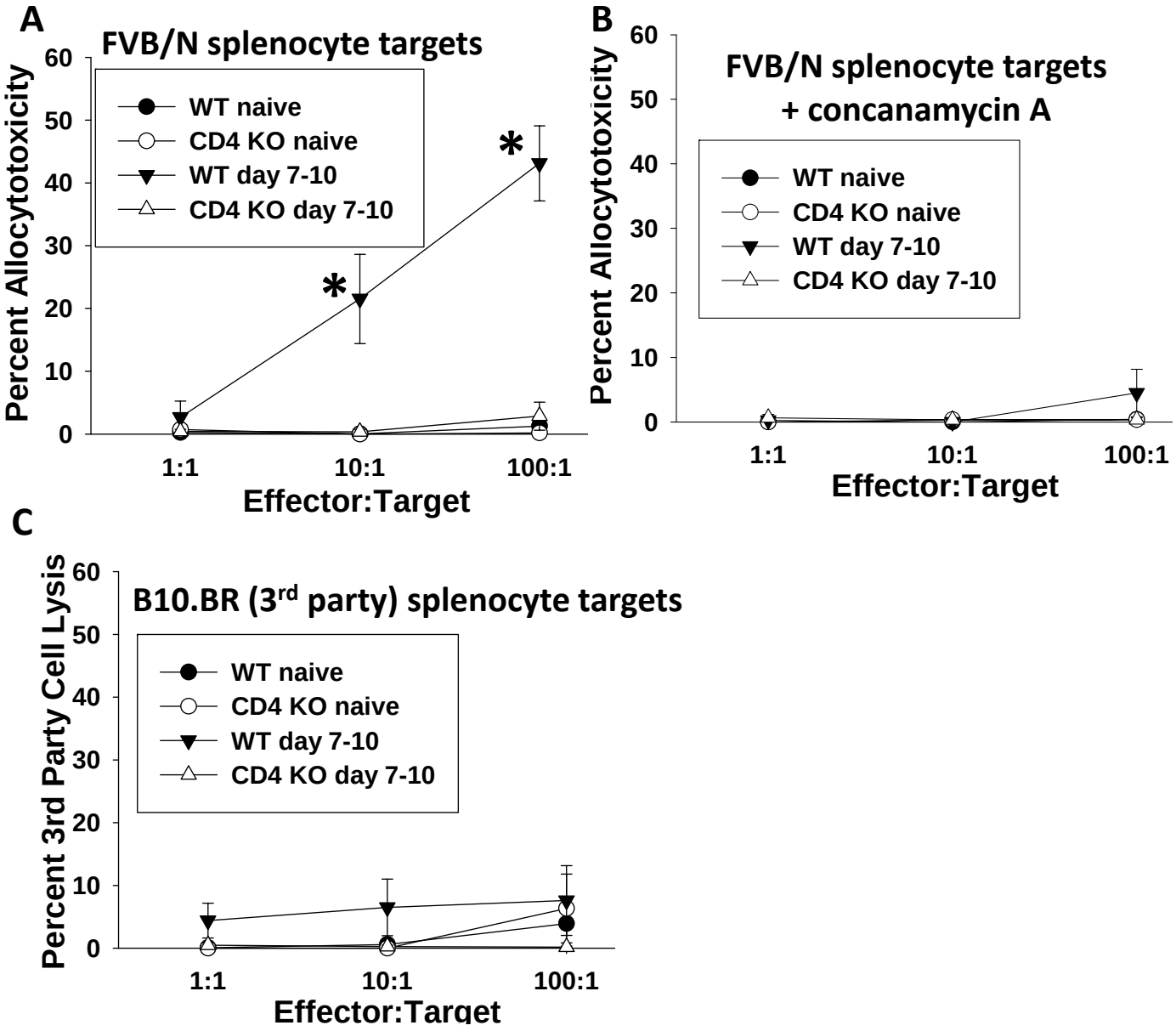
Supplemental Digital Content (SDC), Figure 1



SDC, Figure 1. ***In vitro*** **allospecific perforin-dependent cytotoxicity is readily detected by splenocytes from wild-type, but not CD4-deficient, hepatocellular allograft recipients.** Splenocytes from wild-type (WT; day 7-10) and CD4-deficient (CD4 KO; day 7-10) allogeneic hepatocyte recipients were restimulated *in vitro* with allogeneic hepatocytes for three days followed by co-culture in a standard effector:target cytotoxicity (100:1 to 1:1) assay using allogeneic, syngeneic or third party concanavalin A blasts. Control assays with naïve splenocytes from wild-type or CD4 KO mice were performed. **A)** Standard 4 hour *in vitro* chromium release assays demonstrated cytotoxic activity only by splenocytes from wild-type recipients (2.7±1.1%, 21.5±2.9, and 43.1±2.4% for 1:1, 10:1, and 100:1 effector to target ratios, respectively). **B)** Treatment of effector cells with concanamycin A (CMA, 28 nM; Sigma, St. Louis, MO) during the *in vitro* chromium release assay abrogated cytotoxic activity, suggesting predominance of a perforin-mediated mechanism of *in vitro* cytotoxic effector function. **C)** *In vitro* cytotoxicity by effector splenocytes from wild-type hepatocyte rejectors was allospecific since no target lysis was observed in response to "third party" B10.BR ($H\text{-}2^k$) target cells. Significance is depicted by a "*" ($p<0.05$). Error bars denote the standard error of duplicate experiments (triplicate wells/sample).