Supplementary Information for Dolina *et al.*

Liver-primed CD8$^+$ T cells suppress antiviral adaptive immunity through Gal-9-independent Tim-3 engagement of HMGB-1 in mice

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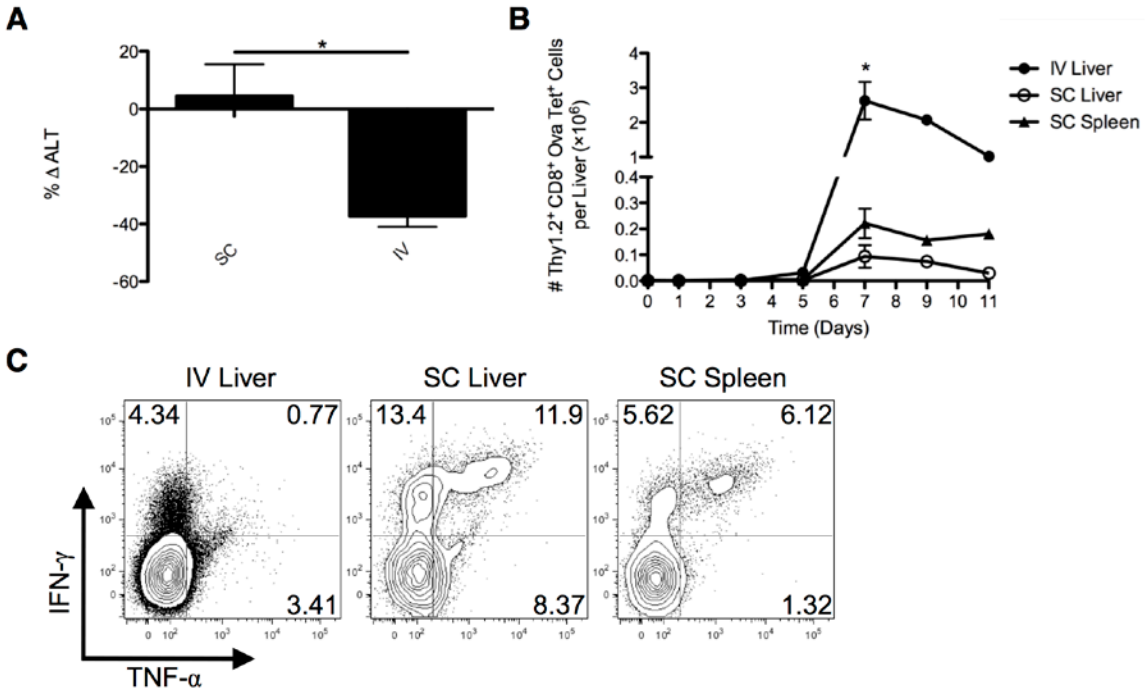
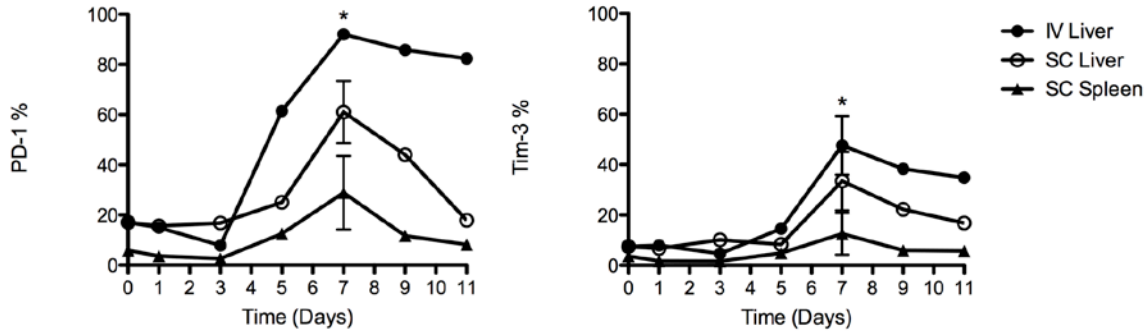
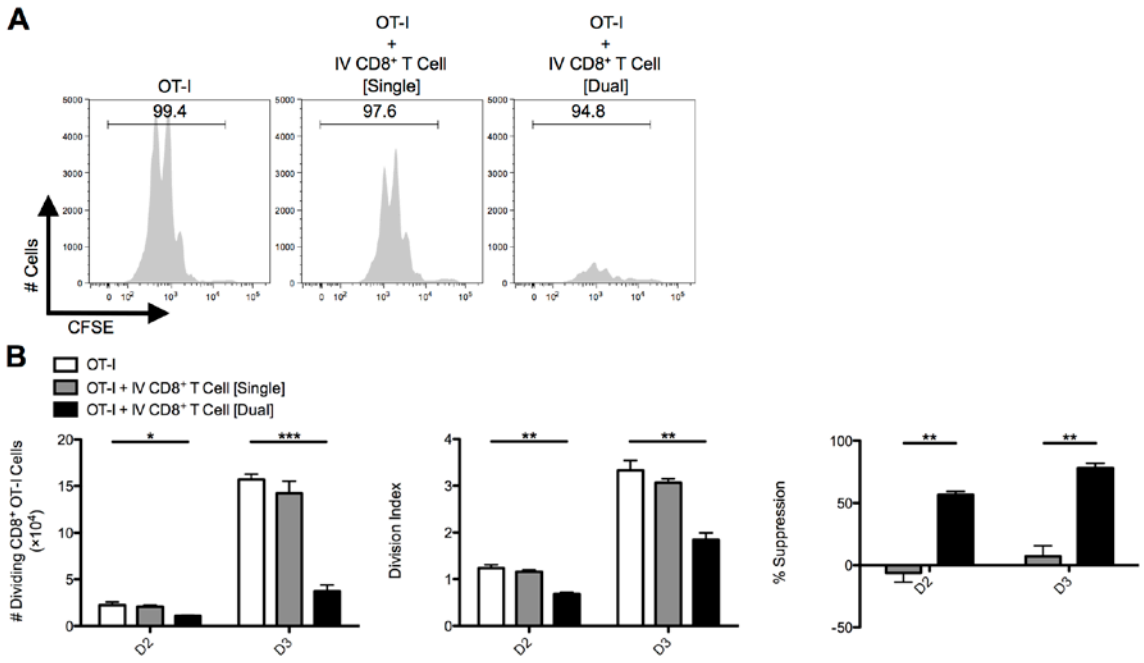


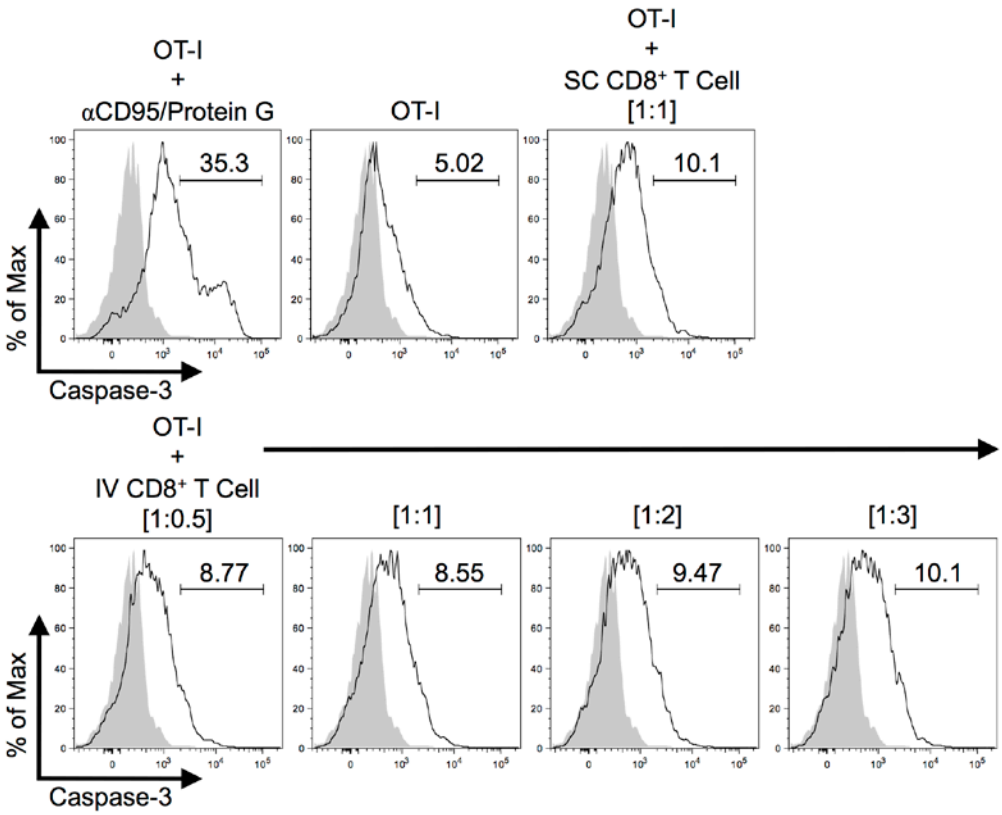
Supporting Fig. 1. Antigen-specific $CD8^+$ T cell absolute number and effector function peak at D7 post-adenovirus infection. C57BL/6 mice were SC or IV infected with 2.5×10^7 IU Ad-Ova. (A) Percent change in serum ALT from D7 to D14 post-adenovirus infection is displayed for each infection group (n = 3 per group). (B) Endogenous liver and spleen Ova-specific $CD8^+$ T cell absolute number was determined from D0 to D11. (C) TNF-α and IFN-γ were quantified at D7 after a 5 hr re-stimulation with 2 μg/mL SIINFEKL peptide (n = 4 per group). Numbers in the scatter plots represent percentages. Mean ± s.e.m.; *P < 0.05.



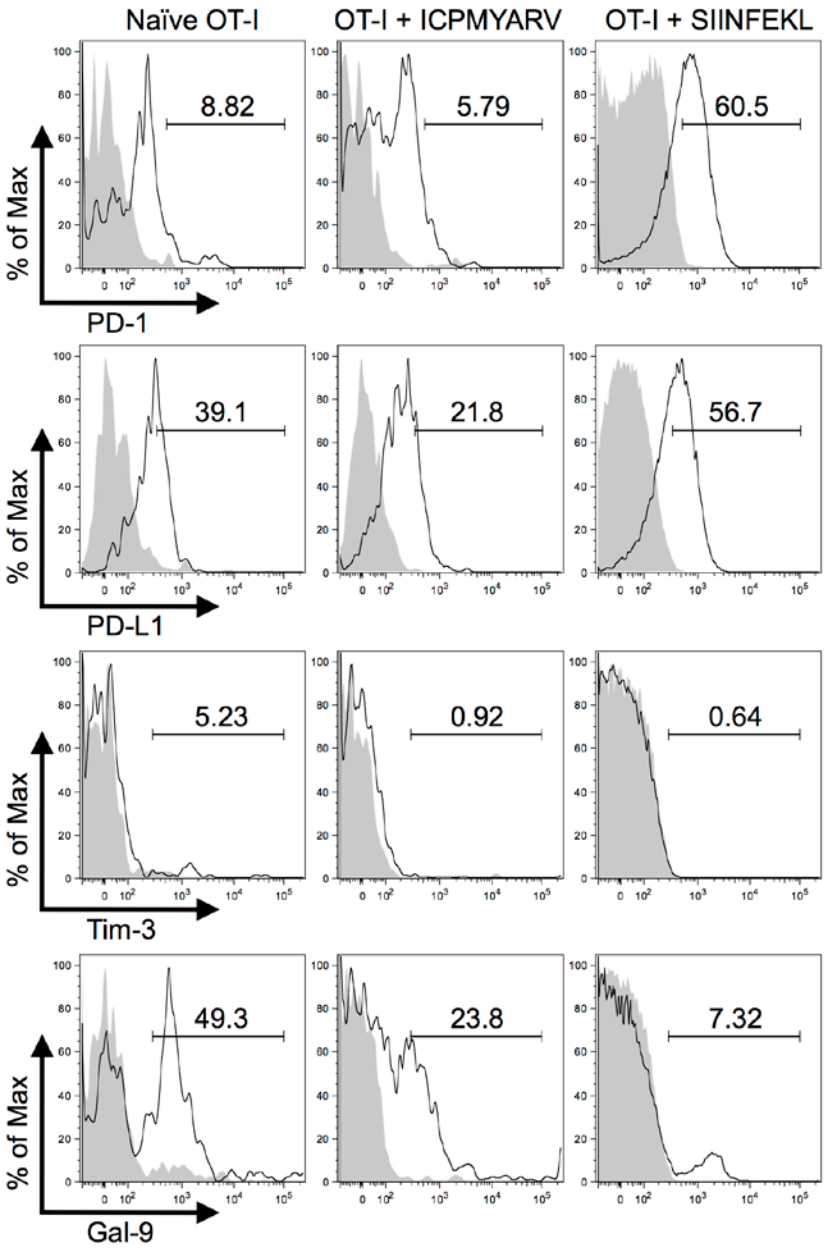
Supporting Fig. 2. PD-1 and Tim-3 inhibitory receptors peak at D7 following IV adenovirus administration. C57BL/6 mice were SC or IV infected with 2.5×10^7 IU Ad-Ova, and percent surface expression of PD-1 and Tim-3 was determined directly *ex vivo* on bulk liver and spleen $CD8^+$ T cells at days 0, 1, 3, 5, 7, 9, and 11 (one-way ANOVA/Tukey's post test; n = 3 per group). Mean ± s.e.m.; **P* < 0.05.



Supporting Fig. 3. Liver-primed $CD8^+$ T_{reg} cells restrain OT-I $CD8^+$ T cell outgrowth in an antigen-specific manner. (A) $CD8^+$ T_{reg} cells were isolated from D7 livers of C57BL/6 mice IV infected with 2.5×10^7 IU Ad-LacZ and cultured with CFSE-labeled naïve $Thy1.1^+CD8^+$ OT-I T cells at a 1:1 ratio. SIINFEKL (Single)- or SIINFEKL/ICPMYARV (Dual)-pulsed BMDCs were used as the source of antigen, and CFSE dilution was determined at D3. (B) The number of dividing OT-I T cells, OT-I T cell division index, and percent suppression by IV $CD8^+$ T_{reg} cells was assessed after D2 and D3 of culture (one-way ANOVA/Tukey's post test; n = 6 per group). Numbers in the histograms represent percentages. Mean ± s.e.m.; **P* < 0.05, ***P* < 0.01, and ****P* < 0.001.



Supporting Fig. 4. Cleaved caspase-3 is unaltered in OT-I T cells co-cultured with SC or IV $CD8^+$ T cells. C57BL/6 mice were SC or IV infected with 2.5×10^7 IU Ad-Ova, and bulk $CD8^+$ T cells were isolated from D7 SC spleens and IV livers then cultured with CFSE-labeled naïve $Thy1.1^+CD8^+$ OT-I T cells at various ratios. SIINFEKL-pulsed BMDCs were used as the source of antigen, and OT-I T cells were analyzed at D2 for the presence of intracellular cleaved caspase-3 (open) compared to FMO (filled). As a positive control for cell death, OT-I T cells alone were treated with anti-CD95 Ab and protein G. Representative cultures are displayed (n = 3 per group). Numbers in the histograms represent percentages.



Supporting Fig. 5. OT-I T cells express PD-1/PD-L1 and lack Tim-3 surface expression. Representative D2 Thy1.1^{+}CD8^{+} OT-I T cell PD-1, PD-L1, Tim-3, and Gal-9 surface expression (open) after co-culture with ICPMYARV- or SIINFEKL-pulsed BMDCs compared to isotype control (filled) is depicted (n = 3 per group).