

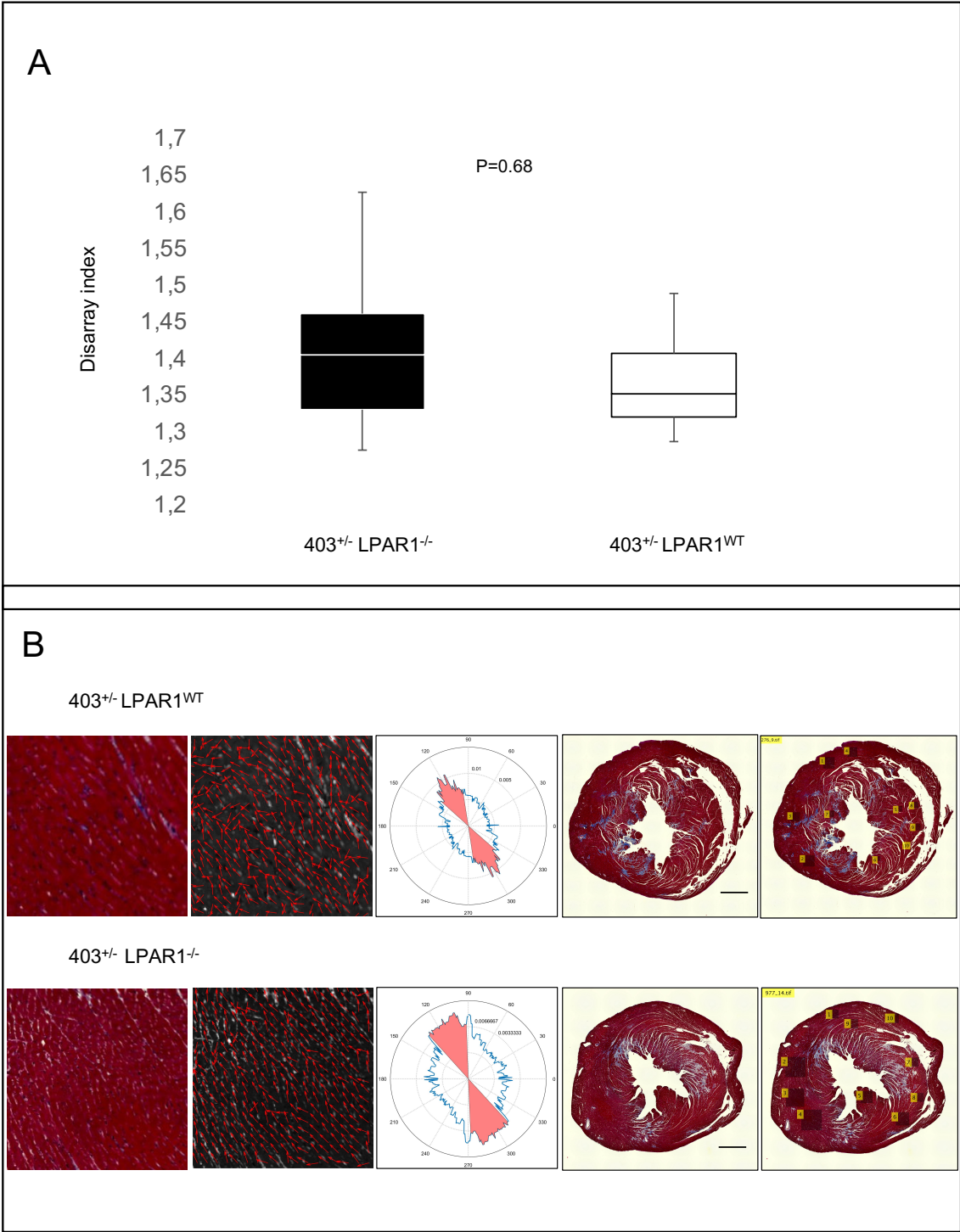
Ablation of Lysophosphatidic Acid Receptor 1 attenuates hypertrophic cardiomyopathy in a mouse model

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SUPPLEMENTAL MATERIAL

Supplemental figures and figure legends

Supplemental Figure 1.



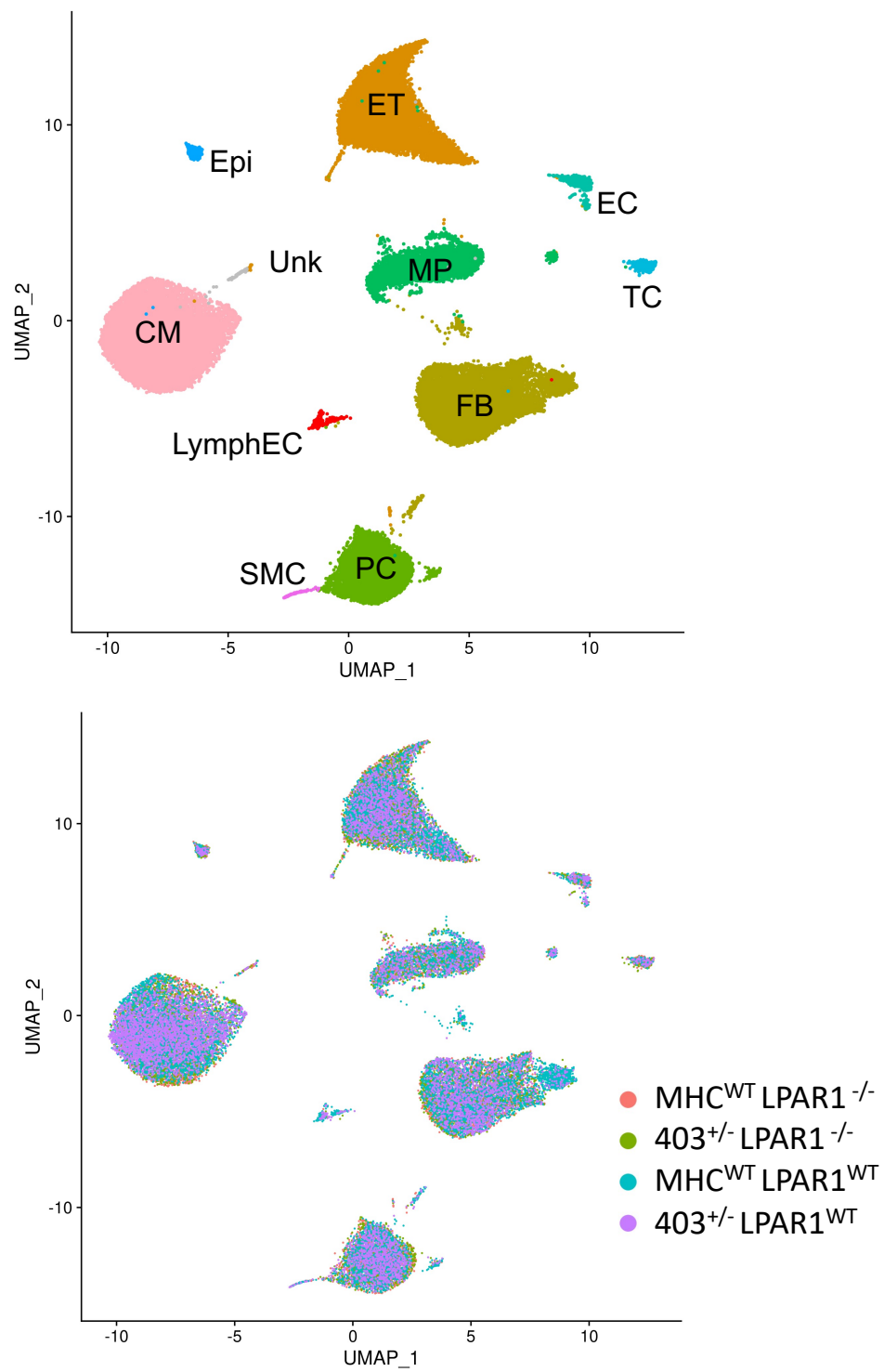
Supplemental figure 1. Disarray of cardiomyocytes is not affected by the ablation of LPA1

(A) Disarray index analyzed for 5 mice, 5 slides per mouse and 10 segments per slide (total 50 segments per mouse). There was no significant difference, per animal ($p=0.79$), per slides ($p=0.68$) and per all segments ($p=0.70$).

(B) Representative regions of analysis of cell orientation and rose plots

Scale bars for higher magnification (white) is 50 μm , and lower magnification (black) is 1mm

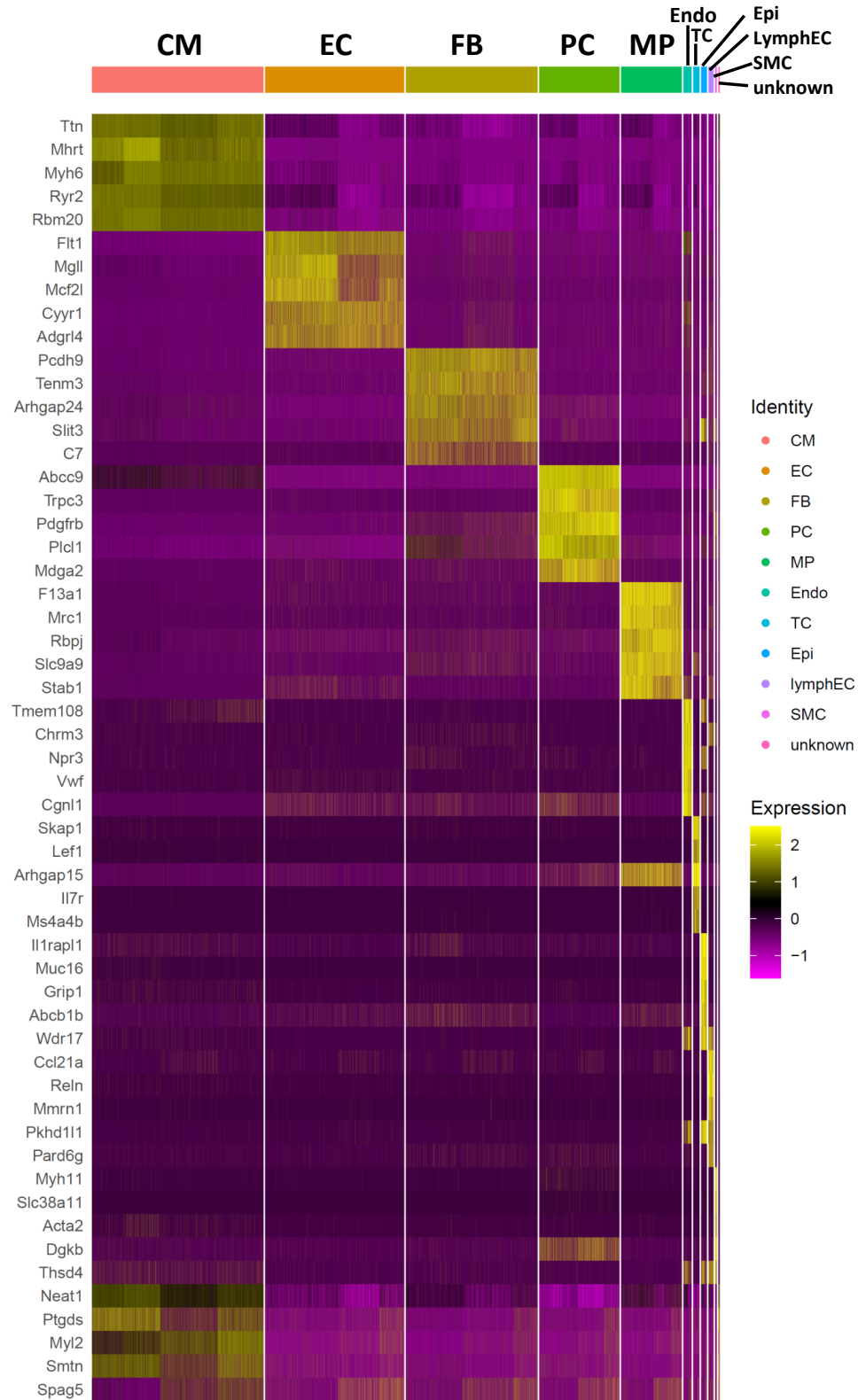
Supplemental figure 2.



Supplemental figure 2. UMAP representation of all cell types in single-nucleus RNA sequencing cluster

UMAP representation of all cell types (A) identified using single-nucleus RNA sequencing overall and (B) in the four different genotypes, $\alpha\text{MHC}^{\text{WT}}\text{LPA1}^{-/-}$, $\alpha\text{MHC}^{403/+}\text{LPA1}^{-/-}$, $\alpha\text{MHC}^{\text{WT}}\text{LPA1}^{\text{WT}}$, and $\alpha\text{MHC}^{+/-}\text{LPA1}^{\text{WT}}$. 10 distinct cell types are identified, including: cardiomyocytes (CM); endocardial cells (EC), endothelial cells (ET); epithelial cells (Epi); fibroblasts (FB); lymphatic endothelial cells (LymphEC); macrophages (MP); pericytes (PC); smooth muscle cells (SMC); lymphocytes (TC), Unknown (Unk).

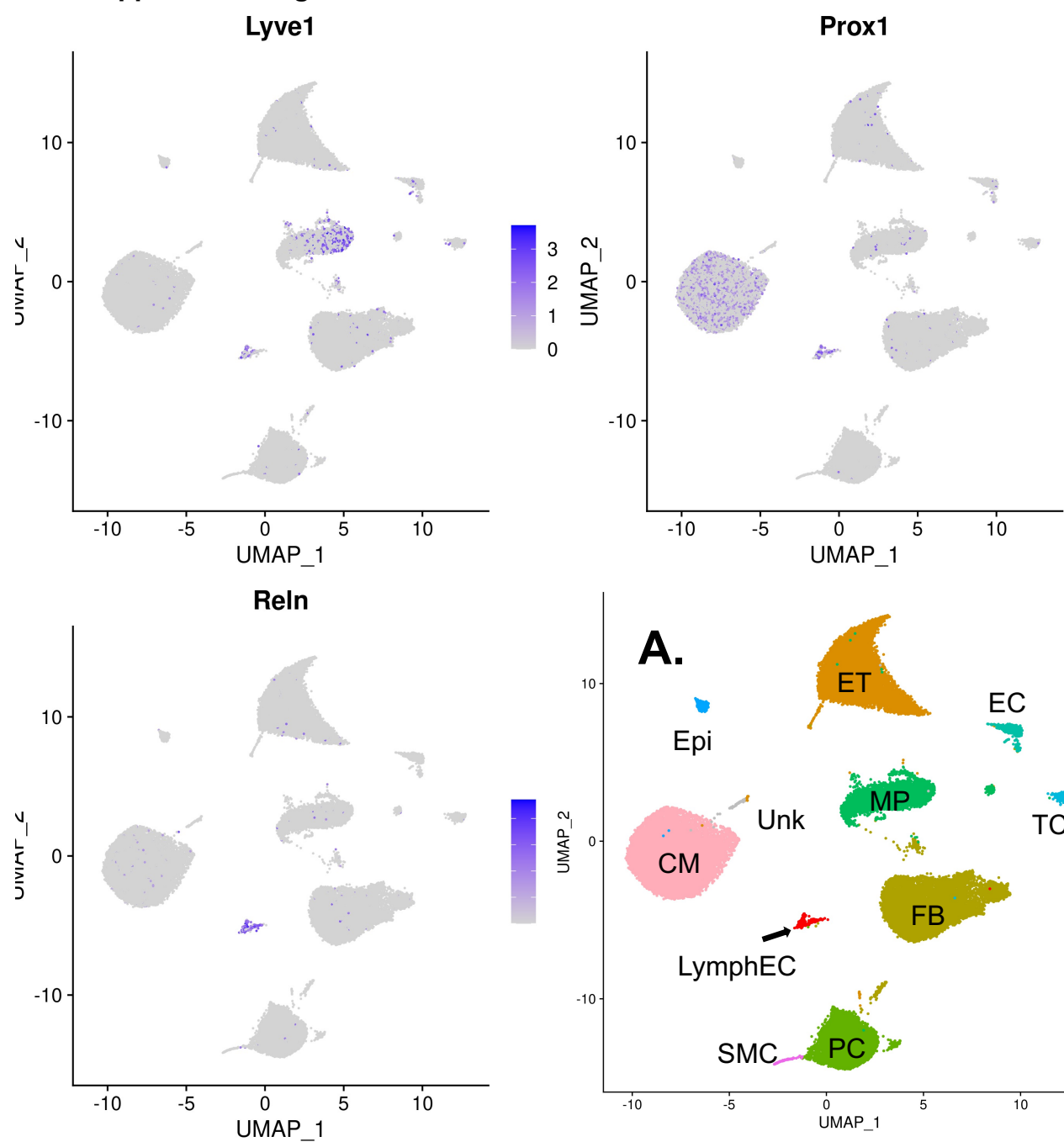
Supplemental Figure 3.



Supplemental Figure 3. Heatmap of all cell types in single-nucleus RNA sequencing

Heatmap of the top 5 significantly enriched genes for each cluster in Fig 4A as found by a Wilcoxon rank sum test.

Supplemental Figure 4.



Supplemental Figure 4. Expression of Lyve1, Prox1 and Reln assessed in different cell types by nuclear RNA sequencing. Note expression of Prox1 in cardiomyocytes (CM) and Lymphatic endothelial cells (Lymph Ec) while Lyve1 is expressed in macrophages (MP) and lymphatic endothelial cells.

Supporting datasets

Dataset 1. Echocardiographic measurements in male mice of different genotypes. BSA denotes body surface area; IVSd intraventricular septum in diastole; LVPWd, left ventricular posterior wall in diastole; LVIDd, left ventricular internal diameter in diastole; LVIDs, left ventricular internal diameter in systole; FS, fractional shortening.

Dataset 2. Total mRNA expression in LV tissue of $\alpha\text{MHC}^{403/+}$ $\text{LPA1}^{-/-}$ and $\alpha\text{MHC}^{403/+}$ LPA1^{WT} mice.

#mRNA levels were determined by RNAseq using polyA selected mRNA (see Methods).

Dataset 3. Cell type distribution by genotype

Dataset 4. Marker genes for each cell type

^Abbreviations: pval, p-value or likelihood that this marker gene is expressed at a different level in this cell type than in all other cell types; ave_logFC, average log fold change or log2 of ratio of expression in this cell type compared to all other cell types; pct.1, percent cells of this cell type with at least one marker gene read; pct.2, percent cells of all other cell types with at least one marker gene read; p_val_adj, p-value corrected for multiple testing of ~28000 genes; cluster, cell type; gene, marker gene.

Dataset 5. RNA levels measured by snRNAseq in $\alpha\text{MHC}^{403/+}$ $\text{LPA1}^{-/-}$, $\alpha\text{MHC}^{403/+}$ LPA1^{WT} , $\alpha\text{MHC}^{\text{WT}}$ $\text{LPA1}^{-/-}$, $\alpha\text{MHC}^{\text{WT}}$ LPA1^{WT} hearts identify 14 Cell Types

#mRNA levels, expressed as normalized read counts, were measured in 14 different cell types

Dataset 6. Non-Redundant Signaling pathways functioning in FB0 and FB1 cells defined by patterns of RNA expression

Dataset 7. Expression of lysophosphatidic acid receptor genes, Reln and Ccl21a by cell type in WT male mouse LV assessed by single nuclear RNAseq

Dataset 8. Expression of lysophosphatidic acid receptor genes, Reln and Ccl21a in human HCM and control LV by cell type assessed by single nuclear RNAseq

Dataset 9: Expression LPAR, RELN and CCL21 human male and female LV assessed by single nuclear RNA sequencing