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Glossary

bioinformatics	Statistical or computational approaches to biological data or research tools.
centrality	A network metric which identifies important vertices.
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).
pathway	A series of biomolecules that produces a particular product or biological function.
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.
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).

Chapter 5

Synthetic Lethal Pathway Structure

Having identified key pathways implicated in synthetic lethal genetic interactions with *CDH1* (in Chapter 4), these were investigated in this Chapter for the synthetic lethal genes within them, and for their relationships to pathway structure. This chapter will focus on the Reactome 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 **??**). 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 is important in cancer because it is involved in 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 well characterised and 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 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*

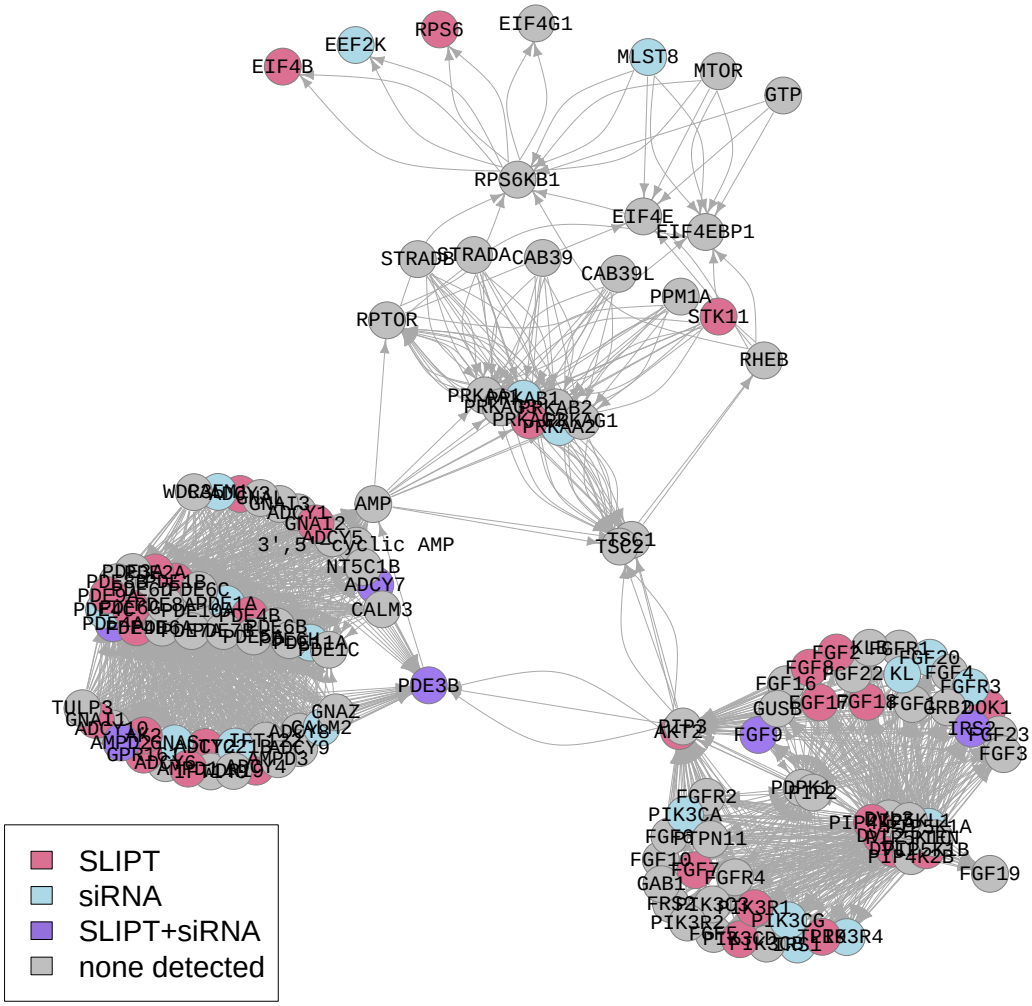


Figure 5.1: **Synthetic lethality in the PI3K cascade.** The Reactome PI3K Cascade pathway with synthetic lethal candidates coloured as shown in the legend.

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

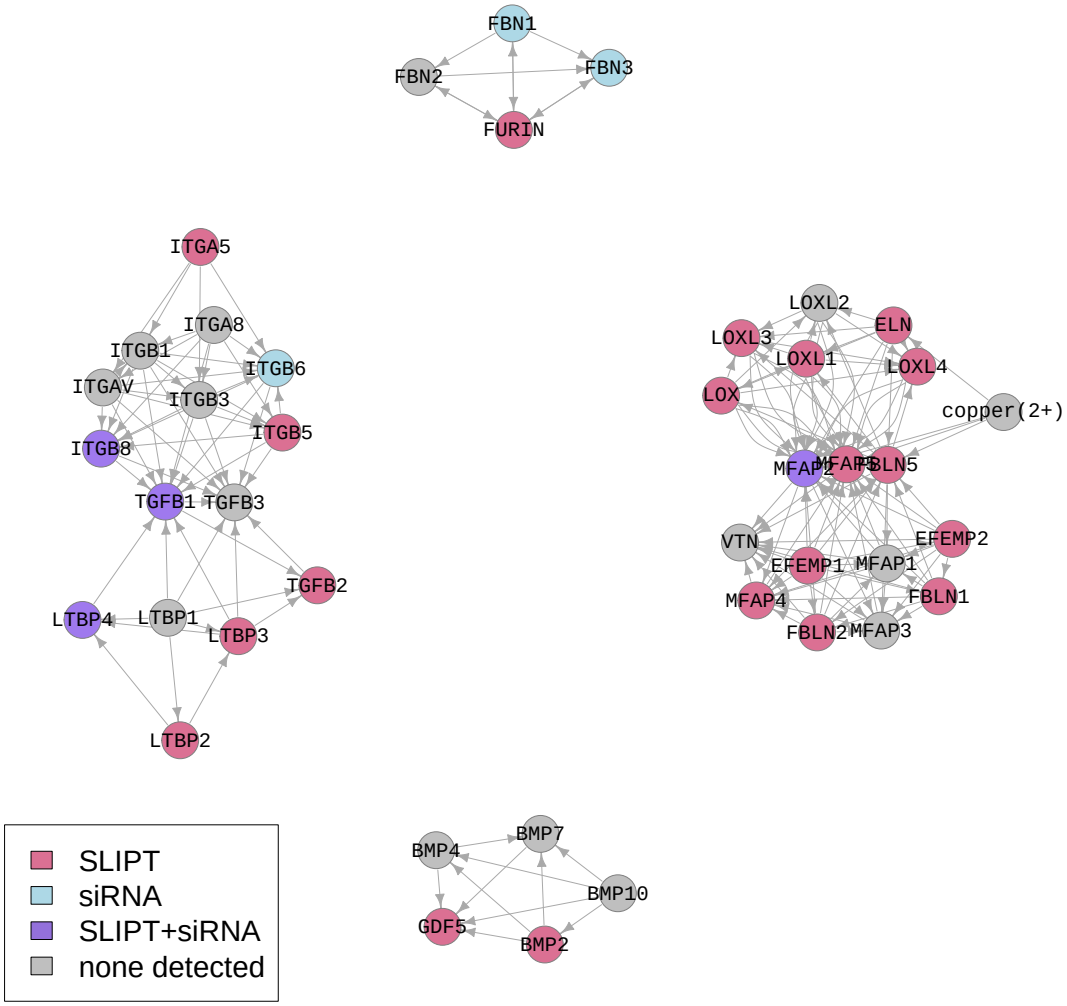


Figure 5.2: **Synthetic lethality in Elastic Fibre Formation.** The Reactome Elastic Fibre Formation pathway with synthetic lethal candidates coloured as shown in the legend.

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

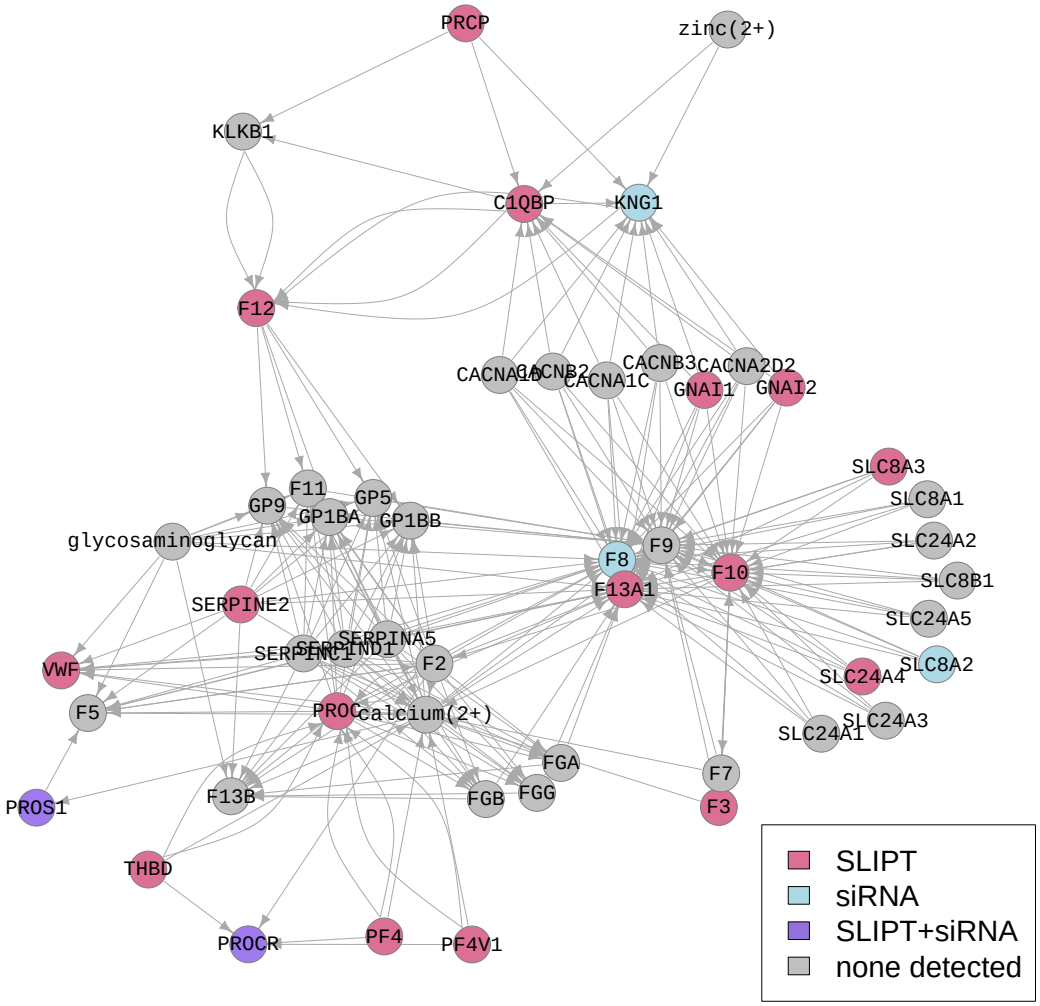


Figure 5.3: **Synthetic lethality in Fibrin Clot Formation.** The Reactome Fibrin Clot Formation pathway with synthetic lethal candidates coloured as shown in the legend.

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 Figure 5.4 and Appendix Figure G.4). 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 over-represented in translational elongation (as shown in Appendix Figure G.5). 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

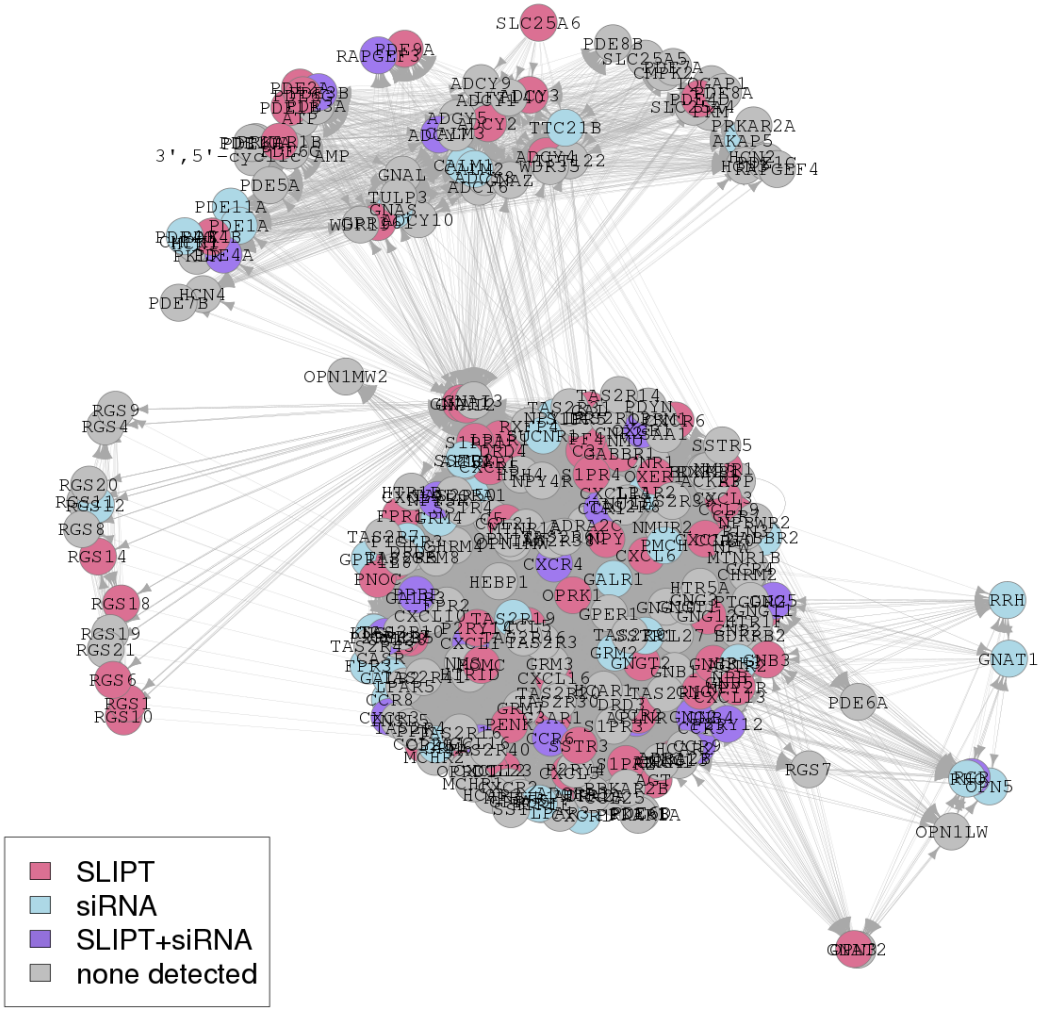


Figure 5.4: **Synthetic lethality in the GPCRs.** The Reactome $G_{\alpha i}$ pathway with synthetic lethal candidates, coloured as shown in the legend.

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.6) or 3′ untranslated region (UTR) mediated translational regulation (shown in Appendix Figure G.7). 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 $G_{\alpha i}$ signalling, a GPCR pathway. This pathway was used to demonstrate deeper network analysis approaches to synthetic lethal candiates within complex pathways it was supported across analyses (in Chapter 4), with significant over-representation in both SLIPT and siRNA screening, and the genes differed considerably between synthetic lethal detection methods (shown in Appendix Figures 5.4). These network metrics were used to measure whether the network properties 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 $G_{\alpha i}$ pathway (shown in Figure 5.5) having pathway relationships with a high number of genes, consistent with the scale-free property of biological networks (Barabási and Oltvai, 2004). The number of connections was similar between gene groups (by synthetic lethal detection). Genes detected by siRNA included those with the fewest connections, despite there being fewer genes that were 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).

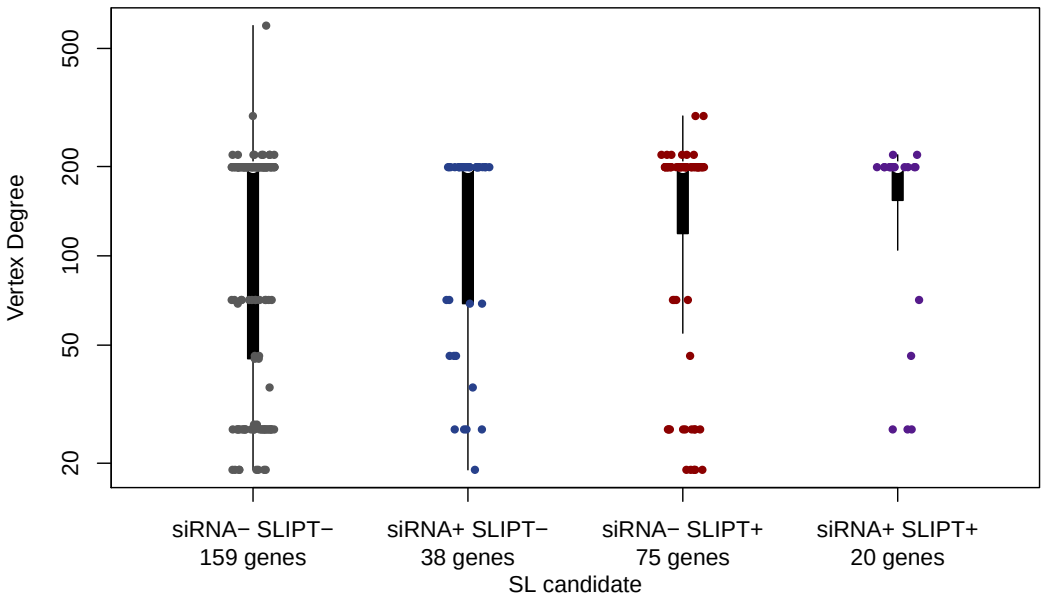


Figure 5.5: **Synthetic lethality and vertex degree.** The number of connected genes (vertex degree) was compared (on a log-scale) across genes detected by SLIPT and siRNA screening in the Reactome $G_{\alpha i}$ cascade pathway. There were no differences in vertex degree between the groups (shown in Table 5.1), 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	21	20.8	0.0030	0.9561
SLIPT	1	16215	16215	2.3722	0.1246
siRNA×SLIPT	1	17	17	0.0025	0.9603

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

The results for the $G_{\alpha i}$ pathway were very similar when testing synthetic lethality against *CDH1* mutation (mtSLIPT). In either case, there was no significant evidence that SLIPT or mtSLIPT-specific genes had higher connectivity than those detected by siRNA screening (shown in Appendix Figure H.1 and Appendix Table H.1). Thus synthetic lethal detection does not discriminate among genes by their connectivity in this 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 in the $G_{\alpha i}$ signalling pathway.

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 in terms of 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 be indicative of the essentiality of genes or proteins (Kranthi *et al.*, 2013).

Within the $G_{\alpha i}$ pathway (shown in Figure 5.6), the information centrality across gene groups detected by either synthetic lethal approach did not differ significantly (shown by Table 5.2). Genes detected by SLIPT span the complete range of PageRank centrality values for this pathway. These findings were replicated (shown in Appendix Figure H.2 and Appendix Table H.2). Thus neither method was unable to detect

synthetic lethal genes in the $G_{\alpha i}$ pathway with particular centrality constraints but they were also not detecting genes with higher centrality than expected by chance.

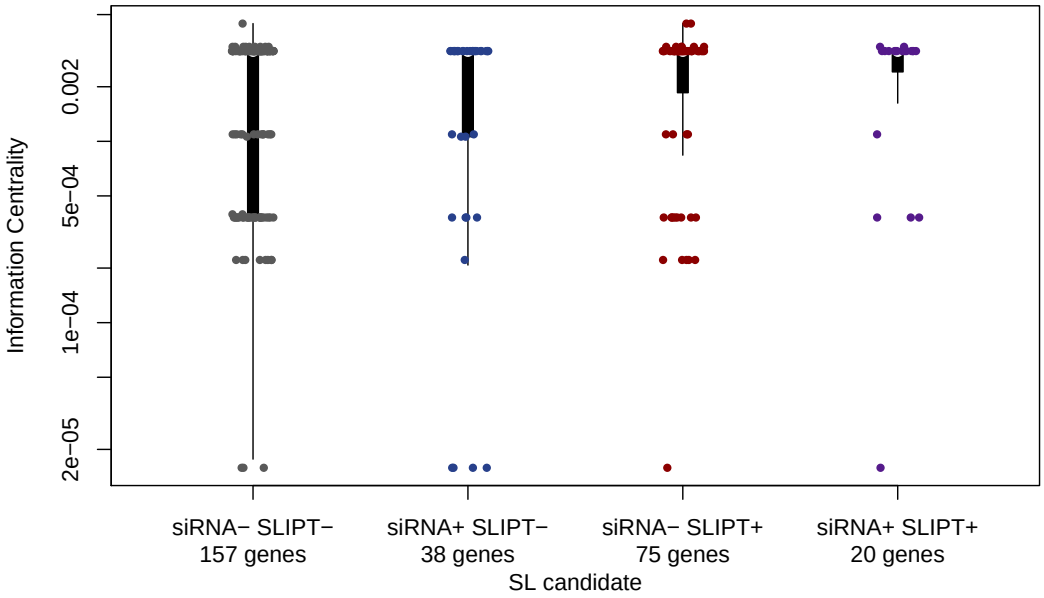


Figure 5.6: **Synthetic lethality and centrality.** The information centrality was compared (on a log-scale) across genes detected by SLIPT and siRNA screening in the Reactome $G_{\alpha i}$ pathway. Genes detected by SLIPT or siRNA did not have higher centrality than other genes (shown in Table 5.2). Genes detected by SLIPT spanned the range of centrality values.

Table 5.2: ANOVA for synthetic lethality and information centrality

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	0.00000000	2.7000×10^{-9}	0.0016	0.96783
SLIPT	1	0.00000548	5.4831×10^{-6}	3.3253	0.06926
siRNA×SLIPT	1	0.00000002	1.8800×10^{-8}	0.0114	0.91511

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 $G_{\alpha i}$ pathway (shown in Figure 5.7), which differs considerably from the information centrality scores (as shown in Figure 5.6). Genes detected by either synthetic lethal approach did not include those with the highest PageRank centrality. There was a significant association between genes detected by SLIPT (which had a lower median) with PageRank centrality (shown by Table 5.3). The genes detected by SLIPT span the range of centrality values of siRNA showing that both approaches were capable of detecting genes of moderately high centrality (as shown for information centrality) and that the lower centrality of SLIPT candidates in $G_{\alpha i}$ pathway may be due to synthetic lethal partners being less critical to the pathway, rather than a limitation of the methodology.

There was not a significant association between siRNA candidates and PageRank centrality. Te significant result for SLIPT was not replicated when testing synthetic lethality against *CDH1* mutation (shown in Appendix Figure H.3 and Appendix Table H.3). However, this may be due to fewer genes being detected by mtSLIPT and siRNA.

Table 5.3: ANOVA for synthetic lethality and PageRank centrality

	DF	Sum Squares	Mean Squares	F-value	p-value
siRNA	1	0.0001059	1.0589×10^{-4}	2.1021	0.14818
SLIPT	1	0.0002881	2.8808×10^{-4}	5.7188	0.01743
siRNA×SLIPT	1	0.0000477	4.7704×10^{-5}	0.9470	0.33131

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

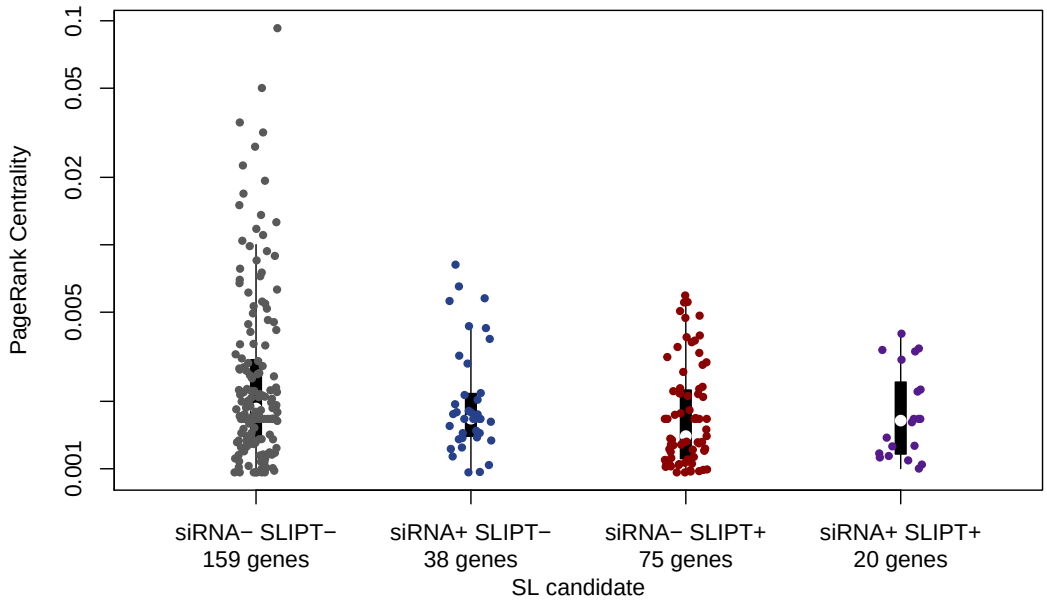


Figure 5.7: **Synthetic lethality and PageRank.** The PageRank centrality was compared (on a log-scale) across genes detected by mtSLIPT and siRNA screening in the Reactome $G_{\alpha i}$ pathway. Genes detected by with either synthetic lethal detection approach had a more restricted range of centrality values but only SLIPT genes had a significant association with centrality (shown in Table 5.3).

5.3 Relationships between Synthetic Lethal Genes

This network analyses so far have tested whether synthetic lethal candidate genes were more connected or important within a pathway structure, such as the $G_{\alpha i}$ pathway. However these metrics do not ascertain whether there were relationships between SLIPT and siRNA candidate partners of *CDH1*. In particular, it is plausible that they may be upstream or downstream of one and other within a pathway.

The direction of a biological pathway is important, particularly those involved in cell signalling which respond to extracellular stimuli and transmit these signals via intermediary proteins to regulate core functions and responses of the cell. These pathways regulate process such as gene expression and protein translation, which are important in the proliferation of cancers (Gao and Roux, 2015). Therefore it is important to determine which synthetic lethal candidates were upstream or downstream in the con-

text of a biological pathway. In particular, pathway structure may be used to identify relationships between SLIPT and siRNA gene candidates.

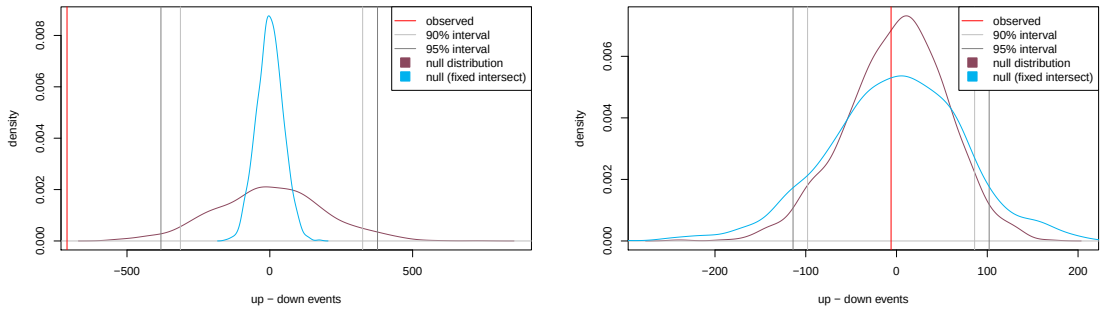
A pathway structure method has been devised to use network structures to identify directional relationships between individual SLIPT and siRNA genes. This pathway structure methodology will be applied (as described in Section 3.4.1) to detect the direction of shortest paths between SLIPT and siRNA gene candidates. This will be used to demonstrate the methodology on the PI3K and $G_{\alpha i}$ pathways, to develop a statistical test for pathway structure between between SLIPT and siRNA gene candidates 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.

5.3.1 Detecting Upstream or Downstream Synthetic Lethality

Shortest paths in a pathway network were used to devise a strategy to detect pathway structure between SLIPT and siRNA gene candidates 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 procedure enables the detection of directional relationships between SLIPT and siRNA gene candidates.

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 candidates 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 candidates 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 candidates partners (as described in Sections 2.3.6 and 3.4.1.1).

This resampling procedure was performed for the $G_{\alpha i}$ and PI3K pathways to generate a null distribution for the difference in the number of "up events" and "down events" for these pathway structures (as shown in Figures 5.1 and 5.4). The resulting



(a) Resampling in $G_{\alpha i}$ signalling

(b) Resampling in the PI3K cascade

Figure 5.8: **Structure of synthetic lethality resampling.** 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 each pathway. To assess significance, the observed events (with shortest paths) were compared to the 90% and 95% intervals for the null distribution (shown in blue). Genes detected by both methods were not fixed to the same number as observed for the alternative null distribution (shown in violet), although the significance of the observed number of events (red) was changed in either case. The genes detected by both approaches were included in computing the number of shortest paths (in either direction) between SLIPT and siRNA genes. The permutations show (a) a significant pathway relationship for $G_{\alpha i}$ signalling and (b) and non-significant relationship for the PI3K cascade.

null distributions (as shown in Figure 5.8) were used to detect whether genes detected by SLIPT had significantly more upstream or downstream siRNA candidates in either pathway. Therefore it can be shown that siRNA genes were significantly downstream of SLIPT candidate genes by resampling for the $G_{\alpha i}$ signalling pathway (as shown in Figure 5.8a). This demonstrates that pathway relationships can be detected between synthetic lethal candidates by this procedure and that siRNA genes were downstream of gene detected by SLIPT in an example of GPCR signalling expanding on support for synthetic lethality in this pathway (as shown in Chapter 4). These structural relationships may also account for why each the computational and experimental approaches did not detect many of the same specific genes because they are detecting different parts of the pathway.

In contrast, there was not significant evidence of such pathway structure between siRNA and SLIPT candidate genes when resampling within the PI3K cascade pathway (as shown in Figure 5.8b). This indicates that these relationships may be pathway specific rather than a general property of these synthetic lethal detection methods.

These results were robustly reproducible, with similar findings (as shown in Appendix Figure I.1) for each pathway when testing for synthetic lethality against *CDH1* mutation (mtSLIPT). Neither relaxing the fixing the number of genes detected by both approaches (as shown in Figure 5.8 and Appendix Figure I.1) 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 "meta-pathways" constructed in Section 2.4.3) also had little effect on the null distribution generated, despite increasing the computational complexity of the procedure.

5.3.2 Resampling for Synthetic Lethal Pathway Structure

The permutation procedure (as described in Section 3.4.1.1) that was performed in Section 5.3.1 for the $G_{\alpha i}$ and PI3K pathways was also applied to other pathways identified in Chapter 4 and discussed in Section 5.1. In addition to the cell signalling pathways (PI3K/AKT and GCPRs demonstrated in Section 5.3.1), the pathways tested include extracellular matrix (with constituent elastic fibre and fibrin pathways), and translational pathways (with NMD and 3′UTR regulation).

The resampling results across these pathways (as shown in Table 5.4) 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 Figure 5.8b), with the exception of $G_{\alpha i}$ signalling (as shown in Figure 5.8a). However, the exact 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 therefore 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.6). 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 I.1) nor was it supported by the related 3′UTR regulation and translational elongation pathways.

Table 5.4: Resampling for pathway structure of synthetic lethal detection methods

	Graph		States		Observed				Permutation p-value		p-value (FDR)
Pathway	**Nodes**	**Edges**	**SLIPT**	**siRNA**	**Up**	**Down**	**Up−Down**	**Up/Down**	**Up−Down**	**Down−Up**	**Down−Up**
PI3K Cascade	138	1495	38	25	122	128	-6	0.953	0.5326	0.4606	0.6734
PI3K/AKT Signalling in Cancer	275	12882	98	44	779	679	100	1.147	0.3255	0.6734	0.6734
$G_{\alpha i}$ Signalling	292	22003	95	58	836	1546	-710	0.541	0.9971	0.0029	0.0145
GPCR downstream	1270	142071	312	160	9755	9261	494	1.053	0.3692	0.6305	0.6734
Elastic fibre formation	42	175	24	7	1	2	-1	0.500	0.5461	0.3865	0.6734
Extracellular matrix	299	3677	127	29	547	455	92	1.202	0.3351	0.6636	0.6734
Formation of Fibrin	52	243	18	5	12	17	-5	0.706	0.6198	0.3564	0.6734
Nonsense-Mediated Decay	103	102	74	2	0	74	-74	0	1.0000	< 0.0001	< 0.0010
3' -UTR-mediated translational regulation	107	2860	77	1	0	0	0		0.4902	0.5027	0.6734
Eukaryotic Translation Elongation	92	3746	76	0	0	0	0		0.4943	0.4933	0.6734

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 includued 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 (as shown in Figure 5.8a). In contrast to the other pathways $G_{\alpha i}$ signalling showed significant downstream siRNA genes for SLIPT from a large number of shortest paths (in Table 5.4). This would indicate that SLIPT detects upstream regulators of genes experimentally validated by siRNA in this pathway. This result was pathway was also the strongest result in mtSLIPT results (Appendix Table I.1), although it was not significant after adjusting for multiple testing in this case.

There is insufficient evidence to determine whether there is pathway structure, that genes were 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 most of the signalling pathways (with the exception of $G_{\alpha i}$ signalling) upon which the rationale for pathway structure hypotheses were based. 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), many of these did not show relationships with respect to pathway structure. Despite the design of a robust resampling approach to test relationships between gene groups, the detection of structural relationships between SLIPT and siRNA gene candidates did not generalise across pathways (and was specific to a few). Such structural relationships may apply more

broadly to gene networks as different biological pathways were more over-represented among SLIPT and siRNA gene candidates. 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 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 (Croft *et al.*, 2014) . 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 SLIPT and siRNA. The differences between these 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.

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 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.

A measure of pathway structure between individual SLIPT and siRNA candidate genes within a pathway was 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. This approach successfully detected a statistically robust relationship between SLIPT and siRNA candidate genes on the $G_{\alpha i}$ signalling pathway, despite there being few differences between these genes with respect to network metrics of connectivity or centrality.

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 existed 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, apart from structural relationships specific to $G_{\alpha i}$ signalling. In this pathway, synthetic lethal candidates did not exhibit significant differences in network connectivity or centrality measures. These network analyses were also unable to ascertain whether the candidates detected by either method stratified into upstream and downstream genes on the pathway.

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 did not detect many structural relationships in the synthetic lethal pathways identified in Chapter 4. Overall, support for pathway structure between SLIPT and siRNA gene candidates was weak and the direction was inconsistent between pathways. Therefore pathway structure does not appear to generally account for the differences between the SLIPT and siRNA gene candidates, although it may apply in specific pathways as demonstrated with $G_{\alpha i}$ signalling. It was possible to detect some pathway relationships between synthetic lethal candidates in synthetic lethal pathways, in addition to the significantly over-represented genes shared between SLIPT and siRNA (as identified in Chapter 4).

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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