Supplemental Information

Pioneer factor Foxa2 enables ligand-dependent activation of type II nuclear receptors FXR and LXRα.

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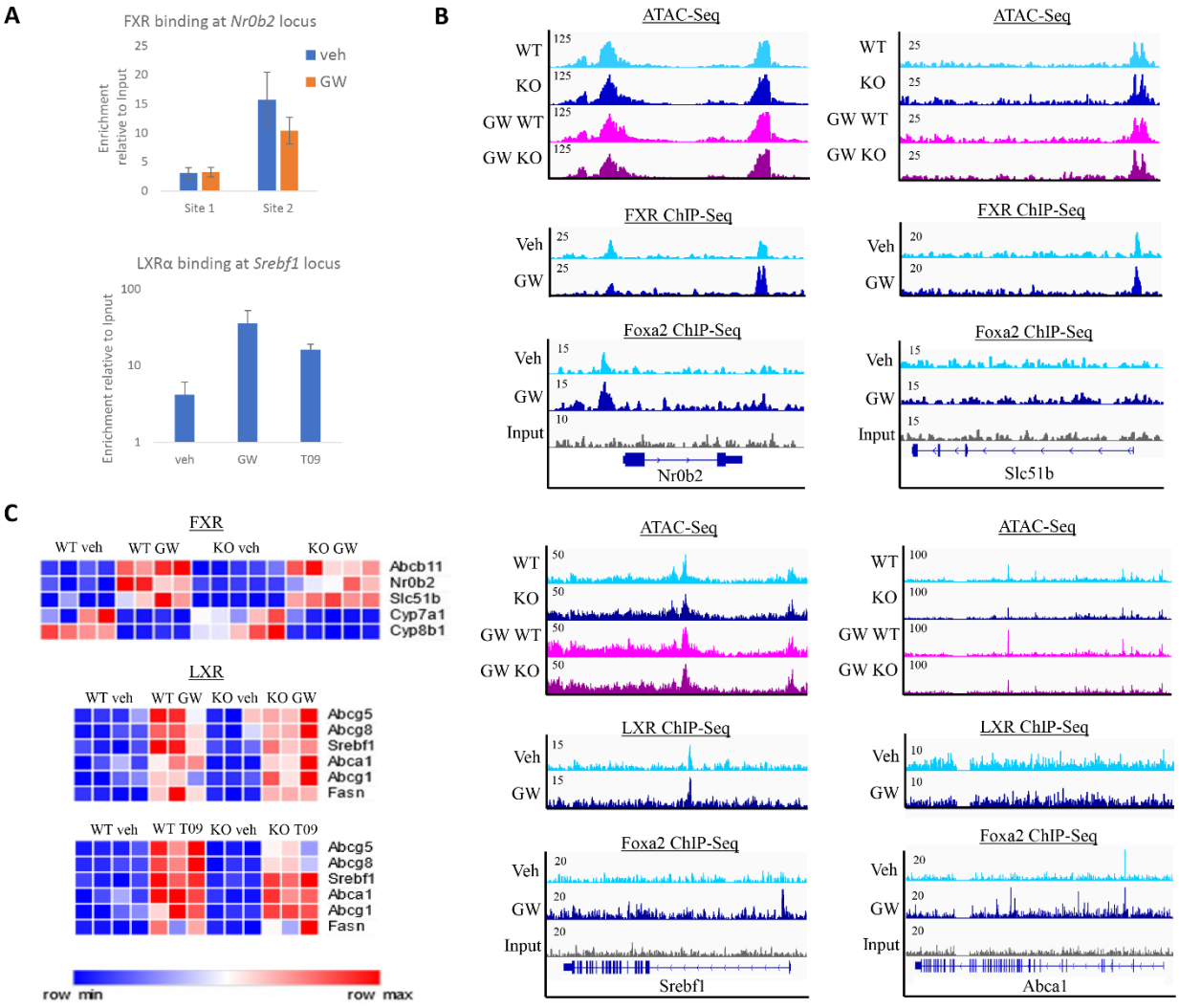
*equal contribution

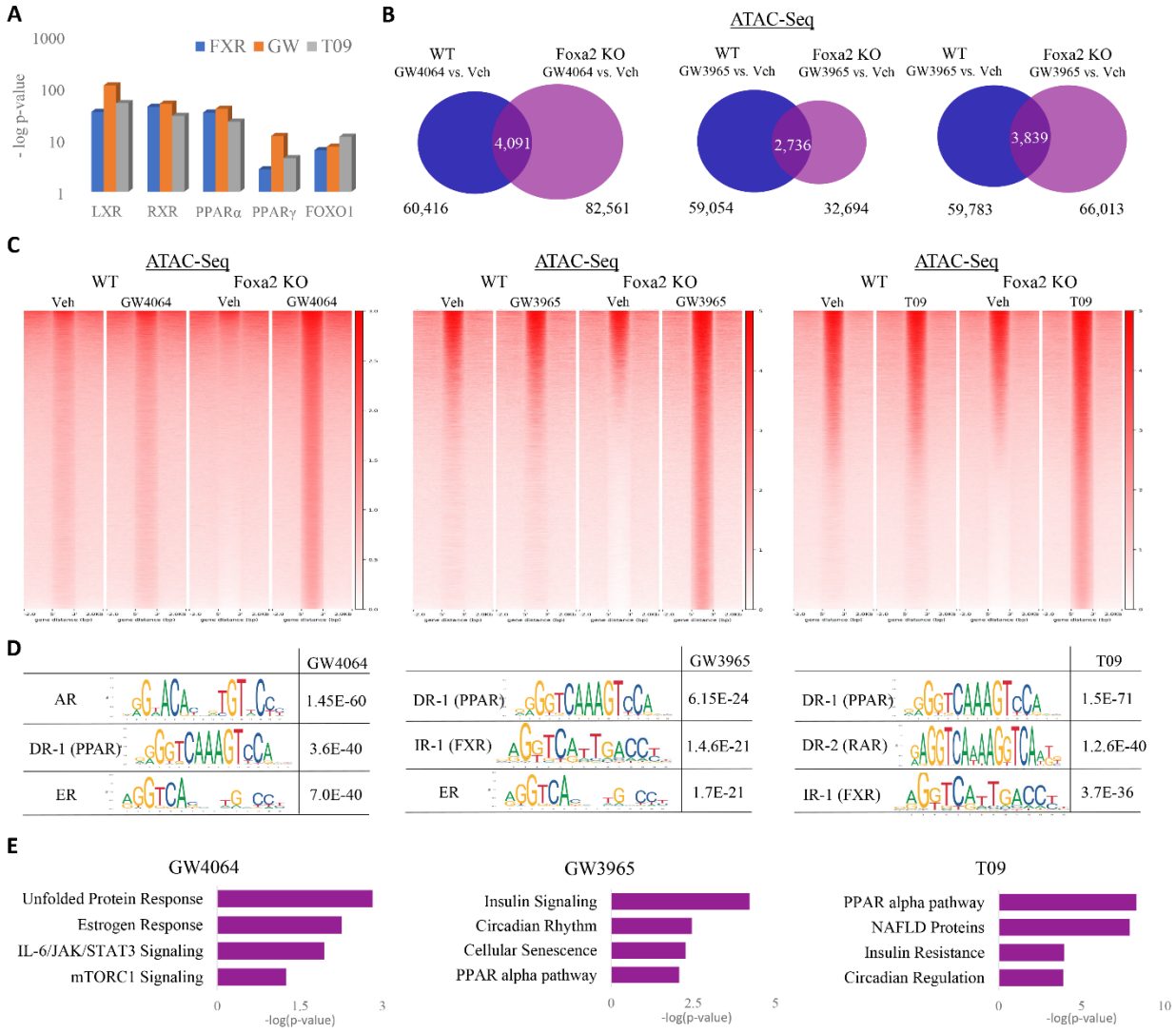


Supplemental Figure 1

Supplemental Figure 1 Classical ligand-independent FXR and LXRα targets

(**A**) Q-PCR validation of FXR ChIP in livers of wildtype mice treated with vehicle or GW4064 at two sites at Shp/*Nr0b2* locus (top panel). Q-PCR validation of LXRα ChIP in livers of wildtype mice treated with vehicle, GW3965 or T09 at *Srebf1* locus (bottom panel). (**B**) IGV view of chromatin accessibility (ATAC-Seq), FXR ChIP-Seq, and Foxa2 ChIP-Seq signal for classical FXR targets Shp/*Nr0b2* and Ostb/*Slc51b* (top panel) and LXRα targets *Srebf1* and *Abca1* (bottom panel, track of Input reads is provided for comparison). Chromatin accessibility does not change at these loci with addition of the ligand in wildtype controls and *Foxa2* mutants. FXR (top panel)

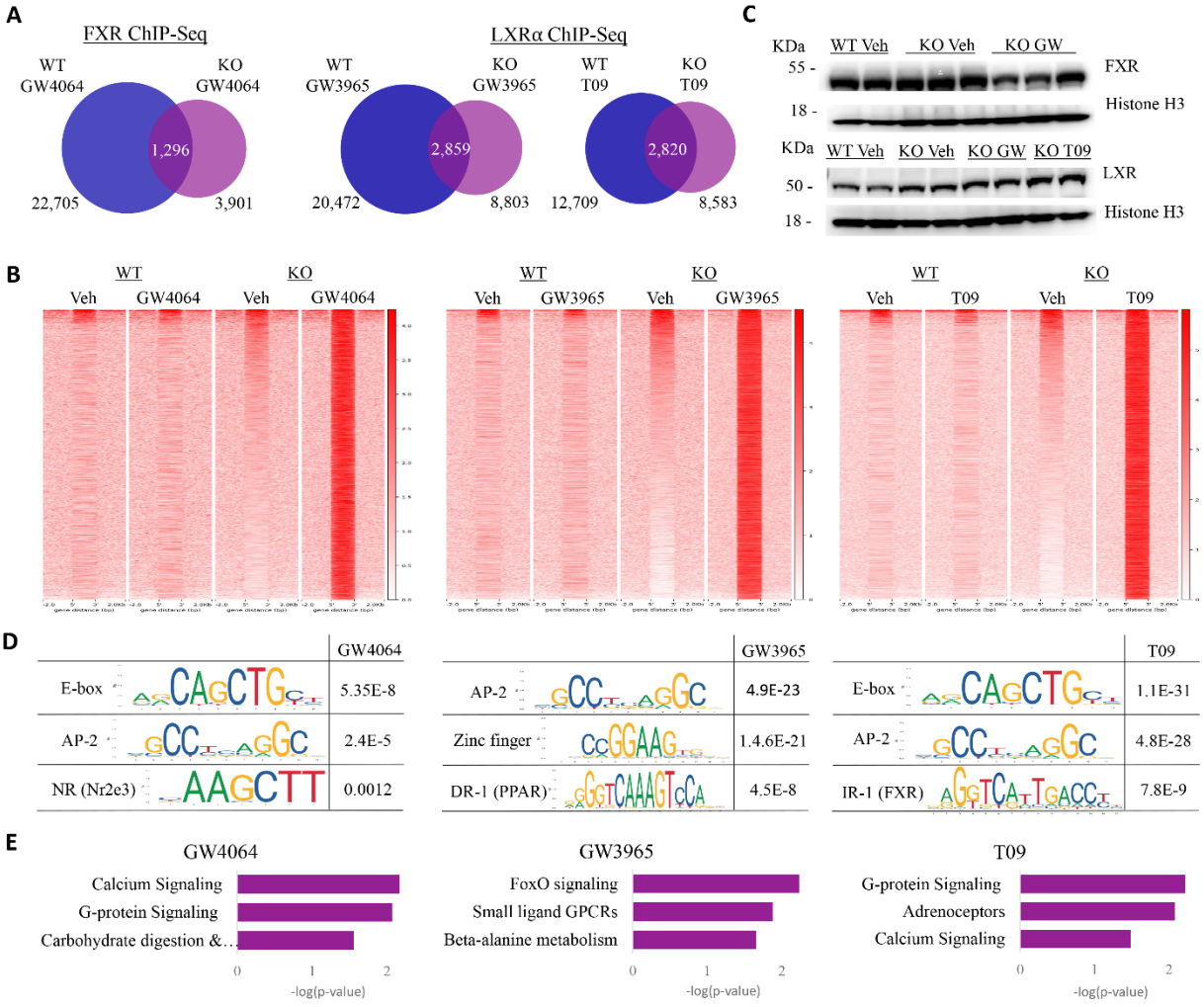
and LXRα binding (bottom panel) is ligand-independent for these targets. (C) Heatmap (RNA-Seq gene expression) of classical FXR target genes with ligand-independent binding (top panel). Ligand-dependent activation or repression of these targets is Foxa2-independent. Heatmap (RNA-Seq gene expression) of classical LXR target genes with ligand-independent binding. (GW23965 middle panel, T09 bottom panel). Ligand-dependent activation of these targets is Foxa2-independent.



Supplemental Figure 2

Supplemental Figure 2 Changes in chromatin accessibility in *Foxa2* mutants.
(A) Overlap analysis between regions of ligand-dependent chromatin in wildtype controls and the binding data present in ChEA database (ChEA analysis in enrichR). Regions of increased accessibility for both FXR and LXRα activation were enriched for known binding sites of nuclear receptors binding sites of nuclear receptors LXR, RXR, PPARα, PPARγ, and *forkhead* factor FOXO1. FXR ChIP-Seq data is not available in ChEA database. (**B**) Venn diagrams comparing changes in chromatin accessibility in wildtype controls and *Foxa2*-deficient mice treated with FXR agonist GW4064 (left panel) and LXR ligands (GW3965, middle panel; T09, right panel). The

overlap analysis between increased chromatin accessibility in wildtype controls and *Foxa2* mutants determined only 4,091 regions for GW4064. 2,736 for GW3965, and 3,839 for T09. (**C**) Heatmaps comparing ATAC-Seq signal in wildtype controls and *Foxa2* mutants treated with FXR agonist GW4064 (left panel) and LXR ligands (GW3965, middle panel; T09, right panel). (**D**) Scanning motif of positional weight matrices in Jaspar and TRANSFAC databases in regions of newly open chromatin during acute ligand activation (FXR ligand GW4064 on the left, LXR ligands GW3965 and T09 on the right) in *Foxa2* mutants. PscanChIP identified DR-1 element, bound by PPAR factors, as highly enriched in all three conditions. (**E**) enrichR analysis reported "Unfolded Protein Response", "Stat3 signaling' and "mTORC1 Signaling" as significantly enriched pathways in *Foxa2*-deficient mice on ligand treatment. In addition, "PPAR alpha pathway" were highly overrepresented in newly opened regions in *Foxa2* mutants.



Supplemental Figure 3

Supplemental Figure 3 FXR and LXRα binding in ligand-treated *Foxa2* mutants
(A) Venn diagrams comparing FXR and LXRα binding in wildtype controls and *Foxa2*-deficient mice treated with FXR agonist GW4064 (left panel) and LXR ligands (GW3965, middle panel; T09, right panel). The overlap analysis between nuclear receptor binding upon ligand activation

in wildtype controls and *Foxa2* mutants determined only 1,296 regions for FXR (GW4064), 2,859 and 2,820 for LXRα (GW3965 and T09). (**B**) Heatmaps comparing FXR ChIP-Seq signal in wildtype controls and *Foxa2* mutants treated with FXR agonist GW4064 (left panel) and LXR signal in wildtype controls and *Foxa2* mutants treated with LXR ligands (GW3965, middle panel; T09, right panel). (**C**) Western blot analysis of protein nuclear extracts from two wildtype control livers treated with vehicle, three Foxa2 KO livers treated with vehicle and 3 Foxa2 KO livers treated with FXR agonist GW4064 with antibodies to FXR and histone H3 (loading control, top panel). Western blot analysis of protein nuclear extracts from two wildtype control livers treated with vehicle, two Foxa2 KO livers treated with vehicle, 2 Foxa2 KO livers treated with LXR agonist GW3965 and 2 Foxa2 KO livers treated with LXR agonist T09 with antibodies to LXR and histone H3 (loading control, top panel). (**D**) IR-1 and DR-4 motifs for FXR and LXR, respectively, were not overrepresented in scanning motif analysis in regions occupied by these factors in *Foxa2* mutants. Instead, motifs for PPAR nuclear receptors (DR-1 element) as well as other factors (AP-2, zinc fingers) were enriched in these targets. (**E**) enrichR analysis of bound regions bound in *Foxa2* mutants identified highly enriched pathways including "Calcium Signaling" and "G-protein Signaling", that were not found in nuclear receptor targets in wildtype controls.

Supplemental Table 1 Differential gene expression in wildtype controls and *Foxa2* mutants treated with FXR agonists

A list of genes differentially expressed in wildtype mice and *Foxa2* mutants treated with GW4064 (Tab 1, Tab 2, FDR < 5%) used to generate the Venn Diagram in **Figure 5**

Supplemental Table 2 Differential gene expression in wildtype controls and *Foxa2* mutants treated with LXR agonists

A list of genes differentially expressed in wildtype mice and *Foxa2* mutants treated with GW3965 (Tab 1, Tab 2, FDR < 5%) and wildtype controls and *Foxa2*-deficient mice treated with T09 (Tab 3, Tab 4, FDR < 5%) used to generate the Venn Diagram in **Figure 6**.

Supplemental Table 3 Gene lists associated with heatmaps

Gene names associated with heatmaps in Figure 5 and 6.