Table S1. Summary of published work on cell potency for *in vitro* cell lines

Cells used	Media	*In vitro* differentiation assays	Method of chimera generation	Numbers of cells introduced into host embryos	Observed contribution to multiple lineages at the blastocyst stage	Observed contribution to later embryonic/fetal and extra-embryonic lineages	Chimeric offspring	Reference(s)
ESCs (CP1 and CP3)	Serum (on feeders)	N.A.	Blastocyst injection	10-15 cells	N.A.	At E10.5, 15/16 chimeras showed ESC contribution to embryonic tissues, and three of these also exhibited VE or PE contribution. An additional two of these showed trophoblast contribution, and one embryo showed contribution to all three lineages. One embryo showed trophoblast contribution only.	N.A.	Beddington and Robertson (1989)
				Single cells	N.A.	At E10.5, 12/12 chimeras show ESC contribution to the embryonic tissues, one of them also showed contribution to the PE.	N.A.	
ESCs (N1, D3 ESCs; LacZ promoter trap)	Serum (on feeders)	Embryoid bodies	Blastocyst and morula injection	15-20 cells	34/53 blastocysts showed ESC contribution to the ICM and TE. 13/53 showed contribution to the ICM only and 6/53 to the TE only.	From E5.5 to E9.5, ESC contribution to the embryonic, but not extra-embryonic, lineages was observed.	N.A.	Lallemand and Brûllet (1990)
				Single cell	6/14 blastocysts show ESC contribution to the ICM, 8/14 show contribution to TE only and 0/14 show contribution in both lineages.	N.A.	N.A.	
ESCs (Hhex-Venus; CAG-βgeo), SSEA1$^+$	SL	Embryoid bodies	Morula aggregation and blastocyst injection	N.A.	N.A.	At E6.5, 12/120 embryos derived from Hhex$^+$/SSEA1$^+$ ESCs showed VE/PE contribution with modest contribution to the embryo proper and 65/120 showed low-moderate embryonic contribution, whereas 52/69 Hhex$^-$/SSEA1$^+$ ESCs showed high levels of embryonic contribution.	N.A.	Canham et al. (2010)
ESCs (MERVL-Tomato; CMV-GFP or EF1α-GFP)	SL, KOSR, 2iL	N.A.	Morula and blastocyst injection	4 cells	From morula injections, 3/5 chimeric blastocysts showed MERVL$^+$ ESC contribution to both the ICM and TE. 1/5 showed contribution to the TE only.	From blastocyst injections at E12.5, MERVL$^+$ ESCs contributed to the embryonic lineages, yolk sac and placenta.	N.A.	Macfarlan et al. (2012)
ESCs (Zscan4-Emerald)	SL	N.A.	Blastocyst injection	10-15 cells and single cells	N.A.	N.A.	Zscan4$^-$ ESCs support live-born chimeric mice at high frequencies (75% for multiple cells, 31% for single cells), whereas Zscan4$^+$ ESCs support reduced levels of chimera formation (31% for multiple cells, 0% for single cells). Multiple ESC lines were tested with significant *n* values.	Amano et al. (2013)
ESCs (Zscan4-ERT2 overexpression)	SL	N.A.	4N blastocyst injection	10-15 cells and single cells	N.A.	N.A.	Overexpression of Zscan4-ERT2 restored the ability to form chimeras in high passage ESCs in multiple clones, with up to 43% live-born chimeric mice, compared to 0-3% for controls. For single-cell injections, a similar enhancement was observed (5% vs 0-1% for controls). Multiple ESC lines were tested with significant *n* values.	
ESCs (Hhex-Venus;CAG-LacZ), PECAM1$^+$ and (Hhex-Venus;H2B-Tomato), PECAM1$^+$	2iL	Trophoblast and endoderm differentiation	Morula aggregation and morula injection	8-10 cells	3/7 blastocysts generated from HhexVenus$^+$ ESCs show contribution to embryonic and extra-embryonic lineages.	At E6.5, 30/55 chimeric embryos generated with HhexVenus$^+$ 2iL ESCs show contribution to both embryonic and extra-embryonic lineages. Chimeric embryos from HhexVenus$^-$ 2iL ESCs also showed embryonic and extra-embryonic contribution, but at a much lower rate (18/60 embryos). At E9.5, 3/8 embryos derived from HhexVenus$^+$ ESCs showed placenta and visceral yolk sac contribution (assessed by histology).	N.A.	Morgani et al. (2013)
				Single cells	N.A.	At E6.5, 13/23 chimeric embryos from single HhexVenus$^+$ ESCs showed contribution to both the Epi and VE or Epi and TE. Three of them showed contribution to all three lineages. Staining for KRT7 (TE) and GATA6 (VE and PE).	N.A.	
ESCs (H2B-Tomato)	SL and 2iL	N.A.	Morula injection	3-8 cells	30/30 blastocysts showed ESC contribution to Epi, 2/30 to the TE and 3/30 to the PrE (ESCs in the TE and PrE did not co-stain with CDX2 and SOX17, respectively).	N.A.	Two chimeric embryos out of six pups from SL-injected embryos. Live-born chimeras were generated following imaging to show that embryos survived time -lapse.	Alexandrova et al. (2016)

Cells used	Media	*In vitro* differentiation assays	Method of chimera generation	Numbers of cells introduced into host embryos	Observed contribution to multiple lineages at the blastocyst stage	Observed contribution to later embryonic/fetal and extra-embryonic lineages	Chimeric offspring	Reference(s)
ESCs (CAG-H2B-Tomato and CAG-Kozac-Venus) derived from F1 129S6;C57BL/6N	2iL and KOSR	Trophoblast and endoderm differentiation	2C embryo injection	Single cells	N.A.	N.A.	Single ESCs grown in 2iL support high contribution chimera formation (up to 100%, 26/87). ESCs grown in KOSR also generate high contribution chimeras (3/8). ESC-derived chimeric mice were judged by coat color, organ composition and germ-line competence.	Martin Gonzalez et al. (2016)
			Morula injection	Single cells (also 5 cells, not included in this table).	N.A.	N.A.	Single ESCs grown in 2iL support high contribution chimera formation (up to 100%, 4/9). ESCs grown in KOSR also generate high contribution chimeras (2/15). ESC-derived chimeric mice were judged by coat color, organ composition and germ-line competence.	
ESCs (EF1α-GFP), PECAM1^{+}, PDGFRA^{+} and double positives)	KOSR	Trophoblast and endoderm differentiation	Blastocyst injection	6-8 cells	N.A.	At E6.5, 4/8 chimeric embryos generated with double-positive cells showed contribution to both Epi and PrE derivatives. 15/18 chimeric embryos generated from PDGFRA^{+}/PECAM1^{-} ESCs showed contribution to PrE derivatives, and 12/12 chimeric embryos from PDGFRA^{-}/PECAM1^{+} ESCs showed contribution to Epi.	N.A.	Nigro et al. (2017)
ESCs (miR34a$^{-/-}$; GFP)	SL and 2iL	Embryoid bodies	Morula and blastocyst injection	4 cells	28/46 blastocysts from miR34a$^{-/-}$ ESCs showed contribution to the ICM and TE, 2/46 showed only TE contribution. Blastocysts from wild-type ESCs only contributed to the ICM (23/23).	N.A.	N.A.	Choi et al. (2017)
				Single cells	21/61 blastocysts from miR34a$^{-/-}$ ESCs showed contribution to the ICM and TE, and 20/61 showed contribution only to the TE.	N.A.	N.A.	
				10-15 cells	N.A.	Chimeras generated with miR34a$^{-/-}$ ESCs had both embryonic and extra-embryonic contribution: 4/10 embryos at E9.5, 5/8 embryos at E12.5 and 12/15 at E14.5. Wild-type ESCs did not contribute to extra-embryonic tissues at any stage. TPBPA and MTP1 staining used to identify trophoblast derivatives.	N.A.	
mEPS cells (LCDM-EPS cells; CAG-tdTomato) and hEPS cells (LCDM-EPS cells; CAG-tdTomato)	LCDM	N.A.	8C embryo injection	Single cells	86/261 blastocysts showed EPS cell contribution to the ICM and TE. Staining for NANOG and OCT4 (ICM), GATA3 and CDX2 (TE). Single mESCs only contributed to the ICM.	Chimeras generated with single EPS cells showed both embryonic and extra-embryonic contribution: 21/90 embryos at E10.5, 10/63 embryos at E12.5 (with one embryo with solely extra-embryonic) and 13/94 embryos at E17.5. CK8, PLF and TPBPA staining used to identify trophoblast derivatives.	59/113 live-born chimeras with high levels of germ-line transmission (>67%).	Yang et al. (2017a)
			Morula and blastocyst injection	10-15 TSC-like and ESC-like EPS cells (derived from embryos injected with a single EPS cell at the 8-cell stage)	N.A.	EPS-ESCs contributed only to the embryos; EPS-TSCs contributed only to placental tissue (at E13.5).	N.A.	
			4N blastocyst injection	Single mEPS cells	N.A.	N.A.	Seven mEPS single cell-derived mice were live-born (out of 311 injected embryos).	
			8C embryo injection	Single hEPS cells	51/345 blastocysts showed hEPS cell contribution to mouse ICM and TE, 24/345 showed hEPS contribution only to the TE, and 43/345 showed only ICM contribution. Human ESCs or primed iPSCs did not contribute to mouse blastocysts (n =143). Staining for NANOG and OCT4 (ICM), GATA3 and CDX2 (TE).	N.A.	N.A.	

Cells used	Media	*In vitro* differentiation assays	Method of chimera generation	Numbers of cells introduced into host embryos	Observed contribution to multiple lineages at the blastocyst stage	Observed contribution to later embryonic/fetal and extra-embryonic lineages	Chimeric offspring	Reference(s)
			Morula and blastocyst injection	10-15 hEPS cells	N.A.	Chimeras generated with hEPS cells at E10.5 showed contribution to the mouse embryo (24/54) or placenta (9/54), or both (6/54).	N.A.	Yang et al. (2017a)
EPSCs (CAG–mCherry, EF-1α-H2B–mCherry or CAG-H2B–mCherry)	EPSC	TSC and XEN cells derived from EPSCs. Additional *in vitro* differentiation to embryonic lineages.	Morula and 8C embryo injection	6-8 cells	9/17 blastocysts had EPSC contribution to both the ICM and TE. 1/17 showed TE contribution only. ESCs showed only ICM contribution and CDX2 staining was used to identify TE.	At E6.5, 78/225 embryos generated with EPSCs showed contribution to both the embryo proper and the extra-embryonic ectoderm. 4/225 showed only extra-embryonic contribution. ELF5 staining was used to identify extra-embryonic ectoderm. At E14.5, chimeras generated from EPSCs showed contribution to the yolk sac and placenta, based on histology (placental staining for TFAP2C). Donor cells were also sorted from chimeric placentas, followed by RT-PCR and staining for specific markers (TFAP2C, GCM1, Ezrin and CK7).	Germ-line contribution from one male chimera.	Yang et al. (2017b)
			8C embryo injection	Single cell	N.A.	At E14.5, 28/190 chimeras were produced from single EPSCs. Some showed contribution to the extra-embryonic lineages.	N.A.	
Blastoids (generated from a combination of ESCs and TSCs)	SL+TX media in microwells	Derivation of ESCs (2iL) and TSCs from blastoids	Blastoid transfer	25 blastoids	N.A.	10% of blastoids generated deciduas (*n* =5 mice). Staining of deciduas and inside structures for CDX2, ELF5, TEAD4, ASCL2, HAND1, PLF and ALDH3A1 at E7.5.	N.A.	Rivron et al. (2018)
			Morula injection of blastoid-derived TSCs and ESCs	12-15 cells	N.A.	At E6.5, chimeras were generated with blastoid-derived ESCs contributing to the Epi and blastoid-derived TSCs contributing to the TE. Placental contribution of blastoid-derived TSCs is also shown at E11.5.	N.A.	
EPS-blastoids (generated from LCDM mEPS cells)	EPS-blastoid media (50%KSOM, 25% N2B27 and 25% TSC) in aggrewells	Derivation of ESCs (2iL), TSCs and XEN cells from EPS-blastoids	EPS-blastoid transfer	20 blastoids	N.A.	7.3% of EPS-blastoids generated deciduas (*n* =300). Analysis of post-implantation stages indicated that 4/9 deciduas contained malformed embryo-like structures. Stained for CK8, GATA4, EOMES and OCT4.	N.A.	Li et al. (2019)
			Blastocyst injection of ESCs, TSCs and XEN cells derived from EPS-blastoids	15 cells	N.A.	In post-implantation embryos, EPS-blastoid-derived TSCs generated chimeric placental tissues (CK8 staining), while EPS-blastoid-derived XEN cells contributed to the yolk sac.	EPS-blastoid-derived ESCs could generate adult chimeric mice, although with low levels of chimerism judged by coat color.	
EPS-blastoids (generated from TSCs and LCDM mEPS cells)	LCDM+TX media in aggrewells	N.A.	EPS-blastoid transfer	N.A.	N.A.	Decidualization assessed 4 days post-transfer. Staining of deciduas and inside structures for PTGS2, Ki67, CDX2, KRT18, PLF, SOX2 and EOMES.	N.A.	Sozen et al. (2019)
ETX-embryoids (generated from a combination of ESCs, TSCs and XENs)	ETX-embryo medium (39% RPMI, 39% DMEM, 17.5% FBS)	N.A.	36 h ETX-embryoid transfer to E3.5 dpc pseudo-pregnant females	20 embryoids	N.A.	87/97 mice contained implantation sites after ETX-embryoid-transfer. 20-30% of ETX-embryoids generated deciduas (*n* =5 experiments). 48 hours post transfer, 2/20 deciduas contained embryo-like structures. Staining of deciduas and inside structures for COX2, PL1, CK, GATA4 and LAMININ.	N.A.	Zhang et al. (2019)
iBLC (generated by reprogramming mEpiSCs that contain a MERVL-RFP reporter)	CTSFES medium (DMEM F12, N2B27 and ascorbic acid)	ESC- and TE-like cells	iBLC transfer	N.A.	N.A.	10/149 iBLCs generated deciduas. 24/186 deciduas formed when iBLCs were co-transferred with blastocysts. Histology of deciduas and staining for PL1, TPBPA and TROMA1.	N.A.	Kime et al. (2019)
ESCs [Dppa2 overexpression (OE) and Pias4 knockout (KO); mRuby2]	SL	N.A.	8C embryo injection	4 cells	16/40 blastocysts showed ESC contribution to the TE and ICM, and 3/40 to the TE only from Dppa2 OE cells. 16/42 blastocyts had dual contribution from Pias4 KO cells. No contribution to the TE was reported for wild-type ESCs (*n* =19).	N.A.	N.A.	Yan et al. (2019)
2CLCs (miR-344-GFP or MERVL-GFP both activated by CRISPR$_{SAM}$)	SL	N.A.	8C embryo injection	Single cells (or up to 3)	11/74 blastocysts had ESC contribution to the TE and ICM from ESCs expressing *miR-344,* with 2/74 exhibiting only TE contribution. 10/66 blastocysts showed ESC contribution to the TE and ICM from ESCs in which MERVL was activated. No TE contribution observed from WT ESC (*n* =66).	At E12.5, chimeras from miR-344- and MERVL-activated ESCs contributed to the placenta (7/11 and 8/11, respectively). Staining for TBPA and PLF was used to identify trophoblast derivatives.	N.A.	Yang et al. (2020)

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ESCs (NELFA-Strep-HA-P2A-EGFP reporter; mCherry), sorting NELFA^{high} and NELFA^{low} ESCs	SL	N.A.	8C embryo or E3.25 blastocyst injection	5-7 cells	12/73 blastocysts showed contribution from NELFA^{high} ESCs to both the ICM and TE. NELFA^{low} cells contributed only to the ICM (n=74). Staining for CDX2 was used to identify the TE.	N.A.	N.A.	Hu et al. (2020)
ESCs (H2B-eGFP)	2iL, EPSC (or L-EPSC), and LCDM (or D-EPSC)	TSC	Morula aggregation	6-8 cells	Approximately 20% of chimeric embryos had both Epi and TE-localized contribution from cells cultured in EPSC media (n=34) and LCDM (n=40). Donor cells did not stain for the TE marker CDX2.	At E6.5, approximately 5% of chimeras derived from cells cultured in 2iL or LCDM media (n=14 and 23, respectively) showed Epi and TE contribution, whereas cells grown in EPSC media had higher levels (20%) of dual lineage contribution (n=26). None of the TE-localized contributing cells expressed either TFAP2C or ELF5 and continued to express OCT4. At E12.5, in chimeras from cells grown in EPSC media, there was no evidence of placental contribution (staining for TFAP2C).	N.A.	Posfai et al. (2020)

This table is not meant to be exhaustive, but includes all reports of ESCs or ESC-like cells with extra-embryonic potential that we are aware of. It includes the experiment type, n values for experiments, stages assessed and types of analysis. Not all experiments in every referenced paper are listed, only those directly relevant to the discussion in this Primer. 2C, 2-cell stage embryo; 2CLCs, 2C-like cells; 2iL, 2iLIF; 4N, tetraploid; Epi, epiblast; EpiSCs, epiblast-stem cells; EPS cells, extended pluripotent stem cells generated in LCDM media; EPSCs, expanded potential stem cells; ESC, embryonic stem cell; hEPS, human EPS; hESCs, human ESCs; iBLC, induced blastocyst-like cysts; ICM, inner cell mass; KOSR, knockout serum replacement; m, mouse; N.A., not assessed; PE, parietal endoderm; PrE, primitive endoderm; SL, serum LIF; TE, trophectoderm; TSC, trophoblast stem cell; TX, TSC media; VE, visceral endoderm; XEN, extra-embryonic endoderm stem cell.