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Supplemental Information

Matched Developmental Timing of Donor Cells with the Host Is Crucial for Chimera Formation

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SUPPLEMENTAL INFORMATION

A. SUPPLEMENTAL DATA:

B. SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

Figure S1:

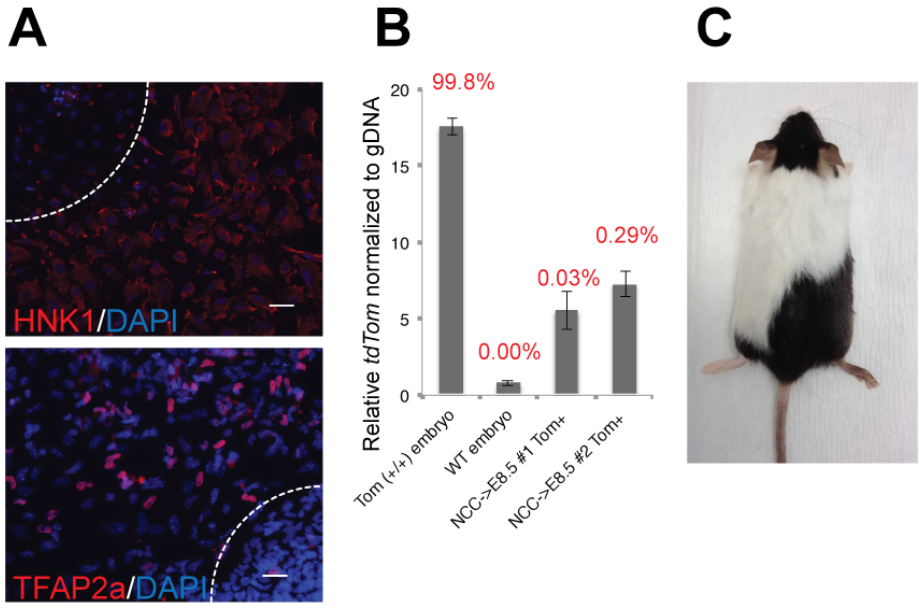

Figure S1. NC chimera formation.

Related to **Figure 1** and **Table 1**.

(**A**) Primary NCCs derived from E8.5 C57Bl/6 mouse embryos. NCCs identity was confirmed by immunostaining. About two 45% of cells were found positive for the NCCs marker HNK-1 and TFAP2a (Scale bar =100 μm; Dashed arches mark the borders of neural tube explants). (**B-C**) NCCs injected into E8.5 host mouse embryos contribute to form chimeras. (**B**) E10.5 embryos were tested for chimeric contribution by FACS and fluorescence positive cells were sorted (% of positive cells are indicated in red) and analyzed for *TdTomato* gene by qPCR for examining chimeric contribution, along with appropriate negative and positive controls, as indicated. For full statistical analysis of injected embryos see **Table 1.** Data are represented as mean ± SD. (**C**) NCCs injected into E8.5 W^{sh}/W^{sh} mouse embryos contribute to form NC chimeras, as indicated by extensive coat color contribution.

Figure S2:

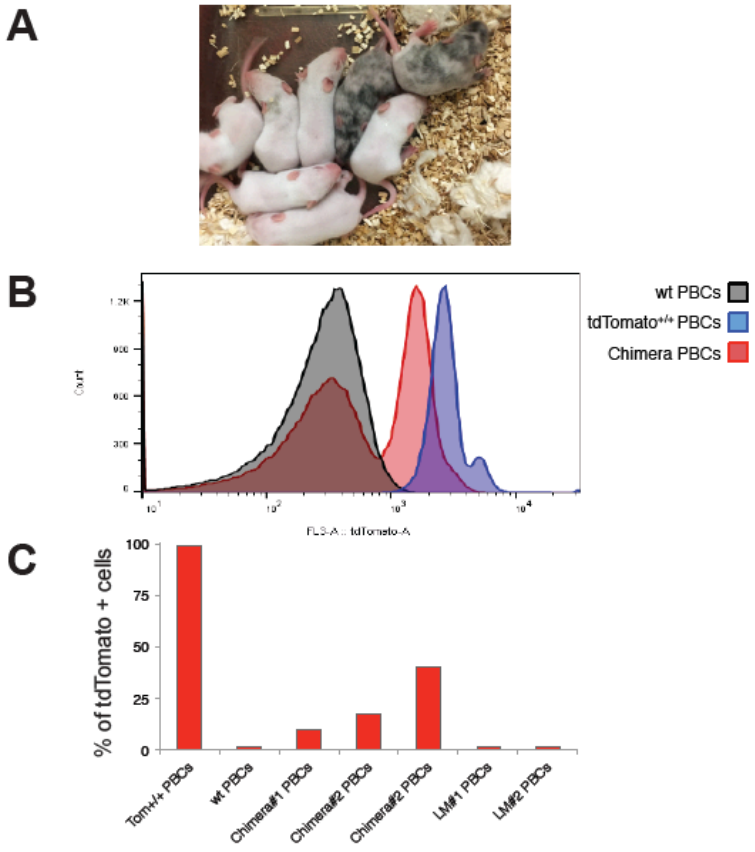

Figure S2. Pluripotent mESCs overexpressing *Bcl2*, rather than *in vitro* differentiated NCCs, contribute to blastocyst chimeras.

Related to **Figure 2** and **Table 1**.

C57Bl/6;*R26*$^{(tdTomato/M2rtTA)}$;FUW-Teto::*Bcl2*-T2A-*PuroR* mESCs differentiated to NCCs were injected into CD1 mouse blastocysts in the presence of Dox (2mg/ml ; E 3.5 - E 9.5) to generate chimeras. (**A**) A representative litter showing some chimeric mice with coat color contribution of donor cells. For full statistical analysis of injected embryos see **Table 1**. (**B**) The identity of donor cells contributing to chimeras upon *Bcl2* overexpression was tested. Thus, we monitored peripheral blood cells (PBCs) from the resulting chimeras and from their littermates, along with appropriate negative and positive controls for tdTomato-fluorescence of by FACS. (**C**) All PBCs derived from chimeras

were found to be chimeric, indicating a pluripotent chimeric contribution to multiple linages, rather a restricted neural crest only contribution.

Figure S3:

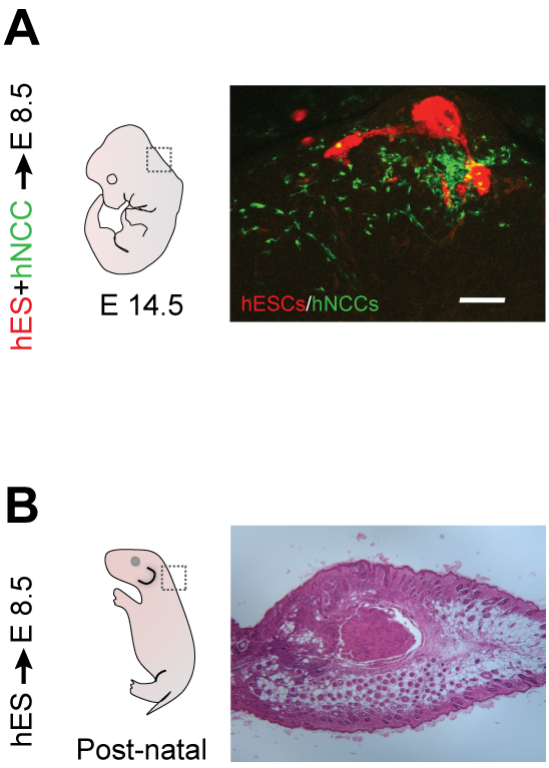

Figure S3. Human ESCs fail to contribute to chimera formation in E8.5 embryos. Related to **Figure 1**.

(**A**) Tomato-labeled hESCs were co-injected with GFP-labeled NCCs into E8.5 host mouse embryos (1:1 ratio). The presence of the donor cells was examined 6 days post-injection. Tomato-labeled hESCs formed clusters of cells whereas GFP-labeled NCCs were found migrating as expected from cell contributing to the host neural crest lineages (Scale bar = 200 μm). (**B**) hESCs injected to E8.5 mouse embryos failed to functionally contribute to chimera formation, but rather formed teratoma outgrowth in postnatal mice. Dashed squares in the illustrations indicate the area within the hosts the cells were found. For full statistical analysis of injected embryos see **Table S1**.

Table S1. Injections of hESCs into E8.5 mouse embryo:

Related to **Figure 1** and **Figure S3**

Cells	# E8.5 embryos injected	# Total embryos dissected	# Embryos fluorescence positive	% Embryos fluorescence positive
hESCs *AAVS1*$^{(CAAGS::GFP)}$	90	81	17	20.98%
Co-injection of hESCs *AAVS1*$^{(CAAGS::tdTomato)}$ + hNCCs *AAVS1*$^{(CAAGS::GFP)}$	44	34	17	50%

Table S1. Injections of hESCs into E8.5 mouse embryo. E 8.5 mouse embryos injected with hESCs or co-injected with hNCCs were isolated between E 9.5-E 14.5. The number of injected and dissected embryos as well as the number of embryos with fluorescent cells are presented. In all fluorescence-positive embryos injected with GFP-hESCs, cell aggregates rather than chimeric contribution were observed. For the embryos co-injected with tdTomato-hESCs and GFP-hNCCs (1:1 ratio), 94.11% of fluorescence-positive embryos were found to be positive for both GFP and tdTomato cells. While NCCs were found migrating and contributing to the host development, hESCs formed cell aggregates (**see Figure S3 A**).

B. SUPPLEMENTAL EXPERIMENTAL PROCEDURES:

1. ES cell line

Blastocysts from a cross between a B6;129Sv/Jae*Col1a1*$^{(CAG\text{-}eGFP)Jae}$ male and a C57Bl/6J female were collected and mESCs were derived, and were termed C57Bl/6;*Col1a1*$^{(GFP)}$. Blastocysts from a cross between a B6;129S6-*Gt(ROSA)26Sor*$^{tm9(CAG\text{-}tdTomato)Hze}$/J (Jax#007905) male and a B6.Cg-*Gt(ROSA)26Sor*$^{tm1(rtTA*M2)Jae}$/J (Jax#006965) female were collected and ES cells were derived. Cre-mediated recombination allowed constitutive expression of tdTomato in the mESCs and their derivatives. Those mESCs were termed C57Bl/6;*R26*$^{(tdTomato/M2rtTA)}$.

2. Antibodies list:

TFAP2a (1:50, # 3B5, DSHB)

HNK-1 (1:100, mouse monoclonal, # CBL519, EMD Millipore)

Alexa Fluor dye conjugated secondary antibodies (1:1000, # A10037, ThermoFisher)

3. Primers list:

tdTomato primers:

Fwd *CAGTTCATGTACGGCTCCAA*; Rev *CCGTCCTCGAAGTTCATCAC*.

UCNE $^{TFAP2A\#463}$ primers:

Fwd *AAC AAT GGG TTC AGC TGC TT*; Rev *CCC AGG CGT ATT TTT GTT CT*.

Bcl2 primers:

Fwd *GAGGATTGTGGCCTTCTTTG;* Rev *GCCGGT TCAGGTACTCAGTC.*

GAPDH primers:

Fwd *AGGTCGGTGTGAACGGATTTG;* Rev *TGTAGACCATGTAGTTGAGGTCA*