

4% PFA fixed brain tissue
free-floating sections

		1	2	3	4
Day 1	1. 60 µm sections				
	2. wash in PBS, 4 x 10min				
	3. heat mediated antigen retrieval in citrate buffer (citrate buffer: 10mM sodium citrate buffer, pH 6.0, adjust with citric acid)				
	3a. 5min in 10mM citrate buffer, pH 6.0				
	3b. 3h in 10mM citrate buffer, pH 6.0 at 80°C (water bath)				
	3c. let sections cool down in citrate buffer, 30min at RT				
	4. blocking step: 2h, room temperature, on a shaker (blocking solution: PBS, 0.5% Triton X-100, 10% goat serum)	1			
	5. rinse briefly in PBS, 1x				
	6. primary antibody rat CTIP2 (rat anti-CTIP2 , #ab18465), 1:1000 in carrier solution primary antibody mouse SATB2 (mouse anti-SATB2 , #ab51502), 1:200 in carrier solution (carrier solution: PBS, 0.5% Triton X-100, 1% goat serum)				
	incubate over night, room temperature, on a shaker				
Day 2	1. rinse in PBS, 4x 10min	1	2	3	4
	2. secondary antibody anti-mouse 568 or 488 (highly cross absorbed), 1:750 in carrier solution secondary antibody anti-rat 647, 1:750 in carrier solution (carrier solution: PBS, 0.5% Triton X-100, 1% goat serum)				
	incubate for 6h, room temperature, on a shaker, in the dark				
	3. rinse in PBS, 4x 10min				
	4. mount sections on object slides, air dry sections, coverslip using Vectashield mounting medium store mounted sections at 4°C				