Supplementary Information

Supplementary Figure 1.

NOTCH1

>gi|27894368|ref|NP_060087.2| notch1 preproprotein [Homo sapiens]

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TLTEYKCRCP PGWSGKSCQQ ADPCASNPCA NGGQCLPFEA SYICHCPPSF HGPTCRQDVN ECGQKPGLCR HGGTCHNEVG SYRCVCRATH TGPNCERPYV PCSPSPCQNG GTCRPTGDVT
HECACLPGFT GQNCEENIDD CPGNNCKNGG ACVDGVNTYN CRCPPEWTGQ YCTEDVDECQ LMPNACQNGG TCHNTHGGYN CVCVNGWTGE DCSENIDDCA SAACFHGATC HDRVASFYCE
CPHGRTGLLC HLNDACISNP CNEGSNCDTN PVNGKAICTC PSGYTGPACS QDVDECSLGA NPCEHAGKCI NTLGSFECQC LQGYTGPRCE IDVNECVSNP CQNDATCLDQ IGEFQCICMP
GYEGVHCEVN TDECASSPCL HNGRCLDKIN EFQCECPTGF TGHLCQYDVD ECASTPCKNG AKCLDGPNTY TCVCTEGYTG THCEVDIDEC DPDPCHYGSC KDGVATFTCL CRPGYTGHHC
ETNINECSSQ PCRHGGTCQD RDNAYLCFCL KGTTGPNCEI NLDDCASSPC DSGTCLDKID GYECACEPGY TGSMCNINID ECAGNPCHNG GTCEDGINGF TCRCPEGYHD PTCLSEVNEC
NSNPCVHGAC RDSLNGYKCD CDPGWSGTNC DINNNECESN PCVNGGTCKD MTSGYVCTCR EGFSGPNCQT NINECASNPC LNQGTCIDDV AGYKCNCLLP YTGATCEVVL APCAPSPCRN
GGECRQSEDY ESFSCVCPTG WQAGQTCEVD INECVLSPCR HGASCQNTHG GYRCHCQAGY SGRNCETDID DCRPNPCHNG GSCTDGINTA FCDCLPGFRG TFCEEDINEC ASDPCRNGAN
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DCAEHVPERL AAGTLVVVVL MPPEQLRNSS FHFLRELSRV LHTNVVFKRD AHGQQMIFPY YGREEELRKH PIKRAAEGWA APDALLGQVK ASLLPGGSEG GRRRRELDPM DVRGSIVYLE
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MONO MW: **272394**, pI: **4.95**
Database = **/home/sorcerer/fasta/RefSeqHuman_082508.fasta**
Links: Google Google Scholar NCBI UniProtKB

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1213-1235	2450.1	GTQGVHCEINVDDCNPPVDPVSR
1289-1297	1121.44	VNDFHCECR
1600-1608	1055.61	VLHTNVVFK
1651-1662	1099.56	ASLLPGGSEGGR
1928-1938	1138.61	TGETALHLAAR
2080-2088	1083.58	VLLDHFANR
2265-2273	986.52	LAFETGPPR

NOTCH2

>gi|24041035|ref|NP_077719.2| notch 2 preproprotein [Homo sapiens]

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LCMPGFKGVH CELEINECQS NPCVNNGQCV DKVNRFQCLC PPGFTGPVCQ IDIDDCSSTP CLNGAKCIDH PNGYECQCAT GFTGVLCEEN IDNCDPDPCH HGQCQDGIDS YTCICNPGYM
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PCENAAVCKE SPNFESYTCL CAPGWQGQRC TIDIDECISK PCMNHGLCHN TQGSYMCECP PGFSGMDCEE DIDDCLANPC QNGGSCMDGV NTFSCLCLPG FTGDKCQTDM NECLSEPCKN
GGTCSDYVNS YTCKCQAGFD GVHCENNINE CTESSCFNGG TCVDGINSFS CLCPVGFTGS FCLHEINECS SHPCLNEGTC VDGLGTYRCS CPLGYTGKNC QTLVNLCSRS PCKNKGTCVQ
KKAESQCLCP SGWAGAYCDV PNVSCDIAAS RRGVLVEHLC QHSGVCINAG NTHYCQCPLG YTGSYCEEQL DECASNPCQH GATCSDFIGG YRCECVPGYQ GVNCEYEVDE CQNQPCQNGG
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VASNMPDGFI CRCPPGFSGA RCQSSCGQVK CRKGEQCVHT ASGPRCFCPS PRDCESGCAS SPCQHGGSCH PQRQPPYYSC QCAPPFSGSR CELYTAPPST PPATCLSQYC ADKARDGVCD
EACNSHACQW DGGDCSLTME NPWANCSSPL PCWDYINNQC DELCNTVECL FDNFECQGNS KTCKYDKYCA DHFKDNHCDQ GCNSEECGWD GLDCAADQPE NLAEGTLVIV VLMPPEQLLQ
DARSFLRALG TLLHTNLRIK RDSQGELMVY PYYGEKSAAM KKQRMTRRSL PGEQEQEVAG SKVFLEIDNR QCVQDSDHCF KNTDAAAALL ASHAIQGTLS YPLVSVVSES LTPERTQLLY
LLAVAVVIIL FIILLGVIMA KRKRKHGSLW LPEGFTLRRD ASNHKRREPV GQDAVGLKNL SVQVSEANLI GTGTSEHWVD DEGPQPKKVK AEDEALLSEE DDPIDRRPWT QQHLEAADIR
RTPSLALTPP QAEQEVDVLD VNVRGPDGCT PLMLASLRGG SSDLSDEDED AEDSSANIIT DLVYQGASLQ AQTDRTGEMA LHLAARYSRA DAAKRLLDAG ADANAQDNMG RCPLHAAVAA
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VSDTTSSPMI TSPGILQASP NPMLATAAPP APVHAQHALS FSNLHEMQPL AHGASTVLPS VSQLLSHHHI VSPGSGSAGS LSRLHPVPVP ADWMNRMEVN ETQYNEMFGM VLAPAEGTHP
GIAPQSRPPE GKHITTPREP LPPIVTFQLI PKGSIAQPAG APQPQSTCPP AVAGPLPTMY QIPEMARLPS VAFPTAMMPQ QDGQVAQTIL PAYHPFPASV GKYPTPPSQH SYASSNAAER
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MONO MW: **265226**, pI: **4.95**
Database = **/home/sorcerer/fasta/RefSeqHuman_082508.fasta**
Links: Google Google Scholar NCBI UniProtKB

Position	Mass	Peptide
53-65	1537.64	CPEGFLGEYCQHR
53-70	2109.87	CPEGFLGEYCQHRDPCEK
127-140	1550.64	DTYECTCQVGFTGK
260-268	1082.46	NIDDCPNHR
357-368	1257.52	VASFSCMCPEGK
409-432	2465.92	GADCTEDVDECAMANSNPCEHAGK
475-487	1372.63	IGGFTCLCMPGFK
666-678	1403.6	YSCVCSPGFTGQR
829-849	2160.94	NCQTVLAPCSPNPCENAAVCK
1049-1058	1027.45	CSCPLGYTGK
1059-1069	1249.59	NCQTLVNLCSR
1082-1111	3100.35	KAESQCLCPSGWAGAYCDVPNVSCDIAASR
1211-1218	819.34	CSCPPGTR
1219-1231	1449.62	GLLCEENIDDCAR
1245-1251	754.34	IGGYSCR
1333-1341	890.41	CPPGFSGAR
1353-1365	1368.66	KGEQCVHTASGPR
1366-1372	808.34	CFCPSPR
1394-1410	1886.81	QPPYYSCQCAPPFSGSR
1568-1578	1207.7	ALGTLLHTNLR
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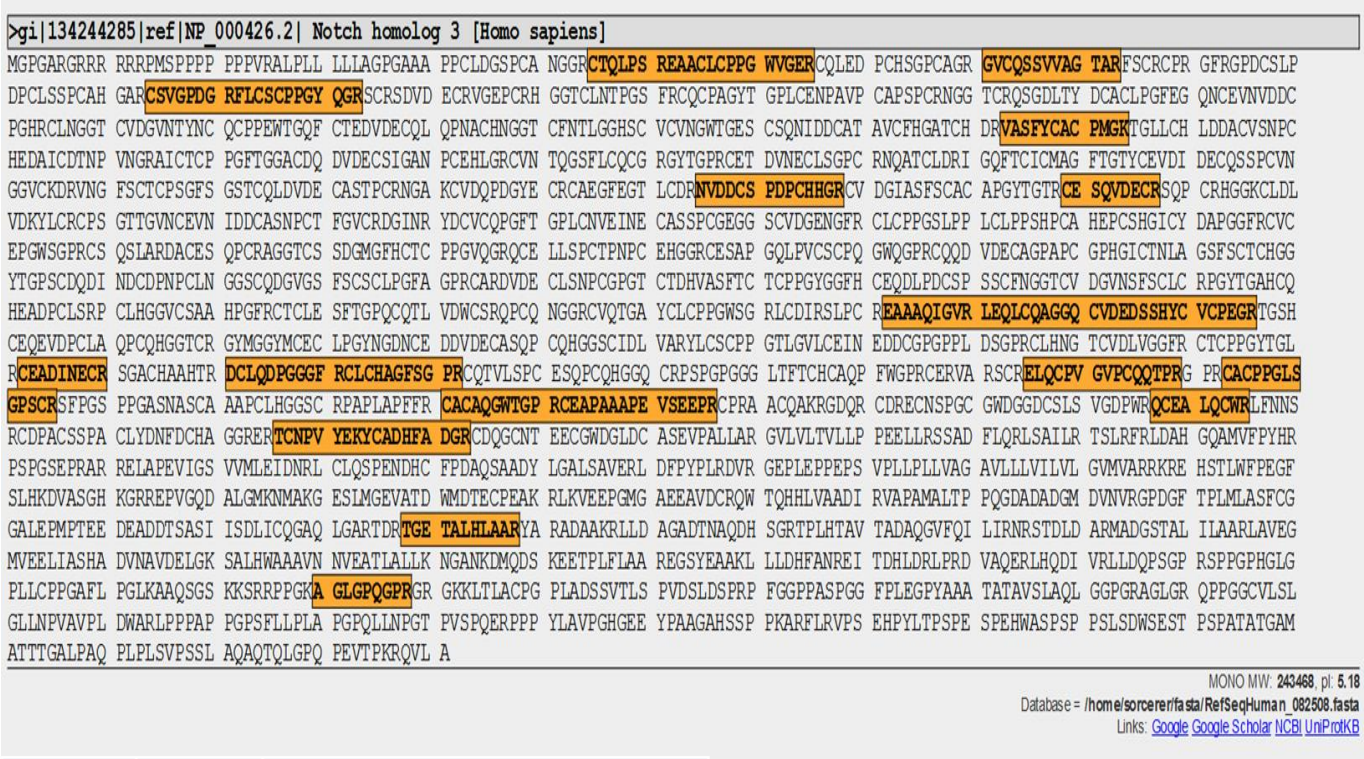
NOTCH3



Position	Mass	Peptide
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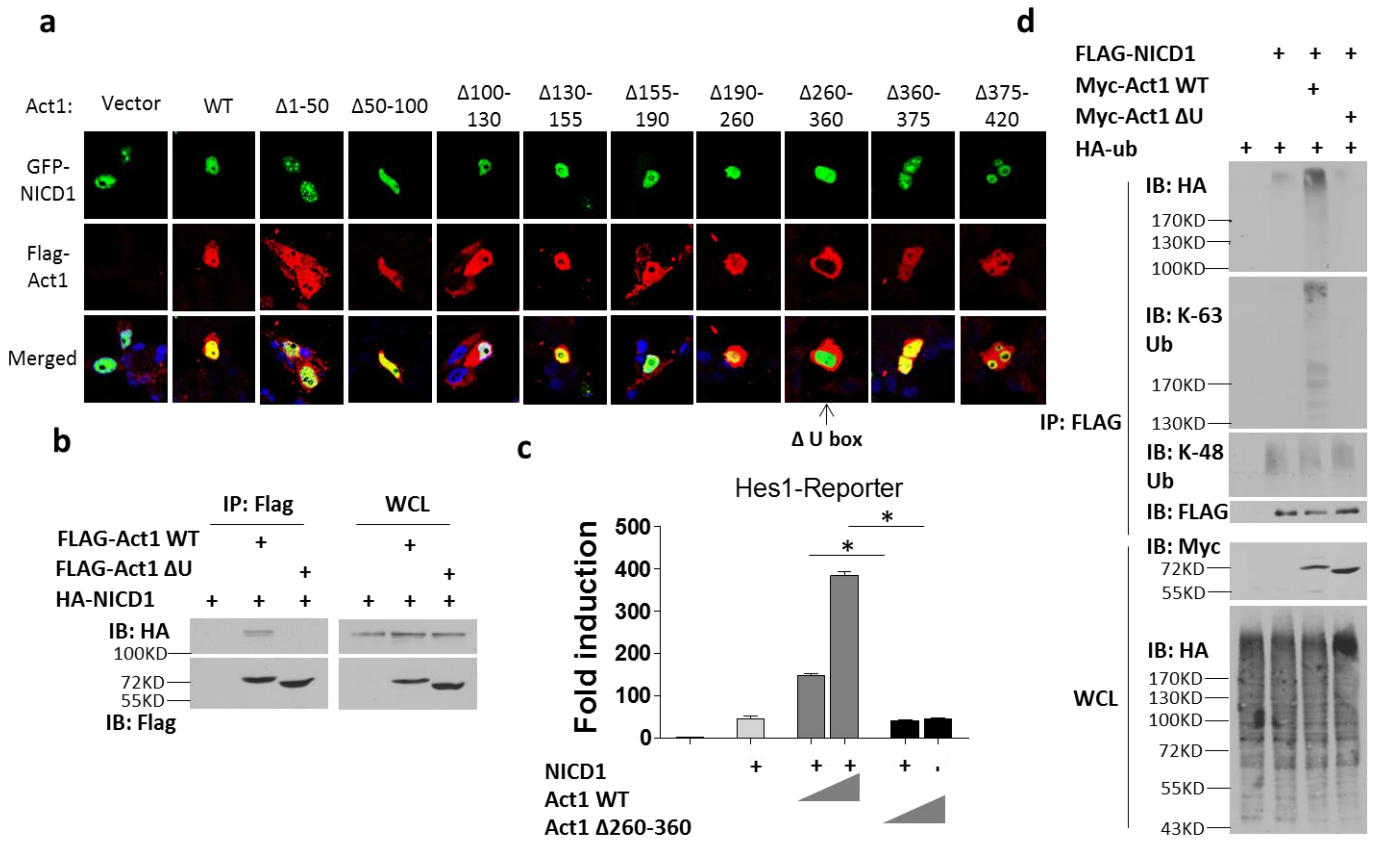
142-153	1326.58	FLCSCPPGYQGR
333-344	1275.55	VASFYCACPMGK
545-558	1550.6	NVDDCSPDPCHHGR
579-587	1067.4	CESQVDECR
1042-1050	913.5	EAAAQIGVR
1051-1076	2825.15	LEQLCQAGGQCVDEDSSHYCVCPEGR
1202-1210	1051.41	CEADINECR
1221-1231	1163.5	DCLQDPGGGFR
1232-1242	1146.51	CLCHAGFSGPR
1295-1309	1653.8	ELQCPVGVPCQQTPR
1313-1325	1246.53	CACPPGLSGPSCR
1361-1371	1148.49	CACAQGWTGPR
1372-1386	1554.7	CEAPAAAPEVSEEPR
1427-1435	1135.49	QCEALQCWR
1466-1473	952.43	TCNPVYEK
1474-1483	1153.46	YCADHFADGR
1838-1848	1138.61	TGETALHLAAR
2070-2078	851.46	AGLGPQGPR

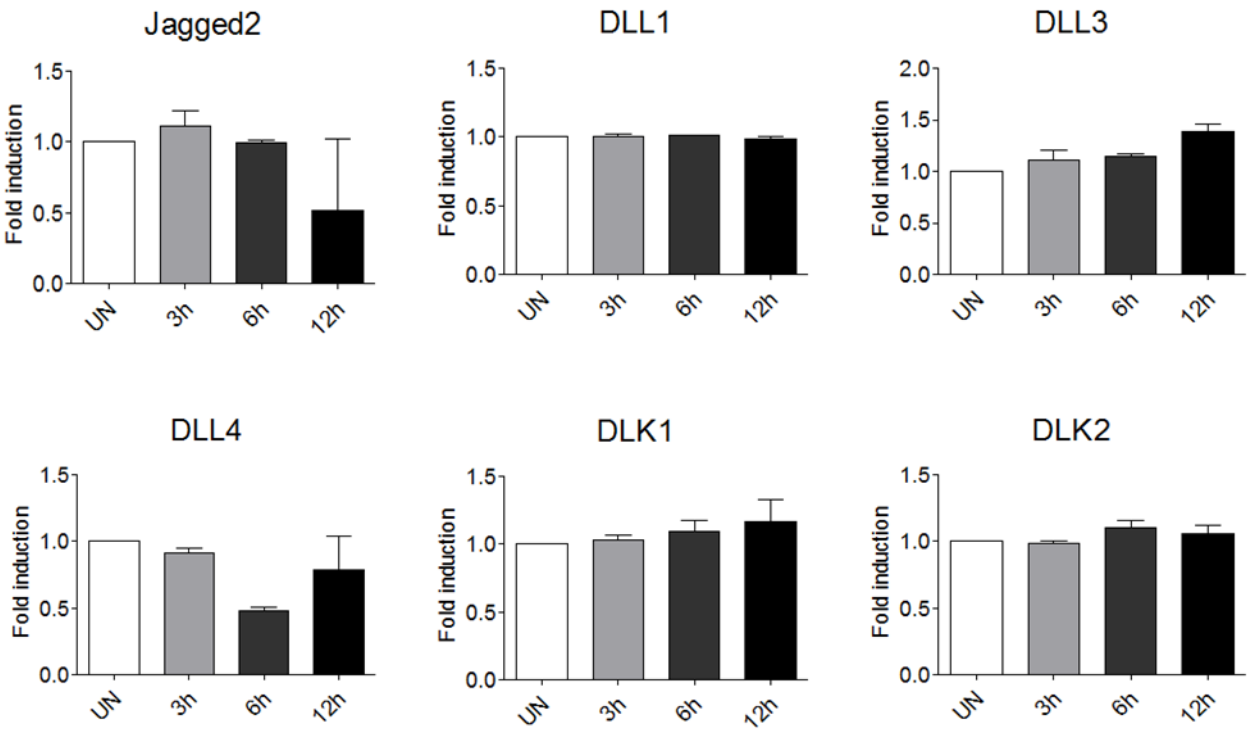
Supplementary Figure 1. **Mass spectrometry identification of NOTCH family proteins as Act1-interacting proteins.** HeLa cell lysates were immunoprecipitated with anti-Act1 antibody followed by mass spectrometry analysis of co-precipitated proteins. Co-immunoprecipitated proteins were identified by mass spectrometry. Shown are the NOTCH1, NOTCH2 and NOTCH3 peptides identified in the analysis.

Supplementary Figure 2.



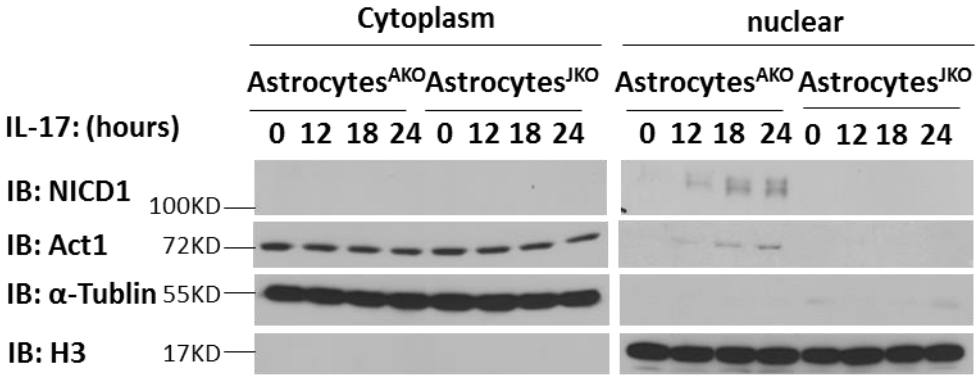
Supplementary Figure 2. E3 ligase activity of Act1 is indispensable for engaging NOTCH pathway. (a) HeLa cells were transfected with GFP-NICD and indicated Flag tagged wild-type Act1 or Act1 deletion mutants. Transfected cells were fixed and stained with anti-Flag antibody followed by analysis with confocal microscope under a 63X objective. Arrow indicates U box deletion mutant of Act1 (*Δ 260-360*) (b) HeLa cells were transfected with HA-NICD and vector, wild-type Act1 or U-box deletion mutant of Act1 (Act1 *ΔU*). Cell lysates were immunoprecipitated with anti-FLAG antibody, followed by immunoblot analysis for indicated proteins. IB: immunoblotting; IP: Immunoprecipitation, WCL: whole cell lysates. (c) HeLa cells were transfected with Hes1–luciferase reporter (100ng) alone or in combination with the different amounts of wild-type Act1 (200ng and 500ng) or Act1 *ΔU* (200ng and 500ng) and NICD1 (200ng). Data is reported as fold induction of luciferase activity in indicated transfection over Hes1-reporter transfection alone. Data are repeated two different experiments. (d) HeLa cells were transfected with HA-Ub in combination with wild-type myc-Act1, myc-Act1 *ΔU* and FLAG-NICD as indicated. Cell lysates were immunoprecipitated with anti-FLAG antibody, followed by immunoblot analysis for indicated proteins. IB: immunoblotting; IP: Immunoprecipitation, WCL: whole cell lysates. Data are representative of two independent experiments.

Supplementary Figure 3.



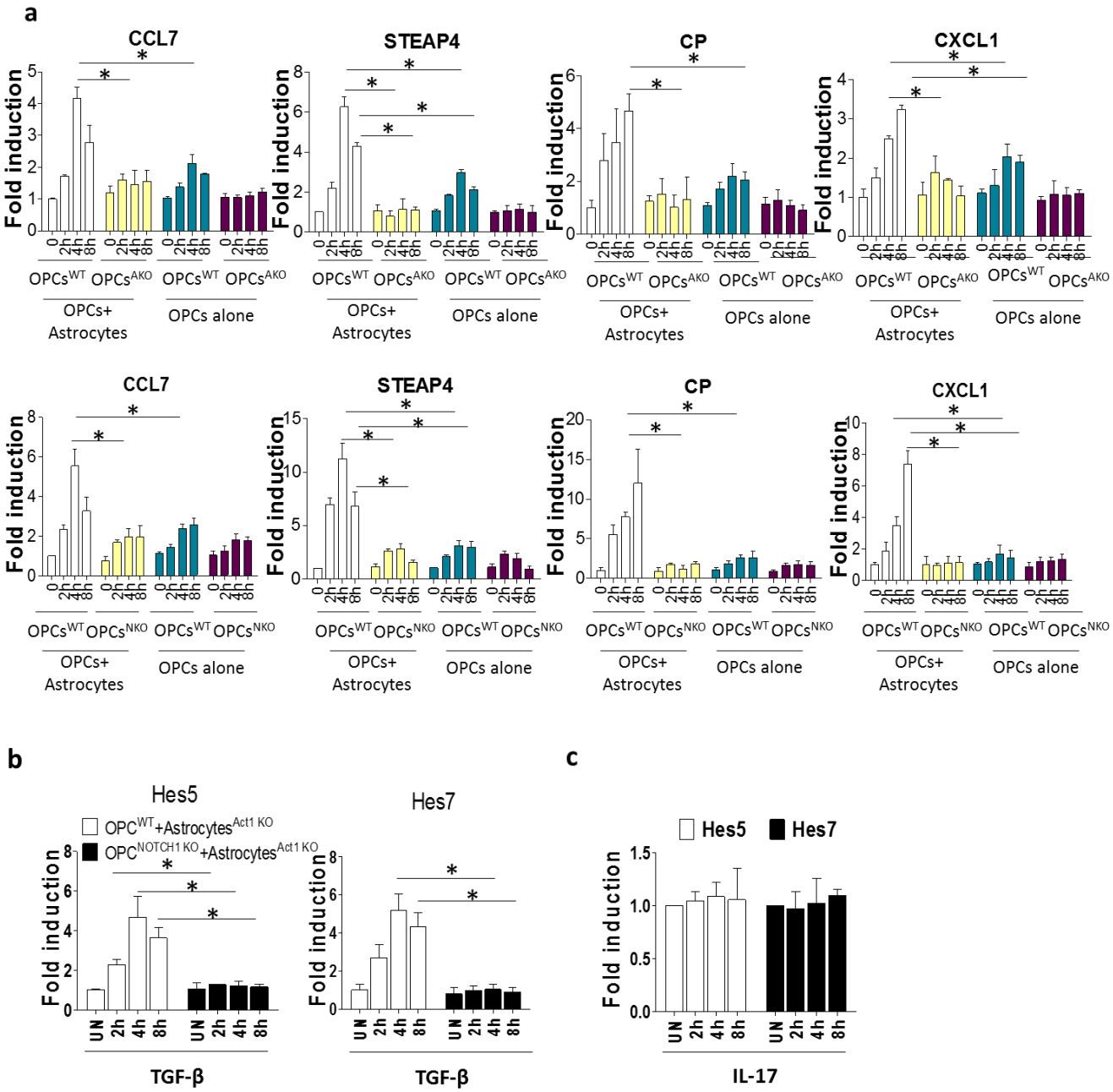
Supplementary Figure 3. IL-17 stimulation does not induce NOTCH1 ligand expression in astrocytes. Astrocytes were stimulated with IL-17 (50ng ml^{-1}) for indicated time. Stimulated cells were subjected to RT-PCR analysis for the expression of indicated NOTCH ligand. Data are representative of two experiments.

Supplementary Figure 4.



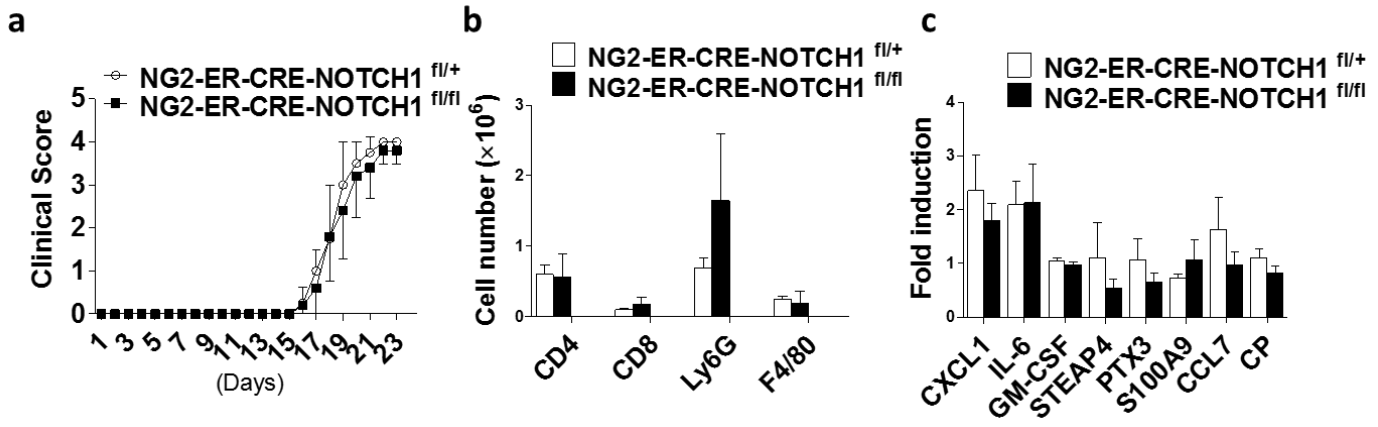
Supplementary Figure 4. Jagged1 expressed in astrocytes is required for IL-17-induced Act1 nuclear translocation and OPCs maturation. OPCs co-cultured with Act1 KO or Jagged1 KO astrocytes were left untreated or treated with IL-17 (50ng ml^{-1}) for indicated time, followed by cell fractionation for cytoplasmic and nuclear proteins. Cell fractions were analyzed by immunoblot analysis for indicated proteins. Data are representative of two independent experiments.

Supplementary Figure 5.



Supplementary Figure 5. Differential regulation of gene expression by canonical IL-17 pathway and IL-17-NOTCH axis. (**a**) Act1- and NOTCH1 -dependent genes. Wild-type, Act1 KO or NOTCH1 KO OPCs co-cultured with Act1 KO astrocytes were treated with IL-17 for indicated time, followed by real-time PCR analysis of different gene induction. (**b**) Wild-type or NOTCH KO OPCs co-cultured with Act1 KO astrocytes were treated with TGFβ for indicated time, followed by real-time PCR analysis of different gene induction. **(c)** OPCs co-cultured with Act1 KO astrocytes were stimulated with IL-17 for indicated time, followed by RT-PCR analysis for indicated genes. Error bars represent SEM of technical replicates. *P < 0.05 based on Mann-Whitney U test. Data are representative of three independent experiments.

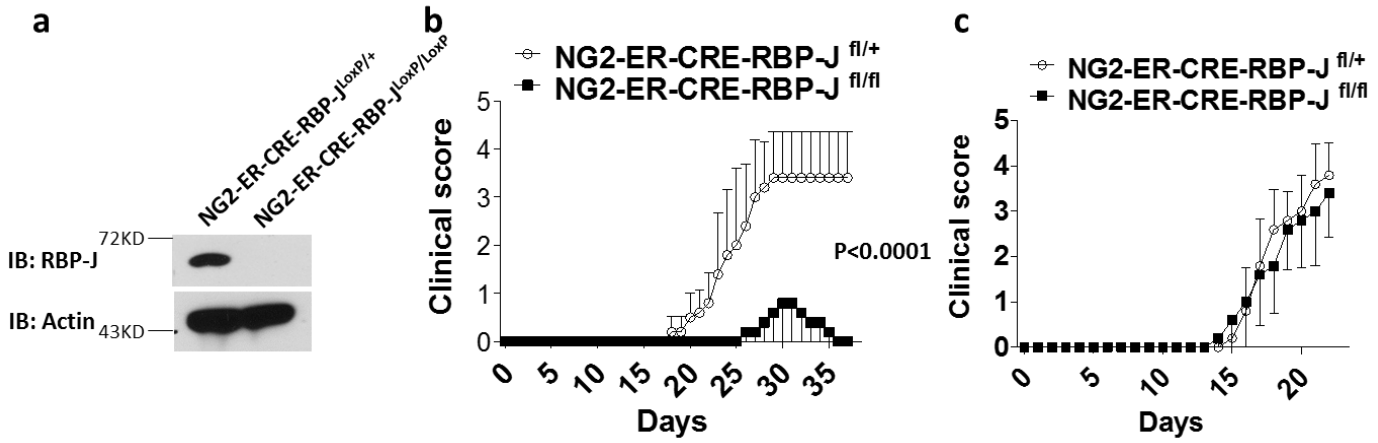
Supplementary Figure 6



Supplementary Figure 6. NOTCH1 deficiency in NG2+ cells does not affect Th1-induced EAE.

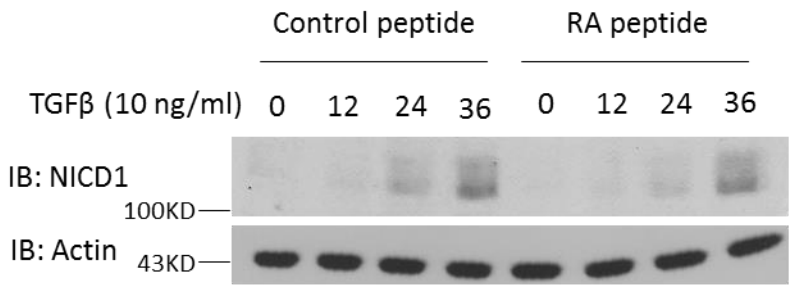
(a) Mice (n=15) of indicated genotypes were adoptively transferred with MOG-reactive Th1 cells. Clinical scores of EAE symptoms are graphed over experimental time course. **(b)** Infiltrating cells from the brain of mice with Th1 induced EAE (n=5) were analyzed by flow cytometry at the peak of the disease. **(c)** RT-PCR analysis of inflammatory gene expression in spinal cords of mice (n=5) with Th1 induced EAE. All error bars represent SEM of biological replicates. *: $p<0.05$ based on Mann-Whitney U test. Data are representative of three independent experiments.

Supplementary Figure 7



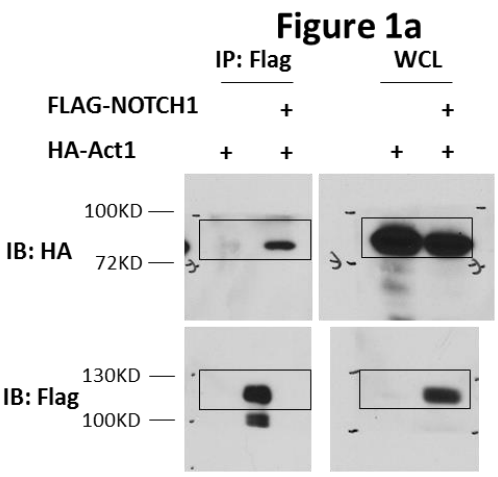
Supplementary Figure 7. Ablation of RBP-J in OPCs ameliorates Th17- but not Th1-induced EAE. (a) Immunoblot analysis of RBP-J expression in $NG2^+$ cells sorted from brains of indicated mice. **(b)** Mice of indicated genotypes were adoptively transferred with MOG-reactive Th17 cells (n = 5) to induce EAE. Clinical score of symptoms are graphed over the experimental time course. **(c)** Mice of indicated genotypes were adoptively transferred with MOG-reactive Th1 cells (n = 5) to induce EAE. Clinical score of symptoms are graphed over the experimental time course. Data are representative of three independent experiments.

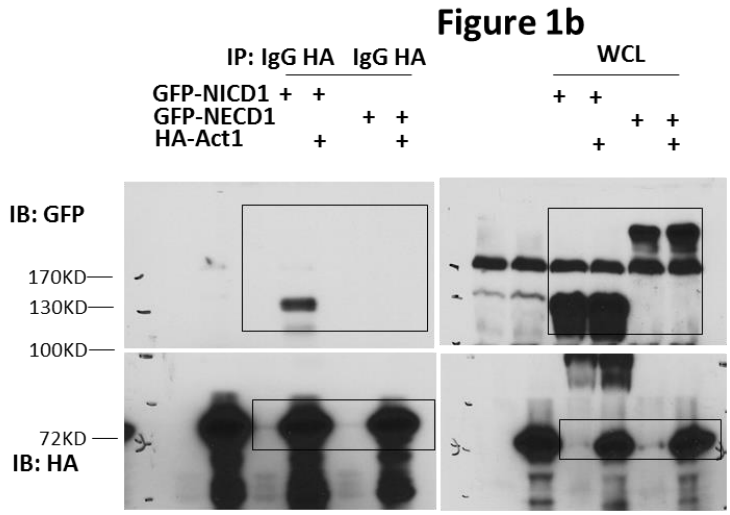
Supplementary Figure 8

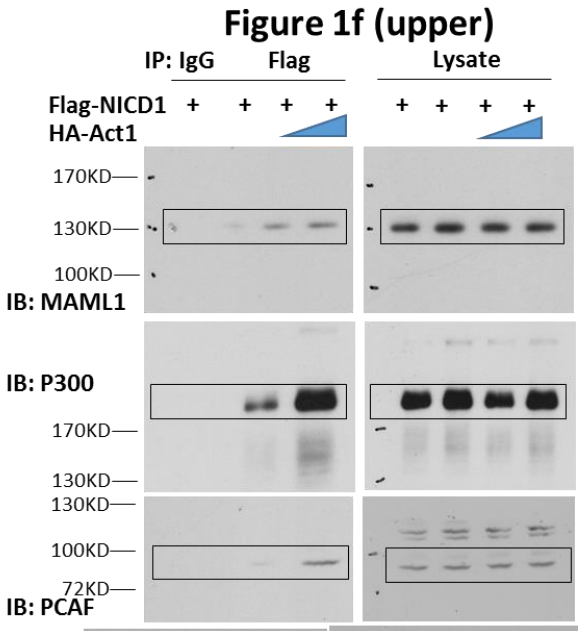


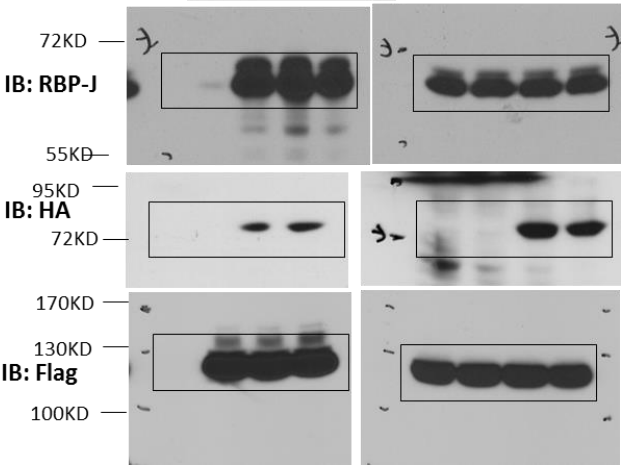
Supplementary Figure 8. IL-17RA decoy peptide does not block TGFβ-induced NOTCH1 activation in OPC-astrocyte co-culture. OPCs co-cultured with Act1 KO astrocytes were left untreated or treated with recombinant TGFβ (10ng ml^{-1}) for indicated time, followed by immune blot for NICD1 and Actin. Data are representative of two independent experiments.

Supplementary Figure 9. Uncropped image of films for all the Figures.









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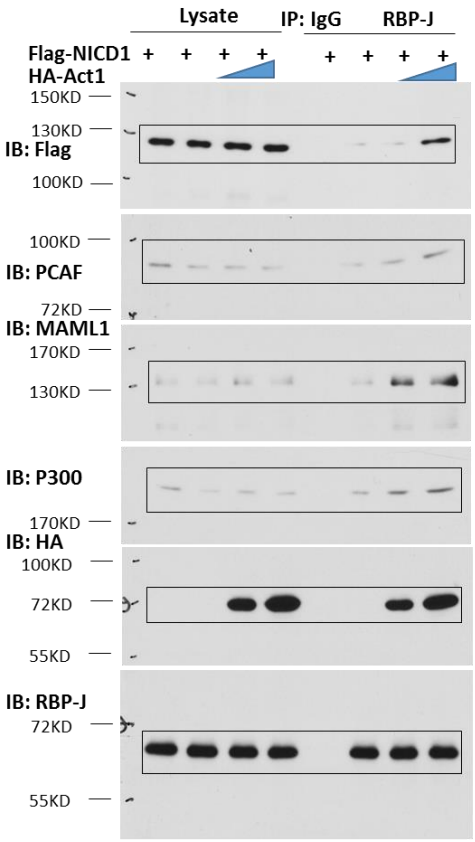


Figure 2a

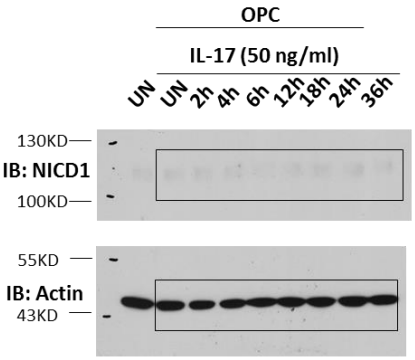


Figure 2c

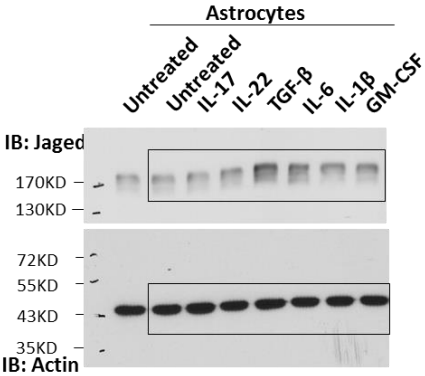


Figure 2b

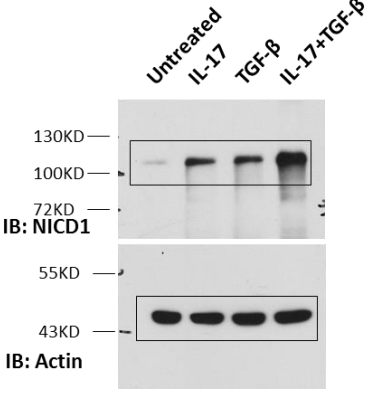
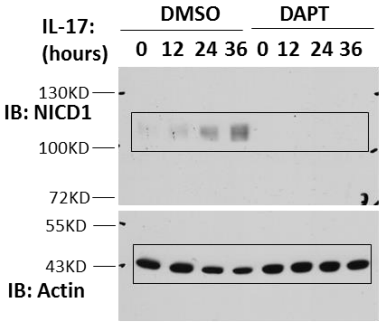


Figure 2d (left)



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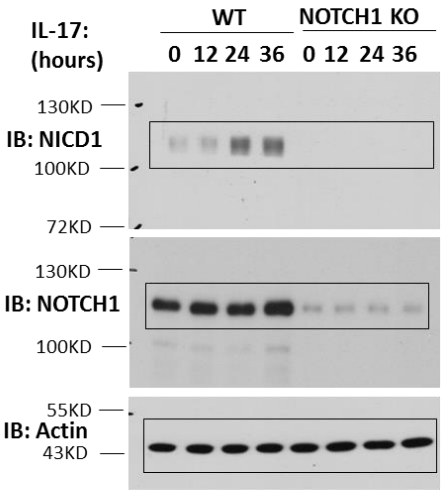


Figure 2f

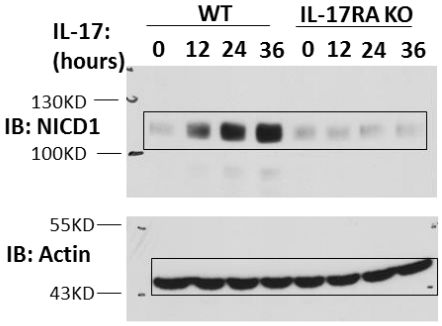


Figure 2h

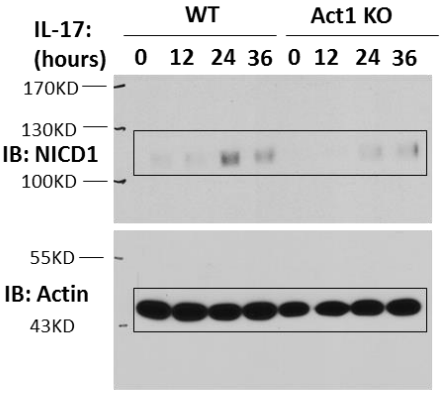


Figure 2e

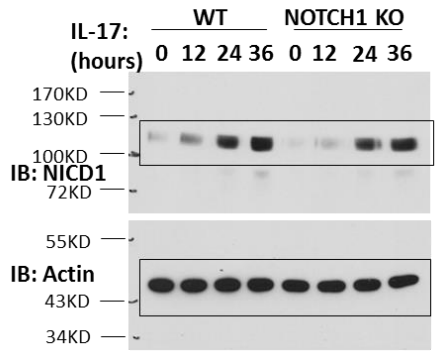


Figure 2g

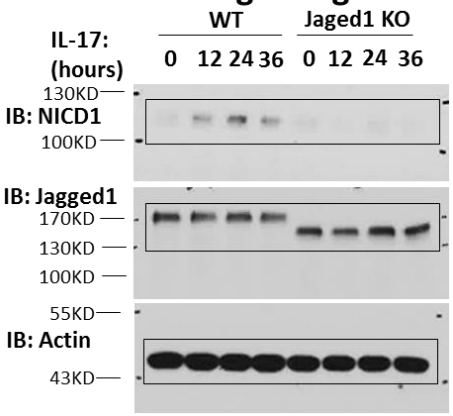


Figure 2i

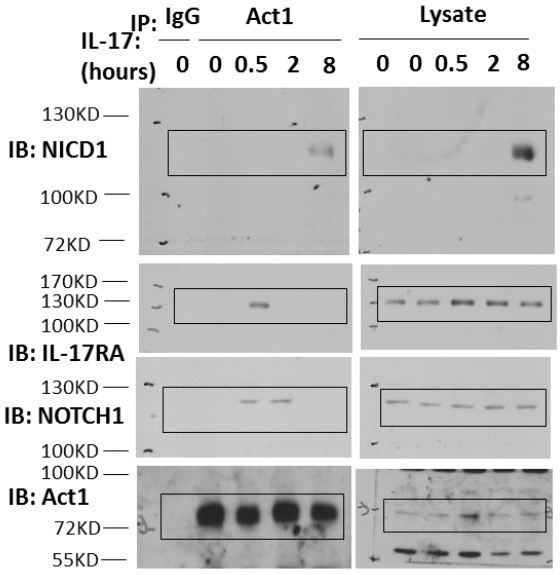


Figure 3a

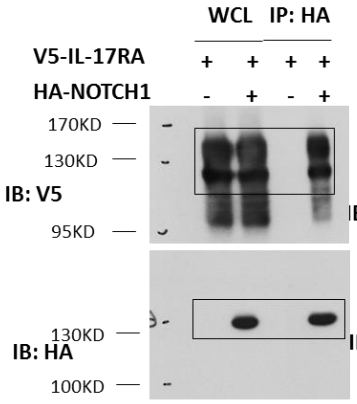


Figure 3c left

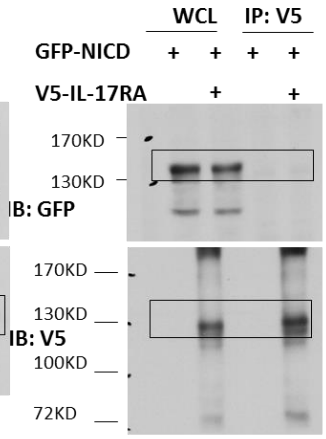


Figure 3d

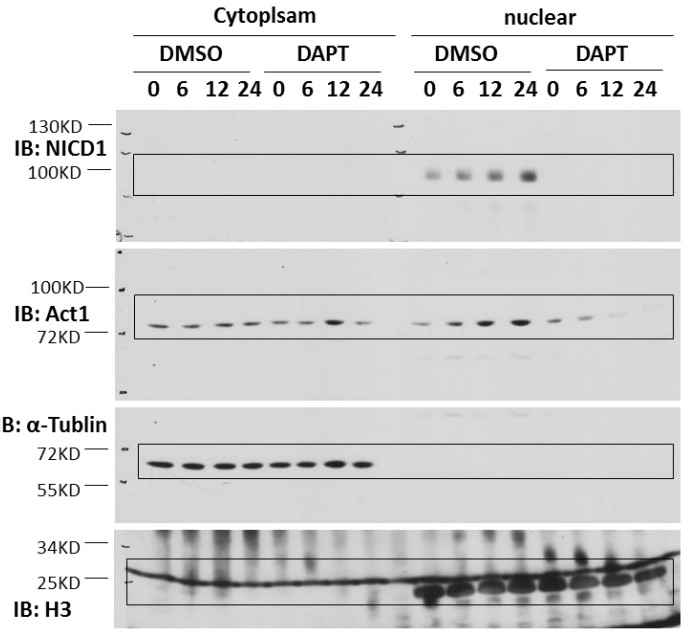


Figure 3c right

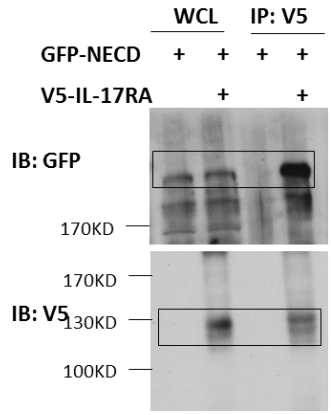
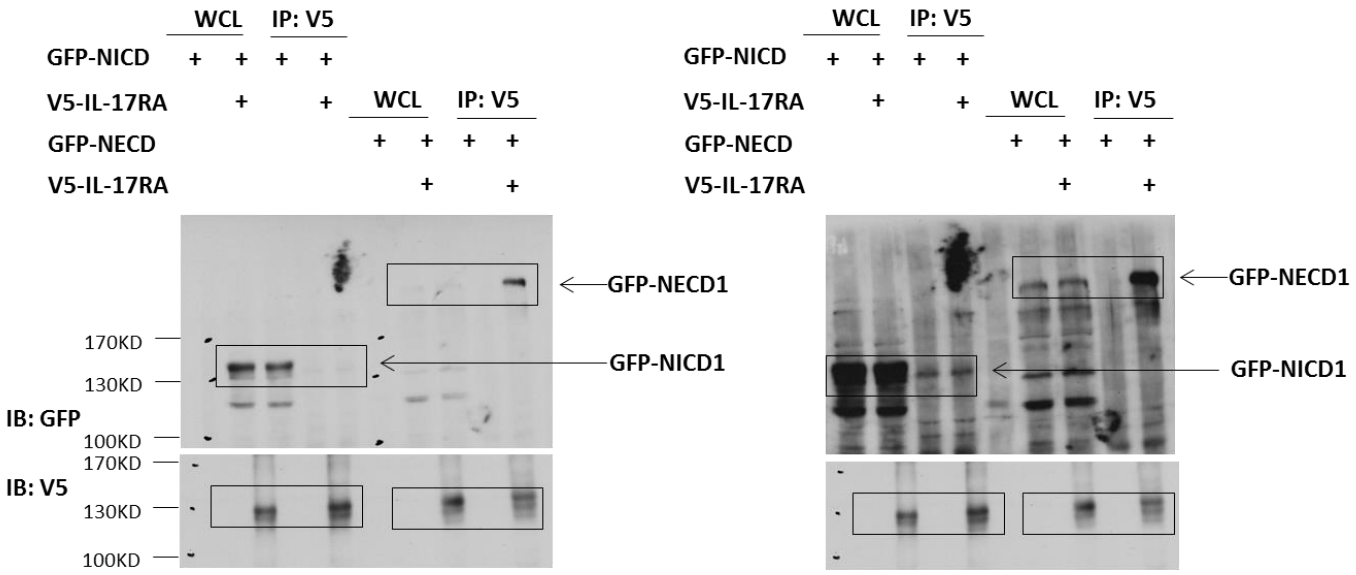


Figure 3c



Short exposure **Longer exposure**

Figure 3e

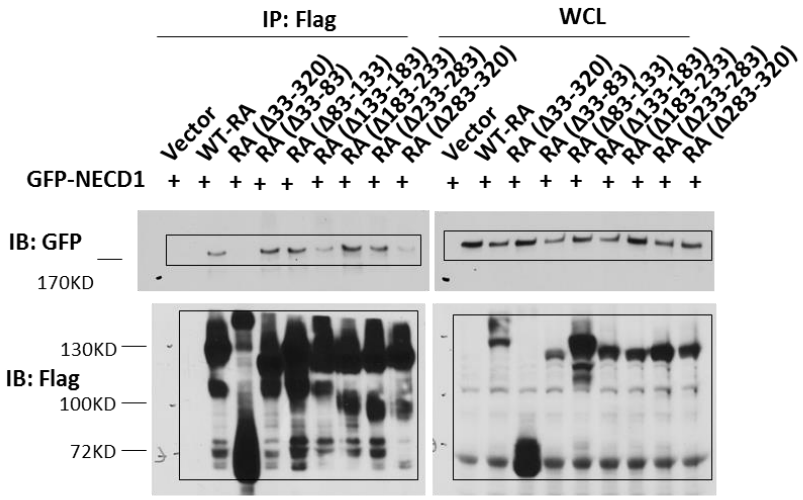


Figure 6a

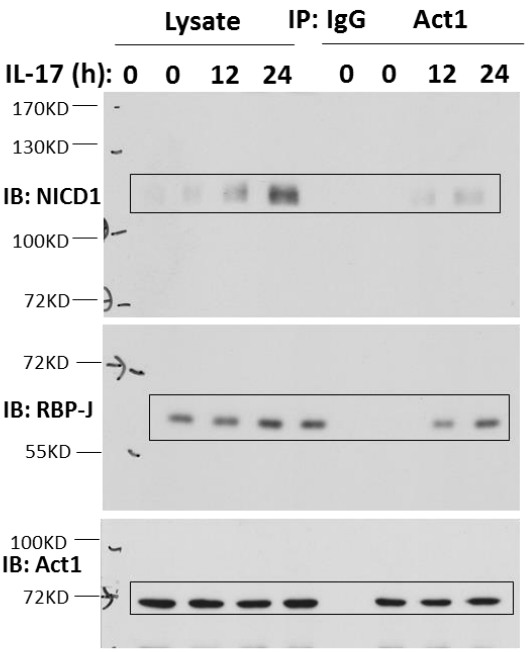


Figure 6d

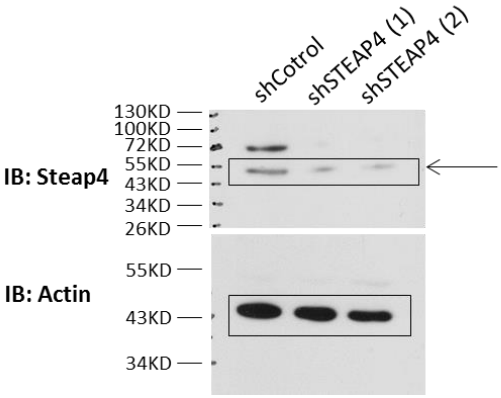


Figure 9a

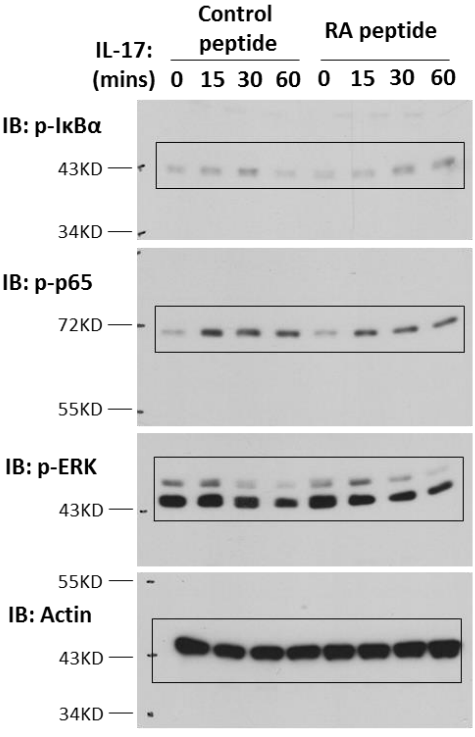


Figure 9b

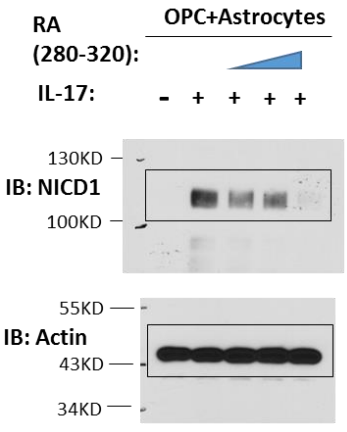
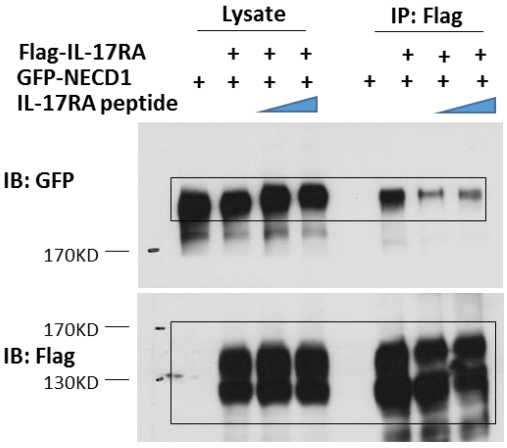
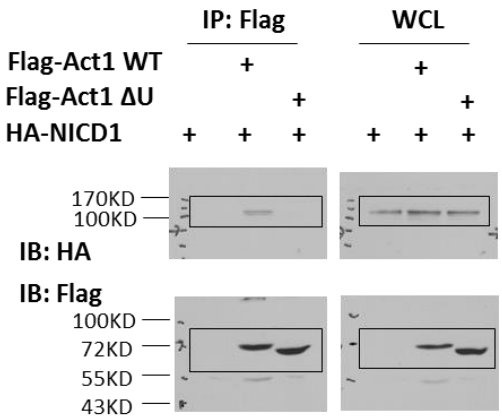


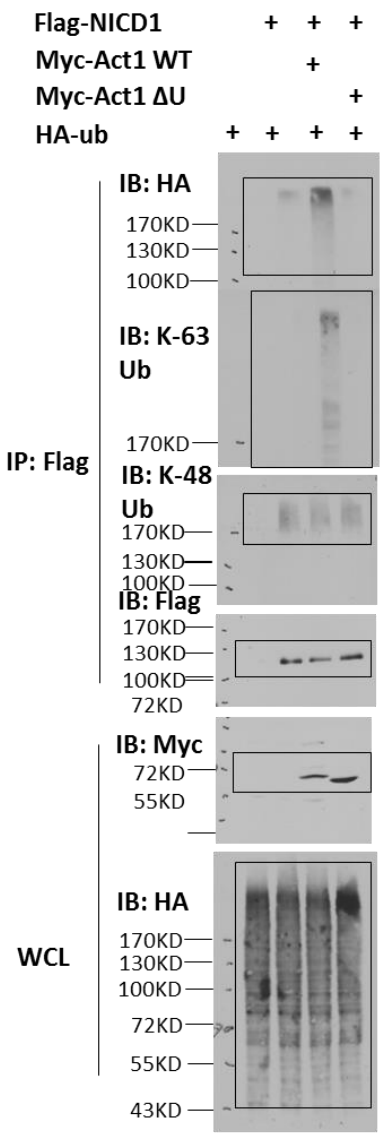
Figure 9c



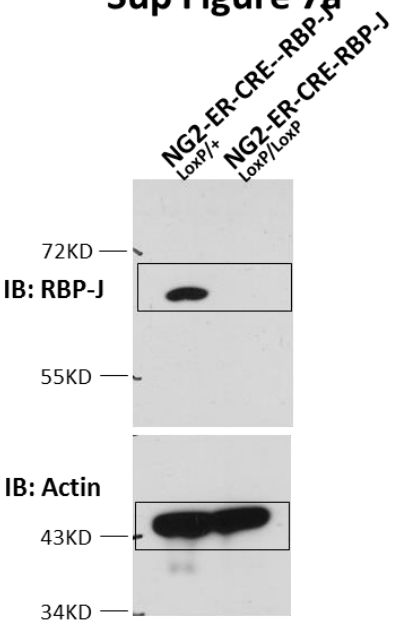
Sup Figure 2a



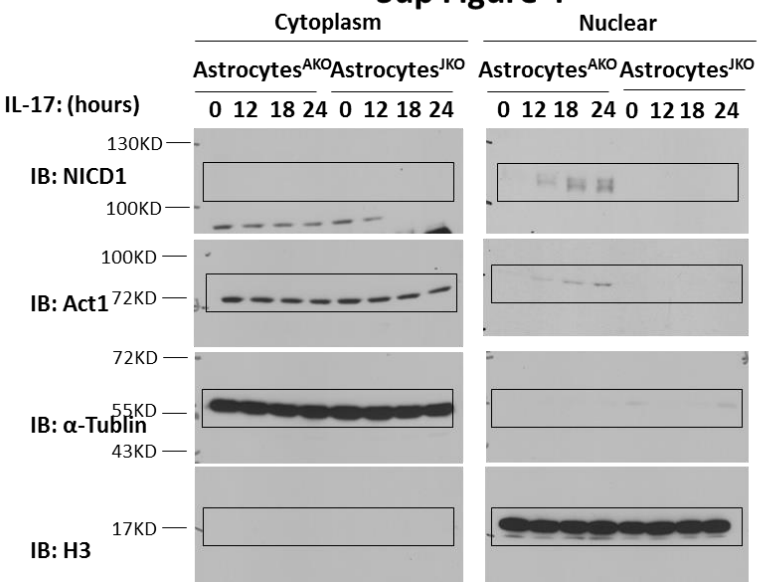
Sup Figure 2c



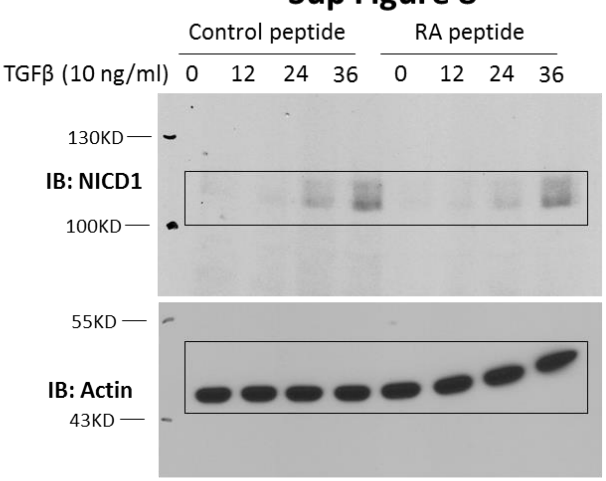
Sup Figure 7a



Sup Figure 4



Sup Figure 8



Supplementary table 1: Primer sequence

Gene name	Forward primer	Reverse Primer
Ccl7	5’-accagtagtcggtgtccctg-3’	5’-ctatagcctcctcgacccacttc-3’
Steap4	5’-gaacactagatgcaagccgg-3’	5’-gagagatccttggtccagtgg-3’
Ptx3	5’-gggtggactcctacagattgg-3’	5’-ctgagaacccgatcccagat-3’
Csf2	5’-ctaacatgtgtgcagacccg-3’	5’-gtctggtagtagctggctgtc-3’
Ccl20	5’-cacaagacagatggccgatg-3’	5’-ccctttcacccagttctgc-3’
Cxcl1	5’-ccagagcttgaaggtgttgc-3’	5’-tgaaccaagggagcttcagg-3’
Cp	5’-gtgactgtaacaagccctcg-3’	5’-agtgaagatgtccgtcccag-3’
Mmp9	5’-cgacatagacggcatccagt-3’	5’-gataggccgtgggaggtatag-3’
S100a9	5’-ggccaacaaagcaccttctc-3’	5’-tccttcttgctcagggtgtc-3’
Hes5	5’-tgcaccaggactacagcgag-3’	5’-cgctggaagtggtaaagcag-3’
Hes7	5’-catcaaccgcagcctagaag-3’	5’-cgaactccagtatctccgct-3’
Mbp	5’-cttcagaccatccaagaagacc-3’	5’-gtacttgctgtggccaggtact-3’
Mag	5’-tcaaggagaagcagatcctagc-3’	5’-tctcagccacacaccagtattc-3’
Mog	5’-ccttctcaagagtggttcacct-3’	5’-gggtaacctttccctcactgat-3’

Gene name	Forward primer	Reverse Primer
IL-17RA Δ33-320	5’ -CTGGGCGTGCTGGCCCCGGGTGGC GCCTCCGTGTACTGGTTCATCACGG GCATCTCCATCC-3’	5’ -GGATGGAGATGCCCGTGATGAACCAGTA CACGGAGGCGCCACCCGGGGCCAGCACGC CCAG-3’
IL-17RA Δ33-83	5’ -CGTGCTGGCCCCGGGTGGCGCCTCC CACACCCAACAAGGAGACCTGTTCC -3’	5’ -GGAACAGGTCTCCTTGTTGGGTGTGGGAG GCGCCACCCGGGGCCAGCACG-3’
IL-17RA Δ83-133	5’ -GGACCTGCAGATCCAGCTGCACTTT AAACTGAGGCATCACCACAGGCGGT -3’	5’ -ACCGCCTGTGGTGATGCCTCAGTTTAAAG TGCAGCTGGATCTGCAGGTCC-3’
IL-17RA Δ133-183	5’ -GTGCGTCAGGTTTGAGTTTCTGTCC GACTGTGAGCACGCCAGGATGAAGG -3’	5’ -CCTTCATCCTGGCGTGCTCACAGTCGGAC AGAAACTCAAACCTGACGCAC-3’
IL-17RA Δ183-233	5’ -CCAGTCCAAGAATTTCCTTGTGCCT CTGACCAGTTTTCCGCACATGGAGA- 3’	5’ -TCTCCATGTGCGGAAAACTGGTCAGAGGC ACAAGGAAATTCTTGGACTGG-3’
IL-17RA Δ233-283	5’ -CGAATCTACCCATTACCAGATCCTG CAGCCCTTCTTCAGCAGCTGCCTCA- 3’	5’ -TGAGGCAGCTGCTGAAGAAGGGCTGCAG GATCTGGTAATGGGTAGATTCG-3’
IL-17RA Δ283-320	5’ -GGTGCTGTCGCCACCAAGTGCAGAT CGTGTACTGGTTCATCACGGGCATC T-3’	5’ -AGATGCCCGTGATGAACCAGTACACGATC TGCACTTGGTGGCGACAGCACC-3’

Gene name	Forward primer	Reverse Primer
Act1 Δ50-100	5’ -AGAACCACCTGCTCCAAATATAAGGGAAGACAGTTTCTGCAGGAGACACC-3’	5’ -GGTGTCTCCTGCAGAAACTGTCTTCCCTTATATTTGGAGCAGGTGGTTCT-3’
Act1 Δ100-130	5’ -GCACTCAAGTTCTGGAGGACGTTGGAGCCCTCCCTGCAGA-3’	5’ -TCTGCAGGGAGGGCTCCAACGTCCTCCAGAACTTGAGTGC-3’
Act1 Δ130-155	5’ -GCGAGCCTGCGTCTGAGTCTGCGGCTTCTCCTGACACTGG-3’	5’ -CCAGTGTCAGGAGAAGCCGCAGACTCAGACGCAGGCTCGC-3’
Act1 Δ155-190	5’ -GTAATCAATGGCTGGTATCTCAGCTTCAGCCTCACAGGAACCGAGCAGGCC-3’	5’ -GGCCTGCTCGGTTCCTGTGAGGCTGAAGCTGAGATACCAGCCATTGATTAC-3’
Act1 Δ190-260	5’ -CGGTAGCCAGGAGATGGTGCAACGGCCTCCCAATCTTTCCCCACATGCTC-3’	5’ -GAGCATGTGGGGAAAGATTGGGAGGCCGTTGCACCATCTCCTGGCTACCG-3’
Act1 Δ260-360	5’ -GAGGTATCCAGCATGTGCACAGATGGCTGGTGCTCCTGGGGAGTCCTTGG-3’	5’ -GCTGGGGAACCTGTGGATTAGGTGGCTGGTGA-3’
Act1 Δ360-375	5’ -TCACCAGCCACCTAATCCACAGGTTCCCCAGC-3’	5’ -AGATGCCCGTGATGAACCAGTACACGATCTGCACTTGGTGGCGACAGCACC-3’
Act1 Δ375-420	5’ -GGAGTCCTTGGAGTGCCCTGCAGAGCTGGCTATGGAGGTGGTGAAATTCGTGA-3’	5’ -TCACGAATTTCACCACCTCCATAGCCAGCTCTGCAGGGCACTCCAAGGACTCC-3’

Supplementary table 2 : ChIP primer sequence

Gene name	Forward primer	Reverse Primer
CXCL1 ChIP	5’ -TTGGCTCCTGAGTCTAAGAA-3’	5’ -GTAAAACTGAGTCTTTGAATATT-3’
S100A9 ChIP	5’ -GGTTGCCAACTGTGCTTCCA-3’	5’ -ATGGCCAACAAAGCACCTTCT-3’
PTX3 ChIP	5’ -AGGGTAGTAGCCCTGCCATC-3’	5’ -GAACCACTCCCAGAGGATACC-3’
STEAP4 ChIP	5’ -TGGCATCCATTTAGGTAACAT-3’	5’ -CTCTGTGTTCTCCAGGGCT-3’
CCL7 ChIP	5’ -CCCTGCCCTTGCCTAATACTC-3’	5’ -AAGTGTCCAGGCACATAGGC-3’
CP ChIP	5’ -TATACATTTAGATACATGCCAC-3’	5’ -GAATGATTTTACTCAACTGGAA-3’
Control ChIP	5’ -TCGATATCCACGTGACATCCA-3’	5’ -GCAGCATTTTTTTACCCCCTC-3’