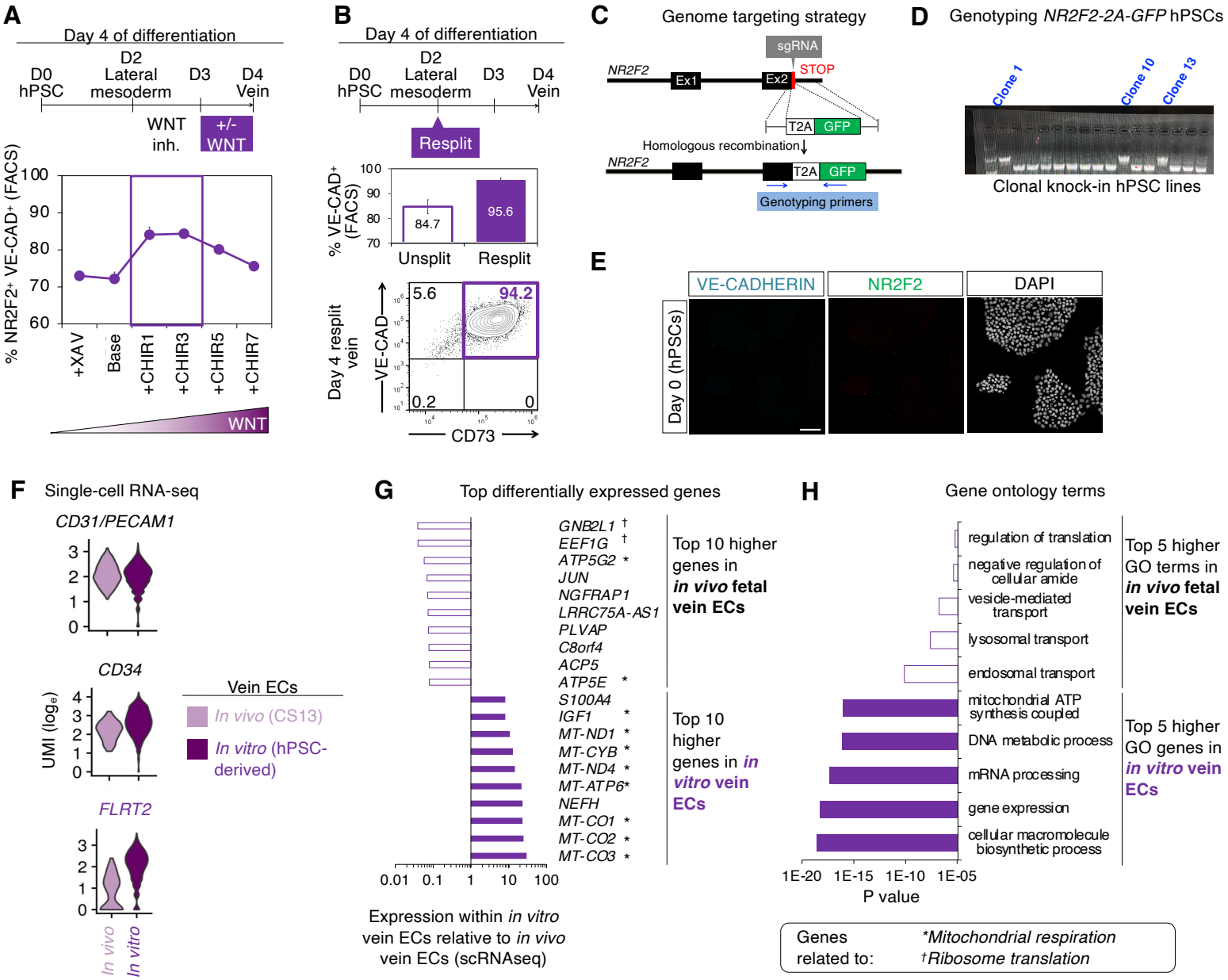
Figure S3


A
Day 4 of differentiation
D0 hPSC
D2 Lateral mesoderm
D3
D4 Vein
WNT inh.
+/- WNT
% NR2F2+ VE-CAD+ (FACS)
+XAV
Base
+CHIR1
+CHIR3
+CHIR5
+CHIR7
WNT
B
Day 4 of differentiation
Resplit
% VE-CAD+ (FACS)
84.7
95.6
Unsplit
Resplit
Day 4 resplit vein
VE-CAD
CD73
5.6
94.2
0.2
0
C
Genome targeting strategy
sgRNA
NR2F2
Ex1
Ex2
STOP
T2A
GFP
Homologous recombination
Genotyping primers
D
Genotyping NR2F2-2A-GFP hPSCs
Clone 1
Clone 10
Clone 13
Clonal knock-in hPSC lines
E
VE-CADHERIN
NR2F2
DAPI
Day 0 (hPSCs)
F
Single-cell RNA-seq
CD31/PECAM1
CD34
FLRT2
UMI (log$_e$)
Vein ECs
In vivo (CS13)
In vitro (hPSC-derived)
In vivo
In vitro
G
Top differentially expressed genes
GNB2L1 †
EEF1G †
ATP5G2 *
JUN
NGFRAP1
LRRC75A-AS1
PLVAP
C8orf4
ACP5
ATP5E *
S100A4
IGF1 *
MT-ND1 *
MT-CYB *
MT-ND4 *
MT-ATP6*
NEFH
MT-CO1 *
MT-CO2 *
MT-CO3 *
Top 10 higher genes in in vivo fetal vein ECs
Top 10 higher genes in in vitro vein ECs
Expression within in vitro vein ECs relative to in vivo vein ECs (scRNAseq)
H
Gene ontology terms
regulation of translation
negative regulation of cellular amide
vesicle-mediated transport
lysosomal transport
endosomal transport
mitochondrial ATP synthesis coupled
DNA metabolic process
mRNA processing
gene expression
cellular macromolecule biosynthetic process
Top 5 higher GO terms in in vivo fetal vein ECs
Top 5 higher GO genes in in vitro vein ECs
P value
Genes related to:
*Mitochondrial respiration
†Ribosome translation