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Supplemental Information

Single-Cell Resolution and Quantitation of Targeted Glucocorticoid Delivery in the Thymus

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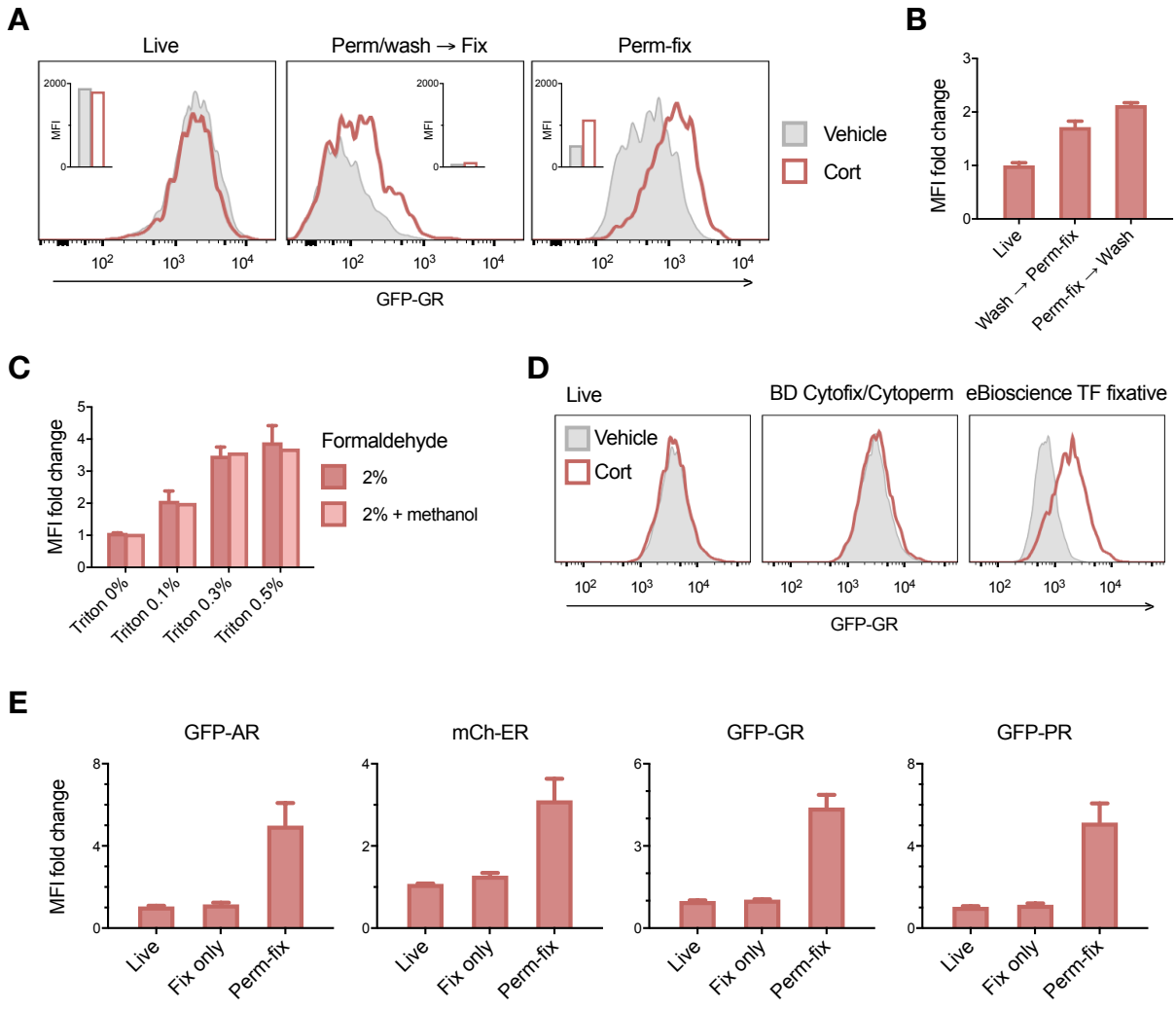

Figure S1. ***Simultaneous permeabilization and fixation (perm-fix) retains liganded glucocorticoid receptor.*** *Related to Figure 1 and 3.*

(**A**) 3617 cells expressing a GFP-glucocorticoid receptor (GFP-GR) fusion protein were cultured in steroid-free medium, incubated for 60 min with or without 10^{-6} M corticosterone (Cort), and trypsinized. Cells were then kept on ice (Live), briefly washed in permeabilization buffer (eBioscience) and fixed with the eBioscience transcription factor fixation reagent (Wash → perm-fix), or fixed and washed in permeabilization buffer (Perm-fix → wash). GFP-GR median fluorescence index (MFI) values in insets. Data were acquired by flow cytometry.
(**B**) Relative change in GFP-GR MFI values after corticosterone treatment, for (a).
(**C**) Relative change in 3617 cell GFP-GR MFI values after corticosterone treatment, simultaneous perm-fix with the indicated permeabilization buffer and methanol-free or methanol-containing formaldehyde, and acquisition by flow cytometry.
(**D**) GFP-GR expressing 3617 cells were incubated in the absence or presence of corticosterone for 15 min, then kept on ice ("Live"), fixed with BD Cytofix/Cytoperm, or fixed with eBioscience FoxP3 transcription factor fixative reagent. Cell were washed and GFP-GR quantified by flow cytometry.
(**E**) 3617 cells expressing a GFP-androgen receptor (GFP-AR), mCherry-estrogen receptor (mCh-ER), GFP-GR, or GFP-progesterone receptor β (GFP-PR) fusion proteins were cultured in steroid-free medium, incubated for 20 min with or without 10^{-6} M steroid (testosterone, 17β-estradiol, corticosterone, or progesterone, respectively), then kep on ice ("Live"), fixed, or perm-fixed. Cell were quantified by flow cytometry. Data are presented as the relative increase in fluorescence of steroid-treated versus untreated samples.

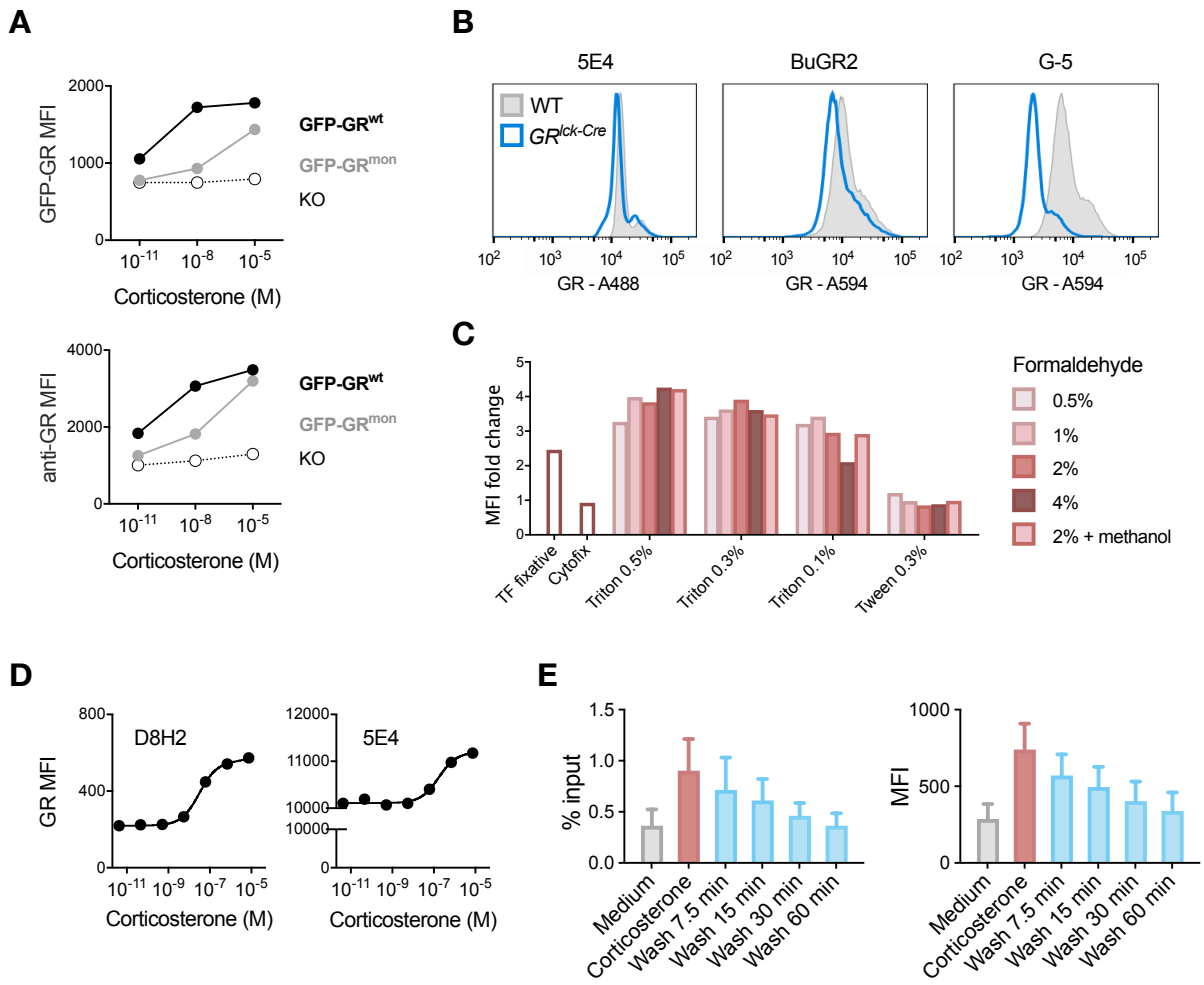

Figure S2. ***Antibody GR staining and permeabilization-fixation conditions in mouse thymocytes.*** *Related to Figure 4.*

(**A**) 3617 cells expressing the wild-type GR (GFP-GR^{wt}), dimerization-impaired GR (GFP-GR^{mon}), or deficient for GR (KO) were treated with different concentrations of corticosterone, perm-fixed, stained with anti-GR antibody (clone G-5) and a secondary Alexa 647-conjugated anti-mouse antibody, and data acquired by flow cytometry. Live cell GFP-GR MFI values are shown in Fig. 2c).
(**B**) WT and *GR$^{lck-Cre}$* mouse thymocytes were surface stained, fixed, and stained for GR using the specified monoclonal antibodies. The 5E4 antibody was directly conjugated to FITC, while BuGR2 and G-5 were detected with a secondary Alexa 594-conjugated anti-mouse antibody. $CD4^+8^+$ DP thymocytes are shown.
(**C**) WT mouse thymocytes were incubated with or without 10^{-6} M corticosterone, perm-fixed with the indicated combination of permeabilization buffer and formaldehyde, and stained for GR using an Alexa 488-conjugated G-5 monoclonal antibody. The relative increase in MFI after corticosterone treatment is shown.
(**D**) WT mouse thymocytes were incubated in medium containing the specified amount of corticosterone, surface markers were stained, cells perm-fixed, and GR stained with D8H2-Alexa 488 or 5E4-FITC monoclonal antibodies (G-5-Alexa 488 shown in Fig. 4b).
(**E**) WT mouse thymocytes were incubated for 20 min in medium containing 10^{-7} M corticosterone, washed twice and incubated in steroid-free medium, and collected at the indicated times. One aliquot was fixed in 1% formaldehyde and ChIP-qPCR performed to quantitate GR binding to the *Tsc22d3* (Gilz) gene promoter. Another aliquot was perm-fixed, GR stained, and counted by flow cytometry.

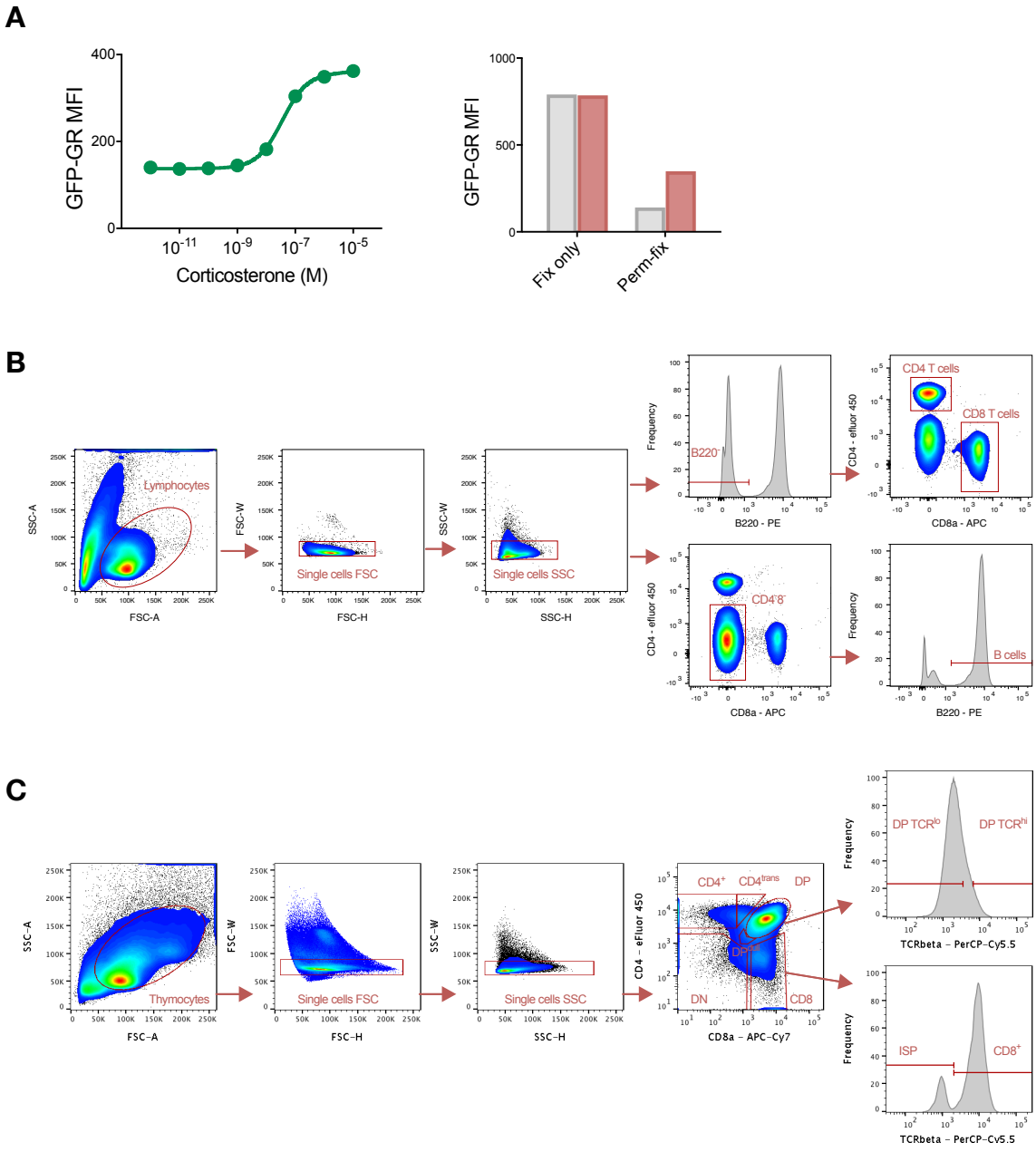

Figure S3. ***Dose-response of perm-fixed GFP-GR mouse thymocytes and flow cytometry gating strategies.*** *Related to Figures 6 and 7.*

(**A**) Primary thymocytes from GFP-GR knockin mice were incubated in the indicated corticosterone concentrations for 20 min, fixed or perm-fixed, and cells measured by flow cytometry.
(**B**) Flow cytometry gating strategy for blood and spleen lymphocytes (adult mouse blood sample shown).
(**C**) Flow cytometry gating strategy for thymocyte subsets (neonatal mouse thymus shown).