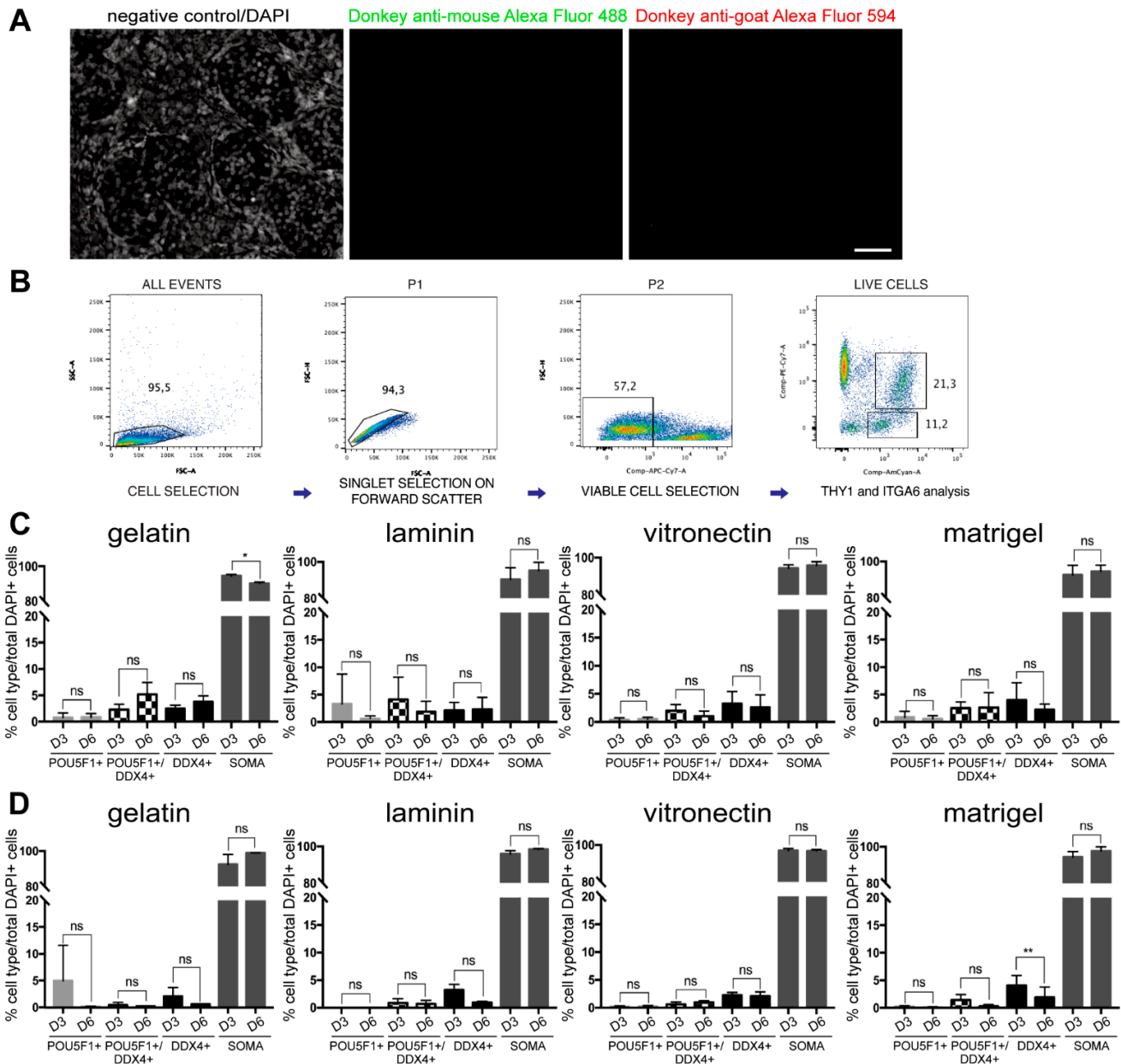


Supplementary Materials:



**Supplementary Figure S1. Negative controls, FACS gating strategy and visualization associated with Table 1.** **A)** Negative control for the immunofluorescence using secondary antibodies only in male gonads of 16.5 WPF. Scale bar: 50  $\mu$ m. **B)** FACS gating strategy showing representative dot-plots for the gates used to select population 1 (P1), singlets (P2), viable cells and finally the expression analysis of THY1 and ITGA6. **C)** Percentage hFGCs and gonadal somatic cells per DAPI+ cells (N=3 donors) after culture for D3 and D6 in Shinohara-medium on the different substrates (mean  $\pm$  SD). **D)** Percentage of hFGCs and gonadal somatic cells cells per DAPI+ cells (N=2 donors) after culture for D3 and D6 in Zhou-medium on the different substrates (mean  $\pm$  SD). Statistical analysis was performed using the t-test. The graphs are a visualization of the mean percentages shown in Table 1.

**Table S1.** List of antibodies used in this study.

Antibody	Company (Cat. no.)	Dilution
goat anti-DDX4 (VASA)	R&D Systems (AF2030)	IHC, 1:500; ICC, 1:1000
mouse anti-POU5F1 (OCT3/4)	Santa Cruz Biotechnologies (SC5279)	IHC, 1:50; ICC, 1:100
LIVE/DEAD APC.Cy7(viability)	Invitrogen (L-34975)	FC, 1:1000
mouse anti-CD90 (THY1) PE.Cy7	BD Biosciences (561558)	FC, 1:50
rat anti-CD49f (ITGA6) BV510	BD Biosciences (563271)	FC, 1:50
rabbit anti-Ki67	Epredia (RM-9106-S0)	IHC, 1:500; ICC, 1:500
donkey anti-mouse Alexa fluor 647	Invitrogen (A-31571)	IHC, 1:500; ICC, 1:500
donkey anti-goat Alexa fluor 546	Invitrogen (A-11056)	IHC, 1:500; ICC, 1:500
donkey anti-rabbit Alexa fluor 488	Invitrogen (A-21206)	IHC, 1:500; ICC, 1:500
donkey anti-mouse Alexa fluor 488	Invitrogen (A-21202)	IHC, 1:500; ICC, 1:500
donkey anti-goat Alexa fluor 594	Invitrogen (A-11058)	IHC, 1:500; ICC, 1:500

ICC, immunocytochemistry; IHC, immunohistochemistry; FC, flow cytometry; Cat. No., catalogue number.

**Table S2.** Total number of DAPI+ cells counted in each biological donor (associated with Table 1).

	donor 1	donor 2	donor 3	Mean DAPI+ cells $\pm$ SD
<b>Shinohara-medium, with EGF, FGF2, GDNF, LIF, estrogen and progesterone</b>				
<b>D3</b>				
gelatin	395	1172	809	792,0 $\pm$ 388,8
laminin	792	672	2314	1259,3 $\pm$ 915,3
matrigel	2026	804	448	2252,3 $\pm$ 356,6
vitronectin	1847	2518	2392	1092,7 $\pm$ 827,7
<b>D6</b>				
gelatin	2839	1934	1312	2028,3 $\pm$ 767,9
laminin	2127	1486	5180	2931,0 $\pm$ 1973,9
matrigel	3544	1880	1569	1405,0 $\pm$ 694,3
vitronectin	2147	1297	771	2331,0 $\pm$ 1061,9
<b>Zhou-medium (adapted), with RA, BMP4 and ActA</b>				
<b>D3</b>				
gelatin	673	1448		1060,5 $\pm$ 548,0
laminin	1348	509		928,5 $\pm$ 593,3
matrigel	1347	1427		1148,5 $\pm$ 7,8
vitronectin	1143	1154		1387,0 $\pm$ 56,6
<b>D6</b>				
gelatin	471	1235		853,0 $\pm$ 540,2
laminin	3125	542		1833,5 $\pm$ 1826,5
matrigel	3231	2407		1955,5 $\pm$ 170,4
vitronectin	2076	1835		2819,0 $\pm$ 582,7