SUPPLEMENTARY INFORMATION
Vaccines targeting the inflammasome protein ASC reduce inflammation and downstream pathology in multiple disease models

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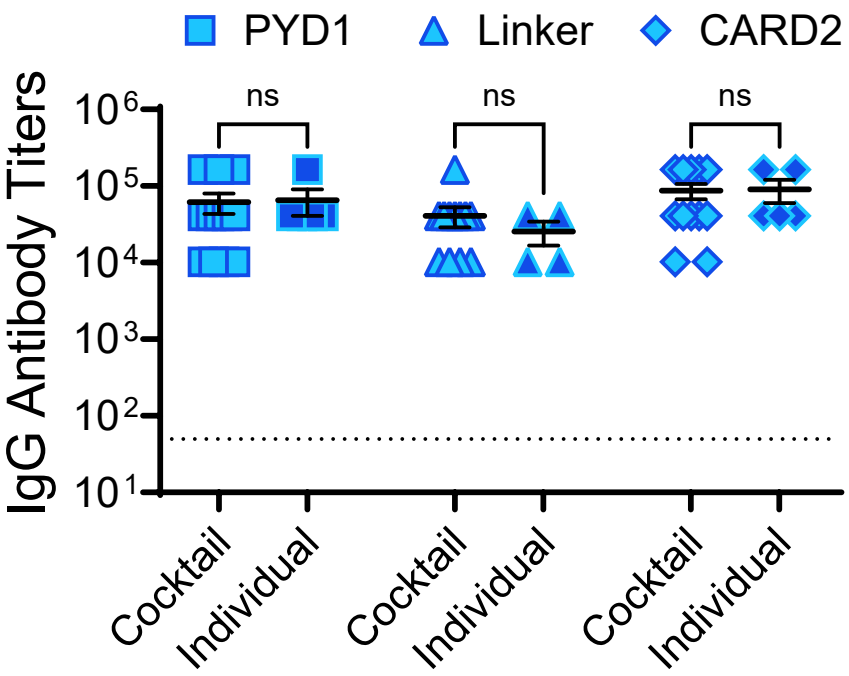
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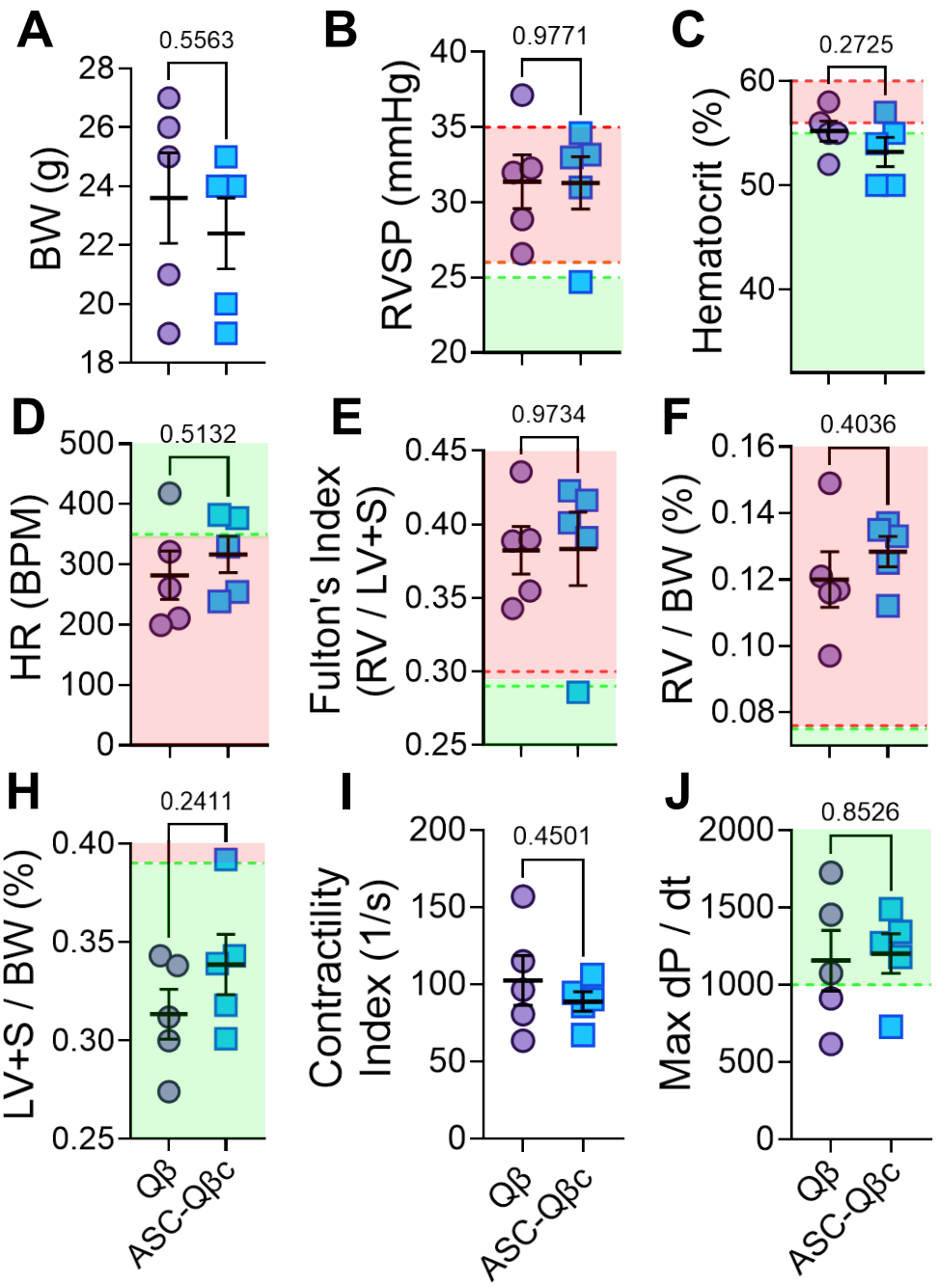
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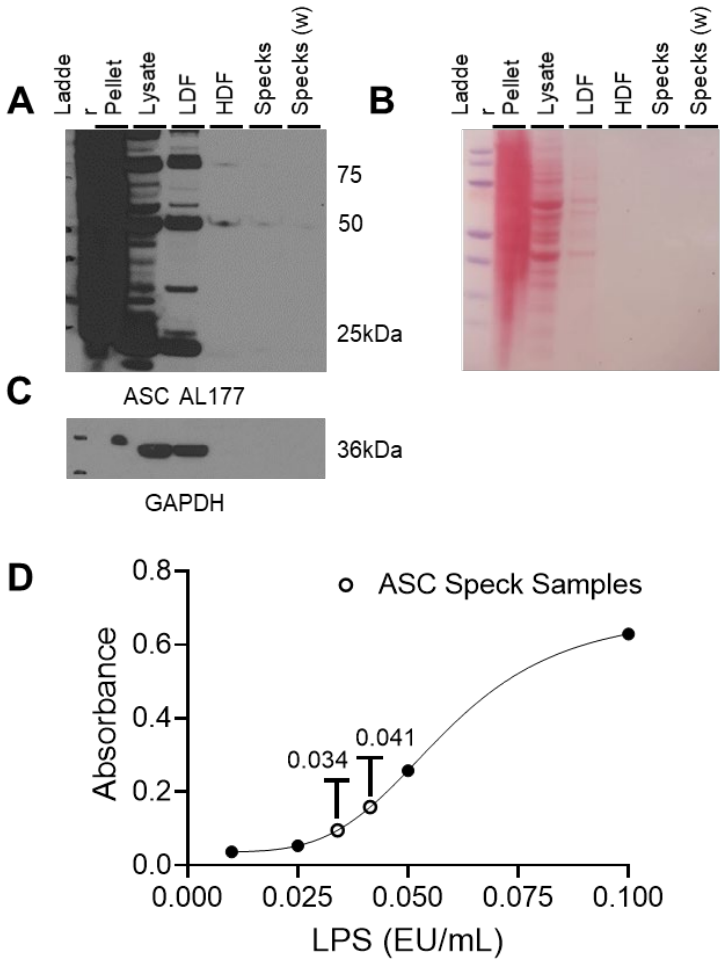
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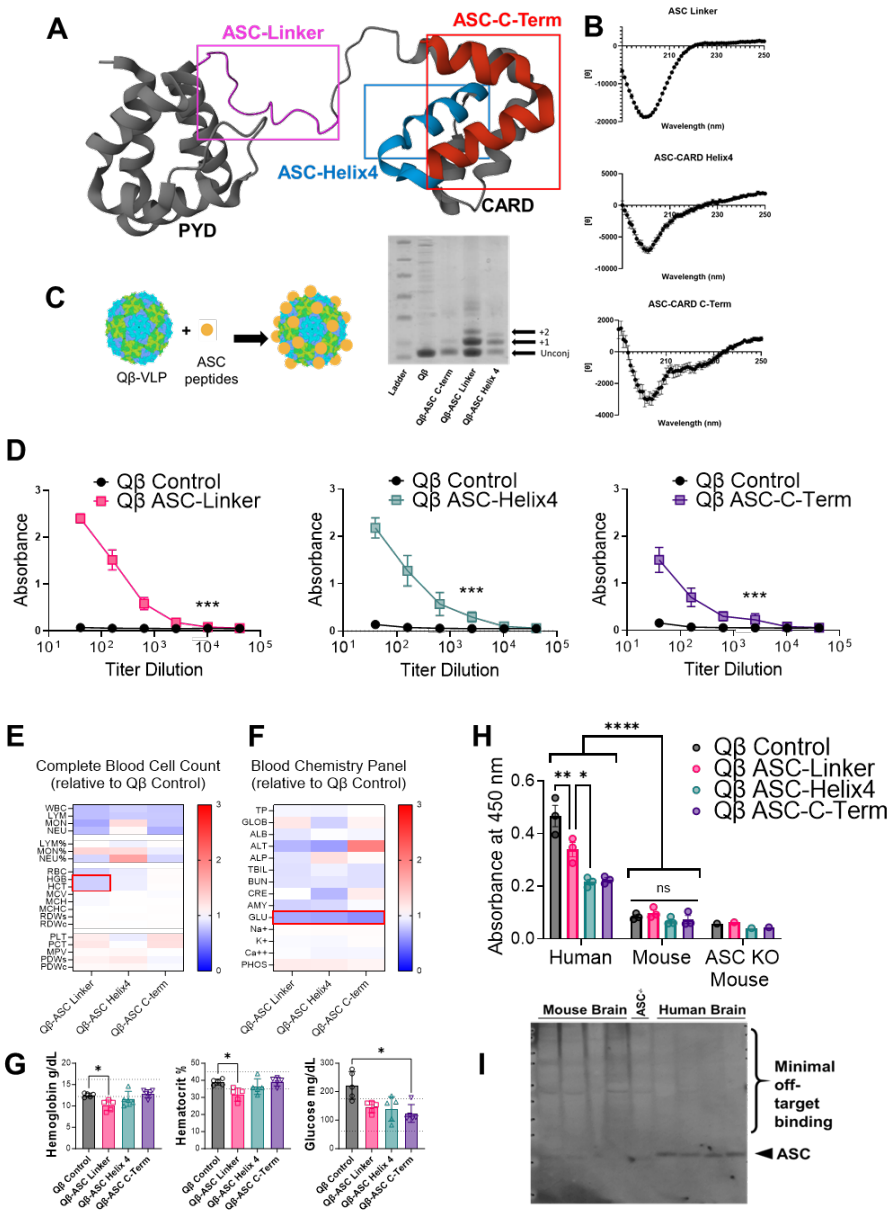
Supplementary Figure 1: Antibody titers against the PYD1, Linker, and CARD2 peptides are not significantly different when administered in a cocktail vaccine in the same animal compared to individually in different animals. Serum IgG antibody titers were assessed by ELISA in animals vaccinated with ASC-Qβc (cocktail containing PYD1, Linker, and CARD2 mASC-Qβ VLPs) or in animals that were individually vaccinated with the respective mASC-Qβ VLPs. Two-way ANOVA with Šídák's multiple comparisons. Data presented as mean ± SEM. n=12.



Supplementary Figure 2: Qβ-vaccination does not exacerbate chronic hypoxia-induced pulmonary hypertension. C57BL/6 mice were vaccinated twice with either unconjugated Qβ (n = 5) or ASC-Qβc (n = 5) prior to subjecting them to chronic hypoxia. Hypoxia was induced in vaccinated mice by placing the mice in a hypobaric chamber (380mmHg). **A, B, C, D, E, F, G, H, I.** Body weight, hematocrit, and indices of pulmonary hypertension were assessed after 21 days of hypoxic exposure. BW = body weight; RVSP = right ventricular systolic pressure; Hematocrit = % red blood cells of total blood volume; HR (BPM) = Heart rate (beats per minute); Fulton's Index = weight of right ventricle (RV)/weight of left ventricle + septum (LV + S); dp/dt = maximum rate of left ventricular pressure rise. Green shaded area = typical range values in normal normoxic C57BL/6j mice; Red shaded area = typical range values in hypoxic conditions. Data shown as mean ± SEM; Unpaired *t-test*.



Supplementary Figure 3: Validation of ASC speck purification shows no evidence of contaminating proteins or endotoxin in purified ASC speck samples. (**A**) Western blot validation of ASC purification by density fractionation showing presence of ASC in final sample preparation (**B**) Ponceau S staining of Western blot showing no additional proteins present in the ASC speck preparation after density fractionation. (**C**) GAPDH staining of the Western blot confirming the absence of small cytosolic proteins in the ASC speck preparation after density fractionation (**D**) Endotoxin assay of ASC speck preparations with estimated values at 0.03-0.04 EU/mL quantifying LPS contamination of the final ASC speck stock preparations after density fractionation at <0.005 ng/mL. LDF=low density fraction; HDF=high density fraction; Specks=ASC specks after resuspension in CHAPS; Specks(w)=ASC specks after washing step.



Supplementary Figure 4: ASC-Qβ VLPs targeting human ASC are safe and immunogenic in mice but preferentially bind to human ASC over mouse ASC. A. Schematic of ASC protein showing targeted regions. **B**. Circular dichroism spectroscopy of human ASC peptides showing random coil/unstructured secondary structure. **C**. Schematic of conjugation and mobility shift gel electrophoresis showing successful conjugation of human ASC peptides to Qβ VLPs. **D**. IgG antibody titer dilution curves from sera of human ASC-Qβ VLP vaccinated mice against their respective peptide targets. **E**. Complete blood cell counts of human ASC-Qβ VLP vaccinated mice relative to unconjugated Qβ vaccinated mice. **F**. Comprehensive diagnostic profiles of human ASC-Qβ VLP vaccinated mice relative to unconjugated Qβ vaccinated mice. **G**. Hemoglobin, hematocrit, and blood glucose levels showed significant changes identified during blood profiling. **H**. Sandwich ELISA of human and mouse brain lysate using vaccine immune sera as the detection antibody for ASC protein, demonstrating increased binding to human ASC over mouse ASC. **i**. Western blot of human and mouse brain lysate using vaccine immune sera as the detection antibody for ASC protein, demonstrating specific binding to human but not mouse ASC. **D, E, F, G** One-way ANOVA. **H**. Two-way ANOVA. Data presented as mean ± SEM. *p<0.05; **p<0.01; ***p<0.001; ****p<0.0001. **A**. Protein data base. **C**. Created using BioRender.com.