

## Nuclear factor- $\kappa$ B inducing kinase is required for graft-versus-host disease

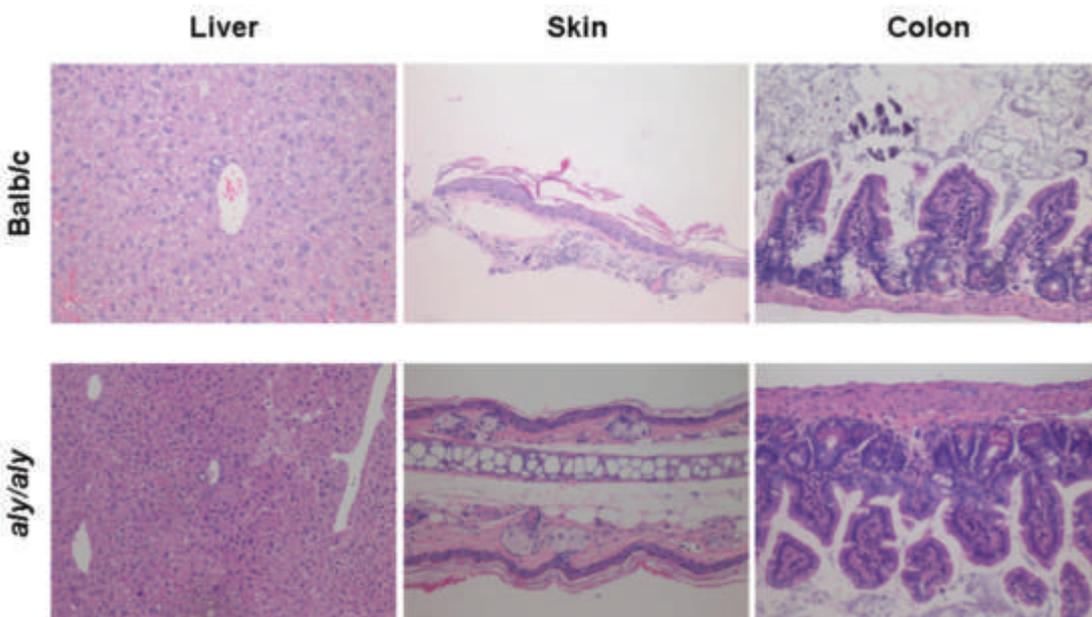
Carmen Sánchez-Valdepeñas,<sup>1\*</sup> Lucía Casanova,<sup>2\*</sup> Isabel Colmenero,<sup>3</sup> Mar Arriero,<sup>2</sup> África González,<sup>2</sup> Nieves Lozano,<sup>3</sup> Marta González-Vicent,<sup>2</sup> Miguel A. Díaz,<sup>2</sup> Luís Madero,<sup>2</sup> Manuel Fresno,<sup>1\*</sup> and Manuel Ramírez<sup>2\*</sup>

<sup>1</sup>Centro de Biología Molecular “Severo Ochoa” (CSIC-UAM). Universidad Autónoma de Madrid, Madrid, and <sup>2</sup>Oncohematología y Trasplante, and <sup>3</sup>Pathology, Hospital Universitario Niño Jesús. Madrid, Spain

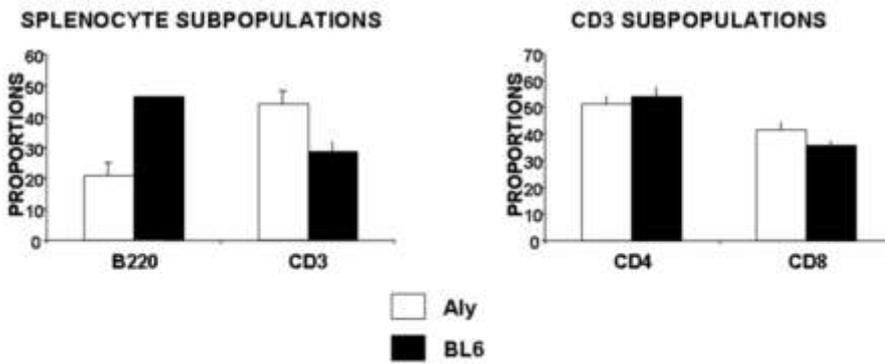
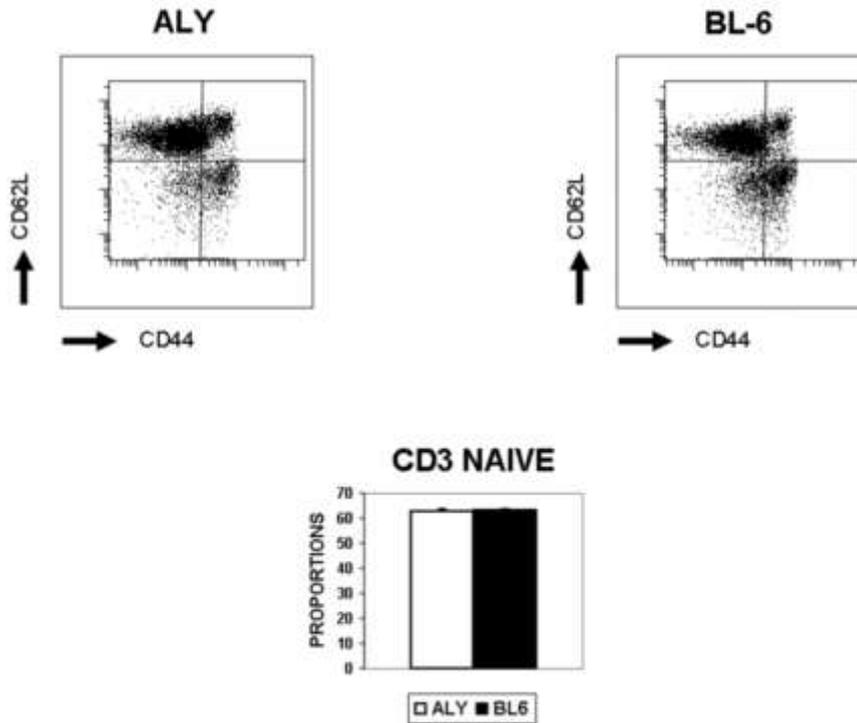
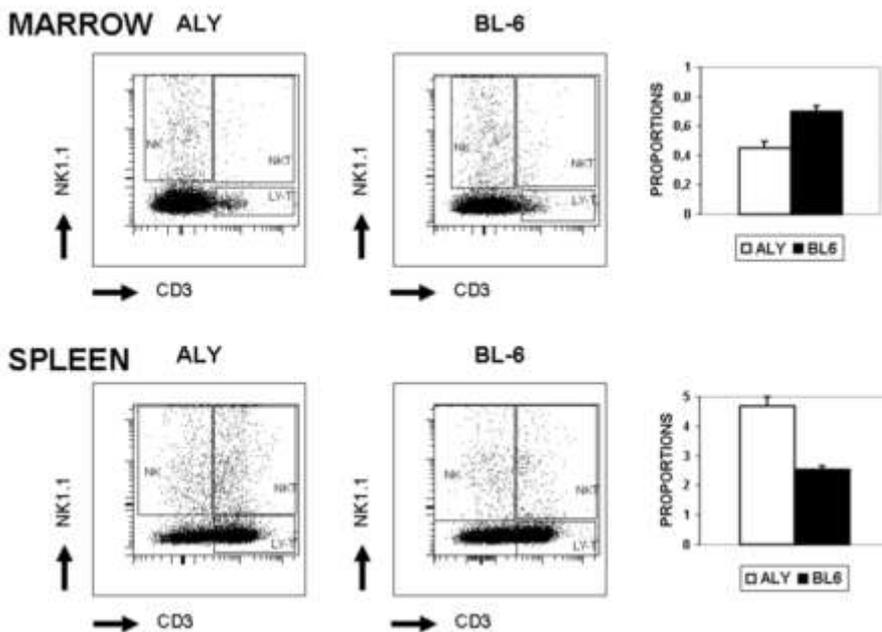
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**Online Supplementary Table S1.** NIK protein expression in peripheral blood lymphocytes of patients with acute GVHD. Peripheral blood T lymphocytes from patients with acute GVHD were stained with an anti-NIK monoclonal antibody. +: 25% positive cells; ++: 25-50%; +++: 50-75%, ++++: 75-100%.

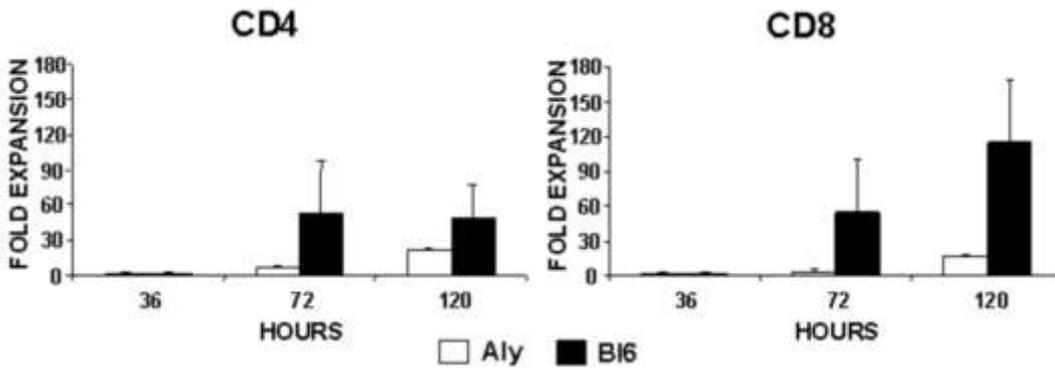
	NIK
Patient #1	
CD8 T lymphocytes	+++
Patient #2	
CD3 lymphocytes	++++
Patient #3	
CD4 T lymphocytes	+++
CD8 T lymphocytes	+++
Patient #4	
CD3 lymphocytes	++++
Patient #5	
CD3 lymphocytes	++++
Positive control	
Activated T lymphocytes	++++
Negative control	
Resting T lymphocytes	-



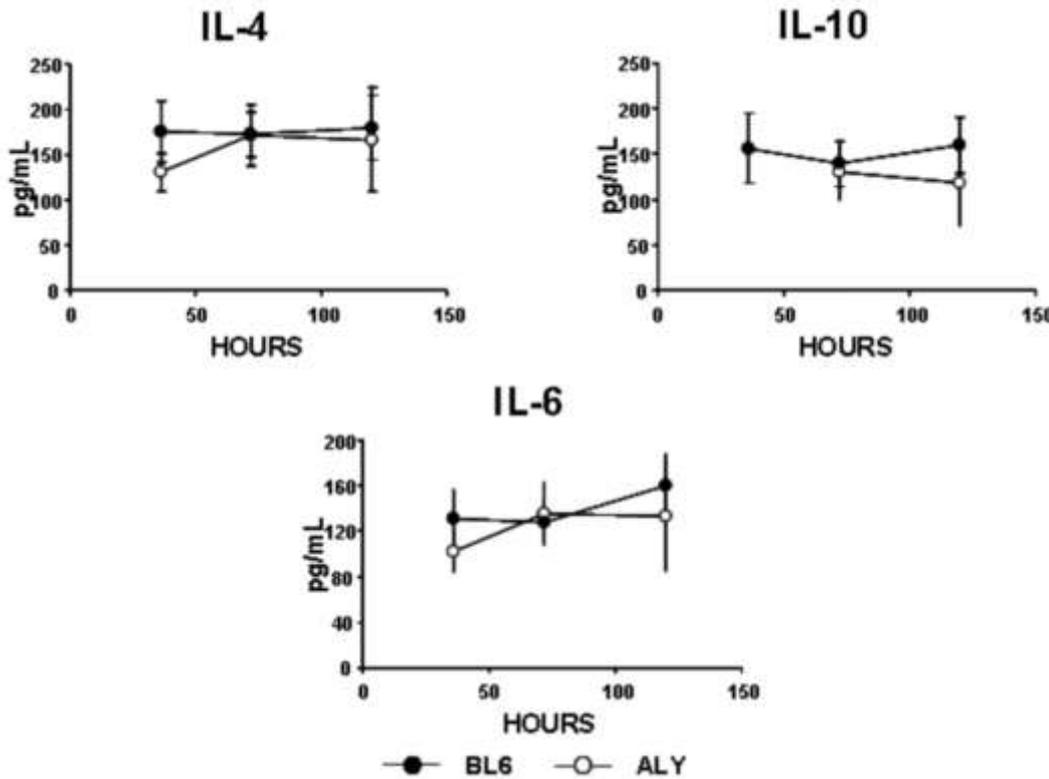
**Online Supplementary Figure S1.** Transplantation of *aly/aly* marrow and spleen cells into Balb/c mice did not cause pathological GVHD. Liver, skin and colon samples obtained from Balb/c mice 3 months after receiving marrow and spleen cells from syngeneic (above) or allogeneic *aly/aly* (below) donors. All samples showed normal tissue and cellular features.

**A****B****C**

Online Supplementary Figure S2. Major cell subsets transplanted from *aly/aly* and BL6 mice were not different. (A) Proportions of lymphocyte subpopulations in the spleens of *aly/aly* and C57BL/6 (BL6) mice. Mean  $\pm$  standard error from four mice per group. (B) Naive (CD62L<sup>+</sup>CD44<sup>-</sup>), central memory (CD62L<sup>+</sup>CD44<sup>+</sup>) and effector (CD62L<sup>-</sup>CD44<sup>+</sup>) T lymphocytes from *aly/aly* (above, left) and C57BL6 (above, right) mice. Proportions of the three subpopulations in *aly/aly* and C57BL6 mice (below, mean  $\pm$  standard error from four mice per group). (C) NKT (CD3NK1.1<sup>+</sup>) cells in marrow (above) and spleen (below) from *aly/aly* and C57BL6 mice. Proportions represent the mean  $\pm$  standard error from six mice per group.



Online Supplementary Figure S3. T cell expansion early during acute GVHD is impaired with donor *aly/aly* mice. Fold expansion was calculated based on donor T lymphocyte numbers recovered from the spleens of Balb/c mice transplanted with T cells from C57BL/6 (solid bars) or *aly/aly* (open bars) mice. Three mice per timepoint per group, mean  $\pm$  standard error. Values normalized to those obtained at 36 h. CD4 and CD8 subpopulations.



Online Supplementary Figure S4. IL-4, IL-10 and IL-6 cytokine production is not affected in mice transplanted with *aly/aly* T lymphocytes. The levels of circulating IL-4, IL-10 and IL-6 cytokines in Balb/c mice transplanted with C57BL/6 (black circles) or *aly/aly* (white circles) T lymphocytes. Three mice per timepoint per group, mean  $\pm$  standard error.