Supplemental Digital Content

Table S1 Demographics of patients included in the study

Demographics of patients included in the donor study.			
Variable	**Living (n=37)**	**DBD (n=82)**	**DCD (n=38)**
Age (years)[a]	53 (23-69)	47 (9-72)	53 (17-65)
ECD donor (%)	19	15	18
Gender (% Female)	65	52	42
Cause of death (%): CVA Trauma Other	NA	 65 27 8	 53 26 21
Duration of BD (min)	NA	679 (348-1814)	NA
WIT1 (min)	4 (2-8)	NA	18 (9-37)

Donor, recipient and transplant characteristics of patients included in the paired recipient study.			
Variable	**Living (n=34)**	**DBD (n=67)**	**DCD (n=29)**
Donor age (years)[a]	53 (23-69)	52 (10-76)	52 (18-66)
ECD donor (%)	18	16	14
Donor gender (% Female)	65	51	41
Cause of death (%): CVA Trauma Other	NA	 75 15 10	 45 31 24
Duration of BD (min)	NA	602 (184-3325)	NA
WIT1 (min)	4 (2-8)	NA	16 (9-35)
Recipient age (years)[a]	45 (15-73)	56 (19-73)	59 (25-68)
Recipient gender (% Female)	32	46	24
Recipient transplants (% first)	85	90	97
Cold ischemia time (min)[a]	153 (87-203)	1096 (461-1817)	956 (517-1500)
DGF no. (%)	6	33	79
PNF no. (%)	0	1	7

Donor, recipient and transplant characteristics of patients included in the unpaired recipient study.				
Variable	**DBD (n=43 T2)**	**DBD (n=38 T3)**	**DCD (n=24 T2)**	**DCD (n=35 T3)**
Donor age (years)[a]	55 (12-74)	53 (17-72)	46 (11-68)	42 (9-65)
ECD donor (%)	30	32	17	17
Donor gender (% Female)	70	58	38	51
Cause of death (%): CVA Trauma Other	 77 12 11	 71 18 11	 50 25 25	 40 34 26
Duration of BD (min)	569 (227-2127)	607 (220 – 2850)	NA	NA
WIT1 (min)	NA	NA	19 (9-26)	18 (9-33)
Recipient age (years)[a]	55 (26-70)	56 (23-71)	55 (35-70)	59 (22-75)
Recipient gender (% Female)	44	47	33	37
Recipient transplants (% first)	93	84	96	94
Cold ischemia time (min)[a]	987 (542-2024)	1005 (600-1430)	1001 (560 – 1429)	998 (579-2092)
DGF (%)	16	34	67	77
PNF no. (%)	0	5	4	3

[a]Median (range).

Abbreviations: BD; brain death, WIT1; warm ischemia time 1, NA; not applicable, DGF; delayed graft function

Table S2a: KEGG pathways enriched in differentially expressed genes between DBD donor kidneys (n=82) and living donor kidneys (n=37, FC ≥ 1.1). No additional pathways were revealed using a cut off of FC ≥ 1.3.

Pathway	Gene count	Benjamini FDR
Glycolysis/gluconeogenesis	28	1,5E-06
Pyruvate metabolism	19	1,7E-04
Proteasome	21	2,0E-04
Drug metabolism	24	3,0E-04
Tryptophan metabolism	18	4,9E-04
Pathogenic E. coli infection	22	5,8E-04
Ascorbate and aldarate metabolism	11	6,9E-04
Metabolism of xenobiotics by cytochrome P450	22	1,1E-03
Beta-Alanine metabolism	12	1,2E-03
Amino sugar and nucleotide sugar metabolism	18	1,2E-03
Arginine and proline metabolism	20	1,2E-03
Histidine metabolism	14	1,3E-03
Butanoate metabolism	15	1,7E-03
Complement and coagulation cascades	23	2,0E-03
Alanine, aspartate and glutamate metabolism	14	2,0E-03
Glycine, serine and threonine metabolism	14	2,0E-03
Glutathione metabolism	18	3,9E-02
Pentose and glucuronate interconversions	10	4,1E-03
Fructose and mannose metabolism	14	5,0E-03
Drug metabolism	16	5,6E-03
Glyoxylate and dicarboxylate metabolism	8	2,5E-02
Propanoate metabolism	12	3,1E-02

Table S2b: GSEA analysis between DBD (n=82) and living (n=37) donor kidneys using pathway definitions of the KEGG. Abbreviations: ES; enrichment score, NES; normalized enrichment score.

Gene Set	Genes	p-value	q-value	ES	NES
KEGG_PROTEASOME	48	0,00	0,04	0,78	1,90
KEGG_BASAL_TRANSCRIPTION_FACTORS	36	0,01	0,08	0,62	1,83
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	69	0,00	0,25	0,54	1,70
KEGG_PATHOGENIC_ESCHERICHIA_COLI_INFECTION	59	0,00	0,00	0,71	2,11
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	72	0,01	0,16	-0,52	-1,75
KEGG_BETA_ALANINE_METABOLISM	22	0,01	0,17	-0,74	-1,79
KEGG_HISTIDINE_METABOLISM	29	0,00	0,04	-0,74	-1,97

Table S3a: KEGG pathways enriched in differentially expressed genes between DCD donor kidneys (n=38) and living donor kidneys (n=37, FC ≥ 1.1). No additional pathways were revealed using a cut off of FC ≥ 1.3.

Pathway	Gene count	Benjamini FDR
Tryptophan metabolism	18	9,0E-05
Glycolysis/gluconeogenesis	23	1,0E-04
Glycine, serine and threonine metabolism	15	2,3E-04
Pyruvate metabolism	17	2,5E-04
Histidine metabolism	13	2,0E-03
Alanine, aspartate and glutamate metabolism	13	2,2E-03
Complement and coagulation cascades	21	2,2E-03
Butanoate metabolism	14	2,2E-03
Beta-Alanine metabolism	11	2,4E-03
Fatty acid metabolism	15	2,4E-03
Pathogenic E. coli infection	18	3,6E-03
Ascorbate and aldarate metabolism	9	5,4E-03
Drug metabolism	18	9,2E-03
Propanoate metabolism	12	9,9E-03
Arginine and proline metabolism	16	1,1E-02
Valine, leucine and isoleucine degradation	14	1,4E-02
Metabolism of xenobiotics by cytochrome P450	17	1,5E-02
Prion diseases	12	1,8E-02
Limonene and pinene degradation	7	3,4E-02
Lysine degradation	13	3,8E-02
Glycerolipid metabolism	13	4,4E-02

Table S3b: GSEA analysis between DCD (n=38) and living (n=37) donor kidneys using pathway definitions of the KEGG. Abbreviations: ES; enrichment score, NES; normalized enrichment score.

Gene Set	Genes	p-value	q-value	ES	NES
KEGG_COMPLEMENT_AND_COAGULATION_CASCADES	69	0,00	0,14	0,55	1,89
KEGG_LEISHMANIA_INFECTION	70	0,01	0,21	0,57	1,80
KEGG_SYSTEMIC_LUPUS_ERYTHEMATOSUS	139	0,00	0,21	0,52	1,77
KEGG_BASAL_TRANSCRIPTION_FACTORS	36	0,00	0,19	0,70	1,75
KEGG_ECM_RECEPTOR_INTERACTION	83	0,01	0,29	0,49	1,68
KEGG_PROTEASOME	48	0,05	0,28	0,66	1,66
KEGG_BETA_ALANINE_METABOLISM	22	0,04	0,29	-0,71	-1,68
KEGG_DRUG_METABOLISM_CYTOCHROME_P450	72	0,01	0,13	-0,54	-1,81
KEGG_HISTIDINE_METABOLISM	29	0,00	0,02	-0,77	-2,01

Table S4: KEGG pathways enriched in differentially expressed genes between all left ór all right deceased (DBD n=19 and DCD n=12) donor kidneys from the same deceased donor versus living donor kidneys (n=37, FC ≥ 1.1). No additional pathways were revealed using a cut off of FC ≥ 1.3.

Left deceased kidney versus living donor kidney

Pathway	Gene count	Benjamini FDR
Glycolysis/gluconeogenesis	23	4,7E-05
Pathogenic E. coli infection	19	3,6E-03
Complement and coagulation cascades	20	7,7E-03
Proteasome	16	8,3E-03
Alanine, aspartate and glutamate metabolism	12	1,4E-02
Pyruvate metabolism	13	3,5E-02
Amino sugar and nucleotide sugar metabolism	13	7,3E-02
Butanoate metabolism	13	7,8E-02
Glyoxylate and dicarboxylate metabolism	7	8,5E-02
Arginine and proline metabolism	17	9,4E-02
Tryptophan metabolism	15	1,7E-01
Histidine metabolism	11	1,7E-01

Right deceased kidney versus living donor kidney

Pathway	Gene count	Benjamini FDR
Glycolysis/gluconeogenesis	22	6,1E-05
beta-Alanine metabolism	11	1,9E-03
Tryptophan metabolism	15	2,9E-03
Arginine and proline metabolism	17	3,2E-03
Butanoate metabolism	13	3,9E-03
Proteasome	15	6,0E-03
Complement and coagulation cascades	19	6,1E-03
Histidine metabolism	11	1,1E-02
Alanine, aspartate and glutamate metabolism	11	1,8E-02
Ascorbate and aldarate metabolism	8	1,9E-02
Amino sugar and nucleotide sugar metabolism	13	2,5E-02
Pathogenic E. Coli infection	15	2,9E-02
Pyruvate metabolism	12	3,1E-02
Glyoxylate and dicarboxylate metabolism	7	3,8E-02
Glycine, serine and threonine metabolism	10	4,4E-02

Table S5a: KEGG pathways enriched in differentially expressed genes between T3 (n=34) and T1 (n=34) biopsies of living donor kidneys (FC ≥ 1.3). No additional pathways were revealed using a cut off of FC ≥ 1.1.

Paired/unpaired	Pathway	Gene count	Benjamini FDR
Paired (n=34)	MAPK signaling pathway	18	1,0E-06
	P53 signaling	7	7,2E-03

Table S5b: GSEA analysis between between T3 and T1 biopsies of living donor kidneys using pathway definitions of the KEGG. Abbreviations: ES; enrichment score, NES; normalized enrichment score.

Gene Set	Genes	p-value	q-value	ES	NES
KEGG_ASTHMA	30	0,004	0,107	-0,693	-1,846

Table S6a: KEGG pathways enriched in differentially expressed genes between T2 and T3 biopsies of DBD donor kidneys (FC ≥ 1.1 and ≥ 1.3).

FC ≥ 1.1

Paired/unpaired	Pathway	Gene count	Benjamini FDR
Paired (n=67)	Lysosome	33	2,5E-02
Unpaired (n=43 vs 38)	Parkinson's disease	41	2,1E-06
	Oxidative phosphorylation	40	5,7E-06
	Huntington's disease	48	1,9E-05
	Alzheimer's disease	44	3,4E-03
	Ribosome	25	3,7E-03
	MAPK signaling pathway	54	8,5E-03
	GPI-anchor biosynthesis	11	1,3E-02

FC ≥ 1.3

Paired/unpaired	Pathway	Gene count	Benjamini FDR
Paired (n=67)	NOD-like receptor signaling pathway	12	2,8E-05
	MAPK signaling pathway	22	1,1E-04
	Prion diseases	6	4,2E-02
	Cytokine-cytokine receptor interaction	16	4,3E-02
Unpaired (n=43 vs 38)	NOD-like receptor signaling pathway	12	1,2E-03
	SLE	14	2,8E-03
	MAPK signaling pathway	22	2,0E-02
	Complement and coagulation pathways	10	2,3E-02
	Metabolism of cytochrome p450	9	3,2E-03

Table S6b: GSEA analysis between T2 and T3 biopsies of DBD donor kidneys using pathway definitions of the KEGG. Abbreviations: ES; enrichment score, NES; normalized enrichment score.

Gene Set	Genes	p-value	q-value	ES	NES
KEGG_PYRIMIDINE_METABOLISM	97	0,032	0,276	-0,445	-1,583
KEGG_RIBOSOME	87	0,047	0,241	-0,698	-1,610
KEGG_AMINOACYL_TRNA_BIOSYNTHESIS	41	0,036	0,232	-0,616	-1,630
KEGG_PROTEASOME	48	0,043	0,260	-0,651	-1,632
KEGG_OXIDATIVE_PHOSPHORYLATION	120	0,026	0,262	-0,664	-1,650
KEGG_LYSOSOME	121	0,027	0,141	-0,518	-1,734
KEGG_GLYCOSYLPHOSPHATIDYLINOSITOL_GPI_ANCHOR_BIOSYNTHESIS	25	0,010	0,168	-0,669	-1,739
KEGG_N_GLYCAN_BIOSYNTHESIS	46	0,000	0,038	-0,608	-1,913
KEGG_DNA_REPLICATION	36	0,004	0,040	-0,605	-1,945
KEGG_NUCLEOTIDE_EXCISION_REPAIR	44	0,000	0,024	-0,622	-2,038

Table S7a: KEGG pathways enriched in differentially expressed genes between T2 and T3 biopsies of DCD donor kidneys (FC ≥ 1.3). No additional pathways were revealed using a cut off of FC ≥ 1.1.

Paired/unpaired	Pathway	Gene count	Benjamini FDR
Paired (n=29)	MAPK signaling pathway	21	2,1E-08
	NOD-like receptor signaling pathway	8	8,6E-04
	Chemokine signaling pathway	10	2,9E-02
Unpaired (n=24 vs 35)	MAPK signaling pathway	22	3,9E-05
	NOD-like receptor signaling pathway	11	5,4E-05
	Antigen processing and presentation	9	2,0E-02

Supplementary Table 7b: GSEA analysis between between T3 and T2 biopsies of DCD donor kidneys using pathway definitions of the KEGG. Abbreviations: ES; enrichment score, NES; normalized enrichment score.

Gene Set	Genes	p-value	q-value	ES	NES
KEGG_NOD_LIKE_RECEPTOR_SIGNALING_PATHWAY	59	0,000	0,022	0,604	2,056
KEGG_N_GLYCAN_BIOSYNTHESIS	46	0,002	0,107	-0,603	-1,869
KEGG_OTHER_GLYCAN_DEGRADATION	15	0,020	0,299	-0,712	-1,686
KEGG_DNA_REPLICATION	36	0,002	0,076	-0,612	-1,962

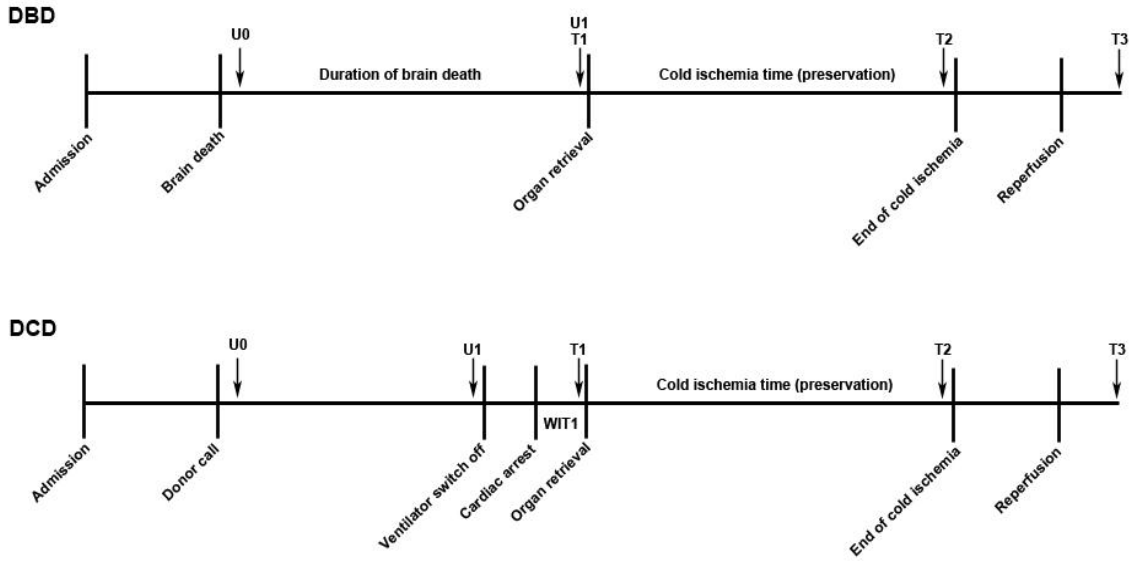

Figure S1: Overview of the biopsies and urine samples taken from deceased donors at different time points in the study. In DBD donors, a T1 biopsy and U1 urine sample were taken after the brain death period, before cessation of blood flow and organ retrieval. A paired U0 urine sample was taken directly after diagnosis of brain death. In DCD donors, a T1 biopsy was taken after the first warm ischemia time (WIT1) between cardiac arrest and organ retrieval. Paired urine samples were taken at the moment of organ call to Eurotransplant (U0) and before ventilator switch off (U1). In recipients, a T2 biopsy was taken at the end of cold ischemia and a T3 biopsy 45-60 minutes after reperfusion.

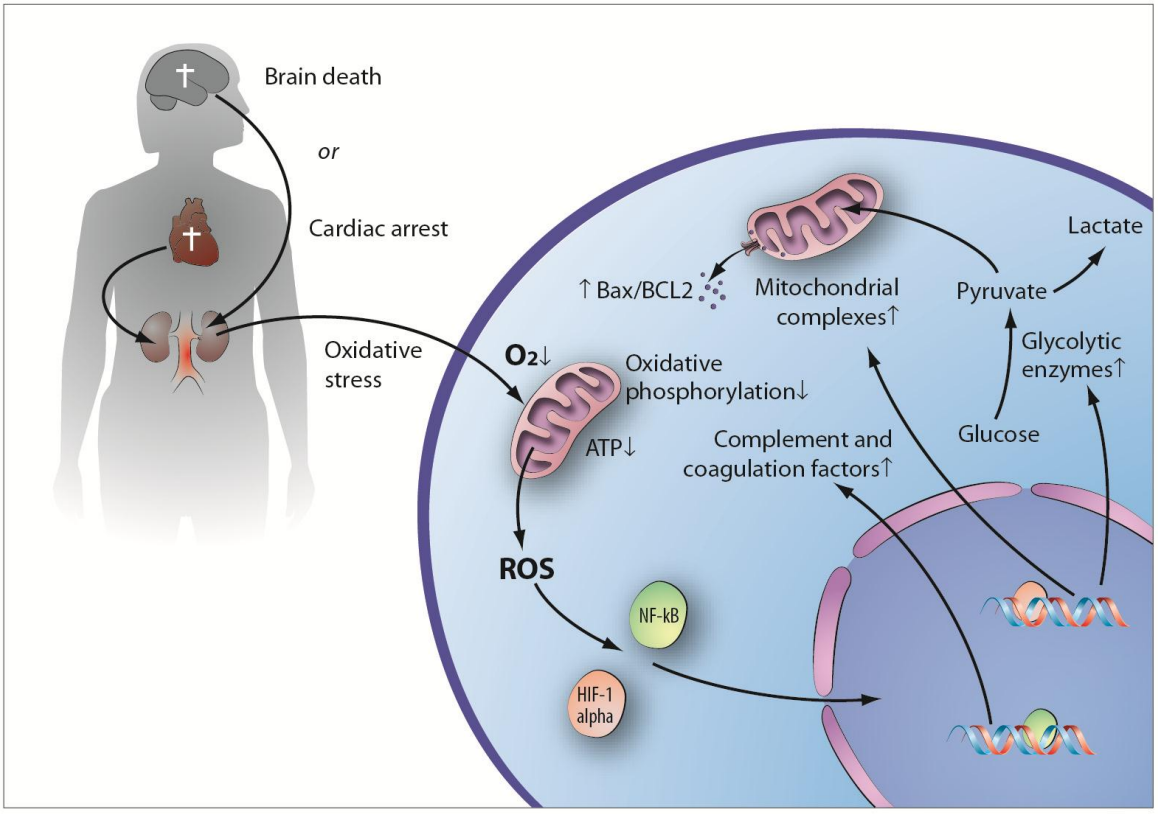

Figure S2: Hypothetical schematic overview of the underlying cellular processes in kidneys from deceased organ donors. Brain death or cardiac arrest in the donor results in hypoxia in the kidney. Hypoxia in renal cells leads to inefficient mitochondrial oxidative phosphorylation and the release of reactive oxygen species (ROS). This triggers a highly conserved evolutionary mechanism via stabilization of hypoxia inducible factor 1, alpha subunit (HIF1A). HIF1A translocates to the nucleus and induces the expression of glycolytic enzymes and mitochondrial complexes. This way, inefficient mitochondrial ATP generation is compensated by increased glycolytic ATP generation. Moreover, fine-tuning of mitochondrial respiration by upregulation of mitochondrial complexes will lead to additional ATP generation. Translocation of NF-κB to the nucleus will enhance the transcription of HIF1a (highly upregulated in DBD and DCD donor kidneys) and complement and coagulation factors. Hypoxia also results in the accumulation of misfolded proteins, which initiates the unfolded protein response, among which the upregulation of the proteasome and induction of chaperones (not shown). Finally, DNA damage by hypoxia results in apoptosis reflected by an increased BAX/BCL2 ratio.

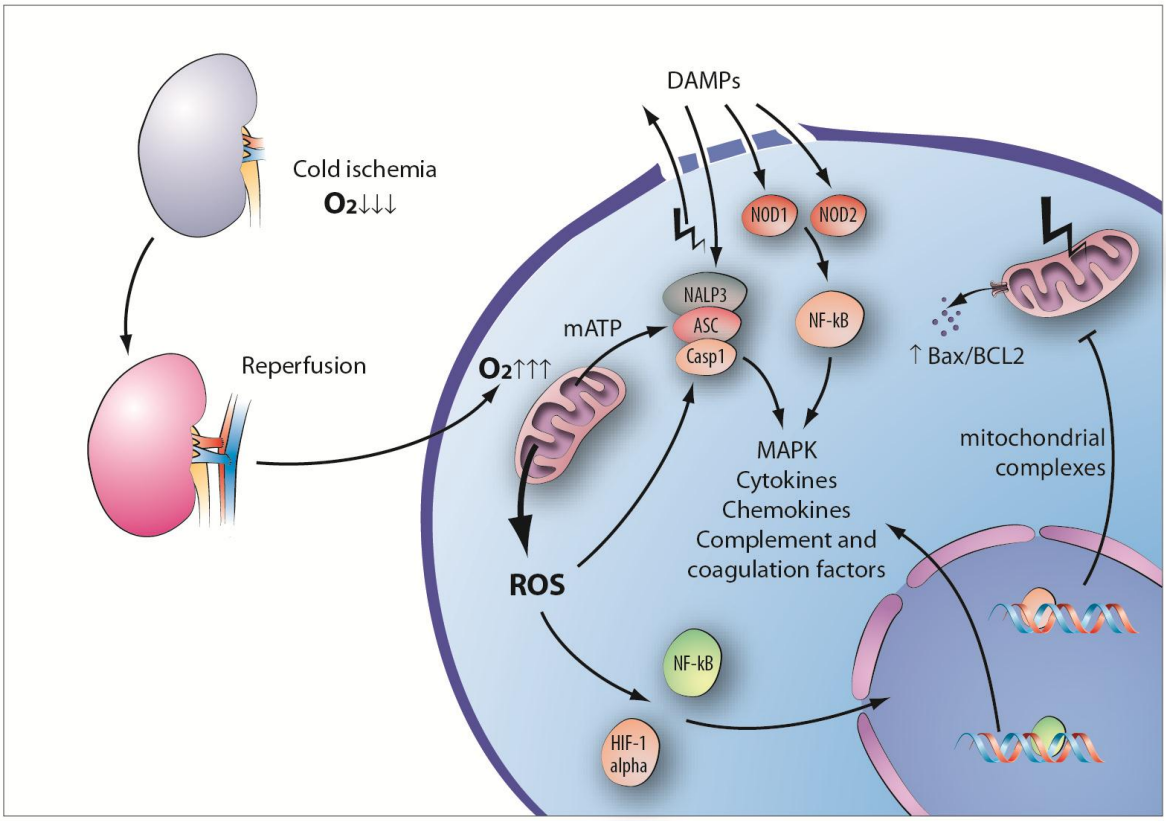

Figure S3: Hypothetical schematic overview of the underlying cellular processes after reperfusion of deceased donor kidneys. After reperfusion of a cold ischemic graft, renal vascular cells are reintroduced to oxygen from the very first moment. Excess oxygen cannot be utilized efficiently by discordant respiratory chain enzymes in ATP depleted mitochondria. This leads to a rapid release of ROS into the cytosol and stabilization of HIF1A and NF-κB. Subsequently, enhanced transcription of inflammatory genes and shut-down of mitochondrial complex genes is initiated. This efficient cell program decreases electron transport and limits further overproduction of ROS during hypoxia. Second, ATP utilization during hypoxia is reduced through decreased transcription of ribosome genes and dissociation of ribosomes from the ER, thereby reducing protein synthesis. Reperfusion results in the release of danger associated molecular patterns (DAMPs) that can activate the NOD-like receptor pathway by activation of NOD1 and 2, which eventually activate NF-kB and MAPK. Furthermore, DAMPs can activate the oligomerization of the NLRP3 inflammasome that leads to the release of cytokines.