# A clinically relevant murine model unmasks a 'Two-Hit' mechanism for reactivation and dissemination of cytomegalovirus following kidney transplantation

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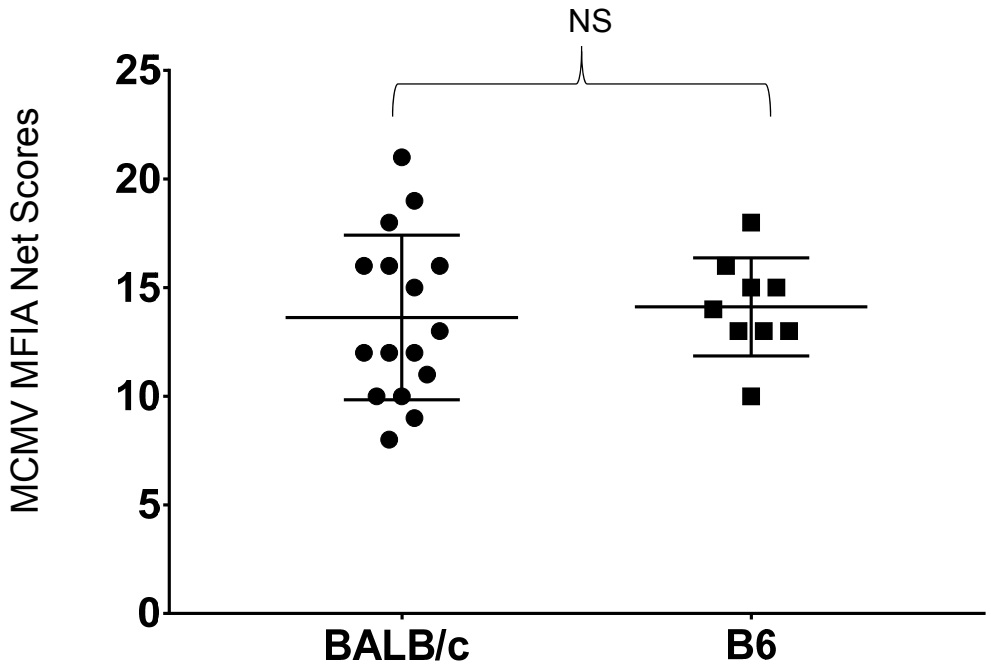
## SUPPLEMENTAL DATA

**Supplement Figure 1**



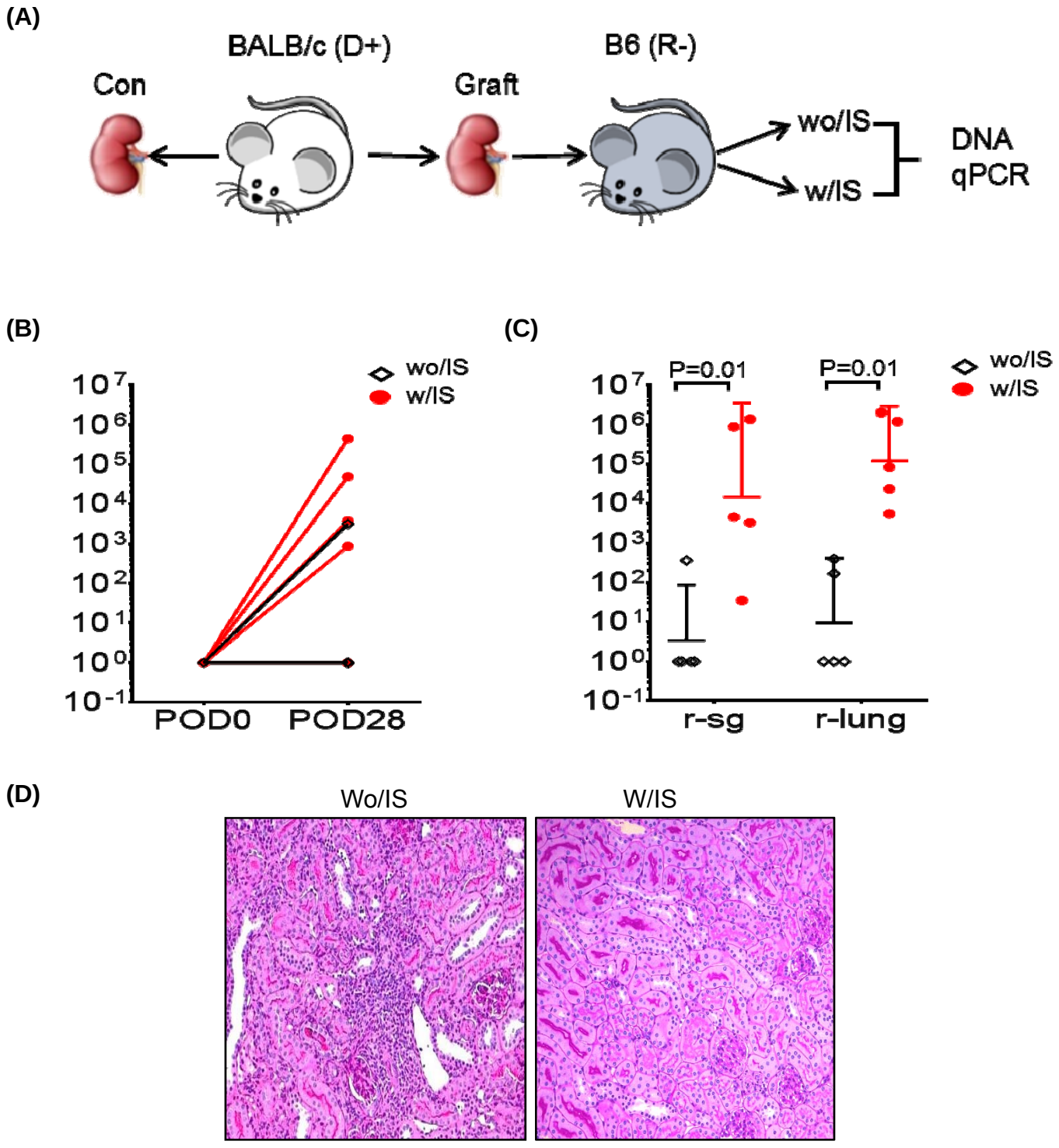

**Supplement Figure 1, Donor mcmv latency were confirmed by serological analysis (EZ spot) and no difference in MFIA score was noted between BALB/c and B6.** Plasma were collected at BALB/c or B6 mice at 6 months post infection. MCMV Multiplexed Fluorometric Immune Assay (MFIA) was performed by Charles River Research Animal Diagnostic Services (Wilmington MA ). Interpretation of MFIA/ELISA Net Scores: Provided that the Tissue Control score < 2.0, 1 is Negative, 2 is Equivocal and 3 or higher is Positive .

**Table 1. D+/R- kidney transplant mediate MCMV reaction and productive infection at POD28.**

| Recipient ID | DNA copies per million cells | | | | **Plaques (pfu/SG)** |
|---|---|---|---|---|---|
| | Donor Controls* | | Recipients at POD28 | | |
| | Kidney | SG | Transplanted kidney | Recipient SG | |
| RT259 | 30 | 0 | 8614 | 482013 | **$9 \times 10^3$** |
| RT260 | 0 | 45 | 70063 | 123727 | **$7.6 \times 10^4$** |
| RT261 | 0 | 0 | 40378 | 357178 | **$6 \times 10^3$** |
| RT262 | 61 | 0 | 30806 | 328730 | **$1 \times 10^3$** |
| RT263 | 0 | 0 | 15475 | 41419 | **$2.2 \times 10^4$** |

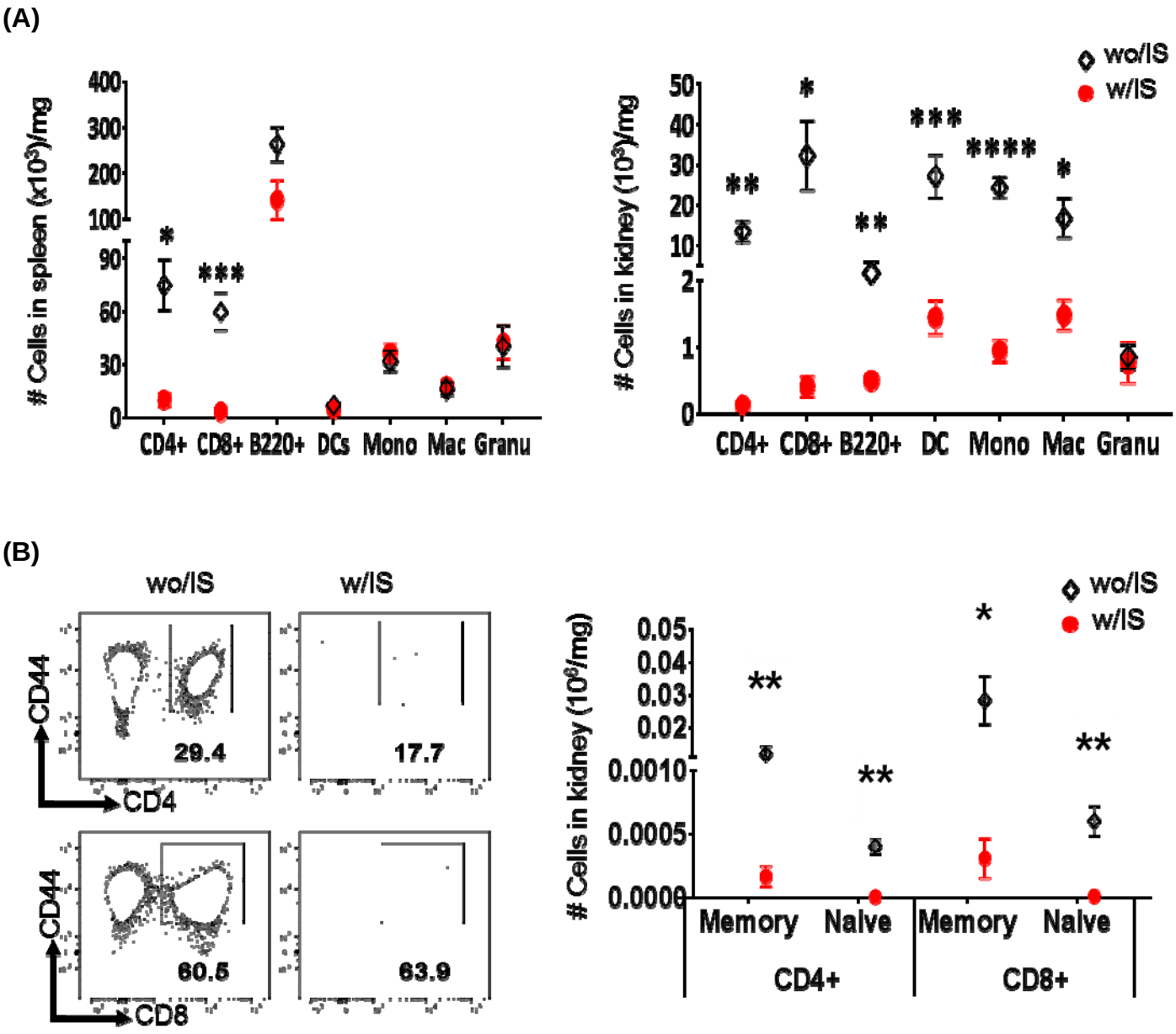
* Contralateral latent kidney and salivary gland.

**Supplemental figure 2**




**Supplement Figure 2. MCMV reactivation and systemic dissemination were also detected in BALB/c (D+) to B6 (R-) allogeneic transplants treated w/IS**. Kidneys from latently infected BALB/c were transplanted into B6 mice respectively.
**(A):** Schematic of experimental setup..
**(B):** DNA abundance POD0 (contralateral donor kidney) and POD28 in kidney grafts.
**(C):** DNA abundance at day 28 post-transplant recipients' salivary gland (r-SG) and lungs (r-lung).
**(D):** Representative images showing histology of the kidnay allograft without IS (wo/IS, left) or with IS (w/IS, right) at POD28.

**Supplement figure 3**

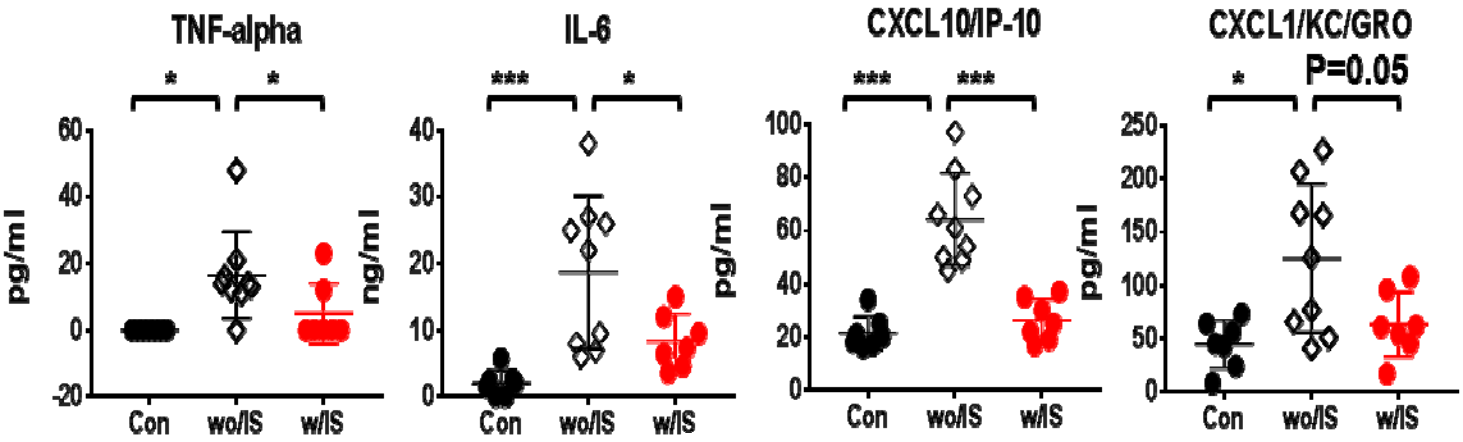



**Supplement Figure 3. Effect of IS on immune phenotypes in spleens and kidney allograft following D+/R- kidney transplant at day 7.** Cells isolated from spleens and renal grafts of D+/R- kidney allografts w/IS (n=4) or wo/IS (n=4) at POD 7 were analyzed by flow cytometry. Frequencies of recipients' T cells (gated on $CD45.2^+CD3^+$ cells), and myeloid cells (gated on $CD45.2^+CD11b^+$ cells), including DC ($CD11c^+MHCII^+$ dendritic cells), Mac ($CD11b^+F4/80^+$ macrophages), Mono ($CD11b^+Ly6C^+$ monocytes), and Granu ($Ly6G^+$granulocytes) were determined based on total number of live cells normalized by tissue weight (mg). Data in bar graphs are expressed as mean value with SEM. *p ≤ 0.05; **p < 0.01; ***p < 0.001; ****p < 0.0001.

**(A)**: Absolute number of cells in spleens (left) and kidney grafts (right

**(B)**: Percentage (left) and absolute number (right) of infiltrating $CD44^+CD4^+$ T cells and $CD44^+CD8^+$ T cells in kidney grafts (/mg), gated on $CD45^+CD3^+$ cells. Data are expressed as mean value with SEM.

**Supplemental figure 4**




**Supplement Figure 4 Plasma proteomic analysis following transplantation. P**lasma samples were collected from D+ to R- kidney allografts treated w/IS or wo/IS at 48hr post-transplant (n=6-8/group). Plasma samples from naïve B6 mice were used as controls (con). *P<0.05, **P<0.01, ***P<0.001