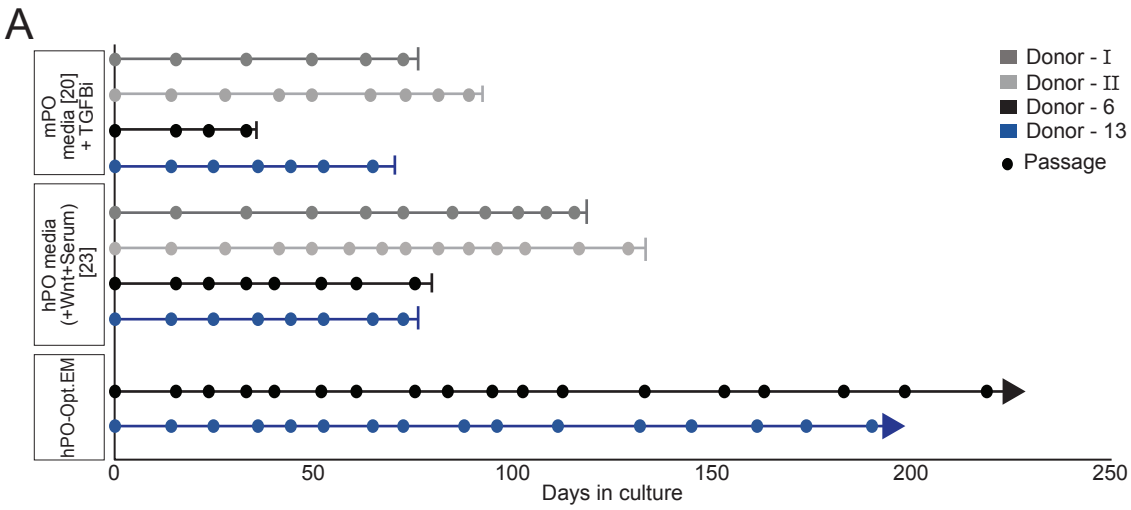
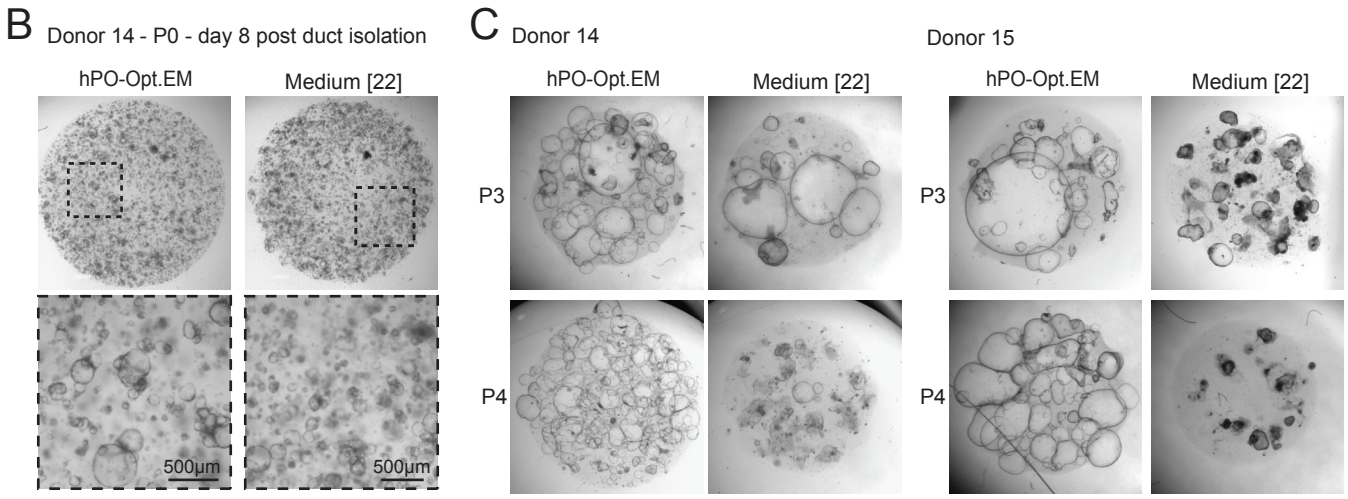
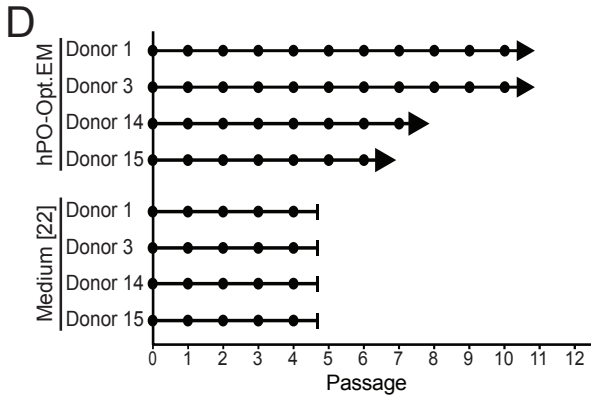
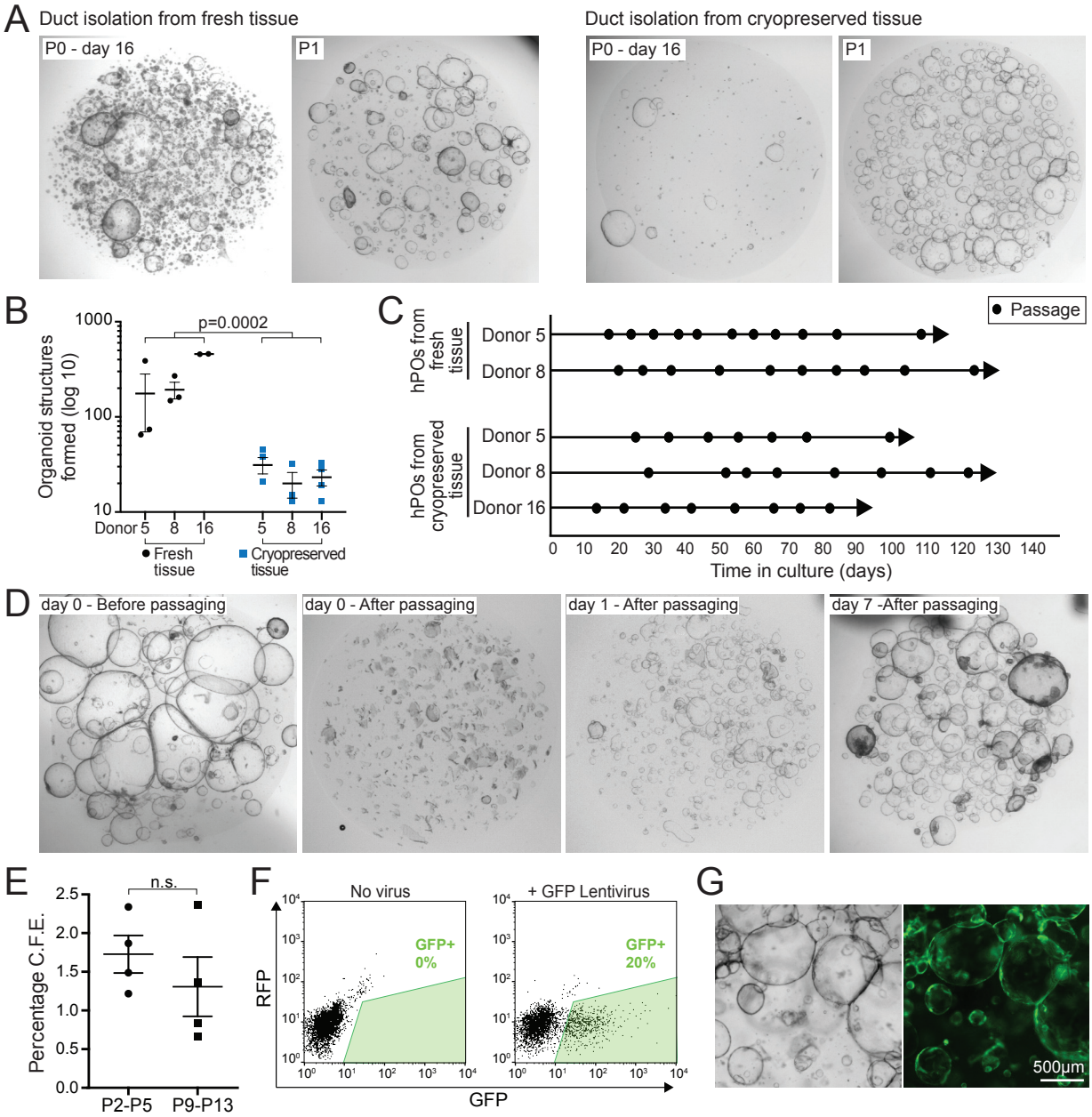
Supplementary Figure 1 - Optimisation of hPO-Opt.EM culture medium and its expansion potential compared with published pancreatic organoid culture systems.



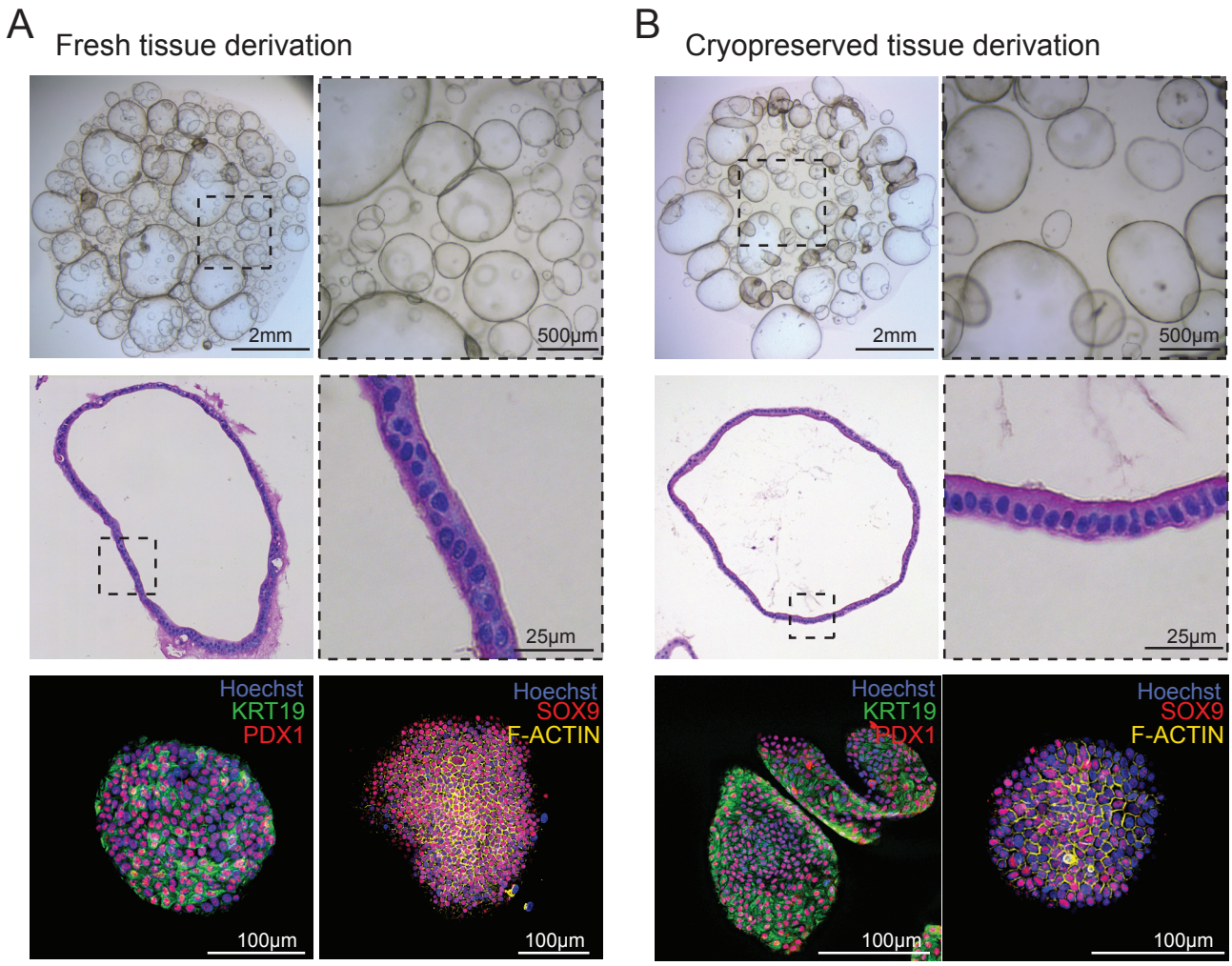




Supplementary Figure 2 - Human pancreas organoids (hPOs) can be derived from fresh and cryopreserved pancreas tissue and are amenable for genetic manipulation.



Supplementary Figure 3 - hPOs derived from fresh and cryopreserved samples expand as a single cell-layer epithelium of ductal cells and are phenotypically indistinguishable.

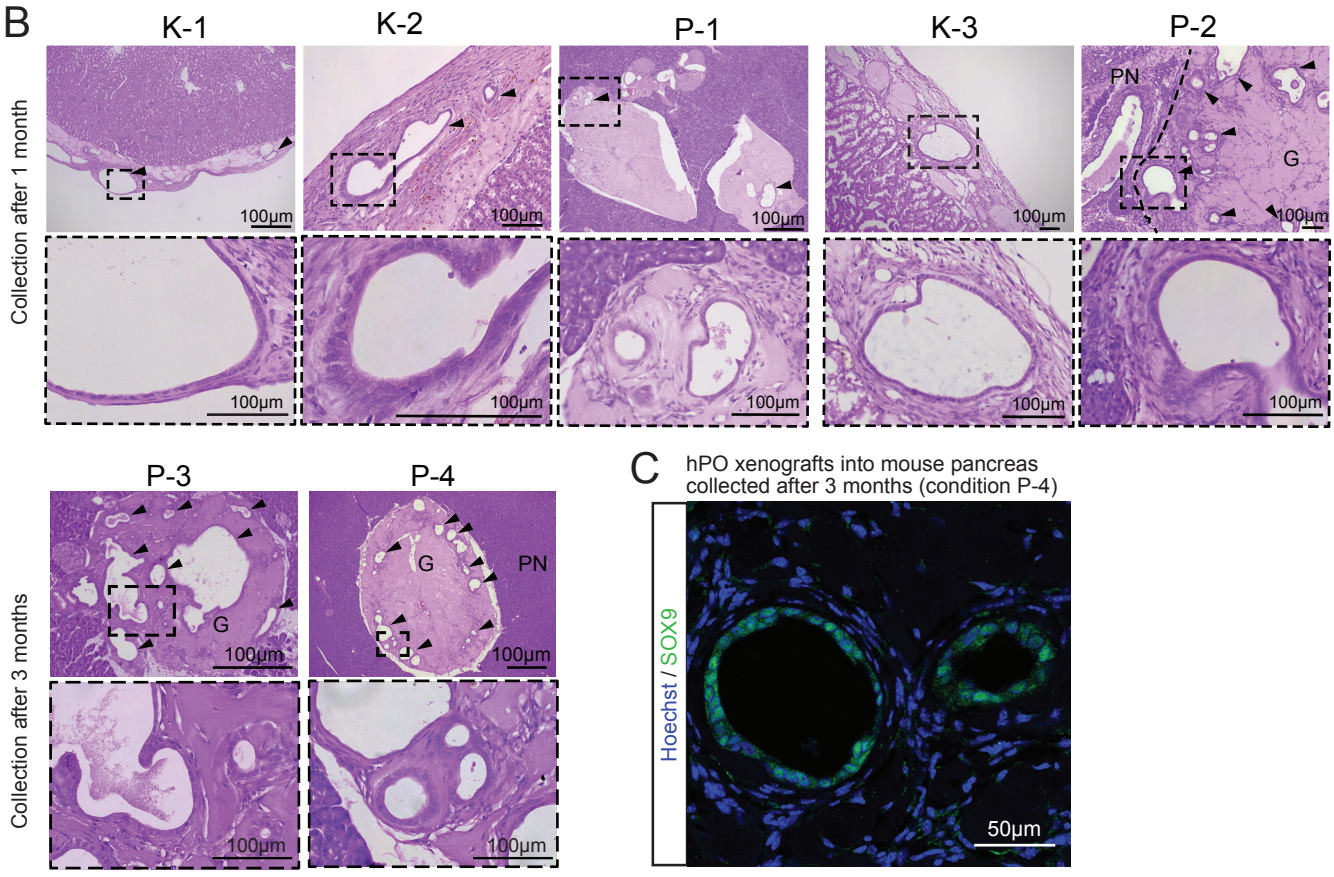


Supplementary Figure 4- Optimisation of hPO transplantation.

A

Condition	K-0	K-1	K-2	P-1	K-3	P-2	P-3	P-4
Location	Kidney	Kidney	Kidney	Pancreas	Kidney	Pancreas	Pancreas	Pancreas
Matrix type	BME 2	BME 2	BME 2	BME 2	Matrigel	Matrigel	Glycosil Hyaluronic Acid	Matrigel
Matrix (%)	100	100	30	100	30	30	50	30
VEGF	✗	✗	✓	✗	✓	✗	✓	✓
Rho Kinase inhibitor	✗	✓	✓	✓	✓	✓	✓	✓
hPO-Opt.EM Medium	✗	✗	✗	✗	✓	✗	✓	✓
Engraftment Success (1 month)	4/20	2/3	2/3	3/3	1/3	3/3	1/1	1/1
Engraftment Success (3 months)	0/23	NT	NT	NT	NT	NT	3/3	3/3
Time of *in vitro* expansion	35 mice- 60 days 8 mice- 135 days	60 days			60 days		40 days	

B



C

hPO xenografts into mouse pancreas collected after 3 months (condition P-4)

Supplementary Figure 5 - hPOs cultured in optimised hPO-Opt.EM medium grow in a chemically defined hydrogel.