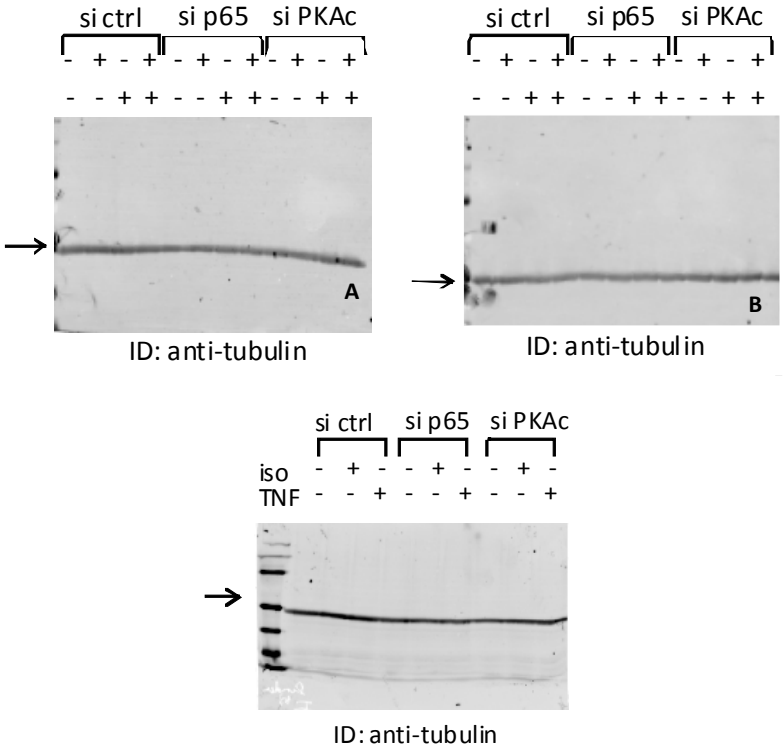
Table 1

Cell type studied	antibody	technique	MW	Reference
L929sA	non-commercial (Dr.Cohen, Dundee)	IF, ChIP	NA	Vermeulen L et al (2003) *Embo J* 22: 1313-1324.
KBM-5	Rockland #100-401-264	WB	?	Takada Y *et a*l (2004) *J Biol Chem* 279: 15096-15104.
melanoma	CS #3037	WB	?	Kuphal S *et al* (2004) *Oncogene* 23: 8509-8519.
MDA-MB-231	CS #3037	WB	?	Ho WC *et al* (2005). *Cancer Res* 65: 4273-4281.
MEFs	Abcam #ab30623	WB	?	Jacque E *et al* (2005). *Proc Natl Acad Sci U S A* 102: 14635-14640.
Hela, 293T	?	WB, ChIP	?	Chen LF *et al* (2005) *Mol Cell Biol* 25: 7966-7975.
liver	CS #3037	IHC	NA	Mackay H *et al* (2005) *Clin Cancer Res* 11: 5526-5533.
muscle	CS #3037	IHC	NA	Messina S *et al* (2006) *Am J Pathol* 168: 918-926.
Raw274.7	CS #3037	WB	?	Chen BC *et al* (2006) *J Immunol* 177: 681-693.
CV-1	CS #3037	WB	?	Hargett D *et al* (2006) *J Virol* 80: 10565-10578.
C2C12	CS #3037	WB	?	Kefaloyianni E *et al* (2006) *Cell Signal* 18: 2238-2251.
L929sA	CS #3037	WB	130 kDa	Vanden Berghe W *et al* (2006) *Cancer Res* 66: 4852-4862.
retina	CS #3037	WB	?	Miki K *et al* (2007) *Exp Eye Res* 84: 285-292.
chondrocytes	CS #3037	WB	?	Dossumbekova A *et al* (2007) *Arthritis Rheum* 56: 3284-3296.
synovial fibroblasts	CS #3037	WB	?	Tang *et al* (2007) *J Immunol* 179: 5483-5492.
lung, HUVEC	CS #3037	WB	?	Gorska MM et al (2007) J Exp Med 204: 1637-1652.
Hela	CS #3037	WB	?	Horion J *et al* (2007) *J Biol Chem* 282: 15383-15393.
chondrocytes	CS #3037	WB	?	Hsu HC *et al* (2007) *Cell Signal* 19: 2317-2328.
U937	CS #3031?	WB, ChIP	?	Jamaluddin M *et al* (2007) *Cell Signal* 19: 1419-1433.
BEAS-2B	Chemicon #AB3375	WB	?	Kim YM *et al* (2007) *Cell Signal* 19: 538-546.
skin	CS #3037	WB	?	Lee JC *et al* (2007) *Carcinogenesis* 28: 1491-1498.
A549	CS #3037	WB	?	Liu P *et al* (2007) *J Virol* 81: 1401-1411.
K562	CS #3037	WB	?	Oh SM *et al* (2007) *J Immunol* 179: 5686-5692.
Raw264.7	CS #3037	WB	?	Olson CM *et al* (2007) *Infect Immun* 75: 270-277.
keratinocytes	?	WB	?	Otkjaer K *et al* (2007) *J Invest Dermatol* 127: 1326-1336.
HUVEC	CS #3037	WB	?	Partridge J *et al* (2007) *Faseb J* 21: 3553-3561.
astrocytes	Abcam #ab30623	WB, IF	?	Saha RN et al (2007) J Immunol 179: 7101-7109.
A549	CS #3037	WB	130 kDa	Beck IM *et al* (2008) *Embo J* 27: 1682-1693.
airway smooth muscle cells	CS #3037	WB	?	Clarke DL et al. (2008) J Immunol 181: 3503-3514.

mononuclear cells	CS #3037	WB	?	Deree J *et al* (2008) *Clinics* 63: 321-328.
U266, L363	CS #3037	WB	80 kDa	Gerlo S *et al* (2008) *Cell Signal* 20: 1489-1496.
Ad5	CS #3037	WB	?	Guan H *et al* (2008) *J Virol* 82: 40-48.
osteoblasts	CS #3037	WB	?	Hou CH *et al* (2008) *Cell Signal* 20: 978-988.
MEFs	CS #3037	WB	?	Laszlo CF *et al* (2008) *Photochem Photobiol* 84: 1564-1568.
Hela	Santa Cruz?	WB, ChIP	80 kDa	Nowak DE *et al* (2008) *Mol Cell Biol* 28: 3623-3638.
glioma	CS #3037	WB, IF	?	Nozell S *et al* (2008) *Mol Cell Biol* 28: 6632-6645.
intestinal epithelial cells	CS #3037	WB, IHC	?	Onizawa M *et al* (2009) *Am J Physiol Gastrointest Liver Physiol* 296:G850-9.
Hela	CS #3037	WB	?	Ishinaga H et al (2009) Biochem J 417: 583-591.
Raw264.7	CS #3037	WB	?	Wijayanti N et al (2008) Free Radic Biol Med 44: 699-710.
Hela	CS #3037	WB	?	Williams LM et al (2008) Mol Immunol 45: 2446-2454.
Schwann cells	CS #3037	WB	?	Yoon C et al (2008) J Neurosci 28: 3738-3746.
Raw264.7	CS #3037	WB	?	Furusawa J et al (2009) Cell Signal 21: 778-785.
Raw264.7	CS #3037	WB	?	Furusawa J et al (2009) Int Immunopharmacol 9: 499-507.
bone marrow neutrophils	CS #3037	WB	?	Lorne E et al (2009) Am J Respir Cell Mol Biol. [Epub ahead of print]
lung fibroblasts	CS #3037	WB	70-80 kDa	Reber L et al (2009) PLoS ONE 4: e4393.

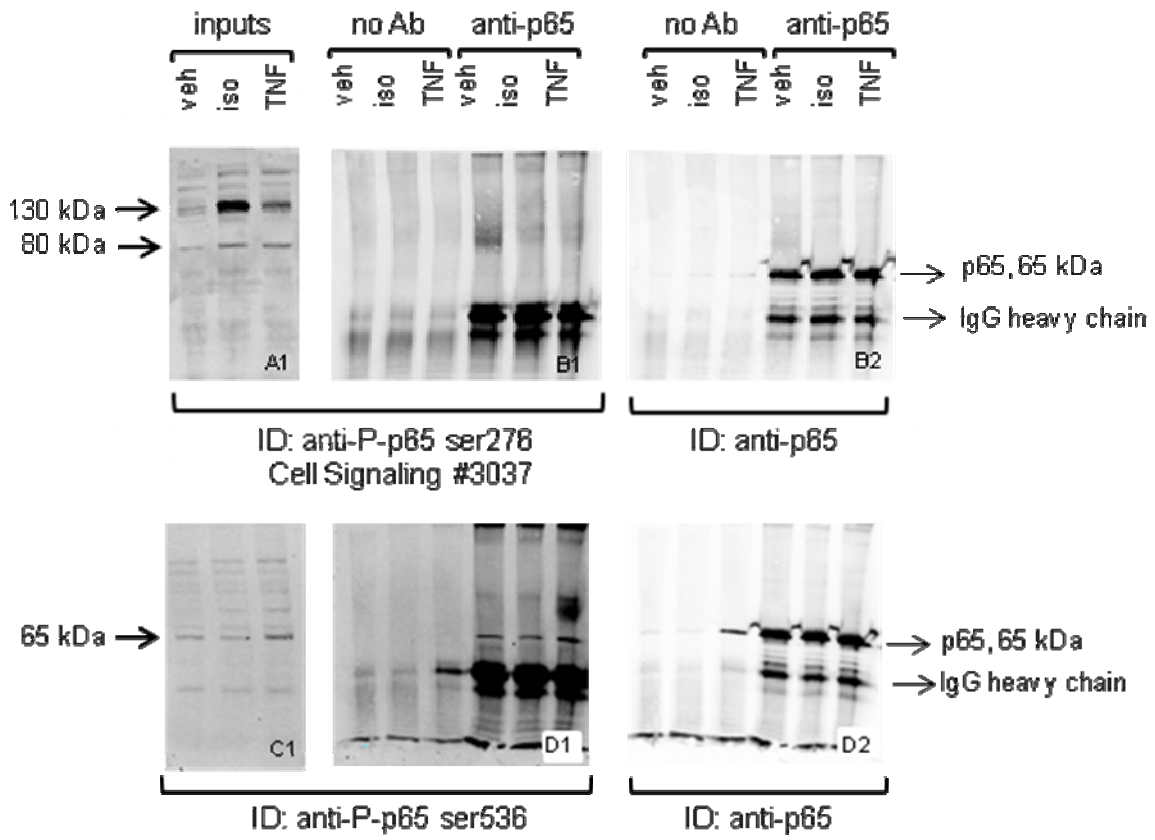
Suppl. table 1. Summary of reports that have used phospho-specific antibodies to show phosphorylation of p65 at the Ser276 residue.

In the table the cell types in which p65 phosphorylation was investigated, and the antibody-based techniques used to study p65 phosphorylation, are indicated. All corresponding authors were contacted via E-mail and where available, information (from these personal communications) on the MW of the immunoreactive band shown on anti-P-p65 Ser276 Western Blots in the paper is indicated. (NA: not applicable, CS: Cell Signaling, WB: Western Blot, IF: immunofluo, IHC: immunohistochemistry, ChIP: chromatin immunoprecipitation).



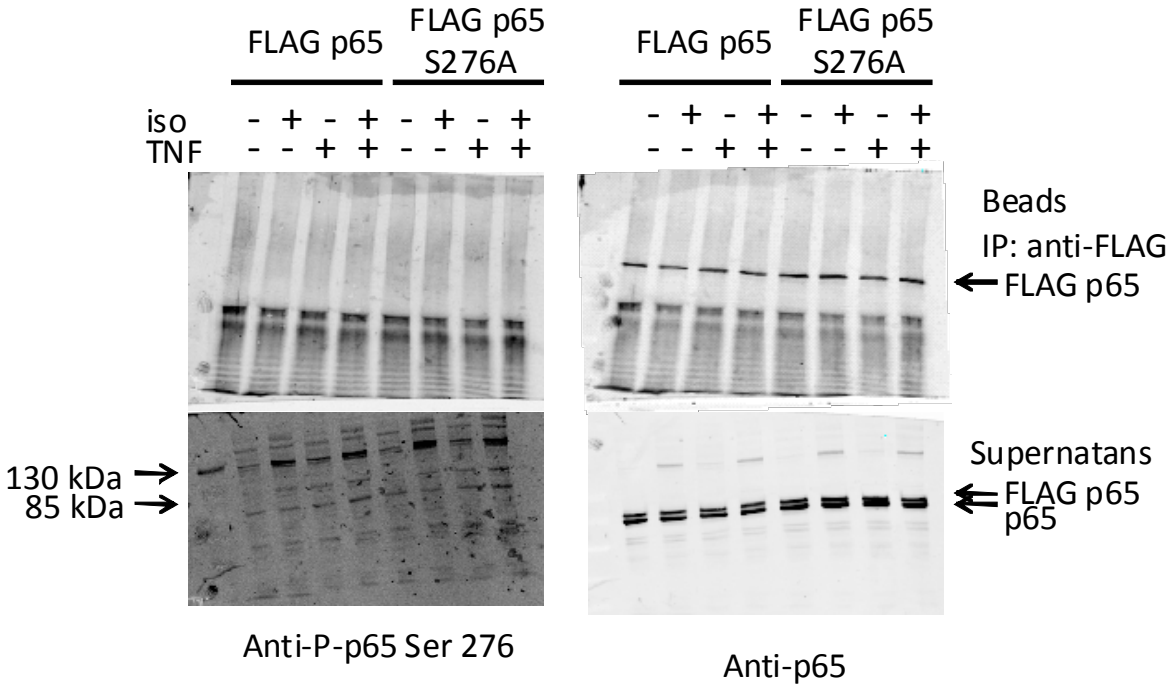
Suppl. Fig. 1. Tubulin loading controls to Fig. 3.

Presence of tubulin in cell lysates was analysed by Western Blot. After anti-p65 + anti-PKAc detection (Fig. 3, A2, B2, C2), blots were reprobed with anti-tubulin to assess equal protein loading.



Suppl. Fig. 2. Anti-P-p65 Ser276 is not detectable in p65-enriched immunoprecipitates.
1321N1 cells were induced for 30 minutes with vehicle, isoproterenol (iso) or TNF. A. Cell lysates were subjected to immunoprecipitation with beads or anti-p65 (p65 C20). Presence of anti-P-p65 Ser276 immunoreactivities in inputs (A1) and immunoprecipitates (B1) was investigated by Western Blotting using Cell Signaling #3037 (A1, B1). Presence of anti-P-p65 Ser536 in the same inputs (C1) and immunoprecipitates (D1) was investigated by Western Blotting using anti-P-p65 Ser536. Blots B1 and D1 were washed extensively and reprobed with anti-p65 (B2, D2).

IP protocol: Briefly, cells (2 x 10^6 cells per 10 cm dish) were scraped and lysed in 500 µl RIPA lysis buffer (150 mM NaCl, 50 mM Tris HCl pH 7,5, 1% NP40, 0,01% SDS, 1 mM EDTA, 1 mM EGTA, 0,5% sodium deoxycholate) supplemented with leupeptine, β-glycerophosphate, Aprotinin, Pefabloc, sodium fluoride and sodium orthovanadate. After 1h of rotation, lysates were centrifuged at 14000 rpm for 10 min and supernatant was used for immunoprecipitation. 30 µl of supernatant was isolated before immunoprecipitation and used as input control. After 2 hours of incubation with antibody, 20 µl of A-Beads was added and lysates were rotated overnight. Subsequently beads were washed 3 times with 1 ml of RIPA buffer supplemented with inhibitors. Samples were denatured in 20 µl of RIPA, 25 µl of 2× Laemmli buffer and 2,5 µl of 1M DTT for 10 min at 95°C and used for western blotting as described in materials and methods.



Suppl. Fig. 3. Immunoprecipitation of overexpressed wild type FLAG p65 or FLAG p65 S276A mutant does not lead to recovery of the 130 kDa or 80 kDa immunoreactive bands. 1321N1 cells were induced for 30 minutes with vehicle, isoproterenol (iso) or TNF. A. Cell lysates were subjected to immunoprecipitation with anti-FLAG beads. Presence of anti-P-p65 Ser276 immunoreactivities in inputs (bottom panels) and immunoprecipitates (top panels) was investigated by Western Blotting using Cell Signaling #3037. Blots were washed extensively and reprobed with anti-p65. IP protocol was the same as in Suppl. Fig. 2.