Supporting Information

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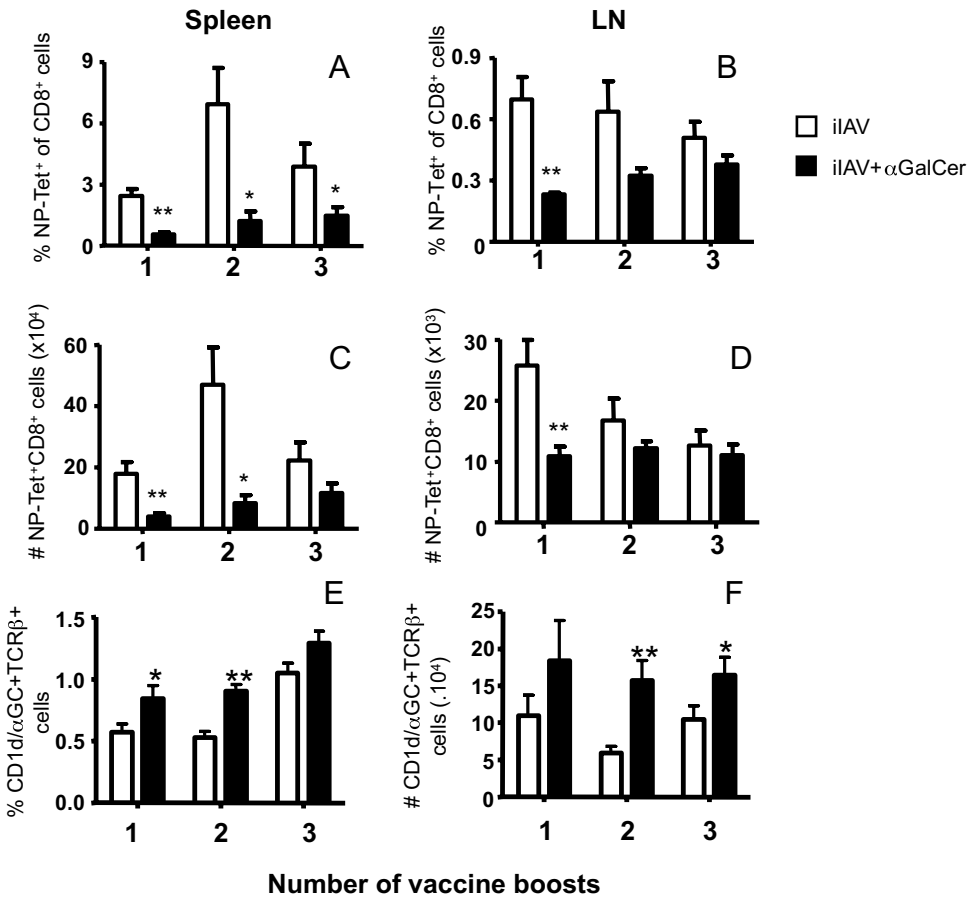

Fig. S1. Repeat boosting does not improve DbNP366. $^{+}CD8^{+}$ T cell immunity. The B6 mice were immunized 3 times at 2-week intervals with iIAV ± α-GalCer. Splenocytes were harvested and stained at 7 days after the last immunization. Shown are the percentage of tetramer-positive cells (*A* and *B*) and absolute number of cells (*C* and *D*) for spleen (*A* and *C*) and BLN (*B* and *D*). The results are expressed as mean ± SD for groups of 5 mice, with each boost representing an independent experiment repeated twice. $*P \leq 0.05$ and $**P < 0.01$ comparing iIAV with α-GalCer to iIAV alone. (*E* and *F*) The spleen was harvested and analyzed by flow cytometry for the presence of TCRβ+α-GalCer/CD1d tetramer-specific NKT cells after vaccination. The proportion (*E*) and absolute number (*F*) of CD1d/α-GalCer tetramer-positive NKT cells are presented as the mean ± SD for groups of 5 mice. $*P \leq 0.05$ and $**P < 0.01$ comparing iIAV with α-GalCer to iIAV alone.

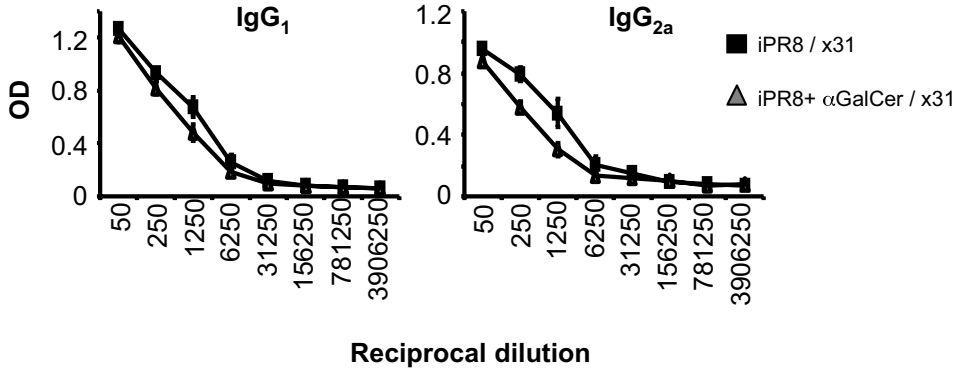

Fig. S2. Humoral response after heterologous infection. The sera of mice was sampled 7 days after challenge with x31 virus, and the level of IgG1 and IgG2a isotypes evaluated using an ELISA. Inactivated whole PR8 virus was used to coat the ELISA plates. Results are expressed as mean optical density for serial dilution ± SD.

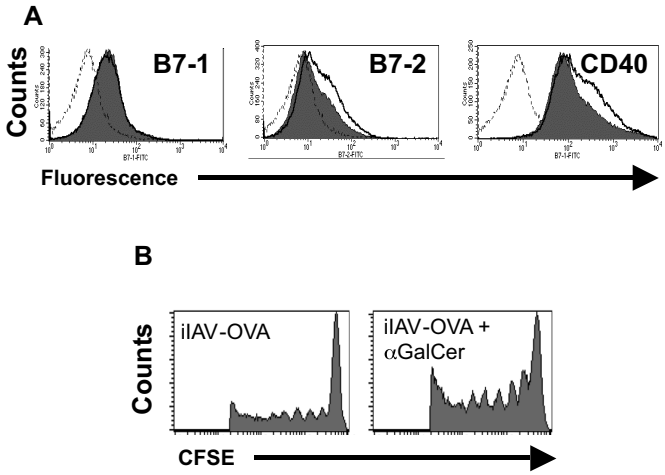

Fig. S3. α-GalCer does not impair DC antigen presentation. (*A*) The B6 mice were immunized with iIAV ± α-GalCer, and lymphoid cells from the draining BLN were harvested 3 days later. The DCs were enriched and stained for CD80, CD86, and CD40. The shaded histogram is iIAV alone, the black line iIAV + α-. GalCer, and the dotted line the isotype control. (*B*) 1 × 106 carboxyfluorescein succinimidyl ester (CFSE)-labeled OT-I cells. (CD45.1) were transferred i.v. into congenic B6 mice (CD45.2), then immunized 1 day later with iHKx31-OVA ± α-GalCer. Three days after infection, the draining BLN was isolated and processed. The transferred OT-I cell population was identified by staining for CD45.1, and proliferation of this population was assayed by measuring the loss of CFSE fluorescence. The data are representative of 3 animals per group and 2 independent experiments.