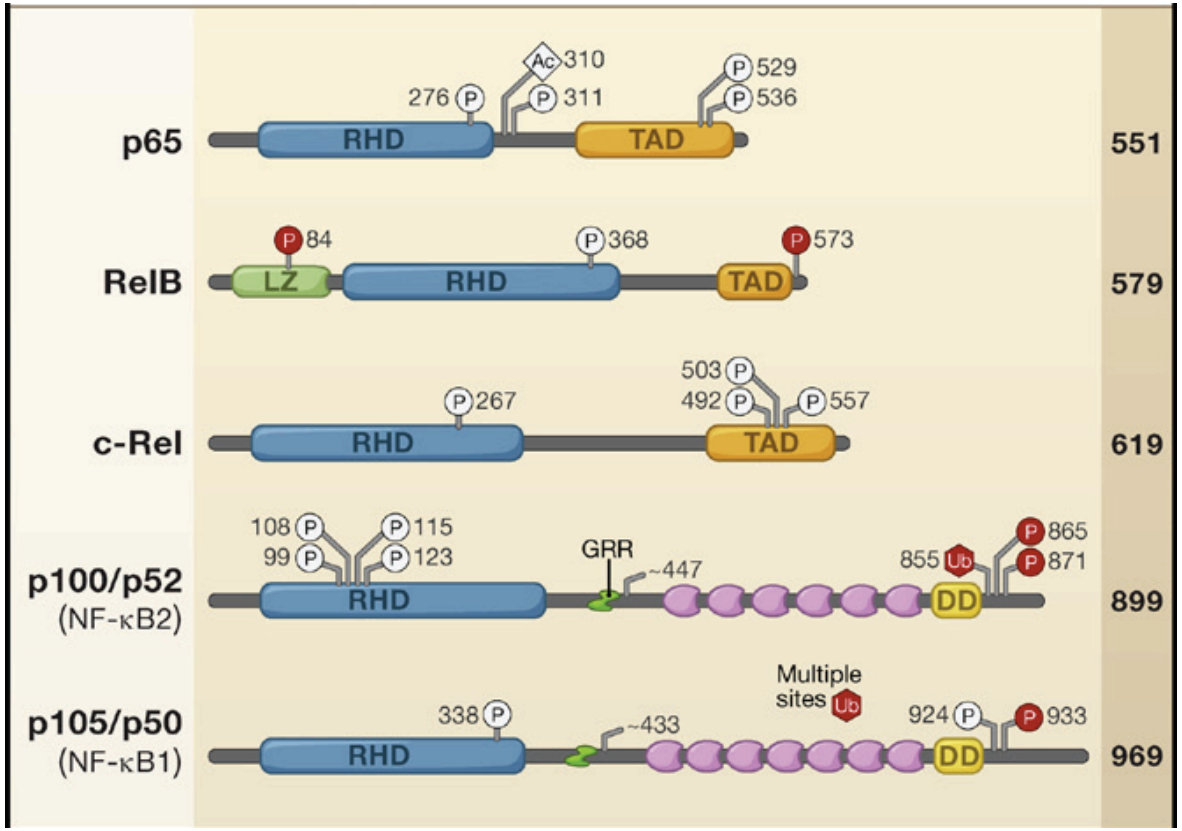
Characterization of a Direct NF-{kappa}B Ligand

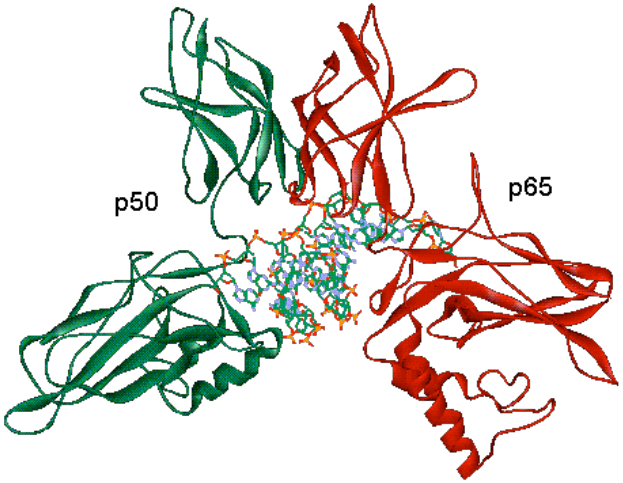
Amalchi Castillo-Rodriguez

08/07/09

Five proteins of the NF-{kappa}B Family

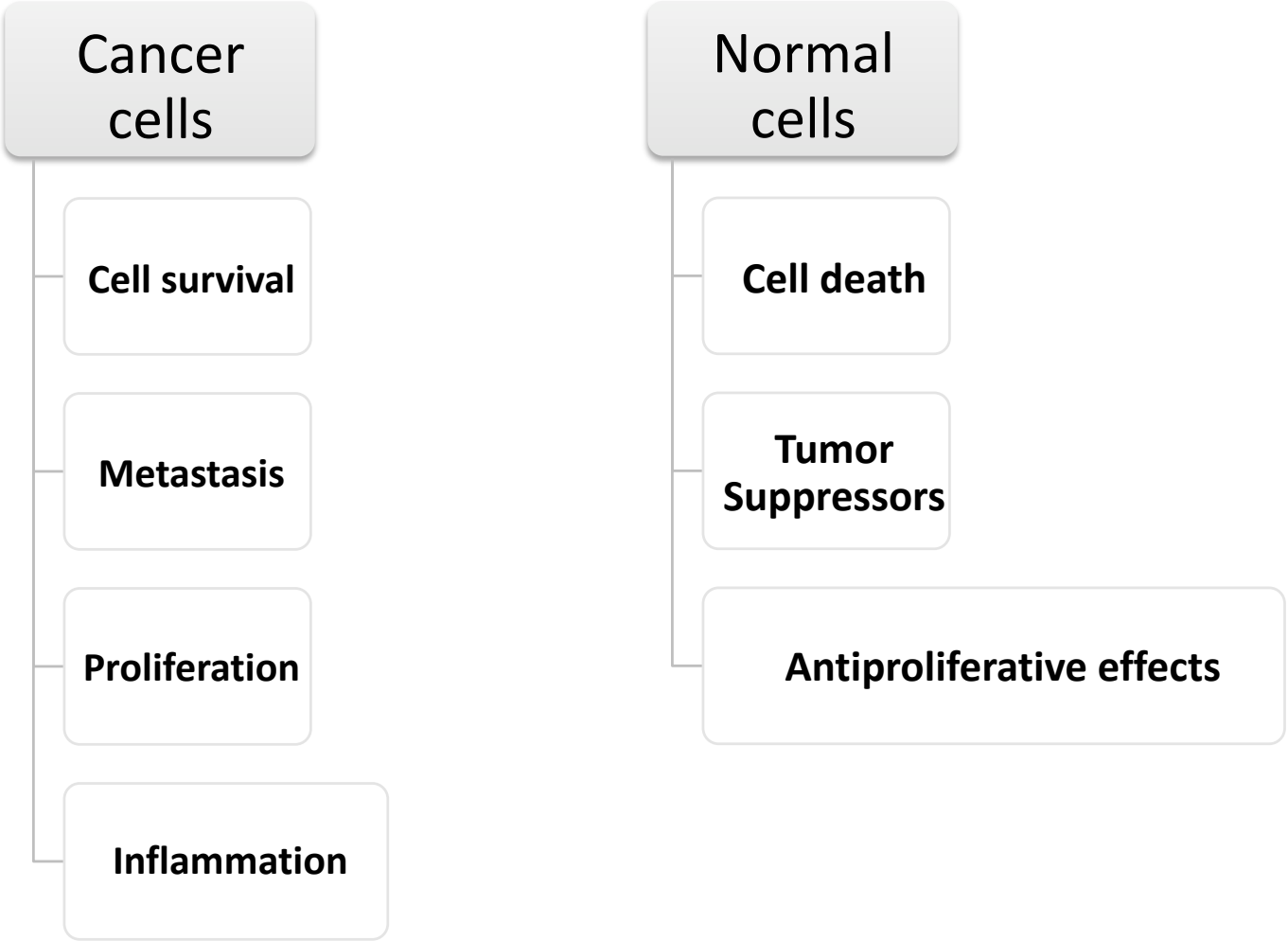


Cell. 2008 Feb 8;132(3):344-62.

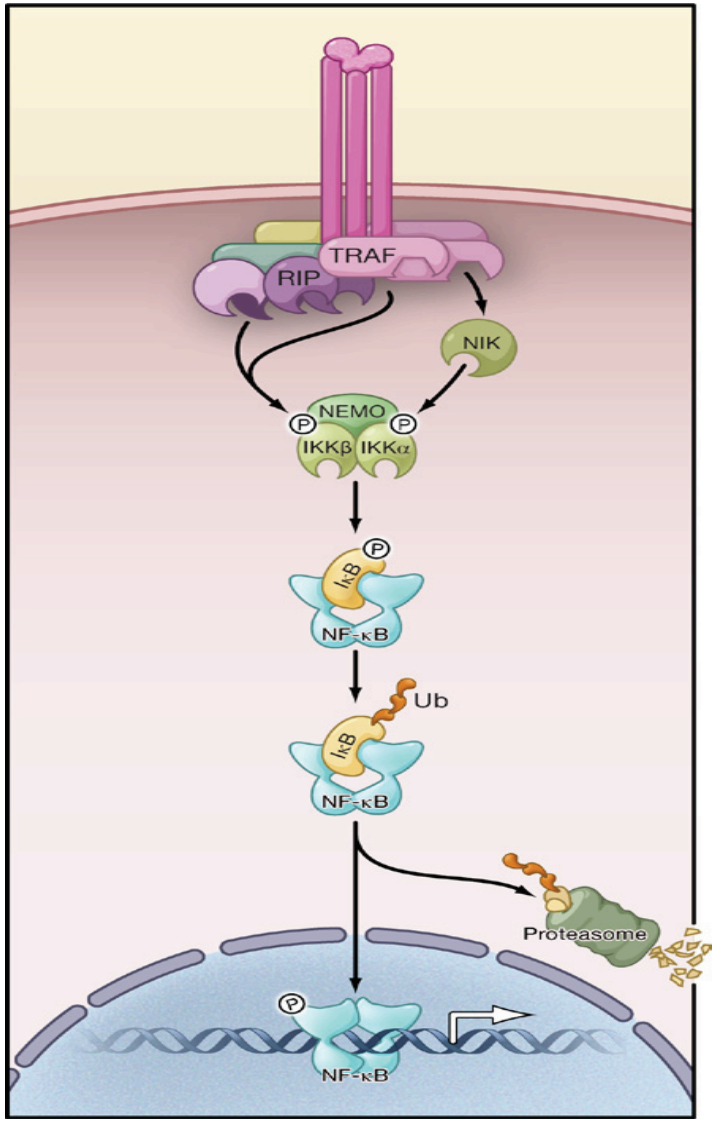


Crystal structure of NF-kB p50/p65 heterodimer bound to DNA
Nature 391, 410-413 (22 January 1998) | doi:10.1038/34956

The two faces of NF-{kappa}B in gene regulation



The NF-{kappa}B canonical pathway



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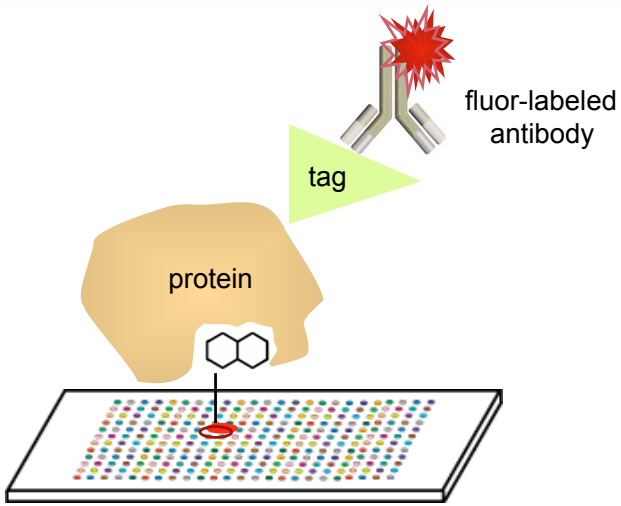
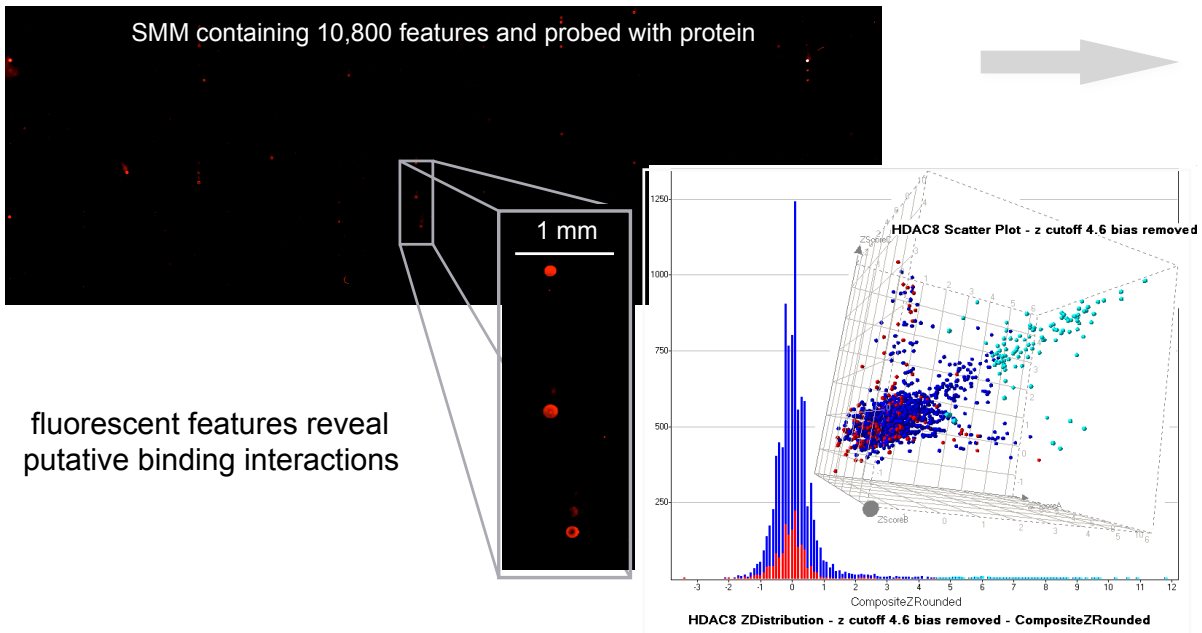
Small-molecule microarrays (SMMs)

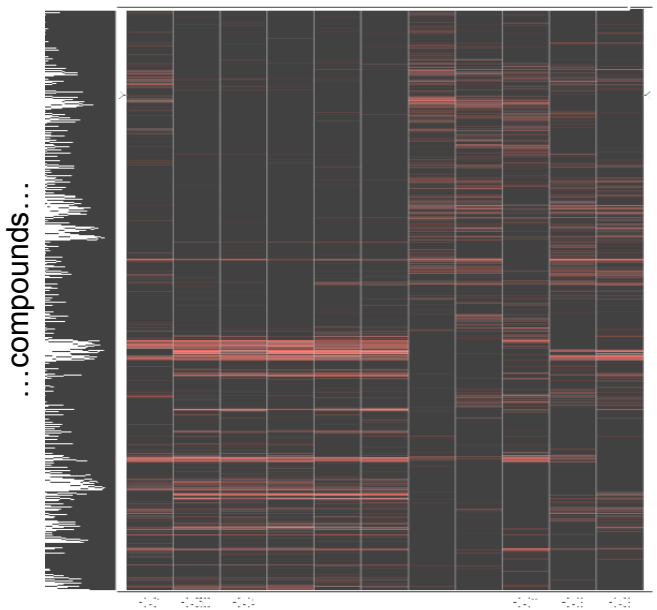




fluorescent features reveal
putative binding interactions

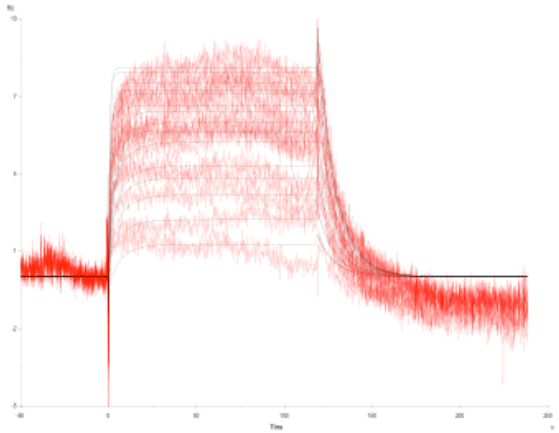
compute composite Z-scores

specificity heat map



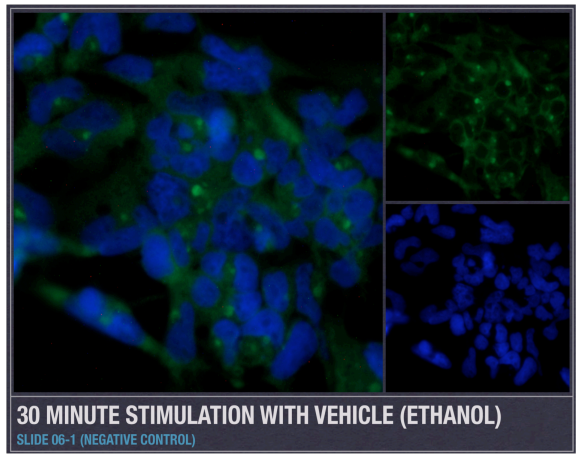
AGA is a stronger NF-{kappa}B binder as shown by surface plasmon resonance

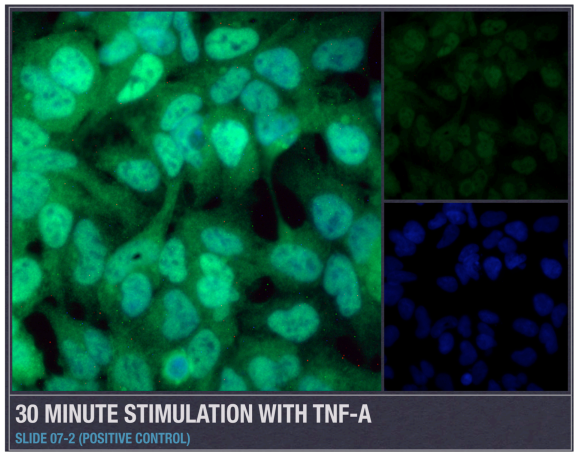
	80% BRD-K92758126		AGA
	80% pure	**purified**	
p50-SPR	K_D = 226 nM	K_D = 2,300 nM	K_D = 76 nM
P65-SPR	not tested	K_D = 13,000 nM	K_D = 6,340 nM

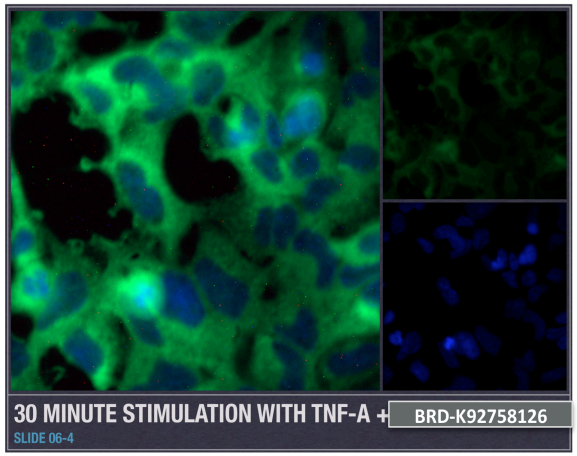


AGA inhibits NF-{kappa}B translocation

Translocation assay, p50 Ab (green) and Hoechst (blue)







Edgar Ibarra
Rakhee Busanelli

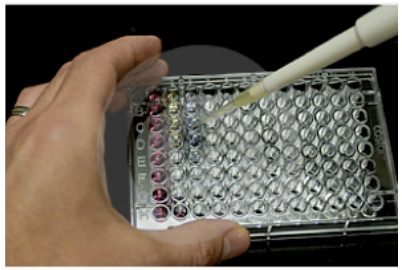
Main goals

- To study the inhibitory effect of AGA on all five NF-{kappa}B proteins.

- To determine the effect of AGA on cell viability.

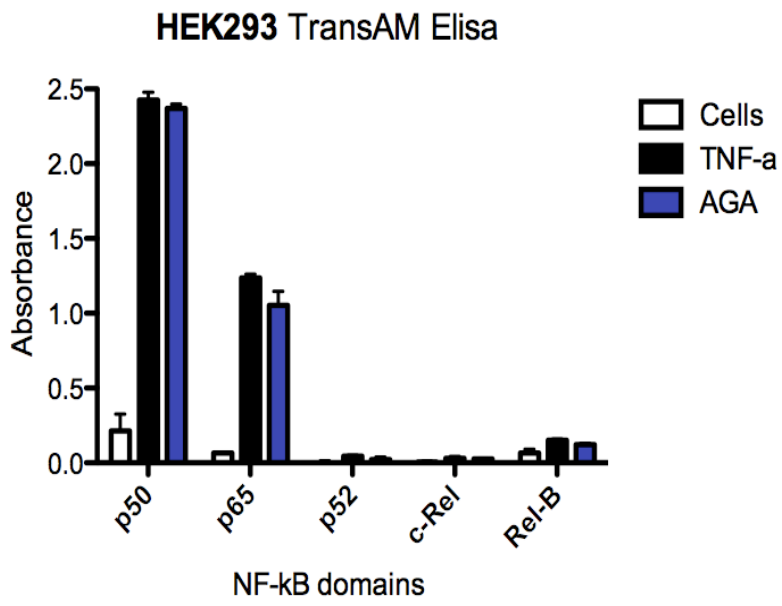
Measuring the effect of AGA in NF-{kappa}B protein translocation

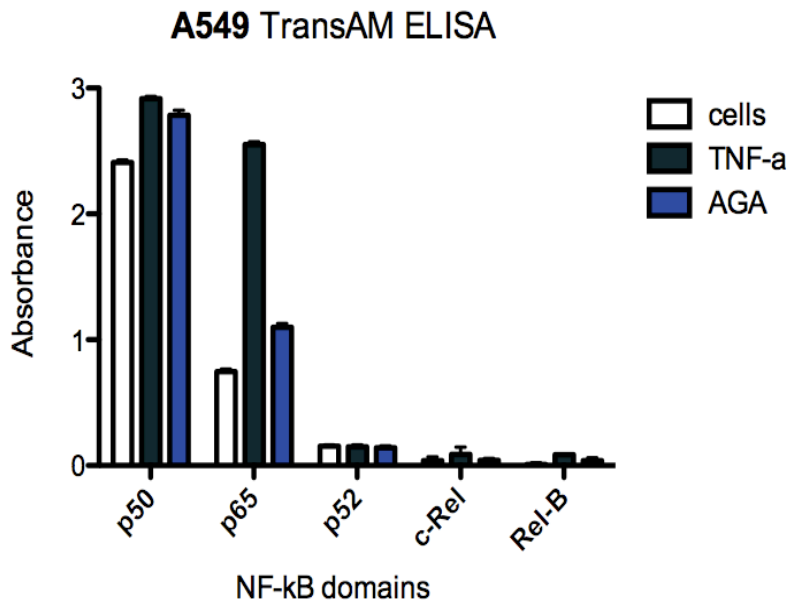
Nuclear extraction



1. Non-stimulated cells
2. TNF-a stimulated
3. TNF-a stimulated + AGA (10nM)

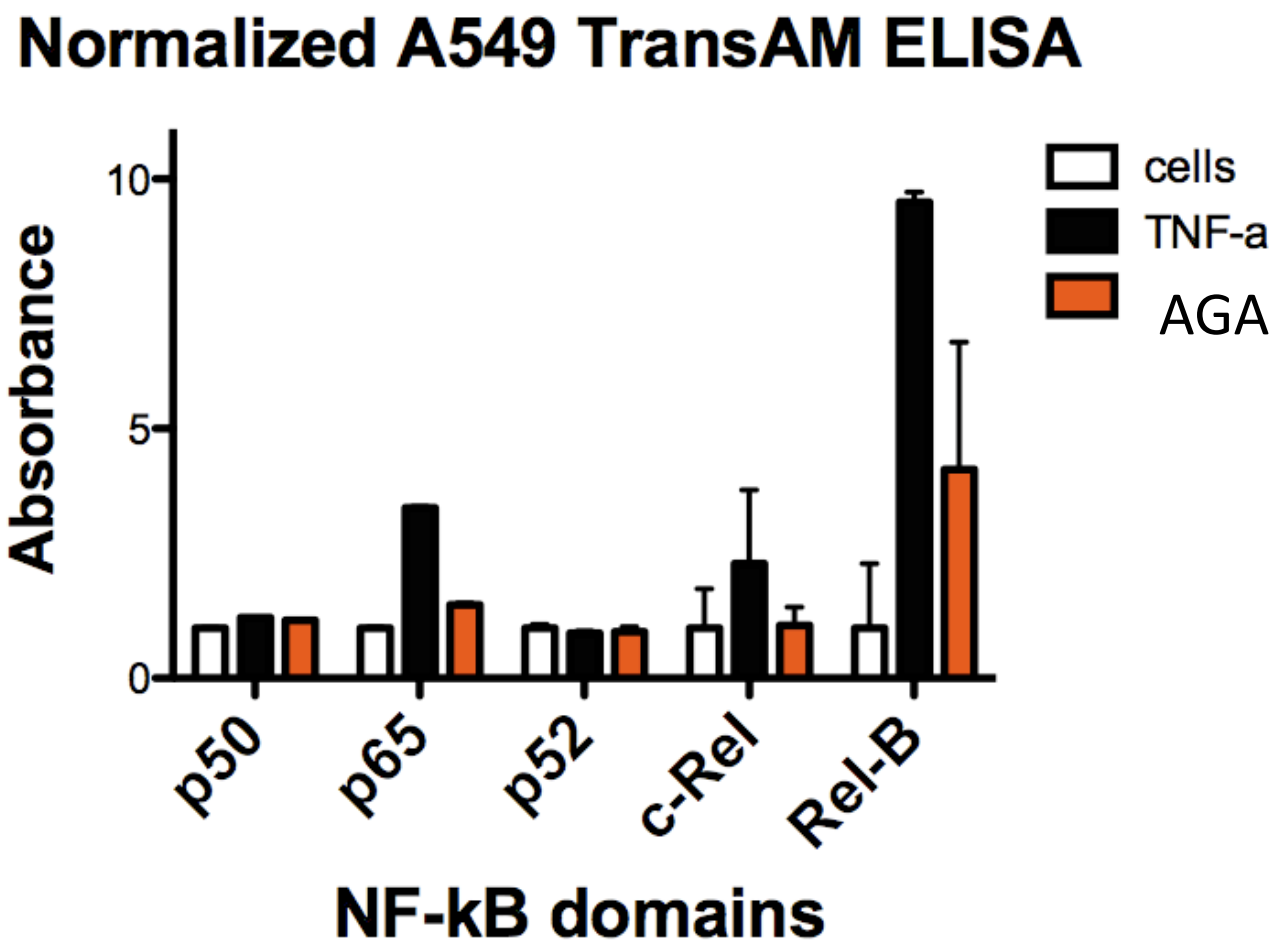
AGA TransAM has a preference for p65



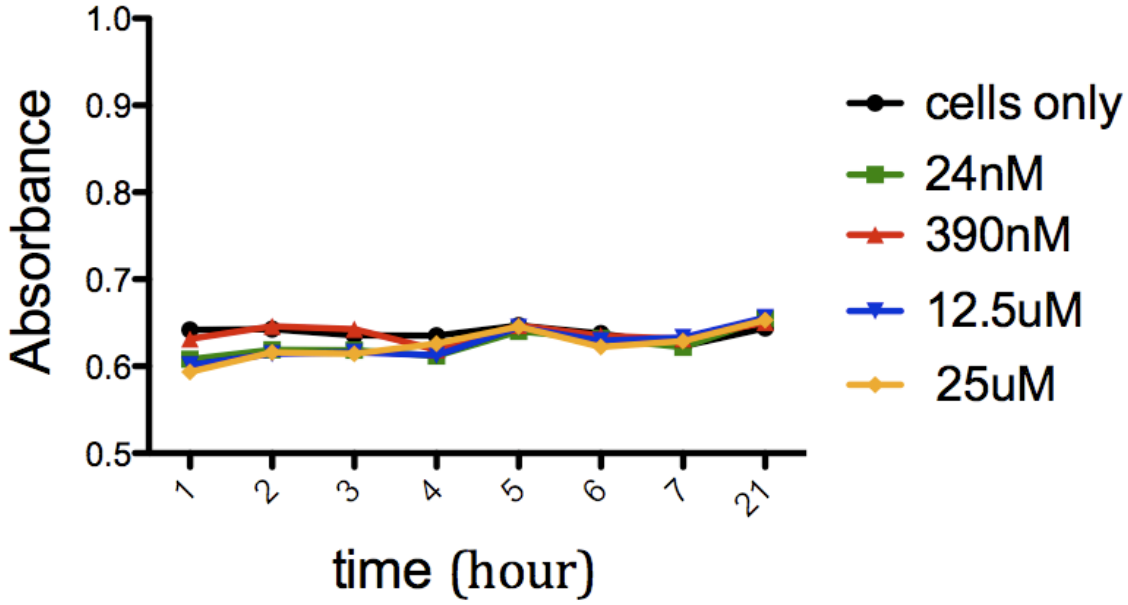


1 hour stimulation

10nM AGA concentration


Normalized A549 TransAM ELISA
Absorbance
10
5
0
cells
TNF-a
AGA
p50
p65
p52
c-Rel
Rel-B
NF-kB domains

HEK293 cell viability is not affected upon addition of AGA



Conclusions

- AGA temporarily inhibits NF-{kappa}B translocation to the nucleus.
- The inhibitory effect of AGA appears to vary in different cancer cell lines, but might have a preference for p65.
- AGA does not affect cell viability.

Future directions

- Use of a gene expression database to explain ELISA data.

- Use of higher concentrations of AGA to test for inhibition.

- Synergy studies involving compounds that target other parts of the NF-kB pathway.

- Docking experiments will help theorize sites of interaction.

Acknowledgements

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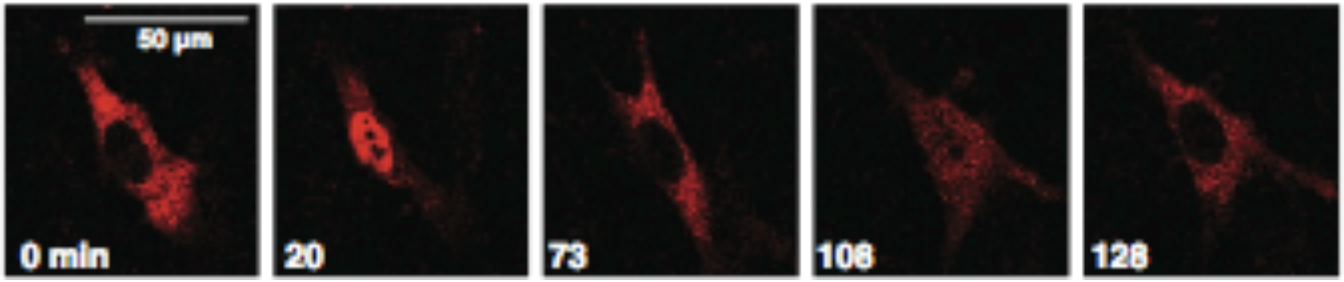
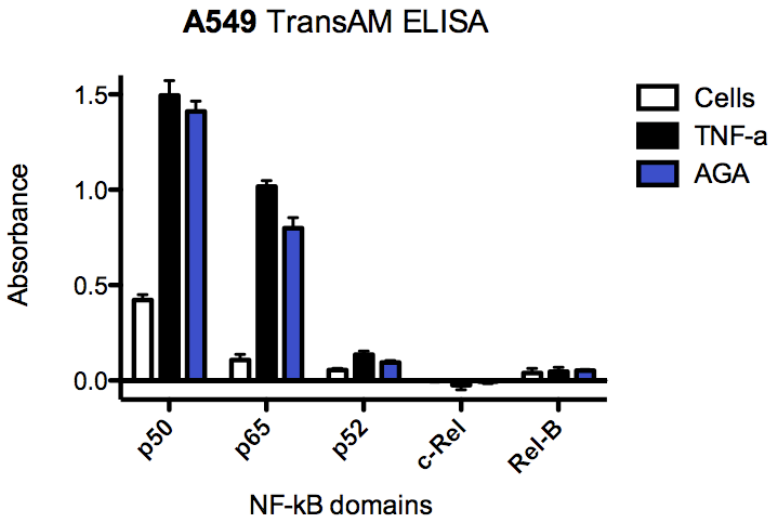
SRPG program

BROAD INSTITUTE

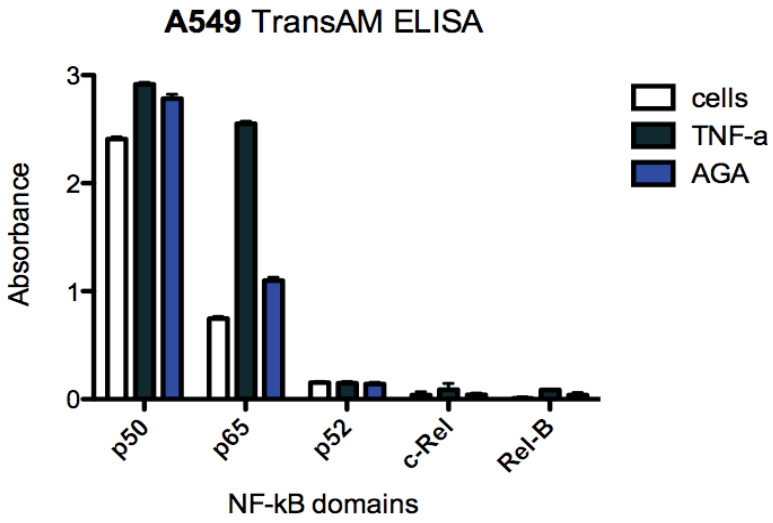
There is no significant increase in absorbance upon 30 minute incubation over 1 hour incubation.



Pulsatile Stimulation Determines Timing and Specificity of NF-kB-Dependent Transcription.
L. Ashall, C. A. Horton. Science 324, 242-246



30 minute incubation



1 hour incubation

Varying degree of AGA selectivity among different cancer cells