

Supplementary material

Interferon Regulatory Factor 9 Promotes Lung Cancer Progression via Regulation of Versican

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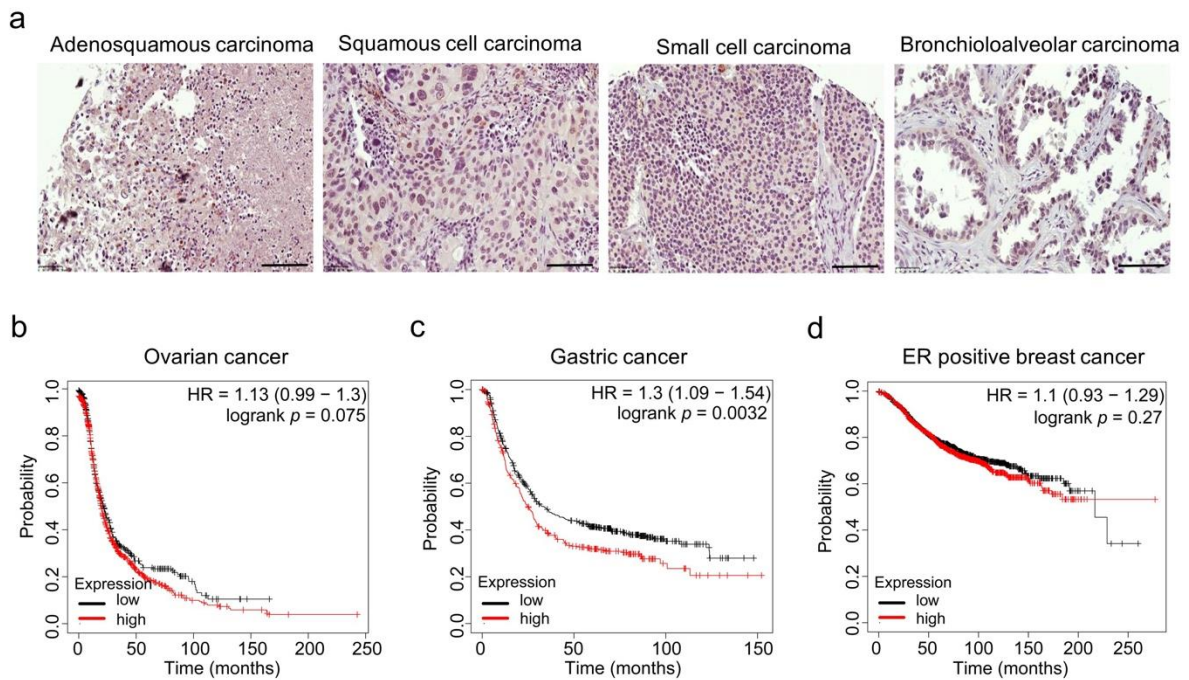


Figure S1. (a) IRF9 expression in different types of lung cancer types and its association with survival in various cancer types. IRF9 immunohistochemistry images from adenosquamous carcinoma ($n = 7$), squamous cell carcinoma ($n = 32$), small cell carcinoma ($n = 6$), and bronchioloalveolar carcinoma. ($n = 2$). Scale bar 100 μm . Kaplan–Meier curves for IRF9 expression associated with the survival of patients with (b) ovarian cancer, (c) gastric cancer and (d) ER positive breast cancer, divided by the median into high and low expression. Data obtained from Kaplan Meier plotter (Gyorffy et al. 2013).

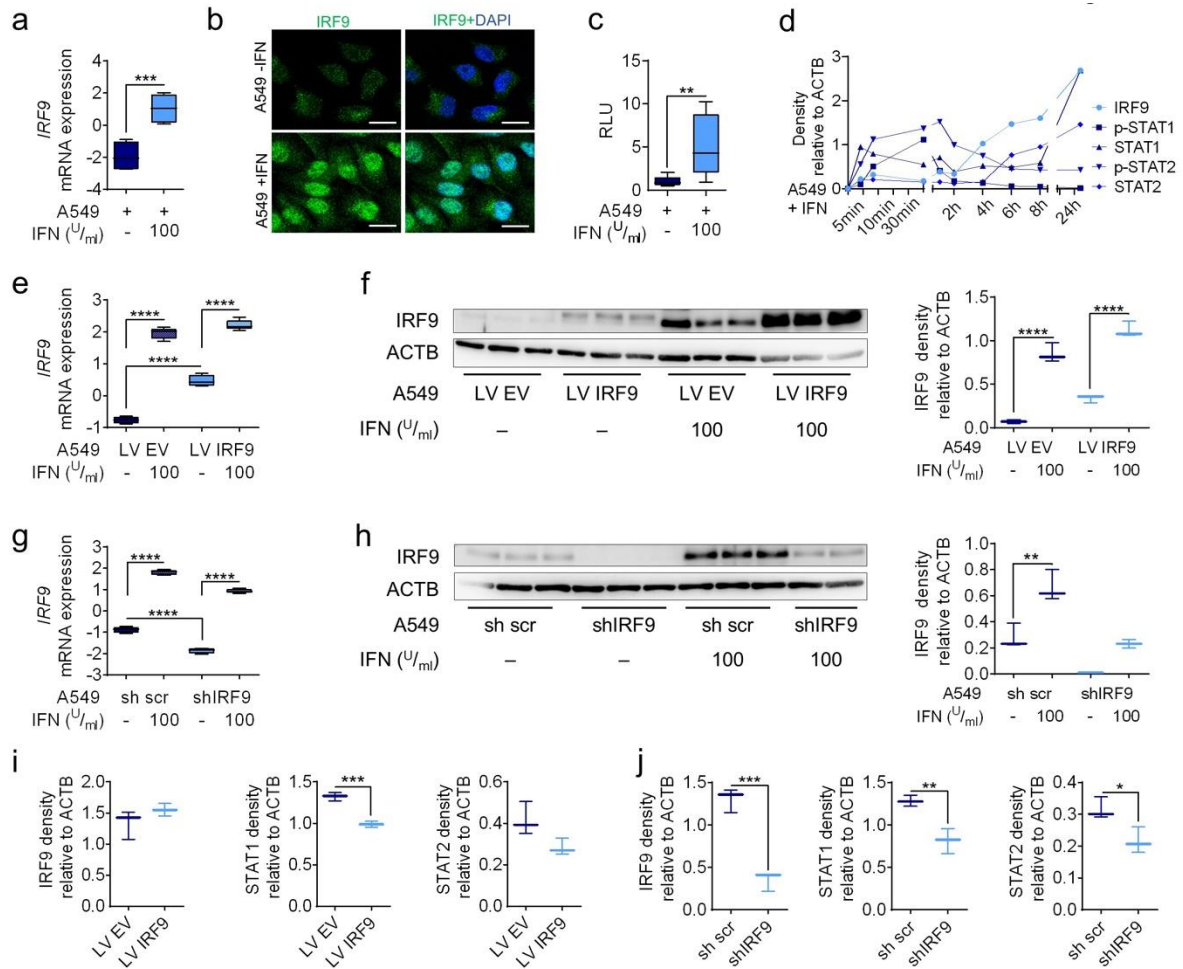


Figure S2: IFN stimulation leads to increased expression and activity of IRF9. A549 cells were stimulated with 100 U/mL Type I IFN for 24 h and expression of IRF9 was evaluated on (a) mRNA level and (b) cellular level via immunocytochemistry staining using IRF9 antibody and revealed by an AlexaFlour 488 secondary antibody (green). DNA was stained with 4',6-diamidino-2-phenylindole (Dapi, blue), scale bars, 50 μm ($n = 3$). (c) Activity of IRF9 was measured using a luciferase reporter gene assay with Interferon stimulated response element (ISRE) containing luciferase vector ($n = 3$). (d) Quantification of protein expression via western blot densitometry analysis of IFN stimulated A549 cells at several time points. (e,g) Transduced IRF9 tumor cells were stimulated with 100 U/mL Type I IFN for 24 h and expression of *IRF9* was evaluated on mRNA level ($n = 2-3$) and (f,h) protein level, RNA expression was calculated as $\Delta\Delta Ct$. A549 cells treated with IFN compared to A549 without IFN, LV IRF9 compared to LV EV and shIRF9 to sh scr respectively. (i,j) Protein expression of IRF9, STAT1 and STAT2 genes in transduced cell lines were quantified via western blot analysis measuring densitometry compared to loading control ACTB ($n = 3$). Data are shown as mean \pm standard error of the mean using Student's *t*-test or one-way ANOVA. p -values ≤ 0.05 were considered statistically significant for all analyses. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$.

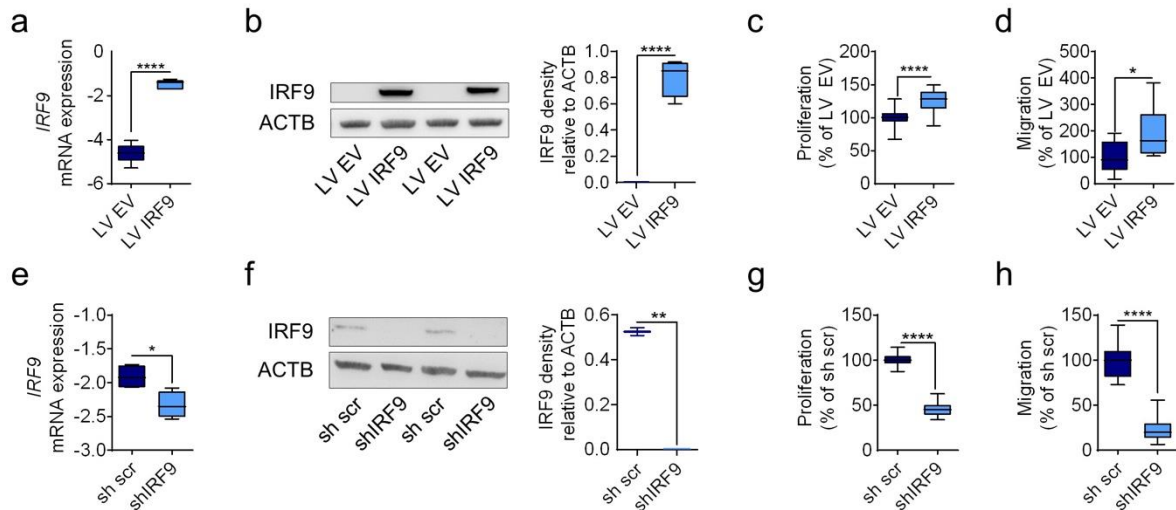


Figure S3: IRF9 transduced A427 cells reveals oncogenic phenotype. (a,e) A427 cells were transduced to overexpress IRF9 (LV IRF9) or silence (shIRF9) validated by qRT-PCR. Empty vector (LV EV) or scrambled sequence (sh scr) were used as controls respectively. Western Blot analysis confirmed the (b) overexpression or (f) silencing of IRF9 at protein level. (c,g) Proliferation and (d,h) migration of transduced A427 were evaluated as percentage of control respectively. Data are shown as mean \pm standard error of the mean using Student's *t*-test. * $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$; were considered statistically significant for all analyses ($n = 3$).

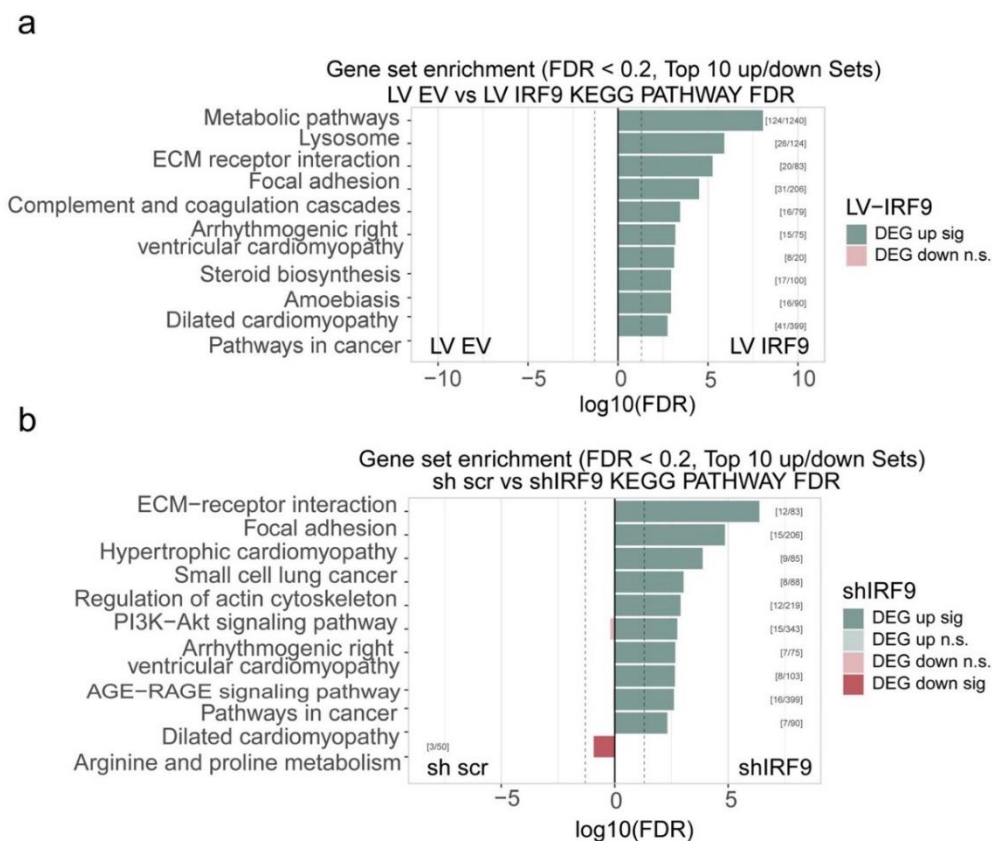


Figure S4: Gene set enrichment analysis of IRF9 overexpressing and silenced A549 cells. (a,b) Gene set enrichment analysis of KEGG pathway genes in LV IRF9 and shIRF9 transduced A549 cells compared to control vector transduced A549 cells.

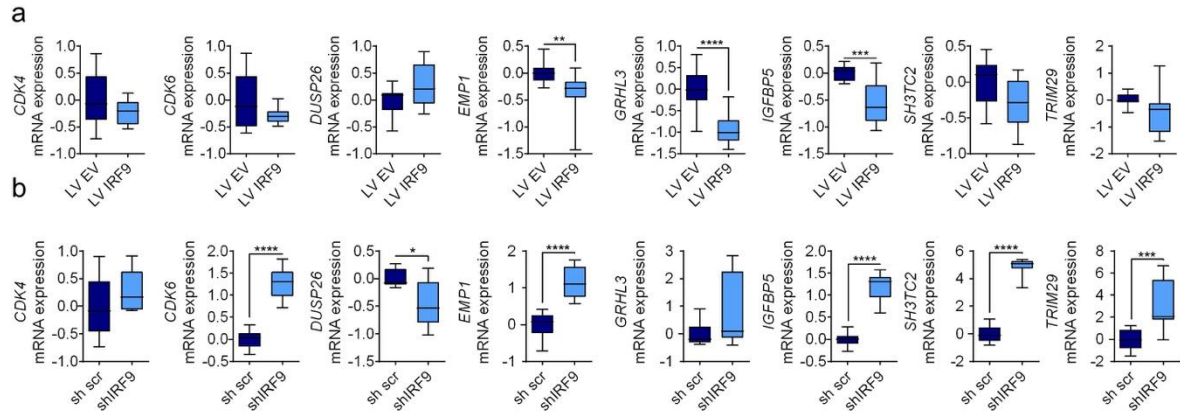


Figure S5: Validation of RNA seq target genes in transfected A549 cells. mRNA expression of potential target genes (*CDK4*, *CDK6*, *DUSP26*, *EMP1*, *GRHL3*, *IGFBP5*, *SH3TC2*, *TRIM29*) from RNA-seq were validated using qRT-PCR in IRF9 (a) overexpressing and (b) knockdown cells, calculated as $\Delta\Delta Ct$ ($n = 2-3$). LV IRF9 compared to LV EV and shIRF9 to sh scr respectively. Data are shown as mean \pm standard error of the mean using Student's *t*-test. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$; **** $p < 0.0001$; were considered statistically significant for all analyses.

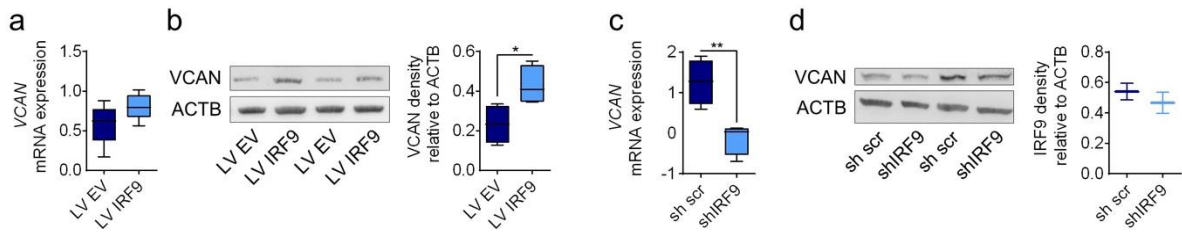


Figure S6: VCAN expression in IRF9 transfected A427 lung cancer cells. Expression of VCAN was evaluated on (a,c) mRNA level and (b,d) protein level in each cell set of IRF9 transfected A427. LV IRF9 compared to LV EV and shIRF9 to sh scr respectively. Data are shown as mean \pm standard error of the mean using Student's *t*-test. * $p < 0.05$; ** $p < 0.01$; were considered statistically significant for all analyses ($n = 3$).

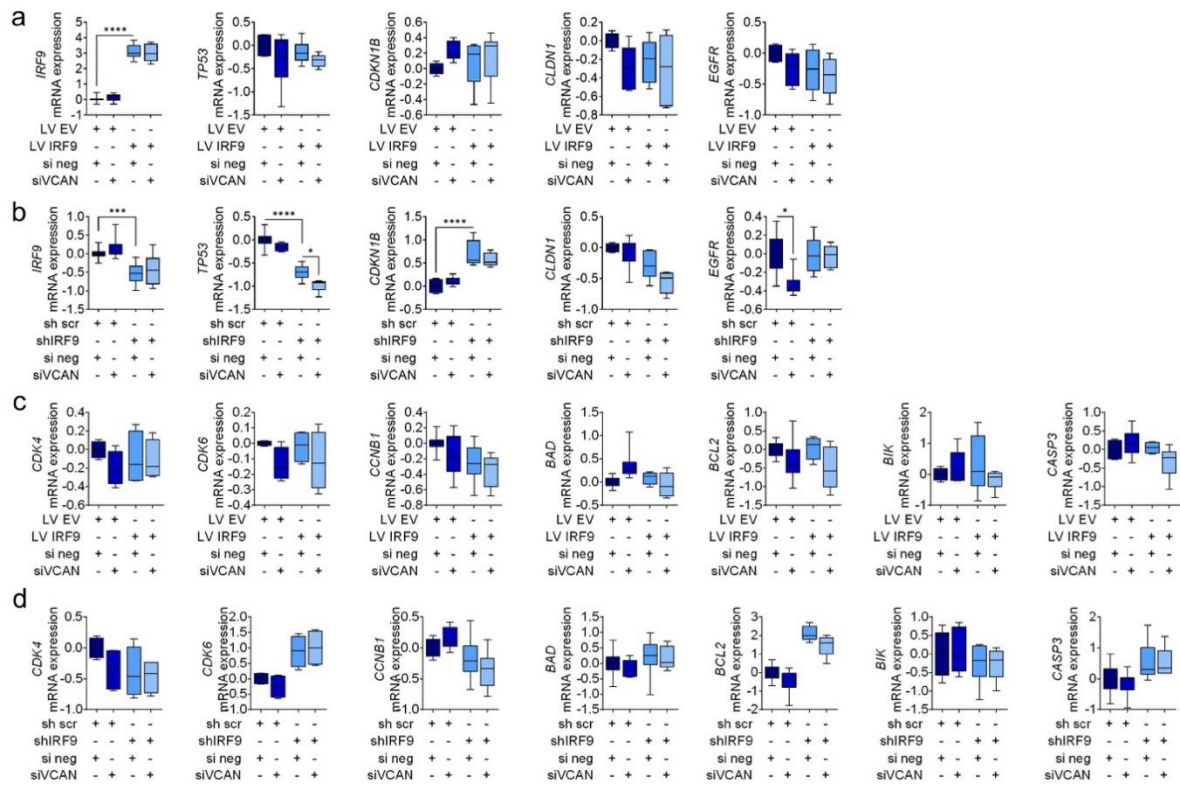


Figure S7: Knockdown of VCAN does not alter genes that are involved in EGF, cell cycle and apoptosis pathways. IRF9 transduced A549 cells were transfected with siRNA against VCAN (siVCAN) to knockdown VCAN expression. A non-targeting sequence was used as transfection control (si neg). After knockdown, the expression of indicated genes (*TP53*, *CDKN1B*, *CLDN1*, *EGFR*, *CDK4*, *CDK6*, *CCNB1*, *BAD*, *BCL2*, *BIK* and *CASP3*) was evaluated via qRT-PCR, in (a,c) overexpressing A549 and (b,d) knockdown A549 compared to A549 LV EV si neg or A549 sh scr si neg respectively. mRNA expression was calculated as $\Delta\Delta Ct$. Data shown as mean \pm standard error of the mean using one-way analysis of variance. * $p < 0.05$; *** $p < 0.001$; **** $p < 0.0001$; were considered statistically significant for all analyses ($n = 3$).

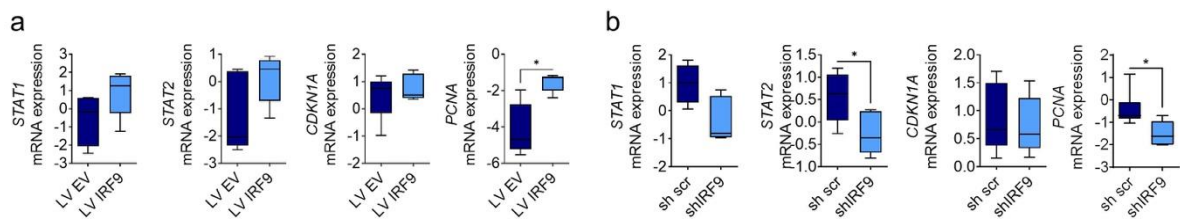
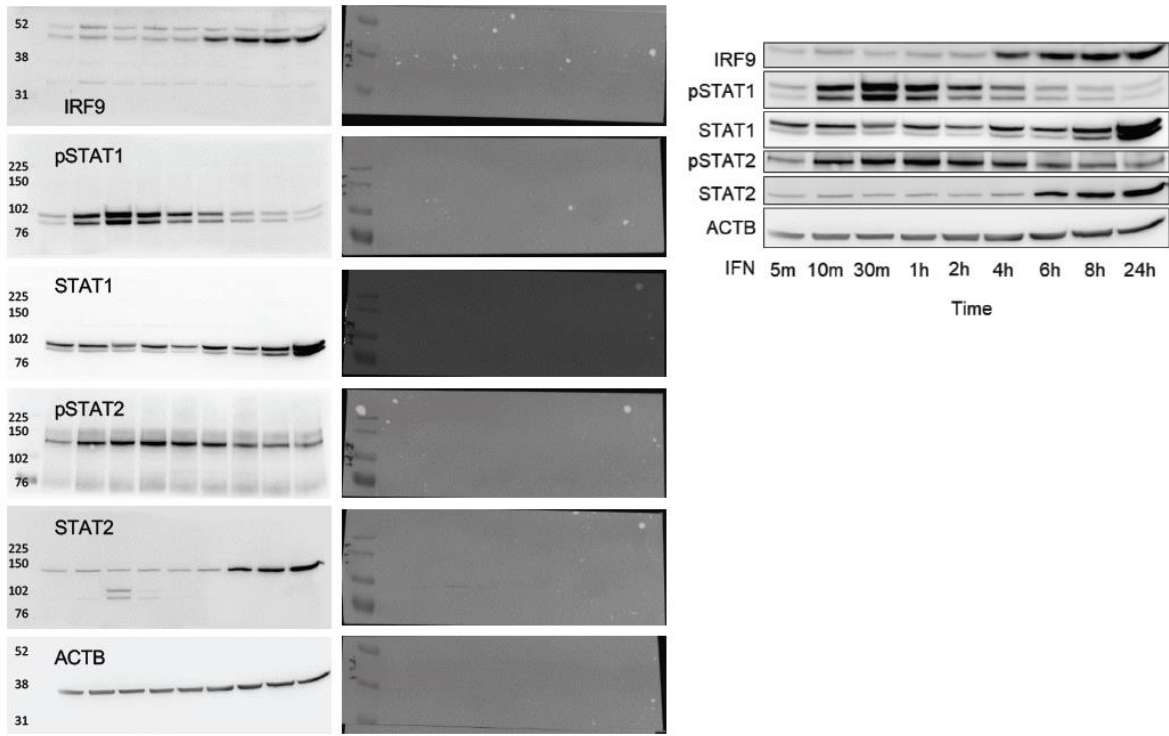
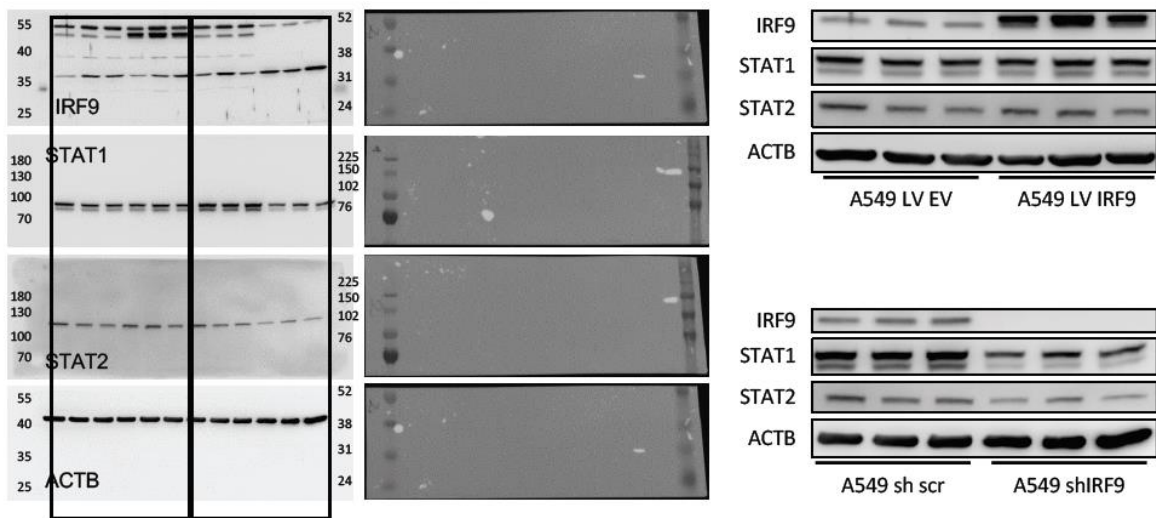


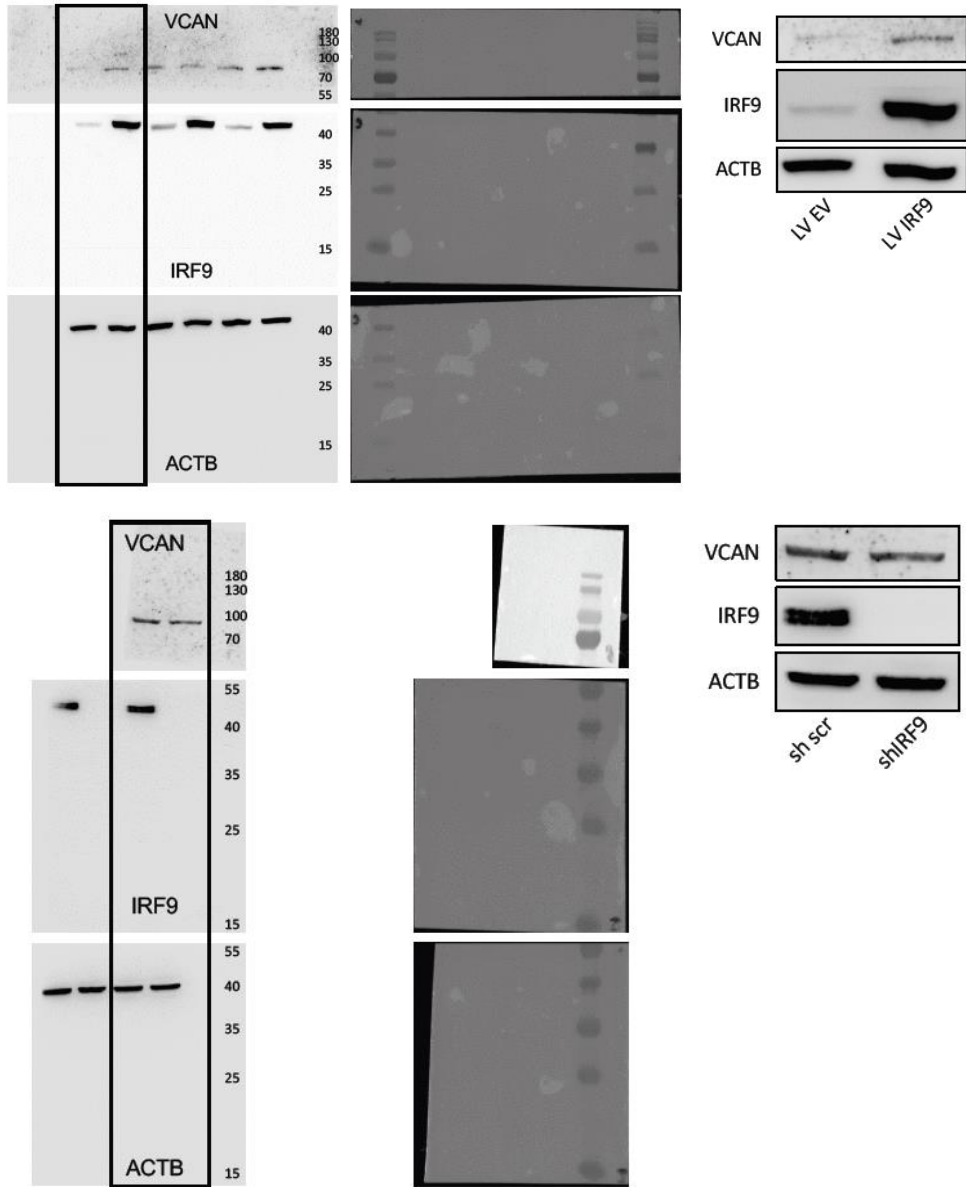
Figure S8: Evaluation of IRF9 associated genes in IRF9 overexpressed or silenced tumors. mRNA expression of *STAT1*, *STAT2*, *CDKN1A* and *PCNA* from tumor homogenate of IRF9 overexpressing (a) and silenced (b) tumors. Data are shown as mean \pm standard error of the mean using Student's *t*-test. * $p < 0.05$; were considered statistically significant for all analyses ($n = 5$).



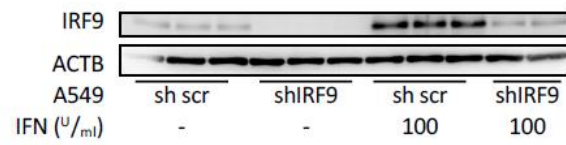
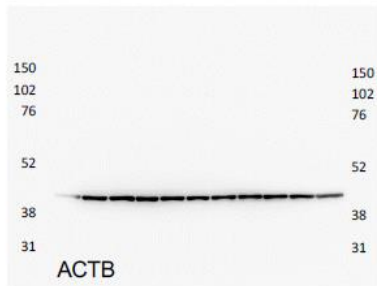
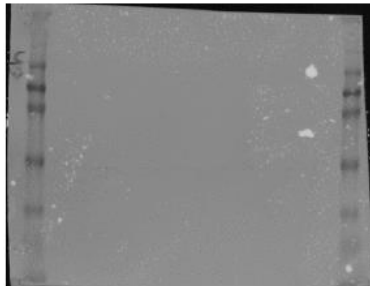
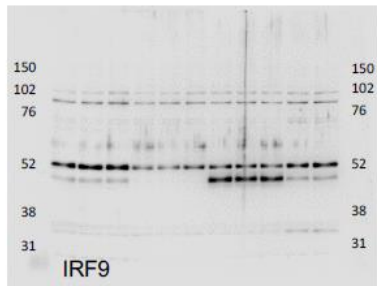
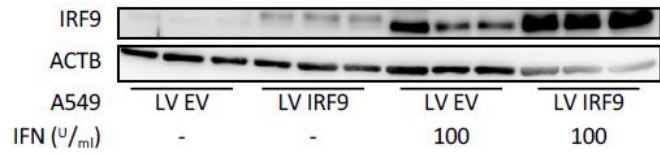
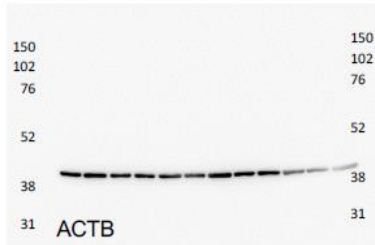
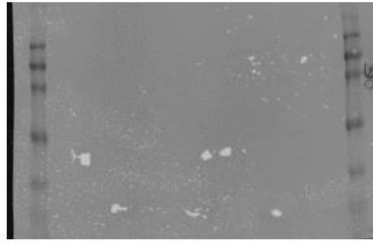
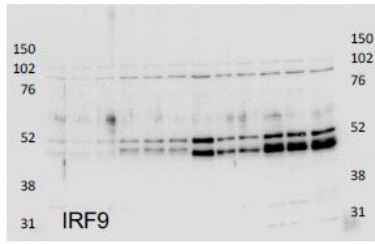
Detail information about Figure 2a.



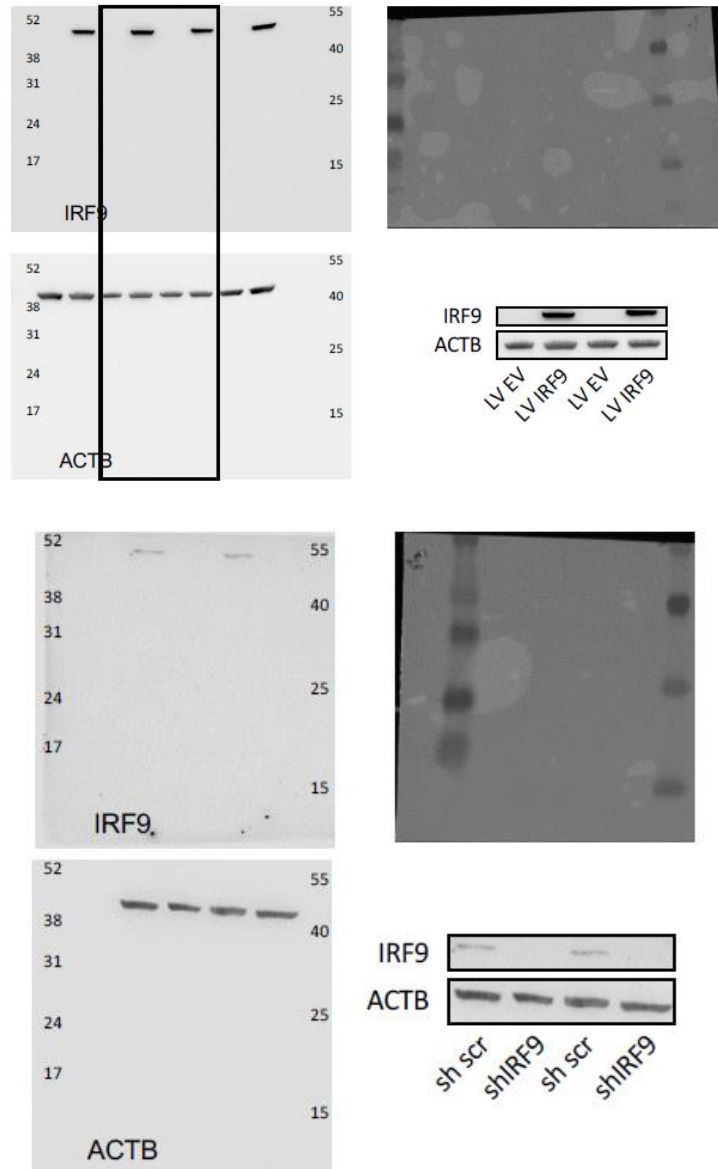
Detail information about Figure 3b,h.



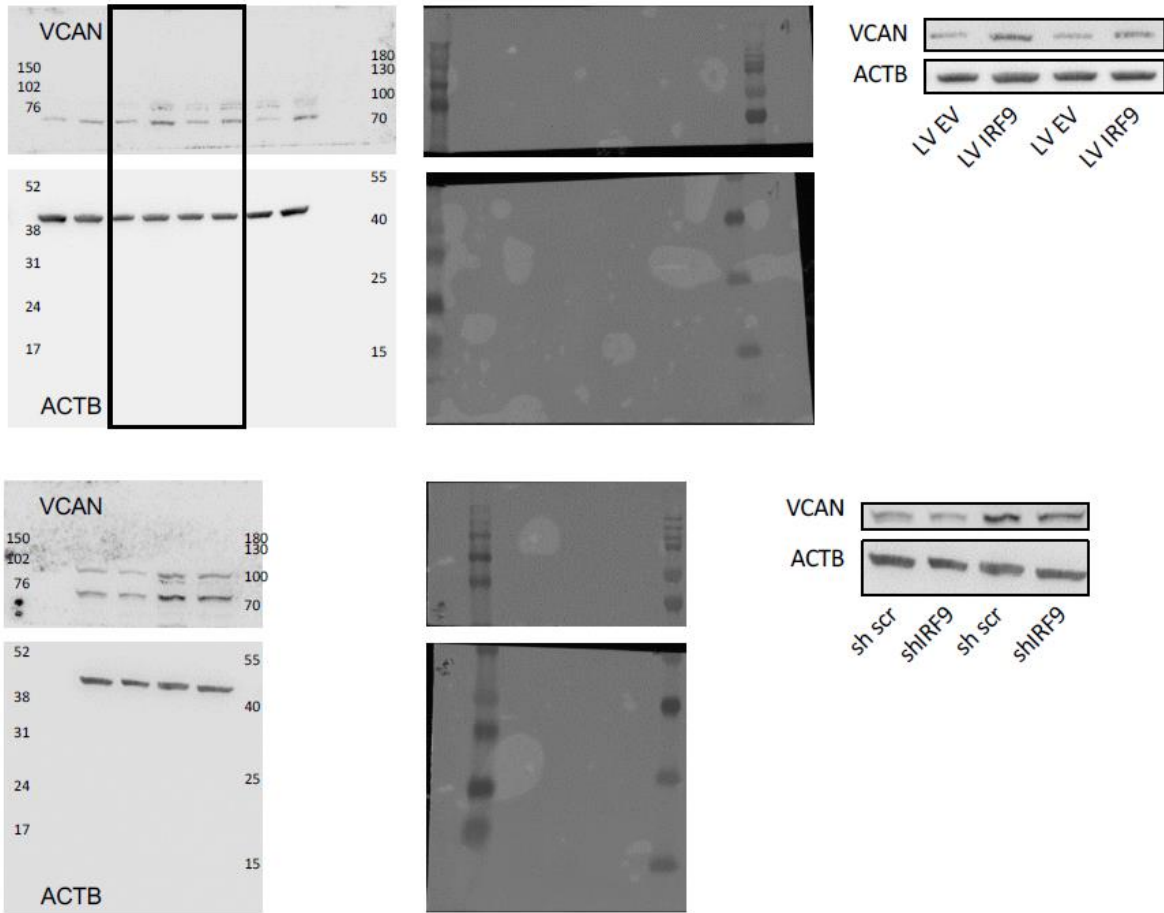
Detail information about Figure 4h,j.



Detail information about Figure S2f,h.



Detail information about Figure S3b,f.



Detail information about Figure S6b,d.



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