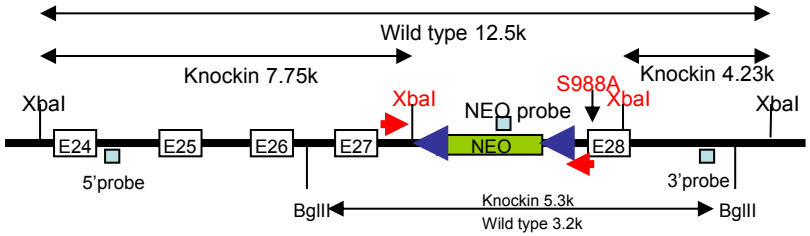
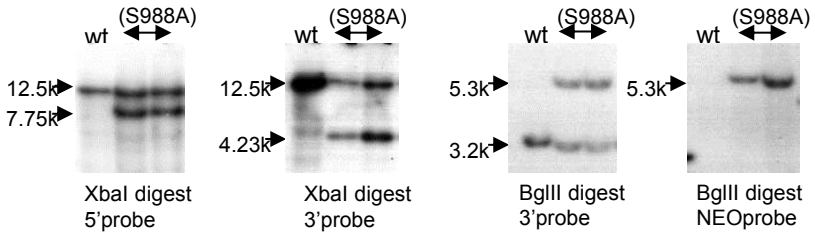
Knock-in construct



Southern blot

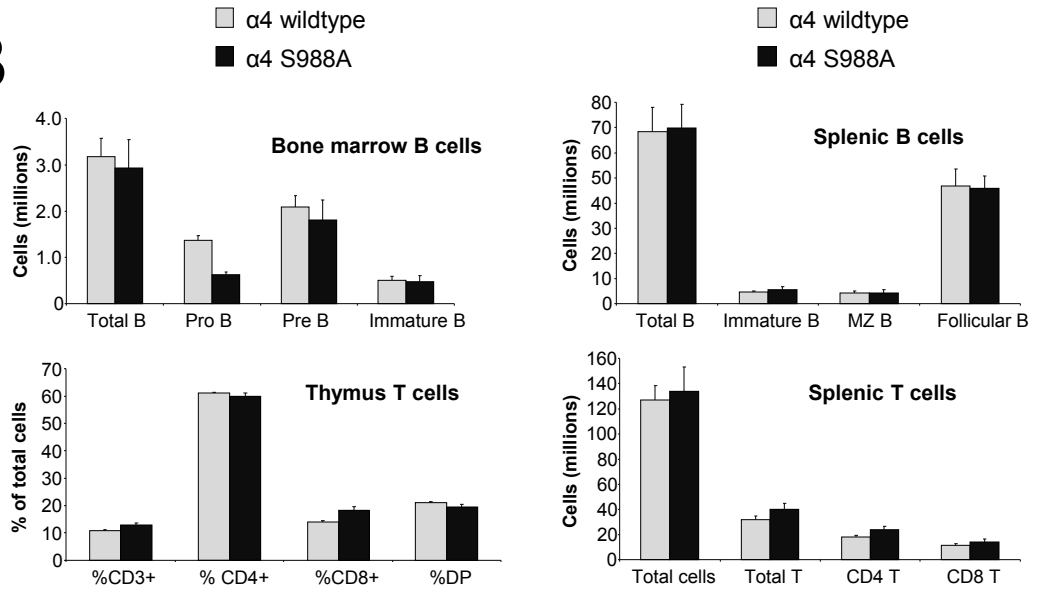


S1

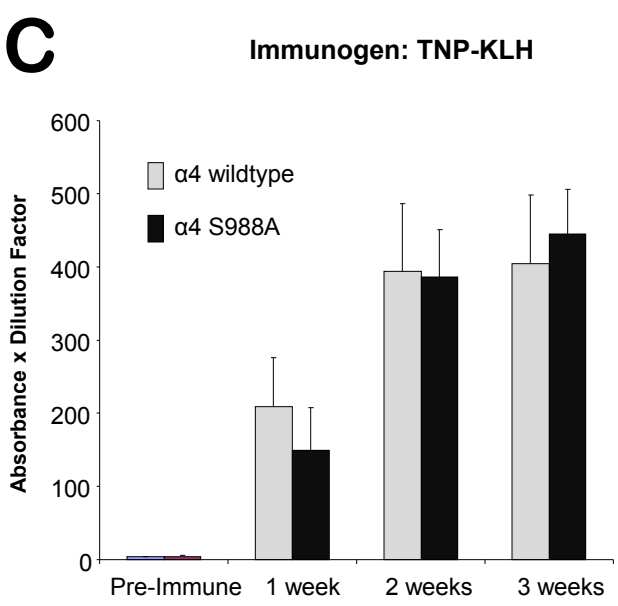
A

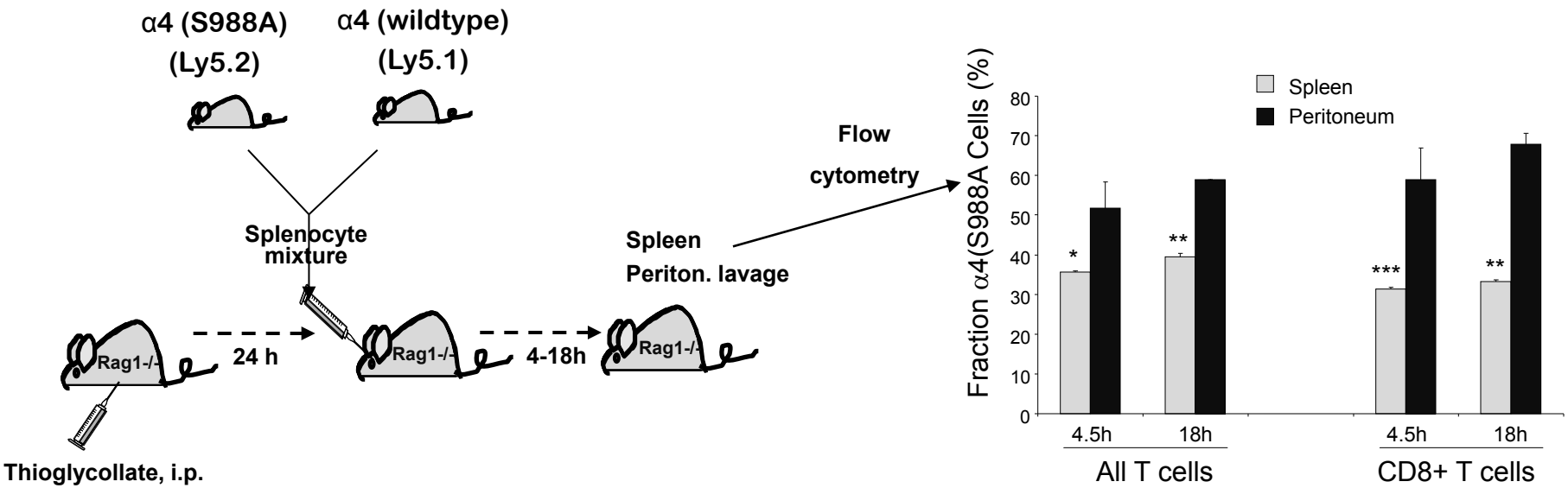
	WBC (10^3/μL)	Neut (%)	Lymphs (%)	Monos (%)	Eos (%)	Baso (%)	Platelet (10^3/μL)	RBC (10^6/μL)	HgB (g/dL)	HCT (%)	MCV (fL)	MCH (pg)	MCHC (g/dL)
α4(S988A)	**10.3**	**29**	**68**	**3.6**	**0.1**	**0.04**	**1021**	**10**	**17**	**44**	**43**	**17**	**39**
Std. Dev.	0.25	7.3	7.3	0.29	0.11	0.04	25	0.17	0.49	0.51	0.66	0.38	0.8
α4 (wt)	**10.1**	**20**	**75**	**4.3**	**0.1**	**0.02**	**982**	**9.5**	**17**	**44**	**46**	**18**	**39**
Std. Dev.	0.17	4.2	4.3	0.73	0.06	0.03	78	0.16	0.82	0.86	0.53	0.59	1.4
p-value	0.28	0.095	0.12	0.11	0.92	0.56	0.37	0.001	0.49	0.48	0.001	0.04	0.60

B

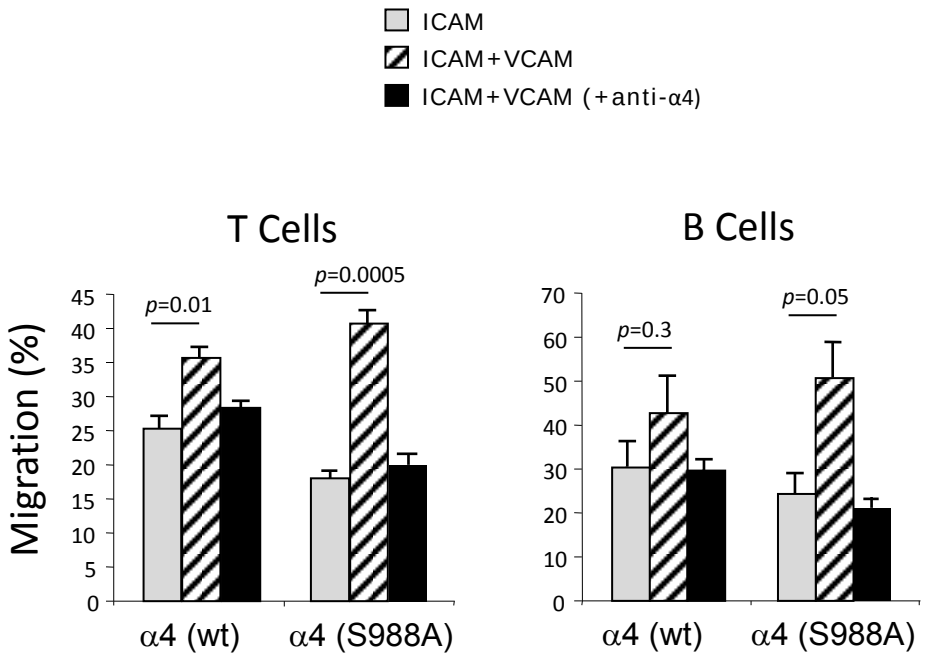


C

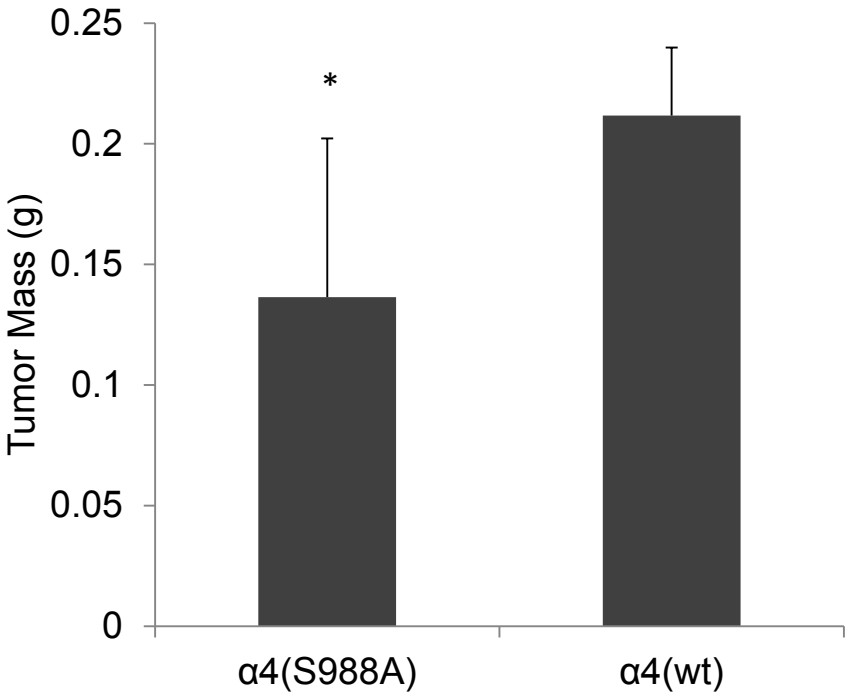



α4 (S988A)
(Ly5.2)
α4 (wildtype)
(Ly5.1)
Splenocyte
mixture
Rag1-/-
24 h
Rag1-/-
4-18h
Rag1-/-
Thioglycollate, i.p.
Spleen
Periton. lavage
Flow
cytometry
Fraction α4(S988A Cells (%)
Spleen
Peritoneum
80
70
60
50
40
30
20
10
0
*
**
***
**
4.5h
18h
4.5h
18h
All T cells
CD8+ T cells

S3

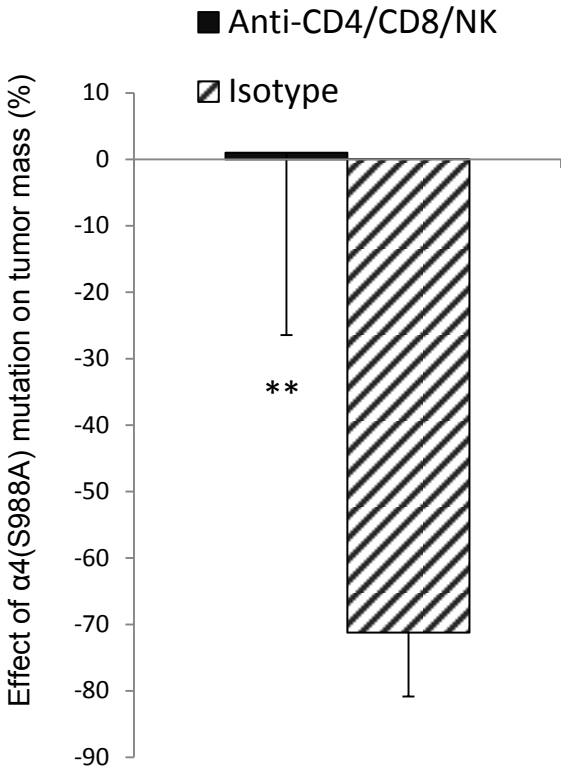

ICAM
ICAM+VCAM
ICAM+VCAM (+anti-α4)
T Cells
B Cells
Migration (%)
p=0.01
p=0.0005
p=0.3
p=0.05
45
40
35
30
25
20
15
10
5
0
70
60
50
40
30
20
10
0
α4 (wt)
α4 (S988A)
α4 (wt)
α4 (S988A)

S4

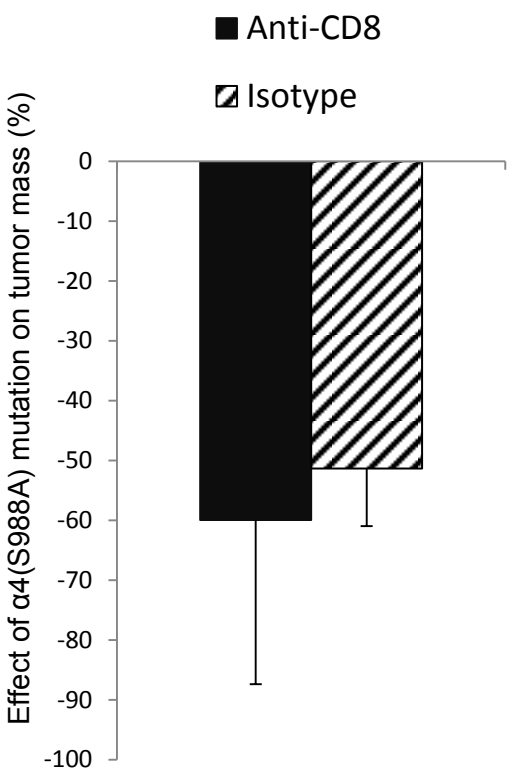


S5

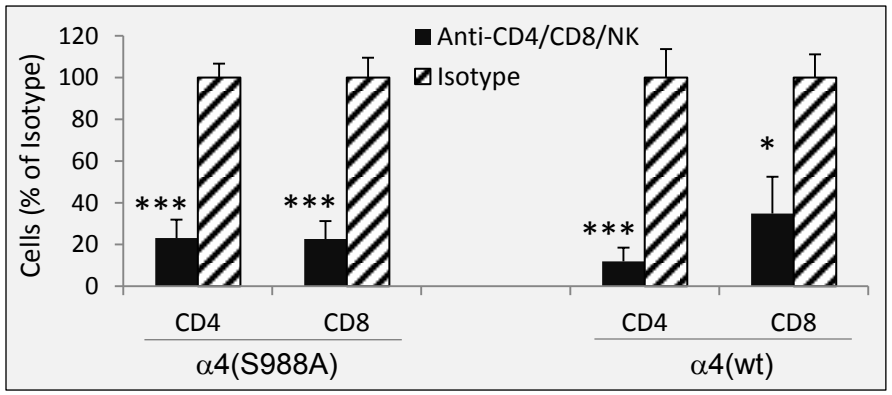
A

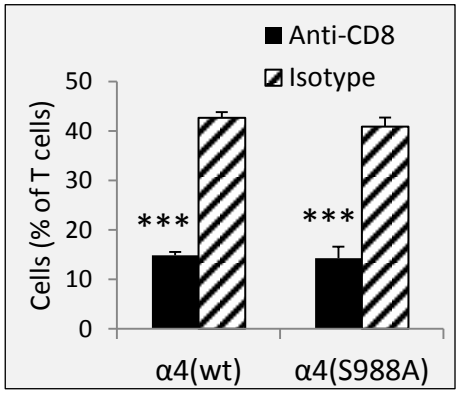


B

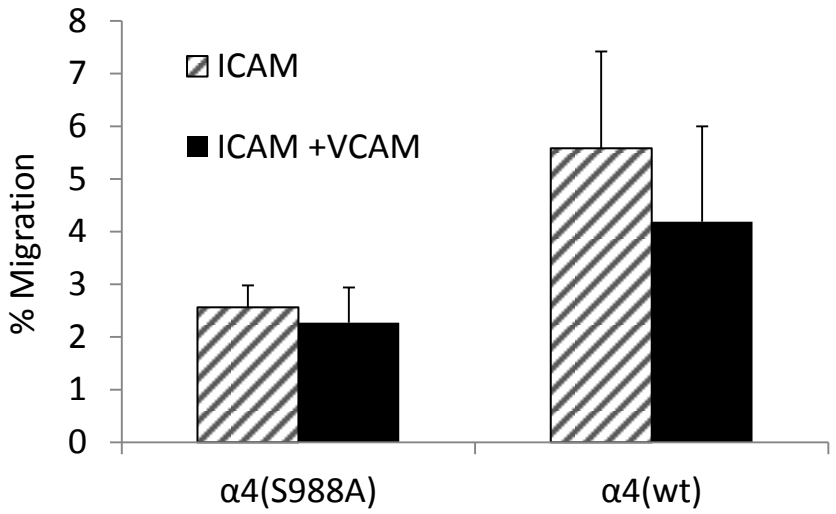


Depletion efficiency





S6



S7

Supplemental Figure Legends

Supplemental Figure 1 *Generation of the α4(S988A) mouse.* A DNA fragment encoding α4(S988A) integrin with a Neomycin resistance gene was transfected into ES cells. ES clones heterozygous for knock-in were confirmed by treatment of restriction-digested DNA with P32-labeled DNA probes specific for 3' or 5' flanking sequences or neomycin resistance cassette. One α4(wt) sample is compared with DNA from two α4(S988A) ES clones. ***(B)*** *Hematological analysis*. Error bars are S.E.M. of n=5 for each group. ***(C)*** *Selectively increased lymphocyte migration in α4(S988A) mice.*

Supplemental Figure 2. ***(A)*** *Hematological analysis*. Blood was collected from adult (>9 weeks) α4(S988A) or control α4(wt) mice and analyzed by an automated cell counter and manual differential cell counting of stained smears. Error bars are S.E.M. of n=4 mice for each group. Two parameters (RBC and MCV) were statistically significant different, but fell within normal ranges for BL6 mice. ***(B)*** *Naïve lymphoid organ lymphocyte subsets*. Bone marrow, thymus, and spleen were isolated from adult (>9 weeks) α4(S988A) or control α4(wt) mice, made into single-cell suspensions, treated with RBC lysis buffer, and counted. Bone marrow cells were stained with combinations of antibodies for the following B cell subsets and analyzed by flow cytometry: Total B (B220+) Pro-B (CD43+ B220+), Pre-B (B220+ IgM- IgD-), and Immature B (B220+IgM+IgD-). Likewise, thymocytes were stained for T-cell subsets: Total T-cells (CD3+), CD4 SP (CD3+CD4+CD8-), CD8 SP (CD3+CD8+CD4-), and DP (CD4+CD8+). Splenic T and B cells subsets were identified as: Total B (B220+), Immature B (CD21/35-CD23-), Marginal Zone (MZ) B (CD21/35hi CD23-), Follicular B (CD23+ CD21/35lo), Total T (CD3+), CD4 T (CD3+CD4+CD8-), and CD8 T (CD3+CD8+CD4-). ***(C)*** *Humoral immune response*. Adult α4(S988A) or control α4(wt) mice were immunized i.p. with 100 μg Trinitrophenol-Keyhole Lympet Hemocyanin (TNP-KLH) emulsified in Complete Freund's Adjuvant (CFA). Plasma from pre-immune and weeks 1, 2, and 3 bleeds were tested by direct specific ELISA for the presence of anti-TNP IgG. Similar results were seen with anti-IgM responses. Error bars are S.E.M. of n=4 for each group

Supplemental Figure 3. *Intrinsic homing advantage of α4(S988A) splenocytes.* Splenocytes from α4(S988A) or control α4(wt) Ly5.1 congenic mice were mixed and injected i.v. into *Rag1*$^{-/-}$ mice that had been challenged 24h earlier with 1ml thioglycollate medium. Mice were sacrificed

at 4.5h or 18h after splenocyte transfer; peritoneal lavage and spleen were counted, stained with fluorochrome-conjugated antibodies, and analyzed by flow cytometry. The ratio of α4(S988A) to control α4(wt) splenocytes was determined by measuring the ratio of Ly5.1-negative to Ly5.1-positive T-cells (CD3+). Errors bars indicate SEM from n=3 (4.5h) and n=2 (18h) mice per group. *p< 0.02, **p<0.01, ***p<0.04 (one-tailed t-test

Supplemental Figure 4. Migration of α4(S988A lymphocytes in vitro. B and T-cells were purified from spleens of α4(S988A) or control α4(wt) mice. Chemotactic migration towards SDF-1α (15ng/ml) was assessed using a modified Boyden Chamber assay in wells coated with ICAM-1 (5 μg/ml) +/- VCAM-1 (0.02 μg/ml). For anti-integrin antibody blocking, cells were treated with 10μg/ml of anti-α4 or integrin prior to the assay. Error bars are S.E.M. of n=4 for each group

Supplemental Figure 5. Lewis Lung Carcinoma Tumor Growth. Lewis Lung Carcinoma (LLC) tumor cells ($1x10^6$) were injected subcutaneously in the hind flank of α4(S988A) or control α4(wt) mice. On day 15, mice were sacrificed and tumors weighed. Error bars are S.E.M. from 10-11 mice per group. *p<0.02 (one-tailed Mann-Whitney test). Similar results were obtained with inoculation of $5x10^6$ tumor cells.

Supplemental Figure 6. Tumor growth after depletion of lymphoid cells in α4(S988A) mice. B16 tumors were grown in α4(S988A) or control α4(wt) mice as in Figure 3. Two days before and 5 days after tumor cell inoculation, anti-CD8 (***A***), or a combination of anti-CD4, anti-CD8, and anti-NK1.1 (***B***) depleting antibodies were injected i.p. On day 15, excised tumors were weighed and compared between α4(S988A) and control α4(wt) genotypes in depleted or in antibody isotype-treated mice. Upper bar graphs show percentage change in tumor mass in α4(S988A) vs. α4(wt) mice. Lower graphs are depletion efficiency of T cells in the spleen on day 15. n ≥ 5 mice per group. *p<0.03, **p<0.015, ***p<0.001 (one-tailed)

Supplemental Figure 7. Migration of α4(S988A) macrophages. Macrophages were differentiated from bone marrow of α4(S988A) or control α4(wt) mice by culture for one week in 30% L929 supernatant medium. Chemotactic migration towards SDF-1α and MCP-1 (15ng/ml each) was assessed using a modified Boyden Chamber assay in wells coated with VCAM-1 alone (2 ug/ml), or ICAM-1 (5 ug/ml) +/- VCAM-1 (0.02 ug/ml). Bar graphs summarize migration from n= 3 mice per group; no integrin transregulation or migrational differences were observed.