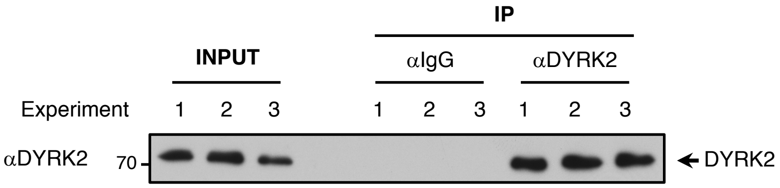
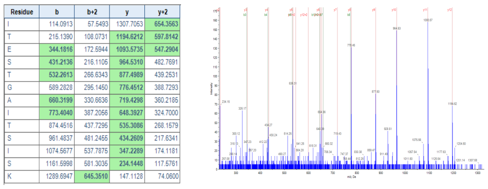
A



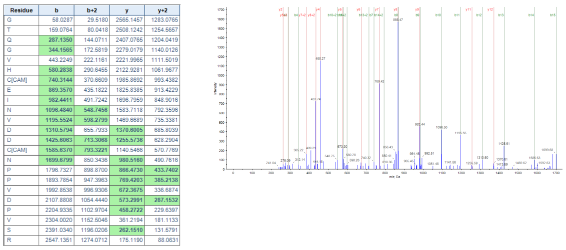
Representative Accession	Name	Control IGs Peptides	IP DYRK2 Peptides	Unused ProtScore	Total ProtScore	Confident Peptides	%Seq Cov - peptides >95% conf
sp|Q92630|DYRK2_HUMAN	Dual specificity tyrosine-phosphorylation-regulated kinase 2 OS=Homo sapiens GN=DYRK2 PE=1 SV=3	**0|0|0**	**18|8|11**	28,06	28,08	18	32,4
sp|P46531|NOTC1_HUMAN	Neurogenic locus notch homolog protein 1 OS=Homo sapiens GN=NOTCH1 PE=1 SV=4	**0|0|0**	**11|10|11**	14,60	25,07	12	33,1
sp|Q04721|NOTC2_HUMAN	Neurogenic locus notch homolog protein 2 OS=Homo sapiens GN=NOTCH2 PE=1 SV=3	**0|0|0**	**22|44|19**	54,31	54,31	44	23,3
sp|Q9UM47|NOTC3_HUMAN	Neurogenic locus notch homolog protein 3 OS=Homo sapiens GN=NOTCH3 PE=1 SV=2	**0|0|0**	**13|8|9**	21,95	21,99	13	6,1

Q92630 - DYRK2_HUMAN



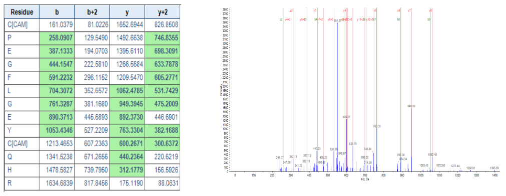
Residue	b	b+2	y	y+2
I	114.0913	57.5493	1307.7053	**654.3563**
T	215.1390	108.0731	**1194.6212**	**597.8142**
E	**344.1816**	172.5944	**1093.5735**	**547.2904**
S	**431.2136**	216.1105	**964.5310**	482.7691
T	**532.2613**	266.6343	**877.4989**	439.2531
G	589.2828	295.1450	**776.4512**	388.7293
A	**660.3199**	330.6636	**719.4298**	360.2185
I	**773.4040**	387.2056	**648.3927**	324.7000
T	874.4516	437.7295	**535.3086**	268.1579
S	961.4837	481.2455	**434.2609**	217.6341
I	1074.5677	537.7875	**347.2289**	174.1181
S	1161.5998	581.3035	**234.1448**	117.5761
K	1289.6947	**645.3510**	147.1128	74.0600

P46531 - NOTC1_HUMAN



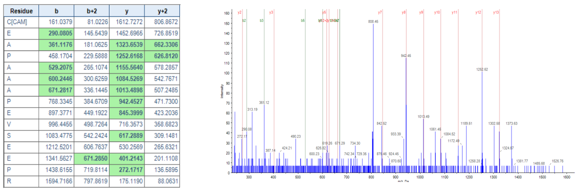
Residue	b	b+2	y	y+2
G	58.0287	29.5180	2565.1457	1283.0765
T	159.0764	80.0418	2508.1242	1254.5657
Q	**287.1350**	144.0711	2407.0765	1204.0419
G	**344.1565**	172.5819	2279.0179	1140.0126
V	443.2249	222.1161	2221.9965	1111.5019
H	**580.2838**	290.6455	2122.9281	1061.9677
C[CAM]	**740.3144**	370.6609	1985.8692	993.4382
E	**869.3570**	435.1822	1825.8385	913.4229
I	**982.4411**	491.7242	1696.7959	848.9016
N	**1096.4840**	**548.7456**	1583.7118	792.3596
V	**1195.5524**	**598.2799**	1469.6689	735.3381
D	**1310.5794**	655.7933	**1370.6005**	685.8039
D	**1425.6063**	**713.3068**	**1255.5736**	628.2904
C[CAM]	**1585.6370**	**793.3221**	1140.5466	570.7769
N	**1699.6799**	850.3436	**980.5160**	490.7616
P	1796.7327	898.8700	**866.4730**	**433.7402**
P	1893.7854	947.3963	**769.4203**	**385.2138**
V	1992.8538	996.9306	**672.3675**	336.6874
D	2107.8808	1054.4440	**573.2991**	**287.1532**
P	2204.9335	1102.9704	**458.2722**	229.6397
V	2304.0020	1152.5046	361.2194	181.1133
S	2391.0340	1196.0206	**262.1510**	131.5791
R	2547.1351	1274.0712	175.1190	88.0631

Q04721 - NOTC2_HUMAN



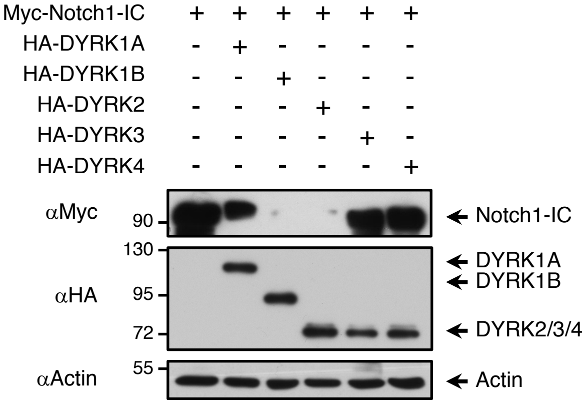
Residue	b	b+2	y	y+2
C[CAM]	161.0379	81.0226	1652.6944	826.8508
P	**258.0907**	129.5490	1492.6638	**746.8355**
E	**387.1333**	194.0703	1395.6110	**698.3091**
G	**444.1547**	222.5810	1266.5684	**633.7878**
F	**591.2232**	296.1152	1209.5470	**605.2771**
L	**704.3072**	352.6572	**1062.4785**	**531.7429**
G	**761.3287**	381.1680	**949.3945**	**475.2009**
E	**890.3713**	445.6893	**892.3730**	446.6901
Y	**1053.4346**	527.2209	**763.3304**	**382.1688**
C[CAM]	1213.4653	607.2363	**600.2671**	**300.6372**
Q	1341.5238	671.2656	**440.2364**	220.6219
H	1478.5827	739.7950	**312.1779**	156.5926
R	1634.6839	817.8456	175.1190	88.0631

Q9UM47 - NOTC3_HUMAN



Residue	b	b+2	y	y+2
C[CAM]	161.0379	81.0226	1612.7272	806.8672
E	**290.0805**	145.5439	1452.6965	726.8519
A	**361.1176**	181.0625	**1323.6539**	**662.3306**
P	458.1704	229.5888	**1252.6168**	**626.8120**
A	**529.2075**	265.1074	**1155.5640**	578.2857
A	**600.2446**	300.6259	**1084.5269**	542.7671
A	**671.2817**	336.1445	**1013.4898**	507.2485
P	768.3345	384.6709	**942.4527**	471.7300
E	897.3771	449.1922	**845.3999**	423.2036
V	996.4455	498.7264	716.3573	358.6823
S	1083.4775	542.2424	**617.2889**	309.1481
E	1212.5201	606.7637	530.2569	265.6321
E	1341.5627	**671.2850**	**401.2143**	201.1108
P	1438.6155	719.8114	**272.1717**	136.5895
R	1594.7166	797.8619	175.1190	88.0631

B



C

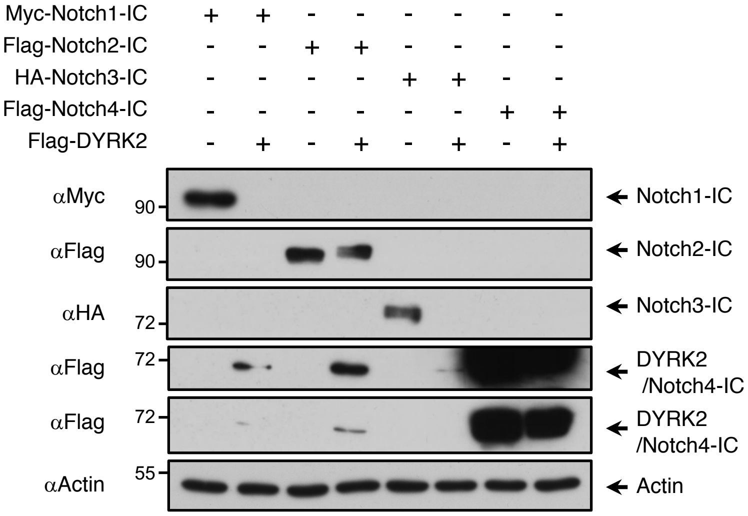


Fig. S1

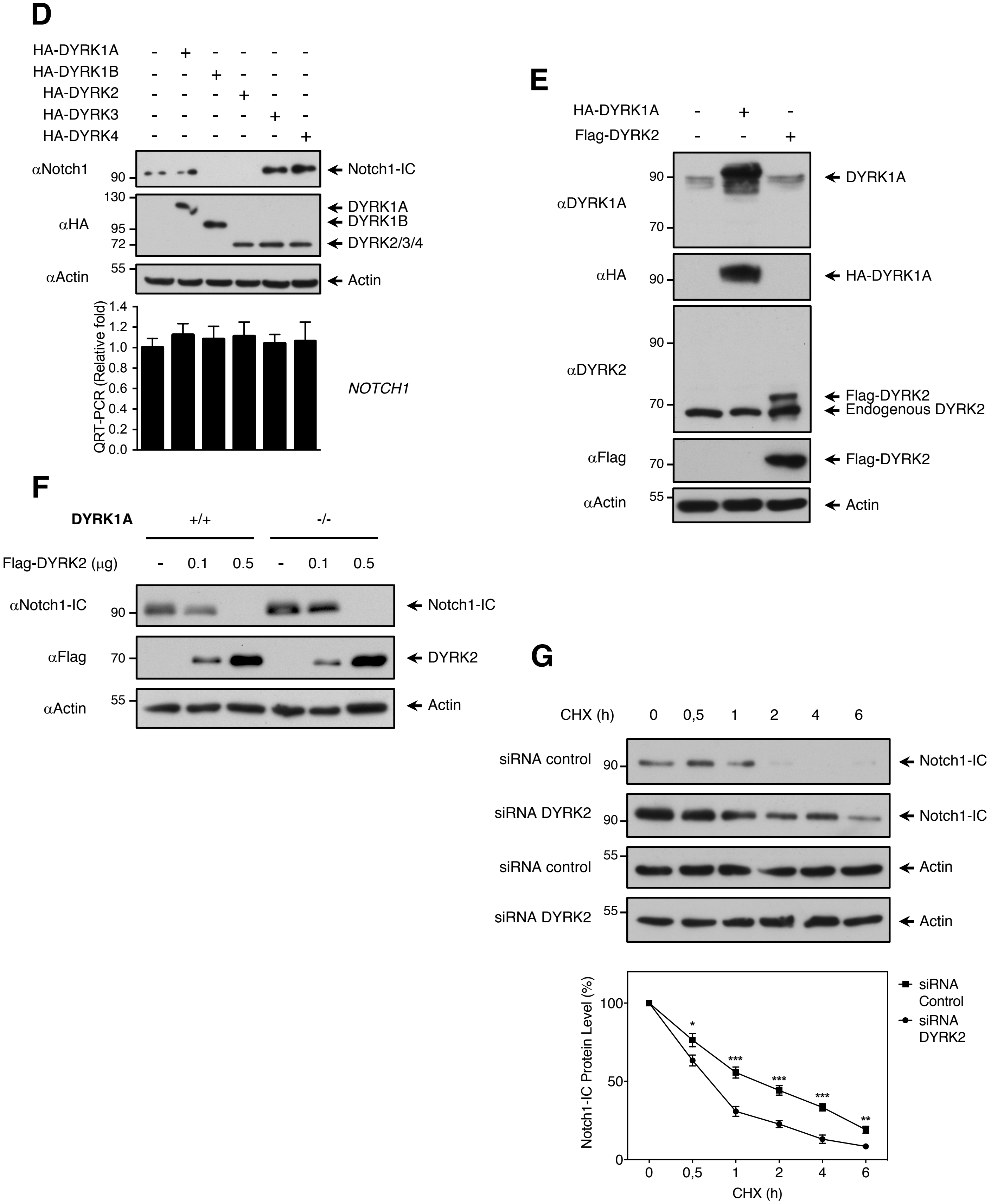
D
HA-DYRK1A - + - - - -
HA-DYRK1B - - + - - -
HA-DYRK2 - - - + - -
HA-DYRK3 - - - - + -
HA-DYRK4 - - - - - +
αNotch1
90
Notch1-IC
αHA
130
95
72
DYRK1A
DYRK1B
DYRK2/3/4
αActin
55
Actin
QRT-PCR (Relative fold)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
NOTCH1
E
HA-DYRK1A - + -
Flag-DYRK2 - - +
αDYRK1A
90
70
DYRK1A
αHA
90
HA-DYRK1A
αDYRK2
90
70
Flag-DYRK2
Endogenous DYRK2
αFlag
70
Flag-DYRK2
αActin
55
Actin
F
DYRK1A
+/+
-/-
Flag-DYRK2 (μg) - 0.1 0.5 - 0.1 0.5
αNotch1-IC
90
Notch1-IC
αFlag
70
DYRK2
αActin
55
Actin
G
CHX (h) 0 0,5 1 2 4 6
siRNA control
90
Notch1-IC
siRNA DYRK2
90
Notch1-IC
siRNA control
55
Actin
siRNA DYRK2
55
Actin
Notch1-IC Protein Level (%)
0
50
100
*
***
***
***
**
0
0,5
1
2
4
6
CHX (h)
siRNA Control
siRNA DYRK2

Fig. S1

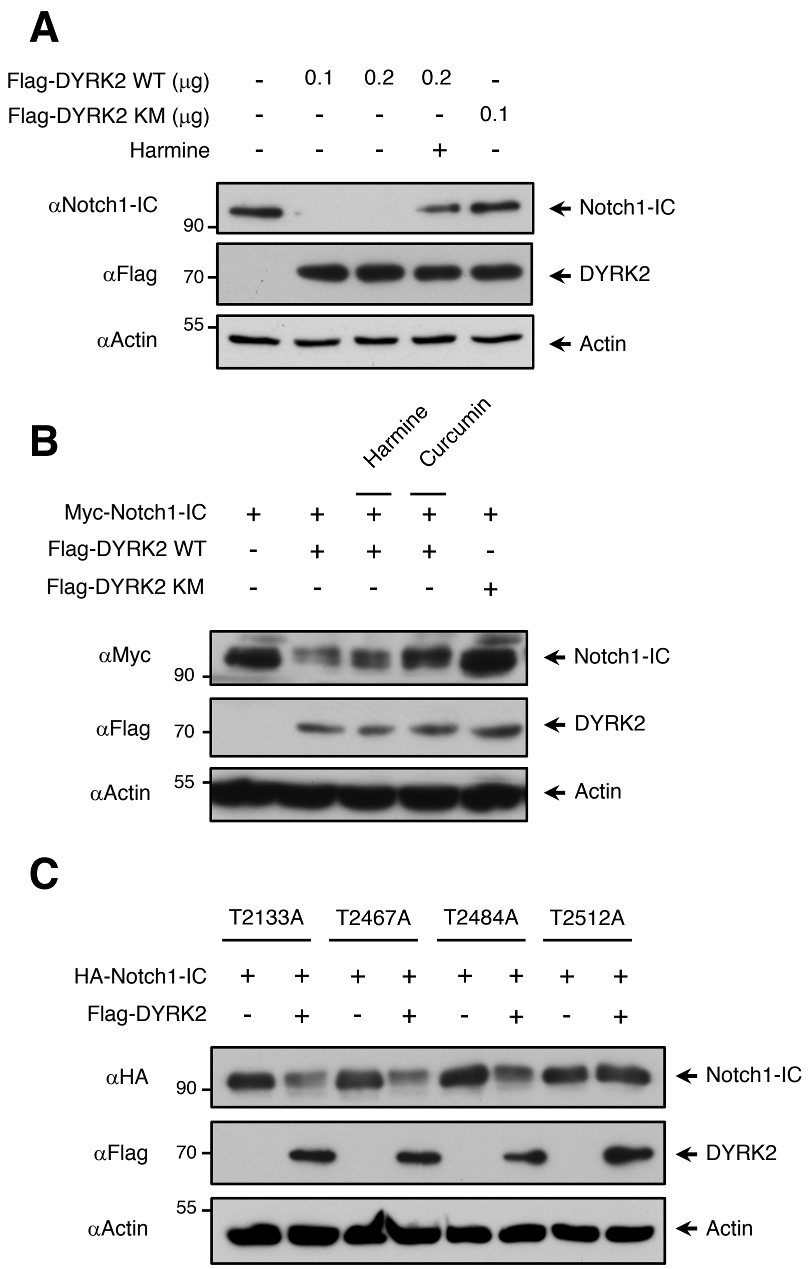
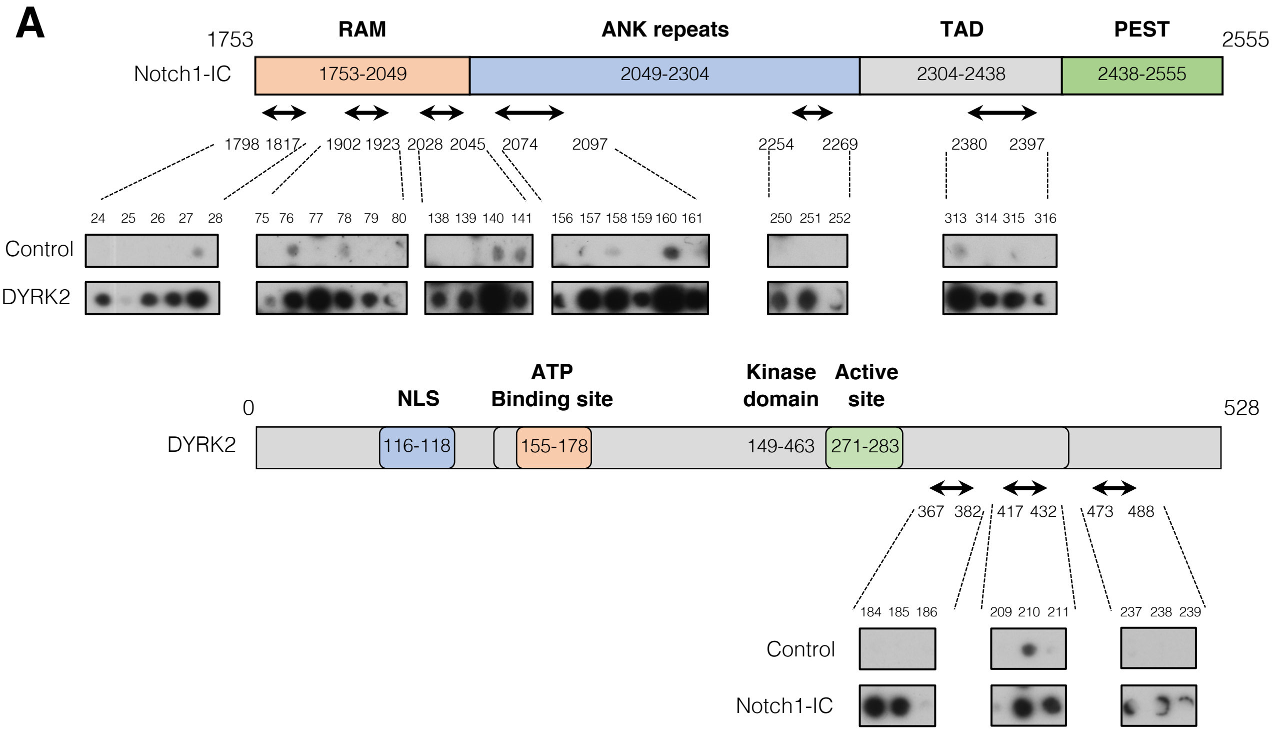

A
Flag-DYRK2 WT (μg) - 0.1 0.2 0.2 -
Flag-DYRK2 KM (μg) - - - - 0.1
Harmine - - - + -
αNotch1-IC
90
Notch1-IC
αFlag
70
DYRK2
αActin
55
Actin
B
Harmine
Curcumin
Myc-Notch1-IC + + + + +
Flag-DYRK2 WT - + + + -
Flag-DYRK2 KM - - - - +
αMyc
90
Notch1-IC
αFlag
70
DYRK2
αActin
55
Actin
C
T2133A
T2467A
T2484A
T2512A
HA-Notch1-IC + + + + + + + +
Flag-DYRK2 - + - + - + - +
αHA
90
Notch1-IC
αFlag
70
DYRK2
αActin
55
Actin

Fig. S2



B

Notch-IC

24	KNASDGALMDDN
25	ASDGALMDDNQN
26	DGALMDDNQNEW
27	ALMDDNQNEWGD
28	MDDNQNEWGDED
75	EEEDAPAVISDF
76	EDAPAVISDFIY
77	APAVISDFIYQG
78	AVISDFIYQGAS
79	ISDFIYQGASLH
80	DFIYQGASLHNQ
138	GKSALHWAAAVN
139	SALHWAAAVNNV
140	LHWAAAVNNVDA
141	WAAAVNNVDAAV
156	YETAKVLLDHFA
157	TAKVLLDHFANR
158	KVLLDHFANRDI
159	LLDHFANRDITD
160	DHFANRDITDHM
161	FANRDITDHMDR
162	NRDITDHMDRLP
250	EMAALGGGGRLA
251	AALGGGGRLAFE
252	LGGGGRLAFETG
313	VQTQQVQPQNLQ
314	TQQVQPQNLQMQ
315	QVQPQNLQMQQQ
316	QPQNLQMQQQNL

DYRK2

184	MPSQKLLDASKR
185	SQKLLDASKRAK
186	KLLDASKRAKNF
209	PPESREWGNALK
210	ESREWGNALKGC
211	REWGNALKGCDD
237	EKTSVKRITEST
238	TSVKRITESTGA
239	VKRITESTGAIT

Fig. S4

C

HA-Notch1-RAM1:
1798 1817
KNASDGALMDDNQNEWGDED WT
KNASDGAL**AAA**NQNEWGDED Mutant

HA-Notch1-RAM2:
1902 1923
EEEDAPAVISDFIYQGASLHNQ WT
EEEDAPAVI**AAA**IYQGASLHNQ Mutant

HA-Notch1-RAM3:
2028 2045
GKSALHWAAAVNNVDAAV WT
GKSALHWAA**KEA**NVDAAV Mutant

HA-Notch1-ANK1:
2074 2097
YETAKVLLDHFANRDITDHMDRLP WT
YETAKVLL**AAAA**ANRDITDHMDRLP Mutant

HA-Notch1-ANK2:
2254 2269
EMAALGGGGRLAFETG WT
EMAALG**AAA**RLAFETG Mutant

HA-Notch1-TAD1:
2380 2397
VQTQQVQPQNLQMQQQNL WT
VQTQQVQ**AAA**LQMQQQNL Mutant

D

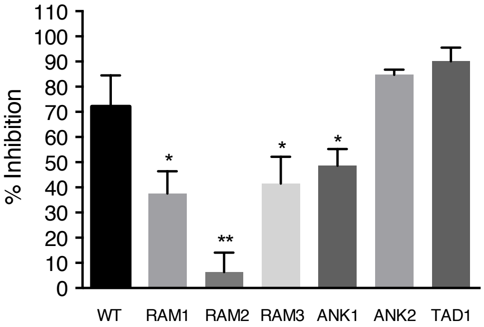


Fig. S4

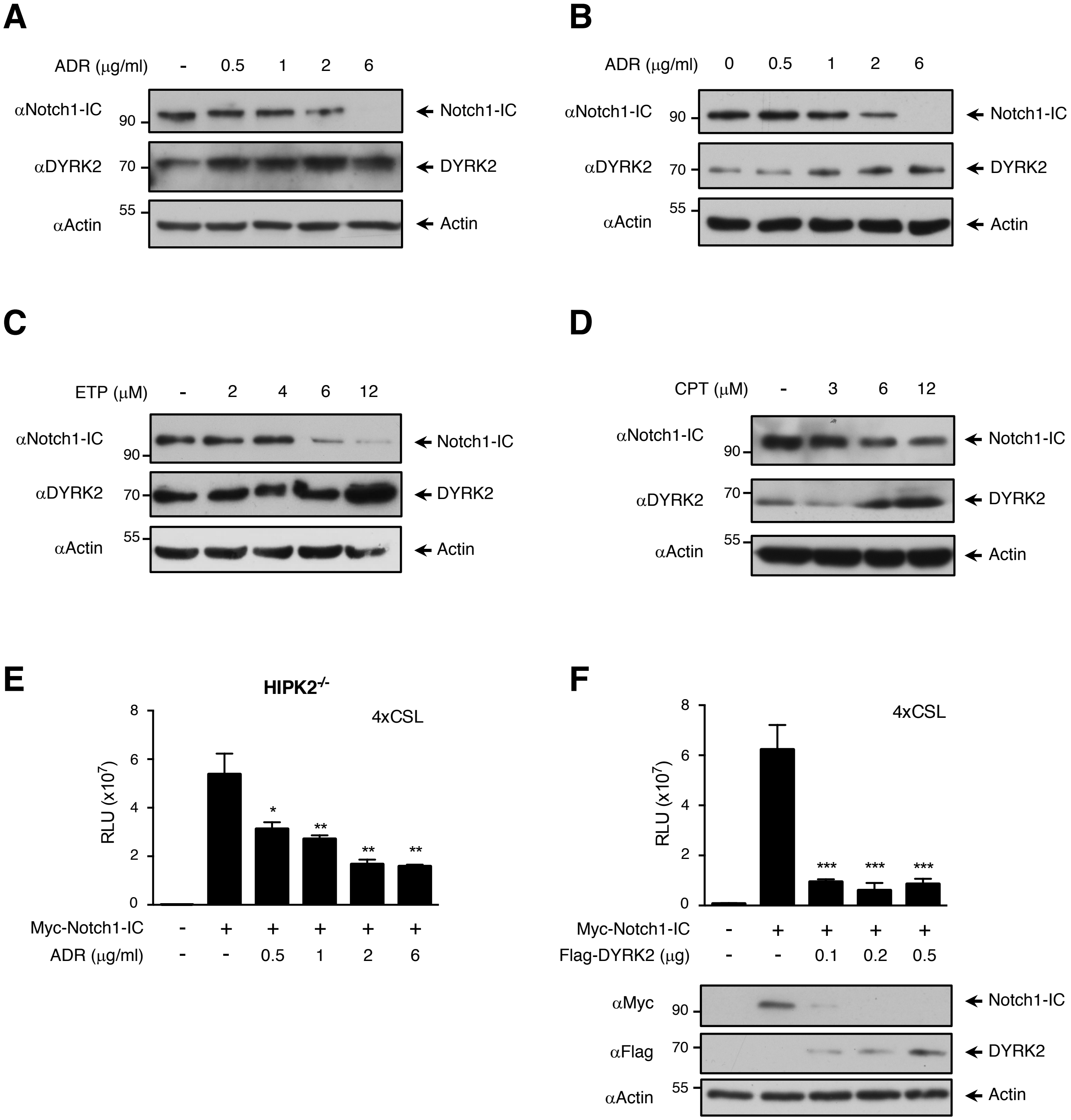

A
ADR (μg/ml) - 0.5 1 2 6
αNotch1-IC 90
αDYRK2 70
αActin 55
Notch1-IC
DYRK2
Actin
B
ADR (μg/ml) 0 0.5 1 2 6
αNotch1-IC 90
αDYRK2 70
αActin 55
Notch1-IC
DYRK2
Actin
C
ETP (μM) - 2 4 6 12
αNotch1-IC 90
αDYRK2 70
αActin 55
Notch1-IC
DYRK2
Actin
D
CPT (μM) - 3 6 12
αNotch1-IC 90
αDYRK2 70
αActin 55
Notch1-IC
DYRK2
Actin
E
HIPK2-/-
4xCSL
RLU (x10^7)
0
2
4
6
8
*
**
**
**
Myc-Notch1-IC - + + + + +
ADR (μg/ml) - - 0.5 1 2 6
F
4xCSL
RLU (x10^7)
0
2
4
6
8
***
***
***
Myc-Notch1-IC - + + + +
Flag-DYRK2 (μg) - - 0.1 0.2 0.5
αMyc 90
αFlag 70
αActin 55
Notch1-IC
DYRK2
Actin

Fig. S5

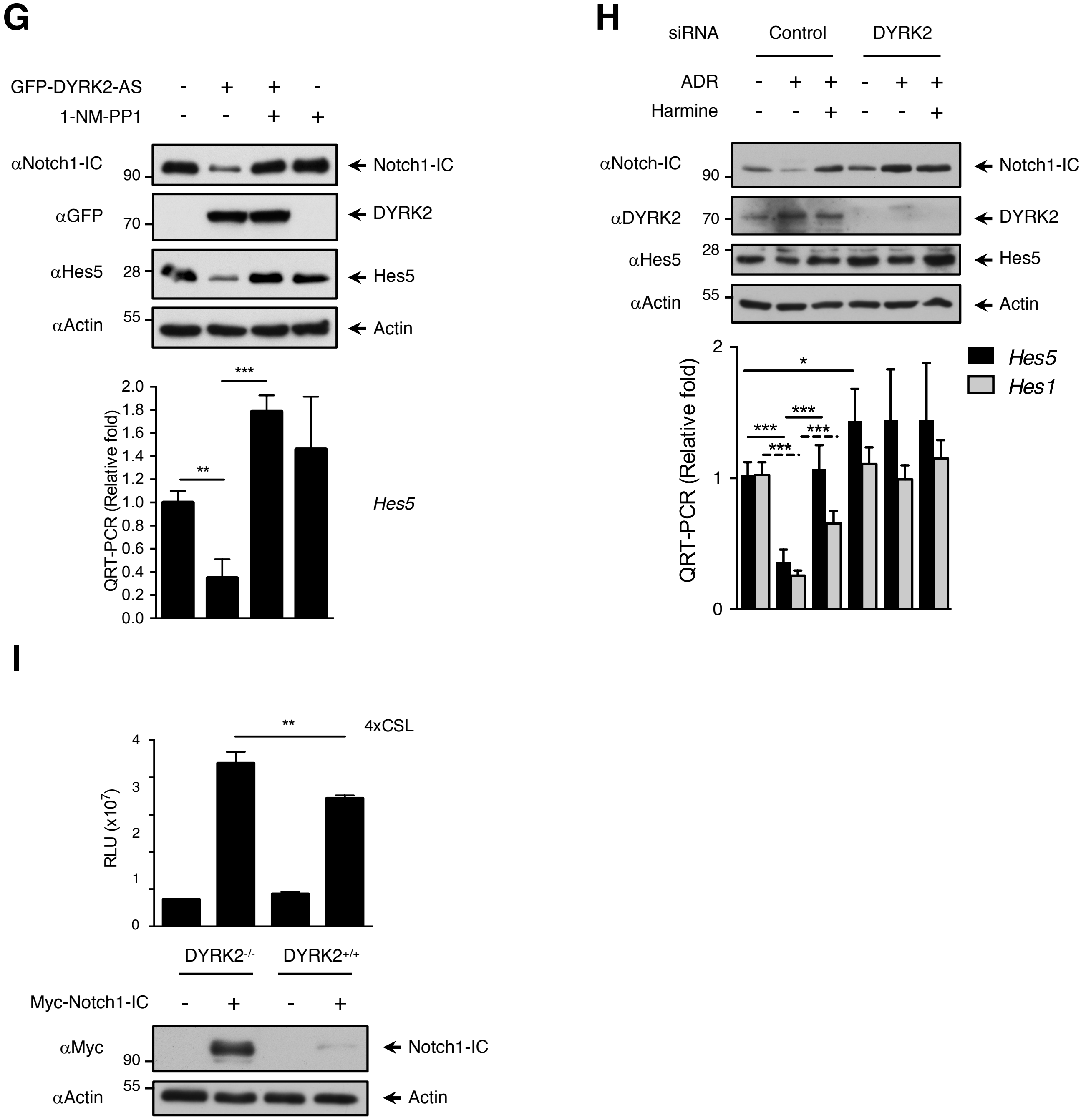

G
GFP-DYRK2-AS - + + -
1-NM-PP1 - - + +
αNotch1-IC
90
Notch1-IC
αGFP
70
DYRK2
αHes5
28
Hes5
αActin
55
Actin
QRT-PCR (Relative fold)
2.0
1.8
1.6
1.4
1.2
1.0
0.8
0.6
0.4
0.2
0.0
**
***
Hes5
H
siRNA
Control
DYRK2
ADR - + + - + +
Harmine - - + - - +
αNotch-IC
90
Notch1-IC
αDYRK2
70
DYRK2
αHes5
28
Hes5
αActin
55
Actin
QRT-PCR (Relative fold)
2
1
0
*
***
***
***
***
Hes5
Hes1
I
4xCSL
**
RLU (x10^7)
4
3
2
1
0
DYRK2-/-
DYRK2+/+
Myc-Notch1-IC - + - +
αMyc
90
Notch1-IC
αActin
55
Actin

Fig. S5

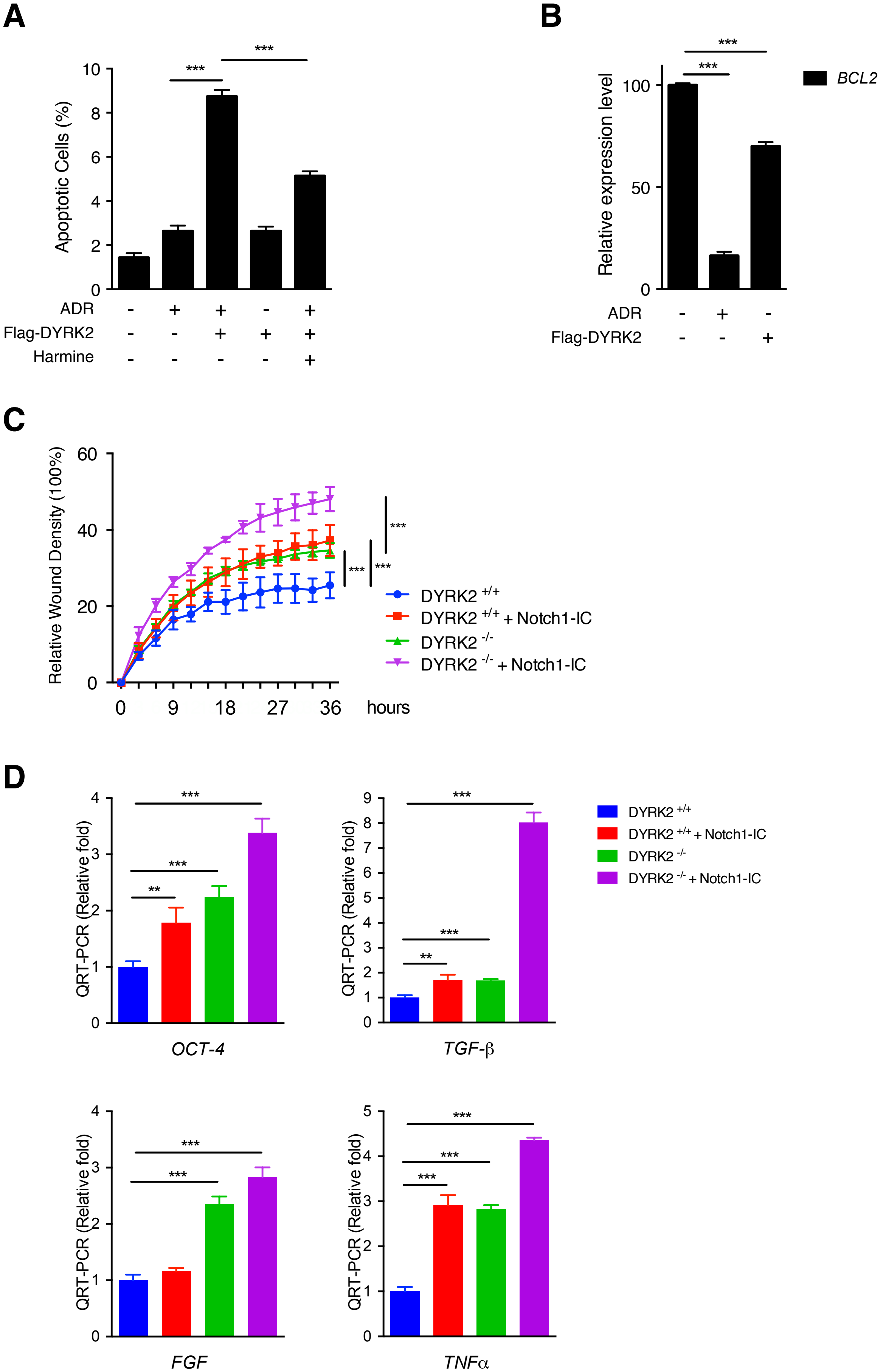

A
B
C
D
Apoptotic Cells (%)
10
8
6
4
2
0
***
***
ADR - + + - +
Flag-DYRK2 - - + + +
Harmine - - - - +
Relative expression level
100
50
0
***
***
BCL2
ADR - + -
Flag-DYRK2 - - +
Relative Wound Density (100%)
60
40
20
0
0
9
18
27
36
hours
***
***
***
DYRK2 +/+
DYRK2 +/+ + Notch1-IC
DYRK2 -/-
DYRK2 -/- + Notch1-IC
QRT-PCR (Relative fold)
4
3
2
1
0
***
***
**
OCT-4
9
8
7
6
5
4
3
2
1
0
***
***
**
TGF-β
DYRK2 +/+
DYRK2 +/+ + Notch1-IC
DYRK2 -/-
DYRK2 -/- + Notch1-IC
4
3
2
1
0
***
***
FGF
5
4
3
2
1
0
***
***
***
TNFα

Fig. S6