

Figure S1. 4T1-induced Gr1⁺CD11b⁺ cells have the phenotype of granulocytic MDSC: CD11b⁺Gr1⁺Ly6G^{hi}Ly6C^{neg-low}IL-4R α ⁺ Arginase⁺ iNOS⁻ CD115^{low} F4/80^{low} PDL1^{low} CD80⁺ CD86⁺ ROS⁺.

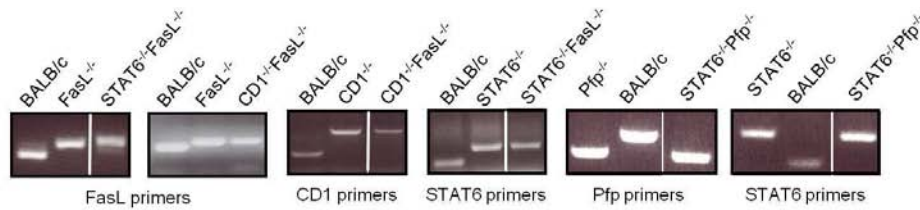


Figure S2. Genotyping of STAT6^{-/-}FasL^{-/-}, CD-1^{-/-}FasL^{-/-}, and STAT6^{-/-}Pfp^{-/-} mice. CD1 primers: CD1 P3 - 5'GAATGACACCTGCCCCCTATTTGT 3', CD1 P4 - 5'TAGAACCCAAGAGTGGCTGTCAGT3', and Neo C5 - 5' CCGCTTCCTCGTGCTTTACGGTAT 3'. CD1 PCR conditions: Initialization at 94°C for 2 min; denaturation at 94°C for 1 min; annealing at 65°C for 30 sec; extension at 72°C for 30 sec. The cycle from denaturation to extension was repeated 34 times with a final extension at 72°C for 5 mins and then storage at 4°C. BALB/c and CD1^{-/-} PCR products were 217-bp and 600-bp, respectively. STAT6 primers: Common 97 - 5' TGAGGTGGGGACCAGCCGG 3', BALB 99 - 5' GTGACCAGGACACACAGCGG 3' and STAT6 98 - 5' GCTACCCGTGATATTGCTGAAGAG 3'. STAT6 PCR conditions: The same as for CD1 primers, except initialization was for 1 min, extension for 30 se, and denaturation to extension was repeated 29 times. Wild type BALB/c and STAT6^{-/-} PCR products were 100-bp and 225-bp, respectively. FasL primers: FasL F3 - 5' TCTGATCAATTTTGAGGAATCTAAGGCC3', FasL R3 - 5'CATGAGGTCTTTGTGGCTCATGTA 3'. Primers produce an Eco147I (StuI) restriction site in wild type DNA. FasL PCR conditions: Initialization at 94°C for 4 min; denaturation at 94°C for 30 sec; annealing at 60°C for 60 sec; extension at 68°C for 90 sec. The denaturation to extension cycle was repeated 40 times with final extension at 72°C for 10 min and storage at 4°C. The PCR product was digested with 1ml of Eco 147I at 37°C for 2h to overnight, and run on 4% agarose gel at 70V for 45 mins. Wild type and FasL^{-/-} mice have bands of 150 and 175 bp, respectively; however, a faint band of 175bp was sometimes present in wild type mice due to incomplete digestion. Perforin primers: 597 - 5' CGTGAGAGGTCAGCATCCTTC 3', 598 - 5' TGGCCTAGGGTTACATCCAG 3', 599 - 5' ATATTGGCTGCAGGGTTCGCTC 3'. Perforin PCR conditions: The same as for CD1^{-/-} primers except extension was for 90 sec. and the cycle from denaturation to extension was repeated 30 times. BALB/c and Perf^{-/-} PCR products were 500-bp and 350-bp, respectively.

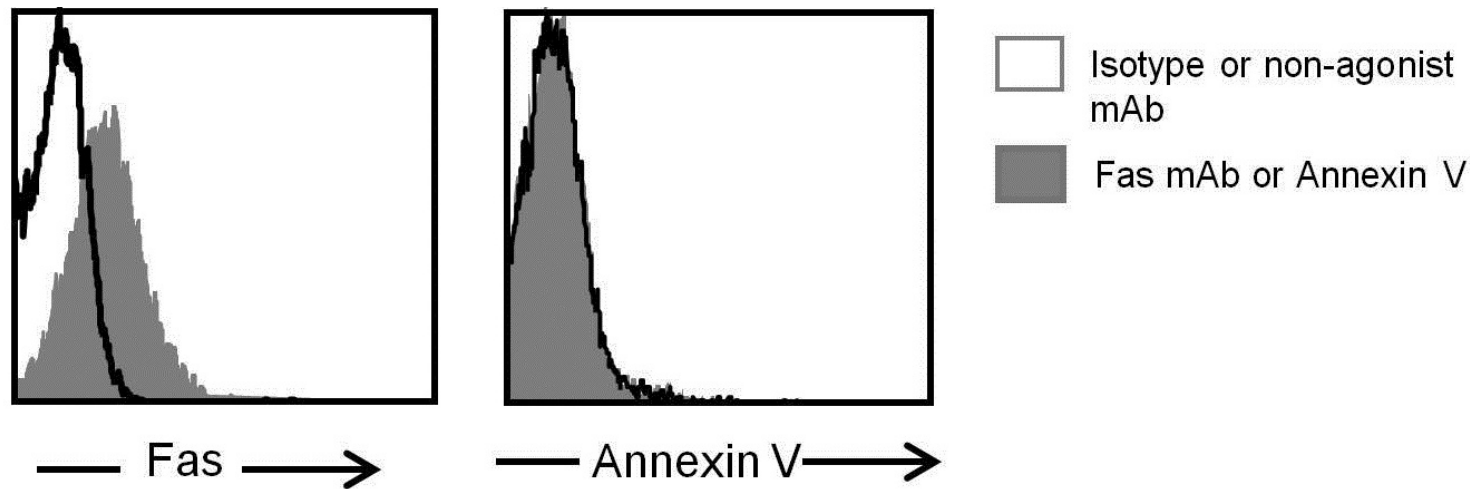


Figure S3. 4T1 tumor cells express Fas but are not susceptible to Fas-FasL-mediated apoptosis. Left panel: 4T1 tumor cells were labeled for Fas, or isotype control mAb, and analyzed by flow cytometry for expression of Fas. Right panel: 4T1 tumor cells were cultured with agonist Jo2 mAb or non-agonist control mAb for 24h, then labeled with Annexin V-FITC, and analyzed by flow cytometry. Data are from one of three independent experiments.

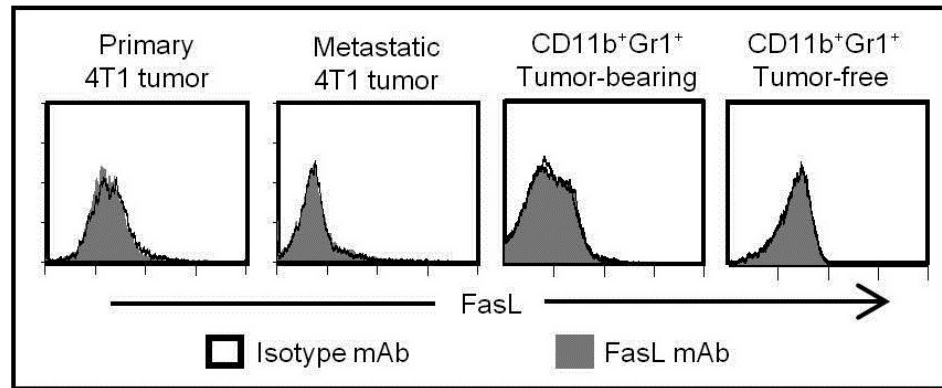


Figure S4. Primary and metastatic 4T1 tumor cells, and Gr1⁺CD11b⁺ MDSC from tumor-bearing and tumor-free mice do not express FasL. Left two panels: BALB/c mice were inoculated on day 0 in the abdominal mammary gland with 4T1 tumor cells and primary and metastatic (lung) tumors were removed on day 34 and dissociated into single cell suspensions. Resulting cells were stained with FasL or isotype control mAbs. Right two panels: Peripheral blood leukocytes from tumor-free and 4T1 tumor-bearing BALB/c mice were stained with mAbs to CD11b, Gr1, and FasL, or isotype mAb, and the gated Gr1⁺CD11b⁺ cells were analyzed for FasL.

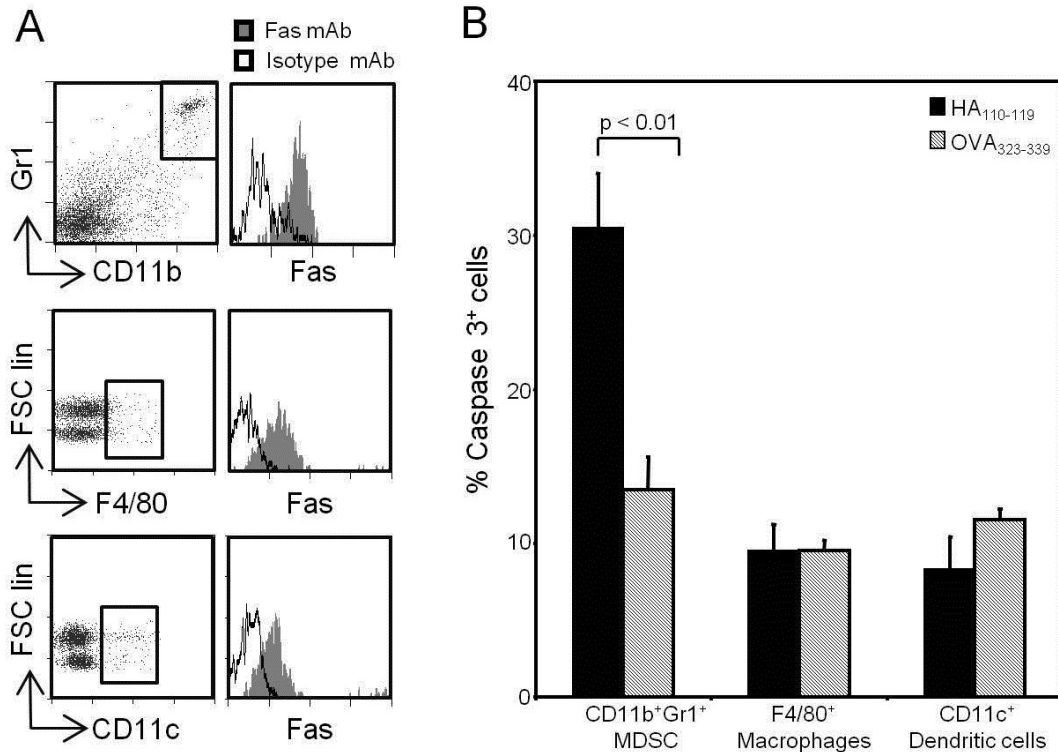


Figure S5. Macrophages and dendritic cells are Fas⁺ but do not apoptose in response to activated FasL⁺ T cells. **A.** Splenocytes from tumor-free BALB/c mice were stained with CD11b, Gr1, F4/80, CD11c and Fas mAbs, and gated cells were analyzed for Fas expression. **B.** Splenocytes of TS1 transgenic mice injected with specific peptide (HA₁₁₀₋₁₁₉) or nonspecific peptide (OVA₃₂₃₋₃₃₉) were stained with CD11b, Gr1, F4/80, CD11c and cleaved caspase 3 mAbs and the gated cells were analyzed for expression of cleaved caspase 3. Average percent of Caspase 3 positive cells from 3 mice per group.