Parallel states of pathological Wnt signaling in neonatal brain injury and colon cancer

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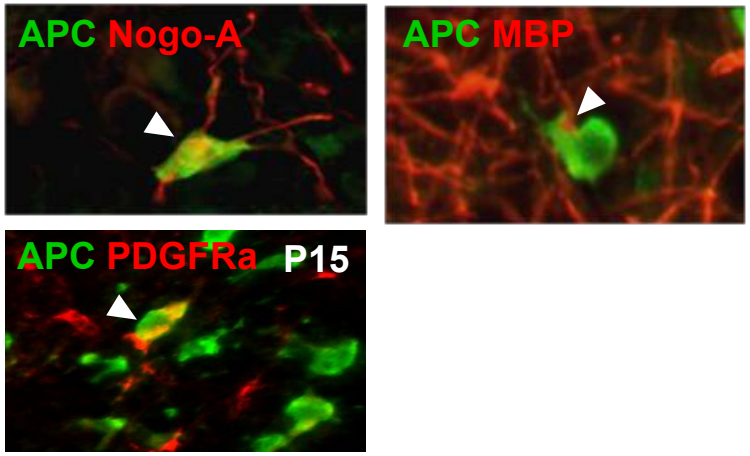
Supplementary Table 1

	DACat4Day	cancer_MSI_CIM_	cancer_Invasive_	cancer_CIN_	ratio of publications up vs down
NKD1	2.15326961	-1.187492958	0.268234973	0.735827652	1.8
SPS	1.35164388	-0.179730047	0.135237705	-0.56084375	1.8
RNF43	1.27681478	0.925871831	1.44855082	1.693268182	30.0
CXCR4	1.13879569	0.003253521	-1.119980874	-1.242996212	2.6
TNFRSF19	1.05399847	-3.376295305	-3.092336612	-3.3230375	2.4
AXIN2	1.05155404	2.002986385	3.353209836	3.361271591	1.7
ALDH1A1	0.87498347	1.246887324	0.917912568	0.578806818	7.3
HMGA2	0.81217225	-2.132453521	-2.257460656	-1.852943182	2.2
APCDD1	0.7292846	1.819406103	2.855081967	2.998541667	2.4
FBLN1	0.72676277	0.20286513	-0.068401366	0.232103134	10.0
LEF1	0.69896825	-2.16553662	-2.270909836	-2.302727727	2.3
ST8SIA2	0.64613851	0.471557512	0.464635246	0.337263258	80.0
DUSP4	0.6103816	-0.450434272	-1.255248634	-2.000073864	2.7
DACT1	0.57239755	1.576161972	1.01368306	0.916638258	10.0
CASP3	0.54810699	0.696852113	0.63481694	0.534070076	3.6
FOXO1	0.53022814	0.956692488	0.522325137	0.936318182	2.4
NPY	0.52115917	-1.574400235	-1.424512295	-1.393332386	1.9
LCK	0.50406345	-1.004746479	-1.338377049	-1.923738636	4.8
BMP7	0.50390832	-1.252988156	-0.550494536	-0.650221935	10.0
TOM1L1	0.48764817	0.554816901	0.548622951	0.334	10.0
GLI1	0.46906487	-0.127306338	-0.564987705	-0.128786932	2.5
ARG2	0.46265141	-1.222792254	-1.135512295	-1.364207386	20.0
WNK2	0.46007029	0.669385563	1.001493852	0.90227983	10.0
GSG1L	0.45056407	-0.561400939	-0.48997541	-0.369286364	10.0
MEG3	0.43609079	-1.248426761	-1.498690164	-1.220784091	10.0
CSNK1E	0.42580986	-1.029188967	-0.836928962	-0.742447917	2.7
PDLIM4	0.42524529	-1.355130282	-1.937959016	-1.533644886	3.0
L1CAM	0.42437331	-1.183111033	-1.358076685	-1.126030429	4.5
DLG4	0.40905682	0.209431925	0.107387978	-0.002647727	10.0
EPHB2	0.40768401	2.984338028	3.670614754	3.436107955	2.9
CDC42EP4	0.40687513	0.320133803	0.280691803	0.197705682	10.0
ZMYM3	0.40660858	-0.977265845	-0.952178279	-0.912524148	10.0
IGFBP5	0.40522098	-0.101815064	-0.988356379	-0.483596344	10.0
CDKSR1	0.40212059	-0.113606103	-0.220713115	-0.061015909	3.3
NLN	0.39856784	-0.358211268	-0.315968306	-0.287456944	30.0
ETS2	0.39089648	2.384816901	2.826502342	2.696047078	1.8
PITX2	0.3879029	2.411637324	3.142163934	3.189880682	40.0
RSPO3	0.38527584	3.905366197	2.395878142	2.49806553	10.0
AHI1	0.38320287	-0.176198357	-0.052740437	-0.071079545	10.0
FOXP1	0.37856102	0.830501761	0.574877049	0.46537642	10.0
SBK1	0.37324111	-0.008881455	-0.099061475	-0.024214962	10.0
EPHA3	0.36349988	-1.173481534	-1.269153734	-1.06981089	3.3
IKZF4	0.36260017	0.053894366	0.01917418	0.04003125	1.8
CDKN1A	0.36253611	0.720468813	0.849758782	0.376497565	2.6
EYA1	0.34542799	-2.788622066	-2.118102186	-1.792515152	10.0
MAF	0.33229367	-1.402315336	-2.194112022	-1.804558712	2.4
MERTK	0.32761097	0.271257042	-0.02077459	0.397019886	2.6

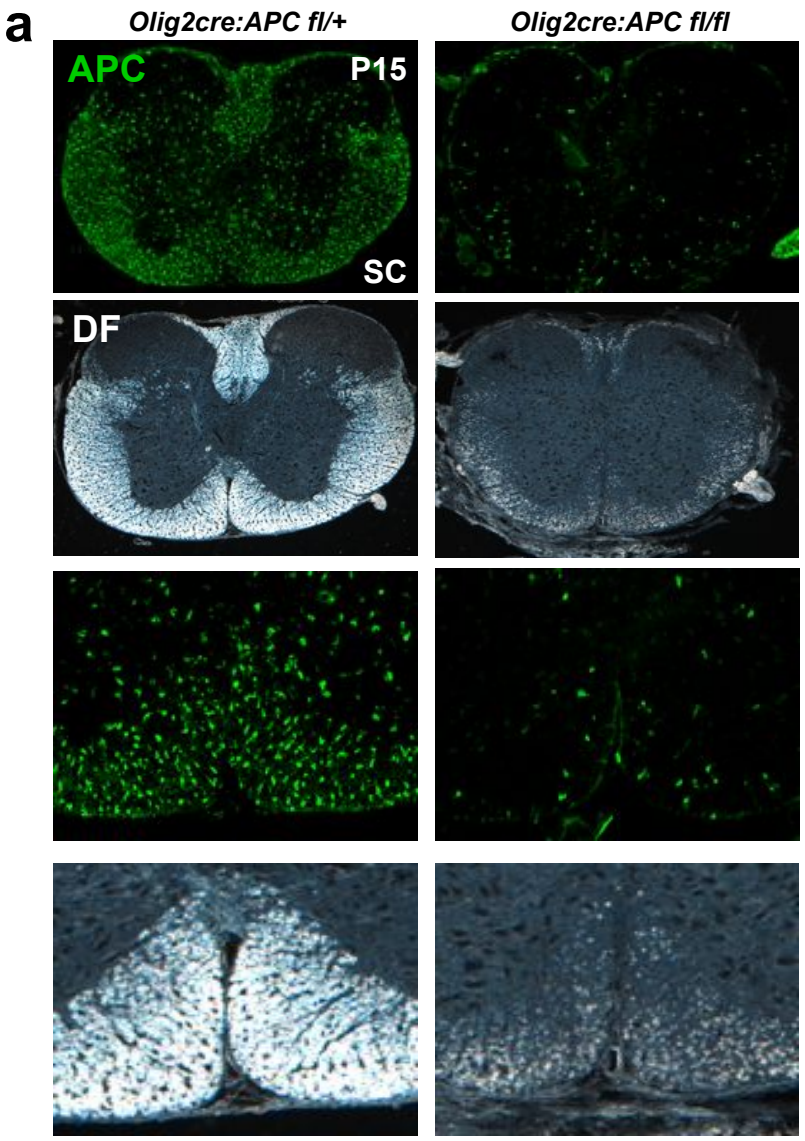
Suppl. Table 1: Comparison of transcripts upregulated in Wnt-activated OPCs and colon cancer. To define markers for a putative high activity Wnt signaling state across various tissues, we compared mRNA transcripts upregulated in P4 mouse spinal cord (DACat4Day) Wnt-activated OPCs (Olig2cre/DA-Cat: (15, *Fancy et al. 2009 Genes and Development*)(Microarray data are available on the GEO website, GSE19403) and human colon carcinomas (3, *Nature 2012*). We retrieved log(2) fold-change expression values from a previous study using Olig2-Cre/DA-Cat mice (15, *Fancy et al. Genes and Dev. 2009*) and another on colon cancer RNA profiling of three colon cancer categories MSI, invasive, and CIM (3, *Nature, 2012*) (MSI= microsatellite instablity, CIM= CpG island methylation). Transcripts elevated in either data set or with a positive normalized "domain knowledge score" (DKS) were selected. Normalized DKS was defined as the ratio of the number of articles in PubMed supporting upregulation vs. downregulation of each transcript in colon cancer.

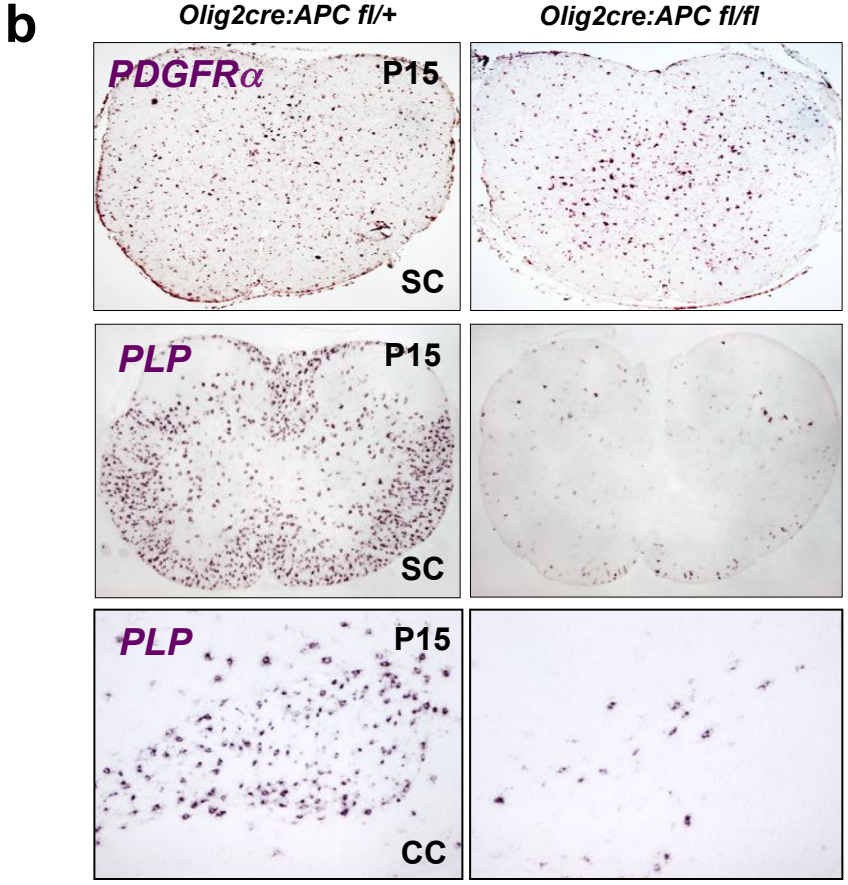
Supplementary Fig. 1



Suppl. Fig.1: APC expression in oligodendrocyte lineage. In the adult mammalian CNS, *Adenomatous polyposis coli* (APC) expression is specific to mature oligodendrocytes, and APC protein co-localizes with mature oligodendrocyte markers Nogo-A and MBP. During murine developmental myelination, APC is one of the earliest markers that an OPC is beginning to differentiate, and can be seen to co-localize on occasion with PDGFRα in OPCs.

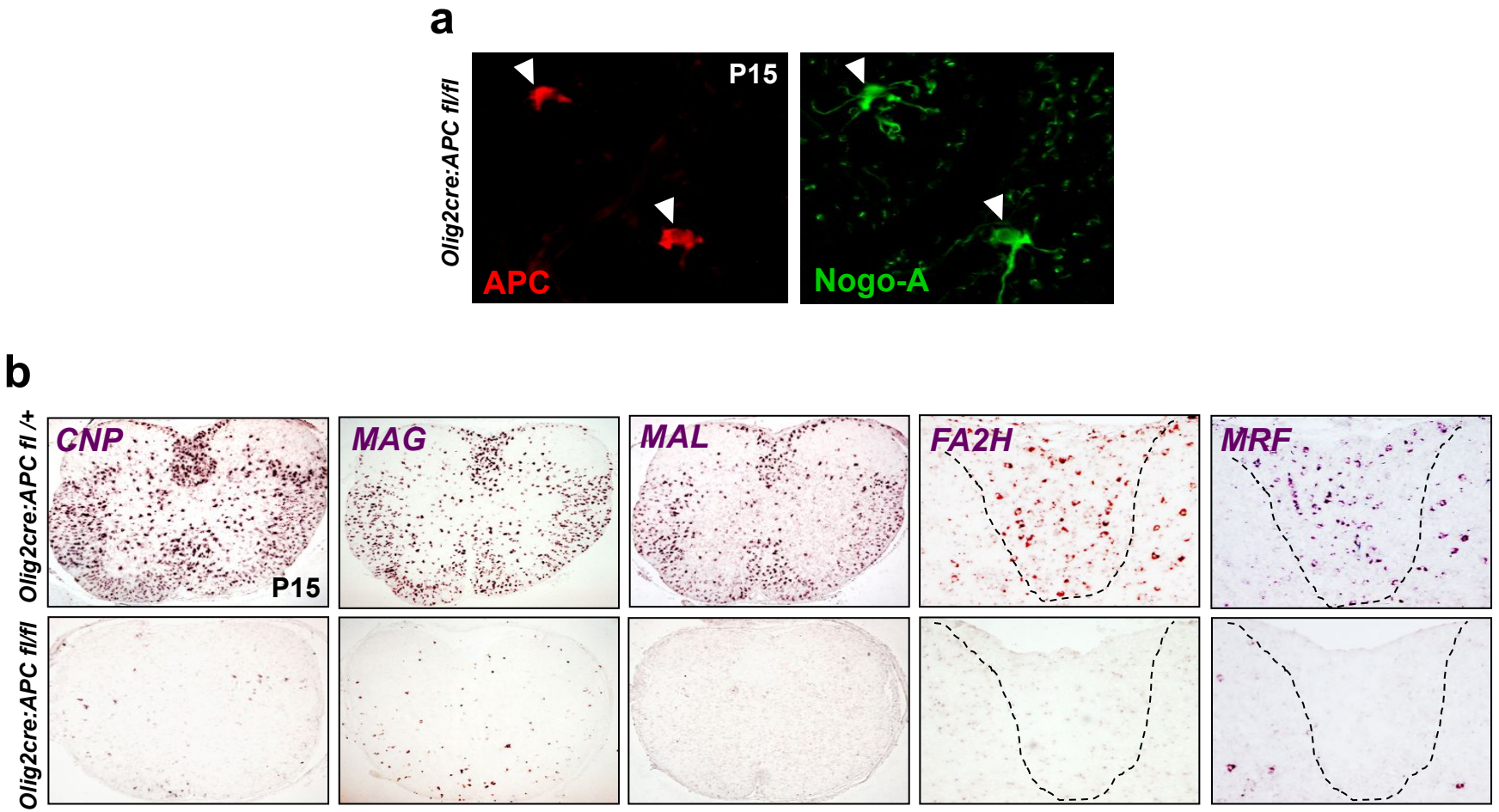
Supplementary Fig. 2





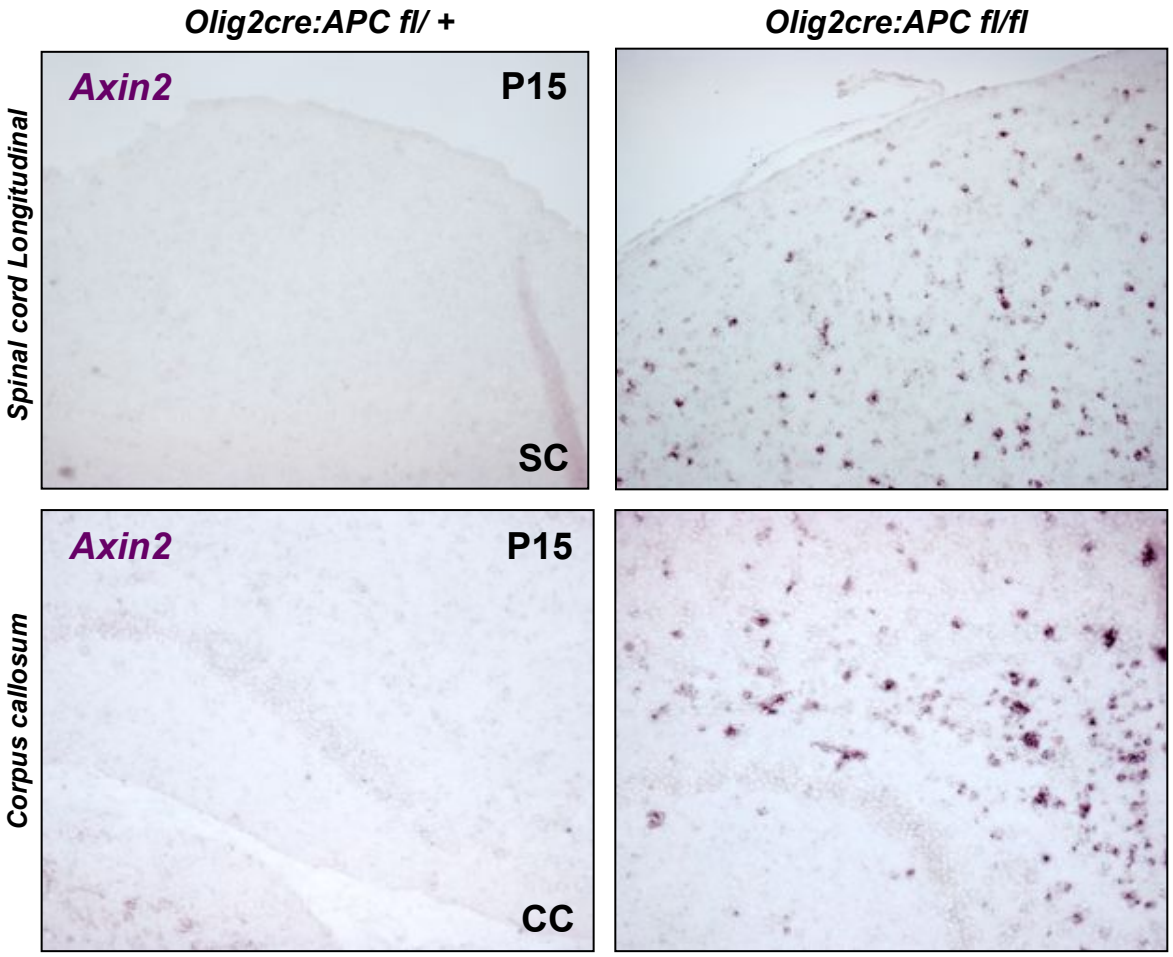
Suppl. Fig.2: Loss of APC causes significant deficits in developmental myelination. (a) Generation of Olig2cre: APCfl/fl mice resulted in ~85% efficiency of APC protein loss in OPCs and ~15% "escapees" that failed to delete APC in P15 developing spinal cord (SC) which lead to a severe hypomyelination as seen on dark field (DF) compared to control Olig2cre: APCfl/+ littermates. (b) While numbers of OPCs expressing PDGFRα were normal in spinal cord (SC) of Olig2cre: APCfl/fl at P15, APC-deficient OPCs showed differentiation arrest and failed to express mature oligodendrocyte marker *PLP* in SC or corpus callosum (CC) at P15.

Supplementary Fig. 3



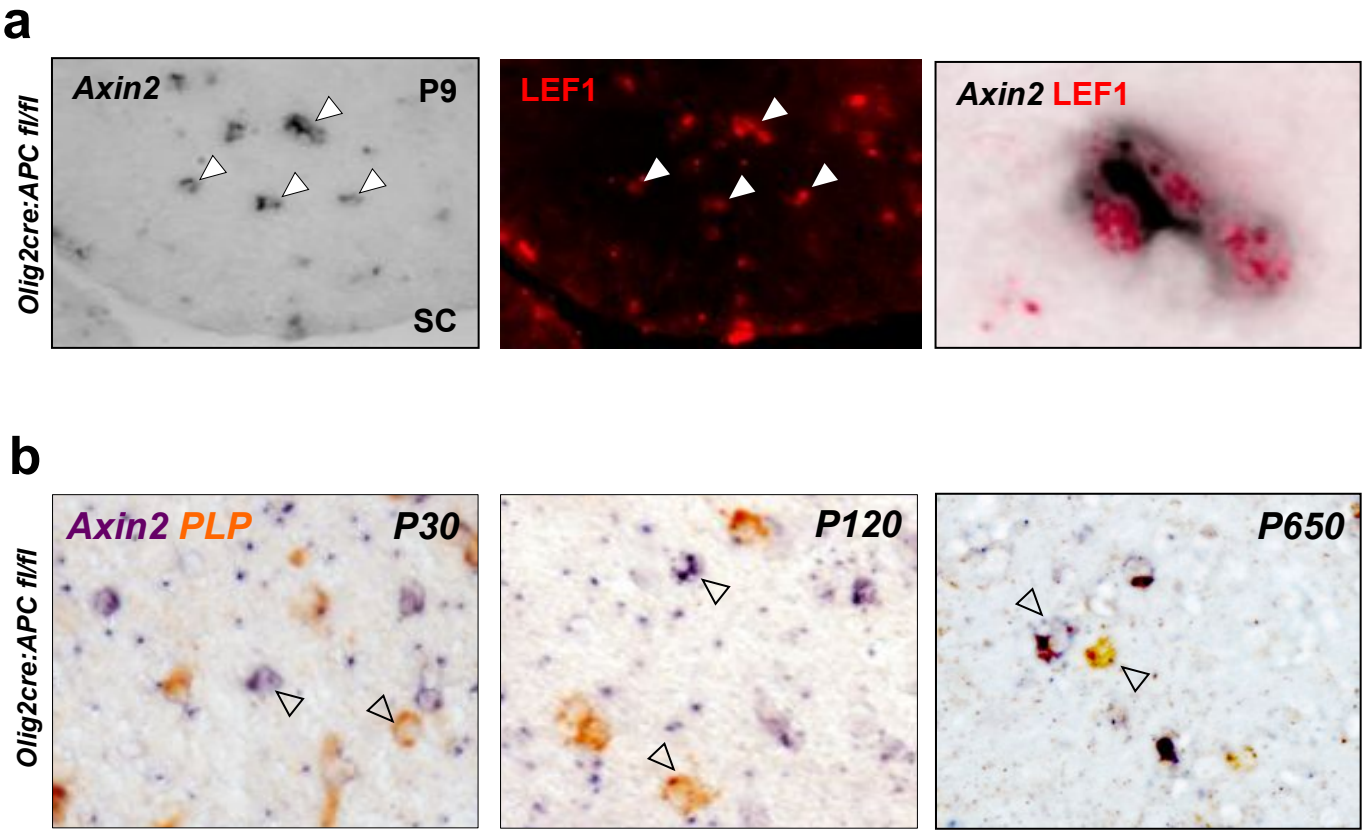
Suppl. Fig.3: Loss of APC causes OPC differentiation failure. APC-deficient OPCs showed maturation arrest and failed to express the mature oligodendrocyte genes, myelin regulatory factor (*MRF*), or the mature oligodendrocyte markers *Cnp, Mag*, *Mal and Fa2h* at P15 in spinal cord of Olig2cre: APCfl/fl mice (b).
(a) Only "escapees" of Olig2-cre activity that expressed APC were capable of differentiation to NOGO-A-positive mature oligodendrocytes.

Supplementary Fig. 4



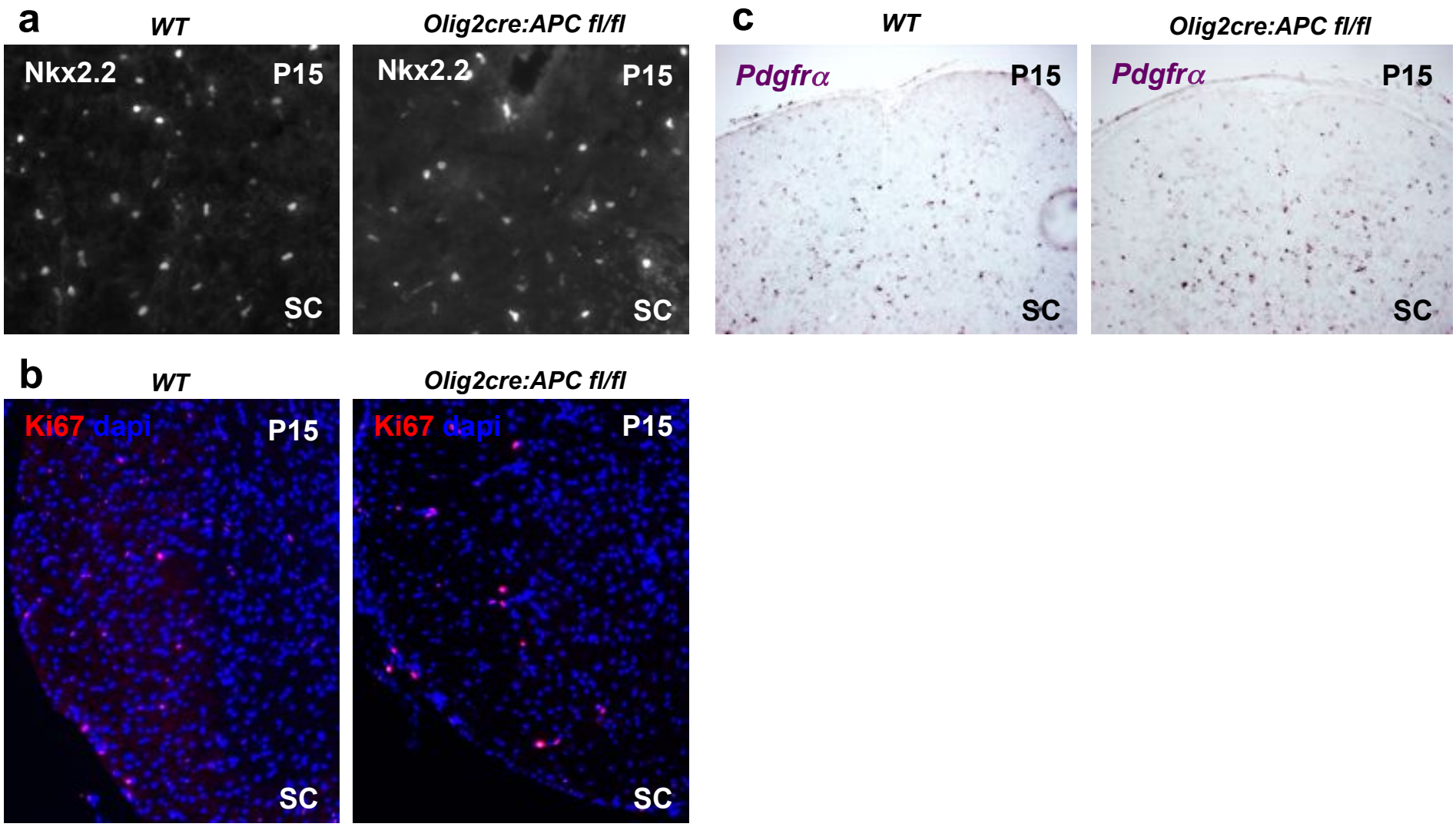
Suppl. Fig.4: APC-deficient OPCs expressed greatly elevated levels of the Wnt transcriptional target Axin2. This can be seen at P15 in spinal cord (SC) and corpus callosum (CC) in Olig2cre: APCfl/fl mice compared to Olig2cre: APCfl/+ littermates.

Supplementary Fig. 5



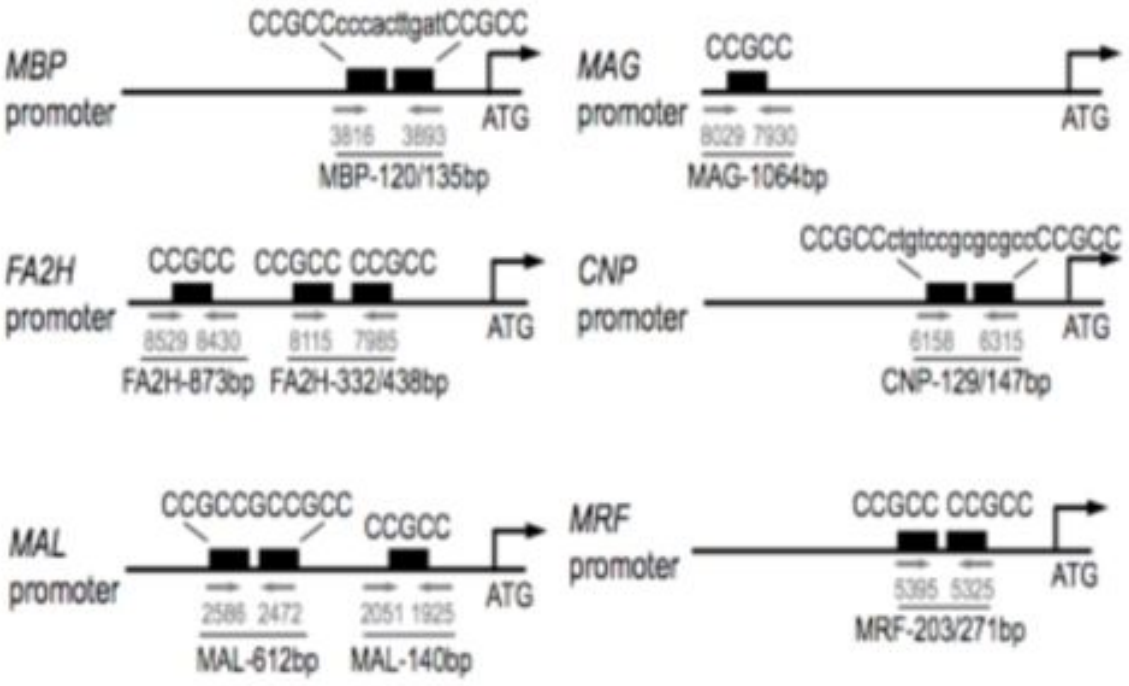
Suppl. Fig.5: Lef1 expression is activated in OPCs in a state of high activity Wnt signaling. (a) High-activity Wnt signaling in colon cancer involves a switch from a TCF4-b-catenin to a LEF1-b-catenin complex. Although Lef1 is not expressed during normal oligodendrocyte development, we observed robust Lef1 expression in the spinal cord of Olig2-cre, APCfl/fl animals at P9, where it co-localizes with the highly upregulated *Axin2* mRNA. (b) Such cells expressed high levels of *Axin2* and remained persistently undifferentiated (failing to express mature oligodendrocyte marker *PLP*) at postnatal day 30 (P30), P120, and P650.

Supplementary Fig. 6



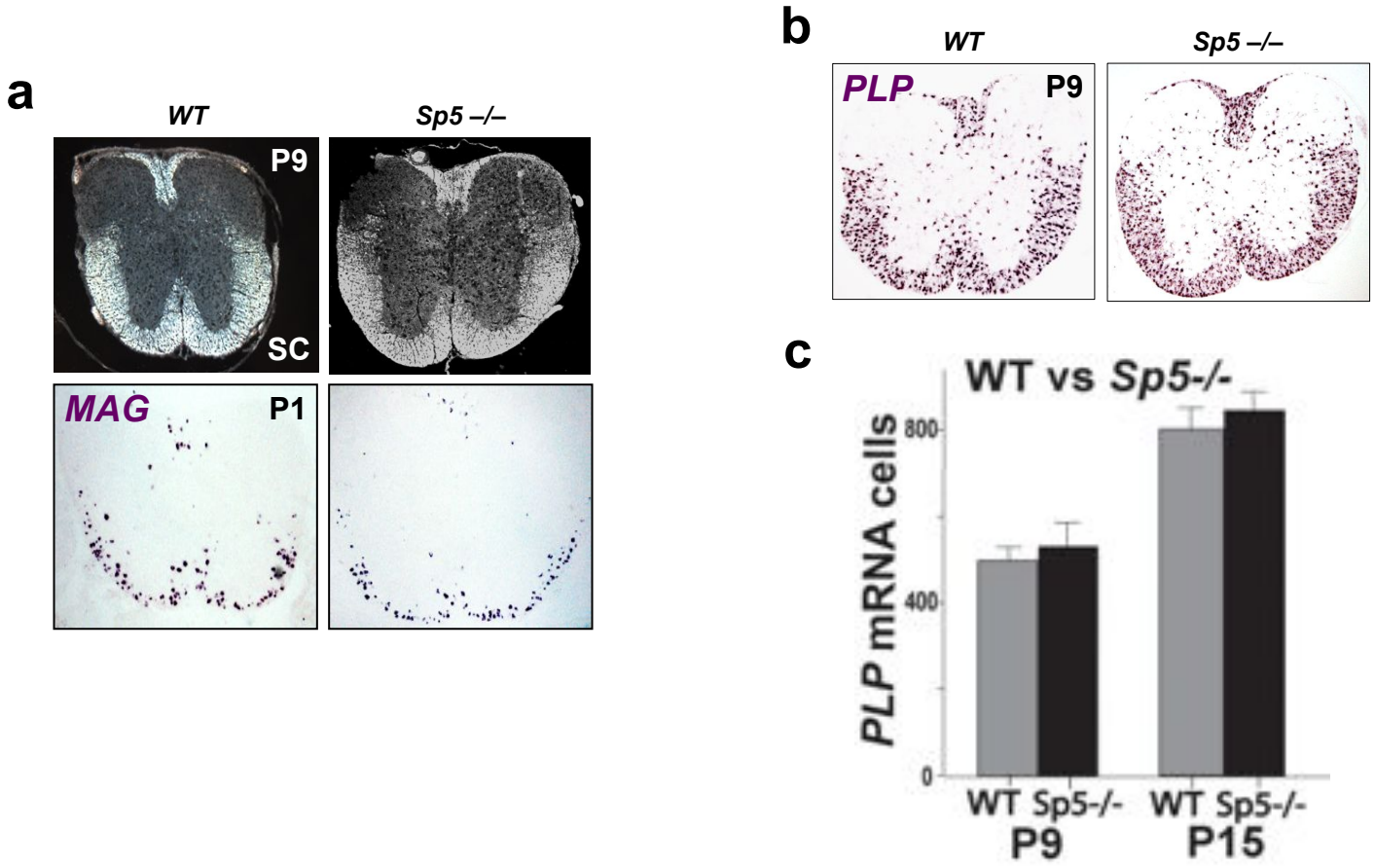
Suppl. Fig.6: High activity Wnt signaling in OPCs does not lead to hyperproliferation. Unlike Wnt-driven cancers of gut and hematopoetic systems, which respond to mitogenic Wnt signaling, we do not observe hyperproliferation of OPCs in Olig2cre:APC fl/fl mice. There is no observable difference in (a) the number of Nkx2.2+ OPCs, (b) the number of Ki67+ dividing cells, or (c) the number of *PDGFRα*+ OPCs in spinal cords of wildtype or Olig2cre:APC fl/fl mice at postnatal day 15.

Supplementary Fig. 7



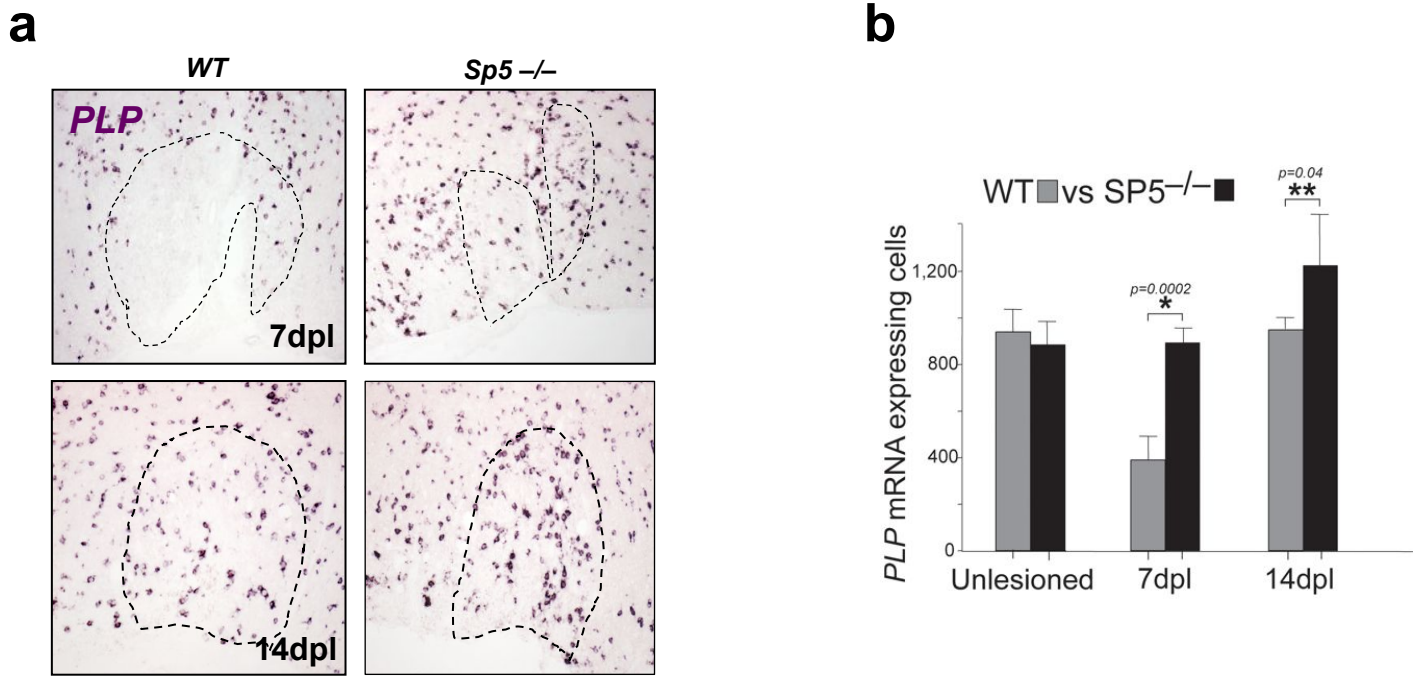
Suppl. Fig.7: Predicted Sp5 binding sites in multiple mature oligodendrocyte gene promoters. There are predicted binding sites for the transcription factor SP5 in the promoter regions of multiple mature oligodendrocyte/ myelin genes, including *myelin basic* protein (MBP), *myelin associated glycoprotein (MAG), fatty acid-2-hydroxylase (FA2H), 2'3'cyclic-nucleotide 3'-phosphodiesterase (CNP), myelin and lymphocyte protein (MAL) and myelin regulatory factor (MRF).*

Supplementary Fig. 8



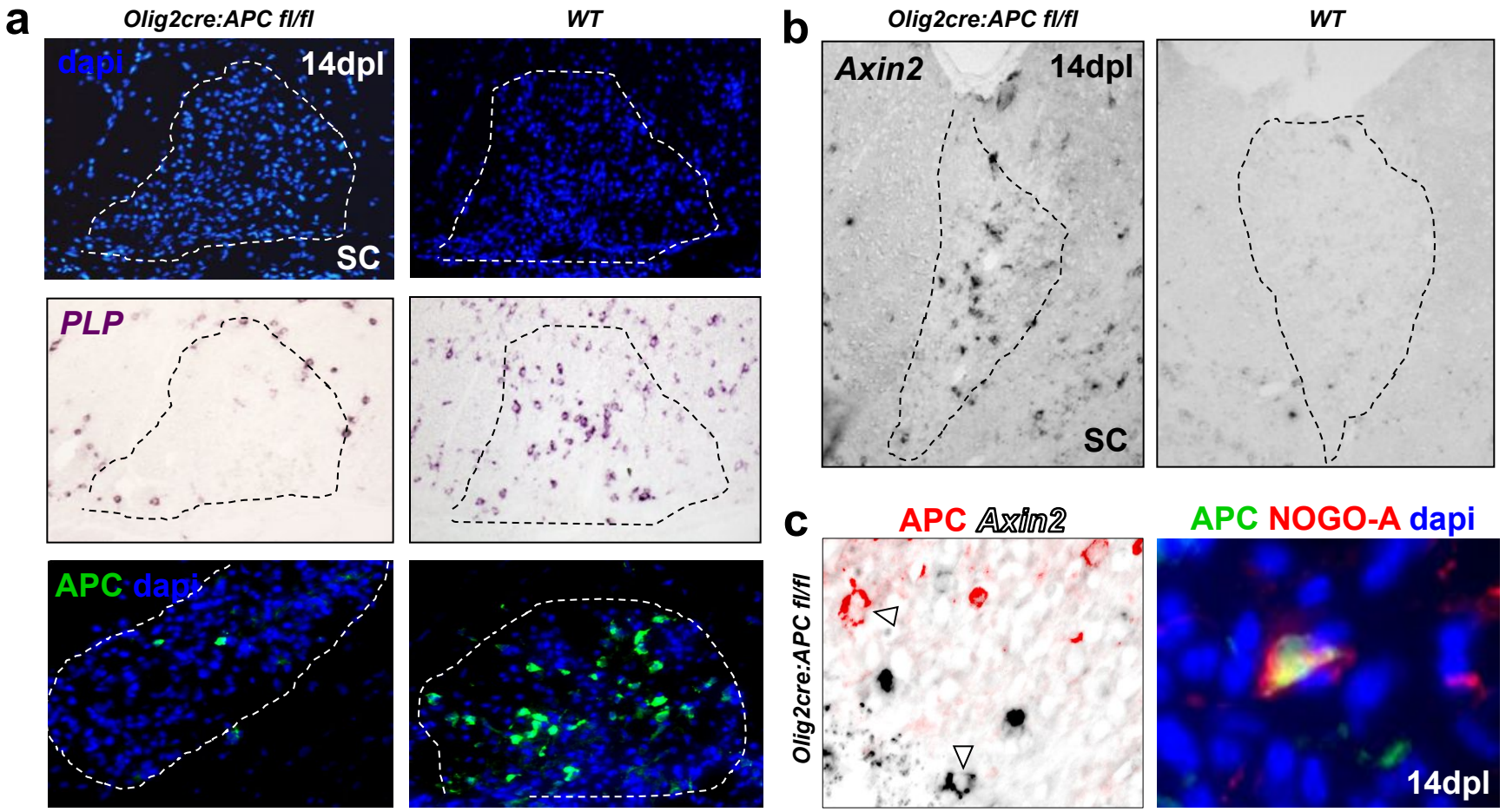
Suppl. Fig.8: Oligodendrocyte development proceeds normally in SP5 null animals. (a) Oligodendrocyte development proceeds normally in SP5-/- animals with normal white matter thickness on dark field (DF) in spinal cord (SC) at P9 and normal onset of expression of mature oligodendrocyte gene *MAG* at P1, and normal numbers of mature oligodendrocytes expressing mature marker *PLP* at P9 (b,c) and P15 (c) in SC.

Supplementary Fig. 9



Suppl. Fig.9: Precocious OPC differentiation in SP5 null remyelination. (a, b) SP5-/- animals showed acceleration of OPC differentiation in the mouse model of remyelination using focal injection of lysolecithin into adult spinal cord white matter, with significant increases in number of cells expressing PLP mRNA at 7 days post lesioning (7dpl) and 14dpl (* $p< 0.01$, ** $p<0.05$).

Supplementary Fig. 10



Suppl. Fig.10: Loss of APC in OPCs leads to significant remyelination deficits. (a) Loss of APC in OPCs during remyelination (lysolecithin spinal cord focal injection) leads to a failure of differentiation and onset of mature oligodendrocyte marker *PLP* in lesions at 14dpl. (b) Loss of APC in OPCs during remyelination results in a massive upregulation of the Wnt pathway target *Axin2* mRNA, signaling a loss of Wnt repressor tone. (c) A small percentage of OPCs escape the Olig2-cre floxing activity and express APC in remyelinating lesions (a), and these are the only OPCs which do not express the Wnt target *Axin2* and are capable of differentiating to NOGO-A mature remyelinating oligodendrocytes.