Supplementary figure 1

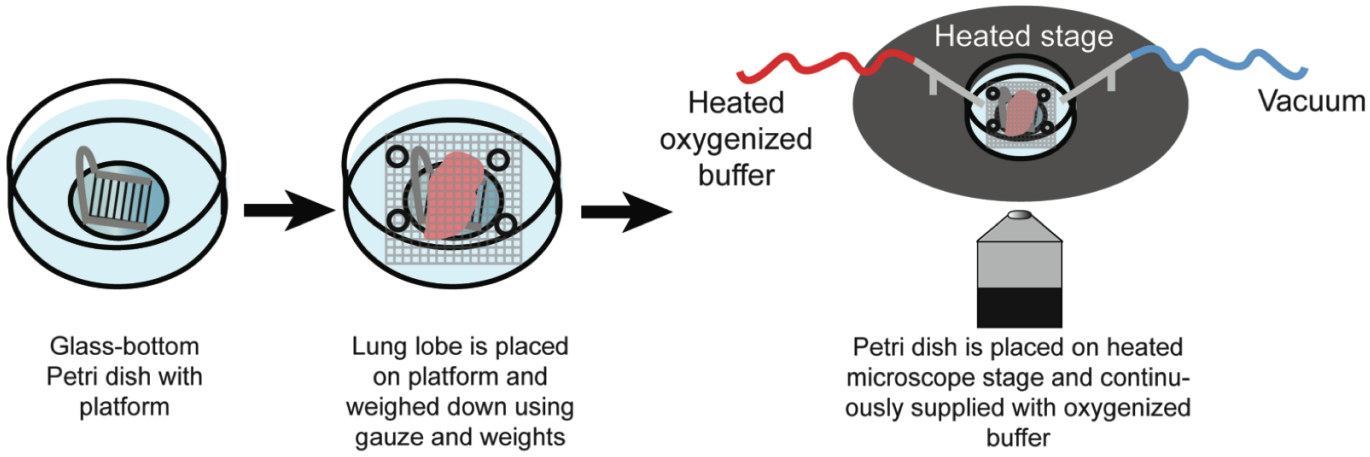

Figure S1: **Schematic diagram representing the platform used for imaging of lung explants using an inverted microscope setup.** This platform is composed of a slightly raised apparatus that is then placed on a glass bottom microwell dish (Mat Tek Corporation). The imaging platform is enclosed in a chamber maintained at 37ºC by warmed air. Pre-warmed phenol-free PRMI 1640 (Lonza) is circulated via a pump into the imaging dish (Bioscience Tools), while being continuously supplied with a 95% oxygen/5% CO2 mixture.

Supplementary figure 2

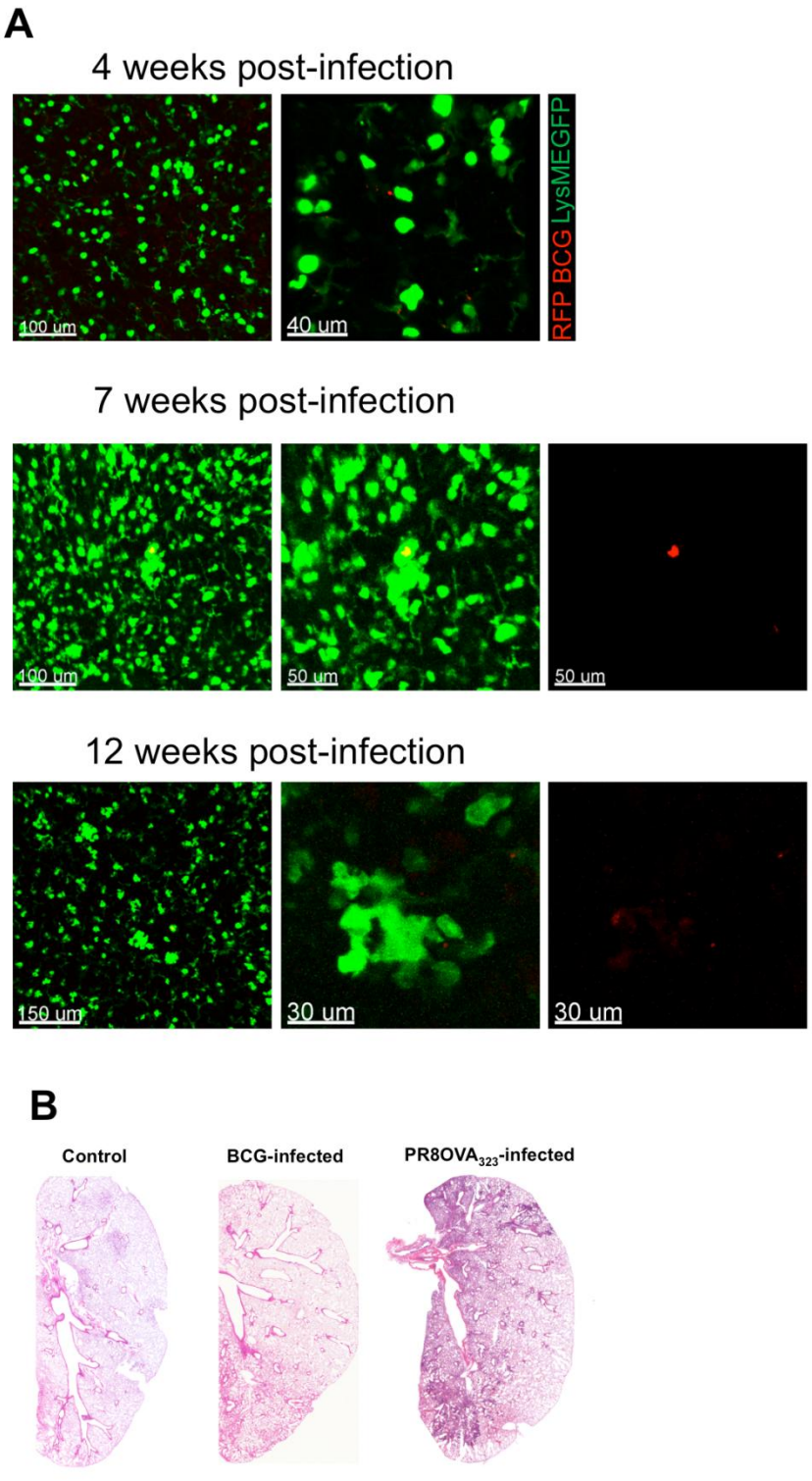

Figure S2: **Time course of BCG infection and gross pathology associated with BCG and PR8OVA$_{323}$ infection**. *A*, LysM-EGFP mice were infected with 5x10^3 CFUs BCG expressing the red fluorescent protein (RFP). Lungs were harvested at the indicated time points and confocal images acquired. Images are z stack projections of approximately 40-50μm volumes. Colors of the words correspond to colors in the image. *B*, Representative hematoxylin and eosin stains of the left lobe of an uninfected animal, an animal infected with 5x10^3 CFUs BCG 5 months previously, and an animal infected with PR8OVA$_{323}$ 7 days previously.

Supplementary figure 3

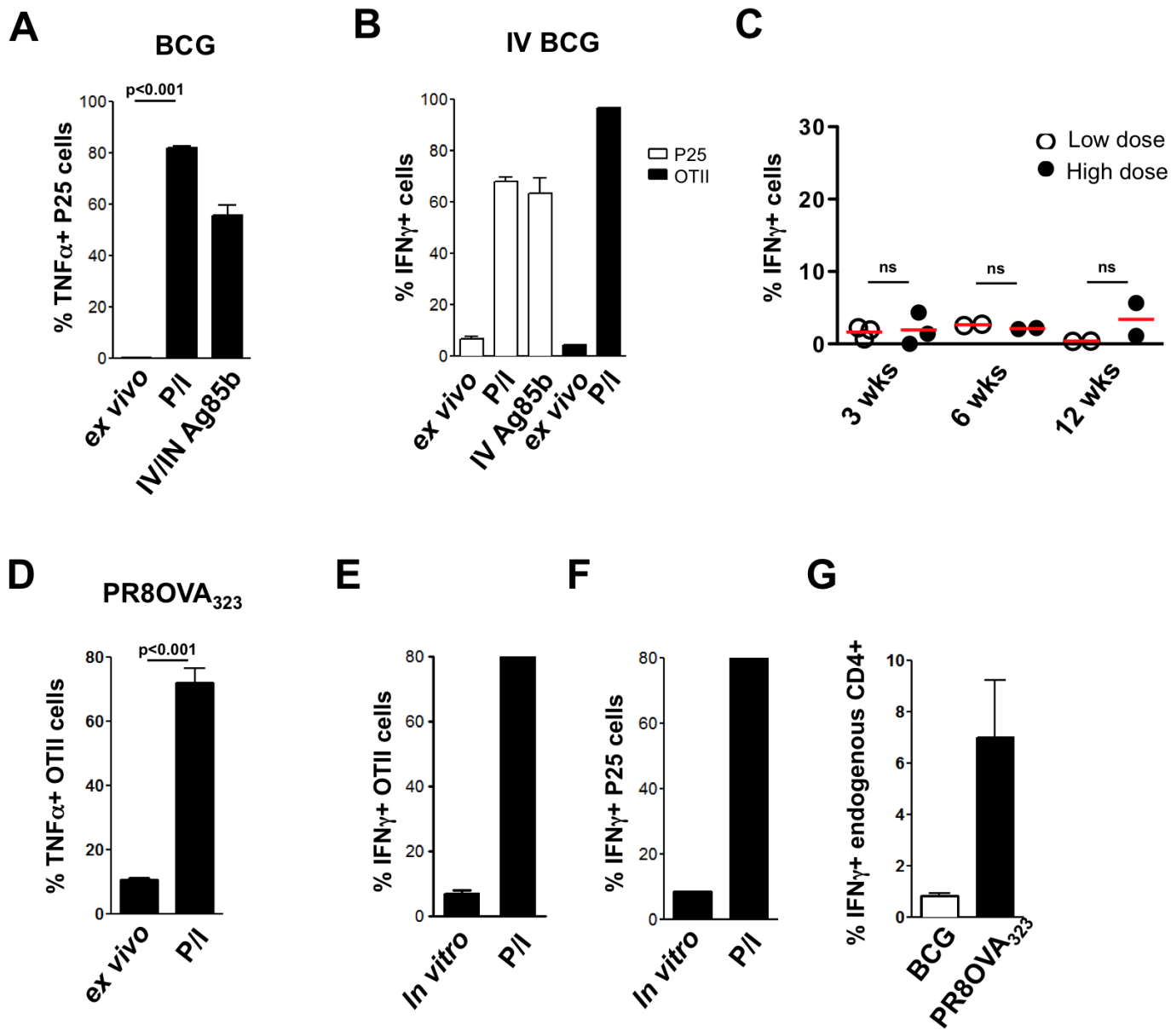

Figure S3: Cytokine production profile of antigen specific T cells. *A,* Percentage of P25 T cells producing TNFα, as measured by intracellular cytokine stain, directly *ex vivo* from lungs of BCG-infected mice, without further restimulation. *B,* Quantification of the percentage of IFNγ+ P25 T cells or OTII T cells, when taken directly *ex vivo* from the lungs of mice infected with $5x10^5$ CFUs of BCG administered intravenously 2 weeks prior, after 4 hours of *in vitro* PMA+ionomycin (P/I) stimulation, or 2 hours after *in vivo* administration of cognate antigen. C, Quantification of the percentage of IFNγ+ P25 T cells, when taken directly *ex vivo*, at the indicated time points post infection, from the lungs of mice infected with $5x10^3$ (low dose) or $5x10^5$ (high dose) CFUs of BCG administered intranasally. Horizontal lines indicate the mean. *D,* Percentage of OTII T cells producing TNFα, as measured by intracellular cytokine stain, directly *ex vivo* from lungs of influenza-infected mice, without further restimulation. *E and F,* Percentage of OTII and P25 T cells producing IFNγ prior to transfer into the animals, after 7 days of *in vitro* culture, without further restimulation (*in vitro*) and after PMA+ionomycin stimulation. *G,* Percentage of CD4+ T cells actively secreting IFNγ in the lung tissue of mice infected with BCG or $PR8OVA_{323}$, gated on all CD4+ T cells, excluding transferred T cells. Data represented as means with standard error of the mean.

Supplementary figure 4

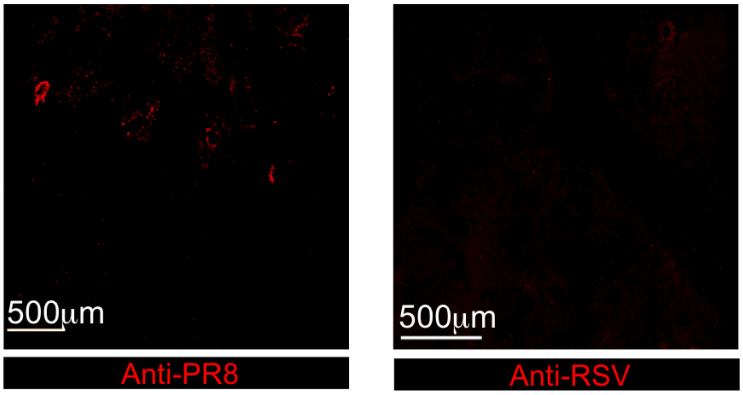

Figure S4: Influenza-infected lungs stained with anti-PR8 or with anti-RSV antibody as a specificity control. Immunofluorescent staining of influenza-infected mouse lung with anti-PR8 antibody or anti-RSV antibody, as acquired by confocal microscopy. Colors of the words correspond to colors in the image.