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Supplemental information

mRNA-delivery of IDO1 suppresses T cell-mediated autoimmunity

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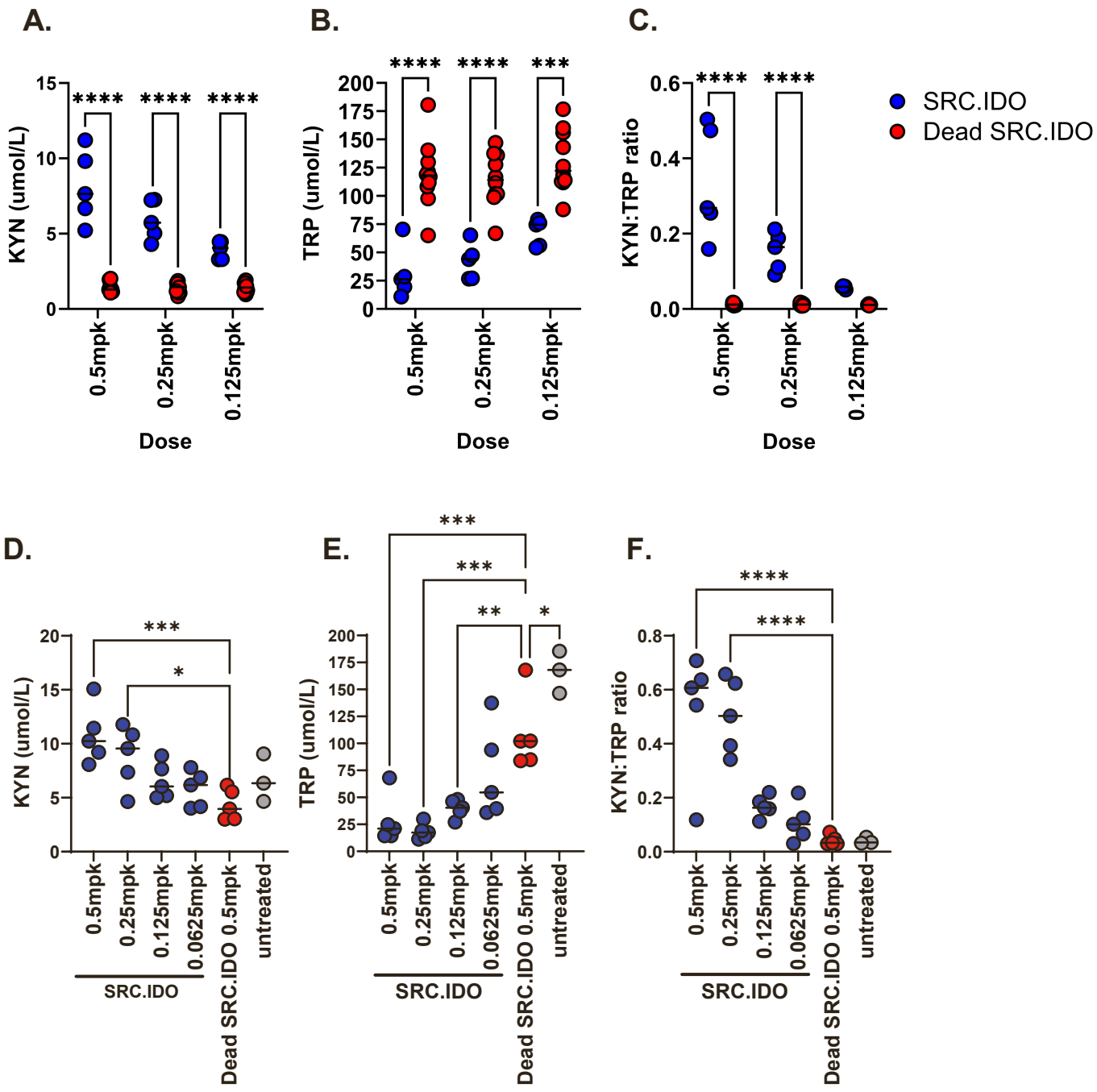
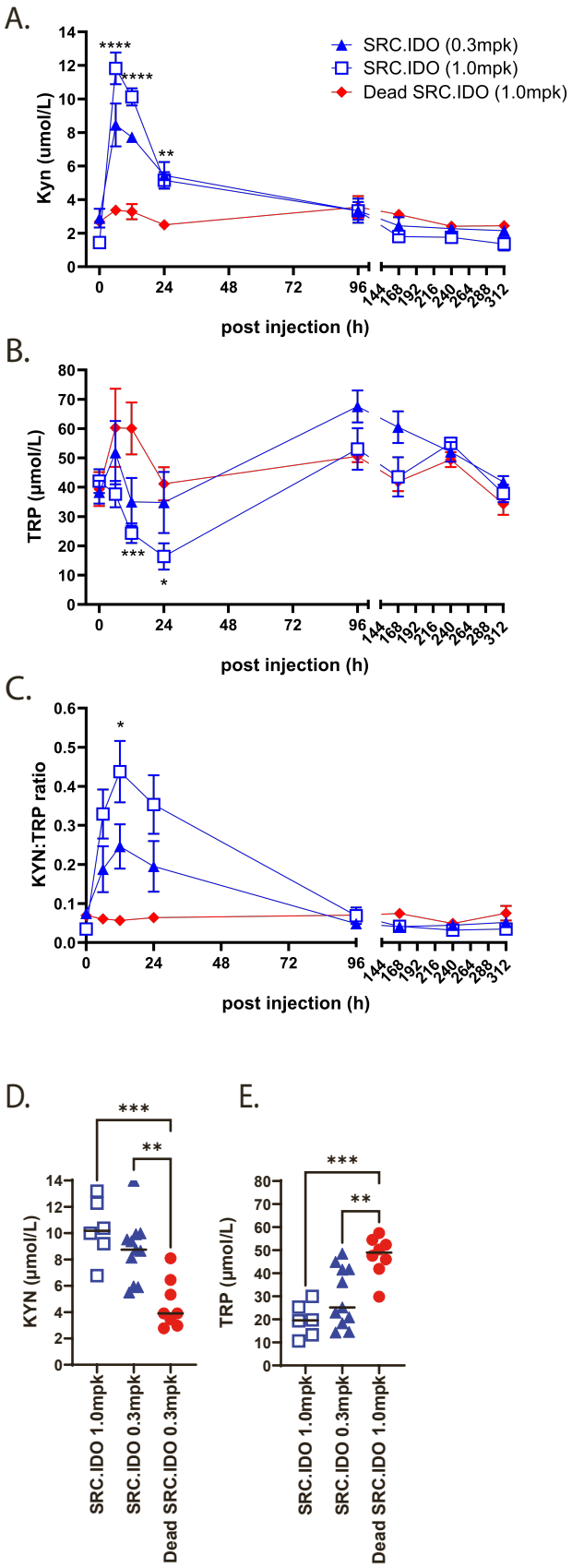
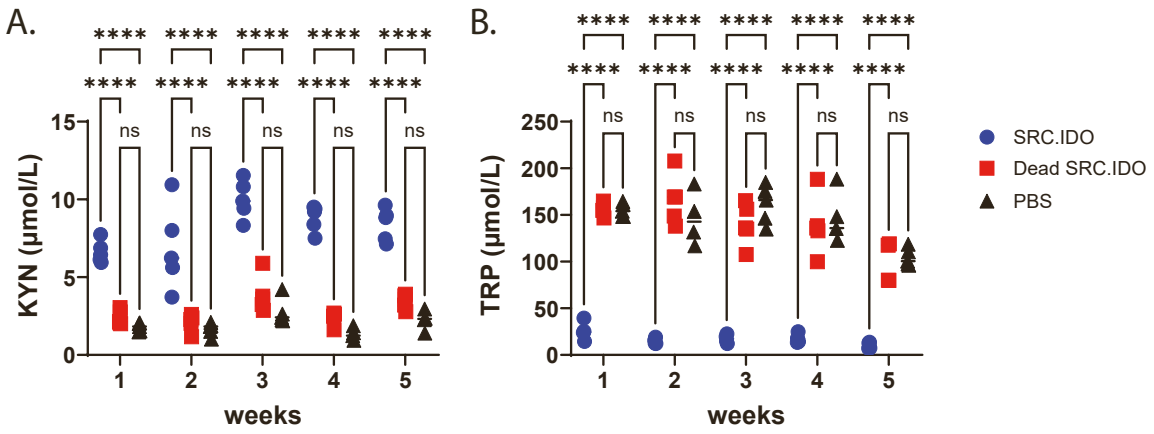


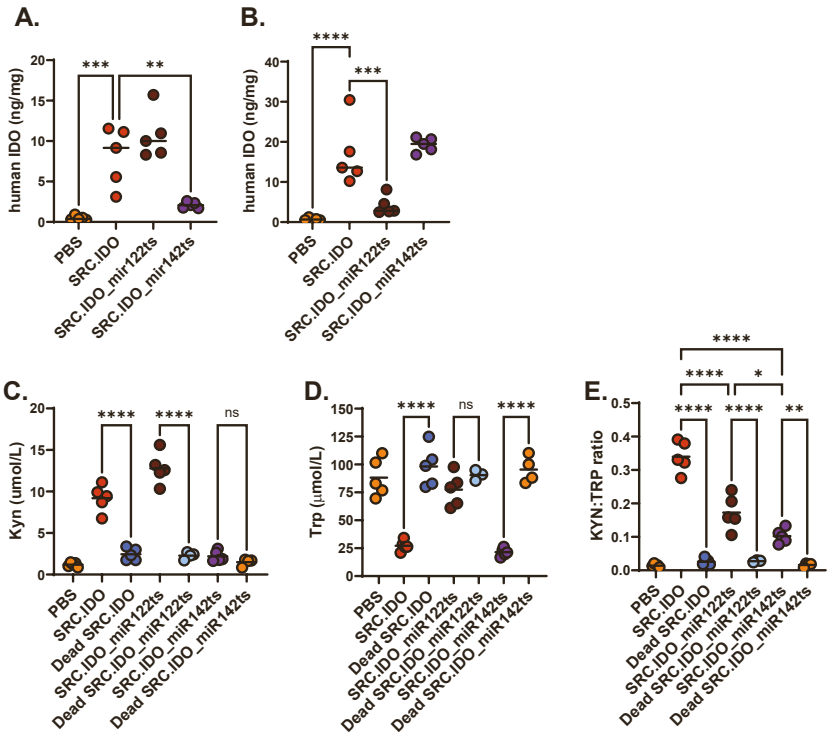
Supplemental Figure 1. Engineered IDO1 induces dose-dependent changes in serum TRP and KYN in mouse and rat. Related to Fig 2. (**A**–**C**) Naïve C57BL/6 mice were injected i.v. with 0.5, 0.25, or 0.125 mg/kg of LNP A-formulated mRNA. Serum (**A**) KYN, (**B**) TRP, and (**C**) KYN:TRP ratios were determined at 72 h by ELISA. Significance was determined by one-way ANOVA compared with Dead SRC.IDO controls with secondary Sidak's multiple comparisons test. ****p<0.0001. (**D**–**F**) Naïve Sprague Dawley rats were injected i.v. with 0.5, 0.25, 0.125 or 0.0625 mg/kg of LNP A-formulated mRNA and plasma (**D**) KYN, (**E**) TRP, and (**F**) KYN:TRP ratios were determined at 72 h by ELISA. Data are individual rats and medians of n=3–5 animals/group and representative of 2 similar experiments. Significance was determined by one-way ANOVA compared with Dead SRC.IDO controls with secondary Dunnett's multiple comparisons test. *p<0.05, **p<0.005, ***p<0.001, ****p<0.0001.



Supplemental Figure 2. mRNA-delivered anchored IDO1 is bioactive in NHP. Related to Fig 2. (**A**, **B**) NHP were infused over 60 mins along with 0.3 or 1.0 mg/kg of LNP B-formulated mRNA. Blood samples were obtained at 6, 12, 24, 96, 168, 240, and 312 h post-injection. (**A**) KYN and (**B**) TRP were measured in the serum by ELISA. N=3 NHP/group. Data are mean and s.e.m. and are representative of similar experiments. Significance was determined by two-way ANOVA compared with Dead SRC.IDO controls using Dunnett's multiple comparisons tests. *p<0.05, **p<0.005, ***p<0.0005, ****p<0.0001. (**C** & **D**) (**C**) KYN levels at 6 h and (**D**) TRP levels at 24 h post-injection determined by ELISA from serum. N=5–11 NHP/group. Data are individual NHP and median and are pooled from 3 similar experiments. Significance was determined by one-way ANOVA compared with Dead SRC.IDO controls using Dunnett's multiple comparisons tests. **p<0.005, ***p<0.0005.



Supplemental Figure 3. In vivo function is sustained with repeat dosing of SRC.IDO. Related to Fig 2. Naïve C57BL/6 mice were injected i.v. with 0.5 mg/kg of LNP A-formulated mRNA every 7 days for 5 weeks. Serum (**A**) KYN and (**B**) TRP were determined at 24 h post each administration by ELISA. Significance was determined by two-way ANOVA with Tukey's multiple comparisons test. ****p<0.0001. Data are individual mice and medians of 3-5 mice/groups and representative of 2 similar experiments.



Supplemental Figure 4. Protein expression and changes in metabolite levels in SRC.IDO_miR122ts- and SRC.IDO_miR142ts-treated mice. Related to Fig 6. Naïve C57BL/6 mice were injected i.v. with 0.5 mg/kg of LNP A-formulated mRNA. Human IDO1 protein expression in (**A**) spleen and (**B**) liver lysates was determined at 24 h by ELISA. Significance was determined by one-way ANOVA compared to SRC.IDO with Dunnett's multiple comparisons. **p<0.005, ***p<0.0005, ****p<0.0001. Serum (**C**) KYN, (**D**) TRP and (**E**) KYN:TRP ratio were determined at 24 h by ELISA. Significance was determined by one-way ANOVA with Tukey's multiple comparisons. *p<0.05, **p<0.005, ****p<0.0001. Data are individual mice and medians of 5 mice/groups and representative of 4 similar experiments.

Supplemental Table 2. Lists the antibodies used for the flowcytometry analysis used for aGVHD studies, related to the Flowcytometry for aGVHD section of the STAR Methods.

Color	Antigen	Clone	Company	Order number	Dilution
APC-eFluor780	Viability	n/a	Invitrogen	65-0865-14	1:5000
BUV805	CD3	145-2C11	BD biosciences	749276	1:400
BV785	CD4	GK1.5	Biolegend	100453	1:400
BUV737	CD8	53-6.7	BD Biosciences	612759	1:400
AF700	CD19	6D5	Biolegend	115528	1:800
PE	H2-Kd	SF1-1.1.1	Invitrogen	12-5957-82	1:400
BV421	H2-Kb	AF6-88.5	Biolegend	116525	1:400
AF647	FoxP3	150D	Biolegend	320014	1:200
BV605	Ki67	16A8	Biolegend	652413	1:200
FITC	Caspase-3	C92-605	BD Biosciences	559341	1:500