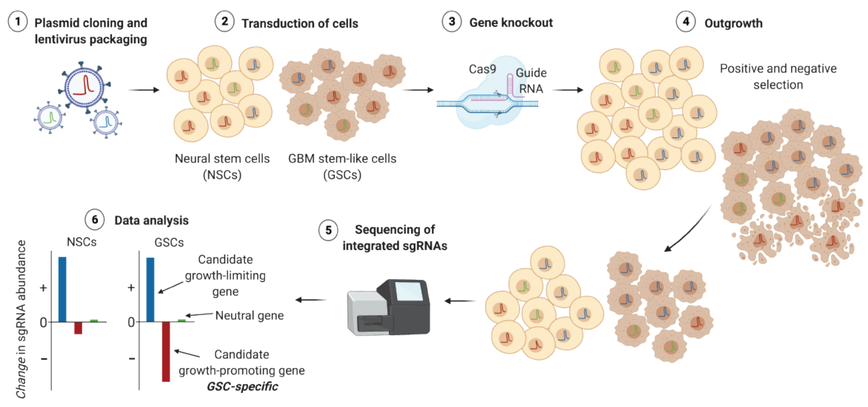
FBXO42 is a cancer-specific genetic vulnerability that is involved in proper chromosome alignment during mitosis

Pia Hoellerbauer[1,2], Megan Kufeld[1], Sonali Arora[1], Emily J. Girard[3], Lucas Carter[1], Philip Corrin[4], Ryan Basom[4], Jacob A. Herman[1,5], Jeffrey J. Delrow[4], James M. Olson[3], and Patrick J. Paddison[1,2].

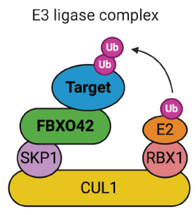
[1]Human Biology Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA [2]Molecular and Cellular Biology Program, University of Washington, Seattle, WA, USA [3]Clinical Research Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA [4]Genomics and Bioinformatics Shared Resources, Fred Hutchinson Cancer Research Center, Seattle, WA, USA [5]Basic Sciences Division, Fred Hutchinson Cancer Research Center, Seattle, WA, USA

Background

FBXO42 was a gene hit identified in pooled CRISPR/Cas9 screens comparing patient-derived glioblastoma stem-like cell (GSC) isolates to normal human neural stem cells (NSCs):



- FBXO42 encodes an F-box protein that serves as the substrate-recognition component of an SCF (SKP1-CUL1-F-box protein)-type E3 ubiquitin ligase complex.
- Scored as highly specifically required by one of three patient-derived isolates in our outgrowth screens.
- Thus far very poorly studied.
- One group previously described a role for FBXO42 in the destabilization of p53, similar to MDM2 (Sun et al. 2009, Sun et al. 2011).



Overall Questions:
- Disease relevance and cancer type specificity for gene requirement?
- Ubiquitin ligase activity or other function responsible for requirement?
- Cause of viability loss in sensitive cells?

The ubiquitin ligase role of FBXO42 is responsible for the gene requirement through a novel, unknown substrate

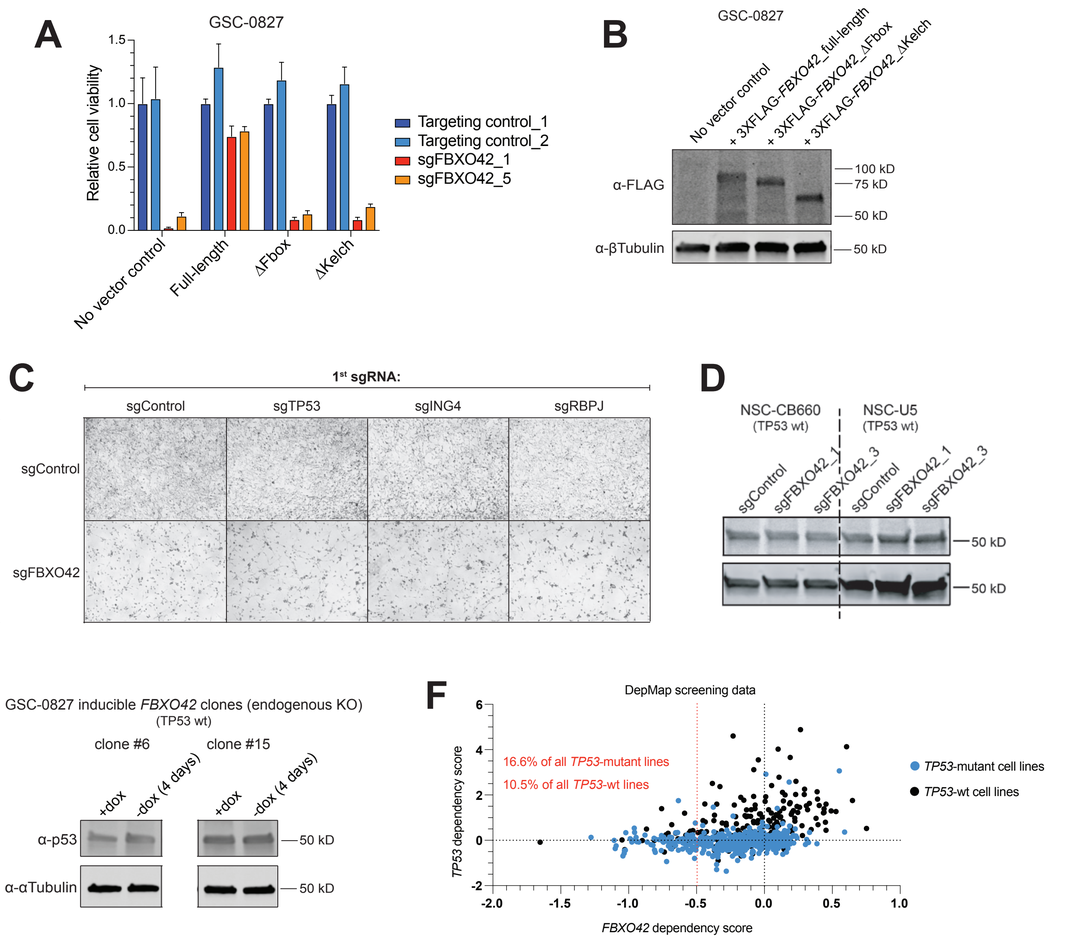


Figure 3: (A) Relative cell viability (normalized to targeting control sgCD8A) for GSC-0827 transduced with full-length, F-box domain deletion mutant, or Kelch domain deletion mutant versions of 3XFLAG-FBXO42 and then nucleofected with CRISPR RNPs targeting FBXO42, measured at 8 days post nucleofection. The lentiviral expression constructs are resistant to the FBXO42 sgRNAs used here. Untransduced GSC-0827 are shown for comparison. (B) Western blot for FLAG tag in transduced GSC-0827 used for viability assay in (A). (C) Representative images of GSC-0827 nucleofected with CRISPR RNPs targeting the published FBXO42 targets/interactors TP53, ING4, or RBPJ in combination with sgControl or sgFBXO42, taken at 5 days post nucleofection. (D) Western blot for p53 levels in NSCs nucleofected with CRISPR RNPs targeting FBXO42. (E) Western blot for p53 levels in the GSC-0827 doxycycline-inducible FBXO42 clones. (F) Cell line functional genomic screening data from DepMap showing FBXO42 dependency score vs. TP53 dependency score. TP53-mutant cell lines are marked in blue.

FBXO42 is required in a subset of cancer cells in vitro and in vivo but is nonessential in NSCs in vitro

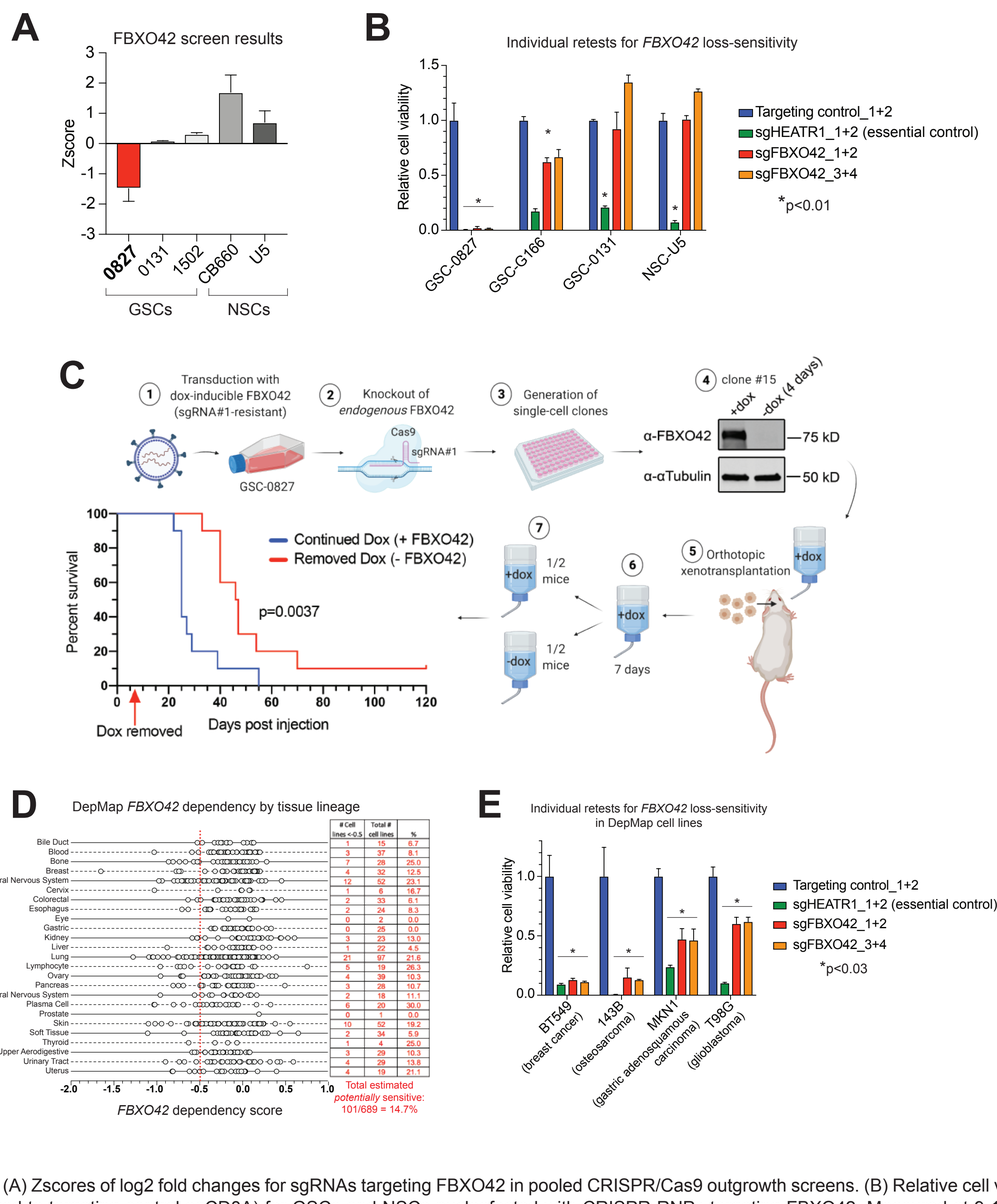


Figure 1: (A) Zscores of log2 fold changes for sgRNAs targeting FBXO42 in pooled CRISPR/Cas9 outgrowth screens. (B) Relative cell viability (normalized to targeting control sgCD8A) for GSCs and NSCs nucleofected with CRISPR RNPs targeting FBXO42. Measured at 9-11 days post nucleofection (depending on doubling time). (C) In vivo experiment using immunocompromised mice which were orthotopically xenografted with a clone of GSC-0827 that was engineered to express doxycycline-inducible FBXO42 (endogenous FBXO42 had been knocked out). All mice were initially kept on doxycycline, and then doxycycline was removed for half the mice on Day 7 post injection. n=10 mice per group.(D) Breakdown of FBXO42 dependency by tissue type for cell lines screened in DepMap database. Each circle corresponds to a cell line. Summary of proportion of potentially sensitive cell lines (score <-0.5) by tissue lineage is shown to the right. (E) Relative cell viability (normalized to targeting control sgCD8A) for 4 cell lines that were nucleofected with CRISPR RNPs targeting FBXO42. Lines had been predicted FBXO42 loss-sensitive based on DepMap data. Measured at 8-12 days post nucleofection (depending on doubling time).

Sensitive cells suffer an extended metaphase arrest upon FBXO42 loss due to prolonged spindle assembly checkpoint activation

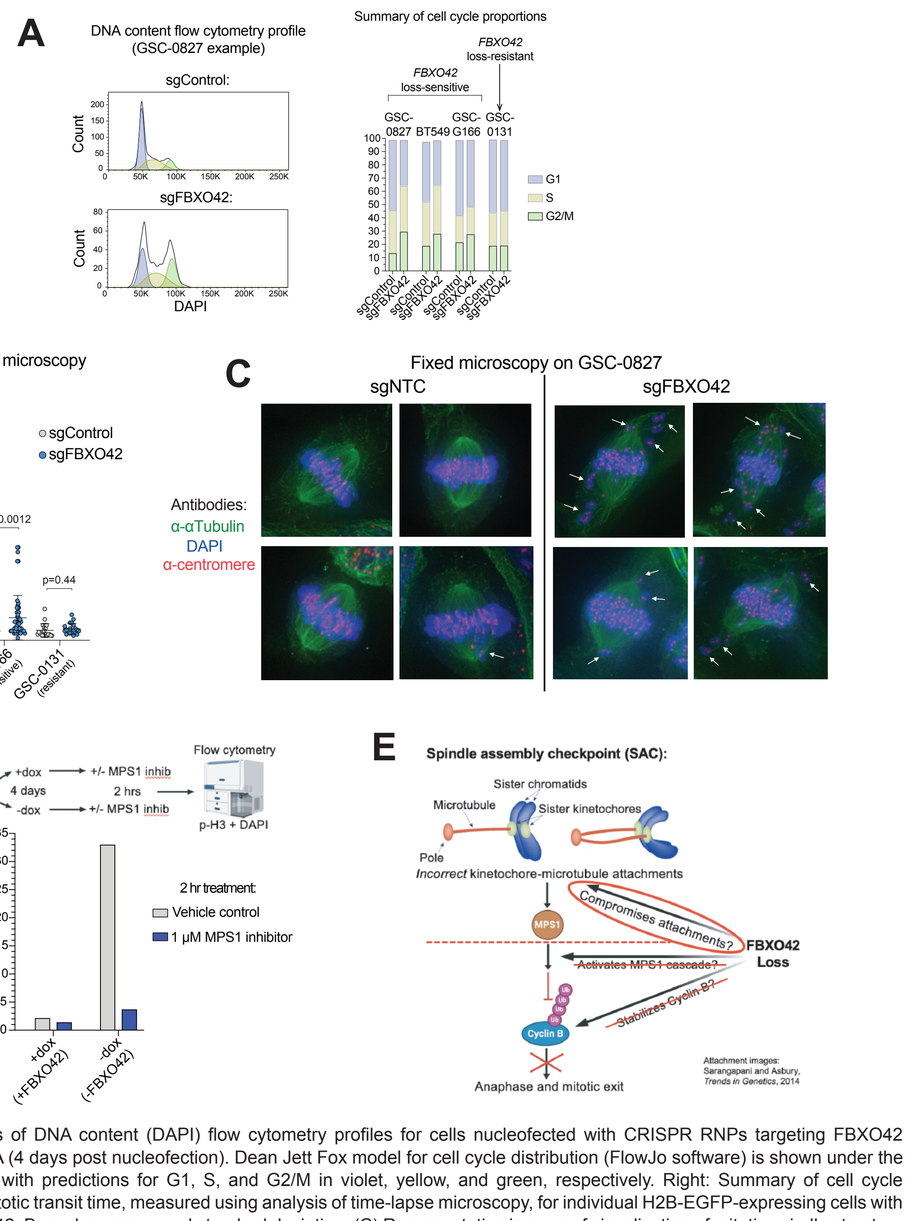


Figure 4: (A) Left: Examples of DNA content (DAPI) flow cytometry profiles for cells nucleofected with CRISPR RNPs targeting FBXO42 compared to a control sgRNA (4 days post nucleofection). Dean Jett Fox model for cell cycle distribution (FlowJo software) is shown under the histogram for each sample, with predictions for G1, S, and G2/M in violet, yellow, and green, respectively. Right: Summary of cell cycle proportion predictions. (B) Mitotic transit time, measured using analysis of time-lapse microscopy, for individual H2B-EGFP-expressing cells with or without knockout of FBXO42. Bars show mean and standard deviation. (C) Representative images of visualization of mitotic spindle structure and chromosome alignment at metaphase upon FBXO42 knockout. Images are a result of 3D projections of multiple Z-stacks taken using a DeltaVision Ultra High Resolution Microscope. (D) Percent mitotic cells (based on DAPI and p-H3 (Ser10) flow cytometry profiles) for GSC-0827 doxycycline-inducible FBXO42 clone kept in +/- doxycycline for 4 days and then treated with vehicle or an Mps1 inhibitor (NMS-P715) for 2 hours. (E) Schematic showing the effect of FBXO42 loss in sensitive cells.

FBXO42 and CCDC6 are both necessary to promote viability in FBXO42 loss-sensitive cells

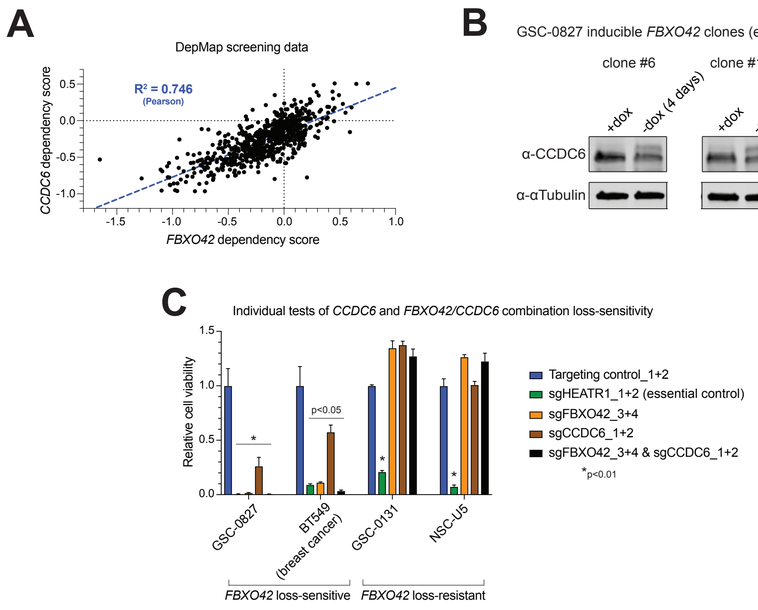


Figure 2: (A) Cell line functional genomic screening data from DepMap showing FBXO42 dependency score vs. CCDC6 dependency score. Each dot represents a cell line. Dotted blue line shows linear regression fit. (B) Western blot for CCDC6 levels in doxycycline-inducible clones of GSC-0827 (described in Figure 1), +/- doxycycline. (C) Relative cell viability (normalized to targeting control sgCD8A) for cells nucleofected with CRISPR RNPs targeting FBXO42, CCDC6, or both. Measured at 8-11 days post nucleofection (depending on doubling time).

Conclusions

- FBXO42 is *not* a pan-essential gene but is required in a subset of cancer cells, independent of tissue of origin. This suggests broad therapeutic potential for targeting FBXO42.
- FBXO42 loss-sensitive cells also require CCDC6 for survival, but FBXO42 and CCDC6 are *not* synthetic lethal in non-sensitive cells, indicating that these two proteins likely work together rather than redundantly.
- The ubiquitin ligase role of FBXO42 is responsible for the sensitivity phenotype, but the published targets TP53, ING4, and RBPJ are *not* primarily responsible.
- Loss of FBXO42 in sensitive cells causes chromosome misalignment, which triggers activation of the spindle assembly checkpoint and prolonged mitotic arrest, eventually leading to cell death.

Outstanding Questions:

What is the cause of sensitivity vs. resistance? What is/are the FBXO42 target(s) responsible for the pheonotype?

References and Acknowledgements

Li, X. et al. Proteomic analyses reveal distinct chromatin-associated and soluble transcription factor complexes. Mol Syst Biol 11, 775-775 (2015).672-685 (2015); Nagler, A. et al. A genome-wide CRISPR screen identifies FBXO42 involvement in resistance toward MEK inhibition in NRAS-mutant melanoma. Pigment Cell & Melanoma Research n/a (2019); Sun, L. et al. JFK, a Kelch domain-containing F-box protein, links the SCF complex to p53 regulation. Proceedings of the National Academy of Sciences of the United States of America 106, 10195-10200 (2009); Sun, L. et al. Substrate phosphorylation and feedback regulation in JFK-promoted p53 destabilization. Journal of Biological Chemistry 286, 4226-4235 (2011); Yan, R. et al. SCFJFK is a bona fide E3 ligase for ING4 and a potent promoter of the angiogenesis and metastasis of breast cancer. Genes Dev 29, 672-685 (2015).

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E-mail: phoeller@fredhutch.org