

Cullin-5 adaptor SPSB1 controls NF- κ B activation downstream of multiple signalling pathways

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Supplementary Material

Figure S1

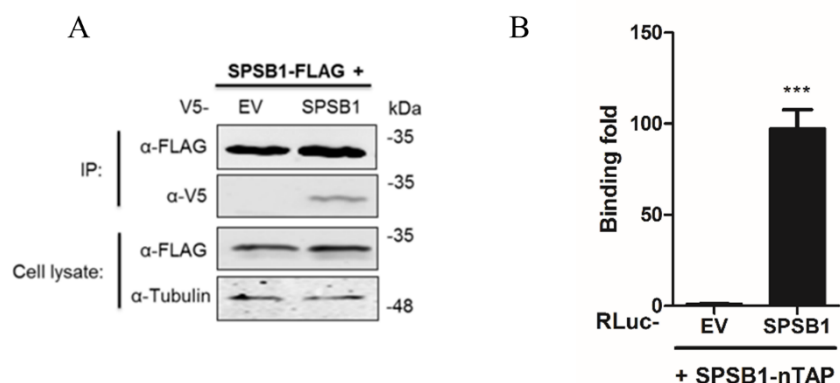


Figure S1. SPSB1 self-associates. (A) HEK293T cells were co-transfected with V5-tagged SPSB1, or an empty vector (EV) and FLAG-tagged SPSB1. The cells were lysed in IP buffer and subjected to FLAG IP. The IP and cell lysates were loaded onto an SDS-PAGE gel and immunoblotted against V5, FLAG and α -tubulin. (B) HEK293T cells were co-transfected in triplicates with RLuc-tagged SPSB1, or RLuc-only (EV) as a control and SPSB1-nTAP. Cells were lysed in IP buffer supplemented with protease inhibitors and lysates subjected to AP using streptavidin beads. The beads were eluted with biotin and the luciferase activity was measured both in lysates and eluates. The binding fold was calculated as an eluate/lysate ratio and this was normalised to the EV control. The experiments have been repeated three times independently. Means and standard deviations are shown and statistical significance was determined by using an unpaired Student's *t*-test (***) $p \leq 0.001$.

Figure S2

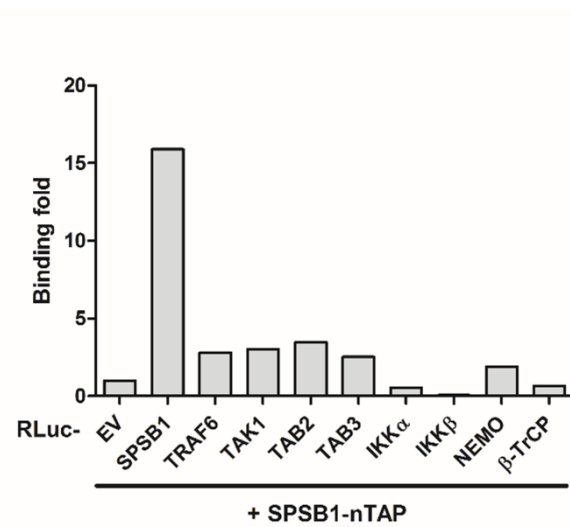


Figure S2. SPSB1 does not associate with any of the NF-κB components tested in LUMIER screens. HEK293T cells were co-transfected with SPSB1-nTAP and different components of the NF-κB pathway fused to RLuc, or an RLuc-only (EV) control. SPSB1 self-association was used as a positive control. Cells were treated as in Fig. S1 and the binding fold was calculated as an eluate/lysate ratio and this was normalised to the EV control. Data are representative of 2 experiments showing similar results.

Figure S3

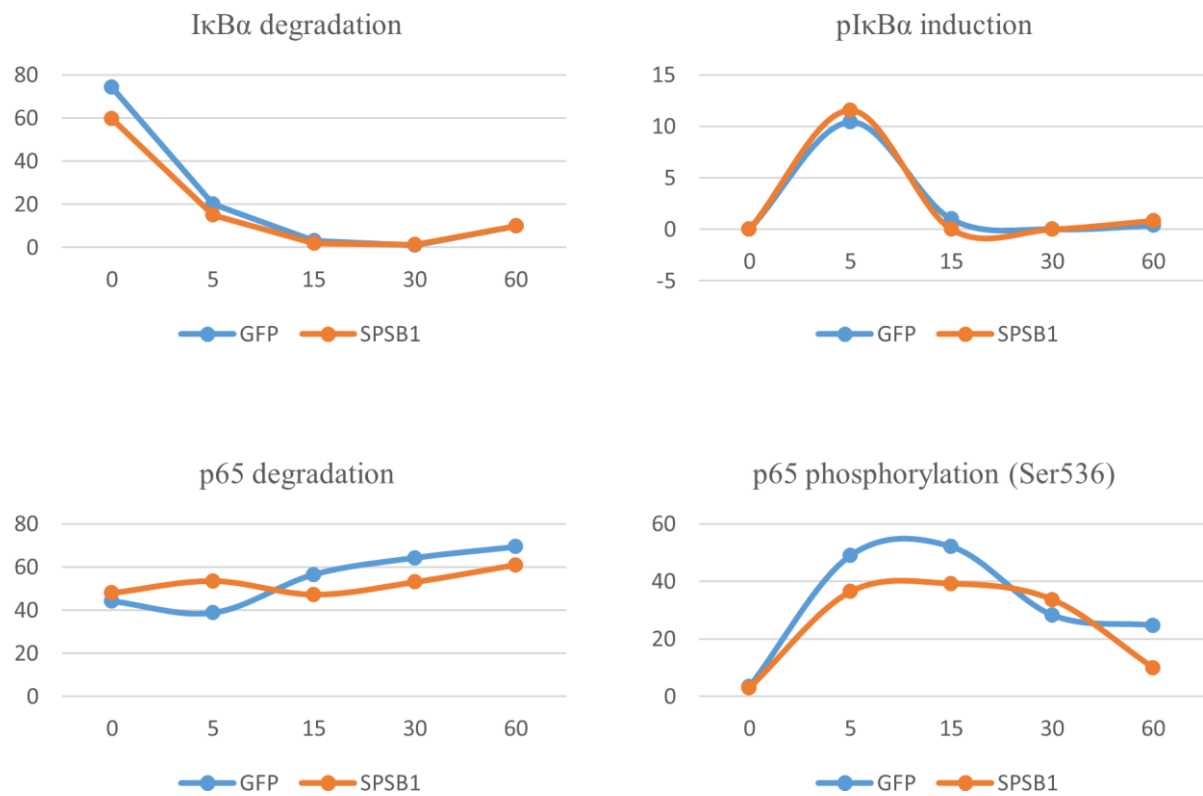


Figure S3. Densitometric analysis of the phosphorylation and degradation of I κ B α and p65 in GFP- or SPSB1-expressing A549 cells. Immunoblotting results from Fig. 6 were quantitated (arbitrary units) and plotted over time.

Table S1. RNAi sequences employed for the specific knock-down of CRL5 substrate adaptor molecules.

Gene Symbol	Accession number	Duplex Number	Sequence
FBXW11	NM_033645	D-003490-02	GCACAUUGGUGGAACAUUC
FBXW11	NM_033645	D-003490-03	GUUAGUGGAUCAUCAGAU
FBXW11	NM_033645	D-003490-04	GAGCAAGGCUUAGAUCACA
FBXW11	NM_033645	D-003490-17	AGAAGUAAAUCGACCGUCA
ASB1	NM_001040445	D-017197-01	GAAGAAAAGUGGACCCUGA
ASB1	NM_001040445	D-017197-02	GAGCCAACCUGAAUCUAGU
ASB1	NM_001040445	D-017197-03	GCAAACACCGGCUUCAUCU
ASB1	NM_001040445	D-017197-04	GCGCGAACCCUGACUUCAA
ASB2	NM_016150	D-009575-01	GAAAGGCCAUUGGGAAAUA
ASB2	NM_016150	D-009575-02	GCACGAGGCCGCAUACUAU
ASB2	NM_016150	D-009575-03	GAACAUCGACGCCUAUAUC
ASB2	NM_016150	D-009575-04	UUAGAUACCUGAAAUAACGA
ASB3	NM_016115	D-017457-01	GGACAAAGCUACACCCUUG
ASB3	NM_016115	D-017457-02	GAAAGCUUGAGCAUACUUA
ASB3	NM_016115	D-017457-03	GAACGUCUACGGUCUGACA
ASB3	NM_016115	D-017457-04	CUUUGGAGUUUACUAAUUG
ASB4	NM_016116	D-013339-01	CUACAAAGCCGAAGUCAAU
ASB4	NM_016116	D-013339-02	CCAAGUUAGUUAAGAGAAA
ASB4	NM_016116	D-013339-03	CAAGGUUACUGGUUGCCUA
ASB4	NM_016116	D-013339-04	CCACAAUGCUACAAUCAAC
ASB5	NM_080874	D-017458-02	GGAUAUUGCUUCAACAUGA
ASB5	NM_080874	D-017458-03	GCCGUUUGCUCAACAAUUA
ASB5	NM_080874	D-017458-04	CAAGGAAGCGGCAAGGAUA
ASB5	NM_080874	D-017458-17	UGUAAUGGAUAACGUGCAU
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ASB6	NM_017873	D-013355-02	GGAGGAUCAUCUUCGAGUA
ASB6	NM_017873	D-013355-03	GCAACGCCCUGCUCAAGAU
ASB6	NM_017873	D-013355-04	CUGAAGAGCUUUAACUGC
ASB7	NM_024708	D-012930-01	GUGCACGGAUGUUAUAUAA
ASB7	NM_024708	D-012930-02	GGAGCGACAUCAUUAUUGC
ASB7	NM_024708	D-012930-03	GCGAUACGCCGUGAUCAAA
ASB7	NM_024708	D-012930-04	CGAACACACGGAACUAUGA
ASB8	NM_024095	D-014301-01	GGGCAGCCUUUAAGAACAA
ASB8	NM_024095	D-014301-02	GCGCUUAAUCCGAACAAUU
ASB8	NM_024095	D-014301-03	GCGCAGAGGUCAGAGUCAU
ASB8	NM_024095	D-014301-04	CUGGAUGGGUAUAACCGAA
ASB9	NM_024087	D-014295-01	GCAUCAGGCUUCUUUCAA
ASB9	NM_024087	D-014295-02	GCAUGGAGCUCAGGUGAAU
ASB9	NM_024087	D-014295-03	GCACGGAGCCAGCGUUCAA
ASB9	NM_024087	D-014295-04	GAUGGGCGAUGCUGUGUCU
ASB10	NM_080871	D-007725-01	CAACAUCGCUGACCAGGAU

ASB10	NM_080871	D-007725-02	CAACAUCCGUGCUCUGAGA
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RAB40A	NM_080879	D-008924-05	GGGUAUGGAUCGAUGGAUU
RAB40B	NM_006822	D-008353-01	CGGCAUUGAUCGAUGGAUU
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