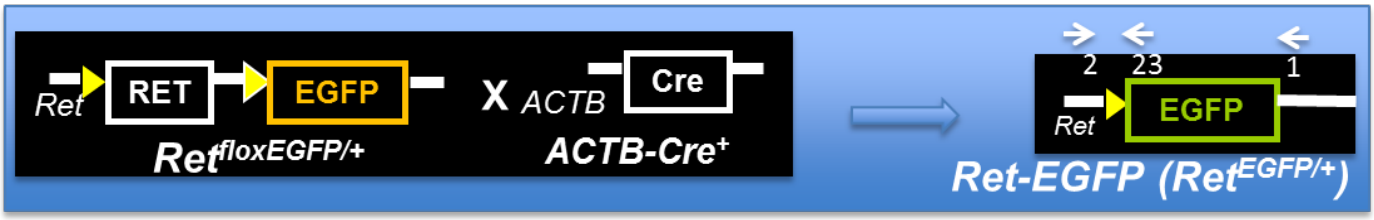
Characterization and Analysis of Ret-EGFP Reporter Mice (Jain lab, Washington University, St. Louis)

Background. RET is a receptor tyrosine kinase that is activated upon binding to a complex formed by one of the four members of the glial cell line-derived neurotrophic factor family ligands (GFLs: GDNF, NRTN, ARTN, PSPN) and GFRalpha coreceptors (GFRa1-4) [1]. *RET* mutations result in a number of defects and diseases in humans and rodents including maldevelopment of the sympathetic, parasympathetic, sensory and enteric nervous systems, kidneys, ureters and testes. The human developmental diseases with causative *RET* mutations include Hirschsprung disease (intestinal aganglionosis), hereditary oncogenic syndromes (MEN2A, 2B) and CAKUT. CAKUT in mice or humans with RET defects include renal agenesis, hypoplasia, dysplasia, duplication, megaureter and vesicoureteral reflux. In the developing urinary system Ret expression is reported in the Wolffian duct (nephric duct), common nephric duct (CND), ureteric bud, UB tips. In the peripheral nervous system Ret is expressed in sympathetic, parasympathetic, enteric, sensory and motor neurons.

Strain Information.
Strain name: Ret-EGFP
Background: C57B6
Reporter: enhanced green fluorescent reporter (EGFP)
Locus: Ret
Alteration at locus: knockin-knockout at the *Ret* locus

Description: The *Ret-EGFP* reporter mice were derived from *Ret* conditional reporter mice (*RetfloxEGFP)* that express floxed human RETcDNA followed by the EGFP reporter ($Ret^{tm13.1Jmi}$; MGI:3691588) [2,3]. The engineered targeting construct was introduced in the first exon by homologous recombination thereby disrupting endogenous *Ret* expression and instead expressing the RETfloxedEGFP under the control of the native mouse *Ret* locus. RetfloxEGFP mice were bred with ACTB-Cre mice to excise the floxed RET cDNA from the germline thereby positioning EGFP reporter directly under the control of *Ret* promoter resulting in strong expression in all tissues known to express Ret [2-4]. The resulting Ret-EGFP mice were backcrossed with C57B6 mice for more than 15 generations and the Cre allele was crossed out. Selected data are shown below and more details can be found in the above papers.



Genotyping: Ret-EGFP mice

CAGCTACCCGCAGCGACCCGGTTC		1		WT Reverse (Intron 1)
CAGCGCAGGTCTCTCATCAGTACCGCA		2		Forward (Ret Promoter)
WT PCR				
Rxn volume	25			
		1X		
10X Buffer (25mM MgCl2)		2.5	10	**94 hold 4 min**
10 mM dNTP (.08 mM final)		0.5	2	
DMSO		1.25	5	**94, 45s; 60, 45s; 72, 45s**
Primer **1** (10 uM)		1	4	**35 cycles**
Primer **2** (10 uM)		1	4	
Taq		0.2	0.8	**72 hold 5 min**
Water		16.55	68.2	
DNA		2		**WT band ~300bp**

CAGCGCAGGTCTCTCATCAGTACCGCA		2		Forward (Ret Promoter)
GCCGTTTACGTCGCCGTCCAGCTCGACCAG		23		Reverse (EGFP cDNA)
EGFP ret removed				
Rxn volume	25			
		1X	4X	
10X Buffer		2.5	10	**94 hold 4 min**
10 mM dNTP		0.5	2	
DMSO		1.25	5	**94, 45s; 60, 45s; 72, 45s**
Primer **2** (10 uM)		1	4	**35 cycles**
Primer **23** (10 uM)		1	4	
Taq		0.2	0.8	**72 hold 5 min**
Water		16.55	66.2	
DNA		2		**Band ~300bp**

Expression of EGFP in Ret-expressing tissue

Live EGFP expression in whole Ret-EGFP embryos. Live detection of EGFP signal in E11.5 Ret-EGFP embryo shows strong EGFP expression (denotes Ret expression) in known sites of Ret expression (Fig. 1).

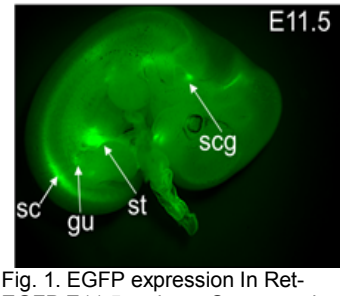


Fig. 1. EGFP expression In Ret-EGFP E11.5 embryo. Scg, superior cervical ganglion; gu, genitourinary; st, stomach; sc, spinal cord.

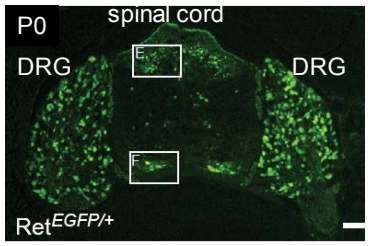


Fig. 2. EGFP expression In all sensory neurons in dorsal root ganglia (DRG) and their projections in the spinal cord (E, white box) and in motor neurons (F, white box).

EGFP expression in sensory and motor neurons. Live detection of fluorescence signal from Ret-EGFP mice from a new born mouse (P0) showing expression in sensory and motor neurons (Fig. 2).

EGFP expression in early stages of developing urinary system and sensory neurons in Ret-EGFP mice. Whole mount detection of EGFP signal using antiEGFP antibodies shows appearance of Ret expression (green) in DRGs as early as E9.5 along with strong expression in the Wolffian duct (WD) (Fig. 3). At E10.5 strong Ret expression is visualized in the WD especially the caudalmost part where the UB will emerge. Note expression in caudal DRGs (white dashed circles). At E13.5, note exact overlap between EGFP and antiRet immunostaining signal in DRGS indicating EGFP recapitulates Ret expression in Ret-EGFP mice.

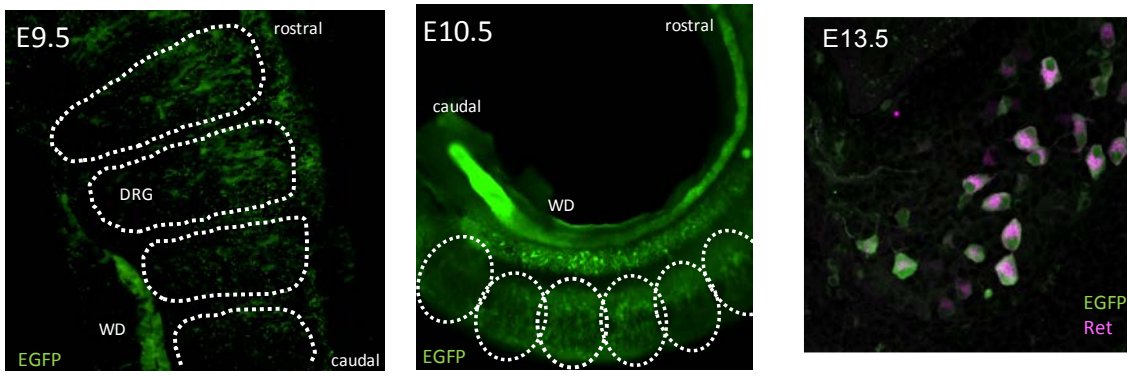


Fig. 3. Early embryonic expression of EGFP in DRG sensory neurons and Wolffian duct (WD) in Ret-EGFP mice using confocal microscopy.

Ret expression (EGFP signal) in the developing urinary system in Ret-EGFP mice (Fig. 4).

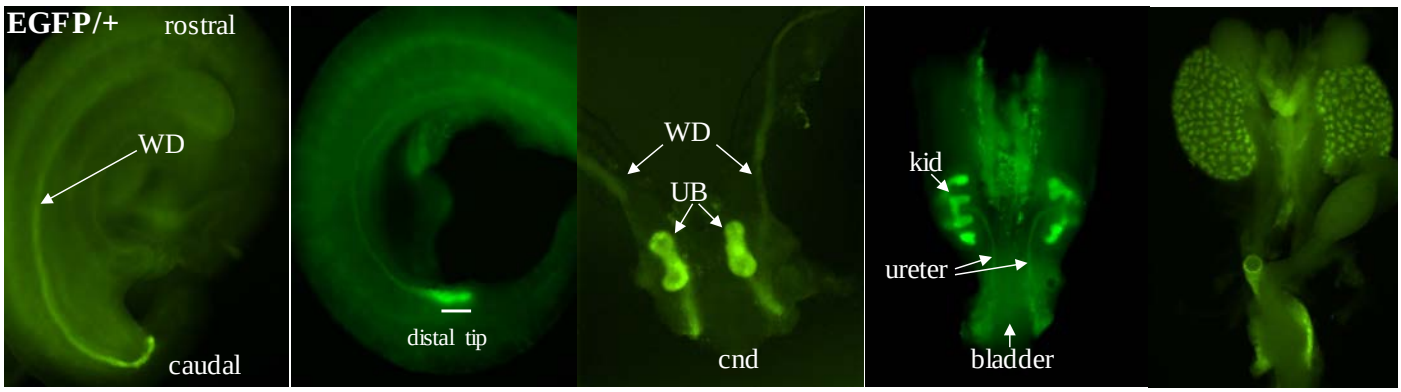


Fig. 4. Whole mount EGFP immunofluorescence signal denotes sites of Ret expression in Ret-EGFP mice. WD, Wolffin duct; UB, ureteric bud; cnd, common nephric duct.

REFERENCES

1. Jain, S. The many faces of RET dysfunction in kidney. *Organogenesis* **5**, 1-14 (2009).
2. Golden, J.P., Hoshi, M., Nassar, M.A., Enomoto, H., Wood, J.N., Milbrandt, J., Gereau IV, R.W., Johnson, Jr., E.M., Jain, S. RET Signaling is Required for Survival and Normal Function of Non-Peptidergic Nociceptors. *Journal of Neuroscience* **30**, 3983-3994 (2010).
3. Jain, S. *et al.* RET Is Dispensable for Maintenance of Midbrain Dopaminergic Neurons in Adult Mice. *J. Neurosci.* **26**, 11230-11238 (2006).
4. Hoshi, M., Batourina, E., Mendelsohn, C. & Jain, S. Novel mechanisms of early upper and lower urinary tract patterning regulated by RetY1015 docking tyrosine in mice. *Development* **139**, 2405-15 (2012).