

10. Supporting Information

10.1. Figure S1: Evaluation of cardiac abnormalities in A/J mice immunized with BCKD_k

111-130. Groups of mice were immunized with or without BCKD_k 111-130; after 20 days, animals were euthanized, hearts were weighed, and the heart weight (wt) to body wt ratios were then determined. Mean \pm SEM values representing heart and body wts and heart wt/body wt ratios for a group of mice are shown (n=3/group). The *p* values were determined using Student's *t*-test (**P* < 0.05 and ***P* < 0.001).

10.2. Figure S2: BCKD_k peptides that induce T cell responses, but not myocarditis in

immunized animals. Groups of A/J mice were immunized with the indicated peptides in CFA, and after three weeks, animals were euthanized to prepare LNCs from the draining lymph nodes. Cells were stimulated with the immunizing peptides or RNase 43-56 (control) for two days, and after pulsing with tritiated-thymidine for 16 hours, proliferative responses were measured as cpm. Mean \pm SEM values obtained from two individual experiments, each representing 2 to 3 mice, are shown. The *p* values were determined using Student's *t*-test (**P* < 0.05 and ***P* < 0.001 between indicated doses).

10.3. Figure S3: Proliferative responses of lymphocytes from naive animals to BCKD_k

peptides. Lymphocytes containing a mixture of LNCs and splenocytes obtained from naive A/J mice were stimulated with the indicated peptides or RNase 43-56 (control) for two days, and after pulsing with tritiated-thymidine for 16 hours, proliferative responses were measured as cpm. Mean \pm SEM values obtained from five mice are shown.

10.4. Figure S4: Cytokine responses induced by BCKD_k 71-90 and BCKD_k 141-160. LNCs

were prepared from animals that received CFA/peptide emulsions twice, and the cells were stimulated with or without immunizing peptides or RNase 43-56 (control). Supernatants

collected on day 3 poststimulation were analyzed using cytokine-capture beads and detection antibodies based on cytometric bead array analysis. Left panel **A**): BCKD_k 71-90; right panel **B**): BCKD_k 141-160. Mean \pm SEM values obtained from three to four individual experiments are shown. The *p* values were determined using Student's *t*-test (**P* < 0.05, ***P* < 0.005 and ****P* < 0.0005). ND; not detectable.

Table S1: List of overlapping peptides of BCKD_k used to determine their immunogenicity

Peptide	Sequence
BCKD _k 1-20	STSATDTHHVELARERSKTV
BCKD _k 11-30	ELARERSKTVTSFYNQSAID
BCKD _k 21-40	TSFYNQSAIDVAAEKPSVRL
BCKD _k 31-50	VAAEKPSVRLTPTMMLYSGR
BCKD _k 41-60	TPTMMLYSGRSQDGSHELLKS
BCKD _k 51-70	SQDGSHELLKSGRYLQQELPV
BCKD _k 61-80	GRYLQQELPVRIAHRIKGFR
BCKD _k 71-90	RIAHRIKGFRSLPFIIGCNP
BCKD _k 81-100	SLPFIIGCNPTILHVHELYI
BCKD _k 91-110	TILHVHELYIRAFQKLTDFP
BCKD _k 101-120	RAFQKLTDFPPIKDQADEAQ
BCKD _k 111-130	PIKDQADEAQYCQLVRQLLD
BCKD _k 121-140	YCQLVRQLLDDHKDVVTLA
BCKD _k 131-150	DHKDVVTLAEGLRERKHI
BCKD _k 141-160	EGLRESRKHIQDEKLVRYFL
BCKD _k 151-170	QDEKLVRYFLDKTLTSRLGI
BCKD _k 161-180	DKTLTSRLGIRMLATHHLAL
BCKD _k 171-190	RMLATHHLALHEDKPDFVGI
BCKD _k 181-200	HEDKPDFVGIICTRLSPKKI
BCKD _k 191-210	ICTRLSPKKIIEKWVDFARR
BCKD _k 201-220	IEKWVDFARRLCEHKYGNAP
BCKD _k 211-230	LCEHKYGNAPRVRINGHVAA
BCKD _k 221-240	RVRINGHVAARFPFIPMPLD
BCKD _k 231-250	RFPFIPMPLDYILPELLKNA
BCKD _k 241-260	YILPELLKNAMRATMESHLD
BCKD _k 251-270	MRATMESHLDTPYNVPDVVI
BCKD _k 261-280	TPYNVPDVVITIANNIDILI
BCKD _k 271-290	TIANNIDILIIRISDRGGGI
BCKD _k 281-300	IRISDRGGGIAHKDLDRVMD
BCKD _k 291-310	AHKDLDRVMDYHFTTAEAST
BCKD _k 301-320	YHFTTAEASTQDPRINPLFG
BCKD _k 311-330	QDPRINPLFGHLDMHSGGQS
BCKD _k 321-340	HLDMHSGGQSGPMHGFGL
BCKD _k 331-350	GPMHGFGLPTSRA YAEYL
BCKD _k 341-360	PTSRA YAEYLGGS LQLQSLQ
BCKD _k 351-370	GGSLQLQSLQGIGTDVYLRL
BCKD _k 361-380	GIGTDVYLRLRHIDGreesF

Table S2: T cell responses induced by peptides of BCKD_k

Pools	Peptides	Fold difference†	
		10 (µg/µl)	100 (µg/µl)
I	RNase 43-56 (control)	0.93	0.99
	BCKD _k 1-20	0.97	1.18
	BCKD _k 11-30	0.86	1.16
	BCKD _k 21-40	0.82	1.35
	BCKD_k 31-50	1.16	1.34
	BCKD _k 41-60	1.06	1.21
II	RNase 43-56 (control)	1.04	1.08
	BCKD_k 51-70	1.28	1.47
	BCKD_k 61-80	1.46	1.80
	BCKD_k 71-90	1.49	2.55
	BCKD _k 81-100	1.08	1.81
	BCKD_k 91-110	1.86	2.05
III	RNase 43-56 (control)	1.04	1.06
	BCKD_k 101-120	1.21	1.46
	BCKD_k 111-130	1.21	1.75
	BCKD_k 121-140	1.41	1.84
	BCKD _k 131-150	1.08	1.53
	BCKD_k 141-160	1.58	1.66
IV	RNase 43-56 (control)	1.05	1.12
	BCKD _k 151-170	1.08	1.46
	BCKD_k 161-180	1.24	1.88
	BCKD _k 171-190	0.98	1.12
	BCKD _k 181-200	1.03	1.22
	BCKD _k 191-210	1.14	1.40
V	RNase 43-56 (control)	0.92	0.97
	BCKD_k 201-220	1.18	1.17
	BCKD_k 211-230	1.17	1.22
	BCKD_k 221-240	1.13	1.26
	BCKD_k 231-250	1.37	1.64
	BCKD_k 241-260	1.11	1.28
VI	RNase 43-56 (control)	0.88	1.10
	BCKD _k 251-270	1.12	1.23
	BCKD_k 261-280	1.51	1.41
	BCKD_k 271-290	1.36	1.74
	BCKD _k 281-300	1.16	1.18
VII	RNase 43-56 (control)	1.16	1.24
	BCKD_k 291-310	2.04	3.02
	BCKD_k 301-320	1.89	2.21
	BCKD _k 311-330	1.05	1.46
	BCKD_k 321-340	1.49	2.15
VIII	RNase 43-56 (control)	1.00	1.25
	BCKD_k 331-350	1.63	1.65
	BCKD_k 341-360	1.51	1.57
	BCKD_k 351-370	1.39	1.59
	BCKD _k 361-380	1.21	1.25

†Represents fold difference in T cell proliferative responses induced with BCKD_k peptides in relation to medium controls. Significant differences for BCKD_k peptides vs. RNase 43-56 are bolded ($P \leq 0.005$).

Table S3: Echocardiographic assessment of cardiac abnormalities in mice immunized with BCKD_k 111-130

Parameters	Naïve	BCKD _k 111-130
Interventricular septal thickness at diastole (mm)	0.70 ± 0.06	0.93 ± 0.03*
LV internal diameter at diastole (mm)	3.50 ± 0.03	3.20 ± 0.06
End diastolic volume (μl)	110.00 ± 3.33	86.60 ± 3.33
End systolic volume (μl)	30.00 ± 10.00	20.00 ± 0.00

Data represent mean ± SEM with **P* < 0.05 vs. naïve group.

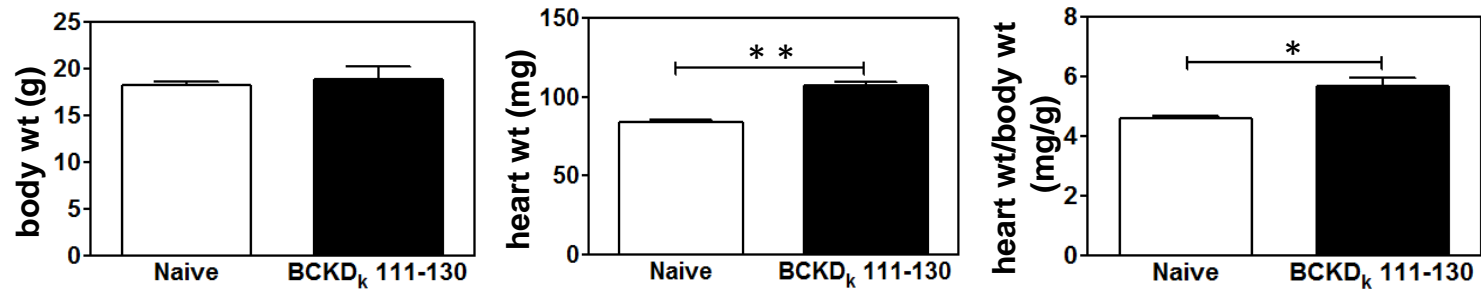


Figure S1: Evaluation of cardiac abnormalities in A/J mice immunized with BCKD_k 111-130

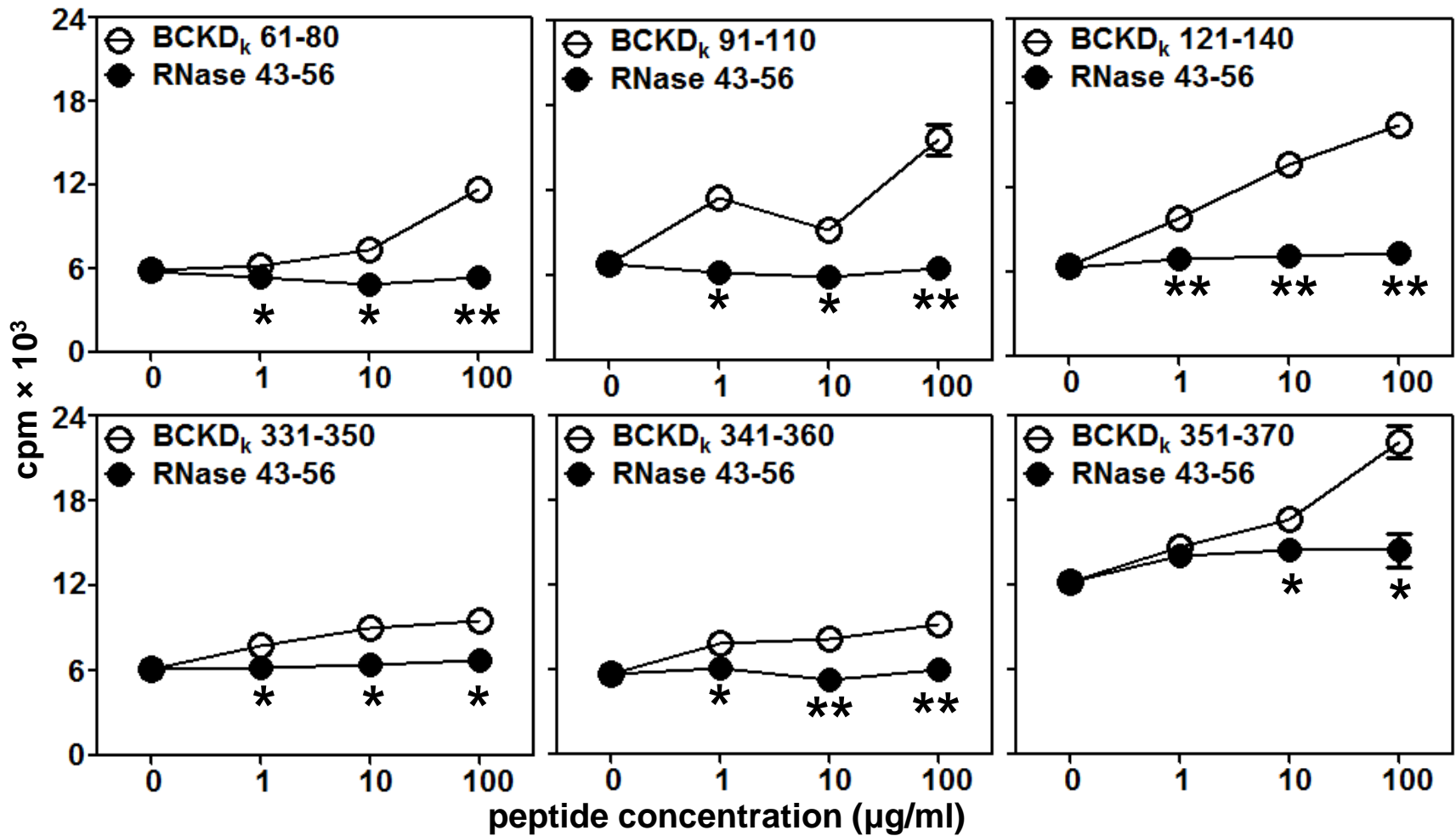


Figure S2: BCKD_k peptides that induce T cell responses, but not myocarditis in immunized animals

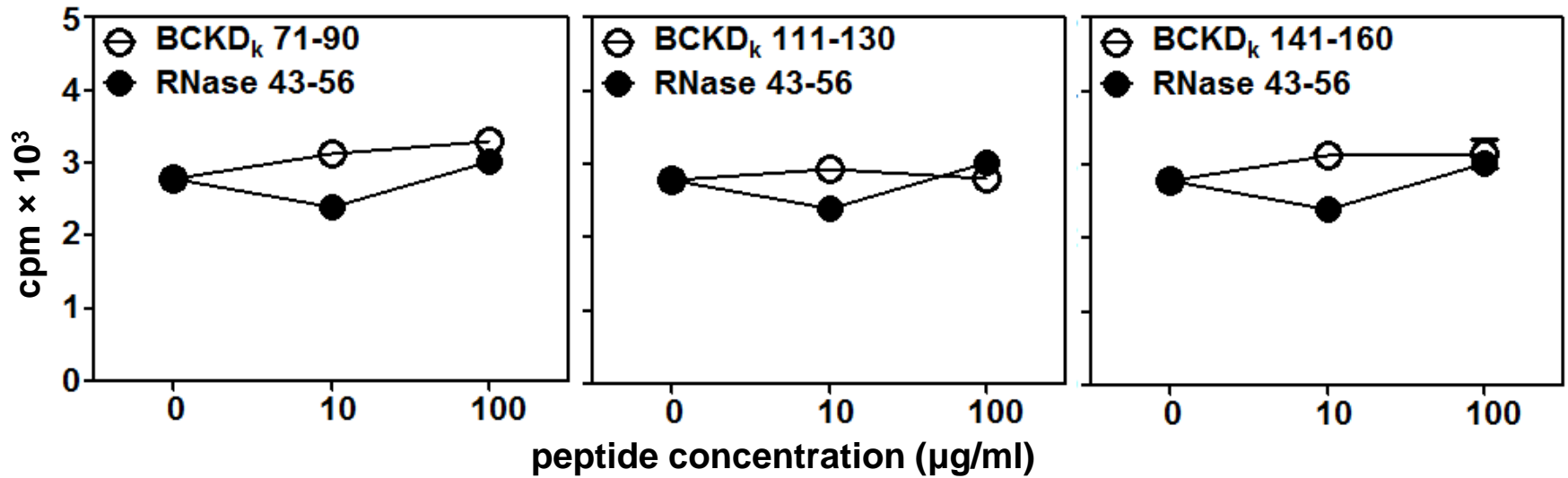


Figure S3: Proliferative responses of lymphocytes from naïve animals to BCKD_k peptides

