# Combinational Immunotherapy with Allo-DRibble Vaccines and Anti-OX40 Co-Stimulation Leads to Generation of Cross-Reactive Effector T Cells and Tumor Regression

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# Supplementary data

**Figure S1 Anti-OX40 co-stimulation promoted the proliferation and expansion of memory precursor (MPEC), transitional cells, short-lived memory T cells (SLEC), and memory T cells.**

**Figure S2 Purification of DRiPs from tumor cells with a Vx3GFP fusion protein.**

(**A)** A schematic representation of Vx3GFP, a fusion protein comprising an engineered, novel, tandem ubiquitin-interacting motifs (tUIMs-Vx3) and GFP, binds polyubiquitinated proteins with high affinity and selectivity toward lys63 linkage. (**B)** Efficient isolation of polyubiquitinated proteins (DRiPs) from lysate of tumor cells that were pretreated with a proteasome inhibitor. 10, 30, 100μg Vx3GFP protein were added to C57MG lysate and DRiPs were pulled down with Ni-Sepharose excel resin. The flow through fraction was saved and DRiPs binding the resin were washed with Tris-NaCl buffer containing 5mM imidazole. Bound DRiPs were eluted with Tris-NaCl buffer containing 250mM imidazole. Samples from input, flow through, and elution were separated by SDS-PAGE and purification of DRiPs was demonstrated by western blot analysis with rabbit anti-ubiquitin antibody (1:2,000; Sigma).

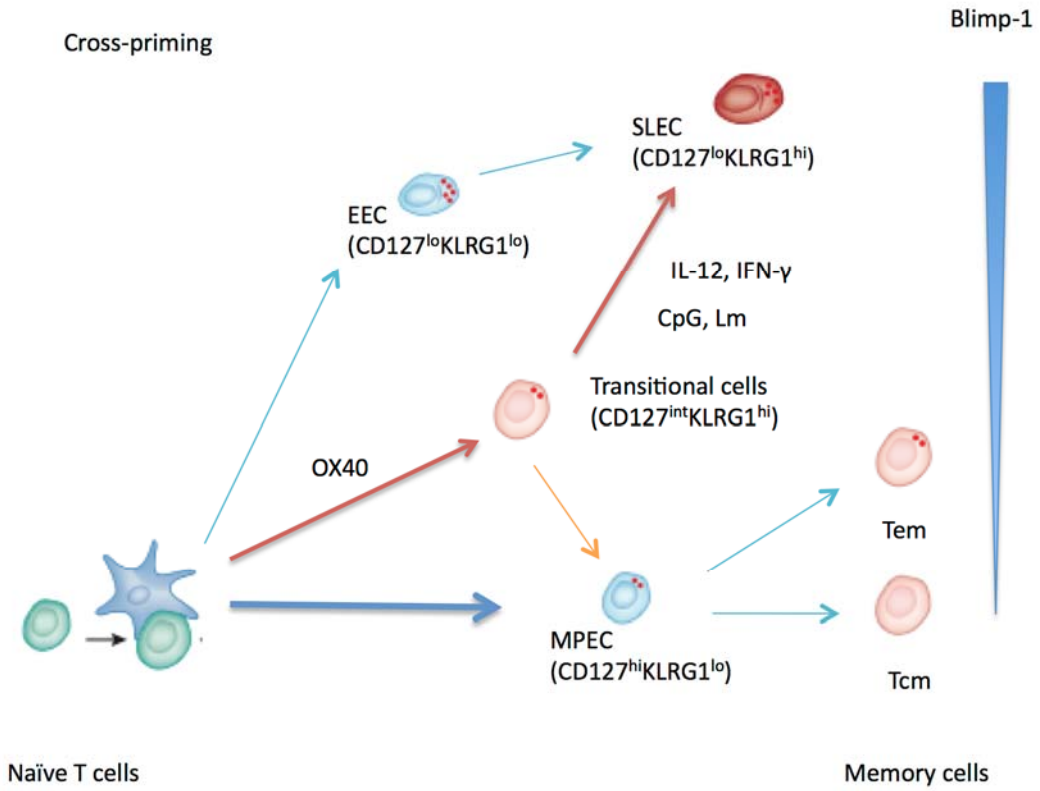
**Figure S3 Isolated DRiPs stimulate antigen-specific T-cell proliferation efficiently when loaded onto dendritic cells.**

To determine whether they could stimulate antigen-specific T cell proliferation, we isolated DRiPs from B78H1 cells that expressed R-GFP-OVA protein after pretreatment with bortezomib. DRiPs from B78H1 cells that express GFP, but not OVA, and liver tissues were included as specificity

controls. Mutu-1940 DCs were loaded with aluminum nanoparticles plus the indicated amount of DRiPs. CFSE-labeled OT-I transgenic T cells were added after a 6-hour incubation. T-cell activation was assessed by flow cytometry analysis of CFSE dilution of labeled T cells at day 6. Mutu-1940 DCs pulsed with antigen-expressing ubiquitinated DRiPs were efficient stimulators of OT-I $CD8^+$ T cells. Ubiquitinated DRiPs from normal liver tissue or antigen-free B78H1 cells barely stimulated OT-I $CD8^+$ T cells. *, $P < 0.05$; **, $P < 0.01$.

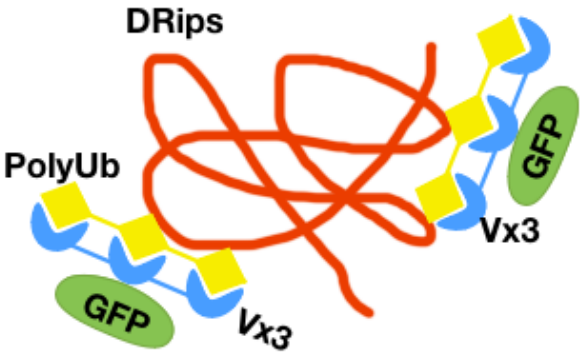
**Figure S4 Schematic illustration of a possible pathway for the selective accumulation of DRiPs and generation of DRibbles.**

# Supplementary Figure 1

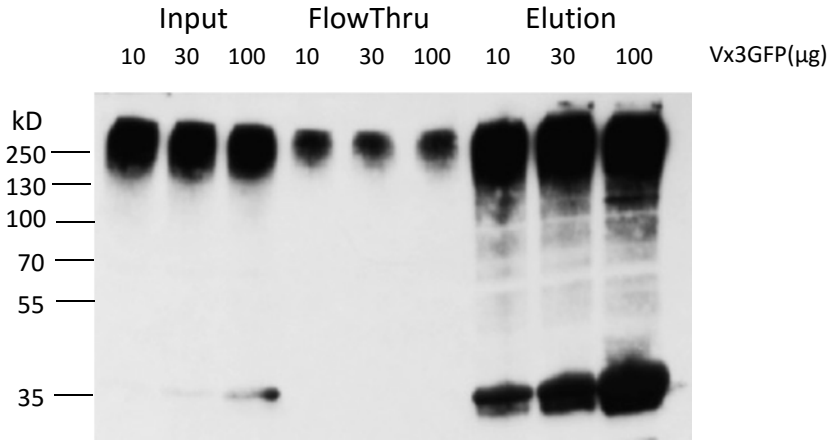


# Supplementary Figure 2

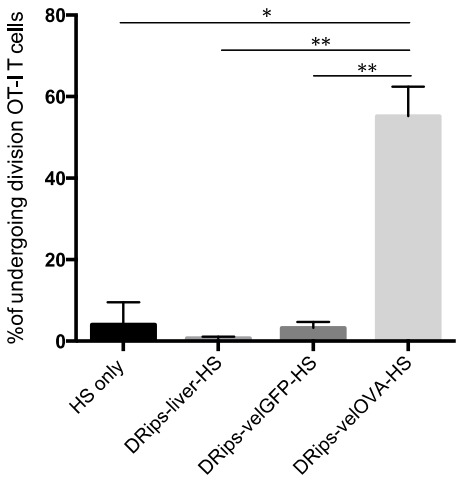
**A**




**B**



# Supplementary Figure 3



# Supplementary Figure 4