Contents

List of Figures

List of Tables

Glossary

bioinformatics	Statistical or computational approaches to biological data or research tools.
centrality	A network metric which identifies important vertices.
E-cadherin	Epithelial cadherin (calcium-dependent adhesion), a cell-adhesion protein encoded by *CDH1*.
edge or link	A relationship connecting a pair of elements of a graph structure or network, may be weighted or directional.
essential	A gene which is required to be functional or expressed for a cell or organism to be viable, grow or develop.
gene expression	A measure of the relative expression of each gene from the mRNA extracted from (pooled) cells.
graph or network	A mathematical structure modelling or depicting the relationships between elements.
hub	A central or highly connected component of a network.
information centrality	A network centrality metric which uses the impact of removing a vertex or node on connections in the network.
metagene	A consistent signal of expression for a collection of genes such as a biological pathway, derived from singular value decomposition.
mutation	A change in DNA sequence that disrupts gene function.

non-oncogene addiction	The dependence of a cancer cell on functioning non-mutant genes.
oncogene	A gene that potentially causes cancer, typically by over-expression or mutant gene variants.
oncogene addiction	The dependence of a cancer cell on a specific oncogenic pathway.
PageRank centrality	A network centrality metric which uses eigenvectors with a scaling factor (Brin and Page, 1998).
scale-free	A property of a network which has a power law vertex degree distribution, that is several highly connected hub genes and many with very few connections.
shortest path	A path with the fewest possible edges which connects two particular vertices.
synthetic lethal	Genetic interactions where inactivation of multiple genes is inviable (or deleterious) which are viable if inactivated separately.
tumour suppressor	A gene potentially causes cancer, typically by disruption of functions which protect the cell from cancer.
vertex degree	A network metric of connectivity of vertices which uses the number of edges connected to each vertex or node.
vertex or node	An element of a graph structure or network.

Acronyms

AMP	Adenosine Monophosphate.
AMPK	AMP-activated Protein Kinase.
ANOVA	Analysis of Variance.
BioPAX	Biological Pathway Exchange.
BMP	Bone Morphogenic Protein.
CXCR	Chemokine Receptor.
EMT	Epithelial-Mesenchymal Transition.
GPCR	G Crotein Coupled Receptor.
JAK	Janus Kinase.
mtSLIPT	Synthetic Lethal Interaction Prediction Tool (against mutation).
NMD	Nonsense-Mediated Decay.
PDE	Phosphodiesterase.
PI3K	Phosphoinositide 3-kinase.
PIP_2	Phosphatidylinositol-(4,5)-bisphosphate.
PIP_3	Phosphatidylinositol-(3,4,5)-trisphosphate.
RGS	G-protein Signalling.
RHO	Ras Homolog Family.
RNA	Ribonucleic Acid.
siRNA	Short Interfering RNA.
SLIPT	Synthetic Lethal Interaction Prediction Tool.
TCGA	The Cancer Genome Atlas (genomics project).
TGFβ	Transforming Growth Factor β.

UTR Untranslated Region (of mRNA).

WNT Wingless-Related Integration Site.

Chapter 5

Synthetic Lethal Pathway Structure

Having identified key pathways implicated in synthetic lethal genetic interactions with *CDH1* (in Chapter 4), these were investigated for the synthetic lethal genes within them and their relationships to pathway structure in Reactome pathways. This chapter will focus on the pathway structure of biological pathways detected across analyses in Chapter 4. Specifically, investigations were performed to determine whether synthetic lethal candidates, detected by SLIPT or siRNA, exhibited differences with respect to metrics of pathway structure of network connectivity and importance (as described in Sections 2.4.4 and 3.5.3). The relationships between synthetic lethal candidates, detected by either approach, were also examined to determine whether SLIPT candidate genes were upstream or downstream siRNA candidate genes. These directional relationships were tested by resampling (as described in Sections 3.4.1 and 3.4.1.1) and comparisons to the pathway hierarchical score based on biological context (as derived in Section 3.4.1.2). Together these investigations into structural relationships demonstrate how a combination of network biology and statistical techniques can be performed with genes identified by a bioinformatics analysis.

5.1 Synthetic Lethal Genes in Reactome Pathways

The graph structure for Reactome pathways was obtained from Pathway Commons via Biological PAthway eXchange (BioPAX) (as described in Section 2.4.2). The pathways describe the (directional) relationships between biomolecules, including genes that encode proteins in biological pathways. These relationships include cell signalling (e.g., kinase phosphorylation cascades), gene regulation (e.g., transcription factors, chromatin modifiers, RNA binding proteins), and metabolism (e.g., the product of an enzyme being the substrate of another). Together these relationships describe the known functional pathways in a human cell with a reasonable resolution, from a curated database supported by publications documenting pathway relationships.

Pathway structures from the Reactome network (as described in Section 2.4.3) were used to derive the graph structure of each biological pathway. The synthetic lethal candidate genes for notable pathways discussed in Chapter 4, including candidate synthetic lethal pathways of *CDH1*, were examined to show the SLIPT and siRNA candidates within these pathways. The synthetic lethal genes considered here are those candidates detected by SLIPT (as described in Section 3.1) in The Cancer Genome Atlas (TCGA) breast cancer expression and mutation data (Koboldt *et al.*, 2012) in comparison to the candidate gene partners from the siRNA screening in breast cell lines (Telford *et al.*, 2015).

5.1.1 The PI3K/AKT Pathway

The phosphoinositide 3-kinase (PI3K) cascade signalling pathway exhibited unexpected results with metagene analyses (as discussed in Section D). This pathway is also of interest because mediating signals between the G protein coupled receptors and regulation of protein translation have both been strongly implicated to be synthetic lethal pathways with loss of *CDH1* function (in Chapter 4). These pathways have are all subject to dysregulation in cancer (Courtney *et al.*, 2010; Dorsam and Gutkind, 2007; Gao and Roux, 2015). Thus the PI3K cascade will be examined along with the most supported synthetic lethal pathways (as identified in Chapter 4).

The phosphoinositide 3-kinase (PI3K) pathway is also an ideal pathway in which to test pathway structure because it has an established direction of signal transduction from extracellular stimuli (and membrane bound receptors) to the inner mechanisms of the cell, namely, the regulation of protein translation. The production of proteins is necessary for the growth of the cell so it is reasonable to suggest that these processes may be subject to (non-oncogene) addiction in some cancer cells which rely upon them for sustained protein production and cell growth. This is also supported by the oncogenes *PIK3CA* and *AKT1* being involved with the PI3K cascade and related PI3K/AKT pathway which may be subject to oncogene addiction when these proto-oncogenes are activated.

The PI3K cascade was not supported across SLIPT in TCGA breast expression data and the siRNA primary screen by over-representation (in Section 4.2.5) or resampling (in Section 4.2.5.1) but genes were detectable by either approach (as shown in Figure 5.1). While few genes were identified by both approaches, they include genes

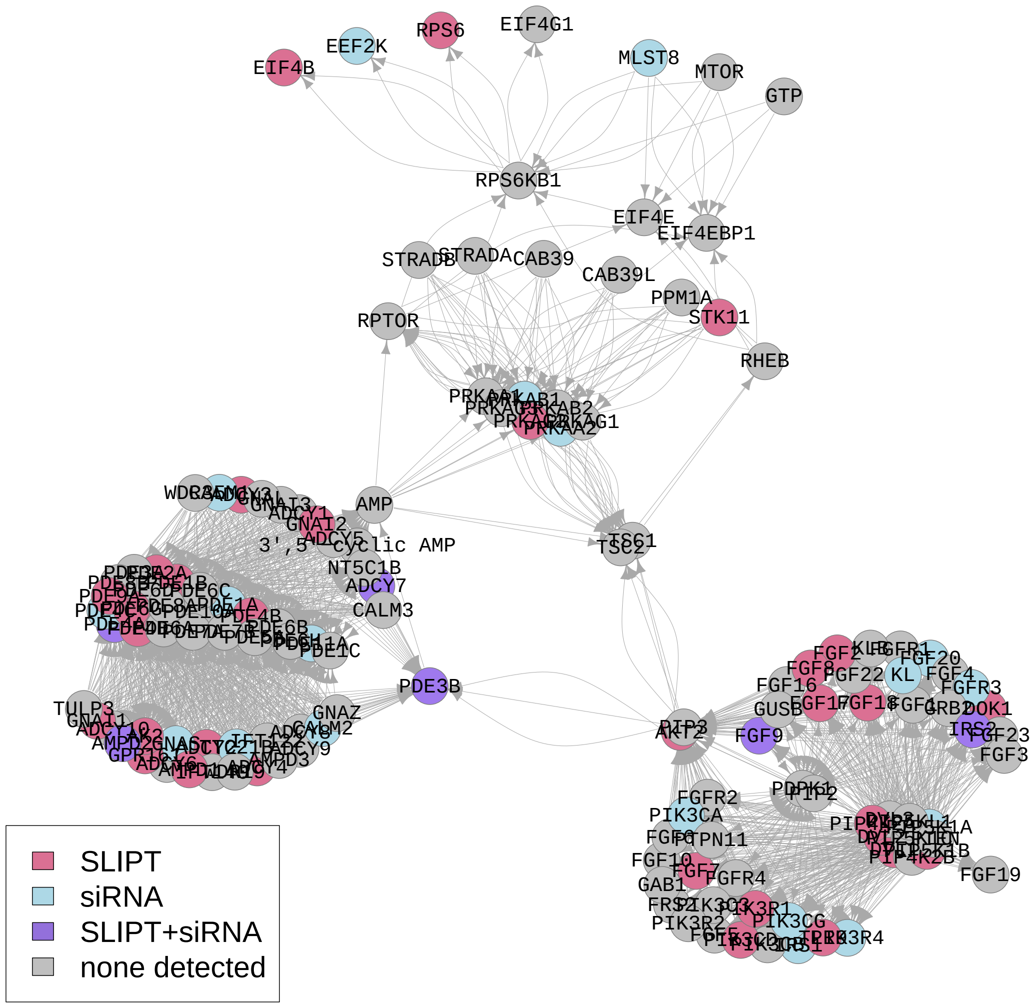


Figure 5.1: **synthetic lethality in the PI3K cascade.** The Reactome PI3K Cascade pathway with synthetic lethal candidates coloured as shown in the legend.

that are highly connected in the PI3K cascade and are hubs to information transmission such as *FGF9*,*PDE3B*, and *PDE4A*. The key upstream genes *PIK3CA* and *PIK3CG* were detected by siRNA whereas the downstream *PIK3R1* and *AKT2* genes were detected by SLIPT. Gene detected by either method were also prevalent in the PI3K, phosphodiesterase (PDE), and AMP-activated protein kinase (AMPK) modules, in addition to the downstream translation factors and ribosomal genes (*EIF4B*, *EEF2K*, and *RPS6*). Together these suggest that there may be further structure between the SLIPT and siRNA candidate partners of *CDH1* in pathways as illustrated by PI3K. As such, pathway structure will be investigated to detect differences in the upstream and downstream gene candidates of those detected by either method. Pathway structure may account for the disparity between SLIPT and siRNA genes, even in pathways such as PI3K where they did not significantly intersect. For instance, SLIPT gene partners may be downstream of siRNA candidates rather than replicating them directly.

This disparity between SLIPT and siRNA gene candidate synthetic lethal partners of CDH1 (i.e., a high number of genes detected by either approach with few detected by both) was replicated in the related PI3K/AKT pathway and the "PI3K/AKT in cancer" pathway (shown in Appendix Figures G.1 and G.2). Many synthetic lethal candidates were at the upstream core of these pathway networks and the downstream extremities. It is particularly notable that the many genes important in cell signalling and gene regulation were detected by either synthetic lethal detection approach. These include *AKT1*, *AKT2*, and *AKT3*, the Calmodulin signalling genes *CALM1* and *CAMK4*, and the forkhead family transcription factors *FOXO1* (a tumour suppressor) and *FOXO4* (an inhibitor of EMT).

5.1.2 The Extracellular Matrix

The extracellular pathways "elastic fibre formation" and "fibrin clot formation" (shown in Figures 5.2 and 5.3 respectively) were both supported across analyses (in Chapter 4). These pathways were identified by both SLIPT (for TCGA breast cancer) and siRNA gene candidates as they had significant over-representation and resampling.

Particularly for elastic fibres (Figure 5.2), the vast majority of genes were detected by either approach in addition to a significant proportion of genes detected by both approaches (as determined in Section 4.2.5). The genes detected by both approaches also appeared to have a non-random distribution in the network, with *TFGB1*, *ITGB8*, and *MFAP2* exhibiting high connectivity, and having a central role in their respective pathway modules. In addition to a structural role in the extracellular matrix and connective tissue (including the tumour microenvironment), these proteins including Furin, transforming growth factor β (TGFβ), and the bone morphogenic proteins (BMPs), are also involved in responses to endocrine signals and interact with the cellular receptors for signalling pathways. Therefore it is plausible that *CDH1* deficient tumours will be subject to non-oncogene addiction to the extracellular environment and growth signals arising from this pathway. The pathway structure also indicated that the genes detected by siRNA (or by both approaches) may be be downstream of those detected by

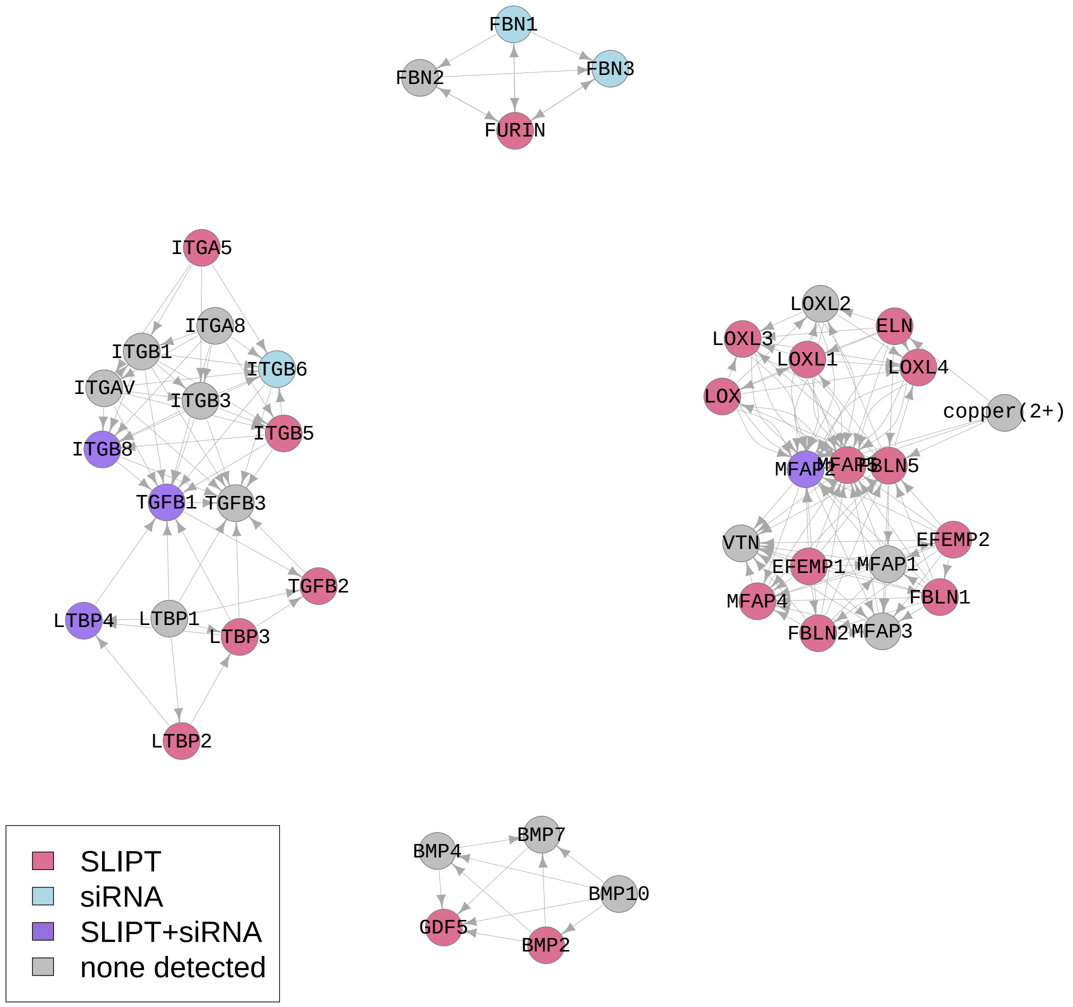


Figure 5.2: **synthetic lethality in Elastic Fibre Formation.** The Reactome Elastic Fibre Formation pathway with synthetic lethal candidates coloured as shown in the legend.

SLIPT, in addition to whether connectivity or centrality is higher for synthetic lethal candidates than other genes in the pathway.

Genes detected as synthetic lethal partners of *CDH1* by SLIPT or siRNA screening were also common in the Fibrin clot formation pathway (shown in Figure 5.3). This is consistent with the established pleiotropic role of *CDH1* in regulating fibrin clotting. It is also notable that the genes detected by either method appear to be highly connected such as *C1QBP KNG1*, *F8*, *F10*, *F12*, *F13A*, and *PROC* (including many of the coagulation factors). Synthetic lethal candidates also include *SERPINE2* and *PRCP*,

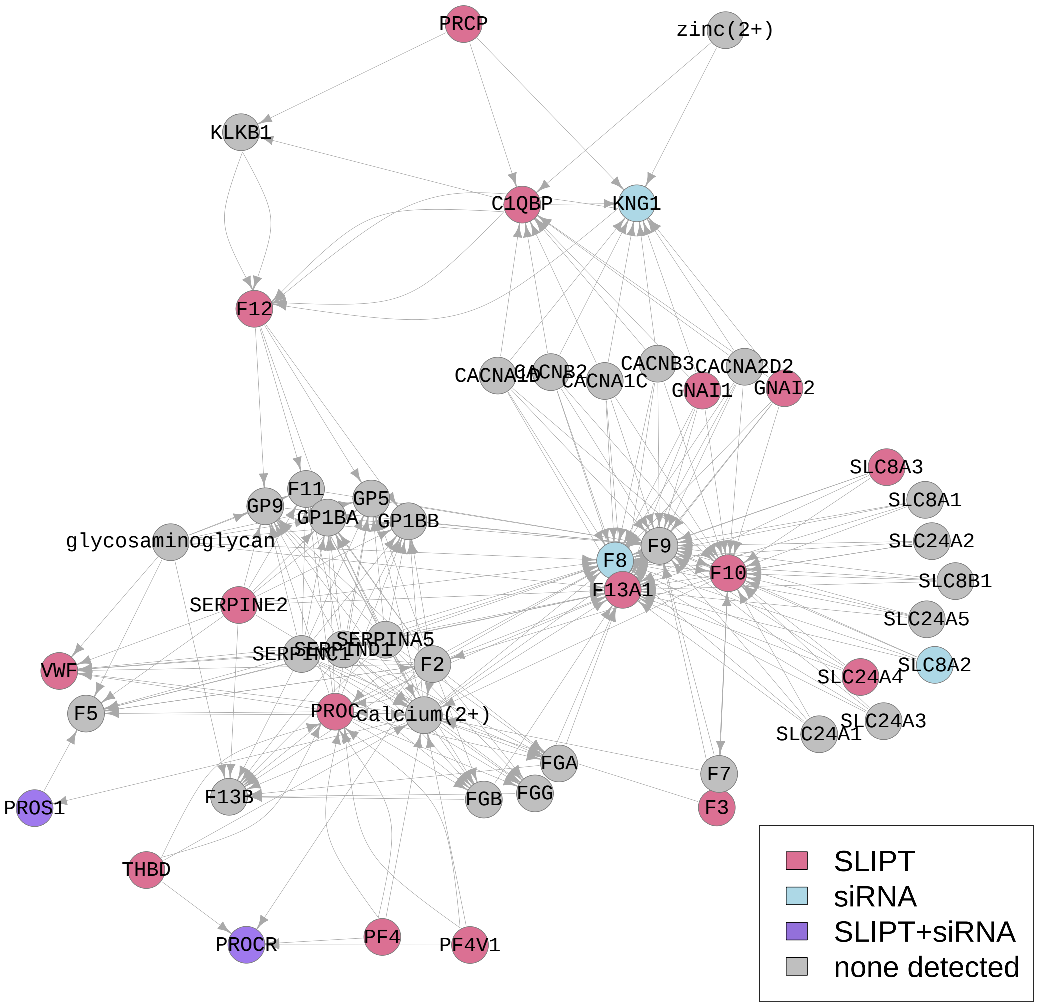


Figure 5.3: **Synthetic lethality in Fibrin Clot Formation.** The Reactome Fibrin Clot Formation pathway with synthetic lethal candidates coloured as shown in the legend.

which only affect downstream genes, in addition to *PROCR* and *VWF*, which are only affected by upstream genes.

Many of these genes are involved in the larger Extracellular Matrix pathway (shown in Appendix Figure G.3), including many of the synthetic lethal candidates discussed for elastic fibres. The number of SLIPT candidate genes outnumbers those identified by siRNA, as expected from an isolated cell model. However, the endocrine response genes (e.g., *TGFB1* and *LTBP4*) which are potentially artifacts of the cell line growth process were replicated with SLIPT analysis in patient tumours (TCGA breast cancer data). There is also additional support for synthetic lethal genes (e.g., *ITGB2*, *MFAP2*, and *SPARC*) being highly connected networks hubs of the pathway. The complexity of the extracellular matrix pathway lends credence to the need for formal network analysis approaches to interpret the pathway structure of synthetic lethal candidates. Furthermore statistical approaches are needed to determine whether structural relationships are unlikely to be observed between synthetic lethal candidates by chance

5.1.3 G Protein Coupled Receptors

G protein coupled receptor (GPCR) pathways are highly complex (as shown in Appendix Figures G.4 and G.5). Many of genes in these pathways were synthetic lethal candidates, detected by either SLIPT or siRNA screening, including genes frequently detected with both approaches, consistent with these pathways being supported by prior analyses (in Sections 4.2.5 and 4.2.5.1). Synthetic lethal candidates include the PDE and Calmodulin genes (as discussed in Section 5.1.3) in addition to others such as the regulators of G-protein signalling (RGS), chemokine receptors (CXCR), Janus kinase (JAK), and the Ras homolog family (RHO) genes. These are important regulatory signalling pathways necessary for cellular growth and cancer proliferation. Thus the GPCR pathways (and downstream PI3K/AKT signals) are a potentially actionable vulnerability against *CDH1* deficient cancers, particularly since many existing drug targets exist among these signalling pathways, some of which have been experimentally validated (Telford *et al.*, 2015). While a statistically significant number of genes in GCPR pathways was detected by both approaches (in Sections 4.2.5 and 4.2.5.1), the complexity of GPCR networks (containing hundreds of genes) further support the needs for a rational network-based approach to the relationships between SLIPT and experimental candidates.

5.1.4 Gene Regulation and Translation

While very few synthetic lethal genes were detected in translational pathways in an experimental screen against *CDH1* (Telford *et al.*, 2015), these were highly overrepresented in translational elongation (as shown in Appendix Figure G.6). These SLIPT genes include many ribosomal proteins and the regulatory "elongation factors" which may be subject to responses in the upstream signalling pathways. This observation further indicates that pathway structure may be used to identify relationships between synthetic lethal candidates detected by SLIPT and siRNA. The computational approach with SLIPT may exhibit the ability to detect downstream genes in the core translational processes, which experimental screening did not identify. The experimental screening may similarly detect upstream regulatory genes less sensitive to inactivation, that is, genes that are less likely to be indiscriminately lethal to both genotypes at high doses of inactivation.

Many of these SLIPT candidate genes are also among the nonsense-mediated decay (NMD) pathway (shown in Appendix Figure G.7) or 3′ untranslated region (UTR) mediated translational regulation (shown in Appendix Figure G.8). While genes in these pathways were also supported by experimental screening with siRNA, there were differences in which genes were detected within the pathway structures. In particular, *UPF1* was detected in the siRNA screen and is the focal downstream gene for the entire NMD pathway showing that (in this case) siRNA genes are downstream effectors of those detected by SLIPT. 3′ UTR mediated translational regulation has a similar structure with two modules connected solely by *RPL13A*, giving an example of SLIPT candidate genes with high connectivity, although there were many ribosomal proteins detected by SLIPT. However, the detection of *EIF3K*, a regulatory elongation factor (not essential to ribosomal function) was replicated across SLIPT and siRNA screening, while the majority of the elongation factors were not detected by either approach. Regulatory genes, being more amenable to experimental validation, also support further investigation into pathway structure. The SLIPT candidates may support experimental candidates in biological pathways by detecting downstream genes, which may not be detectable by experimental screening with high dose inhibitors. This difference between the approaches may explain the greater number of SLIPT candidate partners of *CDH1* than those experimentally identified.

5.2 Network Analysis of Synthetic Lethal Genes

Genes detected as synthetic lethal partners of *CDH1* with the SLIPT computational approach and the siRNA screen (Telford *et al.*, 2015) were compared across network metrics in the example of the PI3K cascade pathway (where the genes differed considerably between synthetic lethal detection methods). These were used to test whether network metrics differed between groups of genes detected by either or both approaches. These analyses serve to test both whether synthetic lethal gene candidates had higher connectivity or importance in a network and whether either detection approach is biased towards genes with different network properties.

5.2.1 Gene Connectivity and Vertex Degree

Vertex degree (the number of connections) for each gene is a fundamental property of a network. The vast majority of genes had a relatively modest number of connections, each with only a few genes in the PI3K pathway (shown in Figure 5.4) having pathway relationships with a high number of genes, consistent with the scale-free property of biological networks (Barabási and Oltvai, 2004). There were few differences in the number of connections between gene groups (by synthetic lethal detection), although genes detected by siRNA included those with the fewest connections. The median connectivity of genes detected by both approaches was marginally higher.

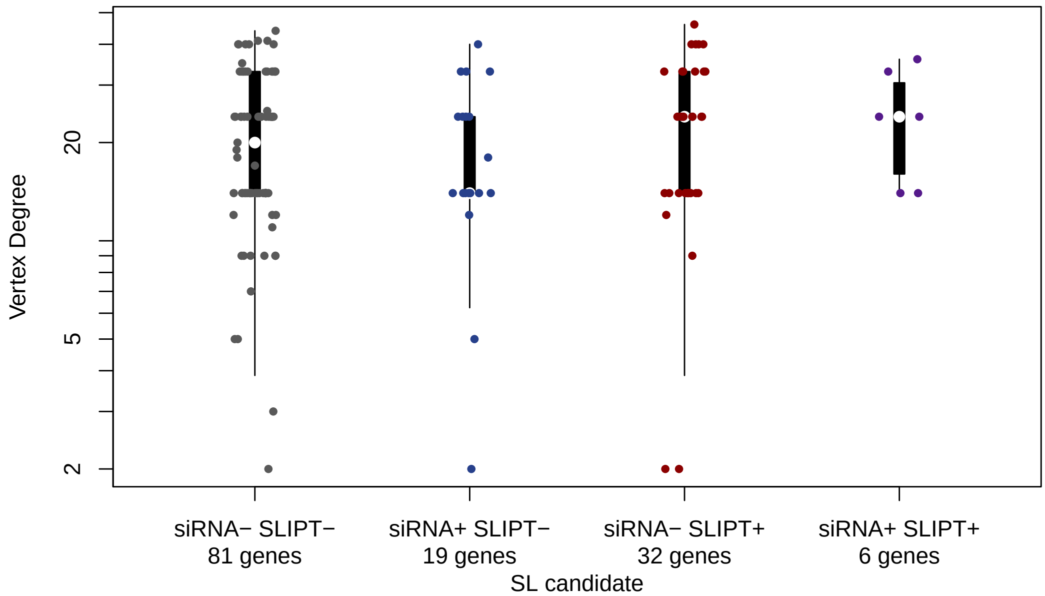


Figure 5.4: **Synthetic lethality and vertex degree.** The number of connected genes (vertex degree) was compared (on a log-scale) across genes deteced by SLIPT and siRNA screening in the Reactome PI3K cascade pathway. There were very few differences in vertex degree between the groups, although genes detected by siRNA included those with the fewest connections.

Table 5.1: ANOVA for synthetic lethality and vertex degree

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	15	15.50	0.0134	0.9082
SLIPT	1	506	506.01	0.4378	0.5105
siRNA×SLIPT	1	0	0.05	0.0000	0.9947

Analysis of variance for vertex degree against synthetic lethal detection approaches (with an interaction term)

The results for the PI3K pathway were very similar when testing synthetic lethality against *CDH1* mutation (mtSLIPT). In this case, there is also indication that mtSLIPT-specific genes may have higher connectivity than those detected by siRNA screening (shown in Appendix Figure H.1).

However, these apparent differences in vertex degree may be due to fewer genes being detected by either approach. There was no statistically significant effect of either computational or experimental synthetic lethal detection method on vertex degree, as determined by analysis of variance (ANOVA) (shown by Table 5.1 and Appendix Table H.1). Thus synthetic lethal detection does not discriminate among genes by their connectivity in a pathway network, nor is either approach constrained to detecting highly connected genes. Both approaches have been demonstrated to detect genes with many and very few connections.

5.2.2 Gene Importance and Centrality

5.2.2.1 Information Centrality

Information centrality is a measure of the importance of nodes in a network by how vital they are to the transmission of information throughout the network. This applies well to biological pathways, partcularly gene regulation and cell signalling. The nodes with the highest information centrality are not necessarily the most connected, as they may also include nodes that pass signals between highly connected network hubs. Information centrality therefore provides a distinct metric for the connectivity of a gene in a pathway, which has the added benefit of being directly related to the disruption of pathway function were it to be inactivated or removed.

Information centrality has also been suggested to indicate essentiality of genes or proteins (Kranthi *et al.*, 2013). The information centrality for each gene was computed across the entire Reactome network (as discussed in Appendix I). Reactome contains substrates and cofactors in addition to genes and proteins. In support of centrality as a measure of essentiality or importance to the network, a number of nodes with the highest centrality (shown in and Appendix Table I.1) were essential nutrients, including Mg^{2+}, Ca^{2+}, Zn^{2+}, and Fe.

Genes important in development of epithelial tissues and breast cancer were also detected with relatively high information centrality (as shown by the distribution across the Reactome network in Appendix Figure I.1). Interleukin 8 (encoded by *IL8*) is a chemokine important in epithelial cells, the innate immune system, and binding GPCRs. *GATA4* is an embryonic transcription factor involved in heart development, epithelial-mesenchymal transition (EMT), and has been shown to be recurrently mutated in breast cancer (Koboldt *et al.*, 2012). β-catenin (encoded by the proto-oncogene *CTNNB1*) is a regulatory protein which binds to E-cadherin, being involved in cell-cell adhesion and Wingless-related integration site (WNT) signalling. Together these show that information centrality identifies nodes of importance to biological functions in pathway networks, including those relevant to *CDH1* deficient breast cancers.

Within the PI3K pathway, genes detected by siRNA did not include those with lower centrality (shown in Figure 5.5), although the median information centrality across gene groups detected by either synthetic lethal approach did not differ. The genes with the highest information centrality included the synthetic candidates *PDE3B* (detected by SLIPT and siRNA) and *AKT2* (detected by SLIPT) which were markedly higher than most other genes in the pathway. The higher centrality of these genes is consistent with their known biological role in PI3K/AKT signalling and the pathway structure (shown in Figure 5.1). Other biomolecules with high centrality included the *RPS6KB1* and *RPTOR* genes, adenosine monophosphate (AMP), phosphatidylinositol-(4,5)-bisphosphate (PIP_2), and phosphatidylinositol-(3,4,5)-trisphosphate (PIP_3).

These findings were replicated (shown in Appendix Figure H.2) when testing synthetic lethality against *CDH1* mutation (mtSLIPT). The differences in network centrality between gene groups detected by either method were not statistically significant as determined by ANOVA (shown by Table 5.2 and Appendix Table H.2). Thus neither method was unable to detect synthetic lethal genes with particular centrality constraints, although they were also not detecting genes with higher centrality than expected by chance.

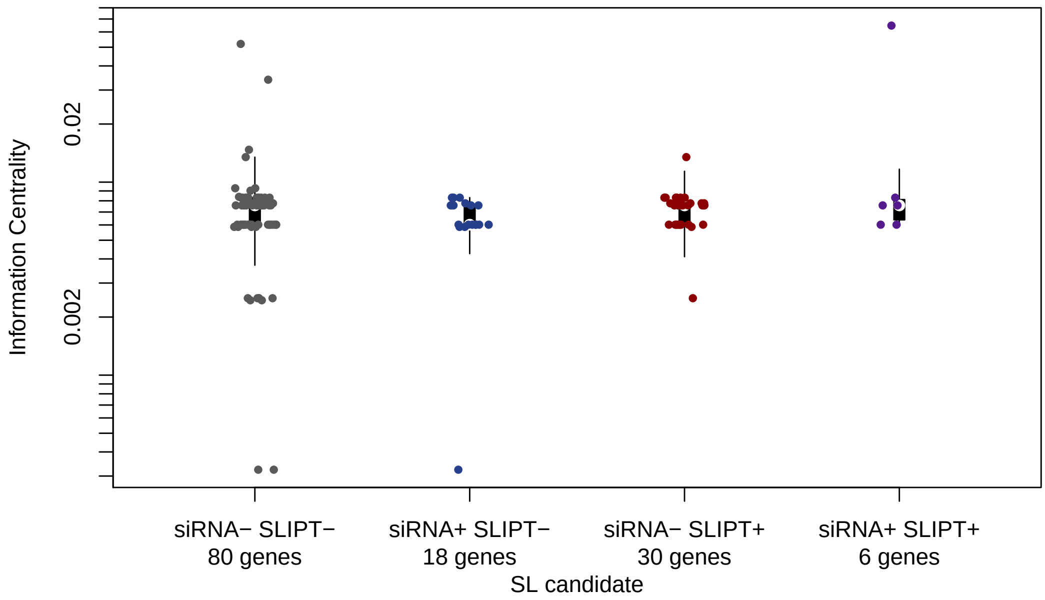


Figure 5.5: **Synthetic lethality and centrality.** The information centrality was compared (on a log-scale across genes deteced by SLIPT and siRNA screening in the Reactome PI3K cascade pathway. Genes detected by SLIPT or siRNA did not have higher connectivity than other genes. The gene with the highest centrality was detected by both approaches.

Table 5.2: ANOVA for synthetic lethality and information centrality

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	0.000256	0.0002561	0.1854	0.6682
SLIPT	1	0.003827	0.0038275	2.7717	0.1008
siRNA×SLIPT	1	0.000804	0.0008036	0.5820	0.4483

Analysis of variance for information centrality against synthetic lethal detection approaches (with an interaction term)

5.2.2.2 PageRank Centrality

PageRank centrality is another network analysis procedure to infer a hierarchy of gene importance from a network using connections and structure (Brin and Page, 1998). In contrast to the information centrality approach of removing nodes, PageRank uses the eigenvalue properties of the adjacency matrix to rank genes according to the number of connections and paths they are involved in.

This distinction is immediately clear within the PI3K pathway (shown in Figure 5.6), which differs considerably from the information centrality scores. Genes detected by SLIPT span the complete range of PageRank centrality values for this pathway, which was replicated when testing synthetic lethality against *CDH1* mutation (shown in Appendix Figure H.3). However, the genes detected by both SLIPT and siRNA screening have a higher median PageRank centrality, although the differences in PageRank centrality between these methods were not statistically significant as determined by ANOVA (shown by Table 5.3 and Appendix Table H.3).

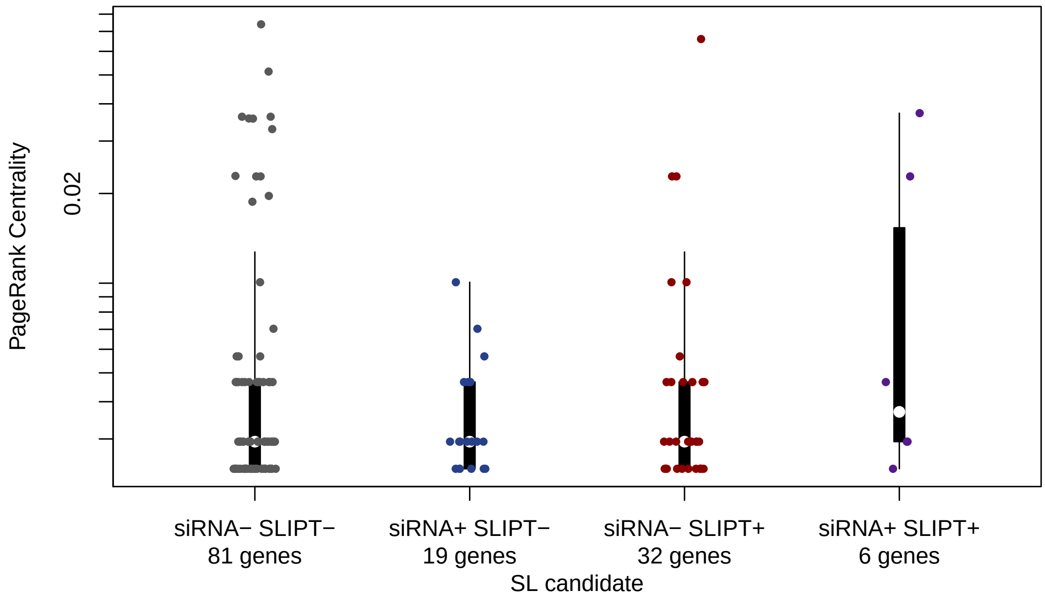


Figure 5.6: **Synthetic lethality and PageRank.** The PageRank centrality was compared (on a log-scale across genes deteced by mtSLIPT and siRNA screening in the Reactome PI3K cascade pathway. Genes detected by siRNA had a more restricted range of centrality values (which may be constrained experimental detection in a cell line model) than other genes not detected by either approach, although these groups also had fewer genes and a higher median.

Table 5.3: ANOVA for synthetic lethality and PageRank centrality

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	0.0002038	2.0385×10^{-4}	1.1423	0.2892
SLIPT	1	0.0000208	2.0752×10^{-5}	0.1163	0.7342
siRNA×SLIPT	1	0.0000137	1.3743×10^{-5}	0.0770	0.7823

Analysis of variance for PageRank centrality against synthetic lethal detection approaches (with an interaction term)

5.3 Relationships between Synthetic Lethal Genes

5.3.1 Hierarchical Pathway Structure

5.3.1.1 Contextual Hierarchy of PI3K

A contextual hierarchy of genes in the PI3K pathway was performed (as described in in Section 3.4.1.2) to assign scores for their relative order in the pathway. In the case of PI3K (shown in Figure 5.7), this orders genes from the upstream genes, which respond to signals from extracellular stimuli, to the downstream genes which transmit these to the gene expression (translation) responses of the cell. The directionality of this pathway is evident in transmitting signals from the PI3K complex, via AKT, PDE, and mTOR to the ribosomal regulatory proteins. This hierarchical procedure enables testing whether the biological context of a gene in a pathway is relevant to detection as a synthetic lethal candidate by either computational SLIPT analysis or experimental siRNA screening.

5.3.1.2 Testing Contextual Hierarchy of Synthetic Lethal Genes

This pathway hierarchy in the PI3K cascade was tested for differences between genes detected across SLIPT and siRNA screening. The synthetic lethal candidates for *CDH1* detected by either method (as shown by Figure 5.8a) did not differ, each being distributed throughout the pathway. When adjusted for being more numerous, there was little indication that SLIPT candidate genes are more frequently upstream or downstream of siRNA candidate genes (as shown by Figure 5.8b) and were more frequent at moderate hierarchies which contained more genes. Synthetic lethal candidates from both methods were less frequently detected in the downstream effectors of the pathway (e.g., the mTOR complex), although core pathway genes (e.g., *AKT2* and *PDE3B*) were detectable as synthetic lethal candidates (as discussed for Figures 5.1 and 5.6).

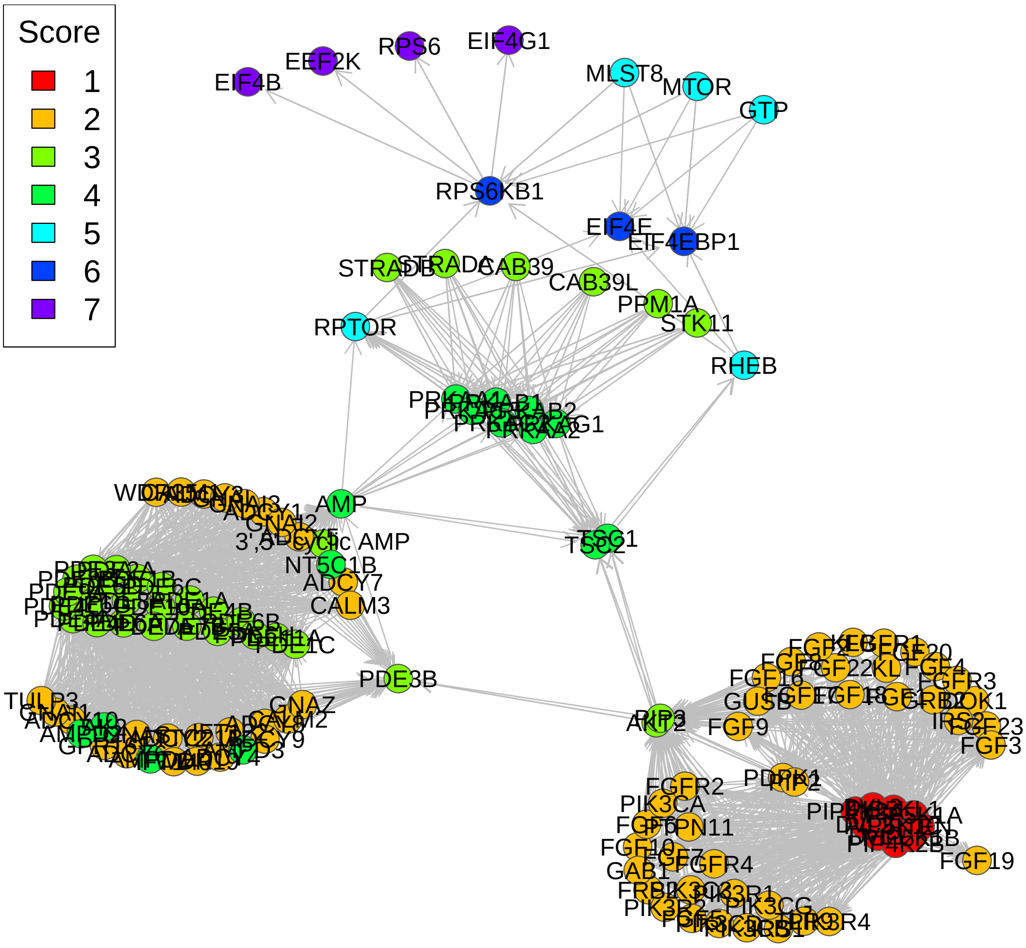
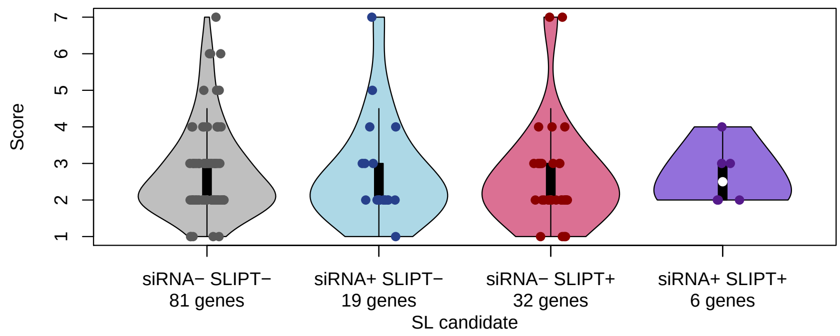


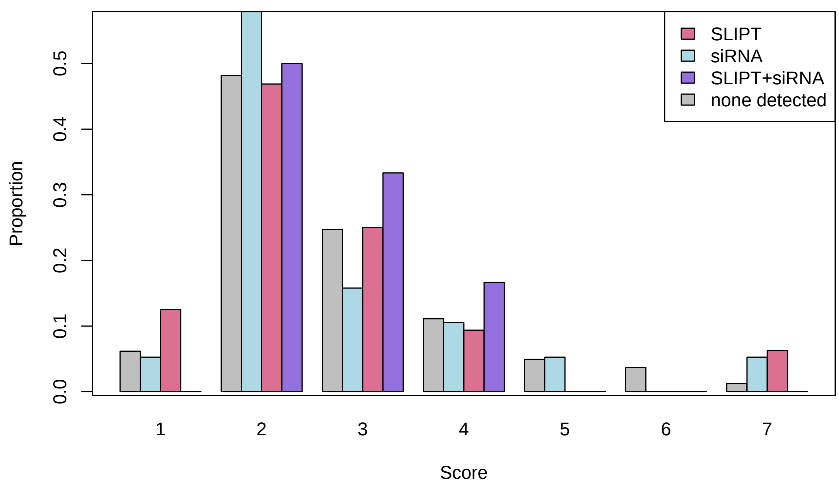
Figure 5.7: **Hierarchical structure of PI3K.** A contextual score was used for ranking genes within the PI3K Cascade to demonstrate a pathway structure analysis to examine whether genes detected by either SLIPT or siRNA were more frequently upstream or downstream in the PI3K pathway.

Similarly, when testing synthetic lethality against *CDH1 mutation* (mtSLIPT), the hierarchical score for the PI3K pathway did not differ between mtSLIPT-specific and siRNA-specific gene candidates (as shown by Appendix Figure J.1). The median among genes detected by both approaches was marginally elevated such that these genes may be further downstream in the pathway that other synthetic lethal candidate partners of *CDH1*. There were fewer genes overall with higher scores (shown in Appendix

Figure J.2). While these were more frequently detected by both SLIPT and siRNA, there was no significant effect variation in pathway hierarchy (shown by ANOVA in Table 5.4 and Appendix Table J.1) accounted for by SLIPT or siRNA detection in the PI3K pathway (as shown in Figure 5.1). Thus these hierarchical scores may be observed by sampling variation and there is no indication that SLIPT or siRNA detection differs



(a) Hierarchical Distance Score



(b) Proportion of Genes

Figure 5.8: **Hierarchy score in PI3K against synthetic lethality in PI3K.** The hierarchical distance scores were similarly distributed across SLIPT and siRNA genes. The number of SLIPT and siRNA genes against the hierarchical distance scores showing no significant tendency for either method to either of the pathway upstream or downstream extremities.

along the direction of the pathway. Genes detected by either method are no more or less common among upstream or downstream of the pathway.

Table 5.4: ANOVA for synthetic lethality and PI3K hierarchy

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	0.001	0.00066	0.0004	0.9842
SLIPT	1	0.456	0.45605	0.2740	0.6016
siRNA×SLIPT	1	0.019	0.01878	0.0113	0.9156

Analysis of variance for PI3K hierarchy score against synthetic lethal detection approaches (with an interaction term)

[remove this paragraph and Figures 5.9 and J.3?]

Furthermore the pathway hierarchical scores did not exhibit different more or less SLIPT than siRNA genes above or below the given threshold. Since the ideal threshold to detect pathway structure is unclear, an exploratory analysis was performed, with χ^2-test for the SLIPT or siRNA candidate genes upstream or downstream of each gene. It is unsurprising that these χ^2 tests were highest when the gene used as a threshold was in the middle of the pathway (as shown in Figure 5.9). However, there was no statistically significant support for pathway structure by this approach, as none of the χ^2 values were high enough to detect pathway structure between SLIPT and siRNA gene candidates. Nor was structure detectable for mtSLIPT testing synthetic lethality against *CDH1* mutation (as shown in Appendix Figure J.3).

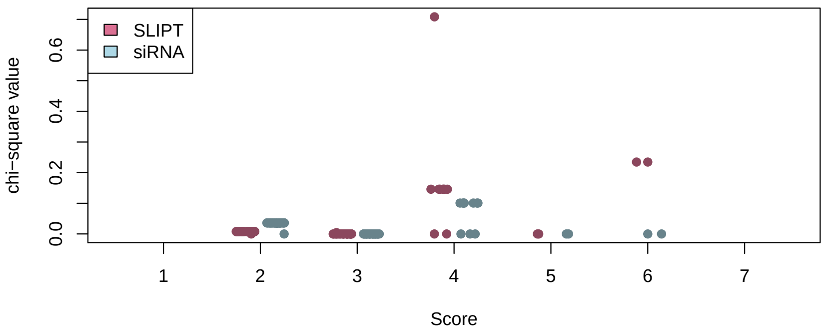


Figure 5.9: **Structure of synthetic lethality in PI3K.** The number of SLIPT and siRNA genes upstream or downstream of each gene in the Reactome PI3K pathway were tested (by the χ^2-test). These are plotted as a split jitter stripchart against the hierarchical distance scores showing no significant tendency for either method to either of the pathway upstream or downstream extremities.

5.3.2 Upstream or Downstream Synthetic Lethality

This approach does not ascertain whether SLIPT and siRNA candidate partners of *CDH1* are upstream or downstream of one and other within a pathway such as the PI3K cascade. The hierarchical approach is designed to detect differences in pathway location between gene groups. An alternative pathway structure method has been devised to use network structures to identify directional relationships between individual SLIPT and siRNA genes. This pathway structure methodology will be applied (as described in Section 3.4.1) to detect the direction of shortest paths between SLIPT and siRNA gene candidates. This will be used to demonstrate the methodology on the PI3K pathway, to develop a statistical test for pathway structure between between SLIPT and siRNA gene candidate using resampling (as described in Section 3.4.1.1), and to apply this test for pathway structure among synthetic lethal gene candidates to the pathways identified in Chapter 4 and discussed in Section 5.1.

5.3.2.1 Measuring Structure of Candidates within PI3K

Shortest paths in a pathway network were used to devise a strategy to detect pathway structure between SLIPT and siRNA gene candidate partners of *CDH1* (as described in Section 3.4.1). Thus we can determine whether individual SLIPT genes have upstream or downstream siRNA candidates (scored as “up” or “down” events respectively). This

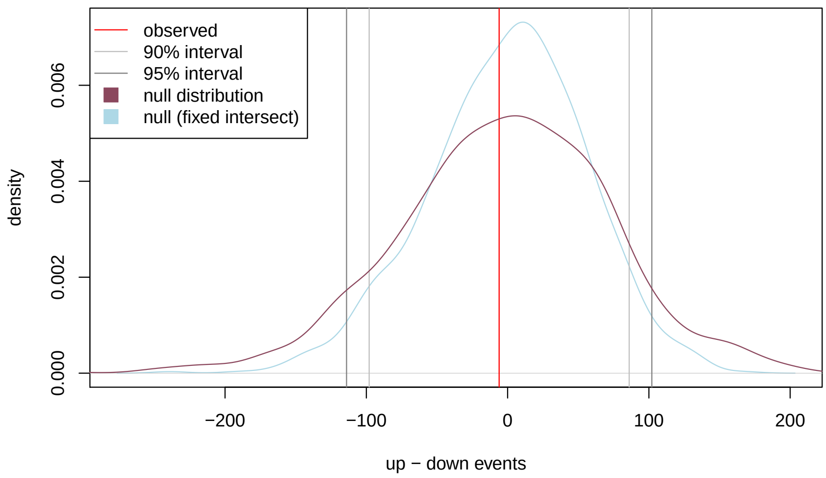


Figure 5.10: **Structure of synthetic lethality resampling in PI3K.** A null distribution with 10,000 iterations of the number of siRNA genes upstream or downstream of SLIPT genes (depicted as the difference of these) in the PI3K pathway. To assess significance, the observed events (with shortest paths) were compared to the 90% and 95% intervals for the null distribution (shown in violet). Genes detected by both methods were fixed to the same number as observed for the alternative null distribution (shown in blue), although the observed number of events (red) was not significant in either case. In both cases, these genes detected by both approaches were included in computing the number of shortest paths (in either direction) between SLIPT and siRNA genes.

procedure enables the detection of directional relationships between SLIPT and siRNA gene candidates (in contrast to the hierarchical approach).

The total number of gene candidate pairs in either direction can be compared within a pathway network to assess the overall directional relationships in a pathway. This directionality is detectable by the difference between the number of SLIPT candidate genes with upstream and downstream siRNA gene partners. However, this measure alone is not sufficient to determine whether there is evidence of pathway structure between SLIPT and siRNA gene candidate partners of *CDH1* in a pathway network. Nevertheless, it does serve to measure the magnitude (and direction) of the consensus of directional relationships (upstream and downstream) between SLIPT and siRNA gene candidate partners. This measure of pathway structure can be used for testing for statistical significance of pathway structure by resampling, using a permutation procedure to test whether these relationships are detectable among randomly selected gene groups rather than the detected SLIPT and siRNA gene candidate partners (as described in Sections 2.3.6 and 3.4.1.1).

This resampling procedure was performed for the PI3K network to generate a null distribution for the difference in the number of "up events" and "down events" for this pathway (as shown in Figure 5.1). Resampling yields a distribution to detect whether genes detected by SLIPT had significantly more upstream or downstream siRNA candidates. While there was modest indication that siRNA genes were downstream of SLIPT candidate genes, resampling for the PI3K pathway (as shown in Figure 5.10) did not detect a significant number of siRNA genes upstream or downstream.

In contrast, when testing synthetic lethality against *CDH1* mutation (mtSLIPT) there was modest indication that siRNA genes were upstream of SLIPT candidate genes. However, resampling (as shown in Appendix Figure J.4) was also unable to detect a significant number of siRNA genes upstream or downstream of mtSLIPT candidates. Neither fixing the number of genes detected by both approaches (as shown by the blue line in Figure 5.10 and Appendix Figure J.4) nor excluding these jointly detected genes altered the findings of this approach. These genes were included in the analysis because they can disproportionately count towards siRNA genes being upstream (or downstream) of SLIPT genes as they may still have different proportions of gene detected by either approach upstream (or downstream) of them. Furthermore, expanding the range of shortest paths to consider links in related pathways (using the "metapathways" constructed in Section 2.4.3) also had little effect on the null distribution generated, despite increasing the computational demands of the procedure.

5.3.2.2 Resampling for Synthetic Lethal Pathway Structure

The permutation procedure (as described in Section 3.4.1.1) that was performed in Section 5.3.2.1 for the PI3K cascade was also applied to other pathways identified in Chapter 4 and discussed in Section 5.1. These include extracellular matrix (with constituent elastic fibre and fibrin pathways), cell signalling (by PI3K/AKT and GCPRs), and translational pathways (with NMD and 3′UTR regulation). The resampling results across these pathways (as shown in Table 5.5) had limited support for association between pathway structure and detection of synthetic lethal genes, with the majority of these being non-significant as shown for PI3K (in Appendix Figure J.4). However, the distribution for these pathways will differ depending on their structure, the number of genes they consist of, and the proportion of synthetic lethal candidates among them (including a higher frequency of genes detected by both methods for the pathways identified in Section 4.2.5.1). This resampling is an appropriate procedure to use to detect structural relationships across pathways as it does not assume an underlying test statistic distribution.

Pathway structure was supported for the NMD pathway (which is consistent with siRNA being downstream in Appendix Figure G.7). However, this observation rests upon a single gene and was not replicated when testing synthetic lethality (mtSLIPT) against *CDH1* mutation (as shown in Appendix Table J.2) nor was it supported by the related 3′UTR regulation and translational elongation pathways.

Table 5.5: Resampling for pathway structure of synthetic lethal detection methods

	Graph		States		Observed				Permutation p-value	
Pathway	**Nodes**	**Edges**	**SLIPT**	**siRNA**	**Up**	**Down**	**Up−Down**	**Up/Down**	**Up−Down**	**Down−Up**
PI3K Cascade	138	1495	38	25	122	128	-6	0.953	0.5326	0.4606
PI3K/AKT Signalling in Cancer	275	12882	98	44	779	679	100	1.147	0.3255	0.6734
G$_{\alpha i}$ Signalling	292	22003	95	58	836	1546	-710	0.541	0.9971	0.0029
GPCR downstream	1270	142071	312	160	9755	9261	494	1.053	0.3692	0.6305
Elastic fibre formation	42	175	24	7	1	2	-1	0.500	0.5461	0.3865
Extracellular matrix	299	3677	127	29	547	455	92	1.202	0.3351	0.6636
Formation of Fibrin	52	243	18	5	12	17	-5	0.706	0.6198	0.3564
Nonsense-Mediated Decay	103	102	74	2	0	74	-74	0	1.0000	< 0.0001
3' -UTR-mediated translational regulation	107	2860	77	1	0	0	0		0.4902	0.5027
Eukaryotic Translation Elongation	92	3746	76	0	0	0	0		0.4943	0.4933

Pathways in the Reactome network tested for structural relationships between SLIPT and siRNA genes by resampling. The raw p-value (computed without adjusting for multiple comparisons over pathways) is given for the difference in upstream and downstream paths from SLIPT to siRNA gene candidate partners of *CDH1* with significant pathways highlighted in bold. Sampling was performed only in the target pathway and shortest paths were computed within it. Loops or paths in either direction that could not be resolved were excluded from the analysis. The gene detected by both SLIPT and siRNA (or resampling for them) were included in the analysis and the number of these were fixed to the number observed.

There does not appear to be a consensus on the directionality of SLIPT and siRNA candidates across pathways as distinct pathways showed stronger tendency for siRNA genes to be either upstream or downstream. Even related pathways such as PI3K and PI3K/AKT signalling showed directional events in opposite directions. The strongest pathway (among those tested) with support for directional pathways structure is G$_{\alpha i}$ signalling which showed significant downstream siRNA genes for both SLIPT and mtSLIPT from a large number of shortest paths (in Table 5.5 and Appendix Table J.2). This would indicate that SLIPT detects upstream regulators of genes experimentally validated by siRNA. However, these results are borderline significant (with raw permutation p-values) and are unlikely to be detected after adjusting for multiple comparisons across the 10 pathways presented here (nor in the 1652 Reactome pathways used previously in Chapter 4).

Therefore, there is insufficient evidence to determine whether there is pathway structure, gene detected upstream or downstream by either method, between the SLIPT and siRNA candidates in many of the synthetic lethal pathways (identified in Chapter 4). In particular, directional structure among synthetic lethal candidates for *CDH1* was not strongly supported in signalling pathways upon which the rationale for pathway structure hypotheses were based on. Despite the design of a robust resampling approach to test relationships between gene groups, this did not detect many structural relationships between SLIPT and siRNA gene candidates, although it may apply more broadly to gene networks. Furthermore, the pathway relationships are unlikely to be statistically supported by resampling when testing across the search space of Reactome pathways and adjusting for multiple comparisons. While there is statistically significant over-representation of many of these pathways in genes detected by both SLIPT and siRNA (as described in Chapter 4), these did not consistently show pathway structure. Furthermore, pathway structure did not account for the discrepancy between SLIPT and siRNA gene candidates which did not significantly intersect such as the PI3K cascade.

5.4 Discussion

These investigations used a functional pathway network that encapsulates protein complexes and functional modules. The Reactome network (Croft *et al.*, 2014) uses curated, experimentally identified pathways to determine relationships between genes and does not have the limitation of relying solely on protein binding or text-mining which are prone to false positives. While it is not documented whether these relationships are activating or inhibitory, the Reactome network (Croft *et al.*, 2014) is sufficient to test pathway relationships with directional information.

Synthetic lethal genes and pathways (for *CDH1* loss in cancer) were identified across gene expression and mutation datasets in Chapter 4. These pathway structure investigations extend those investigations into synthetic lethal gene candidates including exploring the discrepancy between SLIPT and siRNA candidate genes in a pathway such as PI3K in which they did not significantly intersect. Pathways with replicated synthetic lethal genes across these detection methods, breast and stomach cancer data, and patient and cell line data were also investigated including pathways from the extracellular microenvironment to core translational pathways and the signalling pathways between them.

Synthetic lethal gene candidates in the context of pathway structures can also be interpreted to provide additional mechanisms and support for belonging to a synthetic lethal pathway. Gene candidates with known mechanisms are ideal for triage of targets specific to *CDH1* deficient tumours and for further experimental validation in preclinical models. This chapter presents computational methods to use pathway structure in an attempt to detect genes with importance in a pathway and reconcile the differences between SLIPT and siRNA candidate genes with pathway relationships (e.g., one group being downstream of the other).

Many genes were detected by either method and the differences between the computational and experimental screening approaches could feasibly lead to differences in which genes within a synthetic lethal pathway are identified. Genes detected by synthetic lethal detection strategies included those of biological importance within synthetic lethal pathways, those which are actionable drug targets, and those with functional implications for the biological growth mechanisms or vulnerabilities of *CDH1* deficient tumours. It appeared that genes detected by both approaches were highly connected (or of importance) in the network structure or some pathways and that there may be some structure with SLIPT and siRNA upstream or downstream of each other. However, the complexity of biological pathways meant that relationships between gene candidates were difficult to discern without formal mathematical and computational approaches and thus these were used to analyse large biological networks.

Network analysis techniques were therefore applied to formalise and quantify the connectivity and importance (centrality) of genes within pathways (using PI3K as an example). However, these network techniques were unable to identify distinct differences in the network properties of genes detected as synthetic lethal candidates by computational or experimental methods. These network metrics support the application of synthetic detection across pathways (and the findings using pathways as gene sets in Chapter 4) as neither synthetic lethal detection approach was biased towards genes of higher importance or connectivity and neither approach was insensitive to genes of lower importance or connectivity. SLIPT is therefore not biased towards genes with more crucial role in the pathway as inferred by pathway connectivity and centrality measures and detects genes irrespective of pathway structure.

Similarly, a network hierarchy based on biological context (ordered from receiving extracellular stimuli to affecting downstream gene expression and cell growth) was devised to test whether PI3K genes of a particular upstream or downstream level were more frequently detected as synthetic lethal candidates. However, this approach was unable to ascertain whether genes detected by either method were further upstream or downstream in the pathway and there was no statistical evidence that either method differed in which levels of this structure were detected.

A measure of pathway structure between individual SLIPT and siRNA genes within a pathway was also devised using the direction of shortest paths in a directed graph structure. This is amenable to detecting the consensus directionality of the pathway across pairs of genes detected by either method. The pathway structure methodology developed here is generally applicable to comparison of node groups (allowing overlapping) including genes in biological pathways and their detection by different methodologies. While the pathway structure measure alone is not able to detect structural relationships between gene groups (e.g., SLIPT and siRNA gene candidates), it is amenable to resampling to determine whether these relationships are statistically significant.

5.5 Summary

Together these analyses of biological pathways, network metrics, and statistical procedures devised specifically for this purpose were applied to Reactome pathway structures to test whether structural relationships exist between synthetic lethal candidates. Of particular interest was whether these relationships relate to the differences between the computational (SLIPT) and experimental (siRNA) synthetic lethal candidate partners of *CDH1* (in the pathways discussed in Chapter 4).

While biologically relevant relationships were observed in specific pathways, there were few detectable structural relationships between SLIPT and siRNA gene candidates. These candidates did not exhibit significant differences in network connectivity or centrality measures. Network analyses were also unable to ascertain whether the candidates detected by either method stratified into upstream and downstream genes on the pathway and they likely do not.

A statistical resampling procedure was applied to shortest path analysis to test whether pairs of SLIPT and siRNA gene candidates were more likely to be upstream or downstream of each other. This approach detected very few structural relationships in the synthetic lethal pathways identified in Chapter 4. Overall, support for pathway structure between SLIPT and siRNA gene candidates is weak and the direction is inconsistent between pathways. Therefore pathway structure does not account for the differences between the SLIPT and siRNA gene candidates, although this does support the validity of gene set analyses in Chapter 4 and the synthetic lethal pathways identified.

Furthermore, the resampling procedure demonstrated in this chapter is more widely applicable to gene states in network structures and may be of further utility in the analysis of biological pathways or networks. This approach was able to quantify structural relationships that were otherwise difficult to interpret and to conclusively exclude many potential relationships. In this respect, the network resampling methodology may also be applicable to triage of experimental validation.

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