Supplemental Figure 1. **Validation of *Ret CFP*-knock-in allele**

Heterozygous Ret *CFP*-knock-in mice (*Ret*$^{CFP/+}$) were obtained by crossing *Ret*$^{flox/+}$ mice with β-actin-Cre mice to examine patterns of RET expression. (A) complete overlap between cells expressing RET protein (red) and those expressing CFP (green) in the P0 trigeminal ganglion, validating CFP expression as a marker to reliably detect Ret-expressing cell populations in mice carrying the Ret CFP-knock-in allele. (B and C) CFP expression was detected in all regions endogenously expressing RET (DRGs, and enteric nervous system shown as examples) at E13.5. Scale bar, 10 μm.

Supplemental Figure 2. ***Ret***$^{CFP/CFP}$**mouse fetuses display an absence of kidney and ENS precursors**

Whereas CFP-fluorescent kidneys and ENS precursors are easily identifiable in *Ret*$^{CFP/+}$ fetuses (A), no functional kidneys (arrows) and ENS develop in *Ret*$^{CFP/CFP}$ fetuses (B, E12.5). Scale bar: 20 μm.

Supplemental Figure 3. **Branching morphogenesis in the kidneys of *Ret*$^{+/CFP}$, *Ret*$^{fl/CFP}$, *Ret*$^{9/CFP}$ fetuses**

Ureteric buds were visualized by CFP fluorescence at E15.5. No obvious changes were detected in the fetuses.

Supplemental Figure 4. **Migration of enteric neural crest cells in the gut of various Ret mutant fetuses.**

Guts dissected from E10.5 $Ret^{+/CFP}$ (A), $Ret^{fl/CFP}$ (B), $Ret^{9/CFP}$ (C), and $Ret^{CFP/CFP}$ (D) fetuses were stained for CFP. In E10.5 $Ret^{+/CFP}$, $Ret^{fl/CFP}$, and $Ret^{9/CFP}$ fetuses, the front of migrating CFP^{+} ENS progenitors (arrowheads) has reached the midgut. By contrast, in the gut of $Ret^{CFP/CFP}$ fetuses, the wavefront of migrating ENCDCs (arrowhead) was located more rostrally, in the foregut. White lines indicate demarcation between the midgut and the hindgut. Fg, foregut; Mg, midgut; Hg, hindgut. Scale bar, 200 μm in (A-F); 20 μm in (G-I).

Supplemental Figure 5. **Migratory behavior of enteric neural crest cells in $Ret^{fl/CFP}$ was indistinguishable from that in wild type fetuses.**

Whole mount Sox10 staining of the gut from wild type (A, B) and $Ret^{fl/CFP}$ (C, D) fetuses at E12.5 (A, C) and E13.5 (B, D). Arrowheads depict the most caudal regions where migrating enteric neural crest cells are found. Whole mount GFP staining of the

gut from *Ret*$^{fl/CFP}$ fetuses at E12.5 (E) and E13.5 (F). No abnormality was detected in the migration of ENCDCs of *Ret*$^{fl/CFP}$ fetuses. In the front of migrating Sox10^{+} cells in *Ret*$^{fl/CFP}$ fetuses, Ret-expression (as revealed by CFP) was confirmed in almost all Sox10^{+} cells (G-I). Mg, midgut; Ce, caecum; Hg, hindgut. Scale bar: 200 μm.

Supplemental Figure 6. Disappearance of CFP^{+} cells in *Ret*$^{9/CFP}$ fetal colon reflects actual cell loss.

After time-lapse observation of E15.5 *Ret*$^{9/CFP}$*gut*, the tissue explant was examined by TuJ1 and Phox2b immunohistochemistry. No TuJ1^{+} enteric neurons were found remaining in the gut regions. Scale bar, 100 μm.

Supplemental Movies 1 and 2

Time-lapse imaging of enteric neurons in the distal colon of E15.5 *Ret*$^{9/CFP}$ fetus. Images were acquired every 10 minutes under minimal light exposure period (less than 100 ms) with low fluorescent light. Progressive loss of GFP-positive enteric neurons was observed (boxed areas). No phototoxic reaction (cell disappearance) was observed in control gut (*Ret*$^{fl/CFP}$, not shown).

Supplemental Table 1 Incidence and severity of impaired colonization by ENCCs in *Ret*$^{9/CFP}$ embryos

Phenotype (E15.5)	*Ret*$^{9/CFP}$	
	Males	Females
Normal	9	7
Colonic hypoganglionosis	4	1
Colonic aganglionosis	7	7
Incidence (%)	55.0	53.3

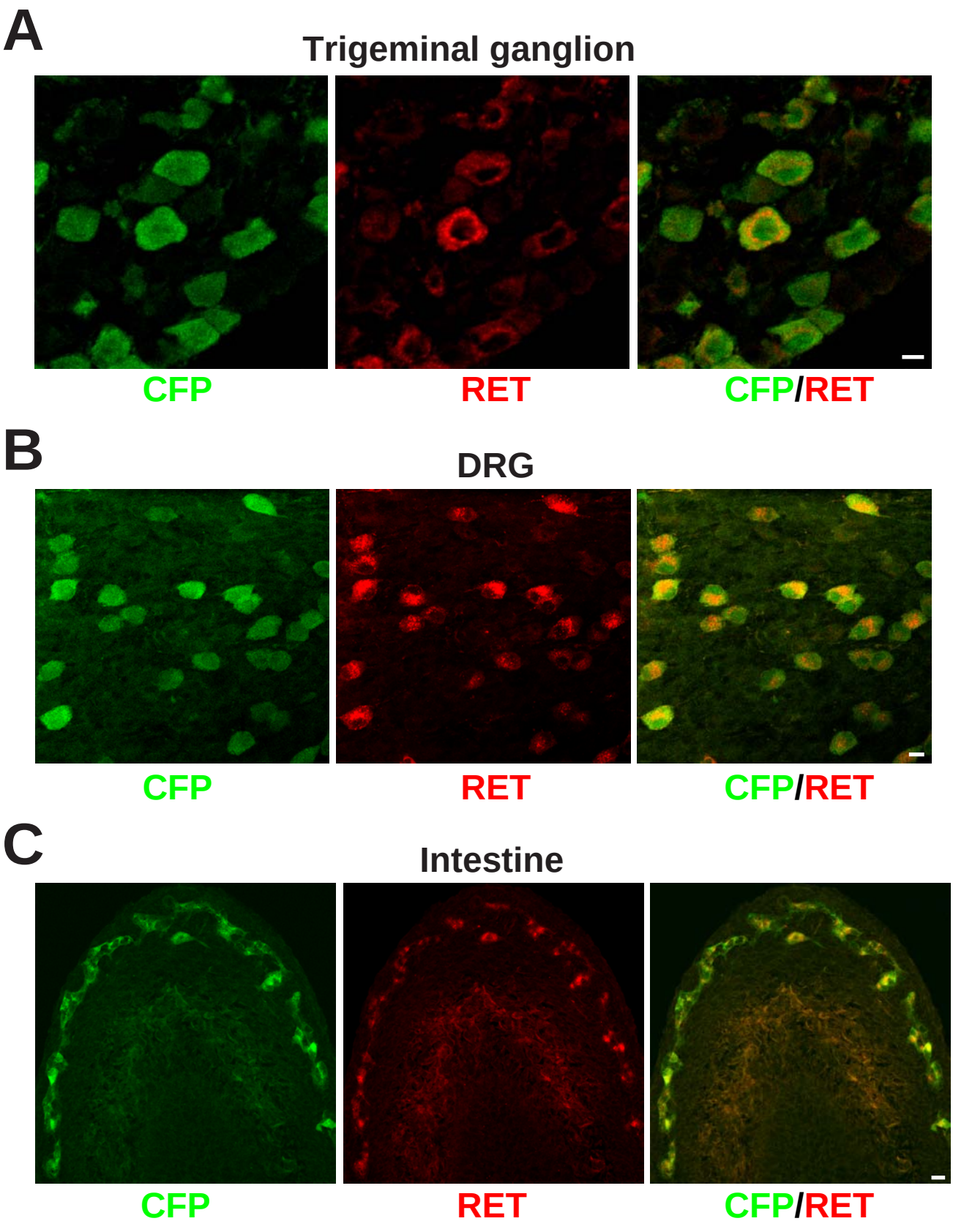
The term ‘colonic hypoganglionosis’ identifies embryos displaying more sparsely distributed ENCCs.
The term ‘colonic aganglionosis’ identifies embryos displaying either total colonic aganglionosis or distal colonic aganglionosis.

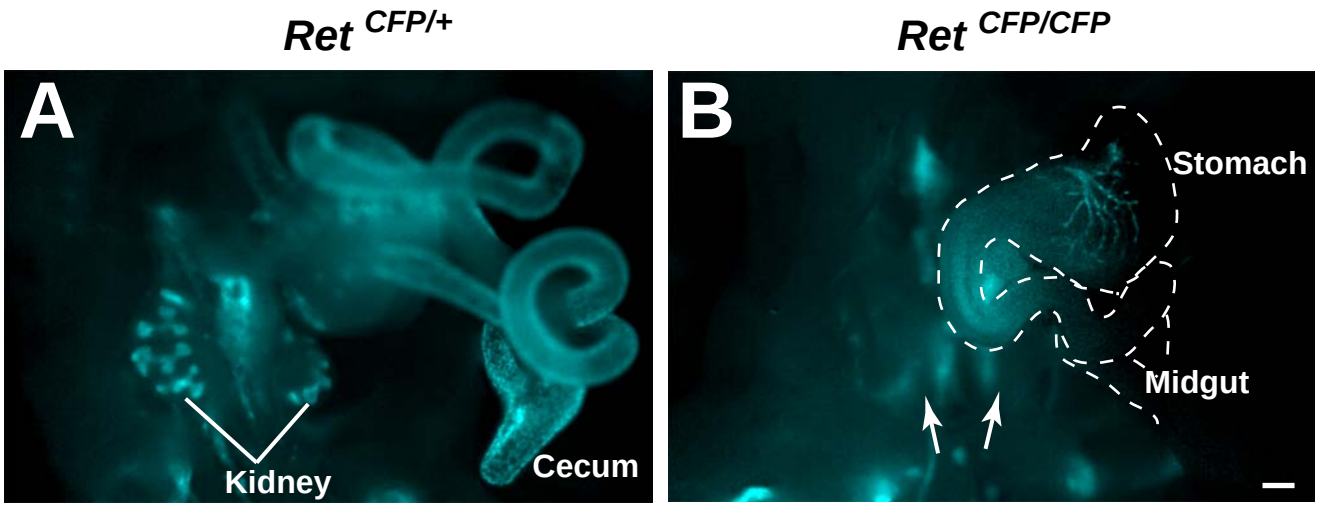
Supplemental Table 2 Primary antibodies used for immunostaining

Antibody	Host	Source	Dilution
GFP	Chicken	Aves Labs	1:1000
RET	Goat	Neuromics	1:1000
PGP9.5	Rabbit	Ultra Clone	1:500
Neurofilament 200kDa	Rabbit	Millipore	1:250
TuJ1	Mouse	Covance	1:500
Phox2b	Rabbit	Brunet JF. (Pattyn et al., 1997)	1:500

Reference

Pattyn, A., Morin, X., Cremer, H., Goridis, C. and Brunet, J.F. (1997) Expression and interactions of the two closely related homeobox genes Phox2a and Phox2b during neurogenesis. *Development* 124, 4065-4075.


A
Trigeminal ganglion
CFP
RET
CFP/RET
B
DRG
CFP
RET
CFP/RET
C
Intestine
CFP
RET
CFP/RET


Ret CFP/+
Ret CFP/CFP
A
B
Kidney
Cecum
Stomach
Midgut

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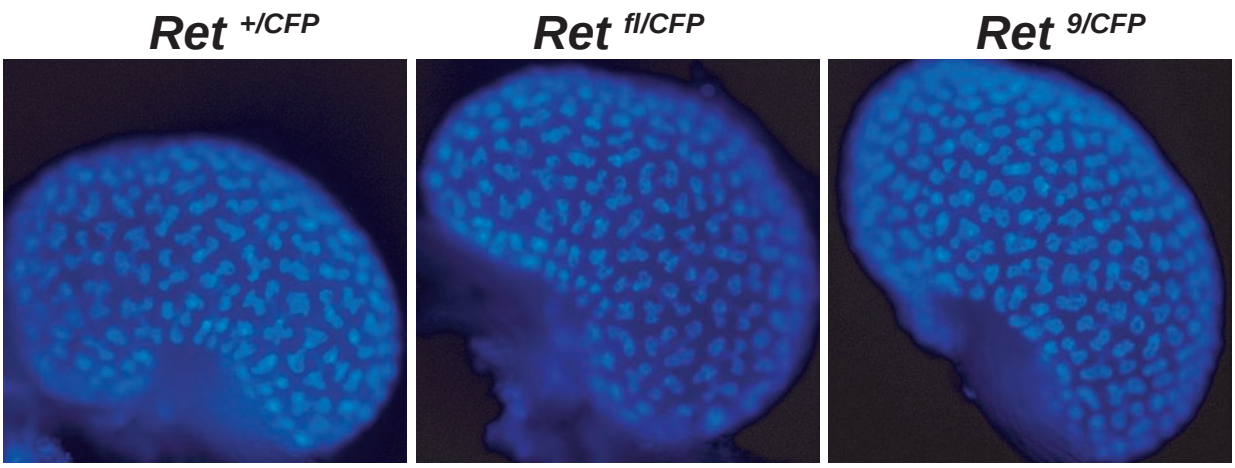
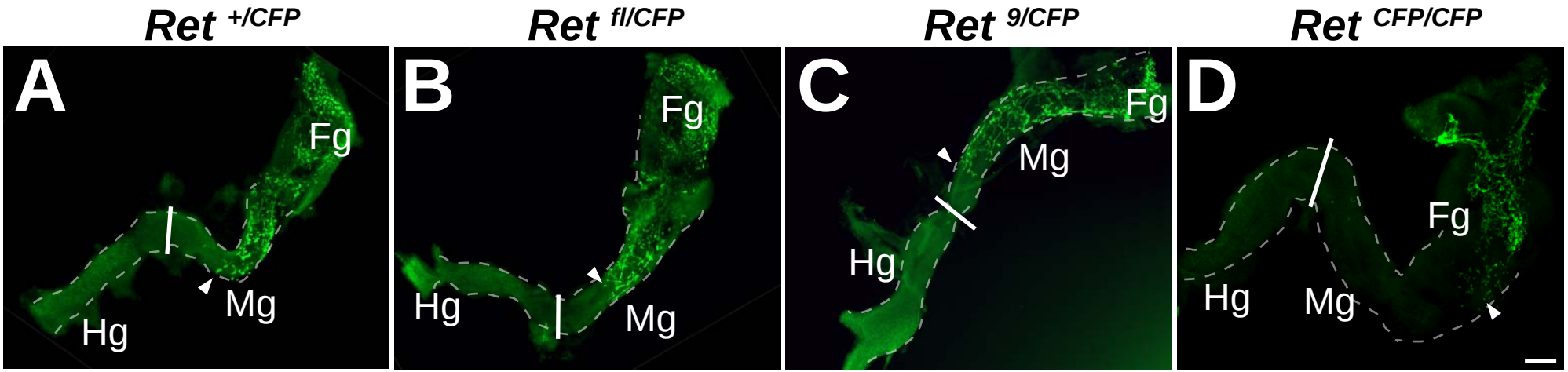
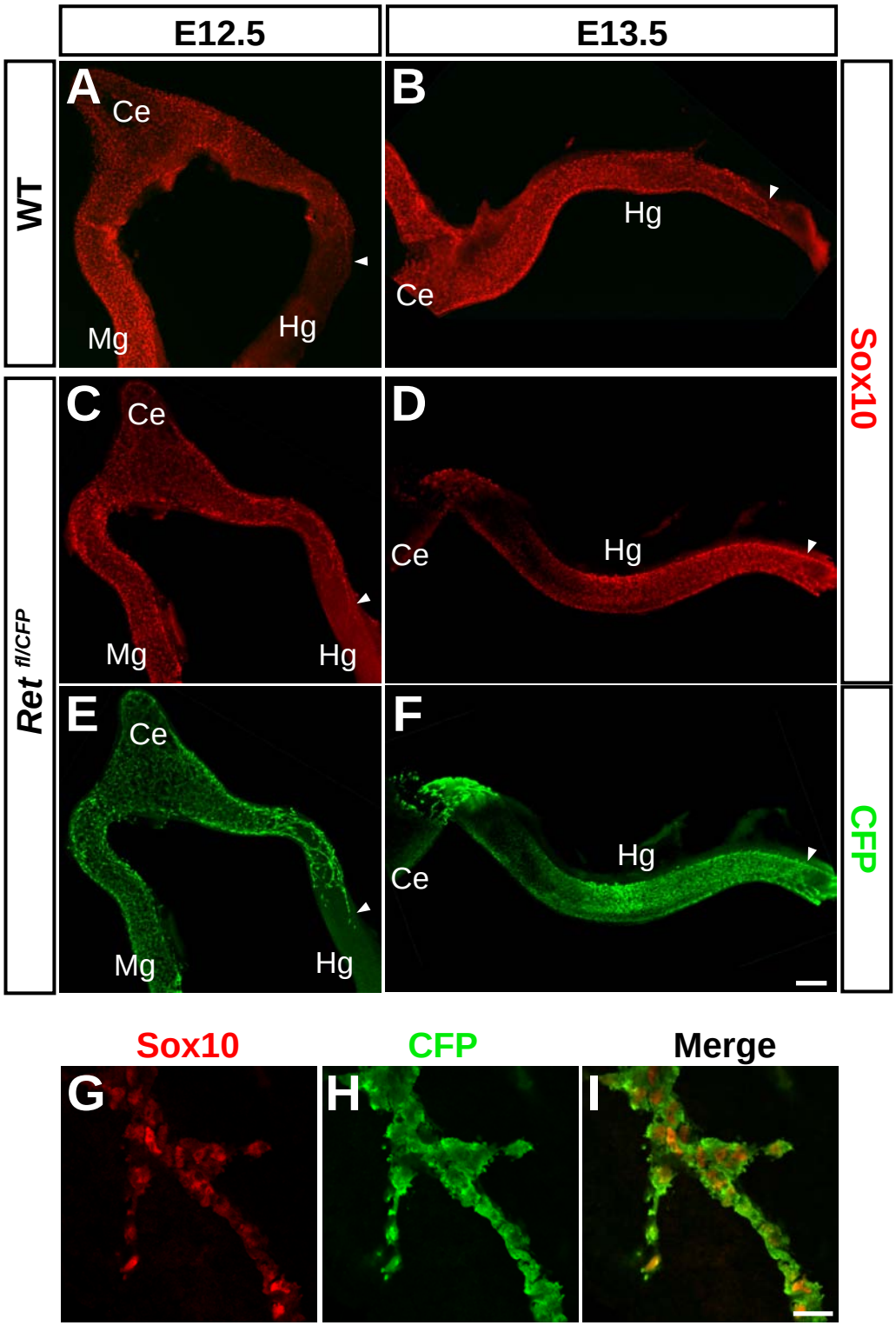



Ret +/CFP
Ret fl/CFP
Ret 9/CFP
Ret CFP/CFP
A
B
C
D
Fg
Mg
Hg
Fg
Mg
Hg
Fg
Mg
Hg
Fg
Mg
Hg


E12.5
E13.5
WT
Ret fl/CFP
Sox10
CFP
A
Ce
Mg
Hg
B
Hg
Ce
C
Ce
Mg
Hg
D
Ce
Hg
E
Ce
Mg
Hg
F
Hg
Ce
Sox10
CFP
Merge
G
H
I

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