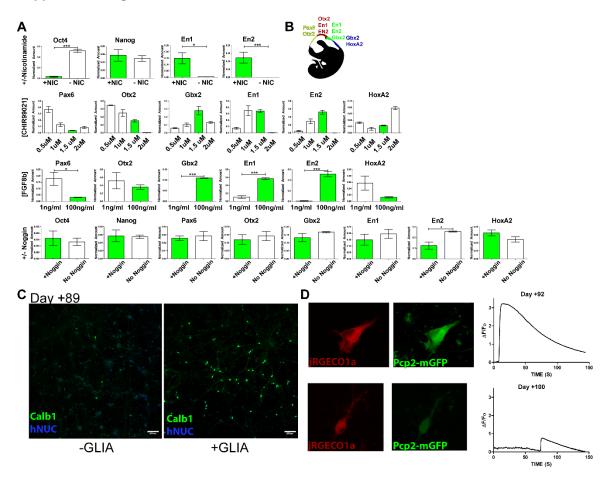
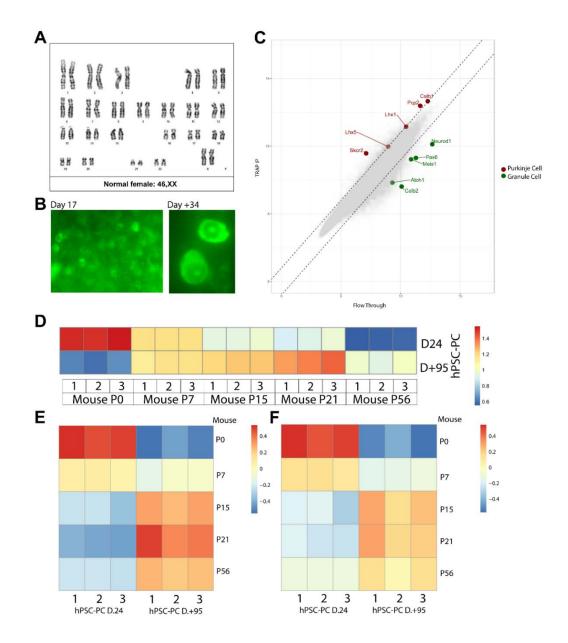
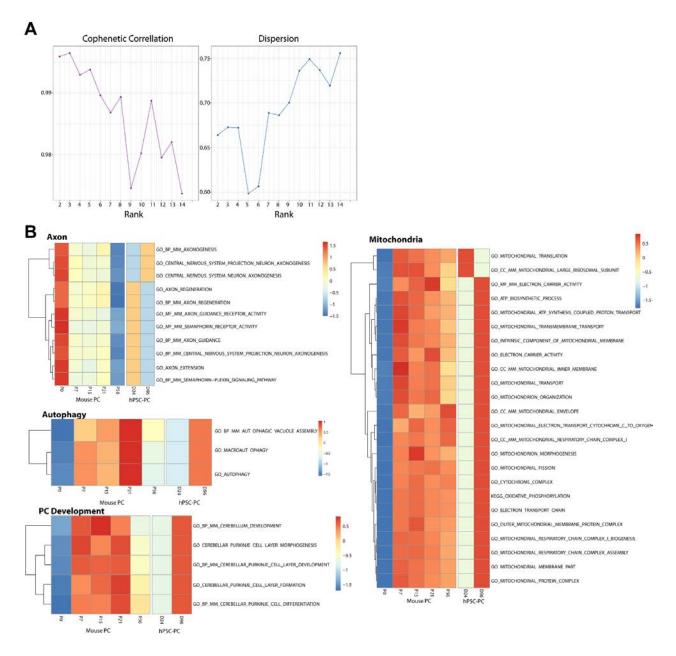
Supplemental Figures



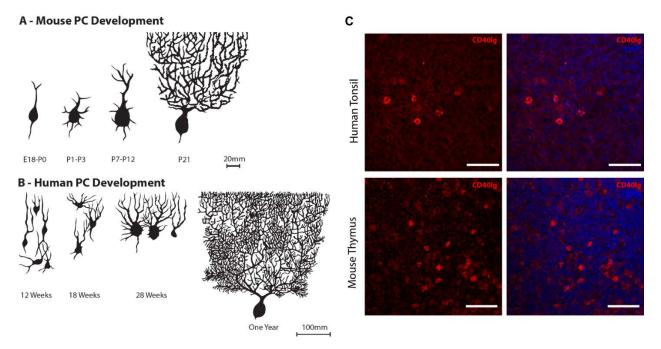
Supplemental Figure 1. Differentiation of hPSCs to PCs. A. qPCR analysis at day 6 of differentiation in the presence or absence of differentiation factors. Green bars represent optimized conditions used subsequently. Student's T-Test P-values *<0.05 ***<0.001. Data were collected from three rounds of differentiation and error bars show standard error of the mean. **B.** Schematic of neurodevelopmental patterning depicting rostral-caudal marker genes. Cerebellar genes and the cerebellar primordium are in green. **C.** Live imaging of genetically encoded calcium indicator jRGECO1a and hPSC-PC reporter Pcp2-mGFP after 92 and 100 days of co-culture with mouse glia and granule cells and traces of the change in fluorescence (Δ F/Fo) over time.



Supplemental Figure 2. Translational Profiling in hPSC-PCs and Mouse PCs. A. Karyotype analysis of RUES2-PCP2-L10a-EGFP stem cell line. **B.** Live imaging of the L10a-EGFP TRAP reporter after 17 days of differentiation and an additional +34 days of differentiation following isolation and co-culture. **C.** Test for enrichment of PC markers and depletion of GC markers from TRAP isolation of RNA from a P7 *Tg(Pcp2-L10a-Egfp)* TRAP mouse, dotted lines represent Log₂ fold change. **D.** Heat map depicting expression levels in mouse PCs over postnatal development of gene sets defined as log₂ four-fold change between Day 24 and Day +95 in hPSC-PCs following subtraction of mouse genes from hPSC-PC data using an in silico human-mouse reference genome (34). Day 24 hPSC-PCs are most similar to P0 mouse PCs (p=5.26x10⁻¹⁷). Day +95 PCs are most similar to P21 mouse PCs (p=3.73x10⁻⁵). **E.** Heat map depicting expression levels in hPSC-PCs of mouse gene sets defined as the 100 most expressed genes per time point. **F.** Heat map depicting expressed genes per time point. **F.** Heat map depicting expressed genes per time point. **F.** Heat map depicting expressed genes per time point following subtraction of mouse genes from hPSC-PCs of mouse genes from hPSC-PC data using an in silico human-mouse reference genes per time point following subtraction of mouse genes from hPSC-PCs of mouse genes per time point. **F.** Heat map depicting expressed genes per time point following subtraction of mouse genes from hPSC-PC data using an in silico human-mouse reference genome (34).



Supplemental Figure 3. NMF metagene analysis of mouse PC TRAP Data. A. Cophenetic correlation and dispersion by rank (metagene number). Rank 5 showed high cophenetic correlation with low dispersion. **B.** Heat maps depicting all gene ontology terms for the gene ontology signatures (Axon, Autophagy, PC Development, Mitochondria) in Figure 3.



Supplemental Figure 4. Species differences. A. Schematic of mouse PC development. (Adapted from (76)and (77)). **B.** Schematic of human PC development (Adapted from(27)). **C.** Positive controls for anti-human CD40LG antibody and anti-mouse CD40LG antibody. Scale bars = 50µm.

Gene	Forward	Reverse	Source
Oct4	CAGTGCCCGAAACCCACAC	GGAGACCCAGCAGCCTCAAA	(78)
Nanog	CAGAAGGCCTCAGCACCTA C	ATTGTTCCAGGTCTGGTTGC	(78)
Pax6	TCACCATGGCAAATAACCTG	CAGCATGCAGGAGTATGAGG	Designed on Primer3
Otx2	ACAAGTGGCCAATTCACTCC	GAGGTGGACAAGGGATCTGA	Designed on Primer3
Gbx2	GTTCCCGCCGTCGCTGATG AT	GCCGGTGTAGACGAAATGGCC G	Designed on Primer3
En1	GAGCGCAGGGCACCAAATA	CGAGTCAGTTTTGACCACGG	Primerbank (126090908c 1)
En2	GGCGTGGGTCTACTGTACG	TACCTGTTGGTCTGGAACTCG	Designed on Primer3
HoxA2	CGTCGCTCGCTGAGTGCCT G	TGTCGAGTGTGAAAGCGTCGA GG	(24)
GPI3'	GGACCACGAGCCCTTAGC	AACACTTCAGCCAATTCTAACA C	(65)
GPI5'	CGTCATCAACATTGGCATTG G	GGGACCTCCTGAAGAGTATGG	(65)
HMBS	TGCTATCTGGGGAGTGATTA CC	GGCTGTTGCTTGGACTTCTC	(65)
GAPD	AGCAAGAGCACAAGAGGAA GAG	GAGCACAGGGTACTTTATTGAT GG	(65)

Supplementary Table 1. qPCR Primer Sequences.

Antibody	% Triton X-100	Dilution	Source
En1	0.3	1:10,000	Gift of T. Jessell
Otx2	0.3	1:2000	Millipore Cat# AB9566, RRID:AB_2157186
Kirrel2	0.3	1:250	R&D Systems
Corl2(Skor2)	0.3	1:200	Atlas Antibodies Cat# HPA046206, RRID:AB_2679588
Pcp2(L7)	0-0.1	1:500	Takara Cat#M202
Calb1	0.3	1:250	Swant Cat# 300, RRID:AB_10000347
Calb1	0.3	1:500	Swant Cat# CB38, RRID:AB_2721225
GD3	0	1:200	BioLegend Cat# 917701, RRID:AB 2565200
NCAM(CD56)	0	1:200	BioLegend Cat# 304601, RRID:AB 314443
hNUC	0.3	1:200	Millipore Cat# MAB1281, RRID:AB 94090
Syn1	0.3	1:500	Sigma-Aldrich Cat# S193, RRID:AB 261457
GFP	0.3	1:4000	Aves Labs Cat# GFP- 1020, RRID:AB_10000240
Human CD40lg	0.05 cell culture, 0.1 paraffin	1:50	R and D Systems Cat# MAB617, RRID:AB_2291414
Mouse CD40lg	0.05	1:50	R and D Systems Cat# AF1163, RRID:AB_35463

Supplemental Table 2. Antibody List.