

Supporting Information for

Cancer immunotherapy *via* targeting cancer stem cells using vaccine nanodiscs

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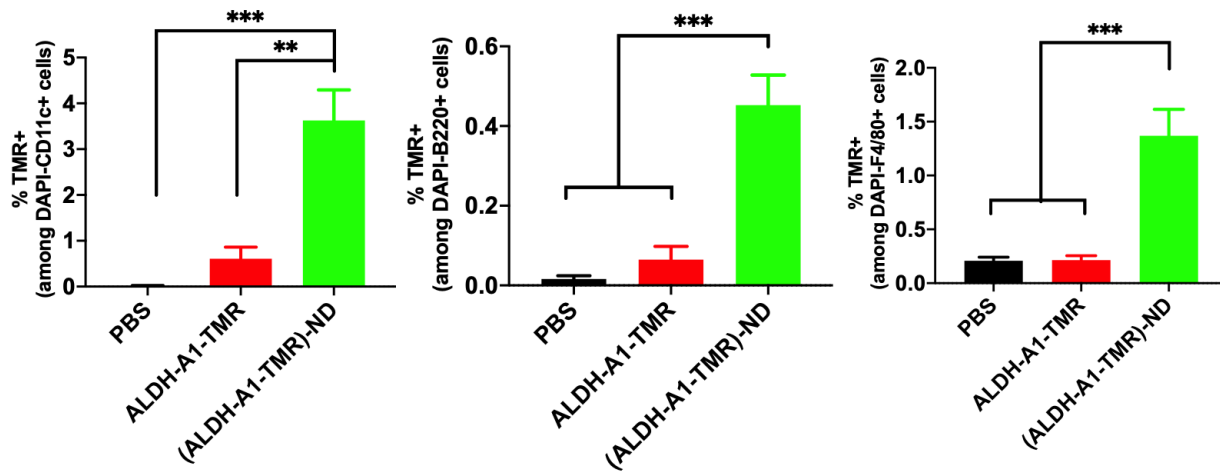


Figure S1. ND is internalized by APCs in draining lymph nodes. C57BL/6 mice were administered s.c. with 15.5 nmol TMR-tagged ALDH-A1 peptides as a soluble or ND form. After 24 h, TMR taken up by APCs in dLNs was quantified by flow cytometry analysis. The data show mean \pm SEM. Statistical significance was calculated by one-way ANOVA, followed by the Tukey's multiple comparisons post-test. ** $p < 0.01$, *** $p < 0.001$, and **** $p < 0.0001$.

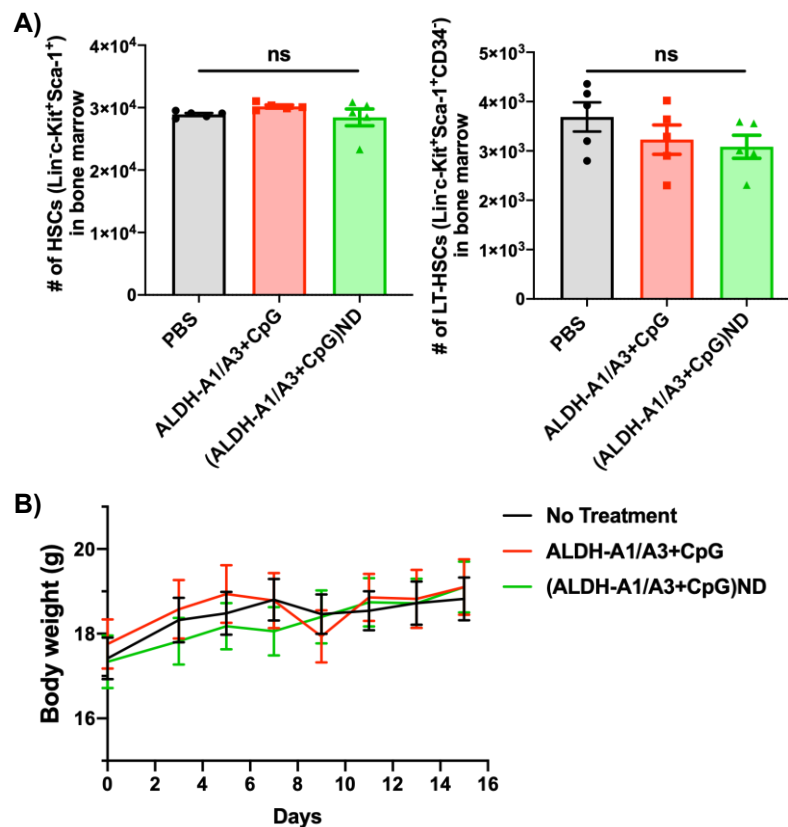


Figure S2. Effects of ALDH vaccination on hematopoietic stem cells and animal body weight. C57BL/6 mice were immunized with (ALDH-A1/A3-CpG)-ND or a soluble mixture of ALDH-A1/A3 and CpG (15.5 nmol/dose each Ag peptide and 15 μ g/dose CpG) on days 0 and 7. Shown are the **A)** numbers of hematopoietic stem cells (HSC, Lin⁻c-Kit⁺Sca-1⁺) and long-term HSCs (LT-HSC, Lin⁻c-Kit⁺Sca-1⁺CD34⁺) in bone marrow on day 15 and **B)** changes in animal body weight over time. The data show mean \pm SEM. Statistical significance was calculated by A) one-way ANOVA, or B) two-way ANOVA, followed by the Tukey's multiple comparisons post-test. ns = not statistically significant.

	No Treatment	ALDH-A1/A3+CpG	(ALDH-A1/A3+CpG) ND	Normal Range
Creatinine (CREA, mg/dl)	0.4 ± 0.01	0.4 ± 0.1	0.4 ± 0.03	0.09 - 0.4
Glucose (GLUC, mg/dl)	247.6 ± 34.9	298.6 ± 33.7	241.2 ± 30.0	79.4 - 354.7
Albumin (ALB, g/dl)	3.4 ± 0.1	3.0 ± 0.16	3.3 ± 0.1	2.7 - 4.2
TRPO, g/dl	5.0 ± 0.2	5.6 ± 0.2	5.5 ± 0.2	4.6 - 7.2
Calcium arsenazo (CALA, mg/dl)	9.8 ± 0.2	8.9 ± 0.4	9.8 ± 0.6	9.0 - 12.4
Alanine transaminase (ALT, U/L)	47.4 ± 7	71.4 ± 37.1	47.4 ± 6.4	24.3 - 115.3
Total bilirubin (TBIL, mg/dl)	0.3 ± 0.1	0.3 ± 0.09	0.2 ± 0.1	0.1 - 0.6
Blood urea nitrogen (BUN, mg/dl)	31.6 ± 5.7	39.2 ± 4.7	32.4 ± 4.4	5.2 - 30.7
Alkaline phosphatase (ALP, U/L)	190.0 ± 20.0	158.6 ± 13.2	164.4 ± 15.7	65.5 - 364.2

Table S1. C57BL/6 mice were immunized as in **Figure S2**, and serum biochemical markers were quantified on day 15.

	No Treatment	ALDH-A1/A3+CpG	(ALDH-A1/A3+CpG)ND	Normal Range
White blood cells (WBC, K/uL)	7.6 ± 1.9	10.6 ± 1.1	6.9 ± 1.2	1.8 - 10.7
Neutrophils (NE, K/uL)	0.9 ± 0.3	1.1 ± 0.2	1.3 ± 0.3	0.1 - 2.4
Lymphocytes (LY, K/uL)	6.5 ± 1.8	5.7 ± 1.0	9.1 ± 1.0	0.9 - 9.3
Monocytes (MO, K/uL)	0.1 ± 0.04	0.1 ± 0.06	0.2 ± 0.1	0 - 0.4
Eosinophil (EO, K/uL)	0.01 ± 0.008	0.008 ± 0.01	0.006 ± 0.005	0 - 0.2
Basophil (BA, K/uL)	0.004 ± 0.009	0 ± 0	0 ± 0	0 - 0.2
Neutrophils (NE, %)	12.1 ± 1.8	15.6 ± 2.3	12.3 ± 2.6	6.6 - 38.9
Lymphocytes (LY, %)	85.6 ± 2.1	82.6 ± 2.0	85.5 ± 1.9	55.8 - 91.6
Monocytes (MO, %)	2.0 ± 0.9	1.7 ± 0.7	2.1 ± 1.1	1.10 - 7.5
Eosinophil (EO, %)	0.2 ± 0.1	0.1 ± 0.1	0.05 ± 0.03	0.0 - 3.9
Basophil (BA, %)	0.08 ± 0.1	0.02 ± 0.03	0.03 ± 0.02	0.0 - 2.0
Red blood cells (RBC, M/uL)	9.4 ± 0.5	8.1 ± 3.4	9.1 ± 0.4	6.36 - 9.42
Hemoglobin (HB, g/dL)	13.4 ± 0.8	11.5 ± 4.7	13.3 ± 0.4	11.0 - 15.1
Hematocrit (HCT, %)	40.9 ± 2.5	42.4 ± 1.5	40.2 ± 1.2	35.1 - 45.4
Mean Corpuscular Volume (MCV, fL)	43.4 ± 0.4	43.3 ± 0.4	44.2 ± 1.4	45.4 - 60.3
Mean corpuscular hemoglobin (MCH, Pg)	14.2 ± 0.2	14.2 ± 0.3	14.7 ± 0.5	14.1 - 19.3
Mean corpuscular hemoglobin concentration (MCHC, g/dL)	32.8 ± 0.5	32.7 ± 0.9	33.1 ± 0.7	30.2 - 34.2
Red cell distribution (RDW, %)	15.9 ± 0.5	17.7 ± 0.4	18.8 ± 1.7	12.4 - 27.0
Platelets (PLT, K/uL)	714.4 ± 69.0	923.8 ± 53.5	716.4 ± 130.0	592 - 2972
Mean platelet volume (MPV, fL)	4.5 ± 0.1	4.4 ± 0.0	4.8 ± 0.1	5.0 - 20

Table S2. C57BL/6 mice were immunized as in **Figure S2**, and the complete blood counts were quantified on day 15.