## **Supplementary Information**

## Combining Exosomes Derived from Immature DCs with Donor Antigen-Specific Treg Cells Induces Tolerance in a Rat Liver Allograft Model

Ben Ma<sup>1<sub> $\triangle$ </sup></sub>, Jing-Yue Yang<sup>2<sub> $\triangle$ </sub></sub>, Wen-jie Song<sup>1<sub> $\triangle$ </sub></sub>, Rui Ding<sup>1<sub> $\triangle$ </sub></sub>, Zhuo-chao Zhang<sup>1</sup>, Hong-chen Ji<sup>1</sup>, Xuan</sup></sup></sup></sup>

Zhang<sup>1</sup>, Jian-lin Wang<sup>1</sup>, Xi-sheng Yang<sup>1</sup>, Kai-shan Tao<sup>1</sup>\*, Ke-feng Dou<sup>1</sup>\*, Xiao Li<sup>1</sup>\*

1 Department of Hepatobiliary Surgery, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi Province, China.

2 Department of Clinical Oncology, Xijing Hospital, Fourth Military Medical University, Xi'an, Shaanxi Province, China

 $\triangle$  These authors contributed equally to this work.

Correspondence should be addressed to XL (lixiao0076@163.com), K-F D (gdwkgwx@fmmu.edu.cn) or K-S T (taokaishan0686@163.com).

The authors declare no competing financial interest.

Sample	% CD4 <sup>+</sup> CD25 <sup>+</sup>	% CD4 <sup>+</sup> CD25 <sup>+</sup> FOXP3 <sup>+</sup>	% CD4/CD25/CD127
A1	91	69.4	14.9
A2	83.5	63.1	10.1
A3	92.9	73.1	13.7
MST/SEM	89.1±2.9	68.5±2.9	12.9±1.4
B1	92.2	88.2	7.1
B2	87.8	81.3	5.5
B3	93.1	91.3	9.1
MST/SEM	91.0±1.6	86.9±3.0	7.2±1.0
Sample	% CD8 <sup>+</sup>		
C1	88.7		
C2	84.9		
C3	93.8		
MST/SEM	89.1±2.6		

Samples A: fresh isolated CD4<sup>+</sup>CD25<sup>+</sup> T cells

Samples B: donor SDCs co-cultured CD4<sup>+</sup>CD25<sup>+</sup>T cells

Samples C: isolated  $CD8a^+T$  cells



**Supplementary Fig. S1. Additional phenotype identification of Treg.** (A) To further identify the statue of two fraction of Treg (fresh isolated Tregs and Tregs co-cultured with donor SDCs), we detected the expression of CTLA-4, Ki67 and ICOS with FCM. One representative result of three is shown. (B) Mean fluorescence intensity (MFI) was analysed. Ki67 expression was higher in co-cultured Tregs than fresh isolated Tregs, p < 0.05, n = 3. \* indicates p < 0.05; \*\* indicates p < 0.01; ns indicates no significant difference.



Supplementary Fig. S2. Process diagram of survival analysis in fig. 3.



Supplementary Fig. S3. Process diagram of anti-donor cell response analysis and pathological analysis in fig. 4/5.



Supplementary Fig. S4. Process diagram of immunofluorescence analysis in fig. 6.



Supplementary Fig. S5. Process diagram of donor-specific Treg proliferation assays in vivo in fig. 7.