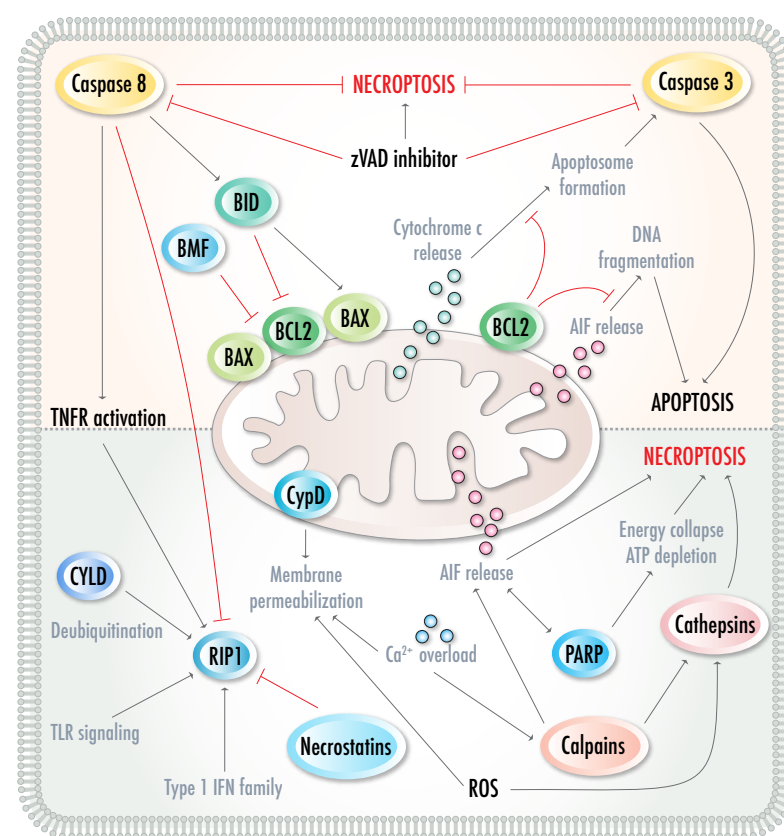


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### Cell Death



**Fig. 1. Crosstalk between apoptosis and programmed necrosis (necroptosis).** Caspase 8-mediated degradation of RIP1 (receptor-interacting protein kinase 1) is a major molecular switch between apoptosis and necroptosis. Necroptosis centers on the activation of RIP1. As opposed to apoptosis, necroptosis does not engage apoptotic regulators such as caspases, BCL2 family members, or cytochrome c (1-7).

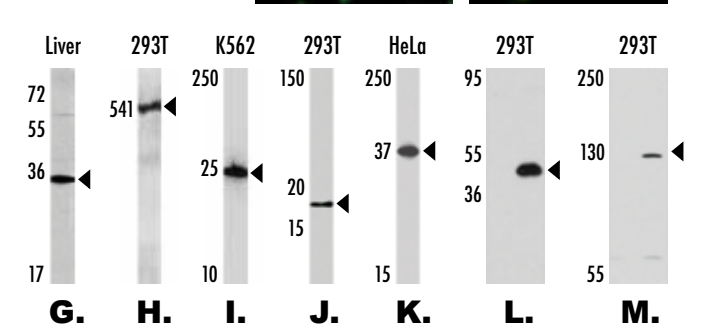
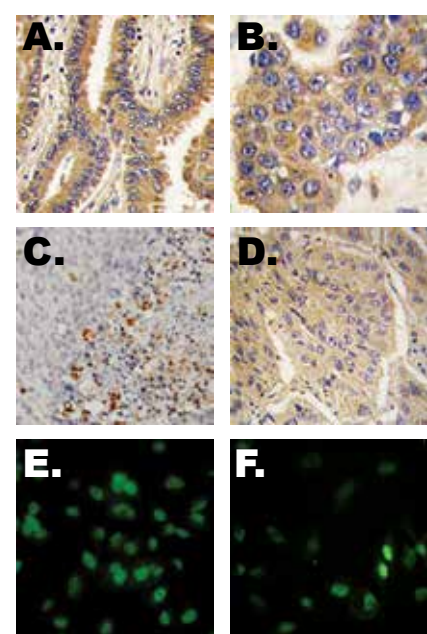
### Comparison of different cell death programs

CHARACTERISTICS	APOPTOSIS	NECROPTOSIS	AUTOPHAGIC	CALCIUM-MEDIATED	AIF/PARP-DEPENDENT	ONCOSIS
Morphology	Chromatin condensation, nuclear fragmentation, apoptotic bodies	Mitochondrial dysfunction, membrane rupture, ER swelling, increase of ROS	Autophagic vacuoles, membrane rupture	Membrane whorls	Mild chromatin condensation	Cellular swelling
Triggers	Oxidative stress, death receptors, viral infections, hypoxia, etc.	Troptotoxicity, TNF, damage-induced lesions, ischemia, antimycin A	Serum, amino acid starvation, protein aggregates	Calcium entry, CDK5 signaling, deg mutants	DNA damage, glutamate, NO	Ischemia, excitotoxicity
Mediators	Caspases, BH family, etc.	ERK2, NUR77	Atg orthologs	Calpains, cathepsins	PARP, AIF	JNK
Inhibitors	Caspase inhibitors, TOP1 inhibitors, survivin, VEGF, zVAD, NO, etc.	Necrostatins, Ca <sup>2+</sup> chelators, PARP inhibitors, U0126, DN NUR77, CypD inhibitors	3-Methyladenine, bafilomycin A1, mTOR, JNK inhibitors?	Calreticulin, calpain inhibitors	PARP inhibitors	JNK inhibitors, glycine
Examples	Type I and nuclear pcd	Type III and cytoplasmic pcd	Type II pcd	<i>C. elegans</i> deg mutants	Some excitotoxic pcd	Ischemic pcd

**Table 1. Alternative programmed cell death (pcd) processes.** Apoptosis is a cellular mechanism of necrotic cell death induced by apoptotic stimuli under conditions where apoptotic and/or autophagic execution are prevented. **Abbreviations** for Fig. 1 and Table 1: **AIF**, apoptosis-inducing factor; **BAX**, BCL2-associated X protein; **BCL2**, B-cell CLL/lymphoma 2; **BID**, BH3 interacting domain death agonist; **BMF**, BCL2 modifying factor; **CDK5**, cyclin-dependent kinase 5; **CYLD**, cylindromatous (turban tumor syndrome); **CypD**, cytochrome P; **deg**, degenerate; **ERK2**, mitogen-activated protein kinase 1; **IFN**, interferon; **JNK**, mitogen-activated protein kinase 8; **mTOR**, mechanistic target of rapamycin; **NO**, nitric oxide; **NUR77**, nuclear receptor; **DN NUR77**, dominant negative Nur77; **PARP**, poly (ADP-ribose) polymerase; **ROS**, reactive oxygen species; **TLR**, Toll-like receptor; **TNF**, tumor necrosis factor; **TRAF**, tumor necrosis factor receptor; **TOP1**, DNA topoisomerase 1; **U0126**, inhibitor of MEK kinase; **zVAD**, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone, a caspase inhibitor (1-7).

### Selected Abgent Products

Figure	Target	Tissue/Cell line	Cat #
A.	BAD	Human lung carcinoma	AP1314b
B.	PUMA	Human breast carcinoma	AP1317a
C.	CASP1	Human hepatocarcinoma	AT1400a
D.	CASP3	Human lung carcinoma	AP7563c
E.	DNMT1	Human carcinoma (Hela)	AT1805a
F.	CBX5	Human carcinoma (Hela)	AT1411a
G.	CASP6	Mouse liver tissue	AP7563b
H.	MLL3	Transfected 293T cells	AP6184a
I.	AIF1	Human leukemia cells K562	AT1077a
J.	BIRC5	Transfected 293T cells	AT1299a
K.	SPP1	Human carcinoma (Hela)	AT4028a
L.	JUN	Transfected 293T cells	AP1984d
M.	PARP1	Transfected 293T cells	AP6373a



### Protein Associations

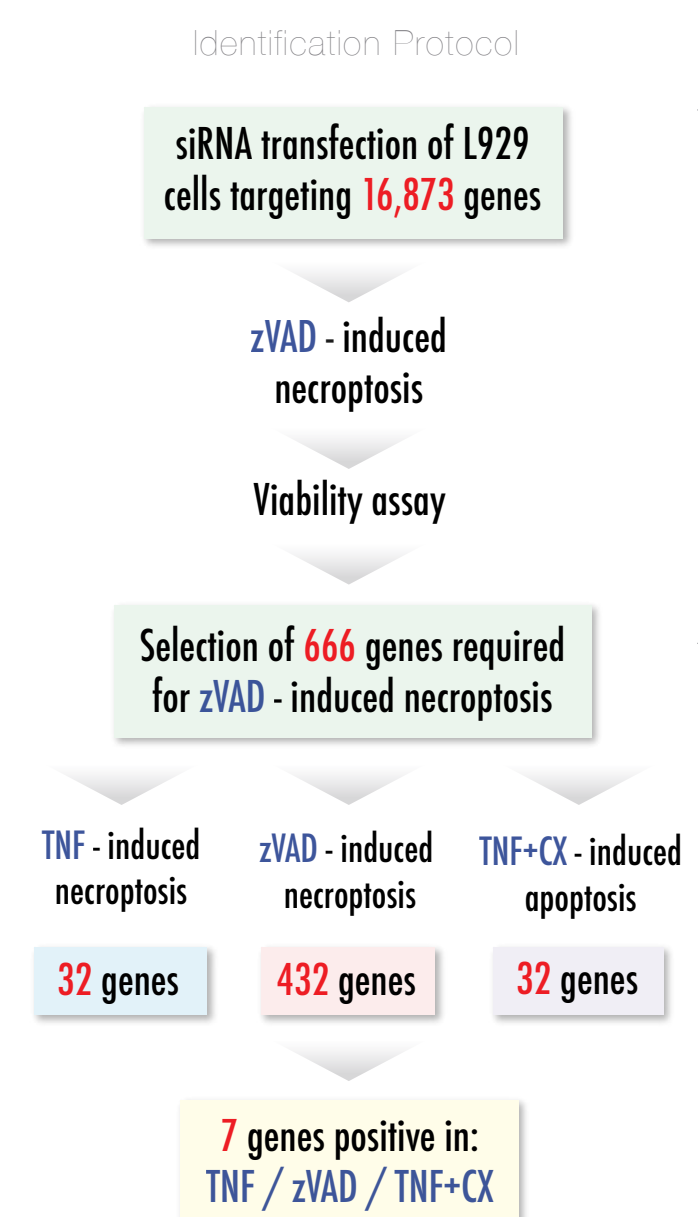


Fig. 2

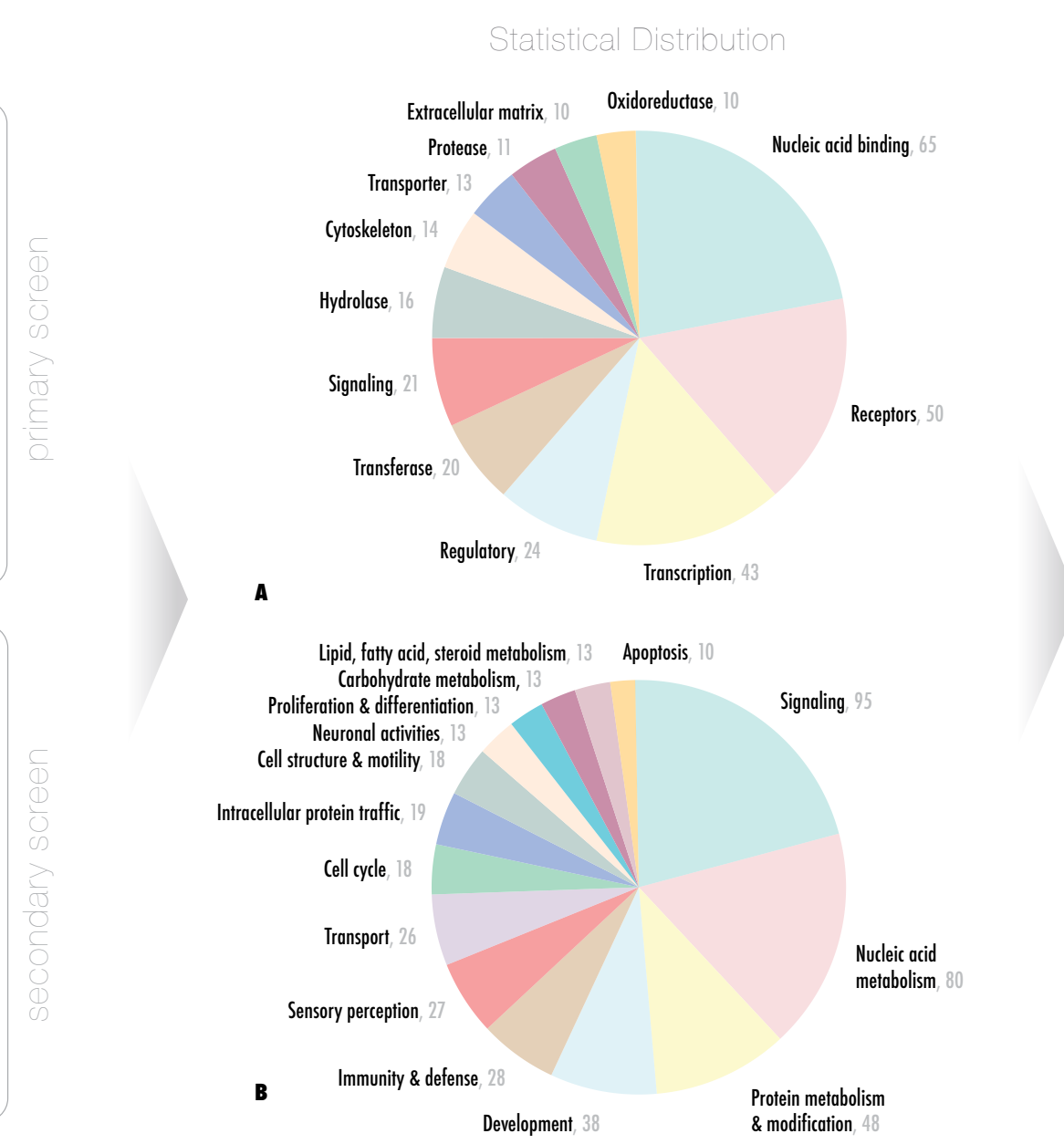


Fig. 3

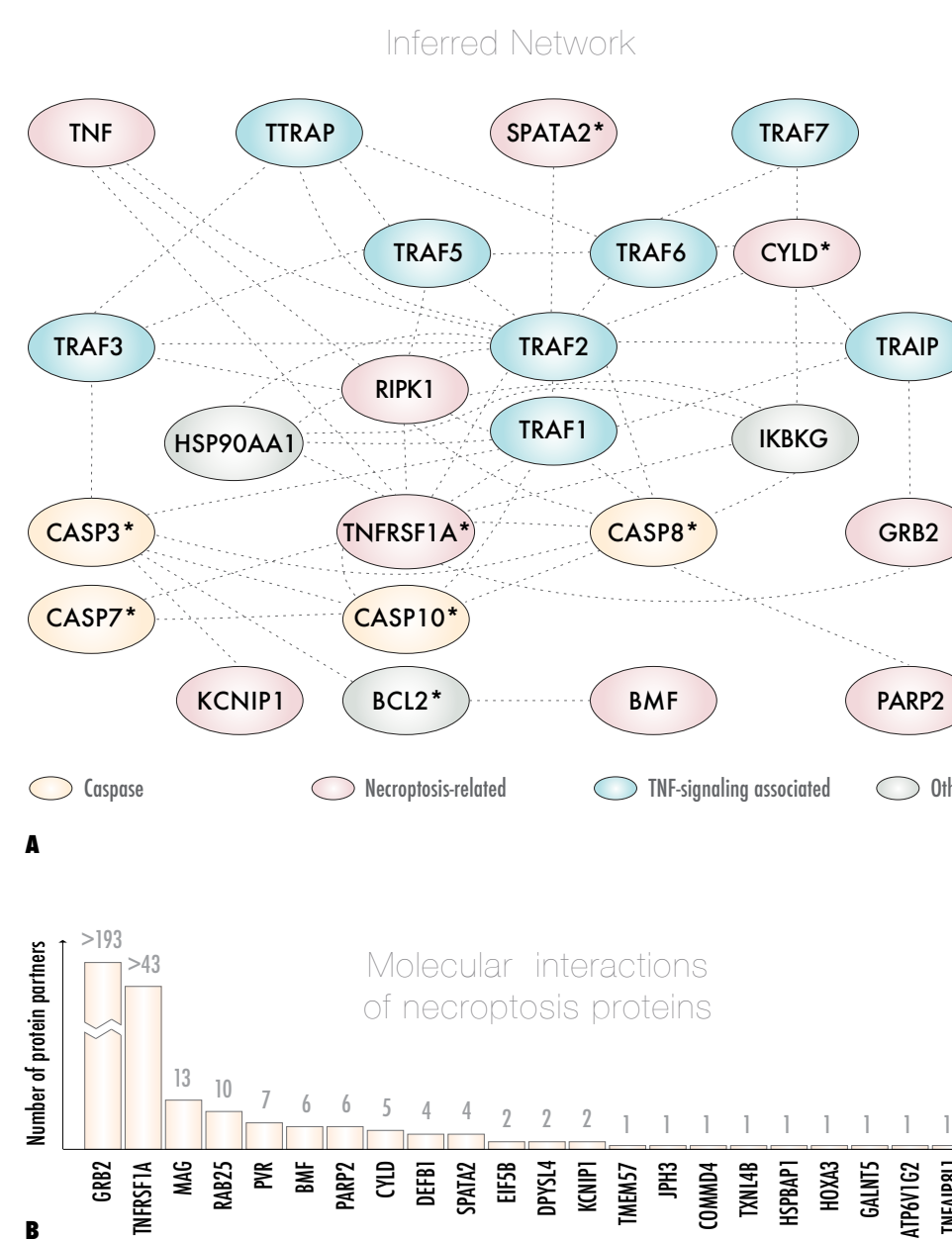


Fig. 4

**Fig. 2 siRNA screening for genes required in necroptosis.** siRNA screen of the mouse genome for regulators of necroptosis identified a set of 432 genes that regulate necroptosis, a subset of 32 genes that are associated with RIP1 kinase, 32 genes required for apoptosis, and 7 genes involved in both necroptosis and apoptosis: **zVAD**, carbobenzoxy-valyl-alanyl-aspartyl-[O-methyl]-fluoromethylketone; **CX**, cycloheximide (7).

**Fig. 3 Classification of 432 selected proteins from the necroptosis screen into (A) molecular function and (B) biological process categories.** Genes for which no annotations could be assigned were excluded from the analysis. Categories with at least ten genes are displayed. The number of genes assigned to each category are shown in gray (7).

**Fig. 4 Molecular interactions of TNF-induced necroptotic proteins.** A, Crosstalk between necroptosis-related proteins, positively identified in the secondary screen, with apoptotic (\*) and other signaling molecules. The network was generated by using data curated from the literature. B, Number of protein binding partners for necroptotic proteins selected from the secondary screen. **Protein abbreviations:** **ATP6V1G2**, vacuolar ATP synthase subunit G2; **CASP**, caspase; **COMMD4**, COM domain containing 4; **EIF5B**, eukaryotic translation initiation factor 5B; **DEFB1**, defensin, beta 1; **DPYSL4**, dihydropyrimidinase-like 4; **JPH3**, junctional protein 3; **HOXA3**, homeobox A3; **HSPBAP1**, heat shock associated protein 1; **GALNT5**, N-acetylglucosaminyltransferase 5; **GRB2**, growth factor receptor-bound protein 2; **KCNIP1**, Kv channel interacting protein 1; **MAG**, myelin associated glycoprotein; **RAB25**, RAB25, member RAS oncogene family; **SPATA2**, spermatogenesis associated 2; **TMEM57**, transmembrane protein 57; **TNFAIP8L1**, tumor necrosis factor, alpha-induced protein 8-like 1; **TNFRSF1A**, tumor necrosis factor receptor superfamily, member 1A; **TRAF1**, TRAF interacting protein; **TRAF2**, TNF receptor-associated factor 2; **TRAF3**, TNF receptor-associated factor 3; **TRAF4**, TNF receptor-associated factor 4; **TRAF5**, TNF receptor-associated factor 5; **TRAF6**, TNF receptor-associated factor 6; **TRAF7**, TNF receptor-associated factor 7; **TRAIP**, TRAF interacting protein; **TRAF1**, TNF receptor-associated factor 1; **TTRAP**, TRAF and TNF receptor associated protein; **TXNL4B**, thioredoxin-like 4B.

### Protein Markers

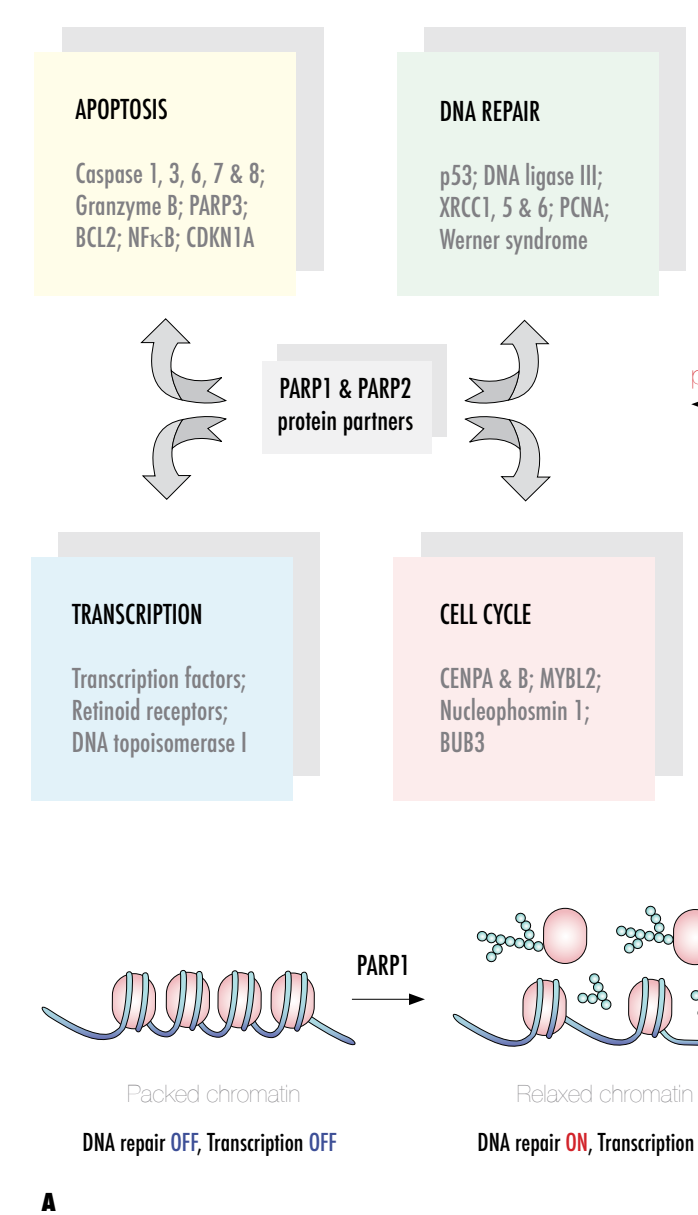


Fig. 5

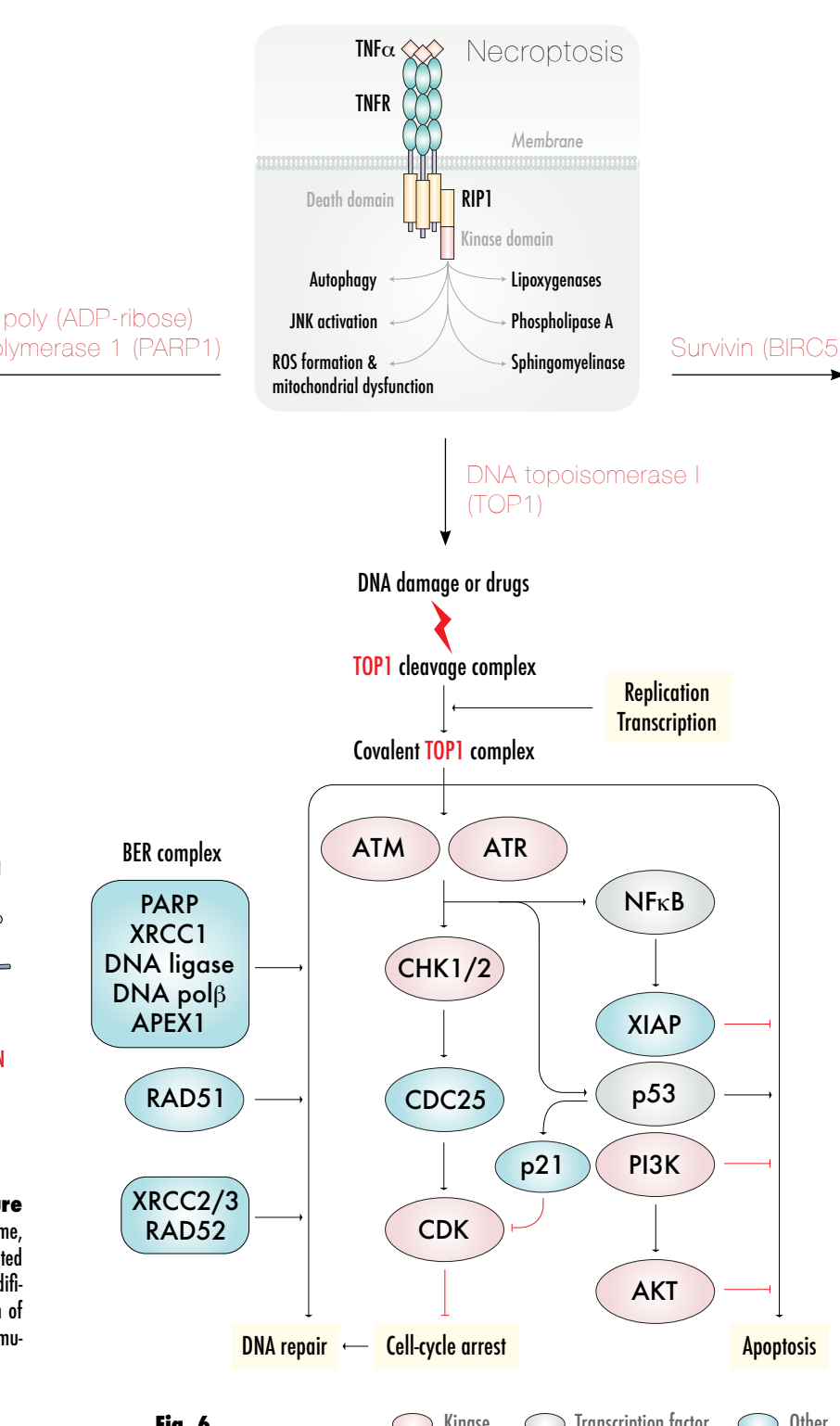


Fig. 6

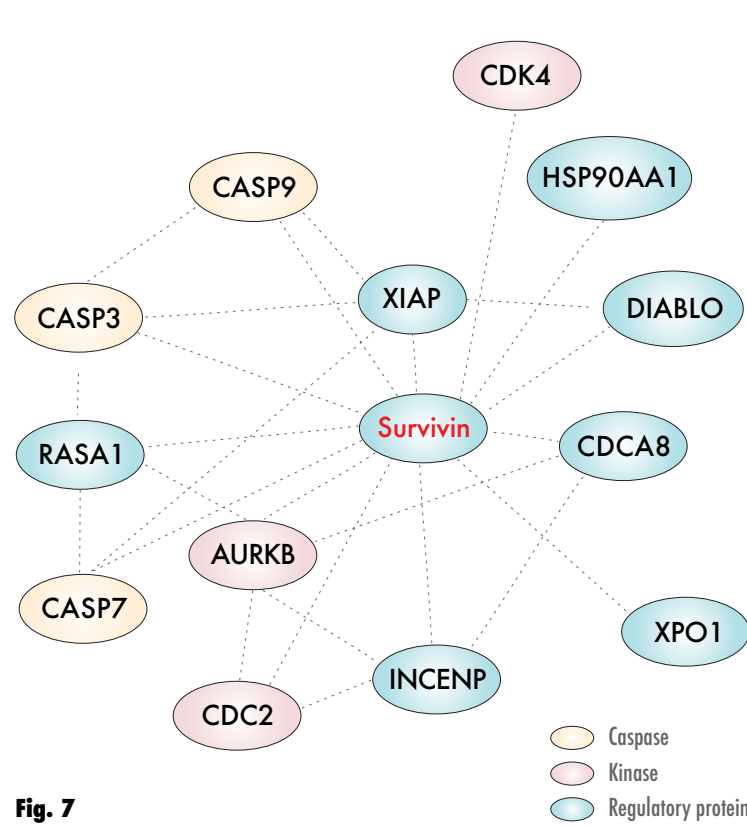


Fig. 7

**Fig. 6 Signaling events associated with TOP1.** DNA damage and drugs induce reversible TOP1 cleavage complexes, subsequently converted to irreversibly by replication and transcription. Irreversible TOP1 complexes can also be formed during apoptosis. DNA repair engages multiple signaling events, including the BER (base excision repair) complex and the RADS1/RAD52-associated homologous recombination. Cell-cycle arrest facilitates DNA repair and involves ATM (ataxia telangiectasia mutated) and ATR (ataxia telangiectasia and RAD3 related) kinases, as well as the checkpoint molecules **CHK1** and **CHK2**. Additional molecules, associated with checkpoint regulation, include CDC25, histone H2AX, MKN complex, and BRCA1. p53 activates apoptosis both directly and by transactivating pro-apoptotic genes. p21 is a p53-inducible gene, which inhibits CDKs (cyclin-dependent kinases). TOP1 inhibitors suppress apoptosis by activating nuclear factor NF- $\kappa$ B, which induces the expression of anti-apoptotic genes (12).

**Fig. 7 Molecular interactions between survivin and its protein partners.** The network was generated by using protein-protein interaction data curated from the literature.

**Protein abbreviations:** **AKT**, v-akt murine thymoma viral oncogene homolog; **APEX1**, nucleic acid excision repair protein; **AURKB**, aurora kinase B; **BCL2**, B-cell CLL/lymphoma 2; **BUB3**, budding uninhibited by benzimidazoles 3 homolog; **CASP**, caspase; **CDK**, cell division cycle; **CDKN1A** or **p21**, cyclin-dependent kinase inhibitor 1A; **CENPA**, centromere protein A; **DIABLO**, diablo homolog; **INCENP**, inner centromere protein antigens; **MYBL2**, v-myb myeloblastosis viral oncogene homolog (avian)-like 2; **PCNA**, proliferating cell nuclear antigen; **PI3K**, phosphoinositide-3-kinase; **RAD51**, RAD51 homolog; **XIAP**, X-linked inhibitor of apoptosis; **XPO1**, exportin 1; **XRCC**, X-ray repair complementing defective repair in Chinese hamster cells.

### Product Abbreviations

- BAD**: BCL2-associated agonist of cell death; BCLX/BCL2 binding protein; BCL2-binding component 6; BBC2
- PUMA**: BCL2 binding component 3; BBC3
- CASP1**, -3, -6: caspase 1, -3, -6
- DNMT1**: DNA (cytosine-5)-methyltransferase 1; CXX finger protein 9; DNA methyltransferase 1
- CBX5**: chromobox homolog 5 (HP1 alpha homolog, Drosophila); heterochromatin protein 1 homolog alpha
- MLL3**: myeloid/lymphoid or mixed-lineage leukemia 3; histone-lysine N-methyltransferase, H3 lysine-4 specific
- AIF1**: allograft inflammatory factor 1; interferon gamma responsive transcript; ionized calcium-binding adapter
- BIRC5**: baculoviral IAP repeat-containing 5; apoptosis inhibitor 4; survivin variant 3 alpha
- SPP1**: secreted phosphoprotein 1; bone sialoprotein 1; osteopontin
- JUN**: jun oncogene; Jun activation domain binding protein; v-jun avian sarcoma virus 17 oncogene homolog
- PARP1**: poly (ADP-ribose) polymerase 1; ADP-ribosyltransferase (NAD+); poly (ADP-ribose) polymerase

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