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RESEARCH ARTICLE

Figures and figure supplements

Reconstructing human pancreatic differentiation by mapping specific cell populations during development

Cyrille Ramond *et al*

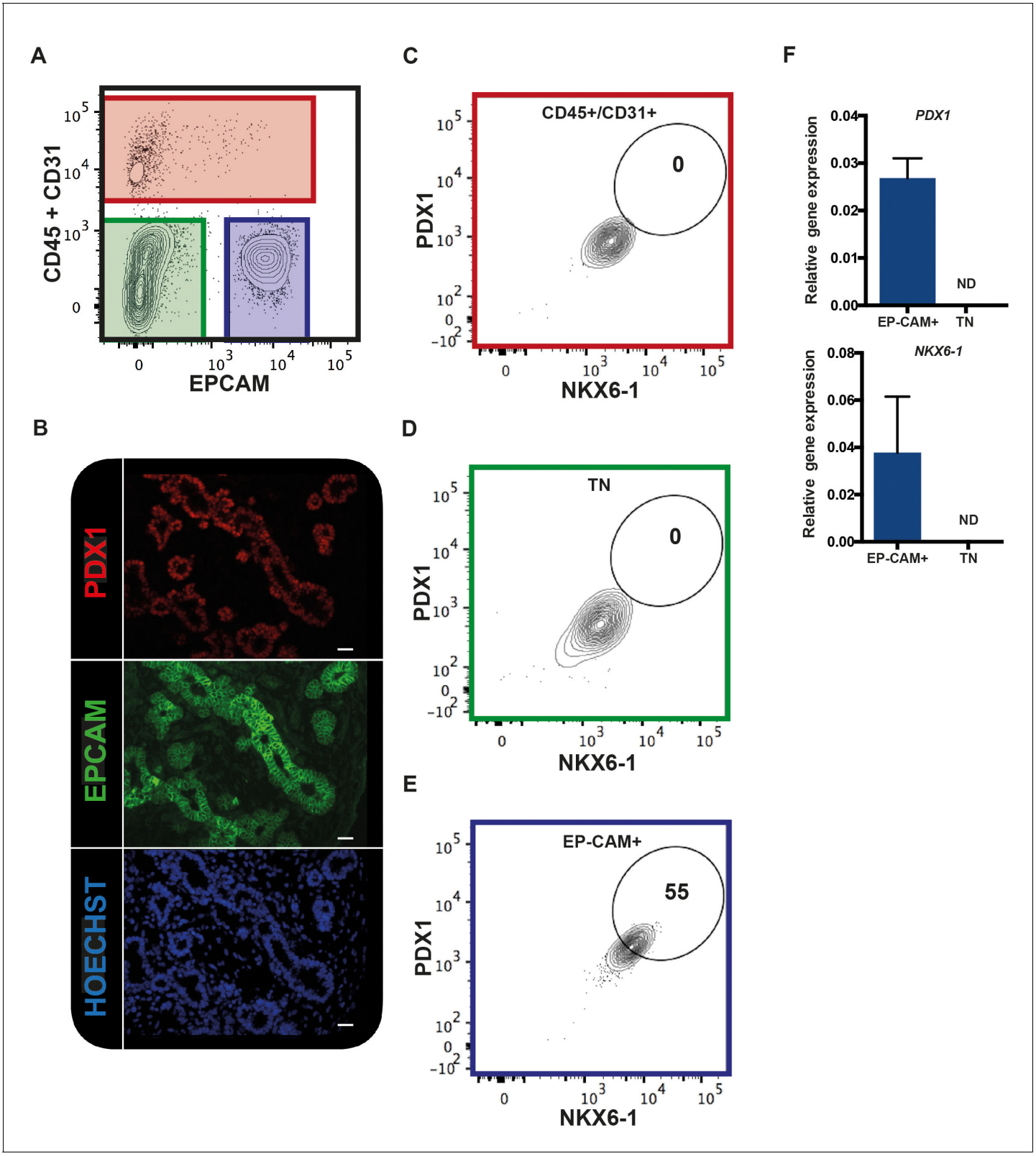

Figure 1. EPCAM expression in the human fetal pancreas. (**A**) The flow cytometry plot represents CD45 and CD31 expression against EPCAM gated on live human fetal pancreatic cells (9.7WD), n = 9. (**B**) Immunohistochemistry for PDX1 and EPCAM on pancreatic section (9WD), n = 3. Scale bar = 100 µm. (**C–E**) Flow cytometry plots of PDX1 and NKX6-1 expression at 9.4WD on $CD45^{+}/CD31^{+}$ cells (red square), $CD45^{-}CD31^{-}EPCAM^{-}$ cells (TN = triple

Figure 1 continued on next page

Figure 1 continued

negative green square) and CD45$^-$CD31$^-$EPCAM$^+$ cells (blue square). (**F**) RT-qPCR analysis of *PDX1* and *NKX6-1* expression on sorted CD45$^-$CD31$^-$EPCAM$^+$ and TN cells. ND = Not Detected.

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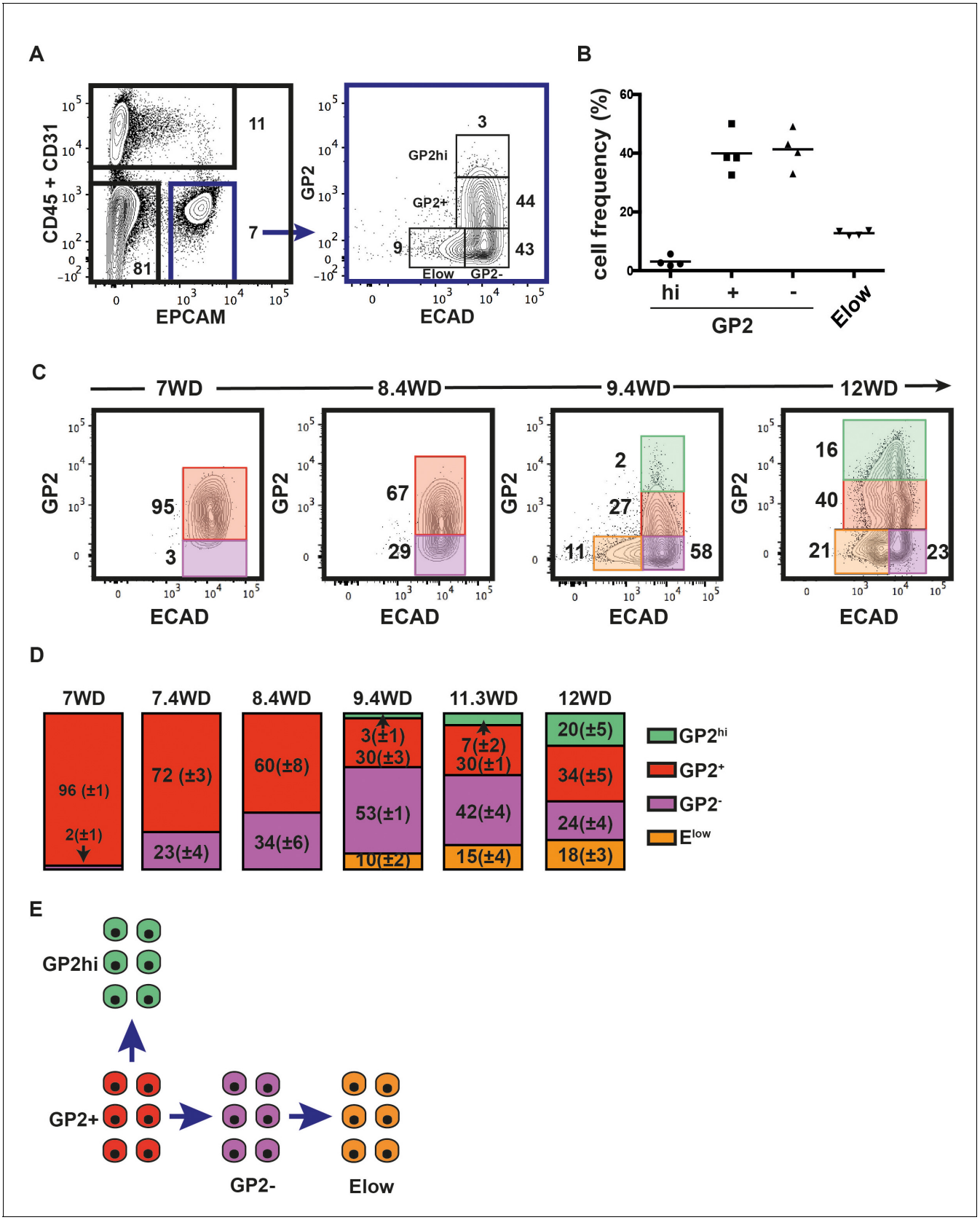

Figure 2. GP2 and ECAD expression in the human fetal pancreatic epithelium. GP2 and ECAD expressions were assayed by flow cytometry during development. (**A**) FACS plots display the expression at 9.4WD of CD45 and CD31 against EPCAM (left plot) and GP2 and ECAD gated on

Figure 2 continued on next page

Figure 2 continued

$CD45^-CD31^-EPCAM^+$ (right plot). n = 4 (**B**) Cell frequencies of the $GP2^{hi}$ ($GP2^{hi}ECAD^+$), $GP2^+$ ($GP2^+ECAD^+$), $GP2^-$ ($GP2^-ECAD^+$) and E^{low} ($GP2^-ECAD^{low}$) populations at 9.4WD. n = 4 (mean ±SEM) (**C**) GP2 and ECAD expressions on fetal pancreases at 7-12WD gated on $CD45^-CD31^-EPCAM^+$ cells. 7WD n = 2, 8.4WD n = 9, 9.4WD n = 4, 12WD n = 5. (mean ±SEM) (**D**) Cell frequencies of the $GP2^{hi}$, $GP2^+$, $GP2^-$ and E^{low} populations at 7-12WD. Cell frequencies were calculated from three independent experiments for each time point. (**E**) Scheme that represents the development of $GP2^{hi}$, $GP2^+$, $GP2^-$ and E^{low} populations.

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The following source data is available for figure 2:

Source data 1. Cell frequency at 9.4WD by flow cytometry.

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Source data 2. Cell frequency during development.

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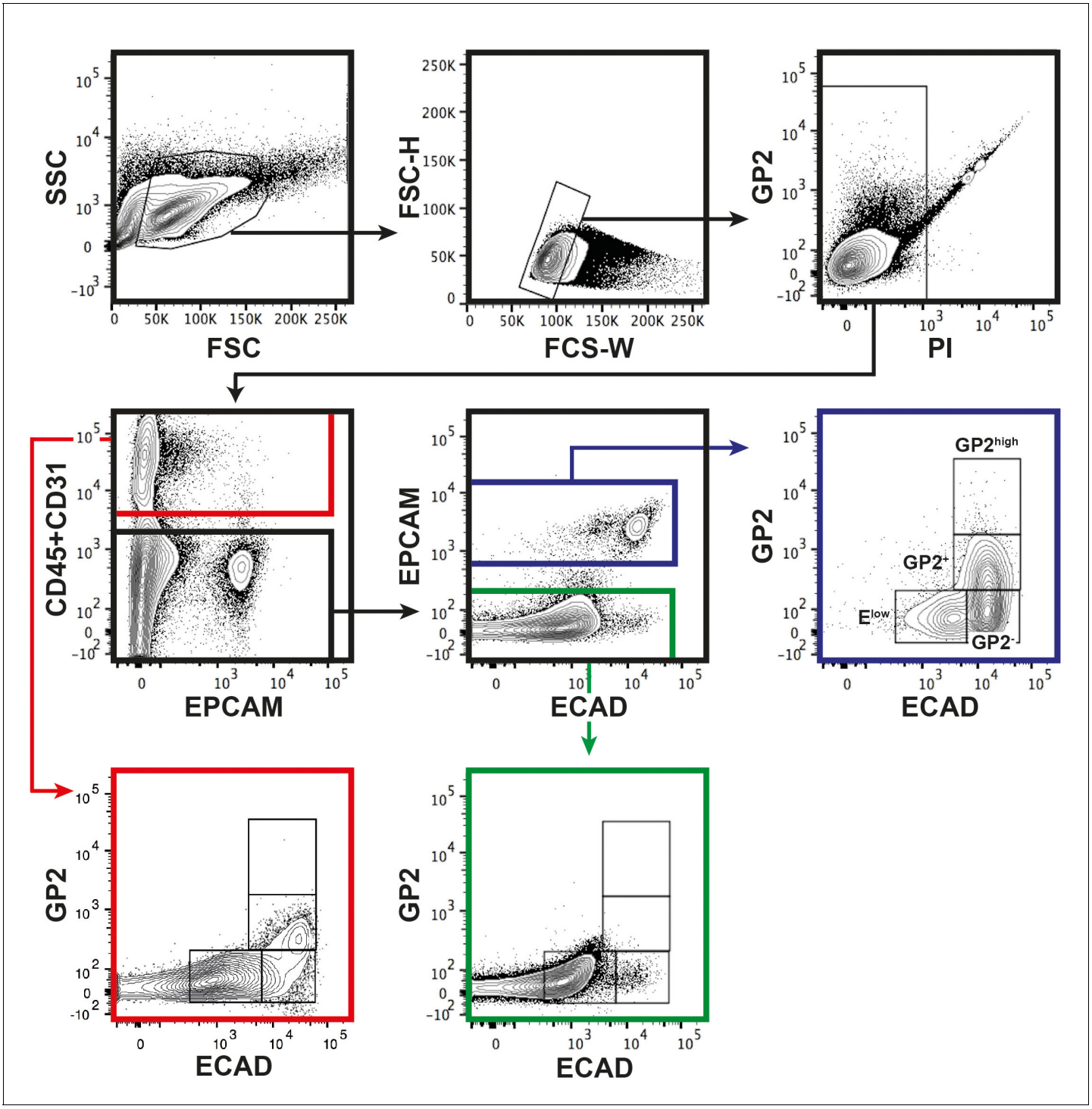

Figure 2—figure supplement 1. Gating strategy for GP2 and ECAD. Human fetal pancreas at 9WD was stained for CD45, CD31, EPCAM, ECAD and GP2. Doublets cells were excluded from the analysis with FSC-H and FSC-W (middle top plot). Propidium iodide was used to exclude dead cells as shown in the right top plot in the diagonal. GP2 and ECAD expressions were analyzed in $CD45^+/CD31^+$ (red square), $CD45^-CD31^-EPCAM^-$ (green square) and $CD45^-CD31^-EPCAM^+$ (green square) compartments. $CD45^-CD31^-EPCAM^-$ population was used as negative control to set up the $GP2^-ECAD^+$ (named $GP2^-$) and $GP2^+ECAD^+$ (named $GP2^+$) gates. $GP2^+ECAD^+$ population was used to set up the gate for ECAD levels. This experiment is representative of 5 independent stainings at 9WD. This gating strategy was applied to each pancreatic stage.
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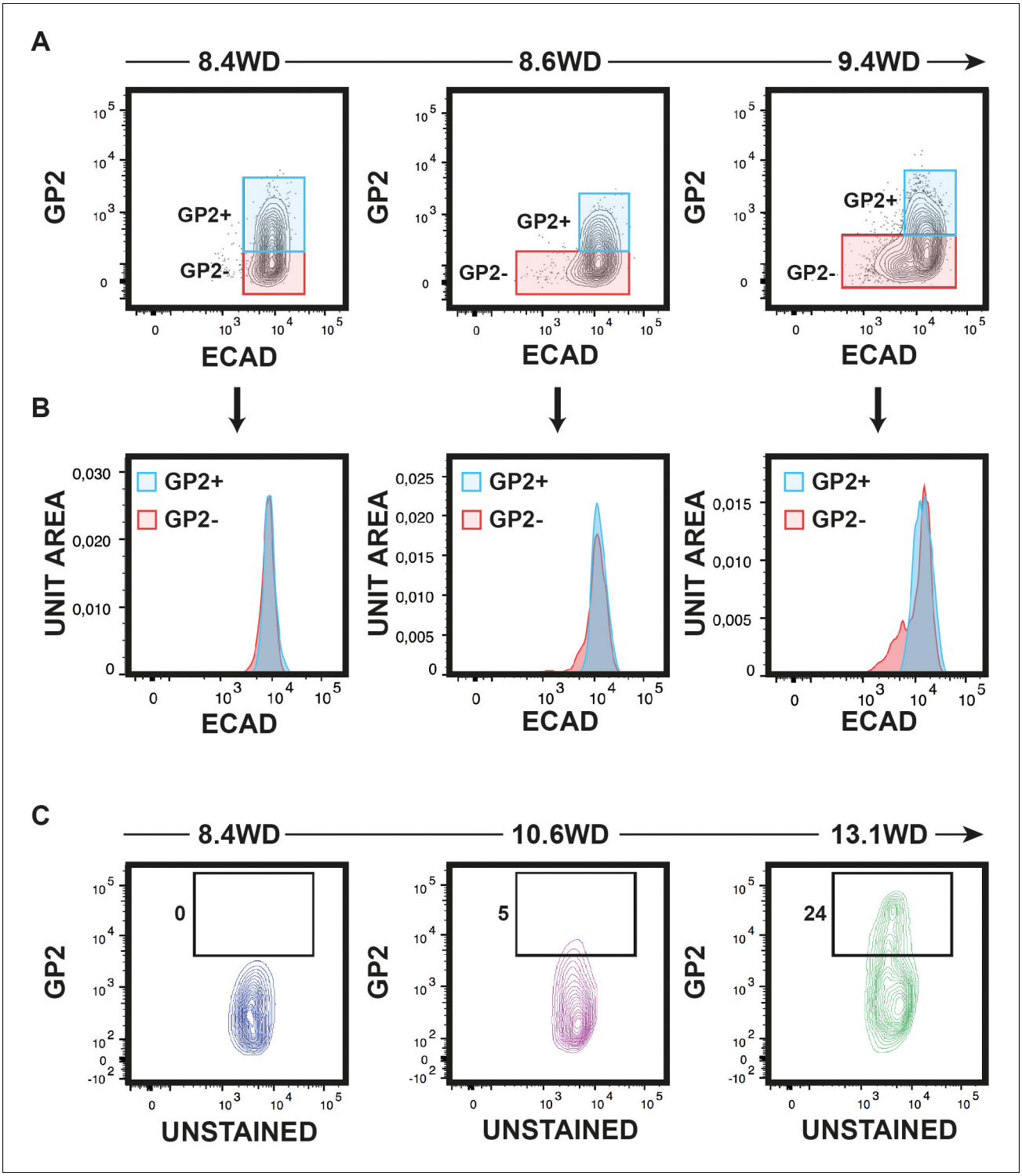

Figure 2—figure supplement 2. Expression of ECAD and GP2 during development. (**A**) GP2 and ECAD expression by flow cytometry on $CD45^{-}CD45^{-}EPCAM^{+}$ population at 8.4, 8.6 and 9.4WD. (**B**) ECAD expression in the $GP2^{+}$ (in blue) and all $GP2^{-}$ ($GP2^{-}ECAD^{+}$+$GP2ECAD^{low}$, in red) populations at 8.4, 8.6 and 9.4WD. (**C**) GP2 expression by flow cytometry on $CD45^{-}CD31^{-}EPCAM^{+}$ at 8.4, 10.6 and 13.1WD. $GP2^{hi}$ gates were fixed at 13.1WD for the three stages. (**A, B**) 8.4WD n = 7, 8.6WD n = 7 and 9.4WD n = 3. (**C, D**) 8.4WD n = 7, 10.6WD n = 3, 13.1WD n = 2.
DOI: 10.7554/eLife.27564.007

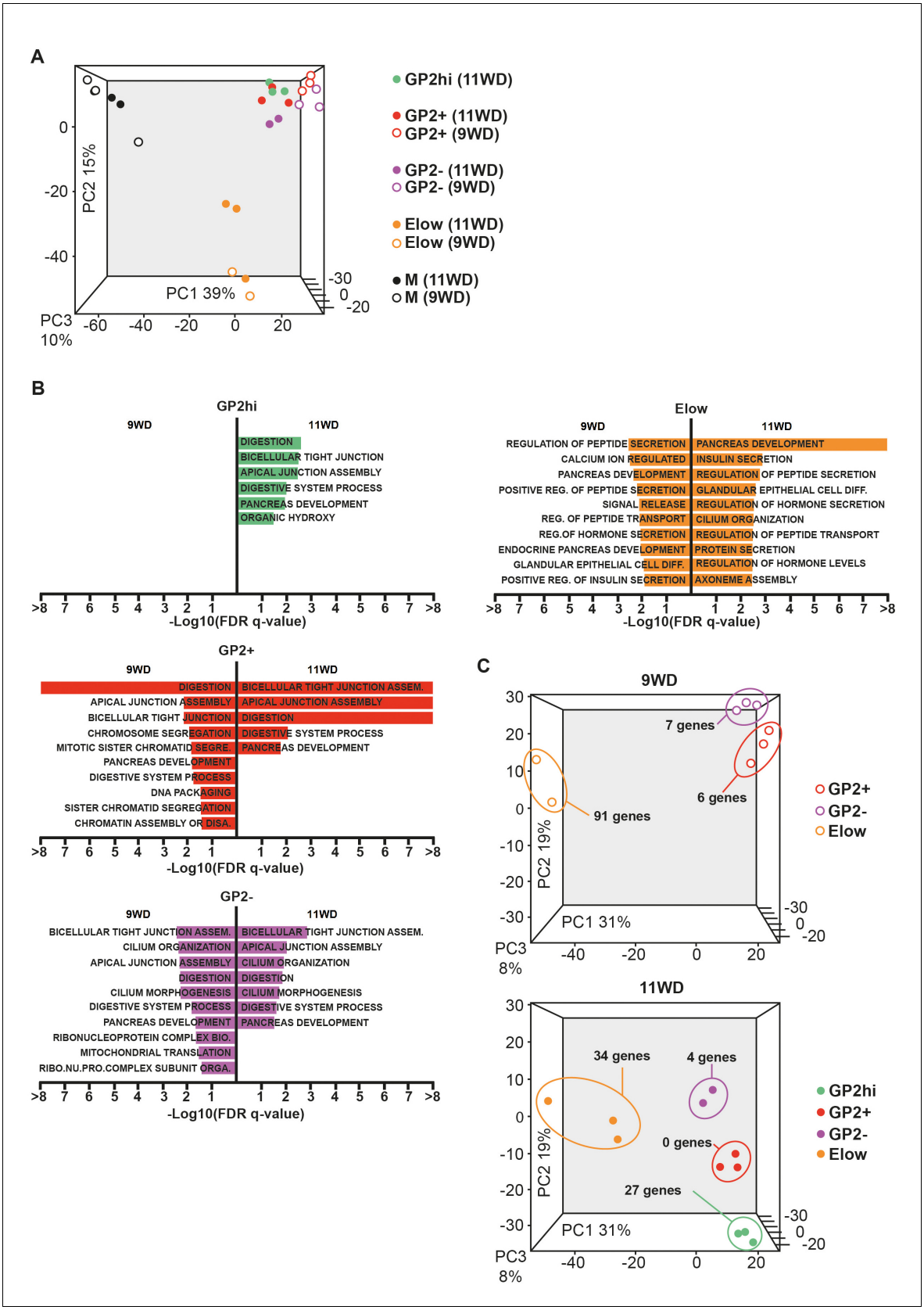

Figure 3. Transcriptomic analysis of the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations. (**A**) PCA map of sorted pancreatic cells (epithelium and mesenchyme). (**B**) Top enriched biological processes in each cell population compared to the mesenchyme. Results were obtained with GSEA software using the GO

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Figure 3 continued

database. (**C**) PCA map of epithelial-sorted cells ($GP2^{hi}$, $GP2^{+}$, $GP2^{-}$ and E^{low} populations) from 9 (top map) and 11WD (bottom map). The number of specifically genes enriched in each population in each population ($p<0.05$) is displayed. PCA maps are displayed in 2D with the three Principal Components on figure ***Figure 3—figure supplement 1***) and the Gene Ontology lists in ***Supplementary file 1a***.

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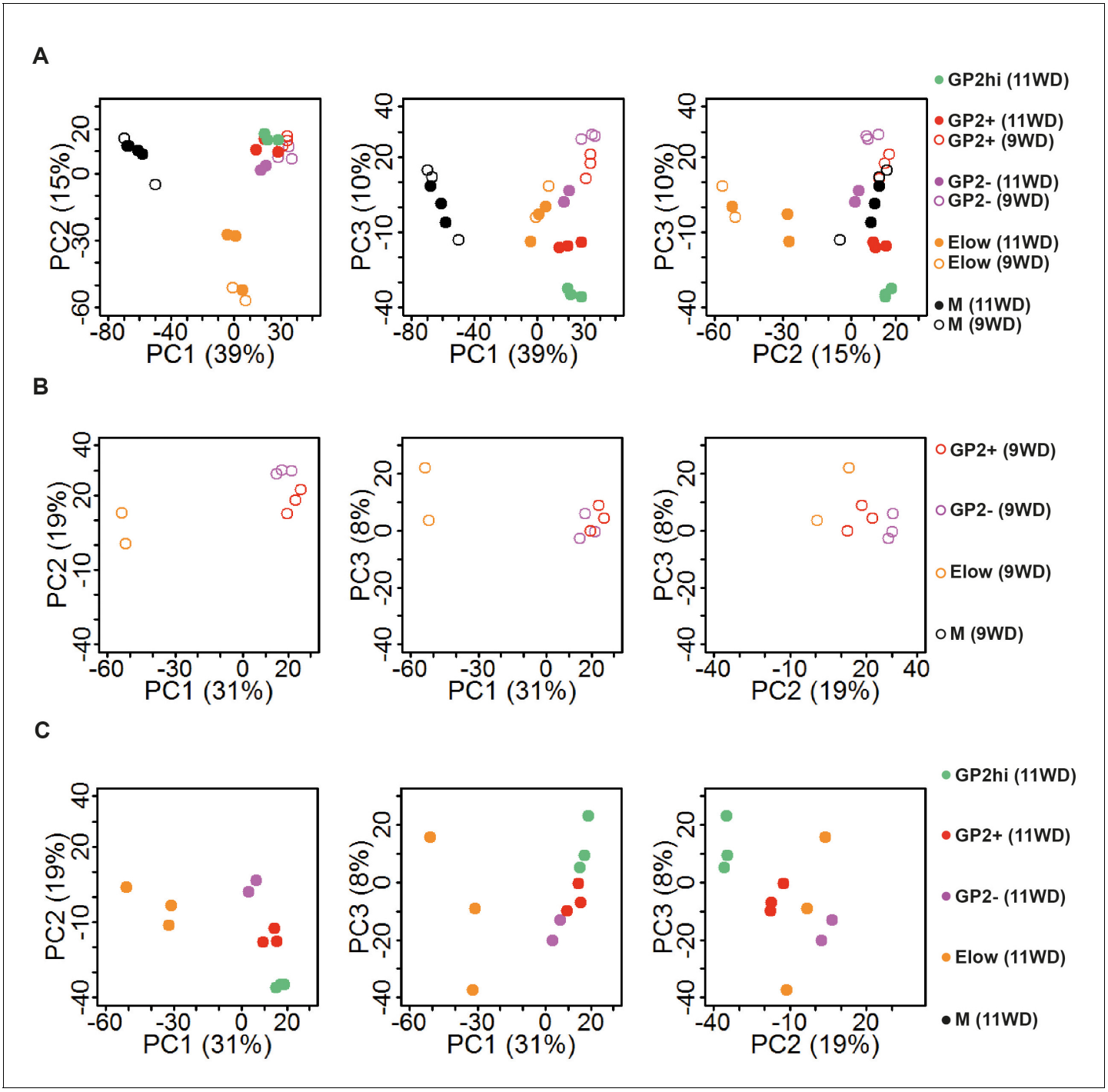

Figure 3—figure supplement 1. Principal component analysis. (**A**) 2D PCA maps of sorted pancreatic cells (epithelium and mesenchyme) displaying the first three Principal Components (PC1 against PC2, PC1 against PC3 and PC2 against PC3) from ***Figure 3A***. (**B**) 2D PCA maps of sorted epithelial cells ($GP2^{hi}$, $GP2^{+}$, $GP2^{-}$ and E^{low} populations) at 9WD displaying the first three Principal Components (PC1 against PC2, PC1 against PC3 and PC2 against PC3) from ***Figure 3C*** (top map). (**C**) 2D PCA maps of sorted epithelial cells ($GP2^{hi}$, $GP2^{+}$, $GP2^{-}$ and E^{low} populations) at 11WD displaying the first three Principal Components (PC1 against PC2, PC1 against PC3 and PC2 against PC3) from ***Figure 3C*** (top map) from ***Figure 3C*** (bottom map).
DOI: 10.7554/eLife.27564.009

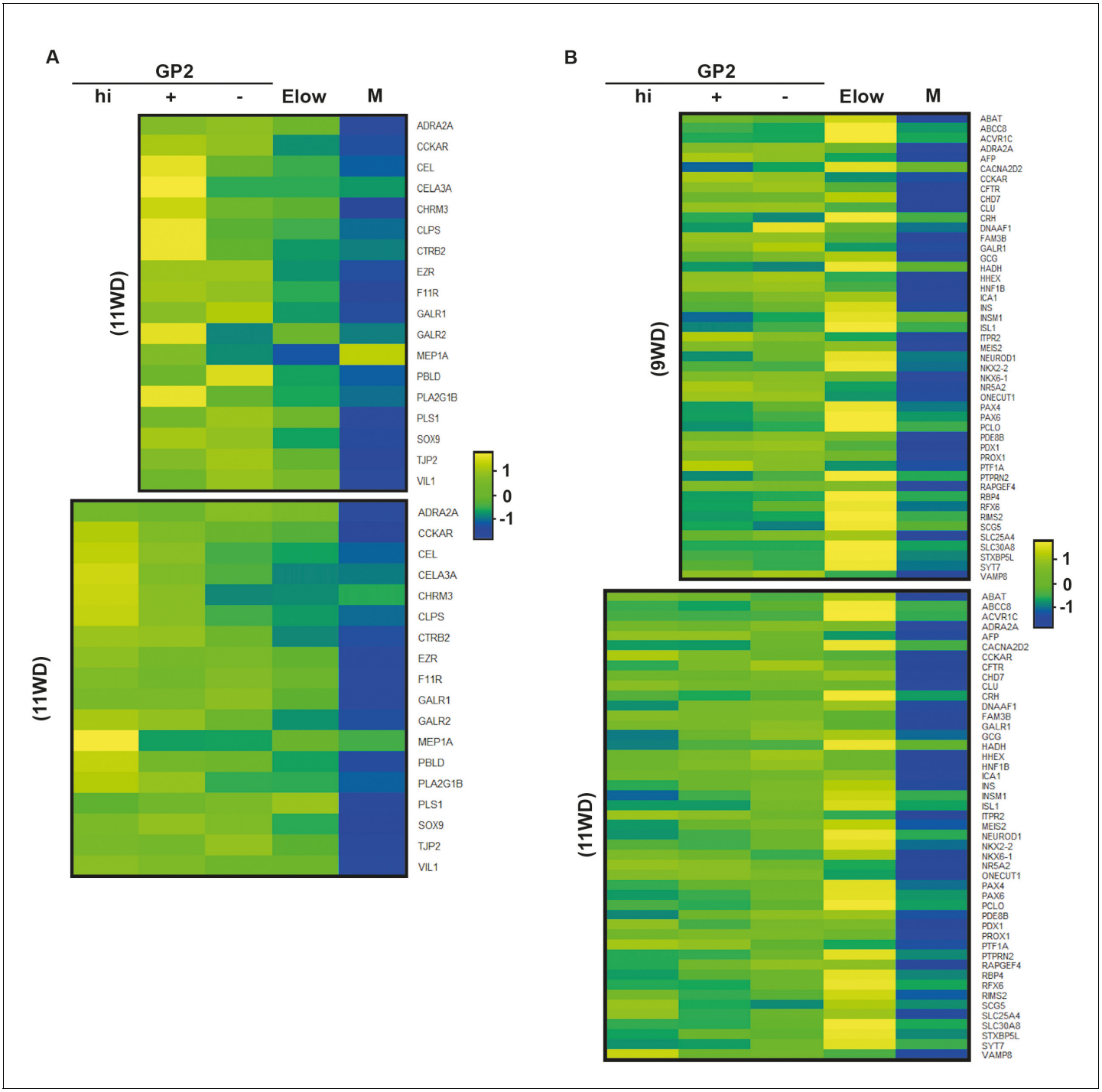

Figure 3—figure supplement 2. Heatmaps of genes from GO Biological processes digestion, insulin secretion and pancreas development. Enriched genes from GO biological processes « digestion » (**A**), « insulin secretion » and «pancreas development» (**B**) were used to generate heatmaps. They display expression of these genes at 9 and 11WD in the $GP2^{hi}$, $GP2^{+}$, $GP2^{-}$, E^{low} and Mesenchyme populations. Heatmaps were generated by the 'heatmap2' function from gplots R package on standardized log2 expression values, with Pearson correlation as the distance function.
DOI: 10.7554/eLife.27564.010

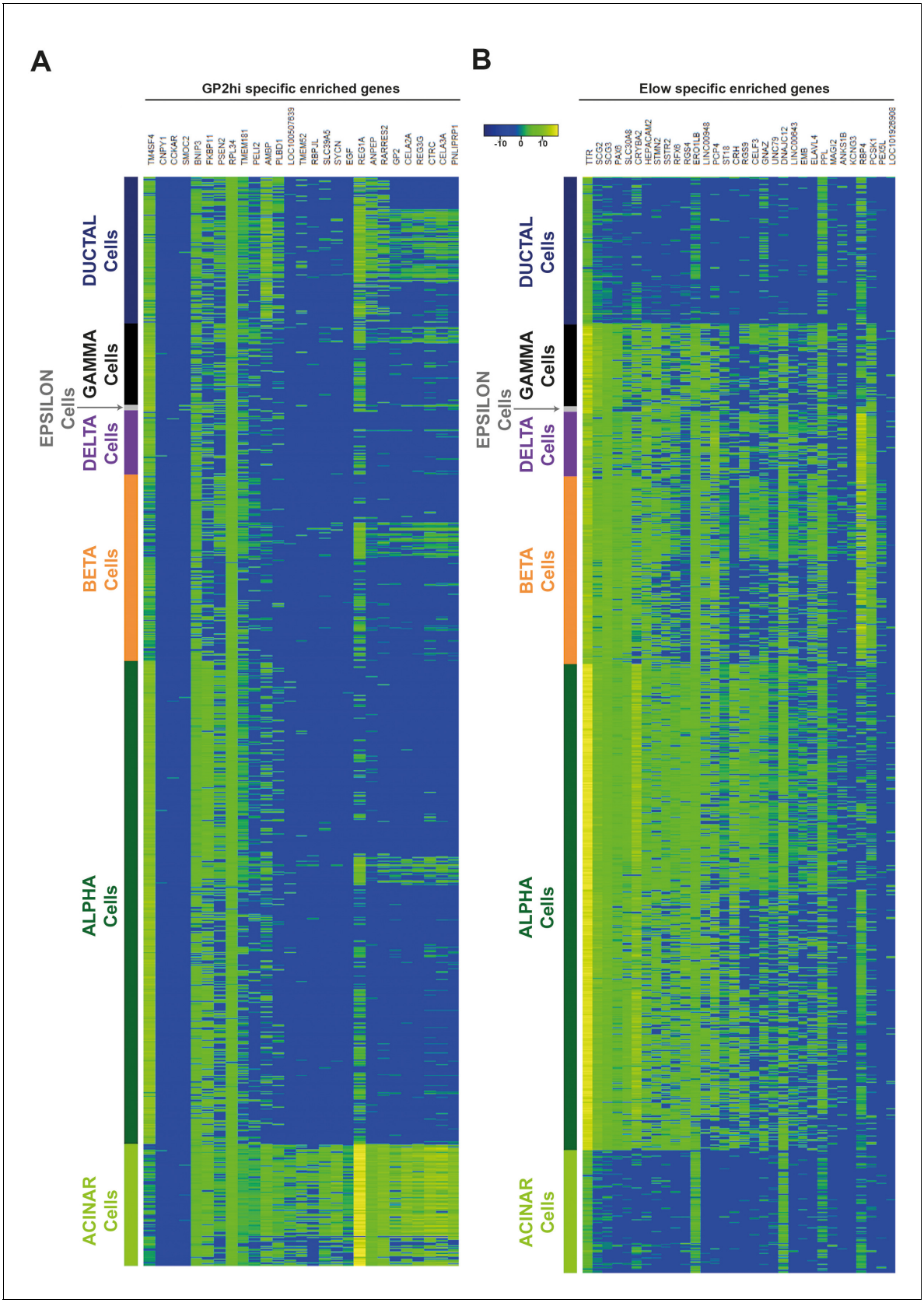

Figure 3—figure supplement 3. The GP2^{hi} and E^{low} populations are enriched in acinar and endocrine markers respectively. Comparative analyses using data from ***Segerstolpe et al. (2016)*** indicate that (**A**) Genes found enriched in the GP2^{hi} population, are preferentially expressed in the human

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Figure 3—figure supplement 3 continued

adult pancreas in acinar cells (**B**) Genes found enriched in the E^{low} population, are preferentially expressed in the human adult pancreas in endocrine cells. Heatmaps were obtained with Log2 RPKM values of the 1000 characterized single cells from (***Segerstolpe et al., 2016***).

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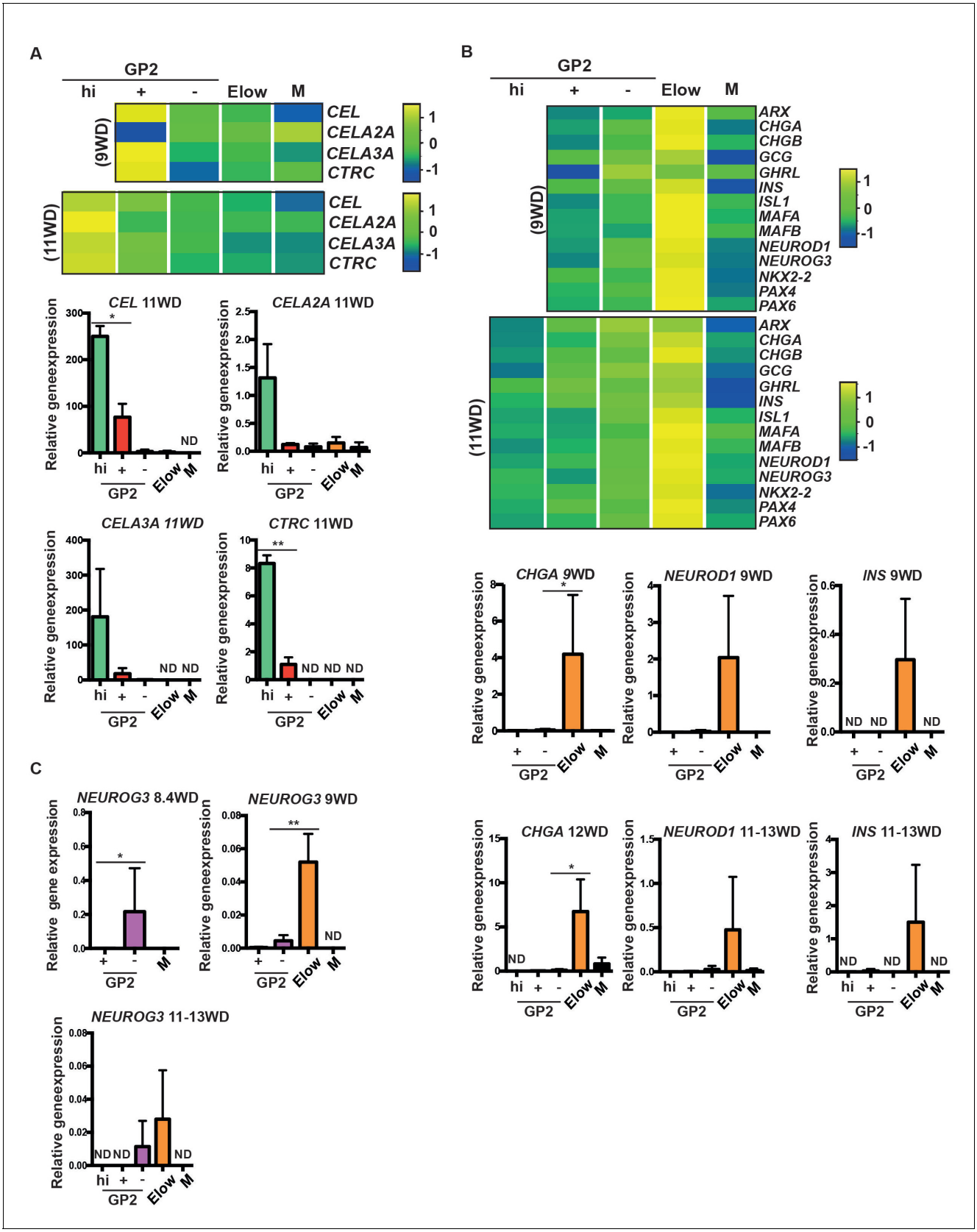

Figure 4. Characterization of the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations. (**A**,**B**) Expression of acinar (**A**) and endocrine (**B**) markers in the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations by global transcriptomic analyses and by RT-qPCR. (**C**) Expression of *NEUROG3* by RT-qPCR in the GP2^{hi}, GP2^{+}, GP2^{-} and M

Figure 4 continued on next page

Figure 4 continued

populations. Heat maps and RT-qPCR are representative of 3 independents experiments. ND = Non Detected. M = $CD45^{-}CD31^{-}EPCAM^{-}$. *p<0.05, **p<0.001, *t* test. (mean ± SEM).

DOI: 10.7554/eLife.27564.012

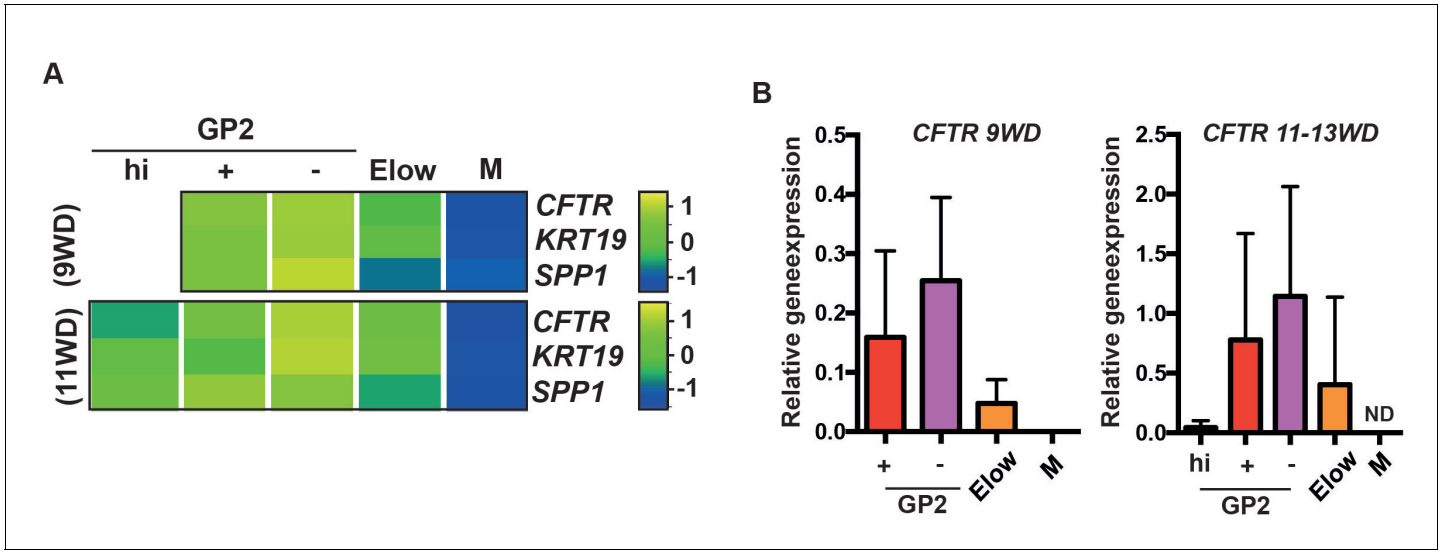

Figure 4—figure supplement 1. Expression of ductal markers in the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations. Expression of ductal markers in the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations by transcriptomic analysis (**A**) and RT-qPCR (9-13WD) (**B**). ND = Not Detected. M = CD45CD31^{-}EPCAM^{-}. Heatmaps and RT-qPCR are representative of 3 independents experiments. (mean ± SEM).

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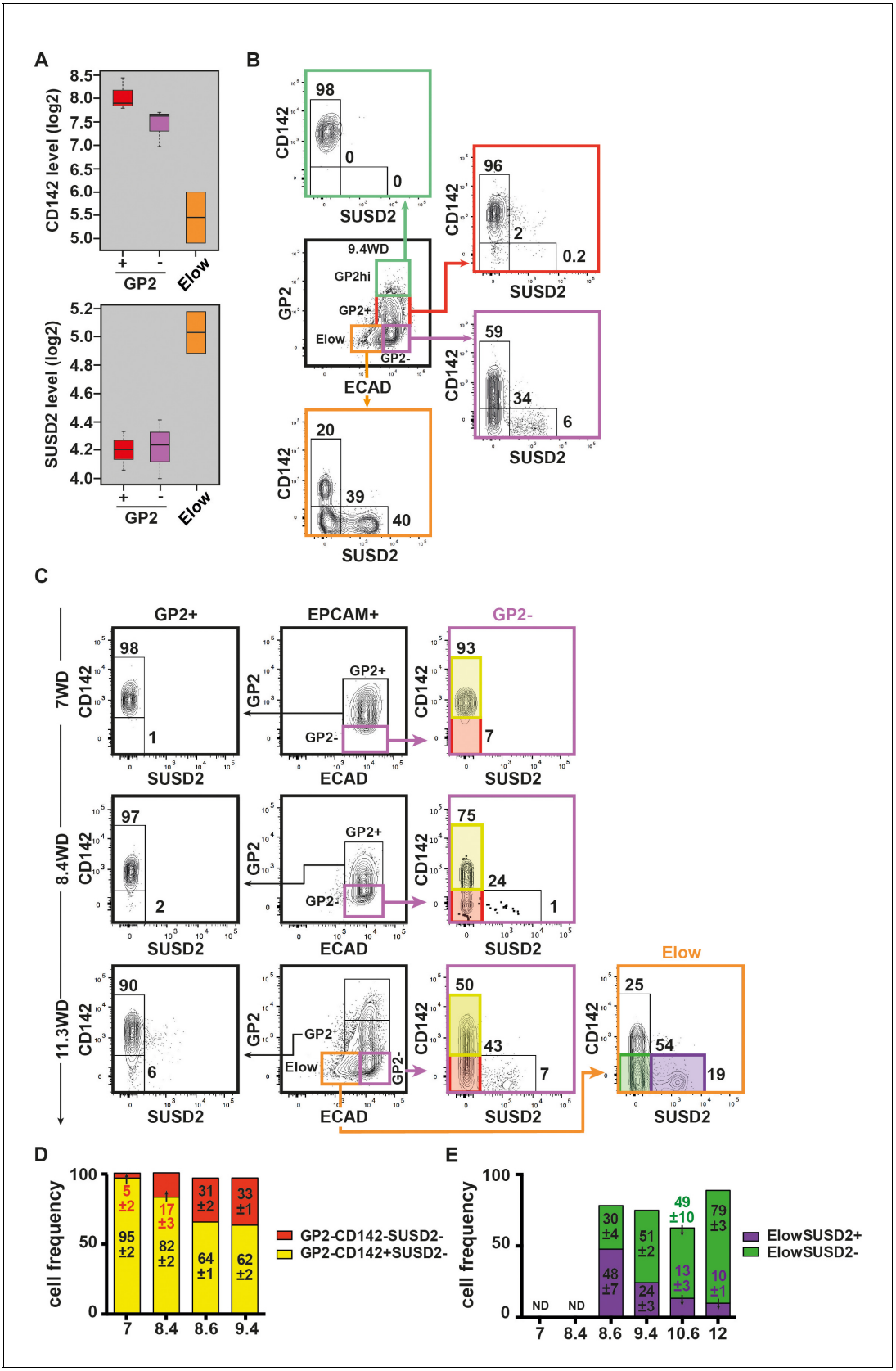

Figure 5. Expression of CD142 and SUSD2 in the GP2^{hi}, GP2^{+}, GP2^{-} and E^{low} populations. (**A**) Expression of *CD142* and *SUSD2* in GP2^{+}, GP2^{-} and E^{low} populations at 9WD by microarray analysis. Boxplots were obtained using standardized log2 expression values. (**B**) Expression of CD142 and SUSD2 in

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Figure 5 continued

the GP2^{hi} (in green), GP2^{+} (in red), GP2^{-} (in purple) and E^{low} (in orange) populations at 9.4WD by flow cytometry. GP2 and ECAD expressions were gated on live CD45^{-}CD31^{-}EPCAM^{+} cells. (**C**) Expression of CD142 and SUSD2 in the GP2^{+} (left plot), GP2^{-} (right plot) and E^{low} (far right plot) populations at 7WD, 8.4WD, and 11.3WD. (**D**) Cell frequencies of the GP2^{-}CD142^{+}SUSD2^{-} (named CD142^{+}SUSD2^{-} in yellow) and the GP2^{-}CD142^{-}SUSD2^{-} (CD142^{-}SUSD2^{-} in red) subsets from 7WD to 9.4WD. (mean ± SEM). (**E**) Cell frequencies of the E^{low}GP2^{-}CD142^{-}SUSD2^{+} (named E^{low}SUSD2^{+}, in purple) and the E^{low}GP2^{-}CD142^{-}SUSD2^{-} (E^{low}SUSD2^{-}, in green) subsets from 7 to 12WD. (mean ± SEM). (**A**) n = 2–3, (**B**) n = 3; **C**) 7WD n = 2, 8.4WD to 9.4WD n = 3. (**D**) n = 3.

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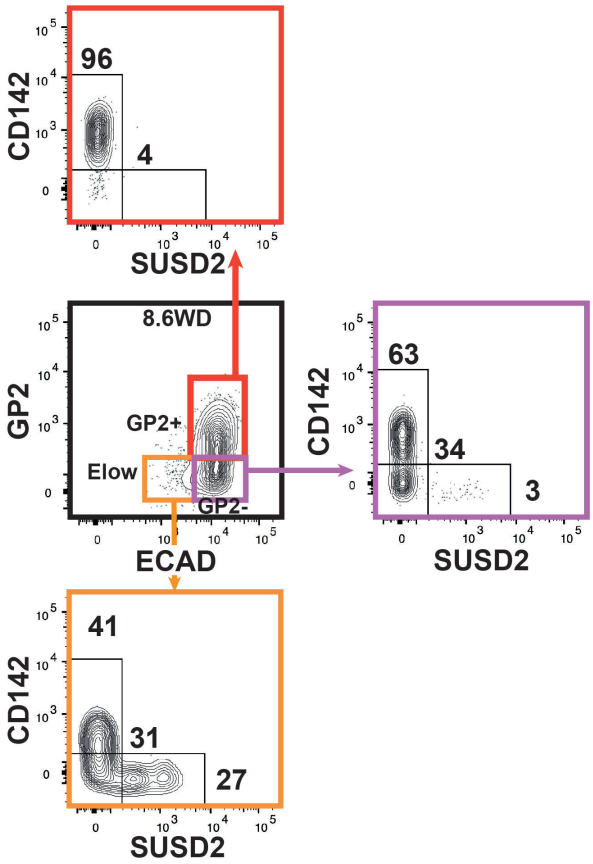
96
4
CD142
SUSD2
8.6WD
GP2
GP2+
Elow
GP2-
ECAD
63
34
3
CD142
SUSD2
41
31
27
CD142
SUSD2

Figure 5—figure supplement 1. Expression of CD142 and SUSD2 in the $GP2^+$, $GP2^-$ and E^{low} populations at 8.6WD. Expression of CD142 and SUSD2 in the $GP2^+$ (in red), $GP2^-$ (in purple) and E^{low} (in orange) populations at 8.6WD by flow cytometry. GP2 and ECAD expressions were gated on live $CD45^-CD31^-EPCAM^+$ cells. n = 7.

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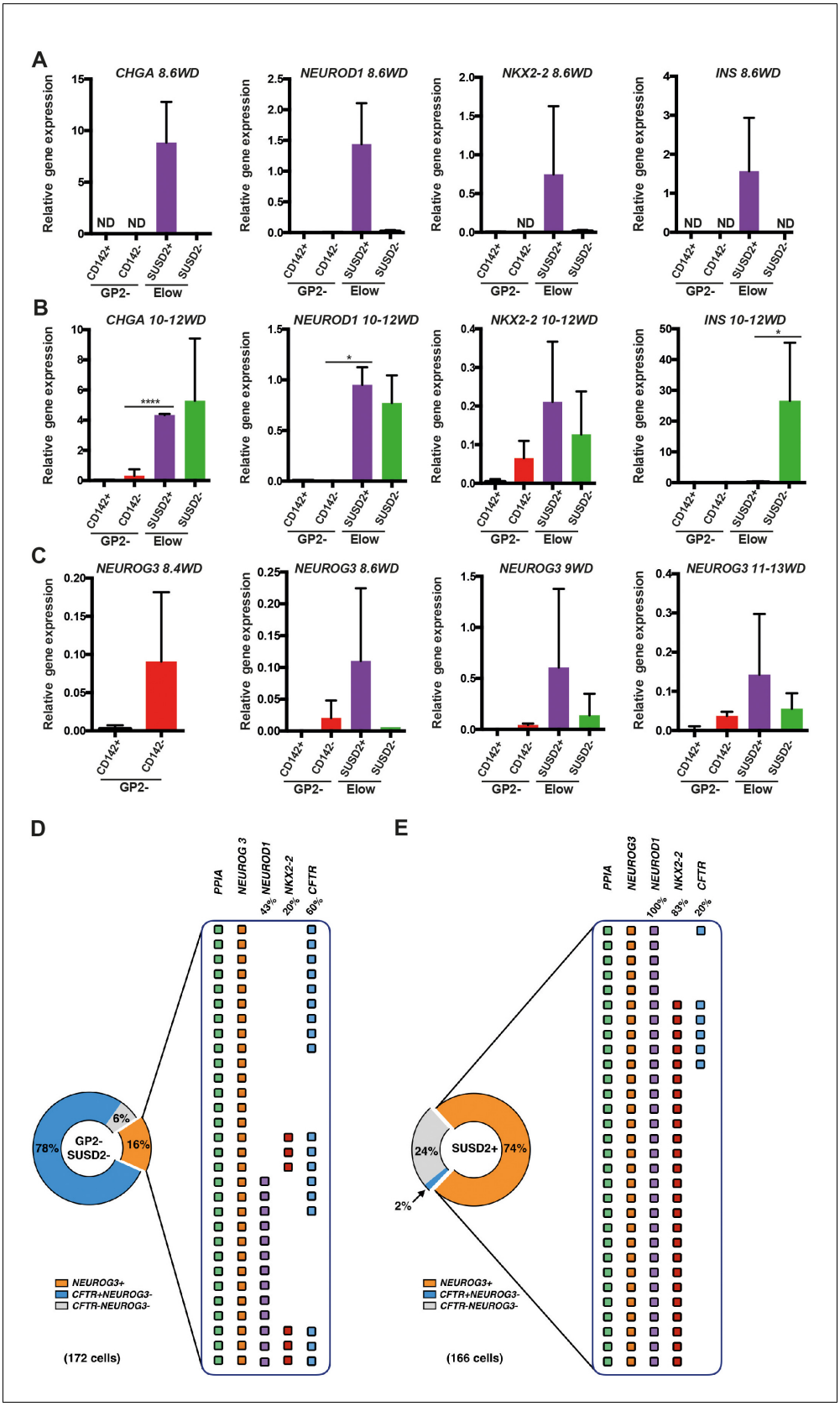

Figure 6. Molecular characterization of the GP2$^-$CD142$^-$SUSD2$^-$ and E^{low}GP2$^-$CD142$^-$SUSD2$^-$ subsets. (**A, B**) RT-qPCR for *CHGA*, *NEUROD1*, *NKX2-2* and *INS* on sorted GP2$^-$CD142$^+$SUSD2$^-$ (GP2$^-$CD142$^+$), GP2$^-$CD142$^-$SUSD2$^-$ (GP2$^-$CD142$^-$), E^{low}GP2$^-$CD142$^-$SUSD2$^+$ (E^{low}SUSD2$^+$) and E^{low}GP2$^-$CD142$^-$SUSD2$^-$

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Figure 6 continued

($E^{low}SUSD2^-$) subsets at 8.6 and 10-12WD. (**C**) RT-qPCR for *NEUROG3* at 8.4, 8.6, 9 and 11-13WD in the $GP2^-CD142^+SUSD2^-$ ($GP2^-CD142^+$), $GP2^-CD142^-SUSD2^-$ ($GP2^-CD142^-$), $E^{low}GP2^-CD142^-SUSD2^+$ ($E^{low}SUSD2^+$) and $E^{low}GP2^-CD142^-SUSD2^-$ ($E^{low}SUSD2^-$) subsets. (**D, E**) Single cell RT-qPCR at 9WD on 172 $GP2^-CD142^+SUSD2^-$ ($GP2^-SUSD2^-$, left panel) and 166 $E^{low}GP2^-CD142^-SUSD2^+$ ($SUSD2^+$, right panel) cells for the expression of *PPIA*, *NEUROG3*, *NEUROD1*, *NKX2-2* and *CFTR*. Pie charts represent the percentage of $NEUROG3^+$ (in orange), $NEUROG3^-CFTR^+$ (in blue) and $NEUROG3^-CFTR^-$ cells (in grey). For $NEUROG3^+$ the percentages of $NEUROD1^+$, $NKX2\text{-}2^+$, and $CFTR^+$ cells are displayed. Each line represents one cell. $PPIA^-$ cells were excluded from the analysis. (**A–C**) n = 3, (**D, E**) n = 2. *p<0.05, ****p<0.0001, *t* test. (mean ±SEM).

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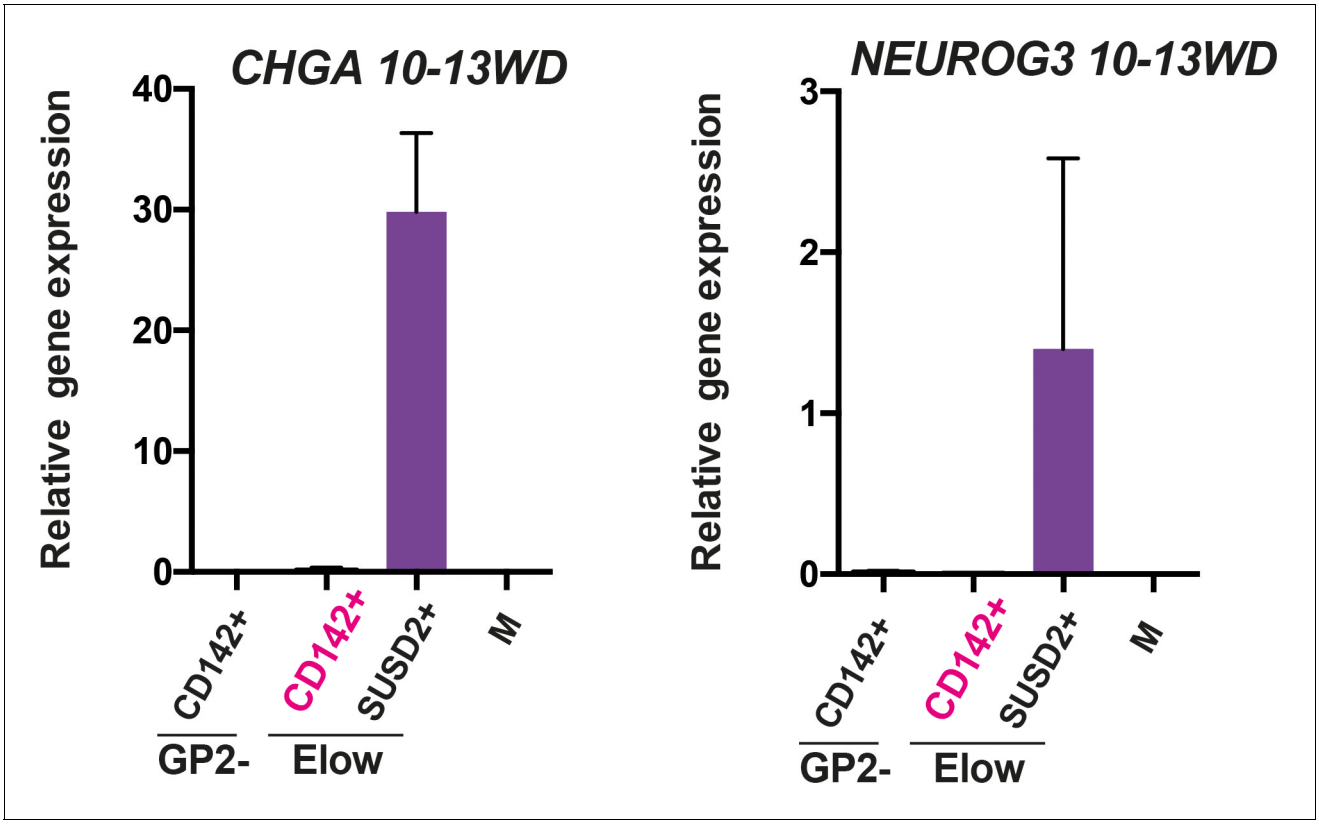

Figure 6—figure supplement 1. Expression of *CHGA* and *NEUROG3* in the $E^{low}CD142^{+}$ subsets. RT-qPCR for *CHGA* and *NEUROG3* in the $GP2^{-}CD142^{+}SUSD2^{-}$ (named $GP2^{-}CD142^{+}$), $E^{low}CD142^{+}$, $E^{low}GP2^{-}CD142^{-}SUSD2^{+}$ (named $E^{low}SUSD2^{+}$) and M ($CD45^{-}CD45^{-}EPCAM^{-}$) populations. Heatmaps and RT-qPCR are representative of 3 independents experiments. (mean ± SEM).

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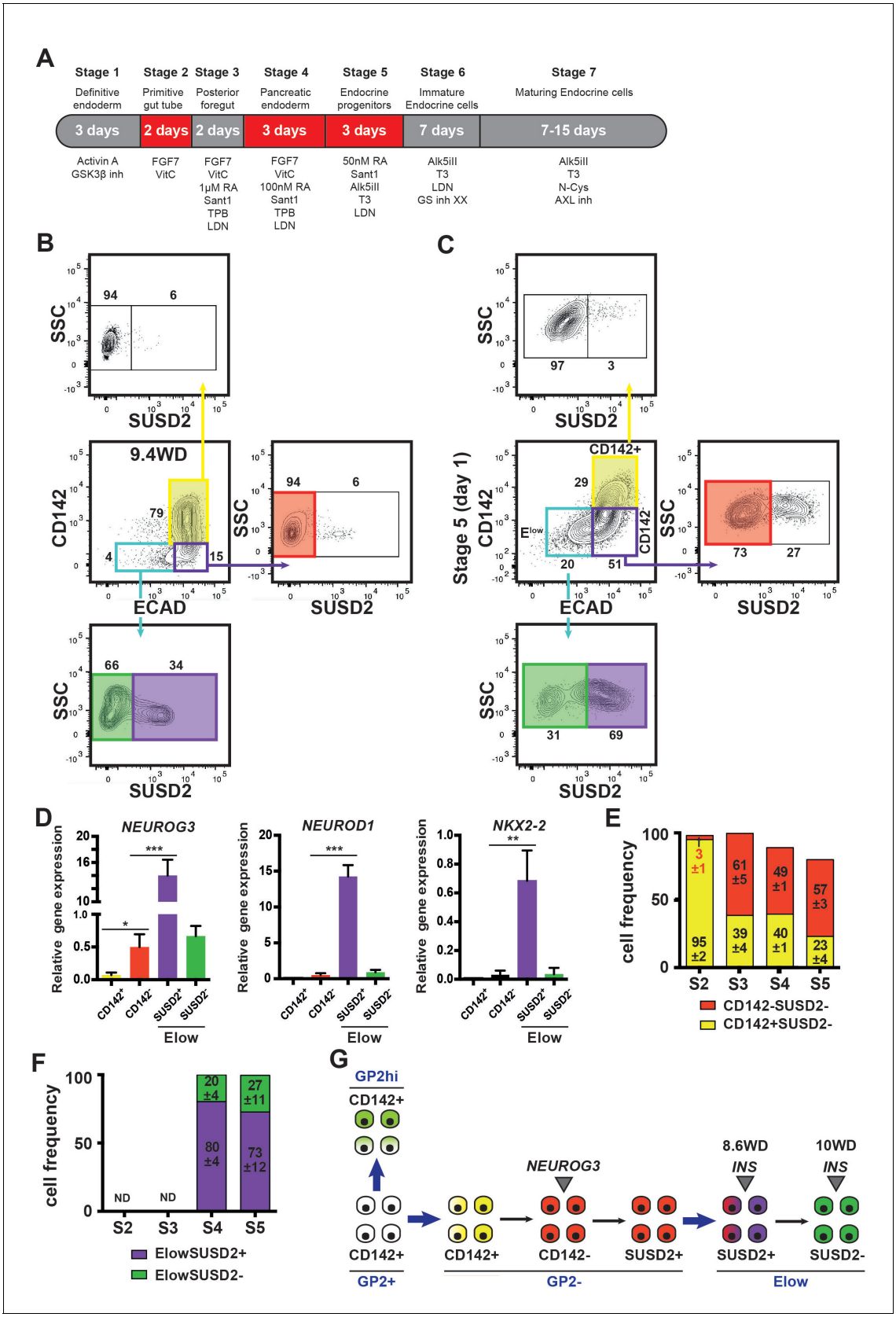

Figure 7. Expression of CD142, ECAD and SUSD2 in pancreatic endocrine cells derived from hPSCs. (**A**) Scheme of in vitro differentiation of hPSCs into endocrine pancreatic cells. (**B, C**) Expression of CD142, ECAD (gated on lived $CD45^{-}CD31^{-}EPCAM^{+}$ cells for the human) and SUSD2 in $CD142^{+}$, $CD142^{-}$

Figure 7 continued on next page

Figure 7 continued

and E^{low} population by flow cytometry in the human fetal pancreas at 9.4WD (in B) and in pancreatic endocrine cells derived from hPSCs (AD3.1 iPSC) at stage 5 (in C). (**D**) RT-qPCR on $CD142^{+}E\text{-}CAD^{+}SUSD2^{-}$ (named $CD142^{+}$), $CD142^{-}E\text{-}CAD^{+}SUSD2^{-}$ (named $CD142^{-}$), $CD142^{-}E\text{-}CAD^{low}SUSD2^{+}$ (named $E^{low}SUSD2^{+}$), $CD142^{-}E\text{-}CAD^{low}SUSD2^{-}$ (named $E^{low}SUSD2^{-}$) from hPSCs (AD3.1 iPSC) at stage five for *NEUROG3*, *NEUROD1* and *NKX2-2*). (**E**) Cell frequencies of $CD142^{+}SUSD2^{-}$ (in yellow) and $CD142^{-}SUSD2^{-}$ (in red) from hPSCs (SA121 hESC, AD2.1 iPSC, AD3.1 iPSC) from stage 2–5. (**F**) Cell frequencies of $E^{low}SUSD2^{+}$ and $E^{low}SUSD2^{-}$ from hPSCs (SA121 hESC, AD2.1 iPSC, AD3.1 iPSC) from stage 2–5. (**G**) Scheme representing human pancreatic differentiation across development using cell surface markers. (**B–F**) n = 3. *p<0.05, **p<0.001, ***p<0.005, *t* test.

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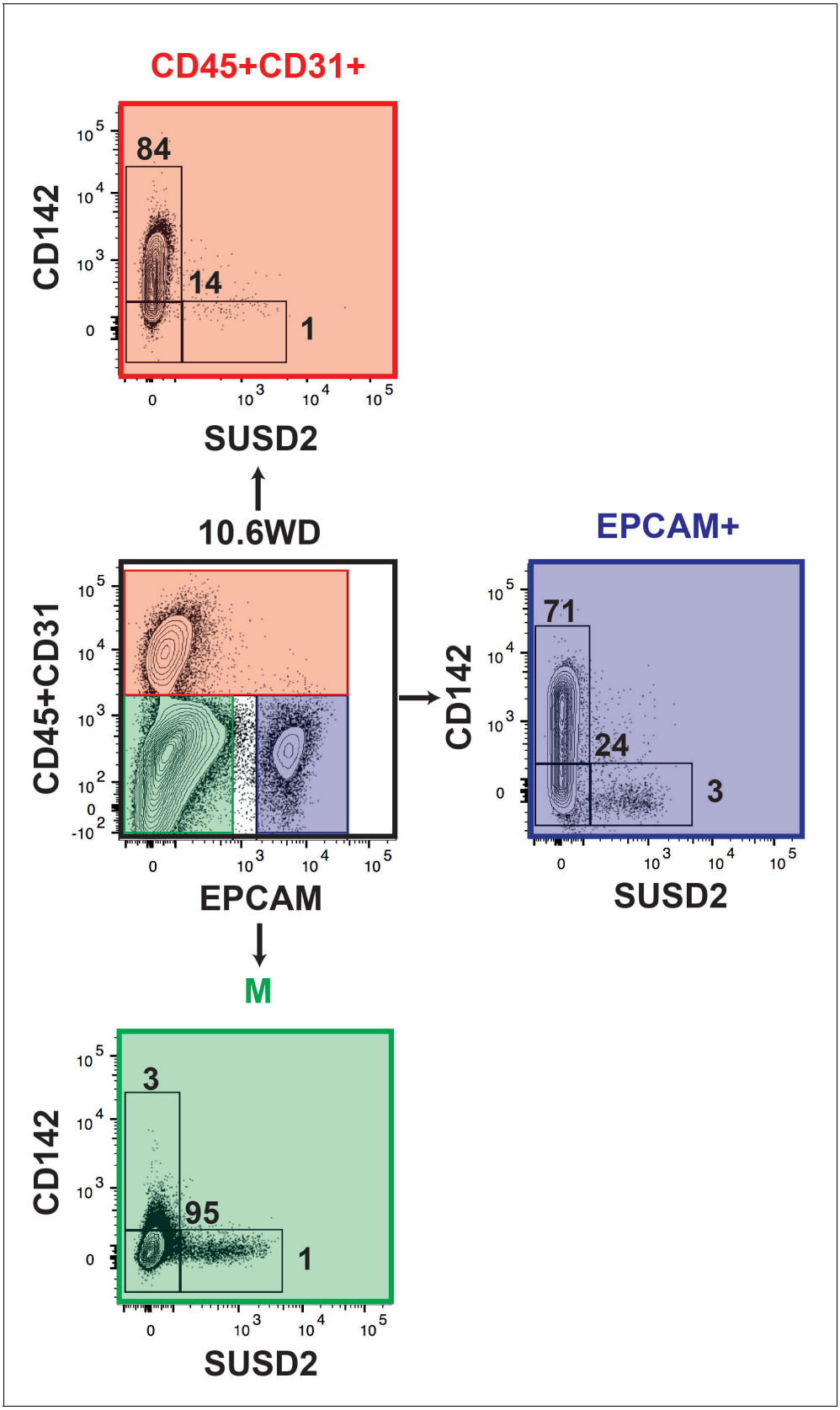
CD45+CD31+
84
14
1
CD142
SUSD2
10.6WD
EPCAM+
71
24
3
CD142
SUSD2
CD45+CD31
EPCAM
M
3
95
1
CD142
SUSD2

Figure 7—figure supplement 1. Expression of CD142 and SUSD2 in the mesenchyme, the endothelial/hematopoietic and the epithelial compartments. Human fetal pancreas at 10.6WD was stained for CD45, CD31, EPCAM, CD142 and SUSD2. CD142 and SUSD2 expressions are displayed in the $CD45^{+}/CD31^{+}$ (in red), $CD45^{-}CD31^{-}EPCAM^{-}$ (in green) and in $CD45^{-}CD31^{-}EPCAM^{+}$ (in blue) populations on lived cells. n = 5.
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