SUPPLEMENTAL MATERIAL

Joyce-Shaikh et al., http://www.jem.org/cgi/content/full/jem.20090516/DC1

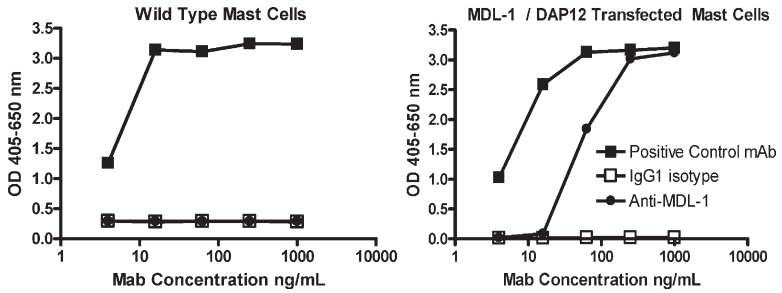

Figure S1. A mast cell degranulation assay was performed to demonstrate the agonistic activity of anti–MDL-1 mAb. DT830 is a mast cell line in which MDL-1 is transduced. These cells were stimulated with anti–MDL-1 (clone DX163) or isotype control antibodies. Anti–MDL-1, but not isotype control, induced mast cell degranulation in MDL-1 transfectants. Clone DX89 (anti-CD200R), a potent mast cell degranulating antibody, was utilized as a positive control.

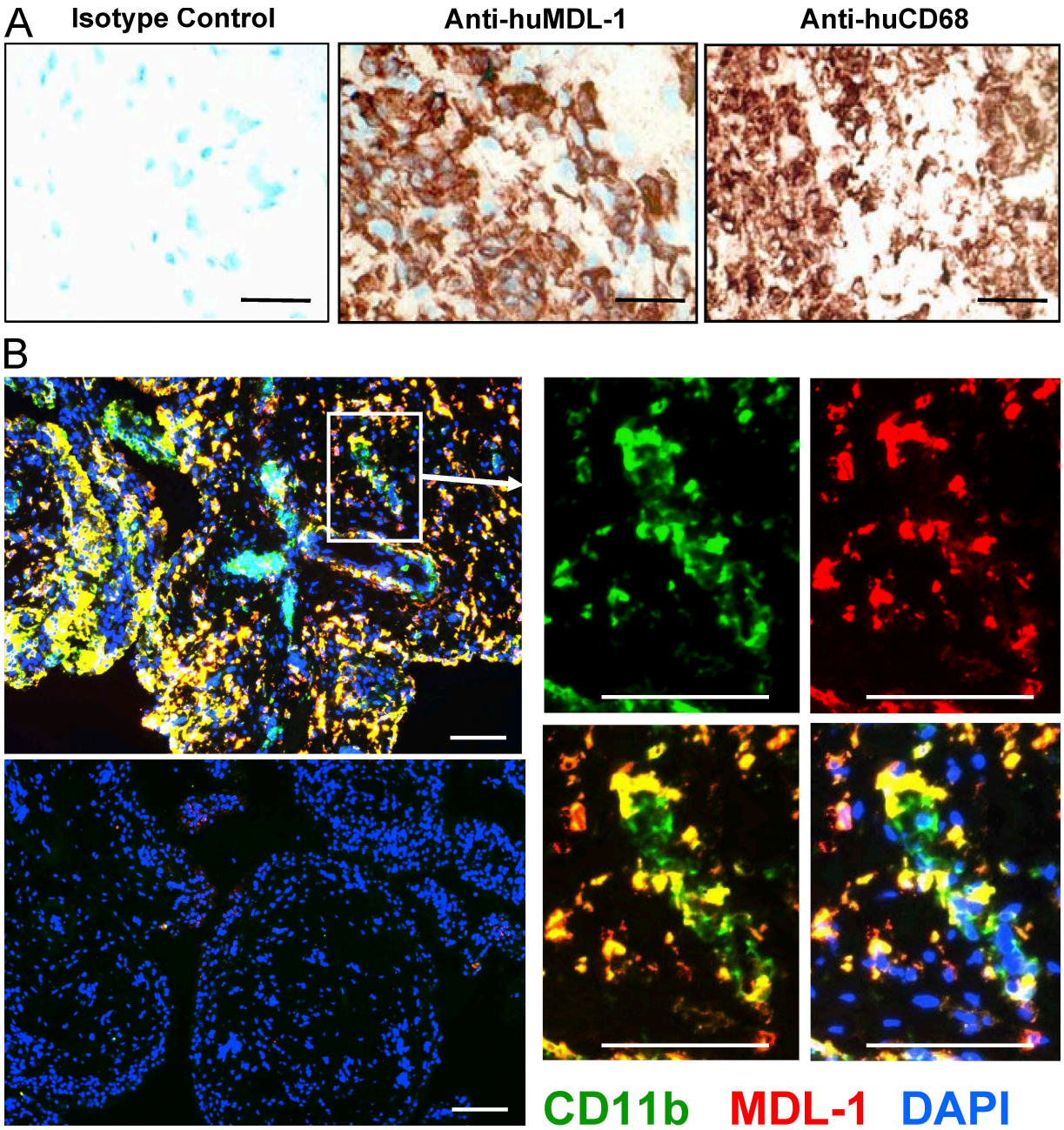

Figure S2. CD68⁺ CD11b⁺ macrophages in human RA joints express MDL-1. (A) MDL-1 expression is elevated in human arthritis tissues. Serial sections from RA synovial tissues were stained by IHC using anti–hMDL-1 (clone DX246) or anti-CD68. Staining patterns revealed extensive MDL-1 expression on a subset of CD68 positive macrophages in the area of pannus formation and tissue damage. Results are representative of four patient samples. Bar, 33 µm. (B; top left) two-color fluorescent immunostaining of human RA inflamed synovium demonstrate colocalization of MDL-1(DX246) to a major subset of CD11b⁺ cells. (bottom left) DAPI nuclei stain (blue) plus FITC and PE-conjugated isotype controls demonstrates virtually no background staining. (right) Higher magnification of area boxed in white rectangle. Note that most, but not all, of the CD11b⁺ cells (green) co-express MDL-1 (red) as depicted in the merged immunofluorescent image (yellow). Results are representative of four patient samples. Bar, 400 µm.

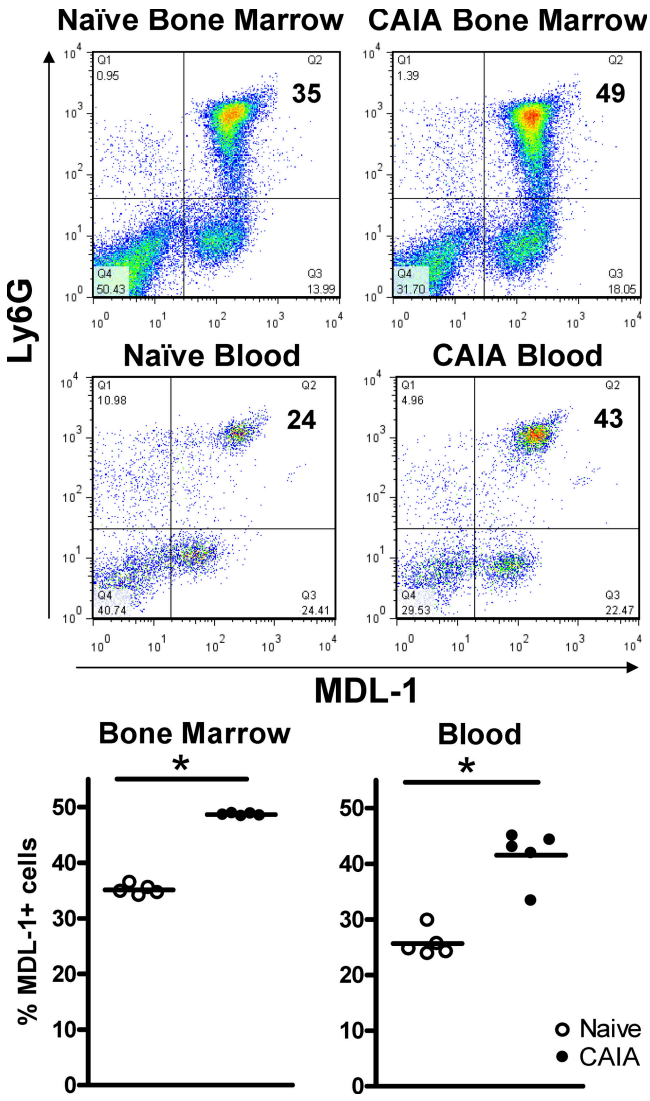

Figure S3. Proportion of MDL–1⁺ cells increased during CAIA in bone marrow and peripheral blood. Cells were isolated 7 d after induction of CAIA for flow cytometric analysis. CD11b⁺/ Ly6G⁺ bone marrow and blood leukocytes were compared for MDL-1 expression.

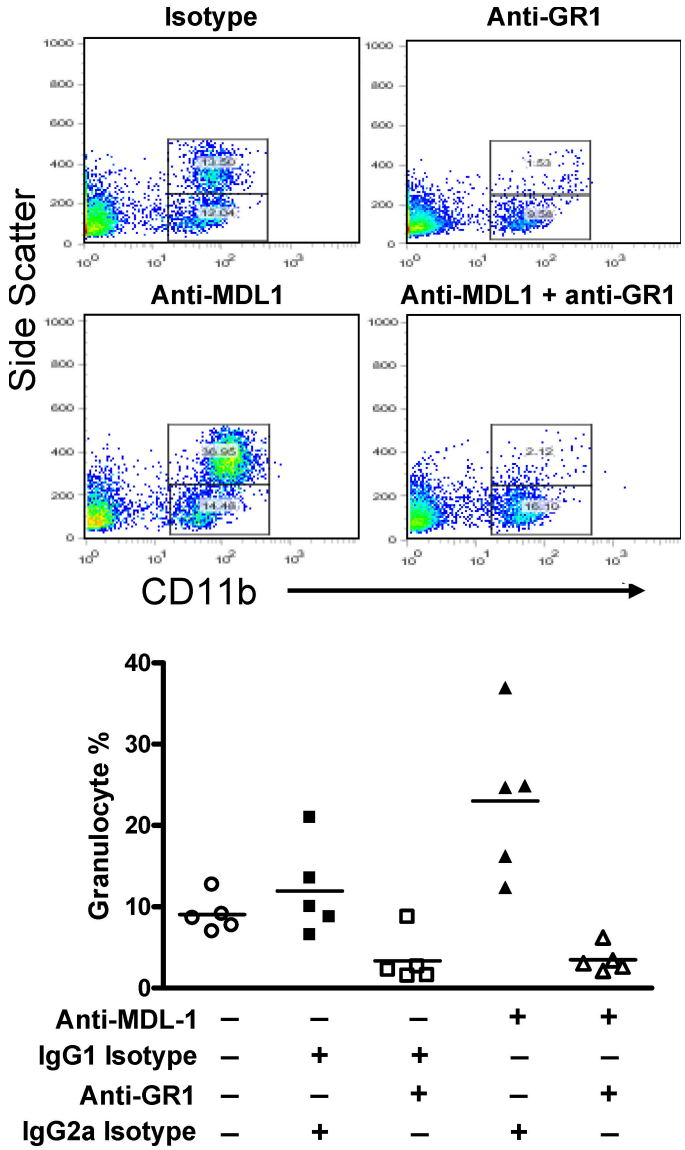

Figure S4. Anti-GR1 depletion was initiated at day −1 in B10.RIII mice and CAIA was induced on day 0. Blood analysis was performed on day six after disease induction. After red cell lysis, cells were stained with CD11b and analyzed by flow cytometry. Mice that received anti-GR1 antibody showed depletion of $CD11b^{+}$ side $scatter^{Hi}$ granulocyte population.

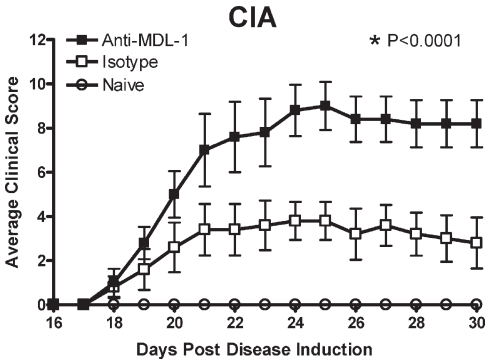

Figure S5. MDL-1 activation enhanced T cell– and myeloid cell–dependent arthritis. B10RIII mice were immunized with bovine type II collagen emulsified in CFA at day 0 to induce collagen-induced arthritis. Anti–MDL-1 agonist (clone DX163) was given on day 18 of immunization. Statistical significance was determined by ANOVA analysis. Results are representative of two studies.

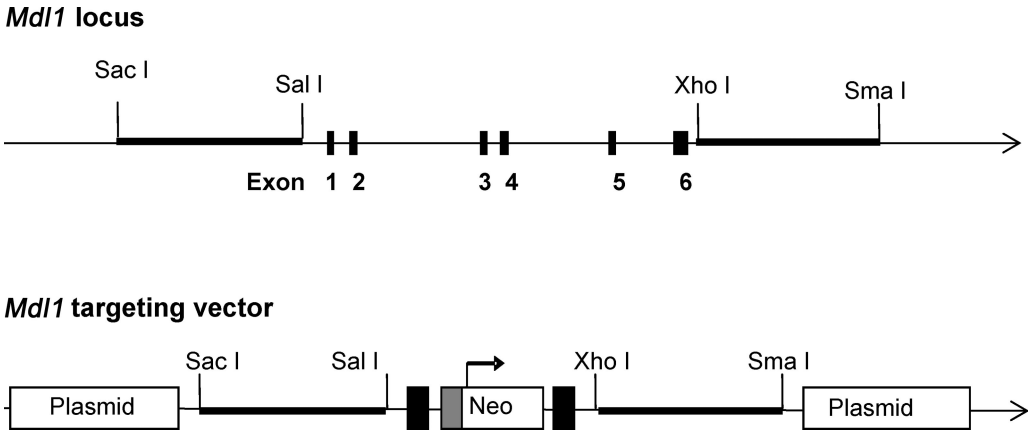

Figure S6. *Mdl1*$^{-/-}$ mice were generated by homologous recombination using the above targeting vector in C57BL/6 ES cells.

		Naïve WT	WT AIA	Naïve DAP12	DAP12 AIA	Naïve MDL-1	MDL-1 AIA
1	MCP-1	28	336	15	53	12.8	40
2	MMP9	128	762	207	221	171	249
3	LIX	12	34	11	6	8	3
4	IL-6	5	136	8	30	10	7
5	IL1b	26	578	55	131	55	47
6	RANKL	6	88	8	25	6	15
7	TIMP-1	1257	16925	1184	4386	1054	2058
8	DAP12	1040	3563	0.8	0.3	1503	1547
9	MDL-1	56	118	68	79	0.9	0.2
10	TRAP	471	3025	712	1188	610	1291
11	OPN	5617	13925	4911	6784	3942	5725
12	c-fos	15	603	27	139	47	265
13	CD14	65	207	26	125	23	89
14	CD11b	39	329	43	186	55	117
15	RANK	10	118	24	52	8	45
16	CTSK	86	1198	112	384	137	463
17	c-fms	46	109	47	43	41	43
18	calcitonin R	0.6	3	0.2	0.2	0.2	0.2
19	NFATc1	40	332	34	129	46	168
20	TNF	9	84	18	10	8	6

Figure S7. Hind paws from naïve and arthritic mice were prepared for gene expression analysis. Ubiquitin levels were used to normalize the data by the $\Delta - \Delta$ Ct method, using the mean cycle threshold (Ct) value and the genes of interest for each sample (Ct ubiquitin − Ct gene of interest) × 10^4 was used to obtain the normalized values. This result corresponds to the heat map shown in Fig. 3B.

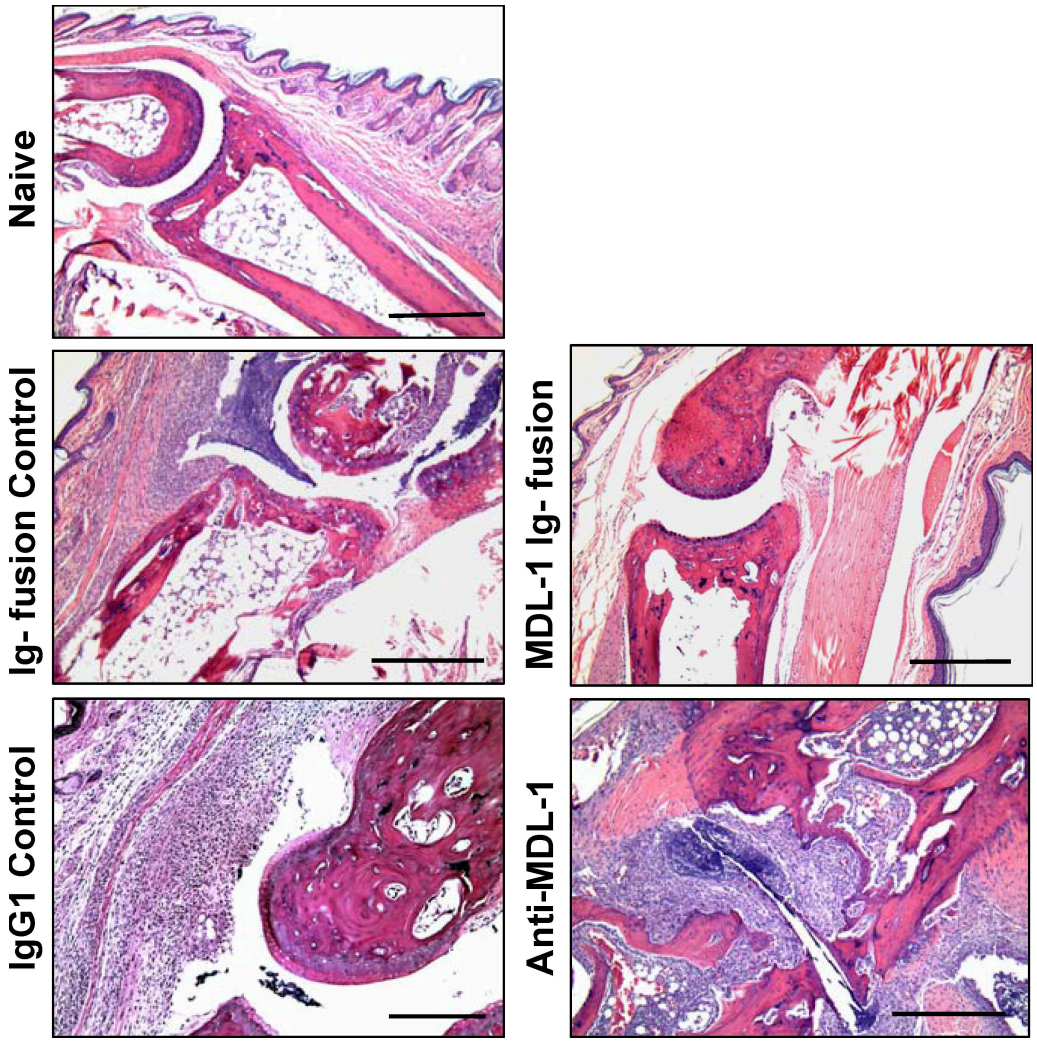

Figure S8. Representative H&E stained micrographs of metatarsal-phalange joints from the study shown in Fig. 3D. Tissue samples were taken at day 11 of the experiment. MDL-1–Ig fusion protein treatment group showed less damage as compared to controls. Bar, 400 µm.