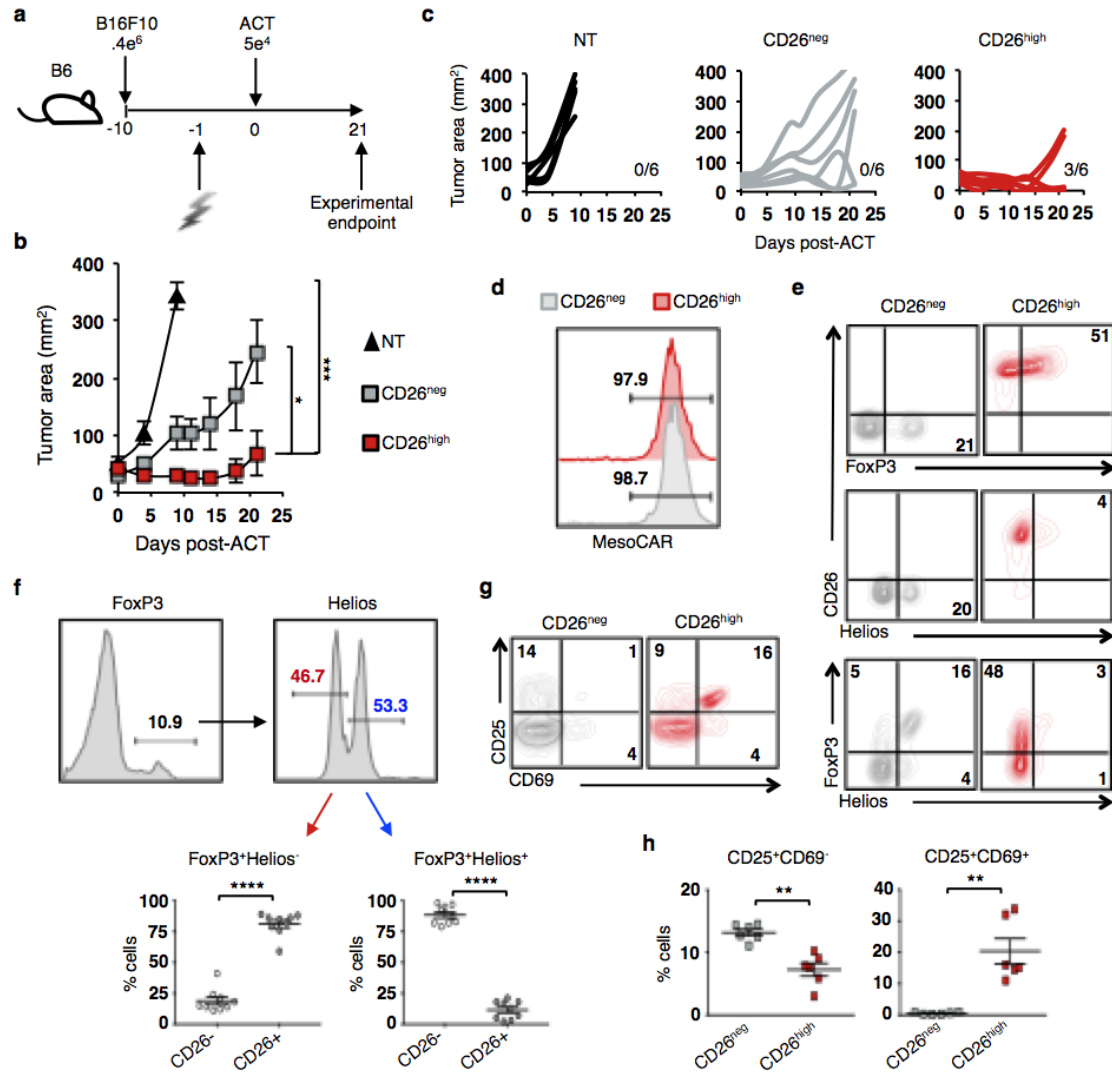
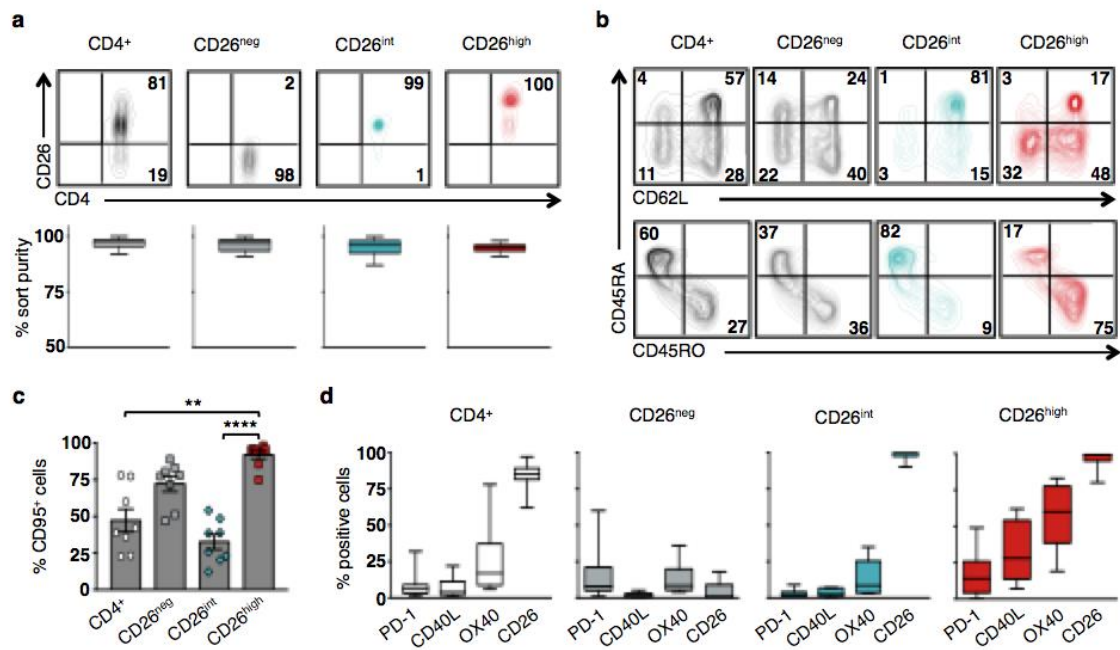


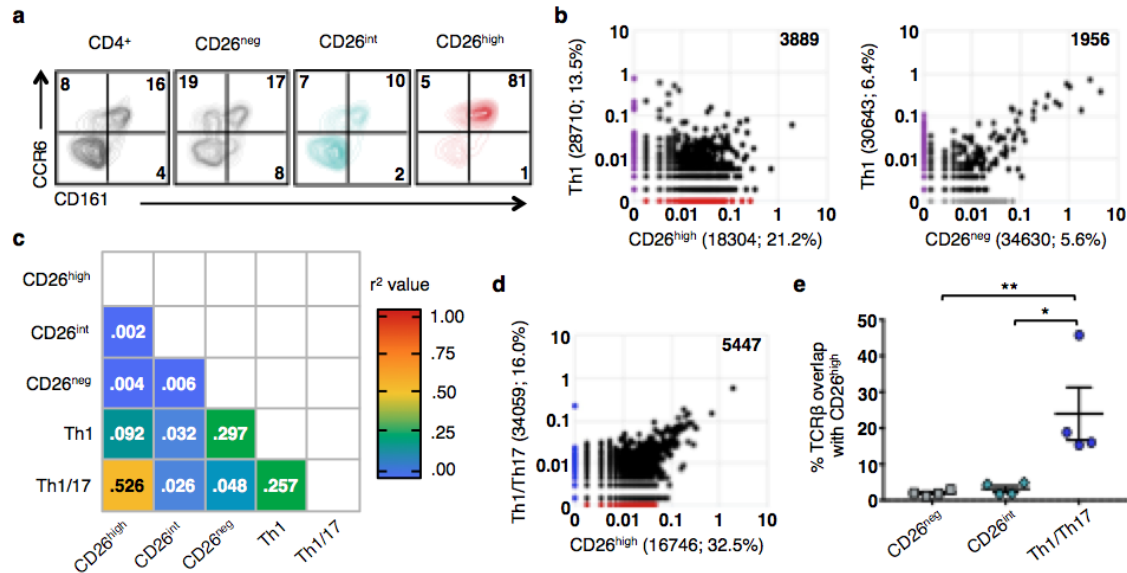


**Supplementary Figure 1. CD26<sup>high</sup> T cells are activated and regress malignancy to a greater extent than CD26<sup>neg</sup> T cells.** Splenocytes from transgenic TRP-1 mice were isolated and cultured with 1μl/ml TRP-1 peptide and feeder cells at a ratio of 1 feeder:5 TRP-1 cells. TRP-1 CD4<sup>+</sup> T cells were programmed to a Th17 phenotype using polarizing cytokines (10ng/ml hIL-1β, 100ng/ml hIL-21, 100ng/ml hIL-6, 30ng/ml hTGFβ, 10μg/ml αm-IFNγ, 10μg/ml αm-IL-4) and expanded with 100IU/ml IL-2. After six days expansion, Vβ14<sup>+</sup>CD4<sup>+</sup> Th17 cells were sorted by CD26 into CD26<sup>neg</sup> (bottom ~10%) and CD26<sup>high</sup> (top ~5%). **a-c**, B6 mice bearing B16F10 melanoma established for 10 days were left untreated (NT; No Treatment) or infused with 5e<sup>4</sup> CD4<sup>+</sup>Vβ14<sup>+</sup> CD26<sup>neg</sup> or CD26<sup>high</sup> T cells. All mice were lymphodepleted with 5 Gy total body irradiation one day prior to ACT (N=6 mice/group, representative of two individual experiments). P values for the tumor curve were calculated using One-way ANOVA with a Kruskal-Wallis comparison on the last day that all mice from compared groups were alive (NT vs. CD26<sup>high</sup>=day 9, CD26<sup>neg</sup> vs. CD26<sup>high</sup>=day 21). **d**, Representative histograms of human CD26<sup>neg</sup> and CD26<sup>high</sup> T cells after being sorted for MesoCAR expression, yielding ~98% pure population **e**, Representative FACS plots of transcription factors from sorted human CD4<sup>+</sup> T cells prior to bead stimulation. **f** Summary graphs of CD26 expression on CD4<sup>+</sup>FoxP3<sup>+</sup>Helios<sup>-</sup> and CD4<sup>+</sup>FoxP3<sup>+</sup>Helios<sup>+</sup> T cells using back gating on FlowJo

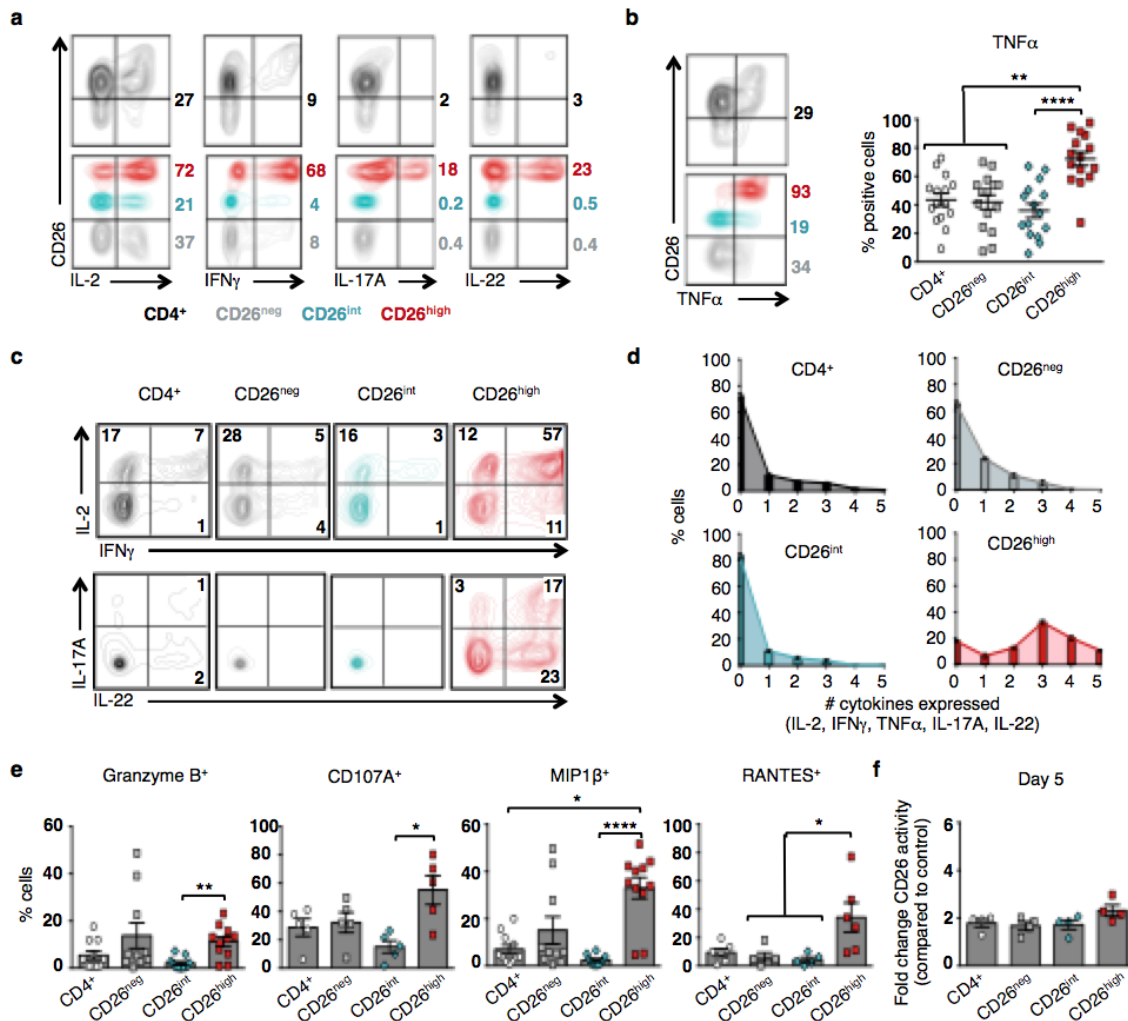
software from the bulk CD4<sup>+</sup> T cell subset. **g** and **h**, Representative FACS plots (**g**) and summary data (**h**) of activation markers from six individual donors. One-way ANOVA with Kruskal-Wallis comparison was performed on **f** and **h**. Data with error bars represent mean  $\pm$  SEM. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ .; \*\*\*,  $P < 0.001$ ; \*\*\*\*,  $P < 0.0001$ .



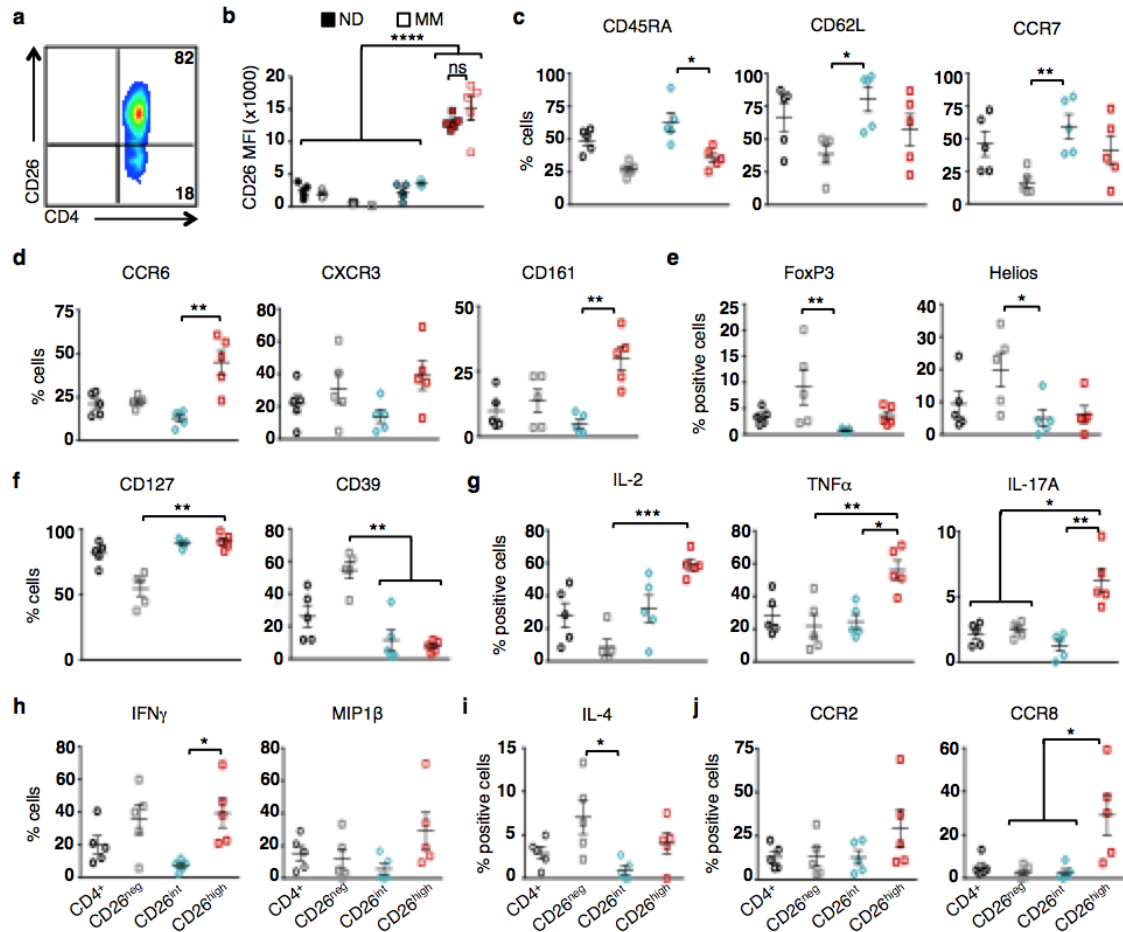
**Supplementary Figure 2. CD26-sorted subsets are pure and have unique profiles.** Human CD4<sup>+</sup> T cells from healthy donors were sorted by CD26 expression. **a**, Post-sort phenotype (top, representative of 26 donors) and purity (bottom, N=26) of all sorted subsets was determined using flow cytometry. **b-d**, Representative FACS plots of memory (**b**; N=26) and graphical representation of CD95 expression (**c**; N=8) or co-stimulatory/co-inhibitory markers (**d**; N=8-12) by flow cytometry prior to cell activation. One-way ANOVA with Kruskal-Wallis comparison was performed on **c**. Data represented as mean  $\pm$  SEM. \*\*, P < 0.01; \*\*\*\*, P < 0.0001.



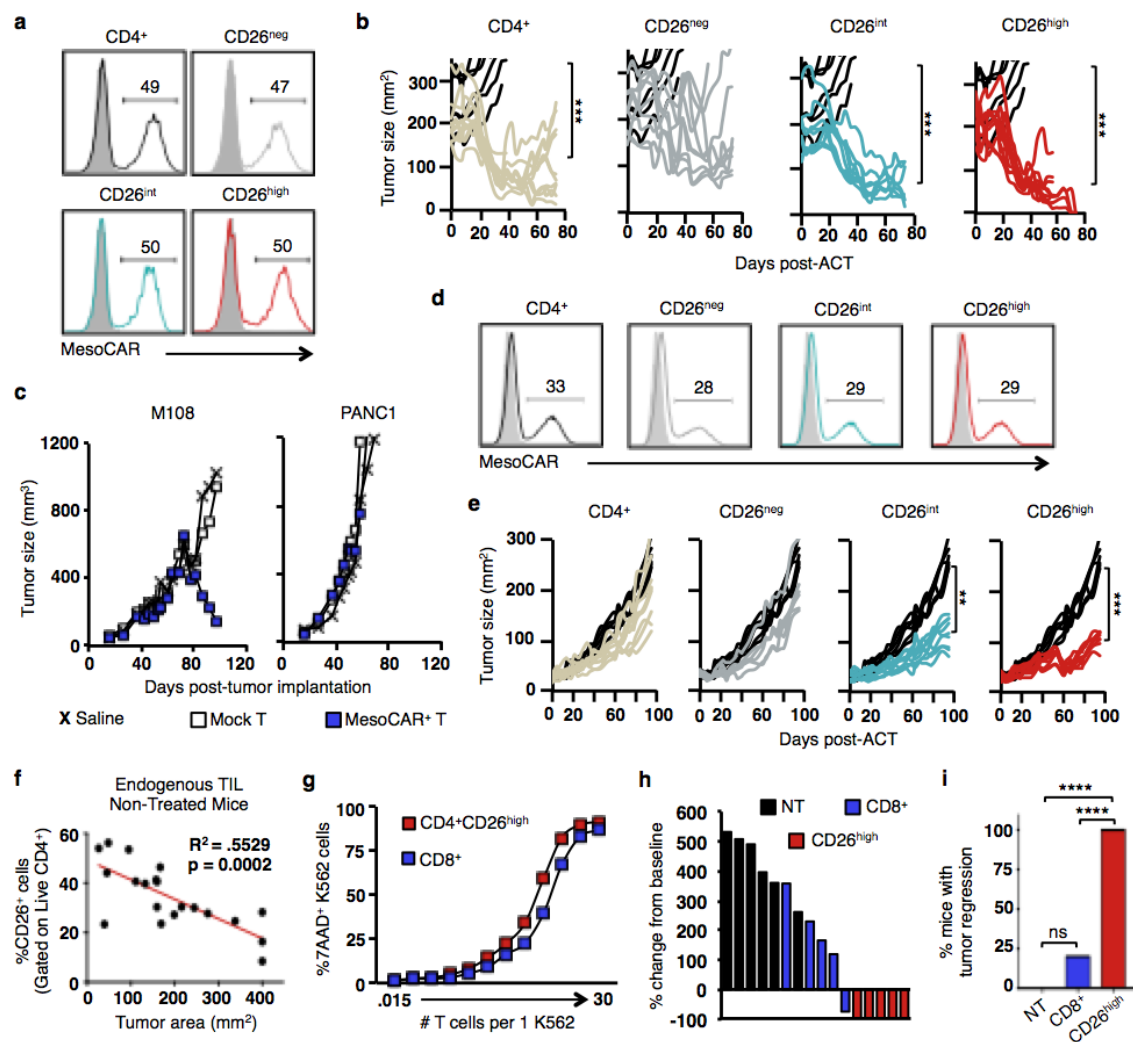
**Supplementary Figure 3. TCR $\beta$  sequences of CD26<sup>high</sup> T cells overlap with Th1/Th17 cells, but not Th2.** **a**, Human CD4<sup>+</sup> T cells were sorted by CD26 and analyzed for CCR6 and CD161 expression by flow cytometry (representative of 26 donors). Human CD4<sup>+</sup> T cells were sorted into bulk CD4<sup>+</sup>, CD26<sup>neg</sup>, CD26<sup>int</sup>, CD26<sup>high</sup>, Th1 (CXCR3<sup>+</sup>CCR6<sup>-</sup>), Th2 (CCR4<sup>+</sup>CCR6<sup>-</sup>), Th17 (CCR6<sup>+</sup>CCR4<sup>+</sup>) and Th1/Th17 (CXCR3<sup>+</sup>CCR6<sup>+</sup>). DNA was isolated from sorted subsets and TCR $\beta$  sequencing was performed using an immunoSEQ kit and subsequent analysis. **b**, Graphical representations of TCR overlap between Th1 cells and CD26<sup>high</sup> or CD26<sup>neg</sup> (N=4). **c**, Correlation of TCR expression ( $r^2$ ) determined and graphed using a heatmap diagram (N=4). **d** and **e**, TCR overlap between Th1/Th17 and CD26<sup>high</sup> (**d**) and CD26<sup>high</sup> compared to CD26<sup>neg</sup>, CD26<sup>int</sup> and Th1/Th17 cells (**e**) was defined via immunoSEQ software (N=4). One-way ANOVA with Kruskal-Wallis analysis was performed on **e**. Data represented as mean  $\pm$  SEM. \*,  $P < 0.05$ , \*\*,  $P < 0.01$ .



**Supplementary Figure 4. CD26<sup>high</sup> T cells are highly functional and cytotoxic.** CD4<sup>+</sup> T cells from the blood of healthy individuals were sorted by CD26 expression (**Fig. 2A**). **a-e**, Sorted cells were activated with PMA/Ionomycin and Monensin for 4 hours prior to intracellular staining (N=5-26). In **d**, three independent donors were analyzed by FlowJo software and graphed to display the percentage of cells simultaneously secreting 0-5 cytokines (IL-2, IFN $\gamma$ , TNF $\alpha$ , IL-17A, IL-22). **f**, 1e<sup>5</sup> sorted cells were incubated with the CD26 ligand gly-pro-P-nitroanilide at 37°C for 2 hours and enzyme activity was determined using a colorimetric assay (N=5). One-way ANOVA with Kruskal-Wallis comparison was performed on **b**, **e** and **f**. Data with error bars represent mean  $\pm$  SEM. \*, P < 0.05; \*\*, P < 0.01; \*\*\*\*, P < 0.0001.



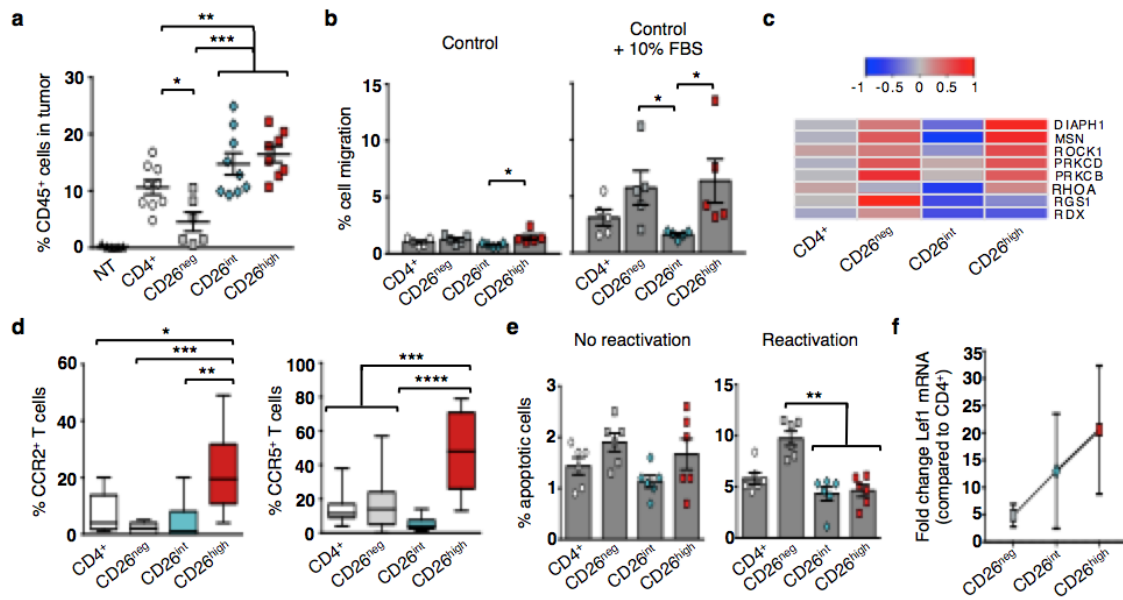
**Supplementary Figure 5. CD26-sorted subsets in patients with metastatic melanoma have a similar functional and phenotypic profile as those enriched from healthy donors.** The biologic properties of CD26<sup>neg</sup>, CD26<sup>int</sup> and CD26<sup>high</sup> T cells from the blood of patients with metastatic melanoma were analyzed via flow cytometry (N=5). **a**, Representative FACS plot of CD26 expression on CD4<sup>+</sup> T cells. **b**, Graphical representation of CD26 MFI on sorted T cells from cancer patients (N=5) and healthy individuals (N=5). **c-j**, Scatter plots of sorted T cells from melanoma patients analyzed by flow cytometry. Intracellular stains were performed following activation with PMA/Ionomycin and Monensin for four hours (N=5). One-way ANOVA with Kruskal-Wallis comparison was performed for **b-j**. Data represents mean  $\pm$  SEM. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; \*\*\*\*, P < 0.0001.



### Supplementary Figure 6. CD26<sup>int</sup> and CD26<sup>high</sup> T cells regress multiple tumors.

CD4<sup>+</sup> lymphocytes from healthy donors were sorted by CD26 expression and stimulated with CD3/ICOS beads (1 bead : 5 T cells). Cells were transduced at 36 hours post-activation to express a 1<sup>st</sup> generation mesothelin-specific CD3ζ CAR and expanded in IL-2 (100IU/ml) for 10 days. **a**, MesoCAR transduction efficiency was determined using flow cytometry prior to ACT. **b**, Individual tumor curves for each treatment group compared to no treatment (black lines) for M108-bearing NSG mice discussed in Fig. 5 (N=7-9 mice/group). P values for tumor curves were calculated by Mann-Whitney on the final day when mice from all comparison groups were still alive (NT vs. all groups=day 38). **c**, NSG mice bearing either mesothelioma or pancreatic cancer were infused with 12.5e<sup>6</sup> non-specific mock T cells or 12.5e<sup>6</sup> transduced T cells (~40% MesoCAR<sup>+</sup>) on days 49 (PANC1) or 75 (M108) post-tumor inoculation. **d**, MesoCAR transduction efficiency of sorted T cell subsets were determined by flow cytometry prior to ACT into

PANC1-bearing mice. **e**, Individual tumor curves for each treatment group compared to no treatment (black lines) for NSG mice with pancreatic cancer (6-9 mice/group). P values for tumor curves were calculated by Mann-Whitney on the final day when mice from all comparison groups were still alive (All groups=day 84). **f**, TIL were isolated from un-treated, melanoma-bearing mice and assessed for CD26 expression via flow cytometry (N=20). **g-i**, MesoCAR<sup>+</sup> CD4<sup>+</sup>CD26<sup>high</sup> and CD8<sup>+</sup> T cells were assessed for their ability to eradicate cancer cells *in vitro* (**g**) and *in vivo* (**h-i**, N=6 mice/group). Data in **h** and **i** represent 60 days post-ACT. One-way ANOVA with Kruskal-Wallis component was performed on **i**. Data with error bars represent mean  $\pm$  SEM. \*\*, P < 0.01; \*\*\*, P < 0.001; \*\*\*\*, P < 0.0001.



**Supplementary Figure 7. Stem memory CD26<sup>high</sup> T cells persist and migrate to the tumor.** **a**, Tumors from PANC1-bearing mice were harvested and the percentage of CD45<sup>+</sup> T cells was determined by flow cytometry (N=7-9). **b**, 7.5e4 bulk CD4<sup>+</sup>, CD26<sup>neg</sup>, CD26<sup>int</sup> and CD26<sup>high</sup> T cells were assayed for migration towards control media (top) and media supplemented with 10% FBS (bottom) using a transwell assay (N=5). **c**, Expression of migration-related genes in pre-activated, sorted T cells was determined via gene array and graphed as a (+/-) log2-fold change (N=3-5). **d**, Chemokine receptor expression on inactivated, sorted T cells was assessed via flow cytometry (N=15). **e**, Ten-day expanded T cells were analyzed for apoptosis using Annexin V/PI staining in the presence or absence of overnight activation with anti-CD3 antibody (N=6). **f**, Lef1 expression in sorted T cell subsets was determined by qPCR analysis (N=4). One-way ANOVA with Kruskal-Wallis comparison was performed for **a**, **b** and **d-j**. Data represents mean  $\pm$  SEM. \*, P < 0.05; \*\*, P < 0.01; \*\*\*, P < 0.001; \*\*\*\*, P < 0.0001.

| Target                  | Clone     | Fluorophore  | Provider       |
|-------------------------|-----------|--------------|----------------|
| $\alpha$ m-CD4          | GK1.5     | APC Cy7      | BD Biosciences |
| $\alpha$ m-V $\beta$ 14 | 14.2      | FITC         | BD Biosciences |
| $\alpha$ m-CD26         | H194-112  | PE           | BioLegend      |
| $\alpha$ h-CD26         | C5a5b     | PE           | ↓              |
| $\alpha$ h-CD4          | OKT4      | APC Cy 7     |                |
| $\alpha$ h-CD25         | BC96      | FITC         |                |
| $\alpha$ h-CD45RA       | HI10U     | PerCP Cy5.5  |                |
| $\alpha$ h-CD45RO       | UCHL1     | APC          |                |
| $\alpha$ h-OX40         | ACT35     | PE Cy7       |                |
| $\alpha$ h-CD154/CD40L  | 24-31     | AF488        |                |
| $\alpha$ h-CCR6         | G034E3    | PE Cy7       |                |
| $\alpha$ h-CXCR3        | G025H7    | BV421        |                |
| $\alpha$ h-CD127        | A019D5    | PE Cy7       |                |
| $\alpha$ h-CD39         | A1        | PE Cy7       |                |
| $\alpha$ h-CD45         | HI30      | Pacific Blue |                |
| $\alpha$ h-FoxP3        | 150D      | AF647        |                |
| $\alpha$ h-Helios       | 22F6      | Helios       |                |
| $\alpha$ h-IL-22        | 516406    | AF647        |                |
| $\alpha$ h-TNF $\alpha$ | MAb11     | PE Cy7       |                |
| $\alpha$ h-Granzyme B   | 515403    | FITC         |                |
| $\alpha$ h-RANTES       | VL1       | PerCP Cy5.5  |                |
| $\alpha$ h-CD4          | RPAT4     | V500         | BD Biosciences |
| $\alpha$ h-PD1          | M1H4      | FITC         | ↓              |
| $\alpha$ h-CD28         | CD28.2    | APC H7       |                |
| $\alpha$ h-CCR7         |           | PE Cy7       |                |
| $\alpha$ h-CCR2         | 48607     | AF647        |                |
| $\alpha$ h-CCR5         | 2D7       | V450         |                |
| $\alpha$ h-IL-2         | MQ1-17H12 | PE Cy7       |                |
| $\alpha$ h-IFN $\gamma$ | B27       | V450         |                |
| $\alpha$ h-IL-17A       | N49-653   | PerCP Cy5.5  |                |
| $\alpha$ h-CD107A       | H4A3      | APC H7       |                |
| $\alpha$ h-MIP1 $\beta$ |           | PerCP Cy5.5  |                |
| $\alpha$ h-IL-4         |           | FITC         | ↓              |
| Streptavidin            |           | V500         |                |
| $\alpha$ h-CD69         | FNS0      | APC          | eBioscience    |
| $\alpha$ h-ICOS         | ISA-3     | Biotin       | ↓              |
| $\alpha$ h-CD161        | HP-3G10   | PerCP Cy5.5  |                |
| $\alpha$ h-CCR4         |           | FITC         | R&D Industries |
| $\alpha$ h-CCR8         |           | PerCP Cy5.5  | R&D Industries |

**Supplementary Table 1. Complete list of flow cytometry antibodies.**