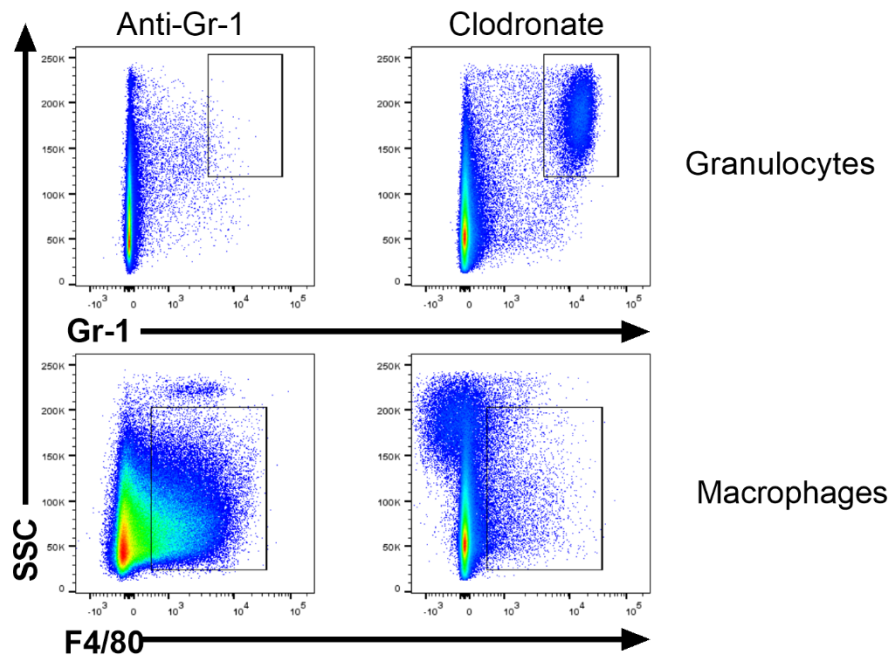Contribution of antibody-mediated effector functions to the mechanism of efficacy of vaccines for opioid use disorders.

SUPPLEMENTAL MATERIAL

Figure S1

Supplemental Figure S1. Verification of granulocyte and macrophage depletion by flow cytometry. Mice were treated with liposome-embedded clodronate to deplete macrophages, or with an anti-Gr-1 mAb to deplete granulocytes. To verify successful treatment of macrophage and granulocyte populations, spleen, cervical, inguinal, brachial, and axillary lymph nodes were collected at the conclusion of the experiment, and processed to obtain single cell suspensions. Red blood cells were lysed with ammonium-chloride-potassium (ACK) lysis buffer (Gibco, Gaithersburg, MD) and the remaining white blood cell pellet was washed and stained for CD90.2 (PerCP-e710, clone 30H12), Gr-1 (APC-eF780, clone RB6 8CS), and F4/80 (Pe-Cy7, clone BMB) at 1:100 dilution in a sample volume of 100 µL. Cells were fixed with BD sample fixation buffer (BD Biosciences). Samples were analyzed on an LSR Fortessa (BD Biosciences, San Jose, CA) flow cytometer, and data analyzed using FlowJo software (FlowJo LLC, Ashland, OR).

Figure S2
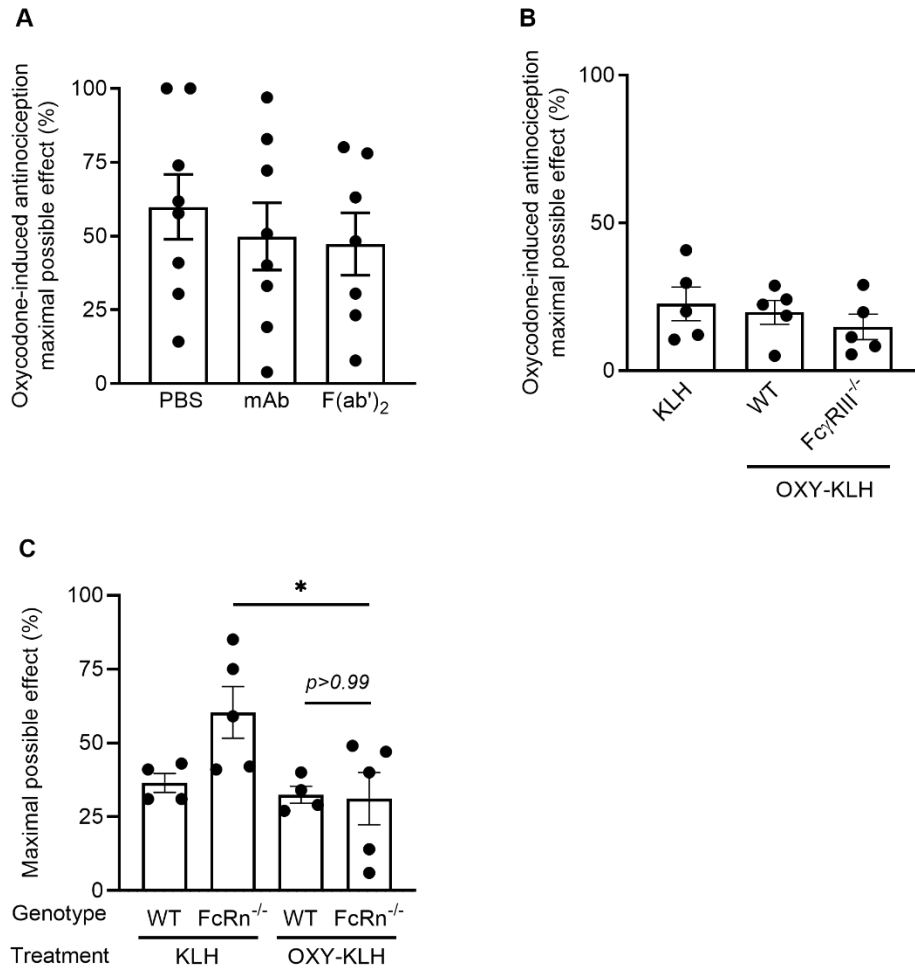
Supplemental Figure S2. Oxycodone-induced antinociception responses. A) Balb/c mice were passively immunized with anti-oxycodone mAb or F(ab')2 at 24 hours before a 2.25 mg/kg oxycodone challenge, and antinociception was measured on a hot plate. B) C57BL/6 wild-type or FcγRIII$^{-/-}$ mice were immunized with either OXY-KLH or unconjugated KLH on days 0, 14, 28, and 42. In addition, mice receiving OXY-KLH were also co-administered with an anti-IL-4 mAb (αIL-4) on days -2 and +1. Oxycodone-induced antinociception was analyzed 7 days after the 3rd immunization (day 35). Mice were challenged s.c. with 2.5 mg/kg oxycodone to test for vaccine efficacy in reducing oxycodone-induced antinociception measured on a hot plate. Mice were re-challenged after the 4th vaccination (Figure 3). C) C57BL/6 wild-type or FcRn$^{-/-}$ mice were immunized with either OXY-KLH or unconjugated KLH on days 0, 14 and 28, and on day 35 mice were challenged s.c. with 5.0 mg/kg oxycodone, and antinociception was measured on a hot plate. Data are mean ± SEM. Sample size: n=5 mice in each treatment group. Statistical symbols: * p≤ 0.05 as compared by brackets.

Figure S3
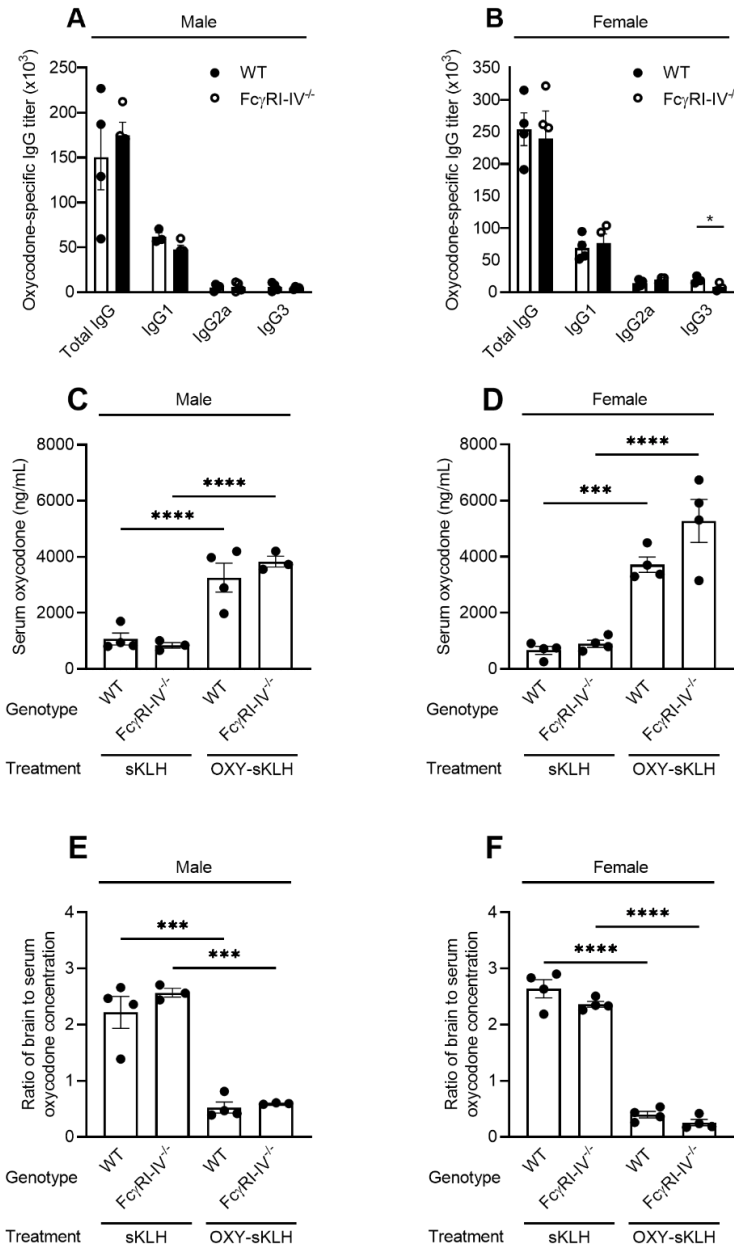
Supplemental Figure S3. Subset analysis of vaccine efficacy in male vs female wild-type and FcγRI-IV^{-/-} mice. Wild-type or FcγRI-IV^{-/-} male and female mice were immunized with OXY-KLH or KLH control on days 0, 14 and 28. On day 35, mice were challenged with 5.0 mg/kg oxycodone in order to evaluate vaccine efficacy. Pre-challenge oxycodone-specific IgG and subclass titers were measured by ELISA in male (A) and female (B) mice. At 30 minutes post-challenge, drug concentration in serum in male (C) and female (D) mice, and ratio of brain to serum drug concentration in male (E) and female (F) mice was assessed by LC-MS. Data are mean ± SEM. Sample size: *n*=4/treatment group. Statistical symbols: *, ***, **** p≤ 0.05, 0.001, 0.0001.