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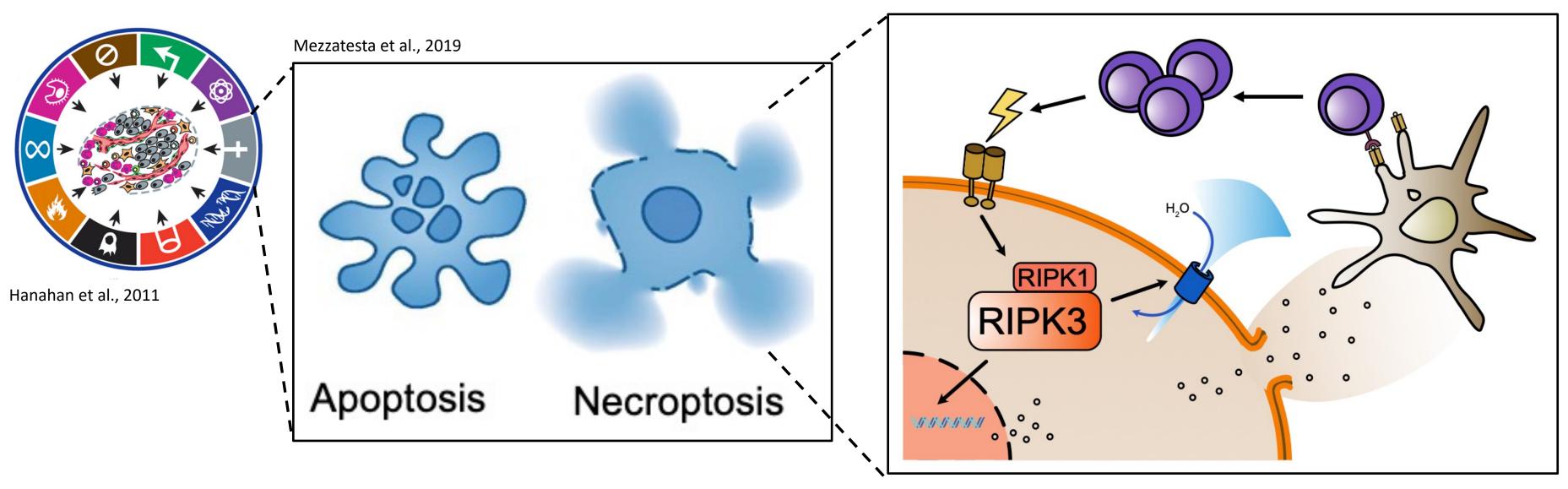
Klinikum rechts der Isar

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Immunogenic cell death establishes an inflammatory tumor immune microenvironment acting as repressor in lung adenocarcinoma.

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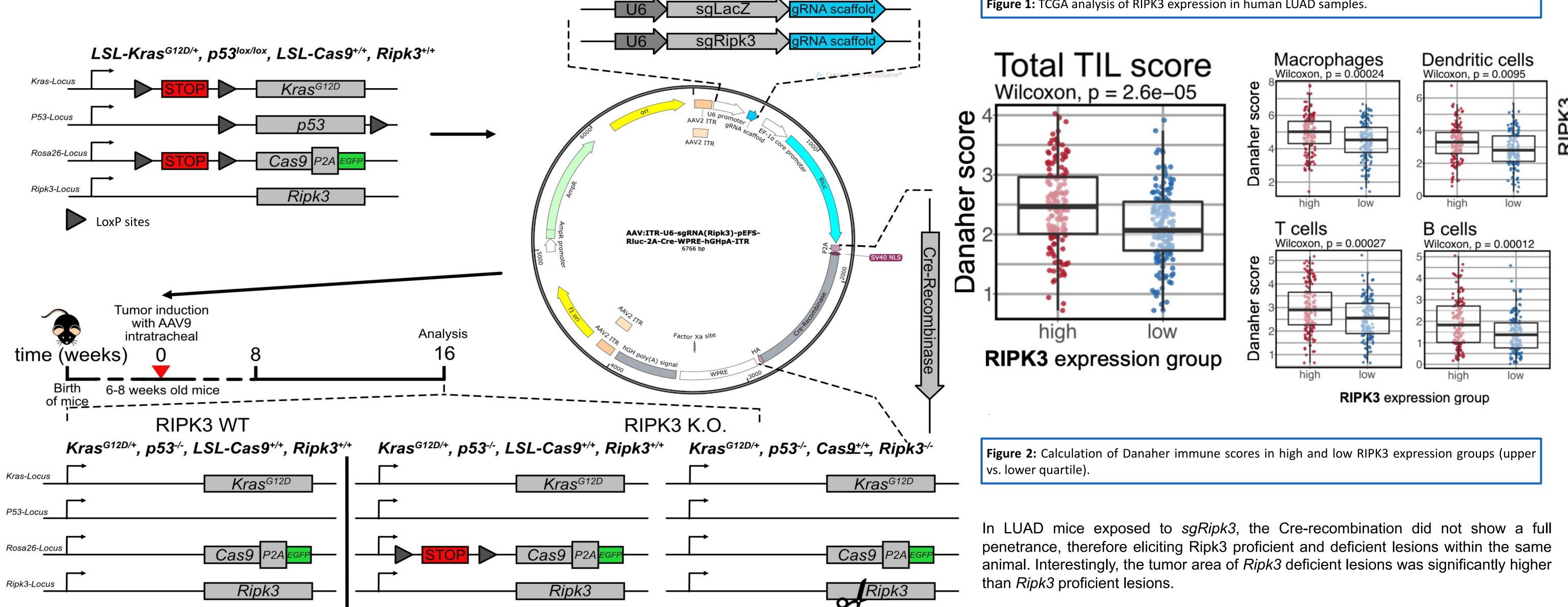3. Cancer Evolution and Genome Instability Laboratory, The Francis Crick Institute, London, UK
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Background:

Receptor-Interacting Serin/Threonin-Protein Kinase 3 (RIPK3) mediates necroptosis, a programmed form of cell death, which elicits the release of intracellular contents (DAMPs) and cytokines. These substances attract and activate anti-tumoral immune cells, rendering necroptosis highly immunogenic. In lung adenocarcinomas (LUAD), RIPK3 is a tumor suppressive aberrational target, which has been shown to induce immune suppression in the tumor microenvironment.

Material and Methods:

To investigate RIPK3 in human LUAD, we took advantage of The Cancer Genome Atlas program (TCGA) and primary samples. We further studied RIPK3 in a LUAD mouse model, carrying $Kras^{G12D}$ oncogene as well as Cas9 (both under a floxed stop codon) and floxed p53 tumor suppressor. Tumor induction was obtained through Cre recombination, which induced Cas9 and $Kras^{G12D}$ activation as well as p53 deletion. Together with Cre we delivered single guide RNA for Cas9 activity directed against Ripk3 (sgRipk3) or the negative control LacZ (sgLacZ).

Results:

Expression of RIPK3 was decreased in human LUAD tumors compared to paired healthy tissue, both at mRNA and protein levels. Moreover, RIPK3 expression was further decreased in advanced stage tumors. Leveraging the Danaher scores, as an index of immune infiltration, we identified that high RIPK3 expression correlates with an overall enrichment of tumor infiltrating leukocytes, such as T-cells, B-cells, macrophages, and dendritic cells.
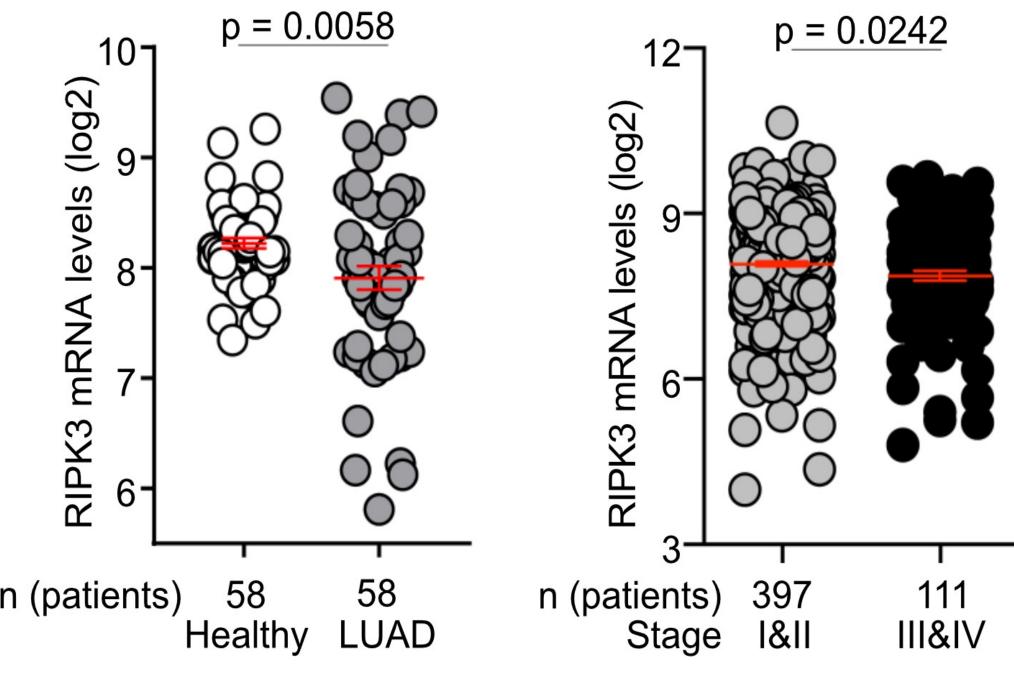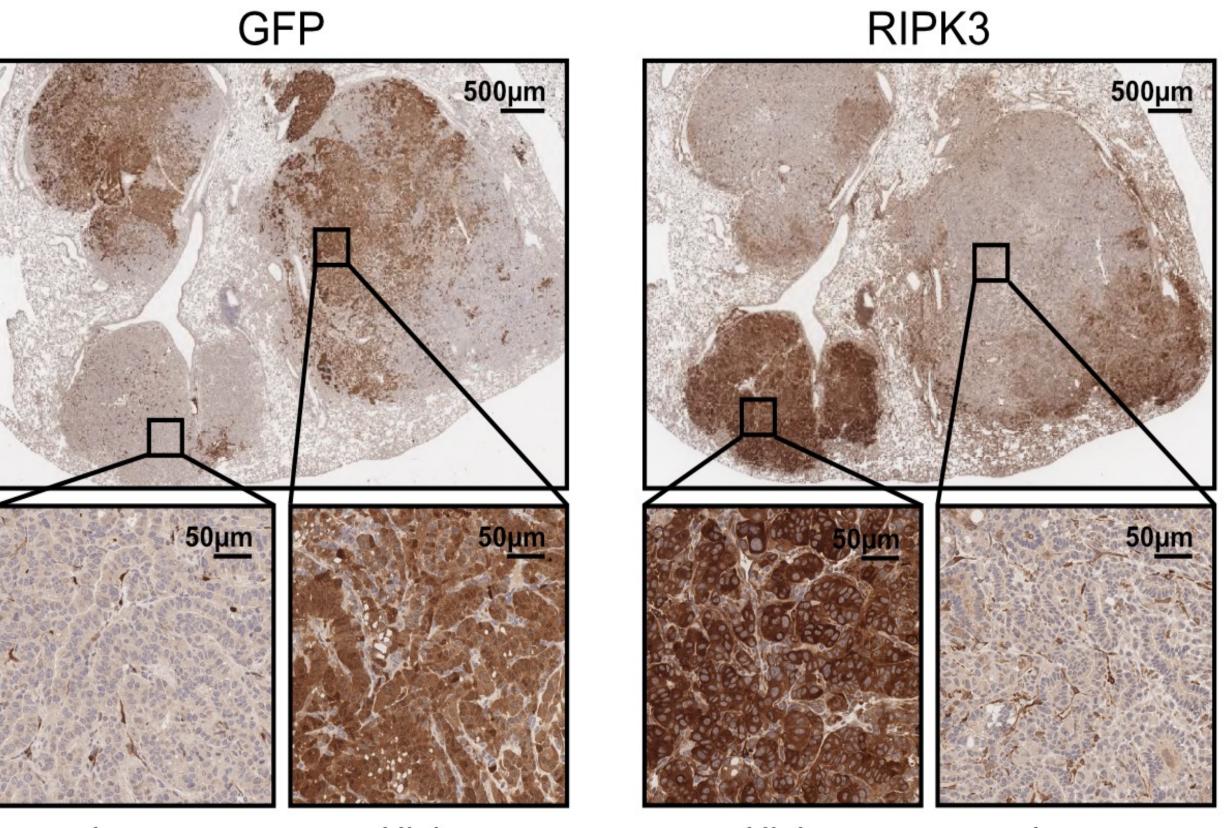

Figure 1: TCGA analysis of RIPK3 expression in human LUAD samples.

Figure 2: Calculation of Danaher immune scores in high and low RIPK3 expression groups (upper vs. lower quartile).

In LUAD mice exposed to sgRipk3, the Cre-recombination did not show a full penetrance, therefore eliciting Ripk3 proficient and deficient lesions within the same animal. Interestingly, the tumor area of Ripk3 deficient lesions was significantly higher than Ripk3 proficient lesions.

Figure 3: Representative LUAD lesions with IHC stains against GFP (Cas9 – reporter gene) and RIPK3 (knockout control). Cas9 mediated Ripk3 knockouts in LUAD lesions increased tumor size and number of tumors.

Analysis of the sgLacZ group revealed an heterogenous Ripk3 expression profile in tumors, where more advanced and bigger tumors showed weaker expression of RIPK3, suggesting that tumors actively evade RIPK3 expression.

Figure 4: Advanced LUAD lesions decreased RIPK3 expression (BH = Bronchial hyperplasia, AAH = Atypical adenomatous hyperplasia, Ad, AC = Adenoma, Adenocarcinoma). RIPK3 high expressing Adenoma, Adenocarcinoma showed smaller tumor sizes then low RIPK3 expressing LUAD.

Conclusion

Taken together our data clearly point to RIPK3 as a tumor suppressor in LUAD. This effect may be driven by the inhibition of necroptotic cell death, which in turn alters immune cell recruitment.

The presenting author has no conflicts of interest to declare.
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Platt, R.J., et al., *CRISPR-Cas9 knockin mice for genome editing and cancer modeling.* Cell, 2014. **159**(2): p. 440-55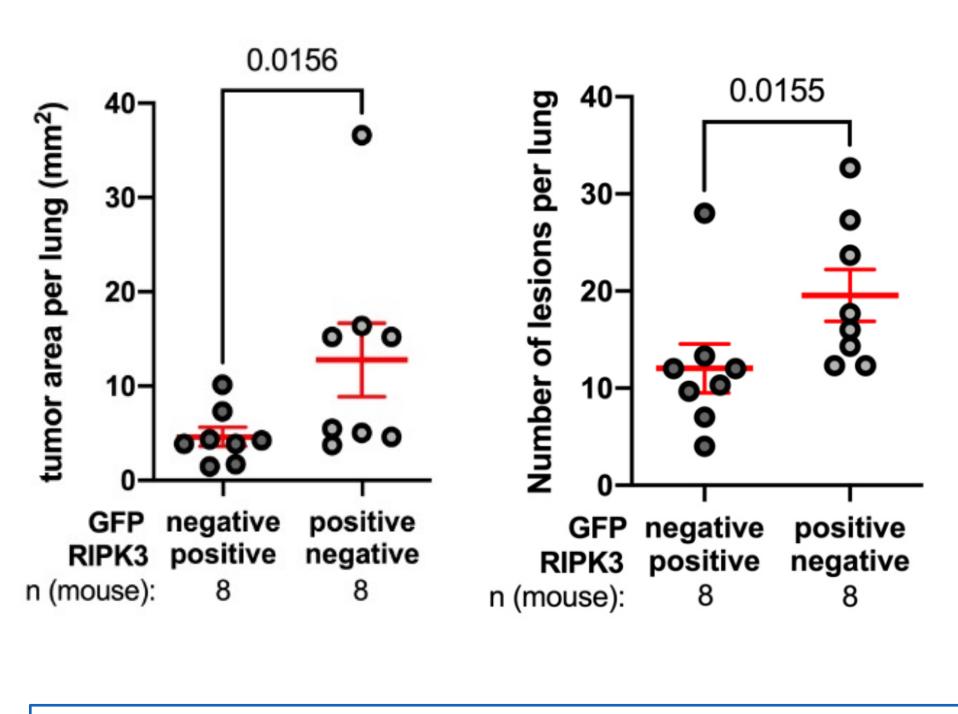.