

Supplemental Data

Cryopreserved Interleukin-4-Treated Macrophages Attenuate Murine Colitis in an Integrin β 7-Dependent Manner

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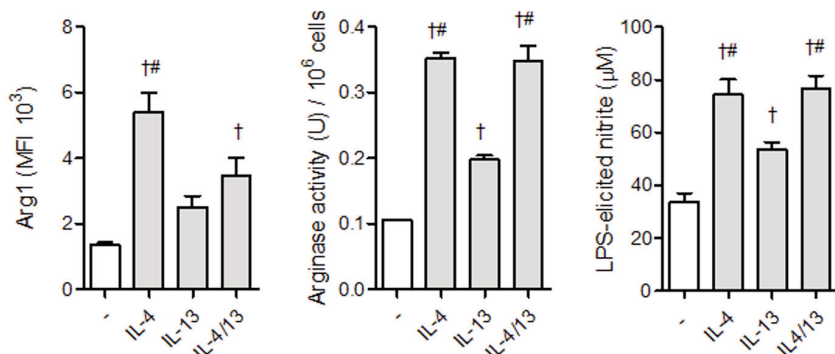
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Supplementary Table S1. Alternative activation of murine and human M(IL-4) vs. M(IL-4+IL-13) by real time PCR.

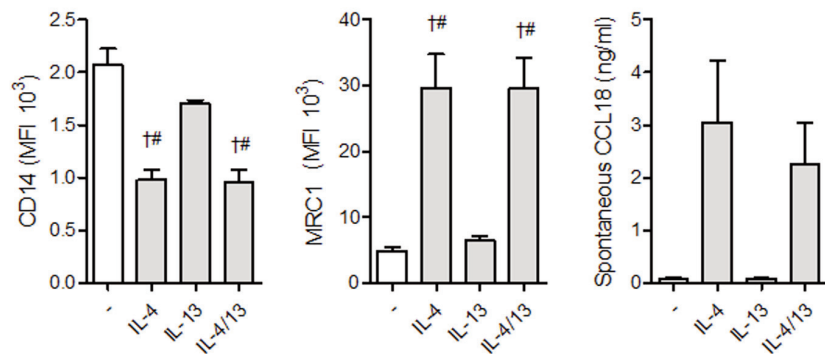
Species	Gene	Fold change over M(-)		
		M(IL-4)	M(IL-13)	M(IL-4+IL-13)
Mouse	<i>Arg1</i>	72328 ± 19415	13959 ± 4627	279295 ± 118883 ^{†#}
	<i>Nos2</i>	8.18 ± 1.80	4.62 ± 2.00	11.63 ± 3.99 [†]
	<i>Chi3l3</i> (Ym1)	7256 ± 4311	2699 ± 1402	18990 ± 6944 ^{†#}
Human	<i>CD14</i>	0.148 ± 0.0347 ^{†#}	0.598 ± 0.191 [†]	0.0899 ± 0.0273 ^{†#}
	<i>MRC1</i>	3.98 ± 1.70	2.98 ± 1.05	8.43 ± 4.21
	<i>CCL18</i>	19.23 ± 9.99	0.534 ± 0.217	5.70 ± 3.93

Mouse bone marrow-derived M(IL-4)s and M(IL-4+IL-13)s were compared in their ability to up-regulate AAM markers *Arg1*, *Chi3l3*, and *Nos2* (a marker of M(IFN γ)) as a negative control. Likewise, monocyte-derived macrophages from healthy blood donors were evaluated for their ability to downregulate *CD14* in response to IL-4, and upregulate *MRC1* and *CCL18* expression. Mouse (n=7), human (n=6). M(-), undifferentiated macrophage. $p < 0.05$: †, compared to M(-); #, compared to M(IL-13).

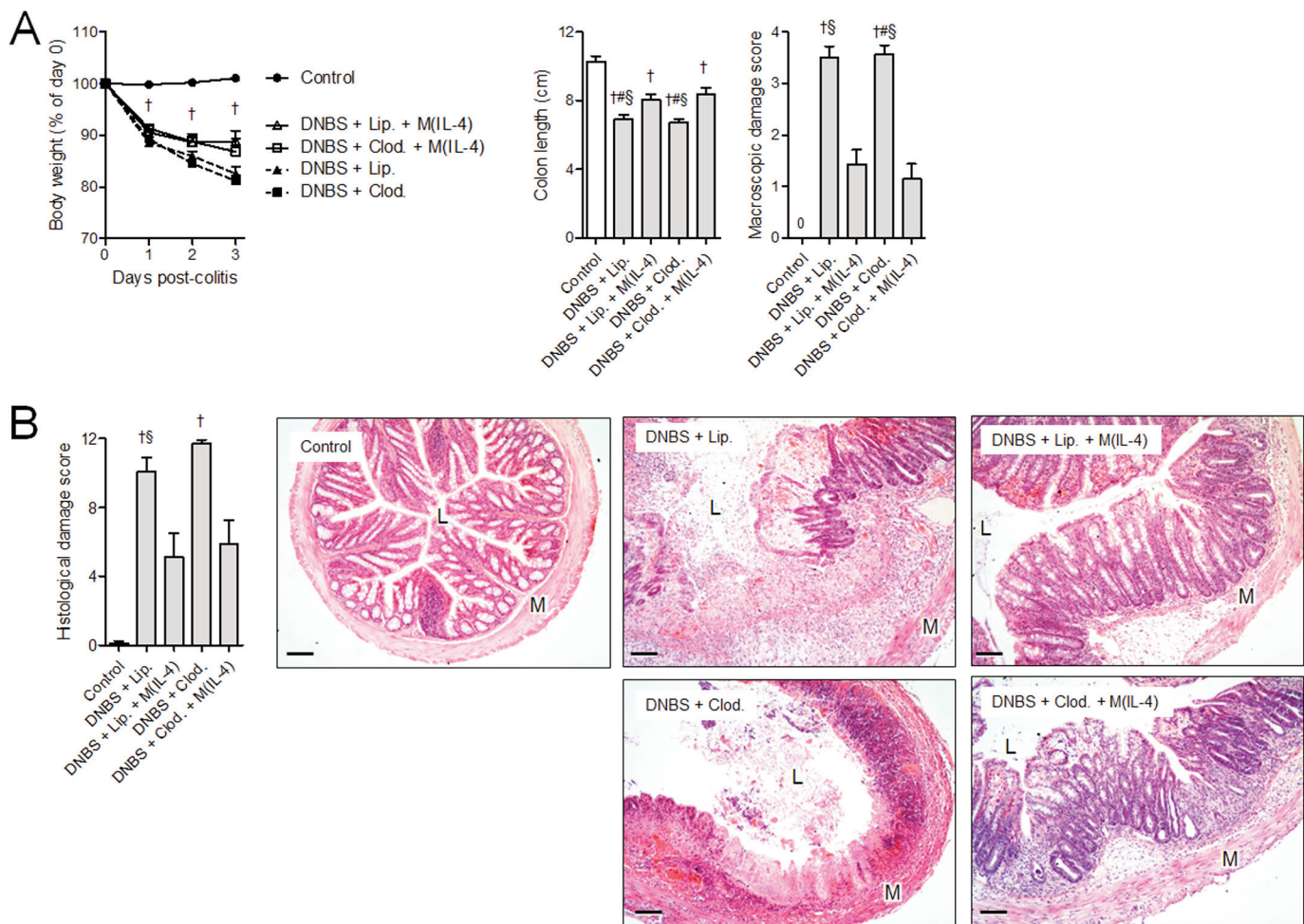
A Mouse



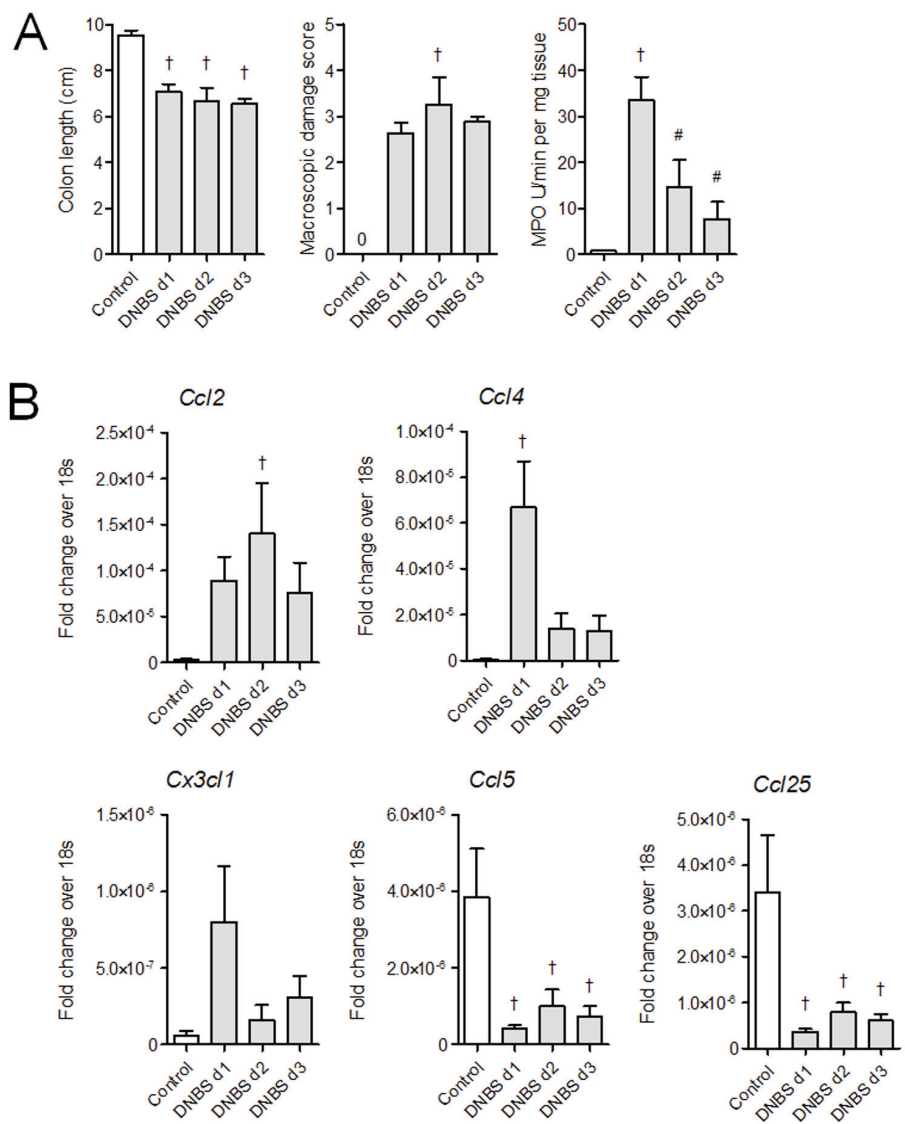
B Human



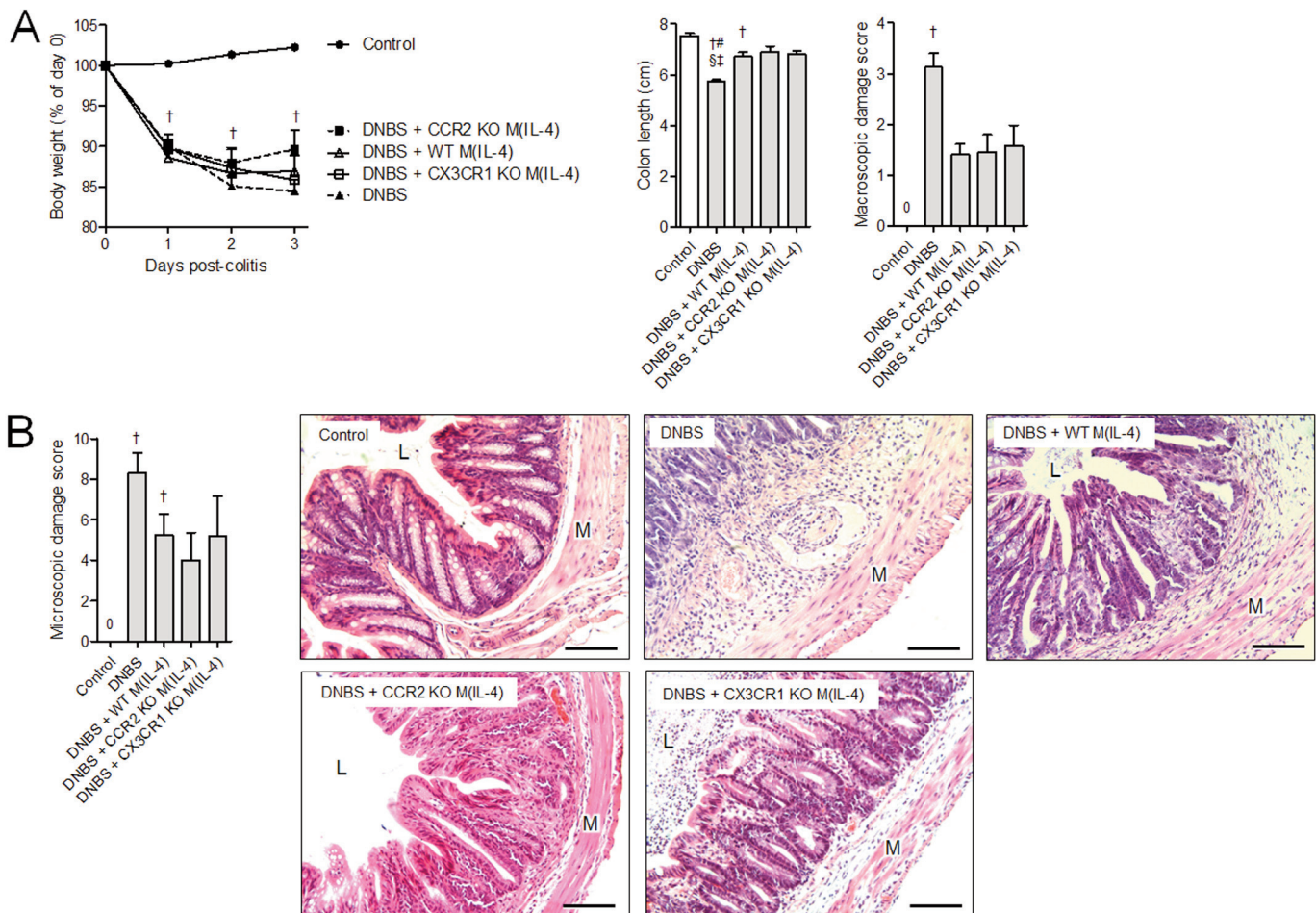
Supplementary Figure S1. Addition of IL-13 with IL-4 does not enhance the alternatively activated phenotype of murine and human macrophages. (A) Bone marrow-derived mouse macrophages from naive control BALB/c mice were compared in their capacity to upregulate arginase 1 (Arg1) by flow cytometry, arginase activity, and nitrite levels following LPS stimulation as a measure of nitric oxide synthase activity (all n=3). (B) Human monocyte-derived M(IL-4)s and M(IL-4+IL-13)s from healthy control donors were similarly evaluated by their ability to downregulate CD14 and upregulate MRC1 as measured by flow cytometry (n=3), and spontaneously secrete CCL18 as measured by ELISA (n=4). MFI, mean fluorescence intensity, *p*<0.05: †, compared to M(-); #, compared to M(IL-13). Data are represented as the mean ± SEM.



Supplementary Figure S2. Endogenous peritoneal macrophages are not required for protection against DNBS colitis by M(IL-4) transfer. Mice were pre-treated with clodronate-loaded liposomes (Clod.) i.p. to ablate macrophages, or liposomes alone (Lip.) as a control. Clodronate was administered 3 and 5 d before the administration of DNBS. Depletion of macrophages by clodronate i.p. did not affect the ability of M(IL-4)s to protect against DNBS colitis. (A) Colitis was assessed by body weight loss, colon shortening, macroscopic damage, and (B) microscopic damage. Experiments were repeated twice: naive control (n=4), DNBS + Lip. (n=7), DNBS + Lip. + M(IL-4) (n=8), DNBS + Clod. (n=8), DNBS + Clod. + M(IL-4) (n=10). $p < 0.05$: †, compared to naive control; #, compared to DNBS + Lip. + M(IL-4); §, compared to DNBS + Clod. + M(IL-4). Bar represents 50 μm ; M, muscularis; L, lumen. Data are represented as the mean \pm SEM.



Supplementary Figure S3. Colonic damage by DNBS is significant from 24 h post-administration and is associated with an increase in chemokine expression. Colonic damage due to the introduction of DNBS i.r. was significant as measured by (A) colon shortening, macroscopic damage, and MPO activity, the latter of which was most significant on d 1 post-colitis. (B) This inflammation was associated with a specific increase in mRNA expression in the colon of *Ccl2* and *Ccl4*, while expression of *Ccl5* and *Ccl25* were significantly decreased compared to colons treated with PBS only. All groups n=7, experiments were repeated twice. $p < 0.05$ by Dunnett's post-test: †, compared to naive control; #, compared to d 1 DNBS. Data are represented as the mean \pm SEM.



Supplementary Figure S4. Lack of CCR2 or CX3CR1 on transferred macrophages does not affect their ability to block colitis. Wild-type, CCR2 KO, and CX3CR1 KO M(IL-4)s were administered to mice 48 h prior to the induction of DNBS colitis i.r. (A) In these experiments, all mice treated with DNBS lost a significant amount of body weight, however all WT and KO M(IL-4)s were able to attenuate the severity of colitis as measured by increased colon length, reduced macroscopic damage, and (B) microscopic damage. Experiments were repeated 1-2 times: naive control (n=6), DNBS (n=8), DNBS + WT M(IL-4) (n=10), DNBS + CCR2 KO M(IL-4) (n=11), DNBS + CX3CR1 M(IL-4) (n=6). $p < 0.05$: †, compared to naive control; #, compared to DNBS + WT M(IL-4); §, compared to DNBS + CCR2 KO M(IL-4); ‡, compared to DNBS + CX3CR1 M(IL-4). Bar represents 50 μm; M, muscularis; L, lumen. Data are represented as the mean ± SEM.