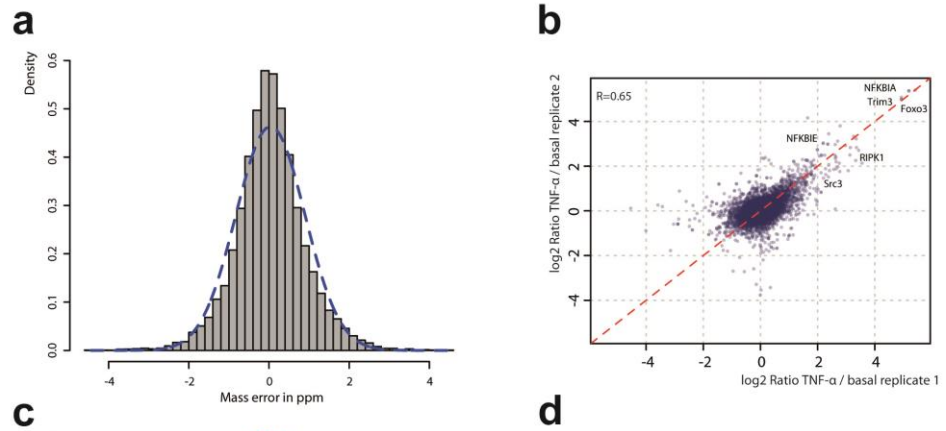
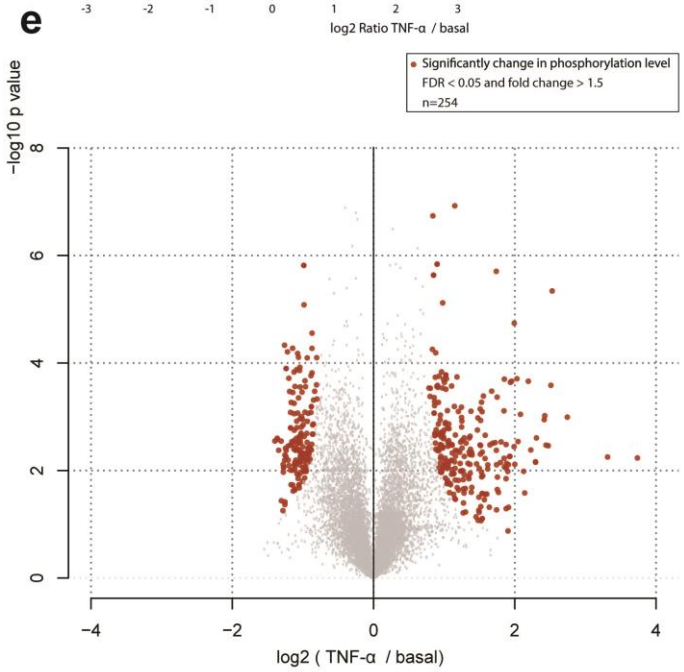
Supplementary Figure 1



d

Proteins	7429
Quantified in at least one Exp.	6024 (81 %)
Phosphoproteins	4616
Phosphopeptides	31639
Phosphosites	24773
Phosphosites Quantified	22662 (92 %)
Phosphosites Quantied in all 4 experiments	10656 (40 %)

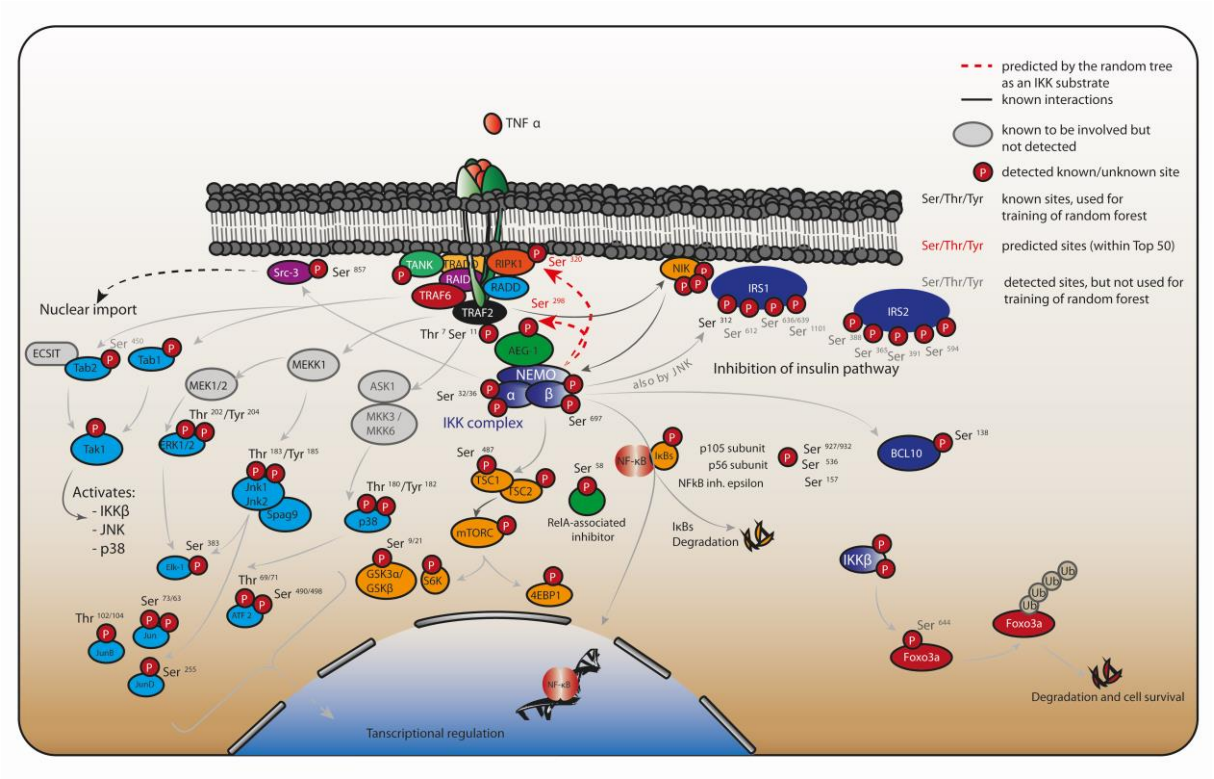


Uniprot ID	Gene name	Position (phospho)	Log2 fold change in response to TNF-α	p value (FDR < 0.05)
O95999	BCL10	138	1.64	1.32
P35568	IRS1	312	1.34	1.57
Q9NQC7-2	CYLD	418	1.61	1.58
Q04206	RELA	536	0.98	1.77
P19838	NFKB1	932	1.48	1.88
P19838	NFKB1	927	1.55	2.07
O14920	IKBKB	697	1.65	2.11
Q92574-2	TSC1	487	1.06	2.15
Q9Y6Q9-5	NCOA3	857	1.63	2.17
P25963	NFKBIA	36	5.19	2.24
P25963	NFKBIA	32	5.19	2.24
O00221	NFKBIE	157	3.13	2.78
P35568	IRS1	312	1.34	1.57
Q99759	MAP3K3	166	0.69	2.18
P01106	MYC	62	1.33	2.23
P26358	DNMT1	143	2.65	2.23
Q13546	RIPK1	320	2.20	1.88
Q6ZVM7	TOM1L2	457	3.26	1.88
Q96BY7	ATG2B	497	1.18	1.90
Q9HCH5-8	SYTL2	649	1.61	1.99
Q86UE4	MTDH	298	2.07	1.59

Supplementary Figure 1: Statistical analysis of the phosphoproteome and proteome dataset. (a) Histogram indicating the mass errors of detected phosphorylation sites and 95 % of all detected phosphopeptides fit less than 2 ppm. Blue broken-line shows Gaussian distribution. (b) Correlation between the biological replicates was determined by Pearson

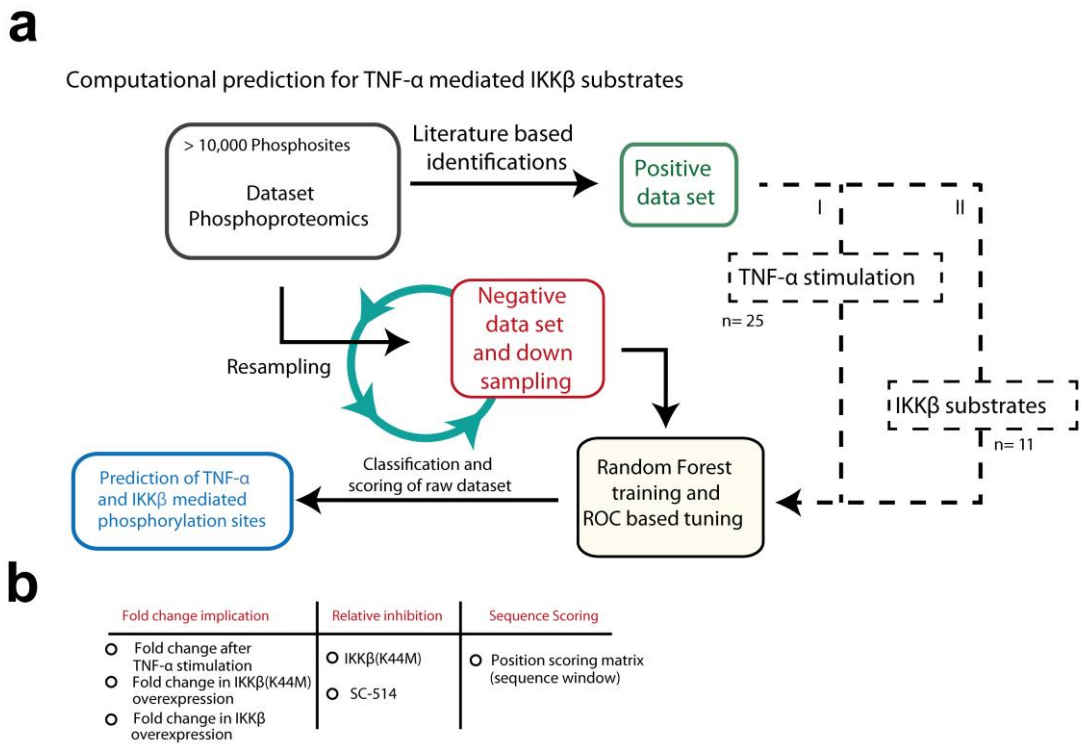
correlation coefficient. Known phosphoproteins involved in the TNF-α-mediated signalling pathway are indicated. (c) Expression changes were analysed from the identified proteins and 96.8 % of detected proteins did not show changes in protein expression levels upon TNF-α stimulation. (d) Number of identified and quantified proteins and phosphopeptides from the global phosphoproteomics screen are represented in the table. (e) Volcano plot showing the fold change in response to TNF-α stimulation compared to p- values from a two-sided t-test. Red dots represent significant changes at a FDR cut off of 0.05 (parameters: s0=0.6, permutations: 500). The table depicts selected candidates for up-regulated phosphorylation sites in response to TNF-α stimulation.

Supplementary Figure 2



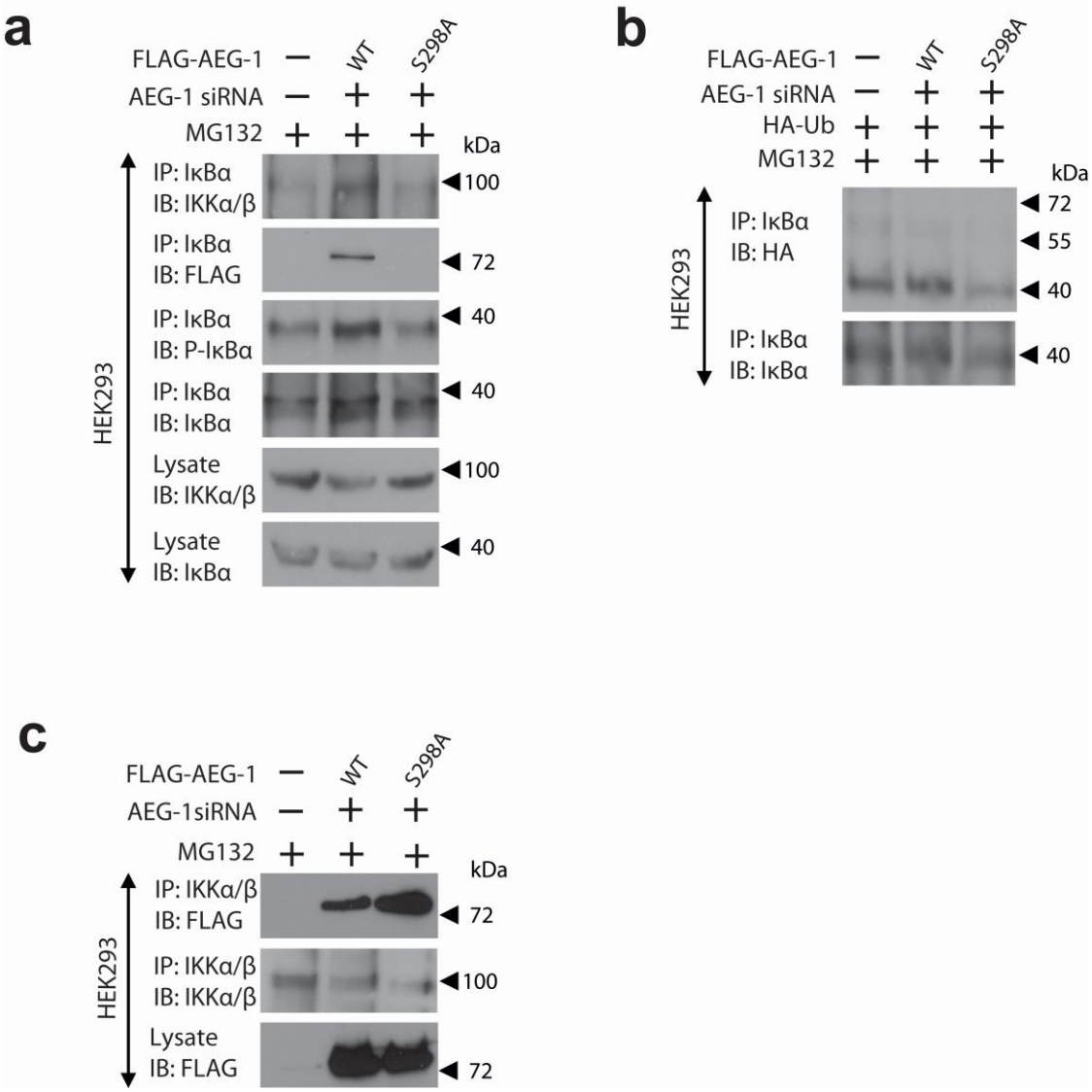
Supplementary Figure 2: TNF-α phosphoproteomic network. Indicated are the known substrates of IKKβ as well as the activated proteins upon TNF-α stimulation. Novel potential IKKβ substrates were shown in red arrows. Identified phosphorylation sites / phosphoproteins which were not included in random forest analysis were represented in pale grey.

Supplementary Figure 3



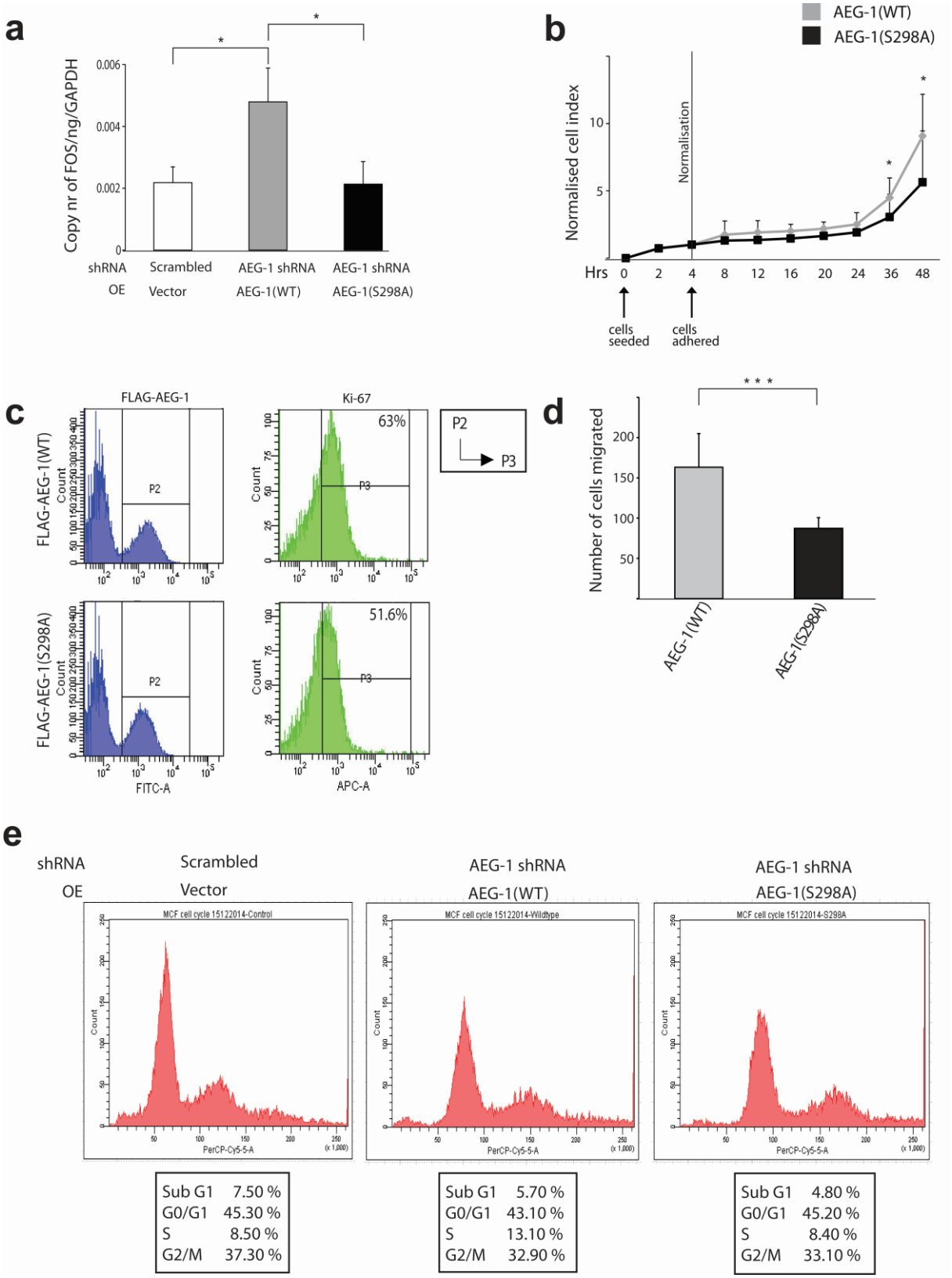
Supplementary Figure 3: Schematic overview of computational workflow. (a) Random forest approach with down sampling was used for the prediction of IKKβ substrates and TNF-α mediated phosphorylation sites. Positive phosphorylation sites were identified by literature search that were utilized to train the classifiers. Resampling of the remaining dataset was practiced to create a negative data set. Both classifiers were then utilized to predict the substrates of IKKβ and TNF-α dependent phosphorylation sites. (b) Table representing features of each phosphorylation site used. Sequence scoring matrix was applied for the assessment of amino acid based identification of the IKKβ substrates.

Supplementary Figure 4



Supplementary Figure 4: Phosphorylation of AEG-1 serine 298 is required for IKKβ-mediated IκBα phosphorylation, ubiquitination and degradation. (a) HEK293 cells were transfected with control or AEG-1 siRNA. After 24 hours, the cells were transfected with either vector or FLAG-tagged wild-type (WT) or S298A mutant of AEG-1. 48 hours later, cells were treated with MG132 (20μM) for 2 hours and lysed. IκBα was immunoprecipitated from cell lysates. The precipitates were immunoblotted using respective antibodies (b) HEK293 cells were transfected as in (a) in combination with HA-tagged ubiquitin. IκBα was immunoprecipitated as described above. The precipitates were immunoblotted using anti-HA antibody. (c) HEK293 cells were treated as above. Protein complex from the precipitates were immunoblotted using anti-FLAG and anti IKKα/β antibodies.

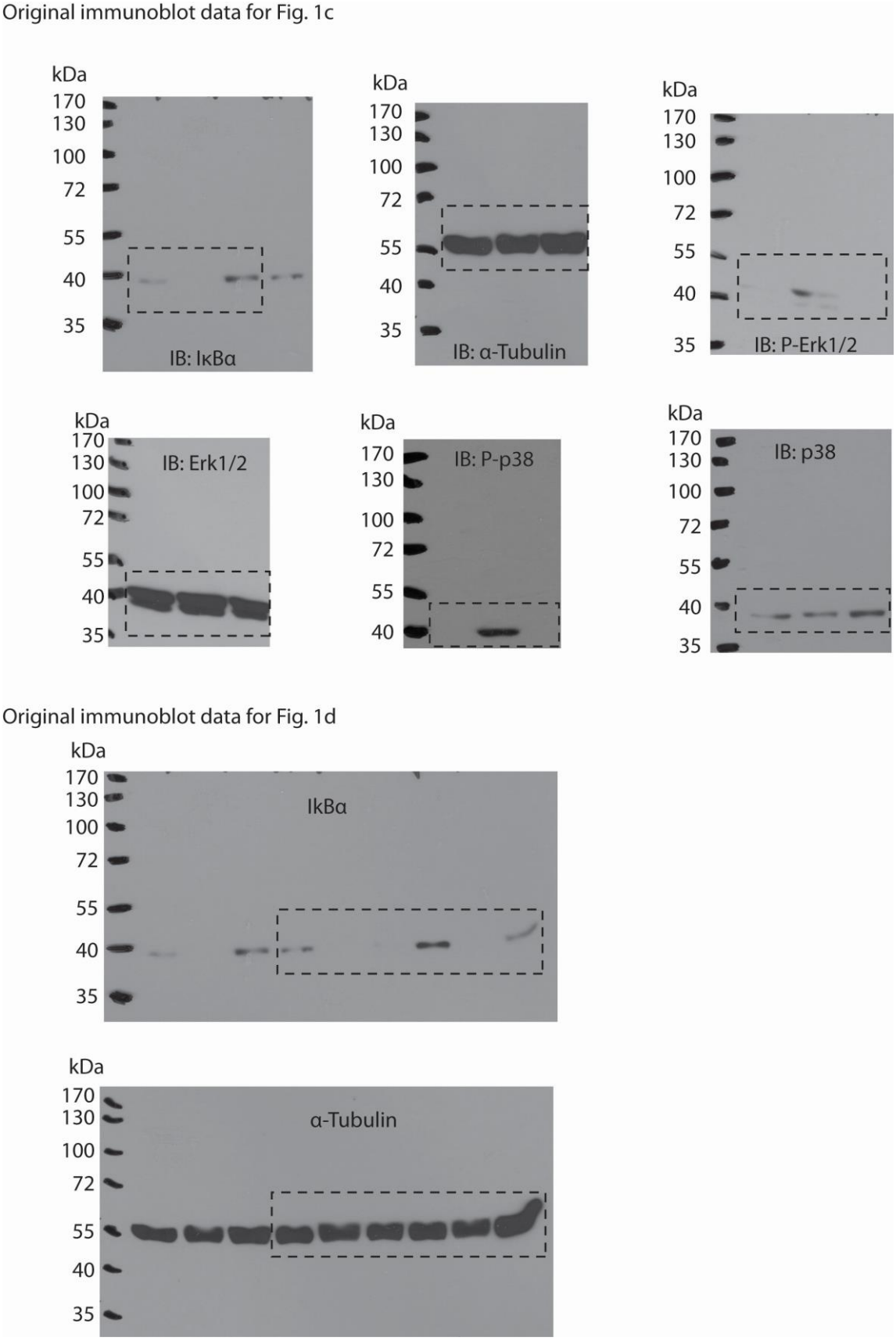
Supplementary Figure 5



Supplementary Figure 5: Role of serine 298 in NF-κB-mediated gene expression, cell proliferation and migration. (a) RNA from stable 4T1 cells was isolated and expression of NF-κB-regulated Fos gene was determined (b) MCF-7 cells transfected with wild-type (WT)

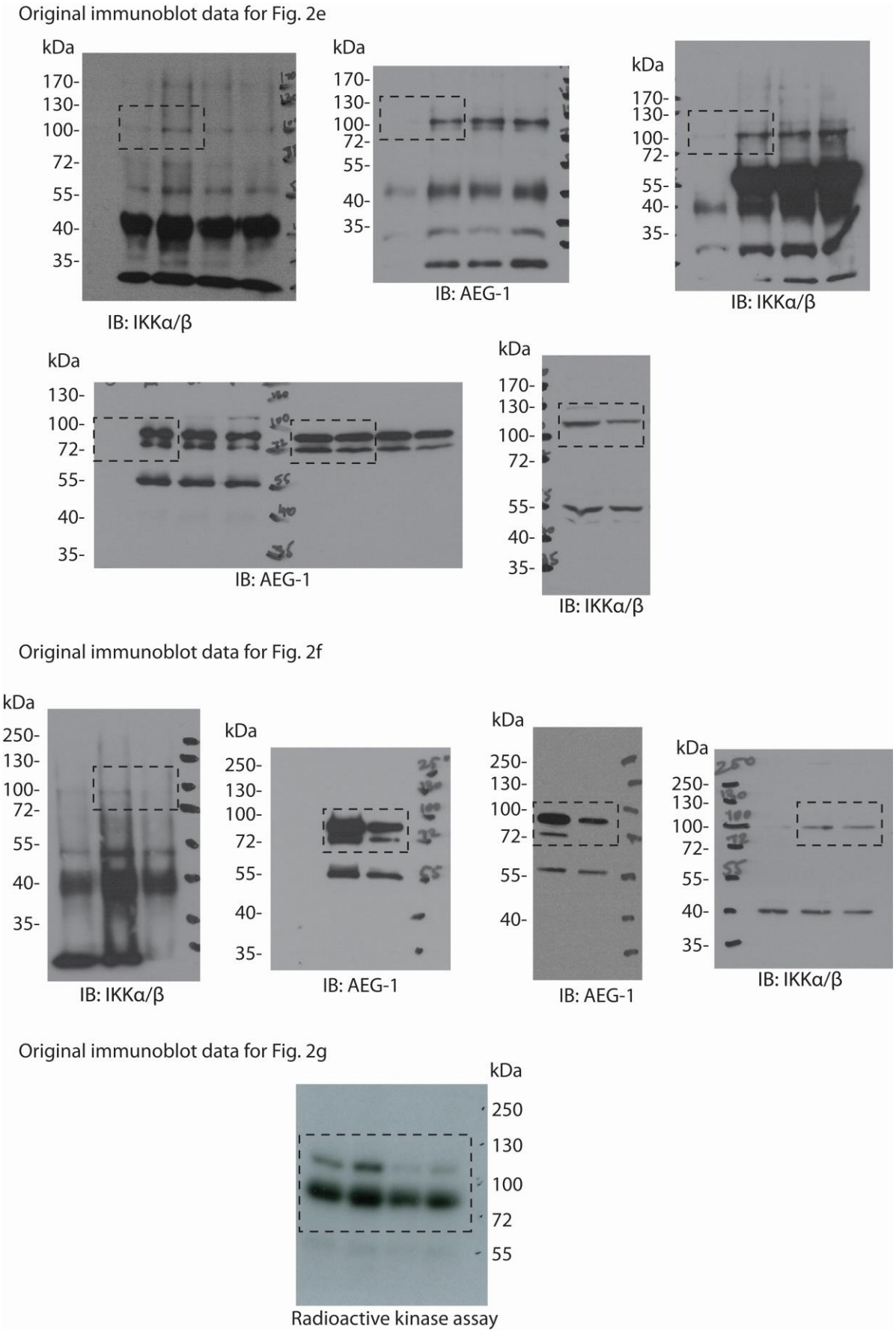
or mutant (S298A) AEG-1 were seeded onto the E-Plate. Real-time, label-free impedance measurement was carried out for 48 hours. (c) Wild-type or S298A AEG-1 was overexpressed in HEK293 cells. After starvation and stimulation with TNF-α for 12 hours, cell pellets were collected, fixed with 4% PFA and immunostained using anti-FLAG-FITC and anti-Ki-67. The percentage of Ki-67-positive cells in the FLAG-positive group was quantified. (d) MCF-7 cells transfected with wild-type or S298A AEG-1 were trypsinized and counted. 3×10^5 cells in RPMI containing 0.5% FBS were seeded into the upper chamber of a transwell plate. Cells were allowed to migrate for 24 hours. Migrated cells were then fixed, stained and counted as described in Methods. (e) Cell cycle distributions of 7-AAD stained stable MCF-7 cells. All plots represent mean values -/+ SD from triplicates. *, $P < 0.05$.

Supplementary Figure 6



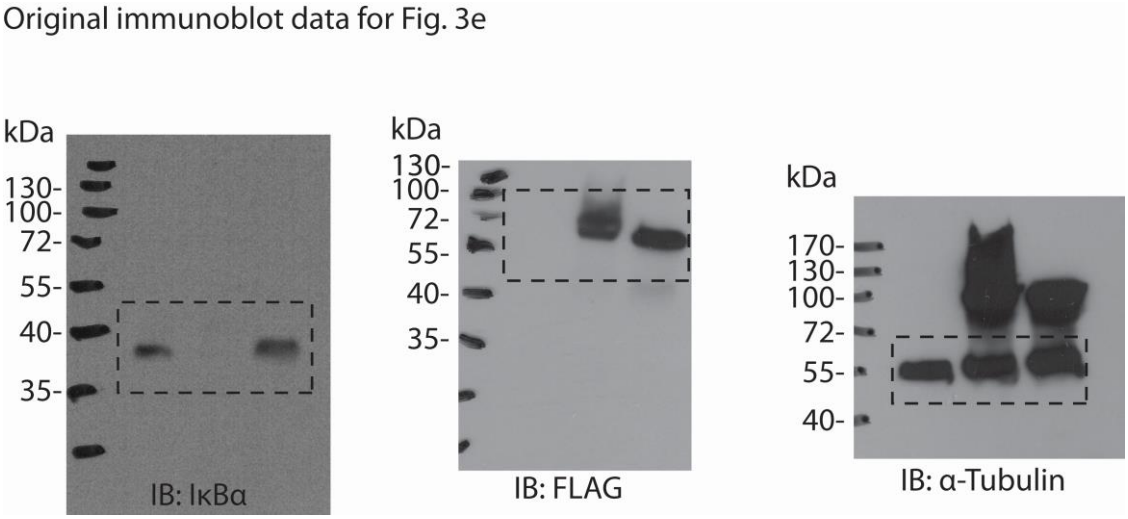
Supplementary Figure 6: Original Western blots for Figure 1

Supplementary Figure 7



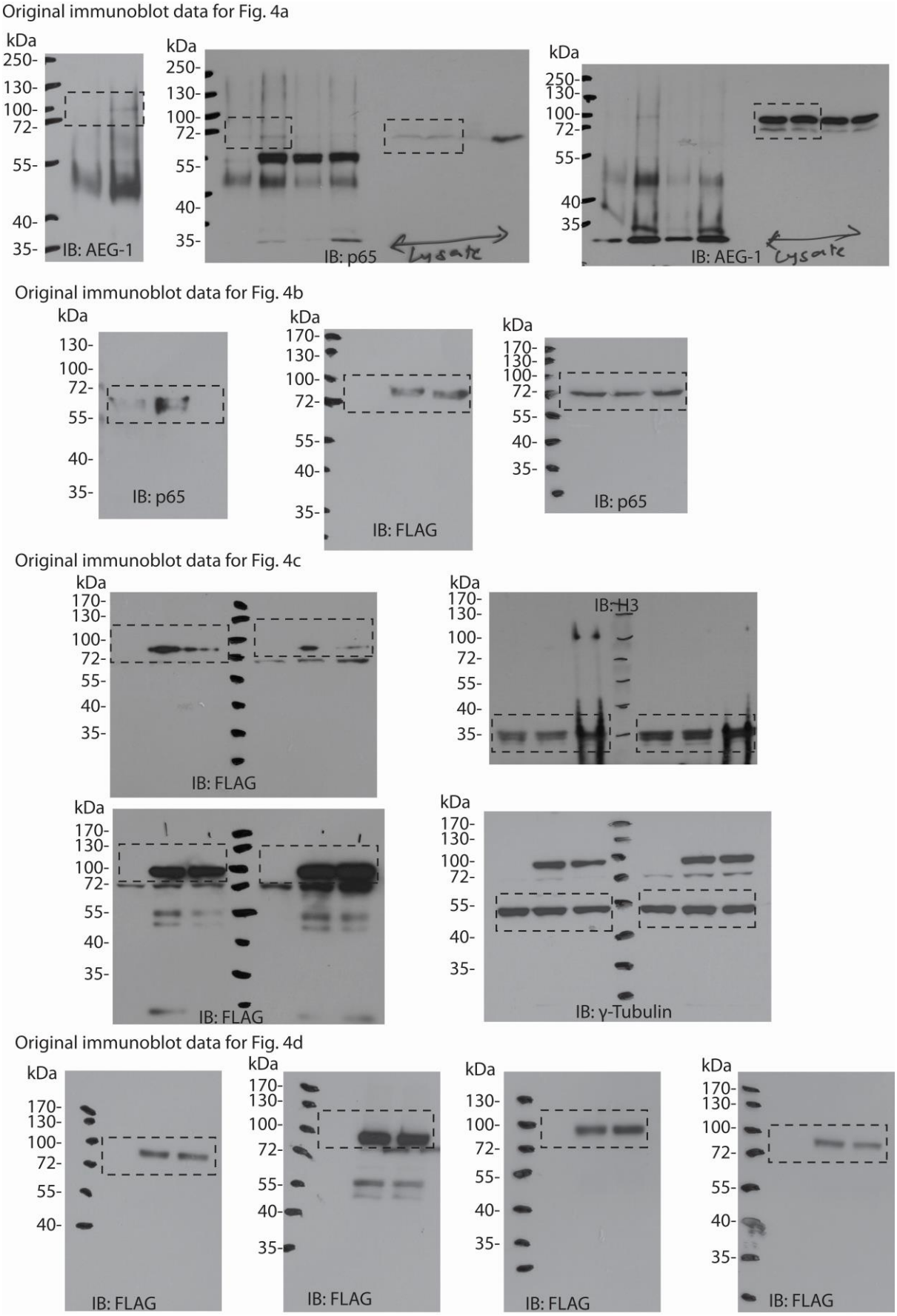
Supplementary Figure 7: Original Western blots for Figure 2

Supplementary Figure 8



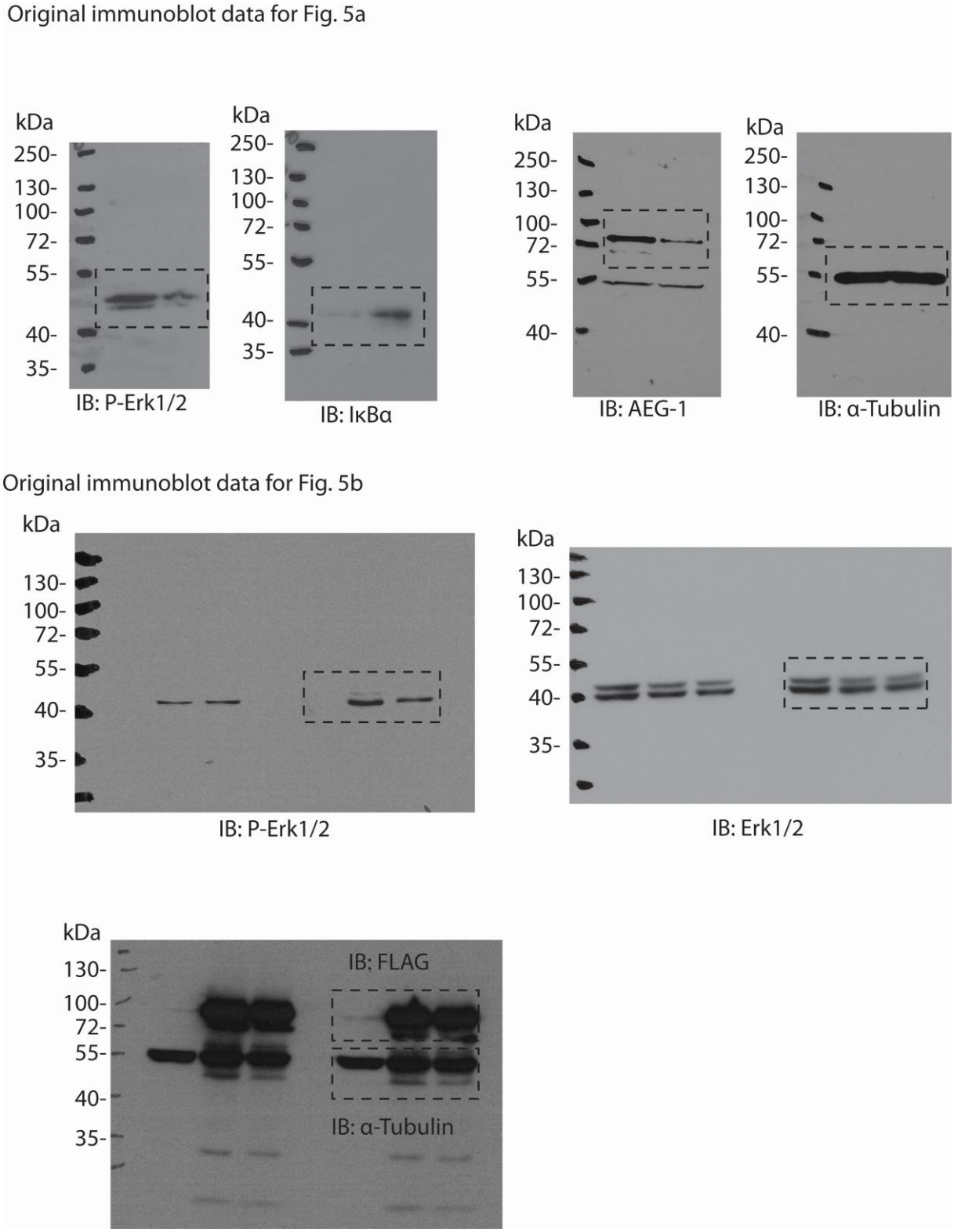
Supplementary Figure 8: Original Western blots for Figure 3

Supplementary Figure 9



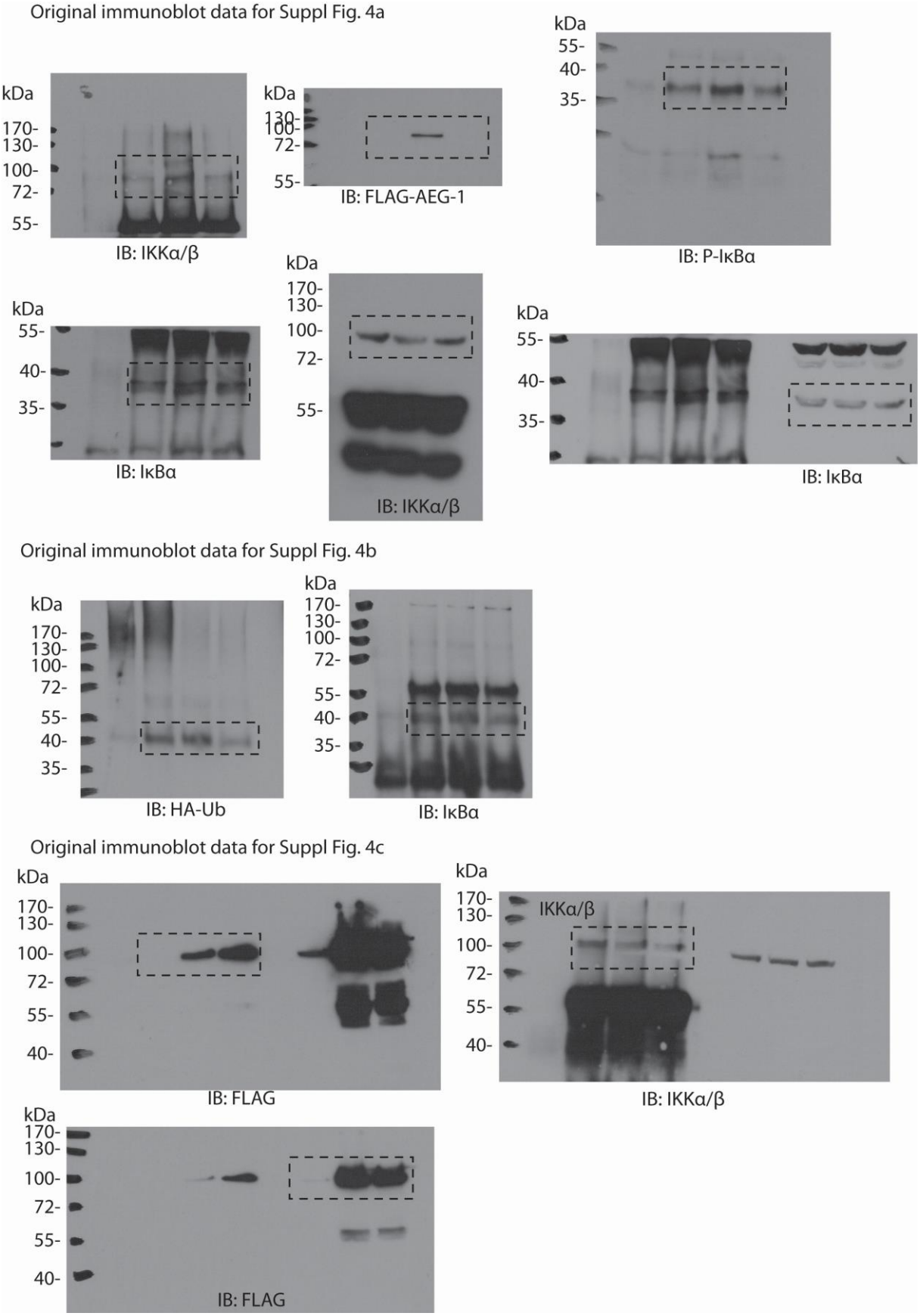
Supplementary Figure 9: Original Western blots for Figure 4

Supplementary Figure 10



Supplementary Figure 10: Original Western blots for Figure 5

Supplementary Figure 11



Supplementary Figure 11: Original Western blots for Supplementary Figure 4