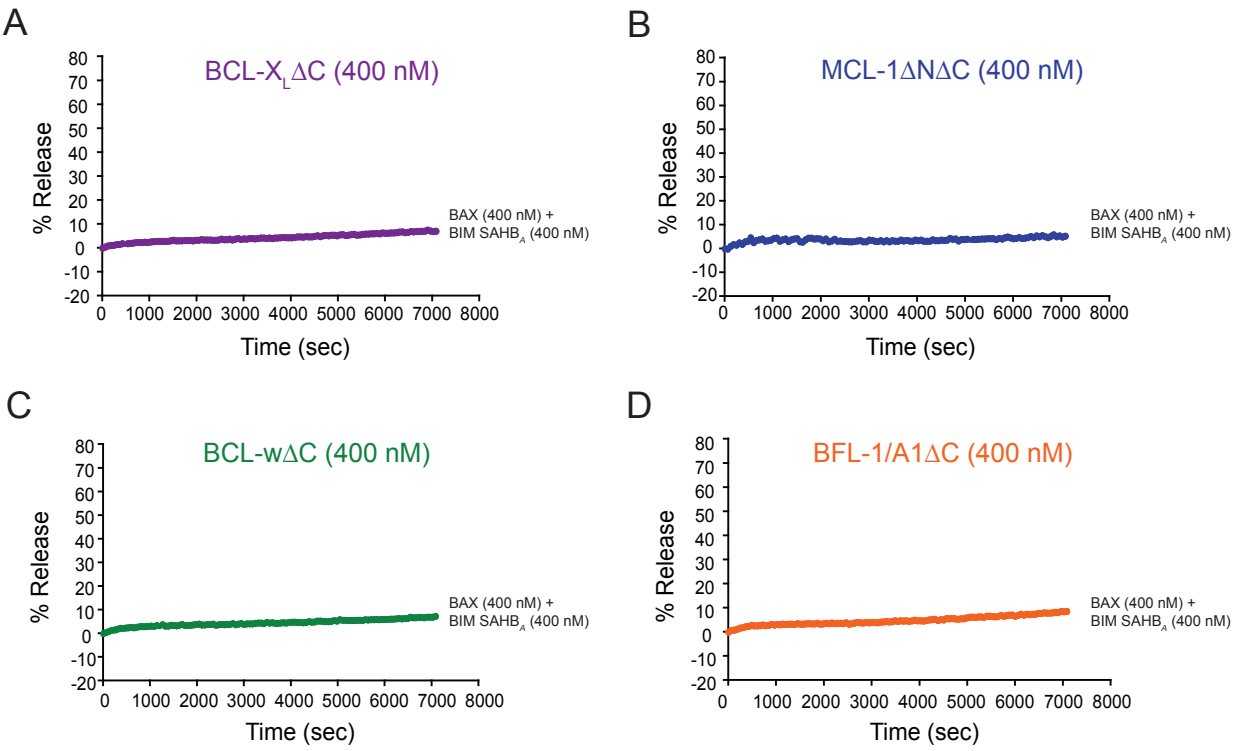

Supplementary Figure 2
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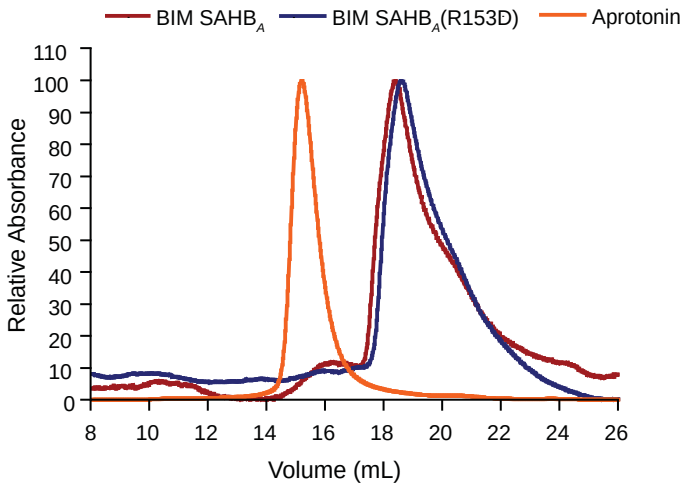

Supplementary Figure 3
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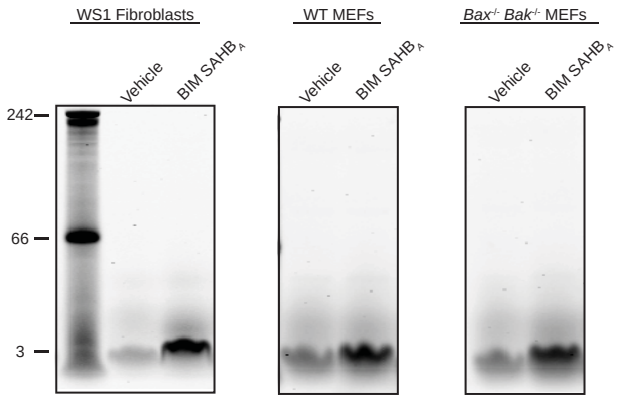

Supplementary Figure 4
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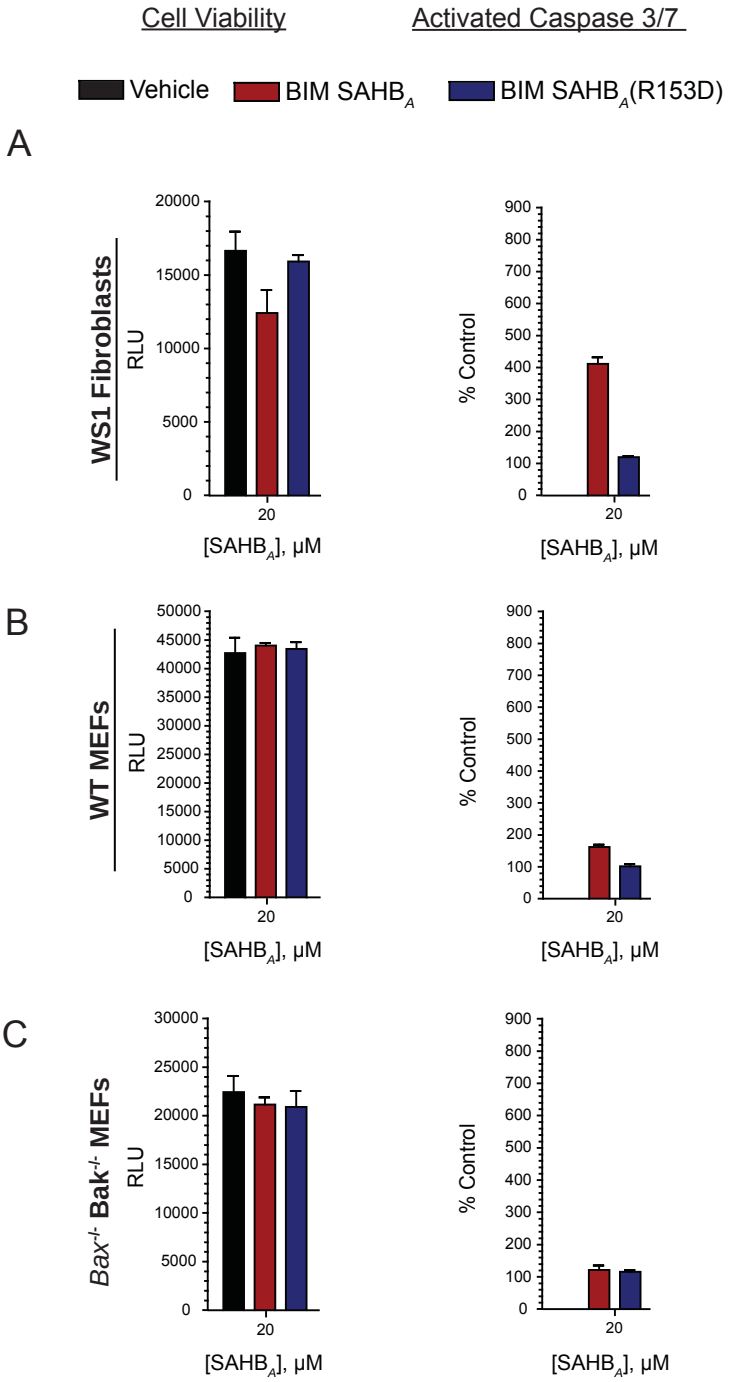

Supplementary Figure 5
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Treatment	WBC (x 10^3 cells/μL)	% Neutrophils	% Lymphocytes	% Monocytes
Vehicle	5.0 (± 1.8)	30.4 (± 5.8)	41.8 (± 5.0)	25.8 (± 5.7)
BIM $SAHB_A$	3.7 (± 1.2)	29.1 (± 7.2)	40.8 (± 7.1)	28.8 (± 5.3)
BIM $SAHB_A$(R153D)	2.8 (± 1.0)	32.5 (± 4.6)	39.6 (± 7.1)	27.1 (± 3.5)
BIM $SAHB_A$ + ABT-263	5.1 (± 4.6)	27.8 (± 10.5)	47.3 (± 13.5)	22.7 (± 8.5)
BIM $SAHB_A$(R153D) + ABT-263	2.7 (± 1.0)	21.7 (± 6.3)	56.2 (± 7.8)	21.6 (± 2.7)

Supplementary Figure 6
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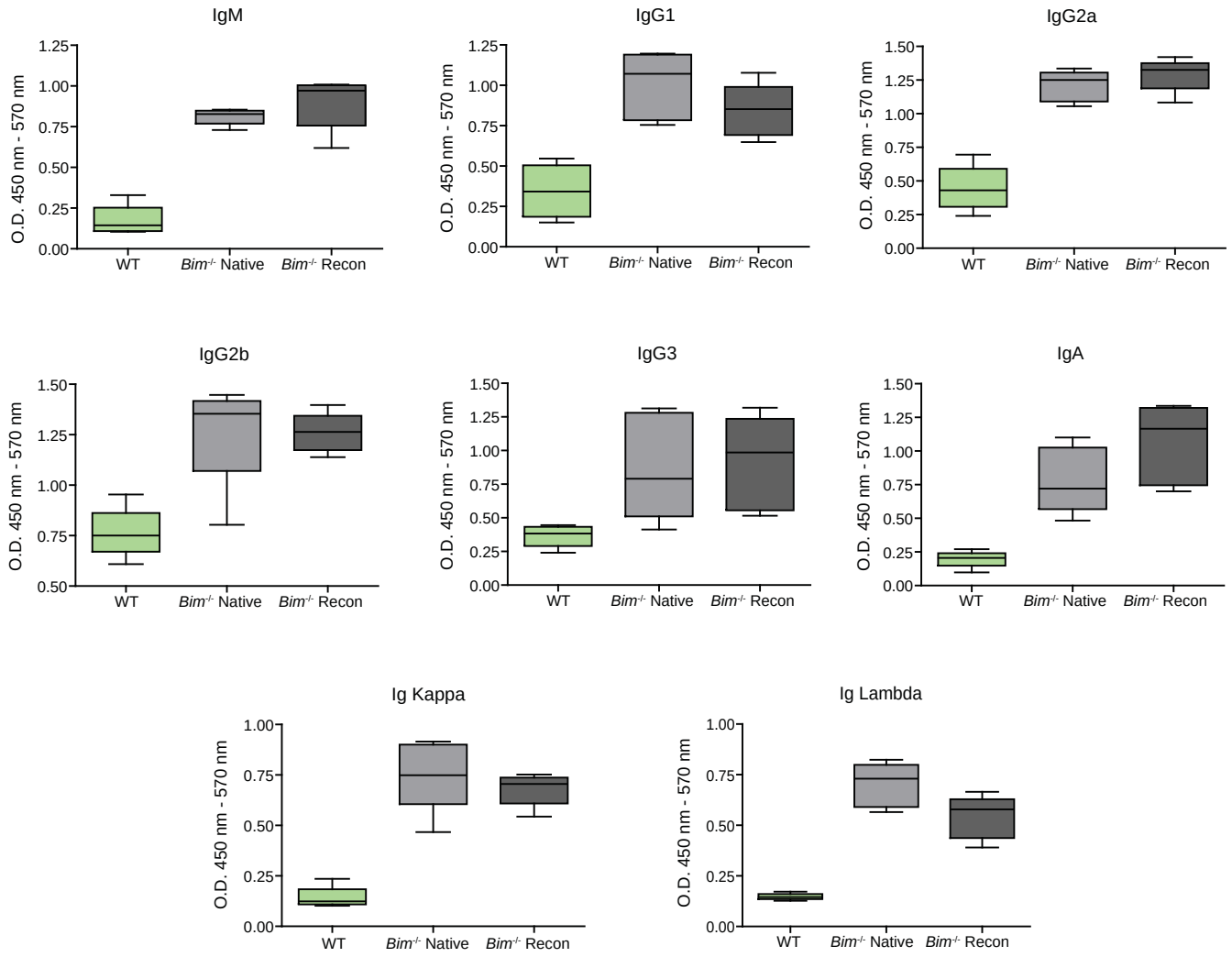
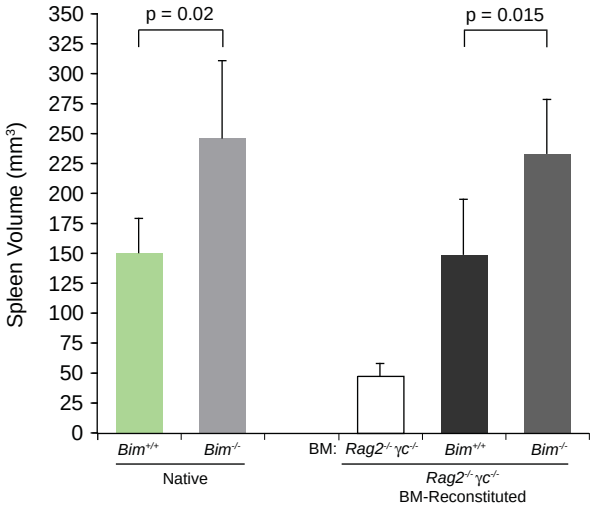
A



B



Supplementary Figure 7
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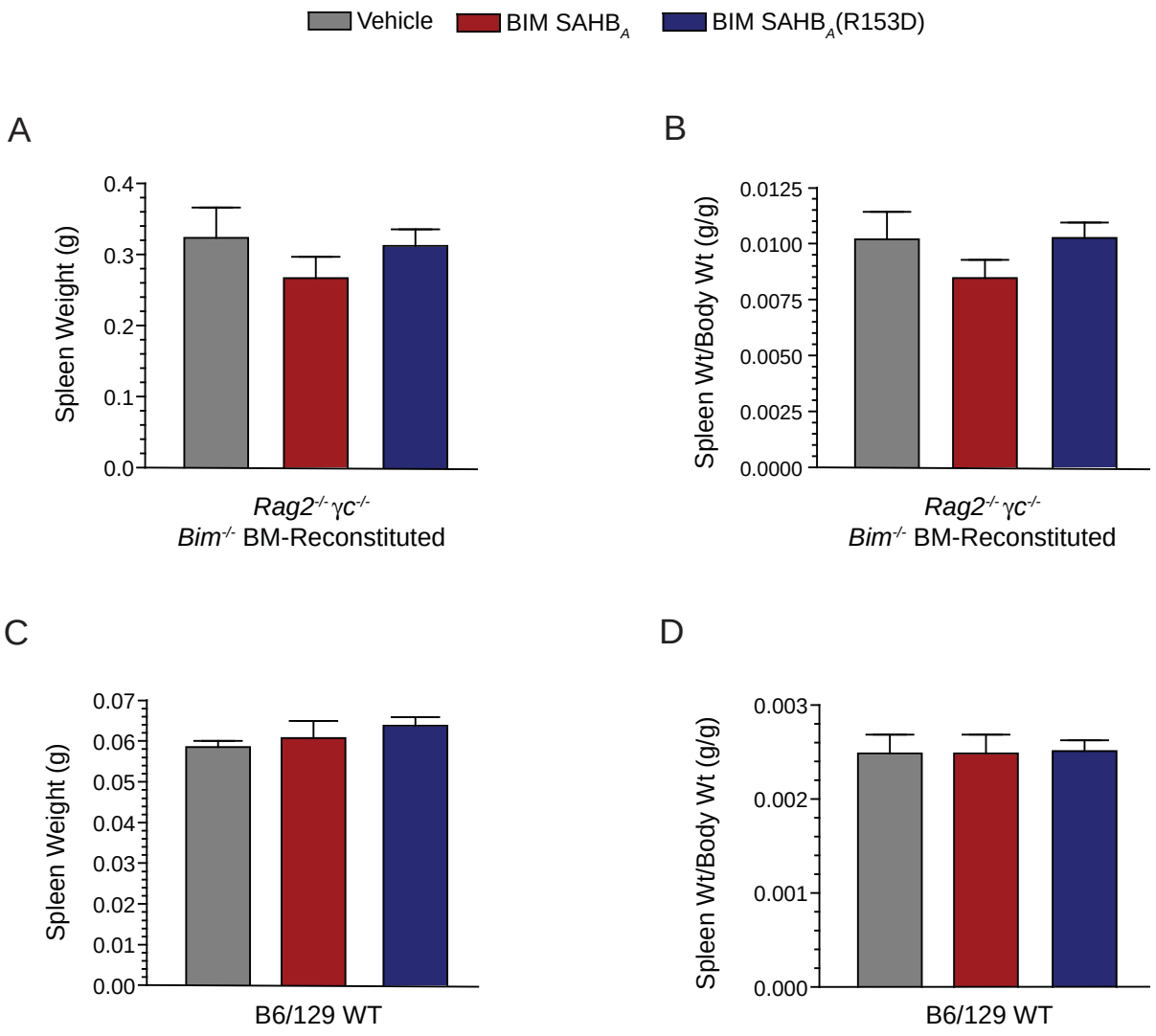

Supplementary Figure 8
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A

	Treatment	WBC (x 10^3 cells/μL)	% Neutrophils	% Lymphocytes	% Monocytes
Pre-Treatment	Vehicle	4.7 (± 0.9)	8.4 (± 1.5)	87.6 (± 0.9)	1.8 (± 0.7)
	BIM $SAHB_A$	5.6 (± 1.9)	9.0 (± 2.0)	87.0 (± 2.4)	1.9 (± 0.4)
	BIM $SAHB_A$(R153D)	5.4 (± 0.6)	9.4 (± 1.6)	86.0 (± 1.7)	1.9 (± 0.2)
Post-Treatment	Vehicle	6.2 (± 1.1)	9.8 (± 2.3)	82.5 (± 2.8)	1.6 (± 0.5)
	BIM $SAHB_A$	7.6 (± 1.1)	10.4 (± 3.0)	80.7 (± 5.4)	2.9 (± 1.5)
	BIM $SAHB_A$(R153D)	8.1 (± 2.7)	6.4 (± 1.5)	85.4 (± 2.4)	1.2 (± 0.4)

B

		Lymphocytes (x 10^3 cells/μL)		
		CD4+	CD8+	B220+
Peripheral Blood	Vehicle	0.8 (± 0.1)	1.1 (± 0.3)	2.8 (± 0.9)
	BIM $SAHB_A$	1.0 (± 0.2)	1.0 (± 0.2)	3.6 (± 0.5)
	BIM $SAHB_A$(R153D)	1.3 (± 0.3)	1.7 (± 0.4)	5.2 (± 1.9)

C

		Lymphocytes (%)		
		CD4+	CD8+	B220+
Spleen	Vehicle	26.1 (± 1.5)	11.7 (± 0.3)	62.8 (± 0.8)
	BIM $SAHB_A$	24.3 (± 0.9)	10.4 (± 0.8)	63.1 (± 2.0)
	BIM $SAHB_A$(R153D)	28.3 (± 1.5)	11.1 (± 0.5)	60.0 (± 1.4)

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