Supplementary Methods

Reagents

PMA, ionomycin, DAPI, cycloheximide were from Calbiochem (La Jolla, CA, USA). PHA was from Roche (Indianapolis, IL, USA). Lithium chloride and ketamine/Xylaxine were from Sigma (St. Louis, MO, USA). MMP2 inhibitor I (OA-Hy (*cis*-9-Octadecenoyl-N-hydroxylamide Oleoyl-N-hydroxylamide)) and MMP2/MMP9 inhibitor IV were from Calbiochem (La Jolla, CA, USA). Human recombinant SDF1α, mouse recombinant TNFα and IFNγ were from Peppro Tech (Norwalk, CT, USA). Texas red Streptavidin was from Vector Lab (Burlingame, CA, USA). Anti-Flag M2 and anti-α-tubulin mAbs were from Sigma (St. Louis, MO, USA). Anti-β-catenin mAb was purchased from BD Transduction Laboratories (San Diego, CA, USA). Anti-ICAM-1 was obtained from the Developmental Studies Hybridoma Bank (University of Iowa, Iowa City, IA, USA). The control Ab HB64 was isolated from the hybridoma clone from the American Type Culture Collection (ATCC, Manassas, VA, USA). Hamster anti-mouse CD3, and Alexa 488-conjugated goat anti-mouse were from Invitrogen, and biotin-conjugated rat anti-mouse CD31 mAbs were from BD Pharmingen (San Diego, CA, USA). Soluble Fz5-Fc was from R&D (Minneapolis, MN).

Determination of transfection efficiency

To determine percentage of cells expressing GFP, dot plots were generated for GFP (x axis) vs. FL2, which was not used (y axis). Auto-fluorescence falls on the diagonal and a gate was drawn around control cells on this diagonal. All GFP-transfected cells outside of the gate (below the diagonal) were counted as GFP positive. This method allowed us to see cells expressing low levels of GFP that are normally “lost” in the auto-fluorescent peak when histograms are plotted.

RT-PCR

Total RNA from $2x10^6$ effector T cells or $1x10^7$ resting T cells was extracted using Trizol (Invitrogen) and treated with RQ1 DNase (Promega, Irvindale, CA, USA).

cDNA was synthesized from 2μg of RNA using Superscript II reverse transcriptase (Invitrogen). Control reactions were run without RT to confirm lack of genomic DNA contamination. PCR amplification was performed with GAPDH, MMP2- and MMP9-specific primers that cross intron-exon boundaries, for 35 cycles at annealing temperatures of 61ºC. For each primer set, a cycle number was chosen to ensure that results fell in the linear range of amplification. Primer sequences:

GAPDH	upper	5'-ACCACAGTCCATGCCATCAC-3'
	lower	5'-TCCACCACCCTGTTGCTGTA-3'
MMP2	upper	5'-TGCGGCACCACTGAGGACTACGAC-3'
	lower	5'-TCCGGGAGCTCAGGCCAGAATGT-3'
MMP9	upper	5'-CGCGGGCGGTGATTGACGAC-3'
	lower	5'-GTGGTGCAGGCGGAGTAGGATTGG-3'

Quantitative RT-PCR

Quantitative Real-time PCR was performed as previously described (Fruman et al., 2002). Relative Transcript levels of GAPDH, MMP2 and Cyclin D1 in T cells were determined using a standard curve generated from the cDNA of HUVEC stimulated with 10nM PMA, 10ng/mL TNFα and 25μM Forskolin. Primer sequences were:

GAPDH	upper	5'- TCGACAGTCAGCCGCATCTTCTT-3'
	lower	5'- GCGCCCAATACGACCAAATCC-3'
MMP2	upper	5'- CGCAGTGACGGAAAGATGTGGT-3'
	lower	5'- AGAGCTCCTGAATGCCCTTGATGT-3'
CyclinD1	upper	5'- AGAACACGGCTCACGCTTACCTC-3'
	lower	5'- CTTGCCCCATCACGACAGACA-3'

Time-lapse videomicroscopy

Effector T cells were plated in collagen IV-coated 24-well plates in the presence of SDF1α(100ng/ml). Wnt3A-CM or control medium was added. After 12hrs, the

plate was transferred into a CO_2 incubating system pre-warmed at 37 ºC. 2D phase-contrast images were acquired at 15-s intervals and recorded by METAMORPH software (Universal Imaging). Cell tracking was performed with NIH ImageJ software.

Protein purification

HEK293T cells (2 $X10^6$) were plated in 10cm dishes and transiently transfected on the following day with 5μg of recombinant Sfz5-Fc using Lipofectamine 2000 (Invitrogen) according to the manufacturer's protocol. The medium was changed to serum-free LGM3 (Clonetics) and conditioned for 72 hrs at 37°C. Medium was then changed and cultured for an additional 48 hrs. SFz5-Fc-conditioned-medium was then stored at 4°C until purification. sfz5-Fc protein was purified using protein-G-conjugated agarose beads (Pierce). 50mL of conditioned-medium was run over 500μL of beads and washed with 5-column volumes of PBS. Pure protein was then eluted off the column in 500 μL fractions with 0.1M Glycine 150mM NaCl, pH2.8, and immediately neutralized with 50μL of 1M Tris pH 9.5. Column fractions were then buffer exchanged and concentrated in PBS using centrifugation (Millipore). Protein concentration was determined using a standard BCA protein assay (Sigma).

Luciferase assay

Lysates from 1x10^6 T cells were analyzed with the Promega luciferase assay system. Luciferase activity was measured on a Sirius luminometer (Berthold detection systems, Pforzheim, Germany). Relative luciferase activity was normalized to protein content, which was determined by Bio-Rad protein assay kit.

Supplementary Legends

Supplementary Figure 1. Wnt3A moderately increases T cell motility

(A) Effector T cells were added to collagen IV gels in the presence of control *(left)* or wnt3A-conditioned medium *(right)*, and movement was analyzed by time-lapse microscopy with images collected every 15sec. Tracks for 30 randomly selected T cells are shown with their origins normalized to (0,0). (B) Data from A plotted to show the distribution of instantaneous velocities measured from point-to-point tracks at 15sec intervals (n=1200). A *t*-test of the mean instantaneous velocities for each condition gave $p<0.01$ (*t*-test). (C) A plot of the mean displacement of individual T cells away from their starting points approximates a straight line under both conditions, suggesting that migration is random.

Supplementary Figure 2. Wnt signaling does not augment T cell migration in the absence of collagen.

Effector T cells were allowed to migrate across EC-free, uncoated wells in response to SDF1-α in the presence of control or wnt3A-conditioned medium. Mean and SD for triplicate wells, no statistically significant difference.

Supplementary Figure 3. Wnt signaling induces MMP2 expression in effector T cells

Effector T cells were treated with control or wnt3A-CM overnight and RNA was harvested for qRT-PCR using cyber green for product detection. Standard curves were prepared and fold increase in expression of MMP2 and the known wnt target gene cyclinD1 was calculated.

Supplementary Figure 4. sFz5 blocks wnt-induced MMP2 and MMP9 expression in effector T cells

Effector T cells were treated with control or wnt3A-CM overnight in the presence or absence of 10μg/ml sFz5-Fc. RNA was harvested after 17 hours for qRT-PCR using cyber green for product detection. Standard curves were prepared and fold

increase in expression of MMP2 and MMP9 was calculated. Mean and SD for triplicate samples, $p<0.05$ (*t*-test) for BSA v sFz5.

Supplementary Figure 5. sFz5 blocks wnt-induced MMP2 and MMP9 expression in effector T cells

Effector T cells were transfected with MMP2-Luc along with either a control vector or expression vectors for CA-LRP5 (LRP5-ΔN) or CA-LRP6 (LRP6-ΔN). Cells were harvested 24 hours later for analysis of luciferase expression. Mean and SD for triplicate wells, $p<0.005$ (*t*-test) for control v LRP5-ΔN or LRP6-ΔN.

[E] Figure

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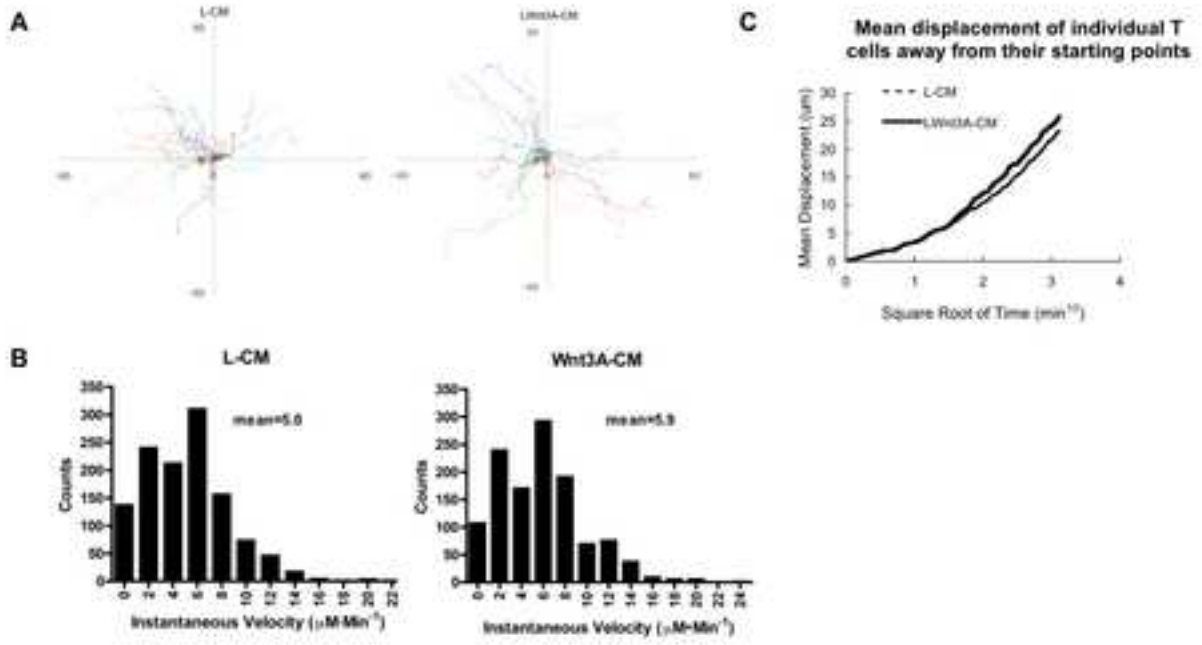
Fig. S1



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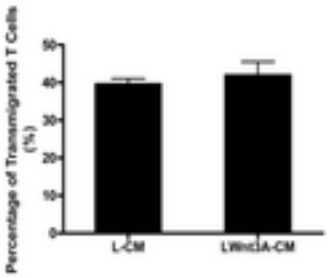

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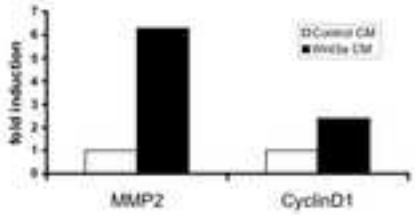

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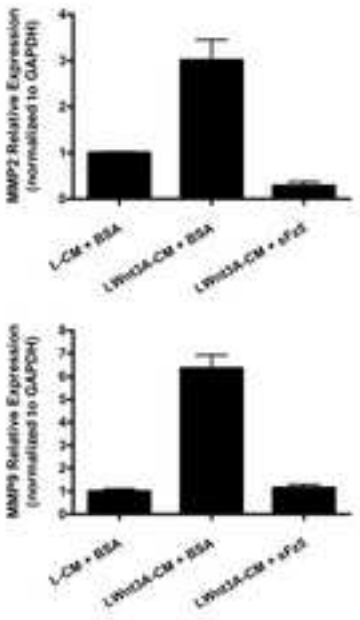

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Fig. S5