

A sequence-specific DNA binding small molecule triggers the release of immunogenic signals and phagocytosis in a model of B-cell lymphoma.

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Supplemental Information: Figures and Tables

- 1) Full chemical structures of polyamides
- 2) Polyamide mass spectrometry data
- 3) CRT histogram of dose-dependence and controls.
- 4) Table of motifs and associated e-values for Bind-n-Seq of biotin-conjugate polyamide **1b**.
- 5) Table of cell death categorization by flow cytometry after different treatments for 24 hours.
- 6) Table of time course of cell death categorization after treatment with **1**.
- 7) Representative flow cytometry data for phagocytosis assay of Raji cells by peripheral blood macrophages after treatment with polyamide **1**.

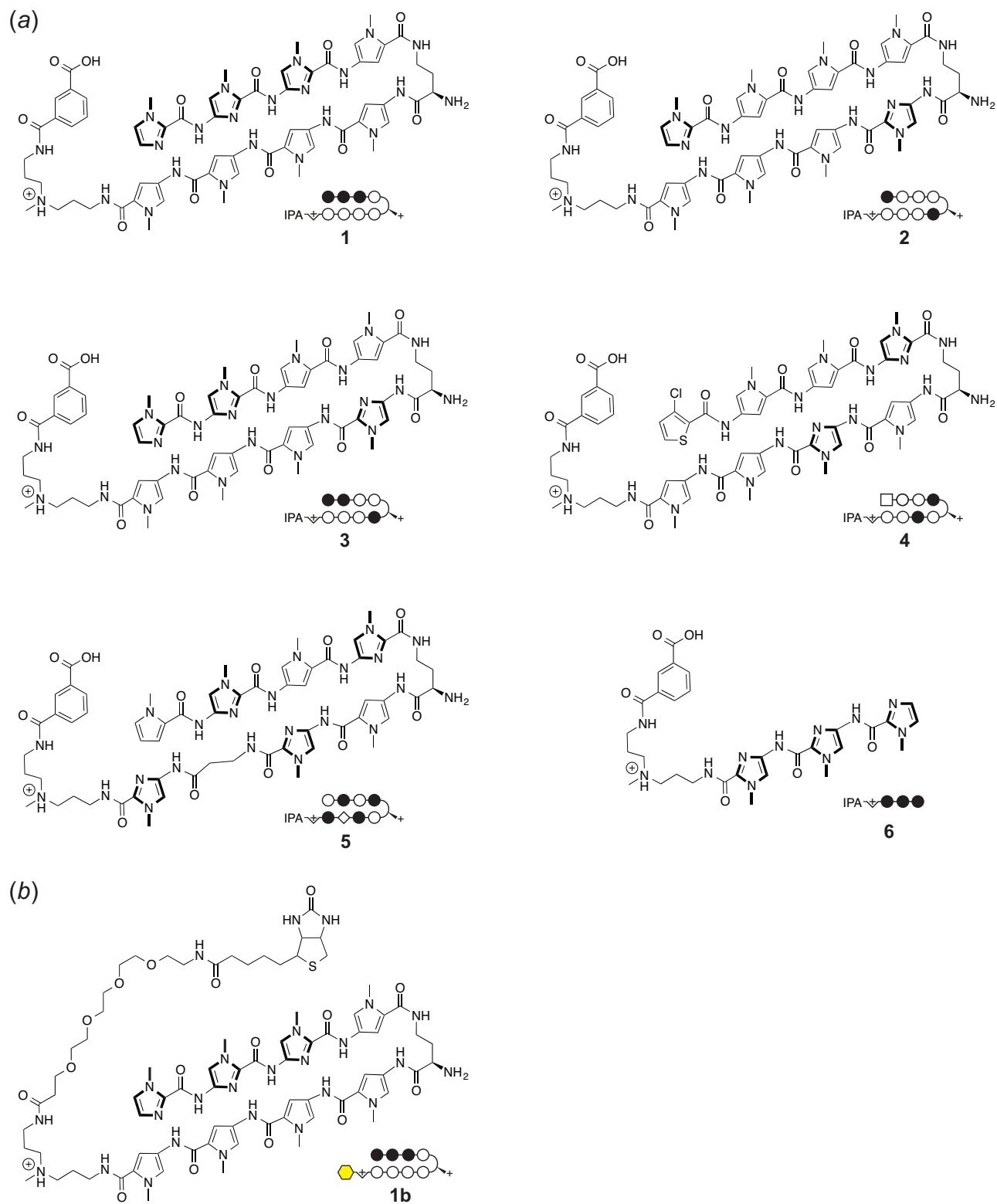
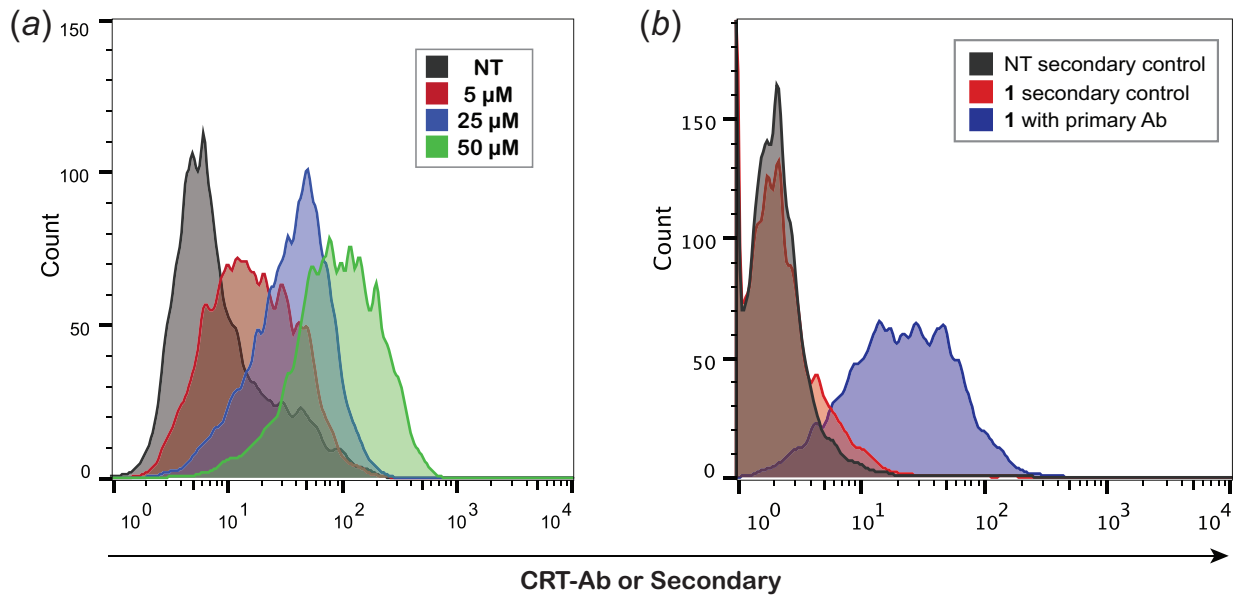


Table S2. Mass spectrometry (MALDI-TOF) for Py-Im polyamides.

Polyamide	Molecular formula	[Mass + H]	Found Mass
ImImImPy-(R) ^{α-NH₂} γ -PyPyPyPy-(+)-IPA (1)	C ₆₄ H ₇₆ N ₂₃ O ₁₂ ⁺	1358.6	1358.5
ImPyPyPy-(R) ^{α-NH₂} γ -ImPyPyPy-(+)-IPA (2)	C ₆₅ H ₇₇ N ₂₂ O ₁₂ ⁺	1357.6	1357.1
ImImPyPy-(R) ^{α-NH₂} γ -ImPyPyPy-(+)-IPA (3)	C ₆₄ H ₇₆ N ₂₃ O ₁₂ ⁺	1358.6	1358.0
CtPyPyIm-(R) ^{α-NH₂} γ -PyImPyPy-(+)-IPA (4)	C ₆₄ H ₇₃ ClN ₂₁ O ₁₂ S ⁺	1394.5	1394.1
PyImPyIm-(R) ^{α-NH₂} γ -PyIm β Im-(+)-IPA (5)	C ₆₀ H ₇₄ N ₂₃ O ₁₂ ⁺	1308.6	1309.0
ImImIm-(+)-IPA (6)	C ₃₀ H ₃₈ N ₁₁ O ₆ ⁺	648.3	648.8
ImImImPy-(R) ^{α-NH₂} γ -PyPyPyPy-(+)-4PEG-Biotin (1b)	C ₇₇ H ₁₀₇ N ₂₆ O ₁₆ S ⁺	1683.8	1683.1



SI Figure 3. CRT dose-dependence data and controls. (a) Representative histogram of CRT dose-dependence after treatment with polyamide **1** at 5, 25, and 50 μM , as measured by flow cytometry. (b) Antibody specificity control: secondary antibody with or without primary antibody after treatment with **1**.

SI Table 4. Table of top motifs and associated e-values for three Bind-n-Seq analyses of biotin-conjugate polyamide **1b**.



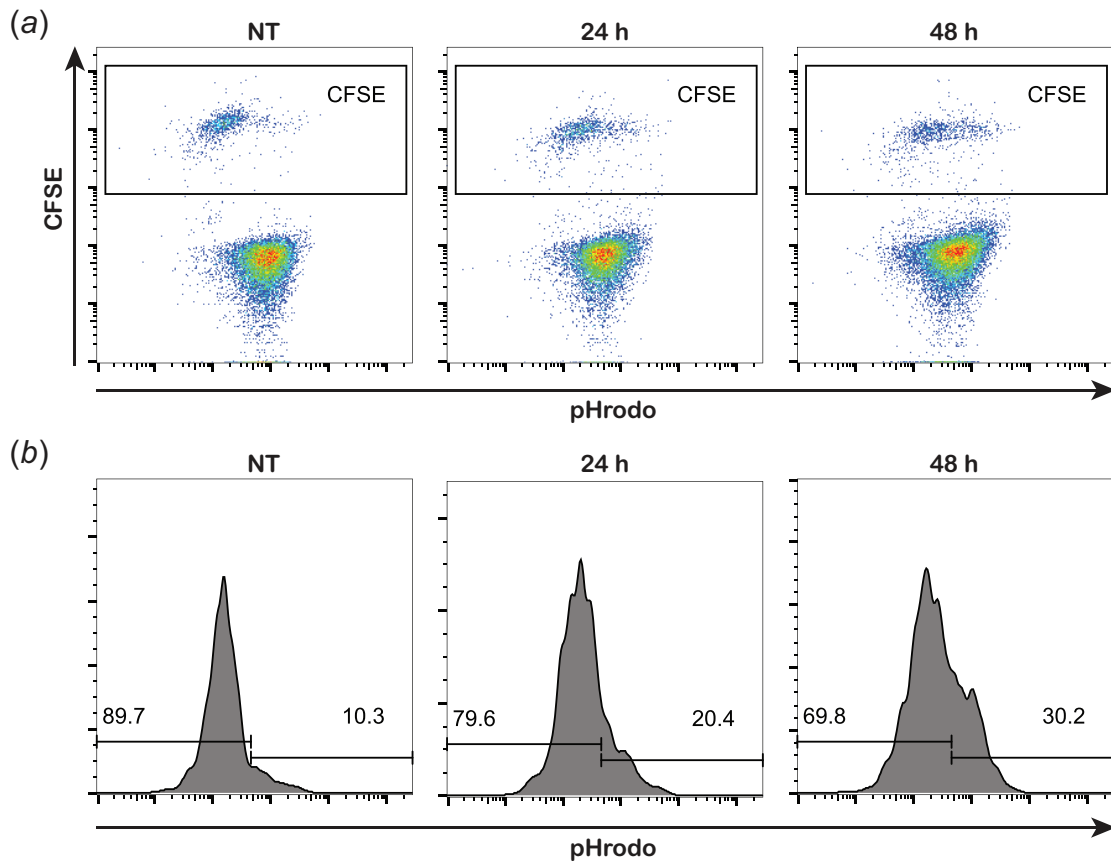
Concentration	Primary motif	E-value	Secondary motif	E-value
250 nM		2.6e-5582		4.5e-207
25 nM		7.3e-509		1.1e-028
25 nM		3.9e-129		9.1e-008

SI Table 5. Table of cell death flow cytometry after different treatments for 24 hours.

	Live	Necrotic	Apoptotic	2° Necrotic
NT	86.9 (± 1.6)	2.0 (± 0.4)	5.8 (± 1.3)	5.3 (± 0.3)
1	57.0 (± 2.2)	11.9 (± 1.1)	12.8 (± 1.2)	18.4 (± 1.2)
2	88.1 (± 1.0)	2.3 (± 0.3)	5.2 (± 0.9)	4.5 (± 0.2)
Eto	26.7 (± 0.7)	5.3 (± 0.2)	32.7 (± 1.1)	35.3 (± 1.0)

SI Table 6. Table of cell death flow cytometry after treatment with **1** over time.

	Live	Necrotic	Apoptotic	2° Necrotic
NT	95.7 (± 0.5)	1.7 (± 0.4)	1.1 (± 0.1)	1.5 (± 0.1)
12 h	47.5 (± 1.1)	19.6 (± 2.0)	14.4 (± 1.1)	18.5 (± 0.5)
24 h	59.7 (± 4.8)	15.6 (± 1.0)	11.8 (± 1.0)	12.9 (± 3.0)
48 h	79.4 (± 1.5)	7.7 (± 1.1)	6.7 (± 0.3)	6.2 (± 0.4)



SI Figure 7. Representative flow cytometry data for phagocytosis assay of Raji cells by peripheral blood macrophages after treatment with polyamide **1**. (a) Raw data showing gate of CFSE+ population, peripheral blood macrophages. (b) Histogram of pHrodo dye in gated macrophage population of (a).