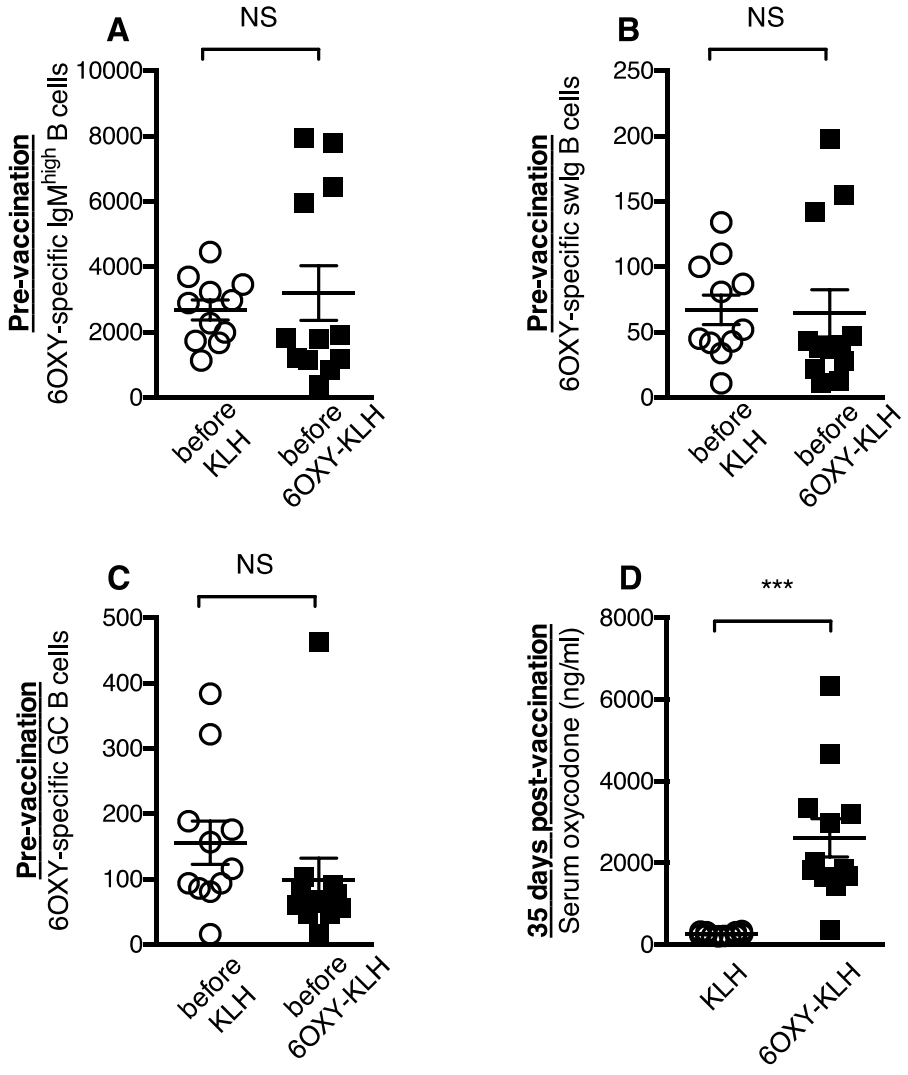
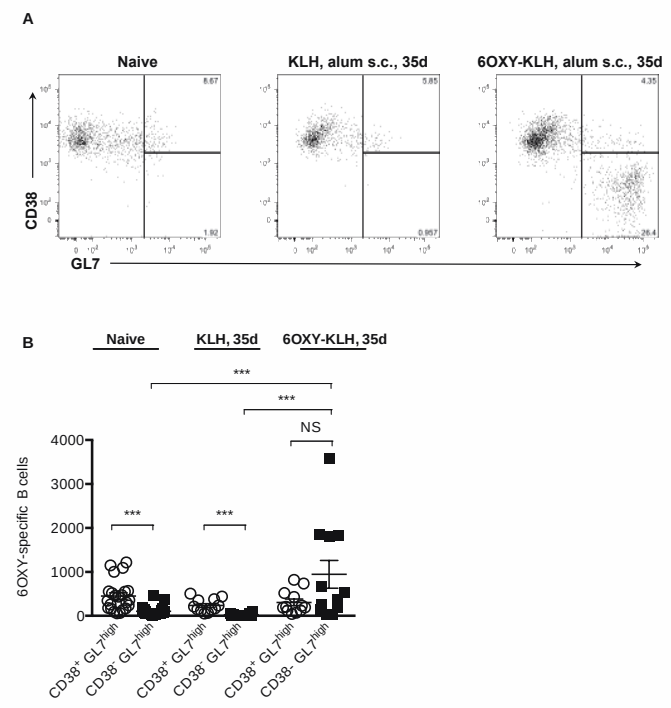
**Supplemental Figures and Legends**
**Supplemental figure 1**



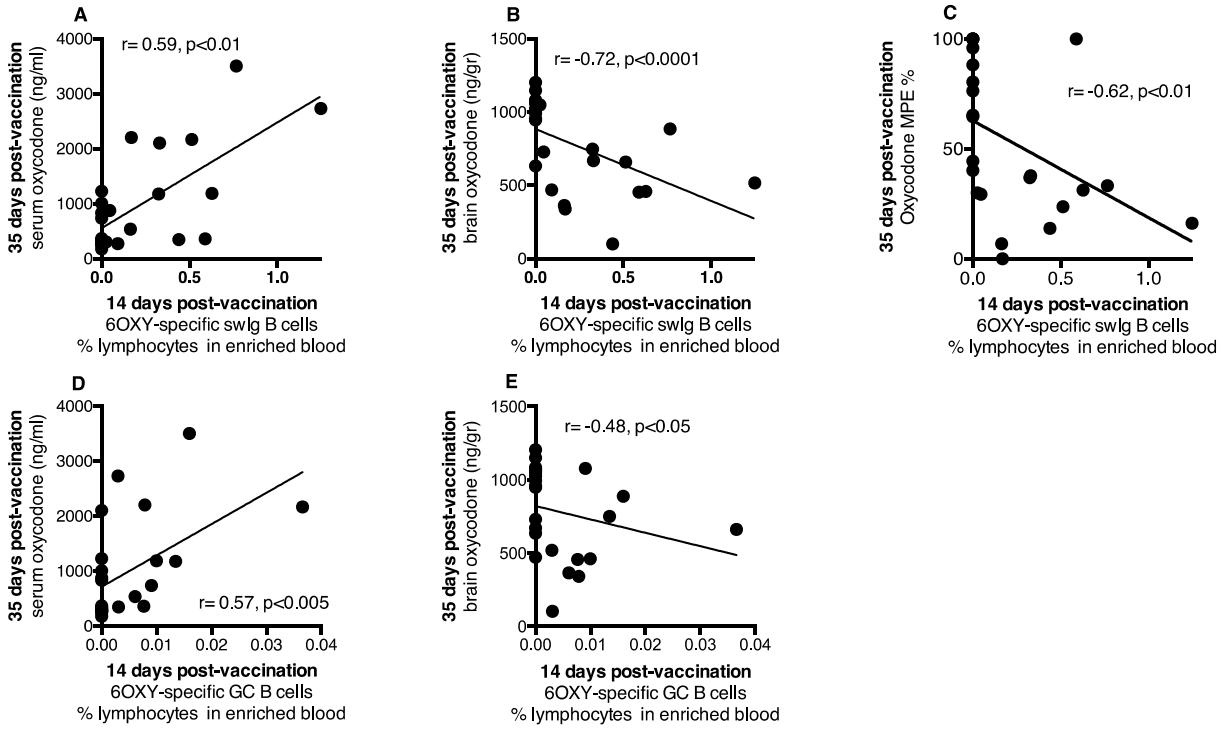

*Supplemental figure 1. The pre-immunization splenic hapten-specific B cell repertoire is not different between groups.* B cell analysis was performed by means of spleen biopsy in a naïve cohort of mice (n=24) prior to vaccination and then randomly assigned to either KLH or 6OXY-KLH (n=12 each group). Before vaccination: A) 6OXY-specific $IgM^{high}$ B cells; B) 6OXY-specific switched immunoglobulin B cells; and C) 6OXY-specific $GL7^{high}$ B cells. After vaccination: D) serum oxycodone concentrations. Data are the number of 6OXY-specific B cells in spleen biopsy samples from each mouse.

**Supplemental figure 2**




*Supplemental figure 2. Vaccination with 6OXY-KLH elicits germinal center activation*. Vaccination s.c. at days 0, 14 and 28 with either 6OXY-KLH or unconjugated KLH adsorbed on alum elicits genuine 6OXY-specific $CD38^-$ $GL7^{high}$ GC B cells. A) Gating strategy to classify 6OXY-specific $GL7^{high}$ B cells as $CD38^+$ $GL7^+$ (GC-independent pathway) or $CD38^-$ $GL7^+$ (GC-dependent). Data are representative dot plots from a naïve mouse, or mice immunized with either 6OXY-KLH or unconjugated KLH on days 0, 14 and 28. B cell analysis was performed by means of spleen biopsy 7 days after the third immunization. B) 6OXY-specific $GL7^{high}$ B cells as $CD38^+$ $GL7^+$ (GC-independent pathway) or $CD38^-$ $GL7^+$ (GC-dependent) in naïve mice or mice immunized with either 6OXY-KLH or KLH at 0, 14 and 28 days. Data are the number of 6OXY-specific B cells in spleen biopsy samples from each mouse. *** $p<0.001$ as indicated by brackets.

**Supplemental figure 3**




*Supplemental figure 3. Early-activated 6OXY-specific B cells in blood correlates to vaccine efficacy.* The frequency of 6OXY-specific swIg B cells 14 days after the first immunization correlated to vaccine efficacy after the third immunization determined as A) oxycodone serum distribution, B) oxycodone distribution to brain, and C) oxycodone antinociception. The frequency of 6OXY-specific GC B cells 14 days after the first immunization correlated to vaccine effect on oxycodone distribution to D) serum, and E) to the brain.

*Supplemental table I. Summary of results*

| B cell subset | Analysis timepoint* | Spleen biopsy analysis vs vaccine efficacy# | Blood analysis vs vaccine efficacy |
|---|---|---|---|
| $IgM^{high}$ | before | $r^2$= 0.47, p<0.05 vs serum IgG titers<br>$r^2$= 0.35, p<0.05 vs brain oxycodone | $r^2$= 0.44, p<0.05 vs brain oxycodone |
| $IgM^{high}$ | after | No significant correlation | r= 0.59, p<0.01 vs serum oxycodone<br>r= -0.66, p<0.001vs brain oxycodone<br>r= -0.52, p<0.05 vs antinociception |
| swIg | before | $r^2$= 0.47, p<0.05 vs serum IgG titers<br>$r^2$= 0.28, p= 0.08 vs brain oxycodone | No significant correlation |
| swIg | after | No significant correlation | r= 0.59, p<0.01 vs serum oxycodone<br>r= -0.72, p<0.001vs brain oxycodone<br>r= -0.62, p<0.05 vs antinociception |
| GC | before | No significant correlation | No significant correlation |
| GC | after | r= 0.75, p<0.0001 vs serum oxycodone | r= 0.57, p<0.005 vs serum oxycodone<br>r= -0.48, p<0.05 vs brain oxycodone |
| ASC | before | No significant correlation | No significant correlation |
| ASC | after | r= 0.46, p<0.05 vs serum oxycodone | No significant correlation |

*Shown the correlation (Pearson's coefficient, $r^2$) between numbers of hapten-specific B cells before immunization and vaccine efficacy in the 6OXY-KLH group, and the correlation (Spearman's coefficient, r) between numbers of hapten-specific B cells 14 days after immunization and vaccine efficacy in all immunized mice. #Mice were immunized s.c. with 6OXY-KLH or KLH in alum on days 0, 14 and 28. Serum antibodies and vaccine effects on drug distribution and behavior were determined a week after the last immunization.