Supplemental Material

Mast cells promote nitrogen mustard mediated toxicity in the lung associated with proinflammatory cytokine and bioactive lipid mediator production

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Table S1. Targeted lipidomics yields changes in both arachidonic acid and linoleic acid pathways observed in C57BL/6J mice compared to Kit$^{W\text{-}sh}$ mice after nitrogen mustard exposure.

Compound	Control C57BL/6J (pg/mL)	Treated C57BL/6J (pg/mL)	Control Kit$^{W\text{-}sh}$ (pg/mL)	Treated Kit$^{W\text{-}sh}$ (pg/mL)
12-HETE	1893.124 ± 2691.323	599.950 ± 320.817	2154.246 ± 764.643	715.911 ± 416.973
14-HDHA	271.0467 ± 87.422	171.625 ± 63.761	485.761 ± 438.781	125.981 ± 33.141
7(epi)Maresin R1	N.D	N.D	N.D	N.D
7(R)Maresin	N.D	N.D	N.D	N.D
12-HEPE	30.0936 ± 67.291	N.D	N.D	N.D
15-HETE	80.134 ± 69.879	45.143 ± 23.084	63.832 ± 46.953	51.533 ± 16.284
13-HOTrE	22.939 ± 26.087	35.635 ± 39.193	59.601 ± 81.700	60.346 ± 51.058
15-HETrE	18.479 ± 17.844	7.513 ± 11.698	9.442 ± 14.234	10.687 ± 13.501
10,17-DiHDoHE	N.D	N.D	N.D	N.D
17-HDHA	170.645 ± 81.336	172.717 ± 73.113	298.744 ± 270.854	142.936 ± 43.919
17R-RVD1	N.D	N.D	N.D	N.D
RVD1	N.D	N.D	N.D	N.D
RVD2	N.D	N.D	N.D	N.D
RVD3	N.D	N.D	N.D	N.D
15-HEPE	N.D	N.D	N.D	N.D
18-HEPE	N.D	N.D	N.D	N.D
RVD5	N.D	N.D	N.D	N.D
13-HODE	341.723 ± 421.009	382.084 ± 450.512	N.D	578.963 ± 463.379
13-OxoODE	74.653 ± 51.723	23.132 ± 36.080	N.D	N.D
5,15-DiHETE	N.D	N.D	N.D	N.D
LTB4	8.162 ± 18.250	N.D	N.D	N.D
LXA4 Isomers	N.D	N.D	N.D	N.D
LXB4	N.D	N.D	N.D	N.D
5-HEPE	N.D	N.D	N.D	N.D
5-HETE and 14(15)-EET	53.699 ± 36.270	38.091 ± 4.258	57.0341 ± 27.702	55.0773 ± 25.845
LTD4	33.110 ± 74.0359	N.D	86.161 ± 107.771	N.D
LTE4	162.504 ± 245.866	N.D	773.532 ± 803.804	N.D *
12-HHTrE	167.123 ± 209.783	N.D	N.D	N.D
(11B)PGF2a	N.D	N.D	N.D	N.D
6a-PG I1	12.260 ± 19.344	N.D	N.D	N.D
6-keto-PGF1a	229.598 ± 133.611	171.611 ± 44.616	102.382 ± 104.583	103.735 ± 90.041
Carb-TBX A2	N.D	N.D	N.D	N.D
PGD2	N.D	22.422 ± 6.685 * †	N.D	10.013 ± 8.336 * †
PGE2	49.895 ± 31.441	44.194 ± 53.429	N.D	24.815 ± 39.861

PGF2a Isomers	60.328 ± 51.353	46.883 ± 11.492	60.112 ± 33.0140	36.991 ± 10.241
TXB2	14.584 ± 32.611	N.D	N.D	N.D
11(12)-EET	N.D	N.D	N.D	N.D
8(9)-EET	N.D	N.D	N.D	N.D
17(18)-EpETE	N.D	N.D	N.D	N.D
12(13)-EpOME	531.140 ± 485.892	1433.888 ± 719.723	378.579 ± 217.317	1355.0243 ± 636.334
9(10)-EpOME	235.0409 ± 124.974	426.844 ± 217.916	140.911 ± 38.503	390.312 ± 181.540
RVE1	N.D	N.D	N.D	N.D
11,12-DiHETrE	13.122 ± 21.604	56.574 ± 9.826 *	N.D	36.620 ± 30.977
14,15-DiHETrE	13.480 ± 22.0881	36.913 ± 40.692	N.D	34.159 ± 38.557
5,6-DiHETrE	N.D	N.D	N.D	N.D
19,20-DiHDPA	N.D	N.D	N.D	N.D
17,18-DiHETE	N.D	28.653 ± 22.531	N.D	24.635 ± 31.607
12(13)-DiHOME	262.509 ± 208.400	1111.651 ± 619.806 *	238.826 ± 277.326	959.842 ± 481.733
9(10)-DiHOME	471.313 ± 367.974	1449.590 ± 693.227 *	455.0292 ± 350.0160	1160.823 ± 520.889
9-HODE	353.535 ± 323.284	366.612 ± 176.895	173.0497 ± 25.933	478.724 ± 253.292
9-OxoODE	83.154 ± 17.111	106.182 ± 27.913	11.638 ± 23.276	105.502 ± 67.872 *
11-HETE	N.D	N.D	N.D	N.D
8-HETE	15.343 ± 6.363	17.0941 ± 7.583	27.300 ± 16.319	24.298 ± 6.286
8-iso-15R-PGF2a	N.D	N.D	N.D	N.D
8-iso-PGF2a	N.D	N.D	N.D	N.D
11-HDoHE	270.310 ± 107.723	166.0250 ± 71.165	437.722 ± 381.798	136.714 ± 90.838

To further assess lipid mediators in both strains we analyzed the BALF utilizing mass spectrometry. There were increases in NM treated C57BL/6J mice compared to its control in the following compounds that were not seen in Kit$^{W\text{-}sh}$ mice: 11,12-DiHETrE, 12(13)-DiHOME, and 9(10)-DiHOME. 9-OxoODE was significantly increased NM treated Kit$^{W\text{-}sh}$ mice compared to control while it was not in C57BL/6J mice. Data is presented as the mean ± SM (n=4-6 animals/ group); one way ANOVA with Tukey's *post hoc* test, *$p \leq 0.05$ compared to its respective control, † $p \leq 0.05$ compared between treated strains.

Supplemental Figures

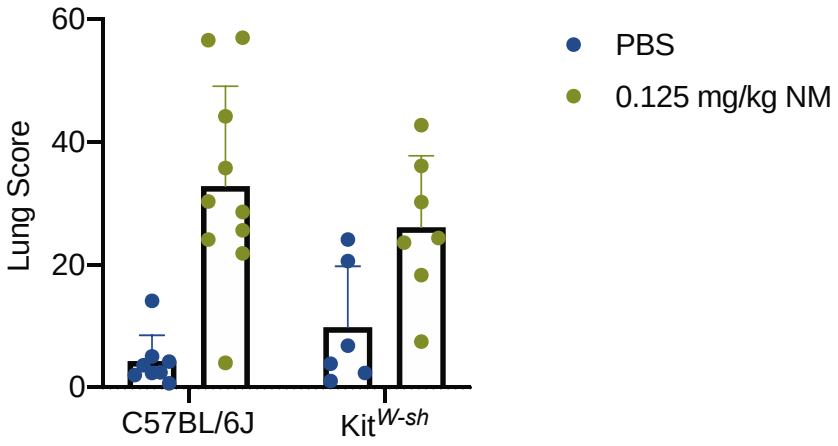

Figure S1. Damage quantified through lung scoring for NM exposed C57BL/6J and Kit$^{W\text{-}sh}$ mice.

To further confirm damage, lungs were scored to determine the degree of injury that C57BL/6J exhibited compared to Kit$^{W\text{-}sh}$ mice. C57BL/6J mice trended toward a higher injury score compared to Kit$^{W\text{-}sh}$ mice. Data is presented as the mean ± SD (n=5-9 animals/ group); Two - way ANOVA with Tukey's *post hoc* test, *$p \leq 0.05$.

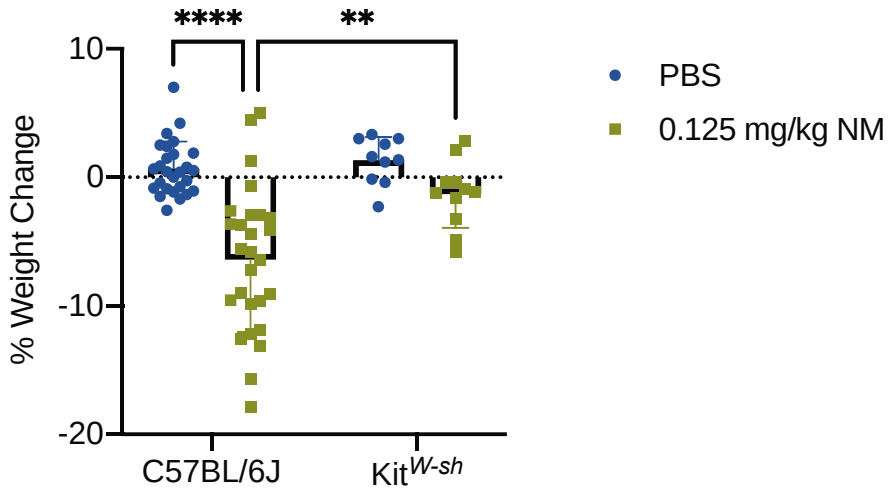

Figure S2. Nitrogen mustard exposure caused major weight changes in C57BL/6J compared to Kit$^{W\text{-}sh}$ mice.

Mice exhibited major weight differences after being dosed with NM for 72 hrs. Mice were weighed prior to dosing and weighed right after sacrificing. Treated C57BL/6J mice lost significantly more weight than Kit$^{W\text{-}sh}$ mice. Data is presented as the mean ± SEM (n=11-27 animals/ group); two way ANOVA with Tukey's *post hoc* test, *$p \leq 0.05$ compared to respective controls, † $p \leq 0.05$ compared between treated strains.

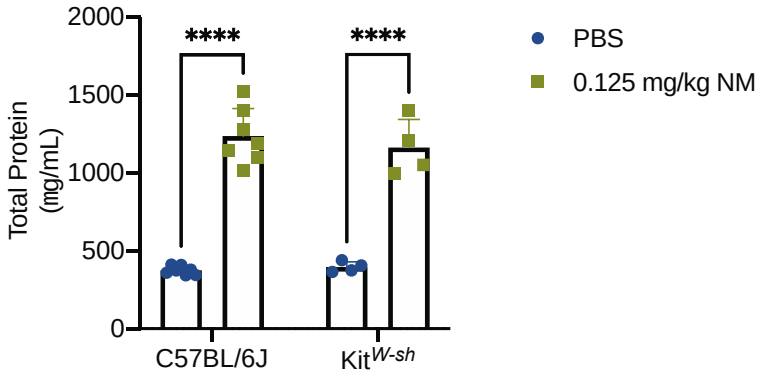

Figure S3. Nitrogen mustard causes damage in both C57BL/6J and Kit $^{W\text{-}sh}$ mice measured by protein presence in the bronchoalveolar lavage fluid.

To measure damage, we looked at total protein in the BALF of both C57BL/6J and Kit$^{W\text{-}sh}$ mice after 72 h NM exposure. Both C57BL/6J and Kit$^{W\text{-}sh}$ strains exhibited cellular damage compared to its respective control. Data is presented as the mean ± SD (n=4-8 animals/ group); Two - way ANOVA with Tukey's *post hoc* test, *$p \leq 0.05$, ** $p \leq 0.01$, *** $p \leq 0.001$, **** $p \leq 0.0001$.

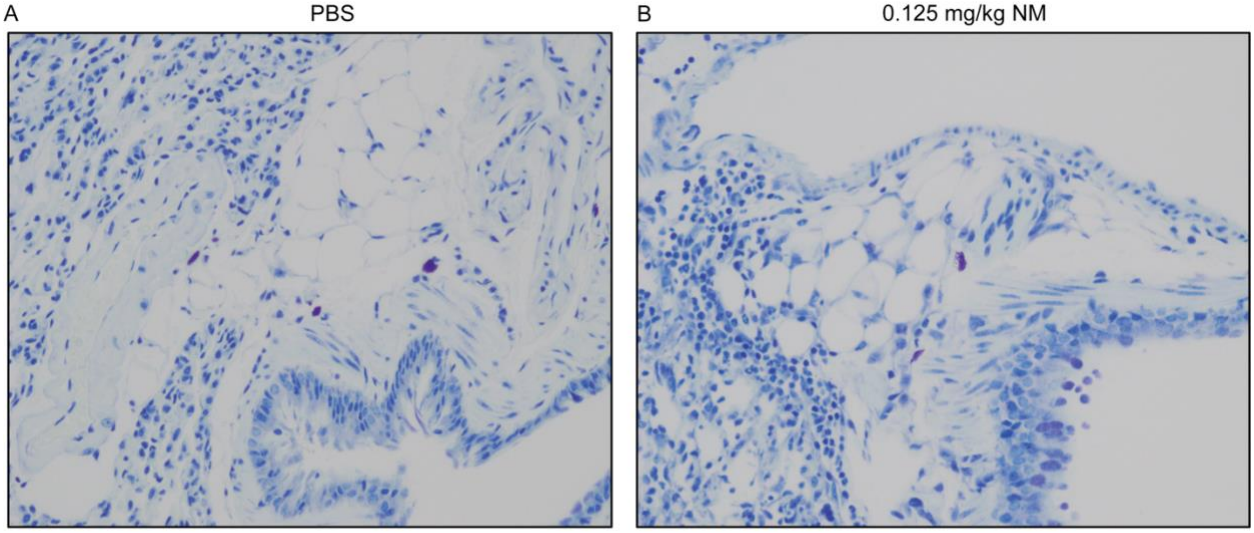

Figure S4. Nitrogen mustard exposure causes mast cell degranulation in vivo.

Representative histological findings in mice treated via oropharyngeal aspiration with PBS (control) or nitrogen mustard (0.125 mg/kg) for 72 hrs (N = 5-9 mice/group). The left lobe of the lung was removed and stained with toluidine blue. Black arrows are pointing to the mast cells present in the lung. Images were obtained using an Olympus light microscope at the 20x objective (indicated by 50 μm scale).

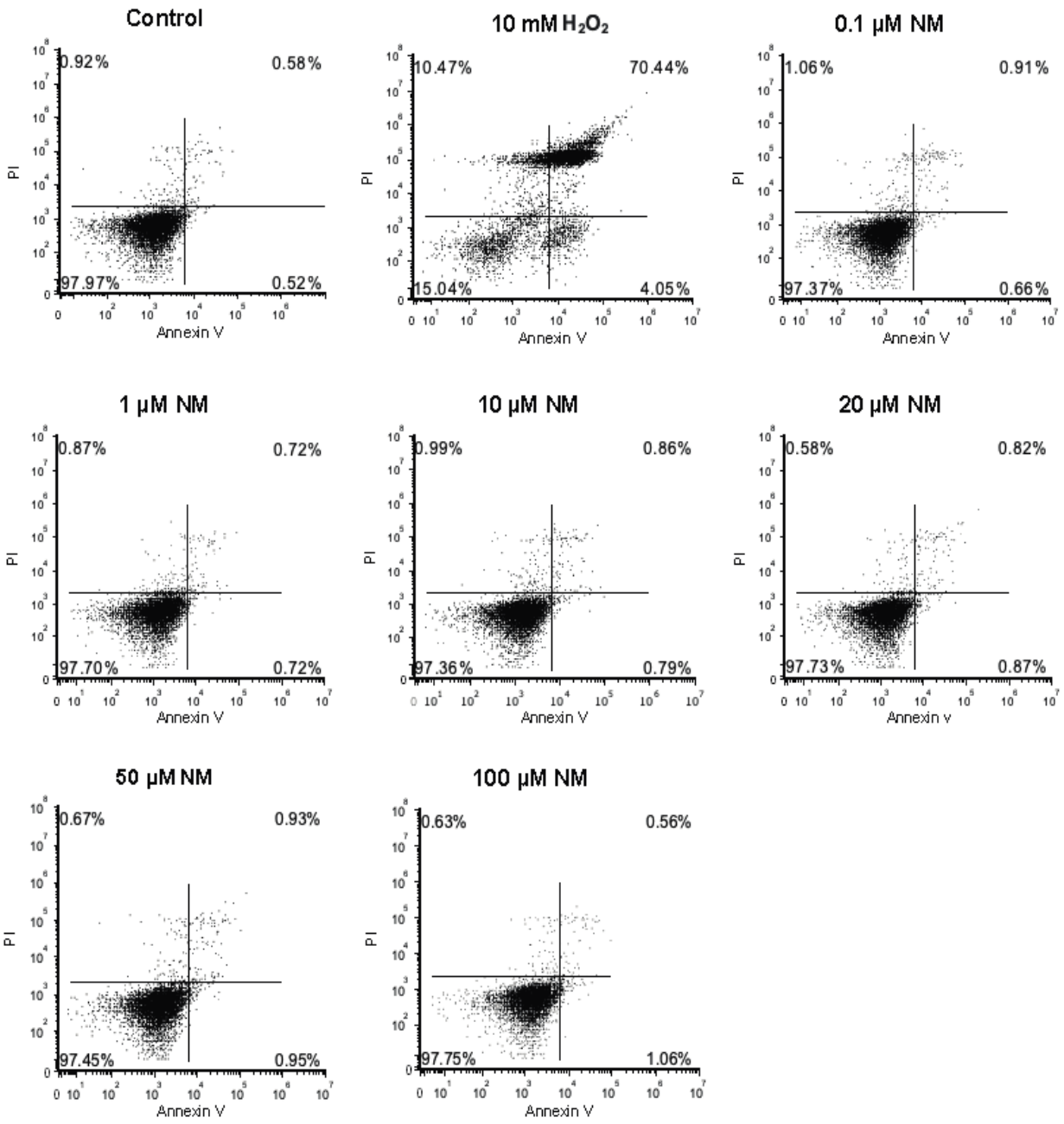

Figure S5. Nitrogen mustard exposure for 1 h does not cause cell death in BMMCs.

Cell death was measured by staining BMMCs with propidium iodide (PI) which measures necrosis and Annexin V which measures apoptosis after NM exposure. BMMCs were exposed to nitrogen mustard (concentrations 0.1 µM - 100 µM) for 1 h. The cells were washed, stained and processed for flow cytometer. Plots are a representation of the biological replicates ($n \geq 3$).

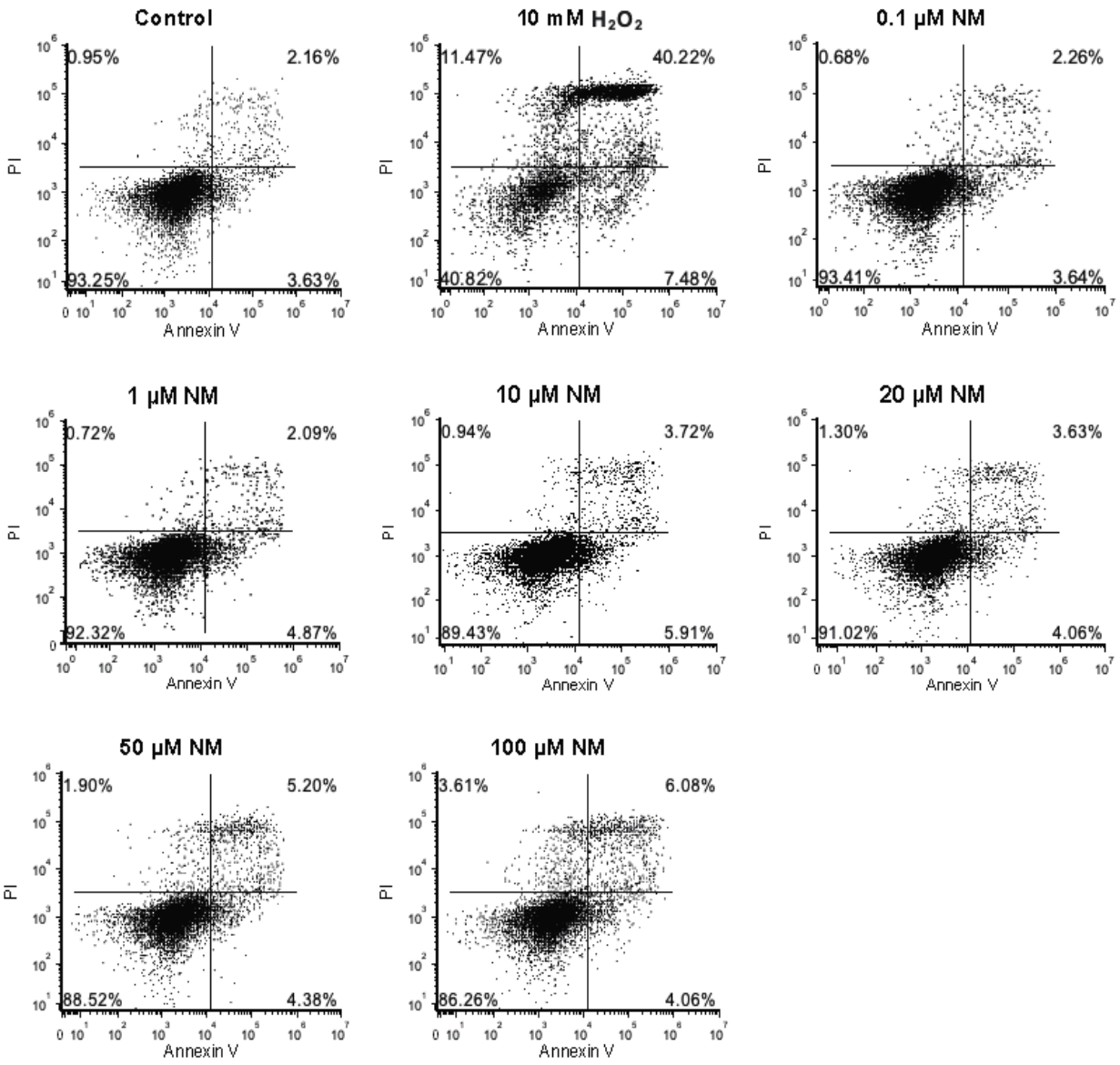

Figure S6. Nitrogen mustard exposure for 6h does not cause cell death in BMMCs

Cell death was measured by staining BMMCs with propidium iodide (PI) which measures necrosis and Annexin V which measures apoptosis after NM exposure. BMMCs were exposed to nitrogen mustard (concentrations 0.1 µM - 100 µM) for 6 h. The cells were washed, stained and processed for flow cytometer. Plots are a representation of the biological replicates (n ≥ 3).

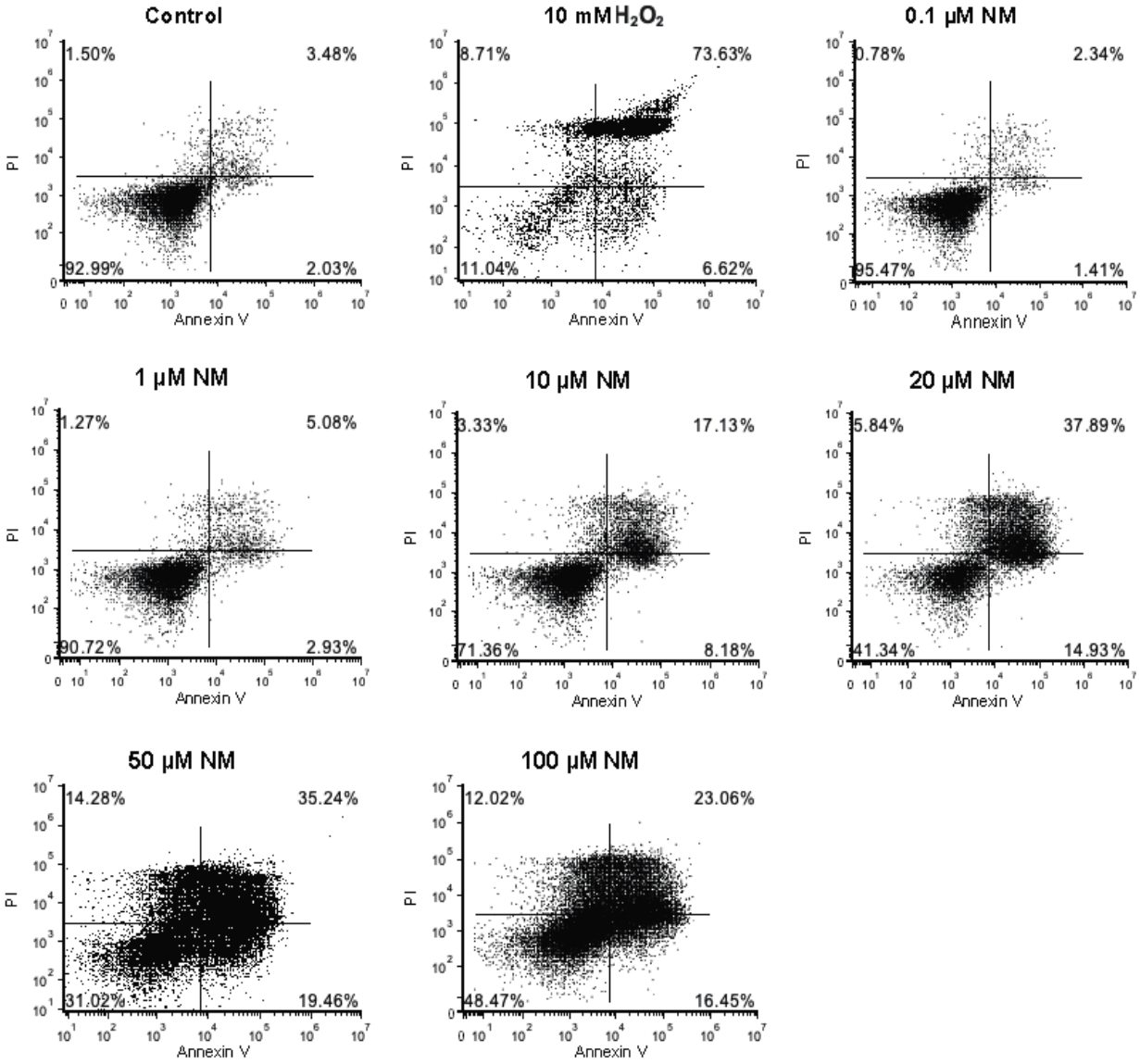

Figure S7. Nitrogen mustard exposure for 24h caused cell death in BMMCs.

Cell death was measured by staining BMMCs with propidium iodide (PI) which measures necrosis and Annexin V which measures apoptosis after NM exposure. BMMCs were exposed to nitrogen mustard (concentrations 0.1 μM - 100 μM) for 24 h. The cells were washed, stained and processed for flow cytometer. Plots are a representation of the replicates (n ≥ 3).

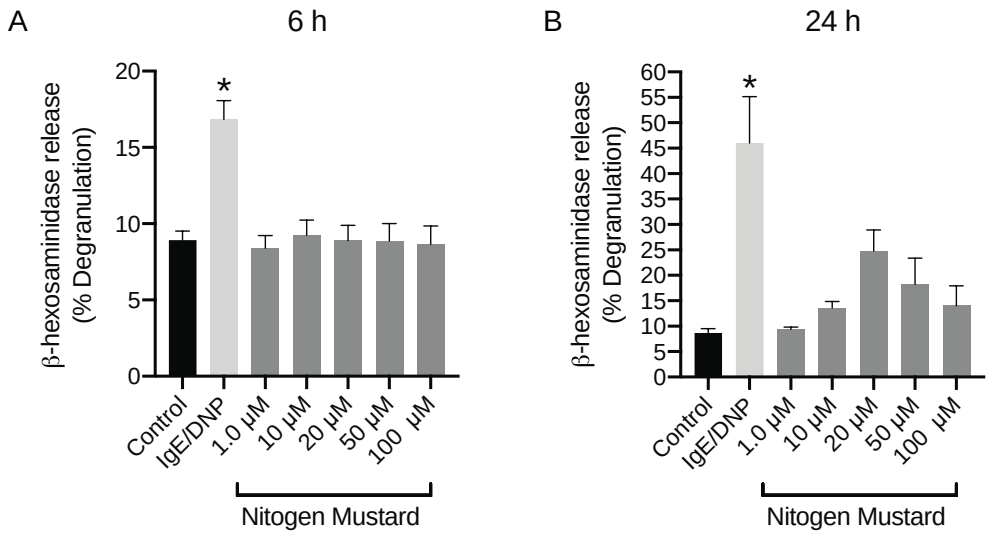

Figure S8. Nitrogen mustard does not cause degranulation at 6 h but degranulation is observed at 24 h at toxic doses *in vitro*.

Degranulation was assessed through the release of β-hexosaminidase (lysosomal enzyme) which was measured in supernatants relative to lysed cells in BMMCs. (A) At 6 h NM exposure, β-hexosaminidase exhibited no differences compared to control. (B) At 24 h there was an increasing trend in degranulation at 20 μM – 50 μM . Data is presented as the mean ± SEM (n=3); one way ANOVA with Dunnett's *post hoc* test, $*p \leq 0.05$ treatments compared to control.