



**SUPPLEMENTARY FIG. S3.** Naïve splenocytes do not expand after challenge in mice that have been previously vaccinated with the heterologous prime-boost regimen. (A) C57BL/6 mice were immunized, adoptively transferred with GFP<sup>+</sup> cells, and challenged as depicted. The mouse groups were the same described in the legend of Fig. 1. (B) Splenic cells were collected at day 55 and cultured in the absence (Med) or presence of the VNHRFTLV peptide (Pep), anti-CD28, BdGolgiPlug, and monensin. After 12 hr, the cells were stained with anti-CD8, anti-IFN $\gamma$ , and anti-TNF. (C) The GFP<sup>+</sup> cells were localized among the CD8<sup>+</sup> cells, and the frequency of the CD8<sup>+</sup> GFP<sup>+</sup> IFN $\gamma$ <sup>+</sup> cells was estimated. (D) Frequencies of the GFP<sup>+</sup> IFN $\gamma$ <sup>+</sup> splenic cells cultured in the absence (Med) or presence of the VNHRFTLV peptide (Pep). (E) Same as panel B (individual mouse results). (F) Same as panel D (individual mouse results).