Sample	Age	Raw	Clean	Cell # (after	UM	II numb	er distri	bution of	Mito% Ribo%		Correlation			
		reads	reads (1)	filtering)	Mean	5%	25%	Median	75%	95%	(2)	(3)	coefficient (4)	
Human- D1-T1	40y	124.9M	57.8M	808	7218	1771	2983	5114	10341	18365	5.39%	3.91%	0.9967	
Human- D1-T2		72.1M	34.2M	777	6184	1721	2795	4614	8396	15099	6.06%	3.66%	0.9907	
Human- D2-T1	45y	81.3M	37.4M	420	6305	1908	2863	4914	9041	14037	0.33%	2.55%	0.9105	
Human- D2-T2		107.0M	43.8M	549	6036	2063	3072	5234	8200	13290	0.30%	2.53%	0.9982	
Mouse-D1	4m	70.1M	37.3M	591	8472	1663	3717	7259	11975	19647	1.48%	1.83%	0.9655	
Mouse-D2	4m	179.5M	78.0M	1002	9308	1669	3436	7466	13484	23744	1.00%	2.28%		

Table S1. General sample information across scRNA-seq replicates, Related to Figure 1.

Note: (1): The clean reads were determined by having valid barcode, UMI and linker (W1 sequence) sequences, and the mRNA region of the read was uniquely mapped to the reference genome.

(2): Mito% stands for the ratio of total UMI count of mitochondria genes versus total UMI counts of all genes.

(3): Ribo% stands for the ratio of total UMI count of ribosomal protein genes versus total UMI counts of all genes.

(4): Pearson correlation coefficient between the average gene expression profiles of biological/technical replicates.

Table S2. Detected gene numbers across scRNA-seq studies of human developmental systems,Related to Figure 1.

scRNA-seq sample / Publication	Accession Number	scRNA- seq method	Cell cluster name (1)	Cell number		Gene num	ber distrib	Gene number per cluster by	Gene number per sample			
					Mean	5%	25%	Median	75%	95%	bootstrap 1000 cells (3)	by bootstrap 1000 cells (4)
Human adult	GSE112013	10X	SSCs	294	4528	2524	3572	4447	5254	6539	16186	
testicular		Genomics	Diff. Sg	320	3971	2129	3005	4023	4817	6162	14697	
cells / Guo		scRNA-	Early Sc	560	3324	2259	2794	3149	3584	5191	14420	
et al, Cell		seq	Late Sc	319	4041	1892	2866	3854	5298	6330	14391	15783
Research,			RS	509	4074	1915	2964	4067	5101	6371	14746	15705
2018			ES	876	3470	1569	2355	3334	4364	6002	14904	
			Sperm-1	920	1843	1035	1358	1733	2248	2998	12535	
			Sperm-2	1025	1320	858	1034	1211	1481	2122	11576	
			Macrophaga	241	1815	1350	1970	2385	2909	2414	13300	
			Endothelia	341	2405	1450	1843	2220	2039	4646	12442	13951
			Sertoli	28	2495	1282	1947	2229	3194	3656	11522	15751
			Levdig	607	1747	1202	1473	1685	1955	2464	12809	
Human adult	GSE117498	InDrop	HSC	1282	883	533	743	854	988	1304	11200	
bone	002117170	mbrop	MPP	215	980	646	841	965	1080	1330	12322	
marrow			MLP	123	1377	929	1122	1250	1435	2357	14348	
hematopoieti			PreB/NK	592	987	491	760	919	1166	1678	12238	
c cells /			MEP	1211	922	593	701	825	1048	1632	11096	
Pellin et al,			CMP	1576	1068	478	834	996	1255	1808	11433	11703
Nature			GMP	1012	1432	650	974	1326	1814	2553	12176	
Communicat			CD34+CD164+	6343	1057	510	723	966	1305	1891	11195	
ion, 2019			CD34-CD164high	4434	941	243	434	790	1284	2146	10505	
			CD34lowCD164high	4266	1683	693	1270	1712	2079	2663	11679	
Ilumon	CSE76291	Eluidiam	CD34NegCD164I0W	358	000	128	360	239	2026	1568	10347	
embryonic	GSE/0381	C1	hDA1	47	23/8	908	1211	2006	2030	2635	11555	
midbrain /		System	hDA2	37	1887	1027	1370	1689	2029	3573	10816	
Le Manno et		and	hEndo	77	2428	1273	1866	2276	2841	3923	12917	
al. Cell.		protocols	hGaba	59	1952	1098	1572	1809	2334	3012	12551	
2016		from	hMgl	19	2025	1113	1460	1828	2334	3323	10092	
		Islam et	hNProg	100	1714	945	1257	1661	2099	2824	12433	
		al., 2014	hNbGaba	22	2100	928	1399	1950	2440	4683	10626	
			hNbM	91	1922	975	1218	1806	2376	3328	12392	
			hNbML1	52	2133	1018	1457	2110	2629	3466	11651	
			hNbML5	47	1864	972	1236	1790	2272	3352	11428	
			hOMTN	106	2515	1339	1928	2494	3091	3712	13133	14172
			hOPC	29	2221	1215	1668	2277	2759	3028	11184	14173
			hDrogDD	191	2211	1115	10/1	2188	2085	2228	13/73	
			hProgEPI	54	2033	1544	2200	2694	3212	3752	11984	
			hProgFPM	82	2005	1255	1621	1960	2361	3038	12163	
			hProgM	67	2259	1083	1639	2256	2903	3531	12329	
			hRN	40	1833	850	1306	1703	2183	3235	10760	
			hRgl1	39	2375	1358	1844	2189	2955	3518	11771	
			hRgl2a	210	2550	1375	1990	2549	3086	3791	13750	
			hRgl2b	77	1983	1259	1544	1880	2416	2931	12753	
			hRgl2c	26	2326	1226	1686	2350	2847	3614	11028	
			hRgl3	51	2616	1301	2124	2559	3142	4067	12463	
	h.u	F1 ' 1'	hSert	14	2762	1315	1850	2457	3330	4793	9943	
Human	nttps://cells.	Fluidigm	Choroid	46	2568	1262	2012	2449	3260	4162	0211	
cortex /	ucsc.edu/?d	Dren	EN-PEC	242	2314	1402	1621	2490	2830	3003	13967	
Nowakowski	<u>s-curtex-</u>	System	EN-V	424	2360	1214	1808	2311	2837	3601	14567	
et al.	<u>uev#</u>	bystein	Endothelial	30	2477	1375	1730	2483	3028	3775	11533	
Science,			Glyc	77	2395	1016	1498	2155	3207	4403	13569	
2017			IN-CTX-CGE	301	2198	1049	1581	2142	2736	3470	14321	
			IN-CTX-MGE	287	2196	1120	1606	1995	2615	3808	14083	
			IN-STR	83	2307	1212	1735	2175	3002	3596	12948	
			IPC-div	87	2426	1226	1792	2304	2891	3930	13212	
			IPC-nEN	183	2244	1237	1641	2177	2738	3692	13691	
			MGE-IPC	146	2526	1195	1661	2319	3165	4292	15867	
			MGE-RG	114	2270	1065	1627	2183	2877	3866	14134	14652
			Microalia	ן כ דר	2239	1229	1520	1856	2797	4057	12809	
			Mural	19	2057	961	1495	2099	2724	2900	9538	
					2001	201	1.75	_0,,,		2700	1000	

		OPC	37	2440	1332	1719	2119	2913	4432	11730	
		RG-div	257	2380	1115	1694	2208	2926	3946	14161	
		RG-early	53	2603	1128	1958	2655	3045	4201	12343	
		U	191	3609	1823	2652	3618	4516	5498	14691	
		nEN-early	507	2404	1221	1804	2330	2891	3772	14792	
		nEN-late	321	2207	1121	1683	2055	2639	3795	14522	
		nIN	314	2125	1089	1482	1906	2647	3638	13820	
		oRG	65	2372	1002	1556	2200	2847	4332	12822	
		tRG	100	2304	1257	1745	2274	2685	3739	13177	
		vRG	97	2113	1207	1556	1891	2472	3737	14057	
Human	GSM314360 10X	CnT	80	2175	1237	1684	2015	2510	3713	12354	
embryonic	1 Genom	ics DTLH	67	2425	1182	1656	2010	2830	4970	12566	
kidney,	scRN	A- End	223	1736	953	1186	1524	2186	3224	12291	
week 16 /	seq	ErPrT	338	1926	1130	1552	1854	2182	3065	12608	
Hochane et		ICa	653	1476	943	1110	1317	1643	2630	12213	
al, PLoS		ICb	677	1237	944	1057	1172	1344	1747	11411	
Biology,		IPC	177	1836	1070	1429	1771	2140	2965	12439	
2019		Leu	65	1457	791	1005	1300	1740	2812	10443	
		Mes	37	1278	893	1118	1232	1419	1777	9170	
		NPCa	522	1251	974	1094	1207	1343	1674	11473	
		NPCb	296	1550	1027	1268	1456	1722	2452	12127	12256
		NPCc	596	1521	1034	1256	1467	1690	2225	12003	15550
		NPCd	280	2178	1358	1747	2104	2515	3277	12936	
		PTA	459	2424	1165	2018	2381	2860	3576	13109	
		Pod	430	1590	1136	1259	1414	1786	2722	11734	
		Prolif	85	1021	745	856	961	1107	1581	9074	
		RVCSBa	367	1915	1245	1559	1828	2142	3045	12693	
		RVCSBb	312	1688	1056	1314	1552	1898	2802	12431	
		SSBm/d	151	2374	1393	1607	1826	2755	4957	12987	
		SSBpod	257	1789	1238	1516	1741	1968	2486	11938	
		SSBpr	213	2415	1425	1825	2171	2814	4365	13350	
		UBCD	317	2305	1282	1719	1985	2725	4213	13183	

Note: (1): The cell cluster names were extracted from the original publication.

 (2): The gene counts only considered protein-coding genes, thus some numbers may be slightly different from the original publication.
(3): The number represents the number of detected genes of a corresponding cell cluster. Gene expression in a single cell was defined as >0 UMI/TPM count. Gene expression in a cell cluster was defined as having expression in at least 5 cells from a random sampling (with replacement) of 1000 cells. Then the presented number of genes indicates the average detected gene number through bootstrap sampling (with replacement) of 1000 cells for 100 times. Such a strategy allows an overall fair comparison of gene numbers across studies which have different total cell numbers.

(4): Same as determining the expressed gene number of a cell cluster in (3), but instead work with the mixture of all single cells from a tissue/sample type. Colormap: The colormap represents the relative gene number abundance in each column across all cell clusters/samples.

Gene number: Because of random sampling and/or averaging, the gene numbers in this table were rounded to the nearest integer in case necessary.

Table S3. Human gene names across gene clusters defined by germ cell expression pattern, Related to Figure 1 and 2. The gene name lists correspond to the human gene clusters defined by germ cell expression pattern in Figure 2D-E.

Table S4. Mouse gene names across gene clusters defined by germ cell expression pattern, Related to Figure S2 and STAR Methods. The gene name lists correspond to the mouse gene clusters defined by germ cell expression pattern in Figure S2C-D.

Table S5. Gene lists of bidirectional gene pairs and all upstream-confounded genes,Related to Figure 3 and STAR Methods.

Table S6. Human gene names across gene groups defined by germ cell expression levels,Related to Figure 5 and STAR Methods. The gene name lists correspond to the gene groupsdefined by germ cell expression levels as shown in Figure 5B.

Table S7. Gene Ontology (GO) terms analysis of the unexpressed genes (sheet 1) and highly-expressed genes (sheet 2) during human spermatogenesis, Related to Figure 7. The GO term analysis was done by GOrilla (Eden et al., 2009). 'FDR q-value' is the corrected p-values for multiple testing using the Benjamini and Hochberg method (Benjamini and Hochberg). Enrichment (N, B, n, b) is defined as 'Enrichment = (b/n) / (B/N)'. N, total number of genes; B, total number of genes associated with a specific GO term; n, number of genes in the input list; b, number of genes in the intersection. The highlighted GO terms were displayed in Figure 7A.