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Supplemental Information

Circular RNA circSnx5 Controls Immunogenicity of Dendritic Cells through the miR-544/SOCS1 Axis and PU.1 Activity Regulation

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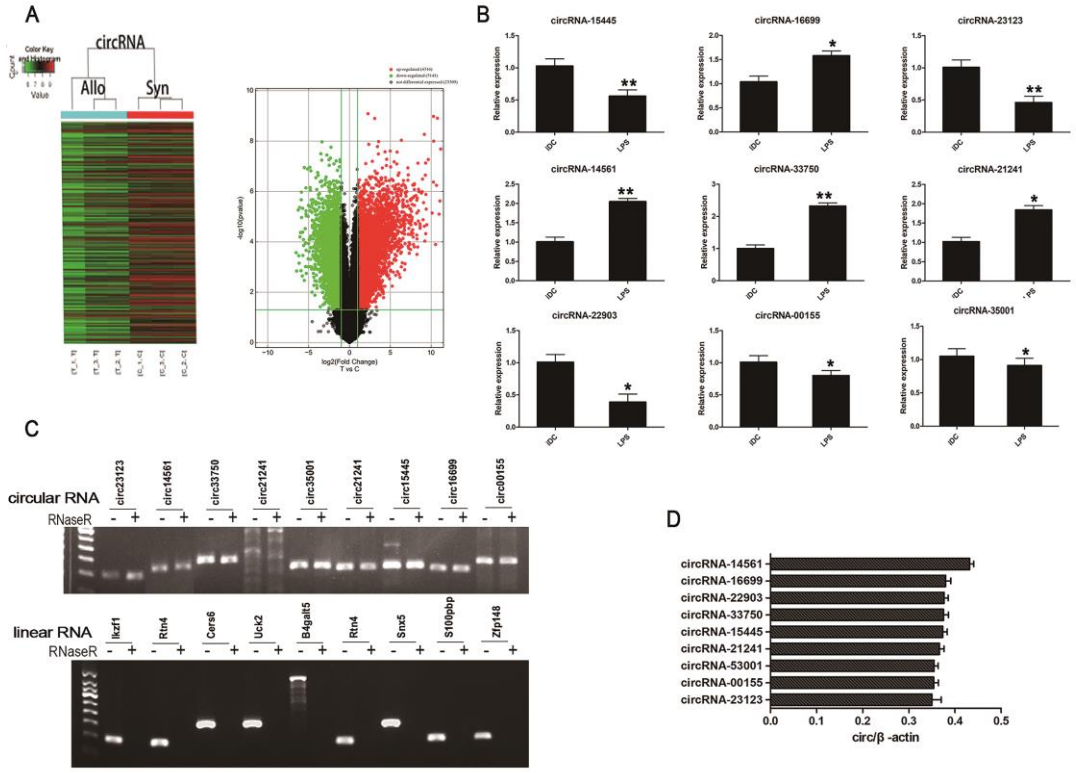

Figure S1 Identification and validation of DC-enriched circRNAs

(A) Heat map analyses of microarray data showing that differentially expressed circRNA (left) with fold changes >2.0 and P < 0.05. The expression levels are presented in different colors indicating expression levels above and below the median expression level across all samples. The volcano plot was constructed using fold-change values and P-values. Red represents increased expression whereas green represents decreased expression. (B) Bone marrow was flushed from the femur and tibia of 8- to 14-week-old and cultured in IL-4 and GM-CSF cultured media for 5 days .DCs were treated with or without 200 ng/ml LPS for 12h at day 5.The expression level of selected 9 strong candidates for circRNAs in the two guoups mentioned above by RT-qPCR analysis. (C) agarose gel electrophores is validation of circRNAs and their linear isoforms from RNaseR-treated RNA samples isolated from Immature DCs (iDC). (D) The expression level of indicated circRNAs in iDCs was accessed by RT-qPCR analysis. The expression of circRNAs was normalized to β-action. Data are shown as mean ± sd, derived by Student's t-test, *p < 0.05, **p < 0.01. N=3.

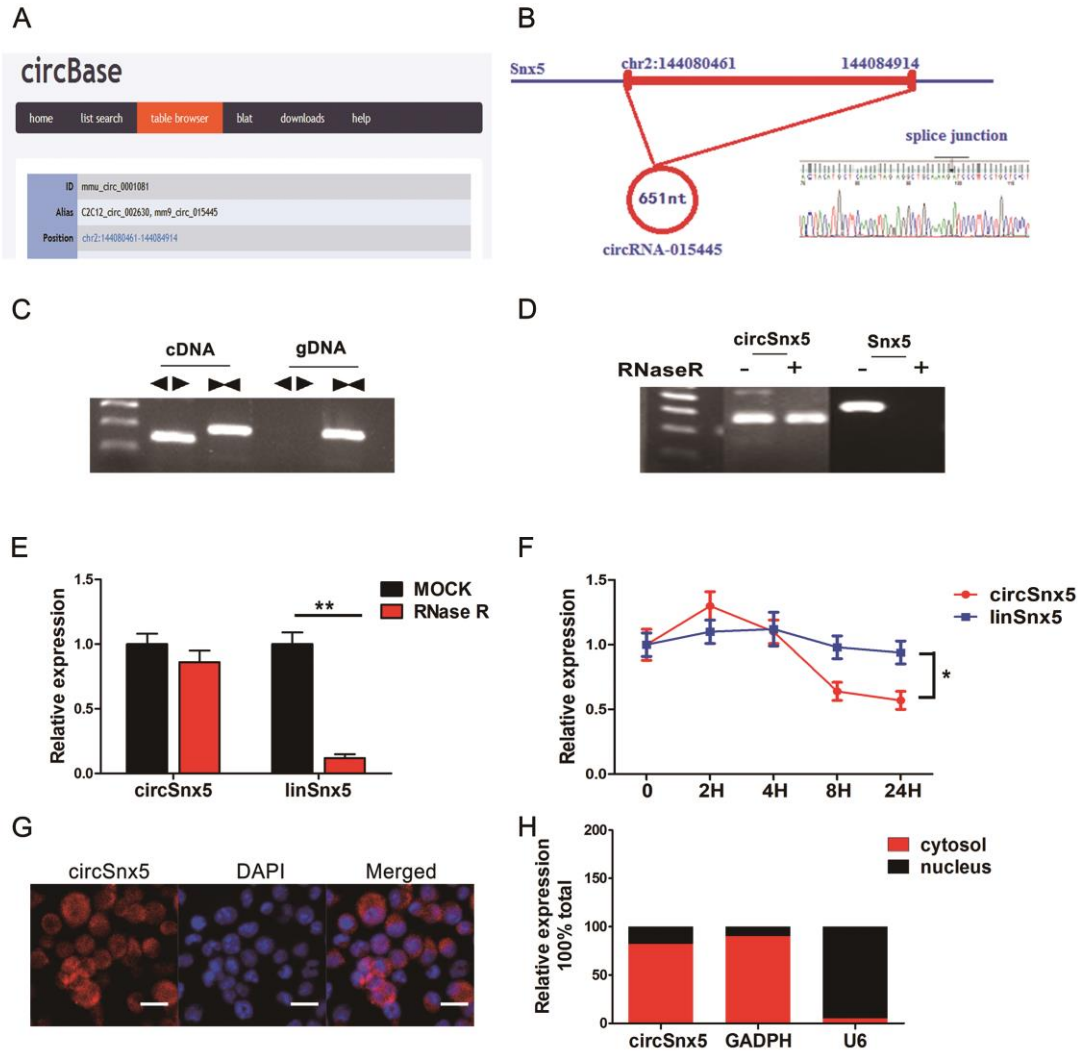

Figure S2 Characterization of circSnx5 RNA in DCs

DCs were treated with or without 200 ng/ml LPS for 12h at day 5. (A)ID number and alias of circSnx5(mmu_circ_00001081) from the circBase database. (B) The schematic diagram of genomic location and splicing pattern of circSnx5. The splice junction of specific primers was verified by direct sequencing. (C)circSnx5 was validated by qRT-PCR in DCs using divergent primers(←→) in complementary DNA (cDNA) and genomic DNA (gDNA). (D-E) qRT-PCR analysis for the abundance of circ and their linear isoforms Snx5 mRNA in DCs with or without RNase R treatment. PCR product of circSnx5 was identified by 1.5% agarose gel electrophoresis(D). The expression of circSnx5 and mRNA were normalised to β-action(E). (F) A time course of circSnx5 expression in DCs. qRT-PCRs were conducted to detect circSnx5 expression in the presence of 200ng/ml LPS after 0,2, 4, 8and 24 hours. circSnx5 expression was decrease after a slight increase. (G) RNA fluorescence in situ hybridization FISH was performed to detect the expression of circSnx5 in DCs. Nuclei were stained with 4,6-diamidino-2-phenylindole (DAPI). Images were taken by confocal microscopy (×63 magnification). Scale bar=50 μm. (H) qRT-PCR data indicating the abundance of circSnx5 and Snx5 mRNA in either the cytoplasm or nucleus of iDCs. The amounts of circSnx5 mRNA were normalized to the value measured of nuclear control transcript (U6), cytoplasm control transcript (GAPDH). Data are shown as mean ± sd, derived by Student's t-test, *p < 0.05, **p < 0.01. N=6 independent DC

preparations.

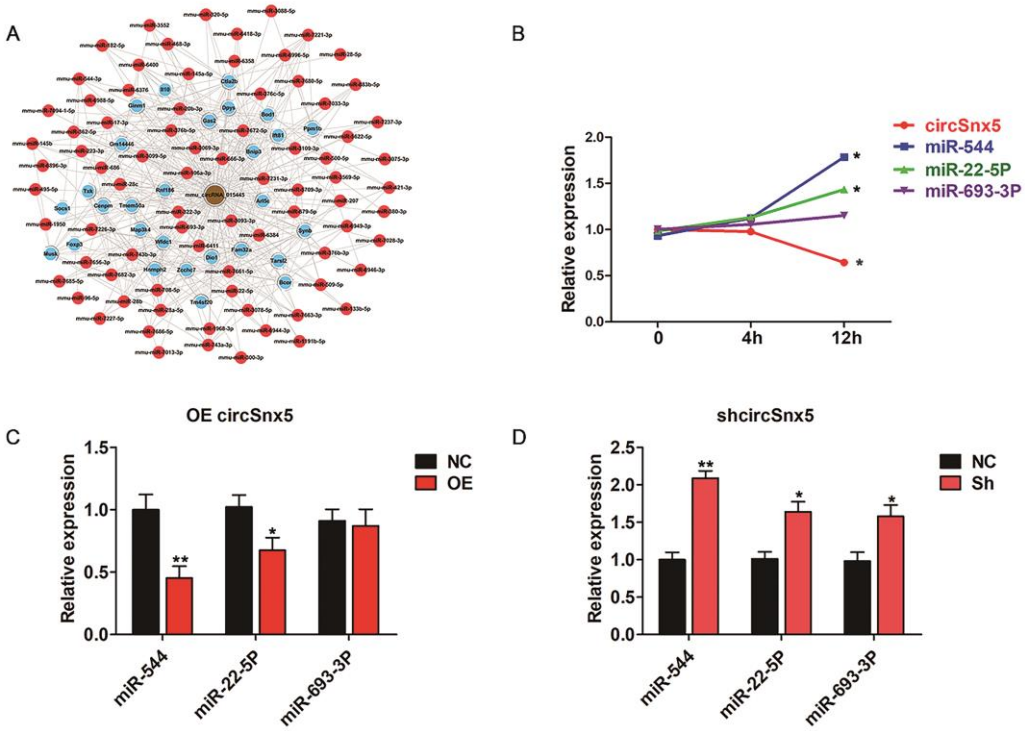

Figure S3 circSnx5-associated-ceRNA etworks in cardiac allograft mice

(A)The ceRNA network shows 80 circSnx5-associated-miRNAs and 39 immunity related differentially expressed mRNAs were selected under the criteria of P-value<0.01 and Fold Change>3. (B) RT-qPCR analysis of circSnx5, miR-544, miR-22-5p and miR-693-3p expression in LPS-DCs. *P<0.05 vs. 0h group. (C) RT-qPCR analysis of miR-544, miR-22-5p and miR-693-3p expression in DCs transfected with circSnx5 vector. (D) RT-qPCR analysis of the expression of miR-544, miR-22-5p and miR-693-3p in DCs transfected with a circSnx5 shRNA. Data are shown as mean ± sd, derived by Student's t-test, *p < 0.05, **p < 0.01. N=6 independent DC preparations.

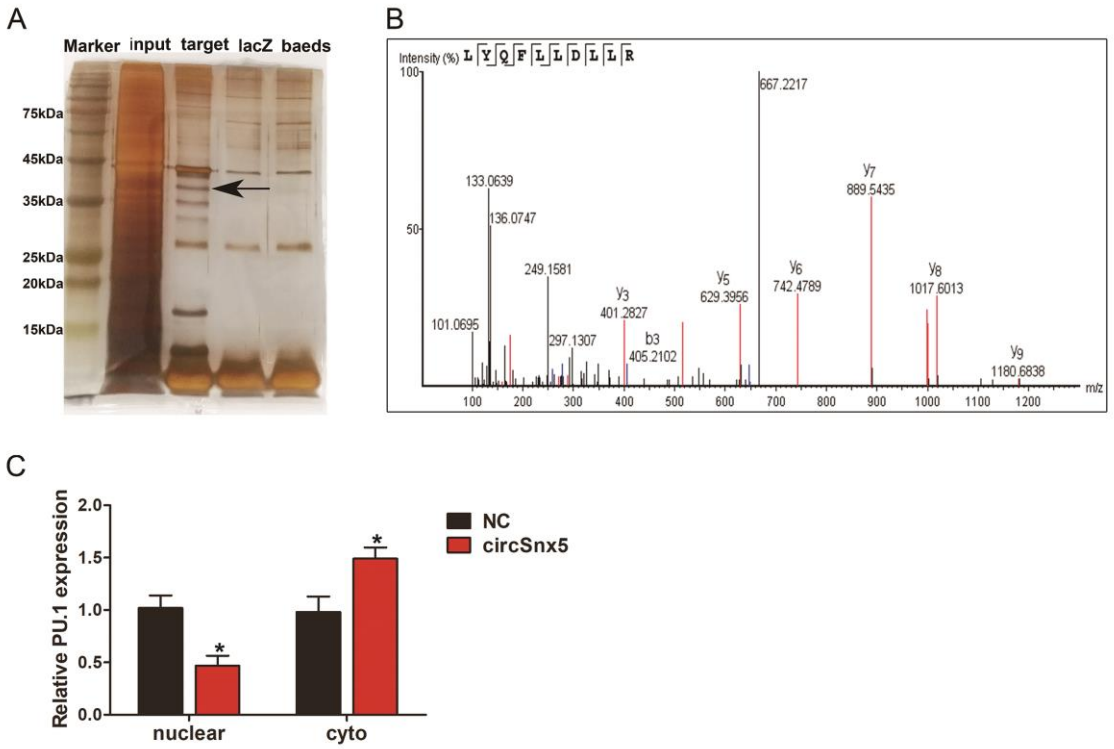

Figure S4 circSnx5 binds to PU.1

(A) SDS-PAGE silver staining of circSnx5 pull-down samples in DCs. The target group contained a circSnx5 specific pull-down probe. Input represents the positive control. LacZ and baeds represents negative control. (B) Representative mass spectral maps of PU.1. (C) PU.1protein were detected in cytoplasm and nucleus by Western blotting after transfection with or without circSnx5 overexpression. Data are shown as mean ± sd, derived by Student's t-test, *p < 0.05, **p < 0.01. N=3 independent DC preparations.

Table S1. Primers used in study

PCR Primers (5' to 3')		
circSnx5 (circ15445)	Forward	CATAGAGGCTGCAAAGATCC
	Reverse	CAGTTCTTGCTTCATCTTGGC
circ22903	Forward	ATGAGGGTCAAGACATGTC
	Reverse	CATTGTCTTCAGGTTATCCATG
circ14561	Forward	GAGCTGAATAAAACTTCAGATGAG
	Reverse	TCTTGACCAGACGAAACAGT
circ33750	Forward	GGCATATTTCCTCTCTGATTC
	Reverse	TTGGAGAGCCCTTCTAATCT
circ21241	Forward	GTGCCGACAATCTCGTCTTC
	Reverse	GACTCGGTAGAAGCTATCCTG
circ35001	Forward	GTCCAGTTTCTTGGCAGTGAAC
	Reverse	CTGCTGTTCCTCTTGGCATAG
circ23123	Forward	GTTGCCATATCAGAGGAATTGG
	Reverse	GAGCAAAAAGGGTCTCATCTTC

circ16699	Forward	AGGGGACTCTAACCAAGATATG
	Reverse	CAAGGAGTCATCCAACTCATC
circ00155	Forward	GCACTCAATGTCCCTGATAG
	Reverse	GAGTTTCTACCTTTCACAGGC
Ikzf1	Forward	TCCGAGGTGGTGCCAGTCATC
	Reverse	CGGCGTCCTGTGCTGAATGG
Rtn4	Forward	ACTGGAAGGACTCGGTGGTGTG
	Reverse	AGTTGTCGGACAGAATGTGACTTGAC
Cers6	Forward	TGAACTGCTTCTGGTCTTACCTGATTG
	Reverse	TCCGTTGGTGGTTGTTGAAGAGTG
Uck2	Forward	GTGTAGCCGTTGAGATAGCCGTTC
	Reverse	GTGTAGCCGTTGAGATAGCCGTTC
B4galt5	Forward	GGCGGAGAAGATGACGACTTGTG
	Reverse	CGATGGTGGTGAGGAATGGACTTG
Snx5	Forward	GCTCCTCCGATACTACATGCTCAAC
	Reverse	CCTGCTGATGAGTCTCTGCCAAC
Zfp148	Forward	GCAGCAGGCTTTGGACAGAA
	Reverse	GCGGATTTGGAAGGGTCTGG
miR-544	Forward	GGAACTCGAGCCGCTGCCTTAAGTCACTCTCTAT
	Reverse	GGAAGGATCCCACAACTGACCACAGTTT
hnRNP C	Forward	CGTGTACCTCCTCCTCCTCCTATTG
	Reverse	TCTCTACTGCTTGTTTGCTCTGTTCC
PU.1	Forward	TCTGGTGGGTGGACAAGGAC
	Reverse	TCTTCTTGCGGTTGCCCTTC
β-catin	Forward	GCTTCTAGGCGGACTGTTAC
	Reverse	CCATGCCAATGTTGTCTCTT
RIP Primers (5' to 3')		
site a	Forward	AGGCATGCACCACCAC
	Reverse	GCGGATTTCTGAGTTCGAG
site b	Forward	GAAGAGATAGCAATCAGTGCTCTG
	Reverse	CAAGGTCTACAAAGTGAGTTCCAG
site c	Forward	GAGCTCCTCCGATACTACATG
	Reverse	CAACATTCCTCTCTAACCCAG
site d	Forward	GCAATAGAGACACATGCATATGC
	Reverse	CTGGAAAGATTAACAATCATGATG
others		
miR-544-3P inhibiter	primer	mmu_circRNA_015445-1 GAGGCTGCAAAGATCCCTCCT mmu_circRNA_015445-2 GCTGCAAAGATCCCTCCTGCT
shSOCS1	primer	GACAATGCAGTCTCCACAGCA
shhnRNP C	primer	GCAACAAATTGATGAGCAATG
si PU.I	primer	GGATTTCTCCGCACACCATGT

circSnx5 shRNA	primer	GAGGCTGCAAAGATCCCTCCT

Table S2. The miRNAs data obtained from circSnx5-associated ceRNA networks

miRNA	Sites total	Context+
mmu-miR-22-5p	2	-0.072
mmu-miR-544-3p	2	-0.284
mmu-miR-693-3p	2	-0.088
mmu-miR-6400	2	-0.454
mmu-miR-7028-3p	2	-0.41
mmu-miR-7685-5p	2	-0.166
mmu-miR-17-3p	1	-0.086
mmu-miR-20b-3p	1	-0.104
mmu-miR-28b	1	-0.238
mmu-miR-28c	1	-0.275
mmu-miR-28a-5p	1	-0.203
mmu-miR-28-5p	1	-0.275
mmu-miR-106a-3p	1	-0.086
mmu-miR-133b-5p	1	-0.388
mmu-miR-145b	1	-0.245
mmu-miR-145b	1	-0.245
mmu-miR-145a-5p	1	-0.245
mmu-miR-182-5p	1	-0.15
mmu-miR-207	1	-0.123
mmu-miR-223-3p	1	-0.33
mmu-miR-300-3p	1	-0.197
mmu-miR-320-5p	1	-0.126
mmu-miR-322-3p	1	-0.163
mmu-miR-362-5p	1	-0.178
mmu-miR-376b-3p	1	-0.126
mmu-miR-376b-5p	1	-0.08
mmu-miR-376c-5p	1	-0.09
mmu-miR-380-3p	1	-0.129
mmu-miR-421-3p	1	-0.272
mmu-miR-468-3p	1	-0.152
mmu-miR-495-5p	1	-0.165
mmu-miR-500-5p	1	-0.168
mmu-miR-509-5p	1	-0.204
mmu-miR-666-3p	1	-0.197
mmu-miR-679-5p	1	-0.323
mmu-miR-686	1	-0.132
mmu-miR-708-5p	1	-0.157
mmu-miR-743b-3p	1	-0.144

mmu-miR-743a-3p	1	-0.123
mmu-miR-883b-5p	1	-0.331
mmu-miR-1191b-5p	1	-0.158
mmu-miR-1950	1	-0.366
mmu-miR-1968-3p	1	-0.365
mmu-miR-3069-3p	1	-0.187
mmu-miR-3075-3p	1	-0.22
mmu-miR-3078-5p	1	-0.14
mmu-miR-3088-5p	1	-0.09
mmu-miR-3093-3p	1	-0.16
mmu-miR-3099-5p	1	-0.304
mmu-miR-3109-3p	1	-0.251
mmu-miR-3552	1	-0.164
mmu-miR-3569-5p	1	-0.503
mmu-miR-5622-5p	1	-0.336
mmu-miR-5709-3p	1	-0.242
mmu-miR-6358	1	-0.197
mmu-miR-6376	1	-0.169
mmu-miR-6384	1	-0.196
mmu-miR-6411	1	-0.218
mmu-miR-6418-3p	1	-0.339
mmu-miR-6896-3p	1	-0.071
mmu-miR-6944-3p	1	-0.126
mmu-miR-6946-3p	1	-0.079
mmu-miR-6949-3p	1	-0.343
mmu-miR-6988-5p	1	-0.378
mmu-miR-6996-5p	1	-0.258
mmu-miR-6996-5p	1	-0.258
mmu-miR-7013-3p	1	-0.182
mmu-miR-7033-3p	1	-0.312
mmu-miR-7094-1-5p	1	-0.112
mmu-miR-7221-3p	1	-0.323
mmu-miR-7226-3p	1	-0.202
mmu-miR-7227-5p	1	-0.288
mmu-miR-7231-3p	1	-0.142
mmu-miR-7237-3p	1	-0.199
mmu-miR-7656-3p	1	-0.113
mmu-miR-7661-5p	1	-0.051
mmu-miR-7663-3p	1	-0.205
mmu-miR-7672-5p	1	-0.286
mmu-miR-7682-3p	1	-0.235
mmu-miR-7680-5p	1	-0.112
mmu-miR-7686-5p	1	-0.233

Supplemental Table 3. Sequences of probes used in the RNA pull down experiment

Gene name	Label	Probe sequence
circSnx5	Biotin labeling	CAGGAGGGATCTTTGCAGCC