



**SUPPLEMENTARY FIG. S2.** Readministration of rAAV2/5 in adult life is more efficient after initial fetal than neonatal vector delivery. rAAV2/5-fLUC ( $1.5 \times 10^{10}$  GC/animal) and rAAV2/5- $\beta$ -Gal ( $1.0 \times 10^{10}$  GC/animal) were delivered to fetal mice on E18 and to neonatal pups on day 3. At 3 months of age, a second vector dose was given by intratracheal instillation to approximately half of the animals, whereas the other half of the group was kept as non-readministration controls. An adult control group was included to control for the maximum amount of gene expression to be expected in the upper and lower airways after intratracheal instillation of rAAV2/5-fLUC and rAAV2/5- $\beta$ -Gal. fLUC expression was visualized (photos at *bottom*) and quantified (A–F) over time, using BLI. The pseudocolor scale of BLI images depicts the photon flux per second, per square centimeter per steradian (p/sec/cm<sup>2</sup>/sr). Total photon flux (p/sec) was quantified at various time points (1–4 months) for nose and lung. Measurements of individual animals were plotted as single values and the average BLI signal per group per time point is depicted. The gray circles represent all the different animals from the experiment, including the ones killed at various time points (1–3 months). The black arrow depicts the first dose received as fetus/neonate; the red arrow readministration in adult life. Red circles, BLI signal after readministration; blue triangles, non-readministration controls. (C and F) The lung and nose signal of animals that received a second vector dose (i.e., readministration) after perinatal gene delivery was compared with the signal measured in adult control animals that received a single vector dose, 1 month postinjection. Comparisons between groups at specific time points were performed by one-way ANOVA followed by a Tukey HSD post-hoc test, \* $p < 0.05$ , \*\* $p < 0.01$ . *Abbreviations:* Fetal-R or Neonatal-R, initial fetal or neonatal vector delivery followed by readministration at 3 months.