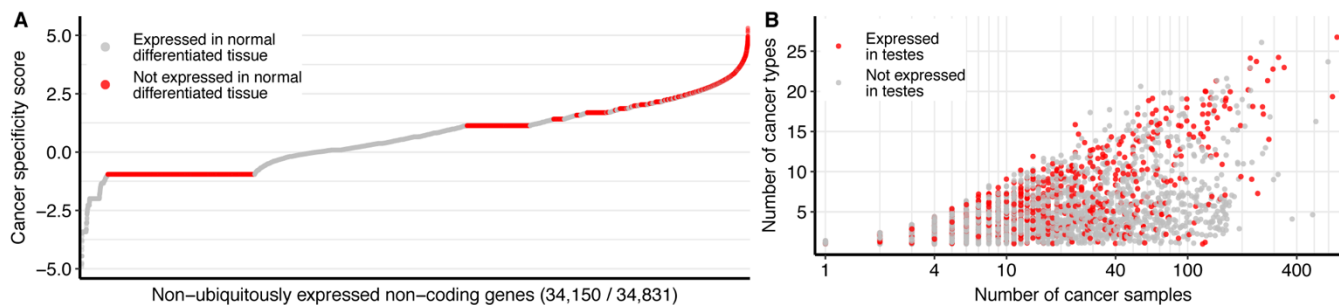# SUPPLEMENTAL FIGURES

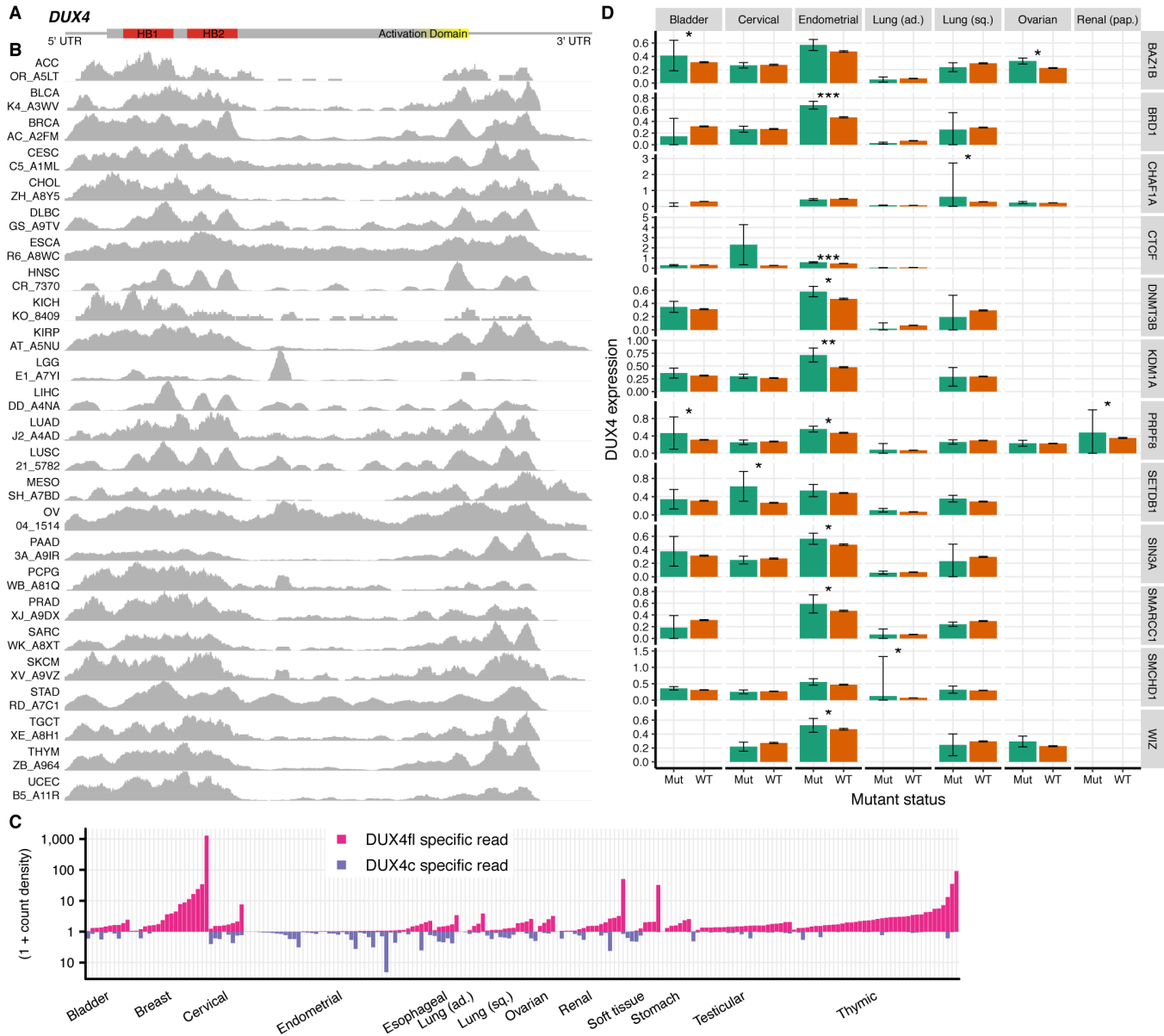## Figure S1 – related to Figure 1

Figure S1 – related to Figure 1. Cancer specificity of non-coding genes.
(**A**) As **Fig. 1A**, but for non-coding genes that are not expressed in all tissue types.
(**B**) As **Fig. 1C**, but for non-coding genes.

Figure S2 – related to Figure 2

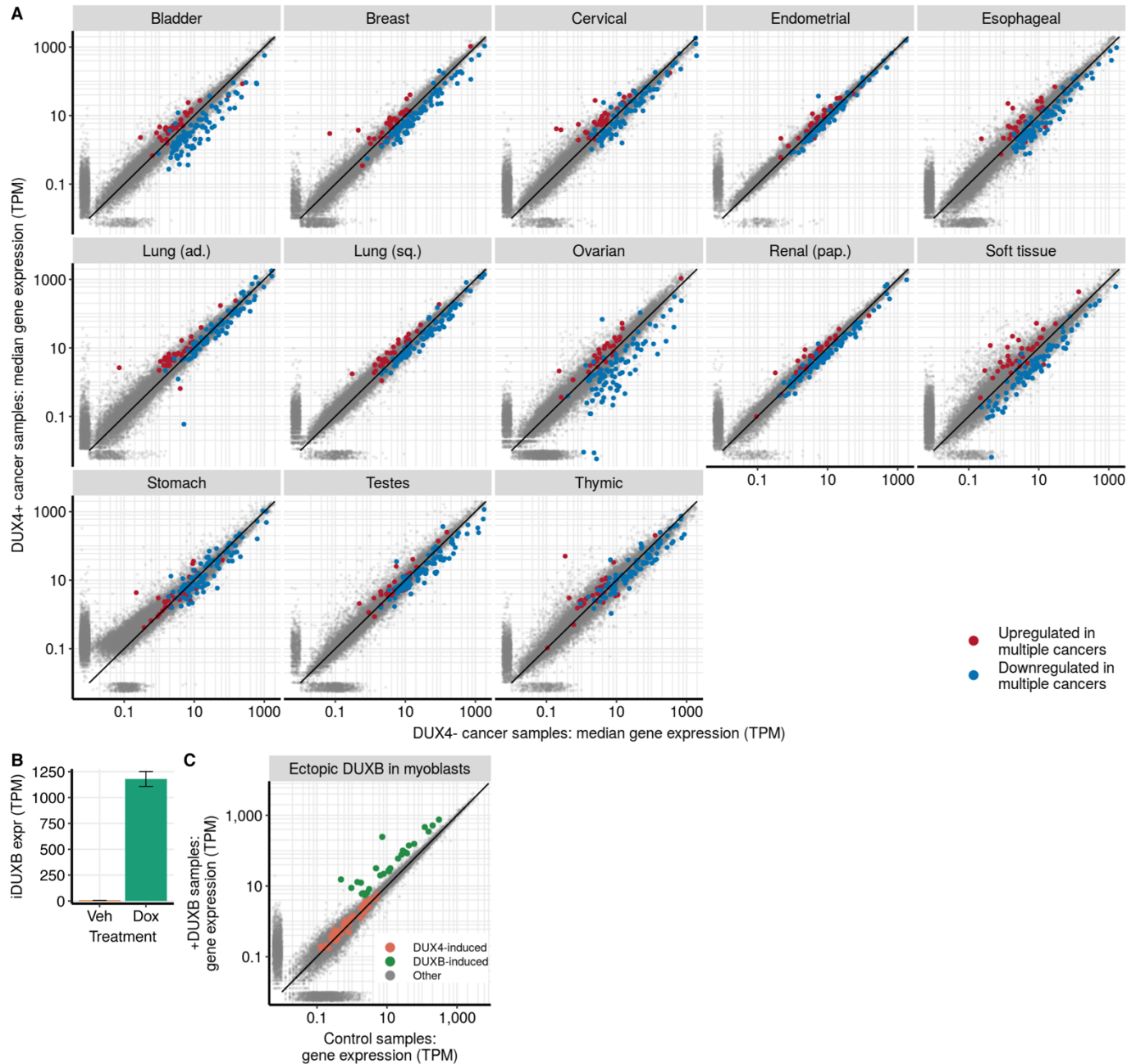**Figure S2 – related to Figure 2. DUX4 is expressed as a full-length mRNA in solid cancers.**
(**A**) Schematic of DUX4 isoform that encodes the full-length transcription factor. Red, sequence encoding the DNA-binding homeodomains (HB1 and HB2); yellow, sequence encoding the C-terminal activation domain.

(**B**) As **Fig. 2C**, but including data from additional representative DUX4+ samples from each analyzed cancer type.

(**C**) Counts of RNA-seq reads mapping uniquely to the DUX4 mRNA encoding the full-length transcription factor (DUX4fl) or the DUX4C mRNA (DUX4c). Plot restricted to DUX4+ samples in cancer types with ≥5 DUX4+ samples.

(**D**) Median DUX4 mRNA levels (TPM) in cancer samples with (Mut) or without (WT) predicted loss-of-function mutations affecting the indicated genes. */**/***, p < 0.05/0.01/0.001 by the one-sided Mann-Whitney U test. See also **Fig. 2G**. Error bars, standard deviations estimated by bootstrapping. TPM, transcripts per million.

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Figure S3 – related to Figure 3

Figure S3 – related to Figure 3. DUX4 expression is associated with decreased immune activity.
(**A**) Scatter plots of median gene expression of DUX4- and DUX4+ samples. Differentially expressed genes that are up- and down-regulated in DUX4+ samples in multiple (≥8) cancers are indicated in red and blue, respectively.
(**B**) Mean levels of DUXB mRNA following treatment of MB135 myoblasts engineered to contain a doxycycline-inducible *DUXB* expression construct with vehicle (Veh, water) or doxycycline (Dox) for 24 hours. Error bars, upper and lower values from two biological replicates.
(**C**) Differentially expressed genes in myoblasts treated with vehicle or doxycycline to induce *DUXB* expression. Red, high-confidence DUX4-induced genes (from **Fig. 3A**). Green, DUXB-induced genes based on this analysis, defined as genes exhibiting a fold-change of >2 and associated Bayes factor > 10 in both biological replicates. TPM, transcripts per million.

Figure S4 – related to Figure 4
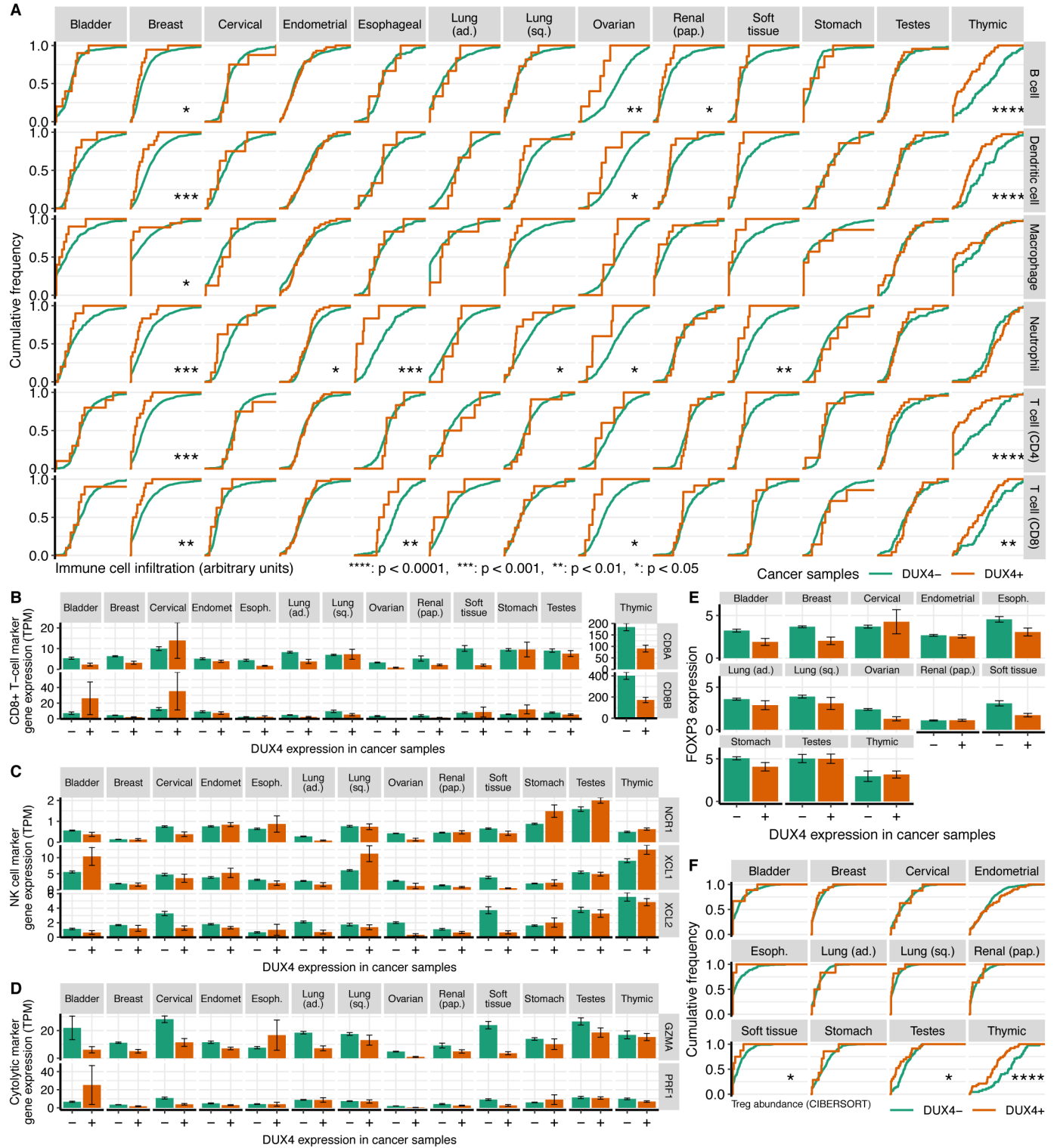
Figure S4 – related to Figure 4. DUX4+ cancers exhibit reduced immune cell infiltration.
(**A**) Estimated infiltration of different immune cell types in DUX4- and DUX4+ cancers, where infiltration was estimated with the TIMER method (Li et al., 2017). */**/***, $p < 0.05/0.01/0.001$ by the one-sided Mann-Whitney U test.
(**B**) Mean mRNA levels of the indicated markers of CD8+ T cells in DUX4- and DUX4+ cancers. Error bars, standard deviation of the mean estimated by bootstrapping.
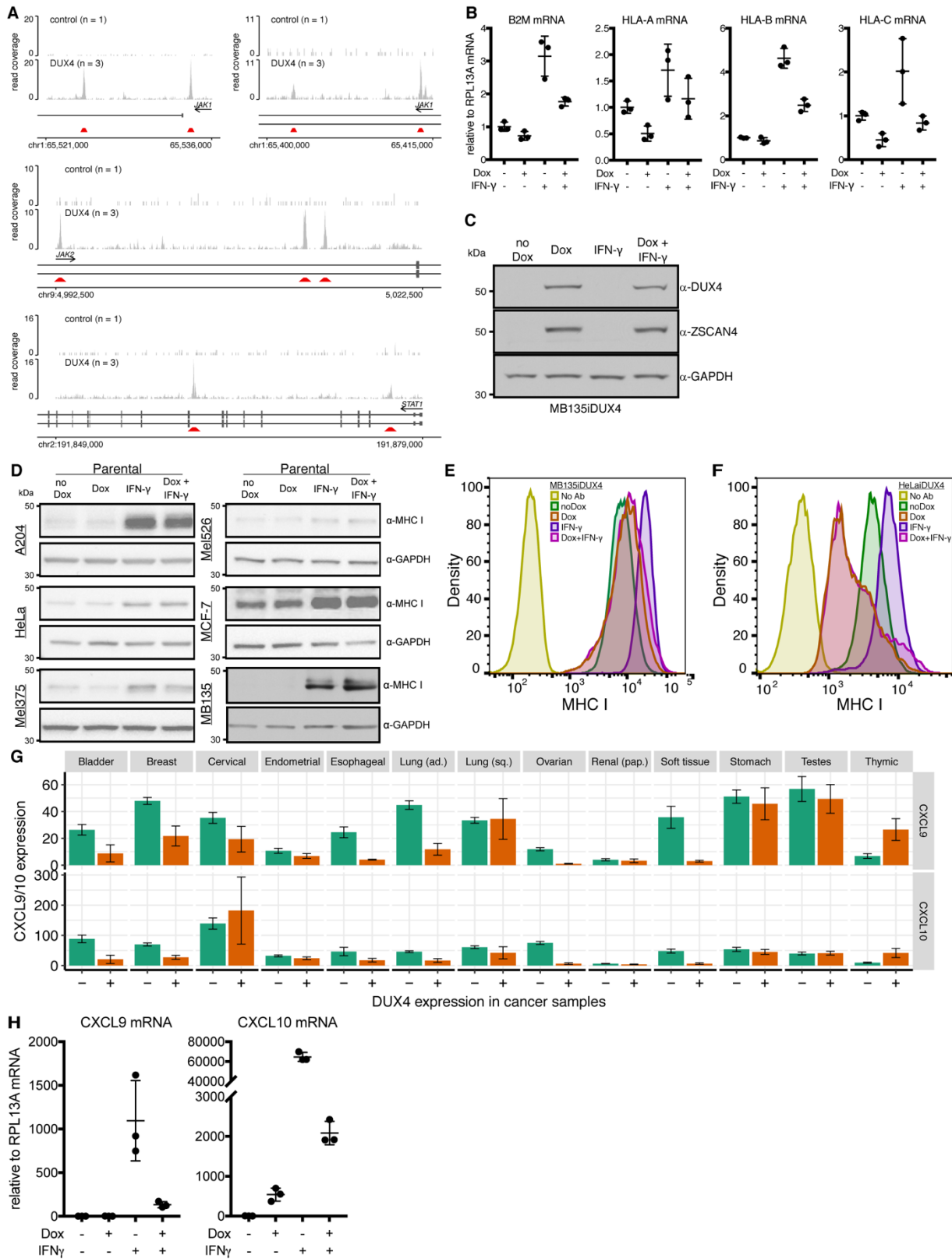(**C**) As (B), but illustrating NK cell markers.
(**D**) As (B), but illustrating *GZMA* and *PRF1* expression. See also **Fig. 4C**.
(**E**) As (B), but illustrating *FOXP3* expression.
(**F**) Estimated infiltration of regulatory T cells in DUX4- and DUX4+ cancers as estimated by CIBERSORT (Newman et al., 2015). */**/***, $p < 0.05/0.01/0.001$ by the one-sided Mann-Whitney U test.

Figure S5 – related to Figure 5

Figure S5 – related to Figure 5. DUX4 blocks interferon-γ-mediated induction of MHC Class I.
(**A**) Read coverage from DUX4 ChIP-seq experiments following acute *DUX4* expression in cultured myoblasts (Geng et al., 2012). Red blocks indicate peaks called with MACS (Zhang et al., 2008). n, number of replicates.

(**B**) Levels of B2M, HLA-A, HLA-B, and HLA-C mRNA following treatment of MB135iDUX4 with IFN-γ and/or doxycycline (Dox) to induce DUX4. Error bars, standard deviation across biological replicates.

(**C**) Levels of DUX4, ZSCAN4, and GAPDH protein following treatment of MB135iDUX4 cells with interferon-γ (IFN-γ) and/or doxycycline (Dox) to induce DUX4.

(**D**) Levels of MHC Class I and GAPDH protein following treatment of the indicated parental cell lines (without an inducible DUX4 construct) with interferon-γ (IFN-γ) and/or doxycycline (Dox). α-MHC I, pan-MHC Class I probe. These data serve as a control for **Fig. 5D-I** to confirm that doxycycline treatment alone does not block interferon-γ-mediated induction of MHC Class I.

(**E**) As Fig. 5J, but including the no antibody control (No Ab).

(**F**) As Fig. 5K, but including the no antibody control (No Ab).

(**G**) Mean CXCL9 and CXCL10 mRNA levels in DUX4- and DUX4+ cancers. Error bars, standard deviation of the mean estimated by bootstrapping.

(**H**) As (B), but illustrating CXCL9 and 10 mRNA levels.