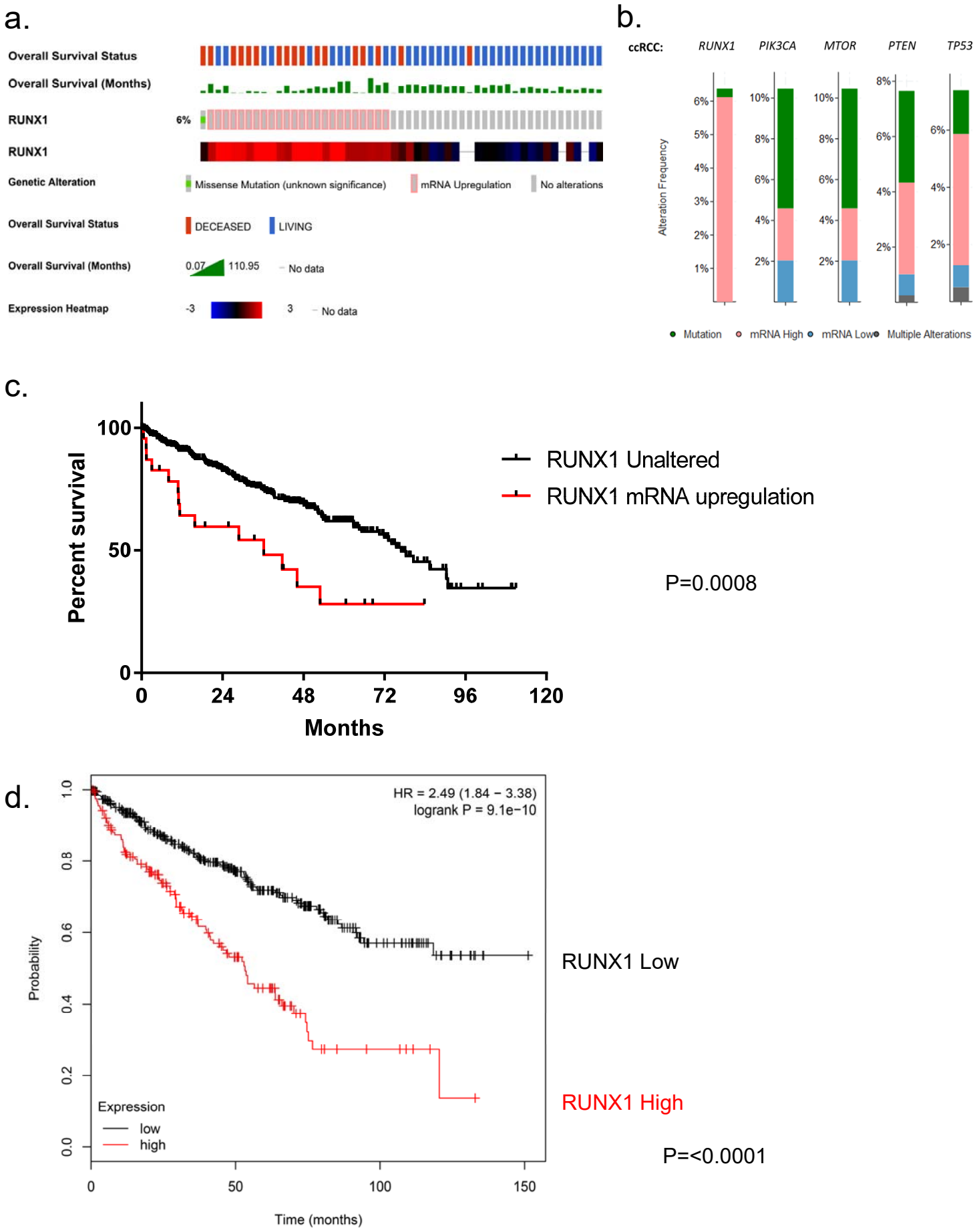
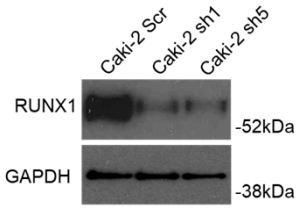


Supplementary Figure 1. *In Silico analysis of RUNX1 expression in human kidney cancer.* **a,** Data from TCGA Nature 2013 ccRCC dataset acquired using cBioportal, *RUNX1* is altered in 6% of ccRCC cases. **b,** The majority of *RUNX1* genetic alterations (96%) are mRNA upregulation in comparison to a mix of alterations for other ccRCC genes (*PIK3CA, MTOR, PTEN, TP53*). **c,** TCGA Nature 2013 ccRCC survival data show patients with *RUNX1* mRNA upregulation have poorer overall survival, Log-rank P=0.0008, *RUNX1* unaltered n=390, median=76.98 months; *RUNX1* mRNA upregulation n=24, median=36.21 months. **d,** Data from KM-plotter analysis of 530 ccRCC patients, those with high *RUNX1* expression have poorer survival, Log-rank P<0.0001, *RUNX1* High, n=155, *RUNX1* Low n=375.

a.



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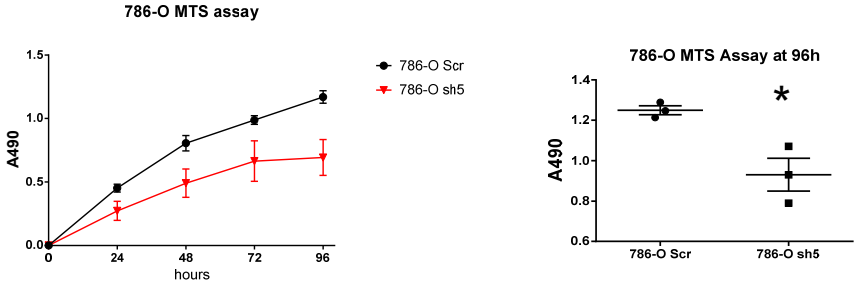

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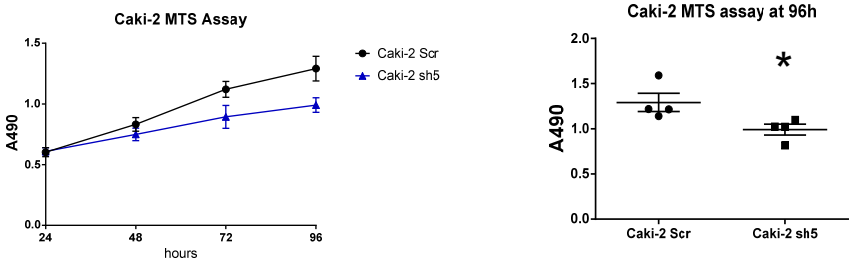

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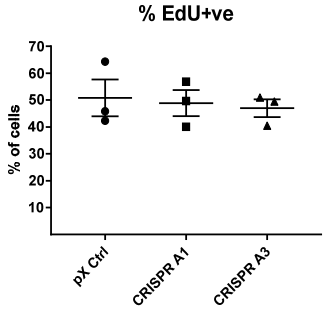

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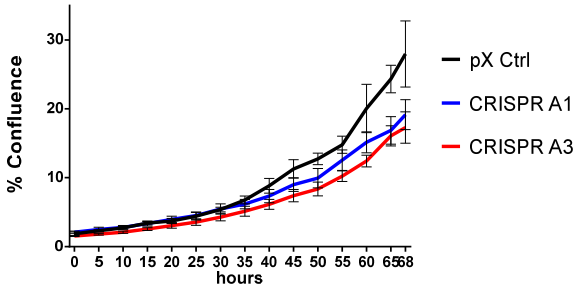
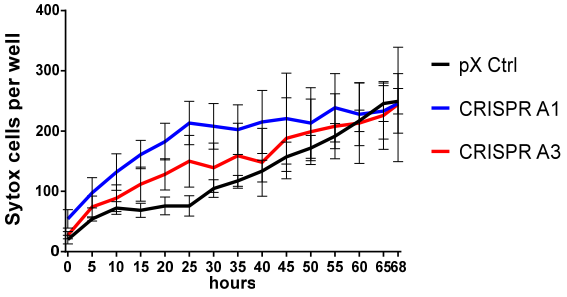
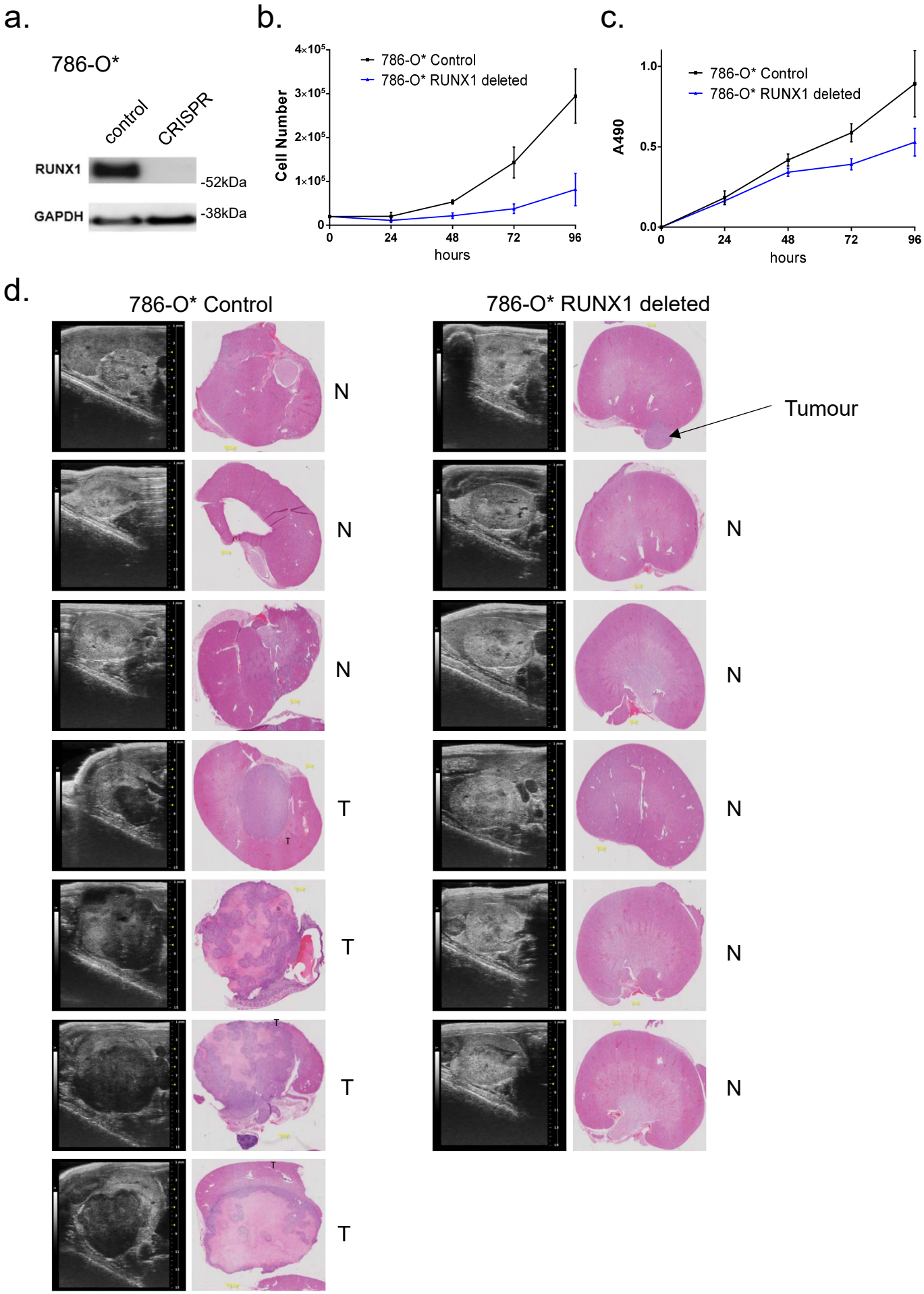


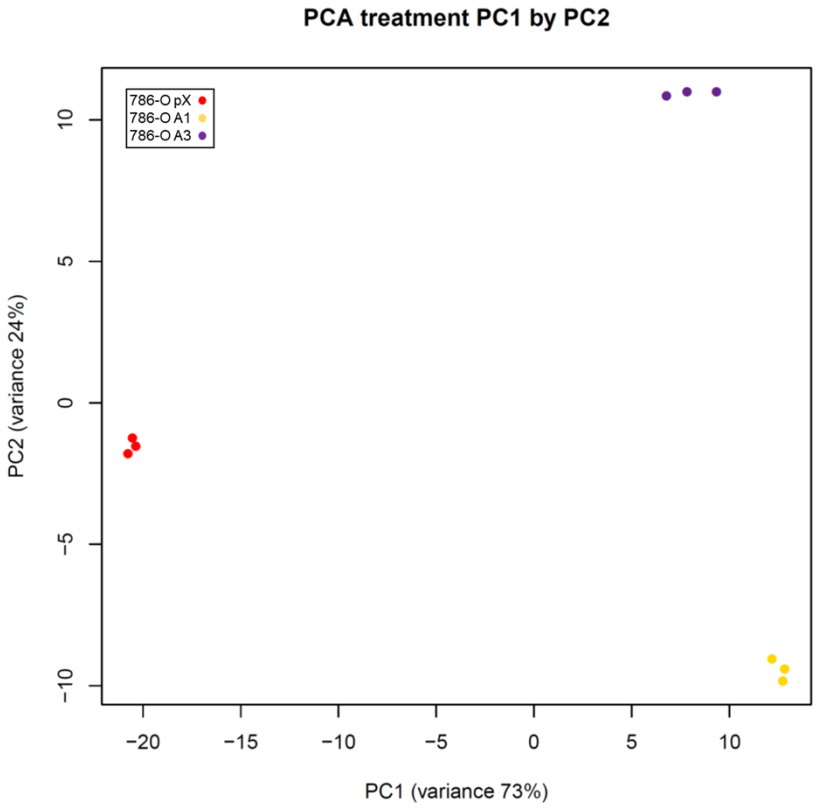
f.



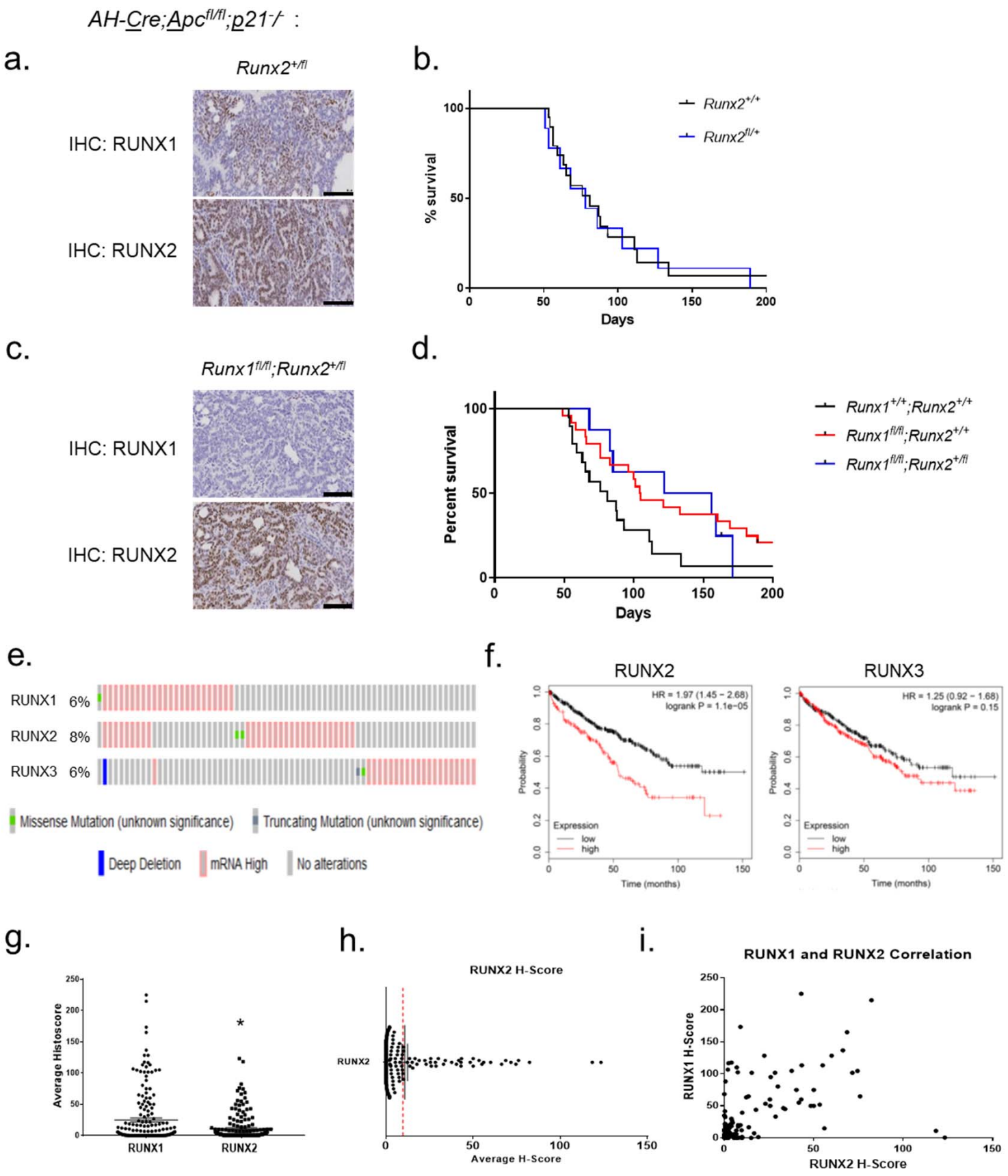
Supplementary Figure 2. *Knockdown of RUNX1 with shRNA causes a growth defect in two ccRCC cell lines.* **a,** Representative immunoblot of Caki-2 cells confirms partial knockdown of RUNX1 in shRUNX1 transduced cells (sh1 and sh5) compared to scrambled control (Scr). Blot stripped and re-probed for GAPDH loading control. **b,** MTS assay shows reduced viability for shRUNX1 -5 (786-O sh5) vs Scr control cells (786-O Scr). T-test at 96 hours shows statistically significant decrease in absorbance at 490nm in sh5 cells compared to Scr (right panel), *P=0.0191. **c,** MTS assay shows reduced viability for shRUNX1 -5 (Caki-2 sh5) vs Scr control (Caki-2 Scr). T-test at 96 hours shows statistically significant decrease in absorbance at 490nm in sh5 cells compared to Scr (right panel), *P=0.0436. All MTS assays performed in quadruplicate a minimum of 3 independent times. Error bars -/+ SEM. **d,** Average % of EdU+ve cells at T0. **e,** Average % confluence of control (pX Ctrl) and RUNX1-deleted (CRISPR A1 and CRISPR A3) 786-O cells cultured in the presence of SYTOX® Green. **f,** The average number of SYTOX® Green positive cells per well, ANOVA P values: A1 P= 0.0001, A3 P= 0.015. SYTOX experiments (N=4) were performed in quadruplicate and analysed using Incucyte software, error bars -/+ SEM.



Supplementary Figure 3. *RUNX1 deletion in 786-O* cells and orthotopic xenograft*. **a,** Representative Immunoblot of 786-O* vector control and RUNX1 CRISPR cells confirms deletion of RUNX1. GAPDH loading control. **b,** Cell counting growth curve shows reduced cell number in RUNX1-deleted 786-O* cells, T-test at 96h P= 0.0421, error bars are -/+ SEM, N=3 independent experiments. **c,** MTS assay for RUNX1-deleted compared to control 786-O* cells, y axis is absorbance at 490nm, error bars are -/+ SEM, N=4 independent experiments. **d,** Representative examples of whole kidney H&E and endpoint ultrasound images of injected kidneys – control group on the left and RUNX1-deleted on the right. The small tumour region present in 1/6 RUNX1-deleted kidneys is indicted by an arrow. T=tumour, N=no tumour.



Supplementary Figure 4. *Principle component analysis.* Gene expression variances between control (786-O pX, Red) and RUNX1 CRISPR cells (786-O A1, yellow and 786-O A3, purple).



Supplementary Figure 5. *Heterozygous deletion of RUNX2 in a GEM model of kidney cancer does not affect survival.* **a,** Representative image of a *CAP;Runx2*$^{fl/+}$ kidney tumour immuno-stained for RUNX1 and RUNX2, scale bars=100μm. *CAP* mice are *AH-Cre;Apc*$^{fl/fl}$*;p21*$^{-/-}$. **b,** Kaplan-Meier curve showing no effect on survival in *CAP;Runx2*$^{fl/+}$ mice (n=9) compared to *CAP;Runx2*$^{+/+}$ mice (n=16). **c,** Representative image of a *CAP;Runx1*$^{fl/fl}$*;Runx2*$^{fl/+}$ kidney tumour immuno-stained for RUNX1 and RUNX2, scale bars=100μm. **d,** Kaplan-Meier curve showing no effect due to heterozygous deletion of *Runx2* on a *Runx1*$^{fl/fl}$ tumour background. *CAP;Runx1*$^{fl/fl}$*;Runx2*$^{fl/+}$ kidney tumour mice (blue line, n=7) do not have altered survival compared to *CAP;Runx1*$^{fl/fl}$ mice (red and black lines are the same as from Fig5C). **e,** Data from TCGA Nature 2013 human ccRCC dataset acquired using cBioportal, *RUNX2* is altered in 8% of ccRCC cases compared to 6% for RUNX1 and RUNX3 respectively. **f,** Data from KM-plotter analysis of 530 human ccRCC patients, those with high *RUNX2* expression have poorer survival, Log-rank P<0.0001, RUNX2 High n=142, RUNX2 Low n=388. Survival is unaffected in patients with high RUNX3 expression. **g,** Comparison of average RUNX1 and RUNX2 H-Scores in human RCC TMA. RUNX1 H-Score=25.1, RUNX2 H-Score=10.9, t-test P=<0.0001. **h,** Quantification of RUNX2 expression by RUNX2 H-Score for the full TMA, the dashed red line represents the cut off for RUNX2 Low (Quartile 1-3, H-Score: 0 to 9.3, n=139) and RUNX2 High (upper quartile, H-Score 9.7 to 123.3, n=44). **i,** Positive correlation between average RUNX1 and RUNX2 H-Score, Pearson r coefficient= 0.5639, P= <0.0001.